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SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS OF NOVEL 
PSEUDOSTATIONARY PHASES IN MICELLAR CAPILLARY 
ELECTROPHORESIS FOR SEPARATION OF CHIRAL AND ACHIRAL 
COMPOUNDS

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 
Cevdet Akbay 
B.S., İnönü University (Malatya, Turkey), 1990 
M.S., Louisiana State University, 1999 
August, 2002
Dedication

I want to dedicate this work to my wife and best friend Müzeyyen. You have been very supportive and understanding throughout all our hard times. I hope I can repay you in kind in my lifetime. I would also like to dedicate this to my sons, Ahmet Hüsrev and Muhammed Mus’ab, who have kept asking me “Father, when are you going to graduate?” Well, sons! I think I made it!
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It has been a long, hard and tedious journey, but it was worthy beginning and joyful being able to finish it at last. All praise be to God, the Cherisher and Sustainer of the worlds, Who has said in His Noble Book, “say: ‘O my Lord! Increase me in knowledge” (The Holy Qur’an, 20:114). Without help and mercy of God and support of my family and friends, none of this would have been possible.

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<tr>
<td>α-CD</td>
<td>Alpha cyclodextrin</td>
</tr>
<tr>
<td>ACE</td>
<td>Acenaphthene</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
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<td>Acenaphthylene</td>
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<td>ANTH</td>
<td>Anthracene</td>
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<td>β-DC</td>
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<td>BaA</td>
<td>Benz[a]anthracene</td>
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<tr>
<td>BbF</td>
<td>Benzo[b]fluoranthene</td>
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<td>BGE</td>
<td>Background electrolyte</td>
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<td>BkF</td>
<td>Benzo[k]fluoranthene</td>
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<td>BNA</td>
<td>1,1'-binaphthyl-2,2'-diamine</td>
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<td>BNP</td>
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<td>CAC</td>
<td>Critical aggregation concentration</td>
</tr>
<tr>
<td>CD</td>
<td>Cyclodextrin</td>
</tr>
<tr>
<td>CHRY</td>
<td>Chrysene</td>
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<tr>
<td>CMC</td>
<td>Critical micellar concentration</td>
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<tr>
<td>CoPM</td>
<td>Co-polymerized molecular micelle</td>
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<td>Abbreviation</td>
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<tr>
<td>CPyrCl</td>
<td>Cetylpyridinium chloride</td>
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<tr>
<td>C_{surf}</td>
<td>Concentration of surfactant</td>
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<tr>
<td>DEHP</td>
<td>Di(2-ethylhexyl)phosphate</td>
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<td>DiBahA</td>
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<td>Mono-</td>
<td>Monomeric</td>
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Poly-L₆S₄  Co-polymerized molecular micelle of 60 mM SUL and 40 mM SUS
Poly-L₈S₂  Co-polymerized molecular micelle of 80 mM SUL and 20 mM SUS
Poly-SDUT  Polymerized sodium diundecenyl tartarate
Poly-SUL  Polymerized sodium N-undecanoyl L-leucinate
Poly-SUS  Polymerized sodium undecenyl sulfate
PYR    Pyrene
SDS    Sodium dodecyl sulfate
SDUT   Sodium diundecenyl tartarate
SUA    Sodium 10-undecylenate
SUL    Sodium N—undecanoyl L-leucinate
SUS    Sodium undecenyl sulfate
TM-β-CD  Trimethyl beta cyclodextrin (or heptakis (2,3,6-tri-O-methyl)-β-CD)

Instrumentation and method

$\alpha_{CH_2}$  Methylene (or hydrophobic) selectivity
CD-MCE  Cyclodextrin modified micellar capillary electrophoresis
CE    Capillary electrophoresis
CEC   Capillary electrochromatography
CGE   Capillary gel electrophoresis
CIEF  Capillary isoelectric focusing
CITF  Capillary isotachophoresis
CZE   Capillary zone electrophoresis
EOF   Electroosmotic flow
FSCE  Free zone capillary electrophoresis
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<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>LSER</td>
<td>Linear solvation energy relationship</td>
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<td>Micellar capillary electrophoresis</td>
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<td>MEKC</td>
<td>Micellar electrokinetic chromatography</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>SSFQ</td>
<td>Steady-state fluorescence quenching</td>
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Abstract

The research presented in this dissertation involves the synthesis, characterization, and the use of novel surfactants, including both micelles and vesicles, as pseudostationary phases in micellar capillary electrophoresis (MCE) for the separation of achiral and chiral compounds. Separation of environmental pollutants such as 2 to 6-ring polycyclic aromatic hydrocarbons (PAHs) was achieved using poly(sodium undecylenic sulfate). A baseline separation of all 16 PAHs was possible for the first time in MCE by a single-surfactant system. In addition, a surfactant with a phosphated head group, i.e., di(2-ethylhexyl)phosphate (DEHP), was also introduced as a novel pseudostationary phase for separation of 21 weakly and strongly hydrophobic neutral compounds. Acetonitrile at a concentration of 30% (v/v) in combination with 100 mM DEHP gave optimum separation for a mixture of 21 benzene derivatives and PAHs in under 16 minutes. An application of cyclodextrin modified MCE was used for separation of twelve mono-methylbenz[a]anthracene positional isomers using a combination of poly-SUS and β-CD, γ-CD or β-CD derivatives.

Tartaric acid based vesicular surfactants were synthesized and utilized as novel pseudostationary phases in MCE. Linear solvation energy relationship model was applied to understand the fundamental nature of the solute-surfactant interactions and to investigate the effect of the type and the composition of pseudostationary phases on the retention mechanism and selectivity in MCE. The solute size has the largest influence on the solute retention in MCE. The hydrogen bond accepting ability of the solute is the second most important factor on retention and is the largest contributor towards the selectivity differences between pseudostationary phases used.
Another study conducted was the synthesis of sodium N-undecanoyl L-leucinate and co-polymerization of SUL with SUS to make a variety of co-polymerized molecular micelles having both chiral (leucinate) and achiral (sulfate) head groups. These surfactants were applied as novel pseudostationary phases in MCE for separation of chiral and achiral compounds. Aggregation numbers and partial specific volumes of these surfactant systems were determined using fluorescence spectroscopy and densitometry, respectively. Thermodynamic parameters such as enthalpy, entropy, and Gibbs free energy changes upon transfer of analyte(s) from aqueous phase to the pseudostationary phase were also determined.
Chapter 1.
Introduction to Surfactants, Capillary Electrophoresis, Chirality, and Applied Characterization Techniques

1.1. Surfactants and Micelles

Surfactants, also called surface-active agents, amphiphiles or detergents, are among the most versatile molecules available. They have applications in many areas, including chemistry (for kinetic studies), biology (as membrane mimics), and pharmacy (as drug delivery agents) (1-3). Surfactants contain both a hydrophobic, water-insoluble long-chain hydrocarbon “tail” and a hydrophilic water-soluble, usually ionic or polar “head” group. In polar solvents, for example water, surfactants arrange themselves into organized molecular assemblies known as micelles (Figure 1.1).

![Simplified illustration of polymerizable surfactant molecule and its micelle.](image)

Figure 1.1. Simplified illustration of polymerizable surfactant molecule and its micelle.

The hydrophobic (water hating) part of the micelle forms the core of the micelle, while the hydrophilic (water loving) head groups are located at the micelle-water interface in contact with water molecules. A micelle is made up of different numbers of surfactant monomers,
depending on the detergent type, and this number is referred to as its aggregation number (N). Each micelle is composed of about 40-140 molecules (4). The aggregation process depends on the surfactant type and the conditions of the system in which the surfactant are dissolved. Several methods are available to determine the N value of a micelle. These include light scattering, diffusion, viscosity, sedimentation velocity, ultra filtration, nuclear magnetic resonance (NMR) and fluorescence (5,6). The N values of the surfactants used in this dissertation were determined using a static fluorescence quenching technique. Depending on the chemical structure of the surfactant, its micelle can be cationic, anionic, ampholitic (zwitterionic), or nonionic (7). The concentration and the critical temperature above which micelles form are called the critical micelle concentration (CMC) and the Kraft point (T_K), respectively.

Above the CMC, monomers and micelles exist in dynamic equilibrium. The CMC can be measured by the change in the physicochemical properties of the surfactant solutions as the concentration of the amphiphilic molecules is increased (Figure 1.2). Many techniques have been used for determination of CMC including surface tension, osmotic pressure, turbidity, conductivity, NMR, capillary electrophoresis (CE), light scattering, and fluorescence (8-18). The CMC value may vary depending on the variation in hydrophobicity, counterion, or electrolyte concentration (8). Several generalizations can be made about the aggregation number and the CMC value. First, all conditions being approximately equal, the aggregation number N increases as the length of the hydrocarbon chain increases. An increase in the CMC is also observed with branching of the hydrophobic tail. Addition of a double bound to the end of the hydrophobic tail decreases the hydrophobicity of the surfactant and thus increases the CMC value by a factor of two (19). Second, the factors that increase N tend to lower the CMC. Third,
at and above CMC, micelles are roughly spherical and relatively dispersed. Fourth, in the presence of an organic solvent, the CMC of the surfactant increases. Fifth, in general, nonionic surfactants have lower CMC values than ionic surfactants. This is due to an increase in the hydrophobicity of nonionic surfactants as compared to ionic surfactants. Finally, the addition of salt lowers the CMC of ionic micelles and hence increases N. This is because ions decrease the repulsive forces among the charged head groups of the micelle, and less energy is required for micelle formation.

![Figure 1.2. Measurement of CMC based on several solution parameters.](image)

The structure of the micelle is dictated by equilibrium between the repulsive forces among hydrophilic “head” groups and attractive forces among hydrophobic “tail”. Several structures have been proposed for micelles. McBain proposed a coexistence of the spherical and the lamellar micelles (20). Hartley suggested that micelles are spherical with charged groups located at the micellar surface (21). The Hartley model successfully describes many micellar system properties. According to this model, counterions are bound to the charged head groups of
the surfactants. This explains the drop in conductance of the surfactant solution at the CMC. In addition, Hartley proposed that the inside core of the micelle has properties of liquid hydrocarbons; thus, micelles are able to solubilise hydrophobic molecules that are otherwise insoluble (7, 22). Debye and Anacker proposed rod-shaped micelles rather than spherical or disk-like ones (23). Based on NMR and kinetic studies, Menger reports that micelles are more disorganized with chain looping, nonradial distribution of chains, and contact of terminal methyl groups with water (24). According to the Menger model, micelles have a rough surface with water-filled pockets. In general, the spherical form is accepted as a true representation of the micelle (Figure 1.3).

![Different proposed structures of the micelle.](image)

With increasing surfactant concentration, the shape of the ionic micelles changes in sequence to spherical-cylindrical-hexagonal-lamellar (Figure 1.4) (7). Additional factors that
affect the micelle shape are the optimal head group area, the volume, and the chain length of the tail (8). The net aggregation of an ionic micelle is found to be less than the degree of micellar aggregation. This indicates that large fractions of counterions remain associated with the micelle. These counterions form the Stern layer at the micellar surface (7). In ionic micelles, the Stern layer, which resembles a concentrated electrolyte solution, consists of bound ionic surfactant head groups, counterions, and water molecules. The water is present as both free molecules and water of hydration. Figure 1.5 shows different regions of a spherical micelle. The thickness of the Stern layer is usually only a few angstroms. The layer just beyond the Stern layer is a diffuse layer (known as the Guoy-Chapman layer) extending outward to several hundred angstroms.

![Figure 1.5. Different regions of a spherical micelle.](image)

**Figure 1.5. Changes in micelle shape with respect to change in surfactant concentration**

The inner core of the micelle is usually divided into two regions. The hydrophobic tail of the surfactant forms a water free region (inner core). Moving outward from the inner core of the micelle, there is a hydrated region between the inner core and the polar head group of the micelle. This hydrated region is called the palisade layer and is viewed as liquid hydrocarbon.
The radius of the inner core and the palisade layer is approximately equal to the length of the fully extended hydrocarbon chain (25).

![Diagram of micellar regions]

**Figure 1.5. Important micellar regions.**

There are two dominant models commonly used to explain the behavior of surfactants in solution: the mass action model (8) and the phase equilibrium model (26). The mass action model considers micellization as a “chemical reaction”, whereas the phase equilibrium model treats micellization as a phase separation phenomena. The phase separation model treats the micelle as a separate, but soluble phase. This model suggests that the concentration of the monomeric species remains constant above the CMC. In solution, micelles exist in dynamic equilibrium with the monomers from which they are formed. Therefore, micelles are generally considered as polydispersed species due to dynamic equilibrium. As will be discussed in the later chapters, such polydispersity can result in a range of micelle migration velocities in CE,
resulting in band broadening which can be detrimental to electrokinetic separations. In order to better understand the nature of micelles and micellization, kinetics and thermodynamic factors should be discussed. Therefore, the concepts of surfactant/micelle equilibrium are discussed next.

1.1.1. Kinetics of Micelle Formation

The formation of micelles from ionic surfactants occurs as a result of the balance needed between hydrophobic tail attraction and electrostatic head repulsion. As discussed in some kinetic studies, micelles are involved in a highly dynamic equilibrium (9). These studies have shown that an equilibrium exists between the surfactant molecules and the micelle. In addition to this dynamic equilibrium, it is important to understand another equilibrium interaction between a micelle and its guest solute. The complexation of the micelle with a given guest solute is also a dynamic interaction. These two equilibria are illustrated in Figure 1.6.

Aniansson and Wall have suggested a kinetic model for micelle formation that is generally accepted as the basis for the relaxation process of micelles (27, 28). According to their model, the following equilibria are observed:

\[
\begin{align*}
S_1 + S_1 & \rightleftharpoons S_2 & K_1 = \frac{[S_2]}{[S_1]^2}, \\
S_2 + S_1 & \rightleftharpoons S_3 & K_2 = \frac{[S_3]}{[S_1][S_2]}, \\
S_{n-1} + S_1 & = S_n & K_n = \frac{[S_n]}{[S_1][S_{n-1}]}.
\end{align*}
\]

where \( S_1 \) and \( S_2 \) are surfactant monomers and dimer, respectively. \( S_n \) refers to a micelle with \( n \) numbers of monomers (n-mer). In the above equations, \( K_1, K_2, \) and \( K_n \) are the equilibrium constants. From the stepwise formation process, it is evident that a micellar solution will contain
different degrees of aggregations. Therefore, normal micelles are considered as polydispersed aggregates. The law of mass action implies that a continuous distribution of species should be present (26). Increasing the micelle concentration should shift the equilibrium in Equation 1.1, 1.2, and 1.3 towards the right. In other word, an increase in surfactant concentration will increase $S_2$, $S_3$, and $S_n$ concentrations.

1.1.2. Thermodynamics of Micellization

To understand the thermodynamics of micellization, let us assume a simple association equilibrium between the surfactant monomers (S) and the micelle ($M_n$) with an aggregation number of $n$ (29), we can write

$$nS \rightleftharpoons M_n.$$  \hspace{1cm} 1.4

The micellization constant $K$ is written as
The total concentration of surfactant ($C_t$) becomes

$$C_t = [S] + nK[S]^n.$$  \hspace{1cm} 1.6

The free energy of micellization for a surfactant is expressed as

$$-\Delta G = RT \ln K,$$  \hspace{1cm} 1.7

where $K$ is the micellization constant defined in Equation 1.5. Combining Equations 1.5 and 1.7 leads to

$$-\Delta G = RT(\ln[M_n] - n \ln[S]).$$  \hspace{1cm} 1.8

The free energy for inserting one monomer unit into the micelle can be expressed by dividing Equation 1.8 by the numbers of monomers

$$-\Delta G^0 = \frac{-\Delta G}{n} = \frac{RT}{n} \ln[M_n] - RT \ln[S].$$  \hspace{1cm} 1.9

If the value of $n$ is large, then

$$\Delta G^0 \approx RT \ln[S].$$  \hspace{1cm} 1.10

The concentration of free surfactant monomers is equal to the CMC value if the added surfactant monomers form new micelles above the CMC. As a result, $[S] = \text{CMC}$, and Equation 1.10 can be rewritten as

$$\Delta G^0 \approx RT \ln \text{CMC}.$$  \hspace{1cm} 1.11

1.2. Vesicles

The micelle-forming amphiphiles were discussed in detail previously. Other surfactants possessing two or more hydrophobic tails per monomer usually form bilayers in water. When exposed to ultrasonic radiation, these bilayers form a closed bilayer structures like spherical
bags, referred to as vesicles (schematically depicted in Figure 1.7 (30, 31). Lipid molecules, i.e., double-chain amphiphiles, are the basic building blocks of all biological membranes (32, 33). Their self-aggregation in water is the result of the hydrophobic effect, as in the case with surfactants (34). This self-organization depends also on the relative proportion of hydrophobicity and hydrophilicity of the lipid, as well as on its geometry (35). Depending on the water content, homogeneous, smectic phases of parallel lipid bilayers (lyotropic phases) and heterogeneous dispersions of multi-lamellar or single-walled liposomes can be formed. In addition, for low water content and high temperature, other lyotropic liquid-crystalline phases exist, such as the hexagonal, the cubic, and the ribbon phase (36). Most of the naturally occurring lipids are zwitterionic (e.g., lecithin), anionic (e.g., phosphatidic acid) or uncharged (glycolipids) (37). In addition to the classical double-chain lipids discussed above, bilayer-forming amphiphiles with only one (38) or three (39) hydrophobic alkyl chains also exist.

Figure 1.7. Vesicle formation by ultrasonic treatment of bilayer membrane.
Vesicles are spherically closed lipid bilayers, which, in analogy to the cell membrane, enclose an aqueous compartment (40). Vesicles can be prepared by variety of methods, which lead to the formation of completely different vesicle systems (41). These differ in diameter (20 nm to 100 \( \mu \)m) as well as in the number of bilayers. The sonication of lipid suspensions in water leads to small unilamellar vesicles with a diameter ranging from 20 to 100 nm. Vesicles are suitable for a large number of biophysical and biochemical investigations including measurement of membrane permeability (40), reconstitution of active membrane proteins (42), study of surface recognition reactions (43) or dynamic membrane processes (44), and usage as drug carriers (45). Interactions of drugs and nucleic acids with liposomal membranes have been extensively studied (46-48).

1.3. Capillary Electrophoresis

Electrophoresis (from the Greek words \( electron = \) electron and \( phoresis = \) carrying) is defined as the differential movement of charged molecules under the influence of an electric field (49). The experiments of Arne Tiselius on moving boundary electrophoresis during 1930s are considered as the root of modern electrophoresis (50). Studies on partial separation of protein mixture (i.e., \( \alpha-, \beta-, \) and \( \gamma-\)-globulin) contributed to Tiselius’s receipt of the Nobel Prize in 1948 (51). Over the next two decades, research continued on development of several modes of electrophoresis, moving boundary electrophoresis, zone electrophoresis, and isotachophoresis. In the late 1960s and 70s, several researchers tried to develop CE as a microanalytical separation tool (52-54). The modern era of CE was initiated with a series of papers by Jorgenson and Lukacs using fused silica capillaries with internal diameter (I.D.) of 75 \( \mu \)m to achieve separation (55-57).
A simple schematic of a CE instrument and its components are shown in Figure 1.8. A CE instrument consists of: 1) a high-voltage power supply (0 to 30 kV), 2) a capillary (externally coated with polyimide to give flexibility) with an internal diameter ranging from 20 to 200 μm, 3) two buffer reservoirs that house the capillary ends, 4) two electrodes connected to the power supply, and 5) a detector. To perform a CE separation, the capillary is filled with a desired electrolyte solution. Both ends of the capillary and the electrodes are placed into buffer reservoirs and a voltage is applied to the system. Upon application of voltage, ionic species in the capillary experience an electric field (E). In such a field \( E \), an ion with a charge of \( q \) experiences a force magnitude \( F_E \) of

\[
F_E = qE .
\]
An anion in this field migrates toward the positive electrode (anode), and a cation migrates toward the negative electrode (cathode). As the ion moves through the buffer solution, it experience a frictional retarding force \( F_F \),

\[
F_F = 6\pi \eta r \nu ,
\]

where \( \eta \) is the viscosity of the solution, \( r \) is the hydrodynamic radius of the particle. The directions of the two forces, \( F_E \) and \( F_F \), are opposite; therefore, the charged particles quickly reach a terminal speed \( \nu \), (59), where \( \nu \) is

\[
\nu = \frac{qE}{6\pi \eta r} .
\]

The ion velocity can also be expressed as

\[
\nu = \mu_e E ,
\]

where \( \mu_e \) is the electrophoretic mobility of the charged particle. Combining equation 1.14 and 1.15 will give the relationship between \( \mu_e \) and the charge and the size of the ion, as well as the viscosity of the solution as follows:

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

From Equation 1.16, it noted that smaller and more highly charged molecules will have higher mobilities than larger, less charged species. In addition, the electrophoretic mobility increases as the viscosity of the solution and the hydrodynamic radius of the ion decreases. The movement of a charged species through the capillary is also a function of the movement of the bulk solution. The movement of the bulk solution is known as the electroosmotic flow (EOF)(60). The walls of a fused-silica capillary contain silanol groups (Si-OH). When a fused silica capillary is rinsed with a buffer above pH 3, the inner surface of the capillary acquires a negative charge due to ionization of the silanol groups on the walls of the capillary. The negatively charged capillary
wall then attracts positively charged species from the running buffer. Consequently, a fixed positive layer forms at the capillary wall. This results in a potential difference at the capillary wall known as the zeta potential ($\zeta$). Because these cations are not sufficient to neutralize all the negative charges, an outer mobile layer of positive ions forms (Figure 1.9).

![Diagram of the capillary and the ionic layer](image)

**Figure 1.9. Diagram of the capillary and the ionic layer**

When an electric field is applied across the capillary, the outer layer of positively charged ions is pulled toward the negative electrode (i.e., cathode) (60). A schematic of this process is depicted in Figure 1.10. This movement of the cationic layer will drag the bulk buffer solution with it, thus causing EOF. Because the magnitude of the EOF toward the cathode is very large, anions are also swept toward the cathode under the influence of the EOF. In normal CE mode,
smaller cationic species with a large charge/radius ratio elute first, followed by the larger, less charged cations. Anions with smaller charge/radius ratios elute earlier than anions with larger

Figure 1.10. Development of electroosmotic flow. A) Fused silica capillary tube with silanol groups. B) Partial dissociation of hydroxyl group. C) Complete dissociation of hydroxyl group leaving negative charge on the inside of capillary. Positive ion layer flows toward the cathode causing electroosmotic flow.
charge/radius ratio. Neutral species, however, migrate at the rate of the EOF due to the lack of a charge. The differential solute migration of the various species is depicted in Figure 1.11. The EOF is proportional to $\zeta$, which is proportional to the thickness of the double layer. This relationship can be formulated as following:

$$\zeta = \frac{4\pi \delta e}{\epsilon},$$

1.17

where $\delta$ is the thickness of the double layer, $e$ is the charge per unit surface area, and $\epsilon$ is the dielectric constant of the buffer. The velocity of the EOF, $v_{\text{EOF}}$, is given by

$$v_{\text{EOF}} = \frac{\epsilon \zeta E}{4\pi \eta},$$

1.18

where $E$ is the applied electric field in volts/cm. The EOF mobility, $\mu_{\text{EOF}}$, of the running buffer is given by

$$\mu_{\text{EOF}} = \frac{\epsilon \zeta}{4\pi \eta}.$$  

1.19

As shown in Equation 1.19, dielectric constant, zeta potential and the buffer viscosity influence

![Figure 1.11. Differential solute migration in capillary zone electrophoresis.](image)
the value of $\mu_{\text{EOF}}$. The factors affecting zeta potential are surface charge density, silanol dissociation, pH and the concentration of the buffer.

The EOF is a major factor that controls the retention time of a given solute. Therefore, it is imperative to control the EOF by alteration of the capillary surface or the viscosity of the running buffer. Decreasing the applied voltage can decrease the EOF; however, this will result in an increase in analysis time. Other ways to modify the EOF are by adjusting the pH and the ionic strength of the buffer and by modification of the capillary wall. Modification of the capillary wall may increase, decrease, or reverse the EOF.

The solute velocity, $v$, and the apparent solute mobility, $\mu_{\text{app}}$, are calculated using the following equations

$$v = \frac{L_d}{t},$$  \hspace{1cm} 1.20

$$\mu_{\text{app}} = \frac{v}{E} = \frac{L_d L_t}{t E t V},$$ \hspace{1cm} 1.21

where $L_d$ is the effective capillary length (to the detector), $L_t$ is the total capillary length, $t$ is the migration time, and $V$ is the applied voltage. The effective mobility $\mu_{\text{ep}}$ can be calculated as follows

$$\mu_{\text{ep}} = \mu_{\text{app}} - \mu_{\text{EOF}}.$$ \hspace{1cm} 1.22

As seen in Equation 1.22, $\mu_{\text{app}}$ will be positive for cations and negative for anions. Since the neutral species do not have any charge, their electrophoretic mobility is zero. The EOF is usually measured using neutral species (i.e., methanol, acetonitrile, dimethyl sulfoxide, mesitylene oxide, etc.) that move at the velocity of the EOF.

One of the major advantages of CE over conventional separation techniques such as high performance liquid chromatography (HPLC) and gas chromatography (GC) is the flat flow
profile. The flat profile is a result of uniform distribution of the driving force along the capillary wall, therefore, there is no pressure drop within the capillary. The flat profile in CE increases the separation efficiencies as compared to pressure driven HPLC (59). A comparison of the flow profile observed in HPLC to the flow profile seen in CE is illustrated in Figure 1.12.

\[ N_{TP} = \frac{\mu_{ep} V}{2D_0}. \]  

1.23

According to Equation 1.23, the higher theoretical plates are achieved when ions have large mobilities. However, as the speed of the migration increases, resolution decreases, because there is not enough time for the components to be resolved.
1.3.1. Modes of Capillary Electrophoresis

The main modes of CE that have been developed include capillary zone electrophoresis (CZE), also known as free-solution CE (FSCE), capillary isoelectric focusing (CIEF), capillary isotachophoresis (CITF), capillary gel electrophoresis (CGE), capillary electrochromatography (CEC), and micellar capillary electrophoresis (MCE), commonly known as micellar electrokinetic chromatography (MEKC or MECC) (61, 62). The main advantage of CE is that the same instrument can be used, with minor modifications, in any mode listed above.

The CZE mode of separation has a simple arrangement of the background electrolyte (BGE). The separation capillary is filled with BGE and upon application of a voltage, V, the solutes are separated due to their different charge and/or size. In CIEF, a charged molecule is electrophoretically driven through a pH gradient until it encounters a pH at which it carries a zero net charge. At this point, it experiences zero electromotive force and stops moving. Most of the proteins stop migrating at their isoelectric point ($pI$) at which they possess a zero net charge and separate according to their $pI$ values (63, 64). In CITF, a sample is injected between a discontinuous electrolyte system formed by the leading and terminating electrolytes. The leading electrolyte contains the ion with the highest mobility, whereas the terminating electrolyte contains the ion with lowest mobility. When a sample is introduced into the capillary between the leading and terminating electrolytes, the ions with mobilities that are between those of the leading and terminating ions will migrate isotachophoretically and create typical stacked isotachophoretic zones with sharp boundaries.

One of the major drawbacks with CE separation of either nucleotides or DNA molecule is their constant linear charge density, that is, a ten base pair and hundred base pair DNA molecule both have the same charge/mass ratio. As a result, a sieving medium is often used to separate
such molecules based on size, not based on charge/mass ratio. In CGE, cross-linked or non-cross-linked matrices have been used (65, 66) for the separation of a variety of macromolecules. In CGE, it is the size-dependent retardation of the solute that is a primary factor of the separation of large molecules. If the solute is a polion holding the same amount of charge per unit, the total electrostatic force on the moving ion is assumed to be constant per molecular weight unit. Thus, the amount of SDS molecules surrounding the protein depends on the protein’s molecular weight (MW). Subsequently, the higher the MW of the protein, the more SDS is attached and separation will be based on the MW of the biopolymer (67-69).

The CEC mode is a hybrid of HPLC and CE. In CEC, separation is achieved on the basis of differential partitioning into a stationary phase. The mobile phase is pumped electrically and thus the analytes are carried through the column by the EOF. In essence, CEC has a similar experimental setup to that of CE except that the capillary is packed with stationary phase particles. Since there is no pressure limitation in CEC, the stationary phase particle diameter can be reduced to the submicrometer levels. Furthermore, larger theoretical plates can be achieved by increasing the column length which is not practical in conventional HPLC systems. The laminar flow profile (Figure 1.12) in pressure-driven HPLC contributes to zone dispersion and causes low efficiencies. In contrast, one expects to achieve higher efficiencies due to electrically driven plug profile in CEC. However, major problems are encountered with CEC during column preparation. These problems include the preparation of the frits (which prevent the stationary phase from migrating out of the capillary), the column packing method, reproducibility between columns, and Joule heating which may cause bubble formation in the column as well as band broadening. Finally, MCE (commonly known as MEKC) will be discussed below in detail because of its use in this dissertation.
1.4. Micellar Capillary Electrophoresis

The primary application of CE is the separation of ionic species. Due to the lack of a net charge, neutral analytes cannot be separated with conventional CE. In an effort to extend the separation power of CE to charged as well as uncharged solutes simultaneously, Terabe and co-workers introduced the use of ionic surfactants in the buffer solutions for CE (70,71). This mode of CE is known as micellar capillary electrophoresis (MCE), micellar electrokinetic chromatography (MEKC), or micellar electrokinetic capillary chromatography (MECC). This technique is regarded as a chromatographic technique because the separation mechanism is due to the differential partitioning of the analytes into the micellar (pseudostationary) phase. The MCE mode can be viewed as a hybrid of CZE and reversed-phase HPLC (RP-HPLC) because electrokinetic migration, partitioning mechanism, and hydrophobic interactions govern the migration and the separation of the analytes. The MCE mode uses the same instrumental setup as CE, but charged organized media such as micelles are added to the buffer as the separation medium for uncharged solutes. Charged micelles move under an applied voltage across the separation capillary column at an electrophoretic velocity that is proportional to their charge/size ratio. Uncharged solutes can be separated based on their micelle-water partition coefficients, $P_{mw}$. The parameter $P_{mw}$ is defined as

$$P_{mw} = \frac{C_m}{C_w}, \quad 1.24$$

where $C_m$ and $C_w$ are the concentration of the solute in micellar phase and in aqueous phase, respectively. Figure 1.13 illustrates the migration of neutral species with anionic and cationic micelles. In addition, as shown in this figure, all neutral solutes are separated between the migration time of an unretained solute, $t_0$, and the migration time of micelle, $t_{mc}$. Thus, there is a limited elution window in MCE. Since MCE is a hybrid of CZE and RP-HPLC, it offers a
combination of unique features of CZE and RP-HPLC such as high efficiencies, rapid analysis, small sample size, small solvent consumption, and excellent selectivity.

The MCE mode is the only CE mode that can separate charged and uncharged solutes simultaneously. It offers larger theoretical plates (i.e., higher efficiency) than RP-HPLC and

![Diagram of MCE separation](image)

Figure 1.13. Migration of uncharged solutes in MCE using anionic (top) and cationic (middle) micelles. Separation of solutes $S_A$, $S_B$, and $S_C$ is achieved due to their differential partitioning into the micellar phase. The uncharged solutes are eluted within an elution window ($t_{mc}/t_{eo}$) (bottom).
CEC. Another major advantage of MCE over conventional chromatographic techniques is the flexibility of changing the chemical composition of the pseudostationary phase and/or the mobile phase. This flexibility of controlling and easily modifying key parameters leads to improved separations and better method development.

1.4.1. Pseudostationary Phases in MCE

A variety of pseudostationary phases have been used in MCE since the introduction of the technique. These include 1) ionic alkyl chain monomeric and polymeric surfactants (70-87), 2) liposomes and vesicles (88-93), 3) bile salts (94-100), and 4) dendrimers (101-105).

1.4.1.1. Monomeric Surfactants and Vesicles

Monomeric surfactants used in MCE can be anionic, cationic, zwitterionic; however, the majority of the pseudostationary phases used in MCE are anionic surfactants. Although limited in utility, fluorinated surfactants have also been used in MCE (106). The location of the solubilized solutes within the micelle may be in any of the several regions of the micelle (107). Ionic species oppositely charged from the polar head of the surfactant may bind tightly to the polar head via coulombic attraction (108-112). Nonpolar species with polarizable electrons such as aromatic hydrocarbons reside near polar head group rather than deep within the core of the micelle (113,114). Hydrophobic alkanes are believed to penetrate deeper into the core of the micelle (108, 110, 115). Finally, solutes with amphiphilic character may have special interaction with the micelle and align themselves with the nonpolar section of the molecule directed towards the hydrophobic core of the micelle and the more polar end of the molecule directed to the bulk aqueous phase (113, 115, 116). Figure 1.6 depicts some of the possible solubilization sites of surfactant.
Some attempts to use vesicles, as alternative to micelles, as pseudostationary phases have been carried out (88-93). Liposome-like vesicles formed from a mixture of cationic and anionic surfactant (cetyltrimethylammonium bromide / sodium dodecyl sulfate, CTAB/SDS) have been also used for the CE separation of hydrophobic analytes (93). It has been shown that liposome-like surfactant vesicles gave larger migration time windows and better selectivities than the normal SDS micelle.

It is important to note that there are chemical differences between micelles and vesicles. As shown in Figure 1.14, a vesicle in aqueous solution displays nine different regions for solute interaction (31). These regions include the outer bulk water region, the hydration sphere, the hydrophobic membrane close to the outer head groups, the similar four inner regions in the water pool direction, and the hydrophobic core of the vesicle. In contrast, micelles provide less available sites for solute localization/ solubilization. Interaction of a given solute with normal micelles is restricted to the first four regions mentioned above and may be to the hydrophobic core of the micelle (30).

**Figure 1.14. Available sites for solute interaction/solubilization in vesicles and micelles.**
1.4.1.2. Mixed Micelles

The use of mixed micelles in MCE has dramatically increased over the past few years (117-122). Different types of surfactant offer different retention behavior and selectivity. One type of surfactant may not be suitable choice for certain complex mixture of structurally similar solutes. This may be due to a lack of selectivity and/or a narrow elution window. In these situations, mixed micelles can enhance separation, change selectivity, and increase the elution window. Wallingford and co-workers observed that selectivity and efficiency increased significantly using mixed SDS-sodium octyl sulfate for a group of borate-complexed catechols (118). Mixed chiral and achiral surfactants have also been used to enhance selectivity of MCE separation for optical isomers (120). Rasmussen et al. reported selectivity changes between benzene and benzaldehyde upon addition of a nonionic surfactant, Brij-35®, to SDS (123). The two analytes coeluted at different concentrations of SDS; however, they were readily separated with mixed Brij-SDS micelles.

1.4.2. Modifiers

Modifiers such as organic solvents (127-133), cyclodextrins (130, 134-141), and urea and glucose (135, 142-147) are included in the micellar solutions of MCE for adjusting the migration factor, manipulation of selectivity, and extension of the elution window. These additives influence EOF velocity, elution of micelles, and partition coefficients of solutes into pseudostationary phases.

1.4.2.1. Organic Solvents

Organic modifiers such as methanol, ethanol, 2-propanol, acetonitrile, and tetrahydrofuran offer a wide range of polarity and selectivity and also improve the separation of highly hydrophobic compounds that elute near or with micelles (124-133). The main role of
organic solvents in MCE has been to reduce the capacity factor, $k'$, of highly hydrophobic analytes to a reasonable range. Typically addition of organic modifiers leads to a reduction in EOF, a change in velocity of the micelle, and hence an increase in the size of the elution window.

In addition to regular organic modifiers, the use of other modifiers such as urea and glucose has also been employed in MCE. It has been reported that urea increases the separation window and increases the solubility of the highly hydrophobic solutes in the mobile phase. Kenata et al. have reported that glucose as additive to the mobile phase increases the resolution in MCE (147).

1.4.2.2. Cyclodextrins

Cyclodextrins (CDs) are cyclic oligomers of $\alpha$-D-glucose unites formed by the action of certain enzymes on starch. Three CDs are commonly available: $\alpha$-CD, $\beta$-DC, and $\gamma$-CD, with six, seven, and eight glucose units, respectively. These three CDs are generally referred to as native CDs. Many covalently modified CDs have been prepared from these native forms. Figure 1.15 shows the chemical structures of $\alpha$-CD, $\beta$-DC, and $\gamma$-CD.

The glucose unites of cyclodextrins are connected through glycosidic $\alpha$-1,4 bonds (Figure 1.15 D). These molecules typically take the shape of a truncated cone with an open cavity, relatively hydrophobic and an outside hydrophilic due to the presence of hydroxyl groups (Figure 1.15 E). As shown in Table 1.1, the physical properties of the three CDs are quite different; however, they possess the same depth (148-155). As can be seen in Table 1.1, the solubility of $\beta$-CD is very low compared to that of the other native CDs. The solubility of $\beta$-CD can be increased using additives such as organic solvents, urea, high pH, and chemical modification of $\beta$-CD (148, 156).
Figure 1.15. Chemical structure of (A) α–CD, (B) β–CD, (C) γ–CD, (D) glucose units, and (E) the shape of CD.
Table 1.1. Physicochemical properties of native CDs.

<table>
<thead>
<tr>
<th>Property</th>
<th>α-CD</th>
<th>β-CD</th>
<th>γ-CD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of glucose units</td>
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<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Empirical formula (anhydrous)</td>
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<td>C_{42}H_{70}O_{35}</td>
<td>C_{48}H_{80}O_{40}</td>
<td></td>
</tr>
<tr>
<td>Molecular weight, g/mol</td>
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<td>1134.99</td>
<td>1297.14</td>
<td></td>
</tr>
<tr>
<td>Cavity depth, Å</td>
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<td>8</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>Cavity diameter, Å (approximately)</td>
<td>~5.2</td>
<td>~6.6</td>
<td>~8.4</td>
<td>100</td>
</tr>
<tr>
<td>Solubility (water, 25°C), mol/L</td>
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<td>0.0163</td>
<td>0.168</td>
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<tr>
<td>[α]_D, deg</td>
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<td>+162.0</td>
<td>+177.4</td>
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</tr>
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<td>Heat capacity (anhyd solid), J/mol K</td>
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<td>1342</td>
<td>1568</td>
<td>103</td>
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<tr>
<td>Heat capacity (infinite diln), J/mol K</td>
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<td>1783</td>
<td>2070</td>
<td>103</td>
</tr>
<tr>
<td>pK_a (25°C)</td>
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<td>12.20</td>
<td>12.08</td>
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</tr>
<tr>
<td>ΔH° (ionization), kcal/mol</td>
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<td>9.98</td>
<td>11.22</td>
<td>105</td>
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<tr>
<td>ΔS° (ionization), cal/mol K</td>
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<td>ΔH° (solution), kcal/mol</td>
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<td>8.31</td>
<td>7.73</td>
<td>101</td>
</tr>
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<td>ΔS° (solution), cal/mol K</td>
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<td>11.7</td>
<td>14.7</td>
<td>101</td>
</tr>
</tbody>
</table>

Since its first application by Terabe et al. for the separation of very hydrophobic compounds, CD modified MCE (CD-MCE) has been extensively used for the separation of both chiral (138, 139, 157, 158) and achiral (134, 140, 159) solutes. Figure 1.16 shows a proposed migration model for CD-MCE.

![Figure 1.16. Schematic illustration of the separation principle in CD-MCE.](image_url)
The hydrophobic cavity of CDs provides an alternative site of interaction to micelles for the hydrophobic solutes. Analytes are then distributed between micelles and CDs. Since uncharged CDs migrate with the EOF velocity and in the opposite direction of charged micelles, the net retention time of analytes decreases in the presence of CDs. As a result, hydrophobic analytes that interact strongly with the micelle can be better separated and enhancement in resolution would be achieved. An important feature of this technique is that CDs introduce a shape selective effect that is beneficial for the separation of structural, geometrical, and optical isomers. The inclusion complex between the CDs and solutes depends on the size and shape of molecules, as well as the interior size of the CDs. Based on the published data it seems that the cavity of β-CD is appropriate to host variety of chemical compounds. The many attractive properties of CDs have lead many researchers to develop novel CD derivatives. More attention has been given to the less soluble β-CD. Thus, many derivatives of β-CD have been synthesized by substitution of the primary and secondary hydroxyl groups with different groups (160). The hydroxyl groups present on the rim of the CDs can be easily modified by chemical reactions. A wide number of CD derivatives have currently been used in CE for chiral and achiral analysis. Among them, uncharged methylated-, hydroxyethylated-, hydroxypropylated-, acetylated-CDs and charged ones such as methylamino-, sulfobutylether-, carboxymethylated-, sulfated-, phosphated-CD can be mentioned.

Modified CDs can have very different properties than native CDs. These include: increased solubility, possibility for different secondary bonds, different hydrophobicity of the cavity, potentiality for the analysis of highly hydrophobic and uncharged compounds, etc. For example, comparing β-CD and dimethyl-β-CD (DM-β-CD), it can concluded that the presence
of methoxy groups increases both the depth and the solubility of the derivative $\beta$-CD. Derivatives of $\beta$-CD can be easily used for improving the selectivity of CD-MEKC separations.

The formation of inclusion complexes between solutes and CDs in solution is described by the following relationship

$$CD + S \rightleftharpoons CD-S,$$ 1.25

where CD, S, and CD-S represent the cyclodextrin, solute, and inclusion complex of CD and the solute, respectively. The association constant ($K_a$) and the dissociation constant ($K_d$) are described by the following equations:

$$K_a = \frac{[CD-S]}{[CD][S]},$$ 1.26

$$K_d = \frac{1}{K_a} = \frac{[CD][S]}{[CD-S]},$$ 1.27

where $[CD]$, $[S]$, and $[CD-S]$ are the equilibrium concentrations of the CD, solute, and inclusion complex, respectively. In CD-MEKC, the distribution of solute between aqueous (aq) CD, and micelle (M) phases can be expressed in terms of the micellar-aqueous ($K_M$), CD-aqueous ($K_{CD}$), and CD-micellar ($K_{CD-M}$) distribution coefficients as depicted below.

$$S_{(aq)} \rightleftharpoons S_{(M)}, \quad K_M = \frac{[S_{(M)}]}{[S_{(aq)}]},$$ 1.28

$$S_{(aq)} \rightleftharpoons S_{(CD)}, \quad K_{CD} = \frac{[S_{(CD)}]}{[S_{(aq)}]},$$ 1.29

$$S_{(M)} \rightleftharpoons S_{(CD)}, \quad K_{CD-M} = \frac{[S_{(CD)}]}{[S_{(M)}]},$$ 1.30

The exchange of the solute between CD and micellar phases can occur in a stepwise fashion involving the processes labeled $K_M$ and $K_{CD}$ in which case...
$$K_{CD-M} = \frac{K_{CD}}{K_M}. \quad (1.31)$$

Alternatively, direct exchange of the solute between electroosmotically migrating CD and electrophoretically hindered micelle can occur. In either case, the value of $K_{CD-M}$ is directly related to the stability of a CD-S complex and the relationship between the $K_{CD-M}$ values of the solute should determine elution order (140).

### 1.4.3. Molecular Micelles in Capillary Electrophoresis

There are some problems with conventional micelles used in MCE. First, conventional micelles such as SDS can tolerate only up to 20-30% organic solvents before micelle formation is deteriorated. The use of high concentration of organic modifier is often necessary, especially for the separation of highly hydrophobic compounds such as polycyclic aromatic hydrocarbons (PAHs) that interact strongly with the micellar phase (76, 81, 161). Second, applied voltage across the capillary often causes Joule heating, especially when high concentrations of surfactant are used. Joule heating will increase the temperature inside the capillary. Since CMC can be affected by temperature (76, 161), change in CMC will have a significant effect on separation in MCE (162-165). This will result in irreproducible run times and poor peak shapes and efficiencies. Third, the dynamic equilibrium between micelles and surfactant monomers has a significant effect on the shape and the size of the micelle. This limits the flexibility of the technique in terms of the choice of the analytical conditions.

An ideal pseudostationary phase for MCE should: 1) provide stability and desirable chromatographic selectivity under a wide variety of separation conditions; 2) have high electrophoretic mobility in order to provide a wide elution window; 3) have zero or very low CMC to minimize Joule heating; 4) be monodisperse and provide a fast mass transfer of the solutes between aqueous buffer phase and the pseudostationary phase to achieve high efficiency.
Although they are useful for many applications of MCE, conventional micelles will not meet all of these criteria. This has led many researchers to development of new types of pseudostationary phases. Polymeric micelles seem to satisfy most of criteria and be candidate as ideal pseudostationary phases.

Polymeric micelles have several advantages over conventional micelles. First, the covalent linkage among the monomer units of the polymeric micelle provides a rigid structure. This can improve the mass transfer rate between the polymeric micelle and the solute, consequently, reducing peak broadening. Second, polymeric micelles have zero CMC. Thus, the polymeric micelle can be used over a wide range of concentrations than the monomer, e.g. below the CMC of the unpolymerized surfactant. Third, the dynamic equilibrium between the micelles and the monomers is eliminated. This minimizes problems often associated with the conventional micelles in MCE (166). Fourth, polymeric micelles are stable in the presence of relatively high content of organic modifiers and inclusion molecules, such as CDs. In contrast, normal micelles are disrupted in presence of high content of organic solvents and inclusion compounds (167-169). Finally, certain properties of polymeric micelle can be fixed through the polymerization process. Figure 1.17 illustrates three models that represent proposed polymerized surfactant structure and the polymerized vesicle.

The first reports of the use of a polymeric micelle as pseudostationary phase in MCE were those of Palmer and coworkers (72, 161). They used polymeric micelle of sodium 10-undecylenate (SUA) as for the separation of alkyl phthalates and PAHs in presence of high content of organic modifier (50% v/v methanol or 45% v/v acetonitrile). The stability of the polymer in the presence of high concentration of organic solvents was its greatest advantage. However, a major problem reported regarding this polymer is that the anodic buffer becomes
cloudy after several runs. This problem was attributed to the carboxylic acid head groups and hydrolysis of the anodic buffer. To eliminate this problem, Palmer and Terabe introduced the sulfate analog of SUA, sodium undecenyl sulfate (SUS) as the pseudostationary phase (72, 170, 171). The monomer of SUS has a double bond at the end of the aliphatic chain with a sulfate head group.

Shamsi et al. have also utilized this polymer as a pseudostationary phase in MCE for the separation of PAHs. Palmer et al. polymerized the micellar solution of SUS using a chemical polymerization procedure. Alternatively, T-type polymeric micelles were produced by Shamsi et al. with gamma radiation induced covalent linkage of the monomers at concentrations above the CMC (81, 84, 172).

Figure 1.17. Illustration of polymeric surfactant models A) molecular micelle, B) regional micelle, C) local micelle, and D) polymerized vesicle.
1.4.4. Migration in Micellar Capillary Electrophoresis

The representative migration scheme for uncharged solutes in MCE using an ionic and a cationic surfactants in an untreated fused silica capillary is illustrated in Figure 1.13. Anionic micelles migrate in the opposite direction of the EOF in an uncoated fused capillary under typical conditions (e.g., pH greater than 6). The EOF is stronger than the electrophoretic velocity of the micelles. Thus, anionic micelles are carried toward the cathode, the negative electrode. When cationic micelles are used as pseudostationary phases, the negatively charged capillary wall is coated with positively charged surfactants, which reverses the direction of EOF. Therefore, It is imperative to reverse polarity of the electrodes in the CE setup when using cationic surfactants.

The elution window (or separation window) is defined by two extremes in MCE. Analytes that do not interact with the pseudostationary phase ($P_{mw} \sim 0$) spend their entire time in running buffer and migrate at the EOF velocity. These are generally neutral polar solutes such as methanol or acetonitrile that are used as EOF markers, $t_{eo}$. The other extreme is defined by the elution of the analytes that spend almost all of their time inside the micellar phase ($P_{mw} \sim \infty$). These types of analytes (e.g., Sudan III, and dodecanophenone) are highly hydrophobic and used as $t_{mc}$ markers.

The retention factor, $k'$, in MCE is defined, as in chromatography, as the ratio of the number of moles of analyte in the micellar pseudostationary phase, $n_{mc}$, to that in the bulk aqueous buffer phase, $n_{aq}$. The $k'$ is directly proportional to the micelle-water partition coefficient, $P_{mw}$, and the phase ratio, $\Phi$, as

$$k' = \frac{n_{mc}}{n_{aq}} = P_{mw} \Phi .$$

1.32
The \( k' \) in MCE can be determined from \( t_{eo} \), \( t_{mc} \) and the retention time of neutral solute, \( t_R \) using the following equation (70, 71):

\[
k' = \frac{t_R - t_{eo}}{t_{eo} \left[ 1 - \frac{t_R}{t_{mc}} \right]}.
\]

1.33

This is similar to the equation for \( k' \) in conventional chromatography except the additional term 
\( (1-t_R/t_{mc}) \) in the denominator which indicates that the pseudostationary phase is actually mobile. If \( t_{mc} \) approaches infinity, the extra term in denominator is omitted and \( k' \) becomes the same as that in conventional chromatography

\[
k' = \frac{t_R - t_{eo}}{t_{eo}}.
\]

1.34

The fundamental resolution equation for uncharged solutes in MCE has the same format as that for conventional chromatography.

\[
R = \left( \frac{N^{1/2}}{4} \right) \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k_2'}{1 + k_2'} \right) \left[ \frac{1 - (t_{eo} / t_{mc})}{1 + (t_{eo} / t_{mc})k_1'} \right],
\]

1.35

Equation 1.35 indicates that resolution depends on three terms related to efficiency, selectivity, retention, and elution window, represented by the first, second, third, and the fourth term, respectively (71).

The peak capacity, \( n \), can be increased with wider elution windows:

\[
n = 1 + \sqrt[4]{\frac{N_{TP}}{4}} \ln \frac{t_{mc}}{t_{eo}},
\]

1.36

where \( N_{TP} \) is the number of theoretical plates. The \( n \) value determines the maximum number of components that could be resolved within a chromatographic run (173). The optimum capacity
factor or retention factor, $k'_{opt}$ for achieving maximum resolution is approximately related to the square root of the elution window size (174):

$$k'_{opt} = \frac{t_{mc}}{t_{eo}}. \quad 1.37$$

The concentration of the surfactant can be adjusted to achieve optimum $k'$ for a better resolution. According to Terabe et al. (71), the relationship between $k'$ and surfactant concentration, $C_{surf}$, can be expressed as follows:

$$k' = \frac{\bar{v}(C_{surf} - CMC)}{1 - \bar{v}(C_{surf} - CMC)} P_{mw}, \quad 1.38$$

where $\bar{v}$ is the partial specific volume of the surfactant, $C_{surf}$ is the concentration of the surfactant, CMC is the critical micelle concentration, and $P_{mw}$ is the partition coefficient of a solute between an aqueous phase and micellar phase. The second term in the denominator (i.e., $C_{surf}-CMC$) becomes negligible at low micelle concentrations of surfactant. Thus, a linear relationship between $k'$ and surfactant concentration can be described as follow:

$$k' = P_{mw} \bar{v}(C_{surf} - CMC). \quad 1.39$$

Selectivity, $\alpha$, between two solutes is simply defined as the ratio of their $k'$ values. The $\alpha$ value is approximately equal to the ratio of the $P_{aw}$ values:

$$\alpha = \frac{k'_{2}}{k'_{1}} \equiv \frac{P_{mw2}}{P_{mw1}}. \quad 1.40$$

1.4.5. Chemical Selectivity in Micellar Capillary Electrophoresis: Characterization of Solute-Pseudostationary Phase Interactions

It has been widely believed that retention in MCE is due to the hydrophobicity of the solute. It is well known that hydrophobic interaction plays a major role in solute-pseudostationary phase interaction in MCE. However, the composition of the micellar solution,
especially the type of surfactant, has a great influence on the overall retention behavior and separation. This indicates that there are other types of interactions besides hydrophobic interaction.

To achieve a better understanding of the factors that control selectivity and retention, one should first understand the nature of the interactions. This is crucial for appropriate method developments. Complex mixtures can be separated using various surfactant systems in MCE. However, the standard practice for choosing the right pseudostationary phase solution has been trial and error or analysts’ own experience. Stationary phases in gas chromatography (GC) or solvents in HPLC can be selected on the basis of the Rohrschneider-McReynolds scale (175) or Snyder’s selectivity triangle (176, 177). Similar efforts are beneficial for MCE studies. One of the most popular methods used for characterization of selectivity and retention is linear solvation energy relationships (LSERs) (178-183). The LSER model involves regressing the experimentally observed retention factor of a set of solutes against their known solvation and size parameters. The solvation parameter model that is suitable for use in MCE is formulated below:

\[
\log k' = c + mV_x + rR_2 + s\pi_2^1 + a\sum\alpha_2^1 + b\sum\beta_2^0. 
\]

In this equation, \(V_x\), \(R_2\), \(\pi_2^1\), \(\sum\alpha_2^1\), and \(\sum\beta_2^0\) are the solute descriptors. The \(V_x\) represents the characteristic volume of the solute (in \(\text{cm}^3 \text{ mol}^{-1} / 100\)). It is divided by 100 to bring it to scale with the other solute descriptors. The excess molar refraction (in \(\text{cm}^3 / 10\)) is represented by \(R_2\); and is divided by 10 for rough scaling with the other parameters. The polarity / polarizability of the solute is represented by \(\pi_2^1\). The descriptor \(\sum\alpha_2^1\) is the solute’s effective hydrogen bond donating ability; and \(\sum\beta_2^0\) is solute’s effective hydrogen bond accepting ability. The subscript 2 indicates that these parameters represent solute values.
The system constants (m, r, s, a, and b) are related to the contribution of the pseudostationary phase toward each type of interaction. The relative ease of cavity formation and general dispersion interactions for the solute in the pseudostationary phase and mobile phase are related to m. The r coefficient determines the ability of pseudostationary phase to interact with the solute’s n- or π-electrons. The s term represents the dipolarity of the micellar phase. The a and b constants are the measures of the hydrogen-bond-accepting ability and hydrogen-bond-donating ability of the pseudostationary phase, respectively. The regression constant, c, is the phase ratio of the separation system.

1.5. Chirality and Chiral Recognition

A molecule that is not superimposable on its mirror image and rotates plane-polarized light is considered to be chiral. Optically active molecules that rotate light to the right and left are called dextrorotatory (D) and levorotatory (L), respectively (184). These descriptor letters (e.g., D and L) are often used to describe the physical differences of the various stereoisomers of chiral compounds known as enantiomers. The equal proportion mixture of enantiomers is known as a racemic mixture. Enantiomers have the same physical and chemical properties, except for the rotation of plane-polarized light. Many enantiomers may exhibit different biological activities. A well-known example that individual enantiomers of a chiral drug may have different pharmacological properties is thalidomide. Thalidomide is a drug whose racemic mixture had been used as a sedative, anti-nausea, and sleep inducing drug for pregnant women in 1950’s. It was later found that this drug caused serious birth defects. In 1960’s, it was discovered that only one enantiomer (R) of thalidomide was beneficial, while the other enantiomer (S) was causing the birth defects (185). In addition to pharmaceutical products, demand for optically pure compounds is growing in agrochemical, food, and electronic industries.
Therefore, there has been a great demand for development in chiral separation techniques. Advances have been made in chiral analysis using gas chromatography (187, 188), supercritical flow chromatography (189, 190), and high performance liquid chromatography (HPLC) (191-195). However, varying the temperature during separation is a major problem for the first two techniques; and poor efficiency, time-consuming and method development are major problems of the last technique. Capillary electrophoresis (CE), on the other hand, has shown a great promise for chiral compounds. The major advantages of CE over other conventional chromatographic techniques are the use of minimal sample, mobile phase, and chiral selector (pseudostationary phase) consumption, and high efficiency (196).

The mechanism of chiral separation is still not completely understood. Three-point interaction rule was proposed by Dalgliesh to explain the chiral recognition. This rule proposes that chiral recognition requires a minimum of three simultaneous interactions between at least one enantiomer and the chiral selector; and at least one of these three interactions should be stereoselective (197). The other enantiomer, however, can only achieve two of these interactions due to the spatial restrictions (198, 199). A diagram illustrating the three-point rule is shown in Figure 1.18.

Chiral separation by MCE was first reported by Zare et al. using Cu(II) complexes of histidine to separate dansylated amino acid enantiomers (200). Since then, several natural (201-203) and synthetic (204, 205) chiral selectors have been used in MCE to separate chiral solutes. Cohen et al. reported the first synthetic chiral surfactant, N,N-dodecyl-L-alaninate, for the separation of dansylated amino acids’ enantiomers using MCE (206). Dobashi et al. utilized a valine based synthetic monomeric chiral surfactant to separate N-3,5-dinitrobenzoylated amino acid isopropyl ethers (207, 208). Other monomeric chiral surfactants with different head groups
such as glutamate (209), serine (210), tartarate (211), and threoninate (212) attached to a hydrophobic dodecanoyl group have been also used for separation of a variety of chiral solutes.

Figure 1.18  The three-point rule for chiral recognition.

As noted earlier, normal micelles are dynamic aggregates and the dynamic micellar system may have a negative influence on the efficiency of the chiral interaction. To overcome this problem, Wang and Warner introduced the first polymerized chiral surfactant, N-undecanoyl L-valinate, for the separation of chiral solutes (213). Since the initial report by Wang and Warner, several other papers exploring the potential of polymerized chiral surfactants for enantiomeric separation with MCE have been reported by Warner’s group (214-224).

1.6.  Applied Characterization Techniques

1.6.1.  Fluorescence Spectroscopy

Luminescence is the result of the emission of photons after the relaxation of an electronically excited molecule, atom, or ion into a lower energy level (225, 226). There are three categories of luminescence: fluorescence, phosphorescence, and chemiluminescence. Fluorescence occurs more rapidly than fluorescence and chemiluminescence and is generally
complete after about \(10^{-5}\) seconds or less from the time of excitation. Phosphorescence emission, on the other hand, takes place over periods longer than \(10^{-5}\) seconds (may continue for minutes even hours). When the excited species is the product of a chemical reaction, this type of luminescence is called chemiluminescence.

The CMC, micropolarity, microviscosity, micellar size and shape, aggregation number can be determined using fluorescence techniques. In this dissertation, fluorescence techniques are used to determine the polarity and aggregation numbers of the monomeric and polymeric surfactants.

1.6.1.1. Polarity Measurements

The polarity of the surfactant can be measured using a fluorescence probe that interacts strongly with the surfactant and sensitive to the polarity of the microenvironment (227). Pyrene, a fluorescence probe, has been extensively used for this purpose (228, 229). This molecule has a very distinctive fluorescence emission spectrum that consists of five vibronic bands. The intensities of these bands depend on the polarity of the surrounding microenvironment. An increasing polarity of the environment will cause an increase in the peak intensity at 372 nm (band I) and a decrease in the peak intensity at 383 nm (band III) (227). Therefore, the ratio of the intensity of band I and band III is often used to determine the polarity of a given environment (230, 231). This ratio is about 1.6 for water, the most polar solvent, and is about 0.6 for the least polar solvents such as methylcyclohexane.

1.6.1.2. Steady-State Fluorescence Quenching Technique

Any process that results in reduction or elimination of the fluorescence quantum yield is called fluorescence quenching, and any species that causes quenching is called quencher (226). There are two types of quenching: 1) time-resolved or dynamic fluorescence quenching, and 2)
steady-state or static quenching. Time-resolve fluorescence quenching (TRFQ) occurs when the fluorophore and the quencher collide in the excited state. On the other hand, steady-state fluorescence quenching (SSFQ) occurs when the fluorophore and the quencher form a complex in the ground state and inhibits the excitation of the fluorophore. Since SSFQ was chosen for the quenching studies in this dissertation, it will be explained in detail. The SSFQ involves measurements of the fluorescence emission intensity at increasing quencher concentration, using a sensitive spectrofluorometer. The SSFQ measurements are much easier to perform and to analyze than TRFQ measurements. This is the reason it has been extensively used for measuring aggregation number, \( N \), values. However, there are some restrictive assumptions involved in SSFQ (232-235): 1) both the fluorophore molecule and the quencher molecule are exclusively solubilised into micelles obeying the Poisson distribution among micelles (236), and 2) \( k_Q / k > 1 \), where \( k_Q \) is the second-order quenching rate constant and \( k \) is the probe decay rate constant. In SSFQ, we assume a purely dynamic quenching and consider that no intermicellar migration of the probe and quencher takes place on the time scale of the experiment (233, 234). Using a probe concentration much smaller than the micelle concentration, \([M]\), and increasing the quencher concentration, \([Q]\), such that the ratio \([Q]/[M]\) varies between, e.g., 0 and 2, the variation of the fluorescence emission intensity is given by the following equation (10, 237):

\[
\frac{I_\lambda}{I_\lambda^0} = \exp \left( \frac{[Q]}{[M]} \right). \tag{1.42}
\]

Equation 1.42, which is valid for micellar solutions, can be compared to Stern-Volmer equation, which describes quenching in homogeneous solutions:

\[
\frac{\tau_0}{\tau} = 1 + K_{sv}[Q], \tag{1.43}
\]
where $\tau_0$ and $\tau$ are the probe fluorescence lifetime in the absence ($\tau_0 = 1/k$) and the presence of the quencher, respectively. The difference between Equation 1.42 and 1.43 reflects the compartmentalization of the probe and quencher in the micelles. The plot of $\ln(I_0/I_Q)$ against $[Q]$ at constant surfactant concentration permits the determination of the micelle aggregation number, $N$, from

$$N = \frac{C_{\text{surf}} - CMC}{[M]},$$

1.44

where $C_{\text{surf}}$ and CMC are the total surfactant concentration and critical micelle concentration.

### 1.6.2. Determination of Partial Specific Volume Using Densitometer

Due to the difficulty of measuring the exact volume of a particle, instead, partial specific volume, $\bar{v}$, is often used for the characterization of the substances of interest. The $\bar{v}$ is defined as the increase in volume upon dissolving 1 gram of a dry material (e.g., surfactant) in a large volume of a solvent (e.g., water) when the mass of solvent, temperature, and pressure are held constant. The $\bar{v}$ can be measured by analytical ultracentrifugation (238) or can be determined from a plot of the inverse of the density, $1/\rho$, of the aqueous surfactant solution versus the weight fraction, $W$, of the surfactant according to Equation 1.45:

$$\frac{1}{\rho} = \bar{v} + W\frac{\partial(1/\rho)}{\partial W}. $$

1.45

The $W$ value is defined as:

$$W = \frac{m_w}{m_w + m_s}, $$

1.46

where $m_w$ and $m_s$ represent the masses of water and the surfactant, respectively. Several different surfactant solutions are prepared in either buffer solution or in deionized water for density measurements. The $\bar{v}$ value is then obtained as the y-intercept of the $1/\rho$ versus $W$ plot.
A high-precision digital densitometer was used to perform density measurements. The principle of the technique, in brief, is: first, the period of oscillation \( T_1 \) of a borosilicate glass U-shaped tube containing the sample is measured; then, the period of oscillation \( T_2 \) of the U-shaped tube containing a reference material (e.g., water or air) with known density is measured. Equation 1.47 shows the relationship between the density difference between two media \( \rho_2 - \rho_1 \) and periods \( T_1 \) and \( T_2 \):

\[
\rho_2 - \rho_1 = k(T_2^2 - T_1^2),
\]

where \( k \) is an instrument constant. This constant is determined from instrumental calibration using doubly distilled water and air.

### 1.7. Scope of This Dissertation

The focus of this dissertation is to synthesize, characterize, and to utilize novel micellar and vesicular surfactants as pseudostationary phases in MCE, and develop methods for separation of achiral and chiral compounds such as polycyclic aromatic hydrocarbons (PAHs), mono-methylbenz[a]anthracene (MBA) isomers, neutral benzene derivatives, alkyl phenyl ketones, benzodiazepines, binaphthyl derivatives. Cyclodextrins (CDs), in addition to surfactants, were also utilized as organized media for separations of environmental pollutants. Several studies were carried out to understand the fundamental nature of the interaction between analyte and the micellar phase as well as the retention mechanisms in micellar capillary electrophoresis (MCE).

The first chapter is an introduction to relevant topics related to this dissertation such as surfactants, capillary electrophoresis (CE), MCE, and chirality. A brief discussion about applied characterization techniques is also presented in the first chapter. In part I of Chapter 2, a polymerized surfactant (molecular micelle) with a sulfate head group, namely, poly(sodium
undecylenic sulfate) (poly-SUS) was synthesized and applied for separation of 16 PAHs categorized by the U.S. Environmental Protection Agency (EPA) as priority pollutants using MCE. Parameters such as pH, concentration of poly-SUS, and the use of organic modifiers were investigated to follow the retention trends of PAHs. Poly-SUS, the unpolymerized SUS as well as sodium dodecyl sulfate (SDS) were compared under similar conditions. Because of the high purity (97-99%), poly-SUS is stable even at higher concentration of organic solvents, unlike SUS and SDS, making this methodology particularly useful for the separation of highly hydrophobic compounds. In second part of Chapter 2, a double alkyl chain di(2-ethylhexyl)phosphate (DEHP) was introduced as a potential anionic micellar pseudostationary phase for a wide range of benzene derivatives and/or PAHs. Several parameters such as concentration of DEHP, type and concentration of organic solvents (acetonitrile, isopropanol, and methanol) as well as applied separation voltage were optimized to enhance resolution, efficiency and selectivity as well as to maximize peak capacities. The migration times and selectivity order for a number of PAHs differed significantly, depending on the type of organic solvent added to the DEHP surfactant.

Recently, PAHs have evoked considerable attention due to the fact that they are well known as serious environmental contaminants and some are believed to contribute to the cancer in living organisms. Methylated PAHs such as MBAs are among the most biologically active alkylated aromatic compounds found in the environment. The carcinogenicity of MBA depends mostly on the position of methyl group on the benz[a]anthracene molecule. In the first part of Chapter 3, a method for the separation of twelve MBA isomers using poly-SUS surfactant by means of MCE is described. Several parameters such as concentration of acetonitrile, pH, as well as applied voltage were studied to optimize the MCE separation. The results of this study suggest that molecular length of MBA rather than length-to-breath ratio plays an important role.
in the elution order of some isomers. However, due to their structural similarities, baseline separation of all isomers was not achieved using poly-SUS in combination with acetonitrile. A few isomers were coeluted. In the second part of Chapter 3, CD modified MCE (CD-MCE) was investigated for the same isomers (i.e. MBAs) to increase the resolution and the selectivity of all twelve isomers using two native CDs (i.e., β-CD and γ-CD) and three derivatives of β-CD (i.e., dimethyl-, trimethyl-, and hydroxypropyl-β-CD) in combination with poly-SUS. Each CD was found to provide different resolution and selectivity. In addition, retention of MBA isomers was found to be dependent on the type and concentration of CD additives.

Solute-solvent interactions play a major in the development and optimization of analytical separations. Since retention prediction and selectivity optimization are very critical in rapid method development in MCE, it is imperative to achieve a better understanding of the factors that control selectivity to choose an appropriate pseudostationary phase. In the last several years, linear solvation energy relationship (LSER) model has been given a significant amount of attention for the characterization of retention and selectivity differences between different pseudostationary phases in MCE. In Chapter 4, sodium di(undecenyl) tartarate monomer (mono-SDUT), a vesicle forming amphiphilic compound possessing two hydrophilic carboxylate head groups and two hydrophobic undecenyl chains, was synthesized and critical aggregation concentration and aggregation number were determined using surface tensiometer and fluorescence quenching method, respectively. Poly-SDUT was prepared by exposing mono-SDUT to gamma radiation. The retention behavior of the 36 test solutes (i.e., benzene derivatives) in each pseudostatoinary system was examined and compared using two LSER models, i.e., solvatochromic model and solvation parameter model. Retention factors were determined for the 36 compounds used in this study, and the system constants were calculated by
multiple linear regression. The statistical validity of the LSER models was evaluated through the F test, correlation coefficient (R) and, standard error in the estimate (S.E.). The differences in LSER coefficients indicate the variations in the types of interactions between pseudostationary phases and solutes. Solute interactions with the vesicular and micellar systems occur through a variety of mechanisms such as surface adsorption, coaggregation, or partitioning into the hydrophobic core of micelles or vesicles. Due to these different mechanisms, the LSER constants for different set of solutes and different surfactant systems are not identical.

In Chapter 5, SUS, an achiral surfactant, and sodium N-undecanoyl L-leucinate (SUL), a chiral surfactant, were synthesized. These two surfactants were then polymerized separately to form poly-SUS and poly-SUL; or together at various molar ratios to make a variety of co-polymerized molecular micelles (CoPMs) holding both chiral (i.e., leucinate) and achiral (i.e., sulfate) head groups. These surfactant systems were applied as novel pseudostationary phases in MCE for separation of chiral and achiral molecules. The physicochemical parameter such as aggregation number, partial specific volume, and hydrophobic selectivity were determined for these surfactant systems. The separation of binaphthyl derivatives, alkyl phenyl ketones, and benzodiazepines were achieved using these surfactants. In addition, the thermodynamic parameters such as enthalpy, entropy, and Gibbs free energy changes for seven alkyl phenyl ketones and three benzodiazepines were determined in each pseudostationary phases using van’t Hoff plots.

1.8. References


60. Li, S. F. Y. *Capillary Electrophoresis Principle, Practice, and Applications*; Elsevier Science Publisher: Amsterdam, 1992.


148. Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akademiai Kiado: Budapest, **1982**.


Chapter 2.

Separation of Polycyclic Aromatic Hydrocarbon Using Micellar Capillary Electrophoresis


2.1. Introduction

After the first publication on micellar capillary electrophoresis (MCE) more than 10 years ago (1), many researchers have explored various types of monomeric surfactants above their critical micelle concentrations (CMCs) as pseudostationary phases for the separation of both ionic and nonionic compounds (2-6). Among the pseudostationary phases investigated, sodium dodecyl sulfate (SDS) has been successful in the MCE separation of many water-soluble solutes (7, 8). However, in the case of highly hydrophobic analytes such as polycyclic aromatic hydrocarbons (PAHs), the binding with SDS micelle is often too strong to permit adequate resolution of these compounds (9, 10).

Polymerized surfactants bearing both chiral (11-14) and achiral (15-18) ionic head groups have been proposed as alternative pseudostationary phases in MCE. This technique has several potential advantages over the use of the normal micelles generated from monomeric surfactants. First, polymerized surfactants have no CMC. In this respect, polymerized surfactants can be effective as pseudostationary phases over a wide range of concentrations. In contrast, normal micelles require higher surfactant concentrations (at least 2-10 times the CMC) for effective separations. Thus, Joule heating is expected to be more serious in conventional monomeric micellar-mediated MCE than in MCE with polymerized surfactants. Second, the elimination of dynamic equilibrium between monomer and micelle, as well as the presence of covalent bonds between these surfactant aggregates, provides enhanced stabilities, enhanced rigidities, and...
controllable sizes to polymerized surfactants. Third, in buffers modified with a higher fraction of organic solvents, the chromatographic selectivity with polymerized surfactant is superior to that with SDS micelle. For example, acetonitrile and methanol can be used at higher concentrations, ~65-75% (v/v) with polymerized surfactants (19, 20), whereas the SDS micelle can only tolerate ~30-40% (v/v) of these solvents (20, 21). Fourth, MCE with polymerized surfactants offers a wider elution window than MCE with normal micelles, resulting in higher peak capacity.

Poly(sodium-10-undecylenate) was the first polymerized achiral surfactant used in MCE (15, 16). Although this surfactant provided high performance separation of a wide range of neutral compounds including some PAHs, its application is limited by the carboxylated head groups, whose ionization influences the electrophoretic mobility and solubility of the polymer at acidic or neutral pH values. Furthermore, problems such as erratic migration time and cloudiness of the anodic buffer vials after several runs have been reported (16). To overcome these difficulties, our research group (19) and Palmer and Terabe (18, 20) recently synthesized a polymerized surfactant with a sulfate head group, namely, poly(sodium undecylenic sulfate) (poly-SUS). However, the latter studies used potassium persulfate as a free radical initiator for the polymerization process, whereas we used $^{60}$Co γ-irradiation. Two major limitations reported by Palmer with the chemical method of polymerization for poly-SUS were (1) low synthetic yields and (2) contamination of the product with sodium sulfate (22). Our earlier MCE studies with poly(sodium N-undecylenyl-L-valinate) (11) and this study with poly-SUS indicate that these problems can be avoided if γ-irradiation is used to initiate polymerisation. The present studies report the application of poly-SUS for MCE separation of 16 PAHs categorized by the U.S. Environmental Protection Agency (EPA) as priority pollutants. To the best of our knowledge, this is the first report on the simultaneous separation of all 16 PAHs in a single MCE
run. Poly-SUS and the nonpolymerized SUS as well as SDS are compared under similar conditions. Because of the high purity (97-99%), poly-SUS is stable even at higher concentration of organic solvents, making this methodology particularly useful for the separation of highly hydrophobic compounds.

2.2. Experimental

2.2.1. Instrumentation

A Beckman (Fullerton, CA) P/ACE model 5510 capillary electrophoresis (CE) instrument was employed in MCE separation of PAHs. This CE instrument was equipped with (1) a 21-position inlet and 10-position outlet sample carousels for automatic sample/buffer change, (2) a 0-30-kV high-voltage built-in power supply, (3) 200-, 214-, 254-, and 280-nm selectable wavelength filters for UV detection, (4) a liquid thermostated capillary cartridge (capillary 50 μm i.d. x 375 μm o.d. x 47 cm total length, 40 cm to the detector), and (5) software System Gold for system control and data handling. The capillary in the Beckman instrument was thermostated by use of a fluoroorganic fluid. The detector time constant was 0.2 seconds.

2.2.2. Materials

The 16 polycyclic aromatic hydrocarbons (PAHs) were obtained from the following suppliers: 1) naphthalene (NAPH), 2) acenaphthyene (ACY), 3) acenaphthene (ACE), 4) fluorene (FLU), 5) phenanthrene (PHEN), 6) anthracene (ANTH), 7) fluoranthene (FLT), 8) pyrene (PYR), 9) benz[a]anthracene (BaA), 10) chrysene (CHRY), 11) benzo[b]fluoranthene (BbF), 12) benzo[k]fluoranthene (BkF) and 13) benzo[a]pyrene (BaP) from Aldrich (Milwaukee, WI); 14) dibenz[a,h]anthracene (DiBahA), 15) benzo[ghi]perylenne (BghiP), and 16) indeno[1,2,3-cd]pyrene (INPY) from ChemService (West Chester, PA). HPLC grade acetonitrile (ACN) was obtained from Burdick and Jackson (Muskegon, MI). Disodium
tetraborate (Na$_2$B$_4$O$_7$), disodium hydrogen phosphate (Na$_2$HPO$_4$), and sodium carbonate were of analytical grade and were purchased from EM Science (Gibbstown, NJ). The undecylenyl alcohol, alkyl aryl ketone homologues, C$_4$-C$_{14}$ phenones, chlorosulfonic acid (ClSO$_3$H), sodium dodecyl sulfate, and pyridine (PY) were of analytical reagent grade and were obtained from Aldrich.

2.2.3. Synthesis of Poly(Sodium N-andecylenic Sulfate)

The sodium undecylenic sulfate (SUS) monomer was prepared according to Bergstrom's procedure (23). A schematic of the synthesis of poly(sodium undecylenic sulfate) (poly-SUS, d) is shown in Figure 2.1. To sulfate the alcohol, 113.8 mmol (7.5 mL) of ClSO$_3$H was added dropwise to 75 mL of PY in a 250-mL round-bottom flask placed in an ice bath, and the mixture was stirred vigorously. Similarly, a solution of 82.3 mmol (16.5 mL) of ω-undecylenyl alcohol (a) and 75 mL of PY was slowly added to the above solution, and cooling and stirring were continued. The contents of the flask were refluxed with heat (heating mantle with transformer set on 40 V) for about 3 h until a clear yellow solution was formed. The product was undecylenic sulfuric acid (USA) (b). The sodium salt of USA (i.e., SUS, c) was formed by adding USA solution to 600 mL of deionized water containing 4 g of NaOH and about 80-100 g of sodium carbonate. The solution was stirred overnight. The resulting SUS surfactant solution was extracted twice using n-butanol in a separatory funnel. The organic phase on the top contained the product. Evaporating the organic solvents (PY, butanol) by rotary evaporation followed by vacuum desiccation produced a dry product. Purification of SUS surfactant was performed by dissolving the product in water and extracting with ethyl ether. This was followed by distillation and lyophilization which resulted in a dry white powder. Recrystallization was performed by dissolving the dry powder in isopropanol using heat. The solution was filtered,
cooled to room temperature, and refrigerated for recrystallization. The crystals were dried in a vacuum desiccator overnight. The final product was SUS monomers.

A 100 mM aqueous solution of SUS monomers was exposed to a $^{60}$Co $\gamma$-ray source for 92 hours for polymerization in a micellar form. After irradiation, the poly-SUS (d) was dialyzed.
against bulk H₂O using a regenerated cellulose membrane with 2000 Da molecular mass cutoff. The purified solution was lyophilized and dried under a vacuum. The various batches of polymers were found to have 97-99% purity, as calculated from elemental analysis. Further characterization, such as molecular weight and partial specific volume of the polymer, is under study in our laboratory.

2.2.4. Capillary Electrophoresis Procedure

All new capillaries were prepared by use of a standard wash cycle of 1 M NaOH for 1 hour before use. Each day, operation was started by purging the capillary with 1 M NaOH (15 minutes), triply deionized water (2 minutes), and the running buffer (10 minutes). Prerun rinsing consisted of 3.0 minutes of the running buffer. Unless otherwise noted, the time for pressure injection was 3 seconds for most separations. Postrun rinse consisted of a 2.0-minutes flush with 0.5 M NaOH. These procedures resulted in improved peak shapes, minimized analyte adsorption on the capillary wall, and a good migration time reproducibility range of 2.0-2.5 % RSD, n = 3.

2.2.5. Preparation of EKC Buffers and Standard Solutions

For all MCE experiments, the final background electrolyte (BGE) consisted of a 12.5 mM mixture of Na₂HPO₄ and Na₂B₄O₇ buffer at pH 9.2. Appropriate percentages of poly-SUS surfactant (w/v) and of ACN (v/v) were added to the BGE, and then the final volume was adjusted with triply deionized water. After a thorough mixing in a sonicator for 10 minutes, the final running buffers were filtered through a 0.45-μm syringe filter (Nalgene, Rochester, NY) by creating a vacuum inside the syringe. All stock standard PAH solutions were prepared in 80/20 (v/v) ACN/H₂O at concentrations of about 3-5 mM each, except for BghiP, BaP, and INPY, which were dissolved in 80/20 (v/v) ACN/CH₂Cl₂. Molar concentrations of the injected test mixture of 16 PAHs ranged from 0.2 to 0.5 mM.
2.2.6. Safety Precautions

Transfer of solid PAHs from the reagent bottle in a volumetric flask and dilution of the stock solutions were performed in a ventilated hood. All PAH solutions were stored in closed containers in a refrigerator. Disposable latex gloves were worn and care was taken to dispose of PAH waste solutions appropriately. For polymerization of SUS monomer, the surfactant solutions prepared in a glass bottle were placed in a container (using protective gloves) and lowered to the 14-ft level for radiation using an electric winch. The $\gamma$-irradiation source is located under a 14-ft pool of water covered by an iron gate. Access to the $^{60}$Co $\gamma$-ray source facility is controlled by key padlocks and a card-reader door. However, it should be noted that irradiation by $\gamma$-rays does not induce radioactivity in the samples.

2.2.7. Calculations

The migration factor, $k'$, of a neutral solute was measured according to the formula (24)

$$k' = \frac{t_R - t_{eo}}{t_{eo} \left[ 1 - \left( \frac{t_R}{t_{mc}} \right) \right]}$$

(2.1)

where $t_R$ is the migration time of a neutral retained analyte, $t_{eo}$ is the migration time of a neutral unretained analyte, and $t_{mc}$ is migration time of the micelles. The void time, $t_{eo}$, was determined by a first solvent disturbance due to a refractive index change. The value of $t_{mc}$ was determined by using the procedure proposed for a series of homologous compounds by Bushey and Jorgenson (25). This procedure consists of five steps: 1) migration times of some homologous series of alkyl aryl ketones ($C_6$-$C_{14}$) were measured at various percentages (20-50 % v/v) of ACN; 2) using the longest migration time of $C_{14}$ phenone as a measured (assumed) $t_{mc}$ value, the $k'$ values of $C_6$-$C_{12}$ phenones were calculated using the above-mentioned equation; 3) from the plot of log $k'$ versus the carbon number, a new $k'$ value for $C_{14}$ phenone was calculated; 4) a new
t_{mc} was then found by rearranging the above equation and substituting the values of new k’ and measured t_R for the C_{14} phenone; 5) all k’ values (C_{6}-C_{12} phenones) are recalculated, and the procedure is reiterated (n=30) until the t_{mc} converges to a value less than 0.1 % from its previous iteration.

2.3. Results and Discussion

The PAHs are ubiquitous organic pollutants with at least two aromatic rings in their basic structure. They are widely distributed in the environment due to incomplete combustion processes (26). The chemical structures of the 16 priority PAHs employed in this study are shown in Figure 2.2. These PAHs range from two to six fused rings, with widely different hydrophobic properties. As discussed in our previous study (27), an equimolar mixture of Na_2HPO_4 and Na_2B_4O_7 buffered at pH 9.2 is an effective BGE for the electrokinetic separation of PAHs. However, the solubility of most of the PAHs in a purely aqueous micellar solution is poor, owing to the strong hydrophobic properties of the former. For this reason, ACN was added as an organic modifier to the BGE containing poly-SUS for PAHs separation.

2.3.1. Comparison of Monomeric and Polymeric Surfactants as Pseudostationary Phases

The selectivity differences for monomeric (SDS and SUS) and polymeric (poly-SUS) surfactants for the separation of 16 EPA priority pollutants are shown in Figure 2.3. Although all three electropherograms were run under similar BGE conditions (i.e., 12.5 mM each of Na_2HPO_4/Na_2B_4O_7, at pH 9.2), the analyte peak shapes are significantly different. With SDS, poor selectivity and peak broadening are evident. Under equivalent buffer and surfactant concentrations, the PAHs showed some improvement in selectivity with monomeric SUS. These improvements in separation with SUS over SDS are probably due to π-π interaction between the PAHs and the terminal double bond of the SUS surfactant. However, the enhanced separations
of PAHs with excellent peak shapes using poly-SUS are clear indicators that the structural integrity of poly-SUS is maintained, even at a very high concentration of organic solvent (e.g., 57% (v/v) ACN used to generate electropherogram in Figure 2.3). In contrast for conventional
Figure 2.3. Comparison between (A) sodium dodecyl sulfate (SDS), (B) nonpolymerized sodium undecylenic sulfate (SUS), and (C) poly-SUS for the separation of PAHs. MCE conditions: 1.5 % (w/v) each of SDS and SUS; 1.0 % (w/v) of poly-SUS in 12.5 mM each of Na₂HPO₄ and Na₂B₄O₇ buffered at pH 9.2 with 57 % (v/v) of acetonitrile; pressure injection for 3 seconds; +30 kV applied voltage for separation; current 55 μA for SDS, 50 μA for SUS, and 38 μA for poly-SUS; UV detection at 254 nm. Peak identifications are same as Figure 2.2.
surfactants (SDS or SUS), the use of such a high content of organic solvents breaks up the micelle. These improved separations of PAHs with poly-SUS are consistent with previous study conducted in our laboratory on enantiomeric separations, in which the polymerized chiral surfactant poly(sodium N-undecylenyl-L-valinate) (11) showed superior MCE separations over the corresponding monomeric surfactant. In addition, the spectroscopic data reported by Paleos et al. (28) indicated that the hydrophobic analyte does not penetrate deeply into the core of polymerized surfactant as it does into normal micelle. Thus, an increase in the mass transfer rate of the PAH to and from the polymerized pseudostationary phase indeed improves the separation efficiency and selectivity. However, certain critical pairs of analytes (mostly isomers), e.g., BaA-CHRY and BbF-BkF, remain unresolved. In addition, partial resolution was obtained for ANTH and PHEN. Thus, the optimization of an MCE method that can separate rapidly and efficiently the isomers of the above-mentioned peak pairs in the test mixture of 16 PAHs in a single run was necessary.

2.3.2. Effect of Poly-SUS Concentration

The purpose of varying the surfactant concentration is to adjust the k’ values to obtain a compromise between resolution and analysis time. Figure 2.4 shows the effect of changing the concentration of the Poly-SUS on the k’ of 16 PAHs. It is noted that the k’ values of PAHs increased proportionally with the poly-SUS concentration from, 0.1 to 0.75 % (w/v). As expected, the k’ values and the slopes of the linear plots increase with increases in the ring size and hydrophobicities of different PAHs. In addition, the linear plot for each PAH passed close to the origin. This observation confirms the fact that the CMC of poly-SUS is zero. Moreover, it can be seen from Figure 2.4 that the separation of all and even the faster-eluting PAHs (e.g., NAPH, ACY, ACE, FLU, Figure 2.4 inset) is still possible at 0.25 % (w/v) poly-SUS (equivalent
to 9.2 mM SUS monomer). Thus, the separation with a micelle polymer is feasible even at lower concentrations much below the CMC (the CMC of SUS is ~32 mM). In contrast, for nonpolymerized micelles, the concentration of the surfactant has to be higher than the CMC in order for it to function as a pseudostationary phase. Table 2.1 shows the values of the elution window defined here as the ratio of $t_{mc}/t_{co}$. As expected, the elution window became wider with increasing concentration of poly-SUS. On going from 0.10 to 0.70 % (w/v) of poly-SUS, the elution range increased by a factor of ~13, and the migration window became infinite, i.e., a true stationary phase was approached when the concentration of poly-SUS was raised to 0.75 % (w/v). With poly-SUS concentrations ≥0.75 % (w/v), the first eight PAHs that elute earlier or in the middle of the electropherogram showed very high resolution. Unfortunately, this gain in

Figure 2.4. Retention factors of the 16 PAHs plotted as a function of poly-SUS concentration. The inset plot in A shows an expanded view of retention factors for the first eight PAHs (peak 1-8). MCE conditions: 12.5 mM each of Na$_2$HPO$_4$ and Na$_2$B$_4$O$_7$ buffered at pH 9.2 with 40 % (v/v) of acetonitrile; Separation voltage, +30 kV; current, 29-52 µA.
resolution with an infinite elution window was accompanied by long analysis times (>150 minutes) for more hydrophobic PAHs, in particular DIBahA, BghiP, and INPY. Furthermore, no peak was observed for C14 phenone (this analyte elutes after BghiP and was used as a tmc marker) even after 5 hours of electrokinetic run. In general, 0.50 % (w/v) of poly-SUS was chosen as the optimum concentration, as this was a best tradeoff between resolution and analysis time for 16 PAHs.

Table 2.1. Data which shows the effect of polymerized anionic surfactant on the elution window in MCEa.

<table>
<thead>
<tr>
<th>% (w/v) poly-SUS</th>
<th>tmc/tco</th>
<th>% (w/v) poly-SUS</th>
<th>tmc/tco</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>1.60</td>
<td>0.60</td>
<td>10.60</td>
</tr>
<tr>
<td>0.25</td>
<td>3.50</td>
<td>0.70</td>
<td>21.32</td>
</tr>
<tr>
<td>0.50</td>
<td>4.30</td>
<td>0.75</td>
<td>α</td>
</tr>
</tbody>
</table>

a) 0.5 % (w/v) poly-SUS, 12.5 mM each of Na2HPO4 and Na2B4O7, pH 9.2.

2.3.3. Effect of Acetonitrile Concentration

The use of polymerized surfactants as a pseudostationary phase provides an opportunity to investigate the role of organic solvents over a wide range of concentrations. The primary role of organic solvents such as ACN in MCE is to shorten the k' values of highly hydrophobic solutes. Dependencies of the k' values of 16 PAHs on the fraction of ACN measured at the optimized poly-SUS concentration (0.5 % w/v) are shown in Figure 2.5. The k’ values for the first 11 PAHs (Figure 2.5 A) decreased sharply as the ACN was raised from 20 to 30 % (v/v) and then decreased gradually in the range 30-40 % (v/v) ACN, and finally they leveled off and became very small at 50 % (v/v). However, in Figure 2.5 B, it can be seen that certain solutes (e.g., DIBahA, BghiP, and INPY) are predominantly hydrophobic and show large k' values. For such lipophilic PAHs, the k’ values show sharp drops at a much higher range of ACN (i.e., 40-50 % v/v). In addition, note that, due to very strong surfactant-analyte interactions, no reliable k'
values can be obtained for BkF and BaP at 20 % (v/v) ACN, as well as for DIBahA, BghiP, and INPY at < 40 % (v/v) ACN. Moreover, it is worth noting that the ACN content in the polymerized surfactant has a distinguished effect on the EOF.

The electroosmotic mobility decreases from 2.26 x 10^{-4} to 1.01 x 10^{-4} cm^2 V^{-1} s^{-1} with an increase in ACN content from 20 to 50 % (v/v). However, despite an increase in t_{eo} values, the migration time and k' values of PAHs showed a continuous drop in the same range. This trend of converging k' with a decrease in polarity of the aqueous phase is very similar to the retention mechanism of reversed-phase HPLC. Since increasing the fraction of ACN in the poly-SUS does not break up the micelle polymer, a progressive decrease in k' values of PAHs is probably related to a synergistic effect of reduced partition coefficient and a change in shape of the

**Figure 2.5.** Retention factors of the 16 PAHs plotted as a function of acetonitrile concentration. The insets in both A and B show an extended view of retention trends. Separation voltage, +30 kV; current, 35-58 μA. Other conditions are same as Figure 2.4.
polymerized surfactant. Table 2.2 summarizes the effect of ACN content on the elution window. As shown, the elution range became narrower with an increase in the volume fraction of ACN. This is attributed to a decrease in EOF and an increase in apparent electrophoretic mobility of the micelle polymer. As $t_{e0}$ rises and $t_{mc}$ drops, a decrease in $t_{mc}/t_{e0}$ values must occur with an increase in ACN content from 20 to 50 % (v/v). A reasonable compromise is found at 40 % (v/v) of ACN, since early-eluting PAHs were baseline resolved and relatively narrow peak spacings were observed for PAHs with large $k'$ values.

**Table 2.2.** Data which shows the effect of acetonitrile concentration on the elution window in MCE\textsuperscript{a}.

<table>
<thead>
<tr>
<th>% (v/v) ACN</th>
<th>$t_{mc}/t_{e0}$</th>
<th>% (v/v) ACN</th>
<th>$t_{mc}/t_{e0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>37.30</td>
<td>45</td>
<td>2.70</td>
</tr>
<tr>
<td>30</td>
<td>9.80</td>
<td>50</td>
<td>2.60</td>
</tr>
<tr>
<td>40</td>
<td>4.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} 0.5 % (w/v) poly-SUS, 12.5 mM each of Na₂HPO₄ and Na₂B₄O₇, pH 9.2.

**2.3.4. Optimized Separation**

Figure 2.6 shows the separation of the 16 PAHs (EPA priority pollutants) in about 30 minutes with optimized poly-SUS and ACN concentrations. The elution orders of most PAHs generally follow an increasing length-to-breadth ratio (29) with ANTH and INPY as the only two exceptions. For even faster separation (15 minutes), the percentage of ACN in the poly-SUS can be raised to as high as 65% (v/v) (data not shown). However, under such conditions, the signals for first eight PAHs of the electropherogram were a little compressed, but the last three PAHs (with high $k'$ values) showed higher efficiencies with improved resolutions.
2.4. Conclusions

A high-purity T-type polymerized surfactant having an undecenyl (C_{11}) and a sulfate head group was prepared from $^{60}$Co $\gamma$-irradiation. This polymer was then used for the MCE separation of 16 PAHs. The methodology offers a valid alternative to gradient high performance liquid chromatography (HPLC) and capillary electrochromatography (CEC). The former requires gradient and large amounts of organic solvents for PAHs eluting from HPLC columns; the later still needs extensive studies on reproducible column preparation and optimization of...
conditions before it is ready for practical application. In contrast, with MCE, a simple manipulation of organic solvent composition and the concentration of polymerized surfactant in the running buffer enables one to realize the inherent benefits of MCE, that is, large number of peaks can be resolved at small k’ values and relatively narrow peak spacings are observed at large k’ values. This advantage is clearly demonstrated in the separation of 16 PAHs with varying hydrophobicities in a single run.

2.5. References


Part II. Phosphated Surfactant as Pseudostationary Phase for Micellar Capillary Electrophoresis: Separation of Neutral Benzene Derivatives and Polycyclic Aromatic Hydrocarbons

2.6. Introduction

The separation of electrically neutral molecules by capillary electrophoresis (CE) is difficult because nonionic organic molecules lack a charge and thus move at the rate of electroosmotic flow through the capillary. Terabe has shown that separation of neutral molecules can be achieved by addition of ionic surfactants that form micelles and act as pseudophase in the CE background electrolyte (1). This mode of CE is now commonly referred to as micellar electrokinetic capillary chromatography (MEKC or MECC) or micellar capillary electrophoresis (MCE). Many researchers have employed various types of micelles such as anionic (2-6), cationic (3, 7-9), nonionic (10-12), and zwitterionic (10, 13), as pseudophases for the separation of both ionic and nonionic compounds. In particular, sodium dodecyl sulfate (SDS)-MCE has been successfully employed in the separation of many water-soluble solutes (14). However, in the case of highly hydrophobic analytes such as polycyclic aromatic hydrocarbons (PAHs), interaction with the micelle is often too strong to permit adequate resolution of these compounds. In addition, it is well established that SDS micelles have a tendency to decompose in the presence of organic solvents (15-17). For these reasons, other anionic surfactants such as bile salts (5, 6), sodium dioctyl sulfosuccinate (DOSS) (17) have been utilized as MCE pseudostationary phases for the separation of hydrophobic compounds.

In the present report, a surfactant with a phosphated head group, di(2-ethylhexyl)phosphate (DEHP), is introduced as a pseudostationary phase for separation of a wide variety of weakly and strongly hydrophobic nonionic compounds. To the best of our knowledge, there has not been a report on the successful use of phosphate micelles either for micellar liquid
chromatography or MCE separation of nonionic analytes. Although the effect of acetonitrile in combination with either DOSS (17) or polymerized sodium undecylenic sulfate (18) to separate PAHs has recently been reported, no attempts were made to compare the effects of various types of organic solvents on the MCE separation of such compounds. In this work, we have studied the influence of several types of organic solvents (acetonitrile, isopropanol, and methanol) in conjunction with DEHP as an anionic micelle to enhance resolution, selectivity, and elution window for a range of neutral compounds.

The chemical structure of DEHP is shown in Figure 2.7. DEHP exists predominately as a monoanion due to the presence of one OH and a double chain ethylhexyl group. The critical micelle concentration (CMC) of DEHP was measured by following the increase of the fluorescence intensity of 6-(p-toluidino)-2-naphthalene-sulfonic acid (PTNS) with an increase in concentration of DEHP. The background electrolyte (BGE) consisted of 10 mM H₃BO₃, 30 mM Na₂HPO₄, at a pH of 7.0 in 10% v/v methanol. From a sharp change in fluorescence of PTNS the CMC for the DEHP was found to be around 10 mM. This value was confirmed by measuring the variation in capacity factor (k') for five neutral phenols as a function of DEHP.

Figure 2.7. Chemical structure of phosphoric acid di(2-ethylhexyl) ester surfactant.
concentration with CE using the same BGE and pH conditions as described for fluorescence measurement. Within the range of 10-100 mM DEHP, linear plots were obtained for each derivative, and the point of intersection on the DEHP axis indicated the CMC of DEHP to be about 10 mM as well. These results are consistent with the CMC of 10 mM for DEHP reported in the literature (19).

Note that the use of organic solvents is essential for both solubility and to obtain selectivity between the PAHs of various ring size and hydrophobicity. However, buffers containing 15 % v/v or more of an organic solvent (20% v/v acetonitrile, 15 % v/v isopropanol, and 30 % v/v methanol) dramatically increase the CMC. Therefore, we recently initiated studies to examine the influence of organic solvent, ionic strength, as well as pH of the background electrolyte on the CMC of DEHP. In binary (aqueous/-organic) solvent, DEHP is anticipated to provide separation based on differential partitioning of the various benzene derivatives and PAHs (Figure. 2.8). We note that DEHP in particular provides much better separation of PAHs than has been previously reported by use of SDS. This is because DEHP is more polar than SDS and has a wider elution window than SDS. Thus, moderate interaction of DEHP with PAHs often results in adequate resolution of such compounds.

2.7. Experimental

2.7.1. Instrumentation

A Dionex CESI Capillary Electrophoresis System (Sunnyvale, CA) equipped with a multiple wavelength UV-Vis detector (254 nm setting) was employed for MCE experiments. The software for control of the instrument and data processing was the AI-450 chromatography workstation. Fused-silica capillaries obtained from Polymicro Technologies (Phoenix, AZ) of 50 µm ID and 51 cm total length (46 cm to the detector) were used in all experiments. Samples
were loaded by gravity injection and separations were performed at ambient temperature (25-30°C).

Figure 2.8. Structures of the 21 neutral compounds studied.
2.7.2. Materials

DEHP was obtained from TCI America (Portland, OR). All organic solvents (acetonitrile, isopropanol, and methanol) purchased from various chemical sources were HPLC grade. Most of the PAHs and neutral compounds (e.g. acetophenone, nitrobenzene, 7,8-benzo-quinoline, azulene, benzophenone, naphthalene, acenaphthylene, acenaphthene, 3-aminofluoran-thene, fluorene, 7,12-dimethylbenz[a]anthracene, benz[a]anthracene, phenanthrene, anthracene, fluoranthene, chrysene, pyrene, perylene, 2,3-benzofluorene, benzo[a]pyrene, benzo[ghi]perylene) were purchased from Aldrich (Milwaukee, WI). Sodium borate was obtained from EM Science (Gibbstown, NJ).

2.7.3. Preparation of Micellar and Analyte Solutions

The micellar solutions were prepared by weighing appropriate amounts of DEHP along with sodium borate and diluting with 20-30 mL of triply deionized water. About 5-20 mL of organic modifier (acetonitrile, isopropanol, or methanol) and ~1 mL of 10 N NaOH solution were added to promote complete dissolution. After about 20 min of ultrasonication in a water bath, the pH was adjusted to a value of 9.0 with 0.5 N NaOH solution. This was followed by dilution of micellar solution in a 250 mL volumetric flask to obtain the desired concentrations. All final operating micellar solutions were filtered through a 0.45 μm syringe filter (Gelman Science, Ann Arbor, MI) by creating a vacuum inside the syringe. All stock standard solutions were prepared in 90 % v/v acetonitrile/water at concentrations of about 3 mM each. However, the stock solutions of more hydrophobic analytes (e.g., chrysene, perylene, benzo[a]pyrene, and benzo[ghi]perylene) were prepared in 90% v/v chloroform/water solution. Since many PAHs are potential carcinogens, all were handled carefully with appropriate safety precautions.
2.7.4. Capillary Conditioning

All new capillaries were prepared by use of a standard wash cycle of 1 N NaOH for 1 hour prior to use. A daily routine procedure also involved flushing the capillary with 1 N NaOH (15 minutes), triply deionized water (15 minutes), and running buffer (15 minutes). Between injections, the capillary was successively flushed for 10 minutes with each of following: triply deionized water, 1 N NaOH, triply deionized water, and then the operation buffer. However, this rinsing procedure can also be reduced to a total minimum period of 11 min (i.e. 5 min flush of 1 N NaOH, and 2 minutes flush with triply deionized water before and after NaOH rinse, and finally 2 minutes with running buffer containing the micellar solution). These procedures resulted in improved peak shapes and minimized analyte adsorption on the capillary wall.

2.8. Results and Discussion

Previous studies in which separation of neutral compounds were performed using DOSS surfactant indicated that a pH of 9.0 is best for separation of PAHs because shorter analysis times and better peak shapes are obtained (17). In addition, our literature survey revealed that PAHs are usually best separated in the pH 9-10 range with shorter and reproducible migration time and improved peak shapes (20, 21). Based on these observations, the work presented here using DEHP surfactant was performed with Na₂B₄O₇ buffered at pH 9.0.

2.8.1. Effect of Surfactant Concentration

The influence of DEHP concentration on the separation of a mixture of 21 neutral aromatic compounds is depicted in Figure.2.9 A-C. In agreement with previously reported MCE studies by use of other pseudostationary phases, a gradual increase in DEHP surfactant concentration provides increased resolution at the expense of longer migration time. This resulted in a wider elution window (tₘᵢᵣ/tₑ₀). The tₑ₀ was measured by the baseline disturbance
Figure 2.9. Effect of DEHP concentration on the separation of 21 neutral compounds. Electrolyte composed of (A) 25 mM DEHP, (B) 50 mM DEHP, (C) 100 mM DEHP in 30 % v/v acetonitrile, 8 mM sodium borate, pH 9.0. Peak identifications are same as Figure 2.8. Gravity injection for 5 seconds. +12 kV is applied for separation. Current varied from 12-31 μA.

cased by methanol, isopropanol or acetonitrile, whereas benzo[ghi]perylene, the most hydrophobic PAH, was used as the \( t_{mc} \) marker. Initially, at 25 mM concentration of DEHP
(Figure 2.9 A), most of the aromatic hydrocarbons coeluted with poor resolution and the window for separation is only 3.0 minutes with \( t_{mc}/t_{co} = 1.4 \). At DEHP concentrations over 50 mM (Figure 2.9 B), separation of relatively less hydrophobic analytes (peak 1-15) improves, as does the resolution and migration window which is extended to ~10.6 minutes with \( t_{mc}/t_{co} = 2.2 \). However, the more hydrophobic PAHs (peak 16-21) are still poorly resolved. A complete resolution of all analytes except for 7,8-benzoquinoline (peak 3) and azulene (peak 4) with a migration window of ~53 minutes and \( t_{mc}/t_{co} = 5.3 \) was only achieved when the DEHP concentration exceeded 100 mM (Figure 2.9 C). Shi and Fritz (17) were able to resolve a similar mixture of neutral compounds using DOSS as an anionic surfactant with a migration window reported to be in the (0.7-18 minutes) range using a field strength of 476 V/cm when varying the DOSS concentration from 10-70 mM. The elution window (1.5-53.4 minutes) reported with our DEHP surfactant seems to be comparable at a field strength of 235 V/cm using 25-100 mM DEHP. However, use of the relatively high concentrations of DEHP, required in order to achieve separation of PAHS, clearly indicates that when organic modifiers are used, high surfactant concentrations are necessary to offset the increase in CMC.

Figure 2.10 shows the effect of electrophoretic (\( \mu_{ep} \)) and electroosmotic (\( \mu_{eo} \)) mobilities as a function of DEHP concentration. A constant reduction in both \( \mu_{ep} \) and \( \mu_{eo} \) (Figure 2.10, inset) are, in fact, not only caused by an increase in viscosity and ionic strength of the running buffer, but also by a decrease in zeta potential at the surface of the capillary. Moreover, concentrations of DEHP above 100 mM result in an undesirable increase in migration time of the analytes. This is due to the direct proportionality of migration time or the \( k' \) value on the thermodynamic partition coefficient (K), i.e. \( k' = K\beta \), where \( \beta \) is the phase ratio of the volume of
the micellar phase to that of the aqueous phase, and is anticipated to increase with increasing DEHP concentration.

![Graph showing electrophoretic mobilities (\(\mu_{ep}\)) of 21 neutral compounds as a function of DEHP concentration in 30% v/v acetonitrile, 8 mM sodium borate, pH 9.0. Separation voltage and injection conditions are same as Figure 2.9.](image)

**Figure 2.10.** Effect of electrophoretic mobilities (\(\mu_{ep}\)) of 21 neutral compounds as a function of DEHP concentration in 30% v/v acetonitrile, 8 mM sodium borate, pH 9.0. Separation voltage and injection conditions are same as Figure 2.9.

### 2.8.2. Effect of Separation Voltage

The applied voltage has a significant effect on the migration time of analytes, number of theoretical plates (\(N_{TP}\)), as well as the resolution (\(R_S\)) between two adjacent peaks. Figure 2.11 shows the separation of 21 nonionic compounds at various applied voltages. As expected, an increase in voltage from 12-25 kV increases the \(\mu_{eo}\) and reduces the migration time of all 21 analyses. When a voltage of 12 kV is applied, the migration time difference between the very first peak (acetophenone) and the very last peak (benzo[ghi]perylene) is nearly 53 minutes, with
Figure 2.11. Electropherograms showing the effect of applied voltage on separation of neutral compounds. Electrolyte composed of 100 mM DEHP, 30 % v/v acetonitrile, 8 mM sodium borate, pH 9.0. Peak identifications are same as Figure 2.8.
a total separation time of 70 minutes. As the voltage is increased from 15 to 20 kV, the migration time of each peak becomes shorter. All compounds were eluted under 40 minutes at 15 kV with a peak window of about 26.5 minutes, and under 16 and 6 minutes with a migration window of 8.2 and 2.5 minutes at 20 and 25 kV, respectively. The inset in Figure 2.11 shows the relationship between current and applied voltage. Note that current is linear up to 20 kV. Since the CE instrument used in this study was not temperature controlled, it is clear that as the voltage is increased above 20 kV, improper heat dissipation inside the capillary leads to broader peaks with poor resolution between the closely migrating compounds. Therefore, a maximum voltage of 20 kV is recommended for separations under such conditions. At or above 25 kV, we noticed that electrical discontinuity through the capillary resulted in nonreproducible migration times, in some cases shutting off the power supply of the electrophoresis system.

Tables 2.3 and 2.4 provide data describing the effect of applied voltage on $N_{TP}$ and $R_S$ for some representative neutral analytes. In general, both $N_{TP}$ and $R_S$, increased to a maximum, then

### Table 2.3. Effect of the applied voltage on the theoretical plates of polycyclic aromatic hydrocarbons\(^a\).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>5 kV</th>
<th>12 kV</th>
<th>20 kV</th>
<th>25 kV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7500</td>
<td>86322</td>
<td>75406</td>
<td>72331</td>
</tr>
<tr>
<td>2</td>
<td>52150</td>
<td>61205</td>
<td>79366</td>
<td>74342</td>
</tr>
<tr>
<td>11</td>
<td>68210</td>
<td>78210</td>
<td>83402</td>
<td>88012</td>
</tr>
<tr>
<td>12</td>
<td>44320</td>
<td>54457</td>
<td>72490</td>
<td>70197</td>
</tr>
<tr>
<td>20</td>
<td>60350</td>
<td>72330</td>
<td>87571</td>
<td>77437</td>
</tr>
<tr>
<td>21</td>
<td>52510</td>
<td>66215</td>
<td>106150</td>
<td>72920</td>
</tr>
</tbody>
</table>

\(^a\) Using 100 mM DEHP, 8 mM sodium borate, 30 % v/v acetonitrile, pH 9.0.
decreased as the voltage was increased. The increase in $N_{TP}$ between 5 and 20 kV might be explained by a decrease in axial diffusion ($H_a$). This $H_a$ term has been found to have some contribution to observed plate height (22). However, as observed by current-voltage plots (Figure 2.11, inset), an increase in voltage above 20 kV decreases the $N_{TP}$ values, probably due to improper heat dissipation inside the capillary. Moreover, the $R_S$ deteriorates only after 12 kV. Note that the conditions for maximum $R_S$ can only be obtained when current and Joule heating are not limiting factors. It is also interesting to note that the $R_S$ for most hydrophobic peak pairs (peak 20 and 21) was improved.

Table 2.4. Effect of the applied voltage on the resolution of polycyclic aromatic hydrocarbons$^a$.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>5 kV</th>
<th>12 kV</th>
<th>20 kV</th>
<th>25 kV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>2.90</td>
<td>3.93</td>
<td>3.40</td>
<td>2.00</td>
</tr>
<tr>
<td>11-12</td>
<td>1.70</td>
<td>2.25</td>
<td>1.81</td>
<td>1.27</td>
</tr>
<tr>
<td>20-21</td>
<td>2.00</td>
<td>2.70</td>
<td>2.90</td>
<td>3.23</td>
</tr>
</tbody>
</table>

$^a$ Using 100 mM DEHP, 8 mM sodium borate, 30 % v/v acetonitrile, pH 9.0.

2.8.3 Influence of Concentration and Type of Organic Modifier

It is difficult to analyze moderately or highly hydrophobic solutes by MCE using only an aqueous buffer system. These kinds of solutes migrate near or with the micelle at $t_{mc}$. This problem can often be solved by increasing the affinity of the solute for the mobile phase. In order to increase the affinity of these solutes for the mobile phase, it is necessary to add the appropriate concentration of organic modifiers such as acetone, acetonitrile, isopropanol, methanol, etc., to the buffer system. The use of organic modifiers will affect the retention as well as the selectivity of the solutes. Three organic solvents (acetonitrile, isopropanol, and methanol) were investigated for the possibility of separating 21 aromatic compounds using 100
mM DEHP, 8 mM Na₂B₄O₇, pH 9.0. Owing to the highly hydrophobic character of most of the solutes studied (e.g. analyte 7-21, Figure 2.8), at least 20 % v/v of one of the three organic solvents were added to the surfactant containing borate buffer.

Figure 2.12 A-C summarizes the effects of various concentrations of acetonitrile, isopropanol, and methanol, respectively, on the relative migration (t/tₑ₀) behavior of PAHs. It is shown that as the volume fraction of acetonitrile and isopropanol was raised from 20 % to 40% or 45% v/v, the migration time of all analyses tended to decrease (Figure 2.12 A-B). In contrast, a sharp increase in migration time of all analytes except for nitrobenzene and acetophenone was observed with an increase in methanol fraction from 5-35 % v/v (Figure 2.12 C). It is well established from data in the literature that the effective mobility of nonionic solutes is a result of solvophobic interaction with the surfactant (17, 23). A higher concentration of organic modifiers solvates the hydrophobic solutes and competitively reduces the solvophobic interaction. It appears that the use of methanol at low concentrations (5-35 % v/v) increases the migration time for two reasons: (i) an increase in viscosity of the running buffer, and (ii) a decrease in zeta potential at the capillary surface. Note that raising the volume fraction of acetonitrile and isopropanol resulted in enhanced viscosity (as evidenced by the increase in the tₑ₀ values (Figure 2.12 A and B, inset). The high solvation ability of the latter two solvents offsets such viscosity contribution, resulting in a gradual decrease in migration time. Nevertheless, at concentrations above 45 % v/v the solvation ability of methanol improves at the expense of reduced selectivity and migration time.

Figure 2.13 is a comparison of the separation obtained under optimised organic fractions of 30 % v/v each of acetonitrile and isopropanol (Figure 2.13 A-B) and 20 % v/v of methanol (Figure 2.13 C). The most glaring differences in the three electropherograms are the selectivity
Figure 2.12. Relative migration ($t/t_{co}$) of 21 neutral analytes as a function of % v/v of organic solvent in 100 mM DEHP, 8 mM sodium borate, pH 9.0. (A) % v/v acetonitrile, (B) % v/v isopropanol, (C) % v/v methanol. In each plot the inset shows the relationship between $t_{co}$ versus % v/v of organic solvent.
Figure 2.13. Effect of the type of organic modifiers on resolution and selectivity of neutral compounds. Electrolytes contained 100 mM DEHP, 8 mM sodium borate, pH 9.0, in (A) 30 % v/v acetonitrile, (B) 30 % v/v isopropanol, (C) 20 % v/v methanol. Peak identifications are same as Figure 2.8. Separation voltage was +20 kV, current 12-42 μA.
changes observed for a number of analyte pairs depending on the type of organic modifiers added to DEHP. For instance, enhancement in selectivity with reversal in migration order between solute pairs such as 7,12-dimethylbenz-[a]anthracene/benz[a]anthracene (peak 11 and 12), pyrene/chrysene (peak 16 and 17), were observed using isopropanol or methanol instead of acetonitrile. On the other hand, the more hydrophobic solutes, e.g. perylene, 2,3-benzofluorene, benzo[a]pyrene, and benzo[ghi]perylene (peaks 18-21), displayed improved resolution and selectivity with acetonitrile as compared to methanol and isopropanol. This differential selectivity and resolution enhancement can be attributed to the unique ability of DEHP surfactant to compartmentalize solutes differently in the presence of various types of organic solvents. In terms of analysis time, the separation performed in the methanol-DEHP hybrid system is on average about 1.5 and 2.0 times longer compared to those for the isopropanol- or acetonitrile-DEHP, respectively.

2.9. Conclusions

In summary, MCE separation of a 21-component mixture of moderately and strongly hydrophobic analytes can be successfully achieved using the DEHP surfactant in the presence of an appropriate amount of organic solvent (20 % v/v methanol, 30 % v/v acetonitrile or isopropanol). However, acetonitrile is the most effective modifier that provides highest peak capacity with rapid analysis time. It appears that the unique structure of the DEHP micelle is more tolerant to the addition of an organic solvent than SDS. This extends the scope of MCE with partially aqueous buffer systems to separate moderately-to-strongly hydrophobic compounds. The successful outcome of this preliminary work has encouraged us to embark on a program to examine the effect of a variety of other phosphated surfactants. Such surfactants are expected to be usable in MCE with partially aqueous buffer systems.
2.10. References


Chapter 3.

Separation of Positional Isomers of Methyl Substituted Benz[a]anthracene

Part I. Separation of Mono-methylbenz[a]anthracene Isomers Using an Anionic Molecular Micelle by Means of Micellar Capillary Electrophoresis

3.1. Introduction

Recently, polycyclic aromatic hydrocarbons (PAH) have evoked considerable attention \{1-5\}. PAHs and their alkylated derivatives are well known as serious environmental contaminants and some are believed to contribute to the incidence of cancer in living organisms (6, 7). Methyl-substituted PAHs such as mono-methylbenz[a]anthracenes (MBA) are among the most biologically active alkylated aromatic compounds found in the environment (8, 9). There are 12 possible positional isomers of MBAs (Figure 3.1), whose carcinogenicity depends mostly on the position of the methyl group on benz[a]anthracene molecule. For example, 7-MBA has been found to be the most carcinogenic compound, followed by 6-, 8-, and 12-MBA, which have almost equal carcinogenic activity. The 9- and 11-MBA are the next most carcinogenic compounds; however, 1-, 2-, 3-, 4- and 5-MBA have low carcinogenicity (8, 10-12).

![Figure 3.1. The chemical structure of MBA employed in this study. The numbers show the position of substituted methyl- group on the benz[a]anthracene molecule.](image)

Various methods using gas chromatography (GC) on conventional nonpolar phases and high performance liquid chromatography (HPLC) have been published on the separation and
identification of MBAs (13-16). Several liquid-crystalline alkene compounds based on the biphenylcarboxylate ester were evaluated as stationary phases for separation of MBAs in capillary GC, although some isomers were not resolved (13). Garrigues and his coworkers have studied the extracts of rock and air particulate matter by high-resolution Shpol’skii spectrometry (HRS). This technique allows identification of isomers of MBA based on the sharpening of the fluorescence emission spectra at low temperature in n-alkane polycrystalline frozen solution (14). However, before HRS analysis, isomers of MBA need to be isolated by use of a highly selective reversed-phase HPLC column. Wise et al. observed a linear relationship between the calculated length-to-breadth ratios (L/B) of PAHs, (including many mono- and di-methylated PAHs) and their reversed-phase LC retention (15, 16). In general, they found an increase in HPLC retention with an increasing L/B of PAHs.

Capillary electrophoresis (CE) of nonionic analytes cannot be performed in a free solution due to lack of electric charges of analytes. This problem can be overcome by employing a charged additive that forms micelles that can be used as a pseudo-stationary phase in fused-silica capillary columns (17, 18). For example, sodium dodecyl sulfate (SDS) has been widely used as a pseudo-stationary phase with an aqueous electrolyte for the micellar capillary electrophoresis (MCE) of water-soluble analytes (19). However, MBA isomers are highly hydrophobic neutral species that are difficult to separate using purely aqueous MCE. Although, a number of recent publications dealing with the use of binary mixtures of acetonitrile (ACN)-water and methanol-water and SDS for the separation of C_{10}-C_{16} alkyl aryl phenones (20) and some PAHs (21) have been reported, its effectiveness as a pseudo-stationary phase remains problematic for the separation of highly hydrophobic PAHs. For these reasons, several different types of anionic surfactants such as bile salts (22, 23), as well as some double chain surfactants
such as disodium 5,12-bis(dodecyloxymethyl)-4,7,10,13-tetraoxa-1,16-hexadecanedisulfate (DBTD) (24), sodium dioctyl sulfosuccinate (DOSS) (25), di(2-ethylhexyl)phosphate (DEHP) (Chapter 2 of this dissertation) have been used in MCE for separation of such compounds.

As an alternative, polymer surfactants (26-29) and micelle polymers (5, 21, 30-35) offer a simple and convenient way to organize surfactant monomers. Two current reviews cover the introduction, development and application of these polymerized surfactants in MCE in great detail (32,33). Recently poly-(sodium undecylenic sulfate) (poly-SUS) has been proposed as an alternative pseudo-stationary phase in MCE (5, 21, 31-35). The monomer of SUS has a double bond at the end of the aliphatic chain with a sulfate head group. Thus, T-type micelle polymers (poly-SUS) are produced by γ-radiation induced covalent linkage of the monomers at concentrations above the critical micelle concentration (CMC) (5). In this study, the feasibility of poly-SUS as a pseudo-stationary phase for MCE separation of twelve MBA isomers is demonstrated. To the best of our knowledge, there is only a brief report in the literature on the separation of only four MBA isomers using capillary electrophoresis (CE) (36).

3.2. Experimental

3.2.1 Instrumentation

A Beckman P/ACE Model 5510 CE instrument (Fullerton, CA) was employed in MCE separation of 12 MBA isomers. This instrument is equipped with 0-30 kV high-voltage power supply, 200, 214, 254 and 280 nm selectable wavelength filters for UV detection, a liquid thermostated capillary cartridge, and System Gold software for system control and data acquisition. The fused-silica capillary was obtained from Polymicro Technologies (Phoenix, AZ) and had the following dimensions: 57 cm total length, 50 cm to detection window, 51 µm
ID, and 361 \( \mu m \) OD. The capillary was mounted in a cartridge and thermostatet at 23 \(^\circ\)C by use of a fluoroorganic fluid. The detection time constant was 0.2 seconds.

3.2.2 Materials

The scheme for the synthesis of poly-SUS is reported in Chapter 2 of this dissertation. The average molecular weight of the monomer units per polymer of the poly-SUS surfactant was estimated to be 32. This number was determined by use of average molecular weight (8,780), which was determined by analytical ultracentrifugation. The twelve isomers of MBA (each with a purity ranging from 94 to 99\%) were a gift from Midwest Research Institute (Kansas City, Missouri)/National Cancer Institute Chemical and Physical Carcinogenesis Branch (Bethesda, MD). HPLC grade ACN was obtained from Burdick and Jackson (Muskegon, MI). Disodium tetraborate (\( Na_2B_4O_7 \)) and disodium hydrogen phosphate (\( Na_2HPO_4 \)) were purchased from EM Science (Gibbstown, NJ). Sodium hydroxide (\( NaOH \)) was from Curtin Matheson Scientific (Houston, TX).

3.2.3 Preparation of MCE Buffers and Standards

For all MCE experiments, the final background electrolyte (BGE) consisted of a 12.5 mM mixture of \( Na_2HPO_4 \) and \( Na_2B_4O_7 \) buffered at various pHs. The desired pH value was obtained by using either 1 M \( NaOH \) or 1 M \( HCl \). The pHs of all BGE were adjusted before addition of ACN and poly-SUS. Therefore, pH values reported in this study are actually the pHs before the addition of ACN. The running MCE solutions were prepared by addition of various % (w/v) of poly-SUS surfactant to the appropriate % (v/v) of ACN and BGE. After ultrasonication for about 5-10 minutes, the final EKC buffers were filtered through a 0.45 \( \mu m \) Nalgene syringe filter (Rochester, NY). All stock standard MBA solutions were prepared in ACN at a concentration of 1 mg/mL (ca. 4 mM). Since MBAs are carcinogens, stock solutions
were handled in a ventilated hood and stored in a closed container in refrigerator. Disposable latex gloves were worn while working with MBA standards and care was taken to dispose of the waste solutions appropriately.

3.2.4. Capillary Electrophoresis Procedure

A new capillary was prepared by use of a standard wash cycle of 1 M NaOH for 3 hours at 50 °C before use. A daily routine procedure also involved flushing the capillary with 0.1 M NaOH (10 minutes), triply dionized water (5 minutes), and running MCE buffer (5 minutes). In between injections, the capillary was flushed for 3 minutes each with 0.1 M NaOH and EKC buffer. These procedures resulted in improved peak shape and minimized analyte adsorption on the capillary wall. At least four injections were made for each measurement under identical conditions. The RSD of migration time was not larger than 2.5 % for the pH range of 7.0 to 9.5; however, RSD at pH 9.75 and 10.0 was ca. 8 % (n=3). The molar concentration of 12 MBAs was ca. 0.3 mM injected by use of pressure method (typically 1s, 0.5 psi). The wavelength of 254 nm was selected for UV detection. The separation voltage was applied over a 0.17-minute ramp to prevent any possible current breakdown.

3.3. Results and Discussion

In our previous studies, 0.5% (w/v) of poly-SUS was found to be the optimum concentration for separation, as this was a best trade-off between resolution and analysis time for 16 priority PAHs (5, 26). Similarly, Palmer et al. (21, 30) and Tanaka et al. (28, 29) have also used low concentration of polymerized surfactants in their studies. In this work, various concentrations of poly-SUS, ranging from 0.1 to 1.0% (w/v), were studied and 0.5% (w/v) was found to be the best concentration for the separation of MBA isomers (data not shown).
3.3.1. Influence of ACN Concentration on the Separation

In general, the use of organic solvents as a mixture with water alters the polarity and the viscosity of the bulk electrolyte in MCE. In addition, they may also increase the solubility of the analytes and decrease the partitioning between the solutes and the pseudo-stationary phase. As a consequence, the use of organic solvents is favorably applied to enhance the selectivity of MCE by influencing the EOF and effective mobility of the analyte. Since the solubility of MBAs in a purely aqueous micellar solution is poor, ACN was added as an organic modifier to the BGE containing poly-SUS for separation of MBA isomers. In our earlier MCE study, 40 % (v/v) ACN and 0.5 % (w/v) poly-SUS provided a baseline separation of all 16 priority PAHs in about 30 minutes (Chapter 2). With this in mind, we first tried 40 % (v/v) ACN; however, resolution, especially that of later eluting MBA isomers, was not good. In contrast, 30 % (v/v) ACN resulted in very long migration times (first peak appeared at around 120 minutes at 30 kV). Thus, various % (v/v) of ACN fractions with a range of 33 to 40 were investigated. Dependencies of the relative migration (tR/teo) values of twelve MBAs on the fraction of ACN, measured at the optimized 0.5 % (w/v) of poly-SUS, are shown in Figure 3.2. As seen, when the volume fraction of ACN was raised from 33 % to 40 % (v/v), there was a sharp decrease in migration times for all MBAs. In contrast, the migration time of the electroosmotic flow (i.e., teo) continued to increase in the same range (Figure 3.2 inset). This trend of converging tR/teo with a decrease in polarity of the bulk electrolyte is very similar to the retention mechanism of reversed phase HPLC. Note that an increase in the fraction of ACN in the poly-SUS cannot disrupt the micelle polymer. This is because the covalent linkage formed between the monomers prevents the polymerized surfactant to dissociate into individual monomers. In addition, the partial specific volume of poly-SUS was found to be 0.7584 and 0.7685 in 35% (v/v) ACN and
pure water, respectively. These data show that there is no significant change in polymer structure at high volume fraction of ACN. Hence, the decrease in \( t_R/teo \) values of MBAs is probably related to a synergistic effect of reduced binding between the analyte and poly-SUS. The result obtained in this study is consistent with our previous findings with PAHs (Chapter 2). In addition, Seifar et al. (20) and Palmer et al. (30) have observed a similar behavior for the CE separation of hydrophobic analytes using ACN. Although the \( t_R/teo \) values for MBA isomers are too large at 33 % (v/v) ACN; at or above 40 % (v/v) ACN resolution suffered. Thus, 35 % (v/v) ACN was chosen as the optimum for the separation of the MBA isomers.

3.3.2. Influence of pH on Separation

The effect of the pH range of 7.0 to 10.0 in 12.5 mM phosphate-borate buffer with 0.5 % (w/v) poly-SUS, 35 % (v/v) ACN was examined. Since the MBAs are electrically neutral and
poly-SUS is fully charged in this pH range, the electrophoretic mobility of both the analyte and the pseudo-stationary phase should not be affected by a change in pH. However, if the pH of the bulk electrolyte is adjusted, there will be a change in the ionic strength of the electrolyte solution. We observed almost the same current value of 29 µA at pH 7.0 and 8.0. As the pH of the buffer system was increased to 10.0 (using 1 N NaOH), current also increased to 48 µA as a result of higher ionic strength produced by Na\(^+\) and OH\(^-\) as well as an increase in % of borate and phosphate. Such an increase in ionic strength causes the EOF to decrease due to a decrease in zeta potential. Our data are consistent with the study on ionic strength effects on EOF reported by Vindevogel et al. (39, 40). In addition, variation in ionic strengths may also affect the partition coefficient of the analytes between the aqueous and the micellar phase. Thus, an increase in pH from 7.0 to 10.0 provides a sensible increase in \(t_{co}\) and \(t_R/t_{co}\) values (Figure 3.3 and inset A of Figure 3.3). However, note that both \(t_{co}\) and \(t_R/t_{co}\) increase much more rapidly between pH 9.1 to 10.0 as compared to pH 7.0 to 9.1. We believe that at higher pH values (pH 9.1 and above) changes in the conformation of poly-SUS provide an open and hydrophobic structure, which causes a stronger interaction with MBA isomers. Chu et al. have also reported better separation at pH 10.0 than pH 9.0. They attributed this behavior to a more open structure of the polymer at higher pH (41). Separation at pH ≤9.5 is advantageous in terms of shorter retention time compared to that obtained at higher pH values (pH 9.75 and 10.0). However, better resolution for neighboring peak pairs of MBA was gained at higher pH values as shown in the inset B of Figure 3.3.

### 3.3.3. Effect of Applied Voltage

According to theory, use of a higher voltage will produce greater theoretical plates and shorter migration times of sample components (42, 43). Figure 3.4 A shows the theoretical plate
number as a function of applied voltage. There is a gradual increase in theoretical plate number as voltage increased from 10 to 30 kV. As shown in the inset of Figure 3.4 A, there is an increase in current from 10 to 20 kV, then slightly deviation from linearity at 30 kV due to Joule heating effects. At lower voltage (10 kV), diffusion seems to be the dominant factor in band broadening resulting in lower efficiencies. Moreover, at 10 kV, elution of all MBAs took more than 250 minutes, with a slightly better resolution compared with higher voltages (20 and 25 kV) (Figure 3.4 B). Shorter elution time with slightly higher resolution between the adjacent peaks was observed at 30 kV than 10 kV. Therefore, in this study, a maximum voltage of 30 kV was chosen to achieve faster separation.

3.3.4. Optimized Separation

Figure 3.5 shows the separation of the twelve MBA isomers, with optimized poly-SUS and 35 % (v/v) ACN concentrations at pH 9.5. It is well known that in HPLC with polymeric
stationary phases the elution order of most PAHs generally follow increasing L/B (4). To investigate the relationship between $t_R/t_{co}$ and shape parameters (i.e., length and L/B of MBA isomers, not shown), $t_R/t_{co}$ values of isomers were plotted against the length and L/B values. Although linear relationship with lower correlation coefficients was observed for both parameters, the correlation with L/B was much more lower than that with the length (data not shown). It appears that the length of MBA plays an important role in elution order, more so than

Figure 3.4. Effect of applied voltage on (A) theoretical plates (inset: current change vs. applied voltage); (B) resolution of MBAs. Separation conditions are same as Figure 3.2 except a fixed 35 % v/v acetonitrile was used.
L/B using polymerized surfactant in MCE mode. It should be noted that 1-, 7- and 12-MBAs eluted faster than other methyl derivatives. Our MCE data are also consistent with the earlier ultraviolet photoelectron studies published by Akiyama et al. (44) in which the electron densities at the 7- and 12- positions of MBA molecule were found to be very high compared to the other substitution positions. It appears that the higher electron density of 7- and 12-MBAs might cause electronic repulsion with the ionic head of poly-SUS resulting in shorter migration times for these isomers.

3.3.5. Comparison of Poly-SUS and SDS

The separation of the MBA isomers using SDS, the most widely used surfactant in MCE, under similar BGE conditions, (i.e., 12.5 mM each of Na$_2$B$_4$O$_7$ and Na$_2$HPO$_4$ with 35% (v/v) ACN at a pH of 9.5) was not successful as compared to poly-SUS (Figure 3.6 A and B). With
18.4 mM SDS (equivalent to 0.5 % poly-SUS) ca. 1.0-minute elution window was generated and only three MBAs, out of twelve, were partially resolved (Figure 3.6 A). As the concentration of SDS is increased to 36.8 mM, the elution window was increased to 15-minute, but only five MBAs were resolved in 72 minutes (Figure 3.6 B). Further increases in SDS concentration to 54 mM produced no elution of any MBA isomers even in 300 minutes (data not shown). The poor resolution of MBAs with SDS can be explained by the disruption of the formation of SDS micelles at high concentrations of organic solvent (in this case 35 % ACN). In contrast, the structural integrity of poly-SUS is preserved at high content of organic solvents. Thus, this comparison indicated the superiority of poly-SUS over the SDS for the separation of MBAs.

3.4. Conclusions

In conclusion, a partial separation of MBA isomers can be successfully achieved by use of poly-SUS, a T-type micelle polymer with sulfate head groups, in the MCE mode in the

Figure 3.6. Electropherograms showing the separation of twelve MBA isomers using A) 0.5 % w/v (18.4 mM) of SDS and B) 1 % w/v (36.8 mM) of SDS. Separation conditions and peak identifications are same as Figure 3.5.
presence of 35 % (v/v) of ACN at pH range of 9.1-10.0. These improved separations of MBA with poly-SUS are consistent with our previous study on 16 EPA priority PAHs (Chapter 2). In addition, it should be noted that spectroscopic data reported by Paleos et al. indicated that hydrophobic analytes do not penetrate as deeply into the core of the polymerized surfactant as into normal micelle (45). Further studies such as the combined use of poly-SUS with β- and γ-cyclodextrins are done to increase the peak resolution of co-eluting MBAs in the second part of this chapter.

3.5. References


3.6. Introduction

As discussed in Part I, mono-methylbenz[a]anthracene (MBA) isomers are of great environmental concern owing to their carcinogenicity and biologically activity (1, 2). The MBA has twelve positional isomers. The position of the methyl functional group on benz[a]anthracene molecule plays a significant role on the carcinogenicity of the compound (1, 3-6).

Gas chromatography (GC) and high performance liquid chromatography (HPLC) (7-10) have been used for the separation and identification of the MBA isomers. However, neither of these techniques provides separation of all MBA isomers. Due to the lack of a charge, MBAs cannot be separated in a free solution by capillary zone electrophoresis (CZE). This obstacle can be overcome by using micellar capillary electrophoresis (MCE), which employs a charged micelle forming surfactant (i.e., sodium dodecyl sulfate, SDS) as pseudo-stationary phase (11,12). The technique of MCE is more common for water-soluble analytes (13); however, in general, polycyclic aromatic hydrocarbons (PAHs) are highly hydrophobic compounds and are difficult to separate using purely aqueous MCE. Therefore, other separation strategies have been used to overcome this problem: the use of bile salts (14, 15), addition of an organic solvent (16, 17), cyclodextrins (CDs) (18-20) or urea (21) to micellar solution. Since high content of organic modifier disrupts the micelles (22, 23), it is not appropriate to use very high concentrations of organic solvent (e.g. >30% acetonitrile) with normal micelles.

The CDs are cyclic oligosaccharides and consist of 6 (α-), 7 (β-) and 8 (γ-) glucopyranose units linked together by α-1,4 linkages. Since native CDs are neutral compounds and migrate with the electroosmotic flow (EOF), their separation abilities are limited. Thus, native CDs function as a pseudostationary phase for neutral compounds only when used with
charged micelles in MCE. Introduced by Terabe and co-workers (24, 25), CD modified MCE (CD-MCE) has been demonstrated to be useful for the separation of both chiral (25-28), and achiral (18-20, 24, 29) compounds. However, there is a disadvantage of CD-MCE. Normal surfactant monomers in the running buffer will likely form inclusion complexes with CD molecules (30). Thus, complexation of free surfactant monomer with CD will possibly interfere with complexation between the analyte and the CD, which may result in a poor separation. Another disadvantage of CD-MCE is that the concentration of surfactant used in CD-MCE has to be above the critical micellar concentration (CMC) to achieve effective separation. Apparently, a surfactant with a high CMC requires very high concentration of a charged surfactant in the MCE buffer. This in turn generates excess Joule heating in the capillary. The heat production will inhibit the desired optimum separation.

Polymeric surfactants (or molecular micelles) have been proposed as alternatives to normal micellar systems (31-36). As discussed previously, the polymeric surfactants have several advantages over normal micelles. First, they do not have a CMC. Thus, the use of polymerized surfactant as pseudostationary phase even at low concentrations is an advantage over normal micelles. This advantage can possibly be used to minimize Joule heating. Second, covalent linkage between the individual monomers in polymeric surfactants enhances the stability of polymer in high content of organic modifiers. Furthermore, unlike a normal micellar system, the covalent linkage in polymeric surfactant diminishes the formation of normal inclusion complex between monomers of surfactant and CDs (28). Third, due to the high rigidity of polymeric surfactants, the mass transfer rate of the analyte between the polymeric surfactant and the bulk solution is expected to be relatively faster as compared to a normal (unpolymerized)
surfactant system. This is because solutes cannot penetrate as deeply into the hydrophobic core of a polymeric surfactant as in normal micelles (37-40).

The aim of this work was to study the possibilities of using two native CDs (β-CD, and γ-CD) and three derivatives of β-CD (dimethyl-, trimethyl-, and hydroxypropyl-β-CD) in combination with a polymeric surfactant, poly(sodium 10-undecenyl sulfate) (poly-SUS), to increase the selectivity for the separation of twelve MBA isomers. At present, there are only a few reports on the separation of MBA isomers. The separation of only four MBA isomers has been reported by Ding and Fritz (41). In part I of this chapter, six out of twelve MBA isomers (1-, 4-, 7-, 10-, 11-, and 12-MBA) were baseline separated using 0.5% (w/v) poly-SUS, 35% (v/v) acetonitrile, 12.5 mM phosphate/borate buffer at a pH of 9.5. Three pairs of the MBA isomers, that is, 2-MBA/8-MBA, 5-MBA/6-MBA and 3-MBA/9-MBA, co-eluted under these conditions. In the present study, the background electrolyte composition was the same as reported in our previous work. The concentrations of native β-CD and γ-CD as well as β-CD derivatives were varied at pH of either 9.5 or 9.75 to improve the resolution and selectivity of isomers. Shorter analysis times were achieved using a combination of poly-SUS and β-CD derivatives. However, better selectivity and resolution of twelve MBA isomers were gained by a combination of 5 mM γ-CD and 0.5% (w/v) poly-SUS, 35% (v/v) acetonitrile at a pH of 9.75.

3.7. Experimental

3.7.1. Instrumentation

All of the CE experiments were performed on a Beckman P/ACE model 5510 CE instrument (Fullerton, CA, USA). A fused silica separation capillary (57 cm X 51 µm I.D., 361 µm O.D., 50 cm to detector) obtained from Polymicro Technologies (Phoenix, AZ, USA) was
installed in a capillary cartridge and thermostated at 23°C by use of a fluoroorganic fluid. The detection of MBA isomers was carried out at 254 nm.

3.7.2. Materials

The detailed syntheses and polymerization of sodium 10-undecenyl sulfate (SUS) monomer is reported in Chapter 2. Hydroxypropyl-β-CD (HP-β-CD) with an average degree of substitution of 0.8 hydroxypropyl groups per cyclodextrin ring was purchased from Aldrich (Milwaukee, WI, USA). Heptakis (2,6-di-O-methyl)-β-CD (DM-β-CD) and heptakis (2,3,6-tri-O-methyl)-β-CD (TM-β-CD) were obtained from Sigma (St. Louis, MO, USA). The β- and γ-CDs were a gift from American Maize-Products (Hammond, IN, USA). The HPLC grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI, USA). Disodium tetraborate and disodium hydrogen phosphate were obtained from EM Science (Gibbstown, NJ, USA) and sodium hydroxide was purchased from Curtin Matheson Science (Houston, TX, USA). The monomethyl-benz[a]anthracene (MBA) isomers were kindly provided by Harold Seifred (Chemical and Physical Carcinogenesis Branch, NCI, Rockville, MD).

3.7.3. Capillary Electrophoresis Procedure

A new capillary was conditioned with 1 M NaOH for about 3 hours at 40°C and then washed with water for about 30 minutes. Between injections, the capillary was conditioned for 3 minutes with the running buffer. The mixture of the MBA isomers was loaded on the capillary by applying 0.5 psi pressure on the injection vial for 1 second.

3.7.4. Buffer and Standard Preparation

The pH of the stock solution of disodium tetraborate and disodium hydrogen phosphate (12.5 mM each) was adjusted to either 9.5 or 9.75 using 1 M NaOH and used as a background electrolyte (BGE). The running MCE solutions were prepared by dissolving 0.5% (w/v) poly-
SUS and an appropriate amount of CD in a 12.5 mM borate/phosphate buffer. The pH values of all BGE samples were adjusted before addition of acetonitrile, poly-SUS and CD. Appropriate amounts of acetonitrile and triply deionized water were added to make final running buffer solutions. The running EKC solutions were sonicated and filtered through a 0.45 μm Nalgene Syringe filter (Rochester, NY, USA) before use. The stock standard MBA solutions were prepared in acetonitrile at a concentration of ca. 4 mM. The final concentration of each MBA isomer in a test mixture was ca. 0.3 mM. Due to the carcinogenicity of the MBA isomers, precautions were taken while handling stock solutions. The solutions of MBA isomers were prepared and diluted in a ventilated hood and stored in a closed container in a refrigerator. Disposable latex gloves were worn while working with MBA standards and care was taken to dispose of the waste solutions.

3.8. Results and Discussion

Cyclodextrins (CDs) are used as additives in EKC to solubilize hydrophobic compounds that migrate closer to the micelle and cannot be separated by the micellar solution alone. The addition of CD into the micellar solution alters the partitioning of the solute between the micellar phase and the CD phase. A variety of neutral and charged organic and inorganic molecules form highly selective molecular inclusion complexes with CDs. Since CDs are electrically neutral they move with the EOF. When the hydrophobic solute is introduced to the CD-MCE system, it will partition with either CD or micelle. Due to the high hydrophobic character, the MBA isomers retain in either the CD or the micellar phase more than in the aqueous phase. The ratio of an MBA isomer incorporated into the micelle phase depends on its hydrophobicity; however, the inclusion complex formation between an MBA isomer and CD depends on the cavity size and hydrophobicity of CD. In addition, MBA isomers may possibly exchange directly between
electroosmotically migrating CD and electrophoretically migrating micelle. We believe that this latter mechanism is dominant in this study and should be governing the stability of CD-isomer inclusion complex and the micelle-isomer interaction strength. Furthermore, the ratio of MBA distribution between CD-phase and micellar phase should determine the elution order and electrophoretic mobility of the analytes. Similar behaviors were observed by Copper and Sepaniak (29) using CDs as modifiers to SDS. Since the measurement of \( t_{mc} \) in the presence of CD is difficult, due to the inclusion complex of tracers of the micelle such as Sudan III or dodecanophenone with CD, relative migration times (\( t_R/t_{co} \)), instead of \( k' \), were used in this study.

3.8.1. Separation Selectivity of MBA Isomers Using \( \beta \)-CD Derivatives

Our previous study indicated that the use of 0.5% (w/v) poly-SUS as micelle polymer, 35% (v/v) acetonitrile, 12.5 mM mixture of NaHPO\(_4\) and Na\(_2\)B\(_4\)O\(_7\) buffered at pH 9.5 were optimum conditions for the separation of MBA isomers (Chapter 3, part I). In order to separate the individual components of a mixture containing twelve MBA isomers, various concentrations of three \( \beta \)-CD derivatives, HP-\( \beta \)-, DM-\( \beta \)-, and TM-\( \beta \)-CDs were added as modifiers to the micelle polymer solution. As a first step in our study on the modification of separation selectivity, several concentrations ranging 0 to 50 mM HP-\( \beta \)-, DM-\( \beta \)-, TM-\( \beta \)-CD were investigated.

3.8.1.1. Effect of Concentration of \( \beta \)-CD Derivatives

Figure 3.7 A-C shows the effect of the concentration of three \( \beta \)-CD derivatives on relative retention time (\( t_R/t_{co} \)). It is clear from Figure 3.7 A-C that larger \( t_R/t_{co} \) values are obtained at low concentration of any \( \beta \)-CD derivative. This indicates that at zero or slightly higher concentrations of the \( \beta \)-CD derivative, the interaction between MBA isomers and the poly-SUS micelle is stronger than that of \( \beta \)-CD derivatives. Note that, as the concentration of \( \beta \)-
CD derivatives increased from 0 mM to 10 mM DM-β-CD and 0 mM to 20 mM TM-β-CD, t_R/t_eo values decreased sharply. Further increase in concentration (>20 mM) of these two β-CD derivatives showed only a slight decrease in t_R/t_eo values. The use of DM-β-CD as an additive to
poly-SUS reduces the \( t_R/t_{eo} \) values of MBA isomers to a greater extent than for TM-\( \beta \)-CD. No significant decrease in \( t_R/t_{eo} \) vs. mM HP-\( \beta \)-CD was observed. In fact, a gradual decrease in relative retention time was noticed as the concentration of the HP-\( \beta \)-CD increased from 0 mM to 50 mM (Figure 3.7 C). As seen in Figure 3.7A and B insets, the \( t_{eo} \) values increased significantly between 0-10 mM, then level off between 10-30 mM using DM-\( \beta \)-CD or TM-\( \beta \)-CD. The increase in \( t_{eo} \) values between 30-50 mM for DM-\( \beta \)-CD was much more noticeable, whereas the increase in \( t_{eo} \) for TM-\( \beta \)-CD was slight. In addition, a gradual increase in \( t_{eo} \) values is observed between 0-30 mM HP-\( \beta \)-CD. However, the unexpected decrease in \( t_{eo} \) between 30-50 mM HP-\( \beta \)-CD is not clear. The reduction in \( t_R/t_{eo} \) values of MBA isomers is not surprising, because these \( \beta \)-CD derivatives are neutral and move with EOF. As the concentration of \( \beta \)-CD derivative increases, stronger interaction between MBA isomers and CD will occur. Thus, faster separation (i.e., reduction in \( t_R/t_{eo} \) values) is expected. Furthermore, changes in selectivity of MBA isomers were observed when different concentrations of \( \beta \)-CD derivatives were added to the running buffer solution (Figure 3.8 A-C). Figure 3.8 A-C shows not only the change in selectivity, but also the maximum number of peak resolved at each concentration of \( \beta \)-CD derivative. Overall, the maximum resolved MBA isomers at 0, 5, 10, 20, 30 and 50 mM \( \beta \)-CD derivative are: 9, 7, 7, 8, 10 and 10 with DM-\( \beta \)-CD; 9, 6, 5, 7, 8, and 8 with TM-\( \beta \)-CD; and 9, 5, 10, 7, 8, and 8 with HP-\( \beta \)-CD.

3.8.1.2. Effect of Type of \( \beta \)-CD Derivatives

Figure 3.9 A-C shows the separation profiles of the MBA isomers under the optimum conditions of each \( \beta \)-CD derivative. The optimum concentrations were found to be 30 mM each of DM-\( \beta \)-CD and TM-\( \beta \)-CD, and 15 mM HP-\( \beta \)-CD. Better resolution with shorter analysis time
was observed with DM-β-CD compared to TM-β-CD at the same concentration (Figure 3.9 A and B). The total number of peaks resolved was 10 and 8 using 30 mM each of DM-β-CD and TM-β-CD, respectively. TM-β-CD is relatively more methylated than DM-β-CD. It is more likely that MBA isomers have relatively stronger interaction with the hydrophobic cavity of DM-β-CD than with TM-β-CD, most likely due to the steric effect of the methyl groups on the CD.
molecule. It is assumed that the presence of more methyl groups may have given TM-β-CD unfavorable structure than DM-β-CD for best complexation with the MBA isomers. Hydroxypropyl groups of HP-β-CD might have prevented the MBA isomers from penetrating into the CD cavity. Thus, the MBA isomers interact more strongly with poly-SUS than with HP-

Figure 3.9. Electropherograms showing the separation of twelve MBA isomers using optimum concentration of β-CD derivatives. (A) 30 mM DM-β-CD, (B) 30 mM TM-β-CD, and (C) 15 mM HP-β-CD. Peak identifications: 1) 1-MBA, 2) 2-MBA, 3) 3-MBA, 4) 4-MBA, 5) 5-MBA, 6) 6-MBA, 7) 7-MBA, 8) 8-MBA, 9) 9-MBA, 10) 10-MBA, 11) 11-MBA, and 12) 12-MBA. MCE conditions are the same as in Figure 3.7 except fixed concentration of each β-CD derivative was used.
β-CD. As a result, longer migration times are observed even with 15 mM HP-β-CD (Figure 3.9 C) as compared to 30 mM DM-β-CD or TM-β-CD (Figure 3.9 A, B). It should be noted that, under optimum conditions, the separation window (i.e., retention time from the first MBA peak to the last MBA peak) was wider (~15 minutes) with HP-β-CD than that with DM-β-CD (~2.7 minutes) and TM-β-CD (~2.0 minutes). Moreover, some selectivity differences were observed using DM-, TM- or HP-β-CD. For example, the resolution and selectivity between 4-MBA and 2-MBA and that between 1- or 7-MBA vs. 11-MBA isomers is significantly higher with DM-β-CD than with TM-β-CD. In addition, 12-MBA isomer comigrated with 1- and 7-MBA as the second to last peak with DM-β-CD. On the other hand, the same isomer eluted faster (i.e., ahead of 1- and 7-MBA) but comigrated with 6- and 8-MBA isomers using TM-β-CD. Moreover, enhancement in selectivity with reversal in migration order of 4- and 5-MBA was observed using HP-β-CD instead of DM-β-CD of TM-β-CD. Note that, 2-MBA eluted much later (as second to last peak) with HP-β-CD than with DM-β-CD. As discussed in a recent review paper, the chemical modification of native CDs has significant effects on the hydrogen bond ability and physical properties as well as on the shape and size of their cavities (42). Thus, it is more likely that different substituted groups on native β-CD would give different selectivity and retention behavior due mostly to steric effects of substituted groups. As shown in Figure 3.9, a complete separation of all twelve isomers was not successful with any of the β-CD derivatives.

### 3.8.2. Separation Selectivity of MBA Isomers Using Native β-CD and γ-CD

Since MBA isomers are large hydrophobic compounds, β-CD and γ-CD were chosen for their larger cavity diameters (6.6 Å and 8.4 Å, respectively). As reported earlier without β-CD or γ-CD, nine of twelve MBA isomers were resolved using poly-SUS. By addition of 2 mM of
β-CD to the mixture of 0.5% (w/v) poly-SUS, 35% (v/v) acetonitrile and 12.5 mM each of borate and phosphate buffer containing running solution, $t_R/t_{eo}$ value reduced slightly and a shift in migration order of some MBA isomers was observed (Figure 3.10A). However, no improvement in overall resolution was observed. Although 2-MBA/8-MBA and 5-MBA/6-MBA isomer pairs were resolved at 2 mM β-CD, some other isomers co-migrated. For instance, 8-MBA co-eluted with 4-MBA and 6-MBA (fourth peak); 10-MBA co-eluted with 11-MBA.

Figure 3.10. Relative migration ($t_R/t_{eo}$) (A) and migration order (B) of twelve MBA isomers as a function of β-CD concentration. MCE conditions: 0.5% w/v poly-SUS and 35% v/v ACN in 12.5 mM each of Na$_2$HPO$_4$ and Na$_2$B$_4$O$_7$ buffered at pH 9.75; pressure injection for 1 s, +30 kV applied voltage; UV detection at 254 nm. Peak identifications are shown on the right of the plot. The inset in (A) shows the relationship between $t_{eo}$ and β-CD concentration.
Addition of 5 mM \(\beta\)-CD increased \(t_R/t_{eo}\) values resulting in the separation of ten MBA isomers and resolution of 9-MBA/3-MBA isomers. Note that at this concentration of \(\beta\)-CD, 8-MBA was also separated from 4-MBA and 6-MBA. However, still the peak pairs of 4-MBA/6-MBA and 2-MBA/11-MBA could not be resolved. Although \(t_R/t_{eo}\) values were decreased with further increases in \(\beta\)-CD concentration to 7 mM and leveled off at 10 mM, the number of resolved peaks was decreased to 9 and 7, respectively (Figure 3.10 B). The unexpected behavior of \(t_{eo}\) and \(t_R/t_{eo}\) using >2 mM \(\beta\)-CD is not clear. One possible source can simply be the uncertainties in measuring \(t_R\) that lead to significant error in determining \(t_R/t_{eo}\) values (Figure 3.10 A and inset).

It is worth mentioning that the relative standard deviation (RSD) of migration time reproducibilities in presence of \(\beta\)-CD was much higher (~10%) compared to other CDs. The RSD values for DM-\(\beta\)-CD, TM-\(\beta\)-CD, HP-\(\beta\)-CD were < 2% and that for \(\gamma\)-CD was 4.6%. The optimum \(\beta\)-CD concentration was found to be 5 mM, which resulted in a separation of 10 MBA isomers (Figure 3.10 B).

Addition of 2 mM \(\gamma\)-CD to BGE showed a decrease in \(t_R/t_{eo}\) values, but did not improve the resolution of the MBAs (Figure 3.11 A). The isomers of 12-MBA, 1-MBA, and 7-MBA eluted as the first, second, and third peak, respectively, and remained in the same order at all \(\gamma\)-CD concentrations. At 0 mM \(\gamma\)-CD, 2-MBA co-migrated with 8-MBA (fifth peak) and 5-MBA co-migrated with 6-MBA (seventh peak). The migration order changed at 2 mM \(\gamma\)-CD. For example, 8-MBA co-migrated with 11-MBA (fourth peak) and 5-MBA co-migrated with 10-MBA (seventh peak). On the other hand, 6-MBA and 2-MBA eluted separately as sixth and eighth peak, respectively (Figure 3.11 A, B). Upon addition of 3 mM \(\gamma\)-CD, migration order remained the same except 5-MBA, 10-MBA, and 3-MBA were resolved and eluted as the
seventh, eighth, and ninth peak, respectively, whereas 9-MBA and 2-MBA co-migrated as the tenth peak. However, at 5 mM $\gamma$-CD all but two of the MBA isomers were baseline resolved. The 9-MBA and 2-MBA were partially resolved (Figure 3.12 B). A further increase in $\gamma$-CD concentration to 7 mM deteriorated the resolution of some MBA isomers. As seen in Figure 3.11 A, an increase in $\gamma$-CD concentration from 2 mM to 7 mM caused an increase in both $t_R/t_{eo}$ and $t_{eo}$. The increase in $t_{eo}$ can be attributed simply to an increase in viscosity as the concentration of

Figure 3.11. Relative migration time (A) and migration order (B) of twelve MBA isomers as a function of $\gamma$-CD concentration. The MCE conditions are same as Figure 3.10. Peak identifications are shown on the right of the plot. The inset in (A) shows the relationship between $t_{eo}$ and $\gamma$-CD concentration.
\( \gamma \)-CD increased (Figure 3.11 A inset). In general, an increase in \( \gamma \)-CD concentration decreases the retention time (18); however, for some hydroxylated benz[a]anthracene isomers the opposite behavior was observed (19) which is consistent with our present study.

The significant differences in the electropherograms shown in Figure 3.12 A and B are the selectivity changes observed for a number of MBA isomers depending on the type of native CD added to BGE. For instance, reversal in migration order of 4-MBA and 11-MBA as well as
3-MBA and 9-MBA isomers, were observed using γ-CD as an additive (Figure 3.12 B) instead of β-CD (Figure 3.12 A).

3.8.3. Effect of pH on Resolution of MBA Isomers

The electropherograms of twelve MBA isomers at pH 9.5 and pH 9.75 are compared in Figure 3.12 B and Figure 3.13, respectively. As seen, an increase in pH from 9.5 to 9.75 resulted in baseline resolution of all MBA isomers except 9-MBA and 2-MBA isomers, which were partially resolved. However, a substantial increase in retention times was observed with increasing pH. This behavior was explained previously (Chapter 3, Part I). That is, at higher pH values, poly-SUS has a relatively more open and hydrophobic structure, which causes a stronger interaction between poly-SUS and MBA isomers, resulting in an increase in retention time. Furthermore, an increase in pH increases the ionic strength of the buffer solution, which reduces the zeta potential on the capillary surface. Similar behavior was also observed when β-CD was

![Electropherogram showing the separation of twelve MBA isomers using 5 mM γ-CD at pH of 9.75. The MCE conditions are same as Figure 3.7 except fixed γ-CD concentration was used at pH of 9.75. Peak identifications are same as Figure 3.9.](image)

Figure 3.13. Electropherogram showing the separation of twelve MBA isomers using 5 mM γ-CD at pH of 9.75. The MCE conditions are same as Figure 3.7 except fixed γ-CD concentration was used at pH of 9.75. Peak identifications are same as Figure 3.9.
added to poly-SUS. However, under such conditions an increase in pH did increase the retention
time, but did not increase the number of resolved MBA isomers (data not shown). An attempt to
increase the resolution of the last two peaks (i.e., 2-MBA and 9-MBA) by addition of 0.5-2 %
(v/v) isopropanol or n-butanol to running EKC buffer (5 mM γ-CD, 0.5% poly-SUS) was not
successful. For example, addition of 1% isopropanol to the running MCE buffer (containing 34
% (v/v) acetonitrile) increased the total analysis time to ~200 minutes. However, a slightly
better resolution of 9-MBA and 2-MBA was observed (data not shown).

3.9. Conclusions

A combination of poly-SUS and three β-CD derivatives (i.e., DM-β-CD, TM-β-CD, and
HP-β-CD) as well as native β-CD and γ-CD was investigated to separate twelve MBA isomers.
The β-CD, γ-CD and three β-CD derivatives were found to have different resolution and
selectivity. Additionally, the analysis time of isomers was found to be dependent on the type and
concentration of the CD additives. Relatively shorter analysis times were achieved using β-CD
derivatives comparing to native β-CD and γ-CD. This is an indication of a stronger
complexation between MBA isomers and β-CD derivatives, which can be attributed to the fact
that β-CD derivatives have deeper cavities compared to native β-CD (43). The \( t_R/t_{eo} \) values were
decreased as the concentration of β-CD derivatives increased, whereas the opposite effect was
observed with native β-CD and γ-CD. A combination of 5 mM γ-CD, 0.5 % (w/v) poly-SUS, 35
% (v/v) acetonitrile at a pH of 9.75 provided the best selectivity and resolution of the twelve
MBA isomers. However, a total separation time was about 110 minutes. Alternatively,
combined use of poly-SUS and DM-β-CD resulted in a relatively faster separation (ca. 16
minutes) of MBA isomers. This occurred only at the expense of co-migration of some MBA
isomers.
3.10. References


Chapter 4.
Characterization of Chemical Selectivity in Micellar Capillary Electrophoresis Using Linear Solvation Energy Relationship Models

4.1. Introduction

Since its introduction by Terabe (1), micellar capillary electrophoresis (MCE), also known as micellar electrokinetic chromatography (MEKC), has been extensively used for the separation of both charged and uncharged solutes. A major advantage of MCE over most of the separation techniques is the feasibility of changing the chemical composition of the MCE system by simply rinsing the capillary with a solution of a new pseudostationary phase. Thus, the selectivity of the technique can be easily manipulated and controlled by proper selection of the surfactant type or addition of modifiers such as organic solvents or cyclodextrins (2,3). In MCE uncharged solutes are separated based on their differential partitioning into the pseudostationary phase. The hydrophobic interaction between solutes and the pseudostationary phase is a major driving force behind the solute retention in MCE. However, some other types of interactions between solutes and the pseudostationary phases also influence solute retention and the selectivity. Therefore, one should first understand the nature of the interactions.

Solute-solvent interactions play a major role not only in the development and optimization of analytical separations but also many other areas of chemistry, including chemical synthesis, spectroscopic methods, coating developments, and pharmaceuticals. Since retention prediction and selectivity optimization are very critical in rapid method development in MCE, it is imperative to achieve a better understanding of the factors that control selectivity. In the last several years, linear solvation energy relationship (LSER) model has been given a significant amount of attention for the characterization of retention and selectivity differences between different pseudostationary phases in micellar capillary electrophoresis (MCE) (3-19). The basic
The concept of LSER model known as solvatochromic model was first developed by Kamlet, Taft and their co-workers (20-24). They have shown that chemical systems involve some properties that are linearly related to either a free energy of reaction, a free energy of transfer, or an activation energy. These properties such as logarithmic retention factor \( \log k' \) can be correlated with various fundamental molecular properties of the solvents or solutes involved in the chemical processes. The Kamlet-Taft solvatochromic model was first employed by Chen et al. (4) and Yang and Khaledi (5) to determine the selectivity of a number of surfactant systems in MCE. In Equation 4.1, \( \log k' \) is correlated to known solute descriptors, \( V_1, \pi^*, \beta, \) and \( \alpha \):

\[
\log k' = c + mV_1 + s\pi^* + b\beta + a\alpha .
\]  

4.1

The descriptor \( V_1 \) is the intrinsic volume of the solute and is divided by 100 to bring it to scale with the other terms. The solute polarity and polarizability are represented by the \( \pi^* \) term. The \( \beta \) and \( \alpha \) represent the solute hydrogen bond accepting and solute hydrogen bond donating abilities, respectively. The system coefficients \( (m, s, b, \) and \( a) \) in Equation 4.1 reflect differences in the two bulk phases, the aqueous and the pseudostationary phases, between which the solute is transferring and are obtained by multivariable, simultaneous, linear regression (25). These coefficients provide quantitative information about solute-pseudostationary phase, solute-buffer interactions and selectivity of the bulk buffer in MCE. The constant \( c \) represents the intercept and includes information about the separation phase ratio (26). The \( m \) term is a measure of the relative ease of cavity formation and general dispersion interactions for the solute with the pseudostationary phase and the bulk aqueous phase, respectively. The difference in dipolarity/polarizability between the pseudostationary phase and the bulk aqueous phase is represented by the coefficient \( s \). The \( b \) and \( a \) terms represent the hydrogen bond donating ability and hydrogen bond accepting ability of the pseudo phase, respectively.
The solvation parameter model (Equation 4.2), another expression of LSER, is introduced by Abraham et al. (24,26-28) and is a revised form of Kamlet-Taft solvatochromic model:

$$\log k' = c + mV_x + rR_2 + s\pi_H^n + a\sum \alpha_H^n + b\sum \beta_2^0.$$  \hspace{1cm} 4.2

In this model $V_x$ represents the McGowan solute characteristic volume (in cm$^3$/mol$^{-1}$/100) (29). In the solvation parameter model the $R_2$ represents the excess molar refraction of the solute (in cm$^3$/10) (27). In order to obtain a rough scaling with the other descriptors, $V_x$ and $R_x$ have been divided by 100 and 10, respectively. The subscript 2 denotes that these parameters are solute properties. The $m$, $b$, and $a$ coefficients for the solvation parameter model contain the same information as for the solvatochromic model. The $r$ coefficient determines the difference in ability of the pseudostationary phase and separation buffer (mobile phase) to interact with the n- or $\pi$-electrons of the solute. The dipolarity/polarizability differences between the pseudostationary phase and separation buffer is represented by the $s$ coefficient. It is important to note that the Kamlet-Taft solvatochromic model does not contain the $R_2$ solute descriptor. In addition, the solvatochromic model uses the intrinsic volume of the solute instead of the McGowan characteristic volume. Despite the numerical differences in the values for the two models, major discrepancies in overall trends predicted by both models are rare. However, exact agreement in quantitative aspects cannot be expected.

From all previous studies, the solute size was found to have the largest influence on the solute retention in MCE. The hydrogen bond accepting ability of the solute is the second most important factor on retention and is the largest contributor towards the selectivity differences between pseudostationary phases. Many spectroscopic studies have shown that most solutes interact with the micellar palisade and Stern layers (30-32); therefore, the headgroup of the pseudostationary phase and the counterion may have a significant influence on the solute
Retention and selectivity in MCE. In this study, two different headgroups (i.e., sulfate and carboxylate) have been investigated. Several MCE systems using sodium dodecyl sulfate (SDS), sodium di(undecenyl) tartarate (mono-SDUT), poly sodium di(undecenyl) tartarate (poly-SDUT) as well as the mixture of SDS/mono-SDUT, SDS/poly-SDUT, and mono-SDUT/poly-SDUT as pseudostationary phases were characterized using previously mentioned two LSER models.

4.2. Experimental

4.2.1. Instrumentation

All MCE experiments were performed on a Beckman (Fullerton, CA) P/ACE model 5510 capillary electrophoresis (CE) instrument equipped with a 0-30 kV power supply, a 21-position inlet and 10-position outlet sample carousels for automatic sample/buffer change, 200-, 214-, 254-, and 280-nm selectable wavelength filters for UV detection, a liquid thermostated capillary cartridge (capillary 50 μm i.d. x 375 μm o.d. x 67 cm total length, 60 cm to the detector), and software System Gold for system control and data handling. The capillary in the Beckman instrument was thermostated by use of a fluoroorganic fluid. The detector time constant was 0.2 s. The detector was operated at 200 nm for LSER test solutes and at 254 for homologues series of alkyl phenyl ketones. All experiments were carried out at 25 °C. A voltage of 25 kV was applied throughout the experiments.

4.2.2. Materials

All of the LSER test solutes, SDS, 10-undecenoyl chloride, DL-tartaric acid and alkyl phenyl ketone homologues were purchased from Aldrich (Milwaukee, WI). Sodium hydrogenphosphate and sodium dihydrogenphosphate were obtained from EM Science (Gibbstown, NJ). Deionized water was obtained by a water purification system from Millipore Corp. (Milford, MA).
4.2.3. Synthesis of Sodium di(Undecenyl) Tartarate

The sodium di(undecenyl) tartarate monomer, a vesicle forming amphiphilic compound possessing two hydrophilic carboxylate headgroups and two hydrophobic undecenyl chains, was prepared according to a procedure reported by Kunitake and Okahata (33) (Figure 4.1). Briefly,

Figure 4.1. Synthetic scheme for mono-SDUT and poly-SDUT.
0.04 mol of 10-undecenoyl chloride (a) in toluene was added to a pyridine solution of 0.02 mol of DL-tartaric acid (b) at 10 °C over a period of 30 minutes. The mixture then was stirred vigorously at room temperature for 1 hour. The precipitates formed were filtered and the solution containing the product was concentrated. The resulting solid product, i.e., di(undecylenic) tartaric acid (DUTA) (c), was recrystallized twice from hexane. In order to prepare the sodium salt of DUTA (i.e., SDUT), a desired amount of DUTA was placed in aqueous sodium bicarbonate solution (e.g., 0.02 mol DUTA and 0.04 mol sodium bicarbonate in 150 ml water). The cloudy mixture was stirred until the acidic product (i.e., DUTA) was completely neutralized by alkaline solution. After the mixture turned into a clear solution, which indicates the completion of the titration, the SDUT surfactant solution was freeze-dried to yield a white powder. Recrystallization was performed by dissolving the white powder in an aqueous methanol solution and refrigerated. The crystals were dried in a vacuum desiccator overnight. The final product was SDUT monomers (d).

Polymerization of the SDUT monomers (e) was achieved by preparing a 20 mM solution of the surfactant in water and exposing the sample to a 60Co γ-ray source (~680 rad/h) for a week. After radiation, the solution was filtered and lyophilized to yield a white powder. Polymerization was confirmed by the disappearance of the double bond using nuclear magnetic resonance and infrared spectroscopic methods.

4.2.4. Determination of Critical Aggregation Concentration of Mono-SDUT

Surface tension method was used to determine the critical aggregation concentration (CAC) of mono-SDUT surfactant. A 30 mM stock solution of mono-SDUT surfactant was prepared in deionized water (18 MΩ). Twelve different concentrations ranging from 1.0 to 30.0 mM were prepared from the stock solution. A Du Nüoy type tensiometer was used to measure
the surface tension. The measured surface tension, in millinewtons per meter (mN/m), was plotted against the surfactant concentration (in mM) (Figure 4.2). The CAC was determined as

![Graph showing variation of surface tension with concentration of mono-SDUT](image)

**Figure 4.2.** Variation of the surface tension with the concentration of mono-SDUT in aqueous solution at room temperature. The inset is an enlargement of the region of interest.

the crossing point (ca. 3.94 mM) of the two straight lines that fit the experimental values before and after the abrupt change of slope.

### 4.2.5. Determination of Aggregation Number and Polarity of Surfactants

#### 4.2.5.1. Determination of Aggregation Number

The aggregation number of the surfactants was determined by the method proposed by Turro and Yekta (34), using following expression:

\[
\ln \left( \frac{I_0}{I} \right) = \frac{N[Q]}{[S_{tot}]-CAC},
\]

\[4.3\]
where $I_0$ and $I$ are the emission intensities at a certain wavelength in the absence and presence of $[Q]$ concentrations of the fluorescence quencher, respectively. The $[S_{tot}]$ is the total surfactant concentration and the CAC is the critical aggregation concentration of the surfactant.

Fluorescence measurements were performed on a SPEX model F2T211 spectrophotometer. Pyrene and cetylpyridinium chloride (CPyrCl) were used as fluorescent probe and quencher, respectively. A $1 \times 10^{-3}$ M stock solution of pyrene was prepared in methanol. A $2.8 \times 10^{-3}$ M stock solution of CPyrCl and a 1.5 % (w/v) of each of SDS, mono- and poly-SDUT stock solutions were prepared separately in deionized water. A known volume of pyrene stock solution was pipetted into a clean volumetric flask, methanol was evaporated by nitrogen gas, and aqueous surfactant solution was added. At this step, the concentrations of pyrene and surfactant were $2.0 \times 10^{-5}$ M and 1.5 % (w/v), respectively (solution 1). After sonicating for 90 minutes, solution 1 was stored in a dark area overnight to equilibrate. Solution 1 was then divided in half. One half was diluted with deionized water to give a $1.0 \times 10^{-5}$ M pyrene and 0.75 % (w/v) surfactant (solution 2), while the other half was mixed with quencher stock solution to make $1.4 \times 10^{-3}$ M CPyrCl, $1.0 \times 10^{-5}$ M pyrene, and 0.75 % (w/v) surfactant (solution 3). Solution 3 was added to solution 2 in increasing increments of 25 µL and allowed 20 minutes for equilibration after addition of each quencher solution before fluorescence measurement. The decrease in emission spectra of pyrene was recorded at 393.0 nm after each aliquot of the quencher solution (solution 3) was added and the logarithm of the intensity ratio $I_0/I$ was plotted against the quencher concentration. The aggregation number, $N$, is obtained from the slope of the plot of $\ln (I_0/I)$ vs. $[Q]$ (i.e., $N = \text{Slope} \times [S_{tot}] - \text{CAC}$). The aggregation number measurement plots for SDS, mono- and poly-SDUT are shown in Figure 4.3.
Figure 4.3. Degree of aggregation measurement for (A) SDS, (B) mono-SDUT, and (C) poly-SDUT.
4.2.5.2. Determination of Polarity

As summarized in chapter 1 of this dissertation, the polarity of the aggregated surfactant core can be measured using a fluorescence molecule that stays in the core and is sensitive to the polarity of the environment. Pyrene is a fluorescent molecule that has been used extensively for this purpose. The emission spectrum of pyrene molecule is sensitive to the environment in which it is dissolved. Pyrene has characteristic fluorescence emission spectra that consist of five vibronic bands. The intensities of these vibronic bands depend on the polarity of the environment in which pyrene is dissolved. An increase in the intensity of the band I at 372 nm is accompanied by a decrease in the intensity of the band III at 383 nm 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 or vesicular core.

4.2.6. Capillary Electrophoresis Procedure

All new capillaries were activated by the following washing sequence: 1 hour of 1 M NaOH and 20 minutes of triply deionized water. Prior to each separation with the same surfactant the capillaries were rinsed with triply deionized water (5 minutes), 0.1 M NaOH (3 minutes), and separation buffer (3 minutes). When the surfactant was changed the capillaries were reconditioned for 15 minutes with deionized water, 10 minutes with 0.1 M NaOH, and 5 minute with the separation buffer. Unless otherwise noted, the time for pressure injection was 3 seconds for most separations.

4.2.7. Preparation of Separation Buffers and Standard Solutions

A 100 mM stock solution of phosphate buffer (pH 7.0) was prepared by dissolving appropriate amount of sodium hydrogenphosphate and sodium dihydrogenphosphate and refrigerated after each use. The solutions of SDS, mono-SDUT, and poly-SDUT were prepared
by first dissolving 0.1 gram of surfactant in 5.0 mL of deionized water. Two mL of a 100 mM phosphate stock buffer was then added to this solution. Lastly, the final volume was adjusted to 10.0 mL with deionized water. The same sequence was followed for the preparation of mixed surfactant solutions except 0.05 gram of each surfactant (e.g., 0.05 gram SDS and 0.05 gram mono-SDUT for SDS/mono-SDUT mixed surfactant system) was dissolved in 5.0 mL of water to keep final surfactant concentration at 1.0 % (w/v). The corresponding molar concentrations of the pseudostationary phases were 34.7 mM SDS, 19 mM mono-SDUT, and 19 mM equivalent monomer concentration of poly-SDUT. The mixed surfactants contained 17.35 mM SDS and 9.5 mM mono- or poly-SDUT for SDS/mono-SDUT or SDS/poly-SDUT, and 9.5 mM each of mono- and poly-SDUT for mono-SDUT/poly-SDUT mixture. After a thorough mixing in a sonicator for 10 minutes, the final running buffers were filtered through a 0.45-μm syringe filter (Nalgene, Rochester, NY) then sonicated for 3 more minutes before capillary electrophoretic experiments. All stock LSER solute solutions were prepared in methanol at concentrations of ca. 4-7 mM each. Molar concentrations of the injected standard solute mixture were about 0.5 mM.

4.2.8. Calculations

The capacity factor (also known as retention or migration factor), $k'$, of a neutral solute was measured according to the following formula (1):

$$
k' = \frac{t_R - t_{co}}{t_{co} \left[ 1 - \left( \frac{t_R}{t_{psh}} \right) \right]},
$$

where $t_R$, $t_{co}$ and $t_{psh}$ are the migration times of a neutral retained analyte, the electroosmotic flow (EOF), and the pseudostationary phase, respectively. Methanol was used as the $t_{co}$ marker and was measured from the time of injection to the first deviation from the baseline. Decanophenone was used as tracer for $t_{psh}$. 

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The elution range is defined as \( t_{\text{psp}}/t_{\text{eo}} \). The apparent electrophoretic mobility of pseudostationary phase (\( \mu_{\text{app}}, \text{cm}^2 \text{V}^{-1} \text{s}^{-1} \)) is calculated according to the Equation 4.5:

\[
\mu_{\text{app}} = \frac{l_t l_d}{V t_{\text{psp}}},
\]

where \( l_t \) is the total length of the capillary (in cm), \( l_d \) is the length of the capillary from injector to detector (in cm), \( V \) is the applied voltage (in V), and \( t_{\text{psp}} \) is in seconds (s). To calculate the electroosmotic mobility of the buffer solution (\( \mu_{\text{eo}}, \text{cm}^2 \text{V}^{-1} \text{s}^{-1} \)), \( t_{\text{psp}} \) term in Equation 4.5 is replaced with \( t_{\text{eo}} \). The relationship between effective electrophoretic mobility of the pseudostationary phase (\( \mu_{\text{ep}} \)), \( \mu_{\text{app}} \), and \( \mu_{\text{eo}} \) is shown as:

\[
\mu_{\text{ep}} = \mu_{\text{app}} - \mu_{\text{eo}}.
\]

The methylene selectivity (hydrophobic selectivity), \( \alpha_{\text{CH}_2} \), was calculated from the antilogarithm of the slope of the regression line of \( \log k' \) vs. carbon number of alkyl phenyl ketone homologous series.

**4.3. Results and Discussion**

**4.3.1. Physicochemical Properties of Pseudostationary Phases**

The primary structural difference between pseudostationary phases used in this study is their head groups. Carboxylate (mono-SDUT, poly-SDUT, and mono-SDUT/poly-SDUT), sulfate (SDS), and the mixture of carboxylate and sulfate (SDS/mono-SDUT, SDS/poly-SDUT) were examined as head groups. Sodium is a counterion and alkyl chain (C11 for mono- and poly-SDUT, C12 for SDS) is their hydrophobic moiety for all surfactants. Physicochemical properties of pseudostationary phases are compared in Table 4.1. Aggregation number of mono-SDUT (275 monomers per vesicle) is higher than that of SDS (71 monomers per micelle) and poly-SDUT (71 monomer per vesicle). Possessing two double alkyl chains, mono-SDUT and
poly-SDUT tend to form vesicles. However, during polymerization of mono-SDUT, the number of aggregates seems to be decreased resulting in smaller aggregation number of the poly-SDUT. This may be due to the slower and continuous rearrangement of the monomers in the vesicle during the polymerization process that took a week. The intensity of $\gamma$-radiation source ($^{60}$Co)

### Table 4.1. Comparison of physicochemical properties of six pseudostationary phases.

<table>
<thead>
<tr>
<th>Physicochemical property</th>
<th>SDS/ Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/Mono-SDUT</th>
<th>SDS/Poly-SDUT</th>
<th>Mono-SDUT/Poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of aggregation, $N$</td>
<td>71</td>
<td>275</td>
<td>71</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Molecular weight, $M$ ($10^4$ g mol$^{-1}$)</td>
<td>2.05</td>
<td>14.5</td>
<td>3.74</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Critical aggregation concentration, CAC (mM)</td>
<td>8.00</td>
<td>3.94</td>
<td>0.00</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Electroosmotic mobility, $\mu_{eo}$ ($10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$)</td>
<td>5.60</td>
<td>4.79</td>
<td>4.97</td>
<td>4.77</td>
<td>5.09</td>
</tr>
<tr>
<td>Apparent electrophoretic mobility, $\mu_{app}$ ($10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$)</td>
<td>1.55</td>
<td>1.18</td>
<td>1.32</td>
<td>1.13</td>
<td>1.23</td>
</tr>
<tr>
<td>Effective Electrophoretic mobility, $\mu_{ep}$ ($10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$)</td>
<td>-4.05</td>
<td>-3.61</td>
<td>-3.65</td>
<td>-3.64</td>
<td>-3.86</td>
</tr>
<tr>
<td>Mobility ratio, $R_{m}$</td>
<td>0.723</td>
<td>0.754</td>
<td>0.734</td>
<td>0.763</td>
<td>0.758</td>
</tr>
<tr>
<td>Methylene-group selectivity, $\alpha_{CH2}$</td>
<td>2.57</td>
<td>3.31</td>
<td>2.72</td>
<td>2.97</td>
<td>2.78</td>
</tr>
<tr>
<td>Migration-time window, $t_{pp}/t_{eo}$</td>
<td>3.61</td>
<td>4.06</td>
<td>3.77</td>
<td>4.22</td>
<td>4.14</td>
</tr>
<tr>
<td>Polarity (Pyrene I/III)</td>
<td>1.09</td>
<td>1.31</td>
<td>0.98</td>
<td>1.05</td>
<td>1.04</td>
</tr>
</tbody>
</table>

---

*a* Determined in water by fluorescence quenching method; *b* Data not available; *c* Calculated from degree of aggregation shown above; *d* From reference 35; *e* Determined in water by surface tension measurement; *f* Critical aggregation concentration of the polymerized surfactant is assumed to be zero; *g* Data were collected with 67 cm (60 cm effective length) x 50 µm ID capillary with an applied voltage of +25 kV using a 20 mM phosphate buffer at pH of 7.0, final surfactant concentration was 1.0 % w/v; *h* Calculated using Equation 5.5, $t_{pp}$ was replaced with $t_{eo}$; *i* Calculated using Equation 5.5; *j* Calculated from $\mu_{eo}=\mu_{app}-\mu_{eo}$; *k* Calculated from $-\mu_{ep}/\mu_{eo}$; *l* Calculated from the antilogarithm of the slope of the regression line of log $k$ vs. carbon number of alkyl phenyl ketones (C10-C14); *m* Determined from the ratio of the intensity of band I and band III of pyrene in presence of 0.75 % w/v surfactant using fluorescence spectroscopy.
used for the polymerization in this study was ~0.7 krad/h. The flux of the gamma rays from $^{60}$Co was probably not strong enough to provide polymer with aggregation numbers similar to the monomer. Shorter polymerization period with a stronger radiation source may result in higher aggregation numbers of poly-SDUT. Previous studies have shown that the intensity of the radiation source used for polymerization has a significant effect on the number of the repeat units of polymers (36). Using a relatively stronger gamma radiation source (143 krad/h) than the source used in this study, Paleos et al. have obtained polymers with the same size as the micelles by polymerization of sodium 10-undecenoate (37).

The order of pseudostationary phases from grater to smaller value for each physicochemical parameter is shown in Table 4.2.

**Table 4.2.** The order of pseudostationary phases for each physicochemical properties.

<table>
<thead>
<tr>
<th>Physicochemical property</th>
<th>Order (from greater to smaller value)</th>
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<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Degree of aggregation, N</td>
<td>M</td>
</tr>
<tr>
<td>Molecular weight, M</td>
<td>M</td>
</tr>
<tr>
<td>Critical aggregation concentration, CAC</td>
<td>S</td>
</tr>
<tr>
<td>Electroosmotic mobility, $\mu_{eo}$</td>
<td>S</td>
</tr>
<tr>
<td>Apparent electrophoretic mobility, $\mu_{app}$</td>
<td>S</td>
</tr>
<tr>
<td>Effective Electrophoretic mobility, $\mu_{ep}$</td>
<td>MP</td>
</tr>
<tr>
<td>Mobility ratio, $R_m$</td>
<td>SM</td>
</tr>
<tr>
<td>Methylene-group selectivity, $\alpha_{CH2}$</td>
<td>M</td>
</tr>
<tr>
<td>Migration-time window, $t_{psp}/t_{co}$</td>
<td>SM</td>
</tr>
<tr>
<td>Polarity (Pyrene I/III)</td>
<td>M</td>
</tr>
</tbody>
</table>

S = SDS; M = mono-SDUT; P = poly-SDUT; SM = SDS/mono-SDUT; SP = SDS/poly-SDUT; MP = mono-SDUT/poly-SDUT; $^{a}$SDS and poly-SDUT have the same degree of aggregation; $^{b}$Data not available; $^{c}$SDS/mono-SDUT and mono-SDUT/poly-SDUT have the same electroosmotic mobility.
SDS system has the highest \( \mu_{eo} \) value (5.60 x 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{s}^{-1}) followed by SDS/poly-SDUT and poly-SDUT systems with the values of 5.09 x 10^{-4} and 4.97 x 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{s}^{-1}, respectively. SDS/mono-SDUT and mono-SDUT/poly-SDUT systems have the same \( \mu_{eo} \) value (4.77 x 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{s}^{-1}), and Mono-SDUT vesicles have a slightly higher \( \mu_{eo} \) (4.79 x 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{s}^{-1}) than these two surfactants. The difference in \( \mu_{eo} \) values for each pseudostationary phase is due probably to the difference in Zeta potentials of the capillary wall (\( \zeta_{cw} \)) and pseudostationary phase (\( \zeta_{psp} \)) as well as the viscosity of the separation buffer (Equations 4.7 and 4.8).

\[
\mu_{eo} = -\frac{\varepsilon \zeta_{cw}}{\eta}, \quad 4.7
\]

\[
\mu_{ep} = \frac{\varepsilon \zeta_{psp}}{\eta}, \quad 4.8
\]

where \( \varepsilon \) is the dielectric constant of bulk solution, \( \eta \) is the viscosity of the buffer solution. The \( \mu_{ep} \) values of all pseudostationary phases are negative. This indicates that all pseudostationary phases migrate against EOF due to their negative charges. However, as a result of strong EOF, pseudo phase is swept toward the detector. Thus, the net electrophoretic mobility, or \( \mu_{app} \), of the micelle or vesicle is a vector sum of \( \mu_{ep} \) of micelle (or vesicle) and \( \mu_{eo} \), i.e., \( \mu_{app} = \mu_{ep} + \mu_{eo} \).

Different negative values of \( \mu_{ep} \) are related to net charge and the size of the aggregates (vesicles or micelles) as well as the viscosity of the buffer solution according to Equation 4.9.

\[
\mu_{ep} = -\frac{q}{6\pi\eta r}, \quad 4.9
\]

where \( q \) is the charge on the particle (vesicle or micelle), \( \eta \) is the viscosity of the buffer solution, and \( r \) is the Stokes’ radius of the particle. According to Equation 4.9, it is fairly evident that mono-SDUT/poly-SDUT has largest, and SDS has the smallest \( q/r \) value (Table 4, \( \mu_{ep} \) values).
Recently, Chen and Terabe introduced a new parameter, the mobility ratio, $R_m$ (38, 39). The ratio of the $\mu_{ep}$ (Equation 4.8) to the $\mu_{eo}$ (Equation 4.7) is known as $R_m$ and expressed as:

$$R_m = -\frac{\mu_{ep}}{\mu_{eo}}.$$  \hspace{1cm} 4.10

The negative sign in Equation 4.9 represents the direction of EOF. The combination of the equations 4.7, 4.8, and 4.10 will result in the following expression:

$$R_m = \frac{\mu_{ep}}{\mu_{eo}} = \frac{\zeta_{psp}}{\zeta_{cw}}.$$  \hspace{1cm} 4.11

According to Equation 4.11, the value of the $R_m$ determines the ratio of $\zeta_{psp}$ to $\zeta_{cw}$. Being a measure of the $\mu_{ep}$ relative to the $\mu_{eo}$, $R_m$ is an important parameter in MCE. It has the advantage of reflecting the effect of the Zeta potential of the pseudostationary phase and the Zeta potential of the capillary wall regardless of any changes in the dielectric constant and the viscosity of the buffer. However, slight viscosity changes may be expected due to the nature and the composition of each pseudostationary phase system. As seen in Table 4, $R_m$ decreases through the following trend: SDS/mono-SDUT > SDS/poly-SDUT > mono-SDUT > mono-SDUT/ poly-SDUT > poly-SDUT > SDS. It should also be noted that migration-time window ($t_{psp}/t_{eo}$) follows the same trend as $R_m$. The relationship between $t_{psp}/t_{eo}$, $\mu_{eo}$, $\mu_{app}$, and $\mu_{ep}$ can be formulated as:

$$\frac{t_{psp}}{t_{eo}} = \frac{\mu_{eo}}{\mu_{app}} = \frac{\mu_{eo}}{\mu_{eo} + \mu_{ep}}.$$  \hspace{1cm} 4.12

Thus, $t_{psp}/t_{eo}$ and $R_m$ can be related as:

$$\frac{1}{t_{psp}/t_{eo}} + R_m = 1.$$  \hspace{1cm} 4.13
The hydrophobic or methylene selectivity, $\alpha_{CH_2}$, measurements indicate that all vesicular pseudostationary phases have more hydrophobic character than SDS micellar phase, under the experimental conditions studied. Mono-SDUT is the most hydrophobic phase and SDS the least hydrophobic phase with $\alpha_{CH_2}$ values of 3.31 and 2.57, respectively. The polarity and the methylene selectivity orders of pseudostationary phases are expected to be opposite, i.e., one would anticipate mono-SDUT to be least polar. On the contrary, fluorescence polarity measurement showed mono-SDUT as the most polar surfactant. This divergence is probably due to the fact that pyrene, fluorescent probe used in polarity measurement, is dissolved in the relatively polar region of the mono-SDUT vesicle. As discussed in Chapter 1 of this dissertation, vesicles are spherically closed bilayers, which, in analogy to the cell membrane, enclose an aqueous compartment (40, 41). When pyrene is dissolved in or near this polar aqueous region, the ratio of band I (at 372 nm) to band III (at 383 nm) intensities of pyrene’s spectra increases, which indicates a more polar environment. Same analogy may be true for the rest of vesicular pseudostationary phases.

4.3.2. Linear Solvation Energy Relationship

The retention behavior of the 36 test solutes in each pseudostationary system was examined and compared using two LSER models, i.e., Kamlet and Taft’s solvatochromic model and Abraham and coworker’s solvation parameter model. The test solutes and their descriptors used in this study are given in Tables 4.3 and 4.4.

A set of solutes with known descriptors is required to determine the coefficients of Equations 4.1 and 4.2 accurately. Some recommendations for selecting an appropriate set of solutes are given in the literature (3): 1) mathematically, a minimum number of seven solutes is needed to solve a multiple linear regression equation for six unknowns (five system constants
Table 4.3. Test solutes and their solvation descriptors\(^a\) for the solvatochromic model.

<table>
<thead>
<tr>
<th>#</th>
<th>Solute</th>
<th>(V_1)</th>
<th>(\pi^*)</th>
<th>(\beta)</th>
<th>(\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene</td>
<td>0.491</td>
<td>0.590</td>
<td>0.100</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>Toluene</td>
<td>0.592</td>
<td>0.550</td>
<td>0.110</td>
<td>0.000</td>
</tr>
<tr>
<td>3</td>
<td>Ethylbenzene</td>
<td>0.668</td>
<td>0.530</td>
<td>0.120</td>
<td>0.000</td>
</tr>
<tr>
<td>4</td>
<td>Propylbenzene</td>
<td>0.769</td>
<td>0.510</td>
<td>0.120</td>
<td>0.000</td>
</tr>
<tr>
<td>5</td>
<td>p-Xylene</td>
<td>0.668</td>
<td>0.510</td>
<td>0.120</td>
<td>0.000</td>
</tr>
<tr>
<td>6</td>
<td>Chlorobenzene</td>
<td>0.581</td>
<td>0.710</td>
<td>0.070</td>
<td>0.000</td>
</tr>
<tr>
<td>7</td>
<td>Bromobenzene</td>
<td>0.624</td>
<td>0.790</td>
<td>0.060</td>
<td>0.000</td>
</tr>
<tr>
<td>8</td>
<td>Iodobenzene</td>
<td>0.671</td>
<td>0.810</td>
<td>0.050</td>
<td>0.000</td>
</tr>
<tr>
<td>9</td>
<td>4-Chlorotoluene</td>
<td>0.679</td>
<td>0.670</td>
<td>0.080</td>
<td>0.000</td>
</tr>
<tr>
<td>10</td>
<td>Biphenyl</td>
<td>0.920</td>
<td>1.180</td>
<td>0.200</td>
<td>0.000</td>
</tr>
<tr>
<td>11</td>
<td>Naphthalene</td>
<td>0.753</td>
<td>0.700</td>
<td>0.150</td>
<td>0.000</td>
</tr>
<tr>
<td>12</td>
<td>1-Methylnaphthalene</td>
<td>0.851</td>
<td>0.660</td>
<td>0.160</td>
<td>0.000</td>
</tr>
<tr>
<td>13</td>
<td>Acetophenone</td>
<td>0.690</td>
<td>0.900</td>
<td>0.490</td>
<td>0.040</td>
</tr>
<tr>
<td>14</td>
<td>Benzonitrile</td>
<td>0.590</td>
<td>0.900</td>
<td>0.370</td>
<td>0.000</td>
</tr>
<tr>
<td>15</td>
<td>Nitrobenzene</td>
<td>0.631</td>
<td>1.010</td>
<td>0.300</td>
<td>0.000</td>
</tr>
<tr>
<td>16</td>
<td>Methyl benzoate</td>
<td>0.736</td>
<td>0.750</td>
<td>0.390</td>
<td>0.000</td>
</tr>
<tr>
<td>17</td>
<td>Ethyl benzoate</td>
<td>0.834</td>
<td>0.740</td>
<td>0.410</td>
<td>0.000</td>
</tr>
<tr>
<td>18</td>
<td>4-Chloroanisole</td>
<td>0.720</td>
<td>0.730</td>
<td>0.220</td>
<td>0.000</td>
</tr>
<tr>
<td>19</td>
<td>4-Nitrotoluene</td>
<td>0.729</td>
<td>0.970</td>
<td>0.310</td>
<td>0.000</td>
</tr>
<tr>
<td>20</td>
<td>4-Chloroacetophenone</td>
<td>0.780</td>
<td>0.900</td>
<td>0.450</td>
<td>0.060</td>
</tr>
<tr>
<td>21</td>
<td>Methyl 2-methylbenzoate</td>
<td>0.834</td>
<td>0.710</td>
<td>0.400</td>
<td>0.000</td>
</tr>
<tr>
<td>22</td>
<td>Phenyl acetate</td>
<td>0.736</td>
<td>1.140</td>
<td>0.520</td>
<td>0.000</td>
</tr>
<tr>
<td>23</td>
<td>3-Methylbenzyl alcohol</td>
<td>0.732</td>
<td>0.950</td>
<td>0.530</td>
<td>0.390</td>
</tr>
<tr>
<td>24</td>
<td>Phenethyl alcohol</td>
<td>0.732</td>
<td>0.970</td>
<td>0.550</td>
<td>0.330</td>
</tr>
<tr>
<td>25</td>
<td>Benzyl alcohol</td>
<td>0.634</td>
<td>0.990</td>
<td>0.520</td>
<td>0.390</td>
</tr>
<tr>
<td>26</td>
<td>Phenol</td>
<td>0.536</td>
<td>0.720</td>
<td>0.330</td>
<td>0.610</td>
</tr>
<tr>
<td>27</td>
<td>4-Methylphenol</td>
<td>0.634</td>
<td>0.680</td>
<td>0.340</td>
<td>0.580</td>
</tr>
<tr>
<td>28</td>
<td>4-Ethylphenol</td>
<td>0.732</td>
<td>0.660</td>
<td>0.350</td>
<td>0.580</td>
</tr>
<tr>
<td>29</td>
<td>4-Fluorophenol</td>
<td>0.562</td>
<td>0.730</td>
<td>0.280</td>
<td>0.650</td>
</tr>
<tr>
<td>30</td>
<td>4-Chlorophenol</td>
<td>0.626</td>
<td>0.720</td>
<td>0.230</td>
<td>0.670</td>
</tr>
<tr>
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<td>4-Bromophenol</td>
<td>0.669</td>
<td>0.790</td>
<td>0.230</td>
<td>0.670</td>
</tr>
<tr>
<td>32</td>
<td>4-Chloroaniline</td>
<td>0.653</td>
<td>0.730</td>
<td>0.400</td>
<td>0.310</td>
</tr>
<tr>
<td>33</td>
<td>3-Chlorophenol</td>
<td>0.626</td>
<td>0.770</td>
<td>0.230</td>
<td>0.690</td>
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<tr>
<td>34</td>
<td>3-Methylphenol</td>
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<td>0.680</td>
<td>0.340</td>
<td>0.580</td>
</tr>
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<td>35</td>
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<td>0.840</td>
<td>0.230</td>
<td>0.690</td>
</tr>
<tr>
<td>36</td>
<td>3,5-Dimethylphenol</td>
<td>0.732</td>
<td>0.640</td>
<td>0.350</td>
<td>0.560</td>
</tr>
</tbody>
</table>

\(^a\)Solvatochromatic parameter values from reference 24.
Table 4.4. Test solutes and their solvation descriptors\textsuperscript{a} for the solvation parameter model.

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<tr>
<th>#</th>
<th>Solute</th>
<th>$V_x$</th>
<th>$R_2$</th>
<th>$\pi^H_2$</th>
<th>$\sum \alpha^H_2$</th>
<th>$\sum \beta_0^0$</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Benzene</td>
<td>0.716</td>
<td>0.610</td>
<td>0.520</td>
<td>0.000</td>
<td>0.140</td>
</tr>
<tr>
<td>2</td>
<td>Toluene</td>
<td>0.857</td>
<td>0.601</td>
<td>0.520</td>
<td>0.000</td>
<td>0.140</td>
</tr>
<tr>
<td>3</td>
<td>Ethylbenzene</td>
<td>0.998</td>
<td>0.613</td>
<td>0.510</td>
<td>0.000</td>
<td>0.150</td>
</tr>
<tr>
<td>4</td>
<td>Propylbenzene</td>
<td>1.139</td>
<td>0.604</td>
<td>0.500</td>
<td>0.000</td>
<td>0.150</td>
</tr>
<tr>
<td>5</td>
<td>p-Xylene</td>
<td>0.998</td>
<td>0.613</td>
<td>0.520</td>
<td>0.000</td>
<td>0.160</td>
</tr>
<tr>
<td>6</td>
<td>Chlorobenzene</td>
<td>0.839</td>
<td>0.718</td>
<td>0.650</td>
<td>0.000</td>
<td>0.070</td>
</tr>
<tr>
<td>7</td>
<td>Bromobenzene</td>
<td>0.891</td>
<td>0.882</td>
<td>0.730</td>
<td>0.000</td>
<td>0.090</td>
</tr>
<tr>
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<td>Iodobenzene</td>
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<td>0.830</td>
<td>0.000</td>
<td>0.120</td>
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<tr>
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<td>4-Chlorotoluene</td>
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<td>0.670</td>
<td>0.000</td>
<td>0.070</td>
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<td>Biphenyl</td>
<td>1.324</td>
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<td>0.000</td>
<td>0.220</td>
</tr>
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<td>11</td>
<td>Naphthalene</td>
<td>1.085</td>
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<td>0.920</td>
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<td>0.480</td>
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<tr>
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<td>1.110</td>
<td>0.000</td>
<td>0.330</td>
</tr>
<tr>
<td>15</td>
<td>Nitrobenzene</td>
<td>0.891</td>
<td>0.871</td>
<td>1.110</td>
<td>0.000</td>
<td>0.280</td>
</tr>
<tr>
<td>16</td>
<td>Methyl benzoate</td>
<td>1.073</td>
<td>0.733</td>
<td>0.850</td>
<td>0.000</td>
<td>0.460</td>
</tr>
<tr>
<td>17</td>
<td>Ethyl benzoate</td>
<td>1.214</td>
<td>0.689</td>
<td>0.850</td>
<td>0.000</td>
<td>0.460</td>
</tr>
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<td>1.038</td>
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<tr>
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<td>0.870</td>
<td>1.110</td>
<td>0.000</td>
<td>0.280</td>
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<td>4-Chloroacetophenone</td>
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<td>0.440</td>
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<tr>
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<td>1.214</td>
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<td>0.000</td>
<td>0.430</td>
</tr>
<tr>
<td>22</td>
<td>Phenyl acetate</td>
<td>1.073</td>
<td>0.661</td>
<td>1.130</td>
<td>0.000</td>
<td>0.540</td>
</tr>
<tr>
<td>23</td>
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<td>0.815</td>
<td>0.900</td>
<td>0.330</td>
<td>0.590</td>
</tr>
<tr>
<td>24</td>
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<td>0.660</td>
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<td>0.803</td>
<td>0.870</td>
<td>0.330</td>
<td>0.560</td>
</tr>
<tr>
<td>26</td>
<td>Phenol</td>
<td>0.775</td>
<td>0.805</td>
<td>0.890</td>
<td>0.600</td>
<td>0.300</td>
</tr>
<tr>
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<td>4-Methylphenol</td>
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<td>0.820</td>
<td>0.870</td>
<td>0.570</td>
<td>0.310</td>
</tr>
<tr>
<td>28</td>
<td>4-Ethylphenol</td>
<td>1.057</td>
<td>0.800</td>
<td>0.900</td>
<td>0.550</td>
<td>0.360</td>
</tr>
<tr>
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<td>4-Fluorophenol</td>
<td>0.793</td>
<td>0.670</td>
<td>0.970</td>
<td>0.630</td>
<td>0.230</td>
</tr>
<tr>
<td>30</td>
<td>4-Chlorophenol</td>
<td>0.898</td>
<td>0.915</td>
<td>1.080</td>
<td>0.670</td>
<td>0.200</td>
</tr>
<tr>
<td>31</td>
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<td>0.950</td>
<td>1.080</td>
<td>1.170</td>
<td>0.670</td>
<td>0.200</td>
</tr>
<tr>
<td>32</td>
<td>4-Chloroaniline</td>
<td>0.939</td>
<td>1.060</td>
<td>1.130</td>
<td>0.300</td>
<td>0.310</td>
</tr>
<tr>
<td>33</td>
<td>3-Chlorophenol</td>
<td>0.898</td>
<td>0.909</td>
<td>1.060</td>
<td>0.690</td>
<td>0.150</td>
</tr>
<tr>
<td>34</td>
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<td>0.916</td>
<td>0.822</td>
<td>0.880</td>
<td>0.570</td>
<td>0.340</td>
</tr>
<tr>
<td>35</td>
<td>3-Bromophenol</td>
<td>0.950</td>
<td>1.060</td>
<td>1.150</td>
<td>0.700</td>
<td>0.160</td>
</tr>
<tr>
<td>36</td>
<td>3,5-Dimethylphenol</td>
<td>1.057</td>
<td>0.820</td>
<td>0.840</td>
<td>0.570</td>
<td>0.360</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Solute descriptors from reference 28.
and the intercept); statistically, between nine to eighteen solutes would be reasonable; however, to obtain a better fit 20 to 40 solutes should be used; 2) there should be an absence of significant cross-correlation among the descriptors, and the clustering of individual descriptor values should be avoided (the cross-correlation matrix for descriptors with respect to one another is listed in Tables 4.5 and 4.6); 3) Since the common detection method in MCE is absorption, the solutes should have a reasonable absorbance between 200 and 250 nm for a convenient detection; 4) solutes should be neutral at working pH. Solutes with similar descriptor values (e.g., $\Sigma \alpha_2^H$ values of a homologous series compounds) can diminish the accuracy in determination of the system constants.

Table 4.5. Cross-correlation matrix ($R^2$) for the solvatochromic model solute parameters.

<table>
<thead>
<tr>
<th></th>
<th>$V_x$</th>
<th>$\pi_2^H$</th>
<th>$\Sigma \beta_2^0$</th>
<th>$\Sigma \alpha_2^H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_x$</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\pi_2^H$</td>
<td>0.0704</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Sigma \beta_2^0$</td>
<td>0.0486</td>
<td>0.2908</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>$\Sigma \alpha_2^H$</td>
<td>0.0968</td>
<td>0.0019</td>
<td>0.0636</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Table 4.6. Cross-correlation matrix ($R^2$) for the solvation parameter model solute parameters.

<table>
<thead>
<tr>
<th></th>
<th>$V_x$</th>
<th>$R_2$</th>
<th>$\pi_2^H$</th>
<th>$\Sigma \alpha_2^H$</th>
<th>$\Sigma \beta_2^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_x$</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_2$</td>
<td>0.1317</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\pi_2^H$</td>
<td>0.0094</td>
<td>0.2448</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Sigma \alpha_2^H$</td>
<td>0.1254</td>
<td>0.0062</td>
<td>0.1382</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>$\Sigma \beta_2^0$</td>
<td>0.1017</td>
<td>0.0196</td>
<td>0.1162</td>
<td>0.0039</td>
<td>1.0000</td>
</tr>
</tbody>
</table>
The solutes in Tables 4.3 and 4.4 can be characterized as non-hydrogen bond donors (NHB) (solutes 1-12, \( \beta \leq 0.20 \)), hydrogen bond acceptors (HBA) (solutes 13-24, \( \beta \geq 0.22 \)), and hydrogen bond donors (HBD) (solutes 25-36, \( \alpha \geq \beta \)). The NHB solutes that include alkyl- and halo-substituted benzenes and polyaromatic hydrocarbons do not have any hydrogen-bonding functional groups; however, due to the aromatic ring(s), they are weak hydrogen bond acceptors (\( \beta \leq 0.2 \)). The HBA solutes possess hydrogen bond accepting functional groups on the aromatic ring, whereas, HBD solutes have hydrogen bond donating functional groups.

4.3.3. Linear Solvation Energy Relationship Results

Retention factors were determined for the 36 compounds used in this study, and the system constants were calculated by multiple linear regression using SAS software (SAS Institute Inc., Cary, NC). The statistical validity of the LSER models was evaluated through the F test, correlation coefficient (R) and, standard error in the estimate (S.E.). The differences in LSER coefficients indicate the variations in the types of interactions between pseudostationary phases and solutes. Solute interactions with the vesicular and micellar systems occur through a variety of mechanisms such as surface adsorption, coaggregation, or partitioning into the hydrophobic core of micelles or vesicles. Due to these different mechanisms, the LSER constants for different set of solutes (e.g., NHB, HBA, or HBD solutes) are not identical. In addition, microenvironments of solutes in pseudostationary phase vary significantly.

4.3.3.1. Solvatochromic Model

The LSER constants and the statistics for all of the pseudostationary phases using Solvatochromic model (Equation 4.1) are listed in Table 4.7. The regression constant, \( c \), is large and negative for all of the pseudostationary phases studied. This constant is related to the phase
ratio, $\Phi$, for the separation system. The phase ratio is related to the molar volume of surfactant, $v$, and to the concentration of aggregated surfactant according to the following expression:

$$\Phi = -\frac{v(S_{\text{tot}} - CAC)}{1 - v(S_{\text{tot}} - CAC)},$$

where $S_{\text{tot}}$ and $CAC$ are the total concentration and the critical aggregation concentration of the surfactant, respectively. At low surfactant concentrations the denominator of Equation 4.14 is close to unity.

Table 4.7. System constants for the six pseudostationary phases in MCE using solvatochromic model.

<table>
<thead>
<tr>
<th>System constants</th>
<th>Pseudostationary phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SDS</td>
</tr>
<tr>
<td><strong>c</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.886 (0.187)</td>
</tr>
<tr>
<td><strong>m</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.545 (0.251)</td>
</tr>
<tr>
<td><strong>s</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.336 (0.153)</td>
</tr>
<tr>
<td><strong>b</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-2.037 (0.176)</td>
</tr>
<tr>
<td><strong>a</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>-0.014</strong> (0.083)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistics</th>
<th>n</th>
<th>36</th>
<th>36</th>
<th>36</th>
<th>36</th>
<th>36</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>0.972</td>
<td>0.891</td>
<td>0.973</td>
<td>0.966</td>
<td>0.975</td>
<td>0.924</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.121</td>
<td>0.286</td>
<td>0.131</td>
<td>0.174</td>
<td>0.131</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>131</td>
<td>30</td>
<td>139</td>
<td>109</td>
<td>151</td>
<td>62</td>
</tr>
</tbody>
</table>

n= number of test solutes; R= correlation coefficient of linear regression; SE= standard error of the y estimate; $F$= Fischer $F$-statistic; underlined values are not statistically significant at the 95% confidence level. Numbers in parentheses indicate the standard deviation for each coefficient.

The $c$ values of the six pseudostationary phases suggest that the molar volumes of mono-SDUT, poly-SDUT, SDS/mono-SDUT, SDS/poly-SDUT, and mono-SDUT/poly-SDUT are...
approximately 2.7-, 2.1-, 2.7-, 2.0-, and 2.6-fold of the molar volume of SDS. This result seems reasonable because all surfactant systems, except SDS, form vesicles, which are larger in size relative to SDS micelles.

The m and b coefficients in Table 4.7 show that solute size and hydrogen bond accepting ability play the most important roles in MCE retention. The positive m values indicate that retention in MCE increases with the size of the solutes. Furthermore, the large positive m values show that the disfavored (endoergic) cavity formation term has the most important effect on retention. The coefficient m is directly related to the difference in the cohesive energies of the aqueous phase and the pseudostationary phase:

\[ m = M(\delta_{\text{aq}}^2 - \delta_{\text{psp}}^2), \]

where \( \delta \) is the Hildebrand solubility parameter and M is a proportionality factor. The \( \delta^2 \) is directly related to the cohesive energy. The subscripts aq and psp represent the aqueous phase and pseudostationary phase, respectively. According to Equation 4.15, the larger the m value, the smaller the cohesive energy of the pseudostationary phase. The positive sign of m indicates that solutes prefer to transfer from the more cohesive aqueous phase to the less cohesive pseudostationary phase. From the Table 4.7, \( m_{\text{SDS/mono-SDUT}} > m_{\text{SDS/poly-SDUT}} > m_{\text{mono-SDUT}} > m_{\text{poly-SDUT}} > m_{\text{SDS}} > m_{\text{mono-SDUT/poly-SDUT}} \); hence, \( \delta_{\text{mono-SDUT/poly-SDUT}}^2 > \delta_{\text{SDS}}^2 > \delta_{\text{poly-SDUT}}^2 > \delta_{\text{mono-SDUT}}^2 > \delta_{\text{SDS/poly-SDUT}}^2 > \delta_{\text{SDS/mono-SDUT}}^2 \). Therefore, mono-SDUT/poly-SDUT provides the most cohesive (more polar) environment and the SDS/mono-SDUT has least cohesive (more apolar) media. The large m values indicate that water is a very cohesive solvent and is not easy to create a cavity for the solute as compared to pseudostationary phase systems employed. The water molecules form a relatively firm hydrogen-bonding network, and hence the creation of any cavity within the aqueous phase takes place at the cost of a significant free energy.
The coefficient \( b \) is the second most important factor in the LSER solvatochromic model in pseudostationary phase systems used in this study. The \( b \) coefficients are all large and negative. The \( b \) coefficient is proportional to the difference in hydrogen bond donating ability (HBD acidity) of the pseudostationary phase and that of the aqueous phase:

\[
b = B(\alpha_{\text{psp}} - \alpha_{\text{aq}}),
\]

where \( \alpha \) and \( B \) are the solvatochromic parameter for measuring solvent HBD acidity and a proportionality factor, respectively. The subscript \( \text{psp} \) represents the pseudostationary phase and \( \text{aq} \) represents the aqueous phase. The negative signs of \( b \) coefficients in Table 4.7 indicate that all pseudostationary phases are less acidic than the aqueous buffer phase (i.e., \( \alpha_{\text{aq}} > \alpha_{\text{psp}} \)). The larger (or less negative) \( b \) coefficient is, the higher HBD strength of the pseudostationary phase. In other words, the pseudostationary phases with larger \( b \) values provide stronger HBD sites for solute interaction. The relative HBD strength of the pseudostationary phases used in this study can be ordered as SDS > mono-SDUT/poly-SDUT > SDS/poly-SDUT > poly-SDUT > SDS/mono-SDUT > mono-SDUT.

The coefficient \( a \) is one of the least important factor in the solvatochromic model in surfactant systems studied here. This coefficient represents the difference in hydrogen bond accepting ability (HBA basicity) of the pseudostationary phase and that of the aqueous phase:

\[
a = A(\beta_{\text{psp}} - \beta_{\text{aq}}),
\]

where \( \beta \) and is the solvatochromic parameter for hydrogen bond acceptor basicity and \( A \) is a proportionality factor. The subscript \( \text{psp} \) represents the pseudostationary phase and \( \text{aq} \) the aqueous phase. A positive coefficient \( a \) means that HBA ability of the pseudostationary phase is greater than the aqueous phase. Based on Table 4.7, pseudostationary phases can be ranked according to their HBA strength as following: mono-SDUT > mono-SDUT/poly-SDUT > poly-
SDUT > SDS/poly-SDUT > SDS/mono-SDUT > SDS. It should be noted that the coefficient \( a \) for SDS, SDS/mono-SDUT, and SDS/poly-SDUT systems is statistically insignificant. In other words, the smaller values for coefficient \( a \) for these three surfactant systems indicate that their hydrogen bond accepting strength is not much different from hydrogen bond accepting strength of aqueous phase. It is noteworthy that all of the surfactant systems holding sulfate headgroups have statistically insignificant coefficient \( a \), whereas surfactant systems with only carboxylate headgroups have larger coefficient \( a \) values. Previous studies have shown that the hydrogen bond accepting ability of the pseudostationary phase may be related to the pK\(_a\) of the surfactant headgroup (14, 16). This can be confirmed by comparing the coefficient \( a \) of mono-SDUT, poly-SDUT, or mono-SDUT/poly-SDUT surfactant system to SDS system. As a result of the carboxylate headgroup, mono-SDUT, poly-SDUT, and mono-SDUT/poly-SDUT surfactant systems display better hydrogen bond accepting characteristics than SDS, SDS/mono-SDUT, and SDS/poly-SDUT surfactant system all of which contain complete or partial sulfate headgroups. Among all surfactant systems mono-SDUT is the strongest hydrogen bond accepting surfactant, and SDS is the weakest hydrogen bond acceptor.

The difference in dipolarity/polarizability of the pseudostationary phase and the aqueous phase is represented by coefficient \( s \):

\[
s = S(\pi_{psp}^* - \pi_{aq}^*),
\]

where \( \pi^* \) and \( S \) are the solvatochromic parameter for dipolarity/polarizability and a proportionality factor, respectively. The subscript psp represents the pseudostationary phase and aq the aqueous phase. The negative signs of coefficient \( s \) mean that the solutes experience a microenvironment that has less dipolar/polarizable characteristics than the aqueous phase. On the contrary, the positive \( s \) values indicate that the solutes find a more dipolar microenvironment.
in the pseudostationary phases than in the aqueous phase. As shown in Table 4.7, the s values are negative for SDS and mono-SDUT/poly-SDUT systems, whereas are positive for mono-SDUT, poly-SDUT, SDS/mono-SDUT, and SDS/poly-SDUT systems. However, it should be mentioned that except SDS systems the s values for all systems are not statistically significant at the 95% confidence level.

Putting the descriptors (Table 4.7) in Equation 4.1, the following fits are obtained for each MCE system:

1.0 % (w/v) SDS (34.7 mM):

\[
\log k' = -1.886(\pm 0.187) + 4.545(\pm 0.251)V_i - 0.336(\pm 0.153)\pi^* - 2.037(\pm 0.176)\beta - 0.014(\pm 0.083)\alpha .
\]

1.0 % (w/v) mono-SDUT (19 mM):

\[
\log k' = -2.875(\pm 0.440) + 4.791(\pm 0.591)V_i + 0.236(\pm 0.360)\pi^* - 3.436(\pm 0.415)\beta + 0.740(\pm 0.195)\alpha .
\]

1.0 % (w/v) poly-SDUT (19 mM, equivalent monomer concentration, emc):

\[
\log k' = -2.692(\pm 0.201) + 4.585(\pm 0.270)V_i + 0.208(\pm 0.164)\pi^* - 3.113(\pm 0.190)\beta + 0.228(\pm 0.089)\alpha .
\]

1.0 % (w/v) SDS/mono-SDUT (17.5 mM SDS / 9.5 mM mono-SDUT):

\[
\log k' = -2.786(\pm 0.268) + 5.597(\pm 0.360)V_i + 0.096(\pm 0.219)\pi^* - 3.228(\pm 0.253)\beta + 0.039(\pm 0.118)\alpha .
\]

1.0 % (w/v) SDS/poly-SDUT (17.5 mM SDS / 9.5 mM poly-SDUT, emc):

\[
\log k' = -2.631(\pm 0.202) + 5.094(\pm 0.271)V_i + 0.027(\pm 0.165)\pi^* - 2.801(\pm 0.190)\beta + 0.077(\pm 0.089)\alpha .
\]

1.0 % (w/v) mono-SDUT/poly-SDUT (9.5 mM mono-SDUT / 9.5 mM poly-SDUT, emc):

\[
\log k' = -2.684(\pm 0.247) + 4.264(\pm 0.331)V_i - 0.087(\pm 0.202)\pi^* - 2.368(\pm 0.233)\beta + 0.491(\pm 0.089)\alpha .
\]

Calculated (or predicted) log k' values of 36 test solutes were computed for each pseudostationary phase system using Equations 4.19 through 4.24. To demonstrate the quality of the fits, the experimental log k' versus the calculated log k' values are plotted in Figure 4.4.
Figure 4.4a. Calculated versus experimental log $k'$ values for (A) SDS, (B) mono-SDUT using solvatochromic model and parameters. Plots (insets) on the right of each figure represent calculated versus experimental log $k'$ values of NHB (A1, B1), HBA (A2, B2), and HBD (A3, B3) solutes. (Fig. con’d.).
Figure 4.4b. (C) poly-SDUT, (D) SDS/mono-SDUT using solvatochromic model and parameters. Plots (insets) on the right of each figure represent calculated versus experimental log k' values of NHB (C1, D1), HBA (C2, D2), and HBD (C3, D3) solutes. (Fig. con’d.).

\[ y = 0.9696x - 0.0182 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]

\[ y = 0.9337x + 0.0154 \quad R^2 = 0.9477 \]
Figure 4.4c. (E) SDS/poly-SDUT, (F) mono-SDUT/poly-SDUT using solvatochromic model and parameters. Plots (insets) on the right of each figure represent calculated versus experimental log k’ values of NHB (E1, F1), HBA (E2, F2), and HBD (E3, F3) solutes.
Experimental log k' versus calculated log k' of 36 solutes for surfactant systems resulted in regression fits with correlation coefficient ($R^2$) ranging from 0.7936 (mono-SDUT) to 0.9510 (SDS/poly-SDUT) (Figure 4.4 A-F). However, when the calculated log k' values of each subset (i.e. NHB, HBA, and HBD) are graphically compared with their experimental log k' values (insets in Figure 4.4 A-F), the NHB solutes gave the best fits for all surfactant systems (except for mono-SDUT) with $R^2$ ranging from 0.9687 (SDS/mono-SDUT) to 0.9908 (SDS/poly-SDUT). In addition, the NHB solutes are more retained (i.e., larger experimental log k' values) than the HBA and HBD solutes for all surfactant system (notice the inset plots in Figure 4.4 A-F). This is due largely to the solute size ($V_1$) which is the most important solute descriptor governing the retention.

The poor correlations between experimental log k' and calculated log k' values are caused primarily by a few outlying solutes. The outliers for each surfactant system were determined by: 1) calculating residual values (experimental log k' minus calculated log k') for each solute, 2) normalizing residual values (dividing residual value by the standard deviation of the residual) for each pseudostationary system, and 3) plotting normalized residual values against solute number (Figure 4.5). Based on Figure 4.5, outliers for all pseudostationary phases are listed in Table 4.8. Note that solutes 22 (phenyl acetate, HBA solute) and 32 (4-chloroaniline, HBD solute) are the major outliers for most of the surfactant systems. The best correlation between experimental log k' and calculated log k' values are obtained when the normalized residuals are zero or close to zero. However, normalized residual range of +2 to −2 is reasonable for statistically sound correlations. As seen in Figure 4.5, normalized residuals are relatively low for NHB solutes, resulting in higher correlation coefficients, as opposed to HBA and HBD solutes.
Table 4.8. Outlier solutes using solvatochromic model for each pseudostationary phase.

<table>
<thead>
<tr>
<th>Pseudostationary phases ▼</th>
<th>Solutes ►</th>
<th>NHB</th>
<th>HBA</th>
<th>HBD</th>
<th>Total number of outliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>-</td>
<td>22</td>
<td>32</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>Mono-SDUT</td>
<td>2, 3, 4</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>Poly-SDUT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>SDS/mono-SDUT</td>
<td>10</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>SDS/poly-SDUT</td>
<td>-</td>
<td>22</td>
<td>32</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Mono-SDUT/poly-SDUT</td>
<td>13, 18, 20</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

2) Toluene, 3) ethylbenzene, 4) propylbenzene, 10) biphenyl, 13) acetophenone, 18) 4-chloroanisole, 20) 4-chloroacetophenone, 22) phenyl acetate, 32) 4-chloroaniline

Figure 4.5. Normalized residuals versus solute number for the six pseudostationary phases using solvatochromic model. Solute numbers are as listed in Table 4.3.
The LSER system constants coefficients for all surfactant systems were recalculated by multiple linear regression using solvatochromic model after excluding the outliers. The new coefficients are listed in Table 4.9.

Table 4.9. Recalculated System constants for the six pseudostationary phases in MCE using sovatochromic model.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>SDS</th>
<th>mono-SDUT</th>
<th>poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>System constants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>-2.008</td>
<td>-2.485</td>
<td>-2.764</td>
<td>-2.281</td>
<td>-2.746</td>
<td>-2.794</td>
</tr>
<tr>
<td></td>
<td>(0.114)</td>
<td>(0.285)</td>
<td>(0.179)</td>
<td>(0.277)</td>
<td>(0.158)</td>
<td>(0.162)</td>
</tr>
<tr>
<td>m</td>
<td>4.508</td>
<td>5.086</td>
<td>4.646</td>
<td>5.003</td>
<td>5.079</td>
<td>4.339</td>
</tr>
<tr>
<td></td>
<td>(0.152)</td>
<td>(0.368)</td>
<td>(0.239)</td>
<td>(0.327)</td>
<td>(0.211)</td>
<td>(0.218)</td>
</tr>
<tr>
<td>s</td>
<td>-0.131</td>
<td>-0.335</td>
<td>0.269</td>
<td>-0.184</td>
<td>0.205</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>(0.096)</td>
<td>(0.237)</td>
<td>(0.146)</td>
<td>(0.224)</td>
<td>(0.133)</td>
<td>(0.132)</td>
</tr>
<tr>
<td></td>
<td>(0.110)</td>
<td>(0.254)</td>
<td>(0.171)</td>
<td>(0.235)</td>
<td>(0.152)</td>
<td>(0.163)</td>
</tr>
<tr>
<td>a</td>
<td>-0.066</td>
<td>0.575</td>
<td>0.237</td>
<td>-0.075</td>
<td>0.037</td>
<td>0.605</td>
</tr>
<tr>
<td></td>
<td>(0.051)</td>
<td>(0.121)</td>
<td>(0.078)</td>
<td>(0.097)</td>
<td>(0.070)</td>
<td>(0.073)</td>
</tr>
<tr>
<td><strong>Statistics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>R</td>
<td>0.9896</td>
<td>0.9663</td>
<td>0.9799</td>
<td>0.9709</td>
<td>0.9854</td>
<td>0.9781</td>
</tr>
<tr>
<td>SE</td>
<td>0.073</td>
<td>0.171</td>
<td>0.115</td>
<td>0.137</td>
<td>0.101</td>
<td>0.104</td>
</tr>
<tr>
<td>F</td>
<td>342</td>
<td>95</td>
<td>181</td>
<td>119</td>
<td>243</td>
<td>149</td>
</tr>
</tbody>
</table>

n= number of test solutes; R= correlation coefficient of linear regression; SE= standard error of the y estimate; F= F-statistic; underlined values are not statistically significant at the 95% confidence level. Numbers in parentheses indicate the standard deviation for each coefficient.

Using the new system constants, the following fits are obtained for the surfactant systems:

1.0 % (w/v) SDS (34.7 mM):

$$\log k' = -2.008(\pm0.114) + 4.508(\pm0.152)V_1 - 0.131(\pm0.096)\pi' - 2.011(\pm0.110)\beta - 0.066(\pm0.051)\alpha.$$  

4.25

1.0 % (w/v) mono-SDUT (19 mM):
\[ \log k' = -2.485(\pm 0.285) + 5.086(\pm 0.368)V_i - 0.335(\pm 0.237)\pi^* - 3.650(\pm 0.254)\beta + 0.575(\pm 0.121)\alpha. \]  \hspace{1cm} \text{(4.26)}

1.0 \% (w/v) poly-SDUT (19 mM, emc):

\[ \log k' = -2.764(\pm 0.179) + 4.646(\pm 0.239)V_i + 0.269(\pm 0.146)\pi^* - 3.218(\pm 0.171)\beta + 0.237(\pm 0.078)\alpha. \]  \hspace{1cm} \text{(4.27)}

1.0 \% (w/v) SDS/mono-SDUT (17.5 mM SDS / 9.5 mM mono-SDUT):

\[ \log k' = -2.281(\pm 0.277) + 5.003(\pm 0.327)V_i - 0.184(\pm 0.224)\pi^* - 2.741(\pm 0.235)\beta - 0.075(\pm 0.097)\alpha. \]  \hspace{1cm} \text{(4.28)}

1.0 \% (w/v) SDS/poly-SDUT (17.5 mM SDS / 9.5 mM poly-SDUT, emc):

\[ \log k' = -2.746(\pm 0.158) + 5.079(\pm 0.211)V_i + 0.205(\pm 0.133)\pi^* - 2.804(\pm 0.152)\beta + 0.037(\pm 0.070)\alpha. \]  \hspace{1cm} \text{(4.29)}

1.0 \% (w/v) mono-SDUT/poly-SDUT (9.5 mM mono-SDUT / 9.5 mM poly-SDUT, emc):

\[ \log k' = -2.794(\pm 0.162) + 4.339(\pm 0.218)V_i + 0.024(\pm 0.132)\pi^* - 2.711(\pm 0.163)\beta + 0.605(\pm 0.073)\alpha. \]  \hspace{1cm} \text{(4.30)}

Statistically, the recalculated fits (Equations 4.25 through 4.30) are noticeably better than the original fits (Equations 4.19 through 4.24) from a chemical sense. Removing the outliers significantly improved the fits resulting in relatively small standard deviations in the system constants and in the y estimate; and provided higher R^2 and F values. However, some surfactant systems (e.g., mono-SDUT, SDS/mono-SDUT, and mono-SDUT/poly-SDUT) yet contain somewhat larger standard deviations in the system constants due probably to too many low experimental log k' values that are likely to produce large experimental errors.

The log k' values were recalculated using Equations 4.25 through 4.30 and obtained calculated log k' values were then plotted against experimental log k' values. The R^2, slope, and the intercept values of the new correlation lines for all pseudostationary phases are listed in Table 4.10. Comparing the R^2, slope, and the intercept values listed in Table 4.10 with those in Figure 4.4 A-F clearly show that eliminating a few outliers improved the correlation significantly. For instance, eliminating phenyl acetate and 4-chloroaniline (solute 22 and 32 in Table 4.3) from SDS system improved the correlation coefficient of calculated (or predicted) log
k' versus experimental log k' plot for 34 solutes from 0.9441 to 0.9793. Similar improvements are seen in other surfactant systems as well as in subset solutes (NHB, HBA, and HBD).

The system constant ratios obtained from solvatochromic model are listed in Table 4.11.

Table 4.10. The correlation coefficient, slope, and the intercept of the calculated log k' versus experimental log k' plot for each surfactant system using Equations 4.25 to 4.30 (solvatochromic model).

<table>
<thead>
<tr>
<th>Pseudostationary phase</th>
<th>Solutes</th>
<th>R²</th>
<th>Slope</th>
<th>Intercept</th>
<th>n</th>
<th>Solutes Excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>All</td>
<td>0.9793</td>
<td>0.9793</td>
<td>0.0086</td>
<td>34</td>
<td>22, 32</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9920</td>
<td>0.9633</td>
<td>0.0347</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9197</td>
<td>0.9652</td>
<td>0.0078</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9795</td>
<td>0.9471</td>
<td>0.0032</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Mono-SDUT</td>
<td>All</td>
<td>0.9337</td>
<td>0.9337</td>
<td>-0.0113</td>
<td>32</td>
<td>2, 3, 4, 32</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9039</td>
<td>0.9204</td>
<td>0.0955</td>
<td>9</td>
<td>2, 3, 4</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9361</td>
<td>0.8105</td>
<td>-0.1408</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9262</td>
<td>0.8338</td>
<td>-0.0462</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Poly-SDUT</td>
<td>All</td>
<td>0.9601</td>
<td>0.9601</td>
<td>-0.0077</td>
<td>35</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9793</td>
<td>0.8927</td>
<td>0.0145</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.8471</td>
<td>0.9997</td>
<td>0.0126</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9588</td>
<td>1.0665</td>
<td>0.0339</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>SDS/mono-SDUT</td>
<td>All</td>
<td>0.9426</td>
<td>0.9426</td>
<td>0.0112</td>
<td>34</td>
<td>10, 22</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9739</td>
<td>0.8741</td>
<td>0.1019</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9313</td>
<td>1.1049</td>
<td>0.0304</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.8397</td>
<td>0.9886</td>
<td>-0.0128</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>SDS/poly-SDUT</td>
<td>All</td>
<td>0.9710</td>
<td>0.9711</td>
<td>0.0040</td>
<td>34</td>
<td>22, 32</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9892</td>
<td>0.9036</td>
<td>0.0384</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.8983</td>
<td>1.0738</td>
<td>0.0255</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9787</td>
<td>1.1129</td>
<td>0.0170</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Mono-SDUT/Poly-SDUT</td>
<td>All</td>
<td>0.9566</td>
<td>0.9566</td>
<td>-0.0173</td>
<td>32</td>
<td>13, 18, 20, 32</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9800</td>
<td>1.0110</td>
<td>0.0297</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9018</td>
<td>0.8099</td>
<td>-0.1675</td>
<td>9</td>
<td>13, 18, 20</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9627</td>
<td>0.8503</td>
<td>-0.0685</td>
<td>11</td>
<td>32</td>
</tr>
</tbody>
</table>

2) Toluene, 3) ethylbenzene, 4) propylbenzene, 13) acetophenone, 18) 4-chloroanisole, 20) 4-chloroacetophenone, 22) phenyl acetate, 32) 4-chloroaniline
Table 4.11. Ratio of the system constants obtained using solvatochromic model for six pseudostationary phases studied.

<table>
<thead>
<tr>
<th></th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>c/m</td>
<td>S₁</td>
<td>-0.415</td>
<td>-0.600</td>
<td>-0.587</td>
<td>-0.498</td>
<td>-0.517</td>
</tr>
<tr>
<td></td>
<td>S₂</td>
<td>-0.445</td>
<td>-0.489</td>
<td>-0.595</td>
<td>-0.456</td>
<td>-0.541</td>
</tr>
<tr>
<td>∆S</td>
<td></td>
<td>0.030</td>
<td>-0.111</td>
<td>0.008</td>
<td>-0.042</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>S₁</td>
<td>-0.074</td>
<td>0.049</td>
<td>0.045</td>
<td>0.017</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>S₂</td>
<td>-0.029</td>
<td>-0.066</td>
<td>0.058</td>
<td>-0.037</td>
<td>0.040</td>
</tr>
<tr>
<td>∆S</td>
<td></td>
<td>-0.045</td>
<td>0.115</td>
<td>-0.013</td>
<td>0.054</td>
<td>-0.035</td>
</tr>
<tr>
<td>s/m</td>
<td>S₁</td>
<td>-0.448</td>
<td>-0.717</td>
<td>-0.679</td>
<td>-0.577</td>
<td>-0.550</td>
</tr>
<tr>
<td></td>
<td>S₂</td>
<td>-0.446</td>
<td>-0.718</td>
<td>-0.693</td>
<td>-0.548</td>
<td>-0.552</td>
</tr>
<tr>
<td>∆S</td>
<td></td>
<td>-0.002</td>
<td>0.001</td>
<td>0.014</td>
<td>-0.029</td>
<td>0.002</td>
</tr>
<tr>
<td>b/m</td>
<td>S₁</td>
<td>-0.003</td>
<td>0.155</td>
<td>0.050</td>
<td>0.007</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>S₂</td>
<td>-0.015</td>
<td>0.113</td>
<td>0.051</td>
<td>-0.015</td>
<td>0.007</td>
</tr>
<tr>
<td>∆S</td>
<td></td>
<td>0.012</td>
<td>0.042</td>
<td>-0.001</td>
<td>0.022</td>
<td>0.008</td>
</tr>
<tr>
<td>a/m</td>
<td>S₁</td>
<td>-0.003</td>
<td>0.155</td>
<td>0.050</td>
<td>0.007</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>S₂</td>
<td>-0.015</td>
<td>0.113</td>
<td>0.051</td>
<td>-0.015</td>
<td>0.007</td>
</tr>
<tr>
<td>∆S</td>
<td></td>
<td>0.012</td>
<td>0.042</td>
<td>-0.001</td>
<td>0.022</td>
<td>0.008</td>
</tr>
</tbody>
</table>

S₁ = System constant ratios obtained with 36 analytes, S₂ = system constant ratios obtained after outlier solute(s) excluded, ∆S = S₁ - S₂

The ratios of the system coefficients (i.e., c, s, b, and a) to the coefficient m are used to compare the relative magnitudes of each system coefficients, and hence individual interactions, between different pseudostationary phases (3, 42, 43). While comparing surfactant systems, it is safer to compare S₁ values rather than S₂ values, because S₁ values are obtained from identical set of solutes. Table 4.11 shows that each surfactant system has a distinct interaction with the solutes. Despite differences between the pseudostationary phases, a few similarities are evident. Specifically, the s/m values of mono-SDUT (0.049) and poly-SDUT (0.045) are evident that these two surfactants have similar polarity/polarizability characteristics. Similarly, b/m values indicate that SDS/poly-SDUT and mono-SDUT/poly-SDUT systems have comparable hydrogen-bond acidities. The ∆S values in Table 4.11 show that excluding some solutes from the solute set (e.g., outliers) has some effect on system coefficients. For example, the s/m value in mono-
SDUT system is 0.049 for 36 solutes; however, excluding four solutes from the solute set (i.e., toluene, ethylbenzene, propylbenzene, and 4-chloroaniline) resulted in a decrease in s/m value to –0.066.

4.3.3.2. Solvation Parameter Model

As discussed earlier, the major difference between solvatochromic LSER model and solvation parameter model is that the latter contains a new rR$_2$ term representing the solute’s excess molar refraction (Equation 4.2). Moreover, as seen in Tables 4.3 and 4.4, there are numerical differences in the values of the solute descriptors. Therefore, exact agreement between the two models cannot be expected; however, radical inconsistencies in overall trends predicted by both models are uncommon. Nevertheless, the solvation parameter model has been favored over solvatochromic model by several researchers for several reasons (3, 9, 15). First, the solute descriptors in solvation parameter model are related to free energy processes. Second, the solute descriptor values are published for a large number of solutes. Finally, solvation parameter model has been found to provide both statistically and chemically sound results. The LSER constants and the statistics for all of the surfactant systems using solvation parameter model (Equation 4.2) are listed in Table 4.12.

The comparison of the coefficients for each surfactant system reveals that the m and b have the largest absolute values among all coefficients for all systems presented here. The large positive values (m >> 0) of the m coefficient show that the cavity contribution is more favorable for solutes to partition to the pseudostationary phases than to aqueous phase. In other words, it requires less energy to create a cavity in the pseudostationary phase to host a solute than in aqueous phase. The large m values suggest also that retention is primarily influenced by the size of solutes. Thus, solutes prefer to transfer from the aqueous phase to the surfactant phase. From
Table 4.12, the surfactant systems can be ordered according to their coefficient m values:

SDS/mono-SDUT > SDS/poly-SDUT > mono-SDUT > SDS > mono-SDUT/poly-SDUT > poly-SDUT.

Table 4.12. System constants for the six pseudostationary phases in MCE using solvation parameter model.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>SDS</th>
<th>mono-SDUT</th>
<th>poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>-2.011 (0.161)</td>
<td>-2.974 (0.397)</td>
<td>-2.515 (0.127)</td>
<td>-2.599 (0.207)</td>
<td>-2.529 (0.128)</td>
<td>-2.875 (0.199)</td>
</tr>
<tr>
<td>m</td>
<td>2.987 (0.181)</td>
<td>3.029 (0.448)</td>
<td>2.750 (0.144)</td>
<td>3.517 (0.233)</td>
<td>3.194 (0.145)</td>
<td>2.838 (0.224)</td>
</tr>
<tr>
<td>r</td>
<td>0.403 (0.127)</td>
<td><strong>0.497</strong> (0.313)</td>
<td>0.802 (0.101)</td>
<td>0.788 (0.163)</td>
<td>0.693 (0.101)</td>
<td><strong>0.281</strong> (0.157)</td>
</tr>
<tr>
<td>s</td>
<td>-0.403 (0.130)</td>
<td><strong>0.125</strong> (0.321)</td>
<td>-0.511 (0.103)</td>
<td>-0.692 (0.167)</td>
<td>-0.546 (0.104)</td>
<td><strong>0.013</strong> (0.161)</td>
</tr>
<tr>
<td>a</td>
<td><strong>-0.055</strong> (0.076)</td>
<td>0.412 (0.187)</td>
<td><strong>0.020</strong> (0.060)</td>
<td><strong>-0.104</strong> (0.097)</td>
<td><strong>-0.057</strong> (0.061)</td>
<td>0.326 (0.094)</td>
</tr>
<tr>
<td>b</td>
<td>-1.845 (0.152)</td>
<td>-2.989 (0.377)</td>
<td>-2.296 (0.121)</td>
<td>-2.431 (0.196)</td>
<td>-2.163 (0.122)</td>
<td>-2.238 (0.189)</td>
</tr>
</tbody>
</table>

System constants

- **c** - c parameter
- **m** - m parameter
- **r** - r parameter
- **s** - s parameter
- **a** - a parameter
- **b** - b parameter

Statistics

- **n** - number of test solutes
- **R** - correlation coefficient of linear regression
- **SE** - standard error of the y estimate
- **F** - F-statistic

The large negative values (b << 0) of coefficient b indicate that the hydrogen-bond-acidity of all pseudostationary phases is much lower than that of the aqueous buffer solution. The mono-SDUT has the least acidity whereas SDS has the most acidity among all the systems. The poly-SDUT, SDS/mono-SDUT, SDS/poly-SDUT, and mono-SDUT/poly-SDUT systems

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have an acidity intermediate between SDS and mono-SDUT systems. The acidity of all systems can be ranked from the most acidic to the least as following: SDS > SDS/poly-SDUT > mono-SDUT/poly-SDUT > poly-SDUT > SDS/mono-SDUT > mono-SDUT.

The coefficient a is small as compared to m and b coefficients. This means that solute’s hydrogen-bond-donating acidity has a small or no effect on retention. Despite being statistically insignificant, the negative coefficient a values of SDS, SDS/mono-SDUT, and SDS/poly-SDUT systems mean that the aqueous buffer phase is more basic than these pseudostationary phases. As seen in Table 4.12, mono-SDUT and mono-SDUT/poly-SDUT systems have the largest positive coefficient a values, thus they are the most basic than all other surfactant systems studied and the aqueous phase. It is worth noting that only these two systems (i.e., mono-SDUT, and mono-SDUT/poly-SDUT) have statistically significant coefficient a values. Comparing the coefficient a values will provide the following order of acidity of all surfactant systems: SDS/mono-SDUT > SDS/poly-SDUT > SDS > poly-SDUT > mono-SDUT/poly-SDUT > mono-SDUT.

All pseudostationary phases except mono-SDUT and mono-SDUT/poly-SDUT have negative s coefficient values that are statistically significant (Table 4.12). Since the coefficient s is related to the difference in the $\pi_2^H$ of the pseudostationary and aqueous buffer phases (Equation 4.18), the negative sign of this coefficient shows that the aqueous buffer phase is more dipolar than the surfactant systems except mono-SDUT and mono-SDUT/poly-SDUT, which have small positive coefficient s indicating that these two surfactant systems are slightly more dipolar than aqueous phase. An increase in the solute dipolarity/polarizability decreases retention slightly with the pseudostationary phases that have negative coefficient s.
As discussed earlier, the \( r \) coefficient represents the ability of the surfactant system to interact with \( n \)- and \( \pi \)-electron pairs of solute and hence become polarized. All pseudostationary phases have positive coefficient \( r \) (Table 4.12). The large positive values of this coefficient indicate that the surfactant system can interact with or become polarized by adjoining solute’s \( n \)- and \( \pi \)-electrons more easily. According to Table 4.12, the polarizability ability of pseudostationary phases is ranked as: poly-SDUT > SDS/mono-SDUT > SDS/poly-SDUT > SDS. The coefficient \( r \) is statistically insignificant for mono-SDUT and mono-SDUT/poly-SDUT systems.

The coefficients in Table 4.12 show that the surfactant systems with large absolute values of coefficients \( a \) and \( b \) (e.g., mono-SDUT) would be very convenient to separate mixtures of solutes with dissimilar hydrogen-bond acidity. Among all pseudostationary phases, SDS/mono-SDUT, which has a relatively larger absolute coefficient \( s \) value, would be comparatively better system of choice to separate compounds by their polarity. Similarly, poly-SDUT would be convenient system to separate solutes by their polarizability (coefficient \( r \)). All surfactant systems show a similar strength to separate compounds according to their size, because all systems have similar coefficient \( m \) values.

Placing the descriptors obtained with solvation parameter model (Table 4.12) in Equation 4.2, the following fits are attained for each MCE system:

1.0 \( \% \) (w/v) SDS (34.7 mM):

\[
\log k^* = -2.011(\pm 0.161) + 2.987(\pm 0.181)V_x + 0.403(\pm 0.127)R_x^2
\]

\[-0.403(\pm 0.130)\pi_x^{11} - 0.055(\pm 0.076)\sum \alpha_x^{11} - 1.845(\pm 0.152)\sum \beta_x^{11}.
\]

1.0 \( \% \) (w/v) mono-SDUT (19 mM):
log \( k' = -2.974(\pm 0.397) + 3.029(\pm 0.448)V_x + 0.497(\pm 0.313)R_x^2 \)
\[ +0.125(\pm 0.321)\pi_x^{\mu} + 0.412(\pm 0.187)\sum \alpha_x^{\mu} - 2.989(\pm 0.377)\sum \beta_x^\mu. \]

1.0 % (w/v) poly-SDUT (19 mM, emc):

\[
log \( k' = -2.515(\pm 0.127) + 2.750(\pm 0.144)V_x + 0.802(\pm 0.101)R_x^2 \)
\[ -0.511(\pm 0.103)\pi_x^{\mu} + 0.020(\pm 0.060)\sum \alpha_x^{\mu} - 2.296(\pm 0.121)\sum \beta_x^\mu. \]

1.0 % (w/v) SDS/mono-SDUT (17.5 mM SDS / 9.5 mM mono-SDUT):

\[
log \( k' = -2.599(\pm 0.207) + 3.517(\pm 0.233)V_x + 0.788(\pm 0.163)R_x^2 \)
\[ -0.692(\pm 0.167)\pi_x^{\mu} - 0.104(\pm 0.097)\sum \alpha_x^{\mu} - 2.431(\pm 0.196)\sum \beta_x^\mu. \]

1.0 % (w/v) SDS/poly-SDUT (17.5 mM SDS / 9.5 mM poly-SDUT, emc):

\[
log \( k' = -2.529(\pm 0.128) + 3.194(\pm 0.145)V_x + 0.693(\pm 0.101)R_x^2 \)
\[ -0.546(\pm 0.104)\pi_x^{\mu} - 0.057(\pm 0.061)\sum \alpha_x^{\mu} - 2.163(\pm 0.122)\sum \beta_x^\mu. \]

1.0 % (w/v) mono-SDUT/poly-SDUT (9.5 mM mono-SDUT / 9.5 mM poly-SDUT, emc):

\[
log \( k' = -2.875(\pm 0.199) + 2.838(\pm 0.224)V_x + 0.281(\pm 0.157)R_x^2 \)
\[ +0.013(\pm 0.161)\pi_x^{\mu} + 0.326(\pm 0.094)\sum \alpha_x^{\mu} - 2.238(\pm 0.189)\sum \beta_x^\mu. \]

Using Equations 5.31 through 5.36, the predicted (or calculated) log \( k' \) values of 36 test solutes were computed for each pseudostationary phase system. The experimental log \( k' \) versus the calculated log \( k' \) values are plotted in Figure 4.6. The correlation coefficients (\( R^2 \)) in Figure 4.6 A-F range from 0.8321 (mono-SDUT) to 0.9806 (SDS/poly-SDUT). These values are better than those obtained previously with solvatochromic model (Figure 4.4 A-F). As seen in insets of Figure 4.4 A-F, when the calculated log \( k' \) values of each subset solutes (i.e., NHB, HBA, and HBD) were plotted against their experimental log \( k' \) values, the subset solutes gave relatively better correlations (except mono-SDUT system) as compared to the whole set of 36 solutes.
Figure 4.6a. Calculated versus experimental log k' values for (A) SDS, (B) mono-SDUT using solvation parameter model and parameters. Plots (insets) on the right of each figure represent calculated versus experimental log k' values of NHB (A1, B1), HBA (A2, B2), and HBD (A3, B3) solutes. (Fig. con’d.).
Figure 4.6b.  (C) SDS/mono-SDUT, (D) SDS/poly-SDUT using solvation parameter model and parameters. Plots (insets) on the right of each figure represent calculated versus experimental log k' values of NHB (C1, D1), HBA (C2, D2), and HBD (C3, D3) solutes. (Fig. con’d.).

\[ y = 0.9648x + 0.0035 \quad R^2 = 0.9867 \]

\[ y = 1.0017x - 0.0032 \quad R^2 = 0.9867 \]
Figure 4.6c. (E) SDS/poly-SDUT, (F) mono-SDUT/poly-SDUT using solvation parameter model and parameters. Plots (insets) on the right of each figure represent calculated versus experimental log k' values of NHB (E1, F1), HBA (E2, F2), and HBD (E3, F3) solutes.
More specifically, the $R^2$ values for NHB subset solutes (Figure 4.6. A1, B1, C1, D1, E1, and F1) range from 0.6799 for mono-SDUT system to 0.9885 for SDS system. The major reason behind the lower $R^2$ value for mono-SDUT (Figure 4.6 B1) is that there are several NHB outlying solutes in this particular surfactant system resulting a smaller $R^2$.

As can be seen in Figure 4.6 A-F, each plot contains one or more outliers that lead to poor correlations between experimental log $k'$ and calculated log $k'$ values. The outliers for each surfactant system are determined through Figure 4.7. Based on Figure 4.7, outliers for all pseudostationary phases are listed in Table 4.13. Note that five out of six surfactant systems have at least one HBA solute outlier, while there is only one system (SDS/mono-SDUT) that has an HBD outlier (4-methylphenol, solute 27). The NHB solutes are outliers in only two
pseudostationary phases, that is, mono-SDUT (solute 3, 4, and 5) and SDS/mono-SDUT (solute 10). The total number of outliers in each surfactant is presented in the last column of Table 4.13.

**Table 4.13. Outlier solutes using solvation parameter model for each pseudostationary phase.**

<table>
<thead>
<tr>
<th>Pseudostationary Phases ▼</th>
<th>Solutes ►</th>
<th>NHB</th>
<th>HBA</th>
<th>HBD</th>
<th>Total number of outliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>-</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Mono-SDUT</td>
<td>3, 4, 5</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Poly-SDUT</td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>SDS/mono-SDUT</td>
<td>10</td>
<td>-</td>
<td>27</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>SDS/poly-SDUT</td>
<td>-</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Mono-SDUT/poly-SDUT</td>
<td></td>
<td>15, 18, 22</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

3) Ethylbenzene, 4) propylbenzene, 5) p-xylene, 10) biphenyl, 15) nitrobenzene, 18) 4-chloroanisole, 22) phenyl acetate, 27) 4-methylphenol

Comparison of Tables 4.8 and 4.13 shows that based on the LSER model employed the type and the number of the outlier(s) may vary. More specifically, in solvatochromic LSER model 4-chloroaniline (solute 32 in Table 4.3) is an outlier for all pseudostationary phases except SDS/mono-SDUT (Table 4.8), whereas, the same solute is not among the outliers in solvation parameter model (Table 4.13). In both LSER models there are a total number of four outliers in mono-SDUT; however, the outliers in solvatochromic model are toluene, ethylbenzene, propylbenzene, and 4-chloroaniline (solute 2, 3, 4, and 32, respectively), whereas in solvation parameter model these outliers are ethylbenzene, propylbenzene, p-xylene, and 4-chloroanisole (solute 3, 4, 5, and 18, respectively). Further differences can be seen in Table 4.8 and Table 4.14.

After the elimination of outliers for all surfactant systems, system constants were recalculated by multiple linear regression using solvation parameter model. The new
coefficients are listed in Table 4.14. Comparing Tables 4.12 and 4.14 shows that the standard errors in the system constants and y estimate, $R^2$, and F-statistic values are statistically better in Table 4.14 than those in Table 4.12. It is worth noting that removing phenyl acetate from the solute set increased the absolute value of coefficient $a$, and hence made it statistically significant, as it was not significant at the 95% confidence level in Table 4.12.

Using the new system constants (Table 4.14), the following fits are obtained for the surfactant systems:

Table 4.14. Recalculated system constants for the six pseudostationary phases in MCE using sovation parameter model.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>SDS</th>
<th>mono-SDUT</th>
<th>poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>c</strong></td>
<td>-2.100 (0.091)</td>
<td>-3.056 (0.245)</td>
<td>-2.486 (0.121)</td>
<td>-2.334 (0.178)</td>
<td>-2.577 (0.110)</td>
<td>-2.878 (0.134)</td>
</tr>
<tr>
<td><strong>m</strong></td>
<td>3.063 (0.103)</td>
<td>3.517 (0.309)</td>
<td>2.690 (0.138)</td>
<td>3.307 (0.193)</td>
<td>3.235 (0.123)</td>
<td>2.756 (0.152)</td>
</tr>
<tr>
<td><strong>r</strong></td>
<td>0.221 (0.075)</td>
<td><strong>0.270</strong> (0.208)</td>
<td>0.790 (0.095)</td>
<td>0.709 (0.132)</td>
<td>0.596 (0.090)</td>
<td><strong>0.165</strong> (0.110)</td>
</tr>
<tr>
<td><strong>s</strong></td>
<td>-0.182 (0.078)</td>
<td><strong>-0.042</strong> (0.204)</td>
<td>-0.445 (0.101)</td>
<td>-0.725 (0.133)</td>
<td>-0.428 (0.094)</td>
<td><strong>0.242</strong> (0.122)</td>
</tr>
<tr>
<td><strong>a</strong></td>
<td>-0.133 (0.044)</td>
<td>0.524 (0.116)</td>
<td><strong>-0.021</strong> (0.060)</td>
<td><strong>-0.052</strong> (0.079)</td>
<td><strong>-0.098</strong> (0.053)</td>
<td>0.231 (0.069)</td>
</tr>
<tr>
<td><strong>b</strong></td>
<td>-1.827 (0.086)</td>
<td>-3.239 (0.248)</td>
<td>-2.308 (0.114)</td>
<td>-2.338 (0.156)</td>
<td>-2.153 (0.103)</td>
<td>-2.199 (0.126)</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>35</td>
<td>32</td>
<td>35</td>
<td>34</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td><strong>R</strong></td>
<td>0.9932</td>
<td>0.9703</td>
<td>0.9908</td>
<td>0.9835</td>
<td>0.9929</td>
<td>0.9830</td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td>0.059</td>
<td>0.158</td>
<td>0.079</td>
<td>0.107</td>
<td>0.071</td>
<td>0.086</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>421</td>
<td>84</td>
<td>311</td>
<td>166</td>
<td>401</td>
<td>155</td>
</tr>
</tbody>
</table>

n= number of test solutes; R= correlation coefficient of linear regression; SE= standard error of the y estimate; $F$= $F$-static; underlined values are not statistically significant at the 95% confidence level. Numbers in parentheses indicate the standard deviation for each coefficient.
1.0 % (w/v) SDS (34.7 mM):

\[
\log k' = -2.100(\pm 0.091) + 3.063(\pm 0.103)V_x + 0.221(\pm 0.075)R_x^2
\]

\[
-0.182(\pm 0.078)\pi^H_2 - 0.133(\pm 0.044)\sum \alpha^H_2 - 1.827(\pm 0.086)\sum \beta^H_2 .
\]

1.0 % (w/v) mono-SDUT (19 mM):

\[
\log k' = -3.056(\pm 0.245) + 3.517(\pm 0.309)V_x + 0.270(\pm 0.208)R_x^2
\]

\[
-0.042(\pm 0.204)\pi^H_2 + 0.524(\pm 0.116)\sum \alpha^H_2 - 3.239(\pm 0.248)\sum \beta^H_2 .
\]

1.0 % (w/v) poly-SDUT (19 mM, emc):

\[
\log k' = -2.486(\pm 0.121) + 2.690(\pm 0.138)V_x + 0.790(\pm 0.095)R_x^2
\]

\[
-0.445(\pm 0.101)\pi^H_2 - 0.021(\pm 0.060)\sum \alpha^H_2 - 2.308(\pm 0.114)\sum \beta^H_2 .
\]

1.0 % (w/v) SDS/mono-SDUT (17.5 mM SDS / 9.5 mM mono-SDUT):

\[
\log k' = -2.334(\pm 0.178) + 3.307(\pm 0.193)V_x + 0.709(\pm 0.132)R_x^2
\]

\[
-0.725(\pm 0.133)\pi^H_2 - 0.052(\pm 0.079)\sum \alpha^H_2 - 2.338(\pm 0.156)\sum \beta^H_2 .
\]

1.0 % (w/v) SDS/poly-SDUT (17.5 mM SDS / 9.5 mM poly-SDUT, emc):

\[
\log k' = -2.577(\pm 0.110) + 3.235(\pm 0.123)V_x + 0.596(\pm 0.090)R_x^2
\]

\[
-0.428(\pm 0.094)\pi^H_2 - 0.098(\pm 0.053)\sum \alpha^H_2 - 2.153(\pm 0.103)\sum \beta^H_2 .
\]

1.0 % (w/v) mono-SDUT/poly-SDUT (9.5 mM mono-SDUT / 9.5 mM poly-SDUT, emc):

\[
\log k' = -2.878(\pm 0.134) + 2.756(\pm 0.152)V_x + 0.165(\pm 0.110)R_x^2
\]

\[
+0.242(\pm 0.122)\pi^H_2 + 0.231(\pm 0.069)\sum \alpha^H_2 - 2.199(\pm 0.126)\sum \beta^H_2 .
\]

The log k’ values were recalculated using Equations 4.37-4.42 and obtained log k’ (i.e., calculated log k’) values are then plotted against experimental log k’ values. The R^2, slope, and the intercept values of the new correlation lines for all pseudostationary phases are listed in Table 4.15.
Table 4.15. The correlation coefficient, slope, and the intercept of the calculated log $k'$ versus experimental log $k'$ plot for each surfactant system using Equations 4.37 to 4.42 (solvation parameter model).

<table>
<thead>
<tr>
<th>Pseudostationary phase</th>
<th>Solutes</th>
<th>$R^2$</th>
<th>Slope</th>
<th>Intercept</th>
<th>n</th>
<th>Solutes excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>All</td>
<td>0.9864</td>
<td>0.9862</td>
<td>0.0058</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9893</td>
<td>1.0241</td>
<td>-0.0168</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9689</td>
<td>0.9383</td>
<td>0.0220</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9777</td>
<td>0.9113</td>
<td>0.0098</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Mono-SDUT</td>
<td>All</td>
<td>0.9415</td>
<td>0.9415</td>
<td>-0.0143</td>
<td>32</td>
<td>2, 3, 4, 18</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9136</td>
<td>1.0050</td>
<td>0.0158</td>
<td>9</td>
<td>2, 3, 4</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9295</td>
<td>0.7996</td>
<td>-0.1268</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9473</td>
<td>0.8933</td>
<td>-0.0303</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Poly-SDUT</td>
<td>All</td>
<td>0.9817</td>
<td>0.9817</td>
<td>-0.0036</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9866</td>
<td>0.9572</td>
<td>0.0075</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9580</td>
<td>1.0323</td>
<td>0.0065</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9434</td>
<td>0.9659</td>
<td>-0.0038</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>SDS/mono-SDUT</td>
<td>All</td>
<td>0.9673</td>
<td>0.9673</td>
<td>0.0064</td>
<td>34</td>
<td>10, 27</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9708</td>
<td>0.8996</td>
<td>0.0685</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9468</td>
<td>0.9571</td>
<td>-0.0010</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9108</td>
<td>1.0481</td>
<td>0.0051</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>SDS/poly-SDUT</td>
<td>All</td>
<td>0.9858</td>
<td>0.9858</td>
<td>0.0020</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9897</td>
<td>0.9498</td>
<td>0.0208</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9703</td>
<td>1.0623</td>
<td>0.0070</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9617</td>
<td>1.0438</td>
<td>0.0118</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Mono-SDUT/Poly-SDUT</td>
<td>All</td>
<td>0.9663</td>
<td>0.9664</td>
<td>-0.0113</td>
<td>32</td>
<td>15, 18, 22</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.9857</td>
<td>1.0887</td>
<td>0.0134</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HBA</td>
<td>0.9345</td>
<td>0.9015</td>
<td>-0.0621</td>
<td>9</td>
<td>15, 18, 22</td>
</tr>
<tr>
<td></td>
<td>HBD</td>
<td>0.9731</td>
<td>0.8390</td>
<td>-0.0689</td>
<td>12</td>
<td>-</td>
</tr>
</tbody>
</table>

$n$) number of test solutes, 3) Ethylbenzene, 4) propylbenzene, 5) p-xylene, 10) biphenyl, 15) nitrobenzene, 18) 4-chloroanisole, 22) phenyl acetate, 27) 4-methylphenol

Comparing the $R^2$, slope, and the intercept values listed in Table 4.15 with those in Figure 4.6 A-F (with insets) clearly show that eliminating a few outliers improved the correlation significantly for each surfactant system. For instance, eliminating phenyl acetate from SDS
system improved $R^2$ of calculated log $k'$ versus experimental log $k'$ plot for 35 solutes from 0.9595 to 0.9864. Likewise, similar improvements are seen in the subset solutes NHB (0.9885 to 9893), HBA (0.8782 to 0.9689), and HBD (0.9681 to 0.9777).

The ratio of system constants to coefficient $m$ obtained through solvation parameter model is presented in Table 4.16. The $S_1$ values (obtained from the same set of solutes for all pseudostationary phases) show the differences in interactions between each surfactant system and solutes. It is obvious from $S_1$ values in Table 4.16 that each surfactant system has a distinct interaction with the solutes. Some $S_1$ values indicate a few similarities among pseudostationary phases in some system constants such as both SDS and SDS/poly-SDUT systems have the same

Table 4.16. Ratio of system constants obtained using solvation parameter model for six pseudostationary phases.

<table>
<thead>
<tr>
<th></th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$</td>
<td>-0.673</td>
<td>-0.982</td>
<td>-0.915</td>
<td>-0.739</td>
<td>-0.792</td>
<td>-1.013</td>
</tr>
<tr>
<td>$S_2$</td>
<td>-0.686</td>
<td>-0.869</td>
<td>-0.924</td>
<td>-0.706</td>
<td>-0.797</td>
<td>-1.044</td>
</tr>
<tr>
<td>$\Delta S$</td>
<td>0.013</td>
<td>-0.113</td>
<td>0.009</td>
<td>-0.033</td>
<td>0.005</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_1$</td>
<td>0.135</td>
<td>0.164</td>
<td>0.292</td>
<td>0.224</td>
<td>0.217</td>
<td>0.099</td>
</tr>
<tr>
<td>$S_2$</td>
<td>0.072</td>
<td>0.077</td>
<td>0.294</td>
<td>0.214</td>
<td>0.184</td>
<td>0.060</td>
</tr>
<tr>
<td>$\Delta S$</td>
<td>0.063</td>
<td>0.087</td>
<td>-0.002</td>
<td>0.010</td>
<td>0.033</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_1$</td>
<td>-0.135</td>
<td>0.041</td>
<td>-0.186</td>
<td>-0.197</td>
<td>-0.171</td>
<td>0.005</td>
</tr>
<tr>
<td>$S_2$</td>
<td>-0.059</td>
<td>-0.012</td>
<td>-0.165</td>
<td>-0.219</td>
<td>-0.132</td>
<td>0.088</td>
</tr>
<tr>
<td>$\Delta S$</td>
<td>-0.076</td>
<td>0.053</td>
<td>-0.021</td>
<td>0.022</td>
<td>-0.039</td>
<td>-0.083</td>
</tr>
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<tr>
<td>$S_1$</td>
<td>-0.018</td>
<td>0.136</td>
<td>0.007</td>
<td>-0.030</td>
<td>-0.018</td>
<td>0.115</td>
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<tr>
<td>$S_2$</td>
<td>-0.043</td>
<td>0.149</td>
<td>-0.008</td>
<td>-0.016</td>
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<td>0.084</td>
</tr>
<tr>
<td>$\Delta S$</td>
<td>0.025</td>
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<td>0.015</td>
<td>-0.014</td>
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</tr>
<tr>
<td>$S_1$</td>
<td>-0.618</td>
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<tr>
<td>$S_2$</td>
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<td>-0.798</td>
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<tr>
<td>$\Delta S$</td>
<td>-0.021</td>
<td>-0.066</td>
<td>0.023</td>
<td>0.016</td>
<td>-0.011</td>
<td>0.009</td>
</tr>
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</table>

$S_1$ = System constant ratios obtained with 36 analytes, $S_2$ = system constant ratios obtained after outlier solute(s) excluded, $\Delta S = S_1 - S_2$
value of –0.018 for a/m ratio. This is an indication of similar hydrogen-bond-accepting ability of these two surfactant systems. Similarly, r/m values of SDS/mono-SDUT (0.224) and SDS/poly-SDUT (0.217) are close to each other and evident that these surfactant systems have fairly close abilities to interact with the n- or π-electrons of the solutes. The $S_1$ and $S_2$ values in Table 4.16 show that removing a few solutes from the solute set has some effect of the system constants. The $\Delta S$ values in Table 4.16 indicate that major differences are noticed in mono-SDUT system.

**4.3.4. Effect of Number of Solutes on System Constants**

To further investigate the effect of solutes on the system constants, solute with the largest residual was eliminated from solute set (i.e., 36 solutes) for each surfactant system and the system constants were then determined using multiple linear regression. Next, among the remaining 35 solute set, the second outlier with the largest residual value was omitted and the system constants were determined for each pseudostationary phase. This process of stepwise solute elimination was repeated until correlation coefficient (R) for each surfactant system was 0.9900 or better. Solutes omitted from solute set are listed in Table 4.17 (for solvatochromic model) and Table 4.18 (for solvation parameter model). As seen in Tables 4.17 and 4.18, a total of fifteen solutes were omitted from the solute set for each system; however, the eliminated solutes are not identical for all surfactant systems and for the two LSER models for the same surfactant system. Thus, the effect of solutes on system constants for each surfactant system as well as for each LSER model is expected to be different. In Tables 4.17 and 4.18 are also shown the total eliminated NHB, HBA, and HBD solutes. For some pseudostationary phase systems an equal number of each subset solutes are eliminated (SDS/poly-SDUT, 5 each of NHB, HBA, HBD, Table 4.18) whereas in some cases uneven number of solutes are excluded (e.g., SDS, 3 NHB, 8 HBA, and 4 HBD; poly-SDUT, 8 NHB, 5 HBA, and 2 HBD; and mono-SDUT/poly-
SDUT, 2 NHB, 9 HBA, and 4 HBD in Table 4.17). The largest number of NHB solutes is the outliers in poly-SDUT and SDS/mono-SDUT (Table 4.17) and mono-SDUT (Table 4.18) systems. The SDS and mono-SDUT/poly-SDUT systems have a large number of HBA outliers in both LSER models (Tables 4.17 and 4.18). The least number of NHB solutes are omitted in mono-SDUT/poly-SDUT system in both LSER models (2 solutes, solutes 1 and 10) and SDS system (3 solutes, solutes 1, 2, and 9) in solvatochromic model. The mono-SDUT has three HBA solutes as outliers (solutes 17, 18, and 20). The least number of HBD solutes are eliminated in poly-SDUT (solutes 29 and 32) and SDS/mono-SDUT (solutes 27, 29, and 32) in Table 4.17, and SDS (solutes 30, 31, and 32) in Table 4.18.

Table 4.17. The outliers eliminated from solute set for solvatochromic model.

<table>
<thead>
<tr>
<th>Order</th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
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</tr>
<tr>
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<td>3 HBD</td>
<td>5 HBD</td>
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Table 4.18. The outliers eliminated from solute set for solvation parameter model.

<table>
<thead>
<tr>
<th>Order</th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
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The system constants and ratios of system constants to coefficient m are plotted against number of solutes in Figure 4.8 (solvatochromic model) and Figure 4.9 (solvation parameter model). As seen in Figure 4.8 A-F, the number of solutes has an effect on approximately every coefficient and the ratio of coefficients. The coefficients c, m, and b seem to experience the major variations as the number of solutes is varied. It is worth noticing that the variations in constant m value are inversely proportional to those in constants b and c values, that is, as the value of m increases (or becomes more positive) depending on the number of solutes, the values of coefficient b and c decrease (or become more negative) accordingly. Similarly, as seen in
Figures 4.8 and 4.9 (insets A1-F1), an increase in a/m ratio results in a decrease in b/m ratio; similar relationship is observed between s/m and c/m, i.e., as s/m is increased c/m is decreased accordingly. In addition, there is an obvious inverse relationship between coefficients r and s (Figure 4.9 insets A1-F1). The insets A2 through F2 in Figures 4.7 and 4.8 represent the number of solutes versus the F-statistic. The F value increases proportionally as more outliers are eliminated from the solute set. For instance, the F value increased from 131 to 3038 (Figure 4.8) or from 140 to 4581 (Figure 4.9) as number of solutes decreased from 36 to 21 by eliminating the outliers subsequently. Similar increases are observed for each surfactant system studied.

To examine the effect of solutes on the calculated log k' values, the log k' values of all 36 solutes were computed using the coefficients obtained for each set of solutes. There are sixteen solute sets in which number of solutes range from 21 to 36. The calculated log k’ values then plotted against the number of solutes. Plots for each surfactant system are shown in Figure 4.10 (solvatochromic model) and Figure 4.11 (solvation parameter model). Each point on the x-axis of the plots in Figures 4.10 and 4.11 represents a set of solutes with certain number of solutes, e.g., 36 on x-axis means a solute set with 36 solutes. In Figures 4.10 and 4.11 are shown the calculated log k’ values of fifteen representative solutes. These fifteen solutes consist of five of each subset (i.e., NHB, HBA, and HBD). The NHB solutes represented in Figures 4.10 and 4.11 are: benzene, propylbenzene, chlorobenzene, iodobenzene, and naphthalene; the HBA solutes are: nitrobenzene, 4-chloroanisole, 4-nitrotoluene, phenyl acetate, and phenethyl alcohol; and the HBD solutes are: phenol, 4-methylphenol, 4-fluorophenol, 4-chloroaniline, and 3-bromophenol. It should be noted that some of these solutes such as propylbenzene, nitrobenzene, phenyl acetate, 4-chloroanisole, 4-methylphenol are among the outliers as seen in Tables 4.8 and 4.14 and included intentionally to examine their calculated log k’ trends.
Figure 4.8. Effect of number of solutes on system constants for A) SDS, B) mono-SDUT, C) poly-SDUT, D) SDS/mono-SDUT, E) SDS/poly-SDUT, and F) mono-SDUT/poly-SDUT systems using solvatochromic model. Insets are the enlargement of s, a, c/m, s/m, a/m, and b/m (A1-F1), and F-statistics versus number of solutes (A2-F2). Legends are shown on the top of the plots.
Figure 4.9. Effect of number of solutes on system constants for A) SDS, B) mono-SDUT, C) poly-SDUT, D) SDS/m mono-SDUT, E) SDS/poly-SDUT, and F) mono-SDUT/poly-SDUT systems using solvation parameter model. Insets are the enlargement of s, a, c/m, s/m, a/m, and b/m (A1-F1), and F-statistics versus number of solutes (A2-F2). Legends are shown on the top of the plots.
Figure 4.10. Calculated log k' using solvatochromic model versus number of solutes for MCE system with A) SDS, B) mono-SDUT, C) poly-SDUT, D) SDS/mono-SDUT, E) SDS/poly-SDUT, and F) mono-SDUT/poly-SDUT. Legends are shown on the top of the plots.
Figure 4.11. Calculated log $k'$ using solvation parameter model versus number of solutes for MCE system with A) SDS, B) mono-SDUT, C) poly-SDUT, D) SDS/mono-SDUT, E) SDS/poly-SDUT, and F) mono-SDUT/poly-SDUT. Legends are shown on the top of the plots.
Calculated log k' values of the majority of the solutes seem to stay constant for entire solute sets (Figures 4.10 and 4.11). However, there are several noticeable deviations in log k' (calculated) values of some solutes. The log k' values of propylbenzene, nitrobenzene, 4-nitrotoluene, phenyl acetate, and phenethyl alcohol are inconsistent in SDS system for five sets (with 32, 33, 34, 35, and 36 solutes) using solvatochromic model (Figure 4.10 A). The solvation parameter model provided similar results with an additional solute, 4-chloroaniline (Figure 4.11 A). Except propylbenzene (NHB) and 4-chloroaniline (HBD), all these solutes are members of HBA subset.

In mono-SDUT system, except a few solutes such as nitrobenzene, 4-nitrotoluene, 4-methylphenol and 4-chloroaniline, most of the solutes have inconsistencies to some extent for a wide range of solute sets (Figure 4.10 B). However, largely NHB (e.g., benzene, propylbenzene, chlorobenzene, iodobenzene and naphthalene) and HBD solutes such as 3-bromophenol and 4-fluorophenol have major variations in their log k' values. The usage of solvation parameter model improved the consistency of log k' for most of the solutes in mono-SDUT system (Figure 4.11 B); however, prophylbenzene still remained the same with large variation in its log k' value.

Comparison of Figure 4.10 C and Figure 4.11 C shows that solvation parameter model is relatively better than solvatochromic model in providing consistent log k' values with poly-SDUT system. Mainly NHB (e.g., benzene, propylbenzene, chlorobenzene, iodobenzene) and HBA solutes (nitrobenzene, and phenyl acetate) show modest to relatively large variations in log k' values using solvatochromic model (Figure 4.10 C). However, solvation parameter model improves the results significantly as seen in Figure 4.11 C.

Although there are not many solutes with large deviations in log k' values with SDS mono-SDUT system, some differences are apparent in both LSER models Figures 4.10 D
and 4.11 D. For instance, solvatochromic model gives slightly better log k' values for propylbenzene and naphthalene (Figure 4.10 D) than solvation parameter model (Figure 4.11 D), but, in general, the later model still seems to be relatively better. Similar analogy is true for SDS/poly-SDUT system, that is, with solvatochromic model, the log k' values of propylbenzene, iodobenzene, and naphthalene (all are NHB solutes) are constant throughout the solute sets (Figure 4.10 E), whereas, deviations in log k' values increase when solvation parameter model is used as shown in Figure 4.11 E. Nevertheless, the opposite is true with the HBA solutes (e.g., nitrobenzene, 4-nitrotoluene, and phenyl acetate) where solvation parameter model produces relatively sound log k' values.

Mono-SDUT/poly-SDUT system, similar to mono-SDUT, generates large divergence in log k' values for a significant number of solutes (Figures 4.10 F and 4.11 F). In this surfactant system, the solvatochromic model gives slight variations in log k' values of benzene, phenyl acetate, 3-bromophenol, and phenol (Figure 4.10 F), whereas the solvation parameter model provides relatively larger deviations in log k' values of nitrobenzene, 4-chloroanisole, 4-nitrotoluene, phenyl acetate, 4-chloroaniline, where the last solute is HBD and the rest are HBA solutes (Figure 4.11 F).

### 4.3.5. Determination of System Coefficients Using NHB, HBA, and HBD Subset Solutes

The retention behaviors of twelve solutes in each subset of NHB, HBA, and HBD in six surfactant systems were examined independently. The system coefficients were determined using solvatochromic (Equation 4.1) and solvation parameter (Equation 4.2) models. The obtained system constants using NHB, HBA, and HBD solutes are listed in Tables 4.19, 4.20, and 4.21, respectively. Each table contains system coefficients obtained by both solvatochromic model and solvation parameter model. Tables 4.19 through 4.21 show that the size and basicity
Table 4.19. Comparison of system coefficients for both LSER models using NHB solutes.

<table>
<thead>
<tr>
<th>Surfactants</th>
<th>Solvatochromic model</th>
<th>Solvation parameter model</th>
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</thead>
<tbody>
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<td>c</td>
<td>-2.133 (0.072)</td>
<td>-2.829 (0.756)</td>
</tr>
<tr>
<td>m</td>
<td>4.934 (0.158)</td>
<td>4.263 (1.655)</td>
</tr>
<tr>
<td>s</td>
<td>-0.230 (0.070)</td>
<td>0.759 (0.735)</td>
</tr>
<tr>
<td>a¹</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>b</td>
<td>-2.967 (0.387)</td>
<td>-4.507 (4.049)</td>
</tr>
<tr>
<td>n</td>
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<td>12</td>
</tr>
<tr>
<td>R</td>
<td>0.998</td>
<td>0.819</td>
</tr>
<tr>
<td>SE</td>
<td>0.037</td>
<td>0.389</td>
</tr>
<tr>
<td>F</td>
<td>604</td>
<td>5</td>
</tr>
</tbody>
</table>

n= number of test solutes; R= correlation coefficient of linear regression; SE= standard error of the y estimate; F= Fischer F-statistic; underlined values are not statistically significant at the 95% confidence level. Numbers in parentheses indicate the standard deviation for each coefficient. The coefficient a has been set to 0, because \( \alpha \) and \( \sum \alpha_z \) values for this set of solutes are 0.
Table 4.20. Comparison of system coefficients for both LSER models using HBA solutes.

<table>
<thead>
<tr>
<th>Surfactants</th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>-0.083 (0.828)</td>
<td>-2.077 (0.761)</td>
<td>-1.301 (0.701)</td>
<td>-0.670 (0.735)</td>
<td>-0.711 (0.719)</td>
<td>-1.377 (0.924)</td>
</tr>
<tr>
<td>m</td>
<td>3.128 (0.864)</td>
<td>5.178 (0.794)</td>
<td>3.114 (0.730)</td>
<td>3.441 (0.766)</td>
<td>3.221 (0.749)</td>
<td>3.611 (0.963)</td>
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<tr>
<td>s</td>
<td>-1.357 (0.489)</td>
<td>-0.602 (0.499)</td>
<td>-0.606 (0.413)</td>
<td>-1.143 (0.434)</td>
<td>-1.019 (0.424)</td>
<td>-0.904 (0.545)</td>
</tr>
<tr>
<td>a</td>
<td>0.096 (0.449)</td>
<td>0.195 (0.413)</td>
<td>0.177 (0.380)</td>
<td>0.095 (0.398)</td>
<td>0.112 (0.390)</td>
<td>0.269 (0.501)</td>
</tr>
<tr>
<td>b</td>
<td>-1.721 (0.724)</td>
<td>-4.049 (0.665)</td>
<td>-2.193 (0.612)</td>
<td>-2.011 (0.642)</td>
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<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
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<td>-1.961 (0.569)</td>
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<td>-1.812 (0.575)</td>
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<td>m</td>
<td>2.474 (0.607)</td>
<td>3.772 (0.578)</td>
<td>2.326 (0.388)</td>
<td>2.708 (0.433)</td>
<td>2.499 (0.392)</td>
<td>2.794 (0.554)</td>
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<tr>
<td>r</td>
<td>1.566 (0.635)</td>
<td>0.818 (0.604)</td>
<td>1.040 (0.405)</td>
<td>1.428 (0.452)</td>
<td>1.391 (0.410)</td>
<td>1.318 (0.579)</td>
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<td>-1.328 (0.352)</td>
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<td>-3.658 (0.607)</td>
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<td>-1.895 (0.455)</td>
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<td>-2.268 (0.582)</td>
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</table>

n= number of test solutes; R= correlation coefficient of linear regression; SE= standard error of the y estimate; F= Fischer F-statistic; underlined values are not statistically significant at the 95% confidence level. Numbers in parentheses indicate the standard deviation for each coefficient.
Table 4.21. Comparison of system coefficients for both LSER models using HBD solutes.

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<th>Surfactants</th>
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<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
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<tr>
<td>c</td>
<td>-0.649 (0.233)</td>
<td>0.008 (0.674)</td>
<td>-0.577 (0.273)</td>
<td>-0.376 (0.612)</td>
<td>-0.815 (0.241)</td>
<td>-0.281 (0.252)</td>
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<tr>
<td>m</td>
<td>4.430 (0.184)</td>
<td>5.003 (0.533)</td>
<td>3.610 (0.216)</td>
<td>3.991 (0.484)</td>
<td>4.070 (0.191)</td>
<td>4.248 (0.199)</td>
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<tr>
<td>s</td>
<td>0.088 (0.115)</td>
<td>0.628 (0.333)</td>
<td>0.622 (0.135)</td>
<td>0.383 (0.303)</td>
<td>0.300 (0.119)</td>
<td>0.469 (0.125)</td>
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<td>a</td>
<td>-1.548 (0.175)</td>
<td>-2.424 (0.506)</td>
<td>-1.696 (0.205)</td>
<td>-1.818 (0.459)</td>
<td>-1.460 (0.181)</td>
<td>-2.127 (0.189)</td>
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<tr>
<td>b</td>
<td>-3.842 (0.243)</td>
<td>-7.939 (0.704)</td>
<td>-5.202 (0.285)</td>
<td>-4.786 (0.639)</td>
<td>-4.229 (0.252)</td>
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<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
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<tbody>
<tr>
<td>c</td>
<td>-2.582 (0.330)</td>
<td>-4.510 (0.807)</td>
<td>-3.717 (0.350)</td>
<td>-3.659 (0.771)</td>
<td>-3.214 (0.361)</td>
<td>-3.923 (0.497)</td>
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<tr>
<td>m</td>
<td>3.254 (0.164)</td>
<td>3.520 (0.402)</td>
<td>2.500 (0.174)</td>
<td>3.259 (0.384)</td>
<td>2.926 (0.180)</td>
<td>3.205 (0.247)</td>
</tr>
<tr>
<td>r</td>
<td>0.132 (0.199)</td>
<td>0.366 (0.487)</td>
<td>0.084 (0.211)</td>
<td>-0.940 (0.465)</td>
<td>0.067 (0.218)</td>
<td>-0.151 (0.300)</td>
</tr>
<tr>
<td>s</td>
<td>0.259 (0.254)</td>
<td>1.045 (0.623)</td>
<td>1.034 (0.270)</td>
<td>1.787 (0.595)</td>
<td>0.627 (0.278)</td>
<td>1.144 (0.383)</td>
</tr>
<tr>
<td>a</td>
<td>-0.255 (0.182)</td>
<td>0.647 (0.444)</td>
<td>0.433 (0.193)</td>
<td>-0.015 (0.424)</td>
<td>0.137 (0.199)</td>
<td>0.210 (0.274)</td>
</tr>
<tr>
<td>b</td>
<td>-1.754 (0.288)</td>
<td>-2.446 (0.706)</td>
<td>-1.272 (0.307)</td>
<td>-1.314 (0.674)</td>
<td>-1.425 (0.316)</td>
<td>-2.106 (0.435)</td>
</tr>
<tr>
<td>n</td>
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<td>12</td>
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<td>R</td>
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<td>SE</td>
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<td>0.087</td>
<td>0.041</td>
<td>0.056</td>
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<td>F</td>
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<td>133</td>
<td>117</td>
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</table>

n= number of test solutes; R= correlation coefficient of linear regression; SE= standard error of the y estimate; F= Fischer F-statistic; **underlined** values are not statistically significant at the 95% confidence level. Numbers in parentheses indicate the standard deviation for each coefficient.
of the solutes i.e., coefficients m and b, are the two predominant factors that govern the retention for all the subsets, similar to the main set, in the six surfactant systems. The phase ratio term c obtained from NHB solutes is similar to that obtained from complete solute set (Tables 4.7 and 4.12) and more negative than the c term obtained from HBA and HBD solutes. This shows that distribution of solutes of each subset between pseudostationary phase and the mobile phase (i.e., buffer solution) is not identical.

For the NHB subset solutes the m system constants are larger than those obtained from the complete set and the HBA and HBD subsets. However, the m coefficient in mono-SDUT system is smaller than those attained from the main set and the HBA and HBD subsets. This means that NHB solutes interact with relatively less nonpolar region of mono-SDUT while HBA and HBD solutes interact with slightly more nonpolar environment of the surfactant. The larger m values for NHB subset show that the microenvironments of pseudostationary phases for these solutes are more nonpolar (i.e., less cohesive). The surfactant systems are ranked according to the magnitude of system constants obtained from NHB, HBA, and HBD subset solutes as well as the complete solute set using solvatochromic and solvation parameter models in Tables 4.22 and 4.23, respectively. It is obvious from these two tables that the order of the surfactants for each coefficient is not exactly the same for neither solute set. This indicates that solute number and type has an influence on the magnitude of the system constants and this influence may vary for the same surfactant system as the type and number of solute are differed. Comparing the m values for the NHB subset reveals that SDS/mono-SDUT system provides somewhat less cohesive microenvironment for NHB solutes. On the contrary, mono-SDUT/poly-SDUT system offers relatively more cohesive environment for the same set of solutes.
For the HBA bases subset (Table 4.20), the coefficient m values are smaller than those obtained from NHB, HBD, and main sets. These smaller m values suggest that the HBA solutes interact with the relatively polar regions of pseudostationary phases. However, the HBA solutes seem to be located in less cohesive (more nonpolar) environments of mono-SDUT surfactant (large m value).

Table 4.22. Order of the surfactant systems in MCE according to the magnitude of system constants obtained from complete solute set (Table 4.7) as well as subset solutes (Tables 4.19-4.21) using solvatochromic model.

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<td>SP</td>
<td>M</td>
<td>P</td>
<td>S</td>
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<td>SP</td>
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</tr>
<tr>
<td>b</td>
<td>S</td>
<td>MP</td>
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*From largest (1, least negative) to the smallest (6, most negative) value; * the coefficient a is zero for all surfactant systems because α is zero for NHB solutes; S) SDS, M) mono-SDUT, P) poly-SDUT, SM) SDS/mono-SDUT, SP) SDS/poly-SDUT, MP) mono-SDUT/poly-SDUT; the coefficient is not statistically significant for the underlined surfactant system.
Table 5.23. Order of the surfactant systems in MCE according to the magnitude of system constants obtained from complete solute set (Table 4.12) as well as subset solutes (Tables 4.19-4.21) using solvation parameter model.

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</tr>
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Complete solute set (36 solutes)

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NHB solutes (12 solutes)

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HBA solutes (12 solutes)

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HBD solutes (12 solutes)

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* From largest (1, least negative) to the smallest (6, most negative) value; the coefficient a is zero for all surfactant systems because $\sum \alpha_n^a$ is zero for NHB solutes; S) SDS, M) mono-SDUT, P) poly-SDUT, SM) SDS/mono-SDUT, SP) SDS/poly-SDUT, MP) mono-SDUT/poly-SDUT; the coefficient is not statistically significant for the underlined surfactant system.

For the HBD acids (Table 4.21), the coefficient m values are larger than those obtained from HBA solutes but smaller than those obtained from NHB and the main set. This means that
the HBD acids are located in a slightly more nonpolar environment of the pseudostationary phases than HBA bases; however, NHB solutes experience relatively more nonpolar environments in the surfactant systems than HBD solutes.

The negative coefficient b for all surfactant systems indicates that all pseudostationary phases are less acidic than the aqueous buffer solution. For the HBA solutes the b coefficients are less negative compared to those obtained for NHB, HBD, and main set solutes using solvatochromic model. This indicates that as the HBA strengths of solutes increase, their interactions with the surfactant systems increase accordingly due to the less negative b coefficients. Among all, mono-SDUT has the most and SDS has the least negative value of coefficient b, thus more HBA basic solutes favor relatively more acidic surfactant (i.e., SDS) than the least acidic one (i.e., mono-SDUT). However, solvation parameter model does not provide the same trend for coefficient b. In general, HBD solutes provide less negative b values. It should be mentioned that HBD solutes have both acidic and basic character (i.e., $\sum \alpha_2^\alpha$ and $\sum \beta_2^\beta$ values in Table 4.2). The more positive r coefficients for HBA solutes show that the surfactants interact more strongly with the solutes than the aqueous buffer solution and hence pseudostationary phases become more polarized by n- or $\pi$-electrons of the HBA solutes.

The solvatochromic and solvation parameter models provide comparable s coefficients for NHB, HBA, and HBD subsets. For the NHB solutes, the s coefficient is negative and statistically significant only for SDS system. As mentioned earlier, the negative s coefficient verifies that the solutes experience a less dipolar/polarizable environment than the aqueous phase. Thus, NHB and more importantly HBA solutes find less dipolar environment in pseudostationary phases. However, with the positive s values, the HBD solutes experience more dipolar/polarizable environment in surfactant systems than in the aqueous buffer solution.
The coefficients \( a \) are 0 (zero) for NHB solutes and are not statistically significant for HBA solutes. Solvation parameter model provides only one positive coefficient \( a \) value that is statistically significant for poly-SDUT system. This means HBD solutes experience slightly more basic environment in/on poly-SDUT surfactant than in the aqueous phase. However, coefficients \( a \) are statistically significant for all surfactant systems using solvatochromic model (Table 4.21). Thus, all surfactant systems, according to solvatochromic model, provide relatively weaker basicity than aqueous solution for HBD solutes. It is worth noting that better correlation coefficients were observed for three subsets (Tables 4.19-4.21) as compared to that for the main set (Tables 4.7 and 4.12)

### 4.3.6. Prediction of log \( k' \) Values Using NHB, HBA, and HBD Subset Solutes

The predicted log \( k' \) values of thirty-six solutes were determined by putting the system constants in Tables 4.19-4.21 into the Equations 4.1 and 4.2. Then, these predicted log \( k' \) values obtained from solvatochromic model and solvation parameter model were plotted against experimental log \( k' \) values, Figure 4.12 and Figure 4.13, respectively. Each subset was used separately for the prediction of log \( k' \) for the same subset, other two subsets as well as for the main set. Figure 4.12 A shows three plots of calculated (or predicted) log \( k' \) versus experimental log \( k' \) values for SDS system. In Figure 4.12 A1, the calculated log \( k' \) values obtained from twelve NHB solutes for each subset and the main set are plotted against the experimental log \( k' \) values of the same subset (i.e., NHB, squares), the remaining two subsets (i.e., HBA, triangles; and HBD, circles), and the main set (dashed line). As seen in Figure 4.12 A1, high correlation \( (R^2 = 0.9956, \text{ slope } = 0.9957, \text{ y-intercept } = 0.0035) \) exists between the calculated and the experimental log \( k' \) values for NHB solutes. However, relatively poorer correlations are observed between the calculated and the experimental capacity factors for HBA \( (R^2 = 0.8252, \text{ slope } = 0.8252, \text{ y-intercept } = 0.0035) \).
slope = 0.9543, y-intercept= -0.2389), HBD (R^2 = 0.8790, slope= 1.1086, y-intercept= -0.2329), and the main set (R^2 = 0.9218, slope = 1.1295, y-intercept = -0.2085). This is due mainly to the fact that NHB solutes do not represent HBA and HBD solutes. Therefore, using system constants obtained from NHB solutes cannot provide accurate calculated capacity factors for HBA and HBD solutes. As seen in Figures 4.12 and 4.13, always higher correlations are observed when a certain type of solute set (e.g., NHB) is used for prediction of capacity factors of the same type of solute set (e.g., NHB). In other words, using the system constants obtained from NHB solutes will provide better-predicted capacity factors for NHB solutes (Figure 4.12 or 4.13 A1) for each surfactant system. Similarly, HBA solutes will offer better-predicted capacity factors for HBA solutes (Figure 4.12 or 4.13 A2) and HBD solutes for HBD set solutes (Figure 4.12 or 4.13 A3). It is worth noting that using only twelve NHB or HBA solutes, it is possible to predict capacity factors for thirty-six solutes with a correlation of 0.9568 (slope = 1.088, y-intercept = -0.0297) or 0.951 (slope = 0.9685, y-intercept = -0.0007), respectively, using solvation parameter model (Figure 4.13 C1 and C2, dashed lines) with poly-SDUT surfactant system. Comparison of Figure 4.13 C1-C2 and Figure 4.12 C1-C2 shows that solvation parameter model provides better correlations between predicted and experimental capacity factors than solvatochromic model. However, HBD solutes provide lower correlation between predicted and experimental log k' for the main set with both solvatochromic model (R^2 = 0.6437, slope = 1.5499, y-intercept = 0.8772) and solvation parameter model (R^2 = 0.3639, slope = 0.4352, y-intercept = -0.4348). As seen in Figure 4.13 A3, the HBD solutes in SDS surfactant system provide relatively good correlation for thirty-six solutes using solvation parameter model (R^2 = 0.8946, slope = 0.9342, y-intercept = 0.006). However, solvatochromic model does not give a good correlation (Figure 4.12 A3).
Figure 4.12a. Calculated capacity factors obtained by solvatochromic model versus experimental capacity factors for MCE systems with A) SDS, B) mono-SDUT using NHB (A1, B1), HBA (A2, B2), and HBD (A3, B3) subsets. Legends: ■ = NHB solutes, ▲ = HBA solutes, and ● = HBD solutes. Dashed line represents correlation line for the main set (36 solutes). Regression equations for each line are shown in the plots. (Fig. con’d.)
Figure 4.12b. C) poly-SDUT, D) SDS/mono-SDUT using NHB (C1, D1), HBA (C2, D2), and HBD (C3, D3) subsets. Legends: ■ = NHB solutes, ▲ = HBA solutes, and ● = HBD solutes. Dashed line represents correlation line for the main set (36 solutes). Regression equations for each line are shown in the plots. (Fig. con’d.).
Figure 4.12c. E) SDS/poly-SDUT, and F) mono-SDUT/poly-SDUT using NHB (E1, E3), HBA (E2, F2), and HBD (E3, F3) subsets.  Legends: ■ = NHB solutes, ▲ = HBA solutes, and ● = HBD solutes.  Dashed line represents correlation line for the main set (36 solutes).  Regression equations for each line are shown in the plots.
Figure 4.13a. Calculated capacity factors obtained by solvation parameter model versus experimental capacity factors for MCE systems with A) SDS, B) mono-SDUT using NHB (A1, B1), HBA (A2, B2), and HBD (A3, B3) subsets. Legends: ■ = NHB solutes, ▲ = HBA solutes, and ● = HBD solutes. Dashed line represents correlation line for the main set (36 solutes). Regression equations for each line are shown in the plots. (Fig. con’d.)
Figure 4.13b. C) poly-SDUT, D) SDS/mono-SDUT using NHB (C1, D1), HBA (C2, D2), and HBD (C3, D3) subsets. Legends: ■ = NHB solutes, ▲ = HBA solutes, and ● = HBD solutes. Dashed line represents correlation line for the main set (36 solutes). Regression equations for each line are shown in the plots. (Fig. con’d.).
Figure 4.13c. E) SDS/poly-SDUT, and F) mono-SDUT/poly-SDUT using NHB (E1, F1), HBA (E2, F2), and HBD (E3, F3) subsets. Legends: ■ = NHB solutes, ▲ = HBA solutes, and ● = HBD solutes. Dashed line represents correlation line for the main set (36 solutes). Regression equations for each line are shown in the plots.
4.3.7. Energy of Transfer Determination for Functional Group Selectivity

Solute-pseudostationary phase interactions in MCE can also be examined by determining the free energy of transfer of the substituted functional groups from the aqueous buffer phase into the pseudostationary phase, $\Delta \Delta G$. The functional group selectivity, $\tau$, can be defined as the ratio of the capacity factor, $k'$, of a substituted benzene (Ph-R) over the $k'$ of benzene (Ph-H):

$$
\tau = \frac{k'_{\text{Ph-R}}}{k'_{\text{Ph-H}}}. \quad 4.43
$$

The $\Delta \Delta G$, then, can be calculated according to Equation 4.44:

$$
\Delta \Delta G = -RT \ln \tau, \quad 4.44
$$

where $R$ is universal gas constant (8.31451 J/mol) and $T$ is the absolute temperature ($0 ^\circ C = 273.15 K$).

The $\Delta \Delta G$ values for various functional groups are listed in Table 4.24. A negative $\Delta \Delta G$ value indicates that the addition of a functional group to benzene ring leads an increase in the interaction with the pseudostationary phases. In contrast, a positive $\Delta \Delta G$ means the addition of a functional group to benzene ring leads a decrease in the interaction with the surfactant systems. The larger negative $\Delta \Delta G$ indicates more favorable interactions between pseudostationary phases and the substituted solute as compared to that between pseudostationary phases and the unsubstituted benzene molecule. As seen in Table 4.24, for NHB functional groups (1-7), the $\Delta \Delta G$ values are all negative for all surfactant systems. As the hydrophobicity and the size of the functional group increases, the interaction with the pseudostationary phase increases accordingly. To be more specific, the negative value of $\Delta \Delta G$ increases consequently as the carbon number of the functional group (1-4) increases. Furthermore, as the size of halogen functional group (5-7) is increased, $\Delta \Delta G$ becomes more negative; hence, interact strongly with the pseudostationary phases. The strength of the interactions between solutes 1-3 in Table 4.24 is less for mono-
SDUT than the other surfactant systems which can be explained by the smallest coefficient $m$ value of mono-SDUT as seen in Table 4.19. However, halogen substituted solutes interact strongly with the mono-SDUT because $\Delta \Delta G$ values for these solutes are relatively more negative in mono-SDUT system.

Table 4.24. Effect of pseudostationary phases on functional group selectivity.

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<th>SDS</th>
<th>Mono-SDUT</th>
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<th>SDS/Poly-SDUT</th>
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<td>-2.522</td>
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<td>2 -CH$_2$CH$_3$</td>
<td>-4.794</td>
<td>-1.767</td>
<td>-4.181</td>
<td>-4.538</td>
<td>-4.786</td>
<td>-3.202</td>
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<td>5 -Cl</td>
<td>-3.197</td>
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<td>-3.370</td>
<td>-2.743</td>
<td>-3.336</td>
<td>-2.244</td>
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<tr>
<td>7 -I</td>
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<td>-6.465</td>
<td>-6.121</td>
<td>-5.468</td>
<td>-5.936</td>
<td>-4.632</td>
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<td>8 -CN</td>
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<td>0.856</td>
<td>1.105</td>
<td>0.101</td>
<td>0.904</td>
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<td>9 -NO$_2$</td>
<td>-0.853</td>
<td>-0.517</td>
<td>0.302</td>
<td>0.412</td>
<td>-0.564</td>
<td>0.601</td>
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<td>10 -C(O)CH$_3$</td>
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<td>0.104</td>
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<td>11 -C(O)OCH$_3$</td>
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<td>12 -C(O)OCH$_2$CH$_3$</td>
<td>-5.610</td>
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<td>13 -CH$_2$CH$_2$OH</td>
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<td>1.105</td>
<td>0.101</td>
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<td>14 -CH$_3$OH</td>
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<td>15 -OH</td>
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<td>1.274</td>
<td>2.624</td>
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The HBA functional groups (8-13) show relatively stronger interactions with the SDS system. Smaller polar functional groups (8, 9) show less favorable interactions with mono-
SDUT, poly-SDUT, SDS/mono-SDUT, mono-SDUT/poly-SDUT systems, while show slightly stronger interactions with the SDS and SDS/poly-SDUT systems. Thus, less hydrophobic solutes such as nitrobenzene and benzonitrile are retained relatively longer than benzene with the SDS and SDS/poly-SDUT systems through hydrogen bonding with the surfactant system. The larger and more hydrophobic functional groups (10-12) have favorable interactions with all surfactant systems. The alcohol functional groups (13, 14 in Table 4.24) interact strongly with the SDS, whereas, weakly with mono-SDUT system. It should be mentioned that alcohol functional groups have negative ΔΔG values in all pseudostationary phases, except number 13 in Table 4.24 is slightly negative in SDS. The hydroxyl group (number 15 in Table 4.24) has a small negative ΔΔG value in only mono-SDUT system that indicates a relatively stronger interaction between phenol and mono-SDUT surfactant than the rest of the surfactant systems. Since phenol is a HBD acid, mono-SDUT acts like a strong basic surfactant, which is evident in Table 4.21 with a more negative coefficient b value. The weakest interaction occurs between phenol and SDS/mono-SDUT system due to the large positive ΔΔG value.

4.3.8. Comparison of log k' for Different Pseudostationary Phases

As mentioned previously, different system constants for each pseudostationary phase using linear solvation energy relationships methodology indicate that the overall migration patterns in all six types of surfactant systems are different. This dissimilarity in migration pattern can be confirmed by plotting the logarithm of capacity factors of 36 test solutes in each surfactant system against log k' values in the SDS system (Figure 4.14). Figure 4.14 shows similarities and differences in retention behaviors between each surfactant system. Some correlations between the migration patterns are obvious since the hydrophobic interaction is the major factor governing the solute-pseudostationary phase interaction.
Figure 4.14a. Solute retention comparison between SDS and A) SDS, B) mono-SDUT, C) poly-SDUT for NHB (□), HBA (△), and HBD (○) solutes. Regression equations for all subset and main set solutes are shown on the right of each plot. Dashed line represents correlation line for the main set (36 solutes). (Fig. con’d.).
Figure 4.14b. D) SDS/mono-SDUT, E) SDS/poly-SDUT, and F) mono-SDUT/poly-SDUT for NHB (□), HBA (△), and HBD (○) solutes. Regression equations for all subset and main set solutes are shown on the right of each plot. Dashed line represents correlation line for the main set (36 solutes).
In Figure 4.14 A is presented the correlation of capacity factors of SDS system to show that when the selectivity of two surfactant systems is identical, the correlation coefficient ($R^2$) and the slope of the correlation line are unity, and the y-intercept is zero. Any deviation in these parameters (i.e., $R^2$, slope, and y-intercept) indicates the selectivity differences between surfactant systems. Figure 4.14 (B-F) clearly indicates that each system has a distinctive selectivity towards the main and/or subset solutes.

As the coefficients a in solvatochromic model (Table 4.7) suggest, the hydrogen bond accepting strengths of pseudostationary phases are ranked as: mono-SDUT > mono-SDUT/poly-SDUT > poly-SDUT; however, coefficients a for SDS, SDS/mono-SDUT, and SDS/poly-SDUT are not statistically significant. In solvation parameter model (Table 4.12), on the other hand, only two surfactant systems (i.e., mono-SDUT and mono-SDUT/poly-SDUT) have statistically significant a coefficients. With the largest positive coefficient a value in both models, mono-SDUT is the strongest hydrogen bond acceptor (i.e., base), followed by mono-SDUT/poly-SDUT and poly-SDUT, respectively. The carboxylate headgroups of mono- and poly-SDUT are weaker Brönsted-Lowry acids as compared to the sulfate headgroup of SDS. It is evident from coefficient a values in Tables 4.7, 4.12, and 4.21 that SDS and SDS containing mixture surfactant systems (i.e., SDS/mono-SDUT and SDS/poly-SDUT) are usually weaker hydrogen bond acceptors as compared to mono-SDUT. Figure 4.14 B confirms these results, that is, the HBD acid solutes show a clear deviation above the dashed line, which represents the linear regression data for the main solute set, indicating a strong interaction between the HBD solutes and mono-SDUT system. The HBA bases, however, show deviation below the dashed line (Figure 4.14 B), indicating a stronger interaction with SDS micelles. This is evident from larger (or less negative) coefficients b for SDS micelles in Tables 4.7, 4.12, and 4.20, which show that
HBA bases find the SDS micelles more acidic than the bulk aqueous buffer solution and mono-SDUT. Although the majority of the NHB solutes show only a slight affinity (i.e., fall on or near the dashed line) towards mono-SDUT and SDS, three solutes, i.e., p-xylene, chlorobenzene, and bromobenzene seem to interact strongly with mono-SDUT while toluene, ethylbenzene, and propylbenzene interact strongly with the SDS system. These phenomena of divergence in the NHB solutes may be due to the combined effect of more than one coefficient. As mentioned earlier, the NHB solutes have basic character due to the $\beta$ (Table 4.1) or $\sum \beta_2^0$ (Table 4.2) descriptor. The solutes with smaller $\beta$ or $\sum \beta_2^0$ values (e.g., chlorobenzene and bromobenzene) are relatively weak bases and tend to interact with mono-SDUT (basic surfactant) stronger than SDS (acidic surfactant). It is worth noting that when only NHB solutes were examined in both LSER models, the coefficient m value was larger in SDS system than in mono-SDUT indicating that NHB solutes find mono-SDUT surfactant more cohesive than SDS (Table 4.19). In other words, SDS provides more nonpolar, hydrocarbon-like sites for NHB solutes as compared to mono-SDUT.

Having the same headgroups as mono-SDUT, poly-SDUT possesses a weaker basic character than mono-SDUT surfactant due maybe to a smaller aggregation number resulting relatively less number of carboxylate headgroups on the surface of the poly-SDUT vesicles. In addition, possible partial hydrolysis of carboxylate headgroups during polymerization process and/or the change in the structural configuration of poly-SDUT may also be effective on the decrease of basicity. As mentioned above, the hydrogen bond accepting strength (i.e., basicity) of poly-SDUT system is less than that of mono-SDUT but more than that of SDS (Tables 4.7 and 4.12). Therefore, when compared to SDS, poly-SDUT interacts relatively more strongly with HBD acids (Figure 4.14 C). However, as seen in Figure 4.14.C, some HBD solutes fall below
the dashed line, that is, they interact with SDS stronger. This can be explained by the dipolarity term. As seen in Table 4.12, SDS provides marginally more dipolar microenvironment (less negative coefficient s) than poly-SDUT, thus, polar HBD solutes prefer SDS than relatively less polar poly-SDUT phase. Table 4.21 shows that the basicity characters of SDS and poly-SDUT obtained from twelve HBD solutes using solvatochromic model ($a_{SDS} > a_{poly-SDUT}$) disagree with those obtained from solvation parameter model ($a_{poly-SDUT} > a_{SDS}$). However, solvation parameter model in Table 4.21 agrees with both models (Tables 4.7 and 4.12) using the main set solutes where $a_{poly-SDUT} > a_{SDS}$. Since the coefficient b for SDS is higher (less negative) than that for poly-SDUT, the HBA bases find microenvironment in the SDS micelles more acidic than aqueous buffer, and retain poorly in poly-SDUT system. Looking at the coefficient m, one would expect slightly stronger interactions between NHB solutes and SDS, since the m value of SDS is close to (Table 4.7) or less (Table 4.12) than that of poly-SDUT. This indicates that there may be multiple types of solute-pseudostationary phase interactions that determine the solute retention. One possible explanation of stronger NHB interaction with poly-SDUT is the ability of this surfactant to interact with $n$- and/or $\pi$-electrons of the solutes easily (larger coefficient r, Table 4.12). Moreover, solvatochromic model using twelve NHB solutes provides larger m coefficient with poly-SDUT than SDS (Table 4.19). This indicates that NHB solutes find poly-SDUT vesicular microenvironment less cohesive (i.e., more nonpolar) as compared to SDS micellar microenvironment.

The coefficients a for SDS, SDS/mono-SDUT, and SDS/poly-SDUT surfactant systems are not statistically significant (Tables 4.7 and 4.12). Thus, as seen in Figure 4.14 D and E, there is a slight difference between NHB and HBD solutes for both surfactant systems. Figure 4.14 D shows that HBD acids show small deviations below the dashed line, that is, have slightly more
interactions with SDS than SDS/mono-SDUT. Likewise, correlation between SDS and SDS/poly-SDUT in Figure 4.14 E clearly shows that majority of the HBD solutes fall on the line indicating about the same selectivity for both surfactant systems. The different selectivity between SDS/mono-SDUT and SDS/poly-SDUT may be influenced partially by coefficient s. The SDS system provides more dipolar microenvironment (i.e., coefficient s is less negative) as compared to SDS/mono-SDUT and SDS/poly-SDUT systems, but SDS/poly-SDUT (s = -0.546) is more dipolar than SDS/mono-SDUT (s = -0.692) (Table 4.12). Consequently, polar HBD solutes tend to interact with SDS and SDS/poly-SDUT relatively stronger than SDS/mono-SDUT, as seen in Figure 4.14 D, E. Coefficients b values in Table 4.12 for SDS (-1.845), SDS/mono-SDUT (-2.431), and SDS/poly-SDUT (-2.163) show that the HBA bases find SDS system more acidic than SDS/mono-SDUT and SDS/poly-SDUT systems. Thus, HBA bases show deviations below the dashed line. The NHB solutes, on the other hand, fall on the line with exceptions of only a few solutes.

Similar to mono-SDUT, mono-SDUT/poly-SDUT mixed surfactant system is a strong hydrogen bond acceptor, as coefficient a suggests (Tables 4.7 and 4.12), thus interacts strongly with HBD acids (Figure 4.14 F). The HBA bases, on the other hand, have stronger tendency of interacting with SDS (i.e., more acidic) than mono-SDUT/poly-SDUT due generally to a less negative b coefficient of the former. This is illustrated in Figure 4.14 F where HBA solutes show an obvious deviation below the dashed line. The NHB solutes find SDS micelles more nonpolar and hence interact strongly due mostly to relatively larger coefficient m value.

The log k' values of the main set (Table 4.25), NHB (Table 4.26), HBA (Table 4.27), and HBD (Table 4.28) solutes for surfactant systems are plotted against each other, and the R^2, slope, and the y-intercept for each correlation line are listed in each respective table. There is no
selectivity difference between any surfactant systems when the $R^2$ and the slope of the correlation line are unity and the y-intercept is zero. As an example, the log $k'$ values of the same surfactant system (e.g., SDS) are plotted in Figure 4.14 A; the $R^2$, slope, and y-intercept values for all pseudostationary phases are listed in Tables 4.25 to 4.28.

Correlation between mono-SDUT and poly-SDUT reveals that NHB solutes interact with poly-SDUT stronger, while HBA and HBD solutes prefer mono-SDUT (plot not shown). As mono-SDUT and SDS/mono-SDUT surfactants compared, NHB solutes tend to be retained in the later and HBD in the former; however, there is no significant selectivity difference between these two surfactant systems for HBA solutes. The SDS/poly-SDUT surfactant system shows almost the same selectivity as SDS/poly-SDUT when compared with each other and mono-SDUT surfactant. A plot of solute retention in mono-SDUT versus mono-SDUT/poly-SDUT system shows that HBD solutes interact stronger with the former and the HBA solutes with the later. However, NHB solutes, with a few exceptions, show no differences in both surfactant systems. Poly-SDUT and SDS/mono-SDUT surfactant systems show almost the same selectivity towards all solute sets. When plotted against poly-SDUT system, SDS/poly-SDUT shows a slight affinity for HBA solutes, but no difference is observed for NHB and HBD solutes. The poly-SDUT, SDS/mono-SDUT, and SDS/poly-SDUT systems show very similar behaviors towards the NHB, HBA, and HBD solutes when each system is compared to mono-SDUT/poly-SDUT system separately. In all cases mono-SDUT/poly-SDUT interacts with the majority of HBA and HBD solutes stronger, but the other surfactants (i.e., poly-SDUT, SDS/mono-SDUT, and SDS/poly-SDUT) interact with NHB solutes and a few HBD (e.g., benzyl alcohol) and HBA (e.g., phenyl acetate, benzonitrile, 3-methylbenzyl alcohol) solutes more.
### Table 4.25. The $R^2$, slope, and y-intercept values of log $k'$ comparison plots (all solutes).

<table>
<thead>
<tr>
<th>Surfactant systems ▼</th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>Slope</td>
<td>Intercept</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS</td>
<td>1.0000</td>
<td>1.0000</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-SDUT</td>
<td>0.6976</td>
<td>1.0233</td>
<td>-0.6174</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly-SDUT</td>
<td>0.9162</td>
<td>1.0588</td>
<td>-0.6323</td>
<td>0.7738</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>SDS/mono-SDUT</td>
<td>0.9389</td>
<td>1.2740</td>
<td>-0.2773</td>
<td>0.6586</td>
<td>1.1620</td>
<td>0.4750</td>
</tr>
<tr>
<td>SDS/poly-SDUT</td>
<td>0.9689</td>
<td>1.1354</td>
<td>-0.3371</td>
<td>0.7052</td>
<td>1.0281</td>
<td>0.3318</td>
</tr>
<tr>
<td>Mono-SDUT/poly-SDUT</td>
<td>0.8624</td>
<td>0.8664</td>
<td>-0.7290</td>
<td>0.8695</td>
<td>0.7949</td>
<td>-0.5312</td>
</tr>
</tbody>
</table>

### Table 4.26. The $R^2$, slope, and y-intercept values of log $k'$ comparison plots (NHB solutes).

<table>
<thead>
<tr>
<th>Surfactant systems ▼</th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>Slope</td>
<td>Intercept</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS</td>
<td>1.0000</td>
<td>1.0000</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-SDUT</td>
<td>0.6160</td>
<td>0.9490</td>
<td>-0.6140</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Poly-SDUT</td>
<td>0.9691</td>
<td>1.1094</td>
<td>-0.5860</td>
<td>0.6969</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>SDS/mono-SDUT</td>
<td>0.9471</td>
<td>1.3680</td>
<td>-0.2540</td>
<td>0.5829</td>
<td>1.2194</td>
<td>0.4724</td>
</tr>
<tr>
<td>SDS/poly-SDUT</td>
<td>0.9795</td>
<td>1.2320</td>
<td>-0.3580</td>
<td>0.6302</td>
<td>1.0959</td>
<td>0.2964</td>
</tr>
<tr>
<td>Mono-SDUT/poly-SDUT</td>
<td>0.9754</td>
<td>0.8850</td>
<td>-0.8080</td>
<td>0.6676</td>
<td>0.7930</td>
<td>-0.6243</td>
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</table>
Table 4.27. The $R^2$, slope, and y-intercept values of log $k'$ comparison plots (HBA solutes).

<table>
<thead>
<tr>
<th>Surfactant systems ▶</th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>$R^2$&lt;br&gt;1.0000</td>
<td>$R^2$&lt;br&gt;1.0000</td>
<td>$R^2$&lt;br&gt;1.0000</td>
<td>$R^2$&lt;br&gt;1.0000</td>
<td>$R^2$&lt;br&gt;1.0000</td>
<td>$R^2$&lt;br&gt;1.0000</td>
</tr>
<tr>
<td>Mono-SDUT</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
</tr>
<tr>
<td>Poly-SDUT</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
</tr>
</tbody>
</table>

Table 4.28. The $R^2$, slope, and y-intercept values of log $k'$ comparison plots (HBD solutes).

<table>
<thead>
<tr>
<th>Surfactant systems ▶</th>
<th>SDS</th>
<th>Mono-SDUT</th>
<th>Poly-SDUT</th>
<th>SDS/mono-SDUT</th>
<th>SDS/poly-SDUT</th>
<th>Mono-SDUT/poly-SDUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>$R^2$&lt;br&gt;1.0000</td>
<td>$R^2$&lt;br&gt;1.0000</td>
<td>$R^2$&lt;br&gt;1.0000</td>
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<td>$R^2$&lt;br&gt;1.0000</td>
<td>$R^2$&lt;br&gt;1.0000</td>
</tr>
<tr>
<td>Mono-SDUT</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
<td>Slope&lt;br&gt;1.0000</td>
</tr>
<tr>
<td>Poly-SDUT</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
<td>Intercept&lt;br&gt;0.0000</td>
</tr>
</tbody>
</table>
4.4. Conclusions

Anionic surfactant systems with carboxylate (mono-SDUT and poly-SDUT) and sulfate (SDS) headgroups as well as mixed surfactant systems (SDS/mono-SDUT, SDS/poly-SDUT, and mono-SDUT/poly-SDUT) were applied as pseudostationary phases in MCE. The mono-SDUT and poly-SDUT were synthesized and characterized using various analytical techniques (Figures 4.1, 4.2, 4.3; and Tables 4.1, and 4.2). Two LSER models, i.e., solvatochromic and solvation parameter models (Equations 4.1 and 4.2) were successfully applied to investigate the effect of the type and composition of pseudostationary phases on the retention mechanism and selectivity in MCE. These models are helpful tools to understand the fundamental nature of the solute-surfactant interactions and to characterize the surfactant systems. The results obtained from the both models provide very comparable information, for example, in both models solute size (coefficient m) and hydrogen bond accepting ability (coefficient b) for all pseudostationary phases play the most important role in MCE retention (Tables 4.7 and 4.12) despite the numerical differences in the values for the solute descriptors and slight differences in the form of the equations for both models. However, when the magnitudes of the coefficients are compared for surfactant systems, some differences are obvious in both models (Tables 4.22 and 4.23). For instance, surfactant systems are ranked according to the values of coefficient m obtained from the main set solutes in solvatochromic model as following: SDS/mono-SDUT > SDS/poly-SDUT > mono-SDUT > poly-SDUT > SDS > mono-SDUT/poly-SDUT. In the solvatochromic model, on the other hand, the order for the first three surfactants is the same, but that for the last three ones is: SDS > mono-SDUT/poly-SDUT > poly-SDUT. It is clear that there is a difference in the order of the last three surfactant systems in both LSER models. As seen in Tables 4.22 and 4.23, analogous differences are seen in all other coefficients as well. Similar divergences
between the two LSER models are also seen when the subset solutes are used instead of the main set solutes (Tables 4.22 and 4.23). Omitting the outliers from the solute set improves the statistics (i.e., R, SE, and F) of the both models significantly (Tables 4.9 and 4.14) as compared to the original main set solutes (Tables 4.7 and 4.12). It is worth mentioning that the type of the outlier may vary in both models, e.g., toluene, ethylbenzene, propylbenzene, and 4-chloroaniline are the major four outliers in mono-SDUT system using the solvatochromic model, whereas ethylbenzene, propylbenzene, p-xylene, and 4-chloroanisole are the key outliers when the solvation parameter model is used (Tables 4.8 and 4.14).

The type and the number of solute have a significant effect on the system coefficients (Figures 4.8 and 4.9) and on the predicted log k' values (Figures 4.10 and 4.11) obtained from these coefficients for both LSER models. Although both models provide the same information, the solvation parameter model is found to provide much better both statistically and chemically sound results. This is evident when comparing the statistics (i.e., R, SE, and F values) of the solvation parameter model results in Table 4.12 with those for solvatochromic model results in Table 4.7. Similar superiority of the solvation parameter model is seen in results obtained from NHB, HBA, and HBD solutes in Tables 4.19-21. A better coefficient of determination for each surfactant system is obtained by solvation parameter model when experimental log k' values are plotted against predicted log k' values (Figure 4.6) than by solvatochromic model (Figure 4.4). Subset solutes (i.e., NHB, HBA, and HBD) were also examined for prediction of log k' values of the subset solutes and the main set solutes (Tables 4.19-21 and Figures 4.12-13). It is obvious from Figures 4.12-13 that always higher correlations are observed when a given type of solute set (e.g., NHB) is used for prediction of capacity factors of the same type of solute set (e.g., NHB). In other words, using the system constants obtained from NHB solutes provides better
predicted log k' values for NHB solutes (Figure 4.12 or 4.13 A1) for each surfactant system. Similarly, HBA solutes offer better predicted log k' values for HBA solutes (Figure 4.12 or 4.13 A2) and HBD solutes offer that for HBD set solutes (Figure 4.12 or 4.13 A3). It is critical to choose an appropriate solute set, which has to represent a wide range of solutes, for LSER methodology. It is worth noting that using only twelve NHB or HBA solutes, it is possible to predict capacity factors for thirty-six solutes with a high correlation of 0.9568 and 0.951 with poly-SDUT surfactant system using solvation parameter model and solvatochromic model, respectively.

The chemical selectivity differences between the six pseudostationary phases used in this study are compared in Figure 4.14 and Tables 4.25 to 4.28, where experimental log k' values of pseudostationary phases are plotted against each other. It is evident from the free energy transfer data for NHB solutes and the results of the two LSER models that hydrophobicity play an important role in solute-surfactant interaction; however, selectivity is mainly influenced by hydrogen bond accepting or donating ability of both pseudostationary phases and the solutes.

4.5. References


5.1. Introduction

Capillary electrophoresis (CE), or more specifically capillary zone electrophoresis (CZE), is a powerful technique for separation of charged molecules. However, the applicability of electrophoretic methods for both charged and neutral molecules simultaneously was achieved by introduction of a new analogous CE mode, i.e., micellar capillary electrophoresis (MCE). Also known as micellar electrokinetic chromatography, MCE was introduced by Terabe and coworkers in the early 1980s (1), and uses micelles as pseudo-stationary phases. The separation principle is based on the differential distribution of molecules between an aqueous buffer solution (so-called mobile phase) and a moving charged pseudostationary phase under the influence of an electric field. Sodium dodecyl sulfate (SDS) has been extensively used as pseudostationary phase in MCE.

Although successfully used as separation carriers for many applications, conventional micelles have some drawbacks as pseudostationary phases in MCE. First, normal micelles require higher surfactant concentrations, at least 2-10 times the critical micelle concentration (CMC), for an effective separation. High concentration of surfactant results in an increase in ionic strength of the system; thus, an applied voltage across the capillary causes Joule heating, which, in turn, causes the temperature inside the capillary to rise. The variation in temperature will cause, e.g., a change in the CMC of the surfactant and the viscosity of the separation buffer. These changes may give rise to serious irreproducibilities in the migration times. Second, the CMC of surfactants is also influenced by the surfactant concentration, pH and ionic strength of
the running buffer, and by additives to the micellar phases (2-4). Thus, any unexpected changes in these parameters will cause a change in micelle’s structure, which can demolish the reproducibility in MCE. Third, the separation of highly hydrophobic compounds is difficult because these compounds require high organic solvent content, which tend to disrupt the formation of the micelles, for the adjustment of the retention factors. Conventional micelles cannot tolerate high organic solvent contents due to the presence of dynamic equilibrium between the micelle and the free monomers (5-8). Fourth, the presence of the surfactants with low molecular weights in the running solution makes mass spectrometric detection difficult, that is, large signals from monomers will interfere with most MCE solutes in the low molecular mass region. In addition, accumulation of surfactants can cause fouling of the ion source, and limit the sensitivity in electrospray ionization/mass spectrometry (9-11). Finally, surfactant monomers in the running buffer will more likely form inclusion complexes with inclusion molecules (12). Therefore, complexation of free surfactant monomers with inclusion molecules will possibly interfere with complexation between the analyte and the inclusion molecules, which may result in a poor separation.

Several types of pseudostationary phases have been developed as alternatives to the conventional surfactant micelles. These include, but not limited to, neutral pseudostationary phase such as cyclodextrin (CD) polymers (13-15) and polyvinyl pyrrolidone (16-18); ionic pseudostationary phases such as anionic (19-21) and cationic (22-25) polymers; proteins (26-28); charged CDs (29-31); calixarenes (33-34); dendrimers (35-37); siloxane polymers (38-40); and achiral (41-49) as well as chiral (50-59) molecular micelles.

Molecular micelles (a.k.a. micelle polymers) have drawn considerable attention as potential pseudostationary phases in MCE. This is due mainly to their distinct advantages over
conventional micelles. These advantages include: molecular micelles 1) can be purified, unlike conventional micelles, due to the presence of covalent linkage between the monomer units; 2) have no or very low CMC, thus, they can be effective over a wide range of concentrations when used as pseudostationary phases; 3) are stable in the presence of high content of organic solvents or inclusion molecules due to the presence of covalent bonds between the surfactant aggregates; 4) can be used with inclusion molecules, e.g., CDs, without interfering with the formation of inclusion complexes between the analyte and the inclusion molecule; 5) can be modified with desired properties through the synthesis and/or polymerization processes.

The first successful application of an anionic molecular micelle, i.e., poly(sodium 10-undecylenate (poly-SUA), as a potential pseudostationary phase for the separation of alkyl phthalates and some polycyclic aromatic hydrocarbons (PAHs) in MCE was reported by Palmer et al. (41, 42). The same pseudostationary phase was also used for the successful separation of EPA’s sixteen priority pollutant PAHs using THF as an organic modifier (60). Although this surfactant provided high performance separation of a wide range of neutral compounds, its electrophoretic mobility is influenced drastically by the ionization of the carboxylated head groups, resulting in irreproducible analysis times. In addition, the solubility of this molecular micelle is limited by pH, i.e., it is not soluble below pH 7.0 due to the carboxylated head groups. To overcome these problems, our research group (61) and Palmer and Terabe (44, 45) synthesized a polymerized surfactant with a sulfate head group, i.e., poly(sodium 10-undecenyl sulfate) (poly-SUS). However, the latter studies used potassium persulfate as a free radical initiator for the polymerization process, which resulted in low synthetic yields and contamination of the product with sodium sulfate (62), whereas we used $^{60}$Co $\gamma$-irradiation in all our studies (46, 47, 61).
The first successful reports of the use of single amino acid based molecular micelles as chiral selectors for MCE were those of Wang and Warner and Dobashi et al. (50, 52). A major advantage of chiral molecular micelles is that different functionalities such as a variety of chiral head groups can be integrated into molecular micelles to manipulate selectivities. In addition, the availability of both D and L optical configurations of amino acid-based pseudostationary phases is particularly advantageous to determine enantiomeric impurities more accurately by reversal of the migration order of two enantiomers.

The main disadvantage of molecular micelles is that generally they are not commercially available; therefore, they must be synthesized. However, synthesis of most of the molecular micelles is straightforward. A major drawback of pseudostationary phases with carboxylated head group is that they are not soluble below pH 7.0, which limits their applicability over a wide pH ranges.

In this study we have synthesized sodium 10-undecenyl sulfate (SUS), an achiral surfactant, and sodium N-undecanoyl L-leucinate (SUL), a chiral surfactant. These two surfactants, then, were polymerized separately to form poly-SUS and poly(sodium N-undecanoyl L-leucinate) (poly-SUL); or together at various given molar ratios to produce a variety of co-polymerized molecular micelles (CoPMs) holding both chiral (i.e., leucinate) and achiral (i.e., sulfate) head groups. These CoPMs were applied as novel pseudostationary phases in MCE for separation of chiral and achiral molecules.

5.2. Experimental

5.2.1. Instrumentation

A Beckman P/ACE model 5510 capillary electrophoresis (CE) instrument (Fullerton, CA) was employed for MCE separations. This CE instrument was equipped with two sample
carousels (a 21-position inlet and 10-position outlet) for automatic sample/buffer change; a 0-30-kV high-voltage power supply; 200-, 214-, 254-, and 280-nm selectable wavelength filters for UV detection; a liquid thermostated capillary cartridge; and System Gold software for system control and data handling. The MCE separations were performed in a 57 cm total length (50 cm effective length) x 50-μm i.d. (367-μm o.d.) fused-silica capillary obtained from Polymicro Technologies (Tucson, AZ). The capillary in the CE instrument was thermostated by use of a fluoroorganic fluid. The detector time constant was 0.2 s.

5.2.2. Materials

Flunitrazepam, nitrazepam, clonazepam, temazepam, diazepam, oxazepam, lorazepam, and L-leucine 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) were also obtained from Sigma. N-hydroxysuccinimide, undecylenic acid, dicyclohexylcarbodiimide (DCC), HPLC grade ethyl acetate, disodium hydrogen phosphate, sodium bicarbonate, and sodium carbonate were all reagent grade and obtained from Aldrich (Milwaukee, WI). The undecylenyl alcohol, alkyl aryl ketone homologues, pyrene, chlorosulfonic acid, sodium dodecyl sulfate (SDS), and pyridine (PY) were of analytical reagent grade and were also purchased from Aldrich. All chemicals were used as received.

5.2.3. Synthesis of Molecular Micelles

5.2.3.1. Synthesis of Poly(Sodium Undecenyl Sulfate)

Sodium undecenyl sulfate (SUS) monomer was prepared according to Bergstrom's procedure (63). Details of the synthesis of SUS and poly-SUS are explained in Chapter 2 (part I) of this dissertation.
5.2.3.2. **Synthesis of Poly(Sodium Undecanoyl L-Leucinate)**

Sodium undecanoyl L-leucinate (SUL) was synthesized according to the procedure reported by Lapidot et al. (64). A schematic of the synthesis of SUL is shown in Figure 5.1. N-hydroxysuccinimide (62 mmol) was dissolved in ca. 280 mL dry ethyl acetate. Undecylenic acid (62 mmol) and a 1 M solution of dicyclohexylcarbodiimide in ca. 60 mL ethyl acetate were then added to the N-hydroxysuccinimide solution. The mixture was mixed overnight at room temperature. The by-product, dicyclohexylurea, a white precipitate, was discarded. Evaporating the product-containing organic solution by rotary evaporation yielded a yellowish oil. The yellowish oily product was then recrystallized using hot isopropyl alcohol.

About 15 mM L-leucine was dissolved in 150 mL doubly deionized water containing 20 mM of sodium bicarbonate. Fifteen mM of N-hydroxysuccinimide ester was dissolved in 150 mL THF and this solution was then added to L-leucine solution. The mixture was stirred vigorously for at least 16 hours at room temperature. After evaporation of organic content, the pH of aqueous solution was adjusted to about 8.0 using sodium bicarbonate. This solution was then filtered and acidified to pH 2.0 with concentrated HCl. The resulting white crystals, undecanoyl leucinic acid (ULA), were filtered and dried under vacuum. The sodium salt of ULA (i.e. SUL) was prepared in deionized water using equimolar amount of sodium bicarbonate. The solution was stirred until a clear solution is formed indicating the formation of SUL. After filtration, the SUL solution then freeze-dried to yield white crystals (SUL).

5.2.3.3. **Polymerization of Sodium Undecenyl Sulfate and Sodium Sodium Undecenoyl L-Leucinate**

Polymerization of the surfactants (i.e., SUS and SUL) was achieved by preparing a 100 mM solution of each surfactant in doubly deionized water. These solutions were then exposed to
a $^{60}$Co $\gamma$-ray source (~680 rad/h) for seven days for polymerization in micellar form. After polymerization is completed, the solutions were filtered to eliminate any solid contaminations.
and then lyophilized to yield white powders (i.e., poly-SUS and poly-SUL). The resulting molecular micelles were used without dialysis or further purifications.

5.2.3.4. Preparation of Co-Polymerized Molecular Micelles

Monomers of SUL and SUS were co-polymerized in micellar form to produce molecular micelles. Six different surfactant solutions were prepared in the following molar ratios of SUL/SUS: 100/0; 80/20; 60/40; 40/60; 20/80; and 0/100. As seen in Table 5.1, the six molar fractions of each surfactant are adjusted so that the final concentration of the solution is 100 mM and the volume is 100 mL. In other words, the sum of SUL and SUS concentrations in the system is 100 mM. To prepare a poly-(L₈S₂) co-polymerized molecular micelle, for example, 8 mmoles of SUL and 2 mmoles of SUS were mixed in 100 mL of deionized water. In this solution, the final concentration of SUL and SUS was 80 and 20 mM, respectively. The total concentration of both surfactants, however, was 100 mM. Similarly, all the six surfactant solutions were prepared and exposed to a ⁶⁰Co γ-ray source (680 rad/h) for seven days for polymerization in micellar forms.

Table 5.1. Molar ratios of SUL and SUS surfactant for polymerization as well as proposed names for each co-polymerized molecular micelle.

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</thead>
<tbody>
<tr>
<td>Mₛᵤₜ (mM)</td>
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<td>80</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Mₛᵤₛ (mM)</td>
<td>0</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

Mₛᵤₜ and Mₛᵤₛ are the molar fractions of SUL and SUS, respectively.

After irradiation, the purified solutions of CoPMs were lyophilized and dried under a vacuum. All CoPMs were applied as pseudostationary phases in MCE without any further purification or dialysis. A proposed configuration for CoPMs is shown in Figure 5.2.
5.2.4. Determination of Aggregation Number

The aggregation number of the surfactants was determined by the method proposed by Turro and Yekta (65), using Equation 4.3.

Fluorescence measurements were performed on a SPEX model F2T211 spectrophotometer. Pyrene and cetylpyridinium chloride (CPyrCl) were used as fluorescent probe and quencher, respectively. A 1.0x10⁻³ M stock solution of the probe was prepared in methanol. A 2.0x10⁻³ M stock solution of the quencher and a 2.0 % (w/v) of each of CoPM, poly-SUL, poly-SUS as well as SDS stock solutions were prepared separately in deionized water. A known volume of the probe stock solution was pipetted into a clean volumetric flask. Methanol was then evaporated by nitrogen gas and aqueous surfactant solution was added. The concentrations of the probe and the surfactant were 2.0x10⁻⁶ M and 2.0 % (w/v), respectively (probe solution 1). After sonicating for 90 minutes, solution 1 was stored in a dark area

Figure 5.2. Scheme for co-polymerization of SUL and SUS surfactants. Asterisk represents the chiral center of the surfactant. The m and n represent the mole fractions of SUL and SUS, respectively, in the mixture.
overnight to equilibrate, then was divided in half. One half was diluted with deionized water to give a 1.0 x 10^-6 M probe and 1.0 % (w/v) surfactant (probe solution 2), while the other half was mixed with quencher stock solution to make 1.0 x 10^-3 M quencher, 1.0 x 10^-6 M probe, and 1.0 % (w/v) surfactant (quencher solution). The quencher solution was added to the probe solution 2 in increasing increments of 25 µL and allowed 20 minutes for equilibration after addition of each quencher solution before fluorescence measurement. The decrease in emission spectra of the probe was recorded at 393.0 nm after each aliquot of the quencher solution was added and the logarithm of the intensity ratio I_0/I was plotted against the quencher concentration. The aggregation number, N, is obtained from the slope of the plot of ln (I_0/I) versus [Q] (i.e., N = Slope x [S_{tot}] – CMC).

5.2.5. Determination of Partial Specific Volume

Due to the difficulty of measuring the exact volume of a particle, instead, partial specific volume, \( \bar{v} \), is often used for the characterization of the substances of interest. The \( \bar{v} \) is defined as the increase in volume upon dissolving 1 gram of a dry material (e.g., surfactant) in a large volume of a solvent (e.g., water) when the mass of solvent, temperature, and pressure are held constant. The \( \bar{v} \) can be measured by analytical ultracentrifugation (66) or can be determined from a plot of the inverse of the density, \( 1/\rho \), of the aqueous surfactant solution versus the weight fraction, \( W \), of the surfactant according to Equation 5.1:

\[
\frac{1}{\rho} = \bar{v} + \rho \frac{\partial (1/\rho)}{\partial W}.
\]  \hspace{1cm} 5.1

The \( W \) value is defined as:

\[
W = \frac{m_w}{m_w + m_s}, \hspace{1cm} 5.2
\]
where $m_w$ and $m_s$ represent the masses of water and the surfactant, respectively. Seven different surfactant solutions (i.e., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 % w/v) were prepared in 20 mM phosphate buffer at pH 8.0 for density measurements. The $\overline{\nu}$ values of all surfactant systems used in this study were obtained as the y-intercept of the $1/\rho$ versus $W$ plots.

A high-precision Anton Paar USA (League City, TX), model DMA 58, digital density meter was used to perform density measurements. The principle of the technique, in brief, is: first, the period of oscillation ($T_1$) of a borosilicate glass U-shaped tube containing the sample is measured; then, the period of oscillation ($T_2$) of the U-shaped tube containing a reference material (e.g., water or air) with known density is measured. Equation 5.3 shows the relationship between the density difference between two media ($\rho_2-\rho_1$) and periods $T_1$ and $T_2$:

$$\rho_2 - \rho_1 = k(T_2^2 - T_1^2), \quad 5.3$$

where $k$ is an instrument constant. This constant is determined from instrumental calibration using doubly distilled water and air. The precision of the temperature-controlled system was better than ±0.005 °C.

5.2.6. Capillary Electrophoresis Procedure

New capillaries were prepared by use of a standard wash cycle of 1 M NaOH for 1 hour and 20 minutes of triply deionized water before use. Prior to each separation with the same surfactant the capillaries were rinsed 5 minutes with triply deionized water, 3 minutes with 0.1 M NaOH, and 3 minutes with separation buffer. Each day, the capillaries were reactivated by rinsing with 1 M NaOH (15 minutes), triply deionized water (2 minutes), and the running buffer (10 minutes). When the pseudostationary phase was changed the capillaries were reconditioned for 15 minutes with deionized water, 10 minutes with 0.1 M NaOH, and 5 minute with the separation buffer. Unless otherwise noted, the time for pressure injection was 2 seconds for most
separations. The cartridge temperature was varied for the separation of benzodiazepines and maintained at 20 °C for the separation of BNA, BNP, and BOH.

5.2.7. Preparation of Separation Buffers and Standard Solutions

Four 100 mM stock solutions of phosphate buffer (pH 3.0, 7.0, 8.0, and 9.0) were prepared by dissolving appropriate amount of sodium hydrogenphosphate or sodium dihydrogenphosphate. Solutions were adjusted to desired pHs using phosphoric acid or NaOH solutions and all solutions were refrigerated after each use. The solution of each pseudostationary phase was prepared by first dissolving 0.1 gram of the surfactant in 5.0 mL of deionized water. Two mL of appropriate 100 mM phosphate stock buffer was then added to this solution. Lastly, the final volume was adjusted to 10.0 mL with deionized water. After a thorough mixing in a sonicator for 10 minutes, the final running buffers were filtered through a 0.45-μm syringe filter (Nalgene, Rochester, NY) then sonicated for 3 more minutes before capillary electrophoretic experiments. All stock analyte solutions were prepared in methanol: deionized water (1:1) at concentrations of ca. 0.15-0.30 mM each.

5.2.8. Calculations

The capacity factor, k’, of neutral solutes was measured according to the following formula (67):

$$k' = \frac{t_R - t_{eo}}{t_{eo} \left[ 1 - \left( \frac{t_R}{t_{psp}} \right) \right]}$$

where \( t_R \), \( t_{eo} \) and \( t_{psp} \) are the migration times of the retained analyte, the electroosmotic flow (EOF), and the pseudostationary phase, respectively. Methanol was used as the \( t_{eo} \) marker and was measured from the time of injection to the first deviation from the baseline. Decanophenone was used as tracer for \( t_{psp} \).
The elution window is defined as \( t_{psp}/t_{eo} \). The apparent electrophoretic mobility of pseudostationary phase is calculated according to Equation 5.5:

\[
\mu_{app} = \frac{l_t l_d}{V t_{pp}},
\]

where \( l_t \) is the total length of the capillary (cm), \( l_d \) is the length of the capillary from injector to detector (cm), \( V \) is the applied voltage (V), and retention times are in second (s). To calculate the electroosmotic mobility of the buffer solution, \( t_{psp} \) term in Equation 5.5 is replaced with \( t_{eo} \). The effective electrophoretic mobility of the pseudostationary phase \( (\mu_{ep}) \) can be calculated from \( \mu_{app} \), and \( \mu_{eo} \) (i.e., \( \mu_{ep} = \mu_{app} - \mu_{eo} \)).

The methylene selectivity, \( \alpha_{CH_2} \), was calculated from the antilogarithm of the slope of the regression line of log \( k' \) vs. carbon number of alkyl phenyl ketone homologous series.

5.3. Results and Discussion

5.3.1. Partial Specific Volume of Pseudostationary Phases

As mentioned above, the exact volume of a particle (e.g., micelle) is difficult to measure. Instead, \( \bar{\nu} \) has been used more often. The \( \bar{\nu} \) values of the seven pseudostationary phases are obtained as the y-intercept of the \( 1/\rho \) versus \( W \) plots. Figure 5.3 shows a representative plot of \( 1/\rho \) versus \( W \) for all the pseudostationary phases at 25 \(^{0}\)C in 20 mM phosphate buffer. The \( \bar{\nu} \) values at different temperatures are listed in Table 5.2. As seen in Table 5.2, the partial specific volume of poly-SUL is the lowest among all surfactant, indicating that this surfactant has a relatively more compact structure than other surfactant systems. The SDS system, on the other hand, has the highest partial specific volume, due particularly to a longer hydrophobic chain (C12), followed by poly-SUS. This indicates that SDS and poly-SUS have more open and flexible structures than CoPMs.
Table 5.2. Partial specific volume (mL/g) of pseudostationary phases as a function of temperature.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>Temperature (°C)</th>
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<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Poly-SUL</td>
<td>0.693</td>
</tr>
<tr>
<td>Poly-L₈S₂</td>
<td>0.727</td>
</tr>
<tr>
<td>Poly-L₆S₄</td>
<td>0.736</td>
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<tr>
<td>Poly-L₄S₆</td>
<td>0.743</td>
</tr>
<tr>
<td>Poly-L₂S₈</td>
<td>0.747</td>
</tr>
<tr>
<td>Poly-SUS</td>
<td>0.763</td>
</tr>
<tr>
<td>SDS</td>
<td>0.844</td>
</tr>
</tbody>
</table>

Figure 5.3. A representative plot of $1/\rho$ as a function of W % for all pseudostationary phases in 20 mM phosphate buffer at 25 °C. Legends are shown in the plot.

Table 5.2. Partial specific volume (mL/g) of pseudostationary phases as a function of temperature.
It is interesting to note that as the $\bar{v}$ values of all CoPMs become larger the molar ratio of SUS increased. In addition, the $\bar{v}$ values are slightly increased as the temperature is elevated from $15$ to $40\,^0\text{C}$, due probably to a decrease in viscosity of the surfactant solution that leads the surfactant to be more flexible (i.e., less compact). However, a few inconsistencies are seen in the $\bar{v}$ values of poly-SUL (at $20\,^0\text{C}$), Poly-L$_2$S$_8$ (at $40\,^0\text{C}$) and poly-SUS (at $30\,^0\text{C}$). Observed discrepancies may be attributed to possible conformational changes in these pseudostationary phases at those particular temperatures.

5.3.2. Aggregation Number of Pseudostationary Phases

The aggregation number, $N$, for each molecular micelle and SDS is obtained from the slope of the $\ln(I_0/I)$ versus $[Q]$ plot (Figure 5.4). Knowing the slope of $\ln(I_0/I)$ versus $[Q]$ plot,
total surfactant concentration and the CMC of the surfactants, the aggregation number can be calculated from Equation 5.6. \( N = \text{Slope} \times (\left[ S_{\text{tot}} \right] - \text{CMC}) \) equation gives the aggregation number.

\[
N = \text{Slope} \times (\left[ S_{\text{tot}} \right] - \text{CMC}).
\]

Aggregation numbers for the seven pseudostationary phases are listed in Table 5.3. Poly-SUL, poly-L_8S_2, poly-L_6S_4, and SDS have similar aggregation numbers (~ 62). Poly-SUS and poly-L_2S_8 have the lowest (~21) and the highest (~68) aggregation numbers, respectively, while poly-L_4S_6 has a moderate aggregation number. There is no significant relationship between the molar concentration of the SUL or SUS and the aggregation numbers of the molecular micelles.

**Table 5.3. Aggregation numbers\(^a\) of the pseudostationary phases used in this study.**

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<thead>
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<tbody>
<tr>
<td></td>
<td>61</td>
<td>61</td>
<td>62</td>
<td>49</td>
<td>68</td>
<td>21</td>
<td>62</td>
</tr>
</tbody>
</table>

\(^a\)Determined in deionized water at room temperature by fluorescence quenching method

### 5.3.3. Methylene-Group Selectivity of Pseudostationary Phases

The methylene selectivity, \( \alpha_{\text{CH}_2} \), of each pseudostationary phase was calculated from the antilogarithm of the slope of the regression line of log \( k' \) versus carbon number of alkyl phenyl ketone homologous series. Figure 5.5 shows a representative plot of log \( k' \) versus carbon number of alkyl phenyl ketones. The \( \alpha_{\text{CH}_2} \) values for the seven pseudostationary phases at six different temperatures (i.e., 15, 20, 25, 30, 35, and 40 °C) are listed in Table 5.4. In general, \( \alpha_{\text{CH}_2} \) decreases as the temperature is increased from 15 to 40 °C. An increase in temperature can have an impact upon: 1) net charge, stability, and configuration of analyte; 2) the pH, viscosity, and
the conductivity of the separation buffer, and; 3) the chemical equilibria such as ionization of capillary surface, micelle-analyte partitioning as well as EOF (69, 70). The SDS micelles provide the most and poly-SUL provides the least hydrophobic environment for alkyl phenyl ketones under studied experimental conditions. It should be noted that SDS has largest $\alpha_{\text{CH}_2}$ values (i.e., more hydrophobic character) around 20-25 $^\circ$C. Similarly, poly- SUS and poly-SUL show relatively higher hydrophobic characters (higher $\alpha_{\text{CH}_2}$ values) around 15-20 and 30 $^\circ$C, respectively. In general, these two sulfated surfactants show relatively higher hydrophobic character while all other surfactants show relatively polar character. The hydrophocity of all CoMPs are very similar despite the different SUL and SUS molar ratios.

Figure 5.5. Linear relationship between log $k'$ versus carbon number of alkyl phenyl ketone homologous series. The MCE conditions: pseudostationary phase concentration is 1.0 % (w/v) each; 20 mM phosphate buffer (pH 8.0); +25 kV applied voltage and 25 $^\circ$C temperature for separation; UV detection at 254 nm. Alkyl phenyl ketones are: acetophenone (C8), propiophenone (C9), butyrophenone (C10), valerophenone (C11), hexanophenone (C12), heptanophenone (C13), and octanophenone (C14).
Table 5.4. Methylene-group selectivity$^a$ of pseudostationary phases as a function of temperature.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>Temperature (°C)</th>
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<tbody>
<tr>
<td></td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>30</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>Poly-SUL</td>
<td>2.14</td>
<td>2.15</td>
<td>2.13</td>
<td>2.20</td>
<td>2.15</td>
<td>2.14</td>
</tr>
<tr>
<td>Poly-L₈S₂</td>
<td>2.26</td>
<td>2.18</td>
<td>2.18</td>
<td>2.16</td>
<td>2.17</td>
<td>2.13</td>
</tr>
<tr>
<td>Poly-L₆S₄</td>
<td>2.20</td>
<td>2.17</td>
<td>2.16</td>
<td>2.16</td>
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<tr>
<td>Poly-L₄S₆</td>
<td>2.22</td>
<td>2.16</td>
<td>2.18</td>
<td>2.15</td>
<td>2.15</td>
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<tr>
<td>Poly-L₂S₈</td>
<td>2.22</td>
<td>2.19</td>
<td>2.15</td>
<td>2.17</td>
<td>2.17</td>
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</tr>
<tr>
<td>Poly-SUS</td>
<td>2.32</td>
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<td>2.25</td>
<td>2.26</td>
<td>2.21</td>
<td>2.19</td>
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<tr>
<td>SDS</td>
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<td>2.60</td>
<td>2.57</td>
<td>2.55</td>
<td>2.53</td>
<td>2.50</td>
</tr>
</tbody>
</table>

$^a$Calculated from the antilogarithm of the slope of the regression line of log $k'$ versus carbon number of alkyl phenyl ketones (C₈-C₁₄).

5.3.4. Mobilities and Migration-Time Window of Pseudostationary phases

The electroosmotic mobility, apparent electrophoretic mobility, and effective electrophoretic mobility of seven pseudostationary phases are shown in Tables 5.5, 5.6, and 5.7, respectively. Migration-time window values are given in Table 5.8. The surfactant systems with sulfate head groups, i.e., poly-SUS and SDS, have largest $\mu_{eo}$ values (4.61x10⁻⁴ and 4.47x10⁻⁴ cm² V⁻¹s⁻¹, respectively). Poly-SUL system, on the contrary, provides least $\mu_{eo}$ (3.95x10⁻⁴ cm² V⁻¹s⁻¹) (Table 5.5). It is noteworthy that the $\mu_{eo}$ increases as the mole fraction of sulfate head group in the CoPMs is increased. The variations in $\mu_{eo}$ for different surfactant systems can be attributed to a variety of parameters including viscosity of the surfactant solution, zeta potential of both capillary walls and pseudostationary phase, and charge density on the capillary wall. An increase in temperature leads an increase in the $\mu_{eo}$ of all surfactant systems due mainly to a decrease in the viscosity of buffer solution and an increase in the charge density on the capillary walls as a result of silanol group ionization.
Table 5.5. Effect of temperature on electroosmotic mobilities\(^a\), \(\mu_{eo}\), of seven MCE systems in 20 mM phosphate buffer, pH 8.0.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>Temperature (°C)</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
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<tbody>
<tr>
<td>Poly-SUL</td>
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<td></td>
<td></td>
<td>3.95</td>
<td>4.38</td>
<td>4.96</td>
<td>5.55</td>
<td>6.20</td>
<td>6.79</td>
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<td>Poly-L(_8)S(_2)</td>
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<td>4.04</td>
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<td>5.71</td>
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<td></td>
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<td>4.06</td>
<td>4.70</td>
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<td>5.96</td>
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<td>4.14</td>
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<td>5.40</td>
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<td>5.06</td>
<td>5.69</td>
<td>6.33</td>
<td>6.96</td>
<td>7.58</td>
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</tbody>
</table>

\(^a\) x10\(^4\) cm\(^2\) V\(^{-1}\) s\(^{-1}\); calculated from Equation 5.5 where \(t_{psp}\) was replaced with \(t_{eo}\); methanol was used for determination of \(t_{eo}\)

Table 5.6. Effect of temperature on apparent electrophoretic mobilities\(^a\), \(\mu_{app}\), of seven MCE systems in 20 mM phosphate buffer, pH 8.0.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>Temperature (°C)</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
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<td>Poly-SUL</td>
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<tr>
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<td></td>
<td>0.82</td>
<td>0.90</td>
<td>1.05</td>
<td>1.24</td>
<td>1.58</td>
<td>1.56</td>
</tr>
<tr>
<td>Poly-L(_8)S(_2)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.97</td>
<td>1.07</td>
<td>1.23</td>
<td>1.46</td>
<td>1.61</td>
<td>1.74</td>
</tr>
<tr>
<td>Poly-L(_6)S(_4)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.93</td>
<td>1.02</td>
<td>1.22</td>
<td>1.35</td>
<td>1.54</td>
<td>1.72</td>
</tr>
<tr>
<td>Poly-L(_4)S(_6)</td>
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<tr>
<td></td>
<td></td>
<td>0.97</td>
<td>1.08</td>
<td>1.28</td>
<td>1.50</td>
<td>1.69</td>
<td>1.83</td>
</tr>
<tr>
<td>Poly-L(_2)S(_8)</td>
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<tr>
<td></td>
<td></td>
<td>0.96</td>
<td>1.12</td>
<td>1.30</td>
<td>1.45</td>
<td>1.65</td>
<td>1.82</td>
</tr>
<tr>
<td>Poly-SUS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.18</td>
<td>1.24</td>
<td>1.47</td>
<td>1.67</td>
<td>1.90</td>
<td>2.13</td>
</tr>
<tr>
<td>SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.39</td>
<td>1.45</td>
<td>1.74</td>
<td>1.96</td>
<td>2.17</td>
<td>2.40</td>
</tr>
</tbody>
</table>

\(^a\) x10\(^4\) cm\(^2\) V\(^{-1}\) s\(^{-1}\); calculated from Equation 5.5; decanophenone was used for determination of \(t_{psp}\)

The \(\mu_{app}\) was calculated using Equation 5.5 (Table 5.6) and \(\mu_{cp}\) was determined from \(\mu_{eo}\) and \(\mu_{app}\) values (\(\mu_{cp} = \mu_{app} - \mu_{eo}\)). As seen in Table 5.7, the \(\mu_{cp}\) values for anionic pseudostationary phases
are negative because micelles move toward the positive electrode, anode, (i.e., the opposite direction of EOF movement). However, the net mobility of micelles is positive (Table 5.6) because the mobility of the EOF (i.e., $\mu_{eo}$, Table 5.5) is larger than the $\mu_{ep}$ of micelles. Thus, stronger EOF drags the micelles toward the negative electrode, cathode.

Table 5.7. Effect of temperature on effective electrophoretic mobilities$^a$, $\mu_{ep}$, of seven MCE systems in 20 mM phosphate buffer, pH 8.0.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>Temperature ($^0$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Poly-L$_8$S$_2$</td>
<td>-3.07</td>
</tr>
<tr>
<td>Poly-L$_6$S$_4$</td>
<td>-3.01</td>
</tr>
<tr>
<td>Poly-L$_4$S$_6$</td>
<td>-3.09</td>
</tr>
<tr>
<td>Poly-L$_2$S$_8$</td>
<td>-3.18</td>
</tr>
<tr>
<td>Poly-SUS</td>
<td>-3.43</td>
</tr>
<tr>
<td>SDS</td>
<td>-3.08</td>
</tr>
</tbody>
</table>

$^a$ $x10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$; calculated from $\mu_{ep} = \mu_{app} - \mu_{eo}$

Table 5.8. Effect of temperature on migration-time window, $t_{psp}/t_{eo}$, of seven MCE systems in 20 mM phosphate buffer, pH 8.0.

<table>
<thead>
<tr>
<th>Pseudostationary phases</th>
<th>Temperature ($^0$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Poly-SUL</td>
<td>4.84</td>
</tr>
<tr>
<td>Poly-L$_8$S$_2$</td>
<td>4.18</td>
</tr>
<tr>
<td>Poly-L$_6$S$_4$</td>
<td>4.24</td>
</tr>
<tr>
<td>Poly-L$_2$S$_8$</td>
<td>4.30</td>
</tr>
<tr>
<td>Poly-SUS</td>
<td>3.90</td>
</tr>
<tr>
<td>SDS</td>
<td>3.22</td>
</tr>
</tbody>
</table>
According to Table 5.7, the $\mu_{ep}$ of poly-SUS is the largest of the pseudostationary phases used in this study; however, due to the larger $\mu_{eo}$, the migration-time window is relatively smaller than those of CoPM systems but larger than that of SDS (Table 5.8). At higher temperatures $\mu_{ep}$ tend to increase for all pseudostationary phases, because $\mu_{eo}$ also increases, the migration-time window does not improve as expected from $\mu_{ep}$ values. Poly-SUL provides a widest migration window, which allows the analysis of a larger number of solutes in each electrophoretic separation, as compared to other pseudostationary phases. The SDS system, on the contrary, provides a narrowest migration-time window (Table 5.8).

### 5.3.5. Application of Molecular Micelles as Chiral Selectors

One of the most tedious and difficult problems in analytical chemistry is the separation of enantiomeric mixtures into optical individuals. Since the first successful separation of diastereomeric crystals of different forms out of a racemate by Louis Pasteur, resolution of the optical antipodes has been attracting a considerable attention. The first direct separation of enantiomeric compounds by gas chromatography (GC) using a chiral stationary phase was reported in 1966 (71). During the 1980s, other separation techniques, e.g., high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC), have also been utilized for chiral separations. In the early 1990s, the search for optimum systems, shorter analysis times, and better efficiencies in enantiomeric separation, modern electromigration methods such as CZE and MCE were emerged and used extensively as promising separation tools. Providing an enormous freedom of choice among various chiral selectors, MCE is increasingly complementing and competing with other chiral separation methods.

The first application of an optically active amino acid derivative synthetic surfactant as an chiral selector was reported by Dobashi et al. using sodium dodecanoyl L-valinate (SD-L-Val)
Another well-known monomeric chiral selector, (R)- or (S)-N-dodecanoyl carbonyl-valine (DDCV), was introduced by Mazzeo and coworkers (74, 75). Molecular (or polymeric) chiral surfactants with a single amino acid as a chiral head group were first introduced as pseudostationary phases for MCE by Wang and Warner and Dobashi et al. (50, 52). In addition, dipeptide molecular surfactants were also introduced by Shamsi and Warner to further investigate the chromatographic performances of amino acid based chiral pseudostationary phases (54). The major drawback of amino acid-based pseudostationary phase is their solubility problems below pH 7.0 due to carboxylate group on the amino acid head groups. Mazzeo et al. introduced a chiral monomeric surfactant with a sulfate head group to overcome this solubility problem (75).

Poly-SUL is a single amino acid-based molecular micelle that is used as chiral selector for enantiomeric separations. The major problem with poly-SUL is that it is not soluble at acidic pHs (pH <6.9). Poly-SUS, on the other hand, is an achiral surfactant that is well soluble in acidic, neutral, and basic pHs. To investigate their applicability over a wide pH range, poly-SUL and the CoPM of SUL and SUS were used as chiral selectors for separation of binaphthyl derivatives, i.e., BNA, BNP, and BOH.

5.3.5.1. **Enantiomeric Separation of Binaphthyl Derivatives**

The chemical structures of Binaphthyl derivatives and their enantiomeric separation using poly-SUL and four CoPMs are shown in Figure 5.6 and Figure 5.7, respectively.

![Chemical structures of binaphthyl derivatives.](image-url)
Figure 5.7. Chiral separation of BNA, BNP, and BOH using A) poly-SUL, B) poly-L₈S₂, C) poly-L₄S₄, D) poly-L₄S₆, and E) poly-L₂S₈. Conditions: 20 mM phosphate buffer (pH 7.0); 25 kV applied voltage; 20 °C temperature; current: 30-43 µA.
As seen in Figure 5.7, all three binaphthyl derivatives were baseline resolved using poly-SUL and poly-L_{8S2} molecular micelles. The resolutions of BNA, BNP, and BOH using poly-SUL (at pH 7.0) are 2.02, 2.05, and 1.59, respectively (Figure 5.7 A). It is interesting to note that Poly-L_{8S2} provides better resolutions for BNP (2.21) and BOH (1.76), but gives a slightly lower resolution for BNA (1.88) as compared to poly-SUL (Figure 5.7 B). As the SUL/SUS (i.e., chiral/achiral) ratio of CoPM decreases, the resolution of binaphthyl derivatives is deteriorated, because the number of chiral sites available within CoPM for interaction with solutes is diminished. The resolution of BNP decreased from 1.95 (poly-L_{6S4}) to 1.30 (poly-L_{4S6}) and 0.74 (poly-L_{2S8}) as seen in Figure 5.7 C-E. Poly-L_{6S4} was able to give a partial resolution of BNA (0.89) and BOH (0.96); however, these two binaphthyl derivatives were resolved by neither poly-L_{4S6} nor poly-L_{2S8}. It is worth noting that BNA and BOH were comigrated in poly-L_{6S4} (Figure 5.7 D), and their migration order is reversed in poly-L_{2S8} (Figure 5.7 E).

### 5.3.5.2. Effect of pH on Enantiometric Separation of Binaphthyl Derivatives

As mentioned earlier, poly-SUL is not soluble in acidic pHs. One of the objectives of this study is to increase the solubility of amino acid-based chiral surfactant (e.g., poly-SUL) in a wide pH range, especially in acidic pHs. To achieve this goal, mixed micelles of SUL and SUS, a highly soluble anionic surfactant, at various molar ratios were prepared and co-polymerized (Table 5.1 and Figure 5.2). The solubilities of poly-SUL, poly-SUS and four CoPMs were tested in acidic pHs. Poly-SUL is not soluble below pH ~6.9. Poly-SUS and poly-L_{2S8}, on the contrary, were soluble in entire acidic pHs. The solubility limits of poly-L_{8S2}, poly-L_{6S4}, and poly-L_{4S6} were pH 4.0, 1.7, and 1.6, respectively. The effect of pH on separation of binaphthyl derivatives is shown in Figure 5.8. As can be seen in Figure 5.8, at pH 9.0, poly-SUL separates BNA, BNP, and BOH successfully with resolutions of 2.54, 2.12, and 1.86, respectively (Figure
As a chiral selector, poly-L₈S₂ works better at pH 7.0 as compared to other pHs studied.

Comparison of poly-L₈S₂ and poly-SUL at pH 7.0 and 8.0 reveals that the former provides

Figure 5.8. Comparison of resolution values in five pseudostationary phases at pH 9.0 (A), 8.0 (B), 7.0 (C), and 3.0 (D) Conditions: 20 mM phosphate buffer; applied voltage of +25 (A-C) or –25 (D) kV; temperature of 20°C.

5.8 A). As a chiral selector, poly-L₈S₂ works better at pH 7.0 as compared to other pHs studied.

Comparison of poly-L₈S₂ and poly-SUL at pH 7.0 and 8.0 reveals that the former provides
relatively better enantiomeric separations of BNP (2.21 vs. 2.05 at pH 7.0 and 2.14 vs. 1.97 at pH 8.0) and BOH (1.76 vs. 1.59 at pH 7.0 and 1.41 vs. 1.05 at pH 8.0), while the later separates BNA slightly better at both pHs (2.02 vs. 1.88 at pH 7.0 and 1.48 vs. 1.40 at pH 8.0). Poly-L₆S₄ performs weaker enantiomeric separations of BNA and BOH at all pHs studied as compared to poly-SUL and poly-L₈S₂. However, resolution of BNP in poly-L₆S₄ (1.95, 1.96, and 1.98 at pH 7.0, 8.0, and 9.0, respectively) is comparable with that in poly-SUL and poly-L₈S₂. Further increase in sulfate head group in CoPM pseudostationary phases results in poorer separations. This can be seen in poly-L₄S₆ and poly-L₂S₈ in which the molar fraction of sulfate is more than that of leucinate. These two CoPMs cannot separate either BNA or BOH, but separates BNP with reasonable resolutions (Figure 5.8 A-D). The electropherograms in Figure 5.7 show that BOH and BNA interact stronger (i.e., elute longer) with pseudostationary phases than BNP. It has been postulated by Billiot and Warner that BOH and BNA are relatively more hydrophobic and interact with the hydrophobic region (i.e., the palisade layer) of the surfactant, whereas BNP prefers the outer layer (i.e., Stern layer) (55). Data presented here are in agreement with their results.

Presence of sulfate head group along with chiral leucinate increases not only the solubility of chiral surfactant but also improves the resolution of chiral analytes. This is true when sulfate molar fraction is lower than that of chiral head group. For example, 20 % sulfate and 80 % leucinate (e.g., poly-L₈S₂) seems promising. Higher concentrations of sulfate head groups; however, diminish chiral separation due mainly to steric effects. Figure 5.8 D shows the enantiomeric separation of BNA, BNP, and BOH at pH 3.0. Only poly-L₆S₄ and poly-L₄S₆ provide the separation of BNP alone. These two CoPMs were not successful in separating BNA and BOH. Poly-SUL and poly-L₈S₂ are not soluble while poly-L₂S₈ does not give any separation
at pH 3.0. This anomalous behavior may be attributed to the fact that the carboxylate group of leucinate is less ionized at lower pHs and may have an effect on the solutes’ ability to interact with the chiral center of the CoPMs. This preliminary separation of BNP in acidic pH shows that these types of surfactants may prove to be effective, particularly in the acidic pH range of 2-5 where most of the cationic drugs are usually separated.

5.3.5.3. Separation of Benzodiazepines

Benzodiazepines are a class of compounds and are important in pharmacological, clinical and forensic studies. They have been widely used in psychotherapy as anticonvulsants, sedatives, muscle relaxants and hypnotics (76). They present some side effects such as dizziness, interaction with alcohol, and their abuse can produce a dry-dependence. In addition, after prolonged use, abrupt cessation of benzodiazepines can cause status epilepticus, a life-threatening condition. Thus, due to their dependence capacity and hazard of abuse, the analysis of such compounds is imperative (77).

A variety of methods for the detection and determination of benzodiazepines exist in the literature. A review of chromatographic methods, e.g., HPLC, GC, and thin layer chromatography (TLC), of these compounds has been presented by Rizzo (78). Capillary electrochromatography (CEC), a hybrid technique that uses features of liquid chromatography and CE, has also been used for separation of benzodiazepines (79-82). As an alternative to CEC, open-tubular CEC (OT-CEC) using a molecular micelle was employed for the separation of seven benzodiazepines (83). The MCE has been more widely used for separation of benzodiazepines than capillary zone electrophoresis and CEC (84-88).

Chemical structures, pKa values, and numerical designations for each of the seven benzodiazepines used in this study are shown in Figure 5.9. All benzodiazepines are neutral at
pH 8.0, as can be seen from their $pK_a$ values, and have similar hydrophobicities. Electropherograms of the seven benzodiazepines using the seven pseudostationary phases are compared at 20 °C in Figures 5.10. A wider elution window between the first and the last eluting benzodiazepines (tL-tF) in 20 mM phosphate buffer (pH 8.0) at 20 °C was obtained with poly-L6S4 (ca. 5.45 minutes) (Figure 5.10 C). The $t_{LB}$-$t_{FB}$ values for poly-SUL, poly-L6S4, poly-L4S6, poly-L2S8, poly-SUL, and SDS are 5.22, 5.09, 4.24, 3.43, 1.89, and 1.14, respectively. Shorter

![Chemical structures](image)

1) Flunitrazepam ($pK_a = 1.4$)
2) Nitrazepam ($pK_a = 3.2, 10.8$)
3) Clonazepam ($pK_a = 1.5, 10.5$)
4) Temazepam ($pK_a = 1.6$)
5) Diazepam ($pK_a = 3.3$)
6) Oxazepam ($pK_a = 1.7$)
7) Lorazepam ($pK_a = 1.3, 11.5$)

**Figure 5.9.** Chemical structures, $pK_a$ data, and numerical designations for each of the seven benzodiazepines examined in this study. Temazepam, oxazepam, and lorazepam are chiral while the rest are achiral. The $pK_a$ values are from References 86, 88, and 89.
elution times of benzodiazepines were obtained with poly-SUL, poly-L₈S₂, and SDS; however, unlike the later, the former molecular micelles provide a better separation of the seven solutes (Figure 5.10 A, B and G). Thus, the interaction between the benzodiazepines and these three pseudostationary phases is relatively weaker. Longer retention times, on the other hand, were achieved with poly-L₆S₄, poly-L₄S₆, and poly-L₂S₈ indicating a stronger interaction with benzodiazepines. Having the same head group, poly-SUS and SDS gave a separation of six out of seven benzodiazepines. However, the former provided a better resolution between adjacent peaks with longer migration times than the latter (Figure 5.10 F and G).

Distinct selectivity differences are observed between pseudostationary phases as seen in Figure 5.10 A-G. The total number of benzodiazepines separated was 7 using poly-SUL, poly-L₈S₂, poly-L₆S₄, poly-L₄S₆, and poly-L₂S₈ (Figure 5.10 A-E), but was 6 using poly-SUS and SDS (Figure 5.10 F and G). The elution order of the seven solutes is flunitrazepam, nitrazepam, clonazepam, temazepam, diazepam, oxazepam, and lorazepam, peaks 1 through 7 in electropherogram shown in Figure 5.10 A. The peak 6 (i.e., oxazepam) eluted faster, that is, ahead of peak 5 (i.e., diazepam) when poly-L₈S₂ was used. Poly-L₆S₄ and poly-L₄S₂ had similar selectivities, however, the resolution between peak 6 and peak 5 is significantly higher with the former, while that between peak 5 and 7 is higher with the later. In poly-SUL, poly-L₈S₂, and poly-L₆S₄, lorazepam (i.e., peak 7) is the most retained solute (Figure 5.10 A-C).

Comparing the electropherograms of poly-L₄S₆ and poly-L₂S₈ shows that lorazepam elutes faster than diazepam, but the resolution between these two solutes is better using poly-L₂S₈ (Figure 5.10 D and E). It should also be mentioned that the resolution between the first and the second peaks (i.e., flunitrazepam and nitrazepam) gets poorer as the ratio of sulfate head groups of CoPM is increased. For example, poly-SUL provided a higher resolution between...
Figure 5.10a. Comparison of A) poly-SUL, B) poly-L₈S₂, C) poly-L₆S₄, D) poly-L₄S₆ for the separation of seven benzodiazepines. The MEC conditions: 1.0% (w/v) each surfactant in 20 mM phosphate buffer (pH 8.0); pressure injection for 2 seconds; +25 kV applied voltage for separation; 20 °C temperature; UV detection at 254 nm. Peak identifications are same as Figure 5.8. (Fig. con’d.).
Figure 5.10b. E) poly-L$_2$S$_8$, F) poly-SUS, and D) SDS for the separation of seven benzodiazepines. The MCE conditions: 1.0 % (w/v) each surfactant in 20 mM phosphate buffer (pH 8.0); pressure injection for 2 seconds; +25 kV applied voltage for separation; 20 °C temperature; UV detection at 254 nm. Peak identifications are same as Figure 5.8.
these two solutes while poly-L$_2$S$_8$ gave a lower resolution (Figures 5.10 A and E). Using SDS improved the baseline resolution of flunitrazepam and nitrazepam (i.e., peaks 1 and 2); however, these two benzodiazepines were coeluted when poly-SUS was used. On the other hand, temazepam and oxazepam (i.e., peaks 4 and 6) were partially resolved in presence of poly-SUS, while no separation of these two solutes was observed using SDS.

5.3.5.3.1. Effect of Temperature on Separation of Benzodiazepines

A substantial reduction in elution times of the solutes is expected as the temperature is increased due to a decrease in the viscosity of the separation buffer. Tables 5.9 to 5.15 provide retention times and their relative standard deviation (% RSD) values at 15 to 40 °C for the seven pseudostationary phases. A reduction in retention times of the benzodiazepines is observed in all

**Table 5.9. Effect of temperature on average retention times (minutes) of benzodiazepines using poly-SUL$^a$.**

<table>
<thead>
<tr>
<th>Benzodiazepines</th>
<th>Temperature (°C)</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flunitrazepam</td>
<td></td>
<td>8.55 (1.52)$^b$</td>
<td>7.48 (0.01)</td>
<td>6.54 (0.76)</td>
<td>5.78 (1.51)</td>
<td>5.05 (0.14)</td>
<td>4.50 (0.40)</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td></td>
<td>9.79 (1.97)</td>
<td>8.47 (0.02)</td>
<td>7.31 (0.88)</td>
<td>6.39 (1.71)</td>
<td>5.51 (0.17)</td>
<td>4.86 (0.47)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td></td>
<td>10.88 (2.35)</td>
<td>9.42 (0.01)</td>
<td>8.10 (0.91)</td>
<td>7.05 (1.88)</td>
<td>6.04 (0.19)</td>
<td>5.30 (0.49)</td>
</tr>
<tr>
<td>Temazepam$^c$ 1</td>
<td></td>
<td>11.97 (2.75)</td>
<td>10.58 (0.06)</td>
<td>9.24 (0.98)</td>
<td>8.13 (2.24)</td>
<td>7.00 (0.26)</td>
<td>6.19 (0.57)</td>
</tr>
<tr>
<td>Temazepam$^c$ 2</td>
<td></td>
<td>12.15 (2.82)</td>
<td>10.74 (0.08)</td>
<td>9.36 (0.98)</td>
<td>8.22 (2.27)</td>
<td>7.07 (0.27)</td>
<td>6.24 (0.54)</td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td>13.43 (3.27)</td>
<td>11.94 (0.14)</td>
<td>10.38 (1.01)</td>
<td>9.12 (2.51)</td>
<td>7.81 (0.29)</td>
<td>6.90 (0.61)</td>
</tr>
<tr>
<td>Oxazepam$^c$ 1</td>
<td></td>
<td>13.86 (3.57)</td>
<td>12.23 (0.20)</td>
<td>10.54 (1.09)</td>
<td>9.18 (2.49)</td>
<td>7.81 (0.29)</td>
<td>6.83 (0.66)</td>
</tr>
<tr>
<td>Oxazepam$^c$ 2</td>
<td></td>
<td>13.96 (3.62)</td>
<td>12.32 (0.20)</td>
<td>10.62 (1.10)</td>
<td>9.22 (2.57)</td>
<td>7.81 (0.29)</td>
<td>6.83 (0.66)</td>
</tr>
<tr>
<td>Lorazepam$^c$ 1</td>
<td></td>
<td>14.24 (3.59)</td>
<td>12.64 (0.21)</td>
<td>10.92 (1.10)</td>
<td>9.54 (2.66)</td>
<td>8.13 (0.34)</td>
<td>7.13 (0.66)</td>
</tr>
<tr>
<td>Lorazepam$^c$ 2</td>
<td></td>
<td>14.32 (3.63)</td>
<td>12.70 (0.22)</td>
<td>10.98 (1.08)</td>
<td>9.57 (2.67)</td>
<td>8.13 (0.34)</td>
<td>7.13 (0.66)</td>
</tr>
</tbody>
</table>

$^a$Separation conditions: 1.0 % poly-SUL (w/v); 20 mM phosphate buffer (pH 8.0); +25 applied voltage; UV detection at 254 nm. Relative standard deviation (% RSD) values were calculated from at least three consecutive runs, RSD values are given in parenthesis; $^c$enantiomers of temazepam, oxazepam, and lorazepam.
Table 5.10. Effect of temperature on average retention times (minutes) of benzodiazepines using poly-L_8S_2\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Benzodiazepines</th>
<th>Temperature (\textdegree C)</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td></td>
<td>9.87 (1.41)\textsuperscript{b}</td>
<td>8.45 (0.39)</td>
<td>7.23 (0.34)</td>
<td>6.39 (0.01)</td>
<td>5.64 (0.17)</td>
<td>4.92 (0.01)</td>
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<tr>
<td>Nitrazepam</td>
<td></td>
<td>10.94 (1.65)</td>
<td>9.25 (0.47)</td>
<td>7.82 (0.41)</td>
<td>6.86 (0.01)</td>
<td>6.00 (0.21)</td>
<td>5.18 (0.02)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td></td>
<td>12.10 (1.74)</td>
<td>10.20 (0.60)</td>
<td>8.56 (0.43)</td>
<td>7.52 (0.06)</td>
<td>6.56 (0.22)</td>
<td>5.62 (0.05)</td>
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<tr>
<td>Temazepam\textsuperscript{c} 1</td>
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<td>13.90 (2.01)</td>
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<td>9.93 (0.47)</td>
<td>8.89 (0.07)</td>
<td>7.82 (0.41)</td>
<td>6.68 (0.14)</td>
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<td>Temazepam\textsuperscript{c} 2</td>
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<td>14.04 (1.99)</td>
<td>11.91 (0.74)</td>
<td>10.02 (0.45)</td>
<td>8.96 (0.08)</td>
<td>7.87 (0.42)</td>
<td>6.68 (0.14)</td>
</tr>
<tr>
<td>Diazepam</td>
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<td>15.46 (2.20)</td>
<td>13.14 (0.76)</td>
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<td>9.84 (0.14)</td>
<td>8.66 (0.47)</td>
<td>7.36 (0.14)</td>
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<td>Oxazepam\textsuperscript{d}</td>
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<td>8.43 (0.47)</td>
<td>7.10 (0.18)</td>
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<tr>
<td>Lorazepam\textsuperscript{d}</td>
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<td>15.98 (2.30)</td>
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<td>10.05 (0.16)</td>
<td>8.81 (0.51)</td>
<td>7.44 (0.14)</td>
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\textsuperscript{a}Separation conditions: 1.0 % poly-L_8S_2 (w/v); 20 mM phosphate buffer (pH 8.0); ±25 applied voltage; UV detection at 254 nm. \textsuperscript{b}Relative standard deviation (% RSD) values were calculated from at least three consecutive runs, RSD values are given in parenthesis; \textsuperscript{c}enantiomers of temazepam; \textsuperscript{d}no enantiomeric separation.

Table 5.11. Effect of temperature on average retention times (minutes) of benzodiazepines using poly-L_6S_4\textsuperscript{a}.

<table>
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<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td></td>
<td>12.29 (1.46)\textsuperscript{b}</td>
<td>10.14 (0.01)</td>
<td>8.66 (0.48)</td>
<td>7.37 (0.63)</td>
<td>6.54 (0.41)</td>
<td>5.75 (0.10)</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td></td>
<td>13.23 (1.70)</td>
<td>10.86 (0.14)</td>
<td>9.16 (0.60)</td>
<td>7.70 (0.68)</td>
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<td>5.94 (0.13)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td></td>
<td>14.47 (1.87)</td>
<td>11.90 (0.08)</td>
<td>10.03 (0.65)</td>
<td>8.39 (0.84)</td>
<td>7.41 (0.52)</td>
<td>6.46 (0.15)</td>
</tr>
<tr>
<td>Temazepam\textsuperscript{c} 1</td>
<td></td>
<td>16.73 (2.12)</td>
<td>13.99 (0.29)</td>
<td>11.95 (0.88)</td>
<td>10.00 (0.82)</td>
<td>8.95 (0.79)</td>
<td>7.87 (0.37)</td>
</tr>
<tr>
<td>Temazepam\textsuperscript{c} 2</td>
<td></td>
<td>16.83 (2.14)</td>
<td>14.07 (0.28)</td>
<td>12.00 (0.90)</td>
<td>10.04 (0.85)</td>
<td>8.97 (0.77)</td>
<td>7.87 (0.37)</td>
</tr>
<tr>
<td>Diazepam</td>
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<td>18.30 (2.24)</td>
<td>15.44 (0.10)</td>
<td>13.14 (0.95)</td>
<td>10.97 (0.81)</td>
<td>9.83 (0.97)</td>
<td>8.62 (0.45)</td>
</tr>
<tr>
<td>Oxazepam\textsuperscript{d}</td>
<td></td>
<td>17.91 (2.29)</td>
<td>14.97 (0.33)</td>
<td>12.71 (0.98)</td>
<td>10.54 (0.84)</td>
<td>9.40 (0.88)</td>
<td>8.21 (0.43)</td>
</tr>
<tr>
<td>Lorazepam\textsuperscript{d}</td>
<td></td>
<td>18.53 (2.29)</td>
<td>15.59 (0.19)</td>
<td>13.25 (1.02)</td>
<td>11.01 (0.83)</td>
<td>9.83 (0.97)</td>
<td>8.62 (0.45)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Separation conditions: 1.0 % poly-L_6S_4 (w/v); 20 mM phosphate buffer (pH 8.0); ±25 applied voltage; UV detection at 254 nm. \textsuperscript{b}Relative standard deviation (% RSD) values were calculated from at least three consecutive runs, RSD values are given in parenthesis; \textsuperscript{c}enantiomers of temazepam; \textsuperscript{d}no enantiomeric separation.
Table 5.12. Effect of temperature on average retention times (minutes) of benzodiazepines using poly-L₄S₆.

<table>
<thead>
<tr>
<th>Benzodiazepines ▼</th>
<th>Temperature (°C)</th>
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</thead>
<tbody>
<tr>
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<td>20</td>
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</tr>
<tr>
<td>Flunitrazepam</td>
<td>13.58(0.85)b</td>
<td>11.24(0.04)</td>
<td>9.57(0.25)</td>
<td>8.06(2.44)</td>
<td>7.20(0.28)</td>
<td>6.31(0.24)</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>14.09(0.99)</td>
<td>11.62(0.09)</td>
<td>9.83(0.31)</td>
<td>8.22(2.55)</td>
<td>7.31(0.30)</td>
<td>6.37(0.25)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>15.06(1.26)</td>
<td>12.49(0.08)</td>
<td>10.56(0.40)</td>
<td>8.83(2.67)</td>
<td>7.86(0.38)</td>
<td>6.85(0.28)</td>
</tr>
<tr>
<td>Temazepam</td>
<td>17.01(1.76)</td>
<td>14.41(0.07)</td>
<td>12.26(0.65)</td>
<td>10.34(2.83)</td>
<td>9.28(0.55)</td>
<td>8.17(0.34)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>18.14(1.98)</td>
<td>15.48(0.16)</td>
<td>13.17(0.72)</td>
<td>11.13(2.81)</td>
<td>10.01(0.61)</td>
<td>8.83(0.33)</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>17.60(1.90)</td>
<td>14.87(0.20)</td>
<td>12.64(0.67)</td>
<td>10.63(2.89)</td>
<td>9.52(0.58)</td>
<td>8.35(0.35)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>18.09(2.02)</td>
<td>15.36(0.23)</td>
<td>13.09(0.73)</td>
<td>11.04(2.83)</td>
<td>9.91(0.62)</td>
<td>8.72(0.35)</td>
</tr>
</tbody>
</table>

Separation conditions: 1.0 % poly-L₄S₆ (w/v); 20 mM phosphate buffer (pH 8.0); +25 applied voltage; UV detection at 254 nm. ^bRelative standard deviation (% RSD) values were calculated from at least three consecutive runs, RSD values are given in parenthesis; ^cno enantiomeric separation

Table 5.13. Effect of temperature on average retention times (minutes) of benzodiazepines using poly-L₂S₈.

<table>
<thead>
<tr>
<th>Benzodiazepines ▼</th>
<th>Temperature (°C)</th>
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<th></th>
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<tbody>
<tr>
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<td>20</td>
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</tr>
<tr>
<td>Flunitrazepam</td>
<td>14.75(0.58)b</td>
<td>12.27(0.94)</td>
<td>10.62(0.26)</td>
<td>9.10(0.27)</td>
<td>7.96(0.50)</td>
<td>7.02(0.15)</td>
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<tr>
<td>Nitrazepam</td>
<td>15.00(0.60)</td>
<td>12.42(0.97)</td>
<td>10.71(0.28)</td>
<td>9.13(0.27)</td>
<td>7.96(0.50)</td>
<td>6.98(0.14)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>15.80(0.66)</td>
<td>13.14(1.07)</td>
<td>11.37(0.40)</td>
<td>9.71(0.33)</td>
<td>8.48(0.54)</td>
<td>7.44(0.20)</td>
</tr>
<tr>
<td>Temazepam</td>
<td>17.59(0.77)</td>
<td>14.84(1.35)</td>
<td>13.00(0.62)</td>
<td>11.20(0.43)</td>
<td>9.88(0.61)</td>
<td>8.74(0.38)</td>
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<tr>
<td>Diazepam</td>
<td>18.50(0.81)</td>
<td>15.69(1.47)</td>
<td>13.78(0.73)</td>
<td>11.90(0.46)</td>
<td>10.53(0.66)</td>
<td>9.34(0.46)</td>
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<td>Oxazepam</td>
<td>17.91(0.81)</td>
<td>15.11(1.42)</td>
<td>13.21(0.68)</td>
<td>11.36(0.45)</td>
<td>10.00(0.64)</td>
<td>8.83(0.41)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>18.33(0.82)</td>
<td>15.53(1.48)</td>
<td>13.62(0.73)</td>
<td>11.74(0.46)</td>
<td>10.37(0.64)</td>
<td>9.18(0.45)</td>
</tr>
</tbody>
</table>

Separation conditions: 1.0 % poly-L₂S₈ (w/v); 20 mM phosphate buffer (pH 8.0); +25 applied voltage; UV detection at 254 nm. ^bRelative standard deviation (% RSD) values were calculated from at least three consecutive runs, RSD values are given in parenthesis; ^cno enantiomeric separation
Table 5.14. Effect of temperature on average retention times (minutes) of benzodiazepines using poly-SUS$^a$.

<table>
<thead>
<tr>
<th>Benzodiazepines ▼</th>
<th>Temperature (°C)</th>
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<th></th>
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<tr>
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</tr>
<tr>
<td></td>
<td>▼</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>13.67 (0.66)$^b$</td>
<td>12.92 (0.46)</td>
<td>10.96 (0.38)</td>
<td>9.26 (1.56)</td>
<td>8.05 (1.43)</td>
<td>7.18 (0.51)</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>13.67 (0.66)</td>
<td>12.92 (0.46)</td>
<td>10.91 (0.37)</td>
<td>9.19 (1.56)</td>
<td>7.97 (1.44)</td>
<td>7.08 (0.52)</td>
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<tr>
<td>Clonazepam</td>
<td>14.00 (0.64)</td>
<td>13.28 (0.49)</td>
<td>11.24 (0.40)</td>
<td>9.49 (1.51)</td>
<td>8.25 (1.33)</td>
<td>7.36 (0.53)</td>
</tr>
<tr>
<td>Temazepam$^c$</td>
<td>14.99 (0.47)</td>
<td>14.36 (0.49)</td>
<td>12.25 (0.53)</td>
<td>10.43 (1.40)</td>
<td>9.17 (0.98)</td>
<td>8.24 (0.50)</td>
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<tr>
<td>Diazepam</td>
<td>15.41 (0.47)</td>
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<td>12.65 (0.59)</td>
<td>10.80 (1.39)</td>
<td>9.53 (0.84)</td>
<td>8.59 (0.50)</td>
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<tr>
<td>Oxazepam$^c$</td>
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<td>14.42 (0.66)</td>
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<td>10.45 (1.40)</td>
<td>9.17 (0.98)</td>
<td>8.24 (0.50)</td>
</tr>
<tr>
<td>Lorazepam$^c$</td>
<td>15.26 (0.43)</td>
<td>14.65 (0.72)</td>
<td>12.50 (0.56)</td>
<td>10.67 (1.37)</td>
<td>9.40 (0.90)</td>
<td>8.46 (0.50)</td>
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</table>

$^a$Separation conditions: 1.0 % poly-SUS (w/v); 20 mM phosphate buffer (pH 8.0); +25 applied voltage; UV detection at 254 nm. $^b$Relative standard deviation (% RSD) values were calculated from at least three consecutive runs, RSD values are given in parenthesis; $^c$no enantiomeric separation

Table 5.15. Effect of temperature on average retention times (minutes) of benzodiazepines using SDS$^a$.

<table>
<thead>
<tr>
<th>Benzodiazepines ▼</th>
<th>Temperature (°C)</th>
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<td>▼</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>12.7 (0.20)$^b$</td>
<td>11.8 (0.81)</td>
<td>10.0 (0.30)</td>
<td>8.7 (0.82)</td>
<td>7.8 (0.51)</td>
<td>7.0 (0.26)</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>12.6 (0.24)</td>
<td>11.6 (0.77)</td>
<td>9.9 (0.30)</td>
<td>8.6 (0.88)</td>
<td>7.7 (0.52)</td>
<td>6.8 (0.36)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>12.8 (0.16)</td>
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<td>10.1 (0.25)</td>
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<td>7.9 (0.51)</td>
<td>7.0 (0.42)</td>
</tr>
<tr>
<td>Temazepam$^c$</td>
<td>13.5 (0.04)</td>
<td>12.6 (0.89)</td>
<td>10.8 (0.09)</td>
<td>9.5 (0.46)</td>
<td>8.5 (0.53)</td>
<td>7.7 (0.33)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>13.7 (0.07)</td>
<td>12.8 (0.91)</td>
<td>10.9 (0.03)</td>
<td>9.6 (0.39)</td>
<td>8.7 (0.52)</td>
<td>7.9 (0.32)</td>
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<tr>
<td>Oxazepam$^c$</td>
<td>13.5 (0.02)</td>
<td>12.6 (0.89)</td>
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<td>9.5 (0.46)</td>
<td>8.5 (0.53)</td>
<td>7.7 (0.33)</td>
</tr>
<tr>
<td>Lorazepam$^c$</td>
<td>13.6 (0.07)</td>
<td>12.7 (0.91)</td>
<td>10.9 (0.02)</td>
<td>9.6 (0.41)</td>
<td>8.6 (0.52)</td>
<td>7.8 (0.27)</td>
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</tbody>
</table>

$^a$Separation conditions: 1.0 % SDS (w/v); 20 mM phosphate buffer (pH 8.0); +25 applied voltage; UV detection at 254 nm. $^b$Relative standard deviation (% RSD) values were calculated from at least three consecutive runs, RSD values are given in parenthesis; $^c$no enantiomeric separation
surfactant systems as the temperature is increased from 15 to 40 °C. This is because the benzodiazepines are not well soluble in relatively more viscose buffer solutions at low temperatures. Thus, stronger interactions of the analytes with the pseudostationary phases are expected, leading to a longer retention of the analytes. At elevated temperatures, however, solubility of the benzodiazepines is increased in the buffer solution. In addition, the hydrogen bonding between benzodiazepines and the pseudostationary phases is weakened. As a result, retention times of the analytes are decreased. Effect of temperature on separation windows (\(t_{p sp} - t_{co}\) and \(t_{L} - t_{F}\)) is shown in Figure 5.11. A gradual decrease in the separation windows is observed

![Graph showing the effect of temperature on separation windows](image)

**Figure 5.11.** Effect of temperature on separation windows: A) difference between \(t_{p sp}\) and \(t_{co}\) and B) difference between last eluted benzodiazepine (\(t_L\)) and the first eluted benzodiazepine (\(t_F\)) as a function of temperature.
as temperature is elevated from 15 to 40 °C (Figure 5.11 A and B). The reduction of separation windows in poly-SUS and SDS, however, is less pronounced than that in CoPMs. This may indicate that the complex formed between benzodiazepines and poly-SUS or SDS is affected less by temperature as compared to CoPMs.

Electropherograms in Figure 5.12 shows the separation of benzodiazepines at 40 °C. Comparison of Figures 5.10 and 5.12 reveals some noticeable differences in migration orders of benzodiazepines. For instance, reversal in migration order of diazepam (peak 5) and oxazepam (peak 6) is observed at 40 °C using poly-SUL (Figure 5.12 A). Although there is no difference in migration order of benzodiazepines using poly-L8S2, the resolution between diazepam and oxazepam (peaks 5 and 6) is increased, while that between lorazepam and diazepam (peaks 7 and 5) is decreased at 40 °C (Figure 5.12 B) as compared to resolutions at 20 °C (Figure 5.10 B). Lorazepam and diazepam are baseline resolved at 20 °C; however, they are coeluted at 40 °C using poly-L6S4 (Figure 5.12 C). In addition, an increase in resolution between lorazepam/diazepam pair and oxazepam, but a decrease in resolution between flunitrazepam and nitrazepam (peaks 1 and 2) is observed at 40 °C using poly-L6S4. Higher temperature, i.e. 40 °C, improved the resolution between lorazepam and diazepam significantly, but resolution between flunitrazepam and nitrazepam suffered using poly-L4S6, as seen in Figure 5.12 D. Migration of flunitrazepam and nitrazepam is reversed by high temperature using poly-L2S8 (Figure 5.12 E).

At 20 °C, the selectivity of poly-SUS and SDS is different than that at 40 °C as can be seen in Figures 5.10 and 5.12 F, G. No change in migration order of the analytes is observed in SDS at both temperatures; however, an increase in resolution between benzodiazepines is evident at 40 °C. In poly-SUS, on the other hand, partial resolution between temazepam and oxazepam is deteriorated, but flunitrazepam and nitrazepam are baseline separated at 40 °C.
Figure 5.12a. Comparison of A) poly-SUL, B) poly-L₈S₂, C) poly-L₆S₄, D) poly-L₈S₂ for the separation of seven benzodiazepines. The MCE conditions: 1.0% (w/v) each surfactant in 20 mM phosphate buffer (pH 8.0); pressure injection for 2 seconds; +25 kV applied voltage for separation; 40 °C temperature; UV detection at 254 nm. Peak identifications are same as Figure 5.8. (Fig. con’d.).
Figure 5.12b. E) poly-L$_2$S$_8$, F) poly-SUS, and D) SDS for the separation of seven benzodiazepines. The MCE conditions: 1.0 % (w/v) each surfactant in 20 mM phosphate buffer (pH 8.0); pressure injection for 2 seconds; +25 kV applied voltage for separation; 40 $^\circ$C temperature; UV detection at 254 nm. Peak identifications are same as Figure 5.8.
The effect of temperature on selectivity for the seven pseudostationary phases is shown in Figure 5.13. Selectivity values were determined as the retention time ratios of a pair of adjacent analytes, i.e. \( t_{R2}/t_{R1} \). Selectivity is 1.00 when \( t_{R2} = t_{R1} \), e.g. solutes comigrate. If \( t_{R2} > t_{R1} \) or \( t_{R2} < t_{R1} \), selectivity is greater or smaller than 1.00, respectively. In Figure 5.13 A, selectivity value for oxazepam 1: diazepam pair remains greater than 1.00 up to 35 °C showing that oxazepam is eluting longer than diazepam. At 35 °C, however, selectivity becomes 1.00 indicating comigration of the two analytes. A reversal in migration order of these two benzodiazepines is observed at 40 °C where selectivity value is less than 1.00. Due to this reversal migration, selectivity value for lorazepam 1: oxazepam 2 in poly-SUL increases as temperature is elevated. Similar trend is seen for the same analyte pairs with poly-L_8S_2 molecular micelle (Figure 5.13 B). Below 20 °C, selectivity is greater than 1.00; however, migration order is reversed and the gap between these two analytes gets wider as the temperature is increased. As mentioned earlier, flunitrazepam and nitrazepam are comigrated in poly-SUS molecular micelle at 15 and 20 °C (Figure 5.13 F). At higher temperatures, however, resolution between these two benzodiazepines increases, flunitrazepam eluting longer than nitrazepam. In general, resolution between temazepam 1 and clonitrazepam increases by increasing temperature in all pseudostationary phases. On the contrary, resolution between flunitrazepam and nitrazepam is decreased by increasing temperature in all pseudostationary phases (Figure 5.13 A-E) except in poly-SUS and SDS, where resolution increases by an increase in temperature (Figure 5.13 F and G). It should be mentioned that resolution between clonazipam : nitrazepam, diazepam : temazepam 2, and lorazepam 1 : oxazepam 2 is affected relatively less by temperature in all pseudostationary phases studied. The effect of temperature on the enantiomeric separation of temazepam, oxazepam, and lorazepam will be discussed below.
Figure 5.13. Selectivity versus temperature plots for A) poly-SUL, B) poly-L$_6$S$_2$, C) poly-L$_6$S$_4$, D) poly-L$_4$S$_6$, E) poly-L$_2$S$_8$, F) poly-SUS, and G) SDS. Legends are shown on the top of the figure.
5.3.5.3.2. Enantioseparation of Temazepam, Oxazepam, and Lorazepam

Among the seven benzodiazepines examined in this study, only three (i.e., temazepam, oxazepam, and lorazepam) have asymmetric carbon (Figure 5.9). Although these three benzodiazepines possess similar aromatic skeletons, the major difference is the number and the type of the substituents attached to the aromatic ring. The only difference between temazepam and the other two benzodiazepines (i.e., oxazepam and lorazepam) is the methyl group located on the nitrogen on the seven-member ring of temazepam and the chlorine on the ortho position of the lower benzene ring of lorazepam.

Out of five chiral molecular micelles (i.e., poly-SUL, poly-L_8S_2, poly-L_6S_4, poly-L_4S_6, and poly-L_2S_8) only the first three provided enantioseparation of temazepam (Figures 5.10 and 5.14 A). However, enantiomers of oxazepam and lorazepam were partially resolved using only poly-SUL under experimental conditions studied (Figures 5.10 and 5.14). Better enantiomeric separations were achieved using poly-SUL at 15 °C. Resolution values for temazepam at 15 °C are 2.74, 1.92, and 1.21, using poly-SUL, poly-L_8S_2, and poly-L_6S_4, respectively. The resolution of enantiomers deteriorates upon increasing temperature due partly to racemization of the analyte. As seen in Figure 5.14, no enantiomeric separation of temazepam is observed at 40 °C. For oxazepam and lorazepam, however, no chiral separation is successful above 25 °C.

5.3.6. Measurement of Thermodynamic Quantities of Micellar Solubilization

The capacity factor, k', (Equation 5.4), is related to the distribution coefficient, K, by Equation 5.7:

\[ k = K \left( \frac{V_{psp}}{V_{aq}} \right), \]

where \( V_{psp}/V_{aq} \) is the phase ratio, \( \beta \), i.e., the volume of the pseudostationary phase to the volume
Figure 5.14. Effect of temperature and pseudostationary phase type on enantiomeric separation of A) temazepam, B) oxazepam, and C) lorazepam. Separation conditions: 1.0 % (w/v) each surfactant in 20 mM phosphate buffer (pH 8.0); +25 kV applied voltage for separation; temperature varied; UV detection at 254 nm.
of the remaining aqueous phase. The $\beta$ can be determined using the following equation (3):

$$\beta = \frac{\bar{v}(C_{psp} - CMC)}{1 + \bar{v}(C_{psp} - CMC)}, \quad 5.8$$

where $\bar{v}$, $C_{psp}$, and $CMC$ are the partial specific volume, the concentration, and the critical micelle concentration of the pseudostationary phase, respectively. The $K$ at different temperatures should follow the van’t Hoff equation:

$$\ln K = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}, \quad 5.9$$

where $\Delta H^0$ and $\Delta S^0$ are the enthalpy and entropy change, respectively, associated with the transfer of the analyte from the aqueous buffer solution to the pseudostationary phase, $R$ is the gas constant, and $T$ is the absolute temperature.

The $\Delta H^0$ and $\Delta S^0$ were determined from the slope ($-\Delta H^0/R$) and y-intercept ($\Delta S^0/R$) of the ln $K$ versus $1/T$ plots. The Gibbs free energy, $\Delta G^0$, associated with the transfer of the analyte from the aqueous solution to the pseudostationary phase can be calculated according to Equation 5.10:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad 5.10$$

A plot of the ln $K$ versus $1/T$ is expected to be linear if the heat capacity change upon solute transfer is zero and the phase ratio, $\beta$, is independent of temperature over the temperature range studied. Plots of most of the benzodiazepines, however, are not linear as seen in Figure 5.15. The nonlinearity of the van’t Hoff plots for the benzodiazepines may also be due to entropy controlled factors involved in the complexation between the analytes and the pseudostationary phases (90). The linearity of the van’t Hoff plots indicate an enthalpy-entropy compensation. In other word, the solute enthalpy change with temperature is counterbalanced by
Figure 5.15. Dependence of ln K of benzodiazepines on the 1/T using A) poly-SUL, B) poly-L₈₅₂, C) poly-L₆₄, D) poly-L₄₆₄, E) poly-L₈₂₈, F) poly-SUS, and G) SDS. Legends are shown in the figure. Separation conditions are same as Figure 5.14.
a commensurate change in entropy (91). As seen in Figure 5.15, at temperatures between 20 and 30 °C distinct deviation from linearity in van’t Hoff plots is observed for almost all pseudostationary phases. Similar behaviors, i.e., deviation from linearity of van’t Hoff plots, have been noticed in HPLC between 25-30 °C (92), 40-50 °C (93), 40-60 °C (94). These divergences have been attributed to the “phase transition” of the bonded octadecyl chains from a less ordered liquid-like state at higher temperatures to a more ordered and extended crystalline-like state at lower temperatures. As seen in Table 5.2, partial specific volumes of pseudostationary phases increase as temperature is elevated. This is due to the structural conformation of the pseudostationary phase at different temperatures. Pseudostationary phases studied here have more compact structures at low temperatures, which are evident from lower partial specific volumes, whereas, they have more flexible structures at higher temperatures where they have higher partial specific volumes. It is obvious from Figure 5.15 that the nonlinearity is more pronounced in van’t Hoff plots of more retained benzodiazepines. For less retained benzodiazepines, i.e., flunitrazepam, nitrazepam, and clonazepam, however, nonlinearity is not a significant problem. Thus, the thermodynamic properties of these less retained benzodiazepines and of seven alkyl phenyl ketones in seven pseudostationary phases are examined.

Figures 5.16 and 5.17 show the van’t Hoff plots of three benzodiazepines and seven alkyl phenyl ketones. The lowest correlation coefficients of ln K versus 1/T plot were observed in poly-SUL for benzodiazepines (ranged from 0.75 to 0.89) and alkyl phenyl ketones (0.56 to 0.90). The highest correlations were obtained in poly-L₂S₈ ranging from 0.98 to 0.99 for benzodiazepines and from 0.87 to 0.98 for alkyl phenyl ketones. The thermodynamic results are shown in Tables 5.16 and 5.17 for benzodiazepines and alkyl phenyl ketones, respectively.
As seen in Table 5.16, all three benzodiazepines have negative enthalpy change (i.e., $\Delta H^0$) values for all seven surfactant systems. This shows that the transfer of these analytes from the aqueous phase into the pseudostationary phases is thermodynamically preferential. The $\Delta H^0$ values of all three benzodiazepines are more negative in SDS and less negative in poly-SUL. This indicates that hydrophobic benzodiazepines prefer SDS more than poly-SUL, because SDS has more hydrophobic character than poly-SUL. The $\Delta H^0$ values of benzodiazepines using sulfated surfactants, i.e., poly-SUS and SDS, are always more negative than the rest of the pseudostationary phases. However, $\Delta H^0$ values are more negative using SDS than poly-SUS because poly-SUS is less hydrophobic than SDS. It should be noted that as the sulfate head group fraction in CoPM is increased, in general, $\Delta H^0$ values become more negative.

Figure 5.16. van’t Hoff plots of three benzodiazepines using seven pseudostationary phases. Separation conditions are same as Figure 5.14. Legends are shown in the figure.

As seen in Table 5.16, all three benzodiazepines have negative enthalpy change (i.e., $\Delta H^0$) values for all seven surfactant systems. This shows that the transfer of these analytes from the aqueous phase into the pseudostationary phases is thermodynamically preferential. The $\Delta H^0$ values of all three benzodiazepines are more negative in SDS and less negative in poly-SUL. This indicates that hydrophobic benzodiazepines prefer SDS more than poly-SUL, because SDS has more hydrophobic character than poly-SUL. The $\Delta H^0$ values of benzodiazepines using sulfated surfactants, i.e., poly-SUS and SDS, are always more negative than the rest of the pseudostationary phases. However, $\Delta H^0$ values are more negative using SDS than poly-SUS because poly-SUS is less hydrophobic than SDS. It should be noted that as the sulfate head group fraction in CoPM is increased, in general, $\Delta H^0$ values become more negative.
Figure 5.17. van’t Hoff plots of alkyl phenyl ketones using A) poly-SUL, B) poly-L8S2, C) poly-L6S4, D) poly-L4S6, E) poly-L2S8, F) poly-SUS, and G) SDS. Legends are shown in the figure. Separation conditions are same as Figure 5.14.
Table 5.16. Distribution coefficients, enthalpies, entropies, and Gibbs free energies for flunitrazepam, nitrazepam, and clonazipam in seven pseudostationary phases.

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<sup>a</sup> at 20 °C; <sup>b</sup> kJ mol<sup>-1</sup>; <sup>c</sup> J mol<sup>-1</sup> K<sup>-1</sup>; <sup>d</sup> kJ mol<sup>-1</sup> at 20 °C

The entropy change (i.e., ΔS<sup>0</sup>) values of benzodiazepines, in contrast to the negative ΔH<sup>0</sup> values, are negative using some pseudostationary phases and positive using others. For example, ΔS<sup>0</sup> values of all three benzodiazepines using poly-SUL are large positive. Furthermore, ΔS<sup>0</sup> values of flunitrazepam are positive using all pseudostationary phases except SDS, in which ΔS<sup>0</sup> value of flunitrazepam is negative. The ΔS<sup>0</sup> values for nitrazepam and clonazepam, however, are negative in all surfactant systems except in poly-SUL. The large positive ΔS<sup>0</sup> of transfer for three benzodiazepines that were found in poly-SUL can be explained by hydrophobic effect. That is, presence of a hydrophobic analyte forces the water molecules surrounding the analyte to form a dense, ordered network of hydrogen bonds with each other to minimize their contact with the hydrophobic analyte. This ordering diminishes the total entropy of the aqueous system.
Table 5.17. Distribution coefficients, enthalpies, entropies, and Gibbs free energies for alkyl phenyl ketones in seven pseudostationary phases.

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</table>

$^a$ at 20°C; $^b$ kJ mol$^{-1}$; $^c$ J mol$^{-1}$ K$^{-1}$; $^d$ kJ mol$^{-1}$ at 20°C
This ordered network of water molecules will disappear upon transfer of hydrophobic analytes from polar aqueous solution into relatively hydrophobic pseudostationary phase. Consequently, the total entropy (disorder) of the aqueous system increases significantly. Since the hydrophobic character of poly-SUL is less than that of other CoPMs, poly-SUS, and SDS (evident from methylene selectivity values in Table 5.4), hydrophobic analytes will remain in aqueous phase (before transferring into the micellar phase) relatively longer in poly-SUL system resulting a denser and more ordered network of water molecules. After the analyte is transferred into the micellar phase, the aqueous phase becomes more disordered (i.e., \( \Delta S^0 \) becomes more positive) in poly-SUL system. Other pseudostationary phases, e.g., poly-SUS and SDS, are relatively more hydrophobic than poly-SUL, hence, analyte does not spend long enough time in aqueous phase to form a denser network of water molecules. Therefore, \( \Delta S^0 \) values for three benzodiazepines used here are less positive (or more negative) in more hydrophobic pseudostationary phases.

Not all changes in \( \Delta H^0 \) or \( \Delta S^0 \) values, however, can be solely explained by the hydrophobic characters of the analytes or pseudostationary phases. For example, \( \Delta H^0 \) values get more negative as the distribution coefficients get larger (i.e., stronger interactions between micelles and the analytes) for three benzodiazepines; however, \( \Delta S^0 \) values decrease (become less positive of more negative). This phenomenon can be explained as follows: Their disorder in the pseudostationary phase is less due probably to the restricted motion of these analytes on the surface of the micelles through dipole-dipole or dipole-induced dipole interactions between the analytes and the pseudostationary phases. Polar group(s) on the analytes may also have strong electrostatic interactions with the anionic head groups of the micelles. These electrostatic interactions occur on or near the surface of the micelles and the analytes are said to be in an “adsorbed” state. When the hydrophobicities of the analytes and the micelles match well,
Analytes will be solubilized in the core of the micelles and analytes are said to be in a “dissolved” state (95). This two-state model can explain variations in $\Delta H^0$ and $\Delta S^0$ for benzodiazepines. Since the benzodiazepines used here are bulky and contain both hydrophobic benzene rings and polar group(s), they may be in both states through special conformations (in analytes and/or in micelles) in pseudostationary phases used in this study. This may have a significant effect on both $\Delta H^0$ and $\Delta S^0$ values of benzodiazepines and similar analytes.

The $\Delta H^0$ values for alkyl phenyl ketones used in this study are all negative. However, only one analyte (i.e., acetophenone) has a very small positive $\Delta H^0$ value using poly-L$_8$S$_2$. As seen in Table 5.17, the $\Delta H^0$ values get more negative (decrease) with an increase in alkyl chain length of alkyl phenyl ketones. This decrease means that transfer of alkyl phenyl ketones from aqueous phase to pseudostationary phase is enthalpically more favorable as the length of alkyl chain of alkyl phenyl ketones is increased. On the contrary, generally, $\Delta S^0$ values increased (became more positive) with an increase in alkyl chain length of alkyl phenyl ketones in all pseudostationary phase used. It should be noted that all $\Delta S^0$ values are positive in all pseudostationary phases with only one exception (acetophenone using SDS). The increase in $\Delta S^0$ values can be explained by a strong contribution from hydrophobic interactions explained above. Alkyl phenyl ketone molecules will have more hydrophobic characters with an increase in alkyl chain lengths. More hydrophobic analytes incorporate into pseudostationary phases stronger, resulting in longer electrophoretic retentions of the analytes in MCE. This is evident from K values, which get larger with an increase in chain length (Table 5.17).

In most partitioning chromatographic systems, the contribution of enthalpy on the distribution coefficient is usually much more pronounced than that of entropy (96). Tables 5.18 and 5.19 show this fact for most of the analytes using most of the pseudostationary phases used.
Table 5.18. Contribution of enthalpies and entropies on Gibbs free energies for flunitrazepam, nitrazepam, and clonazepam in seven pseudostationary phases.

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</thead>
<tbody>
<tr>
<td>Flunitrazepam</td>
<td>$\Delta H^{0a} %$</td>
<td>51.2</td>
<td>82.5</td>
<td>97.3</td>
<td>97.6</td>
<td>93.3</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0b} %$</td>
<td>48.8</td>
<td>17.5</td>
<td>2.7</td>
<td>2.4</td>
<td>6.7</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0c} %$</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>$\Delta H^{0} %$</td>
<td>71.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>29.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>0.0</td>
<td>-4.9</td>
<td>-16.2</td>
<td>-12.8</td>
<td>-7.4</td>
<td>-8.7</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>$\Delta H^{0} %$</td>
<td>72.6</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>27.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>0.0</td>
<td>-10.0</td>
<td>-20.0</td>
<td>-13.4</td>
<td>-6.6</td>
<td>-3.6</td>
</tr>
</tbody>
</table>

$^a$ Percent contribution of $\Delta H^0$ and $^b$ Percent contribution of $\Delta S^0$ on $\Delta G^0$. $^c$ Percent amount of hindered $\Delta H^0$ by entropy effect.

Table 5.19. Contribution of enthalpies and entropies on Gibbs free energies for alkyl phenyl ketones in seven pseudostationary phases.

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Acetophenone</td>
<td>$\Delta H^{0} %$</td>
<td>48.2</td>
<td>0.0</td>
<td>51.3</td>
<td>61.0</td>
<td>58.4</td>
<td>81.2</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>51.8</td>
<td>100.0</td>
<td>48.7</td>
<td>39.0</td>
<td>41.6</td>
<td>18.8</td>
</tr>
<tr>
<td>Propiophenone</td>
<td>$\Delta H^{0} %$</td>
<td>36.9</td>
<td>11.8</td>
<td>47.7</td>
<td>58.1</td>
<td>55.9</td>
<td>77.8</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>63.1</td>
<td>88.2</td>
<td>52.3</td>
<td>41.9</td>
<td>44.1</td>
<td>22.2</td>
</tr>
<tr>
<td>Butyrophenone</td>
<td>$\Delta H^{0} %$</td>
<td>31.0</td>
<td>21.0</td>
<td>42.8</td>
<td>54.9</td>
<td>51.4</td>
<td>78.7</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>69.0</td>
<td>79.0</td>
<td>57.2</td>
<td>45.1</td>
<td>48.6</td>
<td>21.3</td>
</tr>
<tr>
<td>Valerophenone</td>
<td>$\Delta H^{0} %$</td>
<td>27.0</td>
<td>24.4</td>
<td>40.9</td>
<td>55.7</td>
<td>49.7</td>
<td>81.0</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>73.0</td>
<td>75.6</td>
<td>59.1</td>
<td>44.3</td>
<td>50.3</td>
<td>19.0</td>
</tr>
<tr>
<td>Hexanophenone</td>
<td>$\Delta H^{0} %$</td>
<td>28.5</td>
<td>29.7</td>
<td>42.5</td>
<td>57.8</td>
<td>50.6</td>
<td>80.7</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>71.5</td>
<td>70.3</td>
<td>57.5</td>
<td>42.2</td>
<td>49.4</td>
<td>19.3</td>
</tr>
<tr>
<td>Heptanophenone</td>
<td>$\Delta H^{0} %$</td>
<td>25.3</td>
<td>32.0</td>
<td>42.7</td>
<td>56.1</td>
<td>50.2</td>
<td>81.7</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>74.7</td>
<td>68.0</td>
<td>57.3</td>
<td>43.9</td>
<td>49.8</td>
<td>18.3</td>
</tr>
<tr>
<td>Octanophenone</td>
<td>$\Delta H^{0} %$</td>
<td>16.3</td>
<td>34.0</td>
<td>40.6</td>
<td>55.5</td>
<td>51.2</td>
<td>84.9</td>
</tr>
<tr>
<td></td>
<td>$\Delta S^{0} %$</td>
<td>83.7</td>
<td>66.0</td>
<td>59.4</td>
<td>44.5</td>
<td>48.8</td>
<td>15.1</td>
</tr>
</tbody>
</table>

$^a$ Percent amount of hindered $\Delta H^0$ by entropy effect ($\Delta S^{0} %) is -4.0
Entropy contribution on $\Delta G^0$, however, is considerably high for flunitrazepam, nitrazepam, and clonazepam (48.8, 29.0, and 27.4 %, respectively) using poly-SUL molecular micelle system. The contribution of entropy on $\Delta G^0$ is either less or, in most cases, zero for all three benzodiazepines using all remaining pseudostationary phases (Table 5.18). For alkyl phenyl ketones, on the other hand, the contribution of entropy on $\Delta G^0$ is more (up to 100 %) than that of enthalpy using poly-SUL, poly-$L_8S_2$ and poly-$L_6S_4$ systems. The only exceptional alkyl phenyl ketone is acetophenone, for which the entropy contribution is slightly less than the enthalpy contribution using poly-$L_6S_4$ system. For the rest two of CoPMs (i.e., poly-$L_4S_6$ and poly-$L_2S_8$) the contribution of entropy on $\Delta G^0$ is still significant for alkyl phenyl ketones, but enthalpy effect is more dominant. For the two sulfated surfactants (i.e., poly-SUS and SDS), however, the enthalpy effect on distribution coefficient is much more significant than entropy effect. It is interesting to note that the entropy contribution for the first three alkyl phenyl ketones (i.e., acetophenone, propiophenone, and butyrophenone) on $\Delta G^0$ is more distinctive using poly-SUS than SDS. For the rest more hydrophobic alkyl phenyl ketones (i.e., valerophenone, hexanophenone, heptanophenone, and octanophenone), however, the entropy effect is more significant using SDS as pseudostationary phase than poly-SUS (Table 5.19).

As discussed previously, the contributions of either $\Delta H^0$ or $\Delta S^0$ to the free energy change are not similar for all analytes used in this study. For example, $\Delta S^0$ values are negative for the three benzodiazepines in most of pseudostationary phases and for acetophenone in SDS. The negative sign of $\Delta S^0$ indicates that these analytes prefer the aqueous phase entropically due probably to their restricted motion on the surface of the micelles. For example, in the case of clonazepam, $T\Delta S^0$ contributed -10, -20, -13.4, -6.6, -3.6, and -11.6 % to $\Delta G^0$ value at 20 °C using poly-$L_8S_2$, poly-$L_6S_4$, poly-$L_4S_6$, poly-$L_2S_8$, poly-SUS, and SDS. Thus, a strong affinity of
clonazepam for these surfactant systems was significantly hindered by the entropy effect. Similarly, the TΔS\textsuperscript{0} values are -1.9 and -4.0 % of ΔG\textsuperscript{0} for flunitrazepam and acetophenone using SDS. In addition, the TΔS\textsuperscript{0} values for nitrazepam are -4.9, -16.2, -12.8, -7.4, -8.7, and -18.7 % of ΔG\textsuperscript{0} using poly-L\textsubscript{8}S\textsubscript{2}, poly-L\textsubscript{6}S\textsubscript{4}, poly-L\textsubscript{4}S\textsubscript{6}, poly-L\textsubscript{2}S\textsubscript{8}, poly-SUS, and SDS. It is obvious that the incorporation of nitrazepam into the micellar system is prevented more in SDS and poly-L\textsubscript{6}S\textsubscript{4} by entropy.

As shown in Figures 5.18 and 5.19, the ΔH\textsuperscript{0} and ΔS\textsuperscript{0} followed the linear free energy relationship for both benzodiazepines and alkyl phenyl ketones. It should be noted that ΔH\textsuperscript{0} versus ΔS\textsuperscript{0} plot has a positive slope (i.e., ΔH\textsuperscript{0} and ΔS\textsuperscript{0} are proportional to each other) for alkyl phenyl ketones using poly-SUL (Figure 5.18 A), whereas, the slope is negative for the rest of pseudostationary phases (Figure 5.18 B-G). Correlation coefficients of ΔH\textsuperscript{0} versus ΔS\textsuperscript{0} plots for alkyl phenyl ketones are generally low (range from 0.27 to 0.95), however, correlation coefficients for benzodiazepine plots are relatively higher (range from 0.97 to 0.99). As seen in Figures 5.18 and 5.19, not all analytes fall on the straight line. This indicates that those analytes may have different conformations when they are solubilized in the pseudostationary phases. Compensation behavior is observed when the plot of ΔH\textsuperscript{0} versus ΔS\textsuperscript{0} is linear. The slope of this line gives the compensation temperature, $\beta_T$, which is characteristic of the type of mechanism between analyte and pseudostationary phase. The $\beta_T$ values are listed in Table 5.20. The average value for $\beta_T$ values for the three benzodiazepines varies depending on the pseudostationary phase used. For example, $\beta_T$ is highest for poly-SUL (i.e., 419.2 K) and lowest for SDS (i.e., 270.6 K). The y-intercept of ΔH\textsuperscript{0} versus ΔS\textsuperscript{0} plots gives the average Gibbs free energy of all analytes plotted (ΔG\textsubscript{0}\textsubscript{avr}) at $\beta_T$. The ΔG\textsubscript{0}\textsubscript{avr} of three benzodiazepines in poly-SUL is -18.4 kJ mol\textsuperscript{-1}. This value can also be obtained by putting the $\beta_T$ value into Equation 5.10.
Figure 5.18. Enthalpy-entropy compensation in the seven pseudostationary phases for alkyl phenyl ketones. Separation conditions are same as Figure 5.14. Analytes: C8) acetophenone, C9) propiophenone, C10) butyrophenone, C11) valerophenone, C12) hexanophenone, C13) heptanophenone, and C14) octanophenone.
Figure 5.19. Enthalpy-entropy compensation in the seven pseudostationary phases for benzodiazepines. Separation conditions are same as Figure 6.14. Analytes: 1) flunitrazepam, 2) nitrazepam, 3) clonazepam.
Table 5.20. Compensation temperatures for analytes in the seven pseudostationary phases and correlation coefficient values of $\Delta S^0$ versus $\Delta H^0$ plots.

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</thead>
<tbody>
<tr>
<td>Benzodiazepines</td>
<td>$\beta_T^a$</td>
<td>419.2</td>
<td>372.3</td>
<td>356.8</td>
<td>353.8</td>
<td>339.9</td>
<td>292.6</td>
</tr>
<tr>
<td></td>
<td>$R^2^b$</td>
<td>0.98</td>
<td>0.99</td>
<td>0.99</td>
<td>0.98</td>
<td>0.97</td>
<td>0.95</td>
</tr>
<tr>
<td>Alkyl phenyl ketones</td>
<td>$\beta_T$</td>
<td>20.1</td>
<td>485.2</td>
<td>137.0</td>
<td>312.7</td>
<td>217.0</td>
<td>1652</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.27</td>
<td>0.70</td>
<td>0.91</td>
<td>0.95</td>
<td>0.92</td>
<td>0.65</td>
</tr>
</tbody>
</table>

$^a$ Compensation temperature (K), obtained from the slope of $\Delta S^0$ versus $\Delta H^0$ plots; $^b$ Correlation coefficient of $\Delta S^0$ versus $\Delta H^0$ plots.

As seen in Table 5.20, $\beta_T$ values obtained from benzodiazepines seem chemically sound and more reasonable than those obtained from alkyl phenyl ketones. The $\beta_T$ values from alkyl phenyl ketones range from 20 K (too low) to 1652 (too high), which are not in a rational range typically found in MCE (3, 97, 98) and RP-HPLC (99). Peterson and Foley found $\beta_T$ value of 287 to 298 K for hydrophilic analytes and 260 to 307 K for hydrophobic analytes using chiral micellar phases (98). Terabe et al. found a $\beta_T$ value of 203 K for alkylphenols using SDS as micellar phase (3). The $\beta_T$ values found in this study as well as those of Peterson and Foley and Terabe et al. are much smaller than those typically found in RF-HPLC (e.g., 625 K). The smaller $\beta_T$ indicate that the contribution of entropic term is very important in solubilization of the analytes (i.e., benzodiazepines and alkyl phenyl ketones) in the molecular and normal micellar phases. Different values of $\beta_T$ for benzodiazepines and alkyl phenyl ketones for the same type of pseudostationary phase suggest that these two groups of analytes are incorporated into the micellar phase by somewhat different mechanisms.

5.4. Conclusions

In this study we have synthesized an achiral monomeric surfactant, i.e., SUS, and a chiral surfactant, i.e., SUL. These two surfactants were then polymerized separately to form poly-SUS
and poly-SUL and together at various given molar ratios to produce a variety of CoPMs possessing both chiral (i.e., leucinate) and achiral (i.e., sulfate) head groups. These CoPMs, molecular micelles of SUS and SUL as well as SDS were characterized using several analytical techniques. Fluorescence quenching technique was used for determination of aggregation number of these surfactant systems, densitometer for partial specific volume determinations, and MCE to compare their chromatographic performance as novel pseudostationary phases for separation of chiral and achiral molecules. Inclusion of highly soluble sulfate head group into the molecular micelle improved solubility of amino acid based CoPMs significantly over a wide range of pHs. To test their applicabilities as potential chiral selector, several chiral analytes such as binaphthyl derivatives (i.e., BNA, BNP, and BOH) and benzodiazepines (temazepam, oxazepam, and lorazepam) were tested. In addition to chiral benzodiazepines, four additional achiral benzodiazepines (i.e., flunitrazepam, nitrazepam, clonazepam, and diazepam) and seven alkyl phenyl ketones (i.e., acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone) were separated using these seven pseudostationary phases. Each surfactant system found to have different selectivities toward test analytes used.

The results of this study show also that MCE is a useful and reliable method for studying the thermodynamics of analyte-pseudostationary phase interactions. For each surfactant system used in this study, differences in both $\Delta H^0$ and $\Delta S^0$ values were observed among all analytes used. Despite the differences in $\Delta H^0$ and $\Delta S^0$ among the seven surfactant systems, linear enthalpy-entropy compensation plots for benzodiazepines show that there is a similar retention mechanism for these analytes. However, linearity for alkyl phenyl ketones was not as large as for benzodiazepines. The significant entropy differences and wide range of entropy contribution
to the Gibbs free energy change of each surfactant system indicate the selectivity differences of these surfactant systems.

5.5. References


59. Hara, S.; Dobashi, A.; Japan, Patent # 92, 149, 205, **1992**.


Electrokinetic Capillary Chromatography. Pittcon 1996, Chicago, IL, March 3-8, 1996; Poster 84P.


64. Lapidot, Y.; Rappoport, S.; Wolman, Y.; J. Lipid Res. 1967, 8, 142.


81. Kapnissi, C. P.; Thiam, S.; Warner, I. M. submitted for publication


Chapter 6.
Conclusions and Future Research

6.1. Conclusions

Several surfactants with different head groups have been synthesized and applied in MCE as novel pseudostationary phases for separation of chiral and achiral compounds. In the first part of Chapter 2, a high-purity T-type polymerized surfactant having an undecenyl (C₁₁) and a sulfate head group was prepared from $^{60}$Co γ-irradiation. This polymer was then used in micellar capillary electrophoresis (MCE) for separation of 16 polycyclic aromatic hydrocarbons (PAHs). The methodology offers a valid alternative to gradient high performance liquid chromatography (HPLC) and capillary electrochromatography (CEC). The former requires gradient and large amounts of organic solvents for PAHs eluting from HPLC columns; the later still needs extensive studies on reproducible column preparation and optimization of conditions before it is ready for practical application. In contrast, with MCE, a simple manipulation of organic solvent composition and the concentration of polymerized surfactant in the running buffer enables one to realize the inherent benefits of MCE, that is, large number of peaks can be resolved at small k' values and relatively narrow peak spacings are observed at large k' values. This advantage is clearly demonstrated in the separation of 16 PAHs with varying hydrophobicities in a single run. Electrokinetic chromatography (EKC) with poly(sodium undecylenic sulfate) (poly-SUS) is utilized to separate PAHs. Parameters such as pH, concentration of polymeric surfactants, and the use of organic modifiers were investigated to follow the retention trends of PAHs. A baseline separation of all 16 PAHs in about 30 min, using 0.50% (w/v) of poly-SUS/12.5 mM sodium phosphate-borate buffer (pH 9.2) with 40% (v/v) acetonitrile, was possible for the first time in MCE by a single-surfactant system. In the second part of Chapter 2, a double alkyl chain
di(2-ethylhexyl)phosphate (DEHP) is introduced as a potential anionic micellar pseudostationary phase for a wide range of benzene derivatives and/or polycyclic aromatic hydrocarbons (PAHs). Several parameters such as concentration of phosphated surfactant, type and concentration of organic solvents (acetonitrile, isopropanol, and methanol), as well as separation voltage were optimized to enhance resolution, efficiency and selectivity as well as to maximize peak capacities. MCE separation of a 21-component mixture of moderately and strongly hydrophobic analytes were successfully achieved using DEHP surfactant in the presence of an appropriate amount of organic solvent (20 % v/v methanol, 30 % v/v acetonitrile or isopropanol). However, acetonitrile is the most effective modifier that provides highest peak capacity with rapid analysis time. It appears that the unique structure of the DEHP micelle is more tolerant to the addition of an organic solvent than SDS. This extends the scope of MCE with partially aqueous buffer systems to separate moderately-to-strongly hydrophobic compounds. The successful outcome of this preliminary work has encouraged us to embark on a program to examine the effect of a variety of other phosphated surfactants as a future research. Such surfactants are expected to be usable in MCE with partially aqueous buffer systems.

In the first part of Chapter 3, a partial separation of mono-methylbenz[a]-anthracene (MBA) isomers can be successfully achieved by use of poly-SUS, a T-type micelle polymer with sulfate head groups, in the MCE mode in the presence of 35 % (v/v) of ACN at pH range of 9.1-10.0. These improved separations of MBA with poly-SUS are consistent with our previous study on 16 EPA priority PAHs (Chapter 2). In addition, as noted in Chapter 2, hydrophobic analytes do not penetrate as deeply into the core of the polymerized surfactant as into normal micelle. The separation of the MBA isomers using SDS, the most widely used surfactant in MCE, under similar BGE conditions, (i.e., 12.5 mM each of Na$_2$B$_4$O$_7$ and Na$_2$HPO$_4$
with 35% (v/v) ACN at a pH of 9.5) was not successful as compared with poly-SUS. With 18.4 mM SDS (equivalent to 0.5 % poly-SUS) ca. 1.0-minute elution window was generated and only three MBAs, out of twelve, were partially resolved. As the concentration of SDS is increased to 36.8 mM, the elution window was increased to 15-minute, but only five MBAs were partially resolved in 72 minutes. Further increases in SDS concentration to 54 mM produced no elution of any MBA isomers even in 300 minutes. The poor resolution of MBAs with SDS can be explained by the disruption of the formation of SDS micelles at high concentrations of organic solvent (in this case 35 % ACN). In contrast, the structural integrity of poly-SUS is preserved at high content of organic solvents. Thus, this comparison indicated the superiority of poly-SUS over the SDS for the separation of MBAs.

In the second part of Chapter 3, a combination of poly-SUS and three β-CD derivatives (i.e., DM-β-CD, TM-β-CD, and HP-β-CD) as well as native β-CD and γ-CD was investigated to separate twelve MBA isomers, which could not be separated using poly-SUS alone as seen in the first part of Chapter 3. The aim of this second part was to study the possibilities of using two native CDs (β-CD, and γ-CD) and three derivatives of β-CD (dimethyl-, trimethyl-, and hydroxypropyl-β-CD) in combination with poly-SUS to improve the separation of twelve MBA isomers. The β-CD, γ-CD and three β-CD derivatives were found to have different resolution and selectivity. Additionally, the analysis time of isomers was found to be dependent on the type and concentration of the CD additives. Relatively shorter analysis times were achieved using β-CD derivatives comparing to native β-CD and γ-CD. This is an indication of a stronger complexation between MBA isomers and β-CD derivatives, which can be attributed to the fact that β-CD derivatives have deeper cavities compared to native β-CD. The retention times of MBA isomers were decreased as the concentration of β-CD derivatives increased, whereas the
opposite effect was observed with native β-CD and γ-CD. A combination of 5 mM γ-CD, 0.5% (w/v) poly-SUS, 35 % (v/v) acetonitrile at a pH of 9.75 provided the best selectivity and resolution of the twelve MBA isomers. However, a total separation time was about 110 minutes. Alternatively, combined use of poly-SUS and DM-β-CD resulted in a relatively faster separation (ca. 16 minutes) of MBA isomers. This occurred only at the expense of co-migration of some MBA isomers. When a TRIS buffer was used instead of phosphate-borate buffer, a significant reduction in retention times (ca. under 16 minutes, data not shown) of MBA was observed using the combination of poly-SUS and γ-CD. This indicates that buffer selection has a significant effect on separation of MBA solutes.

In Chapter 4, a vesicle forming surfactant possessing two hydrophilic carboxylate head groups and two hydrophobic undecenyl chains, sodium di(undecenyl) tartarate monomer, (mono-SDUT), was synthesized and exposed to a gamma radiation to form its polymeric vesicular form (poly-SDUT). These two surfactants, SDS, and a combination of SDS/mono-SDUT, SDS/poly-SDUT, and mono-SDUT/poly-SDUT were applied as pseudostationary phases in MCE. Two LSER models, i.e., solvatochromic and solvation parameter models were successfully applied to investigate the effect of the type and composition of pseudostationary phases on the retention mechanism and selectivity in MCE. These models are helpful tools to understand the fundamental nature of the solute-surfactant interactions and to characterize the surfactant systems. The results obtained from the both models provide very comparable information, for example, in both models solute size (coefficient m) and hydrogen bond accepting ability (coefficient b) for all pseudostationary phases play the most important role in MCE retention despite the numerical differences in the values for the solute descriptors and slight differences in the form of the equations for both models. However, some differences in magnitude of the
coefficients are obvious in both models. Although both models provide the same information, the solvation parameter model is found to provide much better both statistically and chemically sound results. This is evident when comparing the statistics (i.e., R, SE, and F values) of the solvation parameter model results with those for solvatochromic model results. It is critical to choose an appropriate solute set, which has to represent a wide range of solutes, for LSER methodology. It is worth noting that using only twelve non-hydrogen bonding (NHB) or hydrogen-bond accepting (HBA) solutes, it is possible to predict capacity factors for thirty-six solutes with a high correlation of 0.9568 and 0.951 with poly-SDUT surfactant system using solvation parameter model and solvatochromic model, respectively. The chemical selectivity differences between the six pseudostationary phases used in this study are also compared by plotting the experimental capacity factor (log k') values pseudostationary phases. It is evident from the free energy transfer data for NHB solutes and the results of the two LSER models that hydrophobicity play an important role in solute-surfactant interaction; however, selectivity is mainly influenced by hydrogen bond accepting or donating ability of both pseudostationary phases and the solutes.

In Chapter 5, an achiral monomeric surfactant, i.e., SUS, and a chiral surfactant, i.e., sodium undecanoyl L-leucinate (SUL) were synthesized. These two surfactants were then polymerized separately to form poly-SUS and poly-SUL and together at various given molar ratios to produce a variety of CoPMs possessing both chiral (i.e., leucinate) and achiral (i.e., sulfate) head groups. These CoPMs, molecular micelles of SUS and SUL as well as SDS were characterized using several analytical techniques. Fluorescence quenching technique was used for determination of aggregation number of these surfactant systems, density measurements for partial specific volume determinations, and MCE to compare their chromatographic
performances as novel pseudostationary phases for separation of chiral and achiral molecules. Inclusion of highly soluble sulfate head group into the molecular micelle improved solubility of amino acid based CoPMs significantly over a wide range of pHs. To test their applicabilities as potential chiral selector, several chiral analytes such as binaphthyl derivatives (i.e., BNA, BNP, and BOH) and benzodiazepines (temazepam, oxazepam, and lorazepam) were tested. In addition to chiral benzodiazepines, four additional achiral benzodiazepines (i.e., flunitrazepam, nitrazepam, clonazepam, and diazepam) and seven alkyl phenyl ketones (i.e., acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone) were separated using these seven pseudostationary phases. Each surfactant system found to have different selectivities toward test analytes used.

The results of this study presented in Chapter 5 show also that MCE is a useful and reliable method for studying the thermodynamics of analyte-pseudostationary phase interactions. For each surfactant system used in this study, differences in both $\Delta H^0$ and $\Delta S^0$ values were observed among all analytes used. Despite the differences in $\Delta H^0$ and $\Delta S^0$ among the seven surfactant systems, linear enthalpy-entropy compensation plots for benzodiazepines show that there is a similar retention mechanism for these analytes. However, linearity for alkyl phenyl ketones was not as large as for benzodiazepines. The significant entropy differences and wide range of entropy contribution to the Gibbs free energy change of each surfactant system indicate the selectivity differences of these surfactant systems.

6.2. Future Research

Future studies in this area should focus on synthesis of more CoPM using a variety of amino acids as hydrophilic head groups and chiral selectors. The CoPMs used in this dissertation contain C11 carbon chain (hydrophobic moiety) for both achiral sulfated and chiral
leucinated surfactants. Due to the large size of sulfate head groups, chiral analytes cannot penetrate deep into the chiral centers of leucinate. The usage of either shorter alkyl chain of sulfated surfactant or longer alkyl chain of chiral surfactant would overcome this steric effect. Thus, the presence of sulfate would increase the solubility of the CoPM without blocking the enantiomers interacting the chiral centers of chiral selectors. In this way, a better resolution of chiral compounds is expected at a wide range of pHs. In addition, LSER model should be applied these chiral selectors to find out appropriate pseudostationary phases for right enantiomers to be separated. The LSER model provides useful information about the hydrophobicity, polarity, hydrogen-bond accepting or donating ability of the surfactant. Instead of trial-and-error method, an appropriate surfactant can be synthesized. For example, if surfactant is characterized to be acidic from system coefficients found through LSER, this surfactant would be an ideal pseudostationary phase for basic enantiomers.
Vita

Cevdet Akbay was born in Bingöl, a small city located in Eastern Turkey, on November 27, 1968, to middle class parents. His father is a laborer in construction business and his mother is a housewife. After Cevdet completed his 8 year elementary and middle school in Bingöl, his father decided to move to Osmaniye, a relatively larger city located in Southern Turkey, with a hope of finding better jobs to educate his children. There, Cevdet attended a public high school. Spending half a day in the school and the other half at work in a variety of jobs, he graduated from high school in 1986. After passing the National College Placement Exams, Cevdet attended the Chemical Education Department of İnönü University-Malatya (located in Mid-eastern Turkey) in 1986. In 1990, he received a Bachelor of Science degree in chemical education. He got married in 1990 before he was appointed by the Turkish Ministry of Education to a public high school in the same city (Malatya) to teach science and chemistry to middle and high school students. Along with teaching, he was working on the synthesis of phosphorous compounds in the Chemistry Department of İnönü University in pursuit of Master’s degree in Inorganic Chemistry. In 1993, he passed an exam and was qualified for a full fellowship from the Turkish Higher Education Council for graduate studies abroad. He left the graduate program at İnönü University for a better one abroad. In December 1993, he moved to Austin, Texas, and attended an English language school to improve his English language. After spending some time on language, he attended the Chemistry Department of Louisiana State University in the Fall Semester of 1994. His fellowship was terminated in the Summer Semester of 1996 by the Turkish Higher Education Council and was forced to go back to Turkey based on a series of groundless accusations. In reality, however, his rights were taken away from him because of his personal thoughts, religious beliefs, and ethnic background. He went back to take
legal action against them. Even though he won the case, the Turkish Higher Education Council
did not compensate his rights. To complete his graduate studies at Louisiana State University, he
headed back to the U.S.A. with a financial support from his advisor, Dr. Isiah M. Warner. Under
the direction of Dr. Warner, Cevdet studied the development and applications of novel chiral and
achiral surfactants as pseudostationary phases in micellar capillary electrophoresis.

The following is a list of his publications and studies presented at scientific meetings.

Published studies in scientific journals:

Constantina P. Kapnissi; **Cevdet Akbay**; Joseph, B. Schlenoff; and Isiah M. Warner, “Analytical

**Cevdet Akbay**, Shahab A. Shamsi, and Isiah M. Warner, “Cycloextrin-Modified Electrokinetic

**Cevdet Akbay**, Shahab A. Shamsi, and Isiah M. Warner, “Electrokinetic Chromatography of
Twelve Mono-methylbenz[a]anthracene Isomers Using a Polymerized Anionic Surfactant,”

Shahab A. Shamsi, **Cevdet Akbay**, and Isiah M. Warner, “Polymeric Anionic Surfactant for
Micellar Electrokinetic Chromatography: Separation of 16 Priority Pollutant Polycyclic

**Cevdet Akbay**, Shahab A. Shamsi, and Isiah M. Warner, “Phosphated Surfactant as a Pseudo-
Stationary Phase for Micellar Electrokinetic Chromatography: Separation of Polycyclic

Mary W. Kamande, Constantina Kapnissi, **Cevdet Akbay**, Xiaofeng Zhu, Rezik Agbaria, and
Isiah M. Warner, “Open Tubular Capillary Electrophoresis Using a Polymeric Surfactant
Coating.” *Manuscript submitted for publication.*

Simon M. Mwongela, **Cevdet Akbay**, Janet J. Tarus, and Isiah M. Warner, "A Novel Amino

**Cevdet Akbay** and Isiah M. Warner, "Vesicles, Polymeric Vesicles, and Mixed Surfactants; Part
I: Characterization and Application in Micellar Electrokinetic Chromatography as Novel
Pseudostationary Phases.” *Manuscript submitted for publication.*


Cevdet Akbay and Isiah M. Warner, “Copolymerized Molecular Micelles; Part II: Thermodynamics of Interactions with Benzodiazepines and Alkyl Phenyl Ketones.” Manuscript submitted for publication.

Cevdet Akbay, Nathan Wilmot, Rezik A. Agbaria, and Isiah M. Warner, "Characterization and Application of Sodium di(2-ethylhexyl)phosphate and Sodium di(2-ethylhexyl)sulfosuccinate Surfactant in Micellar Electrokinetic Chromatography as Pseudostationary Phases." Manuscript submitted for publication.

Presentations at numerous professional meetings:


