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Lab-on-a-Chip

Microfluidic Glass Chips with an Integrated Nanospray Emitter for Coupling to a Mass Spectrometer**

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The miniaturization of chemical processes on so-called lab-on-a-chip devices, where reactions are executed not in flasks and tubes but in microchannels and cavities, is an emerging new technology. By use of these integrated microfluidic laboratories, chemical reactions and analyses can be accelerated enormously.[1] Furthermore the development of lab-on-a-chip systems was spurred by the demand of analytical tools for high-throughput screening procedures for the life sciences as well as classical chemistry.[2,3]

The miniaturization of chemical processes features numerous advantages, but highly sensitive detection methods are the most challenging tasks in the development of lab-on-a-chip devices. Sensitive fluorescence detection is often used for the analysis of small amounts of samples in microfluidic systems.[4] With the exception of the native fluorescence detection[5] an often problematic labeling strategy is required.

Therefore the coupling of microfluidic glass chips with mass spectrometry seems to be a very attractive detection method as analytes can be identified using the extracted mass spectra.[6] The first approach for coupling microchips and mass spectrometry was developed in the early stages of lab-on-a-chip research.[7] In this case a very easy design was chosen; the electrospray was generated directly at the blunt end of the microchip. Unfortunately this configuration is characterized by a large emitter area, which causes an undesired dead volume in terms of a big liquid droplet. Additionally, external pressure is necessary to ensure sufficient spray stability.

Based on the fundamentals of nanospray ionization by Mann and Wilm employing drawn glass capillaries, the efficiency of the electrospray process could be improved significantly by decreasing the emitter area.[8] Furthermore, in comparison to the classical electrospray ionization the nanospray ionization with its low flow rate is characterized by a higher spray stability, improved ionization efficiency, and reduced ion suppression.[9]

Currently, the most common approach for coupling microfluidic chips with mass spectrometry relies on manually assembled external emitters.[10] The potential of Chip–MS coupling was demonstrated impressively with this method.[11] The disadvantage of this technique is the time-consuming and error-prone assembly. In addition, a dead volume occurs between the fluidic channel and the capillary emitter. These problems are avoidable by directly integrating the emitter on the microfluidic chip. Such monolithic emitter chips are elegantly producible in polymers, for example by using replicate casting techniques such as injection molding and laser ablation.[12]

As in macroscopic laboratories, glass is the preferred substrate for lab-on-a-chip devices owing to its chemical inertness and optical transparency. The fabrication of integrated emitters on microfluidic chips for electrospray mass spectrometry has been more difficult with glass than with polymers.[13] We have now developed a novel approach to combine a microfluidic glass chip and a nanospray emitter on a single device. Commercially available Borofloat glass chips for microchip electrophoresis were utilized as substrates.[14] In the first fabrication step a little cone with a diameter of only 0.3 mm was milled within 10 min using a CNC machine. The cone encloses the end of the microchannel centrically. The protruding cone with the concentric microchannel was then drawn to a sharp tip utilizing a home-built puller based on a platinum heating coil.[15] A schematic drawing of the completed microfluidic glass chip is shown in Figure 1.

Figure 1. Schematic drawing of the microfluidic glass chip with the integrated nanospray emitter. SO: sample outlet, SI: sample inlet, BI: buffer inlet, MS: mass spectrometer.

Using this approach, very fine tips with tapered structured microchannels can be easily fabricated and reproduced. These tips are comparable to commercially available nanospray needles and have very small emitter dimensions of only a few microns. The sharp tip of a microfluidic chip and a match head are shown for comparison in Figure 2.

These nanospray glass chips were applied successfully in electrospray mass spectrometry. After the microchannels had been filled with an acidified solution of methanol and water, the chip was positioned precisely in front of the orifice of the electrospray mass spectrometer[16] by means of a custom-built interface. When external pressure was applied to the microchip, the electrospray process could be visualized as shown in

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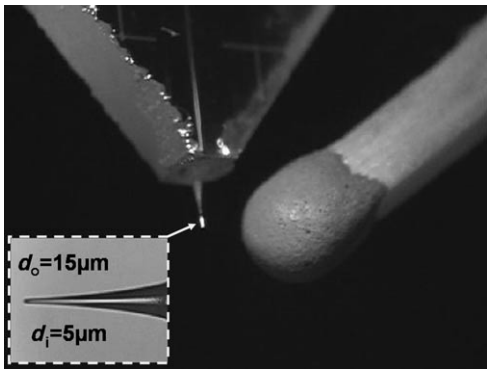

Figure 2. Photograph of the integrated nanospray emitter in comparison to a match head. The inset shows a light-microscopic image of the tip including its dimensions (d_o: outer diameter, d_i: inner diameter).

Figure 3. The distance between the nanospray tip and the MS orifice was 1.5 mm.

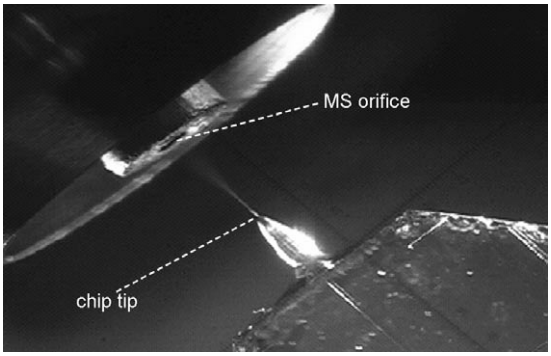

Figure 3. Visualization of the electrospray process using an applied voltage of 4 kV and an external flow of 0.5 µL min^{-1} (MeOH/H_2O 50:50 (v/v) and 0.001 % AcOH).

Like common nanospray needles the presented needles can operate without any external pressure to generate a stable electrospray. Additionally, the flow rate determined of only 25 nL min^{-1} [17] underscores how closely related Chip–MS coupling and nanospray mass spectrometry are. Also, the mass spectra obtained from Chip–MS are comparable to those from commercial nanospray tips (see Figure 4). In this

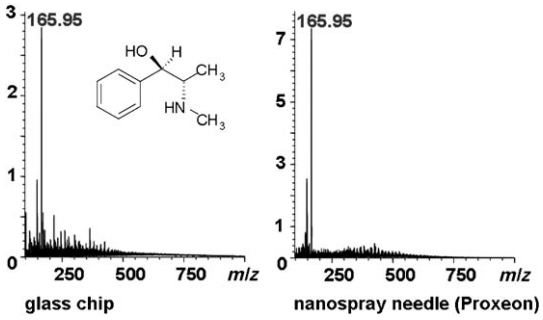

Figure 4. MS signal intensities (×10^6) for a sample of 1 µg mL^{-1} ephedrine in MeOH/H_2O 50:50 (v/v) and 0.001 % AcOH.

experiment 1 µg mL−1 of ephedrine in an acidified MeOH/H_2O solution was sprayed without external pressure into an electric field.

The data from basic electrospray experiments using our glass chip and common nanospray needles are comparable. The tremendous potential of the lab-on-a-chip technology can be shown by implementing chemical processes on the microfluidic structure. For example, chemical reactions inside the microchannels could be followed by mass spectrometry. As a model system from bioanalytical research we decided to use on-chip digest of bovine serum albumin (BSA). The experimental setup of the model system is shown in Figure 5. After

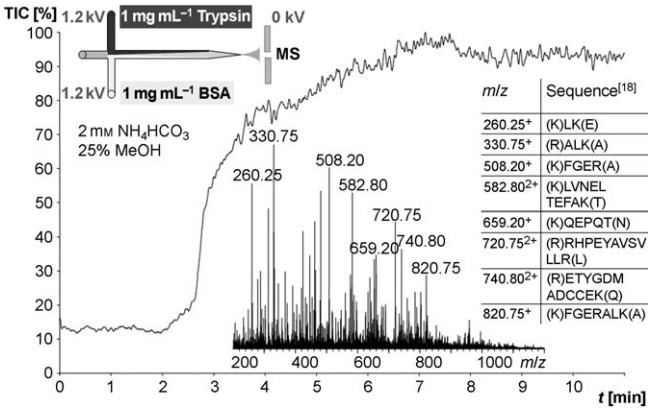

Figure 5. On-chip digest of 1 mg mL^{-1} BSA and 1 mg mL^{-1} trypsin in 2 mM NH_4HCO_3 and MeOH 75:25 (v/v). Schematic illustration of the experiment and classification of dominant MS signals of corresponding peptides. TIC: total ion current.

the protein and enzyme had been pipetted in opposite microvials, the reactants were moved to the tip of the microchip and sprayed into the mass spectrometer using an applied voltage of 1.2 kV. During the process the transport of liquids occurred only by electroosmosis and electrophoresis. After 3 min a strong increase of the total ion current (TIC) was observable in spite of the incomplete mixing of reactants in straight microfluidic channels. The mass spectra could be extracted and correlated to characteristic peptide fragments, which enabled the identification of BSA via databases.[18] Using a more complex microfluidic system, including multiple channels, the identification of many proteins would be possible within a short period of time. Because the reactants were moved only through the applied voltages, no external pumps were necessary.

The development of the microfluidic glass chip with an integrated nanospray emitter was driven by the demand for an efficient coupling of microchip electrophoresis (MCE) and mass spectrometry.[19] Furthermore, MCE, which can be regarded as a miniaturized version of classical capillary electrophoresis, is one of the most successful applications of microfluidics in analytical chemistry.[20] Here, we can introduce the first dead-volume-free coupling of a glass electrophoresis chip with mass spectrometry without any external pressure. The separation and detection of a mixture containing four pharmaceutical compounds is shown in Figure 6. As

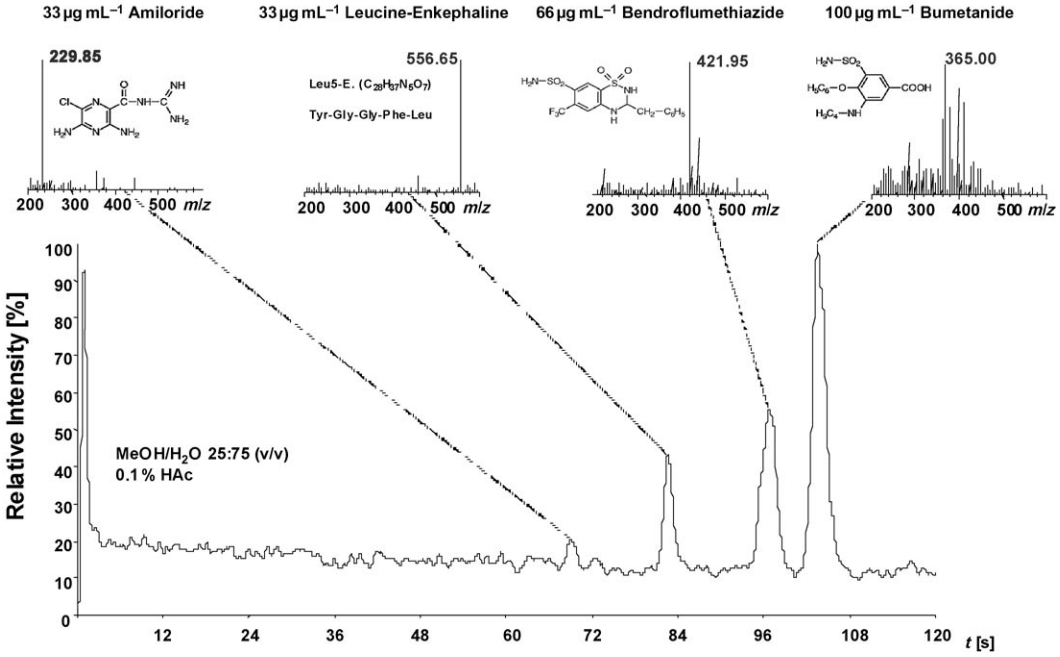

Figure 6. Application of the system for dead-volume-free coupling of microchip electrophoresis and mass spectrometry. Diagram of the total ion current and the extracted MS spectra.[21]

electrolyte medium an aqueous 0.1 % acetic acid solution containing 25 % (v/v) methanol was employed. The total ion current shown in Figure 6 and the corresponding mass spectra document the capability of fast analysis. The electrophoretic separation coupled with MS detection is completed in approximately 100 s; by applying increased field strengths the analysis can be further accelerated.

We have prepared a microfluidic glass chip having a monolithically integrated nanospray tip and coupled it with mass spectrometry. In this first study such chips were used to study a simple biochemical reaction, as well as chip-electrophoresis coupled with mass spectrometry. In the near future the nanospray chips should be implemented as mass-spectrometric couplers of complex lab-on-a-chip systems, particularly to integrate chemical synthesis with subsequent analysis of the products on a chip.[22] The efficient coupling of microfluidic chips with mass spectrometry could solve one of the greatest problems in microfluidics, namely the detection of miniscule sample quantities. In view of the tremendous progress in microsystems technology, the feasibility of even incorporating a mass spectrometer on a chip[23] could fulfil the dream of an entire laboratory on a chip.

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