EVALUATION OF GLYCOSIDIC CHIRAL SURFACTANTS IN ENANTIOMERIC SEPARATIONS BY CAPILLARY ELECTROPHORESIS

By

MEI JU

Bachelor of Science

Tianjin University

Tianjin, P. R. China

1990

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1998

EVALUATION OF GLYCOSIDIC CHIRAL SURFACTANTS IN ENANTIOMERIC SEPARATIONS BY CAPILLARY ELECTROPHORESIS

Thesis Approved:

Thesis Advisor

Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my research advisor, Dr. Ziad El Rassi, whose intelligent guidance, inspiration, and devotion to his work and students have made this work possible.

I would also like to thank my committee members, Dr. K. Darrell Berlin and Dr. Nicholas Kotov, for their support and invaluable suggestions.

My thanks go to my parents Mengqian Ju and Errui Cao for their constant love and support through my educational endeavors and in every aspect of my life. My gratitude also goes to the rest of my family for their support in everything I do.

My deepest gratitude goes to my husband, Jian Liu, for his never ending love and for his good humor through this whole process.

Finally, I would like to thank the Department of Chemistry and all of those whom I befriended for their support while I pursued my dream.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATION

Big CHAP	N, N-bis(3-D-gluconamidopropyl cholamide
$BNPO_4$	1, 1'-binaphthyl-2, 2'-diylhydrogen phosphate
BSA	bovine serum albumin
18C6TCA	18-crown-6-ether-tetracarboxylic acid
CD	cyclodextrin
CE	capillary electrophoresis
CEC	capillary electrochromatography
CGE	capillary gel electrophoresis
CIEF	capillary isoelectric focusing
CITP	capillary isotachophoresis
CMC	critical micellar concentration
CMC_{mix}	CMC of binary surfactant mixtures
2, 4-CPPA	2-(4-chlorophenoxy) propionic acid
CYMAL	cyclohexyl-alkyl maltoside
CYMAL-5	cyclohexyl-pentyl-β-D-maltoside
CZE	capillary zone electrophoresis
Deoxy Big CHAP	N,N-bis(3-D-gluconamidopropyl)-deoxycholamide
DM	decyl-β-D-maltoside
DNP-AA's	dinitrophenyl amino acids

DNP-Lys	DNP-lysine
DNP-Met	DNP-methionine
DNP-Norleu	DNP-norleucine
DNP-Norval	DNP-norvaline
Dns-AA's	dansyl amino acids
Dns-Leu	Dns-leucine
Dns-Met	Dns-methionine
Dns-Phe	Dns-phenylalanine
Dns-Trp	Dns-tryptophan
Dns-Val	Dns-valine
EOF	electroosmotic flow
FLEC	[1-(9-fluorenyl)-ethyl] chloroformate
GC	gas chromatography
GITC	2,3,4,6-tetra-O-acetyl-D-glucopyranosyl isothiocyanate
GS	glycosidic surfactant
HPCE	high-performance capillary electrophoresis
HPLC	high performance liquid chromatography
I.D.	inner diameter
L	total capillary length
l	effective capillary length
μ_a	apparent mobility
μ_e	effective electrophoretic mobility
μ_{EOF}	velocity of EOF

Marfey's reagent	sodium-(2, 4-dinitro-5-fluorophenyl)-L-alaninamide
MECC	micellar electrokinetic capillary chromatography
Met-Trp's	methyl substituted tryptophans
1-Met-Trp	1-methyl-tryptophan
5-Met-Trp	5-methyl-tryptophan
6-Met-Trp	6-methyl-tryptophan
7-Met-Trp	7-methyl-tryptophan
NG	nonyl-β-D-glucoside
OG	octyl-β-D-glucoside
OM	octyl-β-D-maltoside
OSG	S-octyl-β-D-thioglucoside
Poly-L-SUV	poly (sodium-*N*-undecylenyl-L-valinate)
2-PPA	2-phenoxypropionic acid
σ	peak standard deviation
SBE-β-CD	sulfobutyl-ether (IV)-β-cyclodextrin
SC	sodium cholate
SDC	sodium deoxycholate
SDS	sodium dodecyl sulfate
SDVal	sodium dodecanoyl-L-valinate
SFC	supercritical fluid chromatography
STC	sodium taurocholate
STDC	sodium taurodeoxycholate
TLC	thin layer chromatography

t_{R1}	migration time of enantiomer 1
t_{R2}	migration time of enantiomer 2

CHAPTER I

OBJECTIVES, BACKGROUND AND RATIONALE OF THE RESEARCH

Introduction and Objectives

When four different ligands are bound to a tetravalent carbon, an asymmetric molecule is generated in which the carbon is the asymmetric center. As shown in Fig. 1, two optical isomers can be generated due to the different spatial orientations of the ligands around the chiral center. Enantiomers are two stereoisomers which exhibit non-superimposable mirror images. The enantiomers have essentially identical physical and chemical properties, but they do not always possess the same physiological effects.

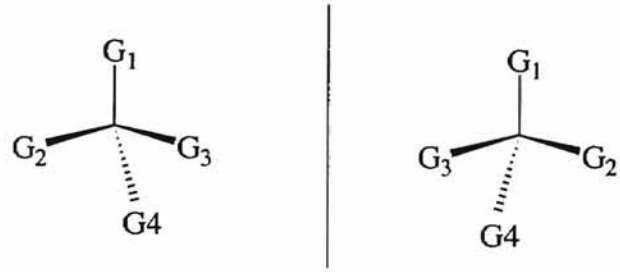


Figure 1. Schematic diagram of a pair of mirror images of chiral molecule.

Thus, enantiomeric separations are important subjects of research in various fields, especially in the pharmaceutical industry. It has been reported recently, that among 1327 synthetic drugs, 39.8% are chiral and only 11.6% of them are sold as single enantiomers (1). In many cases, two enantiomers of the same racemic drug display opposing pharmacological effects. The undesired enantiomer can lower the efficacy of the beneficial enantiomer, and in the worst case it can exhibit adverse side effects. There are several reported cases, such as terbutaline, a drug that is a β2-selective adrenoceptor agonist. (-)-Turbutaline is several thousand times more potent and effective than the (+)-enantiomer. In the case of thalidomide, the drug administered to pregnant women to relieve morning sickness, its (-)-enantiomer is thought to cause birth defects (2).

Regulatory agencies worldwide have realized the potential benefits of pure enantiomer drugs and have published guidelines for the development of chiral drugs. The Food and Drug Administration established a policy regarding the registration of racemates, saying " The stereoisomeric composition of a drug with a chiral center should be known and the quantitative isomeric composition of the material used in pharmacologic, toxicologic, and clinical studies known" (3). This clearly indicates the importance of investigations of the enantiomeric forms of a racemic drug in order to provide improved drug to patients.

Because of the awareness of the harmful effect of racemates, currently there is a trend toward producing single active enantiomers by using highly enantioselective synthetic processes. These synthetic methods often produce single enantiomers containing as little as 0.1 % w/w of the unwanted enantiomer. Since the two enantiomers have identical physical-chemical properties, the analytical chemists are faced with the

challenge of developing methods that have high separation efficiencies, high resolution and sensitivity. In this regard, capillary electrophoresis (CE), which is a microseparation technique, has been shown very effective in the rapid and high resolution separations of enantiomers (4-6).

As will be discussed below, although various direct and indirect CE approaches have been exploited in enantiomeric separations (7-10), each of the different methods provided a solution to a limited number of enantiomers. This has been the driving force for continuous investigation in this area to improve chiral separation by CE and enlarge its scope of applications.

Thus, the aim of this research is (i) to introduce a new class of chiral glycosidic surfactants (GS's), namely cyclohexyl-alkyl-β-D-maltosides (CYMAL's) surfactants for improved enantioseparation by CE, (ii) to assess the effect of the nature of CYMAL's, and (iii) to compare CYMAL's to other GS's that have been previously introduced by our laboratory (11). These various objectives were pursued during the present investigation, and the results are presented and discussed in Chapter II, III and IV. While Chpater II deals with the characterization of cyclohexyl-pentyl-β-D-maltoside (CYMAL-5), Chapter III compares different CYMAL's and Chapter IV compares CYMAL's to other GS's.

The objective of this chapter is to (i) overview CE instrumentation and modes of operation, (ii) provide background of chiral separation and evaluate different CE methods and chiral additives, and (iii) outline the objectives and rationales of this study.

Overview of Capillary Electrophoresis

Separation by electrophoresis is based on the differential migration of solutes under the influence of an applied electric field. In capillary electrophoresis (CE), also known as high-performance capillary electrophoresis (HPCE), the electrophoretic process is performed in narrow-bore capillaries, typically 25 to 100 μm inner diameter (I.D.), which are usually filled with buffers. Use of the capillary format has several advantages, particularly with respect to minimizing the detrimental effect of Joule heating. In fact, capillaries allow fast dissipation of heat, thus minimizing zone distortion arising from Joule heating. In addition, the high electrical resistance of the capillary enables the application of very high electrical fields (up to 30 kV) with only minimal heat generation. The use of the high electrical fields results in short analysis times and high efficiency and resolution. Since the introduction of the first commercial instrument in 1988, CE is now gaining popularity as a powerful microseparation technique for routine analytical applications.

Instrumentation

One of the key feature of CE is the simplicity of its instrumentation. A schematic illustration of an in-house assembled CE instrument similar to that used in our studies, is shown in Fig. 2. It consists of 5 major components: (i) a high voltage power supply capable of delivering up to ± 30 kV, (ii) a buffer filled fused-silica capillary, (iii) an on-column detector (e.g., UV-Vis detector), (iv) a data storage and processing device (e.g., a

computing integrator), and (v) a plexiglass safety box to protect the operator from high voltages.

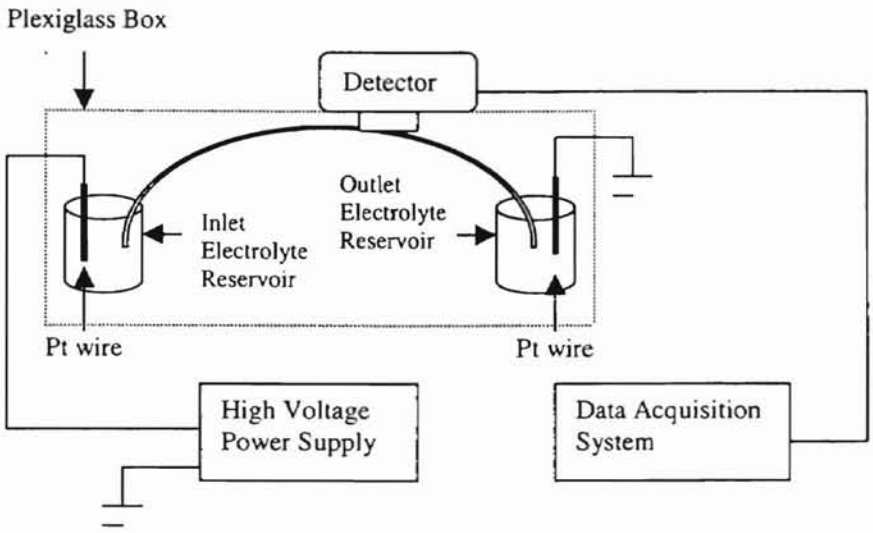


Figure 2. Schematic of an in-house assembled capillary electrophoresis instrument.

Electroosmotic flow

In aqueous or hydro-organic separation media, the surface of fused-silica capillaries possess an excess of negative charges, mainly due to the ionization of the silanol group (SiOH) to the anionic form (SiO^-). As a result, anionic species in the running electrolyte are repelled from the surface, while electrolyte counterions are attracted to the capillary wall. Ions next to the wall are immobile forming a compact layer. Further from the wall is a diffuse and mobile region. Figure 3 illustrates the electric double layer at the surface of the fused-silica capillary.

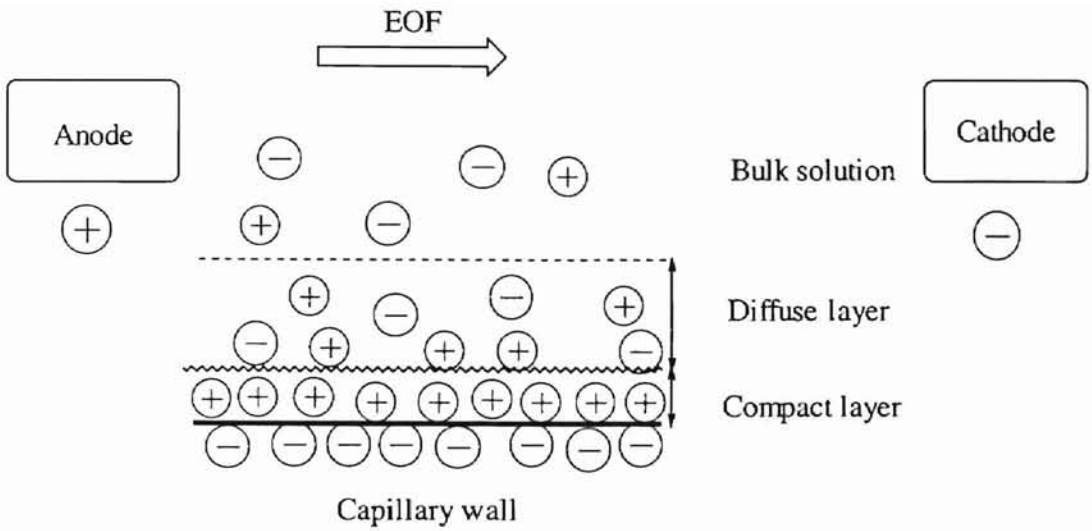


Figure 3. Illustration of the electric double layer at the surface of the fused-silica capillary.

When an electric field is applied across the capillary length, the cations forming the diffuse double-layer are attracted toward the cathode. Since these ions are solvated by water, their movement drags the bulk solution in the capillary toward the cathode. This flow is known as the elecroosmotic flow (EOF) and is the driving force for many modes of CE. The EOF can cause movement of nearly all species, regardless of their charges, in the same direction. Cations migrate fastest since the electrophoretic attraction towards the cathode and the EOF are in the same direction, neutrals are carried at the velocity of the EOF but are not separated from each other, and anions migrate slowest since they are attracted to the anode but are still swept by the strong EOF toward the cathode (12) (13).

Modes of CE

Capillary electrophoresis encompasses various modes of separation. The basic modes of CE are capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MECC), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), and capillary isotachophoresis (CITP). These different modes can be performed on the same basic instrumentation (12, 13).

Capillary zone electrophoresis (CZE) is the most commonly used mode of CE. The separation in CZE is based on the differences in the electrophoretic mobilities of the solutes. Separation of both anionic and cationic species is possible in the presence of EOF. Neutral solutes co-migrate with the EOF.

In MECC, which is a modification of CZE to separate neutral solutes, the running electrolyte contains a surfactant at concentration above its critical micelle concentration (CMC), thus forming micelles. The separation is based on the differential partitioning of the analytes in the micellar "pseudo-stationary" phase, where hydrophobic solutes interact more strongly with the micelle than the less hydrophobic ones. The surfactants and their corresponding micelles are usually charged.

Capillary gel electrophoresis (CGE) has been mainly used for the separation of large molecular weight biopolymers, e. g., proteins and DNA fragments. In CGE, the capillary is filled by a gel, typically polyacrylamide gel, or by a soluble polymer, e.g., polyethylene oxide, hydroxylated cellulose, forming an entangled polymer-filled capillary column (12, 14). The main separation mechanism is based on the differences in solute size as the charged solutes migrate through the pores of gel-filled capillary column.

In CIEF, solutes are separated on the basis of their isoelectric point (pI) values. A pH gradient is formed within the capillary using ampholytes with pI values that cover the desired pH range of the CIEF. Charged species will migrate in the electrical field until they reach the point in the pH gradient where the pH equals the pI. At this point, the various solutes cease to move and they are focused. A mobilization step is then required to pass the focused analytes in front of a detector. This is usually achieved by pumping mobilization.

Capillary isotachophoresis (CITP) uses a combination of two buffer systems in order to create a state in which the separated zones all move at the same velocity. The zones remain sandwiched between the so-called leading electrolyte (contains ions with higher mobility than the solute) and terminating electrolyte (contains ions with lower mobility than the solutes). In a single CITP experiment, either cations or anions can be analyzed, a fact that can be regarded as either advantageous or disadvantageous.

Among the various modes described above, CZE was used as the mode for performing our studies. CZE in the presence of chiral selectors in the running electrolyte is a well suited approach for chiral separations (4, 15, 16).

Chiral Separation

Traditionally, chromatographic techniques, such as gas chromatography (GC) (17), supercritical fluid chromatography (SFC) (18), and high performance liquid chromatography (HPLC) (19) have been frequently used for chiral separations. GC is most useful for small, volatile molecules that can not be separated by HPLC. Compared

with HPLC, SFC displays faster analysis times, column equilibration, and method development with reduced generation of hazardous waste. SFC's disadvantage is its limited polarity range. It can be used only with compounds that dissolve in methanol or less-polar solvents. Compared with GC, SFC allows the use of lower temperatures, and therefore, stationary phase or analyte racemization is less likely to occur (17). Capillary electrophoresis (CE) has grown rapidly as a powerful alternative and promising technique. CE provides an enormous freedom of choice among various chiral selectors, without direct limitations on thermal stabilities of either solutes or chiral recognition agent. Compared to HPLC, CE is an ultramicroscale analytical technique. A few nL of sample solutions are injected, and a few mL of solvent is sufficient. This permits the use of expensive reagents and also makes CE environmentaly friendly. CE offers very high separation efficiency as more than 100,000 theoretical plates can be easily obtained. In HPLC, a different separation column is required for each type of chiral selector. Since HPLC columns are relatively expensive, chiral separation by HPLC can be very costly. Conversely, a unique property of CE is the ease in changing separation media in the capillary column. This enables one to alter quickly and effectively the run buffer to screen various separation media (9, 20, 21).

Separation of enantiomers by CE techniques was first reported in 1985 (22). Gassmann and co-worker separated a number of dansyl amino acids by the diastereomeric interaction between the DL-amino acid and the copper (II) complex of L-histidine present in the running electrolyte. This genuine work has paved the way to other important approaches for enantiomeric separations by CE.

Different CE methods for chiral separation

Separation of enantiomers is a difficult task because they possess similar physico-chemical properties. When an electric field is applied, the enantiomers will move with the same velocity in an achiral running electrolyte due to the identical electrophoretic mobilities. Therefore, we must put the racemic mixture into a chiral environment to form labile diastereoisomers which have different effective mobilities. There are two types of separation methods: indirect separation methods and direct separation methods.

Indirect methods. Indirect separation of enantiomers involves the formation of stable diastereoisomers, before the electrophoretic run, via a chemical reaction (or precolumn derivatization) as shown below:

$$\text{(D, L)-En} + \text{D-Sel} \Rightarrow \text{(D)-En-(D)-Sel} + \text{(L)-En-(D)-Sel}$$

where En is the racemic compound that contains at least one derivatizable functional group, e.g., amino ($-NH_2$), carboxyl ($-CO_2H$) or carbonyl group (R-CO-R) (2), Sel is the chiral selector, and D and L are dextro- and levorotatory, respectively. The chiral reagent reacts with the two enantiomers to give two diastereoisomers through the formation of covalent bonds, e. g., (D)-En-(D)-Sel and (L)-En-(D)-Sel. The diastereomeric pairs formed can be separated in an achiral environment.

An ideal derivatization reagent should fulfil several requirements (23). First, it should be stable and give rapid reactions in high yields at low temperatures and the reaction products should be sufficiently stable. Excess reagent should not disturb the separation. Second, the reagent should be selective for the target analytes. Third, the

reagent should contain or produce a strong chromophore or fluorophore. The reagent should be commercially available or easily synthesized in good yields, and it is desirable that they are available in the L-isomer as well as the D-isomer, so that elution orders can be reversed when required. Fourth, the reagent should be inexpensive.

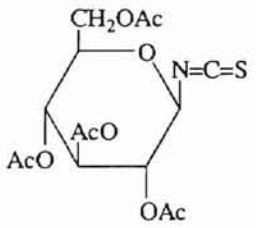
The main advantage of this method is the possibility of analyzing the derivatized sample in common achiral electrolytes. The additional benefit is the improved detectability of certain compounds which lack a strong chromophore or fluorophore in their structures (2, 24).

The disadvantages can be summarized as follows (1): (i) the method is time-consuming since it involves a sample pretreatment; (ii) the analytes should contain reacting groups susceptible to reaction with chiral selectors; (iii) the chiral selector has to be approximately 100% pure in order to avoid any side reactions; (iv) the two enantiomers should react with the reagent at similar rates; and (v) the diastereoisomers formed should have the same detector response.

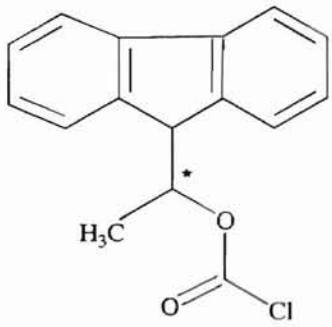
The indirect method can offer a valuable solution in cases where the direct method fails to work. Some small molecules and aliphatic compounds do not exhibit suitable stereoselective interactions with chiral selectors. In this case, the indirect method is the only way for chiral recognition.

A number of derivatization reagents have been developed for applications in connection with chiral separations. Nishi et al. (25) have utilized 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl isothiocyanate (GITC) (**I**) as a chiral reagent for the chemical reaction with 21 racemic amino acids. The GITC reacts rapidly with primary and secondary amino groups to form diastereoisomeric thiourea derivatives.

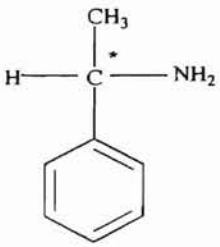
Another chiral derivatizing reagent is [1-(9-fluorenyl)-ethyl]chloroformate (FLEC) (**II**); the fluorenyl skeletal structure enhances UV absorption and has strong fluorescence properties. FLEC has been applied to chiral analysis of D- and L-carnitine (3-hydroxy-4-*N*, *N*, *N*-trimethylammonium buturate). The L-carnitine is very important in the metabolism of long fatty acids, while the D-isomer is toxic in biochemical processes. It is difficult to separate D-isomer from L-isomer by direct method. The separation is performed after derivatization with FLEC. In this application, the background electrolyte consisted of a phosphate buffer at pH 2.6. The addition of tetrabutylammonium bromide improved the diastereoisomer resolution (26).



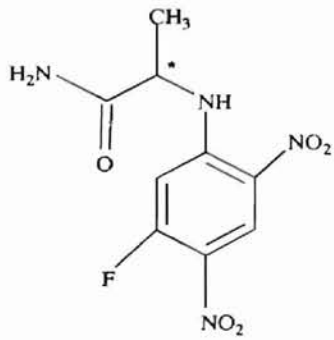
(**I**) GITC



(**II**) FLEC



(**III**) S-(-)-1-phenylethylamine



(**IV**) Marfey's reagent

Figure 4. Structures of some chiral derivatizing agents used in indirect methods.

Noe and Freissmuth (27) have reported the chiral separation of aldohexoses and aldotrioses after derivatization with S(-)-1-phenylethylamine(**III**). Since sugars are not easily accessible to direct resolution methods, this approach makes a valuable contribution to chiral sugar analysis. Marfey's reagent (28) [or sodium-(2,4-dinitro-5-fluorophenyl)-L-alaninamide] (**IV**) is a useful compound that readily reacts with amines, particularly amino acids to form diastereoisomers. An interesting aspect of these derivatives is that by varying the pH of the buffer and reversing the electrical polarity, the diastereoisomeric elution order can be altered. This is particularly useful when the analysis of a diastereoisomer that elutes after the main compound is overshadowed by peak tailing.

Direct methods. Unlike the indirect separation methods, which can only be used in a limited number of chiral separations, the direct separation methods are successfully applied in CE for the enantiomeric separations of several classes of compounds. The chiral selector can be added to the background electrolyte (29), bound to the capillary wall (30), or included in and/or bound to gel matrix (31). The chiral selector interacts with the two enantiomers to form labile diastereoisomers. Various interactions are involved in this process: hydrogen bonds, hydrophobic, π-π and dipole-dipole interactions. The transient diastereoisomers migrate under an applied potential at a different velocity if they possess different stability constant. The direct separation method eliminates all the disadvantages of indirect method. It is rapid, since it does not need additional reaction step. The chiral purity of the selector is not so critical as in the indirect separation. The direct method avoids the danger of sample destruction due to

derivatization. The only serious drawback of the direct methods is the need to find a chiral selector which is appropriate both in enantioselectivity and electrophoretic conditions (1, 24). Several types of chiral selectors and resolution mechanisms will be discussed below.

Different chiral additives used in direct methods

Inclusion-complexations. Inclusion-complexation represents the most frequently used approach for chiral separation by CE. Up to now, three classes of chiral selectors have been used, namely native and modified cyclodextrins (CDs), crown ethers, and antibiotics.

Cyclodextrins are cyclic oligosaccharides consisting of six, seven, or eight glucopyranose units leading to the formation of α-CD, β-CD and γ-CD, respectively. They are obtained by enzymatic reaction with starch. In addition, CD's can be modified by substituting neutral or charged groups on the hydroxyl groups of the molecule. CDs possess a truncated cone with a hydrophobic cavity and a hydrophilic surface. Figure 5 shows the shape of CD. The chiral recognition mechanism is based on inclusion of a hydrophobic part of the molecules, usually aromatic moieties, in the hydrophobic cavity of the CD. An additional requirement is that the analyte and the hydroxyl or modified hydroxyl groups at the rim of the CD form secondary bonds to stabilize the inclusion-complexes (32) (see Fig. 6). In 1988, CDs were used for the first time as chiral additives to the electrolyte in chiral separation. The study was performed in isotachophoresis (33). Fanali (29) was the first researcher to report the application of CDs in CZE and

investigated the chiral separation of several sympathomimetic drugs. In this study, β-CD, heptakis (2,6-di-O-methyl)-β-CD and heptakis (2,3,6-O-methyl)-β-CD have been evaluated. So far, the most widely used and successful additives for enantiomeric separations by CE are CDs and their derivatives. This is due to the fact that CDs are of an optimal size to form inclusion complexes with a significant number of chiral compounds. Another contributing factor is the ability to derivatize the secondary hydroxyls groups, which improve solubility and provide unique selectivity for separations (34, 35).

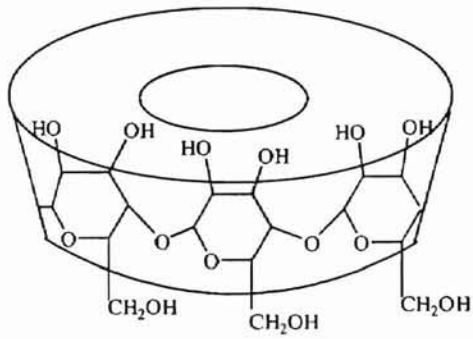


Figure 5. Structure of CD.

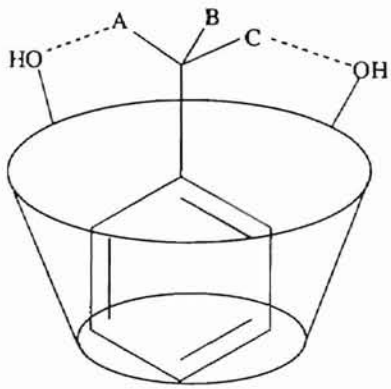


Figure 6. Scheme of inclusion-complexation using CD.

The type of CD used as a chiral additive is an important parameter for the chiral resolution. Amino acids such as tyrosine and phenylalanine have been resolved using an α-CD-based system (36). Attempts to use other cyclodextrins proved unsuccessful primarily because of the poor fit of the molecule within the cavities of β-CD and γ-CD. Resolution and migration time of analytes are strongly influenced by the CD concentration. Migration time and resolution generally increase with increasing concentration of CD. Wren and Rowe (37, 38) postulated a separation model and showed that an optimum selector concentration exists.

The selectivity of the enantiomeric separation with CD's may also be affected by organic modifiers. The use of a running electrolyte at 10% v/v of methanol caused an increase in selectivity and in turn resolution of nine from twelve racemic dansyl-amino acids (31). Another example has been shown by Fanali (39) with the resolution of propranolol obtained by adding 30% of methanol to the running electrolyte containing a buffer (pH 2.5), 4 M of urea and 40 mM of β-CD. Adding methanol at concentration above 30% did not improve resolution.

The effect of the pH of the background electrolyte has been discussed by Sepaniak (40). The resolution of dansyl-DL-phenylalanine has been studied with buffer in the pH range of 3-11 containing hydroxypropyl-β-CD. By lowering the pH, the resolution was substantially enhanced.

The charge of CD can play an important role in the resolution mechanism. In ionized state, charged CDs migrate with their own electrophoretic mobility. The main advantages for using such chiral selectors are better solubility and the ability to display additional electrostatic interactions (4). Sulfobutyl-ether(IV)-β-cyclodextrin (SBE-β-

CD) has been used for the enantiomeric separation of several classes of compounds. As shown in Fig. 7, the SBE-β-CD contains four modified primary hydroxyl groups with butyl chain and sulfonic groups. The modified CD is negatively charged at any commonly used pH in CE. These features allows its use in a charged mode over a wide pH (2-11) range. Operating at pH 2.5 allowed the enantiomeric separation of several basic compounds of pharmaceutical interest, including thalidomide and dimethindene. It also proved useful for the enantiomeric separation of uncharged phenyl-alcohol (41).

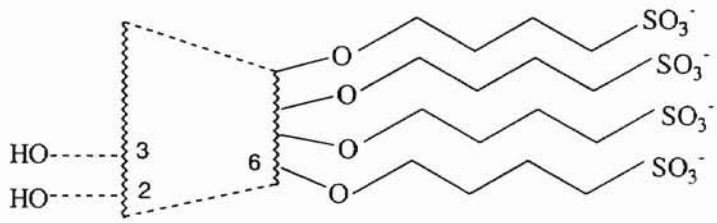


Figure 7. Structure of SBE-β-CD.

Carboxymethylated-β-CD can be either uncharged or negatively charged. At pH > 5, the modified CD had its own mobility. Good enantioselectivity was obtained for the separation of drugs such as hexobarbital and oxazolidinone (42). Terabe's group (43) used positively charged mono (6-β-amino-ethylamino-6-deoxy)-β-CD for the enantiomeric separation of some dansyl amino acids.

Despite the fact that CDs are the most widely used chiral selectors, each type of CD can be used for only a certain number of enantiomeric separations. This fact provides the rationale for introducing new chiral selectors.

Another class of compounds capable of forming inclusion-complexes is crown-ether derivatives. These compounds are able to form stable host-guest complexes with

alkali metals, alkaline earth metals and primary amine ions. The chiral separation mechanism is not the same as for CDs. Here the analyte fits in the crown-ether's cavity with its hydrophilic part (protonated amino group) by forming ion-dipole bonds with the oxygen atoms of the chiral selector. The inclusion complex is stabilized by secondary bonds between substituent groups on the asymmetric center of the analyte and the carboxylic groups of the crown ether (1). Kuhn et al. have used (+)-18-crown-6-ether-tetracarboxylic acid (18C6TCA) for the chiral separation of amino acids. Figure 8 shows the structure of 18C6TCA and the host-guest complex. Separations were carried out at low pH (pH 2-2.5) to protonate the amino groups in the amino acids (36). The 18C6TCA also showed excellent enantioselectivity for amino alcohols (44). Crown ethers have also been used for the chiral resolution of racemic dipeptides (45) and some tripeptides (46). One of the major disadvantage of crown ethers is the complexation with metal (e.g., alkali and alkaline earth), so that the electrolyte should be free of complexing metal ion in order for the chiral solute to undergo interaction with the cavity.

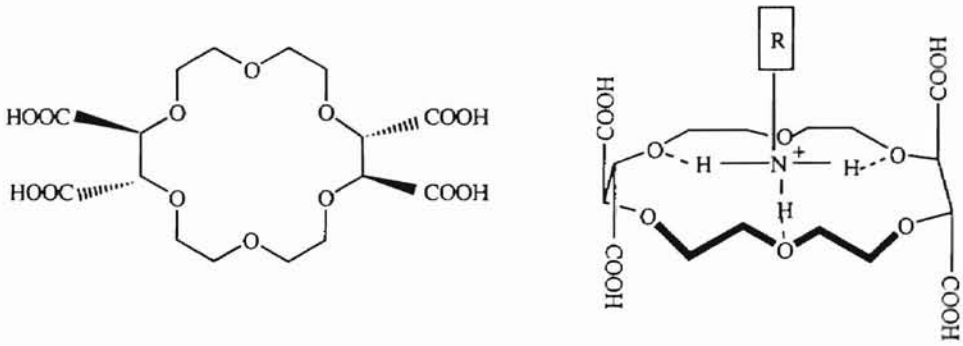


Figure 8. Structure of 18C6TCA and the host-guest complex.

Macrocyclic antibiotics (e.g., vancomycin and ristocetin A) are another class of chiral selectors, which were first introduced for chiral separations in CE by Armstrong

and co-workers (47). Macrocyclic antibiotics have a variety of functional groups such as hydrogen-bonding groups, hydrophobic pockets, aromatic groups, and amide linkages, which are ideal for providing multiple stereo-selective interactions (48). The primary interactions of macrocyclic antibiotics are assumed to be charge-charge or ionic interactions. The secondary interactions are hydrogen bonding, steric repulsion, hydrophobic, dipole-dipole and π-π interactions (49). Two structurally related glycopeptide antibiotics - vancomycin and ristocetin A – were suitable for resolving nonsteroidal anti-inflammatory drugs, antineoplastic compounds, *N*-derivatized amino acids, and various other racemates (50).

Although macrocyclic antibiotics proved useful for several enantiomeric separations, their UV absorptivity is problematic in direct methods. Partial-filling techniques (51) are used to overcome the problem. With this method, a coated capillary was used to suppress the EOF and keep the UV-absorbing macrocyclic antibiotic from passing the detector window. The column is filled with a run buffer containing vancomycin, and then the sample is loaded into the capillary. When voltage is applied, the chiral selector migrates in the opposite direction from the solute, thus removing any background absorbance caused by macrocyclic antibiotic. This kind of operation (i.e., partial filling) requires high precision instrumentation to produce reproducible separations. Another disadvantage is that the optimum antibiotic concentration for maximum resolution is very narrow and situated at low concentration, a fact that renders the optimization of separation to be rather a difficult task.

Linear polysaccharides. The good UV transparency has made polysaccharides a desirable group for use as chiral selectors. In the CE separation of enantiomers using polysaccharides, the conditions for optimal enantiomer separation can be manipulated by changing the buffer solution containing the polysaccharide chiral selector. The pH of the buffer and the concentration of the chiral selector (polysaccharide) are two important factors affecting the enantioselectivity.

Heparin is a naturally occurring mucopolysaccharide that is used clinically as an anticoagulant. The role of heparin as a chiral additive was first investigated by Stalcup (52). A wide range of drugs, including antimalarial drugs and antihistamines, were enantioresolved in 10 mM phosphate buffer. A greater number of analytes were enantioresolved at pH 4.5 in comparison to those separated at pH 5. It was concluded that chiral discrimination arose from a combination of ionic, hydrogen bonding and hydrophobic interactions.

Chondroitin sulfates are linear mucopolysaccharides with *N*-acetylchondrosine as a repeating unit and one sulfate group per disaccharide unit. There are three forms of chondroitin sulfate, namely A, B, C. Nishi and Terabe (53) used chondroitin sulfate C as a chiral additive in phosphate-borate buffer systems. In this system, and at pH 2.4, enantioresolution of diltiazem and clentiazem enantiomers was achived. The same research group further compared chondroitin sulfate A to C for the chiral separation of a range of basic drugs including diltiazem, verapamil and propanolol. Enantiomeric separations were readily realized with both chondroitin sulfate A and C in similar phosphate buffer system. Greater resolution was achieved in every case with chondroitin sulfate C.

Although polysaccharides have shown to be effective for the enantioseparation of a wide number of chiral molecules, in most cases, a capillary washing process is required. Due to the adsorption of polysaccharides onto the capillary wall, the capillary needs to be washed with KOH and NaOH after each run to obtain reproducible migration times (54).

Proteins. Proteins, by their very diverse nature, provide a wide variety of options for chiral selectivity in CE. Proteins can be either added to the background electrolyte to perform affinity CE or immobilized within the capillary (e.g., the protein grafted onto a silica support, or the protein is immobilized within a gel structure) to perform affinity capillary electrochromatography (CEC).

The simplest and most common way of using proteins as chiral selectors in CE is to dissolve the protein in the background electrolyte. The solution completely fills the separation capillary and is present in both buffer reservoirs. The chiral separation of leucovorin stereoisomers was investigated by Barker et al. using bovine serum albumin (BSA) (55). The major disadvantage of filling the buffer reservoirs and the whole capillary with protein is that it will always be present in the detection region. Partial-filling methods provide a partial solution to the problem of protein interference with detection, but impose limitations upon the experimental design.

Two electrochromatographic approaches which have been taken are to immobilize the protein in a gel within the capillary, or use capillaries packed with an immobilized-protein stationary phase. Such systems have some significant advantages: the protein can be immobilized such that there is no interference with detection, and the protein mobility is well defined (i. e., zero). Birnbaum and Nilsson (56) first described

CE separation using a protein selector, in the resolution of tryptophan enantiomers. Their protein (BSA) was immobilized within the capillary by cross-linking with glutaraldehyde. The buffered reaction mixture containing BSA and glutaraldehyde was pumped into the capillary from the inlet end until just before the detection window. The mixture was then allowed to gel, and thus a protein gel plug was made, filling the capillary up to the detection point.

Packed capillary electrochromatography (CEC) with protein phases has been reported with both α_1-acid glycoprotein (57) and human serum albumin (58). This work has been carried out with commercially available silica-based packing materials of 5-7 μm diameter. The packing is performed in a predominantly aqueous solvent to avoid denaturation of the protein.

Because of their large molecular weight and the multiplicity of their chiral sites, proteins in free solution (CZE) or immobilized within the capillary (CEC) yield broad peaks and consequently low separation efficiencies.

Chial surfactants

Many surfactants are commercially available and some of them are chiral. The currently available chiral surfactants can be divided into two main groups: (i) detergents derived from naturally occurring molecules, e.g. bile salts, digitonin, and CHAPS derivatives and (ii) synthetic surfactants derived from simple chiral molecules such as amino acid enantiomers, derivatized glucose, and other charged chiral compounds (2).

Chiral surfactants in MECC. The use of chiral surfactants in CE was first introduced by Terabe et al. for the separation of neutral compounds (59). It is mainly used for the separation of uncharged compounds, and the technique is referred to as micellar electrokinetic capillary chromatography (MECC). The separation system in chiral MECC consists of two phases, the aqueous background electrolyte and a charged chiral surfactant forming micelles moving with their own electrophoretic mobility. A strong EOF pushes both neutral analytes and micelles to the detector. The neutral compounds are interacting with the micellar and aqueous phases and the migration order is a function of the distribution coefficient of the analytes between the two phases. The chiral discrimination mechanism is not well understood. However, most polar solutes are supposed to interact with the optically active surface of the micelle through polar-polar interactions in addition to hydrophobic interactions with the core of the micelle (1, 6). Therefore, a surfactant having a chiral center in the polar head group will be suitable for the separation of enantiomers, because most chiral compounds have polar groups near their chiral centers.

Bile salts are an abundant source of chiral surfactants. They are charged chiral compounds with a helical structure able to interact with relatively flat and rigid compounds where electrostatic, hydrogen and hydrophobic interactions are involved in the separation mechanism (60). The commonly used bile salts are sodium cholate (SC), sodium deoxycholate (SDC), sodium taurocholate (STC), and sodium taurodeoxycholate (STDC) (see Fig. 9). The bile salts derivatized with taurine groups dissolve easily in water, while the others require buffers at pH $\geq$ 7. The STDC is the most widely used of all the bile salts because it has the broadest enantioselectivity. Examples of applications

of STDC include resolution of dansyl-amino acid racemates, binaphthyl derivatives, and some polycyclic compounds (60, 61).

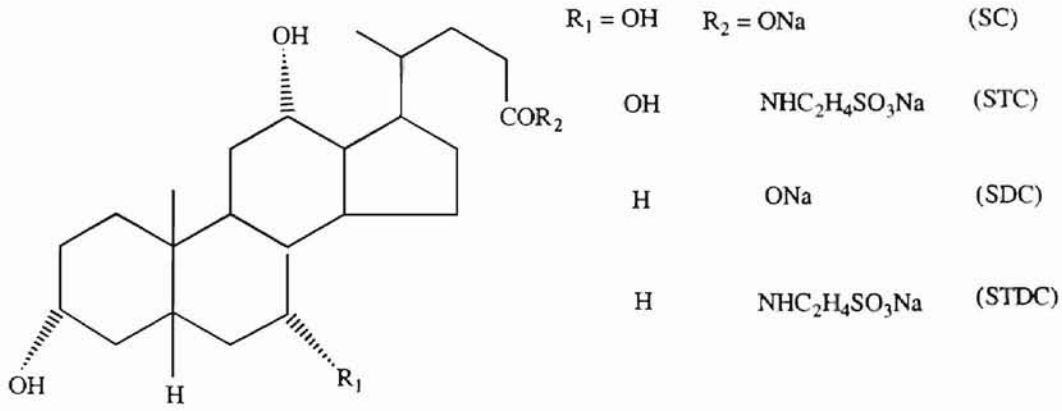
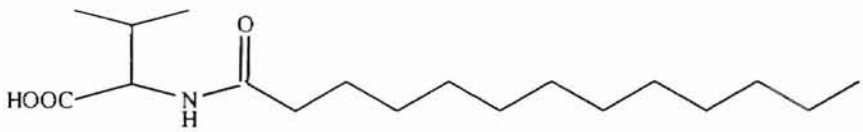


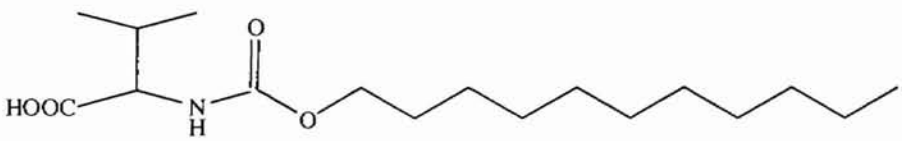
Figure 9. Structure of bile salts.

Over the last few years there has been some activity in the synthesis of chiral surfactants consisting of an amino acid moiety attached to a hydrophobic alkyl chain. One of the earliest examples of this type of surfactant involved the use of chiral derivatized amino acids such as sodium dodecanoyl-L-valinate (SDVal) (**V**) in MECC for the enantiomeric separation of amino acid derivatives (62, 63). The addition of MeOH and urea enforce interaction with the micelle, thus improving peak shapes as well as resolution. Pharmaceutical compounds, like warfarin were enantioresolved using a mixed micelle system consisting of SDVal and sodium dodecyl sulfate (SDS) at pH 9 in the presence of 10% methanol and 5 M urea (64). *N*-Dodecoxy-carbonylvaline (**VI**) S or R configuration, was synthesized and used in MECC at pH > 7 for the enantiomeric separation of several compounds of pharmaceutical interest. Atenolol, bupivacaine,

ephedrine, and terbutaline were successfully resolved into their enantiomers using a phosphate/borate buffer at pH 8.8 and 25 mM *S*-*N*-dodecoxy-carbonylvaline (65). El Rassi and Mechref employed *N*,*N*-bis(3-D-gluconamidopropyl)-cholamide (Big CHAP) and -deoxycholamide (Deoxy Big CHAP) for the separation of enantiomers by MECC (66). Those two new chiral selectors displayed two chiral portion namely the steroidal and the polar polyhydroxyl moieties. The charge of the chiral micelles was adjusted by changing the pH and the borate concentration. Lowering the capillary temperature and the addition of 10-15% methanol improved the enantiomeric resolution of compounds like dansyl amino acids and herbicides. Big CHAP-borate micelles were less stereoselective than Deoxy Big CHAP-borate micellar systems under identical conditions. Due to their properties, the new chiral surfactants could broaden the application in chiral pharmaceutical analysis.



(**V**) SDVal



(**VI**) *N*-Dodecoxy-carbonylvaline

Figure 10. Structure of synthesized chiral surfactants.

Other surfactants were introduced including digitonin, glycyrrhizic acid and β-casein. However, these surfactants have found limited applications and required the use of SDS to separate uncharged chiral solutes. Very recently, Tickle et al. (67) modified dodecyl glucopyranoside by substituting a phosphate group or a sulphate group on the C4 and C6 or C6, respectively. This yielded charged surfactants that exhibited chiral interaction with Troger's base, fenoldopam and metoprolol.

The use of chiral micelle polymers is a very interesting approach in MECC. Enhanced stability and rigidity are the main advantages of the polymers in comparison to the monomer. These properties arise from the covalent bonds formed between surfactant aggregates after polymerization. The presence of these covalent bonds eliminates the normal dynamic equilibrium between the surfactant monomers and the micelles, thus simplifying and enhancing the process of binding between the micelle and the solute. Furthermore, the polymer can be used at any concentration because the polymicelles have no CMC (68). Dobashi used poly (sodium-*N*-undecylenyl-L-valinate) (Poly-L-SUV) for the separation of enantiomeric 3, 5-dinitrobenzoylated amino acid isopropyl esters and amines (69). Cationic chiral drug, laudanosine, a biosynthetic precursor of morphine, and its two analogs, laudanosoline and norlaudanosoline, were separated recently with the same polymeric surfactant Poly-L-SUV (70). Under neutral and alkaline conditions, laudanosoline and norlaudanosoline were partially resolved whereas laudonosine was fully resolved (R_s = 1.2) at pH 11. As in the case of proteins, the UV absorbing of polymicelles represent a difficulty and in addition, the kinetic of solute-polymicelles interaction seems to be slow as manifested by the obtention of broad peaks.

Chiral surfactants in CZE. Capillary zone electrophoresis technique and MECC are increasingly complementing each other in chiral separations. The separation principle of CZE is the difference in electrophoretic mobilities. As enantiomers have identical electrophoretic mobilities, some chiral selectors must be added to the separation buffer to form diastereomeric pairs that are sufficiently dinstinct electrophoretically. At low pH, EOF is minimal and uncharged chiral surfactant micelles do not migrate. However, under these conditions, basic compounds become protonated and move toward the detector by virtue of their charges. When the solute interacts with chiral micelles, its mobility is greatly reduced. If the two enantiomers have different stability constants, one enantiomer will migrate more slowly than the other and chiral resolution will be achieved. Under neutral or alkaline conditions, weakly acidic solutes are negatively charged and move in the opposite direction to the EOF. The strong EOF sweeps micelles to the detector and, when the anionic solute interacts with the migrating micelle, its electrophoretic migration velocity is reduced. Therefore, the enantiomer with the most interaction is eluted before the other stereoisomer, i. e., the reverse elution order to that at low pH is obtained.

Recently, our laboratory has reported for the first time the use of uncharged alkylglucoside chiral micelles in the enantioseparation of negatively charged phenoxy acid herbicides and other anionic enantiomers, e. g., dansyl amino acids and 1,1,-binaphthyl-2,2'-diylhydrogen phosphate) (11, 71, 72). While the neutral micelles of the alkylglucoside surfactants migrate at the velocity of EOF, the electrophoretic mobility of anionic analytes is opposite in direction to the cathodal EOF. Thus, the effective electrophoretic mobility of an anionic solute will decrease as the magnitude of its

association with the micelle increases. The enantiomeric resolution is achieved when the two enantiomers exhibit different association constants with the chiral micelles. The use of uncharged micelles in CZE of charged enantiomers offers a great deal of advantages in terms of manipulating the enantioselectivity by various operational variables such as the surfactant concentration, the ionic strength and pH of the running electrolyte and the capillary temperature. This study is a continuation of our laboratory's previous efforts in the area of chiral capillary electrophoresis.

Conclusions

It is clear from the above review of pertinent literature concerning enantiomeric separations by CE, that there is still an ample room for improvement in chiral CE. The present dissertation addresses this need by introducing and evaluating novel chiral surfactants, namely cyclohexyl-alkyl-β-D-maltosides (CYMAL's), over a wide range of electrophoretic conditions. To better assess the utility of CYMAL's in enantioseparation by CE, the CYMAL surfactants were compared to other glycosidic surfactants (GS's) that were very recently evaluated in our laboratory.

CHAPTER II

EVALUATION OF CYCLOHEXYL-PENTYL-β-D-MALTOSIDE IN ENANTIOSEPARATION BY CAPILLARY ELECTROPHORESIS

Introduction

As stated in Chapter I, an emerging trend in the pharmaceutical industry is the development of drugs as pure enantiomers rather than as racemic mixtures. It is expected that many commercial racemic drugs will soon be available as pure enantiomers. Therefore, improved methods for determining optical purity are needed. The currently available methods can only provide solutions for a limited number of enantiomers, and no single approach or chiral selector exists that can accommodate all enantiomeric separations. This has been the driving force for the search for new chiral selectors to allow the enantiomeric resolution of a wider range of racemates.

Recently, a series of three papers from our laboratory (11, 71, 72) has shown the advantages of chiral glycosidic surfactants (GS's) in the enantiomeric separation of several compounds. These GS's possessed straight alkyl chains as the hydrophobic tails. It is expected that GS's with different hydrophobic tails will lead to

different enantioselectivity. On this basis, we investigated cyclohexyl-pentyl-β-D-maltoside (CYMAL-5) as the chiral selector. CYMAL-5, which has not been evaluated before, possesses a cyclohexyl group at the end of the straight pentyl chain.

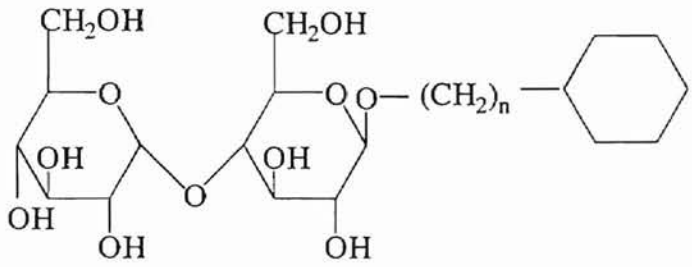
To characterize CYMAL-5, we chose derivatized amino acids as the model chiral solutes. The major rationale for using amino acids resides in their important biological functions. Most amino acids, except phenylalanine, tryptophan, and tyrosine, lack a strong chromophore such that direct UV or fluoresence detection of these amino acids is not possible. We used commercially derivatized dansyl and dinitrophenyl amino acids and methylated tryptophans as model chiral solutes.

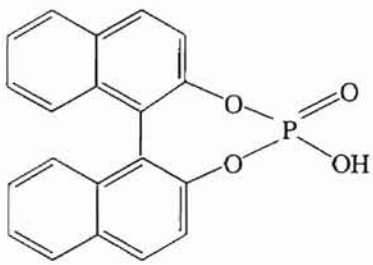
Experimental

Reagents

Cyclohexyl-pentyl-β-D-maltoside (CYMAL-5) surfactant was purchased from Anatrace (Mumee, OH, USA). The structure and critical micellar concentration (CMC) of CYMAL-5 is shown in Table I. 1,1'-Binaphthyl-2,2'-diylhydrogen phosphate ($BNPO_4$) was purchased from Aldrich (Milwaukee, WI, USA). All dansyl amino acids (Dns-AA's), including Dns-tryptophan (Dns-Trp), Dns-phenylalanine (Dns-Phe), Dns-leucine (Dns-Leu), Dns-methionine (Dns-Met), and Dns-valine (Dns-Val), were purchased from Sigma (St. Louis, MO, USA). All methyl substituted tryptophans (Met-Trp's), namely, 1-methyl-tryptophan (1-Met-Trp), 5-methyl-tryptophan (5-Met-Trp), 6-methyl-tryptophan (6-Met-Trp), and 7-methyl-tryptophan (7-Met-Trp), and all

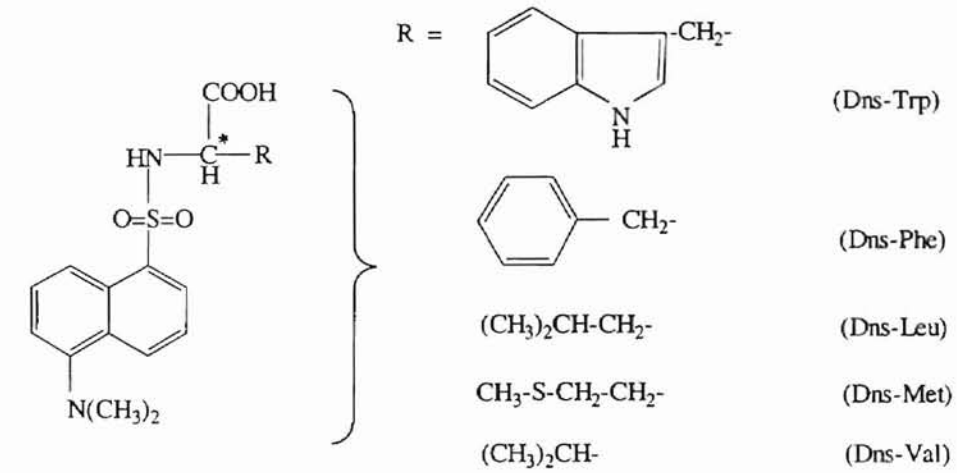
Table I. Structures and CMCs of chiral glycosidic surfactants (GS's) used in this study.

Structure and name of surfactant	abbreviation	CMC (mM)
		
n = 1: Cyclohexyl-methyl-β–D-maltoside	CYMAL-1™	340
n = 2: Cyclohexyl-ethyl-β–D-maltoside	CYMAL-2™	120
n = 4: Cyclohexyl-butyl-β–D-maltoside	CYMAL-4™	7.6
n = 5: Cyclohexyl-pentyl-β–D-maltoside	CYMAL-5™	2.4
n = 6: Cyclohexyl-hexyl-β–D-maltoside	CYMAL-6™	0.56

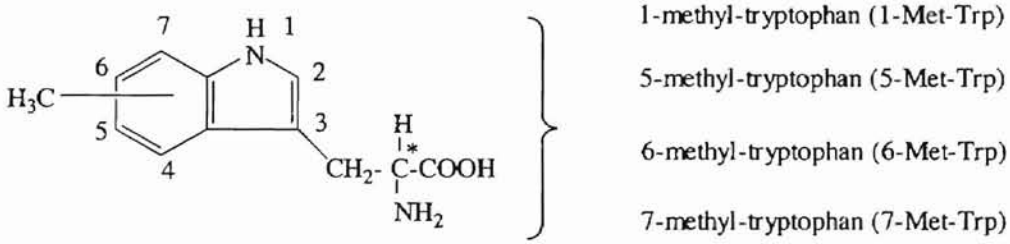


1, 1'-Binaphthyl-2, 2'-diyl-hydrogen phosphate ($BNPO_4$)

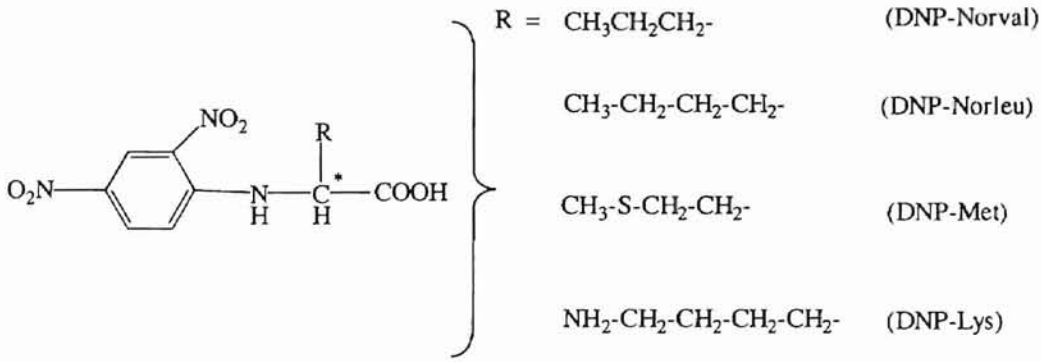
Figure 1. Structures of the chiral solutes used in this study.



Dansyl amino acids (Dns-AA's)



Methyl-tryptophans (Met-Trp's)



Dinitrophenyl amino acids (DNP-AA's)

Figure 1. Continued.

dinitrophenyl amino acids (DNP-AA's), namely, DNP-norvaline (DNP-Norval), DNP-norleucine (DNP-Norleu), DNP-methionine (DNP-Met) and DNP-lysine (DNP-Lys), were also purchased from Sigma. Sodium phosphate monobasic was obtained from Mallinckrodt Specialty Chemical Co. (Paris, KY, USA). Sodium acetate was from Sigma. Phosphoric acid was purchased from EM Science (Cherryhill, NJ, USA). Sodium phosphate dibasic was from Fisher Scientific (Pittsburgh, PA, USA).

Capillary electrophoresis instrument

The capillary electrophoresis instrument was assembled in-house from commercially available components. It consisted of two 30-kV d.c. power supplies of positive and negative polarity, Models MJ 30P400 and MJ 30N400, respectively, from Glassman High Voltage (Whitehouse Station, NJ, USA) and a UV-Vis variable wavelength detector, Model 200, from Linear Instrument (Reno, NV, USA) equipped with a cell for on-column detection. Detection was performed at 254 nm for all solutes. The electropherograms were recorded with a Shimadzu data processor Model CR601 (Kyoto, Japan). Fused-silica capillaries were obtained from Polymicro Technology (Phoenix, AZ, USA) with 50 μm I.D. and 360 μm O.D.. The total length of the capillary was 80 cm, the distance from injection end to the detection point was 50 cm.

Results and Discussion

The CYMAL-5 surfactant was evaluated with various chiral solutes over a wide range of conditions including surfactant concentration, ionic strength and pH of the

running electrolyte. To assess the selectivity of CYMAL-5 as a chiral selector, dansyl amino acids (Dns-AA's), DNP-amino acids (DNP-AA's), methyl substituted tryptophans (Met-Trp's), and 1,1'-binaphthyl-2, 2'-diylhydrogen phosphate ($BNPO_4$) were used as model enantiomers.

Effect of surfactant concentration

Dansyl amino acids. As all surfactants, CYMAL-5 exhibits self-aggregation in aqueous solution at concentration above its CMC. In a micelle, the surfactant molecules are organized in a way that the hydrophobic tails are in contact with each other in the micellar core and the hydrophilic head groups form a shell in contact with the aqueous solution. The polar and hydrophobic interaction between solute and micelle lead to the enantiomeric separation. The neutral micelles of CYMAL-5 migrate at the velocity of EOF. For charged enantiomers, the effective electrophoretic mobility of a given solute will decrease with increasing solute-micelle association. The effective electrophoretic mobility was calculated from the electropherograms using the following equations:

$$\mu_a = \mu_e + \mu_{EOF}$$

$$\mu_e = \mu_a - \mu_{EOF} = \frac{Ll}{Vt_R} - \frac{Ll}{Vt_o} = \frac{Ll}{V}\left(\frac{1}{t_R} - \frac{1}{t_o}\right)$$

where μ_a is the apparent mobility, μ_e is the effective electrophoretic mobility, μ_{EOF} is the velocity of EOF, V is the applied voltage, L is the total capillary length, l is the effective capillary length (length to the detector) and t is the migration time. As can be seen in Fig. 2, the effective electrophoretic mobility of the Dns-AA's under investigation decreased as the concentration of the surfactant increased from 6 mM to 18 mM. At the pH of the

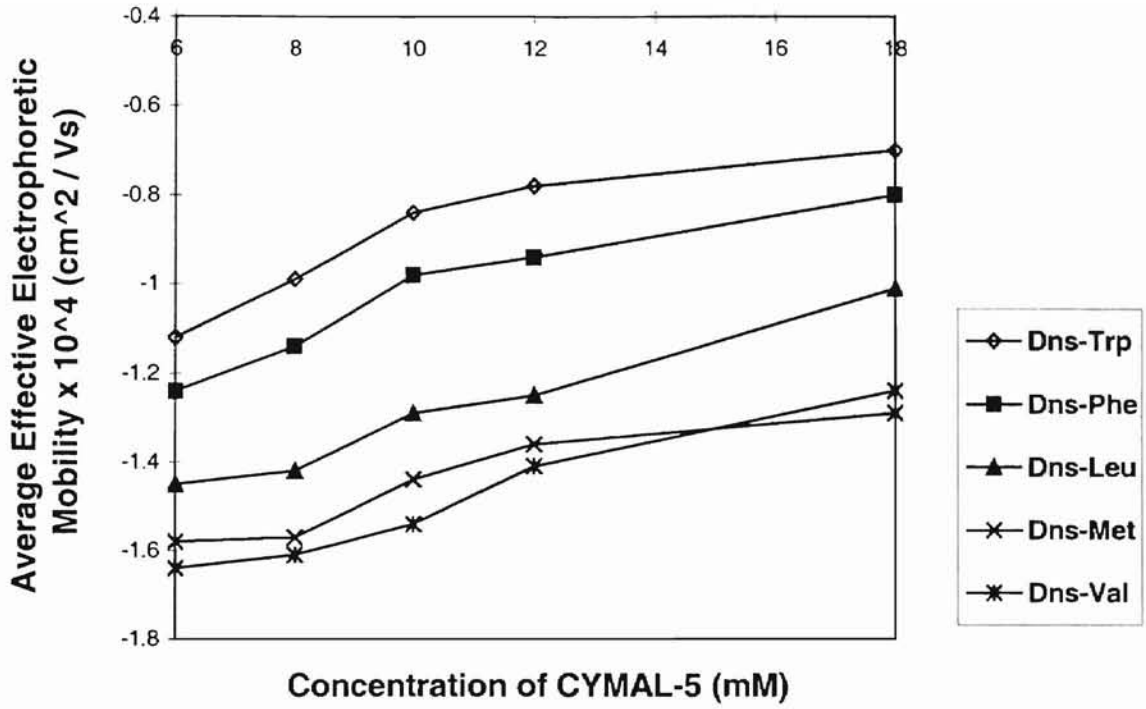


Figure 2. Effect of CYMAL-5 concentration on the average effective electrophoretic mobility of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentrations of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

experiment, the hydrophobicity of Dns-AA's decreases in the order: Dns-Trp > Dns-Phe > Dns-Leu > Dns-Met > Dns-Val.

The effect of CYMAL-5 concentration on the enantiomeric resolution of Dns-AA's is shown in Fig. 3. Resolution was calculated from the eletropherogram using the following equation:

$$R_s = (t_{R2} - t_{R1}) / (2\sigma_1 + 2\sigma_2)$$

where t_{R1} and t_{R2} are the migration times of enantiomers 1 and 2, respectively, and σ_1 and σ_2 are the peak standard deviations of enantiomers 1 and 2, respectively. The optimum surfactant concentration for maximum enantiomeric resolution of Dns-Leu was in the range of 10 to 12 mM, and for that of Dns-Met and Dns-Val, was reached above 18 mM. Dns-Leu is relatively more hydrophobic, and therefore maximum resolution was obtained at low surfactant concentration. Dns-Met and Dns-Val were relatively less hydrophobic, thus demanding higher surfactant concentration for maximum enantiomeric resolution. Dns-Phe showed only slight separation, and its maximum resolution was at low surfactant concentration, because it is as hydrophobic as Dns-Leu. Figures 4 and 5 show the electropherograms of Dns-Met and Dns-Leu in the presence of CYMAL-5, respectively. The reduction of electroosmotic flow by decreasing the pH to 2.5 produced good separation for Dns-Trp which was not obtainable under other experimental conditions. Figure 6 shows the effect of CYMAL-5 concentration on the resolution of Dns-Trp. As can be seen in Fig. 6, resolution increased first, passed through a maximum that spans from 20 to 40 mM surfactant concentration and then decreased as the surfactant concentration was increased. Figure 7 illustrates the electropherogram of Dns-Trp in the presence of CYMAL-5.

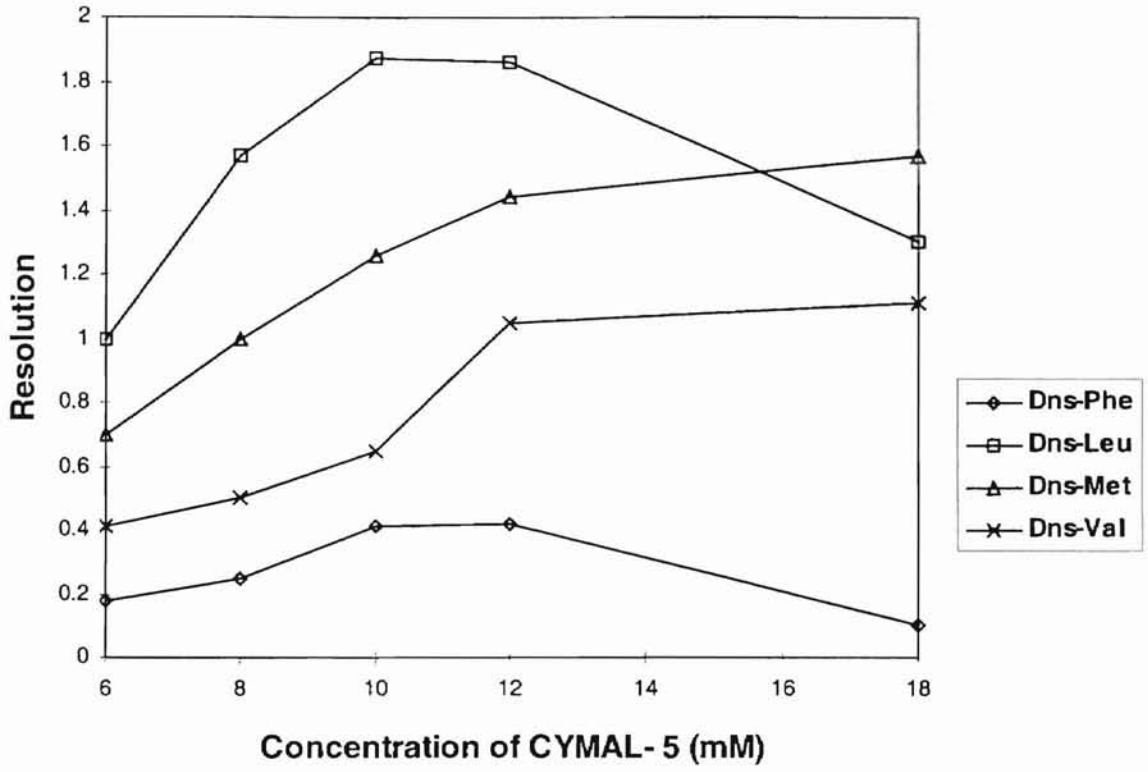


Figure 3. Effect of CYMAL-5 concentration on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentrations of CYMAL-5; voltage, 20 kV, capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

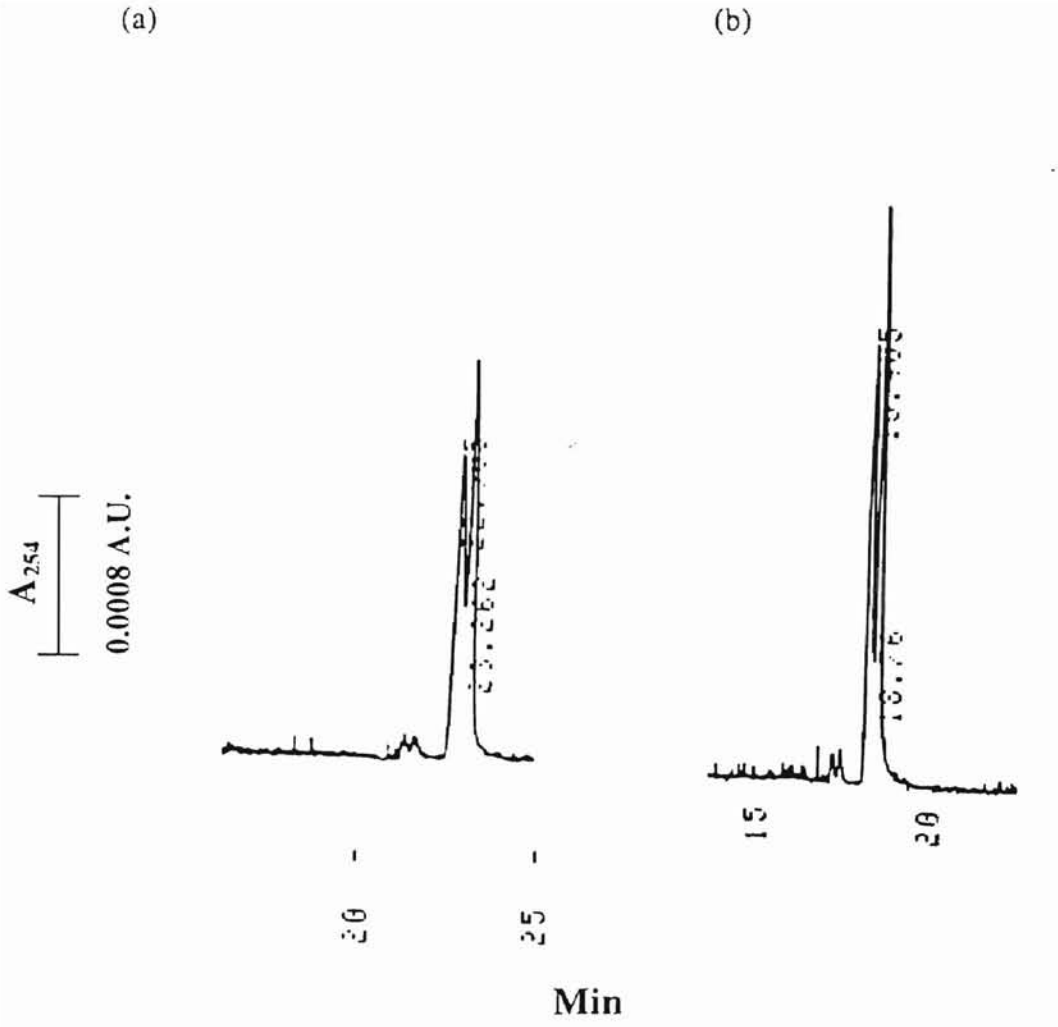


Figure 4. Electropherograms of Dns-Met obtained with CYMAL-5. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing (a) 8 mM CYMAL-5, (b) 18 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

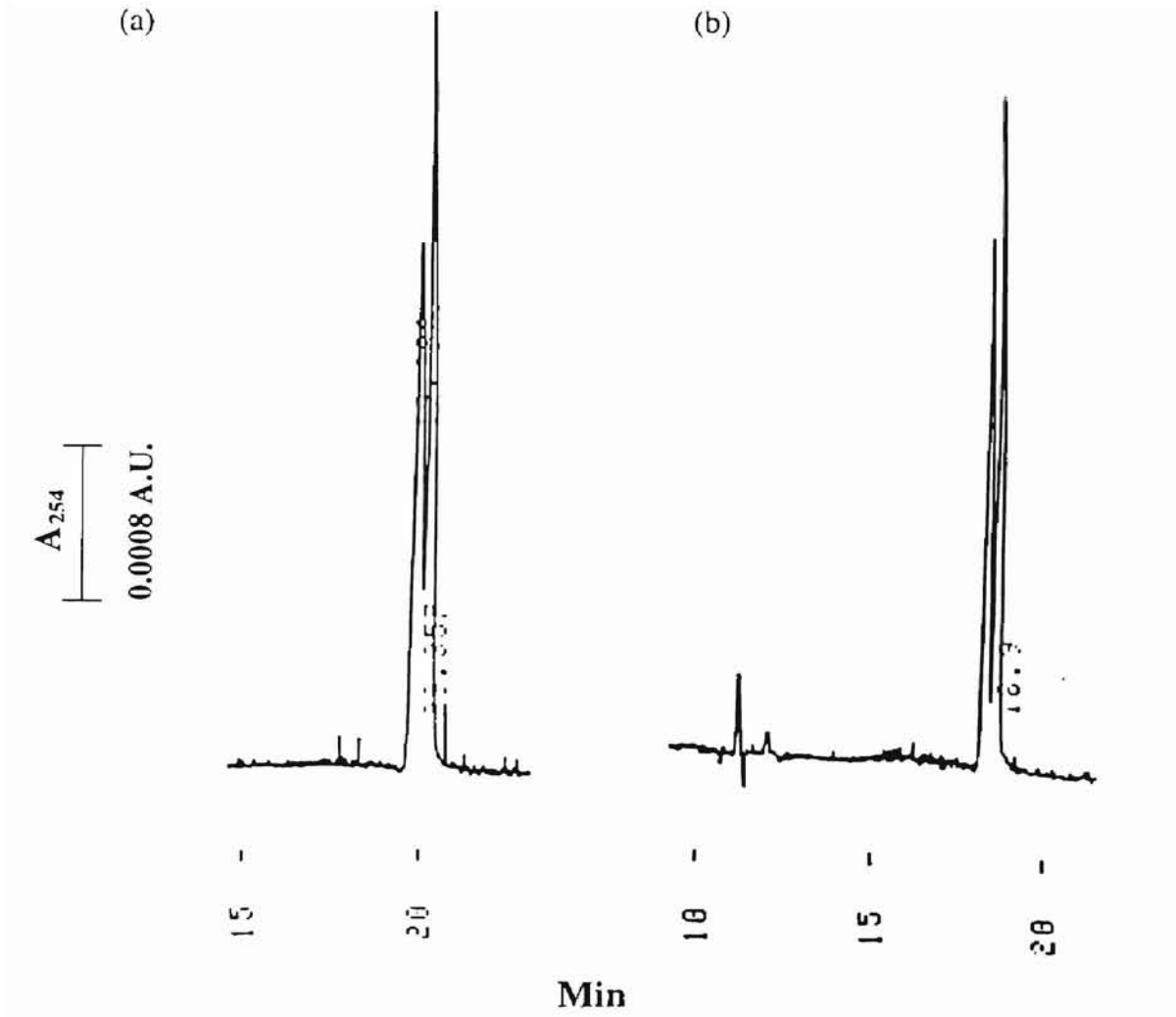


Figure 5. Electropherograms of Dns-Leu obtained with CYMAL-5. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing (a) 8 mM CYMAL-5, (b) 12 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

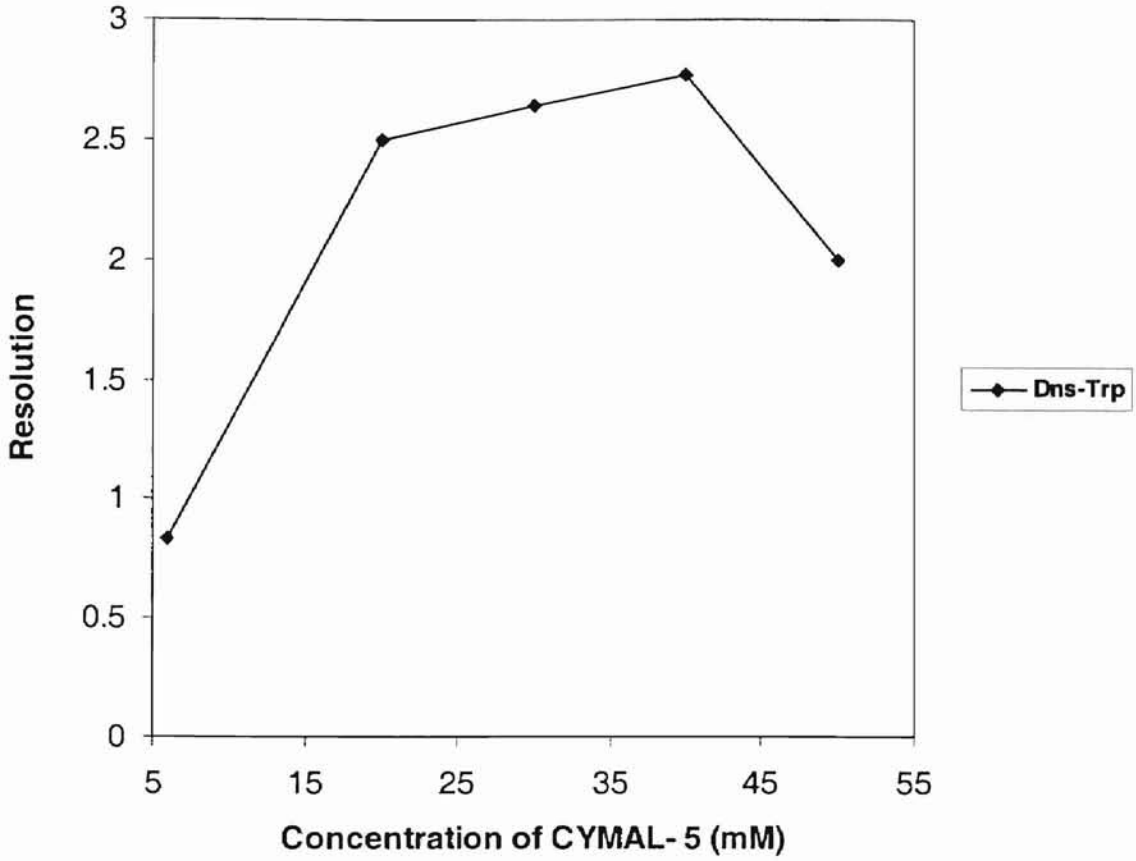


Figure 6. Effect of CYMAL –5 concentration on the enantiomeric resolution of Dns-Trp. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing various concentrations of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

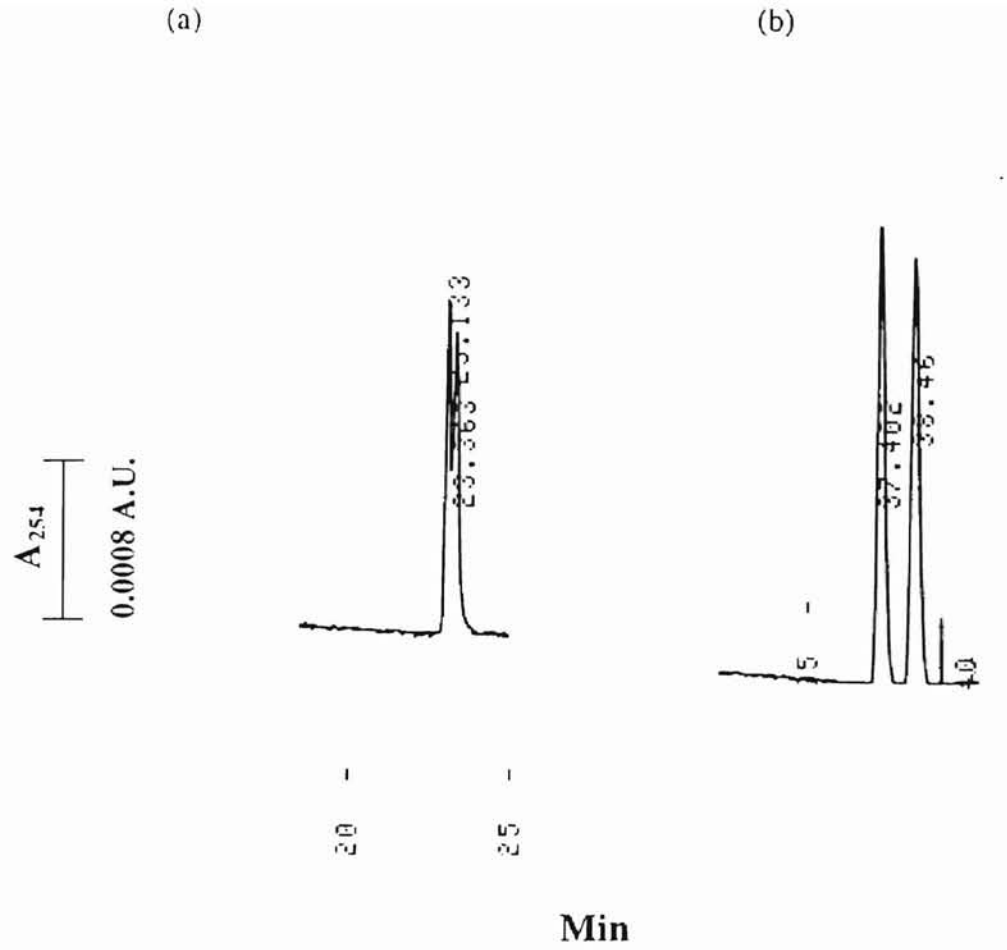


Figure 7. Electropherograms of Dns-Trp obtained with CYMAL-5. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing (a) 6 mM CYMAL-5, (b) 40 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Based on the above results, CYMAL-5 seems to yield enantiomeric resolution of the amino acids that are relatively hydrophobic, e. g., Phe, Trp, Leu, Met, and Val. This indicates that hydrophobic association of the chiral solute with the micelle is essential for chiral recognition.

Methyl substituted tryptophans. Under the chiral separation conditions of Dns-AA's, no noticeable enantiomeric separation was observed for Met-Trp's. Since Met-Trp's are relatively hydrophobic, their interaction with the micelles is relatively strong, thus leading to rapid migration. This is especially true for electrolytes of pH > 4.0, whereby the EOF is increased. Conversely, at pH as low as 2.5, the EOF is negligible and Met-Trp's, which are positively charged, migrate toward the cathode. Under these conditions, enantiomeric resolution is achieved by the difference in the association constant with the chiral micelle. Due to the position of the methyl substituent on the indole ring, 7-Met-Trp, 6-Met-Trp and 5-Met-Trp showed different resolution but with no significant differences in their apparent mobilities. This indicates that there is not enough difference in shape among the various isomers to bring about their achiral separation. 1-Met-Trp can not be resolved enantiomerically. As can be seen from the structure of 1-Met-Trp, the chiral center is in a position where the substituted methyl hinders its accessibility. Blocking the amine function by the substituted methyl group in 1-Met-Trp suggests that the amino group is essential for achieving chiral recognition. As can be seen in Fig. 8, since there is no EOF, the higher the surfactant concentration the more retarded are the solutes and therefore, resolution keeps increasing with increasing

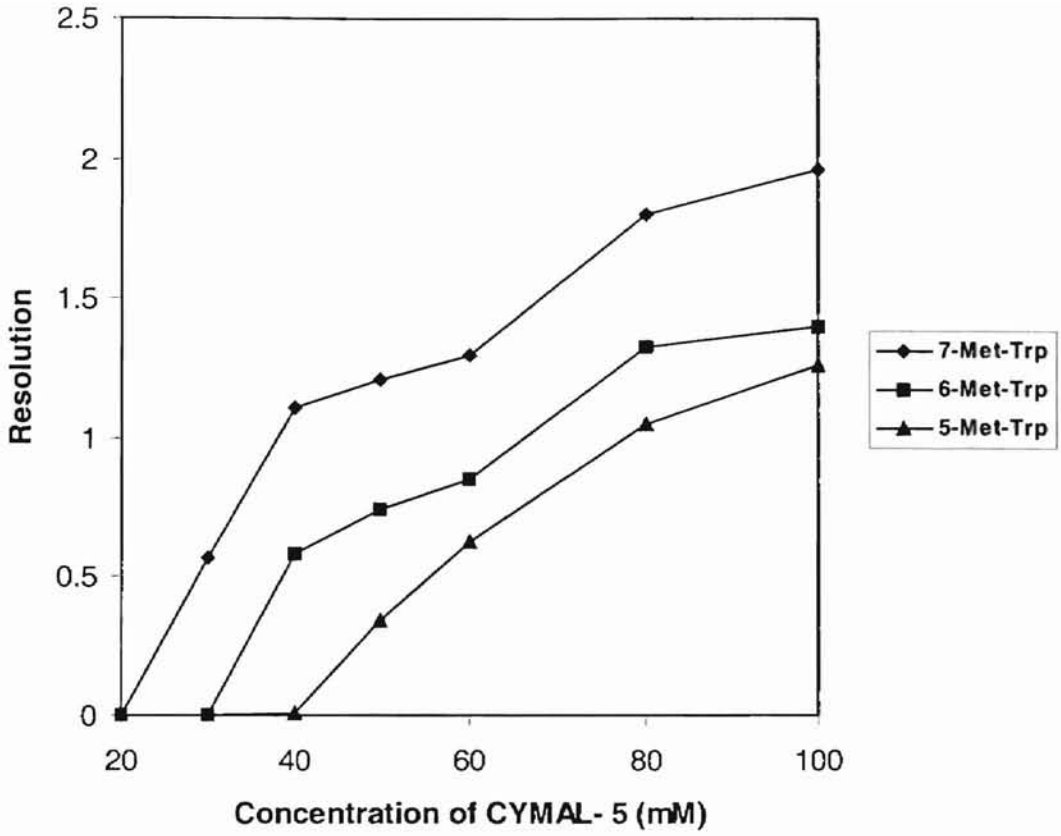


Figure 8. Effect of CYMAL-5 concentration on the enantiomeric resolution of Met-Trp's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing various concentrations of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

migration time. As expected, for chiral solutes that are also positional isomers such as Met-Trp, the enantioresolution among the various isomers almost parallel each other as a function of surfactant concentration. Figures 9 and 10 illustrate the electropherograms of 6-Met-Trp and 7-Met-Trp, respectively.

DNP-amino acids. Figure 11 shows the resolution of DNP-AA's at various concentration of CYMAL-5. The maximum resolution was achieved at about 10 mM CYMAL-5. While maximum resolution for DNP-Norval and DNP-Norleu occured at exactly 10 mM, that of the less hydrophobic DNP-Met extended over a wider surfactant concentration range. As expected, with the exception of DNP-Lys, the effective electrophoretic mobility of all solutes decreased as the concentration of surfactant increased. This is shown in Fig. 12. The effective electrophoretic mobility of DNP-Lys kept constant in the concentration range studied, see Fig. 12. This indicates that DNP-Lys did not associate with the CYMAL-5 micelle.

1,1'-Binaphthyl-2, 2'-diylhydrogen phosphate. Figure 13 shows that the optimum enantiomeric resolution of $BNPO_4$ was achieved at relatively low surfactant concentration. Because of its relatively high hydrophobicity, the resolution of $BNPO_4$ decreased with surfactant concentration. Baseline separation can be reached even at surfactant concentration lower than the CMC value, but due to the broad and asymmetric peak shape, it is hard to measure resolution. Figure 14 shows the dependence of the $BNPO_4$ effective electrophoretic mobility on surfactant concentration. As expected, the effective electrophoretic mobility decreases with increasing surfactant concentration.

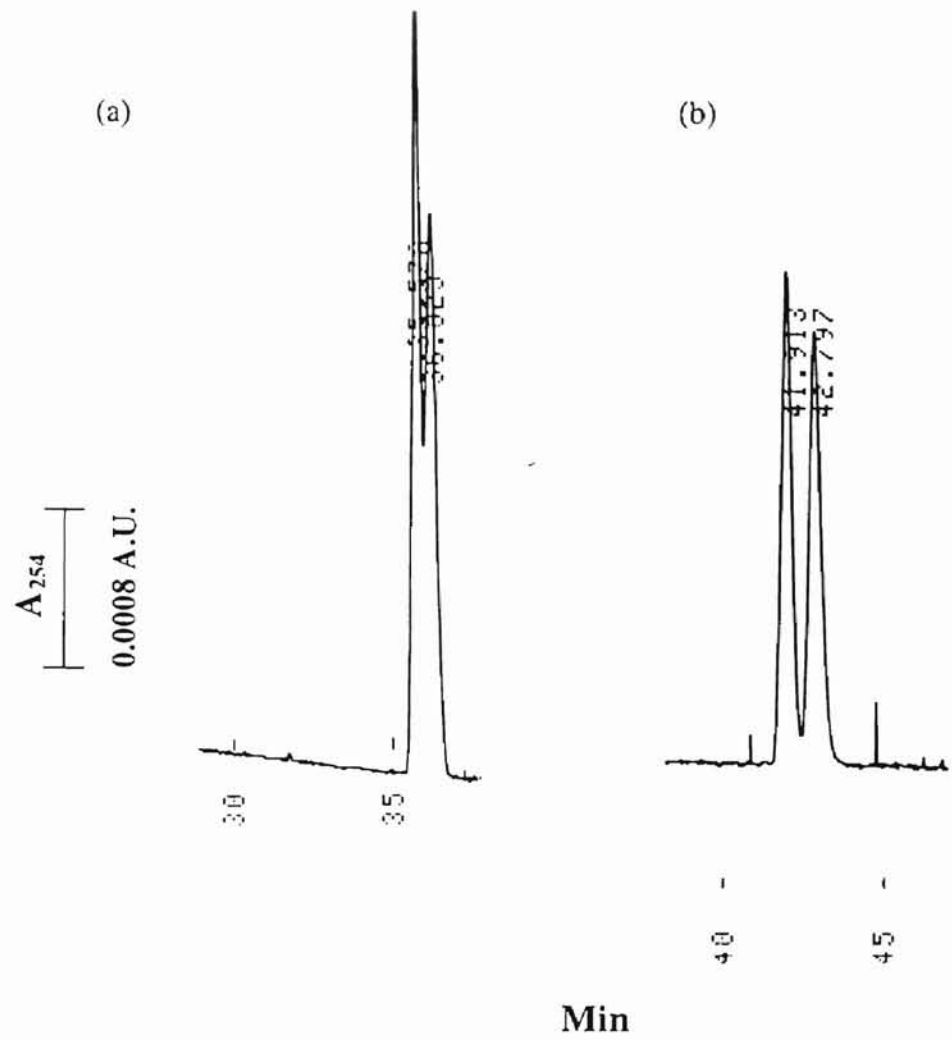


Figure 9. Electropherograms of 6-Met-Trp obtained with CYMAL-5. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing (a) 60 mM CYMAL-5, (b) 100 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

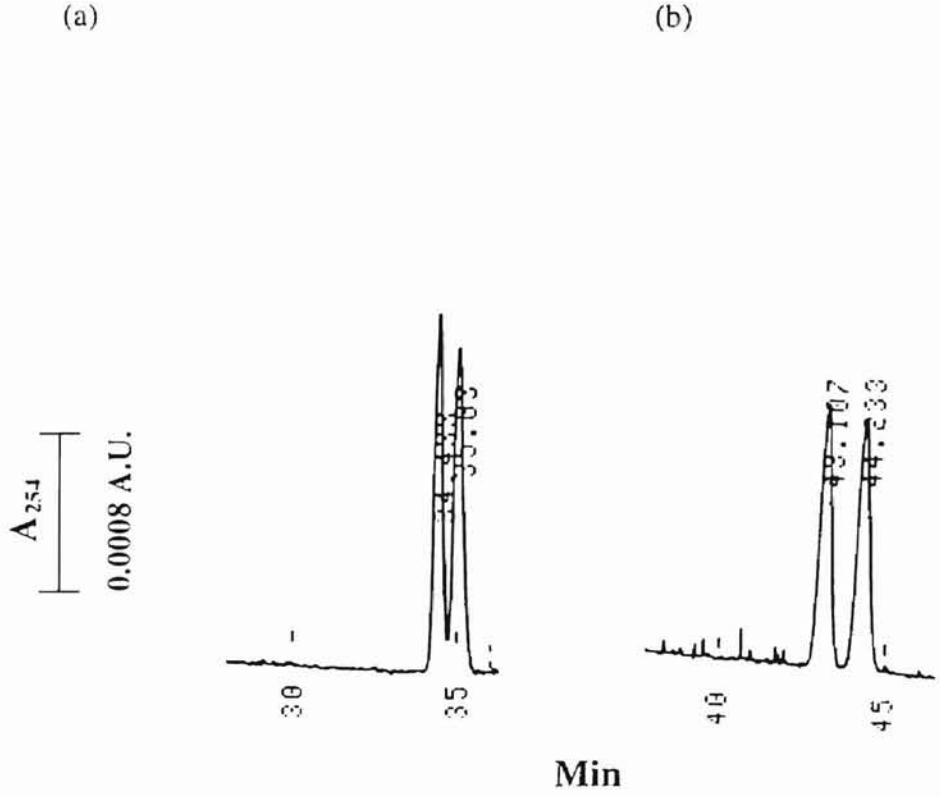


Figure 10. Electropherograms of 7-Met-Trp obtained with CYMAL-5. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing (a) 60 mM CYMAL-5, (b) 100 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

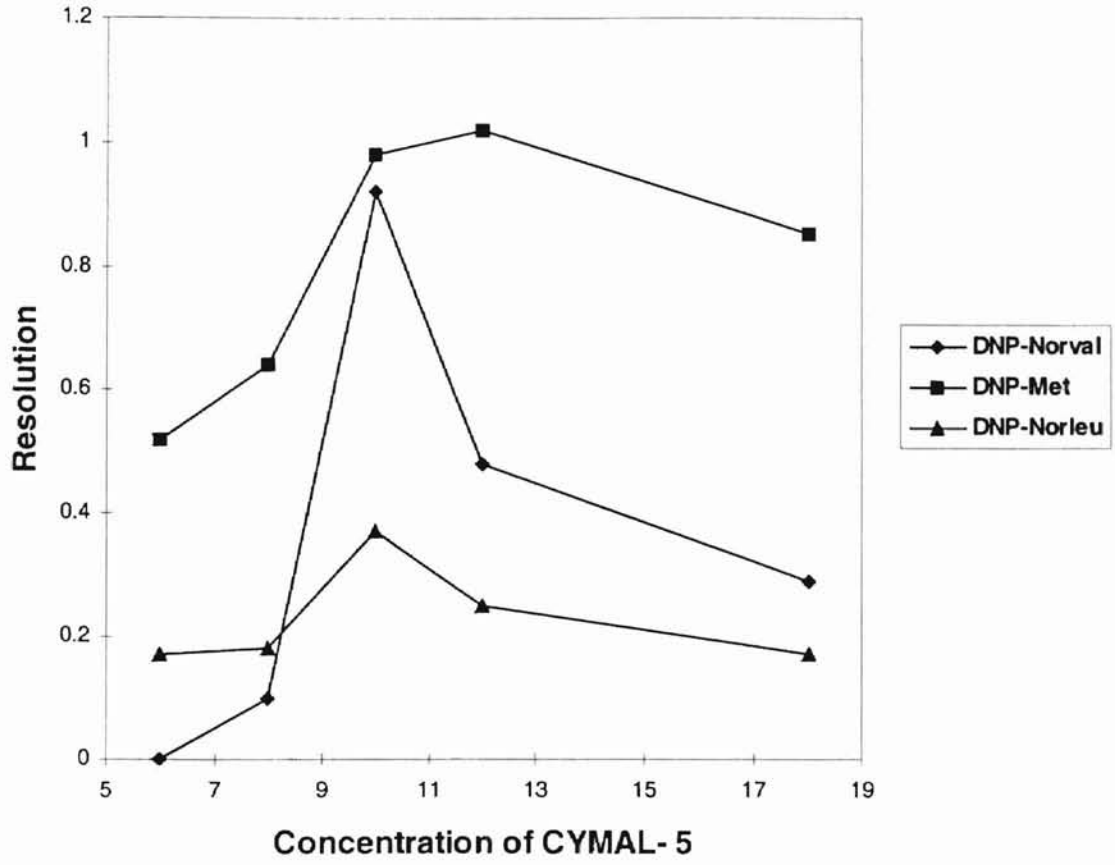


Figure 11. Effect of CYMAL-5 concentration on the enantiomeric resolution of DNP-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentrations of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

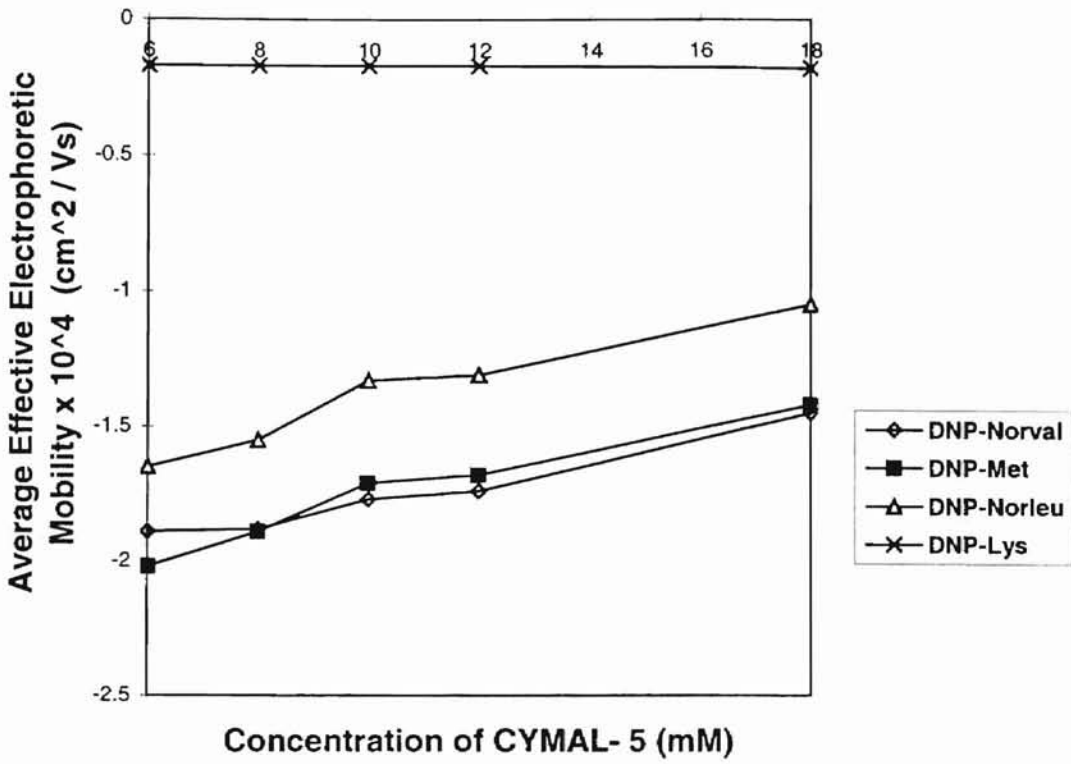


Figure 12. Effect of CYMAL-5 concentration on the effective electrophoretic mobility of DNP-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentrations of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

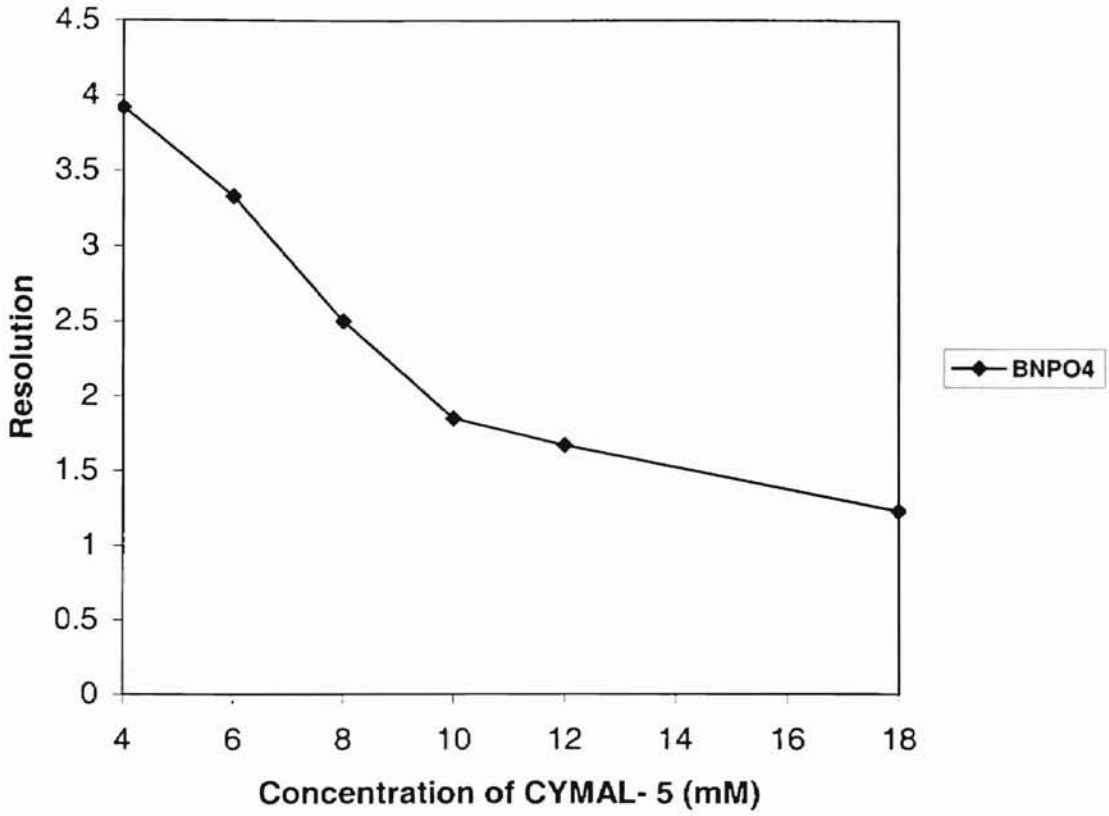


Figure 13. Effect of CYMAL-5 concentration on the enantiomeric resolution of $BNPO_4$. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentrations of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

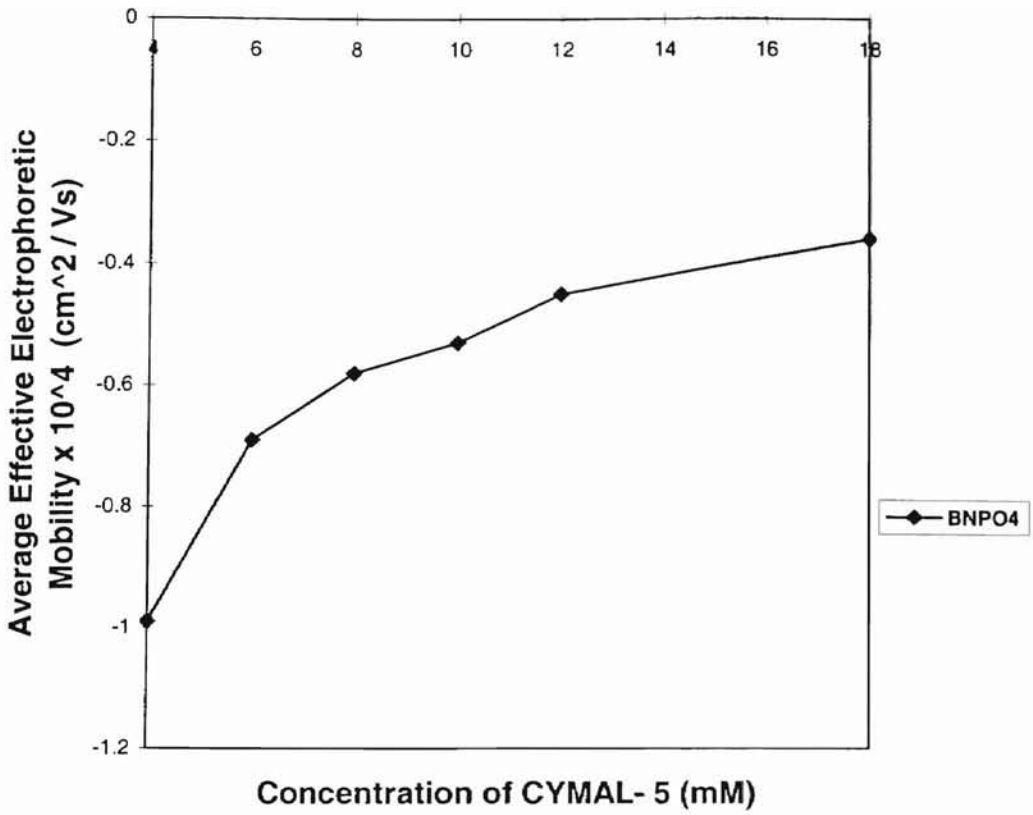


Figure 14. Effect of CYMAL-5 concentration on the effective electrophoretic mobility of $BNPO_4$. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentrations of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Effect of pH and ionic strength

Dansyl amino acids. For charged solutes, such as Dns-AA's, changing the composition of the background electrolyte, e.g. pH and ionic strength, can influence the electrophoretic system. As shown in Table II, most solutes can be separated at pH 6.5. But Dns-Trp can only be resolved at pH 2.5 or 3. At low pH (e.g., pH 2.5 or 3), Dns-Leu exhibited resolution with increasing surfactant concentration. Dns-Trp showed enantiomeric resolution at low pH because of the absence of EOF at pH 2.5, a fact that allows the solute to spend more time associating with the micelle. Amino acids are ampholytes, and therefore the pH of the running buffer will determine their electric charges. At low pH, Dns-AA's are in the fully protonated form and the predominant ionic species are cations, and as a result, they migrate towards the cathode in the absence of EOF. Figure 15 shows the effective electrophoretic mobility at various pH whereby the solute changes from positively to negatively charged passing through neutral as the pH is increased. The pI values of these solutes seem to be around pH 3.6.

The effect of the ionic strength of the running electrolyte on the enantiomeric resolution is illustrated in Fig. 16. Increasing the ionic strength of the running electrolyte seems to decrease the enantiomeric resolution of all analytes. Since the work was performed on a home made instrument without thermostating device, the ionic strength could not be greater than 150 mM due to undesirable Joule-heating effects. At elevated ionic strength, conductivity is increased, and, consequently, Joule heating. This probably explains the decrease in enantiomeric resolution of all analytes at higher ionic strength.

Table II. Effect of pH on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, at various pH, containing 6 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Resolution

Dns-AA	pH 2.5	pH 3	pH 4	pH 5	pH 6.5	pH 7	pH 8
Dns-trp	**0.83**	**0.93**	0	0	0	0	0
Dns-phe	0	0	0	0	**0.2**	0	0
Dns-leu	0	0	0	**0.73**	**1.0**	**0.53**	0
Dns-met	0	0	0	0	**0.7**	0	0
Dns-val	0	0	0	0	**0.41**	0	0

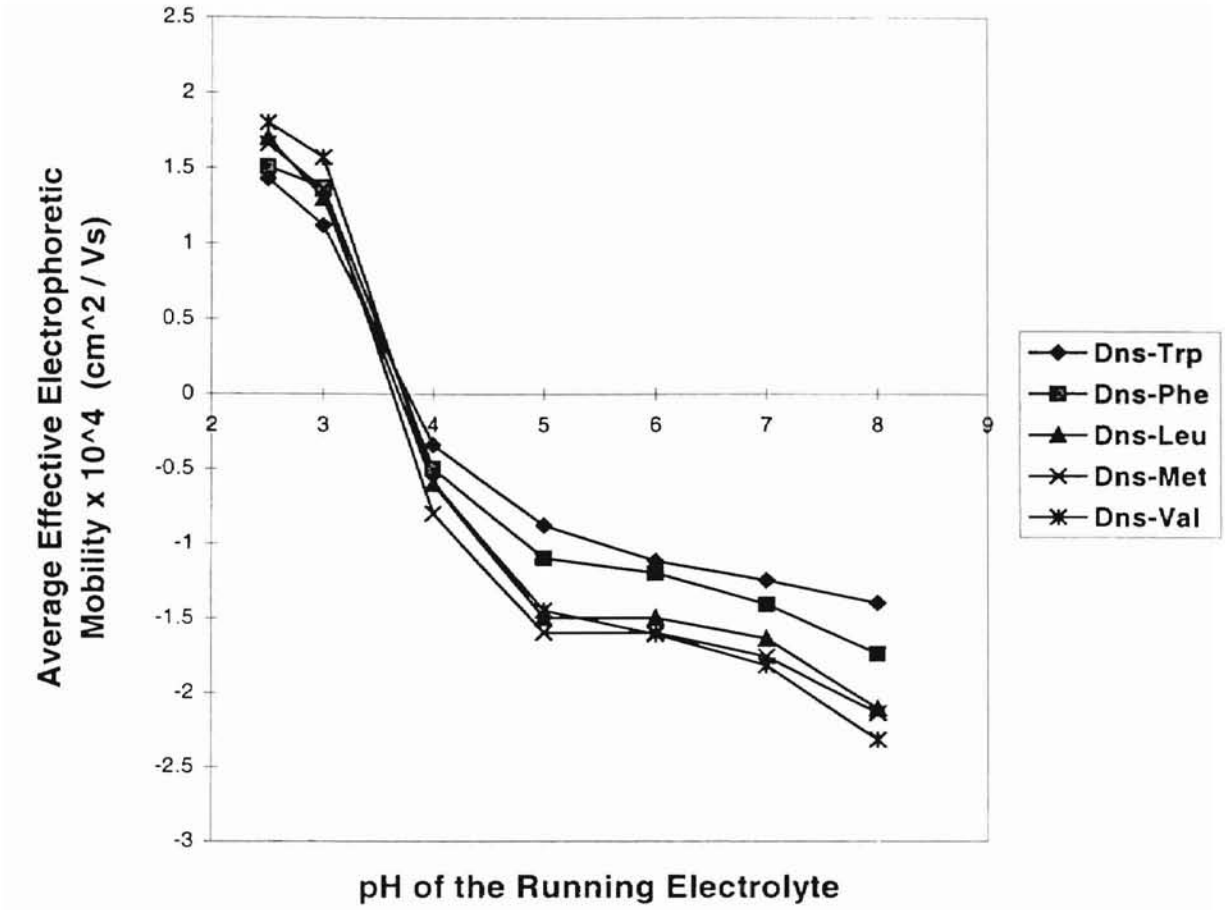


Figure 15. Effect of pH on the effective electrophoretic mobility of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, at various pH, containing 6 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

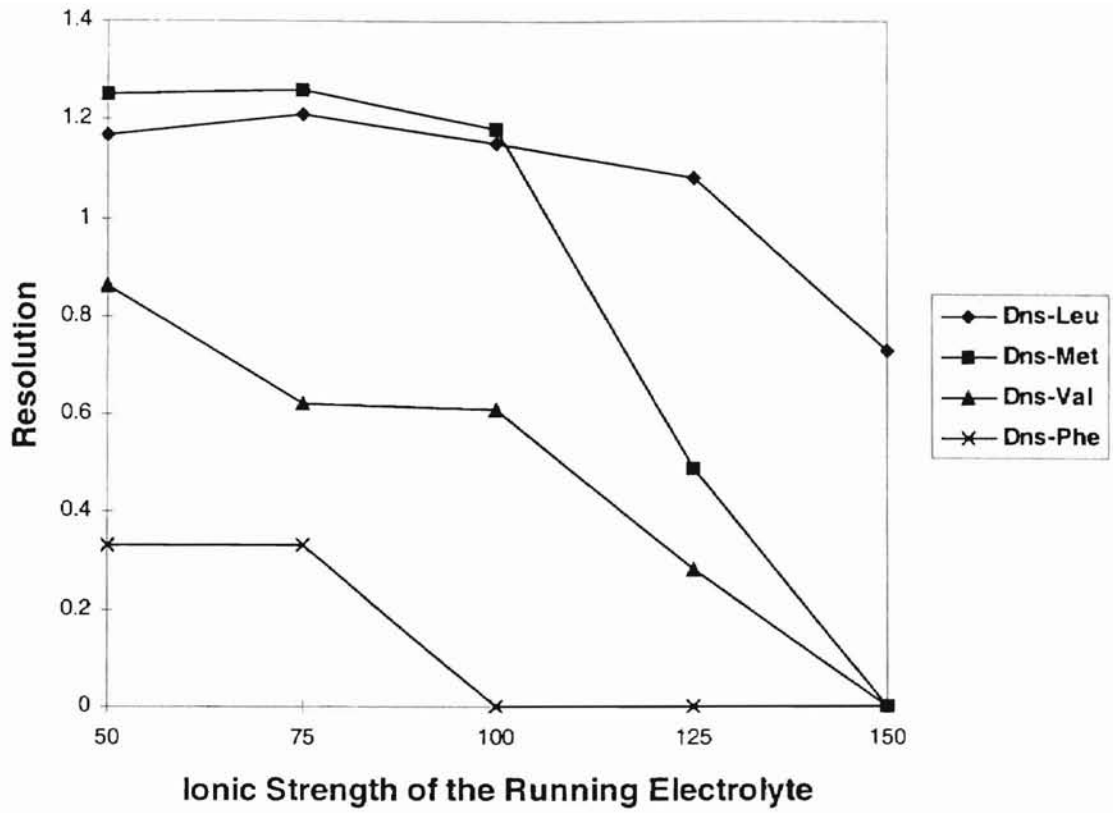


Figure 16. Effect of the ionic strength of the running electrolyte on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, various concentrations of sodium phosphate, pH 6.5, containing 10 mM CYMAL-5; voltage, 20 kV, capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Methyl substituted tryptophans. Because of the ionic nature of the Met-Trp's, the pH has a strong influence on their migration and interaction with the CYMAL-5 micelle. The enantiomeric resolution of Met-Trp's is achieved in a narrow pH range. They showed no separation from pH 6 to 12 even when the concentration of CYMAL-5 surfactant is as high as 120 mM. At pH 2.5, baseline separation was obtained. This may due to decreasing solute-micelle interaction arising from increasing the ionization of the acidic solutes with increasing pH. Also, at pH 2.5, the EOF is very weak and the solute can associate with the micelle for longer time.

As can be seen in Fig. 17, increasing the ionic strength of the separation electrolyte increased the enantiomeric resolution of 5-Met-Trp to a maximum and then decreased. The resolution of 7-Met-Trp and 6-Met-Trp kept constant when the ionic strength of the running electrolyte increased from 75 mM to 125 mM, and then decreased as the ionic strength continued to increase. Increasing ionic strength of the running electrolyte is known to lead to decreasing the CMC and increasing the aggregation number of the CYMAL-5 micelle (73, 74). The increase in aggregation number and decrase in CMC lead to an increase in the micellized surfactant concentration, [S]-CMC, for a given surfactant concentration [S]. This in turn will increase the association of the solute with the micelle, a fact that increasing the ionic strength enhances solute-micelle interaction. On the other hand, it should be noted that there is a limit for increasing the ionic strength due to Joule heating. Under the conditions of Fig. 17, 125 mM phosphate was the limit to which the ionic strength could be increased in order to improve resolution of 5-Met-Trp.

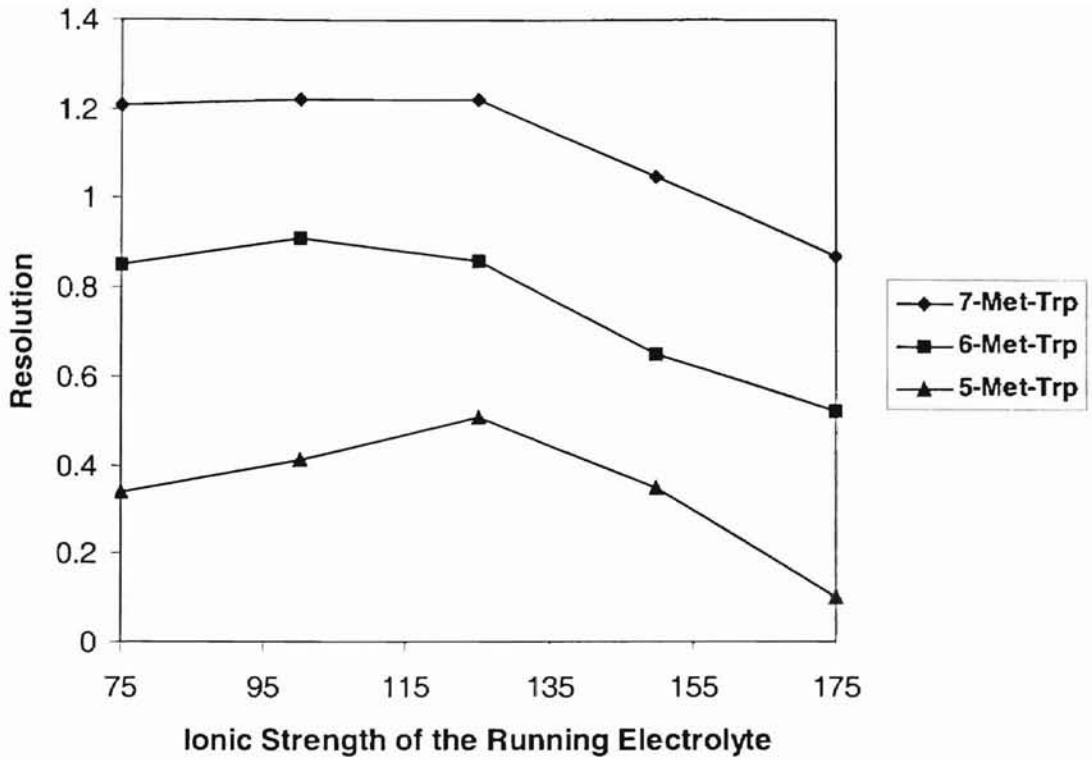


Figure 17. Effect of the ionic strength of the running electrolyte on the enantiomeric resolution of Met-Trp's. Conditions: running electrolyte, various concentrations of sodium phosphate, pH 2.5, containing 50 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

1,1'-Binaphthyl-2,2'-diylhydrogen phosphate. Figure 18 illustrates the relationship between the pH of the running electrolyte and the enantiomeric resolution of $BNPO_4$. Again, and because of its strong hydrophobic character, $BNPO_4$ exhibited much higher enantiomeric resolution at low pH (e.g. pH 4.0) than at higher pH. This is due to the low EOF at low pH. Decreasing the pH of the separation electrolyte decreased the EOF as well as the effective electrophoretic mobility of the analyte with an overall decrease in the apparent mobility of the analyte. The analyte spent more time interacting with the CYMAL-5 micelles and thus attained higher resolution, but the separation time was much longer at low pH. A compromise between enantiomeric resolution and analysis time was reached at pH 6.5.

Conclusions

We have shown that CYMAL-5 is an effective chiral surfactant for the enantiomeric separation of chiral charged solutes by capillary electrophoresis. The enantioresolution of the CYMAL-5 can be manipulated over a wide range of electrolyte composition, e.g., pH, ionic strength and surfactant concentration. For solutes that exhibit strong interaction with the CYMAL-5 micelle, a lower pH yielded better enantioresolution than a high pH. At low pH, the EOF is very weak, a fact that allows the solute to interact longer with the micelle, thus increasing its enantioresolution. With only a few exceptions, in all cases, there is an optimum surfactant concentration for maximum enantioresolution, which is located at low surfactant concentration for

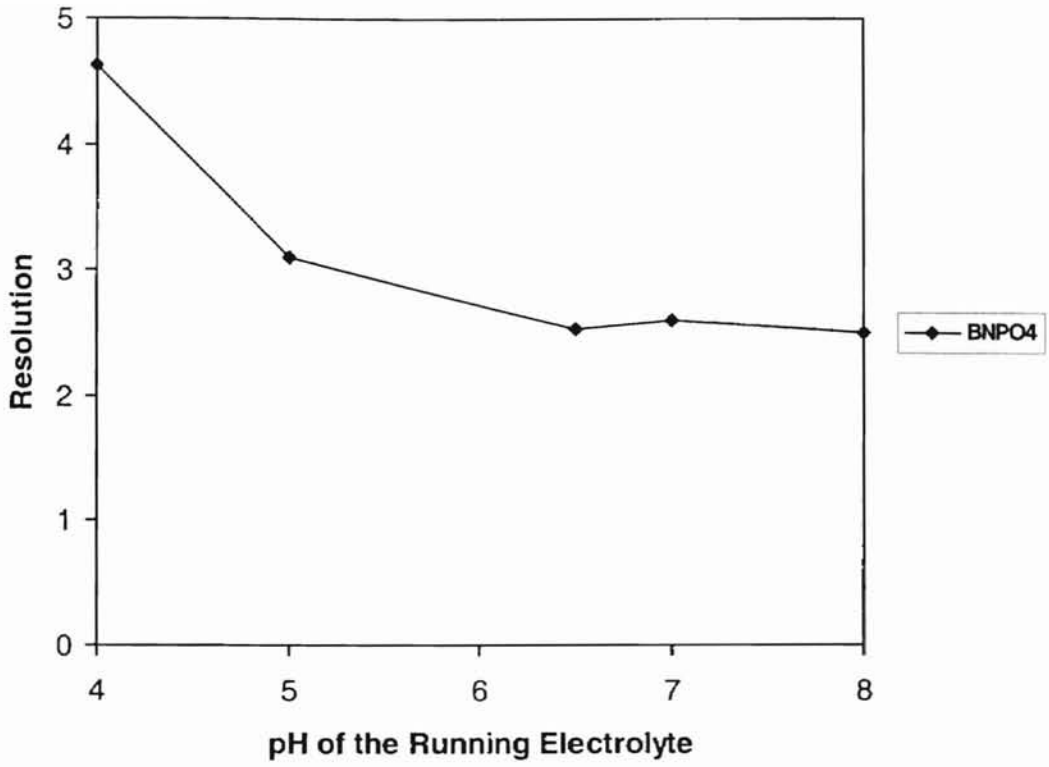


Figure 18. Effect of pH of the running electrolyte on the enantiomeric resolution of $BNPO_4$. Conditions: running electrolyte, 75 mM sodium phosphate, at various pH, containing 6 mM CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

strongly hydrophobic solutes and at high surfactant concentration for relatively hydrophilic solutes. The CYMAL-5 proved useful for the separation of several enantiomers.

CHAPTER III

COMPARISON OF CYCLOHEXYL-ALKYL-β-D-MALTOSIDES SURFACTANTS IN ENANTIO-SEPARATION BY CAPILLARY ELECTROPHORESIS

Introduction

As shown in the previous chapter (Chapter II), CYMAL-5 surfactant is a useful chiral selector to perform enantiomeric separations by CE. This chapter is concerned with the comparison of the enantioselectivity of three different glycosidic surfactants (GS's), namely, cyclohexyl-butyl-β-D-maltoside (CYMAL-4), cyclohexyl-pentyl-β-D-maltoside (CYMAL-5), and cyclohexyl-hexyl-β-D-maltoside (CYMAL-6) (see Chapter II for structures), toward chiral dansyl amino acids (Dns-AA's) and evaluating mixed micelle systems of CYMAL-6 / CYMAL-1 and CYMAL-6 / CYMAL-2. CYMAL surfactants have in common the same chiral head group but differ in the hydrophobic tail, thus allowing the assessment of the effect of the hydrophobic tail on selectivity.

It is possible to employ combinations of different chiral selectors to alter separation selectivity. Recently, our laboratory has reported the enantiomeric separation of phenoxy acid herbicides using electrolyte systems based on mixed cyclodextrins (CDs) (75). Since the various CDs exhibited different chiral selectivity toward the 7-

aminonaphthalene-1,3-disulfonic herbicides, it is obvious that the electrolyte systems composed of mixed CDs should yield a unique chiral selectivity that can not be achieved by either of the CDs alone.

Thus far, enantiomeric separations by CE using chiral micelles have involved mixed chiral/achiral system, or CD-modified micellar system. In the first approach, Otsuka and Tarabe (76) used a nonionic chiral surfactant, digitonin, with anionic sodium dodecyl sulfate (SDS) to form mixed micelles. Under acidic conditions (pH 3.0), phenylthiohydantoin-derivatized amino acids were optically resolved with a 25 mM digitonin-50 mM SDS solution, although a long separation time was required (90 minutes). A mixed micelle consisting of glycyrrhizic acid, octyl-β-D-glucoside, and SDS has been used to separate the enantiomers of several Dns-AA's (77). In the second approach, a CD-modified SDS micellar phase was introduced for the separation of derivatized amino acids. Dansyl-amino acids have been resolved using SDS and γ-CD (78). Nishi et al. showed the separation of chiral drugs thiopental and pentobarbital in their enantiomers at pH 9 in the presence of SDS and γ-CD (61). Since CDs can not be solubilized in the SDS micelle and migrate with the same velocity of the EOF, solutes are distributed among three phases, the bulk solution, the CD and the negatively charged SDS micelle (4).

As shown in Chapter II, and will be shown in this chapter, the CYMAL surfactants do not need to be mixed with SDS or other charged achiral surfactants for the separation of charged enantiomers. This fact is one of the various advantages of CYMAL's which include, among other things, high solubility in aqueous solution,

transparency in the UV, ability of complexing with borate thus leading to the formation of in situ charged micelles.

Experimental

Reagents

Cyclohexyl-alkyl-β-D-maltoside surfactants, namely CYMAL-1, 2, 4 and 6 were purchased from Anatrace (Mumee, OH, USA). For structures, see Table 1 in Chapter II. All other chemicals and reagents were as in Chapter II.

Capillary electrophoresis instrument

Same as in Chapter II.

Results and Discussion

To assess the effect of the hydrophobic tail of CYMAL surfactants on enantiomeric resolution, three different CYMAL's possessing hydrophobic tails of different size were compared over a wide range of surfactant concentration. Furthermore, to alter the enantioselectivity of the CYMAL surfactants, mixed micelles were studied. In all cases, Dns-AA's were used as the model chiral solutes.

Comparison of CYMAL-4, 5 and 6

As can be seen in Figs 1 and 2 as well as in Fig. 3 in Chapter II, the dependence of the enantiomeric resolution on the concentration of CYMAL-4 or CYMAL-6 followed the same trend as that observed with CYMAL-5 in Chapter II. The enantiomeric resolution of Dns-Leu was best achieved at 6 mM CYMAL-6, while the maximum resolution of the same chiral compound was only attained at 10-12 mM CYMAL-5 and 18 mM CYMAL-4. Figure 3 shows the electropherograms of Dns-Leu in the presence of the different CYMAL surfactants. As can be seen in Fig. 3, Dns-Leu was baseline separated at 6 mM CYMAL-6, and only slightly separated at 10 mM CYMAL-4. Since the size of the nonpolar tail of the surfactants increases in the order: CYMAL-4 < CYMAL-5 < CYMAL-6, it is obvious that the CMC decreases in the order: CYMAL-4 > CYMAL-5 > CYMAL-6. CYMAL-6 has a CMC value of 0.56 mM while the CMC values of CYMAL-5 and CYMAL-4 are relatively high reaching 2.4 and 7.6 mM, respectively. The fact that CYMAL-6 is more effective in enantioseparation may indicate that solute-micelle association via polar and hydrophobic interactions is important component for the enantiomeric separation. The enantiomeric resolution is achieved when the two enantiomers exhibit different association constants with the chiral micelles.

Due to its strong hydrophobic character, Dns-Phe did not separate well, especially with the longer hydrophobic tail CYMAL-6, see Fig. 2.

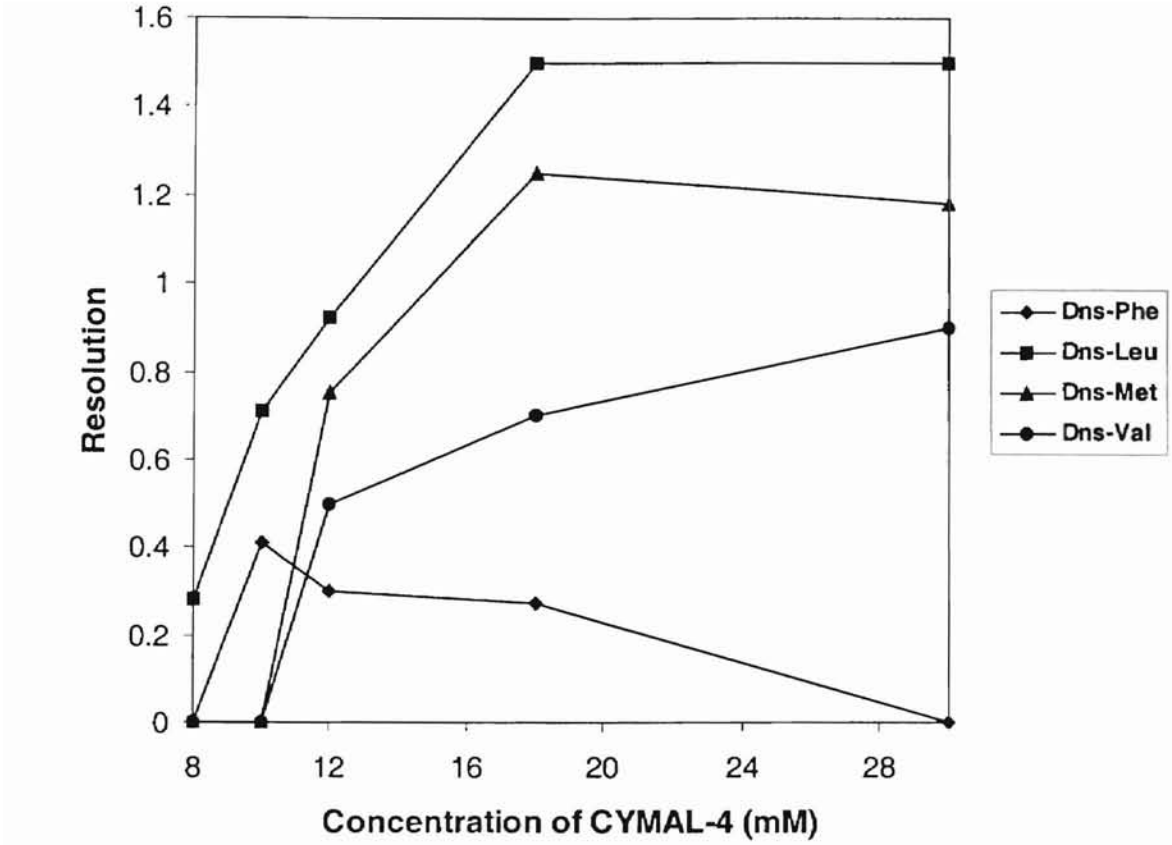


Figure 1. Effect of CYMAL-4 concentration on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentrations of CYMAL-4; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

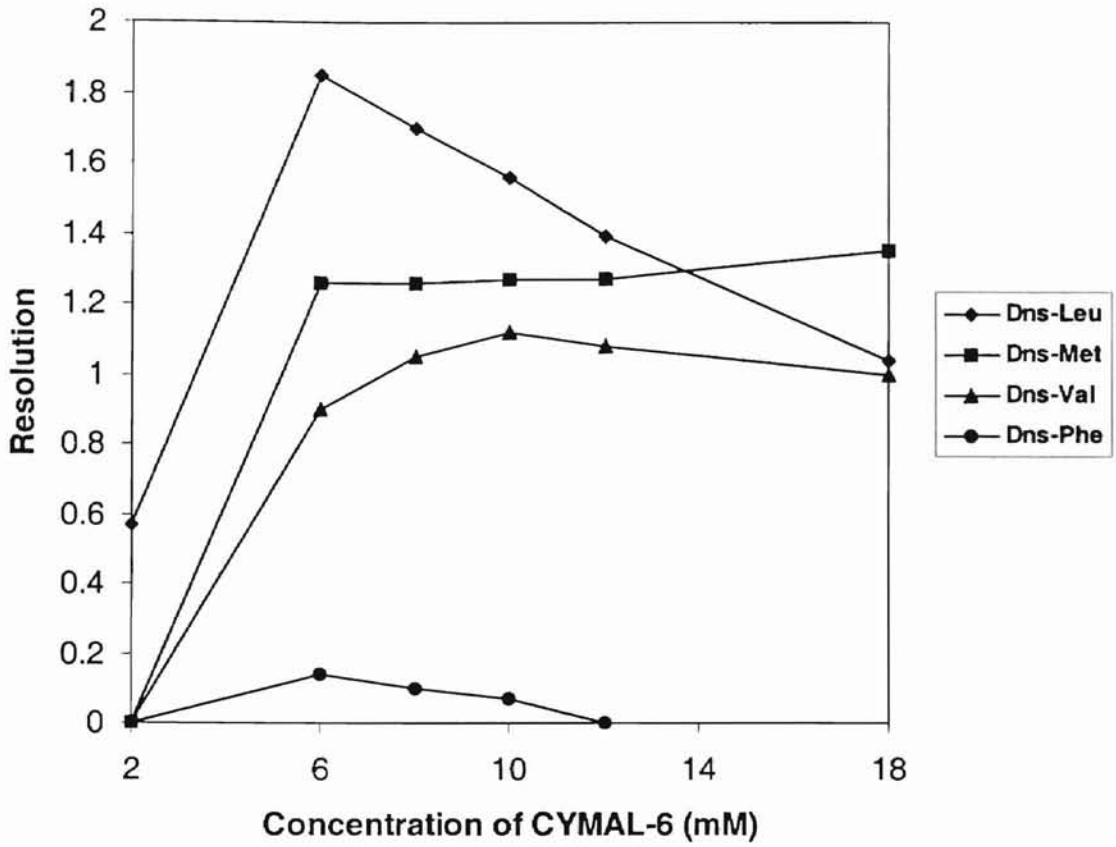


Figure 2. Effect of CYMAL-6 concentration on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentrations of CYMAL-6; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

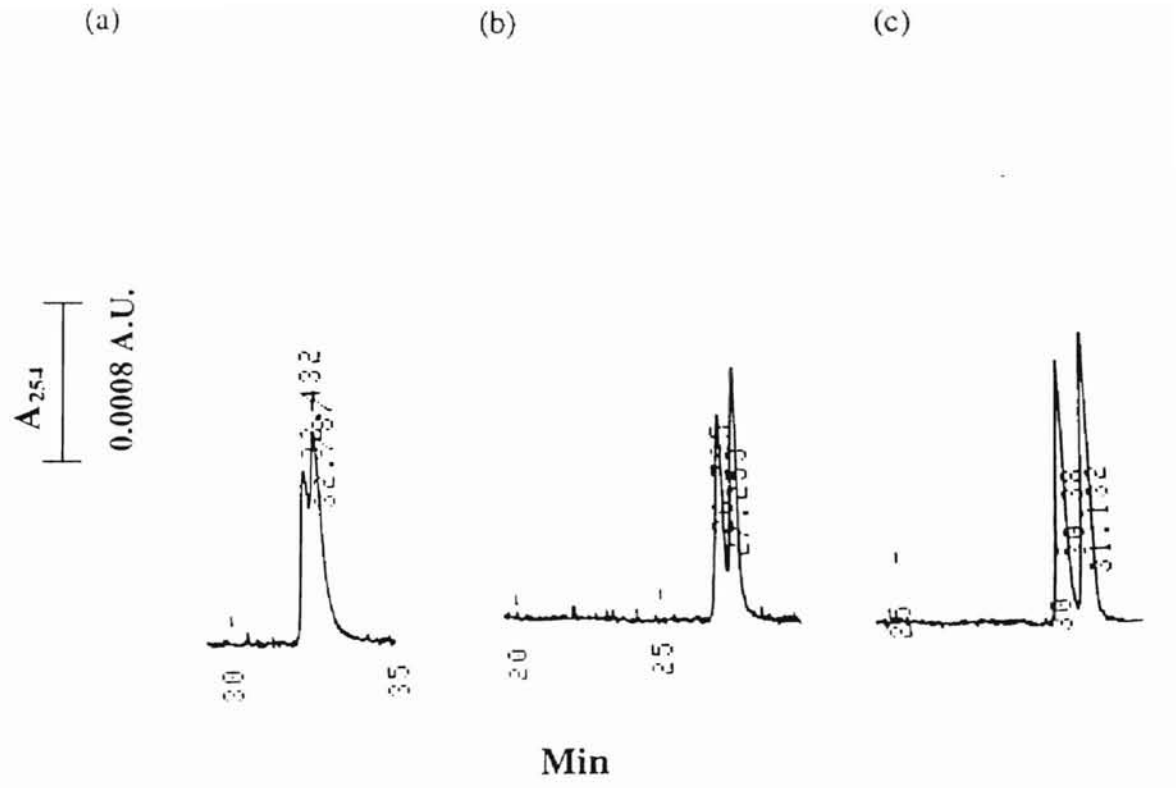


Figure 3. Electropherograms of Dns-Leu obtained with CYMAL-4, 5 and 6. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing (a) 10 mM CYMAL-4 (b) 6 mM CYMAL-5 (c) 6 mM CYMAL-6; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Mixed chiral micelles

Since the various CYMAL surfactants (e.g., CYMAL-4, 5 and 6) exhibited different dependence between resolution and surfactant concentration (e.g., the concentration range for maximum resolution), it was interesting to evaluate the effect of mixing CYMAL surfactants of shorter hydrophobic tail (e.g., CYMAL-1 and 2) with CYMAL-6 in order to extend the concentration range over which the maximum enantioresolution can be achieved. It is well established that the addition of a co-surfactant changes the physical properties of the micelle, e.g., CMC and aggregation number. The CMC of binary surfactant mixtures (CMC_{mix}) may be less than (synergism), an intermediate of, or greater than (negative synergism) the CMC of the two components (79, 80). For neutral micelles (e.g., CYMAL's), the CMC_{mix} is usually an intermediate value between the two individual surfactants, but it is disproportionally influenced by the lower CMC component, i.e., by the longer hydrophobic tail surfactant CYMAL-6. Mixed micelle formation in aqueous solutions arises from hydrophobic and electrostatic interactions. While mixed cationic-anionic micelles are termed nonideal because of electrostatic attraction between dissimilar charges, micelles formed from surfactants of like charges behave ideally. Thus, mixed micellar systems composed of neutral surfactants (e.g., CYMAL-6 / CYMAL-1 or CYMAL-2) will obey ideal mixing behavior (81).

CYMAL-6 / CYMAL-1. Improvement in the selectivity of an enantiomeric separation can be achieved by employing mixed chiral micellar systems. CYMAL-1 has a CMC of 340 mM. When CYMAL-1 monomers are mixed with CYMAL-6 micelles,

they can associate in between the surfactant molecules of the CYMAL-6 micelle, thus modifying the micelles and providing different selectivity. With the addition of CYMAL-1 to CYMAL-6, resolution increased first, reached a maximum and then decreased. As shown in Fig. 4, it seems that too much CYMAL-1 (> 50 mM) monomer leads to decreasing the aggregation of CYMAL-6 micelle and consequently resolution. Figures 5 and 6 illustrate the electropherograms of 6-Met-Trp and 7-Met-Trp in the mixed micelles of CYMAL-6 / CYMAL-1, respectively.

CYMAL-6 / CYMAL-2. The CMC of CYMAL-2 is 120 mM. One carbon difference leads to 3 times decrease in CMC value when going from CYMAL-1 to CYMAL-2. Figure 7 shows that the enantiomeric resolution of the three model solutes is very much improved even at higher concentration of CYMAL-2. CYMAL-1 or 2 can be regarded as suitable additives for manipulating the resolution of chiral system. Mixing CYMAL-2 with CYMAL-6 seems to provide real mixed micelles. This is contrary to the obtained behavior with CYMAL-1 / CYMAL-6 system. Figures 8 and 9 show the electropherograms of 5-Met-Trp and 6-Met-Trp in CYMAL-6 / CYMAL-2 system, respectively.

Conclusions

We have compared three different CYMAL surfactants, namely CYMAL-4, -5, and -6. Varying the size of the hydrophobic tail of CYMAL surfactants changes the optimal surfactant concentration at which maximum enantioresolution is obtained.

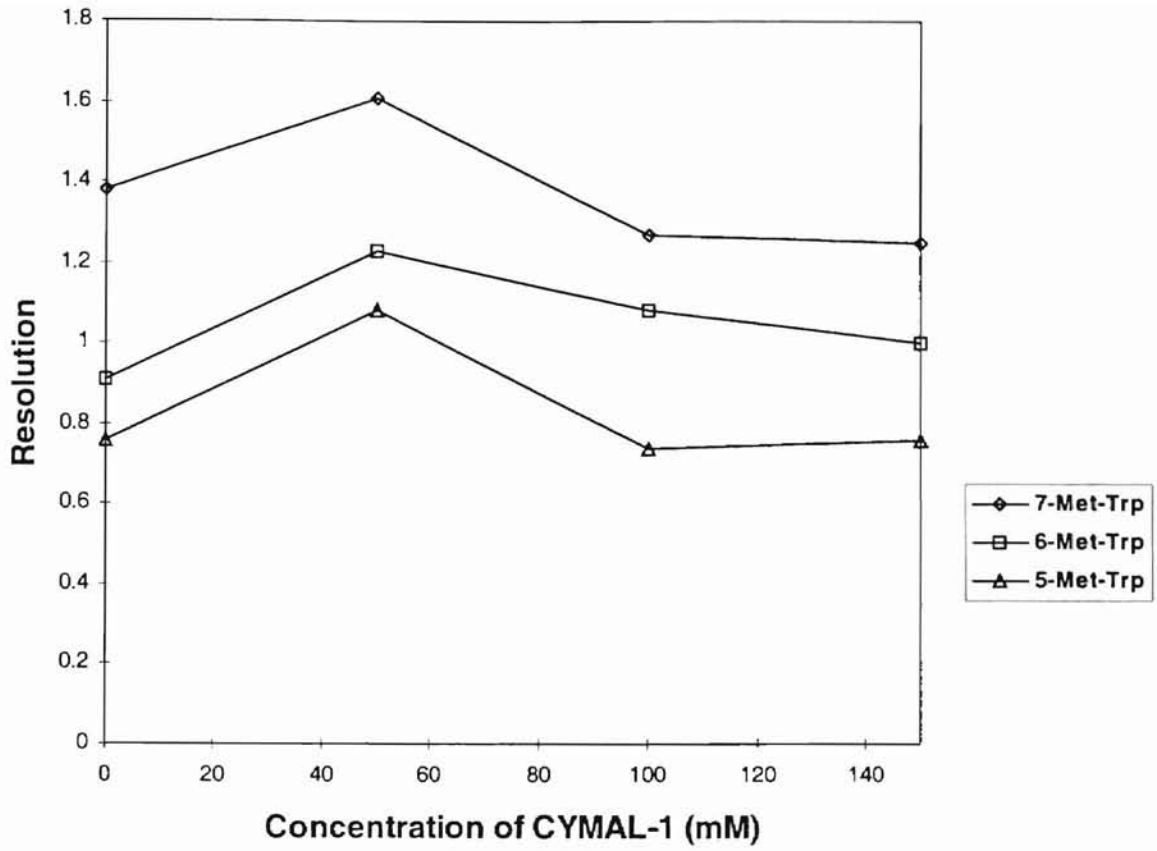


Figure 4. Effect of CYMAL-1 concentration on the enantomeric resolution of Met-Trp's in the mixed CYMAL-6 / CYMAL-1 system. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing 50 mM CYMAL-6 and various concentration of CYMAL-1; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

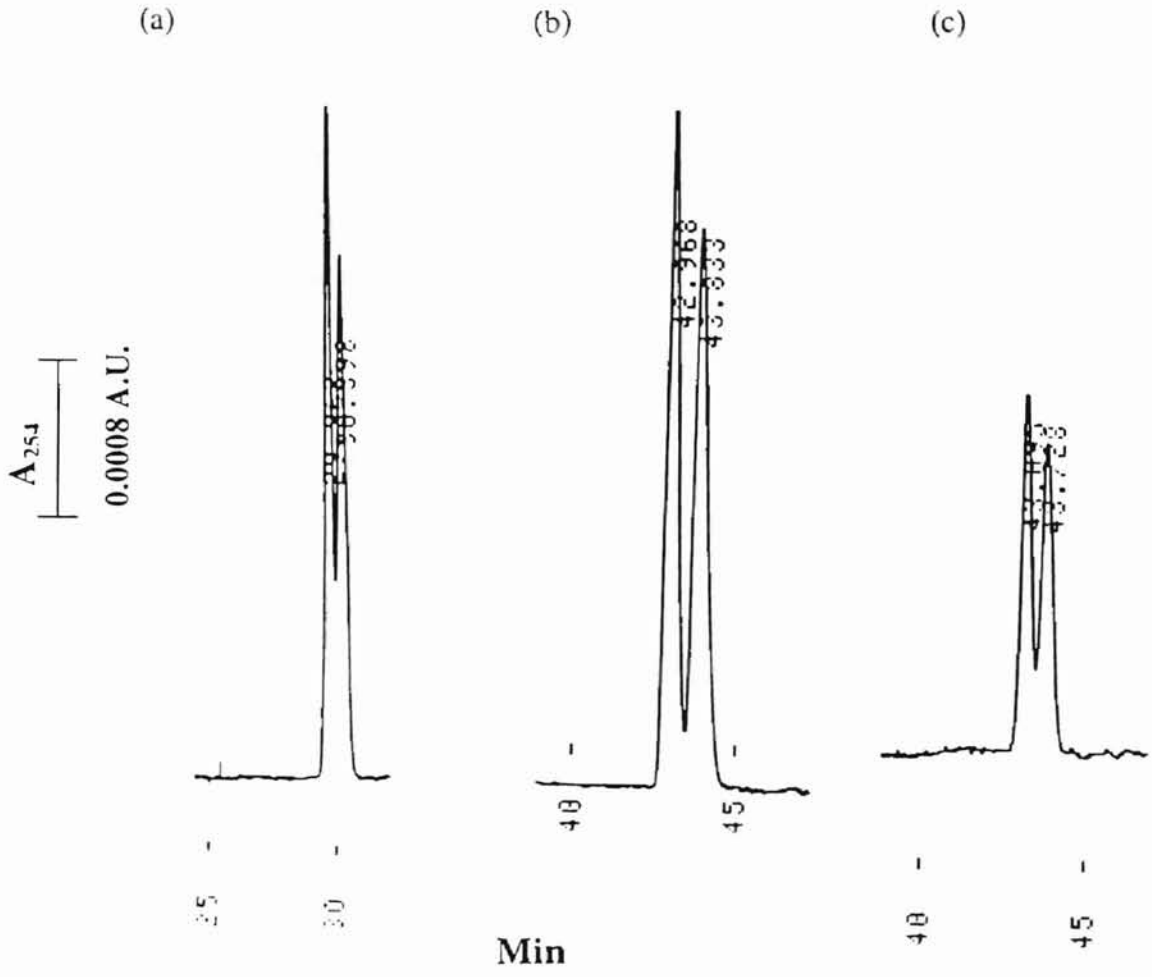


Figure 5. Electropherograms of 6-Met-Trp obtained with the mixed CYMAL-6 / CYMAL-1 system. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing 50 mM CYMAL-6 and (a) 0 mM (b) 50 mM and (c) 150 mM CYMAL-1; voltage, 20 kV; capillary. bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

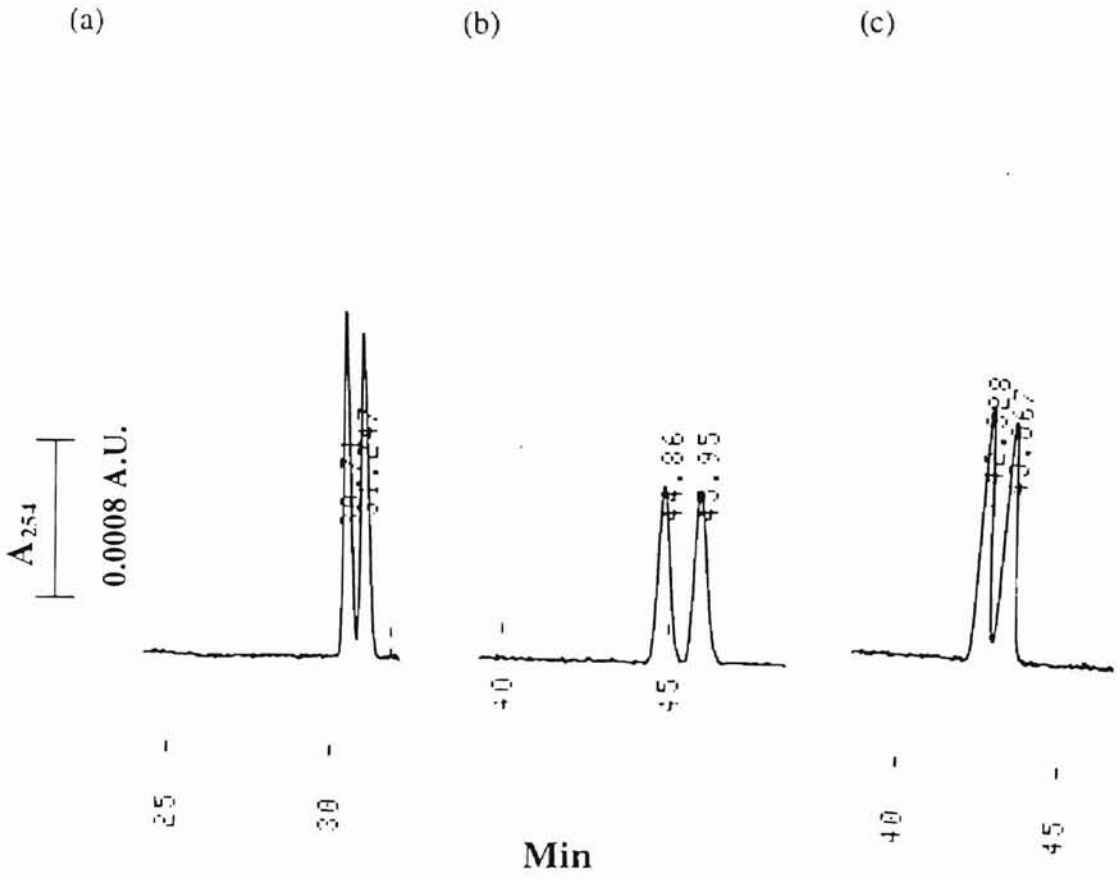


Figure 6. Electropherograms of 7-Met-Trp obtained with the mixed CYMAL-6 / CYMAL-1 system. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing 50 mM CYMAL-6 and (a) 0 mM (b) 50 mM (c) 150 mM CYMAL-1; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

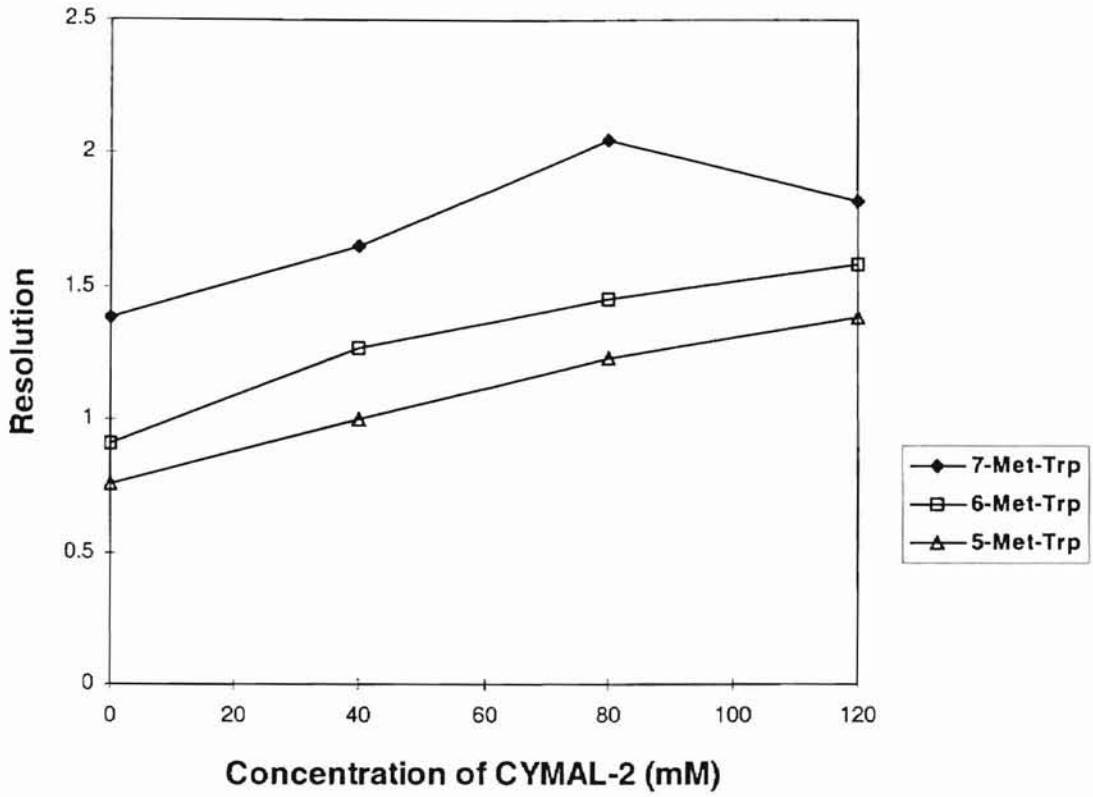


Figure 7. Effect of CYMAL-2 concentration on the enantomeric resolution of Met-Trp's obtained with the mixed CYMAL-6 / CYMAL-2 system. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing 50 mM CYMAL-6 and various concentration of CYMAL-2; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

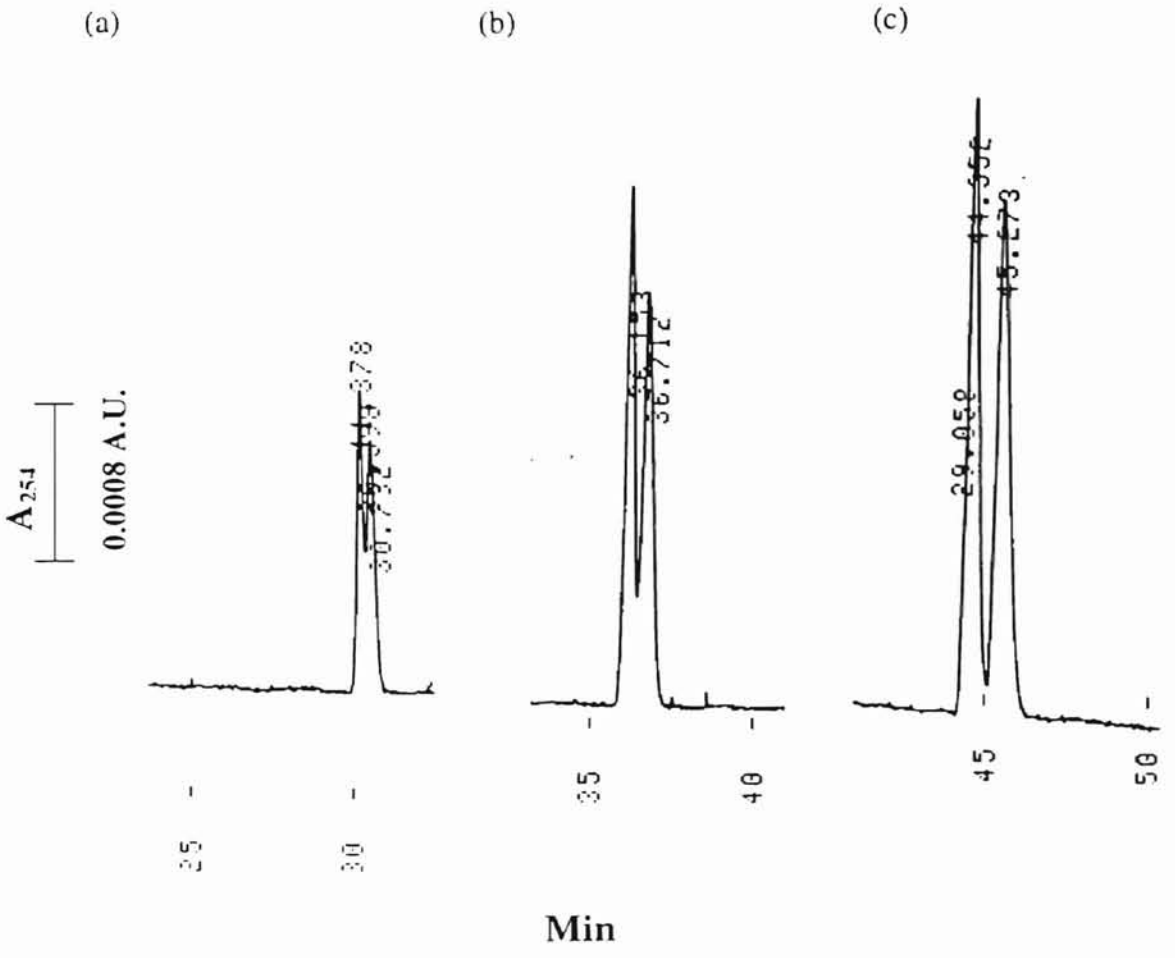


Figure 8. Electropherograms of 5-Met-Trp obtained with the mixed CYMAL-6 / CYMAL-2 system. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing 50 mM CYMAL-6 and (a) 0 mM (b) 40 mM (c) 120 mM CYMAL-2; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

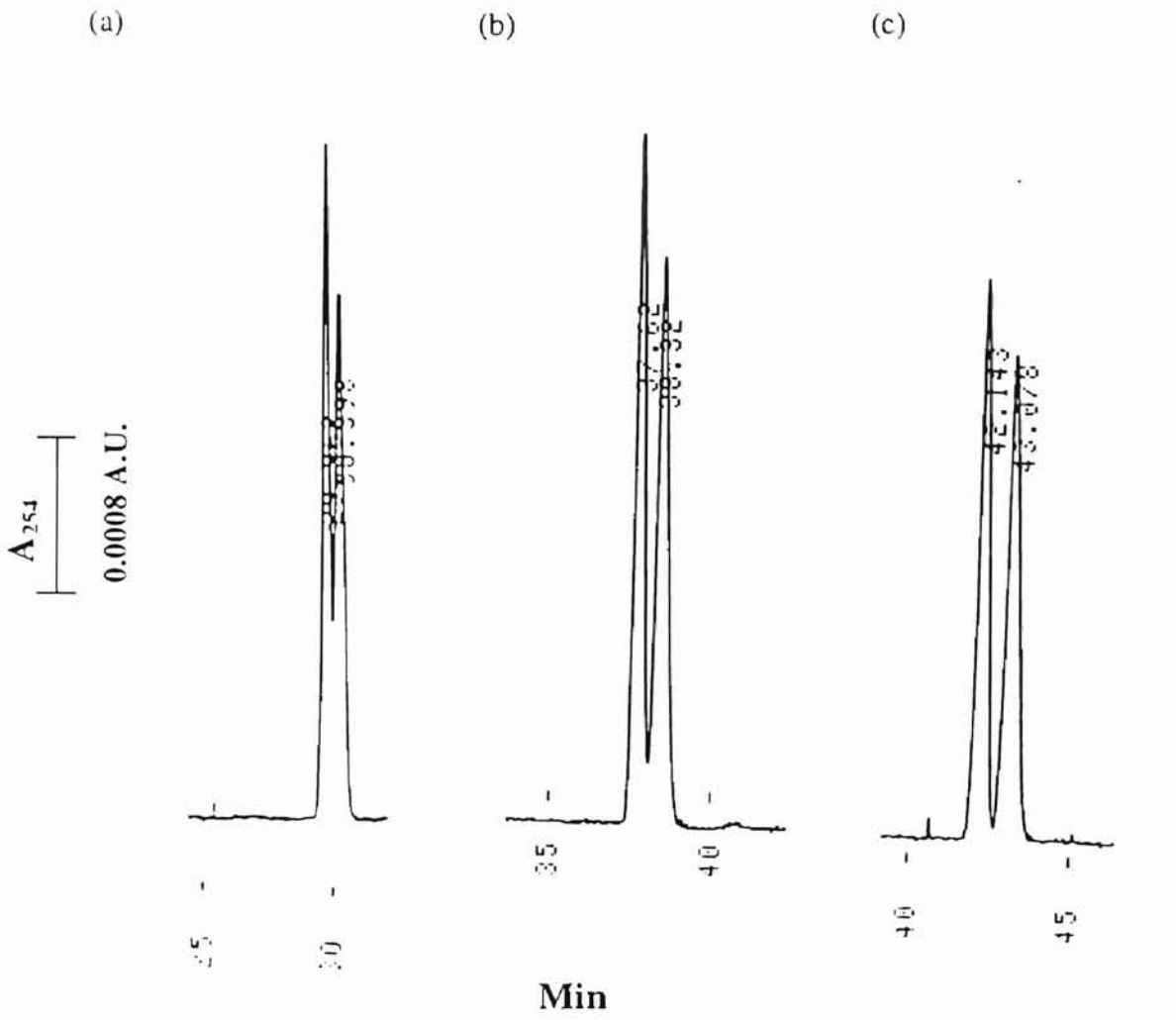


Figure 9. Electropherograms of 6-Met-Trp obtained with the mixed CYMAL-6 / CYMAL-2 system. Conditions: running electrolyte, 75 mM sodium phosphate, pH 2.5, containing 50 mM CYMAL-6 and (a) 0 mM (b) 40 mM and (c) 120 mM CYMAL-2; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

In other words, for a given set of solutes, maximum enantioresolution is obtained at lower surfactant concentration with the CYMAL of larger hydrophobic tail (e.g., CYMAL-6) than with the CYMAL of smaller hydrophobic tail (e.g., CYMAL-4). However, maximum resolution is located over a narrow range of surfactant concentration in the case of CYMAL-6, which makes the optimization of separation rather tedious because a narrow concentration range leads to either hit or miss the optimal concentration. This limitation observed with CYMAL of larger size hydrophobic tail, e.g., CYMAL-6, was alleviated by mixing CYMAL-6 with CYMAL's of small hydrophobic tails, e.g., CYMAL-1 and CYMAL-2, of relatively large CMC values.

CHAPTER IV

COMPARISON OF VARIOUS CHIRAL GLYCOSIDIC SURFACTANTS IN CAPILLARY ELECTROPHORESIS OF ENANTIOMERS

Introduction

This chapter is to provide an evaluation of the effect of the nature of glycosidic surfactants (GS's) on enantioresolution. Four different GS's, namely cyclohexyl-pentyl-β-D-maltoside (CYMAL-5), decyl-β-D-maltoside (DM), octyl-β-D-glucoside (OG), and S-octyl-β-D-thioglucoside (OSG) were compared in terms of enantiomeric resolution using Dns-AA's as model chiral compounds.

Cyclohexyl-pentyl-β-D-maltoside and DM have in common the same chiral head group but differ in the hydrophobic tails, thus allowing the assessment of the effect of the hydrophobic tail on enantioselectivity. Both OG and OSG have the same alkyl tails, but they differ in the presence or absence of thioether linkage between the sugar and the hydrophobic tail. The maltoside-based GS's (e.g., CYMAL-5 and DM) which have in common the maltosyl residue as the polar head group would allow the examination of the effect of the nature of the chiral head group when compared to the alkylglucoside surfactants (e.g., OG and OSG).

Experimental

Reagents

Decyl-β-D-maltoside (DM), octyl-β-D-glucoside (OG), and S-octyl-β-D-thioglucoside (OSG) were purchased from Anatrace (Mumee, OH, USA), for structures, see Fig. 1. All other chemicals and reagents were as in Chapter II.

Capillary electrophoresis instrument

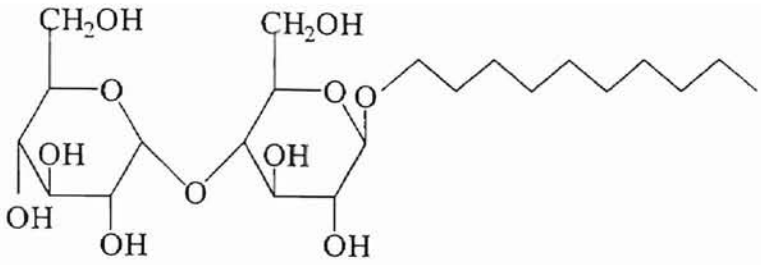
Same as in Chapter II.

Results and Discussion

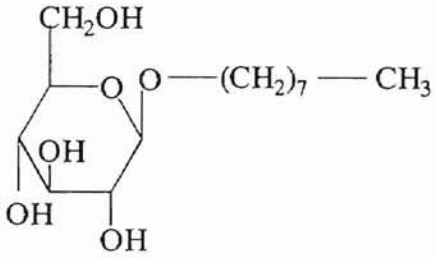
Comparison of CYMAL-5 and DM

CYMAL-5 and DM both have a chiral polar head group composed of two glucose units, i.e., a maltosyl residue. They differ only in their hydrophobic tails. DM has a straight decyl group, while CYMAL-5 possesses a cyclohexyl group at the end of the straight pentyl chain. The rationale for considering CYMAL-5 resides in its bulky cyclohexyl-pentyl tail, which is expected to yield different distribution coefficient for a given set of solutes when compared to the GS's with n-alkyl tail.

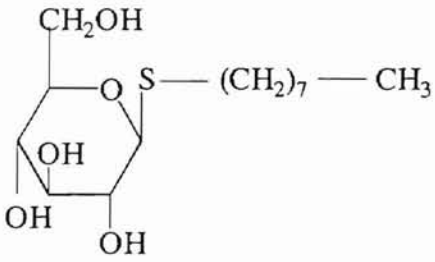
The values of the enantiomeric resolution of some Dns-AA's obtained with CYMAL-5 and DM are summarized in Tables I and II, respectively. The CMC values for CYMAL-5 and DM are 2.4 mM and 1.8 mM, respectively. As shown in Chapter III,



Decyl-β-D-maltoside (DM) CMC = 1.8 mM



Octyl-β-D-glucoside (OG) CMC = 25 mM



1-S-octyl-β-D-thioglucoside (OSG) CMC = 9 mM

Figure 1. Structures and CMCs of the chiral surfactants used in this study.

Table I. Effect of CYMAL-5 concentration on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentration of CYMAL-5; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Resolution

	6 mM	8 mM	10 mM	12 mM	18 mM
Dns-Leu	1	1.57	1.87	1.86	1.3
Dns-Met	0.7	1	1.26	1.44	1.57
Dns-Val	0.41	0.5	0.55	1.05	1.11

Table II. Effect of DM concentration on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentration of DM; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Resolution

	6 mM	8 mM	10 mM	12 mM	18 mM	22 mM	26 mM
Dns-Leu	0.41	0.52	0.52	0.52	1.13	1.13	0.39
Dns-Met	0.25	0.48	0.74	0.74	1.04	1.31	0.71
Dns-Val	0	0.34	0.34	0.39	1.09	1.34	0.78

less surfactant concentration is needed for a surfactant which has a lower CMC to achieve maximum enantioresolution. But in this study, Dns-Val, which can be enantiomerically separated at 6 mM CYMAL-5, required higher DM concentration for achieving similar enantioresolution. Also, the maximum enantiomeric resolution of Dns-Leu was reached at 10-12 mM CYMAL-5, while it was only attainable at 18-22 mM DM. Figure 2 illustrates the electropherograms of Dns-Leu obtained with the same concentration of CYMAL-5 and DM, respectively. For Dns-Leu and Dns-Met, CYMAL-5 showed a wider surfactant concentration window for maximum enantiomeric resolution, which spanned from 8 mM to 18 mM. In the case of DM, the maximum resolution of Dns-Leu and Dns-Met can only be achieved in the concentration range of 18 to 22 mM surfactant. Resolution decreased sharply as the concentration of DM increased to 26 mM. With the two chiral micellar systems under investigation, CYMAL-5 showed a higher resolution for Dns-Leu and Dns-Met over a wider surfactant concentration window.

Since the most stable conformation of a cyclohexane ring is the chair conformation (82), which is shorter in overall length than a n-butyl tail, the tail of CYMAL-5 which is composed of eleven carbon atoms is shorter than DM's decyl chain. Due to geometric and packing constraints, the bulky cyclohexyl group keeps the surfactant molecules from packing very tightly with consequent increase in space available for solubilization between the surfactant molecules in the palisade layer (79). Under these conditions, it is believed that CYMAL-5 would allow hydrophobic solutes to undergo more equitable penetration into the palisade layer of the micelle so that the solute chiral group will interact with the surfactant chiral head group in the micelle, thus leading to enantioresolution.

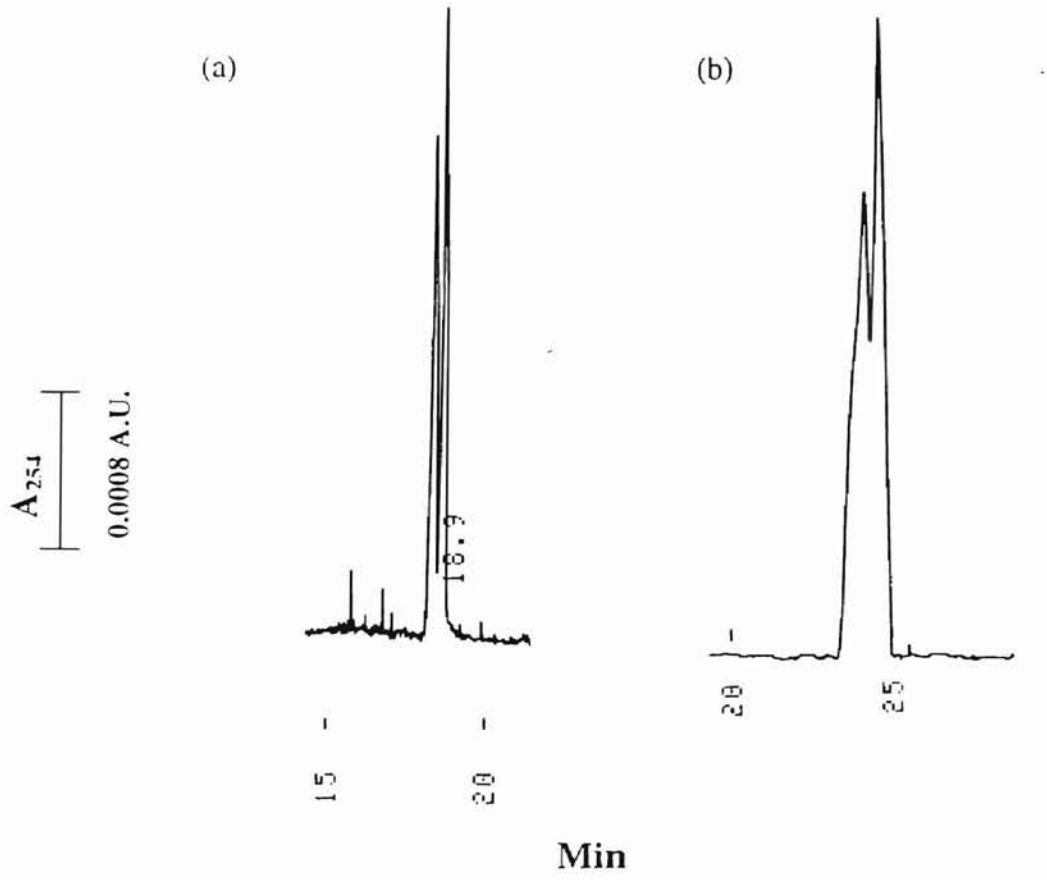


Figure 2. Electropherograms of Dns-Leu obtained with CYMAL-5 and DM, respectively. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing (a) 12 mM CYMAL-5 (b) 12 mM DM; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Comparison of OG and OSG

Within the groups of surfactants that differ in the presence or absence of a thioether bond, e.g., OSG and OG, we are expecting that the presence of a thioether group will eventually result in enforcing hydrophobic interaction, thus providing more equitable interaction with the palisade layer for weakly hydrophobic compounds. OG and OSG were carefully selected to address the importance of structure variable on the enantiomeric resolution. Dns-AA's were used as model chiral solutes.

As can be seen from Tables III and IV, only OSG exhibited some enantiomeric resolution at 10 mM surfactant concentration. This may be attributed to the fact that OSG has a CMC value of 9 mM while the CMC value of OG is 25 mM. Going from OG to OSG, the CMC decreased by almost 3-folds by the addition of a thioether. Thus, for these chiral surfactants to exhibit enantioselectivity toward optical isomers, they must be at a concentration above their CMC, i.e., the surfactant must be in the micellar form.

In both chiral micellar systems, a significant decrease in migration time is observed when the surfactant concentration was increased (results not shown). The migration time of Dns-Leu exceeded 40 min at 10 mM OSG and decreased substantially to 25 min at 30 mM OSG. The neutral micelles of the alkylglucoside surfactants migrate at the velocity of the cathodic EOF. Since the electrophoretic mobility of Dns-AA's is opposite in direction to the EOF, the stronger the interaction between the analyte and the micelle, the faster the analyte migration toward the cathode. In addition, the analysis time with OSG is much shorter than that with OG (see Figs 3 and 4). This is because the solute associated stronger with OSG and moved quicker with EOF. As can be seen in

Table III. Effect of OSG concentration on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentration of OSG; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Resolution

	10 mM	20	25	30	50
Dns-Leu	1.26	1.77	2.14	1.39	1.37
Dns-Met	0.80	1.84	2.13	1.50	1.36
Dns-Val	0.1	1.58	2.24	1.39	1.09

Table IV. Effect of OG concentration on the enantiomeric resolution of Dns-AA's. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing various concentration of OG; voltage, 20 kv; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Resolution

	10 mM	30 mM	50 mM
Dns-Leu	0	1.16	1.33
Dns-Met	0	1	1.30
Dns-Val	0	0.68	1.20

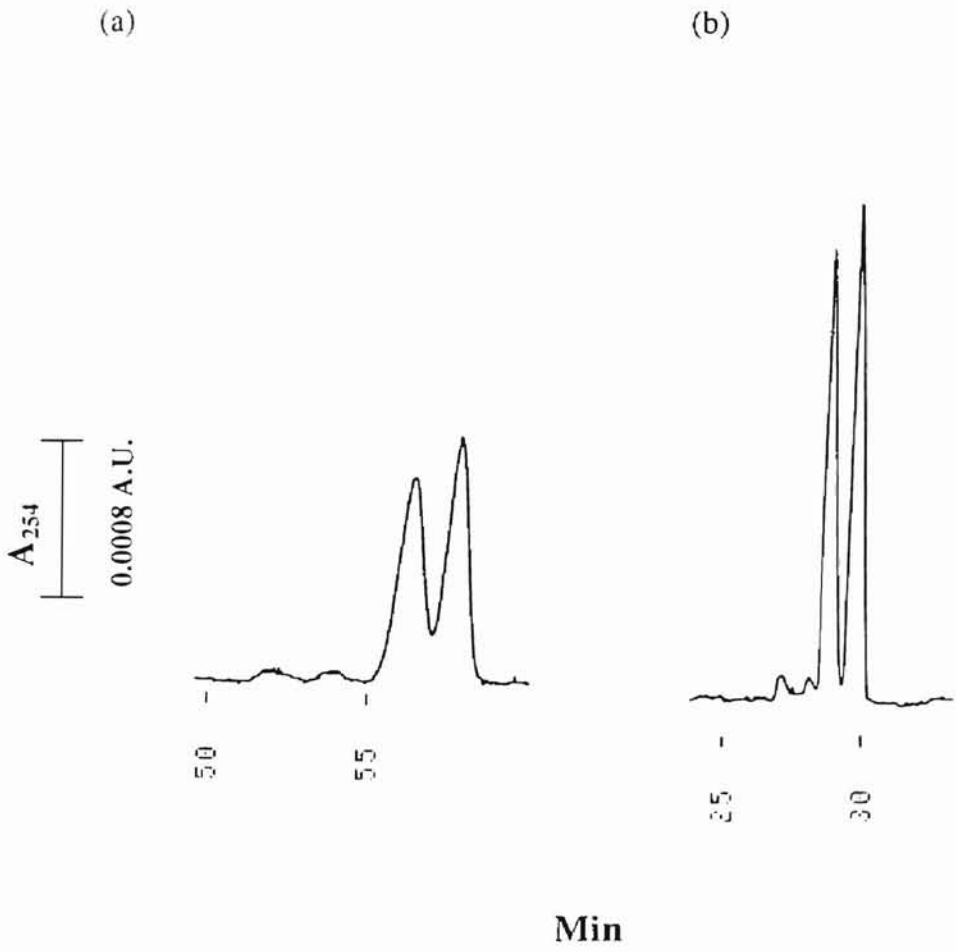


Figure 3. Electropherograms of Dns-Met obtained with OG and OSG, respectively. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing (a) 30 mM OG (b) 30 mM OSG; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

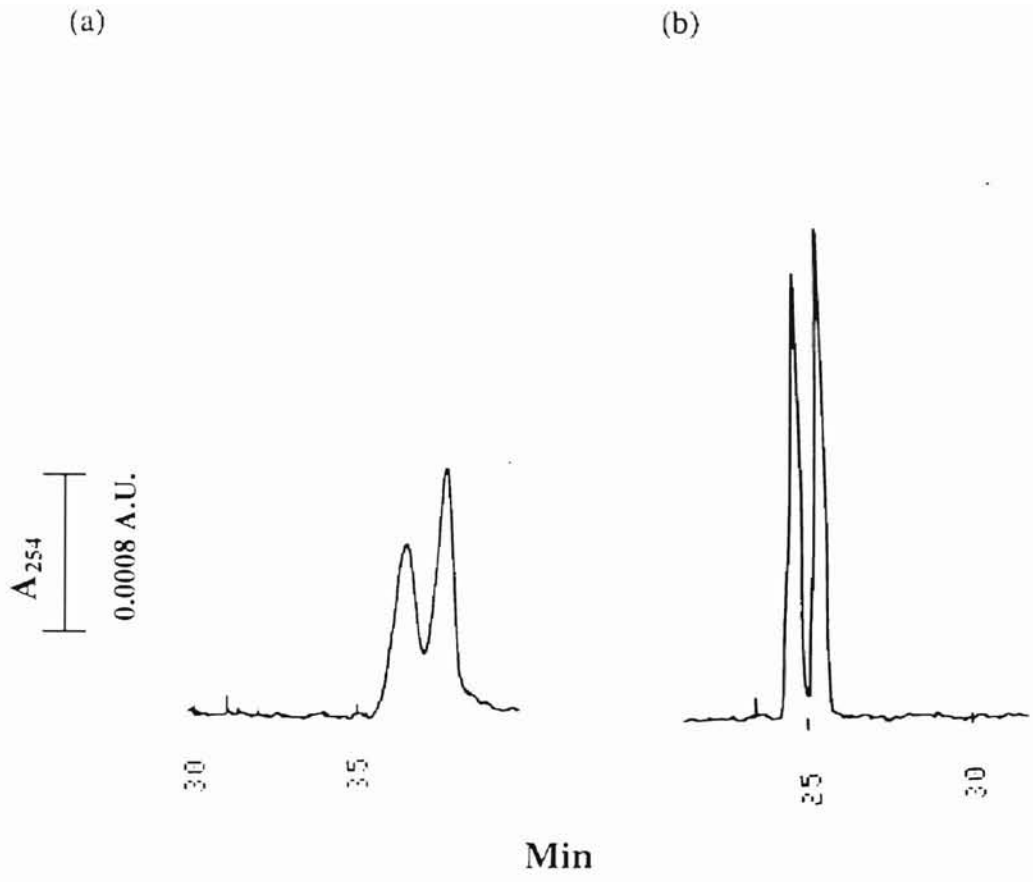


Figure 4. Electropherograms of Dns-Leu obtained with OG and OSG, respectively. Conditions: running electrolyte, 75 mM sodium phosphate, pH 6.5, containing (a) 30 mM OG (b) 30 mM OSG; voltage, 20 kV; capillary, bare fused-silica, 80 cm (total length) x 50 μm I.D. with detection window at 50 cm.

Figs 3 and 4, OSG yielded sharper peaks than OG, which indicates higher separation efficiency obtained with OSG.

The maximum enantiomeric resolution of Dns-Leu, Dns-Met and Dns-Val was achieved at 25 mM OSG. The resolution did not reach the same value even at 50 mM OG. Again, this can be explained by the lower CMC of OSG. A thioether is equivalent to a two-third methylene group in its effect on the CMC of a nonionic surfactant (79). A generally used rule for nonionic surfactants is that the CMC is decreased by a factor of 3 by the addition of one methylene group to a straight chain hydrophobic group attached to a single terminal hydrophilic group. The smaller decrease in CMC discovered by changing an ether to a thioether group may be due to the closeness of thioether to the head group where the surfactant is highly hydrated.

Comparison of alkylglucosides and maltoside-based surfactants

Our laboratory have evaluated the enantioselectivity of three GS's, namely OG, nonyl-β-D-glucoside (NG), and octyl-β-D-maltoside (OM) toward several Dns-AA's (11). As a continuation of these investigations, we evaluated the enantioselectivity of CYMAL and OG.

In both previous work and this current study, the presence of GS's in the micellar form is a must for any chiral selectivity to be achieved. Thus, the enantiomeric resolution exhibited by GS's is based on hydrophobic and polar interactions of solute with the micellar phase rather than with the surfactant monomers.

From previous finding in our laboratory, OM allowed the enantiomeric separation of Dns-Trp, while neither OG nor NG did (11). This coincides with our current observation, that CYMAL-5 permitted the chiral separation of Dns-Trp at low pH and relatively higher surfactant concentration.

Generally, solute solubilization is believed to occur at a number of different sites in the micelle (79): (i) on the surface of the micelle, i.e., at the micelle-solvent interface; (ii) between the hydrophilic head groups; (iii) in the so-called palisade layer of the micelle between the hydrophilic groups and the first few atoms of the micellar interior; (iv) more deeply in the palisade layer; and (v) the inner core of the micelle. Due to their ionic nature, the Dns-AA's are likely to be solubilized between the individual molecules of the surfactant in the palisade layer with varying degrees of penetration. Depth of penetration in the palisade layer will depend on the ratio of polar to nonpolar structures in the Dns-AA molecules, with the less polar compounds penetrating more deeply than the more polar Dns-AA's. The relatively strong hydrophobic character of Dns-Trp may have enforced its nonpolar interaction with the alkyl tail of the surfactant so that on the average the Dns-Trp spent more time deeper in the palisade layer of the micelle. This will distance the chiral center of Dns-Trp from interacting with the polar chiral center of the alkylglycoside surfactant, and may explain the absence of enantioselectivity with OG and NG at any surfactant concentration. Due to the more hydrophilic character of the maltosyl residue, CYMAL-5 and OM yielded enantioresolution at a higher surfactant concentration.

In addition, the resolution value of Dns-Phe can reach 1 in the presence of OG or OM. However, Dns-Phe was only slightly separated at any concentration of CYMAL

surfactants. This difference in enantioselectivity may be attributed to the difference in the extent of solute solubilization in the various alkylglycosidic micelles and / or the presence of cyclohexyl group in CYMAL.

Conclusions

We have shown that maltoside-based surfactants, e.g., DM and CYMAL-5, yielded different enantioselectivity than the alkylglucoside surfactants, e.g., OG and OSG. Moreover, CYMAL-5 which has a cyclohexyl-pentyl hydrophobic tail was more effective in enantioresolution than DM. Due to its strong hydrophobic character, OSG yielded maximum enantioresolution at lower surfactant concentration than that observed with OG.

The above study has indicated the need for further investigation in the area of chiral micellar systems to (i) introduce other chiral surfactants, (ii) shed some lights on the underlying of chiral separation and (iii) enlarge the scope of applications of chiral surfactants in enantioseparation by CE.

REFERENCES

1. S. Fanali, Identification of chiral drug isomers by capillary electrophoresis, *J. Chromatogr. A*, 735 (1996) 77-121.

2. G. Okafo, in: P. Camilleri (Ed.) *Capillary Electrophoresis*, 2nd Edition, CRC Press: London, 1998 pp183-184.

3. T. J. Ward, Chiral media for capillary electrophoresis, *Anal. Chem.*, 66 (1994) 633A-639A.

4. G. Gubitz and M. G. Schmid, Chiral separation principles in capilllary electrophoresis, *J. Chromatogr. A*, 792 (1997) 179-225.

5. B. Chankvetadze, Separation selectivity in capillary electrophoresis with charged selectors (review), *J. Chromatogr. A*, 792 (1997) 269-295.

6. S. Terabe, K. Otsuka and H. Nishi, Separation of enantiomers by capillary electrophoretic techniques, *J. Chromatogr. A*, 666 (1994) 295-319.

7. H. Nishi, Enantiomer separation of basic drugs by capillary electrophoresis using ionic and neutral polysaccharides as chiral selectors, *J. Chromatogr. A*, 735 (1996) 345-351.

8. J. W. Ward, Chiral media for capillary electrophoresis, *Anal. Chem.*, 66 (1994) 633A-640A.

9. M. Novotny, H. Soini and M. Stefansson, Chiral separation through capillary electromigration methods, *Anal. Chem.*, 66 (1994) 646A-655A.

10. R. Vespalec and P. Bocek, Chiral separations by capillary electrophoresis: Present state of the art, *Electrophoresis*, 18 (1997) 843-852.

11. Y. Mechref and Z. El Rassi, Comparison of alkylglycoside surfactants in enantioseparation by capillary electrophoresis, *Electrophoresis*, 18 (1997) 1-7.

12. P. D. Grossman and J. C. Colburn, *Capillary Electrophoresis Theory and Practice*, Academic Press, Inc.: San Diego, 1992.

13. S. F. Y. Li, *Capillary Electrophoresis, Principles, Practice and Applications*, Elsevier: Amsterdam, 1992.

14. R. S. Dubrow, Capillary gel electrophoresis, in: P. D. Grossman and J. C. Colburn (Eds.), *Capillary Electrophoresis Theory and Practice*, Academic Press, Inc.: San Diego, 1992.

15. W. Nashabeh and Z. El Rassi, Capillary zone electrophoresis of linear and branched oligosaccharides, *J. Chromatogr.*, 600 (1992) 1193-1200.

16. S. Fanali, L. Ossicini, F. Foret and P. Bocek, Resolution of optical isomers by capillary zone electrophoresis. Study of enantiomeric and diastereoisomeric Co (II)-complexes with ethylenediamine and amino acid ligands, *J. Microcol. Sep.*, 1(1989) 190-194.

17. R. E. Majors, The separation of chiral compounds by GC, SFC, and CE, *LC.GC*, 15 (1997) 412-422.

18. C. Wolf and W. H. Pirkle, Enantiomer separation by supercritical fluid chromatography on packed column, *LC.GC*, 15 (1997) 352-363.

19. W. Lindner (Ed), Chiral separations. Fundamental aspects and applications, *J. Chromatogr. A*, Vol. 666, 1994.

20. S. Terabe, N. Chen and K. Otsuka, in: A. Chrambach, M. J. Dunn, B. J. Radola (Eds.) *Advances in Electrophoresis*, VCH Publishers, Inc.: New York, 1994 pp89-153.

21. T. J. Ward and T. M. Oswail, Enantioselectivity in capillary electrophoresis using the macrocyclic antibiotics, *J. Chromatogr.* A, 792 (1997) 309-325.

22. E. Gassmann, J. Kuo and R. Zare, Electrokinetic separation of chiral compounds, *Science*, 230 (1985) 813- 814.

23. R. Weinberger, *Practical Capillary Electrophoresis*, Academic Press: Boston, 1993, p.84.

24. S. Fanali, M. Cristalli, R. Vespalec and P. Bocek, in: A.Chrambach, M. J. Dunn, B. J. Radola (Eds.), *Advances in Electrophoresis*, vol 7, VCH Publishers, Inc.: New York 1994, pp 3-86.

25. H. Nishi, T. Fukuyama and M. Matsuo, Resolution of optical isomers of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) derivatised DL-amino acids by micellar electrokinetic chromatography, *J. Microcol. Sep.*, 2 (1990) 234-240.

26. C. Vogt, A. Georgi and G. Werner, Enantiomeric separation of D/L-carnitine using HPLC and CZE after derivatization, *Chromatographia*, 40 (1995) 287-295.

27. C. R. Noe and J. Freissmuth, Capillary zone electrophoresis of aldose enantiomers: separation after derivation with S-(-)-1-phenylethylamine, *J. Chromatogr. A*, 704 (1995) 503-512.

28. C. Dette, H. Waetzig and A. Aigner, Analysis of acetylcysteine by capillary electrophoresis (CE), *Pharmazie*, 49 (1994) 245-250.

29. S. Fanali, Separation of optical isomers by capillary zone electrophoresis based on host-guest complexation with cyclodextrins, *J. Chromatogr.*, 474 (1989) 441-446.

30. S. Mayer and V. Schurig, Enantiomer separation by electrochromatography on capillaries coated with Chirasil-Dex, *J. High Resolut. Chromatogr.*, 15 (1992) 129-131.

31. A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg and B. L. Karger, Use of complexing agents for selective separation in high-performance capillary electrophoresis, *J. Chromatogr.*, 448 (1988) 41-53.

32. S. Fanali, Controlling enantioselectivity in chiral capillary electrophoresis with inclusion -complexation, *J. Chromatogr. A*, 792 (1997) 227-267.

33. J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, Use of cyclodextrins in isotachophoresis, *J. Chromatogr.*, 438 (1988) 211-218.

34. I. E. Valko, H. A. H. Billiet and J. Frank, Effect of the degree of substitution of (2-hydroxy) propyl-β-cyclodextrin on the enantioseparation of organic acids by capillary electrophoresis, *J. Chromatogr.* A, 678 (1994) 139-144.

35. R. J. Trait, D.O. Thompson and V. J. Stella, Sulfobutyl ether β-cyclodextrin as a chiral discriminator for use with capillary electrophoresis, *Anal. Chem.*, 66 (1994) 4013-4018.

36. R. Kuhn, F. Stoecklin and F. Erni, Chiral separations by host-guest complexation with cyclodextrin and crown ether in capillary electrophoresis, *Chromatographia,* 33 (1992) 32-36.

37. S. A. C. Wren and R. C. Rowe, Theoretical aspects of chiral separation in capillary electrophoresis, *J. Chromatogr.*, 603 (1992) 235-241.

38. S. A. C. Wren and R. C. Rowe, Theoretical aspects of chiral separation in capillary electrophoresis, *J. Chromatogr.*, 635 (1993) 113-118.

39. S. Fanali, Use of cyclodextrins in capillary zone electrophoresis, *J. Chromatogr.*, 545 (1991) 437-444.

40. M. J. Sepaniak, R. O. Cole and B. K. Clark, Use of native and chemically modified cyclodextrins for the capillary electrophoretic separation of enantiomers, *J. liq. Chromatogr.*, 15 (1992) 1023-1040.

41. S. Fanali, *An Introduction to Chiral Analysis by Capillary Electrophoresis*, Bio-Rad Laboratories: New York, 1995.

42. T. Schmitt and H. Engelhardt, Charged and uncharged cyclodextrins as chiral selectors in capillary electrophoresis, *Chromatographia*, 37 (1993) 475-483.

43. S. Terabe, H. Otsuka and T. Audo, Electrokinetic chromatography with 2-O-carboxymethyl-β-cyclodextrin as a moving "stationary " phase, *J. Chromatogr.*, 332 (1985) 211-217.

44. E. Hohne, G. J. Krauss and G. Gubitz, Capillary zone electrophoresis of the enantiomers of amino alcohols based on host-guest complexation with a chiral crown ether, *J. High Resolut. Chromatogr.*, 15 (1992) 698-700.

45. M. G. Schmid and G. Gubitz, Capillary zone electrophoretic separation of the enantiomers of dipeptides based on host-guest complexation with a chiral crown-ether, *J. Chromatogr. A*, 709 (1995) 81-88.

46. R. Kuhn, D. Riester, B. Fleckenstein and K. H. Wiesmuller, Evaluation of an optically active crown ether for the chiral separation of di- and tripeptides, *J. Chromatogr. A*, 716 (1995) 371-379.

47. D. W. Armstrong, K. Rundlett and G. L. Reid, Use of a macrocyclic antibiotic, rifamycin B and indirect detection for the resolution of racemic amino alcohols by CE, *Anal. Chem.*, 66 (1994) 1690-1695.

48. T. J. Ward, Macrocyclic antibiotics-the newest class of chiral selectors, *LC.GC*, 14 (1996) 886-894.

49. M. P. Gasper, A. Berthod, U. B. Nair and D. W. Armstrong, Comparison and modeling study of vancomycin, ristocetin A and teicoplanin for CE enantioseparations, *Anal. Chem.*, 68 (1996) 2501-2512.

50. K. L. Rundlett and D. W. Armstrong, Effect of micelles and mixed micelles on efficiency and selectivity of antibiotic-based capillary electrophoretic enantioseparations, *Anal. Chem.*, 67 (1995) 2088-2095.

51. J. Reilly and D. Risley, The separation of enantiomers by countercurrent capillary electrophoresis using the macrocyclic antibiotic A82846B, *LC. GC*, 16 (1998) 170-176.

52. M. Stalcup and N. M. Agyei, Heparin: a chiral mobile-phase additive for capillary zone electrophoresis, *Anal. Chem.*, 66 (1994) 3054-3059.

53. R. M. Sutton, K. L. Sutton and A. M. Stalcup, Chiral capillary electrophoresis with noncyclic oligo-and polysaccaride chiral selectros, *Electrophoresis*, 18 (1997) 2297-2304.

54. H. Nishi, Enantioselectivity in chiral capillary electrophoresis with polysaccharides, *J. Chromatogr. A*, 792 (1997) 327-347.

55. G. E. Barker, P. Russo and R. A. Hartwick, Chiral separation of lencovorin with bovine serum albumin using affinity capillary electrophoresis, *Anal. Chem.*, 64 (1992) 3024-3028.

56. S. Birnbaum and S. Nilsson, Protein-based capillary affinity gel electrophoresis for the separation of optical isomers, *Anal.Chem.*, 64 (1992) 2872-2874.

57. S. Li and D. K. Lloyd, Direct chiral separations by capilary electrophoresis using capillaries packed with an α_1-acid glycoprotein chiral stationary phase, *Anal. Chem.*, 65 (1993) 3684-3690.

58. D. K. Lloyd, S. Li and P. Ryan, Protein chiral selectors in free-solution capillary electrophoreis and packed capillary electrochromatography, *J. Chromatogr. A*, 694 (1995) 285-296.

59. S. Terabe, K. I. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, Electrokinetic separations with micellar solution and open-tubular capillaries, *Anal. Chem.*, 56 (1984) 111-121.

60. S. Terabe, H. Shibata and Y. Miyashita, Chiral separation by electrokinetic chromatography with bile salt micelles, *J. Chromatogr.*, 480 (1989) 403-411.

61. H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, Chiral separation by cyclodextrin-modified micellar electrokinetic chromatography, *J. Chromatogr. A*, 553 (1991) 503-516.

62. A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, Optical resolution of enantiomers with chiral mixed micelles by electrokinetic chromatography, *Anal. Chem.*, 61 (1989) 1984-1986.

63. A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, Enantioselective hydrophobic entanglement of enantiomeric solutes with chiral functionalized micelles by electrokinetic chromatography, *J. Chromatogr.*, 480 (1989) 413-420.

64. K. Otsuka, J. Kawahara, K. Tatekawa and S. Terabe, Chiral separations by micellar electrokinetic chromatography with sodium N-dodecanoyl-L-valinate, *J. Chromatogr.*, 559 (1991) 209-214

65. J. R. Mazzeo, E.R. Grover, M.E. Swartz and J. S. Petersen, Novel chiral surfactant for the separation of enantiomers by micellar electrokinetic capillary chromatography, *J. Chromatogr. A.*, 680 (1994) 125-135.

66. Z. El Rassi and Y. Mechref, Micellar electrokinetic capillary chromatography with in-situ charged micelles VI. Evaluation of novel chiral micelles consisting of steroidal-glycoside surfactant-borate complexes, *J. Chromatogr. A*, 724 (1996) 285-296.

67. D. Tickle, G. N. Okafo, P. Camilleri, R. F. D. Jones and A. J. Kirby, Glucopyranoside-based surfactants as pseudostationary phases for the chiral separations in capillary electrophoresis, *Anal. Chem.*, 66 (1994) 4121-4126.

68. S. A. Shamsi and I. M. Warner, Monomeric and polymeric chiral surfactants as pseudo-stationary phases for chiral separations, *Electrophoresis*, 18 (1997) 853-872.

69. A. Dobashi, M. Hamada and Y. Dobashi, Enantiomeric separation with sodium dodecanoyl-L-amino acidate micelles and poly (sodium (10-undecenoyl)-L-valinate) by electrokinetic chromatography, *Anal. Chem.*, 67 (1995) 3011-3017.

70. K. Agnew-Heard, M. S. Pena, S. A. Shamsi and I. M. Warner, Studies of polymerized sodium N-undecylenyl-L-valinate in chiral micellar electrokinetic capillary chromatography of neutral, acidic and basic compounds, *Anal. Chem.*, 69 (1997) 958-964.

71. Y. Mechref and Z. El Rassi, Capillary electrophoresis of herbicides II. Evaluation of alkylglucoside chiral surfactants in the enantiomeric separation of phenoxy acid herbicides, *J. Chromatogr. A*, 757 (1997) 263-273.

72. Y. Mechref and Z. El Rassi, Capillary electrophoresis of herbicides. III. Evaluation of octylmaltopyranoside chiral surfactant in the enantiomeric separation of phenoxy acid herbicides, *Chirality*, 8 (1996) 518-524.

73. R. M. M. Brito and W. L. C. Vaz, Determination of the critical micelle concentration of surfactants using the fluorescent probe N-phenyl-1-naphthylamine. *Anal. Biochem.*, 152 (1986) 250-255.

74. A. Chattopadhyay and E. London, Fluorimetric determination of critical micelle concentration avoiding interference from detergent charge, *Anal. Biochem.*, 139 (1984) 408-412.

75. Y. Mechref and Z. El Rassi, Capillary electrophoresis of herbicides. I. Precolumn derivatization of chiral and achiral phenoxy acid herbicides with fluorescent tag for electrophoretic separation in the presence of cyclodextrins and micellar phases. *Anal. Chem.*, 68 (1996) 1771-1777.

76. K. Otsuka and S. Terabe, Enantiomeric resolution by micellar electrokinetic chromatography with chiral surfactants, *J. Chromatogr.*, 515 (1990) 221-226.

77. Y. Ishihama and S. Terabe, Enantiomeric separation by micellar electrokinetic chromatography using saponins, *J. Liq. Chromatogr.*, 16 (1993) 933-944.

78. Y. Miyashita and S. Terabe, *Application Data DS-767*, Beckman Instruments, 1990.

79. M. Rosen, *Surfactants and Interfacial Phenomena.* 2nd Edition, Wiley: New York, 1989.

80. D. Rubingh and P. Holland, *Cationic Surfactants*, vol. 37, p. 141, Marcel Dekker: New York, 1992.

81. J. F. Rathman and J. K. Scamehorn, Counterion binding on mixed micelles: Effect of surfactant structure. *Langmuir*, 3 (1987) 372-377.

82. R. T. Morrison and R. N. Boyd, *Organic Chemistry*, Allyn and Bacon, Inc.: Boston, 1983.

VITA

MEI JU

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF GLYCOSIDIC CHIRAL SURFACTANTS IN ENANTIOMERIC SEPARATIONS BY CAPILLARY ELECTROPHORESIS

Major Field: Chemistry

Biographical:

Personal Data: Born in Beijing, P. R. China, December 12, 1967, the daughter of Mengqian Ju and Errui Cao.

Education: Graduated from Beijing No. 171 High School, Beijing, P. R. China in 1986; received Bachelor of Science degree in Polymer Engineering from Tianjin University, Tianjin, P. R. China, in July 1990. Completed the requirements for the Master of Science degree at Oklahoma State University in December, 1998.

Experience: Employed as a graduate teaching assistant at Oklahoma State University, 1996-present. Employed as a chemical engineer at China National Petrochemical Corporation, 1990-1996.

Professional Membership: American Chemical Society.