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# Strategy for selecting separation solutions in capillary electrophoresis–mass spectrometry

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**Abstract**

A simultaneous determination of cationic, neutral and anionic analytes was performed by capillary zone electrophoresis–mass spectrometry (CZE–MS). An ammonium acetate buffer (pH 6.0) was used as a weakly acidic running solution, an ammonium phosphate buffer (pH 7.0) was used as a neutral one, and an ammonium carbonate buffer (pH 8.5) was used as an alkaline one. The best CZE separation was achieved using the alkaline running solution because several analytes having acidic hydroxyl groups were deprotonated near their p$K_a$ values. The ammonium phosphate buffer could also be used as the running solution in CZE–MS without a deterioration of the detection sensitivity. As for a sheath liquid, a mixture of the alkaline buffer (pH 8.5)–methanol (1:1) was suitable because a stable negative electrospray was obtained with an alkaline solution for the detection of anionic analytes by negative ion mode. Electrokinetic chromatography–MS (EKC–MS) was performed for the separation of neutral analytes. Not only sodium dodecyl sulfate (SDS) but also sulfobutyl ether β-cyclodextrin [β-CD-SBE(IV)] and 4-sulfonated calix[6]arene (Cx-$SO_3$) were employed as pseudo-stationary phases. The interference of the MS detection by SDS was investigated using the alkaline running solution (pH 8.5), but the deterioration of the detection sensitivity of analytes was not observed. A significantly different selectivity was achieved when β-CD-SBE(IV) and Cx-$SO_3$ were used as pseudo-stationary phases in comparison with SDS micelles. Furthermore, no background signal on the MS detection caused by the elution of the pseudo-stationary phases was observed. The charged CD and the charged calixarene were also useful for the separation of neutral analytes by EKC–MS. 

*Keywords:* Capillary electrophoresis–mass spectrometry; Buffer composition; Micellar electrokinetic chromatography–mass spectrometry

## 1. Introduction

Capillary electrophoresis–mass spectrometry (CE–MS) has become a new attractive analytical tool because MS detection provides high sensitivity, excellent selectivity and information about molecular mass of analytes. A number of successful applications of CE–MS have been reported in recent years [1–3]. In capillary zone electrophoresis (CZE), phosphate and/or borate buffers are conventionally employed, whereas CZE–MS requires use of volatile buffer electrolytes compatible with a CE–MS interface and/or with a MS instrument. The limitation of available buffer electrolytes to formate or acetate causes some difficulties in optimizing separation conditions because several CZE separations are successful only in alkaline pH. Furthermore, the use of acidic running solutions brings about the extension of analysis times for anionic analytes due to the reduction of the electroosmotic flow (EOF). Nielen

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[4] employed an ammonium acetate buffer (pH 8.9–10.0) for the separations of phenoxy acid herbicides and anionic surfactants, Tsai and Her [5] employed a 2-[*N*-cyclohexylamino]ethanesulfonate (CHES) buffer (pH 10.0) for the separation of phenol derivatives, and Wheat et al. [6] employed an ammonium carbonate buffer (pH 8.0–10.4) for the separations of peptide mixtures, some basic drugs, and anionic food dyes. Anionic analytes were detected with high sensitivity by negative ion mode using an alkaline running solution and an alkaline sheath liquid. The use of an alkaline running solution also leads to an additional merit on CZE separation due to the increased EOF, where both cationic and anionic analytes can be determined simultaneously. Therefore, we tried a simultaneous determination of cationic, neutral and anionic analytes by CZE–MS. In this study, an ammonium acetate buffer (pH 6.0), an ammonium phosphate buffer (pH 7.0), and an ammonium carbonate buffer (pH 8.5) were used as running solutions. The effects of buffer pH and buffer composition are described.

Micellar electrokinetic chromatography (MEKC) has been employed for the separation of neutral analytes, where sodium dodecyl sulfate (SDS) is widely used as a pseudo-stationary phase [7]. However, non-volatile SDS causes several problems in interfacing MEKC to MS. The electrospray ionization of analytes was suppressed by SDS [8]. To overcome the problems, several approaches have been developed [9–16]. Lamoree et al. [9] demonstrated a direct coupling of MEKC to MS by the heart-cut technique using a liquid junction coupling device. MEKC–MS using an atmospheric pressure chemical ionization (APCI) interface was also investigated to avoid the background signals caused by SDS [11,16]. Nelson et al. [12] and Koezuka et al. [15] employed a partial filling technique, where a micellar zone was filled in a part of the capillary and analytes were detected before the micellar zone reached MS instrument. On the other hand, Yang et al. [14] directly manipulated the micellar velocity by the adjustment of the buffer pH not to introduce the SDS micelles into the MS instrument through the reduction of EOF. In this paper, we performed MEKC–MS using ammonium acetate buffer (pH 6.0) to avoid the contamination of SDS into the MS instrument according to the same technique by Yang et al. [14]. Furthermore, we attempted the use of an alkaline buffer in MEKC–MS, where SDS was eluted out of the capillary and subsequently introduced into the MS instrument. The interference of SDS with MS detection is discussed.

To avoid the background signals caused by SDS on MS detection, the use of high-molecular-mass surfactants was also investigated by MEKC–MS [10,13,16]. On the other hand, macrocyclic reagents, such as cyclodextrin (CD) and calixarene, can be also utilized as pseudo-stationary phases to obtain the different selectivity in electrokinetic chromatography (EKC). EKC separations of neutral analytes have been achieved using some anionic CD derivatives [17–19] and some anionic calixarenes [20–22] as buffer additives. In this paper, we demonstrate EKC–MS using sulfobutyl ether β-CD [β-CD-SBE(IV)] and 4-sulfonated calix[6]arene (Cx-$SO_3$) as buffer additives. Some advantages of the use of these additives are described.

## 2. Experimental

### *2.1. Materials*

SDS of biochemical grade was purchased from Wako (Osaka, Japan) and Cx-$SO_3$ was from Lancaster (Morecambe, UK). β-CD-SBE(IV) was kindly donated by Professor J.F. Stobaugh (Center for Drug Delivery Research and Development of Pharmaceutical Chemistry, University of Kansas, KS, USA). Other reagents were of analytical grade or the highest purity commercially available. Buffers were prepared with distilled water of HPLC grade (Kanto, Tokyo, Japan). An untreated fused-silica capillary of 50 μm I.D.×150 μm O.D. (GL Science, Tokyo, Japan) was used for CZE–MS. A capillary of 70 cm in total length was incorporated into a user assembled capillary cartridge. An untreated fused-silica capillary of 80 cm total length×50 μm I.D.×180 μm O.D. (Supelco, Bellefonte, PA, USA) was used for EKC–MS.

### *2.2. Instrumentation*

A Hewlett-Packard 3D CE instrument (Yokogawa Analytical Systems, Tokyo, Japan) was employed for

CE separations. The CE instrument control was performed with a Hewlett-Packard Vectra XM Series 3 (5/120) computer. A Perkin-Elmer Sciex API-300 triple quadrupole MS instrument (Perkin-Elmer Japan, Yokohama, Japan) was employed as a detector. The MS instrument control and data collections were performed with a Macintosh computer (Model 8500/120). A pneumatically assisted electrospray (ionspray) interface supplied by Perkin-Elmer Sciex was employed for the coupling of CE and MS. For the delivery of a sheath liquid, a Harvard Apparatus syringe pump (Model 11, South Natick, MA, USA) was used.

### *2.3. Procedure*

For the optimization of MS conditions, a sample solution (ca. 50 μg/ml) was prepared with a mixture of 40 m*M* ammonium acetate buffer (pH 6.0)–methanol (1:1). The sample solution was infused into the ionspray interface directly at 5 μl/min with a Harvard Apparatus syringe pump. An ionspray voltage was maintained at 5 kV for positive ion mode and at −4.5 kV for negative ion mode. MS conditions were optimized in order to produce the highest signal intensity of each quasi-molecular ion.

In CZE–MS, a 40 m*M* ammonium acetate buffer (pH 6.0), a 50 m*M* ammonium phosphate buffer (pH 7.0), and a 50 m*M* ammonium carbonate buffer (pH 8.5) were used as running solutions, although the buffer capacities were at each pH. As a sheath liquid, a mixture of each running solution–methanol (1:1) was used, but a mixture of a 50 m*M* ammonium carbonate buffer (pH 8.5)–methanol (1:1) was used in the experiments using a 50 m*M* ammonium phosphate buffer (pH 7.0) as a running solution. A sample solution (ca. 100 μg/ml of each analyte) was prepared with a distilled water and methanol. All solutions were filtered through a 0.20-μm syringe type membrane filter prior to use. A sheath liquid was delivered at 2.5 μl/min. The capillary was rinsed with a running solution for 5 min at 94 kPa (940 mbar) prior to each run, and a sample solution was injected at 5 kPa (50 mbar) for 4 s. A constant CE voltage of 20 kV was applied to CZE separations. When the ammonium phosphate buffer (pH 7.0) was used as a running solution, a constant CE voltage of 17 kV was applied. An ionspray voltage was not applied during sample injection and for 1 min from each start of the run, and then 5 kV was applied at another end of the capillary (the net voltage across the capillary was 15 kV or 12 kV). The MS detection was performed in the selected ion monitoring (SIM) mode for each positive quasi-molecular ion. After all cationic and neutral analytes were detected, the ionspray voltage was suspended for 15 s, and the polarity was inverted to −4.5 kV to obtain the negative ionization (the net voltage across the capillary was 24.5 kV or 21.5 kV). The MS detection was performed in the SIM mode for each negative quasi-molecular ion.

In EKC–MS, a 40 m*M* ammonium acetate buffer (pH 6.0) or a 50 m*M* ammonium carbonate buffer (pH 8.5) was used as a running solution. The separation solution was prepared by dissolving additives [SDS, β-CD-SBE(IV) or Cx-$SO_3$] to the running solution. If necessary, methanol was added to both the running solution and the separation solution which contains the additive. A sample solution (ca. 50–100 μg/ml of each analyte) was prepared with a distilled water and methanol. A mixture of each buffer solution–methanol (1:1) was used as a sheath liquid, and was delivered at 4 μl/min. A capillary was rinsed with the running solution for 5 min at 94 kPa (940 mbar) and rinsed with a separation solution at 94 kPa (940 mbar) for 2.5 min to replenish with the separation solution entirely. A sample solution was injected at 5 kPa (50 mbar) for 8 s, the injection end of the capillary was dipped into the running solution, and a constant voltage of 25 kV was applied. The ionspray voltage of 5 kV was applied after 1 min from the start of the run at another end of the capillary (the net voltage across the capillary was 20 kV). Other conditions were the same as CZE–MS described above.

## 3. Results and discussion

### *3.1. Simultaneous determination of acidic, neutral and basic analytes by CZE–MS*

First, we performed a simultaneous determination of cationic, neutral and anionic analytes using a 40 m*M* ammonium acetate buffer (pH 6.0) as the running solution. Thiamine and butylscopolamine

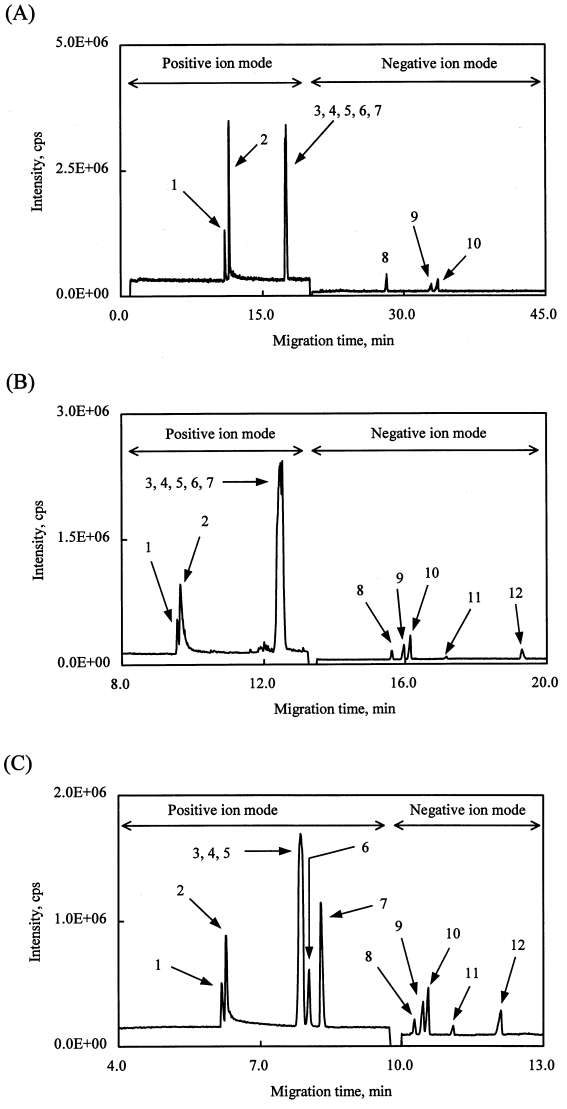


Fig. 1. Reconstructed ion electropherograms by CZE–MS for the simultaneous determination of cationic, neutral and anionic analytes. Samples: 1=thiamine; 2=butylscopolamine; 3=nicotinamide; 4=caffeine; 5=cyanocobalamin; 6=riboflavin; 7=acetaminophen; 8=warfarin; 9=ketoprofen; 10=ibuprofen; 11=L-ascorbic acid; 12=nicotinic acid. Conditions: sample injection, 50 mbar×4 s (100 μg/ml of each analyte); capillary, 70 cm×50 μm I.D. untreated fused-silica capillary; running solution, (A) 40 m*M* ammonium acetate buffer (pH 6.0), (B) 50 m*M* ammonium phosphate buffer (pH 7.0), (C) 50 m*M* ammonium carbonate buffer (pH 8.5); sheath liquid, (A) a mixture of 40 m*M* ammonium acetate buffer (pH 6.0)–methanol (1:1), (B, C) a mixture of 50 m*M* ammonium carbonate buffer (pH 8.5)–methanol (1:1); flow-rate of the sheath liquid, 2.5 μl/min; CE voltage, (A, C) 20 kV, (B) 17 kV; ionspray voltage, 5 kV in positive ion mode, −4.5 kV in negative ion mode; MS detection, *m*/*z* 123.0, 152.1, 195.0, 265.0, 360.0, 377.0 and 678.5 by positive ion mode, *m*/*z* 122.0, 175.0, 205.0, 253.0 and 306.6 by negative ion mode.

were employed as the cationic model analytes; nicotinamide, caffeine, acetaminophen, riboflavin and cyanocobalamin as the neutral model analytes; and L-ascorbic acid, nicotinic acid, ibuprofen, ketoprofen and warfarin as the anionic model analytes. As shown in Fig. 1, the cationic and the neutral analytes were detected as positive quasi-molecular ions. After the neutral analytes were detected, the ionspray voltage was suspended for 15 s. Subsequently, the ionspray voltage of −4.5 kV was applied for the detection of the anionic analytes by the negative ion mode. Fig. 1A shows that cationic analytes were analyzed with high sensitivity using a weakly acidic buffer (pH 6.0) as a running solution, whereas, the analysis of anionic analytes was not suitable under this condition because some analytes could not be detected until 45 min due to the reduced EOF (Fig. 1A).

Second, the CZE separation was performed by using a 50 m*M* ammonium phosphate buffer (pH 7.0) as the running solution, and a mixture of 50 m*M* ammonium carbonate buffer (pH 8.5)–methanol (1:1) was employed as a sheath liquid. In this experiment, a constant CE voltage of 17 kV was applied because the electrophoretic current was higher than 50 μA in the negative ion mode when 20 kV of CE voltage was applied (the net voltage across the capillary was 24.5 kV). As shown in Fig. 1B, five anionic analytes were detected with relatively high sensitivity. In CZE–MS, the flow-rate of the running solution was relatively low compared with the flow of the sheath liquid. We do not know whether the counter-ion, hydrogenphosphate, was eluted out of the capillary or the carbonate ion from the sheath liquid replaced the counter-ion [23], but the phosphate buffer was used as a buffer electrolyte without the deterioration of detection sensitivity. When a mixture of a 40 m*M* ammonium acetate buffer (pH 6.0)–methanol (1:1) was used as the sheath liquid, a similar result in Fig. 1B was obtained, but the detection sensitivity of anionic analytes was slightly

reduced probably due to the decrease of efficiency and stability of the negative electrospray ionization process (data not shown).

As for the use of an alkaline running solution, we employed a 50 m*M* ammonium carbonate buffer (pH 8.5). A mixture of a 50 m*M* ammonium carbonate buffer (pH 8.5)–methanol (1:1) was used as the sheath liquid. At the pH, riboflavin and acetaminophen were slightly negatively charged, and were detected at 8.1 min and 8.4 min, respectively. However, these analytes were detected as positive quasi-molecular ions with high sensitivity. As shown in Fig. 1C, the use of the alkaline running solution was most useful for the simultaneous detection of cationic, neutral and anionic analytes by CZE–MS because several analytes having acidic hydroxyl groups were deprotonated near their p$K_a$ values and were separated.

### *3.2. MEKC–MS using SDS micelles*

For the separation of neutral analytes, MEKC–MS using SDS as a pseudo-stationary phase was performed. Acetaminophen, acetanilide, caffeine, guaifenesine, phenacetin and pyridoxine were used as model samples. As a preliminary experiment, we employed a partial filling technique in the same manner given in as our previous paper on enantiomer separations by CE–MS [24]. However, SDS was eluted from the detection end of the capillary and subsequently introduced into MS instrument when an alkaline running solution at pH 8.5 was used. To avoid the contamination of the CE–MS interface and the MS instrument by SDS, the plug of separation zone containing SDS micelles should be shorten, but the shorter separation zone deteriorated the resolution of the neutral analytes. On the other hand, Yang et al. [14] reported that SDS micelles did not migrate toward MS instrument using a buffer solution at pH 5.9, therefore we chose a 40 m*M* ammonium acetate buffer (pH 6.0) in this experiment. The whole capillary was filled with the separation solution containing SDS micelles, and the ammonium acetate buffer without SDS was used as the running solution in the inlet vial. Fig. 2A shows that the reconstructed ion electropherogram obtained with 20 m*M* SDS dissolved in a 40 m*M* ammonium acetate buffer (pH 6.0) as the separation solution. At the pH, five

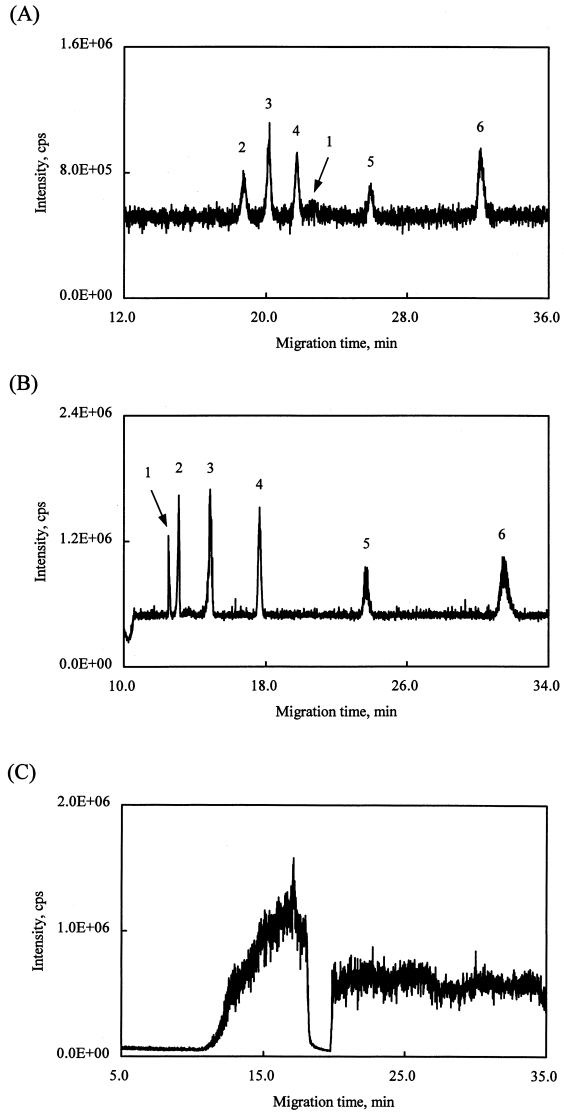


Fig. 2. Reconstructed ion electropherograms by MEKC–MS using SDS micelles as a pseudo-stationary phase. Samples: 1=pyridoxine; 2=acetaminophen; 3=caffeine; 4=acetanilide; 5=guaifenesin; 6=phenacetin. Conditions: sample injection, 50 mbar×8 s (50–100 μg/ml of each analyte); capillary, 80 cm×50 μm I.D. untreated fused-silica capillary; running solution, (A) 40 m*M* ammonium acetate buffer (pH 6.0), (B, C) 50 m*M* ammonium carbonate buffer (pH 8.5); separation solution, (A) 20 m*M* SDS in the running solution, (B, C) 80 m*M* SDS in the running solution; sheath liquid, a mixture of each running solution–methanol (1:1); flow-rate of the sheath liquid, 4 μl/min; CE voltage, 25 kV; ionspray voltage, 5 kV; MS detection, (A, B) *m/z* 135.8, 152.0, 179.6, 195.0 and 199.0 by positive ion mode, (C) *m/z* 284.0 by positive ion mode. Pyridoxine was detected at *m/z* 152.0 as the fragment ion.

analytes were electrically neutral, but pyridoxine was slightly positively charged. All analytes were successfully separated, but the peak shape of pyridoxine was not satisfactory. In this analysis, MS signals at $m/z$ 284 and $m/z$ 301 were additionally monitored to ensure non-elution of SDS toward MS instrument. If SDS was eluted out of the capillary and subsequently introduced into the MS instrument, it must have been detected as the ammonium adduct positive ion (either $[M+NH_4]^+$, $m/z$=284 or $[M+NH_3+NH_4]^+$, $m/z$=301). Since no MS signal caused by SDS was observed during the analysis, SDS micelles must have migrated toward the injection end of the capillary. However, the SDS micelles must have migrated at the different velocity from that of the SDS monomer, because no SDS was supplied from the detection end of the capillary in MEKC–MS.

As for the use of an alkaline buffer, a 50 m*M* ammonium carbonate buffer (pH 8.5) was used as both the running solution and the separation solution instead of a 40 m*M* ammonium acetate buffer (pH 6.0). In this case, 80 m*M* SDS dissolved in the running solution was employed as the separation solution in order to obtain a complete separation of all analytes. SDS was eluted out of the capillary during the analysis due to the increase of EOF, and high background signals, which were the ammonium adduct positive ions of SDS, were observed at $m/z$ 284 as shown in Fig. 2C. Because the positive ion mode was employed in this run, the SDS derived ions were also detected in this mode, although the SDS elution might be detected much easier by the negative ion mode. Nevertheless, the deterioration of detection sensitivity of the analytes was not observed in the SIM mode by the detection of positive ions as shown in Fig. 2B. At the pH, four analytes were electrically neutral, but acetaminophen and pyridoxine were slightly negatively charged. Therefore, the migration order of pyridoxine was changed as compared to that in Fig. 2A. In Fig. 2C, the signals of SDS were not observed until about 12 min. However, it was not considered that SDS was not eluted out of the capillary for 12 min. When the MS detection was performed with full scanning from $m/z$ 100 to $m/z$ 700, high background signals caused by sodium ions in the separation solution were observed (data not shown). Therefore, the ionization of SDS should be repressed during the elution of the sodium ions. The high background signals caused by the sodium ions were also obtained under the same conditions as in Fig. 2A. In this study, we performed the similar determinations continuously, where SDS was eluted out of the capillary during the analysis, but the deterioration of detection sensitivity caused by the contamination by SDS was not observed at all in a day (several runs). The MEKC–MS analyses were performed without specific maintenance to both the CE–MS interface and the MS instrument. However, this procedure can be only applied when the target analytes are detected by the positive SIM mode. It is not suitable for the detection of an unknown analyte because of the high background signals of SDS ions in the total ion monitoring (TIC) mode.

### *3.3. EKC–MS using β-CD-SBE(IV)*

The same samples as used in MEKC–MS were employed as model samples. As shown in Fig. 3A, successful separation of all six analytes was achieved using a 4 m*M* β-CD-SBE(IV) solution in 40 m*M* ammonium acetate buffer (pH 6.0) as the separation solution. At the pH, pyridoxine was slightly positively charged and was detected first. In comparison with MEKC–MS using SDS, a dramatic difference in the migration orders was observed. The interference of sodium ions, the counter-ions of β-CD-SBE(IV), was also observed on MS signals until about 12 min as observed in MEKC–MS using SDS. However, no background signals caused by the elution of β-CD-SBE(IV) were observed from $m/z$ 100 to $m/z$ 1000 in TIC mode. Under this condition, the detection of phenacetin was late due to both the reduction of the EOF and the relatively large complex formation constant between β-CD-SBE(IV) and phenacetin. Therefore, we performed the separation using an alkaline buffer giving reconstructed ion electropherogram (Fig. 3B) using 8 m*M* β-CD-SBE(IV) in 50 m*M* ammonium carbonate buffer (pH 8.5) as the separation solution. Because acetaminophen and pyridoxine were slightly negatively charged at pH 8.5, the migration order of pyridoxine was changed as compared to that in Fig. 3A. The background signals caused by the elution of β-CD-SBE(IV) were not also observed from $m/z$ 100 to $m/z$ 2000 even if the alkaline buffer (pH 8.5) was used. However, it

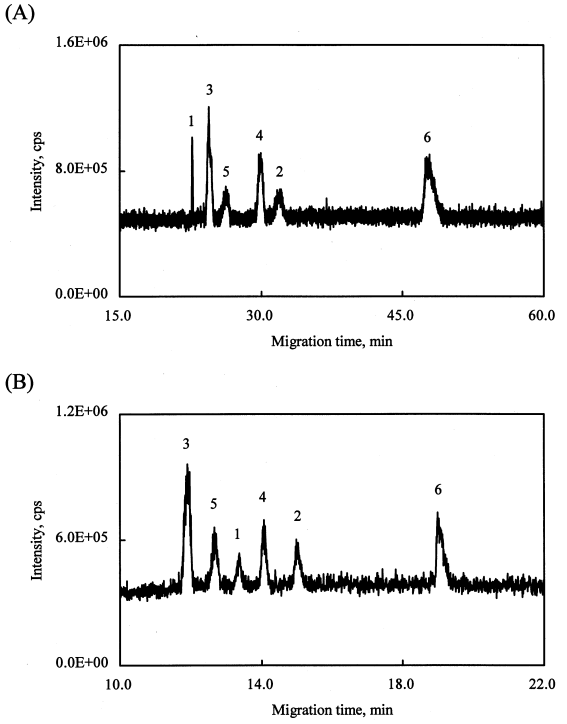


Fig. 3. Reconstructed ion electropherograms by EKC–MS using β-CD-SBE(IV) as a pseudo-stationary phase. Conditions: running solution, (A) 40 m*M* ammonium acetate buffer (pH 6.0), (B) 50 m*M* ammonium carbonate buffer (pH 8.5); separation solution, (A) 4 m*M* β-CD-SBE(IV) in the running solution, (B) 8 m*M* β-CD-SBE(IV) in the running solution. Samples and other conditions as in Fig. 2.

was not certain whether β-CD-SBE(IV) was not eluted out of the capillary due to the high electrophoretic mobility toward the anode or the positive electrospray ionization of β-CD-SBE(IV) was not obtained. An advantage of EKC–MS using charged CDs is that the different selectivity will be obtained easily using different modified CD derivatives. Several ionic CDs, such as carboxymethylated CDs and sulfated CDs, have been already employed for enantiomer separations, and revealed the different selectivity [25–27]. Furthermore, the degree of substitution also influences the complex formation [28,29]. Accordingly, these characteristics will be useful to obtain the different selectivity for the separation of neutral analytes by EKC, where different formation constants between charged CDs and neutral analytes are achieved by changing the type of charged CDs.

### *3.4. EKC–MS using Cx-$SO_3$*

The EKC–MS separation using Cx-$SO_3$ was performed using a 40 m*M* ammonium acetate buffer containing 5% (v/v) methanol as the running solution. As a separation solution, 12 m*M* Cx-$SO_3$ dissolved in the running solution was used. When methanol was not added to both the running solution and the separation solution, the resolution was reduced due to the increase of the EOF. In Fig. 4, pyridoxine was not detected until 60 min because of the strong interaction between Cx-$SO_3$ and pyridoxine. The background signals caused by the elution of Cx-$SO_3$ were not observed at all from $m/z$ 100 to $m/z$ 1200 in TIC mode because Cx-$SO_3$ migrated toward the injection end of the capillary during the analysis. Furthermore, no interference caused by the elution of sodium ions was observed on background signals. Cx-$SO_3$ showed a strong interaction to some cationic analytes. As an example, some cationic analytes were separated using 0.3 m*M* Cx-$SO_3$ dissolved in a 40 m*M* ammonium acetate buffer (pH 6.0) as the separation solution. As shown in Fig. 5, the migration times of bromhexine, chlorpheniramine and thiamine were significantly delayed because of the large formation constants between Cx-$SO_3$ and

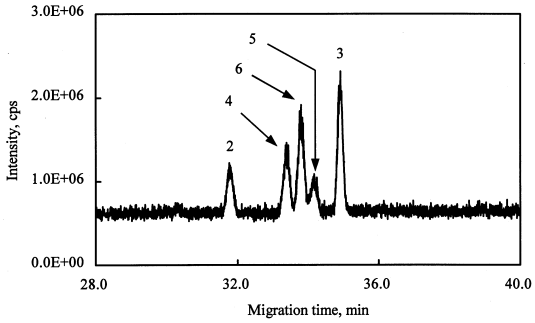


Fig. 4. Reconstructed ion electropherogram by EKC–MS using Cx-$SO_3$ as a pseudo-stationary phase. Conditions: running solution, 40 m*M* ammonium acetate buffer (pH 6.0) containing 5% (v/v) methanol; separation solution, 12 m*M* Cx-$SO_3$ in the running solution; sheath liquid, a mixture of 40 m*M* ammonium acetate buffer (pH 6.0)–methanol (1:1). Samples and other conditions as in Fig. 2.

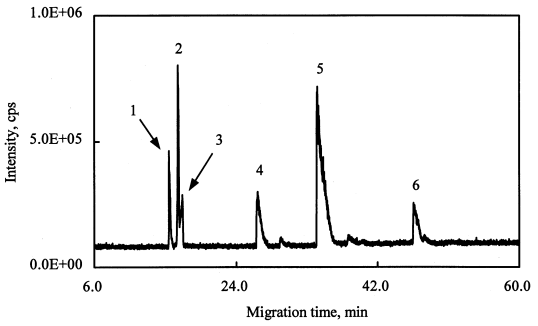


Fig. 5. Reconstructed ion electropherogram by EKC–MS using Cx-$SO_3$ as a pseudo-stationary phase for the separation of neutral and cationic analytes. Samples: 1=pyridoxine; 2=acetaminophen; 3=riboflavin; 4=bromhexine; 5=chlorpheniramine; 6=thiamine. Conditions: running solution, 40 m*M* ammonium acetate buffer (pH 6.0); separation solution, 0.3 m*M* Cx-$SO_3$ in the running solution; MS detection, *m*/*z* 152.0, 170.0, 265.0, 275.0 and 377.0 by positive ion mode. Other conditions as in Fig. 4.

the cationic analytes. Under this condition, the interaction between Cx-$SO_3$ and pyridoxine was not observed yet. The delay of the migration time of pyridoxine caused by the interaction with Cx-$SO_3$ was observed using more than 2 m*M* Cx-$SO_3$ solution as the separation solution. It should be noted that pyridoxine was slightly protonated at pH 6.0 and the migration time depended dramatically on the concentration of Cx-$SO_3$.

## 4. Conclusions

In CZE–MS, the use of the alkaline running solution, a 50 m*M* ammonium carbonate buffer (pH 8.5), was the most suitable for the simultaneous determination of cationic, neutral and anionic analytes. By using the alkaline running solution, anionic analytes were analyzed in a short analysis time due to the increase of EOF. The anionic analytes were also detected with high detection sensitivity because the stable negative electrospray ionization was achieved with the alkaline sheath liquid. Furthermore, some neutral analytes having acidic hydroxyl groups were separated because they were deprotonated near their p$K_a$ values. Although sodium phosphate and/or sodium borate buffers have been employed as the running solution in conventional CZE without MS, an ammonium phosphate buffer (pH 7.0) can be also used as the running solution without deterioration of detection sensitivity in CZE–MS. The use of either an ammonium phosphate or an ammonium carbonate buffer will permit easy transfer of separation conditions from conventional CZE to CZE–MS.

For the separation of neutral analytes, EKC–MS was performed using not only SDS micelles but also β-CD-SBE(IV) and Cx-$SO_3$ as pseudo-stationary phases. When MEKC-MS using SDS was performed with the alkaline buffer solution, SDS was detected as the ammonium adduct positive ion. However, the contamination of SDS did not deteriorate the detection sensitivity of analytes. When β-CD-SBE(IV) and Cx-$SO_3$ were used, no background signal caused by the pseudo-stationary phase was observed. Moreover, the significantly different selectivity was achieved as compared to that of MEKC with SDS micelles. The different selectivity in EKC will be easily obtained using different types of additives.

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