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Chiral separation of drugs by capillary electrophoresis using β-cyclodextrin polymer

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Abstract

The direct separation of enantiomers of trimetoquinol hydrochloride, related substances and some other drugs was investigated by capillary electrophoresis employing six kinds of cyclodextrins (CDs). Enantiomeric recognition of trimetoquinol and related substances was successfully achieved by using β-cyclodextrin (β-CD), heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) and β-CD polymer. β-CD polymer was especially effective for the separation of both enantiomers and different solutes. The effects of the amount of β-CD polymer added to the background electrolyte, the pH of the buffer solution and some additives such as an organic solvent or a surfactant on the resolution of enantiomers were examined. The best enantioseparation was obtained by employing 5–7% β-CD polymer in an acidic solution with a surfactant.

1. Introduction

Capillary electrophoresis (CE) has become a powerful and a popular separation technique because of the fast separation and high resolution achieved [1–3]. Several different separation modes, from capillary gel electrophoresis (CGE) to micellar electrokinetic chromatography (MEKC) [4,5], have been developed and consequently a wide variety of substances such as ions, drugs and biopolymers can now be investigated by CE, according to the physico-chemical properties of the analyte.

The synthesis of chiral compounds and recognition of molecular chirality are important subjects, especially in the pharmaceutical industry, because stereochemistry can have a significant effect on the biological activity of a drug. Further, it is necessary to develop a chiral separation method for the determination of the optical purity of drugs from the viewpoint of quality control, because the antipode of a chiral drug is regarded as one of the impurities. Chromatographic approaches, particularly those using high-performance liquid chromatography (HPLC), are the most successful for the analysis of enantiomers [6,7]. HPLC is also suitable for biomedical samples.

Concerning the separation of enantiomers, CE techniques can take advantage of the ultra-high separation efficiency, easy changes of separation media, extremely small volumes of the sample and the media, etc., in comparison with HPLC. In the development of a CE chiral separation

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method, one can easily alter the separation solution to find the optimum separation medium and can also use an expensive chiral selector because of the small amount required.

Recently much work has been reported on the direct resolution of enantiomers by capillary zone electrophoresis (CZE) employing cyclodextrins (CDs) (CD-CZE) [8–10]. CDs have also been used successfully in chiral separations by CGE [11] or MEKC (CD-MEKC) [12,13]. CDs are cyclic oligosaccharides with truncated cylindrical molecular shapes. Differential inclusion complex formation between CDs and the solute provides differential solute migration and chiral recognition. CD immobilized stationary phases for HPLC or GC are now widely used and most of them are commercially available. Chiral separation by CE using a CD immobilized capillary tube (for GC) has been also reported [14].

In this paper, chiral separation of trimetoquinol hydrochloride and related substances by CD-CZE, especially employing β-CD polymer, is described. Cyclodextrin polymers have been used in chromatographic separations for the improvement of selectivity or optical resolution [15–18]. The effects of β-CD polymer concentration on the migration time and chiral recognition were investigated. The effects of some additives, such as an organic solvent, other CDs or a surfactant, and the buffer pH on the separation of enantiomers were also examined. A comparison between β-CD polymer and β-CD as chiral selectors in CD-CZE is briefly discussed.

2. Experimental

2.1. Apparatus

Most of the experiment was carried out with the following laboratory-made apparatus: a fused-silica capillary of 40 cm length (effective length 25 cm) and 50 μm I.D. (Scientific Glass Engineering, Ringwood, Victoria, Australia) was used as the separation tube, a Model HCZE-30PN0.25 high-voltage d.c. power supply (Matsusada Precision Devices, Kusatsu, Shiga, Japan) delivering from −25 to +25 kV was used to drive the separation, the migrating solutes were detected by the on-column measurement of UV absorption at 220 nm with an SPD-6A spectrophotometer (Shimadzu, Kyoto, Japan) at a time constant of 0.05 s using a laboratory-made cell holder and a slit and a Chromatopac C-R5A (Shimadzu, Kyoto, Japan) was used for data processing. Other apparatus and experimental procedures were the same as reported previously [19]. Some of the experiments were performed on a Beckman P/ACE system 5510 equipped with a UV detector adjusted to 214 nm. A capillary tube of 57 cm total length (effective length 50 cm) and 75 μm I.D. was used in the system for the separation. The separation obtained from the laboratory-made apparatus showed a noisy baseline compared with the P/ACE system.

2.2. Reagents

Five types of CDs, α-CD, β-CD, heptakis-(2,6-di-O-methyl)-β-CD (DM-β-CD), heptakis-(2,3,6-tri-O-methyl)-β-CD (TM-β-CD) and γ-CD, were obtained from Nacalai Tesque (Kyoto, Japan). Water-soluble β-CD polymer, which was synthesized by condensation of β-CD molecules with epichlorohydrin (see Fig. 1), was purchased from Wako (Osaka, Japan). Up to a 10% β-CD polymer solution can be prepared. All other reagents and solvents were of analytical-reagent grade from Katayama Kagaku Kogyo (Osaka, Japan). Water was purified with a Milli-RO 60 water system (Millipore Japan, Tokyo, Japan). CD solution was prepared by dissolving each CD in a 25 m*M* phosphate buffer solution (pH 2.7 or

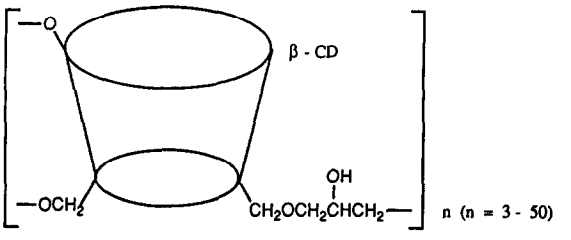


Fig. 1. Structure of β-CD polymer.

6.5) containing 2 *M* urea. The solution was passed through a membrane filter of 0.45-μm pore size (Gelman Science Japan, Tokyo, Japan) and degassed by sonication with a Branson B-2200 ultrasonic cleaner (Yamato, Tokyo, Japan) prior to use.

The structures of samples are shown in Fig. 2. Enantiomers of denopamine, trimetoquinol hydrochloride and the positional isomer of the hydroxy group of trimetoquinol were obtained from Tanabe Seiyaku (Osaka, Japan). Other racemic compounds with structures similar to that of trimetoquinol were purchased from Aldrich (Milwaukee, WI, USA). The samples were dissolved in methanol–water (50:50) at an approximate concentration of 0.1–1 mg/ml so that adequate peak heights could be obtained. Sample injection was performed by siphoning for the laboratory-made apparatus and by the pressure mode for the P/ACE 5510 system.

3. Results and discussion

3.1. Chiral recognition by CDs

The separation model of CD-CZE has been described by Wren and Rowe [20,21]. The difference in electrophoretic mobilities between two enantiomers pairs, $\Delta\mu$, is given by

$$\Delta\mu = \frac{[C](\mu_f - \mu_c)(K_1 - K_2)}{1 + [C](K_1 + K_2) + K_1K_2[C]^2} \quad (1)$$

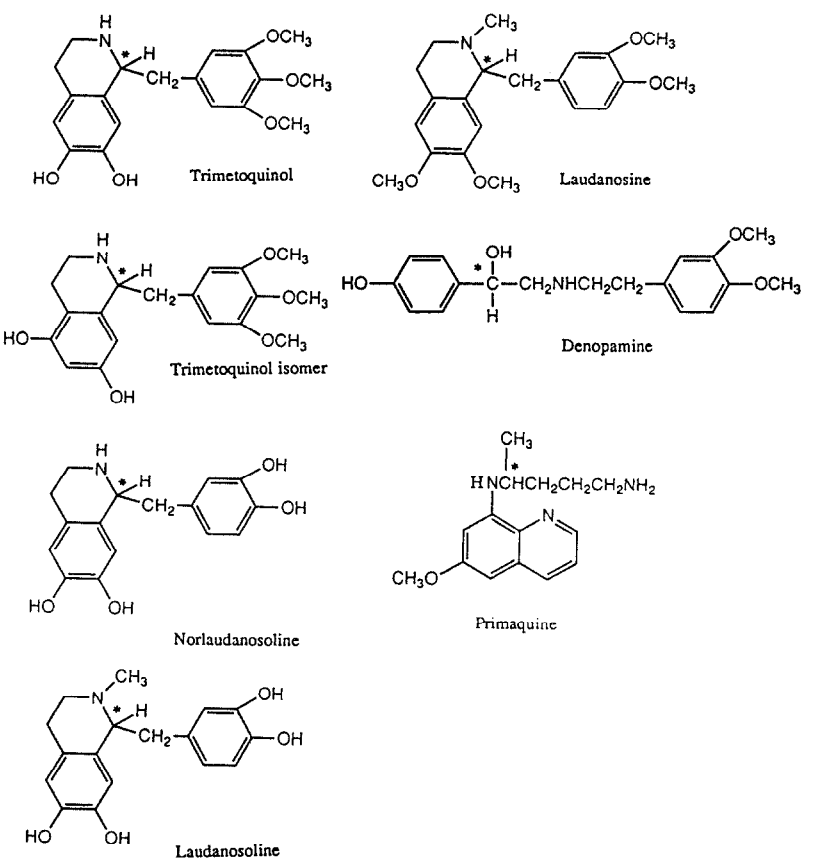


Fig. 2. Structures of samples.

where μ_f is the electrophoretic mobility of the enantiomers in free solution, μ_c is the electrophoretic mobility of the CD-complexed enantiomers, K_1 and K_2 are formation constants of the inclusion complexes of two enantiomers and [C] is the concentration of CD.

Eq. 1 shows the dependence of $\Delta\mu$ on the difference in the mobilities between the free and the CD-complexed enantiomer, $\mu_f - \mu_c$, the formation constants, K_1 and K_2, and the concentration of CD. It is obvious that the larger is the difference $\mu_f - \mu_c$ the greater is $\Delta\mu$. Therefore, an acidic buffer solution was selected to decrease the electroosmotic flow, leading to a small μ_c value. The effect of the type of CD on the chiral recognition of the samples was then investigated by using a 25 m*M* phosphate buffer solution of pH 2.7 containing 2 *M* urea and 20 m*M* of each CD or 1% β-CD polymer. The applied voltage was 15 kV. Urea was added to increase the solubility of the CDs in the aqueous phase [22].

The results are summarized in Table 1 together with the result of MEKC using sodium taurodeoxycholate (STDC) [23]. Chiral recognition was observed with all CDs except α-CD. Chiral recognition depended on the type of CD and DM-β-CD showed the widest enantioselectivity for these solutes. A typical separation using DM-β-CD is shown in Fig. 3. Chiral recognition of denopamine and laudanosine in

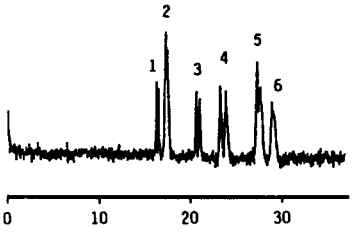


Fig. 3. Separation of enantiomers by CD-CZE using DM-β-CD. Solutes: 1 = primaquine; 2 = laudanosine; 3 = trimetoquinol; 4 = denopamine; 5 = trimetoquinol isomer; 6 = 6-chlorodiltiazem. Conditions: buffer, 25 m*M* phosphate buffer of pH 2.7 containing 2 *M* urea and 20 m*M* DM-β-CD; separation tube; 40 cm (effective length 25 cm) × 50 μm I.D.; applied voltage, 15 kV; detection, 220 cm (0.08 AUFS); temperature, ambient.

CD-CZE was only successful when employing DM-β-CD. Chiral separation of denopamine, primaquine and some other drugs by CD-CZE with DM-β-CD has been reported in detail previously [24].

Separation of the enantiomers of trimetoquinol hydrochloride was achieved by using β-CD, DM-β-CD or β-CD polymer. Chiral separation of trimetoquinol-related compounds was also successful with the same β-type CDs, having a 7–8 Å diameter cavity. However, the chiral recognition of these solutes by TM-β-CD was

Table 1
Chiral recognition by cyclodextrins

CD	Concentration	Solute[a]						
		1	2	3	4	5	6	7
α-CD	20 m*M*	×	–	×	×	×	×	×
β-CD	20 m*M*	○	○	○	○	×	×	×
γ-CD	20 mM	×	–	×	×	△	×	○
DM-β-CD	20 m*M*	○	△	○	○	△	○	○
TM-β-CD	20 m*M*	×	△	×	×	×	△	○
β-CD polymer	1%	○	○	○	○	×	×	×
STDC[b]		○	–	△	×	○	×	–

Buffer, 25 m*M* phosphate (pH 2.7) containing 2 *M* urea and each CD. Conditions: applied voltage, 15 kV; temperature, ambient; detection, 220 nm.

[a] Solutes: 1 = trimetoquinol; 2 = trimetoquinol isomer; 3 = norlaudanosoline; 4 = laudanosoline; 5 = laudanosine; 6 = denopamine; 7 = primaquine. Symbols: ○ = $R_s > 0.5$; △ = $0.5 > R_s > 0.1$; × = not separated; – = not examined.

[b] From Ref. [23].

not successful. This can probably be interpreted by the steric hindrance due to the 3-O-methyl group: it is known that the introduction of a methyl group into a hydroxy group at the 3-position of the glucose unit in CDs causes strain of CD cavity [25], leading to interference with the penetration of the solute into the CD cavity. The cavity size of α-CD (cavity diameter 5–6 Å) and γ-CD (cavity diameter 9–10 Å) was not suited to the molecular size of these substances. The dimensions of CDs and trimetoquinol hydrochloride are summarized in Fig. 4. Chiral separation of laudanosoline, which was not successful by MEKC with STDC [23], was achieved by CD-CZE, although the chiral recognition of laudanosine was decreased.

3.2. Comparison between β-CD and β-CD polymer as a chiral selector

The molecular mass (M_r) of β-CD polymer has a range because of the wide range of the degree of polymerization (3–50) of β-CD (M_r 1135). However, the M_r of one unit of water-soluble β-CD polymer (1135 + 55; see Fig. 1) is almost the same as that of β-CD, indicating that a solution having the same concentration in mass percentage units has almost the same numbers of β-CD units, which is an essential factor for chiral recognition.

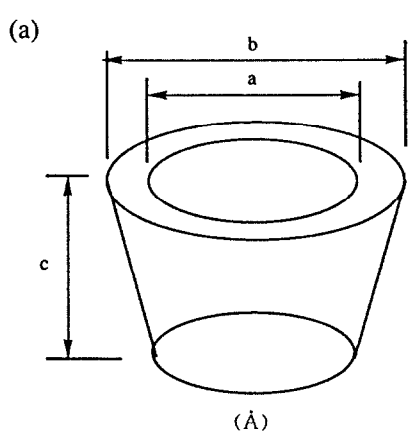
The effect of β-CD and β-CD polymer on the separation of enantiomers of trimetoquinol and related compounds was investigated by using a buffer solution of pH 2.7 containing 2 *M* urea and the following CDs: 20 m*M* of β-CD (0.23 g per 10 ml) and 2% of β-CD polymer. The selectivities (α) and resolution (R_s) between the two enantiomers were calculated with the following equations:

$$\alpha = t_2/t_1 \quad (2)$$

$$R_s = 2\,(t_2 - t_1)/(w_2 + w_1) \quad (3)$$

where t is the migration time and w the peak width. The results obtained for three substances are summarized in Table 2. Good enantiomeric resolution (>1) was obtained by using β-CD polymer, indicating that a polymer-type CD is a useful chiral selector. The high stereoselectivity in the chlorination of aromatic compounds was also observed with α-CD polymer in comparison with α-CD [26].

The great enantioselectivity of β-CD polymer



(Å)

	a	b	c
α	5.7	13.7	7.8
β	7.8	15.3	7.8
γ	9.5	16.9	7.8

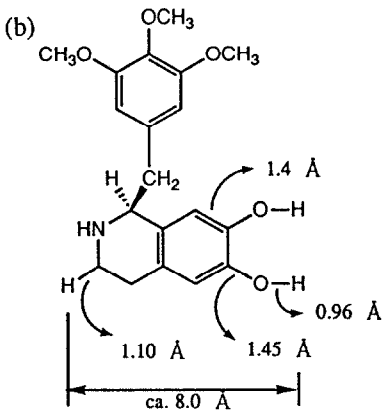


Fig. 4. Dimensions of (a) CDs and (b) trimetoquinol.

Table 2
Comparison between β-CD and β-CD polymer as a chiral sector

Solute	Buffer containing			
	20 m*M* β-CD		2% β-CD polymer	
	α	R_s	α	R_s
Trimetoquinol	1.024	1.1	1.027	1.2
Laudanosoline	1.031	1.3	1.049	1.6
Norlaudanosoline	1.033	0.9	1.053	1.8

Buffer, 25 m*M* phosphate (pH 2.7) containing 2 *M* urea and CDs.

can probably be ascribed to its large M_r. It is clear that the electrophoretic mobility of β-CD polymer is smaller than that of β-CD, and this will cause a greater difference $\mu_t - \mu_c$ (Eq. 1). In addition to M_r, polymerization, i.e., the structure of β-CD polymer, may be an important factor for the enantioselectivity. A decrease in the free rotation of the β-CD unit, a constant distribution of β-CD (regular length interval) or hydrophobic interactions, hydrogen bonding, etc., in the polymeric β-CD network will probably contribute to the chiral recognition. However, the migration times of the enantiomers of trimetoquinol and related substances in CZE with 2% β-CD polymer or 20 m*M* β-CD were almost the same, showing no successful separation of both enantiomers and different solutes. It must be necessary to use a higher concentration of the chiral selector.

3.3. *Influence of concentration of β-CD polymer*

The simultaneous enantiomeric separation of trimetoquinol hydrochloride and related substances was investigated by using β-CD polymer because of its high capability as a chiral selector. The concentration of CD is an important factor influencing the selectivity and chiral recognition, as shown in Eq. 1, and there is an optimum CD concentration for the enantiomeric separation [20]. The effects of β-CD polymer concentration (over the concentration range 1–7%) on the migration time and the chiral recognition were investigated by using 25 m*M* phosphate buffer solution of pH 2.7 containing 2 *M* urea. The results are summarized in Table 3. The resolution and the migration time increased as the concentration increased. However, the separation of enantiomers of laudanosine was not successful. A resolution of >3 was obtained for other solutes by using a 7% β-CD polymer solution. A typical electropherogram using a 5% β-CD polymer solution is shown in Fig. 5. The selectivity for the solutes was considerably improved as the concentration increased, although the separation between laudanosoline and norlaudanosoline was not successful.

Under acidic conditions (pH 2.7), the velocity of electroosmotic flow (EOF) is very low compared with the electrophoretic velocity of cationic solutes. As CD is not charged, CD is transported by the EOF, indicating that a slowly eluted solute is more effectively included in CD. In Fig. 5, the migration time of the positional isomer (5,7-dihydroxy) of trimetoquinol was largest and greatly prolonged as the β-CD polymer concentration increased. On the other had, laudanosine, which has two bulky methoxy groups in the tetrahydroisoquinoline structure (6- and 7-positions), migrated fast. The dependence of the migration time of laudanosine on the β-CD polymer concentration is relatively small compared with other solutes. These results suggest that the molecules of trimetoquinol hydrochloride analogues penetrate the CD cavity from the tetrahydroisoquinoline side. Fig. 6 shows a schematic illustration of a possible

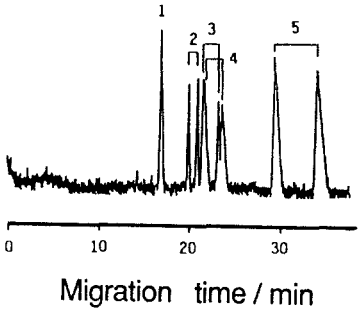


Fig. 5. Separation of enantiomers of trimetoquinol and related compounds by CD-CZE using β-CD polymer. Solutes: 1 = laudanosine; 2 = trimetoquinol; 3 = laudanosoline; 4 = norlaudanosoline; 5 = trimetoquinol isomer. Buffer, 25 m*M* phosphate (pH 2.7) containing 2 *M* urea and 5% β-CD polymer. Other conditions as in Fig. 3.

complex. Trimetoquinol isomer can penetrate more deeply into the CD cavity than trimetoquinol because it is free from steric hindrance at the 6-hydroxy group, as illustrated in Fig. 6.

3.4. Effect of additives on selectivity and chiral recognition

The effects of some additives such as an organic solvent, other CDs or a surfactant on the migration time and the resolution of the enantiomers were investigated to manipulate the selectivity, leading to successful simultaneous enantiomeric separation. As already pointed out previously [21], the addition of an organic modifier is a useful method for manipulating the selectivity. However, the addition of methanol (10–20%) to a 2% β-CD polymer solution of pH 2.7 caused a decrease in the selectivity and theoretical plate number for these solutes, with

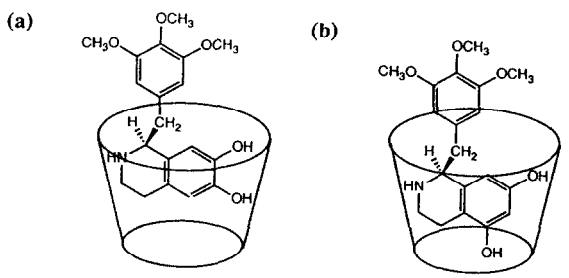


Fig. 6. Schematic illustration of the complexation of (a) trimetoquinol and (b) its isomer in β-CD.

increased migration times. The addition of 20 m*M* DM-β-CD to a 5% β-CD polymer solution of pH 2.7 containing 2 *M* urea did not affect the separation in Fig. 5. In contrast, chiral recognition of denopamine was impaired with the use of both 20 m*M* DM-β-CD and 5% β-CD polymer, compared with Fig. 3.

The addition of a surfactant was investigated by employing STDC, which shows a wide enantioselectivity in MEKC using bile salts [19,27], and sodium dodecyl sulphate (SDS). The separation of four solutes and each enantiomeric separation were successfully achieved in a single run through the addition of 10 m*M* SDS to a 5% β-CD polymer solution of pH 2.7, as shown in Fig. 7. However, the addition of 10 m*M* STDC to the same β-CD polymer solution (5%) was not successful for the separation between laudanosoline and norlaudanosoline. The addition of 10 m*M* STDC was effective for the separation of the two when the concentration of β-CD polymer was 7%. These two were not baseline resolved by only changing β-CD polymer (1–7%). The addition of 30 m*M* STDC to a 7% β-CD polymer solution of pH 2.7 caused a change in elution order, i.e., both enantiomers of laudanosoline eluted faster than those of norlaudanosoline. The results are summarized in Table 4.

The successful separation of both enantiomers and different solutes by CD-MEKC probably depends on the difference in the interaction between the solute and the micelle (micellar

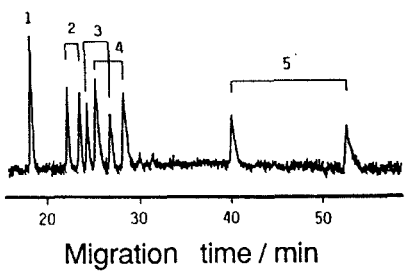


Fig. 7. Simultaneous enantiomeric separation of trimetoquinol and related compounds by CD-MEKC using β-CD polymer and SDS. Solutes numbers and buffer as in Fig. 5, except for the addition of 10 m*M* SDS. Other conditions are as in Fig. 3.

Table 3
Effect of concentration of β-CD polymer on the migration time and chiral recognition

Solute	Concentration of polymer (%)													
	1			2			3			5			7		
	t_R (min)	α	R_s	t_R (min)	α	R_s	t_R (min)	α	R_s	t_R (min)	α	R_s	t_R (min)	α	R_s
Laudanosine	15.11	1.0	–	15.18	1.0	–	15.20	1.0	–	17.86	1.0	–	19.19	1.0	–
Trimetoquinol	15.81	1.018	0.9	16.67	1.026	1.2	17.10	1.032	2.0	21.46	1.053	3.1	24.40	1.075	4.0
	16.10			17.10			17.64			22.60			26.24		
Laudanosoline	16.45	1.033	1.2	17.10	1.044	1.6	17.78	1.064	2.2	23.28	1.085	3.1	27.68	1.094	4.0
	17.00			17.85			18.92			25.25			30.28		
Norlaudanosoline	16.50	1.044	1.3	17.10	1.053	1.8	17.96	1.072	1.8	23.46	1.097	2.9	27.83	1.112	3.9
	17.22			18.00			19.25			25.74			30.95		
Trimetoquinol isomer	–			–			–			29.29	1.132	3.3	38.68	1.150	4.1
										33.17			44.50		

Buffer, 25 m*M* phosphate (pH 2.7) containing 2 *M* urea and β-CD polymer.

Table 4
Effect of surfactant addition on the selectivity of the enantiomer

Solute	Buffer containing								
	5% polymer + 10 m*M* SDS			7% polymer + 10 m*M* STDC			7% polymer + 30 m*M* STDC		
	t_R (min)	α	R_s	t_R (min)	α	R_s	t_R (min)	α	R_s
Laudanosine	18.36	1.0	–	19.81	1.0	–	23.10	1.0	–
Trimetoquinol	22.38	1.060	2.9	24.55	1.069	3.5	28.83	1.046	2.6
	23.72			26.25			30.16		
Laudanosoline	24.49	1.100	4.9	27.79	1.098	4.0	31.21	1.077	3.1
	26.94			30.52			33.62		
Norlaudanosoline	25.38	1.119	3.2	28.40	1.112	3.4	33.75	1.111	5.0
	28.40			31.59			37.50		
Trimetoquinol isomer	40.22	1.312	9.3	41.47	1.232	6.1	49.44	1.500	14.4
	52.76			51.08			74.16		

Buffer, 25 m*M* phosphate (pH 2.7) containing 2 *M* urea and β-CD polymer. Applied voltage, 15 kV; temperature, ambient; detection, 220 nm.

solubilization) in the presence of the surfactant, whose concentration is greater than the critical micelle concentration. Norlaudanosoline interacted more strongly than laudanosoline with the micelle, judging from the increase in the migration time of norlaudanosoline on addition of the surfactant. Anyhow, it was found that the addition of a surfactant to a buffer solution, i.e., MEKC mode, is a useful method for manipulating the selectivity, leading to a simultaneous separation.

3.5. Effect of buffer pH on migration time and chiral recognition

The effects of buffer pH on the migration time and chiral recognition were investigated by using a 25 m*M* phosphate buffer solution of pH 6.5 containing 2 *M* urea and 3–5% β-CD polymer. The solutes tested were laudanosine, trimetoquinol, laudanosoline and trimetoquinol isomer. The results are summarized in Table 5. A typical electropherogram obtained with the P/ACE 5510 system (3% β-CD polymer and an applied voltage of 20 kV) is shown in Fig. 8.

Enantiomers of norlaudanosoline migrated with almost the same velocity as those of laudanosoline. The migration times of the solute decreased with increase in pH. This can be interpreted by the increase in the velocity of EOF at high pH. In Fig. 8, all solutes migrated faster than the velocity of EOF (t_0), which is traced by the negative signal of methanol on the electropherogram. The same enantioselectivity was observed using β-CD at pH 6.5, although the chiral recognition was worse than that in β-CD polymer.

Chiral recognition of trimetoquinol-related compounds was successful even at high pH (6.5), although the resolution was lower than in the separation at pH 2.7. At pH 6.5, the addition of SDS was also effective for improving the selectivity. However, the chiral separation of other solutes, which were enantioresolved by a β-CD polymer solution of pH 2.7, was not achieved at pH 6.5. In previous work [24], the same results were obtained for the basic compounds. That is, the enantioselectivity decreased with increase in pH. Lower pH values gave a higher enantioseparation for basic solutes as discussed regarding Eq. 1. It is recommended to use a solution of low pH, i.e., under the condition of low velocity of EOF, for chiral separation by CD-CZE. However, for trimetoquinol-related compounds, a solution of high pH was better for fast enantioseparation.

Table 5
Effect of buffer pH on the migration time and enantiomer recognition

Solute	Buffer containing								
	3% polymer			5% polymer			5% polymer + 10 m*M* SDS		
	t_R (min)	α	R_s	t_R (min)	α	R_s	t_R (min)	α	R_s
Laudanosine	6.75	1.0	–	6.73	1.0	–	8.52	1.0	–
Trimetoquinol	7.14	1.017	1.1	7.21	1.022	1.2	9.23	1.024	1.3
	7.26			7.37			9.45		
Laudanosoline	7.51	1.027	1.4	7.72	1.028	1.4	9.82	1.038	1.3
	7.71			7.94			10.19		
Trimetoquinol isomer	8.02	1.041	2.2	8.21	1.038	2.5	11.07	1.067	2.9
	8.35			8.52			11.81		
EOF (t_0)	9.90			9.89			12.58		

Buffer, 25 m*M* phosphate (pH 6.5) containing 2 *M* urea and β-CD polymer. Applied voltage, 15 kV; temperature, ambient.

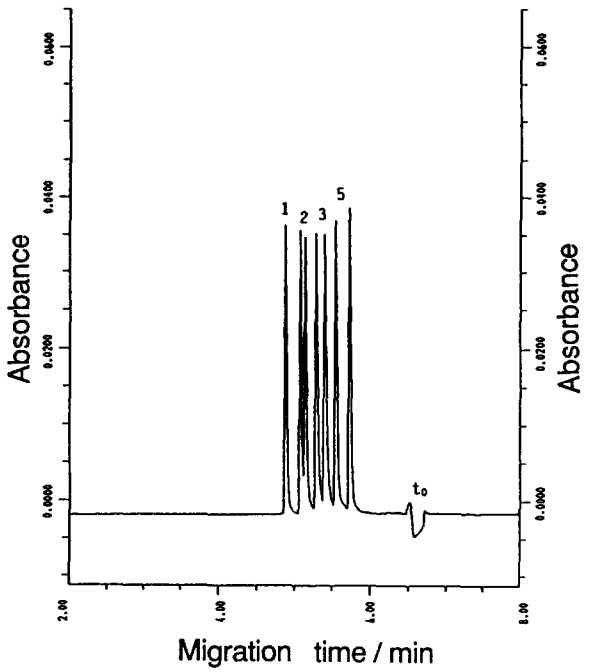


Fig. 8. Separation of enantiomers of trimetoquinol and related compounds by CD-CZE using a pH 6.5 buffer solution. Conditions: buffer, 25 m*M* phosphate (pH 6.5) containing 2 *M* urea and 3% β-CD polymer; separation tube, 57 cm (effective length 50 cm) × 75 μm I.D.; applied voltage, 20 kV; detection, 214 nm (0.06 AUFS); temperature, 23°C. Solute numbers as in Fig. 5.

In conclusion, it was found that β-CD polymer is useful for the enantioseparation of trimetoquinol and related compounds. The higher the concentration of β-CD polymer, the greater is the resolution. The addition of a surfactant was effective for the manipulation of selectivity in CD-CZE using β-CD polymer. The best enantioseparation was obtained by employing 5–7% β-CD polymer in an acidic solution containing the surfactant. On the other hand, the first enantioseparation was achieved by using a high pH solution, although the resolution was slightly decreased. The separation of different solutes and the corresponding enantiomers will be optimized by changing the type and concentration of CD, the pH of the buffer and adding a surfactant.

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