CHARACTERIZATION OF SINGLE AMINO ACID BASED SURFACTANTS UNDECANOIC L-ISOLEUCINE AND UNDECANOIC L-NORLEUCINE IN THE PRESENCE OF DIAMINE COUNTERIONS WITH VARYING CHAIN LENGTHS

A Thesis

by

AMBER KAYLA-LEA MAYNARD-BENSON

BS, Texas A&M University-Corpus Christi, 2017

Submitted in Partial Fulfillment of the Requirements for the Degree of

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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August 2019

ABSTRACT

To understand chiral recognition and discrimination, single amino-acid-based surfactants undecanoic L-isoleucine and L-norleucine were synthesized and examined at various pHs with different counterions. Analysis was conducted utilizing different analytical instruments and techniques such as NMR and Capillary Electrophoresis (CE). Knowledge gained from this research will later be used to develop a database that will recognize behaviors of micelle systems and determine which parameters and materials will be most effective for enantiomeric separation.[1]

The focus of this particular study was to determine how varying the chain length of diamine counterions affects the properties of the surfactant. The following six counterions were investigated: 1,2-ethylenediamine, 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, and sodium. Sodium was employed as the standard counterion for comparison. Early work was performed using arginine and lysine as counterions before shifting focus to the diamines; this data is also included as supplemental information.

Data from this project has shown that the Critical Micelle Concentration (CMC) of surfactants is dependent upon the chain length of the diamine counterions. As the counterion chain length was increased, the CMC decreased. At pH 9 the CMC decreased from 12.19 mM undecanoic L-isoleucine with 1,2 ethylenediamine to a CMC of 2.00 mM for undecanoic L-isoleucine with 1,6 diaminohexane as the counterion. CE data showed that as the chain length of the counterion was increased, the enantiomers were resolved at a lower surfactant-counterion concentration. There was also improved chiral selectivity of BNA enantiomers in the presence of diamine counterions compared to the standard sodium. Based on previous literature, the micellar

size also increased with a decrease in CMC as expected. The average hydrodynamic radius of undecanoic L-norleucine with 1,2-ethylenediamine at pH 10 was 9.17 Å compared to 24.52 Å for L-norleucine with 1,6 diaminohexane at pH 10.

DEDICATION

I would like to dedicate this thesis to my husband N'gai who motivated me to stay the path and to always believe in myself. Thank you for allowing me to trade date nights and family time for late nights in the lab and quiet writing time. I love you.

To my children Myles and Nahla: Thank you for allowing Mommy to work during playtime and for only asking me 10 times if I was ever going to be done with school. May you always remain curious and continue to chase your dreams.

To my sisters Tammera, Jodi, and Jaime who taught me the importance of rivalry and competitiveness. Thank you for always pushing me to do more and be more.

Finally, to the woman who instilled in me the virtue of true perseverance, my mother and best friend, Tonia. You have always been and will always be my champion and for that I owe you the world.

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Many thanks to my fellow graduate students and friends who made my time at Texas A&M University-Corpus Christi an enjoyable and memorable experience. To Sebastian R., Keegan G., Corrie C., Pedro R., Savanna M., Zahra H., and Nhi N. - I couldn't imagine doing this without you. Sharing the ups and downs of research has allowed me to feel less anxious and more normal, if there is such a thing. Thank you for the friendships, the laughs, the frantic texts about deadlines but most importantly, thank you for the support.

I also want to extend my gratitude to my fellow research student and friend, Mariya, for her help with data collection and interpretation. Thank you for always listening to my hypotheses no matter how ridiculous.

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CHAPTER I: INTRODUCTION

1.1 Background

Surfactants are molecules that are amphiphilic in nature and contain both a hydrophobic tail and a polar head group.[2] This allows the surfactant to have both hydrophobic and hydrophilic interactions when it is in solution. When the concentration of surfactant is increased to a specific point known as the critical micelle concentration (CMC), the molecules begin to aggregate with the hydrophobic tails pointing inward, and the hydrophilic head groups facing outward forming a micelle.[3] The micelle is a product of hydrophobic interactions that reduce the amount of free energy in the system.[4]

Surfactants are useful in many different fields such as the petroleum industry, pharmaceuticals, detergents, and cosmetics.[5] There is an increase in demand by manufacturers, consumers and even congress to provide a greener alternative to chemicals in the environment.[6] Alternatives that are less toxic and easily produced with minimal byproducts are often favored. Amino acid based surfactants fit that category since they are biodegradable, antimicrobial and come from a renewable source.[7] They are easily synthesized, environmentally friendly and relatively inexpensive.

The surfactants synthesized for this research were undecanoic L-isoleucine and undecanoic L-norleucine. Isoleucine and norleucine are both isomers of the amino acid leucine; however, only isoleucine is naturally occurring and is often found in meats, fish, cheese eggs and seeds.[8] Norleucine, unlike isoleucine, is an unnatural amino acid that is similar in structure to methionine without the sulfur atom.[9] Both surfactants contain a hydrophobic tail with 11 carbon atoms and a hydrophilic amino acid head group. These surfactants are considered anionic and carry an overall net negative charge in the pH range studied, pH 7 to 11.

1.2 Research Purpose and Proposed Experiments

The purpose of this study was to determine the effect, if any, external factors such as pH and type of counterion play in changing the properties of several amino acid based micellar systems. Some of the properties that were examined include chiral recognition ability, critical micelle concentration, as well as the size of the micelles.

The systems were characterized by utilizing a variety of techniques such as proton NMR with water suppression to determine the critical micelle concentration (CMC) of the surfactant, Diffusion Ordered Nuclear Magnetic Resonance Spectroscopy (DOSY) to study the hydrodynamic radius of the micellar systems and to determine the fraction bound of the surfactants and the various counterions to the micelles and Micellar Electrokinetic Chromatography (MEKC) to examine the effect of pH and counterion type on the chiral recognition ability of these systems.

CHAPTER II: LITERATURE REVIEW

2.1 Effect of Counterion on Critical Micelle Concentration

An article by Rothbauer et al.[10] provides insight into how pH can affect micelle formation. This is a particularly relevant as it highlights the proportional connection between a low CMC and low pH for surfactant-counterion solutions. The article details the binding of surfactants below and above pH 9 as well as its relationship to the hydrodynamic radius of the micelle.

This study looked at the CMC of amino acid-based surfactant, undecylenic L-phenylalaninate (und-Phe) with arginine and lysine as counterions. Even though this study detailed a correlation of a high pH with high CMC and low pH with low CMC as seen in other literature, another important trend was also observed. As the pH of the surfactant with arginine solution increased the fraction of the counterion (arginine) bound to the micelle decreased, which was also seen in a article by Koyama.[11] In that article, Koyama discussed the effect of the counterion species on micelle behavior and the relationship between CMC and fatty acid chain length.[11] The study by Koyama found an inverse relationship between the degree of counterion binding to the CMC of the surfactant solution.[11]

2.2 Critical Micelle Concentration Dependence on Surfactant Chain Length

Another study by Inoue and Yamakawa[12] looked at the micelle formation of nonionic surfactants and how CMC is dependent upon the surfactant chain length. This work described how micellization is an entropy-driven process at lower temperatures but changes to an enthalpy-driven process when the temperature is increased.[12] This is supportive of the hypothesis that the micelle formation is dependent upon the interaction between the hydrocarbon chain of the

surfactant and its aqueous environment, which would also mean that the CMC would then also depend on the chain length of the surfactant.[12] Using ^{1}H NMR, the CMC was determined from the proton chemical shift and plotted against the surfactant concentration. Measurements for CMC were taken at both 25 °C and 30 °C. The logarithm of the CMC was plotted versus the number of carbons in the hydrocarbon chain of the surfactant and a correlation between CMC and chain length was observed. The data plotted from Inoue and Yamakawa's study suggests that the value of the CMC decreases with an increase in hydrocarbon chain length of surfactant. This reflects the idea that the hydrocarbon chain acts as a solvophobic group.[12] The micellar size and aggregation number also decreased as the CMC increased. Their work shows the effect of surfactant chain length on the CMC which can be applied to this research to identify similar trends with counterion chain length effect on CMC.

2.3 The Effect of pH on the Binding of Counterions

Related work on the effect of pH on the binding of counterions with L-undecylenic leucine was performed by Lewis et al.[13] which detailed how increasing the pH of the surfactant solution did not affect viscosity or fraction of surfactant bound. This study has given insight into how counterions can bind to the micelle surface. It was noted that even though larger hydrodynamic radii of undecylenic L-leucine (und-leu) micelles were observed at higher pH levels when Na^{+} was used as a counterion, that same trend was not seen when lysine was used as a counterion. Lysine has two amine groups that are believed to begin losing their positive charge when the pH is above 9. The hydrodynamic radii of und-leu micelles with the counterion lysine remained constant despite changes in pH, suggesting that bridging could occur between two surfactant molecules and the amine groups on lysine.[13] A Rotating-frame Overhauser

Enhancement Spectroscopy (ROESY) experiment was performed to verify this observation and it revealed that the counterion was indeed binding parallel to the micellar surface and forming a bridge-like structure. It was also noted that the counterions seem to bind only when they are cationic but dissociate when they deprotonate.[13]

Studying these related works from Inoue[12] and Lewis[13], a reasonable hypothesis can be made that varying the chain length of the counterion will affect both its CMC and the manner in which it will bind to the surface of the micelle. From Inoue's study it is believed that the increasing the counterion chain length will decrease the CMC by inducing a hydrophobic effect and creating a less soluble micellar complex. From Lewis's study it is hypothesized that the diamines may form a similar bridge-like structure where the two amine groups will bind to two separate polar head groups, as long as the counterion is still cationic. To test this hypothesis, several studies were completed to determine if the same trend was observed while varying the diamine counterion chain lengths at various pHs.

CHAPTER III: INSTRUMENTATION

3.1 Capillary Electrophoresis

Capillary electrophoresis (CE) was originally introduced as a separation technique to separate analytes based on charge and size through the use of an applied electric field.[14] A similar technique was available using gas chromatography (GC) and high-pressure liquid chromatography (HPLC). However, GC worked with volatile substances and was not suitable for high molecular weight analysis and HPLC analysis can result in band broadening and wide peaks. CE has been proven to be more favorable in some instances over GC and HPLC as it is simple to understand and operate, highly effective in its intended purpose and it utilizes a very small sample volume.[15] The small sample volume needed is due in part to the small diameter size of the capillary, approximately 50 μm on average.[14] The sample size used in CE is approximately 1-40 nL whereas HPLC sample sizes are approximately 20 μL and GC uses approximately 1 μL sample size.[14] CE can also be classified as a greener option than HPLC since it does not involve the use of hazardous organic solvents. This type of analysis also produces a small amount of waste because the required amount to analyze is much less than HPLC or GC.[15]

The ability to use CE to better understand chiral recognition will give many researchers the opportunity to predict a selector that would best separate enantiomers.[16] If two molecules are non-superimposable mirror images of one another they are considered enantiomers and will have identical chemical and physical properties in an achiral environment.[17] This is particularly important in the field of pharmaceuticals where administering a drug that is not enantiomerically pure can be detrimental to the patients' health as seen in the case with thalidomide administered to pregnant women from 1957 to 1961. Administering this drug without separating the enantiomers led many children to be born with abnormalities and deformities.[18]

CE is based on the principles of electrophoresis, a separation technique that involves moving a sample through a liquid medium.[16] The effectiveness of electrophoresis is influenced by many factors including viscosity, the buffer chemistry, and the buffer concentration.[19] For this project a buffer that interacts with the sample through non-covalent bonding was chosen.[19] Also required was that the buffer be widely available, inexpensive and able to maintain solubility of the sample. A borate buffer proved to be the best choice for this project and was prepared by dissolving boric acid in ultrapure type I water and adjusting the pH as needed using sodium hydroxide pellets.

The CE column in this research was a fused silica capillary. When the silica comes in contact with an aqueous solution, siloxane bonds hydrolyze into silanol groups which can then ionize when the pH is above 3.[15] Because the buffer solution used here had pH values that were always greater than 3, the abundant silanol (-OH) groups on the surface of the silica capillary were deprotonated and negatively charged silanate ions ($-SiO^-$) were formed[20] as shown in the basic chemical reaction below:

$$SiOH \rightleftharpoons SiO^- + H^+$$

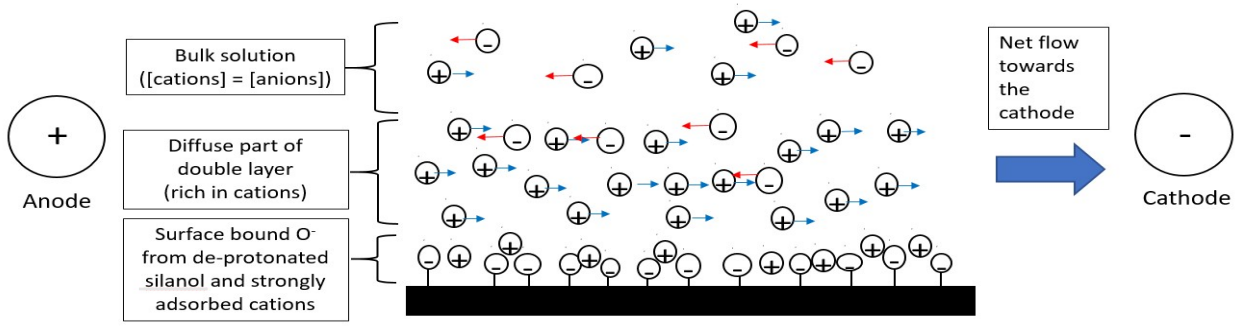


Figure 1. Depiction of electric double layer and movement of the species in solution as shown in Capillary Electrophoresis by Raja [20]

Figure 1 depicts a view of the fused silica capillary after the voltage is applied. When the silanol anions pair with the cations within the buffer solution a double layer rich in cations is

formed on the wall of the capillary. The remainder of the buffer cations are pulled towards the negative electrode and drag the bulk solution with them. This is referred to as electroosmotic flow (EOF).[14] EOF is pH dependent and an increase in pH or a decrease in the buffer's ionic strength will affect the double layer and the EOF will increase.[21] This increase in EOF is caused by an increase in silanate ions on the surface of the capillary. This study required that the EOF be stronger than the electrophoretic mobilities of the solutes in order for the negatively charged species to reach the negative cathode. To measure the EOF, a neutral maker is often injected to determine its migration time to the detector. Here, methanol was used as the neutral marker. Signals detected from the neutral marker and analyte are displayed in the electropherogram. Similar to a chromatogram, the electropherogram is a plot with the time from injection on the *x* axis and the detector signal on the *y* axis.[14]

In addition to EOF, separation by CE is also dependent on the electrophoretic mobility of charged species. Electrophoresis describes the migration and subsequent separation of charged particles (ions) in the presence of an electrical field.[22] When an electric field is applied to the charged particles, the ions will be attracted to the oppositely charged electrode. This is known as electrophoretic mobility.[23] Charged analytes possess an electrophoretic mobility that is dependent on the charge and size of the ion as well as the properties of the buffer solution it is in such as viscosity. Looking at eq 1, where q = ion charge, η = solution viscosity and r = ion radius, it is apparent that smaller, highly charged species have high mobilities whereas large or lesser charged species have low mobilities.[23]

$$\mu_e = \frac{q}{6\,\pi\,\eta\,r} \tag{1}$$

When the outlet buffer vial is located at the negatively charged cathode, as it is in this research, the small highly charged cations will migrate faster than large less charged cations. Additionally, anions will be attracted to the positive inlet (anode), however, the EOF is generally stronger than the electrophoretic mobility of the anion and therefore the anion will still migrate towards the negative outlet (cathode). Figure 2 below is a representation of how different charged species will migrate and spatially separate based on EOF and electrophoretic mobility with the use of an applied electric field.[24] The letter size in Figure 2 represents the size of the ion (large letter A = large ion radius) and superscript indicates the charge of the ion.

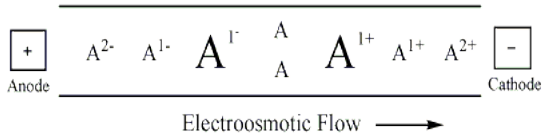


Figure 2. Separation of different charged ions under an applied electric field according to EOF and electrophoretic mobility with pH buffer solution greater than 3.[24]

3.1.1 Micellar Electrokinetic Chromatography (MEKC)

The classic mode of CE as described in section 3.1 *Capillary Electrophoresis* is not capable of separating neutral analytes. The lack of charge on neutral analytes means the analytes lacks self-electrophoretic mobility that results in a migration towards the detector at the same velocity as the electroosmotic flow (EOF).[16] Therefore, no separation occurs with a neutral analyte under classical CE conditions because the EOF and neutral analyte will elute together as one single unresolved peak.[16] In order to separate neutral analytes that do not possess self-electrophoretic mobility, additional separation mechanisms must be added to the classic mode of CE. Possible mechanisms include buffer additives such as surfactants or other chiral selectors

such as bile salts that will solubilize hydrophobic solutes; surfactants are one of the most commonly used buffer additives for separation of neutral analytes.[23]

Micellar electrokinetic chromatography (MEKC) is a mode of CE, more specifically, it is a mode of capillary electrokinetic chromatography (CEKC).[25] Terabe et al.[26] introduced this technique in 1984 which uses electrokinetic migration and chromatographic separation together to separate neutral species. MEKC uses the same instrumental setup as normal CE with the addition of micelles that act as a pseudostationary phase for the uncharged solutes.[2] Surfactants are amphiphilic and therefore are recognized by their head group, anionic for surfactants used here, allowing them to serve as a pseudostationary phase.[2] Adding micelle systems to the solution allows for some of the analytes to interact with the micelles and therefore, migrate as a function of its incorporation with the micelle.[3] An analyte that is more tightly bound with the micelle will be retained longer in the column.

3.1.2 Chiral Separation using Micellar Electrokinetic Chromatography (MEKC)

MEKC, in addition to its ability to separate neutral species, is also a powerful technique for enantiomeric separation of chiral compounds.[27] As previously stated, enantiomers will have identical chemical and physical properties in an achiral environment, therefore placing a chiral compound in a chiral environment will allow enantiomers to interact differently and separate by their differences in affinities for the micelle.[17] Enantiomeric separation usually requires a chiral selector, which in this research is the anionic amino acid-based surfactant. This technique is applicable to both neutral and charged analytes.

3.2 Proton Nuclear Magnetic Resonance (^{1}H-NMR)

NMR is the most widely used technique to identify and analyze compounds. NMR can provide valuable information about the structure of molecules, as well as the environment and dynamics. The magnetic environment of the nuclear spin is influenced by a variety of interactions including hybridization, bonding, charge and polarity.[28] NMR rests on the magnetic properties of the atomic nuclei. When a sample containing protons is placed in a magnetic field, the magnetic moment of the protons interact with the external magnetic field and adopt a specific orientation.[28] Some align parallel with the external magnetic field and others antiparallel. The parallel orientation has lower energy compared to antiparallel and energy is required to flip the nuclei from that lower energy to the higher energy level. The energy required to flip the nuclei comes from electromagnetic radiation in the radiofrequency (RF) region. When the system is exposed to a resonant frequency, energy is absorbed. Absorbing the energy will excite the nucleus and flip it to the higher energy state. At this point, the nuclei are in resonance with the applied radiation, and therefore, the process is called nuclear magnetic resonance.[28] The energy difference increases with higher magnetic field strength. In order to observe an NMR signal, the number of nuclei in the lower energy level must be greater than the number of nuclei in the high energy level.[29] This requirement is explained through thermodynamics and distribution of the two states is given below in eq 2, the Boltzmann relation, where N_α is the number of nuclei in the excited state, and N_β is the number of nuclei in the ground state, ΔE is the energy difference between the two states, k_B is the Boltzmann constant and T is the temperature. [28] Decreasing the temperature will increase signal intensity; however, solubility of the sample will decrease and the solute may precipitate.

$$\frac{N_\alpha}{N_\beta} = e^{-\Delta E/k_B T} \quad (2)$$

NMR operates by injecting a sample into the spectrometer and locking onto a signal from a deuterated solvent. In this study the signal was from 10 % D_2O with 90 % H_2O. This locking operation helps to keep the magnetic field steady.[28] Once the sample is locked it is shimmed to create a more homogenous magnetic field. After shimming, a pulse sequence is performed which excites the nucleus to a higher energy level. The nuclei begin to relax, followed by a time delay that allows their return to a lower energy level. The time delay is followed by an irradiation pulse that causes rapid transition between energy levels and causes the nuclei to realign in equilibrium with the induced magnetic field.[28]

3.2.1 Determining Critical Micelle Concentration using ^{1}H-NMR

There are over 70 different methods for measuring the critical micelle concentration (CMC) of surfactants. Various methods include surface tension, electric conductance, calorimetric measurements, colligative property investigation, refractive properties, light scattering, and diffusion coefficient.[25] NMR was the technique best suited for determining the CMC for the systems studied here as it is easily measured and produces reliable and reproducible data.

Changes in the environment surrounding the nucleus can cause small changes in the resonant frequency of the nuclear spin. The change in resonant frequency is known as chemical shift.[28] If the nucleus is placed in a magnetic field, electron currents will be induced in the molecule perpendicular to the magnetic field. When the currents from the electrons produce a small magnetic field, it partially cancels the applied magnetic field and is known as shielding.[30] If the nucleus is shielded, it will experience a decrease in chemical shift and feels a weaker

magnetic field due to the addition of electron density in neighboring atoms. When the nucleus is shielded it will resonate at a lower frequency and a chemical shift will be seen upfield, meaning the signal will be seen at a lower ppm and lie towards the right side of the spectra.[29]

This chemical shift can be used to determine the CMC of surfactant solutions. Since physical changes occur at the CMC, it is easily measured by observing the proton shifts that depend on the surfactant concentration.[31] In this research, when the micelle begins to form, a change in interactions with nearby atoms causes the amide and chiral hydrogens to become more shielded resulting in an upfield chemical shift.[32] This increase in nuclear shielding could be caused by the reduction in magnetic field experienced by the chiral and amide hydrogens.

3.3 Diffusion Ordered Spectroscopy (DOSY-NMR)

Diffusion ordered spectroscopy (DOSY) is a mode of NMR obtained using ideas from Pulsed Field Gradient NMR (PFG-NMR).[33] DOSY-NMR measures the ability of molecules to translationally diffuse through solution.[33] Smaller molecules diffuse faster through solution and have a larger diffusion coefficient. In contrast, when the surfactant molecules aggregate to form micelles, the micellar complex will be larger than individual surfactant molecules and therefore it will have a smaller translational diffusion coefficient.

DOSY experiments allow for analysis of mixtures and can give insight into molecular interactions within the mixture. DOSY is a reliable source of data for diffusion, structure, and binding information. However, DOSY also has many parameters that affect the reliability of that data. In the Stokes-Einstein equation (eq 3) k_B is the Boltzmann constant, T is the temperature in Kelvin, η is the viscosity of the solution in Pascal seconds and $D_{micelle}$ is the self-diffusion

coefficient of the micelle. In eq 3, D_{micelle} is directly proportional to temperature and inversely proportional to hydrodynamic radii.

$$D_{micelle} = \frac{k_{\text{B}} \bullet T}{6\Pi \bullet \eta \bullet R_{\text{h}}} \quad (3)$$

Therefore, if the temperature is not measured accurately the diffusion coefficient in return is not accurate, as increasing the temperature will also increase the diffusion coefficient. The Stokes-Einstein equation is also specific for spherical particles and is not accurate if the shape is rod-like or ellipsoid shaped. Typical micelles form at the CMC and are spherical in shape, but as the concentration increases, they can grow and change shape. However, micelles may also form at the CMC in other various shapes including a cylinder, bilayer, rod, or other worm-like micelle.

Surfactant systems are "crowded" and the average spacing between solute molecules is very small. The diffusion of micelles and solvent may decrease with increasing surfactant concentration due to the obstruction of the diffusion path and not necessarily because the radius is increasing.[31] Specific details on DOSY experiments and how they were conducted is outlined in section 5.2 Diffusion Ordered Spectroscopy (DOSY) NMR Methodology.

3.3.1 Tetramethylsilane (TMS)

Tetramethylsilane (TMS) is added to all DOSY experiments conducted above the CMC. The main purpose of including TMS in DOSY experiments here is that TMS molecules solubilize inside the und-IL and und-NL micelles. By analyzing the decay in TMS signal, the diffusion coefficient of the micelle, D_{micelle}, can be determined.[10] The chemical formula for TMS is $C_4H_{12}Si$, where all 12 hydrogens atoms are equivalent, as are the 4 carbons. Silicon is less electronegative than carbon and donates electrons to the methyl groups which causes them to become more shielded by the increase in electron density. This increase in shielding produces a

single sharp signal at the right-hand side of the spectrum.[28] This is used as a reference and chemical shifts are measured relative to this line. TMS is relatively cheap and readily available. It is inert and there is no reaction between TMS and the NMR sample. TMS also has a low boiling point and can be removed through evaporation.[28]

CHAPTER IV: SYNTHESIS OF SURFACTANTS

4.1 Synthesis of L-Isoleucine and L-Norleucine based Surfactants

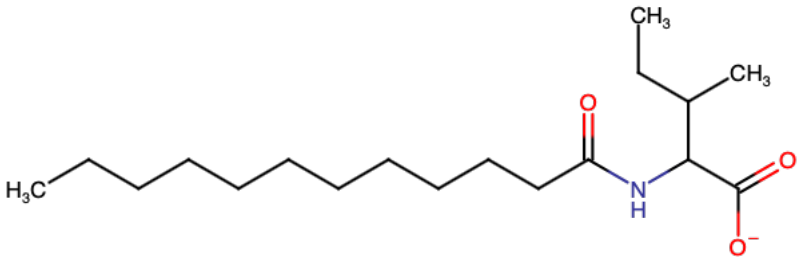
The protocol by Lapidot[34] was used to synthesize the amino acid based surfactants. Approximately 10 g of amino acid powder was added to 10 g of sodium bicarbonate and mixed with 150 mL of ultra-pure type I water in a round bottom flask. The solution was allowed to mix until it became clear. Next, 10 g of undecanoic N-hydroxysuccinimide (NHS) was added to the mixture along with 50 mL of tetrahydrofuran (THF). This solution mixed on a stir plate with a magnetic stir bar for at least 24 hours. Once the reaction was complete and all starting materials were dissolved, the flask was placed on the rotary evaporator to remove residual THF. The solution was then filtered using a Büchner funnel under vacuum to ensure all residual non-soluble THF not previously removed by the rotary evaporator had been removed.

Next, the solution was filtered using a micro-filter to remove any non-water-soluble contaminants. The pH was then lowered using concentrated 12 M hydrochloric acid (HCl) to precipitate the product. HCl was continuously added until there was no visible sign of further precipitate forming. The precipitant (product) was filtered using a clean Büchner funnel under vacuum with the use of ultra-pure type I to rinse the funnel and ensure the maximum amount of product was recovered. The final product on the filter paper was transferred to a beaker which was then placed in the drying chamber of a LABCONCO freeze dry system. The product was dried for a minimum of 24 hours at approximately -50 °C under vacuum.

Once the beaker containing the final product was removed from the freeze dry system, ^{1}H NMR was performed to check for impurities. If impurities were noted in the NMR spectra, the surfactant required purification using petroleum ether. Impurities were observed in the undecanoic L-isoleucine spectra; this product was subsequently purified twice to remove the

impurities. Petroleum ether was chosen due to its ability to remove nonpolar contaminants. Figure 3 below depicts the structures of undecanoic L-isoleucine (A) and undecanoic L-norleucine (B) after synthesis at a high pH.

A.



B.

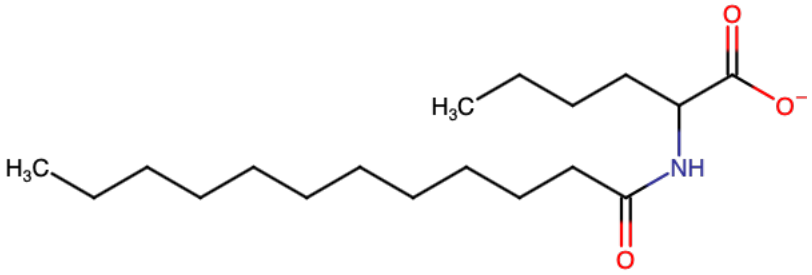


Figure 3. (A) Undecanoic L-Isoleucine and (B**)** Undecanoic L-Norleucine

4.2 Purification with Petroleum Ether

The undecanoic isoleucine product was completely dissolved in THF in a large beaker. After complete dissolution, approximately 300 mL of petroleum ether was added to precipitate the product. After the product formed crystals, it was filtered with a Büchner vacuum filter, once again leaving the product on the filter paper. The product was then transferred to a clean glass jar for storage and an additional ^{1}H NMR experiment was conducted to check for the removal of the impurity.

CHAPTER V: METHODOLOGY

5.1 Proton NMR (^{1}H NMR) Methodology

Sample purity was analyzed using a Bruker 300 MHz spectrometer. Samples were prepared in an NMR tube using a small amount of final product then adding ~1mL deuterated solvent, in this case, deuterated chloroform. This sample was then analyzed by ^{1}H NMR spectra. ^{1}H NMR spectra were also used to determine the critical micelle concentration (CMC) of the surfactants with varying counterions between pH 8 and 11. A 50 mL stock solution was prepared at 50 mM using equal moles of undecanoic amino acid-based surfactant and counterion. The solvent used consisted of 90 % milli-Q water and 10 % deuterium oxide.

After the 50 mM stock was created, it was separated into 4 different centrifuge tubes consisting of 10 mL each. These samples were then adjusted to pH values of 8, 9, 10 and 11. The extra 10 mL remaining was used as needed to assist with pH adjustments. Each of these stock solutions were then diluted to the concentrations 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20 and 50 mM, using 90 % water and 10 % deuterium oxide. ^{1}H NMR spectra of the samples were taken with water suppression; the number of scans was varied based on concentration to get an adequate NMR signal. Shifts in the amide and chiral hydrogens were used to calculate the critical micelle concentration.[6]

5.2 Diffusion Ordered Spectroscopy (DOSY) NMR Methodology

These experiments were described by Lewis[13] in his 2016 study. A series of NMR spectra were collected with increasing magnetic field gradient strength, G, using the bipolar pulse pair longitudinal encode–decode pulse sequence.[10] Typical G values ranged from 2.5 to 30.2 G cm^{-1}. The intensity of NMR resonances in these experiments decayed exponentially with a rate

proportional to the quantity, $(\gamma \bullet G \bullet \delta)^2 \bullet (\Delta - \delta/3 - \tau/3)$, where γ is the magnetogyric ratio, δ is the duration of the magnetic field gradient pulses, τ is the delay between the bipolar gradient pulses, and Δ is the diffusion time.[10] The Δ, δ, and τ values used in this study were 200, 4.0, and 0.20 ms respectively.

A plot of the natural log of peak intensity versus $(\gamma \bullet G \bullet \delta)^2 \bullet (\Delta - \delta/3 - \tau/3)$ produced a regression line with a slope equal to negative surfactant diffusion coefficient (D).[10] Resonances relative to TMS used for calculations of -D were as follows: 1,2-ethylenediamine, 2.60 ppm; 1,3-diaminopropane, 2.90 ppm; 1,4-diaminobutane, 2.74 ppm; 1,5-diaminopentane, 2.73 ppm; 1,6-diaminohexane 2.76 ppm. Resonance for undecanoic L-isoleucine and undecanoic L-norleucine polar head groups were 1.90 ppm and 1.00 ppm respectively.

Once the spectra were processed, the intensities of the TMS and surfactant signals were used to calculate the hydrodynamic radius, fraction bound of the counterions and fraction bound of the surfactant. The hydrodynamic radii were calculated using the eq 3 where D_{micelle} is the micelle diffusion coefficient, k_{B} is Boltzmann's constant, T is absolute temperature, η is the viscosity, and R_{h} is the micelle hydrodynamic radius. Three trials were conducted, consisting of 20 experiments each and the average radius was calculated. The faction of bound counterion was also determined by using eq 4 below:

$$f_{\text{b(CI)}} = \frac{D_{\text{obs(CI)}} - D_{\text{free (CI)}}}{D_{\text{bound(CI)}} - D_{\text{free(CI)}}} \tag{4}$$

Here $D_{\text{obs(CI)}}$ is the slope given by the regression line at 50 mM surfactant with counterion solution, $D_{\text{free (CI)}}$ is the slope of the regression line of the diffusion of counterion free in solution without surfactant, and $D_{\text{bound(CI)}}$ is the regression line of TMS at 50 mM surfactant counterion solution.

5.3 Micellar Electrokinetic Chromatography (MEKC) Methodology

Chiral separations were performed using a Hewlett-Packard (HP) 3D CE model #G7100A. A fused silica capillary was purchased from Agilent Technologies measuring 45 cm in effective length to the detection window, 56 cm in total length with a diameter of 50 µm. Stock solutions of 50 mM surfactant and counterion were prepared using 5 mM aqueous borate buffer at pH 7 as a solvent. The stock solution was then transferred into five separate centrifuge tubes consisting of 15 mL each. Once the solutions were transferred, the pH was then adjusted from 7 through 11 with the use of NaOH to increase pH or HCl to decrease pH. It was important to limit the number of added ions during this step to prevent salt buildup in the column. This was achieved by adding only HCl or NaOH, not a combination of the two to reach the desired pH. Once pH had been modified, various dilutions were made from the stock solution ranging from 50 to 10 mM in 5 mM increments. Prior to CE experiments, solutions were filtered through a 0.45 µm filter using a syringe. The capillary was conditioned for 30 minutes with 0.1 M NaOH followed by triply distilled water for 10 minutes. Analyte standards at 0.1 mg/mL were prepared using 1:1 ratio of methanol to water. The samples were injected at 10 mbar pressure for 5 s. Separation was performed at +30 kV with UV detection at 230 nm. Analytes utilized in this study as identified in Figure 4 were (±)1,1′-bi-2-naphthol (BOH), (±) 1,1′-bi-2-naphthyl-2,2′-diamine (BNA), and (±) 1,1′-bi-2-naphthyl-2,2′-diyl hydrogen phosphate (BNP).[27]

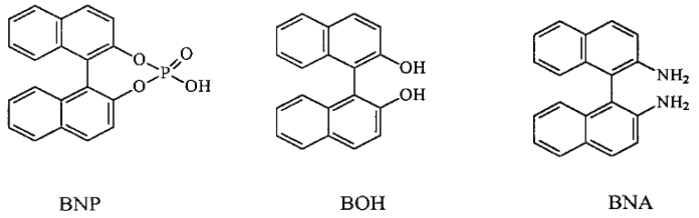


Figure 4. Structures of BNP, BOH, and BNA analytes.[35]

CHAPTER VI: RESULTS

6.1 Overview

To better understand where and how chiral discrimination takes place, undecanoic L-isoleucine (und-IL) and undecanoic L-norleucine (und-NL) were characterized by their CMC, average hydrodynamic radii, enantiomeric retention, and enantiomeric separation resolution. The data provided below gives insight into the micelle size and how steric factors may be affecting the physical properties of the micelles. External factors that were varied in this research included the chain length of the counterion and the pH of the surfactant solution. The temperature for both CE and NMR experiments remained constant at 25 ºC throughout all experiments. Additional variables that were not altered include buffer type and concentration (5 mM borate buffer) and NMR solvent (10 % D_20, 90 % H_20).

Data reported were found to support the hypothesis mentioned previously, that altering the chain length of the counterion lowers the CMC and therefore affects the chiral interactions taking place inside the micelles. Longer counterion chain lengths alter interactions between the polar headgroups which is reflected in the CE and NMR data.

6.2 Diamine Counterions

The counterions utilized in this research were diamines which consist of a hydrocarbon chain that terminates with two amine groups. Most are colorless when stored in unopened amber-colored bottle but tend to have a pale-yellow tinted appearance when exposed to air. The yellow appearance may be caused by oxidation of free radicals from the lone pair of electrons on nitrogen that is producing nitrogen oxides thus causing discoloration which is catalyzed by light leading to photodegradation. The linear diamine counterions used are air sensitive, highly

flammable strong bases. They are also nitrogenous and tend to produce a strong ammonia-like odor when they come in contact with air. Table 1 below details the structure and pKa values for the diamines that were used for this project as well as the standard counterion, sodium.

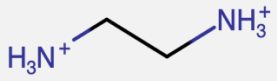
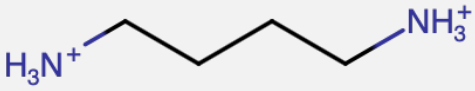
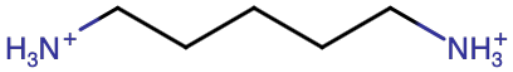
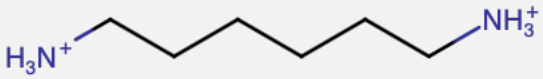
Counterion	***Counterion Structure***	***pKa***
1,2 Ethylenediamine (1,2-ED)		10.71
1,3 Diaminopropane (1,3-DP)		10.94
1,4 Diaminobutane (1,4-DB)		10.80
1,5 Diaminopentane (1,5-DP)		10.51
1,6 Diaminohexane (1,6-DH)		11.86
Sodium (as Sodium Bicarbonate)	Na^+	

Table 1. Properties of diamine counterions including structure at pH 10 and pKa values at 25 °C

6.3 Undecanoic L-Isoleucine (und-IL) Results

Experiments were conducted to study the behavior of und-IL and und-NL in aqueous solution as a function of pH and counterion type. The critical micelle concentration (CMC), average hydrodynamic radii (R_h), and fraction of bound counterions (f_b) were measured by NMR. Micellar Electrokinetic Chromatography (MEKC) was used for enantiomeric separation of (±) 1,1′-bi-2-naphthyl-2,2′-diamine (BNA).

The following sections will detail the observations of und-IL from ^{1}H-NMR and DOSY-NMR experiments followed by the observations of und-NL from ^{1}H-NMR, DOSY-NMR and MEKC experiments. Supplemental data provides additional information about enantiomeric separation of BNA using und-IL micelle systems with arginine and lysine as counterions. This data was compared to the diamine counterion studies because the concentration and pH ranges were different than in the diamine experiments.

6.3.1 Undecanoic L-Isoleucine Critical Micelle Concentration (und-IL CMC)

The critical micelle concentration (CMC) is very important when studying the behaviors of surfactants. A knowledge of the CMC of the surfactant solution is imperative when conducting diffusion studies as well as conducting CE studies. Most experiments need to be performed either above or below the CMC to obtain accurate results.

A series of ^{1} H NMR experiments were conducted using und-IL with diamine counterions of varying chain length to determine the CMC. When the micelles first form they are presumed to be spherical in shape but may grow above the CMC to form different shapes including rods or cylinders. However, it is also possible for different counterions to facilitate the formation of initial non-spherical shaped micelles at the CMC. Table 2 below lists the CMC values of und-IL at pH 10 with different diamine counterions as well as the CMC with Na^{+}. It is apparent that the CMC tends to decrease when the diamine chain length increases.

Undecanoic L-Isoleucine					
Na^+	1,2-ethylenediamine	1,3-diaminopropane	1,4-diaminobutane	1,5-diaminopentane	1,6-diaminohexane
12.95 mM	**12.19 mM**	**10.93 mM**	**5.75 mM**	**3.00 mM**	**2.00 mM**

Table 2. CMC values (mM) for Undecanoic L-Isoleucine at pH 10, 25°C

The highest CMC value seen with und-IL at pH 10 was 12.19 mM with 1,2-ethylenediamine and the lowest CMC seen with und-IL at pH 10 was 2.00 mM with 1,6-diaminohexane. Although this trend is not linear, a decrease in CMC is correlated with increasing counterion length, as all other factors remained constant including temperature, pH, and experimental methodology.

When the chain length of the counterion increases, the hydrophobicity of the counterion also increases. This increase in the overall hydrophobicity likely causes the surfactants to aggregate at lower concentrations. This is partly due to the entropic hydrophobic effect in which nonpolar molecules exclude water; the water molecules become less ordered and allow the hydrophobic entities, in this case the hydrophobic tail, to aggregate and form micelles.[36]

When placed in an aqueous environment, the surfactant solution containing the longer diamine chain causes a larger displacement of the ordered water molecules when compared to the shorter chain counterions; this disruption of ordered water molecules causes an increase in entropy which is more thermodynamically favorable for the formation of micelles.[37] The increase in counterion chain length will also increase the probability that the counterion attaches to two polar head groups through bridging that otherwise could not occur if the chain was shorter. This hypothesis was tested using DOSY-NMR by measuring the hydrodynamic radii of the micelles as the chain length of the counterion was increased.

6.3.2 Undecanoic L-Isoleucine DOSY-NMR Results

Diffusion ordered spectroscopy (DOSY)–NMR experiments were conducted using 50 mM undecanoic L-isoleucine in the presence of varying diamine counterions. Table 3 provides the average hydrodynamic radii (R_h) of the micelles. This data was obtained according to the methodology previously stated in section 5.2 Diffusion Ordered Spectroscopy (DOSY) NMR Methodology

Undecanoic L-Isoleucine	
Counterion	**R_h (Å)**
Sodium	9.68 ± 0.15
1,2-ethylenediamine	9.79 ± 0.23
1,3-diaminopropane	12.58 ± 0.19
1,4-diaminobutane	15.58 ± 0.08
1,5-diaminopentane	14.69 ± 0.32
1,6-diaminohexane	10.63 ± 1.75

Table 3. Average hydrodynamic radii (R_h) of undecanoic L-isoleucine under following conditions: 50 mM surfactant-counterion solution, 25 °C, and pH 10.

The hydrodynamic radius (R_h) is the radius of a hypothetical hard sphere that diffuses similarly to the particle studied. Since the hypothetical hard sphere is non-existent, the R_h value is an apparent size of a dynamic hydrated particle, meaning the radius includes both solvent (hydro) and shape (dynamic) effects.[38] When the diffusion constant is measured, the R_h value includes the radius of the micelle as well as the water molecules that may be attracted to the micelle, therefore it may be possible that a small ion may appear to have a large R_h simply because of an increase in water molecules present.

As the diamine counterion chain length is increased, R_h increases but then decreases at 1,5 diaminopentane and longer. The data reflects a similar micellar size for both sodium and 1,2-ethylenediamine as a counterion at 9.68 Å and 9.79 Å. This could be due to the relatively small size and low molecular weight of sodium and 1,2-ethylenediamine, 23g/mol and 60.1g/mol respectively. In DOSY experiments performed with 1,4-diaminobutane, R_h increases to 15.58 Å which is the highest R_h value recorded for und-IL for all DOSY experiments at pH 10 with 50 mM surfactant-counterion solution. After the micelle reaches its maximum R_h observed, it then begins to decrease slightly in size with 1,5-diaminopentane and decreases further with 1,6-diaminohexane. Numerous explanations are possible for the trend observed in Table 3, two of which are outlined below.

Scenario 1:

Counterion bridging with und-IL surfactants

Table 3 shows the largest R_h value of 15.58 Å for und-IL micelles was observed with 1,4-diaminobutane and may be due to the short chain length of the counterion. When the diamine counterion consists of a shorter chain (< 4 carbons) the distance between the polar headgroups is too large for both ends of the counterion to attach to two separate surfactant polar head groups. However, when the chain length is increased (5 carbons) with 1,5-diaminopentane the counterion is now capable of bridging the distance between two polar headgroups. Looking back to Table 1, the pKa value of 1,5-diaminopentane is equal to 10.51, which indicates that the majority of NH_3^+ on the counterions are still in cationic form at the studied pH of 10. Assuming that the positive charge is still present on the counterion, it is suggested through previous studies conducted by Lewis[13] and Rothbauer,[10] that this is the point at which the counterion may no longer be binding

to the micelle perpendicularly but rather parallel. If the counterion is binding parallel to the micelle it is possible for a single diamine counterion, in this case 1,5-diaminopentane, to attach to two negatively charged surfactant head groups and form a bridge between them as seen in Figure 5.

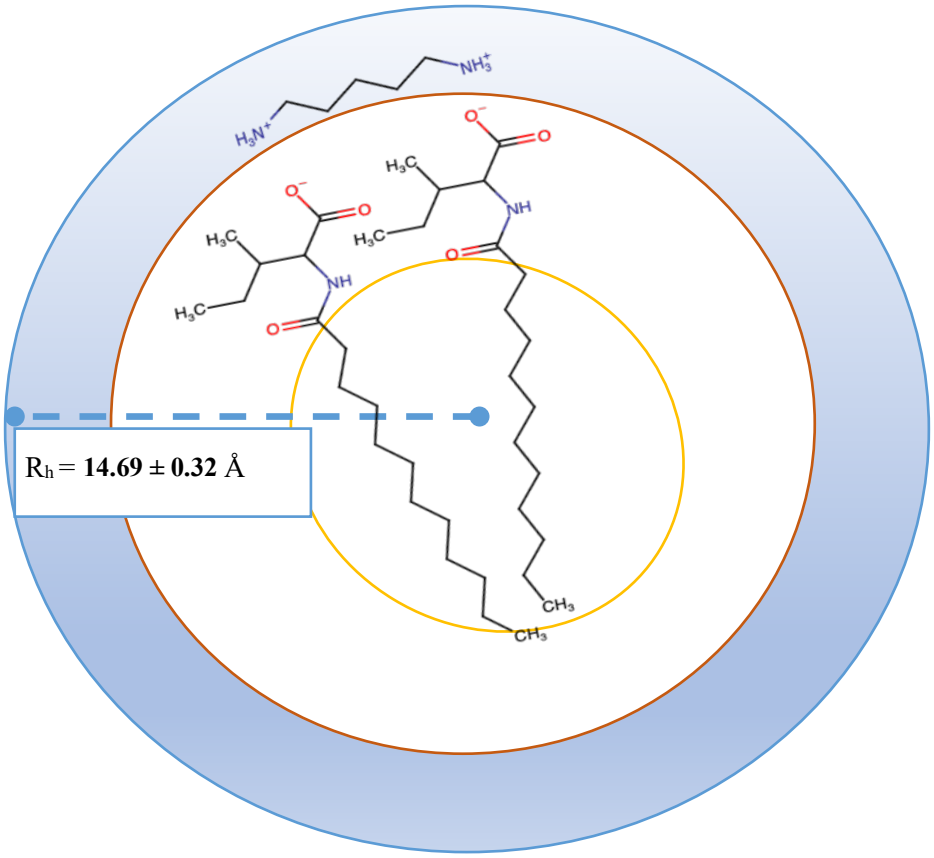


Figure 5. Depiction of 1,5 diaminopentane as a counterion is bridging with two undecanoic L-isoleucine molecules.

Scenario 2:

Hydrophobic burial of counterion

As previously stated, a decrease in R_h was observed in und-IL micelles with 1,5-diaminopentane. One possible explanation for the decrease in R_h is that the hydrophobic chain of the counterion buries itself in the hydrophobic core of the micelle and folds inwards. The terminal diamines would still be interacting with the polar head group by forming a bridge like

structure, however the hydrocarbon chain would be forming a "v"-like structure inside the core as shown in Figure 6. This is often referred to as hydrophobic burial and is driven by the hydrophobic effect, which describes the interaction between the aliphatic surfactant or counterion chain with the water molecules.

The diamine counterions used in this study are very soluble in water, however they become less soluble with an increase in chain length which may increase its hydrophobicity. If the diamine buries itself in order to reduce interaction with the water molecules surrounding the micelles, a reduction in R_h may be seen as the counterion chain is capable of folding several different ways to fit inside the micelle. The majority of NH_3^+ groups are still charged and hydrophilic and may still interact through electrostatic interactions with the negative polar head group. This scenario is an extension of Scenario 1 which includes bridging as well as hydrophobic burial of the counterion chain.

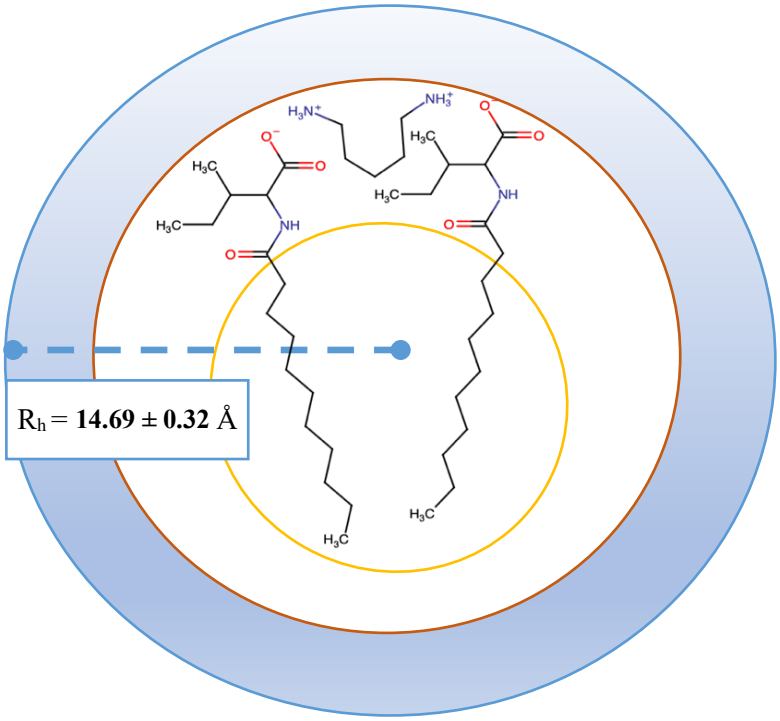


Figure 6. Depiction of 1,5 diaminopentane counterion burying itself inside the hydrophobic core of the undecanoic L-isoleucine micelle.

6.3.3 pH Effect on Hydrodynamic Radius of Undecanoic L-Isoleucine Micelles

To determine the effect pH may have on the average hydrodynamic radius of the micelle, DOSY-NMR experiments were conducted using 50 mM surfactant-counterion solution at 25 °C while increasing pH values of pH 9, 10, and 11. A small observable change in average radius as indicated in Table 4 suggests a similar trend to that described by Lewis et al.[13] The amine groups on the counterions used by Lewis et al.[13] possess two separate dissociation constants that are different from those used in this research and correlations cannot be made above the point of dissociation. However, concepts from the study conducted by Lewis et al. below the pKa may be applied to the interpretation of data from this research that was also conducted below the pKa. The study suggests that pH has minimal effect on the size of the micelle when the counterion is still below its pKa. This could possibly mean the remainder of the counterion that is not binding to the micelle may extending out into the hydration ring and therefore the R_h increases as the counterion chain length increases.

	pH		
	9	10	11
R_h (Å)	10.74	10.63	10.50

Table 4. pH effect on counterion binding with und-IL micelles at 50 mM surfactant-counterion solution and 25 °C.

6.3.4 Fraction of bound Counterion to Undecanoic L-Isoleucine

At concentrations below the CMC, the surfactant molecules and counterions will exist free in solution. At the CMC, the surfactant molecules begin to aggregate together with the hydrophobic tails pointing inward, and the hydrophilic head groups facing outward forming a

micelle.[3] The polar head groups may be experiencing electrostatic repulsion between the negatively charged carboxylate groups, therefore adding a counterion may help to stabilize the micelle.[39] The percent of counterions that are interacting with the micelles is referred to as the fraction of counterion bound, $f_{b(CI)}$. Table 5 shows the $f_{b(CI)}$ values for the various chain length diamine counterions associated with und-IL micelles at pH 10. Sodium was omitted from this comparison as it could not be examined under the same DOSY-NMR experiment.

Fraction of counterion bound to undecanoic L-isoleucine					
Sodium	1,2 Ethylenediamine ($f_{b(1,2\text{-ED})}$)	1,3 diaminopropane ($f_{b(1,3\text{-DP})}$)	1,4 diaminobutane ($f_{b(1,4\text{-DB})}$)	1,5 diaminopentane ($f_{b(1,5\text{-DP})}$)	1,6 diaminohexane ($f_{b(1,6\text{-DH})}$)
N/A	**0.22**	**0.34**	**0.39**	**0.29**	**0.34**

Table 5. Fraction of counterion bound to und-IL micelles at 50 mM surfactant-counterion solution, pH 10, and 25 °C.

The $f_{b(1,2\text{-ED})}$ was determined to be 0.22 which indicates approximately 22% of 1,2-ED counterions were interacting closely with the micelle surface. A maximum in the fraction of counterion bound to und-IL micelles of 0.39 is found with 1,4-diaminobutane (1,4-DB). The fraction of bound counterions continues to decrease with the use of 1,5 diaminopentane (1,5-DP) and 1,6-diaminohexane (1,6-DH).

Figure 7 is a line graph of $f_{b(CI)}$ and counterion pKa values as a function of the varying amount of carbon groups in the chain of the counterion. These results suggest that at pH 10, the counterions 1,5-DP and 1,6-DH may have an overall lower affinity for the und-IL micelles compared to 1,3-DP and 1,4-DB.

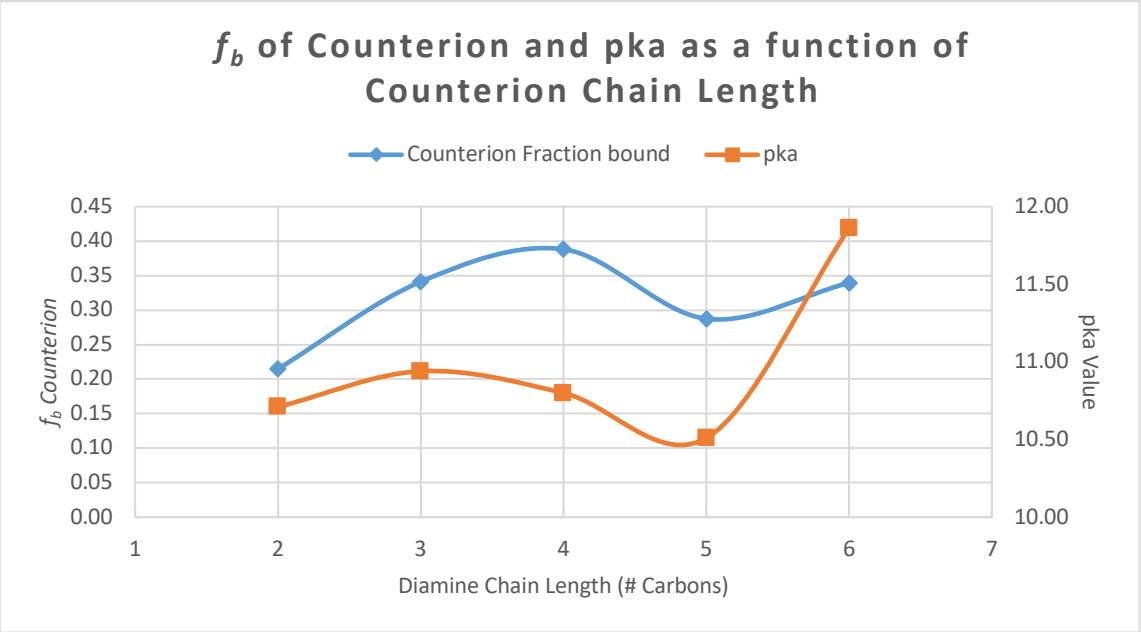


Figure 7. Fraction of bound counterion to und-IL micelles and counterion pka values both as a function of counterion chain length

As previously stated, the percentage of counterions bound to und-IL micelles steadily increases with the shorter chain diamines from 1,2-DB to 1,4-DB. However, a decrease in bound counterions was seen at 1,5-DP which is also the counterion that has the lowest pKa value, 10.51. With 1,5-DP having a pKa value closer to the pH value examined (pH 10), there are less counterions protonated which could explain why a decrease in bound counterions was observed. The increase in $f_{b(CI)}$ observed with 1,6-DH counterions could be in response to an increase in pKa (11.86) for 1,6-DH. The counterions 1,5-DP and 1,6-DH are also suspected of bridging between two surfactant molecules as shown in Figure 5 which may also be causing a decrease in the fraction of bound counterions.

6.4 Undecanoic L-Norleucine Critical Micelle Concentration (CMC) Results

The CMC of undecanoic L-norleucine was determined by the methods described in section 3.2.1. Data was collected over a wide range of concentrations to obtain data both above and below the CMC. The spectra were analyzed, and the chemical shift of the chiral hydrogen as seen in Figure 8 was plotted against the concentration of the surfactant

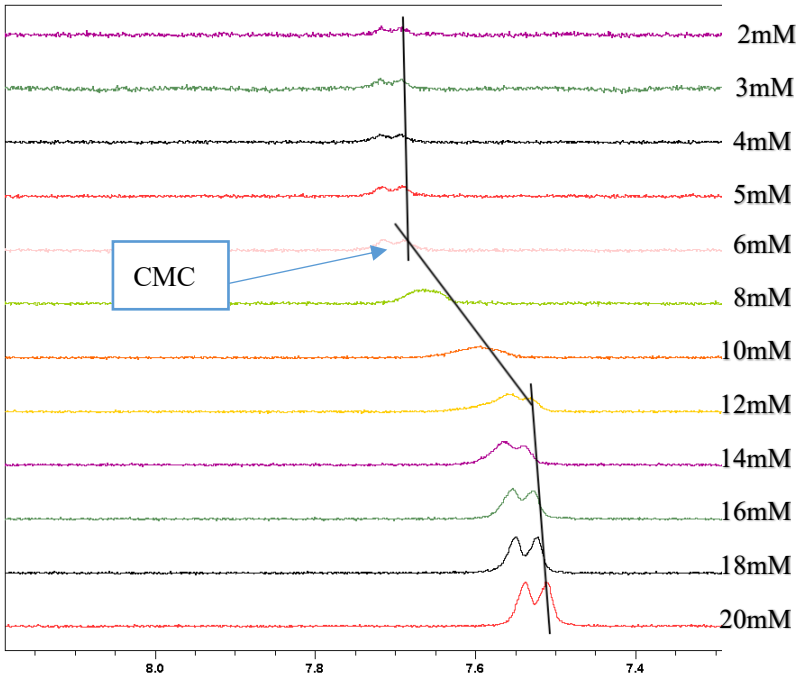


Figure 8. Chemical shift of the undecanoic L-norleucine chiral hydrogen 2 to 20 mM surfactant-counterion solution with 1,5-diaminopentane at pH 10; CMC is 6.08 mM

Two lines from the data series were fitted separately to obtain a line both above and below the CMC based on chemical shift. The CMC was determined as the point of intersection between those lines. Table 6 below shows the CMC values of und-NL with various counterions while other experimental parameters including temperature and pH remain constant

Undecanoic L-Norleucine					
Na^+	1,2-ethylenediamine	1,3-diaminopropane	1,4-diaminobutane	1,5-diaminopentane	1,6-diaminohexane
12.79 mM	**11.53 mM**	**8.97 mM**	**7.16 mM**	**6.08 mM**	**4.89 mM**

Table 6. CMC values (mM) for Undecanoic L-Norleucine at pH 10, 25 °C

As the counterion chain length of the diamine is increased, it appears the CMC is decreasing in response to the extra CH_2 group added. When 1,2-ethylenediamine was used as the counterion with und-norleucine the CMC was 11.53 mM which then decreased to 8.97 mM when it was replaced with 1,3-diaminopropane. This decrease in CMC continued across all the diamines studied and eventually reached 4.89 mM with the longest diamine counterion 1,6-diaminohexane. Although the diamine counterions are very soluble in water and share an attraction for the polar head group of the surfactant, the increase of CH_2 can increase the hydrophobicity of the counterion which likely lowers the CMC.

6.4.1 Undecanoic L-Norleucine DOSY-NMR Results

Once the critical micelle concentration was determined for und-NL, DOSY experiments were performed above and below the CMC to study the affect various chain length diamines have on the size of the micelle. The first step to studying the size of und-NL micelles was to measure the average hydrodynamic radius as a function of counterion. Table 7 lists the R_h values for 50 mM und-NL with diamine counterions at pH 10 as well as with sodium. As the counterion chain length was increased from two CH_2 groups with 1,2-ED to three CH_2 groups with 1,3-DP the R_h value more than doubled from 9.17 Å to 19.56 Å. With the addition of another CH_2 group to the counterion chain, the R_h doubled again from 19.56 Å to 39.38 Å with 1,4-DB. The use of

1,4-DB as the counterion also corresponded to a max R_h in und-NL micelles observed for all studied diamine counterions at the same experimental conditions of pH 10 and 50 mM surfactant-counterion solution. Several explanations are possible for the data observed in Table 7, two of which are outlined following the table below.

Undecanoic L-Norleucine	
Counterion	**R_h (Å)**
Sodium	11.94 ± 0.06 Å
1,2-ethylenediamine	9.17 ± 0.13 Å
1,3-diaminopropane	19.56 ± 0.82 Å
1,4-diaminobutane	39.38 ± 0.28 Å
1,5-diaminopentane	20.28 ± 1.56 Å
1,6-diaminohexane	24.52 ± 0.05 Å

Table 7. Average hydrodynamic radii of undecanoic L-norleucine under following conditions: 50 mM surfactant-counterion solution, 25°C, and pH 10.

Scenario 3:

Hydrophobic Hydration of Nonpolar Surfactant Chains

A possible explanation for the increase in R_h for und-NL micelles with 1,4-DB is the increase in distance between the polar head groups caused by the formation of hydrophobic pockets within the micelle as shown in Figure 9. As the nonpolar tails aggregate together, the hydrophobic R-group on the head group may also interact with one another causing an increase in steric interactions with the adjacent R-group. To elaborate, if the hydrophobic R-group tails on und-NL surfactant molecules angle themselves towards each other in the hydrophobic region this may create a space between the polar head groups. The negatively charged head groups as

previously mentioned may already experience electrostatic repulsion with similarly negatively charged neighboring head groups.[39] The distance between the head groups could make it difficult for the intermolecular hydrogen bonding between two surfactant molecules to remain intact. As proposed by Billiot[39] as well as Bordes,[6] hydrogen bonding occurring between the amide group and the neighboring surfactant carbonyl group has been found to assist in the self-assembly of the molecules. This increase in average hydrodynamic radius could be due to the breaking of some hydrogen bonds which allows water molecules to enter the space between head groups causing a larger hydration ring and causing the micelle to "swell".[13]

There may also be water around the hydrophobic chains as described by Long, et al. in a 2015 publication titled Micelle Structure and Hydrophobic Hydration.[40] Long describes the long standing misconception of a completely dry hydrophobic core within a surfactant micelle. In general, if space allows, water molecules may penetrate below the headgroups and surround interior hydrophobic regions. This study utilized Raman spectroscopy with multivariate curve resolution (Raman-MCR) to suggest the hydrophobic core contains hydrated non-polar cavities that increase in depth with increasing surfactant chain length.[40] This could be an explanation why und-NL micelles have a larger hydrodynamic radius compared to und-IL micelles. The linear hydrocarbon R-group on norleucine may allow hydrophobic pockets to form inside the hydrophobic core. A decrease in R_h is not seen until 1,5-DP is used as a counterion. The micelle might be stabilized through bridging of 1,5-DP to two surfactant molecule polar head groups which in turn might be decreasing the space between polar head groups. This tightening of space between surfactant head groups may allow the hydrogen bonding between the surfactant molecules to be reestablished.

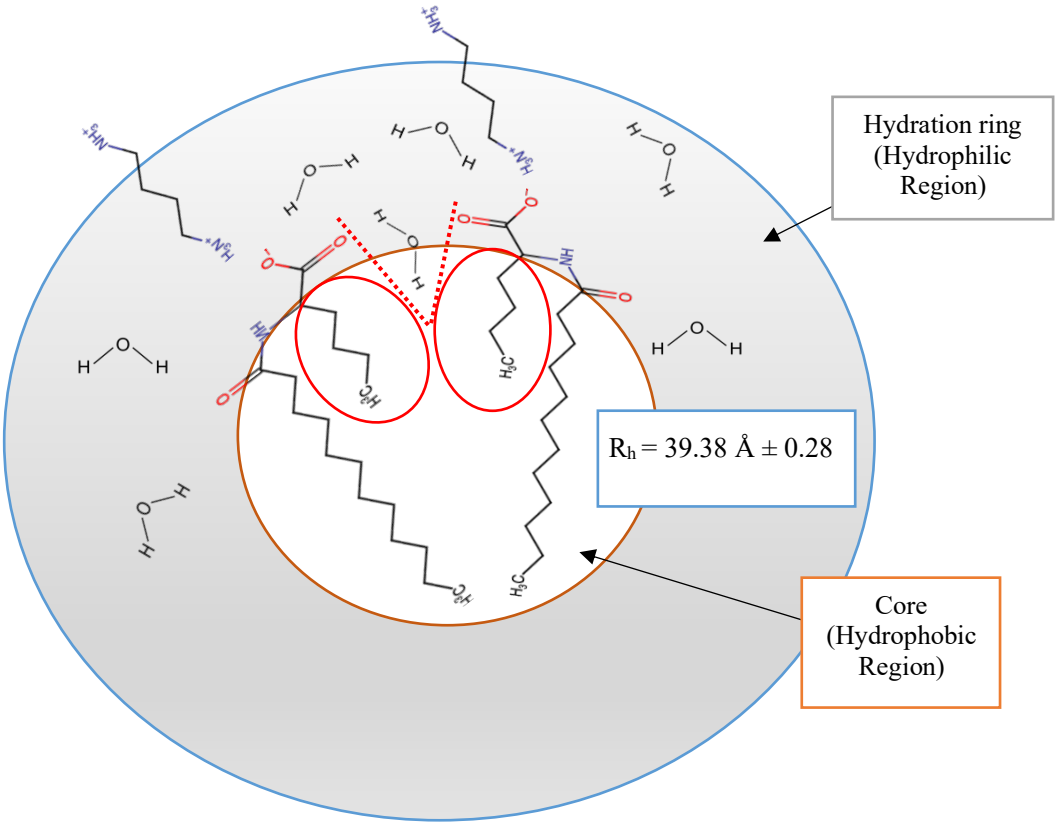


Figure 9. Possible formation of und-NL micelle with linear R-groups facing each other, causing hydrophobic pockets to form inside the micelle with possible hydrophobic hydration indicated by red dashed-line wedge.

Scenario 4:

Non-spherical micelle formation

In addition to Scenario 3, a possible explanation for such a large increase in R_h with 1,4-diaminobutane is that und-NL surfactant molecules may be forming non-spherical shaped micelles. The equation utilized in this research assumes spherical shaped micelles and does not account for the possibility of different structures. There is a possibility that und-NL may be forming into a non-spherical shape causing the appearance of an increased hydrodynamic radius. The hydrophobic R-group on und-NL may allow the formation of a bilayer like structure causing the micelle to elongate in size as depicted in Figure 10. This may correspond to a rise in

hydrodynamic radius as this arrangement would allow more surfactant monomers to aggregate together if they are oriented with their polar heads facing both directions.

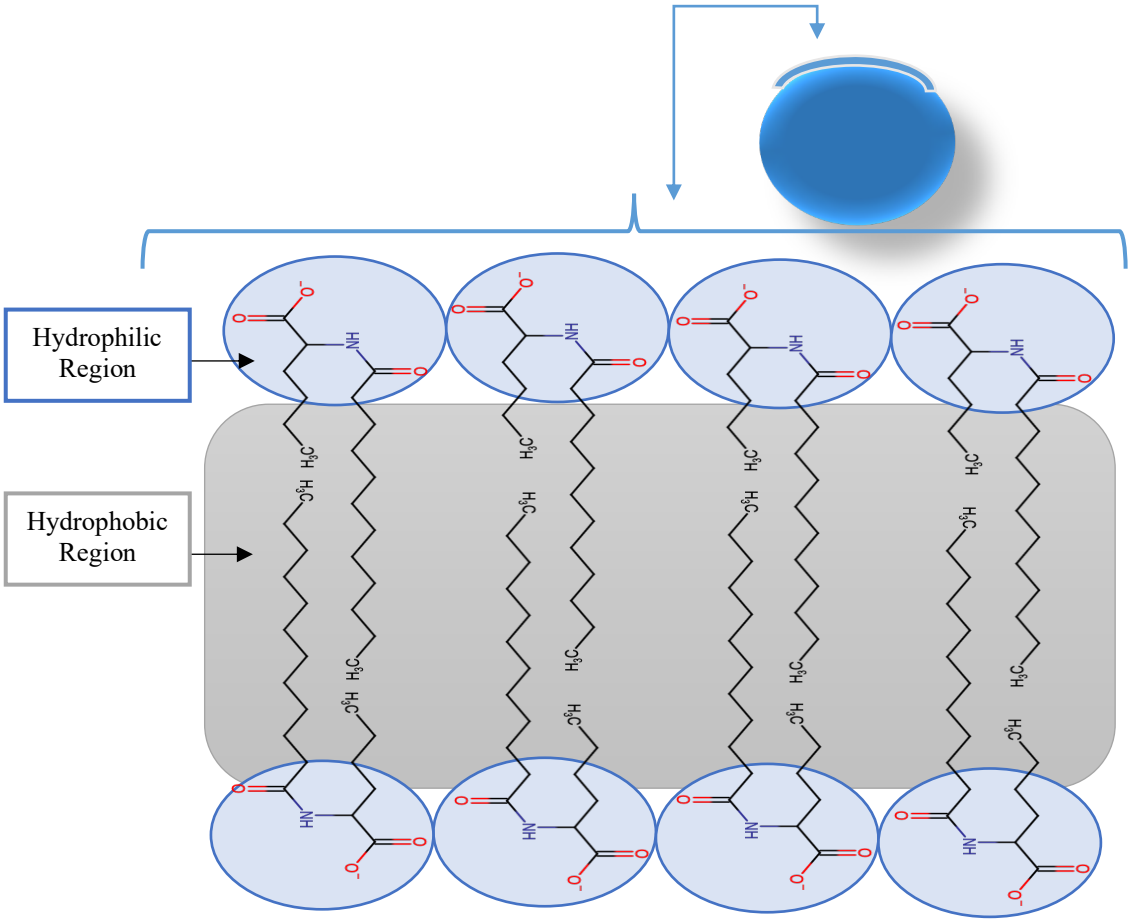


Figure 10. Possible bilayer formation of undecanoic L-norleucine

6.4.2 Fraction of Bound Counterion to Undecanoic L-Norleucine

The fraction of bound (f_b) diamine counterions with varying chain lengths to undecanoic L-norleucine micelles are recorded in Table 8. Sodium was omitted from this comparison as it could not be examined under the same DOSY-NMR experiment. Experiments were performed using methods listed in section 5.2 under the following conditions: 50 mM surfactant-counterion solution, pH 10, and 25 °C. There appears to be no trend with the binding of the counterions to the micelle. The smallest fraction of counterion bound to micelles was not seen in 1,2-

ethylenediamine (1,2-ED) as hypothesized but rather with 1,3-diaminopropane (1,3-DP); $f_{b(1,3\text{-DP})}$ is 0.10. This unpredicted phenomenon should be reinvestigated to determine the validity of experiments containing 1,3-DP. Diffusion experiments using NMR were conducted three times each, however, experiments were performed using the same prepared solution and therefore the standard deviation reported is a reflection of instrument error and not a true standard deviation for the diffusion of the counterion.

Counterion fraction bound to undecanoic L-Norleucine					
Sodium	1,2 ethylenediamine ($f_{b(1,2\text{-ED})}$)	1,3 diaminopropane ($f_{b(1,3\text{-DP})}$)	1,4 diaminobutane ($f_{b(1,4\text{-DB})}$)	1,5 diaminopentane ($f_{b(1,5\text{-DP})}$)	1,6 diaminohexane ($f_{b(1,6\text{-DH})}$)
N/A	**0.24**	**0.10**	**0.45**	**0.28**	**0.32**

Table 8. Counterion fraction bound to undecanoic L-norleucine at pH 10, 50mM surfactant-counterion solution, and 25 °C.

The highest fraction of bound counterions for 50 mM und-norleucine surfactant-counterion solution at pH 10 was seen with 1,4-diaminobutane. There are many possible explanations as to why this occurs with this counterion including the possibility that there is less hydrogen bonding between the amine and the polar head group causing the polar head groups to grow further apart. A sudden decrease in f_b was observed with 1,5-diaminopentane. This decrease in bound counterions could be due to the longer hydrophobic chain of the counterion burying towards the hydrophobic core of the micelle similar to 1,5-DP with und-IL micelles shown in Figure 6. A slight increase in the fraction of bound counterions was then observed with 1,6-diaminohexane. This small increase could be in part to a shift in the shape of the micelle caused by the addition of the longer chain counterion.

6.5 Micellar Electrokinetic Chromatography

The R and S enantiomers of (±) 1,1′-bi-2-naphthyl-2,2′-diamine (BNA) were separated with integration into undecanoic L-isoleucine micelles through the MEKC mode of CE. Various factors influence the successful separation of enantiomers, including optimal pH. The pH of the surfactant-counterion solution was adjusted from 7.0 to 11.0 and experiments were conducted to determine the effect pH would have on chiral selection and enantiomeric separation. The pKa values for the diamine counterions as previously stated range from 10.50 to 11.86. When pH > pKa the counterions are largely deprotonated and the amine groups will no longer carry a positive charge. At higher pHs, the diamine counterions will have the tendency to dissociate from the negative micelle surface and interact more freely in solution due to a net neutral charge.

When the positively charged diamines deprotonate at high pHs, they detach from the negatively charged micelle and also detach from the negatively charged capillary wall. The diamines will become neutrally charged as stated previously and will begin to be replaced on the capillary wall by Na^+ ions. This replacement of diamine counterions with Na^+ ions increase the EOF. Increasing the pH of the surfactant solution also changes the size of the micelles that are formed and can increase the EOF which shifts the EOF marker closer to $T_{eof} = 0$. Figure 11 shows the effect the counterion has on the EOF. As the counterion increases in chain length and size, the EOF slows down and it takes longer for the EOF signal to reach the detector. The neutral marker, methanol, is seen later in 1,6-diaminohexane than with any other counterion. The fastest EOF was noted for Na^+ at 2.847 minutes whereas the slowest EOF was observed with 1,6-diaminohexane at 7.121 minutes.

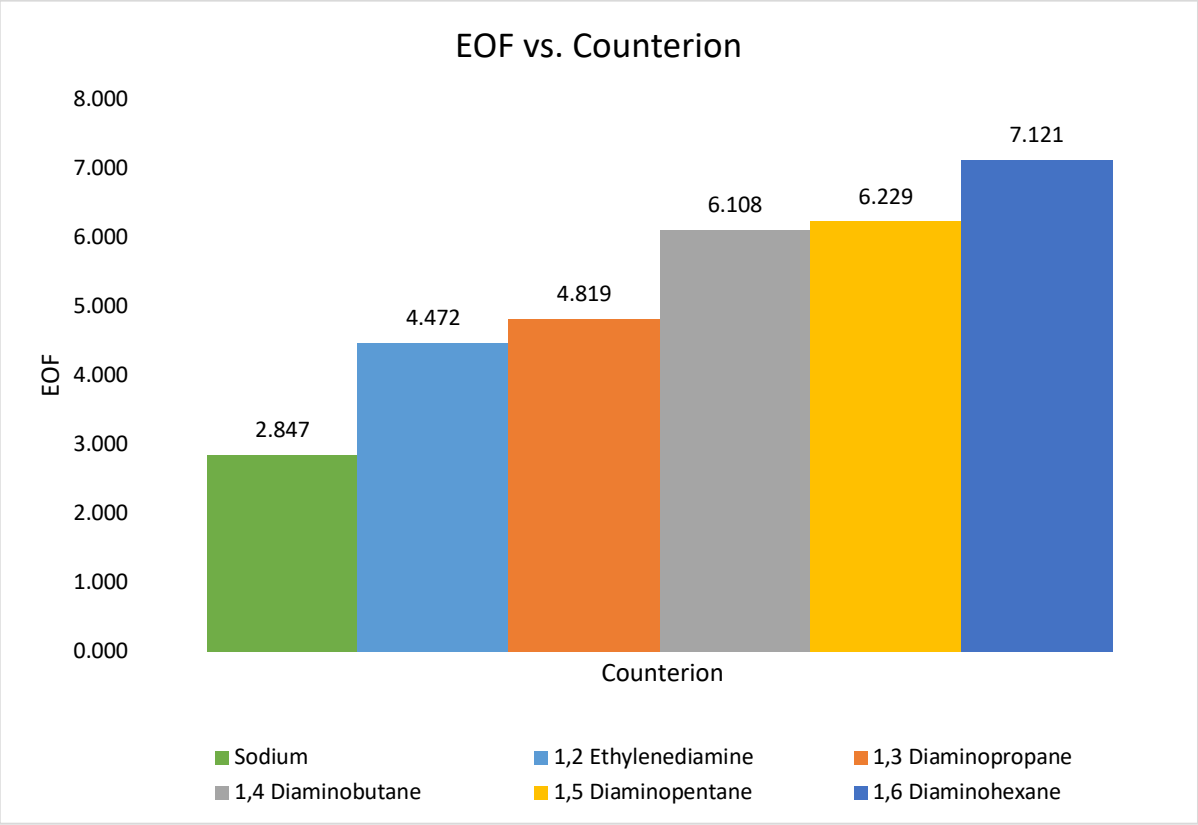


Figure 11. EOF as a function of counterion with BNA and und-IL at pH 9

6.5.1 Enantiomeric Resolution

The resolution is a function of several different components and therefore, changing the surfactant type, surfactant-counterion solution concentration, pH, temperature, type of counterion and applied voltage will influence the resolution values. Resolution can be calculated using eq 5 where w_1 and w_2 refer to the peak widths at the baseline, t_1 and t_2 are the migration times of the analyte.[20] Note that t_2 is observed only if enantiomeric separation is achieved.

$$R_s = \frac{2(t_2 - t_1)}{w_1 + w_2} \quad (5)$$

The highest resolution (R_s) achieved in this research between 1 and 10 mM was recorded at R_s= 10.35 for BNA with 9 mM und-IL and 1,2-ethylenediamine (1,2-ED) at pH 9. At pH 9, the diamine counterions are still predominantly cationic; therefore, the positive charge is attracted to the cathode which helps the solution travel faster than at higher pH values where the diamines are neutrally charged. Figure 12 shows data with this 10.35 resolution value; 1,2-ED is the smallest counterion studied in this research, with the exception of sodium, and is easily pulled through the capillary as it is less bulky and travels faster than the other counterions.

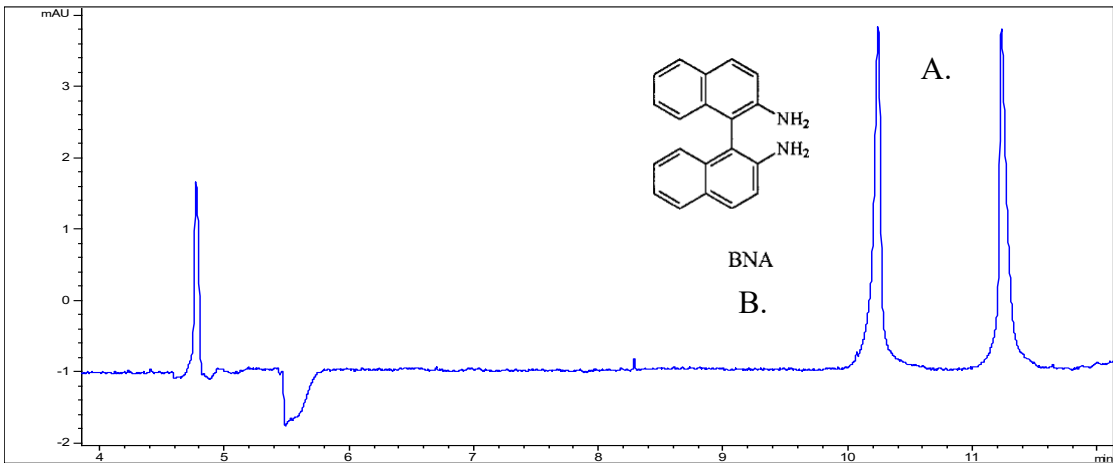


Figure 12. (A) Enantiomeric separation of (B) 1,1′-bi-2-naphthyl-2,2′-diamine (BNA) with 9 mM und-IL with 1,2-ethylenediamine at pH 9, 25°C.

The lowest concentration where enantiomeric separation of BNA was achieved was at 3 mM surfactant-counterion solution and was seen with 1,4-diaminobutane (1,4-DB), 1,5 diaminopentane (1,5-DP), and 1,6-diaminohexane (1,6-DH) counterions. Resolution (R_s) and pH values for 3 mM enantiomeric separation of BNA include: 1,6-DH, pH=8, R_s = 1.90; 1,5-DP, pH=7, R_s= 2.08; 1,4-DB, pH 9, R_s= 1.28. Table 9 lists the resolution values for und-IL with different chain length diamine counterions at pH 9. There appears to be no monotonic trend in resolution for increasing the counterion chain length; however, enantiomeric separation of BNA

was achieved at lower concentrations as the length is increased beyond 1,3-DP. This could be due to many factors including, but not limited to, steric interaction, hydrogen bonding, analyte penetration depth, or electrostatic interactions.[41]

Concentration	Counterion				
	1,2-ethylenediamine	1,3-diaminopropane	1,4-diaminobutane	1,5-diaminopentane	1,6-diaminohexane
3mM	-	-	1.28	2.08	1.50
4mM	-	-	3.35	-	1.90
5mM	-	2.09	3.29	-	1.35
6mM	2.64	2.42	-	-	4.82
7mM	8.45	2.50	5.03	1.63	6.92
* 8mM	9.36	3.12	5.18	4.08	3.55
9mM	10.35	3.17	5.24	-	5.15
10mM	9.10	3.54	4.11	-	-

Table 9. Resolution values (k') for enantiomeric separation of BNA with undecanoic L-isoleucine and various chain length diamine counterions at pH 9

The increase in counterion chain length appears to play a role in the enantiomeric resolution although there is no distinguishable trend. Figure 13 below shows the separation of BNA enantiomers for all diamine counterions used at a surfactant-counterion concentration of 8 mM and pH 9.0. Corresponding resolution values are reported in Table 9. As the chain length is increased, additional side peaks appear on the electropherograms after 1,4-DB. These side peaks continue throughout the remaining diamines 1,5-DP and 1,6-DH and could be occurring for several reasons. For example, an enantiomeric impurity could cause this tailing, as could the formation of diastereomers.

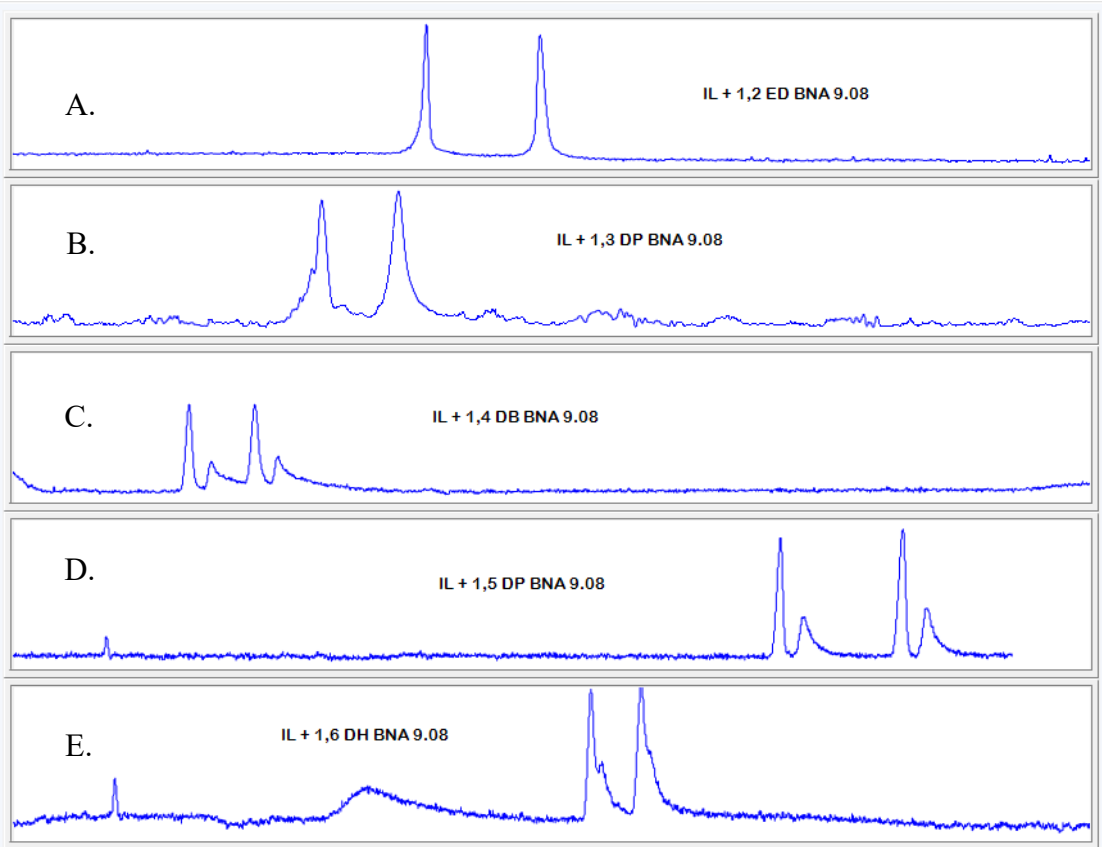


Figure 13. Chiral separation of BNA enantiomers with undecanoic L-isoleucine with various diamine counterions at pH 9, 8 mM; (A.) 1,2-ED (B.) 1,3-DP (C.) 1,4-DB (E.) 1,6-DH

6.5.2 Retention of BNA

The experimental parameter measured in CE is the retention time, t_R which is used to calculate the retention factor *k'* using eq 6. where, t_R is the retention time of the analyte and t_0 is the retention time of the unretained solute; in this research t_0 is the retention time for the neutral marker, methanol. [20]

$$k' = \frac{(t_R - t_0)}{t_0} \quad (6)$$

Values of *k'* for BNA at pH 9 with und-IL and various chain length diamine counterions are listed in Table 10. The retention times increase as the chain length is increased on the counterion. The increase in chain length increases the size of the micelle and it is retained longer in the capillary, equating to a higher EOF and therefore a higher *k'* value. Higher retention values

are reported more often for 1,6-DH than the other counterions, but there appears to be no significant difference observed within the same surfactant solution concentration. In fact, for 3 and 4 mM surfactant-counterion solutions, the *k'* values are lower than other those of 1,3-DP and 1,4-DB.

Concentration	Counterion				
	1,2-ethylenediamine	1,3-diaminopropane	1,4-diaminobutane	1,5-diaminopentane	1,6-diaminohexane
3mM	-	0.49789	0.2654	0.39351	0.1588
4mM	-	0.59734	0.5183	-	0.3348
5mM	-	0.63915	0.8833	-	0.9856
6mM	0.4068	0.67670		-	1.0230
7mM	0.6930	0.70601	0.8555	0.73720	1.1580
8mM	0.9429	0.81385	0.9312	0.9841	1.1219
9mM	1.1458	0.80556	1.0206	-	1.1703
10mM	1.5029	0.86989	1.2148	-	-

Table 10. Retention values for BNA with und-IL and various chain length diamine counterions at pH 9.

Overall, und-IL contains two chiral centers allowing it to better separate neutral species given the right conditions and parameters. For this study, the best enantiomeric separation of BNA was observed using und-IL with 1,2-ED as the counterion. This counterion had similar retention times to others but yielded better separation and higher resolution values. However, the use of 1,2-ED was unable to provide separation below 6mM concentration whereas the counterions 1,4-DB, 1,5-DP and 1,6-DH were capable of separation at a surfactant-counterion solution concentration as low as 3 mM. The lower separation ability of these counterions could be due to their lower CMC values or for their larger size slowing down the EOF and allowing separation to occur.

6.6 Supplementary Data for Early CE studies with Arginine and Lysine

At the beginning of this project, ideas about what counterions would yield positive results were examined and preliminary experiments were conducted using sodium, arginine and lysine counterions with undecanoic L-isoleucine for enantiomeric separation of (±)1,1′-bi-2-naphthol (BOH), (±) 1,1′-bi-2-naphthyl-2,2′-diamine (BNA), and (±) 1,1′-bi-2-naphthyl-2,2′-diyl hydrogen phosphate (BNP). The data collected showed that the counterion has a significant effect on the chiral recognition ability of the micelle. Figure 14 shown at pH 10 and 10 mM surfactant-counterion concentration illustrates that using 1,2-ED as the counterion instead sodium a notable improvement in chiral resolution was observed for enantiomers of 1,1′-bi-2-naphthyl-2,2′-diamine (BNA).

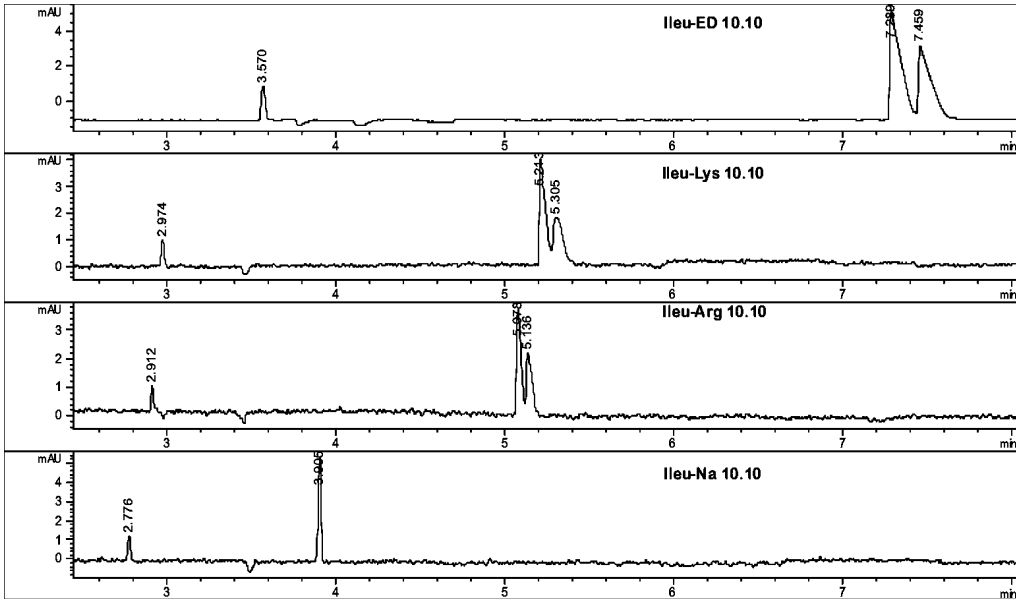


Figure 14. Enantiomeric Separation of BNA with 10 mM und-IL surfactant-counterion solution at pH 10

With the ability to improve resolution at lower concentration, the decision was made to switch from arginine and lysine to diamine counterions of varying chain length in order to determine if the chain length would have an effect on the behavior of the micelle and its ability

of enantiomeric separation. The results from the original study with lysine, arginine and sodium are included below. Table 11 includes data from und-isoleucine with the counterion sodium at pH 9; t_0 is the EOF neutral marker, methanol, t_1 is the signal produced from the analyte and t_2 is the second signal produced from the analyte and is only seen when enantiomeric separation is present.

The increase in pH correlates to an increase in EOF as well as retention. The resolution appears to be similar for all experiments however, a low of 1.76 is observed at 30 mM and a high is noted for 15 mM. Table 12 shows data collected from experiments with lysine as the counterion. Similar trends to sodium are observed with lysine, where the EOF and retention increase as a function of concentration.

Concentration (mM)	t_0 (min)	t_1 (min)	t_2 (min)	R_s	k'
10	2.847	4.005	-	-	-
15	3.431	7.300	7.756	3.74	1.1277
20	3.662	8.968	9.187	2.39	1.4489
25	3.733	9.910	10.134	2.82	1.6547
30	3.556	9.030	9.175	1.76	1.5394
35	3.887	10.538	10.693	3.43	1.7111
40	3.996	11.150	11.301	3.01	1.7903
45	4.040	11.559	11.701	2.81	1.8611
50	4.169	12.330	12.476	2.38	1.9575

Table 11. Undecanoic L-Isoleucine with Sodium for separation of BNA at pH 9

Concentration (mM)	t_0 (min)	t_1 (min)	t_2 (min)	R_s	k’
10	3.310	5.520	-	-	0.6677
15	3.454	7.617	7.838	3.71	1.2053
20	3.543	8.615	8.800	2.43	1.4316
25	3.619	9.306	9.455	1.72	1.5714
30	3.740	10.190	10.347	1.98	1.7246
35	3.829	10.863	11.008	1.86	1.8370
40	3.937	11.900	12.049	0.49	2.0226
45	3.954	11.970	12.103	0.60	2.0273
50	3.964	12.132	12.257	0.56	2.0605

Table 12. Undecanoic L-Isoleucine with Lysine for separation of BNA at pH 9

Figure 15 shows the electropherogram of enantiomeric separation of BNP with undecanoic L-isoleucine in the presence of arginine (A), sodium (B), and lysine (C). Note that the EOF with all three counterions appear to be similar, but the retention times vary. Even though the EOF markers appears to be similar, they do vary slightly; 2.89 min for arginine, 2.96 min for sodium and 3.08 min for lysine. This small shift in EOF can equate to a large difference in retention times. Arginine as a counterion was able to separate BNA enantiomers in a shorter amount of time, but it appears that sodium and lysine had slightly larger resolution values. Lysine was retained longer in the capillary than sodium or arginine.

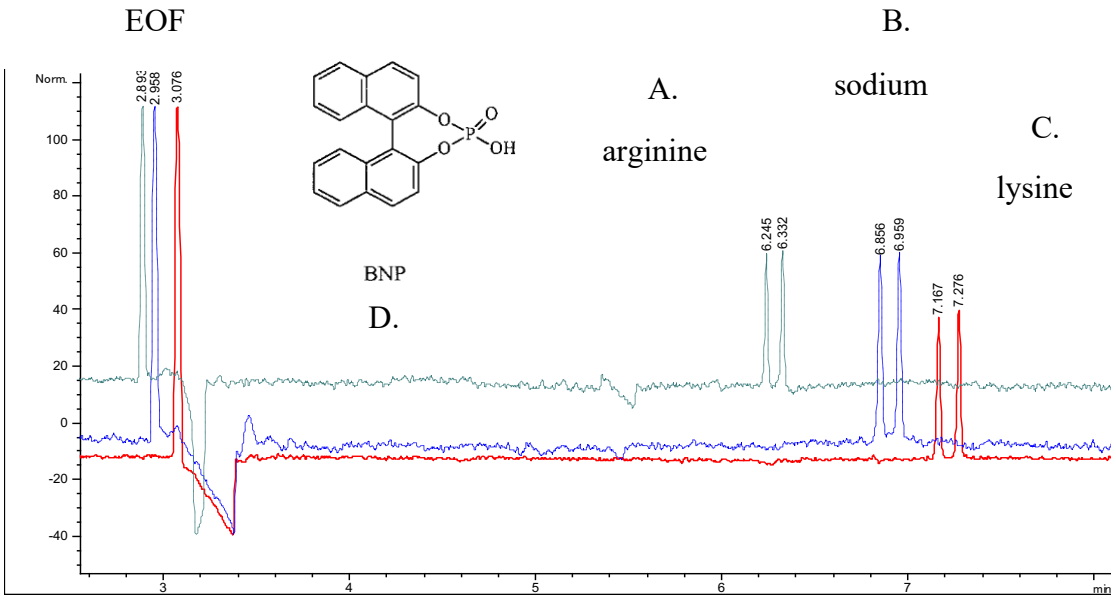


Figure 15. Enantiomeric separation of 1,1′-bi-2-naphthyl-2,2′-diyl hydrogen phosphate (BNP).with 15 mM undecanoic-L-isoleucine and (A) arginine, (B) sodium (C) lysine; (D) Structure of BNP analyte.[27]

Figure 16 shows the effect pH has on EOF when sodium, arginine, and lysine are used as counterions as part of und-IL micellar systems to facilitate the separation of BNP analytes at a surfactant-counterion solution of 15 mM. When sodium was utilized as the counterion it retained a similar EOF despite a change in pH. The EOF of undecanoic isoleucine with arginine as the counterion was the most heavily affected by a change in pH. It is possible that arginine is less likely to interact with the capillary wall as compared to sodium and more likely to stay bound to the micelle. At a lower pH and the same concentration, arginine has more bound counterions on the surface of the micelles when compared to Na^+. This could cause a shift in EOF due to the molecule being larger in size and it would take longer to reach the cathode. As the pH increases above pH 9 there are fewer arginine and lysine molecules attached to the micelle. They dissociate from the surface of the micelle[13] and are replaced with sodium ions from sodium

bicarbonate in solution. This creates a smaller size micelle which will then be pushed faster through the capillary causing arginine and lysine to have similar EOF times as sodium.

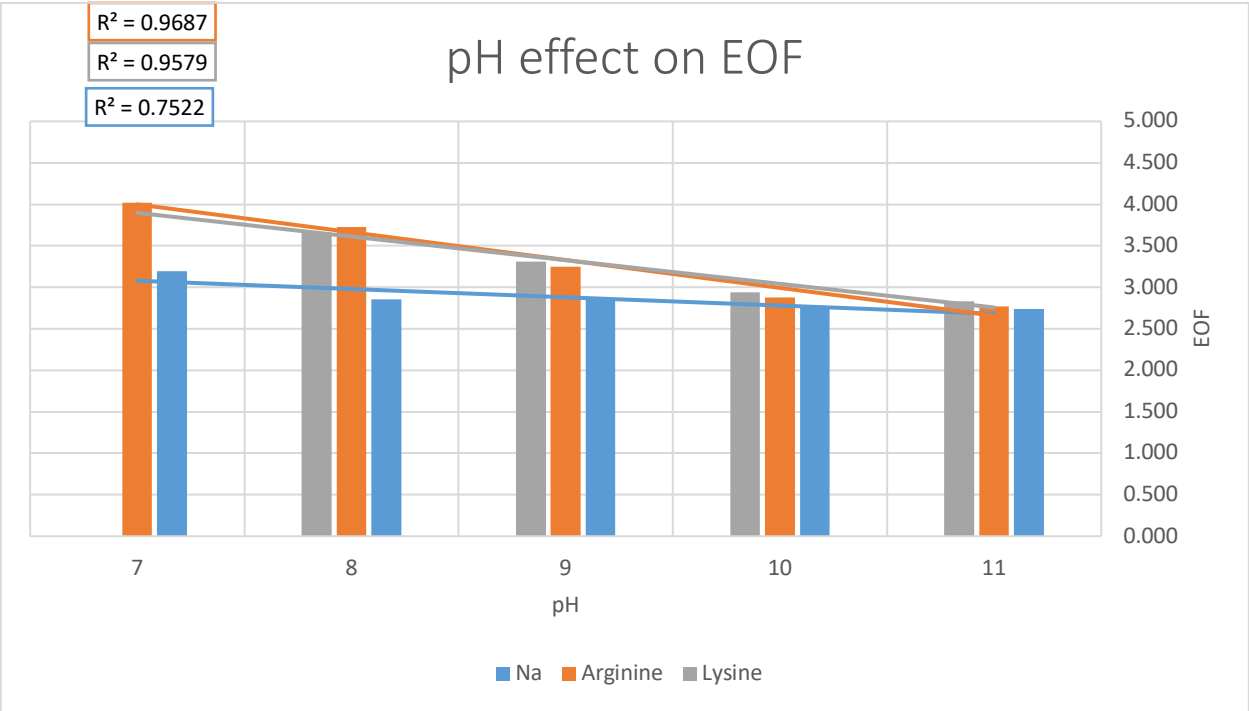


Figure 16. pH effect on EOF for BNP with und-IL and sodium, arginine, and lysine as counterions.

CHAPTER VII: DISCUSSION

7.1 Comparison of Undecanoic L-Isoleucine and Undecanoic L-Norleucine

Above the CMC, hydrogen bonding between the polar headgroups can play a major role in how the micelle is formed. In a 2002 study conducted by Thibodeaux et al.[42] steric factors of L-leucine surfactants with similar hydrophobicities were examined and compared. A significant decrease in enantiomeric resolution was seen with the surfactant containing L-norleucine (L-NL) and is believed to be caused by an increase in the linear R-group side chain's flexibility compared to the more rigid branched R-group on L-isoleucine (L-IL). The linear chain on L-NL may bend or fold in different directions which could prevent enantiomeric overlap and thus block the analyte's access to the chiral center.[42] In contrast, the surfactant containing L-IL was able to separate more chiral compounds with better resolution. In addition to the rigidness of the R-group on L-IL, there is also a second chiral center on the second carbon of the R-group which may allow the analyte better access to the chiral center which may increase enantiomeric resolution. The findings of that study can be somewhat applied to this research as the polar head groups examined are the same, although it should be noted that Thibodeaux et al.[42] utilized polymeric surfactants that are covalently bonded and are much more stable. Based on the studies conducted by Thibodeaux et al. the lack of flexibility as well as the second chiral center in und-IL micellar systems may help improve chiral separation of enantiomers

When the counterions consist of a shorter chain such as 1,2-ethylenediamine or 1,3-diaminopropane there appears to be no significant difference in the hydrodynamic radii between und-NL and und-IL. In fact, the sizes are similar to that observed when sodium was used as the counterion. This could be due to the small size of the counterion and its limited binding sites to

the micelle. In contrast, a significant difference is seen between the average radii of und-IL and undecanoic L-NL for longer chain length counterions. For example, at pH 10 with 1,4-diaminobutane as the counterion, und-IL had an average radius of 15.58 Å whereas the average radius for und-NL was 39.38 Å. One explanation for this could involve the orientation of the R-groups on these surfactant polar head groups. The R-group on und-NL is a linear aliphatic tail whereas und-IL has a branched R-group, leaving the option for und-NL to interact more with the solution. The R-group on und-NL could form its own hydrophobic pocket within the micelle, causing an increase in the size of the micelle. These hydrophobic pockets could appear as bubbles on the inside of the micelle and possibly create a space for the tails to interact with themselves.

CHAPTER VIII: FUTURE RESEARCH

8.1 Continued Characterization Using L-Tert-Leucine

Future research should be conducted with undecanoic L-tert-leucine to compare those results with those of this study. Identifying how altering the R-group of the amino acid headgroup changes the characteristics of the surfactant could give insight to how the CMC, hydrodynamic radii, and capability of counterion to bind to the micelles vary with different steric interactions. A significant difference noted from this study between several characteristics of undecanoic L-norleucine and undecanoic L-isoleucine suggests that using another isomer may also alter the factors which we are trying to characterize.

8.2 Future NMR Studies

NMR studies using ROESY and NOESY should be performed on the above-named surfactants to compare cross peaks and determine how the polar headgroups are oriented in space. This will help identify the separation between headgroups and therefore give more information on the size of the surfactant micelles. That data can be used to test the hypothesis proposed in this study that details a possible explanation for how the differences in characteristics is related to the properties of isoleucine isomers.

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