Enantiomer Separations of Phenylephrine and Synephrine by Capillary Electrochromatography on Bare Silica Stationary Phase Using Hydroxypropyl-β-cyclodextrin as a Mobile Phase Additive

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Abstract: The feasibility of enantiomer separation by capillary electrochromatography (CEC) was shown on 75-μm (i.d.) capillary columns packed with 3-μm bare silica packings. Racemic phenylephrine [3-hydroxy-α-(methylaminomethyl) benzyl alconhol] and synephrine [4-hydroxy-α-(methylaminomethyl) benzyl alconhol] were resolved on bare silica stationary phase using hydroxypropyl-β-cyclodextrin (HPβCD) as a chiral mobile phase additive. The effects of HPβCD concentration, pH of mobile phase, electrolyte concentration, and separation temperature have been investigated. Optimum separations were achieved for phenylephrine using 10 mM Tris–H_3PO_4 buffer (pH 3.12) containing 14 mM HPβCD and those of synephrine with HPβCD concentration 18 mM at 25°C. The chiral recognition mechanisms in these systems were also discussed. J Micro Sep 11: 263–269, 1999

Key words: *enantiomer separations; phenylephrine; synephrine, capillary electrochromatography; bare silica stationary phase*

INTRODUCTION

The enantiomer separation of pharmaceutical compounds is of great importance. It has been reported that often only one of the enantiomers possess biological activity in a racemic drug while the other might have side effects [1]. It has been a challenge for analytical chemists to monitor and determine the enantiomers purity of the drug rapidly and accurately. So far, many advanced separation techniques have been introduced to separate enantiomers, particularly high-performance liquid chromatography (HPLC) [2], gas chromatography (GC) [3], and capillary electrophoresis (CE) [4–7]. Chiral separation by CE is rapidly developed in recent years due to its high separation efficiency, high speed, and lower consumption of solvent and sample. Capillary electrophoresis with different operational modes has been successfully employed for enantiomer separation, such as capillary zone electrophoresis (CZE) [4–7], micellar elecrokinetic capillary chromatography (MECC) [8–10], capillary gel electrophoresis (CGE) [11], and capillary electrochromatography (CEC) [12–15]. Chiral separation by CEC was first demonstrated by Mayer and Schurig [12]. They achieved the enantiomer separation of several nonsteroidal anti-inflammatory drugs, ibuprofen, cicloprofen, flurbiprofen, and etodolac by using 50-μm (i.d.) open tubular columns coated with permethylated β or γ-CD (Chirasil-Dex). Szeman and Ganzler [13] showed the enantiomer separation of epinephrine using CDs-coated capillaries. Li and Lloyd [14, 15] first reported chiral separations by packed-column CEC. They carried out enantiomer separations of neutral and cationic enantiomers on immobilized 5-μm α-acid glycoprotein [14]. Neutral and anionic enantiomers were separated on 5-μm β-CD bonded stationary either by using phosphate buffer for neutral compounds or by reversing the electroosmotic flow with triethylammonium acetate buffer for anionic compounds [15]. Their results seemed discouraging due to the considerably low resolution and unacceptably long separation time. Lelievre et al. [16] reported two approaches to achieving the chiral separation of chlorthalidone by

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packed-column CEC. One is to use a chiral agent, HPβCD, directly in the mobile phase with an achiral stationary phase (3 μm ODS); the other is to use a chiral stationary phase, 5-μm HPβCD bonded silica particles, with an achiral mobile phase. In addition, mianserin, a basic drug, was also separated with the chiral stationary phase. Stalberg [17] recently used open tubular columns coated with C_8 and derivatized cyclodextrins as structure/chiral selectors for separation of several remoxipride analogue rotamers and enantiomers.

Chiral separation using bare silica stationary phase and cyclodextrin as a mobile phase additive in HPLC has also been reported. Walhagen Edholm [18] used silica stationary phase to separate chlorthalidone and oxazepam enantiomers with a mobile phase of β-CD in ammonium acetate buffer. Pullen [19] investigated the chromatographic behavior of the system comprised of β-CD CMPA and bare silica stationary phase. Retention mechanisms of this system were regarded as an ion-exchange, β-CD inclusion and hydrophobic interaction. The technique has a number of attractive features that may broaden the use of chiral mobile phase additives for direct enantiomer separation.

In the present work, the feasibility of chiral separation by CEC on bare silica packed column with HPβCD as a mobile phase additive was shown. The effects of experimental conditions on the separation resolution such as HPβCD concentration, pH of the mobile phase, electrolyte concentration, organic additive content, and separation temperature were also investigated. Optimum separations were achieved for two model chiral compounds.

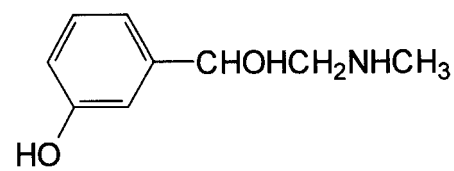
EXPERIMENTAL

Apparatus. Experiments were performed on a P/ACE 5500 capillary electrophoresis system (Fullerton, CA). The packed capillary columns were obtained from Unimicro Technologies, Inc. (Pleasanton, CA), which were packed with 3-μm bare silica (Micra Scientific, Inc., Northbrook, IL) by electrokinetic method [20]. The packed capillary column was 20 cm $\times$ 75 μm i.d. (27 cm total length) 20 cm from the inlet frit to the detection window. The separation column was installed in a Beckman Model 5510 P/ACE capillary cartridge, which was then inserted into the P/ACE instrument after conditioning. Capillary zone electrophoresis experiments under similar experimental condition for the chiral separation were also performed on a P/ACE 5500 capillary electrophoresis. Capillary columns used in CZE experiments were obtained from Yongnia Optical Fibre Factory (Heibei, China). The Capillary columns were 27, 47, and 87 cm total length with 75 μm inner diameter. The effective length of columns is 20, 40, and 80 cm from the inlet to the detection window, respectively. The on-line ultraviolet (UV) detector was operated at 214 mm, detection range was 0.05 a.u.f.s, and rinse time was 0.3 s. A Φ12 pH/ISE meter (Beckman) was used for pH measurements.

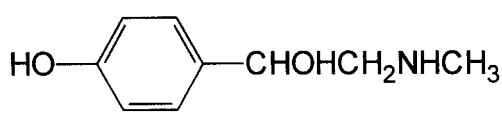
Chemicals. Hydroxypropyl-β-cyclodextrin (HpβCD) was obtained from chiral methods development kits (Beckman). Tris(hydroxymethyl) aminomethane (Tris) was purchased from Shanghai Regent Co. (Shanghai, China). Methanol was HPLC-grade used for organic modifier. Racemic drugs of phenylephrine hydrochloride (I) and synephrine tartrate (II) were kindly provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The structures of the two compounds are shown in Figure 1. All other chemicals were of analytical grade. Water was deionized.

Procedures. The mobile phase was prepared by first adjusting the buffer to the desired pH, then mixing with the appropriate amount of HPβCD. Tris/H_3PO_4 buffer was prepared by mixing different proportional 100 mM Tris solution and 100 mM H_3PO_4 adjusted to the desirable pH. The sample solutions were prepared by dissolving each solute in methanol at an approximate concentration of 0.2–0.5 mg/mL.

The mobile phase was filtered through a 0.25-μm filter and degassed with ultrasonication for about 3 min before it was used. Prior to run, the capillary was preconditioned by the mobile phase through the capillary at a low applied voltage of 5 kV until constant current was achieved. Electrokinectic injections were performed in both CEC and CE experiments at 1 kV for 1 s.



(I)



(II)

Figure 1. *Structures of model compounds.*

Dead times in silica packed column and open tubular column were determinated by using methanol and dimethyl sulfoxide as markers, respectively.

Resolution of the enantiomers (R_s) was calculated by using the following equation

$$R_s = 1.177\frac{t_{R2} - t_{R1}}{W_{0.5(1)} + W_{0.5(2)}}$$

where t_{R1} and t_{R2} are the retention times of the first and second peaks of enantiomers, respectively; $w_{0.5(1)}$ and $w_{0.5(2)}$ are the half-widths of the first and second peaks of enantiomers, respectively.

RESULTS AND DISCUSSION

Enantiomer separations by CEC and CZE. Figure 2(a) shows the electrochromatogram of the enantiomer separation of phenylephrine by CEC using 10-mM Tris–H_3PO_4 buffer (pH 3.12) containing 14 mM HPβCD. The enantiomer separation of synephrine using 10 mM Tris–H_3PO_4 buffer (pH 3.12) containing 18 mM HPβCD are illustrated in Figure 2(b). Poorer resolutions for two model compounds by CZE under the same experimental conditions (i.e., the same buffer and the same length column) were observed compared with those in CEC. It may be attributed to short retention in column due to a fast electroosmotic flow (EOF) velocity in CZE.

In order to compare CE and CEC on a near equal time basis, a 47-cm-length open tubular capillary column was used under 22 kV applied voltage. Although an improved resolution is observed, resolutions for two compounds also were poor, as shown in Figures 3(a) and 3(b). It seemed the packed column did assist the chiral separation. We speculate that the pseudo-chiral stationary formed due to HPβCD sorbed on silica packings, which may assist the chiral separation.

In order to confirm our speculation, the dead times for the separations in CEC and CZE were determined in the same dimension column (27 cm/20 cm) under applied voltage of 15 kV. Dimethyl sulfoxide was selected as a marker in CZE. Methanol was used in CEC because dimethyl sulfoxide may be retained on silica packings [21]. The dead times in CEC and CZE using 10 mM Tris–H_3PO_4 buffer (pH 3.12) containing 18 mM HPβCD were 16.67 and 13.09 mins, respectively. The dead times using 10 mM Tris–H_3PO_4 buffer (pH 3.12) containing 14 mM HPβCD were 15.70 and 12.34 mins, respectively. The dead time in CZE was about 20% shorter than that in CEC, which was different from that in reserved-phase CEC [2]. It is attributed to the larger density of the free silanol groups on silica packings

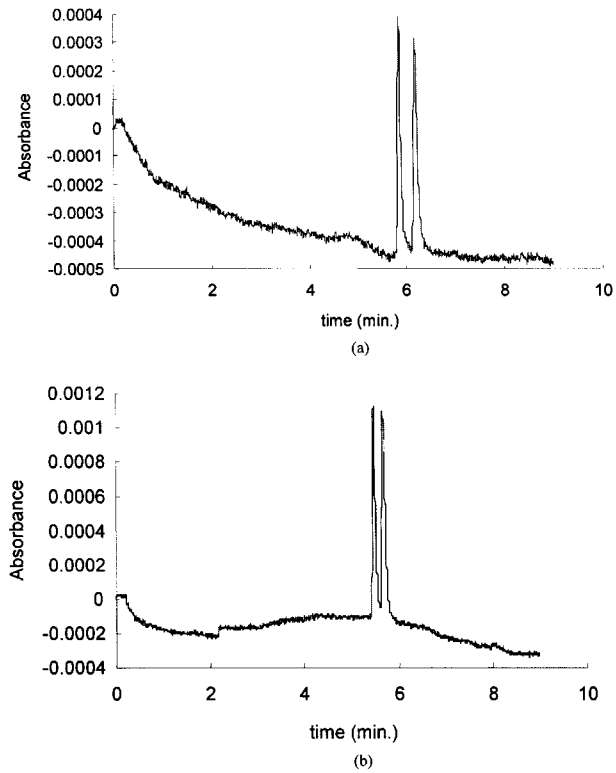


Figure 2. *Electrochromatograms of the enantiomer separations of phenylphrine and synephrine by CEC. Experimental conditions: (a) Phenylephrine: mobile phase, 10 mM Tris/H_3PO_4 (pH 3.12) with 14 mM HPβCD as a additive. (b) Synephrine: mobile phase, 10 mM Tris/H_3PO_4 (pH 3.12) with 18 mM HPβCD as a additive. Sample 0.2 mg/mL in demonized water. Voltage injections, 1 kV/s. Temperature, 25°C. Column, 75-μm (i.d.) × 27 cm/20 cm capillary packed with 3-μm bare silica packings. Applied voltage, 15 kV. UV detection at 214 nm.*

[23]. Obviously, the effect of EOF on resolution cannot be well explained for a good resolution in CEC.

We further increased the column length, and a 87-cm-length column was used under 30 kV applied voltage. The electropherograms of two model compounds are shown in Figures 4(a) and 4(b), respectively. Although retention time is near three times as long as that of CEC under this condition, again, the resolution is less than that in CEC. It indicates that the packed column indeed assists the chiral separation.

HPβCD concentration on resolution. HPβBCD concentration plays an important role in enantiomer separation. The chiral separation only can be achieved when the inclusion-complexation forms between the HPβCD and solutes. Figure 5 gives the

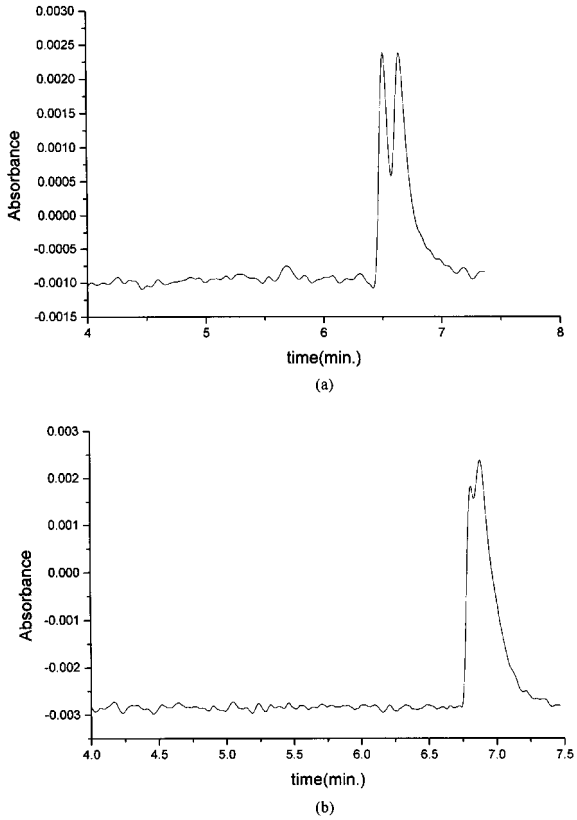


Figure 3. *Electropherograms of the enantiomer separations of phenylphrine and synephrine by CZE: (a) phenylphrine and (b) synephrine. Experimental conditions: column, 75-μm (i.d.) × 47 cm/40 cm open tubular capillary; applied voltage, 22 kV. Other conditions as in Figure 2.*

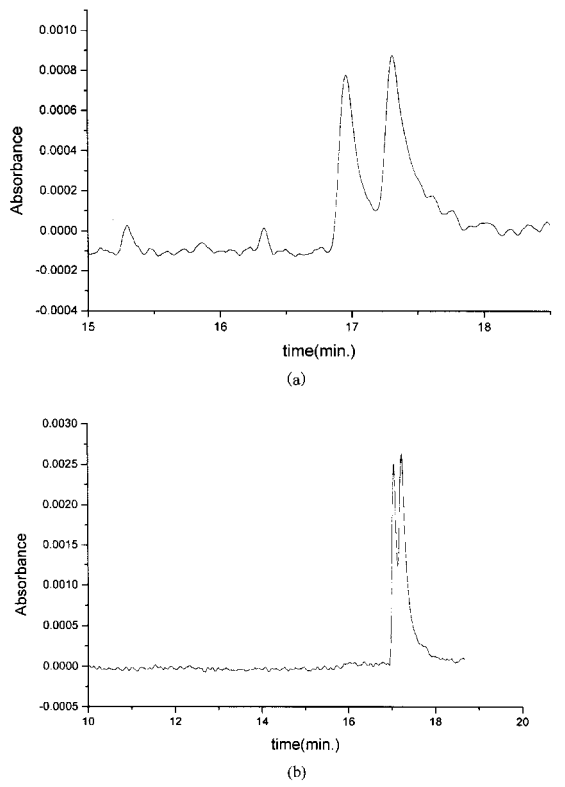


Figure 4. *Electropherograms of the enantiomer separations of phenylphrine and synephrine by CZE: (a) phenylphrine and (b) synephrine. Experimental conditions: column, 75-μm (i.d.) × 87 cm/80 cm open tubular capillary: applied voltage, 30 kV. Other conditions as in Figure 2.*

resolution as a function of HPβCD concentration. For the phenylephrine, when HPβCD concentration is larger than 10 mM, the resolution is less affected by HPβCD concentration. The resolution for synephrine first decreases with the increase of HPβCD concentration, then begins to increase at HPβCD concentration over 14 mM. The different shapes of resolution vs. HPβCD concentration for phenylephrine and synephrine reflect the difference in retention mechanism.

pH of mobile phase on resolution. Separations were studied at pH 2.53, 3.12, 4.21, 5.22, and 6.24. Figure 6 shows the effect of pH on resolutions of two racemic compounds. A best resolution was obtained at pH 3.12 for phenylephrine when pH varied from 2.53 to 6.24. For synephrine, the resolution increased slightly with the increase of pH. The reason is that pH might influence the inclusion-complexation between the HPβCD and solutes. Figure 7 illustrates the relationship between retention time and pH. The retention time decreased with increasing pH from pH 2.53 to 5.22. When pH was above 5.22, retention time began to increase, which was different from that in CZE. The EOF should behave similarly in both CEC and CE since the charge of bare silica surface increases with the increase of pH.

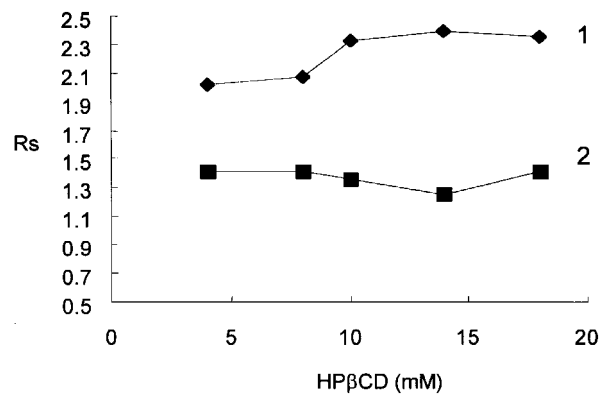


Figure 5. *Effect of HPβCD concentration on resolutions of (1) phenylephrine and (2) synephrine. Experimental conditions: mobile phase, 10 mM Tris/H_3PO_4 (pH 3.12). Other operating conditions as in Figure 2.*

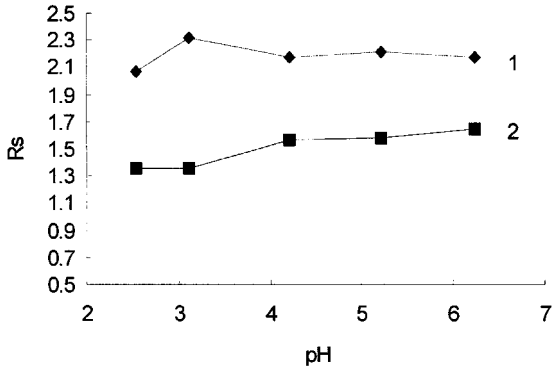


Figure 6. *Effect of pH on resolutions of (1) phenylephrine and (2) synephrine. Experimental conditions: mobile phase, 10 mM Tris/H_3PO_4 with 10 mM HPβCD. Other conditions as in Figure 2.*

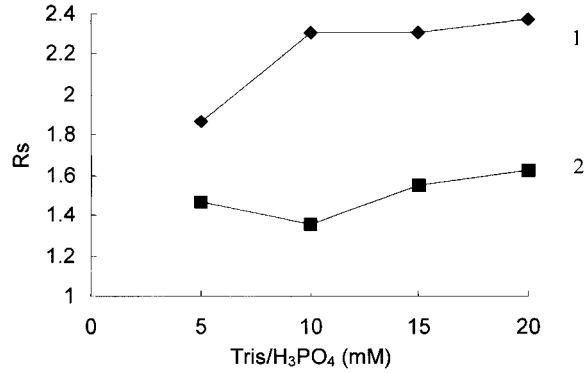


Figure 8. *Effect of ionic strength on retention time of (1) phenylephrine and (2) synephrine. Experimental conditions: mobile phase, 10 mM Tris/H_3PO_4 (pH 3.12) with 10 mM HPβCD. Other conditions as in Figure 2.*

The retention behavior shows a cation exchange mechanism which has been observed in HPLC [24]. Cation exchange mechanism results in a long retention time at higher pH.

Ionic strength on resolution. A high ionic strength electrolyte was often used for achieving chiral separation by CZE. However, a relative low ionic strength buffer was employed in CEC in order to avoid bubble formation in high ionic strength. Figure 8 shows the effect of Tris/H_3PO_4 concentration at pH 3.12 on resolution. The resolution increased with the increase of ionic strength for the phenylephrine while for synephrine it first decreased in Tris/H_3PO_4 concentration from 5 to 10 mM, then increased with the increase of ionic strength. Again, the retention of the two model compounds behaved differently.

Organic additive on resolution. The organic additive can influence enantioselectivity, efficiency, and resolution [25]. The effect of the organic additive is shown in Figure 9. The resolution decreased with the increase of the methanol proportion, which was attributed to the interaction between the solvent and the cyclodextrin cavity. However, a high efficiency and good peak shape were observed with the increase of methanol proportion. Figure 10 shows the relationship between the methanol proportion and theoretical plates per meter. The highest theoretical plate per meter is 186,000 for phenyleprine at 20% (v/v) methanol with 1.61 resolution. The results are very encouraging and indicate the potentiality for chiral separation by using this method.

Temperature effects. Figures 11 and 12 show the effects of the separation temperature on resolution and retention time, respectively. Altria [26] reported that resolution in CZE seemed to correlate with migration time when the separation temperature changed in chiral separation. However, a similar

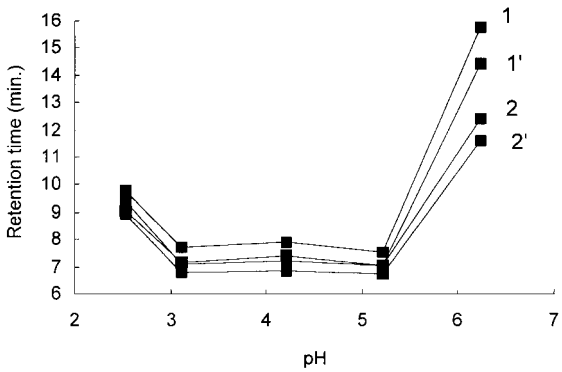


Figure 7. *Effect of pH on retention time of (1 and 1') phenylephrine and (2 and 2') synephrine. Experimental conditions as in Figure 2.*

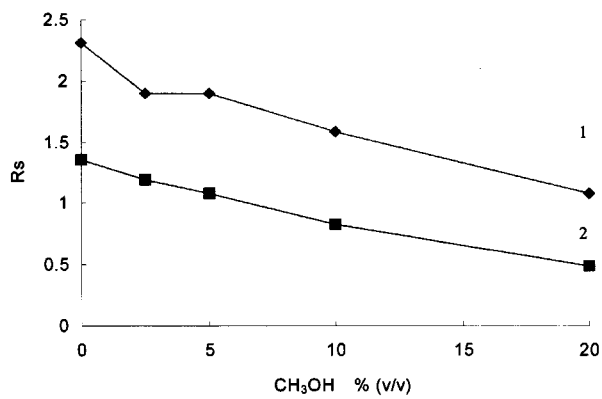


Figure 9. *Effect of organic additive on resolutions of (1) phenylephrine and (2) synephrine. Experimental conditions: mobile phase, 10 mM Tris/H_3PO_4 (pH 3.12) with 10 mM HPβCD. Other conditions as in Figure 2.*

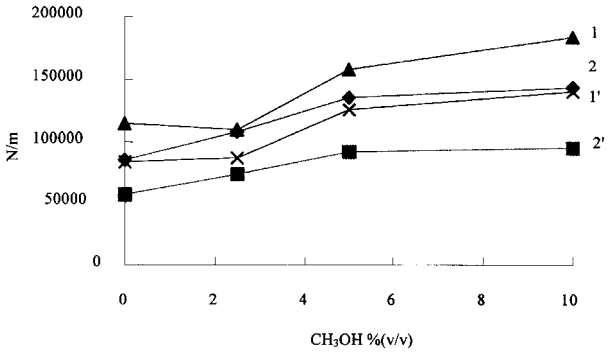


Figure 10. *Effect of methanol proportion on theoretical plates per meter of (1 and 1') phenylephrine and (2 and 2') synephrine. Experimental conditions as in Figure 9.*

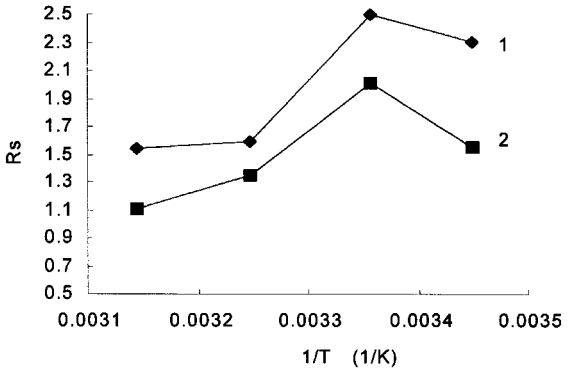


Figure 11. *Effect of separation temperature on resolutions of (1) phenylephrine and (2) synephrine. Experimental conditions: mobile phase, 15 mM Tris/H_3PO_4 (pH 3.12) with 10 mM HPβCD as a additive. Other conditions as in Figure 2.*

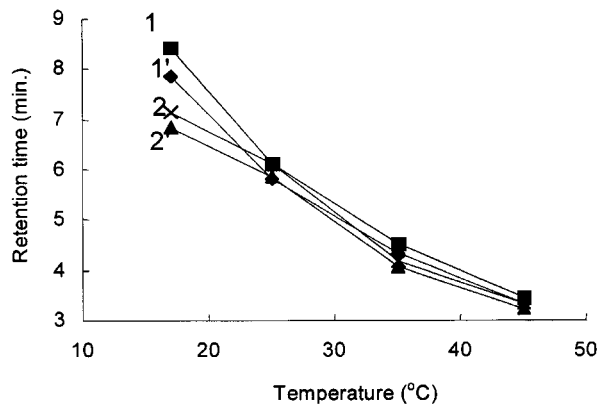


Figure 12. *Effect of separation temperature on retention time of (1 and 1') phenylephrine and (2 and 2') synephrine. Experimental conditions as in Figure 11.*

tendency cannot be observed in our work. The different results reflect the discrimination of retention behaviors between enantiomer separations by CEC and CZE. For both solutes, the best resolution were obtained at 25°C in the separation temperature investigated. The reason temperature affects resolution is not clear. Further investigation is required for exploring the retention mechanism in these systems.

CONCLUSIONS

The feasibility of enantiomer separation by CEC on bare silica stationary using HPβCD as the chiral additives was demonstrated in CEC on capillary columns packed with 3-μm bare silica packings. Two basic racemic drugs were resolved successfully. The effects of experimental parameters on resolution were investigated. Optimum separations were achieved for phenylephrine using 10 mM Tris–H_3PO_4 buffer (pH 3.12) containing 14 mM HPβCD and those of synephrine with HPβCD concentration 18 mM at 25°C. This new method provided several advantages: (1) HPβCD worked very well in CEC because it was retarded in the packed column due to retention, (2) the loadability should be more improved in CEC than that in CE because of the much higher surface in CEC due to packing materials, (3) sensitivity may be improved in CEC by the possibility of using relatively larger internal diameter columns because of the much lower current due to the packing materials and organic solvent in the mobile phase, and (4) higher column efficiency was achievable in CEC compared with that in HPLC.

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