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Research Article

A sheathless poly(methyl methacrylate) chip-CE/MS interface fabricated using a wire-assisted epoxy-fixing method

Using a wire-assisted epoxy-fixing method, a sheathless CE/MS interface on a poly(methyl methacrylate) (PMMA) CE chip has been developed. The sheathless chip-CE/MS interface utilized a tapered fused-silica tip and the electrical connection was achieved through a layered coating of conductive rubber. The wire-assisted method provided facile alignment of channels between the PMMA CE chip and an external capillary sprayer without the need for micromachining. Because the wire was in the channel during fixing, the risk of channel blockage by the epoxy was avoided. This chip CE device has minimal dead volume because the interstitial spaces were filled by a fast-fixing epoxy resin. The performance of the chip-CE-ESI-MS device was demonstrated with the analysis of peptide mixtures.

Keywords:
CE / Electrospray / MS / Microchip

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1 Introduction

Miniaturization of chemical analysis systems using microchips is an emerging new technology. Microfluidic devices, particularly CE microchips, have attracted a great deal of attention, owing to advantages such as high degree of integration, portability, minimal solvent and reagent consumption, high performance, and rapid separation [1, 2]. In addition, high-throughput analysis can be easily achieved by multiplexing the separation channels through the design on a compact device [3, 4]. Because of its great potential, the CE microchips have been commercialized and applied to many areas, such as environmental monitoring, biomedical and pharmaceutical analysis, clinical diagnostics, and forensic investigations.

In the early stages, microdevices were fabricated with glass substrates by a microelectromechanical process. The motivation for using glass was that photolithographic methods for microfabrication in glass were well established and the chromatographic properties of glass have been well characterized. However, glass chips are difficult to produce on a large scale because of the time-consuming manual fabrication and the need for high temperature bonding. Recently, a polymer-based CE microchip has been developed. While plastic substrates are more versatile and easier to machine than glass [5, 6], it is worthy to mention that some of their properties, such as thermal conductivity, dielectric strength and organic solvent compatibility, are inferior to glass. Simple fabrication methods, such as hot-imprinting [7], injection molding [8], casting [9], and laser ablation [10], have been applied to a wide range of polymeric materials. Polymers including poly(methyl methacrylate) (PMMA) [7, 11], PDMS [9], polycarbonate (PC) [12], and polystyrene (PS) [13] have been used to fabricate microchips for CE separations. Unlike the glass microchip, the polymer microchip can be massively produced because only a master chip is needed. Thus the production costs are greatly reduced.

As the field of microchip separation continues its rapid growth, there is an urgent need to develop compatible detection methods. Up to now, optical detection remains the primary detection technique. Most reports on microchip CE rely on LIF to detect the analytes at the end of the separation channel. In recent years, because of high specificity, high sensitivity, and the ability to unambiguously identify analytes, MS has gained increased attention in chip-based CE analysis [14, 15].

For on-chip detection systems, such as UV absorption, fluorescence, or electrochemical detection, the solution never leaves the microdevice. However, when a mass spectrometer is used as the detector, a suitable interface is needed to transfer the solution to the ionization source. Because of good sensitivity for most biochemicals, ESI is currently the method of choice for chip-CE/MS combination. The strategy for performing electrospray in early

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Abbreviation: PMMA, poly(methyl methacrylate)

reports was direct spraying from the flat edge of a chip [16]. The major drawback of this approach is the tendency for the liquid to spread over the surface of the chip. During the past few years, several attempts have been made to couple chip CE to the ESI-MS. These techniques can be generally classified into two different categories. The first refers to designs where the ESI sprayer is integrated within the CE microchip [17, 18]. An example of this type of microdevice using an integrated nebulizer has been reported [17]. In this instance, the solution was sprayed directly from the exit port formed by cutting the chip using a dicing saw. In recent years, several integrated microchip devices have been proposed [19–23]. Although the data presented in these papers were mainly from infusion experiments [19–21] or chip-LC/MS [22, 23], they may have the potential for chip-CE/MS application.

The second category refers to designs where the capillary sprayer is attached to the CE microchip, which at present is the more widely used approach. An advantage of attaching an external ESI sprayer is that many interfaces developed for capillary CE/MS can be implemented to chip-CE/MS with minimal modification. For microchip-CE-ESI-MS applications, several interfaces had been reported including sheathless [24–28], sheath flow [24], and liquid junction [17, 29–34]. In one example, a tapered tip was inserted and fixed into a precreated hole to produce a sheathless interface [24]. Another design used a coaxial sheath flow to pick up the solution from the exit of the channel [30]. Recently, the attachment of microchip-CE with an external sprayer is still under development [27, 28, 32–34]. For example, a fused-silica capillary emitter was inserted into the exit of a glass CEC chip [27] or a PDMS CE chip [28]. A sprayer was attached (screwed) to a precreated hole in a glass microchip using a polyether ether ketone (PEEK) screw [32–34].

For the attachment of an external capillary sprayer, two critical aspects with the connection are the dead volume and interchannel alignment. Although liquid junction approach has the advantage of easy alignment, these interfaces usually have a dead volume in the junction. For sheathless and sheath liquid interface, it is possible to eliminate or greatly reduce the dead volume if great care is exercised during the fabrication of an interface. For example, Harrison and coworkers [35] have reported a sheathless interface by inserting a tapered tip into the chip through a precreated hole followed by fixing glue (Crystal Bond). It was shown that a hole with flat bottom had a smaller dead volume than a hole with a tapered bottom. Furthermore, in order to make proper alignment, the hole must be carefully drilled and centered on the exit of the separation channel. This method required dedicated micromachining and could not be applied to a polymer-based microdevice, because, compared to a glass device, polymers have lower transition temperatures and cannot withstand the high temperatures induced by drilling. Two other examples showed that delicate etching [27] or accurate imprinting [28] was required for inserting a capillary sprayer into the outlet of the chip.

In this report, we present a simple method for making a sheathless chip-CE/MS device. The ESI interface was constructed using a tapered capillary sprayer with a wire-assisted epoxy-fixing method. This method provides a low-cost, simple, and almost zero dead volume connection for attaching a sprayer to polymer-based CE microchip. Comparing to other methods of attaching an external sprayer [24–28], it is not necessary to precisely drill a flat-bottomed hole at a smooth edge of the channel outlet [24] or to accurately etch or imprint a bigger channel to fit the outer diameter of the capillary [27, 28]. The potential of this simple and minimal dead volume chip-CE/MS device is demonstrated.

2 Materials and methods

2.1 Materials and sample preparation

The PMMA plates were obtained from Chi Mei (Tainan, Taiwan). The conductive rubber was purchased from Chemtronics (Kennesaw, GA, USA), and fast-fixing epoxy resin (4 min) was obtained from Kuo Sen (Taipei, Taiwan). Formic acid and acetic acid were obtained from Fluka Chemical (Milwaukee, WI, USA), 48% HF was obtained from Sigma–Aldrich Chemical (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from J. T. Baker (Phillipsburg, NJ, USA). The peptide standards, GY (MW 238), VYV (MW 379), YGGFL ([Leu]^5-enkephalin, MW 555), YGGFM ([Met]^5-Enkephalin, MW 573), DRVYIHPF (angiotensin II, MW 1046), RPKPQQFFGLM (substance P, MW 1347), and RPKPQQFFPLM ([Pro^9]-substance P, MW 1387) were purchased from Sigma–Aldrich Chemical. Another peptide standard, RPPGFS (bradykinin (1–6), MW 659), was purchased from Bachem (Torrance, CA, USA). The 18 MΩ·cm deionized water was from a Milli-Q water purification system (Millipore, MA, USA). The running buffer used throughout the experiments was 25% MeOH, 1% acetic acid at pH 3.7. Before use, the solution was filtered through a 0.22-μm syringe filter (Pall Gelman Laboratory, Ann Arbor, MI, USA). All peptide samples were dissolved (1 mg/mL) in water as stock solution and an aliquot of each peptide was mixed with the buffer before analysis.

2.2 Microchip fabrication

The PMMA chip, shown in Fig. 1a, consisted of two PMMA plates ($65 \times 25 \times 2$ mm) with a 45 mm separation channel (45 mm from the injection cross to the channel exit) and a 15 mm injection channel (7.5 mm from the sample reservoir to the injection cross and 7.5 mm from the injection cross to the sample waste reservoir). The width of the separation channel and the injection channel was 50 μm, and the channel depth was also 50 μm. The exit of the separation channel is connected to a tapered and conductive rubber coated microsprayer (Fig. 1c).

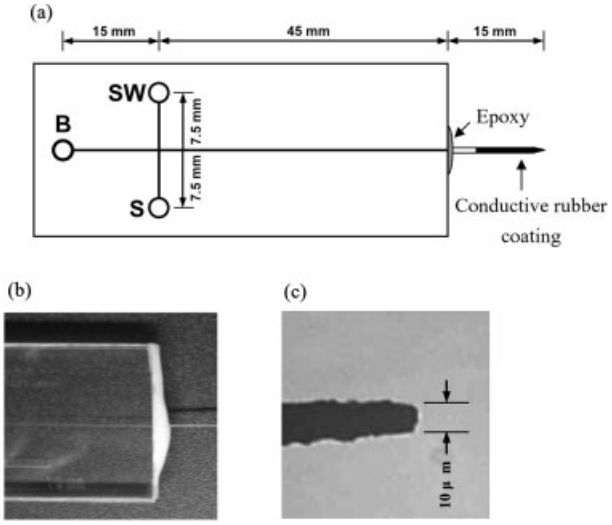

Figure 1. (a) Schematic diagram of the chip-CE-ESI-MS device (B, buffer reservoir; S, sample reservoir; SW, sample waste reservoir). (b) Photograph of the ESI interface. (c) The 10-μm electrospray tip with a conductive rubber coating.

The PMMA chip has two channels with a cross intersection. A silicon master has been used as the template to make the CE chip [7]. To make a silicon master, a photomask of the microchannel system was designed using AutoCAD software (Autodesk, San Rafael, CA, USA) and printed onto a transparency film using a 5000 dpi laser photoplotter. The master was created using standard photolithographic procedures and deep reactive ion etching (DRIE) performed in a Surface Technologies Systems machine (STS, Newport, UK). A 4″-diameter, 525 μm thick p-doped silicon (100) wafer (Woodruff Tech., Arcadia, CA, USA) was used as the starting material. The wafer was first subjected to dehydration for 30 min at 150°C, followed by a surface coating with hexamethyldisilazane (HMDS) to improve adhesion of the photoresist. Next, the AZ4620 (Clariant, Somerville, NJ, USA) positive photoresist was spin coated on the wafer to achieve a thickness of about 5 μm. To facilitate adhesion of the photoresist and drive off the residual solvent, the wafer was baked at 100°C for 2 min. The photoresist was then exposed to UV radiation through a photomask using a PLA-501F mask aligner (Canon, Tokyo, Japan). Following exposure, the photoresist was developed with AZ400K developer (Clariant, Somerville, NJ, USA), and then postbaked at 120°C for 2 min. After postbaking, the patterned wafer was ready for DRIE etching. After etching, the photoresist was removed with acetone. The resulting structure was a raised 3-D image of the channels.

To imprint the channels, the silicon template with protruding features was used to imprint the channel pattern into 2.0-mm thick PMMA substrates. The hot-imprinting and bonding processes were performed in a GC oven (GC-17A, Shimadzu, Japan). Before the imprinting, the PMMA surfaces were cleaned by soaking the plate in isopropanol (IPA) for 10 min, and rinsed thoroughly with deionized water in an ultrasonic bath. The blank PMMA plate and the silicon master were sandwiched between two glass plates (5 mm thick) by a home-made clamp device, and were placed into an oven at 125°C for 10 min. The glass transition temperature (T_g) of the PMMA used is 112°C. To make sure that the resulting channel dimensions are the exact mirror of the silicon template, a temperature of 125°C was chosen for imprinting the channels. This temperature is higher than the T_g of PMMA but lower than the temperature of bubble formation (above 145°C). Prior to bonding, three access holes (2 mm in diameter) were drilled through the cover plate by a drill operated at low rpm to preclude melting of the polymer material. The two PMMA pieces were bonded by application of appropriate pressure and heat (98°C) using the same clamp device. After 10 min, the clamp device was removed, and the bonded chip was annealed at 98°C for another 5 min. Following cooling to room temperature, the PMMA chip was ready for coupling the ESI sprayer.

2.3 Attachment of an external sprayer

The connection between the exit of the microchannel and the ESI sprayer was made by means of a wire-assisted epoxy-fixing method. A 10-cm tungsten wire (30 μm diameter, S.I.S., Ringoes, NJ, USA) was first cleaned with methanol, and then inserted throughout a 3 cm long fused-silica capillary (id = 50 μm, od = 375 μm, Polymicro Technologies, Tucson, AZ, USA). One end (about 1 cm) of the wire was inserted into the outlet of the PMMA channel. As shown in Fig. 1b, the 3-cm capillary was then fixed on the chip using fast-fixing epoxy resin. After the epoxy was cured, the wire was withdrawn from the device.

Next, a small weight (about 50 g) was attached to the capillary end of the microdevice using a binder clip, and the capillary was drawn to a taper using a propane–butane microtorch. To make a tip of 10 μm od, the tapered tip was etched in 48% aqueous hydrogen fluoride solution for ~3 min. Following etching, the capillary was washed with deionized water and dried by a nitrogen flow. To apply ESI voltage, conductive rubber was coated onto the tapered tip. After a small amount of conductive rubber was applied to the tapered tip, the tip was immediately blown by a strong nitrogen flow (70 psi) to smooth the coating surface. During the coating, a nitrogen gas was continuously flown through the channel to prevent clogging. Finally, the device was placed in a 50°C oven for 5 min to accelerate hardening of the conductive rubber.

2.4 Electroosmotic flow measurement

The EOF of the chip was measured by a modified current monitoring technique [36]. Briefly, the sprayer (inserted into a reservoir), the buffer reservoir and the channel were filled with the desired running buffer. The content of the inlet reservoir was then replaced with a buffer of reduced ionic

strength and current was monitored with a digital multimeter (PROVA, Taipei, Taiwan) with 0.01-μA resolution placed in series between the low-voltage reservoir and ground. The EOF velocity was determined according to the equation: $v_{eof} = L \cdot t^{-1}$, where L is the channel length and t is the time required for the lower-concentration buffer to fill the microchannel. The field intensity was 250 $V \cdot cm^{-1}$.

2.5 Experimental procedure

The reservoirs were filled with the sample solution or the running electrolyte, and the electrophoresis voltages were supplied by platinum electrodes immersed in the electrolyte from two high-voltage power supplies (CZE1000R & CZE2000, Spellman, Hauppauge, NY, USA). The ESI voltage was supplied by the linear IT (LTQ) mass spectrometer. Two different sets of voltages were applied to the various reservoirs during the injection and separation steps of chip operation. During injection (pinched method), the voltages to the four ends were as follows: reservoir B,+1.5 kV; reservoir S, +1.5 kV; ESI, +1.5 kV; reservoir SW, 0 V. The voltages during separation were as follows: reservoir B, +7.0 kV; reservoirs S and SW, +4.0 kV; ESI, +2.5 kV. All chip-CE-ESI-MS analyses were carried out using a computer controlled power supply and a relay arrangement through an in-house-written LabView program (National Instruments, TX).

The mass spectrometer used for this study is an LTQ equipped with a nano-ESI source (ThermoFinnigan, San Jose, CA, USA). The microchip device was mounted on the nanoelectrospray source, and fine positioning of the spray tip was achieved by using a XYZ stage. System control and data collection were done by Xcalibur software version 1.4 SR1. On-line chip CE/MS was performed in the positive-ion full-scan mode (m/z 200–1500). For all experiments, the maximum ion injection time was 50 ms, and one scan consists of one microscan.

3 Results and discussion

3.1 Device fabrication

The device which was constructed and studied is shown in Fig. 1. The channel on the PMMA microchip was fabricated by the hot-imprinting method. The layout of the chip is shown in Fig. 1a. The channel plate was closed by thermally bonding to a covering PMMA plate. In the bonding process, we have found that the annealing step described in Section 2.5 was indispensable for extending the life of the chip. Without the annealing step the bonded chip was often disrupted after 2–3 days due to stress release. The annealing step after the thermal bonding also makes the device capable of withstanding the high pressure caused by the high flow rate used during the washing of the channel [37, 38].The sheathless chip-CE/MS interface was constructed by attaching a 3-cm capillary to the exit of the separation channel. To address the alignment between the separation channel and the external sprayer and to avoid blockage during fixing of the capillary with epoxy resin (Fig. 2a), a wire-assisted method was proposed. The metal wire serves two major functions. First, the wire inserted into the channels provides a simple method for interchannel alignment. Second, during the fixing process, because the wire is in the channel, the epoxy resin cannot block the channel. Very often, after the bonding process, the dimension of the channel may be slightly reduced. Thus, to ensure that the metal wire could be inserted into the channel, a wire with a smaller diameter than the id of the channel was used. The id of the separation channel and the attached capillary is 50 μm and we have found that a wire with 30 μm od can be inserted into the channels after the bonding process. Figure 2b is an enlarged photograph of the epoxy-fixing junction, formed upon removal of the 30-μm alignment wire. With the help of a 30-μm metal wire, it was not necessary to drill a flat-bottomed hole at the edge of the channel outlet [24] or to accurately imprint a bigger channel to fit the outer diameter of the capillary [28].

To connect the ESI sprayer, we used epoxy resin to fix the sprayer to the chip and to fill the interstices at the junction. Epoxy is known to be unstable in organic solvent. However, it was found to be relatively stable under the condition we used (25% MeOH), because the shape of the channel did not change after 6 h operation. In order to keep the length of the channel with epoxy wall (epoxy channel) short as compared to the PMMA channel, the viscosity of the epoxy resin must be controlled so that the epoxy will have the fluidity to fill the interstices at the junction but not enough to reach the interstices between the wire and the PMMA channel. Another reason for keeping a short epoxy channel is that the wire can be readily withdrawn from the channel. As expected, the shorter the length of the metal wire adhered to the epoxy resin, the easier it is to withdraw the metal wire from the chip. The study of the fixing time of the capillary with the fast epoxy resin showed that a 3.5 min time lag after mixing provided the best result.

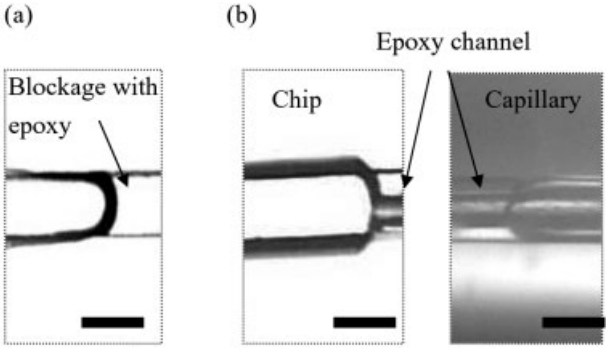

Figure 2. (a) Photograph of channel blockage without the use of wire for fabrication. (b) Photograph of epoxy junction fabricated by the wire-assisted method. The length of the 30-μm epoxy channel was less than 300 μm. Scale bar 50 μm.

A sheathless CE/MS interface was chosen for the microdevice. As shown in Fig. 1c, the capillary was tapered to 10 μm. It has been shown that the size of the sprayer determines the optimal flow rate of a sprayer [39]. For this microdevice, the flow rate generated by the EOF was measured to be about 65 nL/min. Based on the study of the flow rate *versus* response, for a 10-μm tip, optimal sensitivity was obtained at flow rates above 80 nL/min (Fig. 3a). Thus, to match the EOF of the microdevice (65 nL/min), the size of the orifice should be slightly smaller than 10 μm. However, our experience showed that a tip with an od smaller than 10 μm was easily broken or clogged. Considering the sensitivity and ease of fabrication and operation the sprayer, a tip with 10-μm od was chosen in this study. With a 10-μm sprayer, the sensitivity was about 90% of the maximum sensitivity (Fig. 3a). Another important issue which occurs when operating at a flow rate less than the optimal flow rate is spray stability. The spray stability of this device was studied by an infusion experiment. As shown in Fig. 3b, the signal was quite stable for a 10 min operation which is long enough for most of the chip CE experiments.

As described in Section 2.5, the capillary was tapered after connecting to the chip. This method was employed because of the difficulty of connecting a prefabricated 10 μm sprayer to the chip. To connect a 10-μm pretapered sprayer, the od of the wire would need to be about 10 μm. We have found that a 10-μm wire does not have the physical strength to be inserted into the channels. Furthermore, if a 10-μm wire is used, the channel around the interface would have a dimension of 10-μm. This dimension is much smaller than the 50 μm PMMA channel and may result in undesirable effects. Therefore, the 10-μm sprayer was fabricated after the attachment of a 3 cm fused-silica capillary to the CE chip.

After tapering the capillary, the ESI voltage was applied to the sprayer through a conductive layer coated on the tapered tip. In this device, conductive rubber was used instead of carbon [40] or silver [41] to coat the tip. The conductive materials in the rubber are mainly carbon and silver powder, and the resistance was less than 20 Ω. The major advantages of the conductive rubber over carbon or silver coating include longer lifetime and robustness [42]. These characteristics greatly reduce time, attention, and effort in the setup of a CE/MS interface.

3.2 Chemical interference

The PMMA channel, the epoxy resin, and the conductive rubber coating were all contacted with organic solvents (25% MeOH) during operation; therefore, interference from these materials was a concern. The extent of the interference was evaluated in an infusion experiment using a running buffer containing a 0.5 μM peptide standard as the test solution. The mass spectrum (Fig. 4a) was obtained from the direct infusion of the running buffer through a 30 cm tapered fused-silica capillary and without conductive coating. The spray voltage was applied to the inlet of the capillary, and the

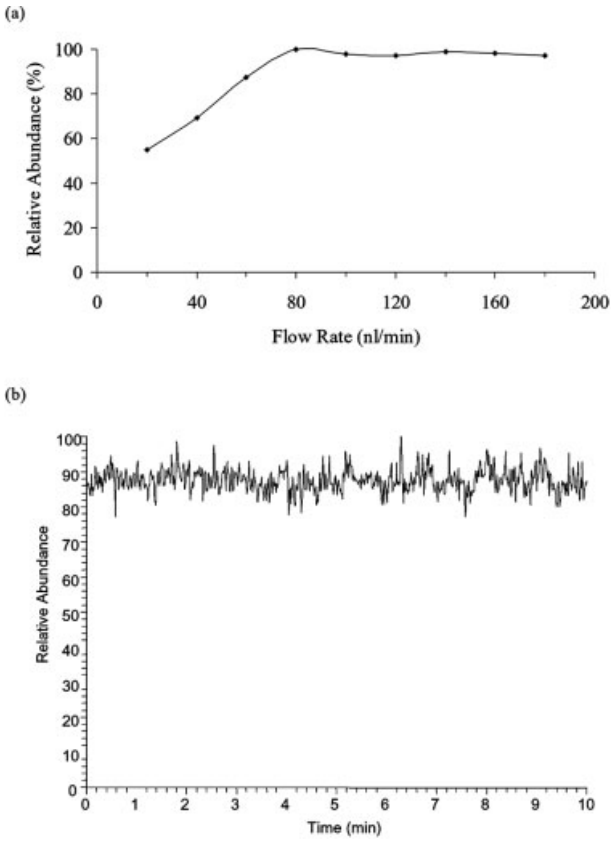

Figure 3. (a) Relative abundance *vs.* flow rate with a 10-μm electrospray tip. The solution was 0.5 μM of a peptide standard ([Leu^5]-enkephalin, *m/z* 556.3) in buffer solution, and was delivered by a syringe pump. (b) The stability of the microdevice with a 0.5 μM peptide standard ([Leu^5]-enkephalin, *m/z* 556.3). The solution was pumped by EOF, and the total ion electropherogram was acquired in the mass range of 200–2000.

solution was pumped by a syringe pump. With this setup, the solution did not flow through the PMMA channel, the epoxy or the conductive rubber. The mass spectrum in Fig. 4b was obtained using the same setup as in Fig. 4a but with conductive rubber coating. The *m/z* 578.3 is the sodium adduct ion of the reference standard, other ions at low mass are from the solvent. Because of the conductive coating on the sprayer, a spray voltage was applied to the rubber. The ions and intensity observed in Figs. 4a and b were basically the same. Thus, chemical interference from the conductive rubber coating was not a concern. Interference from epoxy has been reported [43]. Based on our experience, we can say that the major source of the chemical interferences was indeed due to the epoxy resin, and the interferences vary according to the epoxy resin used. Without washing the device, the intensity of the chemical interference can be about one order of magnitude higher than the peptide standard observed in Figs. 4a and b. Thus, before the analysis, washing the channel with running buffer for 30 min was essential. After the washing procedure, background ions (Fig. 4c)

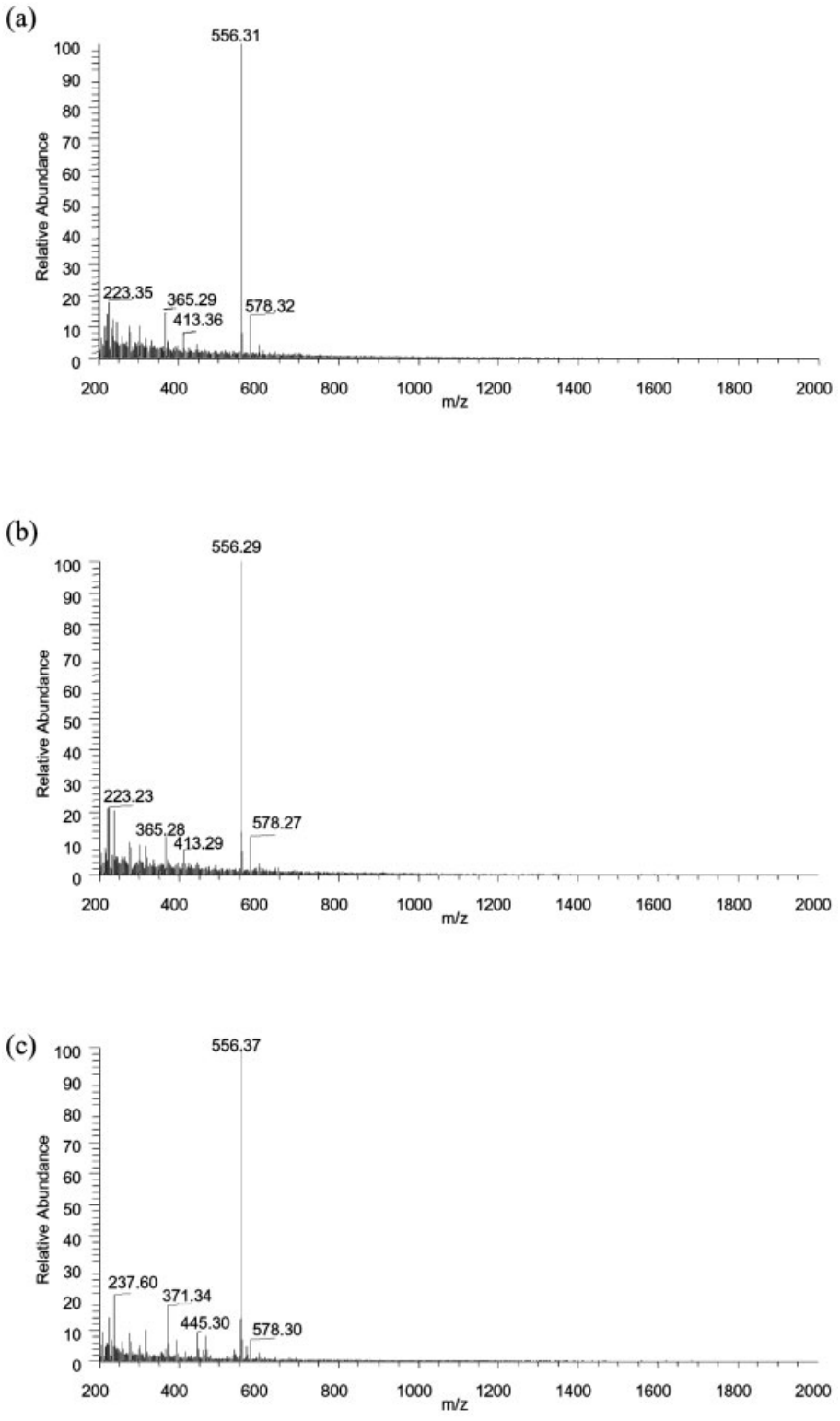

Figure 4. Mass spectra of chemical interference under different conditions. (a) Infusion through a tapered fused-silica capillary without conductive coating. (b) Infusion through a tapered fused-silica capillary with conductive rubber coating. (c) Infusion through a PMMA chip-CE-ESI-MS device. The solution was a 0.5 μM peptide standard ([Leu^5]-enkephalin) in 25% MeOH, 1% HOAc.

different from Figs. 4a and b were still observed; however, the interference was not considered serious because the intensity of the background ions had similar intensity as in Figs. 4a and b. Moreover, with the buffer used (25% methanol), the epoxy is quite stable. The intensity of the interference was not significantly increased after 6 h operation.

3.3 Chip-CE-ESI-MS analysis

In chip CE experiments, it was observed that some peptides showed poor sensitivity if the loading time was less than 5 s. This is more likely due to the different electrophoretic mobility of the peptides. To ensure that all the peptides could reach the intersection, a 20 s loading time was used. Replicate injections of a 20 μM peptide standard were used to evaluate the reproducibility of the chip-CE/MS system. As shown in Fig. 5, for three consecutive injections, the RSDs of the peak height, peak area and migration time were 3.0, 1.3 and 2.5%, respectively.

The device was evaluated for the analysis of an eight-peptide standard mixture. To obtain better separation efficiency and sensitivity, the sample was prepared in a ten-fold dilution of the separation buffer to achieve a stacking effect. The extracted ion electropherograms corresponding to eight peptide standards are shown in Fig. 6. The separation efficiency was calculated to be 495–1755 plates. Dead volume, and the hydrophobic interaction between peptides and PMMA surface are the two more likely causes for the relatively poor separation efficiency. In terms of dead volume, the areas that may have a dead volume are the junctions between the separation channel and the external sprayer.

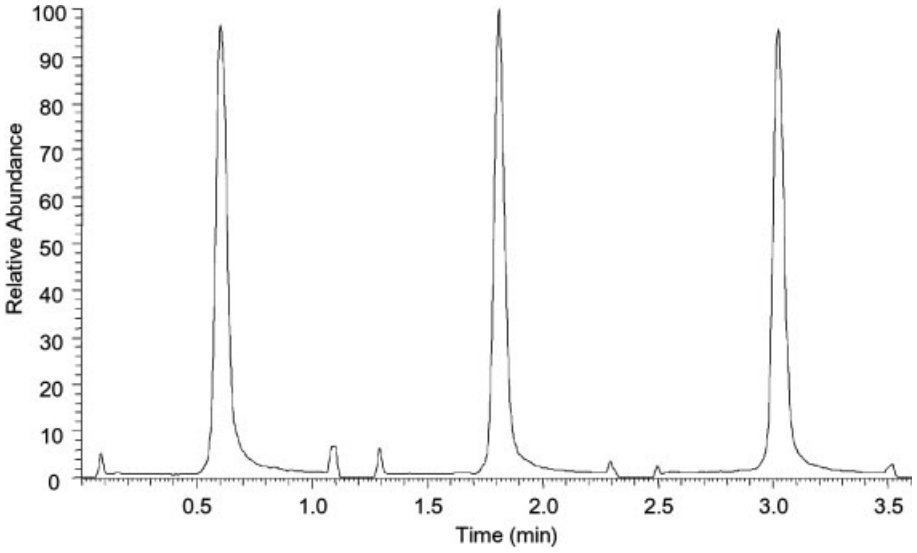

Figure 5. Three consecutive injections of a 20 μM peptide standard ([Leu^5]-enkephalin, *m/z* 556.6). All injections were made with the pinched-injection method. BGE, 1% v/v acetic acid in 25% v/v methanol/water.

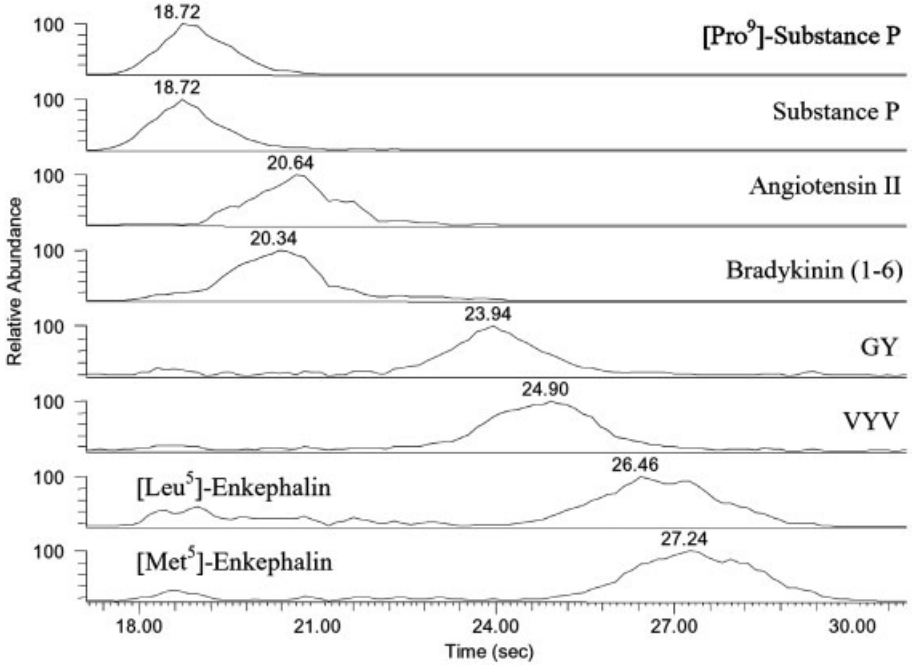

Figure 6. Chip-CE-ESI-MS analysis of a mixture of eight peptides. The electropherograms were acquired under full-scan mode. The extracted ion electropherograms: [Pro^9]-Substance P (*m/z* 694.8), Substance P (*m/z* 674.8), angiotensin II (*m/z* 524.3), bradykinin(1–6) (*m/z* 660.7), GY (*m/z* 239.2), VYV (*m/z* 380.5), [Leu^5]-enkephalin (*m/z* 556.6), and [Met^5]-enkephalin (*m/z* 574.7). Conditions: The injection was performed with pinched-injection method. BGE, 1% v/v acetic acid in 25% v/v methanol/water. Injection time: 20 s.

However, the picture of the interface (Fig. 2b) does not indicate that there is a dead volume in the intersection of connecting channel/separation channel and connecting channel/sprayer. Absorption is another potential cause of the poor separation efficiency. Absorption (hydrophobic interaction) has been proposed as the major reason for the poor separation efficiency in the analysis of peptides using a non-modified PMMA chip. Dramatic improvement was observed [44] after the surface was dynamically coated with quaternary ammonium starch derivatives. Another possible cause for the poor separation efficiency is that the connecting channel is narrower than the separation channel and the sprayer, therefore, there is a possibility that separation efficiency may be degraded. A similar phenomena has been investigated in a paper published by Trust *et al.* [45], and the result showed that a narrower channel in a separation channel did not significantly affect the separation efficiency. On the basis of the above information, absorption was postulated as the major cause for the poor separation efficiency. Although the peptides were not completely resolved, the comigrating components could be resolved by their difference in mass. The LOD (rms) was found to be 100 nM which is significantly inferior to the result (8.20 nM) reported by Li *et al.* [46]. Proper wall coating (glass-coated with BCQ) and larger injection volume (0.5 nL *vs.* 0.125 nL) may explain part of the difference.

4 Concluding remarks

We have developed a chip-CE-MS device using a wire-assisted epoxy-fixing method in the connection of an external ESI sprayer. Except for the imprinting master, this is an inexpensive chip-CE-MS method. More importantly, this method provides a simple way to attach an external ESI sprayer to a CE chip without the need for complicated fabrication. The wire-assisted method can easily achieve interchannel alignment between the PMMA chip and an external capillary tip. The method also provides a low dead volume connection and avoids the risk of channel blockage by the epoxy. The reproducibility and the performance have been evaluated by the analysis of peptide mixtures. These results suggest that this device could have the potential for protein identification by chip-CE-ESI-MS.

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