INTERFACIAL STUDIES OF FATTY ACID MONOLAYERS: STRUCTURE, ORGANIZATION, AND SOLVATION BY SUM FREQUENCY GENERATION VIBRATIONAL SPECTROSCOPY

DISSERTATION

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By

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ABSTRACT

Marine aerosols have direct effects on the physics and chemistry of marine atmosphere. In a global dimension, marine aerosols are a key factor in controlling the global climate change by scattering and absorbing solar radiations. Because of limited understanding of interfacial molecular structure and heterogeneous chemistry, model studies of fatty acid monolayers at the air-liquid interface are capable of providing new insight into the aerosol chemistry. In this dissertation, a broad bandwidth sum frequency generation (BBSFG) vibrational technique was used to investigate surface structure, organization, and solvation of monolayer systems on aqueous surfaces. The first molecule of interest is palmitic acid (PA, C_{16}). One of the key findings is that deprotonation can be initiated by ionic binding to the fatty acid headgroups, even at neutral pH. The binding affinity increases in the order that $Na^+ \sim Mg^{2+} < K^+ < Ca^{2+}$. However, the binding of these four cations has little effect on the order and the orientation of the acyl chain in PA with respect to pure water. In addition, the interfacial water structures underneath the PA monolayers also reveal considerable spectral transformations when exposed to Mg^{2+} and Ca^{2+} . At low concentration (0.1M), three bands were observed in the hydrogen bonding region: ~3600 cm⁻¹ (hydrogen-bonded fatty acid headgroups), ~3400 cm⁻¹ (weakly hydrogen-bonded water molecules), and ~3200 cm⁻¹ (strongly hydrogen-bonded water molecules). At 0.3 M, the intensities of these three bands start to decrease for Mg^{2+} and Ca^{2+} . However, in concentrated Mg^{2+} and Ca^{2+} solutions (~2.0 M), the ~3400 cm⁻¹ band and the ~3200 cm⁻¹ band start to converge and to peak at 3300 cm⁻¹ with enhanced intensity. This may suggest that there is significant water restructuring in the course of increasing concentration due to charge neutralization effects at the surface. More importantly, at concentrated conditions, the already disrupted hydrogen-bonding network reorganizes and reverts to its original hydrogen-bonding network as appeared at the neat solution interface. Finally, the observed spectral intensity trends are consistent among the probed regions from 1300 cm⁻¹ to 3800 cm⁻¹ that encompasses the stretching vibrational modes of COO⁻, C=O, C-H, and O-H.

In the structural studies of monounsaturated isomers of oleic acid (OA) and elaidic acid (EA) at the air/liquid interface, we determined that the methyl-sided alkyl chain in OA and EA is responsible for the initial molecular interactions among neighboring molecules; on the other hand, the carboxyl-sided alkyl chain is accountable for the tighter packing as it adopts a near all-trans conformation and positions closer to the surface normal. More importantly, considerable degrees of conformational ordering already start to emerge at 3 mN/m in both OA and EA alkyl chains at the carboxyl side; moreover, an EA monolayer is capable of being tightly packed with more enhanced conformational order than OA at the same physical conditions.

Dedicated to my family

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FIELDS OF STUDY

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TABLE OF CONTENTS

| ABSTRACTii |
|--|
| ACKNOWLEDGMENTS v |
| VITAvi |
| PUBLICATIONSvi |
| FIELDS OF STUDY vii |
| TABLE OF CONTENTS |
| LIST OF TABLES |
| LIST OF FIGURES |
| CHAPTER 1 1 |
| INTRODUCTION |
| 1.1 Motivation |
| 1.2 Dissertation Highlights7 |
| CHAPTER 2 11 |
| SURFACE VIBRATIONAL SUM FREQUENCY GENERATION |
| 2.1 Theory of Surface Vibrational Sum Frequency Generation |
| 2.2 Broad Bandwidth Sum Frequency Generation Instrumentation |

| CHAPTER 3 | |
|---|---------------|
| IONIC BINDING OF Na ⁺ VERSUS K ⁺ TO THE CARBOXYLIC AC | ID HEADGROUP |
| OF PALMITIC ACID MONOLAYERS | |
| 3.1 Introduction | |
| 3.2 Experimental | |
| 3.3 Results and Discussion | |
| 3.4 Conclusions | |
| CHAPTER 4 | |
| IONIC BINDING OF Mg ²⁺ VERSUS Ca ²⁺ TO THE CARBOXYLIC | ACID |
| HEADGROUP OF PALMITIC ACID MONOLAYERS | 50 |
| 4.1 Introduction | 50 |
| 4.2 Experimental | |
| 4.3 Results and Discussion | |
| 4.4 Conclusions | 66 |
| CHAPTER 5 | |
| STRUCTURAL INVESTIGATIONS OF MONOUNSATURATED IS | SOMERS: OLEIC |
| ACID AND ELAIDIC ACID MONOLAYERS | |
| 5.1 Introduction | 77 |
| 5.2 Experimental | |

| 5.3 Results and Discussion | 80 |
|---|-----|
| 5.4 Conclusions | 93 |
| CHAPTER 6 | 113 |
| INTERFACIAL WATER STRUCTURE AT HYDROPHILIC INTERFACES | 113 |
| 6.1 Introduction | 113 |
| 6.2 Experimental | 114 |
| 6.3 Results and Discussion | 115 |
| 6.4 Conclusions | 125 |
| LIST OF REFERENCES | 139 |

LIST OF TABLES

| Table 3.1. Peak assignments of VSFG spectra of PA monolayers on neat water, NaCl, |
|---|
| and KCl solutions (0.2 and 0.6 M) at equilibrium spreading pressure (ESP) for |
| polarization combinations ssp, sps, and ppp ^a |
| Table 5.1. Fitting results for the ssp VSFG spectra of the OA monolayer on water at |
| surface pressures of 3, 15, and 25 mN/m in C-H stretching region |
| Table 5.2. Fitting results for the ssp VSFG spectra of the EA monolayer on water at |
| surface pressures of 3, 15, and 25 mN/m at the C-H stretching region |
| Table 5.3. Fitting results for the ssp VSFG spectra of the D_{17} - OA monolayer on water |
| at surface pressures of 3, 15, and 25 mN/m in C-H stretching region 101 |
| Table 5.4. Fitting results for the ssp VSFG spectra of the D_{17} - EA monolayer on water at |
| surface pressures of 3, 15, and 25 mN/m in C-H stretching region 105 |
| Table 5.5. Fitting results for the ssp VSFG spectra of the D_{17} - OA monolayer on water |
| at surface pressures of 3, 15, and 25 mN/m in C-D stretching region 107 |
| Table 5.6. Fitting results for the ssp VSFG spectra of the D_{17} - EA monolayer on water at |
| surface pressures of 3, 15, and 25 mN/m in C-D stretching region 109 |
| Table 5.7. Fitting results for the component peak intensities of the v_sCD_3 and v_aCD_3 in |
| the ssp SFG spectra of the D_{17} - OA monolayer on water at surface pressures of 3, 15, and |
| 25 mN/m in C-D stretching region |

| Table 5.8. Fitting results for the component peak intensities of the v_sCD_3 and v_aCD_3 in |
|---|
| the ssp SFG spectra of the D_{17} - EA monolayer on water at surface pressures of 3, 15, and |
| 25 mN/m in C-D stretching region |
| Table 6.1. Fitting results for the ssp VSFG spectrum of neat water at 23°C in O-H |
| stretching region |
| Table 6.2. Fitting results for the ssp VSFG spectra of the neat $MgCl_2$ solutions (0.1, 0.3, |
| and 1.5 M) at 23°C in O-H stretching region |
| Table 6.3. Fitting results for the ssp VSFG spectra of the neat $CaCl_2$ solutions (0.1, 0.3, |
| and 1.5 M) at 23°C in O-H stretching region |
| Table 6.4. Fitted results for the ssp VSFG spectrum of the PA monolayer at equilibrium |
| spreading pressure (ESP) on water at 23°C in O-H stretching region |
| Table 6.5. Fitted results for the ssp VSFG spectra of the PA monolayers at equilibrium |
| spreading pressure (ESP) on MgCl ₂ solutions (0.1, 0.3, 1.5, and 2.6 M) at 23°C in O-H |
| stretching region |
| Table 6.6. Fitted results for the ssp VSFG spectra of the PA monolayers at equilibrium |
| spreading pressure (ESP) on $CaCl_2$ solutions (0.1, 0.3, and 1.5 M) at 23°C in O-H |
| stretching region |
| |

LIST OF FIGURES

Figure 2.1. Energy level diagram of the sum frequency generation process. $|g\rangle$ is the Figure 3.1. Surface compression isotherms (π -A) of PA monolayer at 23°C on aqueous surfaces: (A) neat water and NaCl (0.2 and 0.6 M), (B) neat water and KCl (0.2 and 0.6 Figure 3.2. VSFG spectra of PA monolayers on aqueous NaCl and KCl (0.2 and 0.6 M) solutions and neat water at ESP under three polarization combinations: (A) ssp, (B) sps and (C) ppp; blue, green and red colors denote subphase of neat water, aqueous 0.2 M and 0.6 M salt solutions, respectively. Vibrational modes of v_sCH_2 , v_sCH_3 , and $v_{FR}CH_3$ in (A), v_aCH_3 in (B), and v_sCH_3 , v_aCH_2 and v_aCH_3 in (C) are shown in the spectra. Dash Figure 3.3. ssp VSFG spectra of D₃₁-PA monolayers on water with pH values at 1.0 and Figure 3.4. ssp VSFG spectra of D₃₁-PA monolayers on salt solutions: (A) 0.2 and 0.6 M NaCl solutions; (B) 0.2 and 0.6 M KCl solutions. The individual fitted curves are shown

Figure 3.5. ssp VSFG spectra of PA monolayers on water at pH values: (A) 1.0; (B) 6.0, Figure 3.6. ssp VSFG spectra of PA monolayers on salt solutions: (A) 0.2 and 0.6 M NaCl solutions; (B) 0.2 and 0.6 M KCl solutions. The individual fitted curves are shown Figure 3.7. ssp VSFG spectra of neat water and pure salt solutions (without monolayer) showing the dangling OH of surface water molecules: A. neat water; B. 0.6 M NaCl Figure 3.8. ssp VSFG spectra PA monolayers on salt solutions showing the OH of the PA carboxylic acid. PA spread on: A. 0.2 M NaCl solution; B. 0.6 M NaCl solution; C. Figure 3.9. ssp VSFG spectra PA monolayers on neat water at pH 13.7 showing the Figure 4.1. Surface pressure-area isotherms (π -A) of PA monolayer at 23°C on aqueous surfaces: (A) neat water with Brewster angle microscopy images in the G-TC coexistence region and the TC homogeneous phase region (B) neat water and MgCl₂ (0.1 and 0.3 M), Figure 4.2. ssp VSFG spectra of PA monolayers on aqueous solutions at 10 mN/m: (A) Figure 4.3. ssp VSFG spectra of D_{31} -PA monolayers on water with pH values of 2.1, 6.0,

Figure 4.4. ssp VSFG spectra of D_{31} -PA monolayers on salt solutions: (A) 0.1 and 0.3 M MgCl₂; (B) 0.1 and 0.3 M CaCl₂ solutions. The individual fitted curves are shown as solid lines. The peak intensities in Figure A are enhanced five times for a comparison Figure 4.5. Pictorial illustrations of four possible metal-carboxylate complexes in the Figure 4.6. ssp VSFG spectra of D₃₁-PA monolayers on 0.1 M CaCl₂ solutions in a time series: (A) 5 min, (B) 25 min, (C) 60 min, and (D) 70 min. The individual fitted curves Figure 4.7. ssp VSFG spectra of D₃₁-PA monolayers on 0.3 M CaCl₂ solutions in a time series: (A) 5 min, (B) 25 min, (C) 60 min, and (D) 70 min. The individual fitted curves Figure 4.8. ssp VSFG spectra of PA monolayers on water with pH values of 1.0, 6.0, and Figure 4.9. ssp VSFG spectra of PA monolayers on salt solutions: (A) 0.1 and 0.3 M MgCl₂; (B) 0.1 and 0.3 M CaCl₂. The individual fitted curves are shown as solid lines . 76 Figure 5.1. Simplified structures of oleic acid (cis), elaidic acid (trans), D₁₇-oleic acid Figure 5.2. Langmuir isotherms (π -A) of oleic acid, elaidic acid and the D₁₇ -labeled OA and EA on water: (A) OA and EA and (B) D_{17} – OA and D_{17} – EA. The markers denote the phases of monolayers at each surface pressure: L-G – liquid and gas coexistence

Figure 5.3. ssp VSFG spectra of OA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 1 min acquisition in Figure 5.4. ssp VSFG spectra of the EA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 1 min acquisition in Figure 5.5. ssp VSFG spectra of the D_{17} - OA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 3 min acquisition in CH stretching region. Solid curves represent the fitted spectra......100 Figure 5.6. ssp VSFG spectra of the palmitic acid and D_{17} -OA monolayers on water at 3 mN/m. Each spectrum corresponds to 1 min acquisition in C-H stretching region. 102 Figure 5.7. Schematics for the proposed transformation of the v_aCH_2 IR transition moment during compression. The arrow represents the v_aCH_2 IR transition moment. 103 Figure 5.8. ssp VSFG spectra of the D_{17} - EA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 3 min Figure 5.9. ssp VSFG spectra of the D_{17} - OA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 3 min Figure 5.10. ssp VSFG spectra of the D_{17} - EA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 3 min

Figure 5.11. CD₃ orientation angle simulation curve of $\left(\frac{\chi_{eff,ssp}^{(2)}(v_sCD_3)}{\chi_{eff,ssn}^{(2)}(v_aCD_3)}\right)^2$ vs θ . The red '+' represents the CD₃ orientation angles of D₁₇-EA at 3, 15, and 25 mN/m (from right to left); the blue 'x' represents the CD₃ orientation angles of D_{17} -OA at 3, 15, and 25 mN/m Figure 6.1. ssp VSFG spectrum of neat water at 23°C in O-H stretching region. Solid curve represents the fitted spectrum. Three component bands are depicted as gray solid Figure 6.2. ssp VSFG spectra of the neat MgCl₂ solutions (0.1, 0.3, and 1.5 M) at 23°C Figure 6.3. ssp VSFG spectra of the neat CaCl₂ solutions (0.1, 0.3, and 1.8 M) at 23°C in Figure 6.4. ssp VSFG spectrum of the PA monolayer at equilibrium spreading pressure (ESP) on water at 23°C in O-H stretching region. Solid curve represents the fitted Figure 6.5. ssp VSFG spectra of the PA monolayers at equilibrium spreading pressure (ESP) on MgCl₂ solutions (0.1, 0.3, 1.5, and 2.6 M) at 23°C in O-H stretching region. Figure 6.6. ssp VSFG spectra of the PA monolayers at equilibrium spreading pressure (ESP) on CaCl₂ solutions (0.1, 0.3, and 1.8 M) at 23°C in O-H stretching region. Solid

CHAPTER 1

INTRODUCTION

1.1 Motivation

The key components of our research interests described in this dissertation are focused on fatty acid Langmuir monolayers at the air/liquid interface. The objectives are to strive for an in-depth understanding on molecular structure, organization, and solvation of these monolayers at this unique interface, as well as ionic binding behavior of metal cations towards fatty acid headgroups. To accommodate this research, an in-house broad bandwidth sum frequency vibrational spectroscopic laser setup was chosen to study these objectives. This particular instrumentation has the ability to provide surface specificity and sub-monolayer sensitivity that are essential for interfacial investigations.^{1,2}

Marine aerosols are widely present in the atmosphere, making a significant contribution to the global organic aerosol load. Photosynthesis by marine bioorganisms, especially phytoplankton, produces ~30-60 Pg of organic carbon annually.³ On average, typical fatty acid concentration, both dissolved and in particulates, in seawater is around 3 to 200 μ g/l.⁴ Fatty acids, along with long-chain alkanes and total hydrocarbons prefer to reside at the air/seawater interface, forming an oily microlayer. The formation of marine aerosol is via a bubble bursting mechanism, in which sea salt particles are ejected and coated with surface active organic molecules.⁵⁻⁷ In these primary marine aerosols,

the organic molecules are arranged in an inverted micelle structure with the hydrophilic headgroups in direct contact with the aqueous core, while the hydrophobic tails oriented towards the air phase.⁸ In the marine environment, the $C_{12} - C_{19}$ fatty acid are commonly found, with saturated fatty acids being dominant.^{4,7,9} Moreover, unsaturated fatty acids are also observed in the marine troposphere, but with much less abundance compared to the saturated ones.⁹

Organic molecular makeup significantly influences aerosol properties; hence this could directly modulate aerosol functions in the environment. For instance, it is known that organics can change the hygroscopic properties of aerosols and their optical properties. At the same time, inorganic salt particles can clearly deliquesce and effloresce in a fashion that marine aerosols with a high organic content do not undergo sharp phase transitions and can maintain some water at low relative humidity.¹⁰ Reduced hygroscopicity directly hinders growth under subsaturated conditions and hence affects sunlight scattering, as well as weakening their ability to act as cloud condensation nuclei (CCN) and then, as a consequence, affects cloud properties and climate as a whole.

While in the atmosphere, marine aerosol composition and properties are susceptible to change via various chemical transformations. Among which, the heterogeneous uptake of oxidative species such as OH radical, ozone, NO₂, NO₃, and halogen atoms is considered predominant and results in oxidation of surface residing organics. Chemical transformations constitute aerosol aging. The aging processes have been investigated extensively in many laboratory and field studies.¹¹ Namely, quantifying the rate of chemical transformation and aging of aerosol particles are the primary objectives. Earlier laboratory studies on aging focused mostly on measurements

of reactive uptake coefficients and some characterization of first-generation products. Recently, multicomponent aerosols that better represent atmospheric particles have been key models in laboratory studies for an in-depth understanding of the complex products and changes that occur in the particles. For example, oleic acid (OA) has become a benchmark for studying the heterogeneous reaction of ozone with organic particles. The mixture of a reactive species in an unreactive matrix, in which only a fraction of the organics can react, is more representative of the composition of real aerosols. Therefore, by studying the heterogeneous reaction of ozone with films of saturated fatty acid and oleic acid mixtures, more representative results have been obtained in relation to the real aerosols values.^{12,13}

As stated, one major interest in marine aerosol research is to understand its formation and transformation mechanisms during the atmospheric transport. It is believed that oxidative processing play a key role in the aerosol growth and the ultimate formation of the cloud condensation nuclei (CCN).¹¹ Since surface structure and aggregation of organic molecules are known to be the critical factors in the transformation process, a model study using Langmuir monolayer films at the vapor/liquid interface has benefits not only in mimicking the actual aerosol surface, but also in elucidating surface phenomena in a controlled manner.

Even though significant progress has been made in recent years in the areas of aerosol research such as source origin, formation and transformation, physical and chemical states, and theoretical modeling of the atmosphere, many questions still remained unanswered.^{10,11} For instance, of interest in this research are to shed light on questions such as how the inorganic cations interact with the fatty acid headgroup at the

vapor/aerosol interface, what the trend is like in the binding affinity of four major cations $(Na^+, K^+, Mg^{2+}, and Ca^{2+})$ towards the fatty acid headgroups, whether ionic binding of cations affects the structure of the surface fatty acid layer, and how the interfacial water structures are perturbed underneath the surface fatty acid layer with and without the presence of cations.

Moreover, the key questions that were addressed in this research also have relevant biological implications. Na⁺, K⁺, Mg²⁺, and Ca²⁺ are the four most abundant cations in biological systems.¹⁴ The reason is because the sea is the original source of life, the same abundance of these four cations in seawater naturally translates to the biological organisms during evolution. Na⁺ and K⁺ are the major ionic components of the extra and intra-cellular fluids, respectively. Their functions encompass the active Na⁺ and K⁺ exchange pump that drives most of the secondary transport systems to bring nutrients and metabolites into the cell. However, the selective nature of the potassium ion channel preferring potassium over sodium and vice versa in the sodium channel are still not well understood.¹⁵ One plausible explanation is the difference in protein structure makeup in the channel pores that have varied strength in stripping away solvating water molecules during ion transport.¹⁵

Likewise, divalent cations are essential to the cellular physiology of living organisms. Most importantly, they play a multitude of functions ranging from assisting in protein folding, maintaining protein structures, being cofactors to the cellular nucleotides, modulating enzyme activities, and promoting signal transduction.¹⁶ Because divalent cations such as Mg²⁺ and Ca²⁺ participate in diverse biological processes, there is considerable and sustained interest in understanding their role at a fundamental level such

as that in cellular regulatory mechanisms.¹⁷⁻¹⁹ Mg^{2+} is known as an antagonist in actions of Ca^{2+} in cellular physiology; for instance, Mg^{2+} deficiency impairs Ca^{2+} metabolism. Low Mg^{2+} concentrations concomitantly raise intracellular levels of Ca^{2+} , creating an $Mg^{2+}:Ca^{2+}$ imbalance. As a result, such imbalance can cause a perpetual vasoconstriction state in smooth vascular muscle cells. This condition is generally known as hypertension.²⁰

In this dissertation we investigate the interaction specificity of these four cations with biological ligands at the molecular level. By recognizing their unique interaction specificity with biological ligands, implications of their functions, selectivities, as well as potential binding sites in more complex systems such as trans-membrane proteins can be established. To effectively investigate the cationic binding affinity with biological ligands at an interface, Langmuir monolayers and Langmuir-Blodgett (LB) films are used. The monolayers and LB films serve as a proxy for the cell membrane and continue to be the prevailing model systems adopted by the surface science community.

Historically, surface phenomena have been explored in studies of proxy systems where molecular organization,²¹ surface aggregation,¹⁹ film morphologiy,²² and molecular interactions²³ are examples of past studies. Surface techniques applied to these research areas have advanced considerably. Among them, Langmuir trough instruments have been traditionally used for surface pressure-area (π -A) isotherms to reveal macroscopic phase behavior of surface films. Brewster angle microscopy and fluorescence microscopy have been widely applied in studies of film morphology of Langmuir monolayers at aqueous surfaces;²² likewise, atomic force microscopy and scanning electron microscopy are candidates of choice for LB films on solid substrates.^{24,25} X-ray diffraction and neutron scattering techniques have also become more frequently employed in studies of surface molecular structures and phase behavior of surface films in crystalline states, benefitting from their duality at both aqueous and solid surfaces.²¹ Infrared reflection absorption spectroscopy (IRRAS) has been the predominant, in-situ technique that is capable of providing molecular structure information of surface molecules. By identifying vibrational signatures of functional groups and analyzing spectral components, molecular conformation and chain orientation of amphiphilic molecules can be deduced.²⁶

To date, IRRAS is established as an analytical technique in applied studies that involve ionic binding of metal cations to surfactant headgroups at the air-aqueous interface or at the air-solid interface as in LB films. It is commonly observed that the presence of certain cations in aqueous solution promotes LB film transfer efficiency and film integrity; for instance, empirical evidence suggests that Cd^{2+} and Pb^{2+} have these characteristics.²⁷ To gain more insight into ionic binding behavior of various cations ranging from alkaline earth metals, transition metals, and main-group metals, to longchain fatty acids at the air-aqueous interface, Hühnerfuss and coworkers determined the possible binding configurations of the coordination complexes produced. They evaluated the competition behavior of the metal cations with hydronium ions in increasingly acidic environments.²⁸ With the addition of polarization modulation to IRRAS, Calvez et al. studied both divalent and monovalent cations $(Cd^{2+}, Ca^{2+}, Mg^{2+}, and Na^{+})$ with respect to a deuterated arachidic acid $(D_{39}$ -AA, $C_{20})$ monolayer at the air-aqueous interface under different pH conditions.²⁹ Later, more studies of similar systems were conducted by other groups. These studies incorporated more metal cation species and different

surfactant molecules; in certain cases, polarization-modulated IRRAS was employed to improve signal quality and to assist spectral analysis. However, the binding behavior of alkali and alkaline earth cations is still not well understood. These cations have a weaker binding affinity to the carboxylate (COO⁻) group relative to transition metal or main group metal cations. Due to their noble-gas electron configuration, these cations were considered by some researchers to be purely ionic in nature when exposed to negatively charged ligands such as COO⁻, and thereby rendering their interactions with COO⁻ nonspecific. On the contrary, Dutta and coworkers concluded that Mg²⁺ could interact so strongly with COO^{-} in the heneicosanoic acid (C_{22} , HA) monolayer at the air-aqueous interface that it promoted formation of a superlattice structure in the monolayer film, similar to the outcome induced by Cd^{2+} and Pb^{2+} .³⁰ In addition, the results presented here are in opposition to two recent X-ray absorption spectroscopic (XAS) findings reported separately by Saykally, Cohen, and coworkers,³¹ and Winter, Jungwirth, and coworkers.³² These disparate views indicate that further studies are necessary to elucidate the intermolecular and interionic interactions.

In this dissertation, identifying the ionic binding affinity of these four cations towards biological ligand such as carboxylate in the fatty acid headgroup has unique implications for their functions, selectivities, as well as potential binding sites in more complex systems such as trans-membrane proteins.

1.2 Dissertation Highlights

Chapter 3 reports that alkali metal cations, Na^+ and K^+ , have various degrees of binding affinities towards palmitic acid (C₁₆, PA) headgroup. Deprotonation of the

headgroup occurs when aqueous phase contains Na^+ and K^+ , albeit at neutral conditions. Two unique mechanisms could initiate deprotonation. First, it could be caused by the long-range electrostatic interaction between the hydrated cations and the headgroup; second, the similar effect could as well result from an ionic complex formation between nonhydrated cation and the headgroup. Our data imply that Na^+ favors the first mechanism, while K^+ tends to favor the second mechanism. This is consistent with their differences in hydration parameters: surface charge and hydration radii. More importantly, K^+ manifests greater binding affinity towards the carboxylic headgroup than Na^+ , thereby causing more deprotonation. This is in line with the formation of 1:1 ionic complexes between K^+ and the carboxylate group.

Chapter 4 reports a disparate cationic binding specificity of alkaline earth cations $(Ca^{2+} \text{ and } Mg^{2+})$ with biological-relevant ligands such as carboxylate (COO⁻) at the air/liquid interface. The empirical evidence strongly support that Ca^{2+} binds much more strongly to the carboxylate group than Mg^{2+} . We concluded that at a neutral pH, the mechanism that governs Ca^{2+} binding to COO⁻ is accompanied by commensurate deprotonation of the carboxyl headgroup. In addition, surface molecular structure and ion concentration are also influencing cation binding behaviors at the air/liquid interface. For example, at low concentration (0.1 M Ca^{2+}), Ca^{2+} initially favors forming ionic complexes in a bridging configuration (2 Ca^{2+} :1 COO⁻) but gradually transforms to a chelating (bidentate) complex (1 Ca^{2+} :1 COO⁻) as the equilibrium species. On the other hand, as the Ca^{2+} concentration rises to 0.3 M, the primary complex species exists primarily in the bridging configuration even though sufficient time was given for

structural reorganization as seen in the 0.1 M. Therefore, direct identification of these unique trends in Ca^{2+} has great biological implications.

Chapter 5 reports spectroscopic evidence that allows a more in-depth look into the conformational ordering mechanisms during film compression in monolayers of unlabeled and isotopic-labeled oleic acid (OA) and elaidic acid (EA). It was found that the methyl-sided alkyl chain in OA and EA is responsible for the initial molecular interactions among neighboring molecules; on the other hand, the carboxyl-sided alkyl chain is accountable for the tighter packing as it adopts near all-trans conformation and positions closer to the surface normal. In addition, spectral evidence shows that near all-trans conformation already starts to emerge at 3 mN/m.

The methyl orientation calculation points out that the methyl group in EA is pointing more towards to the surface normal as the surface pressure increases than those of OA at the same surface pressure. This also indicates that EA monolayer is capable of being tightly packed with a more enhanced conformational order than OA at the same physical conditions.

Chapter 6 reports the interfacial hydrogen-bonding network that uniquely exists in between the PA Langmuir monolayer and the underneath surface water molecules. In particular, we identified perturbations due to cation binding of Mg^{2+} and Ca^{2+} . At first, the polar ordering of the interfacial water molecules under the influence of the surface field of the dissociated PA headgroups is confirmed. We concluded that only a small fraction of negative charges can induce considerable polar ordering on the surface water molecules. On the other hand, we found Ca^{2+} has greater impact on the interfacial hydrogen-bonding network than Mg^{2+} on the basis that Ca^{2+} has much greater binding affinity towards the carboxylate group relative to Mg^{2+} . Therefore, the transition point at which surface water structures reorganize occurs at a much lower concentration for Ca^{2+} as compared with Mg^{2+} . More importantly, at concentrated conditions, the already disrupted hydrogen-bonding network reorganizes and reverts to its original hydrogen-bonding network as appeared at the neat solution interface.

CHAPTER 2

SURFACE VIBRATIONAL SUM FREQUENCY GENERATION

2.1 Theory of Surface Vibrational Sum Frequency Generation

In depth descriptions of the second-order optical processes are presented in a great abundance in literature.³³⁻³⁶ Therefore, only a concise introduction is given in this section. Sum frequency generation is a second-order optical phenomenon that originates from material nonlinear response in the event of intense optical radiations. This process is made possible by introducing high power coherent light source such as lasers. Sum frequency generation vibrational spectroscopy (SFG-VS) is a surface vibrational technique that utilizes this nonlinear process for spectroscopic investigations at surface or interface between two bulk media. In conventional SFG-VS, two laser beams at different frequencies, commonly one at a fixed visible frequency and the other with tunable infrared frequency, are coupled both spatially and temporally in medium to generate a third optical beam at a frequency that is the sum of the former two. This process is known as upconversion in which the signal is converted to a higher frequency such as optical frequency for easy signal detection, as shown in Figure 2.1.

The surface SFG signal is generated from the second-order nonlinear polarization in medium that is induced by the coupling of two incident fields $E(\omega_1)$ and $E(\omega_2)$ at frequencies of visible and infrared, respectively, (equation 2.1)

$$P^{(2)}(\omega_s) = \epsilon_0 \chi^{(2)} \colon E(\omega_1) E(\omega_2)$$
(2.1)

where $\chi^{(2)}$ is known as the surface nonlinear susceptibility. Under the electric dipole approximation, a lack of inversion symmetry is an imperative condition in generating the second-order optical process;³³ therefore, any surface or interface separating the two adjacent isotropic media naturally meets this condition, giving SFG-VS the surface specificity. In theory, the SFG intensity is proportional to the square of $P^{(2)}(\omega_s)$, which then makes the measurable SFG intensity proportional to the intensities of the two incident laser beams and the modulus square of $\chi^{(2)}_{eff}$ of the molecular system of interest, as shown in equation 2.2

$$I(\omega_{s}) = \frac{8\pi^{3}\omega^{2}sec^{2}\beta}{c_{0}^{3}n_{1}(\omega_{s})n_{1}(\omega_{1})n_{1}(\omega_{2})} \left|\chi_{eff}^{(2)}\right|^{2} I(\omega_{1})I(\omega_{2})$$
(2.2)

with

$$\chi_{eff}^{(2)} = [L(\omega_s \cdot \hat{e}(\omega_s)] \cdot \chi_{ijk}^{(2)} : [L(\omega_1 \cdot \hat{e}(\omega_1))][L(\omega_2 \cdot \hat{e}(\omega_2)]$$
(2.3)

Here, ω_s , ω_1 and ω_2 are the frequencies of the sum frequency (SF), visible and infrared electric fields, respectively. $n_i(\omega_i)$, corresponds to index of refraction of the bulk medium at frequency ω_i ; β_i is the incidence or reflection angle of the laser beam of ω_i with respect to the surface normal. $I(\omega_i)$ is the intensity of the laser beam of ω_i , represented by the SF, visible, and infrared fields. Next, $\hat{e}(\omega_i)$ is the unit electric field vector of the laser beam of ω_i . In general, the polarization of the electric field can be either s or p. If we define xz plane as the plane of incidence for both the incident and output fields in the laboratory coordinate systems, xyz, then s polarization is defined to be perpendicular to the plane of incidence or parallel to the y axes while p polarization is in the plane of incidence, having field components at both x and z axes. $L(\omega_i \cdot \hat{e}(\omega_i))$ is the Fresnel factor in the tensorial form. It functions as an electric field correction factor when beams propagate across the boundary separating the interface and the adjacent isotropic medium, which is similar to the transmission coefficient at the boundary between two isotropic media. In a review, Shen has clearly demonstrated the complete derivation of all three tensorial Fresnel factors, $L_{xx}(\omega_i)$, $L_{yy}(\omega_i)$, and $L_{zz}(\omega_i)$.³⁷

 $\chi^{(2)}_{ijk}$ is a third-ranked tensor that has 27 macroscopic susceptibility components, where i, j, and k each represent Cartesian coordinates (x, y, and z) in the laboratory reference frame. Under the symmetry constraint, for example, an achiral rotationally isotropic interface with $c_{\infty v}$ has only 7 non-vanishing components.³⁸ Because the interface is defined to be in the xy plane, x and y becomes equivalents; hence the underlying relations for these 7 components are $\chi^{(2)}_{xxz} = \chi^{(2)}_{yyz}, \ \chi^{(2)}_{xzx} = \chi^{(2)}_{yzy}, \ \chi^{(2)}_{zxx} = \chi^{(2)}_{zyy}$ and $\chi^{(2)}_{zzz}$ according to the symmetry rules. In experiment, what is measured is $\chi^{(2)}_{eff}$. By selectively choosing pertinent polarization combinations of the incident beams and the generated SF beam, the aforementioned four unique $\chi^{(2)}_{ijk}$ can be empirically determined. For instance, four polarization combinations are possible in SFG experiment to assist evaluating each unique $\chi_{ijk}^{(2)}$. In practice, polarization combination is assigned in the order of increasing wavelength of the participating laser beams. As an example, ssp polarization denotes the polarizations of the SF, visible, and infrared to be s, s, and p, respectively. Same order is also obeyed in the other three polarization combinations, namely, sps, pss, and ppp. The detailed relations between each measured $\chi_{eff}^{(2)}$ and the corresponding macroscopic $\chi_{ijk}^{(2)}$ are shown in equations 2.4 – 2.7.³⁹

$$\chi_{eff,ssp}^{(2)} = L_{yy}(\omega_s) L_{yy}(\omega_1) L_{zz}(\omega_2) \sin \beta_2 \chi_{yyz}^{(2)}$$
(2.4)

$$\chi_{eff,sps}^{(2)} = L_{yy}(\omega_s) L_{zz}(\omega_1) L_{yy}(\omega_2) \sin\beta_1 \chi_{yyz}^{(2)}$$
(2.5)

$$\chi_{eff,pss}^{(2)} = L_{zz}(\omega_s) L_{yy}(\omega_1) L_{yy}(\omega_2) \sin\beta \chi_{zyy}^{(2)}$$
(2.6)

$$\chi_{eff,ppp}^{(2)} = -L_{xx}(\omega_{s})L_{xx}(\omega_{1})L_{zz}(\omega_{2})\cos\beta\cos\beta_{1}\sin\beta_{2}\chi_{xxz}^{(2)} -L_{xx}(\omega_{s})L_{zz}(\omega_{1})L_{xx}(\omega_{2})\cos\beta\sin\beta_{1}\cos\beta_{2}\chi_{xzx}^{(2)} +L_{zz}(\omega_{s})L_{xx}(\omega_{1})L_{xx}(\omega_{2})\sin\beta\cos\beta_{1}\cos\beta_{2}\chi_{zxx}^{(2)} +L_{zz}(\omega_{s})L_{zz}(\omega_{1})L_{zz}(\omega_{2})\sin\beta\sin\beta_{1}\sin\beta_{2}\chi_{zzz}^{(2)}$$
(2.7)

 $\chi_{ijk}^{(2)}$ is the macroscopic quantity that is intrinsically related to microscopic hyperpolarizability tensorial element, $\beta_{i'j'k'}^{(2)}$, in the molecular coordinate system. The general relation is based on the ensemble average of the Euler angle transformation between the molecular coordinate and the laboratory coordinate, as expressed in equation 2.8.

$$\chi_{ijk}^{(2)} = N \sum_{ijk} \langle \mu_{ijk:i'j'k'} \rangle \beta_{i'j'k'}^{(2)}$$
(2.8)

where N is the number density of the molecular groups and $\langle \rangle$ is the ensemble average operator. In SFG-VS, $\beta_{i'j'k'}^{(2)}$ can be expressed into two main terms when the frequency of the incident infrared beam is resonant with the vibrational dipole transition of molecules. The detailed expression is shown in the following:

$$\beta_{i'j'k'}^{(2)} = \beta_{NR}^{(2)} + \sum_{v} \frac{A_{v}}{\omega_{IR} - \omega_{v} + i\Gamma_{v}}$$
(2.9)

Here, $\beta_{NR}^{(2)}$ represents the non-resonant component; A_v is the transition moment strength; ω_{IR} is the incident infrared frequency; ω_v is the frequency of the vibrational transition, and Γ_v is the line width of the vibrational transition, also known as the half-width at the half maximum (HWHM). Depending on the nature of medium, $\beta_{NR}^{(2)}$ contribution can vary tremendously; for instance, its contribution is almost negligible in the dielectric medium, whereas it becomes predominant in metals or semi-conductors when electronic transitions become accessible.³³ On the other hand, the resonant effect in SFG-VS becomes dominant when ω_{IR} matches vibrational transition, or the $\omega_{IR} - \omega_v$ term approaches zero, giving the smallest denominator. This condition provides a resonant enhancement in the SFG signal.

In the event of a single resonance, $\beta_{i'j'k'}^{(2)}$ is fundamentally connected to both the IR and Raman transition moments of that particular vibrational mode. The following mathematical expression explicitly indicates the underlying relation.

$$A_{v} = \langle \mathcal{G} | a_{i'j'} | v \rangle \langle v | \mu_{\mu'} | \mathcal{G} \rangle$$
(2.10)

Here, the $\langle v | \mu_{\mu'} | g \rangle$ is the infrared transition moment, and $\langle g | a_{i'j'} | v \rangle$ is the Raman transition moment, where g and v represents the vibrational ground and excited states, respectively. Therefore, in order that the SFG signal is nonzero, the vibrational transition of the mode considered has to be both IR and Raman allowed. This is the inherent transition selection rule in SFG-VS.

2.2 Broad Bandwidth Sum Frequency Generation Instrumentation

The broad bandwidth VSFG spectrometer setup has been thoroughly described elsewhere.^{1,2,40} Here, only a concise introduction is given, and a diagram of the instrument is given in Figure 2.2. A titanium:sapphire oscillator (Spectra-physics, Tsunami) with an optimal center-wavelength at 785 nm and a sub-50 fs pulse-width seeds 1-kHz regenerative amplifiers (Spectra-physics, Spitfire, femtosecond and two picosecond versions) that are pumped by a solid state Nd:YLF laser (Spectra-physics, Evolution 30) at 527 nm. The resulting laser beams from the two respective regenerative amplifiers are 85 fs pulses at 785 nm (22 nm bandwidth) and 2 ps pulses at 785 nm (17 cm⁻¹ bandwidth). For the tunable broadband infrared laser generation in an optical parametric amplifier (Light Conversion, TOPAS), the broadband fs laser pulses are used to generate amplified parametric waves (signal and idler) via a BBO crystal using three general steps: superfluorescence generation, pre-amplification, and power-amplification of the signal beam. The amplified signal and idler beams are then used to create an infrared beam via an AgGaS₂ crystal in the nonlinear difference-frequency generation system (Light Conversion, NDFG connected to the TOPAS). On an average, the spectral bandwidth (FWHM) of the resultant broadband infrared beam is 200 cm⁻¹ in the spectral regions under investigation, and because it is a broadband infrared pulse, good signal-tonoise-ratio VSFG spectra are acquired for durations ranging from 30 to 300 seconds depending on the cross-sections of the vibrational modes of interest. The output of the infrared beam can easily cover from 1000 to 4000 cm⁻¹ with sufficient energy outputs as predicted by tuning curves for BBO (type II phase matching) and AgGaS₂ (type I phase matching) crystals and the crystal nonlinear efficiencies. For instance, the average

infrared energies at the sample stage in this study were measured to be 8, 9, 14, and 12 μ J in spectral regions of v_s COO⁻ (1350-1550 cm⁻¹), vC=O (1600-1800 cm⁻¹), vC-H (2800-3000 cm⁻¹), and vO-H (3000-3800 cm⁻¹), respectively. Furthermore, in order to minimize an energy loss of the infrared beam and an overwhelming spectral interference from water vapor absorption, 95% of the infrared beam path was purged with dry nitrogen gas (in-house). Additionally, 300 and 350 μ J visible energies were utilized.

As mentioned in the previous section, the probe beams consisting of the 785 nm visible beam and the broadband infrared beam need to overlap at the sample surface spatially and temporally for the SFG process. The generated VSFG signal from the sample surface is detected in reflection via a combination of two optical components: first, a monochromator (Acton Research, SpectraPro SP-500 monochromator with a 1200 g/mm grating blazed at 750 nm) for the spectral dispersion, and then, a liquid-nitrogen cooled CCD camera (Roper Scientific, 1340 × 400 pixel array, LN400EB back illuminated CCD) for signal collection. In some of studies, polarization investigation was also implemented. Polarization combinations of ssp (s-SFG; s-visible; p-infrared), sps, and ppp were used. To arrive at the final VSFG spectra presentation, backgroundsubtracted VSFG spectra were normalized against the broadband infrared beam energy profile using a non-resonant VSFG spectrum from a GaAs crystal (Lambda Precision Optics, Inc.). The purpose of doing the normalization is to eliminate the spectral distortion caused by the infrared beam energy distribution associated with each frequency in the spectral region of interest. Spectral calibration of VSFG peak positions was completed by comparing the polystyrene absorption bands obtained from a non-resonant GaAs spectrum to reference FTIR spectra. By doing this, the VSFG peak positions reported are accurate to 1 cm⁻¹.


Figure 2.1. Energy level diagram of the sum frequency generation process. $|g\rangle$ is the ground state, $|v\rangle$ is a vibrationally excited state, and $|s\rangle$ is a virtual state.



Figure 2.2. Broad bandwidth sum frequency generation laser system.

CHAPTER 3

IONIC BINDING OF Na⁺ VERSUS K⁺ TO THE CARBOXYLIC ACID HEADGROUP OF PALMITIC ACID MONOLAYERS

3.1 Introduction

Alkali cation binding events in aqueous solution are of great interest in biology, and more specifically, in neuroscience. Sodium and potassium are the major ionic components of intra- and extra-cellular fluids, and their function is critical in electrical communication across cell membranes.¹⁵ However, even though they possess similar chemical properties by belonging to the same chemical group, pronounced selectivity differences are observed in cross-membrane transport and remain to be addressed.^{14,15} To this end, Langmuir monolayers at the air-aqueous interface have long been a popular model system for biological membranes.⁴¹ For instance, studies that encompass molecular structures,^{21,23,42} film morphologies,^{22,43,44} and molecular interactions have been attempted.^{23,28} Additionally, long-chain fatty acids are most frequently used as standard materials in applied surfactant monolayer studies.

In this study, we have applied the VSFG technique to systematically investigate the ionic binding events of biologically relevant cations, Na⁺ and K⁺, to palmitic acid (C₁₆, PA) monolayers at air-aqueous interfaces. VSFG provides nonlinear vibrational resonant enhancement and surface specificity.³³ Vibrational modes investigated in this study consist of the symmetric stretch of the carboxylate anions (v_s COO⁻), the carbonyl stretch (ν C=O), the C-H stretch (ν C-H), and the O-H stretch (ν O-H) of water (dangling OH) and of PA headgroups. Of primary importance is the finding of the distinct K⁺ binding characteristics to the PA monolayer as opposed to Na⁺. To complement the existing knowledge on ionic binding at air-aqueous interfaces, the findings obtained in this study may provide new insight on ionic binding of simple alkali ions during transport across biological membranes. We find that K⁺ is much more effective at binding to the carboxylic acid relative to Na⁺. A 1:1 ionic complex of K⁺ with deprotonated COO⁻ is directly observed from VSFG spectra.

3.2 Experimental

Materials. Palmitic acid (>99% purity) and acyl chain deuterated palmitic acid (>98% purity) were purchased from Sigma-Aldrich and Cambridge Isotope, Inc, respectively. Both corresponding solutions were prepared in the 1.5 mM concentration range by dissolving in spectroscopic-grade chloroform that was purchased from Sigma-Aldrich. Sodium chloride (certified ACS, 99% purity) and potassium chloride (EP/BP/USP/FCC, 99% purity) were purchased from Fisher Scientific to prepare stock solutions by dissolving in deionized water (18.2 M Ω ·cm resistivity) from a Barnstead Nanopure system at pH of 6.0.

Stock solutions of sodium chloride and potassium chloride were filtered using Whatman Carbon-Cap activated carbon filter to eliminate potential organic contaminants. The concentrations of the filtered stock solutions were standardized based on the Mohr titration technique,⁴⁵ in which silver nitrate (reagent grade) and potassium chromate (99.5% purity) were applied as a titrate and an indicator, respectively; their respective

suppliers were Fisher Scientific and E.M. Science. To replicate standard saline concentrations in seawater and biological systems, 0.6 and 0.2 M salt solutions were chosen respectively in this study and then prepared by dilutions of desired amounts of stock solutions. In the pH studies, manipulation of pH values in the water subphase was controlled by mixing an appropriate amount of concentrated HCl or NaOH solution (reagent grade, Fisher Scientific) by direct pH meter readings (Accumet Basic AB15, Fisher Scientific). In addition, all solutions were conditioned at room temperature (23 ± 1 °C) over 24 hrs.

Methods. *Langmuir Film Balance*. The surface compression isotherm (π -A) was acquired by a KSV minitrough (KSV, Finland) with a dimension of 176.5mm × 85mm. The trough and the two barriers are made of Teflon and Delrin, respectively. During compression, the π -A isotherms were recorded in real-time by the Wilhelmy plate method. After 24 hrs equilibration at room temperature, the monolayer–subphase systems were maintained at 23 °C. The surface was compressed quickly and examined for any sign of surface pressure increase to ensure negligible organic contamination prior to spreading the PA monolayer. After confirming the surface purity, tens of micro-liters of PA-chloroform solution were spread in a drop-wise fashion by a micro-syringe (Hamilton) for homogeneous spreading. 10 minutes was allowed for complete solvent evaporation. During compression, a constant rate of 5 mm/min. of both barriers was employed.

Monolayer at Equilibrium Spreading Pressure. Monolayers at equilibrium spreading pressure (ESP) were spread over the various solutions in Petri-dishes, which underwent a stringent cleaning procedure: first soaked in concentrated sulfuric acid with an addition of

strong oxidizer, ammonium peroxydisbisulfate, for 2-3 hrs; then rinsed thoroughly with copious amount of nanopure water before drying in an oven at 125 °C. The monolayers of PA at ESP on neat water and the salt solutions were able to attain a mean molecular area (MMA) coverage of ~21 Å²/molecule, which are generally assumed to be in a highly ordered phase. After spreading, 10 minutes was also allowed for solvent evaporation and monolayer stabilization. Then, VSFG spectra were acquired in the spectral region of interest for both structural and chemical information.

3.3 Results and Discussion

3.3.1 Palmitic Acid Compression Isotherms

Phase information of Langmuir monolayers is revealed in their corresponding compression isotherms. In this study, the compression isotherms of PA monolayers spread on aqueous surfaces were investigated. The subphases include pure (neat) water, Na⁺ solutions (0.2 and 0.6 M), and K⁺ solutions (0.2 M and 0.6 M). Figure 3.1A and 3.1B shows the respective compression isotherms of PA on the Na⁺ and K⁺ solutions. For comparison, the PA isotherm on neat water is given as a reference. In looking at the isotherms, similarities exist. For instance, the observed trend of the PA monolayer phase transitions under compression (right to left in the isotherm) follows this order: the gas (G) – tilted condensed (TC) coexisting phase \rightarrow the TC phase \rightarrow the untilted condensed (UC) phase \rightarrow the collapsed phase. A second order phase transition appears as a kink on the isotherms when the PA monolayers transition from the TC \rightarrow UC phase.⁴⁶ During the compression, the initial surface pressure rise occurs at 21 Å²/molecule on the neat water and Na⁺ solutions; however, a slight deviation is shown for the 0.2 and 0.6 M K⁺

solutions in which a slightly larger mean molecular area (MMA) is observed at 22 and 23 $Å^2$ /molecule, respectively. In addition, the surface pressure at collapse increases when the subphase is varied from the neat water to the Na⁺, and K⁺ solutions. The surface pressure at collapse also increases when the concentration of the Na⁺ and K⁺ solutions is increased. Upon taking a closer look, the reverse trend is found for the surface pressure where the TC to the UC phase transition occurs. At this point, the surface pressure slightly decreases for the Na⁺ solutions, and decreases more for the K⁺ solutions with respect to the neat water subphase. The same trend, decreasing surface pressure at collapse, also correlates with decreasing concentration of same salt solutions.

Because having only a single saturated hydrocarbon chain, PA can exhibit relatively high compressibility characteristics. In addition, PA forms a highly ordered and compact molecular monolayer in the TC and UC phases as reported in the literature.⁴⁷ An MMA of 21 Å²/molecule is typical for saturated long-chain fatty acids,⁴⁸ which indirectly reflects the close-packed nature of the chain when it is subjected to compression. PA is assumed to orient perpendicular to the water surface in the UC phase.²¹ In evaluating the slight difference in MMA values of the PA monolayers on the K⁺ solutions with respect to water and the Na⁺ solutions, it is likely that K⁺ and Na⁺ interact differently with the headgroup of PA, which constitutes the essence of this study. Furthermore, the different interaction behaviors between the two cations with regard to the headgroup could account for the observed trend of decreasing surface pressure at the TC to UC phase transition as shown in the isotherms.

It is important to note the distinct behavioral contrast of these two cations in cross-membrane transport. It has been postulated that the ionic size and the corresponding binding sites provided by chelating ligands work synergistically in governing the observed selectivity and kinetics differences as reported in the literature.¹⁵ Both cations are conventionally thought to be buried deeply into the bulk and surrounded by hydration shells, but clearly the isotherms are suggestive of differing interfacial activity in the presence of a monolayer.

3.3.2 VSFG Spectroscopic Data of PA Monolayers

To decipher the underlying governing factors that have resulted in the small discrepancies revealed in the compression isotherms of the PA monolayers on both neat water and the salt solutions, VSFG was employed in this study for its surface specificity and molecular-level sensitivity. In the following studies, spectral regions of interest in relation to the dominant normal modes of vibration of PA are systematically probed. An in-depth understanding is then gained based on spectral differences. The investigated vibrational modes consist of the vC-H, v_sCOO^- , vC=O, and vO-H.

C-H Stretching Region $(2800 - 3000 \text{ cm}^{-1})$

As a first step, the VSFG spectra of the PA monolayers in the C-H stretching region were acquired on neat water, Na⁺, and K⁺ solutions, respectively. All three polarization combinations (ssp, sps, and ppp) were implemented. As shown in Figure 3.2, at equilibrium spreading pressure (ESP), the PA monolayers reveal many dominant spectral peaks both on water and the salt solutions. The observed peaks, in the order of increasing vibrational frequency, are described in Table 3.1. Using the ssp polarization combination, three peaks with varying intensities are revealed in each spectrum. These peaks correspond to the methylene symmetric stretch (ν_s CH₂), the methyl symmetric stretch (ν_s CH₃) and the methyl Fermi resonance (ν_{FR} CH₃) at frequencies of 2842, 2872,

and 2940 cm⁻¹, respectively. The shoulder at 2960 cm⁻¹ in column A is attributed to the methyl asymmetric stretch (v_aCH_3). In the sps polarization spectra, only one dominant peak occurs at 2960 cm⁻¹, which corresponds to the v_aCH_3 . Upon taking a closer look, a small peak is barely resolvable at 2910 cm⁻¹ where the methylene asymmetric stretch (v_aCH_2) is considered to be the main contributor. In the ppp polarization spectra, the v_aCH_3 peak is most intense while the peak intensities from the v_sCH_3 and v_aCH_2 vibrational modes are fairly weak. Overall, the absolute peak intensities of the aforementioned vibrational modes are similar even though different subphases are used, namely, the neat water, Na⁺, and K⁺ solutions. This observation is consistent with the mostly similar results obtained from the compression isotherms, in which an overall resemblance exists between Na⁺ and K⁺, yet small discrepancies are also noticeable. Given the small variations on peak intensities, it is difficult to infer any firm conclusion regarding a behavior difference on the interactions between Na⁺ and K⁺ with the headgroup of PA based solely on these spectra.

By direct observation of the spectra in Figure 3.2, it is clear that the v_sCH_2 peak is barely noticeable in the spectra collected from water, the Na⁺, and the K⁺ solutions at ESP. To explain this finding, the VSFG selection rules are used. As a reminder, SFG is not active in a centrosymmetric medium; therefore, the observation of low intensity in the v_sCH_2 mode could be explained by assuming formation of centrosymmetry between any adjacent pair of CH₂ groups when the chains are in an all-trans conformation. With an even number of CH₂ groups in PA, the pairing is more complete than another fatty acid with odd number of CH₂ groups. Hence, this explanation has been widely accepted in the VSFG community based on similar findings reported in other studies.⁴⁹ Furthermore, to reconfirm formation of a highly compact structure of the PA monolayer at ESP with close to surface normal orientation of the tails, the peak intensity ratio of v_sCH_3/v_sCH_2 in the ssp polarization combination is frequently used as a qualitative indicator to determine the orientation order of the PA tails with respect to the surface normal.^{50,51} Based on our realtime in-situ compression study coupled with VSFG, the acquired spectra of PA monolayers on water, the Na⁺, and the K⁺ solutions reveal almost identical spectral features while obtained separately in the UC and collapse phase (spectra not shown). This confirms that monolayers at ESP are representative of the UC phase.

Carboxylate Symmetric Stretching ($v_s COO^{-}$) Region (1400 – 1500 cm⁻¹)

Spectral investigations in the carboxylate stretching region have long been a hallmark in studies related to ionic binding of metal ions with fatty acid headgroups, as often demonstrated by the IRRAS technique.^{28,29,52} For the same purpose, special attention is paid to the PA monolayers on the Na⁺ and K⁺ solutions in this spectral region. First, to eliminate spectral contributions from the C-H bending modes, perdeuterated acyl chains of PA (D₃₁-PA) were employed. In addition, a direct investigation of the deprotonated form of the carboxylate headgroups (COO⁻) was also implemented; namely, a pH study of the D₃₁-PA monolayers on a water surface was undertaken with pH values of 1.0, 6.0, and 13.3.

Initially ssp VSFG spectra of the D_{31} -PA monolayers on the water surface with different pH values were obtained separately, as illustrated in Figure 3.3. In looking at the spectrum at pH 13.3, an intense and symmetric peak centered at 1410 cm⁻¹ is clearly observed. This is a direct indication of COO⁻ groups within the interface. Under this highly basic condition, the carboxylate headgroups of D_{31} -PA should all be in the

deprotonated form since the pKa values of fatty acid homologues are generally reported in the range of 5-8.^{29,53} In theory, in order to observe an SFG intensity, the molecules in the interface must show some degree of structural order and lack of inversion symmetry. Therefore, based on the observed peak intensity, even at pH 13.3, the deprotonated form of the D₃₁-PA monolayer at ESP maintains its ordered structure with respect to the carboxylate headgroups. The hydrophobic tails are highly likely to impede any formation of cyclic dimer within the monolayer as is observed in acetic acid-water mixtures within the interface.⁵⁴ According to both bulk transmission and surface reflection IR studies reported in literature, the $v_s COO^2$ peak is usually reported in the spectral range of 1400 – 1500 cm⁻¹ for long-chain fatty acid monolayers in the deprotonated form on aqueous salt solutions that contain metal cations. However, in this spectral range, the reported IRRAS spectra are usually congested with multiple peaks originating from both the C-H bending modes and $v_s COO^-$ modes in different metal-specific coordination environments.^{28,52} Here, taking advantage of VSFG and the isotopic substitution of PA, the 1410 cm⁻¹ peak is solely visible. It is, therefore, reasonable to attribute this peak to the hydrated species of the COO⁻ groups that is prevalent in this environment. The aqueous solution is highly basic, as is the aqueous interface. (Note that the high pH studies utilize NaOH to control pH, but these concentrations are orders of magnitude smaller than those used in the salt The assignment to the hydrated species of the COO⁻ groups is in good studies.) agreement with the work done by Miranda et al. on hexacosanoic acid (HA, C₂₆) under similar conditions.⁵⁵ At pH 1.0, there is no obvious spectral intensity from the D_{31} -PA monolayer in Figure 3.3. This can be easily explained by the fact that the majority of D_{31} -PA molecules exist in the protonated form at this pH, and therefore, having no

contribution in this spectral range. In addition, the D_{31} -PA spectral response at pH 6.0 (data not shown) is similar to the one shown in pH 1.0, which may imply that the majority of monolayer constituents are still in the protonated form at this pH.

In Figure 3.4A and 3.4B, ssp VSFG spectra of the D_{31} -PA monolayers obtained from the Na⁺ and K⁺ solutions reveal considerable intensities in this spectral region. Two peaks with respective center-wavelength positions at 1414 and 1475 cm⁻¹ are shown. In the absence of contributions from the C-H bending modes, the source of contributions to these two peaks are most likely from the COO⁻ groups that manifest into two distinct species. Upon considering the close proximity of the 1414 cm⁻¹ peak to the one at 1410 cm⁻¹ as identified previously, it is reasonable to assume that the former is also derived from the same molecular species that are characterized by the hydrated form of the COO⁻ groups. Even though the 1414 cm⁻¹ peak appears to be broader than that observed at pH 13.3 in Figure 3.3, this could be accounted for by postulating that there exists a relatively fewer number of the COO⁻ groups that somehow are inter-dispersed among these protonated species to cause a population dispersion.⁵⁶ Furthermore, the presence of cations could also affect the hydration shells around the COO⁻ groups to induce a similar spectral broadening.

The higher frequency peak at ~1475 cm⁻¹ shown in Figure 3.4A and 3.4B has also been found in similar studies by IRRAS, as demonstrated by Gerick and Hühnerfuss et al.²⁸ This peak is mainly dominant with the K⁺ solutions (Figure 3.4B). Since complete isotopic substitution has been emphasized in our study, it is sensible to rule out the δ -CH₂ contribution, which leaves the only choice to the ionic complex form of the COO⁻ groups having interactions with Na⁺ or K⁺. Previously, ionic complexes and coordinated species have been identified in studies of metal ions binding to fatty acid monolayers using IRRAS.^{28,29,57}

Most importantly, in looking at Figure 3.4A and 3.4B, it is apparent that the 1414 cm⁻¹ peak only increases in the Na⁺ solutions when the concentration is increased from 0.2 to 0.6 M, while the 1475 cm⁻¹ peak solely increases in the K^+ solutions after following the same increase in concentration. According to our postulates, each peak is assigned to a different COO⁻ species, the 1414 cm⁻¹ peak for the hydrated COO⁻ species, and the 1475 cm⁻¹ peak for the complexed COO⁻ species. In order to clarify these two assignments, some classical theories seem to work well. It is obvious that both Na⁺ and K⁺ belong to the alkali metals. In their common state of ionization, they share closed shell electronic structures that show noble-gas-like chemistry. Therefore, it is important to note that when they interact with the headgroup, they act like point charges with no distinguishable chemistry, which further dictates the interaction to be purely electrostatic. Because the charge density on K^+ is about half the value on Na^+ , 0.045 vs. 0.088 Coulomb/Å³,⁵⁸ respectively, it is natural to imply that K^+ binds less tightly with surrounding water molecules than Na⁺, which can be further supported by the difference in Stokes hydration radii of these two cations, 3.3 and 2.4 Å with respect to Na^+ and K^+ .⁵⁸ Given this unique physical property, K⁺ is more likely to interact with the headgroup than Na⁺ during the diffusion-controlled process. Once a charge-dipole interaction is experienced both by a K^+ and a headgroup, they are likely to bind strongly to allow proceeding of the inner sphere substitution of water molecules proceeds. Ultimately, the head group is able to replace most of the water molecules in the first hydration shell of K^+ . Then, deprotonation occurs, and a 1:1 ionic complex forms, K^+ :COO⁻. This favors

surface neutrality. In addition, the inner sphere substitution rate of the first hydration shell around K^+ is slightly faster than Na^{+,58} Therefore, the selective intensity trends depicted in Figure 3.4A and 3.4B seem reasonable in the systems that are investigated here.

Two points can be used to summarize the interesting observations discussed above. First, deprotonation of the headgroup can be initiated by the presence of metal cations in the aqueous solution. Second, the extent of ion complex formation is cation specific and also follows a nearly linear relationship with the cation concentration in the bulk with respect to K^+ . This linearity indicates a 1:1 complex formation between K^+ and a COO⁻ group. In later sections of this chapter, spectral evidence from other spectral regions also confirms these findings.

Carbonyl Stretching (vC=O) Region (1600 – 1800 cm⁻¹)

In order to further support the spectral findings in the v_sCOO^- stretching region presented in the previous section, VSFG spectra of the PA monolayers were acquired from the water surface under the same set of pH conditions (1.0, 6.0, and 13.3) in the C=O stretching region. Then, the same spectral investigations were conducted on the Na⁺ and K⁺ solutions, respectively.

One strong symmetric peak with its center-wavelength position at 1720 cm⁻¹ is shown in Figure 3.5A and 3.5B, while there is no spectral intensity in Figure 3.5C. This is opposite to the observation shown in the v_s COO⁻ stretching region with respect to pH. First, it is important to point out that the only difference among these spectra is the pH value where (A) is the most acidic at pH 1.0, (C) is the most basic at pH 13.3, and (B) is close to neutral at pH 6.0. According to spectral assignments based on IR and Raman studies,⁵⁹ this peak is attributed to the C=O stretching mode in the protonated form of carboxylate headgroups (COOH). The presented spectra are consistent with the molecular constituents in the headgroups as expected at these pH conditions. As mentioned previously, the PA headgroup at pH values of 1.0 and 6.0 should be mostly protonated, and the degree of dissociation should be correlated with pH. Therefore, the population of the protonated headgroups at pH 1.0 should be higher than that at pH 6.0 even though at the near neutral pH, the majority of headgroups are still protonated. Indeed, this trend is strongly confirmed by observing higher peak intensity in Figure 3.5A than that in Figure 3.5B. At pH 13.3, due to near complete deprotonation, PA headgroups are mostly transformed to the COO⁻ groups. Therefore, there should be no intensity in the C=O spectral region as shown in Figure 3.5C.

ssp VSFG C=O spectra of the PA monolayers acquired from the Na⁺ and K⁺ solutions are presented in Figure 3.6A and 3.6B. Clearly the single C=O peak appears in the Na⁺ and K⁺ solutions at 0.2 and 0.6 M, respectively. The respective spectral features of the observed peaks are identical in terms of the peak position and HWHM as denoted by Γ_i among the fitting parameters shown in eq 2.9. The peak intensity of the C=O peak in both Na⁺ solutions are almost identical, irrespective of the concentration difference. This indicates that the degree of deprotonation of the PA monolayers on the Na⁺ solutions may have an upper limit because having a strong hydration shell around each Na⁺ is a roadblock for forming an ionic complex with the headgroup. On the other hand, the peak intensity variations observed in the K⁺ solutions show concentration dependence. For instance, by varying the concentration from 0.2 to 0.6 M in the K⁺ solutions, a significant peak intensity decrease takes place. It is reasonable to assume that this is mainly caused

by deprotonation of the headgroup due to complex formation. To calculate the percent loss of the C=O groups when the concentration of K^+ solution is increased from 0.2 to 0.6 M, we can normalize the overall peak intensity difference existing in the respective spectra over the one in the reference spectrum (here, the vC=O spectrum at 0.2 M). Because SFG intensity is proportional to N² (number density squared), the square root ratio of this normalized intensity is needed to quantify the corresponding percent loss in the number density. The calculated percent loss is 50 %. On the other hand, this percent loss in theory should be consistent with the value that corresponds to the percent increase of the COO⁻ groups in the same solutions. To confirm this point, the same logic is applied to the spectra collected in the 1400-1500 cm⁻¹ region, but it is necessary to bundle the two identified COO⁻ groups into one to account for the overall effect. The estimated value of the percent increase of the COO⁻ groups due to K⁺ binding is 60 %, which is slightly higher than the fitting value of 50 % from the C=O stretching region.

O-H Stretching Region $(3500 - 3800 \text{ cm}^{-1})$

As a complementary probe, the O-H stretch at the higher frequency side of the hydrogen bonding stretch region was also investigated. There is some consensus that a continuum of hydrogen bonding strengths exists from about 3000 to 3600 cm⁻¹ as described in VSFG studies. In addition, the dangling O-H oscillators exist at \sim 3700 cm⁻¹. ^{56,60,61} In this part of our investigation, first, the dangling O-H peak is used as a common reference in evaluating if there exists a different response of this mode to the separate presence of Na⁺ and K⁺ in the bulk aqueous solutions; second, special emphasis is placed on the broad 3590 cm⁻¹ O-H stretching peak that is uniquely demonstrated in the aqueous interface with fatty acid coverage.

In Figure 3.7, ssp VSFG spectra of the neat water and the pure 0.6 M Na⁺ and K⁺ solutions in the dangling O-H stretching region of water are presented. A sharp peak with ~20 cm⁻¹ (HWHM) at 3702 cm⁻¹ is observed in all three spectra. These observed peaks are attributed to the dangling O-H oscillators of water molecules that reside at the very top layer of the hydrogen-bonded water network. These three spectra are almost identical, showing similar intensities. With this finding in mind, there is negligible surface perturbation from Na⁺ and K⁺ to the dangling O-H oscillators of water molecules, which is consistent with the long-standing perception in classical theories that the alkali cations favor strong hydration shells and are inclined to be buried in the bulk. Therefore, no distinction exists in the pure Na⁺ and K⁺ solutions. However, by simply introducing a monolayer coverage of PA on these two solutions, interestingly, K⁺ demonstrates a stronger complexion ability by causing more deprotonation in the headgroup as shown in the spectra presented in the previous sections. Additional spectral evidence is provided below confirming deprotonation induced by the ionic binding.

ssp VSFG spectra of PA monolayers on the pure Na⁺ and K⁺ solutions (at 0.2 and 0.6 M) in the dangling O-H stretching region are shown in Figure 3.8. The common feature shared in these spectra is the manifestation of one broad peak which decreases in intensity, and its center-wavelength becomes blue-shifted (from 3590 to 3620 cm⁻¹) as shown in Figure 3.8. In previous SFG studies, the assignment of this peak has been unclear. For instance, this peak was first assigned to the OH stretch of weakly interacting OH groups between fatty acid (C₂₆) and water molecules by Miranda, et al.,⁵⁵ and recently was modified as the OH stretch of weakly or non-hydrogen-bonded water molecules by Johnson et al. in studies of acetic acid molecules.⁵⁴ Here, supported by

more concrete spectral evidence acquired in this study, the former assignment is more reasonable. We assign this peak to the OH stretch of an isolated hydrogen-bonded OH of the COOH group and the hydrogen-bonded water OH. This mode arises only between the carboxylic acid headgroup and water molecules. An absence of either of these deems the disappearance of this unique spectral signature. It is more important to address the aspect of deprotonation first before discussing this peak assignment.

Because this specific peak has OH stretch contributions from the PA headgroups (COOH), this broad peak can also be used as a probe for monitoring the deprotonation event. In looking at the PA spectra acquired from the Na⁺ solutions at 0.2 and 0.6 M, it is evident that the peak intensity of the OH stretch is slightly stronger in the 0.2 M solution than that in the 0.6 M solution. The peak position at 3590 cm⁻¹ does not shift. The loss of signal is attributed to the loss of OH oscillators, which is a direct result of deprotonation of the headgroup. In the Na⁺ solutions, deprotonation is unfavorable. However, the headgroups that are deprotonated exist predominantly in their hydrated form. These headgroups should continue to contribute to the SFG signal in this region. This observation is consistent with the spectral data that we have observed in other spectral regions presented above.

The spectra of PA monolayers collected from the K⁺ solutions (Figure 3.8C and 3.8D) show a more abrupt intensity decrease and a significant blue-shift of the peak position as the concentration is increased from 0.2 to 0.6 M. The significant intensity decrease has two related causes: first, the loss of the OH oscillators in the headgroup due to deprotonation, and second, by the complex formation. K⁺ favors forming a 1:1 complex with the COO⁻ group as proposed previously. This is unlike Na⁺. Once the

complex is formed, it would act like a single entity that further prevents hydrogenbonding with the surrounding water molecules.

In further evaluation of Figure 3.8, a 30 cm⁻¹ blue-shift of the OH stretching peak in the K⁺ spectra relative to the Na⁺ spectra is observed. This is an indication of increasingly weaker hydrogen bonds between PA headgroups and water molecules. This observed shift could be caused by perturbations from the complexed K⁺:COO⁻ species on nearby protonated PA molecules hydrogen-bonded with water molecules.

The VSFG spectrum of the PA monolayer on the water surface at pH 13.7, as shown in Figure 3.9, is critical for the assignment of the 3590 cm⁻¹ peak and to confirm the ionic complex formation. A peak at 3590 cm^{-1} is evident with intensity similar to the near neutral pH (0.2 M K⁺ spectrum in Figure 3.8). A peak at ~3700 cm⁻¹ is also observed, the dangling surface OH. The 3590 cm⁻¹ peak is clearly not from the headgroup alone because in this highly basic solution, the majority of headgroups are in the deprotonated form. There is no COOH. However, due to hydration, the 3590 cm⁻¹ peak is observed, which indicates a direct and strong interaction between the deprotonated headgroups and surrounding water molecules. On the other hand, as explained previously, a weaker or even disappearance of this peak in the case of an ionic complex could be well reasoned by assuming that once an ionic complex is formed the interaction between the headgroup and water molecules is greatly suppressed. The bound water molecules have been replaced by the cation in these cases. Given this reasoning, we come to the conclusion that the 3590 cm⁻¹ peak is the unique product arising from water molecules directly interacting with the PA headgroups.

3.4 Conclusions

Ionic binding between simple alkali metal cations and the fatty acid head groups of palmitic acid can take place at the air-aqueous interface. Our findings indicate that alkali metal cations, in the case of Na⁺ and K⁺, have various degrees of binding affinities with the carboxylic acid headgroups of PA. On the neat water surface at a neutral pH, the majority of PA molecules are present in their protonated form, and deprotonation only occurs at relatively high basic conditions; however, this trend no longer holds when the aqueous phase contains simple alkali metal cations such as Na^+ and K^+ , albeit at neutral conditions. Presence of these cations initiates deprotonation of the carboxylic acid headgroups via two unique mechanisms. First, the deprotonation of PA headgroups could be caused by the long-range electrostatic interaction between the hydrated cations and the headgroup; second, a similar interaction could as well directly result from ionic complex formation between nonhydrated cations and the headgroup. To confirm these two unique mechanisms, our data imply that Na^+ favors the first mechanism, while K^+ tends to favor the second mechanism. This is consistent with the slight differences in their hydration parameters: surface charge and hydration radii. Moreover, the degree of overall acid deprotonation is significantly greater with K⁺ than Na⁺ because K⁺ is more likely to form a 1:1 ionic complex with the COO⁻ groups.

Based on the significant findings presented in this study, it is important to note that K^+ binds more strongly to the headgroup of PA relative to Na⁺ at the air-aqueous interface. Rather than direct charge interactions found in real biological systems controlled by physiological pH conditions, alkali metal cations such as K^+ , can initiate

deprotonation. This can occur at neutral pH. The K^+ ion then undergoes ionic complexation with biologically relevant chelating ligands, such as carboxylate groups.

| | $v_{s}CH_{2}$ (cm ⁻¹) | υ _s CH ₃ (cm ⁻¹) | v _a CH ₂ (cm ⁻¹) | υ _{FR} CH ₃ (cm ⁻¹) | v _a CH ₃ (cm ⁻¹) | |
|-----|-----------------------------------|---|---|--|---|--|
| ssp | 2842 | 2872 | | 2940 | | |
| sps | | | 2910 | | 2960 | |
| ppp | | 2872 | 2910 | | 2960 | |

Table 3.1. Peak assignments of VSFG spectra of PA monolayers on neat water, NaCl, and KCl solutions (0.2 and 0.6 M) at equilibrium spreading pressure (ESP) for polarization combinations ssp, sps, and ppp^a

^{*a*} s, symmetric stretch; a, asymmetric stretch; FR, Fermi resonance



Figure 3.1. Surface compression isotherms (π -A) of PA monolayer at 23°C on aqueous surfaces: (A) neat water and NaCl (0.2 and 0.6 M), (B) neat water and KCl (0.2 and 0.6 M)



Figure 3.2. VSFG spectra of PA monolayers on aqueous NaCl and KCl (0.2 and 0.6 M) solutions and neat water at ESP under three polarization combinations: (A) ssp, (B) sps and (C) ppp; blue, green and red colors denote subphase of neat water, aqueous 0.2 M and 0.6 M salt solutions, respectively. Vibrational modes of v_sCH_2 , v_sCH_3 , and $v_{FR}CH_3$ in (A), v_aCH_3 in (B), and v_sCH_3 , v_aCH_2 and v_aCH_3 in (C) are shown in the spectra. Dash lines are provided as a guide for the eye.



Figure 3.3. ssp VSFG spectra of D_{31} -PA monolayers on water with pH values at 1.0 and 13.3. The fitted curve for pH 13.3 spectrum is shown as a solid line.



Figure 3.4. ssp VSFG spectra of D_{31} -PA monolayers on salt solutions: (A) 0.2 and 0.6 M NaCl solutions; (B) 0.2 and 0.6 M KCl solutions. The individual fitted curves are shown as solid lines.



Figure 3.5. ssp VSFG spectra of PA monolayers on water at pH values: (A) 1.0; (B) 6.0, and (C) 13.3. The individual fitted curves are shown as solid lines.



Figure 3.6. ssp VSFG spectra of PA monolayers on salt solutions: (A) 0.2 and 0.6 M NaCl solutions; (B) 0.2 and 0.6 M KCl solutions. The individual fitted curves are shown as solid lines.



Figure 3.7. ssp VSFG spectra of neat water and pure salt solutions (without monolayer) showing the dangling OH of surface water molecules: A. neat water; B. 0.6 M NaCl solution; C. 0.6 M KCl solution.



Figure 3.8. ssp VSFG spectra PA monolayers on salt solutions showing the OH of the PA carboxylic acid. PA spread on: A. 0.2 M NaCl solution; B. 0.6 M NaCl solution; C. 0.2 M KCl solution; D. 0.6 M KCl solution.



Figure 3.9. ssp VSFG spectra PA monolayers on neat water at pH 13.7 showing the hydrogen-bonded OH to PA and the dangling OH of surface water.

CHAPTER 4

IONIC BINDING OF Mg²⁺ VERSUS Ca²⁺ TO THE CARBOXYLIC ACID HEADGROUP OF PALMITIC ACID MONOLAYERS

4.1 Introduction

Divalent cations are essential to the cellular physiology of living organisms.¹⁴ Most importantly, they play a multitude of functions ranging from assisting in protein folding, maintaining protein structures, being cofactors to the cellular nucleotides, modulating enzyme activities, and promoting signal transduction.²⁰ Because divalent cations such as Mg²⁺ and Ca²⁺ participate in diverse biological processes, there is considerable and sustained interest in understanding their role at a fundamental level such as that in cellular regulatory mechanisms.^{16,62,63} Mg²⁺ is known as an antagonist in actions of Ca^{2+} in cellular physiology; for instance, Mg^{2+} deficiency impairs Ca^{2+} metabolism. Low Mg^{2+} concentrations concomitantly raise intracellular levels of Ca^{2+} , creating an Mg²⁺:Ca²⁺ imbalance. As a result, such imbalance can cause a perpetual vasoconstriction state in smooth vascular muscle cells. This condition is generally known as hypertension.⁶³ In this research we investigate the interaction specificity of these two cations with biological ligands at the molecular level. By recognizing their unique interaction specificity with biological ligands, implications of their functions, selectivities, as well as potential binding sites in more complex systems such as transmembrane proteins can be established. To effectively investigate the cationic binding affinity with biological ligands at an interface, Langmuir monolayers and Langmuir-Blodgett (LB) films are used. The monolayers and LB films serve as a proxy for the cell membrane and continue to be the prevailing model systems adopted by the surface science community.¹⁷

In this study, we applied the VSFG technique to systematically investigate the ionic binding specificity of biologically relevant cations Mg^{2+} and Ca^{2+} to a palmitic acid (PA, C₁₆) monolayer at the air-aqueous interfaces. VSFG provides surface specificity and molecular moiety sensitivity. The vibrational modes investigated encompassed both head and tail groups of the PA molecules. The vC-O/ δ O-H, v_sCOO⁻, vC=O, vC-H of PA were probed. Of primary importance is the identification of the ionic binding specificity of Ca²⁺ to COO⁻ relative to Mg²⁺.

4.2 Experimental

Materials. Palmitic acid (>99%, Sigma-Aldrich) and acyl chain deuterated palmitic acid (>98%, Cambridge Isotope Laboratories) were used to prepare solutions at ~1.5 mM by dissolving in spectroscopic-grade chloroform (>99.9, Sigma-Aldrich). Magnesium chloride (99%, Fisher Scientific) and calcium chloride (99%, Fisher Scientific) were used to prepare stock solutions by dissolving in deionized water (18.2 M Ω ·cm resistivity) from a Barnstead Nanopure system at pH of 6.0.

Stock solutions were filtered to eliminate potential organic contaminants using Whatman Carbon-Cap activated carbon filter. The concentrations of the filtered stock solutions were standardized based on the Mohr titration technique in which silver nitrate (reagent grade, Fisher Scientific) and potassium chromate (99.5%, E.M. Science) were used as a titrate and an indicator, respectively.⁴⁵ 0.1 and 0.3 M inorganic salt solutions

were used in this study and were prepared by dilution. On the other hand, aqueous pH values were controlled by mixing an appropriate amount of concentrated HCl or NaOH solution (reagent grade, Fisher Scientific) based on a pH meter (Accumet Basic AB15, Fisher Scientific). In addition, all solutions were conditioned at room temperature $(23 \pm 1 \, ^{\circ}C)$ over 24 hrs before use.

Methods. Langmuir Film Balance. A KSV minitrough (KSV, Finland), 176.5mm × 85mm, was used to acquire surface pressure-area (π -A) isotherms. The trough and the two barriers are made of Teflon and Delrin, respectively. During compression, the π -A isotherms were recorded in real-time by the Wilhelmy plate method. The subphase temperature was maintained at 23 °C after 24 hrs equilibration in a climate-controlled room (23 °C). All π -A isotherms were collected in a Plexiglas box to eliminate any possible contaminant. The aqueous surface was compressed and examined for any sign of surface pressure increase to ensure negligible organic contamination prior to spreading the PA monolayer. After confirming the surface purity, tens of micro-liters of PAchloroform solution were spread in a drop-wise fashion by a micro-syringe (Hamilton) for homogeneous spreading. 10 minutes was allowed for complete solvent evaporation. During compression, a constant rate of 5 mm/min. of both barriers was employed. In SFG experiments that interested in vibrational information of monolayers at different surface pressures, the mini-trough was placed on a height-adjustable sample stage to allow incidence of two laser beams. Next, the same monolayer spreading protocol was followed to prepare monolayer films. VSFG spectra were taken while monolayers were maintained at the predetermined surface pressures.

Monolayer at Equilibrium Spreading Pressure. Monolayers at equilibrium spreading pressure (ESP) were spread over the various solutions in clean Petri-dishes. The monolayers of PA at ESP on neat water and the salt solutions were able to attain a mean molecular area (MMA) coverage of ~21 Å²/molecule, which are generally assumed to be in a highly ordered phase. After spreading, 10 minutes was also allowed for solvent evaporation and monolayer stabilization. Then, VSFG spectra were acquired.

Brewster Angle Microscopy. The Langmuir trough was used in morphology study of surface monolayers using our home-made Brewster angle microscope (BAM). The whole setup is sitting on a vibration-proof laser table, being enclosed in a completely dark Plexiglas housing. The trough is positioned onto a sample stage located in the middle of two optomechanical arms in a home-made goniometer that provides both height and angle adjustment. A collimated laser He:Ne diode module (633 nm, Research Electro Optics, Inc) with polarization ratio of 500:1 is incident onto the air/water interface at the Brewster angle (53°). First, this laser beam is further polarized with a Glan-Thompson polarizer. The reflected beam is collected by an industrial Nikon 20x objective mounted on the detection arm, and then via a tube-lens directed on a CCD camera (512 x 512 pixels, DV412, Andor,). No additional image processing is implemented. On average, this BAM has resolution close to 10 μ m that is ideal for looking at film domains at the air/water interface.

4.3 Results and Discussion

4.3.1 Palmitic Acid: Brewster Angle Microscopy and Compression Isotherms

 π -A isotherms of Langmuir monolayers on aqueous surfaces are frequently used to reveal the underlying phase information of the monolayer being subject to constant compression.²¹ The macroscopic phase behavior using the isotherms of PA monolayers on aqueous alkaline earth solutions were investigated and are shown in Figure 4.1 along with Brewster angle images of the pure PA surface to confirm the phase assignments. Figure 4.1B and 4.1C show the π -A isotherms of PA monolayers on aqueous Mg²⁺ and Ca²⁺ solutions at 0.1 and 0.3 M, respectively. To facilitate a direct comparison, the isotherm on neat water is shown in these plots. Being saturated single acyl chains, PA molecules are fairly compressible, and ultimately, can be packed in a highly ordered structure at high surface pressures. The isotherm of the PA monolayer on neat water reveals this behavior. In the order of decreasing mean molecular area (MMA), the PA monolayer undergoes multiple phase transitions that include the gas and tilted condensed (G-TC) coexistence phase to the tilted condensed (TC) phase followed by the TC to the untilted condensed (UC) phase, then it collapses.

Brewster angle microscopy images of the PA film are shown as insets to Figure 4.1A. The image obtained at a MMA of 46 Å²/molecule reveals the existence of domains, which indicates two dimensional aggregation of the monolayer and thus the assignment of a coexistence region of a tilted condensed (TC) phase with the gas (G) phase. Another image of the film was obtained just after the G-TC to TC transition. This image reveals a homogenous film consistent with the TC phase assignment. The transition from the TC phase to the untilted condensed (UC) phase is regarded as a second order phase transition. Similar to other fatty acid homologues, the PA monolayer shows finite surface pressure at a relatively small MMA of 21 Å²/molecule, which is typical for monolayers
of saturated fatty acids.⁴⁸ In addition, the second order phase transition occurs at 24 mN/ m with a corresponding MMA of 17 Å²/molecule, and the collapse pressure occurs at 50 mN/m, consistent with previous studies.¹⁸

In the presence of Mg^{2+} cations, some deviations are found in comparison with the isotherm obtained from the neat water surface. Figure 4.1B shows that the PA monolayers collapse at a much higher surface pressure at 70 mN/m on 0.1 and 0.3 M Mg^{2+} . This kind of behavior is caused by an increase on the surface tension in inorganic aqueous solutions. The second order phase transition occurs at a lower surface pressure on the aqueous Mg²⁺ than on neat water. This trend has also been observed in the PA monolayers on aqueous alkali solutions. Clearly, each PA molecule in the monolayers occupies a slightly larger MMA on the Mg²⁺ in the UC phase. The average MMA is 17 $Å^2$ /molecule on neat water as opposed to 19 $Å^2$ /molecule on the Mg²⁺. To address the observed discrepancy, cationic interactions with headgroups in the form of charge screening have been suggested. Nevertheless, it is evident that at the same surface pressure the PA monolayers on the Mg²⁺ undergo less compression than on neat water. Because the surface pressure directly correlates with the Van der Waals interaction between adjacent acyl chains, an orderly packed structure can maximize interactions with an all-trans conformation.¹⁹ Therefore, the presence of Mg²⁺ at 0.1 and 0.3 M could induce the PA monolayers to be in the all-trans conformation at MMA of 19 $Å^2$ /molecule. Additionally, the corresponding shift on the MMA in the TC phase could be explained by the electrostatic repulsion between neighboring charged species as a result of deprotonation.

Figure 4.1C shows the π -A isotherms of the PA monolayers on the Ca²⁺ at 0.1 and 0.3 M, respectively. The π -A isotherms acquired from the monolayers on Ca²⁺ are different from that from neat water and the Mg²⁺. First, the TC phase disappears, as well as the second order phase transition; second, the PA monolayers directly transition from the G-TC coexistence phase to the UC phase, and then collapses. This behavior has been attributed to the condensing effect of metal cations as a result of forming fatty acid salts.⁶⁴ In the UC phase, the PA monolayers are similar between the Ca²⁺ and Mg²⁺. The disappearance of the TC phase is further explored below.

4.3.2 VSFG Spectroscopic Data of PA Monolayers

A molecular-level understanding is the prerequisite to decipher the observed disparity between the π -A isotherms on the aqueous Mg²⁺ and Ca²⁺. To gain insight, VSFG spectra of four spectral regions were obtained. These regions encompass the stretching vibrations (v) of the C-H at 2800 – 3000 cm⁻¹, the CO (mixed with the bending mode of OH) at 1300 – 1400 cm⁻¹, the COO⁻ at 1400 – 1500 cm⁻¹, and the C=O at 1700 – 1800 cm⁻¹.

C-H Stretching Region $(2800 - 3000 \text{ cm}^{-1})$

VSFG spectra in the C-H stretching region directly reveal the conformational order of the acyl chains in the PA monolayers by analyzing the relative intensity of the CH₃ and the CH₂ peaks.⁶⁵ By selectively controlling the surface pressure, structural information of the PA monolayers in each distinct phase can be interrogated and compared. Figure 4.2A, 4.2B, and 4.2C show the ssp VSFG spectra of the PA monolayers on the neat water surface, the Mg²⁺ and the Ca²⁺ (0.1 and 0.3 M) at 10 mN/m. Four vibrational modes are assigned, the methylene symmetric stretch (ν_s CH₂) at 2842

cm⁻¹, the methyl symmetric stretch (v_s CH₃) at 2874 cm⁻¹, and in the larger asymmetrically shaped peak at higher frequency consisting of the methyl Fermi resonance (v_{FR} CH₃) at 2940 cm⁻¹ and the methyl asymmetric stretch (v_a CH₃) at 2960 cm⁻¹. Among them, the v_s CH₃ and the v_{FR} CH₃ peaks are strong while the v_s CH₂ and the v_a CH₃ peaks remain relatively weak as lower and higher frequency shoulders respectively. There are only small differences observed between each spectrum.

In Figure 4.2A, the v_s CH₂ peak is much weaker than the v_s CH₃. This observation is indicative of the conformational ordering of the acyl chains at 10 mN/m. Based on the dipole approximation, SFG is not active in a centrosymmetric environment.³³ Therefore, a weak v_sCH_2 peak accompanied by a strong v_sCH_3 peak reflects the formation of centrosymmetry between adjacent CH₂ groups when the acyl chains are near the all-trans conformation. Compared to the spectrum on the water surface, the PA spectrum on the 0.1 M Mg²⁺ (Figure 4.2B) is similar. But on the 0.3 M Mg²⁺, the v_s CH₃ peak is slightly stronger, and concomitantly the v_sCH_2 peak becomes slightly weaker relative to those on the 0.1 M. (Although the y axis is in arbitrary units, the scales can be compared.) These intensity changes suggest that the acyl chain of PA is more ordered when the Mg²⁺ concentration is increased from 0.1 to 0.3M at the same surface pressure. However, this trend is not followed on the Ca^{2+} , as evidenced in Figure 4.2C. For instance, both v_sCH_2 and v_sCH_3 peak intensities remain constant irrespective the concentration increase, and the overall peak intensity and the spectral shape match those on the 0.3 M Mg^{2+} , revealing more order, that is, minimal contribution from gauche defects.

As shown in the π -A isotherms, Ca²⁺ demonstrates the condensing effect on the acyl chains, which consequently leads to the absence of the TC phase. Similarly, the

respective VSFG spectra on the Ca^{2+} consistently affirm this effect, depicting a larger intensity ratio of the v_sCH_3 over the v_sCH_2 than the ratio on the neat water surface at 10 mN/m. Qualitatively, the larger the ratio, the more ordered the acyl chains revealing near all-trans conformation. In comparison, the structural ordering of the PA monolayers on the Mg²⁺ and Ca²⁺ is similar at 0.3 M in the TC phase, but not at 0.1 M. Hence, the concentration of Mg²⁺ affects the acyl chain ordering.

Unlike the spectral features demonstrated in the spectra obtained at 10 mN/m, the overall spectral intensity and shape becomes indistinguishable when the PA monolayers are in the UC phase, surface pressures above 24 mN/m for the aqueous Mg²⁺ subphases, but above 0 mN/m for the Ca²⁺. This result implies that in the UC phase the degree of conformational ordering is similar irrespective of the subphase. This observation agrees with the high compressibility of PA monolayers at the air-aqueous interface. To summarize, the spectral variations are small in the C-H stretching region. Therefore, direct evidence revealing cation binding behavior to the headgroups is imperative in clarifying the isotherm differences.

Carboxyl and Carboxylate Symmetric Stretching Region $(1300 - 1500 \text{ cm}^{-1})$

Because of the significant contribution from the C-H scissoring mode around 1400 cm⁻¹, it is ideal to separate it out by using isotopic substitutions to eliminate any spectral interference.²⁹ Hence, PA molecules with per-deuterated acyl chains (D_{31} -PA) were used for analysis of this spectral region.

To accurately assign the $v_s COO^-$ peak in the spectral region around 1400 cm⁻¹, a control experiment was first performed by selectively adjusting the pH in the bulk. Figure 4.3 shows the ssp VSFG spectra of the D₃₁-PA monolayers spread on aqueous surfaces at pH 2.1, 6.0, 8.2, and 13.0, respectively, at the equilibrium spreading pressure (ESP) of D_{31} -PA. A relatively small intensity peak at 1300 cm⁻¹ is present with almost equal strength with the pH 2.1 and 6.0 solutions, whereas an intense and symmetric peak at 1410 cm⁻¹ appears with the pH 13.0 solution. At the pH 8.2, both peaks are observed. According to literature, the 1300 cm⁻¹ peak is assigned to the C-O stretch of the protonated carboxyl group.⁶⁶ This peak has been observed previously in a VSFG study of interfacial acetic acid solutions and IRRAS studies on fatty acid monolayer systems.^{66,67} The 1410 cm⁻¹ peak is commonly ascribed to the ionic or hydrated form of COO⁻ in aqueous solution;⁶⁸ in addition, this peak has also been identified by Miranda et al. on hexacosanoic (C_{26}) acid film covered surface at pH 12.0 using VSFG.⁵⁵ The pKa value of carboxylic acid group is \sim 4.85 in the bulk, whereas it rises to \sim 8.7 for long-chain fatty acids at the air-liquid interface.^{29,69} Therefore, our result is consistent with the fact that the D_{31} -PA in the monolayers are protonated at pH of 6 and below, yet are fully deprotonated when the pH reaches 13.0; and moreover, at pH of 8.2 we observe both protonated and deprotonated species consistent with the surface pKa.

Next, a direct interrogation of the underlying binding behavior of Mg^{2+} and Ca^{2+} to D_{31} -PA headgroup was undertaken. Figure 4.4A and 4.4B show respectively the ssp spectra of D_{31} -PA monolayers on the Mg^{2+} and Ca^{2+} at 0.1 and 0.3 M. By direct observation, the spectra of Figure 4.4A (magnified 5 times for clarity) reveals two resonances, a dominant peak centered at 1417 cm⁻¹ and a weak shoulder centered at 1475 cm⁻¹. In addition, there is a slight increase of the peak intensities when the concentration is increased from 0.1 to 0.3 M. In contrast, the spectral features shown in Figure 4.4B are very different, only bearing a small resemblance to the spectra collected from the aqueous

 Mg^{2+} . One intense, symmetric peak positioned at 1435 cm⁻¹ is observed from the 0.3 M Ca^{2+} solution, whereas a similar peak but less intense is seen at the same spectral position from the 0.1 M Ca^{2+} solution; however, in the 0.1 M spectrum a small shoulder at 1475 cm⁻¹ is also evident similar to the 0.1 M Mg^{2+} solution. In evaluating the spectral differences exhibited in Figure 4.4A and 4.4B, not only are different peak positions found, but the overall intensity is significantly enhanced with the Ca^{2+} subphase compared to that of the Mg^{2+} subphase.

Because of the complete isotopic substitutions of the acyl chain, there should not be a CH₂ scissoring mode contribution in the spectra of Figure 4.4. Hence, the observed peaks at three distinct spectral positions are assigned here solely to the COO⁻ in three different chemical environments or three different COO⁻ complexes. Blue shifting of the dominant peak from 1417cm⁻¹ to 1435 cm⁻¹ is consistent with degree of deprotonation. The spectral evidence reveals a stark contrast between the degrees of deprotonation on the carboxylic headgroup induced by Mg²⁺ versus Ca²⁺. Based on the spectral intensity alone, Ca²⁺ clearly induces greater degrees of deprotonation than Mg²⁺. We confirm this by additional spectral evidence as discussed below.

The 1417 cm⁻¹ peak on Mg^{2+} (Figure 4.4A) resembles the 1410 cm⁻¹ peak as shown on the pH 13.0 aqueous solution in terms of the peak position; therefore, we assign the former to the hydrated species of COO⁻ as previously stated. Very importantly, a similar peak has also been observed from D₃₁-PA monolayers on aqueous Na⁺ and K⁺ solutions, with spectral features similar between the Na⁺ from that study and the Mg²⁺subphase studied here. Because both Na⁺ and Mg²⁺ favor full hydration, a solvent barrier is likely present during ionic interactions between the ion and the

headgroup. Despite this, the peak occurs at 1414 cm⁻¹ on alkali salt solutions. Similar spectral broadening also takes place on the aqueous Mg²⁺ solution due to a population broadening when the deprotonated and protonated D₃₁-PA molecules coexist at the interface. Furthermore, the surface charge density of Mg²⁺ is much higher than Na⁺, which then implies that Mg^{2+} tends to possess a much stronger hydration shell than that of Na⁺ and behaves predominantly as a hydrated entity in the bulk, which minimizes any direct ionic interaction or formation of ionic complex with COO⁻. According to Figure 4.4A, Mg^{2+} behaves much like a hydrated entity when it binds to the headgroup. Nevertheless, the peak at 1475 cm⁻¹ is also present in the spectra, indicating the existence of other possible COO⁻ species. According to conclusive evidence obtained from a similar study of the D_{31} -PA monolayers on K⁺ solutions, the 1475 cm⁻¹ peak was assigned to the 1:1 ionic complex of K^+ :COO⁻ that exists in a greater proportion on 0.6 M K^+ . Therefore, the small shoulder positioned at 1475 cm⁻¹ is accordingly attributed to the 1:1 ionic complex of Mg^{2+} :COO⁻ and only counts for ~5% of the total COO⁻ population. Hence, on aqueous Mg^{2+} , the predominant species of COO⁻ in the D₃₁-PA monolayers exists as a hydrated species accompanied by a smaller fraction of the 1:1 ionic complex.

In contrast to the spectra shown in Figure 4.4A, the ssp VSFG spectra of D_{31} -PA on the Ca²⁺ are starkly different (Figure 4.4B). The single intense peak at 1435 cm⁻¹ on the 0.3 M Ca²⁺ is about 8 times stronger than the corresponding spectra on the 0.3 M Mg²⁺. Even though spectral peaks in close proximity to 1435 cm⁻¹ sparsely appear in IRRAS spectra collected from similar systems at the air-liquid interface, no in-depth elaboration has been given regarding these peaks due to weak signals.⁶⁶ According to

infrared selection rules, the asymmetric carboxylate stretching ($\upsilon_a COO^-$) peak is inherently stronger than the $\upsilon_s COO^-$ peak, as evidenced in IRRAS spectra. Thus, most spectral analyses were expended on the $\upsilon_a COO^-$ and δCH_2 peaks in obtaining molecular structural information. But because the $\upsilon_a COO^-$ was not detected using VSFG, no direct comparison can be made between them.

It is commonly accepted that Ca^{2+} favors binding to oxygen atoms, especially in biologically relevant ligands like carboxylate, carbonate, and phosphate groups, to form insoluble complexes that play critical roles in biological processes.⁷⁰ For instance, postulates such as matching ionic size, dynamic hydration shell, and ion-water affinity between Ca^{2+} and COO^{-} have been given to explain their strong binding affinities.⁷¹ However, the true nature of how Ca^{2+} interacts with COO⁻ continues to remain as a puzzle without further support of concrete experimental evidence. In IRRAS studies, the difference in the spectral peak positions between the $v_a COO^-$ and $v_s COO^-$, or the socalled Δ value, has been regularly employed to determine the possible coordination type between the metal cations and the headgroup.⁷²⁻⁷⁶ So far, four types of complex species have been determined: ionic complex, monodentate complex, chelating (bidentate) complex, and a bridging complex.⁷⁷ Because Ca^{2+} has a low Pauling electronegativity and a complete electronic shell, Ca^{2+} is thought to bind mostly as an ion with COO^{-} though this binding may possess some covalent binding character.⁷¹ According to Figure 4B, the intensity ratio of $v_s COO^- 0.3$ M over $v_s COO^- 0.1$ M is close to 2.1. Because the VSFG signal intensity is proportional to the molecular number density square (N^2) for all trans configuration, by taking the square root of this ratio, the ratio in terms of the number density of COO⁻ is close to 1.5 assuming no orientation change of the COO⁻ at

both concentrations. On the other hand, the corresponding concentration ratio of the Ca^{2+} is 3; therefore, the ratio between the number of Ca^{2+} per COO⁻ or per one deprotonation event is 2:1, indicating a 2:1 ($Ca^{2+}:COO^{-}$) bridging ionic complex as opposed to the 1:1 chelating ionic complex that exists between K^+ and COO^- as shown in the previous study. Although this kind of ionic ratio has never been identified for Ca^{2+} , under high concentration of Ca^{2+} as used in this study this type of ionic complex could be dominant. By adopting this type of ionic complex geometry, the condensing effect observed in the π -A isotherms appears to be consistent with the bridging configuration. In addition, the Δ -value (difference between the ss and the as) analysis also supports our assignments. From previous studies, the hydrated ionic complex has a Δ -value of 168 cm⁻¹ with the $v_{s}COO^{-}$ at 1410 cm⁻¹,⁶⁸ whereas this value tends to be much smaller in the 1:1 chelating complex and slightly smaller in the 2:1 bridging complex. Hence, in evaluating the v_s COO⁻ mode in this study (we do not observe the v_{as}), the 1475 cm⁻¹ and 1435 cm⁻¹ peaks consistently follow this convention in terms of coordination configurations. Figure 4.5 illustrates these configurations and their associated frequencies as observed here.

To ensure that the D_{31} -PA monolayers exist as stable monolayers on both Mg²⁺ and Ca²⁺ (0.1 and 0.3 M), a time evolution study of the v_s COO⁻ was carried out. Among all, only spectra acquired from the 0.1 M Ca²⁺ exhibit a dynamic nature of the peak evolution while spectra from other solutions remain almost unchanged during the time of spectral acquisition. Figure 4.6 shows the ssp VSFG spectra of D₃₁-PA on 0.1 M Ca²⁺ at four instants with respect to the initial time zero set to be the instant after 10 minutes waiting directly after the spreading of monolayers. The trend shows that the 1435 cm⁻¹ peak intensity gradually decreases while the 1475 cm⁻¹ peak evolves into shape. After 60 minutes, the overall spectral intensity becomes constant as the system reaches equilibrium.

On the contrary, Figure 4.7 shows the ssp spectra of the D_{31} -PA monolayer on the 0.3 M Ca²⁺ where the 1435 cm⁻¹ spectral intensity decreases somewhat with time as a result of film relaxation, but the 1475 cm⁻¹ shoulder stays relatively constant throughout the time of spectral acquisition. These results show the dynamic nature of the ionic complex species that exists on the aqueous Ca²⁺ solutions. As previously stated, the 1435 cm⁻¹ peak is assigned to the 2:1 bridging ionic complex, and the 1475 cm⁻¹ peak to the 1:1 chelating ionic complex. According to the spectra in Figure 4.6 and Figure 4.7, the 2:1 bridging ionic complex exists as the sole species for the 0.3 M Ca²⁺ subphase irrespective of allowing sufficient time for possible structural rearrangement; however, at 0.1 M Ca²⁺, the 2:1 bridging complex is predominant from the beginning, although contribution from the 1:1 chelating ionic complex gradually emerges as a secondary species as time elapses.

To elucidate this markedly different behavior exhibited by Ca^{2+} , it is important to realize that on the 0.3 M Ca^{2+} aqueous subphase, the majority of D_{31} -PA molecules are deprotonated. Complete deprotonation not only indicates the strong binding affinity that exists between Ca^{2+} and COO^{-} , but also signals that an excess of Ca^{2+} is present at the interface. With sufficient Ca^{2+} , the 2:1 bridging ionic complex is favored. On the contrary, the D_{31} -PA monolayer is only partially deprotonated on the 0.1 M Ca^{2+} subphase based on the spectral evidence obtained in the other spectral regions; therefore, even though the 2:1 bridging ionic complex is the dominant species at 0.1 and 0.3 M Ca^{2+} , the 1:1 chelating ionic complex could well be the secondary channel. This would maximize the overall interaction between a Ca^{2+} ion and the headgroup when Ca^{2+} is limited in quantity at the interface. In conclusion, the interplay between the cation species and the concentration level strongly dictates which stable ionic complexes are formed between the cation and the headgroup.

Carbonyl Stretching Region $(1700 - 1800 \text{ cm}^{-1})$

Because protonated and deprotonated forms of carboxylic acid group have unique vibrational signatures, known as vC=O and v_sCOO^- modes respectively, spectral investigations in the vC=O were considered important in confirming the deprotonation events induced by the ionic binding of Mg²⁺ and Ca²⁺ to the PA headgroup. However, to clearly identify the exact spectral position of the vC=O mode, an additional pH control experiment was performed in this spectral region. Figure 4.8 shows the ssp VSFG spectra of the PA monolayers on aqueous solutions at pH 1.0, 6.0, and 13.3. At the two extreme pH conditions, PA molecules are either primarily protonated or deprotonated. The vC=O spectra presented in Figure 4.8 are consistent with the v_sCOO^- spectra in Figure 4.3 at the corresponding pH. By observation, at pH 1.0 Figure 4.8 shows a symmetric peak centered at 1720 cm⁻¹, which is attributed to the hydrated carbonyl group in the carboxylic acid headgroup at this low pH. On the other hand, the same peak becomes hardly detectable at pH 13.3, but only weakens slightly at pH 6.0, consistent with our observations from above.

According to the previous results obtained from the $v_s COO^-$, Ca^{2+} demonstrates much stronger ionic binding affinity to the COO⁻ relative to Mg²⁺, which then correspondingly induces greater extent of deprotonation on the PA monolayers. To confirm this phenomenon, we again evaluate the spectra with Mg²⁺ and Ca²⁺ subphases, but now in the vC=O region. Figure 4.9A and 4.9B shows the ssp VSFG spectra of the PA monolayers on Mg^{2+} and Ca^{2+} (0.1 and 0.3 M), respectively. The spectra representing the Mg^{2+} at 0.1 and 0.3 M reveal a broad, slightly asymmetric peak centered at 1720 cm⁻¹. Even though a concentration difference exists in the Mg^{2+} spectra, the vC=O peak intensities are constant in the concentration range considered. This observation is in good agreement with the v_sCOO⁻ spectral trend seen in the previous section.

The spectra representing the Ca²⁺ show a significant decrease of the peak intensity when Ca²⁺ is increased from 0.1 to 0.3 M. This is consistent with the observation in the v_s COO⁻. Figure 4.9B shows significant deprotonation of the PA monolayers on the 0.1 M Ca²⁺, and much more on the 0.3 M. This latter peak intensity becomes almost negligible. Quantitatively, the percent increase in the number of deprotonation is 96% from the 0.1 to 0.3 M Ca²⁺ using the corresponding vC=O peak intensities; likewise, a similar value (100%) is estimated if based on the v_s COO⁻ data on the same Ca²⁺ solution. The two spectral regions are in a good agreement with respect to an increase in the v_s COO⁻ peak intensity corresponding to a decrease in the vC=O peak intensity.

4.4 Conclusions

In the present investigation, we have demonstrated a disparate cationic binding specificity of alkaline earth cations (Ca^{2+} and Mg^{2+}) with biological relevant ligands such as carboxylate at the air-liquid interface. The empirical evidence obtained strongly support the notion that Ca^{2+} has stronger binding affinity towards many protein surfaces in biological systems than its counter-part, Mg^{2+} . We conclude that at a neutral pH, the mechanism that governs Ca^{2+} binding to COO^{-} is accompanied by a concomitant deprotonation of carboxyl headgroup. Since the majority of intracellular processes

depend on a neutral pH, this might explain why there exists such considerable Ca²⁺ Therefore, direct identification of this concentration gradient across cell membrane. unique behavior in Ca²⁺ has great biological implications. In addition, surface molecular structure and ion concentration are also important factors influencing cation binding behaviors at the air/liquid interface. For example, at low concentration (0.1 M Ca^{2+}), Ca^{2+} initially favors forming ionic complexes in a bridging configuration (2 Ca^{2+} :1 COO⁻) but gradually transforms to a chelating (bidentate) complex (1 Ca^{2+} :1 COO⁻) as the equilibrium species. On the other hand, as the Ca^{2+} concentration rises to 0.3 M, the primary complex species exists in the bridging configuration even though sufficient time was given for structural reorganization as seen in the 0.1 M. This concentrationdependent binding behavior could suggest that Ca²⁺ binding on the protein surface are intrinsically complex due to structural complexity at the protein surface and the availability of Ca^{2+} in the aqueous phase. Therefore, theoretical modeling is critical in providing more in-depth understanding regarding the interactions of Ca^{2+} with biological ligands at a small scale and protein surfaces at a large scale.



Figure 4.1. Surface pressure-area isotherms (π -A) of PA monolayer at 23°C on aqueous surfaces: (A) neat water with Brewster angle microscopy images in the G-TC coexistence region and the TC homogeneous phase region (B) neat water and MgCl₂ (0.1 and 0.3 M), (C) neat water and CaCl₂ (0.1 and 0.3 M).



Figure 4.2. ssp VSFG spectra of PA monolayers on aqueous solutions at 10 mN/m: (A) neat water, (B) 0.1 and 0.3 M MgCl₂, and (C) 0.1 and 0.3 M CaCl₂.



Figure 4.3. ssp VSFG spectra of D_{31} -PA monolayers on water with pH values of 2.1, 6.0, 8.2 and 13.0. Fitted curves are shown as solid lines.



Figure 4.4. ssp VSFG spectra of D_{31} -PA monolayers on salt solutions: (A) 0.1 and 0.3 M MgCl₂; (B) 0.1 and 0.3 M CaCl₂ solutions. The individual fitted curves are shown as solid lines. The peak intensities in Figure A are enhanced five times for a comparison purpose.



Figure 4.5. Pictorial illustrations of four possible metal-carboxylate complexes in the order of decreasing $v_s COO^-$ frequency.



Figure 4.6. ssp VSFG spectra of D_{31} -PA monolayers on 0.1 M CaCl₂ solutions in a time series: (A) 5 min, (B) 25 min, (C) 60 min, and (D) 70 min. The individual fitted curves are shown as solid lines. Each spectrum corresponds to a 5 min acquisition time.



Figure 4.7. ssp VSFG spectra of D_{31} -PA monolayers on 0.3 M CaCl₂ solutions in a time series: (A) 5 min, (B) 25 min, (C) 60 min, and (D) 70 min. The individual fitted curves are shown as solid lines. Each spectrum corresponds to a 5 min acquisition time.



Figure 4.8. ssp VSFG spectra of PA monolayers on water with pH values of 1.0, 6.0, and 13.3. The fitted curve for pH 1.0 spectrum is shown as a solid line.



Figure 4.9. ssp VSFG spectra of PA monolayers on salt solutions: (A) 0.1 and 0.3 M MgCl₂; (B) 0.1 and 0.3 M CaCl₂. The individual fitted curves are shown as solid lines.

CHAPTER 5

STRUCTURAL INVESTIGATIONS OF MONOUNSATURATED ISOMERS: OLEIC ACID AND ELAIDIC ACID MONOLAYERS

5.1 Introduction

Unsaturated fatty acids are major constituents of lipid molecules that are essential in cellular membrane.⁷⁸ For example, they comprise near half of all the acyl chains in phospholipids in order to maintain membrane's fluidity and permeability via conformational changes on the acyl chains. It is known that the degrees of conformation of the unsaturated acyl chains are directly correlated to the number and position of the double bond.⁷⁹ To this end, a molecular-level understanding of structures of unsaturated fatty acid monolayers at the air/liquid interface is indispensible to relate to its functional relationship that are present in membrane lipids. In specific, studies on the molecular ordering during film compression are of great importance in understanding of the polymorphic transformation from gel to lipid-expanded phase in cellular membranes.⁷⁹

One of the most common unsaturated fatty acids found in living organisms is oleic acid (OA, *cis*-9-octadecanoic acid). It is a monosaturated fatty acid with a C=C at the C₉ and C₁₀ positions in the alkyl chain, which naturally give rise to a bent shape structure. Because of this structural hindrance, crystallization does not occur at room temperature, whereas its trans-isomer, elaidic acid (EA), can be in a crystalline state at room temperature.

To date, OA and EA have been of great interest in spectroscopic studies for their complex polymorphic structural behavior in the solid phase.⁸⁰ However, structural understanding of them as surface molecular films at the vapor/liquid interface continues to remain incomplete due to both technical challenges and their disordered structural features at room temperature.

5.2 Experimental

Materials. cis-9-octadeconoic acid (OA) (99%, Sigma-Aldrich) and trans-9-octadeconoic acid (EA) (99%, Sigma-Aldrich) were used to prepare solutions at ~1.5 mM by dissolving in spectroscopic-grade chloroform (>99.9, Sigma-Aldrich). Both D₁₇- OA and D17-EA were synthesized by our collaborator, Dr. Gabriel Oba in Professor David Hart's research group. The D₁₇-OA compound is extremely pure: ¹H NMR (CDCL₃, 250 MHz) δ 1.2-1.4(m, 8H), 1.4-1.5 (t, *J* = 7.2 Hz, 2H), 1.9-2.1 (m, 2H), 2.3 (t, *J* = 7.2 Hz, 2H), 5.3-5.4 (m, 2H), 11.0-12.0 (m, 1H); ¹³C NMR (CDCl₃, 100.6 MHz) δ 24.7, 27.26, 29.05, 29.08, 29.16, 29.7, 34.1, 129.7, 129.9, 180.6; The determined m/z value is 322.3531 with respect to the calculated reference 322.3523 for C₁₈H₁₇D17O₂ + Na. On the other hand, D₁₇-EA has a 10% mixing of D₁₇-OA due to isomerization: mp 39-41°C (literature 42-44°C); ¹H NMR (CDCL₃, 250 MHz) δ 1.1-1.4(m, 8H), 1.5-1.7 (m, 2H), 1.8-2.1(m, 2H), 2.2-2.4 (t, *J* = 7.6 Hz, 2H), 5.3-5.4 (m, 2H), 10.5-11.5 (m, 1H); ¹³C NMR (CDCl₃, 100.6 MHz) δ 24.7, 28.9, 29.0, 29.1, 29.6, 32.6, 34.1, 130.19, 130.44, 180.5. In similar fashion,

both D₁₇-OA and D₁₇-EA were prepared at ~1.5 mM by dissolving in spectroscopic-grade chloroform (>99%, Sigma-Aldrich). When not in use, all these four solutions were stored at -15°C. Deionized water (18.2 M Ω ·cm resistivity) was obtained from a Barnstead Nanopure system and was conditioned at room temperature (23 ± 1 °C) over 24 hrs before use.

Methods. Langmuir Film Balance. A KSV minitrough (KSV, Finland), 176.5mm × 85mm, was used to acquire surface pressure-area (π -A) isotherms. The trough and the two barriers are made of Teflon and Delrin, respectively. During compression, the π -A isotherms were recorded in real-time by the Wilhelmy plate method. The subphase temperature was maintained at 23 °C after 24 hrs equilibration in a climate-controlled room (23 °C). All π -A isotherms were collected in a Plexiglas box with a dry N₂ purge to eliminate any possible contaminant and oxidation. The aqueous surface was compressed and examined for any sign of surface pressure increase to ensure negligible organic contamination prior to spreading the PA monolayer. After confirming the surface purity, tens of micro-liters of PA-chloroform solution were spread in a drop-wise fashion by a micro-syringe (Hamilton) for homogeneous spreading. 10 minutes was allowed for complete solvent evaporation. During compression, a constant rate of 5 mm/min. of both barriers was employed. In SFG experiments that are interested in vibrational information of monolayers at different surface pressures, the mini-trough was placed on a heightadjustable sample stage to allow incidence of two laser beams, and in addition was enclosed in a closed box with dry N₂ purge to eliminate potential oxidations. Next, the same monolayer spreading protocol was followed to prepare monolayer films. VSFG

spectra were taken while monolayers were maintained at the predetermined surface pressures.

5.3 Results and Discussion

Upon isotopic labeling of the methyl-sided chain segment and at the same time keeping the carboxyl-sided chain segment unchanged, concrete empirical evidence was obtained and used to elucidate the detailed structural ordering mechanisms of these monolayers during compression. For reference, Figure 5.1 illustrates the OA and EA structures and the deuteration schemes utilized. In the following paragraphs, a detailed account of this in-situ investigation that coupled a Langmuir trough with SFG-VS is presented.

5.3.1 Surface Compression Isotherms

Initially, Langmuir isotherms (π -A) of OA and EA on water were used to reveal their corresponding physical states as surface monolayers during compression. Figure 5.2A presents their respective π -A isotherms at 23°C. In the order of decreasing MMA, both OA and EA monolayers exist first in the gas-liquid (G-L) coexistence phase before transitioning to the L phase at MMA of 52 and 50 Å²/mol, respectively. Then, the final collapsed pressures occur at 32 and 26 mN/m for OA and EA, respectively. , These two isotherms are consistent with reported isotherms in the literature.⁸¹⁻⁸³

At sub-freezing temperatures, both molecules tend to pack in multi-lamellae films with all-trans conformation at both sides of the olefin bond.⁷⁹ Even though there is a significant difference in the bulk melting temperatures between OA and EA (13°C vs.

44°C),⁷⁹ EA closely resembles OA and exists in the L phase at the vapor/liquid interface, showing conformational disorder in the alkyl chain. As a result, the MMAs of EA and OA at the point of collapse are 24 and 27 Å²/mol, respectively. In addition, the π -A isotherms of the D₁₇-EA and D₁₇-OA monolayers shown Figure 5.2B are depicted for the purpose of chemical validation. The overall features agree well with the isotherms of protonated species in Figure 5.2A, thereby confirming their purity levels.

5.3.2 VSFG Studies of OA and EA in C-H Stretching Region

To systematically investigate any potential structural change in the alkyl chains during compression, surface VSFG spectra in C-H stretching region were acquired in-situ at three different surface pressures (3, 15, and 25 mN/m) associated with the L phase. In each acquisition, the surface film was maintained at one of the specific surface pressures. Figure 5.3 presents ssp VSFG spectra of the OA monolayer at the three predetermined surface pressures. As the surface pressure increases, an overall peak intensity increase is observed in the spectra. In detail, the methyl symmetric stretch (v_sCH_3 , ~2876 cm⁻¹) and the methyl Fermi-resonance ($v_{FR}CH_3$, ~2939 cm⁻¹) show the most significant increase, whereas the methylene symmetric stretch (v_sCH_2 , ~2846 cm⁻¹) only increases to a small extent and no significant changes take place in the methylene asymmetric stretch (v_aCH_2 , ~2926 cm⁻¹) and the olefin stretch (vCH, ~3009 cm⁻¹). In addition, the methyl asymmetric stretch (v_aCH_3 , ~2960 cm⁻¹) peak also appears as a small shoulder in the spectra. Therefore, all these peaks were used to fit the overall spectra and the detailed fitting results are shown in Table 5.1.

As a general rule, SFG intensity directly interrogates the surface molecular number density, polar orientation, and order.⁶⁰ The significantly large increase of the v_sCH_3 intensity in relation to the v_sCH_2 affirms this rule. This trend is much more evident in the saturated amphiphiles.² As a qualitative indicator for conformational order in the alkyl chains, the intensity ratio of the v_sCH_3 over the v_sCH_2 has proved useful. The greater the ratio, the more ordered the chain is in conformation. Increased order is analogous with approaching an all-trans methylene conformation and a decrease in the number of gauche defects. Our data indicate that the conformational order in the OA monolayer is strengthened rapidly during the 3 to 15 mN/m transition, and then slackened during the 15 to 25 mN/m transition. A discussion on the possible cause of this physical trend will be given later. However, it is important to note that the gauche defect or conformational disorder continues to be dominant even at a surface pressure near the collapse as indicated by the increasing v_s CH₂ intensity during compression. According to previous solid-phase studies at sub-freezing temperatures, this behavior is very similar to the proposed molecular structure for OA in conditions that just exceed the surface melting temperature at -2°C.^{80,84}

In previous low temperature studies using infrared and Raman, the olefinic stretch in OA is considered to be highly sensitive to the surrounding environment of the olefin bond as molecular conformation changes in carbons connecting to it.^{80,84,85} Its frequency was observed in the range of 3003 to 3015 cm⁻¹ in the disordered phase (α -phase) in Raman and IR. Additional spectral shifts were observed in the other two more ordered phases (γ - and β -phase).⁸⁵ Thus, the spectral position of this peak identified in Figure 5.3 perfectly overlaps with the spectral assignment for the α -phase of OA; this is consistent with the postulate made previously regarding the disordered conformation in OA alkyl chain at the vapor/liquid interface, even near the collapse.

The same experiment was performed on the EA monolayer on water as the one previously described in the OA monolayer study. Figure 5.4 presents ssp VSFG spectra of the EA monolayer on water at the same three surface pressures: 3, 15, and 25 mN/m, and the fitted results are shown in Table 5.2. Pronounced spectral features are in most part similar to those in the OA spectra, except the absence of the olefinic stretch. By adhering to the SFG selection rule based on the dipole approximation, this spectral observation is completely in-line with the symmetry of two C-Hs in the transconformation of the olefin bond. By positioning at the opposite side of the C=C, the two C-Hs naturally form a centrosymmetry that consequently results in SFG inactivity of the ν CH. This kind of spectral phenomenon due to forming an inversion center in the molecular chains is also pronounced for the CH₂ groups in the saturated fatty acid acyl chains similar to palmitic acid in the condensed phase.²

Aside from the absence of the olefinic stretch, the trend of increasing peak intensities in the v_sCH_3 and $v_{FR}CH_3$ continues to be followed in the EA spectra during the surface pressure increase. However, on the contrary, the v_sCH_2 reverses the trend, depicting a decrease in peak intensity. This behavior could in turn affect the peak intensity ratios of the v_sCH_3 over v_sCH_2 . By combining these two effects uniquely demonstrated in the EA spectra, we can postulate that the conformational ordering in the EA monolayer is considerably more enhanced than that in the OA monolayer, and this is supported by the EA π -A isotherm that reveal lower MMA at the collapse with respect to that of OA. But, the ultimate questions still remain: during the compression process, which part of alkyl segments is contributing more to the overall chain conformational ordering, and how exactly are they oriented at the interface?

In an effort to address these two questions, D_{17} -OA and D_{17} -EA were used to separate the methyl-sided alkyl segment from the one at the carboxyl side. Hence, independent probes at both segments can be implemented. Here, spectroscopic studies in both C-H and C-D stretching regions were conducted at the aforementioned surface pressures. Figure 5.5 presents ssp VSFG spectra of the D_{17} -OA monolayer on water at surface pressures of 3, 15, and 25 mN/m. The spectral region of interest is the C-H region; therefore, only the polymethylene segment at the carboxyl side was investigated. By observation, three spectral peaks are pronounced in the spectra, which are each attributed to the $v_s CH_2$ (~ 2846 cm⁻¹), $v_a CH_2$ (~2926 cm⁻¹), and v CH (~3003 cm⁻¹). The fitted results are shown in Table 5.3. In addition, a fourth peak positioned at $\sim 2888 \text{ cm}^{-1}$ is discernable though it is weak. This peak was previously assigned to the Raman-active v_aCH₂.⁸⁶ Being Raman-active and infrared-forbidden, the observation of this peak was also identified in the SFG study of polyethylene film in Shen's group. They proposed that this phenomenon is caused by the electric-quadrupole excitation by the IR field in the bulk, which is a bulk contribution to the SFG signal.⁸⁷ But in our case D₁₇-OA exists as a monolayer film, a contribution from the bulk is unlikely. Based on the recent ab initio calculation results, the methylene groups at the carboxyl side are strongly coupled to the carboxylic group, which as a consequence influences the vibrational frequencies of these

 CH_2 groups.⁸⁸ Thus, the observation of this mode could well arise from a particular CH_2 group that has different electronic structure than the rest of CH_2 groups in the chain.

Surprisingly, during compression the v_sCH_2 intensity remains unchanged while the v_aCH_2 intensity gradually decreases and the vCH intensity only slightly increases. To clearly assess the relative strength of the v_sCH_2 intensity in the D₁₇-OA molecules, the VSFG spectra of a palmitic acid monolayer on water at the same surface pressures and acquisition time were chosen as references. For instance, at 3 mN/m the spectra shown in Figure 5.5 reveal almost equal intensities from the v_sCH_2 between the PA and D₁₇-OA monolayers after spectral analysis. It is important to note that at this particular surface pressure, PA molecules are already in the tilted-condensed phase where conformational order is prevalent in the acyl chains.² Further increase in surface pressure only results in a small attenuation on the v_s CH₂ intensity as acyl chains undergo a tighter packing and the dipole cancellation is further enhanced. Therefore, by comparison the polymethylene chain at the carboxyl side of OA is deemed to be as conformationally ordered as the PA acyl chain while considering there is even fewer number of CH₂ groups in D₁₇-OA relative to PA (7 vs 14). This observation is consistent with the empirical findings in the OA polymorphism studies, and also validates the postulate that asserts the carboxyl-sided of alkyl chain is ordered in conformation while the methyl-sided alkyl chain shows the opposite, being disordered above the surface melting temperature. Therefore, the OA molecule as a whole shows disordered conformation as evidenced in the protonated OA spectra discussed above.

Moreover, it is interesting to observe that the intensity decrease of the v_aCH_2 is more significant during the 3 to 15 mN/m transition than that of the 15 to 25 mN/m transition while the v_sCH_2 intensity remains constant during the same transitions. To better explain these observations, the perpendicular nature of their IR transition moments According to the previous solid-phase IR and Raman studies, the is considered. polymethylene chain in OA favors a pseudo-orthorhombic subcell packing in which the skeletal plane of methylene groups in an individual OA is parallel to that of the next nearest neighbor in the γ -phase.^{80,85} This parallel nature between adjacent polymethylene skeletal planes remains intact even during the phase transition from the γ - to α -phase; only slight modifications occur to the subcell structure.⁸⁴ Therefore, in a 2D environment at the vapor/liquid interface, parallel packing among the polymethylene planes is more favorable than random packing in order to achieve a small MMA as shown in the isotherms. At 3 mN/m, the carboxyl-sided polymethylene chain is naturally closer to the surface plane than that at a higher surface pressure based on the corresponding MMA. Thus, the IR transition dipole moment of the v_aCH₂ is considered to be more perpendicular to the surface plane than that of the v_sCH_2 . As the OA monolayer gradually adopts a tighter packing via compression, the orientation of the former transition moment becomes more parallel to the surface plane, as illustrated in Figure 6.6. On account of this kind of transformation on the dipole transition moment, attenuation of the v_aCH_2 intensity with the ssp mode is expected irrespective of an accompanying increase on the CH₂ number density since the incident IR polarization is p-polarized. In other words, the IR excitation of the v_aCH₂ mode becomes less favorable during

compression. On the other hand, this packing mechanism has little effect on the v_sCH_2 dipole transition moment because its orientation is maintained to be near the surface plane during compression. In this case, the observation of the constant v_sCH_2 intensity mirrors the results obtained from the saturated fatty acid monolayers where conformational order in the condensed phase has minimal effect on the v_sCH_2 intensity. Aside from the v_sCH_2 and v_aCH_2 , the vCH is also observed in these spectra, but with a slight shift of its peak position relative to that of the completely protonated OA (3003 cm⁻¹ vs 3009 cm⁻¹). This spectral shift could arise from the chemical asymmetry around the olefin bond instead of conformational asymmetry as proposed in past studies. In summary, our spectral evidence also support the notion that the polymethylene chain at the carboxyl side of OA could become as ordered as the acyl chain in saturated fatty acids at small MMAs.

In Figure 5.7, ssp VSFG spectra of D_{17} -EA monolayer on water are presented. Being similar to the D_{17} -OA spectra on water, each of these spectra shows three main peaks that correspond to the v_sCH_2 (~ 2846 cm⁻¹) and the v_aCH_2 (~2928 cm⁻¹) in the order of increasing frequency. However, instead of seeing the constant intensity levels of the v_sCH_2 throughout the D_{17} -OA spectra, only the 3 to 15 mN/m transition reveals the same result, but not the 15 to 25 mN/m transition. The latter shows a slight decrease of the v_sH_2 intensity. Nevertheless, the v_aCH_2 intensity decreases at both transitions, which is consistent with the D_{17} -OA spectra. Next, it is important to note that the v_sCH_2 intensity is about equal in spectra of D_{17} -EA and D_{17} -OA at 3 and 15 mN/m. According to the previous discussion, it is logical to imply that the polymethylene chain at the carboxyl side of D_{17} -EA is also significantly ordered at these two surface pressures. Moreover, the further decrease of the v_sCH_2 intensity could only suggest that the EA monolayer is capable of more conformational ordering at high surface pressures relative to OA. In fact, this attenuation of the v_sCH_2 intensity also agrees with the similar trend shown in the EA spectra that correspond to the 15 to 25 mN/m transition in Figure 5.3. On the other hand, the D_{17} -EA spectrum at 3 mN/m reveals stronger v_aCH_2 intensity with respect to the D_{17} -OA spectrum at the same surface pressure. More distinctively, the extent of intensity reduction of the v_aCH_2 is more considerable in the D_{17} -EA spectra than those in the D_{17} -OA spectra as the surface pressure increases. Taking into account these unique features shown in the D_{17} -EA spectra as well as its π -A isotherm, the polymethylene chain in the carboxyl side of D_{17} -EA is intrinsically more ordered than that of D_{17} -OA at small MMAs.

To complete the comprehensive study on the conformations of OA and EA in their respective monolayers, the methyl-sided alkyl chain was independently studied. Both D₁₇-OA and D₁₇-EA monolayers on water were probed at the same surface pressures (3, 15, and 25 mN/m) in C-D stretching region. Figure 5.8 depicts the ssp VSFG spectra of the D₁₇-OA monolayer at these three surface pressures. By inspection, all three spectra reveal six vibrational peaks that are v_sCD_3 (~ 2076 cm⁻¹), v_sCD_2 (~ 2104 cm⁻¹), $v_{FR}CD_3$ (~ 2124 cm⁻¹), $v_{FR}CD_2$ (~ 2143 cm⁻¹), v_aCD_2 (~ 2205 cm⁻¹), and v_aCD_3 (~ 2223 cm⁻¹) in the order of increasing vibrational frequency. The detailed fitting results are shown in Table 5.5. The assignments for these vibrational peaks are well-known in literature except the rare appearance of the $v_{FR}CD_2$ in past SFG studies.²³ Here, we were able to detect this mode despite its weakness in the D₁₇-OA monolayer.

First, the overall intensity increase across the spectrum is evident when the surface pressure increases. In particular, v_sCD_3 , $v_{FR}CD_3$, and v_aCD_3 show more pronounced enhancement than the others in the spectra. Second, the v_sCD_2 also demonstrates a slight intensity increase in the spectra. This observation clearly explains the fact that the overall v_sCH_2 intensity increase in the OA spectra shown in Figure 5.2 is directly derived from the polymethylene chain at the methyl side since this intensity remains constant at the carboxyl side. This finding further confirms the solid-phase study results that state when crystalline OA is just above the surface melting temperature (- 2° C), the polymethylene chain at the carboxyl side continues to maintain an all-trans conformation while the one at the methyl side adopts a more disordered arrangement with gauche defects.^{80,85} Likewise, the intensity ratio of the v_sCD_3 over the v_sCD_2 also is enhanced along with the surface pressure increase. This trend also suggests that the polymethylene chain at the methyl side is gradually becoming more ordered when D_{17} -OA molecules are forced to pack at high surface pressures; however, this does not rule out the presence of gauche defects in the chain. Finally, it is interesting to find that the v_sCD_3 intensity is already stronger than that of the v_sCD_2 at 3 mN/m, which is opposite to the trend shown in the OA spectrum in Figure 5.2. This unique finding is a direct consequence of only the methyl-sided chain being isotope-labeled, in which case the VSFG intensity is 75% weaker than that of the completely isotope-labeled alkyl chain while assuming the SFG intensity is solely based on the number density squared.

Therefore, this result is consistent with the physical nature of the molecular system considered. At 15 and 25 mN/m, the v_sCD_3 intensities are much stronger than that at 3 mN/m. Thus, we performed molecular orientation calculations to demonstrate how these intensity increases correspond to the methyl group orientation.

In Figure 5.9, ssp VSFG spectra of D_{17} -EA monolayers on water at the surface pressure of 3, 15, and 25 mN/m are presented. The overall spectral features and intensity trends are closely similar to those of D_{17} -OA spectra shown in Figure 5.8. Namely, the same number of vibrational peaks are identified and assigned to the v_sCD_3 , v_sCD_2 , $v_{FR}CD_3$, $v_{FR}CD_2$, v_aCD_2 , and v_aCD_3 ; the pronounced spectral intensity increases also occurs to the v_sCD_3 , $v_{FR}CD_3$, and v_aCD_3 . However, the v_sCD_2 and $v_{FR}CD_2$ also show a more obvious intensity increase than those of in the D₁₇-OA spectra. Therefore, the methyl-sided polymethylene chain is responsible for the initial increase of the v_sCH_2 intensity at the 3-15 mN/m transition, while the carboxyl-sided polymethylene is then responsible for the apparent attenuation of the v_sCH_2 intensity at the 15 – 25 mN/m transition. By this notion, it is logical that the methyl-sided alkyl chain is responsible for the initial molecular interactions among neighboring molecules, and the intensity increase of the v_sCH_2 is primarily a result from the unit number density increase as molecules undergo packing; on the other hand, the carboxyl-sided alkyl chain is accountable for the tighter packing as it adopts near all-trans conformation and positions closer to the surface normal. These two mechanisms combined naturally result in a reduction in the SFG signal response as shown Figure 5.3.
As a final note, the SFG-VS technique has the ability to accurately determine the polar orientation angles of some commonly encountered molecular groups. However, the prerequisite for arriving at an accurate estimation is the pre-knowledge of the hyperpolarizability tensor ratio, in particular, $\frac{\beta_{aac}^{(2)}}{\beta_{ccc}^{(2)}}$. In practice, this ratio can be empirically deduced from the Raman depolarization ratio that is directly measured in the polarized Raman experiment. VSFG angle calculation methodology and procedures have been comprehensively reviewed and documented in literature, ^{35,39,42,89-91} and in addition, many hyperpolarizability ratios are commonly reported for molecular groups such as CH₃, CH₂, OH, and so forth in various organic compounds.³⁹ Here, the general functional relationship between the measured second-order nonlinear susceptibility ($\chi_{eff}^{(2)}$) and the molecular hyperpolarizability ($\beta^{(2)}$) are given:

$$\chi_{eff,ssp}^{(2)} = L_{yy}(\omega_s) L_{yy}(\omega_1) L_{zz}(\omega_2) \sin \beta_2 \chi_{yyz}^{(2)}$$
(5.1)

Here, $L_{ii}(\omega_i)$ are component Fresnel factors corresponding to frequency ω_i , β_2 is the incident angle of the IR field, and $\chi^{(2)}_{yyz}$ is the second-order nonlinear susceptibility tensor component.

In the case of D_{17} -OA and D_{17} -EA, the orientation of the terminal CD₃ group, the angle between the primary C₃ rotational axes and the surface normal of the laboratory coordinates, is determined via following relations for the ssp polarization combination.

$$\chi_{yyz}^{(2)}(v_s - CD_3) = \frac{1}{2} N_s \beta_{ccc}^{(2)} [(1+R)\langle \cos\theta \rangle - (1-R)\langle \cos^3\theta \rangle]$$
(5.2)

$$\chi_{yyz}^{(2)}(v_a - CD_3) = -N_s \beta_{aca}^{(2)}(\langle \cos \theta \rangle - \langle \cos^3 \theta \rangle)$$
(5.3)

The $\chi^{(2)}$ of the $v_s CD_3$ and the $v_a CD_3$ are related by θ , the orientation angle, N_s , the molecular number density, R, the hyperpolarizability ratio of $\frac{\beta_{aac}^{(2)}}{\beta_{ccc}^{(2)}}$. Given the literature value of r (2.3),⁹² the C-D bond derivative polarizability ratio, then $\frac{\beta_{aca}^{(2)}}{\beta_{ccc}^{(2)}}$ is determined to be 1.875 using a bond polarizability derivative model described by Wang.³⁹ In practice, the $\frac{\beta_{yyz}^{(2)}(v_s CD_3)}{\chi_{yyz}^{(2)}(v_a CD_3)}$ ratio is obtained by taking square root of the $v_s CD_3$ and $v_a CD_3$ peak intensities.

To ascertain the validity of this r value, the fitted D_{31} -PA ssp spectrum at 25 mN/m was used to calculated θ . The final result was found to be 38°. By using simple geometric relation, $|\theta - 35^{\circ}|$, for the acyl chain orientation when the chain is in all-trans conformation, the chain is ~ 3° to the surface normal. This result is consistent with the actual orientation of PA molecules in the condensed phase.²¹ Once validation is complete, the fitted component peaks of v_sCD_3 and v_aCD_3 of the D_{17} -OA and D_{17} -OA spectra at surface pressures of 3, 15, and 25 mN/m were calculated and they are listed in Table 5.7, and 5.8, and the corresponding methyl orientation angle, θ , are denoted on Figure 5.10. Based on the data trend appeared on the simulation curve, it is concluded that the θ for the methyl group of the D_{17} -EA is pointing more towards to the surface pressure. This implies that EA monolayer is capable of being tightly packed with a more enhanced conformational order than OA at the same physical conditions.

5.4 Conclusions

According to the spectroscopic evidence presented in this study, it is clear that the isotopic-labeling on the methyl-sided polemethylene chains in both EA and OA allows a more in-depth look into the conformation ordering mechanisms during structural packing in monolayers. It is found that the methyl-sided alkyl chain is responsible for the initial molecular interactions among neighboring molecules; on the other hand, the carboxyl-sided alkyl chain is accountable for the tighter packing as it adopts near all-trans conformation and positions closer to the surface normal. In addition, near all-trans conformation already starts to emerge at 3 mN/m.

The methyl orientation results are also consistent with the slight disparity in molecular structures between OA and EA. Based on the data trend appeared on the simulation curve, the methyl group of the EA is pointing more towards to the surface normal as the surface pressure increases than those of OA at the same surface pressure. This is in-line with π -A isotherm trends. Thus, EA monolayer is capable of being tightly packed with a more enhanced conformational order than OA at the same physical conditions, and that is the reason why it has slightly higher surface melting temperature than OA.



Figure 5.1. Simplified structures of oleic acid (cis), elaidic acid (trans), D_{17} -oleic acid (cis), and D_{17} -elaidic acid (trans) in the order from the top to the bottom.



Figure 5.2. Langmuir isotherms (π -A) of oleic acid, elaidic acid and the D₁₇ -labeled OA and EA on water: (A) OA and EA and (B) D₁₇ – OA and D₁₇ – EA. The markers denote the phases of monolayers at each surface pressure: L-G – liquid and gas coexistence phase; L – liquid phase; and Collape – the collaped phase.



Figure 5.3. ssp VSFG spectra of OA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 1 Min. acquisition in C-H stretching region. Solid curves represent the fitted spectra.

| π (mN/m) | Fitting Parameters | $\upsilon_s CH_2$ | $\upsilon_s CH_3$ | $\upsilon_a CH_2$ | $\upsilon_{FR}CH_3$ | $\upsilon_a CH_3$ | υCH (olefin) |
|-------------|------------------------------|-------------------|-------------------|-------------------|---------------------|-------------------|-----------------|
| | A_{ssp} | 2.90 | 1.60 | 2.60 | 0.90 | -0.60 | 1.10 |
| 3 | $\omega_{IR} (cm^{-1})$ | 2850 | 2876 | 2926 | 2939 | 2961 | 3009 |
| | Γ (cm ⁻¹) | 8 | 8 | 12 | 9 | 9 | 9 |
| | A _{ssp} | 3.10 | 2.85 | 1.99 | 1.70 | -0.90 | 1.30 |
| 15 | $\omega_{IR} (cm^{-1})$ | 2848 | 2876 | 2924 | 2939 | 2961 | 3009 |
| | Γ (cm ⁻¹) | 8 | 8 | 11 | 9 | 9 | 9 |
| | A_{ssp} | 3.19 | 3.96 | 1.60 | 2.80 | -1.00 | 1.30 |
| 25 | $\omega_{IR} (cm^{-1})$ | 2848 | 2876 | 2923 | 2939 | 2960 | 3006 |
| | Γ (cm ⁻¹) | 8 | 8 | 10 | 9 | 9 | 9 |

Table 5.1. Fitting results for the ssp VSFG spectra of the OA monolayer on water at surface pressures of 3, 15, and 25 mN/m in C-H stretching region.



Figure 5.4. ssp VSFG spectra of the EA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 1 min acquisition in C-H stretching region. Solid curves represent the fitted spectra.

| π (mN/m) | Fitting Parameters | $\upsilon_s CH_2$ | $\upsilon_s CH_3$ | $\upsilon_a CH_2$ | $\upsilon_{FR} CH_3$ | $\upsilon_a CH_3$ |
|----------|-----------------------------------|-------------------|-------------------|-------------------|----------------------|-------------------|
| | A_{ssp} | 3.10 | 1.50 | 2.50 | 1.00 | -0.55 |
| 3 | $\omega_{IR} (cm^{-1})$ | 2848 | 2876 | 2924 | 2939 | 2961 |
| | Γ (cm ⁻¹) | 8 | 8 | 11 | 9 | 9 |
| | A_{ssp} | 3.04 | 3.62 | 2.10 | 2.60 | -0.80 |
| 15 | ω_{IR} (cm ⁻¹) | 2848 | 2876 | 2924 | 2939 | 2961 |
| | Γ (cm ⁻¹) | 8 | 8 | 11 | 9 | 9 |
| | A_{ssp} | 2.45 | 4.55 | 1.60 | 3.20 | -0.80 |
| 25 | ω_{IR} (cm ⁻¹) | 2848 | 2876 | 2924 | 2939 | 2961 |
| | Γ (cm ⁻¹) | 8 | 8 | 11 | 9 | 9 |

Table 5.2. Fitting results for the SFG spectra of the EA monolayer on water at surface pressures of 3, 15, and 25 mN/m at the C-H stretching region.



Figure 5.5. ssp VSFG spectra of the D_{17} - OA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 3 min acquisition in C-H stretching region. Solid curves represent the fitted spectra.

| π (mN/m) | Fitting Parameters | $\upsilon_s CH_2$ | $\upsilon_a CH_2$ | υCH (olefin) |
|----------|------------------------------|-------------------|-------------------|--------------|
| | A _{ssp} | 3.35 | 3.75 | -2.00 |
| 3 | $\omega_{IR} (cm^{-1})$ | 2846 | 2928 | 3003 |
| | Γ (cm ⁻¹) | 7 | 15 | 12 |
| | A_{ssp} | 3.35 | 3.01 | -2.00 |
| 15 | $\omega_{IR} (cm^{-1})$ | 2846 | 2926 | 3003 |
| | Γ (cm ⁻¹) | 7 | 15 | 12 |
| | A _{ssp} | 3.30 | 2.65 | -1.8 |
| 25 | $\omega_{IR} (cm^{-1})$ | 2846 | 2925 | 3003 |
| | Γ (cm ⁻¹) | 7 | 15 | 10 |

Table 5.3. Fitting results for the ssp SFG spectra of the D_{17} - OA monolayer on water at surface pressures of 3, 15, and 25 mN/m in C-H stretching region.



Figure 5.6. ssp VSFG spectra of the palmitic acid and D_{17} -OA monolayers on water at 3 mN/m. Each spectrum corresponds to 1 min acquisition in C-H stretching region.



Figure 5.7. Schematics for the proposed transformation of the $\nu_a CH_2$ IR transition moment during compression. The arrow represents the $\nu_a CH_2$ IR transition moment.



Figure 5.8. ssp VSFG spectra of the D_{17} - EA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 3 min acquisition in C-H stretching region. Solid curves represent the fitted spectra.

| π (mN/m) | Fitting Parameters | $\upsilon_s CH_2$ | $\upsilon_a CH_2$ |
|--------------|-----------------------------------|-------------------|-------------------|
| | A _{ssp} | 3.35 | -3.50 |
| 3 | ω_{IR} (cm ⁻¹) | 2846 | 2928 |
| | Γ (cm ⁻¹) | 7 | 11 |
| | A _{ssp} | 3.30 | -2.90 |
| 15 | ω_{IR} (cm ⁻¹) | 2846 | 2928 |
| | Γ (cm ⁻¹) | 7 | 11 |
| | A _{ssp} | 2.90 | -2.80 |
| 25 | $\omega_{IR} (cm^{-1})$ | 2846 | 2928 |
| | Γ (cm ⁻¹) | 7 | 16 |

Table 5.4. Fitting results for the ssp SFG spectra of the D_{17} - EA monolayer on water at surface pressures of 3, 15, and 25 mN/m in C-H stretching region.



Figure 5.9. ssp VSFG spectra of the D_{17} - OA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 3 min acquisition in C-D stretching region. Solid curves represent the fitted spectra.

| π (mN/m) | Fitting Parameters | $\upsilon_s CD_3$ | $\upsilon_s CD_2$ | $\upsilon_{FR}CD_3$ | $\upsilon_{FR}CD_2$ | $\upsilon_a CD_2$ | $\upsilon_a CD_3$ |
|-------------|------------------------------|-------------------|-------------------|---------------------|---------------------|-------------------|-------------------|
| | A _{ssp} | 1.70 | 2.55 | 1.20 | 0.20 | -0.90 | -1.70 |
| 3 | $\omega_{IR} (cm^{-1})$ | 2076 | 2104 | 2124 | 2143 | 2205 | 2223 |
| | Γ (cm ⁻¹) | 7 | 12 | 10 | 7 | 9 | 10 |
| | A _{ssp} | 2.80 | 2.61 | 1.65 | 0.30 | -0.80 | -2.20 |
| 15 | $\omega_{IR} (cm^{-1})$ | 2076 | 2104 | 2124 | 2143 | 2205 | 2223 |
| | Γ (cm ⁻¹) | 7 | 10 | 10 | 7 | 9 | 10 |
| | A_{ssp} | 3.55 | 2.70 | 2.00 | 0.30 | -0.60 | -2.40 |
| 25 | $\omega_{IR} (cm^{-1})$ | 2076 | 2103 | 2124 | 2143 | 2204 | 2223 |
| | Γ (cm ⁻¹) | 7 | 10 | 10 | 7 | 9 | 10 |

Table 5.5. Fitting results for the ssp SFG spectra of the D_{17} - OA monolayer on water at surface pressures of 3, 15, and 25 mN/m in C-D stretching region.



Figure 5.10. ssp VSFG spectra of the D_{17} - EA monolayer on water at three surface pressures: 3 mN/m, 15 mN/m, and 25 mN/m. Each spectrum corresponds to a 3 min acquisition in C-D stretching region. Solid curves represent the fitted spectra.

| π (mN/m) | Fitting Parameters | $\upsilon_s CD_3$ | $\upsilon_s CD_2$ | $\upsilon_{FR}CD_2$ | $\upsilon_{FR}CD_3$ | $\upsilon_a CD_2$ | $\upsilon_a CD_3$ |
|-------------|------------------------------|-------------------|-------------------|---------------------|---------------------|-------------------|-------------------|
| | A _{ssp} | 2.16 | 2.00 | 1.35 | 0.30 | -1.10 | -1.50 |
| 3 | $\omega_{IR} (cm^{-1})$ | 2076 | 2105 | 2124 | 2143 | 2204 | 2223 |
| | Γ (cm ⁻¹) | 7 | 10 | 10 | 8 | 9 | 10 |
| | A_{ssp} | 3.80 | 2.50 | 2.04 | 0.50 | -0.80 | -2.20 |
| 15 | $\omega_{IR} (cm^{-1})$ | 2076 | 2105 | 2124 | 2143 | 2204 | 2223 |
| | Γ (cm ⁻¹) | 7 | 10 | 10 | 8 | 9 | 10 |
| | A_{ssp} | 4.80 | 2.80 | 2.40 | 0.80 | -0.80 | -2.50 |
| 25 | $\omega_{IR} (cm^{-1})$ | 2076 | 2105 | 2124 | 2143 | 2204 | 2223 |
| | Γ (cm ⁻¹) | 7 | 10 | 10 | 8 | 9 | 10 |

Table 5.6. Fitting results for the ssp SFG spectra of the D_{17} - EA monolayer on water at surface pressures of 3, 15, and 25 mN/m in C-D stretching region.



Figure 5.11. CD₃ orientation angle simulation curve of $\left(\frac{\chi_{eff,ssp}^{(2)}(v_sCD_3)}{\chi_{eff,ssp}^{(2)}(v_aCD_3)}\right)^2$ vs θ . The red '+'

represents the CD₃ orientation angles of D_{17} -EA at 3, 15, and 25 mN/m (from right to left); the blue 'x' represents the CD₃ orientation angles of D_{17} -OA at 3, 15, and 25 mN/m (from right to left).

Table 5.7. Fitting results for the component peak intensities of the v_sCD_3 and v_aCD_3 in the ssp SFG spectra of the D_{17} - OA monolayer on water at surface pressures of 3, 15, and 25 mN/m in C-D stretching region.

| π (mN/m) | $I_{v_s C D_3}(s s p)$ | $I_{v_a CD_3}(ssp)$ | $\frac{I_{v_sCD_3}(ssp)}{I_{v_aCD_3}(ssp)}$ |
|----------|------------------------|---------------------|---|
| 3 | 1.246 | 0.858 | 1.452 |
| 15 | 3.381 | 1.436 | 2.354 |
| 25 | 5.434 | 1.709 | 3.179 |

Table 5.8. Fitting results for the component peak intensities of the $\upsilon_s CD_3$ and $\upsilon_a CD_3$ in the ssp SFG spectra of the D_{17} - EA monolayer on water at surface pressures of 3, 15, and 25 mN/m in C-D stretching region.

| π (mN/m) | $I_{v_s CD_3}(ssp)$ | $I_{v_a CD_3}(ssp)$ | $\frac{I_{v_sCD_3}(ssp)}{I_{v_aCD_3}(ssp)}$ |
|----------|---------------------|---------------------|---|
| 3 | 2.202 | 0.688 | 3.012 |
| 15 | 6.227 | 1.436 | 4.336 |
| 25 | 9.935 | 1.855 | 5.356 |

CHAPTER 6

INTERFACIAL WATER STRUCTURE AT HYDROPHILIC INTERFACES

6.1 Introduction

Organic Langmuir monolayer at the air/liquid interface has been commonly studied as potential models for biological membranes, aerosol surfaces, and biomimetic material surfaces.^{8,17,93} For the most part, it is of great significance to know the detailed molecular structure of these monolayers. More importantly, studying the hydrogen-bonding structure of surface water molecules presiding underneath the monolayer could provide a more in-depth molecular-level understanding about the interaction of water molecules with the polar groups of organic monolayers. With incorporations of divalent cations such as Mg²⁺ and Ca²⁺ in the aqueous subphase, the dynamic natures of the interfacial hydrogen-bonding network due to the presences of the inorganic cations become more pertinent to the real systems. At the end, it may help making predictions regarding surface phenomena that are commonly found at both physical and biological interfaces.

In this study, we use sum frequency generation vibrational spectroscopy to study the molecular structure of interfacial hydrogen-bonding network underneath a palmitic acid (C_{16}) monolayer. By varying the cation concentrations, a comprehensive surface water restructuring mechanisms were identified due to the surface charge neutralization effect. We found that at lower concentrations, surface neutralization is the prominent effect; however, at higher concentrations, post reaching the point zero charge transition, the already disrupted hydrogen-bonding network reorganizes and reverts to the original structure at the neat solution interface for both cations.

6.2 Experimental

Materials. Palmitic acid (>99%, Sigma-Aldrich) was used to prepare solutions at ~1.5 mM by dissolving in spectroscopic-grade chloroform (>99.9, Sigma-Aldrich). Magnesium chloride (99%, Fisher Scientific) and calcium chloride (99%, Fisher Scientific) were used to prepare stock solutions by dissolving in deionized water (18.2 M Ω ·cm resistivity) from a Barnstead Nanopure system at pH of 6.0.

Stock solutions were filtered twice to eliminate potential organic contaminants using Whatman Carbon-Cap activated carbon filter. The concentrations of the filtered stock solutions were standardized based on the Mohr titration technique in which silver nitrate (reagent grade, Fisher Scientific) and potassium chromate (99.5%, E.M. Science) were used as a titrate and an indicator, respectively.⁴⁵ 0.1,0.3, 1.5, 1.8 M inorganic salt solutions used in this study were prepared by dilution, and 2.6 M Mg²⁺ was prepared by evaporation of the stock solution in a water bath. All solutions were conditioned at room temperature (23 ± 1 °C) over 24 hrs before use. To confirm solution to be devoid of any possible organic contaminant, VSFG testing in the C-H stretching region was performed on all solutions used. All samples shown no resonance VSFG responses, therefore, their purity levels were validated.

Methods. *Monolayer at Equilibrium Spreading Pressure.* Monolayers at equilibrium spreading pressure (ESP) were spread over the various solutions in clean Petri-dishes. The monolayers of PA at ESP on neat water and the salt solutions were able to attain a mean molecular area (MMA) coverage of ~21 Å²/molecule, which are generally assumed to be in a highly ordered phase. After spreading, 10 minutes was also allowed for solvent evaporation and monolayer stabilization. Then, VSFG spectra were acquired.

6.3 Results and Discussion

Of primary focus in this study is the interfacial hydrogen-bonding network that exists uniquely between palmitic acid (PA) Langmuir monolayer and surface water molecules at the air/liquid interface. More importantly, divalent cation binding towards the PA headgroup and its direct effect on the interested hydrogen-bonding network were systematically investigated. In the following, we present vibrational spectral evidence that reveals a dynamic nature of this interfacial hydrogen-bonding network.

Initially, ssp VSFG spectra of neat water and aqueous divalent salt solutions are presented for references. Figure 6.1 shows ssp VSFG spectrum of neat water in O-H stretching region $(3000 - 3900 \text{ cm}^{-1})$ that reveals three distinct vibrational bands that are individually attributed to the predominant hydrogen-bonded water structures at the air/liquid interface. In the order of increasing vibrational frequency, or decreasing hydrogen-bonding strength, two broad and one sharp bands are identified at spectral positions of ~3200, ~3400, and 3700 cm⁻¹, respectively. Even though a definitive assignment is still missing due to a collective nature of potentially inter- and intra-

molecular interactions of O-H oscillators both in the bulk and at the interface,^{40,60,94-98} one may generally agree that the low frequency band (~3200 cm⁻¹) has more ordered hydrogen-bonding structure than that of the mid-frequency band (~3400 cm⁻¹). In practice, they are commonly assigned to the symmetrically and the asymmetrically hydrogen-bonded water molecules, respectively.⁹⁹ In addition, the ~3700 cm⁻¹ sharp band, generally known as the dangling or free O-H band, is ascribed to the uncoupled O-H oscillators that protrude to the air phase, leaving the other halves in water to participate in the hydrogen-bonding network at the interface.¹⁰⁰ Moreover, according to the recent phase study results published by Shen's group, the former two broad bands are opposite in phase, that is, the average polar orientation of O-H oscillators appear at 3155 and 3235 cm⁻¹ using a global restraint spectral fitting routine developed in our lab. The actual fitting results are shown in Table 6.1.

Next, Figure 6.2 presents VSFG spectra of neat Mg^{2+} aqueous solution interfaces at three different concentrations, namely 0.1, 0.3, and 1.5 M. Upon addition of inorganic ions into subphase, the interfacial water structures are deemed to be transformed. These changes have been previously observed not only in bulk, but also at solution interfaces according to vibrational spectroscopic results reported in other studies.^{40,61,102-104} Likewise, the common variations seen in the spectra in Figure 6.2 include a small attenuation of the ~ 3200 cm⁻¹ intensity on the 0.1 and 0.3 M Mg²⁺ aqueous solutions relative to that of neat water, but an equal intensity of the same band on the 1.5 M Mg²⁺ in the same comparison; furthermore, the ~3700 cm⁻¹ band remains unchanged in the course of concentration increase. Most notably, the enhancement of the \sim 3400 cm⁻¹ intensity gradually intensified as the bulk concentration increases from 0.1 to 1.5 M. This particular trend is consistent with previous VSFG spectra of other inorganic salt solutions at the air/liquid interface.¹⁰⁵

Phenomenologically, a slight decrease of the ~ 3200 cm⁻¹ band with commensurate drastic increase of the ~3400 cm⁻¹ band has always been present in both Raman and IR spectra of inorganic salt solutions as the bulk concentration increases.¹⁰²⁻ ^{104,106} Therefore, assigning the \sim 3400 cm⁻¹ band to the hydrogen-bonded water molecules participating in ion solvation shells has been adopted to interpret both bulk and interfacial water structures. Based on the recent molecular dynamics simulation results, Mg^{2+} is capable of extending polar ordering effect on solvating water molecules well beyond the second solvation shell both in bulk and at interface.¹⁰⁷ This may explain why there is such drastic increase of the \sim 3400 cm⁻¹ intensity as the Mg²⁺ concentration increases. On the other hand, spectral position shifts are evident for the \sim 3200 cm⁻¹ and \sim 3400 cm⁻¹ bands that appear to shift in the opposite directions. The 3200 cm⁻¹ band tends to be blue-shifted while the ~3400 cm⁻¹ band being red-shifted. This spectral trend has been previously identified as a band-narrowing effect in highly concentrated Mg²⁺ solutions (> 2.1M).¹⁰³ According to the fitting results presented in Table 6.2, these two bands also share an opposite phase compared to those shown in the neat water spectrum. The detailed band position shifts are noticeable, including a red-shift from 3440 to 3420 cm⁻¹ and a blue-shift from 3160 to 3180 cm⁻¹ with concomitant increases on the bandwidth as Mg^{2+} concentration is varied from 0.1 to 1.5 M.

In addition, interfacial study of water structures on the neat Ca^{2+} solutions at concentrations of 0.1, 0.3, and 1.8 M was also incorporated. Figure 6.3 shows VSFG spectra that correspond to the hydrogen-bonding structures at these Ca²⁺ aqueous solution interfaces. As compared to Mg^{2+} , Ca^{2+} shows less enhancement on the ~3400 cm⁻¹ intensity as the bulk concentration increases, yet the same trends on the ~3200 and ~3700 cm⁻¹ bands. This variation on the \sim 3400 cm⁻¹ intensity between Mg²⁺ and Ca²⁺ spectra may suggest their different ordering effects on the surrounding water molecules in the solvation shells. Since this band corresponds to the water molecules in the primary solvation shells, any change on the cation species has direct consequence on the dipolar ordering effect on water structures. In fact, there are pronounced differences on both ionic radii and the electron affinities between Mg^{2+} and Ca^{2+} . Mg^{2+} has both shorter ionic radius and higher electron affinity than Ca^{2+,71} Taking into account these two physical properties, Mg^{2+} veritably exerts more influence on its solvation shells than Ca^{2+} in terms of the degrees of polar ordering and the numbers of solvation shells. According to the fitting results presented in Table 6.3, the two bands that represent O-H oscillators in the hydrogen-bonding network also share an opposite phase. The detailed band position shifts include a red-shift from 3450 to 3425 cm⁻¹ and a blue-shift from 3175 to 3200 cm⁻¹ with similar accompanying increases on the bandwidth as compared to those of the Mg²⁺ spectra.

The interfacial hydrogen-bonding network is susceptible to perturbations. According to the findings by Miranda et al., considerable disruption occurs to the hydrogen-bonding network of the interfacial water molecules underneath a neutral fatty acid layer; but a charged surface, as a result of the dissociation of fatty acid headgroups at high pH (\geq 7.0), reestablishes the hydrogen-bonding network due to aligning effect of the surface field on the interfacial water molecules.⁶⁵ Accordingly, Figure 6.4 shows ssp VSFG spectrum of the PA Langmuir monolayer covered water surface at a neutral pH (6.0). In detail, four vibrational bands with enhanced intensities are observed across the spectrum. In the order of increasing frequency, these bands are separately located at \sim 2940, \sim 3200, \sim 3450, and \sim 3600 cm⁻¹. These spectral features are in agreement with the reported VSFG spectrum obtained by Miranda. First, the appearance of the ~2940 cm⁻¹ $(v_{FR}CH_{3})$ affirms the presence PA monolayer. Second, in comparison, the two bands with intermediate frequencies are identical with those appeared in the neat water spectrum at similar frequencies; therefore, they are the same O-H oscillator modes that are associated with the interfacial hydrogen-bonded water molecules. Last, the ~ 3600 cm⁻¹ band is relatively strong in intensity. This mode has been previously ascribed to the hydrogenbonded OH oscillators that exist uniquely between surface water molecules and the PA headgroups.^{2,65} The detailed fitted results are presented in Table 6.4.

Considering the overall intensity enhancement in the spectrum as shown in Figure 6.4 relative to the neat water spectrum, surface field effect could best explain the ordering of hydrogen-bonded water structures at interface. At pH 6.0, the majority of PA headgroups is protonated at the surface according to the reported surface pKa (~8.7) of long-chian fatty acids. This value has been confirmed based on the spectral data presented in v_s COO⁻ in this study. However, a small degree of deprotonation still exists according to the small reduction of vC-O and vC=O intensities as pH increases from ~2.0

to 6.0. Because of the presence of small negative charges as the result of dissociations of PA headgroups, the surface field effect is pronounced, as revealed by the enhanced spectral response from the PA and water interface in relation to the neat water surface.

Yet, ionic perturbations on the interfacial hydrogen-bonding network are apparent in the presence of ionic species in the bulk. Figure 6.5 exhibits ssp VSFG spectra of PA monolayers spread over Mg^{2+} solution surfaces at four different bulk concentrations (0.1, 0.3, 1.5, and 2.6 M). The fitted results are shown in Table 6.5. The most pronounced effect with respect to the spectrum of PA on the water surface is the overall decrease of band intensities as the bulk concentration is increased from 0.1 to 1.5 M, and then is followed by the reemergence of both \sim 3200 and \sim 3400 cm⁻¹ bands at 2.6 M. Even though intensity attenuation is not overwhelmingly significant at 0.1 M, the reductions of \sim 3200 and \sim 3600 cm⁻¹ intensities are noticeable, and more so at 0.3 and 1.5 M with additional decrease of the \sim 3450 cm⁻¹ band. Considering the \sim 3600 cm⁻¹ band alone, its progressive intensity attenuation during the concentration increase is consistent with the spectral evidence presented in the other spectral regions ($\nu C=O$ and $\nu_s COO$). This trend once again affirms the basic rule that cation binding to the carboxylic headgroup directly correlates to the cation concentration in the bulk.² Even though this mechanism is insignificant at low concentrations, at higher concentrations, it can become much more enhanced. Based on the obtained spectral evidence, Mg²⁺ does bind to COO⁻ but the binding strength is relatively weak. At 0.1 M, the degree of deprotonation of PA headgroups is insignificant due to weak binding strength and insufficient numbers of Mg^{2+} ; but, at a higher concentration of Mg^{2+} (0.3M), a small population of chelating ionic

complexes starts to evolve. As a consequence, the 3600 cm^{-1} band starts to attenuate as a sign of decreasing numbers of protonated headgroups. As a general rule, a decrease of the ~3200 cm⁻¹ band may reflect a possible disruption of the symmetrical hydrogenbonding network, and an increase of ~3400 cm⁻¹ band may reveal an augment of the asymmetrically hydrogen-bonded, or solvation shell, water structures. Therefore, the reduction of the ~3200 cm⁻¹ band as shown in Figure 6.5 can be attributed to the disruption of the symmetrically hydrogen-bonding network. However, the ~3450 cm⁻¹ band does not faithfully obey the similar trend demonstrated by the ~3200 cm⁻¹ band. At first, there is no apparent reduction of this intensity at 0.1 M Mg²⁺ in comparison to Figure 6.4. Then, significant reductions take place at 0.3 and 1.5 M, and later being followed by the reappearance of its intensity as equally strong as that of the neat Mg²⁺ solution at 1.5 M. To correctly interpret these unique physical trends observed in the spectra, the underlying ionic binding mechanism is considered to be the primary factor dictating the spectral outcomes as seen.

In principle, cation binding to COO⁻ has an equivalent effect of surface charge neutralization; therefore, more binding events tend to produce more of this effect. In consequence, this de-charging mechanism directly affects the signal response of the \sim 3200 and \sim 3450 cm⁻¹ bands from the hydrogen-bonding network; in particular, the most pronounced effect is the attenuations of band intensities as the zero-point-charge (ZPC) is approached at the interface. For instance, the same effect has been separately demonstrated in studies of fatty acid salt adsorption on CaF₂ surface and hydrophobic adsorption on a modified silica surface at the liquid-solid interface by Richmond and

Shen, respectively.¹⁰⁸⁻¹¹⁰ Hence, it is consistent to observe relatively small reduction of the \sim 3450 cm⁻¹ band at 0.1 M Mg²⁺, and yet large reductions at 0.3 and 1.5 M with respect to the spectrum obtained from the PA monolayer on the water surface. Inherently, Mg²⁺ favors strong solvation shells, and thus it is energetically unfavorable to remove its solvation shells before making a bound complex with COO⁻, as is proved by the ab initio calculation results.¹⁰⁷ At 0.1 M, the formation of bound ionic complexes (Mg²⁺:COO⁻) is unlikely, in which case surface charge neutralization is insignificant because only a small fraction of Mg^{2+} is interacting with COO, and furthermore, the majority of these interacting Mg^{2+} has its solvation shell intact. As a result, the overall spectral intensity, especially the \sim 3450 cm⁻¹ band, remains as strong as that of the spectrum corresponding to the PA monolayer on water owing to the surface field effect. Nevertheless, the progressive reductions of the overall spectral intensity at 0.3 and 1.5 M are indicative of surface charge neutralization as formation of chelating ionic complexes is considerably accentuated, as evidenced by the significant increase of the $v_s COO^{-1}$ intensity at 1475 cm⁻¹ at 1.5 M Mg²⁺. More importantly, the noticeable resurgence of the \sim 3375 cm⁻¹ band at 1.5 M Mg²⁺ clearly marks a critical transition in the interfacial water structures, at which spectral characteristics in the neat Mg²⁺ solution interface start to emerge in the hydrogen-bonding continuum $(3000 - 3600 \text{ cm}^{-1})$. By implication, we can postulate that at this transition concentration, the primary contributions to the hydrogenbonding network consist of water molecules participating in the solvation shells of inorganic ions and the un-dissociated PA headgroups.

As surface charge neutralization surpasses the PZC, the reversal trend is in effect. At 2.6 M, the \sim 3600 cm⁻¹ band becomes significantly reduced compared to that of lower concentrations. Such a reduced intensity is accompanied by an increasing presence of bound ionic complexes at the interface since this band signifies the presence of After surface charge neutralization as a result of forming unprotonated headgroups. bound ionic complexes in the presence of excess Mg^{2+} , the interfacial hydrogen-bonded water molecules reorganize and revert to the hydrogen-bonding structure as the one shown in the neat Mg^{2+} solution interface. This suggests that the water structures underneath the PA Langmuir monolayer at concentrated conditions are exhibiting the similar hydrogen-bonding network as those in neat systems. The only exception is that the dangling O-H band completely disappears while being replaced by the solvated carboxylic O-H band. Unlike those similar studies at the solid/liquid interface, the reversal effect is not in terms of recharging the surface.^{109,110} If that is the case, much more enhanced spectral intensity is deemed to appear across the spectrum as that in Figure 6.4. Therefore, the unique physical picture can be described as the interfacial hydrogen-bonded water molecules surrounding the bound PA headgroups retain almost original hydrogen-bonding structure as appeared in the neat Mg²⁺ solution interfaces when the majority of the headgroups becomes bound with Mg^{2+} . With respect to Table 6.2, the fitted results shown in Table 6.5 also support this conclusion. Moreover, with the presence of $v_{FR}CH_3$ band, any dissolution possibility can be validly ruled out.

Similarly, Ca²⁺ was also interrogated in the hydrogen-bonding region for a direct comparison. Figure 6.6 depicts ssp VSFG spectra of the PA monolayers spread over Ca²⁺

solution surfaces with corresponding bulk concentrations at 0.1, 0.3, and 1.8M. According to Figure 6.6, it is evident that the overall signal strength is much weaker relative to that in Figure 6.5 at low concentrations (0.1 and 0.3 M). Likewise, at a concentrated condition (1.8 M), a spectrum similar to that of neat Ca^{2+} also reemerges, except the disappearance of dangling O-H band. By comparison, this is consistent with the spectral data exhibited in Figure 6.5. To this end, we could imply that Ca^{2+} and Mg^{2+} tend to behave similarly when considering their influence on the interfacial hydrogenbonding network at high concentrations. More or less they share some common points. But the most distinct variation points to the complete disappearance of the \sim 3600 cm⁻¹ band at 1.8 M. This is indicative of complete deprotonation that results in the PA headgroups due to Ca^{2+} binding, which is much stronger than Mg^{2+} . In general, this deprotonation effect also appears as a function of Ca^{2+} concentration since the degrees of deprotonation are progressively enhanced as Ca²⁺ concentration increases, as evidenced in the spectra. On the other hand, the disappearance of $\sim 3600 \text{ cm}^{-1}$ band also agrees consistently with the spectral evidence presented in $v_s COO^-$ and vC=O spectral regions. Collectively, they manifest a complete deprotonation of the PA headgroups due to the formation of bound ionic complexes. Taking into account the drastic attenuation of the \sim 3200 and \sim 3450 cm⁻¹ bands in the spectra associated with 0.1 and 0.3 M Ca²⁺, strong binding affinity of Ca^{2+} to COO^{-} inclines to neutralize the surface charge more efficiently. For instance, this effect already becomes dominant only at 0.3 M Ca^{2+} as compared to that of 1.5 M Mg^{2+} . Thus, it supports the notion that Ca^{2+} interacts much more strongly with COO^{-} than Mg^{2+} at the air/liquid interface. This may also explain why

 Mg^{2+} and Ca^{2+} compression isotherms are distinctively different in terms of charge screening ability of cations considered. For reference, detailed fitted results are shown in Table 6.6. In summary, Ca^{2+} has stronger binding affinity towards COO^{-} than Mg^{2+} so that it is capable of neutralizing surface charge at a much lower concentration than Mg^{2+} . This is further evidenced by spectral results that obey surface charge neutralization effects on the hydrogen-bonding network at the air/liquid interface.

6.4 Conclusions

In this study, we investigated the interfacial hydrogen-bonding network that uniquely exist in between the PA Langmuir monolayer and the underneath surface water molecules, and more importantly, we identified that cation binding of Mg^{2+} and Ca^{2+} has considerable impacts on this hydrogen-bonding network. At first, a significant enhancement of the overall spectral intensity appeared on the spectrum that represents the interface consisting of the PA monolayer and the surface water molecules. This effect reveals the polar ordering of the interfacial water molecules under the influence of the surface field of the dissociated PA headgroups. We conclude that only a small fraction of negative charges can induce considerable polar ordering in the surface water molecules. On the other hand, we found Ca^{2+} has greater impact on the interfacial hydrogen-bonding network than Mg^{2+} on the basis that Ca^{2+} has much greater binding affinity towards the carboxylate group relative to Mg^{2+} . Therefore, the transition point at which surface water structures reorganize occurs at a much lower concentration for Ca^{2+} as compared with Mg^{2+} . More importantly, at concentrated conditions, the already disrupted hydrogen-

bonding network reorganizes and reverts to its original hydrogen-bonding network as appeared at the neat solution interface. As a final note, an in-depth understanding of the dynamic natures of the interfacial hydrogen-bonding network that exists beneath the PA Langmuir monolayer in the presence divalent cations such as Mg²⁺ and Ca²⁺ is of great importance in making predictions regarding surface phenomena that are commonly found at both physical and biological interfaces.


Figure 6.1. ssp VSFG spectrum of neat water at 23°C in O-H stretching region. Solid curve represents the fitted spectrum. Three component bands are depicted as gray solid curves.

| | Fitting Parameters | Symmetric Hydrogen- bonding OH | Asymmetric Hydrogen- bonding OH | Dangling OH |
|------------|-----------------------------------|--------------------------------------|---------------------------------------|----------------|
| | A_{ssp} | 28.5 | -40.0 | -10.8 |
| Neat Water | ω_{IR} (cm ⁻¹) | 3155 | 3435 | 3702 |
| | Γ (cm ⁻¹) | 85 | 105 | 20 |

Table 6.1. Fitting results for the ssp VSFG spectrum of neat water at 23° C in O-H stretching region.



Figure 6.2. ssp VSFG spectra of the neat $MgCl_2$ solutions (0.1, 0.3, and 1.5 M) at $23^{\circ}C$ in O-H stretching region. Solid curves represent the fitted specta.

| Mg ²⁺ (aq) | Fitting Parameters | Symmetric Hydrogen- bonding OH | Asymmetric Hydrogen- bonding OH | Dangling OH |
|-----------------------|-----------------------------------|--------------------------------------|---------------------------------------|----------------|
| 0.1 M | A _{ssp} | 23.5 | -35.0 | -8.9 |
| | ω_{IR} (cm ⁻¹) | 3160 | 3440 | 3702 |
| | Γ (cm ⁻¹) | 85 | 90 | 18 |
| 0.3 M | A_{ssp} | 22.5 | -39.8 | -8.9 |
| | $\omega_{\rm IR} (\rm cm^{-1})$ | 3170 | 3430 | 3702 |
| | Γ (cm ⁻¹) | 85 | 90 | 18 |
| 1.5 M | A_{ssp} | 34.0 | -56.0 | -9.0 |
| | $\omega_{\rm IR} (\rm cm^{-1})$ | 3180 | 3420 | 3702 |
| | Γ (cm ⁻¹) | 110 | 100 | 18 |

Table 6.2. Fitting results for the ssp VSFG spectra of the neat $MgCl_2$ solutions (0.1, 0.3, and 1.5 M) at 23°C in O-H stretching region.



Figure 6.3. ssp VSFG spectra of the neat $CaCl_2$ solutions (0.1, 0.3, and 1.8 M) at 23°C in O-H stretching region. Solid curves represent the fitted specta.

| Ca ²⁺ (aq) | Fitting Parameters | Symmetric Hydrogen- bonding OH | Asymmetric Hydrogen- bonding OH | Solvated Carboxylic OH |
|-----------------------|-----------------------------------|--------------------------------------|---------------------------------------|------------------------------|
| 0.1 M | A_{ssp} | 22.5 | -32.0 | -9.2 |
| | ω_{IR} (cm ⁻¹) | 3175 | 3450 | 3700 |
| | Γ (cm ⁻¹) | 85 | 85 | 18 |
| 0.3 M | A_{ssp} | 23.5 | -32.8 | -9.7 |
| | $\omega_{\rm IR} (\rm cm^{-1})$ | 3175 | 3450 | 3700 |
| | Γ (cm ⁻¹) | 85 | 85 | 18 |
| 1.8 M | $\mathbf{A}_{\mathrm{ssp}}$ | 29.0 | -40.5 | -9.7 |
| | $\omega_{\rm IR} (\rm cm^{-1})$ | 3200 | 3425 | 3700 |
| | Γ (cm ⁻¹) | 100 | 90 | 18 |

Table 6.3. Fitting results for the ssp VSFG spectra of the neat $CaCl_2$ solutions (0.1, 0.3, and 1.8 M) at 23°C in O-H stretching region.



Figure 6.4. ssp VSFG spectrum of the PA monolayer at equilibrium spreading pressure (ESP) on water at 23°C in O-H stretching region. Solid curve represents the fitted spectrum. Four component bands are depicted as gray solid curves.

| | Fitting Parameters | v _{FR} CH ₃ | Symmetric Hydrogen- bonding OH | Asymmetric Hydrogen- bonding OH | Solvated Carboxylic OH |
|-----------------------|------------------------------|---------------------------------|--------------------------------------|---------------------------------------|------------------------------|
| | A _{ssp} | 6.2 | 61.5 | -61.0 | -33.0 |
| PA - H ₂ O | $\omega_{IR} (cm^{-1})$ | 2940 | 3185 | 3470 | 3612 |
| | Γ (cm ⁻¹) | 10 | 105 | 110 | 60 |

Table 6.4. Fitted results for the ssp VSFG spectrum of the PA monolayer at equilibrium spreading pressure (ESP) on water at 23° C in O-H stretching region.



Figure 6.5. ssp VSFG spectra of the PA monolayers at equilibrium spreading pressure (ESP) on MgCl₂ solutions (0.1, 0.3, 1.5, and 2.6 M) at 23° C in O-H stretching region. Solid curves represent the fitted spectra.

| Mg ²⁺ (aq) | Fitting Parameters | Symmetric Hydrogen- bonding OH | Asymmetric Hydrogen- bonding OH | Solvated Carboxylic OH |
|-----------------------|----------------------------------|--------------------------------------|---------------------------------------|------------------------------|
| | A_{ssp} | -20 | -75.5 | -20.5 |
| 0.1 M | $\omega_{\rm IR} (\rm cm^{-1})$ | 3300 | 3455 | 3590 |
| | Γ (cm ⁻¹) | 110 | 115 | 65 |
| 0.3 M | A _{ssp} | -8 | -42 | -23 |
| | $\omega_{\rm IR} (\rm cm^{-1})$ | 3300 | 3475 | 3590 |
| | Γ (cm ⁻¹) | 110 | 115 | 65 |
| 1.5 M | A_{ssp} | 9 | -36 | -26 |
| | $\omega_{\rm IR} (\rm cm^{-1})$ | 3250 | 3375 | 3590 |
| | Γ (cm ⁻¹) | 110 | 110 | 85 |
| 2.6 M | A _{ssp} | 42 | -47.7 | -19 |
| | $\omega_{\rm IR} (\rm cm^{-1})$ | 3160 | 3380 | 3610 |
| | Γ (cm ⁻¹) | 110 | 85 | 80 |

Table 6.5. Fitted results for the ssp VSFG spectra of the PA monolayers at equilibrium spreading pressure (ESP) on MgCl₂ solutions (0.1, 0.3, 1.5, and 2.6 M) at 23°C in O-H stretching region.



Figure 6.6. ssp VSFG spectra of the PA monolayers at equilibrium spreading pressure (ESP) on CaCl₂ solutions (0.1, 0.3, and 1.8M) at 23° C in O-H stretching region. Solid curves represent the fitted spectra.

| Ca ²⁺ (aq) | Fitting Parameters | Symmetric Hydrogen- bonding OH | Asymmetric Hydrogen- bonding OH | Solvated Carboxylic OH |
|-----------------------|-----------------------------------|--------------------------------------|---------------------------------------|------------------------------|
| | A _{ssp} | -5 | -30 | -24 |
| 0.1 M | ω_{IR} (cm ⁻¹) | 3300 | 3465 | 3590 |
| | Γ (cm ⁻¹) | 110 | 115 | 80 |
| 0.3 M | A_{ssp} | -10.00 | -21 | -20 |
| | ω_{IR} (cm ⁻¹) | 3300 | 3420 | 3608 |
| | Γ (cm ⁻¹) | 110 | 115 | 80 |
| 1.8 M | A _{ssp} | 53 | -49 | |
| | ω_{IR} (cm ⁻¹) | 3180 | 3418 | |
| | Γ (cm ⁻¹) | 110 | 85 | |

Table 6.6. Fitted results for the ssp VSFG spectra of the PA monolayers at equilibrium spreading pressure (ESP) on $CaCl_2$ solutions (0.1, 0.3, and 1.8 M) at 23°C in O-H stretching region.

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