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Analytical Chemistry at the Laboratoire d'Electrochimie Physique et Analytique

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Abstract: The Laboratoire d'Electrochimie Physique et Analytique (LEPA) has moved to the new Energypolis campus in Sion. This laboratory is involved in energy research in particular by studying charge transfer reactions at soft interfaces and developing interfacial redox electrocatalysis, by pioneering the concept of photo-ionic cells and by integrating redox flow batteries for the production of hydrogen at the pilot scale. Nonetheless, this laboratory has a long tradition in analytical chemistry with the development of microfabrication techniques such as laser photo-ablation, screen-printing and more recently inkjet printing for the design and fabrication of biosensors and immunosensors. As shown in the present review, the laboratory has recently pioneered new technologies for electrochemical and mass spectrometry imaging and for the screening of allergy in patients. The role of the laboratory in the Valais landscape will be to foster the collaboration with the HES to develop teaching and research in analytical chemistry as this field is a major source of employment for chemists.

Keywords: Analytical chemistry · Electrostatic spray ionization mass spectrometry · Immunoaffinity capillary electrophoresis · Scanning electrochemical microscopy

Scanning Electrochemical Microscopy

Scanning electrochemical microscopy (SECM) is a versatile scanning probe microscopy (SPM) technique with a (sub) micrometer resolution for the spatial characterization and manipulation of various interfaces through the detection or generation of redox active species at an ultramicroelectrode (UME). The UME is immersed in an electrolyte solution and vertically approached or laterally scanned in close proximity to a sample surface while recording the electrochemical signal influenced by the UME-substrate distance and the local surface reactivity of the sample. SECM has found a wide range of applications, for example the investigation of electron and ion transfer processes at liquid/liquid interfaces, the determination of the kinetics of homogeneous and heterogeneous reactions and the reactivity mapping of various samples including combinatorial catalyst libraries and living cells. However, as with most SPM techniques, SECM suffers from some drawbacks related to long imaging times due to restricted probe translation rates (otherwise convective disturbances are induced), the necessity to immerse the sample completely in an electrolyte solution and to keep a constant working distance during SECM imaging to avoid topographic artifacts and a mechanical probe–sample contact. While many of the proposed approaches to overcome such limitations are based on the combination of SECM with other SPMs or by implementing additional signal acquisition techniques to keep a constant working distance, LEPA in collaboration with the group of Professor Gunther Wittstock from the University of Oldenburg (Germany) has introduced a new soft stylus probe concept to tackle such issues. The soft stylus probes consist of an UME embedded in thin, bendable polymeric films. Such probes are brought upon mechanical contact with the substrate, to scan over the sample surface in a brushing like way while keeping a constant working distance. The principle has been demonstrated first for a soft carbon microelectrode probe (the so-called Soft Stylus Probe) to scan rough and tilted substrates without obtaining topographic artifacts.[1,2]

Initially, the probe fabrication was carried out by UV laser ablation on a thin polyethylene terephthalate film to form a microchannel that was filled subsequently with a conductive carbon paste and sealed with an insulating covering layer. Later, other microfabrication techniques such as aerosol jet printing,[3] inkjet printing, Parylene coating of Pt wires[4] and sealing Pt wires in glass sheaths[5] have increased the versatility of the newly designed probes and applications for soft SECM contact mode imaging. The imaging time has been significantly reduced and the screened area has been enlarged to square centimeters by manufacturing and employing soft linear arrays of microelectrodes.[3,5–9] Additionally, due to very weak forces that the probes exert onto the sample surface, scanning of delicate substrates such as micro-contact printed self-assembled monolayers was performed without inducing scratches in the monolayer.[8]

Furthermore, LEPA has realized the scanning of dry surfaces by implementing microfluidic channels into the soft SECM probe body in order to maintain a nanoliter droplet in the gap between the sensing microelectrode and the sample. The investigated area is embedded in the electrochemical cell formed by the droplet, whereas the rest of the substrate is kept dry. This concept was demonstrated first for the Fountain Pen Probe containing one electrolyte-delivering microchannel.[10] Afterwards, the concept was extended with two microchannels as the Microfluidic Push-Pull Probe,[11,12] which has been shown to be very useful to extract not only electrochemical information but also to get the chemical information from the sample at the same time and location. For instance, the chemical products from enzymatic reactions, which are produced locally over a sample surface, can be aspirated through the microchannels and detected by SECM and mass spectrometry (MS).[12] This has been successfully demonstrated for the electrochemical imaging of a human fingerprint and the simultaneous chemical analysis to identify an explosive contaminant adsorbed on the studied fingerprint, which was in this particular case picric acid.[12]

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Additionally, SECM using soft probes with integrated microfluidics shows great potential, in particular to investigate relevant biological samples such as adherent cancer cells and cancer tissues. In addition to classical SECM imaging, it can also be employed to stimulate the microenvironment of living cells and to read their metabolic response (Fig. 1). As a perspective, the SECM probes developed in LEPA could provide alternative ways for the rapid diagnostics of biological samples.

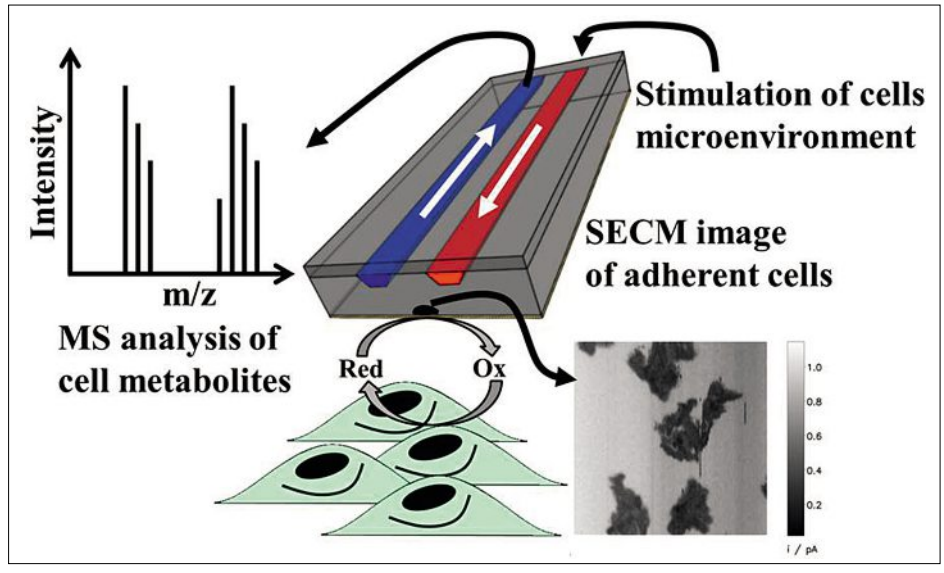

Fig. 1. Schematic representation of the Microfluidic Push-Pull Probe concept for the simultaneous SECM and MS imaging of *in situ* stimulated living cells.

Electrostatic Spray Ionization Mass Spectrometry

MS is a technique to analyze the mass-to-charge ratio (*m/z*) of ions. A typical mass spectrometer includes mainly the ion source generating ions from a sample, the mass analyzer separating the ions according to their *m/z*, and the detector identifying the ions. In LEPA, the electrochemical aspect of ionization methods of MS has been studied.[13] Under this topic, LEPA has used microfluidic chips to combine the electrochemical/chemical redox reactions with MS analysis,[14,15] investigated the photo-electrochemical principle of matrix-assisted laser desorption/ionization (MALDI),[16] and developed a number of ionization devices for MS.[17]

Recently, there is a wide interest in the development of ambient ionization methods that produce ions from untreated samples under ambient conditions for high throughput analysis and to maintain the original state of the sample during analysis.[18] Electrostatic spray ionization (ESTASI) is an ambient ionization method, which has been developed by LEPA since 2012 and which is characterized by inducing the spray by electrostatic forces in order to reduce significantly the influence of the applied high voltage (HV) on the sample.[19] The ESTASI device is illustrated in Fig. 2 showing the electrode connected to the HV, an insulator isolating the sample from the electrode and a counter-electrode that is normally the MS inlet. A pulsed HV square wave from 0 V to 2–10 kV at 0.1–100 Hz frequency is normally applied. There are two main operations depending if the sample is dry or immersed in a solution. If the sample is liquid, ESTASI is realized directly from the sample under ambient conditions. In order to analyze a solid substrate, a droplet of extraction solution is added to extract and dissolve target molecules from the sample for ESTASI-MS analysis. Applying a positive HV at the electrode and grounding the MS inlet (Fig. 2a), the charge separation inside the droplet is caused by the electric field, with cations accumulated close to the MS inlet, and anions close to the electrode. When the charge density on the tip of the droplet is large enough that the electrostatic pressure exceeds the Laplace pressure, a spray of cations is generated while the anions stay inside the droplet. Afterwards, by grounding both the electrode and MS inlet, the anions can be released from the bottom of the droplet, leading to the spray of anions (Fig. 2b). Therefore, after one cycle the electroneutrality of the droplet is provided. By repeating this procedure, a continuous bipolar spraying can be realized for both cation and anion analysis.

With the ionization method, fast *in situ* analysis of samples under ambient condition is achieved.[20] It was used for the rapid characterization of perfume, where the perfume sample was nebulized directly on top of a fragrance-testing strip, and the strip was placed under the MS inlet for direct ESTASI-MS analysis.[21] It was also used for the fast quantification of caffeine contained in various drinks.[22] The caffeine-containing drink was deposited on a plastic strip with a known amount of caffeine for quantitative analysis based on standard addition calibration.

ESTASI is also used to couple MS with various separation techniques for bioanalytical applications. Peptides and antibiotics could be separated by capillary electrophoresis, where the fraction was collected and dried on a plastic plate to form arrays of sample spots. For the analysis, an acidic solution was added on each spot followed immediately by ESTASI-MS.[19,23] The peptides or proteins separated by isoelectric focusing (IEF) in polyacrylamide gels can also be detected *in situ* by ESTASI-MS.[24] By adding a droplet of acidic solution on top of the sample band, protons could diffuse into the gel and protonate the peptides or proteins inside. Afterwards, by applying the HV, the positively charged sample was extracted from the gel into the droplet for ESTASI-MS analysis. With this method, a limit of detection at 1 pmole of sample loading amount was achieved for

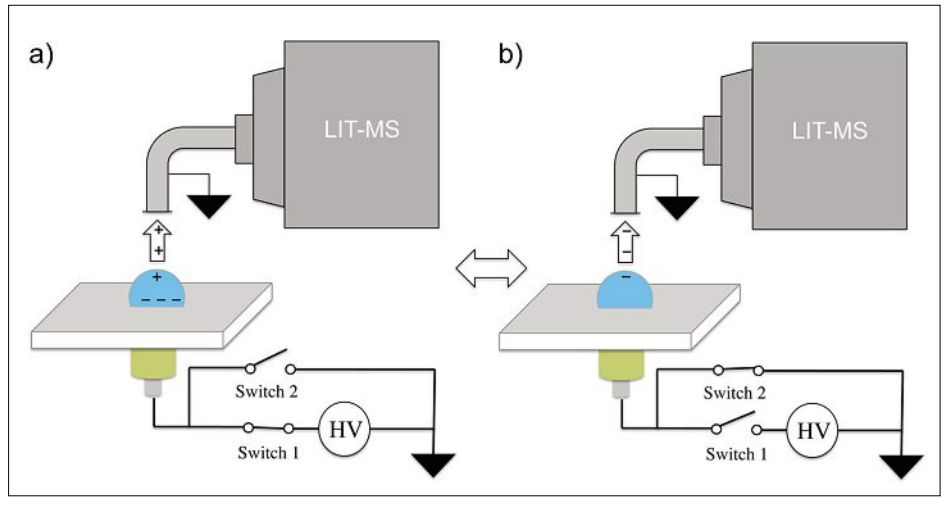

Fig. 2. Schematic illustration of the ESTASI device and principle, with a) positive spray and b) negative spray. LIT-MS: linear ion trap mass spectrometer.

proteins and peptides demonstrating that the ESTASI technique can be more sensitive than the classic Coomassie Brilliant Blue staining for gel visualization as well as providing directly the molecular mass information.

ESTASI was further used for MS imaging.[25] A capillary (I.D. 50 µm, O.D. 150 µm or 363 µm) was used to deliver a solution onto the sample surface in order to extract locally the molecules to be analyzed. A 2D travel stage was used to move the sample in *x* or *y* direction for ESTASI-MS scanning of the whole sample surface. During scanning, the position of electrode, capillary and MS inlet was fixed in line. The ESTASI-MS imaging method has been applied to read proteins, peptides, printed inks and living cells from a plastic surface (Fig. 3). Since the ionization is carried out under ambient conditions the ionization does not destroy the samples. Generally, we have shown that the ESTASI MS technique is very promising and offers great opportunities for the analysis of biological samples such as living cells and tissues. Combined with other SPMs such as SECM it will enable the simultaneous, non-invasive detection of chemical and electrochemical information.

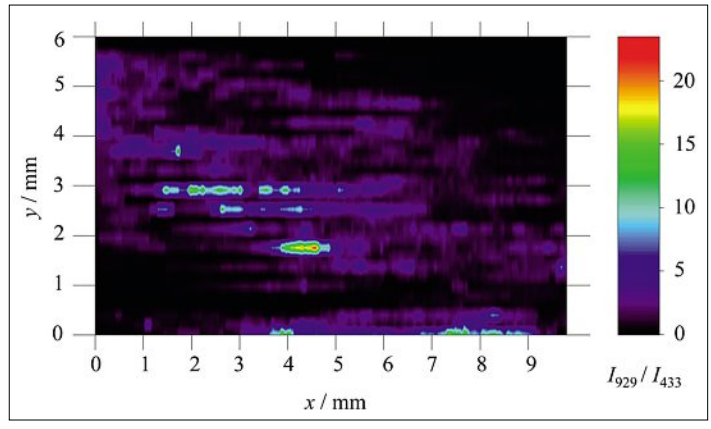

Fig. 3. ESTASI-MS imaging of living cells on a Petri dish. The peak at *m/z* = 929 was selected as a characteristic peak of the cell. I_{929}: ion current intensity at *m/z* = 929. I_{433}: ion current intensity at *m/z* = 433 for the internal standard of angiotensin I (1.5 µM in 50% water, 49% methanol, and 1% HAc, delivered by the capillary).

Immunoaffinity Capillary Electrophoresis

Immunoaffinity capillary electrophoresis (IACE) is a hybrid analytical technique that combines the immunoextraction of a sample with its electrophoretic separation. Typically, the IACE analysis consists of three steps: 1) immunoaffinity-based adsorption of the target compounds from the sample; 2) subsequent recovery of the analytes from immobilized immunoaffinity ligands; 3) electrophoretic separation of the enriched compounds and their detection. UV-adsorption, fluorescence or laser-induced fluorescence (LIF) are normally used as detection techniques for IACE. Due to the high selectivity and sensitivity of the immunoextraction step, and the high resolving power of electrophoretic separation, different complex biological samples can be easily analyzed by IACE.

In LEPA, the main application for IACE is food allergen detection and food allergy diagnosis. This is an important topic, because this disease, defined as an abnormal response of human immune system to otherwise harmless antigens, is an emerging health problem, especially in developed and industrialized countries. In particular food allergy affects around 2% of adults, but being especially acute in childhood, it touches 6–8% of children and infants.[26] As food allergy can easily cause such life-threatening allergic reactions as anaphylaxis, early allergy diagnosis and recognition are very important for patients' health and to improve their quality of life.

The main allergy biomarker is a special type of antibody, produced in response to allergens and referred to as immunoglobulins E or IgE antibodies. When the total concentration of these antibodies in the blood serum of the patient is higher than 240 ng/mL, the person is regarded as allergic. LEPA has developed a sensitive and fast method of IgE antibodies quantification in human blood serum for allergy diagnosis using IACE with LIF detection.[27] This technique, employing magnetic beads coated with anti-human IgE antibodies as immunoaffinity sorbent, allows effective extraction, separation and detection of IgE antibodies to be performed within less than 50 min from only 1 µL of the patient blood serum.

The described procedure provides the value of the total concentration of IgE antibodies resulting in a general allergy diagnosis. For precise diagnosis, correct allergy treatment, monitoring and prognosis of the disease development, it is necessary to access the concentration of specific IgE antibodies, produced against particular allergens in the blood serum of the allergic patient. This type of allergy diagnosis is called component-resolved diagnostics (CRD) and is particularly important in the case of food allergies, as many food products are the sources of more than five different allergenic molecules. Recently, LEPA has presented a CRD of cow's milk allergy by IACE with UV and MALDI-MS detection.[28] The workflow of this diagnostic procedure is schematically shown in Fig. 4.

During the first step (Fig. 4.1), the

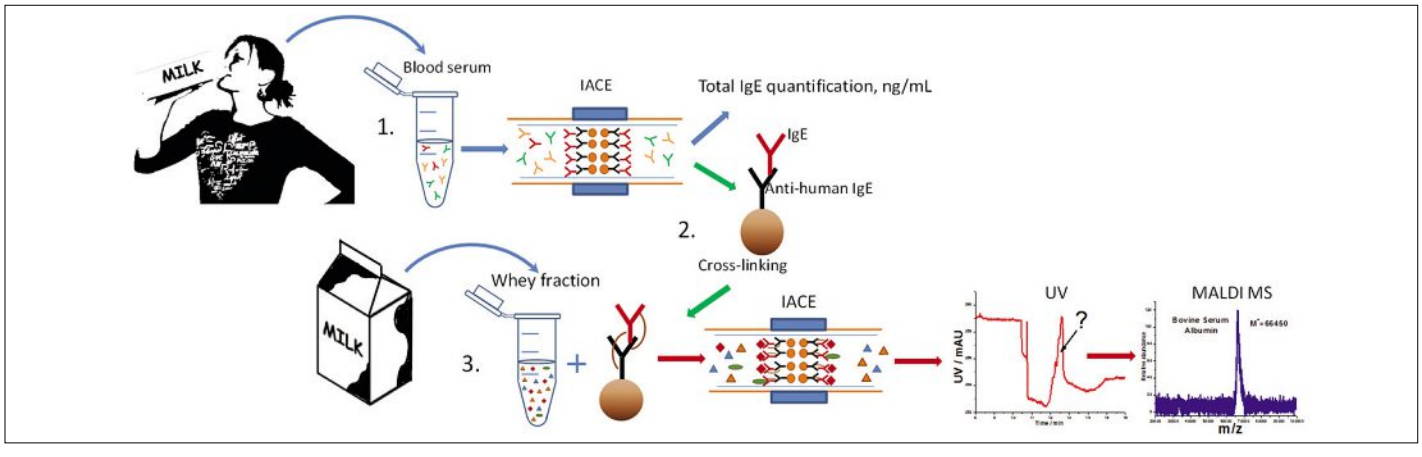

Fig. 4. Experimental workflow of the allergy CRD by IACE. 1) Quantification of total IgE antibodies in blood serum of the milk allergic patient by IACE-UV analysis. 2) Extraction of IgE antibodies using the IACE protocol followed by their further fixation on the surface of magnetic beads *via* a cross-linking reaction. 3) CRD of cow's milk allergy by IACE analysis with UV and MALDI-MS detection using magnetic beads with a cross-linked immunocomplex. Adapted with permission from *Anal. Chem.* **2014**, *86*, 6337. Copyright 2014 American Chemical Society.

IACE-UV analysis was performed for the quantification of total IgE antibodies in the patient blood serum with the limit of detection (LOD) of 0.24 ng/mL. For this step magnetic beads coated with anti-human IgE antibodies were used as immunoaffinity support inside the CE separation capillary. As a second step (Fig. 4.2), the same IACE protocol was used to extract on-line patient IgE antibodies on the surface of magnetic beads *via* the formation of immunocomplex between anti-human IgE-antibodies and patient IgE antibodies. Then, this complex was stabilized by chemical cross-linking. During the third step (Fig. 4.3), CRD of cow's milk allergy was performed by IACE-UV/MALDI-MS applying newly obtained immunosupport for extraction, separation and detection of 'culprit' allergenic proteins. The allergen identification for the chosen patient was realized not only from the individual solutions of milk proteins, but also directly from food extract, *i.e.* milk fractions (whey and caseins), that was possible only due to the application of MS as a detection method. Such direct implementation of food extract in combination with MS detection allows the characterization of allergen mass and structure, providing the possibility to diagnose rare allergies and identify previously unknown allergens. Moreover, the whole analytical procedure requires only 2 µL of the patient blood serum for all manipulations and can easily be extended to any type of allergy.

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