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Enantiomeric separation of amphetamine and phenylephrine by cyclodextrin-mediated capillary zone electrophoresis

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Abstract

The enantiomeric separation of amphetamine and phenylephrine was investigated using cyclodextrin (CD)-mediated capillary zone electrophoresis. The enantiomers of amphetamine can be baseline separated by using 50 m*M* Tris–H_3PO_4 buffer (pH 2.3) containing 12 m*M* heptakis(2,6-di-O-methyl)-β-CD and those of phenylephrine by using 50 m*M* Tris–H_3PO_4–6 *M* urea–100 m*M* β-CD (pH 2.3) buffer. CD type and concentration, buffer composition, organic modifier and capillary length all play a role in chiral separation.

Keywords: Enantiomer separation; Buffer composition; Amphetamine; Phenylephrine; Cyclodextrins; Methamphetamine

1. Introduction

The separation of drug enantiomers is of primary importance because enantiomers of the same compound frequently differ in their physiological activity. For the purpose of chiral separation, various chromatographic techniques, particularly high-performance liquid chromatography (HPLC) [1,2] and gas chromatography (GC) [3,4], can be used; however, GC is limited to volatile compounds and chiral HPLC often shows poor efficiency and is relatively expensive. Recently, capillary electrophoresis (CE), because of its high separation efficiency, relatively simple instrumental set-up and several different separation modes, has shown rapid developments in chiral separations [5,6]. There are several advantages of CE compared with HPLC for chiral separations. Direct chiral separations using CE can be performed easily by adding chiral compounds or chiral surfactants, which interact with enantiomeric solutes, to the buffer solution without changing the capillary tube. Because of the low volume of the CE system, the amount of chiral selector consumed during analysis is small, making chiral separations by CE relatively inexpensive. Various kinds of chiral selectors, including cyclodextrins (CDs) [7–10], bile salts [11], crown ethers [12], maltodextrin [13], proteins [14] and chiral surfactants [15] have been used as buffer additivies in capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC). Capillary electrochromatography (CEC) [16] and capillary gel electro-

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phoresis (CGE) [17] have also been explored for the separation of chiral compounds. In addition, some investigators have considered the theoretical [18,19] and quantitative [20,21] aspects of chiral CE.

Recently, Rizzi et al. [22] studied the enantiomeric separation of amphetamine, methamphetamine and ring-substituted amphetamines by HPLC with a β-CD chiral stationary phase. However, amphetamine, the most frequently encountered drug, could not be well resolved without derivatization. Lurie [23] separated enantiomers of amphetamine, methamphetamine, and their precursors using MEKC after derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glycopyranosyl isothiocyanate. However, he did not succeed in separating the enantiomers of non-derivatized amphetamine by chiral CE. The enantiomers of phenylephrine were investigated by Nishi et al. [9] in phosphate buffer solution (pH 3.0) containing 2 *M* urea and 20 m*M* of each CD. No separation was observed with β-CD and poor resolution with heptakis(2,6-di-O-methyl)-β-CD (DM-β-CD).

Here, we report the successful enantiomeric separation of both amphetamine and phenylephrine by using CZE with CDs as chiral selector.

2. Experimental

2.1. Apparatus

Experiments were carried out on a laboratory-assembled CE system. An uncoated fused-silica capillary of 62 cm length (effective length 41 cm) × 75 μm I.D. × 375 μm O.D. (Yongnian Optical Fibre Factory, Hebei, China) was used as a separation tube. A laboratory-made high-voltage power supply that can provide voltages from 0 to 30 kV was used to drive the separation. On-column detection was performed at the cathode on a CV^4 UV detector (ISCO, Lincoln, NE, USA) at 210 nm with a rise time of 0.8 s. Electropherograms were recorded on an SE 120 recorder (ABB Goerz Instruments, Vienna, Austria). A small fan was used to dissipate the Joule heat generated by power. A pHs-3C pH meter with an E-201-C combination electrode (Rex Instrument Factory, Shanghai, China) was used for pH measurements.

2.2. Chemicals

α-, β- and γ-CD were purchased from TCI (Tokyo, Japan). DM-β-CD was obtained from Sigma (St. Louis, MO, USA). Tris(hydroxymethyl)aminomethane (Tris) and urea were purchased from Fluka (Buchs, Switzerland). Enantiomeric drugs amphetamine, methamphetamine and phenylephrine hydrochloride (structures are shown in Fig. 1) were kindly provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other chemicals were of analytical-reagent grade. Water was doubly distilled.

2.3. Procedures

Tris–H_3PO_4 buffer (50 m*M*) (pH 2.3) was prepared by dissolving 3.025 g of Tris in water, titrating it to pH 2.3 with phosphoric acid and diluting the solution to volume in a 500-ml volumetric flask. CDs were dissolved in the above buffer and, when necessary, an appro-

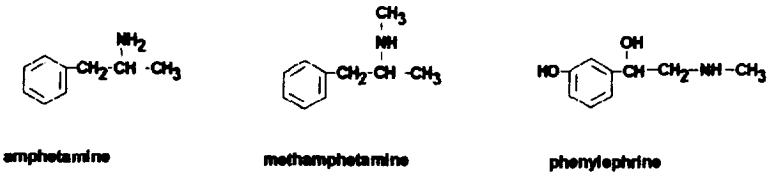

Fig. 1. Structures of the solutes investigated.

priate amount of urea was added. The buffers were filtered through 0.45-μm membrane filters and degassed by sonication prior to use. The sample solutions were prepared by dissolving each solute in Tris–H_3PO_4 buffer at an approximate concentration of 0.1 mg/ml so the adequate signals could be obtained.

The new capillary was vacuum rinsed with 0.5 *M* NaOH and water for 30 min each in order to activate the silica on the wall and then equilibrated with the operating buffer for 10 min. Between two consecutive injections, the capillary was rinsed with 0.1 *M* NaOH for 2 min, water for 2 min and the operating buffer for 5 min. Samples were injected by the electrokinetic method at the anode and CZE operations were run under constant voltage and at ambient temperature (about 14–17°C).

Resolution between enantiomers was evaluated by the equation

$$R_s = 1.177(t_2 - t_1)/[w_{1/2(1)} + w_{1/2(2)}]$$

where t_2 and t_1 arc the migration times (min) of the two enantiomers and $w_{1/2(1)}$ and $w_{1/2(2)}$ are the peak widths of each peak at the half-height (min).

3. Results and discussion

3.1. Selection of buffer pH

Buffer pH is an important parameter in CZE, since alterations in pH can affect the solute charge, depending on the solute properties, and change the electroosmotic flow (EOF), which generally increases as the pH is increased, thus influencing the resolution. It has been shown [6] that generally, successful chiral separation of basic drugs is achieved under acidic buffer conditions when using neutral CDs as chiral selector. Under acidic conditions, basic drugs and their CD complexes will migrate towards the cathode and free CDs are uncharged and will move with the velocity of the EOF, which also moves from the anode to the cathode in the same direction as the electrophoretic mobility. In this study, an acidic buffer at about pH 2.3 was used in all the experiments. At this pH, the basic racemic drugs will be more positively charged and the EOF will be much less than at higher pH, thus providing analytes with a longer time for interaction with CD moieties as they migrate through the capillary. Moreover, at pH 2.3, which is near the pK_{a1} value of H_3PO_4 (2.1), the buffer has a high buffer capacity to resist the pH changes caused by electrolysis effect in the CZE process [24].

3.2. Effect of CD type

CDs have been widely used as chiral selectors for enantioseparation in CE. α-, β- and γ-CD are non-ionic cyclic oligosaccharides which contain six, seven and eight glucose units, respectively. They have the shape of a hollow truncated cone with a hydrophobic inner cavity and hydrophilic outer surface. The larger opening of the cavity is lined with secondary hydroxyl groups and the smaller opening with primary hydroxyl groups. In addition, they are chiral molecules where each glucose unit contains five chiral atoms. Chiral selectivity results from inclusion of a hydrophobic portion of the solute in the cavity and also from the hydrogen bonding to the hydroxyl moieties. CDs, particularly β-CD, have been derivatized with various functional groups to improve solubility and provide unique selectivity for separation.

In this study, α-, β- and γ-CD and DM-β-CD were investigated for the chiral recognition of the three enantiomeric drugs indicated in Fig. 1 by using 50 m*M* Tris–H_3PO_4 buffer solution (pH 2.3) containing 12 m*M* of each CD. When α- and γ-CD were used, no chiral recognition was observed for any of the three drugs tested; when β-CD and DM-β-CD were used, enantiomeric separations of different degrees were achieved for amphetamine and phenylephrine (see Figs. 2 and 3), but not for methamphetamine. As can be seen in Figs. 2 and 3, baseline separation was achieved for phenylephrine when using DM-β-CD, and it was also well separated using β-CD; the enantiomers of amphetamine were better separated with β-CD than DM-β-CD.

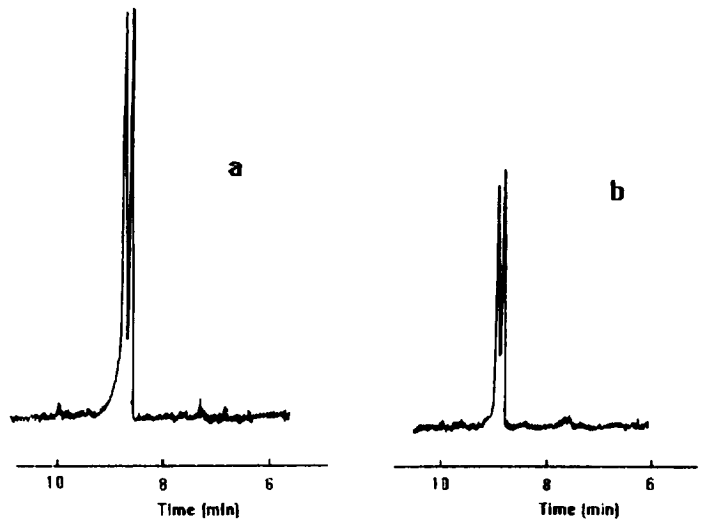

Fig. 2. Electropherograms of the separation of racemic amphetamine (a) and phenylephrine (b). Conditions: background electrolyte (BGE), 50 m*M* Tris–H_3PO_4 containing 12 m*M* β-CD (pH 2.3); separation tube, 62 cm (41 cm to detector) × 75 μm I.D. × 375 μm O.D.; running voltage, 22 kV; detection, 210 nm (0.005 AUFS); temperature, ambient (about 15°C).

3.3. Effect of buffer composition and methanol addition on chiral separation

Altria et al. [8] found that citric acid–sodium acetate buffer has a specific effect on the enantiomeric separation of picumeterol, with the best resolution being achieved in this buffer. We found here that when 50 m*M* NaH_2PO_4–H_3PO_4 buffer (pH 2.3) containing 12 m*M* β-CD, instead of 50 m*M* Tris–H_3PO_4 buffer (pH 2.3) containing 12 m*M* β-CD, was used, the enantiomeric resolution of phenylephrine declined to zero

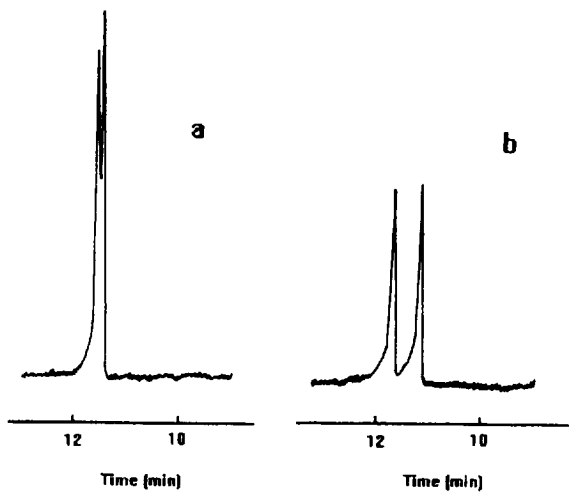

Fig. 3. Electropherograms of the separation of racemic amphetamine (a) and phenylephrine (b). Conditions: BGE, 50 m*M* Tris–H_3PO_4 containing 12 m*M* DM-β-CD (pH 2.3); other conditions as in Fig. 2.

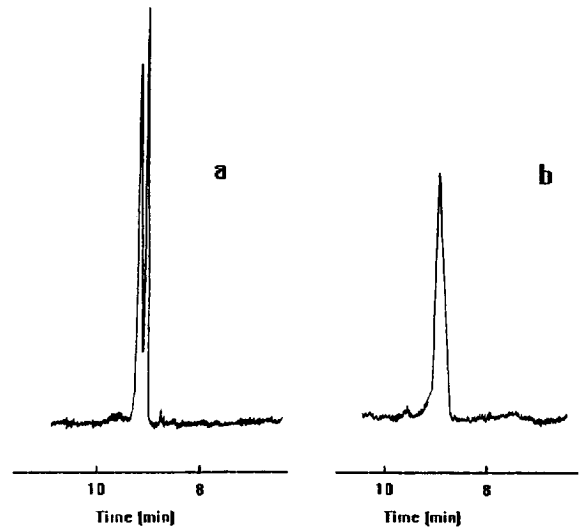

Fig. 4. Electropherograms of the separation of racemic amphetamine (a) and phenylephrine (b). Conditions: BGE, 50 m*M* NaH_2PO_4–H_3PO_4 containing 12 m*M* β-CD (pH 2.3); other conditions as in Fig. 2.

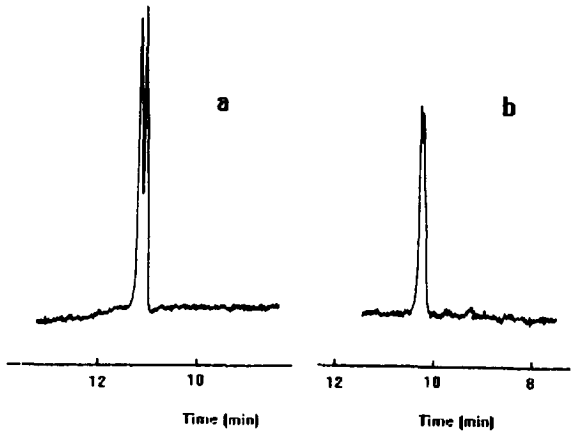

Fig. 5. Electropherograms of the separation of racemic amphetamine (a) and phenylephrine (b). Conditions: BGE, methanol–50 m*M* Tris–H_3PO_4 containing 12 m*M* β-CD (pH 2.3) (10:90); other conditions as in Fig. 2.

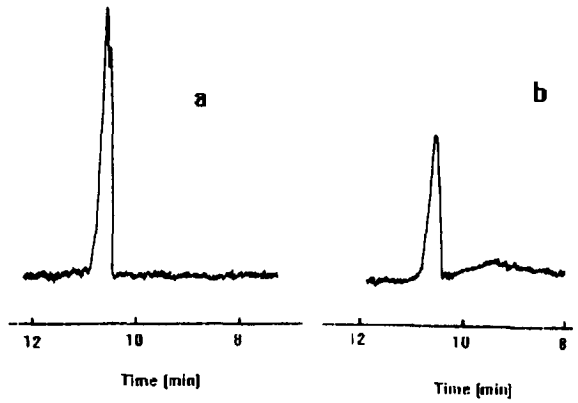

Fig. 6. Electropherograms of the separation of racemic amphetamine (a) and phenylephrine (b). Conditions: BGE, methanol–50 m*M* NaH_2PO_4–H_3PO_4 containing 12 m*M* β-CD (pH 2.3) (10:90); other conditions as in Fig. 2.

while the separation for amphetamine remained almost uninfluenced (compare Fig. 4 with Fig. 2). Considering that the only difference between the two buffers is the buffer cation, one being the Tris and the other Na^+, the result suggests that when Tris was selected as buffer cation, because of its lower mobility and therefore a smaller Joule heating effect relative to smaller ions, it can alter the buffer selectivity for certain specific chiral separations.

Wren and Rowe [18,19] showed that the use of methanol and other organic solvents can lead to either an increase or a decrease in chiral separation. Fanali [7] found that the chiral separation for propranolol was only achieved using CZE with 30% methanol in the background electrolyte (4 *M* urea–40 m*M* β-CD, pH 2.5). In our study, methanol was tested for the chiral separation of the investigated drugs by adding 10% of methanol to buffers consisting of 50 m*M* Tris–H_3PO_4–12 m*M* β-CD (pH 2.3) and 50 m*M* NaH_2PO_4–H_3PO_4–12 m*M* β-CD (pH 2.3), and the results are shown in Figs. 5 and 6. Fig. 5 shows that when 10% of methanol was added to the Tris-containing buffer, the enantiomeric separations of both amphetamine and phenylephrine were poor compared with Fig. 2. Comparing Fig. 6 with Fig. 4, it can be seen that when 10% of methanol was added to the NaH_2PO_4-containing buffer, the separation of amphetamine was also adversely affected, but to a larger extent than in the former case, while phenylephrine still remained unresolved. The resolution

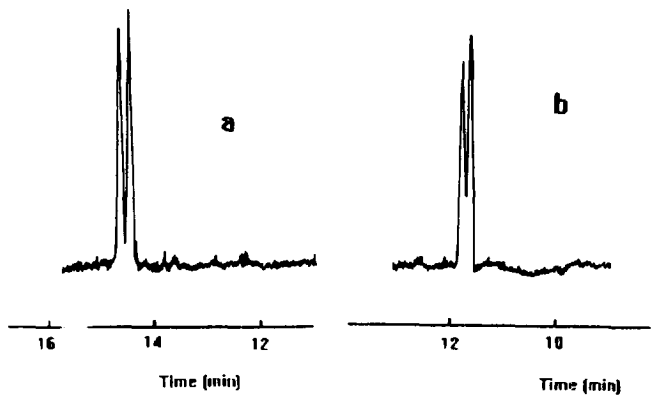

Fig. 7. Electropherograms of the separation of racemic amphetamine (a) and phenylephrine (b). Conditions: BGE, 50 m*M* Tris–H_3PO_4 containing 4 *M* urea and 60 m*M* β-CD (pH 2.3); running voltage, 25 kV; other conditions as in Fig. 2.

differences between Figs. 5 and 6 may further indicate selectivity differences in chiral separation between the two buffer systems, viz., the Tris–H_3PO_4 system and the NaH_2PO_4–H_3PO_4 system, both of which have been widely used for chiral separations in CE.

3.4. Chiral separation at high β-CD concentrations with urea added

Previous work [9,19] has shown that both the concentration of CD and the addition of urea can influence chiral separations. In this study, in order to obtain better separations for the investigated solutes, we explored separations at high β-CD concentrations where the addition of an appropriate amount of urea is necessary for high β-CD solubilities. First, the buffer 50 m*M* Tris–H_3PO_4 containing 4 *M* urea and 60 m*M* β-CD (pH 2.3) was examined and the results are shown in Fig. 7. Compared with the result obtained at low β-CD concentration with no urea added (see Fig. 2), the resolution of amphetamine was improved whereas that of phenylephrine was almost unchanged and the migration times of both compounds were increased. The above results encouraged us to use a much higher concentration of β-CD for further improvement of the separation of amphetamine. Fig. 8 illustrates that when 50 m*M* Tris–H_3PO_4 containing 6 *M* urea and 100 m*M* β-CD (pH 2.3) was used, the resolution of amphetamine was further improved to give baseline separation whereas that of phenylephrine was only slightly improved. From Fig. 8, it also can be seen that under these conditions, there is an anomalous baseline ca. 12 min after sample injection. However, this did not interfere with the detection of the compounds investigated.

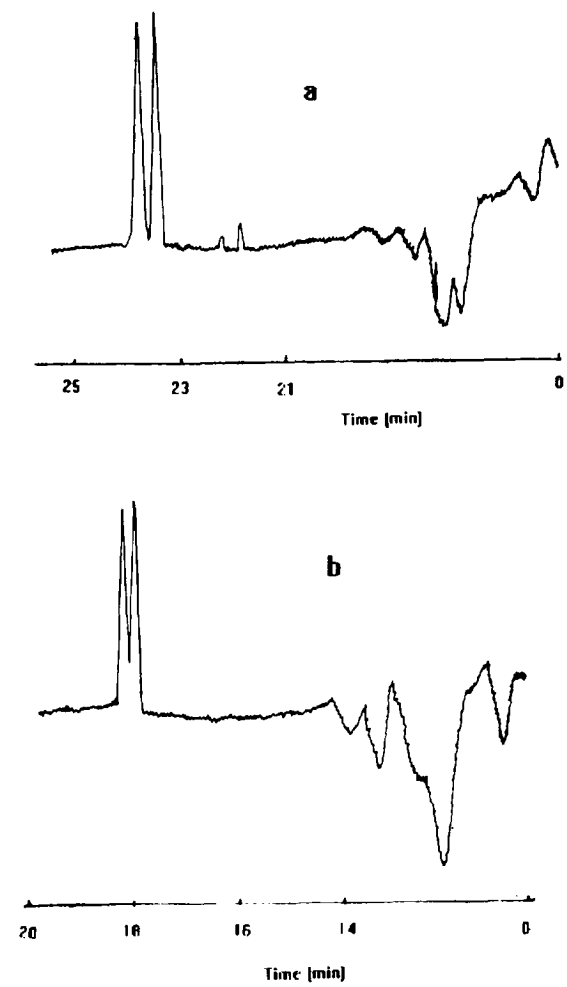

Fig. 8. Electropherograms of the separation of racemic amphetamine (a) and phenylephrine (b). Conditions: BGE, 50 m*M* Tris–H_3PO_4 containing 6 *M* urea and 100 m*M* β-CD (pH 2.3); other conditions as in Fig. 7.

3.5. Effect of capillary length on separation

Fig. 9 indicates that when a longer capillary tube (80 cm to the detector) was used, an improved resolution of both amphetamine and phenylephrine was achieved at the cost of much longer migration times compared with Fig. 2. It appears that appropriately increasing the capillary length would be an approach of choice for improving resolution in CZE with CDs. Of course, a prolonged analysis time would be needed.

4. Conclusion

We have successfully resolved the enantiomers of amphetamine and phenylephrine using CD-mediated CZE. CD type and concentration, buffer composition, organic solvent and capillary

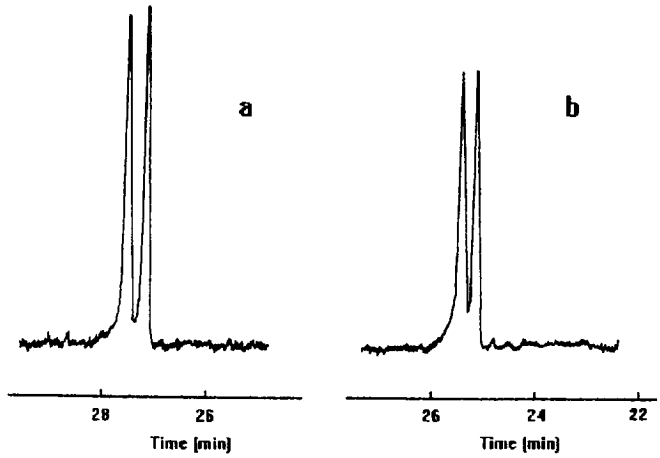

Fig. 9. Electropherograms of the separation of racemic amphetamine (a) and phenylephrine (b). Conditions: separation tube, 102 cm (80 cm to detector) × 75 μm I.D. × 375 μm O.D.; running voltage, 26.5 kV; other conditions as in Fig. 2.

length influence the separation. However, the enantiomers of methamphetamine, an amphetamine analogue, could not be resolved under any of the experimental conditions examined. This suggests that the solute also plays an important role in enantioselectivity. CZE offers an attractive and inexpensive approach for chiral separation. However, at present, chiral separations are mainly achieved by trial and error methods and it is difficult to predict with which chiral selector and under what conditions a successful chiral separation can be achieved for a certain solute.

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