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Anion selective exhaustive injection-sweep–micellar electrokinetic chromatography

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Abstract

A selective on-line sample concentration technique of anion selective exhaustive injection (ASEI)-sweep–micellar electrokinetic chromatography (MEKC) was evaluated using a cationic surfactant. To suppress the electroosmotic flow, a polyacrylamide-coated capillary was introduced. Some aromatic carboxylic acids, dansyl amino acids, and naphthalenedisulfonic acids were used as test analytes. About 1000- to 6000-fold increases in detection sensitivity were obtained in terms of peak heights by ASEI-sweep–MEKC.

Keywords: Anion selective exhaustive injection; Injection methods; Micellar electrokinetic chromatography; Sample stacking; Sweeping; Surfactants; Carboxylic acids; Amino acids; Naphthalenedisulfonic acids

1. Introduction

Since first introduced by Terabe et al. [1], micellar electrokinetic chromatography (MEKC) has become widely popular as a powerful separation technique for improving separation efficiency not only for neutral analytes but also for many ionic ones by using a capillary electrophoresis (CE) instrument without any modification. MEKC can take advantage of minimum requirements of sample and separation media as well as the easy exchange of separation solution. However, the main drawback of UV detection MEKC, which is common to other CE modes, is the low concentration sensitivity resulting from a limited volume of sample injected and a short pathlength equal to the capillary diameter. To surmount this problem, many focusing techniques have been reported, some based on instrumental alteration (e.g., powerful detectors or modified detection cells) and others on off-line and on-line preconcentration procedures.

Two on-line sample concentration techniques, sample stacking and sweeping, are known to be attractive approaches for enhancement of the concentration sensitivity in MEKC [2–12]. Sample stacking occurs as ions cross a boundary that separates regions of the high electric field sample zone and the low electric field background solution (BGS) zone. Since the electrophoretic velocity of ions in the sample zone is higher than in the BGS zone, ions slow down when they reach the BGS zone. The analyte is focused at the boundary of the two zones. However, this technique cannot be applied to neutral analytes because neutral analytes have no electro-

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phoretic mobility. In MEKC, to give effective electrophoretic mobilities to neutral analytes charged pseudostationary phases are employed. Sweeping, a new technique for the on-line sample concentration of neutral or charged analytes in MEKC, is defined as a phenomenon where analytes are picked up and concentrated by the pseudostationary phase that penetrates the sample zone without pseudostationary phase [5,6]. The sample solution is not required to be prepared in a low conductivity matrix, but a conductivity similar to that of the BGS is favored. More than 100-fold and 5000-fold enhancements in detection sensitivity in terms of peak heights were obtained by sample stacking and sweeping, respectively, for highly hydrophobic compounds under suppressed electroosmotic flow (EOF) or acidic conditions [4,5].

In most of the papers concerning on-line sample concentrations for MEKC, anionic surfactants [2–10] were used except for only a few papers [11,12]. In our previous reports, to expand the applicability of these on-line sample concentration techniques, we performed on-line sample concentration in MEKC with cationic micelles under strong EOF conditions [11] and suppressed EOF conditions [12]. Sweeping of some anionic analytes gave high concentration factors up to 1000-fold without any preconcentration step [11].

Recently, a combination of sample stacking and sweeping, cation selective exhaustive injection and sweeping (CSEI-sweep), achieved almost million-fold enhancements in detector response for the cationic hydrophobic analytes [9]. On-line concentration of positively charged analytes with anionic sodium dodecyl sulfate (SDS) micelles provided high sensitivity enhancements because of the strong interaction between oppositely charged analytes and the SDS micelle. Therefore, to effectively concentrate negatively charged analytes the use of the cationic micelles is straightforward.

In this paper, we show about 1000- to 6000-fold increase in UV detector response for negatively chargeable analytes in MEKC. This was accomplished by the proper combination of sample stacking with electrokinetic injection (field-enhanced sample injection, FESI) and sweeping, which we name as anion selective exhaustive injection and sweeping (ASEI-sweep). To suppress the EOF, a polyacrylamide (PAA)-coated capillary can be employed in the presence of cationic surfactants for ASEI-sweep–MEKC. Aromatic carboxylic acids, dansyl amino acids, and naphthalenedisulfonic acids were used as test analytes.

2. Principle of ASEI-sweep–MEKC

The ASEI-sweep–MEKC model is illustrated in Fig. 1, which is basically the same as CSEI-sweep–MEKC reported by Quirino and Terabe [9], but the procedure was modified as follows. The column is a PAA-coated capillary that is filled with a nonmicellar BGS. In step A, a zone of a high-conductivity buffer devoid of micelles (HCB) followed by a short water plug is introduced hydrodynamically. In step B, the anion sample prepared in a low-conductivity solution is injected electrokinetically at the negative polarity. The sample ions enter the capillary through the water plug with high velocities. Once the sample ions reach the interface between the water and HCB zones, they will slow down and focus at this interface. The continued electrokinetic injection builds up a long concentrated sample zone, which is too long to give high resolution unless it is refocused. It should be noted that the direction of the suppressed EOF is toward the cathode but the anionic analytes is toward the anode. In step C, once the separation voltage is applied at the positive polarity with the micellar BGS in the inlet vial, cationic micelles will enter the capillary and sweep the analytes. The stacked anions are completely swept by the micelle and are separated by MEKC in the reverse migration mode.

3. Experimental

3.1. Apparatus

A Hewlett-Packard ^{3D}CE System (Waldbronn, Germany) with a UV absorbance detector was used for all experiments. Fused-silica capillaries (50 μm I.D.×360 μm O.D.) were purchased from Polymicro Technologies (Phoenix, AZ, USA) and used with a surface modification, i.e., PAA coating [12]. The capillary temperature was thermostated at 25°C.

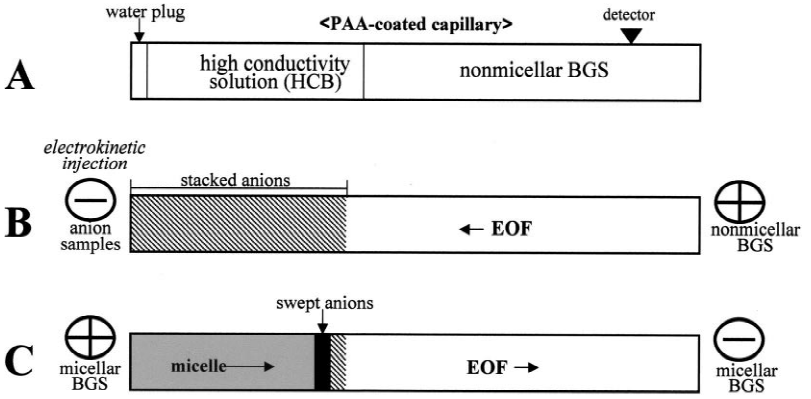

Fig. 1. Schematic diagrams of the ASEI-sweep–MEKC model. (A) PAA-coated capillary is conditioned with a nonmicellar BGS, then a high conductivity buffer devoid of micelles (HCB) is injected, followed by the injection of a short water plug. (B) Electrokinetic injection for a long time at the negative polarity of anionic analytes prepared in a low conductivity matrix wherein anionic analytes focus at the interface between the water and HCB zones. (C) Micellar BGSs are placed at both ends of the capillary followed by the application of the voltage at the positive polarity.

Samples were introduced by pressure (5 kPa) or electrokinetic injection. An optimum detection wavelength was selected for each analyte based upon the UV spectra recorded by the diode-array detector. Conductivity of sample and separation solutions was measured using a Horiba ES-12 conductivity meter (Kyoto, Japan).

3.2. Chemicals

Tris(hydroxymethyl)aminomethane (Tris), cetyltrimethylammonium chloride (CTAC), salicylic acid, 2,6-naphthalenedisulfonic acid disodium salts (2,6-NDSA), and *p*-methoxycinnamic acid were purchased from Wako (Osaka, Japan). 2,7-Naphthalenedisulfonic acid disodium salts (2,7-NDSA) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). 2,5-Dihydroxybenzoic acid was purchased from Aldrich (Milwaukee, WI, USA). Three dansyl amino acids including dansyl-leucine (Dns-Leu), dansyl-valine (Dns-Val), and dansyl-serine (Dns-Ser) were obtained from Sigma (St. Louis, MO, USA). Other reagents were purchased from Nacalai Tesque (Kyoto, Japan). All reagents were of analytical-reagent grade and used without further purification. Water used for the matrix and sample preparations was obtained by using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Buffers were prepared from stock solutions of Tris and hydrochloric acid (HCl). Stock solutions of aromatic carboxylic acids were prepared in 50% aqueous methanol or methanol. Stock solutions of dansyl amino acids were prepared in 50% aqueous methanol. Stock solutions of naphthalenedisulfonic acids were prepared in purified water. Buffer solutions were sonicated and filtered through 0.45-μm filters before use.

3.3. CE procedure

New PAA-coated capillaries were pretreated by rinsing at pressure (ca. 1 bar) with purified water for 20 min and BGS for 10 min. To ensure reproducibility, at the end of each run the capillary was rinsed with methanol for 2 min, followed by with purified water for 3 min and then with the BGS for 3 min.

The volume of samples in injection vials was 40 μl. We did not obtained any degradation when a small volume (40 μl) of sample in the vial due to disturbances from the electrophoresis. To estimate the plug lengths of the injected zones by pressure, a neutral marker was injected into the capillary, and then the 5 kPa pressure was applied until a response was detected. From the velocity that was obtained, the lengths of the zones were computed given the injection time.

4. Results and discussion

4.1. Introduction of PAA-coated capillary

The addition of cationic surfactants to the BGS caused the reversal of EOF owing to positively charged capillary wall by the adsorption of cationic surfactants [13,14]. The reversed EOF directs towards the anode, whereas the cationic micelle has the electrophoretic mobility in the opposite direction. In CSEI-sweep–MEKC, EOF was suppressed significantly under acidic conditions with anionic SDS micelles [9,10]. However, the EOF 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 surface of the fused-silica capillary [11]. In our previous report, EOF was successfully suppressed even under the neutral pH, when a PAA-coated capillary was employed [12]. Therefore, we employed a PAA-coated capillary in the presence of cationic surfactant for ASEI-sweep–MEKC. The magnitude of suppressed EOF was $0.13 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} with a micellar BGS consisting of 50 m*M* CTAC–75 m*M* Tris–HCl–15% acetonitrile, pH 7.0, which is almost the same magnitude as observed for tetradecyltrimethylammonium bromide under the same conditions [12].

4.2. Effect of an HCB zone on ASEI-sweep–MEKC

Effect of an HCB that was injected before the water plug on ASEI-sweep–MEKC is illustrated in Fig. 2. The water plug provides a high electric field at the tip of the capillary in sample stacking by electrokinetic injection, which will eventually improve the sample stacking procedure [15]. Fig. 2A shows the electropherogram of conventional MEKC with a 0.55 mm injection. The concentration of the test analytes, *p*-methoxycinnamic acid and 2,5-dihydroxybenzoic acid, in Fig. 2B and C is 10 000-fold dilution of the corresponding sample solution in Fig. 2A. In Fig. 2B and C, a PAA-coated capillary is conditioned with a nonmicellar BGS. Fig. 2B shows the electropherogram of ASEI-sweep–MEKC without the HCB injection. As shown in Fig. 2B, only unseparated broad peak was observed. On the other hand, when an HCB zone followed by a short water zone was injected, separation and peak shapes were improved (Fig. 2C). This finding is consistent with

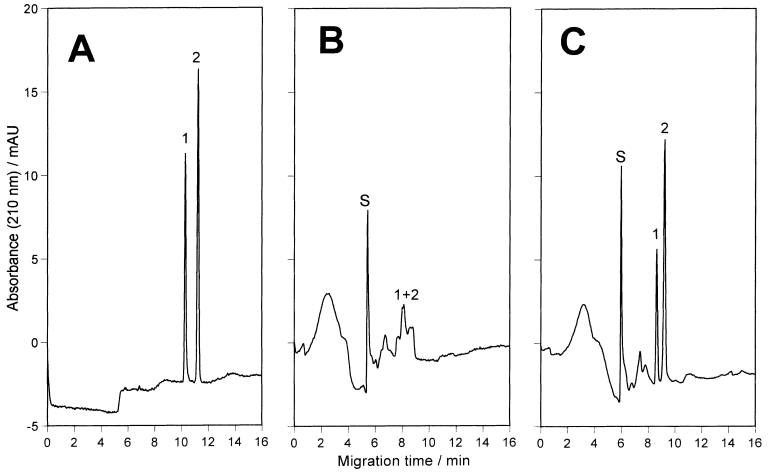

Fig. 2. Effect of a high conductivity buffer on ASEI-sweep–MEKC. Nonmicellar BGS, 100 m*M* Tris–HCl (pH 7.0) containing 20% acetonitrile; micellar BGS, 75 m*M* Tris–HCl (pH 7.0) containing 50 m*M* CTAC and 20% acetonitrile; HCB, 200 m*M* Tris–HCl; sample matrix, 2.5 m*M* Tris–HCl; conditioning solution in the capillary before injection, (A) micellar BGS, (B, C) nonmicellar BGS; injection scheme, (A) 0.55 mm, (B) 3.3 mm pressure injection of water, followed by −13 kV electrokinetic injection of sample for 250 s, (C) 2.8 cm pressure injection of HCB, then 3.3 mm pressure injection of water, followed by −13 kV electrokinetic injection of sample for 250 s; separation voltage, +13 kV with the micellar BGS at both ends of the capillary; detection, 210 nm; PAA-coated capillary, 42 cm total (33.5 cm to detector). Other conditions as described in the Experimental section.

the CSEI-sweep–MEKC technique we developed before [9]. The HCB zone provides a trap in which the analytes are stacked prior to being swept and separated in the micellar BGS [9,16]. The system peak that appeared before the peaks of interest in Fig. 2B and C resulted from the change in composition of the solution that passed the detector. The peak height observed with the injection of an HCB was about five times higher than that without the HCB injection.

4.3. ASEI-sweep–MEKC of anionic analytes

Fig. 3 shows the ASEI-sweep–MEKC analysis of aromatic carboxylic acids using CTAC micelles. To achieve baseline separation of the target analytes, 15% (v/v) acetonitrile was added to the BGS. Fig. 3A shows the electropherogram of conventional MEKC with 0.64 mm injection. Fig. 3B shows the electropherogram of the test analytes by ASEI-sweep–MEKC. The concentration of the test analytes in Fig. 3B is 10 000-fold dilution of the corresponding sample solution in Fig. 3A. In comparison to the peak heights obtained with 0.64 mm injection, as shown in Fig. 3B, about 2700-fold enhancements of peak heights were achieved without loss in resolution. It should be noted that the neutral pH was employed for the micellar solution to secure complete ionization of the carboxylic acids and EOF was suppressed ($0.13 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}) even under the neutral pH with the PAA-coated capillary.

The relative standard deviations (RSDs) and sensitivity enhancement factors in terms of peak heights (SEF_{height}) obtained for the aromatic carboxylic acids in ASEI-sweep–MEKC are summarized in Table 1. Fresh sample solutions were always used for each injection. Acceptable reproducibility was achieved, as RSD values obtained with three consecutive runs in the migration times. However, RSD values in the corrected peak areas (peak area divided by the migration time) and peak heights ranged from 4 to 15% for all analytes. The poor reproducibility was mainly caused by the long electrokinetic injection time, which was confirmed by additional experiments on prolonged electrokinetic injection under the same condition as ASEI-sweep–MEKC without sweeping. Salicylic acid solution was electrokinetically injected for 2, 50, 200 s and RSDs ($n=3$) of the peak area were 1.5, 8.9 and 12.6%, respectively. The results showed clearly that the

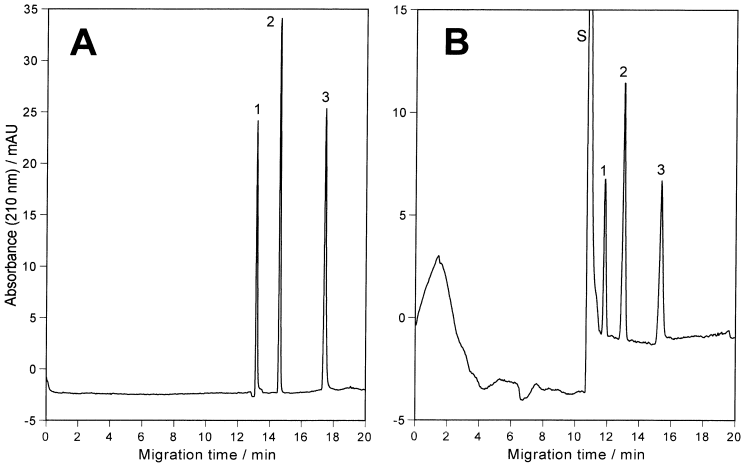

Fig. 3. Conventional MEKC and ASEI-sweep–MEKC of aromatic carboxylic acids. Nonmicellar BGS, 100 m*M* Tris–HCl (pH 7.0) containing 15% acetonitrile; micellar BGS, 75 m*M* Tris–HCl (pH 7.0) containing 50 m*M* CTAC and 15% acetonitrile; HCB, 200 m*M* Tris–HCl; sample matrix, 2.5 m*M* Tris–HCl; conditioning solution before injection, (A) micellar BGS, (B) nonmicellar BGS; concentration of samples, (A) 2-naphthoic acid (peak 1, 216 ppm), salicylic acid (peak 2, 230 ppm), cinnamic acid (peak 3, 236 ppm), (B) 10 000-fold dilution of samples in A; injection scheme, (A) 0.64 mm, (B) 2.8 cm pressure injection of HCB, then 3.3 mm pressure injection of water, followed by −17 kV electrokinetic injection of sample for 250 s; separation voltage, +17 kV with the micellar BGS at both ends of the capillary; PAA-coated capillary, 59 cm total (50.5 cm to detector). Other conditions as described in Fig. 2.

Table 1
RSDs and SEF_{height} for aromatic carboxylic acids in ASEI-sweep–MEKC[a]

	2-Naphthoic acid	Salicylic acid	Cinnamic acid
RSD (%, $n=3$)			
(a) Migration time	1.3	1.0	0.72
(b) Corrected peak area	3.7	13.2	7.6
(c) Peak height	9.0	14.6	10.4
SEF_{height}[b]	2700	2700	2400

[a] Conditions as in Fig. 3B.

[b] $SEF_{height} = \frac{\text{peak height obtained with ASEI-sweep–MEKC}}{\text{peak height obtained with normal MEKC injection}} \cdot \text{dilution factor}$

longer injection times brought about poor reproducibility in peak area. The sensitivity enhancement was about three and five times greater than that obtainable by sweeping under strong EOF [11] and suppressed EOF [12] conditions, respectively. Here, sensitivity enhancement factors were calculated by simply getting the ratio of the peak heights obtained from ASEI-sweep–MEKC and normal injection and the quotient multiplied by the dilution factor.

The optimized ASEI-sweep–MEKC analysis of dansyl amino acids is illustrated in Fig. 4. It was found that a BGS with 100 m*M* Tris–HCl containing 50 m*M* CTAC and 25% acetonitrile gave good separation of the test analytes (Fig. 4A). Fig. 4A shows a representative electropherogram of the baseline separation of the sample mixture injected by the hydrodynamic injection mode (50 mbar for 0.58 mm). The samples used in Fig. 4B is 1000-fold dilution of the samples used in Fig. 4A. The electropherogram obtained after ASEI-sweep–MEKC is depicted in Fig. 4B. About 1000-fold increase in detector response compared to normal injection was achieved for the test analytes. This value was about three times and 30 times greater than that obtainable by sweeping (about 300-fold enhancement) and stacking with reverse migrating micelles (about 35-fold enhancement) of the same analytes, respectively. The electrokinetic injection time of 200 s was

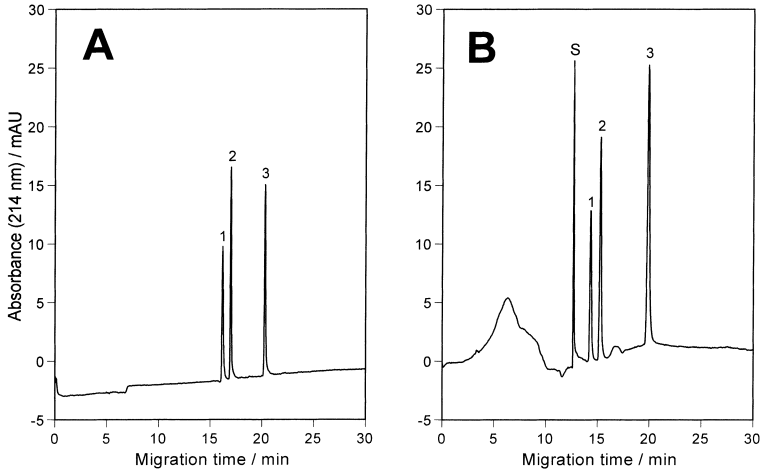

Fig. 4. Conventional MEKC and ASEI-sweep–MEKC of dansyl amino acids. Nonmicellar BGS, 100 m*M* Tris–HCl (pH 7.0) containing 25% acetonitrile; micellar BGS, 100 m*M* Tris–HCl (pH 7.0) containing 50 m*M* CTAC and 25% acetonitrile; HCB, 200 m*M* Tris–HCl; sample matrix, 2.5 m*M* Tris–HCl; conditioning solution before injection, (A) micellar BGS, (B) nonmicellar BGS; concentration of samples, (A) Dns-Leu (peak 1, 156 ppm), Dns-Val (peak 2, 156 ppm), Dns-Ser (peak 3, 144 ppm), (B) 1000-fold dilution of samples in A; injection scheme, (A) 0.58 mm, (B) 4.4 cm pressure injection of HCB, then 3.3 mm pressure injection of water, followed by −15 kV electrokinetic injection of sample for 200 s; separation voltage, +15 kV with the micellar BGS at both ends of the capillary; detection, 214 nm. Other conditions as described in Fig. 3.

Table 2
LODs, RSDs, and SEF_{height} for dansyl amino acids in ASEI-sweep–MEKC[a]

	Dns-Leu	Dns-Val	Dns-Ser
Calibration line[b]	$y=0.0689x-0.0043$	$y=0.0957x+0.0769$	$y=0.1092x+0.7601$
Correlation coefficient (r)	0.9995	0.9994	0.9999
LOD ($S/N=3$)			
(a) ppb	1.2	0.9	0.8
(b) $\cdot 10^{-9}$ M	2.6	2.0	1.8
RSD (%, $n=4$)			
(a) Migration time	0.50	0.52	0.80
(b) Corrected peak area	8.2	6.1	2.3
(c) Peak height	3.8	2.3	6.9
SEF_{height}[c]	1150	1000	1430

[a] Conditions as in Fig. 4B.
[b] Calibration line: peak height (mAU) = slope·concentration (ppb) + y-intercept. Concentration range: 200–20 ng/ml.
[c] See Table 1.

chosen since longer injections did not produce further increase in relative peak heights. Table 2 lists the method validation for three dansyl amino acids in ASEI-sweep–MEKC analysis. The linearity of the present ASEI-sweep technique was checked for peak heights against concentrations. Calibration lines with good linearity were achieved. Relatively high intercept of calibration line of Dns-Ser could be explained by the high RSD of peak height. To calculate the limits of detection (LODs), three times the standard deviation of noise level was used as the signal-to-noise ratio 3 ($S/N=3$). We obtained the LODs of the test analytes in the range from 1.2 to 0.8 ppb or 2.6 to $1.8\cdot10^{-9}$ M with UV detection. Under optimized condition, the RSDs for migration times, corrected peak areas, and peak heights were less than 8.2% for all analytes.

Two naphthalenedisulfonic acid (NDSA) isomers were also used as test samples for ASEI-sweep–MEKC analysis. Fig. 5A shows the electropherogram

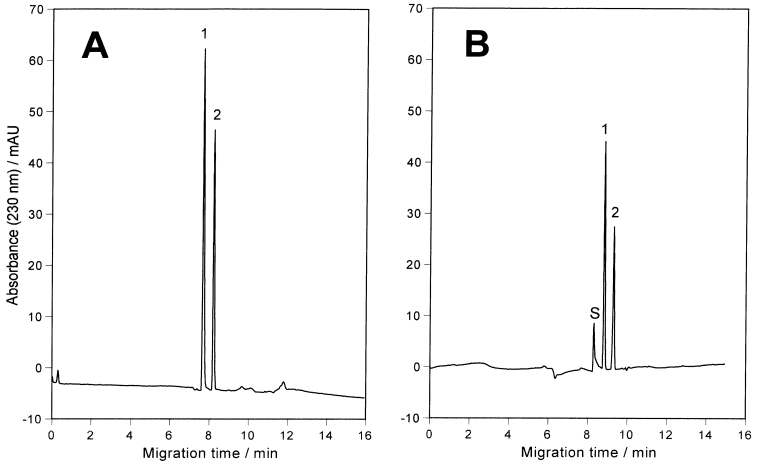

Fig. 5. Conventional MEKC and ASEI-sweep–MEKC of NDSAs. Nonmicellar BGS, 100 mM Tris–HCl (pH 7.0) containing 20% acetonitrile; micellar BGS, 75 mM Tris–HCl (pH 7.0) containing 50 mM CTAC and 20% acetonitrile; HCB, 200 mM Tris–HCl; sample matrix, 2.5 mM Tris–HCl; conditioning solution before injection, (A) micellar BGS, (B) nonmicellar BGS; concentration of samples, (A) 2,7-NDSA (peak 1, 400 ppm), 2,6-NDSA (peak 2, 223 ppm), (B) 10 000-fold dilution of samples in A; injection scheme, (A) 0.55 mm, (B) 4.4 cm pressure injection of HCB, then 3.3 mm pressure injection of water, followed by −20 kV electrokinetic injection of sample for 220 s; voltage, +20 kV with the micellar BGS at both ends of the capillary; detection, 230 nm. Other conditions as described in Fig. 3.

of conventional MEKC analysis with 0.55 mm injection. Note that the concentrations of analytes in Fig. 5B are 10 000-fold dilution of those in Fig. 5A. As shown in Fig. 5A, the optimum concentration of acetonitrile was 20%, under that conditions the two NDSA isomers was successfully separated. Fig. 5B illustrates the electropherogram of the test analytes by ASEI-sweep–MEKC. When the sample solution was injected in greater volumes, no improvement in peak heights was observed. Furthermore, peaks showed broad or asymmetric shapes. The on-line concentration results for two NDSA isomers are listed in Table 3. As shown in Table 3, we obtained the LODs of the test NDSAs in the range from 80 to 60 ppt or 2.4 to $1.8 \cdot 10^{-10}$ M ($S/N=3$) with UV detection. These LODs are much lower than that reported in previous papers, where off-line preconcentration procedure was used in combination with a powerful laser-induced fluorescence detector for the analysis of naphthalenesulfonic acids [17,18]. Because of the long electrokinetic injection time, as mentioned above, poor reproducibility was generated as shown in RSD values for the corrected peak areas and peak heights (12.4~17.8% for the test analytes). Good reproducibility was achieved, as RSD values obtained with five successive experiments in migration times. Compared to the usual injection, the SEF_{height} values were improved about 6000-fold by ASEI-sweep–MEKC. This value was about eight times greater than that obtainable by sweeping of the same analytes only.

In conclusion, although only a preliminary result is shown, it is expected that ASEI-sweep will be a powerful technique to increase sensitivity in MEKC analysis of negatively chargeable analytes. However, the sensitivity enhancements achieved in this work is modest compared to CSEI-sweep–MEKC. Based on the presented results only it is difficult to state that the reason why the modest enhancements were achieved in ASEI-sweep–MEKC. The exact reasons should be studied in detail.

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Table 3
LODs, RSDs, and SEF_{height} for naphthalenedisulfonic acids in ASEI-sweep–MEKC[a]

	2,7-NDSA	2,6-NDSA
Calibration line[b]	$y=1.1130x+0.1239$	$y=1.6419x+0.0760$
Correlation coefficient (r)	0.9951	0.9980
LOD ($S/N=3$)		
(a) ppt	80	60
(b) $\cdot 10^{-10}$ M	2.4	1.8
RSD (%, $n=5$)		
(a) Migration time	0.87	0.76
(b) Corrected peak area	14.3	13.2
(c) Peak height	12.4	17.8
SEF_{height}[c]	5900	5800

[a] Conditions as in Fig. 5B.
[b] See Table 2. Concentration range: 10–1 ng/ml.
[c] See Table 1.

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