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Enantioseparation of chiral sulfoxides and sulfinate esters by capillary electrophoresis

Forty-one chiral sulfoxides and sulfinate esters were separated using sulfated β -cyclodextrin and carboxymethyl β -cyclodextrin as chiral selectors. Binding constants of some analytes to both chiral selectors were measured in order to examine and help explain the observed migration behavior and enantioselectivity trends. Overall, sulfated β -cyclodextrin separated a greater number of compounds, and had better separating capabilities than did carboxymethyl β -cyclodextrin for these analytes. This was true even though all of the analytes showed much stronger binding to carboxymethyl β -cyclodextrin than to sulfated β -cyclodextrin. General procedures to optimize the separation, by varying pH, selector concentration, and organic modifier concentration were examined and discussed. Chiral selector concentration had the greatest effect on enantioseparation, with higher concentrations of selector giving better peak-to-peak separations. Organic modifier had an adverse affect on resolution, with increasing amounts giving lower mobility differences. Lastly, pH had only a minimal effect on separation.

Keywords: Binding constants / Capillary electrophoresis / Carboxymethyl / Chiral separation / Cyclodextrins / Sulfated cyclodextrins / Sulfoxides
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1 Introduction

Molecules having sulfoxide functionality have been used in the asymmetric synthesis of many natural products and biologically active compounds [1]. In some instances, the sulfoxide precursors are biologically active as well [2–6]. For these reasons, sulfoxides and the closely related sulfinate esters are of great interest to the pharmaceutical industry. In some cases the sulfur is a stereogenic center. The separation of these chiral sulfoxides into their respective enantiomers is of particular interest, because often the enantiomers have different biological effects and/or dispositions.

Bayer and co-workers [7] were the first to directly separate unmodified chiral sulfoxides using gas chromatography. They separated five different racemates using Chiral-sil-Val stationary phases. Other early work, pioneered by Farina and co-workers [8], involved the chiral separation of sulfoxides by liquid chromatography (LC) on α -lactose. Later studies progressed to using HPLC with various chiral stationary phases (CSPs) [8, 9]. Welch and co-workers [10] used a π -complex normal phase chiral stationary phase, while Montanari and Cass [11] used four different CSPs (arylcarbamates of cellulose and amylose polysaccharides coated onto 3-aminopropylsilica gel) to

separate a number of sulfoxides. Other chiral separation techniques that did not use chromatographic approaches soon followed. Bortolini *et al.* [12] developed a procedure to optically resolve sulfoxides by inclusion in a host of dehydrocholic acid. Despite all of these studies, very little has been done on the enantiomeric separation of chiral sulfoxides and sulfinate esters, by capillary electrophoresis. In addition, no study has examined the large number and wide diversity of compounds considered in this work.

2 Materials and methods

2.1 Materials

Sulfated β -cyclodextrin (SBC) was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Carboxymethyl β -cyclodextrin (CMBC) was obtained from American Maize Products (Hammond, IN, USA). The chiral sulfoxides and sulfinate esters were the gift of W.S. Jenks, Department of Chemistry, Iowa State University. Sodium phosphate, HPLC-grade methanol, sodium hydroxide and 85% phosphoric acid were all purchased from Fisher Scientific (St. Louis, MO, USA). The capillaries used in these experiments were from Polymicro Technologies (Phoenix, AZ, USA). Mesityl oxide was obtained from Fluka (Buchs, Switzerland).

2.2 Methods

The CE system used for all SBC experiments was a Waters Quanta 4000 with a fixed wavelength lamp. Samples were injected hydrostatically. The capillary used with

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Abbreviations: **CSP**, chiral stationary phase; **CMBC**, carboxymethyl β -cyclodextrin; **SBC**, sulfated β -cyclodextrin

this instrument had a 50 μm ID that was 39.5 cm in length (32 cm to the detector). The analytes were detected at a wavelength of 254 nm with the exceptions of numbers 26 and 27, which were detected at 214 nm. Burning off the polyamide coating from a 1 cm portion of the capillary created a window for detection. Before the first experiment, the new capillary was conditioned for 1 min using purified water, then for 3 min using 1 N phosphoric acid, and finally with 0.1 M sodium hydroxide for 5 min. Before each of the following experiments, the capillary was rinsed with 0.1 M sodium hydroxide for 1 min and then with the running buffer for approximately 30 s. Flushing the capillary was necessary to prevent coating of the capillary wall by the SBC and to ensure reproducible EOFs [16]. A P/ACE MDQ system from Beckman Coulter was used for all of the CMBC experiments. It contained a capillary that had a 50 μm ID, a 30 cm total length, and a 20 cm length to the detector. All analytes were detected at a wavelength of 214 nm with this machine. Samples were injected and rinsing was done at a pressure of 0.5 psi. New capillaries were first conditioned with water for 1 min, then with 1 M sodium hydroxide for 5 min, and lastly with water for 5 min. From then on it was rinsed with water for 1 min, 1 M sodium hydroxide for 1 min, water for 2 min, and finally with running buffer for 2 min, in between every run. All SBC running buffers were prepared by dissolving the required amount of SBC in an aliquot from a 10 mM sodium phosphate stock solution. The pH was adjusted by adding aqueous sodium hydroxide or aqueous phosphoric acid. The buffers were degassed by sonication for 15 min prior to usage. Organic modifier was added on a volume-to-volume basis. Samples for injection were prepared by a variety of methods. For sulfoxide numbers 1–6, 8, 13, 17, 19–20, 22–29, and 30–41, pure samples of the compounds were dissolved in 10 mM sodium phosphate solution at a concentration of approximately 0.1 mg/mL. Sulfoxides 7, 9, 11, 12, and 15 were dissolved by placing roughly 0.2 mg in 3 mL of 10 mM phosphate buffer and then adding just enough methanol for total dissolution. The remaining sulfoxides (10, 14, 16, 18, 21) had severe solubility problems and in order to dissolve these samples, a saturated solution in methanol had to be prepared, and then a drop from this solution was placed in 3 mL of 10 mM sodium phosphate. These samples were then analyzed very quickly before they precipitated.

3 Results and discussion

3.1 Structure – enantioselectivity relationship

SBC and CMBC were used as the chiral selectors for this investigation. These specific derivatives were selected for several reasons. First, neutral chiral analytes require a

charged chiral selector to obtain a separation in CE. Also, negatively charged chiral selectors offer the largest window for interaction with the analyte. A countercurrent motion of the analyte (traveling with the EOF) and the chiral selector (opposing the EOF) is known to accentuate CE enantioseparations. Finally, SBC is known to be a powerful chiral selector that has been used to separate a number of chiral compounds [13–15]. Table 1 shows the structures, migration times, resolutions and mobility differences obtained for the 41 chiral sulfoxides and sulfinate esters separated using SBC as a run buffer additive. Table 2 gives the same information for the separations that used CMBC as the chiral additive. Compounds not listed in Table 2 gave no separation. Most of the compounds in this study could be separated using SBC and many of them gave baseline or better resolution. CMBC separated far fewer compounds. Compounds that could be separated with both chiral selectors usually had better resolution with the SBC. Only in one case (for compound # 7) could a separation be achieved with CMBC but not SBC (Tables 1 and 2). Though much less CMBC was used than SBC, these were the optimized conditions for each selector, the importance of this will be discussed subsequently.

The large number of closely related compounds in this study provided a means to examine the effect of small structural changes on enantioseparation. Several compounds are members of a homologous series, others are structural isomers, while still others differ from each other by a single substituent (Tables 1 and 2). Figure 1 shows the separation (using identical conditions) of a series of compounds that differ from one another only in the group that is the *para*-substituent of the phenyl ring. The size of this group had an effect on resolution and peak-to-peak separation, which can be seen by comparing the electropherograms for compounds 1 and 2. When a methyl group was added to the phenyl ring, where no group was previously present, the separation slightly decreased. Comparing compounds 3–6 shows this effect as well, by showing the compound with the largest CF_3 substituent giving the worst separation (Fig. 1). Comparing sulfoxides 6 and 2 shows that electronegativity also plays a role in the degree of separation. The methyl group and the trifluoromethyl group are of a comparable size; however, the compound with the much more electronegative trifluoromethyl group gave a poorer separation. Overall, for this set of compounds, larger groups with more electronegative *para* substituents tended to give worse separations. These effects can also be seen by comparing the mobility differences listed in Tables 1 and 2.

Figure 2 gives examples in which the size of the substituent next to the stereogenic sulfur affects the separation. The substituents vary in size from the small methyl and

Table 1. Separations with SBC^{a)}














Structure	t_{m1}	t_{m2}	R_s	[SBC]	$\Delta\mu$
1 	11.81	12.61	2.2	150	0.76
2 	10.22	10.71	1.4	150	0.57
3 	8.90	9.19	1.3	150	0.58
4 	14.69	15.39	0.8	150	0.58
5 	15.19	15.88	1.7	150	0.41
6 	9.50	9.63	0.7	150	0.22
7 	No separation				
8 	17.68	20.52	5.4	150	1.10
9 	14.56	16.79	6.1	150	1.28
10 	5.16	6.75	6.0	20	3.40
11 	14.50	16.28	5.0	150	1.06
12 	6.18	6.63	2.1	30	0.66
13 	12.83	15.03	7.0	150	1.60

Table 1. Continued















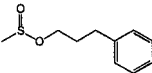
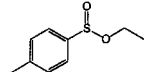
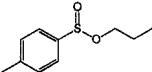
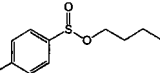
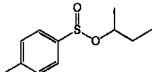
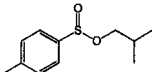

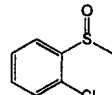

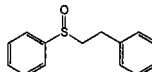
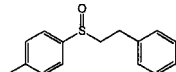
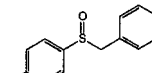


Structure	t_{m1}	t_{m2}	R_s	[SBC]	$\Delta\mu$
14 	No separation				
15 	18.55	22.01	4.2	75	0.98
16 	13.49	13.93	1.7	125	0.43
17 	9.33	10.33	4.5	150	1.46
18 	13.34	13.71	1.4	125	0.37
19 	8.36	9.05	2.1	150	1.28
20 	8.89	11.32	8.3	150	3.40
21 	18.20	18.79	1.6	125	0.29
22 	11.62	14.52	6.7	100	1.97
23 	No separation				
24 	10.32	10.82	1.9	103	0.63
25 	11.87	12.29	1.5	44	0.52
26 	10.98	11.60	1.2	150	0.37
27 	8.57	8.90	1.0	130	0.33

Table 1. Continued

Structure	t_{m1}	t_{m2}	R_s	[SBC]	$\Delta\mu$
28 	No separation				
29 	8.82	9.85	3.3	80	1.50
30 	9.45	10.48	4.0	100	1.46
31 	10.66	11.57	2.3	100	1.03
32 	10.45	11.15	–	100 ^{b)}	
33 	10.63	11.69	3.9	100	1.19
34 	10.00	10.65	1.4	150	0.46
35 	7.35	7.71	2.0	100	0.48

vinyl groups, to *t*-butyl and diphenyl moieties. In this case, the observed effect was different from what was seen for compounds 1–6, where the substituent being examined was attached to a phenyl ring, rather than directly to the stereogenic sulfur. The much bigger *t*-butyl group gave a better separation than either of the smaller methyl or olefinic groups. Compounds 9 and 11 also have very large groups attached to the same site on the stereogenic sulfur, though they are a little further displaced from it by alkyl groups. Nevertheless, this bulk served to enhance the separation of enantiomers just as the *t*-butyl group did. Compound 10 had the greatest enantioseparation with the lowest concentration of chiral selector in the running buffer. Only 20 mg/mL of SBC was required to obtain a resolution of 6.0 and a mobility difference of 3.4 cm²/kV·min. This compound has a diphenyl substituent, providing more bulk next to the stereogenic sulfur than any other compound. This large hydrophobic substituent also caused solubility problems in the aqueous run buf-

Table 1. Continued

Structure	t_{m1}	t_{m2}	R_s	[SBC]	$\Delta\mu$
36 	12.62	13.07	0.7	150	0.20
37 	8.63	9.66	2.9	100	0.74
38 	8.20	9.10	2.5	100	0.72
39 	15.50	18.70	5.4	100	1.55
40 	10.91	11.80	2.7	150	0.96
41 	12.29	13.70	3.1	150	1.18

a) All samples were run at a pH of 8.3 in 10 mM phosphate buffer and +9.00 kV, with the exceptions of 12, 15 and 22, which were run at 13 kV, 11 kV, and 11 kV respectively. SBC concentration is in mg/mL. EOFs were typically in the range of 16 cm²/kV × min.

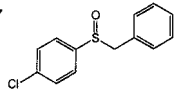
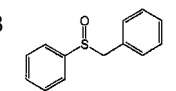
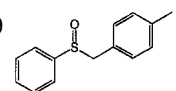
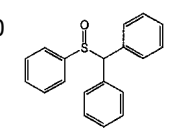
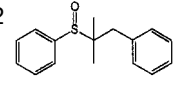
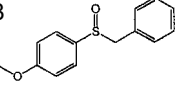
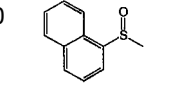
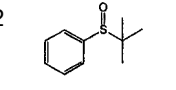
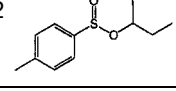
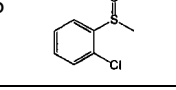
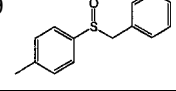
b) This compound had two chiral centers and peaks could not be identified. The two main peak times are listed here.

fers and resulted in the poor electropherogram shown. Clearly though, large, bulky groups in this position have a strong enhancing effect on enantioseparation.

The phenyl group on methyl phenyl sulfoxides can be substituted in the *ortho*-, *meta*-, or *para*-position to produce a series of structural isomers (Fig. 3); see for example, compounds 2, 19, and 41 (methyl substituent); compounds 5, 36, and 40 (bromo substituent), and compounds 4, 34, and 35 (chloro substituent). In every case, the position of the substituent affects enantioresolution (Fig. 3 and Table 1). However, there were no consistent trends except that the *para*-substituted isomer usually gave poorer enantioseparation than the other structural isomers. Thus, both the position and the nature of the substituent (*e.g.*, size, electronegativity, *etc.*) affected the enantioseparation.

A very interesting case arises when compounds 7, 8, 13, and 39 are compared. The electropherograms for each are shown in Fig. 4. These compounds differ only in the

Table 2. Separations with CMBC^{a)}

Structure	t_{m1}	t_{m2}	R_s	[CMBC]	$\Delta\mu$
	22.85	23.45	1.2	20.0	0.040
	17.61	17.94	0.8	20.0	0.038
	20.98	21.43	1.2	20.0	0.036
	29.90	31.33	1.6	20.0	0.056
	32.05	32.55	0.5	20.0	0.017
	20.37	20.84	1.4	20.0	0.040
	17.17	17.95	2.6	20.0	0.091
	27.23	27.66	0.6	20.0	0.021
	10.32	10.82	–	20 ^{b)}	–
	22.83	23.22	0.8	20.0	0.026
	19.08	19.62	1.3	20.0	0.052

a) All samples run at a pH of 5.2, +15 kV, and 10 mM phosphate run buffer. Typical EOFs under these conditions were about $13.5 \text{ cm}^2/\text{kV} \times \text{min}$.

b) This compound had two chiral centers and the peaks could not be identified. The two main peak times are listed here.

type of substituent present on the phenyl ring of each. The presence or absence of a *para*-substituted methyl group or methoxy group (compounds 39, 13, and 8) has a small effect on electrophoretic mobility, but almost no effect on

enantioseparation. However, the presence of a *para*-chloro substituent (compound 7) eliminates any separation. This occurrence provides a reasonable illustration of the factors that govern the enantioselectivity for the compounds in this study. Since the running buffers are all aqueous, it is accepted that a hydrophobic inclusion complex will occur [17, 18]. All of the compounds in this group have, at most, two possible sites for inclusion complexation (*i.e.*, the two organic substituents attached to the stereogenic sulfur (Table 1)). In some cases (*e.g.*, the methyl phenyl sulfoxides, compounds 1–6, 34–36, 14, 40 and 41) it can be argued that the single aromatic substituent is the only possible (or at least a much more favored) site for inclusion complexation.

There are two other noninclusion interaction sites that are identical for all of the chiral sulfoxides and sulfinate esters in this study. They are the oxygen moiety and the lone pair of electrons on the stereogenic sulfur (see structures in Table 1). These are available for hydrogen bonding interactions, for example. Compounds that have two aromatic substituents, or one aromatic substituent plus a comparable (in size or hydrophobicity to an aromatic ring) alkyl substituent, will have two hydrophobic binding or complexation sites. If an assumption is made that the enantioselectivity of the derivatized cyclodextrins for these compounds is different when it complexes with one aromatic substituent *versus* the other, then the enantiomeric separation will be governed largely by two factors (assuming optimal experimental conditions). They are: (i) the difference in enantioselectivity when the cyclodextrin binds to one aromatic group on the chiral sulfoxide *versus* the other substituent, and (ii) the relative amount of time that one aromatic moiety of the sulfoxide molecule is complexed (included) *versus* the other substituent. Any number of possible scenarios could be imagined for this model. Since these are dynamic systems, all of the possible interactions and all of the magnitudes of these various interactions can be treated statistically. In fact, this has been done using statistical mechanics for cyclodextrins and other chiral selectors [19]. While it is beyond the scope of this paper to determine the magnitude and enantioselectivity of every possible interaction between cyclodextrin and a specific chiral sulfoxide, the general model remains useful. For example, an examination of the electropherograms in Fig. 4 reveals several things. Obviously the enantioselectivity of the chloro-substituted compound 7 is considerably less than the other structurally similar compounds. This is entirely due to the presence of the chloro substituent, which mainly affects the enantioselective binding, and therefore, the migration of the second eluting enantiomer (see Fig. 4 and Table 2). It is known from previous reports that halogenated aromatic rings are often preferred for inclusion complexation over

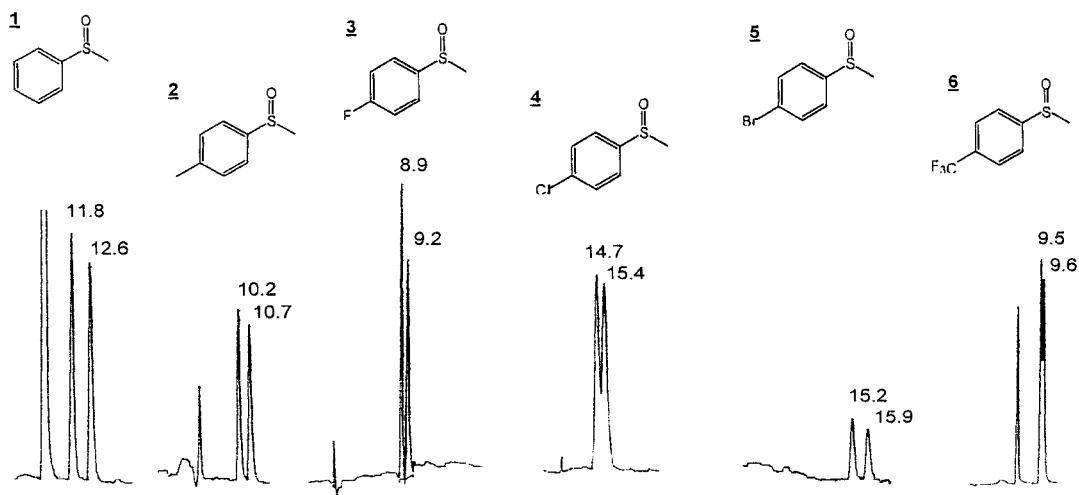


Figure 1. Electropherograms of compounds 1–6. Experimental conditions for all compounds except compound No. 4: 150 mg/mL SBC in 10 mM phosphate buffer, pH 8.3; +9 kV. Compound No. 4 was run at +7 kV.

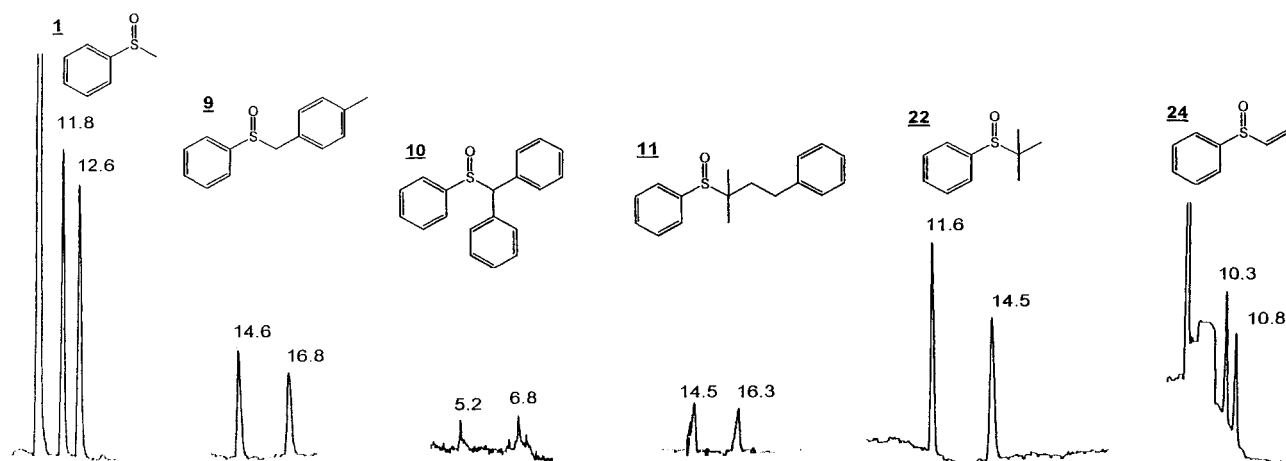


Figure 2. Electropherograms of compounds 1, 9, 10, 11, 22, and 24. See Fig. 1 for experimental conditions.

analogous unsubstituted rings or rings substituted with other groups [18, 20]. Thus, it is likely that when SBC binds to the benzyl substituent of all of these compounds (Fig. 4), it provides good enantioselectivity, while the presence of the chlorophenyl group in compound 7 both reduces the fractional binding to the benzyl group and provides much less pronounced or opposite enantioselectivity.

3.2 Effect of chiral selector concentration

Compounds 9 and 22 can be separated using either SBC or CMBC. Their behavior as a function of chiral selector concentration is representative of this class of chiral compounds. As can be seen from Table 3, both selectors give

better resolution and larger mobility differences when their concentration is increased. Migration times increased as well, since additional amounts of chiral selector increase the ionic strength, increase the viscosity of the solution, and cause the analyte to spend more time in the slower moving (moving opposite to the EOF) complex form [21].

The experimental results obtained here agree well with the discussion and experiments done by Wren and Rowe [21, 22] concerning chiral selector concentration in a simple system, such as the one presented here. The system used in their experiments consisted of a neutral selector and a charged analyte, whereas our system consisted of a charge selector and neutral analytes. However to a first approximation, some of the trends observed for their sys-

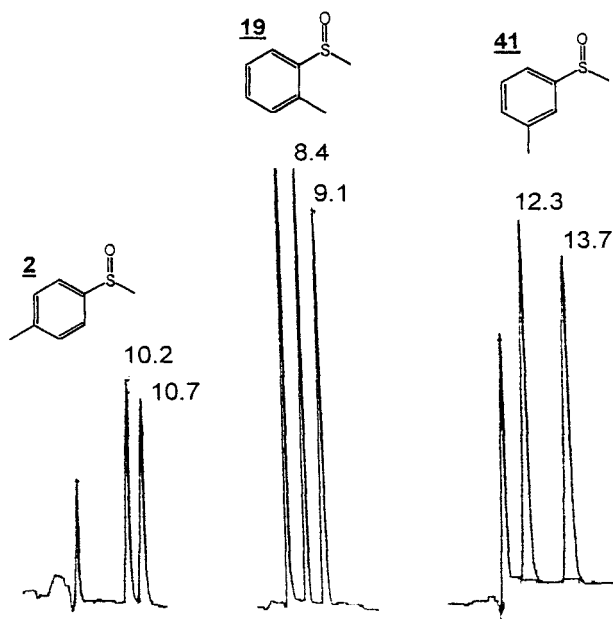


Figure 3. Electropherograms of compounds 2, 19 and 41. See Fig. 1 for experimental conditions.

tem and using their model are analogous to what we observed in the current study. In a recent review, Rizzi [25] outlines the complex equilibria for charged selectors and neutral analytes in depth. Wren and Rowe [21, 22] derived an equation that relates the difference in mobilities of two enantiomers to their association constants, as well as to the chiral selector concentration present in the run buffer.

$$\Delta\mu = \frac{[C](\mu_1 - \mu_2)(K_1 - K_2)}{1 + [C](K_1 - K_2) + K_1K_2[C]^2} \quad (1)$$

As can be seen from the above equation, when [C] is very large or when [C] is equal to zero, the difference in mobilities will be zero as well. A maximum exists for each specific analyte depending on the magnitude of the association constants of the enantiomers. When the association constant between the analyte and chiral selector is small, more chiral selector will be necessary to reach the optimum mobility difference [21]. As discussed previously, about 25–35 mg/mL was the optimum amount of CMBC, while the optimum amount of SBC was 150 mg/mL.

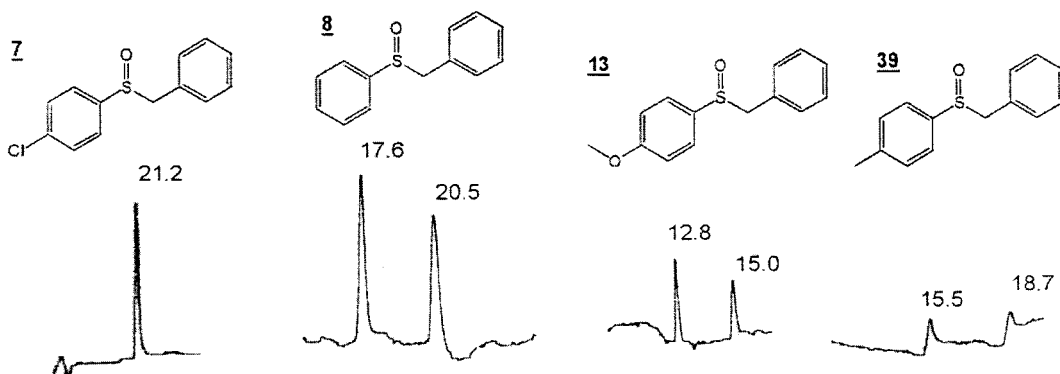


Figure 4. Electropherograms of compounds 7, 8, 13, and 39. See Fig. 1 for experimental conditions.

Table 3. Chiral selector concentration effect^{a)}

	SBC					CMBC				
	(mg/mL) ^{b)}	t_{m1}	t_{m2}	R_s	$\Delta\mu$	(mg/mL) ^{b)}	t_{m1}	t_{m2}	R_s	$\Delta\mu$
	44	6.16	6.38	0.80	0.71	5	3.84	3.87	0.50	0.1
	66	7.83	8.30	1.30	0.91	15	8.81	9.06	0.80	0.16
	88	8.71	9.40	1.40	1.06	20	16.00	16.90	1.20	0.17
	44	7.42	7.93	1.30	1.1	5	4.18	4.26	0.50	0.20
	66	8.25	9.12	1.40	1.46	15	10.20	10.50	0.70	0.13
	88	11.3	13.40	2.20	1.75	20	18.00	19.10	1.00	0.15

a) SBC conditions: +10 kV and pH 8.3. CMBC conditions: +12 kV and pH 5

b) Compound 22 migration times became very long above these concentrations.

Higher concentrations of CMBC required an experimental switch to the reverse polarity mode, in order to observe the peaks. Under these conditions the resolution and mobility difference greatly decreased (data not shown). Additional amounts of SBC led to extremely high currents and very noisy baselines. The sulfated β -cyclodextrin (SBC) is ionized at all pHs and because of the multiple ionogenic groups (and the counter ions) on each cyclodextrin, they contribute significantly to the conductivity of the running buffer. The CMBC has fewer substituents and is weakly acidic. Therefore, due to limiting factors such as conductivity and differing optimal selector concentrations, the two chiral selectors could not be compared under identical conditions. These results also imply that there should be a significant difference in the binding of these chiral sulfoxides to SBC *versus* CMBC.

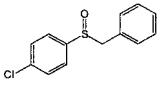
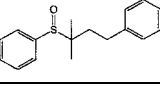
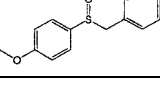
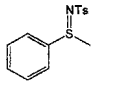
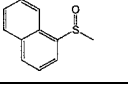
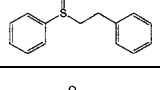
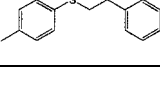
3.3 Binding studies

Binding constants were measured using the methods described by Rundlett and Armstrong [24]. The mobility of the free solute (m_f) and the mobility of the solute at various selector concentrations $[L]$ (m_b) were calculated and plotted using the y -reciprocal and double-reciprocal

methods. The binding constants were obtained directly from these plots. While these methods do not take into account the fact that the chiral selectors are actually mixtures of closely related homologues and isomers or the differences in binding that result from things like increased ionic strength, it does provide a rough estimate of the average binding constants. The results for several selected compounds are shown in Table 4. The ratio of the individual binding constants gives α (the enantioselectivity factor), and provides a means to directly compare the separating capabilities of each chiral selector. The binding constants for the enantiomeric compounds to SBC were relatively small and within a narrow range (*i.e.*, $K_b = 10$ – 60). Binding constants of the same analytes to CMBC were approximately an order of magnitude larger (Table 4). The estimated average binding constants seem reasonable given the large difference between the binding constants of these sulfoxide analytes with the two chiral selectors. Also, they provide the most accurate way to evaluate enantioselectivity (*i.e.*, *via* calculation of α).

The binding constant results support many of the experimental observations. As mentioned in the previous discussion, compounds that display smaller binding con-

Table 4. Binding constants for selected compounds

Compound	Method	SBC			CMBC		
		K_1	K_2	α	K_1	K_2	α
7 	y -reciprocal	20	–	–	640	738	1.15
	double-reciprocal	20	–	–	560	658	1.18
11 	y -reciprocal	27	31	1.15			
	double-reciprocal	29	33	1.14			
13 	y -reciprocal	19	22	1.16			
	double-reciprocal	18	21	1.17			
17 	y -reciprocal	39	40	1.03			
	double-reciprocal	33	34	1.03			
20 	y -reciprocal	15	20	1.33	201	226	1.13
	double-reciprocal	22	28	1.27	175	205	1.17
37 	y -reciprocal	51	61	1.20			
	double-reciprocal	34	40	1.18			
38 	y -reciprocal	20	23	1.15			
	double-reciprocal	19	22	1.16			

starts require larger amounts of chiral selector to reach an optimum mobility difference. Indeed, CMBC with a much larger binding constant required significantly less selector to achieve optimum separation conditions (Tables 1 and 2). The effective mobilities obtained using CMBC were also much longer than those obtained with SBC (not shown). This was also due to the larger binding constants, which caused the compounds to spend more time as the slower moving CMBC/analyte complex, than as the free analyte. On the other hand, the sulfoxide compounds exhibited poorer enantioseparation due to the smaller difference in the binding constants of the enantiomers to CMBC. This clearly shows that it is not the magnitude of the binding constants that make effective chiral selectors, but rather the difference in the magnitude of the enantiomeric binding constants (*i.e.*, α) as well as other factors that affect efficiency. Indeed, SBC which exhibited smaller overall binding constants, separated a greater number of the chiral compounds examined in this study, and usually with higher efficiency and greater selectivity than did the more strongly binding CMBC (Tables 1, 2, and 5).

3.4 Organic modifier effect

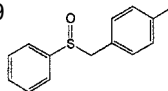
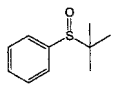
Addition of organic modifier is also a well known way to affect chiral separation in CE [22]. In fact, in some cases in which it is the only way to obtain a separation [23]. While the resolution change with added organic modifier was not as significant as when the chiral selector concentration was altered, the mobility difference was very evident. As can be seen from Table 5, when no methanol is present a fairly good separation is obtained, but upon addition of methanol, the resolution decreases and continues to worsen slightly as more methanol is added. The same trend is even more evident for the mobility differ-

ences. This is the case for both chiral selectors. This phenomenon can be explained by referring back to the association constants and the work of Wren and Rowe [22]. As discussed in the previous section, when more chiral selector is added to the run buffer the analyte spends more time as the complexed form. If the optimum concentration has not been reached, then the analyte requires more chiral selector (more time as the complexed form). If the optimum concentration has been passed, then the analyte requires less time as the complexed form. It has been established that addition of organic modifier to the run buffer decreases the association constant for some analyte/chiral selector systems (especially these where hydrophobic interactions are important). Hence, if the optimum chiral selector concentration has not been reached, the addition of more organic modifier will decrease the mobility difference. The opposite trend is observed if the optimum concentration has been passed [22]. The concentration of both chiral selectors that were used in this study were below the optimum concentration, so a decrease in the mobility difference was not unexpected.

3.5 pH effect

pH can affect the separation by changing the charge of the analyte and/or the chiral selector. It can also slow the EOF, which affords more time for a separation to occur. As mentioned earlier, the compounds in this study lacked any ionizable groups, therefore it was necessary to use charged chiral selectors. SBC can be protonated only at pH below ~ 2 . The carboxy groups on CMBC have a pK_a of about 5. Of course, going to this low a pH is counter productive since it would drastically lower the EOF (which

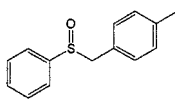
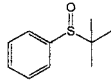
Table 5. Organic modifier effect^{a)}

	SBC					CMBC				
	[methanol] ^{b)}	t_{m1}	t_{m2}	R_s	$\Delta\mu$	[methanol] ^{b)}	t_{m1}	t_{m2}	R_s	$\Delta\mu$
	0%	6.40	6.70	1.70	0.77	0%	5.53	5.62	0.60	0.14
	10%	10.80	11.20	1.00	0.42	10%	5.29	5.35	0.50	0.11
	20%	16.14	16.43	0.80	0.14	20%	7.17	7.23	0.50	0.06
	0%	7.03	7.53	2.90	1.19	0%	6.15	6.28	0.60	0.17
	10%	13.50	14.50	1.10	0.65	10%	5.90	6.00	0.50	0.14
	20%	15.70	16.30	0.80	0.30	20%	7.88	8.04	0.40	0.12

a) SBC conditions: +10 kV, 60 mg/mL SBC in 10 mM phosphate, pH 8.3. CMBC conditions: +12 kV, 10 mg/mL CMBC in 10 mM phosphate, pH 5

b) By volume

Table 6. pH effect^{a)}

	SBC					CMBC				
	pH	t_{m1}	t_{m2}	R_s	$\Delta\mu$	pH	t_{m1}	t_{m2}	R_s	$\Delta\mu$
9 	5.8	10.40	11.40	2.00	1.01	3.0	8.47	8.70	0.70	0.16
	6.9	9.57	10.70	1.50	1.39	5.0	5.76	5.87	0.70	0.16
	8.3	8.71	9.40	1.40	1.07	8.0	2.58	–	0.00	0.00
22 	5.8	15.40	18.90	2.50	1.52	3.0	8.96	9.19	0.60	0.14
	6.9	11.70	14.10	2.20	1.89	5.0	7.61	7.79	0.60	0.15
	8.3	11.30	13.40	2.20	1.72	8.0	2.78	–	0.00	0.00

a) SBC conditions: 88 mg/mL SBC in 10 mM phosphate, +10 kV. CMBC conditions: 10 mg/mL CMBC in 10 mM phosphate, +12 kV

is necessary to detect the compounds in a reasonable amount of time) and the selector would no longer produce an enantiomeric separation (neutral analytes require charged selectors). This combination of factors led to the conclusion that changing pH (in the range of 5–9) would not have a large effect on the separation of these compounds. Table 6 shows the results. There was no noticeable improvement in resolution; however, migration times continually shortened as pH was raised, due to the increase of the EOF velocity. There is no clear trend in the mobility differences.

4 Concluding remarks

Forty-one chiral sulfoxides and sulfinate esters were examined using SBC and CMBC. SBC was able to separate a far greater number of compounds than CMBC. Since such a large number of compounds could be separated using SBC, several structural effects relating to separation could be observed. The most notable effect was the addition of large bulky groups next to the stereogenic sulfur, which greatly enhanced the enantiomeric peak to peak separation. The binding of these compounds to each chiral selector was also studied, and it was found that though SBC had much smaller binding constants to the chiral sulfoxides, it had greater selectivity than CMBC. Higher concentrations of chiral selector enhanced mobility differences for both selectors until a maximum was reached. Addition of organic modifier slightly decreased resolution and mobility differences, since the chiral selector concentration was at or below the optimum level. Altering pH and did not have a significant effect on enantioseparation (at least in the ranges used in this study).

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