

# On-line sample preconcentration in micellar electrokinetic chromatography using ion-pair reagents

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## Abstract

To improve the detection sensitivity of some aromatic carboxylic acids and naphthalenesulfonic acids, the use of ion-pair reagents was examined in sweeping micellar electrokinetic chromatography (MEKC) with an anionic sodium dodecyl sulfate (SDS) micelle. Tetraalkylammonium (TAA) salts were used as ion-pair reagents to improve the sweeping efficiency. The effects of the alkyl chain length of the TAA groups and the TAA salt concentration on sweeping were examined. Under optimized conditions, about 400-fold enhancement in detection sensitivity was obtained in terms of peak heights by addition of ion-pair reagents in sweeping MEKC. This value was about 10 times greater than that obtainable by the SDS micelle used alone.

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## 1. Introduction

Micellar electrokinetic chromatography (MEKC) is a mode of capillary electrophoresis (CE) which effectively separates neutral analytes [1] and also provides enhanced selectivity for separations of ionic species [2–4]. Moreover, this technique offers faster analysis times and greater efficiencies than those achieved by high-performance liquid chromatography (HPLC). However, the low concentration sensitivity with photometric detection is one of the major drawbacks of MEKC, as in other modes of CE, due to the short optical pathlength, and many

studies have been carried out to improve the sensitivity.

Sweeping was developed for on-line sample preconcentration in MEKC [5,6] and has been demonstrated to be a useful technique to improve the detection sensitivity [7–13]. The sweeping mechanism has been described as a process where analytes are picked up and concentrated by the pseudo-stationary phase that penetrates the sample zone devoid of pseudostationary phase. In our previous reports [7–9], test anionic analytes were effectively concentrated by sweeping MEKC using cationic surfactants.

Ion-pair reagents have previously been used for the selectivity enhancement of ionic analytes in capillary zone electrophoresis (CZE) [14] and MEKC [15]. The purpose of this study was to improve the detection sensitivity of some anionic analytes when using ion-pair reagents in sweeping MEKC with an anionic sodium dodecyl sulfate

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(SDS) micelle. Anionic analytes, such as carboxylates, normally possess weak affinity for anionic SDS micelles owing to electrostatic repulsion. However, when an ammonium salt is added to the background solution (BGS), the ammonium cation will interact with the carboxylate anion to form an ion-pair. The paired ions thus formed are electrically neutral and are partitioned into the micelle to a greater extent than the free carboxylate form.

In this study, tetraalkylammonium (TAA) salts were used as ion-pair reagents to improve the sweeping efficiency of the test analytes. The effects of the alkyl chain length of the TAA groups and the TAA salt concentration on sweeping were examined.

## 2. Experimental

### 2.1. Apparatus

All electrophoresis experiments were performed using a Hewlett-Packard 3D CE (Waldbronn, Germany) instrument equipped with a UV absorbance detector. Fused-silica capillaries were 60 cm (51.5 cm to detector)  $\times$  50  $\mu$ m I.D., from Polymicro Technologies (Phoenix, AZ, USA). The capillary temperature was maintained at 25 °C. Samples were introduced by pressure (5 kPa) injection. An optimum detection wavelength was selected for each analyte based upon the UV spectra recorded by the diode-array detector. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). Conductivities of sample and separation solutions were measured using a Horiba ES-12 conductivity meter (Kyoto, Japan).

### 2.2. Chemicals

Cetyltrimethylammonium chloride (CTAC), sodium dihydrogenphosphate dihydrate, sodium tetraborate, phthalic acid, isophthalic acid, 1-naphthalenesulfonic acid sodium salt (1-NSA), and 1,5-naphthalenedisulfonic acid disodium salt (1,5-NDSA) were purchased from Wako (Osaka, Japan). SDS, tetraethylammonium chloride (TEAC), tetrabutylammonium chloride (TBAC), and tetrapentylammonium chloride (TPAC) were obtained from Nacalai Tesque (Kyoto, Japan). All reagents were of

analytical-reagent grade and used without further purification.

Buffers were prepared from stock solutions of sodium tetraborate and sodium dihydrogenphosphate dihydrate. Stock solutions of phthalic and isophthalic acids were prepared in 50% aqueous methanol. Stock solutions of naphthalenesulfonic acids were prepared in purified water. All solutions were sonicated and filtered through 0.45  $\mu$ m filters prior to CE experiments.

### 2.3. CE procedure

A new capillary was pretreated by flushing at pressure (ca. 100 kPa) with 1.0 M sodium hydroxide for 20 min, followed by methanol for 20 min, 0.1 M sodium hydroxide for 20 min, purified water for 20 min, and finally the BGS for 10 min. To ensure reproducibility, at the end of each run the capillary was rinsed with 0.1 M sodium hydroxide for 2 min, methanol for 2 min, followed by purified water for 2 min and then with the BGS for 2 min. Capillaries were reconditioned at the start of each day's experiments by flushing with 1.0 M sodium hydroxide and purified water for 10 min each.

For the sweeping experiments, test analytes prepared in borate–phosphate solutions with a conductivity similar to that of the BGS were pressure injected into the capillary at the anodic end. The BGS vials were then set at both ends of the capillary and the separation voltage was applied at positive polarity. Other experimental conditions are described in the text or figure legends.

## 3. Results and discussion

### 3.1. Sweeping of aromatic carboxylates using a cationic micelle

Generally, the addition of cationic surfactants to the BGS causes anodic electroosmotic flow (EOF) owing to the capillary wall being positively charged by the adsorption of cationic surfactants [16,17]. Here, therefore, negative polarity was applied at the inlet electrode to detect the analytes.

The electropherogram obtained after the sweeping (80 s injection) of aromatic carboxylates using the

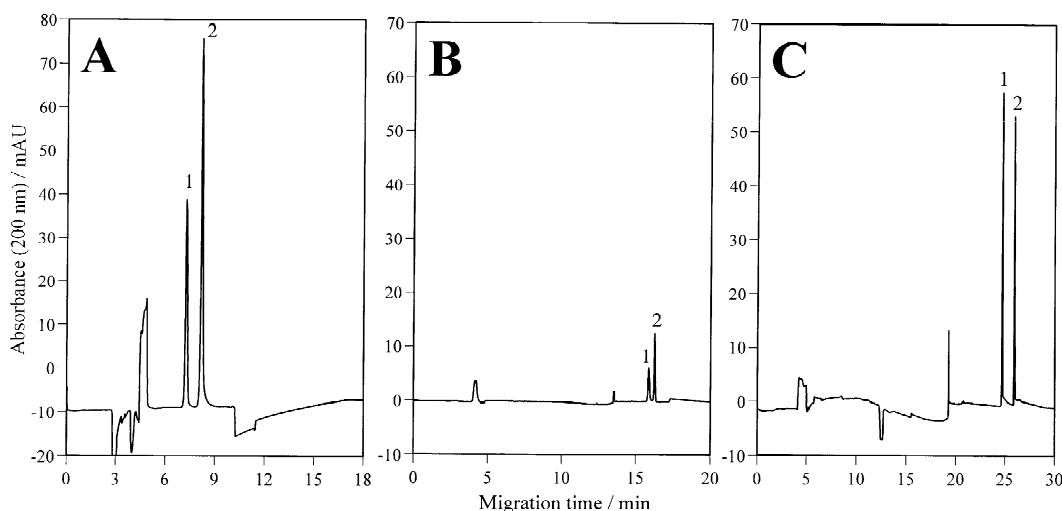


Fig. 1. Sweeping MEKC of aromatic carboxylates using (A) a cationic CTAC micelle, (B) an anionic SDS micelle, (C) a SDS micelle and TBAC. BGS, 40 mM borate–phosphate buffer (pH 9.0) containing (A) 80 mM CTAC and 15% acetonitrile, (B) 80 mM SDS, (C) 80 mM SDS and 20 mM TBAC; sample solution, borate–phosphate buffer (pH 9.0) with a conductivity similar to that of the BGS; concentration of samples, (A) phthalic acid (peak 1, 10 ppm), isophthalic acid (peak 2, 10 ppm), (B,C) phthalic acid (1 ppm), isophthalic acid (1 ppm); injection time, (A) 80 s, (B) 40 s, (C) 120 s; voltage, (A) –20 kV, (B,C) +20 kV; capillary, 60 cm total length (51.5 cm to detector). Other conditions as described in Experimental.

cationic CTAC micelle is depicted in Fig. 1A. To obtain a baseline separation between phthalic acid and isophthalic acid, 15% (v/v) acetonitrile was added to the BGS. An injection time of 80 s was chosen since longer injections did not produce further enhancement in relative peak heights. Note that the concentrations of analytes in Fig. 1A are 10-fold dilutions of those in conventional MEKC (1 s injection). Table 1 summarizes the results of the

Table 1  
RSDs and  $SEF_{\text{height}}$  for aromatic carboxylates in sweeping MEKC

	Phthalic acid		Isophthalic acid	
	CTAC <sup>a</sup>	SDS <sup>b</sup>	CTAC <sup>a</sup>	SDS <sup>b</sup>
RSD (% , $n=4$ )				
(a) Migration time	1.8	0.20	2.0	0.17
(b) Corrected peak area <sup>c</sup>	3.8	6.0	3.3	2.2
(c) Peak height	5.6	10.2	5.7	9.1
$SEF_{\text{height}}$ <sup>d</sup>	20	40	30	60

<sup>a</sup> Conditions as in Fig. 1A.

<sup>b</sup> Conditions as in Fig. 1B.

<sup>c</sup> Corrected peak area = peak area/migration time.

<sup>d</sup>  $SEF_{\text{height}} = \frac{\text{peak height obtained with sweeping MEKC}}{\text{peak height obtained with normal MEKC injection}}$

· dilution ratio

relative standard deviations (RSDs) and sensitivity enhancement factors in terms of peak heights ( $SEF_{\text{height}}$ ) obtained for the test analytes with sweeping MEKC. Acceptable reproducibility was achieved as the RSD values, obtained for four successive runs with respect to migration times, corrected peak areas (peak area divided by migration time), and peak heights. The detection sensitivity can be enhanced about 20- to 30-fold. Sensitivity enhancement factors were calculated simply from the ratio of the peak heights obtained from sweeping and normal injection (1 s) and correction by the dilution ratio (10).

### 3.2. Sweeping of aromatic carboxylates using an anionic micelle

Fig. 1B shows the electropherogram of a sweeping MEKC (40 s injection) using an anionic SDS micelle. Unlike with the use of a cationic surfactant, good baseline separation was obtained without acetonitrile. The concentration of the test analyte in Fig. 2B is a 100-fold dilution of the corresponding sample solution in conventional MEKC (1 s injection). The results for the RSDs and  $SEF_{\text{height}}$  obtained for the test analytes with sweeping MEKC

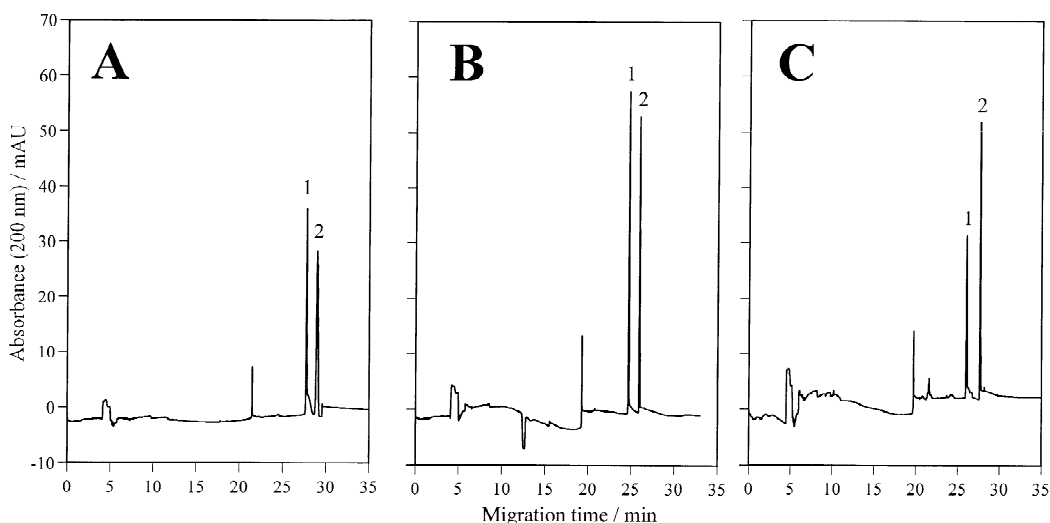


Fig. 2. Effect of ion-pair reagents on peak heights. BGS, 80 mM SDS in 40 mM borate–phosphate buffer (pH 9.0) containing (A) 20 mM TEAC, (B) 20 mM TBAC, (C) 20 mM TPAC; sample solution, borate–phosphate buffer (pH 9.0) with a conductivity similar to that of the BGS; concentration of samples, phthalic acid (peak 1, 1 ppm), isophthalic acid (peak 2, 1 ppm); injection time, 120 s. Other conditions as described in Fig. 1.

are listed in Table 1. Detector responses were improved about 40- to 60-fold in terms of peak heights. These values are two times greater than those obtained with a CTAC micelle. From the viewpoint of electrostatic interaction, however, it was unexpected that the  $SEF_{\text{height}}$  obtained with the SDS micelle would be much higher than that of the CTAC micelle used. Hydrophobic interactions may contribute more strongly to the incorporation of the aromatic carboxylates by the SDS micelle.

### 3.3. Sweeping of aromatic carboxylates using a SDS micelle and ion-pair reagents

#### 3.3.1. Effect of ion-pair reagent on peak heights

Fig. 2 shows the effect of ion-pair reagents on peak heights in sweeping MEKC. The BGS was 80 mM SDS in 40 mM borate–phosphate buffer. The concentrations of analytes in Fig. 2 are the same as in Fig. 1B (1 ppm each). TEAC, TBAC, and TPAC were added to the BGS. The optimum TBAC concentration (5–30 mM) was found to be 20 mM in terms of separation and sensitivity.

#### 3.3.2. Sweeping of aromatic carboxylates using a SDS micelle and TBAC

Fig. 1C shows the sweeping MEKC of aromatic carboxylates using an anionic SDS micelle and TBAC with an injection time of 120 s. To obtain a greater detector response, different injection times were tested. The best results were obtained for an injection time of 120 s. Injections much greater than this in volume resulted in broad peaks. In comparison with the peak heights obtained with 1 s injection, about 400-fold improvements in peak heights were achieved without loss of resolution. The results for the RSDs and  $SEF_{\text{height}}$  obtained for the aromatic carboxylates with sweeping MEKC are summarized in Table 2. Acceptable reproducibility was achieved as the RSD values obtained for three consecutive runs. The sensitivity enhancement was about 10 times greater than that obtainable with the SDS micelle used alone.

#### 3.4. Sweeping of naphthalenesulfonic acids using SDS micelle and ion-pair reagents

Fig. 3 shows conventional and sweeping MEKC of naphthalenesulfonic acids using SDS micelle and

Table 2  
RSDs and  $SEF_{\text{height}}$  for aromatic carboxylates in sweeping MEKC<sup>a</sup>

	Phthalic acid	Isophthalic acid
RSD (% , $n=3$ )		
(a) Migration time	2.0	2.2
(b) Corrected peak area <sup>b</sup>	7.1	0.76
(c) Peak height	5.5	3.8
$SEF_{\text{height}}$ <sup>c</sup>	450	380

<sup>a</sup> Conditions as in Fig. 1C.

<sup>b</sup> See Table 1.

<sup>c</sup> See Table 1.

ion-pair reagents. Fig. 3A shows the electropherogram of a conventional MEKC (1 s injection). Focusing of these analytes by sweeping (80 s injection) with SDS was not very successful, as shown in Fig. 3B. 1-NSA (peak 1) appeared as a very broad peak, whereas 1,5-NDSA (peak 2) was focused about 50-fold. However, when TBAC (Fig. 3C) or TPAC (Fig. 3D) was added to the SDS solution, a further sensitivity enhancement of 1,5-NDSA by sweeping (80 s injection) was obtained. In spite of the addition of ion-pair reagents, 1-NSA still appeared as a broad peak due to weak interaction. Note that the concentration of naphthalenesulfonic acids in Fig. 3B–D

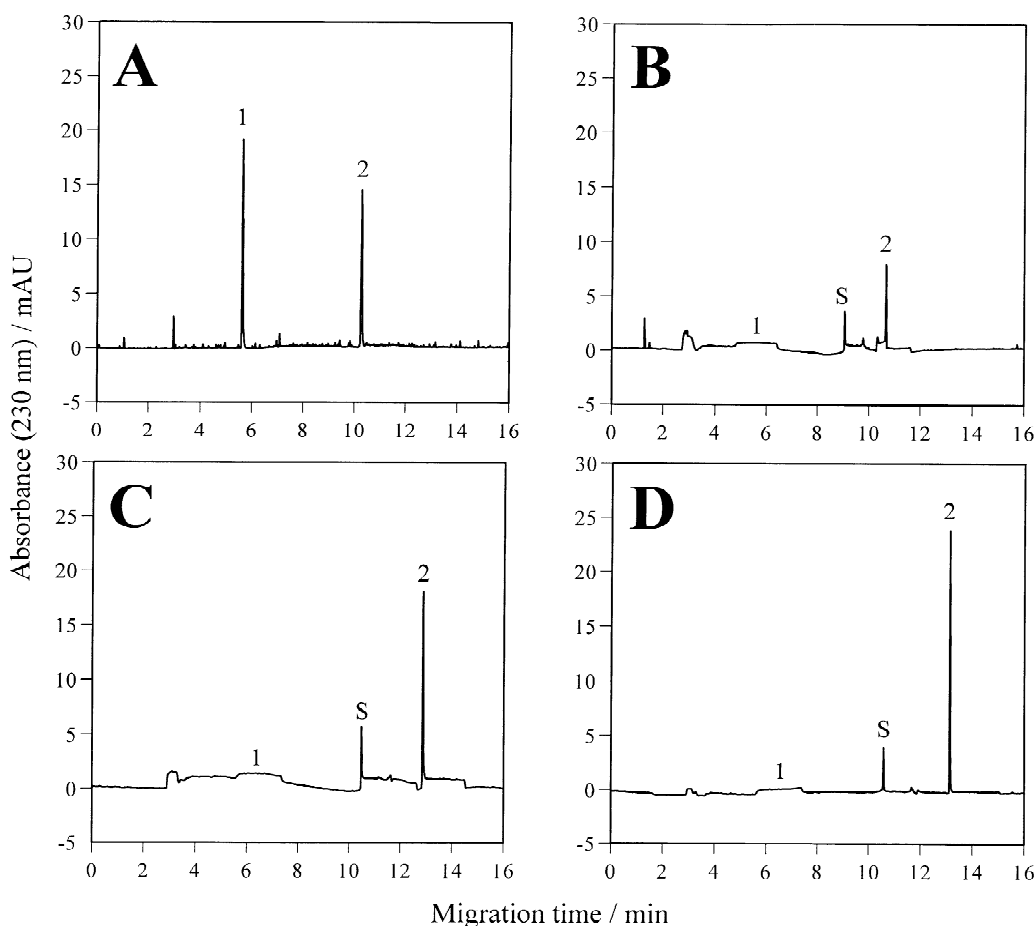


Fig. 3. Conventional MEKC and sweeping MEKC of naphthalenesulfonic acids using SDS micelle and ion-pair reagents. BGS, 80 mM SDS in 20 mM borate–phosphate buffer (pH 9.0) (A, B) containing (C) 20 mM TBAC, (D) 20 mM TPAC; sample solution, (A) BGS, (B) borate–phosphate buffer (pH 9.0) with a conductivity similar to that of the BGS; concentration of samples, (A) 1-NSA (peak 1, 100 ppm), 1,5-NDSA (peak 2, 100 ppm), (B) 100-fold dilution of samples in (A); injection time, (A) 1 s, (B, C, D) 80 s; voltage, +25 kV; S, system peak. Other conditions as described in Fig. 2.

is a 100-fold dilution of the corresponding sample solution in Fig. 3A. Compared with conventional injection, detector responses for 1,5-NDSA were enhanced by about 120- and 140-fold by the addition of TBAC and TPAC, respectively.

In conclusion, in order to improve the detection sensitivity of some anionic analytes, the use of ion-pair reagents was examined in sweeping MEKC. About 400-fold sensitivity enhancements were obtained in terms of peak heights by sweeping combined with ion-pair reagents. It has been demonstrated that the sweeping technique using ion-pair reagents is a useful method to increase the sensitivity in the MEKC analysis of some anionic analytes.

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