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Polymer Microspray with an Integrated Thick-Film Microelectrode

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A microfabrication process leading to a sheathless electrospray interface for mass spectrometry analysis is described. Photoablation is performed on a polymer substrate, allowing the integration of a thick-film conductive track in a sealed microchannel. High voltage is supplied close to the outlet, through an embedded microelectrode. The microspray is generated directly from the edge of the substrate without any tip addition. The flexibility of this technology provides a wide range of dimensions for the probe and the microelectrode design, including location, shape, and conductive material used. Thanks to the thick-film microelectrode and the hydrophobicity of the polymer, which avoids solution spreading at the outlet, the device has been found to be an efficient ionization source providing a stable MS signal through time. Moreover, the same device can be used several times without failure. The performance of the microspray has been studied in simple infusion mode for proteins and reserpine MS analyses. The detection limit of reserpine was found to be at the picomolar level in full-scan MS mode. It implies also that ~500 zmol was read consumed during 3 min of infusion. A dynamic range from pico- to millimolar level is also underlined.

Micro total analysis systems (μTAS) have been extensively developed for use in applications such as genetic analysis. In particular, miniaturized devices, which incorporate separation techniques such as capillary electrophoresis, have been widely investigated for DNA analyses but must be adapted for protein separation purposes. The increasing number of microchip implications in pharmaceutical and biochemical analyses has led to a growing demand for detection methods, which are more sensitive, more selective, and better able to provide structural information. This last trend is particularly true in protein analysis that must be more and more efficient, due to the increase of interest in proteomics and the concomitant complexity of the analysis.[1]

In parallel to the development of microchips, progress in mass spectrometry (MS) instrumentation has opened the way to the analysis of large biomolecules,[2] mainly by the implementation of two soft ionization techniques: matrix-assisted laser desorption/ionization (MALDI) and the electrospray ionization (ESI). The latter is a very useful on-line interface between liquid-phase separation systems and mass spectrometry. ESI stands out as the easiest technique for ionization in terms of flexibility and technical requirements.

During the 1990s, miniaturized electrospray interface fabrication was widely investigated in order to couple CE devices to mass spectrometers. Actually, it was established that micro- and nanospray interfaces provide better sensitivity than standard electrosprays.[3,4] For the spray designs, the critical points remaining are the application of the high voltage, the coupling to a CE microdevice, and the dead volume minimization.

Among all the designs, small-diameter glass capillary tips are widely used as sheathless emitters. It requires several steps to obtain the final shape, such as pulling and HF etching, and to include an electrical connection. To achieve the latter, gold is usually sputtered at the outlet of the tip.[5−7] However, this conductive layer is rapidly damaged due to the high voltage applied. Several strategies have been investigated to overcome this problem. Valaskovic and Mclafferty[8] improved the mechanical

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stability of the gold layer by overcoating the emitter with a SiOx insulating layer, inducing an extended life span. Barnidge and coworkers[9] could obtain a longer one by fixing 2-μm gold particles with polyimide on glass tips.

Others configurations have been proposed, where the electrical connection is obtained thanks to a metallic wire inserted at the tip.[10,11] The drawback in this case could be the flow perturbation due to the wire and the electrochemical events that take place on it, such as bubble formation.[12] The design proposed by Hsieh et al.[13] prevents this flow perturbation: it consists of a pulled capillary wherein a sheath liquid capillary and a stainless steel wire for the high-voltage connection are inserted. In this case, the sheath liquid delivers high voltage to the emitter and prevents an analyte counterflow during CE experiment.

Due to the emergence of separation microsystems, pioneer work on glass chips has been presented by the Karger[14] and Ramsey[15] groups for on-line coupling to a mass spectrometer through a microspray interface. The spray was generated at an open end of a microchannel on the edge of glass chips by applying voltage at a reservoir, without any pneumatic assistance. However, they reported troubles in the spray stability due to the hydrophilic nature of the glass substrate. Derivatization of the chip edge was found to be one way to overcome this problem. Glass or silicon microchips are therefore often interfaced to a spray tip by means of glue, which yet renders the fabrication tedious.[16−18] In all these cases, the high voltage for the spray process is applied at an inlet reservoir, preventing any control on the spray current.

A liquid junction has also been included in several microdevices, allowing the modification of solution composition to yield an easier spray process[19] but also to supply the spray voltage.[20,21] During the last years, polymeric substrates have been also investigated. A wide range of fast prototyping techniques has been used to fabricate microfluidic chips, such as replica molding,[22] embossing,[23] automated cutting,[24] and photoablation.[25,26] One advantage of these substrates, compared to glass or silicon, is the hydrophobic nature, which favors spray generation. The convenience of these materials allows also the microfabrication of multichannel emitters with different configurations[24,26,27] but also the complete integration of a sharp emitter in the microfluidic chip.[25]

In this paper, a novel microspray interface is presented, which is based on different microchip designs developed previously[28,29] and polymer photoablation. The present polymer device includes a carbon ink microelectrode located inside a sealed photoablated microchannel, close to the outlet of the probe. Some features of this system, such as stability and ionization efficiency, have been evaluated performing μESI-MS analyses on protein and reserpine solutions.

EXPERIMENTAL SECTION

Chemicals. Reserpine and myoglobin from horse heart were purchased from Sigma Chemical Co. (St. Louis, MO) and used as received. All the samples were diluted in 50/49/1% (v/v) methanol/water/acetic acid. Methanol was purchased from Merck and acetic acid from Fluka (Buchs, Switzerland), and both were used without any further purification. Deionized water (18.5 MΩ) was prepared using a Milli-Q system from Millipore (Bedford, MA). The reserpine stock solution was 1 mg/mL. Fresh solutions were diluted to obtain the desired concentrations.

Microchip Fabrication. For the fabrication of the device, a UV excimer laser (ArF 193 nm, Lambda Physics LPX 205i) was used to photoablate poly(ethylene terephthalate) (PET) substrates (100-μm-thick Mylar A sheets from Dupont). With the use of photomasks and a translation stage (Microcontrol), one can obtain several geometries, such as microchannels or microholes and reservoirs.

Photoablation allows good control of the width, the depth, and the length of drilled microchannels by adjusting parameters such as pulse frequency, translation stage speed, and laser energy.[28]

The fabrication procedure used for the integration of the microelectrode was performed as previously described[30] and is shown in Figure 1. Briefly, an L-shaped channel is machined and filled with an Electrador carbon ink (Electra Polymer & Chemicals Ltd.). It is cured and then protected with a 25/10-μm laminated PE/PET composite sheet (Morane), where PE acts as the sealing agent.

A feeding microchannel is drilled on the other side of the substrate in order to reach the bottom of the previous conductive track. Thus, a microelectrode with controlled dimensions is generated at the bottom of the channel. The contact between the power supply and the generated microelectrode is obtained through the conductive track and pad. The feeding channel is then sealed by lamination.

Finally, the chip edge is cut by photoablation, thereby producing the microspray outlet and also fixing the distance between the microelectrode and the open end (here 2.5 mm).

In the present microspray, the feeding microchannel has a trapezoidal cross section, due to the photoablation process, with an average width of 40 μm and a depth of 40 μm. The resulting microelectrode area is 30 × 85 μm^2, preferably chosen here in order to lower the current density during the spraying and hence minimize the bubble formation due to water electrolysis. However, the dimensions can be varied from 200 to 8000 μm^2 depending on photoablation parameters.

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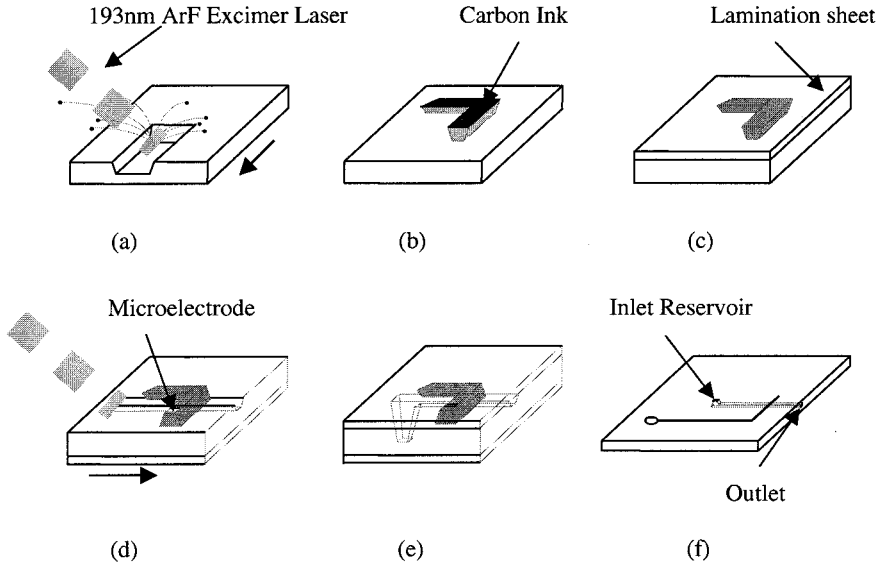

Figure 1. Fabrication scheme of microspray. (a) The substrate is placed on a translation stage and dynamically photoablated using a rectangular mask. (b) It is filled with carbon ink; 1 h at 90 °C is used for ink curing. (c) The resulting track is then sealed with a lamination sheet (130 °C, 3 bar). (d) The feeding microchannel is drilled on the other side of the substrate in order to expose the bottom of the first microchannel, resulting in an embedded microelectrode with controlled dimension; (e) The feeding microchannel is closed with a lamination sheet. (f) A photoablation is performed though all the substrate thickness, generating the microspray outlet.

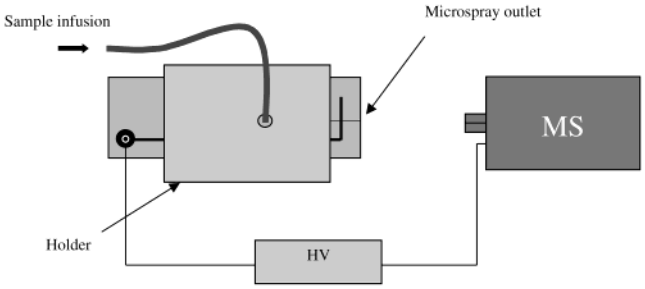

Figure 2. Schematic experimental setup. The sample is introduced by simple infusion via a syringe pump. The high voltage is supplied by the LCQ Duo source. The ink pad allows the microelectrode electrical connection to the supplier using a crocodile clip.

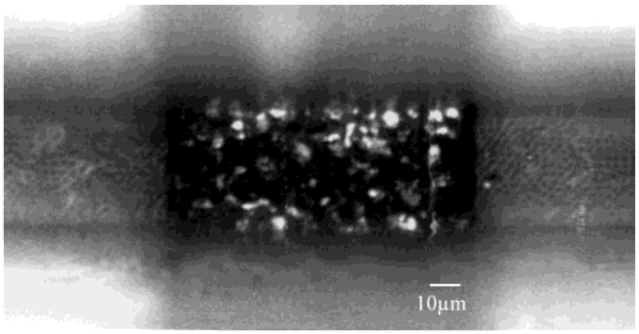

Figure 3. Microscope photograph of the microelectrode at the bottom of the feeding microchannel. One can distinguish the intersection between the conductive track and the main microchannel.

Experimental Setup. All the experiments are carried out using the simple infusion configuration. First, the chip is inserted in a Plexiglas holder in order to facilitate solution delivery to the microspray. The ESI interface of an LCQ DUO ion trap (Finnigan, San Jose, CA) is then removed, and the microchip holder is mounted on the probe slide adapter of the mass spectrometer without any other modification of the latter. The voltage supplier of the MS is connected to the microelectrode by the carbon ink pad on the microchip. The experimental setup is illustrated in Figure 2. A syringe pump (Cole Parmer 74900) is used to apply the desired flow rate to feed the microspray.

The applied voltage is 7−8 kV, resulting in a spray current of ∼100 nA. The heated capillary of the MS is maintained at 200 °C, and the target value for full-scan MS is fixed to 5×10^7. All the spectra are obtained in full-scan MS. The distance between the microspray outlet and the MS entrance (0.5−2 cm), the applied high voltage, and the flow rate (50−1000 nL/min) are varied to obtain the best stability and the highest intensity of the base peak.

Microscopy Analyses. Scanning electron microscopy (SEM) was performed on a JEOL 6300 apparatus. The acceleration voltage was 5 kV. Samples were gold sputtered prior to SEM measurements.

RESULTS

Aspect of the Structure. In this microdevice, the electrode is inserted in the top of the feeding channel. The photoablation process provides a precise positioning of the electrode with respect to the microspray outlet. Figure 3 illustrates the location of the electrode at the bottom of the main microchannel. Embedding the microelectrode avoids perturbations in solution flow contrary to the cases where a wire is placed in the capillary end. Moreover, in this particular configuration, the electric field lines are forced through the solution, hence focusing the spray. The thickness of the substrate is less than 170 μm, which favors cone formation with a lower dead volume. Figure 4 shows a SEM picture of a microspray outlet after fabrication. One can identify the two lamination sheets and the PET substrate where the feeding microchannel has been drilled. It is worth noticing the efficient sealing obtained with the lamination process. The appearance of the spray outlet is similar to the ones made in glass and presented by Ramsey and Ramsey.[15]

Spray in Operation. Figure 5 shows a top view of the Taylor cone obtained at the microspray outlet. In a first approximation, the cone volume is calculated to be ∼2 nL. One can see that it occurs near to the microchannel aperture but not at the outlet. This is attributed to a protruding tip on the substrate edge, where

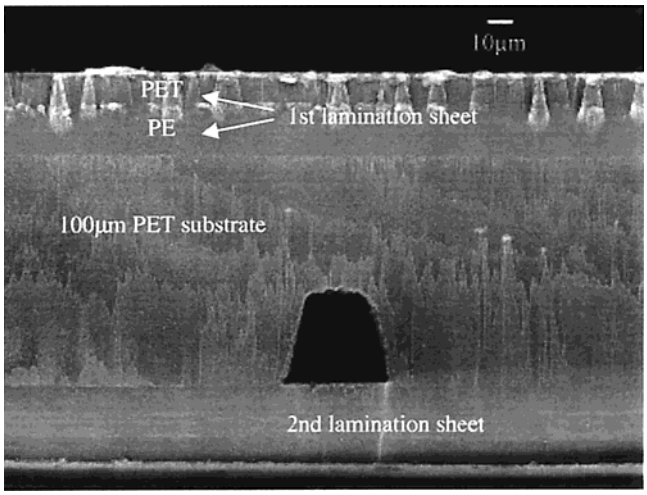

Figure 4. SEM picture of the microspray outlet, obtained following the fabrication scheme described in Figure 1 (front view).

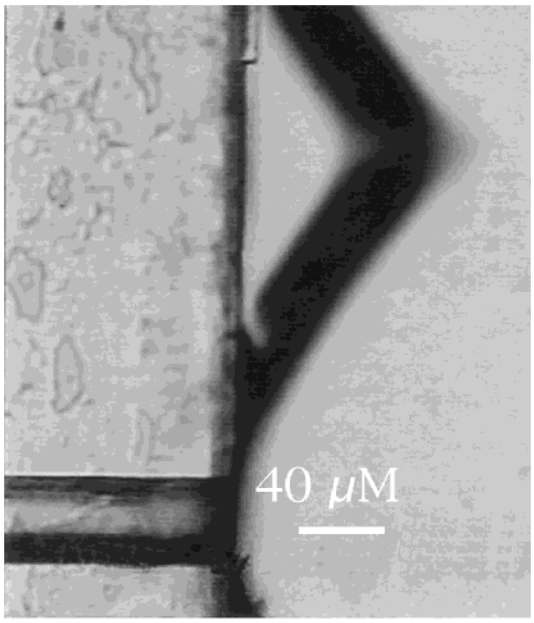

Figure 5. Microscope photograph of a Taylor cone formed at the outlet of the microspray (top view).

the electric field is greatly increased due to the small dimensions of the tip, which acts as an "electric field densifier". Referring to Figure 4, this tip could be one of the rough patches observed.

The hydrophobic character of the polymer body, improved by the photoablation process,[31] prevents the use of nondurable derivatization which is commonly used on glass microchips to obtain a stable spray.[14] It helps to prevent solution spilling at the outlet of the chip as already mentioned. Further surface modifications, such as plasma treatment, has been shown to induce a durable enhancement of the hydrophobic nature of the polymeric substrates.[26,32] This improvement combined with a thinner substrate should induce a smaller cone volume and thus increase the sensitivity and resolution using the present interface. To adapt it to a separation device, a main critical parameter consists of this cone volume, where strong convection occurs,[33] which mixes the

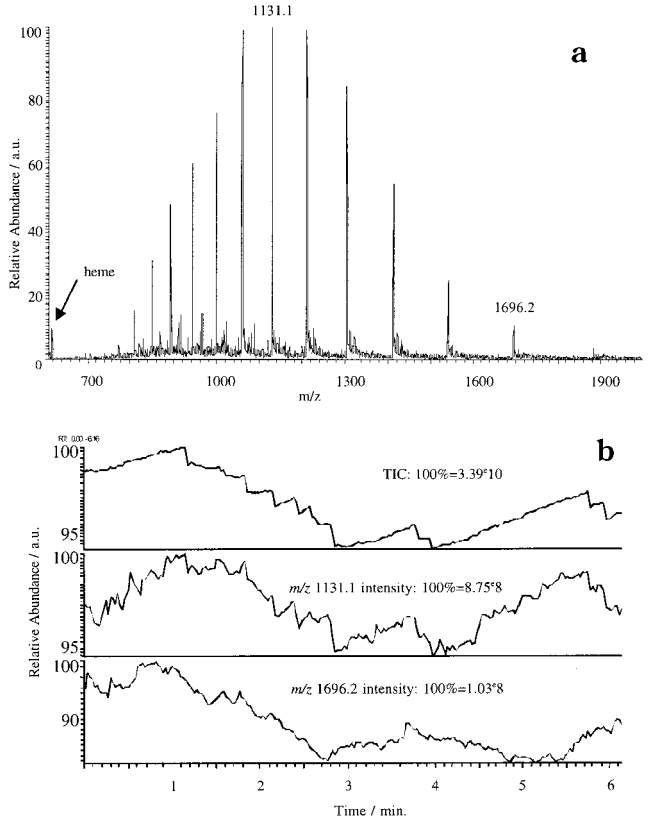

Figure 6. (a) Full-scan MS spectrum for 5 μM horse heart myoglobin in 6-min simple infusion experiment at 233 nL/min. (b) Evolution of total ion current, *m*/*z* 1131.1 peak intensity, and *m*/*z* 1696.2 peak intensity with time on a 6-min simple infusion experiment at 233 nL/min (average of 10 scans).

present molecules. Actually, if the cone volume is too large, a well-resolved separation could be completely lost. The individual separated analytes should be sequentially spaced enough to avoid overlapping during mass analysis. Therefore, in this configuration, the injection plug size and the separation length must be adapted with this limitation.

Stability in ESI-MS. The stability of the spray has first been tested with the infusion of 5 μM myoglobin. Figure 6a shows the charge-state distribution of the protein and the peak corresponding to the heme group (m/z 616.15). The deconvoluted mass is 16 951.57 ± 0.94 Da (theoretical mass 16 951.48 Da). Figure 6b illustrates the spray stability over 6 min by plotting the total ion current (TIC), the +15 intensity, and the +10 charge peak versus the acquisition time. TIC is in 95−100% full-scale range, which indicates a highly stable spray process. Moreover, the stability for the different peak intensities illustrates the ionization stability of the interface. It was verified that the microspray operates for at least 1 h without interruption. It can also be used several times, even with different analytes, and no contamination was observed. It is also worth noticing that once the microspray is on, no contamination peak due to the polymer is observed.

Calibration of the Microspray. We also evaluated the detection limit of the device. To achieve this, solutions of different concentrations of reserpine were infused, from 1 pg/mL (1.64 pM) to 1 mg/mL. Once the microspray was on and stable, the spectrum acquisition was done during 3 min. Figure 7 shows that a good signal of the base peak $[M + H]^+$ is obtained in the full-scan MS

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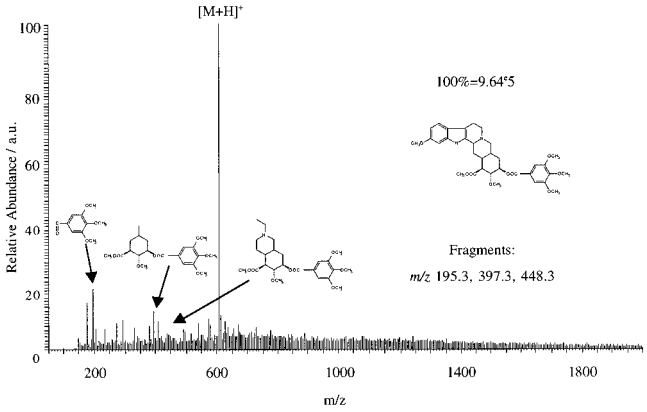

Figure 7. Full-scan MS spectrum for 1 pg/mL (1.64 pM) reserpine (average on 100 scans); simple infusion experiment at 200 nL/min.

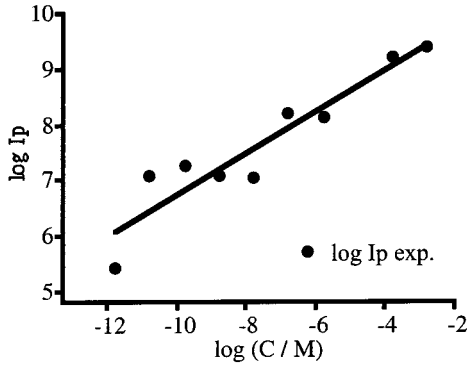

Figure 8. Logarithm of base peak intensity (counts/s) versus logarithm of reserpine concentration.

mode at the picomolar level. One can notice that peaks corresponding to reserpine molecular ion fragmentation are also detected. These fragment peaks can be also considered as a part of the signal due to the analyte. Considering the experimental conditions and results, we are able to calculate the ion transmission ratio, i.e., the ratio between injected and detected ions. During the infusion of the 1 pg/mL solution at 200 nL/min, 3290 molecules/s are consumed by the microspray interface. Considering a scan time of 1 s, a 3×10^5 gain value, and the peak intensity (8.8×10^5 counts), one calculates an ion transmission ratio of 9×10^{-4}, meaning that ~1 molecule for 1000 consumed reached the detector. It should be pointed out that only 3.28×10^{-19} mol/min reserpine have been injected at that concentration, which means that subattomole detection has been obtained; During a 3-min experiment at 100 nL/min (data not shown), only 492 zmol is consumed. The same level of detection was obtained for neurotensin infusion with the microelectrospray interface developed by Andren and co-workers[34] and Lazar et al.[17] It is worth notice that field strengths used by Lazar and Andren are very low (0.2 kV/cm), however, compared to our conditions (4−8 kV/cm). The efficiency of the system can be attributed to the high electric field applied, inducing high electrophoretic separation close to the outlet but also to the small generated Taylor cone volume. Both of these phenomena could result in a preconcentration process at the tip of the spray.

Results for all the concentrations tested are summarized in Figure 8. The base peak intensity has been plotted versus concentration. Within experimental and calculation errors, the relationship obtained is $\log(I_p) = 0.37 \log(C) + 10.4$ ($R^2 = 0.95$).

Enke[35] showed that the ion response is highly dependent on flow rate and spray current I_{spray}. Moreover, depending on solution concentration, a linear trend could be predicted for low analyte concentration compared to electrolyte. However, this model is not adapted to a protonation process and our experimental conditions, in terms of electric field, induce a nonequilibrium situation. This could explain the divergence of our calibration curve from a linear tendency.

Considering the uncontrolled spray current and the varied flow rates applied, some discrepancies are observed between 10^{-5} and 10^{-9} M. It must be stressed here that every single point has been performed with different chips to avoid contamination and the optimization procedure such as flow rate value, positioning, and tuning of the MS has been repeated for each experiment. Nevertheless, a dynamic range of 9 orders is obtained, which indicates the high integration potential of this interface. Furthermore, the injected concentration can be well evaluated.

CONCLUSION

In this paper, a novel microspray interface has been presented. The design is easily obtained thanks to the flexibility of the photoablation process. The presented interface has been shown to provide a highly stable and durable spray ionization, the embedded location of the microelectrode preventing flow perturbations. The detection limit with this device has been found to be at the picomolar level. A dynamic range of 9 orders of magnitude was also described. Taking advantage of the hydrophobic nature of PET substrates, multichannels could be drilled on the same chip to perform automated MS analyses. In this case, no further modification of the microspray outlet is necessary to prevent sample-to-sample cross-contamination due to solution spilling; however, the location of the spray generation has to be taken into account for the design.

The present interface can be integrated into a microdevice for LC or CE analysis, and a capillary for prior separation step can also be easily connected. With the presented fabrication process, several microelectrodes, including pseudoreference electrodes, can be placed in a microchip, thereby allowing one to decouple and control the different applied potentials. Besides, electroosmotic flow and CE systems in photoablated PET have been previously reported,[28,29] meaning that all the conditions are fulfilled to develop an efficient CE-microspray chip in future work.

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