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Robust and simple interface for microchip electrophoresis–mass spectrometry

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Abstract

A robust and simple interface for microchip electrophoresis–mass spectrometry (MCE–MS) was developed using a spray nozzle connected to the exit of the separation channel of the microchip. The spray nozzle was attached to the microchip using a polyether ether ketone screw without adhesive, thus allowing easy replaced. Sample injection and electrophoretic separation was performed by control of the voltage only. The analysis of a few basic drugs was performed using the optimized MCE–MS system. The separation was improved by using a high-viscosity separation buffer and a spray nozzle with a small bore size. This system was also applied to the separation of peptides and protein–trypsin digests. Sample adsorption was minimized by adding acetonitrile to the separation buffer when using a quartz microchip.

Keywords: Interfaces, CE–MS; Chip technology; Peptides

1. Introduction

Recently the micro total analysis system (μ-TAS), which performs a series of chemical analysis operations, such as pretreatment, reaction, separation and detection on a microchip, has attracted much attention. Since microchip electrophoresis is effective as a separation technique, microchip electrophoresis (MCE) equipment is commercially available and it already goes into the practical application stage. Microchip electrophoresis has advantages such as rapid separation, versatile channel designs possible and small sample volumes compared with conventional capillary electrophoresis, but high sensitivity detection methods are required. Laser induced fluorescence detection has been widely used in microchip electrophoresis. However, in many cases analytes are not fluorescent and derivatization is needed. On the other hand, mass spectrometry detection is highly sensitive and does not need derivatization. Moreover, MS has an advantage that structural information of the analytes can be acquired. MCE–MS is valuable, in particular, for the analysis of complex mixtures such as biomedical samples, since the analytical capability in MS and MS–MS is greatly improved by

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separating and concentrating the sample before MS analysis. Thus, it is expected to be used in wide fields such as gene analysis, proteomics, and medicine development.

A conventional capillary electrophoresis–mass spectrometry (CE–MS) system is used widely, and represents a high-performance technique especially for biomedical analysis [1–5]. In many cases, the electrospray ionization (ESI) method is used as an interface of CE–MS. Sheath liquid, spray gas, a tapered spray nozzle, etc. are used in order to generate a stable electrospray. Nano spray, which uses a tapered spray nozzle, is suitable for low flow-rates and analysis of small amounts of analytes.

An interface for MCE–MS is based on the same principle as CE–MS. Since the scale is small, fine and precise processing is required to fabricate such interface. In many cases, ESI ionization method is used for MCE–MS as an interface (MCE-ESI–MS) like for conventional CE–MS. Development of the interface for MCE-ESI–MS is performed by several groups such as Karger's [9–13], Ramsey's [14–16], and Harrison's [17–21]. Review articles were also published [3–8]. There are a few technical problems, such as minimization of dead volume, a method to apply ESI voltage, and a method to maintain stable flow-rate, etc.

Dead volume is mainly generated by the formation of a droplet at the channel outlet and at a connecting joint between spray nozzle and microchip. Xue et al. [9], Ramsey et al. [14] tried to generate electrospray directly from the vertical surface of the channel outlet. Xue et al. [9] maintained flow velocity of 100–200 nl/min with a syringe pump to generate stable electrospray, and obtained mass spectra of proteins and peptides [9]. Although Ramsey et al. [14] generated flow electroosmotically and obtained mass spectra, a droplet of 12 nL volume was formed at the channel outlet section on the vertical surface of the microchip [14]. Zhang et al. [10] developed two different types of devices, which made it possible to perform separation by electrophoresis. One was a system which used a tapered fused-silica capillary and the other incorporated a nebulizer into the microchip. An efficiency of about 47 000 plates was obtained by the former system, and 7700 plates by the latter in separation of angiotensin peptides using an 11-cm channel length microchip. Recently, the system using a tapered fused-silica capillary as a spray nozzle has gained popular acceptance. To reduce the dead volume produced at the connection section between the microchip and the spray nozzle, Being et al. [17] made the connection section to the spray nozzle flat. In evaluation by LIF detection, 98% of the predicted theoretical plate number was obtained.

There are two methods to apply ESI voltage. One is through a liquid junction and the other is at a spray nozzle having conductive coating. Karger and co-workers [10–13] used a liquid junction, while Harrison and co-workers [18–21] used a gold-coated spray nozzle. Li et al. [19] tried to apply ESI voltage by a unique system using a glass membrane. To maintain a stable flow-rate, Karger and co-workers [10–13] prepared the subatmospheric ESI chamber between the outlet of the spray nozzle and MS orifice. They coated the channel wall with polyacrylamide or polyvinyl alcohol to prevent sample adsorption and to suppress EOF. The flow-rate was stabilized by adjusting pressure inside the ESI chamber. They separated angiotensin peptides and obtained 31 000 plates using an 11-cm channel length microchip [11]. Theoretical discussion about the liquid junction using a subatmospheric ESI chamber was also given [12]. They also attached a micro plate wells and constructed a system which could perform sample injection and channel rinsing automatically [13]. In this system, they separated a main part of the microchip from the reservoir section and simplified the manufacturing process. Harrison and co-workers [18–21] performed MCE-ESI–MS using a microchip, in which the channel wall was coated by [(acryloylamino)propyl]trimethylammonium chloride. They stabilized the flow by adding an auxiliary flow through a side channel using a syringe pump, and connected the microchip to the interface using a transfer capillary. In the interface, ESI was assisted by a sheath liquid and a nebulizer gas. High reproducibility was realized in terms of migration time and peak area [18]. In the system using a gold coated spray nozzle, sample concentration was performed by solid-phase extraction and stacking. Using standard peptides as samples, limit of detection was 2.5 nM in solid-phase extraction, and sub-nM in stacking [19]. They detected carnitine in human urine [20]. They also attached auto sampler to their

system and identified proteins using peptide mass-fingerprint database searching [21].

In this study, we developed a robust and simple interface for MCE–MS. A tapered spray nozzle was used to generate stable electrospray. The spray nozzle was attached to the microchip using a polyether ether ketone (PEEK) screw without glue, thus allowing easy exchange. ESI voltage was applied through a liquid junction, because conductive coating on the spray nozzle had extremely short lifetime. Liquid flow and sample injection were performed by the control of the voltage only, and the quartz microchip was uncoated. We evaluated performance of the device using Rhodramine B and basic drugs as analytes, and tried to apply it to the analysis of peptides and trypsin digests of a protein.

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 SIM mode (sampling rate 0.1 s) and scanning mode (sampling rate 0.4 s).

The detailed structure of an ESI interface is shown in Fig. 1B. A quartz microchip (Shimadzu, Kyoto, Japan), 33.5 mm in length × 12.5 mm in width × 6.05 mm in thickness, with simple cross channels was employed. The channel width, depth and separation channel length were 50 μm, 50 μm and 22.9 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. 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. It is easy to replace the interface block with another type. The spray nozzle was a tapered fused-silica capillary, 360 μm O.D. × 20 μm I.D. × 10 μm I.D. at the tip, or 360 μm O.D. × 50 μm I.D. × 15 μm I.D. at the tip (PicoTip FS360-20-10-N, FS360-50-15-N, New Objective, Cambridge, MA, USA), cut to a length of 25 mm. It was attached to the guide hole bottom and fixed using a PEEK screw (Shimadzu, Kyoto, Japan) and a PEEK tube 380 μm I.D. (F-185 Micro SLV, Upchuech Scientific, Washington, DC, USA) without adhesive. The spray nozzle was also easy to replace 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 junction reservoir. The solution at the liquid junction is the same as that of separation buffer, and pressure was not applied. 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).

The microdevices arranged for the laser-induced fluorescence (LIF) detection is shown in Fig. 1C. The spray nozzle of MS interface was removed and the liquid junction block was used as a reservoir block. LIF detection system was constructed in our laboratory [22]. Argon ion laser (488 nm) was used as a light source, detection wavelength 600 nm and detection point was set 0.5 mm from the end of the separation channel.

2.2. Reagents

Pindolol was purchased from Wako (Tokyo, Japan), nicardipine and trimipramine were from Sigma (St. Louis, MO, USA), sulpiride was from Research Biochemical (Natick, MA, USA), Rhodamine B was from Nacalai Tesque (Kyoto, Japan). L-1-Tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin, the sample peptides and proteins were obtained from Sigma. Water was purified with a Milli-Q Labo system (Nihon Millipore, Tokyo, Japan). All other reagents were of analytical or HPLC grade.

2.3. Procedure

Sample preparation

Rhodamine B was dissolved at 1 m*M* in water.

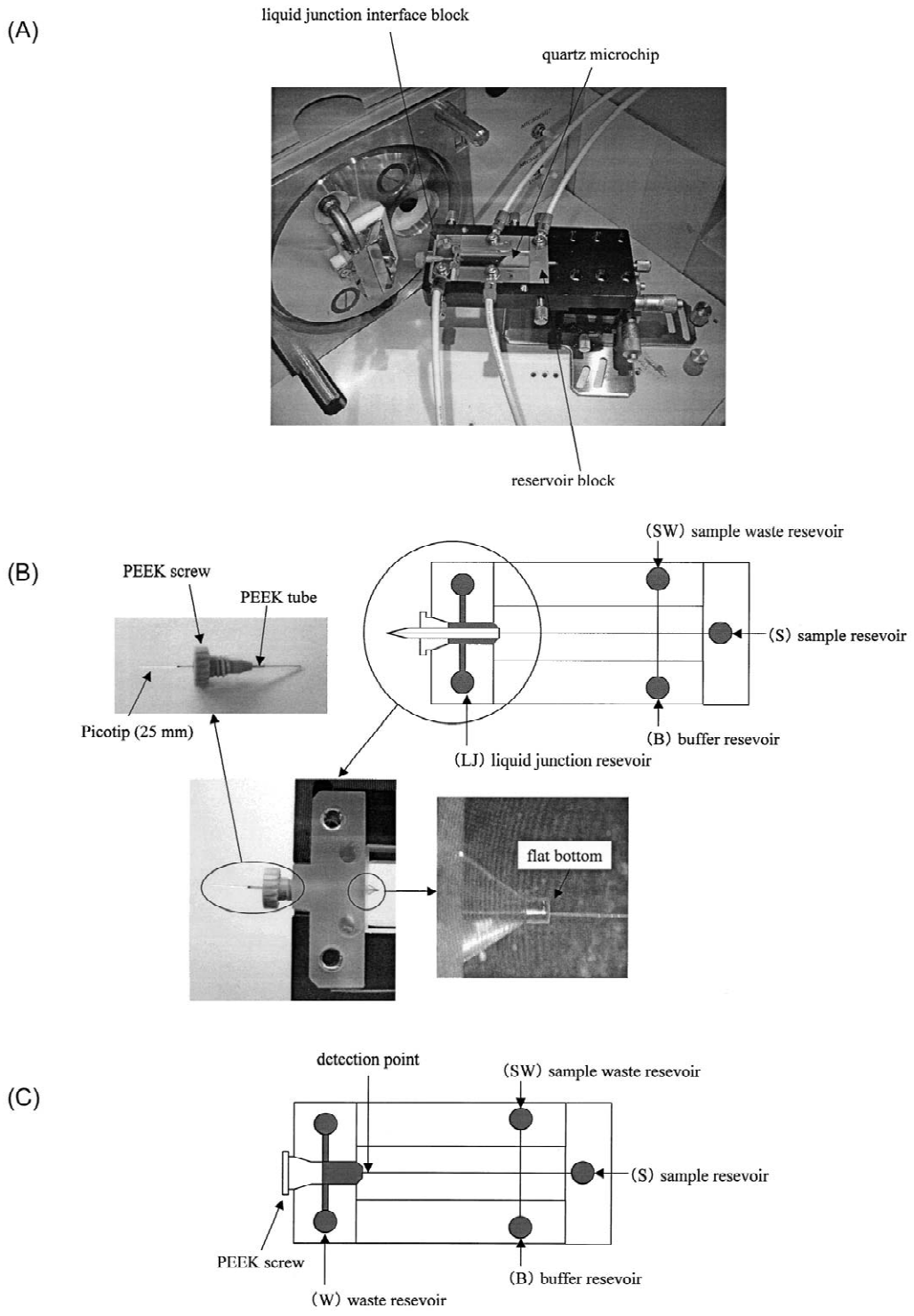

Fig. 1. The outline of equipment. (A) Photograph of the microdevice used in this study. Quartz microchip, ESI-MS interface and reservoir blocks were attached to the x–y–z translation stage. (B) Schematic diagram of the microdevice and photographs of ESI-MS interface. (C) Schematic diagram of the microdevice arranged for LIF detection.

Stock solutions of the other test analytes were prepared at 1000 ppm concentration each: pindolol and sulpiride in water–methanol (50:50, v/v) solution, trimipramine and nicardipine in methanol, tripeptides and angiotensin peptides in water. They were diluted to the required concentrations with the separation buffer prior to use.

2.4. Electrophoresis condition

A 50-m*M* acetic acid–ammonium acetate buffer (pH 5.0, 5.7, 6.0), 50 m*M* ammonia–ammonium acetate buffer (pH 7.4) and 50 m*M* ammonium carbonate–ammonium hydrogen carbonate buffer (pH 8.7) containing 30% (v/v) methanol or acetonitrile were used as separation buffers. The microchip channel was rinsed before use with 0.1 *M* NaOH for 30 min, water for 2 min, methanol or acetonitrile for 2 min, water for 2 min, and the separation buffer for 5 min by applying pressure at the reservoir with a syringe. After every five runs, it was washed with the separation buffer by applying the same procedure. Sample stacking was performed using 50 m*M* acetate buffer (pH5.7) containing 30% acetonitrile as the separation solution. Sample stock solutions were diluted with 5.0 m*M* acetate buffer (pH 5.7) containing 30% acetonitrile and injected for 2.0 s with gated injection.

In MS detection, the applied voltages were 4256 V at a sample reservoir (S), 4416 V at a buffer reservoir (B), 3416 V at a waste reservoir (W), 3000 V at a liquid junction reservoir (LJ). The electric-field strength was 400 V/cm in the separation channel. Injection was performed by the gated injection [23] and the applied voltage was 4256 V to (S), 3929 V to (B), 3903 V to (W), 3000 V to (LJ).

In LIF detection, the following voltages were applied: 1256 V at (S), 1416 V at (B), 416 V at sample waste reservoir (SW) and (W) was grounded. The electric-field strength was 400 V/cm during the separation. Injection was performed by gated injection and the voltages applied were 1256 V at (S), 929 V at (B), 903 V at sample waste reservoir (SW) and (W) was grounded. Detection point was 0.5 mm from the end of the separation channel, and the effective channel length was 22.4 mm.

2.5. Cytochrome c TPCK–trypsin digestion

First, 50 m*M* ammonium carbonate was adjusted to pH 8.2 by the addition of 50 m*M* ammonium hydrogencarbonate. Then, TPCK-treated trypsin was added to a 1000 ppm cytochrome *c* solution at the enzyme–substrate ratio of 1:50 (w/w), and the solution was heated 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.

3. Results and discussion

Migration times and peak profiles of a fluorescent analyte, Rhodamine B, were compared using LIF and MS detection as shown in Fig. 2. Electrophoretic conditions are given in Section 2, and injection time was 0.2 s. In LIF detection, the effective channel length was 22.4 mm. In MS detection, the 25-mm long spray nozzle was connected to the 22.9-mm long separation channel. Spray nozzles with 50 μm I.D. or 20 μm I.D. were used to investigate the effect of spray nozzle diameter on separation efficiency. Inside the spray nozzle, a laminar flow was generated due to negative pressure at the tip during the ESI operation. Although a shorter spray nozzle will not deteriorate the separation efficiency significantly, it was difficult to make the length of the nozzle less than 25 mm because of the configuration of the equipment used in this work. At pH 8.7 used in LIF

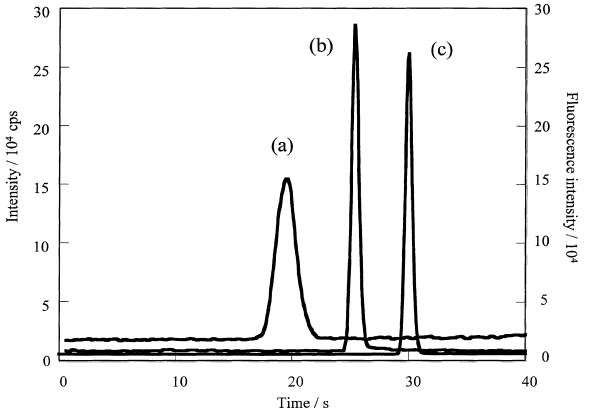

Fig. 2. Peak shape of fluorescent analyte Rhodamine B. (a) MS detection using 50 μm I.D. spray nozzle. (b) MS detection using 20 μm I.D. spray nozzle. (c) LIF detection. Separation buffer: 50 m*M* ammonium carbonate–ammonium hydrogencarbonate buffer (pH 8.7) containing 30% (v/v) acetonitrile; sample concentration: 100 μ*M*; injection time: 0.2 s; electric field strength: 400 V/cm; ESI voltage: 3.0 kV; MS detection mode: SIM.

detection, Rhodamine B was neutral and hence, its migration velocity must be equal to that of electroosmotic flow (EOF). The total variance of the peak in LIF detection (σ^2_{LIF}) was mainly generated by the electrophoretic process within a microchip. In MS detection, σ^2_{MS} can be described as the sum of variances due to the interface (σ^2_{int}) and σ^2_{LIF}:

$$\sigma^2_{MS} = \sigma^2_{LIF} + \sigma^2_{int} \quad (1)$$

In MS detection, the laminar flow profile inside the spray nozzle is considered to be the main reason for the considerable increase in peak width, whereas plug-like flow in microchip channel if the migration is solely due to EOF. In the case of laminar flow, the variance in an open tubular capillary can be calculated by Eq. (2):

$$\sigma^2 = \frac{r^2}{24D}\, uL \quad (2)$$

where D is the diffusion coefficients of sample molecules, u is flow velocity, r is capillary radius, L is the capillary length.

Table 1 compares peak parameters of a fluorescent analyte Rhodamine B detected with LIF and MS. The average migration time with LIF detection was 31.2 s, while with MS detection, the average value was determined to be 25.4 and 19.6 s using spray nozzle having 20 and 50 μm I.D., respectively, despite a longer path length compared to the former. These reduced migration times were due to the enhanced flow-rate assumed by the negative pressure generated at the tip of the ESI spray nozzle. The peak variance (σ^2) was calculated using Eq. (3), assuming Gaussian peak shape:

$$\sigma^2 = \left(\frac{1}{4}\cdot W\right)^2 = \left(\frac{1}{2.354}\cdot W_{1/2}\right)^2 \quad (3)$$

where W is the peak width at base line and $W_{1/2}$ is the peak width at half height. As shown in Table 1, σ^2_{LIF} was 0.10 s^2, σ^2_{MS} was 1.08 s^2 (50 μm I.D. nozzle) and 0.25 s^2 (20 μm I.D. nozzle). The peak variances with 20 and 50 μm I.D. spray nozzle were 2.5- and 10.8-times higher than that observed with LIF detection, respectively. Using Eq. (1), the value of σ^2_{int} was determined to be 0.98 s^2 and 0.15 s^2 for 50 and 20 μm I.D. nozzle, respectively. The variance σ^2_{int} decreased by 85% by using a 20 μm I.D. nozzle compared with 50 μm I.D. one. According to Eq. (2), the variance inside the spray nozzle is expected to decrease by as much as 87% when the spray nozzle of 20 μm I.D. was used instead of 50 μm I.D. Experimental results were in good agreement of this prediction. In this study, there were many complex factors: channel shape of the microchip was different from that of the spray nozzle, the influence of the negative pressure by the electrospray has reached in the microchip channel, and liquid enters the spray nozzle through the liquid junction. Although it was difficult to calculate the contribution of each factor with precision, change of the flow profile in the spray nozzle was considered to be the main reason in the increase of peak variance.

Fig. 3 shows the separation of four basic drugs, pindolol, trimipramine, sulpiride and nicardipine. The separation buffer was carbonate buffer (pH 8.7) containing 30% (w/w) acetonitrile (Fig. 3A) or methanol (Fig. 3B). Table 2 shows a comparison of migration time, peak width, theoretical plate number, and resolution of pindolol and sulpiride. The peak

Table 1
Comparison of peak parameters of fluorescent analyte Rhodamine B detected by LIF and MS

Detector	Spray nozzle I.D. (μm)	Migration time (s)	Flow rate (cm/s)	Peak width (s)	Plate no.	Variance (s^2)
LIF	–	31.2	0.07	0.73[a]	10 200	0.10
MS	20	25.4	0.19	2.02[b]	2600	0.25
MS	50	19.6	0.24	4.16[b]	360	1.08

Experimental conditions as in Fig. 2.
[a] Peak width at half height.
[b] Peak width at baseline.

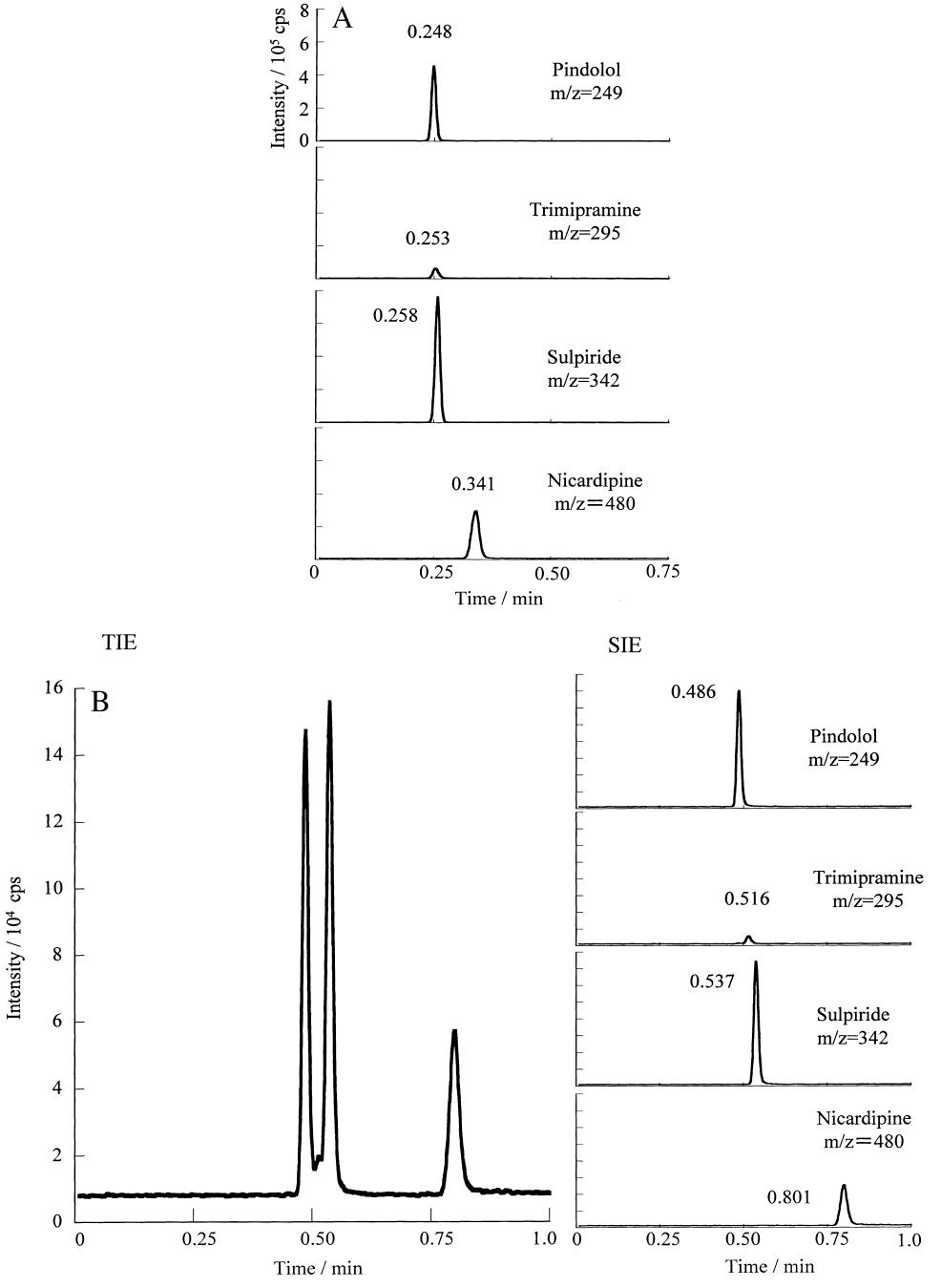

Fig. 3. Separation of basic drugs. Separation buffer: 50 m*M* ammonium carbonate–ammonium hydrogencarbonate buffer (pH 8.7) (A) containing 30% (v/v) acetonitrile, (B) containing 30% (v/v) methanol; spray nozzle: 20 μm I.D.; sample concentration: 100 ppm; injection time: 0.2 s; electric field strength: 400 V/cm; ESI voltage: 3.0 kV; MS detection mode: SIM. (A) Selected ion electropherogram, (B) TIE, total ion electropherogram (Σ4 ions); SIE, selected ion electropherogram. The migration time of each peak is given in its respective SIE.

Table 2
Peak parameters of pindolol and sulpiride

Spray nozzle I.D. (μm)	Organic solvent	Analyte	Migration time (s)	Peak width[a] (s)	Plate no.	Variance (s^2)	R_s[b]
50	30% CH_3CN	Pindolol	16.3	3.4	360	0.73	0.29
		Sulpiride	17.3	3.7	350	0.87	
20	30% CH_3CN	Pindolol	18.3	1.7	1900	0.18	0.85
		Sulpiride	19.8	1.8	1900	0.21	
20	30% CH_3OH	Pindolol	29.0	2.3	2700	0.32	1.26
		Sulpiride	31.9	2.4	2900	0.35	

Experimental conditions as in Fig. 3.
[a] Peak width at baseline.
[b] Resolution between pindolol and sulpiride.

variance decreased by 75% for pindolol and by 76% for sulpiride when the spray nozzle of 50 μm I.D. was replaced with 20 μm I.D., hence, separation was improved significantly (not shown in Fig. 3). Theoretical plate numbers of pindolol and sulpiride were about 1900, when using a spray nozzle of 20 μm I.D. (Fig. 3A).

When the separation buffer contained 30% (v/v) methanol, migration times became longer but peak broadening was less pronounced compared with that when acetonitrile was used (Fig. 3B). Theoretical plate numbers of pindolol and sulpiride were about 2700 and 2900, respectively, and three components except trimipramine were separated completely. When methanol was added to water at the ratio of 3:7 (v/v), the coefficient of viscosity of the resulting solution was about 1.6 times higher compared with pure water at 25 °C. For this reason, the linear flow velocity u inside the spray nozzle decreased and the peak variance was suppressed as predicted by Eq. (2), resulting in improved separation.

One of the targets of MCE–ESI-MS is peptide analysis in this project. Five tripeptides were used as test compounds. Gly–Gly–His contains a basic amino acid residue, Tyr–Gly–Gly and Met–Leu–Phe contain neutral amino acid residues only, while Glu–Val–Phe contain an acidic amino acid residue. Pro–Leu–Gly amide has a structure that the terminal carboxyl group is amidated. Fig. 4A shows the separation of four tripeptides Gly–Gly–His, Tyr–Gly–Gly, Met–Leu–Phe and Pro–Leu–Gly amide using acetate buffer (pH 5.7) containing 30% acetonitrile. Although separation of Gly–Gly–His from Pro–Leu–Gly amide, and Tyr–Gly–Gly from Met–Leu–Phe were unsuccessful, the four tripeptide signals were detected reasonably well. Separation was carried out using buffers having different pH of 5.0, 5.7, 6.0, 7.4, and 8.7. Although separation was incomplete as in the case of pH 5.7, the four tripeptides were detected at pH 5.7, 6.0 and 7.4. At pH 5.0, only Gly–Gly–His and Pro–Leu–Gly amide were detected. It is probably because the electrokinetic injection was not successful due to a weak EOF. At pH 8.7, only Pro–Leu–Gly amide could be detected, probably due to slow migration velocity caused by the dissociation of the carboxyl group. Glu–Val–Phe which contains an acidic amino acid residue was not detected with any of the separation buffers.

Fig. 4B shows separation of five tripeptides injected by sample stacking. The separation solution was 50 m*M* acetate buffer (pH 5.7) containing 30% acetonitrile, and the sample concentration was adjusted to 100 ppm with a low concentration buffer, 5.0 m*M* acetate buffer (pH 5.7) containing 30% acetonitrile. Sample solution was injected for 2.0 s with gated injection. All five tripeptides could be detected within 1 min. However the peak intensity of the acidic Glu–Val–Phe was very low, due to positive electrophoretic mobility.

Adsorption of proteins and peptides on the channel wall is a common problem in microchip electrophoresis. Using a separation buffer containing 30% (v/v) methanol, the peptides peaks were broad, tailed, and gated injection was difficult because of unstable EOF due to the adsorption of the analytes on the channel wall. Substitution of methanol with acetonitrile minimized adsorption.

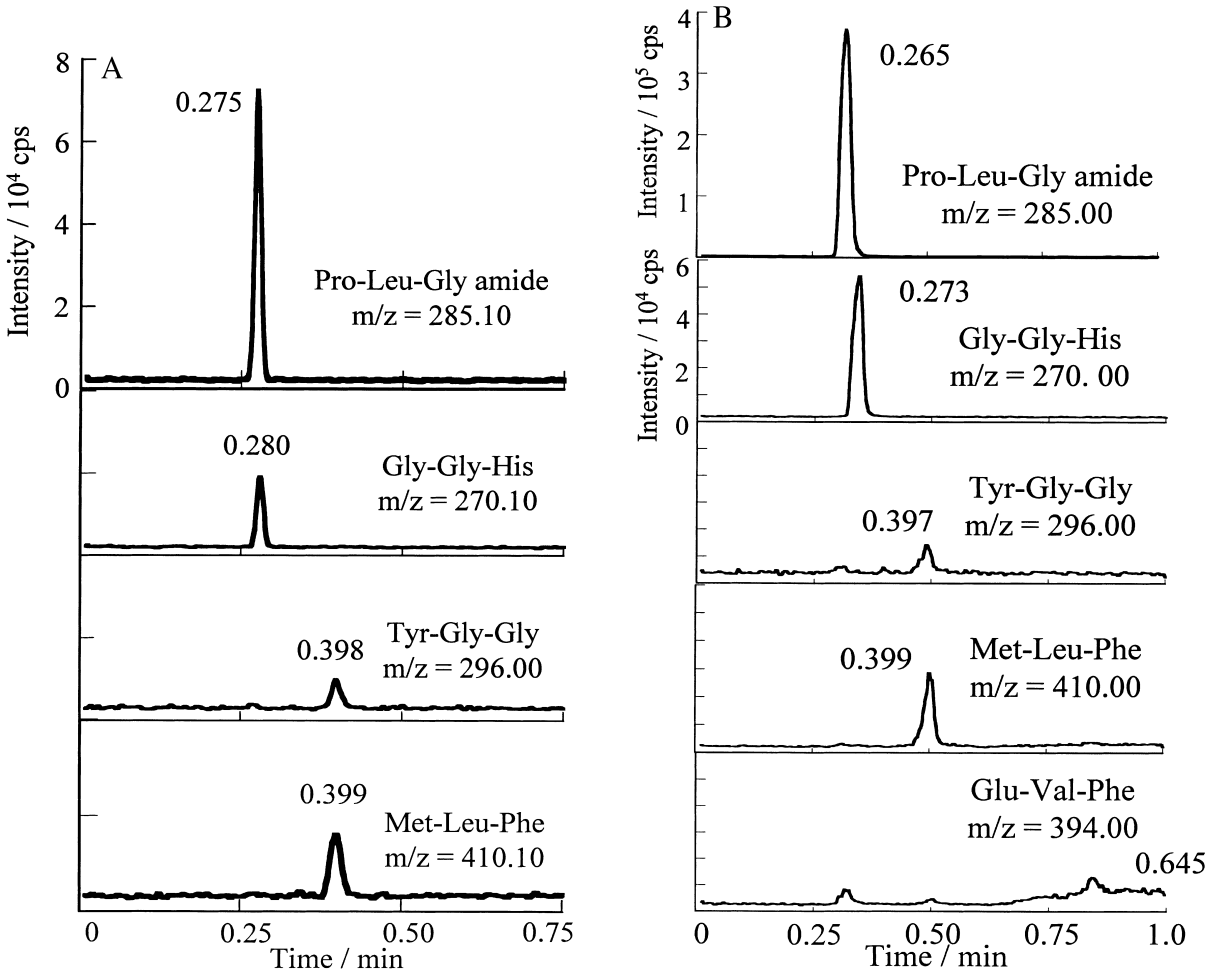

Fig. 4. Separation of tripeptides. Separation buffer: 50 m*M* acetic acid–ammonium acetate buffer (pH 5.7) containing 30% (v/v) acetonitrile; sample concentration: 100 ppm; electric field strength: 400 V/cm; ESI voltage: 3.0 kV; MS detection mode: SIM; injection time: 0.2 s (A), 2.0 s (B). (B) Separation was performed following sample stacking. Other conditions are the same as in Fig. 3.

Fig. 5 shows the separation of peptides consisting of 7–10 amino acid residue using acetate buffer (pH 5.7) containing 30% (v/v) acetonitrile as separation buffer. Detection was performed with scan mode and injection time was 0.5 s. Angiotensin I, angiotensin II, [Ser^1,Ala^8]angiotensin II, [Val^4]angiotensin III, bradykinin were used as a model mixture. As all of these analytes contain basic amino acid residues, it was easy to inject electrokinetically and they could be detected within 35 s as $[M+2H]^{2+}$. In SIM mode, a theoretical plate number of 1600 was obtained for bradykinin injected for 0.2 s by gated injection.

Fig. 6 shows the separation of peptides generated by tryptic digestion of cytochrome *c* using acetate buffer (pH 5.7) containing 30% (v/v) acetonitrile. Although separation was not complete, seven peptides (GITWK, IFVQK, YIPGTK, MIFAGIK, KYIPGTK, TGPNLHGLFGR, TGQAPGFTYTDANK) were detected within 40 s. The first four were detected as $[M+H]^+$ and the last three were detected as $[M+2H]^{2+}$.

4. Conclusion

We have developed a robust and simple interface

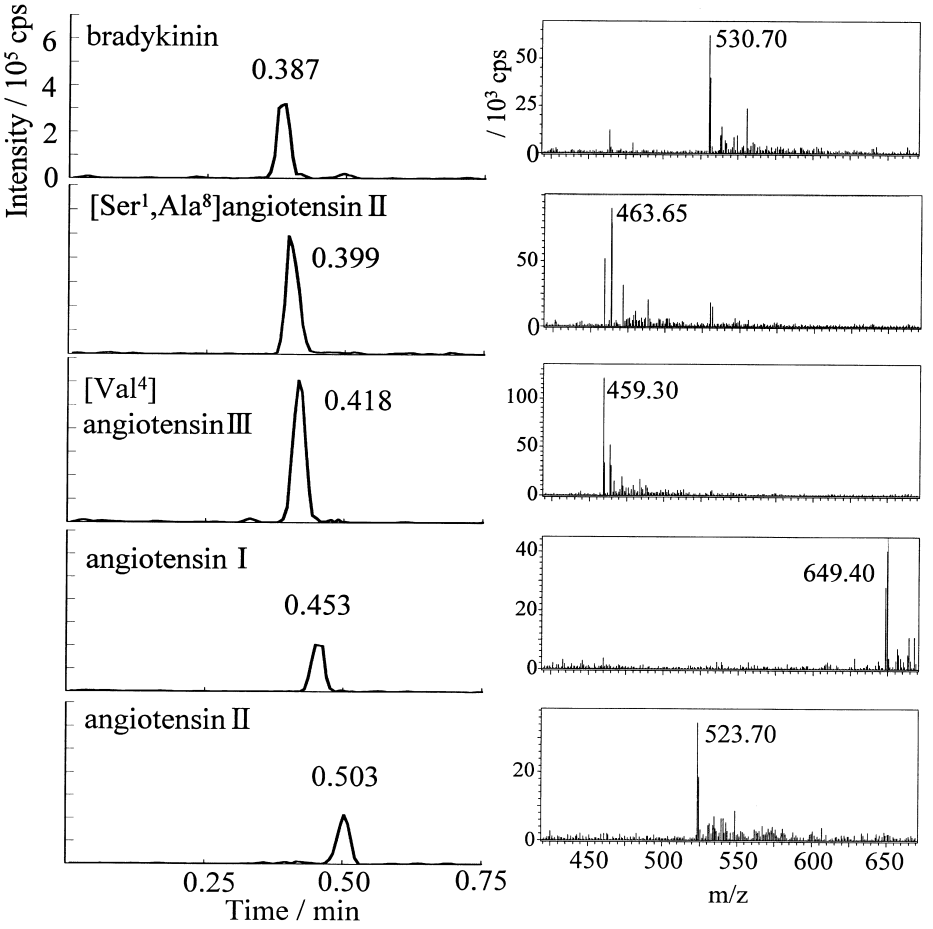

Fig. 5. Separation of peptides with 7–10 amino acid residue. Separation buffer: 50 m*M* acetic acid–ammonium acetate buffer (pH 5.7) containing 30% (v/v) acetonitrile; sample concentration: 100 ppm; injection time: 0.5 s; electric field strength: 400 V/cm; ESI voltage: 3.0 kV; MS detection mode: scan (m/z 420–670). Other conditions are the same as in Fig. 3.

for microchip electrophoresis–mass spectrometry. For basic drugs, separation was improved significantly using a small I.D. spray nozzle and a separation buffer with high viscosity. Separation and detection conditions of peptides were examined using tripeptides as test compounds. Separation of peptide standards and cytochrome *c*–trypsin digests were performed. Although the quartz microchip was not coated, adsorption of peptides was suppressed by adding acetonitrile to the separation buffer.

In MCE–ESI-MS, the negative pressure generated at the tip of the ESI nozzle affects the flow-rate inside the microchip channel, causing reduced separation efficiency. For better results, it is necessary to minimize this detrimental influence. In this research, incomplete separation of the test analytes was obtained because the microchip used had the short channel length, but this could be remedied by using a longer channels.

Although electrokinetic injection by gated injection has advantages that equipment can be simplified compared with pressure injection and sample plug length can be adjusted easily, its disadvantage is that EOF is necessary for stable injection and it is biased

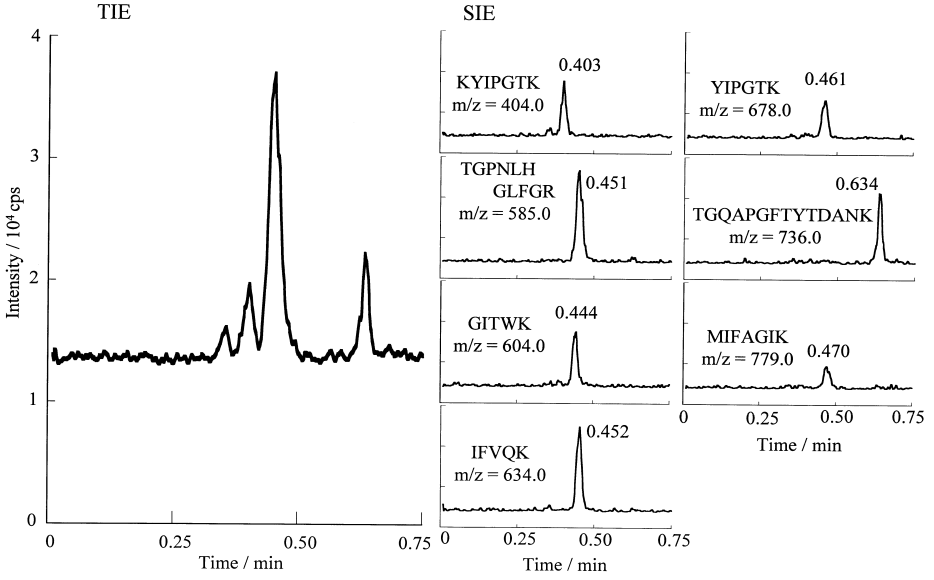

Fig. 6. Separation of cytochrome *c* TPCK-treated trypsin digest. Separation buffer: 50 m*M* acetic acid–ammonium acetate buffer (pH 5.7) containing 30% (v/v) acetonitrile; injection time: 0.5 s; electric field strength: 400 V/cm; ESI voltage: 3.0 kV; MS detection mode: SIM. TIE, Σ7 ions and SIE. Other conditions are the same as in Fig. 3.

against samples of low electrophoretic mobilities. A wider range of samples will be injected even at low pH by adjusting the EOF using channel wall coating.

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