Remodelling, Optimization and Characterisation of Absorption Columns to Precipitate Hydrogen Chloride and Sulphur Dioxide

Daniel Mesitscheck
Hochschule für Technik und Architektur, Perolles 80, 1705 Fribourg, Switzerland

The aim of the first part of this thesis is to create an extensive safety documentation for the operation of two absorption columns and a reactor, i.e. the drawing up of checklists, the revision of operating instructions as well as designing a reactions-, resistance- and a HAZOP-list. Besides, a comprehensive substance-data-collection for HCl and SO₂ has been made for the planned use in absorption. A further aspect deals with the rebuilding of glass-raschig-ring-columns to modern high-capacity random and structured packed columns. For the description of these packings an overview of the applied measuring technique and analyses will be given.

The last part of this dissertation explores mass-transfer and hydraulicsof the new baffles. For the mass transfer the investigation of the number of mass transfer units that the new packings provide for the system HCl/H₂O is of immediate importance.

Deposition of Fragrance Precursors on Fabrics

Pitt Allmendinger
ZHAW Zürcher Hochschule für Angewandte Wissenschaften Technikumstrasse 9, 8401 Winterthur, Switzerland

The purpose of this work was to consider the influence on deposition during a laundering. The deposition on cotton was measured under different washing conditions. A broad variety of molecules was investigated. An HPLC analytical method was developed to quantify the interesting substances in a washing emulsion. Adsorption of these substances to the vessel surfaces distorted the detection rate. Due to the addition of liquid detergents the problem could get overcome and adsorption was avoided. As a result, the recovery rate was between 90 to 100 % allowing for a reliable analysis. Since there are no interdependencies between the different substances, the deposition could be analyzed for several substances in the same experiment.

The following interrelations were found: An increasing deposition comes along with an increased substance concentration in the washing liquid. This was observed in the case of liquid detergent as well as with a fabric softener. If the liquid detergent concentration was increased, the deposition decreased. This behavior is caused by the formation of micelles, being formed if the liquid detergent concentration is high enough to allow for. The micelles are able to include the substances.

The ratio of the washing liquid to textile mass influences the deposition also. Using more liquid lowered the deposition. The influence of the molecular structure is not clear. Two different mechanisms have been determined. The first refers on solubility in the washing liquid, taking in account the polarity of the substances. The second refers on interaction between substance and substrate by conjugated pi-bond systems.

Current Position of GC-MS and LC-MS in Clinical and Forensic Toxicology

Hans H. Maurer
Department of Experimental and Clinical Toxicology, Saarland University, D-66421 Homburg (Saar), Germany, e-mail: hans.maurer@uks.eu

Reliable analytical data are a prerequisite for competent expertises in clinical and forensic toxicology. Nowadays, hyphenated mass spectrometric techniques, particularly gas chromatography/mass spectrometry (GC-MS) and liquid chromatography/mass spectrometry (LC-MS), are indispensable tools in clinical and forensic toxicology due to their high sensitivity and specificity. They are used for screening, library-assisted identification, and quantification of drugs, poisons and their metabolites, prerequisites for competent expertises in these fields. In addition, they allow studying metabolism of new drugs or poisons as a basis for developing screening procedures in biological matrices, most notably in urine, or toxicological risk assessment. Concepts and procedures using GC-MS and LC-MS techniques in these areas with special focus on multi-analyte procedures will be presented and discussed [1-7]. The presentation will close with a short discussion of the future position of GC-MS and LC-MS in these fields.

Analytical Chemistry is a field of research which is continuously expanding into a wide variety of interdisciplinary fields of science. The state of the art use of analytical instrumentation is a prerequisite for supporting other fields of research or further development of instrumentation. However, education of students is dominantly hosted in Chemistry and depends significantly on the existing infrastructure. In addition, research in this field becomes highly specialised and is so diverse that lab courses for students are lacking adequate instrumentation which makes “first-hand” education difficult to maintain excellence in education of Analytical Chemistry.

Reviewing the different Universities and Universities of applied sciences within Switzerland indicate however, that we have a large pool of resources missing. Based on this lack of interaction and the potential for improving the teaching, Division of Analytical Chemistry supports the formation of a platform where lectures for students can be “offered and booked”. The lecture topics should be focused on techniques and instrumentation and should be combined with some “educational supportive” examples. Further details how to enter this platform, the benefit from participation will be presented.

Cytochrome P450 enzymes (CYPs) play a central role in the oxidative metabolism of drugs and other xenobiotics. Considering the increasingly large number of chemical compounds in the pipeline of pharmaceutical companies, it is important during the drug discovery/development processes to have rapid, low-cost and automated CYP-based metabolism studies at hand. We have developed a method for such studies and tested it for kinetics and inhibition experiments on CYP2D6. The O-demethylation of dextromethorphan into dextrorphan was thereby used as a probe of the enzyme’s activity. Our approach uses the capillary electrophoresis technique requiring only 100-200 nl of enzyme and substrate/inhibitor solutions for the enzymatic assays. The produced metabolites are detected off-line by UPLC-MS allowing automated and rapidly performed consuming very low amounts of enzyme compared to standard techniques. The proposed approach is thus suited for early developmental processes.

Isotope ratios measured with a multi-collector-inductively coupled plasma mass spectrometer (MCICPMS) always differ from the true isotopic composition of the sample and need to be corrected by internal and/or external standardizations even for interference-free samples. In a study to identify instrumental sources of mass bias, a significant dependence of the measured Nd isotope ratios on the ICP operating parameters was observed (Fig. 1). For conventional nebulization, a range of carrier gas flow rates, sampling depths and acceleration voltages was found for which the absolute mass bias is increased, but more stable upon small variations of the respective parameter than under conditions where maximum signal intensity is measured. Operating conditions that maximize sensitivity resulted in successively heavier isotopic ratios in dependence on the concentration of a matrix (0.1-10 ppm Ho). If measurements were repeated at a higher carrier gas flow rate, the relative deviations between the matrix sample and bracketing standards could be reduced up to 6 times (Fig. 2).

In-capillary enzymatic assays for automated drug metabolism studies on the nanoliter-scale.

Raffaele Curcio, Raul Nicoli, Serge Rudaz, Jean-Luc Veugly
Laboratory of Pharmaceutical Analytical Chemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Boulevard d’Yvoy 20, 1211 Geneva 4, Switzerland

Cytochrome P450 enzymes (CYPs) play a central role in the oxidative metabolism of drugs and other xenobiotics. Considering the increasingly large number of chemical compounds in the pipeline of pharmaceutical companies, it is important during the drug discovery/development processes to have rapid, low-cost and automated CYP-based metabolism studies at hand. We have developed a method for such studies and tested it for kinetics and inhibition experiments on CYP2D6. The O-demethylation of dextromethorphan into dextrorphan was thereby used as a probe of the enzyme’s activity. Our approach uses the capillary electrophoresis technique requiring only 100-200 nl of enzyme and substrate/inhibitor solutions for the enzymatic assays. The produced metabolites are detected off-line by UPLC-MS allowing automated and rapidly performed consuming very low amounts of enzyme compared to standard techniques. The proposed approach is thus suited for early developmental processes.

Isotope ratios measured with a multi-collector-inductively coupled plasma mass spectrometer (MCICPMS) always differ from the true isotopic composition of the sample and need to be corrected by internal and/or external standardizations even for interference-free samples. In a study to identify instrumental sources of mass bias, a significant dependence of the measured Nd isotope ratios on the ICP operating parameters was observed (Fig. 1). For conventional nebulization, a range of carrier gas flow rates, sampling depths and acceleration voltages was found for which the absolute mass bias is increased, but more stable upon small variations of the respective parameter than under conditions where maximum signal intensity is measured. Operating conditions that maximize sensitivity resulted in successively heavier isotopic ratios in dependence on the concentration of a matrix (0.1-10 ppm Ho). If measurements were repeated at a higher carrier gas flow rate, the relative deviations between the matrix sample and bracketing standards could be reduced up to 6 times (Fig. 2).

In-capillary enzymatic assays for automated drug metabolism studies on the nanoliter-scale.

Raffaele Curcio, Raul Nicoli, Serge Rudaz, Jean-Luc Veugly
Laboratory of Pharmaceutical Analytical Chemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Boulevard d’Yvoy 20, 1211 Geneva 4, Switzerland

Cytochrome P450 enzymes (CYPs) play a central role in the oxidative metabolism of drugs and other xenobiotics. Considering the increasingly large number of chemical compounds in the pipeline of pharmaceutical companies, it is important during the drug discovery/development processes to have rapid, low-cost and automated CYP-based metabolism studies at hand. We have developed a method for such studies and tested it for kinetics and inhibition experiments on CYP2D6. The O-demethylation of dextromethorphan into dextrorphan was thereby used as a probe of the enzyme’s activity. Our approach uses the capillary electrophoresis technique requiring only 100-200 nl of enzyme and substrate/inhibitor solutions for the enzymatic assays. The produced metabolites are detected off-line by UPLC-MS allowing automated and rapidly performed consuming very low amounts of enzyme compared to standard techniques. The proposed approach is thus suited for early developmental processes.

Isotope ratios measured with a multi-collector-inductively coupled plasma mass spectrometer (MCICPMS) always differ from the true isotopic composition of the sample and need to be corrected by internal and/or external standardizations even for interference-free samples. In a study to identify instrumental sources of mass bias, a significant dependence of the measured Nd isotope ratios on the ICP operating parameters was observed (Fig. 1). For conventional nebulization, a range of carrier gas flow rates, sampling depths and acceleration voltages was found for which the absolute mass bias is increased, but more stable upon small variations of the respective parameter than under conditions where maximum signal intensity is measured. Operating conditions that maximize sensitivity resulted in successively heavier isotopic ratios in dependence on the concentration of a matrix (0.1-10 ppm Ho). If measurements were repeated at a higher carrier gas flow rate, the relative deviations between the matrix sample and bracketing standards could be reduced up to 6 times (Fig. 2).
A large number of biological species have intrinsic fluorescence excited in the UV region of 260-280 nm. UV laser excitation is an attractive alternative to tag these compounds with fluorescence labels excited at visible region. In this contribution we present a deep UV fluorescence lifetime microscopy system based on a mode-locked diode-pumped picosecond deep UV laser. The described setup is well-suited for biological applications for ultrasensitive detection of intrinsic fluorescence. (1) Label-free detection of single protein molecules. We investigated the bursts of autofluorescence photons from tryptophan residues in β-Galactosidase molecules from Escherichia coli (Ecβ Gal) and fluorescence correlation spectroscopy of Ecβ Gal. The results demonstrate that deep UV laser-based fluorescence lifetime microscopy is useful for identification of biological macromolecules at the single molecule level using intrinsic fluorescence. (2) Label-free detection of antibody/antigen and protein/drugs interactions. A label free method for detection of Ecβ Gal/anti- Ecβ Gal interactions and protein/drugs interactions have been demonstrated by means of steady-state and time-resolved fluorescence spectroscopy. The interaction can be monitored by fluorescence lifetime changes between free components in the interaction system and corresponding complex. Energy transfer between tryptophan and bound drug in protein-drugs complexes has been observed. (3) One-dimension miniaturized polyacrylamide gel electrophoresis with native fluorescence detection. The mixture of three biological compounds (β-Galactosidase from Escherichia coli, apo-Transferrin and bovine serum albumin) have been separated using miniaturized gel electrophoresis and a staining free detection limit below 80 pg per band has been achieved.


---

Environmental proteomics for the analysis of stress response in Chlamydomonas reinhardtii (green algae)

Marc J-F Suter, Holger Nestler, René Schönökerberger, Victor J Nesaty
Eawag - Swiss Federal Institute of Aquatic Science and Technology
Überlandstrasse 133, 8600 Dübendorf, Switzerland

Multidimensional protein identification technology (mudPIT) has become a popular tool for analyzing complex protein extracts [1]. It is capable of detecting subtle changes in the proteome of an organism in response to multiple stressors, and is a promising tool for ecotoxicological risk assessment. In addition to identifying new protein biomarkers, it can also help to provide insights into modes of toxic action. The green alga Chlamydomonas reinhardtii presents an attractive model for studying multiple stressor effects. Exposure to the herbicides diuron and paraquat in combination with UV radiation were analyzed in triplicates using X!Tandem and The Open Mass Spectrometry Search Algorithm (OMSSA). MudPIT analysis of the protein extracts from control samples showed significant variance in protein identification and reproducibility. This clearly improved when acquiring charge state +1 in addition to +2 and +3. Exposure to various herbicides and UV radiation showed significant changes in protein levels in C. reinhardtii. As expected from the underlying mode of action, paraquat exposure resulted in the induction of both chloroplastic (Fe) and mitochondrial (Mn) superoxide dismutases. Also, exposure with photosensitizer Rose Bengal, known to induce oxidative stress via singlet oxygen generation in C. reinhardtii, led to increased levels of the glutathione peroxidase GPX5, confirming gene expression results previously published. Other changes on the proteome level have been observed, but more careful investigation using pathway and cluster analysis is required in order to understand the underlying mechanisms of stress response.

Chemical cross-linking in combination with mass spectrometry has emerged as a powerful tool for the structure elucidation of tertiary or quaternary structures in proteins. Despite many applications, only a few studies [1, 2] concerning the reactivity and selectivity of cross-linkers towards certain amino acids have been reported so far. The commonly applied N-hydroxy succinimide esters (NHS esters) are usually described to be selective for only observed for the lysine. For the peptides Fmoc-EGGXGZGGE with \((X,Z) = \text{His,Tyr}; \text{His,Ser}, \text{Tyr} \text{and Ser}\) showed high reactivity due to arginine were clearly identified by MS/MS measurements as reaction sites besides the N-terminus and lysine. In most cases, the reaction of Tyr, Ser, Thr and Arg was only observed as intra-link with a primary amine. Our data imply that the neighboring amino acids play an important role for the reactivity and selectivity of chemical cross-linkers.

---

**Perfluorinated chemicals (PFCs) are widely used and are being detected in the environment, wildlife and humans. Conventional wastewater treatment has limited effectiveness in removing PFCs from aqueous waste streams, and, thus, wastewater treatment plants (WWTPs) act as point sources to the aquatic environment. Some PFCs such as perfluorooctane sulfonate (PFOS) sorb partially onto sewage sludge and therefore the sewage sludge produced in a WWTP may be an important sink for PFCs. A survey of anaerobically stabilized sewage sludges in the Canton of Zurich was performed to determine the levels of PFCs and to find possible hotspots. An analytical method was developed based on negative liquid solvent extraction and analysis by HPLC coupled to a tandem mass spectrometer using negative electrospray ionization. For total perfluorocarboxylates (e.g., PFOA), the concentrations ranged from 14 to 50 µg/kg. The concentrations for total perfluorosulfonates (mainly PFOS) ranged from 15 to 610 µg/kg. Among the twenty studied samples, the levels of six were above 100 µg/kg. These data indicate the widespread occurrence of PFCs in municipal WWTPs.**

---

**Probing switch peptides by tandem mass spectrometry:**

**on/off state quantification and applications**

Hisham Ben Hamidane, Enrico Condemi, Adrien Schmid, Horst Vogel, Manfred Mutter, Yury O. Tsybin

Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

Protein aggregation and formation of toxic oligomeric species is a fundamental biological problem related to many neurodegenerative diseases. Switch peptides that mimic the primary structure of amyloid \(\beta\), a neuropeptide involved in Alzheimer’s disease, allow triggering or inhibiting peptide oligomerization in a controlled manner [1]. We apply tandem mass spectrometry to monitor and improve characterization of switch peptide transition states and oligomerization kinetics.

Switch peptides containing molecular switches (pH and UV dependent) and derived from the aggregation promoting sequence of amyloid \(\beta\) were synthesized by solid phase FMOC biochemistry. “On” and “Off” states were monitored by solution phase circular dichroism measurements and gas phase tandem mass spectrometry (MS/MS) using an 11 T Fourier transform ion cyclotron resonance mass spectrometer with simultaneous electron capture dissociation (ECD) and infrared multiphoton dissociation (IRMPD).

MS data indicates that peptide oligomerization is a process involving the formation of dimers and trimers. MS/MS applied to monomers allows quantitating the amount of peptide in the “On” state relative to the “Off” state. The use of ECD is of particular importance in the current application due to high repeatability and reliability of the method towards relative product ion abundance quantitation. Further correlation between secondary structures and fragmentation patterns is under investigation. Advantages of adding MS and MS/MS data to classical aggregation/oligomerization kinetics studies will allow us to better understand the complexity of low molecular aggregates and correlate peptide morphology with toxicity in neurodegenerative diseases.

The stochastic fluctuations in gene expression can lead to different phenotypes within isogenic cell populations. Thus, a single cell approach to the cause of the difficulties to detect the wide chemical variety constituting the metabolome and due to the minute amount of sample available in each cell, the detection of metabolites in single cells is very challenging.

We exploit matrix-assisted laser desorption/ionization – mass spectrometry (MALDI-MS) to detect endogenous cell metabolites in single yeast cells. MS can cope with the diversity of the molecules under study and a specially adapted MALDI method can reach the sensitivity necessary to detect the small amount of sample contained in a single cell. Microfluidic devices are employed for cell sampling, handling and preparation. The coupling of microfluidics devices to MALDI-MS detection allows to perform a microscale preparation that can deal with the needs in terms of sensitivity, sample consumption, and high throughput for single yeast cell detection of metabolites. Using the method presented, MALDI-MS spectra of an extract of a single yeast cell lysate are obtained and detection of endogenous metabolites was achieved. The coupling of microfluidics and MALDI results in a powerful analytical tool for system biology that, by monitoring metabolome dynamics at the single cell level, allows to observe and analyze the stochasticity of biochemical processes giving a novel view into the cell behavior.

Analytical Chemistry

**Microfluidic Chip for Mass Spectrometric Detection of Metabolites in Single Cells**

Andrea Amantonico¹, Ralph Streicher², Nils Goedecke² and Renato Zenobi³

¹ Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zürich, Switzerland; ² Department of Biosystems Science and Engineering, ETH Zürich, 4058 Basel, Switzerland

Fetti formation (pM) during Fe-redoxcycling driven by photo-reduction of Fetti-complexes - Implications for ROS production.

Adrian A. Ammann⁴ and Kathrin Barbeau⁴

a) Environmental Toxicology, EAWAG, Überlandstr 133., 8600 Dübendorf, Switzerland.
b) Scripps Institution of Oceanography, Univ. of California San Diego, CA, 92037 La Jolla, USA

Analytical Chemistry

**Fast analysis of doping agents by UPLC-QTOF-MS**

**Part I: Screening analysis**

Flavia Badoud¹, Elia Grata¹,², Laurent Perrenoud¹, Lidia Avois¹, Martial Sausy¹, Serge Rudaz², Jean-Luc Veuthey¹.

¹Swiss Laboratory for Doping Analysis, Institut Universitaire de Médecine Légale, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Chemin des Croisettes 22, 1066 Epalinges, Switzerland

²Laboratory of Analytical Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Boulevard d’Yvoy 20, 1211 Geneva 4, Switzerland

More than 150’000 urine samples are analysed per year over the world for doping control. During major sporting events, results are required within 24 or 48 hours after samples reception meaning that the time delivery response must be as short as possible. Therefore, the entire analytical process, including sample preparation, analyte separation, selective detection and data mining needs to be optimized. The aim of this study is to analyze more than 100 doping agents excreted under their free form in urine, including diuretics, masking agents, β-blockers, stimulants, narcotics, and so on. Regarding sample preparation, the dilute and shoot was selected as the simplest and fastest sample preparation method. Because UPLC offers the opportunity to obtain short analysis time, while maintaining or even enhancing efficiency and sensitivity, it was used for separating analytes with a linear gradient of water/acetonitrile containing 0.1% formic acid in 6 minutes. Due to the peaks thinness (6 sec), high acquisition rates detector afforded by a quadrupole time of flight (QTOF) MS is required for selective detection by measuring exact mass of analytes.

Method sensitivity was evaluated by measuring the LOD of more than 100 substances in urine. An average value of 20 ng/mL was reached. The matrix effect was evaluated and the method validated.

**Analytical Chemistry**

**Improving Ion Transmission for Inductively Coupled Plasma Mass Spectrometry (ICPMS) – Back to Basics.**

Tatiana Egorova, Rolf Dietiker, Bodo Hattendorf, Detlef Günther

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

Inductively coupled plasma mass spectrometry (ICPMS) is an established technique for trace and ultra-trace element determinations in a large range of applications. It offers very high sensitivity for almost the entire periodic table with a wide dynamic range and a flexible coupling to many existing sample introduction techniques. Nonetheless the detection efficiency of current instruments is severely limited and only one ion is detected from 5000 – 50000 atoms introduced into the ion source [1]. This is mainly a result of the design of the interface required to transfer the ions from the ICP to the high vacuum in the mass spectrometer in its classical sampler-skimmer configuration.

To improve ion transmission, a new interface configuration is currently under investigation. It involves a specially designed transfer stage incorporating a so called “ion funnel” [2]. This ion funnel is composed of a stack of ring electrodes with decreasing inner diameter, located downstream a sampler cone. The transmission properties of such a device, when connected to a plasma ion source are studied in detail. Dependence of ion beam characteristics on pressure and applied fields will be presented.

**Analytical Chemistry**


Nuclear receptors, such as the retinoic acid receptor (RAR), interact not only with their ligands but also with other types of receptors. Previous biological analyses (gel-shift) have shown that two coactivators and a specific DNA sequence bind to the receptor but also induce a partial dimerization of RAR. Mass spectrometry (MS) has been shown to be a powerful technique to analyze changes such as these. Nondenaturing nanoelectrospray (nanoESI) was used to study these interactions. The RAR protein was incubated with either coactivator peptides (PF108 and PF124) or with various DNA sequences (DR5, As and C3) and then analyzed with MS. We were able to detect RAR alone as well as complexed with the two coactivator peptides.

A complex between protein RAR and the double strand DR5 was detected after cross-linking of the high-mass MALDI. Further, nanoESI showed that RAR binds the single strand DR5. Moreover, DNA induced the dimerization of RAR. We were able to detect the formation of RAR dimers, indicating that two RAR molecules bind to the DNA sequence.

First, the importance of the uncertainties in initial concentrations is shown for the acid-catalysed reaction of benzophenone with phenylhydrazine in THF repeatedly investigated. We then present an experimental validation of the method using the acid-catalysed reaction of benzophenone with phenylhydrazine in THF repeatedly investigated. We were able to cover an important proportion of the observed standard deviation in the rate constants obtained from all experiments. Differences between results obtained from UV-vis and mid-IR spectroscopy, as well as the importance of the doping rate in the design of semi-batch experiments are discussed. We then present an experimental validation of the method using the acid-catalysed reaction of benzophenone with phenylhydrazine in THF repeatedly investigated.

Analytical Chemistry

Comparison of non-matrix matched calibration using ns- and fs-LA-ICP-MS in geochronology

Karin Birbaum, Detlef Günther

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

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has become an important technique in the analysis of solid samples. As a spatial resolved, ‘quasi’ non-destructive and sensitive technique it is used for geochronology. The use of glass standards (NIST 610) for age determination in Zircons (ZrSiO₄) has been discussed in the literature, but the reported results are contradictory to each other [1-3]. Therefore, we show the insights into that discrepancy. The ablation behavior of Si, Zr, Pb and U in the two matrices, glass and Zircon, was studied using ns- and fs laser ablation. For the comparison of the two laser systems, the detection efficiencies (detected ions/ablated atoms) were determined. For the estimation of the number of ablated atoms, the ablation rate and the material density were considered. To broaden the scope of materials, the same procedure was carried out with minerals having different concentrations of Si.

Analytical Chemistry

Rapid Detection of Explosives on Human Skin by Neutral Desorption Extractive ElectrospRAY ionization (ND-ESI) Mass Spectrometry

Huanwen Chen1,2, Bin Hu1, JianQiang Li1, Konstantin Chingin1*, Renato Zenobi2

1 East China Institute of Technology, Fuzhou, 34400 China
2 Department of Chemistry and Applied Biosciences, ETH Zürich, CH 8093 Switzerland

Low picograms of explosives 2,4,6-Trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), triacetone triperoxide (TATP) and nitroglycerin (NG) were successfully sampled from human skin in vivo, using a novel neutral desorption (ND) device1 with a nitrogen gas beam, for sensitive detection and rapid identification by extractive electrospray ionization (EESI) tandem mass spectrometry. Without any sample pre-treatment, the fragile metal complexes of explosives were gently sampled from skin. For most explosives on a skin surface, ND-ESI-MS provided a LOD (limit of detection) in the low picogram range. A linear dynamic range of 4 orders of magnitude (0.01 ng-100 ng) was found for RDX using the characteristic fragment (m/z 237), generated from (RDX+ CH3COO) under CID conditions. The signal intensity for either RDX or TNT was maintained at the same level when the length of the sample transfer line varied from 2 cm to 200 cm. No serious sample carryover effect was found using a Teflon tube (200 cm length; 3 mm I.D.) as the sample transfer line. Less than 1 second was required to record a spectrum when a 200 cm-length tube was used to transport the sample plume generated by the ND process. Furthermore, the EESI source requires no optimization when changing samples, facilitating high throughput analysis of complex samples. The capability of ND-ESI-MS for remote analysis of explosives provides an alternative way for convenient fast screening of explosives under hazardous environment.

Reference:

Analytical Chemistry

Electrochemical Detection of Tagged Proteins by SEC

Fernando Cortés-Salazar, Jean-Marc Busnel and Hubert G Girault1.
Laboratoire d’Electrochimie Physique et Analytique, Ecole Polytechnique Fédérale de Lausanne, Station 6, CH-1015 Lausanne, Switzerland.

Electrochemical detection of proteins by scanning electrochemical microscopy (SEC(M)) has been performed mainly by coupling it with common protein detection techniques like immuno-detection or metal staining. 1,2 Thus, general or specific protein detection can be achieved in separate experiments. Here in, we propose a new protein detection method based on the protein tagging with benzoinonine. This new methodology is less time consuming than immuno-detection or metal staining and can be used for general or specific protein detection, thanks to the pH dependent specificity of the tagging reaction.3 Thus, by using this method relevant information for protein identification and sensitive protein quantification (5 ng per band) can be obtained at the same time.

The electrochemical detection principle of tagged proteins is based on the electrochemical reduction of a redox mediator (ferrocyanide) on a scanning electrode surface and its chemically recycling by the quinone-protein adducts on the polypyrilène fluoride (PVDF) membrane (see Figure 1).

Figure 1. a) Electrochemical detection principle of tagged proteins with benzoinonine by mediated reduction of quinone-protein adducts. b) Constant height SEC(M) image of a tagged protein spot (BSA, 500 ng) over PVDF with benzoinonine in a solution of K4[Fe(CN)6] 2.9 mM with KNO3 0.11 M. Working electrode Pt (20 μm diameter); Counter electrode Pt, quasi-reference electrode (QRE) Ag, EAg = +0.1 V vs QRE. Probe-substrate distance = 4 μm, step size = 50 μm and translation rate = 50 μm/s.

Analytical Chemistry

Stable isotope labeling-based protein quantitation probed by top down mass spectrometry

Thibaut Douche, Adrien Schmid, Michael Affolter, Martin Kussmann, Yury O. Tsybin
Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland
Nestlé Research Centre, 1000 Vers-chez-les-Blanc, Switzerland

The stable isotope-based protein quantitation method, ANIBAL, implemented in bottom-up mass spectrometry, is based on protein chemical derivatization with two chemical tags, aniline and benzoic acid, targeting carboxyl- and amino side chains, respectively, at protein level [1]. Here, we extend the application of the ANIBAL method to top-down mass spectrometry to distinguish protein isoforms and deliver more complete protein characterization.

Chemical derivatization with carbodiimide chemistry was employed to label proteins, e.g. ubiquitin, first in 1M pyridine pH 5.0 containing either light or heavy aniline, followed by the addition of an EDC solution and second in HEPES 200 mM pH 8.0 containing light or heavy benzoic acid, respectively. The extent of derivatization was monitored by MALDI TOF MS. In-depth sample analysis was performed on an 11 T LTQ FT-ICR MS/MS. The extent of derivatization was monitored by MALDI TOF MS.

Top-down high-resolution mass spectrometry of ANIBAL stable-isotope labelled proteins revealed deep insights into the obtained protein population and provided information complementary to bottom-up mass spectrometry. Preliminary analysis of derivatized ubiquitin reveals a population of 10- to 12-fold aniline labelled proteins, which corresponds to 83% to 100% yield of theoretically possible derivatization. Complementary to bottom-up MS, the top-down MS approach allows for localization of ANIBAL labels on the protein sequence in heterogeneous mixtures of labeled proteins thereby indicating protein structural preferences for chemical derivatization. The two main challenges are the reduced solubility of the intact protein after derivatization and the observed impurities.

Analytical Chemistry

Continuous nitrous oxide isotopomer measurements based on quantum cascade lasers

Joachim Mohl, Helen Waechter, Bela Tuzson, Markus W. Sigrist, Lukas Emmenegger

(1) Empa, Ueberlandstrasse 129, CH-8600 Dübendorf, Switzerland
(2) ETH Zurich, Schaffnattstr. 16, CH-8093 Zürich, Switzerland

The intramolecular distribution of $^{15}$N in N$_2$O can be used to obtain important information on the geochemical cycle of N$_2$O because isotopic fractionation is characteristic for different processes. The isotopomers $^{15}$N$^{15}$NO and $^{15}$N$^{14}$NO (or $^{15}$NO and $^{14}$NO) have the same mass and can only be determined by mass spectrometry (IRMS) through the complex analysis of NO$_2$ and N$_2$O. In contrast, laser spectroscopy offers the inherent advantage of site selectivity combined with high sensitivity and time resolution.

We present a laser spectrometer consisting of a thermoelectrically (TE) cooled, pulsed quantum cascade laser (QCL) at 4.6 µm, a multipass cell with a path length of 56 m and a TE cooled IR detector, allowing continuous, liquid nitrogen-free operation. With this instrument, the isotopemixing ratios of NO$_2$ can be determined with high precision. Using a prototype laser, a precision of 3 ‰ was achieved.

Online Control of Ethanol Fermentation by a MFC-type Biosensor

Marie-France Favre, Raphael Ducommun, Fabian Fischer
Life Technologies Institute, HES-SO Valais, Route du Rawyl 47, 1950 Sion, Switzerland, E-mail: fabian.fischer@hevs.ch

A microbial fuel cell type biosensor integrated in a 500 mL fermenter was constructed and employed for ampero- (µA) and potentiometric (mV) measurements. The aim was to non-invasively monitor the fermentation process by saccharomyces cerevisiae and to detect the end of sugar consumption. Three different sensor setups were tested to register online electrochemical signals produced by the ethanolic metabolism of glucose and fructose (artificial wine). First set-up: a reference electrode was used to record potentiometric values, which rose from 0.26 to 0.5 Volt in about 10 hours during the growth phase. In a second set-up a combination of ampero- and pseudo potentiometric measurements delivered a maximum voltage of 35 mV. In a third type of arrangement a reference electrode was added to the anodic fermentation compartment to record separate ampero- and potentiometric measurements. In this case the reference potential rose to 0.44 Volt while the current maximum recorded by the working electrodes, reached 27 µA. To compare the electrochemical signals with standard values, the fermentation was also monitored by optical density (600 nm) analysing biomass production. HPLC with an Amimon column and RI detector was used for fructose and glucose conversion, and ethanol production was analyzed by GC with methanol as internal standard.

Analytical Chemistry

Extractive Electrospray Ionization Mass Spectrometry of Breath for Monitoring Intake of Pharmaceuticals in Real-Time: Valproic Acid

Gerardo Gamez, Liang Zhu, Konstantin Chingin, Huanwen Chen, Renato Zenobi*

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

Monitoring the levels of pharmaceuticals and their metabolites is of utmost importance. This is especially true when there are changes in dosage, clinical condition or concomitant medications. For example, if the antiepileptic drug valproic acid is below therapeutic levels it will not manage seizures efficiently and above a certain threshold adverse effects become more frequent. Thus, frequent blood tests are required to supervise the plasma concentration. However, blood sampling is painful, invasive, requires specialized personnel, and produces hazardous waste. An alternative that overcomes these disadvantages is breath analysis. Recently, extractive electrospray ionization (EESI) mass spectrometry was successfully applied in our laboratory for analysis of breath. Basically, the volunteers exhale through a tube that guides the breath to the area where a pure solution is electrosprayed onto a MS sampling interface. Here, the compounds in the breath can be ionized and later analyzed. This technique requires no sample storage or pre-treatment, thus analysis can be performed in real-time. We have found that unique features in the EESI mass spectral fingerprints of individuals under valproic acid can be used for monitoring its intake and perform pharmacokinetic studies. Current work towards identifying these biomarkers and how they relate to the valproic acid plasma concentration is underway. However, it is evident that EESI MS of exhaled breath allows the real-time measurement of drugs intake in a pain-free and non-invasive manner, which will ultimately permit better patient therapy management in the clinical setting.
For doping control, the analysis of samples is achieved in two steps: a rapid screening and, in case of a positive result, a confirmatory analysis. The latter is dedicated to unambiguously determine the presence of a forbidden analyte. To definitively incriminate an athlete, following the WADA regulations, different samples have to be simultaneously analyzed: urine spiked with the corresponding standard, blank urine and the suspect sample. All materials should be submitted to the entire analytical process. After a specific sample preparation, the putative compound is analyzed on a short UPLC column (50 mm) and detected by mass spectrometry to obtain accurate mass of parent and fragment ions. Following the WADA expectations, spectra obtained for the spiked and suspect urine must fit in the number of fragments and intensity ratio. Whereas, in the blank urine, no specific fragment ions had to be found.

A highly selective method was developed for each doping agent (more than 100 substances) by setting the cone voltage and the collision energy. Thanks to the QTOF analyser, it was possible to obtain in the same chromatographic run, a TOF-MS and a QTOF-MS/MS information. At the expected retention time an acquisition of parallel scanning at two voltages allows to obtain molecular ions (precursor) and fragment pattern with high mass precision (<5 ppm).

Nowadays, one of the main objectives of analytical laboratories is to develop rapid and efficient procedures for performing qualitative and quantitative analyses. The pharmaceutical industry is interested to cope with a large number of samples and to reduce the time response delivery, particularly for quality control of pharmaceutical formulation. A simple strategy consists in decreasing column length since analysis time is directly proportional to the latter. Because efficiency is also affected, a simultaneous particle size reduction is mandatory to limit resolution decrease. Therefore, ultra-short columns (i.e. 10 mm) filled with 1.9 µm particles were packed and evaluated. Basic chromatographic performance (efficiency, backpressure, etc) were evaluated through Van Deemter curves and compared with 150 mm, 5 µm and 50 mm, 1.9 µm columns packed with identical stationary phase chemistries. Several simple pharmaceutical formulations (e.g. local anaesthetic, spasmolytic, anti-hypertension drugs) were selected and a method was developed and validated for each of these substances following ICH guidelines. This study demonstrates that 10 mm columns packed with 1.9 µm particles represents a good alternative to conventional columns for simple pharmaceutical formulations analysis. Equivalent quantitative performance with a significant analysis time reduction (up to 60-fold) was obtained with the use of an optimized chromatographic system, to avoid extra-column dispersion.


Analytical Chemistry

Ultra-fast separations with 10mm columns packed with sub-2µm particles: Some qualitative and quantitative perspectives in pharmaceutical analysis

Davy Guillarme, Cedric Schelling, Serge Rudaz, Jean-Luc Veuthey

Laboratory of Analytical Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Boulevard d’Yvoy 20, 1211 Geneva 4, Switzerland

Very efficient separations in isocratic and gradient modes using UHPLC technology at ambient and high temperature.

Davy Guillarme, Elia Grata, Gaëtan Glauser, Jean-Luc Veuthey, Jean-Luc Wolfender, Serge Rudaz

1 Laboratory of Analytical Pharmaceutical Chemistry, 2 Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva 4 Switzerland.

In isocratic mode, experimental conditions to reach the highest possible efficiency were determined using the kinetic plot representation (ΔPmax=1000 bar). By limiting the column length to 450 mm, the maximal plate count (around 100’000) with the lowest analysis time was achieved using a mobile phase temperature of 90°C. An excellent agreement was found between experimental efficiency values and predicted values from kinetic plots, for many different set of conditions. For a given gradient length in UHPLC, the longest column does not necessarily provide the maximal peak capacity. Therefore, a compromise should be found between column length and efficiency to reach the highest peak capacity. We used the (N, tR) data from the kinetic plot method to demonstrate that a 150 mm column should ideally be selected for gradient lengths up to 100 min, while the 3x150 mm columns coupled in series was attractive only for tR>350 min. At higher temperature (90°C), peak capacities were increased by about 30% vs. 30°C, for a constant gradient length. Finally, some separations of standardized complex plant extracts produced at the industrial level were carried out in gradient mode using a UPLC-TOF-MS instrument. These separations clearly demonstrated the benefits of working in optimal conditions to obtain the best resolution per unit of time.
Analytical Chemistry

Contactless Conductivity Detection for Microseparation Techniques

Peter C. Hauser,
Xiao Yang Gong, Aiping Schuchert-Shi, Worapan Pornsila,
Department of Chemistry, University of Basel, Spitalstrasse 51, 4056 Basel, Switzerland

Capacitively coupled contactless conductivity detection (C4D) for capillary electrophoresis has gained considerable popularity over the last few years. The method generally allows the facile detection of all charged analytes with good sensitivity, including those species which cannot be quantified with optical means. The principles have been thoroughly investigated and a comprehensive understanding of the fundamental properties has been reached.

In our laboratory, a range of projects based on C4D are carried out. In clinical analysis the method is suitable for the determination of inorganic electrolytes as well as of organic species of interest which are not UV-active. Further applications are in therapeutic drug monitoring and also possible is the use of the method in enzymatic assays for neutral species such as urea and ethanol.

A new portable and all-battery powered CE-instrument has been designed and tested in the Tasmanian wilderness. A further instrumental advance has been made by coupling CE with sequential injection analysis (SIA) for automated injection and capillary flushing. The approach should be useful in process analysis. Highly rapid separations in short conventional capillaries are also feasible with this arrangement.

Contactless conductivity detection was furthermore shown to be suitable for detection in HPLC for the quantification of non-UV-absorbing species. The method is, within limits, compatible with gradient elution and particularly suitable for detection when using micro-scale monolithic columns.

Analytical Chemistry

Applications of Capillary Electrophoresis with Contactless Conductivity Detection in Clinical Analysis

Worapan Pornsila, Qi Jin Wan, Gamze Belin, Wai Siang Law, Peter C. Hauser

Department of Chemistry, University of Basel, Spitalstrasse 51, 4056 Basel, Switzerland

Clinical analysis of inorganic and small organic species in biological fluids has been successfully carried out with capillary electrophoresis using contactless conductivity detection. The inorganic cations (potassium, ammonium, sodium, calcium and magnesium) and anions (nitrate and sulfate), which play an important role in the composition of human body fluids, were successfully determined in human serum and urine [1]. The determination of valproic acid, a small organic molecule which is an anticonvulsant and mood-stabilizing agent, was also achieved [2], and the method can be used in therapeutic drug monitoring (TDM). Similarly the antibiotic tobramycin was determined in plasma samples [3]. Currently being investigated is the determination of small native organic ions of clinical interest such as uric acid, lactate and pyruvate.


Analytical Chemistry

Monitoring of Enzymatic Reactions with Capillary Electrophoresis Using Contactless Conductivity Detection

Aiping Schuchert-Shi, Peter C. Hauser

Department of Chemistry, University of Basel, Spitalstrasse 51, 4056 Basel, Switzerland

The use of capillary electrophoresis with contactless conductivity detection was evaluated for the monitoring of enzymatic reactions. The non-ionic species ethanol, glucose, ethyl acetate and ethyl butyrate were made accessible for analysis by capillary electrophoresis via charged products or byproducts obtained in enzymatic conversions using hexokinase, glucose oxidase, alcohol dehydrogenase and esterase [1]. Two of the reactions, namely the conversion of glucose with glucose oxidase and that of ethylacetate with esterase, were also successfully demonstrated on a microchip-device. The determination of urea in human blood as clinical application of this method was investigated. The results were compared with the established methods and were found to be very close [2].

The digestion of proteins with pepsin and trypsin, important in food chemistry and in proteomics, can also be successfully monitored by capillary electrophoresis with contactless conductivity detection. The method was furthermore applied to monitor the enantioselective hydrolysis of esters of amino acids with lipases. The enantiomeric excess (e.e.) as well as the product yield were studied. Lipases from porcine pancreas and wheat germ were compared with regard to their efficiency for the hydrolysis and enantioselectivity. Studies are also in progress on the application of the method for the investigation of acetylenoholsterase inhibitors such as the drug galantamine.


Analytical Chemistry

Separating Stereoisomers Using Capillary Electrophoresis with Contactless Conductivity Detection

Xiao Yang Gong, Peter C. Hauser

Department of Chemistry, University of Basel, Spitalstrasse 51, 4056 Basel, Switzerland

The separation and quantification of enantiomers is an important application of capillary electrophoresis as it is possible to use even expensive chiral reagents due to the low buffer volumes employed. However, non-UV-active or non-fluorescent compounds usually have to be chemically derivatized in order to allow detection. Conductivity detection makes such compounds directly accessible. It was found possible to determine many small amines in their protonated form by using a combination of a non-charged cyclodextrin and a chiral crown-ether as selectors in the separation buffer [1-3]. Also successful with this approach was the separation of the stereoisomers of di-, tri- and tetrapeptides which are otherwise difficult to distinguish [4]. Currently investigated is the separation of the enantiomers of small organic acids of biological importance, such as lactic acid.

Protein kinases have emerged as a major drug target in the last years and therefore tools are required for a rapid classification of inhibitors according to their affinity for a certain target. We are comparing different nanoelectrospray mass spectrometry (nanoESI-MS) based methods to quantify binding affinities and qualitatively determine, by competition experiments, the relative affinity of several clinical inhibitors. Method 1 is based on monitoring the noncovalent complex signals of two different inhibitors competing for binding to protein [1]. The binding affinity order obtained for p38 was: BIRB796 > VX-745 > SB202190 > BIRB analogue 5 > PD-173074, and for Lck: CGP076030 > BIRB796 > PP1 ≈ PP2 > CGP062464. Method 2 is based on ligand depletion [2]. The signal of two inhibitors is monitored for different concentrations of protein. The binding affinity order obtained for p38 was: VX-745 > SB202190 > BIRB analogue 5 ≈ Bay43-9006 > BIRB analogue 4 > PD-173074, and for Lck: Bay43-9006 > CGP062464 ≈ CGP076030 > PP2 > PP1 > Tarceva.

With few exceptions, the results of both methods agree well. The advantages and disadvantages of the used methods will be discussed. The qualitative binding orders obtained are compared to standard IC50 measurements. Sample consumption, speed of the measurements and ease-to-use of the nanoESI-MS based methods versus IC50 measurements will also be compared.


Lysozyme (14.4 kDa), BSA (66.4 kDa) and beta-galactosidase (116 kDa) were successfully separated and determined directly within 80 seconds using a laboratory-made miniaturized capillary electrophoresis apparatus provided with a confocal fluorescence spectrometer. Several parameters controlling on the detection limits, including focusing effect, laser power and buffer composition were tested and optimized. Separation buffer was 10 mM phosphate containing 4 mM CTAB at pH 2.5. The LOD values for lysozyme, beta-galactosidase and BSA were found as 9.0, 13.0 and 55 fg/ul, respectively. This miniaturized CE system offers a lot of advantages for protein analysis. First; it provides reproducible separation reducing wall-adsorption effects at low pH. Second, the analysis time observed from our system is in the range of chip electrophoresis applications. And finally, LOD values for standard proteins are much more lower than that of obtained from traditional gel electrophoresis method.

The potential of laser sampling has been continuously described in numerous studies [1]. The high spatial resolution sampling and high sensitivity makes LA-ICP-MS an attractive technique for determining major, minor and trace elements in solid samples. The capabilities of the technique has been successfully applied in the analysis of precious, gold-matrix-based samples [2-3]. New gold reference materials were characterized (element distribution and composition) and tested for investigation of Pre-Columbian gold artifacts. The reference materials (NA1, NA2) were produced by Norddeutsche Affinarie AG, Germany. The compositions (1 % Ag, 99 % Au for NA1 and 5.5 % Ag and 94.5 % Au for NA2) allowed matrix-matched solid calibration for the analysis of the gold objects. The evaluation of the reference materials were performed using nano- and femtosecond laser ablation systems in combination with liquid and gold standard-based solid calibration (FAU7 – NIST 8053, 8054, 8055, FAU10 – NIST 8062, 8063, 8064). The obtained results were compared to the element concentration values determined by the manufacturer and were in agreement for most of the elements. Liquid calibration and gold standard-based solid calibration was found to be applicable for the analysis of gold artifacts. The differences between the two calibration strategies were in the order of 10 % for most of the elements. Figures of merit and the results will be discussed in this presentation.

The ability to perform tip-enhanced Raman spectroscopy (TERS) in liquids is of great importance, for example for the in-vivo visualization of membrane proteins in a cell. Knowledge of the movements and activities of these proteins would provide much-awaited answers to the mechanisms of cell growth, transformation and transport. However, TERS experiments have been carried out only in air or vacuum so far. These are unrealistic environmental conditions for most biological systems.

In this work, TERS has been performed in liquid for the first time. TERS of a self-assembled monolayer of thiophenol on a gold surface was successfully carried out in water as the proof-of-principle study. TERS was then used to investigate erythrocyte membranes and the membrane proteins were identified with high spatial resolution. Our results pave the way for detailed nanoscale chemical analysis of lipid bilayers and other biologically important molecules in their natural physiological environment, which will give valuable insight into the working mechanisms of a cell.

Analytical Chemistry

Tip-Enhanced Raman Spectroscopy of Membranes in Liquids

Grace Jiahui Leong, Boon Siang Yeo and Renato Zenobi*
Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland

How to Generate Peak Capacity in HPLC

Veronika R. Meyer
EMPA St. Gallen, Lерchenfeldstrasse 5, 9014 St. Gallen

Mass Spectrometry Analysis of Phospholipids Complexation Reactions in Biphasic Systems

Michel Prudent, Manuel A. Méndez, Bin Su and Hubert H. Girault
Laboratoire d’Electrochimie Physique et Analytique, Ecole Polytechnique Fédérale de Lausanne, Station 6, CH-1015, Lausanne, Switzerland

Mass spectrometry analysis of species formed in a biphasic system is proposed. This was achieved by using a dual-channel microsprayer [1], where channels filled with different immiscible phases meet at the Taylor cone. As a first approach, the interfacial complexation of aqueous lead (II) ions by 1,4,7,10-tetraazacyclododecane (TTCD) was studied [2]. In complete agreement with previously reported electrochemical data, the formation of the 2:1 complex between the ligand and the metallic ion was observed. In the next stage, complexes formation between copper, calcium and small peptides (Phe-Phe, Angiotensin III and Leu-Enkephalin) and L-α-dipalmitoyl phosphatidylethanolamine (DPPC) were also observed and correlated with electrochemical experiments. In this way, the association between metallic cations, small peptides and phospholipids could not only be assessed from electrochemical measurements at the liquid-liquid interface, but also from biphasic mass spectrometry experiments. Moreover, complementary and highly valuable information, like phospholipid oligomers formation and association stoichiometry values, were obtained from this rather complex system. Indeed, using this methodology, the interaction between membrane disrupting peptides and phospholipids will be addressed in the near future.

Indigo naturalis (Quingdai) is used in the Traditional Chinese Medicine (TCM) to treat chronic diseases such as psoriasis, and various cancers. The drug is obtained from indigoferous plants such as Acanthaceae, Isatis indigotica (Brassicaceae) or Polygonum tinctorium (Polygonaceae) via a fermentative extraction process. Indigo naturalis contains indigo (1) and indirubin (2). Indirubin is a kinase inhibitor, mainly of CDK5/GSK2 [1]. A proposal for a European Pharmacopoeia monograph for Indigo naturalis has been recently published, whereby 1 (minimum content 2.0%) and 2 (minimum content 0.13%) should be determined by HPLC [2]. The remaining 97% are undefined. We determined the indigo content of eight different Indigo naturalis samples via quantitative 1H-NMR. A comparison with the results of the proposed pharmacopoeia method clearly revealed, that the HPLC assay consistently gave much lower indigo concentrations due to poor solubility of indigo. NMR spectra showed that one Indigo naturalis sample contained significant amounts of sucrose as formulating agent. All Indigo naturalis samples contained large amount of inorganic material (mainly Ca2+ and carbonate). Minor organic compounds in Indigo naturalis were identified by HPLC-PDA-MS.

2 Monograph “Natural indigo”, Pharmeuropa 2008, 20, 1, 118.

Analytical Chemistry

Tandem Mass Spectrometry of Oligonucleotide-Cisplatin Adducts

Adrien Nyakas, Michael Eymann and Stefan Schürch

Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland

Cis-diamminedichloroplatinum(II) (cisplatin, cis-DPP) is a cornerstone of anticancer therapy and became one of the most widely used drugs for the treatment of various epithelial malignancies. The cytotoxicity of cisplatin is mainly based upon its affinity to adjacent guanines in nucleic acids, resulting in the formation of 1,2-intrastrand adducts. In this study the gas-phase dissociation of DNA- and RNA-cisplatin adducts is investigated by electrospray ionization (ESI) tandem mass spectrometry (MS/MS). The fundamental mechanistic aspects of fragmentation are elucidated in order to provide the basis for the tandem mass spectrometric determination of binding motifs and binding sites of this important anticancer drug. It is shown that the binding of cisplatin to vicinal guanines drastically alters the gas phase fragmentation behavior of oligonucleotides. The 3′-C-O bond adjacent to the G-G sequence is preferably cleaved, leading to an extensive formation of the corresponding w-ion. This observation was even made for oligoribonucleotides, which usually tend to form c- and y-ions under CID conditions. The absence of counter ions of equal abundance indicates that oligonucleotide-cisplatin adducts are following more than one dissociation pathway in the gas-phase. Several mechanisms that explain the increased cleavage of the 3′-C-O bond and the lack of a complementary a-ion are proposed. Results of additional MS/MS experiments on methylphosphonate-oligodeoxynucleotides confirmed the proposed mechanisms.

Analytical Chemistry

Qualitative and Quantitative Analysis of Indigo Naturalis Samples by LC-PDA-MS and qNMR

Natalie Sedlacek, Inken Plitzko, Tobias Mohn and Matthias Hamburger

Institute of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Indigo naturalis contains indigo (1) and indirubin (2). Indirubin is a kinase inhibitor, mainly of CDK5/GSK2 [1]. A proposal for a European Pharmacopoeia monograph for Indigo naturalis has been recently published, whereby 1 (minimum content 2.0%) and 2 (minimum content 0.13%) should be determined by HPLC [2]. The remaining 97% are undefined. We determined the indigo content of eight different Indigo naturalis samples via quantitative 1H-NMR. A comparison with the results of the proposed pharmacopoeia method clearly revealed, that the HPLC assay consistently gave much lower indigo concentrations due to poor solubility of indigo. NMR spectra showed that one Indigo naturalis sample contained significant amounts of sucrose as formulating agent. All Indigo naturalis samples contained large amount of inorganic material (mainly Ca2+ and carbonate). Minor organic compounds in Indigo naturalis were identified by HPLC-PDA-MS.

2 Monograph “Natural indigo”, Pharmeuropa 2008, 20, 1, 118.

Analytical Chemistry

High-Mass MALDI MS: Characterization of Large Molecular Size Hemoglobin-Based Oxygen Carriers

Tatiana Pimenova1, Claudia Pereira2, Dominik Schauer3 and Renato Zenobi1

1Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland;  
2Medical Clinic Research Unit, University of Zurich, Zurich, Switzerland

Hemoglobin-based oxygen carriers (HBOCs) are blood substitutes based on chemically modified hemoglobin (Hb) of either bovine or human origin. Unprotected Hb quickly dissociates into its constituent dimers that are filtered by the kidney, resulting in nephrotoxicity. Extracellular Hb is bound and detoxified by the plasma protein haptoglobin (Hp) which also promotes clearance by the Hb scavenger receptor pathway [1]. Investigation of the complex formation between Hp and differently modified Hbs contributes to the overall understanding of specific toxicity and clearance of HBOCs. Here we report on the first use of high-mass MALDI-TOF MS to study large hemoglobin/haptoglobin complexes. From the data obtained, it is clearly seen that HbA2 and chemically modified Hbs bind Hp with different affinity. Further, multimeric state of HBOCs with covalent modifications involving their α–globin subunits is characterized. Structural similarities of these HBOCs and oxidized Hb were found. The obtained results provide valuable information on mass and stoichiometry of haptoglobin binding, which might help in rational design of HBOCs with limited Hb toxicity.

1 Schaer D.J.; Alayash A.I.; Buehler P.W. Journal Antioxidants & redox signaling. 2007, 9, 991.

Analytical Chemistry

Coupling UPLC with MS: Possibilities and Issues.

Application to the Analysis of CYP450 Substrates for Drug Metabolism

Julie Schappler, Davy Guillarme, Raul Nicoli, Dao Nguyen, Serge Rudaz, Jean-Luc Veuthey

Laboratory of Analytical Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Boulevard d’Yvoy 20, 1211 Geneva 4, Switzerland

In liquid chromatography (LC), there is a need for high throughput separations, particularly in the pharmaceutical analysis. The decrease in the average particle size of chromatographic supports represents an attractive approach to achieve faster analysis time without reduction of overall performance. Recently, several companies have commercialized columns packed with sub-2 µm particles and chromatographic systems able to withstand ultra high pressures (UPLC, up to 1000 bar). With the recent development of separation technologies, there has been a strong development of universal, sensitive and selective detection techniques, such as mass spectrometry (MS). The new generation of MS should be fully compatible with fast and even ultra-fast separations (i.e. duty cycles lower than 5 and 1 minute, respectively). For this purpose, full scan acquisition rates have been significantly improved (up to 10,000 uma/s) and dwell time drastically reduced (5 ms).

In this work, the coupling of UPLC with a new generation of single quadrupole instrument was investigated for the analysis of several CYP450 probe substrates and their metabolites, detected in both positive and negative modes. The effect of numerous operating parameters (e.g. mobile phase flow rate, pH, gradient length, Scan/SIM mode, dwell time, polarity switching, etc) on sensitivity and acquisition rate was studied. Limits of quantitation (LOQ) were determined for the drug mixture in optimal conditions.
Biological systems can be highly heterogeneous on the nanometer scale. Our combined setup allows the investigation of the exactly same part of such samples by confocal laser scanning microscopy (CLSM, ca. 500 nm resolution), Raman spectroscopy (provides chemical information without staining), and AFM (imaging with nanoscale resolution). Additionally, tip-enhanced Raman spectroscopy (TERS) with laser-illuminated, metal-coated AFM tips provides Raman spectroscopic information with 20–50 nm lateral resolution. An AFM-CLSM study of river-water biofilms has demonstrated their heterogeneity at the nanometer scale [1]. The polysaccharide alginate was used as a first model system for TERS experiments on biofilm matrix constituents [2]. Spectroscopic features of these weakly Raman scattering polymers and specific marker bands for their identification in biological systems will be discussed. Additionally, the protein cytochrome c was investigated, whose Raman spectra when excited by visible-light lasers are usually dominated by strong heme bands overwhelming the weak amino acid signature. Only TERS was able to display both, heme and apoprotein bands in one spectrum [3]. Our combined setup is and will be applied to the investigation of biofilms, biomaterialization, and artificial lipid membranes.


**Assessment of Diesel exhaust particulate exposure and surface characteristics in association with levels of oxidative stress biomarker**

Ari Setyan1, Jean-Jacques Sauvain1, Michael Riediker1, Michel J. Rossi2, Michel Guillenmin1

1 Institute for Work and Health, University of Lausanne and University of Geneva, Rue du Bugnon 21, CH-1005 Lausanne, Switzerland
2 Swiss Federal Institute of Technology, Air and Soil Pollution Laboratory, CH-1015 Lausanne, Switzerland

Exposure to PM_{2.5} and PM_{2.5} (particulate matter with aerodynamic diameter smaller than 10 µm and 2.5 µm, respectively) is associated with a range of adverse health effects. Surface characteristics (chemical reactivity, surface area) are considered of prime importance to understand the mechanisms which lead to harmful effects. A hypothetical mechanism to explain these adverse effects is the ability of components (organics, metal ions) adsorbed on these particles to generate Reactive Oxygen Species (ROS), and thereby to cause oxidative stress in biological systems. The aim of the present research project is to test whether there is a correlation between the exposure to Diesel Exhaust Particulate (DEP) and the oxidative stress status. For that purpose, a survey has been conducted in real occupational situations where workers were exposed to DEP (mechanical yards in bus depots).

Different exposure variables have been considered: particulate number, size distribution and surface area; particulate mass (PM_{2.5} and PM_{10}); elemental and organic carbon; total adsorbed heavy metals (iron, copper, manganese); surface functional groups present on aerosols.

An oxidative stress biomarker (8-hydroxy-2'-deoxyguanosine) has been determined in urine of volunteers, and urinary levels of this biomarker will be compared to exposure variables in order to gain a better understanding of the relation between the particulate characteristics and the formation of ROS by-products.

3 T. A. Schmitz, G. Gamez, P. D. Setz, L. Zhu, R. Zenobi, submitted

**Towards nanoparticle molecular analysis and chemical imaging at atmospheric pressure by near field laser ablation mass spectrometry**

Thomas A. Schmitz, Gerardo Gamez, Liang Zhu, Renato Zenobi

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

Techniques for spatially resolved chemical analysis with high resolution have become of great relevance in the past few years. Methods that allow samples to be investigated at ambient conditions are especially suitable for the chemical characterization of biological samples such as tissues or even live cells.

In previous work [1], our group already demonstrated for the first time that near-field laser ablation at atmospheric pressure can in principle be coupled to mass spectrometry (SNOM-MS). We now present an improved setup that combines near-field laser ablation at atmospheric pressure with an ion-trap/time-of-flight mass spectrometer, which was developed for this application. [2]

With this instrument, spatially resolved molecular analysis yielding full mass spectral information for samples at atmospheric pressure could be shown for the first time with a lateral resolution on the low µm scale. [3]

By further improvements in sensitivity, this setup will ultimately allow chemical imaging on the nanoscale at atmospheric pressure to be performed for various applications in material and life sciences.

3 T. A. Schmitz, G. Gamez, P. D. Setz, L. Zhu, R. Zenobi, submitted

**An automatic titration device for the determination of chemical constants using 2D NMR. Application to the determination of pKa’s in complex mixtures**

Rupali Shivapurkar, Bruno Vitorge, Damien Jeannerat

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

We have developed an automatic titration system allowing chemists to study changes in chemical shifts upon addition. Depending on the application, these changes can be translated into pKa’s, binding constants, etc. In order to illustrate how complex mixtures can be studied using this method, we follow signals in 2D HSQC spectra instead of simple 1D 1H spectra so that signal overlap can be resolved in the carbon dimension. The HSQC spectra were acquired using 10.00 ppm window in the carbon dimension in order to reach high resolution in 20 times less time than normal full spectra would require. This allows to measure carbon chemical shifts changes with high precision and accuracy in 30-minute experiments making it possible to run a full titration overnight. In this poster, we demonstrate the use of the device for the determination of the pKa of acids.

![Diagram of the titration device](image)

The titrant is added using a computer-controlled push-syringe (a) into the mixing chamber (b). A peristaltic pump (c) insures the proper mixing of the solution.
Analytical Chemistry

Screening for New Scaffolds for IMAC Purification

Martin Smiesko, Steven Knecht, Beat Ernst
Institute of Molecular Pharmacy, Pharmazentrum Basel, Klingelbergstr. 50, CH-4056 Basel, Switzerland

For immobilized metal ion affinity chromatography (IMAC), Ni$^{2+}$ immobilized on nitrilotriacetic acid (NTA) is most often used for the purification of proteins carrying either a C- or N-terminal histidine (His)-tag [1, 2]. More recently, 1,10-phenanthroline was successfully implied as a tag suitable for the purification of peptides synthesized on solid-phases [3].

In our approach, we searched for new tags based on picolinic acid to be used in IMAC for the purification of synthetic products.

Structural, thermodynamic and electronic properties of different picolinic acid derivatives alone and complexed with Ni-NTA were investigated using computational methods based on the Density Functional Theory in both gas and solvent (water) phases. The results were then correlated with the binding data (binding affinity $K_D$ and kinetic constants $k_{on}$ and $k_{off}$) determined by surface plasmon resonance experiments.

References:

Analytical Chemistry

Development of an HPLC-MS method for the differentiated quantification of the 15 major human bile acids in serum

C. Steiner, I. Burkard, A. von Eckardstein, K.M. Rentsch
1Institute for Clinical Chemistry, University Hospital Zürich, Carine.Steiner@usz.ch

Background and hypothesis: Bile acids are the major degradation products of cholesterol and they undergo considerable structural modification through hepatic and intestinal metabolism. They are biologically important as mediators of dietary lipid absorption as well as ligands of the nuclear receptor Farnesoid X Receptor (FXR) and hence regulators of lipid and carbohydrate metabolism. The aim of our research is to develop a method based on liquid chromatography coupled to mass spectrometry (HPLC-MS) for the differentiated quantification of bile acids in serum.

Methods: The quantification of these compounds requires a highly sensitive method since bile acids are present at micromolar concentrations in serum. An HPLC-MS method was developed using reverse-phase chromatography and methanol/ammonium acetate buffer 10mM, pH 6.8 as a mobile phase. Analysis of the compounds is performed using electrospray ionization (ESI) in the negative mode.

Results: By selecting ESI as the ionization technique and optimizing the chromatographic conditions our newly developed method allows the separation of the 15 major human bile acids in less than 20 minutes and the quantification of 14 bile acids in serum samples of healthy volunteers. In addition, we found that bile acid concentrations show considerable intra-individual variation which, depending on whether they are conjugated or not, is related to food intake or circadian rhythm.

Conclusions: We developed and validated a novel sensitive and fast method for the quantification of bile acids in serum. The high degree of intra-individual variation in serum concentrations render the preanalytical phase difficult to be controlled.

High Spatial Resolution Chemical Investigation of Inorganic Nanostructures

Johannes Stadler, Thomas Schmid, Boon-Siang Yeo, Renato Zenobi
ETH Zürich, Department of Chemistry and Applied Biosciences, 8093 Zürich, Switzerland

The challenge to gather information with nanometer spatial resolution from different types of samples gained importance due to the ongoing miniaturization of production processes. Several techniques can be applied for high resolution information such as SEM, STM, AFM. Yet all these techniques mostly show topographic information. The structural information is very important to make statements about chemical composition and distribution of different substances within heterogeneous materials. Titanium dioxide can form three different crystalline phases namely Brookite, Anatase and Rutile. During the formation of TiO$_2$, mono- and polycrystalline particles can appear. These phases differ in the occupation of different crystalline sites as well as in bulk properties. The aim of designing particles and their bulk properties make it necessary to determine the structure of the nanoparticles formed in a reaction. Using topographic methods only like high resolution AFM (down to around 10 nm lateral resolution) modifications to the particles cannot be distinguished. With additional data from confocal Raman microscopy, we are able to determine the chemical composition of the particles as well as their structure on a submicrometer scale. Similar experiments have been done using tip-enhanced Raman spectroscopy on organic substances, dyes [1] and other mostly resonant model systems [2]. We have now extended the use of these methods to non-resonant inorganic samples allowing us to discern and locate inorganic nanoparticles.

References:

The Laser Ablation Glow Discharge Time Of Flight Mass Spectrometry (LA-MS-TOF-MS) and its capability for high spatial resolution analysis

M. Tarik, G. Lotito, J. Whitby, J. Koch, D. Günther
1 ETH Zurich, Laboratorium für Anorganische Chemie, Wolfgang-Pauli-Str. 10, 8093 Zürich, Switzerland
2 EMPA, Feuerwerkerstrasse 39, 3602 Thun, Switzerland

Laser Ablation (LA) – Glow Discharge (GD) – Time Of Flight (TOF) Mass Spectrometry (LA-GD-TOF-MS) will be presented as a new combination of two analytical techniques along with its design and preliminary experimental results. The LA was used for direct solid sampling into a GD plasma for the ionization of ablated material. The measurements were performed by using a pulsed GD coupled to a TOF-MS. Various ablation parameters were selected to change the mass load of the GD. Furthermore, the material was introduced within different temporal GD regions. The results indicate, that the direct ionization of the laser-generated microplasma can be significantly enhanced (factor 7) when ablating the material into the afterpeak GD regime. Furthermore, laser energy, sampling position and gas atmosphere were studied in detail and figures of merit will be discussed.
Towards Determination of Affinities Between Adenovirus Fiber Knobs of Different Serotypes and Soluble CD46

Huong Viet Trinh1, Venus Chemannparampil2, Stefan Schauer1, Urs Greber3, Silvio Hemmi1

1Institute of Molecular Biology, 2Functional Genomic Center Zurich, 3Institute of Zoology, University of Zurich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

Adenoviruses (Ads) are among the best-characterized viruses. They were discovered in the early 1950s as “adenoid-degenerative agent” [1]. More recently, research on Ad-based vector technologies has increased, and Ads have become one of the most widely used vectors for gene therapy of genetic diseases, cancer treatment and vaccination [2]. To date, 52 human Ad serotypes have been identified and classified into six distinct species A-F. The use of species C-derived Ad vectors in clinical applications is limited due to side effects and low efficiencies of transfection. Ad vectors derived from B species promise to overcome some of the clinical drawbacks of species C Ads [4]. Recently, several groups including ours identified membrane cofactor CD46 as an attachment receptor for species B Ads [5].

CD46 involved in binding of the four different species B serotypes 3, 7, 11 and 35. Affinities of recombinant fiber knobs (FKs) of different Ad serotypes to CD46. We have produced five different histidine-tagged Ad-FKs using Baculovirus mediated expression in insect cells, and affinity purification on Ni-agarose. These soluble FKS are currently characterized by virus blocking experiments. In the next step, the affinities are measured by surface plasmon resonance analyses. The results will be discussed at the meeting.

Keywords: Adenoviruses; Ads; fiber knobs; FKs


Analitical Chemistry

Studying the Distribution of Dyes in an Electrospray Plume

Rui Wang, Thomas Schmid, Renato Zenobi*

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

Electrospray ionization (ESI)-Mass spectrometry (MS) is one of the most widely used analytical methods in biochemistry, especially in the investigation of noncovalent complexes [1]. The study of the distribution of compounds in the plume is important for developing ESI-MS into a reliable method for the quantitative analysis of non-covalent interactions. A better understanding whether the ion peaks obtained by ESI-MS offer an accurate ‘snapshot’ of the bulk solution is important in this context. In this project, we investigated the distribution of two compounds in the plume using different methods, i.e. laser induced fluorescence spectroscopy [2] and confocal Raman microscopy. Mixtures of dyes, (i.e. nile red, nile blue, rhodamine 6G and coumarin 307) with ionic or nonionic character were studied. The fluorescence measurements showed that nile red, a nonionic compound, can only be observed in the center part of the plume while ionic compounds, e.g. rhodamine 6G, can be observed everywhere. Similar results were also obtained in the spatially resolved Raman measurements. All these results imply that the segregation between ionic and nonionic compounds is apparent in the plume, e.g. nonionic compounds are more likely to stay on the axis of the plume. The ionic or nonionic character of an analyte can thus significantly affect its distribution in the plume and influence the selectivity and sensitivity of ESI-MS measurements.


Quantitative Analysis of Compounds in Breath by Extractive Electrospray Ionization Mass Spectrometry

Simon Weidmann, Gerardo Gamez, Renato Zenobi

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

Extractive Electrospray Ionization (ESI) Mass Spectrometry is a new technique for sample analysis without pretreatment. The sample is directed into an electrospray where the analyte is ionized and then sampled into the mass spectrometer. This method holds great potential for a broad set of applications due to its simplicity and ability to yield information in real-time. Some of the applications explored so far include breath analysis, food spoilage screening, perfume recognition, as well as chemical reaction monitoring. However, most of these previous studies have been limited to qualitative analysis. Thus, the goal of this work was to explore the quantitative capabilities of EESI, especially for breath analysis.

One approach was to generate an aerosol from a solution to simulate breath containing the analyte of interest. This simulated “breath” was analyzed directly with ESI. In this fashion, quantification of nicotine in the breath of a regular smoker and an occasional smoker could be performed. Another substance which was investigated was limonene which is a flavor ingredient in chewing gum. The signal of limonene in breath after chewing different gums was quantified by a calibration via an exponential dilution chamber.

Further investigations will include quantification of additional compounds such as pharmaceuticals or metabolites. Additionally, the ESI Interface will be coupled to a spirometer to control the flow rate and volume of the breath to standardize the analysis.

Analytical Chemistry

Profling of two Chinese medicinal plants, Sophora flavescens and Ligusticum chuanxiong, by off-line LC-NMR and LC-MS

Xinzhou Yang, Inken Plitzko, Matthias Hamburger*

University of Basel, Klingelbergstr. 50, CH-4056 Basel, Switzerland

For the identification of new natural product based lead compounds, we combine initial screening of extract libraries in a range of functional assays with HPLC-based micro-fractionation for activity profiling and chemical profiling by LC-MS and off-line NMR [1]. A 1-mm microprobe with z-gradient was used to measure one and two dimensional NMR spectra [2], and fractions were obtained by peak-based fractionation of a single injection of 40 mg of extract on a semipreparative (10 x 250 mm i.d.) HPLC column. The protocol was applied to two plants used in Traditional Chinese Medicine, Sophora flavescens and Ligusticum chuanxiong, to identify 32 compounds including 1-4, and 5-6, respectively, as structures with promising activity on a CNS-related target.

Analytical Chemistry

NMR Based Search for Monoterpane Aldehydes from Amomum tsao-ko

Xinzhou Yang, Inken Plitzko, Olivier Poterat, Matthias Hamburger*

University of Basel, Klingelbergstr. 50, CH-4056 Basel, Switzerland

Amomum tsao-ko Crevost et Lemaire, a member of the family Zingiberaceae, has been used for centuries as food, spice and perfume in China, Japan and Korea. Its fruits have also found applications in Traditional Chinese Medicine (TCM) for the treatment of stomach illness, digestive disorders and throat infections. In previous phytochemical investigations of the species, bicyclic nonane aldehydes, tsaookin, isotsaookin [1], trans-2,3,3a,7a-tetrahydro-1H-indene-4-carbaldehyde, cis-2,3,3a,7a-tetrahydro-1H-4-carbaldehyde, 4-indancearbaldehyde and 5-indancearbaldehyde [2], had been reported.

We carried out a systematic search for monoterpane aldehydes from the fruits of this species, whereby the isolation process was guided by 1H-NMR of fractions (1 mm microprobe, aldehyde resonance at δH 9-10 ppm) spectra. Two new monoterpenes, rel-(3aS,7R,7aS)-7-hydroxy-2,3,3a,6,7,7a-hexahydro-1H-indene-4-carbaldehyde (1) and 6-hydroxy-4-indancecarbaldehyde (2), were isolated along with known aldehydes such as 3 and 4, and other compounds. Cytotoxicity of the isolates against some human cancer cell lines was determined.

Analytical Chemistry

Can Analyte Dissociation and Fluctuating Carbon Contamination Signals in Surface-Enhanced Raman Spectroscopy Be Prevented?

Boon-Siang Yeo, Thomas Schmid, Weihua Zhang and Renato Zenobi*

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

Signal losses and fluctuating carbon contamination bands are ‘bottlenecks’ in the application of surface-enhanced Raman spectroscopy (SERS) for reliable chemical analysis. They originate mainly from prolonged laser irradiation of the sample during data collection that causes analyte decomposition and/or loss of the enhancing capabilities of the adsorption site. In this work, a laser illumination/signal collection technique, the ‘multiple points collection method’ (MPC) is introduced to circumvent these problems. The MPC is based on the use of a pair of galvanic mirrors to scan the laser beam rapidly and steadily across the sample surface. Each position is irradiated for <10 µs, at a rate of ~0.5 Hz. The SER spectrum is obtained by summing the signals collected from a large array of non-overlapping sample points. The MPC is compared with the conventional ‘single point collection’ method, where the laser beam is statically focused onto a particular spot and the scattered signals acquired. The MPC has the following advantages: (i) illumination and collection efficiencies are not compromised, (ii) Signal losses originating from analyte decomposition and/or alteration of the enhancing capabilities of the adsorption site, are avoided, (iii) high quality SER spectra for analytes such as biomolecules and dipicolinic acid (a common marker for bacteria spores) can be easily obtained, and (iv) the occurrence of broad amorphous carbon bands and the commonly observed temporal fluctuations in SERS are prevented. The success of the MPC is attributed to the reduction of local sample heating, as the time interval between the laser irradiations of a spot is much longer than the actual irradiation time itself.


Calcilytics – A New Treatment for Established Osteoporosis
Leo Widler
Novartis Institutes of BioMedical Research, WKL-136.3.93, CH-4002 Basel, Switzerland

Osteoporosis is characterized by low bone mass and micro-architectural deterioration of bone tissue that leads to fragility and increased risk of fractures. Traditional therapies for osteoporosis inhibit bone resorption and prevent further bone loss. However, many osteoporosis patients have already lost a substantial amount of bone at the time of diagnosis, there is a need for agents that stimulate bone formation. The only anabolic treatment for osteoporosis, approved for the US and EU markets, is Forteo® (Teriparatide, the 1-34 fragment of parathyroid hormone (PTH)) which causes a significant increase in bone mass and reduces vertebral fracture risk substantially. This peptide must be administered by daily subcutaneous injection and the therapy is costly. An orally active, low molecular weight compound with the same efficacy would be a highly attractive alternative for the patient.

Instead of applying exogenous PTH, mobilization of endogenous stores of the hormone can be envisaged. PTH is stored in relatively large amounts in parathyroid cells and its secretion is controlled by a calcium-sensing receptor (PCaR) located on the cell surface. Agonists of PCaR (calcilytics) mimic a state of hypocalcemia and stimulate PTH release to the blood stream.

The starting point for the Novartis calcilytics project was a proprietary structure found in a HTS screen using a functional assay in the FLIPR format based on recombinant human PCaR. Optimization of the series resulted in an increase in vitro potency by a factor of >100. First oral applications in rats with these highly potent calcilytics were rather disappointing with regard to PK/PD parameters. The presentation will focus in the second part on how these limitations were overcome.

Analytical Chemistry

Indicator Displacement Assays as Molecular Timers
Friederike Zaubitzer, Alexei Pozdnoukhov, Kay Severin*
*Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

Indicator displacements assays (IDAs) have emerged as powerful analytical tools. They are based on dyes which compete with analytes for binding to synthetic receptors. So far, IDAs have primarily been used to determine the identity and/or the quantity of certain analytes. We show that a multicomponent assay can also be employed to obtain information about the history of chemical inputs. A simple mixture of three commercially available dyes and the organometallic complex [(Cp*RhCl2)2] is employed to time the addition of ADP and ATP with good resolution [1].

Fig.1.: The ADP and ATP addition times determined by the molecular timer in comparison with the real addition times for 12 test samples. The predictions are shown as filled symbols (5 measurements each) and the real addition times are indicated by empty symbols.

The signal of the timer is read by UV/Vis spectroscopy and the data is analyzed via a multivariate analysis.


Medicinal Chemistry

Total Synthesis of the Resorcylic Lactone-based Kinase Inhibitor L-783277
Tatjana Hofmann, Karl-Heinz Altmann
Institute of Pharmaceutical Sciences, ETH Zurich, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

Kinases have emerged as important drug targets in cancer and inflammatory disease and several low-molecular-weight kinase inhibitors have now been introduced into clinical practice.[1] The natural product L-783277 (1) belongs to the family of resorcylic acid lactones (RALs), which includes compounds such as zearalenone, C292 (LL-Z1640-2), hypoxanthin, or radicicol, and which exhibit a diverse range of biological activities.[2] L-783277 (1) is a potent inhibitor of the Src/Thr kinase MEK.[3] We have accomplished the first total synthesis of macrolactone 1, which is based on the consecutive assembly of the key fragments A, B, and C.[4] The development of an efficient enantioselective synthesis of 1 and a more detailed characterization of its biological effects are the primary goals of this research project. This presentation will discuss the details of the synthesis of 1 and the preparation of a number of analogs. Preliminary data on the in vitro biologically activity of these compounds will also be presented.