SYNTHESIS, CHARACTERIZATION, AND APPLICATION OF POLYMERIC SURFACTANTS IN MICELLAR ELECTROKINETIC CHROMATOGRAPHY: USE OF IONIC LIQUIDS AS MODIFIERS

A Dissertation

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

in

The Department of Chemistry

by

Simon M. Mwongela
B.S., University of Nairobi, Nairobi, Kenya, 1997
May, 2005

Dedication

For my son, Timothy Keli
wife, Tabitha Mueni
mum and dad, Agnes K. Mutungu and James M. Mutungu
my brothers, Samson and Samuel, and my sister, Dorcas
and for all my extended family.

Behold, how good and pleasant
it is when brothers (family) dwell in unity!
It is like the precious oil
upon the head,
running down upon the beard,
upon the beard of Aaron,
running down on the collar of his robes!
It is like the dew of Hermon,
which falls on the mountains of Zion!
For there the LORD has commanded the blessing, life for evermore.
---- King David, *A Song of Ascents, Psalms 133*

Acknowledgments

Before 1993, it had never occurred to me that I would pursue a graduate program in chemistry. My plans were to finish college with a degree in mathematics, a subject that I loved dearly and then get a job. It was Dr. John Owino, one of my undergraduate mathematics professors at the University of Nairobi, Kenya, who inspired me by his words of wisdom about higher education.

My father was really excited about my pursuit of a career in mathematics and also by my words, “I feel as if I have just started the learning process.” At the end of my second year it turned out that I had performed slightly better in chemistry than in mathematics and I was one among the few chosen to pursue a Bachelor of Science degree in chemistry. Even though I really wanted to major in mathematics, I made up my mind to major in chemistry.

Despite my poor high school chemistry background, I started to study the subject with a great passion and commitment in order to pull up my grades in a quest for the honors degree. I graduated in December 1997, and I was excited that I would probably get a great job in one of the chemical industries in Kenya. However, my dreams were shattered by the dying Kenyan economy, as well as the increasing number of unemployed graduates at that time. Desperately in need of something to do and with the big El Nino rains of 1997 and 1998, I ended up on my father's country farm. At the farm, I pulled the ox plow in preparation to plant maize and beans. It was during this time that I started to consider going to graduate school, but it was impossible to perceive how that could ever happen given the hard economic times.

This dissertation had its true beginnings at this farm where I learned great values of patience, perseverance, and doing the best I could without supervision. It was during this time that I started to pray for God to open an opportunity for me to either get a job or get admission to graduate school. Later that year I got a high school teaching job and started my application process for graduate school here in the USA. In 1999 I was admitted to Louisiana State University to pursue a graduate degree in chemistry.

My years in the chemistry graduate program were the most challenging of my entire school life. The long nights spent in the laboratory doing research cannot be forgotten. However, I finally can see a light at the end of the tunnel and gladly say it was worth the effort. All Praise, Glory, and Honor are unto Jehovah God. He said, "I will go before you and will be with you; I will never leave you nor forsake you. Do not be afraid; do not be discouraged" [Deuteronomy 31:8]. His word and promises are true and He is ever faithful to those who love Him. I thank Him for straightening my path and seeing me through the tough graduate school journey. I stand in awe of Him forever, a humble and grateful debtor.

I would like to gratefully acknowledge the following people for their help and support:

My mentor, advisor, and committee Chair, Professor Isiah Warner, without whose direction and encouragement this work would not have come to fruition. His relentless and intuitive guidance and financial support helped in sharpening the focus of this research effort. He made my research experience stimulating, challenging, and rewarding. I also thank him, Professors Steve Watkins, and Joseph Rugut for playing a key role in my admission to LSU.

My mother, Agnes Kamanthe Mutungu, and father, James Mwongela Mutungu, for all their love, constant encouragement, and support during my years of education. I wish to thank them for their unforgettable words, "Trust in the LORD with all your heart and lean not on your own understanding; in all your ways acknowledge Him, and He will make your paths straight" [Proverbs 3:5-6]. Indeed, He has made my paths straight.

My Committee Members, for their helpful comments and for making it possible for me to defend my dissertation successfully.

Warner Research Group, for their special understanding, support, and friendship. Their positive criticisms are highly appreciated. Special thanks to Kristin Fletcher and Aleeta Powe for helping me edit this dissertation.

Istrouma Baptist Church Family, Margaret Hackbee, Willie May, Joy, Allan Davis, the Awanna ministry, George and Patsy Crosby, to mention but a few, for prayers and encouragement, and for their financial and material support during the arrival of my wife and the birth of our son, Timothy. Special thanks to **Nancy and Warren Boren** for being our host family, and for their support in our transition period. May God bless them and continue to fill their hearts with love.

Cate and Daniel Moriasi, Special thanks to them for being a great blessing to me and my family. God brought us together for a reason. I can testify that all the glory belongs to God for He has given us victory. Cate and Daniel both completed their Ph.D. in record time while Cate was working on a masters degree in Agricultural Economics and carrying a baby. I wish to congratulate them for their achievements and for the birth of their baby boy Michael Subira Moriasi.

International Christian Fellowship (ICF), for their care, love, fellowship, financial, and spiritual support at all times. Special thanks to the Moriasi, Liwa, Sang, Otieno, Mungai, Mbuba, and Dawa families to mention but a few, for their support, especially after the birth of Timothy.

My family and extended family, thanks for all their moral and unconditional support. May God bless you.

My loving wife, Tabitha Mueni, her love, care, and words of encouragement are highly appreciated. I thank her for bearing with me during the easy and tough times of graduate school. It is hard to repay her, but I will continue loving her as a token for her perseverance.

Son, Timothy Keli, who brings great joy to my life. May he grow to be a man after God's heart, full of wisdom and knowledge to make the right choices in his life.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Name
ADI	acceptable daily intake
AUC	analytical ultracentrifugation
BEE	benzoin ethyl ether
BGE	background electrolyte
BME	benzoin methyl ether
$BMIMBF_4$	1-ethyl-3-methylimidazolium tetrafluoroborate
BNA	1,1'-bi-2-naphthyl-2,2'-diamine
BNP	1,1-bi-2-naphthyl-2,2'-dihydrogen phosphate
BOH	1,1'-bi-2-napthol
CE	capillary electrophoresis
CEC	capillary electrochromatography
CFCs	chlorofluorocarbons
CGE	capillary gel electrophoresis
CIEF	capillary isoelectric focusing
CITP	capillary isotachophoresis
CMC	critical micelle concentration
CZE	capillary zone electrophoresis
DDT	dichlorodiphenyltrichloroethane
EKC	electrokinetic chromatography
$EMIMBF_4$	1-ethyl-3-methylimidazolium tetrafluoroborate
EMIMCL	1-ethyl-3-methylimidazolium chloride

$EMIMPF_6$	1-ethyl-3-methylimidazolium hexafluorophosphate
$EMIMSO_3F_3$	1-ethyl-3-methylimidazolium trifluoromethanesulfonate
EOF	electroosmotic flow
ER	electromagnetic radiation
FAO	food and agriculture organization
GC	gas chromatography
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
$HMIMBF_4$	1-hexyl-3-methylimidazolium tetrafluoroborate
ILs	ionic liquids
MEKC	micellar electrokinetic chromatography
NMR	nuclear magnetic resonance
NVP	N-vinyl-2-pyrrolidone
$OMIMBF_4$	1-octyl-3-methylimidazolium tetrafluoroborate
OT-CEC	open-tubular capillary electrochromatography
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PEM	polyelectrolyte multilayer
Poly-L-SOLV	poly sodium olely-L-leuclyvalinate
Poly-SUA	polysodium 10-undecylenate
Poly-L-SULV	poly sodium *N*-undecanoyl-L-leucylvalinate
Poly-SUS	polysodium undecylinic sulfate

PVP	polyvinyl pyrrolidone
PVP/VA	polyvinyl pyrrolidone/vinyl acetate
Rs	resolution
RSD	relative standard deviation
SDS	sodium dodecyl sulfate
SFC	supercritical fluid chromatography
THF	tetrahydrofuran
TRIS	tris (hydroxymethyl) aminomethane
VA	vinylacetate
V_{bar}	partial specific volume
VOCs	volatile organic solvents
WHO	World Health Organization
2-Pyr	2-pyrrolidone

Abstract

The goal of the research presented in this dissertation is to synthesize, characterize, and apply a novel chiral amino acid-based surfactant and achiral sulfate-based surfactants for the effective separations of chiral and achiral compounds in micellar electrokinetic chromatography (MEKC). The first part of this research involves novel synthesis, characterization, and application of polysodium oleyl-L-leucyl-valinate (poly-L-SOLV) for the separation of chiral compounds in MEKC. Surface tensiometry, proton nuclear magnetic resonance, infrared spectroscopy, densitometry and analytical ultracentrifugation were used as the characterization techniques. Optimal MEKC separating conditions were established by varying surfactant concentration, buffer concentration and pH, applied voltage, and capillary temperature. Poly-L-SOLV was used successfully in the separation of various enantiomers of neutral, acidic, and basic analytes.

The second part of this research focuses on the synthesis, characterization, and application of poly sodium undecylenic sulfate (poly-SUS) for the separation of achiral compounds. The use of ionic liquids (ILs) as modifiers in the separation of achiral and chiral analytes in MEKC was also investigated. In this study, polymeric surfactants and ILs were added to a low-conducting buffer solution. Also, select ILs were compared with select organic solvents as modifiers for the separation of chiral compounds in MEKC. The results indicated that ILs could stabilize the current and at the same time lead to faster and better separations.

Finally, another surfactant, sodium dodecyl sulfate (SDS), was used to develop a method for the separation of 2-pyrrolidone, vinylacetate, and N-vinyl-2-pyrrolidone

monomer residues in polyvinylpyrrolidone (PVP) homo-polymer and polyvinylpyrrolidone/vinyl acetate (PVP/VA) copolymer in MEKC. A capillary electrophoresis (CE) high-sensitivity cell was used for these separations because the conventional CE cell could not achieve the required sensitivity. The method developed was then evaluated for quantitative analysis of the monomer residues from the polymer samples. Although the method was not versatile for the quantitative analysis of the three monomer residues, it was robust, fast, and economical for the separation of these monomer residues.

Chapter 1. Introduction

Part 1. Surfactants, Characterization Techniques, and Chirality

1.1 Surfactants: Definition and Classification

Surfactants, also know as surface-active-agents, amphiphiles, or detergents, are substances which lower the surface tension of the medium in which they are dissolved and/or the interfacial tension with other phases. Accordingly, they are positively adsorbed at the liquid/vapor and/or at other interfaces. The name "amphiphile" is derived from the Greek word *amphi*, meaning both, and the term relates to the fact that all surfactant molecules consist of at least two moities, one of which is soluble in a specific fluid (the lyophilic part) and one which is insoluble (the lyophobic part).[1] When the fluid is water, we refer to the hydrophilic and hydrophobic parts, respectively. The hydrophilic part is known as the head group, and the hydrophobic part as the tail, as illustrated in Figure 1.1.

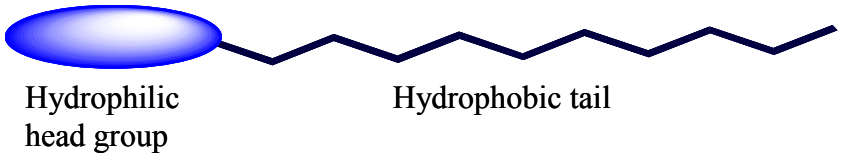


Figure 1.1 Schematic illustration of a surfactant.

Surfactants are grouped into four classes: anionic, cationic, zwitterionic, and non-ionic, based on the charge of the polar head group.[1, 2] Zwitterionic surfactants contain both an anionic and cationic charge under normal conditions and are often referred to as amphoteric surfactants. However, the term "amphoteric" is not always correct, as an amphoteric surfactant is dependent on pH and can be anionic, zwitterionic, or cationic.[1] Most ionic surfactants are monovalent, and the choice of counterion plays an important role in the physicochemical properties.[1] Sodium is a common counterion in anionic surfactants but other

cations, such as potassium, lithium, calcium, and protonated amines, are possible alternatives for special purposes. Cationic surfactants generally have a halide or methyl sulfate counterion, and the hydrophobic group is typically a hydrocarbon (alkyl or alkyl aryl). However, a polydimethysiloxane or fluorocarbon may also be used, particularly for non-aqueous systems.

In this dissertation, I will focus mainly on two categories of anionic surfactants: chiral amino acid-based surfactant, and non-chiral (or achiral) sulfate-based surfactant. These two types have sodium as the counterion and different hydrophobic alkyl tail groups. The monomeric surfactants are polymerized to form polymeric surfactants. I will discuss the synthesis, polymerization, characterization, and applications of the polymeric surfactants in subsequent chapters; however, I will describe universal properties of surfactants in the following sections.

1.2 Surfactants: Characteristics and Properties

A surfactant is characterized by its tendency to adsorb at interfaces and surfaces. The term "interface" denotes a boundary between any two immiscible phases, whereas the term "surface" indicates one of the phases is a gas, usually air.[1, 3] The stronger the tendency of the surfactant to accumulate at an interface, the better the surfactant. However, there is no universally good surfactant because the level of surfactant concentration at a boundary depends on the surfactant structure and the nature of the two phases that meet at the interface. Therefore, the choice of a surfactant is dependent on its application. Another important characteristic of surface-active agents is that unimers (or monomers) in solution tend to spontaneously self assemble. These self assemblies can adopt any shape depending on experimental conditions, but the common self assemblies are micelles. The terms "unimer"

and “monomer” are generally used synonymously to mean free, unassociated surfactant molecules. There are three basic concepts which need to be fully understood to explain the majority of observed phenomena: solubility, adsorption of a surfactant at a surface, and the formation of micelles in solution. These three phenomena differentiate a surfactant from other chemical entities. First, the unique solubility properties of surfactants provide them adsorption at surfaces and micelle formation. Second, the surfactants' adsorption at surfaces properties grant them surface-active effects of forming, wetting, emulsification, solid dispersion, and detergency. Third, micellar properties provide solution and bulk characteristics of surfactants, such as viscosity and solubilization, as well as functional effects such as emulsification and detergency. In this dissertation, I will consider only the phenomena of adsorption and micelle formation in great detail.

1.2.1 Adsorption

Many chemicals produce foams and wet surfaces, but are not considered surfactants, e.g., methanol in aqueous solution. In most cases, a surfactant is at a higher concentration at the interface than in the bulk of a liquid. This phenomenon is called adsorption, and it occurs at a liquid/solid, liquid/liquid, and air/liquid interface.

The adsorption of a surfactant at an air/water surface will result in pronounced physical changes to the water. The more surfactant present at the surface, the greater the physical changes. As shown in Figure 1.2, surfactant monomers can be oriented in various ways; moreover, this orientation depends upon the surfactants' concentration, the nature of the hydrophilic group, and the surface. At very low surfactant concentrations, the monomers lie flat on the surface. As the surfactant concentration increases, the number of monomers on the surface increases. Since there is not enough room for these monomers to lie flat, they

orient in a manner dependent on surface properties. The concentration at which the monomers form a unimolecular layer is called the critical micellar concentration (CMC).[1-3]

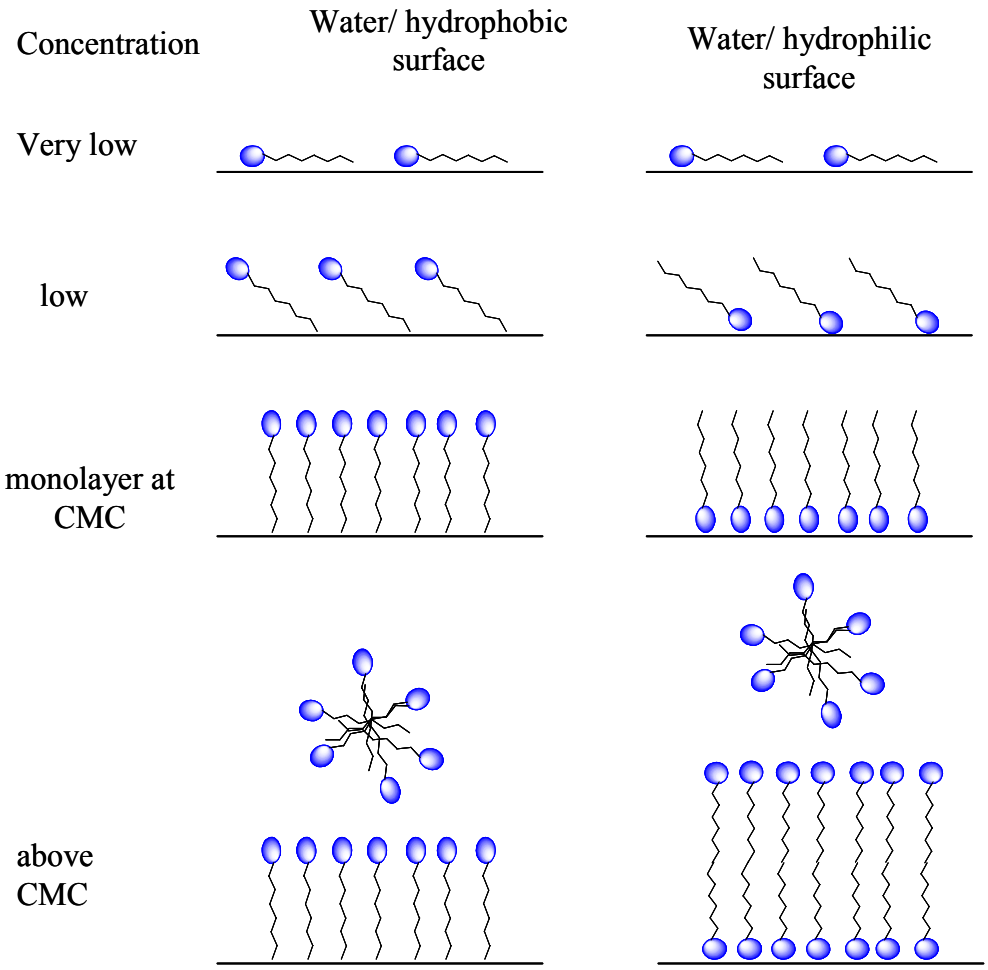


Figure 1.2 Effect of surfactant concentration on adsorption.

For surfactant concentration above the CMC, the monomers in solution will form an ordered structure known as a micelle. Surface adsorption leads to pronounced physical changes of the solution, such as a decrease in surface tension (the force acting over the surface of the aqueous solutions per unit length of the surface perpendicular to the force). At the CMC, the surface tension falls to a minimum, as illustrated in Figure 1.3. Thus, the formation of micelles or monomer multilayers has no significant effect on the surface

tension, although in practice the surface tension does fall very slowly as the surfactant concentration continues to increase.

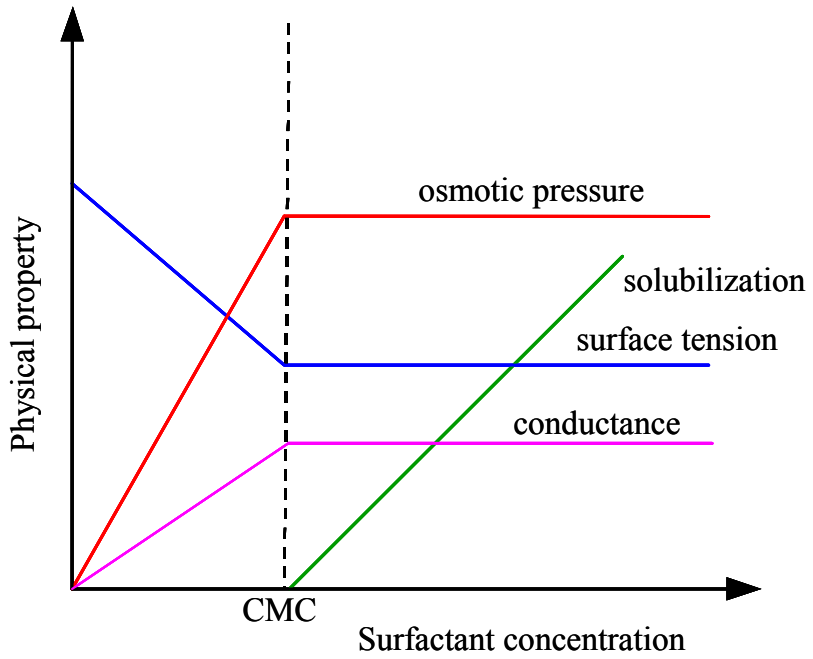


Figure 1.3 Concentration dependence of some physical properties for solutions containing a micelle-forming surfactant.

1.2.2 Critical Micellar Concentration

The critical micellar concentration of surfactants is due to two opposing forces of interaction between surfactant monomers.[3] Force 1 is caused by polar groups in water which repel one another due to mutual charge repulsion. The larger the charge on the polar groups, the greater the repulsion and the lower the tendency to form micelles. For cases where the hydrophilic groups have a strong affinity for water, they disperse to allow a maximum amount of water to solvate the hydrophilic group. Force 2 necessitates the hydrophobic groups to act as if there is a bond attracting them to each other. This complex interaction, called the hydrophobic effect, is due to enthalpy and entropy changes when an alkyl group is transferred from a hydrophobic environment to solution in water.[4, 5] The relative strength of forces 1 and 2 determines the CMC. Figure 1.4 is a diagram illustrating these two forces. At a

certain surfactant concentration, force 1 equals force 2, and thus the molecules will aggregate.

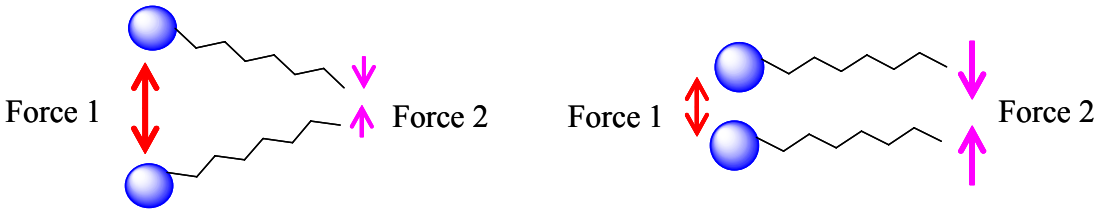


Figure 1.4 Forces between surfactant molecules in solution.

For this to occur, the hydrophobic and hydrophilic effects must be of similar orders of magnitude. In comparing two surfactants, the one with the larger hydrophilic effect will have a higher CMC.

The CMC can be obtained from the plot of physicochemical properties of the surfactant versus the monomer concentration, as shown in Figure 1.3. There are many techniques for the determination of CMC, including monitoring changes in osmotic pressure, surface tension, conductivity, turbidity, fluorescence, or light scattering.[6-11] For this dissertation research, changes in surface tension as surfactant concentration increased was used to determine CMC.

1.2.3 Surface Tension

Surface tension is an extremely sensitive indicator providing information on washability, wetting, emulsification, foaming and other surface-related processes. The progress of various chemical reactions and the presence of solvents or surfactants in a liquid system can be monitored by measurement of surface tension. Surface tension provides information about the quality of water-repellants finishes, stability of emulsions, oxidation, and polymerization.[1, 2] Polymeric surfactants have approximately zero CMC; therefore, surface tension measurements can be used to confirm polymerization of the monomeric

surfactant. The CMCs of the monomeric surfactants synthesized in this research were determined using a surface tensiometer.

The Du Nouy ring and Wilhelmy plate are two methods of determining surface tension by use of a surface tensiometer.

Figure 1.5 Surface tensiometer instrument.

The Du Nouy ring method, named after the French physicist who developed it in the late 1800's, is the most common method and was used to measure surface tension of surfactants used in this research. In this method, a ring (generally) platinum with defined geometry is immersed in the liquid and carefully pulled out through the liquid surface.

The surface tensiometer (Figure 1.5) is composed of a precision microbalance, platinum-iridium ring with defined geometry, and a precision mechanism to vertically move the sample liquid in a glass plate or beaker. The ring hanging from the balance hook is first immersed in the liquid and carefully pulled up through the surface of the liquid. The

microbalance records the force applied on the ring while it is pulled through the surface. The surface tension is the maximum force needed to detach the ring from the liquid surface. Conversely, the Wilhelmy plate method measures the weight of liquid drawn by a plate when the plate is lifted from the surface of the liquid. In this method, the weight of the liquid is proportional to the surface tension.

1.2.4 Micelle Formation

Micelle formation, or micellization, can be viewed as an alternative process to adsorption at the interfaces, which removes hydrophobic groups from contact with water and reduces the free energy of the system.[1] It is an important phenomenon because surfactant molecules in solution behave differently when present in micelles as compared to free monomers. Surface and interfacial tension lowering, as well as dynamic phenomena such as foaming and wetting, are governed by the concentration of free monomers in solution. Micelles may be viewed as a reservoir for surfactant monomers, and the exchange rate of a surfactant molecule between micelle and bulk solution may vary by many orders of magnitude depending on the size and structure of the surfactant.

The number of surfactant monomers that make up the micelle is called the aggregation number (N). Each micelle is typically composed of 40 to 140 molecules, and the aggregation process depends on the type of surfactant and the conditions of the system in which it is dissolved.[12] There are several techniques for determining the value of N. These methods include viscosity, diffusion, ultra filtration, nuclear magnetic resonance, light scattering, fluorescence, and sedimentation velocity.[13, 14]

As mentioned previously, the structure of the micelle depends on the equilibrium between the repulsive and attractive forces among the hydrophilic head groups and

hydrophobic tails, respectively. Since 1913, when McBain proposed the presence of molecular aggregates in soap solutions,[15] the structure of micellar aggregates has been a matter of discussion. In 1936, Hartley proposed micelles were spherical with charged groups located at the micellar surface while McBain, in 1944, suggested spherical and lamellar micellar forms coexisted.[16, 17] Many micellar systems can be described using the Hartley model, which suggests counterions are bound to the charged head groups of the surfactants. This explains the drop in conductance of the surfactant solution at the CMC, as illustrated in Figure 1.3. In addition, Hartley proposed the core of the micelle has properties of liquid hydrocarbons; thus, micelles are able to solubilize hydrophobic molecules otherwise insoluble in polar (water) solvents.[2, 18] Later, Debye and Anacker proposed micelles are rod-shaped rather than spherical or disk-like.[19] Using nuclear magnetic resonance (NMR) and kinetic studies, Menger in 1991 reported that micelles were more disorganized with chain looping, nonradial distribution of chains, and contact of terminal methyl groups with water.[20] Figure 1.6 illustrates several proposed micellar structures mentioned above.

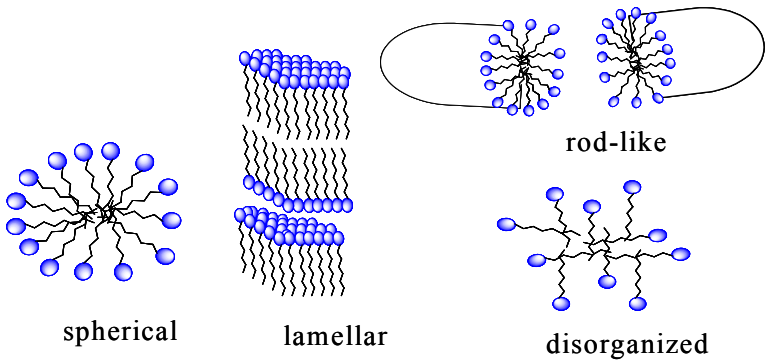


Figure 1.6 Proposed micelle shapes and structures.

In most cases, the spherical form proposed by Hartley and illustrated in Figure 1.6 is accepted as a true representation of the micelle. Recent studies using newly developed research techniques, such as NMR, electron spin resonance, neutron scattering, and quasi-

elastic scattering have significantly clarified the mystery behind the nature and shape of the micelle.

Many micellar systems form the spherical shape proposed by Hartley. However, with increasing surfactant concentration, ionic micellar systems can change their shape in the following sequence: spherical - cylindrical - hexagonal - lamellar (Figure 1.7).[2, 21-23]

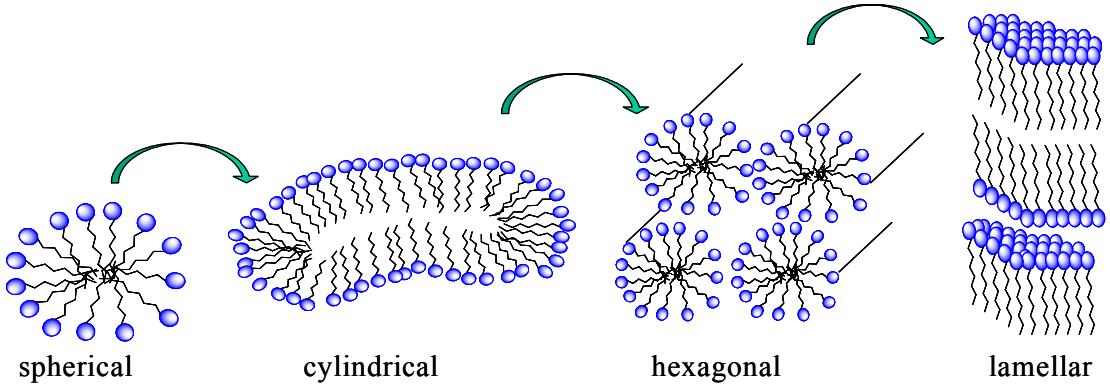


Figure 1.7 Changes in micellar shape and structure with increasing surfactant concentration.

Conversely, for nonionic micellar systems, the shape changes from spherical directly to lamellar with increasing surfactant concentration.[2, 24, 25] Other factors that affect micelle shape are volume, size and structure of head group, chain length of the hydrophobic tail, and nature of the solvent in which the surfactant is dissolved. For ionic micelles the net rate of aggregation is, in most cases, less than the degree of micelle aggregation. This is because large fractions of counter ions remain associated with the micelle and form what is called the Stern layer at the micellar surface.[1, 2] In ionic micelles, the Stern layer resembles a concentrated electrolyte solution consisting of bound surfactant head groups, counter ions, and water molecules. Ionic spherical micelles have two regions, an outer region and an inner region (Figure 1.8). The inner region consists of an inner core and the palisade layer, while the outer region is made of the Stern layer and diffuse layer, also called the Guoy-Chapman

layer. In aqueous systems, the inner core forms a water-free region and is composed of hydrophobic tail groups of the surfactant. The palisade layer, a hydrated region, is viewed as a liquid hydrocarbon. The radius of the inner core and the palisade layer is approximately equal to the length of the fully extended hydrocarbon chain. In addition, the Stern layer is usually a few angstroms, while the diffuse layer extends up to several hundred angstroms.[26]

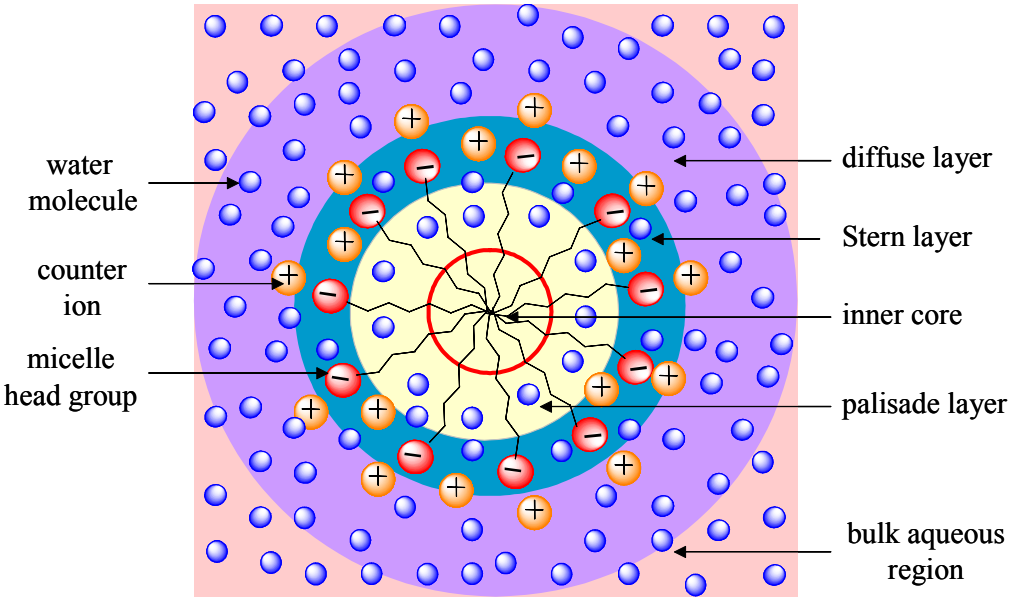


Figure 1.8 Regions of spherical micelles in aqueous solutions.

Micellization is an intermediate stage between phase separation and simple complex formation. This is illustrated by the micellization models in thermodynamic analyses. There are two common models used to explain micelle formation: the phase equilibrium model and the mass action model.[1, 13, 27] The phase equilibrium model treats the micelle as a separate, but soluble phase. It suggests the concentration of monomeric species remains constant above the CMC. Conversely, the mass action model considers micellization as a chemical reaction. It assumes that a single micellar complex is in equilibrium with the monomeric surfactant molecules. In addition, kinetic studies have shown that micelles are involved in a highly dynamic equilibrium.[28] Therefore, micelles are generally considered polydispersed

species due to this dynamic equilibrium. In capillary electrophoresis (CE) such polydispersity can result in a range of micellar migration velocities resulting in band broadening, which is detrimental to micellar electrokinetic separations. The problems of dynamic equilibrium and thermodynamic stability in CE separations can be eliminated by use of polymeric surfactants.

1.3 Polymeric Surfactants

Polymeric surfactants, or surface-active polymers, have attracted much attention over the last two decades. They are used in many different applications, such as rheology control and stabilization of dispersions.[1] They are also of great interest in chromatography where they are used as pseudo-stationary or stationary phases. A polymer with surface-active properties can be produced via three major routes: 1) with hydrophobic chains grafted to a hydrophilic backbone polymer, 2) with hydrophilic chains grafted to a hydrophobic backbone, and 3) with alternating hydrophilic and hydrophobic segments. These manufactured polymers have properties similar to conventional micelles; however, they have rigid structures which can maintain physical properties and show a higher degree of thermodynamic stability.

Polymeric surfactants are common in nature, and three principal types exist in both the plant and animal kingdoms. The hydrophilic segment is often a polysaccharide which may be charged or uncharged. For example, antibodies normally contain carbohydrate residues attached as side chains a distance from the antigen-binding region. The main function of the carbohydrate residue is to improve the hydrophilicity of the relatively hydrophobic protein. Other proteins in the body, such as milk and saliva, exhibit high surface activities, are excellent stabilizers of fat droplets, and contain well-defined amino acid sequences very rich in phosphate groups.[1]

Typical surfactants used in this research have vinyl groups in the hydrophobic tail. In this case, polymerization of the monomeric surfactant is achieved using gamma radiation. This technique is advantageous over other polymerization techniques because it can be performed at room temperature without an additional initiator to start polymerization.[29] Polymerization must be conducted at surfactant concentrations above the CMC for the reaction to proceed to completion.[30] This technique links the surfactant monomers into a rigid geometrical orientation similar to the micelle geometry.[31] As will be discussed in later chapters, polymeric surfactants show superiority over traditional micelles in the separation of both chiral and achiral compounds in micellar electrokinetic chromatography.

1.4 Characterization of Polymeric Surfactants

With the exception of some biological macromolecules, most polymers have a molecular weight distribution which is used to distinguish polymers from low-molecular-weight species. Therefore, in any finite sample of a polymer, the experimental measurement of the molecular weight can only yield an average value. There are three accepted molecular weight distribution ranges: the number average molecular weight (Mn), weight average molecular weight (Mw), and z-average molecular weight (Mz).[32, 33] The Mn of most commercial polymers is in the range of 10,000 to 100,000 g/mol, although some materials have Mn values 10-fold lower or higher. Higher molecular weights can be measured by absolute methods to determine Mw, which is a more useful parameter than Mn in correlating polymer properties such as viscosity or toughness. The ratio Mw/Mn is a measure of the broadness of a distribution and is referred to as the polydispersity index.

To determine Mw, characterization techniques such as membrane osmometry, light scattering, and analytical ultracentrifugation (AUC) can be used.[33-35] A membrane

osmometer uses a physical barrier to create a sharp concentration boundary, whereas light scattering responds to smaller spontaneous osmotic potentials, and AUC uses gravitational potentials to create a smooth but large concentration gradient. All these methods produce absolute molecular weight information because they take advantage of chemical potential. Although osmometry is the most limited in molecular weight range, AUC and light scattering can be applied to practically any macromolecule. Furthermore, the former technique can be used to measure molecular weights of very small molecules such as sugar. Although AUC is extremely powerful for aggregating or associating systems, the disadvantage to this technique is its profound slowness. However, this problem has, to some extent, been overcome in new instruments capable of measuring many samples simultaneously.

Another method for determining the molecular weight is gel permeation chromatography (GPC). Using this technique, the molecular weight distribution in the range of 6,000 to 10,000 g/mol was reported for omega–unsaturated surfactant polymers.[36] Dobashi et al., using pullulans as molecular weight calibration standards in aqueous GPC, estimated the Mw and Mw/Mn of the polymeric surfactant polysodium N-undecanoyl-L-valinate.[37] However, their results were questionable because pullulans, although widely used as standards in GPC, might not be appropriate for the characterization of non-ideal species such as polymeric surfactants. On the contrary, mass spectrometry provides precise measurements of monomer molecular weights; however, the molecular weights of polymeric surfactants synthesized in this research could not be obtained using this technique because of the fragmentation of the covalent linkages at the tail groups.

1.4.1 Analytical Ultracentrifugation

Sedimentation is one of the great classical methods used for polymer characterization.[34, 35] There are two varieties (equilibrium and velocity) which are usually done on the same instrument, an analytical ultracentrifuge. This device can determine the concentration of a polymeric solute as a function of position from the center of a rapidly rotating cell. In the equilibrium method, the particle is not propelled to the bottom of the cell, resulting in "sediment." Rather, a low centrifugal field creates a concentration gradient such that there are more particles in the bottom of the cell than at the top. Since the gradient is opposed by diffusion, dynamic equilibrium is slowly achieved. On the other hand, velocity sedimentation is a transport technique, akin to diffusion or viscosity, where the rate at which a particle sinks is measured. In this method, the particle may sink to the bottom of the cell because the centrifugal field is much higher than in the equilibrium sedimentation method. While velocity sedimentation alone does not produce absolute molecular weights, it does measure friction and is therefore useful for providing information related to shape.[34, 35, 38] In this text, I will consider only sedimentation equilibrium in greater detail.

1.4.1.1 Sedimentation Equilibrium

Over a century ago, Gibbs postulated the general mathematical description of the conditions for equilibrium between phases, either in the presence or absence of an externally applied force.[39] In 1908, Perrin reported the first sedimentation equilibrium experimental observations in an artificial system at the Sorbonne University, Paris, France. Based on this discovery, Perrin won the Nobel Prize for physics in 1926. In the same year, Theodor Svedberg won the Nobel Prize in Chemistry for his work on disperse systems. Svedberg concluded that to produce equilibrium under reasonable experimental conditions for high-

molecular-weight organic polymers and other macromolecules in solution, it was necessary to apply an external field greater than gravity. Later, Fujita provided a simple approach to all basic essential working equations for both sedimentation equilibrium and sedimentation velocity.[40]

One necessary and sufficient condition for a solution to approach equilibrium is the total potential (μ) of the system be constant. The total potential is given by

$$\mu = \mu_c + \mu_2 \qquad 1.1$$

where μ_c is the centrifugal potential of one mole of a given component in the ultracentrifuge cell and μ_2 is the chemical potential of the component. Figure 1.9 is an illustration of the ultracentrifugation cell.

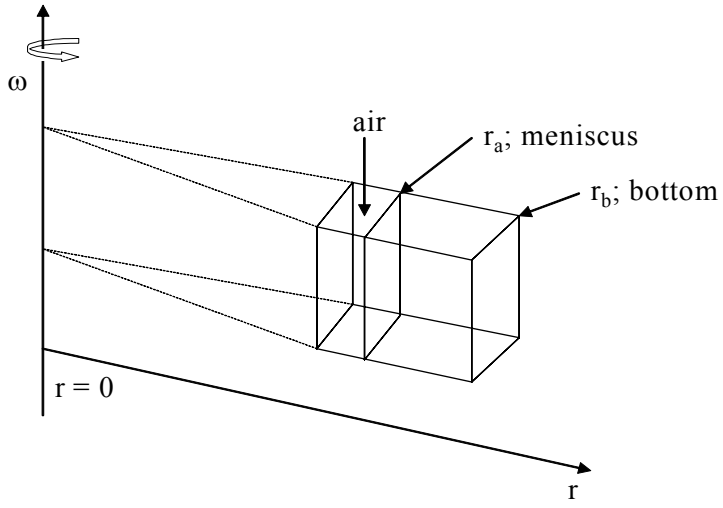


Figure 1.9 Sector-shaped ultracentrifuge cell.

When spun at an angular velocity, ω, a unit of mass of the solution at the radial distance (r) from the axis of revolution, gains a centrifugal potential (μ_c) expressed as,

$$\mu_c = -\int \omega^2 M r dr = -\omega^2 \frac{Mr^2}{2} + \text{constant} \qquad 1.2$$

where M represents the molar mass of the component mentioned in Equation 1.1. At equilibrium,

$$\mu = \mu_c + \mu_2 = \text{constant} \qquad 1.3$$

In principle, this relation holds when each shell in the ultracentrifuge cell is infinitesimally thin (Figure 1.9). In a sedimentation equilibrium experiment, a small volume of uniform solution is centrifuged at a moderate speed (9000 – 30,000 rpm) which is lower than that required in a velocity experiment (30,000 – 60,000 rpm). Under these conditions, the solute begins to sediment toward the bottom of the cell. Consequently, the gravitational field generates a concentration gradient in which the process of diffusion opposes the process of sedimentation. After an appropriate period of time, the two opposing forces reach equilibrium, which is referred to as sedimentation equilibrium.

In a thermodynamically ideal case, the molecular weight of a polymer, M, is calculated by Equation 1.4

$$\ln\frac{A_a}{A_b} = \frac{M\omega^2 r(1-\rho * V_{bar})(r_b^{\,2} - r_a^{\,2})}{2RT} \,,.. \qquad 1.4$$

where A_a and A_b represent the absorbance at the two radii, r_a (meniscus) and r_b (bottom) respectively, ω is the angular velocity, ρ is the solvent density, R is the gas constant, and T is the temperature in Kelvin. The value of V_{bar}, the partial specific volume, is determined externally by a densitometer.

1.4.1.2 Densitometry

The density of a sample material is defined as the quantity of mass of the material to a given volume of the sample. Density (ρ) and specific gravity (sg) are often used interchangeably. While density is a straight mass-to-volume relationship,

$$\rho = \frac{M}{V}, \tag{1.5}$$

specific gravity is defined as the ratio between the density of a given volume of material (ρ_1) to the density of the same volume of water (ρ_2), as shown in Equation 1.6.

$$sg = \frac{\rho_1}{\rho_2} \tag{1.6}$$

There are different types of densitometers, which include mass flowmeters, hydrometers, nuclear, capacitor, and vibration densitometers. In this dissertation, a mass flowmeter was used to determine the density of polymeric surfactant solutions. This type of densitometer determines the density of liquids and gases by measuring the period of oscillation electronically. To do this, the sample is introduced into a U-shaped tube which can be excited to undamped oscillation by electronic means and whose frequency is influenced by the mass of the sample. Both straight sections of the U-shaped tube form the spring element of the oscillator. The direction of oscillation is perpendicular to the plane of the U-shaped tube. The mounting points, which are fixed, limit the oscillating volume. If the U-shaped tube (oscillator) has been filled with the sample to at least the mounting points, then the same known volume of the sample oscillates, and the mass of the sample is proportional to its density. Because filling the oscillator beyond the mounting points has no effect on measurements, the oscillator can also measure densities of samples flowing through it.

Holding the temperature constant, the density can be calculated from the oscillation period by considering a hollow body with mass (m) suspended on a spring with a spring constant (c). The volume (V) of the hollow body is then filled with a sample of density (ρ). The natural frequency (f) of this spring-mass system is expressed as

$$f = \frac{1}{2\pi}\sqrt{\frac{c}{m+\rho \mathrm{V}}} \tag{1.7}$$

and the period (T) is defined as

$$T = 2\pi\sqrt{\frac{m+\rho \mathrm{V}}{c}} \tag{1.8}$$

which can be rearranged to Equation1.9.

$$\rho = T^2\left(\frac{c}{4\pi^2 \mathrm{V}} - \frac{m}{\mathrm{V}}\right) \tag{1.9}$$

Using the abbreviations

$$A = \frac{c}{4\pi^2 \mathrm{V}} \quad \text{and} \quad B = \frac{m}{\mathrm{V}} \tag{1.10}$$

One can deduce

$$\rho = T^2(A - B) \tag{1.11}$$

where A and B are the device constants for each individual oscillation and are derived from two measurements when the oscillator is filled with substances of known density, normally air and water. After the density of the polymeric surfactants is determined at different concentrations, the values are used to determine the partial specific volume of a surfactant.

1.4.1.3 Partial Specific Volume

The partial specific volume (V_{bar}) is defined as the increase in volume when 1g of a dry solute is dissolved in a large volume of solvent. V_{bar} is needed to determine the molecular weight of a polymer or polymeric surfactant using the analytical ultracentrifuge. A binary mixture can be used to envision the calculation of partial specific volume. Consider a binary mixture such as

$$V_{avg} = \frac{v}{n} \tag{1.12}$$

where $n = n_a + n_b$ is the total number of moles and V_{avg} is the average volume per mole. For a binary system, the mole fractions of *a* and *b* components x_a and x_b, respectively, are defined as

$$x_a = \frac{n_a}{n} \tag{1.13}$$

and

$$x_b = \frac{n_b}{n} \tag{1.14}$$

and the partial molar volume (v_b) is defined as follows

$$v_b = (\frac{\partial v_b}{\partial n_b}) n_a(T,P) \, . \tag{1.15}$$

After performing substitutions and derivatization using the chain rule we obtain the expression,

$$V_{avg} = v_b - \frac{\partial x_a}{\partial x_b} V_{avg} \tag{1.16}$$

By definition, the sum of mole fraction is unity, thus,

$$x_a + x_b = 1 \tag{1.17}$$

from Equation 1.16, the following expression is derived

$$\partial x_a = -\partial x_b \tag{1.18}$$

Combining Equations 1.16 and 1.18, the following expression is obtained,

$$V_{avg} = v_b + x_a \frac{\partial V_{avg}}{\partial x_a} \tag{1.19}$$

where v_b is the partial molar volume of component b. The weight fraction (Wa) of the component *a* can be define as

$$W_a = \frac{m_a}{m_a + m_b} \tag{1.20}$$

where m_a and m_b represent the masses of components a and b, respectively. Similar to Equation 1.19, the partial specific volume is related to the average molar volume using the following relationship

$$V_{avg} = \frac{v}{n} = \frac{1}{\rho} = V_{bar} + W_a \frac{\partial \frac{1}{\rho}}{\partial W_a} \tag{1.21}$$

The partial specific volume of each polymeric surfactant is determined by simply plotting the inverse of density ($1/\rho$) of the solutions as a function of the weight fraction (W_a) of the polymeric surfactant.

1.4.2 Absorption Spectroscopy

Spectroscopy is a branch of science which identifies and measures interactions of electromagnetic radiation (ER), whether absorbed, emitted, or scattered, with matter. ER is a form of energy that moves through space at very high speed and possesses both wave properties and discrete-particle properties called photons (packets of energy). It manifests in varying wavelengths and frequencies, i.e., gamma-rays, X-rays, ultraviolet, visible, radiant heat, microwave, and radio-frequency radiation.

The transition of matter from a lower energy state to a higher energy state is the basis of absorption spectroscopy. When a solution absorbs electromagnetic radiation emitted from a given light source, the quantity of radiation absorbed follows definite physical laws.[41] The amount of radiation absorbed by the solution (A) is the logarithm of the ratio of the intensity of the incident radiation (I_o) and the intensity of transmitted radiation (I).

$$A = \log \frac{I_o}{I}. \tag{1.22}$$

Absorbance is linearly related to the concentration of the solution by the Beer-Lambert law, which is expressed as

$$A = \varepsilon bc \qquad 1.23$$

where ε is the molar absorptivity of the absorbing species, b is the light path length, and c is the concentration of the absorbing species solution. The molar absorptivity is the inherent ability of a chemical species to absorb light and is constant for a given wavelength. The path length is the distance light travels through the measured solution. The absorptive process involves a transfer of energy from a photon of energy to a molecule, during which electrons are promoted from the lowest energy level, S_o, to higher energy levels (S_1, S_2 --) called excited states. The energy difference between the ground and excited state is equal to the energy of the incident photon. Normally, the electronic transition takes place in approximately 10^{-15} seconds, which is much faster than nuclear reorganization (>10^{-14} sec). Therefore, the higher energy state is reached without rearrangement of the nuclei. After absorption, a molecule can return to the ground state by radiative and/or non-radiative processes. The radiative processes are fluorescence and phosphorescence, and the non-radiative pathways include vibrational relaxation, internal conversion, intersystem crossing, fluorescence quenching, and other deactivation processes. Absorption spectroscopy was used in this dissertation to obtain the absorption spectra of the surfactants, to determine the maximum wavelength of absorption of the analytes of interest, and as a method of detection in CE.

1.4.3 Fluorescence Spectroscopy

Fluorescence is the result of the emission of a photon following the relaxation of an electronically excited molecule to the lowest vibrational level of the first excited state. The

emission spectrum depends upon the chemical structure and the environment of the molecule. When compared to absorption techniques, the fluorescence technique has a lower limit of detection and is, therefore, more sensitive. Because the emission spectrum of several fluorescent molecules can reflect the polarity and/or viscosity of the environment in which they are solubilized, micellar systems and polymeric surfactants can be studied using these environmentally sensitive fluorescent molecules. Thus, the polarity of the micellar core can be determined by choosing a fluorophore solubilized within the core that is sensitive to the polarity of the environment.[42]

There are two common techniques used in fluorescence spectroscopy to investigate physical and chemical properties of the microenvironment immediately surrounding a fluorophore. These include fluorescence quenching (static or dynamic) and steady-state fluorescence anisotropy. Static fluorescence quenching results from the formation of a ground state complex between the fluorophore and quencher, which reduces the number of fluorophore available for excitation.

Although the dynamic fluorescence quenching technique was not used for this work, I will briefly describe the fundamental theory. Dynamic fluorescence quenching occurs if an excited state fluorophore collides with the quencher. In this case, the dynamic quenching process can be described by the Stern-Volmer equation [45]

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] \qquad 1.24$$

where [Q] is the concentration of the quencher, τ_0 is the lifetime of the fluorophore in the absence of the quencher, F_0 and F are the fluorescence intensities in the absence and presence of the quencher, respectively, and k_q is the bimolecular quenching rate constant.[43] The Stern-Volmer quenching constant (K_D) is expressed as

$$K_D = k_q \tau_0 \quad (1.25)$$

and is the slope resulting from a plot of F_0/F versus [Q], a Stern-Volmer plot. A linear plot can indicate the presence of a single class of fluorophores equally accessible to the quencher while a non-linear plot can be due to either the combination of static and dynamic quenching processes and/or the presence of multiple fluorophore environments.

Static quenching has been used to determine the aggregation number of surfactants.[43, 44] This technique requires both the fluorophore and quencher to be sufficiently hydrophobic to partition into the micellar phase. Different quencher concentrations are prepared, the fluorescence intensity is measured, and based on the following equation

$$\ln \frac{I}{I_0} = \frac{[Q]}{[M]} \quad (1.26)$$

the slope of a plot of $\ln(I/I_0)$ versus [Q] is equal to the reciprocal of the micelle concentration, [M].[45] The micelle concentration, together with surfactant concentration and critical micellar concentration, can be used to calculate the aggregation number (N).

$$N = \frac{[\text{Surfactant}] - \text{CMC}}{[M]} \quad (1.27)$$

Steady-state fluorescence anisotropy occurs when a fluorophore is continuously excited with polarized radiation, resulting in polarized emission.[42] The rotational diffusion of a fluorescent molecule is one cause of the reduction in intensity of a polarized emission. Thus, using polarized excitation, the intensity of the fluorescence emission perpendicular to ($I_\perp$) and parallel to (I_{II}), the direction of excitation, will depend largely on the rotation of the molecule. The degree of depolarization of the fluorophore emission is called anisotropy, r, and is expressed as follows.

$$r = \frac{I_{II} - I_{\perp}}{I_{II} + 2I_{\perp}}. \qquad 1.28$$

Depolarization is a result of photoselection and angular displacement of the absorption and emission dipoles of the fluorophore. When complete depolarization occurs, the anisotropy of the molecule equals zero. However, photoselection leads to an intrinsic anisotropy, (r_o) expressed as

$$r_o = \frac{3\cos^2\theta - 1}{5} \qquad 1.29$$

where θ is the angle between the excitation and emission oscillators or dipoles of the fluorophore. Thus, when θ equal zero, the highest value of anisotropy, 0.4, is realized.

Steady-state fluorescence anisotropy finds many applications in different fields of research.[46] For instance, this technique has been used as a measure of chiral recognition,[47] to study protein-ligand association reactions, protein denaturation, and rotational rates of difussion.[42] In this research, static fluorescence quenching was used to determine the aggregation number of polymeric surfactants.

1.4.4 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) is the resonant absorption of radio frequency radiation by nuclei exposed to a magnetic field. After a radio frequency pulse excites the nuclei, free-induction decay (which is the difference between the applied frequency and the resonance frequency of the nuclei) is detected with a radio coil and stored in a computer for data processing. The time-domain decay signals are converted to a frequency-domain signal by Fourier transformation. When magnetically different nuclei are present, the free-induction decay develops a distinct beat pattern. Thus, the Fourier transform of this pattern displays a frequency-domain spectrum in which different nuclei will possess different chemical shifts.[48]

There are wide applications of NMR spectroscopy. For example, NMR is used for the structural elucidation of proteins and enzymes in solution, for in vivo monitoring of metabolism, and medical diagnosis.[49] NMR spectroscopy was used in this dissertation for structural elucidation of the synthesized monomeric and polymeric surfactants.

1.5 Chirality

The term "chirality" refers to a non-superimposable mirror image phenomenon usually found in nature. A good example is human hands, which are non-superimposable mirror images of each other. Louis Pasteur first recognized it in late 1850s and his findings are well documented.[50] In chemistry, chirality requires the mirror images of a molecule be non-superimposable. In most cases, chirality is a result of an asymmetric carbon atom (a stereogenic center) surrounded, in a tetrahedral spatial arrangement, by four different substituents. However, chiral molecules without a stereogenic center also exist.

There are three types of chirality: axial, helical, and planar. For instance, interconvertible conformational isomers such as the binaphthyl derivatives are atropisomeric and have an axial chirality. Proteins, polysaccharides, and biopolymers have helical chirality, due to their clockwise (right-handed) or anticlockwise (left-handed) arrangement.[51] Planar chirality occurs when a molecule contains a group of bonds in a plane such that chirality arises from the arrangement of the out-of-plane molecules. Two identical molecules that are mirror images of one another, but not superimposable, are deemed enantiomers, and an equal mixture of enantiomers is called a racemate or a racemic mixture. Enantiomers have the same physical properties, except for their equal but opposite rotation of polarized light.[52] Because of the similarity of their physical properties, enantiomers are usually challenging to separate. Early techniques of separation, such as crystallization processes, were limited in their

applications and tedious because several repetitions were necessary to obtain a pure enantiomer.[53]

Increased awareness of the effects of different enantiomers on biological activity became obvious after the use of the drug thalidomide in the 1950s and early 1960s.[54] Thalidomide (n-phythalyl-glutamic acid imide) is a racemic mixture of which the R-(+) enantiomer is therapeutically beneficial while the S-(–) enantiomer is responsible for adverse side effects. This drug was administered to pregnant women to prevent morning sickness but, because it interfered with the development of blood vessels in the fetus, it caused serious birth defects such as stunted or missing limbs.[55] Development of more efficient and effective chiral separation techniques has increased in response to problems of stereoselectivity in pharmaceutical products, as well as the agrochemical, food, and electronic industries.[56, 57]

Some of the chromatographic techniques developed for the simultaneous separation and quantification of enantiomers include high performance liquid chromatography (HPLC),[58, 59] gas chromatography (GC),[60] supercritical fluid chromatography (SFC),[61] and capillary electrophoresis (CE). Other non-chromatographic methods used for quantitative analysis of chiral compounds include polarimetry, calorimetry, NMR, and enzyme techniques.[62] Chiral HPLC is superior to GC due to its ability to resolve a wide variety of non-volatile and thermo-labile analytes as well as very polar analyte mixtures. Also, chiral HPLC has on-line detection and quantification capabilities of both the optical rotation and mass of enantiomers.[63] Some limitations of chiral HPLC are poor separation efficiencies and long analysis times.

Intensive research into chiral separations using micellar electrokinetic chromatography (MEKC) has been performed since the late seventies. This technique is advantageous over

conventional chromatographic techniques because it utilizes an array of chiral selectors, generates high chiral separation efficiencies, and minimizes consumption of mobile phase, pseudo-stationary phase (chiral selector), and sample.[64]

1.6 Mechanism of Chiral Recognition

The mechanism by which chiral recognition occurs is not well understood because of the complexity and multiplicity of the interactions. To distinguish between two enantiomers, a chiral selector must be introduced into the separation media. Further, the chiral selector should be compatible in size and structure to the racemic analyte for separation to occur. Thus, interaction of the chiral selector with the enantiomers of the chiral analyte results in the formation of two transient and/or long-lived diastereomers. These diastereomers differ in their solvation in the mobile phase, thermodynamic stability, and binding constant of the enantiomer chiral selector complex to the solid support. These differences occur because at least three active points of the chiral selector participate in the interaction with corresponding sites of the chiral analyte.

The three-point interaction, described by Easson et al., is necessary for chiral recognition.[65] The rule states that chiral recognition depends on the degree of interaction exhibited between each enantiomer of the chiral analyte and the chiral selector. One enantiomer (eutomer) must interact simultaneously with all three sites and at least one of the interactions should be stereochemically dependent. Consequently, the other enantiomer (distomer) should only achieve two of these interactions due to spatial restrictions. As Figure 1.10 illustrates, while a three-point interaction is possible at K-K, M-M and N-N for the R-(+) enantiomer, the S-(–) enantiomer can only have a two-point interaction at K-K and M-M with the same chiral binding site.[66]

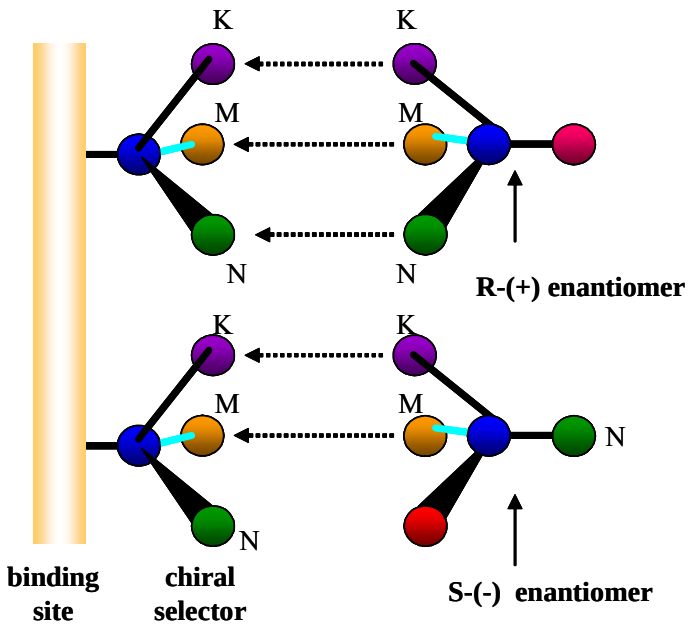


Figure 1.10 Three-point rule for chiral recognition.

According to Figure 1.10, chiral recognition depends on the absence of the N-N fit. The interactions at the three points can be both attractive and repulsive. There are multiple modes of interactions that could be possible, such as dipole-dipole bonds, e.g., hydrogen bonding, which involves secondary amine or carbonyl groups of the chiral selector with the hydroxyl, acidic, or amino groups of the analytes. Also, steric interactions from the bulky non-polar groups attached near the chiral center of the chiral selector provide the conformational control necessary for chiral separation. Electron pair π-donor and π-acceptor aromatic rings, ion-dipole bonds, and van der Waals forces are other interactions that play a remarkable role in chiral recognition. However, not all interactions between enantiomer and chiral selector will meet the three-point rule. The total enantioselectivity is strongly dependent on the composition, temperature, and pH of the mobile phase. Therefore, it is necessary to optimize the separation media in order to maximize the three-point interactions for chiral separations.

Part 2. Introduction to Capillary Electrophoresis

1.7 Electrophoresis and Capillary Electrophoresis

Electrophoresis is defined as the movement of electrically charged particles or molecules in a conductive liquid medium, usually aqueous, under the influence of an electric field.[67] Electrophoresis originated in the 1900s when Teselius introduced it as an analytical technique in 1930.[68] He won a Nobel Prize in 1984 for his pioneering work in this area. Although, electrophoretic separations were developed using tubes, glass, and Teflon, it did not become popular as an analytical techinique until 1981 when Jorgenson and Lukacs showed high resolving power using capillary columns.[69]

Capillary electrophoresis (CE) is a technique which uses narrow-bore capillaries to separate molecules driven by an electric field. These separations are performed on small or large molecules and are facilitated by the application of high voltages, which generate the flow of buffers and analytes through the capillary. CE, a novel separation technique, employs a separation mechanism completely different from that of liquid chromatography and can be used in cases where liquid chromatography is limited. There are several advantages of CE, including high efficiency, fast separations, easy automation, minute sample amounts, and lower reagent consumption also, it can be used for qualitative and quantitative analysis.

Just as HPLC has different modes of chromatography, e.g. normal phase, reverse phase, size exclusion, ion exchange, hydrophobic interaction, chiral, and affinity, CE has several modes of electrophoresis. The most common modes are capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), capillary isotachophoresis (CITP), capillary gel electrophoresis (CGE), capillary electrochromatography (CEC), and electrokinetic chromatography (EKC) or micellar electrokinetic chromatography (MEKC).

Briefly, CZE is relatively simple and applicable to the simultaneous separation of cationic and anionic, but not neutral, analytes. In CZE, the capillary is filled with a buffer of steady composition, as are the source and destination vials. This is contrary to CIEF, where the capillary is filled with a solution that forms a pH gradient, and CITP, where leading and trailing buffers are placed in the capillary. CZE, where the capillary is filled with a free solution to minimize matrix effects, is also unlike CGE, where the capillary is filled with a gel. However, in some cases of CZE, the solute may interact with the capillary wall.[67, 70]

Another method, CEC, is a hybrid technique that combines features of capillary liquid chromatography and CE. For this method of separation, CE capillaries are packed with a stationary phase and filled with a mobile phase (buffer). An applied voltage generates an electroosmotic flow, which transports solutes along the capillary while they partition with the stationary phase packing. The last technique, MEKC, is a mode of CE based on the partitioning of solutes between micelles or surfactants and the running buffer. This mode allows for the separation of neutral and ionized compounds[71-74] and peptides.[75] MEKC is the separation technique primarily used in this dissertation and will be discussed in detail in subsequent sections.

1.7.1 Capillary Electrophoresis Instrumentation

A simple schematic of a CE instrument is shown in Figure 1.11. This instrumentation consists of a separation capillary, inlet and outlet buffer reservoirs and sample reservoirs. It also has two electrodes, a power supply, light source and detector. The separation capillary is placed between the inlet and outlet buffer reservoirs fitted with platinum electrodes and filled with equal volumes of the same aqueous buffer solution. The platinum electrodes are connected to a high voltage power supply.

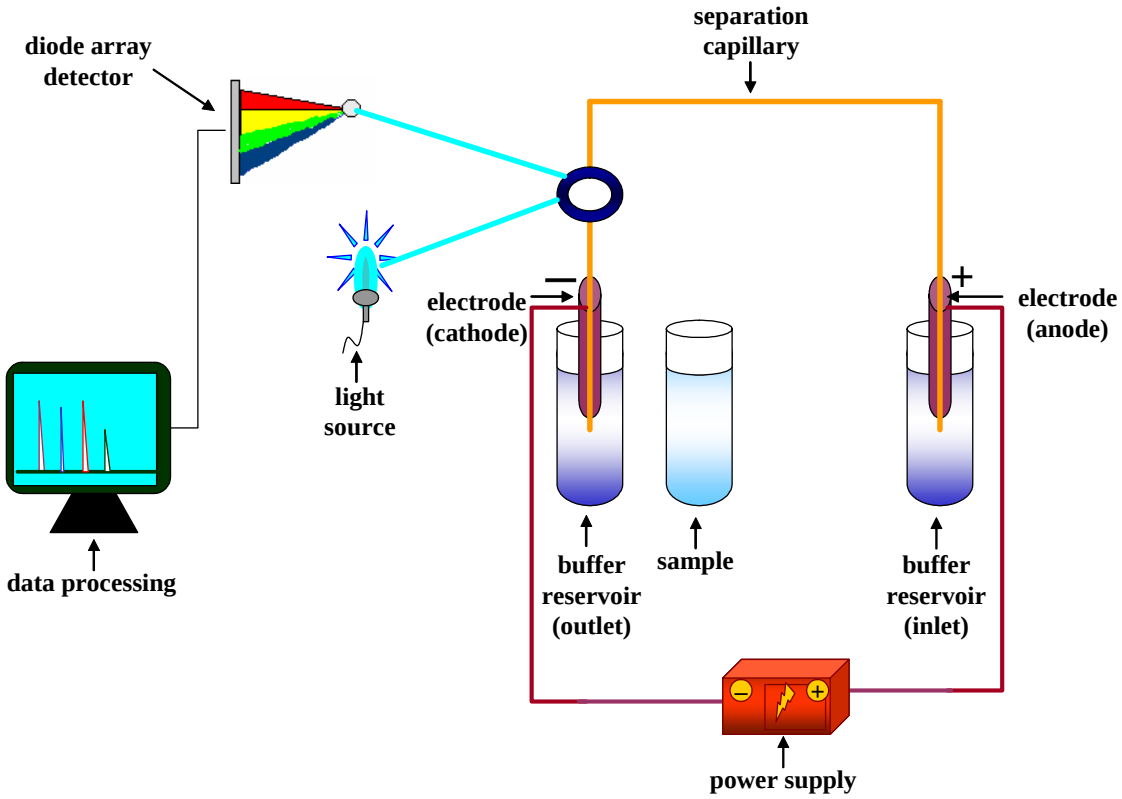


Figure 1.11 Schematic of a CE instrument.

The sample is introduced in the capillary by either hydrodynamic injection (application of external pressure) or by eletrokinetic injection (application of electric field) by switching the sample vial with the inlet buffer vial. Once the analyte is injected, the capillary is then switched back to the inlet buffer. Upon application of a voltage, buffer and analyte ions migrate from the inlet buffer toward the outlet buffer. On the end near the outlet buffer a section of the polyimide coating on the capillary is removed to make a detector window.

The separated analyte molecules are analyzed by the detector, which is aligned with the source (e.g., a UV lamp) by the capillary cassette alignment interface. The power supply provides an electric field across the capillary. Most CE instruments can be operated in constant voltage, constant current or constant power mode and have the ability to reverse the

polarity. Voltages up to 30 kV, currents up to 300 μA, and power up to 6 W are used. It is necessary to have a stable voltage, for any variations in voltage will cause changes in migration times. Thus, the constant voltage mode is most widely employed.

Fused silica capillaries that range from 30 to 100 cm long with inner diameters of 20 to 200 μm and outer diameter of 375 μm are typically used. There are a variety of detectors in use with CE; these include UV/Vis absorbance, fluorescence, laser-induced fluorescence, conductivity, amperometry, radiometry, mass spectrometry, and refractive index. The UV/Vis absorbance detector is widely employed and was used in this dissertation research. Its detection limits are approximately 1 mg/L (ppm) to 1 μg/L (ppb).

1.7.2 Migration in Capillary Electrophoresis

In CE, the mobility of solutes (analytes) in solution is based on their charge-to-size ratio. Further, the velocity of these analytes depends upon the strength of the applied electric field, properties of the solution such as viscosity, temperature, pH, and ionic strength, as well as properties of the analytes, i.e., mass, three-dimensional structure, and charge. The major parameters in CE are migration time (t_m), electric field strength E, V/cm, apparent electrophoretic mobility (μ_{ep}, cm^2/Vs), and electrophoretic velocity (v_{ep}, cm/s). The migration time in CE is the time it takes an analyte to move from the beginning of the capillary to the point of detection.[76] The values of E, v_{ep}, and μ_{ep} can be obtained using the equations below

$$E = \frac{V}{L_t} \tag{1.30}$$

$$v_{ep} = \frac{L_d}{t_m} - \frac{L_d}{t_{nm}} \tag{1.31}$$

$$\mu_{ep} = \frac{vep}{E} = (\frac{Ld}{t_m} - \frac{L_d}{t_{nm}})(\frac{L_t}{V}) \tag{1.32}$$

where V is the applied voltage, L_t is the total capillary length, L_d is the effective capillary length (capillary length from injection end to detection window), and t_{nm} is the migration time of a neutral marker. Equation 1.32 defines the apparent (observed) mobility of an analyte in the capillary and can be employed to calculate the eletrophoretic mobility of a solute. The effective, or actual, mobility is independent of voltage and capillary length and can be determined by subtracting the mobility of the electroosmotic flow (EOF) from the apparent mobility.

When a buffer is placed inside a capillary the inner surface of the capillary acquires a charge. This may be due to ionization of the capillary wall or by the adsorption of ions from the buffer onto the capillary. For fused silica, the silanol (Si-OH) groups are ionized to negatively charged silanoate ($Si\text{-}O^-$) groups at pH above three. This ionization is usually enhanced by passing a basic solution through the capillary followed by the buffer. Often, a new capillary is conditioned by flushing with a KOH or NaOH solution. Figure 1.12 illustrates the formation of EOF; for simplicity, the anions and solvation of the cations are not shown. The negatively charged silanoate groups attract positively charged cations from the buffer, which form a tightly held inner (fixed/Stern) layer of cations at the capillary wall. These cations do not have sufficient density to neutralize the negative charges; therefore, a second outer (mobile/Helmholtz) layer of cations forms. This mobile layer is not tightly held because of its distance from the silanoate groups. A plane of shear develops between the two layers and an electrical imbalance is created at the plane. This electrical imbalance is called the zeta potential (ζ), or the potential difference across the fixed and mobile layers.

When an electric field is applied, the mobile, outer layer of solvated cations is attracted to the negatively charged cathode. These solvated cations drag the bulk buffer with

them, thus, creating EOF. The EOF is proportional to zeta potential, which is proportional to the thickness of the double layer.[67, 77]

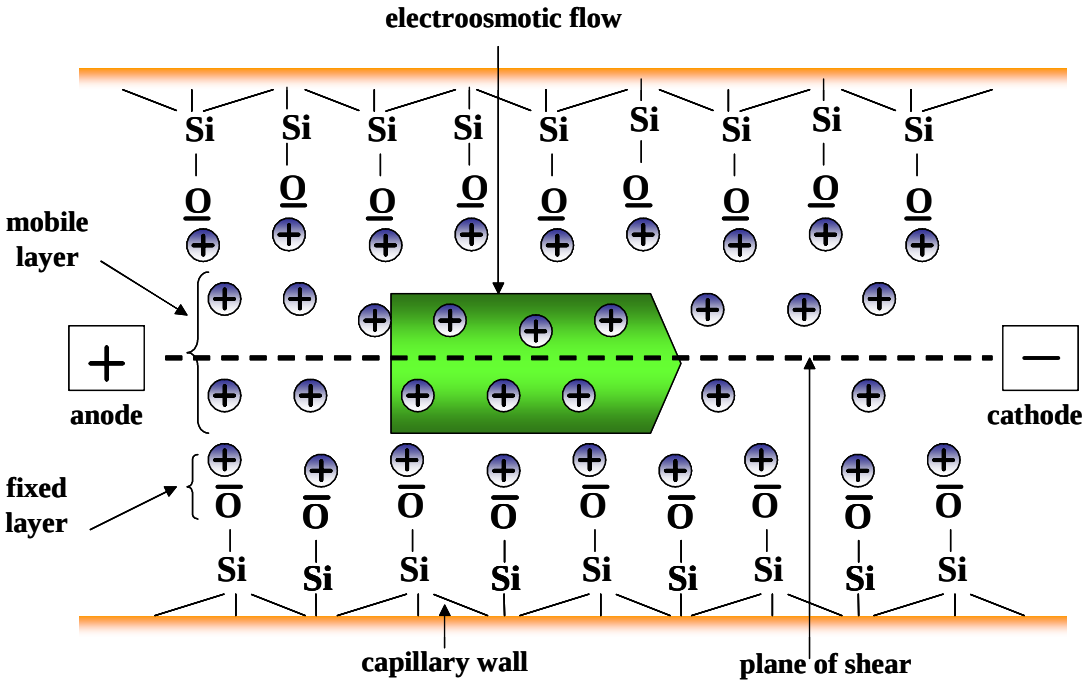


Figure 1.12 Schematic representation of formation of EOF in a capillary.

The zeta potential can be calculated by the following expression

$$\zeta = \frac{4\pi\delta e}{\varepsilon} \qquad 1.33$$

where δ is the thickness of the double layer, e is the charge-per-unit surface area, and ε is the dielectric constant of the buffer. The velocity of the EOF, ν_{EOF}, is given by,

$$\nu_{EOF} = \frac{\varepsilon\zeta E}{4\pi\eta} \qquad 1.34$$

where E is the applied electric field in V/cm and η is the viscosity of the buffer. The electroosmotic mobility, μ_{EOF}, of the buffer is given by Equation 1.35.

$$\mu_{EOF} = \frac{\varepsilon\zeta}{4\pi\eta} \qquad 1.35$$

The electroosmotic mobility depends solely on buffer characteristics, i.e., viscosity, pH, dielectric constant, and concentration (all of which influence the zeta potential) and is independent of the applied electric field. Thus, anything that will cause a change in the right-hand side of Equation 1.34 will cause variation in the EOF.

Because the EOF is evenly distributed along the capillary, the resulting flow profile is essentially flat. The flat or plug flow profile of EOF reduces solute zone dispersion yielding high-efficiency separations. In contrast, the laminar or parabolic flow, generated by pressure-driven systems as in HPLC, causes solutes in the center of the column to migrate faster than those near the wall resulting in relatively broad peaks. Figure 1.13 illustrates the differences in flow profiles generated by hydrodynamic HPLC pumps and by EOF.

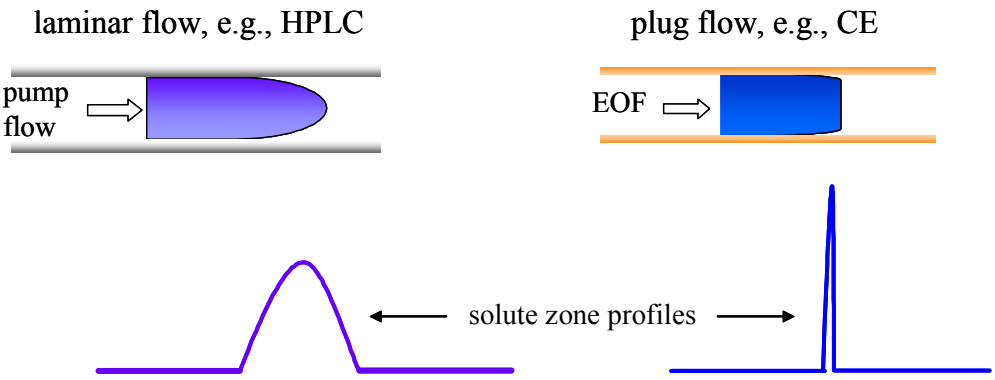


Figure 1.13 Comparison of pressure driven flow profiles and EOF and their corresponding solute zones.

Another benefit of EOF is nearly all species, regardless of charge, are moved in the same direction, usually from anode to cathode. Figure 1.14 depicts the mechanism of ion and neutral molecule separation due to EOF and ion electrophoretic mobility. In CE, because analytes are separated on the basis of their charge-to-size ratios, those analytes with higher charge-to-size ratios have comparatively higher mobilities and velocities. Therefore, the migration of cations will be fastest, neutrals will migrate at the velocity of the EOF, and

anions will migrate slowest. EOF is the primary factor that controls the retention time of a given solute. Several factors influence EOF, i.e., change in applied voltage, alteration of the capillary wall, and a change in the buffer: viscosity, pH, and ionic strength.

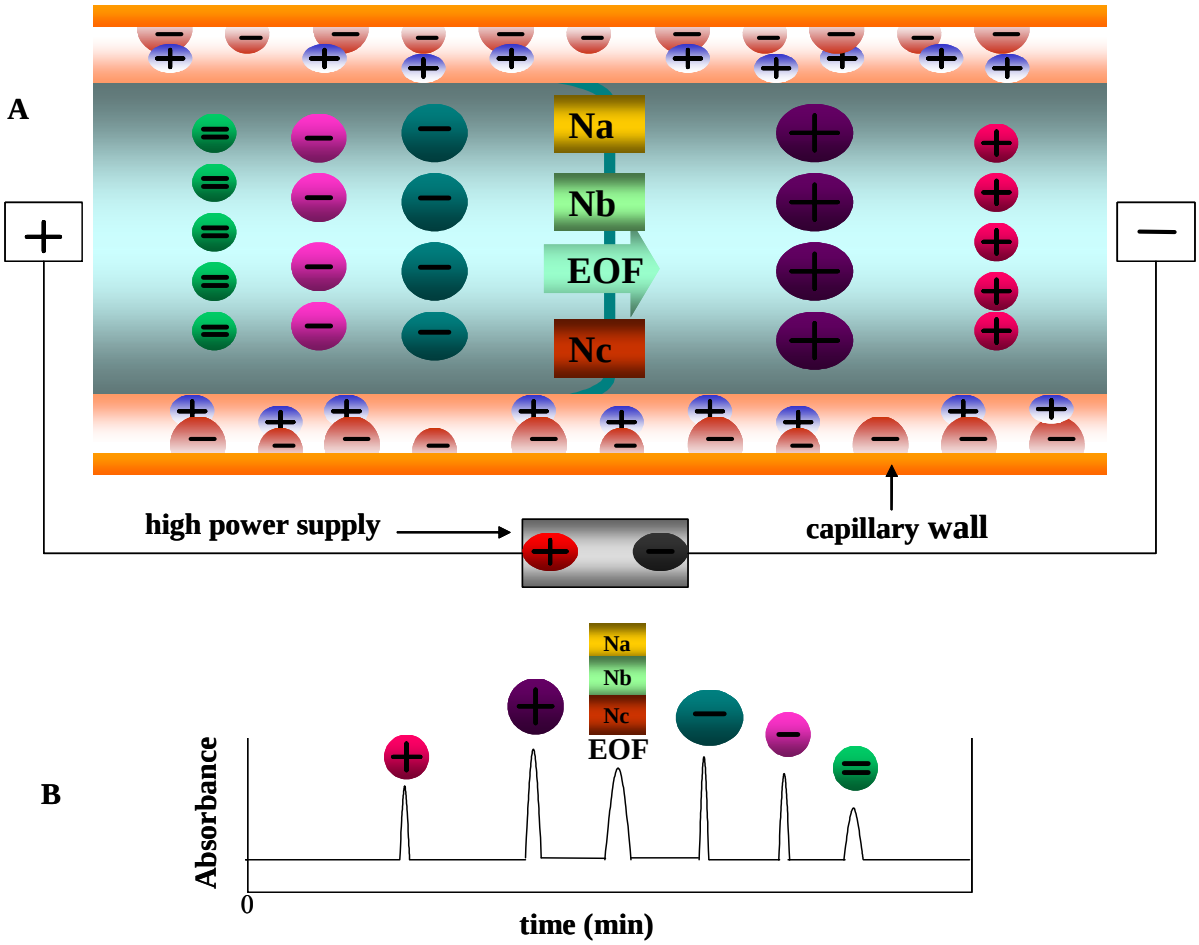


Figure 1.14 (A) Differential solute migration in CE and (B) Solute elution window.

A decrease in the applied voltage leads to a decrease in the EOF and an increase in the analysis time (elution time of the solutes). Modification of the capillary wall may increase, decrease, or reverse EOF. Neutral analytes do not have electrophoretic mobility; therefore, they are swept to the detector by the EOF. To separate neutral molecules, either the capillary surface must be modified or a substance must be added to the buffer to serve as the separation platform. Reminiscent of the biphasic chromatographic system employing a

mobile phase and a stationary phase, the added substance is called a pseudo-stationary phase because it moves at a slower rate than the mobile phase. It possesses either low or no electrophoretic mobility depending upon its charge state. Neutral molecules can now be separated based upon their differential partitioning between the pseudo-stationary phase and the surrounding buffer. This additional partitioning component forms the basis of MEKC.

1.7.3 Efficiency in CE

Separation efficiency, N, is the number of theoretical plates. It can be calculated by measuring the migration time (t_m) and the peak width, w, at the base of the peak.

$$N = 16\left(\frac{t_m}{w}\right)^2 \qquad 1.36$$

If peak width is measured at half height, $w_{1/2}$, the following equation is used to calculate efficiency.

$$N = 5.54\left(\frac{t_m}{w_{1/2}}\right)^2 \qquad 1.37$$

In addition, it has been demonstrated that the separation efficiency, or theoretical plates, in CE depends on the electrophoretic mobility (μ_{ep}), applied voltage (V) and the diffusion coefficient of the ion (D).[69, 78]

$$N = \frac{\mu_{ep} \mathrm{V}}{2D} \qquad 1.38$$

According to Equation 1.38, higher efficiencies are attained with an increase in electroosmotic flow that is in the same direction as the electrophoretic mobility. Efficiency also increases with an increase in voltage. However at higher voltages, a deviation from this linear relationship may occur because of Joule heating which warms the buffer and analyte solution and leads to convective diffusion. Thus, molecules in the warmer center of the

capillary migrate faster than those near the cooler wall, leading to peak broadening. In CE, efficiency is independent of capillary length (if Joule heat is dissipated) unlike liquid and gas chromatography where efficiency is proportional to column length.

1.7.4 Resolution in Capillary Electrophoresis

Resolution, R_s, is how well the components in a mixture are separated. The resolution can be calculated from an electropherogram using the following equation,

$$R_s = \frac{2(t_{r_2} - t_{r_1})}{(w_1 + w_2)}. \qquad 1.39$$

where t_{r_1} and t_{r_2} are the migration times of the former and latter eluting solutes, respectively, and w_1 and w_2 are the peak widths of the former and latter eluting solutes, respectively. Resolution is high when there is a large difference in the electrophoretic mobilities of the solutes, and this difference can be enhanced by optimizing the pH, temperature, and voltage of the buffer. [79] In some instances, the addition of a solvent to the buffer may cause differences in mobilities and improve resolution.[80]

Capillary length also influences the separation. As capillary length is increased, the solutes migrating at different velocities have more time to be separated, assuming the zones are not broadened by diffusion. Terabe et al. have shown that resolution is proportional to the square root of the ratio of the effective capillary length to the total capillary length.[81] However, increasing capillary length leads to longer analysis time. Thus, it is advisable to use the shortest capillary that provides the desired resolution.

1.8 Micellar Electrokinetic Chromatography

Development of micellar electrokinetic chromatography (MEKC) was a major advancement in capillary electrophoresis because it provided a method for separation of

electrically neutral compounds, including chiral compounds. The technique, developed by Terabe et al., involved adding ionic surfactants to buffer solutions.[82, 83] MEKC, a hybrid of chromatography and electrophoresis, employs the separation mechanisms of both methods into one widely used technique. It is the only free-solution CE technique in which both neutral and ionic analytes can be separated in the electric field.

MEKC uses the same instrumental setup as CE, except charged organized media, such as micelles or polymeric surfactants, are added to the buffer as the separation medium for neutral solutes. The charged pseudo-stationary phase moves through the capillary under an applied voltage at an electrophoretic velocity that is proportional to the charge-to-size ratio. The neutral solutes are separated based on their pseudo-stationary phase-water partition coefficients. In the case where a charged micelle serves as the pseudo-stationary phase, this partition coefficient P_{mw} is defined as

$$P_{mw} = \frac{C_m}{C_w} \tag{1.40}$$

where C_m and C_w are the concentration of the solute in the micellar phase and aqueous phase, respectively. Using MEKC, even extremely hydrophobic compounds such as polycyclic aromatic compounds have been separated.[84] Figure 1.15 illustrates the migration of neutral solutes within anionic micelles and the corresponding elution window. All neutral solutes are separated between the migration time of an unretained solute (t_{eo}), and the migration time of the micelle (t_{mc}). The separation of charged solutes is based on their charge-to-size ratio, while that of neutral solutes (Na, Nb, and Nc) is based on their differential partitioning into the micellar phase. For example shown in Figure 1.15, Nc is partitioned into the micelle to a greater extent than Na, thus, has a longer retention time.

Anionic micelles migrate in the opposite direction of the EOF in an uncoated fused capillary for pH greater than 6. However, because the velocity of EOF is greater than the electrophoretic velocity of the micelles, anionic micelles are carried toward the cathode. In the case of cationic micelles it is necessary to reverse the polarity of the electrodes in the CE setup because the negatively charged capillary wall will be modified by the positively charged surfactant, thus, reversing the direction of the EOF.

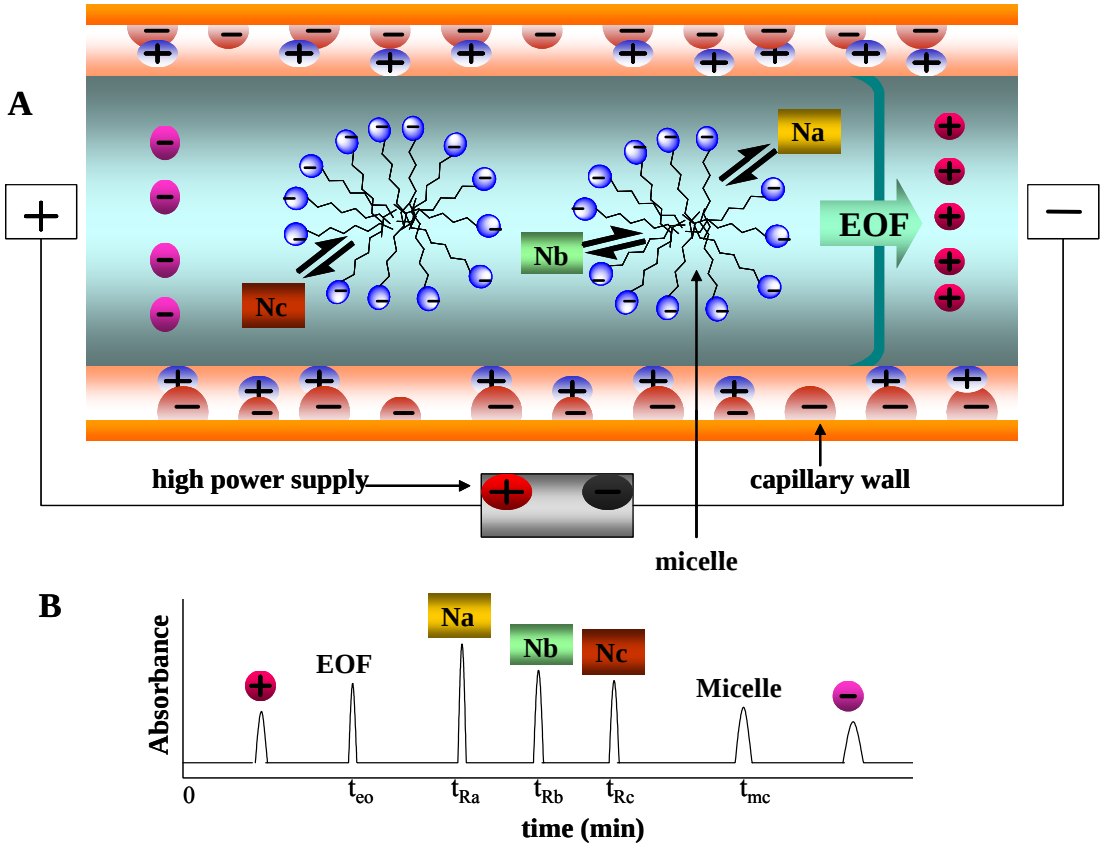


Figure 1.15 (A) Migration of charged and neutral solutes in MEKC and (B) Solute elution time window.

There are two groups of neutral solutes that cannot be separated using MEKC. The first group is made of solutes that do not interact with the pseudo-stationary phase ($P_{mw} \sim 0$) i.e., solutes that spend the entire time in the running buffer and migrate at the velocity of EOF. For instance, neutral polar solutes like methanol or acetonitrile that are commonly used

as EOF markers (t_{eo}) cannot be separated with MEKC. The second group consist of solutes that spend virtually all their time inside the micellar phase ($P_{mw} \sim \infty$). This type of analytes, e.g., sudan III and dodecanophenone, are highly hydrophobic and are used to determine the elution time of the micelle (as t_{mc} markers). The capacity factor, k', describes the differential binding between the neutral solute with the pseudo-stationary phase in MEKC. The k' can be determined from the t_{eo}, t_{mc}, and the retention time of the neutral solute, t_R using the following equation.[82, 83]

$$k' = \frac{t_R - t_{eo}}{t_{eo}\left(1-\left(\frac{t_R}{t_{mc}}\right)\right)} \qquad 1.41$$

This equation is similar to that used to determine k' in conventional chromatography except for the additional term ($1-t_R/t_{mc}$) in the denominator which indicates the pseudo-stationary phase is mobile. If t_{mc} approaches infinity, the extra term in the denominator tends to 1 and k' simplifies to the same equation as that derived for conventional chromatography.

$$k' = \frac{t_R - t_{eo}}{t_{eo}} \qquad 1.42$$

The resolution in MEKC is related to the capacity factors of the solutes as follows

$$R = \left(\frac{N^{0.5}}{4}\right)\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k'_2}{1+k'_2}\right)\left(\frac{1-\left(\frac{t_{eo}}{t_{mc}}\right)}{1+k'_1\left(\frac{t_{eo}}{t_{mc}}\right)}\right) \qquad 1.43$$

where α is the selectivity factor given by k'_2/k'_1 and N is the number of theoretical plates. According to Equation 1.43 the resolution depends on four terms related to efficiency,

selectivity, retention, and elution window, represented by the first, second, third, and fourth terms, respectively.[83]

There are several pseudo-stationary phases of hydrophilic and hydrophobic substances. Examples of substances that have been separated are drugs of abuse,[85] prescription pharmaceuticals,[86, 87] antifungals,[88] peptides,[75, 89] vitamins,[90] enzymes,[91] fatty acids,[92] aromatic hydrocarbons,[93] warfare explosives,[94] industrial chemicals,[95] and environmental pollutants.[96, 97] The pseudo-stationary phases can be categorized into two general groups: dynamic aggregates and covalently-bonded organized assemblies. Examples of dynamic aggregates are vesicles, micelles,[98] and bile salts.[99] Covalently-bonded organized assemblies are dendrimers,[100] calixarenes,[101] crown ethers,[102] cyclodextrins (CD),[103, 104] and polymeric surfactants.[105] In this research the main focus was on the application of polymeric surfactants in MEKC.

1.8.1 Application of Polymeric Surfactants in MEKC

There are problems encountered with conventional micelles used in MEKC. First, conventional micelles do not form in the presence of organic solvents.[106, 107] Second, applied voltage across the capillary often causes Joule heating, especially when high concentrations of monomeric surfactant are used. As previously mentioned, Joule heating leads to an increase in capillary temperature, which in turn may lead to changes in CMC.[106, 108] A change in CMC will have a significant effect on separations in MEKC such as irreproducible run times, erratic poor peak shapes, and poor peak efficiences.[109-111] Third, the dynamic equilibrium between micelles and surfactant monomers has a significant effect on the shape and size of the micelle. This limits the flexibility of the technique in terms of the choice of analytical conditions.

Polymeric surfactants (molecular micelles) have several advantages over conventional micelles. The covalent linkage among the monomer units of the polymeric surfactant provides a rigid structure. Because this removes the dynamic equilibrium between micelle formation and deformation, the mass transfer rate between the polymeric surfactant and solute can be improved, thus, reducing peak broadening. Polymeric surfactants can be used over a wider range of concentrations than monomeric surfactants because they have zero CMC. For these reasons, problems often associated with conventional micelles can be minimized in MEKC.[112] In addition, and perhaps most important, polymeric surfactants are stable in the presence of relatively high concentrations of organic solvents and inclusion compounds.[30, 113] Also, some polymeric surfactant properties can be tuned through the polymerization process.

Palmer et al. first demonstrated the successful use of polymeric surfactants as pseudo-stationary phases for the MEKC separation of achiral molecules.[114] In this work, he used polysodium 10-undecylenate (poly-SUA) to separate alkyl phthalates and polycyclic aromatic hydrocarbons (PAHs). Poly-SUA was found to have a higher selectivity relative to micelles of sodium dodecyl sulfate (SDS). However, the application of poly-SUA was limited by the carboxylate head groups whose ionization influenced its electrophoretic mobility at low pH. In addition, erratic migration times and cloudiness of the anodic buffer vial after several runs were reported.[112, 114] These difficulties were overcome by Palmer and Terabe who synthesized polysodium undecyl sulfate (poly-SUS), a polymeric surfactant with a sulfate head group.[115, 116] This polymer, like its carboxylate analog, when modified with high concentrations of organic solvents exhibited superior separation powers over the conventional SDS micelles. Regardless of the difference in chemistry of their ionic head

groups, the two polymers, poly-SUA and poly-SUS, exhibit analogous chemical selectivity for amine and hydroxyl aromatic compounds. However, the use of potassium persulfate as a free radical initiator for polymerization induced low synthetic yield and resulted in contamination of the product with sulfate.[112, 114]

Shamsi et al. synthesized the same surfactant, poly-SUS, using ^{60}Co gamma irradiation to initiate polymerization.[107] Separation of PAHs and a variety of substituted benzene and naphthalene compounds was possible with the use of this polymer alone and also in combination with beta or gamma-cyclodextrin. Moy et al. also reported the separation of PAHs using polymeric surfactants of sodium undecylenic acid in the presence of acetonitrile, acetone, and tetrahydrofuran (THF).[117] This group separated 16 PAHs with THF as an additive within 48 minutes; however, not all analytes were baseline resolved. Shamsi and coworkers reported more rapid separation of the same PAHs by increasing the acetonitrile concentration in the buffer to 65% (v/v).[107] A complete review of the application of polymeric surfactants in MEKC for the separation of chiral and achiral compounds can be found in recently published reviews.[118-122] In this research, a novel surfactant, polysodium oleyl-L-leucyl-valinate (poly-L-SOLV), was used for the separation of chiral compounds, while poly-SUS, together with ionic liquids (novel mobile phase modifiers) was used in the separation of achiral and chiral compounds.

1.8.2 Modifiers in Micellar Electrokinetic Chromatograpy

Modifiers such as organic solvents,[123-127] cyclodextrins,[128-131] urea and glucose,[74, 88, 132, 133] and ionic liquids[134] are added to the buffer solutions containing pseudo-stationary phase of MEKC for adjusting the capacity factor, manipulation of selectivity, and the

extension of the elution of window. In addition, these additives influence EOF velocity, and partition coefficients of the solutes into pseudo-stationary phases.

Organic modifiers such as methanol, ethanol, 2-propanol, acetonitrile, and THF offer a wide range of polarity and selectivity and also improve the separation of the highly hydrophobic compounds that elute near, or with, micelles.[123-127] The main role of organic solvents in MEKC has been to reduce the capacity factor, k', of the highly hydrophobic analytes to a reasonable range. In most cases, the addition of organic modifiers leads to a reduction in EOF as well as a change in velocity of the micelle, and hence an increase in the length of elution window. Other modifiers such as urea and glucose have been applied in MEKC. Urea is said to increase the separation window and also increase the solubility of the highly hydrophobic solutes in the mobile phase. Glucose, when added to the mobile phase, increases the resolution in MEKC.[74] Part of this research will focus on the investigation of ionic liquids, which are a relatively new class of solvents, as mobile phase modifiers in the separation of achiral and chiral compound in MEKC.

Part 3. Introduction to Ionic Liquids

1.9 Ionic Liquids: Interests and Implications

There is an augmented growth in the interest in ionic liquids (ILs) as reflected by articles in the scientific and popular press.[135-139] This interest in ILs has been driven largely by the perceived opportunities to improve industrial processes using green chemistry principles as well as the desire for more efficient processes with associated overall cost savings.[140, 141] The fundamental concepts behind both green chemistry and ILs is not new; however, the growth of interest in ILs and the influence of new minds, new approaches and enthusiasm are generating new solutions and driving changes in industrial and commercial

practices, proving that green chemistry alternatives exist. However, for industries to adopt new methods and practices, it will be necessary to show that IL processes and technologies are significantly better than existing methods and cost efficient.

Based on the poor societal image of the chemical industry and ever-increasing environmental regulations, there is a need to take proactive steps to demonstrate that chemical technology and the chemical industry are capable of providing clean, efficient technology with limited environmental impact. The common perception of the chemical industry is that it has been responsible for an array of environmental and health related problems such as the birth defects caused by use of thalidomide, along with the contamination and or bioaccumulation of such toxic, non-biodegradable pollutants such as dioxins, dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), chlorofluorocarbons (CFCs), and other persistent materials.[141, 142] The unrecognized reality is that the chemical industry has also led to improvements in society such as antibiotics, fertilizers, pesticides, polymers, and composites on which modern society relies. These perceptions and problems associated the with production, use, and waste of undesirable products within aspects of the chemical industry have led to an enormous growth in environmental legislation and significant moves towards step-wise and process changes within the industry.[141-143]

Chemical research toward green chemistry is vital to allow scientists to reevaluate their perspective on the direction of chemical synthesis and production, with an aim of using simple guidelines in order to increase overall efficiency and eliminate unnecessary waste. These wastes may involve undesirable side products, energy waste, financial waste from insufficient reactions, or purchase and /or disposal costs of expensive reagents, solvents, or

catalysts. Central among these concerns is the move to processes employing greener solvent systems that reduce the reliance on volatile organic compounds (VOCs), especially haloginated organic solvents. Therefore, the growth of interest in ILs as solvents for chemical reactions and separations,[144, 145] as distinct from electrochemical applications,[146, 147] has been fueled by the potential benefits that are offered by ILs as non-volatile replacements for VOCs.[148-150]

Although it is true that ILs have negligible vapor pressure and can be used as alternatives to VOCs as solvents for select synthesis and separations processes,[151, 152] the simple replacements of one solvent with another does not necessarily make a process "green." ILs are much more than just non-volatile solvents, the unique combination of properties allows new innovative chemistry, improved selectivity, and new separation and extraction procedures to be made available. These aspects can enable the development of more efficient and environmentally-friendly processes.

1.9.1 Ionic Liquids: Structure and Properties

The glib definition of an ionic liquid is a liquid that is composed entirely of ions that melts at low temperatures (generally below 100 °C) and may be thought to resemble molten ionic melts such as NaCl at 800 °C. While both ILs and molten salts are composed of ions, the presence of bulky organic cations in ILs interrupts the crystal packing and lowers the melting temperature. Several literature reports have highlighted the cations for use in these systems, including ammonium,[153-155] pyridinium,[156, 157] pyrrollidium,[158] isoquinolinium,[159] and imidazolium,[160, 161] each with the possibility for attaching various alkyl groups to the ring or quaternary cation. Depending on the type of cation investigated and the length of the alkyl chain, the resulting salts may have a melting point above room temperature and several

crystal structures have been reported.[162] Figure 1.16 illustrates common cations and anions that can be used in combination to prepare ILs. Depending on the composition, the resulting IL can be hydrophobic or hydrophilic.

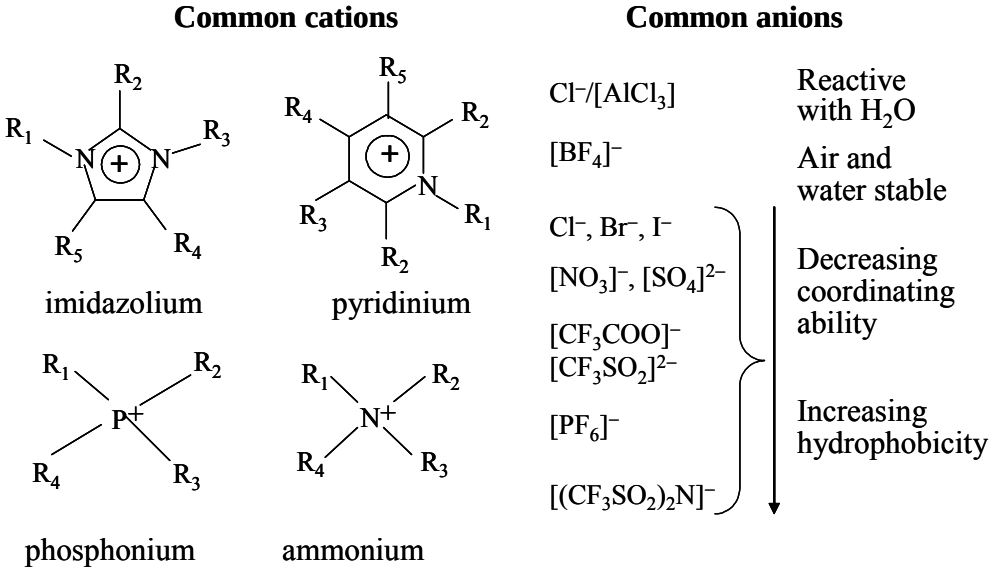


Figure 1.16 Common cations and anions used in combination to make ILs.

A unique property of many ILs is their negligible volatility and easily-manipulated physical properties. Most ILs often have thermal stability to over 350 °C. This minimizes solvent losses to evaporation and environmental release. In addition, ILs are nonflammable. The non-volatility of ILs means they cannot be purified by distillation; however, ILs can be used to recover chemicals by distillation, sublimation, and pervaporation. Other beneficial properties of ILs are good electrical conductivity, and an inherent ionic strength.

Some of the resultant properties of ILs that distinguish them from higher temperature molten salts are the variable coordination through hydrogen-bond donor and acceptor functions, dipole interactions, and CH-aromatic packing.[141] These properties, to some extent, explain the reason for high benzene solubility in many ILs, and variable co-solvent miscibility, from miscible to immiscible, with polar solvents such as water and non-polar

liquids such as hexane, respectively. These properties have been investigated in miscibility with supercritical-CO_2,[163, 164] in liquid-liquid partition and extraction studies,[148, 165, 166] and in solute retention studies using liquid chromatography.[167]

1.9.2 Applications of Ionic Liquids

Ionic liquids are being investigated for use in almost every field of chemistry. The growth in both the interest and accessible variety of ILs stem from electrochemical studies on molten salts.[146, 147] In addition, other studies aim at reducing operating temperatures by utilizing low melting organic salts as alternatives to high temperature molten salts as liquid ionic electrolytes.[141, 142] Research in electrochemical studies continues, producing applications in batteries, capacitors, dissolution, electroplating, and electrosynthesis.[147, 161, 168, 169] Based on these studies, scientists have realized that highly reactive chloroaluminate ILs could be used as both solvents and catalysts for various Lewis acid catalyzed reactions in place of solid heterogeneous catalysts.

Other early IL chemical applications focused on the properties of ILs that led to process improvements, greater reactivity, and ease of product separation.[141, 142] Some examples of such processes are Friedel-Crafts alkylations and acylations,[146] alkylation of olefins,[45] and nickel-catalyzed olefin dimerization reactions.[170, 171] All these processes utilized IL systems as both solvent and catalyst for chemical transformations and took advantage of poor product solubility to facilitate clean separations which could improve process and separation efficiency. In addition, based on electrochemical studies a new class of ILs has been developed which is air and water stable and contains a wide range of anions, including nitrate, tetrafluoroborate, and hexafluorophosphate. In this dissertation, ILs are investigated for use as mobile phase modifiers in achiral and chiral analytes in MEKC.

1.10 Scope of this Dissertation

The research presented in this dissertation focuses on the synthesis, characterization, and application of two types of anionic surfactants (a chiral amino acid-based surfactant and achiral sulfate-based surfactant) for the separation of chiral and achiral analytes in MEKC. These two surfactants are categorized according to the type of anionic head group. They both have sodium as the counterion but have different hydrophobic alkyl tail groups. The monomeric surfactants are polymerized to form polymeric surfactants which were found to be better pseudo-stationary phases in MEKC. Modifiers such as organic solvents and ILs are used to aid in the separation of highly hydrophobic analytes. The advantages of using ILs over organic solvents are also discussed.

Chapter 1 is an introduction to relevant topics related to this dissertation such as surfactants, chirality, CE, MEKC, and ILs. In addition, a brief discussion about techniques applied in the characterization of surfactants is also included in the first chapter. A detailed description of the synthesis, polymerization, characterization, and application of a novel chiral amino acid-based surfactant; poly-L-SOLV is presented in Chapter 2. This polymeric surfactant was useful in the separation of acidic, neutral and basic chiral analytes.

In Chapter 3, a polymerized surfactant with a sulfate head group, poly-SUS, was synthesized and applied towards the separation of a mixture of eight alkyl aryl ketones and seven phenols in MEKC. A commercially available surfactant, sodium dodecyl sulfate (SDS), was also used for the separation of the eight alkyl aryl ketone mixture and compared with the poly-SUS surfactant. Several ILs were investigated, for the first time, as modifiers in the separation of the ketone and phenol mixtures.

In Chapter 4, the effects of using ILs as modifiers for the separation of chiral compounds in MEKC are investigated. From the studies in Chapter 3, the 1,3-dialkylimidazolium tetrafluoroborate-based ILs were found to be more versatile as modifiers. Thus, in this chapter, ILs with different alky subtituents on the imidazolium ring, but the same tetrafluoroborate anion, were investigated. The novel polymeric surfactant poly-L-SOLV, whose synthesis is described in Chapter 2, was used as the chiral selector. A comparison was made between the use of 1,3-dialkylimidazolium-based ILs and the organic solvents methanol, 1-propanol, or acetonitrile as mobile phase modifiers in these chiral separations.

BASF use three HPLC systems to determine 2-pyrrolidone (2-Pyr), vinylacetate (VA), and N-vinyl-2-pyrrolidone (NVP) monomer residues in polyvinylpyrrolidone (PVP) homo-polymer and polyvinylpyrrolidone/vinyl acetate (PVP/VA) copolymer samples. The goal of the research presented in Chapter 5 was to develop an MEKC method for the separation and quantitative analysis of these three monomer residues in the two polymer samples in a single run. A detailed description of the determination of 2-Pyr, VA, and NVP monomer residues in polyvinylpyrrolidone (PVP) homo-polymer and polyvinylpyrrolidone/vinyl acetate (PVP/VA) copolymer using SDS as a pseudo-stationary phase in MEKC is presented in Chapter 5.

Chapter 6 summarizes the previous chapters and gives an account of future directions to consider.

1.11 References

1. Holmberg, K., Kronberg, B., Lindman, B. *Surfactants and Polymers in Aqueous Solution*, 2nd ed.; John Wiley & Sons, Ltd: England, **2003**.

2. Moroi, Y. *Micelles Theoretical and Applied Aspects*; Plenum Press: New York, **1992**.

3. Porter, R. M. *Handbook of Surfactants*, 2nd ed.; Blackie: Glasgow, **1994**.

4. Israelachvili, J. *Intermolecular and Surface Forces*; Academic Press: London, **1992**.

5. Clint, J. H. *Surfactant Aggregation*; Blackie: Glasgow, **1992**.

6. Pinazo, A., Wen, X., Perez, L., Infante, M-R., Franses, E. I. *Langmuir* **1999**, *15*, 1334.

7. Okano, L. T., Quina, F. H., El Seoud, O. A. *Langmuir* **2000**, *16*, 3119.

8. Hiemenz, P. C., Rajagopalan, R. *Principles of Colloid and Surface Chemistry*, 3rd ed.; Marcel Dekker, Inc.: New York, **1997**.

9. Forland, G. M., Samseth, J., Gjerde, M. I., Hoiland, H., Jensen, A. O., Mortensen, K. *J. Coloid Interface Sci.* **1998**, *203*, 328.

10. Choen, J. B., Jeong, Y. I., Cho, C. S. *Polym.* **1999**, *40*, 2041.

11. Attwood, A. W., Florence, A. T. *Surfactant Systems*; Chapman and Hall: London, **1983**.

12. Issaq, H. *J. Instrum. Sci. Tech.* **1994**, *22*, 119.

13. Jover, A., Meijide, F., Nunez, R. E., Tato, V. *Langmuir* **1997**, *13*, 161.

14. Michel, B., Waton, G., Zana, R. *Langmuir* **1997**, *13*, 3111.

15. McBain, J. W. *Trans. Faraday Soc.* **1913**, *9*, 99.

16. McBain, J. W. In *Colloid Chemistry, Theory and Applied*; Reinhol: New York, **1944**.

17. Hartley, G. S. *Aqueous Solutions of Praffin Chain Salt*; Hermann: Paris, **1936**.

18. Christian, S. D., Scamehron, J. F. *Solubilization in Surfactant Aggregates*; Marcel Dekker, Inc.: New York, **1995**.

19. Debye, P., Anacker, E. W. *J. Phys. Colloid. Chem.* **1951**, *55*, 644.

20. Menger, F. M. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1086.

21. Chevalier, Y., Chachaty, C. *Colloid Polym. Sci.* **1984**, *262*, 489.

22. Bendedouch, D., Chen, S-H., Koeler, W. C. *J. Phys. Chem.* **1983**, *87*, 2621.

23. Courchene, W. L. *J. Phys. Chem.* **1964**, *68*, 1870.

24. Birdi, K. S. *Prog. Colloid Polym. Sci.* **1985**, *70*, 23.

25. Kawaguchi, T., Hamanaka, T., Mitsui, . *J. Colloid Interface Sci.* **1983**, *96*, 437.

26. Myers, Y. *Surfactant Science and Technology*; VCH: New York, **1988**.

27. Rosen, M. J. *Surfactants and Interfacial Phenomena*; John Wiley & Sons: New York, **1978**.

28. Aniansson, E. A. G., Wall, S. N., Almgrem, M., Hoffman, H., Kielmann, I., Ulbricht, W., Zana, R., Lang, J., Tondre, C. *J. Phys. Chem.* **1976**, *80*, 5.

29. Dreja, M., Pyckhout-Hintzen, W., Tieke, B. *Macromolecules* **1998**, *31*, 272.

30. Paleos, C. M., Malliaris, A. *Macromol. Chem. Phys.* **1988**, *28*, 403.

31. Hamid, S., Sherrington, D. *J. Chem. Soc. Chem. Commun.* **1986**, *12*, 958.

32. Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, **1953**.

33. Hiemenz, P. C. *Polymer Chemistry: The Basic Concepts*; Marcel Dekker, Inc.: New York, **1936**.

34. Van Holde, K. E. *Physical Biochemistry*; Prentice-Hall: New Jersey, **1928**.

35. Tanford, C. *Physical Chemistry of Macromolecules*; Wiley: New York, **1921**.

36. Lydett, A., Elhachemi, H., Boyer, B., Lamaty, G., Roque, J. P., Scols, D., Snoeck, R., Anderi, G., Ikeda, S., Neyts, Y., Witvroww, M., Declerq, E. J. *Med. Chem.* **1996**, *39*, 1626.

37. Dobashi, A., Hamada, M., Dabashi, Y., Yamaguchi, J. *Anal. Chem.* **1995**, *67*, 3011.

38. Cantor, C. R., Schimmel, P. R. *Biophysical Chemistry*; W. H. Freeman: San Francisco, **1942**.

39. Atkins, P. *Physical Chemistry*, 6th ed.; Freeman and Company: New York, **1997**.

40. Fujita, H. *Foundations of Ultracentrifugal Analysis*; Wiley and Sons: New York, **1975**.

41. Skoog, A. D., Holler, E. J., Nieman, A. T. *Principles of Instrumental Analysis*, 5th ed.; Harcourt Brace & Company: Austin, **1998**.

42. Lakowicz, J. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, **1983**.

43. Turro, N. J., Okuba, T. *J. Am. Chem. Soc.* **1981**, *103*, 7224.

44. Lianos, P., Virito, M.-L., Zana, R. *J. Phys. Chem.* **1998**, *92*, 5580.

45. Abdul Sada, A. K., Atkins, M. P., Ellis, B., Hodgson, P. K. G., Morgan, M. L. M., Seddon, K. R. In *Alkylation Process*; World Patent: W095/21806, **1995**.

46. McCarroll, M., Toerne, K. *Langmuir* **1998**, *14*, 2965.

47. McCarroll, M., Haddadian, F., Warner, I. M. *J. Am. Chem. Soc.* **2001**, *123*, 3173.

48. Silverstein, R. M., Bassler, G. C., Morill, T. C. *Spectroscopic Identification of Organic Compounds*; John Wiley & Sons, Inc.: New York, **1991**.

49. Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon Press: New York, **1987**.

50. Casey, A. F. *Medicinal Chemistry: Stereochemistry and Biological Activity*, 3rd ed.; Wiley Interscience: New York, **1980**.

51. Gestel, V., Schoot, V. D., Michaels, M. A. *J. Macromolecules* **2003**, *36*, 6668.

52. Hong, S.-B., Raushel, F. *Biochemistry* **1999**, *38*, 1159.

53. Subramanian, G. *Chiral Separation Techniques*; Wiley-VCH: New York, **2001**.

54. Bazzoli, A. S., Manson, J., Scott, W. J., Wilson, J. G. *J. Embryol. Exp. Morph.* **1977**, *41*, 125.

55. Helm, F.-C. F., Graudums, I., Flohe, L. *Drugs Res.* **1981**, *31*, 941.

56. Altria, K. D., Kelly, M. A., Clark, B. *Trends in Analytical Chemistry* **1998**, *17*, 214.

57. Frazier, R. A. *Electrophoresis* **2001**, *22*, 4197.

58. Zhang, W., Williams, M. G. *J. Chromatogr. A* **2000**, *871*, 201.

59. Massolini, G., De Lorenzi, E., Calleri, E., Tabolotti, E., Caccialanza, G. *J. Chromatogr. B* **2000**, *738*, 343.

60. Fanali, S. *J. Chromatogr. A* **1996**, *735*, 77.

61. Zosel, K. *Angew. Chem.* **1978**, *90*, 748.

62. Issaq, H. J. *Electrophoresis* **2000**, *21*, 1921.

63. Welch, C. J., Grau, B., Moore, J., Mathre, D. J. *J. Org. Chem.* **2001**, *66*, 6836.

64. Chankvetadze, B. *Capillary Electrophoresis in Chiral Analysis*; John Wiley and Sons, Inc.: New York, **1997**.

65. Easson, L. H., Stedman, E. *Biochem. J.* **1933**, *27*, 1257.

66. Ogston, A. G. *Nature* **1948**, *162*, 963.

67. Baker, D. R. *Capillary Electrophoresis*; John Wiley & Sons, Inc.: New York, **1995**.

68. Tiselius, A. The Moving Boundary Method of Studying the Electrophoresis of Proteins, Ph.D. Thesis, *Nova Acta Regiae Societatis Scientiarum Upsaliensis*, Ser. IV, Vol. 17, No. 4; Almqvist and Wiksell, Uppsala, Sweden, **1930**.

69. Jorgenson, J., Lukacs, K. D. *Anal. Chem.* **1981**, *53*, 1298.

70. Lauer, H. H., McManigill, D. *Anal. Chem.* **1986**, *58*, 166.

71. Nishi, H., Tsumagari, N., Terabe, S. *Anal. Chem.* **1989**, *61*, 2434.

72. Burton, D. E., Sepaniak, M. J., Maskarinec, M. P. *J. Chromatogr. Sci.* **1986**, *24*, 347.

73. Nishi, H., Tsumagari, N., Kakimoto, T., Terabe, S. *J. Chromatogr.* **1989**, *465*, 331.

74. Kaneta, T., Tanaka, S., Taga, M., Yoshida, H. *Anal. Chem.* **1992**, *64*, 798.

75. Yashima, T., Tsuchiya, A., Morita, O., Tarabe, S. *Anal. Chem.* **1992**, *64*, 2981.

76. Weinberger, R. *Practical Capillary Electrophoresis*; Academic Press, Inc.: San Diego, **1993**.

77. Tsuda, T., Nomura, K., Nakagawa, G. *J. Chromatogr.* **1982**, *248*, 241.

78. Giddings, J. C. *Separ. Sci.* **1969**, *4*, 181.

79. McLaughlin, G. M., Nolan, J. A., Lindahl, J. L., Palmieri, R. H., Anderson, K. W., Morris, S. C., Morisson, J. A., Bronzert, T. J. *J. Liquid Chrom.* **1992**, *15*, 961.

80. Harrold, M. P., Wajtusik, J. M., Riviello, J., Henson. P. *J. Chromatogr.* **1993**, *640*, 463.

81. Terabe, S., Yashima, T., Tanaka, N., Araki, M. *Anal. Chem.* **1988**, *60*, 1673.

82. Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A., Ando, T. *Anal. Chem.* **1984**, *56*, 111.

83. Terabe, S., Otsuka, K., Ando, T. *Anal. Chem.* **1985**, *57*, 834.

84. Terabe, S., Miyashita, Y., Shibata, O., Barnhart, R. E., Alexander, L. R., Patterson, D. G., Karger, B. L., Hosoya, K., Tanaka, N. *Organohalogen Compounds* **1990**, *2*, 221.

85. Naess, O., Rasmussen, K. E. *J. Chromatogr. A* **1996**, *760*, 245.

86. Bretnall, A. E., Clarke, G. S. *J. Chromatogr. A* **1996**, *745*, 145.

87. Renou-Gonnord, M. F., David, K. *J. Chromatogr. A* **1996**, *735*, 249.

88. Furuta, R., Doi, T. *J. Chromatogr. A* **1994**, *676*, 431.

89. Peterson, M., Walhagen, K., Nilsson, A., Wahlund, K. G., Nilsson, S. *J. Chromatogr. A* **1997**, *769*, 301.

90. Profumo, A., Profumo, V., Vidali, G. *Electrophoresis* **1996**, *17*, 1617.

91. Loregian, A., Scremin, C., Schiavon, M., Marcello, A., Palu, G. *Anal. Chem.* **1994**, *66*, 2981.

92. Erim, F. B., Xu, X., Kraak, J. C. *J. Chromatogr. A* **1995**, *694*, 471.

93. Ding, W. L., Fritz, J. S. *Anal. Chem.* **1997**, *69*, 1593.

94. Kennedy, S., Caddy, B., Douse, J. M. F. *J. Chromatogr. A* **1996**, *726*, 211.

95. MacLaughlin, G. M., Weston, A., Hauffe, K. D. *J. Chromatogr. A* **1996**, *744*, 123.

96. Rossi, M., Rotilio, D. *J. High Resolut. Chromatogr.* **1997**, *20*, 265.

97. Brumley, W. C., Jones, W. J. *J. Chromatogr. A* **1994**, *680*, 163.

98. Michaelsen, S., Moller, P., Sorensen, H. *J. Chromatogr. A* **1994**, *680*, 299.

99. Brumley, W. C., Brownrigg, C. M. *J. Chromatogr. Sci.* **1994**, *32*, 69.

100. Kuzdzal, S. A., Monnig, C. A., Newkome, G. R., Moorefield, C. N. *J. Chem. Soc., Chem. Commun.* **1994**, *18*, 2139.

101. Mnuk, P., Feltl, L., Schurig, U. *J. Chromatogr. A* **1996**, *732*, 63.

102. Kuhn, R., Riester, D., Fleckenstein, B., Wiesmüller, K. H. *J. Chromatogr. A* **1995**, *716*, 371.

103. Snopek, J., Jelinek, I., Smolkova-Keulemansova, E. *J. Chromatogr.* **1988**, *438*, 211.

104. Fanali, S. *J. Chromatogr.* **1989**, *474*, 441.

105. Wang, J., Warner, I. M. *Anal. Chem.* **1994**, *66*, 3773.

106. Palmer, C. P., Terabe, S. *J. Microcol. Sep.* **1996**, *8*, 115.

107. Shamsi, S. A., Akbay, C., Warner, I. M. *Anal. Chem.* **1998**, *70*, 3078.

108. Palmer, C. P., Khaled, M. Y., McNair, H. M. *J. High Resolut.Chromatogr.* **1992**, *15*, 756.

109. Watzig, H. *Chromatographia* **1992**, *33*, 445.

110. Knox, J. H., Mc Cormack, K. A. *Chromatographia* **1994**, *38*, 207.

111. Varesio, E., Veuthey, J. L. *J. Chromatogr.* **1995**, *717*, 269.

112. Palmer, C. P. *J. Chromatogr. A* **1997**, *780*, 75.

113. Laschewsky, A. *Adv. Polym. Sci.* **1995**, *124*, 3.

114. Palmer, C. P., McNair, H. M. *J. Microcol. Sep.* **1992**, *4*, 509.

115. Palmer, C. P., Terabe, S. *Kuromatoguradi* **1995**, *16*, 98.

116. Peterson, A. G., Foley, J. P. *J. Macrocolumn. Sep.* **1998**, *10*, 633.

117. Moy, T. W., Ferguson, P. L., Grange, A. H., Matchett, W. H., Kelliher, V. A., Brumley, W. C., Glassman, J., Farley, J. W. *Electrophoresis* **1998**, *19*, 2090.

118. Williams, C. C., Shamsi, S. A., Warner, I. M. *Chromatographia* **1997**, *37*, 363.

119. Shamsi, S. A., Warner, I. M. *Electrophoresis* **1997**, *18*, 853.

120. Haynes, J. L., Shamsi, S. A., Warner, I. M. *Anal. Rev.* **1999**, *8*, 6.

121. Haddadian, F., Shamsi, S. A., Warner, I. M. *Electrophoresis* **1999**, *20*, 3011.

122. Yarabe, H. H., Billiot, E., Warner, I. M. *J.Chromatogr. A* **2000**, *875*, 179.

123. Balchunas, A. T., Sepaniak, M. J. *Anal. Chem.* **1998**, *60*, 617.

124. Chen, N., Terabe, S. *Electrophoresis* **1995**, *16*, 2100.

125. Dinelli, G., Vicari, A., Brandolini, V. *J.Chromatogr. A.* **1995**, *700*, 173.

126. Scafroth, M., Thormann, W., Allenmann, D. *Electrophoresis* **1994**, *15*, 72.

127. Sepaniak, M. J., Swaile, D. F., Powell, A. C. *J.Chromatogr.* **1989**, *480*, 185.

128. Chan, K. C., Muschik, G. M., Isaaq, H. J., Snader, K. M. *J. High Resolut. Chromatogr.* **1994**, *17*, 51.

129. Nishi, H., Matsuo, M. *J. Liquid Chrom.* **1991**, *14*, 973.

130. Fanali, S. *J. Chromatogr.* **1991**, *545*, 437.

131. Copper, R. D., Sepaniak, M. J. *Anal. Chem.* **1994**, *66*, 147.

132. Terabe, S., Ishihama, Y., Nishi, H., Fukuyama, T., Otsuka, K. *J. Chromatogr.* **1991**, *545*, 359.

133. Lee, K. J., Lee, J. J., Moon, D. C. *Electrophoresis* **1994**, *15*, 98.

134. Mwongela, S. M., Numan, A., Gill, N. L., Agbaria, R. A., Warner, I. M. *Anal. Chem.* **2003**, *75*, 6089.

135. Freemantle, M. In *Chem. Eng. News*, **2000**, *78*, 37.

136. Adam, D. *Nature* **2000**, *407*, 938.

137. Chang, K. In *New York Times*: New York, **2000**, F2.

138. Renner, R. *Sci. Amer.* **2001**, *265*, 19.

139. Gorman, J. In *Science News*, **2001**, *160*, 156.

140. Anastas, P. T., Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, **1998**.

141. Rogers, R. D., Seddon, K. R. *Ionic Liquids: Industrial Application for Green Chemistry*; American Chemical Society: Washington, **2002**.

142. Rogers, R. D., Seddon, K. R. *Ionic Liquids as Green Solvents*; American Chemical Society: Washington, **2003**.

143. Sheldon, R. A. In *Process Technology: Perspectives for Pollution Prevention*; Kluwer: Dordrecht, **1993**.

144. Seddon, K. R. *J. Chem. Tech. Biotech.* **1997**, *68*, 351.

145. Rodgers, R. D. *Green Chem.* **2000**, *5*, G94.

146. Wilkes, J. S. In *Molten Salt Chemistry, An Introduction and Selected Applications*, Mamantov, G., Marrasi, R., ed.; D. Reichel Publishing: Dordrecht, **1987**.

147. Wilkes, J. S., Levisky, J. A.,Wilson, R. A., Hussey, C. L. *Inorg. Chem.* **1982**, *21*, 1263.

148. Holbrey, J. D., Seddon, K, R. *Clean Prod. Proc.* **1999**, *1*, 223.

149. Welton, T. *Chem. Rev.* **1999**, *99*, 2071.

150. Keim, W., Wasserschied, P. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 3772.

151. Huddleston, J. G., Willauer, H. D., Swatloski, R. P., Visser, A. E., Rogers, R. D. *J. Chem. Soc., Chem. Commun.* **1998**, 1765.

152. Cull, S. G., Holbrey, J. D., Vargas-Mora, V., Seddon, K. R., Lye, G. *Biotech. Bioeng.* **2000**, *69*, 227.

153. Furton, K. G., Morales, R. *Anal. Chim. Acta* **1991**, *246*, 171.

154. Poole, S. K., Shetty, P. H., Poole, C. F. *Anal. Chim. Acta* **1989**, *218*, 241.

155. Sun, J., Forsyth, M., MacFarlane, D. R. *J. Phys. Chem. B* **1998**, *102*, 8858.

156. Robinson, J., Osteryoung, R. A. *J. Am. Chem. Soc.* **1979**, *101*, 323.

157. Gordon, C. M., Holbrey, J. D., Kennedy, A. R., Sedon, K. R. *J. Mater. Chem.* **1998**, *8*, 2627.

158. MacFarlane, D. R., Sun, J., Golding, J., Meakin, P., Forsyth, M. *Electrochim. Acta* **2000**, *45*, 1271.

159. Visser, A. E., Holbrey, J. D., Rogers, R. D. *Chem. Commun.* **2001**, *23*, 2484.

160. Huddleston, J. G., Visser, A. E., Reichert, W. M., Willauer, H. D., Broker, G. A., Rogers, R. D. *Green Chem.* **2001**, *3*, 156.

161. Bonhote, P., Dias, A.-P., Papageorgiou, N., Kalyanasundaram, K., Graetzel, M. *Inorg. Chem.* **1996**, *35*, 1168.

162. Holbrey, J. D., Seddon, K. R. *J. Chem. Soc., Dalton Trans.* **1999**, *13*, 2133.

163. Blanchard, L. A., Hancu, D., Beckman, E. J., Brennecke, J. F. *Nature* **1999**, *399*, 28.

164. Blanchard, L. A., Gu, Z., Brennecke, J. F. *J. Phys. Chem.* **2001**, *105*, 2437.

165. Visser, A. E., Swatloski, R. P., Reichert, W. M., Griffin, S. T., Rogers, R. D. *Ind. Eng. Chem. Res.* **2000**, *39*, 3596.

166. Visser, A. E., Swatloski, R. P., Griffin, S. T., Hartman, D. H., Rogers, R. D. *Sep. Sci. Technol.* **2001**, *36*, 785.

167. Armstrong, D. W., He, L., Lui, Y. S. *Anal. Chem.* **1999**, *71*, 3873.

168. Osteryoung, R. A., Gale, R. J., Robinson, J., Linga, H., Cheek, G. *J. Electrochem. Soc.* **1981**, *128*, C79.

169. Hussey, C. L. *Adv. Molten Salt Chem.* **1983**, *5*, 185.

170. Chauvin, Y., Gilbert, B., Guibard, I. *J. Chem. Soc. Chem. Commun.* **1990**, *23*, 1715.

171. Chauvin, Y., Einloft, S., Olivier, H. *Ind. Eng. Chem. Res.* **1995**, *34*, 1149.

Chapter 2.

Use of Polysodium Oleyl-L-Leucyl-Valinate for the Separation of Chiral Compounds in Micellar Electrokinetic Chromatography

2.1 Introduction

The enantioselectivity of chiral compounds is important to the environment and biological fields, as well as to synthetic chemists and the pharmaceutical industry. In capillary electrophoresis (CE), various types of chiral selectors have been employed for enantiomeric separations. For example, cyclodextrin (natural and derivatized) has been successfully used for enantiomeric separations.[1-4] Other interesting chiral selectors applied in other investigations include polysaccharides,[5, 6] proteins,[7, 8] macrocyclic antibiotics,[9, 10] crown ethers,[11, 12] calixarenes,[13] and micelles.[14-16]

Micellar electrokinetic chromatography (MEKC) has been employed for the chiral separation of various analytes. Although Tarabe et al. first introduced this technique in 1984,[17, 18] Cohen et al.,[19] performed the first successful chiral separation using the MEKC method in 1997. In this study, Cohen and co-workers used a mixed-micelle chiral ligand for their chiral separations. The basic principles and advantages of MEKC were described in Chapter 1 of this dissertation and will not be discussed further.

There are many surfactants available for use in separation science but few are chiral.[20] Despite this limitation, the use of chiral surfactants for enantiomeric separations has recently shown much promise in MEKC.[14, 21-32] In our laboratory, amino acid-based surfactants, typically synthesized using an 11-carbon undecylinic acid chain have been used for separation of various chiral analytes.[29-32] The first polymeric amino acid-based surfactant synthesized in our laboratory for chiral separation in MEKC was reported in 1994.[22] The surfactant synthesized, polysodium N-undecylenyl-L-valinate (poly-L-SUV), was used for

the separation of optical isomers of (±) 1,1'-bi-2-napththol and laudonosine. Elsewhere, Hara and Dobashi have also reported the use of polymeric chiral surfactants for enantiomeric separations using MEKC.[33] In subsequent papers, the use of poly-L-SUV for chiral separation of several other racemic compounds was investigated.[24, 25, 33] Additional studies within our group focused on gaining a better mechanistic understanding of intermolecular interactions between the chiral polymeric surfactants and chiral analytes.[26-28]

Recently, Billiot and co-workers, successfully used 18 monomeric and polymeric chiral surfactants for the MEKC separation of a variety of chiral analytes.[31, 32] The goal of this study was to gain deeper insight into factors governing the enantioselectivity of polymeric amino acid-based surfactants.[31] Among the many polymeric mono and dipeptide amino acid-based surfactants synthesized in our laboratory, polysodium N-undecylenyl-L-leucyl-valinate (poly-L-SULV), has been found to provide a large number of enantiomeric separations for neutral, acidic, and basic compounds by variation of the buffer concentration and pH, surfactant concentration, capillary temperature, and applied voltage.[34]

In this study, a novel chiral amino acid-based monomeric and polymeric surfactant, sodium oleyl-L-leucyl-valinate (L-SOLV) and poly-L-SOLV, respectively, were synthesized and used for chiral separations in MEKC. Both the monomer and the polymer were characterized by various techniques including proton nuclear magnetic resonance (^{1}H NMR) and infrared (IR) spectroscopy for structure elucidation. Surface tensiometry was used to obtain the CMC of the monomer, and densitometry and analytical ultracentrifuge (AUC) were employed to determine the partial specific volume and molecular weight, respectively, of the polymer. The structural difference between this new surfactant and others synthesized in our laboratory is its backbone acid chain which contains 18 carbons with the double bond

located at the 9,10 position as compared to the previously used 11-carbon chain with the double bond at the 1,2 position. The major advantage of this new surfactant is the low CMC of the monomer and high hydrophobicity of the resulting polymer. It is favorable to the interaction with hydrophobic chiral analytes, thus it may provide better chiral recognition ability. This surfactant was used in MEKC for the separation of several chiral analytes whose molecular structures are shown in Figure 2.1. The determination of molecular weight, applications, and advantages of using poly-L-SOLV in MEKC will be discussed in this chapter.

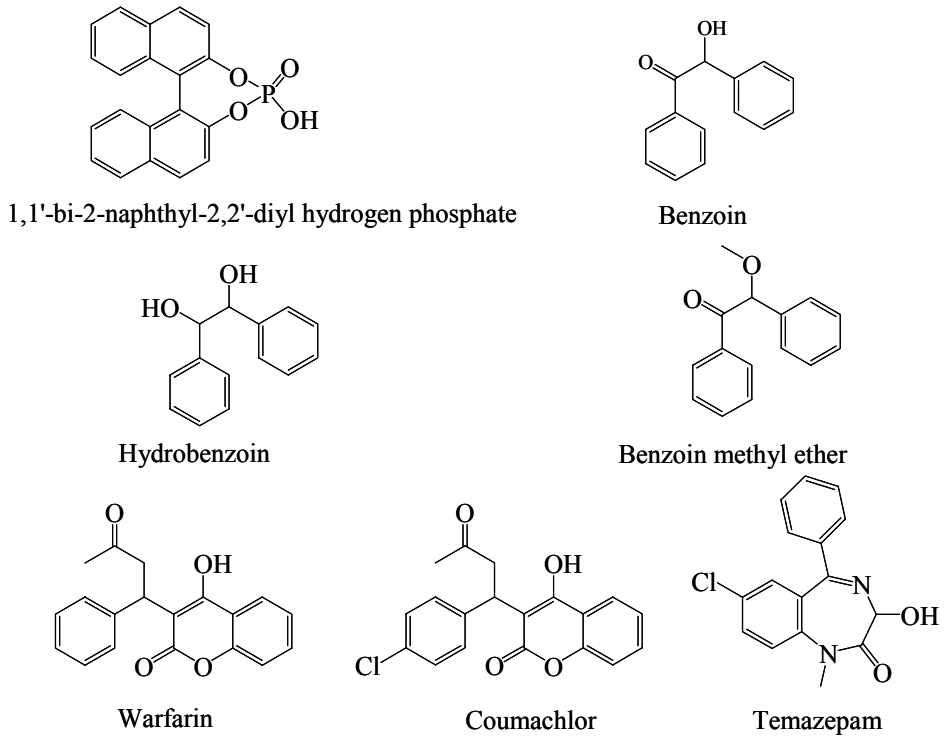


Figure 2.1 Structure of the chiral analytes separated.

2.2 Experimental Methods

2.2.1 Materials

The analytes (±)-1,1'-bi-2-naphthyl-2,2'-diyl hydrogen phosphate (BNP), warfarin, coumachlor, benzoin, hydrobenzoin, benzoin methyl-ether, and temazepam, all of analytical

grade, were purchased from Sigma (St. Louis, MO). The reagents used for synthesis of the surfactant, oleic acid-N-hydroxysuccinimide ester, sodium hydrogen carbonate, and tetrahydrofuran (THF), were purchased from Sigma (St. Louis, MO); while the dipeptide of leucine valine was purchased from BaChem Bioscience, Inc. (King of Prussia, PA). All materials were used as received.

2.2.2 Synthesis of Polysodium Oleyl-L-Leucyl-Valinate

The L-SOLV monomer was synthesized using the guidelines of the procedure previously reported by Wang and Warner[22] with some modifications. The synthetic scheme of L-SOLV and poly-L-SOLV is shown in Figure 2.2.

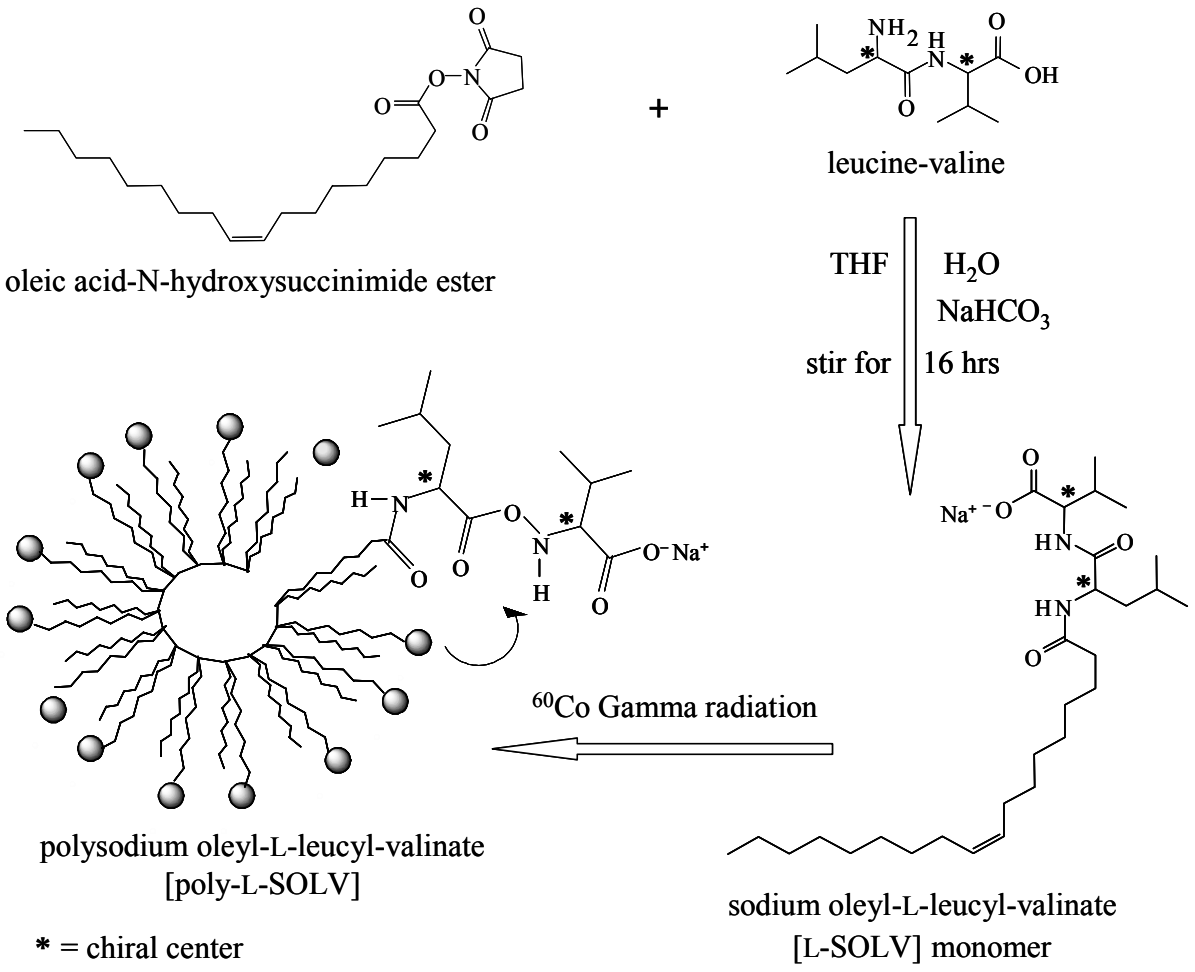


Figure 2.2 Synthesis scheme of polysodium oleyl-L-leucyl-valinate.

To prepare 20 mM of L-SOLV monomer, 1.68 g of $NaHCO_3$ was first dissolved into 200 mL of triply distilled deionized water in a 2000 mL round bottomed flask. In a separate flask, 5 g of oleic acid-N-hydroxysuccinimide ester was dissolved in 200 mL of THF. The ester–THF solution was combined with the $NaHCO_3$ solution and left to stir for 16 hours. Upon mixing the two solutions a white precipitate was observed indicating the start of the reaction. After the 16-hour period the solution was clear, implying the reaction was complete. The clear solution was rotary evaporated at 40 °C to remove the THF and filtered to get rid of any small undissolved impurities.

The filtrate was chilled in an ice bath and its pH was dropped to 1 by titration with 0.1 M HCl. A white, oily, glue-like product was collected, washed with triply distilled deionized water, and dried using a vacuum desiccator. The dry product was weighed and the number of moles calculated by dividing its mass by the molar mass. In order to dissolve the solid product, an equimolar amount of $NaHCO_3$ was added to the solid product and dissolved in a minimum amount of triply distilled deionized water. This solution was stirred overnight, then lyophilized and dried under vacuum. The final dry product was the L-SOLV surfactant monomer.

The CMC of L-SOLV was determined to be 0.9×10^{-3} M using a surface tensiometer from CSC Scientific Company, Inc. (Fairfax, VA). For polymerization, a 6×10^{-3} M aqueous solution of L-SOLV was exposed to a ^{60}Co γ-ray source (70 krad/h) for 7 days. Following polymerization, the aqueous solution was dialyzed in bulk water using a regenerated cellulose membrane with a 2000 Da molecular weight cutoff. The purified solution was lyophilized under vacuum to obtain a dry product of poly-L-SOLV. ^{1}H NMR, and IR spectroscopy were used to determine the molecular structure of the dry product.

2.2.3 Analytical Ultracentrifugation

Analytical ultracentrifugation (AUC) measurements were performed by use of an Optima XLA analytical ultracentrifuge from Beckman Instruments, Inc. (Palo Alto, CA). The instrument has a high-intensity xenon flash light source and a grating monochromator that scans continuously from 190 to 800 nm. The detection system was set to measure absorbance at 220 nm. A toroidally curved holographic diffraction grating was used to select the wavelength and to collimate the beam of light. Four sector cells were used in this experiment. Three of the cells have a sample and solvent chamber; whereas the fourth is a counter-balance cell. The polymer sample concentration was 0.1 g/L in 0.1 M NaCl. Sample volumes in each of the three cells were 110 μL while the solvent volumes were 125 μL. All data were collected at 25 °C and 22,000 rpm. The temperature of the rotor was controlled thermoelectrically to within ± 0.5 °C. The absorbance versus distance from the center of rotation to any position in the sample was collected at 720 min intervals. Successive scans of the cell were compared graphically using the XL-A software to ensure that the samples reached equilibrium. These data were acquired every 10 μm in replicates of 10, were digitized, and displayed as a function of radial distance. Only sedimentation measurements were completed.

2.2.4 Densitometry

A high-precision densitometer (Model DMA58), purchased from Anton Paar USA (League City, TX), was used to perform density measurements. The principles and basic equations for performing these measurements were described in Chapter 1 of this dissertation. Air and water were used for calibration and the precision of the temperature-controlled system was better than ± 0.005 °C.

2.2.5 Capillary Electrophoresis

The MEKC experiments were performed by use of a Hewlett-Packard 3D CE instrument (Foster City, CA). A bare fused silica capillary (effective length 52 cm, 50 μm i.d.) was purchased from Polymicro Technologies (Phoenix, AZ). The surfactant was added to the buffer solution and filtered through a 0.45 μm membrane filter. Separations were performed using a voltage of +30 kV, with UV detection at 220 nm. All analytes were prepared in a 50:50 methanol/water mixture at a concentration of 0.1 mg/mL. Samples were pressure injected for 10 s at 10 mbar. Prior to use, each new capillary was conditioned for 60 min with 1 M NaOH and then for 30 min with 0.1 M NaOH at a temperature of 40 °C. Finally, the capillary was rinsed for 15 min with triply distilled deionized water. Prior to each run, the capillary was flushed with the MEKC buffer for 3 min to condition and fill the capillary.

2.3 Results and Discussions

2.3.1 Determination of Molecular Weight of Poly-L-SOLV

Analytical ultracentrifugation allows one to determine the molecular weight and sedimentation coefficient of a polymer.[36, 37] The basic principles and equations relating to the determination of partial specific volume and molecular weight of a macromolecule using a densitometer and AUC, respectively, were described in Chapter 1 of this dissertation.

Because the exact volume of a substance is a difficult quantity to measure, one often uses partial specific volume $\overline{V}$ (a.k.a V_{bar}), which is defined as the increase in volume when 1 g of dry solute is dissolved in a large volume of solvent. The $\overline{V}$ value is determined as the y intercept from the plot of the inverse of the density ($1/\rho$) of the solution as a function of the weight fraction (W) of the polymer.[29, 35, 36]

$$1/\rho = \bar{V} + W\frac{\partial(1/\rho)}{\partial W} \qquad 2.1$$

In this expression, *W* is obtained by dividing the weight of solvent by the sum of the weight of solvent and weight of solute. Data was collected in triplicate and plotted to determine the partial specific volume of poly-L-SOLV (Figure 2.3). This value was then used in the determination of the polymeric surfactant molecular weight using AUC.

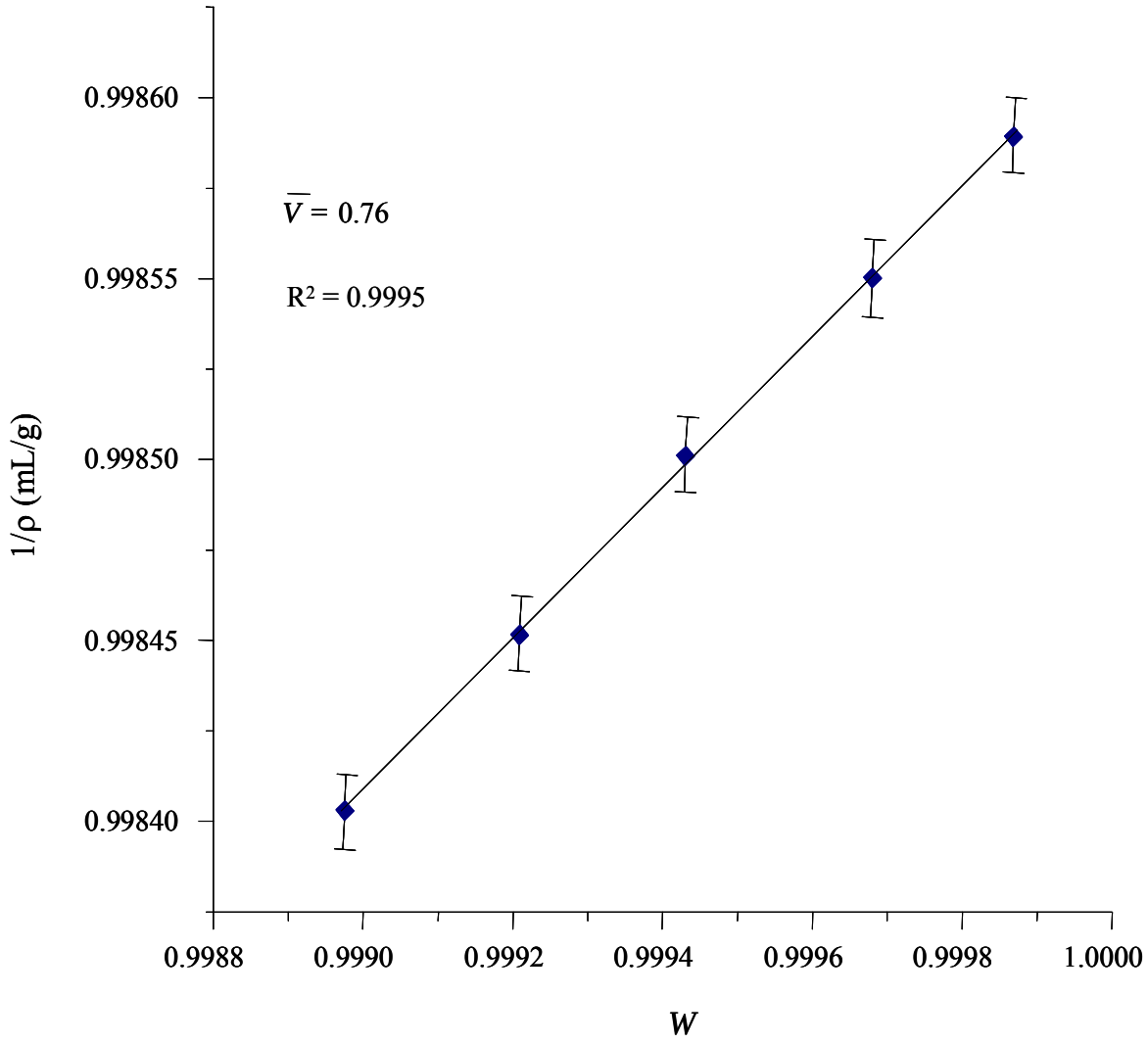


Figure 2.3 Determination of partial specific volume of poly-L-SOLV. Plot of $1/\rho$ (mL/g) as a function of *W*.

Sedimentation equilibrium and sedimentation velocity are the two usual modes of analytical ultracentrifugation. In the thermodynamically ideal case, Equation 2.2 is used in sedimentation equilibrium experiments to calculate the molecular weight of a polymer.

$$\ln \frac{A_a}{A_b} = \frac{M\omega^2 r(1-\rho\bar{V})(r_b^{\,2} - r_a^{\,2})}{2RT} \qquad 2.2$$

where A_a and A_b represent the absorbance at the two radii, r_a and r_b respectively, ω is the angular velocity, ρ is the solvent density, R is the gas constant, and T is the temperature in Kelvin. The value of $\bar{V}$, is determined externally by a densitometer.

An equilibrium concentration distribution of macromolecules can be obtained in the AUC cell if the centrifugal force is small enough to allow the process of diffusion to oppose the process of sedimentation.[36] For an ideal homogeneous sample, the concentration distribution is an exponential function of the buoyant mass of the molecules. According to Equation 2, the molecular weight of a polymer can be determined from the partial specific volume and the slope of the line generated by a plot of ln A vs r. However, it should be noted that this relationship only holds for monodispersed samples. For polydispersed species, the plot deviates from linearity.[35] Therefore, one must analyze the curvature of such a plot in order to obtain the average molecular weight at distance r. From the analytical ultracentrifugation measurements the weight average molecular weight for poly-L-SOLV was calculated using sedimentation equilibrium software and found to be 36102 ± 948. The aggregation number (N) for poly-L-SOLV was calculated by dividing the weight average molecular weight with the molar mass of L-SOLV, and was determined to be 69. This value correlated well with the value of N obtained at room temperature using the fluorescence method described by Billiot et al.[38] Figure 2.4 illustrates the equilibrium distribution of

poly-L-SOLV solute particles at 25 °C. The data points measured around the meniscus and cell bottom were truncated to give a more representative fit. The residuals at the top of the plot indicate how well the data points correlate with the fitting function.

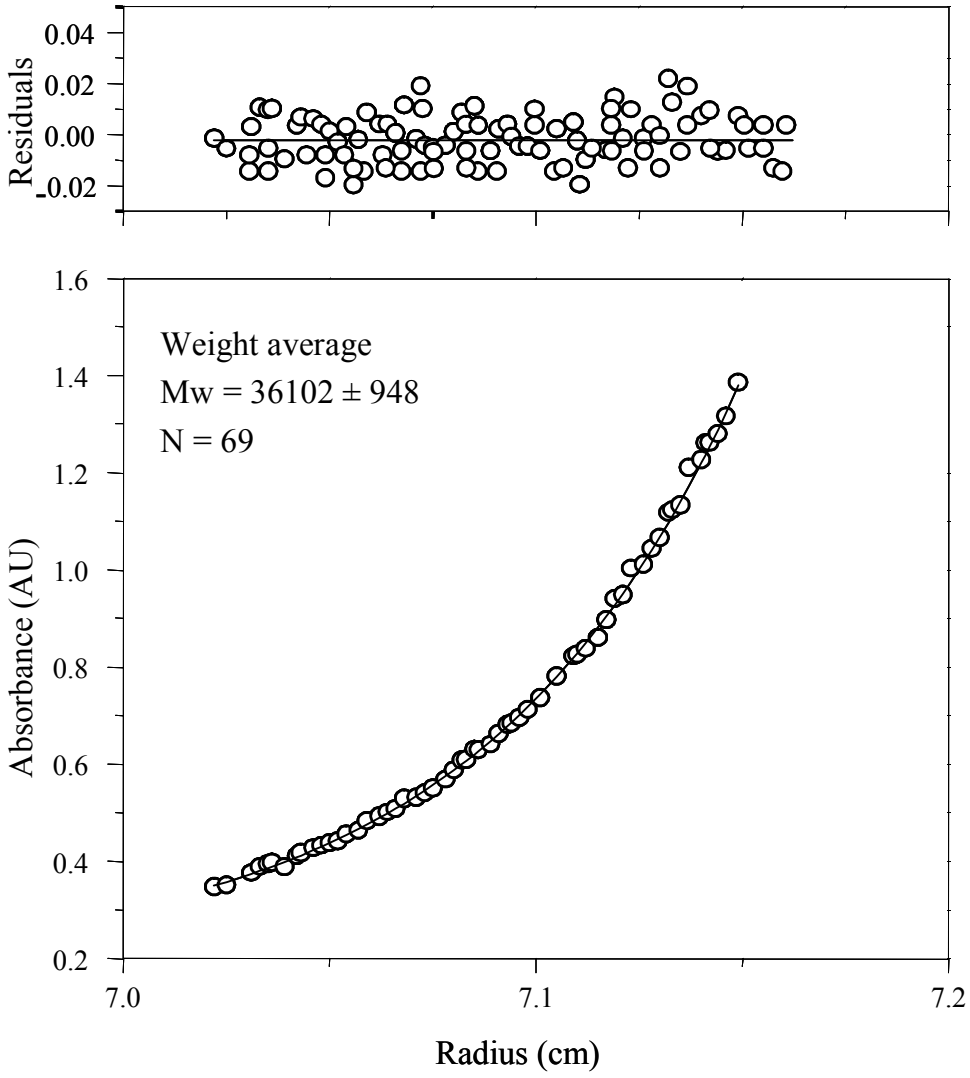


Figure 2.4 **Determination of the weight average molecular weight of poly-L-SOLV. Plots of absorbance and residuals vs. radius of poly-L-SOLV in 0.1 NaCl; wavelength, 220 nm; speed, 22,000 rpm; temperature, 25 °C; $\bar{V}$, 0.76. All data were truncated to avoid the meniscus and to improve the fit.**

2.3.2 Effect of Poly-L-SOLV Concentration on the Separation of BNP

Many molecules are chiral even without an asymmetric carbon center. Good examples are the atropisomeric binaphthyl compounds such as BNP, (molecular structure shown in Figure 2.1). BNP is among a group of molecules that are chiral because they possess an adjacent π-system that cannot adopt a coplanar configuration due to steric hindrances and rotational restrictions around a central bond. (±)-BNP has been used as a chiral shift reagent[25, 39] and for determining the enantiomeric purity of many organic compounds.[25, 40, 41] BNP has also been used as a ligand for dissymmetric catalyst[25, 40] and as building blocks in the synthesis of macrocyclic compounds.[25, 42] In this study, BNP exists in the anionic form due to the basic buffer conditions (pH 10.0) used in these separations. Baseline separation of BNP enantiomers using 25 mM sodium borate buffer and various concentrations of the polymeric pseudostationary phase poly-L-SOLV was achieved as shown in Figure 2.5 (A). A similar study was also completed using the monomer L-SOLV (Figure 2.5 (B)). These results demonstrate the chiral recognition ability of the polymeric surfactant as compared to the monomer micelles. In all cases, the (R)-(+) BNP enantiomer eluted faster than the corresponding S-(–) form. This suggests (S)-(–) BNP has a higher affinity for the pseudo stationary phase (either L-SOLV or poly-L-SOLV). A close inspection of Figure 2.5 reveals that the separation completed using the polymeric surfactant was better than the separation done using the monomer. First, there was a considerable amount of noise in the baseline when using L-SOLV. Peak broadening was also observed when using the monomeric surfactant regardless of the concentration. Furthermore, when the concentration of the monomer was increased to 20 mM, no separation was observed. The resolution of BNP using the polymer and monomer were comparable except at 1mM.

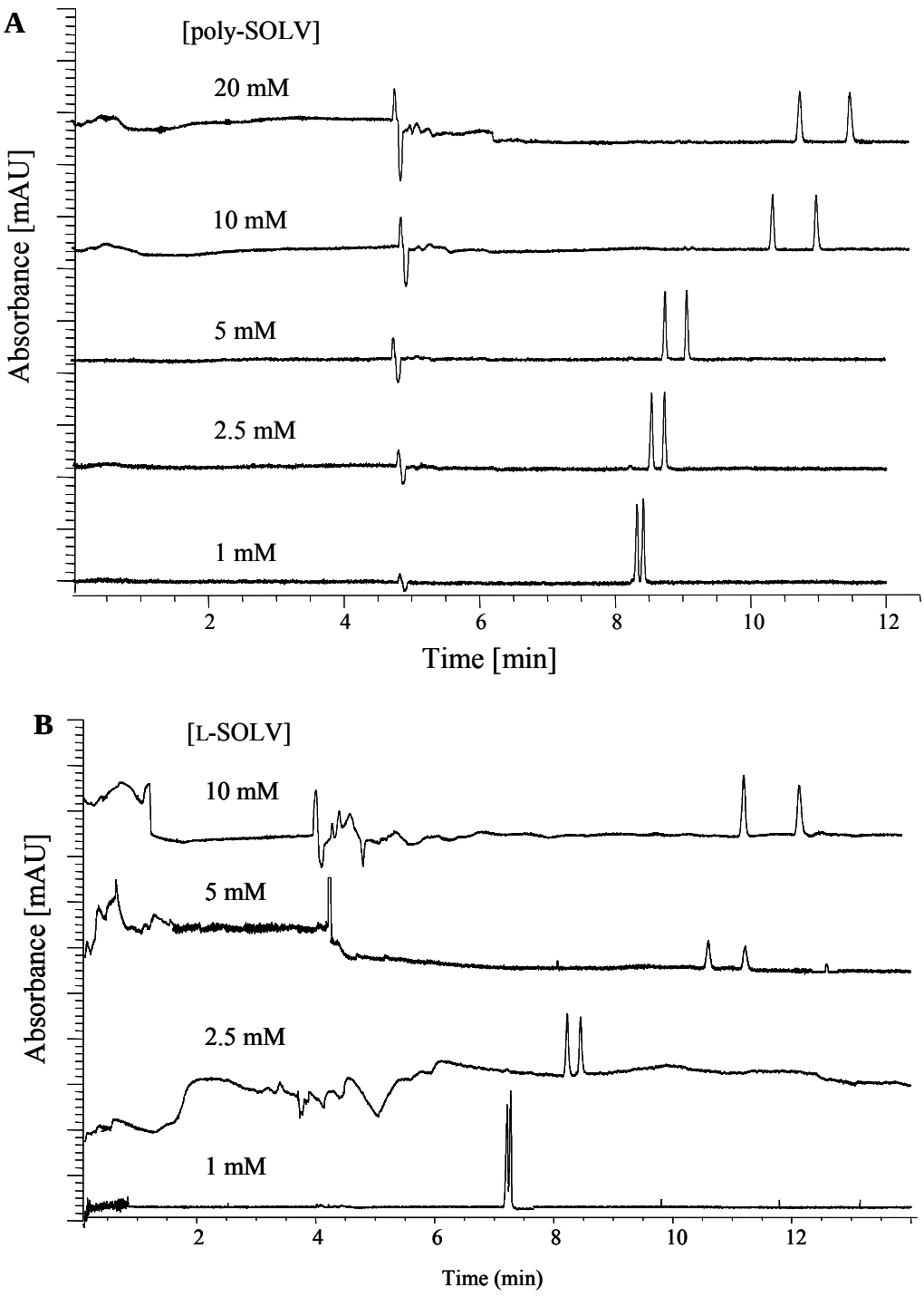


Figure 2.5 **Effect of (A) poly-L-SOLV and (B) L-SOLV monomer concentration on the separation of BNP. Conditions: electrolyte, 25 mM sodium borate; pH, 10.0; temperature, 12 °C; voltage, 30 kV; capillary, 52 cm effective length, 50 μm i.d.; injection size, 10 mbar for 10 s; UV detection λ, 220nm. Concentrations: (A) 20, 10, 5, 2.5, and 1 mM. poly-L-SOLV (B) 10, 5, 2.5, and 1 mM. L-SOLV**

BNP was chosen to investigate the applicability of poly-L-SOLV as a chiral selector because previous studies in our laboratory have shown it to be difficult to separate using the 11 carbon amino-based monomeric and polymeric surfactants.[32] The minimum amount of surfactant that could be used to achieve baseline separation of BNP were determined to be 1 mM for the polymer and 2.5 mM for the monomer.

2.3.3 Effect of Temperature on Separation of BNP

The effect of temperature on the separation of BNP was investigated to determine the thermal stability of both poly-L-SOLV and L-SOLV in MEKC. The polymer was found to be more stable and a better chiral selector even at higher temperatures. Higher resolution values were noted when using the polymer, while more noise was observed in the baseline when using the monomer (Figures 2.6 (A) and (B)). In addition, an increase in temperature led to an enhancement in peak efficiency when poly-L-SOLV was used as the chiral selector. Also, an increase in temperature led to shorter retention times as observed in both Figures 2.6 (A) and (B). This was likely due to an enhancement of the electroosmotic flow (EOF) caused by a decrease in the buffer viscosity. A rise in temperature also causes a decrease in dielectric constant, which suggests a reduction in EOF. However, the decrease in dielectric constant for water is less than the change in viscosity; hence, overall result of a temperature rise is an increase in EOF.

Slightly longer retention times were observed when the monomer was used. Although BNP was resolved at all temperatures, 25 °C was considered optimum. This is because above 25 °C, Joule heating is likely to occur; causing band broadening and poor baselines if the heat is not well dissipated along the capillary. Also, elevated temperatures may cause sample decomposition or boiling buffer, thus, temperature control is always advisable.

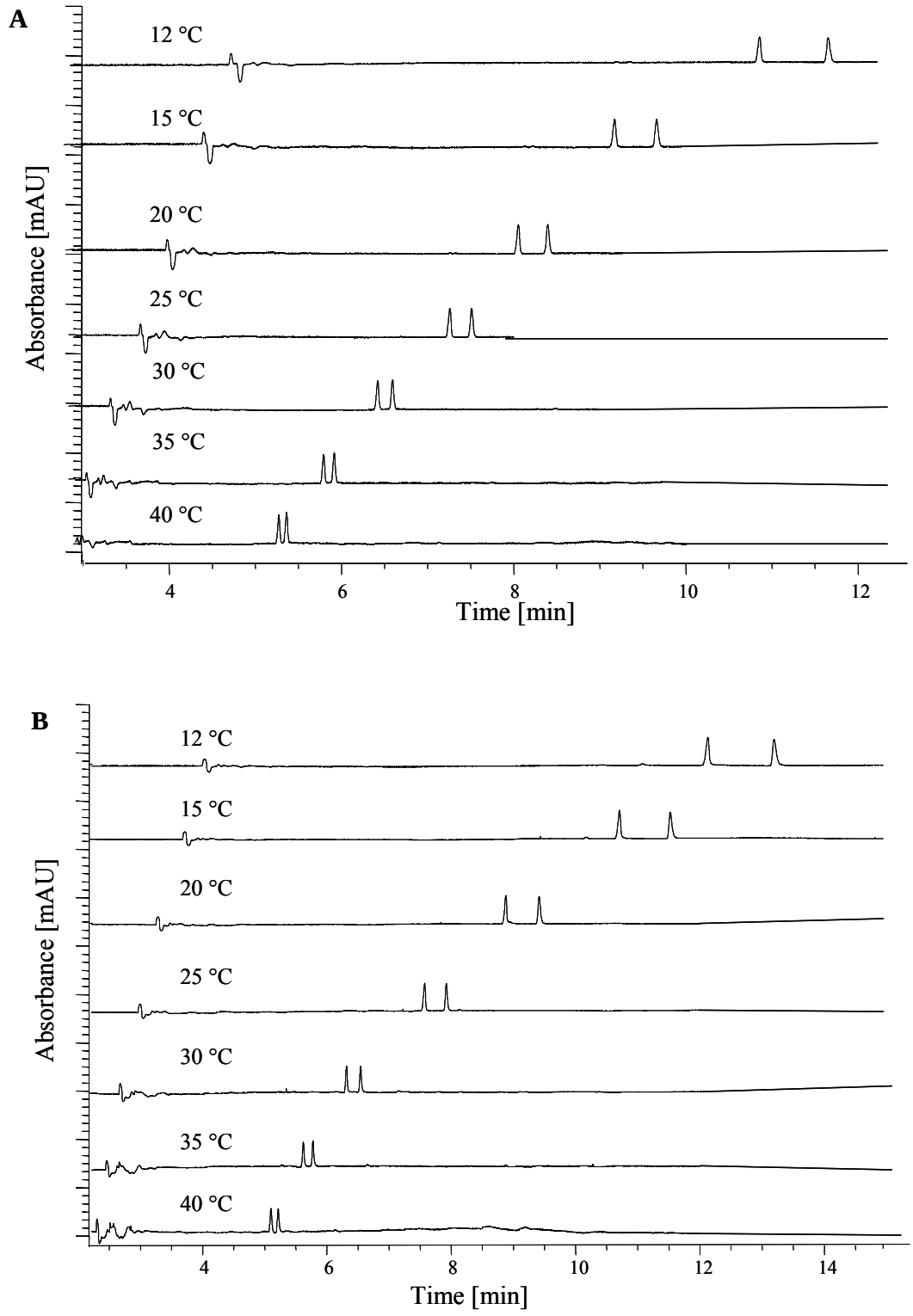


Figure 2.6 **Effect of temperature on separation of BNP using (A) poly-L-SOLV and (B) L-SOLV. Conditions: (A) 5 mM poly-L-SOLV, and (B) 10 mM L-SOLV; other conditions same as Figure 2.5, except the temperature was varied.**

2.3.4 Effect of pH on Separation of BNP

The pH plays a very important role in the separation of chiral compounds. Generally, as pH increases, EOF is expected to increase, mainly because at higher pH, there is more dissociation of Si–OH to Si–O^- on the inner capillary wall. Thus, the increased number of Si–O^- groups causes a greater zeta potential, and consequently, an increase in electroosmotic velocity. Contrary, at lower pH, there is less surface ionization and a lower zeta potential, hence a decrease in the electroosmotic velocity. In addition, the pH of the buffer influences the degree of ionization of the solutes, hence, their electrophoretic mobilities.

For the separation of BNP, pH was varied between 8.5 and 10.0. A decrease in buffer pH led to a slight increase in EOF and a shorter retention time for the analyte as shown in Figure 2.7 (A) and 2.7 (B). The increase in EOF as the buffer pH was lowered was contrary to the general trend explained above, but the reason for this slight increase was unknown. The increase in EOF caused shorter migration times at lower pH. In addition, because the pKa of the OH group on BNP is around 2, the higher the pH the greater the ionization of BNP. Therefore, the shorter migration time at lower pH may also be due to a lower degree of ionization of BNP, thus, allowing it to have a lower electrophoretic mobility towards the anode and migrating faster towards the cathode due to EOF.

Also, for the separation of BNP, the resolution was reduced as the pH was decreased in both cases and at pH of 8.5 no enantoimeric separation was observed when L-SOLV was used. This implies that as the pH is lowered the difference between the electrophoretic mobilities of the R-(+) and S-(–) enantiomers of BNP decreased. Therefore, for the separation of BNP done using L-SOLV, at pH 8.5, the electrophoretic mobilities of the two enantiomers were most likely similar, hence, no resolution was observed.

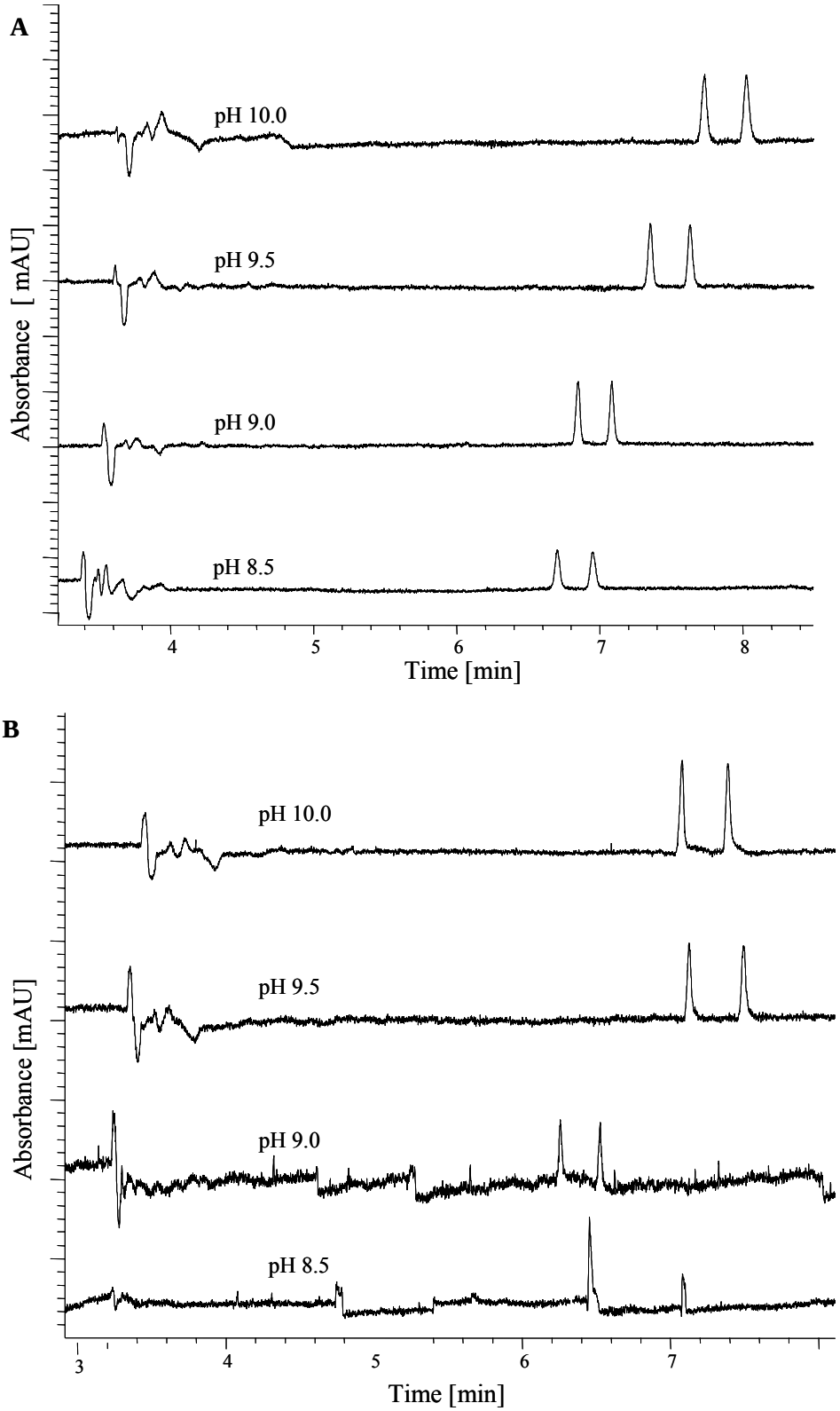


Figure 2.7 Effect of pH on separation of BNP using (A) poly-L-SOLV and (B) L-SOLV. Conditions: same as Figures 2.5 and 2.6, except the pH was varied and the study was done at 25 °C.

2.3.5 Separation of Neutral Compounds

Benzoin and its derivatives are chiral neutral compounds used in the development of antiseptic, astringent, and anti-inflammatory drugs such as Tin-Ben. Unlike BNP which is anionic at basic conditions described above, chiral neutral compounds are challenging to separate; however, they can be separated using a chiral pseudo-stationary phase in MEKC. A mixture of three-benzoin derivatives, benzoin, hydrobenzoin, and benzoin methyl ether, (molecular structures shown in Figure 2.1) were separated using poly-L-SOLV.

The separation conditions were determined by performing optimization experiments for poly-L-SOLV concentration, and buffer type, pH, and concentration. Also, the running voltage, and temperature were optimized. Only the polymeric surfactant was used in these experiments because it gave better separations. Baseline resolution was achieved for the separation of benzoin and hydrobenzoin, while partial enantiomeric separation was observed for benzoin methyl ether as shown in Figure 2.8.

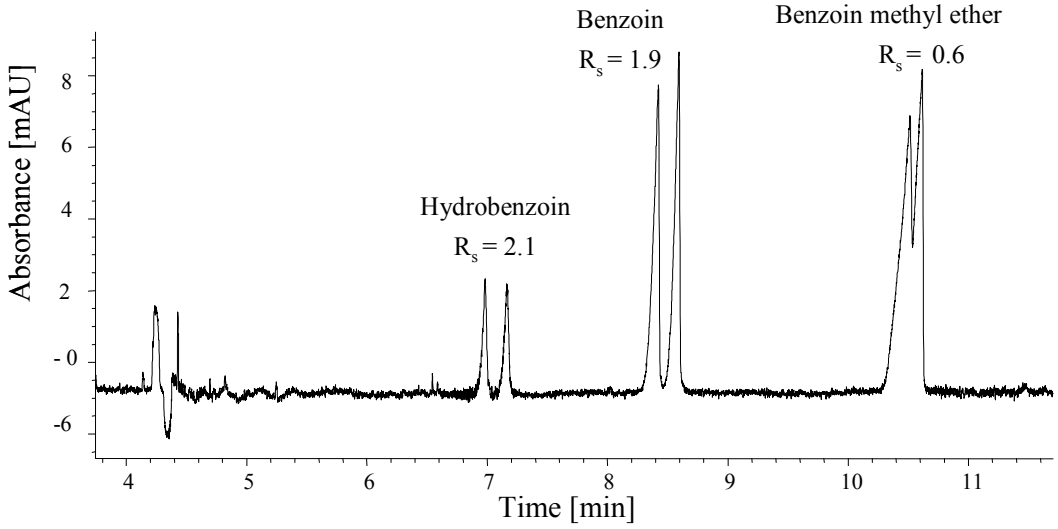


Figure 2.8 Enantiomeric separation of benzoin derivatives using 10 mM poly-L-SOLV. Conditions: electrolyte 20 mM monobasic phosphate + 30 mM dibasic phosphate, pH, 7.2; temperature, 15 °C; voltage, 30 kV; capillary, 52 cm effective length, 50 μm i.d.; injection size, 10 mbar for 10 s; UV detection λ, 254 nm.

2.3.6 Separation of Acidic Compounds

Warfarin is a coumarin anticoagulant drug frequently used in the treatment of thrombeolic diseases.[43] This drug displays a stereoselective metabolism and pharmacokinetics where each enantiomer follows different metabolic pathways.[24, 44] Although it is sold as a racemic mixture, the (S)-(−) enantiomer is more pharmacologically active than its corresponding (R)-(+) form.[24, 43] Coumachlor, an analog of warfarin, has been used in HPLC as an internal standard. Qualitative and quantitative experiments using both warfarin and coumachlor have been documented by use of HPLC and GC.[24, 45, 46] These two drugs, (molecular structures shown in Figure 2.1) are structurally-related, acidic, and electronegative due to their keto-enol groups. The phenolic group on warfarin has a pK_a value of 5.1.[24, 47] Under neutral and basic pH conditions, both drugs are not expected to complex strongly to an anionic chiral selector. As noted by Agnew-Heard et al.,[24] an acidic pH range was better for the separation of these compounds. However, poly-L-SOLV tends to precipitate at a pH lower than 7 due to a decrease in the ionization of its carboxylate functionality. Contrary to previous studies, the separation of these two drugs was achieved at a pH of 7.2 using poly-L-SOLV as the pseudo-stationary phase (Figure 2.9). The separation conditions were determined by optimizing the poly-L-SOLV concentration, buffer concentration and pH, and the running CE parameters, one at a time. Only the polymeric surfactant was used. A resolution of 1.8 and a selectivity of 1.04 were obtained for warfarin while a resolution of 5.8 and a selectivity of 1.11 were obtained for coumachlor. Also, shorter migration times for warfarin and coumachlor were recorded using poly-L-SOLV as compared to those obtained using poly-L-SULV.[34]

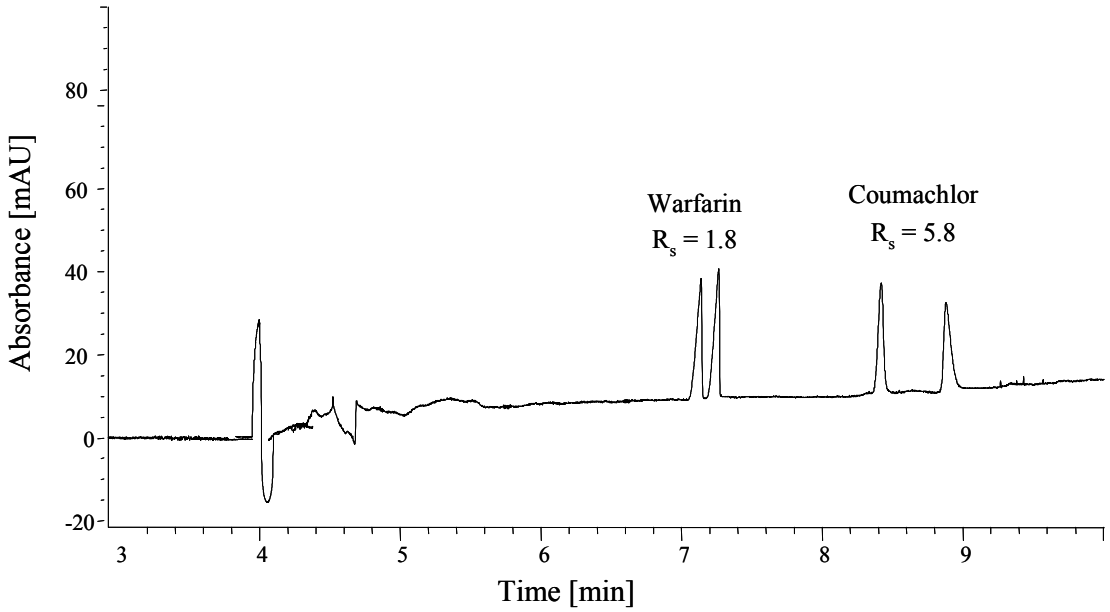


Figure 2.9 Enantiomeric separation of coumarin drugs, warfarin and coumachlor using 20 mM poly-L-SOLV. Conditions: electrolyte 275 mM boric acid + 20 mM monobasic phosphate; pH, 7.2; temperature, 15 °C; voltage, 30 kV; capillary, 52 cm effective length, 50 μm i.d.; injection size, 30 mbar for 3 s; UV detection λ: 220 nm.

2.3.7 Separation of Basic Compounds

Temazepam (molecular structure shown in Figure 2.1) belongs to a class of compounds known as benzodiazepines. These compounds are used as hypotonics, tranquilizers, and anticonvulsants.[48] Although benzodiazepines possess similar aromatic skeletons, the difference lies in the number of substituents attached to the aromatic ring can make the separation of these compounds difficult. Temazepam is one among the benzodiazepines compounds which is chiral and was separated using poly-L-SOLV. The separating conditions were obtained by performing similar optimization experiments as those for the neutral and acidic compounds described previously. Poly-L-SOLV resolved the enantiomers of temazepam with a resolution of 1.2 and a selectivity of 1.06 as shown in Figure 2.10. At the pH which this experiment was conducted, the enantiomers of temazepam

are capable of inter-converting (racemize). Therefore, the deep valley between the two enantiomer peaks of temazepam may be due to the racemization process. Another difficult that may be encountered while separating temazepam was identified by Haddadian et al.[49] According to their studies the methyl group of the temazepam may affect chiral selectivity in two ways, namely, blocking the hydrogen-bonding site of temazepam or by increasing the steric hindrance.

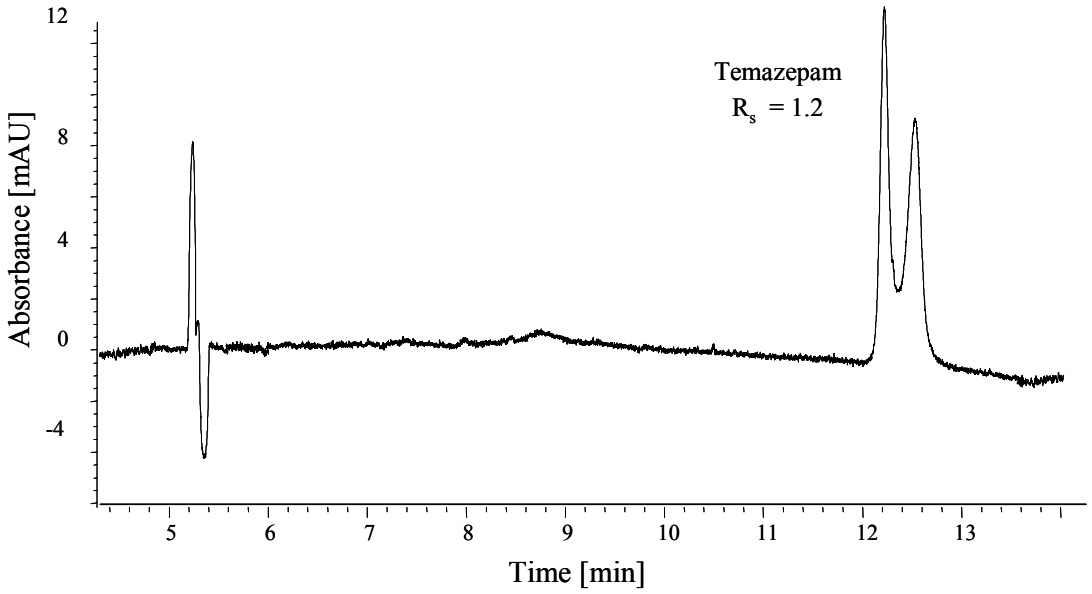


Figure 2.10 Enantiomeric separation of temazepam using 6 mM poly-L-SOLV. Conditions: electrolyte 20 mM monobasic phosphate + 30 mM dibasic phosphate; pH, 8.0; temperature, 12 °C; voltage, 30 kV; capillary, 52 cm effective length, 50 μm i.d.; injection size, 10 mbar for 10 s; UV detection λ, 220 nm.

2.4 Conclusions

Novel monomeric and polymeric surfactant were synthesized and used for chiral separation in MEKC. Both the monomer and the polymer were capable of chiral separation of BNP. However, the polymer provided better separation. In this investigation, poly-L-SOLV was used for the separation of neutral, acidic, and basic chiral compounds. When comparing the monomer of L-SOLV to poly-L-SOLV, several advantages are noted for

polymeric surfactants over normal micelles in chiral separations. First, elimination of the dynamic equilibrium between the monomers and the micelles can enhance chiral recognition of a racemic mixture. Thus, a higher resolution is expected. Second, the lack of a CMC in polymeric surfactants makes them more practical for use in MEKC separations because lack of a dynamic equilibrium eliminates problems associated with current and addition of organic modifiers. Finally, the rigidity of the polymeric surfactant improves the mass transfer rate and thus reduces band broadening.

2.5 References

1. Billiot, E., Thibodeaux, S., Shamsi, S., Warner, I. M. *Anal. Chem.* **1999**, *71*, 4044.
2. Rawjee, Y. Y., Williams, R. L., Vigh, G. *J. Chromatogr.* **1993**, *652*, 233.
3. Biggin, M. E., Williams, R. L, Vigh, G. *J. Chromatogr. A* **1995**, *692*, 319.
4. Wang, F., Khaledi, M. G. *Anal. Chem.* **1996**, *68*, 3460.
5. D'Hurst, A., Verbeke, N. *J. Chromatogr. A* **1992**, *608*, 275.
6. Soini, H., Stefansson, M., Riekkola, M. L., Novotny, M. V. *Anal. Chem.* **1994**, *66*, 3477.
7. Barker, G. E., Russo, P., Harwick, R. A. *Anal. Chem.* **1992**, *64*, 3024.
8. Bucsh, S., Kraak, J. C., Poppe, H. *J. Chromatogr. A* **1993**, *635*, 119.
9. Gasper, M. P., Berthod, A., Nair, U. B., Armstrong, D. W. *Anal. Chem.* **1996**, *66*, 2501.
10. Rundlett, K. L., Armstrong, D. W. *Anal. Chem.* **1995**, *67*, 2088.
11. Kuhn, R., Erni, F., Bereuter, P., Hausler, J. *Anal. Chem.* **1992**, *64*, 2815.
12. Kuhn, R., Riester, D., Fleckenstein, B., Wiesmüller, K. H. *J. Chromatogr. A* **1995**, *716*, 371.
13. Sanchez-Pena, M., Zhang, Y., Thibodeaux, S., McLaughlin, M. L., de la Pena, A. M., Warner, I. M. *Tetrahedron Lett.* **1996**, *37*, 5841.
14. Shamsi, S., Warner. I. M. *Electrophoresis* **1997**, *18*, 853.

15. Nishi, H., Terabe, S. *J. Chromatogr. A* **1996**, *735*, 3.

16. Smith, J. T., Nashabeh, W., El Rassi, Z. *Anal. Chem.* **1994**, *66*, 1119.

17. Terabe, S., Ostuka, K., Ichikawa, K., Ando, T. *Anal. Chem.* **1984**, *56*, 111.

18. Terabe, S., Ostuka, K., Ando, T. *Anal. Chem.* **1985**, *57*, 834.

19. Cohen, S. A., Paulus, A., Karger, B. L. *Chromatographia* **1997**, *24*, 15.

20. Dobashi, A., Hamada, M., Dobashi, Y. *Anal. Chem.* **1995**, *67*, 3011.

21. Williams, C. C., Shamsi, S., Warner. I. M. *Adv. Chromatogr. (NY)* **1997**, *37*, 363.

22. Wang, J., Warner, I. M. *Anal. Chem.* **1994**, *66*, 3773.

23. Wang, J., Warner, I. M. *J. Chromatogr. A* **1995**, *711*, 297.

24. Agnew-Heard, K. A., Sanchez Pena, M., Shamsi, S., Warner, I. M. *Anal. Chem.* **1997**, *69*, 958.

25. Shamsi, S. A., Mocossay, J., Warner, I. M. *Anal. Chem.* **1997**, *69*, 2980.

26. Billiot, E., Mocossay, J., Thibodeaux, S., Shamsi, S., Warner, I. M. *Anal. Chem.* **1998**, *70*, 1375.

27. Billot, E., Agbaria, R. A., Thibodeaux, S., Shamsi, S., Warner, I. M. *Anal. Chem.* **1999**, *71*, 1252.

28. Haddadian, F., Billiot, E., Shamsi, A. S., Warner,. I. M. *J. Chromatogr. A* **1999**, *858*, 219.

29. Yarabe, H. H., Shamsi, S., Warner, I. M. *Anal. Chem.* **1999**, *71*, 3992.

30. Billiot, E., Thibodeaux, S., Shamsi, S., Warner, I. M. *Anal. Chem.* **1999**, *71*, 4044.

31. Billiot, E., Warner, I. M. *Anal. Chem.* **2000**, *72*, 1740.

32. Haddadian, F., Billiot, E., Warner, I. M. *J. Chromatogr. A* **2001**, *922*, 329.

33. Hara, S., Dobashi, A. *Jpn .pat.* 04149205 **1992**.

34. Shamsi, S. A., Valle, B. C., Billiot, F., Warner, I. M. *Anal. Chem.* **2003**, *75*, 379.

35. Durchschlag, H. In *Thermodynamic Data for Biochemistry and Biotechnology*, Hinz, H. J., Ed., Springer-Verlag: New York, **1986**.

36. Fujita, H. In *Foundations of Ultracentrifugal Analysis*; Wiley and Sons: New York, **1975**.

37. Correia, J. J., Shire, S., Yphantis, D. A., Schuster, T. M. *Biochemistry* **1985**, *24*, 3292.

38. Billiot, F. H., McCarroll, M., Billiot, E. J., Rugutt, J. K., Morris, K., Warner, I. M. *Langmuir* **2002**, *18*, 2993.

39. Jacques, J., Fouquey, C. *Tetrahedron Lett.* **1971**, *48*, 4617.

40. Arnold, W., Daly, J. J., Imhof, R., Kyburz, E. *Tetrahedron Lett.* **1983**, *24*, 343.

41. Shao, L., Miyata, S., Muramastu, H., Kaqano, H., Ishii, Y., Saburi, M., Uchida, Y. *J. Chem. Soc., Perkin Trans.* **1990**, *1*, 1441.

42. Castro, P. P., Georgiaddis, T. M., Diederich, F. *Org. Chem.* **1989**, *54*, 5835.

Chapter 3.

Separation of Achiral Analytes Using Polymeric Surfactants with Ionic Liquids as Modifiers in Micellar Electrokinetic Chromatography

3.1 Introduction

Ionic liquids (ILs), are liquids at low temperatures and consist entirely of ions. In the past, ILs were mainly of interest to electrochemists. However, recently it has become apparent that a wide range of chemical reactions can be conducted using ILs.[1] For example, many recent scientific investigations have focused on ILs[2–12] as a new class of solvents for liquid–liquid extraction,[5, 6] organic synthesis,[7–12] electrochemistry,[13–16] catalysis for clean technology,[17–21] ultralow volatility liquid matrixes for matrix-assisted laser desorption/ionization (MALDI) mass spectrometry,[22] and separations.[23–27]

The typical IL consists of a bulky pyridinium or imidazolium cation paired with a variety of anions.[3, 13, 14] ILs have many properties of conventional organic solvents, such as excellent solvation qualities, a low viscosity and a wide temperature range.[3, 16–18] Two characteristics that ILs do not share with conventional organic solvents are volatility, and good electrical conductivities.[3, 4, 7, 16] ILs are environmentally benign, nonvolatile, and nonflammable with a high thermal stability.[4] Their miscibility in water is heavily dependent on the type of anion forming the IL. In addition, the length of the alkyl chain on the pyridinium or imidazolium cation also has a considerable effect on the properties of the IL formed.[19] Wilkes and Zaworotko[7] concluded that the l-ethyl-3-methylimidazolium (EMIM) cation was chemically and electrochemically robust and was generally useful for synthesis of ILs. Fuller and co-workers[28] fully characterized the low-melting salt, l-ethyl-3-methylimidazolium hexafluorophosphate ($EMIMPF_6$, mp 58–60 °C). In Fuller's report, it was

concluded that $EMIMPF_6$ interionic interactions were dominated by cation-anion coulombic attraction with minimal hydrogen bonding. The IL l-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$, mp 15 °C), was found to be a liquid at room temperature and was highly miscible in water.[26, 29]

In addition to the characterization work mentioned above, Armstrong et al. recently characterized ILs on the basis of multiple solvation interactions.[4] It was concluded that ILs were complex solvent systems, as compared to conventional organic solvents. Using their solvation model, Armstrong and co-workers were able to show that the anion had greater effect on the hydrogen bond basicity of the IL, as compared to the cation.

The application of ILs for the separation of various classes of compounds has also been recently recognized. Yanes et al. reported the development of a fairly robust capillary electrophoresis (CE) method for the separation of polyphenols found in grape seed extracts in which the IL tetraethylammonium tetrafluroborate was used as the only background electrolyte (BGE).[25] More recently, Yanes and co-workers developed a CE method for the same analysis using 1-ethyl-3-methylimidazolium-based ILs as the BGE.[26] In another publication, Armstrong et al. examined two ILs (1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$) and 1-butyl-3-methylimidazolium-chloride (BMIMCl)) as stationary phases in gas–liquid chromatography.[27]

In micellar electrokinetic chromatography (MEKC), a surfactant is added into the buffer to separate chiral and achiral analytes. The use of polymeric surfactants bearing both chiral and achiral ionic head groups has been reported.[30–33] In some cases, organic modifiers have been used in the separation of hydrophobic environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs), phenols, and alkyl aryl ketones to help resolve such

mixtures.[33, 34] To date, the use of ILs as modifiers in MEKC using polymeric surfactants has not been investigated. When using ILs, the choice of the BGE is very important because most ILs are highly conductive. There are several advantages for using ILs over organic solvents as modifiers. ILs are soluble in water, have a good electrical conductivity, and act as good electrolytes in CE either when used independently or when mixed with other buffers. ILs are more viscous than organic solvents; therefore, low concentrations may be required for buffer modifications to achieve better separations. In addition, ILs are less volatile, meaning they are environmentally friendly. In contrast, organic solvents are poor conductors of electricity, and high concentrations of organic solvents in the buffer cause current breakdowns in CE. In addition, most organic solvents are highly volatile and harmful to the environment.

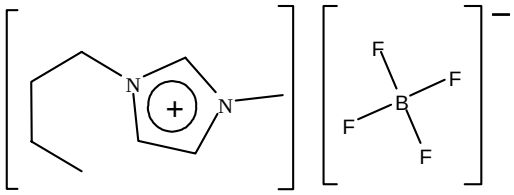
In this paper, ILs were used as modifiers in micellar electrokinetic chromatography (MEKC) using polymeric surfactants to separate achiral compounds. The ILs used in this study were 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), l-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$), l-ethyl-3-methylimidazolium hexafluorophosphate ($EMIMPF_6$), 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ($EMIMSO_3F_3$), and 1-ethyl-3-methylimidazolium chloride (EMIMCl); their chemical structures are shown in Figure 3.1. Two different analyte mixtures were separated using these ILs. Those mixtures included a mixture of eight alkyl aryl ketones and a mixture of seven phenols.

3.2 Experimental Methods

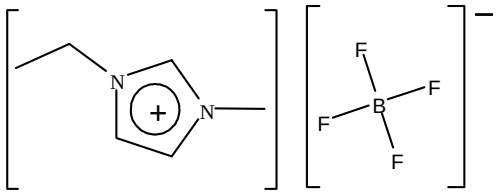
3.2.1 Materials

The alkyl aryl ketones (acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, decanophenone, and

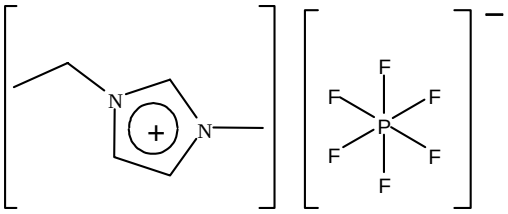
dodecanophenone.), and the phenols (4-chlorophenol, 3-chlorophenol, 4-chloro-3-methylphenol, 2-chlorophenol, 2,4,6-trichlorophenol, 2,4-dichlorophenol, and pentachlorophenol) were purchased from Sigma-Aldrich (Milwaukee, WI) (Figure 3.2).



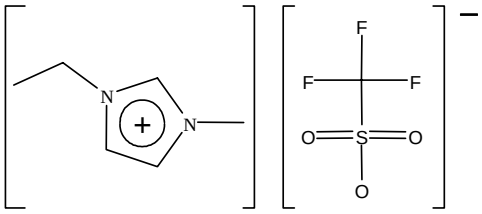
1- butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$)



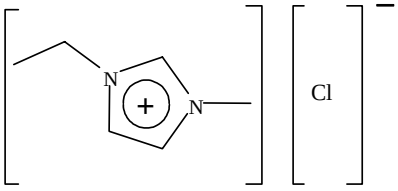
1-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$)



1-ethyl-3-methylimidazolium hexafluorophosphate ($EMIMPF_6$)



1-ethyl-3-methylimidazolium trifluoromethanesulfonate ($EMIMSO_3F_3$)



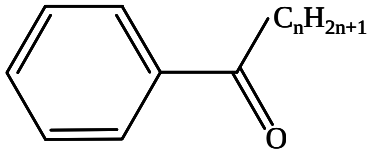
1-ethyl-3-methylimidazolium Chloride (EMIMCl)

Figure 3.1 Chemical structures of the ILs used as modifiers in the MEKC separation experiments.

The undecylenyl alcohol, sodium borate, chlorosulfonic acid, pyridine, sodium hydrogen carbonate, sodium borate, disodium phosphate, tris(hydroxymethyl)aminomethane (TRIS), tetrahydrofuran (THF), sodium dodecyl sulfate (SDS), and the ILs $EMIMBF_4$, $EMIMPF_6$, $EMIMSO_3F_3$, and EMIMCl were also obtained from Sigma-Aldrich. The IL $BMIMBF_4$ was

purchased from Chemada Fine Chemicals Ltd., (Nir Itzhak, Israel). All reagents were of analytical grade and were used as received without further purification.

A



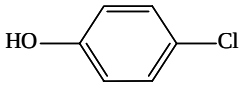
(1) n = 1 Acetophenone (5) n = 5 Hexanophenone

(2) n = 2 Propiophenone (6) n = 6 Heptanophenone

(3) n = 3 Butyrophenone (7) n = 7 Octanophenone

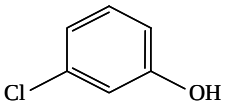
(4) n = 4 Valerophenone (8) n = 9 Decanophenone

B



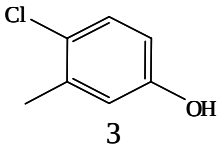
1

4-Chlorophenol



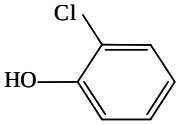
2

3-Chlorophenol



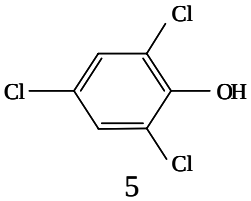
3

4-Chloro-3-methylphenol



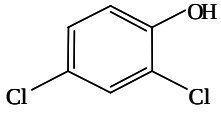
4

2-Chlorophenol



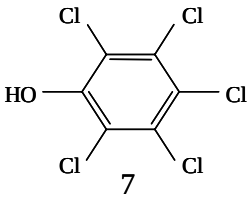
5

2,4,6-Trichlorophenol



6

2,4,-Dichlorophenol



7

Pentachlorophenol

Figure 3.2 (A) Chemical structures of eight alkyl aryl ketones. The elution order is 1-8, except where peaks are co-eluting. (B) Chemical structures of seven phenols. The elution order is 1-7, except where peaks are co-eluting.

3.2.2 Synthesis of Polysodium Undecylenic Sulfate

The sodium undecylenic sulfate (SUS) monomer was prepared according to Bregstrom's procedure[35] and modifications were made according to the procedure described by Shamsi et al.[34] to obtain the polymerized surfactant poly-SUS. A schematic of the synthesis of polysodium undecylenic sulfate (poly-SUS) is shown in Figure 3.3.

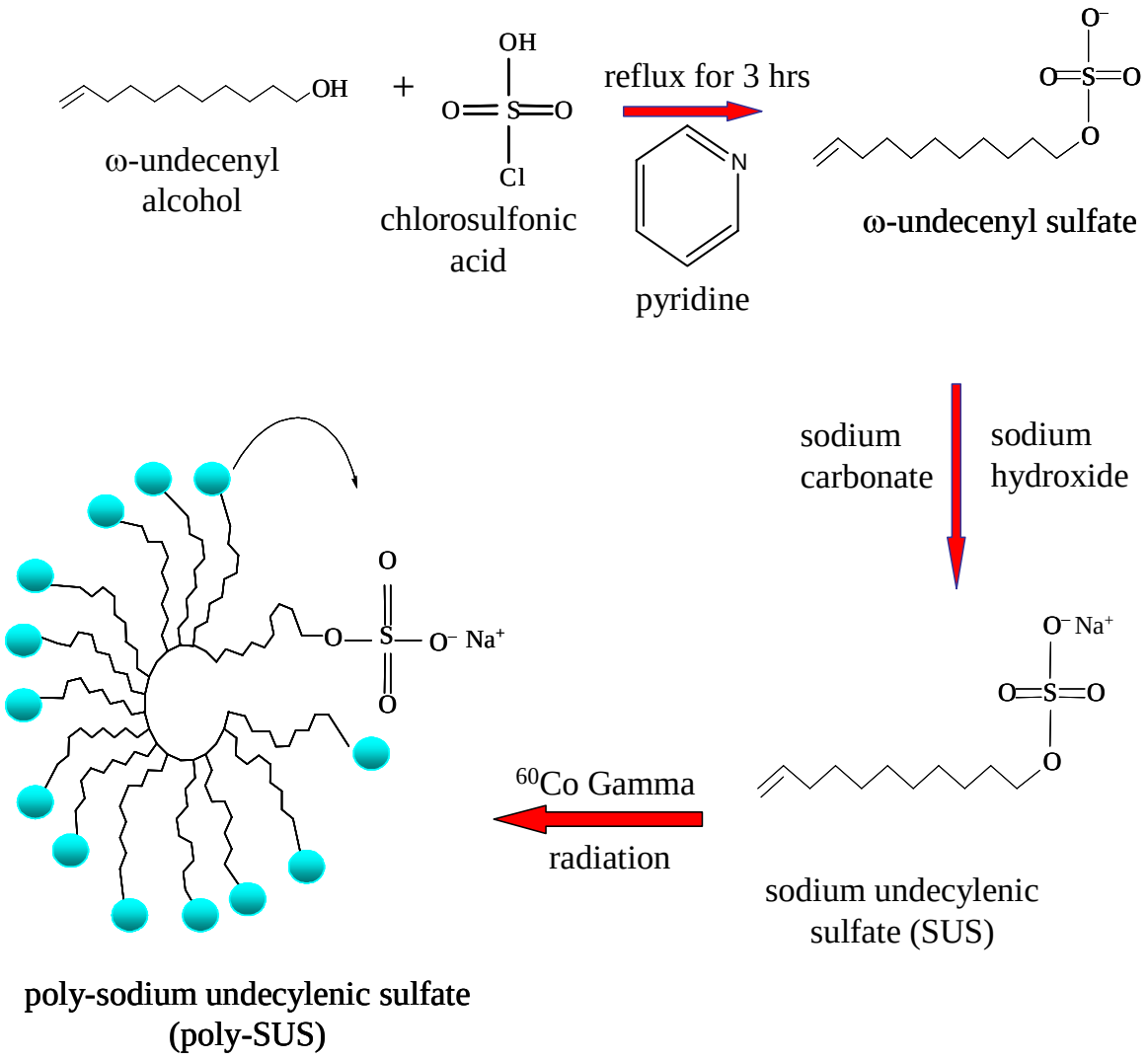


Figure 3.3 Synthetic scheme for polysodium undecylenic sulfate (poly-SUS).

To sulfate the ω-undecylenyl alcohol, 113.8 mmol (7.5 mL) of chlorosulfonic acid was added dropwise to 75 mL of pyridine 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 and 75 mL of pyridine 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 sulfonic acid. 600 mL of deionized water containing 4 g of sodium hydroxide and about 80 to 100 g of sodium carbonate were added to the undecylenic sulfonic acid solution and the solution was stirred overnight. This resulted in the formation of sodium undecylenic sulfate (SUS) surfactant solution. The SUS surfactant solution was then extracted twice using n-butanol in a separatory funnel. The organic phase on the top contained the product. A dry product of SUS surfactant was obtained by evaporation of the organic solvents (pyridine-butanol) using a rotary evaporator followed by a vacuum desiccator. 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 monomeric surfactant. A 100 mM aqueous solution of SUS monomeric surfactant was exposed to a ^{60}Co γ-ray source for 7 days for polymerization. After irradiation, the polysodium undecylenic sulfate (poly-SUS) was dialyzed against bulk water 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.

3.2.3 Preparation of MEKC Buffer Solutions

The BGE used for separation of the alkyl aryl ketones was 100 mM TRIS at pH 10.00 and the BGE used for the phenols was 20 mM sodium borate and disodium phosphate at pH 9.2. The pH of the BGE was adjusted with 1 M NaOH or 1 M HCl, whenever was necessary. An appropriate amount of the polymeric surfactant was added to the BGE and IL was introduced into the BGE as a mobile phase modifier to examine the separation efficiency and resolution of the analytes.

3.2.4 Capillary Electrophoresis

The MEKC experiments were performed using a Hewlett-Packard 3D CE instrument (Foster City, CA) equipped with a UV diode array detector. Bare fused-silica capillaries (effective length 40 cm, 50 μm i.d. for the separation of the ketones and phenol compounds), were purchased from Polymicro Technologies (Phoenix, AZ). The applied voltage ranged from +20 to +30 kV. The detection wavelength was 254 nm. The temperature of the capillary was maintained at 20 °C by the instrument thermostating system, which consisted of a peltier element for forced-air cooling and temperature control.

The ketones and the phenols were prepared in methanol at a concentration of 0.1 mg/mL. The sample injection size varied from 50 mbar for 1 s to 30 mbar for 3 s. Prior to use, each new capillary was conditioned for 30 min with 1 M NaOH and then for 30 min with 0.1 M NaOH. Finally, the capillary was rinsed for 15 min with triply-distilled deionized water. Before each run, the capillary was flushed with MEKC buffer for 3 min to condition and fill the capillary. To change from one mobile phase to another (i.e., buffer containing poly-SUS and an IL to buffer containing poly-SUS and a different IL), the capillary was flushed with 0.1 M NaOH for 10 min and rinsed with water for 5 min, then filled with the

new MEKC buffer containing the IL for 5 min. This was done to minimize any influence of the previous mobile phase.

3.2.5 Calculations

The retention factors for two of the analytes (e.g., acetophenone and decanophenone were determined using Equation 3.1.[36]

$$K' = \frac{(t_r - t_{eo})}{t_{eo}(1 - t_r / t_{mc})} \qquad 3.1$$

where t_{eo} is the migration time of an unretained solute, t_r is the retention of a solute, and t_{mc} is the migration of the micelle or polymeric surfactant. Methanol was used as the electroosmotic flow (t_{eo}) marker and was measured from the time of injection to the first deviation from baseline. The migration time of the micelle or polymeric surfactant was determined using *n*-dodecaphenone as the marker. The resolution (R_s) of the chiral analytes was calculated using Equation 3.2.[37]

$$R_s = 2\frac{(t_{r_2} - t_{r_1})}{(w_1 + w_2)} \qquad 3.2$$

where t_{r_1} and t_{r_2} are the respective migration times of each enantiomer, and w_1 and w_2 are the peak widths at the baseline of each enantiomer.

3.3 Results and Discussions

3.3.1 Separation of Alkyl Aryl Ketones

Experiments were conducted to separate a mixture of eight alkyl aryl ketones first using SDS, and later poly-SUS in the buffer. The separation results obtained at the optimized buffer pH, voltage, temperature, and SDS concentration are illustrated in Figure 3.4 (I). Only five of the eight alky aryl ketones in the mixture were resolved. The elution order for these ketones was from the least hydrophobic analyte acetophenone to the most hydrophobic

analyte decanophenone. The peak numbers are based on the labeling used for Figure 3.2 (A). The optimized SDS concentration was 50 mM. Higher concentrations of SDS led to poor resolution and longer migration times of the analytes, but lower concentrations of SDS did not enhance the separation.

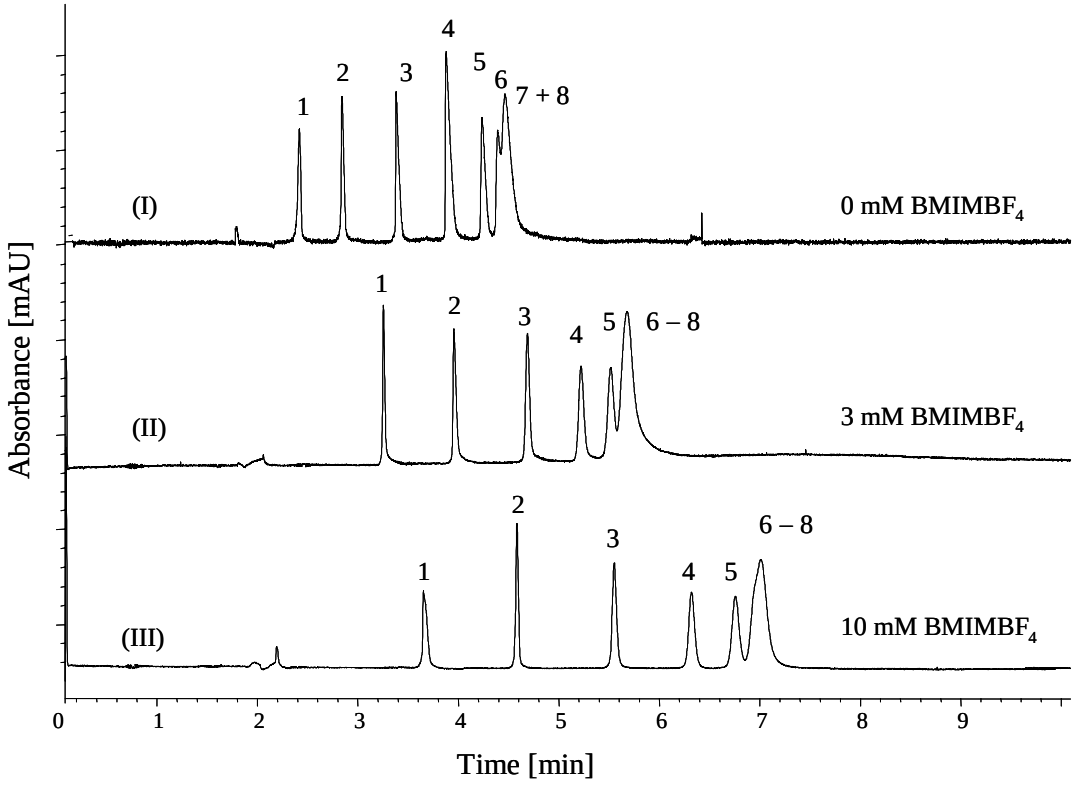


Figure 3.4 Electropherograms showing the separation of eight alkyl aryl ketones: (I) using 50 mM SDS, (II) using 50 mM SDS + 3 mM $BMIMBF_4$, and (III) 50 mM SDS + 10 mM $BMIMBF_4$, Conditions: 100 mM TRIS buffer; pH, 10.00; pressure injection, 50 mbar for 1s; temp., 20 °C; voltage, 30 kV; detection λ, 254 nm.

To enhance the resolution of the analytes, ILs were used as modifiers using the SDS optimized concentration (50 mM). The UV studies showed that the IL had a strong absorbance in the region between 215 and 235 nm as illustrated in Figure 3.5. An IL concentration study was done using 1 to 10 mM of each IL in order to determine the optimum amount to be added to the MEKC buffer composed of 50 mM SDS and 100 mM

TRIS at pH 10.00 Figure 3.4 electropherograms (II) and (III) are representative of the data obtained when ILs were used as modifiers using SDS as the pseudo-stationary phase.

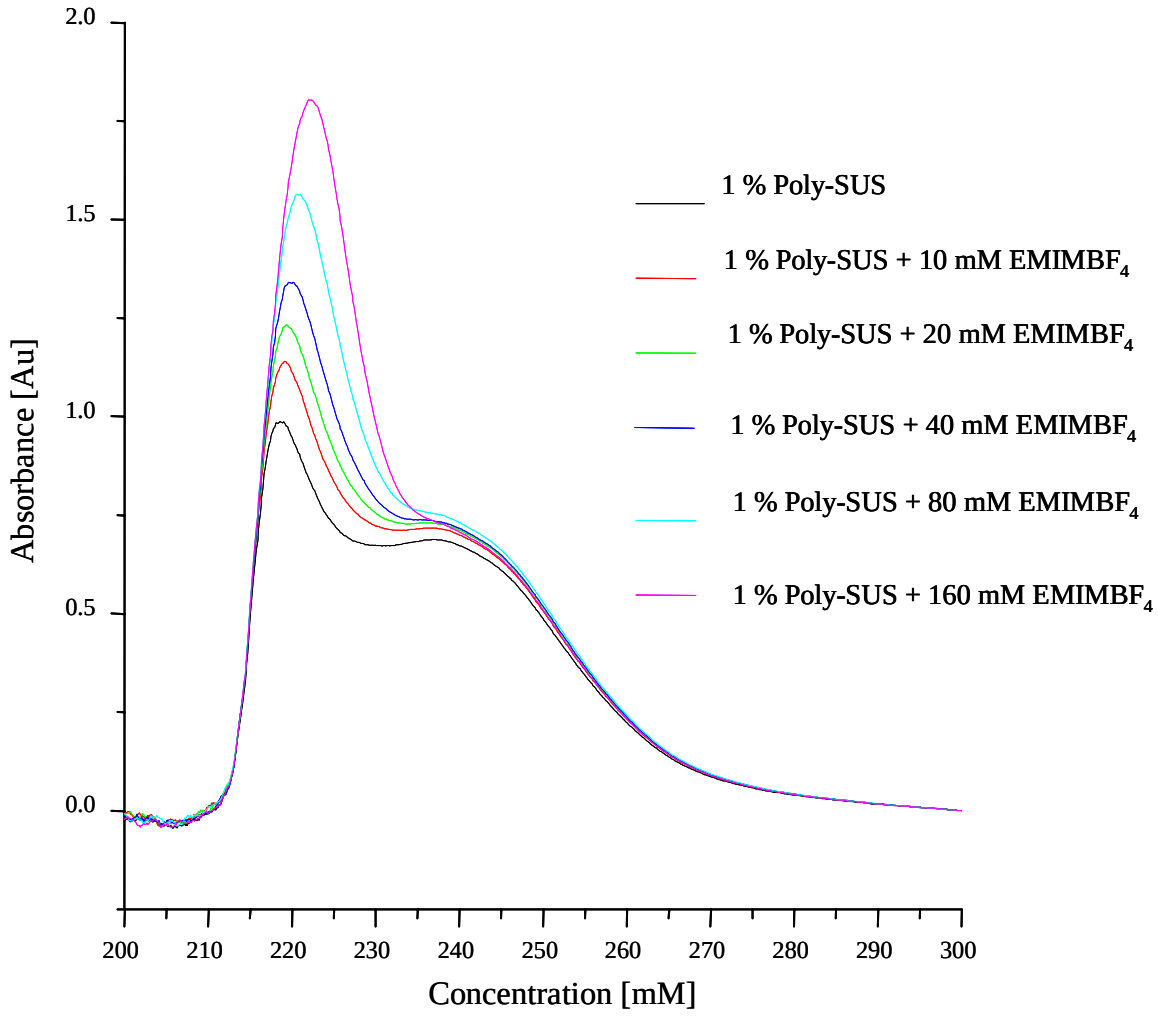


Figure 3.5 UV/Vis. spectra of poly-SUS and poly-SUS with different concentrations of $EMIMBF_4$ in 100 mM TRIS buffer at pH 9.0.

The ILs increased the migration time of the analytes but did not enhance either the resolution or the efficiency for SDS separations of these compounds. The separation of the eight alkyl aryl ketones mixture was also done using poly-SUS. The poly-SUS concentration and separation conditions were optimized. The MEKC buffer consisted of 0.5% poly-SUS and

100 mM TRIS at pH 10.00. Figure 3.6 (I) shows that a better separation was achieved using poly-SUS than SDS for the separation of the alkyl aryl ketones mixture.

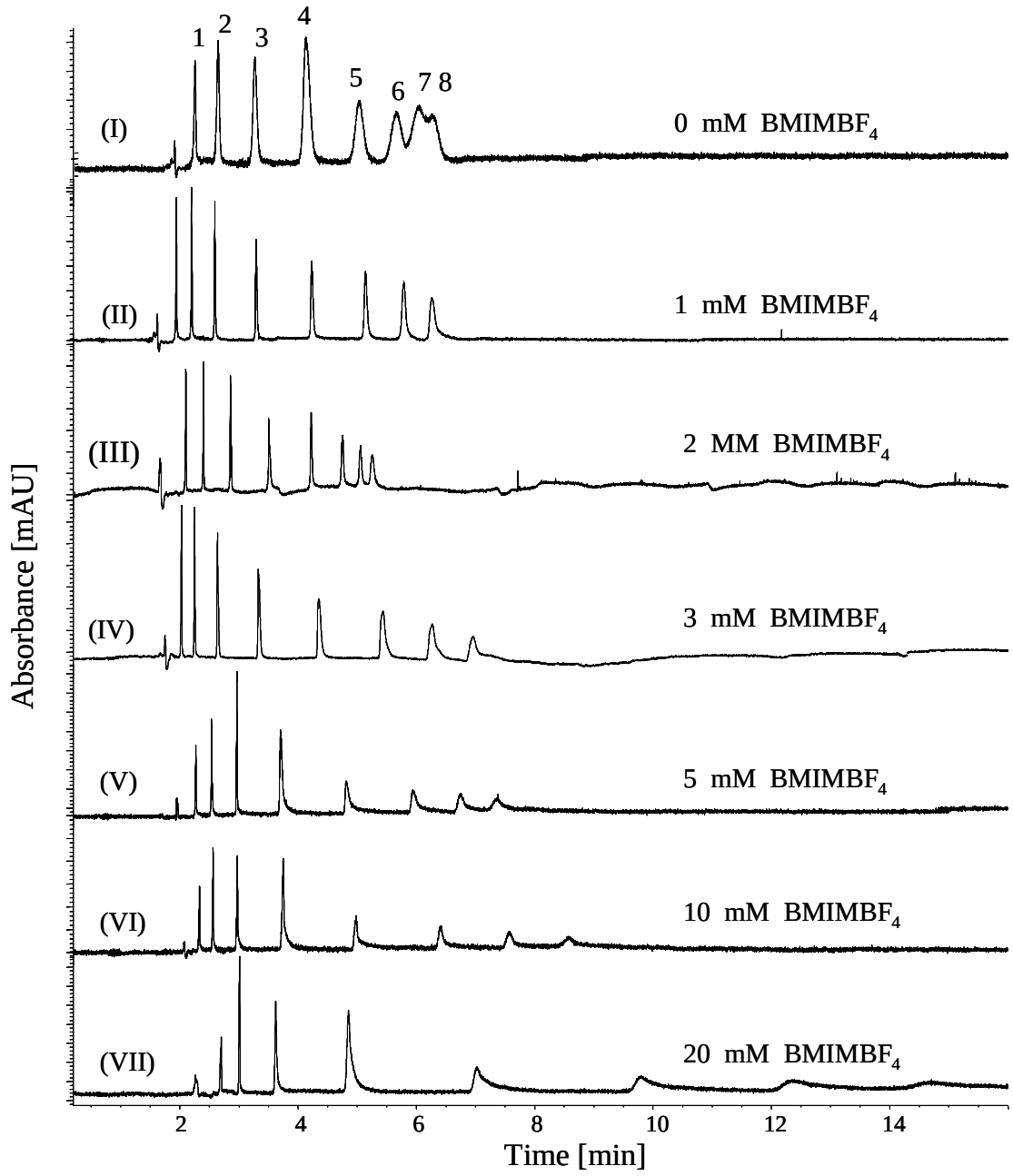


Figure 3.6 **Electropherograms showing the separation of eight alkyl aryl ketones: (I) no modifier; (II-VII), using 1, 2, 3, 5, 10, and 20 mM $BMIMBF_4$ as modifiers. Conditions: 0.5% poly-SUS, 100 mM TRIS buffer, pH 10.00; injection size, 50 mbar for 1s; temp., 20 °C; voltage, 30 kV; detection λ, 254 nm.**

Six of the eight alkyl aryl ketones were well-resolved and eluted in less than ten minutes. However, the last three analytes coeluted. Of the five ILs used as modifiers in the MEKC buffer, $BMIMBF_4$ gave the best results. Figure 3.6 (II-VII) illustrates the effect of $BMIMBF_4$ concentration on the separation of the ketone mixture. The elution order was determined by spiking a small amount of each analyte into the mixture and the peaks are numbered according to the analyte notation in Figure 3.2. Slight changes in the EOF as well as the migration times of the first four analytes when the concentration of $BMIMBF_4$ was increased from 1 mM to 5 mM were observed. However, the peak efficiencies and resolution of these analytes were almost maintained.

Shorter migration times of the last four analytes were observed as the concentration of $BMIMBF_4$ was increased from 0 to 2 mM. However, longer migration times were observed for $BMIMBF_4$ concentrations greater than 2 mM. This trend is observed by viewing the plot of capacity factor (k') of the least hydrophobic analyte acetophenone and the most hydrophobic analyte decanophenone versus the concentration of $BMIMBF_4$, as shown in Figure 3.6. The other ILs, $EMIMSO_3F_3$, and EMIMCl, were used as modifiers in the separation of alkyl aryl ketones, but the separation was not enhanced.

Hydrophobic solutes are known to partition between the aqueous phase and the polymeric pseudo-stationary phase in MEKC. Because of their hydrophobicity, the alkyl aryl ketones are retained in the polymeric pseudo-stationary phase more than the aqueous phase. Figure 3.7 shows that decanophenone interacts more with the polymeric pseudo-stationary phase. ILs are conductive thus, an addition of an ILs to a buffer may enhance its ionic strength under a controlled capillary temperature. This fact was confirmed by an increment in the current as the concentration of $BMIMBF_4$ was increased in the buffer as shown in Figure 3.8.

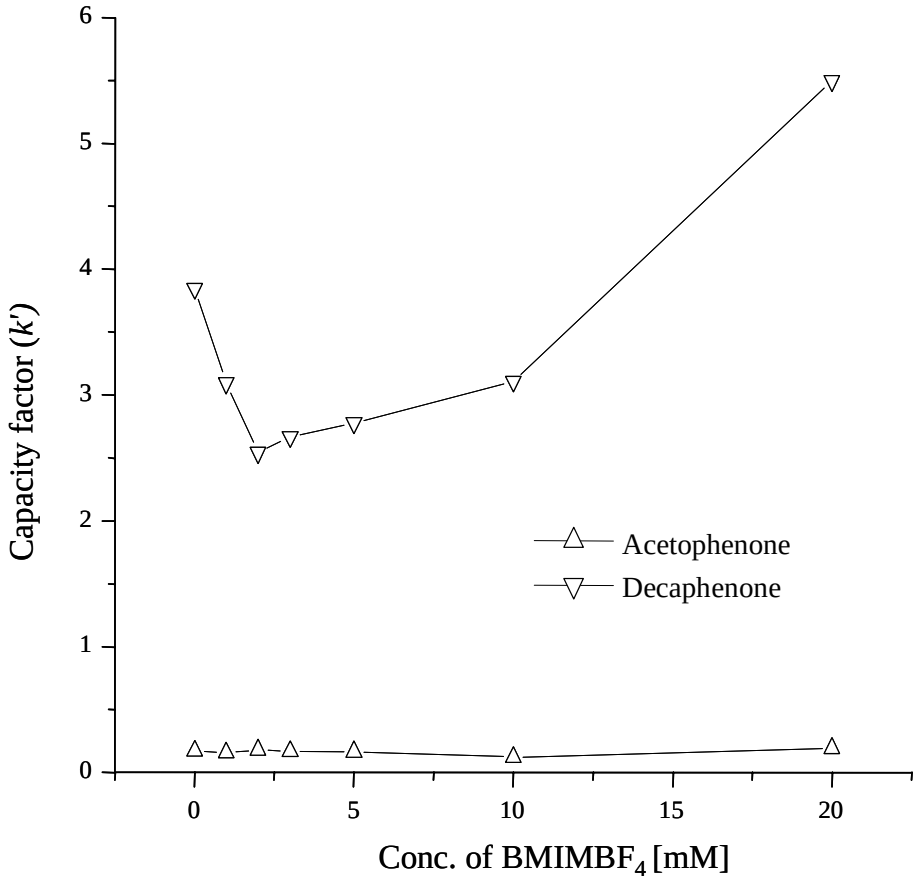


Figure 3.7 A plot of capacity factor of the least hydrophobic and first analyte to elute (acetophenone), and the most hydrophobic and last analyte to elute (decanophenone) versus the concentration of $BMIMBF_4$.

Other parameters held constant, an enhancement in the ionic strength or concentration of the background electrolyte is expected to cause the "value" of EOF to decrease and results in an increase in the migration time of the analytes being separated. However, in looking at Figure 3.8, an abnormality in the EOF versus $BMIMBF_4$ concentration plot is observed. Between 1 and 2 mM, the value of EOF increases with an increase in IL concentration. A plausible explanation given for this trend is that, when 1 to 2 mM of the IL were added to the buffer, we observed an increase in the value of EOF as a result of an enhancement in the current, as shown in Figure 3.8.

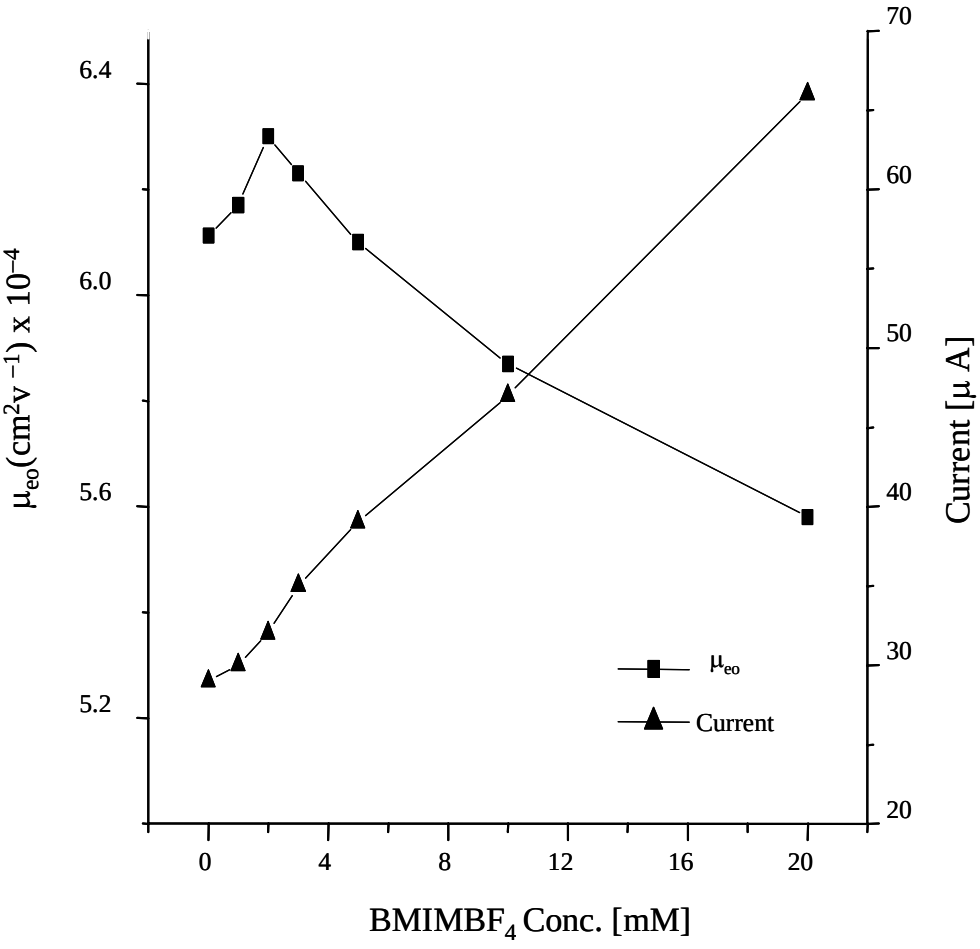


Figure 3.8 Effect of increasing 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), concentration on the eletroosmotic mobility (μ_{eo}) and the observed current for the separations in Figure 3.6.

It was assumed that low concentrations of the IL caused a compression of the mobile layer, making the value of EOF increase. However, the addition of 3 mM or more of the IL in the buffer led to a decrease in the value of EOF, although we observed an enhancement in current. This is because at higher ILs concentrations the (BMIM) cation may coat the capillary wall. Modification of the capillary wall by the IL cation may lead to a change in the EOF. Yanes et al. illustrated that when the IL was used as an electrolyte, it attached to the capillary wall, engendering anodic EOF.[26] In our case, the TRIS buffer was responsible for the cathodic EOF. The addition of 1 to 2 mM of $BMIMBF_4$ led to an increase in the current

and a slight increase in the EOF, which in turn led to shorter migration times and an enhancement in the peak efficiency, especially the last three analytes. At this low concentration of the IL, we suppose that the IL cation moves with the buffer rather than coating on the capillary wall. However, with the addition of 3 mM or higher of $BMIMBF_4$, a reasonable amount of coating of the cation on the capillary wall is possible, which causes a decrease in the EOF value and leads to longer migration times as well as poor peak efficiency of the more hydrophobic analytes, although we observed an enhancement in the current. ILs are complex solvent systems and the mechanism of separation when ILs are used as modifiers in MEKC is still not well understood. Figure 3.9 illustrates a schematic of a possible mechanism between the IL, polymeric surfactant, and analyte. This schematic was modified from reference 26. It should be noted that Figure 3.9 is not intended to imply any sort of ordering on the fused-silica surface.

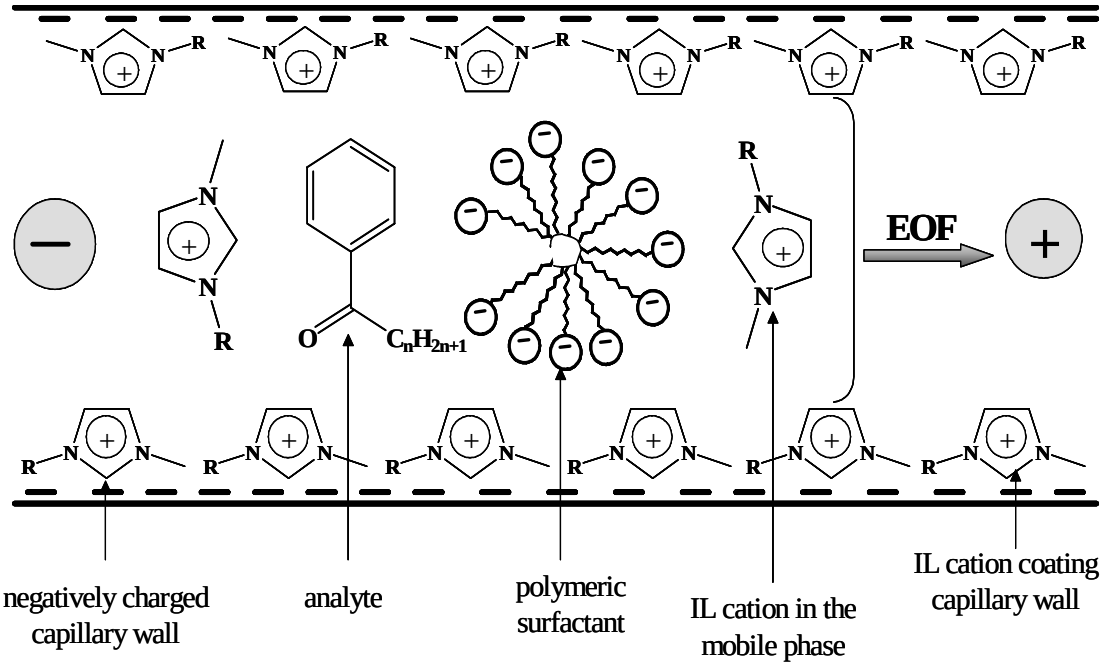


Figure 3.9 Schematic of a possible mechanism of alkyl aryl ketones separation using 1-alkyl-3-methylimidazolium-based ILs. Modified from reference 26.

As illustrated in Figure 3.9, the delay in the migration time and peak tailing of alky aryl ketones, above a concentration of 10 mM IL may be attributed to either the association of the ketones with the positively charged imidazolium cation coating the wall or with the free imidazolium ions in the bulk of the polymeric surfactant buffer solution. This association could in part be driven by hydrophobic, hydrogen bonding or ion-dipole/ion-induced-dipole interactions between the ketones, imidazolium cation, and polymeric surfactant. Further studies, involving separation of more hydrophobic analytes such as PAHs, and using fluorescence spectroscopy or other techniques are needed to achieve a better understanding of the interactions between ILs, polymeric surfactants, and the analytes of interest.

3.3.2 Separation of Phenols

Seven chlorophenols were separated using a plain buffer (i.e., 20 mM $Na_2B_4O_7$ / Na_2HPO_4, pH, 9.2), buffer + poly-SUS, and buffer + poly-SUS + ILs. The results recorded while using the plain buffer are shown in electropherogram (I) of Figures 3.10 (A, and B). Six out of seven of the analytes were well-resolved but peaks 4 and 5 (i.e. 2-chlorophenol and 2,4,6-trichlorophenol) coeluted. The separation of the phenols was possible even with plain buffer because the phenols are slightly charged at pH 9.2 used in this study.

Poly-SUS was then added to the buffer to enhance the separation of the phenols, and the corresponding data is shown in electropherogram (II) in Figure 3.10 (A, B). The presence of the surfactant increased the elution time of the analytes and gave a better resolution of the six analytes as compared to that achieved with the plain buffer. The ILs were used in order to improve the resolution of peaks 4 and 5. Of the five ILs investigated, only $BMIMBF_4$ and $EMIMPF_6$ enhanced the resolution of the peaks as shown in electropherogram (V), Figure 3.10 (A), and electropherogram (III), Figure 3.10 (B), respectively.

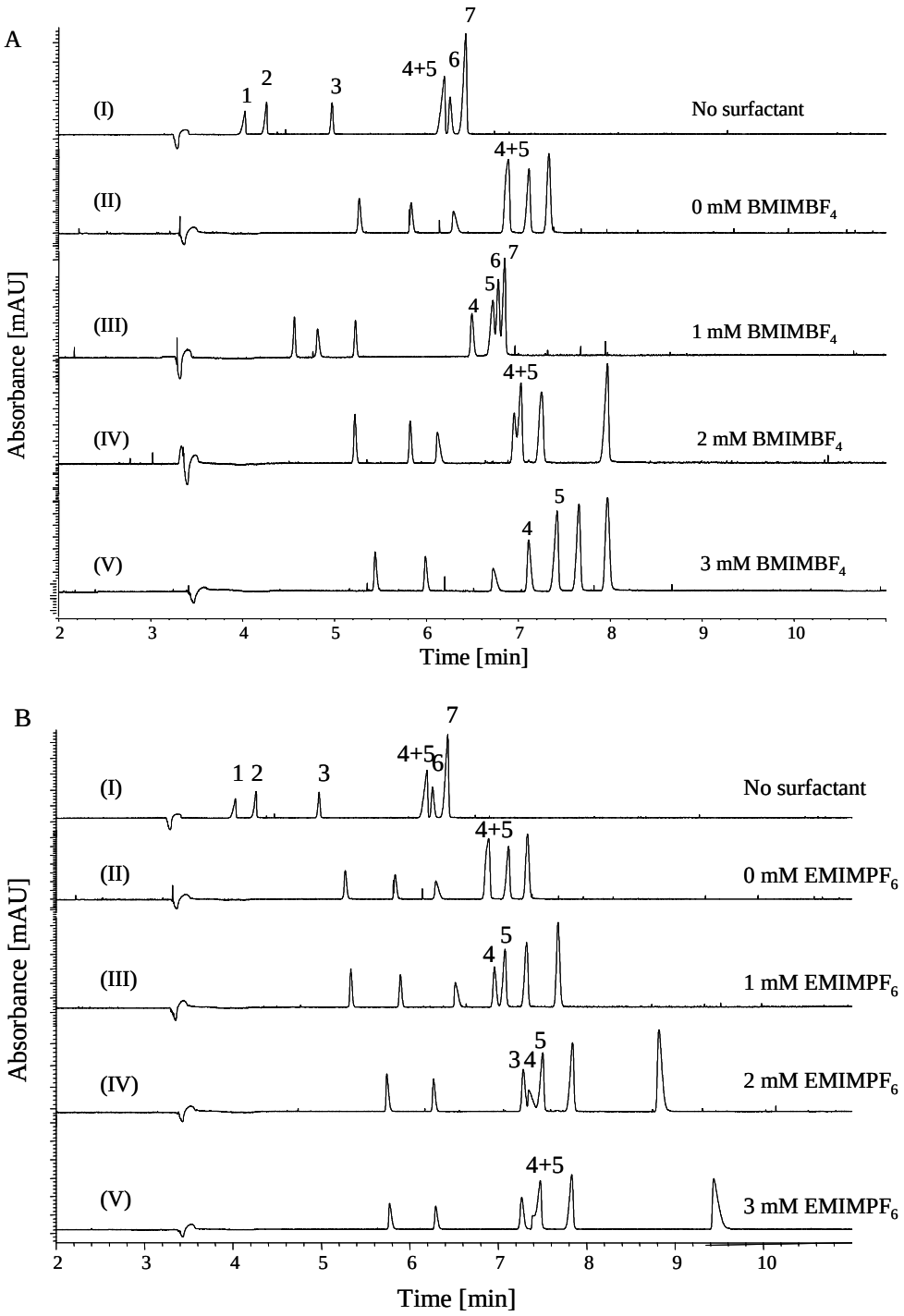


Figure 3.10 Electropherograms showing the separation of seven phenols. (A) (I) no surfactant, (II) 0.5 % poly-SUS, (III-VI) using 0.5% poly-SUS with 1, 2, 3, and 5 mM $BMIMBF_4$, respectively. (B) (I) no surfactant, (II) 0.5 % poly-SUS, (III–V) using 0.5% poly-SUS with 0.5, 2, and 5 mM $EMIMPF_6$, respectively. Conditions: 20 mM $Na_2B_4O_7$ and Na_2HPO_4; pH 9.2; voltage, 20 kV; temp., 20 °C; injection size, 30 mbar for 3 sec, detection λ: 254 nm.

A similar trend was observed for the migration times of the phenols as with the alkyl aryl ketones when the IL $BMIMBF_4$ was used as the modifier. When $EMIMBF_4$, $EMIMSO_3F_3$, and EMIMCl were used as modifiers, longer migration times of the analytes were recorded, although there were no significant changes in the EOF. In addition, the co-eluting peaks (2-chlorophenol and 2,4,6-trichlorophenol) were not resolved. Figure 3.11 shows the electropherograms obtained while using the IL $EMIMBF_4$.

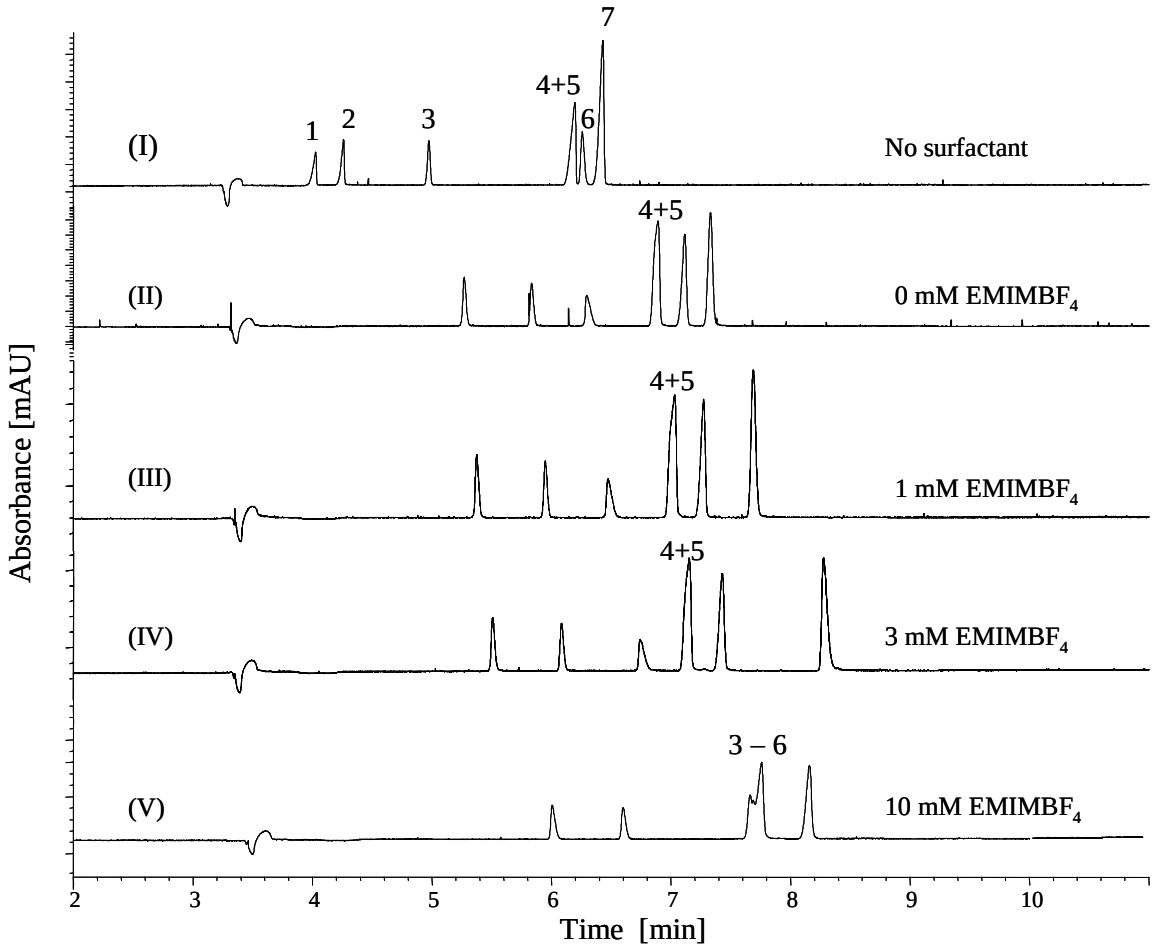


Figure 3.11 Electropherograms showing the separation of seven phenols. (I) no surfactant, (II) 0.5 % poly-SUS, (III-V) using 0.5% poly-SUS with 1, 3, and 10 mM $BMIMBF_4$, respectively. Conditions: 20 mM $Na_2B_4O_7$ and Na_2HPO_4; pH 9.2; voltage, 20 kV; temp., 20 °C; injection size, 30 mbar for 3 sec, detection λ: 254 nm.

3.4 Conclusions

Successful separations of two achiral mixtures have been achieved using ILs as pseudo-stationary phase modifiers. The results obtained were reproducible and the ILs were found to be stable in the background electrolyte solutions. The separation of the analyte mixtures was dependent on the interaction of the analytes with the polymeric surfactants, whereas the ILs influenced the analytes' elution time and peak efficiency. Longer migration times of the more hydrophobic analytes were recorded when high concentrations of the ILs were used as modifiers.

3.5 References

1. Freemantle, M. In *Chem. Eng. News*, **2000**; Vol. 37.
2. Osteryoung, R. A.; Mamantov, G., Marassi, R., Eds.; NATO ASI Ser., Ser. C., **1987**; Vol. 202, pp 329.
3. Fannin, A. A., Floreani, A. D., King, A. L., Landers, S. J., Piersma, J. B., Stech, J. D., Vaughn, L. R., Wilkes, S. J., William, L. J., *J. Phys. Chem.* **1984**, *88*.
4. Anderson, J. L., Ding, J., Welton, T., Armstrong, D. W., *J. Am. Chem. Soc.* **2002**, *124*, 14247.
5. Selvan, M. S., McKinley, M. D., Dubois, R. H., Atwood, J. L., *J. Chem. Eng. Data.* **2000**, *45*, 841.
6. Dai, S., Ju, Y. H., Barnes, C. E., *J. Chem. Soc., Dalton Trans.* **1999**, 1201.
7. Wilkes, J. S., Zaworotko, M. J., *J. Chem. Soc., Chem. Commun.* **1992**, 965.
8. Adams, C. J., Earle, M. J., Roberts, G., Seddon, K. R., *Chem. Commun.* **1998**, 2097.
9. Earle, M. J., McCormac, P. B., Seddon, K. R., *Chem. Commun.* **1998**, 2245.
10. Leadbeater, N. E., Torenius, H. M., **2002**, *67*, 3145.
11. Welton, T. *Chem. Rev.* **1999**, *99*, 2071-2083.
12. Nara, S. J., Harjani, J. R., Salunkhe, M. M., *Tetrahedron Lett.* **2002**, *43*, 2979.
13. Wilkes, J. S., Levisky, J. A., Wilson, R. A., Hussey, C. L., *Inorg. Chem.* **1982**, *21*, 1263.

14. Sanders, J. R., Ward, E. H., Hussey, C. L., *J. Electrochem. Soc.* **1986**, *133*, 325.

15. Dickinson, V. E., Willaims, M. E., Hendrickson, S. M., Masui, H., Murray, R. W., *J. Am. Chem. Soc.* **1999**, *121*, 613.

16. Hussey, C. L. *Pure Appl. Chem.* **1988**, *60*, 1763.

17. Seddon, K. R. *J. Chem. Technol. Biotechnol.* **1997**, *68*, 351.

18. Adams, C. J., Earle, M. J., Seddon, K. R., *Green Chem.* **2000**, 21.

19. Bowlas, C. J., Bruce, D. W., Seddon, K. R., *Chem. Commun.* **1996**, 1625.

20. Erbeldinger, M., Mesiano, A. J., Russell, A. J., *Biotechnol. Prog.* **2000**, *16*, 1129.

21. Mehnert, C. P., Cook, R. A., Dispenziere, N. C., Afeworki, M., *J. Am. Chem. Soc.;* . **2002**, *124*, 12932.

22. Armstrong, D. W., Zhang, L.-K., He, L., Gross, M. L., *Anal. Chem.* **2001**, *73*, 3679.

23. Huddleston, J. G., Willauer, H. D., Swatloski, R. P., Visser, A. E., Rogers, R. D., *Chem. Commun.* **1998**, 1765.

24. Dullius, J. E. L., Suarez, P. A. Z., Einloft, S., de Souza, R. F., Dupont, J., Fischer, J., DeCian, A., *Organometallics* **1998**, *17*, 815.

25. Yanes, E. G., Gratz, S. R., Baldwin, M. J., Robison, S. E., Stalcup, A. M., *Analyst.* **2000**, *125*, 1919.

26. Yanes, E. G., Gratz, S. R., Baldwin, M. J., Robison, S. E., Stalcup, A. M., *Anal. Chem.* **2001**, *73*, 3838.

27. Armstrong, D. W. H., L.; Liu, Y. -S., *Anal. Chem.* **1999**, *71*, 3873.

28. Fuller, J., Carlin, H.C., DeLong, D., Harwoth., *Chem. Commun.* **1994**, 299.

29. Holbrey, J. D., Seddon, K. R., *J. Chem. Soc. Dalton Trans.* **1999**, 2133.

30. Palmer, C. P. *Electrophoresis* **2002**, *23*, 3993.

31. Shamsi, S. A., Palmer, C. P., Warner, I. M., *Anal. Chem.* **2001**, *73*, 140A.

32. Michael, B., Kenndler, E.,,; *Electrophoresis* **2000**, *21*, 3160.

33. Dube, S., Smith, R. M., *Chromatographia* **2003**, *57*, 485.

34. Shamsi, S. A., Akbay, C., Warner, I. M., *Anal. Chem.* **1998**, *70*, 3078.

35. Bergstrom, S. Z. *Physiol. Chem.* **1936**, *236*, 163.

36. Kelly, K. A., Burns, S. T., Khaledi, M. G., *Anal. Chem.* **2001**, *73*, 6057.

37. Terabe, S., Ostuka, K., Ichikawa, K., Ando, T., *Anal. Chem.* **1984**, *56*, 111.

Chapter 4.

A Comparison of Ionic Liquids to Organic Solvents for Chiral Separations in Micellar Electrokinetic Chromatography

4.1 Introduction

There is an increased interest in ionic liquids (ILs) as reflected by articles in the scientific and popular press.[1-5] This great interest in ILs is generating novel solutions and provoking changes in industrial and commercial practice based on green chemistry (environmentally-friendly) principles.[6, 7] Although the fundamental concepts behind both green chemistry and ILs are not new, chemical research involving environmentally benign processes is vital to encourage scientists to reevaluate their perspective on chemical synthesis and production. A major concern is to shift to processes employing cleaner solvents and thus, reduce the reliance on hazardous volatile organic solvents (VOCs). Therefore, the increased interest in ILs as solvents for chemical reactions, such as organic synthesis, catalysis, electrochemistry, and separations has been stimulated by the potential benefits that are offered by ILs as non-volatile replacements for VOCs. In addition, the above-mentioned processes utilize properties of ILs to enhance the performance of the system, for example reduce catalyst leaching, achieve better extractions and reactivity, and improve separations.[7]

An IL is a liquid composed entirely of ions that together melt below 100 °C and are thought to resemble ionic molten salts such as NaCl. Although ILs and molten salts are composed of ions, the presence of organic cations in ILs interrupts the crystal packing and lowers the melting point. ILs have a negligible vapor pressure, a wide liquid range, are non-flammable, and good solvents for a wide range of both inorganic and organic compounds.

The application of ILs for separations was recognized two decades ago by researchers using alkylammonium salts with weak nucleophilic anions as the mobile phase in liquid

chromatography,[8, 9] stationary phase in gas chromatography,[10, 11] and low-melting point tetra-*N*-butylphosphonium salts as the stationary phase in gas-liquid chromatography.[12] Several researchers have recently reported the use of ILs in chromatographic separations.[13-23] Stalcup and co-workers reported the first use of ILs as the background electrolyte in capillary electrophoresis (CE).[15, 16] The use of 1,3-dialkylimidazolium-based ILs as eluent in liquid chromatography,[17] as stationary phase in gas-liquid chromatography,[18] as coating in aqueous CE,[19, 20] as electrolytes in non-aqueous CE,[21, 22] and as modifiers in micellar electrokinetic chromatography (MEKC)[23] have also been reported.

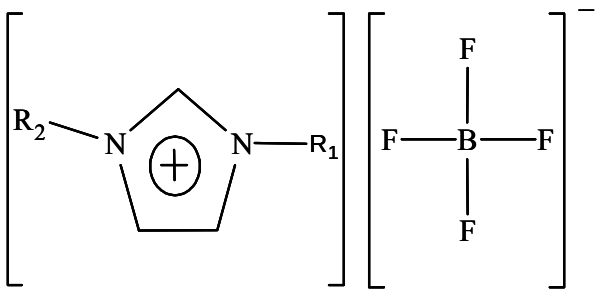
In a previous report, a briefly description on the effect of different ILs on the enantiomeric separation of binaphthyl derivatives was presented.[23] The binaphthyl derivatives (±)-1,1'-bi-2-naphthyl-2,2'-diyl hydrogen phosphate (BNP), 1,1'-bi-2-naphthyl-2,2'-diamine (BNA), and (±)-1,1'-bi-2-naphthol (BOH) belong to a class of compounds that are chiral because they posses an adjacent π system that cannot adopt a coplanar configuration due to steric hindrance and rotational restrictions around a central bond. The chirality of these atropisomers is a result of an asymmetrical plane as opposed to a stereogenic carbon. These analytes were separated in our laboratory using different chiral surfactants.[24, 25] Although the individual enantiomers are easy to separate, enantiomers of BNA and BOH co-elute when separating the mixture. To achieve baseline separation of the four co-eluted peaks, one can increase the surfactant concentration or use either organic solvents or ionic liquids as mobile phase modifiers.

The main role of organic solvents in MEKC is to reduce the capacity factor of the highly hydrophobic analytes. In most cases, the addition of organic modifiers leads to a reduction in the electroosmotic flow (EOF), a change in velocity of the pseudo-stationary

phase, and an increase in the size of the elution window. Besides ILs, other modifiers such as urea and glucose have been applied in MEKC. Urea is said to increase the separation window as well as the solubility of highly hydrophobic solutes in the mobile phase. Glucose, when added to the mobile phase, increases the resolution.[26]

Other chiral analytes investigated in the current study are warfarin, coumachlor, benzoin, benzoin methyl ether (BME) and benzoin ethyl ether (BEE). Warfarin is an anticoagulant drug frequently used in the treatment of thrombeolic diseases.[27] Although it is sold as a racemic mixture, the (S)-(−) enantiomer is more pharmacologically active than its corresponding (R)-(+) form.[27] Coumachlor, an analog of warfarin, has been used in HPLC as an internal standard. Quantitative and qualitative analysis using both drugs have been documented by HPLC and GC.[28, 29] However, adequate separation and quantitation has yet to be reported in CE. Benzoin and its derivatives are used in the development of antiseptic, astringent and anti-inflammatory drugs.

This study compared the effects of select 1,3-dialkylimidazolium tetrafluoroborate ILs with select organic solvents as mobile phase modifiers for the separation of chiral analytes in aqueous buffer in MEKC. The chiral polymeric surfactant, poly-sodium oleyl-L-leucyl-valinate (poly-L-SOLV) was used as the pseudo-stationary phase. The ILs (molecular structures shown in Figure 4.1) were 1-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$), 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), 1-hexyl-3-methylimidazolium tetrafluoroborate ($HMIMBF_4$), and 1-methyl-3-octylimidazolium tetrafluoroborate ($OMIMBF_4$). The significant properties of these ILs: good conductivity, hydrophobicity, and good solubility in aqueous solutions, were considered favorable for the separations investigated here.



(1) $R_1 = C_2H_5$, $R_2 = CH_3$ EMIMBF$_4$

(2) $R_1 = C_4H_9$, $R_2 = CH_3$ BMIMBF$_4$

(3) $R_1 = C_6H_{13}$, $R_2 = CH_3$ HMIMBF$_4$

(4) $R_1 = C_8H_{17}$ $R_2 = CH_3$ OMIMBF$_4$

Figure 4.1 Chemical structures of the ionic liquids (ILs) used as modifiers in the MEKC separation experiments.

The high solubility of the ILs in aqueous solutions is attributed to the tetrafluoroborate anion.[7] The ILs were shown to assist in the separation of hydrophobic chiral analyte mixtures while maintaining adequate background current. On the other hand, the organic solvents: methanol (MeOH), 1-propanol (1PrOH), and acetonitrile (ACN) led to longer migration times and lower background current. High volumes of organic solvents (above 60% vol/vol) in the buffer led to current breakdowns. A comparison was made between the use of 1,3-dialkylimidazolium-based ILs and the selected organic solvents. ILs were shown to be more advantageous than organic solvents for these separations.

4.2 Experimental Methods

4.2.1 Materials

The chiral compounds BNP, BNA, BOH, warfarin, coumachlor, benzoin, BME, and BEE were purchased from Sigma-Aldrich (Milwaukee, WI). The molecular structures of all analytes are illustrated in Figure 4.2.

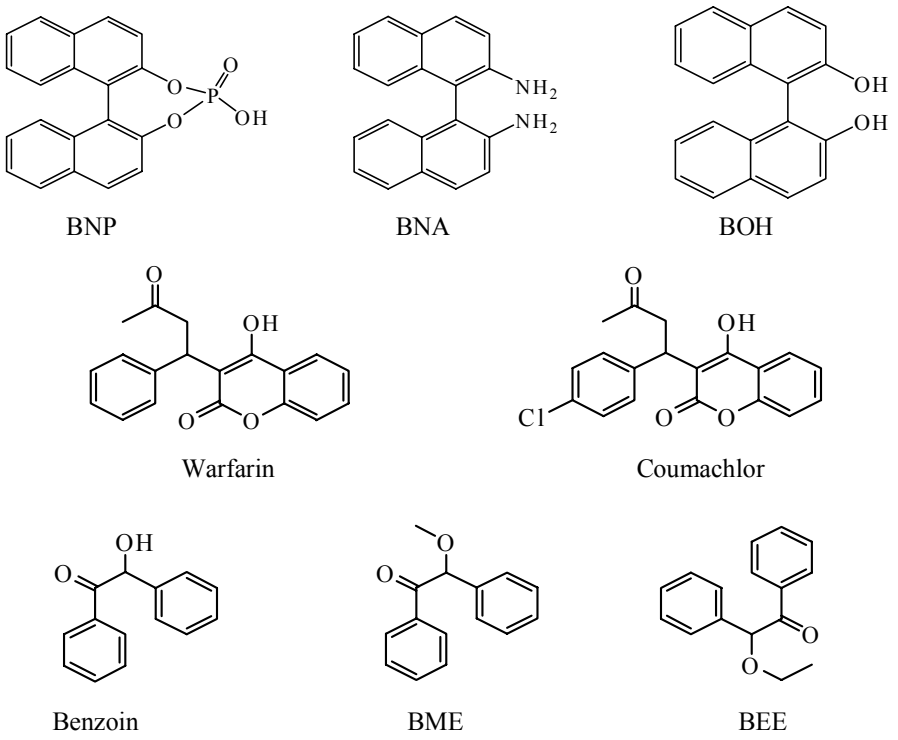


Figure 4.2 Chemical structures of the chiral analytes used in the current study.

The oleic acid-N-hydroxysuccinimide ester, sodium hydrogen carbonate, tris(hydroxymethyl)aminomethane (TRIS), tetrahydrofuran (THF), $EMIMBF_4$, $HMIMBF_4$, and $OMIMBF_4$ were also obtained from Sigma-Aldrich. Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), and sodium borate (NaB_4O_7) were from Amresco (Solon, OH), Mallinckrodt and Baker, Inc. (Paris, KY), and Fisher Scientific (Fair Lawn, NJ), respectively. The organic solvents MeOH, 1PrOH, and ACN were also purchased

from Fisher Scientific. $BMIMBF_4$ was purchased from Chemada Fine Chemicals Ltd., (Nir Itzhak, Israel). The dipeptide leucine-valine was purchased from Bachem Bioscience Inc. (King of Prussia, PA). All the reagents were of analytical reagent grade and used as received.

4.2.2 Synthesis of Poly-L-SOLV

The polymer poly-L-SOLV was synthesized using the modified procedure of Wang and Warner.[30, 31] The synthesis procedure and a schematic of the synthesis scheme of this polymer were recorded in chapter 2. All polymers used in this study were found to be greater than 99% pure as estimated by elemental analysis.

4.2.3 Preparation of MEKC buffer solutions

The background electrolyte (BGE) for separation of the binaphthyl derivatives was 100 mM TRIS mixed with 10 mM sodium borate at pH 10.0 while the BGE for the separation of warfarin, coumachlor, benzoin, BME and BEE was 30 mM sodium monophosphate + 20 mM sodium diphosphate at pH 7.2. The polymeric surfactant, in the concentration range of 0.5% to 1.5% wt/vol, was added to the BGE and the solution was filtered through a 0.45 μm membrane. IL or organic solvent was then added as a mobile phase modifier. The resulting solution was used as the mobile phase in CE separations in order to asses the effects of various modifiers on the separation efficiency and resolution of the analytes.

4.2.4 Capillary Electrophoresis

The MEKC experiments were performed on a Hewlett-Packard 3D CE instrument (Foster City, CA) equipped with UV diode array detector. Bare fused silica capillaries, cut to 60 cm long (52 cm effective length, 50 μm i.d.), were purchased from Polymicro Technologies (Phoenix, AZ). The applied voltage was 30 kV, detection wavelength was 254

nm, and the temperature of the capillary was maintained at 15 °C by the instrument thermostating system. The analytes were prepared in 50:50 methanol/water at concentrations of 0.1 to 0.5 mg/mL depending on the analyte. The samples were introduced into the capillary by hydrodynamic injection at a pressure of 30 mbar for 3 s. Prior to use, each new capillary was conditioned for 60 min with 1 M NaOH followed by a 15 min rinse with triply-distilled deionized water. Before each run, the capillary was flushed with MEKC buffer for 3 min to condition and fill the capillary. When comparing the effect of one IL to another, a new capillary was used in order to eliminate any influence of the previous IL cations. Elution orders were determined by spiking a single pure S-(–) enantiomer into the solution of the corresponding racemic analyte. The resolution (R_s) was calculated using the Equation 4.1.[32]

$$R_s = 2\frac{(t_{r_2} - t_{r_1})}{(w_1 + w_2)} \qquad 4.1$$

where t_{r_1} and t_{r_2} are migration times and w_1 and w_2 are the baseline peak widths for the first and second eluting enantiomer, respectively. The relative standard deviation (RSD) values were calculated using equation 2.[33]

$$RSD = \frac{s}{\bar{x}} \qquad 4.2$$

where s is the standard deviation and $\bar{x}$ is the mean (average) measurement.

4.3 Results and Discussions

4.3.1 Effect of ILs on the Separation of Binaphthyl Derivatives

This group of compounds has a varying degree of hydrophobicity and charge states under the experimental conditions used in this work. Using the polymeric surfactant poly-L-SOLV in a 100 mM TRIS + 10 mM sodium borate buffer, the R-(+) and S-(–) enantiomers

of each binaphthyl derivative could only be resolved individually, not in a mixture as shown in Figure 4.3 electropherogram (I).

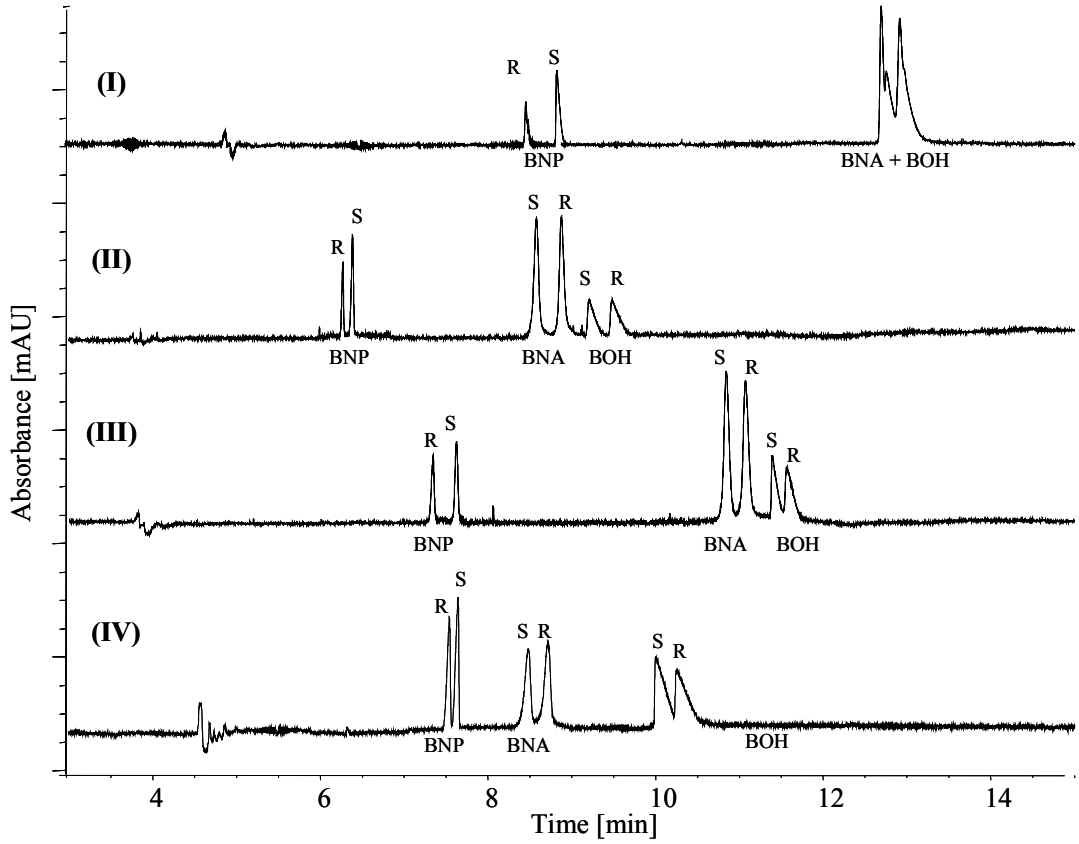


Figure 4.3 Electropherograms showing the separation of the three binaphthyl derivatives BNP, BNA, and BOH using (I) no mobile phase modifier, (II) 3 mM $EMIMBF_4$, (III) 1 mM $BMIMBF_4$, and (IV) 1 mM $HMIMBF_4$ as modifiers respectively. Conditions: 0.5% poly-L-SOLV, 100 mM TRIS + 10 mM sodium borate buffer, pH: 10.0, injection size: 30 mbar for 3 s, temp: 15 ^{o}C, voltage: 30 kV, detectionλ: 254 nm.

We investigated the effect of using several different 1,3-dialkylimidazolium tetrafluoroborate ILs as mobile phase modifiers in the separation of a mixture of enantiomers of the three analytes. A concentration study was done using 0.02% to 0.1% vol/vol of each IL, and a plot of resolution of each analyte versus the IL concentration was generated. Figure 4.4 shows a plot of resolution of the three analytes versus $EMIMBF_4$ concentration and is a representation

of all plots obtained. A slight decrease in the resolution was observed for BNP with increasing concentration of each IL used as a modifier.

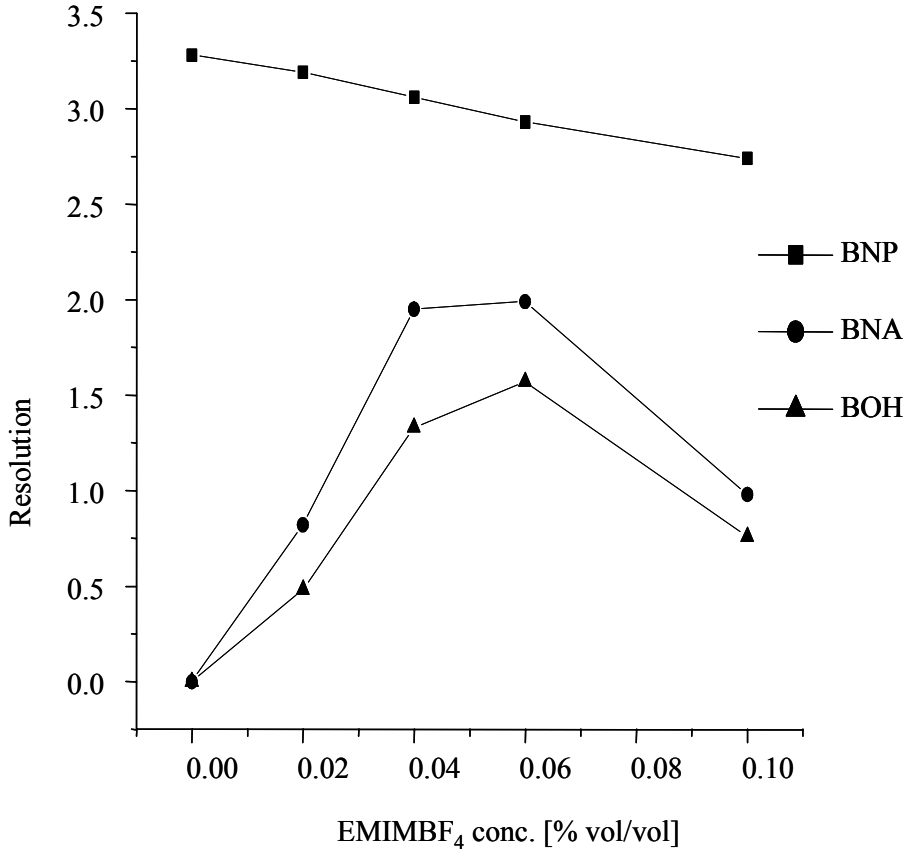


Figure 4.4 Plot of resolution of BNP (–■–), BNA (–●–), and BOH (–▲–) versus $EMIMBF_4$ concentration.

The resolution of BNA and BOH was enhanced with an increase in the IL concentration up to an optimum value based on the particular IL used; however, for concentrations greater than optimum a gradual drop in the resolution was observed. The use of ILs as mobile phase modifiers led to better selectivity values between BNA and BOH with selectivity values increasing as IL concentration was increased from 0.02% to 0.1% vol/vol. Generally, IL concentrations greater than 0.2% vol/vol led to a decrease in the resolution of each analyte but an enhancement of selectivity.

Electropherograms II–IV in Figure 4.3 illustrates the optimum separation of the mixture of the binaphthyl derivatives with the addition of optimum concentrations of $EMIMBF_4$, $BMIMBF_4$, and $HMIMBF_4$, respectively. The R-(+) enantiomer of BNP eluted faster than the S-(–) enantiomer. However, for BNA and BOH the S-(–) enantiomer eluted first. This indicates that the S-(–) enantiomer of BNA and BOH interacted less with the chiral surfactant as compared to the R-(+) enantiomer of the same compounds.

In addition, Figure 4.5 illustrates the enhancement in current observed as the concentration of IL increased.

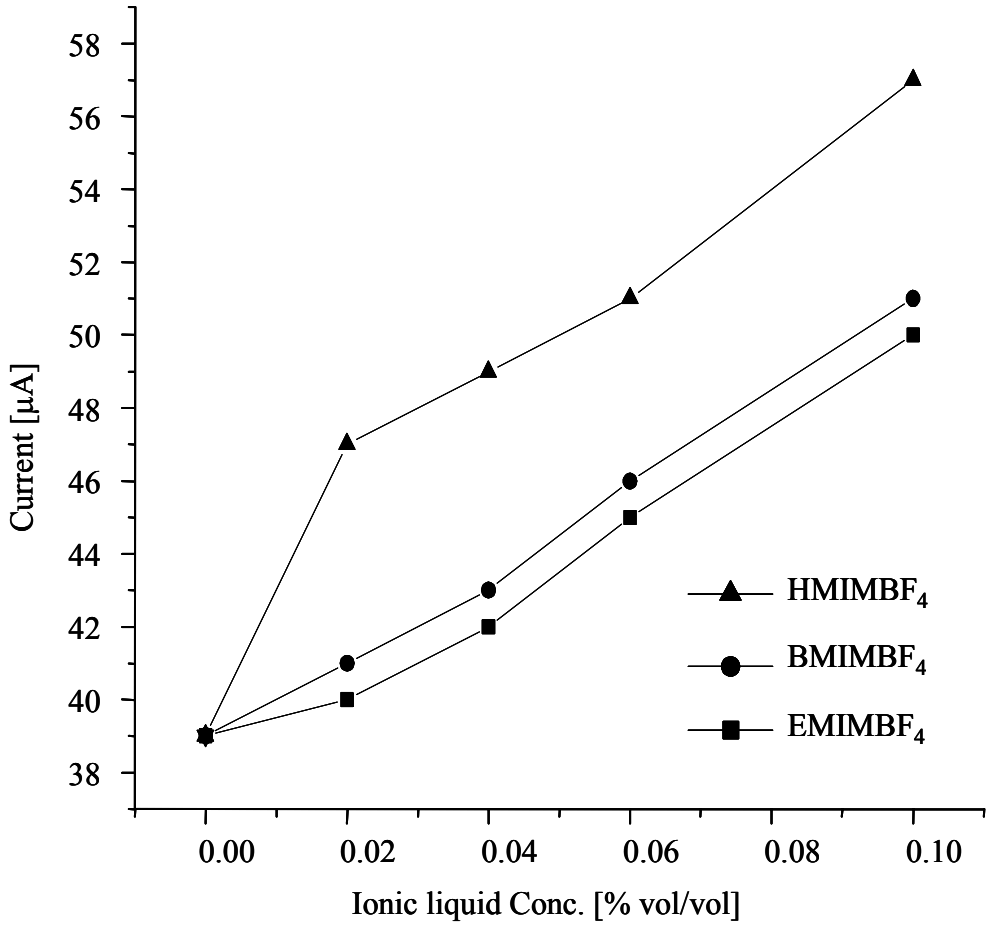


Figure 4.5 Plot of observed current versus concentration of $EMIMBF_4$ (–■–), $BMIMBF_4$ (–●–), and $HMIMBF_4$ (–▲–).

This enhancement was due to the conductive nature of the ILs. Moreover, a slight increase in the EOF was observed when 0.02% to 0.06% vol/vol of IL was added, but above this concentration a gradual drop in the EOF was observed. As was reported in chapter 3, this observance could be due to the ability of the imidazolium-based cation to coat the walls of the capillary for sufficiently high IL concentrations.[23] Alternatively, the IL cation may replace the sodium counterion within the electric double layer. If the capillary surface is modified by IL, the EOF and elution time of the analytes could be altered.

Of the three ILs presented here, 3 mM $EMIMBF_4$ provided the best resolution as well as the shortest elution time as shown in electropherogram (II) Figure 4.3. The optimum concentration of $BMIMBF_4$ and $HMIMBF_4$ was 0.02% vol/vol. The increase in hydrophobicity, viscosity, and conductivity of $BMIMBF_4$ or $HMIMBF_4$ relative to $EMIMBF_4$ could account for the lower concentration required to achieve similar results. The other IL investigated, $OMIMBF_4$, caused peak splitting of BNP. In addition, it resolved the co-eluted peaks of BNA and BOH with partial separation of enantiomers of BNA and poor enantiomeric separation of BOH, as illustrated in Figure 4.6. An increase in the concentration of $OMIMBF_4$ from 0.02% to 0.1% vovl/vol led to the improvement in the peak shape and resolution of BNP. However, peak splitting was observed for BNA and resolution was lost for BOH when 0.1% vol/vol of $OMIMBF_4$ was used. This IL had the same effects on the EOF and current as the other three IL discussed above. In addition, it had the highest hydrophobicity and viscosity. Therefore, these properties may have led to unfavorable interactions between the chiral analytes and chiral surfactant, thus, preventing baseline enantiomeric separation or causing peak splitting.

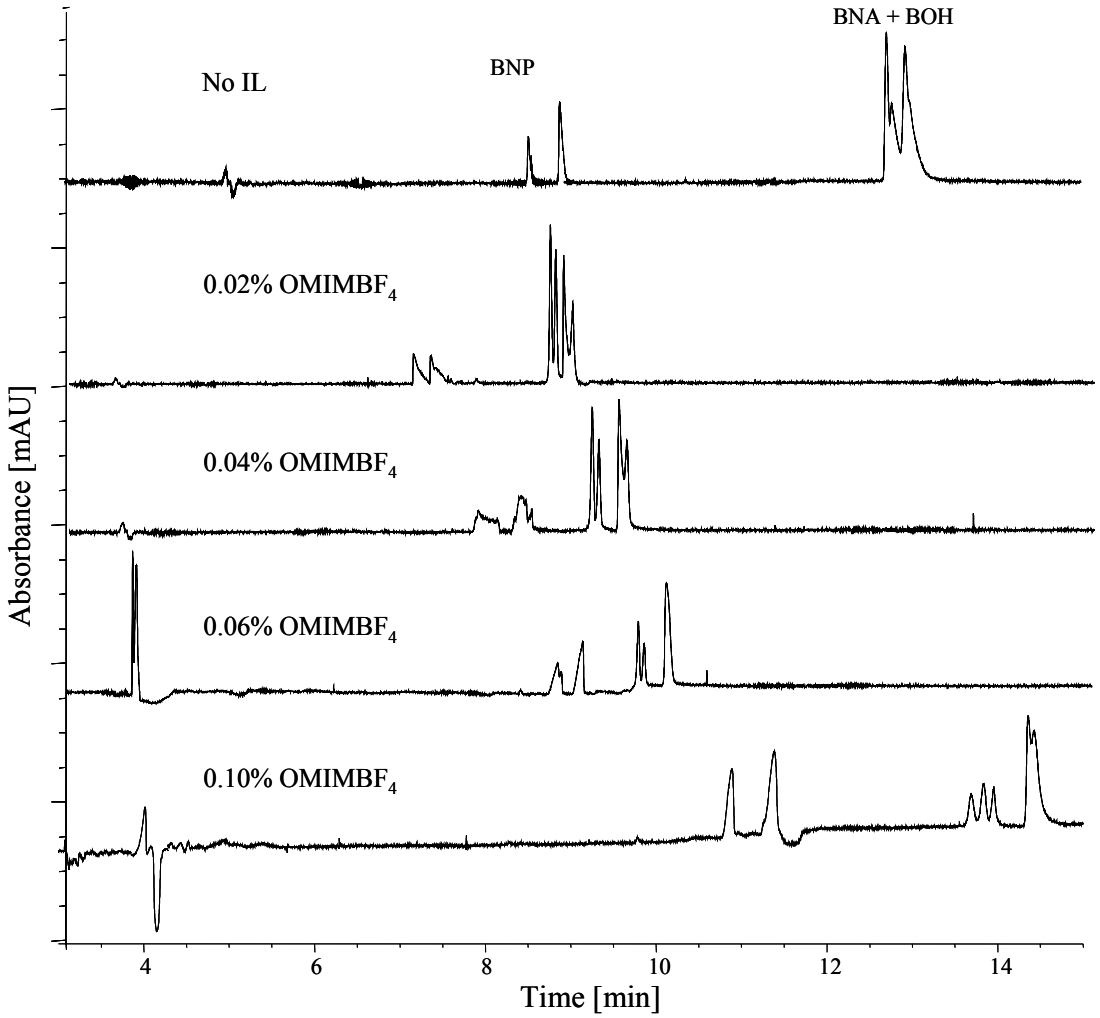


Figure 4.6 Electropherograms showing the separation of three binaphthyl derivatives BNP, BNA, and BOH using no IL modifier, 0.02, 0.04, 0.06, and 0.1 % vol/vol $OMIMBF_4$ as a mobile phase modifier. Conditions: same as Figure 4.3.

4.3.2 Effect of Organic Modifiers on the Separation of Binaphthyl Derivatives

The effect of using organic solvents (MeOH, 1PrOH, or ACN) as mobile phase modifiers in the separation of the three binaphthyl derivatives was also investigated. In order to determine the optimum concentration of each organic solvent, a concentration study was performed using 10, 20, 30 and 50% vol/vol organic solvent/buffer. The optimum concentration of organic solvent was found to be 20% for MeOH and 1PrOH and 10% for ACN (Figure 4.7). MeOH provided the best separation of the three analytes in terms of

resolution and selectivity. However, the addition of ACN provided the best peak efficiencies as well as shortest elution time.

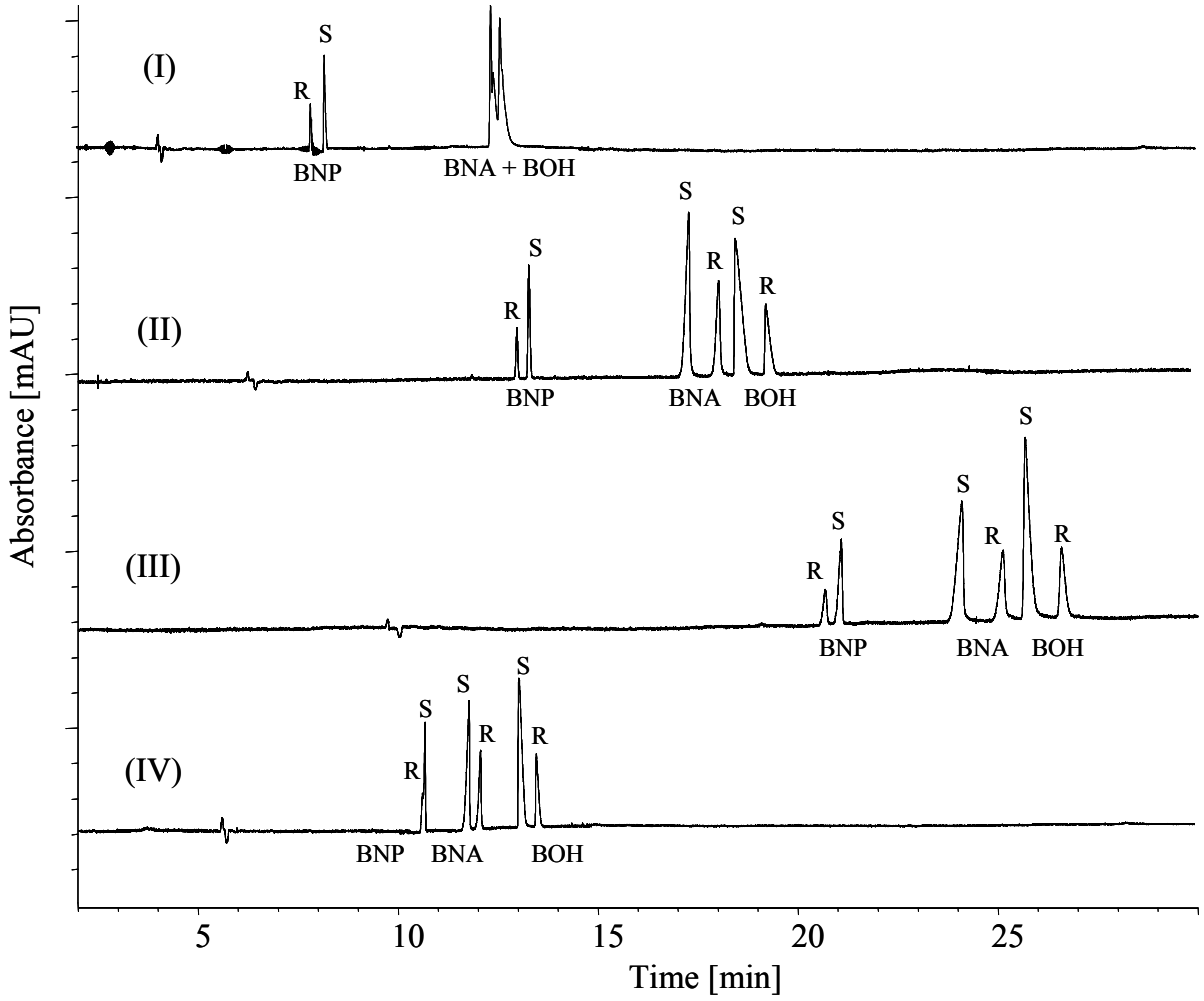


Figure 4.7 Electropherograms showing the separation of three binaphthyl derivatives BNP, BNA, and BOH using (I) no mobile phase modifier, (II) 20% MeOH, (III) 20% 1PrOH, and (IV) 10% ACN as modifiers respectively. Conditions: same as Figure 4.3.

Figure 4.8 is a representation of the effect of the organic solvent concentration on the resolution of the three binaphthly derivatives. Generally, there was a drastic drop in the resolution of BNP but still baseline separation was obtained. Conversely, the resolution of BNA and BOH was slightly enhanced, but after the optimum concentration was surpassed the resolution of these two analytes began to decline. It should be noted that the selectivity

between BNA and BOH was greatly enhanced as the concentration of MeOH, 1PrOH or ACN increased.

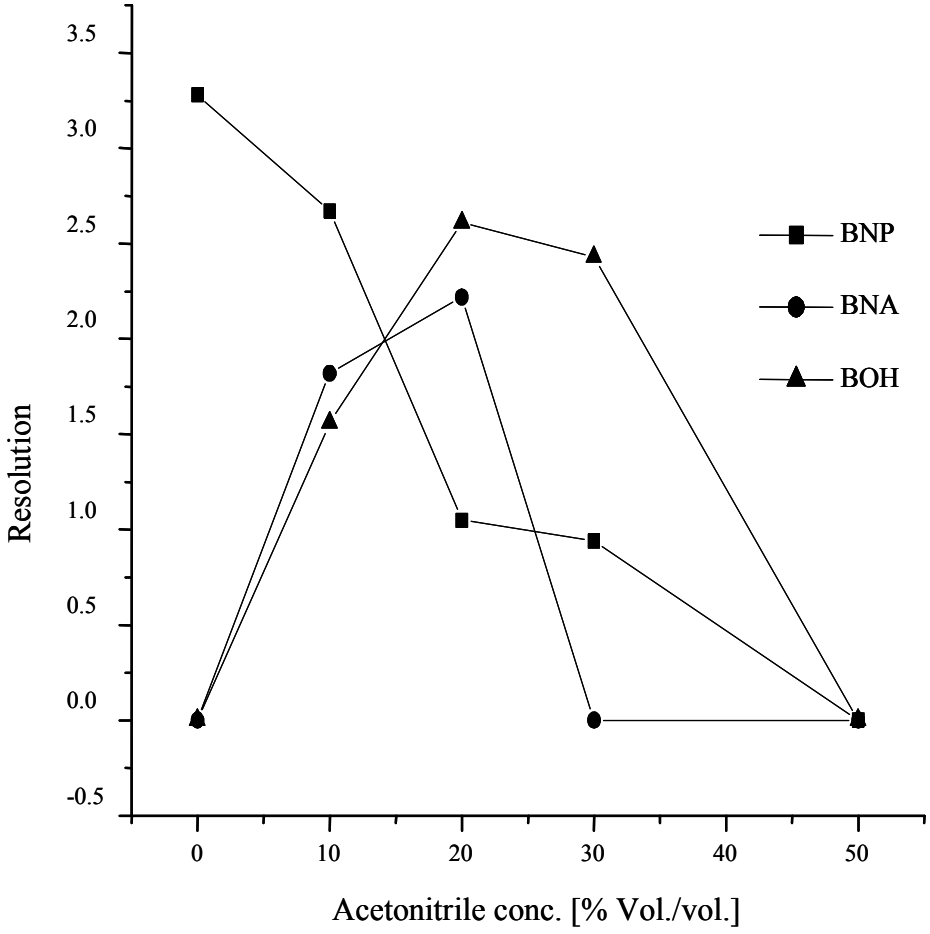


Figure 4.8 Plot of resolution of BNP (–■–), BNA (–●–), and BOH (–▲–) versus ACN concentration.

In addition, increasing the concentration of organic solvent caused a decline in the observed current (Figure 4.9) leading to an increase in the elution time of the three binaphthyl derivatives. The decrease in current is likely due to a reduction in dielectric constant caused by the organic solvents. A summary of the physical properties of the four ILs included in this study, as well as MeOH, 1PrOH, and ACN, are provided in Table 4.1.

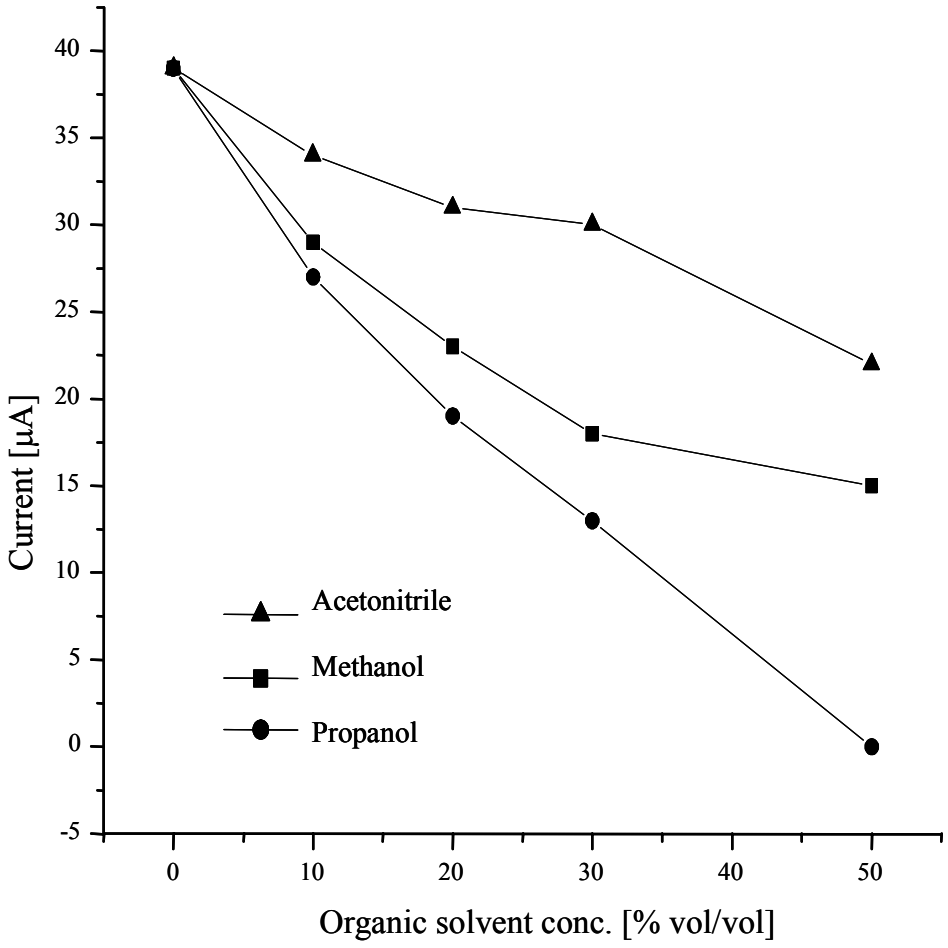


Figure 4.9 Plot of observed current versus concentration of MeOH (–■–) 1PrOH (–●–), and ACN (–▲–).

MeOH, with a moderate dielectric constant (≈ 33 at 20 °C) and the capability of undergoing autoprotolysis, is similar to water. It is a neutral amphiprotic solvent in which solvation is favored due to the formation of hydrogen bonds.[34] 1PrOH is a polar, mobile, water-soluble solvent with medium volatility and a dielectric constant of ≈ 21 at 20 °C. Because of a strong dipole moment, the dielectric constant of ACN (≈ 37 at 20 °C) is higher than that of MeOH and 1PrOH. ACN, a dipolar aprotic solvent with a weak autoprotolysis constant is also very different from MeOH and 1PrOH. The separation results obtained using these organic solvents were consistent with the above-mentioned properties.

Table 4.1 Select physical properties of the ILs and organic solvents used. All organic solvent data was obtained from reference 36. The IL data was obtained from material safety data sheets. na[1] = not available

Compound	Molecular Mass (g/mol)	Density (g/mL)	Melting Point (°C)	Dielectric Constant at 20 °C	Water Solubility
$EMIMBF_4$	198	1.29	15	na[1]	Miscible
$BMIMBF_4$	226	1.22	-71	na	Miscible
$HMIMBF_4$	254	1.15	-82	na	Miscible
$OMIMBF_4$	282	1.12	na	na	Miscible
MeOH	32	0.791	-97.6	33.0	Miscible
1PrOH	60	0.803	-126.1	20.8	Miscible
ACN	41	0.796	-44	36.64	Miscible

When comparing the organic solvents with the ILs as mobile phase modifiers for the separation of the binaphthyl analytes, the elution order of the R-(+) and S-(–) enantiomers remained the same. The ILs led to an enhancement in current, a slight increase in the EOF and a shorter analysis time. The resolution and selectivity of the analytes was dependent on the concentration and type of IL or organic solvents used. However, smaller IL volumes were needed, compared to organic solvents, to achieve equivalent resolution and selectivity. Table 4.2 shows the elution times of the first enantiomer and second enantiomer, t_{r_1} and t_{r_2}, respectively, as well as resolution (R_S), for BNP, BNA, and BOH at the optimum concentration for each mobile phase modifier.

Table 4.2 Elution times of the first enantiomer (t_{r_1}), second enantiomer (t_{r_2}), resolution (R_s) and resolution's RSD of BNP, BNA, and BOH at the optimum concentration (used in Figure 4.3 and 4.7) of each mobile phase modifier.

Modifier at optimum concentration	BNP				BNA				BOH			
	t_{r_1}	t_{r_2}	R_s	RSD	t_{r_1}	t_{r_2}	R_s	RSD	t_{r_1}	t_{r_2}	R_s	RSD
$EMIMBF_4$	6.31	6.46	3.01	0.03	8.99	9.18	2.20	0.03	9.55	9.68	1.18	0.07
$BMIMBF_4$	7.34	7.61	3.26	0.02	10.84	11.07	1.72	0.03	11.38	11.56	1.13	0.04
$HMIMBF_4$	6.89	7.04	1.61	0.08	7.91	8.17	1.53	0.06	9.55	9.68	1.06	0.09
MeOH	12.98	13.28	3.07	0.03	17.28	18.03	3.72	0.08	18.43	19.20	2.68	0.06
1PrOH	20.48	20.88	2.04	0.008	23.79	24.77	3.13	0.08	25.24	26.10	3.13	0.01
ACN	9.37	9.58	2.66	0.02	14.36	14.80	1.81	0.09	15.11	15.54	1.16	0.02

4.3.3 Effects of Ionic Liquids on the Separation of Coumarin Derivatives

Warfarin and coumachlor are both coumarin derivatives. These two structurally related acidic drugs differ only by a chlorine atom on the coumachlor compound (Figure 4.2). $EMIMBF_4$ or $BMIMBF_4$ was added to the buffer, and its effect on warfarin and coumachlor separation was investigated. With the exception of elution time, both ILs gave similar results. The addition of $BMIMBF_4$ resulted in shorter elution times. Figure 4.10 shows the effect of $BMIMBF_4$ concentration on the separation of warfarin. As with the binaphthyl derivatives, increasing the IL concentration enhanced the EOF and led either to shorter elution times or reduced resolution.

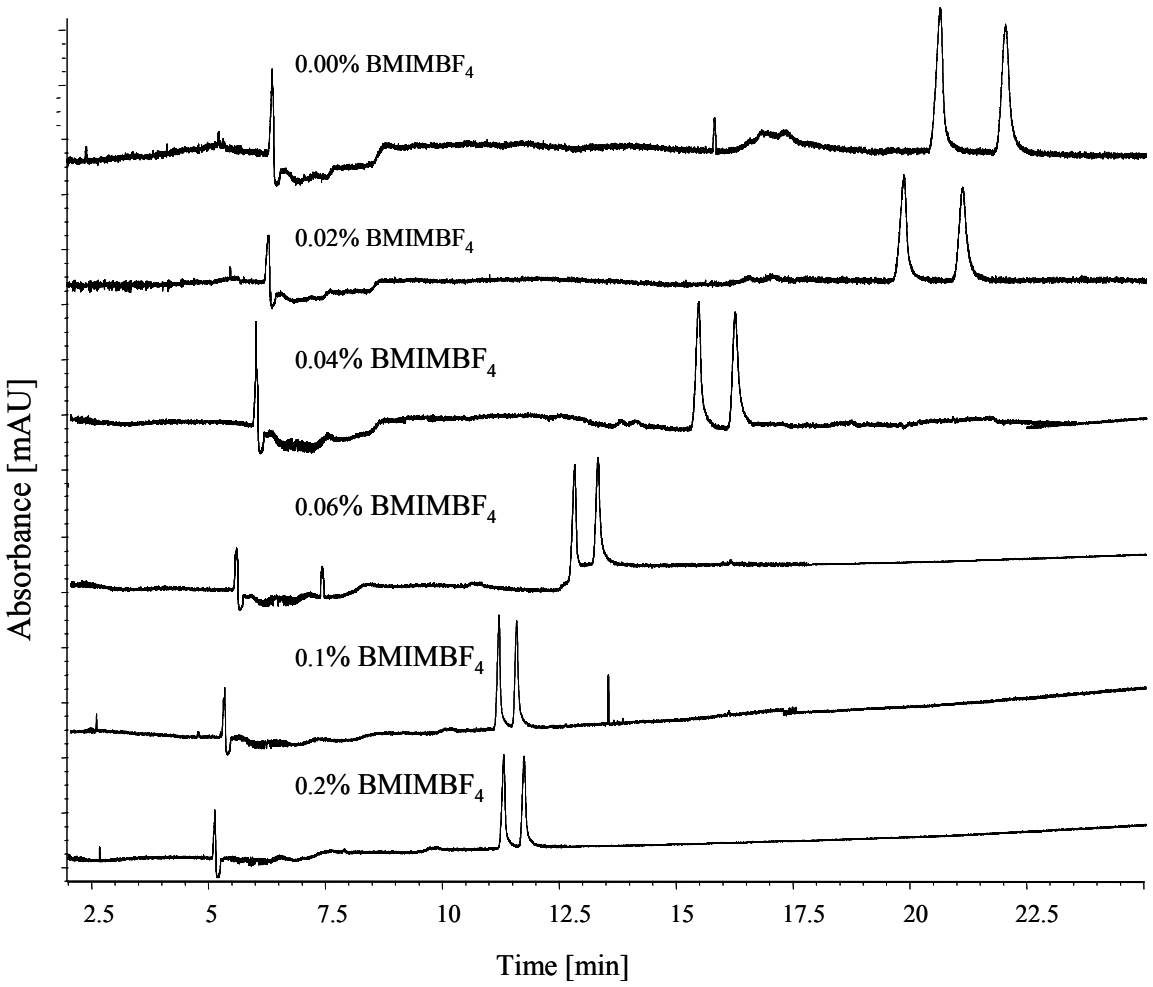


Figure 4.10 Electropherograms showing the separation of warfarin using 0.0, 0.02, 0.04, 0.06, 0.1, and 0.2% vol/vol $BMIMBF_4$ as a mobile phase modifier respectively. Conditions: 1% poly-L-SOLV, 30 mM NaH_2PO_4 + 20 mM Na_2HPO_4 buffer, pH: 7.2, injection size: 30 mbar for 3 s, temp: 15 °C, voltage: 30 kV, detection λ: 254.

Despite a reduction in resolution, baseline separation was obtained over the entire concentration range studied. The reduction in resolution may be due to the increase in the EOF causing the analyte to interact less with the pseudo-stationary phase. At 0.2% vol/vol IL a slight decrease in the EOF and increase in the elution time of warfarin is observed when compared with the separation obtained using 0.1% vol/vol IL. As mentioned previously, the decrease in EOF is likely due to the modification of the capillary wall by the imidazolium-based cation which can affect column performance.

The separation of a mixture of warfarin and coumachlor was also investigated using $EMIMBF_4$ and $BMIMBF_4$ as mobile phase modifiers. The electropherograms in Figure 4.11 illustrate the effect of $EMIMBF_4$ concentration on the separation of the above-mentioned mixture.

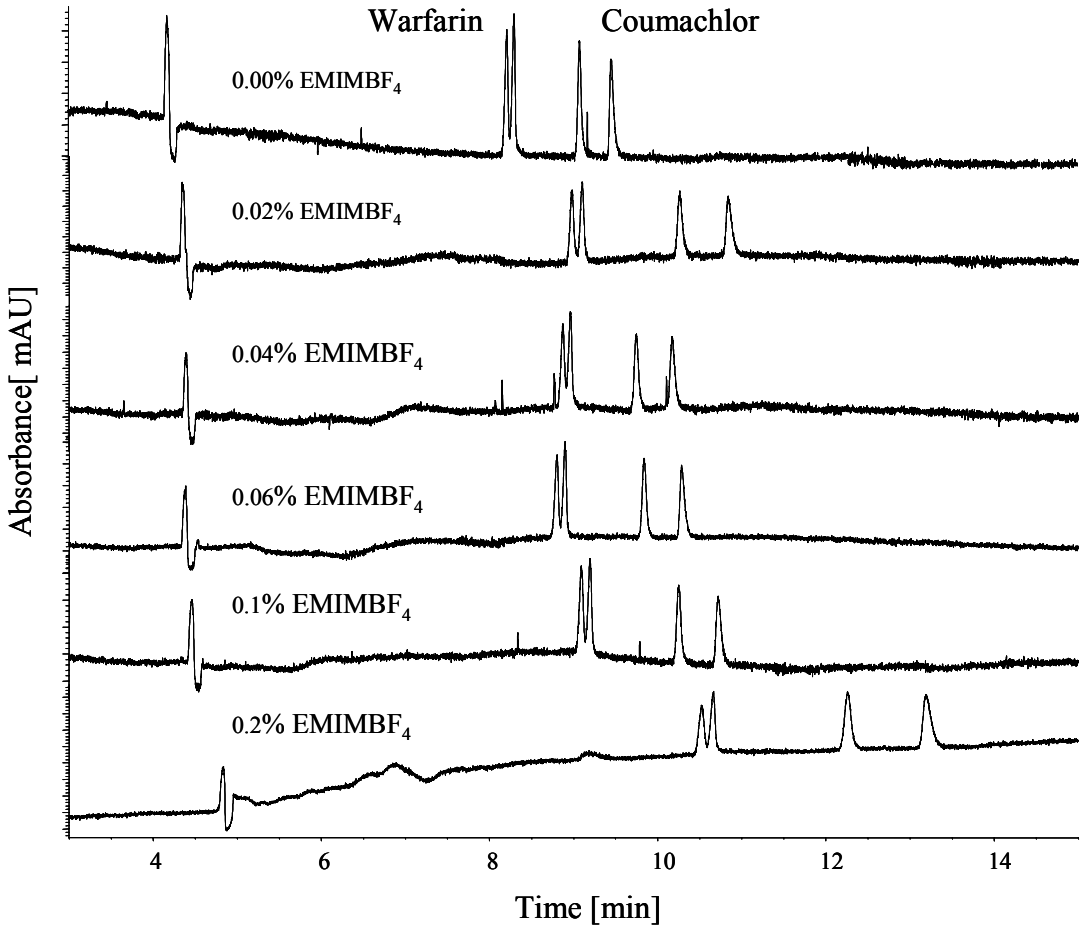


Figure 4.11 Electropherograms showing the separation of warfarin and coumachlor using 0.00, 0.02, 0.04, 0.06, 0.1, and 0.2% vol/vol $EMIMBF_4$ as mobile phase modifier respectively. Conditions: same as Figure 4.10.

When separated individually, the elution time of warfarin was longer than that of the mixture of warfarin and coumachlor as shown by the electropherograms obtained with 0.0% vovl/vol IL in Figure 4.10 and 4.11, respectively. However, the resolution of warfarin in the mixture was greatly reduced. When $EMIMBF_4$ or $BMIMBF_4$ were used as a mobile phase modifier

for the separation of these two coumarin derivatives, results consistent with those observed for the separation of the binaphthly derivatives were obtained. The EOF was enhanced as the IL concentration increased from 0.02% to 0.06% vol/vol as illustrated in Figure 4.11; however, at a concentration of 0.1% vol/vol and above the EOF was slightly reduced leading to longer migration times of the analytes. The resolution of coumachlor was enhanced when 0.2% vol/vol IL was added as shown in Figure 4.11. Thus, addition of these ILs enhanced the interaction of one enantiomer of coumachlor with poly-L-SOLV. However, due to the complex interactions of the enantiomers with the chiral selector and/or IL, the cause or mechanism of this interaction is not well understood.

Organic solvents were also used as modifiers in the separation of a mixture of warfarin and coumachlor. All three organic modifiers, MeOH, 1PrOH, and ACN, led to loss of resolution and selectivity of these analytes. In addition, long migration times and poor baselines were observed. The cause of the observations is unknown; however, it was clear that these organic solvents did not enhance chiral interactions between the coumarin derivatives investigated and the chiral polymeric surfactant.

4.3.4 Effects of Ionic Liquids and Organic Solvents on the Separation of Benzoin Derivatives

Additional chiral analytes benzoin, BME, and BEE were separated using the same conditions as the coumarin derivatives. When no modifier was used, baseline separation of the enantiomers of benzoin and BME was obtained, but poor enantiomeric separation was observed for BEE. ILs and organic solvents, at the same concentration range as before, were used as modifiers in the separation of these analytes; however, no modifier enhanced the enantiomeric separations of the analytes. Figure 4.12 illustrates the effect of EMIMBF$_4$ on the separation of the benzoin derivatives.

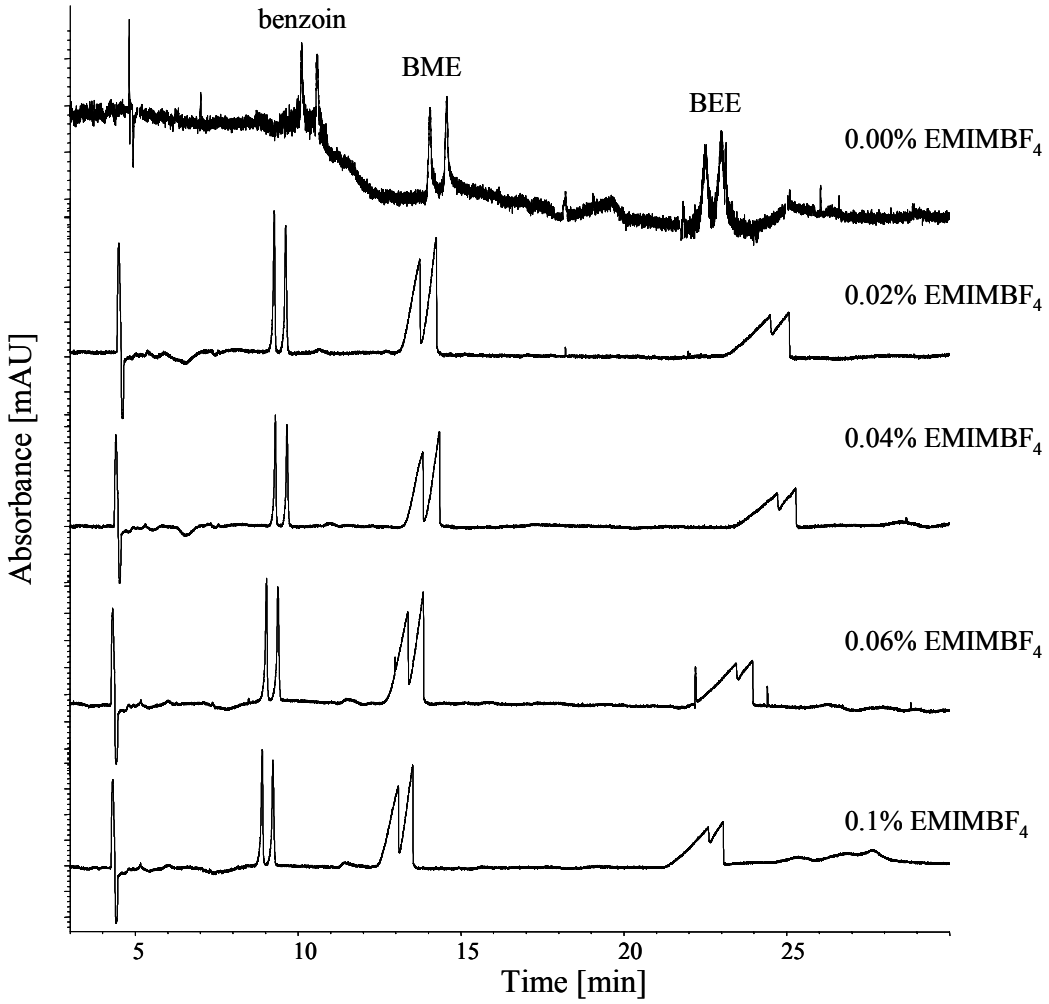


Figure 4.12 Electropherograms showing the separation of benzoin, BME, and BEE using 0.0, 0.02, 0.04, 0.06, and 0.1% vol/vol $EMIMBF_4$ as mobile phase modifier respectively. Conditions: same as Figure 4.10.

The use of $EMIMBF_4$ as a mobile phase modifier led to a reduction in the background noise, and loss of resolution for BME and BEE. In addition, a slight shift in the elution times of the analytes was recorded, which can be explained by the change in EOF and either an increase or decrease in the interactions between the analytes, IL, and chiral surfactant. In addition, increasing the amount of either IL or organic solvent modifier in the running buffer led to a loss of resolution of all the three analytes. Figure 4.13 shows the effect of MeOH on the separation of benzoin, BME, and BEE. Increasing the amount of MeOH in the buffer led

to a decrease in EOF, altered the elution time, and reduced analyte resolution. The mobile phase modifiers may have caused unfavorable chiral interactions, thus, leading to the observed results.

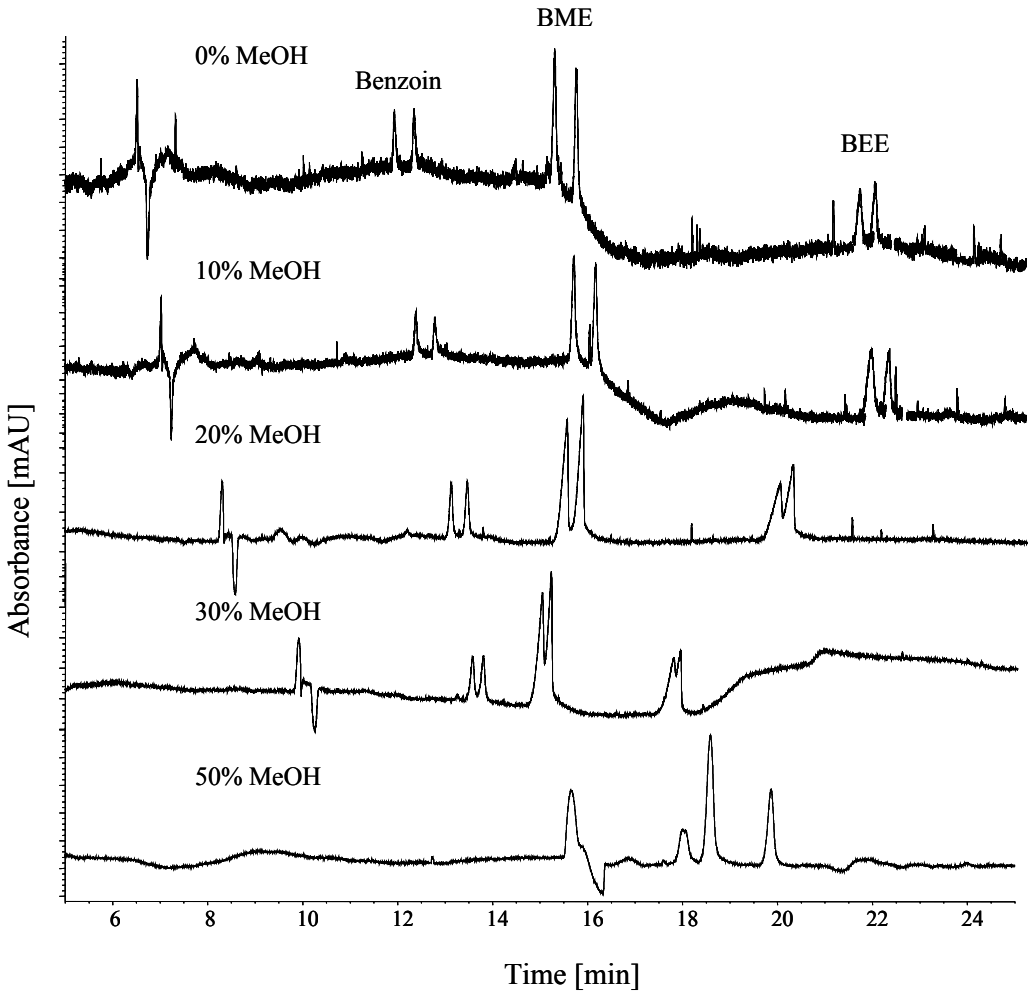


Figure 4.13 Electropherograms showing the separation of benzoin, BME, and BEE using 0.0, 10, 20, 30, and 50% vol/vol MeOH as mobile phase modifier respectively. Conditions: same as Figure 4.10.

We are actively investigating the use of ILs as modifiers in MEKC with the goal of understanding the mechanism of these modifiers in chiral and achiral separations. Also, other techniques, such as steady state and time-resolved fluorescence and fluorescence quenching are being investigated to gain a better understanding of IL properties and their effect on the

behavior of polymeric surfactants and analytes of interest. Because the hydrophobic environment is enhanced at higher IL concentration, it is expected the analyte will experience stronger interactions with the pseudo-stationary phase. If this is the case for both enantiomers this may lead to a loss of chiral resolution. Nevertheless, there are multiple modes of interaction that facilitate chiral separation, such as hydrogen bonding, dipole-dipole interactions, steric interactions, and electron-pair π-donor from analyte aromatic rings, as well as van der Waals forces. Some of these interactions agree with the three-point rule necessary for chiral recognition described by Easson and Stedman.[34] However, total enantioselectivity is strongly dependent on composition, temperature, and pH of the mobile phase. Thus, it is necessary to optimize the separation media in order to maximize the three-point interactions for chiral separations.

4.4 Conclusion

Successful separations of individual chiral analytes and mixtures were achieved for binaphthyl and coumarin derivatives using three ILs ($EMIMBF_4$, $BMIMBF_4$, and $HMIMBF_4$) as modifiers in MEKC. The fourth IL ($OMIMBF_4$) did not provide favorable interactions for chiral separation. Organic solvents were also successfully used as modifiers in the separation of binaphthyl derivatives. Other chiral analytes benzoin, BME, and BEE resolution was not affected by the presence of any of the modifiers. When comparing organic solvents with ILs as mobile phase modifiers, the ILs were found to stabilize the current while at the same time leading to faster separations. The elution order of the enantiomers remained unaltered by the presence of any mobile phase modifier. High volumes of organic solvents in the buffer led to current breakdowns. In addition, the resolution and selectivity of the analytes was dependent

on the concentration and type of IL or organic solvent used. However, smaller IL volumes were needed, compared to organic solvents, to achieve equivalent resolution and selectivity.

4.5 References

1. Adam, D. *Nature* **2000**, *407*, 938.

2. Chang, K. *New York Times* **2000**, April 24, F2.

3. Gorman, J. *Science News* **2001**, September 8, *160*, 156.

4. Freemantle, M. *Chem. Eng. News* **2002**, February 11, *80*, 8.

5. Freemantle, M. *Chem. Eng. News* **2003**, June 30, *81*, 7.

6. Anastas, P. T., Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, **1998**.

7. Rogers, R. D., Seddon, K. R. *Ionic Liquids: Industrial Application for Green Chemistry*; American Chemical Society: Washington, **2002**.

8. Poole, C. F., Kersten, B. R., Ho, S. S. J., Coddens, M. E., Furton, K. G. *J. Chromatogr.* **1986**, *352*, 407.

9. Shetty, P. H., Youngbeg, P. J., Kersten, B. R., Poole, C. F. *J. Chromatogr.* **1987**, *411*, 61.

10. Furton, K. G., Poole, C. F. *J. Chromatogr.* **1987**, *399*, 47.

11. Furton, K. G., Poole, C. F. *Anal. Chem.* **1987**, *59*, 1170.

12. Romaville, R. M., Poole, S. K., Davis, l. J., Poole, C. F. *J. Chromatogr.* **1987**, *411*, 61.

13. Huddleston, J. G., Willauer, H. D., Swatloski, R. P., Visser, A. E., Rogers, R. D. *Chem. Commun.* **1998**, 1765.

14. Dullius, J. E. L., Suarez, P. A. Z., Einloft, S., de Souza, R. F., Dupont, J., Fischer, J., DeCian, A. *Organometallics* **1998**, *17*, 815.

15. Yanes, E. G., Gratz, S. R., Baldwin, M. J., Robison, S. E., Stalcup, A. M. *Analyst* **2000**, *125*, 1919.

16. Yanes, G. E., Gratz, R. S., Baldwin, J. M., Robinson, E. S., Stalcup, M. A. *Anal. Chem.* **2001**, *73*, 3838.

17. He, L., Zhang, W., Zhao, L., Liu, X., Jiang, S. *J. Chromatogr. A* **2003**, *1007*, 39.

18. Armstrong, D. W., He, L., Liu, Y-S. *Anal. Chem.* **1999**, *71*, 3873.

19. Qin, W., Wei, H., Li, S. F. Y. *J. Chromatogr. A* **2003**, *985*, 447.

20. Jiang, T., Gu, Y., Liang, B., Li, J., Shi, Y., Ou, Q. *Analytica Chim. Acta* **2003**, *479*, 249.

21. Vaher, M., Koel, M., Kalijurand, M. *Electrophoresis* **2002**, *23*, 426.

22. Vaher, M., Koel, M., Kalijurand, M. *J. Chromatogr.* **2002**, *979*, 27.

23. Mwongela, S. M., Numan, A., Gill, L. N., Agbaria, A. R., Warner, I. M. *Anal. Chem.* **2003**, *75*, 6089.

24. Haddadian, F., Billot, E., Shamsi, S., Warner, I. M. *J. Chromatogr. A* **1999**, *858*, 219.

25. Yarabe, H. H., Billot, E., Warner, I. M. *J. Chromatogr. A* **2000**, *875*, 179.

26. Kaneta, T., Tanaka, S., Taga, M., Yoshida, H., *Anal. Chem.* **1992**, *64*, 798.

27. D'Hulst, A., Verbeke, N. *Chilarity* **1994**, *6*, 225.

28. Armstrong, D. W., Tang, Y., Ward, T., Nichols, M. *Anal. Chem.* **1993**, *65*, 1114.

29. DeVries, J. X., Schmitz-Kummer, E. *J. Chromatogr. A* **1995**, *644*, 315.

30. Wang, J., Warner, I. M. *Anal. Chem.* **1994**, *66*, 3773.

31. Mwongela, S. M., Akbay, C., Zhu, X., Collins, S., Warner, I. M. *Electrophoresis* **2003**, *24*, 2940.

32. Terabe, S., Ostuka, K., Ichikawa, K., Ando, T. *Anal. Chem.* **1984**, *56*, 111.

33. Skoog, D. A., Holler, J. F., Nieman, T. A. *In Principles of Instrumental Analysis*, 5th ed.; Harcourt Brace College Publishers: Orlando, **1998**.

34. Reichardt, C. *Solvents and Solvents Effects in Organic Chemistry*; VCH Verlagsgesellschaft: Weinheim, **1990**.

35. Easson, L. H., Stedman, E. *Biochem. J.* **1933**, *27*, 1257.

36. Lide, D. R. *Handbook of chemistry and Physics*; CRC Press: Boca Raton, **2001**.

Chapter 5.

Determination of 2-Pyrrolidone, Vinylacetate, and N-Vinyl-2-Pyrrolidone in Polyvinyl Pyrrolidone Homo-Polymer and Polyvinyl Pyrrolidone/Vinylacetate Copolymer Using Micellar Electrokinetic Chromatography

5.1 Introduction

Capillary electrophoresis (CE) has become one of the most advanced separation techniques for pharmaceutical,[1-9] food,[10-12] and chemical analysis.[13-17] It is a useful and reliable alternative or complementary technique to liquid chromatography in many areas, including main component assay,[1, 4, 13] impurity determination,[18-20] enantiomeric separations,[1, 2, 7] identity confirmation,[21-24] and stoichiometric determination.[2, 25-27] CE has several significant advantages which include: very high efficiency (approaching or exceeding 1,000,000 theoretical plates), short analysis time, and small sample volumes (1-5 nL).[28] The introduction of micellar electrokinetic chromatography (MEKC) by Terabe et al. extended the application of CE to the separation of neutral molecules.[29, 30] CE and MEKC techniques were described in detail in Chapter 1 of this dissertation.

N-vinyl-2-pyrrolidone (NVP) is a moderately yellow heterocyclic, reactive vinyl monomer. The inherent properties of NVP, high polarity, low toxicity, water solubility, chemical stability, and pseudo-cationic activity, are imparted to its homo-polymers and copolymers.[31] This vinyl monomer is commonly used as a reactive diluent in ultraviolet and electron-beam curable polymers applied as inks, coatings, or adhesives. In addition, small amounts of NVP are utilized in laboratories for research. Copolymers of NVP are used in the above-mentioned applications and for textile finishes, cosmetics, pharmaceuticals, and as vehicles for hair spray.[31, 32]

One of the NVP-containing polymers, polyvinyl pyrrolidone (PVP), is a white hygroscopic powder with a weak characteristic odor.[33] In contrast to many polymers, it is

readily soluble both in water and in a large number of organic solvents such as alcohol, chlorinated hydrocarbons, amines, acids, amides, and lactams. However, it is insoluble in common esters, ethers, hydrocarbons, and ketones. The most remarkable characteristics of PVP are hygroscopicity and adhesion to different materials. These properties, combined with outstanding film formation, high capacity for complex formation, good stabilizing and solubilizing capacity, insensitivity to pH, radiation-induced crosslinkability, as well as good biological compatibility, have made PVP one of the most frequently applied polymers for agricultural formulations and in the cosmetics and pharmaceuticals industries.[33 31, 32]

NVP and PVP were once declared priority existing chemicals by the national industrial chemicals notification and assessment scheme (NICNAS), due to their potential for high occupational and environmental exposure as well as possible adverse health effects.[34] In the late 1960s and early 1970s, these substances were first evaluated for acceptable daily intake (ADI) for humans by the joint food and agriculture organization (FAO), and the World Health Organization (WHO) expert committees on food additives. Since then, additional toxicology studies have been conducted, and up-to-date safety information is available on material safety data sheets for each individual compound. Now PVP is one of the most frequently investigated classes of materials for use in medicine and other applications interfacing with biological systems.[35-38] The principal reason for successful PVP applications is their extremely low cytotoxicity and excellent biocompatibility with living tissues.[39, 40]

The purpose of this work was to investigate the application of MEKC to determine the monomer species 2-pyrrolidone (2-Pyr), vinylacetate (VA), and NVP in PVP homo-polymer and polyvinyl pyrrolidone/vinyl acetate (PVP/VA) copolymer. These two polymers are products of the BASF Corporation (Geismar, Louisiana). The PVP polymer is

synthesized from the NVP monomer via radical polymerization in an aqueous solution. The analytical requirements for the minimum quantitation limit of these two monomers, NVP and 2-Pyr, are 1 ppm and 1000 ppm, respectively. Currently, BASF uses reversed phase HPLC for the determination of both monomers (NVP and 2-Pyr) in PVP at the concentrations noted above. Different methods are used for each compound, which requires the maintenance of two separate HPLC systems. BASF also manufactures copolymers of PVP/VA. There are several copolymer products produced by varying the ratio of PVP to VA. In each copolymer product, the analytical requirement for the minimum quantitation limit of each residual monomer, VA and NVP, is 10 ppm. VA is also determined by reverse phase HPLC, and thus necessitates a third separate HPLC system. The analysis of these monomer residues using HPLC is not easy because the polymer adsorbs to the analytical column, making it difficult to achieve the required precision during calibrations. Using SDS as the pseudo-stationary phase, a MEKC method was developed for the separation of 2-Pyr, VA, and NVP monomer residues in a single run for the first time. The effects of SDS concentration, temperature, injection size, and voltage were investigated in order to obtain optimum conditions for separation of these analytes. The optimized method was used to develop calibration curves and was evaluated for the quantitative determination of the monomer residues in the two polymers.

5.2 Experimental Methods

5.2.1 Materials

2-Pyr, VA, and NVP monomer standards and PVP homo-polymer and PVP/VA copolymer samples were provided by BASF Corporation (Geismar, LA). The molecular structures of these monomers and polymers are illustrated in Figure 5.1. Sodium borate,

sodium phosphate, tris (hydroxymethyl)-aminomethane (TRIS), and sodium dodecyl sulfate (SDS) were purchased from Fisher Scientific (Fair Lawn, NJ). All reagents were used as received.

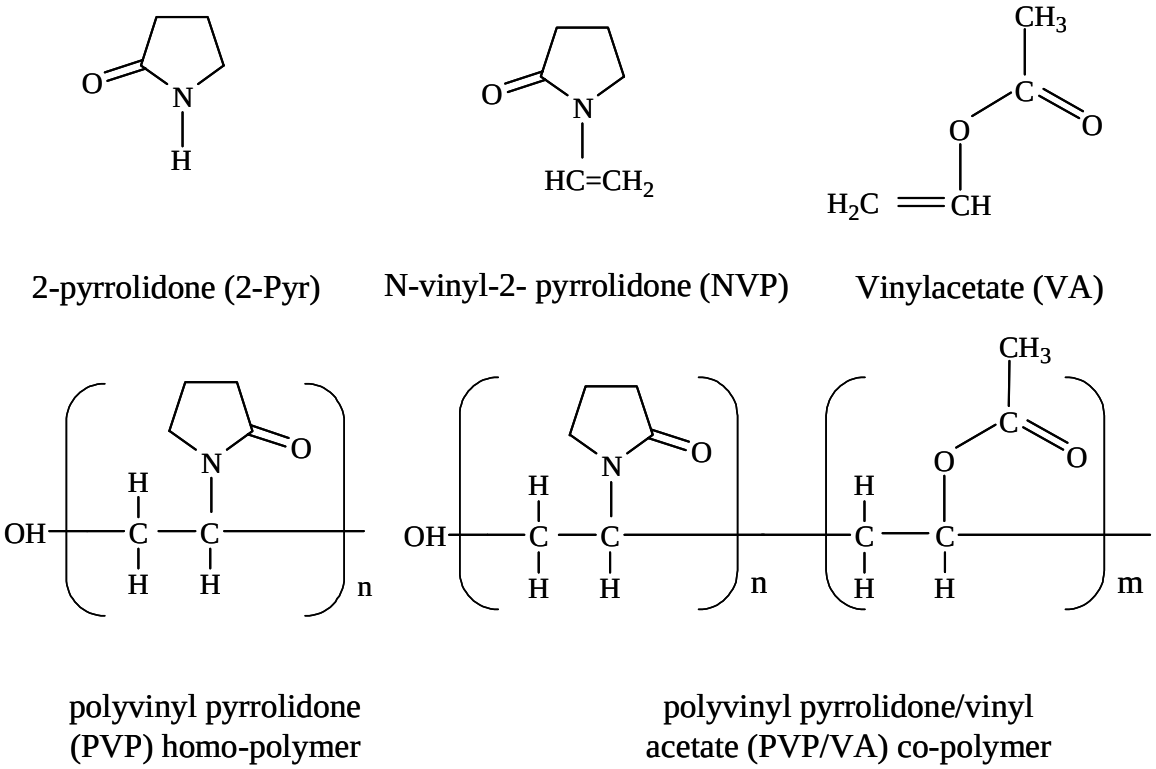


Figure 5.1 Chemical structures of the monomer and polymer analytes used in the current study.

5.2.2 Preparation of MEKC Buffer Solutions and Calibration Standards

The background electrolytes (BGE) investigated for the separation of 2-Pyr, VA, and NVP mixture were 25 mM sodium borate at pH 9.2, 100 mM TRIS at pH 10.0, and 25 mM sodium phosphate pH 7.0. An appropriate amount of SDS surfactant was added to each BGE. Attempts were made to develop a calibration curve using VA as an internal standard at a fixed concentration of 100 ppm and varying the concentration of 2-Pyr and NVP from 50 ppm to 200 ppm.

5.2.3 Capillary Electrophoresis

MEKC experiments were performed on a Hewlett-Packard 3D CE instrument (Foster City, CA) equipped with a UV diode array detector. Bare-fused silica capillaries, cut to 60 cm (effective length 52 cm, 50 μm i.d.) for CE conventional cell, or 70 cm (63 cm effective length, 75 μm i.d.) for CE high-sensitivity cell, were purchased from Polymicro Technologies (Phoenix, AZ). SDS surfactant was dissolved into the buffer solution and filtered through a 0.45 μm membrane for conventional cell CE analysis or a 0.2 μm membrane for analysis using a CE high-sensitivity cell. The applied voltage ranged from 15 to 30 kV, and capillary temperature was varied from 15 to 25 °C. Detection wavelengths for the analytes were 205 nm for 2-Pyr and VA and 235 nm for NVP as determined by UV-VIS. The analytes were prepared in 50:50 methanol/water, and sample injection size was varied from 50 mbar for 1 s to 50 mbar for 7s. Prior to use, each new capillary was conditioned for 30 min with 1 M NaOH and rinsed for 15 min with triply distilled deionized water. Prior to each run, the capillary was flushed with MEKC buffer for 3 min to condition and fill the capillary.

5.3 Results and Discussions

5.3.1 Determination of Analyte Detection Wavelengths

Stock solutions of each analyte were prepared in triply distilled deionized water. 100 ppm standard solutions were prepared from the stock solution and spectra collected on a UV-VIS spectrophotometer (figure 2). The optimum wavelength of absorbance for 2-Pyr and VA was 205 nm and 235 nm for NVP. The homo-polymer and copolymer had similar absorbance spectra to that of 2-Pyr and VA but not NVP, as shown in Figure 5.2.

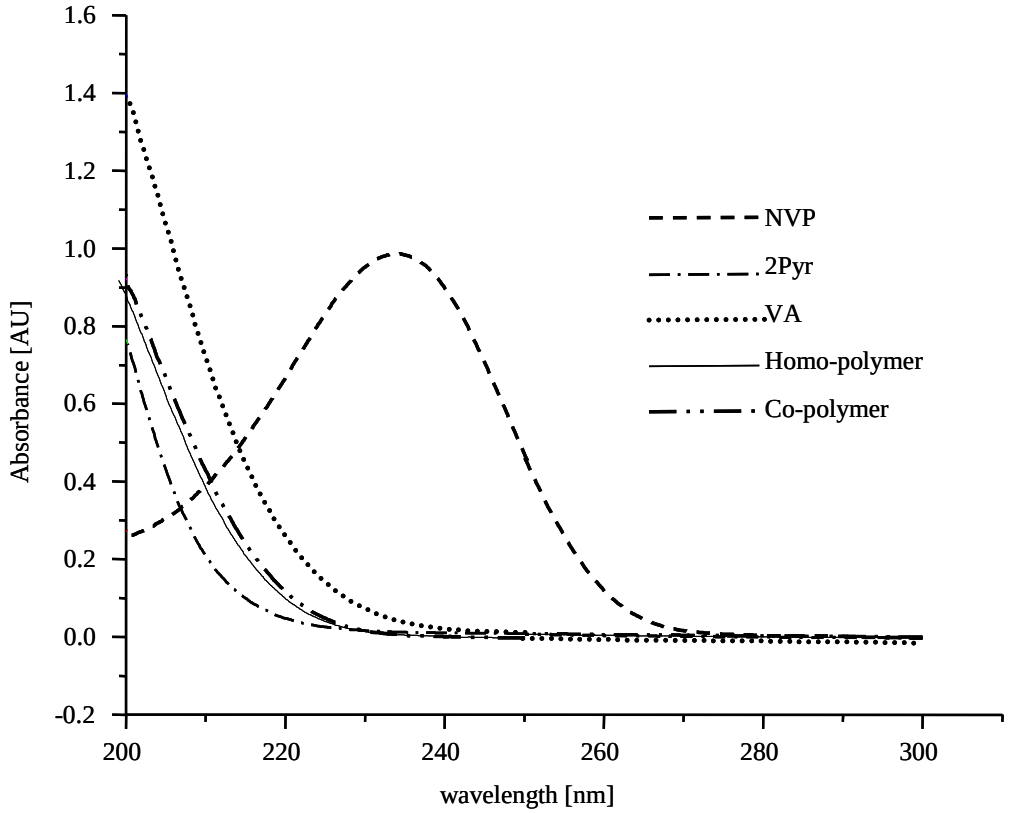


Figure 5.2 UV/Vis spectra of 2-Pyr, VA, and NVP monomer standards, and PVP and PVP/VA polymer samples.

5.3.2 Determination of the Background Electrolyte

Three different electrolytes were investigated in order to determine the best BGE for the separation of the monomer residues in the polymer samples. These electrolytes were 100 mM TRIS buffered at pH 10.0, 25 mM sodium phosphate buffered at pH 7.0 and 25 mM sodium borate buffered at pH 9.2. Each buffer solution contained 50 to 100 mM of SDS as the pseudo-stationary phase for the separation of these neutral molecules. Sodium borate buffer at pH 9.2 provided the best baseline and symmetrical peaks; thus, it was used for the remainder of the separation experiments.

5.3.3 Separation of Monomer Standards Using a Conventional CE Cell

The standard monomers 2-Pyr, VA, and NVP were first separated using a 50 i.d. capillary with an effective length of 52 cm and total length of 60 cm. A mixture of these three monomer standards at a concentration of 1000 ppm was easily separated using 50 mM SDS and the above-mentioned sodium borate buffer, as shown in Figure 5.3 electropherogram (A).

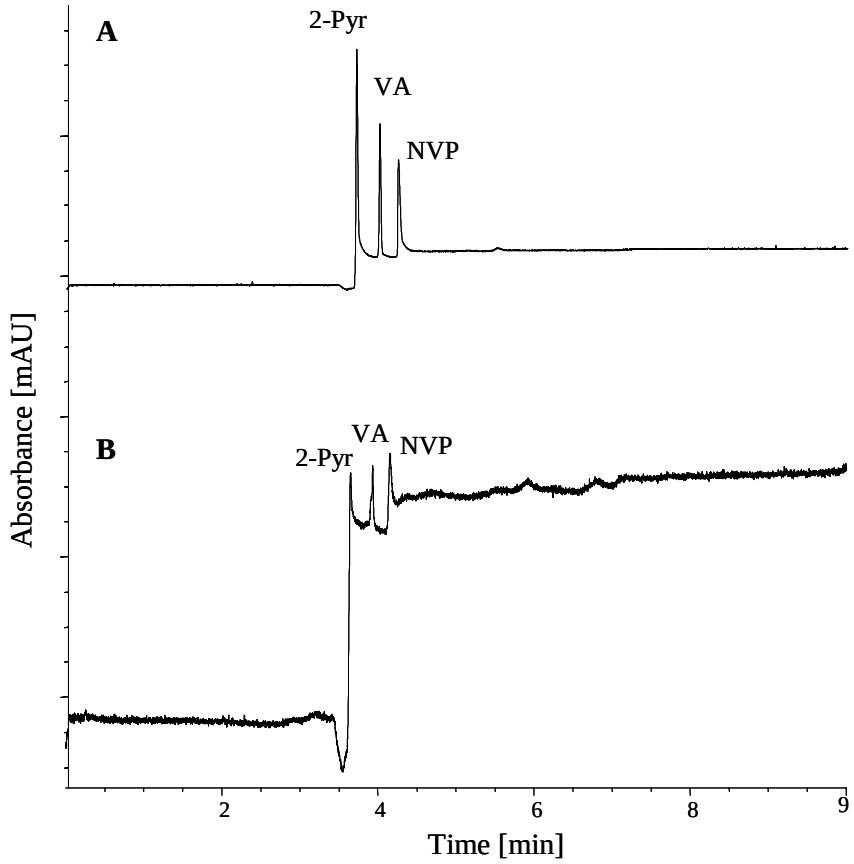


Figure 5.3 Separation of a mixture of 2-Pyr, VA, and NVP standard monomers using conventional CE cell at a concentration of (A) 1000 ppm and (B) 100 ppm. Conditions: 50 mM SDS, 25 mM sodium borate; pH: 9.2, injection size: 50 mbar for 1s; temp.: 20 °C, voltage: 20 kV, detection λ: 205 nm.

2-Pyr eluted first followed by VA and NVP. Peak identification was done by spiking each monomer in the sample mixture. As expected, at a concentration of 100 ppm these analytes

were still separated but the intensity of the peaks was very low. A shift in the baseline was observed, as shown in Figure 5.3 electropherogram A and B, and the cause of this shift was not known. At concentrations below 100 ppm, these analytes could not be detected. Since the requirements for a minimum quantitation limit of NVP and 2-Pyr are 1 ppm and 1000 ppm, respectively, in PVP and 10 ppm VA and NVP in PVP/VA, a conventional CE cell could not be used for quantitative analysis. Therefore, a high-sensitivity cell was used in order to improve the sensitivity and thus achieve the required quantitation limit.

5.3.4 Separation of NVP Monomer Standard Using CE High-sensitivity cell

Compared to the conventional CE cell, the high-sensitivity cell has a longer path length and, based on Beers law (Equation 5.1), increasing the path length will lead to an augmented absorbance

$$A = \varepsilon bc \qquad 5.1$$

where A is absorbance, ε is molar absorptivity, b is path length and c is analyte concentration. The high-sensitivity cell is at least 10 times more sensitive than the conventional CE cell, extends linearity beyond 2000 mAU, and provides unsurpassed spectral fidelity. These improvements are a result of a proprietary micromachined design which increases the detection path length from 75 µm to 1200 µm while dramatically reducing stray radiation.[41] To determine the detection limit of the CE high-sensitivity cell, NVP standard solutions were prepared in the concentration range between 1 ppm and 100 ppm. Figure 5.4 shows electropherograms obtained for NVP standard solutions using the CE high-sensitivity cell. NVP was easily detected at a concentration of 10 ppm; also, NVP could be detected at 1 ppm by increasing the injection size. To determine the optimum conditions for separation of these

analytes using the high-sensitivity cell, SDS concentration, temperature, injection size, and voltage were varied.

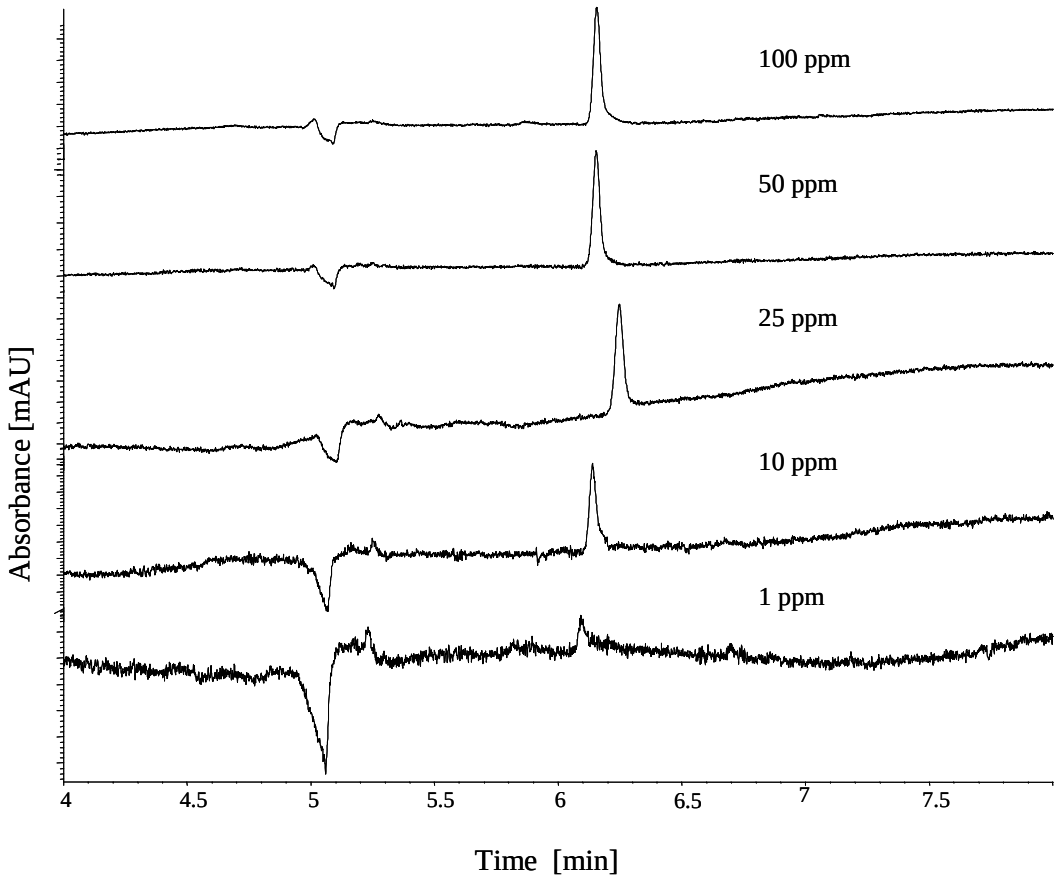


Figure 5.4 Separation of NVP standard using CE high-sensitivity cell. Conditions: 50 mM SDS, 25 mM sodium borate; pH: 9.2, injection size: 50 mbar for 1s; temp.: 20 °C, voltage: 20 kV, detection λ: 235 nm.

5.3.5 SDS Concentration Study

SDS concentration was varied from 10 mM to 100 mM in a 25 mM borate buffer at pH 9.2, temperature of 20 °C, voltage of 30 kV, and injection size of 50 mbar for 1s. As shown in Figure 5.5, a gradual increment in SDS concentration led to a decrease in electroosmotic flow (EOF) and an increase in both resolution and elution time of the analytes. For SDS concentrations above 50 mM, 2-Pyr is resolved from the EOF, making the

quantitative analysis of this analyte possible. As a result, the SDS concentration was fixed at 75 mM for all the subsequent optimization experiments.

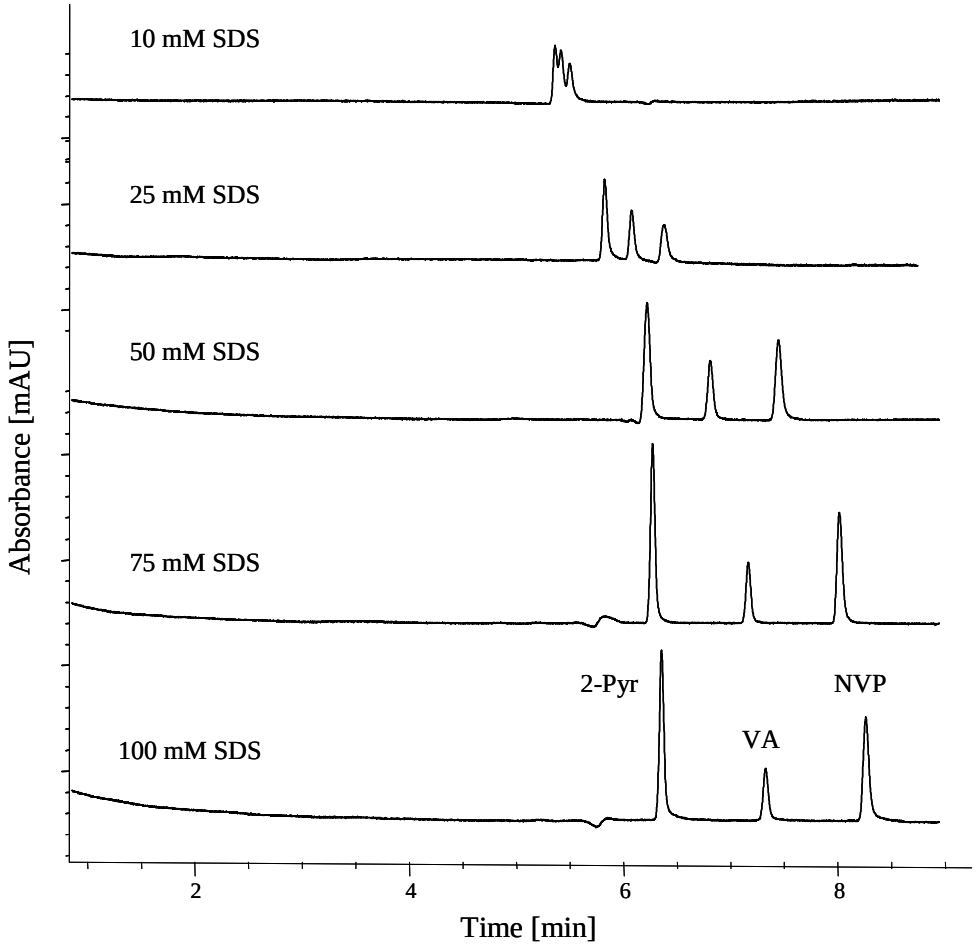


Figure 5.5 Effect of SDS concentration on the separation of 2-Pyr, VA, and NVP using high-sensitivity CE cell. Conditions: 25 mM sodium borate; pH: 9.2, injection size: 50 mbar for 1s; temp.: 20 °C, voltage: 20 kV, detection λ: 205 nm.

5.3.6 Temperature Study

For this investigation, the analyte concentration was 100 ppm, temperature was varied from 15 °C to 30 °C and all the other parameters were held constant. As illustrated in Figure 5.6, all the investigated temperatures gave baseline separation and a good resolution of the analyte peaks. As expected, high temperatures led to an increase in EOF and faster separation

of the analytes. This was likely due to a decrease in electrolyte viscosity. However, a significant decrease in VA peak intensity was observed as the temperature was increased. This may have occurred because VA is very volatile and is easily vaporized at high temperatures. The optimum temperature was therefore determined to be 15 °C.

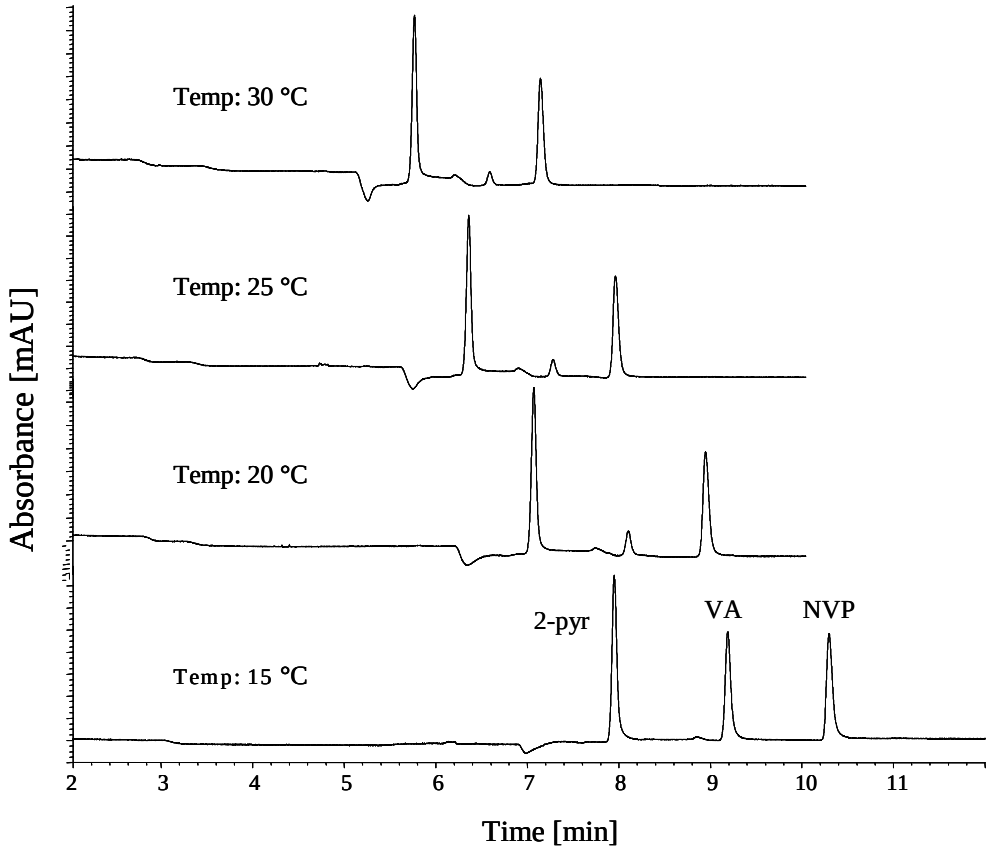


Figure 5.6 Effect of temperature on the separation of 2-Pyr, VA, and NVP using high-sensitivity CE cell. Conditions: 75 mM SDS, 25 mM sodium borate; pH: 9.2, injection size: 50 mbar for 1s; voltage: 20 kV, detection λ: 205 nm.

5.3.7 Injection Size Study

The NVP monomer standard was used to determine the optimum injection size. The concentration of NVP used was 1 ppm and temperature was fixed at 15 °C. All other parameters were fixed and the injection size was varied from 50 mbar for 1 s to 50 mbar for

7s. The peak intensity of NVP increased with an increase in injection size, and a shift in the baseline was observed, as shown in Figure 5.7.

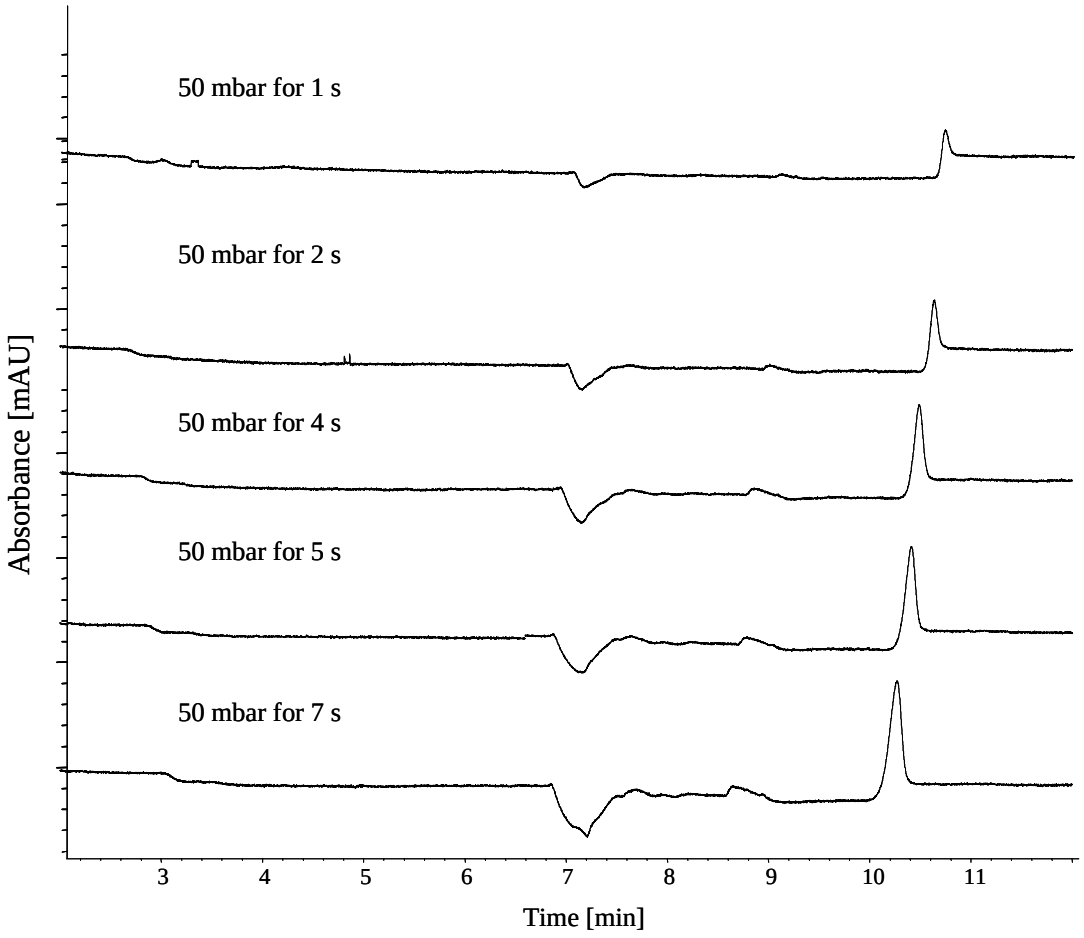


Figure 5.7 Effect of injection size on the separation of NVP using high-sensitivity CE cell. Conditions: 75 mM SDS, 25 mM sodium borate; pH: 9.2, temp.: 20 °C, voltage: 20 kV, detection λ: 235 nm.

However, for a constant injection size, the peak shape and intensity varied with analyte concentration (Figure 5.4). Although 1 ppm of NVP could be detected for all injection sizes, good symmetrical peaks are required for quantitative analysis. Thus, it was difficult to surmise an optimum injection size universal for all concentrations. Therefore, it was concluded that the injection size could be varied based on analyte concentration. Thus, for a

low concentration of the analyte a large injection size could be used, and vice versa, in order to obtain intense symmetrical peaks.

5.3.8 Voltage Study

The influence of voltage on the separation of the NVP monomer standard was evaluated by varying the voltage from 30 kV to 15 kV. NVP concentration was maintained at 1 ppm and an injection size of 50 mbar for 7s was used. All the parameters were fixed at their optimum values as determined above. As shown in Figure 5.8, when a voltage of 30 kV was applied, a sharp symmetrical peak of NVP was obtained in less than 5 minutes.

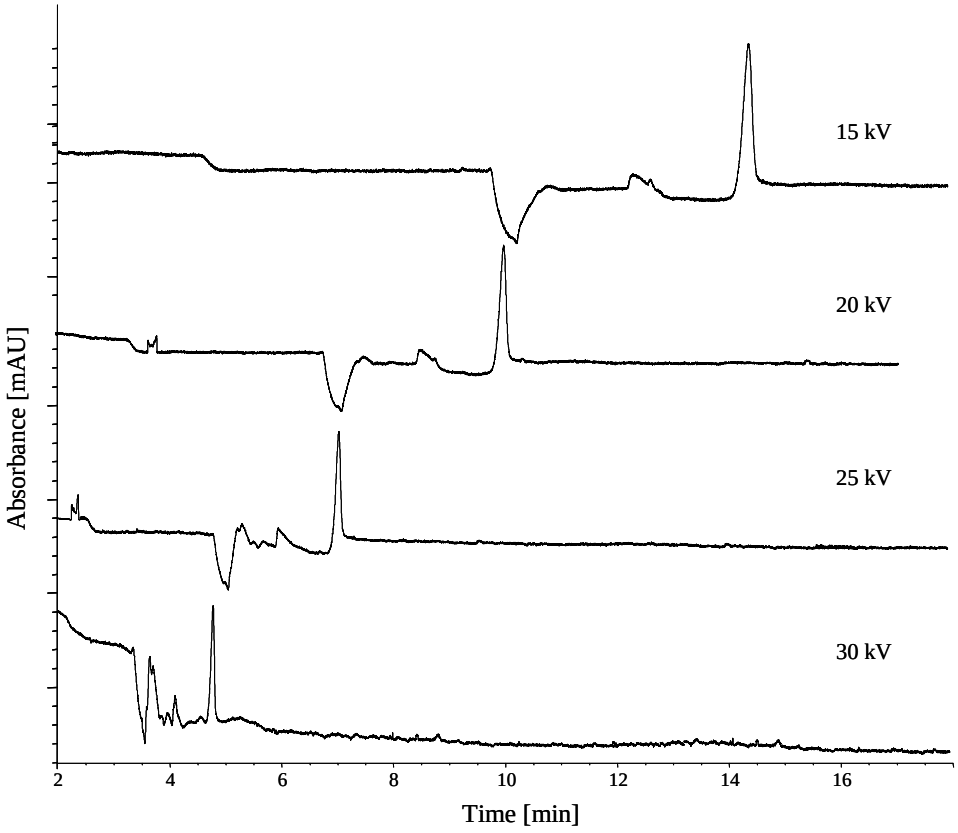


Figure 5.8 Effect of voltage on the separation of NVP using high-sensitivity CE cell. Conditions: 75 mM SDS, 25 mM sodium borate; pH: 9.2, injection size: 50 mbar for 7s; temp.: 20 °C, detection λ: 235 nm.

Although short analysis time is one of the guiding factors in a good analytical method, a voltage of 30 kV was not ideal, especially for the separation of 2-Pyr and VA because their

resolution would greatly be reduced. Thus, these two analytes would either elute very close to the EOF or elute together with the EOF making it difficult to analyze them quantitatively. Hence, 15 kV was chosen to be the optimum voltage because it allowed baseline separation of all three analytes while maintaining a relatively short analysis time.

5.3.9 Separation of Monomer Standards Using Optimized Conditions for the CE High-sensitivity Cell

The optimized conditions achieved from the temperature, injection size, and voltage studies were used to separate a mixture of 2-Pyr, VA, and NVP standard monomers each at a concentration of 100 ppm. Symmetrical and baseline resolved peaks were obtained as shown in Figure 5.9 as compared to the peaks obtained using the conventional CE cell in Figure 5.3 electropherogram (B).

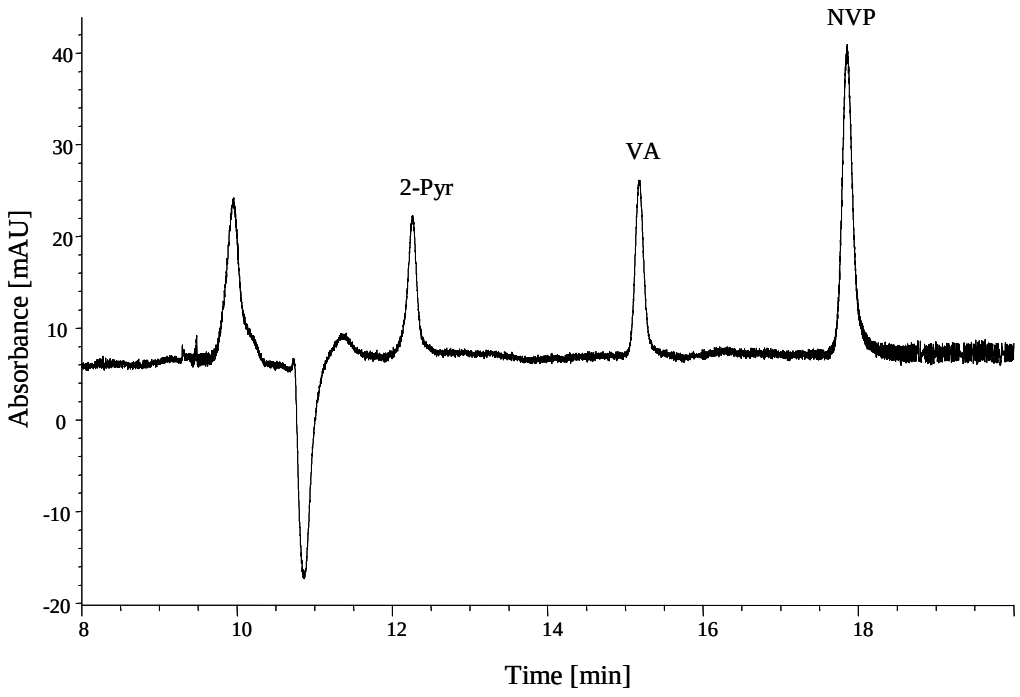


Figure 5.9 Separation of 2-Pyr, VA, and NVP using optimized method for high-sensitivity CE cell. Conditions: 120 mM SDS, 25 mM sodium borate buffer, pH 9.2, Voltage: 15 kV, Temp: 15 °C, Injection size: 50 mbar 1sec.

In addition, no baseline shift was observed for the separations done on the CE high-sensitivity cell. However, a longer elution time was observed because a longer capillary (70 cm and 75 μm i.d.) was required to work with the CE high-sensitivity cell. Although SDS studies (Figure 5) had shown that 75 mM was sufficient to achieve baseline resolution, when all optimum conditions were applied, this SDS concentration was inadequate. Further SDS studies revealed that 120 mM SDS was necessary to resolve 2-Pyr from the EOF. Reproducible results were obtained when this optimized method was repeated more than five times. The reproducibility was evaluated by computing the relative standard deviation (RSD)[42] of the EOF. All RSDs of the EOF were below 1%, and thus, very good run-to-run reproducibilities were observed.

5.3.10 Separation of Monomer Residues from Polymers Samples Using CE High-sensitivity Cell

The developed method was used to detect monomer residues of 2-Pyr, VA, and NVP in PVP homo-polymer and PVP/VA copolymer. 100,000 ppm solutions of the two polymers were prepared in 1:1 methanol/water. For the homo-polymer, 2-Pyr and NVP peaks were detected (Figure 5.10), but only 2-Pyr was detected in the copolymer. In the polymer samples, the 2-Pyr peak eluted very close to the EOF. Therefore, further SDS studies revealed that 200 mM was needed to increase the retention time of 2-Pyr and to provide a smooth baseline. The homo-polymer sample was spiked with 100 ppm of each monomer standard to confirm the observed peaks, as shown in Figure 5.10, electropherogram (A). Two different wavelengths were used to detect 2-Pyr (205 nm, electropherogram (B) and NVP 235 nm, electropherogram (C)) in the homo-polymer sample. An unknown peak eluted after the 2-Pyr peak; however, no attempts were made to identify it. Data for the separation of the

monomer residues in the PVP/VA copolymer is not shown because only 2-Pyr was observed, while the monomers of interest in the copolymer were those of VA and NVP.

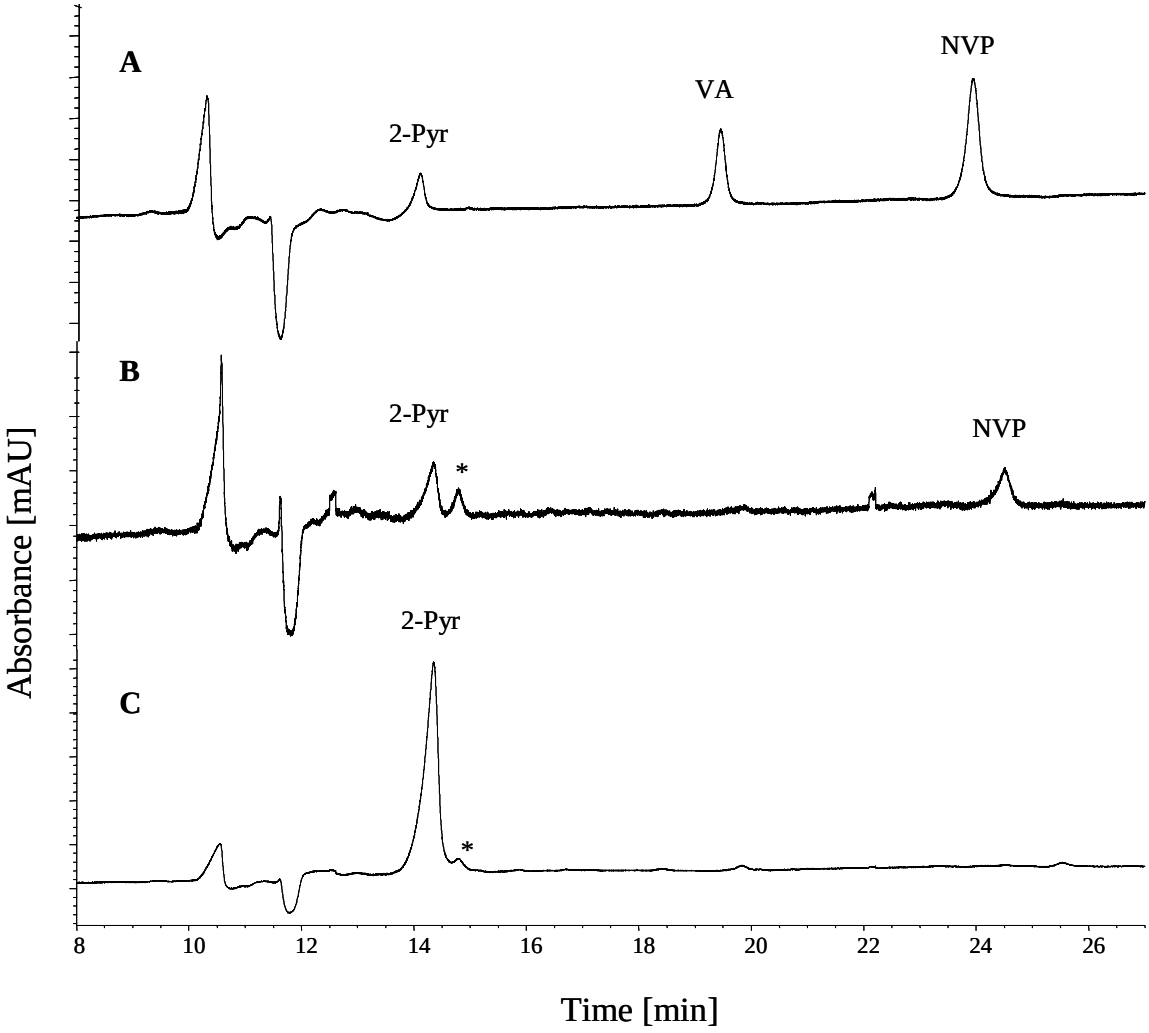


Figure 5.10 Separation of 2-Pyr and NVP monomer residues in a 100,000 ppm PVP homo-polymer sample using optimized method for high sensitivity CE cell. (A) 100 ppm spike of the monomers in the polymer sample detected at 205 nm, (B) NVP detected at 235 nm, and (C) 2-Pyr detected at 205 nm. Conditions: 200 mM SDS, 25 mM sodium borate buffer, pH: 9.2, voltage: 15 kV, temp: 15 °C, injection size: 50 mbar 1sec.

Concentrations above 100,000 ppm of the copolymer were prepared and run using the same method; also, higher injection sizes were tried; however, VA and NVP peaks were not

detected. In addition, the copolymer sample was too viscous at higher concentrations, preventing the analysis of samples greater than 500,000 ppm.

5.3.11 Quantitative Analysis of 2-Pyr and NVP in PVP Homo-Polymer

An attempt was made to develop a calibration curve for 2-Pyr and NVP using VA as an internal standard. It was expected that, by fixing the VA concentration at 100 ppm, the VA peak area would remain constant as the concentration of 2-Pyr and NVP was varied between 50 ppm to 200 ppm. However, the VA peak was irreproducible and its peak area reduced as the concentration of 2-Pyr and NVP were increased over the entire concentration range (data not shown). The primary reason for this fluctuation in peak intensity of VA is not well known, but a possible explanation is that it vaporizes with time because of its high volatility. Research is on-going to develop a reliable method that can be used to accurately determine the amount of 2-Pyr and NVP in the PVP homo-polymer in a single run. This would help BASF to cut the cost of operation by eliminating the use of two HPLC systems for the separation and quantitative analysis of VA and NVP in the PVP homo-polymer. In addition, investigations are being conducted to separate and effectively detect VA and NVP in the PVP/VA copolymer, after which the possibility of quantitative analysis can be evaluated. The success of this project may lead to the separation and quantitative analysis of the three monomer residues in the two polymers using one CE system as compared to three HPLC systems.

5.4 Conclusion

An MEKC method was developed for the separation of 2-Pyr, VA, and NVP, employing SDS as the pseudo-stationary phase and applying a CE high-sensitivity cell. The conventional CE cell was unable to provide the required quantitation limit; thus, the CE high-

sensitivity cell, which has a longer path length, was used to increase the sensitivity. Several parameters, such as SDS concentration, temperature, injection size, and running voltage were varied to optimize the developed method for use with the high-sensitivity cell. Excellent run-to-run reproducibilities were observed (RSD of EOF less than 1%) when the standard monomer analytes were separated using the optimized MEKC method with the CE high-sensitivity cell. The optimized method was successfully used to separate 2-Pyr and NVP monomer residues from the PVP homo-polymer and 2-Pyr from the PVP/VA copolymer. However, the separation of VA and NVP in PVP/VA copolymer was unsuccessful. Also, an accurate calibration curve using VA as an internal standard could not be developed because the VA peak area was irreproducible over the calibration curve concentration range.

5.5 References

1. Lin, C-C., Li, Y-T., Chen, S-H. *Electrophoresis* **2003**, *24*, 4106.
2. Altria, K. D., Elder, D. *Journal of Chromatography, A* **2004**, *1023*, 1.
3. Bertucci, C., Bartolini, M., Gotti, R., Andrisano, V. *Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences* **2003**, *797*, 111.
4. Hartinger, C. G., Timerbaev, A. R., Keppler, B. K. *Electrophoresis* **2003**, *24*, 2023.
5. Li, F., Zhang, C., Guo, X., Feng, W. *Biomedical Chromatography* **2003**, *17*, 96.
6. Petersen, J. R., Okorodudu, A. O., Mohammad, A., Payne, D. A. *Clinica Chimica Acta* **2003**, *330*, 1.
7. Shamsi, S. A. *Electrophoresis* **2002**, *23*, 4036.
8. Chankvetadze, B. *Electrophoresis* **2002**, *23*, 4022.
9. Sung, W-C., Chen, S-H., *Electrophoresis* **2001**, *22*, 4244.
10. Frazier, R. A., Papadopoulou, A. *Electrophoresis* **2003**, *24*, 4095.
11. Recio, I., Ramos, M., Lopez-Fandino, R. *Electrophoresis* **2001**, *22*, 1489.
12. Boyce, M. C. *Electrophoresis* **2001**, *22*, 1447.

13. Bayle, C., Causse, E., Couderc, F. *Electrophoresis* **2004**, *25*, 1457.

14. Lindner, H., Sarg, B., Helliger, W. *Journal of Capillary Electrophoresis and Microchip Technology* **2003**, *8*, 59.

15. Liu, Y-M., Cheng, J-K. *Electrophoresis* **2003**, *24*, 1993.

16. Schmitt-Kopplin, P., Junkers, J. *Journal of Chromatography, A* **2003**, *998*, 1.

17. de Beer, T., Velthorst, N. H., Brinkman, U. A., Gooijer, C. *Journal of Chromatography, A* **2002**, *971*, 1.

18. Lurie, I. S., Hays, P. A., Garcia, A. E., Panicker, S. *Journal of Chromatography, A* **2004**, *1034*, 227.

19. Suess, F., Harang, V., Saenger-van de Griend, C. E., Scriba, G. K. *Electrophoresis* **2004**, *25*, 766.

20. Perez-Maseda, C., Calvet, C., Cuberes, R., Frigola, J. *Electrophoresis* **2003**, *24*, 1416.

21. Mazza, G., Cacace, J. E., Kay, C. D. *Journal of AOAC International* **2004**, *87*, 129.

22. Pryce, T. M., Palladino, S., Kay, I. D., Coombs, G. W. *Medical Mycology* **2004**, *42*, 93.

23. Hsieh, M-C., Lin, C-H. *Electrophoresis* **2004**, *25*, 677.

24. Kato, M., Sakai-Kato, K., Jin, H., Kubota, K., Miyano, H., Toyo'oka, T., Dulay, M. T., Zare, R. N. *Analytical Chemistry* **2004**, *76*, 1896.

25. Girard, M., Turgeon, S. L., Gauthier, S. F. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 6043.

26. Okun, V. M., Kenndler, E. *Electrokinetic Phenomena* **2004**, 109.

27. Tseng, W-L., Chang, H-T. Hsu, S-M., Chen, R-J., Lin, S. *Electrophoresis* **2002**, *23*, 836.

28. Zhao, Y., Lunte, E. C. *J. Chroamtogr. B* **1997**, *688*, 265.

29. Terabe, S., Otsuka, K., Ando, T. *Anal. Chem.* **1984**, *56*, 111.

30. Otsuka, K., Terabe, S., Ando, T. *J. Chroamtogr.* **1985**, *332*, 219.

31. http://www.basf.com/businesses/chemicals/pdfs/n-vinyl2.pdf.

32. http://www.basf.com/businesses/consumer/cosmeticingredients/docs/SOEF97.PDF.

33. http://www.basf.com/businesses/consumer/dispersions/usa/pvp/.

34. http://www.nicnas.gov.au/publications/CAR/PEC/PEC11/PEC11.htm.

35. Migliaresi, C., Nicolais, L., Giusti, P., Chiellini, E. *Polymers in Medicine III*; Elsevier: Netherlands, **1988**.

36. Tomita, N., Tamai, S., Okajima, E., Hirao, Y., Ikeuchi, K., Ikada, Y. *J. Appl. Biomater.* **1994**, *5*, 175.

37. de Queiroz, A., Gallardo, A., San Roman, J., Higa, O., *J. Biomater. Sci. Polym Ed.* **1995**, *7*, 523.

38. de Queiroz, A., Castro, S, J., Higa, O. *J. Biomater. Sci. Polym Ed.* **1997**, *8*, 335.

39. Vijayasekaran, S., Chirila, T.,Hong, Y.,Tahija,S., Dalton, P., Constable, I., McAllister I. *J. Biomater. Sci. Polym Ed.* **1996**, *7*, 685.

40. Mark, E., Okano, T., Kim, S. *Hydrogels in Medicine and Pharmarcy*; CRC Press: Florida, **1987**.

41. http://www.chem.agilent.com/Scripts/PDS.asp?lPage=2753

42. Skoog, D. A., Holler, J. F., Nieman, T. A. *In Principles of Instrumental Analysis*, 5th ed.; Harcourt Brace College Publishers: Orlando, **1998**.

Chapter 6

Summary and Future Directions

6.1 Summary

The research presented in this dissertation focused on the synthesis, characterization, and application of two types of anionic surfactants (a chiral amino acid-based surfactant and achiral sulfate-based surfactant) for the separation of chiral and achiral compounds in MEKC. These two surfactants were categorized according to the type of anionic head group. The monomeric surfactants were polymerized to form polymeric surfactants which were found to be better pseudo-stationary phases in MEKC. Modifiers such as organic solvents and ILs were used to aid in the separation of highly hydrophobic analytes. The advantages of using ILs over organic solvents were also discussed.

Chapter 1 was an introduction to relevant topics related to this dissertation such as surfactants, chirality, CE, MEKC, and ILs. In addition, a brief discussion about techniques applied in the characterization of surfactants was also included in the first chapter. A detailed description of the synthesis, polymerization, characterization, and application of a novel chiral amino acid-based surfactant, poly-L-SOLV, was presented in Chapter 2. Both the monomer (L-SOLV) and the polymer (poly-L-SOLV) were capable of chiral separation. However, the polymer provided better separation. The polymeric surfactant was found to be thermally stable and was successfully used in the separation of neutral, acidic, and basic chiral analytes.

In Chapter 3, a polymerized surfactant with a sulfate head group, namely, poly-SUS, was synthesized and applied towards the separation of a mixture of eight alkyl aryl

ketones and seven phenols in MEKC. A commercially available surfactant, SDS, was also used for the separation of the eight alkyl aryl ketone mixture and compared with the poly-SUS surfactant. Several ILs were investigated, for the first time, as mobile phase modifiers in the separation of the ketone and phenol mixtures. Poly-SUS was more versatile in the separation of all the analytes as compared to SDS. However, baseline separation of all the analytes was not possible when using either SDS or poly-SUS. Therefore, ILs were used for the first time as mobile phase modifiers to improve the separations. The IL $BMIMBF_4$, when used as a mobile phase modifier, provided the best results for the separation of the analytes.

In Chapter 4, the effects of using ILs as modifiers for the separation of chiral compounds in MEKC were investigated. From the studies in Chapter 3, the 1,3-dialkylimidazolium tetrafluoroborate-based ILs were found to be more versatile as modifiers. Thus, in this chapter, ILs with different alky subtituents on the imidazolium ring but the same tetrafluoroborate anion, were investigated. The novel polymeric surfactant poly-L-SOLV whose synthesis was described in Chapter 2, was used as the chiral selector. A comparison was made between the use of 1,3-dialkylimidazolium-based ILs and the organic solvents methanol, 1-propanol, and acetonitrile as mobile phase modifiers in these chiral separations. Successful separations of individual chiral analytes and mixtures were achieved for binaphthyl and coumarin derivatives using three ILs ($EMIMBF_4$, $BMIMBF_4$, and $HMIMBF_4$) as modifiers in MEKC. Organic solvents were also successfully used as modifiers in the separation of binaphthyl derivatives. Other chiral analytes benzoin, BME, and BEE resolution was not affected by the presence of any of the modifiers. When comparing organic solvents with ILs as mobile phase

modifiers, the ILs were found to stabilize the current while at the same time leading to faster separations. The elution order of the enantiomers remained unaltered by the presence of any mobile phase modifier. High volumes of organic solvents in the buffer led to current breakdowns. In addition, the resolution and selectivity of the analytes was dependent on the concentration and type of IL or organic solvent used.

A detailed description of the determination of 2-pyrrolidone, vinylacetate, and N-vinyl-2-pyrrolidone monomer residues in polyvinylpyrrolidone (PVP) homo-polymer and polyvinylpyrrolidone/vinylacetate (PVP/VA) copolymer using SDS as a pseudo-stationary phase in MEKC is presented in Chapter 5. An MEKC method was developed applying the CE high-sensitivity cell because the conventional CE cell was unable to provide the required quantitation limit. Several parameters such a SDS concentration, temperature, injection size, and running voltage were varied to optimize the developed method for use with the high sensitivity cell. Excellent reproducibilities were observed when the standard monomer analytes were separated using the optimized MEKC method with the CE high-sensitivity cell since the RSD values of electroosmotic flow were below 1% for all runs. The optimized method was successfully used to separate 2-Pyr and NVP monomer residues from the PVP homo-polymer and 2-Pyr from the PVP/VA copolymer. However, an accurate calibration curve using VA as an internal standard could not be developed because the VA peak area was irreproducible over the calibration curve concentration range.

6.2 Future Directions

Future studies using the chiral polymeric surfactant (poly-L-SOLV) would be to use it for the separation of more chiral analytes. This could be done either using the

MEKC method or using the open-tubular CEC (OT-CEC) approach. OT-CEC is an alternative method to conventional CEC.[1, 2] In OT-CEC, fused silica capillaries coated with thin films of physically adsorbed charged polymers are developed by use of a polyelectrolyte multilayer (PEM) coating procedure. The PEM coating can be constructed in *situ* by alternating rinses with positively and negatively charged polymers, where the negatively charged polymer would be poly-L-SOLV. The advantage of the OT-CEC approach would be the elimination of problems associated with frits and silica particles in conventional CEC.[1] In addition, this approach is user friendly when coupled with mass spectrometry as compared to the MEKC method because it eliminates the interferences from the MEKC pseudo-stationary phase.

For the studies using ILs, it was shown these liquids have a great potential for use as mobile phase modifiers. ILs are proving to be increasingly promising as viable media for not only potentially "green" synthesis and separation operations, but also for novel applications.[3-5] The unique property set of the IL materials will provide new options based upon different chemical and physical properties. The range and variability in the properties between individual examples within the class of solvents that are known as ILs, however, are both challenges and opportunities for developing new and improved processes. Given the continued interest in ILs, it will be necessary to redesign chemical processes to reduce or eliminate losses of solvents, particularly in situations where these are volatile organic compounds (VOCs). For separation purposes, the trend will be to move towards the use of ILs as mobile phase modifiers,[6,7] as eluent in HPLC,[8] as stationary phase in gas-liquid chromatography,[9] as coating in aqueous CE,[10, 11] as

electrolytes in non-aqueous CE,[12, 13] and as background electrolyte in CE.[14, 15] In addition, the synthesis of chiral ILs will provoke their use in chiral separations.

Finally, for the studies involving the separation of 2-Pyr, VA, and NVP monomer residues in PVP homo-polymer and PVP/VA copolymer, the MEKC technique using the CE high-sensitivity cell gave some promising results. It will be necessary to establish the right internal standard for the quantitative analysis of the monomer residues in the polymer samples. The success of the developed method would lead to separation and quantitative analysis of the monomer residues in the polymer samples in a single run. This would help BASF to cut the costs of operation by eliminating the use of three HPLC systems to using one CE system.

6.3 References

1. Kapnissi, P. C., Valle, B. C., Warner, I. M. *Anal. Chem*. **2003,** 75, 6097.

2. Liu, Z., Otsuka, K., Terabe, S. *Chromatography* **2000**, 21(4), 302.

3. Anastas, P. T., Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, **1998**.

4. Rogers, R. D., Seddon, K. R. *Ionic Liquids: Industrial Application for Green Chemistry*; American Chemical Society: Washington, **2002**.

5. Rogers, R. D., Seddon, K. R. *Ionic Liquids as Green Solvents*; American Chemical Society: Washington, **2003**.

6. Mwongela, S. M., Numan, A., Gill, N. L., Agbaria, R. A., Warner I. M. *Anal. Chem.* **2003**, *75*, 6089.

7. Zhang, W-Z., He, L-J., Liu, X., Jiang, S-X. *Chinese Journal of Chemistry* **2004**, 22(6), 549.

8. He, L., Zhang, W., Zhao, L., Liu, X., Jiang, S. *J. Chromatogr. A* **2003**, *1007*, 39.

9. Armstrong, D. W., He, L., Liu, Y-S. *Anal. Chem.* **1999**, *71*, 3873.

10. Qin, W., Wei, H., Li, S. F. Y. *J. Chromatogr. A* **2003**, *985*, 447.

11. Jiang, T., Gu, Y., Liang, B., Li, J., Shi, Y., Ou, Q. *Analytica Chim. Acta* **2003**, *479*,

12. Vaher, M., Koel, M., Kalijurand, M. *Electrophoresis* **2002**, *23*, 426.

13. Vaher, M., Koel, M., Kalijurand, M. *J. Chromatogr.* **2002**, *979*, 27.

14. Yanes, E. G., Gratz, S. R., Baldwin, M. J., Robison, S. E., Stalcup, A. M. *Analyst* **2000**, *125*, 1919.

15. Yanes, G. E., Gratz, R. S., Baldwin, J. M., Robinson, E. S., Stalcup, M. A. *Anal. Chem.* **2001**, *73*, 3838.

Vita

Simon Muli Mwongela was born in Machakos District, Kenya, on September 9, 1974, to Christian parents, Mr. James Mwongela Mutungu and Mrs. Agnes Kamanthe Mutungu. He grew up in Wenyeani village and attended Kilome primary school and later Matiani primary school. After elementary school, he was admitted to Siakago High School in 1988, but transferred to Machakos High School, where he was a successful student. At Machakos High School, he was a member of the Science Club, an educational society aimed at improving the student's knowledge, skills, and creativity in the sciences. His interest in chemistry began then, and his first creative project was on how to make a mosquito coil from the sap of a cactus tree. Mosquitoes are insects that carry the malaria parasites, and malaria is one of the leading killer diseases in Kenya. He served as the secretary of the Science Club for the 1990-1991 term. He was also involved in sports, especially basketball, and was active in high school debates and drama.

After graduating from Machakos School, he enrolled in the University of Nairobi in 1993 to pursue a bachelor's degree. At the University of Nairobi, he was a member of the University of Nairobi Chemical Club, a science club which helped students to acquire skills and knowledge in activities pertaining to chemistry other than those acquired academically. He graduated in 1997 and worked as a high school teacher at Nunguni High School, in Makueni district, Kenya, for a year.

In August 1999 Mr. Mwongela was enrolled at Louisiana State University (LSU) to pursue a graduate degree in chemistry. He taught Chemistry 2002 before he received a research assistantship from his advisor, Dr. Isiah Warner. Mr. Mwongela received an award for excellence in research and chemical productivity on November 2003 from the

Department of Chemistry at LSU. He is a member of the American Chemical Society and the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers. He was also a member of the International Christian Fellowship at LSU where he served as a recreation ministry leader and as treasurer.

He married Tabitha Mueni on May 26, 2001. They were blessed on January 9, 2004, with a baby boy, Timothy Keli.

Mr. Mwongela's dissertation focuses on the synthesis, characterization, and application of polymeric surfactants in the separation of chiral and achiral compounds using micellar electrokinetic chromatography and the use of ionic liquids as novel mobile phase modifiers.

The papers he has published or submitted for publication from his dissertation work are listed below.

- **Mwongela, S. M**., Akbay, C., Zhu, X., Collins, S.; Warner, I. M., "Use of poly (sodium oleyl-L-leucylvalinate) surfactant for the separation of chiral compounds in micellar electrokinetic chromatography" *Electrophoresis*, **2003**, 24, 2940-2947.

- **Mwongela, S. M**., Numan, A., Gill, N. L., Agbaria, R. A., Warner, I. M., "Separation of Achiral and Chiral Compounds Using Polymeric Surfactants and Ionic Liquids as Modifiers in Micellar Electrokinetic Chromatography." *Anal. Chem.*, **2003**, 75(22), 6089-6096.

- Zhu, X., **Mwongela, S. M**., Kamande, M. W., Thiam, S., Kapnissi, C. P., Warner, I. M., "Open-Tubular Capillary Electrochromatography/Electrospray Ionization Mass Spectrometry Using polymeric Surfactant as a Stationary Phase Coating." *Electrophoresis*, 2004, 25, 562-568.

- Tarus, J., **Mwongela, S.M**, Simuli, L. Rezik, A., Morris, K., Numan, A., Fletcher, K. A., Warner, I. M. "Influence of the Polydispersity of polymeric surfactants on Enantioselectivity of Chiral Compounds in Micellar Electrokinetic Chromatography" *Langmuir* **2004** 20, 6887-6895.

- **Mwongela, S. M.,** Siminialayi, N., Fletcher, K. A., Powe, A., Warner, I. M. "A Comparison of Ionic Liquids to Organic Solvents for Chiral Separations in Micellar Electrokinetic Chromatography" *submitted to analytical chemistry*.

- **Mwongela, S. M**., Numan, A., Fletcher, K. A., Powe, A., Warner, I. M., **"**Determination of 2-Pyrrolidone, Vinylacetate, and N-vinyl-2-pyrrolidone in Poly vinyl pyrrolidone Homo-polymer and Polyvinyl pyrrolidone/vinylacetate Co-polymer using Micellar Electrokinetic Chromatography." In p*reparation.*

He also presented his work in a number of scientific conferences including:

- **Simon M. Mwongela,** Noreen Siminialayi, Xiaofeng Zhu, and Isiah M. Warner "Ionic Liquids as Modifiers in Micellar Electrokinetic Chromatographic Separation of Polycyclic Aromatic Hydrocarbons." The Pittsburgh Conference of Analytical and Applied Spectroscopy, Chicago, Illinois, March 7-12, 2004.

- **Simon M. Mwongela,** Abdulqawi Numan, Rezik A. Agbaria, Nicole Gill, and Isiah M. Warner "Separation of Achiral and Chiral Compounds Using Polymeric Surfactants and Ionic Liquids as Modifiers in Micellar Electrokinetic Chromatography." The Pittsburgh Conference of Analytical and Applied Spectroscopy, Orlando, Florida, March 9-14, 2003.

- **Simon Mwongela**, Cevdet Akbay and Isiah M. Warner, "The Use of Amino-based Poly(sodium oleyl-dipeptide) Surfactants in the Separation of Chiral Compounds Using Micellar Electrokinetic Chromatography." The Pittsburgh Conference of Analytical and Applied Spectroscopy, New Orleans, Louisiana, March 17-22, 2002.

- **Simon Mwongela**, Cevdet Akbay and Isiah M. Warner, "The Use of Poly(sodium oleyl-leucylvalinate) Surfactant in the Separation of Chiral Compounds Using Micellar Electrokinetic Chromatography." Chirality Symposium, Orlando, Florida, July 15-19, 2001.

- **Simon Mwongela**, Cevdet Akbay and Isiah M. Warner, "A Novel Amino Acid-based Surfactant Poly(sodium-oleyl-L-leucylvalinate) for Separation of Chiral Compounds Using Micellar Electrokinetic chromatography." The Pittsburgh Conference of Analytical and Applied Spectroscopy, New Orleans, Louisiana, March 4-9, 2001.

- **Simon M. Mwongela**, Cevdet Akbay, Janet J. Tarus, and Isiah M. Warner, "A Novel Amino Acid Surfactant for Separation of Chiral Compounds Using Micellar Electrokinetic chromatography." Southeastern ACS conference, New Orleans Louisiana, December, 2000.

Simon Muli Mwongela is currently a candidate for the degree of Doctor of Philosophy in analytical chemistry, which will be awarded at the May 2005 Commencement.