

Glass microfluidic devices with thin membrane voltage junctions for electrospray mass spectrometry

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In this study a novel glass membrane was prepared for conducting high voltage (HV) to solution in the channel of a microfabricated device for generation of liquid electrospray. Taylor cone formation and mass spectra obtained from this microdevice confirmed the utility of the glass membrane, but voltage conduction through the membrane could not be successfully explained based solely on the conductivity of the glass itself. This novel method for developing a high-voltage interface for microdevices avoids direct metal/liquid contact eliminating bubble formation in the channel due to water hydrolysis on the surface of the metal. Further, this arrangement produces no dead volume as is often found with traditional liquid junctions. At the same time, preliminary investigations into the outlet design of glass microdevices for interfacing with electrospray mass spectrometry, was explored. Both the exit shape and the use of hydrophobic coatings at the channel exit of the microdevice electrospray interface were evaluated using standard proteins with results indicating the utility of this type of design after further optimization.

1 Introduction

The increased interest in proteomics and analysis of low concentration biological samples has allowed lab-on-a-chip technology for analytical applications to gain more attention in recent years. Microchip methods have a significant advantage in electrophoretic applications with their fast, high-efficiency separations of small sample volumes with low dead volumes.^{1–3} Microfabricated chips for both electrophoretic separation^{2,3} and capillary electrochromatography^{4,5} have been under active development. Technology and methodologies available from the electronics industry have been directly utilized in the construction of microfabricated analytical devices for manipulating nanoliter quantities of sample in integrated designs. The development of disposable devices may lead to large-scale production of such integrated systems.

Microdevices integrated with electrospray mass spectrometry (MS) are becoming popular mainly because of the great sensitivity and accuracy of MS. Compared with other detection systems for microchips such as laser induced fluorescence (LIF), mass spectrometry is a more universal method of detection. In addition, micro- and nanoelectrospray developments for MS are perfectly matched with the flow rate on microchips.⁶ A number of researchers have developed different approaches to optimize the coupling of microfluidic chips to MS.^{7–9} Integration of microchips with mass spectrometry requires solutions for two basic problems: high-voltage application to the solution in the microchannel to create electrospray and minimizing the size of the droplets formed at the microchip outlet into the MS.

A number of methods have been reported for application of high voltage to microchips to generate the required electrospray. Two approaches have been extensively explored: HV applied directly on transfer lines from the microchips, or direct

attachment of nanospray emitters to the microdevices.^{8,10–16} Techniques utilizing a liquid junction either on¹⁷ or off^{10,18} chip have also been reported. Alternatively, the voltage has been applied at the end of the emitter using a conductive material coated on the end of emitter.¹⁹ Porous junctions have been successfully employed in interfacing capillary electrophoresis to mass spectrometry,^{20,21} and this technique has recently been applied to glass microdevices using a porous glass disk.²² Recently a glassy carbon microfluidic device for electrospray MS was developed in which the HV was easily applied on the device due to the conductivity of glassy carbon.²³ Glassy carbon is expensive, however, and it may not match most researcher needs for microfluidic devices. All of these methods complicate the design of the microdevice or the interface and therefore, have been a driving force for new developments in microchip-MS.

To create tiny droplets at the inlet to the MS detector, a number of groups have attached nanospray tips directly to the microdevices at the microchannel outlet.^{10,11} This method shows promising experimental results, but extensive work is required to fabricate and manufacture the chip. As an alternative to coupling an emitter to the microchip for ESI, electrospray directly from the edge of the microdevices was proposed. The low efficiency and sensitivity of ESI with these devices was due to diffusion at the microchip outlet before the onset of electrospray;^{7,24} the Taylor cone volume being on the same order as the channel volume. A method has been reported for eliminating this problem: modification of the exit microchannel to decrease the wettability of the surface.²⁴ This should prevent spreading of the solution along the edge of the device as it exits the channel, resulting in a smaller droplet. Recently a sharp tip on a microchip was developed to form the tiny droplet required for sensitive electrospray, but the devices were made with plastic material^{9,25,26} limiting their ability to

perform protein separations. One other shortcoming of the plastic devices is the inability to generate electroosmotic flow in the polyimide devices that could be used to pump fluid through the system. External pumps must be employed, but the low flow rates required for microdevices are hard to control with current instrumentation.

This experimental work focused on exploring new techniques for addressing these two aspects for integration of glass microdevices with MS. The glass conductivity itself was exploited in this design with direct formation of a glass membrane in the device to apply the HV to the solution in the microchannel for ESI. Two approaches for membrane formation were evaluated with successful application of both methods to ESI of proteins. To eliminate large liquid droplet formation on the flat microdevice exit, from which the infused sample is sprayed, an initial interface design was studied in which the outlet edge of the microchip was sharpened on all sides. To take advantage of the benefits of the polyimide device for electrospray, the sharpened outlet was coated with polyimide to decrease the surface tension of the solution being sprayed.

2 Material and methods

2.1 Reagents

Hydrofluoric acid (HF), nitric acid, acetic acid, and methanol were purchased from Fisher Scientific (Pittsburgh, PA). Diamond tipped drill bits, diamond saw blades, and diamond hand pads were purchased from Crystalite Corp (Lewis Center, OH). Cytochrome c and lysozyme were purchased from Sigma (St. Louis, MO)

2.2 Microchip fabrication

The microchip was fabricated in glass following a procedure previously described.²⁷ Briefly, the chip fabrication consisted of: exposing the photoresist coated glass, etching the glass to the desired depth, then thermally bonding the bottom and top glass layers together. Additional modifications were performed after bonding to produce a final device. Borofloat glass sequentially coated with chrome and photoresist was obtained from Nanofilms (West Lake, CA). Images in a photomask were transferred to the photoresist using a 350 W UV source (OAI, Milpitas, CA). The photoresist was developed and baked, then the exposed chrome removed and the glass etched using a 25% hydrofluoric acid solution containing 10% nitric acid. After etching the glass to the desired depth, the remaining photoresist and chrome were removed from the glass and inlet and outlet reservoirs were drilled in the top glass layer using 1.1-mm diamond tipped drill bits. Thermal bonding was performed at 685 °C for 3.5 h.

Microdevices which contained a glass membrane were fabricated such that the bottom layer of glass was etched 50 μm deep to produce a channel 70 μm wide at the center with a length of 2–4 cm. The top plate was etched 20–50 μm to form side open areas as shown in Fig. 1(a); the depth of this etch set the thickness of the final membrane. Following reservoir drilling, the top plate was bonded over the channel with the open areas between the plates. The membrane was then fabricated over the channel by gluing a small plastic tube

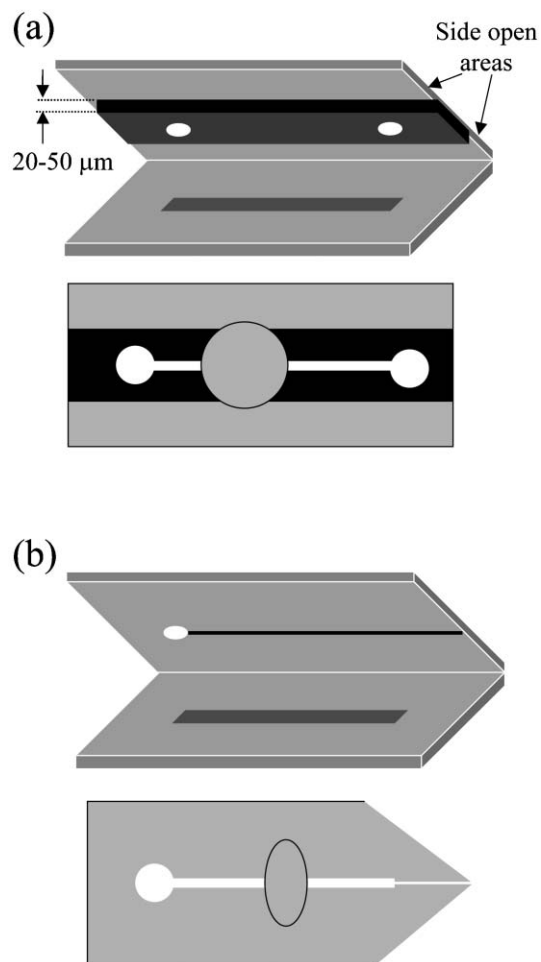


Fig. 1 Microdevice designs utilized in this work: (a) design of the microdevice with an etched voltage reservoir; the etching depth of the top layer determined the membrane glass thickness; (b) design of the microdevices with a drilled voltage reservoir, incorporating a weir and an angled outlet.

(ID ~ 9 mm) to the top of the bonded device; the tubing partly extended over the glass above the open side areas. The tubing was filled with a 48% HF solution and a small magnetic stir bar was used to provide continuous stirring; to expedite the process, the 48% HF solution was changed every 10–15 min. The etch speed of the borofloat glass was $\sim 6 \mu\text{m min}^{-1}$, thus etching to a 50 μm thick membrane in a 1.1 mm glass plate took ~ 3 h. When the HF solution etched through the glass above the side open areas, the membrane had reached the designed thickness. The chip was quickly rinsed with water to stop the etching. The holes into the side open areas were then sealed with epoxy to prevent leakage of the solution (20% acetic acid) used for voltage application into the side channels. Neither the total length of the channel nor the position of the membrane with respect to the channel outlet affected the performance of these devices. For MS interfacing, a hand pulled capillary emitter (10 cm, original ID 75 μm) was attached at the outlet reservoir using an in-house microchip holder and PEEK fittings (Upchurch Scientific, Oak Harbor, WA).

Glass membranes for high-voltage application were also fabricated using drilling. The drill press used for fabricating

reservoirs in the microdevices was set to drill the top layer to within $\sim 150\text{--}300\ \mu\text{m}$ of the bottom glass. A 2.1-mm diameter diamond tipped drill bit, normally used for forming larger reservoirs, was employed. Multiple drillings enlarged the hole to contain sufficient solution for voltage application. This method of voltage application was utilized with microdevices prepared for decreasing the size of the droplet at the microchannel exit. These devices were fabricated with a bottom channel, which was $50\ \mu\text{m}$ deep, but did not extend to the end of the device. The top layer was etched with a single channel $40\ \mu\text{m}$ wide and $10\ \mu\text{m}$ deep, extending to the end of the device as shown in Fig. 1(b). This design provided two important benefits from the use of the smaller channel on the top layer. First, the end of the large bottom channel generated a weir which could be used for packing beads into the bottom channel for desalting or reverse phase separations on the microdevice. Second, the greatly decreased size of the exit channel minimized the size of the droplet initiation point, decreasing the total droplet size. Once bonded, the microdevices were further modified by cutting the outlet end of the microdevice at a $\sim 45^\circ$ angle with the diamond saw used to cut the glass plates into individual devices, then the top and bottom plates angled away from the channel outlet with the diamond hand pad used for sanding glass device edges.

2.3 Taylor cone generation

Taylor cone formation at the outlet of the microdevices was evaluated by infusing buffer (50% methanol–0.5% acetic acid) through the microdevices using a syringe pump (100I Syringe Pump, WPI, Sarasota FL) at a rate of $0.17\ \mu\text{l}\ \text{min}^{-1}$. The syringe was connected to the microdevice using an acrylic holder constructed in-house, and PEEK fittings and tubing from Upchurch Scientific (Oak Harbor, WA). To simulate the ESI source of a mass spectrometer, a voltage (1–10 kV) was applied at the liquid voltage reservoir through a Pt wire while a skimmer, fabricated in the design of the Finnigan TSQ 7000 (Thermo Electron, San Jose, CA) mass spectrometer, was grounded. The distance between the outlet of the chip and the skimmer was adjusted using an in-house constructed three-dimensional motion platform. The platform was placed under a microscope for observation and recording of the Taylor cone formation process.

2.4 Mass spectrometry

Mass spectra were obtained using a LCQ classic or a TSQ 7000 instrument from Thermo Electron. The LCQ classic instrument was tuned using a tuning solution of caffeine, MRFA and Ultramark[™] (Thermo Electron, San Jose, CA) in acetonitrile–methanol–water (50:25:25) with 1% acetic acid. The TSQ 7000 instrument was tuned using a solution of MRFA and myoglobin in 50% methanol–0.5% acetic acid. Except for the ESI voltage, which required adjustment for each experiment, all other parameters, including capillary voltage and tube lens voltage, remained constant after the instrument was tuned with standard solution. The cytochrome c and lysozyme were dissolved at various concentrations in 50% methanol–0.5% acetic acid and used for the evaluation of the microchip designs. For microchips with etched membranes, the

capillary emitter was interfaced to the MS by mounting the end of the emitter to a stage on an in-house constructed three-dimensional motion platform. The end of the capillary was then positioned near the orifice of the MS and high voltage from the MS was applied to the solution above the membrane. For the drilled membranes with the sharpened exit face, the channel outlet was positioned close to the orifice of MS using an in-house constructed three-dimensional motion platform.

3 Results and discussion

3.1 High-voltage (HV) application on microdevices using etched membranes

Application of high voltage to the solution in a microchannel is a significant obstacle for integration of microchips and mass spectrometry. ESI is only possible when the droplet leaving the microchannel is in a high-voltage field. The currently preferred method is to apply the HV on a gold electrode fabricated inside or outside the microchannel on glass devices. Attempts to implement gold electrodes in the current microdevices led to a number of problems. The gold layer, with a chrome seed layer, had to be applied to the glass before thermal bonding to provide contact inside the microchannel. Great care had to be taken in cleaning the glass before the gold layer was applied, and the thermal bonding procedure caused additional problems thus, the gold electrodes were not homogeneous as required. In addition, bubble formation due to electrolysis of the solution on the gold surface caused significant problems with Taylor cone formation and electrospray ionization. This was especially true with non-homogeneous gold coatings where it was easy to see bubbles produced at the gold surface. Stable currents were never observed during high-voltage application with microdevices containing integrated gold electrodes in this work, thus another method for application of the high voltage was investigated.

There are a number of studies about ion conduction in various glass formulations, though the conductivity of glass is quite low under normal conditions.^{28–31} As illustrated by the development of glass electrodes for pH measurement, however, decreasing the thickness of glass can result in conduction through the glass membrane. This has been taken advantage of in fused silica capillaries through the formation of porous junctions to couple CE-MS,^{20,21} for protein concentration³² and for isolation of separation currents from electrochemical and electrochemiluminescent detection.^{33,34} The “porosity” of these membranes has been observed by Janini *et al.*²⁰ using surface scanning to show micrometer sized pores, but cross section scans showed no change in the glass membrane itself in this study. In addition, the etching formation process of these porous junctions has been monitored by measurement of the resistance of the glass membrane as it is etched. A sudden large decrease in resistance is normally seen as the membrane thins and becomes conductive.^{20,21} These results have been interpreted as the membrane having very narrow pores which allow passage of small ions, but not solution or larger ions.³⁴

This etching method was applied to glass microdevices for development of glass membranes which allow application of the high voltage required for ESI to the solution in the microchannel directly through the glass itself. Etching the glass above

the microchannel to thicknesses of 20 and 50 μm was expected to provide a sufficient decrease in the resistance to allow high-voltage application through the glass. To decrease the thickness of glass above the microchannel in a controlled manner, side open areas were initially etched into the bottom of the top glass plate of the device to the desired thickness of the glass membrane above the channel (Fig. 1(a)). By etching the top glass in a region above the channel that overlapped the side open areas, the etch solution leaked out when the glass above the open area was etched through; at this point, the membrane thickness is equivalent to the depth of the side open area etch. After sealing off the open areas, a voltage reservoir was formed which could be filled with an ionic solution and a platinum wire immersed in the solution for electrical connection.

The conductivity of this glass membrane was tested through Taylor cone formation on the capillary emitter attached to the microchip. It is well known that continuous electrospray only appears when a steady Taylor cone is formed at the spray tip, thus testing of the membrane devices was performed by evaluation of Taylor cone formation. Fig. 2 shows the Taylor cone generated on the capillary emitter connected to the outlet of the microchip upon high-voltage application across the glass membrane; this Taylor cone was stable for >30 min. Multiple devices with each membrane thickness were fabricated and tested, and cone formation was found to require a potential of only 1.5–2.8 kV on devices with 20 μm thick membranes, and 2.5–3.5 kV on 50 μm membranes. This stable electrical connection provided a continuous cone, simply through filling the voltage reservoir with ionic solution and applying the voltage through the immersed platinum wire. Since there is no direct contact between the buffer in the voltage reservoir and the solution in the microchannel, there are no contamination issues with this method.

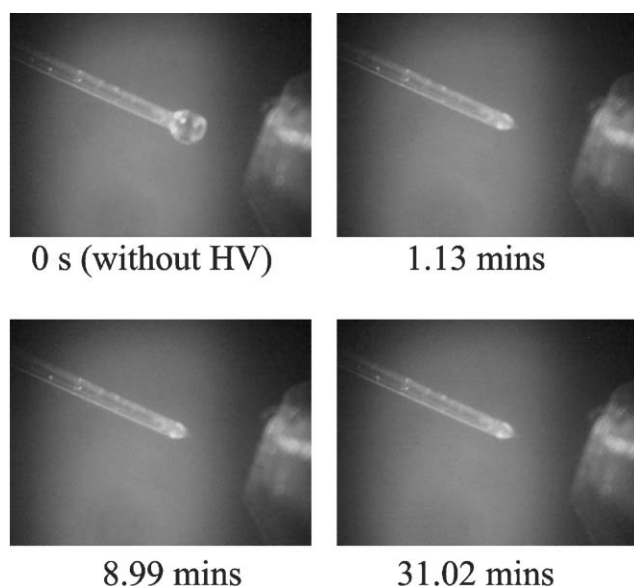


Fig. 2 Exposures from a video of Taylor cone formation on a hand-pulled capillary emitter attached to an etched voltage reservoir microdevice; 50% acetonitrile–0.5% acetic acid was flowed through the microchip at $\sim 0.23 \mu\text{L min}^{-1}$ using a syringe pump. The ESI voltage was 2.10 kV; the membrane glass was $\sim 20 \mu\text{m}$ thick

This chip design achieved good reproducibility for generation of Taylor cones on multiple devices, with application of a relatively low voltage. The borofloat glass used as the substrate for these microdevices is a product of Schott (Elmsford, NY), the conductivity constant of which is $\sim 10^{-12} \Omega^{-1} \text{cm}^{-1}$ at 25 $^{\circ}\text{C}$ when fully hydrated. Based on this conductivity, the resistance between the microchannel contact area (90 $\mu\text{m} \times 9 \text{mm}$) and the liquid filled voltage reservoir should be around $6 \times 10^{11} \Omega$ for a 50 μm thick glass membrane†. The voltage actually applied to generate the Taylor cone was $\sim 2500 \text{V}$, and the current during the nanospray process was $\sim 10^{-6} \text{A}$ based on the ion current in the MS during ESI. This indicates a resistance for the glass membrane of $2.5 \times 10^9 \Omega$, two orders of magnitude lower than predicted by theory. This is not completely unexpected, however. For the porous junctions in the fused silica capillaries discussed above, the resistance dropped to $4\text{--}8 \times 10^9 \Omega$ upon junction formation while fused silica has a conductivity constant of $10^{-14} \Omega^{-1} \text{cm}^{-1}$. While it is known that a hydrated gel forms on the surface of the glass, which can increase the conductivity of the membrane, full evaluation of the specific processes that contribute to the sharp decrease in resistance of silica membranes when the thickness decreases has not been reported.

To test the applicability of this etched membrane glass voltage junction for actual MS detection, the homemade capillary emitter, attached to the outlet of the microdevices as for Taylor cone formation, was positioned at the orifice of a MS and a positive voltage applied to the voltage junction on the microchip. Cytochrome c solution ($0.17 \text{pmol } \mu\text{L}^{-1}$) was flowed through the microchip using a syringe pump at a flow rate of $10 \mu\text{L h}^{-1}$ with $\sim 3\text{--}4 \text{kV}$ potential applied at the microchip to generate the ESI. Fig. 3 compares the results from normal ESI (Fig. 3(a)), using $\sim 10 \mu\text{L min}^{-1}$ flow rate, with those obtained using micro-electrospray ionization from a microdevice (Fig. 3(b)). We noticed that cytochrome c carried more charges when HV was applied through the glass membrane on the chip compared with the normal ESI (Fig. 3(b)). This may be the result of HV being added directly through the membrane glass. Background noise in the spectra from the microchip is also slightly increased relative to the major peaks, possibly due to the hand-pulled capillary emitter, the connection between the emitter and microdevice, unstable flow at the low syringe pump flow rate ($10 \mu\text{L h}^{-1}$) employed, and positioning of the emitter relative to the orifice of MS. These results confirm that the glass membrane allows good conductivity and that high voltage could be applied to the solution in the microchannel to provide direct electrospray into the MS using this method for forming a voltage junction on the microdevice. While there was a slight difference in the ESI voltage required, no obvious difference in the mass spectra of cytochrome c was observed when the voltage reservoir was filled with 5 M NaCl vs. the 8% acetic acid solution since this solution acts simply as a conductor between the platinum electrode and the glass membrane.

Compared with the gold electrode method for voltage application on glass microdevices, this design provides a simple method which avoids bubble formation, since metal/liquid

† Resistance = thickness/(area \times conductivity).

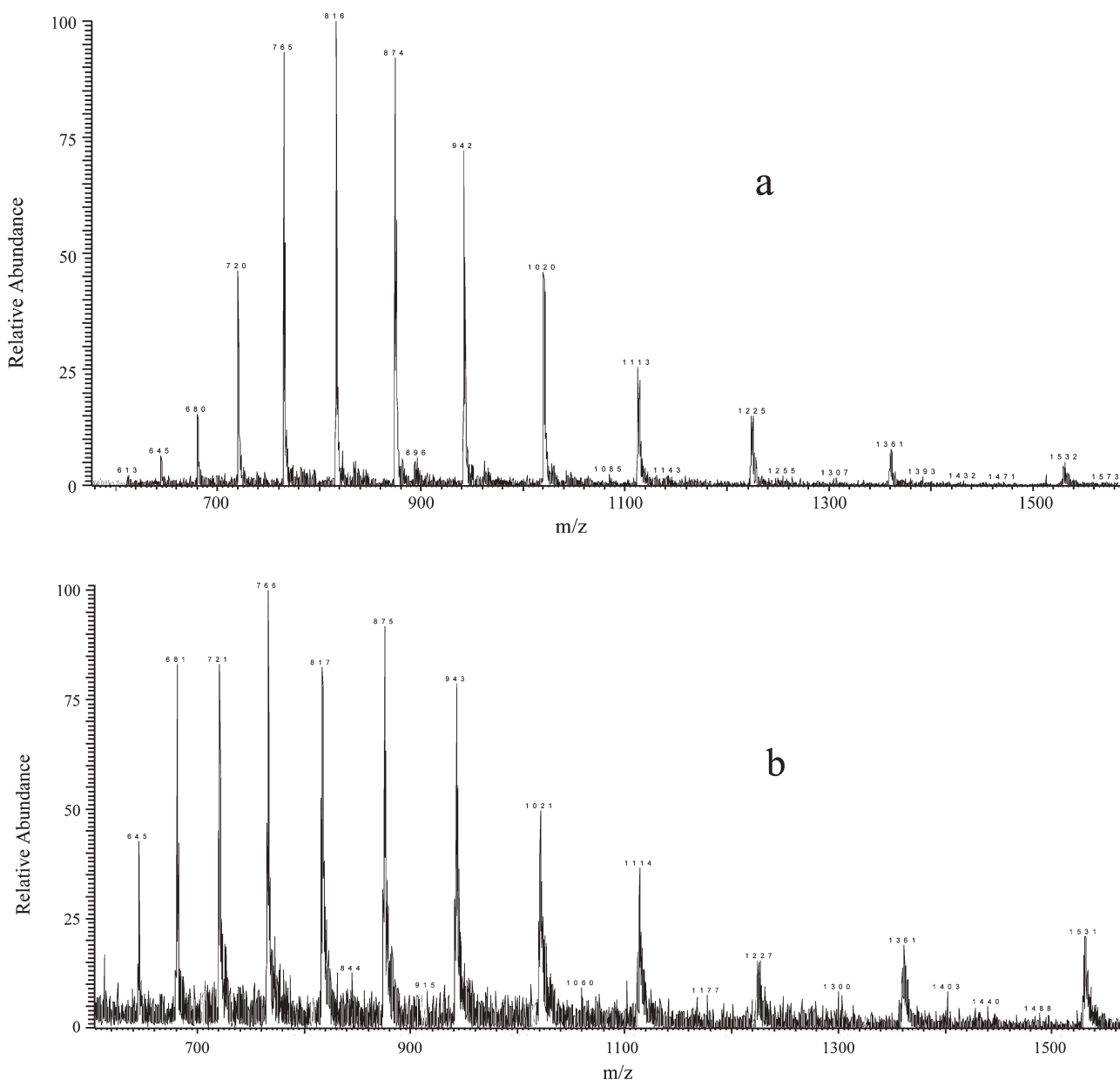


Fig. 3 Mass spectrum from infusion of cytochrome c ($0.17 \text{ pmol } \mu\text{L}^{-1}$) dissolved in 50% methanol–0.5% acetic acid into the TSQ 7000. (a): MS from traditional ESI. The ESI voltage was 5.3 kV and the flow rate was $454 \text{ } \mu\text{L h}^{-1}$. (b) mass spectra of cytochrome c from a microdevice. The ESI voltage was applied through an etched glass membrane ($50 \text{ } \mu\text{m}$ thick) with a hand-pulled capillary emitter attached at the outlet of the microchip. The flow rate was $10 \text{ } \mu\text{L h}^{-1}$ and the ESI voltage was 3.5 kV.

contact occurs outside the microchannel, with no dead volume or contamination. Compared to applying HV directly through microdevices made of a conductive material such as silicon,³⁵ or glassy carbon²³ as the substrate, the method presented here represents a simple, effective, and economic method for high-voltage application to glass microdevices for electrospray.

3.2 High-voltage (HV) application on microdevices using drilled membranes

In addition to etching the glass to produce the thin conductive membrane, a second method was also tested to decrease the thickness of glass above the microchannel, similar to a

procedure described by Whitt *et al.*²¹ for formation of ion porous junctions on fused silica capillaries interfaced with MS detection. The described procedure utilizes both drilling and etching of the capillary wall to produce a thin ($10\text{--}20 \text{ } \mu\text{m}$), ion porous junction in the fused silica, similar to those formed with etching alone. In this work, a hole was drilled part way into the top glass layer of the microchip to form the membrane, and HV was applied through a platinum wire immersed in the solution filling the hole. The benefit of this method was the increased speed of the approach, where drilling part way through the fused silica reduced the etch time required for formation of the junction. The drill depth in the capillary work was monitored through the use of a high-range ohmmeter

($\sim \text{G}\Omega$) to measure the resistance through the silica junction as it was drilled.

A high-range ohmmeter was not available, so the current work relied on the use of a depth gage on a drill press, which was set to leave a thicker membrane ($\sim 150\text{--}200\ \mu\text{m}$) than what was formed by the above etching method or by the drilling method described by Whitt *et al.*²¹ This drill depth was employed because of the lack of precision in the drill press setting and the non-uniform tip surface of the drill bits. The tip of the drill bit abrades the glass beneath it while applying stress to the membrane during drilling, thus cracking of the membrane glass is likely if the membrane is drilled too thin. In addition, the small diameter of the drill bit, 2.1 mm, required multiple drillings to generate a reservoir sufficient for buffer and Pt wire immersion, adding to the likelihood of cracking if drilled too deep. HF etching could have been utilized to further thin these membranes after drilling, but was found not to be required. Taylor cone formation was readily generated when HV was applied at these drilled membranes with both currents and cones stable for >15 min (data not shown). As expected, these thicker drilled membranes had a larger resistance than the etched glass membrane and $\sim 7\text{--}12$ kV potential was required for Taylor cone formation with these devices. However, these membranes could be generated rapidly and consistently through use of the drill press with all devices fabricated performing as required. Thinner membranes should also be possible with this rapid fabrication method using a more precise drill press and other drill bits. In addition, additional etching could also be performed to thin the membranes further if needed.

In comparison with microdevices containing the etched membranes ($20\text{--}50\ \mu\text{m}$), the voltages required with the drilled membrane ($\sim 100\text{--}350\ \mu\text{m}$) were lower than expected. It is possible that the conductivity through these membranes might be based not only on ion conductivity through the glass. An experiment in which the end of the channel was blocked during pressure driven flow resulted in liquid appearing in the voltage reservoir showing that liquid could move through this drilled membrane. The same blocked flow experiment was performed with an etched membrane microdevice but showed no liquid transport through the membrane under these conditions. This suggests possible micropores or microcracks in the membrane produced by the drilling process, which would affect conductivity. A second observation with these drilled membranes was that the voltage required to form a stable electrospray increased with time as these devices were used. This may be due to blocking of the micropores or loss of water from the micropores due to glass heating as the voltage is applied. Again, this effect was not observed with the etched membranes and does not seem to be related to the conductivity of the glass itself. Thus, for single use devices, the rapid drilling procedure has definite advantages in time, but the etched membranes will be more stable and reproducible over repeated usage.

3.3 Microchip interface design for mass spectrometry

The other key to realizing high sensitivity detection with microchip coupled mass spectrometry is the design of the electrospray interface for the microchip. Though capillaries

attached to microdevices as the electrospray emitter is the most common interface,^{8–16} and has been employed in the experiments here with etched glass membranes, this method does not provide sensitive detection for microchip separations due to the large dead volume in the capillary that allows for diffusion. Direct electrospray from the microchip is attractive because it does not require complex fabrication, with the outlet formed simply by cutting the chip into the desired shape. Two studies utilizing this kind of interface design focused on polymeric materials with the obvious reason being that it is much easier to produce a sharp tip on a plastic microchip than a glass chip.^{26,36} Glass microchips normally have a flat face at the outlet resulting in large droplets at the exit edge due to the high surface tension of the liquid with glass. This large droplet results in loss of the resolution of the electrophoretic or reverse phase separation while decreasing the efficiency of electrospray, and also the detection sensitivity with MS.

The second part of this work focused on preliminary investigations into the design of the interface directly on the microchip to decrease the surface area that the droplet contacts as it exits the microdevice. The simplest method involved imitating what was done on polymeric devices by sharpening the exit from the glass microchip (Fig. 4(a)) through cutting and polishing the four sides. A stable Taylor cone was easily formed at this interface (Fig. 4(b)), and the exact angle at which the microchip was cut did not affect the Taylor cone formation or stability at the angles tested (data not shown). This result is consistent with results obtained on plastic microdevices.³⁶ Direct utility of these pointed glass microchips as an ESI interface was shown through stable electrospray into a mass spectrometer (data not shown).

Previous reports suggested that decreasing the surface tension between the glass and liquid droplet should also reduce the droplet size, resulting in better resolution following separation.^{7,13,24} To test this in the current design, the end of a microdevice was coated with polyimide resin (IST Co., Parlin, NJ) using a small paintbrush, then cured following the manufacturer's directions. Fig. 4(c) shows the Taylor cone formed at the sharpened outlet of a microdevice coated with the polyimide resin. Fig. 4(d) and (e) shows application of the coated tip for electrospray into the MS using a drilled membrane voltage junction. A solution of lysozyme, $87\ \text{fmol}\ \mu\text{L}^{-1}$, dissolved in 50% methanol–1% acetic acid, was flowed through the microdevice at a rate of $0.2\ \mu\text{L}\ \text{min}^{-1}$. Fig. 4(d) displays the total ion chromatogram (TIC) of the ESI indicating stable electrospray was achieved for >25 min. The corresponding mass spectrum for lysozyme generated from Fig. 4(d) is presented in Fig. 4(e). The spectrum showed good signal to noise (S/N is ~ 5) at this concentration, but the sensitivity of the interface could be improved by optimizing the positioning of the microchip relative to the orifice of the MS. The sharp peak at 7.8 min in Fig. 4(d) is likely due to a bubble generated at the connector between the inlet capillary from the syringe pump and the microchip.

Unlike the drilling process, the cutting and sanding processes are not expected to create cracks or pores near the outlet. An additional problem which did occur with the sharpened tip, however, was that cutting the glass devices was not an exact process. If the microchannel outlet was not in the

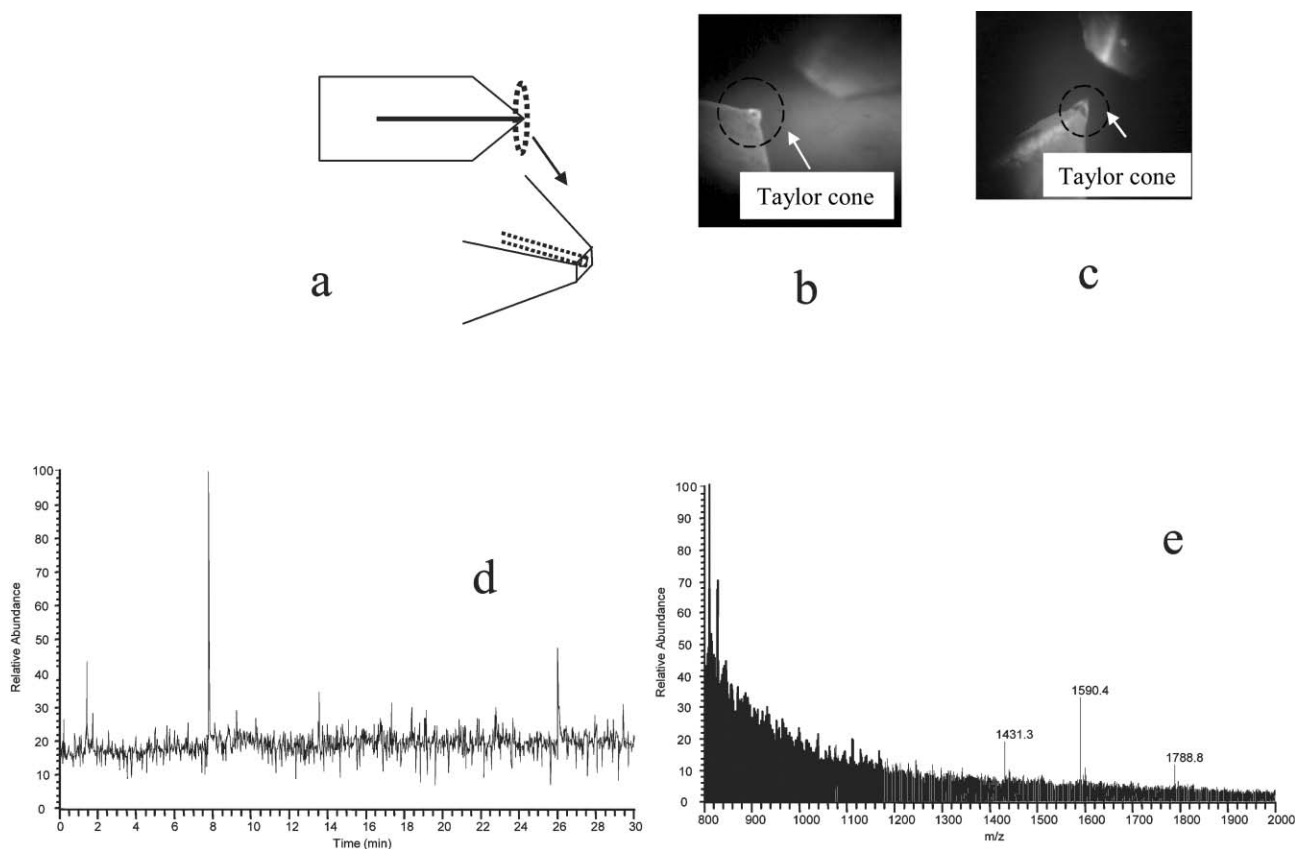


Fig. 4 ESI Process on a sharp tip microdevice interface. The flow rate was $0.2 \mu\text{L min}^{-1}$ with an ESI voltage of 7.5 kV applied through a drilled glass membrane. (a) Diagram of the sharp tip design; (b) Taylor cone formation on the sharp tip; (c) Taylor cone formed on a sharp tip microdevice interface coated with polyimide; (d) TIC from the LCQ classic instrument obtained from the polyimide coated, sharp tip interface microdevice, infusing $87 \text{ fmol } \mu\text{L}^{-1}$ lysozyme in 50% methanol–1% acetic acid. The peak at 7.8 min was due to a bubble; (e) mass spectrum of lysozyme obtained from (d).

center of the sharpened tip, it was not easy to form a stable Taylor cone, and centering is much more difficult in the glass devices than with plastic materials which can be injection molded or the channel fabricated after cutting.^{9,26,36} This raised an issue of the reproducibility of the electrospray signal from multiple devices with this method, thus easier fabrication methods for producing angled glass devices with centered microchannels are being investigated. To circumvent this problem, angling of the exit face of the device away from the microchannel outlet in only two dimensions was also investigated. Fig. 5(a) illustrates this design where only the bottom and top edge of the microchip are angled away from the microchannel exit and polished. Early on, Taylor determined that a drop, elongated by an electric field, became unstable when its length was 1.9 times its equatorial diameter.³⁷ Based on this conclusion, producing the required cone shape might be possible simply by polishing only two sides of the device; decreasing the surface available for spreading in one direction might control spreading in the second dimension. As seen in Fig. 5(b), a Taylor cone was easily formed under an applied HV field with only two sides polished. There are two competing forces in the electrospray process, surface tension and the Coulomb force due to the applied electrical field,³⁸ but the conservation of mass must also be satisfied. The surface tension and Coulombic force will

generate the Taylor cone shape which allows electrospray to occur, but if the rate of electrospray is too slow, the diameter of the cone will grow as liquid from the microchannel is added to the droplet. This decreases the Coulombic force, allowing surface tension forces to spread the droplet further. This was readily apparent in the devices polished on only two sides, where the solution spread laterally along the edge of the microdevice as the flow rate through the microchannel was increased; this generated a second Taylor cone as shown in Fig. 5(c). Under flow conditions where a single stable Taylor cone was formed, the ESI stability into the mass spectrometer was also tested. Fig. 5 also shows the TIC and corresponding mass spectrum generated using the two side polished device for a lysozyme solution ($7.27 \text{ pmol } \mu\text{L}^{-1}$) in 50% methanol–0.5% acetic acid. The TIC was recorded for >10 min in Fig. 5(d) and the corresponding mass spectrum of lysozyme is shown in Fig. 5(e). This is a higher concentration than was observed with the microdevice interface cut and polished to a point, indicating that the sensitivity with this interface is not as good, possibly due to spreading of the solution at the interface.

With both interface designs, it was observed that the linear flow rate also affect Taylor cone formation with higher linear flow rates forming Taylor cones more easily. In addition, the stability with both interfaces was not as long-lived as can be achieved using a capillary emitter directly fabricated into the

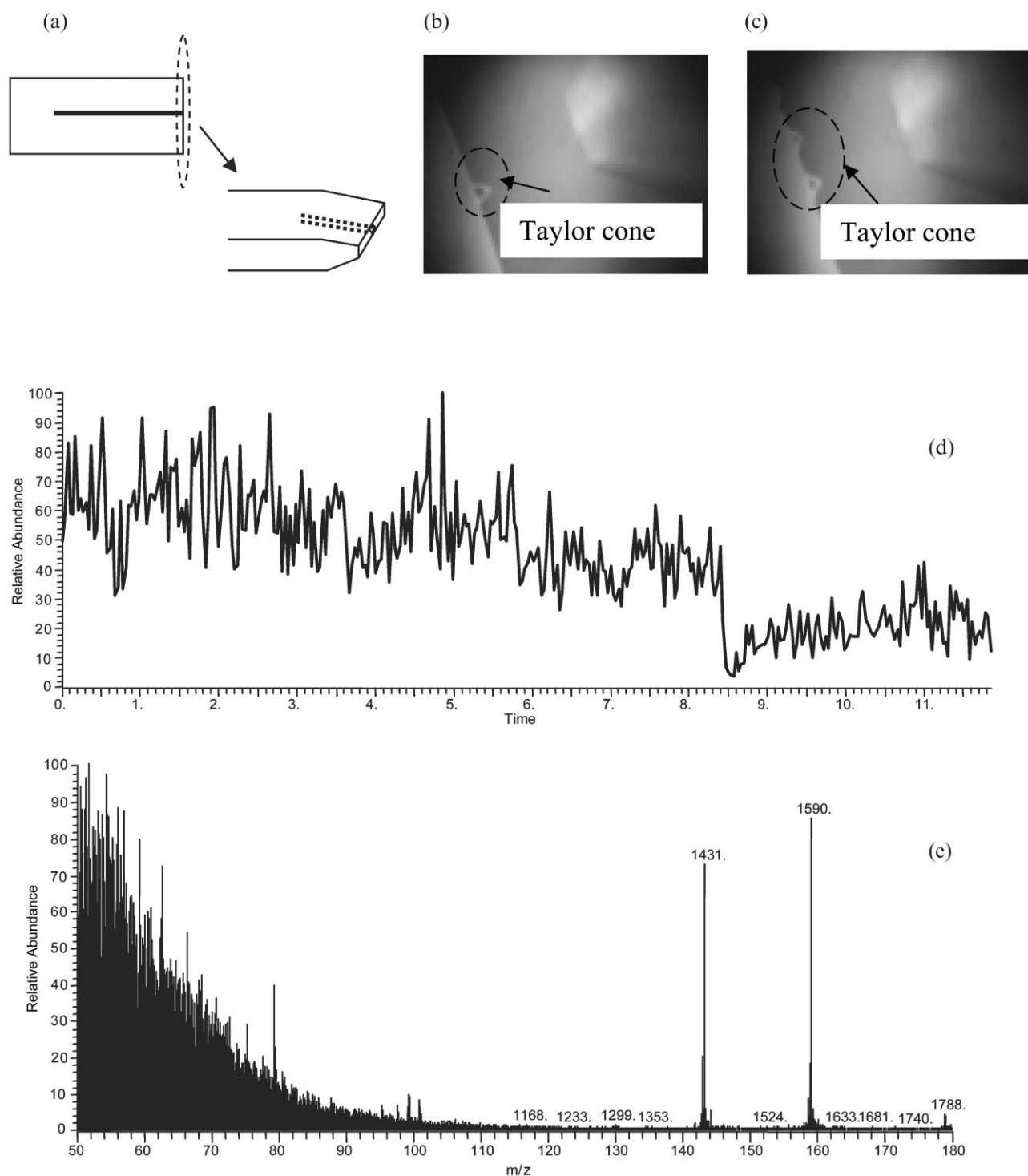


Fig. 5 ESI process on the two side polished microdevice interface with voltage applied through a drilled glass membrane. (a): diagram of this interface design; (b) Taylor cone formation on the sharpened edge ($10 \mu\text{L h}^{-1}$, ESI voltage: 5.87 kV). (c) two Taylor cones formed due to solution spreading along the microchip edge ($0.17 \mu\text{L min}^{-1}$ ESI voltage: 5.87 kV); (d) TIC from the LCQ classic instrument for lysozyme ($7.27 \text{ pmol } \mu\text{L}$ in 50% methanol–0.5% acetic acid) flow rate is $0.30 \mu\text{L min}^{-1}$, ESI voltage: 7.0 kV (e) mass spectrum of lysozyme obtained from (d).

microdevice, this data, however, shows the possibility for this new interface design and is important for studying the mechanism of Taylor cone formation. One problem with the interface on the glass microdevices is that the exit width of the microchannel is much larger than the exit on a capillary

emitter. This problem is due to the isotropic etching of glass and the limitations on the feature size available on the currently used photomasks. The narrowest design that can be printed on the current photomasks is $10 \mu\text{m}$ wide (in these experiments the narrowest width used was $15 \mu\text{m}$). This

limitation means that the minimum width of the microchannel is 40 μm if the channel is etched only 15 μm depth and the outlet will always be trapezoidal in shape vs. circular for the capillary emitter. These exit limitations are not optimal for formation of the small droplets required for efficient electrospray, thus new methods will be required for continued investigational work. In addition, the positioning of the microdevices with respect to the MS inlet was not optimized. As was observed with Taylor cone formation at the skimmer, the position of the microdevice channel exit with respect to the skimmer inlet greatly affected the size of the cone and the electrospray. Additional work on optimizing this positioning should also increase the sensitivity of the analysis.

4 Conclusion

In this work, we focused on two aspects required for integrating glass microdevices with mass spectrometry detection: High-voltage application directly on the microdevice and the design of the device interface for mass spectrometry electrospray. Glass membranes were produced above the microchannel by etching, using etched side areas to control the final thickness of the membrane. Voltage junctions were also fabricated using a drill press to set the membrane thickness; this provided faster construction of devices, but it was much more difficult to control the thickness of membrane. Voltage applied using a platinum electrode in solution above the glass membranes, passed through the membranes and generated electrospray from the solution in the microchannel. These glass membranes supplied a new method for application of HV to the liquid in the microchannel without added components or complicated fabrication steps. It avoids direct metal/liquid contact in the sample solution, completely eliminating bubble formation due to water hydrolysis on the surface of the metal. Furthermore, there is no dead volume as is seen with normal liquid junctions. Though attachment of a spray emitter for ESI can be easily applied to these devices, preliminary designs for fabrication of an MS interface in the microdevice itself were also explored. Two interface designs were functional, with stable Taylor cones formed under applied HV fields. The direct mass spectra data indicated encouraging results, but more optimization of the method is needed to show direct protein separation on microdevices with mass spectrometry detection. Hand fabrication of the interfaces decreases the reproducibility of the method, but additional research will evaluate more automated fabrication for greater repeatability of the design.

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References

- 1 D. I. Papac and Z. Shahrokh, *Pharmacol. Res.*, 2001, **18**, 131–145.
- 2 A. J. Mello, *Lab Chip*, 2001, **1**, 7N–12N.
- 3 R. D. Oleschuk and D. J. Harrison, *Trends Anal. Chem.*, 2000, **19**, 379–388.
- 4 D. J. Harrison, K. Fluri, K. Seiler, Z. Fan, C. S. Effenhauser and A. Manz, *Science (Washington, D. C.)*, 1993, **261**, 859–897.
- 5 S. C. Jacobson, R. Hergenroder, L. B. Koutny and J. M. Ramsey, *Anal. Chem.*, 1994, **66**, 1114–1118.
- 6 M. Wilm and M. Mann, *Anal. Chem.*, 1996, **68**, 1–8.
- 7 R. S. Ramsey and J. M. Ramsey, *Anal. Chem.*, 1997, **69**, 1174–1178.
- 8 D. Figeys, Y. Ning and R. Aebersold, *Anal. Chem.*, 1997, **69**, 3153–3160.
- 9 M. Svedberg, A. Pettersson, S. Nilsson, J. Bergquist, L. Nyholm, F. Nikolajeff and K. Mardides, *Anal. Chem.*, 2003, **75**, 3934–3940.
- 10 L. M. Lazar, R. S. Ramsey, S. Sundberg and J. M. Ramsey, *Anal. Chem.*, 1999, **71**, 3627–3631.
- 11 J. J. Li, P. Thibault, N. H. Bings, C. D. Skinner, C. Wang, C. Colyer and D. J. Harrison, *Anal. Chem.*, 1999, **71**, 3036–3045.
- 12 J. J. Li, T. L. Tremblay, C. Wang, S. Attiya, D. J. Harrison and P. Thibault, *Proteomics*, 2001, **1**, 975–986.
- 13 B. Zhang, H. Liu, B. L. Karger and F. Foret, *Anal. Chem.*, 1999, **71**, 3258.
- 14 D. Figeys and R. Aebersold, *Anal. Chem.*, 1998, **70**, 3721–3727.
- 15 J. H. Chan, A. T. Timperman, D. Qin and R. Aebersold, *Anal. Chem.*, 1999, **71**, 4437–4444.
- 16 Y. Tachibana, L. Otsuka, S. Terabe, A. Arai, L. Suzuki and S. Nakamura, *J. Chromatogr., A*, 2003, **1011**, 181–192.
- 17 B. Zhang, F. Foret and B. L. Karger, *Anal. Chem.*, 2000, **72**, 1015–1022.
- 18 Z. J. Meng, S. Qi, S. A. Soper and P. A. Limbach, *Anal. Chem.*, 2001, **73**, 1286–1291.
- 19 J. J. Li, C. Wang, J. F. Kelly, D. J. Harrison and P. Thibault, *Electrophoresis*, 2000, **21**, 198–210.
- 20 G. M. Janini, T. P. Conrads, K. L. Wilkens, H. J. Issaq and T. D. Veenstra, *Anal. Chem.*, 2003, **75**, 1615–1619.
- 21 J. T. Whitt and M. Moini, *Anal. Chem.*, 2003, **75**, 2188–2191.
- 22 L. M. Lazar, J. J. Li, Y. Yang and B. J. Karger, *Electrophoresis*, 2003, **24**, 3655–3662.
- 23 S. Ssenyange, J. Taylor, D. J. Harrison and M. T. McDermott, *Anal. Chem.*, 2004, **76**, 2393–2397.
- 24 Q. F. Xue, F. Foret, Y. M. Dunayevskiy, P. M. Zavracky, N. E. McGruer and B. L. Karger, *Anal. Chem.*, 1997, **69**, 426–430.
- 25 J. Kameoka, R. Orth, B. Ilic, D. Czaplowski, T. Wachs and H. G. Craighead, *Anal. Chem.*, 2002, **74**, 5897–5901.
- 26 H. Yin, K. Killeen, R. Brennen, D. Sobek, M. Werlich and T. van de Goor, *Anal. Chem.*, 2005, **77**, 527–533.
- 27 J. P. Ferrance, Q. Wu, B. Giordano, C. Hernandez and J. P. Landers, *Anal. Chim. Acta*, 2003, **500**, 223–236.
- 28 A. Bunde, M. D. Ingram and P. Maass, *J. Non-Cryst. Solids*, 1994, **172**, 1222–1228.
- 29 M. Tomozawa and M. Yoshiyagawa, *Glass Sci. Technol. (Frankfurt/Main)*, 1983, **56**, 939.
- 30 A. K. Jonscher, *Nature*, 1977, **267**, 673.
- 31 P. L. Kirby, *Br. J. Appl. Phys.*, 1950, **1**, 193–202.
- 32 W. Wei and E. S. Yeung, *Anal. Chem.*, 2002, **74**, 3899–3905.
- 33 S. Hu, Z. L. Wang, P. B. Li and J. K. Cheng, *Anal. Chem.*, 1997, **69**, 264–267.
- 34 X. B. Yin, H. Qui, X. Sun, J. Yan, J. Liu and E. Wang, *Anal. Chem.*, 2004, **76**, 3846–3850.
- 35 G. A. Schultz, T. N. Corso, S. J. Prosser and S. Zhang, *Anal. Chem.*, 2000, **72**, 4058–4063.
- 36 C. Yuan and J. S. Shiea, *Anal. Chem.*, 2001, **73**, 1080–1083.
- 37 G. Taylor, *Proc. R. Soc. London, Ser. A*, 1964, **280**, 383–397.
- 38 P. Kebarle and M. Peschke, *Anal. Chim. Acta*, 2000, **406**, 11–35.