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Short communication

Novel microfabricated device for electrokinetically induced pressure flow and electrospray ionization mass spectrometry

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Abstract

A novel microchip device for electrospray ionization has been fabricated and interfaced to a time-of-flight mass spectrometer. Fluid is electrokinetically transported through the chip to a fine fused-silica capillary inserted directly into a channel at the edge of the device. Electrospray is established at the tip of the capillary, which assures a stable, efficient spray. The electric potential necessary for electrospray generation and the voltage drop for electroosmotic pumping are supplied through an electrically permeable glass membrane contacting the fluidic channel holding the capillary. The membrane is fabricated on the microchip using standard photolithographic and wet chemical etching techniques. Performance relative to other microchip electrospray sources has been evaluated and the device tested for potential use as a platform for on-line electrophoretic detection. Sensitivity was found to be approximately three orders of magnitude better than spraying from the flat edge of the chip. The effect of the capillary on electroosmotic flow was examined both experimentally and theoretically.
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1. Introduction

Microfabricated fluidic devices are being increasingly recognized as a convenient means of manipulating and analyzing small sample volumes. They have been shown to be highly advantageous for integrating sample pretreatment and separation strategies, and rapidly processing materials. Although laser-induced fluorescence (LIF) has been the primary mode of detection on these devices, microchips have also been recently interfaced with mass spectrometry (MS) [1–14]. Research has focused on electrospray ionization (ESI) interfaces and quadrupole, ion trap, or time-of-flight (TOF) mass spectrometers. On-chip capillary electrophoresis (CE) with ESI-MS detection has also been demonstrated using liquid sheath and liquid junction interfaces for electrospray generation and stabilization [11,13].

We have recently reported on a hybridized microchip nano-ESI source capable of providing zeptomole sensitivity for peptide and protein samples on a TOF-MS detector [14]. The fluid necessary to sustain the electrospray is delivered through the device as a result of capillary action and electrostatic forces at a nanospray tip that is coupled to the microchip. In addition to continuous infusion, the device has been used to inject discrete sample volumes and electrophoretically separate samples. The voltage to generate the spray is applied to a side

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channel that contacts the main separation channel, and is also the terminating voltage for the CE portion of the microchip. In this configuration the bulk electroosmotic flow (EOF) follows the direction of the potential gradient towards the side channel and only a portion of the volumetric flow necessary to sustain a stable electrospray is dispensed by the nanospray tip. In this scheme sample may, therefore, be lost to the side channel and associated reservoir.

Here we describe a new, simple, but robust design for the application of the ESI voltage and closure of the CE electrical circuit. The potential is applied through an electrically permeable porous glass membrane that directs most of the EOF through the electrospray tip. The device is an electroosmotic pump capable of delivering fluid past the point where the ESI voltage (and terminus CE voltage) is applied. In addition, a fused-silica capillary may be used instead of a delicate nanospray emitter, if desired. Unlike some previously reported configurations, external assistance (vacuum [11], pneumatic nebulization [11], syringe pumping [1,4,6,10,13], or external electroosmotic pumping [3,7–9]) is not required to maintain stable electrospray operation. Preliminary results obtained with the device demonstrate sensitive, reliable operation for extended periods of time.

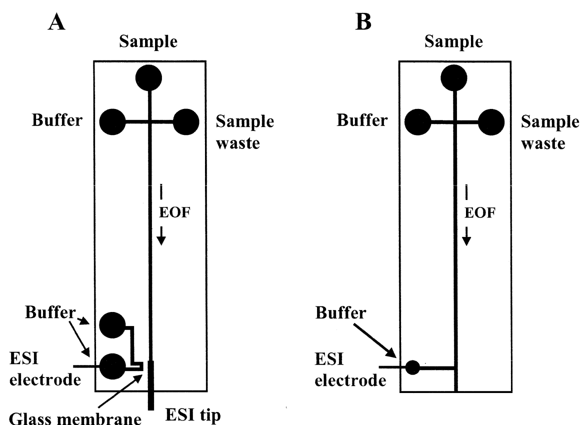

Fig. 1. Schematic representation of the ESI microchips. (A) Capillary tip attached to the channel; (B) channel exit at edge of the device.

2. Experimental

2.1. Instrumentation and methods

2.1.1. Microchip structures

The microchips (Fig. 1) were fabricated using procedures described in previous papers [15–17]. The channels were 20 μm deep and 55–60 μm wide at half-depth. To allow insertion of the ESI capillary (Fig. 1A), the last 3–5 mm of the separation channel was etched to a 55–60 μm depth and 90–110 μm width. A small channel with the same dimensions and relative position was etched into the cover plate, to create an opening that would accommodate the capillary after the substrate and coverplate were bonded. A 10 mm long fused-silica capillary (20 μm I.D.×90 μm O.D., Polymicro Technologies, Phoenix, AZ, USA) was blunt cut at both ends, cleaned with methanol, and inserted into this deeper etched section of the separation channel as shown in Fig. 2. The capillary was secured in place by using a removable adhesive (E6000; Eclectic Products, USA) which permitted easy replacement of the tip. The polyimide coating from the last 2 mm of the capillary was removed prior to conducting ESI experiments (Fig. 2).

2.1.2. ESI-TOF-MS

For the chip design in Fig. 1A the electrospray was generated from the fused-silica capillary that was inserted at the terminus of the separation channel and the electrospray voltage was applied to the U-shaped side channel (Fig. 2). The width of the glass wall between the separation channel and the U-shaped side channel was approximately 4–6 μm. This porous glass membrane was narrow enough to allow efficient application of the ESI voltage [18,19], but still sturdy enough to prevent major EOF leakage and sample losses through the junction. The configuration is similar to one which was utilized for sample preconcentration purposes, but without the potassium silicate layer applied between the substrate and cover plate, as previously described [20]. For the microchip shown in Fig. 1B, the electrospray was generated from the flat edge of the device. To prevent flow into the side channel, the side channel reservoir was physically plugged with the electrode element used for electrospray generation. Potentials from five programmable high voltage power supplies (10A12-

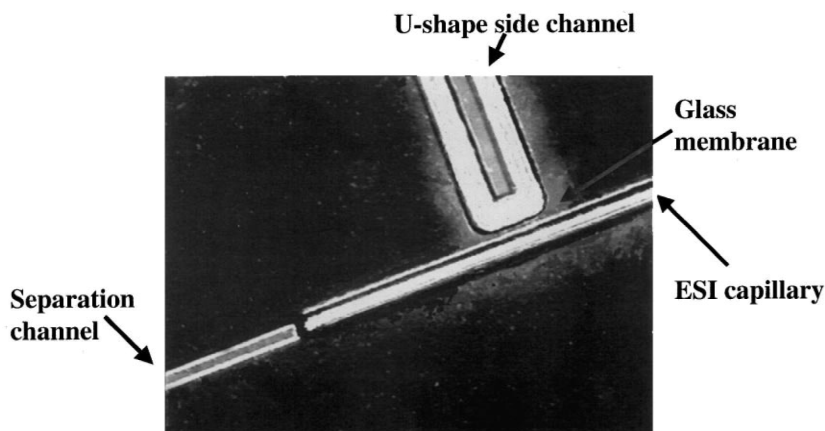

Fig. 2. Image of the porous glass membrane for application of the ESI voltage and capillary chip junction.

P4; Ultravolt, Ronkonkoma, NY, USA) were applied to the fluid reservoirs. The power supplies were computer controlled using multifunction I/O cards (NB-MIO16XL-42; National Instruments, Austin, TX, USA) and Labview 4.1 software (National Instruments).

Electrospray onset and stability were monitored using a VZM 28-180X Color Video System (Edmund Scientific, Barrington, NJ, USA) that included a Model VZM 0.7-4.5X zoom microscope, a Panasonic GP-KR222 CCD (charged coupled device) camera, a Sony monitor, and a fiber optic illuminator source. Samples were continuously infused with a Harvard Apparatus Model 22 syringe pump (South Natick, MA, USA).

A Jaguar TOF-MS instrument (Sensar, Provo, UT, USA) allowed detection at spectral generation rates of 5 kHz and spectral storage rates of 80–100 spectra s^{-1} [21]. The microchip was placed on a three-axis micrometer stage in front of the interface plate, and the ESI fused-silica tip was positioned 3–4 mm away from the sampling nozzle of the instrument. The ion source was heated to 90°C, and the nitrogen curtain gas flow-rate was set at 600–900 ml min^{-1}.

2.1.3. Laser-induced fluorescence detection

The on-chip CE efficiency and EOF rate were determined using LIF and monitoring the migration of a neutral compound (rhodamine B) in sodium borate buffer (pH 9.2). For the other buffer systems at lower pH values, indirect detection was used to measure the EOF by injecting a small plug of buffer into buffer containing a low concentration of rhodamine B (12 μ*M*). Samples were introduced using a gated injection [22]. The instrumentation for fluorescent imaging and for single-point detection has been described previously [15,17]. An argon ion laser (514.5 nm; Evergreen Laser Corp., Durham, CT, USA) was used as the excitation source.

2.2. Reagents

Methanol (HR-GC grade) was purchased from EM Science (Gibbstown, NJ, USA), and acetonitrile (HPLC-grade) was from Baker (Phillipsburg, NJ, USA). Deionized water (18 MΩ cm) was obtained from a NANOpure water system (Barnstead Thermolyne, Dubuque, IA, USA). Glacial acetic acid, ammonium acetate 99.999%, sodium hydroxide 99.99%, and sodium tetraborate 99.998% were obtained from Aldrich (Milwaukee, WI, USA). Gramicidin S was from Sigma (St. Louis, MO, USA) and rhodamine B was purchased from Eastman Kodak (Rochester, NY, USA). Prior to use, samples and buffers were filtered using 0.2-μm Acrodisc syringe filters (Gelman, Ann Arbor, MI, USA).

3. Results and discussion

The objective of this work was to develop a microchip for ESI that could function as a stand-

alone device, without any external assistance for fluid delivery or spray stabilization, that would provide high sensitivity, and that would allow on-line CE analysis. Stable electrospray may be generated from the structure shown in Fig. 1A provided electric field conditions are appropriate and there is sufficient fluid flow through the capillary tip. If the attached capillary is a nanospray emitter capable of withdrawing the necessary liquid flow to sustain electrospray operation then the device can function irrespective of EOF in the microchip. If a fine, blunt cut capillary is used then there must be sufficient EOF in the separation channel to supply the fluid for ESI operation. We were primarily interested in evaluating the performance of microchips of the latter configuration with regard to sensitivity, reliability and suitability for integration with microchip CE separations.

The ESI sensitivity obtained from a chip with an inserted fused-silica capillary was initially compared to that obtained from a device where the spray was established directly at the exit port of a channel at the planar edge of the microchip. Simple infusion experiments were performed where the sample (gramicidin S, 1 μ*M*) was delivered with a syringe pump at low flow-rates through the device. When electrospray was established at the edge of the microchip, the liquid emerging from the channel and the resulting Taylor cone spread over a relatively large area surrounding the exit orifice [2]. The spray was also much less stable as evidenced by pulsation of the Taylor cone. Under these conditions it was necessary to sum a large number of spectra to obtain satisfactory signal/noise ratios for the analyte. Fig. 3A shows data summing 25 600 spectra over a 5 s interval. Obviously, it would be difficult to monitor fast separations on a microchip given such long acquisition times. A relatively large amount of sample (60 fmol) was consumed during this acquisition period. Somewhat better results may have been obtained if the spray could have been stabilized, but efforts to do so by altering the spraying potential or repositioning the microchip in front of the mass spectrometer, with its counter-current flow of drying gas, were unsuccessful. In comparison, much greater ESI stability and sensitivity was obtained when the sample was infused with the aid of a syringe pump through the microchip with the inserted fused-silica

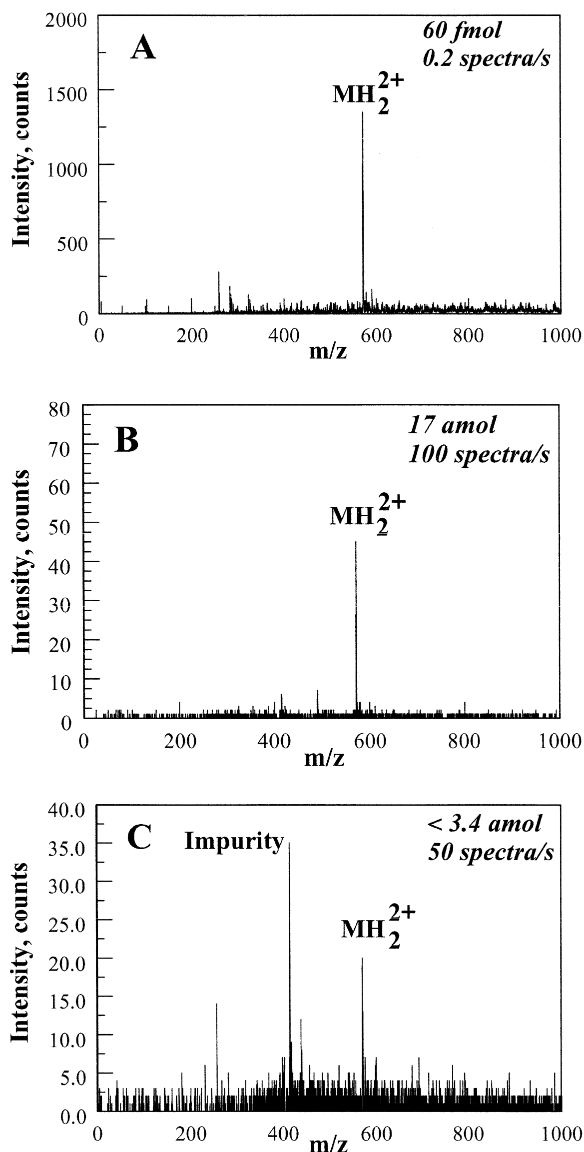

Fig. 3. Microchip-ESI-TOF mass spectra of gramicidin S. Conditions: (A) 1 μ*M* sample in CH_3OH–water–CH_3COOH (50:50:0.5, v/v), continuous delivery through the microchip channel with a syringe pump at 0.7 μl min^{-1}, spray from the flat edge of the chip; TOF-MS data acquisition: 5000 Hz repetition rate, 25 600 summed spectra; (B) 1 μ*M* sample in CH_3OH–water–CH_3COOH (50:50:0.5, v/v), continuous delivery through the microchip channel with a syringe pump at 0.1 μl min^{-1}, spray from an inserted fused-silica capillary; TOF-MS data acquisition: 5000 Hz repetition rate, 50 summed spectra; (C) 0.1 μ*M* sample in CH_3OH–water–CH_3COOH (25:75:0.5, v/v), electroosmotic infusion at 550 V cm^{-1} (glass membrane), spray from an inserted fused-silica capillary; TOF-MS data acquisition: 5000 Hz repetition rate, 100 summed spectra.

tip. Fig. 3B shows the spectrum of the analyte at the same TOF-MS pulsing rate, but summing only 50 spectra over 10 ms, and consuming 17 amol of sample. Electroosmotic infusion instead of syringe pumping was performed using the microchip configuration with a porous glass membrane. A more dilute solution of gramicidin S (0.1 μ*M*), to prevent alteration of the EOF due to adsorption of the peptide on the channel walls, was electrokinetically transported through the device at a field strength of 550 V cm^{-1}. The EOF under these conditions was ca. 30–100 nl min^{-1} as determined from measurements of the migration time of rhodamine B using LIF. Less than 4 amol of gramicidin S could be determined in 20 ms by summing only 100 spectra (Fig. 3C). The signal/noise ratio for the $(M+2H)^{2+}$ ion was approximately 3 (noise defined as peak-to-peak background intensity). The measured RSD for total ion current (0–1500 m/z) was in the range of 3–4% for continuous sample delivery, indicating a high level of stability. In comparison, a microchip with an attached nanospray emitter delivering fluid at similar flow-rates, produced roughly an order of magnitude better detection levels for this peptide [14]. The differences in sensitivity are likely due to characteristics of the emitter rather than mechanisms associated with fluid delivery and chip configuration. Of course, nanospray tips may also be coupled to microchips with glass membranes, in which case similar sensitivity should be obtained.

The effect of the capillary restriction on EOF in the microchip was further examined. An electric field between an inlet channel and the glass membrane on the microchip (Fig. 1A) produces an EOF that becomes a pressure-induced flow below the membrane. The inserted fused-silica capillary will act as a restrictor for the EOF in the channel. Starting from the Fanning (Darcy–Weissbach) equation for the friction induced pressure drop for liquid flowing through a length of tubing, the well known Hagen–Poiseuille equation for isothermal laminar flow (where Re<2300, and Re is a dimensionless number that characterizes the flow regime) can be determined:

$$\Delta p = \frac{128 L \eta F}{\pi d^4} \quad (1)$$

where Δp is the pressure drop across a straight tube which can generate a given flow-rate (F), L is the length of the tubing, d is the internal diameter of the tubing, and η is the fluid viscosity.

The channel–capillary junction could be compared to a junction between a larger diameter (d_1), and a smaller diameter (d_2) capillary, having the lengths L_1, and L_2, respectively. If there is an EOF through the large capillary, but not in the small one, there will be an electrokinetically generated pressure at their junction. This pressure term will generate laminar flow in the two capillaries: back flow through the large capillary (F_{bf}), and forward flow through the small capillary (F), superimposed on the existing EOF. For the case where the EOF exists only in the large capillary, the original EOF (F_{eof}) will be reduced by F_{bf}, and the forward flow will become: $F=F_{eof}-F_{bf}$. Disturbances of fluid flow due to auxiliary components in the fluid flow path are neglected here [23]. Consequently, Eq. (1) can be written as:

$$\Delta p = \frac{128 L_1 \eta F_{bf}}{\pi d_1^4} \text{ and } \Delta p = \frac{128 L_2 \eta F}{\pi d_2^4} \quad (2)$$

Since F and F_{bf} are fractions of F_{eof}, $F=xF_{eof}$ and $F_{bf}=(1-x)F_{eof}$, by replacing F and F_{bf} in Eqs. (2), and then combining the two equations, we obtain:

$$F = F_{eof} \cdot \frac{\frac{L_1}{d_1^4}}{\frac{L_1}{d_1^4} + \frac{L_2}{d_2^4}} \text{ and } F_{bf} = F_{eof} \cdot \frac{\frac{L_2}{d_2^4}}{\frac{L_1}{d_1^4} + \frac{L_2}{d_2^4}} \quad (3)$$

For the case where $d_1=d_2$:

$$F = F_{eof} \cdot \frac{L_1}{L_1 + L_2} \text{ and } F_{bf} = F_{eof} \cdot \frac{L_2}{L_1 + L_2} \quad (4)$$

In cases where there is EOF in both capillaries, and $EOF_2<EOF_1$, the same equations apply except the F_{eof} term is replaced by $F_{eof1}-F_{eof2}$.

The EOF in microchips was experimentally determined prior to and after coupling with an ESI capillary. Data obtained from two different buffer systems at two different field strengths are shown in Table 1. Theoretical calculations, substituting the trapezoidal cross sectional area of the channels with that of a circle of the same area, indicate that the original EOF should be reduced to 23% for an attached 11 mm length of a 20 μm I.D. capillary.

Table 1
Effect of ESI capillary restriction on microchip channel EOF

	Capillary length (mm)	Field strength ($V cm^{-1}$)	Unrestricted $EOF_{experimental}$ ($nl min^{-1}$)	Restricted $EOF_{experimental}$ ($nl min^{-1}$)	Restricted $EOF_{calculated}$ ($nl min^{-1}$)
Sodium borate (10 m*M*), pH 9.26	11	550	408	124	94
Sodium borate (10 m*M*), pH 9.26	11	340	285	75	66
Ammonium acetate (10 m*M*) (CH_3OH–water, 25:75)	11	550	114	43	26
Ammonium acetate (10 m*M*) (CH_3OH–water, 25:75)	11	340	73	32	17

The experimental values for the EOF were somewhat larger, but close to the calculated values. Obviously by maintaining a short ESI capillary the effects on EOF can be minimized.

The suitability of this microfabricated device for on-line electrophoretic detection was also briefly examined. Fig. 4A shows the extracted ion electropherogram (EIE) obtained from an injection of rhodamine B which produces a strong electrospray signal (Fig. 4B). The electrospray voltage was continuously applied to the chip while the sample was introduced using a gated injection scheme. Electrospray was observed throughout the sequence (i.e., loading, injection and run) and was not perturbed by the switching voltages on the front end of the chip that introduced the sample plug. On-line separations were not attempted, however, because of the dead volume associated with the junction between the capillary and the microdevice. A low dead volume connection is essential for maintaining separation efficiency, given the small sample volumes typically injected on microchip devices (0.1–0.5 nl) and the narrow band widths of the resolved constituents (1 s or less). In this device the estimated dead volume of the junction was ca. 1–3 nl. This volume is associated primarily with the large trapezoidal area of the channel that was deep etched to allow insertion of the emitter. The width of the rhodamine peak on the chip was determined by LIF to be ca. 1–1.5 s full width at half maximum (FWHM) and measured 8 s in the EIE. Such a large loss in efficiency is of course unacceptable for performing on-line microchip separations. An alternative approach for creating an opening for attachment of a tip, drilling into the edge of the device as recently reported, for example, may minimize the dead volume and associated losses in efficiency [12]. This approach to capillary attachment is compatible with the porous glass junction.

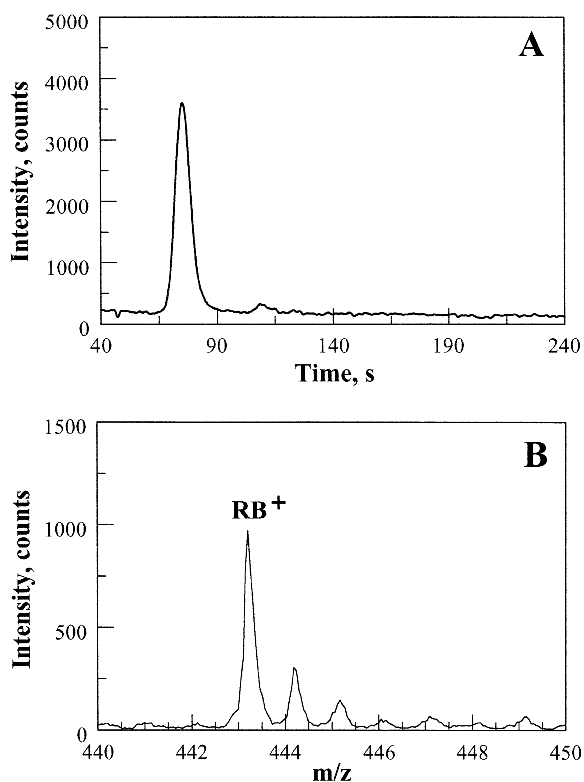

Fig. 4. Extracted ion electropherogram of an injection of rhodamine B. Conditions: (A) 30 μ*M* sample, CH_3OH–water–CH_3COOH (25:75:0.5, v/v) eluent, 1 s injection, 500 $V cm^{-1}$, ESI voltage at porous glass junction; TOF-MS data acquisition: 5000 Hz, 1600 summed spectra, 3.1 spectra s^{-1}; (B) TOF mass spectrum of rhodamine B.

4. Conclusions

A new configuration based on an electrically permeable glass membrane for on-chip electroosmotic pumping and application of ESI voltage for microchip-MS interfacing was developed. The membrane reduces fluid loss relative to previous configurations based on a tee structure [2,14,24] and does not require any surface modification or treatment of the channels. Fabrication is also straightforward and does not require any additional steps in the manufacturing process. External assistance is not required to generate stable electrospray and the microchips can be coupled with nanospray emitters or blunt cut capillaries. Sensitivities for the latter are similar to that obtained from conventional microelectrospray sources [21].. The effect of an inserted fused-silica capillary on EOF was examined. The pressure term introduced by such a capillary reduces the EOF, and consequently, the length of the inserted capillary should be kept to a minimum. The microchip can be easily utilized for continuous electrospray or discrete sample analysis affected by timed injections. Provided a low dead volume channel–capillary junction is constructed, the device can also be utilized for on-line separations. Component adsorption/ion loss at the glass membrane surface was not investigated here. Further studies will be required to examine this phenomenon. In addition to MS interfacing, other microchip applications requiring electroosmotic fluid delivery, indirect adjustment of electric fields in the microchip channels, etc., can be envisioned for this device.

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