ELSEVIER

Journal of Chromatography A, 716 (1995) 183–196

JOURNAL OF CHROMATOGRAPHY A

Use of negatively charged sulfobutyl ether-β-cyclodextrin for enantiomeric separation by capillary electrophoresis

Claudia Desiderio, Salvatore Fanali*

Istituto de Cromatografia del CNR, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo (Rome), Italy

Abstract

A newly modified charged β-cyclodextrin (sulfobutyl ether-β-cyclodextrin) was investigated as a chiral selector in capillary electrophoresis in a study of the enantiomeric separation of a variety of underivatized anionic and cationic compounds of pharmaceutical interest and uncharged phenyl alcohols and dansyl-amino acids. Owing to the presence of four sulfonic groups, the chiral selector is negatively charged at all pH values used (2.5–9) and the complexation caused an increase in migration time for each compound studied. At a relatively low pH (2.5) the chiral selector could only be used at low concentration (0.1–0.5 mg/ml) for basic compounds, whereas at higher pH (6–9) the modified cyclodextrin in the concentration range 0–20 mg/ml was used. The concentration of the chiral selector, the distance from the aromatic group of the asymmetric centre of the analytes and the chemical composition and pH of the background electrolyte influenced the complexation, selectivity and resolution. Good enantiomeric separation was obtained for terbutaline at all pH values studied, whereas for other racemic compounds, warfarin, acenocoumarol, promethazine, bupivacaine and some dansyl-amino acids and phenyl alcohols , the pH range 6–9 was effective for optimizing the chiral resolution. Non-polar substituent groups on the asymmetric carbon of the analytes seem to enhance the complexation and the stereoselectivity.

1. Introduction

The analysis of chiral drugs is a very important field of application, especially in pharmaceutical science, because very often the two enantiomers possess different pharmacological and toxicological properties. Thus rapid, sensitive and high-resolution separation methods need to be optimized for chiral purity control of drugs, pharmacokinetic and/or medical studies, etc.

Analytical methods so far used for chiral separations include high-performance liquid chromatography (HPLC) [1–3], thin-layer chromatography (TLC) [4], gas chromatography (GC) [5–7] and, more recently, capillary electrophoresis (CE) [8–15].

Capillary electrophoresis, which has the above-mentioned characteristics, is becoming very popular for enantiomeric separations mainly using the direct separation method [14]. Among the different separation mechanisms and chiral selectors used in CE, inclusion complexation by cyclodextrin (CD) forming labile diastereoisomeric complexes during the run exhibited successful stereoselective effects, allowing the chiral separation of a wide number of compounds [16].

Among the native CD (α-, β- and γ-) used in CE for chiral resolution, β-CD proved to be the most versatile resolving agent, probably owing to its cavity dimensions, able to accommodate a

* Corresponding author.

SSDI 0021-9673(95)00550-1

wide range of analytes. Unfortunately, its use is limited owing to its relatively low solubility [1.8% (w/v) in aqueous buffer].

Primary and secondary hydroxy groups of the CDs can be modified chemically, leading to CDs with properties different from the native compounds, e.g., increased solubility, different conformation, different dimensions and additional stereoselective bondings.

Chargeable cyclodextrins, first introduced by Terabe [10], represent an interesting field of research in chiral separation by CE [13,17–21]. As previously investigated [19,20], the use of a chiral selector with its own mobility, opposite to that of the electrosmotic flow, showed a strong resolving power, also at very low concentration. Further, the ion-pairing interaction can be advantageously used for the inversion of migration of the two separated enantiomers [13,21].

The aim of this work was to investigate the utility of the negatively charged sulfobutyl ether-β-CD derivative (SBE-β-CD) on the enantiomeric separation by capillary electrophoresis of several basic and acidic compounds of pharmaceutical interest, including antihypertensive, anticoagulant, antihistaminic, anaesthetic and bronchodilator underivatized drugs and several uncharged phenyl alcohols. The effect of the concentration of the chiral selector added to the background electrolyte (BGE) and the effect of the pH of the BGE on the effective mobility, resolution and selectivity were examined.

2. Experimental

2.1. Chemicals and reagents

All solvents used were of HPLC or analytical-reagent grade and were purchased from Carlo Erba (Milan, Italy). Phosphoric acid (85%), sodium hydroxide and tris(hydroxymethyl-aminomethane) (Tris) were obtained from Carlo Erba and hydrochloric acid (30%) and borax (sodium tetraborate) from Merck (Darmstadt, Germany). Sulfobutyl ether-β-cyclodextrin was kindly provided by Perkin-Elmer (San Jose, CA, USA). Warfarin, promethazine, pindolol racemic standard compounds, D,L-2-phenyl-2-butanol (2-Ph-2B), (*S*)-(−)-1-phenyl-1-butanol (1-Ph-1B), (*R*)-(+)-1-phenyl-1-butanol, (*S*)-(+)-1-phenyl-1,2-ethanediol (1-Ph-1,2-diol), (*R*)-(−)-1-phenyl-1,2-ethanediol, (±)-1-phenyl-1-propanol (1-Ph-1P), (*S*)-(+)-2-methoxy-2-phenylethanol (2-Me-2-PhE), (*R*)-(−)-2-methoxy-2-phenylethanol and (±)-α-ethylphenethyl alcohol (α-Et-PhEt) were purchased from Aldrich (Steinheim, Germany) and (*R*)-(+)- and (*S*)-(−)-1-phenylethanol (1-PhEt) from Fluka (Buchs, Switzerland). Racemic acenocoumarol was purchased as a commercial pharmaceutical preparation. Terbutaline, propranolol, atenolol, oxprenolol, bupivacaine, metoprolol were obtained from Sigma (St. Louis, MO, USA). Stock standard solutions (10^{-3} *M*) of the standard compounds were prepared in methanol and then diluted using 10 m*M* of buffer (pH 6) in order to obtain 10^{-4} *M* working standard solutions for injection.

2.2. Instrumentation

Electrophoretic experiments were carried out using a Biofocus 3000 automated capillary electrophoresis apparatus (Bio-Rad Labs., Hercules, CA, USA), equipped with a multi-wavelength UV detector. The output detector wavelength was 206 nm. Electrophoretic runs were performed in uncoated fused-silica capillaries of 40 cm × 50 μm I.D. (35.5 cm effective length) (Polymicro Technologies, Phoenix, AZ, USA) and 50 cm × 50 μm I.D. (45.5 cm effective length) (Bio-Rad). The capillaries were positioned into a Bio-Rad user assembler cartridge after removing the polyimide layer (about 0.5 cm). Injections were made by the pressure method applying 5 p.s.i.·s at the anodic end of the capillary. A constant voltage of 15 kV was applied for the electrophoretic runs. In order to obtain good data reproducibility, washings between runs consisted of four purge cycles: water (50 s), followed by 0.1 *M* sodium hydroxide (50 s), then water (60 s) and BGE (60 s). Background electrolytes of pH 2.5 and 6 were prepared from concentrated phosphoric acid solution (85%, w/v) titrated with NaOH to the final pH value, the final concentration being 50 m*M*,

while 50 m*M* Tris–HCl and 50 m*M* sodium tetraborate–phosphoric acid buffers were used for experiments at pH 8 and 9, respectively.

Electrosmotic flow was measured by detecting for each sample injection the methanol peak as electroosmosis marker.

3. Results and discussion

3.1. Separation of basic and acidic compounds

Several basic compounds of pharmaceutical interest, namely pindolol, propranolol, oxprenolol, atenolol, metoprolol, promethazine, bupivacaine and terbutaline, were analysed by CE using phosphate buffer of pH 2.5. Under the operating conditions, all the analytes were positively charged and moved as a single peak to the cathode. For the study of enantiomeric separation, a new modified β-CD, sulfobutyl ether-β-CD, was added to the BGE.

As can be seen in Fig. 1, the sulfobutyl ether-β-CD possesses four modified hydroxyl groups at position 6 through an ether bond with a butyl chain and with a sulfonic group at the end. The presence of the sulfonic group makes the CD derivative negatively charged at any commonly used pH in CE and more soluble in aqueous buffer in comparison with the parent compound. Owing to its charge, the SBE-β-CD moved in the opposite direction to the analytes. Different amounts of the chiral selector, in the range 0.1–5 mg/ml, were added to the BGE at pH 2.5 and the racemic samples were injected separately for the electrophoretic runs. Among the basic drugs studied, only racemic terbutaline was resolved into its enantiomers. The enantiomeric separation of the other compounds was not successful because broad peaks were obtained with increasing concentration of SBE-β-CD. This effect was probably due to both strong electrostatic interactions between negatively charged CD–analytes and adsorption on the capillary wall. Further, electromigration dispersion and polydispersive effects arising from multiple species in the SBE-β-CD [22] should be taken into account. The tailing recorded for basic compounds confirmed previous findings [19], hence the use of CD concentrations higher than 0.5 mg/ml was not possible, hindering further studies.

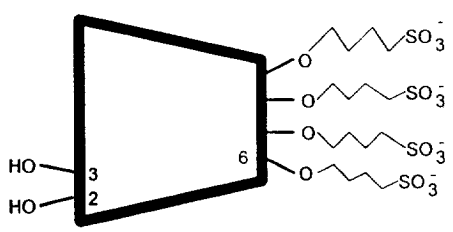

Fig. 1. Structure of sulfobutyl ether-β-cyclodextrin (SBE-β-CD).

Preliminary experiments using a phosphate buffer of pH 6 and containing different amounts of SBE-β-CD were successful for the enantiomeric separation of several compounds with good peak shapes even at concentrations of chiral selector higher than 0.5 mg/ml. Thus a phosphate buffer of pH 6 was selected for further investigations of both basic and acidic analytes (for their structures, see Fig. 2), the former moving to the detector by the electroosmotic flow. In this study, the concentration of SBE-β-CD added to the BGE was in the range 0–20 mg/ml.

Fig. 3a and b show the electropherograms of the separation of racemic terbutaline into its enantiomers at pH 2.5 and 6, respectively, with 0.2 and 1 mg/ml of SBE-β-CD, respectively. Good enantiomeric resolution was achieved under both experimental conditions, but with a noticeable decrease in migration time at pH 6.

The effective mobility, resolution factor and selectivity were calculated using the following equations:

$$\mu_a = \mu_e + \mu_{eof} \quad (1)$$

$$R = 2 \cdot \frac{t_2 - t_1}{w_2 + w_1} \quad (2)$$

$$S = \frac{\Delta\mu}{\mu_m} \quad (3)$$

where μ, R and S represent the mobility, resolution factor and selectivity, respectively, a stands for apparent, e for effective and eof indicates the electroosmotic flow; $\Delta\mu$ and μ_m are

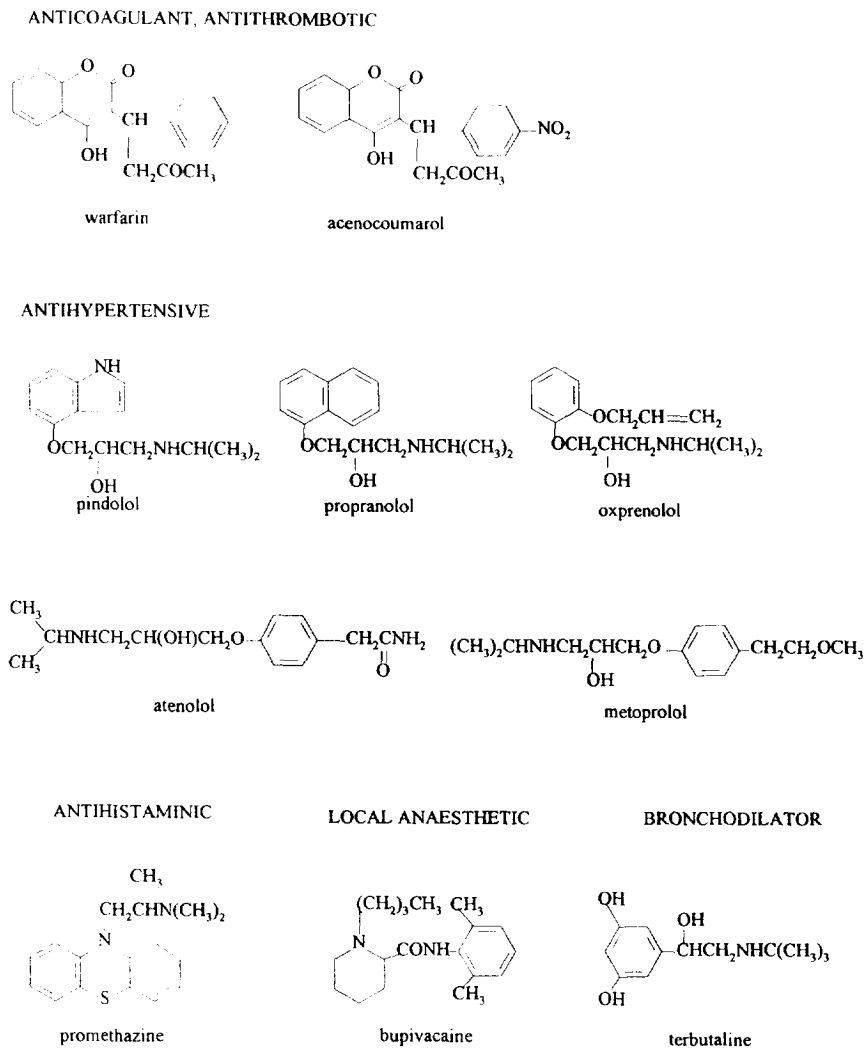

Fig. 2. Structures of the studied compounds of pharmaceutical interest.

the difference in effective mobilities of the two enantiomers and their media, respectively.

The migration times of the analytes were influenced by the concentration of modified CD; a general increase in migration time with increasing CD concentration was recorded owing to the complexation effect towards the analytes.

As can be seen in Fig. 4, both the analytes and chiral selector were carried towards the detector by the electroosmotic flow; the negatively charged CD forming diastereoisomeric complexes caused a retardation of all compounds studied. The apparent mobility (μ_a) for basic and acidic analytes is a combination of the mobility of free and complexed analyte and of the electroosmotic flow. In the case of cationic compounds, the effective mobility (μ_e) was positive and in some instances dropped to a negative value when the concentration of SBE-β-CD was increased. For acidic analytes the sign of the mobility was always negative, i.e., they were migrating behind the electroosmotic flow.

Fig. 5 shows the effect of the concentration of SBE-β-CD, added to the BGE at pH 6, on the effective mobility of the studied underivatized drugs. Owing to the strong complexation, inver-

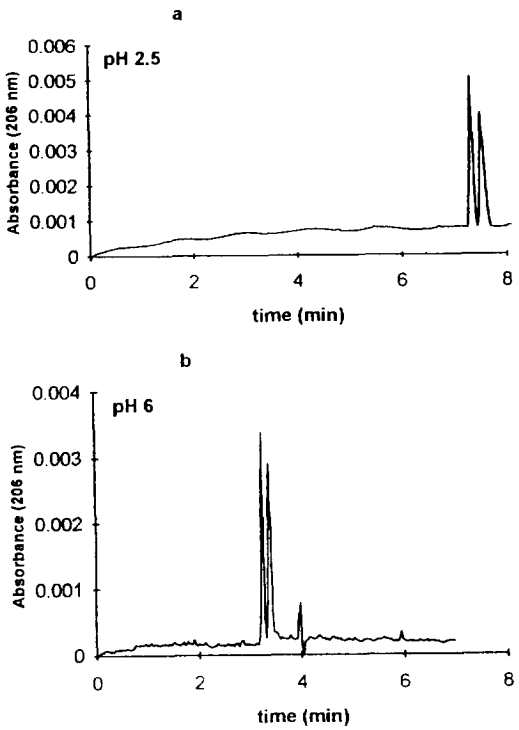

Fig. 3. Electropherograms of the enantiomeric separation of racemic terbutaline. Capillary, 40 (35.5) cm × 0.05 mm I.D. (uncoated). The concentration of SBE-β-CD was 0.2 mg/ml and 1 mg/ml dissolved in 50 m*M* phosphate buffer at pH (a) 2.5 and (b) 6.0, respectively. The concentration of terbutaline was 10^{-4} *M* injected by pressure at 5 p.s.i.·s. Applied voltage, 15 kV.

sion of mobility was obtained for promethazine at 1 mg/ml, propranolol at 3 mg/ml, metoprolol at 6 mg/ml, pindolol and terbutaline at 10 mg/

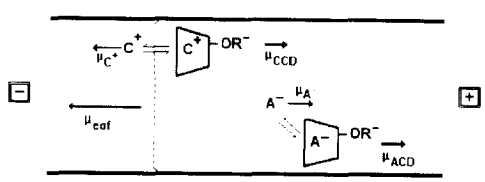

Fig. 4. Electrophoretic separation mechanism of cationic and anionic analytes using a negatively charged cyclodextrin.

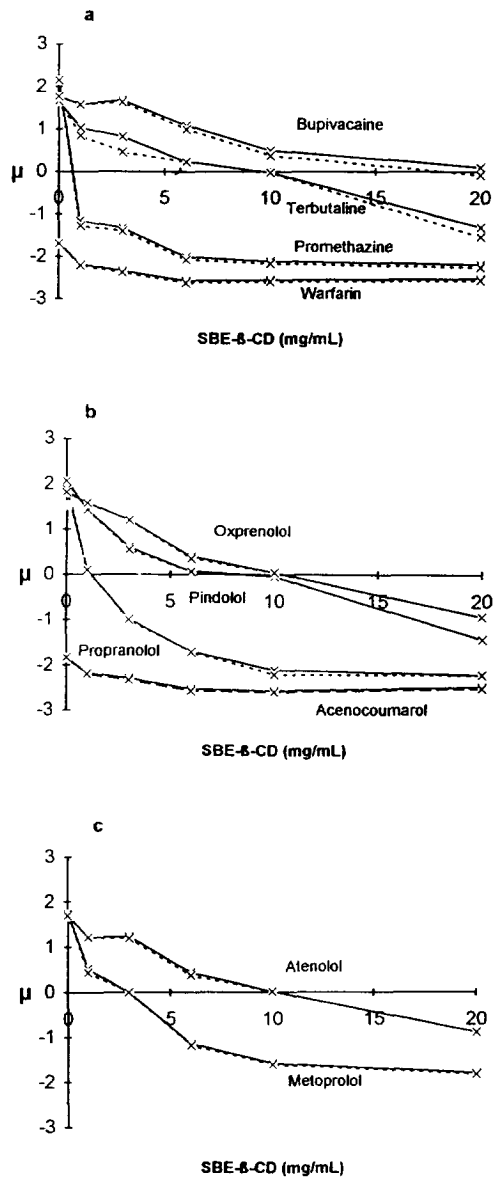

Fig. 5. Effect of the concentration of sulfobutyl ether-β-cyclodextrin (SBE-β-CD) on the effective mobility of the studied compounds of pharmaceutical interest. Background electrolyte, 50 m*M* phosphate buffer (pH 6) containing different concentrations of chiral selector; capillary (uncoated), 50 cm × 0.05 mm I.D. For other experimental conditions, see Table 1.

ml and oxprenolol, bupivacaine and atenolol at 20 mg/ml. Hence the complexation order, based on the decrease or increase in μ_e for cationic and anionic drugs, respectively, was estimated to be

promethazine > propranolol > metoprolol > terbutaline = pindolol > bupivacaine > oxprenolol > atenolol > warfarin = acenocoumarol.

Table 1 gives the calculated resolution factor and selectivity when the analytes were run at pH 6 and the BGE contained increasing concentrations of chiral selector.

Enantiomeric resolution was achieved for warfarin, acenocumarol, promethazine, metoprolol and terbutaline when the lowest concentration of chiral selector was used (1 mg/ml). At this concentration baseline resolution was obtained only for the former compound (terbutaline), while 3 mg/ml of SBE-β-CD were necessary for acenocourmarol and warfarin, 6 mg/ml for promethazine and 10 mg/ml for bupivacaine. Poor enantiomeric resolution was obtained for metoprolol, oxprenolol, atenolol, pindolol and propranolol ($R < 0.5$).

Relatively high selectivity was recorded for terbutaline, metoprolol and promethazine when 1 mg/ml of SBE-β-CD was added to the BGE, whereas for oxprenolol and atenolol a higher concentration of modified CD was necessary in order to obtain comparable results. The increase in the concentration of the chiral selector caused a decrease in selectivity for promethazine, terbutaline and metoprolol; for the other analytes the reverse occurred except for warfarin and acenocoumarol, which showed a maximum of S at 3 mg/ml of CD.

These results indicate the importance of the concentration of the chiral selector for the optimization of the baseline enantiomeric separation of racemic warfarin, acenocoumarol, promethazine and bupivacaine using different concentrations of SBE-β-CD.

Fig. 6 shows the electropherograms of the enantiomeric separation of racemic warfarin, acenocoumarol, promethazine and bupivacaine using the BGE at pH 6 with SBE-β-CD.

In order to study the dependence of the effective mobilities and resolution on pH, phosphate buffer (pH 6), Tris–HCl buffer (pH 8) and borate–phosphate buffer (pH 9) containing 20 mg/ml SBE-β-CD were used. On increasing the buffer pH from 6 to 9, in the absence of a chiral agent, almost all the investigated drugs still exhibited their cationic or anionic behaviour with lower mobilities, close to the electroosmotic flow in same cases, probably in accord with their pK_a values. Electroosmotic flow (EOF) measurement, using methanol as EOF marker, revealed

Table 1
Effect of the concentration of sulfobutyl ether-β-cyclodextrin (SBE-β-CD) on the resolution (R) and selectivity (S) of racemic compounds of pharmaceutical interest (μ_e: 10^{-4} cm^2 V^{-1} s^{-1})

Compound	SBE-β-cyclodextrin (mg/ml)									
	1		3		6		10		20	
	R	S	R	S	R	S	R	S	R	S
(1) Warfarin	<0.5	0.009	1.09	0.017	1.35	0.015	1.69	0.016	2.11	0.016
(2) Acenocoumarol	0.63	0.009	1.09	0.017	1.40	0.016	1.59	0.012	2.13	0.012
(3) Promethazine	<0.5	0.098	0.78	0.051	1.28	0.034	1.45	0.028	2.00	0.027
(4) Bupivacaine	–	–	<0.5	0.030	1.0	0.086	1.36	0.273	–	–
(5) Terbutaline	1.56	0.191	nm	nm	nm	nm	nm	nm	5.10	0.161
(6) Pindolol	–	–	<0.5	0.087	–	–	–	–	–	–
(7) Metoprolol	<0.5	0.189	–	–	<0.5	0.026	<0.5	0.019	<0.5	0.011
(8) Propranolol	–	–	<0.5	0.020	<0.5	0.006	–	–	–	–
(9) Oxprenolol	–	–	–	–	<0.5	0.108	–	–	–	–
(10) Atenolol	–	–	<0.5	0.033	<0.5	0.150	–	–	–	–

Capillary, 50 (45.5) cm × 0.05 mm I.D. (uncoated); background electrolyte, 50 mM phosphate buffer (pH 6) supplemented with the appropriate concentration of chiral selector; applied voltage, 15 kV (constant); injection, pressure 5 p.s.i. · s of 10^{-4} M racemic compounds.

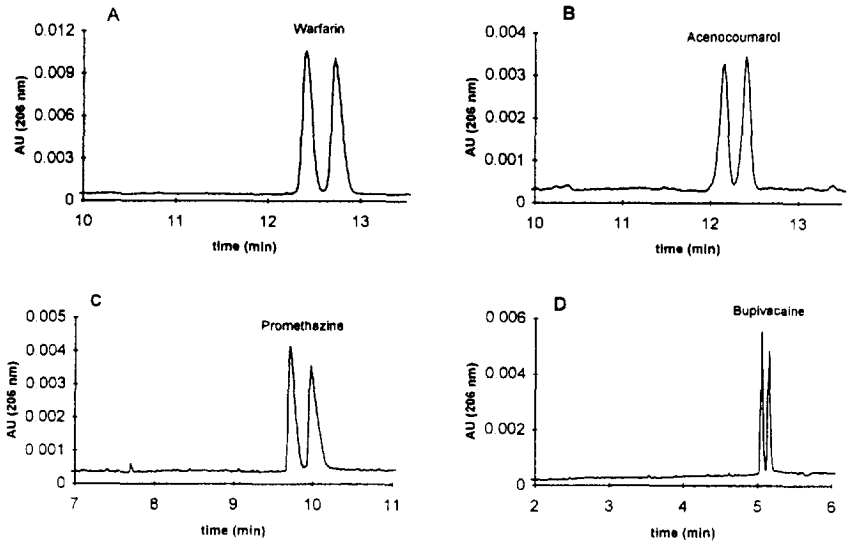

Fig. 6. Electropherograms of the baseline enantiomeric separation of racemic warfarin, acenocoumarol, promethazine and bupivacaine. Background electrolyte. 50 m*M* phosphate buffer (pH 6) containing SBE-β-CD (A, B and C, 6 mg/ml; D, 10 mg/ml); capillary, 50 (45.5) cm × 0.05 mm I.D. For other experimental conditions, see Table 1.

an increase in μ_{eof} from $5 \cdot 10^{-4}$ to $7 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. In the presence of the chiral agent, the electroosmosis increased slowly, from $3.98 \cdot 10^{-4}$ to $5.27 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}.

Table 2 gives the effective mobilities, resolution and selectivity of the drugs at different pH values.

An increase in the pH of the BGE did not influence the enantiomeric resolution of propranolol, bupivacaine and oxprenolol (no resolution at any pH using 20 mg/ml SBE-β-CD). For the other compounds studied a general decrease in R on increasing the pH was recorded, except for atenolol and pindolol, which showed resolution only at pH 9.

From the effective mobilities, we can conclude that at pH 6 the complexation is generally higher than at other pH values (the absolute value of μ_e generally decreased with increase in pH); terbutaline exhibited the highest value at pH 8.

Under the operating experimental conditions, only terbutaline showed a relatively high value of selectivity ($S = 0.161$ at pH 6) that decreased with increase in pH.

The use of a BGE at pH 9 (with 20 mg/ml of chiral selector) but with different chemical composition (Tris–HCl instead of borate–phosphate) was less effective for chiral recognition for all the racemic analytes studied. To explain the different behaviour of the SBE-β-CD using the two buffers we have to consider that the electroosmotic flow with Tris electrolyte was higher than with borate ($5.27 \cdot 10^{-4}$ and $4.02 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}, respectively) and consequently lower migration times were recorded. Comparing the effective mobilities using the two BGEs (results not shown), the different chemical composition caused an increase in absolute effective mobility, using borate buffer, for warfarin, acenocoumarol and bupivacaine, whereas for other analytes a decrease was observed. The better results obtained with borate are probably due to the effect of the counter ion on the selective stability of the inclusion complex and to the longer time spent by the two enantiomers in the CD cavity during the electrophoretic process.

3.2. Enantiomeric separation of uncharged compounds

Several phenyl alcohol derivatives (for their structures, see Fig. 7) were selected as un-

Table 2
Effective mobilities, resolutions and selectivities obtained for pharmaceutical compounds at different pH using 20 mg/ml SBE-β-CD as chiral selector

Compound	pH					
	6		8		9	
	μ_1 R	μ_2 S	μ_1 R	μ_2 S	μ_1 R	μ_2 S
(1) Warfarin	−2.23	−2.57	−2.18	−2.01	−2.17	−2.19
	2.11	0.016	1.00	0.037	1.12	0.009
(2) Acenocoumarol	−2.48	−2.51	−2.34	−2.37	−2.22	−2.25
	2.13	0.012	1.4	0.013	1.52	0.013
(3) Promethazine	−2.20	−2.36	−2.06	−2.12	−2.04	−2.07
	2.00	0.027	1.7	0.029	1.78	0.015
(4) Bupivacaine	0.11	−0.08	−1.06	–	−1.10	–
	nm	nm	–	–	–	–
(5) Terbutaline	−1.31	−1.54	−1.35	−1.56	−1.05	−1.21
	5.10	0.161	5.40	0.144	5.00	0.142
(6) Pindolol	−1.43	–	−1.28	–	−1.11	−1.13
	–	–	–	–	0.90	0.018
(7) Metoprolol	−1.80	−1.82	−1.75	−1.77	−1.50	–
	<0.5	0.011	<0.5	0.011	–	–
(8) Propranolol	−2.21	–	−2.17	–	−1.81	–
	–	–	–	–	–	–
(9) Oxprenolol	−0.93	–	−1.08	–	−0.62	–
	–	–	–	–	–	–
(10) Atenolol	−0.89	–	−0.86	–	−0.78	−0.82
	–	–	–	–	<0.5	0.050
EOF	3.98		5.27		4.02	

Applied voltage 15 kV. For other experimental conditions, see text and Table 1. μ: 10^{-4} cm^2 V^{-1} s^{-1}.

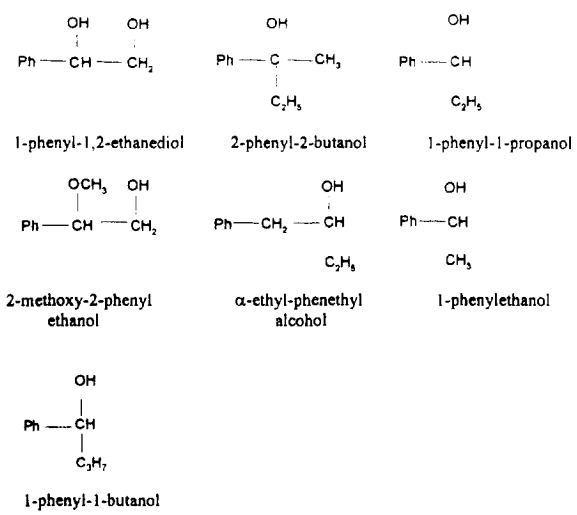

Fig. 7. Structures of the uncharged phenyl alcohols studied.

charged samples in order to study the effect of SBE-β-CD on complexation and enantiomeric separation. The racemic mixtures were separately injected for the electrophoresis using a BGE of pH 6, where they were carried to the detector by the relatively strong electroosmotic flow.

The addition of SBE-β-CD to the BGE caused a general increase in migration time for all the analytes studied owing to both complexation and a decrease in the electroosmotic flow.

Table 3 shows the effect of the concentration of SBE-β-CD in the BGE at pH 6 on the effective mobility, resolution and selectivity of the studied uncharged compounds.

An increased concentration of the chiral selector caused a reduction in the electroosmotic flow, probably due to an increase in the viscosity

Table 3
Effect of the concentration of SBE-β-CD on the effective mobility (expressed as $\mu_e \times 10^{-4}$ cm^2 V^{-1} s^{-1}), resolution (R) and selectivity (S) of uncharged racemic phenyl alcohols

Compound	SBE-β-CD (mg/ml)									
	1		2		6		10		20	
	μ_1 R	μ_2 S	μ_1 R	μ_2 S	μ_1 R	μ_2 S	μ_1 R	μ_2 S	μ_1 R	μ_2 S
2-Phenyl-2-butanol	−0.89	–	−1.60	−1.70	−1.65	−1.73	−1.19	−1.26	−1.92	−1.96
	–	–	<0.5	0.061	1.30	0.047	1.35	0.057	1.40	0.051
1-Phenylethanol	−0.46	–	0.79	–	−0.66	–	−0.11	–	−0.28	–
	–	–	–	–	–	–	–	–	–	–
α-Ethylphenethyl alcohol	−0.74	−0.79	−1.06	–	−1.74	–	−1.72	−1.74	−1.94	−1.95
	<0.5	0.065	–	–	–	–	<0.5	0.012	0.65	0.005
1-Phenyl-1-butanol	−0.72	–	−1.28	–	−1.40	−1.45	−0.90	−0.94	−0.83	−0.86
	–	–	–	–	<0.5	0.035	<0.5	0.043	0.79	0.035
1-Phneyl-1,2-ethanediol	−0.35	–	−0.71	–	−0.39	–	−0.11	–	−0.86	−0.93
	–	–	–	–	–	–	–	–	<0.5	0.078
1-Phenyl-1-propanol	−0.30	–	−0.43	–	−1.16	–	−1.17	−1.22	−1.79	−1.82
	–	–	–	–	–	–	1.09	0.041	1.09	0.017
2-Methoxy-2-phenylethanol	−0.38	–	−0.47	–	−1.42	–	−1.35	–	−1.78	−1.80
	–	–	–	–	–	–	–	–	<0.5	0.010
EOF	4.32		4.52		3.76		3.72		3.11	

Capillary, 40 (35.5) cm × 0.05 mm I.D. (uncoated); background electrolyte, 50 mM phosphate buffer (pH 6) with the appropriate concentration of chiral selector. The concentration of injected racemic compounds was 10^{-4} M. For other experimental conditions, see Table 1. μ: 10^{-4} cm^2 V^{-1} s^{-1}.

of the BGE. Enantiomeric separation was achieved for all the compounds studied except for 1-phenylethanol and the resolution was strongly influenced by the concentration of the modified cyclodextrin. Baseline separation was obtained only for the two enantiomers of 2-phenyl-2-butanol and 1-phenyl-1-propanol when 6 and 20 mg/ml SBE-β-CD were used, respectively, while relatively good resolution was obtained for 1-phenyl-1-butanol ($R = 0.8$) and α-ethylphenethyl alcohol ($R = 0.65$) with 20 mg/ml of chiral selector. 1-Phenyl-1,2-ethanediol and 2-methoxy-2-phenylethanol were poorly resolved with 20 mg/ml of modified CD.

The enantiomeric resolution was generally influenced by the concentration of SBE-β-CD in the BGE and the maximum value was obtained at 20 mg/ml for all the compounds studied.

The chiral resolution capability of SBE-β-CD towards the compounds studied was found to be 2-Ph-2B > 1-Ph-1P > 1 − Ph-1B > α-Et-PhEt > 1-Ph-1,2-diol = 2-Me-2-PhE. The higher enantiomeric separation obtained for 2-Ph-2B and 1-Ph-1P in comparison with the other uncharged analytes is due to the different substituent groups on the asymmetric carbon, responsible for the stereoselective interactions with the chiral selector, causing the formation of stereoisomers with different stability constants. The presence of one ethyl substituent plays a very important role in the stereoselectivity (2-Ph-2B and 1-Ph-1P). In fact, when this group was replaced with a methyl or propyl group (1-PhE and 1-Ph-1B), the resolution was completely lost or decreased, respectively. The explanation for such behaviour is not easy to find without other studies, e.g., NMR spectroscopy, too difficult to perform mainly owing to the nature of the chiral selector (traces of other substituted CD are present). Anyway, we can consider that the C_2H_5 group can either be accommodated in the CD cavity and/or bind with the butyl spacer of the CD. The former could be considered when a comparison is made with a methyl group (less hydrophobic than an ethyl group), but not with the propyl, in which case we could expect an improvement in resolution. This assumption is also supported by the lower resolution of 1-Ph-1,2-diol and 2-Me-2-PhE where the CH_2OH group (less hydrophobic than C_2H_5) was present. Finally, the position of

the asymmetric centre influenced the enantiomeric separation; in fact, in the case of α-Et-PhE, the resolution was lower than for 2-Ph-2B, 1-Ph-1P and 1-Ph-1B but higher than for 1-Ph-1,2-diol and 2-Me-2-PhE.

Three buffer systems of pH 6, 8 and 9, supplemented with 20 mg/ml of chiral selector, were tested in order to study the effect of pH on the enantiomeric resolution of 2-phenyl-2-butanol, 1-phenylethanol, α-ethyl-phenethyl alcohol, 1-phenyl-1-butanol and 1-phenyl-1,2-ethanediol. Table 4 gives the resolutions and selectivities obtained at different pH values. The data shown here at pH 6 are not the same as those in Table 3 because a longer capillary (50 cm) was used.

An increase in pH led to an increase in the electroosmotic flow. The resolution increased with increase in the pH of the BGE for 2-Ph-2B and 1-Ph-1,2-diol whereas it decreased for the other compounds. 1-Phenylethanol was poorly resolved at pH 8 ($R < 0.5$).

The migration order was verified by spiking the racemic mixtures with the separate enantiomers commercially available for 1-phenyl-1-butanol and 1-phenyl-1,2-ethanediol. In both cases the (+)-antipodes moved with shorter migration times than the (−)-antipodes, indicating that the former was less complexed by the SBE-β-CD. As an example, Fig. 8 shows the electropherograms for the enantiomeric separation of some phenyl alcohols using a BGE of pH 9 and containing 20 mg/ml of chiral selector.

3.3. Enantiomeric separation of dansyl-amino acids

The electrophoretic separation of several amino acid derivatives, namely Phe, Glu, Val, nor-Val, Leu, nor-Leu, Thr, Trp, Ser, Asp and Met, in a BGE of pH 8 and in the absence of SBE-β-CD revealed that all these compounds moved behind the electroosmotic flow, clearly exhibiting a negative charge.

The addition of SBE-β-CD to the BGE caused an increase in migration times for all the compounds studied owing to the complexing effect of the chiral additive. Satisfactory enantiomeric resolution was obtained for Leu, nor-Leu, Phe, Met and Trp, whereas Asp, Thr and Val were poorly resolved ($R < 0.5$) but Glu not at all.

As can be seen in Table 5, the absolute effective mobility and resolution increased on

Table 4
Effect of the pH of the background electrolyte on the effective mobility, resolution and selectivity of phenyl alcohols

Compounds	pH					
	6		8		9	
	μ_1 R	μ_2 S	μ_1 R	μ_2 S	μ_1 R	μ_2 S
2-Phenyl-2-butanol	−1.93	−1.99	−2.05	−2.10	−1.92	−1.96
	1.94	0.031	1.18	0.024	2.12	0.021
1-Phenylethanol	−1.30	–	−1.31	−1.33	−1.24	–
	–	–	<0.5	0.015	–	–
α-Ethylphenethyl alcohol	−2.65	−2.67	−2.09	−2.10	−1.92	−1.93
	0.76	0.007	<0.5	0.005	<0.5	0.005
1-Phenyl-1-butanol	−2.37	−2.41	−1.94	−1.98	−1.85	−1.89
	1.47	0.017	1.30	0.021	1.28	0.021
1-Phenyl-1,2-ethanediol	−1.18	–	−0.91	–	−1.21	−1.26
	–	–	–	–	1.35	0.040
EOF	3.94		4.33		3.97	

Capillary, 50 (45.5) cm × 0.05 mm I.D. (uncoated); applied voltage, 15 kV (constant). μ: 10^{-4} cm^2 V^{-1} s^{-1}.

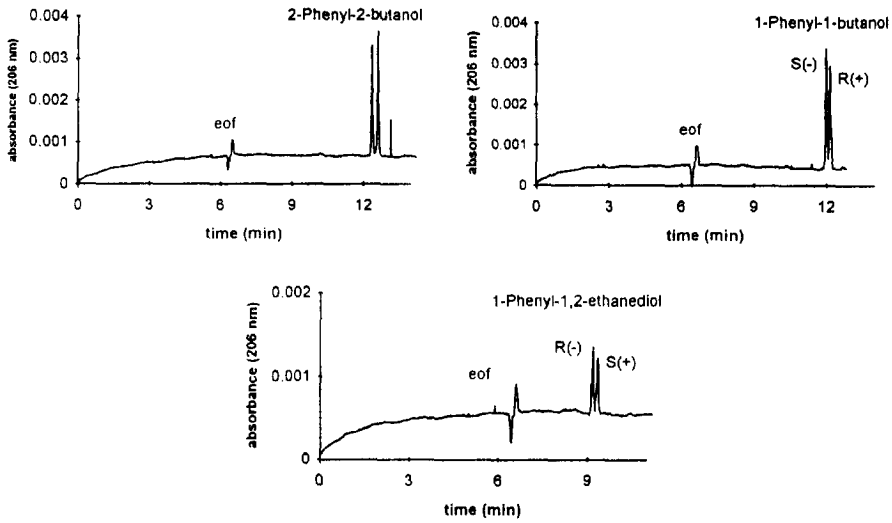

Fig. 8. Electropherograms of the enantiomeric separation of 2-phenyl-2-butanol, 1-phenyl-1-butanol and 1-phenyl-1,2-ethanediol. Background electrolyte, 50 m*M* borate buffer (pH 9) containing 20 mg/ml of SBE-β-CD; capillary, 50 (45.5) cm × 0.05 mm I.D.; applied voltage, 15 kV, 63 μA; injection, 5 p.s.i.·s of 10^{-4} *M* racemic standard.

increasing the concentration of the chiral selector added to the BGE. In some cases an increase in μ_e was observed up to certain concentration of SBE-β-CD and then a decrease (Asp, Thr, Leu), clearly showing a lower complexing effect of the CD in comparison with the other analytes.

The maximum resolution was obtained using 20 mg/ml of modified cyclodextrin for the separated enantiomers, except for Thr, which was poorly resolved only at 10 mg/ml. The resolution order at 20 mg/ml of chiral selector was found to be nor-Leu > Phe > Leu > Met > nor-Val > Trp > Val = Ser = Asp.

Our results indicate that the chiral recognition, also in the case of dansyl-amino acids, is influenced by both the concentration of the chiral selector and the nature of the chain bound to the asymmetric carbon of the amino acid derivative. In fact, amino acids with non-polar chains were better resolved than the others. The migration order was verified by spiking the racemic analytes with their pure L-enantiomer and in all cases the D-enantiomer moved faster than the L-isomer, clearly indicating that the former was the most complexed analyte.

As an example, Fig. 9 shows the enantiomeric separation of several racemic dansyl-amino acids using a BGE of pH 8 and containing different concentrations of chiral selector.

Experiments performed at pH 6 and 9 revealed that a lower pH (6) allowed higher resolution to be obtained for Leu, Trp, Val, nor-Val and Met, whereas at pH 9 the resolution generally decreased. For Asp a change in the pH of the BGE caused no noticeable modification of resolution.

Although the SBE-β-CD was effective for the enantiomeric separation of most of the amino acid derivatives studied, it was not able to separate the racemic mixtures from one another, and further studies are necessary in order to solve this problem, e.g., using surfactant additives to the chiral BGE.

4. Conclusions

Capillary zone electrophoresis using a new chiral selector, sulfobutyl ether-β-cyclodextrin, shows great promise for chiral separations of several classes of enantiomers. The complexation, resolution and selectivity are influenced by

Table 5
Effect of the concentration of SBE-β-cyclodextrin on effective mobility (μ), resolution (R) and selectivity (S) of several dansyl-amino acids

Dansyl-amino acids	SBE-β-CD (mg/ml)											
	0		1		3		6		10		20	
	μ_2 R	μ_1 S	μ_2 R	μ_1 S	μ_2 R	μ_1 S	μ_2 R	μ_1 S	μ_2 R	μ_1 S	μ_2 R	μ_1 S
Phe	−2.05	–	−2.01	−2.03	−2.10	−2.17	−2.29	−2.37	−2.23	−2.36	−2.47	−2.56
	–	–	<0.5	0.010	1.39	0.033	1.78	0.034	3,84	0.057	3.89	0.036
Trp	−1.69	–	−1.90	–	−1.96	–	−2.12	−2.16	−2.11	−2.15	−2.36	−2.40
	–	–	–	–	–	–	0.92	0.019	1.25	0.019	1.28	0.017
Met	−1.73	–	−1.94	–	−1.93	–	−2.02	−2.05	−2.03	−2.07	−2.29	−2.36
	–	–	–	–	–	–	0.72	0.015	1.45	0.019	1.87	0.030
Asp	−3.33	–	−3.38	–	−3.26	–	−3.27	–	−3.19	–	−3.09	−3.10
	–	–	–	–	–	–	–	–	–	–	–<0.5	0.003
Glu	−3.24	–	−3.16	–	−3.14	–	−3.18	–	−3.10	–	−2.79	–
	–	–	–	–	–	–	–	–	–	–	–	–
Val	−1.82	–	−2.04	–	−1.96	–	−2.11	–	−2.16	–	−2.36	−2.37
	–	–	–	–	–	–	–	–	–	–	<0.5	0.004
nor-Val	−1.90	–	−1.96	–	−1.98	–	−2.17	−2.22	−2.06	−2.11	−1.32	−2.37
	–	–	–	–	–	–	0.93	0.023	1.70	0.024	1.69	0.021
Leu	−1.68	–	−1.92	–	−1.96	−2.03	−2.20	−2.28	−2.42	−2.53	−2.21	−2.30
	–	–	–	–	1.00	0.035	1.40	0.036	2.5	0.044	3.54	0.040
nor-Leu	−1.81	–	−2.25	–	−1.98	−2.03	−2.20	−2.29	−2.18	−2.25	−2.45	−2.53
	–	–	–	–	0.64	0.025	1.79	0.040	2.50	0.020	4.28	0.032
Ser	−2.00	–	−2.14	–	−2.00	–	−2.07	–	−2.03	–	−2.24	−2.25
	–	–	–	–	–	–	–	–	–	–	<0.5	0.004
Thr	−1.73	–	−1.93	–	−1.89	–	−2.08	–	−2.24	−2.25	−1.97	–
	–	–	–	–	–	–	–	–	<0.5	0.004	–	–
EOF	5.54		5.24		4.94	4.91	–	–	4.60		4.26	

Conditions: capillary, 50 (45.5) cm × 0.05 mm I.D.; background electrolyte, 50 mM phosphate buffer (pH 8) with the appropriate concentration of modified cyclodextrin; applied voltage, 18 kV; injection, 5 p.s.i. · s of 10^{-4} M racemic mixtures. μ: 10^{-4} cm^2 V^{-1} s^{-1}.

the amount of SBE-β-CD added to the background electrolyte, and the pH of the BGE and the substituent groups on the asymmetric carbon of the analytes. The modified CD being negatively charged, ion-pairing interactions have to be considered in order to optimize the electrophoretic separation mechanism when basic analytes have to be separated into their enantiomers. Owing to this effect, basic compounds exhibited strong complexation and in several instances the mobility was reversed. This effect can be advantageously used in order to reverse the migration order, which is important when a minor component has to be determined in an enantiomeric mixture. However, the coulombic interaction between the SBE-β-CD seems not to be fundamental for the chiral recognition, as demonstrated by the good chiral resolution obtained when negatively charged compounds were studied (warfarin and acenocoumarol). The composition of the chiral BGE has to be chosen so as to avoid a pH and concentration of SBE-β-CD such that the mobility of the two enantiomers is close to the electroosmotic flow in order

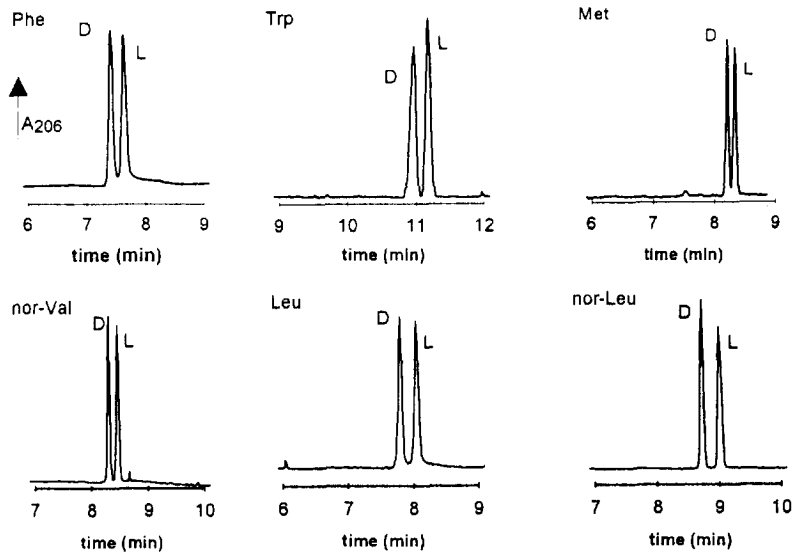

Fig. 9. Electropherograms of the enantiomeric separation of dansyl-amino acids using a BGE of pH 8 (50 m*M* Tris–HCl) and containing different concentrations of SBE-β-CD (Phe, 3 mg/ml; Leu, 6 mg/ml; nor-Leu, nor-Val and Met, 10 mg/ml; and Trp, 20 mg/ml). Capillary, 50 cm × 0.05 mm I.D.; applied voltage, 18 kV; injection, 5 p.s.i.·s of 10^{-4} *M* racemic compounds.

to select the optimum experimental conditions. The CE method is easy and not expensive to perform in comparison with other analytical methods where expensive columns are needed, e.g., HPLC; in fact, the volume of the capillary is only of the order of nanolitres or microlitres.

Table 6
Effect of the pH of the BGE on resolution (*R*) and selectivity (*S*) of enantiomeric separation of dansyl-amino acids

Dansyl-amino acids	pH					
	6		8		9	
	R	*S*	*R*	*S*	*R*	*S*
Phe	4.00	0.035	3.89	0.036	3.44	0.026
Trp	1.89	0.016	1.28	0.017	1.00	0.014
Met	2.96	0.017	1.87	0.030	1.82	0.019
Asp	<0.50	0.003	<0.50	0.003	0.56	0.004
Glu	–	–	–	–	–	–
Val	0.95	0.012	<0.50	0.004	<0.50	0.005
nor-Val	2.71	0.035	1.69	0.021	1.76	0.023
Leu	4.37	0.035	3.54	0.040	2.75	0.032
nor-Leu	3.68	0.032	4.28	0.032	2.27	0.028
Ser	<0.50	0.004	<0.50	0.004	–	–
Thr	–	–	–	–	–	–

Experimental conditions as in Table 5 except the applied voltage, 15 kV, 64 μA, at pH 9.

Acknowledgement

Thanks are due to Perkin-Elmer Applied Biosystems Division (Foster City, CA, USA) for providing the sulfobutyl ether-β-cyclodextrin.

References

[1] J. Debowski, D. Sybilska and J. Jurczak, J. Chromatogr., 282 (1983) 83.
[2] T.J. Ward and D.W. Armstrong, J. Liq. Chromatogr., 9 (1986) 407.
[3] G. Blaschke, J. Liq. Chromatogr., 9 (1986) 341.
[4] D.W. Armstrong, J.R. Faulkner, Jr., and S.M. Han, J. Chromatogr., 452 (1988) 323.
[5] F. Kobor and G. Schomburg, J. High. Resolut. Chromatogr., 16 (1993) 693.
[6] C.P. Granville, B. Gehrcke, W.A. Konig and I.W. Wainer, J. Chromatogr., 622 (1993) 21.
[7] M. Jung, S. Mayer and V. Schurig, LC·GC Mag. Sep. Sci., 12 (1994) 458.
[8] J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, J. Chromatogr., 438 (1987) 211.
[9] A. Guttman, A. Paulus, A.S. Cohen, N. Grinberg and B.L. Karger, J. Chromatogr., 448 (1988) 41.
[10] S. Terabe, Trends Anal. Chem., 8 (1989) 129.
[11] S. Fanali, J. Chromatogr., 474 (1989) 441.
[12] S. Terabe, H. Shibata and Y. Miyashita, J. Chromatogr., 480 (1989) 403.
[13] T. Schmitt and H. Engelhardt, Chromatographia, 37 (1993) 475.
[14] S. Fanali and P. Bocek, Electrophoresis, 11 (1990) 757.
[15] C. Desiderio, Z. Aturki and S. Fanali, Electrophoresis, 15 (1994) 864.
[16] S. Fanali, M. Cristalli, R. Vespalec and P. Bocek, in A. Chrambach, M.J. Dunn and B.J. Radola (Editors), Advances in Electrophoresis, VCH, Weinheim, 1994, p. 1.
[17] A. Nardi, A. Eliseev, P. Bocek and S. Fanali, J. Chromatogr., 638 (1993) 247.
[18] T. Schmitt and H. Engelhardt, J. High Resolut. Chromatogr., 16 (1993) 525.
[19] B. Chankvetadze, G. Endresz and G. Blaschke, Electrophoresis, 15 (1994) 804.
[20] C. Dette, S. Ebel and S. Terabe, Electrophoresis, 15 (1994) 799.
[21] Z. Aturki and S. Fanali, J. Chromatogr. A, 680 (1994) 137.
[22] I.S. Lurie, R.F.X. Klein, T.A. Dal Cason, M.J. LeBelle, R. Brenneisen and E. Weinberger, Anal. Chem., 66 (1994) 4019.