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Effects of the length and modification of the separation channel on microchip electrophoresis–mass spectrometry for analysis of bioactive compounds

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Abstract

Analyses of amino acids and peptides were performed using a quartz microchip and an interface for microchip electrophoresis–electrospray ionization mass spectrometry (MCE–ESI–MS). In MCE–ESI–MS, negative pressure caused by ESI increased band broadening and deteriorated separation. We tried to suppress the negative pressure and improve separation using a microchip with a long separation channel. Separations of peptide standards were compared using two microchips with long separation channel (58.9 mm) and short one (22.9 mm). Theoretical plate numbers and resolution were improved significantly using the former. The theoretical plate numbers of [Val4]angiotensin was 8600 using the former and 1700 using the latter. When background electrolytes of low pH were used in an uncoated quartz microchip, electrokinetic injection was difficult because of weak electroosmotic flow. The use of successive multiple ionic polymer layers coating of the microchip channel stabilized electrokinetic injection and permitted analysis of amino acids and peptides even under low pH conditions. Separation of amino acids was successfully performed using formic acid solution (pH 2.5) as background electrolyte.

Keywords: Chip technology; Multiple ionic polymer layers coating; Instrumentation; Amino acids; Peptides

1. Introduction

Microchip electrophoresis is an effective separation technique for micro total analytical system (μ-TAS) due to the small amount of sample required and rapid separation. Recently, there have been remarkable developments in mass spectrometry (MS) as a detection tool for high performance liquid chromatography and capillary electrophoresis. Its advantages include high sensitivity, no needs for the derivatization of analytes and structural information capability. The use of MS as a detection method of microchip electrophoresis (MCE–MS) has also been explored using electrospray ionization (ESI) interface [1–9]. The analytical capability of MS and MS–MS can be significantly enhanced by separating and concentrating the components, in particular for complex mixtures such as biomedical samples, prior to introduction to the mass spectrometer. Thus, MCE–MS enables high-throughput analyses and is expected to find wide application in genomics, proteomics, metabolomics and drug development.

MCE–MS has been developed by Karger and co-workers' [10,12–15], Ramsey and co-workers' [11,16,17] and Harrison and co-workers' [18–22], mainly for the analysis of proteins and peptides. The interface used employs generally a tapered fused silica capillary as spray nozzle. ESI voltage is applied through a liquid junction or a conductive coating of the spray nozzle. In construction of the MCE–MS system, several factors must be taken into account such as channel design, channel surface modification, sample injection method, spray device to obtain stable spray during separation as discussed below. Karger and co-workers [12–15] prepared a subatmospheric chamber between the outlet of the spray nozzle and the MS orifice to maintain stable spray. Channel surface was coated with polyacrylamide or polyvinyl alcohol to prevent adsorption of analytes.

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Although electroosmotic flow (EOF) was weak, flow was stabilized by adjusting the pressure inside the subatmospheric chamber. Moreover, a micro well plate was attached and an automated system was developed [15]. In this system, they separated the microchip and the reservoir section and simplified the manufacturing process of a microchip. Harrison and co-workers [18,19,21] stabilized the flow by adding an auxiliary solution with a syringe pump from a side channel. Channel surface was coated with [(acryloylamino)propyl]trimethylammonium chloride. Since the surface has positive charge, voltage was applied so that the spray nozzle end has higher potential than that at the inlet. They attached a gold coated tapered fused silica capillary to a microchip. The limit of detection of 2.5 nM was obtained using solid phase extraction and sub-nM was obtained using stacking for peptides standards [19]. Analyses of trace-level tryptic digests for identification of proteins was also performed using a system attached to an auto sampler [21].

Electrokinetic injection and pressure injection are main sample injection modes in capillary electrophoresis and MCE. For MCE, electrokinetic injection is mainly used for simplicity. However, it has the disadvantage that sampling bias occurs according to the difference in electrophoretic mobilities among analytes. Karger's and Harrison's groups both used the double T channel microchip and electrokinetic injection mode. Zhang et al. [13] injected peptides by pressure using a double T channel. In separation of angiotensin peptides, the results were not significantly different from those obtained from electrokinetic injection.

The effects of channel length were investigated using two microchips of separation channel lengths 4.5 and 11.0 cm [13] and it was concluded that quick separation is possible with a short channel, but a long one is required for high separation efficiency.

We have reported a robust and simple structure interface for MCE–ESI–MS [23]. ESI voltage was applied through a liquid junction and a tapered spray nozzle was employed. We used a quartz microchip with simple cross channel. Analytes were injected electrokinetically by gated injection. Gated injection has some advantages, which include easy regulation of the amount of analytes injected, and simplicity of equipment and channel design compared to pressure injection. In our previous work, we have reported that theoretical plate number and separation efficiency deteriorated under the influence of the negative pressure caused by electro spray. Moreover, when EOF or electrophoretic mobility of analytes was weak, electrokinetic injection was difficult, and hence the pH range of background electrolyte (BGE) was limited. In this work, improvement of separation was carried out by suppressing the influence of the negative pressure caused by ESI using a microchip with a separation channel of 2.6 times as long as the previous one [23]. Moreover, successive multiple ionic polymer layers (SMIL) coating of a quartz microchip was performed to stabilize EOF under low pH conditions. SMIL coating developed by Katayama et al. [24–28] is semi-permanent for fused silica capillary. Anionic polyelectrolyte dextran sulfate (DS) and cationic polyelectrolyte polybrene (PB) were passed into a capillary alternately, and multiple polyelectrolyte layer was formed on the capillary surface. When the outer layer was DS, the channel surface was negatively charged [26,27], whereas when the outer layer was PB, positively charged [28]. In the former, stable cathodic EOF was obtained in the range of pH 2–13 by the sulfonyl group of DS. Liu et al. used SMIL coating for modifying the inner surface of a polydimethylsiloxane (PDMS) microchip [29]. With SMIL coating, removal and recoating can be accomplished easily. Moreover, it has excellent chemical stability and durability. These are definite advantages for repeated use of microchips that have specially designed structures.

2. Experimental

2.1. Apparatus

The outline of equipment is shown in Fig. 1A. The mass spectrometer was an LCMS-2010 quadrupole mass-spectrometer (Shimadzu, Kyoto, Japan). The probe used for usual LC–MS was removed, and an *x–y–z* translation stage for microchips was installed. Mass detection was performed in the selected ion monitoring (SIM) mode (sampling rate 0.1–0.2 s).

Two quartz microchips with simple cross channels (Shimadzu, Kyoto, Japan) were employed (Fig. 1B). One was a short chip of 33.5 mm (length) × 12.5 mm (width) × 6.05 mm (thickness) and the other was a long chip 68.5 mm (length). The channel width was 50 μm and depth 20 μm. Separation channel length was 22.9 mm or 58.9 mm, respectively. A guide hole of 370 μm i.d. was prepared at the end of the separation channel, and the spray nozzle was inserted into it. Although the shapes of the guide hole were slightly different, the difference between the two chips did not cause significant differences in the performance of the system. The bottom of the guide hole was fabricated in flat plane to minimize the dead volume. The interface and reservoir sections had block structures made of polychlorotrifluoroethylene resin and the blocks were independent from the quartz microchip, thus making easy to replace the interface block with another type as may be necessary. The spray nozzle was a tapered fused silica capillary, 360 μm o.d., 20 μm i.d., and 10 μm i.d. at the tip (PicoTip FS360-20-10-N, New Objective, Cambridge, MA, USA), cut to a length of 25 mm. It was attached to the bottom of the guide hole and fixed using a polyether ether ketone (PEEK) screw (Shimadzu, Kyoto, Japan) and a PEEK tube 380 μm i.d. (F-185 Micro SLV, Upchurch Scientific, Washington, DC, USA) without adhesive. The spray nozzle was also easily replaceable with a new one. The volumes of reservoirs were about 30 μl. The microchip was placed on the *x–y–z* translation stage. A platinum electrode was connected to each reservoir to apply the voltage. ESI voltage (3.0 kV) was applied through the liquid

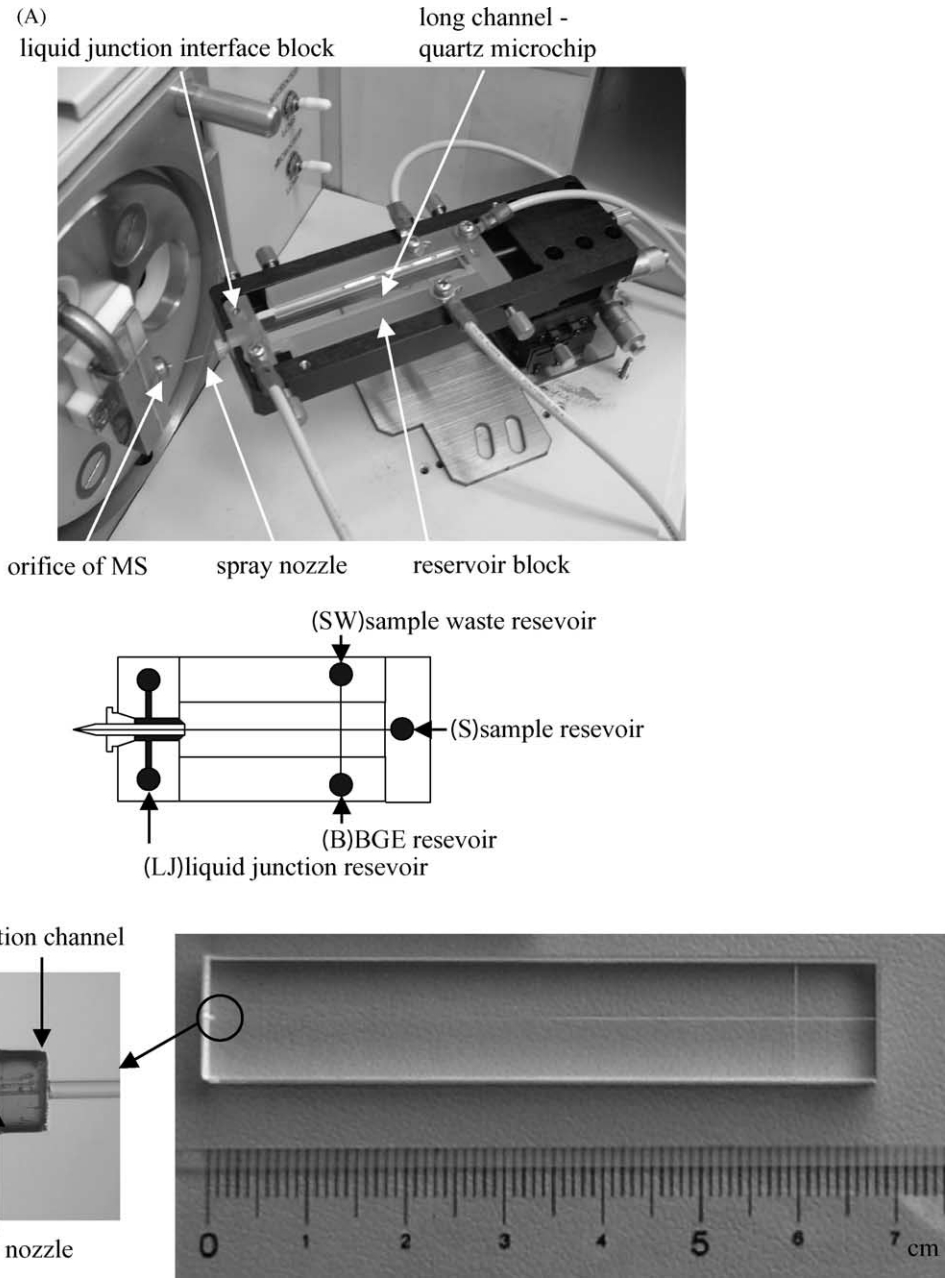

Fig. 1. The outline of equipment. (A) Photograph and schematic diagram of the microdevice used in this study. Quartz microchip, ESI–MS interface and reservoir blocks were attached to the *x*–*y*–*z* translation stage. (B) Photograph of the quartz microchip (long chip) and magnified view of the end of the separation channel connected to the spray nozzle used in this study.

junction reservoir. The solution at the liquid junction is the same as BGE, and pressure was not applied. The distance between the spray nozzle and the MS orifice was set to about 5 mm. The power supply for electrophoresis constructed by Shimadzu was computer-controlled with LabView software (National Instruments, Austin, TX, USA).

A laser-induced fluorescence (LIF) detection system was constructed in our laboratory [30]. Argon ion laser (488 nm) was used as a light source. The detection wavelength was 600 nm, and the detection point was set 0.5 mm from the end of the separation channel.

2.2. Reagent

Pindolol and sodium dextran sulfate (M_w = 500,000) were purchased from Wako (Tokyo, Japan); sulpiride was from Research Biochemical (Natic, MA, USA); Rhodamine B was from Nacalai Tesque (Kyoto, Japan); sulfoRhodamine B was from Tokyo Kasei (Tokyo, Japan); L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin, amino acids, peptides, proteins, nicardipine, trimipramine maleate salt and polybrene were from Sigma (St. Louis, MO, USA). Water was purified with a Milli-Q Labo system

Table 1
Applied voltages for each reservoirs

Microchip	Detection	Reservoir (Fig. 1A)			
		S	B	SW	LJ
Short chip	LIF	1260 (1260)	1420 (930)	420 (900)	0 (0)
	MS	4260 (4260)	4410 (3930)	3410 (3900)	3000 (3000)
Long chip	LIF	2700 (2700)	2860 (2370)	1860 (2340)	0 (0)
	MS	5700 (5700)	5860 (5370)	4860 (5340)	3000 (3000)

Applied voltage: separation (injection) (V).

(Nihon Millipore, Tokyo, Japan). All other reagents were of analytical or HPLC grade. Isoelectric points of peptides were calculated with the site of FUJITSU Kyushu system engineering (http://www.fqs.co.jp/).

2.3. Procedure

2.3.1. Electrophoresis condition

Ammonium carbonate–ammonium hydrogencarbonate buffer (50 mM, pH 8.7), 50 mM acetic acid–ammonium acetate buffer (pH 5.7), 200 mM acetic acid–ammonium acetate buffer (pH 4.0), 50 mM formic acid (pH 2.5) were used as BGEs. Acetonitrile was added to the BGE if necessary. The microchip channel was rinsed before use with 0.1 M NaOH for 30 min, water for 5 min, and BGE for 15 min by applying pressure at the reservoir with a syringe. After every three to five runs, it was washed with the separation buffer following the same procedure. The applied voltages are shown in Table 1. The electric field strength was 400 V/cm in the separation channel. Sample injection was performed by gated injection [31].

2.3.2. Sample preparation

Rhodamine B and sulfoRhodamine B were dissolved at 1 mM in water. Stock solutions of basic drugs were prepared at 1000 mg/l concentration each in methanol. Stock solutions of peptides were prepared at 1000 mg/l concentration in water. They were diluted to the required concentrations with the BGE prior to use. Stock solutions of amino acids were prepared at 1000 mg/l concentration in water except Tyr, which was dissolved at 500 mg/l in 200 mM formic acid. They were diluted to the required concentrations with 200 mM formic acid prior to use.

2.3.3. Protein digestion with TPCK-trypsin

Ammonium carbonate (50 mM) adjusted to pH 8.2 by the addition of 50 mM ammonium hydrogencarbonate was used as the solvent. TPCK-treated trypsin was added to 1000 mg/l cytochrome *c* solution at the enzyme–substrate ratio of 1:50 (w/w), and the solution was incubated at 37 °C for 15 h. The reaction mixture was then stored in a refrigerator, and diluted two-fold with the separation buffer prior to use.

2.4. SMIL coating

SMIL coating was performed based on the method developed by Katayama et al. [26]. Briefly, to activate the silanol groups, the microchip channel was rinsed with 1 M NaOH for 30 min and water for 30 min by applying pressure with a syringe. The first layer coating was applied by rinsing the channel with 5% aqueous PB solution for 15 min and standing for 15 min, after which the channel was flushed with water to remove the excess PB. The second layer coating was applied by rinsing the channel with 3% aqueous DS solution for 15 min and standing for 30 min. The channel was rinsed with water and BGE before use.

3. Results and discussion

The influence of the MS interface on peak shape and separation efficiency was studied with two microchips having different separation channel lengths. Rhodamine B and sulfoRhodamine B were used as test analytes. Samples were injected for 0.2 s and detected with LIF or MS. The results are shown in Fig. 2 and Table 2. In the case of LIF detection, the detection point was set 0.5 mm from the end

Table 2
Comparison of peak parameters of fluorescent analytes Rhodamine B and sulfoRhodamine B detected by LIF and MS

Channel length (mm)	Detection	Rhodamine B			sulfoRhodamine B			R_s
		Migration time (s)	Linear velocity ($cm\,s^{-1}$)	Plate no.	Migration time (s)	Linear velocity ($cm\,s^{-1}$)	Plate no.	
22.9	LIF	15.8	0.142	8200	25.9	0.087	13100	13
	MS	24.4	0.197	2200	40.4	0.119	3200	6.5
58.9	LIF	35.8	0.163	31800	53.2	0.110	34700	18
	MS	44.5	0.189	13600	63.3	0.133	21300	12

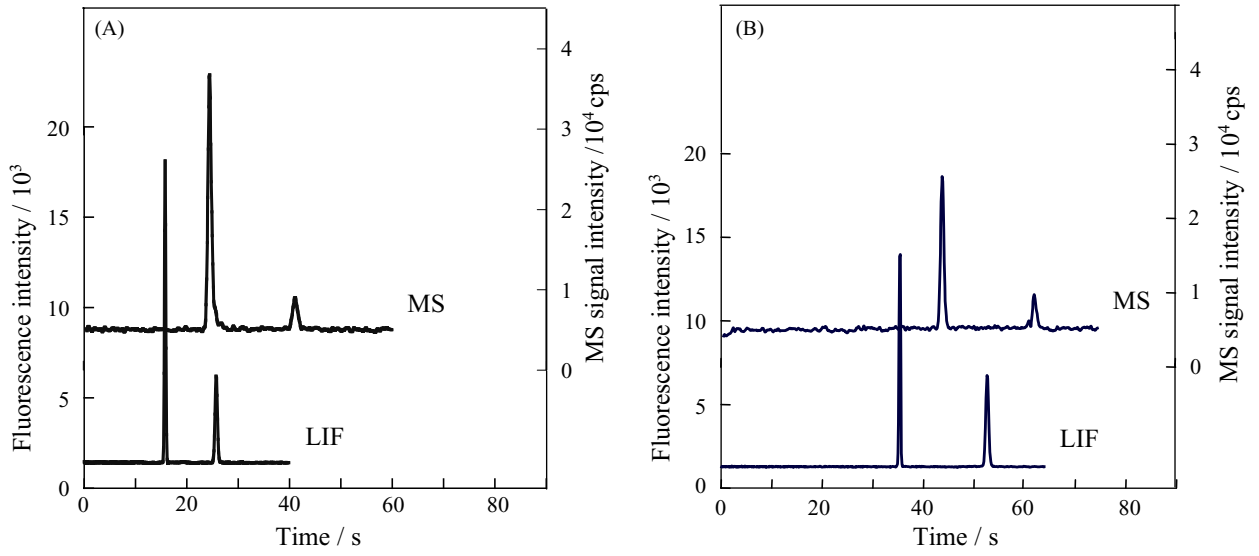

Fig. 2. Separation of fluorescent analytes Rhodamine B and sulfoRhodamine B. (A) Short chip and (B) long chip. Separation buffer, 50 mM ammonium carbonate–ammonium hydrogencarbonate buffer (pH 8.7) containing 30% (v/v) acetonitrile; sample concentration, 10 μM; injection time, 0.2 s; electric field strength, 400 V/cm; ESI voltage, 3.0 kV; MS detection mode, SIM.

of the separation channel. The effective channel length was 22.4 mm for the short chip and 58.4 mm for the long one. In MS detection, a 25 mm-long spray nozzle was employed and the whole channel length was 47.9 mm for the short chip and 83.9 mm for the long one.

In MS detection, the linear velocity was higher compared with LIF detection became of the negative pressure caused by ESI. Hence, peaks were broader and theoretical plate numbers were lower. The enhanced velocity was suppressed and theoretical plate number increased using the long chip compared with the short chip. Theoretical plate number, N, is expressed with Eq. (1).

$$N = \frac{L^2}{\sigma^2} = \frac{L(\mu_{eo} + \mu_{ep})E}{2D} \quad (1)$$

where σ is peak variance, E the electric field strength, L the separation channel length, D the diffusion coefficient of the sample molecule, μ_{eo} the EOF mobility and μ_{ep} the electrophoretic mobility of the sample molecule. Theoretical plate number is proportional to the separation channel length when electric field strength is constant. Since the separation channel length of the long chip is 2.6 times longer than that of the short chip, the theoretical plate number is predicted to increase 2.6 times. In LIF detection, the theoretical plate number was 3.9 times for Rhodamine B and 2.6 times for sulfoRhodamine B using the long chip compared to the short one. In MS detection, it was 6.2 times for Rhodamine B and 6.7 times for sulfoRhodamine B.

$$R_s = \frac{1}{4}\left[\frac{L(\mu_{eo} + \bar{\mu}_{ep})E}{2D}\right]^{1/2} \frac{|\mu_{ep1} - \mu_{ep2}|}{\mu_{eo} + \bar{\mu}_{ep}} \quad (2)$$

Resolution, R_s, is expressed with Eq. (2), where μ_{ep1}, μ_{ep2} are electrophoretic mobilities of the sample components, and $\bar{\mu}_{ep}$ is the average of electrophoretic mobilities of two sample components. Resolution is proportional to the square root of separation channel length when electric field strength is constant. With the long chip, resolution is predicted to be about 1.6 times that of a short channel chip. Resolution of Rhodamine B and sulfoRhodamine B improved 1.6 times in LIF detection and 1.8 times in MS detection. The peak parameters are shown in Table 3.

$$\sigma^2_{MS} = \sigma^2_{LIF} + \sigma^2_{int} \quad (3)$$

Peak variance in MS detection, σ^2_{MS}, can be described as the sum of variances due to the interface, σ^2_{int}, and the total peak variance in LIF detection, σ^2_{LIF}, as shown in Eq. (3). The ratio $\sigma^2_{int}/\sigma^2_{MS}$ was about 0.9 for the short chip and about 0.6–0.7 for the long chip. In a microchip channel, a plug flow was generated by the EOF. When the MS interface was attached, the flow in the spray nozzle was generated by EOF in the microchip channel and the negative pressure caused by ESI. Hence, the flow profile was modified to laminar flow and theoretical plate number was significantly reduced. In the long chip, the influence of the negative pressure caused by ESI was lower compared to the short chip. Although the

Table 3
Comparison of peak variance of Rhodamine B and sulfoRhodamine B

Channel length (mm)	Analyte	σ^2_{MS} (s^2)	σ^2_{LIF} (s^2)	σ^2_{int} (s^2)	$\sigma^2_{int}/\sigma^2_{MS}$
22.9	Rhodamine B	0.269	0.030	0.239	0.89
58.9		0.145	0.040	0.105	0.72
22.9	SulfoRhodamine B	0.510	0.051	0.459	0.90
58.9		0.190	0.082	0.108	0.57

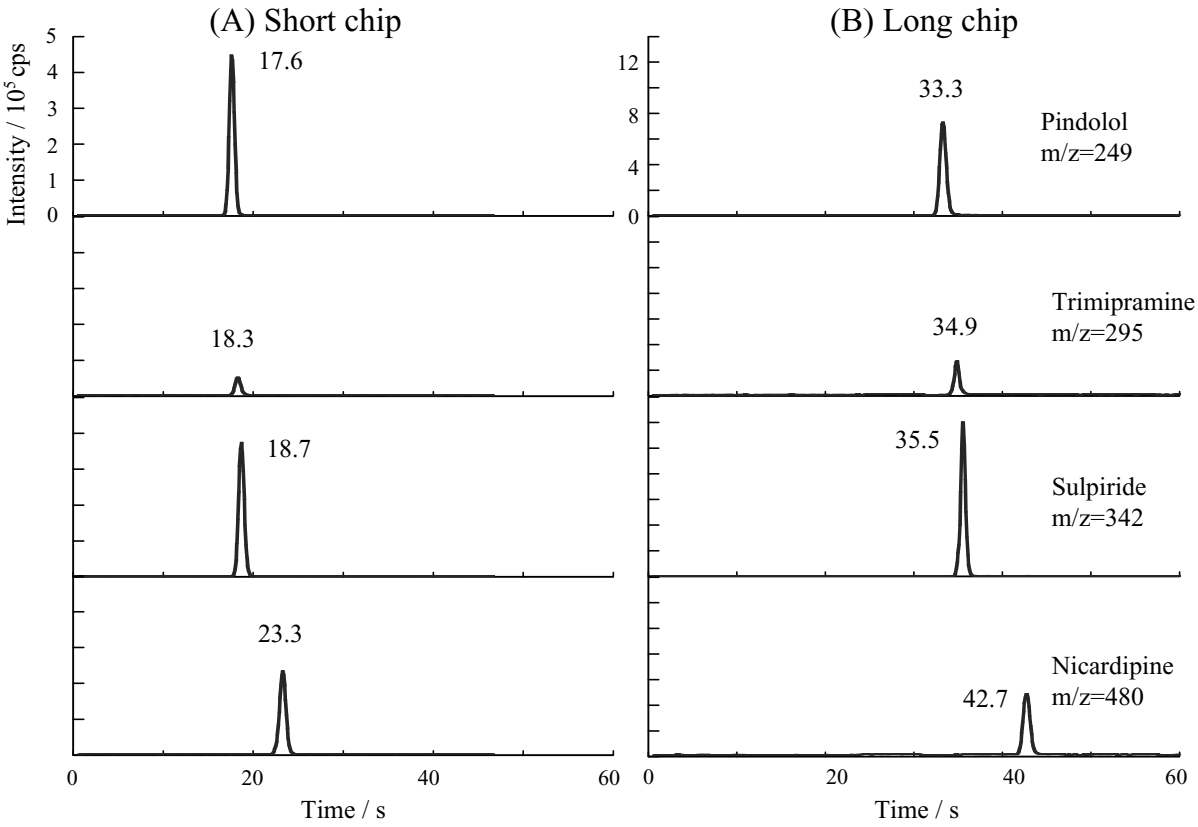

Fig. 3. Separation of basic drugs. (A) Short chip and (B) long chip. Sample concentration, 100 mg/ml. The migration time of each peak is given in its respective selected ion electropherogram (SIE). Other conditions are the same as Fig. 2.

negative pressure affected the flow profile of the microchip channel, the adverse influence was reduced relatively and the theoretical plate number and the separation were improved using the long chip. The length of the injection plug is considered to contribute to the peak variance almost equally in LIF detection and MS detection, although the injection plug length may be a little bit longer in the MS detection than in LIF detection. It is reasonable to assume that the relative contribution of the injection plug length to the total peak variance is much less in the MS detection to compare the difference between σ^2_{LIF} and σ^2_{MS}. Many other complex factors, such as the differences in microchip channel shape from that of the spray nozzle, and the entry of liquid to the spray nozzle through the liquid junction, can cause an increase in peak variance. It is difficult, however, to estimate the contribution of each factor with some degree of accuracy.

Fig. 3 shows MCE–MS of four basic drugs, pindolol, trimipramine, sulpiride and nicardipine using 50 mM carbonate buffer (pH 8.7) containing 30% (w/w) acetonitrile as BGE using the short and long chips. Table 4 shows separation parameters. Whereas 900 and 1000 plate numbers were obtained for pindolol and sulpiride, respectively, using the short chip, 2500 and 3600 were obtained using the long chip, and the separation was improved.

Table 4
Peak parameters of pindolol and sulpiride

Channel length (mm)	Analyte	Migration time (s)	Peak width[a] (s)	Plate no.	R_s[b]
22.9	Pindolol	17.6	2.33	900	0.49
	Sulpiride	18.8	2.32	1000	
58.9	Pindolol	32.6	2.60	2500	0.85
	Sulpiride	34.6	2.30	3600	

[a] Peak width at baseline.
[b] Resolution between pindolol and sulpiride.

Table 5
The list of peptides consisting of 7–10 amino acid residues

Sample	Sequence	*M*	*m/z*	Isoelectric point (p*I*)
Bradykinin	RPPGFSPFR	1060.2	531.0	12.48
[Sar1,Ala8]angiotensin II	Sar-RVYIHPA	926.1	464.0	11.04[a]
[Val4]angiotensin III	RVYVHPF	917.1	459.0	11.04
Angiotensin I	DRVIYHPFHL	1296.5	649.0	7.95
Angiotensin II	DRVIYHPF	1046.2	524.0	7.80

[a] Calculated p*I* as the sequence of GRYYIHPA.

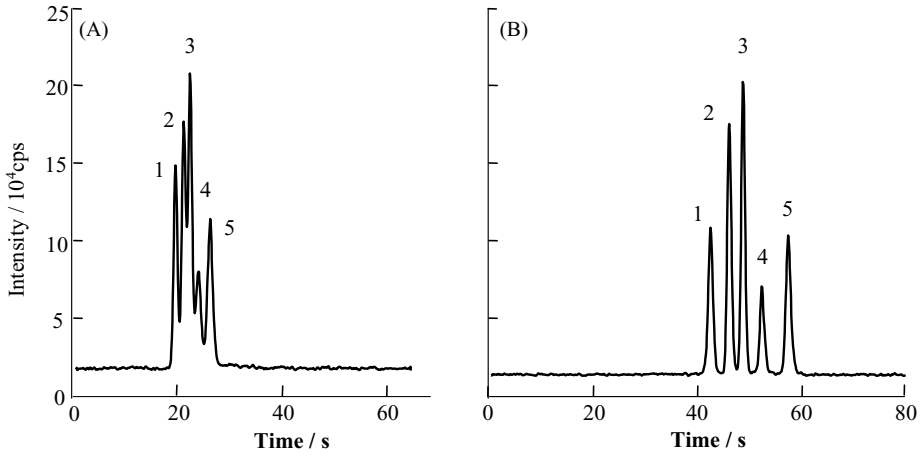

Fig. 4. Separation of peptides consisting of 7–10 amino acid residues, bradykinin, angiotensin I, angiotensin II, [Sar1,Ala8]angiotensin II and [Val4]angiotensin III ($\sum$ five ions). (A) Short chip and (B) long chip. Separation buffer, 50 mM acetic acid–ammonium acetate buffer (pH 5.7) containing 50% (v/v) acetonitrile. Other conditions are the same as Fig. 3.

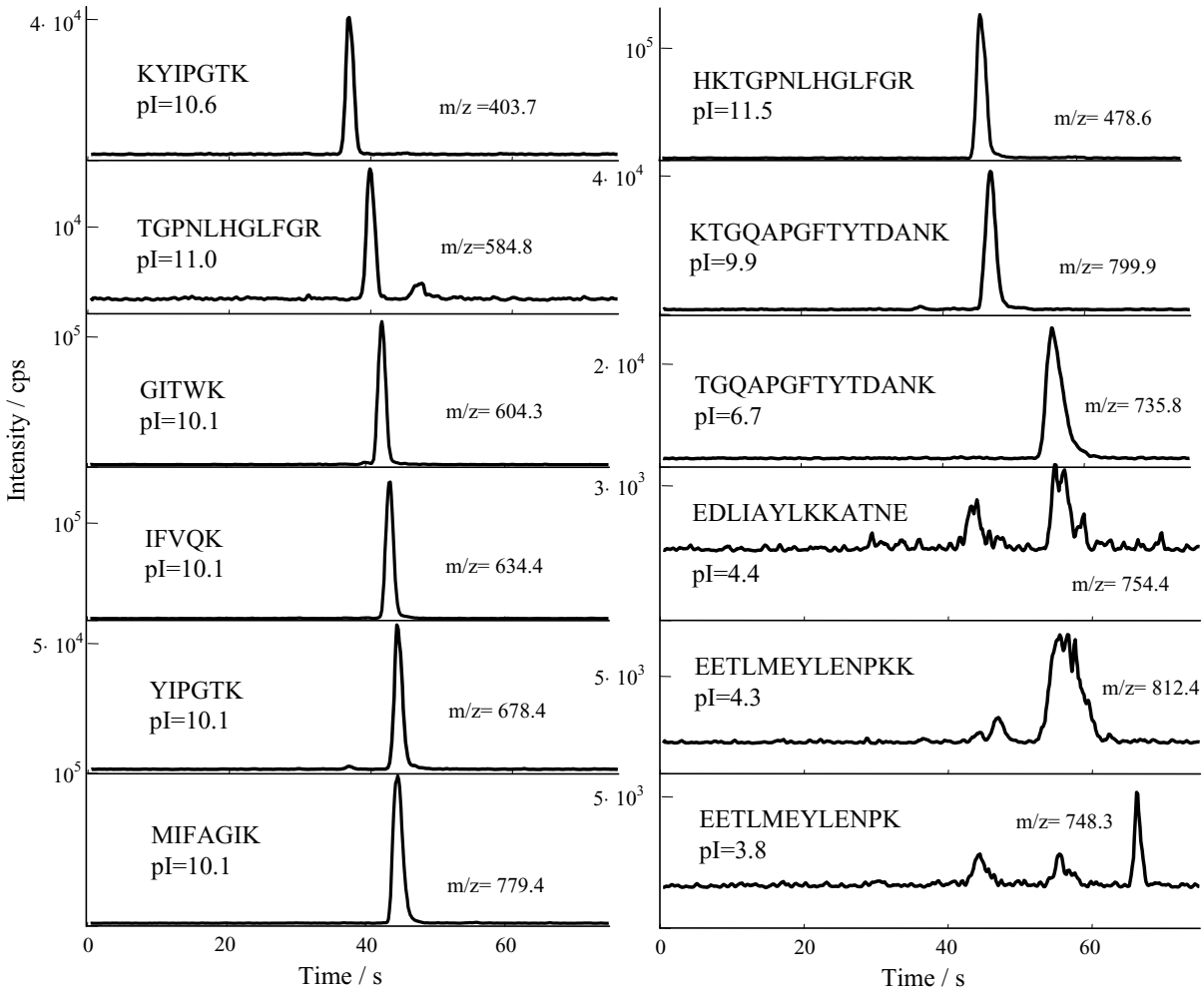

Fig. 5. Separation of TPCK-treated trypsin digests of cytochrome *c* with a long chip. Separation buffer, 200 mM acetic acid–ammonium acetate buffer (pH 4.0); injection time, 1.0 s. Other conditions are the same as Fig. 2.

Fig. 4 shows the separation of peptides consisting of 7–10 amino acid residues (Table 5), bradykinin, angiotensin I, angiotensin II, [Sar1,Ala8]angiotensin II and [Val4]angiotensin III using 50 mM acetate buffer (pH 5.7) containing 50% (w/w) acetonitrile as BGE. Detection was performed in the SIM mode. Unlike in the short chip, complete separation was obtained using the long chip. The theoretical plate number of [Val4]angiotensin III was 1700 with the short chip and 8600 with the long chip.

Fig. 5 shows the separation of cytochrome *c* TPCK-trypsin digest with the long chip using 200 mM acetate buffer (pH 4.0) as BGE. Detection was performed in the SIM mode. Peak broadening due to adsorption of sample molecules to the channel wall was suppressed using high concentration BGE.

In gated electrokinetic injection, the injected amount depends on EOF and the electrophoretic mobility of sample molecules. Under low pH conditions, EOF is reduced and injection of analytes becomes difficult. Improvement of sample injection was studied by enhancing the EOF under low pH condition using SMIL coating of the channel wall. Fig. 6 shows the separation of fluorescent analytes Rhodamine B and sulfoRhodamine B using the short chip and LIF detection. Rhodamine B is cationic under acidic condition while sulfoRhodamine B is anionic. Sample injection became difficult under low pH conditions using the uncoated quartz microchip. The anionic sulfoRhodamine B was hardly detected at pH 4.0, and neither of the samples could be detected at pH 2.8. On the other hand, the anionic sulfoRhodamine B could be detected even at pH 2.8 using the SMIL-coated microchip.

Katayama et al. [26] reported that significant changes in EOF does not occur after more than 200 injections using SMIL-coated fused silica capillary and phosphate buffer (pH 3.0) as BGE. Liu et al. [29] obtained the same result using the SMIL coating PDMS microchip. In this work, durability of SMIL coating was tested using Rhodamine B as analyte by LIF detection. Formate buffer (50 mM, pH 2.8) containing 30% acetonitrile was used as BGE. The migration time of Rhodamine B did not vary significantly after 100 injections. The performance was maintained by rinsing with 3% DS aqueous solution before use. When it was not used for several days, the coating was removed by immersing the chip in 1 M HCl for 15 h and sonicating for 5 min. Recoating was performed by applying the same procedure given earlier.

Analysis of amino acids is important in many fields, such as diagnosis, biochemistry, and food analysis. Unlike in UV

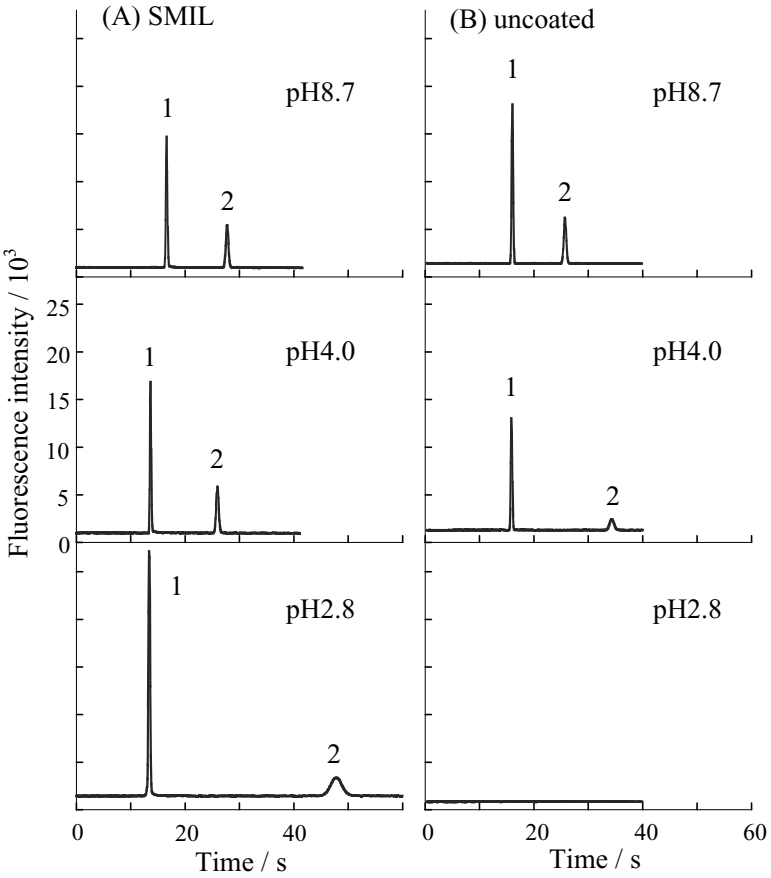

Fig. 6. Separation of fluorescent analytes Rhodamine B (1) and sulfoRhodamine B (2). (A) SMIL coated chip and (B) uncoated chip. Separation buffer, 50 mM ammonium carbonate–ammonium hydrogencarbonate buffer (pH 8.7) containing 30% (v/v) acetonitrile, 50 mM ammonium acetate–acetic acid buffer (pH 4.0) containing 30% (v/v) acetonitrile or 50 mM ammonium formate–formic acid buffer (pH 2.8) containing 30% (v/v) acetonitrile; sample concentration, 10 μM; injection time, 0.2 s; electric field strength, 400 V/cm.

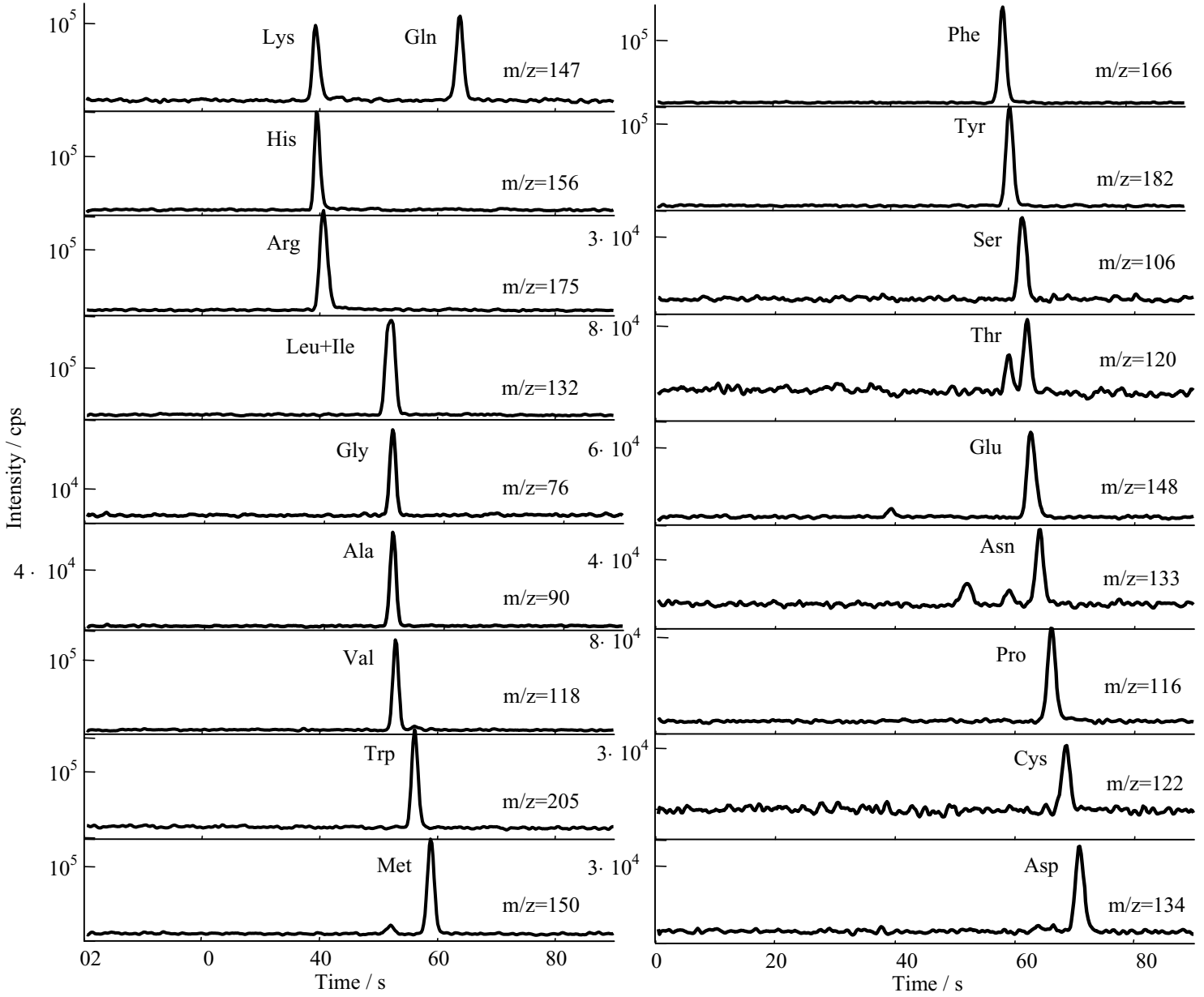

Fig. 7. Separation of amino acids. Separation buffer, 50 mM formic acid–ammonium acetate buffer (pH 2.5) containing 50% (v/v) acetonitrile; sample concentration, 40 mg/l; injection time, 0.2 s; electric field strength, 400 V/cm; ESI voltage, 3.0 kV; MS detection mode, SIM.

or LIF detection where derivatization with phenylthiohydantoin [32] or fluorescein isothiocyanate [33] is required to detect amino acids, derivatization is unnecessary in MS detection. The pK_{a1} values of amino acids are about 2, good separation can be expected under low pH conditions. Soga and Heiger [34] and Schultz and Moini [35] reported separation of amino acids with capillary electrophoresis–mass spectrometry using formic acid as BGE. We tried separation of amino acids under low pH conditions using MCE–MS. When an uncoated quartz microchip was used under low pH condition, EOF was weak and it was difficult to control the flow in the microchip channel. Fig. 7 shows the separation of 20 amino acids at pH 2.5 using the SMIL-coated microchip. EOF was stabilized by SMIL coating, and electrophoretic mobilities of amino acids increased under low pH condition, making stable sample injection possible. Peak shape was improved by incorporating a highly concentration acetonitrile in the BGE probably because of the decrease in adsorption of the amino acids on the channel wall. In the SIEs of Thr and Asn, peaks other than those of the amino acids of interest were obtained. However, these peaks could not be identified, the first peak in the SIE of Asn was considered to be due to Leu and Ile because the scan width was set to 0.25 amu and a slight overlap of signal may have occurred. Although separation is incomplete, 20 amino acids could be identified in less than 75 s.

4. Conclusions

Separation and detection using quartz microchip and the developed interface for MCE–ESI–MS were studied. In MCE–ESI–MS, broadening of peaks and decreased resolution were brought about by the negative pressure caused by ESI. The adverse effects of ESI minimized using a microchip with a long separation channel, and separation efficiency was improved. Separation of drugs and peptides were successfully performed. Electrokinetic gated injection was unstable under low pH condition using the uncoated quartz microchip. Stable EOF was obtained and electrokinetic gated injection became possible even under low pH condition using SMIL-coated quartz microchip. Analysis of amino acids under low pH condition was performed. MCE–ESI–MS can be expected to be used in combination with on-line concentration techniques. The system can be used as an integrated part of μ-TAS.

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