12-1999

Enantioseparation of Alkylaryl Sulfoxides Using Capillary Zone Electrophoresis

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ENANTIOSEPARATION OF ALKYLARYL SULFOXIDES USING CAPILLARY ZONE ELECTROPHORESIS

A Thesis

Presented to

the Faculty of the Department of Chemistry

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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December 1999

ENANTIOSEPARATION OF ALKYLARYL SULFOXIDES

USING CAPILLARY ZONE ELECTROPHORESIS

Date Recommended Oct. 29, 1999

Director of Thesis

Date Approved 12/6/99

Director of Graduate Studies

ACKNOWLEDGMENTS

I would like to thank my research advisor, Dr. Darwin Dahl, for his guidance, knowledge, and patience in assisting me in my research. I also would like to thank Dr. Thomas K. Green for his advice given to my research, Dr. Matthew Kubasik for the use of his flash chromatography apparatus, and Alicia McDaniel for the sulfoxide synthesis and NMR work.

I wish to thank the graduate office for the funding of my chemicals and the Department of Chemistry for their support during my graduate studies here at Western Kentucky University.

Finally, I would like to thank my wife, Ginger, and my parents, Mr. And Mrs. Allen Snyder, who gave me their full support through the years. I would like to thank Dr. and Mrs. Gerald Edds for their support and advice during my stay at Western Kentucky University.

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Chad A. Snyder December 1999 58 pages

Directed by: Dr. Darwin B. Dahl

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Alkylaryl sulfoxides possess a chiral sulfur atom easily identifiable by capillary zone electrophoresis (CZE). Separation of chiral alkylaryl sulfoxides has already been accomplished by a modified method of CZE known as micellar electrokinetic chromatography (MEKC). However, no articles have been published on the enantioseparation of alkylaryl sulfoxides using capillary zone electrophoresis.

A series of sulfoxides were synthesized, purified and identified via NMR. Enantioseparation was performed using CZE employing a 10 mmol. phosphate buffer (pH 4.0, 25% acetonitrile, 2% sulfated-β-cyclodextrin). Synthesis of these sulfoxides will be presented along with the results of the procedure's optimization. Separation of the sulfoxide enantiomers relies on the partitioning between the chiral additive (sulfated-β-cyclodextrin) and buffer solution.

I. INTRODUCTION

A. Asymmetric Sulfoxide

Sulfoxides are usually prepared by controlled oxidation of sulfides. The choice of oxidant and the reaction conditions are critical to avoid further oxidation to the sulfone.[1] Chiral sulfoxides belong to the class of chiral organosulfur compounds, which is most widely used in asymmetric synthesis. Their application as chiral synthons has now become a well-established and reliable strategy.[2] Chiral sulfoxides of high enantiomeric excess have been prepared mainly by (1) diastereomerically pure sulfinates followed by the reaction with organometallic reagents (Andersen-type synthesis)[3] and (2) the direct asymmetric oxidation of prochiral sulfides to sulfoxides by means of modified sharpless oxidant (Kagen oxidant)[4], Davis oxidant[5], a titanium-binapthol catalyst[6], and the oxidation of a sulfide with sodium metaperiodate in the presence of catalytical amounts of bovine serum albumin (BSA).[7] Three main directions for application of chiral sulfoxides are (a) reactions of α-sulfinyl carbanions with a broad variety of electrophiles, (b) reactions of α, β-unsaturated sulfoxides, and (c) introduction of heteroatomic groups to sulfoxides and their transformation.[2]

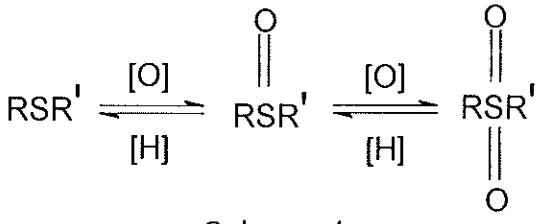
The biological activity varies from class to class of sulfoxides. Herein lies the need to identify and examine their affects biologically, chemically, and environmentally. Condensed thiophenes are commonly found in petroleum and creosote, and some of these compounds have been found to persist in crude oil-contaminated aquatic environments.[8]

These thiophenes can undergo microbial transformation into sulfoxides and sulfones. Some sulfoxides are more mutagenic than their parent thiophenes.

Dimethyl sulfoxide, a non-asymmetric sulfoxide, is therapeutically used as a topical anti-inflammatory. Dimethyl sulfoxide is also converted to sulfuric acid in the atmosphere. Origin of dimethyl sulfoxide arrives from dimethyl sulfide. Dimethyl sulfide, synthesized by phytoplankton, is a primary source of sulfur emitted from the ocean to the atmosphere.[9] Sulfides, sulfoxides and sulfones are interconvertible (Scheme 1), and sulfoxides and sulfones exists as metabolites of thioether-containing chemicals.

Beneficial examples, chemically and biologically, of sulfoxides include the ability to be used for synthesis as a starting material. Compounds such as n-octyl sulfoxides are employed for the synthesis of antitumor antibiotic analogues of the drug sparsomycin. Sparsomycin, a natural product from bacteria, is a potent inhibitor of protein biosynthesis and has attracted considerable interest as an antibiotic and chemotherapeutic agent.[10,11] The drug preferentially binds to "active ribosomes" engaged in polypeptide synthesis on mRNA and blocks the action of peptidyl transferase, a key enzyme integral to ribosomal subunit structure.

The sulfoxidations can be achieved in plants, animals, and microorganisms by essentially two microsomal systems: one involving cytochrome P-450, and the other flavin-containing monoxygenase.[1]



Scheme 1

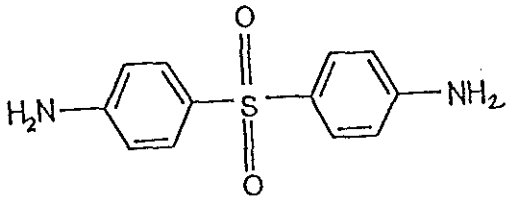
Several sulfoxides and sulfones are biologically active; examples include diaryl sulfones such as dapson (Figure 1). This antibacterial agent acts as a PABA antagonist like the sulfonamide drugs. Other diaryl sulfones, such as tetradifon, are used as acaricides to control spider mites.[1] As can be seen with Scheme 1 sulfur centers are susceptible to oxidation and consequentially biologically active sulfur compounds may undergo metabolic oxidation to the corresponding sulfoxides and sometimes to sulfones. In this oxidation, the sulfur may be part of an aliphatic chain or may be in a ring; thus both the tranquilizer drug chlorpromazine (Figure 1) and the systemic agricultural fungicide carboxin (Figure 1) are converted *in vivo* to the corresponding sulfoxides.[1] Sulfoxides may also be partly reduced *in vivo* to the sulfides (Scheme1), so that in many cases a redox equilibrium is established; this occurs with the antirheumatic sulfoxide drug sulindac (Figure 1).

A procedure is needed to separate enantiomers of sulfoxides for determination of enantiomeric excess. Enantioseparation can be achieved by complexation with a chiral additive such as a cyclodextrin. In order to form a stable complex at least three secondary bonds must form between the analyte and the cyclodextrin. Enantiomers separated in this research were a series of alkylaryl sulfoxides. In the research a sulfur atom was bonded to four groups: (1) oxygen, (2) phenyl, (3) lone pair electrons, (4) an alkyl group. The alkyl group varied in the research while all other groups remained constant. The research emphasis was placed on the ability to separate racemic mixtures of alkylaryl sulfoxides by CZE using sulfated-β-cyclodextrin. The separation occurs due to the partitioning of the analyte between sulfated-β-cyclodextrins and the buffer. The goal of this method is to allow the determination of the enantiomeric excess of a racemic sulfoxide.

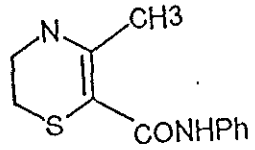
B. Capillary Electrophoresis

The ability to separate individual components of a mixture is a critical requirement in the chemical or biochemical sciences.[12] The science of separating components of a mixture has been dated back to medieval times beginning with filtration, distillation, precipitation, and crystallization. Of today's techniques, one of the most powerful separation techniques is based on the principles of electrophoresis. This process involves the migration, and separation of charged analytes in solution under the influence of an applied electric field. Michaelis coined the term "electrophoresis" in 1909, after discovering that proteins could be separated based upon their isoelectric points.

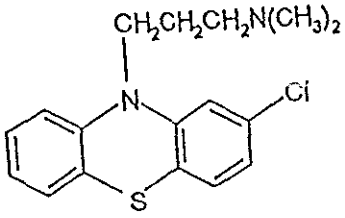
In the early 1970s, two-dimensional electrophoresis was described by Dale and Latner and Macko and Stegemann through the combination of isoelectric focusing (IEF) separation followed by PAGE.[12-14] PAGE stands for polyacrylamide gel electrophoresis and is used for the analyses of characterizing protein mixtures, determination of protein monomer molecular weights, and resolution of oligonucleotides. Also during this time, emphasis was being placed on analyzing small molecules using High Performance Liquid Chromatography (HPLC). Its speed, quantitative results, resolution, and automation made it an invaluable tool in industry and the clinical laboratory. However, HPLC was not without its limitations. The rise in biotechnology, through gene cloning and protein expression, and pharmacology demands led to the realization that neither electrophoresis nor HPLC were adequate for quality control of these new products.[12] Although HPLC was successful with oligonucleotides, peptides, and small proteins, it failed to provide good resolution and recovery with larger molecules.



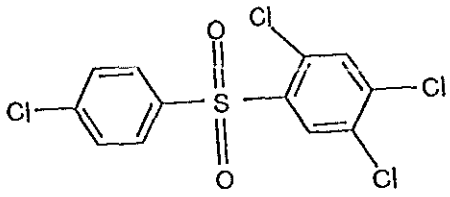
Dapson



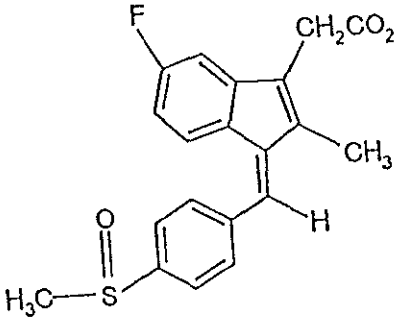
Carboxin



Chlorpromazine



Tetradifon



Sulindac

Figure 1. Some biologically active sulfur compounds.

The increasing demand for high resolution, reproducibility, and quantitative precision of biomolecules prompted the arrival of capillary electrophoresis (CE). Capillary electrophoresis has evolved from rudimentary column electrophoresis techniques that were introduced three decades ago. Early reports, many of which were by Hjerten and Catsimpoolas, employed columns having inner diameters (i.d.'s) on the millimeter scale.[15-17] Hjerten pioneered the work for CE analysis for diverse analytes ranging from small proteins to viruses.[12,18] In 1981, Jorgenson and Lukacs performed the first electrophoretic separations in narrow glass capillaries (< 100 μm i.d.).[15,19] Today, CE is in high demand for analytical separation of a wide variety of analytes and has surpassed previous separation techniques.

CE offers advantages of high efficiency, short analyses time, simple apparatus, small sample and separation buffer volumes, and ease in changing the separation buffer.[15,20]

Once components of mixtures have been successfully separated, the next progression is whether the individual components possess enantiomers that could be separated and identified. The term enantiomers refers to nonsuperimposable mirror-images. The mirror image of two optical isomers is shown in Figure 2. A chiral compound, such as an alkylaryl sulfoxide, possesses such enantiomers. The significant property of enantiomers is represented by the rotation of polarized light in different directions, counter-clockwise (levo-rotatory) and clockwise (dextro-rotatory).[21] Separating a racemic mixture into its enantiomers has profound implications especially in the pharmaceutical industry. Very often the two enantiomers of the same racemic mixture possess very different pharmacological effects. The new guidelines of the U.S. Food and Drug Administration (FDA) in 1992 and similar regulatory policies in China, Europe, and Japan state that only one enantiomer (mirror-image isomer) of a chiral drug should be brought to market.[22]

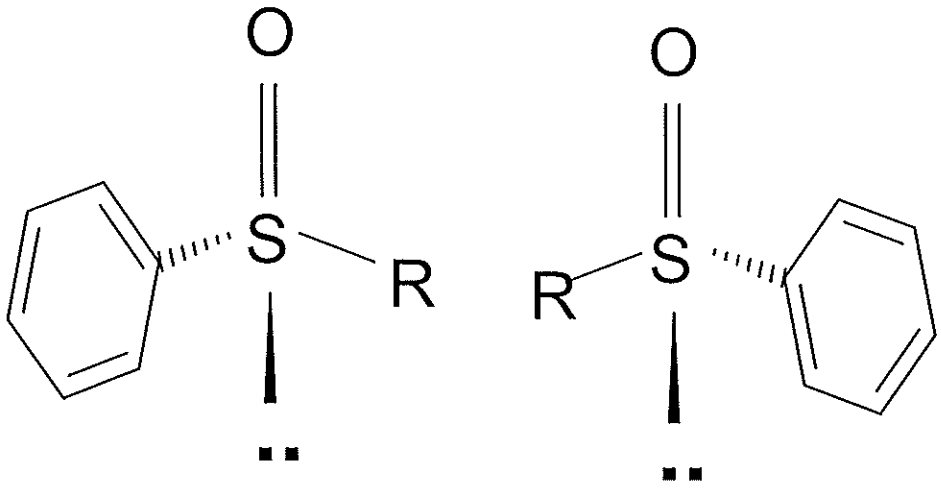


Figure 2. Mirror image of alkylaryl sulfoxide enantiomers.

Such respect is given to stereoselectivity in order to avoid possible unhealthy side effects produced by a particular enantiomer. Traditionally, chiral separations have been performed using high-performance liquid chromatography (HPLC) where, in practice, a different separation column is required for each type of chiral selector. In CE, the selector is part of the buffer and thus makes the system very flexible.[23] While in HPLC several expensive and sensitive columns are needed for this purpose, CE requires only a chiral selector dissolved in buffer.[24]

Understanding the theory of CE operation is critical when attempting analysis. The basis of separation of analytes originates from the polarity that is induced by the inlet and outlet electrodes along with the buffer reservoirs. Normal operation involves the inlet(+) and outlet(-). The polarity can easily be reversed for conditions that would best benefit analytes of interest. To initiate separation, the capillary must be filled with appropriate separation buffer at the desired pH with the sample being introduced at the inlet. Both ends of the capillary and the electrodes are submersed into buffer reservoirs with an adequate amount of applied voltage ranging from 0 to 30 kV. As voltage is applied, analytes will begin to migrate and hence separate based upon their different electrophoretic mobilities and pass the detector as "analyte zones." The fact that under the appropriate conditions all species (net positive, negative, or neutral) pass the detector indicates that a force other than electrophoretic mobility is involved. This phenomenon is known as electroosmotic flow (EOF) and promotes the movement of analytes toward the detector under the influence of an applied electric field. If the applied force were the only force acting on the ions, net positive charged cations substances would pass, while neutral components would remain static, and anionic components would be driven away from the detector.[12] EOF plays a

principle role in many modes of CE.

Electroosmotic flow was first determined in the late 1800's when Helmoltz conducted experiments involving the application of an electrical field to a horizontal glass tube containing an aqueous salt.[12,25] Helmoltz discovered that the silica wall imparted a negative charge to the inner surface of the tube and, when subjected to an applied electric field, promoted a net movement of fluid to the cathode. Interactions of silanol groups (SiO^-) and buffer are shown with Figure 3. The negatively charged silanol groups attract the positive cations of the buffer. The pH of the buffer determines the degree to which silanol groups will be ionized. The ionic layer formed has a positive charge density that decreases exponentially as the distance from the wall increases. The resultant double layer formed closest to the surface is termed the "Inner Helmoltz Layer" or "Stern Layer" and is essentially static. The layer formed more diffuse from the Stern Layer is termed the "Outer Helmoltz Plane" (OHP). Upon application of an applied electric field, cations in the OHP will migrate toward the cathode taking waters of hydration with them. The strength of hydrogen bonding that occurs between water molecules and waters of hydration force the buffer through the capillary toward the cathode. Therefore, EOF behaves as a pumping mechanism that drives all components (cationic, anionic, and neutral) toward the cathode with separation resulting from the different electrophoretic mobilities of the analytes, as illustrated in Figure 4. The velocity of EOF affects the analysis time of analytes. When EOF is fast, migration times are shortened usually resulting in high resolution. When EOF is slow, migration time is slowed and can result in band broadening. At extremely slow EOF velocities, analytes may not reach the cathode within a reasonable analysis time.

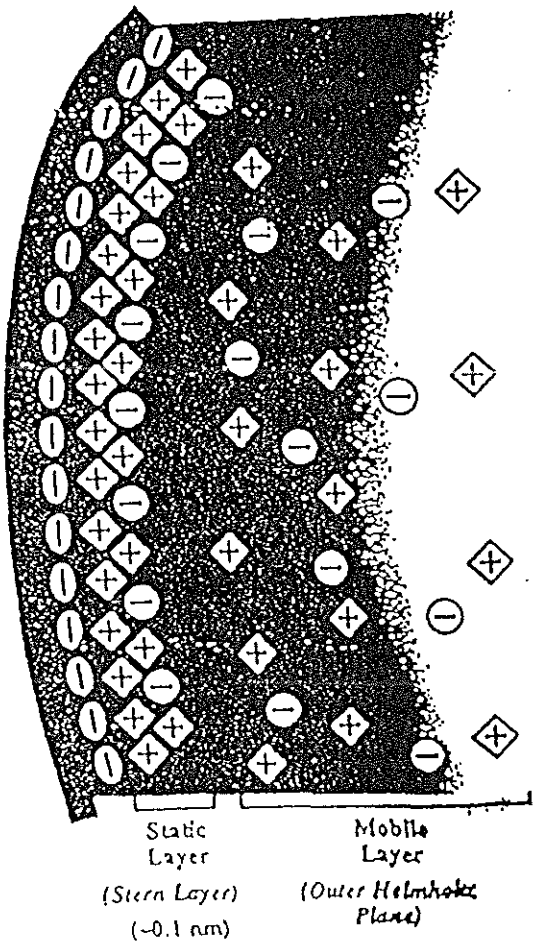


Figure 3. An illustration of the double ionic layer formed in bare silica capillaries in generating electroosmotic flow.[12]

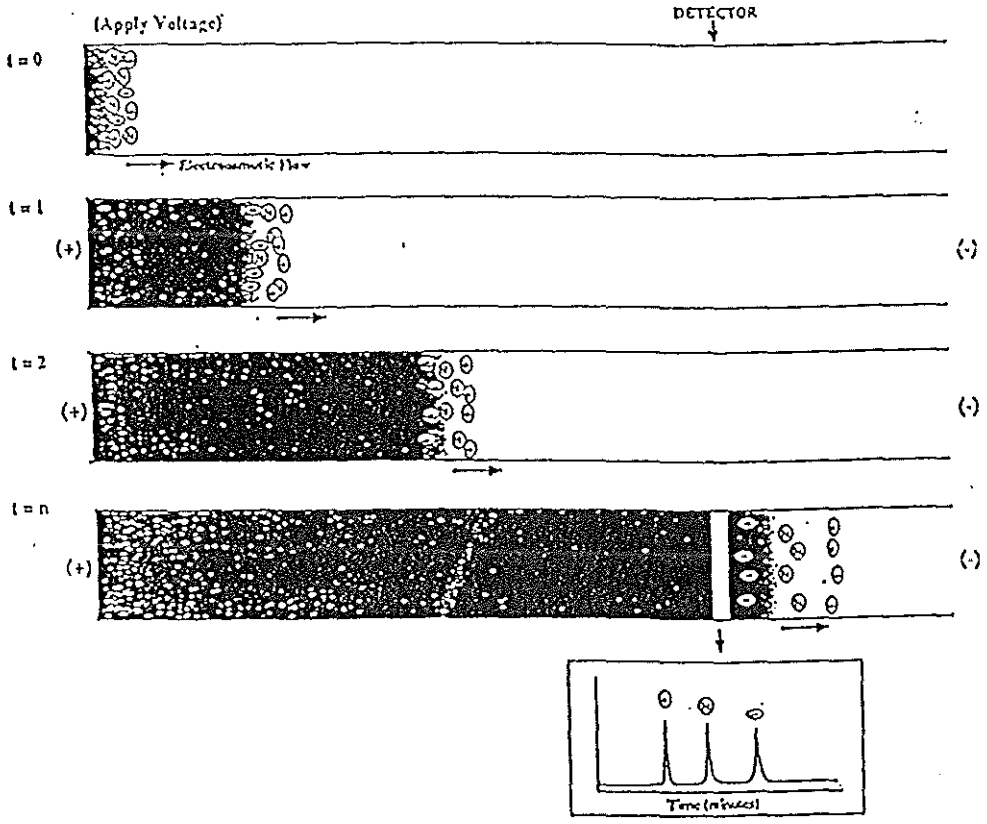


Figure 4. Mobility of charged and uncharged molecules in an applied field.

EOF can be made faster or slower depending upon the desired condition. A variety of factors influence the speed of EOF. Of these many factors polarity, kV, temperature, and buffer are main considerations.

Electrode polarity for normal CE mode is anode (+) at the inlet and cathode (-) at the outlet. In this respect, EOF is driven toward the cathode with cationic species being driven faster than anionic species. However, neutral species will migrate only at the current velocity of the EOF. If the reverse mode is used (cathode-inlet; anode-outlet), EOF is driven toward the inlet and only the negatively charged species that migrate faster than the EOF will pass the detector. This reverse mode has produced promising results for analysis of neutral compound in the presence of chiral additives.

Chiral agents are important to CE as partitioning agents between the analyte and the buffer. It has been pointed out, however, that the maximum opportunity for chiral separation may exist when the electrophoretic mobility of the chiral additives is opposite that of the analyte.[26,27] Very useful chiral additives for enantioseparation are cyclodextrins, particularly sulfated-β-cyclodextrins. The usefulness of sulfonated cyclodextrins, first introduced by Stobaugh, has been further highlighted by Lurie et al. and Terabe et al.[26,29] Blaschke et al. noted the advantage of "countercurrent" flow of the negatively charged additive with respect to the electroosmotic flow.[26,27]

The second major parameter is applied voltage. Results of applying higher voltages are faster EOF, shortened migration time, and in many cases improved peak sharpness. However, if the sample matrix ionic strength is much greater than the buffer ionic strength, and the applied voltage is too high, then joule heating cannot be sufficiently dissipated. High resolution and reproducibility can be retarded under these conditions.

Capillary temperature is the third parameter to consider. Temperature increases can possesses both positive and negative effects. Temperature is indirectly proportional to viscosity, which can be quite useful in shortening analysis time if the buffer and sample are less static. However negative effects of temperature increases can occur by inducing thermal effects on analytes. Temperature-induced conformational changes can occur with protein analytes. Hence, while elevated capillary temperatures shortens analysis times, one should be cognizant of the potential for adverse effects on analyte stability.[12]

The last major parameter to consider is choice of buffer. Once the optimal wavelength for detection has been established, a buffer must be selected that does not interfere with the ability to detect the analytes of interest, that maintains solubility of the analytes, that maintains buffering capacity through the analysis, and that produces the desired separation.[12]

After the buffer is chosen, the appropriate pH needs to be investigated. It is desirable to choose a buffer pH that approximates the pK of the solute mixture. Increasing the pH increases EOF. At high pH more silanol groups along the capillary wall are ionized. The ionic double layer increases and exerts pressure on the buffer solution. The increase in pressure forces buffer through the capillary at a faster rate therby increases EOF. The pH also affects the detection and resolution of analytes. Low pH is usually used to separate cationic drugs, while high pH is used for anionic compounds.[30] Also, capillary temperature can alter buffer pH. As can be understood, optimization of temperature and pH are required to derive useful data. Figure 5 illustrates the discussed parameters and their effects on optimization.

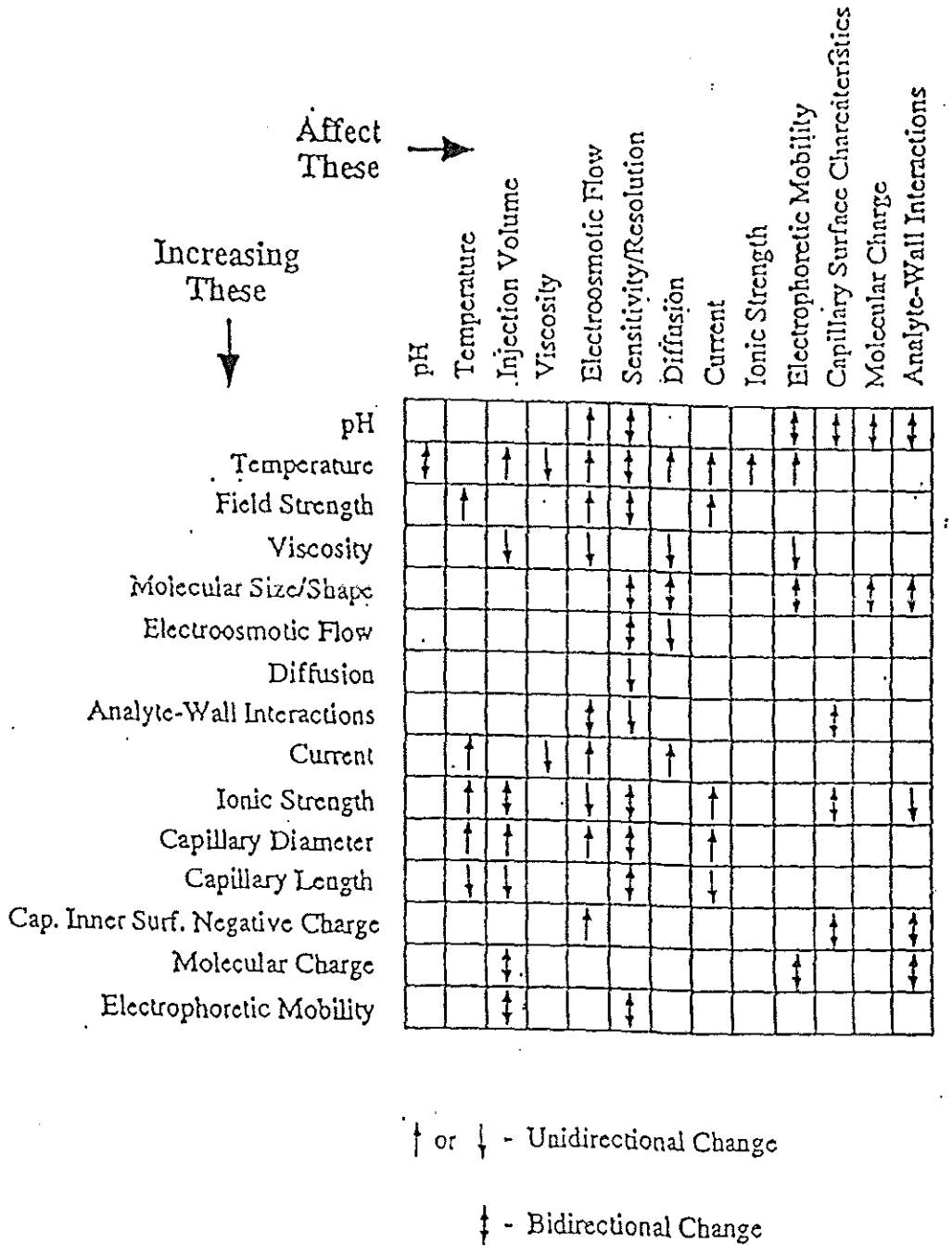


Figure 5. A reference chart for the evaluation of their relationships between variables influencing CE.[12]

As there are many optimization parameters for CE there are also many different modes of CE. Capillary electrophoresis can be modified to a variety of forms each depending on mode of analysis. Main modes of CE include capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), capillary isoelectric focusing (CIEF), capillary gel electrophoresis (CGE), and capillary isotachophoresis (CITP). Of these CZE is the most universal in application. Of these varying modes, two are of interest in the application of enantioseparation of neutral analytes: CZE and MEKC.

CZE, the simplest and most popular form of CE, has performed successfully separating a variety of analytes and continues to gain importance in analytical chemistry. CZE is capable of separating compounds based on their different electrophoretic mobilities.

Electrophoretic mobility (μ) of a charged species can be approximated from the Debye-Huckel-Henry theory

$$\mu = q/6\pi\eta r$$

where q is the charge on the particle, η is the viscosity of the buffer, and r is the Stokes' radius of the particle.[12,31] For neutral analytes, $q = 0$, and separation is not permitted as the analytes possess the same electrophoretic mobility as the EOF. As a means of separating these neutral molecules, MEKC was invented. MEKC was introduced by Terabe et al. in 1984.[32,33] A modification of CE, it separates charged or neutral compounds based on their affinity for the lipophilic interior and/ or the ionic exterior of a micellar pseudo-stationary phase.[32] And as the procedure's name implies, micelles are employed in the migrationofneutral analytes to the detector. Micelles are molecular aggregates of surfactant molecules into which analytes can partition based on hydrophobicity, ionic attraction, and hydrogen bonding.

At low concentrations (<1 m*M*) in water, individual surfactant molecules exist as single entities. As the concentration increases, such as when sodium dodecyl sulfate (SDS)is is increased to 8m*M* in water, individual surfactant molecules begin to interact with each other.[10]

Due to electrophoretic effects, negatively charged micelles formed from anionic surfactants such as SDS migrate at a rate slower than that of the electroosmotic flow. The rate of migration of an analyte therefore depends on its partition coefficient between the micelles and the electroosmotically pumped aqueous phase.[24] In micellar EKC, an ionic surfactant solution is employed instead of a buffer solution at concentrations higher than the critical micelle concentration (CMC).[33] The CMC is the concentration at which the individual surfactant molecules form molecular aggregates. An illustration of these aggregates is shown in Figure 6. MEKC has proven very effective for a variety of analyses including, chiral separation, caffeine, flavonoids, vitamins, explosive residues, organic solvents, nucleic acid constituents, and illegal drugs.[12, 34-42]

Chromatographic techniques such as thin-layer chromatography (TLC), gas chromatography (GC), supercritical fluid chromatography (SFC) and, above all, high-performance liquid chromatography (HPLC), frequently have been used for chiral separation.[43] The first (partial) liquid chromatographic resolution of an optically active sulfoxide on α-lactose was reported in 1959 by Farina.[44,45]

GC enantioseparations have lagged behind the development of HPLC techniques for several reasons: The analyte must be volatile and thermally stable.[46] Although it has been possible to separate racemic sulphoxides by gas chromatography since 1983, few papers have appeared describing this chromatographic approach.[44] The gas chromatograms obtained

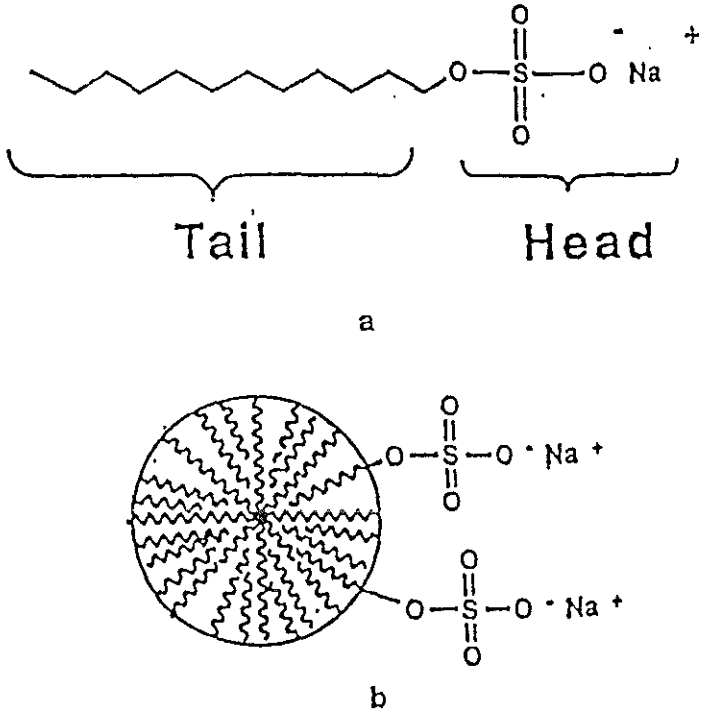


Figure 6. A. Structure of sodium dodecyl sulfate. B. SDS micelle.[12]

indicated poor separation factors and tailing peaks; both due to high polarity of sulfoxides and the result of poorly deactivated column surfaces. Groundwork was laid with these modes when chiral additives, such as cyclodextrins, were employed. When analytes form inclusion complexes with these chiral additives, in a certain respect, they form new compounds capable of being resolved. Resolution of analytes is the result of partitioning between the chiral additive and the buffer system.

C. Enantioseparation and CZE

As previously mentioned, CZE with buffer alone cannot separate neutral analytes unless modified to MEKC. However CZE has been used extensively for enantioseparation of neutral molecules. Whereas isotachophoresis, capillary gel electrophoresis, micellar electrokinetic chromatography, and capillary electrochromatography are used only occasionally for the separation of enantiomers, capillary zone electrophoresis was used in 72% of the references documented in Chirbase/CE as of August 1995.[47-56]

The ability to separate neutral analytes and drive them past the detector is accomplished by addition of a chiral additive (CA) to the running buffer.

The separation of the two enantiomers can take place only if the two diastereomeric complexes, formed during the electrophoretic process, possess different stability complexes. The chiral separation is obtained due to the formation of secondary bonds between the substituent groups on the chiral center of the analytes and those of the chiral selectors positioned outside the cavity (hydroxyl or modified hydroxyl and carboxylic groups for cyclodextrins and crown-ethers, respectively).[21]

The most effective classes of chiral selectors for CE enantioseparations have been cyclodextrins (CDs), macrocyclic antibiotics, and chiral crown ethers.[57-84] The most extensively used CAs are the cyclodextrins. Host-guest complexation of aromatic functional groups by cyclodextrins and various derivatives thereof is by far the most often used and probably the best understood separation principle for this purpose.[24]

Cyclodextrins are neutral, cyclic oligosaccharides composed of several glucopyranose units. There are a total of three neutral cyclodextrins: α-cyclodextrin (6 units), β-cyclodextrin (7 units), and γ-cyclodextrin (8 units). As the CD increases from unit to unit internal diameter increases but depth remains constant. A two-dimensional chemical structure of α-cyclodextrin is shown in Figure 7. Three-dimensional shape resembles a truncated cone. Their cavity is relatively hydrophobic and able to accept guest compounds of different types, particularly those with non-polar groups.[21] The most commonly used cyclodextrin in CE is the β-CD (7 glucose units) which has a cavity diameter of 0.78 nm and a volume of approximately 35nm^3.[12] Further information on native cyclodextrins is given in Table 1.

Two factors that contribute to the success of CDs for enantioseparation include their varying internal diameter and their degree of substitution. Internal diametrer of CDs increase from α<β<γ asthe number of glucose units increase. The question of which CD to use is determined by the relative size of the analytes.

Neutral CDs may not be enough to separate enantiomers even if these analytes complex with CDs. Neutral analytes complexing with neutral CDs is not sufficient to achieve separation. Recent research has employed the use of derivitized CDs, particularly sulfated-β-CD, for successful analysis of neutral compounds.[26]

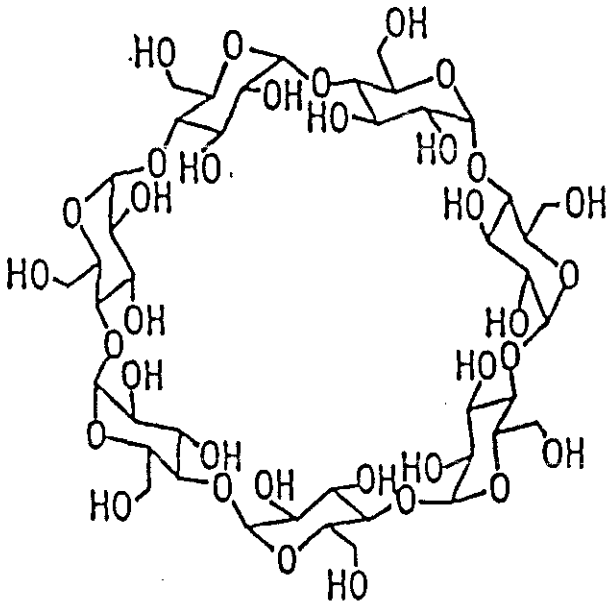


Figure 7. Chemical structure of β-cyclodextrin.[46]

Neutral analytes can form inclusion complexes, with the negatively charged sulfated-β-CDs, which can then migrate at a velocity greater than the EOF and pass the detector. A major requirement that allows for detection is reversing the polarity (cathode-inlet; anode-outlet). In normal mode, electrophoretic mobility of these anionic CDs opposes the EOF. Reversing the polarity, in conjuction with low pH, helps to suppress, and almost totally eliminate, the EOF. In this way, with the electrophoretic mobility of cationic species directed toward the injector and the absence of other mechanisms for transporting cationic or neutral species toward the detector, the only possibility for analyte migration toward the detector is through complexation, possibly stereospecific, with a negatively charged CA.[20]

In this work, a series of alkylaryl sulfoxides was synthesized, purified, and characterized by NMR. These racemic sulfoxides were enantiomerically separated using reverse polarity and optimized variables. The variables studied and optimized were kV strength, pH, organic modifier concentration (acetonitrile), chiral additive concentration (sulfate-β-cyclodextrin), molecular weight, and temperature.

Table. 1 The Main Properties of Native Cyclodextrins.[21,85,86]

Cyclodextrin type	α	β	γ
Number of glucopyranose	6	7	8
Molecular weight	973	1,135	1,297
Internal diameter (nm)	0.47-0.52	0.60-0.64	0.75-0.83
Depth (nm)	0.79-0.80	0.79-0.80	0.79-0.80
Specific rotation [α] 25 D	150.5	162.5	177.4
Melting point (K)	551	572	540
Solubility in water g/ 100 mL 25°C	14.50	1.85	23.20

II. EXPERIMENTAL

A. Reagents

1-bromohexane, 1-bromopentane, 2-bromopentane, 3-bromopentane, 1-bromo-2-methylpropane [isobutylbromide], thiophenol, magnesium sulfate, n-butyl lithium (2.5 M solution in hexane), silica gel (32-63 μm), sand, *d*-chloroform (99.0%), ethyl acetate, and hexane were purchased from Aldrich. 1-bromobutane, 1-bromopropane, *d*-chloroform (99.0%), and tetrahydrofuran (THF) were purchased from Fisher Scientific Company. Sodium periodate (99.0 %) was purchased from Sigma-Aldrich Chemical Company.

B. Synthesis of Sulfide

Synthesis of sulfides is a necessary precursor to forming sulfoxides. The procedure was taken from Yin and Pigeon.[87] In a 100 mL round bottom flask, under a nitrogen atmosphere, 30 mL of anhydrous THF and 6.6 mL n-butyl lithium is added at 0°C. A 1.4 mL volume of thiophenol is then added dropwise to the reaction vessel. A colorless solution immediately forms. The reaction vessel is allowed to warm to room temperature and stirred using a magnetic stirrer for 10 minutes. Then, 0.0136 mol of the desired alkyl bromide is added to the solution. The solution turns yellow after a short time and stirring is continued for 20 minutes. The reaction must be quenched with three-2.0 mL aliquots of distilled water and extracted with hexane. The combined organic layer is then dried over anhydrous

magnesium sulfate. The solvent is removed via rotary evaporation to yield the sulfide. ^{13}C-NMR is used to characterize the sulfide.

C. Synthesis of Sulfoxide

A modified procedure was taken from Leonard and Johnson for the synthesis of sulfoxides.[88] Into a 250 mL round bottom flask, 24.0 mL of a 3:1 methanol/water solution is added at 0°C. Subsequently, 0.898 g (0.0042 mol) of sodium metaperiodate is added and stirred for 10 minutes. While maintaining the reaction vessel at 0°C, 0.004 mol of asymmetric sulfide is added dropwise. A white precipitate immediately forms. Stirring is continued for 24 hours. The reaction mixture, which contains precipitated sodium iodate, is extracted with three-5 mL aliquots of chloroform. The combined organic layers are then dried over anhydrous magnesium sulfate. The solvent is removed by rotary evaporation. The sample is then purified using flash chromatography.

The appropriate solvent system needed for the purification sulfide/sulfoxide mixture using flash chromatography is 30% ethyl acetate in hexane. Using a column of 18.0 cm x 1.5 cm i.d. a small plug of glass wool is added to the bottom of the column body. Carefully, 1- cm of sand is added to generate a smooth layer on top of the glass wool. A slurry of solvent and 32-63 μm silica gel is slowly poured into the column to a height of 15.0 cm. With the stopcock open, the slurry of silica is allowed to pack uniformly under a steady flow of 30% ethyl acetate in hexane. Once packed, another 1 cm layer of sand is placed on top of the slurry. Next, approximately 1 gram of sample is loaded onto the column. After filling the reservoir with 30% ethyl acetate in hexane an applied pressure pump is then attached to the column. Separation occurs at a flow rate of approximately 5 mL per minute. Fractions

are collected at 5-mL intervals and analyzed using TLC. TLC, employing silica and a solvent system of 30% ethyl acetate in hexane allows for the identification of the sulfoxide. The sulfide will travel close to the solvent front while the sulfoxide is retarded. The sulfide will elute first, followed by the sulfoxide. Rotary evaporation is used to remove the solvent and sulfoxides are characterized using ^{13}C-NMR.[89]

D. NMR Preparation

Each sulfide and sulfoxide was characterized by ^{13}C-NMR. In a NMR tube, 50.0 μL of analyte is dissolved in 0.8 mL of *d*-chloroform. The instrument used was a 270 MHZ JOEL GSX, FT-NMR Spectrometer. ^{13}C-NMR spectra of the sulfides and sulfoxides are shown in Figures 8-11.

E. Buffer Preparation

30 mL of a 10 m*M* phosphate buffer containing 25% acetonitrile was prepared for CE operation. The buffer was made by mixing equimolar solutions of NaH_2PO_4 and Na_2HPO_4. In a 250 mL volumetric flask, 0.1725g $NaH_2PO_4 \cdot H_2O$ and 0.1775g Na_2HPO_4 were dissolved to the mark with nanopure distilled water. 7.50 mL of acetonitrile and 2% sulfated-β-cyclodextrins (approximately 0.600 g) is added to the phosphate buffer. The mixture was further titrated, with a 4 m*M* HCl solution, to a pH of 4.0. The buffer was then filtered through a 0.45 μm membrane filter.

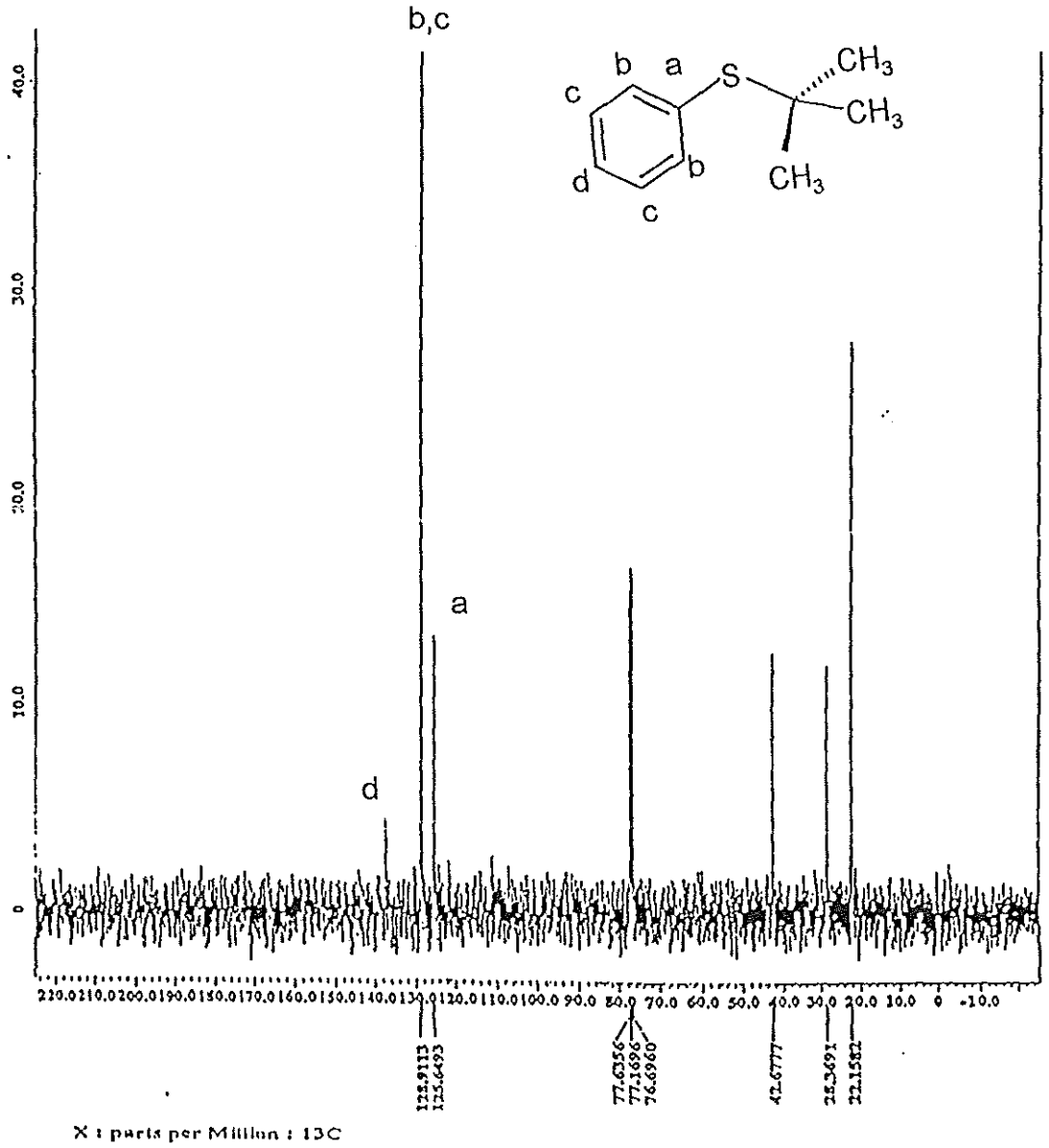


Figure 8. ^{13}C-NMR spectrum of IsoButyl Phenyl Sulfide.

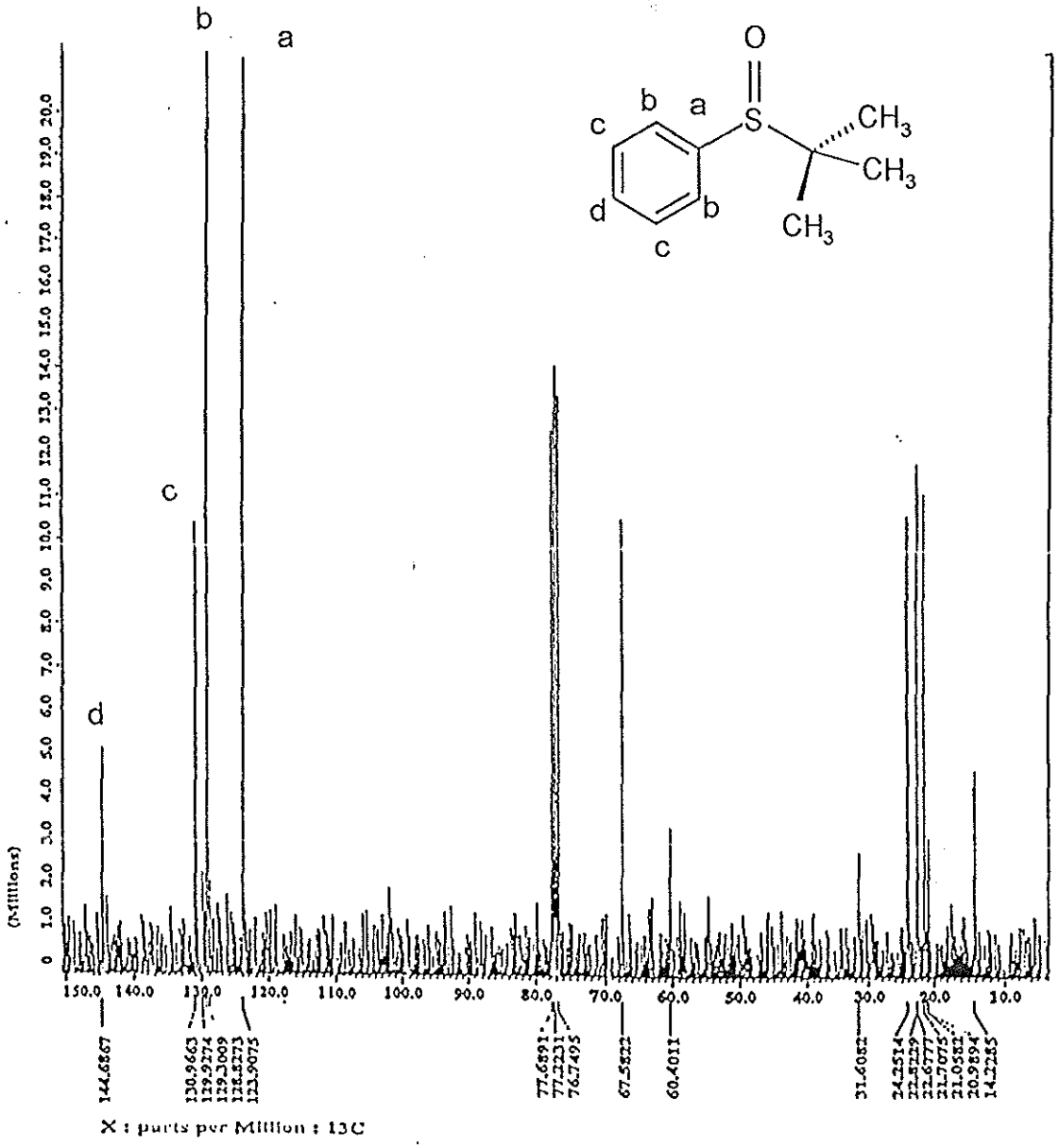


Figure 9. ^{13}C-NMR spectrum of IsoButyl Phenyl Sulfoxide.

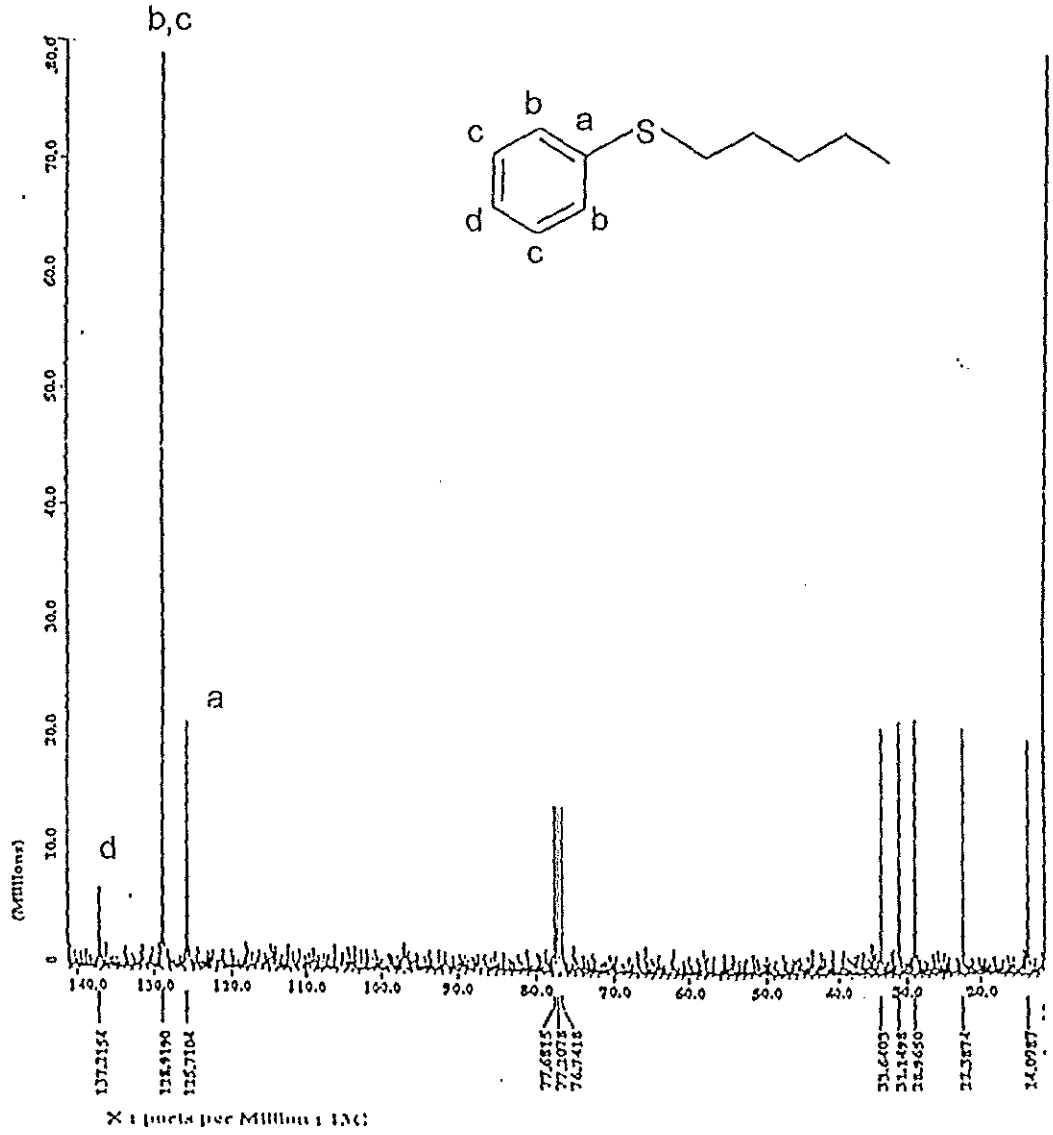


Figure 10. ^{13}C-NMR spectrum of n-Pentyl Phenyl Sulfide.

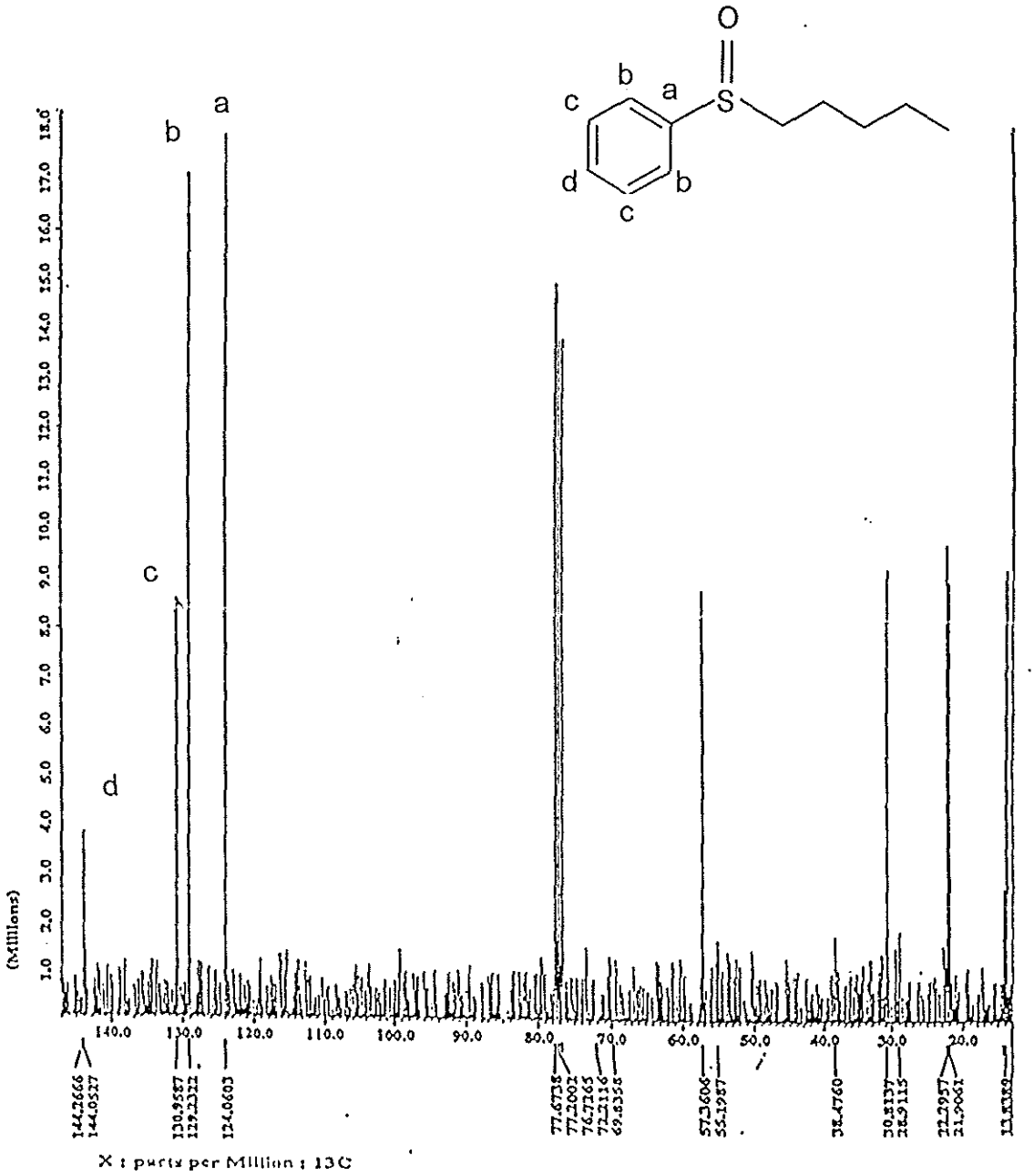


Figure 11. ^{13}C-NMR spectrum of n-Pentyl Phenyl Sulfoxide.

F. Standard Preparation

Stock solutions were prepared by dissolving 5.0 μL sulfoxide in 0.25 mL acetonitrile. The working standard was prepared by diluting the 0.25 mL stock solution to 1.00 mL using the buffer solution. All working standards were filtered through a 0.45 μm membrane filter prior to injection.

G. Instrumentation Conditions

Enantioseparation of the sulfoxides was performed using a BioRad Biofocus 3000 Capillary Electrophoresis system employing a 25.0 cm x 50 μm i.d. uncoated fused-silica capillary column for separation. Samples were introduced by pressure injection mode set at 2 psi*sec. Optimized conditions for partition CE were found to be: 9.50 kV, polarity in the direction of negative to positive, 33°C capillary temperature, and 30°C carousel temperature. Multiple detection wavelengths were set at 214 nm, 220 nm, 236 nm, and 254 nm, for verification of enantiomers. λ_{max} for the sulfoxides are given in Table 2.

H. Operation of CZE

A general schematic diagram of CE is shown in figure 12. Analyte separation occurs within the capillary with working lengths from 25.0 cm or higher. The separation voltage from a high-voltage power supply is applied to platinum electrodes suspended in the reservoirs. The sample mixture is injected into the capillary by replacing the buffer reservoir with the sample vial. Analytes migrate according to their mass to charge ratio and are detected by an on-column UV detector.[76,85]

Table 2. Alkylaryl sulfoxides and their λ_{max}.

Name	λ_{max} (nm)
iso-butyl phenyl sulfoxide	235.5
n-butyl phenyl sulfoxide	219.6
n-pentyl phenyl sulfoxide	219.4, 236.2
2-pentyl phenyl sulfoxide	219.6
3-pentyl phenyl sulfoxide	219.6
n-hexyl phenyl sulfoxide	219.6

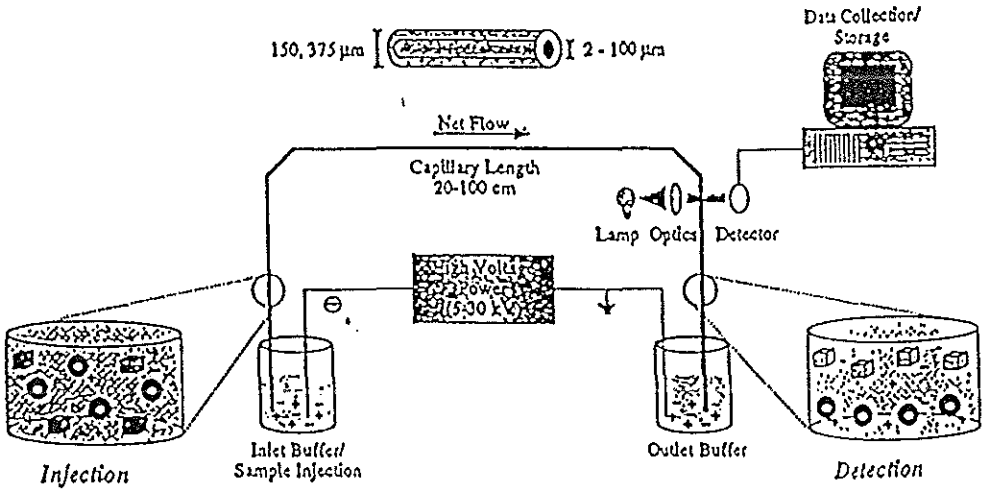


Figure 12. Schematic diagram of CE operation.[12]

III. RESULTS AND DISCUSSION

Six sulfoxides were synthesized and are listed in Table 3. Figures 8-11 show the ^{13}C-NMR spectrum of each individual alkylaryl sulfoxide. There are notable changes from the ^{13}C-NMR spectrum of the sulfide to the sulfoxide.

The n-pentyl phenyl sulfide ^{13}C-NMR spectra exhibits three signals produced by the aromatic ring. These signals arise from aromatic carbons as follows: carbon a (125.7 ppm), carbon's b and c (128.9 ppm), and carbon d (137.2 ppm). Since sulfur has an electronegativity similar to carbon, strong deshielding does not occur and subsequently carbons b and c exhibit the same chemical shift. However, the n-pentyl phenyl sulfoxide spectra exhibits four signals produced by the aromatic ring. The results from the greater electronegativity of the oxygen thereby deshielding carbons b and c therefore experience different chemical shifts and are resolved into two peaks. ^{13}C-NMR provides an excellent means for verifying the successful conversion of sulfide to sulfoxide.

Development of the CZE method involved optimizing the following parameters: polarity, kV, sulfated-β-cyclodextrin concentration, pH, acetonitrile concentration and temperature.

A. Effect of Electrode Polarity

Reverse polarity was employed to overcome strong electroosmotic flow to allow the negatively charged analyte/cyclodextrin complexes to reach the detector.

Normal mode involves inlet (+) and outlet (-). Using reverse polarity (inlet-cathode; anode-outlet) EOF proceeds toward the cathode and the negatively charge complexes migrate toward the anode. The electrophoretic mobility, influenced by net negative charge, is strong enough to overcome the opposing force of the EOF. In normal mode polarity the negatively charged complex is strongly repelled by the cathode thereby never reaching the detector.

B. Effect of Applied Voltage

Increasing the voltage will have a number of effects. It will increase sample migration, electroosmotic flow rate, and shorten analysis time. Also it may increase the sharpness of peaks and improve resolution. However, the advantages associated with increasing the voltage may be lost if the sample matrix ionic strength is much greater than the running buffer ionic strength such that the increased production of Joule heat cannot be efficiently dissipated.

Figure 13 shows that as magnitude of voltage increased migration time decreased. This indirect relationship is well understood for normal operation of CE and obey's Ohm's Law, $V = IR$. Voltage and current are directly proportional. Migration times were considerably longer due to a 45.0 cm working capillary length. Once capillary length was reduced to 25.0 cm, migration time shortened and less kV was needed.

C. Effect of Chiral Additives

A chiral selector can be added to the buffer for enantioseparation. It interacts with the two enantiomers during the electrophoretic process, forming labile diastereomeric complexes. Enantioseparation can take place only if the diastereomers formed possess different stability constants.

Figure 14 shows the effect on resolution of iso-butyl phenyl sulfoxide verses chiral additive concentration. In general increasing the cyclodextrin concentration improves resolution and shortens migration time. Migration time decreases as a result of the analyte complexes electrophoretic mobility increasing. As the charge increases electrophoretic mobility increases. Experimental data also showed that as the concentration of sulfated-β-cyclodextrin was increased, an increase in current would also occur and prove to be difficult to stabilize. Resolution, as well as resulting current, was sufficient at 2% sulfated-β-cyclodextrin.

D. Effect of pH

It is advisable to choose a buffer pH that approximates the pK of the solute mixture. Increasing the pH between 4 and 9 results in an increase in EOF. Figure 15 shows the effect on resolution of 2-pentyl phenyl sulfoxide as pH varied. Resolution was optimized at pH 4.0 At low pH, using reverse polarity, electrophoretic mobility overcomes the effects of EOF. Low EOF results from the mild extent of ionization of silanol groups along the capillary wall. The extent of ionization is pH dependent. As pH decreases less ionization occurs. The ionic double layer within the capillary decreases and less pressure is exerted on the resultant buffer solution. As a result EOF is weakened. The negatively charged analyte

complex can overcome the opposing EOF and reach the anode. Conditions at pH 3.5 and pH 5.0 were investigated but produced no results. No results were observed at high pH. The EOF strength was greater than the electrophoretic mobility of the analytes.

E. Effect of Organic Modifier

Organic solvents such as acetonitrile have the effect of decreasing both the conductivity of the buffer and EOF through their ability to disrupt the ordered structure of the water molecules.[12] In many cases resolution is enhanced due to a combination of the decreased EOF and improved analyte solubility. Figure 16 shows the effect of varying the amount of acetonitrile to determine the effects on resolution of n-pentyl phenyl sulfoxide. Acetonitrile was used due to its solvating ability for the alkylaryl sulfoxides. The sulfoxides investigated tended to be very hard to dissolve in aqueous buffer. For those that did dissolve with agitation, soon thereafter they would precipitate on standing. As the concentration of acetonitrile increased so did resolution. Acetonitrile at a concentration of 25% proved to be optimal. Conditions of 10% and 30% acetonitrile were investigated but produced no results. At 30% acetonitrile conductivity dramatically decreased thus slowing the migration of analytes to the detector. At 10% acetonitrile current fluctuated wildly. This current fluctuation could result from sulfoxides coming out of solution.

F. Effect of Temperature

Increasing the capillary temperature can have both positive effects and negative effects on separation. Elevated temperature increases EOF and analyte electrophoretic mobility. Negative attributes involve thermal effects on the sample.

Electropherograms of the sulfoxides were obtained at 33°C capillary temperature. This temperature remained stable during the experimental runs. Other runs were conducted at 25°C however insufficient coolant flow problems persisted and reproducibility was greatly hindered. It was seen however that the analytes move quickly with the temperature set at 25°C however; 33°C was found sufficient.

G. Effect of Isomers

Table 4 illustrates isomeric effects on migration time. Migration times decreased as branching increased-- the result of better analyte/cyclodextrin interactions and decreased Stokes' radius. According to the Debye-Huckel-Henry theory as particle radius decreases electrophoretic mobility increases.

H. Effect of Molecular Weight

As the molecular weight increases electrophoretic mobility decreases, as shown in Figure 17. This relationship is also a result of the Debye-Huckel-Henry theory. As the particle size increases electrophoretic mobility decreases. Relative migration times of sulfoxides are shown in Table 4.

Capillary zone electrophoresis has proved to be a very useful analytical tool in determining enantiomeric excess, usually by means of modification such as MEKC, of alkylaryl sulfoxides. The electropherograms shown with Figures 18-23 were obtained at a capillary temperature of 33°C, cartridge temperature of 30°C, 9.50 kV, polarity running from negative to positive, and a buffer concentration of 10 m*M* phosphate (pH 4.0)/ 2% sulfated-β-cyclodextrin, 25% acetonitrile. Equimolar concentrations of NaH_2PO_4 * H_2O and

Na_2HPO_4 constituted the phosphate concentration. Good resolution was obtained for the pentane isomers and iso-butyl phenyl sulfoxide. Band broadening was observed with n-butyl phenyl sulfoxide and n-hexyl phenyl sulfoxide, possibly due to routine coolant system problems. Reproducibility and resolution are greatly affected when constant temperature cannot be maintained.

The entire optimization procedure was determined by changing only one variable during each run. Under the optimized conditions described, the enantioseparation of the synthesized sulfoxides was separated successfully. Promising future work for CE separation will employ an inorganic ion, such as copper or nickel, along with a chiral additive. Lewis acid/ base interaction resulting in complex formation with the sulfoxide. The complex would result in a positively charged ion and thereby allow for an easier mode of separation.

Table 3. Alkylaryl sulfoxides.

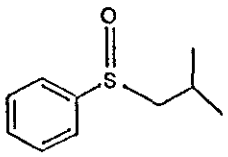
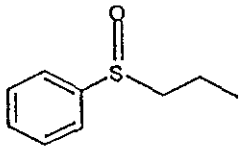
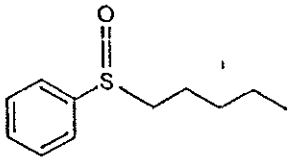
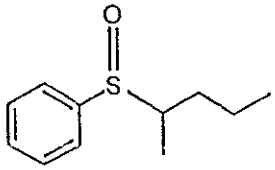
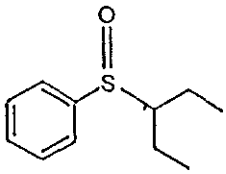
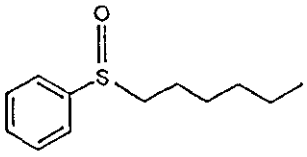
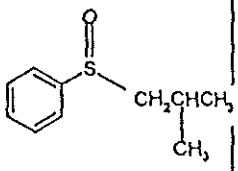
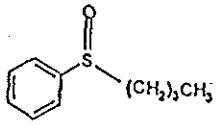
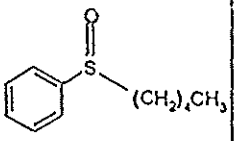
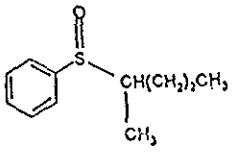
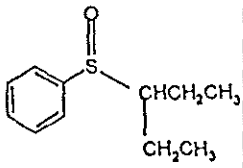
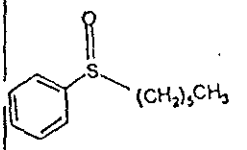
Structure	Name
	Iso-butyl phenyl sulfoxide
	butyl phenyl sulfoxide
	1-pentyl phenyl sulfoxide
	2-penlyl phenyl sulfoxide
	3-pentyl phenyl sulfoxide
	hexyl phenyl sulfoxide

Table 4. Table of alkylaryl sulfoxides.

Order of Migration	Structure	Name	Molecular Mass (g/ mol)	Migration Time (min)	Relative Migration Time
1		Iso-butyl phenyl sulfoxide	182.3	46.4, 59.0	Standard
2		n-Butyl phenyl sulfoxide	182.3	50.2, 54.6	1.08
3		1-Pentyl phenyl sulfoxide	196.3	65.5, 72.3	1.41
4		2-Pentyl phenyl sulfoxide	196.3	52.2, 72.0	1.22
5		3-Pentyl phenyl sulfoxide	196.3	53.2, 68.6	1.15
6		n-Hexyl phenyl sulfoxide	210.3	68.0, 81.7	1.47

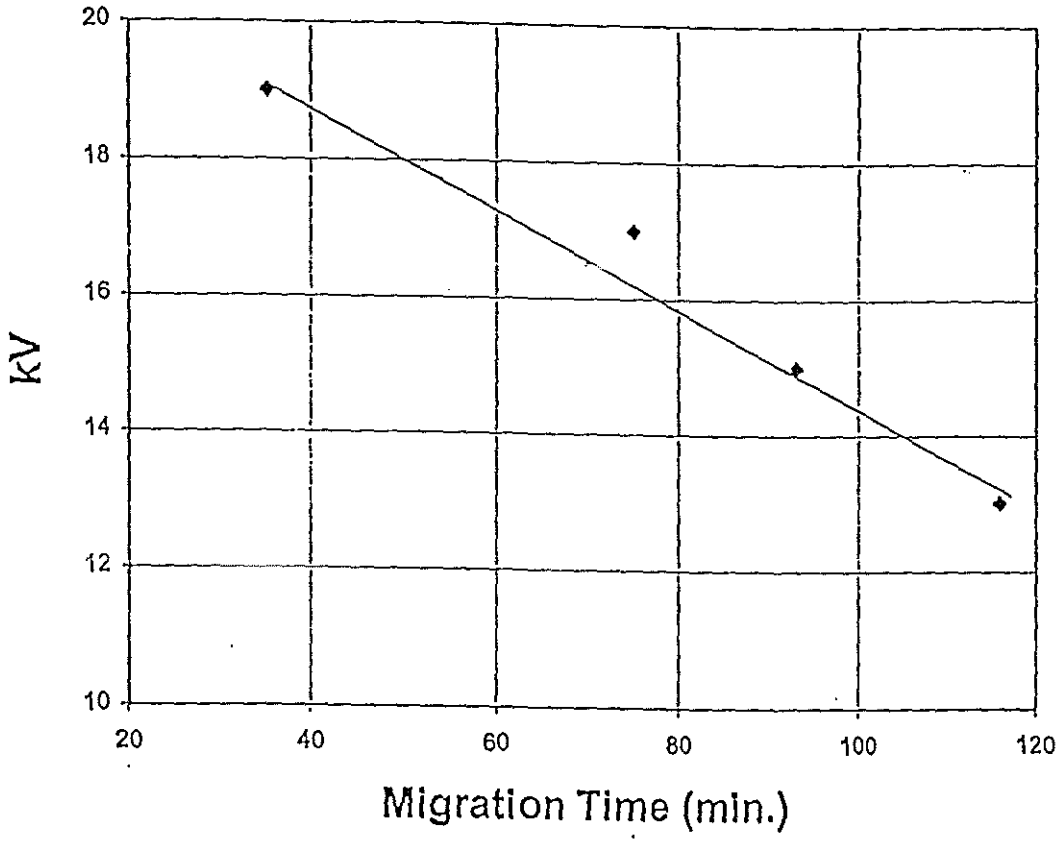


Figure 13. Applied Voltage verses Migration Time (min.).

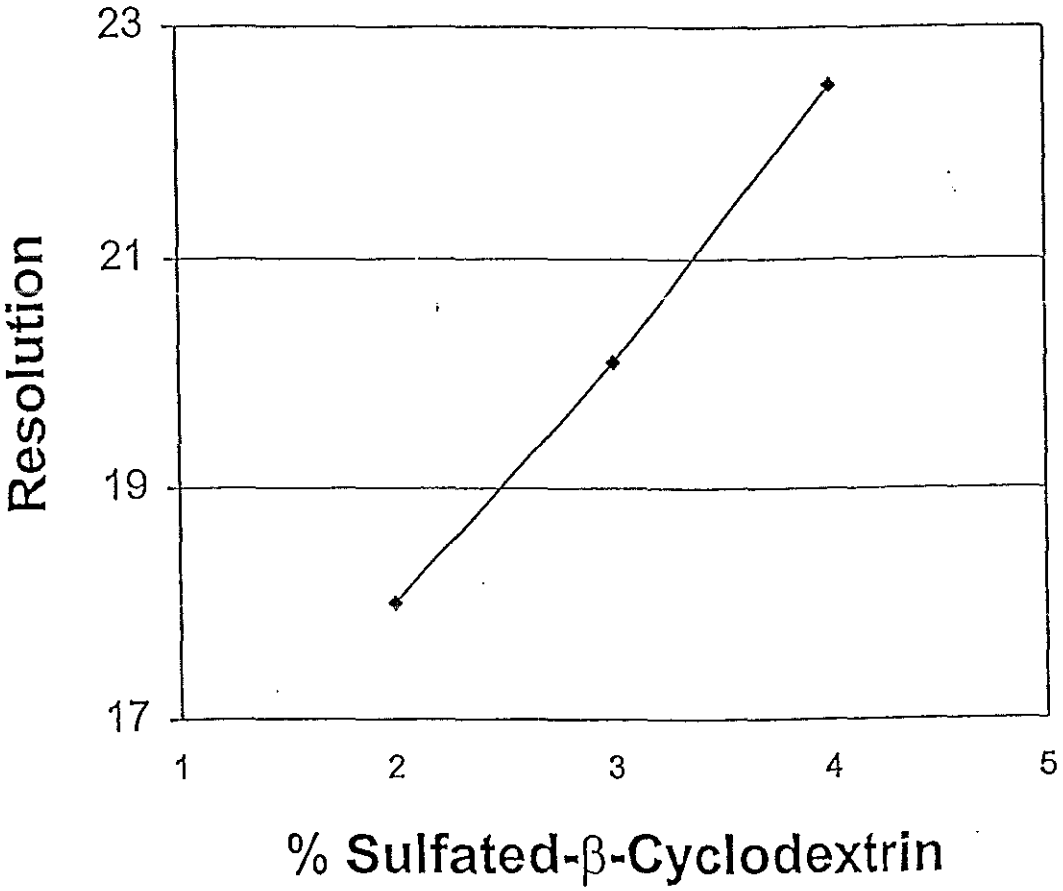


Figure 14. Resolution of IsoButyl Phenyl Sulfoxide verses % Sulfated-β-Cyclodextrin.

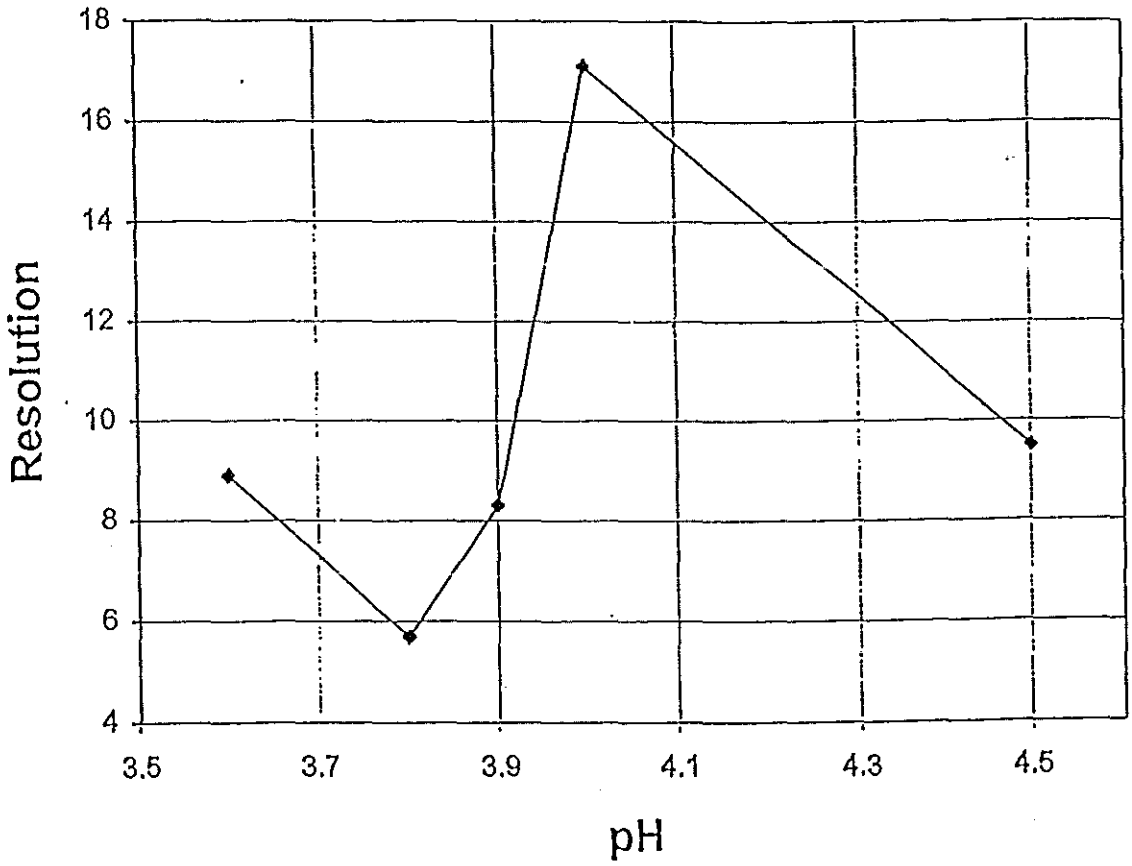


Figure 15. Resolution of 2-Pentyl Phenyl Sulfoxide verses pH.

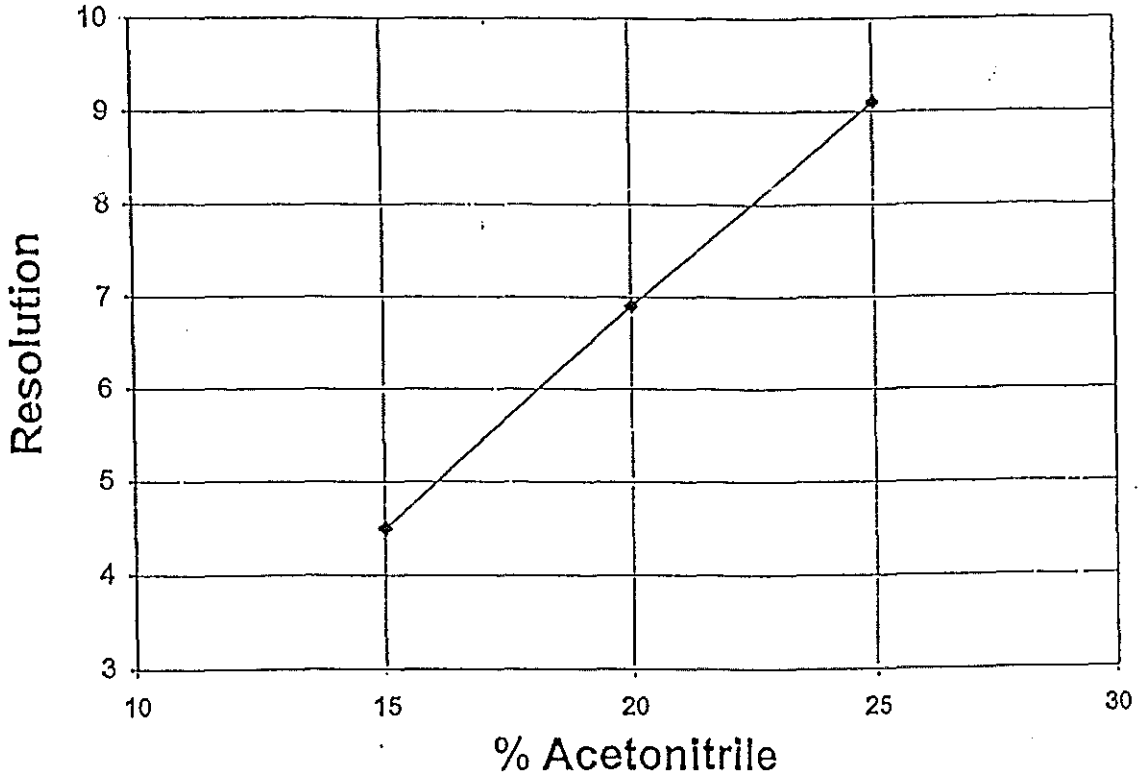


Figure 16. Resolution of 2-Pentyl Phenyl Sulfoxide verses % Acetonitrile.

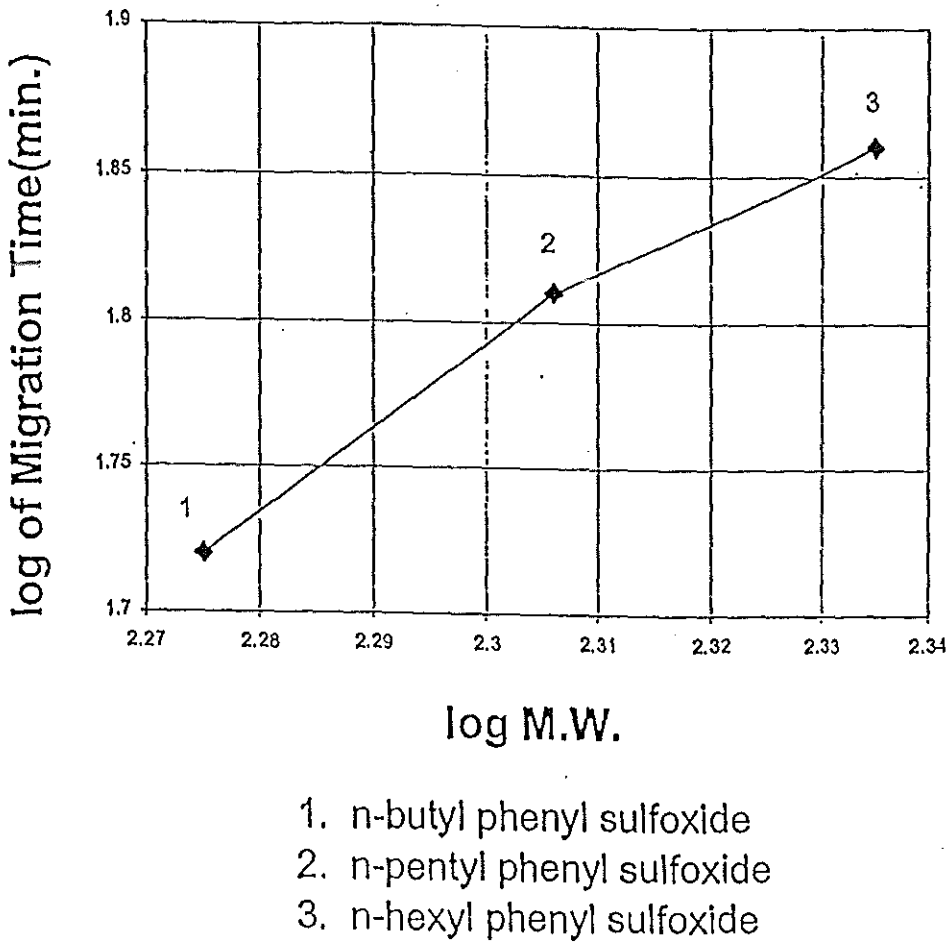


Figure 17. Log of Migration Time (min.) Verses Log of Molecular Weight of Alkylaryl Sulfoxides.

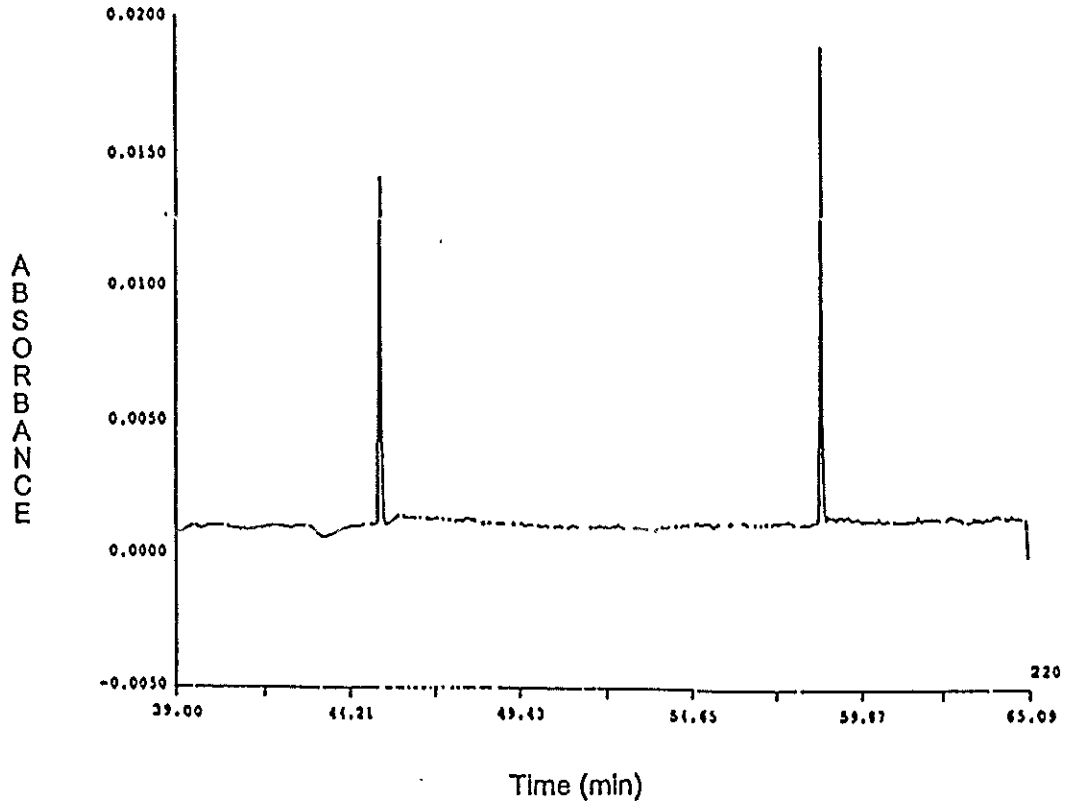


Figure 18. Electropherogram of IsoButyl Phenyl Sulfoxide.

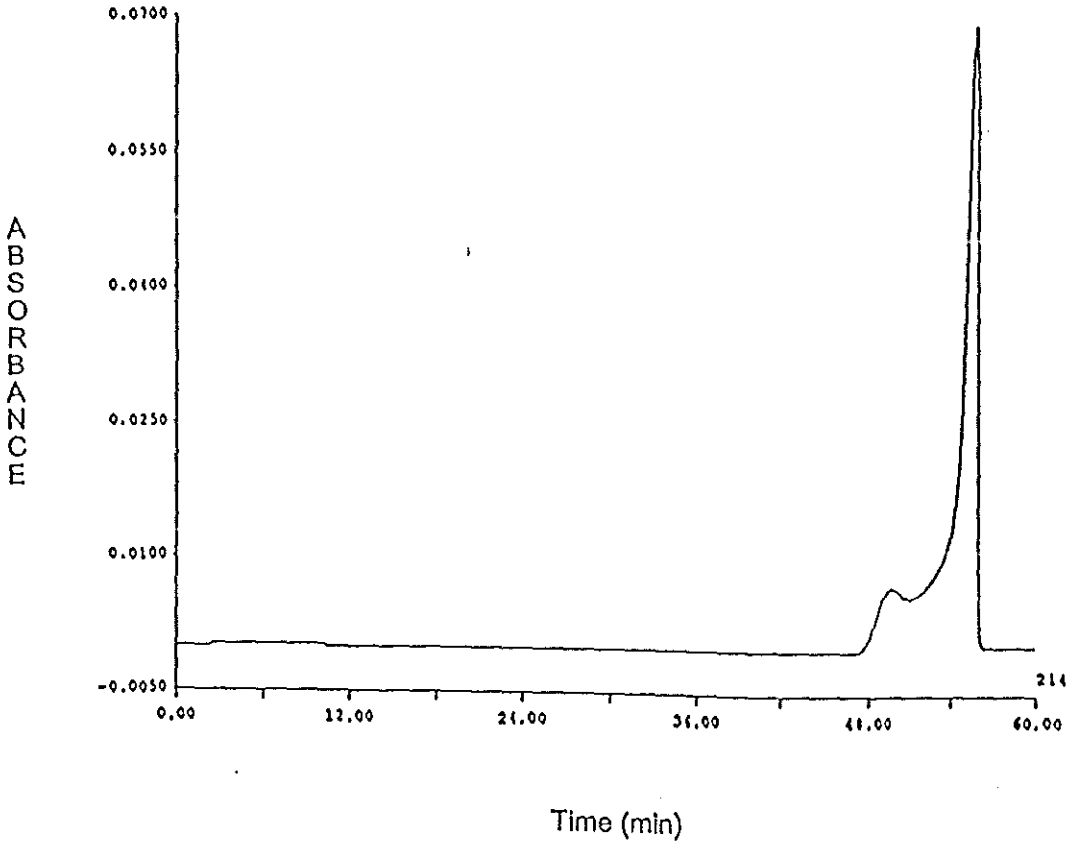


Figure 19. Electropherogram of n-Butyl Phenyl Sulfoxide.

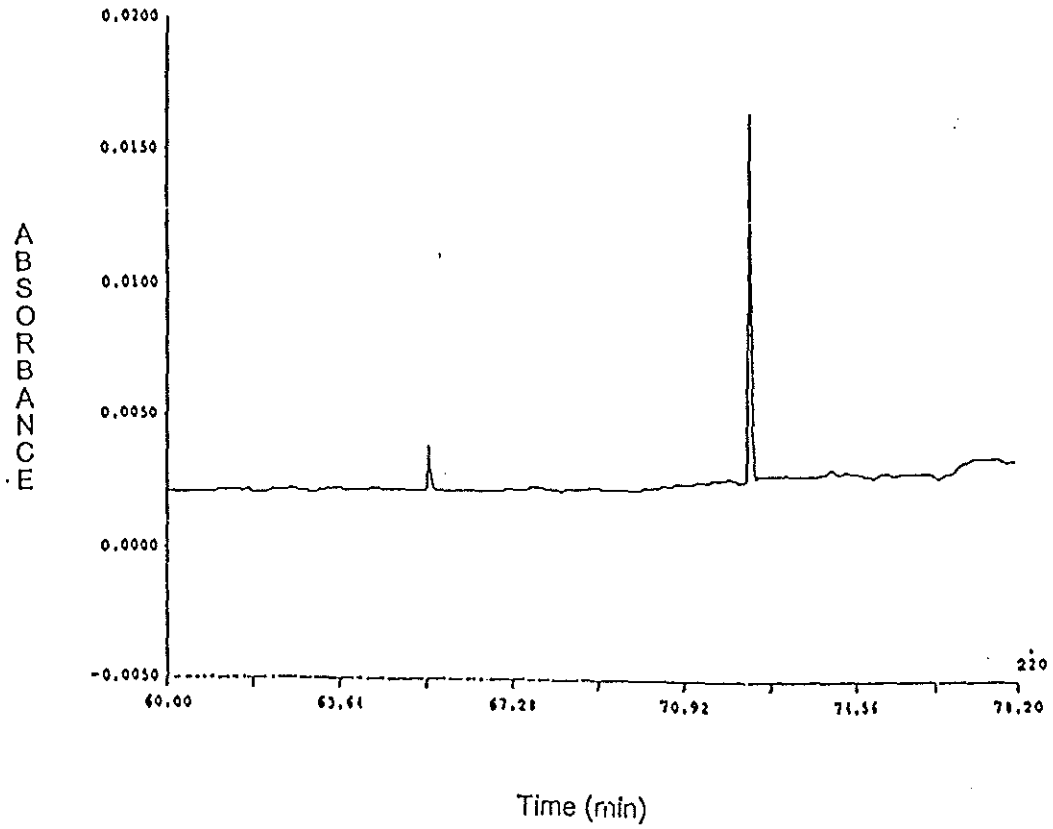


Figure 20. Electropherogram of n-Pentyl Phenyl Sulfoxide.

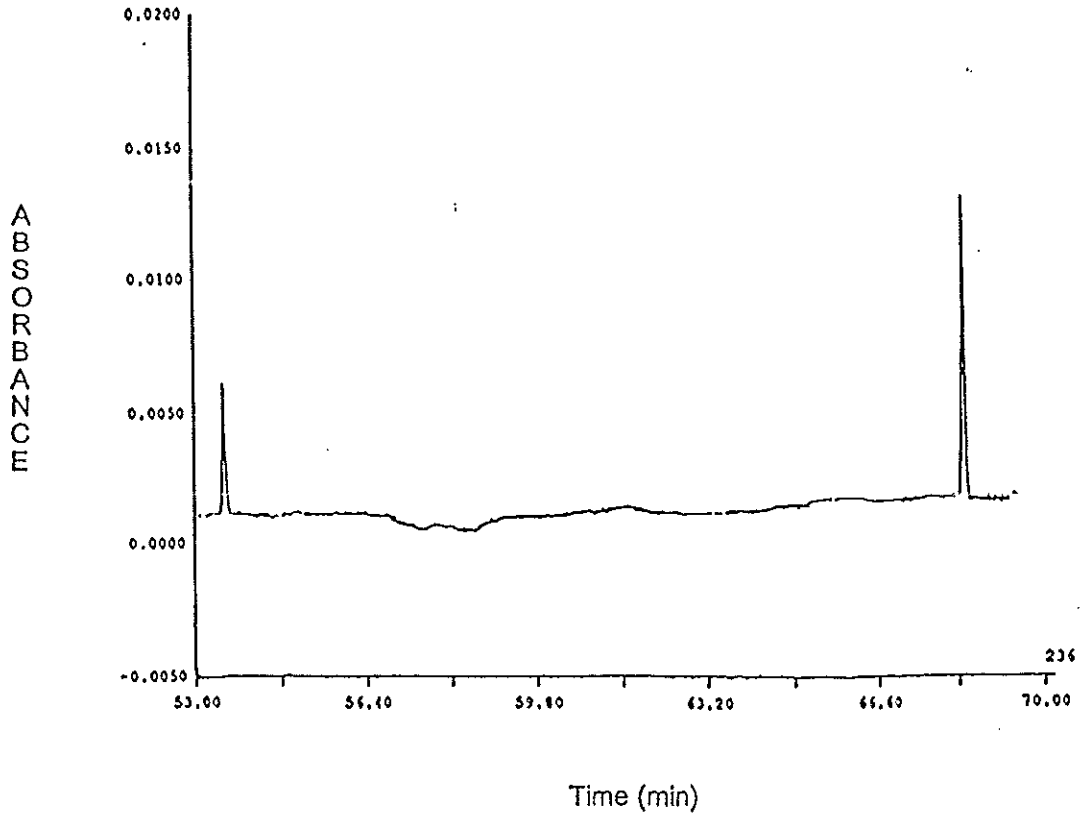


Figure 21. Electropherogram of 2-Pentyl Phenyl Sulfoxide.

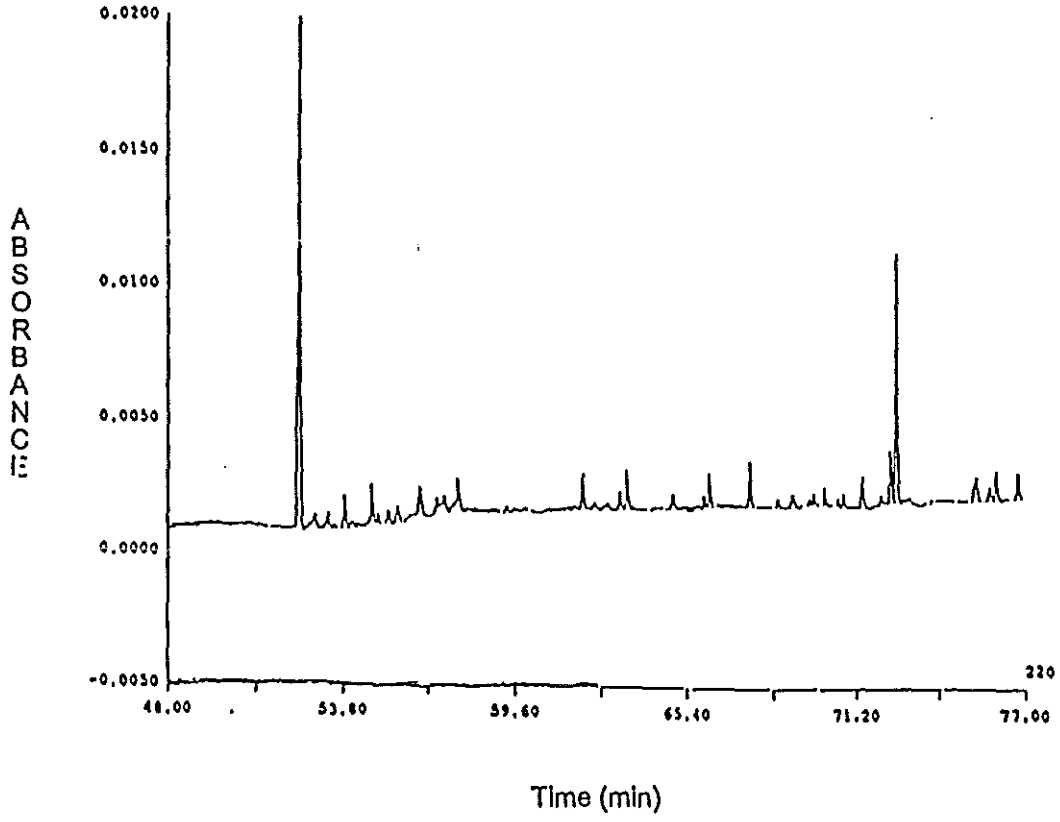


Figure 22. Electropherogram of 3-Pentyl Phenyl Sulfoxide.

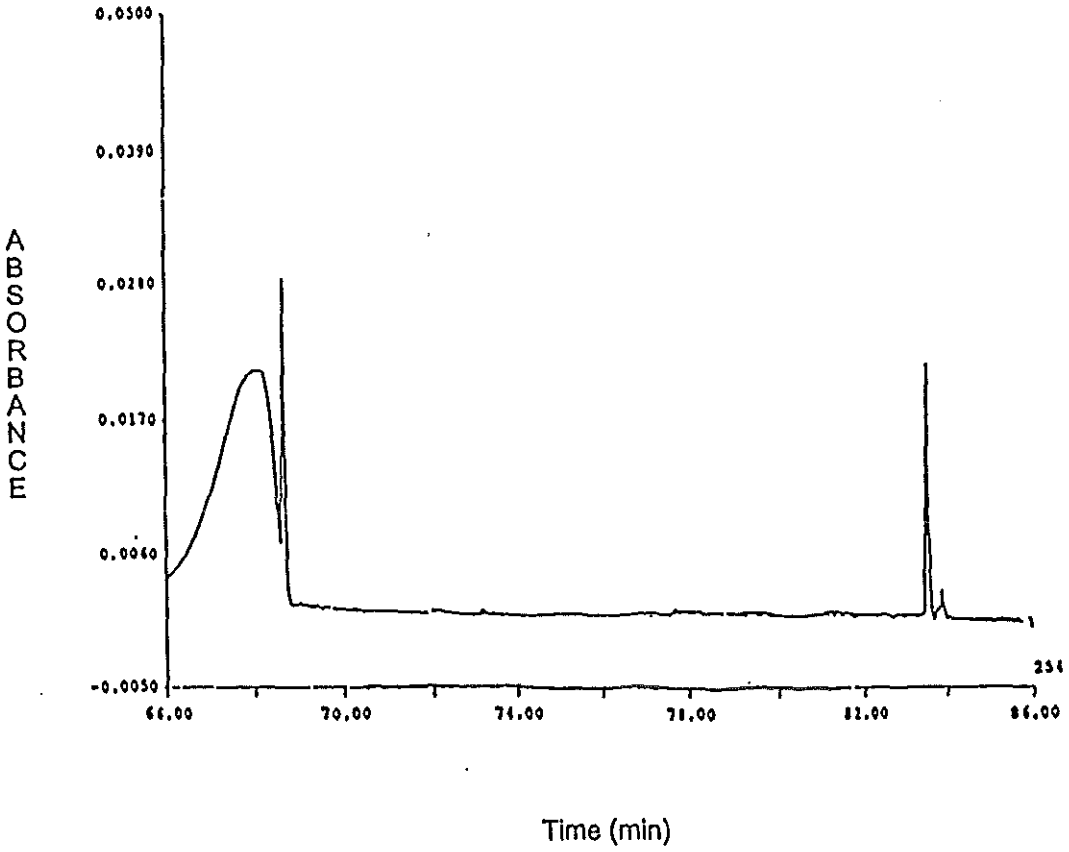


Figure 23. Electropherogram of n-Hexyl Phenyl Sulfoxide.

IV. CONCLUSION

Chiral sulfoxides are widely used in many asymmetric synthetic applications, mainly due to the ease of availability and high asymmetric induction exerted by the chiral sulfinyl group.

The synthesis of these alkylaryl sulfoxides have been shown, followed by a developed method for their enantioseparation using CZE. Other observations and conclusions on the information generated from this study are as follows:

- A successful separation of all synthesized sulfoxides; resolution somewhat less with n-butyl phenyl sulfoxide and n-hexyl phenyl sulfoxide.
- Migration time increased with increasing mass.
- The structural isomers n-pentyl phenyl sulfoxide, 2-pentyl phenyl sulfoxide, and 3-pentyl phenyl sulfoxide show the relationship between increased branching and migration time. As branching increased the migration time decreased. This relationship is presumably due to less surface area of the analyte. A smaller surface area would decrease the degree of partitioning between the analyte and the chiral additive.
- To modify resolution, one can adjust pH, chiral additive concentration, and organic modifier concentration.

- The relative standard deviation of migration times was found to be 0.3% relative standard deviation of peak height was found to be 5.7%.
- Reproducibility of migration times and of peak heights were continuously hindered as a result of cooling system problems.

In summary, partition CZE proved to be an effective tool for the enantioseparation of these sulfoxides. This method offers distinct advantages over other techniques such as HPLC, GC, and NMR. These advantages include high resolution, high efficiency, small sample size, low cost, and ease of operation.

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