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Determination of β-cyclodextrin inclusion complex constants for 3,4-dihydro-2-*H*-1-benzopyran enantiomers by capillary electrophoresis

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Abstract

Chiral separation of 3,4-dihydro-2*H*-1-benzopyran derivatives by capillary zone electrophoresis was achieved by employing β-cyclodextrin (β-CD) as chiral selector. The effects of electrolyte composition (β-cyclodextrin concentration, ionic strength and pH of the buffer) on the migration time, enantioselectivity, peak efficiency and resolution were investigated. As expected, there was an optimum β-CD concentration (C_{opt}) which gave maximum enantioselectivity. The stability constant for the β-CD inclusion complex was determined for each enantiomer of two 3,4-dihydro-2*H*-1-benzopyran derivatives. For each solute, the experimental value of C_{opt} agreed well with the value calculated from the equation $[C]_{opt} = 1/(K_R K_S)^{1/2}$, where we used experimental values for the inclusion complex constants (K_R, K_S). The enantiomeric separation of three 3,4-dihydro-2*H*-1-benzopyran derivatives was achieved using this optimization method, and baseline separation was obtained in less than 15 min with an efficiency of between 300 000 and 600 000 theoretical plates.

1. Introduction

Molecular recognition is a major concept in the understanding of many important chemical interactions such as drug–receptor interactions or enzyme–substrate interactions. For example, many compounds synthetized as potential drugs contain one or several chiral centres; the resulting enantiomers often having different biological activities and different pharmacological effects. Hence it is important to develop chiral separation methods, particularly in the pharmaceutical and medicinal areas, for the determination of the optical purity of drugs.

Chiral separation by capillary zone electrophoresis (CZE) or micellar electrokinetic capillary chromatography (MEKC) is a rapidly developing area owing to the high efficiency and resolution that can be achieved and the option of adding molecular recognition agents to the electrolyte [1–8].

This paper describes the chiral separation of several 3,4-dihydro-2*H*-1-benzopyran derivatives by CZE using β-cyclodextrin (β-CD) as a chiral selector, which is added to the running electrolyte. In CZE, the basic thermodynamic dif-

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ferentiation of enantiomers in many chiral systems is poor, which means that the enantioselectivity value is very close to unity, and consequently the need for a high column efficiency is very important.

In this work, we studied the influence of electrolyte properties (β-CD concentration, ionic strength, pH value) and run voltage on the peak efficiency (N), enantioselectivity (α) and resolution (R_S) between two enantiomers. The main objective was to confirm the capability of a capillary electrophoresis technique to determine the β-CD inclusion complex constant of each enantiomer.

2. Experimental

2.1. Apparatus

All open-tube electrokinetic capillary chromatographic separations were performed with a Spectra-Physics (San Jose, CA, USA) Spectraphoresis 1000 instrument using a 70 cm × 50 μm I.D. silica capillary column. The separations were performed at 25°C at a voltage of +18 kV. The capillaries were conditioned by washing first with 1 *M* sodium hydroxide (5 min) at 60°C, followed by 0.1 *M* sodium hydroxide (5 min) at 40°C, water at 40°C and finally with the electrophoretic buffer (60 min). Between consecutive analyses, the capillary tube was flushed with the electrophoretic buffer (5 min) in order to improve the migration time and the peak-shape reproducibility. Analytes were injected on-column using hydrodynamic injection for 1 s. Data were processed with an IBM PS/2 Model 70 386 computer. Software, operating under IBM OS/2, was supplied by Spectra-Physics. The migrating solutes were detected by on-column measurement of UV absorption at 210 nm.

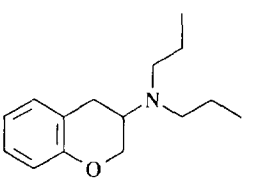
2.2. Reagents

All chemicals were of analytical-reagent grade. Disodium hydrogenphosphate (Fluka, Buchs, Switzerland), sodium tetraborate (Fluka), 1 *M* sodium hydroxide (Fluka), urea (Merck, Darmstadt, Germany) and orthophosphoric acid (Sigma, St. Louis, MO, USA) were used. Water used for dilutions and in buffer solutions was on HPLC grade (Carlo Erba, Milan, Italy). The pH was adjusted with orthophosphoric acid.

The β-CD solutions were prepared by dissolving β-CD in 50 m*M* phosphate–borate buffer containing 8 *M* urea. Finally, the electrophoretic buffer was filtered prior to use through a polypropylene filter (0.22-μm pore size, 25 mm diameter) (Whatman, Maidstone, UK).

Stock standard solutions (100 ppm) were prepared first by dissolving a small amount of each racemic solute in methanol and second by dilution of this methanolic solution in 50 m*M* phosphate–borate solution (pH 7.0).

The effects of β-CD concentration on the migration time and chiral resolution were observed with three different 3,4-dihydro-2*H*-1-benzopyran derivatives, 3-(di-*n*-propylamino)chroman (DPAC), 5-methoxy-3-(di-*n*-



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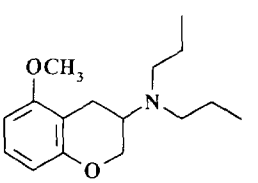

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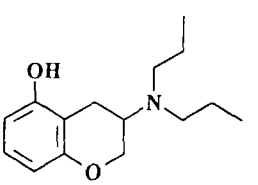

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Fig. 1. Structures of the solutes: 1 = DPAC; 2 = 5-MeO-DPAC; 3 = 5-OH-DPAC.

propylamino)chroman (5-MeO-DPAC) and 5-hydroxy-3-(di-*n*-propylamino)chroman (5-OH-DPAC) (Fig. 1). DPAC and 5-MeO-DPAC were prepared in four steps from salicylaldehyde and 2-hydroxy-6-methoxybenzaldehyde, respectively [9,10]. The O-demethylation of 5-MeO-DPAC by refluxing bromhydric acid in acetic acid produced 5-OH-DPAC with a very good yield [9,10]. Investigations on the pharmacological properties of these products indicated that 5-MeO-DPAC and 5-OH-DPAC act in the n*M* range on 5-HT_{1A} sites but are recognized very poorly on other 5-HT sites. Conversely, the non-5-substituted compound DPAC shows a low affinity for all 5-HT sites [10,11].

The resolution (R_S) between the two enantiomers has been calculated using the equation $R_S = 2(t_{m_R} - t_{m_S})/(w_{b_R} + w_{b_S})$, where t_{m_R} and t_{m_S} are the migration times and w_{b_R}, w_{b_S} the base peak widths of the *R* and *S* enantiomer, respectively. The enantioselectivity (α) was calculated using the equation $\alpha = t_{m_R}/t_{m_S}$ [12,13].

3. Results and discussion

3.1. Theoretical model

Formation of inclusion complexes with a chiral selector is a convenient method to resolve enantiomers by CZE. Cyclodextrins and their alkylated derivatives have been successfully used in CE as chiral selectors added to the electrophoretic buffer [1–8]. For example, β-CD is a well known, commercially available cyclic non-reducing oligosaccharide with seven glucopyranose units and is toroidal with a hydrophobic interior cavity. Several workers have developed theoretical models to describe the variation of electrophoretic mobility versus chiral selector concentration [14–19].

According to the cyclodextrin inclusion complex model, developed by Wren and Rowe [14–16], a pair of cationic enantiomers, R^+ and S^+, interact with the neutral cyclodextrin C producing rapid and dynamic chemical equilibria:

$$R^+ + C \overset{K_R}{\rightleftharpoons} RC^+ \quad (1)$$

$$S^+ + C \overset{K_S}{\rightleftharpoons} SC^+ \quad (2)$$

where m_{R^+} is the electrophoretic mobility of the free enantiomer R^+ and m_{RC^+} is the electrophoretic mobility of the inclusion complex RC^+ between the enantiomer R^+ and the chiral selector C. K_R and K_S are the inclusion complex stability constants of RC^+ and SC^+ complexes. The model assumes that the electrophoretic mobilities of the complex RC^+ and SC^+ are equal.

If the kinetics of complex formation are rapid enough, the electrophoretic mobility $[m_{(R)^+}]$ of enantiomer R^+ (in our study a benzopyran derivative) was determined as the weighted average of the electrophoretic mobility of the free enantiomer (m_{R^+}) and the complexed enantiomer (m_{RC^+}) with the cyclodextrin C, as expressed by the following equation:

$$m_{(R)^+} = \frac{[R^+]}{[R^+] + [RC^+]} \cdot m_{R^+} + \frac{[RC^+]}{[R^+] + [RC^+]} \cdot m_{RC^+} \quad (3)$$

where $[R^+]$ and $[RC^+]$ represent the concentration at equilibrium of the free and complexed enantiomers, R^+ and RC^+, respectively. The apparent mobility of the enantiomer is determined by the proportion of time between when the analyte is free and when it is part of the inclusion complex. The following expression was derived for the calculation of $m_{(R)^+}$:

$$m_{(R)^+} = \frac{1}{1 + K_R[C]} \cdot m_{R^+} + \frac{K_R[C]}{1 + K_R[C]} \cdot m_{RC^+} \quad (4)$$

where K_R is the stability constant of the inclusion complex RC^+ and [C] is the β-CD concentration at equilibrium. Consequently, the variation of electrophoretic mobility $[m_{(R)^+}]$ versus the negative logarithm of the β-CD concentration (p*C* = $-\log[C]$) allows us to determine the stability constant K_R. This plot has an similar shape to an

acid–base titration curve where the abscissa of the inflection point is equal to log K_R [18].

Penn et al. [17] recently proposed a relationship to calculate the equilibrium constant from electrophoretic mobility measurements at different chiral selector concentrations. Rearrangement of Eq. 4 gives the following relationship:

$$K_R = \frac{m_{R^+} - m_{(R)^+}}{(m_{(R)^+} - m_{RC^+})[C]} \quad (5)$$

The difference between the electrophoretic mobilities of the two enantiomers, $\Delta m^+ = m_{(S)^+} - m_{(R)^+}$, can be calculated from the following relationship:

$$\Delta_{m^+} = \frac{(m_{R^+} - m_{RC^+})(K_S - K_R)}{1 + [C](K_S + K_R) + K_R K_S [C]^2} \cdot [C] \quad (6)$$

Thus, chiral recognition will depend on the difference between the electrophoretic mobilities of each free and complexed enantiomer. The separation cannot be achieved if the difference in the mobilities of the free and the complexed solute is too small. Chiral resolution may be achieved if the two enantiomers have different affinities with the chiral selector and if the exchange between free and complexed forms is very rapid and does not give rise to band broadening [14]. Wren and Rowe [14] showed that maximum separation is achieved for a β-CD concentration $[C]_{opt}$ of

$$[C]_{opt} = \frac{1}{(K_R K_S)^{1/2}} \quad (7)$$

From experimental data, the electrophoretic

Table 1
Electrophoretic mobilities of the two enantiomers of 3,4-dihydro-2*H*-1-benzopyran derivatives versus β-CD concentration in the range 1 μ*M*–100 m*M* (not corrected for viscosity)

[β-CD] (μ*M*)	−log[β-CD]	DPAC		5-MeO-DPAC		5-OH-DPAC	
		$m_{epR} \times 10^5$ ($cm^2 V^{-1} s^{-1}$)	$m_{epS} \times 10^5$ ($cm^2 V^{-1} s^{-1}$)	$m_{epR} \times 10^5$ ($cm^2 V^{-1} s^{-1}$)	$m_{epS} \times 10^5$ ($cm^2 V^{-1} s^{-1}$)	$m_{epR} \times 10^5$ ($cm^2 V^{-1} s^{-1}$)	$m_{epS} \times 10^5$ ($cm^2 V^{-1} s^{-1}$)
1	6.00	–	–	8.47	8.47	8.93	8.93
2.5	5.60	–	–	8.47	8.47	8.89	8.89
5	5.30	–	–	8.36	8.36	8.82	8.82
10	5.00	–	–	8.35	8.35	8.76	8.76
25	4.60	–	–	8.61	8.61	8.68	8.68
50	4.30	–	–	8.30	8.30	8.62	8.62
100	4.00	–	–	8.20	8.20	8.59	8.59
250	3.60	–	–	8.28	8.28	8.37	8.37
500	3.30	–	–	7.94	7.94	8.06	7.91
1 000	3.00	–	–	7.60	7.60	7.58	7.30
2 500	2.60	–	–	7.06	6.86	6.37	5.91
5 000	2.30	5.80	5.48	6.19	5.88	5.04	4.48
10 000	2.00	3.86	3.49	4.80	4.39	3.40	2.88
20 000	1.70	1.95	1.67	3.82	3.35	2.37	1.96
30 000	1.52	1.11	0.91	3.17	2.75	1.71	1.35
40 000	1.40	0.72	0.55	2.19	1.86	1.11	0.87
50 000	1.30	0.43	0.31	1.59	1.37	0.83	0.67
60 000	1.22	0.46	0.36	1.23	1.04	0.66	0.50
70 000	1.16	0.15	0.07	1.09	0.93	0.48	0.36
80 000	1.10	0.13	0.13	0.86	0.73	–	–
90 000	1.05	–	–	0.66	0.55	–	–
100 000	1.00	–	–	0.72	0.61	0.35	0.29

Fused-silica capillary column, 70 cm × 50 μm I.D.; applied voltage, −18 kV; buffer, 50 m*M* phosphate–borate (pH 7.0)–β-CD–8 *M* urea; detection at 210 nm; temperature, 25°C.

mobility [$m_{(R)^-}$] of the enantiomer R^- was determined using the following equation:

$$m_{(R)^-} = \frac{L_d \cdot L_T}{V}\left(\frac{1}{t_m} - \frac{1}{t_0}\right) \quad (8)$$

where L_d is the length of the capillary from the inlet to the detector, L_T is the total length of the capillary, V is the applied voltage t_m is the migration time of the enantiomer and t_0 is the migration time of a neutral marker (methanol).

Calculation of binding constants from CE mobility data requires that any change in mobility at higher chiral selector concentration is due to complexation of the analyte to the selector and also to changes in buffer viscosity. Consequently, electrophoretic mobilities have been corrected according to the expression [20]

$$m_{corr.(R)^-} = m_{(R)^-} \cdot \frac{\eta_C}{\eta_0} \quad (9)$$

where η_C and η_0 are the viscosity of electrolyte with and without β-cyclodextrin addition, respectively. The relative viscosity η_C (in cP) of 8 M urea solutions depends on β-CD concentration according to the following equation at 21°C [21]:

$$\eta_C = 1.50 + 9.58[C] \quad (10)$$

where [C] is the β-CD concentration (in M).

3.2. Determination of optimum concentration of β-cyclodextrin

These tertiary amine analytes have ionization constants in the 5–8 pK_a range and consequently their apparent positive charge depends on their pK_a value and on the pH of the running electrolyte. The pH value of the electrolyte (pH 7.0) was selected in order to cause the solutes to be partially protonated. Under these conditions, cationic species moved towards the cathode with the velocity of electrophoretic migration plus that of electroosmotic flow.

The effects of β-CD concentration on the migration time and on the chiral resolution of 5-MeO-DPAC and 5-OH-DPAC enantiomers were investigated using 50 mM phosphate–borate buffer (pH 7.0) containing both 8 M urea and β-CD at concentrations varying from 1 μM up to 100 mM. Urea was added to the cyclodextrin solutions to increase their solubilities. For each of the 22 β-CD concentration values, the separation was performed in triplicate from consecutive injections to obtain an average value for the electrophoretic parameters of each enantiomer (Tables 1 and 2). The influence of the selector concentration on the electrophoretic mobility of each 5-OH-DPAC enantiomer is shown in Fig. 2a. As the β-CD concentration increases, the free enantiomer fraction becomes smaller and consequently the electrophoretic mobility is reduced. At the micromolar level of β-CD concentration (1–100 μM), no enantioseparation occurred because the benzopyran derivative enantiomers were not complexed (Fig. 3a and b). The electrophoretic mobilities of free enantiomers were $8.47 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} for 5-MeO-DPAC and $8.93 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} for 5-OH-DPAC. At the millimolar level of β-CD

Table 2
Complexation degree of each 5-OH-DPAC enantiomer versus β-CD concentration in the range 1 μM–100 mM (not corrected for viscosity)

[β-CD] (μM)	−log[β-CD]	5-OH-DPAC	
		x_R	x_S
1	6.00	0.00	0.00
2.5	5.60	0.45	0.45
5	5.30	1.23	1.23
10	5.00	1.90	1.90
25	4.60	2.80	2.80
50	4.30	3.47	3.47
100	4.00	3.81	3.81
250	3.60	6.27	6.27
500	3.30	9.74	11.42
1 000	3.00	15.12	18.25
2 500	2.60	28.67	33.82
5 000	2.30	43.56	49.83
10 000	2.00	61.93	67.75
20 000	1.70	73.46	78.05
30 000	1.52	80.85	84.88
40 000	1.40	87.57	90.26
50 000	1.30	90.71	92.50
60 000	1.22	92.61	94.40
70 000	1.16	94.62	95.97
100 000	1.00	96.00	96.75

Conditions as in Table 1.

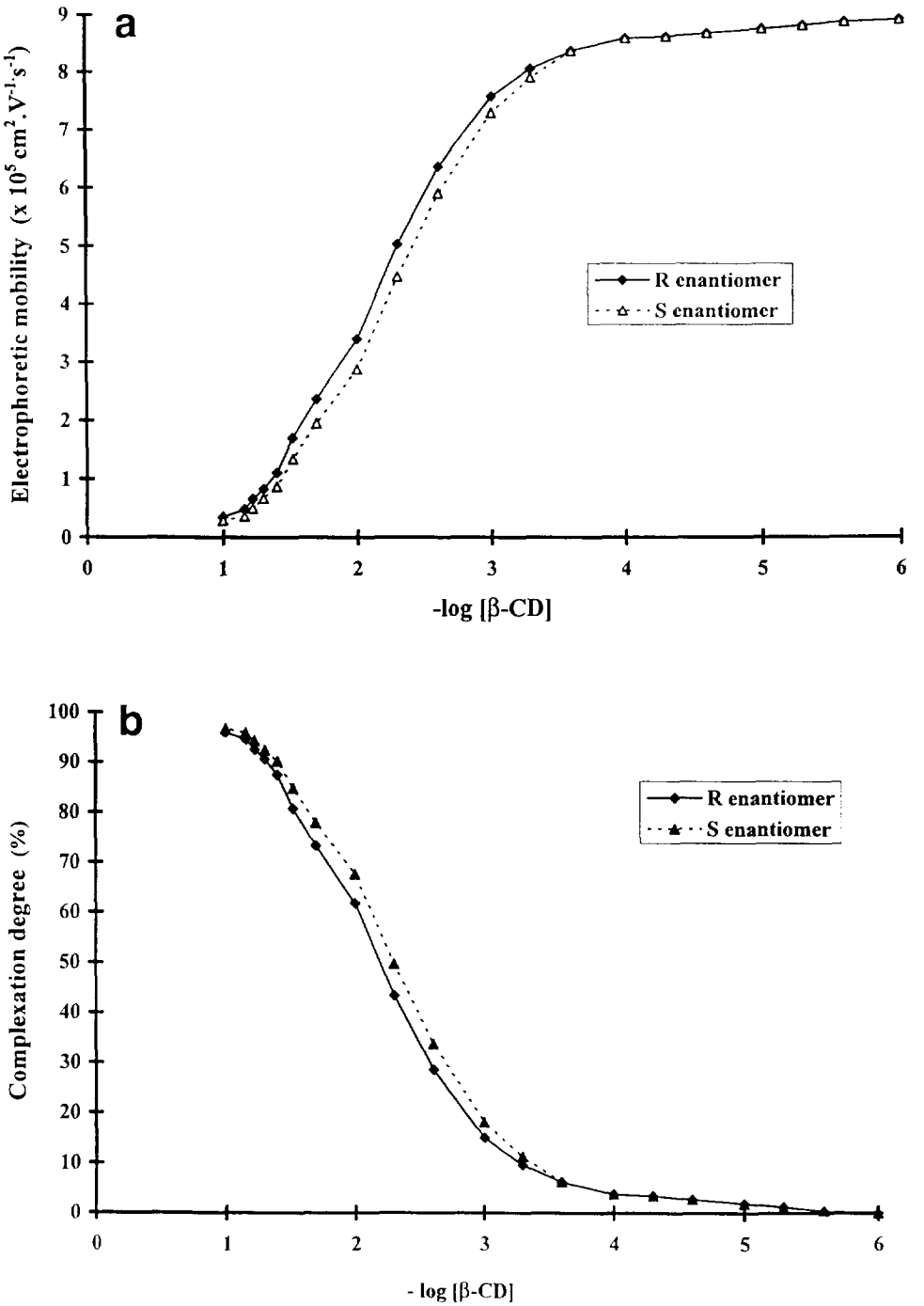

Fig. 2. (a) Variation of the electrophoretic mobility of each the two enantiomers of 5-OH-DPAC versus $-\log[\beta\text{-CD}]$. (b) Variation of the degree of complexation (x) for each enantiomer of 5-OH-DPAC versus $-\log[\beta\text{-CD}]$. Fused-silica capillary column, 70 cm × 50 μm I.D.; applied voltage, +18 kV; buffer, 50 m*M* phosphate–borate (pH 7.0)–β-CD–8 *M* urea; detection at 210 nm; temperature, 25°C.

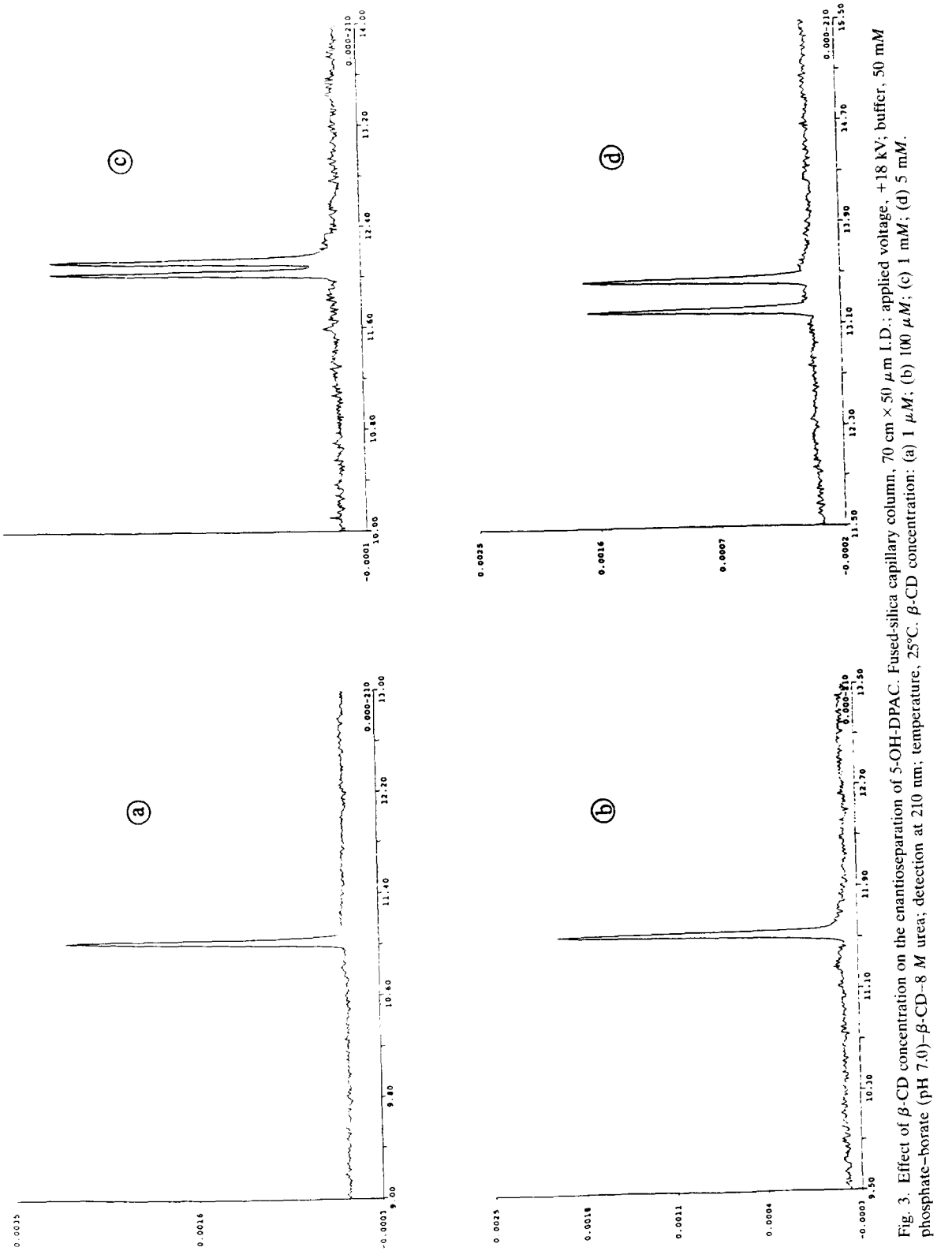

Fig. 3. Effect of β-CD concentration on the enantioseparation of 5-OH-DPAC. Fused-silica capillary column, 70 cm × 50 μm I.D.; applied voltage, +18 kV; buffer, 50 m*M* phosphate–borate (pH 7.0)–β-CD–8 *M* urea; detection at 210 nm; temperature, 25°C. β-CD concentration: (a) 1 μ*M*; (b) 100 μ*M*; (c) 1 m*M*; (d) 5 m*M*.

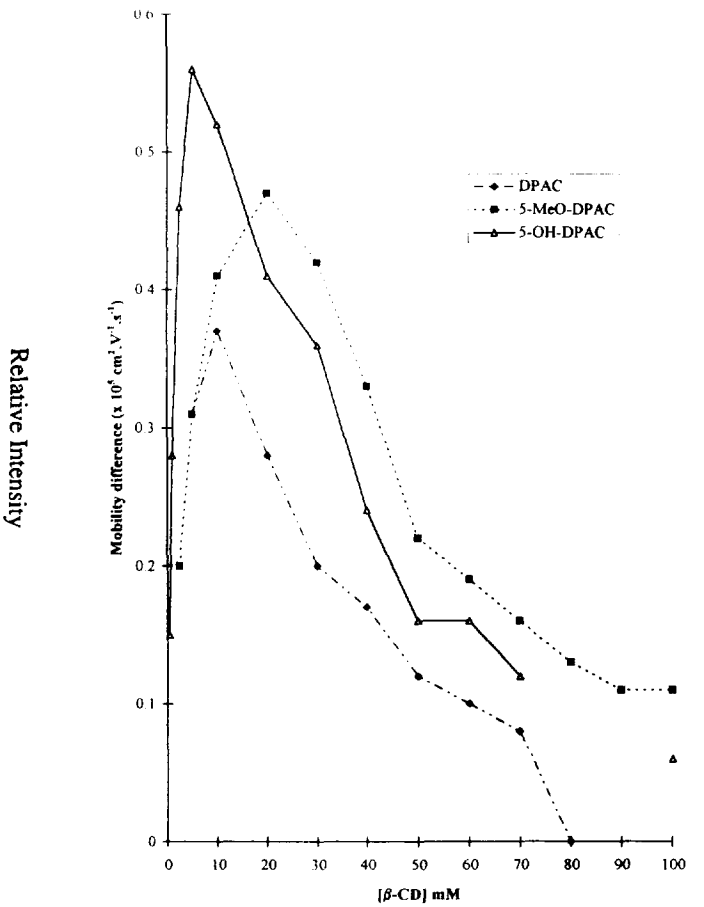

Fig. 4. Determination of the optimum concentration of β-CD for the CZE enantioseparation of three 3,4-dihydro-2*H*-1-benzopyran derivatives. Fused-silica capillary column, 70 cm × 50 μm I.D.; applied voltage, +18 kV; buffer, 50 m*M* phosphate–borate (pH 7.0)–β-CD–8 *M* urea; detection at 210 nm; temperature, 25°C.

concentration (1–5 m*M*), the electrophoretic mobility of each enantiomer decreased with increasing complexation percentage and a baseline resolution of the two enantiomers was achieved (fig. 3c and d). The solubility of β-CD, which is only 16 m*M* in the aqueous phase, can be considerably increased by the addition of urea at a high molar concentration. Therefore, a 100 m*M* concentration of β-CD dissolved in the phosphate–borate buffer (pH 7.0) with an 8 *M* urea concentration was used. Even at this extreme β-CD concentration, the electrophoretic mobility of the two enantiomers of 5-OH-DPAC are not yet equal ($0.35 \cdot 10^{-5}$ and $0.29 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}). In fact, the lower part of the curves in Fig. 2a, which corresponds to the complexed form, could be only reached at chiral selector concentrations higher than 100 m*M*. The complexed forms of 5-OH-DPAC and 5-MeO-DPAC have electrophoretic mobilities of ca. 20 and 12, respectively, times smaller than that of the free form, as a consequence of the smaller effective charge to hydrodynamic radius ratio.

However, the complexation process may be easily followed by the degree of complexation x of the enantiomer R^+, defined as

$$x = \frac{[RC^+]}{[R^+] + [RC^+]} = \frac{m_{R^+} - m_{(R)^+}}{m_{R^+} - m_{RC^+}} \qquad (11)$$

Table 3
β-Cyclodextrin inclusion constants (K_R, K_S) for enantiomers of two 3,4-dihydro-2*H*-1-benzopyran derivatives determinated by capillary electrophoresis. Conditions as in Table 1.

Solute	K_R (M^{-1})	K_S (M^{-1})	C_{opt} [a] (mM)	C_{opt} [b] (mM)
5-MeO-DPAC	58	72	18.2	15.5
5-OH-DPAC	138	166	6.4	6.6

The electrophoretic mobilities were corrected according to Eqns. 9 and 10.
[a] Experimetally determined from Fig. 4.
[b] Calculated from Eq. 7.

where m_{RC^+}, m_{R^+} and $m_{(R)^+}$ are the electrophoretic mobilities of the complexed enantiomer, of the free form and of the partially complexed enantiomer R^+ by the β-CD (concentration [C]), respectively. The variation of degree of 5-OH-DPAC complexation versus −log[β-CD] is shown in Fig. 2b. If two enantiomers are both complexed at 3.8% at 100 μM β-CD concentration, their degrees of complexation become different at 5 mM (44% and 50% for *R*- and *S*-enantiomers, respectively).

The difference between the electrophoretic mobility of each enantiomer (Δm^+) was plotted against the chiral selector concentration for each of the three 3,4-dihydro-2*H*-1-benzopyran derivatives (Fig. 4). The enantiomeric resolution depends on the concentration of the chiral selector in the electrolyte. As expected, there is an optimum β-CD concentration (C_{opt}), which leads to a maximum Δm^+ value and, hence, to an optimum enantioselectivity. The experimentally determined optimum β-CD concentrations for DPAC, 5-MeO-DPAC and 5-OH-DPAC were 9.3, 18.2 and 6.4 mM, respectively. Further increases in β-CD concentration resulted in a decrease in resolution (at 100 mM β-CD, the resolution decreased dramatically). This behaviour agrees well with what is predicted by Eq. 7.

From Fig. 2a, the inclusion complex stability constant can be determined from the *x*-value of the inflection point, according to Eq. 4. The resulting K values are summarized in Table 3. Although the interior cavity size of β-CD fits reasonably well with the steric volume of such solutes having a benzopyran moiety, the inclusion complex of these molecules with β-CD appears to be of moderate stability. The magnitude of the equilibrium constant also influences the migration order. For solutes which are positively charged below pH 7.0, the *R*-enantiomer with the lower equilibrium constant will elute first. These values are approximately two orders of magnitude higher than the equilibrium constants calculated by Valko et al. [19] for the complexation of mandelic acid enantiomers and γ-CD (2.8 and 2.4 M^{-1}). In this particular case, the γ-CD had an interior cavity too large for the mandelic acid enantiomers. A better steric fit was reported by Penn et al. [17] with equilibrium constants for the complexation of tioconazole and hydroxypropyl-β-cyclodextrin of 201 and 231 M^{-1}, respectively. The theoretical model predicts that the optimum concentration for the chiral selector (C_{opt}) depends on the inclusion constant of each enantiomer (K_R and K_S) as expressed by Eq. 7. The experimentally deter-

Table 4
Determination of enantioseparation parameters of three 3,4-dihydro-2*H*-1-benzopyran derivatives at optimum β-CD concentration (C_{opt})

Solute	[β-CD] (mM)	t_{m_R} (min)	t_{m_S} (min)	N_R	N_S	α	R_s	$\Delta(m^+) \times 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)
DPAC	9.3	12.89	13.04	403 000	322 000	1.012	1.72	0.37
5-MeO-DPAC	18.2	11.72	11.88	423 000	423 000	1.014	2.61	0.47
5-OH-DPAC	6.4	13.20	13.44	640 000	604 000	1.018	3.57	0.55

Migration time (t_m), efficiency (N), enantioselectivity (α), resolution (R_s) and the difference in electrophoretic mobilities of two enantiomers (Δ_{m^+}). Conditions as in Table 1.

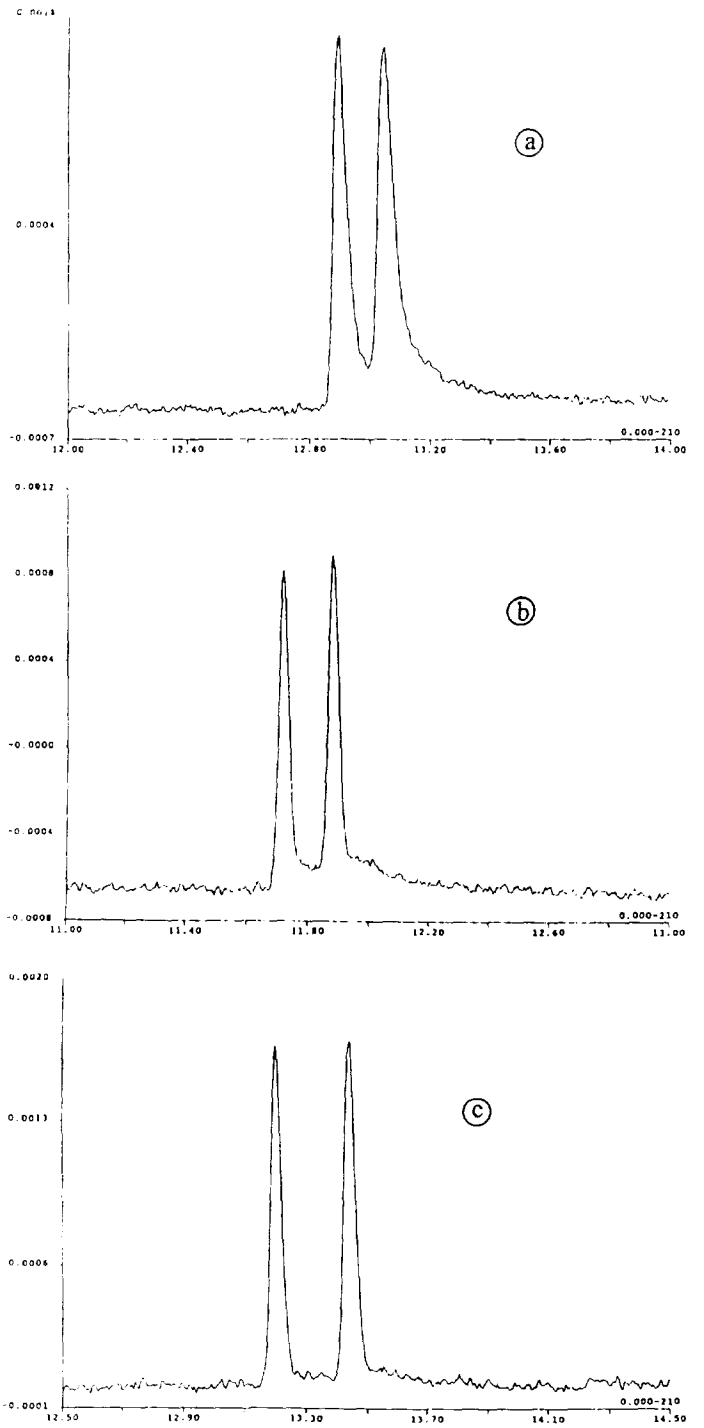

Fig. 5. Enantioseparation of three 3,4-dihydro-2*H*-1-benzopyran derivatives at the optimum β-CD concentration (C_{opt}). Fused-silica capillary column, 70 cm × 50 μm I.D.; applied voltage, +18 kV; buffer, 50 m*M* phosphate–borate (pH 7.0)–β-CD–8 *M* urea; detection at 210 nm; temperature, 25°C. Solutes: (a) DPAC (C_{opt} = 10 m*M*); (b) 5-MeO-DPAC (C_{opt} = 20 m*M*); (c) 5-OH-DPAC (C_{opt} = 6 m*M*).

mined C_{opt} value for 5-OH-DPAC agrees well with the calculated value from the previous equation in which we used experimental values for the inclusion constants (Table 3). The two enantiomers of 5-OH-DPAC have higher K values (138 and 166) than those of 5-MeO-DPAC (58 and 72), which means that for an optimum separation a lower β-CD concentration is required.

The separation parameters, migration time, efficiency, enantioselectivity, resolution and difference in electrophoretic mobilities of the two enantiomers of the three 3,4-dihydro-2*H*-1-benzopyran derivatives, calculated at the optimum β-CD concentration, are given in Table 4. The very high efficiency (320 000–640 000 theoretical plates) of this method allows the separation of these enantiomers (e.g., $R_S = 3.57$ between the two enantiomers of 5-OH-DPAC) with even a small enantioselectivity ($\alpha = 1.018$), which is normally the limiting factor in chiral separations.

Under the optimum analytical conditions, the enantioseparation of each of the 3,4-dihydro-2*H*-1-benzopyran derivatives was achieved in less than 15 min (Fig. 5), with peak efficiencies one order or more higher than are usually encountered in HPLC.

3.3. Influence of phosphate–borate concentration

The effects of buffer ionic concentration on the migration time and chiral resolution of 5-OH-DPAC were evaluated with phosphate–borate buffer (pH 7.0) at the optimum β-CD concentration (6 m*M*). Table 5 shows the variation of migration times, peak efficiency and resolution when the buffer concentration varies from 10 up to 50 m*M*. As expected, an increasing ionic strength results in a longer migration time owing to a slower electroosmotic flow and a reduced electrophoretic mobility of the ionic solute. The migration time of 5-OH-DPAC increases from 8 to almost 12 min as the phosphate–borate buffer concentration increases from 10 to 50 m*M* (Fig. 6a–c). Working with a high ionic strength buffer (50 m*M*) generates a better resolution owing to a large improvement in peak efficiency whilst the enantioselectivity remains approximately constant ($\alpha = 1.014$), as shown in Fig. 6d and e. Using 50 m*M* rather than 10 m*M* phosphate–borate buffer, the electrophoretic parameters were 0.3%, 450% and 193% higher for enantioselectivity, efficiency and resolution, respectively. Since the solutions of 3,4-dihydro-2*H*-1-benzopyran derivatives were dissolved in 50 m*M* phosphate–borate buffer (pH 7.0), no stacking phenomenon occurred as the conductivity of the sample was significantly higher than that of the running buffer. Nevertheless, a buffer of higher ionic strength may perhaps promote the inclusion of the solute hydrophobic moiety into the interior cavity of the β-CD, and hence increase the efficiency.

Further studies are in progress to investigate the effect of the ionic strength of the buffer on the electrophoretic separation performance.

Table 5
Effect of phosphate–borate buffer concentration on the enantioseparation parameters of 5-OH-DPAC resolved by capillary electrophoresis

Buffer concentration (m*M*)	t_{m_R} (min)	t_{m_S} (min)	t_0 (min)	$m_{ep_R} \times 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)	$m_{ep_S} \times 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)	N_R	N_S	α	R_s
10	7.81	7.92	8.90	6.4	5.7	134 000	110 000	1.014	1.18
25	8.70	8.81	10.31	7.3	6.7	403 000	358 000	1.013	1.94
50	11.44	11.64	13.08	4.5	3.9	681 000	658 000	1.017	3.46

Buffer: phosphate–borate (pH 7.0)–6 m*M* β-CD–8 *M*; other conditions as in Table 1.

More information is required for a better understanding of the molecular interactions (hydrophobic or H-bonding types) which influence molecular chiral recognition.

3.4. Influence of buffer pH

The influence of buffer pH on the resolution of 5-OH-DPAC enantiomers was studied in the

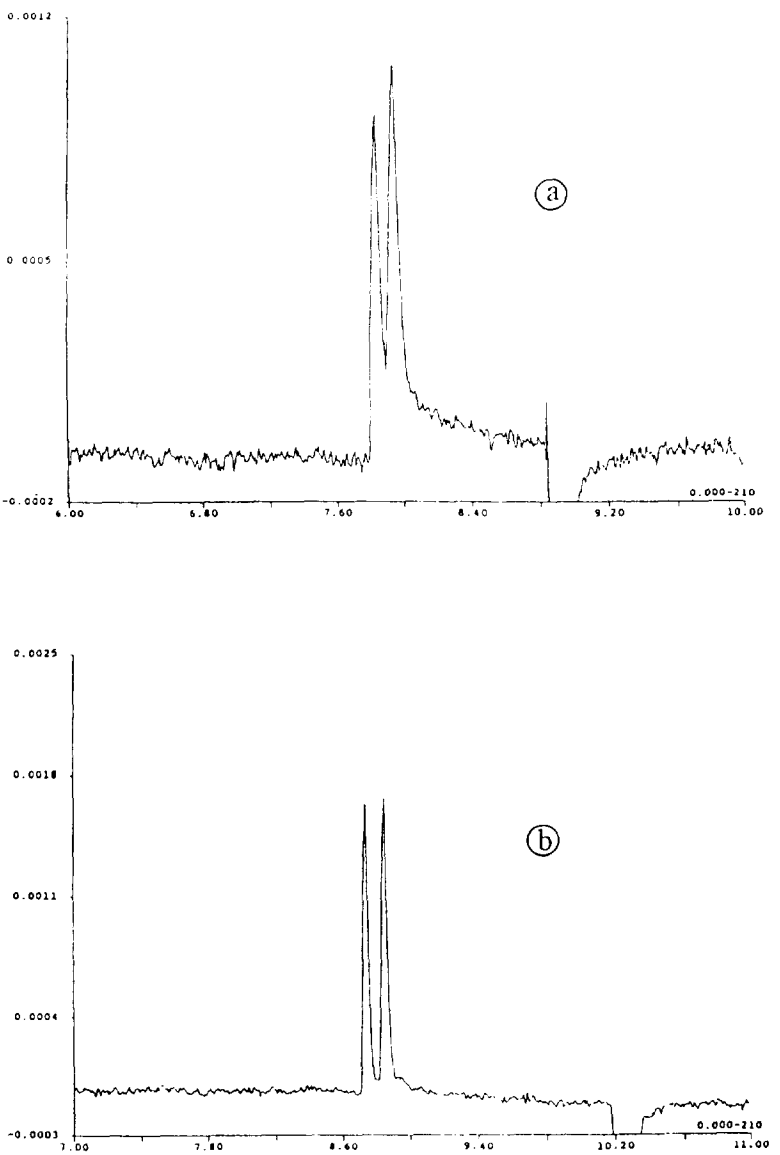

Fig. 6. Effect of phosphate-borate concentration [(a) 10, (b) 25 and (c) 50 m*M*] on enantioseparation of 5-OH-DPAC by CZE. Effect of phosphate–borate concentration on (d) efficiency and (e) resolution of 5-OH-DPAC by CZE. Fused-silica capillary column 70 cm × 50 μm I.D.; applied voltage, +18 kV; buffer, phosphate–borate (pH 7.0)–6 m*M* β-CD–8 *M* urea; detection at 210 nm; temperature, 25°C.

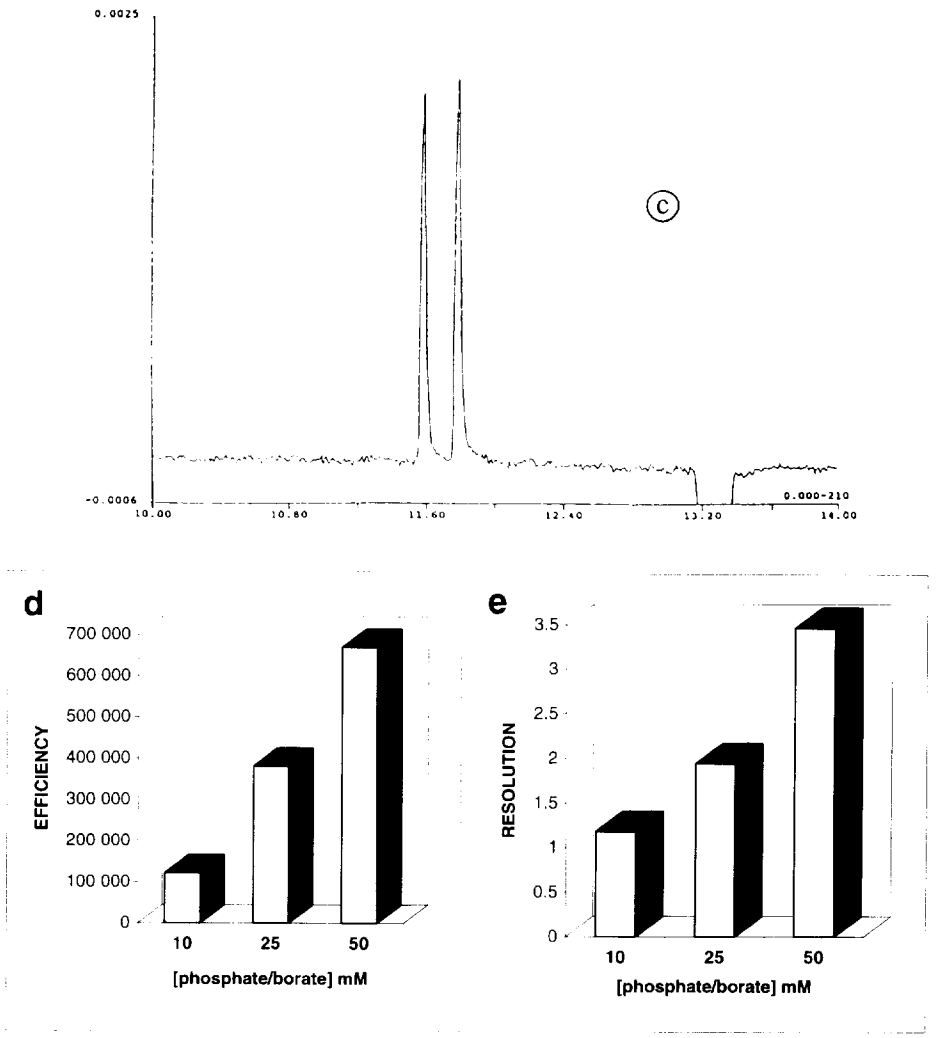

Fig. 6. (*continued*)

pH range 4.5–12, using 6 m*M* β-CD and 8 *M* urea dissolved in 50 m*M* phosphate–borate buffer (Fig. 7); two optimum resolution values were found at pH 7 and 11.75. Table 6 shows the experimental values for the separation parameters of 5-OH-DPAC using different buffer pH values. We observed migration order reversal of the *R*- and *S*-enantiomers on going from acidic to alkaline pH values (Table 6). Enantioseparation was achieved at either pH 5 or 7 when the two enantiomers were in their cationic form and migrated from the anode to the cathode (Fig. 8a and b), and also at pH 11.75 when the solutes were in their anionic form (Fig. 8d). Nevertheless, at pH 7, complete baseline separation of 5-OH-DPAC enantiomers was achieved in less than 15 min with a resolution of 4.02; the peak efficiencies were approximately 640 000 theoretical plates for these enatiomers and the enantioselectivity was found to be weak ($\alpha = 1.021$). Fig. 8c shows an unsuccessfull chiral separation at pH 9 due to the neutral form of this solute.

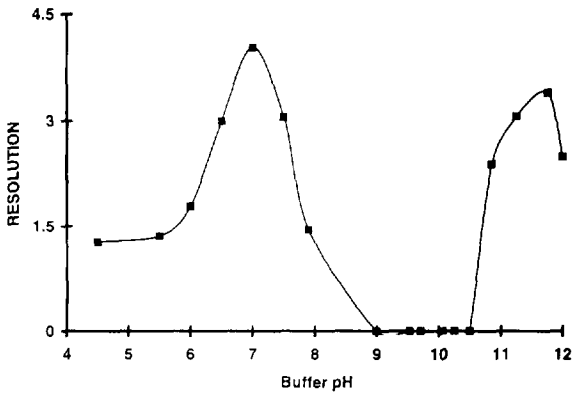

Fig. 7. Variation of the resolution with electrolyte pH during 5-OH-DPAC enantioseparation. Fused-silica capillary column, 70 cm × 50 μm I.D.; applied voltage, +18 kV; buffer, 50 m*M* phosphate–borate–6 m*M* β-CD–8 *M* urea; detection at: 210 nm; temperature, 25°C.

With an acidic background electrolyte, the efficiency for the first peak (*R*-enantiomer) was higher (6%) than that for the second peak (*S*-enantiomer), as expected. However, with the enantiomers in their anionic form, the efficiency for the *R*-enantiomer (second peak) is always higher (4%) than that for the *S*-enantiomer (first peak), owing to lower complexation, as reported previously [19].

4. Conclusion

Using β-CD as a chiral selector, capillary electrophoresis is an attractive method for the enantioseparation of drugs. The optimum separation conditions depend on the chiral selector concentration, the ionic strength and the pH of the buffer.

The β-CD inclusion complex stability constant of each solute enantiomer can be easily determined by capillary electrophoresis by following the variation of the enantiomer electrophoretic mobility versus the β-CD concentration over a wide range (1 μ*M*–100 m*M*). The theoretical model predicts that the optimum concentration for the chiral selector (C_{opt}) depends on the

Table 6
Effect of electrolyte pH on the enantioseparation parameters of 5-OH-DPAC resolved by capillary electrophoresis

pH	t_{m_R} (min)	t_{m_S} (min)	t_0 (min)	$m_{ep_R} \times 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)	$m_{ep_S} \times 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)	N_R	N_S	α	R_s
4.5	18.62	18.80	29.35	8.02	7.81	331 400	258 000	1.010	1.27
5.5	15.00	15.12	22.56	9.12	8.91	555 800	475 500	1.008	1.36
6.0	14.36	14.50	20.76	8.77	8.49	533 100	484 500	1.027	1.79
6.5	14.07	14.30	18.72	7.21	6.74	550 750	518 300	1.016	3.00
7.0	14.10	14.41	16.81	4.63	4.05	658 100	618 000	1.021	4.02
7.5	14.89	15.12	16.16	2.16	1.74	658 400	619 600	1.015	3.05
7.9	15.18	15.32	15.65	0.81	0.56			1.009	1.45
9.0	14.89	–	14.60	−0.54	–		–	1.000	0
9.5	14.81	–	14.46	−0.67	–		–	1.000	0
9.7	15.02	–	14.47	−1.03	–		–	1.000	0
pH	t_{m_S} (min)	t_{m_R} (min)	t_0 (min)	$m_{ep_S} \times 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)	$m_{ep_R} \times 10^5$ ($cm^2\ V^{-1}\ s^{-1}$)	N_S	N_R	α	R_s
10.1	15.71	15.82	14.87	−1.47	−1.65		–	1.007	–
10.3	16.38	16.49	15.26	−1.83	−2.00		–	1.007	–
10.9	18.88	19.24	16.35	−3.35	−3.75	545 400	568 000	1.019	2.38
11.3	19.36	19.68	16.79	−3.23	−3.57	526 800	559 250	1.017	3.06
11.8	22.00	22.44	17.98	−4.15	−4.51	451 400	470 500	1.020	3.39
12.0	25.72	26.14	18.79	−5.86	−6.11	385 400	416 250	1.016	2.49

Buffer: 50 m*M* phosphate–borate–6 m*M* β-CD–8 *M* urea; other conditions as in Table 1.

Fig. 8. Electrophrogram of 5-OH-DPAC enantiomers at different electrolyte pH values. Fused-silica capillary column, 70 cm × 50 μm I.D.; applied voltage, +18 kV; buffer, 50 m*M* phosphate–borate–6 m*M* β-CD–8 *M* urea; detection at 210 nm; temperature, 25°C. buffer pH: (a) 5; (b) 7; (c) 9; (d) 11.75.

stability constant of each enantiomer (K_R, K_S) as expressed by the relationship: $[C]_{opt} = 1/(K_R K_S)^{1/2}$. The experimentally determined C_{opt} value fits well with the calculated value from the previous equation in which we used experimental values for the inclusion complex stability constants. The high efficiencies (400 000–600 000 theoretical plates) of this method allowed the separation of these enantiomers ($R_S = 4.02$) when the enantioselectivity was small ($\alpha = 1.021$), which has been found to be the limiting factor in conventional chiral electrophoretic separations.

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