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Chemical Analysis by Mass Spectrometry in Space - Initial Results from the Comet Churyumov-Gerasimenko

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In situ mass spectrometry in space has its own challenges. Not only has the mass spectrometer to be lightweight and energy efficient, but it also has to withstand a broad temperature range, high vibration levels during launch and a wide variety of pressures. Autonomous operation over long times, immunity to cosmic rays and high compression of data are other prerequisites for successful instruments on board spacecraft. The probably most advanced instrument currently flying aboard a spacecraft is the ROSINA (Rosetta Sensor for Ion and Neutral Analysis) instrument on board the ESA Rosetta spacecraft encountering comet 67P/Churyumov-Gerasimenko. Rosetta is following the comet from almost 4 AU through its perihelion at 1.3 AU and out again for more than 1 ½ years. The closest distances of just a few kilometers to the comet will be reached during the delivery of the lander Philae in November. Rosetta will, at the time of the SCG fall meeting, be less than 30 km from the cometary surface, but still outside of 3 AU from the Sun. The cometary atmosphere consists mostly of water and CO/CO2. However, it is known that comets have quite a diversified organic part in their coma, both as volatiles and as dust. Furthermore, isotopic ratios in water and other molecules can give very strong indications on the formation process of cometary, and therefore solar system material.

In this talk I will give a short overview on mass spectrometry in space and present the ROSINA instruments, the challenges, and the results so far.

Website: http://space.unibe.ch/de/rosina/overview.html

Microscale probing and patterning of biological surfaces using nested hydrodynamic flow confinement and recirculation of sub-microliter volumes of liquid

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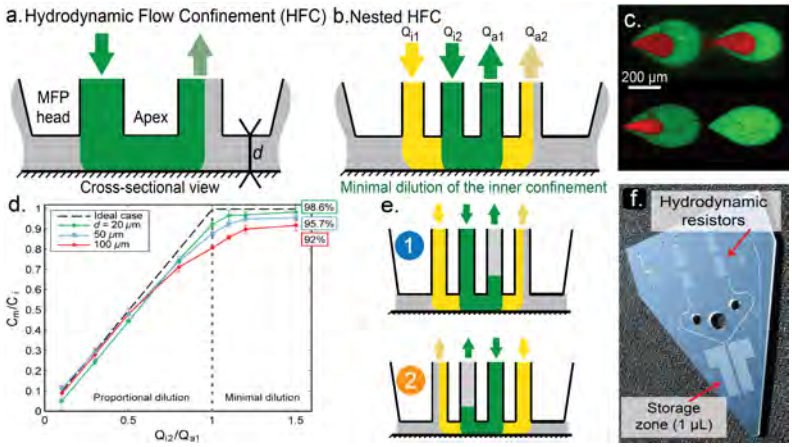
Microscale probing and patterning of biological surfaces plays a significant role in fields ranging from stimulation of adherent cells, microperfusion of brain slices, engineering cellular architectures, modulating stem-cell microenvironments to dispensing chemicals on cells for pharmacology studies [1]. In view of such applications, we developed the microfluidic probe (MFP), which is a non-contact, scanning microfluidic technology to directly operate on microscope slides and Petri dishes without the need for sealed channels/chambers [2]. The MFP is based on the hydrodynamic flow confinement (HFC) of nanoliter volumes of liquids over tens of micrometers of a surface. To exploit the opportunities of HFC, we recently developed nested HFC, wherein multiple layers of liquids are shaped to interact with a surface [3]. In the classical HFC, the asymmetry of injection to aspiration flow rates between two apertures is responsible for the dilution of the liquid of interest (processing liquid) by the immersion liquid, Fig. a. In the nested HFC, we use two extra apertures to “nest” the processing liquid inside a shaping liquid, Fig. b. We illustrated the use of nested HFC by efficiently patterning multiple antibodies on a surface simultaneously, with 5 μm resolution and a 100-fold decrease of reagent consumption compared to microcontact printing, Fig c. Nested HFC not only minimizes the usage of chemicals but also permits efficient retrieval of analytes from a surface, as demonstrated by the minimal dilution (below 2%) of the processing liquid in the inner flow confinement, Fig. d.



We are now developing a strategy to repeatedly use and circulate a defined volume of processing liquid within the MFP head. Combining nested HFC with liquid recirculation allows investigating two critical aspects of microscale surface biochemistry: (a) the efficient removal of analyte, and (b) efficient usage of chemicals on surfaces. In the presentation, we will outline how this new method will allow for new opportunities in microscale surface biochemistry and analytical sciences.

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A Droplet Microfluidic ICP-MS Sample Introduction System

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[1]ETH Zurich

Inductively coupled plasma mass spectrometry (ICP-MS) is an important tool for highly sensitive elemental analysis of single nanoparticles [1] or rare-earth isotope labeled cells [2]. These measurements require a discrete sample introduction system with a high transport efficiency. However, commercially available droplet injection modules are expensive, suffer from a narrow droplet size range, are prone to clogging and are difficult to clean. To overcome these drawbacks a robust, flexible, low cost and low-maintenance sample introduction system for measurements of nanoparticles and single cells by ICP-MS is of interest.

We present a novel method to interface a continuous-flow droplet microfluidic based system with ICP-MS, which enables sensitive elemental analysis of the content of picoliter size droplets containing solutions, cells or nanoparticles. Our system is build around a disposable Polydimethylsiloxane liquid-assisted droplet ejection (LADE) chip (see Figure 1.), which generates aqueous droplets and ejects them in to the ICP-MS. It can measure samples as small as 1 µl and opens the possibility to easily integrate further sample pretreatment steps into the microfluidic device.

In this study, the interface was used for the quantification of the iron content of red blood cells. Additionally, the optimization of the system and its potential for the integration of microfluidic sample pretreatment will be discussed.

In summary, we have demonstrated a droplet-based microfluidic chip as sample introduction system for ICP-MS. Our system is disposable, easy to use and robust. We have shown its use for single cell analysis.

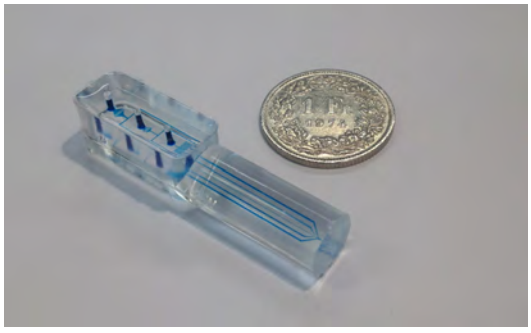

Figure 1. LADE-chip made entirely of Polydimethylsiloxane. The fluid channels are filled with a blue dye for visualization.

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Electrostatic Spray Ionization Mass Spectrometry for Biochemical Analysis and Imaging

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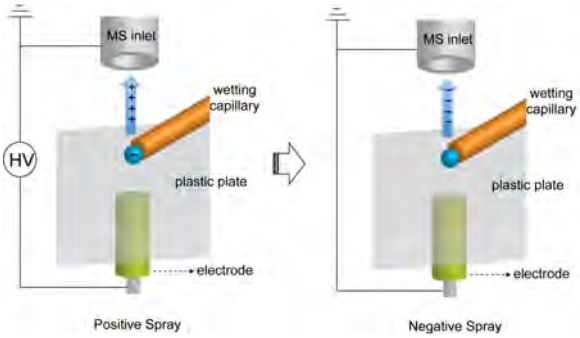
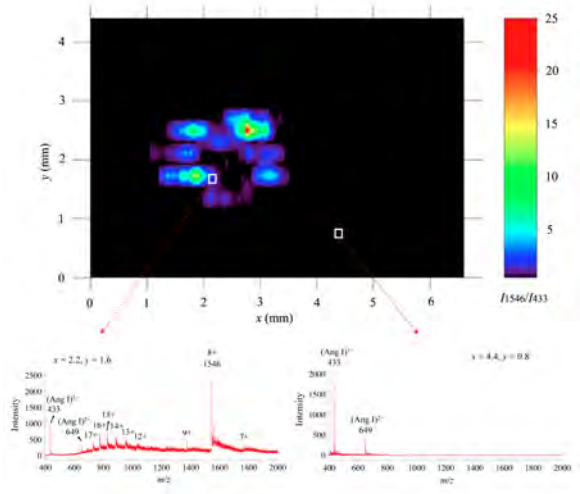
Electrostatic spray ionization (ESTASI) mass spectrometry (MS) is a new ambient ionization MS technique developed in our group.[1] During ESTASI, a sample in electrolyte solution is placed on an insulating plate, and an electrode is placed under the insulating plate. Bipolar spray ionization of samples happens under the application of a pulsed square-wave high voltage between the electrode and MS ion entrance.



Since the invention of ESTASI, we have mainly applied it in (1) interfacing capillary electrophoresis (CE) and MS,[1] (2) interfacing gel electrophoresis and MS,[2] (3) ambient ionization MS for direct analysis of samples with minimal preparation,[3] and (4) MS imaging[4]. The ESTASI is a versatile ambient ionization technique in biochemical analysis.



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2D-algal arrays on-chip as a tool for environmental biosensing

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[1]University of Geneva

Whole-cell biosensors using microalgae as biological component allow fast and sensitive screening of environmental samples [1, 2]. However reproducible immobilization of the living cells as well as maintenance of cell viability, are still challenging [3]. Recent advances in the field of electrokinetic cell handling offer rapid on-chip concentration and immobilization. Such techniques and especially dielectrophoresis (DEP) enable to electrically trap, concentrate and focus suspended cells [4]. The present study focuses on the capture and immobilization in two-dimensional arrays by DEP of green microalga *Chlamydomonas reinhardtii*, a model widely used in ecotoxicology,and in combination with fluorescence detection explores sub-toxic responses such as the production of reactive oxygen species (ROS) upon short term exposure to various micropollutants. Electric field voltage and frequency, as well as collection time were optimized while ROS were used as an indicator of induced oxidative stress on cells and were detected by fluorescent microscopy using a fluorescent dye CellRox Green®. Voltage of 50V and frequency of 100 Hz of AC-current applied for 30 minutes were found to be optimal for the formation of 2D arrays. Regarding the application for environmental biosensing, the results showed an increase in the production of ROS with some of the micropollutants. Indeed, MeHg showed to induce high production of ROS at concentrations of 10^{-9} and 10^{-7} M, while rapid effect on ROS production was observed at 10^{-7} M Hg and a high increase of ROS production was also observed with 10^{-5} M Cu and with both concentrations of CuO-NPs (10 mg/L and 50 mg/L) after 60 min of exposition. However, 10^{-8} M Hg as well as high concentrations of diuron did not show significant impact to the cells.

The study demonstrates the capacity of DEP to trap and assemble cells in 2D arrays without affecting their viability, as well as their applicability to explore the oxidative state of algal cells exposed to different micropollutants and the potential of the 2D-whole cell algal arrays in development of early warning tools for environmental monitoring.

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Quantification of ghrelin and des-acyl ghrelin in human plasma by using cubic selected reaction monitoring LC-MS

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Ghrelin is a gastrointestinal hormone peptide which stimulates appetite and growth. Two forms of the peptide were observed, the acyl-ghrelin with an octanoyl modification on the third serine residue and the des-acyl ghrelin lacking this modification. Quantification using ELISA test may over-estimates ghrelin concentration because the immunoassay may not be selective enough to differentiate ghrelin from desacyl-ghrelin or from a common precursor the prepro-ghrelin. LC-MS analysis is particularly adapted to overcome this selectivity issue especially when using the selected reaction monitoring (SRM) mode for quantitative analysis. Peptides such as ghrelin, are generally ionized, in electrospray, in several charge states (4^+ to 8^+) depending on the mobile phase conditions. Beside the loss in sensitivity the charge state distribution may changes over the concentrations range and sample background which affects the limit of quantification, the accuracy, the precision and the dynamic range of the assay. To overcome these limitations a method based on the sum of multiple charged states and their corresponding fragments was developed for the quantitative analysis of ghrelin and des-acyl ghrelin in human plasma.

However, limited selectivity of the SRM transitions has been observed in human plasma samples at low concentrations (100 pg/mL). Thus, a LC-MS/MS/MS method based on a triple quadrupole linear ion trap was developed, where second generation product ions from multiple charge states peptides are selected and summed (LC-SRM^3/MS). The LC-SRM^3/MS method was found to be linear from 50-75 to 2500 pg/mL for the ghrelins using a 0.5 mL plasma sample. The accuracies and precisions at LOQ for des-acyl ghrelin (50 pg/mL) and ghrelin (75 pg/mL) were found to be better than 91% and 2%, respectively. A partial protein precipitation method combined with a column-switching LC analysis was developed for sample clean-up and analytes separation. Blood and plasma stabilization was found to be essential for good assay performance. Additionally, because ghrelin and des-acyl ghrelin are endogenous analytes bovine plasma was used as surrogate matrix where the sequences in amino acids of the ghrelin peptides are different.

Compared to the LC-SRM/MS method the addition of an additional MS step did significantly improve the selectivity and therefore the sensitivity. The LC-SRM^3/MS method could be successfully applied for the quantification of ghrelin and des-acyl ghrelin in human plasma samples using a generic sample preparation procedure.

Laser based N_2O isotopomer analysis bridges the gap between pure culture studies and field applications

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[1]EMPA Dübendorf

Nitrous oxide (N_2O) is a potent greenhouse gas and the strongest ozone-destroying substance emitted this century. Atmospheric N_2O concentrations have been rising at a rate of 0.2-0.3% per year over the past decades due to anthropogenic emissions, primarily arising from enhanced microbial production in fertilized agricultural soils. N_2O sources are linked to different microbial processes, therefore sources are disperse and highly variable, complicating the development of effective mitigation strategies. Isotopic measurements have great potential to unravel spatial and temporal variations in sources, sinks and chemistry of N_2O. Recent developments in quantum cascade laser spectroscopy [1] allow both the intermolecular distribution of ^{15}N substitutions ('site preference'; $^{15}N^{14}N^{16}O$ versus $^{14}N^{15}N^{16}O$) and the oxygen isotopic composition ($\delta^{18}O$) of N_2O to be measured with a precision of
In a number of laboratory and pilot plant studies we investigated the isotopic signature of distinct microbial and abiotic N_2O production and consumption pathways in wastewater, soil and aqueous solution [e.g. 3]. Specific pathways were favored by selection of the nitrogen substrates and process conditions and their isotopic signatures identified by real-time laser spectroscopic analysis. Results from our laboratory studies are in accordance with pure culture experiments and can therefore be applied to other ecosystems.

Real-time analysis of N_2O isotopic composition in ambient air is feasible by combining high precision laser spectroscopy with automated preconcentration [4]. In the first field campaign measuring real-time N_2O isotopic composition, we monitored an intensively managed grassland in central Switzerland for three months. The responses of the N_2O isotopic composition of soil-emitted N_2O were analyzed with respect to management events and weather influences [5]. In a follow-up project we intend to combine real-time N_2O isotopic analysis at a tall tower in central Switzerland with atmospheric transport simulations and a biogeochemical model of surface fluxes of N_2O isotopomers. The working hypothesis is that this approach will allow us to quantify regional N_2O sources, identify emission hot spots, and constrain source processes, which will be of upmost importance for developing targeted mitigation options.

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Direct voltammetric As(III) sensing in natural waters on a gel integrated renewable gold nanoparticle microelectrode

Romain Touilloux[1], Marie-Louise Tercier-Waeber[1], Eric Bakker[1] *

[1]University of Geneva

Arsenic in the environment is of global concern because of the widespread chronic poisoning found in a number of countries and affecting large populations. Inorganic As(III) and As(V) species are the predominant forms in the water column, and a function of the local biogeochemical conditions, while organic forms are the main arsenic species in aquatic organisms. The As(III) species is 60 times as toxic as the pentavalent salt and several hundred times as toxic as methylated arsenicals. Robust and sensitive analytical tools capable of direct, continuous on-site As(III) sensing are therefore still of prime interest for the assessment of As health risks.

For this purpose, we have developed a microelectrode consisting of a renewable gold nanoparticle plated Ir-based microelectrode (AuNP-IrM) coated by a 350 to 650 µm thick agarose gel [1]. This layer minimizes fouling problems by hindering diffusion of colloids and macromolecules. Ir substrate pre-cleaning, AuNP deposition and renewal are controlled electrochemically. Square Wave Anodic Stripping Voltammetry (SWASV) has been used to characterize i) the flux of As(III) in the agarose gel as a function of the gel thickness and the temperature; and ii) the analytical performance of the sensor in synthetic and natural waters. The results show that the gel-integrated AuNP-IrM fulfills the requirements for direct measurements in freshwaters, i.e.: a mass transport of arsenite species in the gel controlled by pure diffusion ; a gel equilibration time varying with the gel thickness of the gel in accordingly to the theory ; a temperature effect on the SWASV As(III) signal intensities following a Arrhenius behavior and thus readily corrected using a factor defined in laboratory; a nanomolar detection limit at pH 8 ; and negligible Cu interference for Cu(II) to As(III) concentration ratios of ≤ 10. This sensing approach has been applied to environmental fresh water samples.

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Native ESI-MS: Buffer Concentration Effect on Protein-Ligand Binding Affinities

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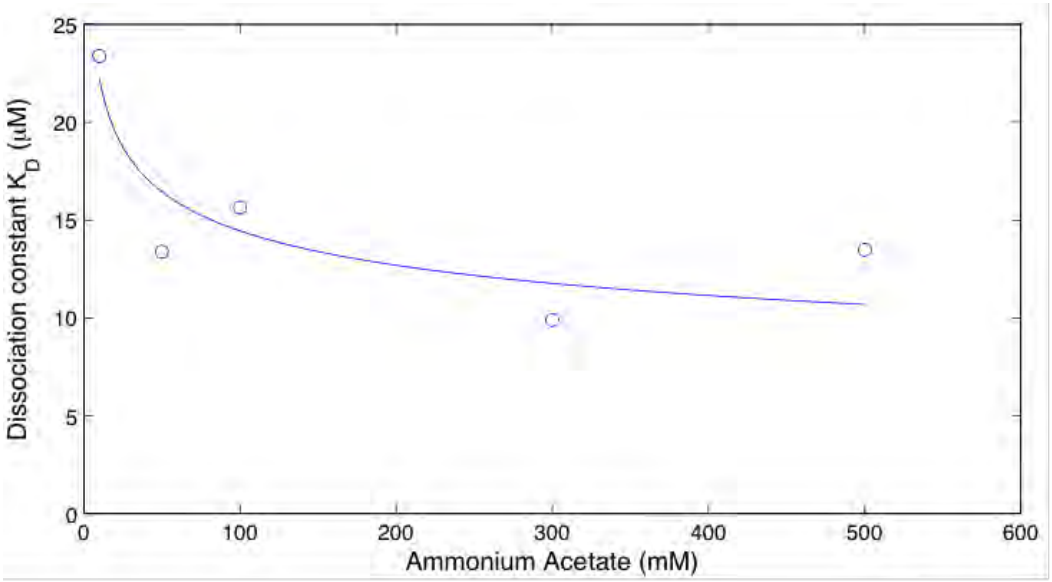
[1]ETH Zurich

The characterization of protein-ligand interactions is important for the understanding of biochemical reactions and pathways as well as for a subsequent design of new therapeutics for treatment of different human diseases, thus it is of crucial importance to study the effect of buffer concentration on the protein-ligand affinities.

A powerful technique for the investigation of noncovalent interactions is native electrospray ionization mass spectrometry (ESI-MS). Ammonium acetate is the most frequent buffer choice for studying non-covalent protein-ligand interactions by native ESI-MS analysis. The buffer concentration used in native ESI-MS titration measurements is generally in the low to high mM range. Previous studies have shown that by varying the buffer concentration the relative abundances of the protein-ligand complex to the free protein can vary (Benkestock et al. 2004; Kapur et al. 2001). However, to date it was not shown to which extent the concentration of the buffer affects the apparent affinity of the ligand to a receptor .
A well studied system (lysozyme-NaG_3) was chosen in order to show to which extent the concentration of ammonium acetate buffer used for nanoESI-MS affects the value of the dissociation constant (K_D). Five different ammonium acetate buffer concentrations were used (10 mM, 50 mM, 100 mM, 300 mM and 500 mM) for this study. It can be seen that the K_D is decreasing with increasing the ammonium acetate concentration (Figure 1).



A general trend is observed that by increasing the ammonium acetate concentration the K_D values are decreasing. The affinity of NaG_3 to lysozyme is altered by a factor of up to 50% with increasing the buffer concentration. .

To date it has not been shown to which extent the buffer concentration affects the value of the dissociation constant (K_D). Different model systems (protein/aptamers-ligands) will be tested in order to investigate this effect.

Instrumentino: An open-source modular Python framework for controlling Arduino based experimental instruments

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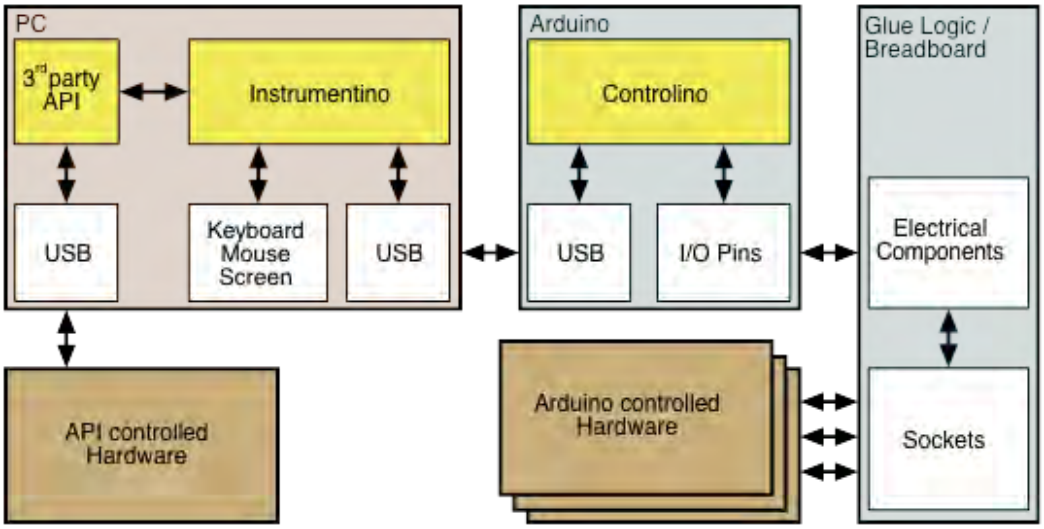
[1]University of Basel, [2]University of Alcalá, Madrid

Instrumentino is an open-source modular graphical user interface framework for controlling Arduino based experimental instruments. It expands the control capability of Arduino by allowing instruments builders to easily create a custom user interface program running on an attached personal computer. It enables the definition of operation sequences and their automated running without user intervention. Acquired experimental data and a usage log are automatically saved on the computer for further processing. The use of the programming language Python also allows easy extension. Complex devices, which are difficult to control using an Arduino, may be integrated as well by incorporating third party application programming interfaces into the *Instrumentino* framework.



In this presentation, the *Instrumentino* [1] infrastructure will be explained and the benefits of its usage will be discussed. A series of example systems in the field of analytical chemistry, already using *Instrumentino*, will be presented as well.

The main goal is to expose the *Instrumentino* freamework for Swiss researchers and promote future collaborations.

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Capillary zone electrophoresis as a capable tool in endotoxin and carbohydrate analysis

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[1]University of Applied Sciences and Arts Northwestern Switzerland, FHNW

Biotechnological products manufactured by Gram-negative bacteria and consumed by humans or animals must be proven to be endotoxin free in order to prevent endotoxic shock, inflammation and / or sepsis. During the development of the production process, determining the endotoxin levels is important to assess the efficiency of endotoxin removal methods. The widely used, FDA approved LAL (*Limulus* Amebocyte Lysate) assay is both time consuming and expensive, especially during the development of endotoxin removal methods due to its high error and small dynamic range, therefore a low cost method would be beneficial. From the chemical structure point of view endotoxins are complex lipopolysaccharides that show high biodiversity, and they are lacking the UV active moieties. Considering these facts two methods are adapted and optimized for their quantitative and qualitative analysis.
In order to determine the total endotoxin content in biopolymers produced by Gram-negative bacteria, based on [1], we have installed and optimized an indirect-UV detection method utilizing CZE-DAD. In the high UV absorbing background electrolyte (BGE), proteins and nucleotides of the crude samples are not detected meanwhile non-UV absorbing native endotoxins provide negative peaks. Due to their specific electrophoretic mobilities endotoxins are sufficiently separated from other non-UV absorbing species like carbohydrates or small ions. Endotoxin standards from different biological sources and of different purity are analyzed; results are compared to each other and to the LAL assay.
For the characterization of the diversity of the polysaccharide (O-antigen) part of the different endotoxins, an on-column reaction based CZE-DAD method was installed and optimized [2]. Liposaccharides or after quantitative hydrolysis the respective monosaccharaides are separated at high pH (12.6). Employing a DAD in the capillary detection window, the carbohydrates undergo an on-column reaction resulting in UV active species which are detectable. They are detected by direct UV-detection at 270 nm with a LOQ of about 10 µg / ml. Detection sensitivity is improved with the application of cationic surface modifiers in the background electrolyte.

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