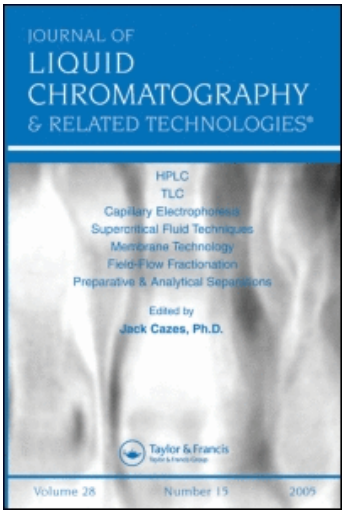

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Capillary Electrophoretic Chiral Separations Using a Sulfated β-Cyclodextrin-Containing Electrolyte

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To cite this Article Wu, Wnhong and Stalcup, Apryll M.(1995) 'Capillary Electrophoretic Chiral Separations Using a Sulfated β-Cyclodextrin-Containing Electrolyte', Journal of Liquid Chromatography & Related Technologies, 18: 7, 1289 — 1315
To link to this Article: DOI: 10.1080/10826079508010414
URL: http://dx.doi.org/10.1080/10826079508010414

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(7), 1289-1315 (1995)

CAPILLARY ELECTROPHORETIC CHIRAL SEPARATIONS USING A SULFATED β-CYCLODEXTRIN-CONTAINING ELECTROLYTE

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ABSTRACT

Sulfated β-cyclodextrin (SO_3-β-CD) was utilized as a chiral additive for capillary zone electrophoresis (CZE). Chiral separations of several uhcharged enantiomers, such as phensuximide, indapamide, etc., which are difficult to separate using neutral CDs, were achieved. The effects of SO_3-β-CD concentration and the pH and ionic strength of the supporting electrolyte as well as the presence of an organic modifier, methanol, were discussed.

INTRODUCTION

In the last decade, capillary zone electrophoresis (CZE) has become an important analytical technique.[1] Like gas chromatography (GC) and high performance liquid chromatography (HPLC), CZE offers some conspicuous advantages over conventional electrophoresis, such as fast

separations, high efficiency, minute sample loading and ease of automation.[1]

CZE has been utilized to analyze a wide variety of charged and uncharged species, such as small ions,[2] simple organic molecules,[3] amino acids,[4,5] peptides,[6] and proteins.[7] One particularly challenging area of separations is that of enantiomeric separations. With identical physical and chemical properties in an achiral environment, enantiomers do not exhibit differences in electrophoretic mobility and have traditionally been considered to be the most difficult among all separations. Nevertheless, the unique high efficiency of CZE and the possibility of enantioselective interactions between the analyte and a chiral additive make CZE well-suited to enantiomeric separations. Separations are usually performed by adding a chiral selector to the buffer electrolyte and the separation is accomplished due to a preferred formation of a complex between the chiral additive and one of the enantiomers. The most commonly used chiral selectors in CE can be divided into three categories, in which different separation principles apply: host-guest complexation (e.g., native and derivatized cyclodextrins,[8,9,10] and crown ethers[11]); ligand exchange complexation[12] and optically active surfactants.[13,14]

Cyclodextrins (CDs) and their derivatives have been successfully used for GC, HPLC and TLC separations of a large number of chiral compounds including drugs and derivatized amino acids.[15,16,17] Although most of the GC and HPLC separations were achieved on immobilized CD chiral

stationary phases (CSPs), many different types of CSPs are required in order to cover a limited range of racemic compounds. The use of CDs as a mobile phase additive provides a flexible alternative for the separation of enantiomers, because separations can be performed on conventional columns which generally have higher efficiencies than CSPs.[18] Both native and derivatized CDs have been utilized as chiral mobile phase additives.[19,20,21]

Accordingly, the use of CDs in CE seems to be a logical extension of existing chiral separation techniques. Two approaches are generally used to achieve the chiral separation on CE. While CD may be immobilized within a polymeric gel,[4] most of the chiral separations in CZE with CDs have been achieved in free solution.

Chiral CZE separations have been achieved with all three native α-, β-, γ-CD and their mixtures.[22,23,24] The proposed separation mechanism seems to be dependent on a difference in stability of the inclusion complexes formed between each enantiomer and the CD coupled with differences in the mobility characteristics of the "host-guest" complexes.[25] Among the three native CDs, β-CD is by far the most widely used for CZE.[26] However, the low solubility of β-CD in water (1.85 g/100ml)[27] limits method optimization. Urea is commonly used as an additive to increase the solubility of the CD.[28,29] Alternatively, incorporation of different functional groups, such as methyl (e.g., dimethyl-β-CD[21,30] or trimethyl-β-CD[10,21]), glycosyl[23], and hydroxypropyl groups,[23,31] onto the CD result in different degrees of

enhanced solubility and enantioselectivity. However, all of these derivatized CDs and native CDs are neutral and therefore migrate with the same mobility as the electroosmotic flow. Hence, the separation "window" is limited to the time between the analyte migration time in the absence of CD and the migration time of the CD.

Nishi et al.[29] reported chiral resolution of electrically neutral compounds by micellar electrokinetic capillary chromatography (MECC), using a buffer containing CDs and negatively charged sodium dodecyl sulfate (SDS). Because the EOF flow was much stronger than the anodic electrophoretic migration of the micelle, the net migration of SDS was also in the direction of cathode, but with a slower velocity than the bulk solution or the CD. The proposed mechanism involved distribution of the solutes between three phases, i.e., the aqueous, the micelle and the CD phase. The retarded migration of the analytes as a result of partitioning into the micelle amplified the enantioselective interaction with the CD, thereby resulting in enhanced enantioselectivity.

Although most reports of CD-based electrokinetic chromatography use neutral CD, Terabe[32] has reported using an ionic derivatized CD, 2-O-carboxymethyl-β-CD to separate the structural isomers of cresols.

In this study, sulfated β-CD (SO_3-β-CD) was used as a chiral additive in the electrolyte. According to the manufacturer, the average degree of substitution is 7 to 11 sulfate/CD, with the substitution pattern unknown. The

presence of the negatively charged groups on the CD should not only slow the velocity of the SO_3-β-CD relative to the native CD, thereby increasing the chiral separation window, but also enhance the solubility of SO_3-β-CD relative to the native CD. It should be noted that derivatization of the CD no doubt changes the chiral recognition ability as well as hydrogen bonding capability of CD with analytes. Therefore, the SO_3-β-CD was anticipated to provide unique enantioselectivity. A variety of neutral racemic compounds were successfully resolved. The influence of experimental factors such as pH, the presence of organic modifier and the concentration of the chiral selector will be discussed subsequently. The contribution of electroosmotic flow may not be easily ascertained in the presence of ionic CD's because the neutral marker may complex with the CD. More importantly, however, the CD's contribute to the overall ionic strength of the buffer. Hence, the difference in the mobility or migration times and resolution of solutes is adopted to evaluate separations.

EXPERIMENTAL

Chemicals

SO_3-β-CD was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Racemic solutes were purchased from Aldrich or Sigma Chemical Company (St. Louis, MO) and dissolved in methanol (ca. 0.5 mg/ml). The methanol was HPLC grade. The buffer solutions were prepared from distilled, doubly deionized water.

Instrumentation

The experiments were carried out on a Water Quanta 4000 Capillary Zone Electrophoresis system equipped with a UV detector (214 nm) and a power supply delivering up to 30 kV. The CZE was operated in a conventional mode with the cathode at the detector end. The fused silica capillary column was 75 μm i.d., 60 cm total length and 52.4 cm capillary length to the detector window. Data acquisition was performed with a Shimadzu Chromatopac CR-501 data station.

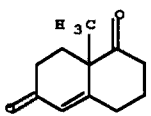
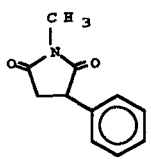
Run Conditions

Run buffers were prepared by dissolving disodium hydrogen phosphate (10-20 mM) and SO_3-β-CD (0-4%) in water, and adjusted with phosphoric acid to the appropriate pH values (5-8). All buffer solutions were filtered through a membrane filter of 0.45 μm pore size prior to use. In all experiments, a constant voltage was applied. The capillary was rinsed before each run with 0.1 M potassium hydroxide for 2 minutes and then buffer for 2 minutes. Samples were injected using the hydrostatic method (2 sec). Methanol was adopted as a marker for EOF.

RESULTS AND DISCUSSION

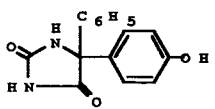
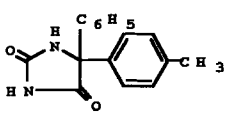
Table 1 lists the compounds used in this study along with results obtained under conditions optimized for each analyte to achieve baseline resolution of enantiomers within the shortest analysis time.

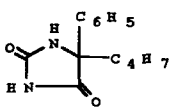
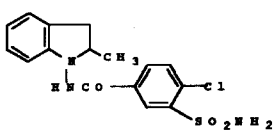
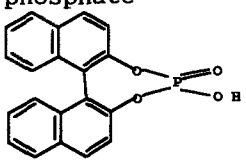
Table 1. Electrophoretic data for recemates resolved using SO-β-CD as CMA in CZE.

No	Compound	t	R_S	Electrophoretic condition	Applied Voltage
1	9-Methyl-$\Delta^{5(10)}$-1,6-dione 	17.88 18.46	2.94	10 mM Na_2HPO_4, 2% CD, pH 6.0	8 kV
2	Phensuximide 	19.32 20.10	1.94	10 mM Na_2HPO_4, 3% CD, pH 7.0	8 kV

(continued)

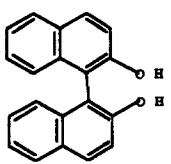
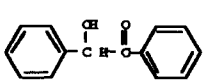
Table 1 (Continued).

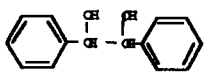
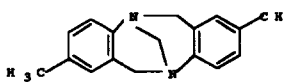
No	Compound	t	R_S	Electrophoretic condition	Applied Voltage
3	5-(4-Hydroxyphenyl) -5-phenyl hydantion 	15.61 16.72	2.94	10 mM Na_2HPO_4, 2% CD, pH 8.0	15 kV
4	5-(4-Methylphenyl) -5-phenyl hydantion 	15.20 16.06	2.96	10% MeOH / (10 mM Na_2HPO_4, 2% CD, pH 8.0)	15 kV

5	5-Cyclobutyl-5-phenyl hydantoin 	11.53 12.36	3.95	10% MeOH / (10 mM Na_2HPO_4, 2% CD, pH 8.0)	15 kV
6	Indapamide 	24.08 24.96	1.50	10 mM Na_2HPO_4, 4% CD, pH 7.0	8 kV
7	1,1'-Binaphthyl-2,2'-diyl hydrogen phosphate 	24.31 25.19	2.43	10 mM Na_2HPO_4, 2% CD, pH 8.0	8 kV

(continued)

Table 1 (Continued).

No	Compound	t	R_S	Electrophoretic condition	Applied Voltage
8	1,1'-Bi-2-naphthol 	9.35 10.17	2.72	10 mM Na_2HPO_4, 2% CD, pH 8.0	15 kV
9	Benzoin 	26.79 28.20	1.77	10 mM Na_2HPO_4, 2% CD, pH 7.0	8 kV

10	Hydrobenzoin 	8.63 10.12	4.40	10 mM Na_2HPO_4, 2% CD, pH 8.0	15 kV
11	Troger's base 	16.81 17.78	2.44	30% MeOH / (10 mM Na_2HPO_4, 2% CD, pH 8.0)	15 kV

From Table 1, it can be seen that most of the solutes resolved in this study either have a chiral center located on a ring (1-7) or have locked ring structures (7, 8, 11). As for many cases in HPLC, formation of an inclusion complex between the relative non-polar interior of the CD cavity and the hydrophobic moiety of the analyte seems to play an important role in chiral recognition with SO_3-β-CD.

Effect of SO_3-β-CD Concentration

The effect of SO_3-β-CD concentration on migration and selectivity was examined. For all compounds in this study, migration times increased as the concentration of SO_3-β-CD increased from 1% to 4%. Figure 1 shows the electrophoregrams for the resolution of 9-Methyl-$\Delta^{5(10)}$-1,6-dione into its enantiomers using different concentrations of SO_3-β-CD. Both the migration times and apparent mobility differences of the enantiomers increased as the concentration of SO_3-β-CD increased. Baseline separation (Rs > 1.5) was achieved when SO_3-β-CD increased to 3% (Figure 1). Although increasing the SO_3-β-CD concentration further improved the selectivity, the migration times also dramatically increased (Figure 1e).

Figure 2 further illustrates the effect of SO_3-β-CD concentration on the migration times and chiral recognition of some racemates in this study. The migration time difference between two enantiomers of each analyte increased as the concentration of SO_3-β-CD increased. It was found that complete enantiomeric resolution was achieved for

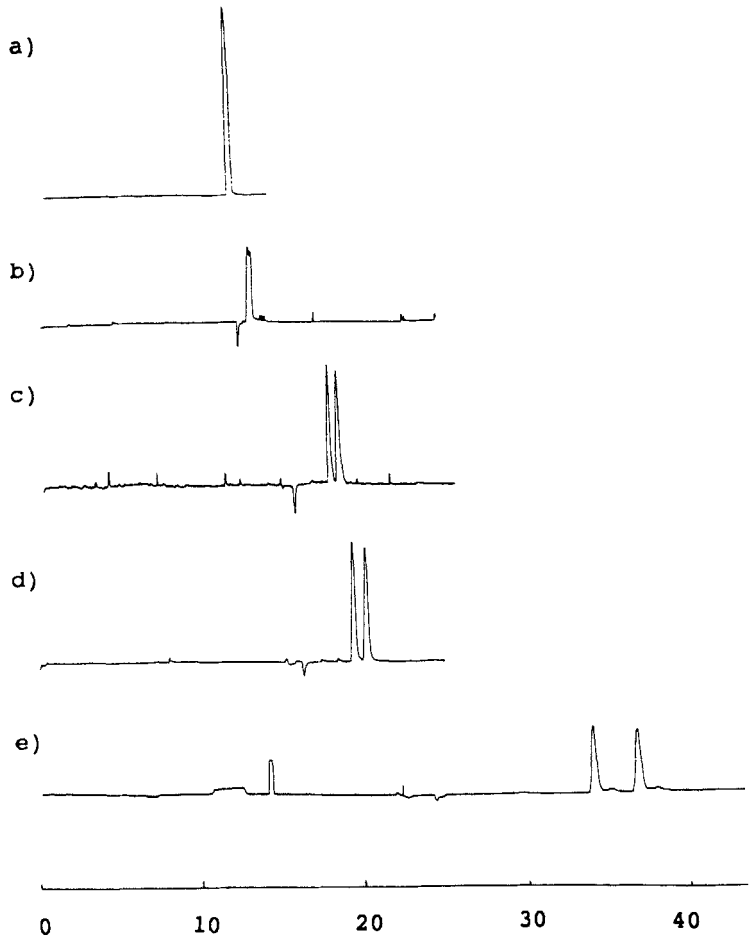


Figure 1 Electropherograms showing the effect of the concentration of SO-β-CD on chiral resolution of phensuximide. Conditions: electrolyte (10 mM Na_2HPO_4, pH 7.0); applied voltage, 8 kV; concentration of SO-β-CD:
a) 0%; b) 1%; c) 2%; d)3%; e)4%.

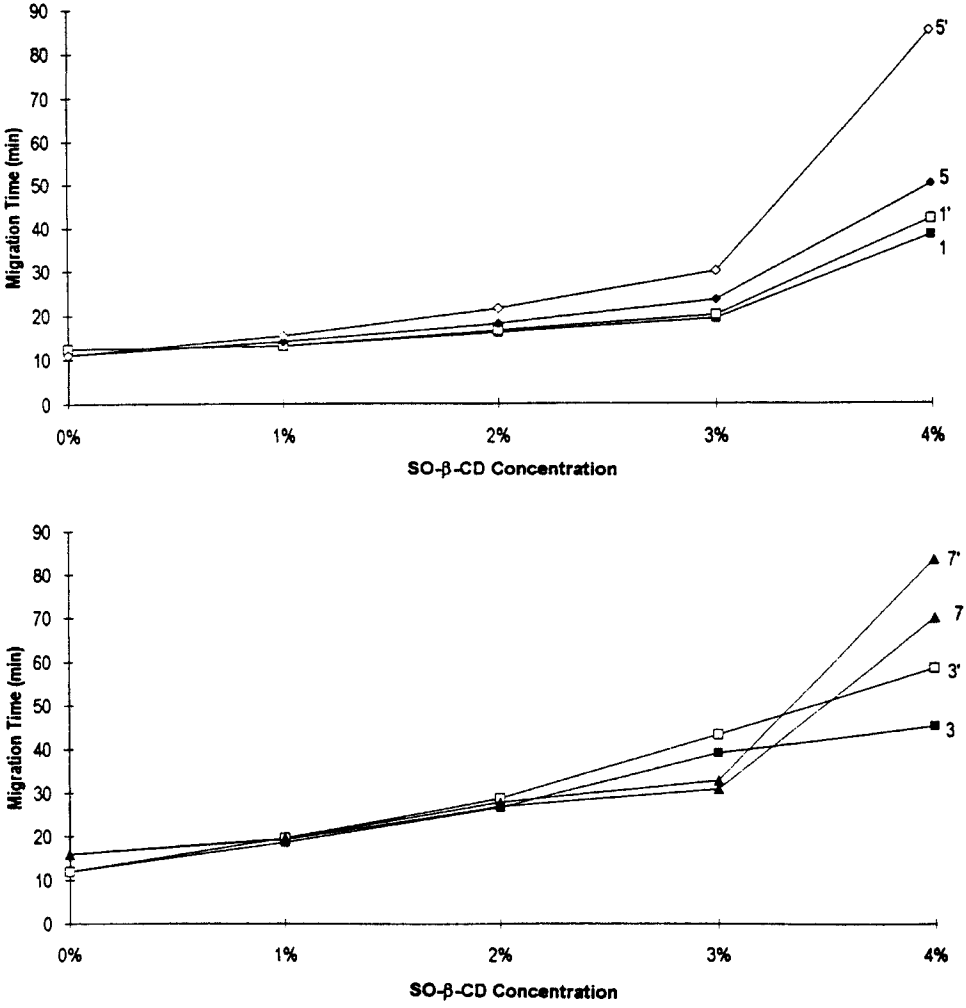


Figure 2 Effect of the concentration of SO-β-CD on the migration times of a) 1,1' = 9-Methyl-$\Delta^{5(10)}$-1,6-dione; 5,5' = 5-Cyclobutyl-5-phenyl hydantoin; b) 3,3' = 5-(4-Hydroxyphenyl)-5-phenyl hydantoin; 7,7' = 1,1'-Binaphthyl-2,2'-diyl hydrogen phosphate. Same conditions as in Figure 1.

analytes 3, 4, 5, 10 and 11 when 1% of SO_3-β-CD was added to the background electrolyte. To completely resolve compounds 2, 7, 8 and 9, 2% of SO_3-β-CD was required. To obtain baseline resolution of compound 1 and 6, it was necessary to add 3% and 4% of SO_3-β-CD to the run buffer, respectively. Although for most solutes, as SO_3-β-CD concentration increased, resolution increased as well (Figure 3), a maximum resolution was observed with compound 3 at 2% SO_3-β-CD.

In the absence of SO_3-β-CD, all analyte species moved toward the cathodic end with migration rates close to EOF. However in the presence of SO_3-β-CD, analytes complex with the SO_3-β-CD and were transported toward the negative electrode at slower velocity, presumably because the negatively charged sulfate groups on SO_3-β-CD electrophoretically attract it to the anodic end. Increasing the concentration of SO_3-β-CD provided more opportunity for analytes to complex with the SO_3-β-CD, therefore further slowing down the migration of analytes toward the detector end. It should also be noted that SO_3-β-CD contributes to the ionic strength and that increases in ionic strength suppresses the electroosmotic flow.

Effect of Electrolyte Ionic Strength

Unlike the native or methyl derivatized CDs, which are neutral in buffer solution, SO_3-β-CD is readily ionized in aqueous solution, and therefore contributes significantly to the ionic strength of background electrolyte. Thus,

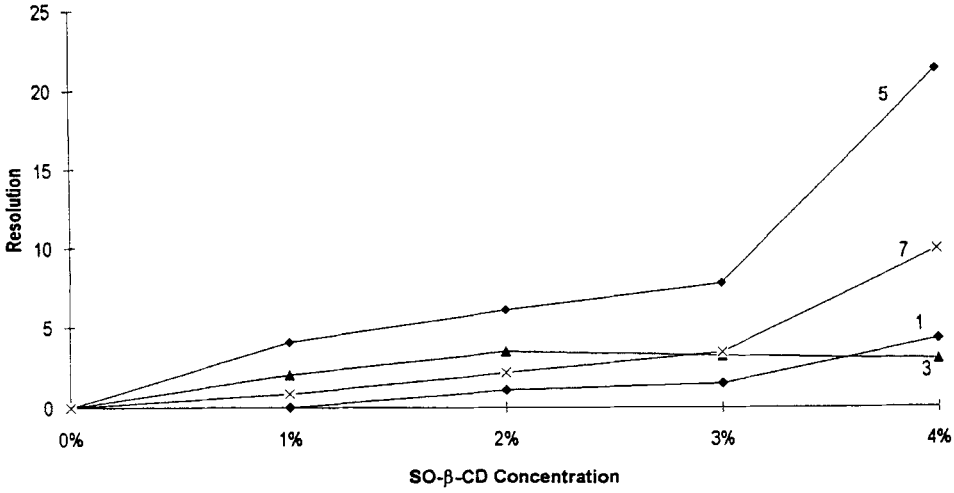


Figure 3 Effect of the concentration of SO-β-CD on R_S of: 1 = 9-Methyl-$\Delta^{5(10)}$-1,6-dione; 3 = 5-(4-Hydroxyphenyl)-5-phenyl hydantoin; 5 = 5-Cyclobutyl-5-phenyl hydantoin; 7 = 1,1'-Binaphthyl-2,2'-diyl hydrogen phosphate. Same conditions as in Figure 1.

increasing the concentration of SO_3-β-CD not only has a considerable impact on the chiral selectivity but on the ionic strength as well. Addition of 1% of SO_3-β-CD to the background electrolyte gave an average of almost 70% increase in current (e.g., current increased from 25.2 μA to 42 μA as SO_3-β-CD increased from 1% to 2%), thereby increasing the amount of Joule heating within the capillary.

To further evaluate the effect of electrolyte ionic strength on migration and chiral recognition, two series of

experiments were carried out at 2% of SO_3-β-CD with pH 8.0, containing 10 mM and 20 mM of phosphate buffer, respectively. The results of some test analytes are summarized in Table 2. It has been rationalized[33] that increased ion concentration reduces the thickness of the double layer on the capillary wall, leading to a decrease of EOF. Indeed, the migration times of most of the selected analytes increased when the phosphate concentration doubled, but the apparent mobility difference between two enantiomers as well as resolution did not show appreciable dependence on phosphate concentration for most analytes. Evidently, in most cases, the effect of phosphate buffer concentration on resolution was non-enantiospecific. Interestingly, the mobility and resolution of compounds 4 and 5 exhibit significant change. From Table 1, compounds 3, 4 and 5 bear similar structures. However, the polarity decreases from compound 3 to 5, which is due to the different substituents at the C5 position. The increase of migration time and resolution from compound 3 to compound 5 as phosphate concentration increased may provide evidence that hydrophobic complexation between the analyte and SO_3-β-CD cavity is important for chiral recognition. However, the overall contribution of phosphate buffer to the ionic strength of the background electrolyte was limited compared to SO_3-β-CD (e.g., current increased from 42 to 50 μA when phosphate concentration increased from 10 to 20 mM). Hence, SO_3-β-CD seemed to be the major contributor to the electrolyte conductivity in this experiment.

Table 2 Effect of phosphate concentration on the solute migration time and resolution. Electrolyte, 2% SO-β-CD, pH 8.0. Applied voltage, 8 kV.

	10 mM phosphate		20 mM phosphate	
Compound	t	Rs	t	Rs
2	15.87	1.17	15.86	1.14
	16.30		16.31	
3	31.78	3.99	32.16	3.46
	34.58		34.58	
4	26.43	3.05	33.64	3.69
	28.71		37.22	
5	16.88	6.21	21.04	11.18
	19.68		24.96	
6	14.95	1.06	16.74	0.74
	15.17		17.00	

Influence of pH

The effect of the run buffer pH was investigated in a pH range of 5 to 8. Generally, the migration times of the test solutes increased as pH decreased. Furthermore, when pH decreased to 5, no peaks were observed for any of the analytes within 120 minutes.

The increased retention at low pH may be attributed, in part, to the reduced EOF. Under low pH conditions, the

dissociation of silanol groups on the fused-silica capillary wall is suppressed. The reduced zeta potential thereby decreases EOF; consequently migration times increase. In aqueous buffer, the sulfate groups (pKa of H_2SO_4 -9) of SO_3-β-CD carry a negative charge. The migration of SO_3-β-CD is under the influence of both EOF and electrophoretic flow, which, in turn, is directly related to the pH of the electrolyte. The electrophoretic mobility of SO_3-β-CD is toward the anodic (injection) side while the EOF is toward the cathodic (detector) end. Presumably, at high pH, the EOF is stronger than the electrophoretic flow, thus the net migration of SO_3-β-CD may be in the direction of the cathode.[34] However, at low pH this may not be the case. Indeed, Stalcup and Agyei demonstrated that the net migration of another sulfated carbohydrate was toward the anode at pH 4.5.[34]

Figure 4 illustrates the effect of pH on the migration time and chiral recognition of compounds 1, 4, and 11. As the pH decreased from 8 to 6, the migration time of all three solutes increased. For compound 1, the increase of migration time for both enantiomers was almost parallel. In this case, the increase in retention seems to be related strictly to the decrease in EOF. This may be due to the fact that compound 1 is neutral and is not affected by the pH of the buffer. With compound 11, the migration time of the second eluting peak increased more than that of the first one at pH 6, resulting in an increase of the apparent mobility difference between the two enantiomers. Although

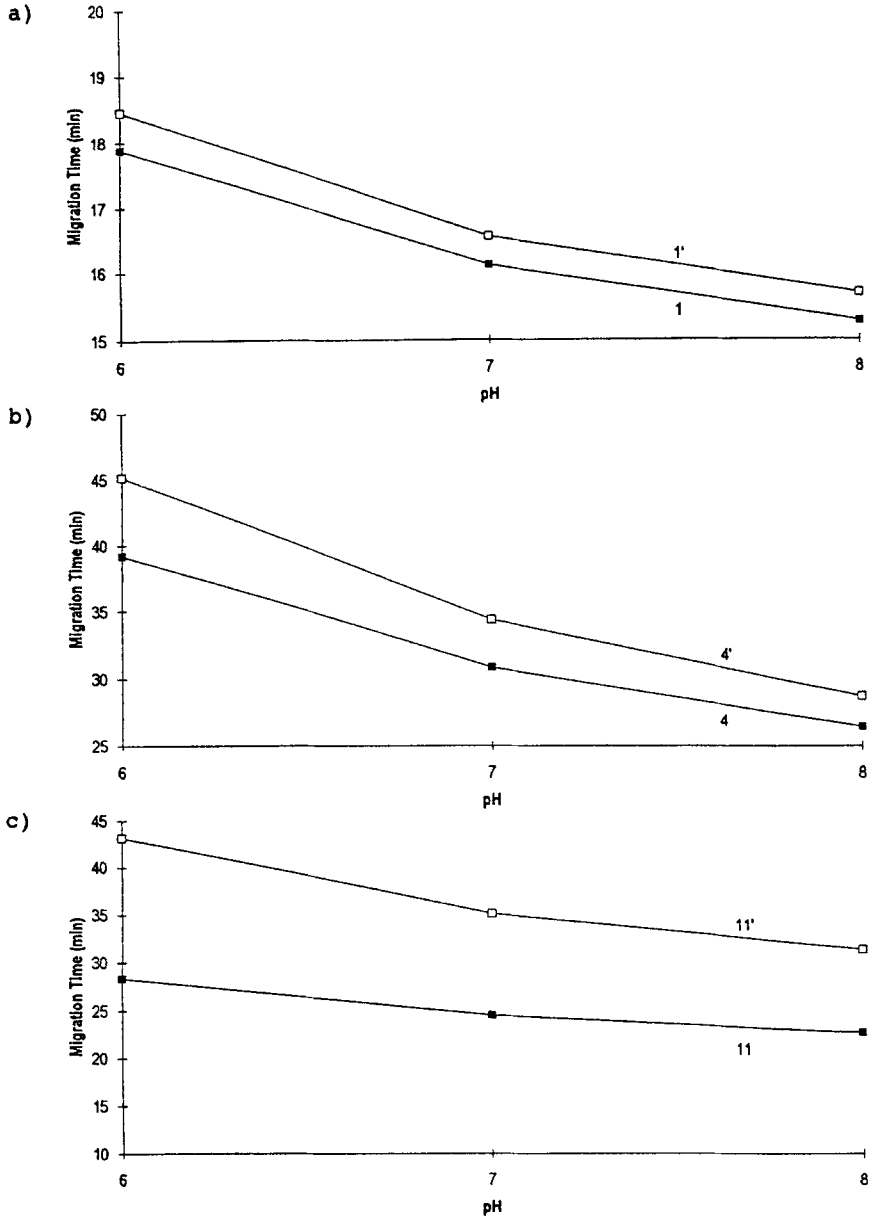


Figure 4 Effect of pH on the migration time of a) 1,1' = 9-Methyl-$\Delta^{5(10)}$-1,6-dione; b) 4,4' = 5-(4-Methylphenyl)-5-phenyl hydantoin; c) 11,11' = Troger's base. Conditions: electrolyte (Na_2HPO_4, 2% SO-β-CD); applied voltage, 8 kV.

apparently the same trend existed for compound 4, it should be noted that compound 11 was protonated under the experimental conditions ($pK_a \approx 10$ for tertiary alkylamine ammonium ion). In this case, at low pH or absense of EOF, the electrophoretic mobilities of the analyte and the additive are in the opposite directions thus further amplifies the differences in binding of the the enantiomers.

Effect of Organic Modifier

The effect of the addition of organic modifier to the run buffer on the performance parameters was also investigated. Methanol and acetonitrile are the two most frequently utilized water-miscible organic solvents.[35] In this study, methanol was added to the electrolyte in increasing proportion within a 0-30% (v/v) range. In all cases, the migration times of solutes increased as the amount of methanol in the electrolyte increased.

Addition of methanol resulted in a decrease of EOF and hence increased migration times of the solutes. It has been pointed out[36] that when methanol is added to the buffer the viscosity of the resulting solution increases while the dielectric constant decreases. Moreover, methanol is thought to interact with the capillary wall thereby masking the surface silanol groups, leading to an increase of the local viscosity in the double-layer.[36] The magnitude of the zeta potential which is generated on the wall of fused-silica capillary therefore decreases. According to equation[37]

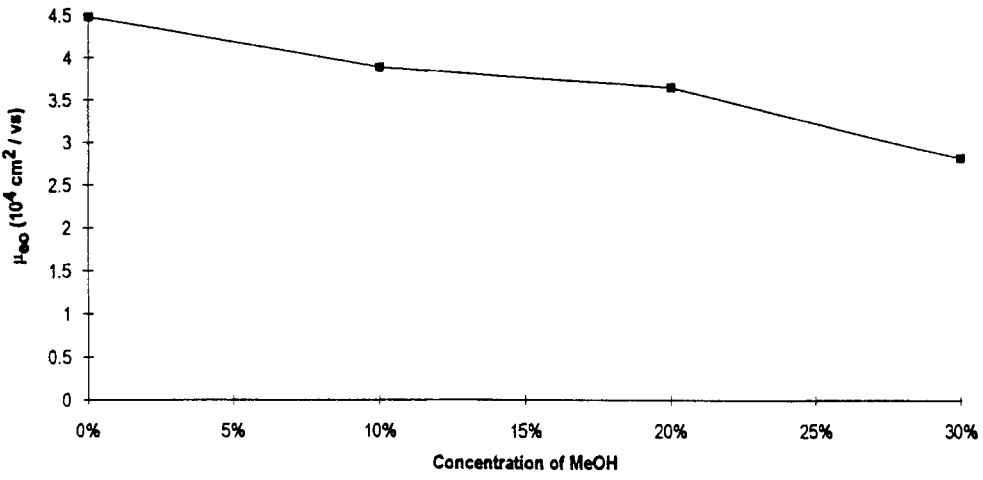


Figure 5 Effect of the concentration of methanol on electroosmotic flow. Electrolyte: 10 mM Na_2HPO_4, 2% SO-β-CD, pH 8.0; applied voltage, 15 kV.

$$v_{eo} = \frac{\varepsilon \xi}{4\pi\eta} E$$

where v_{eo} is the electroosmotic flow velocity; ε is the dielectric constant and η is the buffer viscosity, the EOF is a function of the buffer dielectric constant, viscosity and zeta potential. Figure 5 presents the effect of the addition of methanol on EOF.

Addition of methanol may also affect the CZE chiral separation in another way. Chiral recognition in SO_3-β-CD modified CZE is thought to be due to the host-guest inclusion complexation between the enantiomers and chiral selector. Addition of methanol in the run buffer may weaken the affinity of analytes for SO_3-β-CD, because methanol may

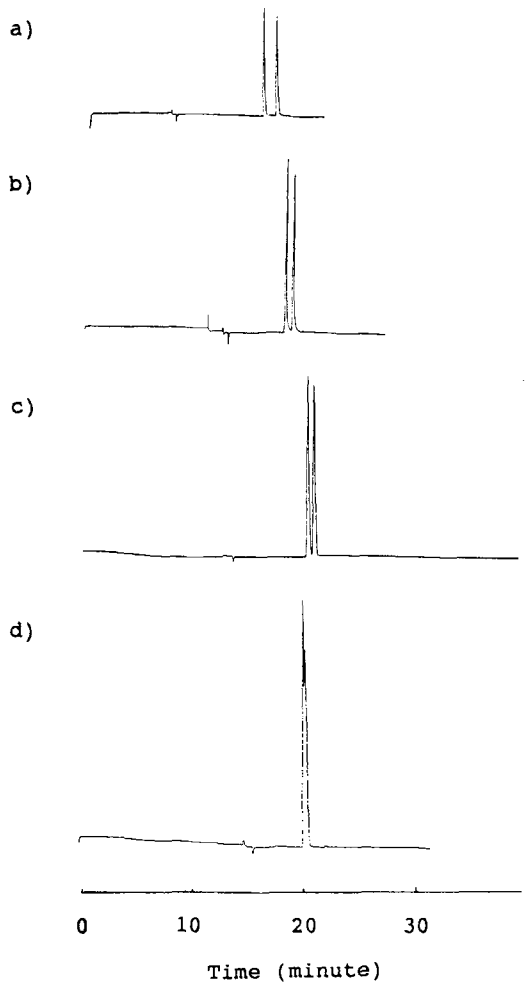


Figure 6 Electropherograms of 5-(4-Hydroxyphenyl)-5-phenyl hydantoin illustrating the effect of methanol concentration migration time and resolution. Same conditions as in Figure 4. Methanol concentration: a) 0%; b) 10%; c) 20%; d) 30%.

compete with solutes for the relatively hydrophobic cavity of SO_3-β-CD, therefore diminishing the chiral recognition. In addition, methanol may provide a more hospitable environment for nonpolar solutes.

It was observed that the resolution of the test enantiomeric compounds gradually deteriorated with the addition of methanol. Typical electropherograms of compound 3 are presented in Figure 6 to illustrate the change in resolution with changing methanol concentration. When the volume concentration of methanol increased to 30%, resolution of racemates 1, 2, 6, 7, 8 and 9 totally diminished and a single peak was observed. Partial resolution was obtained for compound 3, 4, 5 and 10, while base line separation of racemate 11 was still obtained. The case of compound 11 which is ionized under the experimental conditions, may lend support to the premise that the loss of chiral recognition for neutral species with the addition of methanol may be partially attributed to decreased electrolyte polarity.

With increasing volume concentration of methanol from 0% to 30% in solution, currents decreased from 85 μA to 30 μ A. This allowed operation of CZE with a higher electric field strength (15 kV).

CONCLUSIONS

The results demonstrate that SO_3-β-CD has sufficient solubility in an electrolyte to act as a good

enantioselective agent for the resolution of neutral enantiomers, which are difficult to achieve using neutral CDs. The concentration of SO_3-β-CD, pH and ionic strength of electrolyte, and the presence of organic modifier were all shown to exert a profound effect on solute migration and separation. However, the commercially available SO_3-β-CD used in this study is not well characterized. Future work may involve regiospecific sulfation of CD to maximize the separation and understand the separation mechanism. Unless separation and understand the separation mechanism. Unless a thorough understanding of separation mechanism is achieved, the optimization of electrophoretic separation will still be largely an empirical process.

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Received: September 14, 1994
Accepted: October 18, 1994