CHARACTERIZATION OF THE DIPEPTIDE BASED MICELLAR SYSTEMS UNDECANOIC ALANINE-ALANINE AND UNDECANOIC ALANINE-GLYCINE

A Thesis

by

SAVANNA MALDONADO

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

Eugene Billiot, PhD
Chair

Fereshteh Billiot, PhD
Co-Chair/Committee Member

Hussain Abdulla, PhD
Committee Member

December 2018

ABSTRACT

Surfactant are surface-active-agents, meaning surfactants have the ability to lower surface tension. In this research, two dipeptide surfactants undecanoic alanine-alanine and undecanoic alanine-glycine were studied to better understand their micellar systems. Variations of pH, temperature, surfactant concentration, and counterion type were examined to determine what effect, if any, changing these variables would have on micelle formation and chiral recognition. The counter ions examined in this study were di-amine alkanes with a different number of methyl groups separating the amines. These counter ions are pH dependent and preliminary results have shown that pH effects the interaction of these counter ions with the amino acid polar head, and in turn effects the physical properties of the surfactants and their micellar behavior.

Some of the properties examined include: the critical micelle concentration, Krafft temperature, enantiomeric separation of chiral compounds, hydrodynamic radius of the micelles as well as fraction bound of the surfactant and counterions to the micelles. These systems were studied using proton Nuclear Magnetic Resonance (NMR), Diffusion Order Spectroscopy (DOSY), Capillary Electrophoresis (CE) and a LabQuest 2 with attached conductivity and temperature probes. The results here provide a better insight on the behavior of these dipeptide micellar systems which will aid in future research.

Keywords: Surfactant, Nuclear magnetic resonance (NMR), Diffusion order spectroscopy (DOSY), Capillary electrophoresis (CE), Krafft point

DEDICATION

This thesis work is dedicated to my loving wife, Frances Maldonado, who stood by my side through all my failures and successes. This work is also dedicated to my parents, Harvie and Debbie Tubbs, who have provided me with unconditional love and have always encouraged me to strive for more in life. I would not be where I am today without you all in my life.

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Thanks, also go to my fellow graduate and undergraduate research members. Specifically, I would like to thank my undergraduate researcher assistants Olivia Devereaux and Zoe Ramos. You were an unexpendable asset to the success of this research. I wish you both the best of luck in your pursuit of a MS in chemistry.

Finally, I would like to thank all my friends and colleagues and the department faculty and staff for making my time at Texas A&M University-Corpus Christi an enjoyable experience. This research was financially supported by National Science Foundation (NSF), Awards #1709680, #1531526, and #1213532. Welch Departmental grant, Award #634010.

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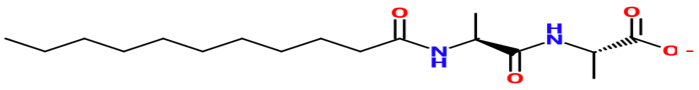
CHAPTER I. INTRODUCTION

1.1 Background

The English word “surfactant” is short for surface-active-agent, meaning surfactants have the ability to lower surface tension. In this research, two dipeptide surfactants undecanoic alanine-alanine (UAA) and undecanoic alanine-glycine (UAG) were studied to better understand their micellar systems. As can be seen in Figure 1 the surfactants being examined contain an 11-carbon chained tail group with a negatively charged dipeptide-based polar head group.

There are many reasons behind why we chose the use amino acid-based surfactants; There is a growing demand for “green” surfactant; Amino acid-based surfactants are biocompatible and biodegradable. [1] They allow for “the synthesis of multifunctional surfactants with chiral properties that can be tailored for specific technological and/or biomedical applications.” [1] Also for our research it is important that we chose a chiral compound for the purpose of chiral separation. Amino acids are the monomers that make up proteins that allow them to be used as a good chiral selector. The amino acids that we chose are also very simple in structure and possess no chromophores that could potentially interfere.

A)



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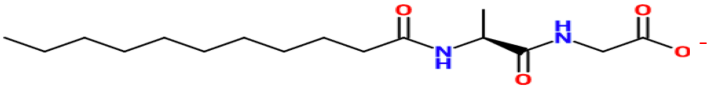


Figure 1: A) Structure of Undecanoic Alanine-Alanine B) Structure of Undecanoic Alanine-Glycine.

Chirality is a concept that explains the existence of the non-superimposable property of mirror-image stereoisomers. [2] Two molecules that are mirror images of one another are considered enantiomeric pairs and have the same physiochemical properties. However, because two enantiomeric drugs are not isotropic, they will have different pharmacologic effects. A classic example of the differences in pharmacological effects is the drug Thalidomide. Thalidomide is a drug that was put on the market in 1957 and was prescribed to pregnant women as a sleeping agent, cure for morning sickness, and depression. In 1961 this drug was banned in the US after it was discovered that many of the offspring of the women who were prescribed thalidomide had serious birth defects. It was later discovered that the (-)(S) form of thalidomide was the cause of the birth defects, while the (+)(R)-thalidomide form is a safe and effective form. [3,4] This discovery brought upon the necessity for researching chirality in the pharmaceutical industry. For this study we examined the enantiomeric separation of binaphthyl derivatives (In Figure 6).

We characterize these micellar systems by looking at their: 1) critical micelle concentration, 4) chiral separation of binaphthyl derivatives, 3) Krafft temperature and Krafft point 4) hydrodynamic radius of the micelles, and 5) fraction bound of the surfactant and counterion to the micelles. The critical micelle concentration (CMC) is the point in which the surfactants form into a micelle. [5] According to Vautier-Giongo and Bales, there are actually two distinguishable measurements that have to be taken when looking at the conductivity results: Krafft point and Krafft temperature. [6] Krafft point being described as the temperature at which the solubility of the monomers become equal to that of the CMC. [7,8,9] First being described by Murray and Hartley, above the Krafft point micelles begin to form which quickly increases the solubility of the surfactant. [10] The Krafft temperature being the temperature where there is an abrupt change in the slope on the conductance versus temperature plot (In Figure 7). [6]

We investigated what effect 1) pH change, 2) temperature change, 3) type of counterion and 4) surfactant concentration had on various physical properties of these micelles. The seven counterions that were investigated include: 1) Sodium, 2) 1,2 Ethylenediamine, 3) 1,3 Propanediamine, 4) 1,4 Diaminobutane, 5) 1,5 Diaminopentane, 6) 1,6 Diaminohexane, and 7) 1,2 DiaminoPropane. The structure for these counterions can be seen in Figure 2. These counterions are di-amine alkanes with a different number of methyl groups separating the amines. These counter ions are pH dependent and preliminary results have shown that pH effects the interaction of these counter ions with amino acid polar head, and in turn effects the physical properties of the surfactants. The analytical techniques that are used in this research include: NMR, DOSY-NMR, and CE. A LabQuest 2 was used with an attached conductivity and temperature probe to examine Krafft point, Krafft temperature and CMC.

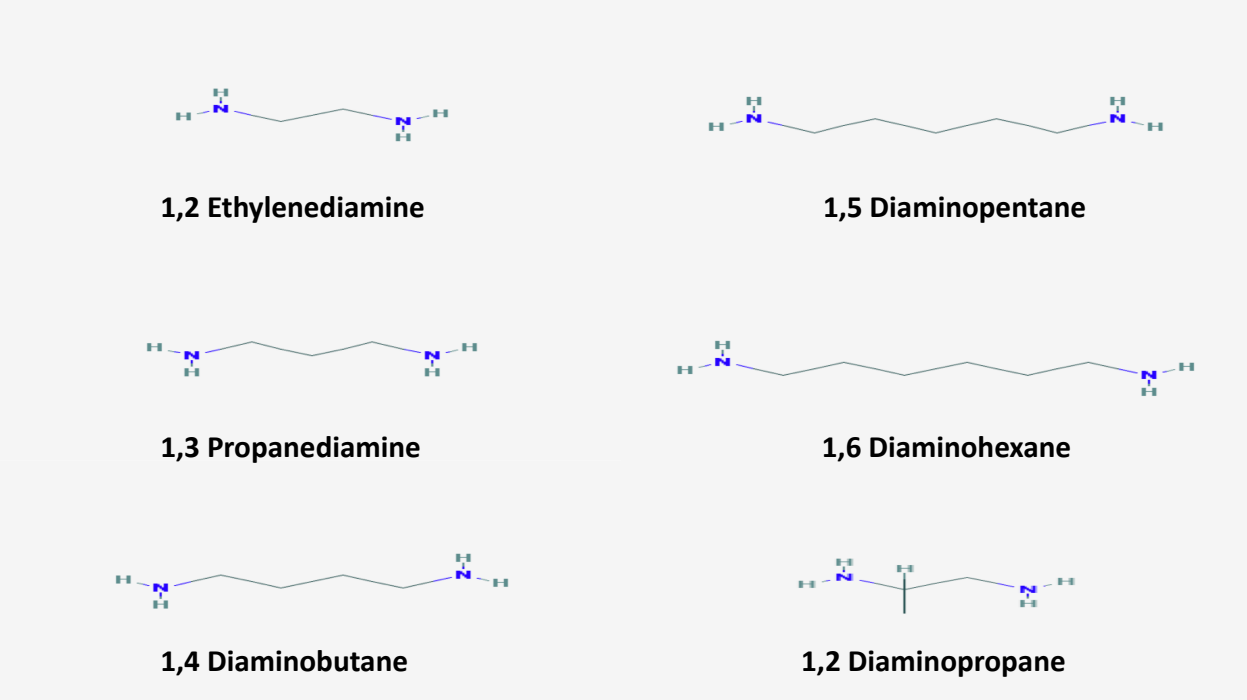


Figure 2: Diamine counterions under study.

1.2 Related Work

A study conducted at the University of Niš by Jelena Ž. Manojlovlc, focused on understanding solution properties of cetyltrimethylammonium bromide (CTAB) so that he could better control the solutions adsorption parameters in order to form uniform self-assembled monolayers. Conductivity as a function of temperature experiments were performed to better understand the CTAB's Krafft temperature. The results of these experiment showed that there were substantial structural changes occurring above and below the Krafft temperature. [11] As well as providing insight on the importance of a surfactants Krafft temperature, this study also gave a foundation on how to perform the conductivity experiments.

There are many studies that used MEKC to understand chiral discrimination that takes place in micellar systems. One study conducted by Billiot et al., dealing with polymeric chiral

surfactants, examined the effect of position, number of chiral centers, amino acid order, and their steric effects on chiral recognition. The results of this study gave insight into the chiral interactions of polymeric dipeptides surfactant. [12] Another study conducted by Haddadian et al. investigated how the number of chiral centers a surfactant has affects chiral recognition. Although the results of this study showed no significant difference between the two, it did however show the importance that hydrogen bonding has on chiral recognition. [13]

In a more recent study, Lewis et al, investigated the relationship between micelle formation and pH [14]. In that study, fluorescence was used to determine the CMC and the aggregation number of surfactant molecules in the micellar form. DOSY was used to determine the hydrodynamic radii and fraction bound of the surfactant and counterion to the micelle. The findings showed that the change in pH greatly affected the fraction bound of surfactants and counterions to the micelle and a micelle's hydrodynamic radius.

1.3 Analytical Techniques

1.3.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR is an analytical technique that can be used to determine the physical and chemical properties of a sample by manipulating the magnetic spin in the nuclei. NMR uses radio frequency to excite the nuclei to a higher energy state and measures the energy gap as the nuclei relaxes back to ground state. [11] For our research, we utilized proton NMR (H-NMR) to determine the purity of our surfactants and to determine the CMC of our surfactants. In addition, **D**iffusion **O**rdered **S**pectroscop**y** (DOSY) NMR was used to determine fraction bound of surfactant molecules to the micelles, and counter ions bound to micelles. DOSY NMR was also used to determine what effect pH and counterion type had on the size of the micelles formed.

DOSY is an NMR technique that utilizes magnetic field gradients to study diffusion related phenomena.

1.3.2 Capillary Electrophoresis

Capillary electrophoresis (CE) is an analytical technique based on the rate at which ions migrate in an electric field. “The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity of the solution, and the hydrodynamic radius of the molecule”. [15] The rate at which the ion moves is directly proportional to the applied electric field--the greater the field strength, the faster the mobility. [15] Neutral species are not affected, only ions move with the electric field” [4]. Our research utilized micelles as the pseudostationary phase. Thus, this CE technique is known as micellar electrokinetic chromatography (MEKC). MEKC is a mode of CE, where the separation is based on partitioning of analyte molecules between the aqueous run buffer and the micelles [3]. In our study, MEKC was used to perform enantiomeric separation of enantiomers of binaphthyl derivatives with the seven counterions (listed in Figure 2) at various pH values (pH 7, 8, 9, 10, and 11) in order to observe and catalog what effect pH and counterion type have on chiral selectivity.

A schematic of the separation principle for MEKC is shown in Figure 3. A high voltage is applied to typically an uncoated fused silica capillary that contains the running buffer generating the electroosmotic flow (EOF). [16] The EOF moves the liquid in the direction of the cathode. Due to the pH that the surfactants being examined are at, the surfactants are anionic. This negative charge on the micelle causes the micelles to oppose the direction of the EOF towards the anode. [16] However, since the EOF is stronger than the pull of the micelle to the anode, the net movement of the micelle is toward the cathode.

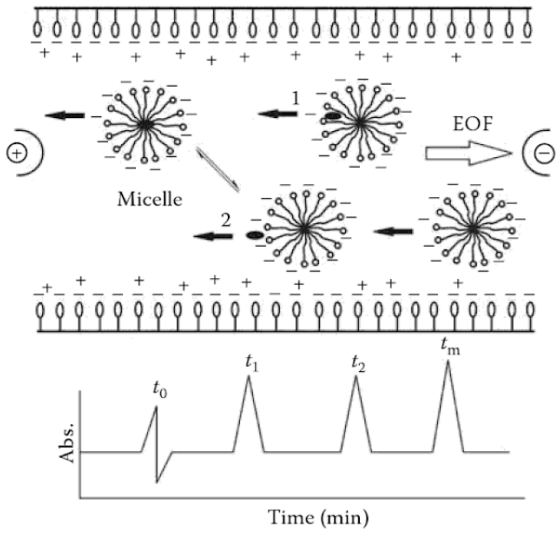


Figure 3. Schematic of principle separation in MEKC. The schematic shows a chromatogram of how the running buffer, analytes, and micelles exit the column. t_0 is the electroosmotic flow, t_1 and t_2 are the enantiomeric pairs of the analyte, and t_m is the micelle.

CHAPTER II: METHODS & PROCEDURES

*2.*1 Surfactant Synthesis

The synthesis of the surfactants started with enough Milli-Q water to dissolve all the sodium bicarbonate. The amount of sodium bicarbonate will vary based off the amount of surfactant being synthesized. Once the sodium bicarbonate was dissolved, roughly 5-7 grams of the desired amino acid / dipeptide was added to the solution. In a separate beaker a designated amount of undecanoic N-Hydroxysuccinimide (und-NHS) and Tetrahydrofuran (THF) was added. Once the und-NHS was dissolved, the two solutions were combined. The new solution was allowed to stir for a minimum of twenty-four hours. After the reaction was complete, the

solution was moved to the rotovap to remove all the THF. The solution was then chilled in an ice bath for several minutes. Next, the solutions were ran through a vacuum filter with a filter size of 100mm, to remove any residual insoluble contaminants. Hydrochloric acid (HCl) was then added to the solution to lower the pH and to get the surfactant to fall out of solution. The solution was then ran through a second vacuum filter and washed with water to remove any water-soluble contaminants. The surfactant was then moved to a freeze dryer and dried for a minimum of twenty-four hours or until the sample was completely dried. The purification of the surfactant was determined using a Bruker 300 UltraShield NMR with a 300MHz/54mm magnet. To prep the sample to run on the NMR, the surfactant was be dissolved in a designated amount of deuterated solvent (methanol or chloroform) and placed in an NMR tube and analyzed on the NMR to ascertain purity of the product.

2.2 Krafft Point

The Krafft point was measured using a conductivity method. A Vernier LabQuest2 measured the time, temperature and conductivity of a series surfactant concentrations (2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50mM). The samples were prepared using a pH 7, 5mM Boric acid buffer, along with the surfactant and counterion that was being investigated. The samples were refrigerated at 4 C^o for 24 hours to ensure all samples had precipitated out before the start of the experiment. Each sample was placed in the conductivity apparatus illustrated below (Figure 3) and heated with stirring until 2-5 degrees °C past the point that at which the solution became clear. Each sample was ran 3 times and chilled between each run. The conductivity values were plotted and the "drastic" change in the conductivity was determined to be the Krafft point. A example of this conductivity change can be seen in Figure 7 located in Section 3.1.

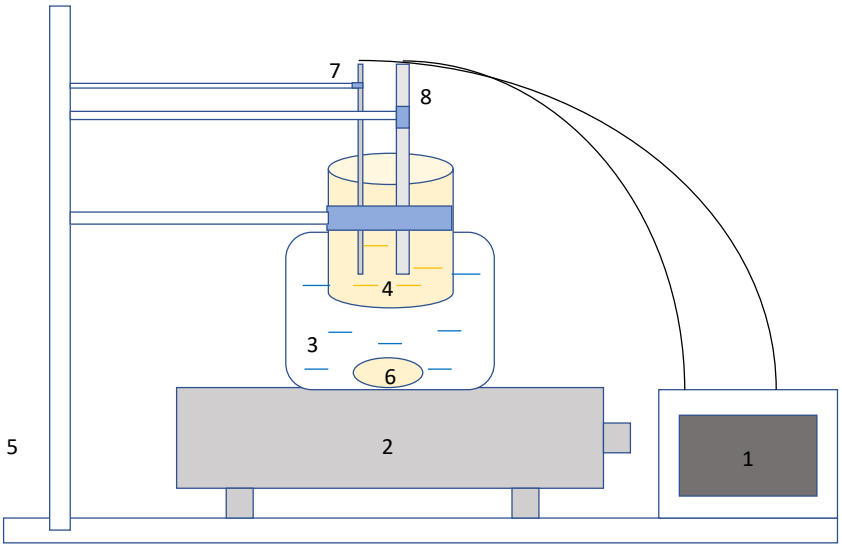


Figure 4: Krafft point set up. 1) Vernier LabQuest 2 2) Hotplate with stir function 3) Water bath 4) Sample holder 5) Stand 6) Stir bar 7) Temperature probe 8) Conductivity probe.

2.3 Diffusion Ordered Spectroscopy

The sample was prepared by making a 50mM surfactant solution with desired counterion in 10% Deuterium oxide (D_2O) 90% H_2O. The solution was vortexed and sonicated until mixture turned clear. Once clear, the solution was filtered using a 0.45-micron syringe filter. The pH was then measured and adjusted to reach pH 7, 9 and 11. Each sample was prepared to make 10mL of each pH set. 100µL of Tetramethylsilane (TMS) was then added to the 50mM sample. The TMS solubilizes inside the core of the micelle and the TMS signal was utilized to measure the micelle diffusion coefficient. The measurements were performed at 25.0℃. The viscosity of the solution was also measured at 25.0℃ using a Viscometer SV-10. For each trial, sixty NMR spectra were collected. This experiment took approximately 8-12 hours depending on the number of scans

chosen in the AcquPars tab on the Topspin software. The intensity of the surfactant, TMS, and counter ion peaks were recorded.

These recordings were then plotted as the natural log of the peak intensities versus (γ G δ)2(Δ-δ/3-$\boldsymbol{\tau}$/2). γ being the magnetogyric ratio, δ the duration of the magnetic field gradient, Δ the diffusion time, and $\boldsymbol{\tau}$ the delay between the bipolar gradients. The slope was then calculated from a linear regression analysis and the resulting slope represented as –D was then used to calculate the surfactant diffusion coefficient represented as D_{obs}, using equation 1 below. $D_{micelle}$ being the weighted average of the slower micelle-bound, D_{free} being the faster free solution surfactant values, and $f_{b,sft}$ being the surfactant monomers bound to the micelle. The hydrodynamic radii of the micelles, which is defined "as the radius of the sphere with the same diffusion coefficient as the micelle", were determined from the diffusion coefficients of the micelle by using the Strokes-Einstein equation [13].

Equation 1: $D_{obs}=f_{b,sft}\ X\ D_{micelle} + \left(1 - f_{b,sft}\right)X\ D_{free}$

Equation 2: $D_{micelle}= \frac{k_B\ X\ T}{6\Pi\ \mathrm{X}\ \mathrm{n}\ \mathrm{X} R_h}$

2.4 Critical Micelle Concentration

There are many different methods that can be used to determine the CMC of a surfactant solution. For this study, NMR was originally the only method intended for the determination of CMC. However, due to solubility issues associated with many of our samples, another method to determine CMC was required. Sections 2.4.1 and 2.4.2 below describe the sample preparation and procedures used for both methods.

2.4.1 NMR

Fifty mM surfactant solutions of UAG and UAA were prepared with equal moles of the counterion to create a stock, that was prepared in 90% water and 10% deuterium

oxide. Each of these stock solutions were then diluted to the concentrations 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, and 45, using 90% water and 10% deuterium oxide. Three concentration sets were made per each surfactant-counterion variation with a pH of 7, 9 and 11. The pH was adjusted using HCl and NaOH pellets. In some instances, the adjustment of the pH affected solubility of the surfactant. Each sample was tested 3 times to ensure reproducibility, with the number of scans varying based on concentration needed to give an adequate NMR signal. Shifts in the amide and chiral hydrogens were observed (Figure 5) and used to calculate a range for the critical micelle concentration. These shifts are referred to as a chemical shift. The observed chemical shifts are the result of the amide and chiral hydrogens at low concentrations, below the CMC, being primarily in an aqueous environment and as the micelles begin to form, the amide and chiral hydrogens are in a more hydrophobic environment within the micellar core. [17]

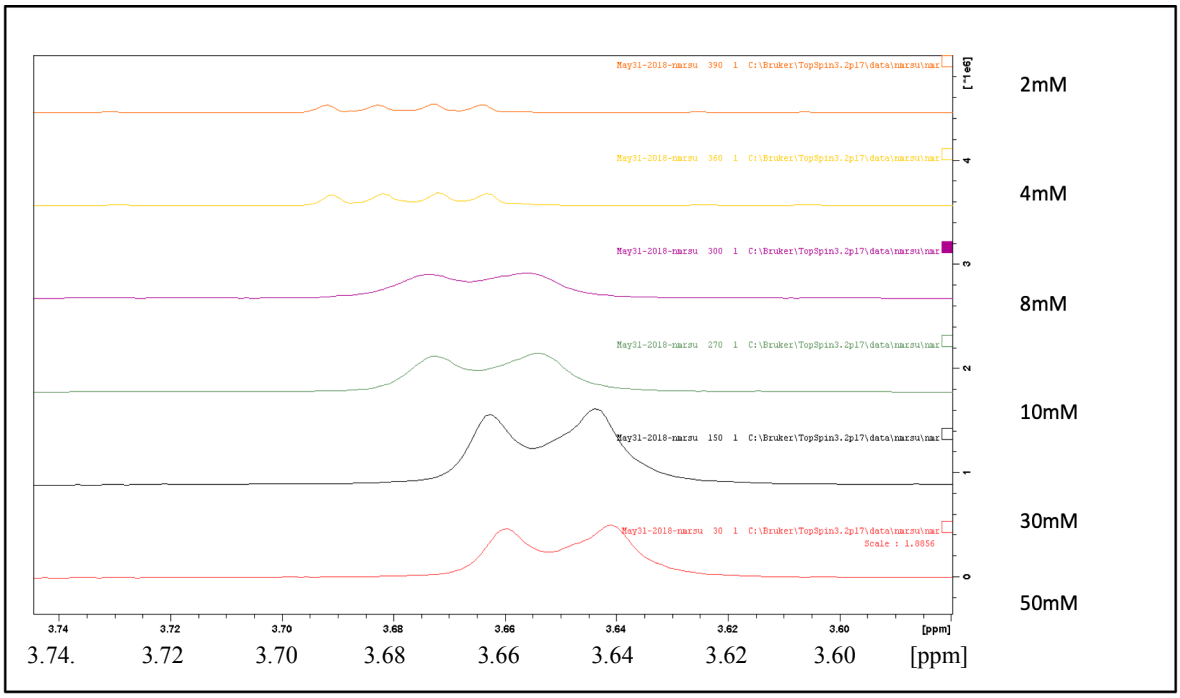


Figure 5: Peak shift of chiral hydrogen for Undecanoic Alanine Glycine the 1,3 propanediamine. Concentrations 2, 4, 8, 10, 30, and 50 mM were shown to illustrate the shift as a function of concentration. Samples were prepared in 10% Deuterium oxide 90% water solution and ran at 25°C.

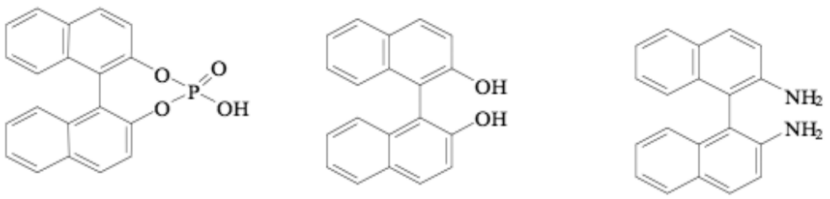
2.4.2 Conductivity

The samples were prepared using a pH 7, 5mM Boric acid buffer, along with the surfactant and counterion that was being investigated. The samples were then diluted to make a series of surfactant concentrations (2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50mM). However, in order to make a more accurate comparison of CMC each sample set was adjusted to the pH 9. Using a Vernier LabQuest2 with an attached conductivity probe, the conductivity of each sample was measured at room temperature (20-22°C). Each sample was measured 3 times for an accurate conductivity and the values were plotted. A “drastic” change in the conductivity, determined by a change in the slope was determined to be the CMC.

2.5 Capillary Electrophoresis

Chiral separation was performed with an Agilent 7100 capillary electrophoresis instrument with Agilent OpenLAB intelligent reporting, following the same technique reported by Billiot et al. [18] MEKC experiments were conducted using an untreated fused silica capillary that had an effective length of 55cm and a diameter of 50 μm (Polymicro Technologies). [18] The capillary was temperature controlled by the instrument's Peltier forced air cooling system, keeping the capillary at 25°C. [18] The detector used was a photo diode array and separations were performed at +30kV with a UV detection at 215 nm. [18] The capillary tube was prepped prior to injection of sample with 1 M NaOH solution for 30 minutes, followed by a 0.1M NaOH solution for 30 minutes, and finally rinsed for 15 minutes with deionized water. The analytes were prepared 50:50 methanol/water mixture at 0.1mg/mL.

The surfactant solutions were prepared at several different surfactant concentrations counterion types and pH's. The samples were injected into the CE for 5 seconds at a pressure of 10mbar. After each run, the capillary tube was reconditioned with a pressure injection of buffer for 2 minutes. The chiral analytes that were examined in these experiments were the binaphthyl derivatives Binaphthyl (BOH), Binaphthyl phosphate (BNP), and Binaphthyl amine (BNA). These analytes were chosen due to their success in previous studies [19]. These chiral analytes are atropisomeric compounds, they have a tetrahedral stereocenter and are nonsurperimposable. [19] These analytes are very rigid and have limited movement, BNP being the most rigid of them all due to its phosphoric acid group. [19]



A B C

Figure 6. A) Structure of Binaphthyl. B) Structure of Binaphthyl Phosphate. C) Structure of Binaphthyl Amine.

CHAPTER III: RESULTS & DISCUSSION

3.1 Krafft Point

The objective of the Krafft studies in this research was to try to get a better understanding of the surfactants being studied and a possible explanation for the solubility issues that were encountered early on in the research. Solubility is important because surfactant solutions cannot be examined in our study if they could not be solubilized in aqueous solution. The three main factors that affect a substances ability to go into solution are: 1) Structure of the solubilizer and solubilized, 2) Concentration of the solubilizer and solubilized, and 3) temperature. [20]

The component being solubilized are the surfactants, while the solubilizers that aid the surfactant molecules into solution are the counterions listed in Figure 2. The structure of both the counterion and surfactant determines how the counterion is able to bind to the surfactant. The

chain length of surfactant tail, substitutions in the carbon chain, and substitutions in the head group are some of the factors that play a role in solubility of the surfactant. As for substitutions in the headgroup, based off preliminary results it appears that the UAG was more soluble than UAA. This difference in solubility is possibly due to the smaller size of glycine in comparison to alanine allowing more water to penetrate into to the core.

The degree to which the counterion binds to the surfactant/micellar system and the size of the counterion is also very important. It is ideal to have more counterion bound to the micelle to assist in solubilization. Investigating the effect of counterions on the properties of micelles, Bijma and Engberts found as the CMC decreases the size of the counterion increases [21]. This is also true for the degree to which the counterions bind to the micelle and the hydrophobicity of the counterion [21].

The temperature also greatly affects when the free surfactant molecules are able to go into solution. The minimum temperature at which the solid free surfactant molecules go into solution forming micelles, is considered the solutions Krafft point. For surfactant that have a Krafft point higher than room temperature, heat is needed to assist the surfactant in reaching its CMC. Below the Krafft point the surfactant's micelles cannot form.

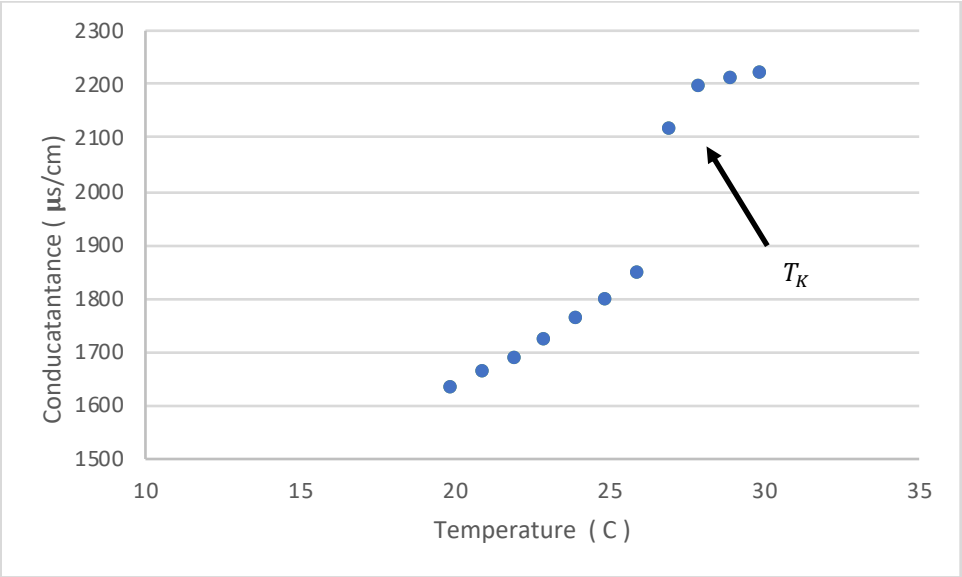


Figure 7: Conductance vs temperature behavior for 40mM undecanoic-alanine-glycine with Na. Krafft temperature represented with T_K. Sample was prepared in a pH 7 5mM boric acid buffer and refrigerated for 24 hours prior to conducting conductivity experiment.

Table 1: Krafft point results at pH 9 for undecanoic-alanine-glycine with sodium, 1,3 propanediamine, 1,5 diaminopentane, and 1,6 diaminohexane. Samples were prepared in a pH 7 5mM boric acid buffer and pH adjusted with sodium hydroxide pellets to pH 9.

Counterion	CMC	Krafft Point (C)
Na	15 ± 1	21.48 ± 1.43
1,3 PD	11 ± 1	14.33 ± 1.15
1,5 DP	7 ± 1	14.30 ± 2.91
1,6 DH	11 ± 1	9.22 ± 0.82

In order to determine the Krafft point, by definition, you must be able to determine the CMC of the solution. The samples where the CMC was able to be determined using either the NMR or conductance are seen in Table 1. UAG samples were prepared with the counterions sodium, 1,3 propanediamine, 1,5 diaminopentane, and 1,6 diaminohexane. For consistency, each of these samples were adjusted to the pH 9 using sodium hydroxide pellets or hydrochloric acid.

Based off the results in Table 1 it appears that the longer the chain is on the counterion

the lower the Krafft point is. 1,6 diaminohexane is the largest counter ion with a Krafft point of 9.22 ± 0.82. Next is 1,3 propanediamine and 1,5 diaminopentane which have a very close Krafft point of 14.33 ± 1.15 and 14.30 ± 2.91 respectively. Finally, sodium being the smallest of all the counterions being examined has not only the highest Krafft point being at 21.48 ± 1.43 but also the highest CMC required out of all four samples.

The Krafft temperature, which does not require the use of the CMC for determination, was determined for all the intended samples of this study with exception of UAA with 1,6 diaminohexane. The reasoning is due to lack of solubility even when heat and stirring was applied. The Krafft temperature for the UAA with sodium is the lowest at 24.91 ± 1.25 and the UAA with 1,2 ethylenediamine is the highest at 49.94 ± 1.43. 1,2 ethylenediamine could have the highest Krafft temperature due to it being the only counterion that branches. This increase in Krafft temperature is also seen in UAG with 1,2 ethylenediamine. For both UAA and UAG is appears that as the chain length of the diamine counterions decreases the Krafft temperature increases. This stands true until UAG reaches 1,3 propanediamine and UAA reaches 1,2 diaminopropane.

Table 2: Krafft temperature results for undecanoic-alanine-alanine and undecanoic-alanine-glycine. Samples were prepared in a pH 7 5mM boric acid buffer and pH adjusted with sodium hydroxide pellets.

Surfactant	Counterion	Krafft Temperature (C)
Undecanoic-Alanine-Alanine	1,2 DP	46.62 ± 2.94
Undecanoic-Alanine-Alanine	1,2 ED	49.94 ± 1.43
Undecanoic-Alanine-Alanine	1,3 PD	48.46 ± 3.92
Undecanoic-Alanine-Alanine	1,4 DB	35.75 ± 0.35
Undecanoic-Alanine-Alanine	1,5 DP	29.50 ± 2.50
Undecanoic-Alanine-Alanine	Na	24.91 ± 1.25
Undecanoic-Alanine-Glycine	1,2 DP	38.80 ± 2.37
Undecanoic-Alanine-Glycine	1,2 ED	52.34 ± 2.48
Undecanoic-Alanine-Glycine	1,3 PD	25.39 ± 0.37
Undecanoic-Alanine-Glycine	1,4 DB	39.05 ± 0.71
Undecanoic-Alanine-Glycine	1,5 DP	22.80 ± 5.76
Undecanoic-Alanine-Glycine	1,6 DH	20.29 ± 4.21
Undecanoic-Alanine-Glycine	Na	27.17 ± 0.76

An interesting question that arose when observing the conductivity results was; *Are there possible structural competition changes occurring at concentrations above CMC and temperatures above the Krafft temperature?* The shape of the conductivity curves were the first indication that there is a possible structural change occurring as the temperature rises above the Krafft temperature. According to the literature, the typical conductivity curve is seen in Figure 8,

which illustrates conductivity versus temperature of primary cetyltrimethylammonium bromide (CTAB) solutions above and below CMC. [11] This curve shape makes sense because we expected that after the solution reaches the Krafft temperature that the aggregates would begin to decrease thus increasing the conductivity as seen below.

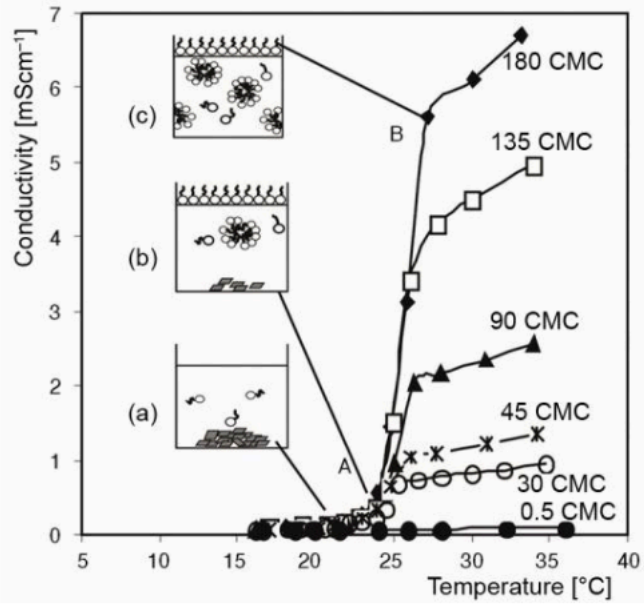


Figure 8: Conductivity versus temperature of CTAB solutions above and below CMC.

However, for some of our conductivity curves have a parabola shape and, in some cases, even the presence of two parabolas as shown in Figure 9. A potential explanation would be a possible shape change is occurring after the sample reaches the Krafft temperature. Another possible explanation is that after the Krafft temperature is reached, the hydrogen bonds begin to break and allow micelles of larger aggregates to form. After examining the literature, it was found that there is actually the possibility of a second CMC that can result in changes in size, shape, polydiversity, and degree of fraction bound of the surfactant and counterion to the micelle [22, 23]. Further research would need to be done to verify that this phenomenon is occurring with these micellar systems.

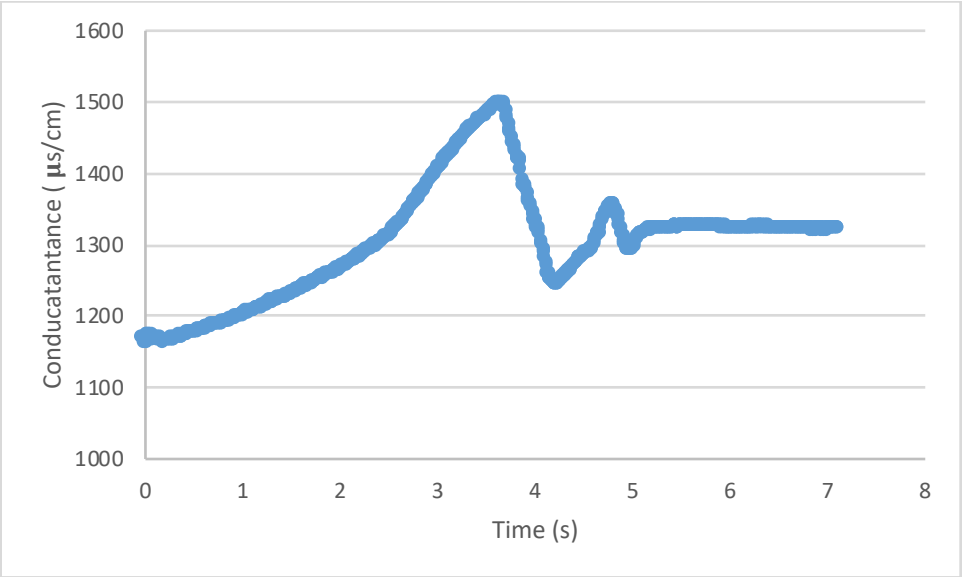


Figure 9: Conductivity as a function of time for 30mM undecanoic-alanine-glycine with 1,2 Ethylenediamine. Samples were prepared in a pH 5mM, 7 boric acid buffer and refrigerated 24 hours prior to running conductivity experiments.

Solubility comparisons were made by running Krafft experiments on samples from 2-50mM. Figure 10 shows the solubility comparisons of UAA with the seven counterions being studied. 1,2 ethylenediamine had the overall highest temperature that was required to solubilize the sample. A possible explanation is that 1,2 ethylenediamine has an extra branch that the other counterions do not. This could potentially make the structure more stable requiring a higher temperature to solubilize. The remaining counterions seem to decrease in stability as the chain length increase; this is true for all expect for 1,3 propanediamine. Figure 11 shows the solubility comparison of UAA with six of the seven counterions being studied. UAA with 1,6 diaminohexane was insoluble even with heat, stirring, and excess counterion added. Figure 11 follows the same trend that Figure 10 did with the exception of 1,3 propanediamine. A possible explanation for why 1,3 propanediamine does not follow the trend would be the way it binds to the surfactant. Although there is no research to support this claim, it is possible that when 1,3

propanediamine binds with UAA it could be bridging reducing solubility and requiring a higher temperature to solubilize.

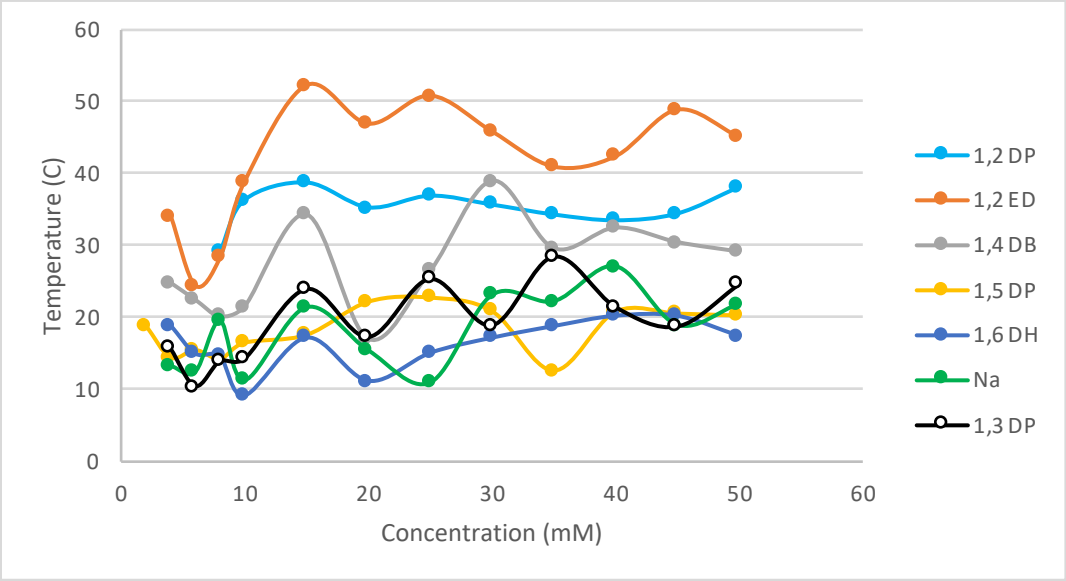


Figure 10: Undecanoic-alanine-glycine solubility comparison. 50mM samples were prepared and concentration was adjusted from 50mM to 2mM using a pH 7, 5mM boric acid buffer. Temperature at which solution became clear was recorded at each concentration

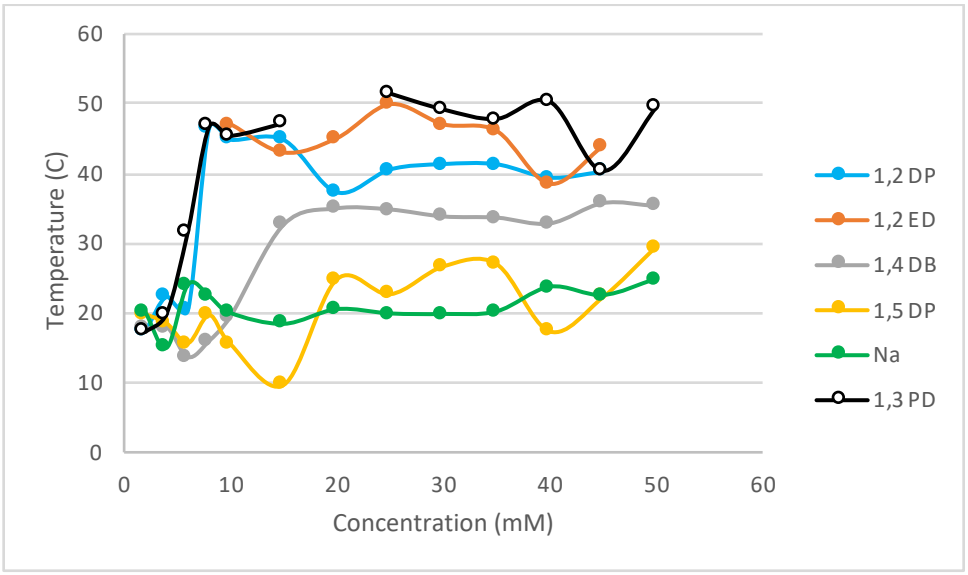


Figure 11: Undecanoic-alanine-alanine solubility comparison. 50mM samples were prepared and concentration was adjusted from 50mM to 2mM using a pH 7 5mM boric acid buffer. Temperature at which solution became clear was recorded at each concentration

3.2 DOSY

The DOSY experiments were performed for two separate purposes: 1) Determination of hydrodynamic radius of the micelles and fraction bound of surfactant and counterion to the micelle for solutions soluble at room temperature at the pH 7, 9 and 11. 2) Comparison of hydrodynamic radius of the micelles and fraction bound of surfactant and counterion to the micelle of solutions at different temperatures was made to try to gain a better understanding of conductivity behavior. The samples that were soluble at room temperature and ran at the pH 7, 9 and 11 were: UAG with sodium and UAG with 1,3 Propanediamine. The solutions that were ran at two different temperatures in order to understand conductivity behavior were: UAG with sodium, UAG with 1,3 Propanediamine, UAG with 1,4 Diaminobutane, UAG with 1,5 Diaminopentane, UAG with 1,6 Diaminohexane, and UAG with 1,2 Diaminopropane. None of the surfactant mixtures with UAA or UAG with 1,2 Ethylenediamine were able to be tested for DOSY due to how rapid they fell out of solution.

As expressed by equation 1, illustrated in Chapter 2, the measured diffusion coefficient is the weighted average of the diffusion coefficients of the surfactant molecules that are bound to the micelles and those that are free (unbound) in the solution. [12] The free surfactant diffusion coefficient is determined by running the surfactant solution below CMC. With a manipulation of this equation the fraction bound of the surfactant monomers to the micelle were calculated. As expressed by equation 2 illustrated in Chapter 2, the micelle diffusion coefficient is calculated using the Stokes-Einstein equation. By a simple modification of this equation we calculated the hydrodynamic radius.

3.2.1 DOSY & ph Change

In order to make a comparison of the data, the surfactant solutions with the sodium counterion was used as the standard. The first thing we looked at when analyzing the binding of the counterion to the UAG micelles, is the fraction of our surfactant in the micellar form. Table 3 shows viscosity, fraction bound (f_b)values, and hydrodynamic radius (R_h) as a function of pH and counterion for 50mM UAG solutions. When looking at our standard counterion (sodium), we see that the $f_{b,suf}$ values fall between 0.826 and 0.946. This tells us that on average, at least 83% surfactant monomers were bound to the micellar form when sodium was the counterion throughout the pH range 7-11. Also, when looking at our standard counterion, we see that there is a modest change in R_h with pH change. The R_h values fall between 6.76 to 7.68 A°, with the largest radii occurring at the highest pH. One possible explanation for this observation is the effect that pH has on hydrogen bonding. At a higher pH there could be a decrease in hydrogen bonding in the polar head group; this decrease in hydrogen bonding would possibly allow for greater distance between the head group which would allow more water to enter the micelle core. This increase in water would cause the micelle to enlarge increasing the R_h value and allowing for an increase in $f_{b,suf}$, which was also observed at the higher pH. This trend can be seen in Figures 12 and 13 below.

Table 3: Viscosity, fraction bound (f_b)values, and hydrodynamic radius (R_h) as a function of pH and counterion for 50mM undecanoic-alanine-glycine solutions. 50mM surfactant solution, 50mM counterion solution, and 6mM surfactant solutions were ran. Samples were prepared in a 10% deuterium oxide 90% water solution with pH adjusted using sodium hydroxide pellets. 100 μL Tetramethylsilane was added to 50mM sample.

Counterion	pH ± 0.1	Viscosity (cP) ± 0.001	f_b Surfactant	f_b Counterion	R_h ($A°$)
Sodium	11	0.96	0.946 ± 0.001	−	7.68 ± 0.070
Sodium	9	0.99	0.960 ± 0.136	−	7.04 ± 0.090
Sodium	7	1.01	0.826 ± 0.004	−	6.76 ± 0.100
1,3 Propanediamine	11	1.01	0.906 ± 0.009	0.967 ± 0.003	6.94 ± 0.310
1,3 Propanediamine	9	0.98	0.977 ± 0.005	0.964 ± 0.002	14.54 ± 3.380
1,3 Propanediamine	7	0.97	0.972 ± 0.006	0.992 ± 0.009	13.02 ± 1.160

The second counterion that was tested was 1,3 Propanediamine. When looking at the $f_{b,suf}$ values, we see that they fall between 0.906 and 0.977. This tells us that on average there was at least 91% surfactant monomers bound to the micellar form throughout the pH range 7-11. When looking at the $f_{b,1,3Propandiamine}$ values we see that they fall between 0.964 and 0.992. This tells us that on average at least 96% of the 1,3 Propanediamine molecules were tightly bound to the surface of the micelle. When looking at change in R_h values we see an interesting trend. From pH 7 to 9 there is an increase in R_h from 13.02 to 14.54 A° but from pH 9 to 11 there is a drastic decrease from 14.54 to 6.94 A°. This corresponds with the change in $f_{b,suf}$ from pH 7 to 9. The $f_{b,suf}$ increases from 0.972 to 0.977 but from pH 9 to 11 $f_{b,suf}$ decreases from 0.977 to 0.906. One possible explanation for this is that at a pH greater than 9 the surfactant molecules begin to break apart and dissociate causing a decrease in $f_{b,suf}$ and R_h (Figure 12 and 13).

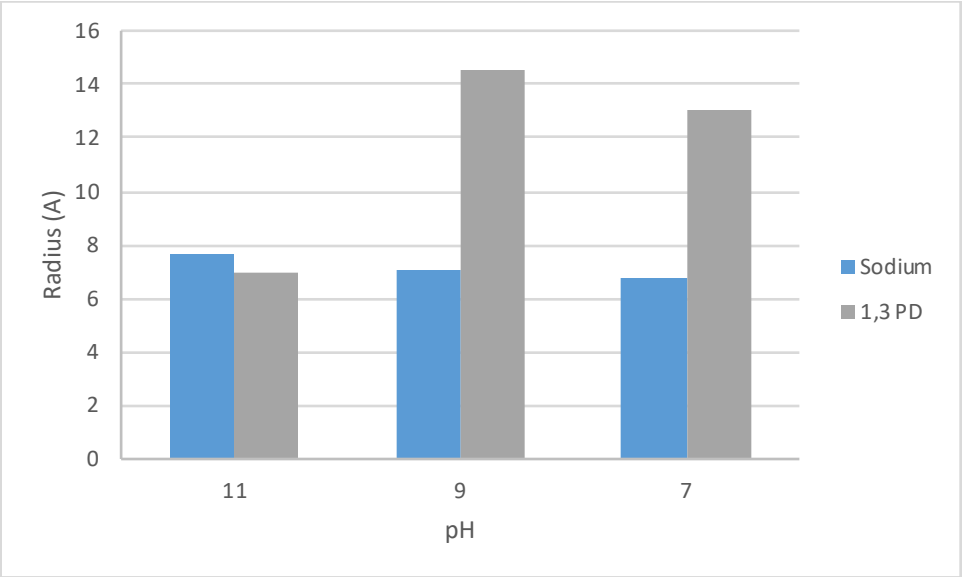


Figure 12: Hydrodynamic radius of undecanoic-alanine-glycine with sodium and 1,3 propanediamine counter ion. Visual representation of data found in Table 3.

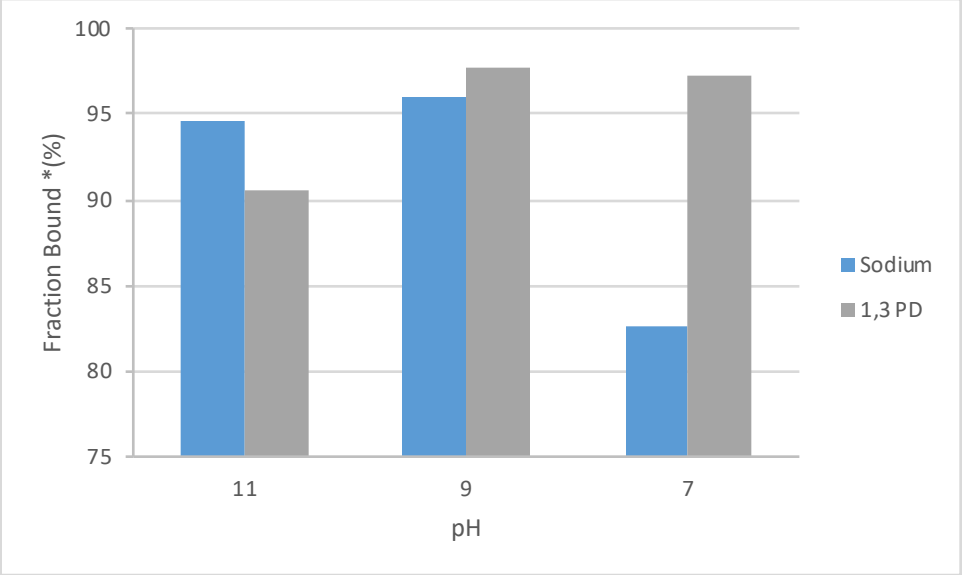


Figure 13: Fraction bound of the surfactant undecanoic-alanine-glycine with sodium and 1,3 propanediamine counterion to the micelle. Visual representation of data found in Table 3.

When comparing the two counterions, we can see that at a lower pH (pH 9 and 7) UAG with 1,3 propanediamine has both a higher fraction bound of surfactants to the micelle and a larger radius. However, when brought to pH 11 the radius of UAG with 1,3 propanediamine

drastically decreases. When UAG is with sodium we see a slight increase in radius as the pH increase. There is also a drastic increase in the surfactant fraction of bound to the micelle as the pH changes from 7 to 9. The linear structure of 1,3 propane diamine may be assisting the micelles form more efficiently at lower pHs compared to sodium. However, as previously stated, the structure of 1,3 propanediamine bound to the surfactant molecules may begin to break apart and dissociate at pHs above 9, causing a decrease in $f_{b,suf}$ and R_h.

3.2.2 DOSY & Temperature Change

The purpose of running DOSY at two seemingly random temperatures is to attempt to explain unexpected conductivity trends seen in the Krafft point experiments. Table 2 shows the results of these DOSY experiments; the temperatures that the samples were ran at were chosen based of the sample's Krafft temperature. Temperature one was close to the Krafft temperature of the corresponding 50mM surfactant solution and temperature two was above the Krafft temperature. For the samples that displayed a parabolic trend, temperature two was the temperature at the bottom right side. For samples that plateaued, temperature two was pulled from the center of the plateau.

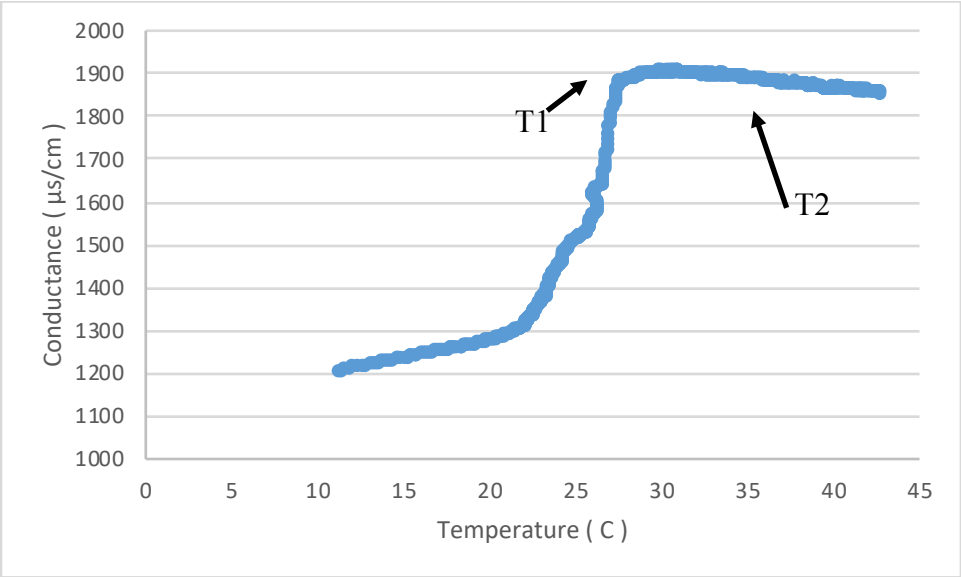


Figure 14: Illustration showing how the two temperatures were chosen for the DOSY experiments of 50mM undecanoic-alanine-glycine with 1,3 Propanediamine. T1 is temperature one that is equal to the Krafft temperature (≈27 °C) and T2 is temperature two is greater than the Krafft temperature and around the center of the plateau (≈35 °C).

Table 4: Viscosity, fraction bound (f_b)values, and hydrodynamic radius (R_h) as a function of temperature (°C) and counterion for 50mM undecanoic-alanine-glycine solutions. 50mM surfactant solution, 50mM counterion solution, and 6mM surfactant solutions were ran. Samples were prepared in a 10% deuterium oxide 90% water solution with pH adjusted using sodium hydroxide pellets. 100 µL Tetramethylsilane was added to 50mM sample.

Counterion	Temperature (°C)	Viscosity (cP) ± 0.001	f_b Surfactant	f_b Counterion	R_h ($\overset{\circ}{A}$)
Sodium	20	0.83	0.888 ± 0.004	–	10.91 ± 0.210
Sodium	25	0.75	1.000 ± 0.046	–	6.87 ± 0.360
1,3 Propanediamine	27	0.91	0.975 ± 0.287	0.302 ± 0.020	7.76 ± 0.520
1,3 Propanediamine	30	0.75	0.979 ± 0.205	0.267 ± 0.026	4.99 ± 0.340
1,4 Diaminobutane	25	0.77	0.869 ± 0.029	0.439 ± 0.024	12.42 ± 0.890
1,4 Diaminobutane	35	0.66	0.705 ± 0.399	0.642 ± 0.068	3.30 ± 0.580
1,2 Diaminopropane	35	0.85	0.330 ± 0.064	0.244 ± 0.093	3.93 ± 0.300
1,2 Diaminopropane	45	0.70	0.907 ± 0.054	0.896 ± 0.017	3.15 ± 0.250
1,5 Diaminopentane	25	1.03	0.563± 0.005	0.936 ± 0.023	8.60 ± 1.140
1,5 Diaminopentane	35	0.88	0.898± 0.134	0.737 ± 0.053	2.42 ± 0.260
1,6 Diaminohexane	19.5	1.18	0.614± 0.532	0.7531 ± 0.460	5.58 ± 4.90
1,6 Diaminohexane	25	1.00	0.918± 0.052	0.888 ± 0.013	14.02 ± 10.64

A common trend that was observed was that the conductivity behavior corresponded with the change in R_h. The samples containing sodium, 1,3 propanediamine, 1,4 diaminobutane, 1,2 diaminopropane, and 1,5 diaminopentane all exhibited parabola behavior during the Krafft experiments. All of these samples also showed a decreased R_h from temperature one to temperature two. One possible explanation is that there could be a shape change that is occurring at higher temperatures for these surfactant solutions. The reasoning behind this is that there is a decrease in R_h but a decrease in conductivity. As explained in one of the previous sections, a decrease in conductivity is not typically seen. In most cases, as temperature is increased the hydrogen bonds in the polar heads of the surfactant begin to break and the aggregation number will begin to decrease and an increase in conductivity is seen. This decrease in conductivity means that the micelles are likely increasing in size with an increase in temperature after the solution has reached its Krafft temperature, but at the same time the R_h value is decreasing. Originally, experimental error was suspected. Three experiments were conducted for each sample and all three experiments resulted in the same conclusion. Somehow, the radius of the micelle is getting smaller while the size of the micelle is increasing. This phenomenon leads us to believe that there is a possibility of shape change from a spherical shape to a rod like shape. One of the best ways to determine shape of a micelle is through static light scattering. Unfortunately, our static light scattering instrument was unavailable (not functional), and this explanation is only theoretical.

3.3 Critical Micelle Concentration

As previously stated, the CMC is the concentration in which the surfactant monomers become concentrated enough to form micelles. At the start of the research, CMC was only going to be determined through the use of the NMR technique. However, the NMR technique did not

form linear trends only allowing us to determine a CMC range, rather than a specific concentration value for CMC. Thus, another technique utilizing conductivity for calculating the CMC was also employed.

3.3.1 NMR Results

The NMR samples that were chosen to be run were based off the Krafft point results; Samples that were soluble at room temperature and had a Krafft point below 25C were ran in the NMR. The Krafft samples were prepared in a boric acid buffer and the NMR samples are prepared in a 10% D_2O mixture, this lead to not all of the samples being soluble at room temperature. After testing both surfactants with the seven counterions for solubility, the only samples that could be run in the NMR were undecanoic-alanine-glycine with sodium and 1,3 propanediamine.

Table 5: CMC results using NMR for undecanoic-alanine-glycine with sodium and 1,3 propanediamine. Samples were prepared in a 10% deuterium oxide 90% water solution and pH was adjusted using sodium hydroxide pellets. All samples were ran at 25C.

Counterion	pH	CMC Range (mM)
Na	7	10 to 15
Na	9	10 to 15
Na	11	10 to 15
1,3 PD	7	6 to 8
1,3 PD	9	10 to 15
1,3 PD	11	15 to 20

Table 5 shows the CMC results for UAG with sodium and 1,3 propanediamine at the pHs 7, 9, and 11. It can be seen that as the pH changes with the sodium counterion, the CMC remains constant between 10 to 15mM. However, for 1,3 propanediamine we see an increase in CMC from 6 - 8 mM for pH 7 to 15-20 mM for pH 11. Although we didn't have more counterions to compare to in this study, this trend is consistent with results from previous studies in this field.

3.3.2 Conductivity Results

The conductivity method is a simple time effective method of determining the CMC of a surfactant solution. The conductivity of each sample was recorded at room temperature (22 °C) and the conductance versus concentration was plotted. The concentration where there is an abrupt change in slope as represented by the arrow in Figure 15 is the CMC. Table 6 shows the CMC results for UAG with sodium, 1,3 propanediamine, 1,5 diaminopentane, and 1,6 diaminohexane at the pH 9. There seems to be no trend in CMC in terms of size of the counterion; 1,6 diaminohexane has the longest structure of the four and maintains the same CMC as 1,3 propanediamine which has a shorter chain. What stands out is that 1,5 diaminopentane has a much lower CMC than the other three. Although it has a lower CMC which is more desirable from a manufacturing standpoint, the counterion issues with its Krafft point and lack of enantiomeric separation abilities makes it less desirable.

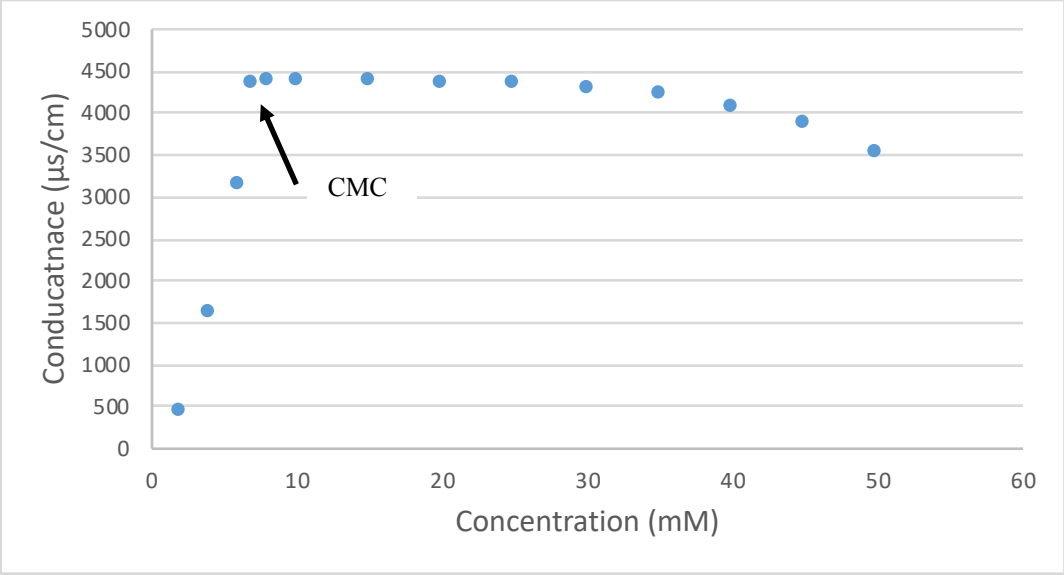


Figure 15: Illustration showing how CMC results were determined using conductance vs concentration plot for undecanoic-alanine-glycine with 1,5 diaminopentane. Sample was prepared using pH 7 5mM boric acid buffer. Samples were ran at laboratory temperature (20-22C).

Table 6: CMC results using conductivity for undecanoic-alanine-glycine with sodium, 1,3 propanediamine, 1,5 diaminopentane, and 1,6 diaminohexane. Sample was prepared using pH 7 5mM boric acid buffer. Samples were ran at laboratory temperature (20-22C).

Counterion	CMC
Na	15± 1
1,3 PD	11± 1
1,5 DP	7± 1
1,6 DH	11± 1

3.4 Chiral Separation

The basis of chiral separation using the CE mode MEKC is based on the differences in stereoselective interactions between the various enantiomeric forms of the chiral compounds and the chiral micelle that acts as the pseudo stationary phase. [16] Not all of the surfactant solutions that were being examined in the study had the capability of being ran in the CE, due to solubility issues. The surfactant that was examined was UAG with the counterions sodium, 1,3 propanediamine, 1,5 diaminopropane, and 1,6 diaminohexane.

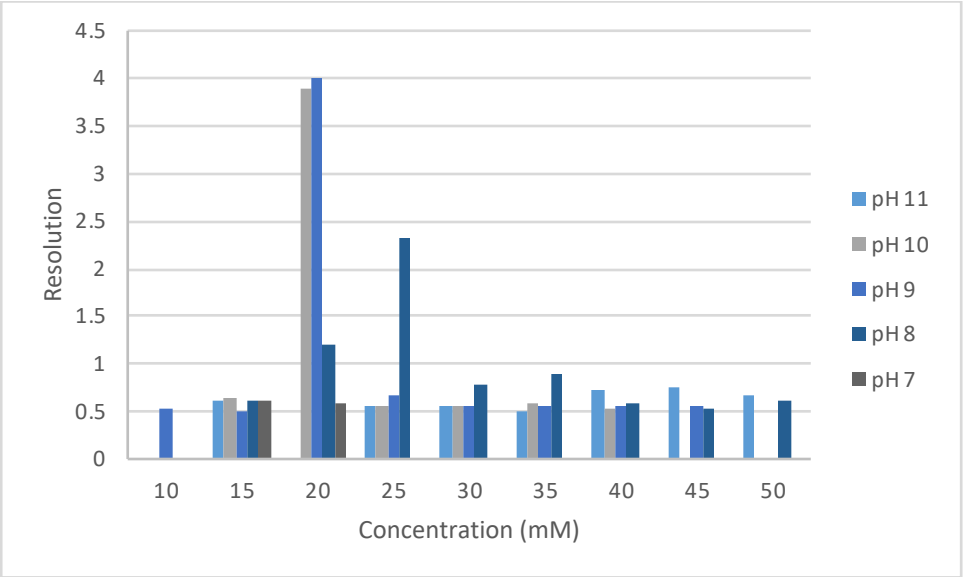


Figure 16: Resolution vs. concentration plot for BOH of undecanoic-alanine-glycine with the sodium counterion. Samples were prepared using pH7, 5mM boric acid buffer and pH adjusted using sodium hydroxide pellets and hydrochloric acid. All samples were ran at 25°C.

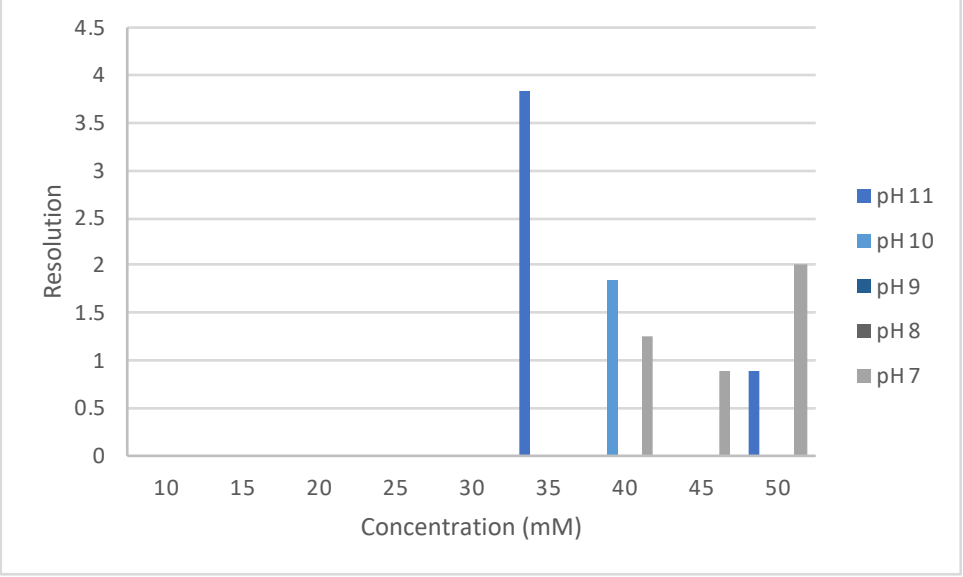


Figure 17: Resolution vs. concentration for BNP of undecanoic-alanine-glycine with the counterion 1,3 propanediamine. Samples were prepared using pH7, 5mM boric acid buffer and pH adjusted using sodium hydroxide pellets and hydrochloric acid. All samples were ran at 25°C.

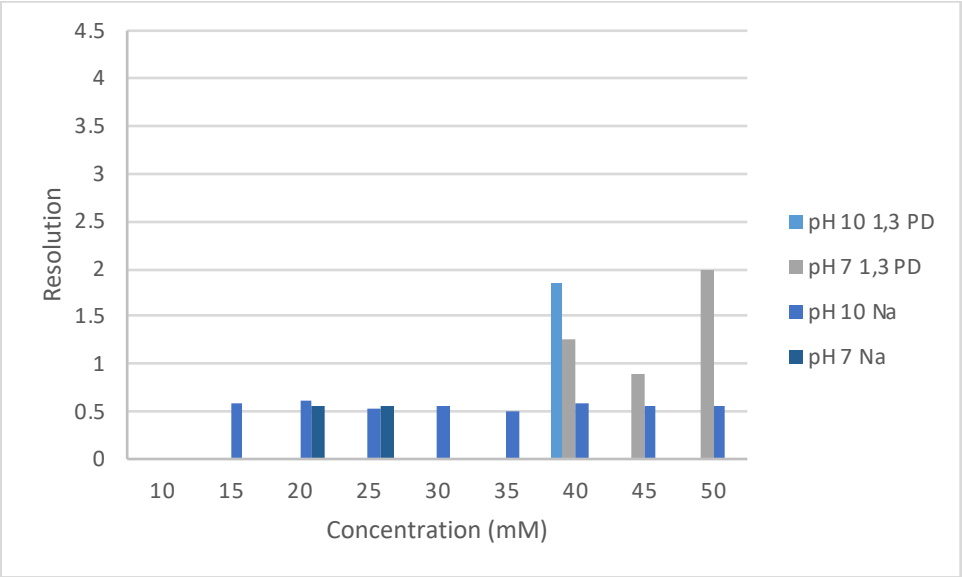


Figure 18: Resolution vs. concentration for BNA of undecanoic-alanine-glycine with sodium and undecanoic-alanine-glycine with 1,3 propanediamine at pH 7 and 10. Samples were prepared using pH7, 5mM boric acid buffer and pH adjusted using sodium hydroxide pellets and hydrochloric acid. All samples were ran at 25°C.

Figure 16 shows the effect that pH and concentration of UAG with sodium as the counterion have on the enantiomeric resolution of BOH. The optimum concentration for the resolution of BOH enantiomers was achieved at 20mM. It was also seen that at the concentration 20mM, the optimum pH range is between 9 and 10 with a resolution between 3.883 and 3.997. UAG with 1,3 propanediamine and UAG with 1,5 diaminopropane showed no enantiomeric separation with BOH.

Figure 17 shows the effect that pH and concentration of UAG with 1,3 propanediamine on the enantiomeric resolution of BNP. The optimum concentration for the resolution of BNP was achieved at 35mM. It is also seen that at the concentration 35mM the optimum pH is 11with a resolution of 3.820. As the concentration decreases below 35mM there is no resolution at any pH. As the concentration increases above 35mM the resolution drops to 2.000 and below with the more optimum pH being 7 at 40mM and 10 at 50mM. Undecanoic-alanine-glycine with

sodium and undecanoic-alanine-glycine with 1,5 diaminopropane showed no enantiomeric separation with BNP.

Figure 18 shows the effect that pH and concentration of UAG with sodium and 1,3 propanediamine have on the enantiomeric resolution of BNA. There are two concentrations that are very close in terms of optimum resolution, 40mM with a resolution of 1.850 and 50mM with a resolution of 2.000. At 40mM the optimum pH is 10 and at 50mM the optimum pH is 7. However, what should be noticed is that both of these samples contained the counterion 1,3 propanediamine. This means that between the concentrations 40-50mM at these pHs 1,3 propanediamine is the preferred counterion of choice between the two. UAG with 1,5 diaminopropane showed no enantiomeric separation with BNA.

There are two main factors that were taken into consideration in order to provide a possible explanation for the enantiomeric resolution: counterion type and chiral interactions. By varying the counterion and keeping the surfactant constant, we can clearly see that the counterion is a major factor in separation. As explained in the conductivity results section, when sodium was the counterion, the highest Krafft points were observed. In addition, when sodium was used as the counterion the longest retention times were observed. This supports the theory that there may be possible bridging occurring with some of the diamine counterions. This bridging could restrict the analytes from penetrating the micelles, thus resulting in a lower retention time, in comparison to sodium.

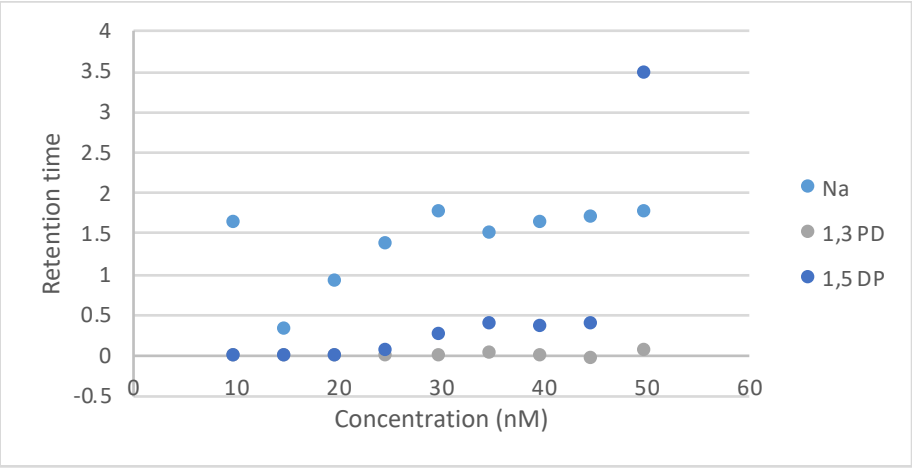


Figure 19: Retention time vs. concentration of undecanoic-alanine-glycine with sodium, 1,3 propanediamine, and 1,5 diaminopentane at pH 10 for BOH. Samples were prepared using pH7, 5mM boric acid buffer and pH adjusted using sodium hydroxide pellets and hydrochloric acid. All samples were ran at 25°C.

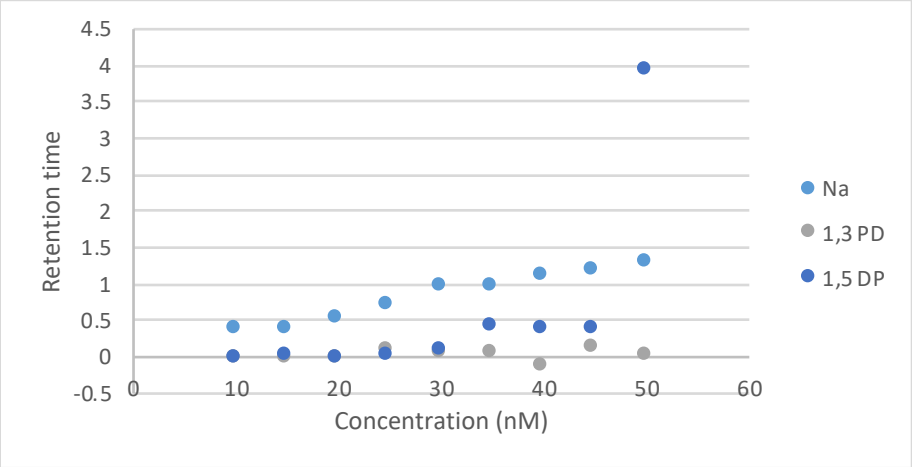


Figure 20: Retention time vs. concentration of undecanoic-alanine-glycine with sodium, 1,3 propanediamine, and 1,5 diaminopentane at pH 10 for BNP. Samples were prepared using pH7, 5mM boric acid buffer and pH adjusted using sodium hydroxide pellets and hydrochloric acid. All samples were ran at 25°C.

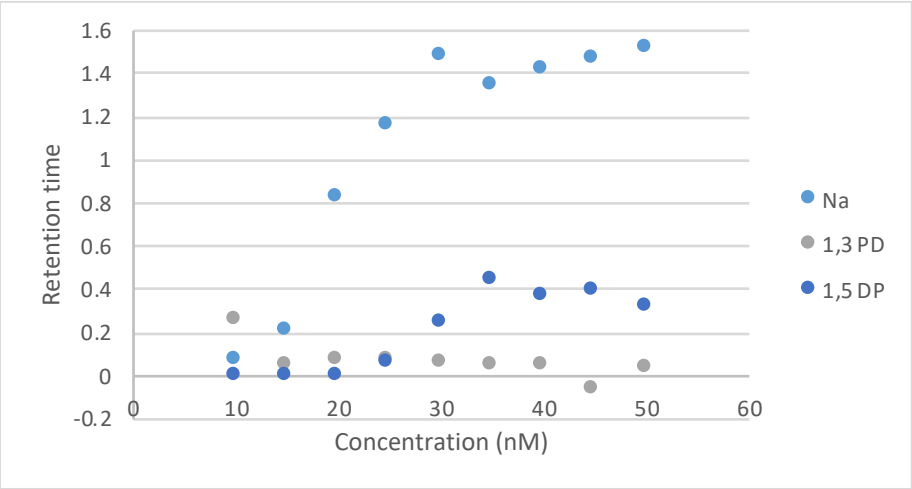


Figure 21: Retention time vs. concentration of undecanoic-alanine-glycine with sodium, 1,3 propanediamine, and 1,5 diaminopentane at pH 10 for BNA. Samples were prepared using pH7 5mM boric acid buffer and pH adjusted using sodium hydroxide pellets and hydrochloric acid. All samples were ran at 25°C.

Another important property looked at was the retention time of the analytes. The retention time is important because it can be an indicator of the stability of the analyte/micelle complex. Enantiomers that form more stable complexes tend to have a longer retention time. [2] In the three figures above, it shows the retention time vs concentration for the binaphthyl derivatives BOH, BNP, and BNA with UAG and the counterions sodium, 1,3 propanediamine, and 1,5 diaminopentane at pH 10. In all three figures (Figures 19-21), we see the same general trend for all three counterions. For the most part, when sodium was the counterion, longer retention times were observed compared to when the counterions were 1,3 propanediamine and 1,5 diaminopentane. However, when the counterion was 1,5 diaminopentane a spike in retention time at 50mM was observed surpassing that when sodium was the counterion. When using 1,3 propanediamine as the counterion, the analyte peak was not observed. This is likely due to the analytes forming too strong of an analyte/counterion/micelle complex thus the analytes are coming at close to the same time as the micelle.

As mentioned in the beginning of this section, the counterion 1,6 diaminohexane was also investigated. As you can see, this counterion does not appear in any of the figures that depict resolution or retention time. This is because no peaks other than the EOF peak on the MEKC electropherogram at any pH or concentration were observed. One possible explanation could be that when the analyte penetrates into the micelle they form an extremely stable complex and do not leave the column in the time frame in which the measurements were made, maximum of one hour.

CHAPTER IV: CONCLUSION

There are many more factors that still must be investigated to have a complete understanding of these systems. We believe however, that the preliminary data presented in this study gives a strong foundation on which to build on. The original intent of this study was to understand the chiral recognition that takes place in the dipeptide micellar systems. Based on previous studies, there was no indication that a surfactant with a single bond tail in place of a double bond tail would cause any issues with solubility.

When faced with these issues, the research had to take a step back and first understand why these systems were behaving in an unexpected manner. The dipeptide micellar systems examined in this study appear to be affected by the size of the C terminal amino acid and how the counterion binds. The surfactant with the smaller polar heads in the C terminal appears to have higher solubility potential that surfactants with larger C terminal amino acids. The results of the Krafft data gave great insight on the stability of the systems, it was originally expected that as the length of the counterion increased that the temperature required to solubilize would also increase. However, the results showed the exact opposite. Excluding 1,2 ethylenediamine, as the counterion became longer, the solubility temperature actually decreased. These results indicate

that the way the counterion binds to the surfactant plays a greater role in the solubility that the size of the counterion.

The DOSY results gave two types of information. First it showed that when consistent temperature was held constant, the pH can have a drastic effect on the hydrodynamic radius and fraction bound of both the surfactant and counterion to the micelle. Secondly, the DOSY results that varied in temperature helped support the theory that these systems are experiencing a structural change as the temperature surpasses the Krafft temperature. The H-NMR and conductivity experiments were then ran to help in the determination of the Krafft point. The results also illustrated that 1,3 propanediamine, 1,5 diaminopentane, and 1,6 diaminohexane had the capability of forming micelles at lower concentrations compared to the widely used sodium counterion.

The CE results gave us information regarding the chiral recognition that is taking place as well as giving us a better understanding of the rigidity of these systems. The diamine counterions do not separate the binaphthyl derivative analytes very well, except at very specific concentrations and pH values. Sodium may be the preferred counterion of choice if there is a need for variable flexibility. Based off the finding of this study, it is suggested to further this research by examining the behavior of the same dipeptide surfactants from this study but with a double bonded tail. It is expected that the double bond on the tail may assist in the solubility issues faced in this study. It is also suggested to procure the required equipment to run samples from this study that fit the temperature limits for CE, light scattering, and fluorescence. By running the samples in these instruments, it would give us a better understanding of the structure and a possible explanation on why we had so many solubility issues.

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