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Evaluation of a microchip electrophoresis-mass spectrometry platform deploying a pressure-driven make-up flow

Xiangtang Li¹, Shulin Zhao^{1,2}, and Yi-Ming Liu^{1,*}

¹Department of Chemistry and Biochemistry, Jackson State University, 1400 Lynch St., Jackson, MS, 39217

²College of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin, 51004, China

Abstract

Integration of a pressure-driven make-up flow (MUF) into a microchip electrophoresis (MCE) platform in order to facilitate its coupling with electrospray ionization-mass spectrometric detection (ESI-MS) is described. In the glass /PDMS hybrid microchip, a MUF channel was made to intersect with the MCE separation channel at an angle of 45°. The MUF was generated by a syringe pump. Microscopic image results from simulation studies showed that the pressure-driven MUF and the potential-driven electroosmotic flow in the MCE separation channel could be run separately without interfering with each other and mixed well at the joint point by adjusting either the MUF flow rate or the potential applied for MCE separation. The MUF had several desirable functions, including making the start of electrospray easy and cleaning the nanoESI emitter continuously when not spraying. High separation efficiency was achieved with the proposed MCE-nanoESI-MS system in separating an amino acid mixture containing glutamine, serine, threonine, phenylalanine, and glutamic acid. All of them were baseline separated from each other within 3 min. Plate numbers of >10,000 (on a 2.5 cm MCE separation channel) were obtained. The analytical platform also showed a linear response for quantification of DOPA with a detection limit (S/N =3) of 0.10 μM. In addition, on-line derivatization of MCE elutes in order to enhance MS detection sensitivity was easily carried out by adding the tagging reagent into the MUF. These results indicated that the present system might have a good potential in MCE-MS applications.

Keywords

Microchip electrophoresis; nano-electrospray ionization-mass spectrometry; glass/PDMS hybrid microchip; make-up flow; electrophoretic separation

1. Introduction

Microfluidic devices coupled with mass spectrometry (MS) have great potentials in bioanalytical applications [1–2]. Study on the coupling interface has been intensive over the past decade. In the majority of these works electrospray ionization (ESI) – MS was employed by virtue of the simplicity of the interface. Various electrospray configurations

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*Corresponding author: Prof. Yi-Ming Liu, Tel: +1-601-979-349; yiming.liu@jsums.edu.

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were reported [3–4]. Microchip electrophoresis (MCE), a miniaturized format of capillary electrophoresis (CE) performed on a microfluidic chip is a powerful separation technique, offering high separation efficiency, high through-put sampling, and many advantageous microfluidic features. Because of the low flow rate of the effluent from MCE separations (normally at ~ 25 nL/min) coupling MCE with ESI-MS is very different from and technically more difficult than coupling microfluidic devices where fluid flows are generated and controlled by using syringe (or air pressure) pumps. Electrospray of a fluid flow at such a low flow rate can become unstable because the Taylor cone generated in the spray process may not fit well at the emitter tip. A straightforward solution to this problem is to use sheath liquid. Two types of sheath liquid interfaces were reported: 1) liquid-junction interface [5–6] and 2) co-axial sheath liquid interface [7]. In these works, a sheath liquid flow that carried MCE effluent to ESI emitter was generated by using a syringe pump at a flow rate of $2\sim 6$ μ L/min. The assay sensitivity was severely compromised because of the dilution of MCE effluent by sheath liquid. To avoid this problem, low sheath flow [8–9] and sheathless [10–11] interfacing was investigated. In sheathless interfacing, MCE microchips with an external ESI spray nozzle and glass microchips with a monolithically integrated spray tip were reported. In these studies, the ESI emitter was carefully fabricated to minimize the emitter area so that an efficient electrospray process could be established even with a liquid flow at a flow rate of the nL/min level. The design was improved by adding an auxiliary channel joining with the separation channel at the outlet ends [12–14]. This channel with a potential applied to its inlet provided an electrical contact for electrospray, and also increased the flow rate of the fluid flow to the ESI emitter by working as an electroosmotic pump [15]. With a poly(dimethylsiloxane) (PDMS) microchip of this design, stable ESI could be established over a range of flow rate from 50 to 1000 nL/min [12]. A review on coupling MCE with ESI-MS was recently given [16]. In an effort to develop MCE-ESI-MS methods with the use of a sheathless interface, referencing to the microchip designs reported in literature and on the basis of our experience with microchip fabrication [17–18] and CE-ESI-MS method development [19–20], we encountered problems / inconveniences such as the difficulty to start electrospray without a pneumatic assistance and easily clogging of the tiny nanoESI emitter. Similar observations were also reported by other research groups [11, 21–22].

We herein report a novel MCE-nanoESI-MS platform. Compared with the platforms reported previously in literature, a new feature of the present platform is the integration of a pressure-driven make-up flow (MUF, versus a potential-driven electroosmotic MUF) into the system. An auxiliary microchannel is made in the microchip to transport a MUF generated by a syringe pump to the ESI emitter. The MUF channel intersects with the MCE separation channel at an angle of 45° for a smooth joining of the MUF and MCE effluent. The difference between the present MUF design and previously reported sheath liquid designs lies in that the MUF flows at ~ 100 nL/min (*versus* sheath liquid at $2\sim 6$ μ L/min). Compared with the low sheath flow interfaces reported previously, the present design integrates the CE separation channel and MUF channel into a single microchip, thus offering a potential advantage of having minimum dead volumes in the system. The proposed MCE-nanoESI-MS system was evaluated by separating amino acid mixtures. The separation efficiency, assay repeatability, detection sensitivity, and linearity of the signal-concentration relationship were investigated. On-line derivatization of amino acids with 7-fluoro-4-nitrobenzoxadiazole (NBD-F) after MCE separation was also demonstrated.

2. Experimental

2.1. Materials

PDMS prepolymer and the curing agent were purchased from Dow Corning (Midland, MI). SU-8 (2010) negative photoresist and SU-8 developer were obtained from Microchem

(Newton, MA). Fused silica capillaries (100 /194 μm and 254 /365 μm) were obtained from Polymicro Technologies (Tucson, AZ). Glass slides (3" $\times$ 3" $\times$.060") were obtained from Silicon Valley Microelectronics (Santa Clara, CA). Hexamethyldisilazane (HMDS) was from Ultra Pure Solutions (Castroville, CA). DOPA, glutamine, serine, threonine, phenylalanine, and glutamic acid and NBD-F were purchased from Sigma–Aldrich Chemical (St. Louis, MO). Milli-Q water was used through out the work. All solutions were filtered through a nylon 0.22 μm syringe filter before use.

2.2. Fabrication of Microfluidic Chip

Design of the microchip is shown in Figure 1A. The chip was composed of a glass substrate and a PDMS cover bearing the channels. The procedure used to create channels in PDMS was based on that described previously [23]. Briefly, to create masters for PDMS device construction, HMDS was transferred onto a silicon wafer with a pipette. The wafer was pinned at 2000 rpm till complete dryness. The wafer was then coated with SU-8 2010 negative photoresist using a spin coater operating at 2500 rpm for 80s. After a pre-exposure bake at 65°C for 30 min, the coated wafer was exposed to a near-UV light source through a negative chrome mask that contained the desired channel features. Following a post exposure bake at 95°C for 10min, the wafer was developed with SU-8 developer. PDMS monopolymer solution prepared by mixing the PDMS prepolymer and curing agent in a 10:1 ratio was poured onto the master. After > 3 hour curing at 50 °C, the PDMS was pilled off from the master to yield a pattern of negatively relieved channels in the PDMS sheet. PDMS cover (thickness, 2 mm) was exposed to an air plasma for 30 s and was placed on to a glass substrate to form a completed microchip. It was then treated in an air plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY) for 5 min (10.5 W and 500mTorr).

2.3. MCE-nanoESI-MS system

The system consisted of an ion trap mass spectrometer (LCQ Deca, ThermoFinnigan, San Jose, CA), the microchip prepared above, a high voltage power supply, and two syringe pumps. One syringe pump was used for sample introduction and the other for MUF delivery. The nanoESI emitter was prepared from a piece of capillary (100 μm ID, 194 μm OD) drawn to ~ 20 μm OD at the tip. The other end was connected to the MUF channel. The sample was infused into the PDMS/Glass microchip from a 100 μL syringe via a fused silica capillary (254 μm ID, 360 μm OD). The sample size was controlled by the syringe pump as reported previously [24–25]. The electrospray potential was applied by a high voltage power supply and was also used to drive MCE separation. Xcalibur software (ThermoFinnigan) was used for MS data acquisition and process. The After tuning the MS detector in positive ion mode with a standard DOPA solution, the MS detection conditions were set as follows: ionization source voltage, 0V; a relative collision energy of 25–30% was used for MS/MS experiments with an isolation width of 1.0 u. and activation time of 30 ms. Figure 1B illustrates the operation of the system.

2.4. MCE-nanoESI-MS analysis

The syringes were filled with sample solution and make-up fluid, respectively. They were then connected to the respective capillaries assembled into the microchip. MCE running buffer was transferred to the buffer reservoir on the chip. The MCE channel was filled with the buffer by applying vacuum at the tip of the nanoESI emitter (i.e. docking the emitter into a piece of plastic tubing connected to vacuum). The microchip was placed on a XYZ-translational stage and positioned in front of the MS orifice so that the nanoESI emitter tip was about 1 mm away from the orifice. The MUF pump was tuned on to generate a flow. A potential of 3150 V was applied at the buffer reservoir through a platinum electrode to start ESI. The sample syringe pump was turned on and then off, making sure that the sample solution filled completely the sample channel in the microchip. Waited for ~3 min till all

solutes migrated out from the MCE channel, and then the sample was injected by switching on the syringe pump for 2 seconds. MS data acquisition was then started. When completed, the potential applied at the buffer reservoir was removed to stop ESI. MUF was maintained for several min to clean the nanoESI emitter before the syringe pump was turned off.

MCE running buffer was a mixture of methanol /water containing a pH adjuster (either 0.1% acetic acid or ammonium acetate buffer at pH 5.5 depending on the separation depending on the sample to be separated). Make-up fluid was a mixture of methanol / water (8:2) containing 0.1% (v/v) acetic acid at a flow rate of 100 nL/min. Post-column on-line derivatization with NBD-F was carried out by using a NBD-F containing make-up fluid which was prepared as following: mixing 63 μ L ammonium acetate /acetic acid buffer (200 mM at pH 9.0) with 75 μ L NBD-F(10 mM in acetonitrile), and diluting this solution to 500 μ L with a methanol /water mixture (80:20).

Safety Information: The high voltage used in the described MCE-MS operations can cause electric shock. Precautions such as current limiting settings on power supplies and isolation of electrical leads must be taken.

3. Results and discussion

3.1. Microchip design

An innovative aspect of the present MCE chip design is the integration of a pressure-driven MUF into the system (see Figure 1 for chip design details). Using a syringe pump to deliver a MUF offered advantages such as convenience in operation and an accurate control of MUF flow rate. To achieve a stable and efficient ESI, the chemical composition of the make-up fluid was investigated. Methanol /water mixtures at ratios 2:8, 5:5, and 8:2 with 0.1% acetic acid were tested, and it was found that the mixture of methanol /water at 8:2 produced the best ESI results in terms of MS signal background. It was, therefore, selected for further studies. With the proposed MCE-nanoESI-MS system, a stable electrospray process could be easily established by applying a potential of ~3200 kV at the buffer reservoir in the microchip after the MUF syringe pump was turned on. It's worth noting that the pressure-driven MUF can be used to flush the microchip channels and the nanoESI emitter whenever needed to maintain the system in good conditions. For simplicity, one voltage approach was used in the present MCE-MS platform. This approach was reported previously, particularly in sheathless MCE-MS systems [3, 11, 16]. The voltage applied (i.e. ~3200 kV in this work) served for both driving MCE separation and generating electrospray. Compared with a more commonly used two-voltage CE-MS interface, a disadvantage of the one-voltage approach is that the voltage can't be optimized separately for ESI performance and separation efficiency. Therefore, the compositions of the MCE running buffer and the MUF solution should be carefully chosen in order to achieve both an effective MCE separation and a stable ESI. In this work, a pressure injection procedure was used to inject samples into the system. Sample injection was executed while a potential was applied at the buffer reservoir. That is, the sample was injected into a flow of the running buffer (EOF). This situation was very similar to those in microfluidic works [25]. The injection approach was effective because clean and narrow sample plugs were produced which was evidenced by sharp and symmetric MCE peaks obtained from separations.

In the proposed microchip design, the MUF channel transporting a pressure-driven flow is connected with the MCE separation channel that transports a potential-driven flow. Therefore, a set of experiments were performed to see whether the pressure-driven MUF would enter into the connected MCE separation channel causing a disturbance on MCE separations. In these experiments, a fluorescent dye, i.e. fluorescein, was added to MUF at a concentration 0.1 mM, and an epi-fluorescence microscope was used to monitor the MUF.

The microchip was placed on the microscope and positioned thus that the joint point of MUF and MCE separation channels was clearly viewed as shown in Figure 2A. The syringe pump was turned on to generate a MUF at a flow rate of 100 nL/min, and then a potential of +3150 V was applied at the buffer reservoir to start electrospray against a piece of grounded metal disc placed ~ 1mm in front of the nanoESI emitter. As can be seen in the microscopic image obtained (Figure 2B), pressure-driven MUF and potential-driven EOF flow in their respective channels and mixed smoothly at the joint point under these experimental conditions. However, when increasing MUF flow rate to 500 nL/min the MUF started to enter the MCE separation channel as clearly seen in Figure 2C. This finding was verified by the image obtained after further increasing MUF flow rate to 750 nL/min (Figure 2D). Further study showed that the MUF back flow into the MCE separation channel could be pushed out by increasing the potential applied at the buffer reservoir. Figures 2E and 2F are images taken when potentials applied at the buffer reservoir increased to 3500 and 4000 V, respectively while MUF flow rate was set at 750 nL/min. These results indicated that in the present microchip pressure-driven MUF and potential-driven EOF were able to flow in their respective channels without interfering with each other and to mix smoothly at the joint point as long as the MUF flow rate and the MCE/ESI potential were set at appropriate values. In another set of experiments, MUF flow rates from 50 ~ 500 nL/min were tested for analyzing a DOPA solution at 1.0×10^{-5} M in order to examine its effects on MCE-nanoESI-MS results. The MCE/ESI potential was set at +3150 V. The TIC electropherograms obtained are shown in Figure 3. As can be seen, peak widths were 12, 8, and 16 seconds with MUF at 50, 100, and 250 nL/min, respectively. Severe peak broadening (a wide baseline elevation) was observed when MUF flow rate was 500 nL/min. Under the experimental conditions, best results were obtained with a MUF flow rate of 100 nL/min. These MCE-nanoESI-MS results were in accordance with those image results from the simulation studies.

3.2. Analytical performance of the proposed MCE-nanoESI-MS system

Assay repeatability was assessed by injecting a DOPA standard solution (1.0×10^{-5} M) consecutively for 20 times, and the TIC signal from ion m/z 198 was recorded continuously. The electropherogram is shown in Figure 4. Relative standard deviation (RSD) of the peak area was calculated to be 5.4% ($n = 20$), which was good for MCE-based analysis. The repeatability can be significantly improved by adding an internal standard to the sample as it's usually done in assays with MS detection. The signal-concentration relationship was investigated by analyzing a series of 5 standard DOPA solutions at concentrations ranging from 0.50 to 100 μ M using m/z 198 $\rightarrow$ 181 SRM MS/MS detection mode. Peak area versus analyte concentration showed a good linear response. The calibration equation was found to be $PA = 5.98 \times 10^5 \text{ Conc} - 2.0 \times 10^3$ with a correlation coefficient of 0.995 (where C was in μ M). Detection limit ($S/N = 3$) was estimated to be 0.10 μ M DOPA. Compared with our previous work where a CE-ESI-MS method was developed for DOPA quantification [20], the present MCE-nanoESI-MS method was more sensitive (LODs: 0.10 μ M versus 0.50 μ M). The increase in assay sensitivity could be attributed to two factors: 1) less dilution of the CE effluent by sheath liquid, and 2) use of nanoESI in the present system [16].

Separation efficiency of the present MCE-nanoESI-MS system was assessed by separating a mixture of 5 amino acids (i.e. glutamine, serine, threonine, phenylalanine, and glutamic acid at 1.0×10^{-5} M each). These five amino acids represented basic, neutral, and acidic all three categories of amino acids. TIC signal from full scan for positive ions was monitored. The electropherogram obtained is shown in Figure 5A. The peaks were identified by MS² spectra (e.g. Figure 5B and 5C). The five compounds were baseline separated from each other within 3 min. All peaks were very narrow ($w_b < 10$ seconds) and had a symmetric peak shape. It is worth noting that in the MCE separation a plate number of >10,000 (calculated

by $N = 16 (t_R/w_b)^2$) was obtained for all of these amino acids. The high separation efficiency might be attributed to two factors: the optimal joining geometry of MCE effluent with MUF and the minimized effects of electrospray aspiration. In the proposed MCE microchip, the MUF channel intersects with the MCE channel at an angle of 45° , and this joint geometry may cause minimum broadening of the MCE bands [6]. Moreover, since the outlet of the MCE separation channel joins the MUF channel about 1.5 cm before the ESI emitter tip and the MUF is generated by a gas-tight system, the aspiration effects from the ESI process on MCE separations might be minimized with the present MCE-nanoESI-MS system.

3.3. On-line post-column derivation on the MCE-nanoESI-MS platform

In some cases when MS detection is used, tagging of analytes with an appropriate organic moiety can significantly improve detection sensitivity [26–28]. The sensitivity enhancement can be due to the introduction of moieties that are of high proton or electron affinity. With the proposed MCE-nanoESI-MS system, on-line derivatization can be conveniently done by adding the tagging reagent to MUF. To demonstrate this, derivatization of amino acids, i.e. Ser and Ala with NBD-F was investigated. NBD-F was added to MUF at a concentration of 1.5 mM. The amino acid mixture was injected into the system, separated by MCE, and mixed with NBD-F containing MUF when derivatization occurred. The MS detector was set up to monitor ions m/z 269 for Ser-NBD and m/z 253 for Ala-NBD simultaneously using SRM MS/MS detection mode. The TIC electropherogram obtained is shown in Figure 6A. As can be seen, both amino acids tested were effectively derivatized on-line. The chemical structures of the derivatives were verified by MS² spectra (Figure 6B and 6C). A comparative study was carried out to analyze this amino acid mixture with or without NBD-F derivatization. The results indicated that with derivatization the assay sensitivity was enhanced by a factor of ~ 50 for the amino acids tested.

4. Conclusions

A glass /PDMS hybrid microchip was fabricated and evaluated for microchip electrophoresis-nano-electrospray ionization-mass spectrometry (MCE-nanoESI-MS) applications. An innovative feature in this new design was the integration of a pressure driven make-up flow (MUF) intersecting with the MCE effluent at an angle of 45° into the microchip. Epi-fluorescence microscopic image results from the simulation studies showed that pressure-driven MUF and potential-driven EOF were able to flow in their respective channels without interfering with each other and to mix smoothly at the joint point as long as the MUF flow rate and the MCE /ESI potential were set at appropriate values. The pressure-driven MUF was found to have desirable functions such as making the start of electrospray easy and stable and cleaning the nanoESI emitter continuously when not spraying. With the proposed MCE-nanoESI-MS system, a plate number of $>10,000$ (on a 2.5 cm MCE separation) was obtained from separation of amino acids. The detection limit was found to be $0.10 \mu\text{M}$ for DOPA. Finally, on-line post-column derivatization of native amino acids was conveniently and effectively done by adding the tagging reagent, NBD-F into the MUF. These results suggested the MCE-nanoESI-MS platform proposed might have a potential in bioanalytical applications.

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Highlights

- Deploying a pressure-driven make-up flow in a MCE-MS platform
- Glass /PDMS hybrid microchip for MCE-MS applications
- Efficient separation of amino acids by MCE-MS
- Facile on-line postcolumn derivatization in MCE-MS analysis

Fig 1. Design of the microchip (A) and an illustration of operating the proposed MCE-nanoESI-MS platform (B).

Fig 2.

Epi-fluorescence microscopic Images obtained from the simulation study on MUF moving direction (see text for details): (A) the joint point of the MUF and MCE separation channels in the proposed microchip; (B) MUF flow rate at 100 nL/min and MCE /ESI potential at +3150 V, showing MUF (the fluorescent band) and EOF mix smoothly at the joint point; (C) MUF flow rate increased to 500 nL /min while MCE /ESI potential remained at +3150 V, showing MUF started to enter the MCE channel; (D) MUF flow rate further increased to 750 nL /min while MCE /ESI potential was still at +3150 V, showing MUF entered into the MCE channel more quickly; (E) MUF flow rate remained at 750 nL/min, but MCE /ESI potential increased to +3500 V, showing MUF was partially pushed out of the MCE channel; and (F) MCE /ESI potential further increased to +4000 V while MUF flow rate remained at 750 nL /min, showing MUF and EOF mixed smoothly without interfering each other.

Fig 3.

Effects of MUF flow rate on MCE-nanoESI-MS analytical results, indicating severe peak broadening occurred at high MUF flow rates. Experimental conditions: sample injected, DOPA standard solution at 1.0×10^{-5} M; MCE running buffer, methanol / water (1:1) containing 0.1% (v/v) acetic acid; MCE potential applied, +3150 V; MUF, methanol / water (8:2) containing 0.1% (v/v) acetic acid; and MS detection, full scan of ion m/z 198. Note: migration times shown in these electropherograms were inaccurate because MS data acquisition started at different times.

Fig 4. Repeatability of the proposed MCE-nanoESI-MS analysis. A sample solution (DOPA standard solution at 1.0×10^{-5} M) was consecutively injected into the analytical system for 20 times. MCE and MS detection conditions were as in Figure 3.

Fig 5.

Separation of a mixture of amino acids (1.0×10^{-5} M each) by using the proposed MCE-nanoESI-MS system: (A) TIC electropherogram (peak identification 1, glutamine, 2, serine, 3, threonine, 4, phenylalanine, and 5, glutamic acid); (B) and (C) MS² spectra for peak 1, confirming the peak identity of glutamine, and peak 4, confirming the peak identity of phenylalanine, respectively. MCE running buffer, methanol /water (1:2) containing 15 mM ammonium acetate buffer at pH 5.5; MCE potential applied, +3050 V; MUF, methanol / water (8:2) containing 0.1% (v/v) acetic acid.

Fig 6. On-line post-column derivatization of amino acids with NBD-F on the proposed MCE-nanoESI-MS platform: (A) TIC electropherogram; (B) MS² spectrum of m/z 269 at 1.26 min from (A); and (C) MS² spectrum of m/z 253 at 1.47 min from (A). [amino acid] = 5×10^{-6} M. MCE and MS conditions were as in Figure 5 except MUF was a mixture of methanol /water (8:2) containing 25 mM ammonium acetate buffer (pH 9.0) and 1.5 mM NBD-F.