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Enantiomeric Separation of Sulfonium Ions by Capillary Electrophoresis Using Neutral and Charged Cyclodextrins

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Capillary zone electrophoresis was successfully applied, for the first time, to the chiral separation of structurally related sulfonium ions, using sodium phosphate buffer pH 2.5 with β-cyclodextrin (β-CD) or sulfated-β-cyclodextrin (S-β-CD) as the chiral selector with tetrabutylammonium bromide (TBA). For this study, a series of structurally related sulfonium ions in which one of the alkyl chains varied in length were synthesized from a common sulfide. The resolution of the ions was found to be dependent on the type of cyclodextrin used, the presence or absence of TBA, and the structure of the sulfonium ion. β-CD was found to be effective only for ions containing two aromatic groups, while the S-β-CD was effective only for ions containing one aromatic group. The chiral separation of thiophenium ions was also studied under the conditions established. Chiral separation of sulfonium ions in a binary buffer system containing methanol was explored as well. Separations were achieved for all but one sulfonium ion and one thiophenium ion. The data presented show the effectiveness of the enantiomeric separation using CZE with cyclodextrin-modified buffer for these types of ions.

The analysis of tertiary sulfonium compounds using currently available methods often encounters drawbacks in terms of efficiency and sensitivity. Previously reported procedures for analysis include ion chromatography,[1] liquid chromatography,[2] paper electrophoresis,[3] and thin-layer electrophoresis.[4] The use of capillary zone electrophoresis (CZE) offers an unprecedented analytical advantage over these published methods. Valenzuela et al. have reported preliminary data concerning the analysis of sulfonium compounds using CZE and determined the technique was both rapid and highly effective.[5]

Little has been reported on the study of chiral sulfonium salts, and only a handful of these compounds have actually been assigned absolute configurations.[6] Although racemic mixtures of sulfonium salts have found certain applications, i.e., cationic initiators of polymerization,[7−9] the importance of chiral sulfonium salts has yet to be fully appreciated. Chirality around a sulfonium center is often found to play an integral role in biological systems. It has been determined that methyltransferases use substrates containing chiral sulfonium centers as their methyl donors.[10] *S*-Adenosyl-L-methionine is an important methyl donor that has such a center, and it has been additionally established that preference is given to one enantiomeric conformation over the other.[11−13] One of the most promising applications investigated so far is the use of optically active sulfonium ions as asymmetric alkylating agents.[14] The synthetic value of such an agent is evident. The synthesis of enriched or optically pure enantiomers requires the use of an analytical method to assess the enantiomeric excess. Conventional methods, i.e., polarimetry, for the analysis may not prove to be satisfactory. Although an NMR procedure for determining enantiomeric excess using chiral shift reagents has been developed,[15] it requires extended sample preparation time and the use of expensive shift reagents.

CZE has been shown to be a very effective analytical technique for a wide range of compounds and, therefore, has received considerable attention in efforts to separate difficult mixtures and racemic compounds. Separation of analytes in CZE is achieved by the difference in the net electrophoretic mobility of charged compounds under the influence of an electric field. The migration time will vary according to the mass-to-charge ratio of the species. Neutral compounds can also be analyzed, but typically this is achieved via different electrophoretic methods, i.e., micellar electrokenetic chromatography (MEKC).[16] A very important area of application is the enantiomeric separation of optically active

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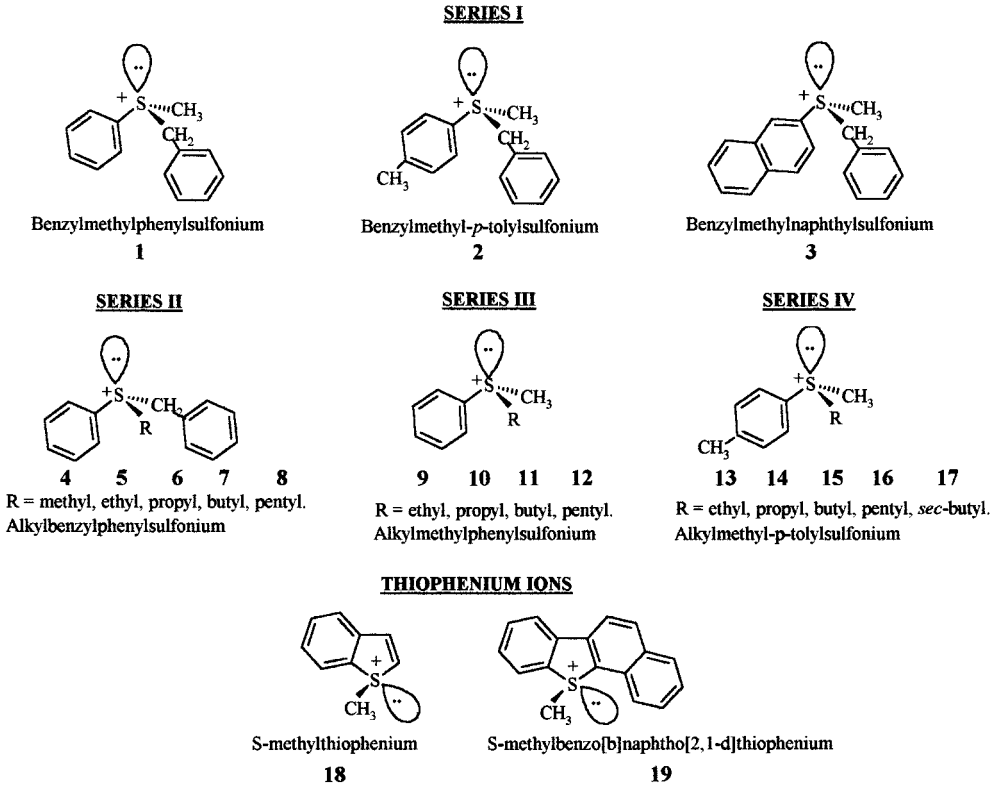

Figure 1. Molecular structures and names for the ions used in the study. All compounds are BF_4^- salts.

compounds. The significance of the different activity of enantiomers in biological systems is well understood. Stereospecific reactions are carried out preferring one enantiomeric form of a substrate or drug over the other. Thus, enantiomeric separations are of principal importance in the pharmaceutical analysis of chiral drugs.[17,18] Enantiomeric separation is typically achieved by the addition of a chiral resolving agent such as chiral surfactants, crown ethers, bile salts, or cyclodextrins to the separation buffer.[19,20]

The most versatile chiral resolving agents used thus far in CZE are the cyclodextrins (CDs). The most common CDs, α-, *β*-, or *γ*-forms, are able to resolve chiral compounds by forming differentiated, transient inclusion complexes with the two enantiomers dissolved in the separation buffer. The degree of the interaction changes the migration time of the enantiomers enough to achieve separation. Cyclodextrins have been used extensively for the enantioseparation of drugs of pharmaceutical importance.[21−25] Although *β*-CD appears to be the most commonly used chiral selector, it is somewhat limited in the types of structural compounds it can resolve. Further derivatization of the native CDs can greatly enhance their selectivity and specificity. For example, typical commercial CDs development kits include *β*-cyclodextrin, 2-hydroxypropyl-*β*-cyclodextrin, heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin, heptakis(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin, and *γ*-cyclodextrin which can be used to optimize specific analyses.[26]

In this report, we describe, for the first time, the effectiveness of *β*-CD-modified enantiomeric separation of chiral sulfonium ions using CZE. In this study, we investigated the use of *β*-CD and sulfated-*β*-cyclodextrin (S-*β*-CD) with buffer additives of tetrabutylammonium bromide (TBA) and methanol in an effort to separate chiral sulfonium ions. Four series of chiral sulfonium ions with varying alkyl chain lengths (Figure 1) were synthesized from a common parent sulfide by alkylation with the appropriate iodoalkane. The extent of enantiomeric resolution for each racemic mixture was found to be dependent on the type of cyclodextrin used, buffer additive, and the general structure of the sulfonium ion. Additionally, the enantiomeric separation of chiral thiophenium ions was also briefly explored using the conditions established for the chiral sulfonium ions.

EXPERIMENTAL SECTION

Materials. *β*-Cyclodextrin, sulfated-*β*-cyclodextrin (degree of substitution 7−11), acetonitrile, HPLC grade methanol, tetrabutylammonium bromide, dichloroethane (DCE), all sulfides, thiophenes, and iodoalkanes were purchased from Aldrich Chemical Co. (Milwaukee WI) and were used without further purification. Silver tetrafluoroborate was purchased from Alfa Aesar (Ward Hill, MA). Sodium phosphate buffer (0.1 M, pH 2.5), was obtained from Bio-Rad (Hercules, CA). Dichloroethane was kept

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dry over 4-Å molecular sieves. HPLC grade water was used for injection sample preparation.

Instrumentation. The CZE separations were accomplished using a BioFocus 3000 capillary electrophoresis system from Bio-Rad. An uncoated fused silica capillary column of 50 μm i.d., with a total length of 50 cm (45.4 cm to detector window), mounted on a user-assembled cartridge was used. Sample injection was achieved using the pressure mode set at 2 psi·s. The working potential was operated at 17 kV (+ to −, constant voltage), and the current limit was set at 100 μA. The cartridge and carousel temperature was preset to 20 °C. The detection wavelength was fixed at 220 nm. The capillary column was cleaned after each run by flushing for 2 min with the running buffer, followed by flushing for 5 min with deionized water. Prior to storage, the column was purged with nitrogen for 2 min. It was necessary to clean the electrodes every five consecutive runs to remove adsorbed salts by dipping them in methanol for 2 min.

Synthesis of Sulfonium Salts. A modification of the procedure developed by Acheson and Harrison[27] was used for the preparation of the sulfonium and thiophenium salts. In this procedure, 1 mmol of the appropriate sulfide or thiophene and 1.2 mmol of the corresponding iodoalkane are dissolved in approximately 2 mL of dry DCE in a 5-mL conical vial. A DCE solution of silver tetrafluoroborate (1.2 mmol of $AgBF_4$ dissolved in 1 mL of DCE) is then added dropwise over a 5-min period. The mixture is then stirred overnight. The yellow AgI precipitate is centrifuged and washed twice with 1 mL of acetonitrile. The solvents are then removed by rotary vaporization at 40 °C. The resulting oil or crystal is dried under a vacuum overnight. After the product is thoroughly dry, the structure is confirmed by NMR analysis using acetone-d_6 as the solvent.

Sample and Buffer Preparation. Stock solutions of the compounds were prepared by dissolving 10−20 mg of the sulfonium salts in 1 mL of acetonitrile. The solutions were stored at 4 °C. The buffers used were prepared by dissolving TBA, β-CD, and S-β-CD, in the required amounts, directly into the standard 0.10 M phosphate buffer (pH 2.5) or the 30% methanol (v/v) 0.10 M phosphate buffer (pH 2.5). The 30% methanol/phosphate buffer required the addition of NaH_2PO_4 to compensate for the dilution of the 0.10 M phosphate buffer by the addition of methanol and the final salt concentration was brought back up to 0.10 M. All buffers made were filtered through a 0.45-μm membrane filter (Gelman Sciences) prior to use. The injection samples were prepared by diluting the necessary aliquot of the stock solution with a 50/50 (v/v) water/appropriate running buffer for each run to yield a final concentration of 2.5 mM. Resolution of selected ions was calculated by using the equation

$$R_s = 2[(t_2 - t_1)/(w_2 + w_1)] \quad (1)$$

where t is the migration time of the enantiomers and w is the peak width.[22]

RESULTS AND DISCUSSION

The general structures for the synthesized series of sulfonium ions and the two thiophenium ions are shown in Figure 1. For convenience, all compounds synthesized included at least one chromophore, this group not only facilitates detection in the UV range but also plays a central role in the currently accepted model of inclusion interaction within the cyclodextrin cavity.[28−31] All sulfonium salts are stable for analysis in an acidic buffer. However, the thiophenium ions, particularly *S*-methylthiophenium, are subject to nucleophilic attack by water molecules[27] and thus slowly revert back to the corresponding thiophene after addition to the buffer. The instability did not present a problem in the time frame of the analysis. It has been shown that a strong interaction occurs between β-CD and dibenzylmethylsulfonium tetrafluoroborate. Studies revealed that the transformations sulfonium ions undergo when subjected to alkaline conditions in the absence or presence of β-CD are different. Dibenzylmethylsulfonium, for example, typically undergoes Stevens rearrangement to give a 1,2-diphenylethane derivative, but in the presence of β-CD it undergoes a Sommelet rearrangement to give a diphenylmethane derivative.[32] As a result of this strong interaction, β-CD was chosen as the resolving agent for chiral separation. The first set of chiral separations attempted were carried out using standard parameters: acidic pH (2.50) and an uncoated capillary with varying concentrations of β-CD in 0.1 M phosphate buffer. Sulfonium ions containing both a benzyl and a phenyl group were initially investigated because these groups are believed to have the strongest interaction within the β-CD cavity.[32]

Under acidic conditions, the electroosmotic flow (EOF) is directed toward the cathode. EOF velocities in phosphate buffers, from pH 2.00 to 8.00, have been well established by St. Pierre and Sentell.[33] They showed that, at low pH, the EOF velocity is relatively low. The uncharged β-CD molecules move with the velocity of the EOF and thus can be considered to form a pseudostationary phase.[21] The net mobility of positively charged species, which also migrate toward the cathode, is thus the summation of the electrophoretic mobility of the ions and the EOF. It has been shown that a number of buffer additives, particularly tetraalkylammonium (TAA) salts and organic solvents, alter the polarity and the viscosity of the buffer system, resulting in a lowering of the EOF.[21,34,] The alteration of the EOF thus affects the net electrophoretic mobility of the analytes, often improving resolution but at the expense of analysis time. Reports show that some chiral separations are achieved only in the presence of these modifiers. Short-chain tetraalkylammonium salts effectively suppress or even reverse the EOF in the low pH range.[21,34] Quang and Khaledi established EOF velocities in the presence of different TAA salts. They found that a phosphate buffer at pH 2.5 containing 50 mM TBA showed a low magnitude reversal in EOF.[34] Under these conditions, the EOF counteracts the migration of the enantiomers, thus improving resolution. Figure 2A shows the electropherogram of a mixture of ions **1** and **2**, from series I, with 5 mM β-CD added to the phosphate buffer. In the absence of TBA, incomplete resolution of these ions results. Figure 2b shows the effect of adding 50 mM TBA for the

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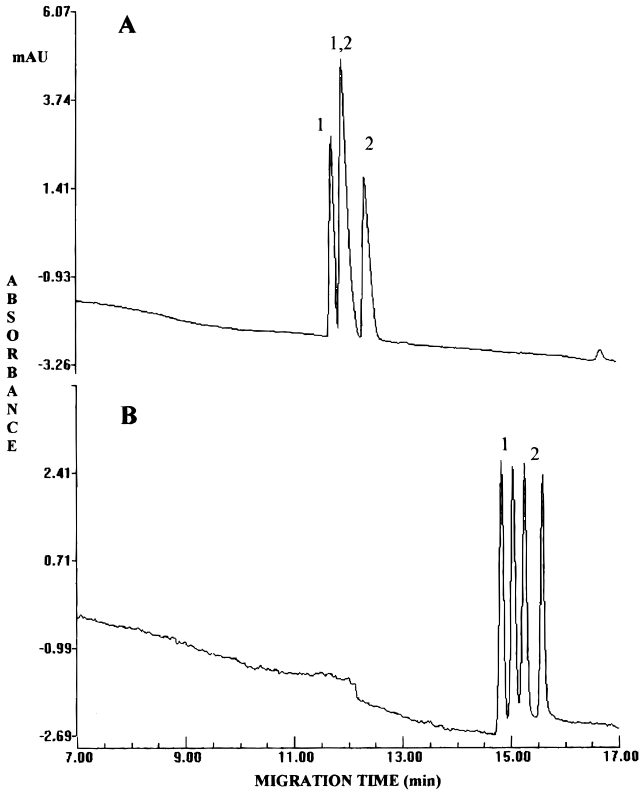

Figure 2. Electropherograms showing the chiral separation of ions **1** and **2**. Conditions: capillary, uncoated fused silica 50 μm i.d. × 50 cm (45.4 cm effective length); voltage, 17 kV; injection, 2 psi•s; current limit, 100 μA; temperature, 20 °C; detection, 220 nm; concentration of sulfonium ions, 2.5 mM; buffer, 5 mM β-CD in 0.1 M sodium phosphate buffer pH 2.5. Separation (A) without TBA and (B) with 50 mM TBA.

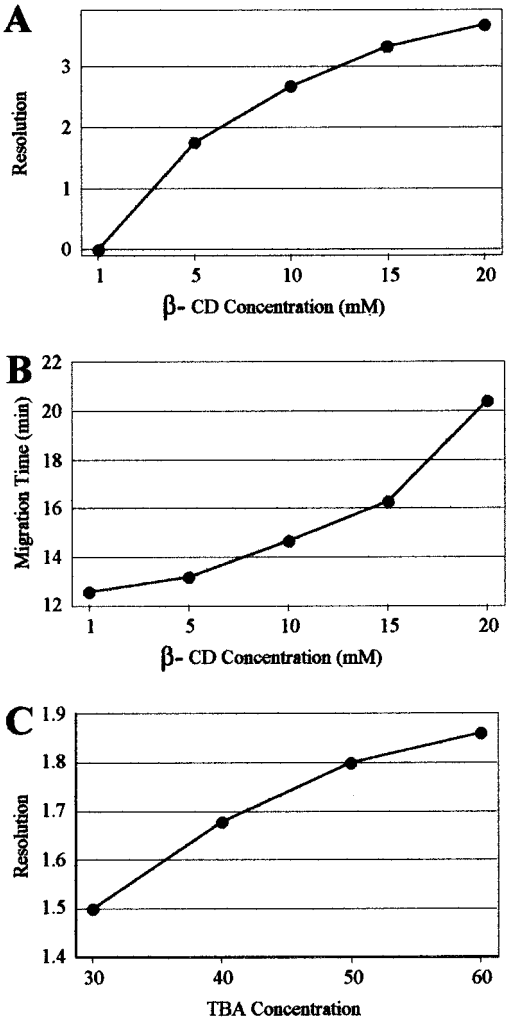

Figure 3. Impact of β-CD concentration on the resolution of ion **2** holding TBA concentration constant at 50 mM, (A) Resolution vs migration time, (B) migration time of the first-eluted enantiomer vs β-CD concentration, and (C) effect of TBA concentration on resolution on ion **2**, plot of the resolution vs migration time, holding β-CD concentration constant at 5 mM. Other conditions are as described for Figure 2.

separation of the same sulfonium ions, **1** and **2**. The two sets of enantiomers are clearly separated, and peak symmetry is dramatically improved, as is the resolution of each pair of enantiomers. The sulfonium ion containing the *p*-tolyl group yielded better resolution, presumably due to a stronger interaction with the β-CD cavity. As expected, an increase in migration time due to the reversed EOF is also observed.

Effects of TBA and β-CD concentration. Several factors make TBA a desirable additive. The low conductivity of the salt allows the use of a wide range of concentrations, it increases the solubility of β-CD and, the low hydrophobicity prevents micelle formation as well as minimizes competitive effects with the positively charged analytes for the β-CD cavity.[21] The combined effects of varying concentrations of the buffer additives were explored. Figure 3A shows the effects of varying β-CD concentrations, while holding TBA constant at 50 mM, on the resolution of a single sulfonium ion, benzylmethyl-*p*-tolylsulfonium ion (**2**). No separation was observed using a 1 mM β-CD concentration; however, baseline resolution can be achieved easily with a β-CD concentration as low as 5 mM. It is evident that the resolution increases substantially as the β-CD concentration is increased. The trade-off between higher resolution and analysis time is clearly seen in Figure 3B, which shows the migration time of the first-eluted enantiomer of ion **2** versus β-CD concentration. The migration time of the enantiomer increases dramatically at higher β-CD concentrations. Figure 3C shows the effects of varying TBA concentrations, while holding β-CD constant at 5 mM, on the resolution of the same ion. A gradual increase in resolution is observed as the concentration of TBA increases as well as a small corresponding increase in migration time.

Substituent Effects on Resolution. The first series of ions studied, **1**−**3**, differ only in the size of one of the aromatic groups. Figure 4 shows the separation of all three ions from this series using 5 mM β-CD and 50 mM TBA. These concentrations were chosen to minimize analysis time while still affording good resolution. Resolution increases in the order phenyl < *p*-tolyl < naphthyl substituent. The enhanced resolution of ion **3** may be attributed to a greater degree of interaction of the hydrophobic naphthyl substituent with the β-CD cavity.

To further study substituent effects, series II, ions **4**−**8**, were synthesized. In this particular series, the aromatic substituents are kept constant while the length of the alkyl chain is increased from methyl to pentyl. Separations of these five ions are shown in Figure 5. Figure 5A shows the separation carried out in the absence of TBA with 5 mM β-CD. Although chiral resolution is

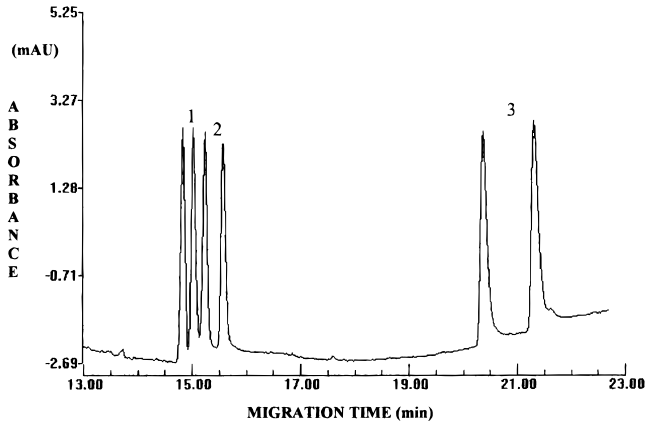

Figure 4. Electropherogram showing the chiral separation of series I, ions **1**−**3**, using 5 mM β-CD and 50 mM TBA. Other conditions are as described for Figure 2.

achieved, peak tailing and only partial resolution for ion **5** is observed. The addition of TBA, Figure 5B, results in improved resolution of the same five ions as expected, as well as increasing the migration time of each set of enantiomers by about 2 min.

A general trend in resolution can be observed under these conditions: that is, as the alkyl chain lengthens, the resolution decreases. The diminishing resolution can be attributed, in part, to decreased interactions within the β-CD as the alkyl chain lengthens. It is possible that the longer alkyl chain sterically hinders the interaction between the aromatic groups with the hydrophobic cavity of β-CD. In the absence of this steric interaction, the resolution should increase as the length of the alkyl group increases due to column residence time alone. However, since resolution clearly decreases, it is concluded that the alkyl chain is actively involved in the interaction.

Effect of Methanol. The general solubility of these particular types of sulfonium ions is not very large in an aqueous buffer system. The addition of organic solvents (10−80%) has been used to enhance the solubility and also to improve CZE separations of highly hydrophobic substances. Among the solvents used are methanol, acetonitrile, 1-propanol, THF, ethanol, and 2-propanol.[35,36] The improved solubility of analytes in a binary buffer system of this type significantly improves peak shape, thus potentially enhancing resolution. An increase of the EOF at low pH has been noted on previous studies when organic modifiers of this type have been employed.[37] The effects of methanol on the chiral separation of the sulfonium ions was briefly explored to determine whether an enhancement on the overall chiral separation was, in fact, observed. Figure 5c shows the separation of ions **4**−**8** using a buffer containing 50 mM TBA, 5 mM β-CD, 30% (v/v) methanol, and 0.1 M phosphate (pH 2.5). It is obvious from Figure 5C that chiral selectivity was completely lost by the addition of methanol. Szökő et al. reported a similar effect: that is, a lowering of binding constants, and thus resolution, was observed for drug enantiomers as the result of increasing the concentration of methanol.[36] The enhanced solubility of the ions in the binary buffer system essentially eliminated the hydrophobic interaction within the β-CD cavity necessary for chiral separation.

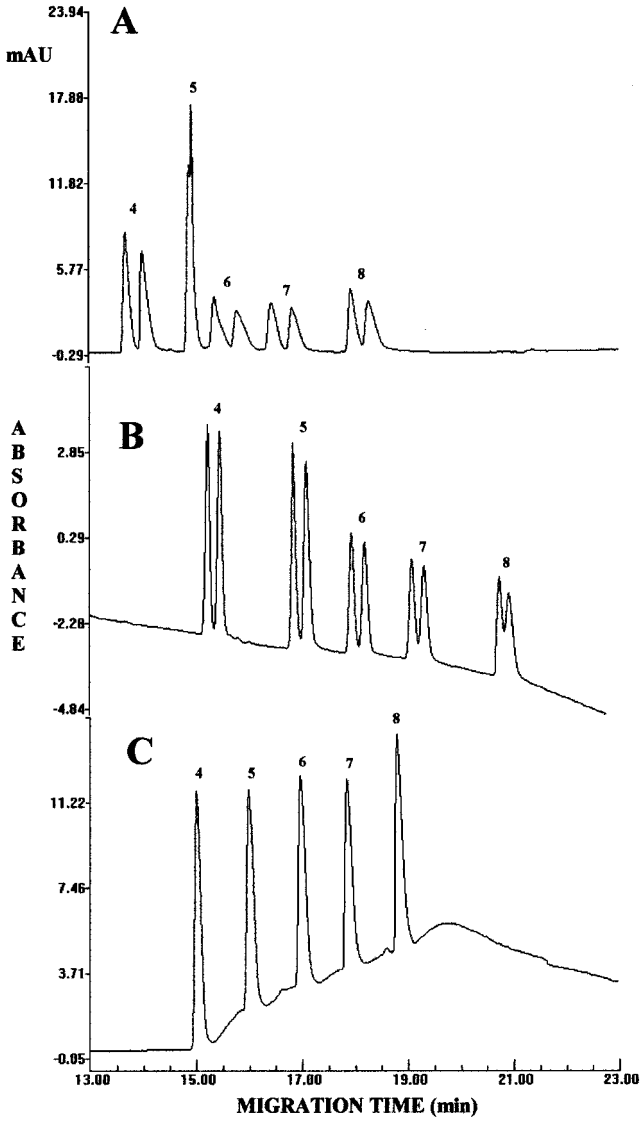

Figure 5. Electropherograms showing the chiral separation of series II, ions **4**−**8**, (A) with 5 mM β-CD, (B) 5 mM β-CD and 50 mM TBA, and (C) with 5 mM β-CD, 50 mM TBA, and 30% methanol. Other conditions are as described for Figure 2.

Use of Sulfated-β-Cyclodextrin. Series III, ions **9**−**12**, and series IV, ions **13**−**17**, were synthesized next to study the extent of interaction of a lone aromatic group and two alkyl substituents with the β-CD cavity. Again, two of the groups were held constant, aromatic and methyl substituents, while the second alkyl chain increased from ethyl to pentyl. Surprisingly, no chiral separation was achieved when using β-CD, even at high concentrations, >20 mM, in the absence or presence of TBA (data not shown). A negatively charged cyclodextrin was postulated to have a greater affinity for sulfonium ions, in general, due to the additional electrostatic attraction of opposite charges. Sulfated-β-cyclodextrin was obtained for evaluation as a potential chiral selector for these particular ions. Figure 6A shows the separation of series III, ions **9**−**12**, with S-β-CD. An approximate concentration of 4 mM was used since the molecular mass is not accurately known due to the nature of the product, a mixture of randomly substituted molecules. It was necessary to keep the concentration relatively low to avoid exceeding the current limit of 100 μA and to keep Joule heating at a minimum. Although it is possible to work at

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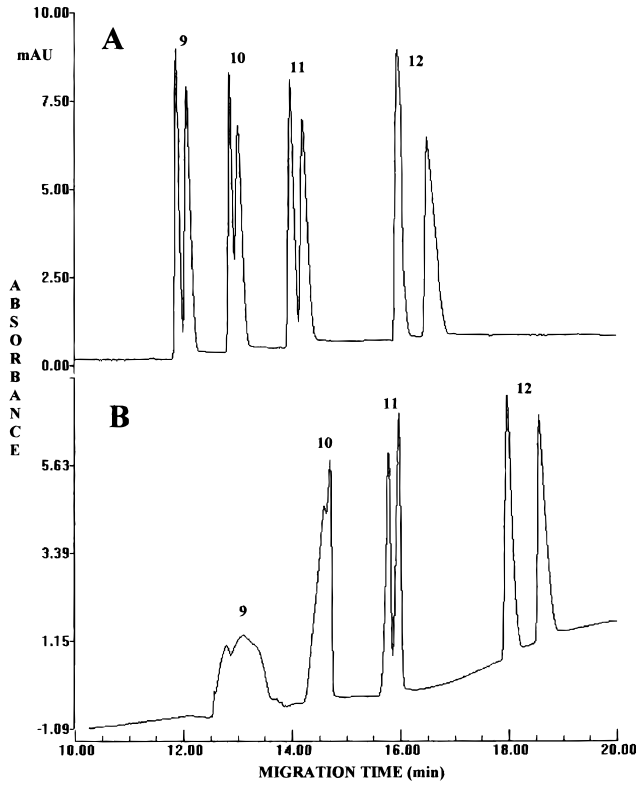

Figure 6. Electropherograms showing the chiral separation of series III, ions **9**−**12**, using (A) 4 mM S-β-CD and (B) 4 mM S-β-CD and 50 mM TBA. Other conditions are as described for Figure 2.

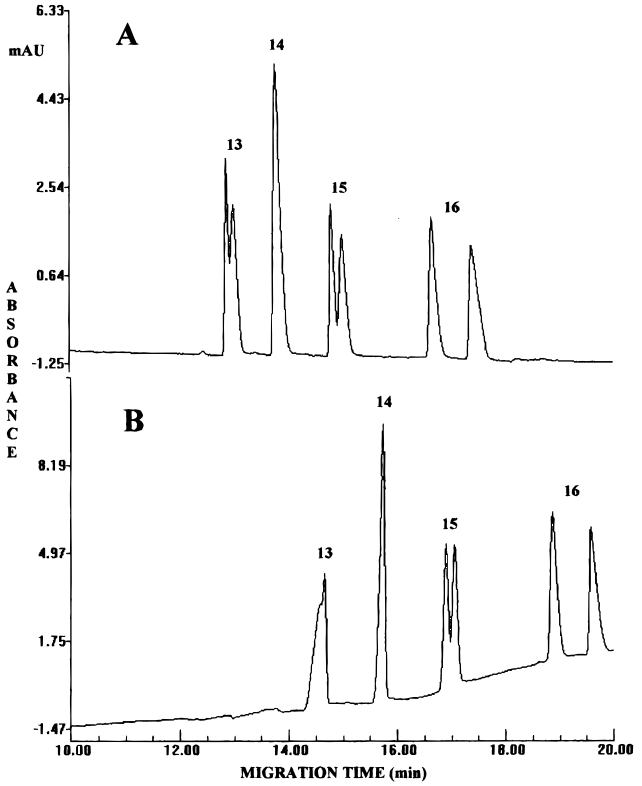

Figure 7. Electropherograms showing the chiral separation of series IV, ions **13**−**16**, using (A) 4 mM S-β-CD and (B) 4 mM S-β-CD and 50 mM TBA. Other conditions are as described for Figure 2.

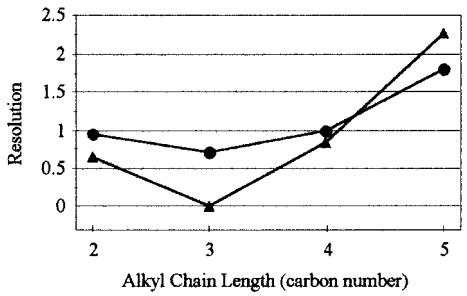

Figure 8. Plot of the resolution vs alkyl chain length for series III (●) and series VI (▲) using the conditions described for Figures 7 and 8.

higher concentrations of S-β-CD by switching to reversed polarity or reversed mode,[38,39] it was deemed unnecessary for this study since, even at this low concentration of 4 mM, S-β-CD was able to adequately resolve each pair of enantiomers. Although some peak tailing is observed, the overall resolution is acceptable. No obvious trend is observed for this series. The resolution decreases in going from ion **9** to **10**, and then it increases, with ion **12** having the greatest resolution, as shown in Figures 6A and 8. Ion **10**, with the propyl substituent, showed the lowest resolution. It possible that the overall diameter of the structure imparts a similar degree of interaction for the two enantiomers with S-β-CD or that there is a reversal in binding constants when going from propyl to butyl substituent. The lack of nonracemic standards prohibited experimental verification of this hypothesis.

TBA was tested to determine whether any improvement is observed upon its addition. Figure 6B shows the separation of the same ions in the presence of 50 mM TBA. The effect was detrimental to the chiral analysis for ions containing the smaller ethyl and propyl substituents. As shown, complete loss of resolution was observed for ions **9** and **10**. The observed results can, perhaps, be caused by competitive effects between the positively charged TBA and the sulfonium ions, having small alkyl substituents, for the negatively charged S-β-CD cavity due to the strong electrostatic attraction. Ions **11** and **12** were relatively unaffected in terms of resolution.

Figure 7A shows the separation of series IV with 4 mM S-β-CD, with the exception of ion **17**, which was analyzed individually.

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The overall separation looks similar to that of series III, in that there is a minimum in resolution when the alkyl group is propyl (ion **14**), as shown in Figures 7A and 8. In fact, there is no chiral resolution for ion **14** at all. This result may be explained by the enantiomers of ions **14** having virtually the same degree of interaction with the S-β-CD. Ion **14** was not resolved under any of the conditions studied thus far. Figure 7B shows the effect of adding 50 mM TBA to the same separation buffer. The resolution remained virtually the same for the analytes aside from ion **13**, having the small ethyl substituent, whereby resolution decreased.

All of the sulfonium ions **1**−**16** possess a chiral sulfur center. The successful resolution of all but one of these sulfonium ion suggested that a sulfonium ions possessing two chiral centers might be resolved into four stereoisomers. To test this hypothesis, ion **17** was synthesized to contain two adjacent chiral centers, an additional chiral carbon present on the *sec*-butyl group. If there is a strong interaction at a chiral carbon, the S-β-CD should be

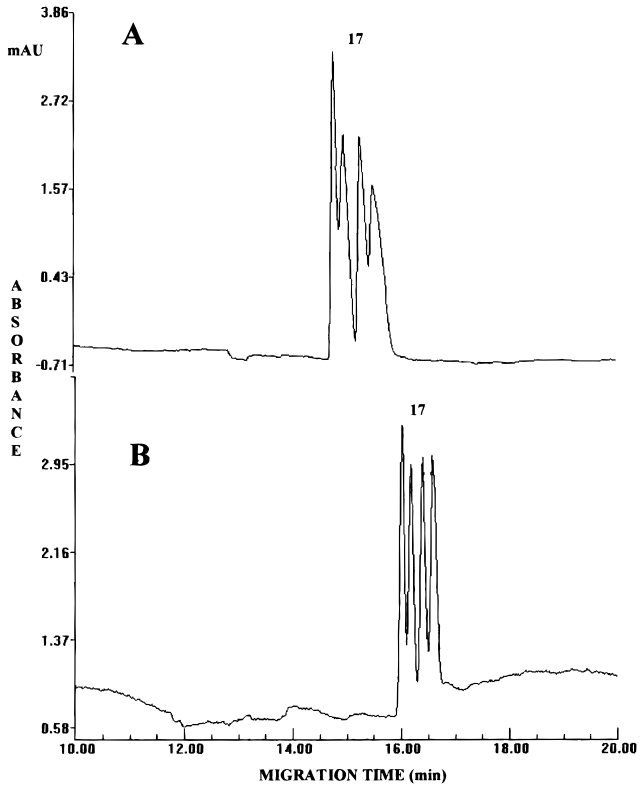

Figure 9. Electropherograms of the chiral separation of ion **17** showing all four enantiomer peaks, (*R*,*R*), (*S*,*S*), (*R*,*S*), and (*S*,*R*), not necessarily in that order, using (A) 4 mM S-β-CD and (B) 4 mM S-β-CD and 50 mM TBA. Other conditions are as described for Figure 2.

able to recognize and resolve all four stereoisomers: (*R*,*R*), (*S*,*S*), (*R*,*S*), and (*S*,*R*). Figure 9A shows the separation of ion **17** using 4 mM S-β-CD. Although the four stereoisomers are separated, they are not well resolved. The addition of 50 mM TBA improved resolution considerably, as shown Figure 9B.

It is important to note that, even though S-β-CD was able to effectively resolve sulfonium ions containing only one aromatic substituent, it failed to resolve the first two series of ions containing two aromatic substituents. Trial analysis showed that, when a 4 mM S-β-CD modified buffer was used to analyze series I and II, the ions failed to elute from the capillary within 1 h. The peaks were so broad that no useful data could be obtained. When a very low concentration of the S-β-CD was employed, <1 mM, peaks were seen at a reasonable time, but they were again broad and showed no chiral resolution (data not shown). This result suggests that the interaction between these ions and the S-β-CD is sufficiently strong that they are detained significantly by the oppositely migrating S-β-CD, thereby limiting chiral separation.

Thiophenium Ion Analysis. Two chiral thiophenium ions, **18** and **19**, were synthesized to determine if the conditions

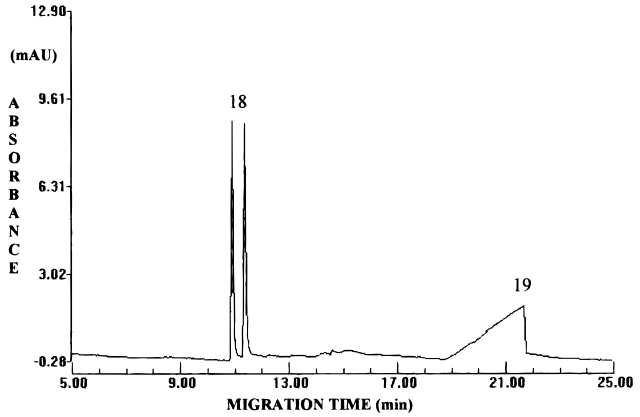

Figure 10. Electropherogram showing the chiral separation for the two thiophenium ions, **18** and **19**, using 4 mM S-β-CD. Other conditions are as described for Figure 2.

established for the chiral separation of sulfonium ions are also applicable. No chiral separation was observed for these ions when using β-CD-modified buffers. However, ion **18** was baseline resolved when using 4 mM S-β-CD-modified buffer, as shown of Figure 10. Ion **19** was not resolved under any of the conditions studied. We are currently investigating the use of different cyclodextrin derivatives which may prove to be better candidates for chiral separation of thiophenium ions.

CONCLUSION

The chiral analysis of a variety sulfonium and thiophenium ions was successful using cyclodextrin/TBA-modified capillary zone electrophoresis employing uncoated fused silica capillaries. Successful chiral resolution of the series of ions analyzed was found to be dependent on the type of substituents on the sulfonium ions, the type of cyclodextrin used, and the presence of TBA. The established parameters can be easily optimized further to obtain baseline resolution of particular ions of interest. The reproducibility of migration time was found to yield a %RSD of 0.37, based on six replicate injections. In conclusion, the use of cyclodextrin-modified CZE for the analysis of sulfonium ions offers an effective, inexpensive, and rapid method of analysis.

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