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Use of cyclodextrins and cyclodextrin derivatives in high-performance liquid chromatography and capillary electrophoresis

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Abstract

Carboxymethyl- and carboxyethyl-β-cyclodextrin have proved to be effective chiral mobile phase additives in HPLC for the resolution of enantiomers of hexobarbital, ephedrine and an aminomethylbenzodioxane derivative. The separations are influenced by a combination of hydrophobic and electrostatic interactions. The type and concentration of the cyclodextrin derivative and the pH and type of base used to adjust the pH can be varied in order to optimize the method. In addition, a coating procedure was developed for capillary electrophoresis. The enantiomers of epinephrine were resolved on a γ-cyclodextrin-coated capillary. The length of the capillary has a significant effect on the resolution. β-Cyclodextrin was found to give no resolution.

1. Introduction

Cyclodextrins (CDs) can be very effective for enantiomer separations in a variety of separation methods. When CDs are used as chiral mobile phase additives in liquid chromatography, at least three factors control the enantioselective separation: differences in the stability constants of the CD complexes, differences in the adsorption of CD complexes on the surface of stationary phase and differences in the adsorption of free solute molecules on the cyclodextrin layer that is adsorbed on the surface [1]. In addition to the hydrophobic complex-forming interaction between the CD cavity and the guest molecule in the case of ionic guests, electrostatic interaction is also involved when using ionic CD derivatives [2]. In previous work we studied the use of ionic CD derivatives as mobile phase additives [3]. We now report on the effect of two statistically substituted ionic β-CD derivatives, namely carboxymethyl-β-CD (CMBCD) and carboxyethyl-β-CD (CEBCD), on the enantiomeric separation of some cationic guests.

Cyclodextrins are also used in capillary electrophoresis. Enantiomeric separation in capillary electrophoresis is usually achieved by the addition of a chiral selection to the buffer solution [4–6]. Using this approach, the chiral selector has to be replaced after each electrophoretic run. Immobilization of cyclodextrins can offer an advantage, in that the chiral selector is bound to the surface of the capillary, thus avoiding the need to replace it. Recently, an immobilized cyclodextrin stationary phase has been developed for coating fused-silica capillaries using

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GLC stationary phases [7]. Here, we report another approach for the immobilization of cyclodextrins.

2. Coating theory

Grafting vinyl monomers to cellulose or other hydroxyl-containing polymers using Ce(IV) or Co(IV) salts or redox catalysts is a well known technique in the textile industry [8–11]. Coating capillaries with cyclodextrins involves a similar mechanism, the difference being that an acrylamide layer is bound to the capillary wall prior to the cyclodextrin coating, to minimize electroosmotic flow and solute–wall interaction. The polymerization of acrylamide is a free radical polymerization, where chain termination is performed either by chain elongation or by charge disproportionation [12]. During acrylamide polymerization, owing to the chain termination step several double bonds develop, thus providing binding sites for the cyclodextrins:

Chain termination of acrylamide:

$$AA(n)^* + AA(m)^* \rightarrow AA(n+m)$$

$$AA(n)^* + AA(m)^* \rightarrow AA(n) + AA(m-1){=}A$$

where AA(n) and AA(m) denote polyacrylamide from n or m segments, respectively, and AA($m-1$)=A represents polyacrylamide with m segments, containing a vinyl group at the end of the chain. During polymerization both termination steps occur, but their ratio is different depending on the conditions [12].

In this coating procedure a cyclodextrin layer is grafted to the double bonds present in the linear acrylamide [13] coating:

Coating reactions:

Initiation:

$$CD{-}OH + Ce^{4+} \rightarrow Complex \rightarrow CD{-}O^* + Ce^{3+} + H^+$$

Propagation:

$$CD{-}O^* + AA(m-1){=}A \rightarrow CD{-}O{-}AA(m)^*$$

Termination:

$$CD{-}O{-}AA(m)^* + Ce^{4+} \rightarrow CD{-}O{-}AA(m) + Ce^{3+} + H^+$$

3. Experimental

3.1. Reagents

Cyclodextrins, CMBCD [Degree of substitution (DS): 3, CY-2006], CEBCD (DS: 3.5, CY-2012) and soluble γ-cyclodextrin polymer (CY-3009) are commercial products of CYCLOLAB (Budapest, Hungary). The racemic solutes tested (Fig. 1) were of pharmaceutical grade. Epinephrine samples [(−)- and (±)-] were purchased from Aldrich (Steinheim, Germany). Eluents were prepared from HPLC-grade solvents (CHEMOLAB). Triethylamine (TEA) was purchased from Carlo Erba (Milan, Italy), cerium(IV) sulphate from Fluka (Buchs, Switzerland) and acrylamide, ammonium peroxodisulphate and TEMED from Bio-Rad Labs. (Hercules, CA, USA). Fused-silica capillaries (50 μm I.D.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). All other reagents were of analytical-reagent grade.

3.2. Apparatus and procedures

HPLC

A Hewlett-Packard HP 1050 HPLC system with multiple wavelength detector and a HPLC

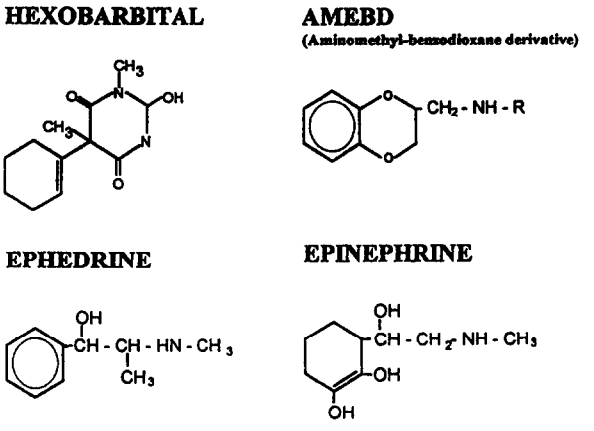

Fig. 1. Structures of the drugs studied.

ChemStation (DOS series) were used. The detector wavelength was 220 nm (band width 4 nm) and the reference wavelength was 350 nm (band width 80 nm). Experiments were carried out using a Nucleosil 300-5 C_4 cartridge column (100 × 4 mm I.D.) (Macherey–Nagel, Düren, Germany). The mobile phase was usually 5 mg/ml aqueous CD solution–ethanol (90:10, v/v); the flow-rate was 1 ml/min.

Capillary electrophoresis

Electrocoating and the electrophoretic runs were performed using a simple laboratory-made capillary electrophoresis system [14] consisting of a power supply and a UV detector (ISCO, Lincoln, NE, USA). Detection was performed at 214 nm.

Coating procedure. Fused-silica capillaries were coated with linear acrylamide (LA) according to the procedure of Hjertén [13] with a slight modification [15]. After polymerization, the excess of LA was pushed out from the capillaries and replaced with 5–10% (w/v) CD solution. CDs were dissolved in the separation buffer. The Ce^{4+} ions were caused to migrate electrophoretically into the capillary to provide the highest grafting ratio (Fig. 2). (Ce solution should be freshly prepared each day.) The coating procedure was completed overnight. The capillary should be washed extensively with the running buffer prior to a run. The stability of the coating was checked under the conditions in ref. 15.

Separation conditions. Electrophoretic separation was performed at room temperature in borate–phosphate buffer (0.02 *M*, pH 7.0) containing 10% of 2-propanol, using a 200 V/cm electric field in each instance. The samples were injected hydrodynamically.

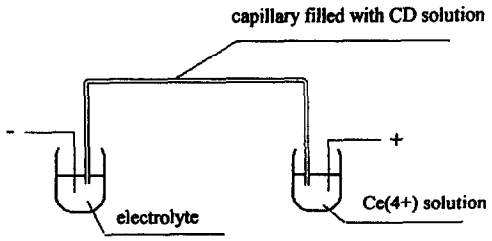

Fig. 2. Electrocoating of the capillary. Conditions: fused-silica capillary filled with CD solution. Applied electric field, 50 V/cm; for other parameters, see Experimental.

4. Results and discussion

4.1. HPLC

Effect of pH and the type of base used to adjust pH on capacity factor and resolution

Hexobarbital enantiomers are resolved using 5 mg/ml CEBCD in the mobile phase at pH 3.3 without using any base to adjust the pH (Fig. 3A

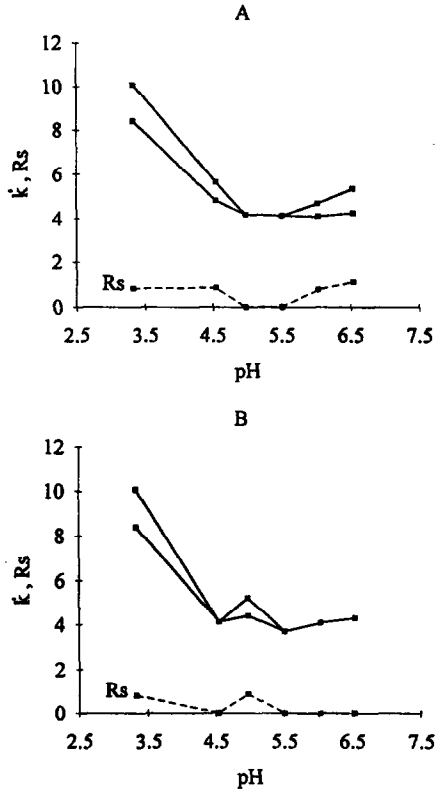

Fig. 3. Effects of pH and the type of base used to adjust pH on the capacity factor and resolution of hexobarbital using CEBCD as mobile phase additive. Mobile phase, 5 mg/ml aqueous CEBCD solution–ethanol (90:10, v/v); pH adjusted with (A) TEA and (B) NaOH. Solid lines, capacity factor of the two enantiomers, dashed lines, resolution.

and B). The capacity factor of hexobarbital decreases on increasing the pH to 5 and increases with further increase in pH when the pH is adjusted with TEA (Fig. 3A). A similar tendency can be observed for resolution. Using NaOH to adjust the pH, enantiomeric separation is observed at pH 5.0. The best resolution, R_s = 1.11, was achieved at pH 6.5 with the CEBCD–TEA eluent (Fig. 4).

Hexobarbital enantiomers could not be resolved using CMBCD when the pH was not adjusted with any base. The use of NaOH to adjust the pH resulted in good enantiomeric resolution at pH 4.5 and 5.5 (Fig. 5B). Although in the literature TEA is usually recommended as a buffer component when chemically bonded CD columns are used, in our experiments the separation of hexobarbital enantiomers cannot be achieved when the pH of CMBCD solution is adjusted with TEA (Fig. 5A).

The enantiomers of another basic guest molecule, an aminomethylbenzodioxane derivative, AMEBD, could also be resolved using 5 mg/ml

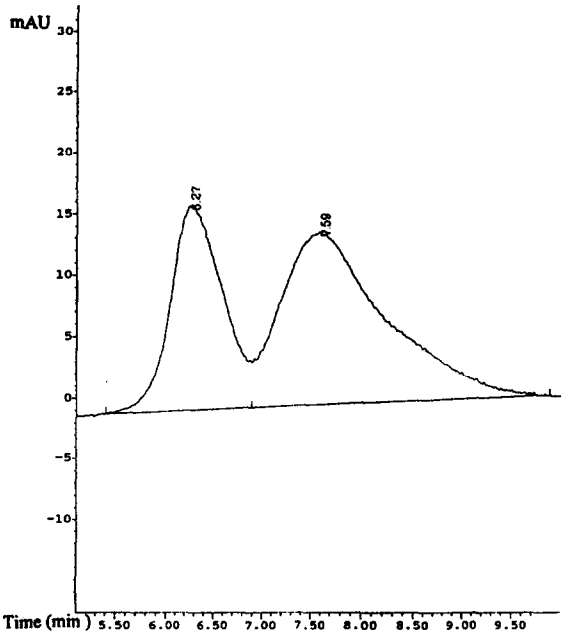

Fig. 4. Separation of hexobarbital enantiomers. Mobile phase, 5 mg/ml aqueous CEBCD solution–ethanol (90:10, v/v); pH = 6.5, adjusted with TEA.

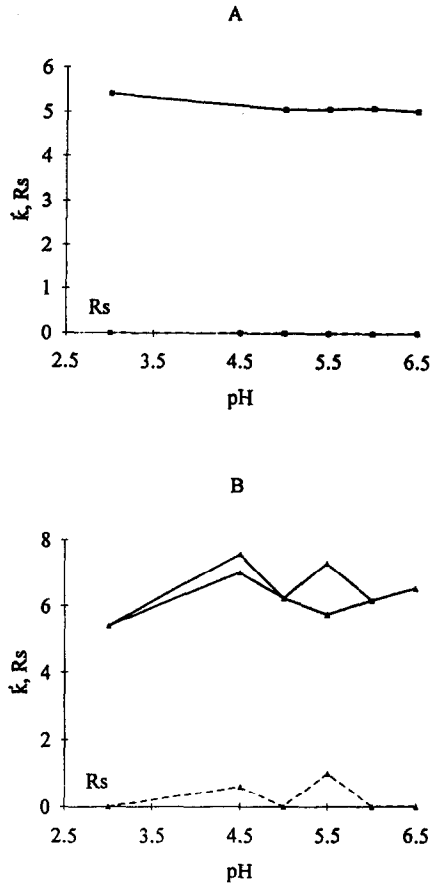

Fig. 5. Effects of the pH and the type of base used to adjust pH on the capacity factor and resolution of hexobarbital using CMBCD as mobile phase additive. Mobile phase, 5 mg/ml aqueous CMBCD solution–ethanol (90:10, v/v); pH adjusted with (A) TEA and (B) NaOH. Solid lines, capacity factor of enantiomers; dashed lines, resolution.

CEBCD at pH 3.3 without using base to adjust the pH. The capacity factor decreases with increasing pH in CEBCD–TEA mobile phases (Fig. 6A); with this, reasonable resolution (R_s = 1.10) was obtained at pH 5.5 (Fig. 7). Resolution could hardly be achieved at all when using NaOH to adjust the pH of CEBCD solution (Fig. 6B). Similarly to the results obtained with hexobarbital, when CMBCD was used as a mobile phase additive, only when NaOH was used as the pH modifier could the resolution of enantiomers of AMEBD be achieved (Fig. 8B).

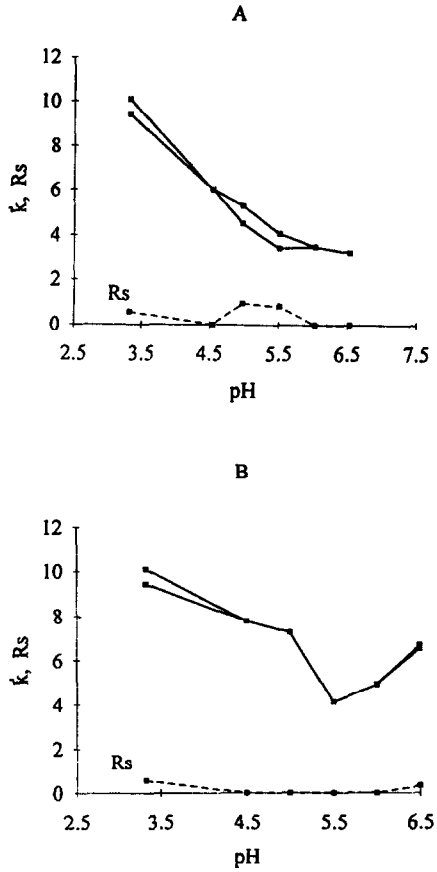

Fig. 6. Effects of the pH and the type of base used to adjust pH on the capacity factor and resolution of AMEBD using CEBCD as mobile phase additive. Mobile phase, 5 mg/ml aqueous CEBCD solution–ethanol (90:10, v/v); pH adjusted with (A) TEA and (B) NaOH. Solid lines, capacity factor of enantiomers; dashed lines, resolution.

The optimum pH is 5.5, where the resolution is 1.11.

The enantiomers of ephedrine were also separated in CEBCD–TEA systems (Fig. 9A) in the pH range 3.3–5.5 (at pH 3.3 no base was added to the CEBCD solution), whereas the CEBCD–NaOH-containing eluents were found not to be effective (Fig. 9B). The capacity factors of ephedrine are lower than 2 when CMBCD is used in the mobile phase; probably the use of a more apolar stationary phase (C_8 or C_{18}) would give better results.

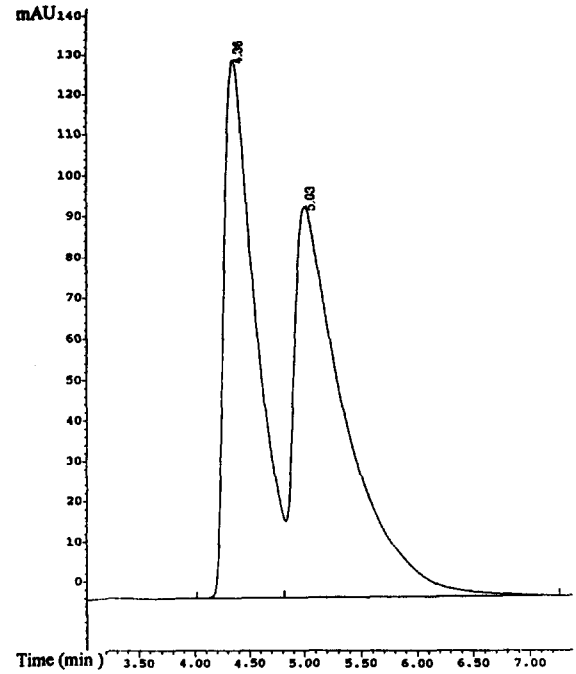

Fig. 7. Separation of AMEBD enantiomers. Mobile phase, 5 mg/ml aqueous CEBCD solution–ethanol (90:10, v/v); pH 5.5, adjusted with TEA.

Effect of CD concentration on capacity factor and resolution

The enantioselectivity generally improves with increasing CD concentration in the mobile phase. However, enhancement of the ionic CD concentration also results in an increase in the ionic strength of the solution. In oppositely charged host–guest systems, electrostatic attractive interactions are weakened by an increase in ionic strength, whereas hydrophobic interactions are strengthened [2]. The resultant of these effects influences the chromatographic behaviour of basic drugs in anionic CD-containing mobile phase systems.

Table 1 summarizes the resolution obtained with different eluent systems. Usually no enantiomeric separation was achieved at 2.5 mg/ml CMBCD or CEBCD concentration. Increasing the CD concentration resulted in a decrease in capacity factor and in an increase in the resolution of enantiomers. However, no enantio-

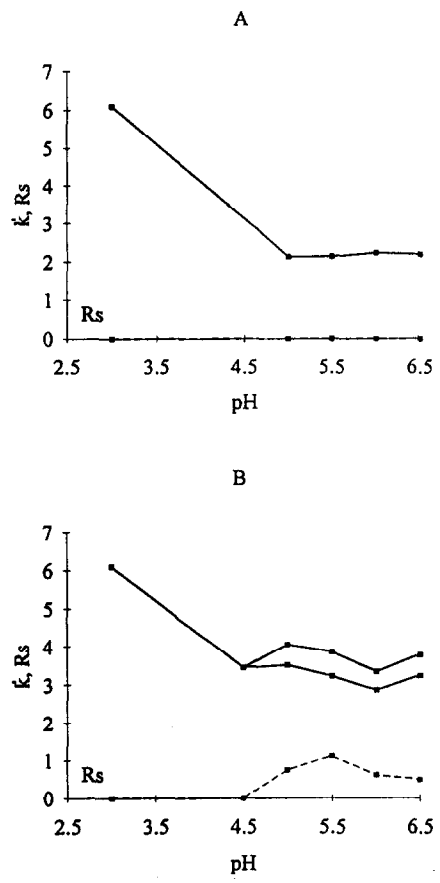

Fig. 8. Effects of the pH and the type of base used to adjust pH on the capacity factor and resolution of AMEBD using CMBCD as mobile phase additive. Mobile phase, 5 mg/ml aqueous CMBCD solution–ethanol (90:10, v/v); pH adjusted with (A) TEA and (B) NaOH. Solid lines, capacity factor of enantiomers; dashed lines, resolution.

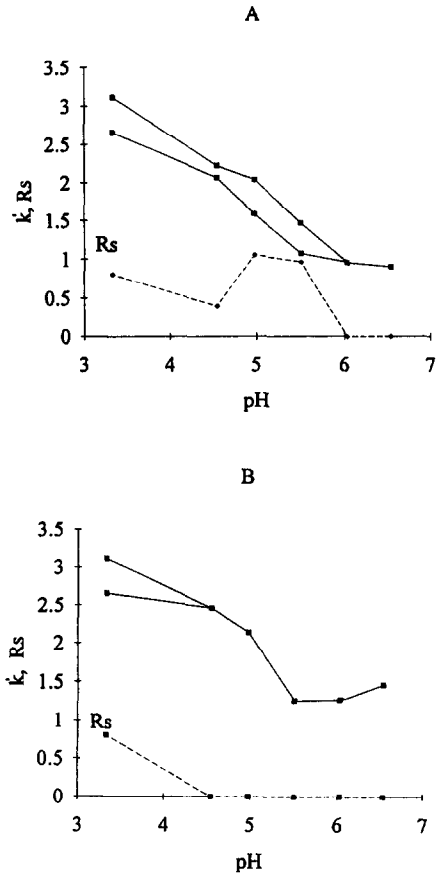

Fig. 9. Effects of the pH and the type base used to adjust pH on the capacity factor and resolution of ephedrine using CEBCD as mobile phase additive. Mobile phase, 5 mg/ml aqueous CEBCD solution–ethanol (90:10, v/v); pH adjusted with (A) TEA and (B) NaOH. Solid lines, capacity factor of enantiomers; dashed lines, resolution.

Table 1
Dependence of resolution on CD concentration in the mobile phase

Solute	Mobile phase	CD (mg/ml)				
		2.5	5	10	20	5 (in 0.05 *M* NaCl)
Hexobarbital	CMBCD–NaOH (pH 5.5)	0	1.01	0.53	0	0
	CEBCD–TEA (pH 5.0)	0	0	0.93	0.87	–
AMEBD	CMBCD–NaOH (pH 5.5)	0.67	0.71	0.61	0	0.43
	CEBCD–TEA (pH 5.0)	0	0.94	0.85	0	–
Ephedrine	CEBCD–TEA (pH 5.0)	0	1.05	0.36	0	–

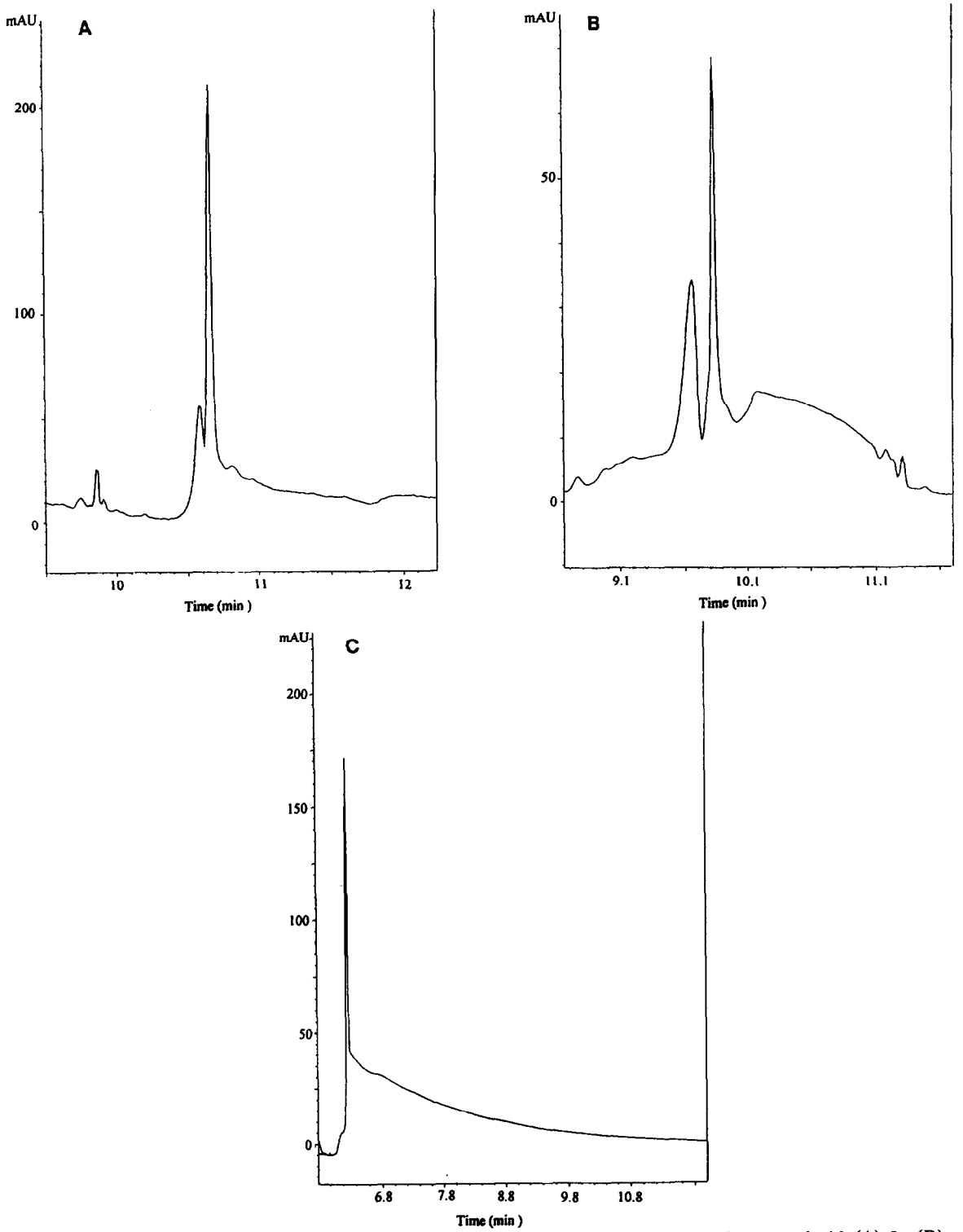

Fig. 10. Effect of CD type on the separation of (±)-epinephrine. Conditions: fused-silica capillary coated with (A) β-, (B) γ- or (C) γ-CD polymer. The effective length of the capillary was 14 cm in each instance. For other parameters, see Experimental.

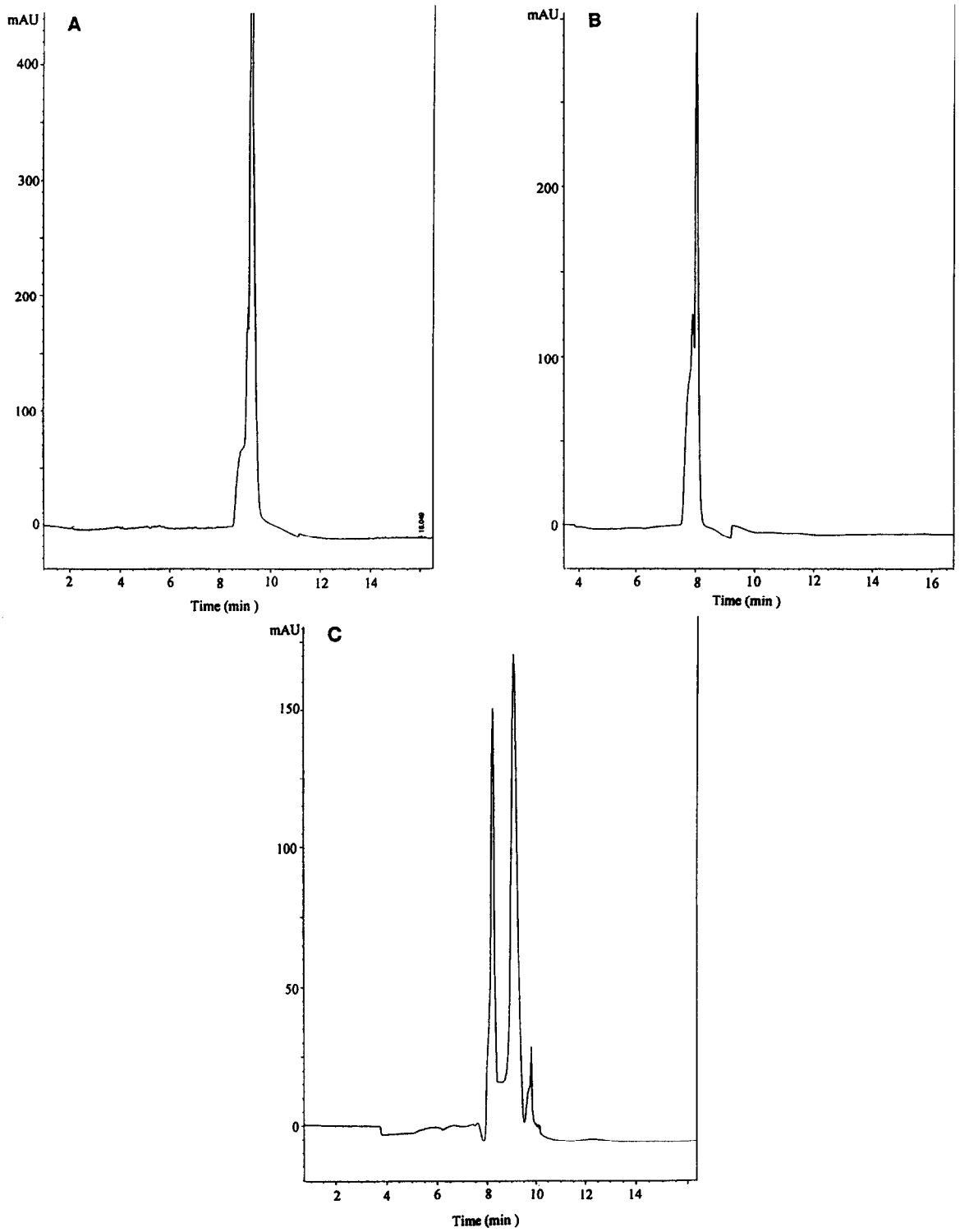

Fig. 11. Effect of the capillary length on the separation of (±)-epinephrine. Conditions: fused-silica capillary coated with γ-C
Effective length: (A) 5 cm; (B) 8 cm; (C) 18 cm. For other parameters, see Experimental.

meric separation was observed at 20 mg/ml CD concentration. The optimum CD concentration was usually 5–10 mg/ml, except for hexobarbital in the CEBCD–TEA system, where a 10 mg/ml CD concentration was necessary for the resolution of enantiomers. On increasing the ionic strength with NaCl (0.05 *M*), the resolution also decreased.

Effect of the ethanol concentration of mobile phase

The capacity factor, as usual, showed a tendency to decrease with increase in ethanol content of the mobile phase, whereas there were only slight differences in the resolution of enantiomers at different ethanol concentrations.

4.2. Capillary electrophoresis

The effect of the type of CD coating on the separation of epinephrine was studied using β- and γ-CD and water-soluble γ-CD polymer-coated capillaries. As shown in Fig. 10B, a γ-CD coating seems to be the best for the separation of epinephrine enantiomers.

The effect of the capillary length was studied with a γ-CD coating using capillaries with effective lengths of 5, 8 and 18 cm. The resolution increases with increasing capillary length (Fig. 11).

5. Conclusions

Ionic CD derivatives are useful chiral mobile phase additives in HPLC methods. The combination of hydrophobic and ionic interactions offers a wide range of possibilities for optimizing separations. In addition to the usual conditions, such as concentration of CD derivatives and pH, the type of ionic substituents on the CD ring (*e.g.*, carboxymethyl or carboxyethyl) also influences enantiomeric separations. The type of base used to adjust the pH is also an important factor. The effect of TEA, which is a widely used additive in RP-HPLC, was unfavourable when CMBCD was used as a chiral selector.

Chiral-selective capillary coatings were prepared by modifying the coating procedure [15] for enantiomeric separations in capillary electrophoresis. The CD coating proved to be stable at neutral pH for up to 50 injections. The length of the capillary has a significant effect on the resolution of enantiomers. Also, the type of chiral selector plays a significant role in the performance of the capillary. Further studies are planned on the use of other CD derivatives and to test the stability of the column over a wider pH range.

6. Acknowledgement

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7. References

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