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Extending the scope of chiral separation of basic compounds by cyclodextrin-mediated capillary zone electrophoresis

Changyu Quang[1], Morteza G. Khaledi*

Department of Chemistry, North Carolina State University, P.O. Box 8204, Raleigh, NC 27695, USA

Abstract

In a previous paper on cyclodextrin-mediated capillary zone electrophoresis, it was shown that the use of short-chain tetraalkylammonium cations leads to a reversal in the direction of the electroosmotic flow without an adverse effect on enantioselectivity. As a result, enantiomeric resolution of basic (cationic) compounds can be improved as the electroosmotic flow counteracts the migration of solute enantiomers. It is demonstrated in this report that the scope of chiral separation of basic compounds can be further extended by a combination of reversing the electroosmotic flow and enhancing enantioselectivity through the chemical modifications of β-cyclodextrin. Therefore, β-cyclodextrin and its derivatives were evaluated as chiral recognition agents for the chiral separations of 22 basic compounds with rather complex molecular structures. The differences in enantioselectivity displayed by β-cyclodextrin and derivatives are discussed in order to achieve a better understanding of the chiral interactions involved in the discrimination of solute enantiomers.

1. Introduction

Cyclodextrin (CD)-mediated capillary zone electrophoresis (CZE) has been successful, versatile, and inexpensive for separation of chiral compounds [1–18]. Chiral separation of dansylated amino acids, catecholamines, and other chiral amines have been reported where CDs have either been incorporated into a gel matrix [5,6], mixed with a micellar phase [7,8], or used in the free buffer solutions. Although chiral separation by CD-mediated CZE has been quite successful, individual separation problems frequently require different types of derivatized CDs (e.g., dimethylated-, trimethylated-β-CD and others). At this time, selections of the appropriate chiral selectors for a given chiral compound are not straightforward since predictions of the enantioselectivity of CDs based on the molecular structures of guest molecules are often difficult. Therefore, it is necessary to evaluate the different enantioselectivity of CDs for a wide range of chiral compounds in order to better understand the chiral interactions involved in the discrimination of solute enantiomers and to effectively utilize them in chiral separations.

In a previous work on CD-mediated CZE, it was shown that the use of short-chain tetraalkylammonium (TAA) cations in the buffer solution leads to a reversal in the direction of the electroosmotic flow (EOF) at the acidic condition of pH 2.5 [1]. As a result, enantiomeric resolution of basic (cationic) compounds can be improved as the EOF counteracts the migration

* Corresponding author.

[1] Present address: Battelle Pacific Northwest Laboratory, M.S. P7-07, P.O. Box 999, Richland, WA 99352, USA.

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of solute enantiomers [1,2]. The advantage of the short-chain TAA cations as compared to the long-chain cationic surfactants is that the formers do not interact strongly with CDs, leaving the chiral recognition sites more accessible for solute enantiomers. On the other hands, the short-chain TAA cations effectively cover the fused-silica capillary surface, leading to a reversal of the EOF at the acidic condition of pH 2.5. It is demonstrated in this report that the scope of chiral separation of basic compounds by CD-mediated CZE can be further extended by a combination of reversing the EOF with enhancing enantioselectivity through the chemical modifications of β-CD.

2. Experimental

2.1. Apparatus

Experiments were carried out on a laboratory-built CZE unit, consisting of a ± 30 kV high-voltage power supply (Series EH; Glassman High Voltage, Whitehouse, NJ, USA), a UV–Vis detector (Model 200; Linear Instruments, Reno, NV, USA) and a Spectra-Physics integrator (Model SP4200; Spectra-Physics, San Jose, CA, USA). Untreated fused-silica capillary tubes (Polymicro Technologies, Phoenix, AZ, USA) of 52 μm I.D. × 360 μm O.D. were used as the separation columns. The total length of the capillary was 62 cm, and 50 cm to the detector. The capillary temperature was maintained at 40°C by jacketing it in light mineral oil using a constant-temperature circulator (Type K2-R; Lauda, Germany). The injection samples were dissolved in water–methanol mixture and introduced into the capillary by gravity, 10 cm height for 5 s. The applied voltage was 20 kV for all the electrophoretic separations.

2.2. Chemicals

Tetramethylammonium (TMA) hydroxide (40% in water) and tetrabutylammonium (TBA) hydroxide (40% in water) were obtained from Aldrich (Milwaukee, WI, USA). Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) was purchased from Sigma (St. Louis, MO, USA). β-CD, dimethyl-β-cyclodextrin (DM-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were gifts from American Maize-Products Co. (Hammond, IN, USA). All racemic samples were commercially available from Sigma, and their chemical structures are listed in Table 1.

2.3. Procedures

A computer program, MIXBUF (a program developed in this laboratory in Turbo Vision), was used to calculate the buffer composition with a given ionic strength at pH 2.50. The appropriate amount of CD was dissolved in the buffer electrolyte, then the solution was filtered with a 0.45-μm filter before use.

3. Results and discussion

3.1. Theoretical considerations

With a buffer electrolyte containing CD, the relationship between the electrophoretic mobility (in short mobility, μ) of a solute and CD inclusion complexation can be expressed as [5]

$$\mu = \frac{\mu^f + K_{CD}[CD]\mu^c}{1 + K_{CD}[CD]} \quad (1)$$

where μ^f and μ^c are the mobilities of the solute in the free and complexed forms, respectively. K_{CD} is the formation constant of inclusion complex. [CD] represents the concentration of CD in the buffer electrolyte.

Resolution, R_s, between two enantiomers in CZE in the presence of electroosmosis can be expressed as [19,20]

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\Delta\mu}{\mu_{av} + \mu_{eo}} \quad (2)$$

where N is the number of theoretical plates, μ_{eo} is the electroosmotic mobility, μ_{av} is the average electrophoretic mobility and $\Delta\mu$ is the mobility difference for a pair of enantiomers. By using Eq. 1, $\Delta\mu$ can be derived as

$$\Delta\mu = \frac{(\mu^f - \mu^c)\Delta K[CD]}{(1 + K_{CD1}[CD])(1 + K_{CD2}[CD])} \quad (3)$$

where ΔK is the difference in formation constants for the two enantiomers (e.g., $K_{CD2} - K_{CD1}$).

Substituting Eq. 3 into Eq. 2 gives

$$R_s = \frac{(\mu^f - \mu^c)\Delta K[CD]}{(1 + K_{CD1}[CD])(1 + K_{CD2}[CD])} \cdot \frac{\sqrt{N}}{4(\mu_{av} + \mu_{eo})} \quad (4)$$

As theoretically indicated by Eq. 4, there are several methods for improving enantiomeric resolution, R_s:

Enhancing enantioselectivity (ΔK)

This approach involves selecting a suitable chiral recognition agent. The selection of chiral selector, however, requires an understanding of the chiral interactions between the chiral selector and solute molecules. For this reason, it is necessary to evaluate the differences in enantioselectivity displayed by β-CD and its derivatives for a wide range of chiral compounds.

Controlling electroosmosis

The term of $\mu_{av} + \mu_{eo}$ in Eq. 4 indicates that both the magnitude and direction (sign) of μ_{eo} relative to those of the mobility of solutes will affect R_s. Maximum R_s could be obtained when $\mu_{eo} = -\mu_{av}$ at the expense of analysis time approaching infinity. Methods of incorporating β-CD into a gel matrix [5], and using coated capillary columns at low-pH conditions [10,11] are intended to eliminate or considerably reduce the EOF. In a bare fused-silica capillary, the migration of basic (cationic) compounds is in the same direction of the EOF. Therefore, it is possible to increase enantiomeric resolution by reversing the EOF to counteract the migration of the basic enantiomers. One limitation of such an approach, however, is the loss of efficiency due to longitudinal diffusion as the migration times of solutes are prolonged.

Optimizing [CD]

For a given chiral compound, as reported by Wren and Row [15,16], R_s can be improved by optimizing the concentration of CD since R_s is a function of [CD]. However, since R_s depends on K_{CD1} and K_{CD2}, the optimum CD concentration may be different from one pair of chiral compounds to another.

Using charged CDs

The term of $(\mu^f - \mu^c)$ in Eq. 4 can be increased by using CDs with the electric charges opposite to that of guest enantiomers, such that the chiral recognition agents and solute enantiomers will move in the opposite directions. Such an example has been reported by Otsuka and Terabe [7] who used a positively charged β-CD for the separation of anionic enantiomers. However, there exists a chance that the introduction of electrostatic interactions would adversely influence the enantioselectivity of β-CDs.

3.2. Effect of short-chain TAA cations

The role of sodium, TMA and TBA cations were compared as the buffer cations in the absence of CD for the non-chiral separation of a mixture of aromatic amines. As shown in Fig. 1, the use of short-chain TAA cations extended the migration range, and obviously the overall resolution was improved at the expense of analysis times. The μ_{eo} values were measured as 1.1, −1.2, and −0.9 ($\cdot 10^{-4}$ cm^2/V s) for the buffer conditions in Fig. 1A (sodium), B (TMA) and C (TBA), respectively. The reversed EOF was determined by reversing the polarity of the applied voltage and injecting neutral solutes. Note that although the EOF was reversed under the buffer conditions in Fig. 1B and C, the magnitude of μ_{eo} values was still smaller than that of the mobility of all the test solutes. Therefore, the aromatic amines still migrate towards and are detected at the negative electrode.

The separations in Fig. 1 also illustrate the effect of the buffer cations on separation selectivity. A baseline separation of isoproterenol and

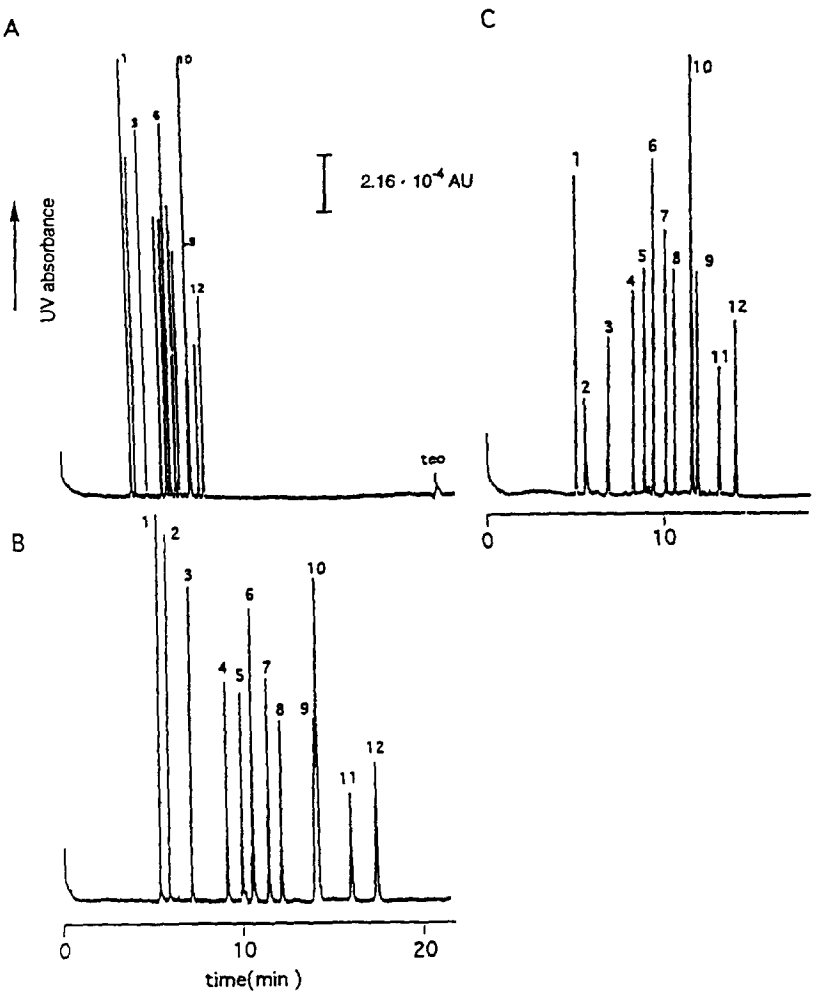


Fig. 1. Effect of the buffer cations on the separation of 12 organic amines. Buffer electrolytes: (A) 50 m*M* sodium phosphate (pH 2.50), (B) 50 m*M* TMA phosphate (pH 2.50) and (C) 50 m*M* TBA phosphate (pH 2.50). Peaks: 1 = imidazole; 2 = nicotine; 3 = doxylamine; 4 = 2-methylphenethylamine; 5 = 1-methylphenylpropylamine; 6 = pseudoephedrine; 7 = norepinephrine; 8 = epinephrine; 9 = isoproterenol; 10 = propranolol; 11 = metoprolol; 12 = nadolol. Capillary: 62 cm (50 cm to the detector) × 52 μm I.D. × 360 μm O.D. The applied voltage is 20 kV, and the current is 37 μA for (A), 34 μA for (B) and 29 μA for (C).

propranolol (peaks 9 and 10) was obtained only with the TBA phosphate buffer (Fig. 1C).

Fig. 2 shows chiral separation of norephedrine, pseudoephedrine and ephedrine. As predicted by the chiral recognition model proposed earlier [1], norephedrine, which can not be resolved by β-CD because of the lack of an alkyl group on the N atom, can be resolved by DM-β-CD that introduces a steric effect between the N atom of the guest molecule and the C-2 positions of β-CD. Again, a selectivity difference was observed between using TMA and TBA as buffer cations as shown in Fig. 2. In the TMA phosphate buffer, (1*S*,2*R*)-norephedrine co-eluted with (1*R*,2*R*)-ephedrine; a better overall separation was provided with the TBA phosphate buffer. The migration order in the above separations can be explained by the proposed model [1] and was confirmed by injecting pure enantiomeric samples.

Fig. 3 shows another example of the effect of buffer cations on chiral separation. Interestingly,

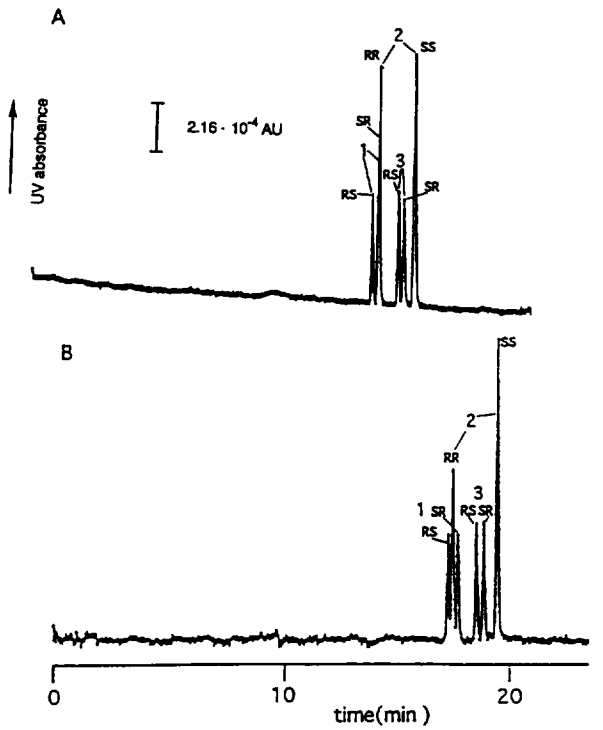


Fig. 2. Chiral separation of (1) norephedrine, (2) pseudoephedrine and (3) ephedrine. Buffer electrolytes: 20 m*M* DM-β-CD in (A) 50 m*M* TMA phosphate (pH 2.50) and (B) (B) 100 m*M* TBA phosphate (pH 2.50). Other conditions as in Fig. 1.

a complete chiral separation of trimipriamine, an antidepressant drug, was obtained only by using a buffer of 100 m*M* TBA phosphate (pH 2.50) containing 20 m*M* HP-β-CD.

As compared to the TMA cations, the TBA cations have many significant features such as providing a different separation selectivity, increasing the solubility of β-CD [1]; and having low conductivity. However, it was found that the TBA cations compete with analytes to some extent for the hydrophobic cavity of β-CDs, which reduces the enantiomeric resolution of some compounds. In this regard, the TMA cations are less competitive due to a shorter alkyl chain, and generally more suitable for chiral separation of basic compounds.

3.3. Effect of chemical modifications of β-CD

Fig. 4 shows chiral separation of norepinephrine, epinephrine, isoproterenol and propranolol with different types of β-CDs. It can be seen that increasing the size of the substituent on the N atom of guest molecule [e.g., H–, CH_3– and $(CH_3)_2CH$–, for norepinephrine, epinephrine and isoproterenol] and alkylating the hydroxyl groups at the C-2 positions of β-CD (e.g., DM-β-CD) leads to higher enantioselectivity (see Fig. 4A and B). Note that the more retained enantiomers have the same stereochemical arrangement, having the *S* absolute configuration, except propranolol, for which this configuration is defined as *R* [10,11].

Using the proposed chiral recognition model [1], it is conceivable that the steric interaction between the substituent on the N atom of guest molecules and the functional groups at the C-2 positions of β-CD generally decrease the stability of inclusion complexes (comparing DM-β-CD with β-CD), but it destabilize the complexes formed by the *S*-enantiomers to a lesser extent as compared to those of *R*-enantiomers. In other words, the steric effect increases the differences in stability of complexes formed by *S*- and *R*-enantiomers, leading to an increase in enantiomeric resolution. A chiral recognition mechanism for propranolol on β-CD stationary phase in HPLC has been described by Armstrong et al. [21].

It is important to note that since β-CD contains 21 hydroxyl groups, the products obtained from chemical modifications are always a mixture of the derivatized β-CD with various degree of substitution [22]. It is known that the partial alkylation of β-CD is more likely to occur at the hydroxyl groups at C-2 and C-6 positions [23,24]. Since DM-β-CD and HP-β-CD are partially alkylated products of β-CD, they may possess similar molecular structures, leading to their similarity in enantioselectivity (see Fig. 4B and D).

We have evaluated β-CD and its derivatives for chiral separation of 22 basic compounds with rather complex molecular structures, including β-blockers, calcium-channel blockers, antihis-

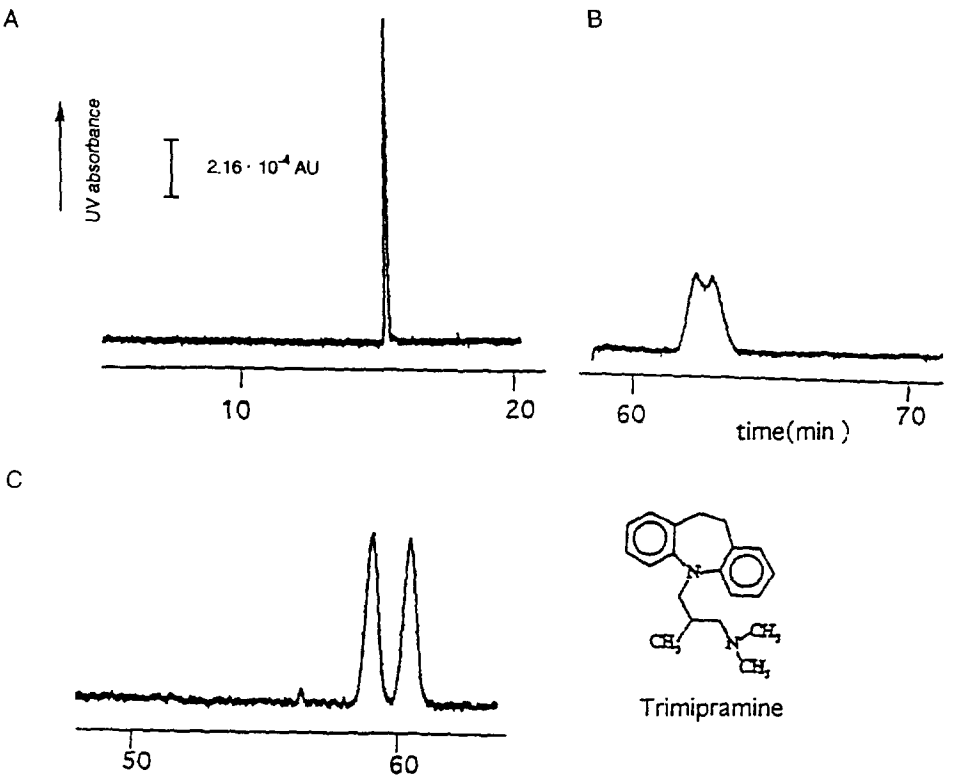


Fig. 3. Chiral separation of trimipriamine. Buffer electrolytes: 20 m*M* HP-β-CD in (A) 50 m*M* sodium phospate (pH 2.5), (B) 50 m*M* TMA phosphate (pH 2.5) and (C) 100 m*M* TBA phosphate (pH 2.50). Other conditions as in Fig. 1.

tamines, catecholamines, and other chiral amines. Their chemical structures are listed and separation data are summarized in Table 1. For each racemate, separation factor, α, was calculated by [5]

$$\alpha = \mu_1/\mu_2 \quad (5)$$

μ_1 and μ_2 are the mobilities of two enantiomers (note: $\mu = \mu_{obs} - \mu_{eo}$). R_s was calculated according to Eq. 2 by assuming N equal to 100 000. Baseline separation is generally obtained when R_s exceeds 1.5.

From the data in Table 1, it appears that HP-β-CD shows a similarity in enantioselectivity to both DM-β-CD and β-CD. This is indicated by the fact that HP-β-CD resolve the same group of the basic compounds as DM-β-CD, and it also resolves compounds such as doxylamine, chlorpheniramine and labetalol that are well resolved by β-CD, which, apparently, is related to its unique molecular structures. According to the manufacture, there are 6–7 mol of hydroxypropyl ($HOCH_2CH_2CH_2-$) per mol of β-CD. In other words, two thirds of the hydroxyl groups remain unreacted on the rim of β-CD. It can be expected that HP-β-CD resembles DM-β-CD by having some of the C-2 and C-6 hydroxyl groups substituted, and differs with DM-β-CD by having longer and polar substituents and by having lesser degree of substitution. The unreacted hydroxyl groups on the D-(+)-glucopyranose units provide HP-β-CD with the characteristics of native β-CD. Consequently, HP-β-CD is generally more selective for basic compounds with a chiral center located at a distance from the hydrophobic moiety such as trimipriamine and some of the β-blockers. On the other hand, DM-β-CD is more suitable for compounds with a chiral center close to the aromatic ring such as

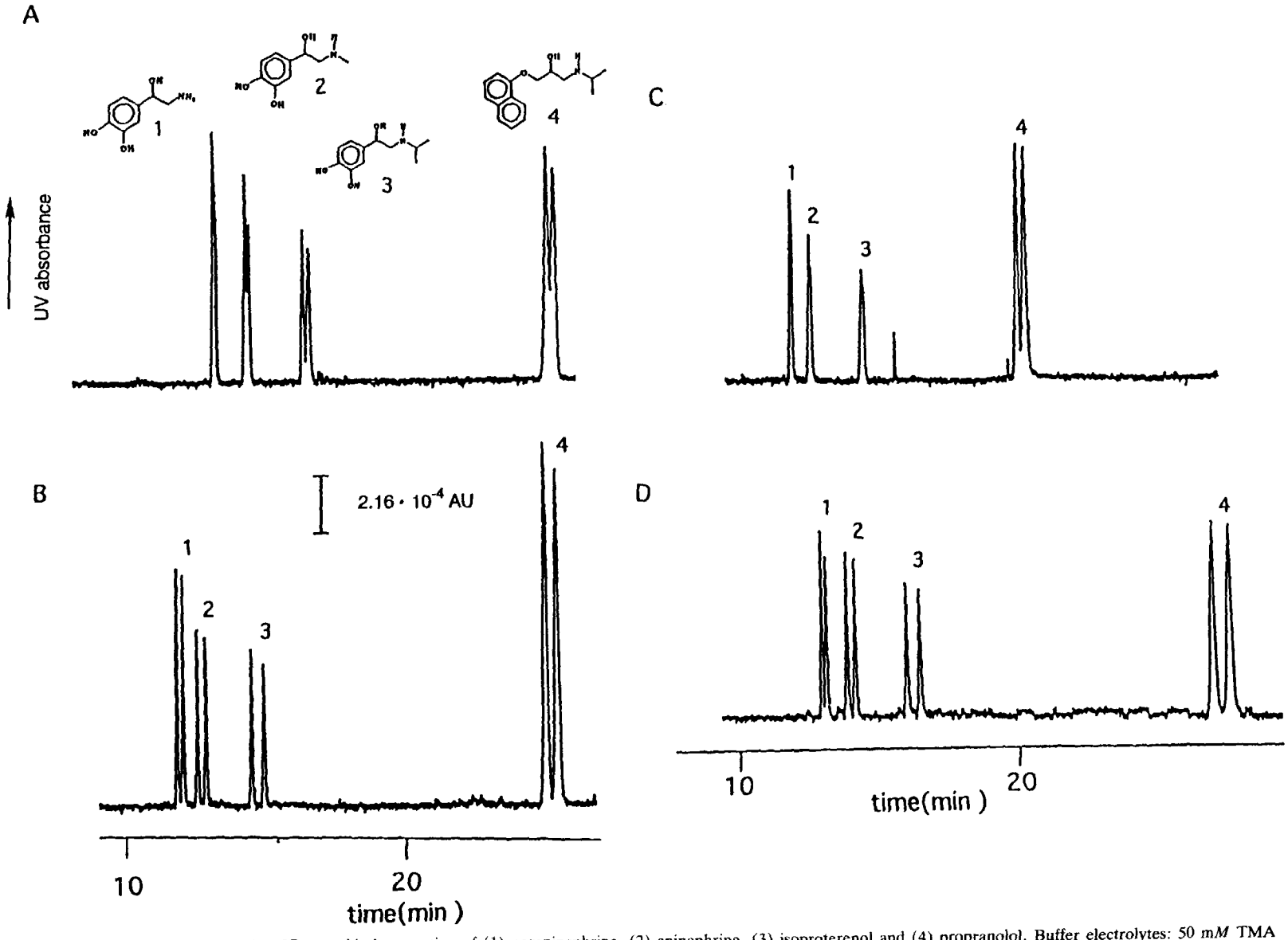
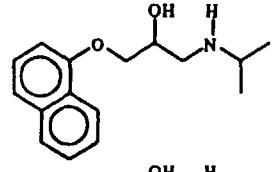
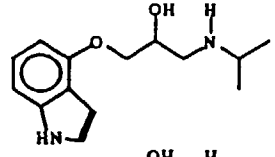
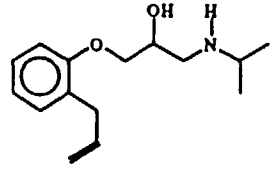
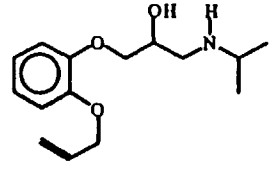
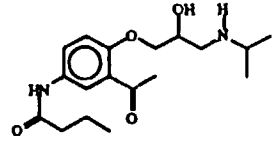
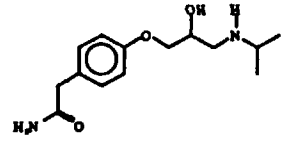
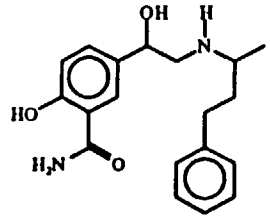
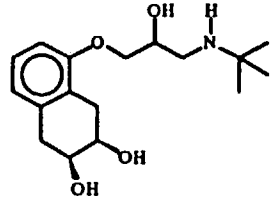
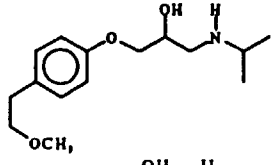
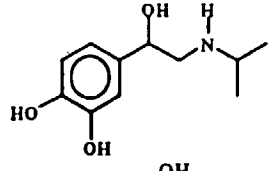
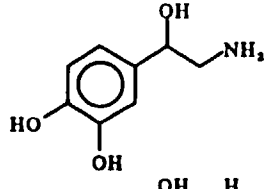
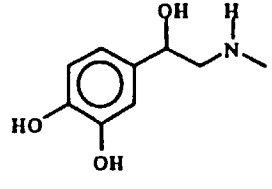


Fig. 4. Effect of the type of β-CDs on chiral separation of (1) norepinephrine, (2) epinephrine, (3) isoproterenol and (4) propranolol. Buffer electrolytes: 50 m*M* TMA phosphate (pH 2.50) containing: (A) 20 m*M* β-CD, (B) 20 m*M* DM-β-CD, (C) 20 m*M* TM-β-CD and (D) 20 m*M* HP-β-CD. Other conditions as in Fig. 1.

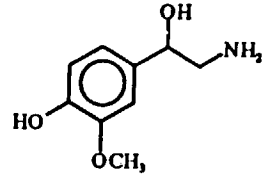
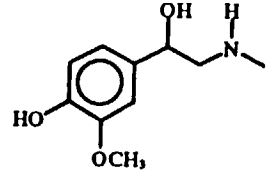
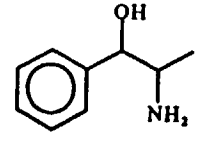
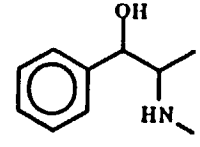
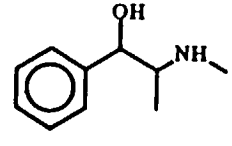
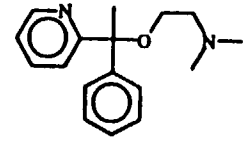
Table 1
Separation data for basic (cationic) compounds

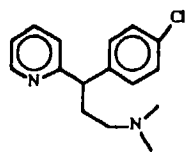
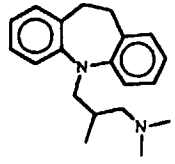
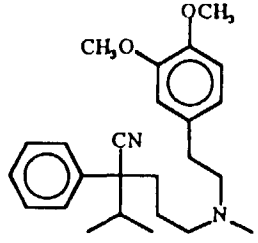
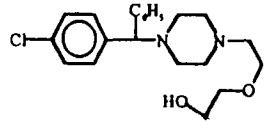
Name	Structure	β-CD			TM-β-CD			DM-β-CD			HP-β-CD		
		t_R	α	R_s	t_R	α	R_s	t_R	α	R_s	t_R	α	R_s
Propranolol		24.83 25.08	1.005	0.79	20.06 20.34	1.007	1.10	25.19 25.62	1.008	1.34	26.83 27.43	1.010	1.75
Pindolol		18.40 18.56	1.005	0.68	15.32	N.S.		17.43 17.71	1.009	1.26	17.61 17.82	1.007	0.94
Alprenolol		33.30 30.60	1.004	0.78	17.54	N.S.		24.68 25.00	1.006	1.02	24.95 25.39	1.008	1.38
Oxprenolol		19.42	N.S.		16.00	N.S.		18.14 18.31	1.005	0.74	17.90 18.09	1.006	0.84
Acebutolol		23.41	N.S.		19.85	N.S.		19.49	N.S.		20.45	N.S.	
Atenolol		27.80	N.S.		16.34	N.S.		16.95 17.07	1.004	0.56	18.68 18.82	1.004	0.59

Labetalol[a]		29.50 29.96	1.007	1.22	22.33	N.S.	27.45 27.52	1.001	0.20	28.67 29.10	1.006	1.18
Nadolol[a]		19.16	N.S.		17.92	N.S.	17.24	N.S.		17.89 18.08	1.006	0.84
Metoprolol		33.20	N.S.		18.13	N.S.	26.36 26.56	1.003	0.60	29.84	N.S.	
Isoproterenol		15.94 16.15	1.008	1.04	14.68	N.S.	14.46 14.90	1.018	2.37	15.64 16.12	1.018	2.39
Norepinephrine		12.76	N.S.		11.88	N.S.	11.80 12.01	1.011	1.40	12.62 12.79	1.008	1.06
Epinephrine		13.89 14.02	1.007	0.85	12.57	N.S.	12.53 12.82	1.015	1.81	13.51 13.80	1.013	1.68

(Continued on p. 262)

Table 1 (*continued*)

Name	Structure	β-CD			TM-β-CD			DM-β-CD			HP-β-CD		
		t_R	α	R_s	t_R	α	R_s	t_R	α	R_s	t_R	α	R_s
Normetanephrine		11.64	N.S.		11.65	N.S.		10.75	N.S.		11.78	N.S.	
Metanephrine		12.38	N.S.		12.32	N.S.		11.33	N.S.		12.42	N.S.	
Norephedrine		14.16	N.S.		11.04	N.S.		12.16 12.45	1.015	1.86	12.92	N.S.	
Ephedrine		15.37 15.63	1.010	1.33	11.51	N.S.		12.97 13.16	1.009	1.15	13.91 14.03	1.005	0.68
Pseudoephedrine		15.28 16.43	1.043	5.73	11.21 11.42	1.012	1.47	11.18 12.73	1.049	5.93	12.83 14.32	1.070	8.68
Doxylamine		9.52 9.75	1.017	1.89	7.59	N.S.		7.85	N.S.		7.70 7.77	1.007	0.72

Chlorpheniramine		13.60 13.93	1.015	1.90	9.29 9.35	1.005	0.51	11.48 11.59	1.006	0.75	10.20 10.37	1.011	1.31
Trimipramine		50.70	N.S.		21.74	N.S.		32.32	N.S.		34.01 34.37	1.004	0.83
Verapamil		49.98	N.S.		23.19 24.30	1.022	3.70	32.32	N.S.		40.20 40.60	1.003	0.78
Hydroxyzine		54.80	N.S.		29.30	N.S.		33.42	N.S.		43.60	N.S.	

Buffer: 50 m*M* TMA phosphate at pH 2.50 containing 20 m*M* chiral recognition agent. Migration times (t_R) are in minutes; $\alpha = \mu_1/\mu_2$ ($\mu = \mu_{obs} - \mu_{eo}$; $\mu_{eo} = -1.2 \cdot 10^{-4}$ $cm^2/V\,s$); R_s was calculated by using Eq. 2, and by assuming $N = 100\,000$. N.S. = No separation.

[a] Diastereomers.

norephedrine, ephedrine, norepinephrine and epinephrine with the exception of pseudoephedrine. Finally, it is important to note that although β-CD and TM-β-CD are less selective for the basic compounds listed in Table 1, they showed remarkable high enantioselectivity for certain groups of compounds, e.g., β-CD for doxylamine, chlorpheniramine and labetalol, and TM-β-CD for verapamil and propranolol, for which the mechanism is not known.

Fig. 5 shows two electropherograms of the separation of 12 chiral amines using (A) 20 m*M* DM-β-CD and (B) 20 m*M* HP-β-CD. Clearly, the separation capability of CZE is extended by using the TMA cations to reverse the EOF, and at the same time by incorporating selective interaction into the solute migration. Not only many of the chiral amines were resolved, but also all the amines were separated from each other. Comparing the above two separations, it is clear that HP-β-CD is a better chiral selector for doxylamine (pair 1) and labetalol (pair 12) for the reasons mentioned earlier, but not for pindolol and oxprenolol (pairs 7 and 8) which co-eluted.

Fig. 6 shows a separation of the same group of chiral amines with a TBA phosphate buffer solution containing 20 m*M* HP-β-CD. In comparison with the separation in Fig. 5B, a better separation was obtained for pindolol and oxprenolol (pairs 7 and 8). However, the presence of TBA cations causes a decrease in the enantiomeric resolution for the early-eluted amines such as doxylamine and norepinephrine due to the competing effect of the TBA cations. Finally, for some partially resolved enantiomers such as those of atenolol, oxprenolol, pindolol (in the case of HP-β-CD), enantiomeric resolution of these compounds can be further improved by lowering the separation temperature and by using higher concentrations of the TMA cations [2].

It is not surprising that the enantiomers of acebutolol could not be resolved. It seems that the presence of a long-chain substituent at the *para* position (see Table 1) prevents the aromatic moiety from penetrating deeply into the β-CD cavity, leaving the chiral center of the guest

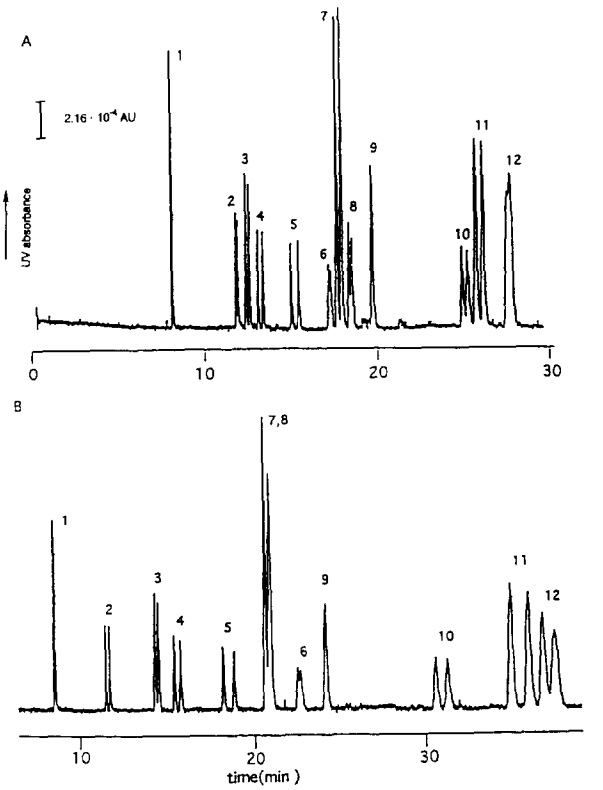


Fig. 5. A comparison ofseparations of 12 chiral amines using two different chiral selectors. Buffer electrolytes: 50 m*M* TMA phosphate (pH 2.50) containing (A) 20 m*M* DM-β-CD and (B) 20 m*M* HP-β-CD. Peaks: 1 = doxylamine; 2 = chlorpheniramine; 3 = norepinephrine; 4 = epinephrine; 5 = isoproterenol; 6 = atenolol; 7 = pindolol; 8 = oxprenolol; 9 = acebutolol; 10 = alprenolol; 11 = propranolol; 12 = labetalol. Other conditions as in Fig. 1.

molecule beyond the reach of the functional groups on the rim of β-CD cavity. Similarly, no chiral separation for normetaphrine and metaphrine was achieved by using any of the β-CDs. This might also be due to the steric effect at the *meta* position of the aromatic moiety, which prevented the functional groups of the guest molecules from interacting with the functional groups on the rim of β-CD. Therefore, it is suggested that the usefulness of β-CD derivatives with longer substituents or other kinds of chiral selectors (e.g., helical amylose oligomers [25]), be explored for the separation of the enantiomers of these compounds.

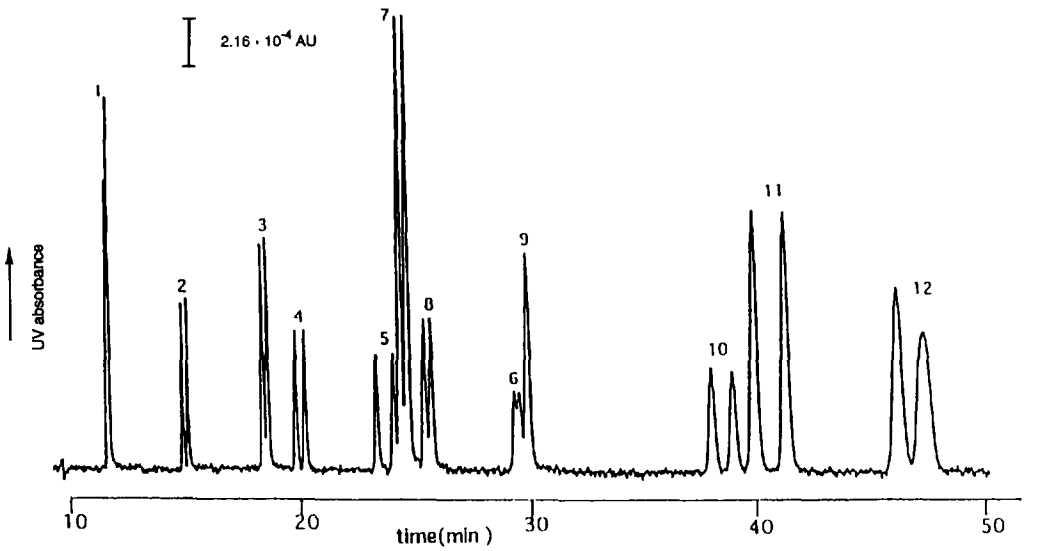


Fig. 6. Effect of TBA cations on the separation of the chiral amines. Buffer electrolyte: 20 m*M* HP-β-CD in 100 m*M* TBA phosphate (pH 2.50). Peak numbers as in Fig. 5, and other conditions as in Fig. 1.

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References

[1] C. Quang and M.G. Khaledi, *Anal. Chem.*, 65 (1993) 3354.
[2] C. Quang and M.G. Khaledi, *J. High Resolut. Chromatogr.*, 17 (1994) 99.
[3] J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 452 (1988) 571.
[4] R. Kuhn and S.H. Kuhn, *Chromatographia*, 34 (1992) 505.
[5] A. Guttman, A. Paulus, A.S. Cohen, N. Grinberg and B.L. Karger, *J. Chromatogr.*, 448 (1988) 41.
[6] I.D. Cruzado and G. Vigh, *J. Chromatogr.*, 608 (1992) 421.
[7] K. Otsuka and S. Terabe, in N.A. Guzman (Editor), *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993, p. 627.
[8] H. Nishi, T. Fukuyama and S. Terabe, *J. Chromatogr.*, 553 (1991) 503.
[9] M.J. Sepaniak, R.O. Cole and B.K. Clark, *J. Liq. Chromatogr.* 15 (1992) 1023.
[10] S. Fanali, *J. Chromatogr.*, 474 (1988) 441.
[11] S. Fanali, *J. Chromatogr.*, 545 (1991) 437.
[12] J. Snopek, H. Soini, M. Novotny, E. Smolkova-Keulemansova and I. Jelinek, *J. Chromatogr.*, 559 (1991) 215.
[13] H. Soini, M.-J. Riekkola and M.V. Novotny, *J. Chromatogr.* 608 (1992) 265.
[14] R. Kuhn, F. Stoecklin and F. Erni, *Chromatographia*, 33 (1992) 32.
[15] S.A.C. Wren and R.C. Rowe, *J. Chromatogr.*, 603 (1992) 274.
[16] S.A.C. Wren and R.C. Rowe, *J. Chromatogr.*, 609 (1992) 363.
[17] M.W.F. Nielen, *Anal. Chem.*, 65 (1993) 885.
[18] K. Otsuka and S. Terabe, *J. Liq. Chromatogr.*, 16 (1993) 945.
[19] J.C. Giddings, *Sep. Sci.*, 4 (1969) 181.
[20] J.W. Jorgenson and K.D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
[21] D.W. Armstrong, T.J. Word, R.D. Armstrong and T.E. Beesley, *Science*, 232 (1986) 1132.
[22] R.P. Frankewich, K.N. Thimmaiah and W.L. Hinze, *Anal. Chem.*, 63 (1991) 2924.
[23] W.A. Konig, *Carbohydr. Res.*, 192 (1989) 51.
[24] V. Schurig and H.-P. Nowotny, *Angew. Chem., Int. Ed. Engl.*, 29 (1990) 939.
[25] C. Quang and M.G. Khaledi, *J. High Resolut. Chromatogr.*, 17 (1994) 609.