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Non-aqueous capillary electrophoresis chiral separations with sulfated β-cyclodextrin☆

Fang Wang, Morteza G. Khaledi*

Department of Chemistry, Box 8204, North Carolina State University, Raleigh, NC 27695-8204, USA

Abstract

This paper reports the application of an anionic cyclodextrin (CD), sulfated β-cyclodextrin with a degree of substitution of four (β-CD-$(SO_4^-)_4$, in chiral separations of pharmaceutical enantiomers by non-aqueous capillary electrophoresis (NACE). Upon complexation with the anionic CD, electrophoretic mobilities of the basic enantiomers decreased, however, both separation selectivity and resolution were enhanced. The advantage of NACE chiral separations over the aqueous CE with the charged CD is that higher electric field strength and higher ionic strength could be applied due to the characteristics of the solvent formamide. The higher ionic strength leads to stacking of peaks and reduces the electrodispersion caused by the mobility mismatch between β-CD-$(SO_4^-)_4$–analyte complexes and the co-ions in the running buffer. As a result, better peak shapes and higher separation efficiency were obtained. Comparing with NACE chiral separations with neutral CDs, lower concentration of β-CD-$(SO_4^-)_4$ was needed due to the fact that the electrostatic attraction caused stronger binding between β-CD-$(SO_4^-)_4$ and the enantiomers. The effects of the experimental parameters, such as concentration of the CD, apparent pH (pH*), degree of substitutions of the CDs, percentage of water in mixed solvent systems, and type of solvents were also studied.

Keywords: Enantiomer separation; Sulfated β-cyclodextrin

1. Introduction

Chiral separations by capillary electrophoresis (CE) depend on the selective interactions between a chiral selector and enantiomers in the buffer solution. The electrophoretic mobility of an enantiomer (μ) is a function of concentration of a chiral selector as [1]:

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

where μ^f and μ^c are the mobilities of the enantiomer in free form (i.e., [CD]=0) and in fully complexed form (i.e., [CD]=∞), respectively, K is the binding constant of CD and the enantiomer.

As described by Wren and Rowe [2,3], separation selectivity, as measured by the mobility difference between two enantiomers, can be expressed as:

$$\Delta\mu = \frac{(\mu^f - \mu^c)\Delta K[CD]}{(1 + K_1[CD])(1 + K_2[CD])} \quad (2)$$

where K_1 and K_2 are the binding constants between CD and two enantiomers, respectively.

Chiral resolution (R_s) between two enantiomers in CE is expressed as [4,5]

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*Corresponding author. Tel.: +1-919-515-2943; fax: +1-919-515-5079.

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$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\Delta K[\mathrm{CD}]}{(1+K_1[\mathrm{CD}])(1+K_2[\mathrm{CD}])}\right)\left(\frac{\mu^{\mathrm{f}}-\mu^{\mathrm{c}}}{\mu_{\mathrm{avg}}+\mu_{\mathrm{eo}}}\right) \quad (3)$$

where N is the number of theoretical plates, μ_{avg} is the average electrophoretic mobility of enantiomers, and μ_{eo} is the electroosmotic mobility, respectively.

There exists an optimum CD concentration at which a maximum separation is reached. The optimum concentration of CD is inversely related to the binding constants of enantiomers as $[\mathrm{CD}]_{\mathrm{opt}} = 1/\sqrt{K_1 K_2}$ [6]. In addition to the chiral selector concentration, separation selectivity is also related to binding constants (K_1, K_2), difference in binding constants (ΔK), and difference in mobilities of the free and complexed forms of enantiomers ($\mu^{\mathrm{f}} - \mu^{\mathrm{c}}$). The type of CDs has a significant effect on separation selectivity due to the fact that it determines the $\mu^{\mathrm{f}} - \mu^{\mathrm{c}}$, ΔK, and K values in the Eq. (2). The application of charged CDs in CE makes it possible to separate neutral enantiomers [7,8]. Anionic CD seem to be also quite effective for separation of chiral amines, where as cationic enantiomer–anionic CD complexes migrate in the opposite direction of EOF. This counter-EOF setup widens the separation window and resolution at the expense of longer analysis times (here the separation window is defined as the time between t^{f} and t^{c}, where t^{f} and t^{c} are the migration times of free and fully complexed forms of enantiomer, or $\mu^{\mathrm{f}} - \mu^{\mathrm{c}}$ in Eqs. (2) and (3)) [8–11]. Stalcup and Gahm reported successful separation of 40 out of 56 compounds at one sulfated β-CD concentration [8]. This is quite remarkable as this CD concentration was not at the optimum value for many of the enantiomers. Other anionic chiral selectors, such as heparin [12,13] and sulfated dextran [13], have been used for chiral separations in aqueous CE. For solutes that bind strongly to the selector, it is crucial to operate at the optimum concentration due to drastic changes in selectivity vs. CD concentration [14]. This is especially pronounced in aqueous CE as hydrophobic solutes can have very large binding constants, whereas in non-aqueous media, the interactions are weakened [14].

Ye and Khaledi [16] first reported NACE chiral separation of trimipramine with β-CD in *N*-methylformamide. Chiral separation of dansyl-amino acids with neutral CD in *N*-methylformamide was achieved by Valko et al. [17]. Stalcup and Gahm [18], and Bjoernsdottir et al. [19] also reported the application of ion-paring interaction in NACE chiral separations in other organic solvents. A detailed study of chiral separations of more basic pharmaceutical compounds with neutral CDs in NACE was recently published [14]. It was found that the binding constants between β-CD and analytes, such as trimipramine and thioridazine, decreased systematically from -10^4 to 10^{-2} M^{-1} in the following five solvent systems: water, 6 *M* urea in water, formamide, *N*-methylformamide, and *N*,*N*-dimethylformamide. In general, lower binding constants between neutral CD and solutes are observed for less polar solvents. Due to smaller binding constants, the optimum CD concentrations shift to higher values and maximum separation selectivity occurs over a wide concentration range. Thus, the selection of the optimum concentration is not as critical as compared to aqueous CE.

In addition to optimization of separation selectivity, chiral resolution can also be improved by adjusting other experimental variables. For chiral separations of basic analytes with neutral CDs in uncoated fused-silica capillaries, compounds are positively charged at low buffer pH and migrate in the same direction as EOF, from anode to cathode. One way to increase chiral resolution is to reduce the ($\mu_{avg} + \mu_{\mathrm{eo}}$) term in Eq. (3). Previously, Quang and Khaledi [15] applied short chain cationic surfactants to reverse the EOF to reduce the $\mu_{avg} + \mu_{\mathrm{eo}}$ term. One can also use an anionic CD to create counter-EOF condition [8]. However, when the anionic CD is used for the counter-EOF setup, higher CD concentration has to be used to achieve a negative net mobility of the enantiomer–CD complexes. Higher concentrations of the charged CD can cause a dramatic increase in electrical current, considering that the degree of substitution of anionic CDs is typically four or larger. As a result, lower electric field strength has to be used to minimize Joule heating. Another advantage of using anionic CDs for chiral separations of basic compounds is that μ^{c} is negative; in this case, $\mu^{\mathrm{f}} - \mu^{\mathrm{c}}$ becomes greater, thus separation selectivity and resolution are enhanced. One can then expect to achieve better separations for

a large number of compounds using anionic CD at non-optimum concentrations [7,8].

In a previous article on NACE chiral separations of basic solutes with neutral CD, we also reported the first chiral separation in formamide with a sulfated CD, β-CD-(SO_4^-) [14]. The focus of this paper is the use of the anionic CD for separation of enantiomeric amines by NACE.

2. Experimental

2.1. Apparatus

All experiments were carried out on a home-built unit. It consisted of a ±30 kV high voltage power supply (Series EH, Glassman High Voltage, Whitehouse, NJ, USA) and a UV–Vis detector (Model 200, Linear Instruments, Reno, NV, USA) operating at certain wavelengths according to the analytes and an electronic integrator (Hewlett-Packard, Avondale, PA, USA). Untreated fused-silica capillary tubes (Polymicro Technologies, Phoenix, AZ, USA) with 50 μm I.D., 363 μm O.D. were used. The total length of the capillary was 42 cm and the length of the capillary to the detector was 31 cm. The capillary was jacketed in light mineral oil to maintain its temperature at 30°C using a constant temperature circulator (Type K2-R, Lauda, Germany). All the injections were done by gravity injection.

2.2. Chemicals

Sulfated β-CD (degree of substitution = 4.0, or β-CD-(SO_4^-)$_4$) was a gift from American Maize-Products Company (Hammond, IN, USA). Tris (hydroxymethyl)amino-methane (Tris) and sulfated β-CD (degree of substitution = 7–11, or β-CD-(SO_4^-)$_{7-11}$) were purchased from Aldrich (Milwaukee, WI, USA). All the racemic samples were purchased from Sigma (St. Louis, MO, USA), and their chemical structures are listed in Fig. 1. Formamide and *N*-methylformamide were purchased from Fluka Chemika-Biochemika (Buchs, Switzerland). *N*,*N*-Dimethylformamide was purchased from Fisher Scientific (Pittsburgh, PA, USA).

3. Results and discussion

Fig. 2 shows the chiral separation for thioridazine (21) with ACE using 1.54×10^{-4} w/v% (~1.0 μ*M*) β-CD-(SO_4^-)$_4$ in 50 m*M* Tris–phosphate aqueous buffer (pH 2.50) (Fig. 2A) and NACE separation with 1.54 w/v% (~10 m*M*) β-CD-(SO_4^-)$_4$ in 100 m*M* Tris–150 m*M* citric acid in formamide (pH* 5.1) (Fig. 2B). Note, the w/v% is used as the concentration unit because the anionic CD from American Maize-Products Co. is a mixture of the β-CDs with a different number of sulfated groups [22]. As can be seen in Fig. 2A, the peaks are severely tailed and the number of theoretical plates for these peaks is around 1000. In aqueous CE, the optimum concentration of native β-CD for chiral separation of thioridazine (21) is about 0.05 m*M* at pH 2.50 due to strong binding between the enantiomers and chiral selector (*K*~30 000). Therefore, it is expected that the optimum concentration of the anionic CD for this compound is at a micromolar level due to the additional electrostatic attraction. The electrodispersion (not Joule heating) resulting from a mobility mismatch between the β-CD-(SO_4^-)$_4$–analyte complex and the co-ions in the running buffer is probably the cause of poor peak shape (Fig. 2A) [20]. In formamide media, higher voltage could be applied because of the overall lower electrical current than that in aqueous buffer. Note that a higher electrolyte concentration was also used in formamide. Baseline separation of the racemate was achieved at 1.54 w/v% (~10 m*M*) β-CD-(SO_4^-)$_4$ and the separation efficiency was around 150 000, which is a similar value to that in aqueous CE chiral separation of racemates with neutral CDs. As the CD concentration increased to 3.85 w/v% (~25 m*M*), the separation efficiency decreased to around 50 000 in the non-aqueous system. Better separation efficiency in formamide can also be attributed to the stacking effect in formamide [21] with higher ionic strength. A combination of higher ionic strength and smaller interactions between β-CD-(SO_4^-)$_4$ and analytes reduced the electrodispersion (Fig. 2B).

Twenty-four basic enantiomers were separated using the anionic CD (Table 1). As compared with neutral CD, lower concentrations of β-CD-(SO_4^-)$_4$ were needed to resolve these racemates in form-

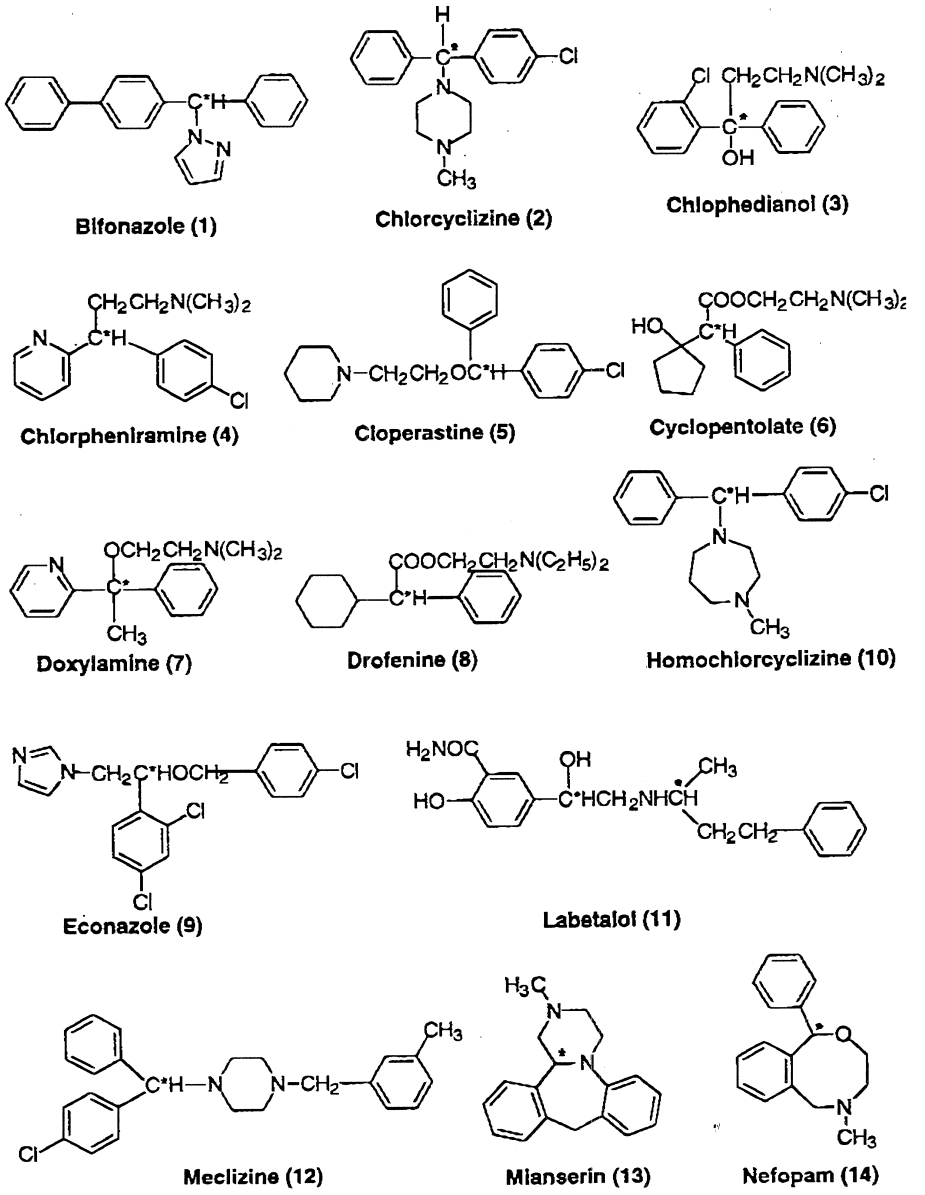

Fig. 1. Structure of chiral compounds.

amide. Some analytes, such as doxylamine (7), labetalol (11), and propranolol (19), which were not resolved by neutral CDs in formamide, were separated by β-CD-$(SO_4^-)_4$ due to the additional electrostatic interaction in formamide. A number of solutes, such as acebutolol, metanephrine, and normetanephrine that weakly bind with β-CD in aqueous media (data not shown in Table 1), could not be separated with β-CD-$(SO_4^-)_4$ at the concentrations used in this study. For polar compounds such as these, using charged chiral selectors in aqueous media would be more effective.

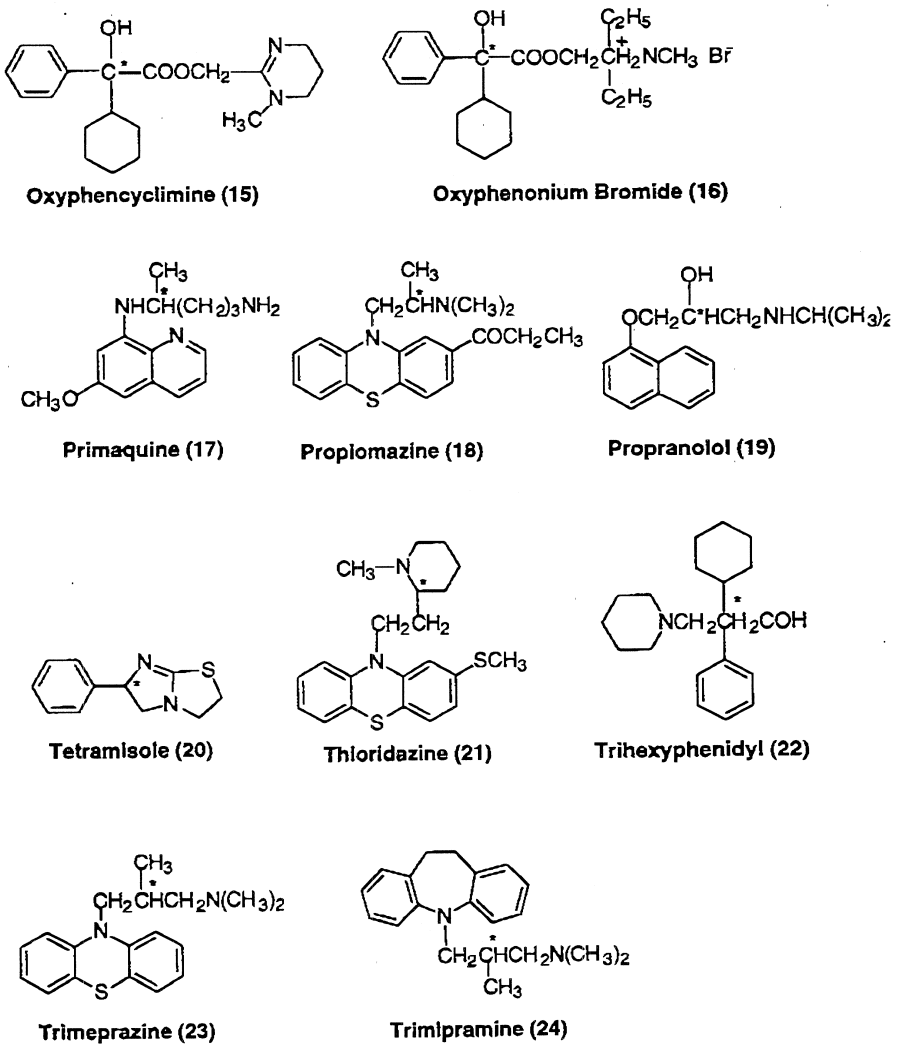

Fig. 1. (*continued*)

3.1. Effect of CD concentration

Fig. 3 shows the NACE chiral separation of labetalol (11), a β-blocker which has two chiral centers, with β-CD-$(SO_4^-)_4$ in Tris–citrate buffer at apparent pH (pH*) 5.1. Under this condition, the analyte was positively charged. Although the pK_a value of HSO_4^- groups on the CD rims in formamide is not known, it is probably fully ionized due to the fact the second pK_a for sulfuric acid in aqueous media is around 2.0 and formamide is a basic solvent. At 0.77 w/v% (~5 m*M*) β-CD-$(SO_4^-)_4$, chiral separation of the diastereomers with two different chiral centers was achieved (Fig. 3A). A further increase in the CD concentration to 1.54 w/v% (~10 m*M*) significantly increased the resolution of the first pair of the enantiomers and slightly improved the chiral separation of the second pair (data not shown). The elution times of the analyte increased with the CD concentration. At the CD concentration higher than 1.54 w/v% (~10 m*M*), chiral separations of both pairs of enantiomers

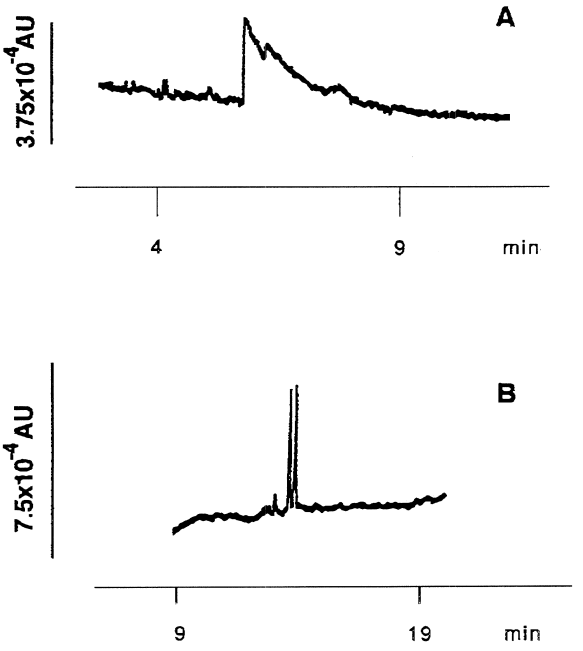

Fig. 2. Comparison of chiral separation of thioridazine (21) with β-CD-$(SO_4^-)_4$ in aqueous and non-aqueous media. Buffer: (A) 1.54×10^{-4} w/v% (~1 μ*M*) β-CD-$(SO_4^-)_4$ in 50 m*M* Tris–phosphate (pH 2.50) aqueous media; (B) 1.54 w/v% (~10 m*M*) β-CD-$(SO_4^-)_4$ in 150 m*M* citric acid–100 m*M* Tris (pH* 5.1) in formamide. Field strength: (A) 357 V/cm, (B) 595 V/cm.

of labetalol (11) were achieved in NACE (Fig. 3B). Under aqueous conditions, only three of four peaks were separated in 0.154 w/v% (~1 m*M*) β-CD-$(SO_4^-)_4$ at pH 2.50 [22]. Chiral separations for compounds such as drofenine (8), oxyphencyclimine (16), and trihexyphenidyl (22), were achieved in β-CD-$(SO_4^-)_4$ concentration range of 0.154–3.08 w/v% (1–15 m*M*).

Previously, three of the four peaks of labetalol isomers were resolved using β- CD-$(SO_4^-)_4$ in aqueous media under the co-EOF setup [22]. No chiral separation of this β-blocker by the counter-EOF setup has been reported. Fig. 4 shows chiral separation of labetalol (11) in 2.31 w/v% (~15 m*M*) β-CD-$(SO_4^-)_4$ by the counter-EOF setup in aqueous buffer at pH 5.11 (the same conditions as those of Stalcup and Gahm [8]). Again, only three peaks could be resolved. The reason that not all four peaks were resolved in ACE with either the co-EOF or counter-EOF setups is the lack of separation selectivity. A complete chiral separation of the labetalol (11) isomers indicated that higher separation selectivity can be achieved in NACE.

Fig. 5 shows the change of the mobilities of thioridazine (21) and labetalol (11) with the increase of β-CD-$(SO_4^-)_4$ concentration in formamide. The electrophoretic mobilities of enantiomers decreased with the increase in concentration of β-CD-$(SO_4^-)_4$. The decrease in mobilities for these basic analytes can be attributed to the complexation between enantiomers and β-CD-$(SO_4^-)_4$. Note that the concentration range of the anionic CD used here was less than that in the native β-CD study [14]. This is because of the stronger binding between the analytes and the anionic β-CD in formamide as compared to the uncharged CD.

Fig. 6 shows the effect of the concentration of the anionic CD on the separation selectivity ($\Delta\mu$). The chiral selectivity for the enantiomers of bifonazole (1) remains constant at the CD concentration higher than 1.54 w/v% (~10 m*M*). For the other analytes, chiral selectivity increased with the increase in the CD concentration. Higher β-CD-$(SO_4^-)_4$ concentration was needed to achieve a better separation selectivity for these analytes.

*3.2. Effect of pH**

The effect of apparent pH (pH*) was studied by the variation of the concentration of acetic acid and Tris while keeping the total concentration of acetic acid and Tris constant. Fig. 7 shows the effect of pH* on the electrophoretic mobilities of the first eluted enantiomers of some test solutes. From Fig. 7, it can be seen that the mobilities of the solutes were positive when pH* was smaller than 10. As pH* increased from 6 to 8, there was no obvious decrease in mobilities. With the further increase of pH*, the mobility of basic solutes decreased. Finally, when pH* was 10.3, negative mobilities were obtained for all enantiomers. This is due to the fact the these amines are neutral at higher pH*. The pH* effect on mobility difference was relatively small in the pH* range of 6–9 since there was no drastic change in the mobilities of the enantiomers (Fig. 7).

Table 1
Chiral separations of basic analytes with β-CD-$(SO_4^-)_4$ in formamide[a]

Analytes	Concentration of β-CD-$(SO_4^-)_4$ (%)	t_1 (min)	t_2 (min)	μ_1[b]	μ_2[b]	$\Delta\mu$[b]	R_s[c]
Bifonazole (1)	3.85	24.51	24.76	0.52	0.49	0.03	0.51
Chlorcyclizine (2)	3.85	25.96	26.86	0.91	0.79	0.12	3.16
Chlorphedianol (3)	3.85	13.44	13.68	2.34	2.28	0.06	0.65
Chlorpheniramine (4)	3.85	18.75	19.00	1.75	1.69	0.06	1.41
Cloperostine (5)	3.85	17.26	17.32	1.23	1.22	0.01	0.16
Cyclopentolate (6)	3.85	20.15	21.05	0.85	0.74	0.11	1.89
Doxylamire (7)	3.85	14.67	14.92	2.06	2.00	0.06	2.12
Drofenine (8)	0.154	11.45	12.00	2.32	2.11	0.21	0.65
Econazole (9)	3.85	16.62	17.04	1.75	1.68	0.07	0.97
Homochlorcyclizine (10)	2.31	17.60	18.40	1.54	1.42	0.12	2.03
Labetalol 1 (11)	3.85	20.42	20.77	1.00	0.95	0.05	0.78
Labetalol 2 (11)	3.85	21.45	21.65	0.87	0.85	0.02	0.35
Meclizine (12)	3.85	24.81	25.30	0.69	0.65	0.04	1.16
Mianserin (13)	3.85	15.04	15.61	1.98	1.87	0.11	1.75
Nefopam (14)	3.85	13.48	13.75	0.78	0.74	0.03	2.95
Oxyphencyclimine (15)	0.154	11.89	12.41	2.41	2.22	0.19	0.82
Oxyphenonium (16)	0.154	10.66	10.75	2.85	2.81	0.04	0.39
Primaquine (17)	3.85	18.00	18.15	1.99	1.97	0.02	0.87
Propiomazine (18)	3.85	19.27	19.40	1.90	1.86	0.04	0.68
Propranolol (19)	3.85	13.81	13.90	2.23	2.21	0.02	0.23
Tetramisole (20)	3.85	10.52	10.67	3.65	3.58	0.07	1.44
Thioridazine (21)	3.85	23.24	24.42	0.84	0.73	0.11	2.87
Trihexyphenidyl (22)	0.77	15.88	16.00	1.28	1.25	0.03	0.22
Trimeprazine (23)	3.85	21.44	22.63	1.48	1.25	0.23	3.19
Trimipramine (24)	3.85	18.64	19.30	1.23	1.14	0.09	3.64

[a] Buffer: 100 m*M* Tris –150 m*M* citric acid, pH* 5.1.
[b] Unit: $cm^2/kV/min$.
[c] R_s was calculated as previously described [24].

3.3. Effect of degree of substitutions of anionic CDs

Fig. 8 shows chiral separation of trimeprazine (23) using two types of sulfated β-CDs with different number of sulfate substituents, β-CD-$(SO_4^-)_4$ (Fig. 8A) (from American Maize-Products Co.) and β-CD-$(SO_4^-)_{7-11}$ (Fig. 8B) (from Aldrich) at the same w/v percentage. Note that higher electrical field strength could be used for the CD with the degree of substitution four than that for 7–11 as the ionic strength and consequently the current generated were higher for the latter. In order to compare the separation with two sulfated CDs, the experimental conditions were kept the same. Ten racemic mixtures, trimipramine (24), trimeprazine (23), thioridazine (21), ethopropazine, nefopam (14), mianserin (13), propranolol (19), labetalol (11), propiomazine (18), and promethazine, were examined. For trimipramine (24) and trimeprazine (23), no chiral separation was achieved in the concentration range from 0.77 to 3.85 w/v% when β-CD-$(SO_4^-)_{7-11}$ was used (Fig. 8B), while chiral separation for ten of these racemic mixtures was obtained in the concentration range from 0.77 to 3.85 w/v% (~0.5–25 m*M*) when the degree of substitution was four (Fig. 8A). For the other eight compounds, chiral separations were basically the same. The mobility for all the compounds was smaller at the same concentration (w/v%) of β-CD-$(SO_4^-)_{7-11}$. This is due to the fact that at the same w/v%, the molar concentration of β-CD-$(SO_4^-)_4$ is higher than

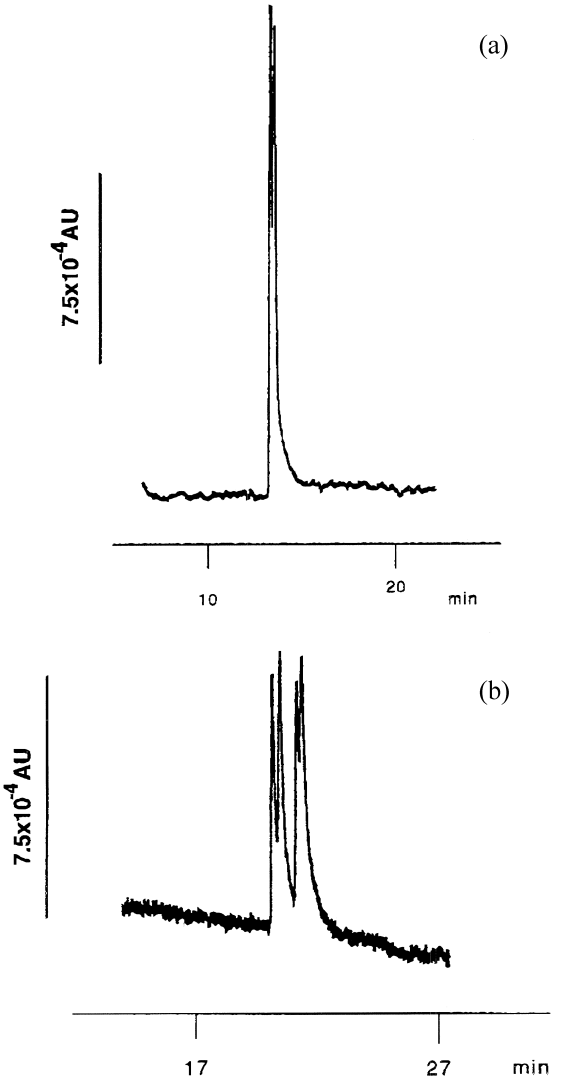

Fig. 3. Effect of β-CD-$(SO_4^-)_4$ concentration on chiral separation of labetalol (11) in formamide. Buffer: (A) 0.77 w/v% (~5 m*M*), (b) 3.08 w/v% (~20 m*M*) β-CD-$(SO_4^-)_4$ in 150 m*M* citric acid–100 m*M* Tris (pH* 5.1) in formamide. Field strength: 595 V/cm.

that of β-CD- $(SO_4^-)_{7-11}$. With the CD concentration relatively below its optimum in formamide, higher CD molar concentrations offered better separation selectivity. Theoretically, a larger number of sulfate groups on CD should give larger negative μ^c values. Thus, larger separation selectivity and resolution should be expected for β-CD-$(SO_4^-)_{7-11}$. The reason

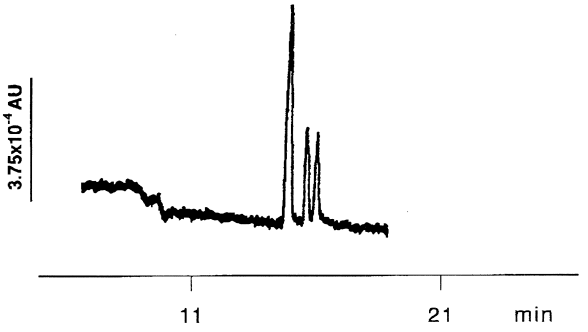

Fig. 4. Chiral separation of labetalol (11) by the counter-EOF setup in aqueous CE. Buffer: 2.31 w/v% (~15 m*M*) β-CD-$(SO_4^-)_4$ in 50 m*M* Tris–citrate (pH 5.11). Field strength: −357 V/cm.

that different degrees of substitutions of CDs causes different separation selectivity is due to the changes in the interaction sites and the steric effects on the rims of the CDs, which can change binding constants of the isomers and their difference. A good example is that some β-blockers showed different chiral selectivity with mono-, di-, and tri-methylated β-CD [23,24] in aqueous CE chiral separations. β-CD-$(SO_4^-)_4$ shows better selectivity for most of the analytes at this weight/volume percentage concentration range, and less current is generated due to smaller degree of substitution.

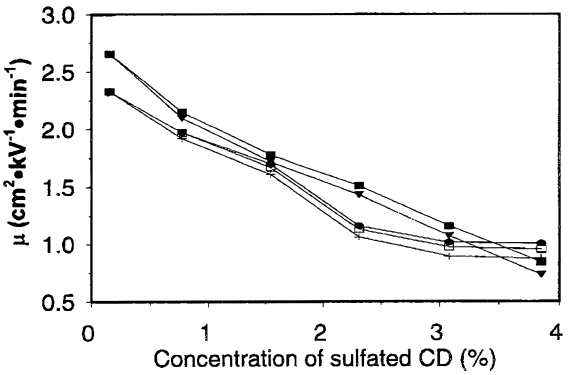

Fig. 5. Effect of β-CD-$(SO_4^-)_4$ concentration on mobility of thioridazine (21) and labetalol (11). Conditions as in Fig. 3. The first (filled square) and second (filled triangle) eluted peaks of thioridazine (21); the first (filled oval), second (empty square), and third (plus) eluted peaks of labetalol (11).

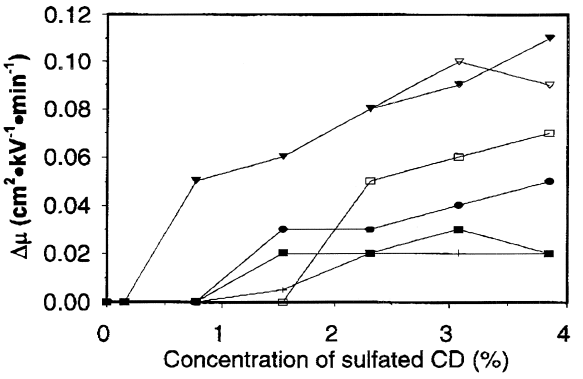

Fig. 6. Effect of β-CD-$(SO_4^-)_4$ concentration on chiral selectivity. Conditions as in Fig. 3. Compounds: bifonazole (1) (filled square), cyclopentolate (6) (empty square), thioridazine (21) (filled triangle), trimeprazine (23) (empty triangle), the first and second eluted peaks of labetalol (11) (filled oval), and the third and the fourth eluted peaks of labetalol (11) (plus).

3.4. Effect of solvents

Previously, pure water, 6 *M* urea in water, formamide, *N*-methylformamide, and *N*,*N*-dimethylformamide were chosen to study the effect of solvents on chiral separations with neutral CDs [15]. The binding constants for three test solutes, trimipramine (24), thioridazine (21). and mianserin (13), decreased with the decrease in the polarity of the solvents. Chiral separations could still be achieved in the *N*-methylformamide system even for solutes with binding constants around 0.1.

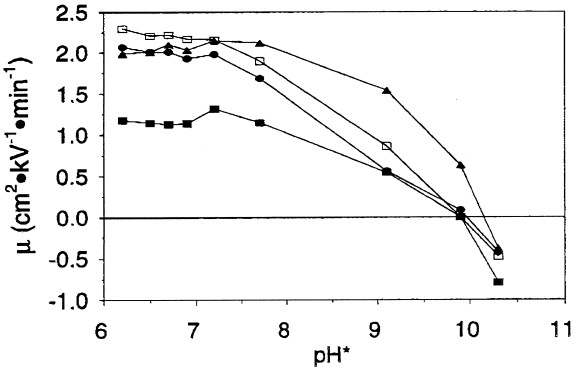

Fig. 7. Effect of pH* on mobility. Buffer electrolyte: 3.08 w/v% (~20 m*M*) β-CD-$(SO_4^-)_4$ in formamide, the total concentration of acetic acid plus Tris was kept at 250 m*M*. Field strength: 595 V/cm. Compounds: trimipramine (24) (filled square), ethopropazine (filled triangle), propiomazine (18) (filled oval), promethazine (empty square).

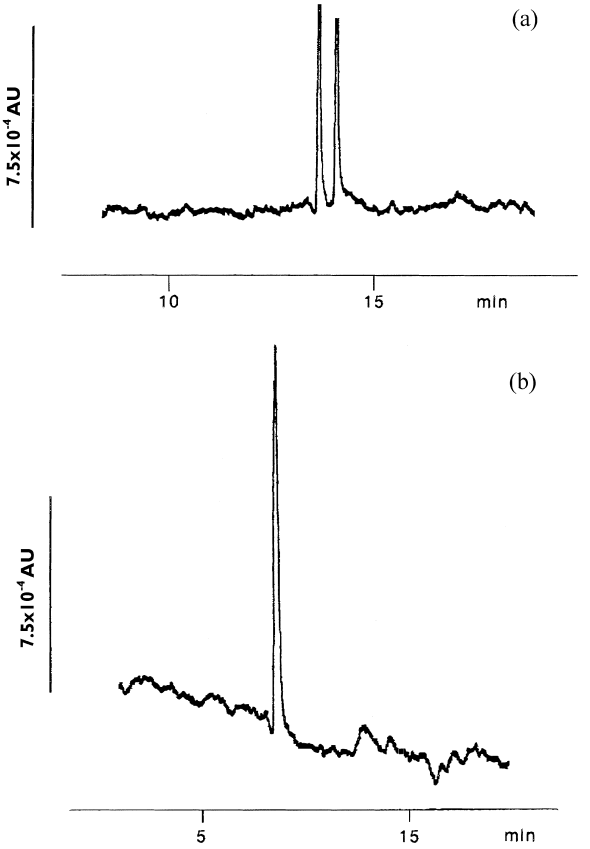

Fig. 8. Effect of degree of substitution on chiral separation of trimeprazine (23). Buffer: (A) 3.08 w/v% (~20 m*M*) β-CD-$(SO_4^-)_4$, (B) 3.08 w/v% β-CD-$(SO_4^-)_{7-11}$ in 150 m*M* citric acid–100 m*M* Tris (pH* 5.1) in formamide. Field strength: 595 V/cm.

Among the compounds tested, the only chiral separation achieved in *N*-methylformamide was drofenine (8) at 3.08 w/v% (~20 m*M*) β-CD-$(SO_4^-)_4$ even though the chiral selectivity was small (separation is not shown). This is due to the fact that the binding ability between solutes and the CD in *N*-methylformamide is smaller than that in formamide, thus a higher CD concentration is required [14]. In *N*,*N*-dimethylformamide, no chiral separation was achieved for any of the test salutes. There is no possibility to increase the concentration of the CD

in *N,N*-dimethylformamide due to the solubility problem. Therefore, for these three solvents, the interactions between the analytes and the anionic CD in formamide are the largest. Formamide offered the best chiral separations for the test analytes. Similar results were observed using neutral CD in these solvents.

3.5. Mixed formamide–water solvent

For aqueous CE chiral separations, some organic modifiers, such as acetonitrile and methanol, have been utilized to enhance chiral separation due to the change of EOF and the change of binding constants between enantiomers and chiral selectors. The problem with these two solvents is that the solubility of most CDs in these solvents is extremely low. β-CD-$(SO_4^-)_4$, for example, can not be dissolved in either methanol nor acetonitrile. In this study, all chiral separations were carried in pure organic media (formamide and *N*-methylformamide). The interactions between the analytes and the CD in formamide were weaker than those in aqueous media. In order to achieve optimum separation selectivity, higher CD concentration has to be used in formamide. From the previous study on the effect of organic solvents [14], binding constants in aqueous media are greater than those in organic solvents. As an alternative to increase the CD concentration, water was added to the running buffer in increasing proportion within the 0 to 20% range to increase the binding between the analytes and the CD. pH* was controlled at 5.1 by the addition of 0.25 *M* Tris stock solution in formamide. Fig. 9 shows the electropherograms for the resolution of labetalol (11) at 1.54 w/v% (~10 m*M*) β-CD-$(SO_4^-)_4$ at 5% (Fig. 9A) and 20% of water (Fig. 9B). The addition of water resulted in the decrease in the migration times of the optical isomers and the t_{eo} due to the fact that the viscosity of the media decreased. The mobilities of three optical isomers first increased with the increase of water percentage and then remain constant at the water percentage of 10% or larger (Fig. 10). Chiral selectivity between the first two peaks decreased at 5% water and then increased with the increase in water percentage while the selectivity between peaks 2 and 3 increased with the increase of water percentage due to the fact that the change in separation media

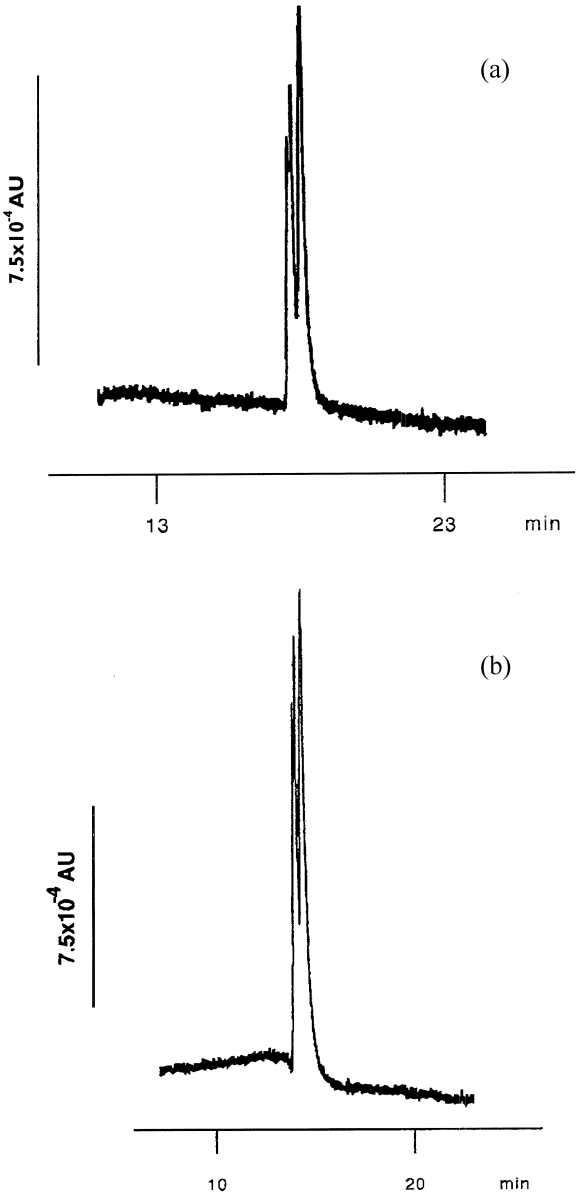

Fig. 9. Effect of water percentage on chiral separation of labetalol (11). Buffer: 10 m*M* β-CD-$(SO_4^-)_4$ in 150 m*M* citric acid–100 m*M* Tris (pH* 5.1) in formamide with (A) 5% and (B) 20% water. Field strength: 476 V/cm.

resulted in the change of the binding constants and/or their difference. Chiral separation of the second pair of enantiomers was not achieved at any water percentages. This is due to the smaller separation selectivity for the second pair of enantiomers in

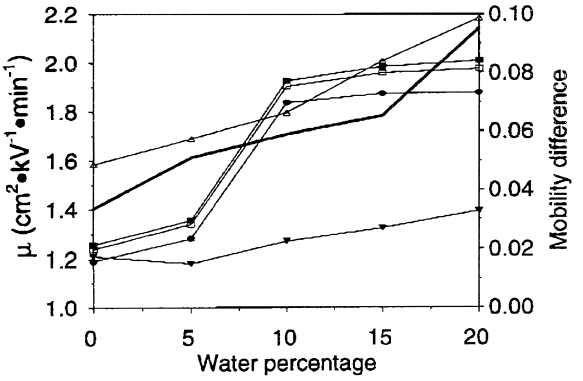

Fig. 10. Effect of water percentage on mobility and chiral selectivity of labetalol (11). Conditions as in Fig. 9. Mobility: the first eluted peak (filled square), the second eluted peak (empty square), the third eluted peak (filled oval), and EOF (solid black line). Mobility difference: the first and second (filled triangle), and the second and third peak (empty triangle).

water. In general, better separations for strongly binding compounds were observed in the purely non-aqueous media.

4. Conclusions

Better chiral separation was achieved in formamide for strong binding compounds due to the smaller mobility mismatch, and/or higher separation efficiency. The interactions between the anionic CDs and analytes are stronger than those between the neutral CDs and analytes in formamide. The degree of substitution has some effect on separation selectivity. Better separation was achieved when the CD with a degree of substitution of four was used for the tested compounds.

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