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Chiral Recognition Using Polymeric and Monomeric Amino Acid Based Surfactants.

Fereshteh Haddadian Billiot

Louisiana State University and Agricultural & Mechanical College

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CHIRAL RECONGNITION  
USING POLYMERIC AND MONOMERIC AMINO ACID BASED SURFACTANTS

A Dissertation
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Chemistry

by

Fereshteh Haddadian Billiot
B.S., Shariff University of Technology, 1989
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M.S., Miami University, 1997
December, 2000

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<td>EKC</td>
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<td>EMC</td>
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<td>GL</td>
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<td>Mono</td>
<td>Monomer</td>
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<td>Oxp</td>
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<td>PFG</td>
<td>Pulse filed gradient</td>
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<td>Poly</td>
<td>Polymer</td>
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<td>Propranolol</td>
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<td>Sodium undecanoyl alaninate</td>
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<td>SUAA</td>
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<td>SUVV</td>
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Abstract

The research presented in this dissertation involves the comparison and characterization of polymeric and monomeric amino acid based surfactants for the enantiomeric separation of chiral compounds. Comparison of the performance of these two kinds of surfactants in EKC suggest that polymers are better chiral pseudostationary phases (CPSP) than the monomers for enantiomeric separation of neutral and cationic analytes, at least for the examined in this dissertation. However, the anionic enantiomers of 1,1'-binaphthyl-2-2'-dihydrogen phosphate (BNP) were better separated using monomeric CPSPs. The depth of penetration of binaphthyl derivatives into the micellar core of chiral dipeptide surfactants (CDS) was examined using various techniques. The results indicate that enantiomers of BNP interact preferentially with the C-terminal amino acid of monomeric CDS, whereas these enantiomers interact with both chiral centers (C- and N-terminal) of polymeric CDS.

In this dissertation, steady state fluorescence anisotropy and pulse field gradient NMR techniques are used to study the chiral interactions of binaphthyl derivatives with these amino acid based surfactants. The results indicate that the anisotropy and diffusion coefficients of various enantiomers are different when complexed to poly sodium undecanoyl leucyl-leucinate. Interestingly, the enantiomer that binds stronger to the CPSP, as evidenced by EKC experiments, has higher anisotropy values and lower diffusion coefficients. The results of this study suggest that these two techniques can be used to gain further insight into chiral recognition.

The physical properties of CDS are examined in Chapters 4 and 5 of this dissertation. The polymeric CDS examined in this dissertation always have lower
aggregation numbers than their monomeric counterparts. As can be deduced from the aggregation numbers, regardless of the size of the polar head, polymeric CDS most likely adopt a spherical shape in solution, while the shape of monomeric CDS depend on the size of the polar head group. In addition, polymeric CDS have a higher effective charge, and are less polar than the monomers. Furthermore, polymeric surfactants have a lower solubilization capacity for neutral organic probes compared to the monomer. This in turn results in faster mass transfer in EKC experiments when polymeric surfactants are used as a CPSP. Finally, the chiral separations of a variety of analytes are examined using polymeric surfactants. Some of the factors examined were number and position of chiral centers, dipeptide amino acid order, and steric effects.
Chapter 1
Introduction to Chirality, Micelle, Capillary Electrophoresis, and Spectroscopy Techniques

From the beginning of the evolutionary process right up to the present diversity of biological species, life has been under the constant influence of chirality. This is because nature is chiral. There is an evolutionary force which leads to most proteins being composed of L-amino acids, and carbohydrates being composed of D-sugars. Therefore, studying chiral interactions assist us to better understand biological systems.¹

A molecule can be considered as chiral if it is non-superimposable on its mirror image. A compound whose molecules are chiral can exist as enantiomers. Enantiomers have opposite signs of optical rotation that arise from an asymmetric plane, center, or axis. Racemates (an equal mixture of the different enantiomers) are common in synthetic drugs. The individual enantiomers of a chiral drug very often have different pharmacological properties.² This phenomenon is exemplified by the well-known case of thalidomide. The racemic form of the drug thalidomide was prescribed to pregnant women during the 1950's as a sedative and antinausea medication. It was soon learned that this drug caused serious birth defects. Later, in 1960's, it was found that the R-enantiomer of thalidomide was responsible for the drugs beneficial therapeutic effect, while the S-enantiomer caused the birth defects.³ ⁴

An additional impact of the thalidomide disaster was that research based on understanding the biological nature of chiral interactions became a high priority. In addition, the United State Food and Drug Administration mandated the testing of the optical isomers of all chiral drugs. As a result, today, pharmaceutical industries are
facing a challenge to ensure the quality of their drug production. In addition to pharmaceutical products, demand for optically pure compounds are growing in agrochemical, food, and electronic industries. Consequently, there has been a great demand for advancement in chiral separation techniques for chiral molecules.

During the early years of research in chiral separations, resolution of chiral compounds was often achieved by forming diastereomeric complexes. The difference between enantiomers and diastereomers is that enantiomers have opposite configurations at all chiral center(s), while diastereomers have opposite configuration at some chiral center(s), but the same configuration at other chiral center(s). In addition, diastereomers have different chemical and physical properties and thus can be resolved by conventional separation techniques. The problem with converting the enantiomer to a diastereomer is that derivatization results in loss of the physical properties of the enantiomer. Therefore, derivatization of enantiomers to achieve chiral separation is not the best approach.

To overcome some of the inherent problems associated with derivatization of enantiomers, chromatographic methods were developed for the separation of enantiomeric mixtures. Chromatographic methods of chiral separation are based on the difference in interaction of the various enantiomers with the chiral selector. The difference in interaction between the enantiomers and the chiral selector is often very small. Therefore, in order to achieve chiral separations, a highly resolving analytical technique is required.

Although, advances are being made in chiral analysis using gas chromatography, and supercritical flow chromatography, varying the
temperature during separation is a major problem. High performance liquid chromatography (HPLC) is another technique that has been used extensively in chiral separation. However, this technique has very poor efficiency. Therefore, in order to obtain chiral separation in HPLC, high selectivities must be achieved. In addition, method development in HPLC can be time consuming. On the other hand, capillary electrophoresis (CE) has shown great promise for the separation of optically active isomers. The advantages of CE over other techniques are the use of minimal sample, small chiral selector consumption, and high efficiency.

It should be noted that the mechanism of chiral separations is still not completely understood. Dalgalish proposed that chiral recognition is achieved based on the three-point interaction rule, which means that chiral recognition requires a minimum of three simultaneous interactions between the chiral selector and the analyte. At least one of these three interactions should be stereoselective. Because of spatial restrictions, the other enantiomer can only achieve two of these interactions. As illustrated in Figure 1.1, one enantiomer has three points of interaction with the chiral selector, while its mirror image would not be able to achieve the same three points of interaction. This model is similar to the lock and key model used to explain enzymatic activity. However, enzymatic activity is often explained via a dynamic model. In the dynamic model, selector and selectant adjust their conformations in order to achieve the best interaction, i.e. the lowest energy configuration for the complex. In this model, the interaction sites are viewed as a spatial environment. First, a complex between the selector and selectant forms. Then conformational adjustments of the two elements occur to optimize the interaction.
Chiral separations in CE are achieved either through the use of an immobilized chiral phase or through addition of the chiral selector as a pseudostationary phase in the running buffer. In this dissertation, chiral amino acid based surfactants/micelles are utilized as chiral pseudostationary phases (CPSP) in CE to separate enantiomers of optically active isomers in different charge states. To be familiar with the topics of discussion in this dissertation, it is necessary to introduce concepts related to

**Figure 1.1 The three-point rule of chiral interaction.**
micelle/surfactant properties and CE. In addition, an overview of the techniques that I used to characterize these CPSPs (fluorescence and nuclear magnetic resonance) also follows.

Part I. Surfactant and Micelle

As illustrated in Figure 1.2, surfactant molecules consist of a polar region (polar head) and a nonpolar region (hydrophobic tail). Surfactants are classified as anionic, cationic, nonionic, and zwitterionic, depending upon the nature of the polar head group. Above a characteristic temperature known as the Kraft temperature and above the critical micelle concentration (CMC), surfactant molecules aggregate to form micelles (Figure 1.2). Surfactants form aggregates in aqueous solution because of the high energy of interaction between water and the surfactant hydrocarbon chain, but the association is limited because of repulsive interactions between the surfactant head groups. The CMC of surfactants can be determined by monitoring the change in

\[ n \]

\[ \text{Surfactant Molecule} \]

\[ \text{Micelle} \]

\[ \text{Polar Head} \]

\[ \text{Hydrophobic Tail} \]

\[ n; \text{aggregation number} \]

Figure 1.2 Surfactant molecule and mechanism of micelle formation.
various physical properties of the solution with increasing concentration of the surfactant as illustrated in Figure 1.3.

![Diagram showing surface tension, conductance, and absorbance maxima against surfactant concentration.]

**Figure 1.3 Determination of CMC.**

In aqueous solutions, the micelle consists of surfactant monomers that are oriented so that their polar region is in maximum contact with water. This is best achieved through a spherical structure. Figure 1.4 shows different regions of a spherical micelle. The region of the micelle, which has a high density of counter ions, is called a Gouy-Chapman double layer. This double layer has a diameter of several angstroms. The area near the interface formed by the polar head groups and the water is known as the Stern layer for ionic surfactants. This layer consists of the ionic surfactant head group, bound and free counterions, and water. Moving outward from the center of the micelle, there is a region called the palisade layer that is viewed as a
liquid hydrocarbon. The radius of the palisade layer is approximately equal to the length of the fully extended hydrocarbon chain.\textsuperscript{27}

The chemical structure of the surfactant determines the size and shape of the micelle. In 1920, McBain\textsuperscript{28} proposed that ionic surfactants form spherical micelles and neutral surfactants form lamellar micelles. Hartley\textsuperscript{29} suggested that surfactant molecules form a spherical micelle in which the radius of the sphere is approximately equal to the length of the hydrocarbon chain. The Hartley model for anionic surfactants is shown in Figure 1.5. This model successfully describes many of the micellar system properties. For example, according to this model, counter ions are
bound to the charged head group of the surfactants.\textsuperscript{30} This explains the drop in conductance of the surfactant solution at the CMC. In addition, Hartley has proposed that the inside core of the micelle, which is composed of the hydrocarbon chain, has properties of liquid hydrocarbon.\textsuperscript{26} This is the reason that micelles are able to solubilize organic molecules.\textsuperscript{31} However, small angle neutron scattering, NMR, and fluorescence probe studies have shown that, due to the motion of the surfactant molecules, the surface of the micelle is not as smooth as what Hartley has proposed.\textsuperscript{32-35}

Above the CMC, as the concentration of the surfactant increases, the shape of the micelle changes from spherical to rod-like and finally to lamellar shaped micelles (Figure 1.6).\textsuperscript{36} These structural changes are due to the presence of attractive forces caused by the hydrophobic affinity of the hydrocarbon chain and repulsive forces such
as electrostatic and steric among the polar head. The aggregation number and CMC of the micelle play an important role in these structural changes.

The value of the CMC depends on the properties and size of the hydrophobic and hydrophilic parts of the surfactant. For example, the CMC decreases as size of the hydrophobic portion of the surfactant increases. In the presence of an organic solvent, the CMC of the surfactant increases. An increase in the CMC is also observed with branching of the hydrocarbon group of the hydrophobic tail. In general, nonionic surfactants have lower CMC values than ionic surfactants. This is due to an increase in the hydrophobicity of nonionic surfactant as compared to ionic surfactants. For example, addition of a double bond to the end of the hydrophobic tail decreases the hydrophobicity of the surfactant and thus increases the CMC by a factor of two. The CMC can be determined by several techniques including surface tension, turbidity, conductivity, fluorescence, NMR, CE, and light scattering. The nature and size of the hydrophobic and hydrophilic parts of the surfactant also determines how many surfactant monomer units aggregate together to form a micelle.

The average number of surfactant molecules per micelle is termed the aggregation number. The aggregation number of the micelle determines the size and geometry of the micelle. Several methods are available to determine the aggregation number of a micelle. These include light scattering, diffusion, viscosity, sedimentation velocity, ultrafiltration, NMR and fluorescence. A static fluorescence quenching technique is used to determine the aggregation number of the amino acid based surfactants examined in this dissertation. Other factors which must
be considered to better understand the nature of micelles are the kinetics and thermodynamic processes involved in micellization. These factors are discussed next.

![Structural changes of micelle.](image)

**Figure 1.6 Structural changes of micelle.**

**KINETICS AND THERMODYNAMICS OF MICELLIZATION**

Kinetic studies of micellization have shown that an equilibrium exists between the surfactant molecules and the micelle. This equilibrium can be described as follows

\[
nS \leftrightarrow M_n
\]

(1.1)

\[
K_m = \frac{[M_n]}{[S]^n},
\]

(1.2)

where \(n\) is the aggregation number, \(S\) and \(M\) are the surfactant and micelle concentrations, respectively, and \(K_m\) is the equilibrium constant.\(^{29}\) From the above
equations it can be seen that the total surfactant concentration, $C_t$ can be calculated from the following equation

$$C_t = [S] + K_m [S]^n. \quad (1.3)$$

Once the micellar equilibrium constant has been determined, the standard Gibbs free energy of micellization can be calculated using the following equation$^{29}$

$$\Delta G^0 = -RT \ln K_m. \quad (1.4)$$

The free energy needed to insert one monomer unit into the micelle can be obtained by dividing equation 1.4 by the aggregation number ($n$).

For a large value of $n$, the free energy can be calculated as follow

$$\Delta G^0 = -RT \ln [S]. \quad (1.5)$$

Above the CMC, where the added surfactant monomer forms a micelle, the concentration of the free surfactant will be constant. Therefore, $[S]$ is equal to the CMC and equation 1.5 can be written as

$$\Delta G^0 = -RT \ln \text{CMC}. \quad (1.6)$$

Plots of $\Delta G^0$ versus $T$ can be used to determine $\Delta S^0$. The value of $\Delta H^0$ can then be calculated using the following equation$^{55}$

$$\Delta G^0 = \Delta H^0 - T \Delta S^0. \quad (1.7)$$

The free energy of the micellization for ionic surfactants can be written as

$$\Delta G^0 = RT \left(2 - \frac{p}{n}\right) \ln \text{CMC}, \quad (1.8)$$

where $p$ is the effective charge of the micelle. $^{56}$
Micelle formation in aqueous systems is commonly endothermic. That micelles spontaneously form above the CMC indicates that the entropy change must be positive. The fact that the entropy change is positive even though the molecules are clustering together shows that there is a contribution to the entropy from the solvent, and that solvent molecules are more free to move once the surfactant molecule forms a micelle. Therefore, examination of the solvent physical properties (i.e. surface tension), indicates a sharp transition at the CMC.

It should be mentioned that micelles are involved in a highly dynamic equilibrium with their monomeric units. Aniansson and Wall have proposed a model in which there is multiple equilibrium between the micelle and the surfactant molecules. This model assumes that micellization occurs through multiple equilibria:

\[ S_1 + S_1 \leftrightarrow S_2 + S_1 \leftrightarrow S_3 + \ldots S_{n-1} + S_1 \leftrightarrow S_n \]

\[ K_1 = \frac{[S_2]}{[S_1]^2}, K_2 = \frac{[S_3]}{[S_1][S_2]}, \ldots K_n = \frac{[S_n]}{[S_1][S_{n-1}]} \]

where \( S_1, S_2, \ldots \text{and} \ S_n \) are the surfactant monomer, dimer,.. and n-mer, and \( K_1, K_2, \ldots K_n \) are the equilibrium constants. Based on the equilibrium shown in equation 1.9, micellar solutions contain aggregates with different degrees of aggregation. Therefore, normal micelles are polydispersed.

Having covered the basics of surfactants and micelle formation it is now appropriate to move on and discuss the various aspects of capillary electrophoresis. That is the purpose of the next section of this chapter.
Part II. Capillary Electrophoresis

Separation by electrophoresis is based on differences in solute velocity in an electric field. When a potential difference is applied across two electrodes in an electrolyte solution, the ions in that solution experience an electric field ($E$). In such a field, an ion of charge $q$ experiences a force magnitude ($F_E$) of

$$F_E = qE.$$  

(1.10)

A cation in this electric field migrates toward the negative electrode, and an anion migrates toward the positive electrode. As the ion moves through the solvent, it experiences a frictional retarding force ($F_F$), i.e.

$$F_F = 6\pi \eta r v,$$  

(1.11)

where $r$ is the hydrodynamic radius and $\eta$ is the viscosity of the solvent. The two forces, $F_E$ and $F_F$, act in opposite directions; therefore, the ions quickly reach a terminal speed $v$, where

$$v = \frac{qE}{6\pi \eta r}.$$  

(1.12)

The ion velocity can also be expressed as

$$v = \mu_e E,$$  

(1.13)

where $\mu_e$ is called the electrophoretic mobility of the ion. Combining equations 1.12 and 1.13 the relationship between the $\mu_e$ and the charge and size of the ion, as well as the viscosity of the solution can be determined by

$$\mu_e = \frac{q}{6\pi \eta r}.$$  

(1.14)
As noted in equation 1.14, the electrophoretic mobility of an ion increases with increasing charge on the ion and decreases by increasing the viscosity and hydrodynamic radius. It is important to note that, although an ion may be relatively small, it may have a large hydrodynamic radius because of the many solvent molecules associated with it. An analytical technique which uses the differences in migration of these charged species in an electric field as a separation tool is electrophoresis.

Modern electrophoresis is based on the studies of free moving boundary electrophoresis by Tiselius in 1930. Tiselius separated α-, β-, and γ-globulin using electrophoresis. He was awarded a Nobel Prize in 1984 for his contribution in separation science. In the 60-70's, Hjerten, Virtanen, and Mikkers tried to develop capillary electrophoresis (CE) as a microanalytical separation tool. In 1981, Jorgenson and Lukas advanced the CE technique by using 75 μm I.D. fused silica capillaries.

Movement of ions in a capillary is not only dependent on the electrophoretic mobility of the ion, but also on the movement of the bulk aqueous solution through the capillary. The movement of the bulk solution is known as the electroosmotic flow (EOF). The EOF results from the effect of the applied electric field on the double layer at the wall of the capillary. The walls of the fused-silica capillary contain silanol groups. The silanol groups are weakly acidic and become ionized in a solution with a pH above 2. This results in formation of a negatively charged capillary wall. Positive ions are attracted to the wall, forming a fixed layer. This in turn results in a potential difference at the capillary wall known as the zeta potential. But the negative
charge is not quite balanced by this fixed layer, so a further diffuse layer of positive ions becomes attracted to the wall, giving an electrical double layer. When a voltage is applied across the capillary, the positively charged ions in the diffuse layer move toward the cathode. Since these positively charged ions are solvated, their movements drag the bulk solution in the capillary toward the outlet. A schematic of this process, is shown in Figure 1.7.

The equation for EOF mobility ($\mu_{EOF}$) can be written as

$$\mu_{EOF} = \varepsilon \zeta \eta,$$

where $\varepsilon$ is the dielectric constant, and $\zeta$ is the zeta potential. The zeta potential which is strongly pH dependent is determined by the charge on the capillary wall. At high pH, where most of the silanol groups are deprotonated, the EOF is significantly greater than at low pH where they become protonated. Figure 1.8 illustrates the effect of pH on EOF for fused silica, Pyrex, and Teflon. Teflon has been used in cases where suppression of the EOF is required. Pyrex capillaries have been used for fundamental studies in capillary electrophoresis.

The double layer formed at the surface of the capillary is a very thin layer relative to the radius of the capillary. In addition, since the driving force of the flow inside the capillary is uniformly distributed, there is no pressure drop within the capillary. Therefore, the flow of the bulk solution inside the capillary is uniform, resulting in an approximately flat profile. The flat profile in CE increases the separation efficiencies as compared to pressure driven separation techniques such as HPLC. 

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Figure 1.7 Electroosmotic flow (a) hydrated cation accumulating near the surface, and (b) bulk flow toward the cathode upon application of the electric field.
The EOF is a major factor that determines the amount of time solutes spend in the capillary. Therefore, it is important to control the EOF. Control of the EOF can be accomplished by alteration of the capillary surface charge or buffer viscosity. The rate of the EOF can be easily decreased by decreasing the electric field as described by equation 1.15. However, lowering the electric field will increase the analysis time. As can be inferred from Figure 1.8, adjusting the pH of the running electrolyte is one of the most practical ways of adjusting the EOF. Adjusting the ionic strength of the buffer also affects the EOF. Finally, the EOF can be controlled by modification of the capillary wall. Either dynamic or covalent coatings can modify the capillary wall. The modification of the capillary wall may increase, decrease, or even reverse the EOF.

A simple schematic of a CE instrument is shown in Figure 1.9. A CE instrument consists of a high voltage power supply, two buffer reservoirs, a capillary, and a detector. In CE, a fused silica capillary is filled with a buffer solution that conducts the electric current and provides buffering capacity. The sample which is a mixture of ions is then introduced (usually by pressure or vacuum) at the end of the capillary away from the detector. The capillary ends are then dipped into a reservoir containing high voltage electrodes and buffer solution. A voltage is applied and the ions migrate either toward the detector or away from the detector depending on the polarity of the applied voltage, the charge of the ion, and on the EOF.

In CE, under the influence of the electric field, cations are attracted toward the cathode, and their speed is increased by the EOF. Since the magnitude of the EOF toward the cathode is very large, anions are also swept toward the cathode under the
Figure 1.8 Effect of pH on EOF with various capillary materials.

Influence of the EOF. As shown in Figure 1.10, in the separation of ions with normal mode CE, cations with a large charge/radius ratio elute first, followed by the cations with small ratios. Neutral species migrate at the rate of the EOF. Lastly, anions with smaller charge/radius ratios migrate earlier than anions with large charge/radius ratios.
In the presence of the EOF, the experimentally measured analyte mobility is called the apparent mobility $\mu_a$, which is expressed as

$$\mu_a = \frac{IL}{tV},$$

(1.16)

where $V$ is the applied voltage; $l$ is the effective capillary length; $L$ is the total capillary length, and $t$ is the migration time of the analyte. The effective mobility $\mu_e$ can be calculated as follows

$$\mu_e = \mu_a - \mu_{EOF}.$$  

(1.17)
Note that the values of $\mu_a$ will be positive for cations and negative for anions. The EOF is usually measured using a neutral species such as methanol, dimethyl sulfoxide or mesitylene oxide, which move at the velocity of the EOF.

![Diagram of ion separation in CE](image)

**Figure 1.10 Separation of ions in CE.**

Jorgenson and Lukas demonstrated that the separation efficiency or theoretical plates ($N$) in CE depends only on the total electrophoretic mobility ($\mu_a$), applied voltage ($V$), and the diffusion coefficient of the ion ($D_0$).\(^6\)

$$N = \frac{\mu_a V}{2D_0} \quad (1.18)$$

Equation 1.18 implies that the highest efficiency is obtained when ions are migrating at the fastest velocity (i.e., have the largest $\mu_a$ value). Therefore, one can increase the EOF to help speed up the separation and to increase the separation efficiency. However, as the speed of the migration increases, resolution decreases, simply because there is not enough time for the components to physically separate from one another.
In CE, resolution between two adjacent eluting species, which is directly proportional to the difference in their mobilities ($\mu_{e1} - \mu_{e2}$) and inversely to the square root of their average total mobility ($\mu_{avg}$), can be expressed as follow\textsuperscript{68}

$$Rs = 0.177(\mu_{e1} - \mu_{e2}) \left[ \frac{V}{D_0(\mu_{avg} + \mu_{EOF})} \right]^{1/2}. \tag{1.19}$$

This equation indicates that as EOF increases, the resolution decreases. Therefore, there are limitations to how fast we can perform a separation and still achieve adequate Rs values.

It should be mentioned that neutral molecules will always elute with the EOF without any separation. It was for this reason that Terabe and his co-workers introduced electrokinetic chromatography (EKC) in 1984.\textsuperscript{69}

**ELECTROKINETIC CHROMATOGRAPHY**

Electrokinetic chromatography is a mode of CE that is capable of separating both charged and neutral compounds simultaneously. The fundamental experimental setup for EKC is similar to conventional CE which was discussed in the previous section. In addition to the buffer used in CE, another component called a pseudostationary phase is added to the buffer solution. Charged and neutral analytes are separated based on their relative affinity for the pseudostationary phase. A variety of materials can be used as pseudostationary phases in EKC. Some examples include; monomeric and polymeric surfactants,\textsuperscript{70-79} microemulsion,\textsuperscript{80-81} macrocyclic and macromolecular phases,\textsuperscript{82-83} vesicles,\textsuperscript{84} dendrimers,\textsuperscript{85} and polymer ions.\textsuperscript{86-89} Micellar EKC (or MEKC) is the term most commonly used when micelles or surfactants are used as the pseudostationary phase.
Micelles, as mentioned earlier in this chapter, are aggregates of surfactant molecules. One of the important properties of micelles is their ability to enhance the solubility of otherwise insoluble analytes and to greatly enhance the solubility of slightly soluble analytes. Therefore, the differential solubility or partitioning of analytes between the aqueous mobile phase and the micellar phase is the basis of separation in MEKC. The partition coefficient (P) of a solute dissolved in a micelle is defined as

\[ P = \frac{C_m}{C_w}, \]

where \( C_m \) and \( C_w \) are the concentration of the solute in micelle and in aqueous phase, respectively. The degree of interaction of solutes with micelles depends on the degree and nature of the charge on the analyte, as well as the micelle, and on hydrophobic interactions. The location of the solubilized analyte in the micelle can be any or all of the micellar regions shown in Figure 1.4. Ionic solutes that have opposite charge from the polar head of the surfactants may bind strongly to the polar head through electrostatic attractions. Solutes with amphiphilic character align themselves in such a way that the more polar end of the molecule is directed toward the bulk aqueous phase and the hydrophobic portion of the molecule is directed toward the hydrophobic core of the micelle. Neutral species, depending on their hydrophobicities, bind to the micelle anywhere between the sterol layer and the micellar core. Highly hydrophobic neutral analytes penetrate deeper into the micelle core as compared to more polar neutral molecules. Small uncharged polar molecules, such as methanol and acetonitrile, do not interact with the micelle to any significant degree. An
illustration of some of the possible solubilization sites of solutes in a micelle is shown in Figure 1.11.

![Figure 1.11](image)

**Figure 1.11** Interaction of analytes with micelles (A) hydrophilic analyte, (B) hydrophilic analyte with opposite charge than the polar head of the surfactant, (C) highly hydrophobic analytes, and (D) moderately hydrophobic analyte.

In MEKC, surfactants are added to the running buffer above the CMC to act as the separation medium. In free zone capillary electrophoresis, charged micelles migrate in the electric field at an electrophoretic velocity that is proportional to their charge/radius ratio. However, in MEKC the mobility of the ion is not only dependent on its charge/radius ratio but also on the mobility of micelle when it is complexed to the micelle. Neutral species, which cannot be separated in free zone electrophoresis,
partition into the micelle and are separated because of differences in partitioning of the various analytes with the micelle.\textsuperscript{70} Figure 1.12 illustrates the migration of neutral species with anionic and cationic micelles. As indicated in this figure, neutral analytes elute between $t_0$ (EOF marker) and $t_{mc}$ (elution time of the micelle).

As illustrated in Figure 1.12, anionic surfactants migrate in the opposite direction of the EOF. Under normal conditions, the EOF velocity is stronger than the migration of the micelle. As a result, the anionic micelles transport toward the cathode. On the other hand, using cationic surfactant, the capillary wall is coated with the micelle resulting in reversal of the EOF. As shown in Figure 1.13, cationic surfactant monomers adhere to the capillary wall through ionic interactions. The positive charges near the capillary wall result from hydrophobic interaction of free surfactant molecules with those bound to the wall. Therefore, the polarity of the electrode should be reversed, when cationic surfactants are employed.\textsuperscript{19}

In micellar EKC, analytes that do not interact with the micelle elute with the EOF. Neutral highly polar molecules, such as methanol, are usually used as an EOF marker. Analytes that interact very strongly with the micelle elute with the micelle. These highly hydrophobic molecules can be used as $t_{mc}$ markers.

The elution window for neutral molecules in MEKC is between $t_0$ and $t_{mc}$, which means that neutral molecules should be separated within this elution window. This limits the peak capacity in MEKC. The elution window can be enlarged by the addition of organic modifiers to the running electrolyte.\textsuperscript{90} The addition of organic modifiers also affects the capacity factor, $k'$. 

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Figure 1.12 Migration of uncharged compounds in MEKC using (a) anionic and (b) cationic pseudostationary phases. The separation of solute S1 and S2 is achieved due to their differential partitioning into the micelle.
As previously stated, the capacity factor in micellar EKC is defined as

\[ k' = \frac{n_{mc}}{n_{aq}} \]  

(1.21)

where \( n_{mc} \) and \( n_{aq} \) are the total number of solute molecules incorporated into the micelle and the total number of solute molecules dissolved in the aqueous phase, respectively. Note that this equation is similar to the partitioning of the solute in a micelle as defined in equation 1.20. The solute migration time \( t_R \) is related to \( k' \) by equation 1.22

\[ k' = \frac{t_R - t_0}{t_0(1 + (t_R/t_{mc})}, \]  

(1.22)

where \( t_R \) is the retention time for the solute, and \( t_{mc} \) is the elution time for the micelle.

Solving equation 1.22 for \( t_R \) results in the following equation
The resolution between two neutral solutes is related to $k'$ and can be expressed as

$$t_R = \frac{(1 + k')t_0}{(1 + (t_0/t_{mc}))(k')}.$$  \hfill (1.23)

The resolution between two neutral solutes is related to $k'$ and can be expressed as

$$R = \left( \frac{N^{1/2}}{4} \right) \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k'}{1 + k'} \right) \left( \frac{1 - (t_{EOF}/t_{mc})}{1 + (t_{EOF}/t_{mc})} \right).$$  \hfill (1.24)

where $N$ is the theoretical plate number.\textsuperscript{19}

In MEKC, $R$ and $N$ are greatly affected by the dynamics of the surfactant/micelle system, as well as micelle/analyte interactions. Thus, the separation efficiency is usually much less in MEKC than in free zone electrophoresis. This is the reason that, in 1992, Palmer et al. used polymeric surfactants for EKC separations.\textsuperscript{91-92}

The following section of this chapter focuses on polymeric surfactants and the advantages of this class of pseudostationary phase over the conventional micelles.

**Polymeric Surfactants in Electrokinetic Chromatography.** As mentioned previously, MEKC is characterized by limited migration time ranges. All neutral analytes have migration times between $t_0$ (migration time for unretained molecule e.g. methanol) and $t_{mc}$. Due to high partitioning coefficients, highly hydrophobic analytes tend to have migration times close to $t_{mc}$ with very high capacity factors. Therefore, adjustment of the capacity factor is necessary for optimum separation.\textsuperscript{89} One way of adjusting the capacity factor is adding an organic modifier to the running electrolyte. However, due to the instability of the conventional micelles in organic solutions, adjusting the capacity factor by addition of organic modifiers to the running buffer is not always possible.\textsuperscript{89}
The capacity factor, $k'$, in MEKC can be defined as

$$k' = K \left( \frac{V_{mc}}{V_{aq}} \right)$$

(1.25)

where $K$ is the distribution coefficient, and $V_{mc}$ and $V_{aq}$ are the volumes of the micelle and the aqueous phase, respectively. Note that the capacity factor is directly proportional to the micellar volume.\textsuperscript{93} The volume of the micelle is given as

$$V_{mc} = \nu (C_{sur} - CMC),$$

(1.26)

where $\nu$ is the partial specific volume of the surfactant and $C_{sur}$ is the total concentration of the surfactant. The CMC changes by varying temperature, pH, electrolyte concentration, and buffer additives including organic solvents.

In micellar EKC, the dependence of the CMC on the temperature can be problematic. The applied electric field across the capillary causes joule heating and an increase in temperature inside the capillary. This change in temperature will cause a change in CMC, and in turn, $K$, and viscosity of the buffer. Because of the dependence of the capacity factor on the CMC and $K$, temperature has a significant effect on separations in MEKC.\textsuperscript{94-98}

Another problem in MEKC that arises from the dynamic equilibrium between the surfactant molecule and micelle is that it limits the flexibility of the technique in terms of the choice of the analytical conditions. Surfactants must have a relatively low CMC and they must be above the CMC in the running buffer. Additionally, the effect of organic modifier on the CMC and the structure of the micelle complicates the analysis of hydrophobic compounds.\textsuperscript{98-103}
An ideal pseudostationary phase for EKC should provide desired chromatographic selectivity under a wide variety of separation conditions. In addition, in order to provide a wide separation window, pseudostationary phases should have high electrophoretic mobilities. To minimize Joule heating, they should have zero or low CMC. To achieve high efficiency, they should provide a fast mass transfer of the analyte between the pseudostationary phase and running buffer. It is obvious that conventional micelles do not meet all of the criteria for an ideal pseudostationary phase. Polymeric surfactants come much closer to satisfying the conditions set forth for an ideal pseudostationary phase.

The fundamental differences between polymeric surfactants and conventional micelle is that polymerization eliminates the dynamic equilibrium between the micelle and surfactant molecules. In other words, the covalent linkage between the surfactant molecules fixes the size and structure of polymeric surfactants. Polymeric surfactants provide very stable pseudostationary phases with zero CMC. Therefore, polymeric surfactants can be used in the presence of high organic modifier concentrations without breaking up the micelle. Since the requirement for self-association is eliminated, polymeric surfactants can be synthesized with any selectivity and electrophoretic mobility.

Palmer and coworkers were the first to use achiral polymeric surfactants in EKC. They used poly sodium-10-undecylate to separate poly aromatic hydrocarbons in buffers contain 50% organic modifier. One of the major advantages of this polymeric pseudostationary phase over conventional micelles is the stability of the polymer at high organic solvent concentrations. However, because of the presence
of the carboxylate group at the polar head, this polymer is not soluble at low pH. To overcome this problem, Terabe and Palmer synthesized sulfate analogs of this polymer, poly sodium undecenyl sulfate. This polymer is similar to the conventional surfactant micelle sodium dodecyl sulfate. The main difference is the presence of the covalent linkage among the hydrophobic tail of the polymer surfactants. Terabe and Palmer have reported that this polymeric surfactant provides better separations due to the greater migration time range compared to its unpolymerized counterpart.\textsuperscript{93,98} In this dissertation, monomeric and polymeric chiral amino acid based surfactants are utilized as CPSP in EKC for the enantiomeric separation of optically active analytes.

**Chiral Separation Using Capillary Electrokinetic Chromatography.**

Electrokinetic chromatography is one of the most common chiral separation modes in CE. Such separations depend on the addition of a CPSP into the buffer. Chiral EKC was first reported by Zare et al. in 1985.\textsuperscript{107} This group utilized Cu(II) complexes of histidine to separate enantiomers of dansylamino acids. Since that time, several natural\textsuperscript{108-111} and synthetic\textsuperscript{112-114} CPSPs have been used in EKC to separate isomers of optically active analytes.

Cohen et al., for the first time, utilized a synthetic chiral surfactant in MEKC.\textsuperscript{19} They separated enantiomers of dansylated amino acids using N,N-dodecyl-L-alaninate in combination with Cu(II) and SDS. The mechanism of chiral separation in this case involves the formation of diastereomeric complexes between Cu(II), the chiral analyte, and the surfactant. Dobashi et al. synthesized a valine based surfactant and used this surfactant to separate N-3,5-dinitrobenzoylated amino acid isopropyl ethers.\textsuperscript{19} In addition, this group stated that the migration time of the analytes increase with
increasing hydrophobicity of the amino acid derivatives. This indicates the important role of the hydrophobic forces in micelle-solute interactions.

As mentioned earlier, normal micelles are dynamic aggregates with a dynamic equilibrium between the micelles and the surfactant monomers. In addition, complexation of micelles with a given solute is also a dynamic interaction, which can be altered by the equilibrium that exists between the micelle and surfactant monomer. Thus, the dynamic micellar system may have a negative influence on the efficiency of the chiral interaction. To overcome this problem, in 1994, Wang in Warner's group introduced polymeric chiral micelles for enantiomeric separations. Using poly sodium N-undecanoyl L-valinate (poly SUV), they reported the enantiomeric separation of (±)-1,1'-2-2'-naphthol and D,L-laudonosine. Shortly thereafter, two papers, one by Dobashi's group and one by Warner's group reported the use of this polymer to separate more chiral analytes. Having determined that polymeric amino acid based surfactants showed some promise as a CPSP, the next logical step was to determine if polymeric dipeptide surfactants would be better than their single amino acid counterparts.

Shamsi et al. compared the chromatographic performance of the polymeric dipeptide surfactant, poly sodium undecanoyl L,L-valy-valinate (poly SUVV), to the previously mentioned single amino acid surfactant poly SUV. The polar head group of poly SUVV contains two amino side chains (CH(CH₃)₂) three carbonyl and two amine moieties, while the single amino acid surfactant possesses only one amino acid side chain, two carbonyls, and one amine group. It should be also mentioned that poly SUV possesses one chiral center and poly SUVV contains two stereogenic centers. In
this work parameters, such as pH, concentration of the polymer, as well as type and concentration of running buffer on chiral separation of enantiomers in different charge states were investigated.

Positively charged enantiomers of propranolol and alprenolol were separated using poly SUVV and poly SUV. It was reported that poly SUVV with two chiral centers provides better chiral recognition for enantiomers of these β-blockers than poly SUV with one chiral center. This may be due to the fact that dipeptide surfactants provide more hydrogen bonding sites on the ionic head group in poly SUVV. Shamsi et al.\textsuperscript{118} concluded that the improved chiral resolution with increase in interaction time of β-blockers using polymeric dipeptide surfactants might be due to the fact that chiral recognition of these analytes are controlled by steric factors. Further comparisons of single amino acid and dipeptide surfactants were conducted on the separation of the negatively charged enantiomers of (±)1,1′-binaphtyl-2,2′-diyl hydrogen phosphate (BNP). Comparing the chiral recognition ability of these surfactants indicates that, dipeptide surfactants perform better than single amino acid surfactants for the separation of BNP enantiomers.

Knowing that a chiral selector's size and shape is important in selectivity,\textsuperscript{119,120} the effect of the order of amino acids in dipeptide surfactants was investigated by Billiot et al.\textsuperscript{121} The two main dipeptide surfactants used in this study were sodium N-undecanoyl (L,L) valyl-leucinate (poly SUVL) and poly sodium N-undecanoyl (L,L) leucyl-valinate (poly SULV). In poly SUVL, valine is the N-terminal amino acid while in poly SULV valine is the C-terminal amino acid of the dipeptide surfactant. A dramatic difference in the chiral selectivity of these two surfactants was observed. In
order to further understand the differences in chiral separation ability of these two surfactants, the single amino acid surfactants of poly SUV and poly sodium N-undecanoyl L-leucinate (SUL), as well as two dipeptide surfactants poly sodium N-undecanoyl valyl-valinate (poly SUVV) and sodium N-undecanoyl leucyl-leucinate (poly SULL) were also examined in this study and their performance compared to poly SULV and poly SUVL.

One of the important factors which needs to be considered when trying to understand the chiral recognition ability of dipeptide surfactants is the configuration of the dipeptide R-groups in the CPSP. Billiot et al. proposed that the lowest energy configuration of dipeptide surfactants in solution was when the larger (more hydrophobic) of the two amino acid's R-group is facing the micellar core and the smaller (less polar) R-group is forced to twist more towards the aqueous layer due to steric constraints.\(^{12}\)

In another study, Billiot et al. proposed that the depth of penetration of the analyte into the micellar core of the polymeric dipeptide CPSP dictates the preferential site of interaction of the analyte with the polar head of the surfactants.\(^{12,3}\) Hydrophobicity, as well as electrostatic interactions of the enantiomers with the surfactant determines the depth of penetration. The amino acid located at the C-terminal position of the dipeptide is in a more hydrophilic environment than the N-terminal amino acid. Hydrophobic enantiomers will penetrate deeper into the micellar core to shield themselves from the aqueous layer. Thus, they interact mostly with the N-terminal amino acid. Hydrophilic or cationic enantiomers interact mostly with the C-terminal amino acid. However, moderately hydrophobic enantiomers will interact...
with both amino acids of the dipeptide CPSP. Billiot et al. have utilized
diastereomeric surfactants of poly sodium N-undecanoyl leucyl-leucinate to evaluate
this phenomena.

Yarabe et al. studied the effect of the heteroatom on chiral recognition with the
single amino acid polymeric surfactant of SUV and sodium undecanoyl threonate
(SUT). In that study, the temperature dependence of the retention factor in EKC is
used to calculate the enthalpy, the entropy, and the Gibbs free energy of the
surfactant/analyte complexes. Poly SUT provided less chiral resolution for
enantiomers of phenylthiohydantion-DL-amino acids examined in that study
compared to poly SUV. Authors stated that this is due to the less free energy changes
during complexation of analytes with poly SUT compared to poly SUV surfactant.

The main focus of this dissertation is to investigate the performance of the
polymeric and monomeric surfactants in terms of chiral separation. In addition, these
two kinds of surfactants are characterized using spectroscopic techniques such as
fluorescence spectroscopy and nuclear magnetic resonance. A brief overview of these
two techniques follows.

**Part III. Fluorescence and Nuclear Magnetic Resonance Spectroscopy**

**FLUORESCENCE SPECTROSCOPY**

Fluorescence is the result of the emission of a photon after the relaxation of an
electronically excited molecule into a lower energy level. The emission spectrum is
dependent upon the chemical structure and the environment of the molecule. A loss of
energy due to radiationless emission of the molecule results in the emission spectrum
shifting to longer wavelengths. The shift in emission wavelength is known as Stokes
Due to the lower detection limit of fluorescence over absorption, the former technique is more sensitive than the latter. In addition, the emission spectrum of many fluorescence molecules reflects the polarity and viscosity of the environment they are in. These environmentally sensitive fluorescent molecules can thus be used to study the environment of micellar systems. In this dissertation, fluorescence techniques are used to determine the polarity and aggregation number of monomeric and polymeric surfactants. In addition, a steady state fluorescence anisotropy technique is used to study the chiral interaction of analytes with polymeric surfactants.

**Polarity Measurements.** The polarity of the micellar core can be measured using a fluorescence molecule that seeks the core and is sensitive to the polarity of the environment. The emission spectrum of this molecule should reflect the environment in which this molecule is dissolved. Pyrene is a fluorescent molecule that has been used extensively for this purpose. This molecule exhibits a characteristic fluorescence emission spectrum that consists of five vibronic bands. Its vibrational band intensities depend on solvent polarity. Of the five vibronic bands, an increase in the peak intensity at 372 nm (band I) is accompanied by a decrease in the peak intensity at 383 nm (band III) with increasing polarity of the environment. The ratio of the intensity of band I to band III is often used to determine the polarity of the micellar core. It should be mentioned that for water, which is the most polar solvent, this ratio is about 1.6, while for hydrocarbon solvents such as methylcyclohexane, this ratio is about 0.6.

**Static Fluorescence Quenching Technique.** Any process that results in reduction of the fluorescence quantum yield is called fluorescence quenching.
When a fluorophore forms a complex with a molecule (called quencher) in the ground-state, which inhibits the excitation of the fluorophore, the fluorophore is said to be statistically quenched.\textsuperscript{130} The quenching process can be described by the Stern-Volmer equation

\[
\frac{F_0}{F} = 1 + k_q \tau_0 [Q],
\]  

(1.27)

where \(F_0\) and \(F\) are the fluorescence intensities in the absence and presence of the quencher, respectively, \(k_q\) is the bimolecular quenching constant, \(\tau_0\) is the life time of the fluorophore in the absence of the quencher and \([Q]\) is the concentration of the quencher.\textsuperscript{131,132} The Stern-Volmer quenching constant (\(K_D\)) is defined as

\[
k_q \tau_0 = K_D.
\]  

(1.28)

A plot of \(F_0/F\) (or \(\tau_0/\tau\)) vs. \([Q]\), is called a Stern-Volmer plot. A linear plot indicates a single class of fluorophores all in the same polarity environment. Therefore, there is an equal possibility for the quencher to interact with the fluorophore. A non-linear plot is the result of either the combination of static and dynamic quenching or the presence of the multiple fluorophore environments.

Static quenching has been extensively employed to determine the aggregation number of surfactants.\textsuperscript{132-135} Turro and Yekta introduced this technique in 1978.\textsuperscript{132} In this technique, both fluorophore and quencher should be sufficiently hydrophobic to partition into the micellar phase. Fluorescence intensity is then measured at different quencher concentrations. According to the following equation

\[
\ln(1/I_0) = \frac{[Q]}{[M]}
\]  

(1.29)
the slope of a plot of \(\ln(I/I_0)\) vs \([Q]\) is equal to the reciprocal of the micelle concentration \([M]\). The slope of this plot can then be used to calculate the aggregation number where

\[
N = \frac{[\text{surfactant}] - \text{CMC}}{[M]}.
\]

(1.30)

**Steady State Fluorescence Anisotropy.** When a fluorophore is excited with plane polarized light, the resulting emission will also be polarized.\(^{125}\) A change in polarization of the fluorescence, during the excitation and emission process, is due to rotation of the molecule. In other words, with the use of polarized excitation, the intensity of the fluorescence emission perpendicular (\(I_\perp\)) and parallel (\(I_\parallel\)) with respect to the excitation will depend on the rotation of the molecule. The degree of depolarization of the fluorophore is called anisotropy. Anisotropy is defined as

\[
\tau = \frac{I_\parallel - I_\perp}{I_\parallel + 2I_\perp}.
\]

(1.31)

It should be mentioned that depolarization results from photoselection and angular displacement of the fluorophore's absorption and emission dipoles. When complete depolarization occurs, the anisotropy of the molecule is equal to zero. However, photoselection leads to a fundamental anisotropy, \(r_0\),

\[
r_0 = \frac{3\cos^2 \theta - 1}{5},
\]

(1.32)

where \(\theta\) is the angle between the excitation and emission oscillators of the fluorophore. The highest value of the anisotropy when \(\theta = 0\) (collinear oscillation), is 0.4. The dependence of fluorescence anisotropies on rotation of the fluorophore results in numerous applications of this technique in different fields of research.\(^{136}\)
For example, this technique has been used to quality protein denaturation, protein-ligand association reactions, and rotational rates of proteins. In Chapter 3 of this dissertation, steady state fluorescence anisotropy is utilized to study interaction of enantiomers with chiral polymeric surfactants.

**NUCLEAR MAGNETIC RESONANCE**

Nuclear magnetic resonance is the resonant absorption of radio-frequency (rf) radiation by nuclei exposed to a magnetic field. After an rf pulse excites the nuclei, a difference in the applied frequency and the resonance frequency of the nuclei is acquired in the form of a free induction decay (FID). This FID is then detected with a radio-receiver coil, and stored in a computer for data processing. The time domain decay signals are then converted to a frequency domain signal by Fourier transformation (FT). When magnetically different nuclei are present, the FID develops a distinct beat pattern. The FT of this pattern displays a frequency domain spectrum in which different nuclei will possess different chemical shifts.

The application of NMR spectroscopy is very broad. Some examples include structural elucidation of protein and enzymes in solution, and in vivo monitoring of metabolism, and medical diagnostics. In this dissertation, NMR spectroscopy is utilized to first identify and then study the structure of the amino acid based surfactants. Second, diffusion, extracted from pulse field gradient nuclear magnetic resonance (PFG-NMR), is used to study the chiral interaction of different enantiomers with amino acid based surfactants.

Pulse field gradient NMR, which is a two-dimensional NMR technique, results from the conventional chemical shift spectra in one-dimension and diffusion spectra in
the other dimension. The diffusion-ordered (DOSY) spectrum is obtained by pulsing the magnetic field after the rf pulse. The DOSY experiments differ slightly in data acquisition compared to the other 2D NMR techniques (NOSY and COSY). The difference is that a pulse field gradient is needed to supply additional magnetic field strength. This pulse is accomplished by placing coils of Cu wire between the sample and the magnetic field.\textsuperscript{139-142}

The most important part of the DOSY technique is the transformation and display of data of the diffusion dimension. The Fourier transform PFG-NMR experiment provides a 2D data of the form

\[
I(K,v) = \sum_n A(v) \exp \left[ -D_n \left( \Delta - \frac{\delta}{3} \right) K^2 \right]
\]  

(1.33)

where \( K = \gamma g \delta \) is the area of the gradient pulse in cm\(^{-1}\), \( \gamma \) is the gyromagnetic ratio, \( g \) and \( \delta \) are the amplitude and duration of the gradient pulses, respectively, and \( D_n \) is the tracer diffusion coefficient of the \( n^{th} \) species. Here \( A(v) \) is the 1D NMR spectrum of the \( n^{th} \) diffusing species where \( g = 0 \). The inverse Laplace transformation of the data results in a conventional spectrum. The PFG-NMR experiments performed in this dissertation were carried out using the bipolar encode-decode pulse sequence shown in Figure 1.14. In order to minimize the effect of eddy currents during the NMR data acquisition, the gradient pulse (G) is varied while, the delay between the dipolar pulse pair, \( \tau \), gradient pulse duration, \( \delta \), diffusion time, \( \Delta \), and eddy current delay time, \( T_e \), are held constant.
FOCUS OF THIS WORK

The focus of this dissertation is to study chiral recognition using polymeric and monomeric amino acid based surfactants. In Chapter 2, the chiral separation of analytes in different charge states using monomeric and polymeric surfactants are presented. The results of that study encouraged me to examine the depth of the penetration of analytes into the micellar core of these two kinds of surfactants. The depth of penetration of binaphthyl derivatives into the micellar core of polymeric and monomeric SULL is discussed in part II of Chapter 2. The differences in chiral separation of binaphthyl derivatives lead to further investigation of chiral recognition of this class of analytes using steady state fluorescence anisotropy techniques. In Chapter 3 of this dissertation, EKC and steady state fluorescence anisotropy are
utilized to examine effect of temperature on chiral recognition of binaphthyl derivatives.

Several factors including aggregation number, hydrophobicity, and effective charge of the micelle are involved in chromatographic performance of micelles and micelle polymers. These parameters for dipeptide surfactants are presented in Chapter 4. In addition, chiral separations in EKC depend upon the strength of the solubilization of the analyte into the micellar core.\textsuperscript{143-144} The solubilization capacity of achiral and chiral organic molecules in the micellar core of the polymers and monomers are discussed in Chapter 5.

Results from previous studies have shown that dipeptide surfactants with two chiral centers provide better chiral selectivity for three out of four analytes, as compared to single amino acid surfactants with one chiral center.\textsuperscript{118} Therefore, in Chapter 6, the chiral recognition ability of the polymeric dipeptide surfactant, sodium undecanoyl isoleucyl-valinate, with three chiral centers is compared with the dipeptide surfactant, sodium undecanoyl leucyl-valinate, with two chiral centers. In addition, knowing that steric factors play a major role in chiral recognition,\textsuperscript{120,145-147} the effect of steric factors around the chiral centers of these two surfactants is examined. In the second part of Chapter 6, the chiral separation of several neutral chiral analytes with eighteen single amino acid and dipeptide polymeric surfactants is discussed. Lastly, Chapter 7 ties together all the findings in this dissertation and suggests future aspects of this research on polymeric surfactants.
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Chapter 2
Monomeric and Polymeric Surfactants in Electrokinetic Chromatography

Part I. Comparison of Monomeric and Polymeric Amino Acid Based Surfactants in Chiral Separation of Analytes in Different Charge States

Although several studies have been published investigating the potential of polymeric surfactants in chiral recognition,\textsuperscript{1-8} not much work has been done comparing the performance of monomeric and polymeric surfactants. Wang and Warner demonstrated some advantages of polymeric chiral surfactants as compared to monomeric chiral surfactants in 1994.\textsuperscript{2} In that study, the authors discussed the enantioselectivity of sodium N-undecanoyl L-valinate (SUV). It was shown that polymeric surfactants of SUV separated the enantiomers of 1,1'-binaphthyl-2,2'-diol better than its corresponding monomer. Billiot et al.\textsuperscript{3} have shown that monomeric sodium N-undecanoyl (L,L) valyl-leucinate (L,L-SUVL) resolved the enantiomers of 1,1'-bi-2-naphthyl-2,2'-diyl hydrogen phosphate (BNP), while the polymer of this surfactant exhibited no enantioselectivity towards BNP. In contrast, if the amino acid order of the dipeptide surfactant is reversed (i.e. sodium N-undecanoyl (L,L) leucyl-valinate), a different behavior is observed. The polymeric form of sodium N-undecanoyl (L,L) leucyl-valinate separated the enantiomers of BNP better than its corresponding monomer.\textsuperscript{3} From the above mentioned studies, it is obvious that more information is needed to better understand the differences in behavior of monomeric surfactants versus polymeric surfactants in enantiomeric separations using electrokinetic chromatography (EKC).
The main purpose of the work presented in this chapter is to compare the performance of a variety of monomeric and polymeric amino acid based surfactants in chiral separations using EKC. The use of polymeric chiral surfactants offers a number of advantages in terms of enantiomeric separations. In general, solutes do not penetrate as deeply into the hydrophobic core of a micelle polymer due to the covalently linked hydrophobic tail. This in turn leads to a possible faster mass transfer and thus an increase in the separation efficiency of the polymer as compared to the monomer micelle.

In order to compare the performance of polymeric and monomeric amino acid based chiral surfactants, a series of three single amino acid and fifteen dipeptide surfactants were synthesized. The single amino acid surfactants under study are sodium N-undecanoyl L-alaninate (SUA), sodium N-undecanoyl L-valinate (SUV), and sodium N-undecanoyl L-leucinate (SUL). The dipeptide surfactants synthesized for this purpose are all possible dipeptide combinations of glycine, L-alanine, L-valine, and L-leucine. The six single chiral center, dipeptide surfactants examined in this study are sodium N-undecanoyl (L,L) glycyl-alaninate (SUGA), sodium N-undecanoyl L-alanyl-glycinate (SUAG), sodium N-undecanoyl L-glycyl-valinate (SUGV), sodium N-undecanoyl L-valyl-glycinate (SUVG), sodium N-undecanoyl L-glycyl-leucinate (SUGL), and sodium N-undecanoyl L-leucyl-glycinate (SULG). In addition, the nine two chiral center dipeptide surfactants used in this study are sodium N-undecanoyl (L,L) alanyl-alaninate (SUAA), sodium N-undecanoyl (L,L) alanyl-valinate (SUAV), sodium N-undecanoyl (L,L) alanyl-leucinate (SUAL), sodium N-undecanoyl (L,L)
valyl-alaninate (SUVA), sodium N-undecanoyl (L,L) valyl-valinate (SUVV), sodium N-undecanoyl (L,L) valyl-leucinate (SUVL), sodium N-undecanoyl (L,L) leucyl-alaninate (SULA), sodium N-undecanoyl (L,L) leucyl-valinate (SULV), and sodium N-undecanoyl (L,L) leucyl-leucinate (SULL). The structures of these surfactants are illustrated in Figure 2.1.

The chiral separations of analytes in different charge states were compared using polymeric and monomeric forms of the aforementioned surfactants. In order to investigate the effect of the double bond at the end of the hydrophobic tail of the monomeric surfactants in chiral separation, methyl (single bond) terminated monomeric surfactants of SUVL and SULL were synthesized. To this end, the enantiomers of BNP were separated using polymeric, single bond, and double bond terminated of SUVL and SULL surfactants.

**EXPERIMENTAL**

**Chemicals.** Single amino acids and dipeptides were obtained from Sigma (St. Louis, MO). The racemates of (±)-1,1'-binaphthyl-2,2'-diamine (BNA), (±)-1,1'-bi-2-naphthol (BOH), (±)-1,1'-binaphthyl-2,2'-dihydrogen phosphate (BNP), lorazepam (LR), temazepam (TM), and propranolol (Prop), were also purchased from Sigma. N-hydroxysuccinimide, undecylenic acid, sodium bicarbonate, and dicyclohexylcarbodiimide (DCC) were all reagent grades and they obtained from Aldrich (Milwaukee, WI). HPLC grade ethyl acetate was also obtained from Aldrich.

**Synthesis of Polymeric Chiral Amino Acid Surfactants.** Single amino acid and dipeptide surfactants were synthesized according to the procedure reported by
Polymerization

single amino acid
X=NH-CH(R1)-C-ONa

\[ O \]

dipeptide
X=NH-CH(R1)-C-NH-C(R2)-C-ONa

\[ O \]

<table>
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<tr>
<th>R1</th>
<th>R2</th>
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<td>H</td>
<td>CH₃</td>
<td>SUGA</td>
</tr>
<tr>
<td>H</td>
<td>CH(CH₃)₂</td>
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</tr>
<tr>
<td>H</td>
<td>CH₂CH(CH₃)₂</td>
<td>SUGL</td>
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<tr>
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</tr>
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<td>CH₂CH(CH₃)₂</td>
<td>CH₂CH(CH₃)₂</td>
<td>SULL</td>
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Figure 2.1 Simple schematic representation of the amino acid based surfactants used in this study and their abbreviations.
Lapidot et al. Scheme 2.1 shows the synthesis of N-hydroxysuccinimide ester from undecylenic acid. N-hydroxysuccinimide (62 mmol) was dissolved in dry ethyl acetate (280 mL). An equimolar amount of undecylenic acid and a 1 M solution of DCC in ethyl acetate (62 mL) were then added to the N-hydroxysuccinimide solution. The mixture was stirred overnight at room temperature under dry atmosphere. The white precipitate that is the by-product, dicyclohexylurea, was filtered. The solvent was evaporated to yield a yellowish oil. The resulting oil was recrystallized using hot isopropyl alcohol.

In order to prepare single amino acid or dipeptide surfactants, the desired amino acid or dipeptide was placed in an aqueous sodium bicarbonate solution (e.g. 18 mM amino acid, 18 mM sodium bicarbonate in 180 mL water). Equimolar amounts of N-hydroxysuccinimide ester (18 mM) was dissolved in 180 mL THF and this solution was then added to the amino acid solution.

\[
\begin{align*}
\text{undecylenic acid} & \quad + \quad \text{N-hydroxy succinimide ester} \\
\text{dry ethyl acetate} & \quad \rightarrow \quad \text{N-hydroxysuccinimide ester of undecylenic acid}
\end{align*}
\]

Scheme 2.1 Synthesis of N-hydroxysuccinimide ester of undecylenic acid.

The solution was stirred for at least 16 hrs at room temperature. The organic solvent was evaporated and the pH of the aqueous solution was adjusted to 7 using sodium bicarbonate.
bicarbonate. This solution was then filtered and acidified to pH 2 with 1 N HCl. The resulting white crystals were filtered and dried under vacuum. The sodium salts of the surfactants were prepared in water using equimolar amounts of sodium bicarbonate, and then freeze dried to yield white crystals. The synthesis of single amino acid surfactants is shown in Scheme 2.2.

![Scheme 2.2 Synthesis of amino acid based surfactants.](image)

Polymerization of the surfactants was achieved by preparing a 100 mM sodium salt solution of the surfactants in water and irradiating the sample for seven days with $^{60}$Co γ-radiation (680 rad/h). After radiation, the solution was lyophilized to yield a white powder. Polymerization was confirmed by the disappearance of the double bond signal at about 5 ppm (the chemical shift of the vinyl proton).

**Capillary Electrophoresis Procedure.** Electrokinetic chromatography separations were performed on a Hewlett Packard (HP) 3D CE model #G1600AX. The fused silica capillary, effective length of 55 cm (to detection window), 50 µm i.d., with a total length of 63.5 cm, was purchased from Polymicro Technologies (Phoenix, AZ)
and mounted in an HP capillary cartridge. The cartridge temperature was maintained at 25 °C for the separation of BNP and BOH and 12 °C for LR, TM, and Prop separations. The running background electrolytes (BGEs) were prepared in triply distilled water, surfactants were added and the pH was adjusted by adding either HCl or NaOH to the BGE. All solutions were filtered through a 0.45 μm membrane filter before use.

A new capillary was conditioned for 30 min with 1 N NaOH at 60 °C, followed by 10 min with triply distilled water. The capillary was then flushed with buffer for 2 min prior to injecting the sample. All analyte standard solutions were prepared in 1:1 methanol:water at 0.1-0.5 mg/mL. Samples were injected for 5 seconds at 10 mbar pressure. Separations were performed at +30 kV, with UV detection at 220 nm.

**Background Electrolyte Conditions.** The EKC conditions for optimum enantiomeric resolution using amino acid based surfactants are as follows: (1) LR and TM: 25 mM TRIS, 25 mM sodium borate, pH 9.2 at 12 °C; (2) BNP, BNA, and BOH: 10 mM sodium borate, 100 mM TRIS, pH 10.0 at 25 °C; (3) Prop: 50 mM sodium borate, 300 mM CAPS, pH 8.5 at 12 °C.

**RESULTS AND DISCUSSION**

Chiral recognition with amino acid based surfactants can largely be attributed to electrostatic, hydrophobic, and steric interactions, as well as hydrogen bonding. Hydrophobic forces dictate the depth of penetration of the analyte into the micellar core. This in turn plays a major role in chiral recognition of charged, as well as neutral enantiomers. Positively charged analytes interact preferentially with negatively
charged surfactants at the surface of the micelle due to electrostatic interactions, while hydrophobic neutral analytes penetrate deeper into the micellar core. Due to electrostatic repulsion, most anionic chiral analytes cannot be enantiomerically resolved with the negatively charged amino acid based surfactants discussed in this dissertation. However, enantiomeric recognition of negatively charged analytes can be achieved with anionic surfactants if the enantiomers are highly hydrophobic (e.g. BNP).

In addition to the differences discussed above, joule heating of unpolymerized micelles can be problematic. For example, I have observed that at equivalent monomer concentrations (EMC), monomeric surfactants produce more current than their corresponding polymers. The polymeric chiral pseudostationary phases (CPSPs) examined in this study always provided lower currents with higher theoretical plate numbers compared to monomeric CPSP. Furthermore, I have observed that at higher surfactant concentrations, normal micelles produce bubbles inside the capillary, resulting in spikes and an unstable baseline during the electrokinetic run. This problem was not observed with the polymers.

To evaluate the chromatographic performance of monomeric and polymeric surfactants in terms of chiral recognition, enantiomeric separations of five test analytes were performed at two different concentrations: 1) the optimum polymer concentrations, and 2) the concentration at which the monomer (unpolymerized micelle) provided optimum selectivity. It should be mentioned that for each analyte, the optimum concentration for all monomers is the same, as well as all polymers. In other words, the optimum concentration is analyte dependent not surfactant dependent.
**Enantioseparation of Neutral Analytes.** In this section, the enantioselectivity of three neutral analytes (LR, TM, and BOH) are examined. Prior to comparison of the various surfactants, optimum monomer and polymer concentrations were determined. Optimum enantiomeric resolution of LR, TM, and BOH for the various polymers was achieved at 12, 20, and 6 mM EMC, respectively. In contrast, the optimum concentrations for the monomers were 45 mM for LR and TM and 50 mM for BOH. The optimum monomer concentration was more than twice the concentration of the corresponding polymers for TM and LR and around eight times greater for BOH. It should be mentioned that the CMC of the single amino acid and dipeptide surfactants were determined to be about 20 and 7 mM, respectively. Although 12 and 20 mM are above the CMC of the dipeptide surfactants, the enantiomers of LR and TM coeluted with the electroosmotic flow (EOF) at this concentration of monomers. In fact, for dipeptide monomeric surfactants at 20 mM, only 65% of surfactants are in the micellar state, while the polymeric surfactants examined in this study are in “micellar” form at any concentration.

Presumably, the diastereomeric complexes formed between the enantiomer and monomeric CPSP are less stable compared to that of the polymeric phase. The success of chiral recognition depends, in part, on the strength of the chiral interaction of the enantiomers with the CPSP. Covalent linkage among the hydrophobic tail of the surfactants results in a more organized phase with greater steric constraints than the unpolymerized phases. This greater structural rigidity of the former may result in enhanced enantioselectivity for neutral analytes.
As shown in Figure 2.2, the polymeric surfactants always provide better chiral separation for LR as compared to the monomeric form of the same surfactant. For example, the polymers of SULV, SUAG and SUVA separated the enantiomers of LR with resolution values of 3.11, 1.83, and 2.94, respectively. However, no chiral recognition of these enantiomers was achieved even when the concentrations of the corresponding monomers were increased to as high as 45 mM. Examination of the data for the single amino acid and dipeptide surfactants investigated in this study, indicate that only six monomers were able to show any chiral recognition for LR (i.e. SUL,
SUAA, SUAL, SUVG, SUVL and SULG). In addition, selectivity data shown in Table 2.1, indicates that the polymeric surfactants always provide better enantioselectivity for enantiomers of LR.

Figure 2.3 shows the chromatographic data for enantiomers of TM. Note the structural differences of LR and TM. The main difference is the methyl group located on the nitrogen in the seven member ring of TM and the chlorine in the ortho position of the lower benzene ring of LR. Examination of the data for the single amino acid surfactants reveals that the polymers of SUV and SUL were able to separate the enantiomers of TM with resolution values of 2.32, and 2.68, respectively, while no chiral recognition was obtained using the monomeric form of the same surfactants. In contrast, mono SUA separated the enantiomers of TM (Rs of 0.36), while no enantiomeric resolution of TM was observed using poly SUA. When comparing single chiral center dipeptide surfactants with the chiral center at the N-terminal position, i.e. SUAG, SUVG, and SULG, monomeric surfactants provided either the same or better chiral selectivity for TM compared to the polymers (Table 2.1). However, as shown in Figure 2.3, the resolution values achieved with the aforementioned polymers are always better than the corresponding monomers. This is due to the better efficiency of the polymers as compared to the monomers. Whereas, the better chiral selectivity of these three monomeric single chiral center monomers might be related to their “loose” structure. The looser configuration of the monomer could allow rearrangement of the polar head group enabling the chiral center of TM to interact stronger with the inside amino acid of the monomeric surfactants as compared to the polymers.
Figure 2.3  Chiral separation of TM enantiomers.

Table 2.1  Chiral selectivity of neutral compounds.

<table>
<thead>
<tr>
<th></th>
<th>LR</th>
<th>TM</th>
<th>BOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poly</td>
<td>mono</td>
<td>poly</td>
</tr>
<tr>
<td>SUA</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SUV</td>
<td>1.009</td>
<td>1</td>
<td>1.021</td>
</tr>
<tr>
<td>SUL</td>
<td>1.014</td>
<td>1.012</td>
<td>1.031</td>
</tr>
<tr>
<td>SUGA</td>
<td>1.005</td>
<td>1</td>
<td>1.031</td>
</tr>
<tr>
<td>SUGV</td>
<td>1.006</td>
<td>1</td>
<td>1.051</td>
</tr>
<tr>
<td>SUGL</td>
<td>1.006</td>
<td>1</td>
<td>1.054</td>
</tr>
<tr>
<td>SUAG</td>
<td>1.023</td>
<td>1</td>
<td>1.019</td>
</tr>
<tr>
<td>SUAA</td>
<td>1.022</td>
<td>1.011</td>
<td>1</td>
</tr>
<tr>
<td>SUAV</td>
<td>1.100</td>
<td>1</td>
<td>1.014</td>
</tr>
<tr>
<td>SUAL</td>
<td>1.029</td>
<td>1.013</td>
<td>1.045</td>
</tr>
<tr>
<td>SUVG</td>
<td>1.039</td>
<td>1.009</td>
<td>1</td>
</tr>
<tr>
<td>SUVA</td>
<td>1.027</td>
<td>1</td>
<td>1.010</td>
</tr>
<tr>
<td>SUVV</td>
<td>1.012</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SUVL</td>
<td>1.025</td>
<td>1.007</td>
<td>1.030</td>
</tr>
<tr>
<td>SULG</td>
<td>1.021</td>
<td>1.010</td>
<td>1.015</td>
</tr>
<tr>
<td>SULA</td>
<td>1.019</td>
<td>1</td>
<td>1.033</td>
</tr>
<tr>
<td>SULV</td>
<td>1.028</td>
<td>1</td>
<td>1.043</td>
</tr>
<tr>
<td>SULL</td>
<td>1.016</td>
<td>1</td>
<td>1.053</td>
</tr>
</tbody>
</table>
Similar to the single amino acid surfactant SUA, some enantioselectivity of TM was observed with the monomeric dipeptide surfactant SUAA, while no chiral selectivity was achieved using poly SUAA. One possible explanation could be the differences in aggregation number of the polymers and the monomers. The aggregation number of monomeric SUAA, determined via steady state fluorescence quenching technique, is more than 350, while the number of repeat units of the polymeric form is about 23 (Chapter 4). Therefore, mono SUAA has a non-spherical structure in solution, while poly SUAA is probably spherical. The non-spherical structure of mono SUAA may provide a better chiral interaction of TM enantiomers with monomeric surfactants as compared to the polymer.

The best enantioseparation for the optical isomers of TM was attained using poly SUAL (Rs of 3.50) with two chiral centers. Except for the surfactants that were discussed earlier, polymeric surfactants always provided better chiral resolution for enantiomers of TM as compared to their monomeric counterparts. The three monomeric surfactants that provided better chiral resolution toward the enantiomers of TM compared to their corresponding polymers were SUA, SUAA, SUVG, and SULG.

The third neutral analyte examined in this study was BOH. The difference between this analyte and LR and TM is that BOH possesses a chiral plane, while the other two analytes contain chiral centers. In addition, BOH is very hydrophobic and the optimum polymeric concentration (ca. 6 mM EMC) for this analyte is significantly lower than for LR and TM (12 and 20 mM EMC, respectively). As illustrated in Figure 2.4, poly SUGA and poly SUAV provided Rs values of 1.32 and 1.36 for the
enantiomers of BOH, respectively, while no chiral recognition was observed using the corresponding monomers. Monomeric surfactants of SUGV and SUGL provided $R_s$ (and $\alpha$) values of 1.71 ($\alpha$ of 1.012) and 0.53 ($\alpha$ of 1.009), respectively, while poly SUGV had a resolution (and $\alpha$) value was 0.77 ($\alpha$ of 1.004), and poly SUGL was not able to enantiomerically resolve BOH. It has been proposed that the enantiomers of BOH preferentially interact with the N-terminal amino acid of polymeric dipeptide surfactants.\textsuperscript{8} It is believed that the looser configuration of the monomers allow the enantiomers of this analyte to interact stronger with the C-terminal amino acids of the monomeric surfactants as compared to the polymers.

A comparison of the selectivity factors for the neutral analytes reported in Table 2.1, indicates that polymers generally provide better enantioselectivity than the corresponding unpolymerized form. From the chromatographic data presented here, it
is clear that polymeric surfactants are a better CPSP for the enantiomeric separation of the neutral compounds examined in this study than the corresponding monomers.

**Enantioseparation of Charged Analytes.** In an effort to compare the chromatographic performance of monomeric and polymeric surfactants for the enantiomeric separation of charged analytes, Prop (positively charged) and BNP (negatively charged) were examined. The optimum enantioseparation of Prop using polymeric surfactants was achieved at 18 mM EMC. However, at this concentration, no chiral separation was observed using monomeric surfactants. Much higher concentrations of monomeric surfactants were needed to achieve optimum separation (i.e. 50 mM). The optimum concentration of both monomeric and polymeric forms of the surfactants for the enantiomeric separation of BNP was determined to be 30 mM.

Comparisons of the enantioresolution of Prop for various surfactants are illustrated in Figure 2.5. Again, in most cases, the Rs values of Prop obtained with polymeric surfactants were higher than those achieved with the corresponding unpolymerized ones. However, when the chiral center of the single chiral center dipeptide surfactant is located at the N-terminal amino acid (e.g. SUVG and SULG) the monomer performed better than the polymer. As can be seen in Figure 2.5, no chiral resolution was observed with the polymer of SUVG, and poly SULG provided a Rs value of 0.3, while the monomers separated the enantiomers of Prop with Rs values of 0.31, and 0.70, respectively. This apparent anomaly is probably due to differences in depth of penetration of Prop into the hydrophobic core of the micelle, as compared to the polymer.
Figure 2.5 Chiral separation of Prop enantiomers

Electrostatic interactions between the positively charged Prop and the negatively charged dipeptide surfactants are likely to be the primary factor in binding of this class of compound to the polar head of the micelle. Thus, chiral selectivity is assumed to be dependent primarily on the C-terminal amino acid. However, steric interactions of the benzene ring of this positively charged analyte with the N-terminal amino acid’s R-group of the dipeptide surfactants need to be considered as well.

Examination of the selectivity factors of Prop enantiomers shown in Table 2.2 indicates that monomers always provide better or approximately the same chiral selectivity for enantiomers of this positively charged analyte. Again, this is possibly
due to the fact that the looser configuration of the monomers allows a better chiral interaction of the Prop enantiomers as compared to the polymers. As pointed out earlier, higher resolution values are usually obtained with polymers due to the increase in efficiency of the polymeric over the monomeric micelles. In addition, lower current and lower concentrations of the polymeric surfactants are encouraging to develop polymeric phases for separation of the cationic enantiomers.

Chromatographic data for the enantiomeric separation of BNP are reported in Figure 2.6. In contrast to the other analytes examined in this study, the optimum monomeric and polymeric concentrations for chiral selectivity of BNP are similar (i.e. 30 mM). As mentioned earlier, so far BNP is the only negatively charged analyte that has been enantiomerically separated in our laboratory using anionic amino acid based surfactants. This is most likely due to the fact that this analyte is an atropisomer and also possesses a very hydrophobic moiety which can penetrate into the micellar core and compete with charge repulsion.

As shown in Table 2.2, both the monomeric and the polymeric forms of ten surfactants (SUL, SUGV, SUGL, SUAL, SUVG, SUVA, SULG, SULA, SULV, and SULL), were able to separate the enantiomers of BNP. Out of these ten surfactants, half of the monomers provided either better or approximately the same chiral recognition for the enantiomers of BNP. In addition, monomers of SUV, SUAG and SUVL separated the enantiomers of BNP with Rs values (and \( \alpha \)) of 0.64 (\( \alpha \) of 1.011), 0.72 (\( \alpha \) of 1.013), and 4.46 (\( \alpha \) of 1.037), respectively, while the corresponding polymers did not show any chiral selectivity toward the enantiomers of BNP. It is
Table 2.2 Chiral selectivity of the charged compounds.

<table>
<thead>
<tr>
<th></th>
<th>Prop</th>
<th>BNP</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>poly</td>
<td>mono</td>
<td>Poly</td>
<td>mono</td>
</tr>
<tr>
<td>SUA</td>
<td>1.005</td>
<td>1.032</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SUV</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.011</td>
</tr>
<tr>
<td>SUL</td>
<td>1.013</td>
<td>1.014</td>
<td>1.019</td>
<td>1.009</td>
</tr>
<tr>
<td>SUGA</td>
<td>1.010</td>
<td>1.011</td>
<td>1.016</td>
<td>1</td>
</tr>
<tr>
<td>SUGV</td>
<td>1.013</td>
<td>1.017</td>
<td>1.027</td>
<td>1.024</td>
</tr>
<tr>
<td>SUGL</td>
<td>1.022</td>
<td>1.023</td>
<td>1.047</td>
<td>1.049</td>
</tr>
<tr>
<td>SUAG</td>
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<td>1</td>
<td>1.013</td>
</tr>
<tr>
<td>SUAA</td>
<td>1.012</td>
<td>1.022</td>
<td>1.008</td>
<td>1</td>
</tr>
<tr>
<td>SUAV</td>
<td>1.014</td>
<td>1.029</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SUAL</td>
<td>1.024</td>
<td>1.025</td>
<td>1.009</td>
<td>1.008</td>
</tr>
<tr>
<td>SUVG</td>
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<td>1</td>
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<td>1.026</td>
</tr>
<tr>
<td>SUVA</td>
<td>1.015</td>
<td>1.024</td>
<td>1.050</td>
<td>1.036</td>
</tr>
<tr>
<td>SUVV</td>
<td>1.019</td>
<td>1.026</td>
<td>1.020</td>
<td>1</td>
</tr>
<tr>
<td>SUVL</td>
<td>1.033</td>
<td>1.038</td>
<td>1</td>
<td>1.037</td>
</tr>
<tr>
<td>SULG</td>
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<td>1.015</td>
<td>1.096</td>
<td>1.097</td>
</tr>
<tr>
<td>SULA</td>
<td>1.011</td>
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<td>1.073</td>
<td>1.102</td>
</tr>
<tr>
<td>SULV</td>
<td>1.011</td>
<td>1.012</td>
<td>1.066</td>
<td>1.036</td>
</tr>
<tr>
<td>SULL</td>
<td>1.018</td>
<td>1.044</td>
<td>1.059</td>
<td>1.042</td>
</tr>
</tbody>
</table>

worth noting that the polymers of SUAA, SUVV, and SUGA separated the enantiomers of BNP with Rs values (and $\alpha$) of 1.3 ($\alpha$ of 1.008), 1.2.11 ($\alpha$ of 1.02), and 0.59 ($\alpha$ of 1.016), respectively, whereas no selectivity was achieved with the monomers of these surfactants. In general, it can be concluded that monomeric surfactants are a better CPSP for BNP as compared to the polymers.

Effect of Double Bond of the Monomeric Surfactants on Enantioselectivity. As discussed earlier, in some cases, monomeric surfactants provide better chiral separation for enantiomers of BNP. For example, in contrast to poly SUVL that provides no enantiomeric resolution of BNP, mono SUVL
separated the enantiomers of BNP with a resolution value of 2.6 (Figure 2.6). It is well known that incorporation of a terminal double bond (terminal methylene) has an effect on the micellization of the surfactants.\textsuperscript{10} The double bond surfactant is more polar than its saturated counterpart. To investigate the effect of double bond in chiral separation, the single bond terminated surfactant of SUVL was synthesized. The critical micelle concentration of the single bond surfactant was determined to be about 3 mM compared to the double bond terminated surfactant (about 7 mM).

A comparison of the enantioseparation of BNP using the single bond, double bond and polymer of SUVL is shown in Figure 2.7a-c. Single bond and double bond SUVL resolved the enantiomers of BNP with resolutions of 2.6 and 4.5, respectively; while no separation with poly SUVL was observed.
The difference in resolution of single bond terminated with double bond terminated SUVL is possibly due to the packing of the polar head in these surfactants. In poly SUVL, the bulky amino acid leucine is located in the C-terminal position. In order for BNP to interact with both chiral centers of this surfactant, a loose configuration for surfactants at the polar head is required. Single bond terminated SUVL possibly has a more packed polar head. The packing of the single bond terminated SUVL may not allow the proper interaction of BNP with the chiral centers of this surfactant.

![Graphs](image)

**Figure 2.7** Chiral separation of BNP using (a) polymeric, (b) single bond terminated monomeric, and (c) double bond terminated monomeric SULV surfactant.

To further investigate the effect of the double bond in chiral separation of BNP enantiomers, single bond terminated SULL was synthesized. Figure 2.8a-c shows the chiral separation of BNP enantiomers with polymeric, double bond and single bond
terminated monomeric surfactants of SULL. Polymeric SULL provided a resolution of 5.8, which is slightly better than single bond and double bond terminated SULL.

![Graph showing chiral separation of BNP](image)

Figure 2.8 Chiral separation of BNP using (a) polymeric, (b) single bond terminated monomeric, and (c) double bond terminated monomeric SULL surfactant.

Both single bond and double bond terminated SULL provided a resolution value of 5.6 for the enantiomers of BNP. From the chromatographic results shown in Figure 2.8, it can be concluded that the presence of the double bond does not significantly affect the chiral separation of BNP enantiomers.

Conclusions. The chromatographic data presented in this section suggest that polymeric surfactants are better chiral selectors for enantiomers of neutral as well as...
cationic compounds. However, better chiral separation of the negatively charged enantiomers of BNP was obtained using monomeric surfactants.

In the next section, one of the factors important in chiral recognition of monomeric and polymeric surfactants, depth of the penetration of the analyte into the micellar core of these two kinds of surfactants, are examined. In the following chapters, I will discuss a variety of techniques to further investigate the interaction of enantiomers with amino acid based surfactants. Some of the techniques include fluorescence and NMR spectroscopy.

**Part II. Investigation of the Depth of Penetration of Binaphthyl Derivatives into the Micellar Core of Monomeric and Polymeric Sodium Undecanoyl Leucyl-Leucinate**

In part I, the performance of chiral monomeric and polymeric amino acid based surfactants in EKC was compared. The chromatographic results indicated that the chiral recognition ability of monomeric and polymeric surfactants is different. These differences are due to the fact that the physical properties of these two classes of surfactants are different. In addition to the physical properties, the depth of penetration of the analyte into the micellar core plays a major role in chiral recognition.

Some of the factors that dictate the depth of penetration of the chiral analyte into the micellar core are the hydrophobicity and the effective charge of the analyte, as well as the micelle. Hydrophobic neutral enantiomers penetrate relatively deeply into the micellar core. Due to the electrostatic interactions, enantiomers with opposite charge than the surfactant interact preferentially at the surface of the micelle.
It should be mentioned that the hydrophobicity of the analyte, the surfactant core, as well as the hydrophobicity of the running buffer plays a major role in analyte-selector interaction.\textsuperscript{11} The hydrophobicity of the running buffer in EKC can easily be varied by either changing the separation temperature or addition of organic modifiers. In addition to their influence on hydrophobicity, temperature and organic modifiers significantly affect kinetic, thermodynamic, and electromigration processes in EKC separations.\textsuperscript{13}

In EKC, chiral separation is achieved because of the differences in mobility of two enantiomers in the running buffer. These differences are due to the formation of transient diastereomeric complexes between the CPSP and chiral analytes. In order for chiral separations to be achieved, the energy of the formation of the diastereomeric complexes must be different for the two enantiomers. Temperature plays a significant role in the formation and stability of these complexes.\textsuperscript{15} Temperature may shift the pK\textsubscript{a} of the CPSP, as well as the enantiomers. This in turn alters the electrostatic interactions. Temperature may also change the structure of the selector and/or the analyte. Change in spatial shape of the complexes may vary the electrophoretic mobilities and chiral interactions. For instance, proteins, which have been used extensively as CPSPs, undergo structural changes in different temperatures.\textsuperscript{15} Furthermore, from the electrokinetic stand point, the viscosity and electroosmotic flow are temperature dependent. In this chapter, I will focus on a different aspect not commonly examined with respect to temperature, depth of penetration of the analyte into the hydrophobic core of the polymeric CPSP.
Similar to temperature, organic modifiers can alter the interaction of chiral analytes with the CPSP. Organic modifiers alter the electrostatic, hydrophobic and hydrogen bonding interactions of the enantiomers with the CPSP. Furthermore, organic modifiers can increase the solubility of hydrophobic enantiomers in the buffer solution. Thus, it increases the mass transfer between the analyte and the surfactant, which results in an increase in separation efficiency. In addition, the EOF decreases linearly by increasing the organic modifier concentrations. Consequently, neutral enantiomers will have more time to interact with the chiral selector. This may result in improvement in chiral separation. In conventional micelles, the concentration of the organic modifier would have to be limited in order to maintain the form of the micelle. However, the use of polymeric micelles provides an opportunity to investigate the role of organic solvents over a wide range of concentrations.

In part I of this chapter, the chromatographic performances of polymeric and monomeric surfactants were studied. That study concluded that the rigidity of the polymeric surfactants may limit penetration of some analytes into the micellar core of the polymeric surfactants as compared to that of the monomers. However, some analytes may penetrate deeper into the core of the polymers as compared to the monomers. In this section, I investigate the role of the depth of penetration of three binaphthyl derivatives into the micellar core of polymeric and monomeric SULL surfactants. In addition, the effect of temperature and organic modifier on the depth of the penetration of these analytes into the micellar core of the polymeric surfactants is examined.
RESULTS AND DISCUSSION

In this study, differences in the chiral selectivity of monomeric and polymeric surfactants were observed. These differences are probably due to the variation in their hydrophobicity and rigidity. The hydrophobic forces dictate the depth of penetration of the chiral analyte into the micellar core\textsuperscript{16} and the rigidity affects the depth of penetration, as well as having an effect on steric interactions. In order to compare the depth of penetration of the chiral analytes into the micellar core of polymeric and monomeric surfactants, enantiomers of BNA, BOH, and BNP were separated using diastereomeric surfactants (in L,L and L,D configuration) of SULL. In L,L-SULL surfactants, both amino acids have L-configurations, while in L,D-SULL, the C-terminal amino acid has an L-configuration and the N-terminal amino acid has a D-configuration.

Enantiomeric Separation of Binaphthyl Derivatives. Figure 2.9 shows the chiral separation of BNA using various SULL surfactants. Both, poly and mono L,L-SULL separated the enantiomers of this analyte with a resolution value of around 1.8 (Figure 2.9 a-b). However, as shown in Figure 2.9 c-d, mono L,D-SULL provided a resolution value of 1.4 while poly L,D-SULL resolved the enantiomers of BNA with a resolution value of only 1.0. Similar results were obtained when the separation of the enantiomers of BOH was examined with SULL surfactants. As can be noted from the electropherograms shown in Figure 2.10a-b, both poly and mono L,L-SULL resolved the enantiomers of BOH equally well. In addition, mono L,D-SULL provided a better resolution for the BOH enantiomers than poly L,D-SULL. This might be due to the
fact that enantiomers of these analytes (BNA and BOH) penetrate deeper into the micellar core of the monomer as compared to the polymeric form of SULL. Therefore, BNA and BOH enantiomers interact stronger with the C-terminal amino acid of poly L,D-SULL as compared to mono L,D-SULL. Since the C-terminal amino acid of L,D-SULL has an opposite configuration than the N-terminal amino acid, chiral resolution of these enantiomers with mono L,D-SULL is higher than poly L,D-SULL. In the electropherograms shown in Figures 2.9 and 2.10,

![Electropherograms](image)

**Figure 2.9** Chiral separation of BNA enantiomers using (a) poly L,L-SULL, (b) mono L,L-SULL, (c) poly L,D-SULL, and (d) mono L,D-SULL.

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Figure 2.10. Chiral separation of BOH enantiomers using (a) poly L,L-SULL, (b) mono L,L-SULL, (c) poly L,D-SULL, and (d) mono L,D-SULL.

The S-enantiomer, which is at half the concentration of the R-enantiomer, always eluted first. From this elution order, it is reasonable to assume that the R-enantiomer interacts stronger with both polymeric and monomeric SULL, than the S-enantiomer.

The enantiomeric separation of BNP with monomeric and polymeric SULL is illustrated in Figure 2.11. Poly L,L-SULL separated the enantiomers of BNP with a
resolution value of 5.8 (Figure 2.11a) which is slightly better than mono L,L-SULL (Rs of 5.5), Figure 2.11b. According to the chromatographic data shown in Figure 2.11, the S-enantiomer of BNP interacts stronger with both polymeric and monomeric L,L-SULL, than the R-enantiomer. The separation of BNP with L,D-SULL is shown in Figure 2.11c-d. As illustrated in Figure 2.11c, polymeric L,D-SULL does not separate the enantiomers of BNP. This is probably due to the fact that BNP interacts similarly with both chiral centers of poly L,D-SULL (which are of opposite chiral selectivity). Interestingly, monomeric L,D-SULL separates the enantiomers of this analyte with a resolution value of 2.3. Note that, in contrast to L,L-SULL, R-BNP interacts stronger with mono L,D-SULL. Under the conditions used for this study (pH 10), BNP enantiomers are negatively charged. Therefore, this analyte is more soluble in the buffer than BNA and BOH. However, the presence of the four fused benzene rings on BNP makes this analyte rigid and hydrophobic as well. These competing factors, hydrophobicity of the aromatic groups and hydrophilicity of the anionic phosphate group, dictate the site of chiral interaction of BNP with the surfactant.

Since no enantiomeric separation of BNP was observed with poly L,D-SULL and a reversal of enantiomeric order was observed with mono L,D-SULL as compared to mono and poly L,L-SULL, it is reasonable to assume that the preferential site of interaction of BNP with mono L,D-SULL is closer to the bulk aqueous phase as compared to poly L,D-SULL. In other words, BNP interacts preferentially with the C-terminal amino acid of monomeric surfactants and approximately the same with the C- and N-terminal amino acids of poly SULL. My hypothesis is consistent with the elution
order of the enantiomers of BNP observed with the monomer and polymer of this surfactant. The reversal of enantiomeric order of BNP with mono L,D-SULL as compared to mono and poly L,L-SULL indicates that the R-enantiomer of BNP interacts preferentially with the C-terminal amino acid (D-configuration) of mono L,D-SULL. In contrast to BNP, the enantiomers of BOH and BNA are highly hydrophobic. Therefore, these enantiomers penetrate deeper into the core of the monomeric CPSP as compared to BNP. Note that the elution order of R- and S-BOH and BNA with the polymers and the monomers of L,L-SULL (Figures 2.9a-b and

![Chiral separation of BNP enantiomers](image)

**Figure 2.11** Chiral separation of BNP enantiomers using (a) poly L,L-SULL, (b) mono L,L-SULL, (c) poly L,D-SULL, and (d) mono L,D-SULL.
2.10a-b) is the same as L,D-SULL (Figures 2.9c-d and 2.10c-d). This suggests that the enantiomers of BOH and BNA probably interact preferentially with the N-terminal amino acid of the dipeptide CPSP (both polymeric and monomeric form). Figure 2.12a-b indicates the proposed preferential site of interaction of these analytes with polymeric and monomeric SULL, respectively.

Although BNA and BOH enantiomers interact preferentially with the N-terminal amino acids, they also interact to some extent with the C-terminal amino acid. This is evident from an increase in chiral recognition of these enantiomers with the corresponding polymers and monomers of L,L-SULL as compared to L,D-SULL. The depth of penetration of the analyte into the core of the micelle is examined further in
next section by looking at the effect of temperature on the preferential site of the interaction.

**Effect of Temperature on Chiral Separation of Binaphthyl Derivatives.**

Enantioseparation of BNP in three different temperatures is shown in Figure 2.13, using poly L,D-SULL as the CPSP. At 12 °C, poly L,D-SULL provided a selectivity factor of 1.005 for enantiomers of this analyte. At intermediate temperature, i.e. 25 °C, no chiral recognition of the BNP was observed, while at higher temperature, i.e. 55 °C, an α value of 1.005 was again observed. Note, in the electropherograms shown in Figure 2.13a, S-BNP, which is at half the concentration of the R-BNP, elutes first, whereas at 55 °C (Figure 2.13c) the S-enantiomer elutes second. In other words, varying the temperature resulted in reversal of the elution order of the BNP enantiomers. At low temperature, BNP enantiomers interact preferentially with the N-terminal amino acid of poly L,D-SULL. This is due to the fact that BNP is less soluble in the bulk solution, therefore it penetrates deeper into the micellar core of the poly SULL surfactants. By increasing the temperature, this analyte becomes more soluble in the bulk solution. At intermediate temperature, BNP interacts similarly with both amino acids of poly L,D-SULL. At 55 °C, BNP enantiomers interact preferentially with the C-terminal amino acid. It should be mentioned that, when mono L,D-SULL was used as a CPSP, R-BNP always eluted first in different temperatures. Figure 2.14 shows the effect of temperature on the chiral separation of BNP using polymeric and monomeric L,L-SULL. The chiral separation of this analyte decreases by increasing the temperature.
The investigation of the chiral separation of BOH and BNA enantiomers at different temperatures indicates that the enantiomers of these analytes preferentially interact with the N-terminal amino acid of the polymeric SULL surfactants in all temperatures examined. As with temperature, the amount of organic modifier in the running buffer can also potentially affect the preferential site of interaction.

**Effect of Methanol on Chiral Separation of Binaphthyl Derivatives.** The chiral interaction of binaphthyl derivatives with SULL surfactants was further investigated by addition of methanol into the running electrolyte. The concentration of poly L,L-SULL was varied from 5 to 35 mM EMC and the concentration
Figure 2.14 Effect of temperature on chiral separation of BNP enantiomers using polymeric and monomeric L,L-SULL surfactant.

of methanol was varied from 0% to 30% (v/v). As mentioned earlier, addition of methanol results in decreased EOF. Above 30% methanol the analysis time significantly increased, therefore, concentrations higher than 30% were not examined.

Addition of methanol to the running electrolyte, resulted in an enhancement of the chiral selectivity for enantiomers of BNA (Figure 2.15). Examination of the $\alpha$ values at 5 mM EMC in various % methanol concentrations indicates that at 0% methanol, an $\alpha$ value of 1.076 was achieved while at 30%, this value increased to 1.152. At 0% methanol, increasing the concentration of surfactant from 5 mM to 35 mM EMC resulted in a decline in the selectivity factor. However, addition of methanol in each concentration improved chiral selectivity of the BNA enantiomers. The capacity factor of BNA enantiomers in different poly L,L-SULL and methanol concentrations is illustrated in Figure 2.16. As can be seen, addition of methanol to the
running buffer decreased the $k'$ values. For example, at 5 mM EMC and 0% methanol, a $k'$ of 0.92 was obtained. However, at this concentration of the surfactant (5 mM EMC), when the concentration of methanol increased to 30%, a $k'$ value of 0.43 was attained. In other words, addition of methanol increased the chiral selectivity and decreased the capacity factor.

The presence of methanol in the running buffer can have several effects on separation parameters. However, in this chapter, as with the effect of temperature, I will focus on one particular aspect not commonly examined with respect to organic modifier; effect of organic modifier on the preferential site of interaction of chiral analytes with polymeric CPSPs. Methanol increases the affinity of the BNA enantiomers for the bulk solution. Thus, as the concentration of methanol increases, the analyte moves closer to the bulk aqueous phase, thereby interacting with both chiral centers on the dipeptide. This change in preferential interaction site results in an increase in chiral selectivity.

To confirm this hypothesis, chiral separation of BNA enantiomers was examined using L,D-SULL and methanol. As the concentration of methanol increased, the resolution of BNA decreased rapidly in contrast to poly L,L-SULL where addition of methanol increased the resolution. The separation of BOH enantiomers was also investigated in different surfactant and methanol concentrations. Similar to BNA, the addition of methanol resulted in a decrease in the capacity factor for poly L,L- and L,D-SULL. However, while an increase in selectivity factor was obtained for L,L-SULL a decrease was observed for L,D-SULL.
Figure 2.15 Chiral selectivity of BNA

Figure 2.16 Effect of concentration of organic modifier and poly SULL on the capacity factor of BNA.
The effect of methanol concentration on the chiral separation of BNP was also examined. In contrast to BOH and BNA, the capacity factor of BNP increased by increasing the methanol concentration, and the selectivity and resolution values decreased. As mentioned earlier, under the conditions used for this study, pH of 10, BNP is anionic. The pKa of this analyte will increase by addition of methanol to the running buffer. Therefore, in the presence of methanol, the phosphate group of BNP is slightly protonated. As the phosphate group becomes more protonated, BNP becomes less soluble in the bulk phase and more soluble in the hydrophobic core of the CPSP. In addition, hydrogen bonding between the phosphate group and amide moieties of the poly SULL results in a stronger complexation of BNP with this surfactant. These factors result in increased k' values. However, the H-bonding of the phosphate group with SULL may not be enantioselective. Therefore, by addition of methanol, the chiral selectivity of BNP enantiomers decreases. In summary, the addition of methanol will assist the chiral separation of BOH and BNA, while that of BNP will be decreased.

Conclusions. The depth of penetration of the analyte into the micellar core of the dipeptide surfactants determines which chiral center(s) the analyte preferentially interacts with. Among the analytes investigated in this study, BOH and BNA preferentially interact with the N-terminal amino acid of the SULL surfactants (both monomeric and polymeric). In contrast, the enantiomers of BNP interact preferentially with the C-terminal chiral center of mono SULL and both chiral centers of poly SULL. Varying the temperature of the running electrolyte resulted in a change in the depth of penetration of the BNP enantiomers. At low temperature, BNP interacts preferentially
with the N-terminal chiral center, while at high temperature, it interacts preferentially
with the C-terminal chiral center of poly SULL. On the other hand, at intermediate
temperature, BNP interacts with both chiral centers of the poly SULL surfactant.
Addition of organic modifier to the running electrolyte resulted in increased chiral
resolution and peak efficiency of the BOH and BNA enantiomers and a decrease in
partition coefficient. The opposite behavior was observed with BNP. In the following
chapter, chiral recognition of BOH and BNP is further investigated using fluorescence
anisotropy technique.

REFERENCES


Chapter 3

Chiral Recognition of 1,1'-Bi-2-naphthol, and 1,1'-Binaphthyl-2,2'-dihydrogen Phosphate Using Electrokinetic Chromatography and Fluorescence Spectroscopy: Effect of Temperature

The separation of enantiomers in electrokinetic chromatography (EKC) is achieved as a result of the different mobilities of the enantiomers under separation conditions. Enantiomers do not differ in their electrophoretic mobilities in free solution. This means that they would be unresolved in free zone capillary electrophoresis. Therefore, in EKC, chiral pseudostationary phases (CPSP), which can recognize both enantiomers stereoselectively, are added to the running buffer. The difference in the mobilities of the enantiomers in EKC is due to the formation of transient diastereomeric complexes between the chiral analyte and CPSP. It should be mentioned that enantiomers bind to the chiral selector with different binding constants. The time, which an enantiomer spends in the capillary column as a transient diastereomeric complex, depends on the type and strength of its interaction with the CPSP. In addition, separation parameters including pH of the running buffer, concentration of the CPSP, and temperature, also play a major role in retention factor of the enantiomers in EKC.

Fluorescence spectroscopy is a very powerful technique to study the formation and interaction of analytes with various complexing agents such as ligands, proteins, and surfactants. Since many chiral analytes contain fluorophores, fluorescence spectroscopy techniques seem like ideal candidates to study chiral interactions. However, very few studies have been reported using fluorescence techniques to examine chiral recognition. Chiral discrimination in excimer formation of a pyrene
derivative was reported by Tran et al. The authors stated that the rate of the excimer formation of this fluorophore is different in a racemic mixture as compared to the pure enantiomers. Kano et al. studied the effect of methyl-β-cyclodextrin on the fluorescence emission of binaphthyl derivatives. In addition, a photophysical investigation of chiral recognition in crown ethers was reported by Tundo et al.

Steady state fluorescence anisotropy measurements reveal the average angular displacement of a fluorophore which occurs between absorption and emission of a photon. This angular displacement is dependent upon the rate and extent of rotational diffusion during the lifetime of the excited state. Presumably, the diffusion motion of the enantiomers in a chiral environment depends upon the strength of interaction of that enantiomer with the chiral selector. According to the three point rule of interaction, a minimum of three simultaneous interactions between the chiral phase and one of the enantiomers are required in order to achieve chiral recognition. At least one of these three points of interactions must be enantioselective. It should be noted that the other enantiomer would not be able to achieve the same three points of interactions. If one enantiomer interacts more strongly with a given chiral selector, with respect to the other enantiomer, then the anisotropy of the two enantiomers should be different when they are measured in the presence of that chiral selector. The enantiomer which interacts more strongly with the chiral selector will have less rotational diffusion as compared to the other enantiomer, leading to greater anisotropy values compared to its mirror image.

In Chapter 2, the effect of temperature on the depth of penetration of binaphthyl derivatives into the micellar core of the polymeric surfactants was
discussed. In this chapter, EKC and steady state fluorescence anisotropy is utilized to further investigate the effect of temperature on chiral recognition of these enantiomers.

Part I

EXPERIMENTAL

Chemicals. The chiral analytes (±)1,1′-bi-2-naphthol (BOH), and (±)1,1′-binaphthyl-2,2′-dihydrogen phosphate (BNP) were purchased from Aldrich (Milwaukee, WI). The amino acid leucine-leucine was purchased from Sigma Chemical Co. (St. Louis, MO). The polymer of sodium N-undecanoyl leucyl-leucinate (poly SULL) was synthesized according to the procedure discussed in Chapter 2.

Electrokinetic Chromatography Measurements. Electrokinetic chromatography separations were performed on a Hewlett Packard (HP) 3D CE model #G1600AX. Fused-silica capillary (50 μm i.d.) was purchased from Polymicro Technologies (Phoenix, AZ). The overall length was 63.5 cm with an effective length to detection window of 55 cm. The capillary was mounted in an HP capillary cartridge and used for the separation of racemic mixtures of binaphthyl derivatives (BOH and BNP). The running background electrolytes were prepared in triply distilled water. A 30 mM equivalent monomer concentration (EMC) solution of poly SULL for BNP (6 mM EMC for BOH) were prepared in pH 10, 100 mM TRIS, and 10 mM sodium borate. All solutions were filtered through a 0.45 μm membrane filter prior use. Before each run, the capillary was flushed for 2 min with buffer prior to injecting the analytes. A new capillary was conditioned for 30 min with 1 N NaOH at 60 °C followed by triply distilled water (for 10 min). Standard stock solutions of
racemic analytes were prepared in methanol:water (1:1 v/v) mixtures at 0.1 mg/mL. Samples were injected for 5 s at 10 mbar pressure and separated with an applied voltage of +30 kV. UV wavelength of 215 nm was used for absorbance detection.

**Fluorescence Measurements.** Fluorescence measurements were performed on a SPEX model F2T211 spectrofluorometer equipped with a thermostated cell housing and a thermo-electrically cooled Hamamatsu R928 photomultiplier tube. The excitation and emission wavelengths used for examination of the chiral analytes were, 317 nm and 410 nm for BNP, and 326 nm and 390 nm for BOH, respectively. A 0.1 mM stock solution of chiral analyte was prepared in methanol. A 100 µL aliquot of the analyte solution was transferred into a vial and methanol was evaporated. Poly SULL was then added to the vial to prepare 30 mM EMC in 3 mL buffer solution. The buffer composition is similar to the condition used in EKC measurements.

Steady state fluorescence anisotropy was measured using L-format optics shown in Figure 3.1. The sample was excited with vertically polarized light. Fluorescence emission was then measured through a polarizer. In order to measure the anisotropy, the intensity of the fluorescence emission was measured when the polarizer was parallel ($I_{VH}$) and when it was perpendicular ($I_{VH}$) to the direction of the polarized excitation light. It should be pointed out that $I_{VH}$ corresponds to vertically polarized excitation and horizontally polarized emission, and $I_{VH}$ corresponds to vertically polarized emission and excitation. In order to calculate the actual values for $I_{VH}$ and $I_{VH}$, the G factor, which is the ratio of the sensitivities of the detection system for vertically and horizontally polarized light, needs to be measured. The G factor for
this measurement was 1.2. This means that the emission monochromator passes vertically polarized light with 1.2-fold greater intensity than the horizontally polarized light. Steady state anisotropy was calculated using equation 3.1.2

\[
\text{anisotropy} = \frac{I_{yy} - 1.2xI_{yH}}{I_{yy} - 2x1.2xI_{yH}}
\]

(3.1)

**Part II**

**RESULTS AND DISCUSSION**

**Effect of Temperature on Electrokinetic Measurements**

Figure 3.2 shows that the chiral resolution of both BNP and BOH decreases with increasing separation temperature. As discussed in the previous chapter, temperature can control chiral separation by its influence on the thermodynamics and kinetics of separation. In addition, temperature can affect electrokinetic parameters. A decrease in buffer viscosity with increasing separation temperature will increase the diffusional band broadening and in turn results in a decline in peak efficiency.
Although the chiral resolution of BOH enantiomers decreases by increasing the temperature, a slight increase in chiral selectivity of these enantiomers was observed by raising the temperature (Figure 3.3). The chiral selectivity of BOH enantiomers increases from 1.016 at 13 °C to 1.021 at 55 °C. As shown in Figure 3.4, the capacity factors for both analytes decreases with increasing temperature. A decline in the capacity factors for BNP and BOH indicates that interaction of these analytes with the polymeric CPSP decreases with increase in temperature.

Although the BOH enantiomers have less time to interact with the polymeric CPSP, an improvement in chiral selectivity is observed at higher temperatures. The capacity factors indicate that BOH interacts stronger with the polymeric CPSP at
lower temperatures. However, the selectivity factor suggests that the enantioselectivity increases slightly with increase in temperature. The increase in

![Graph showing chiral selectivity of BNP and BOH in different temperatures.](image)

**Figure 3.3 Chiral selectivity of BNP and BOH in different temperatures.**

chiral selectivity is possibly due to a decrease in hydrogen bonding of the hydroxyl groups of BOH with the polymeric CPSP at elevated temperatures. These hydrogen-bonding interactions may not be enantioselective. Increasing the temperature disturbs the hydrogen bonding between BOH and the polymeric CPSP leading to better chiral selectivity at higher temperature. As shown in Figure 3.3, temperature has a pronounced effect on the chiral selectivity of BNP, as well. A decline in Rs, k' and α values of BNP with increasing temperature suggest that, in contrast to BOH, a more stable diastereomeric complex forms between the enantiomers of BNP and poly SULL.
at lower temperatures. Dependence of $k'$ on temperature was further examined using equation 3.2

$$\ln k' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \beta$$  \hspace{1cm} (3.2)

![Graph showing the capacity factor of BNP and BOH in different temperature.](image)

**Figure 3.4 Capacity factor of BNP and BOH in different temperature.**

where $R$; gas constant, $T$; temperature in Kelvin, $\Delta H$ and $\Delta S$ are the enthalpy and entropy of the solute transfer from the aqueous to the micellar phase, and $\beta$ is the phase ratio. From this equation, a van't Hoff plot,\(^7\) which is $\ln k'$ vs. $1/T$, for enantiomers of BNP was obtained and is shown in Figure 3.5. The slope of the van't Hoff plot is equal to $\Delta H$ and the intercept with the $y$ axis is equal to $(\Delta S/R) + \ln \beta$. From the slope of the van Hoff plot, $\Delta H$ for the enantiomers of BNP was calculated. Enthalpy values of 6 KJ/mol for R-BNP and 7 KJ/mol for S-BNP were obtained.
Figure 3.5 van Hoff Plot for Enantiomers of BNP.

However, the plots of lnk' vs. 1/T for the BOH enantiomers were not linear. The nonlinearity of the van’t Hoff plot for the enantiomers of BOH is possibly due to entropy controlled factors involved in the complexation of BOH with the polymeric CPSP. As noted earlier, at higher temperatures a more ordered diastereomeric complex may be formed for the enantiomers of BOH.

Steady State Fluorescence Anisotropy. As previously mentioned, if the enantiomers of chiral analytes bind with different strengths to chiral selectors, the measured anisotropy for the two enantiomers in that chiral environment should be different. Thus, steady state fluorescence techniques were used to study the chiral interaction of BNP and BOH with poly SULL surfactants. As shown in Table 3.1, S-BNP has an anisotropy value of 0.106 while the value for the R-BNP enantiomer is 0.087 when they were measured in the presence of poly L,L-SULL. This indicates that S-BNP interacts stronger with poly L,L-SULL as compared to R-BNP.
the anisotropy values are consistent with the ΔH values calculated from the van’t Hoff plot. An enthalpy value of 6 KJ/mol and an anisotropy value of 0.087 was obtained for R-BNP, while S-BNP had values of 7 KJ/mol for the ΔH, and an anisotropy of 0.106. Measurements of the anisotropy in the presence of D,D-SULL resulted in reversal of the anisotropy values which indicates that R-BNP interacts stronger with D,D-SULL. An anisotropy value of 0.092 was obtained for R-BNP, while S-BNP had an anisotropy value of 0.080 (Table 3.1).

In order to confirm the differences in anisotropy of R- and S-BNP in the chiral environment, these values were measured in the presence of an achiral surfactant. Table 3.1 also shows the anisotropy of BNP enantiomers in the presence of the achiral surfactant poly glycinate (poly SUG). Note that no significant difference in anisotropy values of the BNP enantiomers were observed in the achiral environment. Similar results were achieved for enantiomers of BOH. In the presence of poly L,L-SULL, the anisotropy of R-BOH was 0.244. This value is higher than the anisotropy of S-BOH (0.235). Again, the enantiomer that was retained longer in the separation column during EKC measurements (R-BOH) resulted in higher anisotropy values.

Table 3.1 Fluorescence anisotropy values for enantiomers of BNP

<table>
<thead>
<tr>
<th></th>
<th>R-BNP</th>
<th>S-BNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly L,L-SULL</td>
<td>0.087</td>
<td>0.106</td>
</tr>
<tr>
<td>Poly D,D-SULL</td>
<td>0.092</td>
<td>0.080</td>
</tr>
<tr>
<td>Poly SUG</td>
<td>0.099</td>
<td>0.097</td>
</tr>
</tbody>
</table>
Figure 3.6 shows the effect of temperature on the anisotropy of the BNP enantiomers in the presence of poly L,L-SULL. Note that, similar to the chiral resolution shown in Figure 3.2, the anisotropy decreases with increasing temperature. Similar to the EKC results where temperature influences the electrokinetic parameters, in anisotropy measurements, raising the temperature decreases the viscosity of the solution (as well as the viscosity of the micellar core), which in turn results in decreased anisotropy values. As with BNP, the anisotropy of the BOH enantiomers decreased by increasing the temperature (Figure 3.7). However, note that the anisotropy of BOH varies from 0.257 at 10 °C to 0.228 at 40 °C, while BNP
anisotropy varies at a higher rate, 0.142 at 10 °C to 0.047 at 40 °C. Interestingly, this trend is similar to the trend observed for chiral resolution of these two chiral analytes. The chiral resolution of BNP decreases at a higher rate compared to BOH with the same increase in temperature.

![Figure 3.7 Anisotropy of BOH enantiomers in different temperature. ±0.0008 average std. of three consecutive runs.](image)

In Chapter 2, I have shown that varying the temperature from 12 °C to 55 °C in EKC measurements does not change the preferential site of interaction of BOH enantiomers with poly L,L-SULL surfactants significantly. In this temperature range, the enantiomers of BOH preferentially interact with the N-terminal amino acid of this
dipeptide surfactant. The anisotropy data suggests that the microviscosity of the hydrophobic core, where BOH predominantly resides, does not change significantly by varying the temperature. On the other hand, the site of interaction of the enantiomers of BNP does vary by changing the separation temperature. One of the factors involved in the large difference observed in the anisotropy of BNP at different temperatures is most probably due to the change in preferential site of interaction. The fluorescence anisotropy measured for BNP in each temperature is the sum of the anisotropy of the free (the portion that is in bulk solution) and the bound form (the portion that is inside the micelle). The free form of BNP in bulk solution has an anisotropy value close to zero. Raising the temperature increases the contribution of the free BNP in total anisotropy, resulting in a drastic decline in the anisotropy value of these enantiomers. In addition, as discussed in Chapter 2, the enantiomers of BNP interact preferentially with the N-terminal amino acid of poly SULL surfactant at low temperature (i.e. 12 °C), while the preferential site of interaction of these enantiomers changes at higher temperature. At 55 °C, the enantiomers of BNP preferentially interact with the C-terminal amino acid of this polymeric surfactant. Thus, as the enantiomer moves closer to the bulk aqueous phase, it is experiencing a decrease in viscosity due to the fact that the core of the micelle is much more sterically hindered than the surface of the micelle.

Note that in Figure 3.8, differences in the anisotropy of R- and S-BNP decrease with increasing temperature. This indicates that at higher temperatures, less chiral interaction occurs between the enantiomers of this analyte and polymeric SULL. This is consistent with the chiral selectivity results reported in Figure3.3. Therefore, from
Figure 3.8 Differences in anisotropy of R-and S-enantiomer for BNP and BOH in different temperature.

In this study EKC and steady state fluorescence anisotropy is used to investigate chiral interactions of BNP and BOH with poly SULL surfactants. The results suggest that decreasing the temperature improves the chiral interaction of BNP with poly SULL surfactants. In contrast, raising the temperature results in better chiral interaction of BOH enantiomers with this surfactant. Similar results were achieved with both EKC and steady state fluorescence anisotropy techniques. The
results of this study suggest that steady state fluorescence anisotropy can be used to gain further insight into chiral recognition.

In order to gain more of an insight into the interactions involved in chiral selectivity of the polymeric chiral surfactants with the various enantiomers, knowledge of the physical properties of the chiral selector is very useful. Therefore, in following chapter, some physical properties of polymeric and monomeric surfactants are determined using different spectroscopy techniques.

REFERENCES


Chapter 4
Characterization and Physical Properties of Polymeric and Monomeric Amino Acid Based Surfactants

In previous chapters, the chiral recognition of polymeric and monomeric amino acid based surfactants have been investigated. One of the factors responsible for the differences in performances of these two kinds of micelles are the differences in their physical properties. In this chapter, different spectroscopic techniques such as fluorescence and nuclear magnetic resonance are used to study the properties of chiral dipeptide surfactants (CDS).

Part I

EXPERIMENTAL

Chemicals. Dipeptide surfactants were synthesized as described in Chapter 2 of this dissertation. Pyrene was purchased from Aldrich (Milwaukee, WI), and recrystallized from methanol. Other chemicals were reagent grade and were used as received.

Fluorescence Measurements. Fluorescence measurements were performed on a SPEX model F2T211 spectrofluorometer equipped with a thermostated cell housing and a thermo-electrically cooled Hamamatsu R928 photomultiplier tube. A 1 mM stock solution of pyrene was prepared in methanol. An appropriate amount of stock solution was placed in the sample vial, methanol was evaporated, and aqueous solution of surfactant was added. The final concentration of pyrene was 0.1 mM and that of surfactants was 100 mM for monomer and 100 mM equivalent monomer concentration (EMC) for polymer (solution A). Solution A, which contained pyrene and surfactant, was placed in dark area overnight to equilibrate. Then, solution A was

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divided in half. The first half was diluted with water to give a concentration of 50 mM surfactant and 0.05 mM pyrene (solution B). The second half of solution A was mixed with cetylpyridinium chloride (quencher) to provide 1.5 mM quencher, 50 mM surfactant and 0.05 mM pyrene (solution C). In fluorescence quenching measurements, solution C was added to solution B in increasing increments of 50 µL. The decrease in fluorescence intensity of pyrene was measured at 393.0 nm after each aliquot of the quencher was added. The aggregation number of the surfactants was then determined by following the method developed by Turro\(^1\) using the following expression

\[
\ln\left(\frac{I_0}{I}\right) = \frac{N[Q]}{(C_s - CMC)}, \tag{4.1}
\]

where \(C_s\) is the total surfactant concentration, \(I_0\) and \(I\) are the fluorescence intensity of the pyrene at zero and \([Q]\) concentrations of the quencher, respectively. The aggregation number of the surfactants, \(N\), is obtained from the slope of the plot of \(\ln\left(I_0/I\right)\) vs. \([Q]\). This plot for mono SULL is shown in Figure 4.1.

**Nuclear Magnetic Resonance Measurements.** The NMR spectra were recorded on a Bruker ARX 300 MHz spectrometer and the data were processed with Bruker Xwinnmr software operating on a Silicon Graphics Indigo workstation (Silicon Graphics Inc., Bruker Co.). Solutions of CDS at concentrations above and below the CMC were prepared in D\(_2\)O or in a mixed solvent of 90% H\(_2\)O and 10% D\(_2\)O. The D signal of D\(_2\)O was used for frequency-lock and the intensity of the H\(_2\)O resonance was suppressed by presaturation.\(^2\)
Typical one-dimensional (1D) $^1$HNMR spectral acquisition parameters were as follows: Data size, 16K; spectral width, 3500 Hz; 90 radio frequency pulse, 7.0 $\mu$s;

Figure 4.1 Measurements of the aggregation number of mono SULL.

recycling delay between transients, 2.0 s. Adequate signal-to-noise (S/N) ratios in the $^1$HNMR spectra were achieved after 256 transients. Prior to Fourier transformation, the spectra were multiplied by a Lorentz-Gauss window function and zero-filled. Chemical shifts are reported in part per million (ppm) relative to TSP. Coupling constants ($^3J_{H-H}$) were measured directly from the $^1$HNMR spectra. Two-dimensional
(2D) $^1$H-$^1$H correlation spectroscopy (COSY) experiments were measured with suppression of the water resonance by presaturation. The following acquisition parameters were used: temperature, 298 K; recycling delay, 2.0 s; spectral width in both dimensions, 3500 Hz; dummy scans, 4; D0 increments, 3 μs. All 2048 data points were acquired in $t_2$ and 64 transients were coadded at each of 256 $t_1$ increments with zero-filling to 2048 points. Gaussian or shifted sinebell apodization was applied in both dimensions.

**Diffusion Ordered NMR Measurements.** The diffusion ordered NMR experiments were carried out on a Bruker DPX 300 MHz spectrometer equipped with an actively shielded z-gradient coil. The instrument's coil constant of 50.3 G/cm at 100% gradient strength was determined by carrying out pulsed gradient NMR experiments on a 10 mM β-cyclodextrin sample. All pulse field gradient nuclear magnetic resonance (PFG-NMR) experiments were performed with the bipolar pulse pair longitudinal encode-decode pulse sequence. With this experiment, a series (typically 12-15) of NMR spectra were collected as a function of increasing gradient amplitude, G. The areas of the peaks in the resulting spectra, $S(G)$, decay exponentially as a function of $G^2$ as shown in equation 4.2

$$S(G) = S_0 \cdot \exp \left[ -D \cdot \left( \Delta - \frac{\delta}{3} - \frac{\tau}{2} \right) \cdot \gamma^2 G^2 \delta^2 \right]$$

(4.2)

where $S_0$ is the peak integral at zero gradient strength, $D$ is the diffusion coefficient, $\Delta$ is the diffusion time, $\delta$ is the duration of the gradient pulses, $\tau$ is the delay between the bipolar pulses in the BPPLIED experiment, and $\gamma$ is the magnetogyric ratio. In the
PFG-NMR experiments performed here $\Delta$, $\delta$, and $\tau$ were, respectively 250, 2, and 0.2 ms. The gradient amplitudes ranged from 7.55 to 42.8 G/cm.

After the free induction decays were collected, each data set was apodized with 5 Hz line broadening, Fourier transformed, phased, and a spline baseline correction was performed. The methylene resonances (0.5 to 1.5 ppm) in the sample were then integrated. The diffusion coefficient is calculated from the slope of the ln of peak area vs. $(\Delta-\gamma/3-\tau/2)(\gamma G\delta)^2$. This plot for mono and poly SULL is shown in Figure 4.2. It should be mentioned that sample solutions of poly SULL (at 50 mM EMC) and mono SULL (at 50 mM) were prepared at pH 7 in D$_2$O (99.9%). In the SULL monomer experiment, the surfactant sample was spiked with one µL of tetramethylsilane (TMS). The decay of the TMS integral with gradient strength was then monitored and analyzed to obtain the diffusion coefficient of the surfactant micelles. Since the hydrophobic TMS probe is solubilized in the hydrocarbon core of the micelle, this method gives a measure of the diffusion coefficient of the micellar aggregate. If the surfactant signal from the SULL was monitored instead, the resulting diffusion coefficient would be the weighted average of the micellar and free solution values.

**Electrophoretic Mobility Measurements.** The migration time of the micelles were measured with a Hewlett Packard (HP) 3D CE model #G1600AX. The fused silica capillary, effective length of 8 cm (to detection window), 50 µm i.d., with a total length of 63.5 cm, was purchased from Polymicro Technologies (Phoenix, AZ) and mounted in an HP capillary cartridge. The cartridge temperature was maintained at 25 °C during the measurements. The capillary was flushed with buffer for 2 min prior to
injecting the sample. The running background electrolytes (15 mM phosphate) were prepared in triply distilled water; surfactants (at 50 mM for monomer and 50 mM

![Graph a)](image-a)

![Graph b)](image-b)

**Figure 4.2.** PFG-NMR plots for (a) poly SULL and (b) mono SULL surfactant. The slope of the plot is the diffusion coefficient of the surfactant.

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EMC for polymer) were then added to the buffer. The tert-butyl anthracene and/or Sudan III solution were prepared in methanol at 0.1 mg/mL and injected for 5 seconds at 10 mbar pressure from outlet. Electrophoretic measurements were performed at -30 kV, with UV detection at 220 nm.

Part II

RESULTS AND DISCUSSION

Aggregation Number of Chiral Dipeptide Surfactants. The aggregation numbers of the fifteen chiral CDS examined in this study are shown in Table 4.1. These surfactants were classified based on the aggregation numbers of the monomers.

Table 4.1 Aggregation number of monomeric dipeptide surfactant and repeating units of polymeric dipeptide surfactant. ±1 std.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Monomer</th>
<th>Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUGA</td>
<td>380</td>
<td>33</td>
</tr>
<tr>
<td>SUGV</td>
<td>140</td>
<td>23</td>
</tr>
<tr>
<td>SUGL</td>
<td>110</td>
<td>23</td>
</tr>
<tr>
<td>SUAG</td>
<td>270</td>
<td>30</td>
</tr>
<tr>
<td>SUAA</td>
<td>358</td>
<td>26</td>
</tr>
<tr>
<td>SUAV</td>
<td>74</td>
<td>24</td>
</tr>
<tr>
<td>SUAL</td>
<td>65</td>
<td>25</td>
</tr>
<tr>
<td>SUVG</td>
<td>62</td>
<td>22</td>
</tr>
<tr>
<td>SUVA</td>
<td>50</td>
<td>19</td>
</tr>
<tr>
<td>SUVV</td>
<td>62</td>
<td>23</td>
</tr>
<tr>
<td>SUVL</td>
<td>48</td>
<td>19</td>
</tr>
<tr>
<td>SULG</td>
<td>40</td>
<td>21</td>
</tr>
<tr>
<td>SULA</td>
<td>42</td>
<td>18</td>
</tr>
<tr>
<td>SULV</td>
<td>39</td>
<td>18</td>
</tr>
<tr>
<td>SULL</td>
<td>38</td>
<td>19</td>
</tr>
</tbody>
</table>

Class I surfactants are those having aggregation numbers above 100 and class II surfactants are those having aggregation numbers below 100. As can be noted from Table 4.1, the number of repeat units for the polymers are always lower than the
aggregation number of the corresponding monomers. For instance, mono SUAA (class I) and mono SULL (class II) have aggregation numbers of 358 and 38, respectively. However, the number of repeat units for poly SUAA and poly SULL were 26 and 19, respectively. This indicates that, under the conditions used in this study, polymerization results in a change in the size of the "micelle". Previous studies have shown that the intensity of the radiation source used for polymerization can affect the number of repeat units of polymers. The intensity of the gamma radiation source used for the polymerization in this study was about 0.7 krad/h. The flux of the gamma rays from the source was probably not strong enough to provide polymers with "aggregation numbers" similar to the monomer. Thus, the smaller "aggregation numbers" of the polymeric CDS are probably a result of the slower polymerization. It should be mentioned that Paleos et al. have obtained polymers with the same size as the micelles by polymerization of sodium 10-undecenoate with gamma radiation of 143 krad/h.

Other factors that affect aggregation numbers are the size of the polar head group of the surfactants, as well as ionic repulsion and attractive forces caused by the hydrophobic attraction of the hydrocarbon chain. The steric forces caused by the R-groups of leucine in SULL result in micelles with smaller aggregation numbers as compared to SUAA with smaller R-groups. Monomeric surfactants in class I, such as SUAA, have aggregation numbers greater than 100 which is probably indicative of nonspherical micelles. However, polymerization yielded polymeric surfactants with aggregation numbers of around 20-33, which is probably indicative of a spherical polymeric "micelle". Thus, it is reasonable to conclude that, under the conditions used
in this study, regardless of the size of the polar head, the polymeric surfactants examined in this study most likely adopt a spherical shape in solution. On the other hand, in the monomeric state, CDS with smaller polar head groups (i.e. SUAA) have different packing size than the monomeric CDS with bulky polar head groups (i.e. SULL). HNMR spectroscopy was utilized to gain insight into the differences in the observed aggregation numbers of the monomeric CDS.

**Packing of Monomeric Chiral Dipeptide Surfactants.** As with all amino acid based compounds, the amide moieties in CDS are capable of forming strong intermolecular or intramolecular H-bonding. At concentrations below the CMC, they hydrogen bond with water, whereas upon micellization, water is excluded from the hydrophobic core and hydrogen bonding between the polar head groups can then play a major role in the conformation and thus the aggregation number of CDS. As shown in Figure 4.3, CDS contain two amide moieties, C- and N-terminal. The C-terminal amide is closer to the surface of the micelle. No significant difference was observed in the C-terminal NH proton signal of CDS in the monomeric form compared to the micellar form. This is possibly due to the fact that the C-terminal amide interacts with water strongly even in the micellar state. Therefore, in this study, only the amide and Hα of the N-terminal amino acids of CDS are discussed.

The environment of the amide and Hα changes upon micellization. Therefore, 1H NMR measurements were performed at two concentrations, below and above the CMC of the CDS. Considering that the CMC of the CDS are around 7 mM, 1H NMR was conducted at 1 mM (below the CMC) and 50 mM (above the CMC). No difference in chemical shifts of the Class I N-terminal Hα was observed upon
micellization. Therefore, only the $H_\alpha$ signal and NH-CH$\alpha$ coupling constant ($^{3}J_{H-H}$) for Class II surfactants are reported in Table 4.2. The chemical shifts of the N-

![Structure of dipeptide surfactant.](image)

Figure 4.3 Structure of dipeptide surfactant.

terminal $H_\alpha$ were shifted downfield in the micellar state as compared to the monomeric state (Table 4.2). A difference of about 0.1 ppm between the $H_\alpha$ above and below the CMC was observed. Upon micellization, significant chemical shifts were observed in the N-terminal $H_\alpha$ protons of CDS with bulky polar head groups. The change in the chemical shift of the $H_\alpha$ in the presence of a bulky amino acid is possibly due to the fact that the amino acid side chain (R-group) of CDS in micellar state tend to aggregate and twist toward the hydrophobic core to avoid exposure to the water.
Table 4.2 Proton NMR chemical shift for Class II CDS below and above the CMC. ±0.01 the average standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Below CMC (1 mM)</th>
<th>Above CMC (50 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH$<em>{(ppm)}$(J$</em>{Hz}$)</td>
<td>H$_{a}(ppm)$</td>
</tr>
<tr>
<td>SUAV</td>
<td>8.22(6.34)</td>
<td>4.33</td>
</tr>
<tr>
<td>SUAL</td>
<td>8.18(6.48)</td>
<td>4.35</td>
</tr>
<tr>
<td>SUVG</td>
<td>8.13(8.27)</td>
<td>4.20</td>
</tr>
<tr>
<td>SUVA</td>
<td>8.10(8.19)</td>
<td>4.16</td>
</tr>
<tr>
<td>SUVV</td>
<td>7.65*</td>
<td>4.12</td>
</tr>
<tr>
<td>SUVL</td>
<td>8.10(8.38)</td>
<td>4.10</td>
</tr>
<tr>
<td>SULG</td>
<td>8.32(7.45)</td>
<td>4.39</td>
</tr>
<tr>
<td>SULA</td>
<td>8.24(7.51)</td>
<td>4.36</td>
</tr>
<tr>
<td>SULV</td>
<td>8.29(7.41)</td>
<td>4.39</td>
</tr>
</tbody>
</table>

* extracted from COSY spectra

The amide bond is rigid. It has partial double bond character.

aggregation of the side chain causes the bond between C-H$_{a}$ and the adjacent carbonyl to twist to adopt the new conformation. Since the R-group of the amino acid is facing the hydrophobic core, the H$_{a}$ will be forced toward the aqueous phase. The downfield shift of the H$_{a}$ in micellar states can be attributed to the anisotropic effect of the carbonyl group of the amino acid moieties in the dipeptide backbone. As indicated in Table 4.2, the vicinal coupling constant (J$_{H-H}$) values of the N-terminal amino acids are always higher at concentrations above the CMC as compared to below the CMC. This suggests that the R group twists toward the hydrophobic core which causes reorientation of the carbonyl moieties.

The packing of the monomeric CDS in solution was further investigated by comparing the chemical shift of the amide protons below and above the CMC in 90% H$_{2}$O and 10% D$_{2}$O. As shown in Table 4.2, the N-terminal NH of class II surfactants
are shifted upfield in micellar states. My rationale for the upfield shift of the N-terminal NH is that the structure of CDS in the micellar state excludes water resulting in the loss of NH-water hydrogen-bonding. The differences in the chemical shift changes of the N-terminal amide in micellar and monomeric states are indicative of the degree of hydrogen bonding in the CDS. The nature of the amino acid side chain (R-group) at the alpha carbon plays a significant role in hydrogen-bonding; less bulky groups permit closer packing and stronger hydrogen bonding among the amide groups of the polar head.

Shinitzky et al. have proposed the formation of chiral assemblies of amide planes on the micellar surface of N-stearoylsarcosine by examining the circular dichroism spectrum of this surfactant above and below the CMC. This configuration of the amide moieties is supported and aligned by the hydrophobic forces of the surfactant hydrocarbon chain. Since the polar head of the dipeptide surfactants contain more hydrogen bonding sites than single amino acid based surfactants, it is reasonable to assume that upon micellization, stronger hydrogen bonds form among the amides of the polar head of CDS as compared to single amino acid based surfactants. However, bulky polar head groups may not always allow the formation of the strong hydrogen bonds. Examination of the aggregation numbers indicate that, in monomeric surfactants, when a bulky group is located at the N-terminal amino acid of the CDS, lower aggregation numbers are achieved. The unfavorable steric interactions of the bulky side chains prevent the formation of intermolecular hydrogen-bonding between the adjacent amide groups. Figure 4.4-a illustrates the proposed conformation of Class II CDS.
It should be mentioned that no difference in $H_\alpha$ signal of class I N-terminal amide was observed. This is possibly due to the fact that class I surfactants have small polar head groups. Therefore, upon micellization, the configuration of the amides in the polar head may not change significantly. In mono SUGV and mono SUGL, the C-terminal amino acid contains large R-groups. Presumably, the conformation of CDS is more dependent upon the size of the N-terminal amino acids. The presence of valine in SUGV and leucine in SUGL resulted in aggregation numbers of 142 and 110, which is significantly smaller than the other CDSs in class I. The chemical shift for the $H_\alpha$ of glycine does not change upon micellization. If the conformation of SUGV
and SUGL changes upon micellization by orienting the R-group of valine and leucine toward the hydrophobic core, this may not affect the chemical shifts of the glycine's Hα. It should be noted that glycine has two protons at its alpha position and the chemical shift observed with 1H NMR is the average of both protons.

Due to the fast proton exchange of class I N-terminal amide protons, no signal was observed for SUAG, SUAA, and SUGA. In addition, upon micellization, a small down field shift was observed for SUGV and SUGL. The down field shifts of the amide protons in SUGV and SUGL is possibly due to the fact that glycine, which is much more hydrophilic than valine and leucine, is located at the N-terminal position. This may disturb the hydrophobic/hydrophilic balance required for micelle formation. 10 Therefore, the polar head adopts a configuration to balance these hydrophobic forces. However, the reason for the down field shift of these two surfactants (SUGV and SUGL) is not clear at this time.

Although NMR data are not sufficient to propose a conformation for Class I surfactants, from the aggregation number of class I surfactants shown in Table 4.1, it is proposed that there is possible strong hydrogen bonding among the amide moieties of CDS with small polar heads. The model shown in Figure 4.4-b represents the proposed conformation of class I CDS with a small R-group in the N-terminal position. This model is also consistent with the model proposed by Shinitzky et al. for the single amino acid surfactant serine. 9

**Micropolarity.** As discussed in Chapter 1, pyrene exhibits a characteristic fluorescence emission spectrum that consists of five vibronic bands. The intensity ratio of the first to the third peak (I₁/I₃) depends strongly on the polarity of the medium

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in which pyrene is dissolved. The higher the \( I_1/I_3 \) ratio, the more polar the medium. This ratio for polymeric and monomeric surfactants are presented in Figure 4.5. The results indicate that, with the exception of SUAG, SUVG, and SULG, the monomers are always more polar than their corresponding polymers. The hydrophobic tail of the monomeric surfactants examined here are terminated with a vinyl group. The double bond at the end of the hydrophobic tail increases the polarity of the micellar core because there is a dipole between the sp\(^2\) and sp\(^3\) carbons. Upon polymerization, the double bonds are converted to single bond and the hydrophobic tails are covalently linked. Therefore, it can be concluded that the core of the polymeric CDS examined in this study contain less water than the core of the monomers.

Presumably, the presence of the covalent linkage changes the polarity of the “micellar” core of the polymers as compared to the single bond terminated micelles. Therefore, single bond terminated surfactants were synthesized. The single bond terminated class I surfactants have very poor solubility in water. A 2 mM solution of the single bond terminated SUAA formed a gel at room temperature. Therefore, in this study, only the single bond terminated SULL surfactant from class II CDSs was examined. The CMC of this surfactant was around 2.5 mM, which is less than half the CMC of the double bond terminated SULL. This is in agreement with literature, which suggests that the presence of the double bonds increases the CMC of ionic surfactants. However, the aggregation number of single bond terminated SULL was about 45. This value is only slightly higher than the aggregation number of the double bond terminated SULL (38). Comparing the aggregation number of the single bond
Figure 4.5 Micropolarity of CDS based on $I_1/I_3$ ratio of pyrene.

terminated SULL and the number of repeat units in poly SULL indicates that the core of the polymeric micelle has significantly higher space compared to the monomers. In addition, the presence of the covalent linkages among the hydrophobic tail results in a rigid and open "micelle". The polarity of the hydrophobic cores of these three surfactants was measured using the $I_1/I_3$ ratio of pyrene. These values were 0.85, 0.89, and 0.95 for single bond, polymer, and monomer of SULL, respectively. This indicates that the hydrophobic core of the monomer with the terminal double bond is more polar than the core of the polymeric surfactants. On the other hand, the micellar core of the single bond terminated SULL is less polar than the polymer. Therefore, it can be concluded that polymerization results in a more hydrophobic micelle as compare to the double bond terminated monomers.

It was postulated that after polymerization some monomeric units may remain free in solution. The presence of monomeric units may change the aggregation
number of the polymeric surfactants. Therefore, poly SULL surfactants were dialyzed with a 2000 MW cut-off membrane. The aggregation number of the resulting polymer was around 21, which is only slightly higher than the aggregation number of non-dialyzed SULL (19). These results indicate that the amount of the monomers remaining in solution after polymerization is negligible. This assumption was further investigated by comparing $I_1/I_3$ ratio of pyrene for dialyzed and non-dialyzed polymers. Results indicated that dialysis did not change the polarity of the polymeric surfactants.

**Electrophoretic Mobility.** Above pH 6.5, polymeric dipeptide surfactants examined in this study are negatively charged owing to the deprotonation of their carboxylate groups. If the CE instrument is set up such that sample is injected at the anode and the detector is near the cathode and positive voltage is applied, the electroosmotic flow (EOF) will be from cathode toward anode. Thus, these negatively charged polymeric micelles are attracted to the anode and consequently, oppose the EOF in the capillary. Polymeric micelles would still move toward the detector end because their electrophoretic mobilities are not large enough to overcome the EOF.

Due to the presence of the carbonyl groups, polymeric amino acid based surfactants absorb UV light about 215-220 nm, thus they can be detected with the UV-Vis detector. Therefore, these polymers were injected as the sample into the capillary filled with 15 mM phosphate buffer at pH 7. From the migration time, the electrophoretic mobility of fifteen polymeric dipeptide micelles were calculated and the results are shown in Table 4.3. As can be seen from the data, these polymers have similar electrophoretic mobilities.
Table 4.3 Electrophoretic mobility ($
\mu_e$) of polymeric CDS. ±2.5x10$^{-4}$ average standard deviation.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Electrophoretic Mobility x10$^{-4}$ (cm$^2$/Vmin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUGA</td>
<td>218</td>
</tr>
<tr>
<td>SUGV</td>
<td>217</td>
</tr>
<tr>
<td>SUGL</td>
<td>214</td>
</tr>
<tr>
<td>SUAG</td>
<td>220</td>
</tr>
<tr>
<td>SUAA</td>
<td>216</td>
</tr>
<tr>
<td>SUAV</td>
<td>216</td>
</tr>
<tr>
<td>SUAL</td>
<td>218</td>
</tr>
<tr>
<td>SUVG</td>
<td>217</td>
</tr>
<tr>
<td>SUVA</td>
<td>218</td>
</tr>
<tr>
<td>SUVV</td>
<td>213</td>
</tr>
<tr>
<td>SUVL</td>
<td>219</td>
</tr>
<tr>
<td>SULG</td>
<td>220</td>
</tr>
<tr>
<td>SULA</td>
<td>218</td>
</tr>
<tr>
<td>SULV</td>
<td>215</td>
</tr>
<tr>
<td>SULL</td>
<td>214</td>
</tr>
</tbody>
</table>

The electrophoretic mobility of conventional micelles cannot be measured by direct injection of their solution into the capillary. However, the electrophoretic mobility of the conventional micelles can be calculated by measuring the migration time of the micelle ($t_{mc}$) using a highly hydrophobic analyte that strongly partitions into the micellar core and elutes with the micelle. Sudan III has been extensively used to determine the $t_{mc}$. However, no $t_{mc}$ was obtained when Sudan III was injected with these surfactants. Therefore, tert-butyl anthracene was used to obtain the $t_{mc}$. It should be pointed out that the $t_{mc}$ of sodium dodecyl sulfate (SDS) was measured using Sudan III and tert-butyl anthracene. The results indicated that tert-butyl anthracene partitions stronger into the micellar core of the SDS as compared to Sudan III. Similar to SDS, the CDS examined in this study are negatively charged.
Therefore, it is reasonable to assume that tert-butyl anthracene would serve as a suitable \( t_{\text{mc}} \) marker to trace the elution of these amino acid based surfactants.

The \( t_{\text{mc}} \) of monomeric and polymeric CDS was measured with reversed polarity and injecting the tert-butyl anthracene at the detector end making the effective length of the capillary only 8.5 cm. The electrophoretic mobility of monomers and polymers are shown in Figure 4.6. From these values, it can be concluded that polymeric surfactants are retained longer in the capillary compared to their corresponding monomers. The retention of a given solute in the capillary depends upon several parameters including, molecular weight, and effective charge. The molecular weights of the CDS examined in this study are shown in Table 4.1. In the following section, the effective charge of SULL surfactant (both monomer and polymer) is calculated.

**Effective Charge of Amino Acid Based Surfactant.** Although the electrophoretic mobility is a readily measured quantity, its interpretation is considerably more difficult for micelles, as compared to simpler molecules. The charge carried by a micelle is not a known quantity as is the case of simple ions. The charge on small ions can be measured according to the general equation for \( \mu_c \)

\[
\mu_c = \frac{(Ze)}{(6\eta \pi r)}, \quad (4.3)
\]

where \( Z \) is the effective charge of the surfactant; \( e \) is the charge of electron; \( \eta \) is viscosity; and \( r \) is the radius of hydration. One of the difficulties of using this equation for micelles is determining the radius of the hydration. As indicated earlier, Class I monomeric surfactants have a nonspherical shape. In order to use equation 1, ions
should be spherical. Therefore, only the effective charge on SULL surfactant from class II was calculated.

The diffusion coefficient of the spherical micelles is defined by Stokes-Einstein equation

\[ D = \frac{K_B T}{6\pi \eta r} \quad (4.4) \]

where \( K_B \) is Boltzmann constant, \( T \) is Kelvin temperature, and \( \eta \) is the viscosity of the solvent. Equation 4.4 can be written as

\[ r = \frac{K_B T}{6\pi \eta D} \quad (4.5) \]
Combining equation 4.3 and 4.5 gives the equation 4.6

\[ \mu_e = \frac{ZeD}{K_B T} \] (4.6)

Thus, from this equation using combined information from electrophoresis and diffusion experiments would allow evaluating the charge carried by the macroion.\(^{14}\)

As discussed in the experimental section, the diffusion coefficient (D) of SULL surfactant was calculated using PFG-NMR. These values were \(9.37 \times 10^{-7}\) and \(1.31 \times 10^{-6}\) cm\(^2\)/s for poly and mono SULL, respectively. Therefore, the effective charge on the micelles can be calculated using equation 4.6. The effective charge on poly and mono SULL was about 7.2 and 5.1, respectively. From the aggregation number and the effective charge it was determined that 38% of the carboxyl groups on poly SULL were charged at the experimental conditions used, while that of monomeric SULL was only about 13%. Therefore, it can be concluded that under the conditions used in this study, the polymeric surfactants are more charged than the monomers.

**Conclusions.** The results of this study indicate that the number of repeat units for polymeric surfactants is always smaller than the aggregation number of the respective monomers. Also, the results of the aggregation number studies suggest that the polymeric CDS examined in this study most likely adopt spherical shapes in solution while the shape of the monomers are dependent upon the structure of the polar head group. Monomers with small polar head groups, i.e. SUAA, form nonspherical micelles, while monomers with bulky polar head groups, i.e. SULL, form spherical micelles. In addition, using pyrene as a polarity sensitive fluorescent probe, the core of the polymeric surfactants was determined to be less polar than that of the
monomers. The effective charge of the "micelles" was also measured and it was determined that the polymeric CDS are more charged than the monomers.

As discussed in Chapter 2, for the analytes examined, polymeric CDS provided better chiral resolution for the enantiomers of neutral and cationic analytes. However, better separation for the enantiomers of negatively charged BNP was obtained using monomeric surfactants. The results of this study suggest that since polymers are more charged, cationic enantiomers interact more strongly with polymeric surfactants owing to the higher electrostatic interaction that can sometimes favor chiral separation. In contrast, due to the repulsion of similar charges, anionic enantiomers, i.e. BNP, probably interact more strongly with monomeric surfactants as compare to the polymers. In addition, the results of this study suggest that since polymers are more charged, and provide higher electrophoretic mobilities, neutral analytes spend more time interacting with CPSP resulting in improved chiral selectivity of neutral analytes using polymeric surfactants. On of the factors involved in determining the amount of time an analyte spends interacting with the CPSP is the solublization of the micelle. Thus, in a following chapter, the solubilization capacity of the monomeric and polymeric surfactants is determined using pulse filed gradient NMR.

REFERENCES

Chapter 5
Pulsed Field Gradient Nuclear Magnetic Resonance Study of Polymeric and Monomeric Amino Acid Based Surfactants: Diffusion Coefficient and Solubilization of Organic Probes

As discussed in Chapter 1, micelles consist of several regions. The core of the micelle, which is the most nonpolar part of the micelle, is the region in the center that is composed almost entirely of the hydrocarbon moieties of the surfactants. The palisade layer is the region surrounding the core. It consists of hydrocarbon and polar moieties from the polar head of the surfactant and water. The palisade layer becomes more polar as the distance from the core of the micelle increases. The Stern layer, which consists of the surfactant polar heads, is the most polar region. Organic solutes can be solubilized by incorporating into any of these regions. Hydrophobic (or nonpolar) solutes penetrate deeply into the micellar core and are mostly dissolved in the core of the micelle. Moderately polar solutes interact within the palisade layer.\textsuperscript{1,2} The discussion above holds for polymeric surfactants as well.

Polymerization of surfactants results in covalent linkage of the hydrophobic tails within the hydrophobic core of the micelle. Due to their rigidity and high stability, polymeric surfactants have been used extensively in chiral\textsuperscript{3-9} and achiral\textsuperscript{10-19} electrokinetic chromatography (EKC). The ability of a particular surfactant to solubilize organic compounds will obviously play a major role in its performance in EKC separations. Therefore, in this chapter, the solubilization of organic molecules in polymeric and monomeric surfactants is investigated.

In general, molecules in solution exhibit ceaseless, and random motion called Brownian motion. Brownian motion is due to bombardment of the dispersed particles...
by molecules of the medium. Solubilization of the analyte into the micellar core slows this Brownian movement. Therefore, dissolved analytes have slower Brownian motion than that of the free form. The diffusion coefficient of solutes is directly related to their Brownian motion. Thus, when analytes “dissolve” in the micellar core, they diffuse much slower (or have less Brownian motion) than when free in solution. Therefore, the diffusion coefficient can be used to investigate the ability of the micelle to dissolve organic probes.

In this chapter, the results of pulsed field gradient nuclear magnetic resonance (PFG-NMR) experiments used to compare the ability of polymeric and monomeric amino acid based surfactant to solubilize the organic solutes toluene, chlorobenzene, and benzyl alcohol are reported. It is well known that organic solutes partition into the core of micelles based upon their solubilities in water and hydrocarbon media. This phenomenon has been extensively studied for anionic, cationic, and neutral micelles by PFG-NMR and NMR relaxation techniques.\(^\text{20-28}\) When a hydrophobic solute molecule is placed in solution with surfactant micelles, the solute undergoes fast exchange on the NMR time-scale between the bulk solution and the interior of the micelles. Under these conditions, the diffusion coefficient measured for the solute in the PFG-NMR experiment, \(D_{\text{obs}}\), is given by Equation 5.1,\(^\text{28}\)

\[
D_{\text{obs}} = f_b \cdot D_{\text{micelle}} + (1 - f_b) \cdot D_{\text{free}} \tag{5.1}
\]

where \(f_b\) is the fraction of solute molecules associated with the micelles, \(D_{\text{free}}\) is the solute diffusion coefficient in free solution, and \(D_{\text{micelle}}\) is the diffusion coefficient of the surfactant. It should be noted that \(D_{\text{obs}}\) and \(f_b\) values are inversely related. The higher the \(D_{\text{obs}}\) value the lower the \(f_b\) value and vice-versa. The goal of the
solubilization experiments performed in this study was to compare $f_b$ and $D_{obs}$ values for three small aromatic probes associating with polymerized and the corresponding unpolymerized surfactant micelles to gain insight into the relative solubilization capabilities of these two kinds of surfactants.

In this chapter, the $f_b$ and $D_{obs}$ of organic probes are compared with polymers and monomers of two single amino acid surfactants, sodium undecanoyl valinate (SUV), and sodium undecanoyl glycinate (SUG), and three dipeptide surfactants; sodium undecanoyl leucyl-leucinate (SULL), sodium undecanoyl valyl-valinate (SUVV) and sodium undecanoyl glycyl-glycinate (SUGG) using PFG-NMR. In addition, the diffusion coefficients of the enantiomers of 1,1'-binaphthyl-2,2'-dihydrogen phosphate (BNP) in the surfactant poly SULL are calculated and used to investigate the differential binding of the enantiomers to the polymer.

Part I

EXPERIMENTAL

Chemicals. The amino acids and dipeptides were purchased from Sigma (St. Louis, MO). The surfactants were synthesized according to the procedure discussed in Chapter 2. Organic solutes, pure enantiomers of 1,1'-binaphthyl-2,2'-dihydrogen phosphate (BNP), and deuterium oxide were purchased from Aldrich (Milwaukee, WI).

NMR Diffusion Measurements. All NMR experiments were carried out on a Bruker DPX 300 MHz spectrometer equipped with an actively shielded z-gradient coil. The instrument's coil constant of 50.3 G/cm at 100% gradient strength was determined by carrying out pulsed gradient NMR experiments on a 10 mM $\beta$-
cyclodextrin sample. The bipolar pulse pair longitudinal encode-decode (BPPLED) pulse sequence shown in Figure 1.16 was used in all diffusion studies. This experiment has been shown to be effective in minimizing interference from magnetic field gradient induced eddy currents. In addition in the BPPLED experiment, transverse evolution times can be kept to a minimum, allowing for the detection of species with short spin-spin relaxation times. The latter is particularly important in the study of macromolecules where spin-spin relaxation rates can be up to an order of magnitude larger than spin-lattice relaxation rates.

In the BPPLED experiment, the NMR peak intensities, $S$, vary as a function of magnetic field gradient strength, $G$, as shown in Equation 4.2. In a typical NMR diffusion measurement 20-25 NMR spectra were collected with gradient amplitudes ranging from 5.0 to 35.0 G/cm. The diffusion time $\Delta$ was 250 ms, $\gamma$ was 2.0 ms, and $\tau$ was 1.2 ms in all experiments. All NMR studies were carried out at 25.0 °C.

After data collection, the free induction decays were apodized with 5 Hz line broadening, Fourier transformed, and phased with the spectrometer's Xwinmr software package. Resonances from the surfactant micelles, or aromatic solutes were then integrated and the natural log of the peak integrals was plotted versus $(\Delta-\delta/3-\tau/2)(\gamma G \delta)^2$. The PFG-NMR plot for toluene in polymeric and monomeric SULL is shown in Figure 5.1. As discussed in Chapter 4, the diffusion coefficient, $D$, is the negative slope of that line.
In diffusion experiments with amino acid based surfactants, a 200 mM solution of the surfactants was prepared in 99.98% D$_2$O. It has been observed that no aggregation of the polymeric surfactant occurs at this concentration. When investigating the binding of the aromatic solutes to the surfactant, solutions containing 1 mM of the organic probe (toluene, benzyl alcohol, or chlorobenzene) and 200 mM surfactants (200 mM equivalent monomer concentration (EMC) for polymeric surfactants) were prepared in D$_2$O.

In the studies of the solubilization of BNP enantiomers in poly SULL surfactant, 0.6 mg/mL of either the R- or S-enantiomer of BNP and 200 mM of the poly SULL were dissolved in a 0.10 M boric acid buffer at pH 10. The buffer was
prepared gravimetrically by dissolving an appropriate amount of boric acid in D$_2$O and then adjusting the pH with either DCl or NaOD.

Part II

RESULTS AND DISCUSSION

Table 5.1 shows the $D_{obs}$ and the $f_b$ values for each of the three solutes in solution with polymeric and monomeric single amino acid surfactants. Examination of the results shown in Table 5.1 indicate that the order of the hydrophobicity of these probes are benzyl alcohol $>$ toluene $>$ chlorobenzene, where benzyl alcohol is the most and chlorobenzene is the least hydrophilic solute. It should be pointed out that the $D_{obs}$ for the probe with higher solubility is smaller than the probe with lower solubility. For example, in Table 5.1, toluene has a smaller $D_{obs}$ value than benzyl alcohol when they are dissolved in SUV surfactants. From the calculated $f_b$ values ($f_b$ of 0.88 for toluene and $f_b$ of 0.36 for benzene alcohol) toluene is more soluble in SUV than benzyl alcohol.

The unpolymerized surfactant, SUG provided $D_{obs}$ (and $f_b$) values of 6.78 (0.36), 2.89 (0.87), and 2.63 (0.92) for benzyl alcohol, toluene, and chlorobenzene, respectively. The values observed for benzyl alcohol and toluene when they are dissolved in SUG are similar to those observed with the surfactant SUV. However, SUG solubilizes higher amounts of chlorobenzene than SUV surfactants. This is possibly due to the differences in polar head of these two surfactants. The surfactant SUG has two protons at alpha position while in SUV, one of the protons of SUG is substituted with an isopropyl group. The R-group of SUV is hydrophobic, therefore, in micellar form, it tends to aggregate and face the hydrophobic core of the micelle.
This may limit the solubility of a hydrophobic solute such as chlorobenzene in the micellar core of SUV.

From the data shown in Table 5.1, it is clear that organic probes diffuse more freely in the presence of the polymerized micelle compared to unpolymerized form. In other words, unpolymerized micelles dissolve a higher percentage of the organic probes as compared to their polymeric counterparts. Note that $f_b$ values of 0.88, and 0.78 were obtained for toluene, and chlorobenzene, respectively when they were dissolved in SUV surfactant, whereas poly SUV provided $f_b$ values of only 0.15,

<p>| Table 5.1 Solubilization of organic probes in single amino acid surfactants. |
|----------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Organic solute</th>
<th>$D_{obs}$ (cm$^2$s$^{-1}$x10$^{-6}$)</th>
<th>$f_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly SUV</td>
<td>benzyl alcohol</td>
<td>9.46</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>9.14</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>8.98</td>
<td>0.17</td>
</tr>
<tr>
<td>Poly SUG</td>
<td>benzyl alcohol</td>
<td>9.32</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>8.81</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>8.48</td>
<td>0.38</td>
</tr>
<tr>
<td>SUV</td>
<td>benzyl alcohol</td>
<td>6.45</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>2.27</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>3.34</td>
<td>0.78</td>
</tr>
<tr>
<td>SUG</td>
<td>benzyl alcohol</td>
<td>6.78</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>2.89</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>2.63</td>
<td>0.92</td>
</tr>
<tr>
<td>SUG (methyl terminated)</td>
<td>benzyl alcohol</td>
<td>5.29</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>2.41</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>1.6</td>
<td>0.94</td>
</tr>
</tbody>
</table>

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and 0.17 for these probes, respectively. Although SUV provided $f_b$ of 0.36 for benzyl alcohol, poly SUV was not able to dissolve this probe to any significant degree. Similarly, unpolymerized SUG always provided a better solubilization capacity for the organic probes examined in this study as compared to poly SUG.

Presumably if organic probes are solubilized deeply in micellar core, then the hydrophobicity of the core of the micelle should be important in solubilization of the organic probes. In Chapter 4, the $I_1/I_3$ ratio of pyrene was used to compare the hydrophobicity of the methyl (single bond) terminated with vinyl (double bond) terminated surfactants. In that study, it was observed that the methyl terminated surfactant are more hydrophobic than the vinyl terminated ones. In this chapter, I have compared the diffusion coefficients of the vinyl and methyl terminated SUG surfactants. Table 5.1 shows that methyl terminated SUG provided $D_{obs}$ of 5.29 for benzyl alcohol while a value of 6.78 was obtained for vinyl terminated SUG. This indicates that benzyl alcohol diffuses faster (or is less solubilized) in the presence of the vinyl terminated SUG surfactant. Not a significant difference in $D_{obs}$ of the other two probes (benzyl alcohol and toluene) was observed with these two kinds of unpolymerized surfactants. This suggests that the organic probes examined in this study possibly do not penetrate deeply into the micellar core of the unpolymerized micelle. However, the reason for the significant change in $D_{obs}$ for benzyl alcohol in the presence of the double bond terminated SUG is unclear at this time.

The results of the solubility study for dipeptide surfactants are shown in Table 5.2. Note that the dipeptide surfactant SUGG and the single amino acid surfactant SUG provided similar $D_{obs}$ and $f_b$ values for all three probes examined here. However,
SUVV provided better solubility for chlorobenzene compared to SUV. The single amino acid surfactant SUV provided a $D_{\text{obs}}$ (and $f_b$) value of 3.34 (0.78) while a $D_{\text{obs}}$ of 1.28 ($f_b$ of 0.95) was obtained with the dipeptide surfactant SUVV. Comparing the $D_{\text{obs}}$ of this probe with these two surfactants shows that chlorobenzene diffuses much faster when it is dissolved in SUV surfactant compared to SUVV. Solubilization of chlorobenzene in SUV and SUVV resulted in diffusion coefficients of 3.34 and 1.9, respectively. As mentioned earlier in this chapter, among the probes examined here, chlorobenzene is the most hydrophobic. Therefore, it is reasonable to assume that since dipeptide surfactants are less polar than the single amino acid surfactants, in the presence of the single amino acid surfactants, chlorobenzene is more free to move as compared to the dipeptide surfactants.

Again, similar to the results obtained for single amino acid surfactants, polymerization of the dipeptide surfactant reduces their ability to solubilize the organic probes examined in this study. Poly SUGG does not dissolve benzyl alcohol while unpolymerized SUGG provide an $f_b$ value of 0.4 for this probe. The solubility of toluene is more than six times higher in unpolymerized SUGG than the polymerized form.

In Chapter 4, I discussed that polymers always have lower aggregation numbers than their corresponding monomers. As mentioned in the experimental part, the solubility studies were carried out at 200 mM for monomers and 200 mM EMC for polymers. Consequently, the polymeric solutions of these surfactants have a higher micelle concentration as compared to the unpolymerized form. Although the polymeric micelles have a higher concentration of micelles (or hydrophobic pockets)
at 200 mM EMC compared to the monomeric micelles (at 200 mM), the monomers solubilize a higher fraction of the organic solutes than the polymers. It should be pointed out that organic probe incorporated (solubilized) within a micellar core increases the size of the unpolymerized micelle. In other words, the aggregation number of unpolymerized micelle increases upon solubilization of the organic probe.

Table 5.2 Solubilization of organic probes in dipeptide surfactants.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Solute</th>
<th>$D_{\text{obs}}$ (cm²s⁻¹x10⁻⁶)</th>
<th>$f_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly (SUGG)</td>
<td>benzyl alcohol</td>
<td>9.51</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>9.23</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>8.91</td>
<td>0.17</td>
</tr>
<tr>
<td>poly (SULL)</td>
<td>benzyl alcohol</td>
<td>9.51</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>9.72</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>9.49</td>
<td>0.11</td>
</tr>
<tr>
<td>poly (SUVV)</td>
<td>benzyl alcohol</td>
<td>9.48</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>9.31</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>9.50</td>
<td>0.11</td>
</tr>
<tr>
<td>SUGG</td>
<td>benzyl alcohol</td>
<td>6.22</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>2.46</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>1.90</td>
<td>0.95</td>
</tr>
<tr>
<td>SULL</td>
<td>benzyl alcohol</td>
<td>6.79</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>2.04</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>1.86</td>
<td>0.90</td>
</tr>
<tr>
<td>SUVV</td>
<td>benzyl alcohol</td>
<td>4.32</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>1.73</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>chlorobenzene</td>
<td>1.28</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Schematics of the possible interaction of the organic probes with unpolymerized and polymerized micelles are illustrated in Figures 5.2 and 5.3. As shown in Figure 5.2, upon solubilization of the organic probe in unpolymerized

![Figure 5.2 Schematic of the solubilization of organic probe in micellar core of the monomeric surfactant.](image)

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micelle, the size and aggregation number of the micelle increases. The surfactant molecules of the micelle open up and reorganize themselves to provide hydrophobic pockets for the solute. Polymerized micelles, on the other hand, have a rigid structure. Covalent stabilization in polymeric surfactants results in a more structured phase with greater steric constraints than the unpolymerized micelle. This rigidity may diminish
the ability of the polymer to create proper hydrophobic pockets for the solvation of organic probes. As shown in Figure 5.3, monomeric units of the polymers are covalently linked. Therefore, they cannot reorganize themselves or change their size upon solubilization of the organic probe. The polymeric micelle size and aggregation numbers do not change upon solubilization of the organic probes.

**Solubilization of Chiral Solutes.** As mentioned in Chapters 2 and 3, the difference in the observed mobilities of the enantiomers in EKC is achieved due to the formation of transient diastereomeric complexes with different binding strengths. As discussed in Chapter 2, the R-BNP elutes before the S-BNP when poly L,L-SULL is used as the chiral pseudostationary phases (CPSP) in EKC. Therefore, R-BNP interacts less with poly L,L-SULL than S-BNP. In the following chapter, the steady state fluorescence anisotropy values of R- and S-BNP were noted to be different in a chiral environment. The enantiomer that interacts stronger with poly L,L-SULL resulted in higher anisotropy values. Steady state fluorescence anisotropy measurement reveals the rotational motion of the fluorophore. It should be pointed out that the PFG-NMR technique provides a tool for measuring molecular motion as well. Presumably, the enantiomer that interacts stronger with chiral micelle will diffuse more slowly than its mirror image and will therefore have a smaller $D_{obs}$ as compared to the other enantiomer. According to the EKC results, S-BNP interacts stronger with L,L-SULL. Therefore, in PFG-NMR measurements, this enantiomer should have a smaller $D_{obs}$ value compared to R-BNP.

In Figure 5.4, the logarithms of the integrated signal intensities for the aromatic ring resonance of BNP enantiomers are plotted versus $\Delta-\delta/3-t/2(\gamma\delta)^2$. As
can be inferred from this plot, R-BNP diffuses faster than S-BNP. A diffusion coefficient of 3.50 and 3.03 was obtained for R-BNP and S-BNP, respectively.

In contrast to poly L,L-SULL, poly D,D-SULL provided higher $D_{\text{obs}}$ for S-BNP (4.00) than the R-BNP (3.4). Presumably, if S-BNP interacts stronger with poly L,L-SULL, then these enantiomers should interact less with D,D-SULL (with the opposite configuration) as compared to the R-BNP. From the diffusion coefficient values and Equation 5.1, $f_b$ were calculated and the results presented in Table 5.3. Note that poly L,L-SULL provided an $f_b$ value of 0.54 for S-BNP. This value is higher than the $f_b$ value for R-BNP (0.43).

![Figure 5.4 PFG-NMR plots for enantiomers of BNP in the presence of poly SULL surfactant.](image)

**Figure 5.4** PFG-NMR plots for enantiomers of BNP in the presence of poly SULL surfactant.
Table 5.3  Solubilization of BNP enantiomers in poly SULL surfactant.

<table>
<thead>
<tr>
<th></th>
<th>R-BNP</th>
<th>S-BNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly L,L-SULL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_{\text{obs}}$(cm$^2$s$^{-1}$x10$^{-6}$)</td>
<td>3.50</td>
<td>3.03</td>
</tr>
<tr>
<td>$f_b$</td>
<td>0.43</td>
<td>0.54</td>
</tr>
<tr>
<td>Poly D,D-SULL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_{\text{obs}}$(cm$^2$s$^{-1}$x10$^{-6}$)</td>
<td>3.40</td>
<td>4.00</td>
</tr>
<tr>
<td>$f_b$</td>
<td>0.46</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Conclusions. In this chapter, $D_{\text{obs}}$ and $f_b$ values for polymeric and monomeric surfactants are compared. The results indicated that polymerization reduces the solubilization capacity of the polymeric surfactants significantly. This is probably due to the fact that polymers have covalent linkage among their hydrophobic tails and they cannot reorganize their surfactant molecules upon solubilization of the organic probes. Conventional (unpolymerized) micelles, on the other hand, embrace the organic probe by reorganizing their surfactant molecules and increasing their aggregation number. Results of this study indicate that neutral analytes diffuse faster in the presence of the polymerized micelle compared to the unpolymerized form. From these data it can be suggested that polymerized micelles provide faster mass transfer for neutral solutes when used as a CPSP in EKC experiments, as compared to the unpolymerized micelle.

Examination of the diffusion coefficient of chiral organic probes indicated that in a chiral environment, the enantiomer that interacts stronger with the chiral selector diffuses slower as compared to its mirror image. These results are consistent with the results obtained with EKC and steady state fluorescence anisotropy in Chapters 2 and
3. Therefore, it can be concluded that PFG-NMR can be used to gain further insight into chiral recognition.

It should be noted that so far in this dissertation, a comparison of the EKC performances and physical properties of the polymeric and monomeric surfactants suggest that in general, polymeric surfactants are better CPSPs than their monomeric counterparts. Thus, the next chapter is devoted exclusively to polymeric surfactants. In the following chapter, polymeric surfactants are used to separate enantiomers of optically active analytes in different charge states.

REFERENCES


27. Stilbs, P. *Prog. NMR Spectrosc.* 1987, 19, 1.
Chapter 6
Chiral Separation in Electrokinetic Chromatography with Polymeric Surfactants

Part I. Effect of Number of Chiral Centers and Steric Factors on Chiral Separations Using Polymeric Dipeptide Surfactants

Previously, Shamsi et al. compared the single amino acid poly sodium undecanoyl valinate (SUV) with the dipeptide poly sodium undecanoyl valyl-valinate (SUW).\(^1\) Poly SUV has the single amino acid valine with one chiral center as the polar head while poly SUW has the valine-valine dipeptide with two chiral centers as the polar head group. In that study, the authors suggested that the synergistic effect of the multiple chiral centers may have resulted in improved chiral separation using dipeptide surfactants. The results of that study encouraged me to introduce more chiral centers in the polar head of the polymeric surfactants. Therefore, I synthesized two polymeric chiral dipeptide surfactants (PCDS), poly sodium undecanoyl \((L,L)\) isoleucyl-valinate (SUILV) with three chiral centers and poly sodium undecanoyl \((L,L)\) leucyl-valinate (SULV) with two chiral centers. In part I of this chapter, I have utilized these polymeric CPSP to separate enantiomers of chiral analytes in different charge states using electrokinetic chromatography (EKC). In addition, I also evaluated how steric factors located near the chiral center of the N-terminal amino acid of the dipeptide chiral surfactants affects chiral recognition.

The structures of SUILV and SULV are shown in Figure 6.1. As shown, the difference between these two polymers is in the N-terminal position of the dipeptide for each surfactant. The C-terminal amino acids of both polymeric dipeptide surfactants are valine. Therefore, it is reasonable to assign any differences in observed enantioseparation of these two dipeptide surfactants to the change in the N-terminal
amino acid or its impact on the structure of the CPSP. Furthermore, the two amino acids in the N-terminal position have a couple of significant differences which should be taken into account when exploring differences in chiral resolution with these two surfactants. The most obvious difference is the fact that SUILV has three chiral centers while SULV has two chiral centers (Figure 6.1).

Another factor, which must be considered, is steric hindrance. The α-chiral carbon of isoleucine in SUILV is attached to a sec-butyl group, whereas the α-chiral carbon of leucine in SULV is attached to an iso-butyl group. Thus, the N-terminal α-chiral center on the SUILV is more sterically hindered as compared to the N-terminal α-chiral center on SULV.
EXPERIMENTAL

**Chemicals.** The dipeptides ((L,L) isoleucine-valine, and (L,L) leucine-valine), undecylenic acid, N-hydroxysuccinimide, (±)-1,1'-bi-2-naphthol (BOH), (±)-1,1'-binaphtyl-2,2-diamine (BNA), (±)-1,1'-binaphtyl-2,2'-dihydrogen phosphate (BNP), (DL) aminoglutethimide (AGL), (DL) glutethimide (GL), (±) lorazepam (LR), (±)-2,2,2-trifluoro-1-(9-anthryl) ethanol (TFAE), oxazepam (OX), temazepam (TM), propranolol (Prop), alprenolol (Alp) and oxprenolol (Oxp) were purchased from Sigma (St. Louis, MO). The dipeptide surfactants were synthesized according to the procedure discussed in Chapter 2.

**Capillary Electrophoresis Procedure.** The EKC separations were performed on a Hewlett Packard (HP) 3D CE model #G1600AX. The fused silica capillary, effective length of 55 cm (to detection window), 50 μm i.d., with a total length of 63.5 cm, was purchased from Polymicro Technologies (Phoenix, AZ) and mounted in an HP capillary cartridge. The cartridge temperature was maintained at 25 °C for the separation of binaphthyl derivatives and 12 °C for all other enantiomeric separations. The running background electrolytes (BGEs) were prepared in triply distilled water; surfactants were added and the pH adjusted by adding either HCl or NaOH to the BGE. All solutions were filtered through a 0.45 μm membrane filter before use.

A new capillary was conditioned for 30 min with 1 N NaOH at 60 °C followed by 10 min with triply distilled water. The capillary was flushed with buffer for 2 min prior to injecting the sample. All analyte standard solutions were prepared in 1:1
methanol:water at 0.1-0.5 mg/mL. Samples were injected for 5 seconds at 10 mbar pressure. Separations were performed at +30 kV, with UV detection at 220 nm.

**Optimized Conditions.** The EKC conditions, using amino acid based surfactants are as follows: (1) binaphthyl derivatives: BNP; 30 mM equivalent monomer concentration (EMC) of PCDS, BOH and BNA; 6 mM EMC of PCDS, 10 mM sodium borate, 100 mM TRIS, pH 10.0 at 25 °C, (2) β-blockers: (Prop, Alp, Oxp) 18 mM EMC of PCDS, 50 mM sodium borate, 300 mM CAPS, pH 8.5 at 12 °C, (3) GL/AGL: 80 mM EMC of PCDS, 50 mM TRIS, pH 9.2 at 12 °C (4) benzodiazepines: TM; 20 mM EMC of PCDS, LR and OX; 12 mM EMC of PCDS, 25 mM TRIS, 25 mM sodium borate, pH 8.5 at 12 °C, (5) TFAE: 6 mM EMC of PCDS, 30 mM sodium borate, pH 10 at 12 °C.

**RESULTS AND DISCUSSION**

In order to investigate the effect of steric factors and the number of chiral centers on chiral separation of polymeric amino acid based surfactants, enantiomers of twelve analyte were separated. The analytes examined in this study vary in the chemical structure, charge, and degree of hydrophobicities. The discussion on the chiral separation of these chiral analytes follows.

**Enantioseparation of Binaphthyl Derivatives.** The initial set of compounds examined in this study was the binaphthyl derivatives BNP, BOH, and BNA. These compounds are atropisomers and therefore, do not have an asymmetric carbon but rather a chiral plane (C2 symmetry). The three binaphthyl derivatives examined in this study have varying degrees of hydrophobicity and charge states under the
experimental conditions used. For example, BNP is anionic, BOH partially anionic, and BNA is neutral at the optimized pH of 10 used for these studies.

No significant difference in enantiomeric resolution was observed with the three chiral center dipeptide surfactant SUILV compared to the two chiral center surfactant SULV for the enantiomeric separation of BOH and BNA (Figures 6.2 and 6.3). Both SUILV and SULV resolved the enantiomers of BNA with a resolution of about 5.1. Similarly, SUILV and SULV provided respective resolution values of 5.1 and 4.9 for the enantiomers of BOH. In contrast, the three chiral center dipeptide surfactant SUILV separated the enantiomers of BNP with a resolution of 3.5, while SULV with two chiral centers was able to resolve BNP with an enantiomeric resolution of 7.8 (Figure 6.4). From the chromatographic data shown in Table 6.1, it can be concluded that even though poly SUILV interacts stronger with the enantiomers of BNA and BOH than SULV, the enantiomeric resolution of these analytes does not change. In contrast to BOH and BNA, the k' value was higher for SULV compared to SUILV. Furthermore, it is interesting to note that the more sterically hindered, more polar analyte (BNP) showed a significant difference in enantiomeric selectivity using SUILV as compared to SULV.

Enantioseparation of β-Blockers. The β-blockers (Oxp, Alp, and Prop) are a family of compounds that are used for the treatment of hypertension. In most cases, the (S)-enantiomer of these drugs is more potent than the R-enantiomer. The structures of these positively charged compounds are similar. They all possess an alkanolamine side chain attached to one
Figure 6.2 Enantiomeric separation of BNA (a) SUILV, and (b) SULV, CE condition: 6 mM EMC of PCDS, 10 mM sodium borate, 100 mM TRIS, pH 10 at 25 °C, UV detection at 220 nm. Rs values are the average of three consecutive runs.

Figure 6.3 Enantiomeric separation of BOH (a) SUILV and (b) SULV, CE condition: 6 mM EMC of PCDS, 10 mM sodium borate, 100 mM TRIS, pH 10 at 25 °C, UV detection at 220 nm. Rs values are the average of three consecutive runs.
or two aromatic rings (Figure 6.5). As with BOH and BNA, no significant differences in enantiomeric resolution or enantioselectivity of the β-blockers was observed with poly SUILV as compared to SULV. It should be mentioned that although the absolute errors associated with the resolution values listed in Table 6.1 for the β-blockers may be approximately the same as the error observed for other analytes, the relative errors (not listed) are much larger for the β-blockers due to the relatively small resolution values achieved for the β-blockers. Poly SUILV provided enantiomeric resolution values of 1.20, 1.40, and 1.78 for Oxp, Alp, and Prop, respectively. In contrast, poly SULV resolved these enantiomers with resolutions of 0.91, 0.74 and 1.40, respectively (Figure 6.5).
<table>
<thead>
<tr>
<th>Enantiomer</th>
<th>Rs</th>
<th>k'</th>
<th>α</th>
<th>Rs</th>
<th>k'</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP</td>
<td>3.5 ±0.1</td>
<td>1.14</td>
<td>1.06</td>
<td>7.8±0.3</td>
<td>1.22</td>
<td>1.08</td>
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<tr>
<td>BOH</td>
<td>5.1±0.1</td>
<td>1.12</td>
<td>1.10</td>
<td>4.9±0.1</td>
<td>0.98</td>
<td>1.06</td>
</tr>
<tr>
<td>BNA</td>
<td>5.1±0.2</td>
<td>1.16</td>
<td>1.10</td>
<td>5.1±0.3</td>
<td>0.94</td>
<td>1.04</td>
</tr>
<tr>
<td>Alp</td>
<td>0.74±0.44</td>
<td>0.36</td>
<td>1.04</td>
<td>1.4±0.2</td>
<td>0.38</td>
<td>1.04</td>
</tr>
<tr>
<td>Oxp</td>
<td>0.91±0.23</td>
<td>1.12</td>
<td>1.02</td>
<td>1.20±0.46</td>
<td>1.15</td>
<td>1.02</td>
</tr>
<tr>
<td>Prop</td>
<td>1.40±0.31</td>
<td>1.72</td>
<td>1.02</td>
<td>1.78±0.10</td>
<td>1.77</td>
<td>1.03</td>
</tr>
<tr>
<td>AGL</td>
<td>6.02±0.48</td>
<td>0.68</td>
<td>1.08</td>
<td>6.53±0.06</td>
<td>0.68</td>
<td>1.09</td>
</tr>
<tr>
<td>GL</td>
<td>1.50±0.01</td>
<td>1.11</td>
<td>1.01</td>
<td>1.41±0.01</td>
<td>1.12</td>
<td>1.02</td>
</tr>
<tr>
<td>TM</td>
<td>2.01±0.06</td>
<td>1.43</td>
<td>1.04</td>
<td>4.02±0.07</td>
<td>1.24</td>
<td>1.02</td>
</tr>
<tr>
<td>LR</td>
<td>3.49±0.04</td>
<td>1.40</td>
<td>1.04</td>
<td>2.68±0.05</td>
<td>1.13</td>
<td>1.03</td>
</tr>
<tr>
<td>OX</td>
<td>5.43±0.06</td>
<td>1.13</td>
<td>1.06</td>
<td>1.61±0.03</td>
<td>1.31</td>
<td>1.02</td>
</tr>
<tr>
<td>TFAE</td>
<td>1.40±0.03</td>
<td>1.91</td>
<td>1.08</td>
<td>0.74±0.03</td>
<td>2.04</td>
<td>1.02</td>
</tr>
</tbody>
</table>

*±0.01 average standard deviation of three consecutive CE runs.
Previous studies in our research group have shown that electrostatic interaction between the positively charged β-blockers and the negatively charged dipeptide surfactants appears to be the primary factor in the binding of this class of compounds to the polar head of the micelle polymers. Therefore, it is mainly the C-terminal or outside amino acid (valine) which is involved in enantiomeric recognition of these relatively hydrophilic, cationic (i.e., Prop, Alp, Oxp) analytes. In other words, the N-terminal amino acids, i.e., leucine of poly SULV and isoleucine of SUILV, do not contribute significantly to the enantiomeric recognition of the β-blockers. This is consistent with very similar capacity factors and selectivity factors obtained for all three enantiomeric pairs of β-blockers using either poly SUILV or poly SULV.

Figure 6.5 Enantiomeric separations of β-blockers (a) SUILV and (b) SULV, CE condition: 18 mM EMC of PCDS, 50 mM sodium borate, 300 mM CAPS, pH 8.5 at 12 °C, UV detection at 220 nm. Rs values are the average of three consecutive runs.
**Enantioseparation of Glutethimide/Aminoglutethimide.** Glutethimide (GL) and aminoglutethimide (AGL) have been used extensively as anticonvulsant drugs. As shown in Figure 6.6, the difference in the structures of GL and AGL is that AGL has an amine moiety attached to its benzene ring as compared to GL with no functional group on the benzene ring. The structures of these two analytes suggest that GL is more hydrophobic than AGL. This is consistent with the elution order of AGL and GL. A comparison of the enantiomeric separation of AGL and GL using SUILV and SULV is shown in Figure 6.6. The former PCDS provides a resolution of 5.8 for AGL, while the latter resolves the enantiomers of this analyte with a resolution of 6.5. The resolution values for the enantiomers of GL with SUILV and SULV are 1.5 and 1.4, respectively. Note that the enantiomeric resolution of AGL (containing an extra hydrogen bonding site) is always larger than GL using either SUILV or SULV. Furthermore, analyses of the data indicate that the third chiral center of SUILV does not significantly improve the chiral resolution nor does it have much of an impact on the capacity factor and enantioselectivity of GL and AGL.

**Enantioseparation of Benzodiazepines.** The effect of two chiral centers vs. three chiral centers was further investigated with three neutral benzodiazepines (TM, LR and OX). These compounds are used as hypnotics, tranquilizers, and anticonvulsants. Although the benzodiazepine class of analytes possess similar aromatic skeletons, the difference lies in the number and type of substituents attached to the aromatic ring. For example, note the methyl group located on the nitrogen in the seven member ring of TM and the chlorine in the ortho position of the lower benzene ring of LR (Figures 6.7 and 6.8).
Several interesting differences in resolution and selectivity factors were observed for the benzodiazepams. Although TM interacts stronger with SUILV as compared to SULV, the enantiomers of TM are better resolved with the latter (Figure 6.7). Poly SUILV resolved the enantiomers of TM with a resolution of 2.0 and a selectivity factor of 1.04, while SULV was able to separate the enantiomers of TM with a resolution of 4.0 and a selectivity factor of 1.02 (Table 6.1). In contrast, the capacity factor for OX indicates that the enantiomers of this analyte interact stronger with SUILV than SULV resulting in an improvement in enantioselectivity. Note that the capacity factors for OX are 1.06 for SUILV and 1.02 for SULV. Examination of the structures of TM and OX suggests that the latter analyte has more hydrogen bonding sites and is less sterically hindered. The methyl group of TM may affect
chiral selectivity in two ways. First, the methyl group may block the hydrogen
binding site(s) of TM; second, it increases steric hindrance.

Figure 6.7 Enantiomeric separation of TM (a) SUILV and (b) SULV. CE
conditions: 20 mM EMC of PCDS, 25 mM TRIS 25 mM
sodium borate, pH 8.5 at 12 °C. UV detection at 220 nm. Rs
values are the average of three consecutive runs.

Lorazepam is the third benzodiazepine compound investigated in this study. As
shown in Figure 6.8, poly SUILV with three chiral centers provided better chiral
separation for the enantiomers of LR compared to poly SULV with two chiral centers.
Poly SUILV was able to provide a Rs value of 3.2 for these enantiomers while a Rs
value of only 2.7 was obtained with poly SULV. Lorazepam and OX differ by a
chlorine atom located to the ortho position of the free benzene ring of LR. The
presence of the extra chlorine group may limit the movement of the benzene ring
inside the micellar cavity resulting in a decline in enantioselectivity of LR compared
to OX with these two polymeric surfactants.
Enantiomeric separation of LR/OX (a) SUILV and (b) SULV. CE conditions: 12 mM EMC of PCDS, 25 mM TRIS 25 mM sodium borate, pH 8.5 at 12 °C. UV detection at 220 nm. Rs values are the average of three consecutive runs.

Enantioseparation of (±)-2,2,2-Trifluoro-1-(9-anthryl) Ethanol. The enantiomers of TFAE have been used in chiral NMR to resolve the hydrogen signals of various enantiomers. Figure 6.9 compares the separation of the TFAE enantiomers with the two polymeric surfactants, SULV and SUILV. Note the difference in enantiomeric resolution, i.e. a Rs value of 14. with SUILV and 0.7 with SULV. A comparison of $k'$ and $\alpha$ shown in Table 6.1 indicates a weaker interaction and relatively smaller enantioselectivity of this analyte with SUILV compared to SULV. This suggests that steric matching has more of an influence on chiral recognition than the number of chiral centers for TFAE.
Figure 6.9 Enantiomeric separation of TFAE (a) SUILV and (b) SULV. CE conditions: 6 mM EMC of PCDS, 30 mM sodium borate, pH 10 at 12 °C, UV detection at 220 nm. Rs values are the average of three consecutive runs.

Conclusions. Of the twelve chiral analytes examined in this study, LR, OX, and TFAE showed an improvement in chiral recognition with the three chiral center dipeptide surfactant SUILV compared to the two chiral center dipeptide surfactant SULV. In contrast, the enantiomeric resolution of BNP and TM decreased with the former compared to the latter. In addition, no significant differences were observed when comparing the three chiral center surfactants versus the two chiral center surfactants for BOH, BNA, Alp, Oxp, Prop, AGL, and GL. The results suggest that in some cases the presence of sec-butyl group of SUILV may limit access of the analytes.
to the second chiral center of this surfactant, resulting in a decline in chiral recognition of BNP and TM. However, with other analytes, it appears that steric repulsion by the methyl group of the sec-butyl moiety may assist in stereoselectivity of the polymer toward the analytes, resulting in an improvement in the chiral separation of OX, LR, and TFAE. From this study, it can be concluded that the presence of the third chiral center may not affect chiral separation of dipeptides significantly. Therefore, in second part of this chapter, polymeric dipeptide surfactants with one or two chiral center(s) were utilized to separate enantiomers of a wide spectrum of neutral chiral analytes.

**Part II. Chiral Separation of Neutral Enantiomers Using Amino Acid Based Surfactants**

In this part of Chapter 6, chiral selectivities of seven neutral analytes with eighteen amino acid based surfactants are compared. These analytes are divided into two classes, Class I and Class II. Class I analytes (laudanosoline, norlaudanosoline, laudanosine, and chlorthalidone) have the chiral center located on a hydrocarbon ring, which makes the chiral center of these analytes more sterically hindered than Class II. Class II compounds (benzoin, benzoin methyl ether, and benzoin ethyl ether) have the chiral center located in a less sterically hindered, more flexible environment. Several different aspects of the surfactants such as single amino acid versus dipeptide, amino acid order, steric factors, and number and position of the chiral centers on chiral selectivity are investigated.
EXPERIMENTAL

Chemicals. Single amino acids, dipeptides, and racemate mixture of chiral analytes were purchased from Sigma (St. Louis, MO). Synthesis of amino acid based surfactants is discussed in Chapter 2.

Capillary Electrophoresis Procedure. The EKC separations were performed on a Hewlett Packard (HP) 3D CE model #G1600AX. The fused silica capillary, effective length of 55 cm (to detection window), 50 μm i.d., with a total length of 63.5 cm, was purchased from Polymicro Technologies (Phoenix, AZ) and mounted in an HP capillary cartridge. The cartridge temperature was maintained at 12 °C for the separation of all analytes examined in this study. The running background electrolytes which contained 30 mM sodium phosphate were prepared in triply distilled water and pH adjusted to 7. All solutions were filtered through a 0.45 μm membrane filter before use. All analyte standard solutions were prepared in 1:1 methanol:water at 0.3-0.5 mg/mL. Samples were injected for 5 seconds at 10 mbar pressure. Separations were performed at +30 kV, with UV detection at 220 nm.

RESULTS AND DISCUSSION

Under the condition used for this study, pH 7, all the analytes examined in this section are neutral. However, as shown in Figure 6.10, the environments of the chiral centers in these analytes are different. As previously mentioned, Class I analytes have more sterically hindered chiral centers compared to Class II. Optimum chiral selectivity for Class I compounds was determined to be between 6-10 mM equivalent monomer concentrations (EMC) of the polymeric surfactants. On the other hand, optimum selectivities of the Class II analytes examined in this study were achieved.
around 50 mM EMC. It is known that steric forces play a major role in chiral recognition. Since the steric “forces” of class I analytes is relatively large, less concentration of chiral selector is required to achieve optimum chiral selectivity. Class II analytes, on the other hand, do not have strong steric “forces”. Therefore, higher concentrations of polymeric surfactants are required for optimum chiral selectivity.
Tables 6.2 and 6.3 show the chiral selectivity of these analytes with polymeric single amino acid and dipeptide surfactants. The purpose of this study is to compare the chiral selectivity of the Class I and Class II analytes with eighteen polymeric amino acid based surfactants. These surfactants are all possible chiral single amino acid and dipeptide surfactants of glycine, L-alanine, L-valine, and L-leucine. The structure and abbreviations for these surfactants are shown in Figure 2.1. I will begin by comparing the chiral recognition ability of single amino acid surfactants to dipeptide surfactants.

**Single Amino Acid vs. Dipeptide Surfactants.** In this section, the chiral selectivity of three polymeric chiral single amino acids SUA, SUV, and SUL and three PCDS, SUAA, SUVV, and SULL are compared. The single amino acid surfactants examined in this study all possess one chiral center with two carbonyls and one amide moiety, while the dipeptide surfactants contain two chiral centers, three carbonyls and two amide moieties in their polar heads. The differences in polar heads of these two classes of surfactants indicate that dipeptides provide more hydrogen bonding sites, and more possible chiral interaction sites, as compared to the single amino acid surfactants. It should be noted that single amino acids are more polar than dipeptide surfactants. In the following section, the chiral selectivity of Class I and Class II analytes are examined with the aforementioned polymeric surfactants.

**Class I Analytes.** All three single amino acid surfactants examined in this study resolved the enantiomers of norlaudanosoline. Polymers of SUV, and SUL provided $\alpha$ values of 1.136 and 1.127 for the enantiomers of this analyte. These
Table 6.2  Chiral selectivity of Class I analytes with eighteen polymeric single amino acid and dipeptide surfactants.

<table>
<thead>
<tr>
<th></th>
<th>laudanosoline</th>
<th>laudanosine</th>
<th>norlaudanosoline</th>
<th>Chlorthalidone</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUA</td>
<td>1.060</td>
<td>1.018</td>
<td>1.098</td>
<td>1.124</td>
</tr>
<tr>
<td>SUV</td>
<td>1.052</td>
<td>1</td>
<td>1.136</td>
<td>1.094</td>
</tr>
<tr>
<td>SUL</td>
<td>1.057</td>
<td>1</td>
<td>1.127</td>
<td>1.077</td>
</tr>
<tr>
<td>SUGA</td>
<td>1.051</td>
<td>1.016</td>
<td>1.067</td>
<td>1.040</td>
</tr>
<tr>
<td>SUGV</td>
<td>1.016</td>
<td>1.028</td>
<td>1.045</td>
<td>1</td>
</tr>
<tr>
<td>SUGL</td>
<td>1.013</td>
<td>1.014</td>
<td>1.038</td>
<td>1</td>
</tr>
<tr>
<td>SUAG</td>
<td>1.044</td>
<td>1.021</td>
<td>1.058</td>
<td>1.094</td>
</tr>
<tr>
<td>SUAA</td>
<td>1.097</td>
<td>1.020</td>
<td>1.114</td>
<td>1.128</td>
</tr>
<tr>
<td>SUAV</td>
<td>1.038</td>
<td>1.035</td>
<td>1.065</td>
<td>1.096</td>
</tr>
<tr>
<td>SUAL</td>
<td>1.028</td>
<td>1</td>
<td>1.031</td>
<td>1.082</td>
</tr>
<tr>
<td>SUVG</td>
<td>1.027</td>
<td>1</td>
<td>1.069</td>
<td>1.113</td>
</tr>
<tr>
<td>SUVA</td>
<td>1.054</td>
<td>1.021</td>
<td>1.135</td>
<td>1.172</td>
</tr>
<tr>
<td>SUVV</td>
<td>1.014</td>
<td>1.040</td>
<td>1.063</td>
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<tr>
<td>SUVL</td>
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<td>1</td>
<td>1.028</td>
<td>1.159</td>
</tr>
<tr>
<td>SULG</td>
<td>1.022</td>
<td>1</td>
<td>1.047</td>
<td>1.066</td>
</tr>
<tr>
<td>SULA</td>
<td>1.066</td>
<td>1.024</td>
<td>1.143</td>
<td>1.107</td>
</tr>
<tr>
<td>SULV</td>
<td>1.047</td>
<td>1.082</td>
<td>1.111</td>
<td>1.113</td>
</tr>
<tr>
<td>SULL</td>
<td>1.041</td>
<td>1.107</td>
<td>1.081</td>
<td>1.107</td>
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</tbody>
</table>

Table 6.3  Chiral selectivity of Class II analytes with eighteen polymeric single amino acid and dipeptide surfactants.

<table>
<thead>
<tr>
<th></th>
<th>benzoin</th>
<th>benzoin methyl</th>
<th>benzoin ethyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUA</td>
<td>1.022</td>
<td>1.013</td>
<td>1.009</td>
</tr>
<tr>
<td>SUV</td>
<td>1.033</td>
<td>1.016</td>
<td>1.010</td>
</tr>
<tr>
<td>SUL</td>
<td>1.042</td>
<td>1.021</td>
<td>1.014</td>
</tr>
<tr>
<td>SUGA</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SUGV</td>
<td>1.008</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SUGL</td>
<td>1.026</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SUAG</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SUAA</td>
<td>1.019</td>
<td>1.013</td>
<td>1.007</td>
</tr>
<tr>
<td>SUAV</td>
<td>1.025</td>
<td>1.011</td>
<td>1.006</td>
</tr>
<tr>
<td>SUAL</td>
<td>1.031</td>
<td>1.013</td>
<td>1.021</td>
</tr>
<tr>
<td>SUVG</td>
<td>1.021</td>
<td>1.015</td>
<td>1.013</td>
</tr>
<tr>
<td>SUVA</td>
<td>1.037</td>
<td>1.017</td>
<td>1.021</td>
</tr>
<tr>
<td>SUVV</td>
<td>1.035</td>
<td>1.020</td>
<td>1.030</td>
</tr>
<tr>
<td>SUVL</td>
<td>1.054</td>
<td>1.022</td>
<td>1.033</td>
</tr>
<tr>
<td>SULG</td>
<td>1.018</td>
<td>1.018</td>
<td>1.012</td>
</tr>
<tr>
<td>SULA</td>
<td>1.040</td>
<td>1.019</td>
<td>1.011</td>
</tr>
<tr>
<td>SULV</td>
<td>1.046</td>
<td>1.029</td>
<td>1.013</td>
</tr>
<tr>
<td>SULL</td>
<td>1.060</td>
<td>1.042</td>
<td>1.019</td>
</tr>
</tbody>
</table>

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values are significantly higher than the $\alpha$ values obtained with their dipeptide counterparts poly SUVV ($\alpha$ of 1.063), and poly SULL ($\alpha$ of 1.081). However, note that the dipeptide surfactant poly SUAA provided a chiral selectivity of 1.114, while an $\alpha$ value of 1.098 was obtained using poly SUA. Among these six single amino acid and dipeptide surfactants, poly SUV provided the best chiral selectivity for the enantiomers of norlaudanosoline.

Laudanosoline has a very similar structure to norlaudanosoline. As shown in Figure 6.10, the only difference in structure of these two analytes is that norlaudanosoline has a secondary amine while laudanosoline has a tertiary amine. The single amino acid surfactants poly SUV and poly SUL provided $\alpha$ values of 1.052 and 1.057, respectively for enantiomers of laudanosoline. Similar to norlaudanosoline, these values are higher than the $\alpha$ values provided by poly SUVV ($\alpha$ of 1.014) and poly SULL ($\alpha$ of 1.041). However, the dipeptide surfactant poly SUAA provided significantly better chiral selectivity ($\alpha$ of 1.097) as compared to the single amino acid surfactant poly SUA ($\alpha$ of 1.060).

The next analyte examined, laudanosine, also has structure similar to norlaudanosoline and laudanosoline. As shown in Figure 6.10, the difference in structure of laudanosine and laudanosoline is that the hydroxyl groups of laudanosine are methylated. Methylation of the hydroxyl groups of laudanosine result in a more hydrophobic and sterically hindered compound. Table 6.2 shows the chiral selectivity of laudanosine with single amino acid and dipeptide surfactants. Poly SUA is the only single amino acid surfactant that provided some chiral selectivity for enantiomers of
this analyte. Although the polymers of the single amino acid surfactants SUV and SUL did not resolve the enantiomers of laudanosine, a chiral selectivity of 1.040 and 1.107, respectively, was obtained using the dipeptide surfactants poly SUVV and poly SULL. The next class I analyte to be examined is chlorthalidone.

Chlorthalidone is structurally very different from the other three analytes in this group. However, similar to laudanosine and laudanosoline, the dipeptide surfactants provided better chiral selectivity for the enantiomers of chlorthalidone. As shown in Table 6.2, the single amino acid surfactants poly SUA, poly SUV, and poly SUL provided \( \alpha \) values of 1.124, 1.094, and 1.077, respectively. Note that the \( \alpha \) values of the dipeptide surfactants for these amino acids, poly SUAA (\( \alpha \) of 1.128), poly SUVV (\( \alpha \) of 1.156), and poly SULL (\( \alpha \) of 1.107) are always similar to or higher than that of the single amino acid surfactants.

**Class II Analytes.** The class II analytes examined in this study are benzoin derivatives. All polymers of the single amino acid surfactants SUA, SUV, and SUL and the dipeptide surfactants poly SUAA, poly SUVV, and poly SULL provide some chiral recognition for the enantiomers of the benzoin derivatives examined in this study. However, the dipeptides provided better chiral selectivities. A chiral selectivity of 1.060, and 1.042 was obtained for enantiomers of benzoin, and benzoin methyl, respectively, using the dipeptide surfactant poly SULL as the CPSP. These \( \alpha \) values are higher than the \( \alpha \) values obtained with the single amino acid surfactant poly SUL. In addition, among these six single amino acid and dipeptide surfactants, the highest chiral selectivity (\( \alpha \) of 1.030) was achieved for enantiomers of benzoin ethyl when poly SUVV was used as the CPSP.
Examination of the chromatographic data indicates that, with the exception of norlaudanosoline where the single amino acid surfactant poly SUV provided a higher $\alpha$ value than the corresponding dipeptide (poly SUVV), PCDSs are better CPSP for the enantiomeric separation of the analytes examined in this section than the single amino acid surfactants. Note that among the three PCDS SUAA, SUVV, and SULL, the least sterically hindered dipeptide surfactant, poly SUAA, provided the best chiral selectivity for enantiomers of laudanosoline, while the greatest $\alpha$ value for the other chiral analytes in class I and II was achieved with the more sterically hindered surfactants poly SULL and poly SUVV. This indicates that steric factors of the enantiomers, as well as, the steric factors of the polar head of the surfactants are important in chiral recognition. In the following sections, the effect of steric factors on chiral selectivity of these analytes are further investigated using a variety of other PCDS.

**Effect of Amino Acid Order in Chiral Recognition.** Billiot et al. have proposed that the amino acid order of PCDS is important in their performance in terms of chiral recognition. In that study, the authors compared the chiral recognition ability of poly SULV and poly SUVL. Baseline resolution of BNP enantiomers was observed using poly SULV, while no hint of chiral recognition of these enantiomers was obtained using poly SUVL. Note that the difference in the two surfactant polar heads examined by Billiot et al. is that in SULV, the larger amino acid, leucine, is located at the N-terminal position and valine is located at C-terminal position, while in SUVL, the position of the amino acids is reversed; valine is at N-terminal and leucine is the C-terminal amino acid. A similar approach is used in this study. The chiral
selectivity of Class I and Class II analytes with polymers of SUAV, SUAL, and SUVL were compared with that of poly SUVA, poly SULA, and poly SULV, respectively. As before, I will begin the discussion with Class I analytes first.

**Class I Analytes.** As shown in Table 6.2, better chiral selectivity was observed for norlaudanosoline when the larger of the amino acids was located in the N-terminal position of the PCDS. An $\alpha$ value of 1.143 was obtained for the enantiomers of this analyte using poly SULA, with the larger of the amino acid at the N-terminal position, compared to poly SUAL which resolved the enantiomers of norlaudanosoline with an $\alpha$ value of 1.031. Similarly, poly SUAV, and poly SUVL provided selectivity factors of 1.065, and 1.028, respectively, while selectivity factors of 1.135, and 1.111 were obtained with poly SUVA, and poly SULV, respectively. The same trend was observed when comparing the chiral selectivity of laudanosoline and laudanosine. For example, using poly SULV as the CPSP, chiral selectivities of 1.047 and 1.082 were obtained for the enantiomers of laudanosoline and laudanosine, respectively, while poly SUVL did not show any hint of chiral recognition for enantiomers of these analytes. It should be pointed that there was one exception. Poly SUAV, with the larger of the amino acid at the C-terminal position, provided better chiral selectivity for the enantiomers of laudanosine as compared to poly SUVA.

An examination of the effect of the order of the amino acids on chiral selectivity of chlorthalidone indicates that the amino acid order does not significantly affect the chiral selectivity of chlorthalidone. As can be seen in Table 6.2, an $\alpha$ value of 1.107 was observed with poly SULA, while poly SUAL had a selectivity factor of 1.082. However, poly SUVL provided a higher selectivity factor than SULV. Poly
SUVL with the larger of the amino acid at C-terminal position provided an α value of 1.159, while poly SULV, with larger of the amino acid at N-terminal, resulted in an α value of 1.113 for the enantiomers of chlorthalidone. In contrast, poly SUVA (α of 1.172), with the larger amino acid at the N-terminal position is a better CPSP for the enantiomers of this optically active analyte than poly SUAV (α of 1.096), with the larger amino acid in the C-terminal position.

In summary, with the exception of chlorthalidone, better enantioselectivity for sterically hindered analytes (Class I) was obtained when the larger of the amino acids of the PCDS is in the N-terminal position. Billiot et al. proposed a model to explain the interaction of sterically hindered chiral enantiomers with PCDS. According to that model, when the larger of the amino acids of the PCDS is located in the C-terminal position, this limits access of bulky analytes to the N-terminal chiral center of PCDS, thus potentially decreasing its chiral selectivity.

**Class II Analytes.** No consistent trend with regard to amino acid order was observed with class II enantiomers. Benzoin enantiomers were better separated with poly SUVL (α of 1.054) than poly SULV (α of 1.046). In contrast, poly SULA provided an α value of 1.04, while a chiral selectivity of 1.031 was obtained using poly SUAL. Similar to the enantiomers of benzoin, poly SUVL provided a greater α value (1033) for the enantiomers of benzoin ethyl compared to poly SULV (α of 1.013). However the chiral selectivity of these enantiomers was higher with poly SUAL (1.021) compared to poly SULA (1.011). Benzoin methyl, the other chiral analyte in Class II, was better separated with poly SULV (α of 1.029) than poly SUVL.
(α of 1.022). The reason that the enantiomers of the Class II analytes examined in this study do not follow any observable trend with regard to the order of amino acids is possibly due to the structure of this class of analytes. As mentioned earlier, Class II analytes have their chiral centers in a less sterically hindered environment as compared to Class I.

**Effect of Steric Factors on Chiral Selectivity.** The effect of steric factors on chiral selectivity is examined by varying the size of the R-group in the C- and/or the N-terminal position of dipeptide surfactants with two chiral centers. It should be noted that the size of the R-group increases from alanine to valine to leucine. Therefore, the C-terminal amino acid of SUAV (with valine at the C-terminal position) is more sterically hindered than that of SUAA (with alanine in the C-terminal position). In the next couple of sections, the chiral selectivity of Class I and Class II analytes are examined using a series of PDCS.

**Class I Analytes.** The chiral selectivity of laudanosoline enantiomers decreases when the N-terminal amino acid of the PCDS with two chiral centers is kept constant and the size of the C-terminal amino acids increases. As can be seen in Table 6.2, increasing the steric hindrance of PCDS in the series SUAA (α of 1.097), SUAV (α of 1.038), and SUAL (α of 1.028) resulted in a decline in chiral selectivity of the laudanosoline enantiomers. An even greater decline in selectivity of this analyte was observed with polymers of SUVA, SUVV and SUVL. Similarly, the selectivity factor of these enantiomers decreased from poly SULA (α of 1.066), to poly SULV (α of 1.047), to poly SULL (α of 1.041). However, no trend for the chiral selectivity of laudanosoline enantiomers was observed when the size of the C-
terminal amino acid of PCDS was kept constant and size of the N-terminal amino acid was increased. It should be noted that the chiral selectivity of laudanosoline enantiomers is favored by the less sterically hindered dipeptide surfactant poly SUAA ($\alpha$ of 1.097).

Similar to the enantiomers of laudanosoline, the chiral selectivity of norlaudanosoline enantiomers decreases when the size of the C-terminal amino acid of PCDS increases and size of the N-terminal is kept constant. Interestingly, with one exception, when the size of the C-terminal of PCDS is kept constant and the size of the N-terminal amino acid increases, the chiral selectivity of these enantiomers increases also. The exception was observed with poly SUAL and poly SUVL. An $\alpha$ value of 1.031 was obtained with poly SUAL which is slightly larger than the $\alpha$ value obtained with poly SUVL (1.028). Of these surfactants, poly SULA provided the greatest chiral selectivity for the enantiomers of norlaudanosoline.

The effect of steric factors on chiral recognition was different for laudanosine than what was observed for norlaudanosoline and laudanosoline. No significant difference in the chiral selectivity of laudanosine was observed for the polymers of SUAA, SUVA, and SULA. Chiral selectivity values of 1.020, 1.021, and 1.024, respectively, were obtained for the enantiomers of laudanosine. In contrast, the chiral selectivity of laudanosine increased in the series poly SUAV ($\alpha$ of 1.035), poly SUVV ($\alpha$ of 1.040), and poly SULV ($\alpha$ of 1.082). Although poly SUAL and poly SUVL did not provide any chiral selectivity for the enantiomers of laudanosine, an $\alpha$ value of 1.107 was obtained for these enantiomers with poly SULL. It should be pointed out that laudanosine enantiomers do not follow any definite trends with respect to the
steric factors. However, poly SULL with the most sterically hindered polar head provided the best chiral selectivity for these enantiomers. As before, the next analyte to be examined is chlorthalidone.

A decrease in chiral selectivity of the enantiomers of chlorthalidone was observed from SUAA (\( \alpha \) of 1.128), to SUAV (\( \alpha \) of 1.096), to SUAL (\( \alpha \) of 1.082). However, poly SULV provided an \( \alpha \) value of 1.113. This value is higher than the chiral selectivity values obtained with poly SULA (\( \alpha \) of 1.107) and poly SULL (\( \alpha \) of 1.107). Similarly, no trend was observed when the size of the N-terminal amino acid of PCDS was kept constant and the size of the C-terminal amino acid increased. It is interesting to note that the greatest chiral selectivity of these enantiomers was achieved when valine is located at the N-terminal position. Polymers of SUVA, SUVV, and SUVL provided \( \alpha \) values of 1.172, 1.156, and 1.159, respectively. These values are among the highest \( \alpha \) values obtained for these enantiomers.

**Class II Analytes.** An examination of the effect of steric factors on the chiral selectivity of benzoin and benzoin methyl indicates that when the size of the C-terminal amino acid is kept constant and size of the N-terminal amino acid increases, the chiral selectivity of these enantiomers increases. For example, as shown in Table 6.3, the \( \alpha \) values for the enantiomers of benzoin increases from poly SUAA (\( \alpha \) of 1.019), to poly SUVA (\( \alpha \) of 1.037), and poly SULA (\( \alpha \) of 1.040). Interestingly, a similar trend was observed when the size of the N-terminal amino acid was kept constant and the size of the C-terminal amino acid was increased. Poly SULL, the PCDS which has the largest amino acid at both the C- and N-terminal position,
provided $\alpha$ values of 1.060 and 1.042 for the enantiomers of benzoin and benzoin methyl, respectively. It should be mentioned that these are the highest values among the $\alpha$ values shown Table 6.3, for the enantiomers of these analytes. Therefore, it can be concluded that, for the surfactants examined in this study, the chiral selectivity of benzoin and benzoin methyl enantiomers is favored by an increase in steric factors in the polar head group of the PCDS.

Similar to benzoin and benzoin methyl, higher $\alpha$ values for the enantiomers of benzoin ethyl were achieved when the size of the N-terminal amino acid of PCDS was kept constant and the size of the C-terminal amino acid was increased. Note that the $\alpha$ values increase in the series of poly SULA ($\alpha$ of 1.011), poly SULV ($\alpha$ of 1.013) and poly SULL ($\alpha$ of 1.019). However, no trend was observed when the size of the C-terminal amino acids was kept constant and size of the N-terminal amino acid increases. The best chiral selectivity of these enantiomers was achieved using polymers of SUVV ($\alpha$ of 1.030) and SUVL ($\alpha$ of 1.033).

**Effect of the Position and Number of the Chiral Centers on Chiral Selectivity of Polymeric Dipeptide Surfactant.** The effect of the position of the chiral center on chiral selectivity of class I and class II analytes was examined using six single chiral center PCDS; poly SUAG, poly SUVG, poly SULG, poly SUGA, poly SUGV, and poly SUGL. In three of these surfactants, poly SUAG, poly SUVG, and poly SULG, the chiral center is located at the N-terminal position of the PCDS. In the other three surfactants (poly SUGA, poly SUGV and poly SUGL) the chiral center is located at the C-terminal position.
In Chapter 2, I examined the effect of depth of penetration of the analyte into the micellar core of the polymeric and monomeric dipeptide surfactant on chiral recognition was examined. In that chapter, the chiral selectivity of diastereomeric surfactants of SULL was employed to investigate the depth of the penetration of the analyte into the micellar core. The depth of the penetration can also be examined using these surfactants, since only one of the amino acids of the PCDS are chiral. For highly hydrophobic analytes that penetrate deep into the core of the polymeric micelle, little or no chiral selectivity would be expected when the N-terminal amino acid is achiral. Interaction with the C-terminal amino acid is preferred for highly hydrophilic chiral analytes. On the other hand, moderately hydrophobic analytes may interact with both the C- and N-terminal amino acid of the PCDS.

**Class I Analytes.** As shown in Table 6.2, all six single chiral center PCDS (SUGA, SUGV, SUGL, SUAG, SUVG, and SULG) provided some chiral selectivity for the enantiomers of norlaudanosoline. Polymers of SUGV and SUGL, with the chiral centers located at the C-terminal position, provided chiral selectivities of 1.045, and 1.038, respectively, and \( \alpha \) values of 1.069, and 1.047 were obtained, respectively with poly SUVG and poly SULG in which the chiral centers are located at the N-terminal position. Consequently, in can reasonably be concluded that the enantiomers of this analyte interact with both the C- and N-terminal amino acids of the PCDS. Similar results were observed for the enantiomers of laudanosoline.

In the case of laudanosine, poly SUGV and poly SUGL, with chiral center at C-terminal, provided selectivity values of 1.028, and 1.014, respectively, while no chiral selectivity of these enantiomers was obtained using poly SUVG and poly SULG.
(with chiral center at N-terminal). However, α values of 1.016 and 1.021 were obtained using poly SUGA and poly SUAG.

Similar to laudanosine, both poly SUAG and poly SUGA provided some chiral selectivities for enantiomers of chlorthalidone. However, poly SUVG and poly SULG provided α values of 1.113, and 1.066, respectively, for enantiomers of chlorthalidone, while no chiral recognition of these enantiomers was observed using SUGV and SUGL. From this data, it appears that laudanosine and chlorthalidone enantiomers most probably interact with both chiral centers of PCDS.

Class II Analytes. From the enantioselectivity data shown in Table 6.3, it can be reasonably be concluded that benzoin methyl and benzoin ethyl interact preferentially with the N-terminal amino acid of single chiral center PCDS. Poly SUVG and poly SULG provide selectivity factors of 1.015, and 1.018, for enantiomers of methyl benzoin, respectively. In addition, the enantiomers of ethyl benzoin were separated with selectivity values of 1.013 and 1.011, respectively, using poly SUVG and poly SULG. However, no chiral selectivity of the enantiomers of these analytes was achieved with the polymers of SUGV and SUGL. The reason that neither poly SUAG nor poly SUGA are able to enantiomerically resolve the optical isomers of these two analytes is possibly the small size of the polar head of these surfactants. As noted previously, the enantiomeric separation of the benzoin derivatives, examined in this study, appear to be favored by an increase in steric factors.

In contrast to benzoin methyl and benzoin ethyl, examination of the data suggests that the enantiomers of benzoin preferentially interact with both amino acids of the polymeric dipeptide surfactants examined in this study. Poly SULG, with the
chiral center at the C-terminal position, and poly SULG, with the chiral center at the N-terminal position, separated the enantiomers of benzoin with chiral selectivities of 1.026 and 1.018, respectively. In addition, polymers of SUVG and SUGV provided α values of 1.021 and 1.008, respectively. This difference in preferential interact site of benzoin compared to benzoin methyl and benzoin ethyl may be due to the hydrophobicity of these analytes. Benzoin is more hydrophilic than benzoin methyl and ethyl. Therefore, the former chiral analyte interacts closer to the surface of the micelle while the other two chiral analytes penetrate deeper into the micellar core of PCDS and interact preferentially with the N-terminal amino acid.

The effect of the number of chiral centers on the chiral selectivity was also examined using polymers of dipeptide surfactants SUAA, SUVV, and SULL with two chiral centers and their corresponding dipeptide surfactant with one chiral center (SUAG, SUGA, SUVG, SUGV, SULG, and SUGL). The chromatographic results suggest that the chiral selectivity of the analytes examined in this study (both class I and class II analytes) are higher with two chiral centers PCDS. For example, poly SUAA provided selectivity values of 1.097, 1.020, 1.114 and 1.128 for enantiomers of analytes laudanosoline, norlaudanosoline, laudanosine, and chlorothalidone, respectively. These values are higher than the α values obtained with SUAG and SUGA. In addition, neither SUAG nor SUGA recognized the enantiomers of the class II analytes, whereas, poly SUAA provided chiral selectivity values of 1.019, 1.013, and 1.007 for benzoin, benzoin methyl, and benzoin ethyl enantiomers, respectively. Only two anomalies were observed where one chiral center PCDS provided better chiral selectivity than two chiral centers PCDS. Poly SUVG with one
chiral center provided $\alpha$ values of 1.027, and 1.069 for enantiomers of laudanosoline and norlaudanosoline, respectively. These values are higher than the value obtained with poly SUVV surfactant.

**Conclusions.** From the chromatographic data presented here it can be concluded that dipeptide surfactants provided better enantiomeric selectivities for the chiral analytes examined in this study, compared to the single amino acid surfactants. In addition, the preferential site of interaction of these enantiomers were investigated using single chiral center PCDS. The preferential site of interaction in neutral enantiomers depends upon the hydrophobicity and steric hindrance of the analyte. Benzoin, which is more hydrophilic than benzoin methyl and benzoin ethyl, interacts with both C- and N-terminal amino acid, while the latter two enantiomers interact preferentially with the N-terminal amino acid. Enantiomers in Class I interact with both C- and N-terminal amino acids. This is possibly due to the steric hindrance in these analytes and the fact that the micellar core of the polymer is rigid.

It is worth noting that the highest average chiral selectivities of these analytes was obtained with the most sterically hindered surfactants, poly SULL and poly SULV. The average chiral selectivity of the analytes examined in this study was 1.065 for both poly SULV and poly SULL surfactant. Therefore, in the following Chapter, a summary of the chiral separation of a group of analytes with poly SULV surfactant is reported.
REFERENCES


Chapter 7
Conclusions

Part I. Summary

In first part of this dissertation, the differences in chiral recognition of monomeric and polymeric amino acid based surfactants were discussed. The differences in chiral recognition ability of these two kinds of surfactants are due to differences in their physical properties. Polymeric surfactants offer the advantages of being more stable, more rigid, and have no critical micelle concentration (CMC) as compared to conventional micelles.

Chromatographic data indicated that, in general, polymers are better CPSP than the monomers for the enantiomeric separation of the neutral and cationic analytes examined in this dissertation. However, better chiral separation of the anionic enantiomers of 1,1'-binaphthyl-2,2'-dihydrogen phosphate (BNP) was obtained when using monomeric surfactants. In addition, examination of the depth of penetration of the analytes into the micellar core of dipeptide surfactants indicates that BNP enantiomers penetrate deeper into the micellar core of the polymers as compare to the monomers.

In Chapter 3, steady state fluorescence anisotropy was used to gain insight into chiral interactions between binaphthyl derivatives and polymeric amino acid based surfactants. The results indicated that enantiomers that bind stronger to the CPSP, as evidenced by EKC experiments, have higher anisotropy values. The results of this study suggest that steady state fluorescence anisotropy can be used to gain further insight into enantiomeric molecular recognition.
In Chapter 4, fluorescence spectroscopy, and NMR techniques were utilized to learn more about the physical properties and the conformation of these amino acid based surfactants in solution. The polymeric surfactants examined in this dissertation always have lower "aggregation numbers" as compare to their monomeric counterparts. Regardless of the size of the polar head, examination of the data suggests that polymeric surfactants adopt a spherical shape in solution, while the shape of monomeric surfactants depend on the size of the polar head. In addition, polymeric surfactants have a higher effective charge than the monomers. This could possibly explain the better chiral separation of the enantiomers of negatively charged BNP using monomeric surfactant as compared to polymers.

Future work in this area could focus on studying the conformation of these surfactants with atomic force microscopy. Ionic surfactants form aggregates at an interface for the same reason they aggregate in bulk solution. It would be interesting to investigate the conformation of polymeric surfactants at an interface and correlate the results to their conformation in bulk solution. In addition, circular dichorisum could be used to further understand these chiral aggregates.

In Chapter 5, the solubilization capacity of polymer and conventional micelles were examined and compared using pulse field gradient NMR. This technique has been extensively used to study properties of conventional micelles and water soluble polymers. The results of those studies indicated that unpolymerized micelles solubilize a higher fraction of organic molecules than the polymerized form. These results are consistent with the EKC results where polymeric micelles provide faster mass transfer compared to the monomers.
In Chapter 6, the three chiral center dipeptide surfactant poly SUILV was compared with the two chiral center surfactant poly SULV. In some cases, poly SUILV provided better chiral separations than poly SULV. But, in general, no advantage of having the third chiral center on the dipeptide surfactant was observed possibly owing to the fact that the chiral centers of isoleucine in poly SUILV are very close to each other. Future work could be focused on using tripeptide polymeric surfactants with three chiral centers. However, tripeptide surfactants are more hydrophobic than single amino acid and dipeptide surfactants. Single amino acid and dipeptide surfactants synthesized in this dissertation have undecenoate group as a hydrophobic moiety. In order to improve the solubility of the tripeptide surfactants, the tripeptide should be coupled to a shorter hydrocarbon chain, i.e. pentoate moieties.

In the second part of Chapter 6, the enantiomeric separation of several neutral chiral analytes using eighteen amino acid based polymeric surfactants was discussed. Among these eighteen surfactants, poly SULV and poly SULL demonstrated the highest average chiral selectivity for these neutral analytes. Considering this and previous work where poly SULV provided the highest average chiral selectivity of twelve neutral and charged enantiomers,\textsuperscript{11-12} and the fact that the twelve chiral analytes examined in the first part of Chapter 6 showed reasonable chiral separation using poly SULV, a wide spectrum of chiral analytes were separated using this surfactant. The results of that study indicated that poly SULV is capable of providing some chiral recognition for most of the enantiomers of the neutral and cationic analytes examined. A list of the chiral analytes that have been separated using poly SULV is shown in Table 7.1.
Table 7.1 Chiral separation of optically active enantiomers using poly SULV

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Con.*</th>
<th>Rs</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonyl chlorobenzyl hydroxy coumarin (Cumachlor)</td>
<td><img src="image" alt="Structure" /></td>
<td>10</td>
<td>2.13</td>
<td>30 mM phosphate pH 7</td>
</tr>
<tr>
<td>Alprenolol</td>
<td><img src="image" alt="Structure" /></td>
<td>18</td>
<td>1.4</td>
<td>50 mM borate 300 mM CAPS pH 8</td>
</tr>
<tr>
<td>Aminoglutetimide</td>
<td><img src="image" alt="Structure" /></td>
<td>80</td>
<td>6.5</td>
<td>50 mM TRIS pH 9</td>
</tr>
<tr>
<td>Atenolol</td>
<td><img src="image" alt="Structure" /></td>
<td>50</td>
<td>0.7</td>
<td>30 mM phosphate pH 7</td>
</tr>
<tr>
<td>Atropine sulfate</td>
<td><img src="image" alt="Structure" /></td>
<td>30</td>
<td>1.4</td>
<td>30 mM phosphate pH 7</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>Con.</td>
<td>Rs</td>
<td>Buffer</td>
</tr>
<tr>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Benzoin</td>
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<td>50</td>
<td>3.4</td>
<td>30 mM phosphate pH 7</td>
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<tr>
<td>Benzoin ethyl ether</td>
<td><img src="image2.png" alt="Structure" /></td>
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<td>1.2</td>
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</tr>
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<td>Benzoin methyl ether</td>
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<td>2.1</td>
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<td>0.7</td>
<td>10 mM borate 100 mM TRIS pH 9</td>
</tr>
<tr>
<td>1-1'- Binaphthyl -2,2'- diol (BOH)</td>
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<td>4.9</td>
<td>10 mM borate 100 mM TRIS pH 10</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>Con.</td>
<td>Rs</td>
<td>Buffer</td>
</tr>
<tr>
<td>----------------------------------------------</td>
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<tr>
<td>1,1- Bi-2-naphthyl -2,2'-dihydrogen phosphate (BNP)</td>
<td><img src="image1" alt="Structure" /></td>
<td>30</td>
<td>7.8</td>
<td>10 mM borate</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>100 mM TRIS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 10</td>
</tr>
<tr>
<td>1,1- Binaphthyl -2,2-diamine (BNA)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 10</td>
</tr>
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<td></td>
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</tr>
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<td>0.8</td>
<td>30 mM phosphate</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 7</td>
</tr>
<tr>
<td>Epinephrine methyl ether</td>
<td><img src="image5" alt="Structure" /></td>
<td>50</td>
<td>1.5</td>
<td>30 mM phosphate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 7</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>Con.</td>
<td>Rs</td>
<td>Buffer</td>
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<td>0.8</td>
<td>30 mM phosphate pH 7</td>
</tr>
<tr>
<td>Glutethimide</td>
<td><img src="image" alt="Glutethimide structure" /></td>
<td>80</td>
<td>1.4</td>
<td>50 mM TRIS pH 9</td>
</tr>
<tr>
<td>Homatropine HBr</td>
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<td>1.7</td>
<td>30 mM phosphate pH 7</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td><img src="image" alt="Isoproterenol structure" /></td>
<td>20</td>
<td>1.9</td>
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<tr>
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<td><img src="image" alt="Ketamine structure" /></td>
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<td>1.8</td>
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<tr>
<td>Compound</td>
<td>Structure</td>
<td>Con.</td>
<td>Rs</td>
<td>Buffer</td>
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<td>Ladanosoline</td>
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<td>Lorazepam</td>
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<td>2.7</td>
<td>25 mM TRIS 25 mM borate pH 7</td>
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<td>30 mM phosphate pH 7</td>
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<td>Compound</td>
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<td>Con.</td>
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<td>Oxprenolol</td>
<td><img src="image4" alt="Structure" /></td>
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<td>50 mM borate 300 mM CAPS pH 8</td>
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<td>Pentobarbital</td>
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<td>1.6</td>
<td>30 mM phosphate pH 7</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>Con.</td>
<td>Rs</td>
<td>Buffer</td>
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<tr>
<td>Propranolol</td>
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<td>1.78</td>
<td>50 mM borate</td>
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<tr>
<td></td>
<td></td>
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<td>pH 8</td>
</tr>
<tr>
<td>Pseudoephedrine**</td>
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<td>30</td>
<td>1.5</td>
<td>30 mM phosphate</td>
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<td></td>
<td></td>
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<td>pH 7</td>
</tr>
<tr>
<td>Secobarbital</td>
<td><img src="image" alt="Secobarbital Structure" /></td>
<td>30</td>
<td>1.2</td>
<td>30 mM phosphate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 7</td>
</tr>
<tr>
<td>Temazepam</td>
<td><img src="image" alt="Temazepam Structure" /></td>
<td>20</td>
<td>4.0</td>
<td>25 mM borate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 mM TRIS</td>
</tr>
<tr>
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<td>pH 8</td>
</tr>
<tr>
<td>2,2,2-Trifluoro-1-(9-anthryl)ethanol (TFAE)</td>
<td><img src="image" alt="TFAE Structure" /></td>
<td>6</td>
<td>0.74</td>
<td>30 mM borate</td>
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<tr>
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<td><img src="image" alt="Troger Base Structure" /></td>
<td>2</td>
<td>2.31</td>
<td>30 mM phosphate</td>
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<tr>
<td>Warfarin</td>
<td><img src="image" alt="Warfarin Structure" /></td>
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<td>30 mM phosphate</td>
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<tr>
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<td></td>
<td>pH 7</td>
</tr>
</tbody>
</table>

* mM EMC

** four peaks for four enantiomers were observed
Although poly SULV separated enantiomers of a wide spectrum of analytes, it should be mentioned that some analytes could be separated better with other dipeptide surfactants. For example, as shown in Figure 7.1, poly SULV does not recognize enantiomers of verapamil, while baseline separation of these enantiomers were achieved using poly SUAA. It should be mentioned that modification of the running electrolyte with a small percentage of methanol did provide some hint of chiral recognition for enantiomers of verapamil using poly SULV as the CPSP. From my experience with polymeric amino acid based surfactants, the highly hydrophobic enantiomers may be better separated using single amino acid and/or dipeptide surfactants with a small polar head, i.e. poly SUAA. In addition, as discussed in Chapter 6, less sterically hindered surfactants, may provide better chiral recognition for chiral analytes with sterically hindered chiral centers.

Figure 7.1 Chiral separation of verapamil enantiomers. Separation conditions: buffer; 6mM EMC of the PDCS and 30 mM phosphate at pH 7, 12 °C, +30 kV applied voltage, 215 nm UV detection.
Although poly SULV provided some hint of chiral recognition for a variety of chiral analytes, chiral separations of some anionic enantiomers are still problematic. For this reason, the zwitterionic surfactant, poly sodium N-undecanoyl lysinate was synthesized. The synthetic procedure used was similar to the procedure discussed in Chapter 2. The only difference is that lysine has an additional NH$_2$ on its side chain. During the coupling of the lysine with the N-hydroxysuccinimide ester of undecylenic acid, this NH$_2$ group must be protected. After the clean up, the NH$_2$ was unprotected using HCl in dioxane.

In contrast to the other surfactants we have used in our laboratory which fall out of solution at pH around 7, the lysine surfactant is soluble in solution with pH as low as 5. However, no other advantage of using this surfactant over anionic surfactants was observed. This is possibly due to the fact that because of the presence of NH$_2$, the side chain of the lysine is very hydrophilic. Therefore, in micellar solution, this side chain will be facing the water layer, unlike the side chain of valine and/or leucine where they face micellar core. Therefore, at low pH, where side chain of SULys is positively charged, no chiral separation was observed using this polymeric surfactant as CPSP. One other disadvantage of this surfactant at low pH is the adsorption of the cationic side of the surfactant molecule to the negatively charged silanol groups of the capillary wall. At pH above 7, this surfactant performed similar to single amino acid surfactants. Therefore, no advantages of using this surfactant over the other three single amino acid surfactants (SUA, SUV, and SUL) were observed.
In addition to the polymeric surfactants mentioned in previous chapters, mixed polymeric surfactants were also synthesized. Different combinations of single amino acid and dipeptide surfactants were mixed with 1:1 (mol:mol) ratios and polymerized. No advantages of using the mixed anionic surfactants were observed. The only mixed micelle that was interesting was a combination of SUA and SUV. Neither SUA nor SUV separated enantiomers of BNP. However, the mixed micelle SUA:SUV provided some chiral recognition for enantiomers of this analyte. This is possibly due to the fact that SUA with small R-group provides open structure for mixed micelle. Thus, BNP enantiomers can interact with chiral centers of the polymeric surfactant.

Mixed zwitterionic and anionic polymeric surfactants were prepared by polymerization of SULV and SULys 1:1 (mol:mol). The presence of SULys increased the solubility of the polymer at lower pH. Poly SULV is insoluble below pH 7, while the mixed micelle of poly SULV:SULys was soluble at pH 5.5. Interestingly, this

Figure 7.2 Chiral separation of fluorobiprofen using 1.5% (m/v) mixed micelle poly SULV:Lys. Separation conditions: 15 mM phosphate buffer at pH 6, 12 °C, +30 kV applied voltage, 254 nm UV detection.

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mixed micelle provided some hint of chiral recognition for enantiomers of fluorobiprofen at pH 6 (Figure 7.2).

Part II. Future Work

Future work with polymeric surfactants should focus on synthesis of new classes of mixed micelle, where a group of surfactants that have the best selectivity for several enantiomers can be mixed and polymerized. In addition, the mixed polymeric micelle can be designed to improve solubility of the anionic polymer. Zwitterionic, cationic, anionic and neutral surfactants can be polymerized as mixed polymeric micelles to provide a micelle that can resolve enantiomers of chiral analytes in different charge states.

REFERENCES


Appendix
Separation of Free Fatty Acids Using Capillary Zone Electrophoresis and Indirect Photometric Detection

Free fatty acids (FFA) are an important class of naturally occurring compounds that can be found in living cells. These compounds differ in their chain length, branches, degree of unsaturation, position and configuration of their double bonds.\(^1\) The separation of FFAs is important in studying the biological activity of cells. In addition, the analysis of food for FFAs is required for quality control.\(^2\)

Numerous methods employing gas chromatography (GC)\(^3-5\) and high performance liquid chromatography (HPLC)\(^6-9\) have been described for the separation of FFAs. However, both GC and HPLC will often require pre-column derivatizations to enhance volatility and detectability, respectively.

The FFAs contain an acidic hydrogen due to their carboxylic acid functional groups. Therefore, these compounds predominantly exist as anions in basic solutions. Considering the differences in charge to radius ratios, both saturated\(^10-12\) and unsaturated\(^13\) FFAs, can be separated by capillary electrophoresis (CE). Saturated FFAs exhibit weak absorption in the region of 200 nm. Therefore CE with direct UV detection is problematic and results in limited sensitivity. In addition, the use of low wavelengths not only impairs the utility of many organic solvents and buffer system; it also results in increased interference from the biological matrix. Therefore, indirect photometric detection (IPD) can be used to detect these compounds.

The key element for IPD is to maintain a large continuous background absorbance signal at the UV detector by employing a detectable ionic (chromophoric)
species in the running electrolyte. If the concentration of the light absorbing ions remain constant in the electrical double layer in CE columns, a steady of background absorbance translated as a stable baseline is displayed on the electropherogram. When a non-UV detectable ionic species passes the detection window, the original high level of the absorbance signal is decreased due to the dilution of the chromphoric compound by the transparent analyte molecules. This technique provides a simple, easy, and time efficient approach for the detection of FFAs. It should be mentioned that micellar electrokinetic chromatography (MEKC) with direct$^{14}$ or IPD$^{15}$ has also been developed to separate long chain FFAs (C$_8$-C$_{20}$).

The CZE separation of very long chain (C$_{21}$-C$_{31}$) saturated and unsaturated FFAs was difficult using either aqueous or partially aqueous electrolyte. First, C$_{21}$-C$_{31}$ FFAs have poor solubility and they tend to form micelles. Although the use of higher fraction of organic solvents disrupts the micelles, it also results in longer analysis time. Second, the difference in electrophoretic mobility between two consecutive homologues of FFAs rapidly decreases with increased alkyl chain length for those possessing greater than 20 carbon atoms. For these reasons, the use of a nonaqueous electrolyte for CE separation of very long chain FFAs has been recently developed by Drange, et al.$^{10}$ The authors showed that separation of C$_{14}$-C$_{26}$ FFAs could be conveniently accomplished in 15 min using anthraquinone-2-carboxylic acid (ANT) in N-methylformamide (NMF) and dioxane. However, their method was not optimized for the resolution of unsaturated FFAs. In addition, separation of only even chain numbers of C$_{14}$-C$_{26}$ was
reported. The separation of long chain (C_{12}-C_{20}) and very long chain (C_{21}-C_{31}) FFAs differing only by one carbon atom requires the investigation of a new IPD reagent.

Adenosine monophosphate (AMP) has been shown to be a useful chromophore for IPD in aqueous as well as partially aqueous solutions.\textsuperscript{16-19} In this report, we have investigated the potential of AMP as an IPD electrolyte for the separation of saturated FFAs (C_{12}-C_{31}) differing only by one carbon atom. In addition, a partially aqueous CE system was optimized for the separation of a complicated mixture of unsaturated (C_{14}-C_{22}) FFA isomers.

EXPERIMENTAL

**Chemicals.** NMF and dioxane were purchased from Fluka. The monosodium salt of adenosine monophosphate (AMP) (99%), anthraquinone-2-carboxylic acid (ANT), and Trizma\textsuperscript{\textregistered} base (Tris) were all obtained from Sigma (St. Louis, MO). Saturated FFAs such as, lauric acid (C_{12:0}), tridecanoic acid (C_{13:0}), myristic acid (C_{14:0}), pentadecanoic acid (C_{15:0}), palmitic acid (C_{16:0}), heptadecanoic acid (C_{17:0}), stearic acid (C_{18:0}), nonadecanoic acid (C_{19:0}), arachidic acid (C_{20:0}), heneicosanoic acid (C_{21:0}), behenic acid (C_{22:0}), tricosanic acid (C_{23:0}), lignoceric acid (C_{24:0}), pentacosanoic acid (C_{25:0}), hexacosanoic acid (C_{26:0}), heptacosanoic acid (C_{27:0}), octacosanoic acid (C_{28:0}), nonacosanoic acid (C_{29:0}), tricontanoic acid (C_{30:0}), hentriacontanoic acid (C_{31:0}), and unsaturated FFAs such as myristoleic acid (C_{14:1}), palmitoleic acid (C_{16:1}), oleic acid (C_{18:1}), linoleic acid (C_{18:2}), linolenic acid (C_{18:3}), \ensuremath{\gamma}\text{-linolenic acid (}\gamma\text{-C}_{18:3}\), cis-11-eicosenoic acid (C_{20:1}), cis-11, 14-eicosadienoic acid (C_{20:2}), cis-11, 14, 17-eicosatrienoic acid (C_{20:3}), erucic acid (C_{22:1}), cis-13, 16-
docosadienoic acid (C$_{22:2}$), and cis-13, 16, 19-docosatetraenoic acid (C$_{22:3}$) were all obtained from Sigma.

**Capillary electrophoresis procedure.** The CE instrument used was a BioFocus 3000 CE (Bio-Rad, Hercules, CA) equipped with a UV detector. Untreated fused silica capillary (50 μm i.d., 320 μm o.d., 45 cm effective length) was purchased from Polymicro Technologies (Phoenix, AZ).

A new capillary was subjected to a standard wash cycle for 30 min using 1 M NaOH and for 10 min using triply deionized water at room temperature. As a daily routine, a capillary was flushed with 1 M NaOH for 10 min and water for 5 min. Between injections, the capillary was flushed for 2 min with each of the following solutions: 0.1 M NaOH, triply distilled water, and then the running electrolyte. Samples were pressure injected for 1 second. Capillary temperature was controlled with an aqueous coolant. Separations were performed at +20 kV. IPD was performed at 259 nm and 264 nm using AMP$^{18}$ and ANT$^{10}$ respectively.

A running electrolyte solution of AMP and 40 mM Tris buffer was prepared in different % (v/v) of NMF-dioxane. The final buffer was filtered through a 0.45 μm Nalgene Nylon filter (Rochester, NY) and used without any pH adjustment. All FFA standards were dissolved in 4:1 (v/v) NMF-dioxane, sonicated and filtered prior to use.

**RESULTS AND DISCUSSION**

Several parameters were studied to optimize the separation of saturated (C$_{12}$-C$_{31}$) FFAs and unsaturated (C$_{14}$-C$_{22}$) isomers under nonaqueous and partially aqueous
CZE conditions. These parameters include 1) the concentration and the choice of the IPD reagent, 2) the volume fraction of organic modifier, and 3) temperature.

**Type and Concentration of IPD Reagent.** To perform separation of FFAs differing in one carbon, with a reasonable peak capacity and efficiency, an IPD reagent with electrophoretic mobility similar to the analyte ions should be used. Mobility matching between the analyte ions and the IPD reagent reduces peak dispersion, thus two analyte ions with small difference in charge/radius ratio can be resolved.\(^{20}\) The effect of AMP concentration on the sensitivity of FFA signals was studied using 1, 2.5 and 5 mM solutions. With respect to electrophoretic separation and detection point of view, a 2.5 mM AMP solution was found to be a good compromise. In literature, ANT has also been introduced as an IPD reagent for nonaqueous CE.\(^{10}\) We have studied the effect of ANT concentration on the migration behavior of saturated FFAs. At 7 mM concentration, ANT electrolyte completely absorbs the UV light. In addition, ANT provides a poor sensitivity for very long chain FFAs (C\(_n\), n>26). Moreover, AMP provides more rapid separation of FFAs. Therefore we conclude that AMP is a better IPD reagent for the separation and detection of long chain FFAs.

**Effect of Organic Modifier on Separation of FFAs.** To achieve the best separation efficiency for both saturated and unsaturated FFAs, optimization of solvent composition was necessary. The propensity of long chain FFAs to form micelles and their poor solubility in aqueous electrolytes causes serious problems in separation of this group of compounds. The CE separations of FFAs (C\(_n\), n<18) have been reported using 60% methanol.\(^{11}\) In addition, separation of FFAs containing up to twenty
carbons have been achieved using acetonitrile and nonionic surfactants such as Brij.\textsuperscript{15} The use of Brij facilitates the solubility of long chain FFAs. As discussed earlier, Drange, et al. have separated only even chain number FFAs C\textsubscript{14}-C\textsubscript{26} in nonaqueous media.\textsuperscript{10} In order to separate FFAs differing by only one carbon, a better understanding of organic solvent composition is required. Figure a.1 shows the variation of the relative migration time (t\textsubscript{R}/t\textsubscript{0}) of C\textsubscript{12}-C\textsubscript{24} FFAs vs. % (v/v) dioxane in NMF. As shown, at high NMF content, the variation of t\textsubscript{R}/t\textsubscript{0} values of long chain FFAs (e.g. C\textsubscript{24}, and C\textsubscript{23}) are not pronounced as with shorter chains (e.g. C\textsubscript{12}, and C\textsubscript{13}) due to the fast electroosmotic flow (EOF).

Decreasing the percentage of NMF (increasing the percentage of dioxane) decreases the EOF. Additional solvent studies indicated that dioxane improves the solubility of the very long chain FFAs to a certain degree. For example, baseline resolution of C\textsubscript{28} and C\textsubscript{29} were obtained at 40\% (v/v) dioxane. However, dioxane did not affect the resolution of C\textsubscript{30} and C\textsubscript{31}. At concentrations above 50 \% (v/v) dioxane, no significant improvement in the resolution of C\textsubscript{28}-C\textsubscript{31} was observed.

To optimize the CZE conditions for unsaturated FFAs, C\textsubscript{18} isomers were chosen. The t\textsubscript{R}/t\textsubscript{0} of C\textsubscript{18} isomers vs. % (v/v) NMF is shown in Figure a.2. At 100\% NMF, all five isomers coeluted, and at 60 \% (v/v) NMF/40\% (v/v) dioxane maximum difference in t\textsubscript{R}/t\textsubscript{0} values of the five isomers was obtained.

Figure a.3A shows the CE separation of the C\textsubscript{18} isomers using optimized non-aqueous (60\% NMF-40\% dioxane) conditions. All isomers were baseline resolved
except $C_{18:2}$ and $C_{18:3}$. The partial resolution between $C_{18:2}$ and $C_{18:3}$ is possibly due to the aggregation of these two isomers. To achieve baseline resolution and to overcome the aggregation, 10% (v/v) water was added to the running buffer. Under such conditions a near-baseline separation of all five $C_{18}$ isomers was obtained (Figure a.3B). However, water content $>10\%$ resulted in decreased resolution.

**Effect of Temperature.** Dioxane and NMF have relative high boiling points ($102\,^{\circ}C$ and $200\,^{\circ}C$, respectively); therefore, the effects of temperature on separation
of C_{12}-C_{24} FFAs were studied. Different temperatures (20-30 and 40 °C) under optimum conditions (3:2

![Graph showing effect of dioxane/NMF on relative migration time of C18 isomers.](image)

**Figure a.2** Effect of dioxane/NMF on relative migration time of C18 isomers. NMF-dioxane) were compared and results showed that at 40 °C more sensitive signals for short and long chains can be obtained compared to lower temperatures. However, temperature does not influence the resolution of the five unsaturated C_{18} isomers.

Reproducibility for saturated FFA migration times between sequential runs was investigated at several temperatures. The average RSDs for the migration times of five peaks (C_{12}-C_{16}) from ten different runs were found to be 0.8%. The results showed that temperature does not have a significant effect on reproducibility. In addition, a stable baseline at 40 °C was obtained without any significant increase in current.
Figure a.3  Electropherogram of C₁₈ isomers in A) non-aqueous and B) partially aqueous electrolyte.
Separation of Saturated FFAs. Separation of saturated FFAs was performed using 2.5 mM AMP, 40 mM Tris, in 3:2 NMF-dioxane at 40 °C, Figure a.4A. Solubility of the very long chain (C_{26}-C_{31}) FFAs in NMF dioxane was poor. To improve the solubility of C_{26}-C_{31} FFAs and to achieve a resolution between C_{30} and C_{31}, Brij was added to the running buffer. Nonionic surfactants such as Brij have been shown to improve the solubility of long chain FFAs;\textsuperscript{15} however, Brij slows the EOF and increases the viscosity. In this work, we investigated the effect of Brij concentration on the migration time of long chain FFAs. Below the critical micelle concentration of Brij, small surfactant aggregates improved the solubility of the very long chain FFAs. At concentrations below 0.5 %, C_{30} and C_{31} coelutes; while at concentration greater than 0.5 %, elution time of FFAs increases drastically. In addition, Brij content results in peak broadening for FFAs with an alkyl chain length of less than 10 carbon atoms. The optimum concentration of Brij in the running buffer was found to be about 0.5 %, Figure a.4B.

As expected in a mixture of C_{12}-C_{31} FFAs, the longest chain FFA (C_{31}) eluted first and the shortest chain (C_{12}) FFA eluted last. This is because the longer chain FFAs are less mobile and are rapidly swept toward the negative electrode (detection end) by the EOF. It should be reiterated that the difference in electrophoretic mobility of FFAs decreases with an increase in alkyl chain length. Therefore, resolution between C_{26}-C_{31} FFAs is less compared to C_{12}-C_{24} FFAs. Further studies showed that elimination of Brij from the electrolyte, under optimum conditions, resulted in
separation of C5-C31 FFAs using a single CE run. However, long-chain FFAs exhibited poor sensitivity and short-chain FFA peaks were broadened. It should be noted that short chain FFAs (C1-C10) can be conveniently separated in aqueous solution using 2.5 mM AMP and 40 mM Tris.

**Separation of Mixtures of Saturated and Unsaturated Free Fatty Acid Isomers.** As discussed earlier, the use of nonaqueous electrolyte results in partial resolution of C18 and the presence of water improves the resolution of such unsaturated isomers. Therefore, a combination of NMF, dioxane, and water in the ratio of 5:4:1 was required for the separation of complicated mixtures of unsaturated FFAs (C14-C22 with 0, 1, and 2 double bonds). Again, lowering the NMF content in the running electrolyte down to 50 % (v/v) results in a slow separation and loss of peak capacity. In contrast, at 0% NMF (50/50 v/v dioxane-water) the separation time increased to 90 min. Figure a.5 shows the separation of C14-C22 saturated and unsaturated isomers. Isomers with three degree of unsaturations for even chain length (e.g. C12:i) coeluted with the saturated forms of the next even chain length homologues (e.g. C20:0). Similarly, C20:3 and C18:3 coeluted with C18:0 and C16:0, respectively. However, under the same optimum conditions, singly, doubly and triply unsaturated isomers of C18, C20 FFAs can be baseline separated (and C22 isomers nearly so) in one CE run (Figure a.6). In addition, baseline separations of six different isomers of C20 (zero, one, two, three, four, and five double bonds) and five isomers of C22 (zero, one, two, three, four and six double bonds) can be achieved in 25 and 30 min., respectively.

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Figure 2.5: Electropherogram of saturated and unsaturated FFAs (C_{14}-C_{22}).
Figure a.6 Electropherogram of unsaturated FFA (C₁₄-C₂₂).
In conclusion, the advantage of using AMP as an IPD reagent is that AMP is soluble in aqueous, partially aqueous, as well as nonaqueous media. Therefore separation of different saturated and unsaturated FFA homologues is feasible using AMP. In addition, standard mixtures of very long chain saturated (C_{21}-C_{31}) FFAs can be separated in a single run along with long chain (C_{12} - C_{21}) FFAs in the presence of 0.5 % (w/v) Brij in nonaqueous electrolyte.

REFERENCES


Fereshteh Haddadian Billiot was born on April 25, 1965, to Fatemeh Ghazimoradi and Ahmad Haddadian. Fereshteh was raised in a well-educated family, where her father always encouraged his children to pursue the highest degree in the field of their interest. When Fereshteh was in high school, she became interested in physics and chemistry. After high school, she entered Shariff University of Technology in 1983, where she received her bachelor of science degree in chemistry. After graduation, she worked at the same university as a research associate. In 1989 she accepted a position at a pharmaceutical company, Sina Daru.

In order to achieve her goal for higher education, Fereshteh came to America in spring 1993. She joined the master's program at Ball State University in 1993. Her research there focused on synthesis and characterization of phosphine functionalized crown ethers. After receiving her Master's degree from Ball State in 1995, and not wanting to live too far from her new home in America she went to Miami University in Oxford, Ohio, to pursue her doctorate in analytical chemistry. After a short period of time at Miami University she realized that her real interest was in understanding chiral interactions. In perusing the literature, she found the work in Dr. Isiah Warner's laboratory at Louisiana State University to be particularly interesting. Therefore, she quickly finished her second master's, and moved to Louisiana to pursue her doctorate.

At L.S.U., she found a new home and a new family, literally and figuratively. At L.S.U., she joined the Warner "family". There she met Eugene Billiot, and for two years they spent their days and nights in the laboratory in the pursuit of science and finding love along the way. They were married on January 8th, 2000, in the home of
their mentor Isiah Warner, who not only lent the use of his home but also gave the bride away.

At LSU, Fereshteh studied chiral recognition using polymeric and monomeric amino acid based surfactants. The followings are list of her publications. After graduation, she will join her husband, as an assistant professor, at Texas A&M-Corpus Christi University.


“Comparison of Monomeric and Polymeric Amino Acid Based Surfactants as Pseudostationary Phases for Chiral Recognition in Capillary Electrokinetic Chromatography” J. Chromatogr. A, accepted for publication, with E. J. Billiot and I. M. Warner.


DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Fereshteh Haddadian Billiot

Major Field: Chemistry

Title of Dissertation: Chiral Recognition Using Polymeric and Monomeric Amino Acid Based Surfactants

Approved:

[Signature]
Major Professor and Chairman

[Signature]
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signature]

[Signature]

[Signature]

Date of Examination:

July 28, 2000