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On-line sample concentration in micellar electrokinetic chromatography with cationic micelles in a coated capillary ☆

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Abstract

The electroosmotic flow was successfully suppressed even in the presence of cationic surfactants, when a polyacrylamide-coated capillary was employed. Two on-line sample concentration techniques of sample stacking and sweeping were evaluated in micellar electrokinetic chromatography (MEKC) using the polyacrylamide-coated capillary. Cationic surfactants were used as pseudostationary phases in MEKC. At least 60-fold and about 600-fold increases in detection sensitivity were obtained in terms of peak heights by sample stacking and sweeping, respectively. © 2001 Published by Elsevier Science B.V.

Keywords: Micellar electrokinetic chromatography; Sample handling; Sample stacking; Sweeping; Coated capillaries; Background electrolyte composition; Surfactants; Steroids; Carboxylic acids; Naphthalenedisulfonic acids

1. Introduction

Micellar electrokinetic chromatography (MEKC), which was first introduced by Terabe et al. [1], has become popular as a powerful analytical separation technique. MEKC is capable of separating neutral analytes as well as charged ones by using a capillary electrophoresis (CE) instrument without any alteration. The separation of analytes is based on their differential partitioning between the micelle phase and the aqueous phase. As in the other modes of CE, one of the disadvantages of UV detection in MEKC is the low concentration sensitivity resulting from the inherently small dimensions of the capillary and the sample volume capacity. Overcoming the low concentration sensitivity has been the objective of many investigations. On-line sample concentration is known to be an effective approach for enhancement of the concentration sensitivity [2–10].

Two useful and powerful techniques, sample stacking and sweeping, have been developed for on-line sample concentration in MEKC. Sample stacking is a technique frequently used to increase sensitivity and improve peak shape. The difference in migration velocity of micelles within the sample zone and the background solution (BGS) zone is the key to achieving the focusing effect in MEKC. The analyte is focused at the boundary of the two zones. By using sample stacking, at least 10-fold under strong electroosmotic flow (EOF) and more than 100-fold concentration increases under suppressed

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EOF have been achieved [4]. Sweeping in MEKC is defined as the picking and accumulating of analytes by the micelle that penetrates the sample zone [5,6]. The sample is prepared in a matrix with similar conductivity to that of the BGS but without micelles and is introduced to the capillary. More than 5000-fold increases in detector response in terms of peak height were achieved by sweeping of some biologically active compounds [5]. Recently, a combination of sample stacking and sweeping [9], achieved more than 10 000-fold enhancements in detector response for environmentally relevant aromatic amines [10]. In previous results, sample stacking and sweeping were found more effective under suppressed EOF than under high EOF conditions [4,6]. EOF was suppressed significantly under acidic conditions below pH 5.5 with anionic sodium dodecyl sulfate (SDS) micelles [11]. So far, only anionic surfactants, mostly SDS, were employed in on-line sample concentration in MEKC. The analytes effectively concentrated either by stacking or sweeping are hydrophobic or cationic ones because such analytes tend to be strongly incorporated by the micelle. To effectively concentrate anionic analytes the use of the cationic micelles is favorable. However, EOF is reversed and is not suppressed even under acidic conditions in the presence of cationic micelles due to the strong adsorption of the cationic surfactant molecules on the wall. Therefore, in a previous paper, we performed on-line sample concentration in MEKC with cationic micelles under strong EOF conditions [12] and successfully obtained about a 1000-fold increase in sensitivity by sweeping. However, stacking was not very successful under strong EOF. One of the effective methods for EOF suppression is coating of the wall of the capillary with polyacrylamide (PAA) [13–16]. The purpose of this work is to explore the applicability of on-line sample concentration techniques in MEKC with cationic surfactants under suppressed EOF condition using a PAA-coated capillary.

2. Experimental

2.1. Apparatus

All experiments were performed with a Hewlett-Packard 3D capillary electrophoresis system (Waldbronn, Germany). Fused-silica capillaries (50 μm I.D.×360 μm O.D.) were purchased from Polymicro Technologies (Phoenix, AZ, USA) and used with surface modification. Poly(ethylene glycol) (PEG)- and poly(vinyl alcohol) (PVA)-coated capillaries [17] were kindly donated by Dr. Detlev Belder. The capillary temperature was maintained at 25°C. Samples were introduced by pressure injection (50 mbar). An optimum detection wavelength was selected for each analyte based upon the spectra recorded by the diode-array detector. Conductivities were measured with a Horiba ES-12 conductivity meter (Kyoto, Japan).

2.2. Chemicals

Cetyltrimethylammonium chloride (CTAC), salicylic acid, thionyl chloride, tris(hydroxymethyl)aminomethane (Tris), 1,5- and 2,6-naphthalenedisulfonic acid disodium salts (1,5- and 2,6-NDSA), and acrylamide were purchased from Wako (Osaka, Japan). Acrylamide is toxic and should be handled with care; avoid skin contact. Tetradecyltrimethylammonium bromide (TTAB), 2,7-naphthalenedisulfonic acid disodium salt (2,7-NDSA), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), and vinylmagnesium bromide (14% in tetrahydrofuran, THF) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Other reagents were obtained from Nacalai Tesque (Kyoto, Japan). All reagents were of analytical-reagent grade and used without further purification. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

Buffers were prepared from stock solutions of Tris and hydrochloric acid (HCl). Stock solutions of steroids (cortisone, hydrocortisone, testosterone) were prepared with methanol. Stock solutions of naphthalenedisulfonic acids (1,5-, 2,6- and 2,7-NDSAs) were prepared in purified water. Stock solutions of aromatic carboxylic acids (salicylic acid and 2-naphthoic acid) were prepared in 50% methanol or methanol. Buffer solutions were sonicated and filtered through 0.45-μm filters before use.

2.3. CE procedure

The new PAA-coated capillary was rinsed with

purified water for 20 min and BGS for 10 min. To assure reproducibility, at the end of each run the capillary was flushed with methanol for 2 min, followed by purified water for 3 min and then with the BGS (3 min).

For stacking with reverse migrating micelles (SRMM), samples prepared in purified water are injected for much longer time compared to the normal injection, after conditioning the capillary with micellar BGS. Sample solutions were introduced at the anodic end of the capillary at 50 mbar and then high voltage was applied with positive polarity at the injection end. Positive polarity was used due to the higher electrophoretic velocity of the cationic micelles compared to the suppressed EOF. The plug length of the sample solution was optimized in terms of peak shapes by injecting the sample for different times.

For the sweeping experiments, test analytes prepared in Tris–HCl buffer solutions having a conductivity similar to that of BGS were pressure injected into the capillary at the anodic end. The velocities of a liquid in the capillary at 50-mbar pressure were determined by using a neutral marker to approximate the length of the zones injected at different intervals. Then the BGS vials were set to both ends of the capillary and the voltage was applied at positive polarity. Other experimental conditions are described in the text or figures.

2.4. Polyacrylamide coating procedure

Fused-silica capillaries were coated inside with three step reactions (surface chlorination, Grignard reaction, and polyacrylamide coating), which are basically the same as that reported by Cobb et al. [14], but the procedure was modified as follows [15,16].

A fused-silica capillary was first treated with 1 *M* NaOH at room temperature for 1 h, followed by distilled water for 1 h, and dried at 110°C by streaming nitrogen gas for 6 h. Thionyl chloride was passed through the dried capillary for several minutes using a suction pump. Both ends of the capillary were sealed with a propane torch and the sealed capillary was placed in a 70°C heating oven for 6 h. After chlorination the seal was opened, and 0.25 *M* vinylmagnesium bromide solution in THF was introduced into the capillary by a suction pump. The capillary was sealed again and the sealed capillary was kept at 70°C for 6 h to complete the Grignard reaction. After reaction, the seal was opened and the capillary was rinsed with THF for several minutes, then with distilled water for several minutes. An aqueous solution containing 3% (w/v) acrylamide was degassed in an ultrasonic bath. After 40 μl of 10% (v/v) TEMED and 10 μl of 10% (w/v) ammonium peroxodisulfate (APS) were added to 5 ml of the degassed solution, the solution was mixed thoroughly, and introduced into the capillary using a dry syringe. After polymerization at 28±2°C for 1 h, distilled water was passed through the capillary.

2.5. EOF measurement

EOF was measured using the three-injection method introduced by Williams and Vigh [18].

3. Results and discussion

3.1. Measurement of EOF

Measurement of the suppressed EOF in coated capillary is a time-consuming procedure, since very long runs are necessary for an EOF marker to arrive at the detector. Therefore, we measured the velocity of the suppressed EOF using the three-band injection method developed by Williams and Vigh [18]. This method allows precise and reproducible determination of EOF values in a few minutes. The results of the EOF measurements are listed in Table 1 for a TTAB solution at pH 7.0 in PAA-coated capillary and an SDS solution at pH 2.5 in an untreated capillary. EOF velocities observed in an untreated capillary filled with a TTAB solution at pH 7.0 and with an SDS solution at pH 7.0 are shown for comparison. The PAA coating significantly suppressed EOF even with a TTAB solution at a neutral pH. Another two types of polymer coated capillaries, PEG and PVA, were employed to suppress EOF as well. However, EOF in the PEG-coated capillary was not suppressed significantly in comparison with that of the PAA-coated one. Despite the fact that the EOF was suppressed considerably, the stability of PVA-coated capillary was insufficient when cationic sur-

Table 1
Electroosmotic mobilities

Capillary	Surfactant[a]	pH	Direction	Mobility (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%, n=6)
PAA-coated	TTAB	7.0[b]	+	0.12	0.72
Untreated	TTAB	7.0[b]	−	3.2	1.5
Untreated	SDS	7.0[c]	+	2.5	0.59
Untreated	SDS	2.5[c]	+	0.21	0.87

[a] Concentration: 50 m*M*.
[b] 100 m*M* Tris–HCl buffer containing 10% methanol.
[c] 50 m*M* phosphate buffer containing 10% methanol.

factant solutions were introduced. The PAA-coated capillary filled with the cationic micellar solution was stable during 8 days of experiments with 20 injections every day. The addition of cationic surfactants to the BGS causes generally the reversal of EOF owing to positively charged capillary wall by the adsorption of cationic surfactants [19,20]. The strong and reversed EOF was not retarded even under acidic conditions, although EOF was significantly suppressed under acidic conditions when SDS solutions were employed as shown in Table 1. It is interesting that reversal of the EOF was not observed in a PAA-coated capillary although the velocity was very low. The results suggest almost no adsorption of cationic surfactant on the wall. Accordingly, a PAA-coated capillary was employed to explore the effect of EOF on sample stacking and sweeping in MEKC with cationic micelles.

3.2. Stacking with reverse migrating micelles

Fig. 1 shows the SRMM-MEKC analysis of the

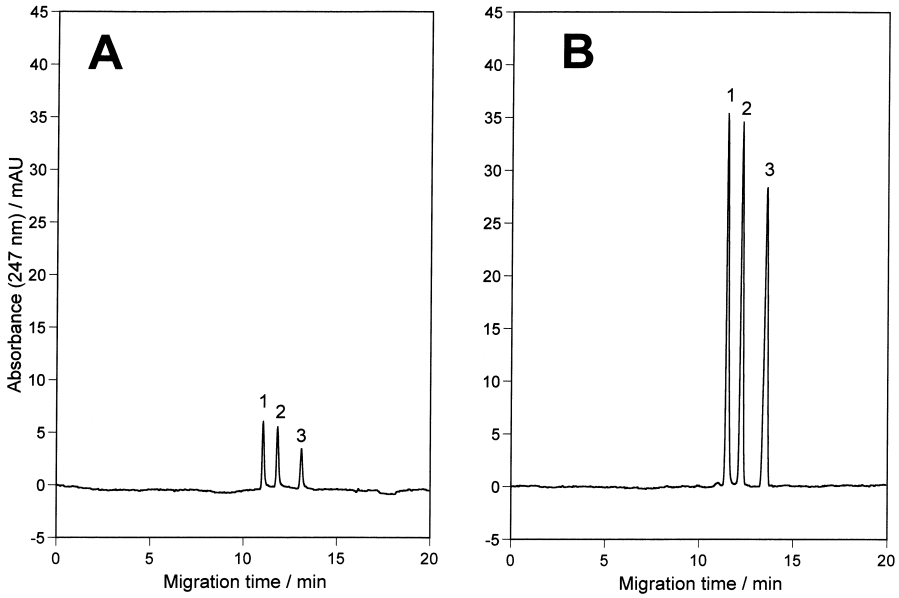

Fig. 1. Separation of the test steroids by (A) conventional MEKC and (B) SRMM-MEKC. BGS, 100 m*M* Tris–HCl buffer (pH 7.0) containing 10% methanol and 30 m*M* CTAC; injection time, (A) 1 s, (B) 60 s; concentration of samples, (A) testosterone (peak 1, 420 ppm), hydrocortisone (peak 2, 422 ppm), cortisone (peak 3, 430 ppm), (B) 10-fold dilution of the samples in A; sample matrix, (A) BGS, (B) water; PAA-coated capillary, 59 cm (50.5 cm to detector)×50 μm I.D.; detection, 247 nm; applied voltage, +18 kV. Other conditions are given in the Experimental section.

test steroids using CTAC micelles. In order to obtain a good baseline separation, 10% (v/v) methanol was added to the BGS. The organic modifier improved the separation of hydrophobic analytes by decreasing the retention factor (*k*) [21]. Fig. 1A shows the electropherogram of conventional MEKC with 1 s injection. The concentration of the test steroids in Fig. 1B is 10-fold dilution of the corresponding sample solution in Fig. 1A. Fig. 1B shows the electropherogram of the test analytes for SRMM when the injection time was 60 s. The injection time of 60 s was chosen since longer injections did not produce further increase in relative peak heights. As shown in Fig. 1B, about 60-fold enhancements of peak heights were achieved. Almost identical results were obtained when TTAB was employed instead of CTAC.

The percentage relative standard deviations (RSDs) and sensitivity enhancement factors in terms of peak heights (SEF_{height}) obtained for the test steroids with SRMM (60 s injection) are listed in Table 2. Excellent reproducibility was achieved, as RSD values obtained with four consecutive experiments in migration times, corrected peak areas (peak area divided by the migration time), and peak heights were less than 2.7% for all analytes. Detection sensitivity can be enhanced more than 60-fold. The sensitivity enhancement factors obtained by different cationic micelles were almost the same level. Sensitivity enhancement factors were calculated by simply getting the ratio of the peak heights obtained from stacking and usual injection and correction by the dilution ratio. In a previous work [22], under suppressed EOF by simply lowering the pH for anionic SDS micelles, about a 100-fold increase in detector response was achieved for phenol derivatives by SRMM. Under these conditions, the EOF increases with increasing sample plug length. On the other hand, using a PAA-coated capillary the EOF is suppressed throughout the whole PAA-coated capillary because capillary surface is totally coated with PAA. Normal stacking mode (NSM) of some neutral analytes in MEKC using a cationic surfactant under strong EOF gave a 15-fold increase in detector response in terms of peak height [12]. The low stacking effect was probably due to the dispersive effect brought about by the local electroosmotic velocity mismatch between the low- and high-conductivity zones. In SRMM using a PAA-coated capillary, however, the mismatch of EOF must not occur. Although the limit of detection (LOD) was not measured for the test steroid samples in this SRMM experiment, they were roughly estimated to be around 100–200 ppb, which were much higher than the values obtained for the same compounds by SRMM with SDS [22]. The lower sensitivity enhancement in SRMM with TTAB compared with SDS is difficult to reasonably explain. It should be mentioned that in SRMM with SDS, EOF was not effectively suppressed in an untreated capillary under acidic conditions and that EOF and the electrophoretic migration of the SDS micelle migrated in opposite directions. However, in this SRMM study with TTAB and the PAA-coated capillary, EOF was more effectively suppressed and EOF migrated in the same direction as the TTAB micelle. Therefore, when the voltage is applies with positive polarity, as shown in Fig. 2B, the micelle from the anodic vial will rapidly reach the concentration boundary (CB) and stack the analytes, while a narrow micelle vacant

Table 2
RSDs and sensitivity enhancement factors (SEF_{height}) for the test steroids in SRMM-MEKC[a]

	Testosterone	Hydrocortisone	Cortisone
RSD (%, $n=4$)			
Migration time	0.074	0.097	0.14
Corrected peak area	1.5	2.7	1.1
Peak height	0.96	2.1	1.1
SEF_{height}[b]	56	60	68

[a] Conditions same as in Fig. 1B.

[b] $SEF_{height} = \frac{\text{peak height obtained with SRMM}}{\text{peak height obtained with usual MEKC injection}} \cdot \text{dilution ratio.}$

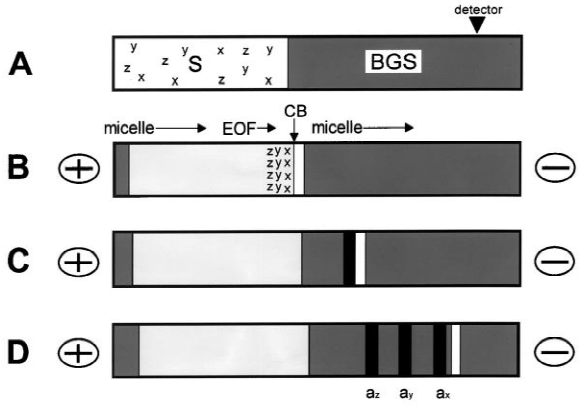

Fig. 2. Schematic diagrams of the SRMM-MEKC model. (A) Sample injection; (B) high-velocity micelles in the sample (S) zone emanating from the anodic vial reach the concentration boundary (CB) carrying some analyte, while a micelle vacant high conductivity zone is formed due to the difference in velocities between BGS and S zones; (C) micelles and neutral analytes stacked at the CB; (D) analyte zones separate by virtue of MEKC. Shaded parts indicate the presence of micelles [concentration of micelles in the BGS (dark-gray parts) is higher than that of the light-gray parts].

zone may be formed because the micelle in the BGS zone will migrate faster than CB which migrates by EOF only. This vacancy zone may cause the loss of stacking efficiency. The detailed mechanism is to be studied further in detail.

3.3. Sweeping

Fig. 3 shows the sweeping MEKC analysis of the three structurally related neutral steroids. Note that Fig. 3A is the electropherogram obtained with normal injection whereas the sample solution used in Fig. 3B is a 100-fold dilution of the sample solution used in Fig. 3A. A usual injection (Fig. 3A) was included for comparison, although it is almost identical to Fig. 1A. Detector responses were improved about 300-fold in terms of peak heights. Table 3 summarizes the results of the LODs, RSDs, and SEF_{height} obtained for the test analytes with sweeping MEKC (12.8 cm injection length). The LODs of the test steroids were in the range from 20 to 9 ppb or 5.5 to $3.1 \cdot 10^{-8}$ M ($S/N=3$). The RSD values

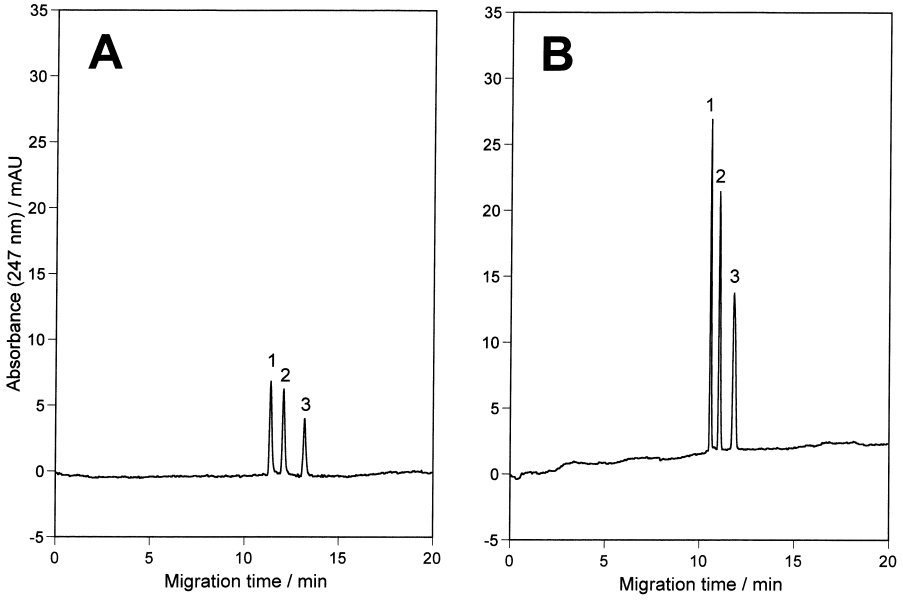

Fig. 3. Separation of the test steroids by (A) conventional MEKC and (B) sweeping MEKC. BGS, 100 mM Tris–HCl buffer (pH 7.0) containing 10% methanol and 50 mM TTAB; sample matrix, (A) BGS, (B) Tris–HCl buffer (pH 7.0) having conductivity similar to that of the BGS (6.9 mS/cm); injected length, (A) 0.58 mm, (B) 12.8 cm; concentration of samples, (A) as Fig. 1A, (B) 100-fold dilution of the samples in A. Other conditions as in Fig. 1.

Table 3
LODs, RSDs, and SEF_{height} for the test steroids in sweeping MEKC[a]

	Testosterone	Hydrocortisone	Cortisone
Calibration line[b]	$y=6.06x-0.87$	$y=4.66x-0.13$	$y=2.69x-0.20$
Correlation coefficient (r)	0.9951	0.9997	0.9992
LOD ($S/N=3$)			
ppb	9	11	20
$\cdot 10^{-8}$ M	3.1	3.0	5.5
RSD (%, $n=4$)			
Migration time	0.15	0.14	0.11
Peak height	3.5	3.2	2.9
Corrected peak area	3.6	3.3	3.0
SEF_{height}[c]	370	300	270

[a] Conditions as in Fig. 3B.
[b] Calibration line: concentration (ppm)=slope×peak height (mAU)+y-intercept.
[c] See Table 2.

obtained with four successive experiments in migration times, corrected peak areas, and peak heights were less than 3.6% for all analytes. The sensitivity enhancements factors were in the range from 270 to 370 and were greater than those obtainable by SRMM. Furthermore, these values are about three-times higher than those of sweeping of the same analytes using a cationic micelle in the presence of EOF [12]. From a comparison with the results it can be stated that the suppressed EOF condition is more favorable for sweeping of neutral steroids than strong EOF condition. However, sweeping MEKC with SDS under acidic conditions gave detection limits one order of magnitude lower than with TTAB in the PAA-coated capillary for the same test steroids. Again it is difficult to explain the discrepancy between the sweeping experiments with SDS and TTAB. The difference in the migration direction between EOF and the electrophoretic migration of the micelle seems not to cause the difference in concentration efficiencies.

Fig. 4 shows that about 600-fold sensitivity enhancement was obtained for two aromatic carboxylic acids. To achieve baseline separation of the target analytes, 20% methanol was added to the BGS. The concentrations of analytes in Fig. 4B are 1000-fold dilution of those of Fig. 4A. Fig. 4A shows the electropherogram of a normal injection MEKC analysis. As can be observed by comparison of Fig. 4A and B, the disturbance of baseline is probably due to change of absorbance when the sample matrix zone (vacancy zone of the micelle) passes the detector. Resolution was a bit poorer with sweeping electropherogram (30 cm injection length) compared to normal injection electropherogram due to the short effective separation zone length caused by long sample injection. Table 4 summarizes the method validation for two aromatic carboxylic acids in sweeping MEKC analysis. The LODs for 2-naphthoic acid and salicylic acid were 0.4 and 3.1 ppb ($S/N=3$), respectively.

Three NDSA isomers were also used as test samples for sweeping MEKC analysis. Fig. 5A shows the electropherogram of conventional MEKC analysis with 0.58 mm injection. Note that the concentrations of analytes in Fig. 5B are 1000-fold dilutions of those in Fig. 5A. As shown in Fig. 5A, the optimum concentration of acetonitrile was 25%, under these conditions the three NDSA isomers were successfully separated. The electropherogram obtained after sweeping (26.1 cm injected) is depicted in Fig. 5B. When the sample solution was injected in higher amounts, peak heights leveled off and peaks showed incomplete separation. This is considered to be a result that the sample zone passed the detector before the complete concentration. The on-line concentration results for three NDSA isomers are summarized in Table 5. Linearity of response spans two

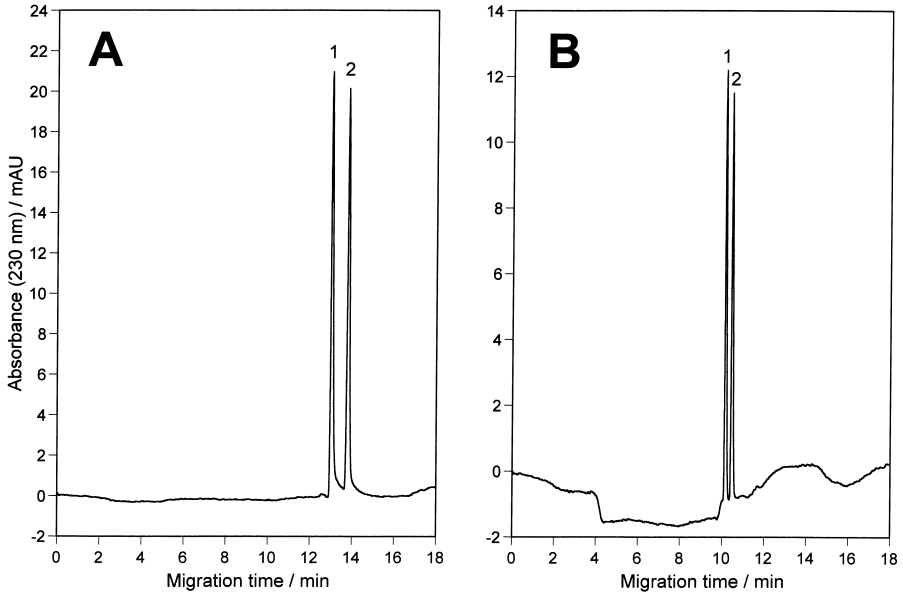

Fig. 4. Separation of aromatic carboxylic acids by (A) conventional MEKC and (B) Sweeping MEKC. BGS, 100 m*M* Tris–HCl buffer (pH 7.0) containing 20% methanol and 50 m*M* CTAC; sample matrix, (A) BGS, (B) Tris–HCl buffer (pH 7.0) having conductivity similar to that of the BGS (5.9 mS/cm); injected length, (A) 0.50 mm, (B) 30 cm; concentration of analytes, (A) 2-naphthoic acid (peak 1, 128 ppm), salicylic acid (peak 2, 933 ppm), (B) 1000-fold dilution of the analytes in A; detection, 230 nm; applied voltage, +18 kV. Other conditions as in Fig. 1.

orders of magnitude. As shown in Table 5, we obtained the LODs of the test NDSA in the range from 2.7 to 0.8 ppb or 7.3 to $2.4 \cdot 10^{-9}$ *M* ($S/N=3$) without any preconcentration step. Acceptable reproducibility was achieved, as RSD values obtained with four successive experiments in migration times,

Table 4
LODs, RSDs, and SEF_{height} for aromatic carboxylic acids in sweeping MEKC[a]

	2-Naphthoic acid	Salicylic acid
Calibration line[b]	$y=0.089x+0.63$	$y=0.012x+0.052$
Correlation coefficient (r)	0.9955	0.9961
LOD ($S/N=3$)		
ppb	0.4	3.1
$\cdot 10^{-8}$ *M*	0.24	2.2
RSD (%, $n=5$)		
Migration time	0.25	0.30
Corrected peak area	3.6	6.2
Peak height	2.9	5.4
SEF_{height}[c]	600	590

[a] Conditions as in Fig. 4B.
[b] See Table 2.
[c] See Table 2.

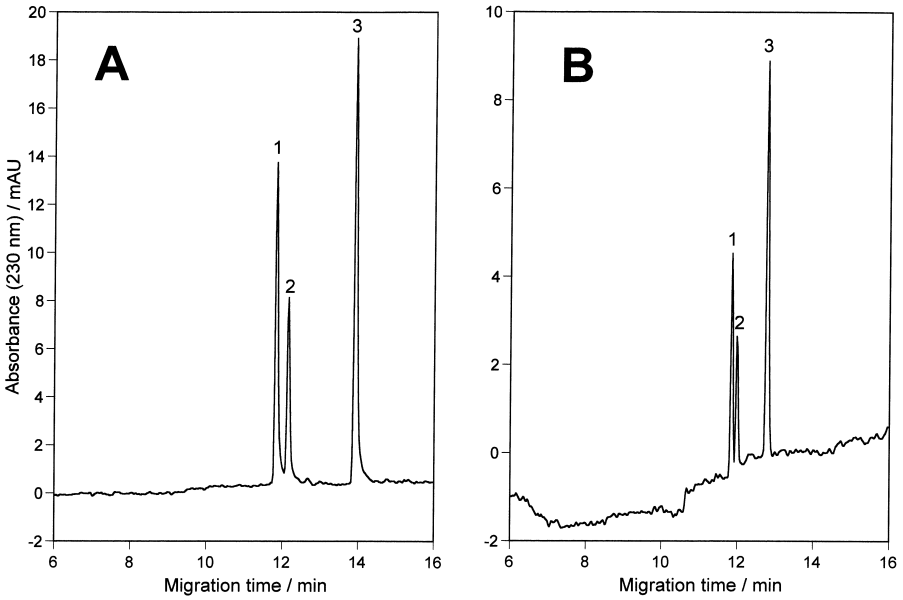

Fig. 5. Separation of naphthalenedisulfonic acids by (A) conventional and (B) Sweeping MEKC. BGS, 88 m*M* Tris–HCl buffer (pH 7.0) containing 25% acetonitrile and 50 m*M* CTAC; sample matrix, (A) BGS, (B) Tris–HCl buffer (pH 7.0) having conductivity similar to that of the BGS (7.5 mS/cm); injected length, (A) 0.58 mm, (B) 26.1 cm; concentration of analytes, (A) 2,7-NDSA (peak 1, 80 ppm), 1,5-NDSA (peak 2, 90 ppm), 2,6-NDSA (peak 3, 89 ppm), (B) 1000-fold dilution of the analytes in A; applied voltage, +16 kV. Other conditions as in Fig. 1.

corrected peak areas, and peak heights were less than 6.4% for the three NDSA isomers. Compared to the usual injection, the SEF_{height} values were improved by around 400-fold by sweeping. In our previous report [12], about 700- to 1000-fold enhancements in detection sensitivity were obtained by sweeping of

Table 5
LODs, RSDs, and SEF_{height} for naphthalenedisulfonic acids in sweeping MEKC[a]

	2,7-NDSA	1,5-NDSA	2,6-NDSA
Calibration line[b]	$y=0.041x+0.99$	$y=0.020x+0.80$	$y=0.070x+1.4$
Correlation coefficient (r)	0.9969	0.9929	0.9974
LOD ($S/N=3$)			
ppb	1.3	2.7	0.8
$\cdot 10^{-9}$ *M*	3.9	7.3	2.4
RSD (%, $n=5$)			
Migration time	1.2	1.3	1.8
Corrected peak area	6.3	5.3	3.9
Peak height	6.4	5.3	4.0
SEF_{height}[c]	360	390	460

[a] Conditions as in Fig. 5B.
[b] See Table 2.
[c] See Table 2.

some negatively chargeable analytes under strong EOF. However, the sensitivity enhancement factors under suppressed EOF were a half levels those of under strong EOF. The reason of the difference is to be clarified.

4. Conclusion

In the present work, we have shown that a PAA-coated capillary can be used in the presence of cationic surfactants for on-line sample concentration in MEKC. The EOF was suppressed significantly even in a neutral pH, when a PAA-coated capillary was used. About 60- to more than 600-fold sensitivity enhancements were obtained with SRMM and sweeping, respectively. In sweeping of some neutral analytes, sensitivity enhancement factor obtained with suppressed EOF were better than those obtained with strong EOF. On the contrary, using some negatively chargeable analytes as test samples, the result under strong EOF was better than under suppressed EOF. In conclusion, the use of PAA-coated capillary is not required, because sweeping with SDS under acidic conditions is superior to sweeping with the cationic micelle under suppressed EOF conditions for neutral analytes and sweeping with the cationic micelle under strong EOF is more efficient than under suppressed EOF for anionic analytes. However, the reason why the suppressed EOF in the PAA-coated capillary caused unexpected results is going to be clarified.

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