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Enantiomer separation by strong anion-exchange capillary electrochromatography with dynamically modified sulfated β -cyclodextrin

A novel mode of capillary electrochromatography (CEC) based on a dynamically modified stationary phase was presented for chiral separation. The capillary column was packed with strong anion-exchange (SAX) stationary phase packing; the sulfated β -cyclodextrin (S-CD), which was added to the mobile phase, was dynamically adsorbed to the packing surface. Separation of enantiomers was achieved by their different abilities to form an inclusion complex with the adsorbed S-CD. The enantiomers of tryptophan, praziquantel, atropine, metoprolol, and verapamil were successfully separated in this system with a column efficiency of 36000–412000 plates/m. The resolution value obtained for atropine was as high as 11.23. The superiority of CEC with a dynamically modified stationary phase over that with a physically adsorbed stationary phase was demonstrated. The influence of ionic strength, S-CD concentration, and methanol content on separation was also studied.

Keywords: Enantiomer separation / Electrochromatography / Strong anion-exchange stationary phase / Dynamically modified stationary phase / Sulfated β -cyclodextrin EL 4222

1 Introduction

Capillary electrochromatography on dynamically modified stationary phase (DMS-CEC) is a novel mode of CEC. This system is established by adding some amount of agent with considerable affinity to the packed stationary phase into the mobile phase. Thus, the agent added will be dynamically adsorbed to the packed stationary phase to form a new layer of stationary phase. The solutes in this system will be separated mainly based on their different interaction with the dynamically adsorbed stationary phase. The long alkyl chain of cetyltrimethylammonium bromide (CTAB) has a strong affinity to the hydrophobic stationary phase. Garner and Yeung [1] have reported that a capillary coated with a hydrophobic stationary phase proved to be a dynamic ion exchanger in the open-tubular electrochromatography when CTAB was added to the mobile phase. Recently, we have developed the packed column based on the DMS-CEC mode [2, 3]. Bare silica and strong cation-exchange (SCX) stationary phase have been used as the packing material and CTAB was used as the dynamically adsorbed agent. Under the

experimental conditions, the surface of the packing is negatively charged and the positively charged quaternary ammonium group of CTAB has a strong affinity to these packing materials due to the electrostatic attraction. Therefore, the CTAB will be dynamically adsorbed to the packing surface to form a hydrophobic layer, and the neutral solutes can be retained by the reversed-phase chromatographic mechanism [2, 3]. Simultaneous separation of the acidic, basic and neutral solutes was achieved successfully in DMS-CEC with SCX packing at low pH because a strong EOF can be obtained in this system [3]. The packing material in DMS-CEC may also be positively charged, and the stationary phase dynamically modified by the anionic agent such as sodium dodecyl sulfate (SDS).

A series of chiral stationary phases including cyclodextrins [4–6], proteins [7–11], cellulose derivatives [12], macrocyclic antibiotics [13], molecular imprint polymers [14, 15] *etc.*, have been successfully used for enantioseparation in CEC. In this work, CEC with a dynamically adsorbed chiral stationary phase is presented. The capillary column was packed with strong anion-exchange (SAX) stationary phase, and the sulfated β -CD (S-CD) was used as additive agent in the mobile phase, which will be dynamically adsorbed on the packing surface to form a layer of β -CD stationary phase. The enantiomers can be separated according to their different interaction to the adsorbed stationary phase. The repeatability of migration times between CEC with physically adsorbed stationary phase and dynamically modified stationary phase was compared. The influences of ionic strength, S-CD con-

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Abbreviations: ASP, physically adsorbed stationary phase; DMS, dynamically modified stationary phase; SAX, strong anion exchange; S-CD, sulfated β -CD; TEA, triethylamine

centration, and methanol content on the separation were also studied.

2 Materials and methods

2.1 Instrumentation and material

All CEC experiments were performed on a P/ACE system MDQ (Beckman, Fullerton, CA, USA); a Spectra-Physics pump (San Jose, CA, USA) was used to pack capillary columns; a 1 mL insulin syringe (Becton Dickinson and Company, Franklin Lakes, NJ, USA) was employed to flush the column. Fused-silica capillaries (50 μm ID, 365 μm OD) were obtained from Yongnian Optic Fiber Plant (Hebei, China). 5 μm Spherisorb-SAX was purchased from Waters Phase Separation (Milford, MA, USA).

2.2 Chemicals and buffers

Praziquantel, atropine, metoprolol, verapamil, benzoin, ibuprofen, warfarin, naproxen, ketoprofen, and fenoprofen were purchased from Sigma (St. Louis, MO, USA). DL-Tryptophan, D-tryptophan, and L-tryptophan were from Shanghai Institute of Biological Chemistry, Academia Sinica (Shanghai, China). All other chemicals used were of analytical or chromatographic grade. The S-CD was obtained from Aldrich (Milwaukee, WI, USA). According to the product comments, the typical substitution is 7–11 moles/mole β -CD. The stock solution of sulfated S-CD with a concentration of 200 mg/mL was prepared by dissolving the appropriate amount of S-CD in water. The stock buffer solution (HAC-TEA) at pH 4.0 was prepared by neutralizing a 100 mM acetic acid (HAC) solution with triethylamine (TEA). The mobile phase for CEC with a physically adsorbed stationary phase (ASP-CEC) was prepared by mixing appropriate volumes of methanol, stock buffer solution, and water. The mobile phase for CEC with a dynamically modified stationary phase (DMS-CEC) was prepared by adding appropriate volumes of a stock S-CD solution to the mobile phase for ASP-CEC. Before running, the mobile phase was degassed in an ultrasonic bath for 30 min. The concentration of the enantiomers was 100–500 $\mu\text{g/mL}$.

2.3 Column preparation and separation conditions

CEC columns were packed in this laboratory by the slurry packing technique as reported [3, 16]. All columns were 31 cm long with an effective length of 10 cm. Prior to the CEC experiment, the column was first flushed with the mobile phase containing S-CD for 30 min by a syringe. Then the column was equilibrated on the instrument with this mobile phase for at least 1 h. The applied voltage first

was ramped from 0 to 10 kV for 10 min and then operated at 10 kV. The separation in DMS-CEC can be carried out directly with this mobile phase. The initial procedure for ASP-CEC was the same as for DMS-CEC. After the column was equilibrated with the mobile phase containing S-CD, the column was conditioned with the mobile phase without S-CD for 30 min. Then the separation was carried out with the mobile phase without S-CD. The temperature was kept at 25°C and the detection wavelength was set at 214 nm. The separation voltage was 10 kV if not otherwise stated.

2.4 k^* -value and separation selectivity

In order to describe the migration process of a charged solute in CEC, the electrochromatographic retention factor (k^*) was defined as follows [17–20]:

$$k^* = (t_r - t_0)/t_0 \quad (1)$$

where t_r is the migration time of a solute and t_0 is the void time, which is marked by the solvent peak in this study. The separation selectivity (α) was calculated as follows:

$$\alpha = (t_2 - t_0)/(t_1 - t_0) \quad (2)$$

where t_1 and t_2 are the migration times of the first eluted and later eluted enantiomer, respectively.

3 Results and discussion

3.1 Comparison of ASP-CEC with DMS-CEC

The surface of SAX packing is positively charged, and the negatively charged compounds can easily be adsorbed to the packing. Chiral separation based on physically adsorbed S-CD in CEC was carried out. The capillary column packed with SAX material was first rinsed with a mobile phase containing 10% methanol and 2.0 mg/mL S-CD in 20 mM HAC-TEA buffer (pH 4.0) for at least 30 min, then conditioned on the electrophoresis instrument with the same mobile phase for 1 h as detailed in Section 2.3. Thus, the negatively charged S-CD will be adsorbed on the packing surface. Then the column was conditioned with a mobile phase without S-CD for 30 min, and the separations were carried out with the mobile phase without S-CD. It was found that tryptophan can be baseline-separated under this condition. The direction of the EOF should be from cathode to anode since the packing surface is positively charged, but we observed that the direction of EOF was reversed. According to the product comments, the substitution is 7–11 moles/mole β -CD, which means that there are 7–11 sulfonic groups on a ring of β -CD. When the sulfonic groups on one side of β -CD are

adsorbed to the packing surface, the sulfonic groups of the other side result in reversal of the EOF. The enantio-separation of tryptophan and the reversal of EOF both indicate the adsorption of S-CD on the packing. An important problem for the ASP-CE is the repeatability of migration time for solutes because of the possible desorption of the adsorbed agent from the packing surface. The repeatability for migration time of tryptophan enantiomers was investigated with 17 consecutive runs (Fig. 1).

It can be seen from Fig. 1 that the void time increased, in other words, the EOF decreased with the increase of the number of runs. The reason is that the adsorbed S-CD on the packing surface was desorbed gradually, which resulted in the decrease of the net negative charge on the packing surface and thereby the decrease of EOF. The migration time of the enantiomers of tryptophan also increased with increase of the runs. The repeatability for the void time, and the migration time of the early- and late-eluted tryptophan enantiomers by 17 consecutive runs was evaluated with relative standard deviation (RSD) values of 11.5, 9.27, and 9.30%, respectively. But the separation selectivity for tryptophan enantiomers did not change obviously with an RSD value of only 0.16%.

In order to improve the repeatability of migration by ASP-CEC, it is necessary to find a way to compensate the desorbed stationary phase on the packing surface. The difference between DMS-CEC and ASP-CEC is the composi-

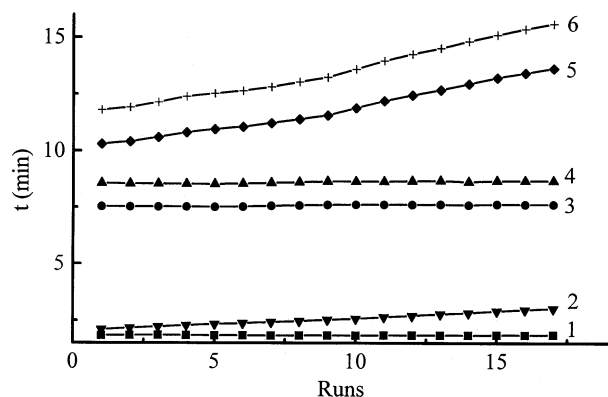


Figure 1. Repeatability of the void time and migration time of tryptophan enantiomers in CEC with physically adsorbed and dynamically modified stationary phase. Conditions: column, 50 μm ID \times 375 μm OD packed by 5 μm SAX with packed/total length = 10/31 cm; applied voltage, 10 kV; detection wavelength, 214 nm; the mobile phase for DMS-CEC was 10% methanol and 2 mg/mL S-CD in 20 mM HAC-TEA buffer (pH 4.0); the mobile phase for ASP-CEC was 10% methanol in 20 mM HAC-TEA buffer (pH 4.0). Lines 1, 3, and 4, void time and the migration time of L-tryptophan and D-tryptophan in DMS-CEC; lines 2, 5, 6, ASP-CEC.

tion of the mobile phase. In DMS-CEC, a certain amount of the adsorbed agent was added into the mobile phase, and the equilibration of added agent between the mobile and stationary phases will be formed. Therefore, the amount of the adsorbed agent on the packing surface will not decrease during the experiments. The repeatability of migration time by DMS-CEC was also investigated with 17 consecutive runs (Fig. 1). The RSD values for the void time, and the migration time of tryptophan enantiomers were calculated as 0.53, 0.62 and 0.69%, respectively, and that for separation selectivity of tryptophan enantiomers was 0.09%. These results showed the superiority of DMS-CEC over ASP-CEC, and a much better repeatability of migration time can be obtained in DMS-CEC. The separation selectivity for tryptophan enantiomers in ASP-CEC was greater than that in DMS-CEC. This means that the presence of S-CD in the mobile phase reduces the separation selectivity. This result is consistent with that of the influence of S-CD concentration on separation selectivity of tryptophan enantiomers in DMS-CEC, which will be discussed later in this paper.

3.2 Chiral separation by DMS-CEC

Resolution of ten racemates of praziquantel, atropine, metoprolol, verapamil, benzoin, ibuprofen, warfarin, naproxen, ketoprofen, and fenoprofen has been performed in this system, and enantiomers of tryptophan and four basic drugs including praziquantel, atropine, metoprolol, and verapamil were successfully separated. However, resolution of four acidic drugs was not achieved, which may be caused by the fact that these acidic drugs are difficult to access into the S-CD cavity to form inclusive complexes due to the electrostatic repulsion between S-CD and these negative solutes. The chromatograms for the resolved enantiomers are shown in Fig. 2.

It can be seen that the baseline of some chromatograms has a step noise, which may be caused by the tiny bubbles formed due to the relatively high Joule heating and different sample solutions injected from the mobile phase. Due to the lack of pure enantiomers, only the migration order of D-tryptophan and L-tryptophan were verified by spiking. The names and the structures of the solutes, the efficiency and resolution data under the given electrochromatographic conditions are listed in Table 1.

Four of the five pairs of enantiomers have been baseline-separated. The resolution value for atropine was as high as 11.2, while that for metoprolol was only 0.82. The column efficiency was only 45 000 plates/m and 36 000 plates/m for early- and late-eluted praziquantel enantiomers, which were relative poor. The column efficiency for tryptophan enantiomers were 133 000 plates/m and

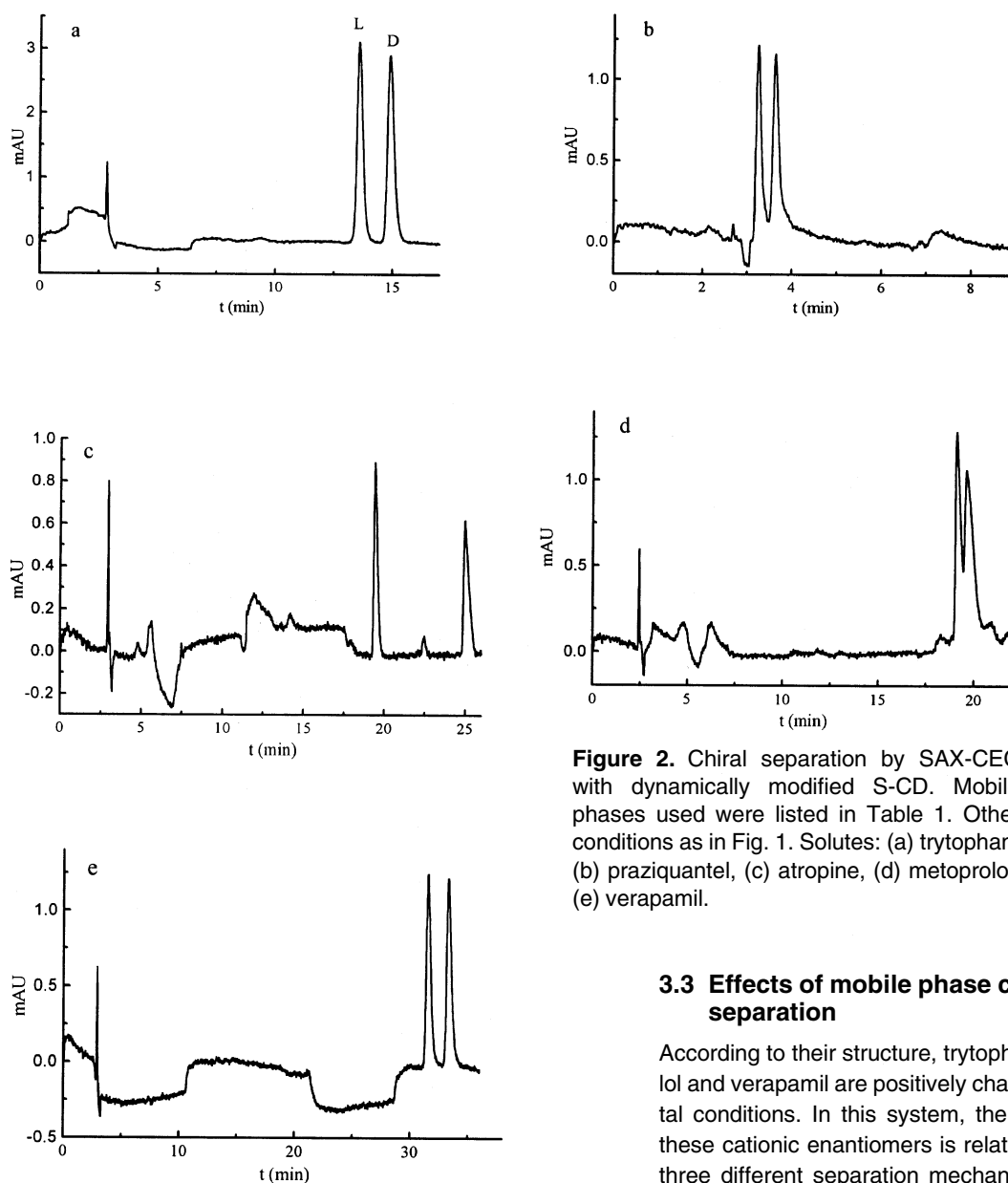


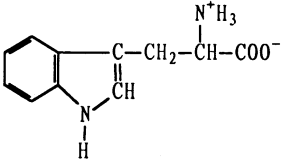
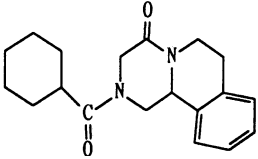
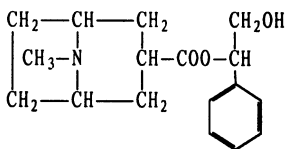
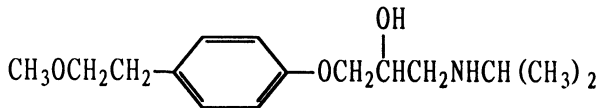
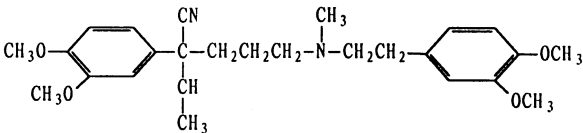
Figure 2. Chiral separation by SAX-CEC with dynamically modified S-CD. Mobile phases used were listed in Table 1. Other conditions as in Fig. 1. Solutes: (a) tryptophan, (b) praziquantel, (c) atropine, (d) metoprolol, (e) verapamil.

3.3 Effects of mobile phase composition on separation

According to their structure, tryptophan, atropine, metoprolol and verapamil are positively charged under experimental conditions. In this system, the migration behavior of these cationic enantiomers is relatively complex. At least three different separation mechanisms contribute to the separation. Firstly, the electrophoresis mechanism contributes to the migration process since the enantiomers are charged. As stated previously, the EOF was from anode to cathode and cationic solutes in an electric field also migrate in the same direction, therefore, these solutes should be eluted before t_0 . But according to the result of our experiment, none of the solutes eluted before t_0 . This means that a strong interaction between the solutes and the stationary phase took place. The packing surface was negatively charged, thereby the ion-exchange mechanism also contributed to the retention of the cationic solutes. But neither of these two mechanisms is responsible for the enantiomer separation. The chiral separation is based on the different inclusion of enantiomers with the adsorbed S-CD. The retention of praziquantel in this sys-

128 000 plates/m, respectively, which were comparable with those in CEC with CD packings [4–6]. However, the efficiency for atropine, metoprolol and verapamil was superior, and about 300 000 plates/m were obtained. Abnormal high efficiency in ion-exchange CEC was reported in [21, 22]; the high efficiency of the enantiomers with strong retention may also result from the strong ion-exchange mechanism in this system. Enantiomers of tryptophan, atropine and verapamil can be separated in a single run using a mobile phase containing 30% methanol, 2.0 mg/mL S-CD in 20 mM HAC-TEA buffer (pH 4.0) with the applied voltage of 15 kV (Fig. 3). A satisfying peak symmetry for the resolved enantiomers can be seen in Figs. 2 and 3.

Table 1 Enantioseparation by SAX-CEC with dynamically modified S-CD

Solute	Structure	Mobile phase	Efficiency ^{a)} Resolution ^{b)}
Tryptophan		30% methanol, 2.0 mg/mL S-CD in 30 mM HAC-TEA buffer (pH 4.0)	$N_1 = 133\ 000$ $N_2 = 128\ 000$ $Rs = 2.67$
Praziquantel		30% methanol and 2.0 mg/mL S-CD and in 30 mM HAC-TEA buffer (pH 4.0)	$N_1 = 45\ 000$ $N_2 = 36\ 000$ $Rs = 1.79$
Atropine		30% methanol and 2.0 mg/mL S-CD and in 30 mM HAC-TEA buffer	$N_1 = 365\ 000$ $N_2 = 288\ 000$ $Rs = 11.23$
Metoprolol		30% methanol and 2.0 mg/mL S-CD and in 30 mM HAC-TEA buffer (pH 4.0)	$N_1 = 266\ 000$ $N_2 = 97\ 000$ $Rs = 0.82$
Verapamil		30% methanol and 2.0 mg/mL S-CD and in 30 mM HAC-TEA buffer (pH 4.0)	$N_1 = 331\ 000$ $N_2 = 324\ 000$ $Rs = 2.45$

a) Efficiencies are given in plate number/m; N_1 and N_2 are the efficiencies for the first eluted and the second eluted enantiomer, respectively.

b) The resolution was calculated by the equation $Rs=1.18 (t_{r,r}-t_{r,l})/(w_{0.5,r}+w_{0.5,l})$, where t_r is the migration time, $w_{0.5}$ is the width of the peak at 50% peak height, and the subscripts r and l indicate the peaks at the right and left side, respectively.

The mobile phase consisted of 30% methanol and 2 mg/mL S-CD in 30 mM HAC-TEA buffer, pH 4.0.

tem is relatively weak, as can be seen from Fig. 2. The reason may be that it is a neutral solute and was retained on the stationary phase only because of the formation of a inclusion complex with the adsorbed S-CD.

The influence of the ionic strength on separation was investigated by varying the buffer concentration in the mobile phase from 10 to 35 mM at the applied voltage of 10 kV. The methanol and S-CD concentrations were kept at 10% and 2.0 mg/mL, respectively. The EOF was found to decrease steadily with increasing ionic strength. The increase of buffer concentration from 10 to 35 mM resulted in a decreased electroosmotic mobility from 18.3 to 15.7 $\text{cm}^2/\text{kV min}$. This behavior is usual in CEC [4, 17, 23]. The influence of buffer concentration on k^* is shown in

Fig. 4. It can be seen that the retention of atropine, metoprolol and verapamil decreased with increase of buffer concentration. As discussed previously, the ion-exchange mechanism contributes to the strong retention of these solutes in this system. The elution strength of the mobile phase increases with increase of the ionic strength in ion-exchange CEC [17, 20], resulting in a reduction of the retention. Although the EOF decreased with the increase of ionic strength, the migration time of these three pairs of enantiomers decreased at high ionic strength because of the reduced retention. For example, the elution time of the late-eluted verapamil enantiomer decreased from 62.1 to 36.4 min when the buffer concentration increased from 10 to 35 mM. Therefore, in order to save analysis time, it is desired to perform separation under high ionic

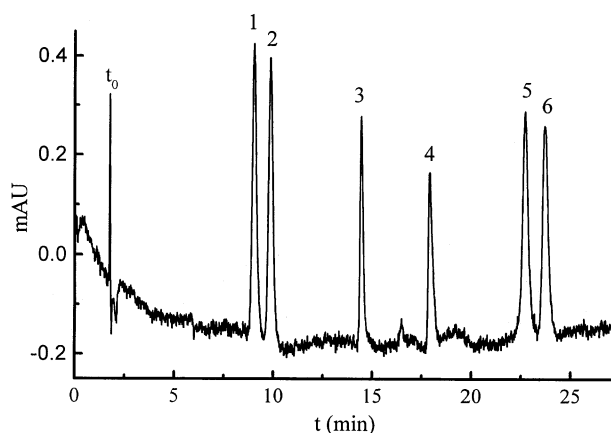


Figure 3. Chiral separation of tryptophan, atropine and verapamil in a single run by strong SAX-CEC with dynamically modified S-CD. Conditions: applied voltage, 15 kV; mobile phase, 30% methanol and 2.0 mg/mL S-CD in 20 mM HAC-TEA buffer (pH 4.0). Other conditions as in Fig. 1. Solutes: (1) L-tryptophan, (2) D-tryptophan, (3) and (4) atropine, (5) and (6) verapamil.

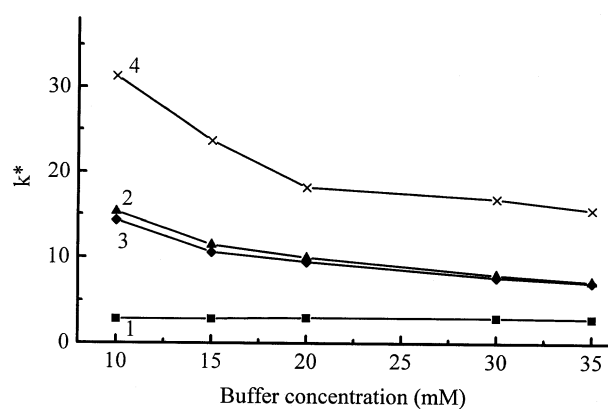


Figure 4. Influence of buffer concentration on the electrochromatographic retention factor. Conditions: mobile phase, 10% methanol and 2.0 mg/mL S-CD in HAC-TEA buffer (pH 4.0). Other conditions as in Fig. 1. Solutes: the first eluted enantiomer for (1) tryptophan, (2) atropine, (3) metoprolol, and (4) verapamil.

strength for these solutes. However, the retention of tryptophan is almost independent of the ionic strength. The reason may relate to other separation mechanisms such as electrophoresis. It was found that the influence of buffer concentration on the separation selectivity for tryptophan and metoprolol was moderate, but a slight reduction was found for atropine and verapamil. Since only the formation of an inclusive complex with CD is responsible for the chiral separation, triethylamine may compete with the solute for inclusion in the CD cavity, resulting in a decrease of the separation selectivity.

The charge on the SAX packing surface had already changed from positive to negative when 2.0 mg/mL S-CD was added into the mobile phase, thereby the net negative charge density should increase when further increasing the S-CD concentration because more S-CD will be adsorbed to the packing surface. Therefore, the velocity of EOF and the retention and separation selectivity of the solutes should also increase. The effect of S-CD concentration on separation was studied by varying the S-CD concentration from 2.0 to 8.0 mg/mL in the mobile phase containing 10% methanol in 20 mM HAC-TEA buffer solution (pH 4.0). Surprisingly, the electroosmotic mobility decreased from 17.6 to 16.3 $\text{cm}^2/\text{kV min}$, and the retention of the basic and neutral solutes decreased by about 15%, when the S-CD concentration increased from

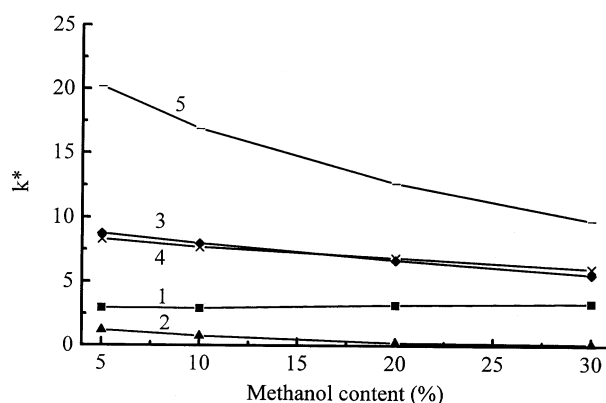


Figure 5. Influence of methanol content on the electrochromatographic retention factor. Conditions: mobile phase, various concentrations of methanol and 2 mg/mL S-CD in 20 mM HAC-TEA buffer (pH 4.0). Other conditions as in Fig. 1. Solutes: the first eluted enantiomer for (1) tryptophan, (2) praziquantel, (3) atropine, (4) metoprolol, and (5) verapamil.

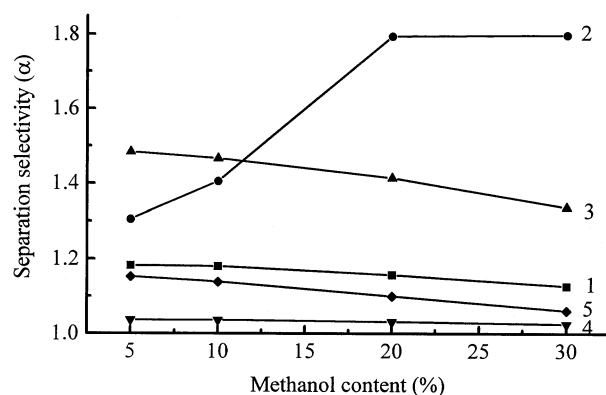


Figure 6. Influence of methanol content on separation selectivity. Conditions as in Fig. 5. Solutes: the enantiomer pairs of (1) tryptophan, (2) praziquantel, (3) atropine, (4) metoprolol, and (5) verapamil.

2.0 to 8.0 mg/mL. These results indicate that the adsorption of S-CD to the packing surface has almost been saturated even at an S-CD concentration as low as 2.0 mg/mL, and the amount of adsorbed S-CD does not significantly increase with increasing S-CD concentration. The reason for the decrease of EOF and retention for cationic solutes may be similar to that of the influence of ionic strength. S-CD is a sodium salt with the substitution of 7–11. This means 7–11 moles sodium ions are added if 1 mole S-CD is added to the solution. The ionic strength increased rapidly with increase of S-CD concentration, which results in the decrease of the retention of a solute based on the ion-exchange mechanism. But the influence of S-CD concentration on the separation selectivity was different from that of the ionic strength. The separation selectivity for tryptophan and metoprolol slightly decreased when the S-CD concentration increased, while the separation selectivity for atropine and verapamil increased with the increase of S-CD concentration. The possible reason is that the S-CD in mobile phase also contributes to the chiral separation.

The influence of methanol content on the separation was studied by varying the fraction of methanol in the mobile phase from 5 to 30%. The mobile phase contained 2.0 mg/mL S-CD and 30 mM HAC-TEA (pH 4.0). It was found that the mobility of the EOF decreased from 18.0 to 10.6 $\text{cm}^2/\text{kV min}$ when the methanol concentration increased from 5 to 30%. The dependence of the electrochromatographic retention factor k^* on the methanol content is shown in Fig. 5.

As can be seen (Fig. 5), an increase in the methanol content results in a decrease in the retention of the solutes. There are at least two reasons for this decrease: (i) as in IE-CEC, the hydrophobic interaction of an organic solute with the stationary phase in ion exchange decreases with the increase of the methanol content [17, 20], thereby the organic solutes retained on the stationary phase decreased; (ii) the methanol competes with the solute for inclusion in the CD cavity. The greater the percentage of methanol in the mobile phase, the more easily a solute is displaced from the CD cavity, resulting in the reduction of retention and separation selectivity for tryptophan, atropine, metoprolol, and verapamil. But the separation selectivity for praziquantel was found to increase with methanol content. The dependence of separation selectivity on methanol content is shown in Fig. 6. The peak shape greatly improves when the methanol content increases.

Figure 7 shows the chromatograms for separation of verapamil with mobile phases containing 5 and 30%

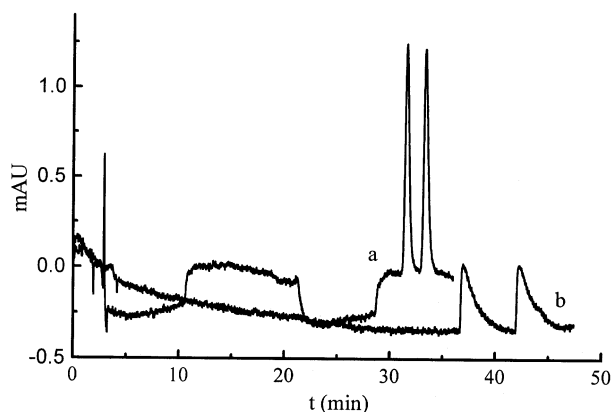


Figure 7. Separation of verapamil at different methanol contents. Conditions: mobile phase, (a) 30% and (b) 5% methanol in 2 mg/mL S-CD and 20 mM HAC-TEA buffer (pH 4.0). Other conditions as in Fig. 2.

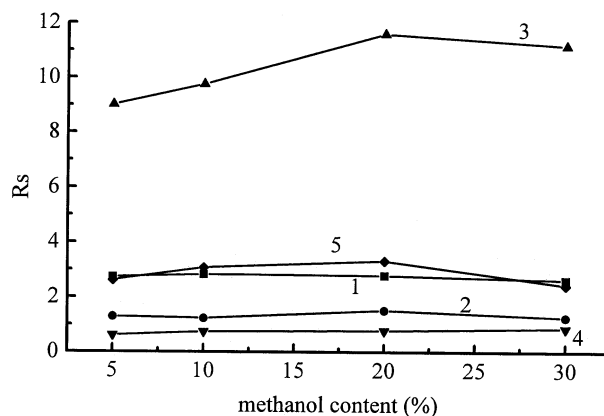


Figure 8. Dependence of resolution values on methanol content. Conditions and solutes were the same as in Fig. 6.

methanol, respectively. The tailing factor for the enantiomers of verapamil decreased from 6.49 and 5.05 to 1.36 and 1.46, respectively. The column efficiency also increased rapidly with increase of methanol content because of the improved peak symmetry. For example, the efficiency for the first eluted enantiomer of atropine increased from 106 000/m to 378 000/m when the methanol content increased from 5 to 30%. The separation selectivity for atropine, metoprolol and verapamil decreased at high methanol content, thereby their resolution should also decrease but instead their resolution increased with increasing methanol content (from 5 to 20%) because of the increase of the column efficiency. Figure 8 shows the dependence of resolution on methanol content. It was observed that the resolution for atropine increased from 9.0 to 11.6 when the methanol content increased from 5 to 20%.

4 Concluding remarks

A novel mode of SAX-CEC with dynamically modified S-CD was developed for resolution of enantiomers. It was observed that the addition of 2 mg/mL S-CD resulted in reversal of EOF from anode to cathode. The enantiomers of tryptophan, praziquantel, atropine, metoprolol, and verapamil were successfully separated in this system with a column efficiency varying from 36 000 to 412 000 plates/m. The retention of the basic enantiomers is simultaneously influenced by the electrophoresis and cation-exchange mechanisms and the inclusion of solutes into the CD cavity, but only the latter contributes to the resolution of enantiomers. The methanol concentration in the mobile phase plays an important role in improving the peak shape and column efficiency.

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5 References

- [1] Garner, T. W., Yeung, E. S., *J. Chromatogr.* 1993, **640**, 397–402.
- [2] Ye, M., Zou, H., Liu, Z., Ni, J., Zhang, Y., *J. Chromatogr. A* 1999, **855**, 137–145.
- [3] Ye, M., Zou, H., Liu, Z., Ni, J., Zhang, Y., *Anal. Chem.* 2000, **72**, 616–621.
- [4] Li, S., Lloyd, D. K., *J. Chromatogr. A* 1994, **666**, 321–335.
- [5] Lelièvre, F., Yan, C., Zare, R. N., Gareil, P., *J. Chromatogr. A* 1996, **723**, 145–156.
- [6] Wistuba, D., Czesla, H., Roeder, M., Schurig, V., *J. Chromatogr. A* 1998, **815**, 183–188.
- [7] Li, S., Lloyd, D. K., *Anal. Chem.* 1993, **65**, 3684–3690.
- [8] Lloyd, D. K., Li, S., Ryan, P., *J. Chromatogr. A* 1995, **694**, 285–296.
- [9] Liu, Z., Zou, H., Ni, J., Zhang, Y., *Anal. Chim. Acta* 1999, **378**, 73–76.
- [10] Liu, Z., Zou, H., Ye, M., Ni, J., Zhang, Y., *Electrophoresis* 1999, **20**, 2891–2897.
- [11] Liu, Z., Zou, H., Ye, M., Ni, J., Zhang, Y., *Chin. J. Chromatogr.* 1999, **17**, 245–248.
- [12] Masruska, A., Pyell, U., *J. Chromatogr. A* 1997, **782**, 167–174.
- [13] Carter-Finch, A. S., Smith, N. W., *J. Chromatogr. A* 1999, **848**, 375–385.
- [14] Schweitz, L., Andersson, L. I., Nilsson, S., *Anal. Chem.* 1997, **69**, 1179–1183.
- [15] Lin, J. M., Uchiyama, K., Hobo, T., *Chromatographia* 1998, **47**, 625–629.
- [16] Zhang, Y., Shi, W., Zhang, L., Zou, H., *J. Chromatogr. A* 1998, **802**, 59–71.
- [17] Ye, M., Zou, H., Liu, Z., Ni, J., *J. Chromatogr. A* 2000, **869**, 385–394.
- [18] Wu, J. T., Huang, P., Li, M. X., Lubman, D. M., *Anal. Chem.* 1997, **69**, 2908–2913.
- [19] Ye, M., Zou, H., Liu, Z., Ni, J., Zhang, Y., *Science in China B* 1999, **42**, 639–648.
- [20] Ye, M., Zou, H., Liu, Z., Ni, J., *J. Chromatogr. A* 2000, **887**, 223–231.
- [21] Smith, N. W., Evans, M. B., *Chromatographia* 1995, **41**, 197–203.
- [22] Li, D., Knobel, H. H., Remcho, V. T., *J. Chromatogr. B* 1997, **695**, 169–174.
- [23] Seifar, R. M., Kraak, J. C., Kok, W. T., Poppe, H., *J. Chromatogr. A* 1998, **808**, 71–77.