Chemical and Physical Changes Unique in and on Water: Implications for Life and Its Origins

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CHEMICAL AND PHYSICAL CHANGES UNIQUE IN AND ON WATER:
IMPLICATIONS FOR LIFE AND ITS ORIGINS

by

ELIZABETH C. GRIFFITH

B.S., University of Maryland Baltimore County, 2009

A thesis submitted to the
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Implications for Life and Its Origins

written by Elizabeth C. Griffith

has been approved for the Department of Chemistry and Biochemistry

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Veronica Vaida

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Joel D. Eaves

Date_____________________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
In this thesis, the unique environment provided by water and its surface is exploited to aid in answering some of the questions surrounding life and its origins. First, the photochemistry of pyruvic acid, a molecule important in modern metabolism and present throughout the modern atmosphere, is explored in the gas phase, atmospheric aerosol particles, and into aqueous solution, illustrating the sensitivity of chemical reactions to environmental conditions. Then, this chemistry is extended to the photochemistry of a single-tailed surfactant of similar chemical functionality, 2-oxooctanoic acid, producing a double-tailed surfactant abiotically followed by spontaneous self-assembly into stable vesicles. This yields a prebiotically plausible synthesis of a primitive enclosure and provides a contribution to the evolution of early membranes in the origin of life.

Further, the water surface is shown to be an advantageous environment for unique chemistry beyond what is available in bulk aqueous solution by selectively concentrating, aligning, and altering the ionization state of useful reactants. In this thesis the ionization state of L-phenylalanine, a natural amino acid, is examined in situ spectroscopically in the surface region, illustrating the change in ionization state at the surface compared with bulk aqueous solution. Further, abiotic peptide bond formation is demonstrated exclusively at the water surface and observed in situ. Condensation reactions such as peptide bond formation are unlikely in bulk aqueous solution on both thermodynamic and kinetic grounds, yet are necessary in many biopolymers essential to modern life. The water surface provides a unique environment for this chemistry.
Finally, mixed surfactant films of differing hydrophobic structure at the water surface are investigated in this thesis. The natural environment is quite complex, stemming from the multifaceted emissions from both biogenic and anthropogenic sources. The specific interactions of water-soluble aromatics (L-phenylalanine, benzoic acid, benzaldehyde) on a stearic acid monolayer film at the air – water interface are studied here relevant to the surfaces of atmospheric aerosol particles and their potential influence on climate. Finally, the same methods are used to explore the interactions of a perturbant (L-phenylalanine) on a model cell membrane (DPPC), contributing to an understanding of the mechanism of disease promoted by the presence of aromatics.
This thesis is dedicated to

my partner in life,

my husband,

Chad Griffith.

"The task is not so much to see what no one has seen, but to think what nobody has yet thought, about that which everybody sees." --- Erwin Schrödinger

"Homo liber nulla de re minus quam de morte cogitat; et ejus sapientia non mortis sed vitae meditatio est." --- SPINOZA's Ethics, Pt. IV, Prop. 67
(There is nothing over which a free man ponders less than death; his wisdom is, to meditate not on death but on life)
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1 Introduction\textsuperscript{1}

The origin of life on Earth remains a scientifically and philosophically intriguing problem. For millennia cultures across the globe have searched for an explanation of the uniqueness of the living state, striving for a deeper understanding of its workings and creating myths and legends to describe its origin. In modern times, the scientific quest to understand life has made great strides; however many key questions remain unanswered. One such key question is how to cross the threshold between a diverse “prebiotic soup” of small, non-living simple molecules and a quite narrow yet complex suite of living organisms seen in modern biology. My thesis work contributes to the search for answers to this particular question, a question answered here with fundamental physical chemistry. Along the way, contributions are made in this thesis to other areas of scientific research (i.e. modern atmospheric chemistry and even chemical biology) using the same techniques and even the same systems that are of interest to origin of life chemistry.

This thesis explores the unique chemical and physical changes possible in aqueous environments in the emergence of increasingly complex structures. Aqueous environments were not only prevalent on the water-rich early Earth, but are the host environment of modern life. In addition, water provides a very special reaction environment [1-4], even having the ability to markedly change a reaction pathway [5-8]. Specifically water-air interfaces (namely the surfaces of lakes, oceans and atmospheric aerosols) are of particular interest due to their advantageous environment for many biophysical processes that are inaccessible in bulk aqueous environments. Amongst these water surfaces, the surfaces of atmospheric aerosol particles (globally distributed

liquid or solid suspensions in air with a broad, power law size distribution) have a global surface area of 1 – 2 orders of magnitude greater than all other water surfaces on Earth today, causing them to be especially advantageous environments for chemistry.

Measurements of the composition of aerosols in the Earth’s contemporary atmosphere at different altitudes and locations have revealed that aerosols consist of rich mixtures of molecular components [9]. Interestingly, a comparison of the periodic table of elements found in aerosols bears a striking resemblance to the periodic table of elements necessary for life [10, 11]. An important finding of these measurements is the large organic content of aqueous aerosols [9, 12]. While the detailed physical and chemical environment 3 - 4 billion years ago is obviously uncertain, some general statements can be made. Then as now, the planet was rotating about a tilted axis with the resulting thermal and pressure gradients producing winds above a liquid ocean. Wind action at the ocean surface results in whitecaps producing aerosols at the air-water interface (illustrated in Fig. 1.1), which would have offered numerous opportunities for natural selection to operate on a vast recycling population of aerosol reaction vessels. Through this mechanism, aerosols are formed having entrained hydrophobic organic material from the ocean surface. Aerosols encapsulated in organic films would have possessed many advantages in providing the sites at which the earliest assemblages of molecules emerged that eventually became life: a role for the ocean surface, aerosols and bubbles having been suggested [13-20].
Figure 1.1: Ocean-atmosphere interaction by atmospheric aerosol (dark grey areas represent surface-active organics, lighter grey represents the aqueous core of the particle). Highlighted are some of the processes affecting aerosols during their atmospheric lifetime.

The distribution of sizes of nascent aerosols is determined on Earth by the competition between their coagulation and possible division and the gravitational deposition of the larger particles, leading to a median aerosol diameter of a few microns. The analogy between the size and structure of atmospheric aerosols and single cell bacteria has been pointed out by Dobson et al. [15]. The air-sea interface would have acted as a two-dimensional concentrator and integrator of the three-dimensional chemistry of the atmosphere and ocean, the meteoric infall and the output from hydrothermal vents on the sea floor, in addition to being the primary generator and recycler of the aerosols and their chemical content. Emerging biomolecules would have found these vast populations of micron and submicron sized particles to be ready-made, adventitious containers.
As a result, organic molecules would have been exposed to a widely varying and fluctuating set of conditions of solar irradiation, temperature, pressure and humidity during the host aerosol’s atmospheric cycle.

Concentration, alignment, and orientation at the surface of water of organic molecules are possible in these environments. Models of the origin of life assume that the chemical monomeric precursors (amino acids, lipids, sugars, purine and pyrimidine bases, phosphates) for biopolymers existed on Earth. These could be chemically synthesized endogenously [21-26] or generated exogenously [27-30] and transported to Earth. Increased efficiency of production of protein-forming amino acids as well as purines and dihydroxy compounds in a Urey-Miller experiment has been demonstrated when aerosols were present [31, 32]. Note that endogenous, chemical synthesis of biomolecules could not have avoided a role for aerosols generated at the Earth’s sea surface from the very start.

The progression from simple chemical monomers to complex biomolecules and biopolymers is an essential step in the origin of life. The synthesis of such necessary biomolecules involves numerous condensation reactions [33], two examples given in Figure 1.2, resulting in both thermodynamic and kinetic challenges in the absence of enzymes in a bulk aqueous environment [15].

![Figure 1.2: Condensation reactions responsible for (a) peptide bond formation and (b) formation of the phosphodiester bond.](image-url)
Aqueous solutions are the preferred reaction media for current biology, and on early Earth, the bulk ocean would have been the environment holding relevant monomers. In such aqueous solutions, the reactions between amino acids to form peptides, and between nucleotides to form nucleic acids, are extremely unfavorable. Accordingly, attempts to synthesize peptides under prebiotic conditions (in the absence of an enzyme) have traditionally only been successful when the reaction is conducted in anhydrous environments [34]. These thermodynamic constraints are alleviated if reaction occurs at the surface of the ocean or on atmospheric aerosols where a water poor environment exists. These reactions also face kinetic constraints in a dilute aqueous environment like the early ocean. Molecules at the surface are known to change ionization state compared with those molecules residing in the bulk [35-37] thereby potentially minimizing unfavorable zwitterion formation of biomolecular monomers. The change in ionization state of surface-residing amino acid molecules (specifically L-phenylalanine) relative to the bulk is demonstrated in Chapter 7 of this thesis, utilizing a molecule that contains dual functionality, having both an acidic and basic group, allowing for sampling of a wide range of ionization states spanning the entire pH range. Environmental interfaces like the ocean surface and the surface of atmospheric aerosols therefore, are demonstrated in this thesis to have the ability to align and concentrate these monomers in addition to altering the polar group ionization states, helping to alleviate both the kinetic and thermodynamic constraints facing polymerization reactions.

As early as 1948, it was known that long-chain amino acid esters spontaneously condense at the water-air interface forming peptides [38]. Many have since confirmed that the water-air interface provides a favorable environment for condensation chemistry [39-41]. Oliver has shown using proton NMR, that amide bond formation occurs preferentially at the interface with kinetic enhancement through surface compression [40, 42]. Using octadecyl esters of amino
acids, Fukuda has also shown with infrared spectra of collected films that polycondensation may be achieved at the air-water interface [43]. Through shifts in the amide I band, they were able to further identify the secondary structure of the polypeptide formed (β-sheet, α-helical, random coil) [44]. In addition, Fukuda showed kinetic enhancement of the polycondensation with the presence of a metal cation (Cu$^{2+}$) in the solution beneath the film [45]. Although the length of the polymers formed has since been disputed [46], the usage of the water surface as a favorable environment of the formation of peptide bonds has been demonstrated. This is a crucial step on the way to biomolecular complexity.

Although the work described above has demonstrated the possibility of peptide bond synthesis at an interface, the techniques used to identify the products formed are not ideal. In all of these studies, the products formed at the surface were collected from the surface and dried to allow for analysis and characterization: the process of drying amino acid solutions onto a substrate could have prompted the formation of peptide bonds. Without an in situ technique, it is impossible to know how much of the polycondensation actually occurred at the interface. Also, there is some question as to the prebiotic relevance of the long chain amino acid esters used in these experiments. They are complex synthetic molecules not found in modern biochemistry, and are not likely to have been abundant on early Earth. The use of more simple, naturally occurring amino acids is necessary to test a plausible origin of life scenario where an interface prompts the emergence of complex biomolecules. This is exactly what is presented in Chapter 8 of this thesis. Utilizing the knowledge gained from the work described above, a short ethyl ester of the amino acid L-leucine was utilized (a water soluble, prebiotically plausible molecule) in conjunction with Cu$^{2+}$ catalysis to promote peptide bond formation specifically at the water surface [47]. In addition, this chemistry was observed in situ using Infrared Reflection-
Absorption Spectroscopy (IRRAS), an instrument that I constructed in the Vaida lab, confirming that this chemistry did indeed occur at the water surface.

In addition to biopolymers, another component necessary for life is an enclosure; containment and organization are central to prebiotic formation, evolution and division of primitive cells. Different models have been proposed for the origin of biological systems [34, 48-53] all of which recognized the need for enclosures within which living systems may have originated [54, 55]. Some studies of prebiotic synthesis yielded lipid-like amphiphilic molecules, long chain hydrocarbons and their derivatives [21, 22, 56]. Elegant work has been reported in the literature regarding the self-assembly and functioning of primitive enclosures, and has shown that the amphiphilic monomers necessary for their self-assembly can be produced endogenously in different environments on ancient Earth [23-26] or transported to Earth through meteoritic and cometary infall [30, 57-60]. The organization of these primitive enclosures is driven by hydrophobic interactions amongst the composite amphiphiles [61, 62]. It is important to note that the sea surface will be the place where both endogenous and exogenous fluxes of prebiotic molecules will accumulate and intermingle, and will furthermore be incorporated into the vast populations of aerosols that are produced through breaking waves (whitecaps). The amphiphiles will not only be concentrated at the sea surface, but also on the surfaces of the aerosols, which can coagulate and divide [16, 17, 63].

Once generated, the synthetic compartments have been found to have many properties analogous to those known to be necessary for the functions of modern life [64-66]. Szostak et al. have shown that when a vesicle is suspended in solution and fatty acid micelles are added, the vesicles spontaneously grow and divide [67]. If a primitive genetic material was present in the vesicle before division, it remained encapsulated during the process [67]. Selective permeability
is important; modern cells use pumps and channels to control diffusion across their membranes, but such machinery would not have been present prebiotically [68]. Without such complex machinery, the double-tailed lipids composing modern cells would not allow for transport into and out of the cell due to the rigidity and stability of the membrane formed – a significant problem for primitive cells, as such exchange is essential [69]. These enclosures are typically composed of single-tailed lipids like fatty acids, forming less stable but more dynamic vesicles, thereby accomplishing many of the functions of a modern cell without any of the complex machinery [68, 69].

In order for these enclosures to have formed in the bulk ocean, equilibrium between the lipids in solution and in the membrane is required, necessitating a very high concentration of monomer [70, 71] (near the critical aggregate concentration), an unlikely scenario on ancient Earth. Without such a high lipid concentration, the protocells will simply disassemble [71]. The work detailed in Chapter 6 of this thesis provides an alternative to this traditional protocell assembly. I show the photochemical synthesis of a double-tailed surfactant from a short (8-carbon long), prebiotically plausible single-tailed surfactant followed by its spontaneous self-assembly into stable vesicles. This result is many faceted, illustrating the advantageous environment provided by the water surface region for concentration and alignment of monomers as well as utilizing the abundant energy-source provided by the early sun for chemistry. As discussed in more detail in Chapter 6 and Appendix B, this chemistry also evades the need for high bulk monomer concentration due to the high local concentration of the water surface region coupled with the utilization of double-tailed surfactants in the resultant vesicular aggregate structure.
Sometimes work performed with the intent of better understanding the origin of life may also shed light on processes important in modern life. For example, the peptide bond formation presented in Chapter 8 may contribute to a better understanding of modern ribosomal peptide bond formation through a deeper knowledge of the reaction conditions necessary for such chemistry to occur in general (thereby hinting at the mechanistic functioning of enzymes). This is also the case with the photochemistry of pyruvic acid presented in Chapters 3 – 5 as pyruvic acid is a prevalent molecule in the modern atmosphere. Although the composition of the atmosphere may have been different before the emergence of life, many of the atmospheric processes, like those shown in Fig. 1.1, would have been the same on the early Earth. Regardless, surface phenomena are widely applicable throughout the ancient and modern atmospheres and even into modern biological processes. Finally, two-dimensional surfaces can be used as proxies for the many three-dimensional surfaces found in the natural environment (the surface of atmospheric aerosol particles, as in Chapters 9 and 10, or biological membranes as in Chapter 11), a fact that is widely used in this thesis.

In summary, this thesis is structured in the following way: Chapter 2 details experimental and theoretical methods used throughout my doctoral work. Chapters 3 – 5 present experimental studies on the photochemistry of pyruvic acid, moving from the influence of environment on the gas phase photochemistry in Chapter 3, through a detailed mechanistic study of the aqueous photochemistry in Chapter 4, and finally with a discussion of the prebiotic implications of the promiscuous photochemistry of pyruvic acid in general in Chapter 5. Then, using the mechanistic understanding of the photochemistry of pyruvic acid in aqueous solution, the same chemistry is applied in the photo-initiated synthesis of a vesicle-forming double-tailed surfactant from the single-tailed surfactant 2-oxooctanoic acid in Chapter 6, now introducing some
advantageous features of the water surface region. The unique properties of the air-aqueous interface are further explored in the remaining chapters. Chapter 7 presents the change in ionization state of the amino acid L-phenylalanine in the water surface region compared with the bulk aqueous phase. Chapter 8 illustrates in situ detection of abiotic peptide bond formation at the water surface – chemistry that only occurs due to the advantageous environment provided by the air-water interface. Finally Chapters 9 – 11 concentrate on morphological changes at the water surface, specifically addressing the effect of aromatics on aliphatic films (L-phenylalanine with stearic acid in Chapter 9, benzoic acid and benzaldehyde with stearic acid in Chapter 10, and L-phenylalanine with DPPC (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine), a double-tailed surfactant common in biological membranes, in Chapter 11). All of the work presented in this thesis illustrates the unique physical and chemical processes available in aqueous environments with their specific implications for life, both modern and ancient.

1.1 References for Chapter 1


2 Experimental and Theoretical Methods

2.1 Introduction
The primary experimental techniques used in this work are surface-sensitive methods allowing for a unique exploration and characterization of processes occurring in the surface region. The Langmuir trough allows for monitoring of surface thermodynamics through probing changes in surface tension. Infrared Reflection-Absorption Spectroscopy (IRRAS) yields chemical signatures of molecules residing in the surface region from their vibrational spectra. Brewster Angle Microscopy (performed in Heather Allen’s group at The Ohio State University) allows for direct visualization of micro-domains at the water surface, allowing for tracking of morphological changes. I also performed Molecular Dynamics (MD) simulations under the supervision of Martina Roeselova (Czech Academy of Sciences) to yield a molecular level picture of the complex processes occurring at and near the water surface, giving insight into specific interactions driving the changes observed experimentally. Finally, a suite of spectroscopic and other analytical techniques were used to further identify specific reaction products and structures formed in this work and will be discussed only briefly here.

2.2 Surface – Sensitive Techniques

2.2.1 Langmuir Trough
A schematic of the Langmuir trough apparatus is shown below in Figure 2.1. The Langmuir trough is equipped with two computer-controlled mechanical barriers to control surface area and a Wilhelmy balance to monitor changes in surface pressure. Both of these are connected to a control box (from KSV-NIMA) that then connects to a user-controlled computer interface.
When molecules are present in the surface region, movement of the mechanical barriers results in restriction of the surface area available. This forces increased contact between molecules residing at the surface, as seen in Figure 2.2, resulting in two-dimensional phase changes detectable in the $\pi - A$ (surface pressure – molecular area) isotherm that is produced.
Figure 2.2: Illustration of compression of the mechanical barriers resulting in a two-dimensional phase transition of surfactants at the water surface

The quantity measured in Langmuir trough experiments is surface pressure ($\pi$),

$$\pi = \gamma_o - \gamma$$  \hspace{1cm} (2.1)

where $\gamma_o$ is the surface tension of the pure solvent (72.8 mN/m for water) and $\gamma$ is the surface tension of the system of interest. Surface tension is a cohesive force due to the unequal environment experienced by molecules at the surface compared with those in the bulk liquid. This difference in environment creates a surface free energy, the result of which at the air-water interface is a net pull downward (into the bulk) on molecules residing at the surface. The surface free energy per unit area is defined as the surface tension. In a Langmuir trough experiment, changes in surface tension are measured using the Wilhelmy method (by the Wilhelmy balance shown in Fig. 2.1) as described below.

There are three forces acting on the plate shown in Figure 2.3: the weight of the plate acting downward, the buoyancy force of the water pushing the plate upwards, and the surface tension acting downwards. Thus, the downward force acting on the plate is given by,
\[ F_{\text{total}} = (\rho_{\text{plate}} lw t) g - (\rho_{\text{liquid}} h wt) g + 2(w + t) \gamma \cos \theta \]  
(2.2)

where \( l \) and \( w \) are the length and width of the plate (as shown in Fig. 2.3), \( h \) is the immersed height of the plate (Fig. 2.3), \( t \) is the thickness of the plate (not shown), \( \rho \) is density, and \( \theta \) is the contact angle of the liquid with the plate (Fig. 2.3).

**Figure 2.3: Schematic of the plate on the Wilhelmy balance used to measure changes in surface tension**

Since surface pressure is measured in these experiments, the negative change in surface tension must be considered rather than absolute surface tension. Taking this into consideration and solving for surface pressure, the following equation results,

\[ \pi = -\Delta \gamma = \frac{-\Delta F_{\text{total}} + \Delta F_{\text{gravity}} - \Delta F_{\text{buoyancy}}}{2(w + t) \cos \theta} \]  
(2.3)

This equation may be simplified further due to the following considerations. First, the change in weight (force due to gravity, \( F_{\text{gravity}} \)) term diminishes, as there is no change in the density of the paper or any of the dimensions of the plate. Second, if a plate composed of filter paper is used (as is the case in all experiments presented in this thesis), the paper is assumed to be completely wetted, yielding a contact angle of zero. Thus, \( \cos(0) = 1 \). Finally, if there is a constant applied...
force acting on the plate (total force term, \(\Delta F_{\text{total}}\), diminishes) and if \(t \ll w\), the equation for surface pressure becomes,

\[
\pi = -\frac{1}{2} \rho_{\text{liquid}} t g \Delta h
\]  

(2.4)

Thus, the measured surface pressure is proportional to the change in immersed height of the plate.

Molecules with hydrophobic character exhibit an increased propensity for the surface region. Such molecules can be insoluble surfactants, such as stearic acid, forming a stable, floating monomolecular film (deemed a Langmuir monolayer) with characteristic two-dimensional phase changes (as will be described below). Or, such molecules can be soluble, in which case they form an adsorbed Gibbs monolayer. In either case, their partitioning to the water surface is driven by the hydrophobic effect [1]. The hydrophobic groups of dissolved surfactants disrupt the water’s hydrogen-bonded network, thus raising the free energy of the system as a whole. To minimize this, hydrophobic species are expelled from the hydrophilic environment of the bulk water, either through aggregation or surface partitioning. Molecules with both hydrophilic and hydrophobic character (amphiphiles) can partition to the air-water interface, with their hydrophilic head groups partitioning to the aqueous phase and their hydrophobic tails partitioning into the gas phase. Once at the surface, the hydrophilic head groups of these surfactants may interact favorably with surface-residing water molecules, yielding the orientation described above.

Insoluble surfactants, such as stearic acid, form ordered, floating, two-dimensional monolayers at the water surface, and can be studied utilizing Langmuir trough methods. The resultant plot, a surface pressure – area (\(\pi\)-\(A\)) isotherm, yields useful thermodynamic information
about the surfactant of interest. Interestingly, the first isotherm was performed by a housewife by the name of Agnes Pockels in her kitchen in the late 19th century. She created the first primitive trough using greasy water in a bowl using a button to measure surface pressure, the results of which were published with the help of Lord Rayleigh in Nature in 1891 [2]. Later, Irving Langmuir studied surface films using a trough very similar to the one used by Pockels, resulting in the name “Langmuir trough” for future such experiments, despite Pockels having the original design.

A typical \( \pi \)-A isotherm of a surfactant, like the one of stearic acid shown in Fig. 2.4, exhibits features (such as plateaus and/or kinks) that indicate the presence of two-dimensional phase transitions, representative of the ordering and interactions of the molecules at the surface [3-6].

![Graph of \( \pi \)-A isotherm](image)

**Figure 2.4:** \( \pi \)–A isotherm of stearic acid illustrating characteristic two-dimensional phase changes as well as collapse pressure (\( \pi_c \)) and molecular footprint (\( A_o \))
Moving from large to small molecular area, the film begins in a two-dimensional, disordered gaseous phase in which there is little order amongst the surfactant molecules at the surface and the surface pressure is negligibly small. At smaller molecular area, a phase transition may be observed to a liquid expanded state in which there is some interaction among the surfactants but still some translational freedom. In the case of stearic acid, however, room temperature is below the triple point and transition to a liquid condensed phase occurs directly (at 24 Å² in Fig. 2.4).

A large number of studies using a variety of techniques have led to a deeper understanding of the various fatty acid liquid condensed phases, which are now characterized as two-dimensional liquid crystalline mesophases that vary in local packing geometry, molecular tilt, etc. [4-6]. The molecules first pass through a tilted liquid condensed phase (seen at 24 Å² in Fig. 2.4) where the surfactant tails are ordered at the surface with varying symmetries depending on the mesophase, but are tilted relative to the surface [4, 6]. Then, if the barriers continue to close, another phase transition may occur to an untilted liquid condensed phase (~20 Å² in Figure 2.4) in which the surfactant molecules transition to occupying a minimum surface area. At even smaller molecular areas, the molecular monolayer becomes unstable with respect to collapse into a three-dimensional interfacial layer. The nature of this collapse has been thoroughly studied and can vary depending on the film composition, the thermodynamic conditions, and the compression rate [7, 8]. For example, some monolayers collapse via activated nucleation and growth of small 3D aggregates [7, 9, 10], while others collapse via the formation of macroscopic “folds” [8]. The collapse pressure ($\pi_c$) is indicated by a sudden decrease in surface pressure, at which point complex, three-dimensional structures begin to form [11]. The regime of the isotherm corresponding to the untilted condensed phase is conventionally extrapolated to zero surface pressure to yield a characteristic molecular area, or footprint ($A_o$). The above description
is relevant to many common fatty acids, like stearic acid, at a temperature below their triple point. However, the phase behavior of every surfactant is different yet characteristic. Any deviation from this phase behavior will be described in more detail in the appropriate chapter.

2.2.2 Infrared Reflection-Absorption Spectroscopy (IRRAS)

The IRRAS technique is a useful tool for chemical characterization of species in the surface region. A schematic of the IRRAS that I constructed in the Vaida lab is shown below in Figure 2.5. The external port of a Bruker Tensor 27 FTIR spectrometer is used to supply unpolarized infrared light. The light is first passed through a focusing CaF$_2$ lens followed by reflection off of a gold 2” mirror that directs the infrared beam towards the water surface (at an angle of 22° relative to the surface normal) of a small Langmuir trough. Then, the reflected light from the water surface is directed towards a liquid nitrogen cooled MCT detector using another 2” gold mirror positioned above the Langmuir trough. The reflected beam from the surface contains vibrational signatures of any species residing in the surface region. The theory behind the IRRAS technique will be briefly summarized below.

Figure 2.5: Schematic of the IRRAS illustrating the infrared light beam path
When light passes from one medium to another, as is the case at the air-water interface, it experiences two things: a reduction in speed and attenuation. The reduction in speed is described by the real refractive index (n) and attenuation is described by the extinction coefficient (k). Taken together, these give you the complex refractive index (n̅):

\[ n = n + ik \]  \hspace{1cm} (2.5)

In an IRRAS experiment, a reflectance-absorbance (RA) spectrum is measured,

\[ RA = -\log \frac{R}{R_o} \]  \hspace{1cm} (2.6)

where R is the reflectivity of the surface of interest and R_o is the reflectivity of the reference surface. Reflectivity (R) is given by,

\[ R = \frac{I_{reflected}}{I_{incident}} = r r^* \]  \hspace{1cm} (2.7)

where the ratio of the intensity of the reflected light to the intensity of the incident light is the quantity that is measured in the IRRAS experiment (eq. 2.6), and the product of the reflection coefficient (r) with its complex conjugate (r*) is the common calculated value of reflectivity (R).

In general, reflection coefficients are given by the Fresnel equations, with the specific coefficients for the three-phase system of a monolayer at the air-water interface presented below.

The reflection coefficients for a three-phase system (air, monolayer, water as seen in Fig. 2.6) in an IRRAS experiment have been detailed by Kuzmin, Michailov, and coworkers [12-14], and thus will be presented here without further derivation.
Figure 2.6: Schematic of reflection and transmission of light in a three-phase system consisting of air, monolayer, and water.

The reflection coefficients for s and p polarized incident light are given, respectively, by:

\[
\tilde{r}_s = \frac{-\sin(\theta_0 - \tilde{\theta}_2) - ik_0\tilde{n}_2^{-1}\sin \theta_0 \tilde{I}_1}{\sin(\theta_0 + \tilde{\theta}_2) - ik_0\tilde{n}_2^{-1}\sin \theta_0 \tilde{I}_1} \tag{2.8}
\]

\[
\tilde{r}_p = \frac{-\sin(\theta_0 - \tilde{\theta}_2) \cos(\theta_0 + \tilde{\theta}_2) - ik_0\tilde{n}_2^{-1}\sin \theta_0 (\tilde{I}_1 \cos \theta_0 \cos \tilde{\theta}_2 - \tilde{I}_2 \sin \theta_0 \sin \tilde{\theta}_2)}{\sin(\theta_0 + \tilde{\theta}_2) \cos(\theta_0 - \tilde{\theta}_2) - ik_0\tilde{n}_2^{-1}\sin \theta_0 (\tilde{I}_1 \cos \theta_0 \cos \tilde{\theta}_2 - \tilde{I}_2 \sin \theta_0 \sin \tilde{\theta}_2)} \tag{2.9}
\]

where,

\[
\tilde{I}_1 = (\tilde{n}_x^2 - \tilde{n}_y^2)d \tag{2.10}
\]

\[
\tilde{I}_2 = \frac{\tilde{n}_x^2 - \tilde{n}_y^2}{\tilde{n}_z^2}d \tag{2.11}
\]

if constant values for the directional refractive indices (\(\tilde{n}_x\) and \(\tilde{n}_y\)) are assumed, and

\[
k_0 = \frac{2\pi}{\lambda} \tag{2.12}
\]
In the IRRAS experimental set-up in the Vaida lab, unpolarized light is used. Thus, if the unpolarized light is assumed to contain equal components of s and p polarized light, the total reflectivity will simply be the average of the individual s and p reflectivities (hence, \( R = 0.5 (R_s + R_p) \)).

The above description applies only to a distinct three-phase system with a neat air phase, monolayer phase, and water phase. This works well for insoluble surfactants (such as stearic acid) deposited on a water surface. However, with soluble surfactants, such as phenylalanine used in Chapters 7, 9 and 11, benzoic acid and benzaldehyde in Chapter 10, or leucine used in Chapter 8, the system becomes much more complex. These soluble surfactants do, in fact, partition to the surface thereby forming a surface excess concentration, but still have a significant concentration in the underlying layers well into the bulk solution. In this situation, the “surface” cannot be treated as such, and must be instead treated as a series of stratified layers with reflection and transmission through each layer [15]. Although the detected reflectance-absorption by the IRRAS is still a ratio of the intensity of the reflected light to the incident light, direct calculation of the reflection coefficients is difficult without aid from simulations and the inclusion of many more assumptions [15].

One other important consideration when using IRRAS as a surface probe is that the IRRAS beam does not only sample the surface layer (defined as the region of non-centrosymmetry). In fact, IRRAS can penetrate as deep as 1-2 microns [15] depending upon the surface and bulk composition. However, as seen in Chapters 7 and 8, the region probed by IRRAS is the reactive region of interest and is thus sufficient to detect unique changes in the surface region when compared with the bulk aqueous phase.
2.2.3 Brewster Angle Microscopy

Complementary to the surface information provided by the Langmuir trough and IRRAS, Brewster Angle Microscopy (BAM) adds morphological information through visualization of surface domains. BAM has been described previously in the literature [16, 17] and thus only a brief description will be given here of its working principle. When p-polarized light impinges on a medium with a different refractive index from the medium it is currently traversing through, it will have no reflection at a specific angle deemed the Brewster angle:

\[
\tan \theta_B = \frac{n_2}{n_0}
\]

For an air-water interface, with the refractive index of air \(n_0\) taken as 1 and the refractive index of water \(n_2\) as 1.33, this angle is 53°. Thus, as shown in Figure 2.7A below, when a BAM image is taken of a bare water surface, the resulting image is dark, as no light was reflected. However, when another molecule is present at the water surface with a different refractive index \(n_1\) than water, a reflected beam is produced and the morphology of the surface is captured in the BAM image (as seen in Fig. 2.7B).
Figure 2.7: Working principle of the Brewster Angle Microscope (BAM); A: no reflection from a bare water surface yielding a dark image and B: reflection from a surfactant-coated interface resulting in an image of the surface domains.

The BAM utilized in this work was in Heather Allen’s group at the Ohio State University. The BAM experiments were carried out on a self-assembled symmetric goniometer system. The laser light source emits 5W p-polarized at 543 nm (Research Electro-Optics, Inc.), which was incident at the Brewster angle of the subphase. The incident beam was then passed through a Glen Thompson polarizer before reaching the liquid surface. An infinity-corrected 10º Nikon lens together with a tube lens was used to form the image. Finally, the BAM image was collected on an Andor back-illuminated electron-multiplier charge-coupled device (EMCCD; Andor DV887) of 512 x 512 pixels.
2.3 Molecular Dynamics Simulations

Classical Molecular Dynamics (MD) is an invaluable tool for exploring molecular level chemical and physical processes. The working principle of classical MD involves computationally solving Newton’s equations of motion for a system of interacting atoms (i = 1…N atoms):

\[ F_i = m_i a_i = m_i \ddot{r}_i \]  \hspace{1cm} (2.14)

where the force, F, can also be defined as the negative derivative of a potential, \( V(r_1…r_N) \) as follows:

\[ F_i = -\frac{\delta V}{\delta r_i} \]  \hspace{1cm} (2.15)

The potential contains all terms describing the interactions between atoms within the system of interest. These interaction potentials may be defined using the “full” potential energy surface from solving the electronic Schrödinger equation with quantum chemistry methods (can only be done for very small systems), using an “on the fly” potential that is calculated for the specific configuration at each time step of the simulation using quantum chemistry methods (e.g. density functional methods), or using an empirical potential (“force field”) that is defined by a model derived from experiment and/or ab initio calculations. In the work presented in this thesis, empirical force fields are utilized. These force fields contain both intramolecular (bonding) and intermolecular (non-bonding) terms. Thus, a simple, pairwise additive, empirical force field would have the general form:

\[ V = V_{bonds} + V_{angles} + V_{dihedrals} + V_{electrostatic} + V_{van der Waals} \]  \hspace{1cm} (2.16)
where the bond, angle and dihedral terms are describing intramolecular interactions and the electrostatic and van der Waals terms are describing intermolecular interactions. Normally, the bond and angle terms are approximated using a harmonic potential, eliminating the possibility of bond breaking events, however a Morse potential may also be used. The dihedral potential is given by a periodic rotational potential (for “proper” dihedrals) and may be supplemented with an improper torsion angle potential (for “improper” dihedrals) to help maintain planarity of groups such as aromatic rings. The non-bonding interactions are typically described by a Coulomb potential for electrostatic interactions and a Lennard-Jones potential for the van der Waals interactions. The details of these and other possible potentials commonly used in empirical force fields are described extensively throughout the literature, with the basics being outlined in reference [18]. These force fields may be modified and customized as needed, with many systems, such as phospholipid films [19, 20], having well-tested force fields freely available.

Once the interactions are defined, Newton’s equations of motion must be solved over time numerically. In practice, one of two algorithms is commonly used: the Leapfrog algorithm [21] or the Verlet algorithm [22]. The Leapfrog algorithm is derived from a Taylor expansion for v(t) and r(t) using small timesteps (Δt), resulting in the following expressions for the propagation of velocity and position respectively:

\[ v_A(t + \Delta t/2) = v_A(t - \Delta t/2) + a_A(t)\Delta t + \cdots \]  \hspace{1cm} (2.17)

\[ r_A(t + \Delta t) = r_A(t) + v_A(t + \Delta t/2)\Delta t + \cdots \]  \hspace{1cm} (2.18)

Similarly, the Verlet algorithm begins with a Taylor expansion of r(t), yielding:
\[ r_A(t + \Delta t) = 2r_A(t) - r_A(t - \Delta t) + \left( \frac{d^2r_A}{dt^2} \right)_t (\Delta t)^2 \]  \hspace{1cm} (2.19)

with the velocity \( v(t) \) given by the difference:

\[ v_A(t) = \frac{r_A(t + \Delta t) - r_A(t - \Delta t)}{2\Delta t} \]  \hspace{1cm} (2.20)

It can be shown that the Leapfrog propagator is formally equivalent to the Verlet propagator, however their use differs slightly in practice. The remaining details pertinent to each simulation will be outlined in their respective chapter of this thesis.

### 2.4 Analytical Techniques

#### 2.4.1 Spectroscopic Techniques

Spectroscopy is a technique that utilizes the interaction of electromagnetic radiation with matter. In the work in this thesis, three types of spectroscopy were used utilizing electromagnetic radiation in the ultraviolet/visible, infrared, and radio range to probe electronic, vibrational, and nuclear structure respectively. Absorption of any electromagnetic radiation by a molecule in solution can be described by Beer’s law,

\[ A = -\log \frac{I}{I_o} = \varepsilon bc \]  \hspace{1cm} (2.21)

where \( I \) and \( I_o \) are the intensity of the transmitted and incident light respectively, \( \varepsilon \) is the molar extinction coefficient of the absorber, \( b \) is the path length of the cell containing the solution of interest, and \( c \) is the concentration of the absorber in solution. When electromagnetic radiation impinges upon a sample, some of that incident radiation is absorbed by the sample and some is transmitted through the sample. Using this equation, the absorbance measured may be used to
determine the concentration (if \( \varepsilon \) is known) or extinction coefficient (if \( c \) is known) of the molecule of interest in solution.

The absorption of light by a molecule induces a transition between energy levels within that molecule. The transition made depends upon congruence between the energy level spacing within the molecule and the frequency of incident light. Ultraviolet radiation has enough energy to induce transitions between electronic energy levels in a molecule, as described in section 2.4.1(a). Infrared light induces vibrational transitions (section 2.4.1(b)) and very low energy radio radiation induces nuclear spin transitions (section 2.4.1(c)).

2.4.1(a) UV/visible Spectroscopy
UV/visible spectroscopy utilizes wavelengths of light within the ultraviolet and visible range to promote electronic transitions within a molecule. In the work presented in this thesis, UV/visible spectroscopy was used to monitor the change in the concentration of a reactant while simultaneously extracting the absorption spectra of products formed during photochemical reaction. Spectra were obtained of aqueous samples using a USB2000 miniature fiber optic UV-vis spectrometer from Ocean Optics.

2.4.1(b) Fourier Transform Infrared Spectroscopy
FTIR spectroscopy utilizes infrared light to promote transitions between vibrational energy levels within a molecule. In this thesis, FTIR spectroscopy is used to chemically characterize species both in their solid state through traditional solid-state FTIR, and in the water surface region through the IRRAS technique (described in more detail in section 2.2.2). Fourier transform (FT) techniques employ an interferometer; the Michelson interferometer is often used to modulate radiation in these instruments and is illustrated schematically in Figure 2.8 below.
Figure 2.8: Schematic of a Michelson Interferometer

Radiation from a broadband source impinges upon a beamsplitter that subsequently reflects half of the light towards a fixed mirror and transmits the other half of the light towards a movable mirror. These two mirrors then reflect the light back towards the beamsplitter that again splits both beams of light, sending half of the light back towards the source and half towards the detector. Before reaching the detector, the two beams of light may then interfere with each other either destructively or constructively, resulting in an interferogram (intensity vs. moving mirror distance). Finally, this interferogram is Fourier transformed into an absorption spectrum (intensity vs. energy). The FTIR spectrometer in this work was a commercial Bruker Tensor 27.

2.4.1(c) Nuclear Magnetic Resonance Spectroscopy

NMR is a technique that utilizes transitions in atomic spin states to elucidate molecular structure. The molecules of interest are exposed to a magnetic field, inducing an energy difference in the spin states of nuclei of specific isotopes. Transitions between these split spin states can then be observed and interpreted. All NMR spectra were obtained by Dr. Richard
Shoemaker in the NMR Spectroscopy Facility at the University of Colorado, Boulder. This NMR facility has instruments in the 300, 400, and 500 MHz ranges with the capability to probe various nuclei including $^1$H, $^{13}$C, and $^{31}$P. Multidimensional spectra can also be obtained, yielding gCOSY (gradient-selected homonuclear correlation spectroscopy), gHMBCad (adiabatic $^1$H-$^{13}$C multiple-bond correlation), gHSQCad (adiabatic $^1$H-$^{13}$C single-bond correlation), and high-resolution 2D-DOSY (diffusion ordered spectroscopy) that can be used for conclusive product identification. In addition, DOSY NMR may be used to identify more complex structures in solution, as is done here to identify confined motion of molecules in vesicular aggregates in Chapter 6.

2.4.2 Mass Spectrometry

Mass Spectrometry (MS) is a technique in which the species of interest is transformed into an ionized gas, followed by separation and detection dependent upon the mass to charge ratio of the ion. In the mass spectra presented in this thesis, a desorption source, specifically electrospray ionization (ESI) was used. ESI is a common ionization technique for biomolecules and is useful in analyzing a wide range of samples due to its operation at atmospheric temperature and pressure. As a soft ionization technique, ESI has the added advantage of minimizing fragmentation, yielding molecular ions that are more easily characterized in mixtures. Once gas phase ions are produced, they are separated by their mass to charge ratio using a mass analyzer. The mass analyzer used in the work presented in this thesis is a Time-of-Flight (TOF) mass analyzer. The TOF mass analyzer uses an electric field to accelerate the ions, resulting in all ions having the same kinetic energy. Then, since the velocity of each particle is inversely proportional to its mass to charge ratio, the ions are separated and detected by their velocities, with the lightest ions being detected first. All mass spectra were obtained by Dan Gu
in the Central Analytical Mass Spectrometry Facility at CU Boulder under the direction of William Old.

2.4.3 Dynamic Light Scattering

Dynamic Light Scattering (DLS) is a technique that utilizes the fluctuations in intensity of scattered light from particulates that are undergoing Brownian motion in solution to determine particle size. Here, the hydrodynamic radius of structures in solution (Chapter 6) was determined assuming spherical particles. DLS was performed in the Biochemistry Shared Instrument Core at CU Boulder with aid from Annette Erbse using a Titan DynaPro instrument.

2.4.4 Fluorescence Microscopy

Fluorescence microscopy is a technique that uses fluorescence to image structures of interest. In this work, Rhodamine 6G was used as the fluorescent probe to image self-assembled vesicles in aqueous solution (Chapter 6), as it partitions preferentially to the otherwise non-fluorescent membrane. All fluorescence microscope images were obtained in the Biofrontiers Advanced Imaging Resource by Kevin Dean.

2.5 References for Chapter 2


3 Pyruvic Acid Gas-Phase Photochemistry: Influence of Environmental Conditions on Chemical Reaction Pathways

3.1 Introduction

The Earth’s atmosphere contains many gas phase species as well as liquid and solid particles of varied composition, exposed to fluctuating conditions such as temperature, pressure, photon flux, and relative humidity [1-4]. Such a diverse environment would logically enable many different chemical reactions, both in today’s atmosphere and in its distant past. One molecule of interest throughout Earth’s history is pyruvic acid, a simple, 3-carbon alpha-keto acid (CH$_3$COCOOH). Today, pyruvic acid is prevalent throughout the atmosphere and is often used in atmospheric models as a proxy for other alpha-keto acids [5-8]. Evolutionarily, pyruvic acid is a very interesting molecule since it is widespread throughout modern biology, acting as an intermediate in many different metabolic cycles, both aerobic and anaerobic [9]. Thus, coupling this molecule with the varying conditions of the natural environment is an interesting test case for the influence of environment on chemistry. In this chapter, the gas phase photochemistry of pyruvic acid is explored in an environmental chamber capable of simulating the natural environment, testing the influence of water and oxygen as well as the presence of aerosol particles on its photochemical reaction pathway. In the following two chapters, this notion is expanded upon, taking the photochemistry of pyruvic acid into aqueous solution [10], further illustrating its diverse chemistry.

Chemistry resulting in decarboxylation of pyruvic acid in the gas phase has been explored in the laboratory extensively in the literature, using thermochemistry, infrared multiphoton pyrolysis, and UV and visible excitation [11-23]. However, in the atmosphere, the
dominating pathway for the destruction of gas phase pyruvic acid is UV photolysis [17, 24, 25]. In the literature, most laboratory studies agree on the reaction scheme shown in Scheme 3.1 below.

![Scheme 3.1: Traditional photochemical reaction mechanism of gas phase pyruvic acid](image)

Pyruvic acid first absorbs light through its carbonyl chromophore ($\lambda_{\text{max}} \approx 350$ nm) [15, 17] producing methylhydroxycarbene (a reactive intermediate) and CO$_2$, as shown in step (1) of Scheme 3.1. This step is thought to occur with a quantum yield approaching unity [12, 15, 23]. Then, as shown in step (2) of Scheme 3.1, the methylhydroxycarbene intermediate quickly rearranges to form acetaldehyde and vinyl alcohol (which eventually also rearranges to form acetaldehyde).

The production of acetaldehyde and CO$_2$ as major photolysis products of gas phase pyruvic acid is well supported in the literature [12, 15, 17, 26], but there are a few interesting exceptions. First, although Yamamoto and Back [15] did see acetaldehyde and CO$_2$ as the only photolysis products in their initial photolysis experiments, when a pressure dependence study was performed (increasing the pressure of air in the reaction cell to 100 torr), the amount of acetaldehyde produced was significantly reduced with increasing pressure despite the amount of CO$_2$ remaining unaffected. This alteration in chemistry was attributed to a reaction of O$_2$ with
the methylhydroxycarbene intermediate. It is important to note that most literature studies have been performed under low pressure, causing this finding by Yamamoto and Back to be especially important. When Berges and Warneck performed photolysis of gas phase pyruvic acid in air at atmospheric pressure, they detected acetic acid in addition to acetaldehyde and CO₂ [23].

The discrepancies in the literature briefly noted above suggest the need for experiments performed in conditions representative of the natural environment in order to truly extend this chemistry to real atmospheric systems and models. To address this, an environmental chamber was used in this work to simulate the natural atmosphere and the effect of additives on the photochemistry of pyruvic acid. The effect of both water and oxygen were explored, confirming the suspicions of Yamamoto and Back [15] that additives do affect the chemistry of the methylhydroxycarbene intermediate. In addition, preliminary experiments were performed using aqueous atmospheric aerosols containing pyruvic acid to introduce a diversity of phase into the chemistry, resulting in the suggestion of a heterogeneous component in the reaction products observed in the gas phase.

3.2 Materials and Methods

Pyruvic acid (98%) was purchased from Sigma-Aldrich, and was distilled under reduced pressure prior to use. Then, pyruvic acid was either directly injected into the atmospheric chamber (described below) in the gas phase or an aqueous solution was prepared followed by nebulization into the chamber, resulting in the injection of aqueous pyruvic acid aerosol particles.
3.2.1 CESAM Chamber

The atmospheric chamber, CESAM (French acronym for Experimental Multiphasic Atmospheric Simulation Chamber), used in this work was housed at the Interuniversity Laboratory of Atmospheric Systems (LISA) at the University of Paris – East under the direction of Jean-François Doussin. The CESAM chamber is described in detail in reference [27]. Briefly, it consists of a 4000-liter stainless steel reaction chamber with injection ports and associated instrumentation for both gas phase and aerosol particle introduction. In addition, it has an attached FTIR spectrometer (Tensor 37, Bruker) that gathers in situ vibrational spectra of gas-phase species within the chamber, with an extended path length of 192 m. Particles can be sampled and analyzed using a light-scattering aerosol spectrometer (WELAS digital 2000, Palas GmbH, Germany) for larger particles in situ (> 1 µm in diameter) and a Scanning Mobility Particle Sizer (SMPS) system (TSI Inc.) for smaller particles analyzed externally. Gas phase species can also be controlled (O₂, N₂, etc.) and relative humidity is manipulated through the injection of water vapor at the base of the chamber. Finally, the chamber is equipped with three 4kW high-pressure Xe arc lamps (MH-Diffusion MacBeam 4000), the equivalent of one-half the photon flux of the contemporary sun. The wavelength output was limited to wavelengths greater than 300 nm through the use of Pyrex filters placed in front of the Xe lamps.

3.3 Results and Discussion

3.3.1 Photolysis of gas phase pyruvic acid

Gas phase pyruvic acid was photolyzed in the CESAM chamber at 1 atmosphere pressure under three different conditions, the results of which are shown in Figure 3.1: dry and without O₂ (100% N₂ atmosphere) in Fig. 3.1 (a.i) and (b.i), dry and under synthetic air (80% N₂,
20% O$_2$) in Fig. 3.1 (a.ii) and (b.ii), and wet (70% relative humidity) and under synthetic air in Fig. 3.1 (a.iii) and (b.iii).

![Figure 3.1: Products formed (acetaldehyde (blue), acetic acid (green), CO (red) and CO$_2$ (black)) from photolysis of gas phase pyruvic acid (gray) under varying conditions in different rows: (a.i, b.i) dry and no O$_2$; (a.ii, b.ii) dry and in synthetic air; (a.iii, b.iii) wet (70% R.H.) and in synthetic air; Columns are: (a.i-a.iii) product formation and pyruvic acid loss over time; (b.i-b.iii) yield plots for formation of products$^2$.](image)

In Fig. 3.1 (a.i), (a.ii), and (a.iii), three stages are shown: injection of pyruvic into the chamber seen as large increases in pyruvic acid concentration with time, then there is a short time of

$^2$ CO$_2$ concentrations shown in (b.i) and (b.iii) have not been corrected for initial conditions.
equilibration allowing for the monitoring of wall loss of pyruvic, and finally the start of the exponential decay of pyruvic once the lamp is turned on. Photolysis products were then identified and monitored spectroscopically using the in situ infrared spectra. Through comparison with literature spectra, the concentrations of all products and the loss of pyruvic acid was determined and plotted over time allowing for the determination of the photolysis rate (assuming simple first order kinetics). In addition, yield plots were constructed comparing the concentration of products with the loss of pyruvic acid from photolysis (accounting for wall loss) allowing for identification of primary and secondary production and losses.

The gas phase photolysis of dry pyruvic acid in the absence of oxygen in the CESAM chamber resulted in the production of acetaldehyde, acetic acid, CO₂ and CO as illustrated in Figure 3.1(a.i). The yield plot shown in Figure 3.1(b.i) shows that acetaldehyde, acetic acid and CO are all primary photolysis products, illustrated by the linear relationship of their production with the decay of pyruvic acid. However, CO₂ exhibits behavior characteristic of a primary and secondary product evidenced by its positive exponential relationship with pyruvic acid decay in Fig. 3.1(b.i). CO may also have a secondary source (there is a slight up-turn in the yield plot), however there is not enough data to confirm this since the photolysis is much slower than in the presence of oxygen (discussed in more detail later).

3.3.1 (a) Influence of oxygen

When synthetic air was used, introducing 20% oxygen into the chamber, no acetaldehyde was detected. Instead, the major products were acetic acid, CO, and CO₂ as shown in Figure 3.1(a.ii). Acetic acid appears, in Fig. 3.1(b.ii), as a primary product but with secondary loss, seen as a rapid decrease in concentration as the concentration of pyruvic acid is depleted. CO
and CO₂ are seen as being primary products but also as having a secondary source (Fig. 3.1(b.ii)).

3.3.1 (b) Influence of water

When the relative humidity is raised in the chamber prior to the injection of gas phase pyruvic acid there is further change to the photolysis products observed. Now, the only easily identifiable product besides CO₂ is acetic acid as shown in Figure 3.1(a.iii). In addition, there appears to be no secondary loss of acetic acid in the presence of water in conjunction with its primary production (Fig. 3.1(b.iii)), but it instead appears to have a secondary source. Consistent with the results in all conditions involving water and oxygen, CO₂ was observed to be both a primary photolysis product and to have a secondary source (Fig. 3.1(b.iii)).

Although acetic acid was the only identifiable product in the wet gas phase photolysis, there are a few unidentified peaks in the vibrational spectrum that arise from photolysis (Fig. 3.2).

![Figure 3.2: FTIR spectra of gas phase pyruvic acid (peaks attributed to PA labeled) with 70% relative humidity and in synthetic air throughout photolysis illustrating evolution of products (acetic acid and unknown product(s) are labeled).](image)
Although these peaks are unidentified, their presence is clearly a product of the influence of water on this photochemical reaction pathway.

### 3.3.1 (c) Summary of gas phase results

The results presented in sections 3.3.1(a) and 3.3.1(b) are summarized below in Table 3.1 with the addition of preliminary photolysis rates calculated from the first order decay of pyruvic acid in each environment.

#### Table 3.1: Summary of preliminary results for photolysis of gas phase pyruvic acid with and without O$_2$ and H$_2$O

<table>
<thead>
<tr>
<th></th>
<th>Dry pyruvic acid, no O$_2$</th>
<th>Dry pyruvic acid, synthetic air</th>
<th>Wet pyruvic acid, synthetic air</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$ present</td>
<td>0%</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>&lt;2%</td>
<td>&lt;2%</td>
<td>70%</td>
</tr>
<tr>
<td>Photolysis rate$^3$ (s$^{-1}$)</td>
<td>0.00006</td>
<td>0.00095</td>
<td>0.00078</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>1° product</td>
<td>not observed</td>
<td>not observed</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>1° product</td>
<td>1° product with 2° loss</td>
<td>1° product with 2° source</td>
</tr>
<tr>
<td>CO</td>
<td>1° product</td>
<td>1° product with 2° source</td>
<td>not observed</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>1° product with 2° source</td>
<td>1° product with 2° source</td>
<td>1° product with 2° source</td>
</tr>
</tbody>
</table>

The rate of photolysis of dry pyruvic acid in the absence of oxygen is an order of magnitude lower than the photolysis rate in the presence of oxygen (both dry and wet). In addition, the presence of oxygen quenches the formation of acetaldehyde as a photolysis product, and enhances the formation of acetic acid. Further, the addition of water quenches the formation of CO, leaving CO$_2$ and acetic acid as identifiable reaction products, and adding a new product (or products) that have yet to be identified. Thus, the gas phase photochemistry of pyruvic acid

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$^3$ When available, the photolysis rate is reported as an average of multiple trials; only no O$_2$, no H$_2$O photolysis rate is the result of a single measurement.
displays the sensitivity of chemical reactions to environmental conditions. The sensitivity to oxygen is important in a prebiotic context due to the likely anoxic atmosphere of early Earth compared with the oxygen-rich atmosphere of modern Earth [28]. In addition, water is now, and was prebiotically, an important component of the atmosphere due to the water-rich surface of the Earth. Thus, the influence of both of these molecules on chemistry is of great interest.

3.3.2 Photolysis of aqueous pyruvic acid aerosols

When pyruvic acid was injected into the chamber as aqueous aerosol particles, the photochemistry is further perturbed. First, it is important to note that once injected into the chamber, the aerosol particles equilibrate with the gas phase. Contrary to traditional thinking, pyruvic acid was observed in this experiment to partition primarily into the gas phase, with an estimated two orders of magnitude more pyruvic acid partitioning into the gas phase when compared with the particle concentration. With such small concentration remaining in the particle phase, aqueous phase pyruvic acid photochemistry (described in detail in Chapter 4) is not expected to play a significant role in the products observed in the gas phase. Thus, this photochemical experiment would be expected to yield the same products as the wet gas phase photochemical experiment presented in section 3.3.1(b).

However, as presented in Fig. 3.3, the presence of particles does influence the photochemistry, exhibiting a very clear enhancement in the products showing features between 850 – 1050 cm\(^{-1}\). This is especially apparent when directly comparing the infrared spectrum after photolysis in the wet gas phase experiment (blue spectrum) with the spectrum after photolysis in the experiment containing particles (red spectrum) shown in Fig. 3.3(b).

---

4 Pyruvic acid concentration in the gas phase was estimated from its FTIR peak intensity and the particle concentration was taken from the dried organic aerosol mass as measured by the Scanning Mobility Particle Sizer (SMPS).
Acetic acid is still a major product in the presence of particles, but there is a clear enhancement in the unknown peaks. This suggests a potential heterogeneous component to the photochemistry in the production of this unknown product(s), implicating a role for the water
However, this product has yet to be identified, limiting the potential for further mechanistic insight.

3.4 Conclusions
Additives, such as oxygen and water, are shown here to compete with the rearrangement of the carbene intermediate and markedly change the reaction pathway. This counterintuitive result necessitates that the carbene intermediate has a long enough lifetime to react with other gas phase molecules, competing with its rearrangement. Thus, the expected result, well supported in low pressure experiments in the literature producing acetaldehyde and CO$_2$ as the dominant photolysis products, is only realistic in a natural environment in the absence of O$_2$ and water. This is important to realize in both a prebiotic context as well as in laboratory studies relevant to the modern atmosphere. Performing experiments in a cell at low pressures may not yield atmospherically relevant results. In conclusion, the gas phase photolysis of pyruvic acid illustrates the diversity of chemistry available in varying reaction conditions that are available in the natural environment, enhancing the appeal of utilizing the atmosphere as a reaction environment throughout the history of the chemistry of life.

3.5 References for Chapter 3


4 Pyruvic Acid: Aqueous Photochemical Mechanism\(^5\)

4.1 Introduction

In the modern environment, predictive models of air quality and climate change require understanding of the role that organic compounds play in aerosol interactions with radiation [1, 2]. Volatile organic compounds (VOC) in the atmosphere oxidize with the products of this chemistry contributing to the formation of secondary organic aerosols (SOA) [3, 4]. Current model predictions, however, do not agree with findings of field measurements and highlight the importance of understanding organic reaction mechanisms which are expected to affect the oxidizing capacity of the atmosphere, the formation of secondary organic aerosol (SOA) and climate-relevant aerosol properties [1-10]. Organic polymers have been identified as important contributors to SOA. These polymeric species do not result from gas phase reactions [11] but rather appear to be products of organic reactions in condensed (aqueous phase) where high molecular weight compounds, polymers and oligomers, can be generated [1, 5, 12-15]. Recent results have mainly addressed radical reactions in aqueous environments [5, 7, 16], with only a few using direct photolysis [13, 17]. The need for understanding organic reaction mechanisms in aqueous solutions representative of aqueous media in the atmosphere (aerosols, cloud droplets, fog, rain) has been recognized [1, 2, 7, 18].

In aqueous environments with sufficiently high organic concentrations, polyfunctional carbonyl compounds react to form high molecular weight species, polymers and oligomers, yet the reaction mechanisms responsible for these aqueous phase reactions remain elusive targets for investigation [5, 6, 13, 19-21]. This investigation provides fundamental lab results and

mechanisms for the photochemistry of pyruvic acid in aqueous solution. Pyruvic acid, an oxidative product of isoprene, is abundant in the modern atmosphere in gas and aerosol phase and has been used in models as a proxy for atmospheric α–dicarbonyls [3, 22-24]. Pyruvic acid undergoes decarboxylation in gas and aqueous phases by different mechanisms. Decarboxylation of gas phase pyruvic acid has been seen to occur thermochemically, through infrared multiphoton pyrolysis, and through UV and visible excitation [13, 25-36]. The main removal pathway of gas-phase pyruvic acid from the troposphere is ultraviolet photolysis with reaction with OH radical providing a minor contribution [31]. The photochemistry (direct photolysis) of pyruvic acid in aqueous solution remains controversial in the literature [37, 38], and is the focus of the present laboratory study. The mechanistic details of this chemistry will be presented in this chapter with the prebiotic implications of pyruvic acid photochemistry being presented in the next chapter (Chapter 5).

Photodecarboxylation of aqueous pyruvic acid is facilitated through excitation of its \( \pi \rightarrow \pi^* \) band, with an absorption maximum of 321 nm [39]. This decarboxylation has been known for many years, but the mechanism and resulting products have been controversial [13, 39-41]. Earlier studies performed by Leermakers and Vesley [39, 40] report 3-hydroxy-2-butanone (acetoin) as the only isolable product from the aqueous photolysis of pyruvic acid. The photochemical products were further found to be medium-dependent, with the photochemistry progressing through two different pathways: decarboxylation and reduction. In water, photochemistry of pyruvic acid progressed through a photodecarboxylation pathway resulting in significant CO\(_2\) production, as well as acetoin as the major aqueous phase product detected. In organic solvents such as methanol, chloroform, and ethyl ether, Leermakers and Vesley did not detect CO\(_2\) with photolysis, and thus suggest that the photochemistry is instead progressing
through a reduction pathway, with the dimer dimethyltartaric acid found to be the major product in all three of these solvents [39]. Closs and Miller [41] then contend that the production of acetoin in aqueous solution is facilitated by the transition through α-acetolactic acid, a β-keto acid which is known to thermally decarboxylate to acetoin [42]. Under similar experimental conditions many years later, Guzman et al. [13] report no detection of acetoin, but rather see two oligomers of pyruvic acid (dimers) as the major products of this photochemistry, one of which (“Product A”) is the same oligomer (dimethyltartaric acid) detected by Leermakers and Vesley [39] in other organic solvents. In this work, the photochemistry of pyruvic acid in aqueous solution is reinvestigated to provide a mechanism for this reaction consistent with the observations reported here and supported by the literature.

4.2 Materials and Methods

Pyruvic acid (98%) was purchased from Sigma-Aldrich, and was distilled under reduced pressure prior to its preparation to a final concentration of 0.1M in distilled water, a typical concentration for atmospheric aerosol particles [5, 6]. Acetoin (≥92%, natural) was also purchased from Sigma-Aldrich, and was prepared to a final concentration of 0.04M in distilled water without further purification. Similarly, lactic acid (racemic, meets USP testing specifications, purchased from Sigma-Aldrich) was prepared to a final concentration of 0.04M in distilled water without further purification.

Aqueous pyruvic acid was photolyzed using a 450W Xe arclamp (Newport) for 1-2 hours, while kept chilled at 4°C in a temperature controlled water bath. A Xe lamp was used due to the similarity of its output to the solar spectrum. The photolyzed pyruvic acid solution was allowed to return to room temperature before any subsequent analysis was performed.
4.2.1 UV/visible Spectroscopy

UV-vis spectra were obtained of aqueous samples using a USB2000 miniature fiber optic UV-vis spectrometer from Ocean Optics.

4.2.2 Gas Phase Infrared Spectroscopy

The gas evolved during photolysis of aqueous pyruvic acid was collected over the solution during two hours of photolysis. Then, after the solution was allowed to return to room temperature, a gas-phase infrared spectrum was obtained of the evolved gaseous products using the internal compartment of a Bruker Tensor 27 FTIR spectrometer, purged with dry house air. Spectra were collected with a 1 cm\(^{-1}\) resolution, and were averaged over 50 scans.

4.2.3 Nuclear Magnetic Resonance

NMR spectra were acquired using a Varian INOVA 500 MHz NMR spectrometer operating at 499.60 MHz for \(^1\)H observation. All samples were prepared with a volume of 0.8mL in 5mm NMR Tubes. 0.050 ml of 98% D\(_2\)O was added to provide a field-frequency lock, making the final solvent 95% H\(_2\)O, 5% D\(_2\)O. To fully characterize the key components of the photolysis products, a combination of standard high-resolution NMR experiments such as 1-dimensional \(^1\)H NMR, 2-dimensional gCOSY, gHMBCad (adiabatic \(^1\)H-\(^{13}\)C multiple-bond correlation), gHSQCad (adiabatic \(^1\)H-\(^{13}\)C single-bond correlation), and high-resolution 2D-DOSY (Diffusion Ordered Spectroscopy) were performed. NMR spectra were obtained both before and after photolysis for comparison.

4.2.4 Computation

Electronic structure calculations used the CBS-QB3 composite method [43], as implemented in the Gaussian 03 suite of programs [44].
4.3 Results

Both aqueous and gas phase products were detected using FTIR and UV spectroscopy, NMR, and mass spectrometry. The photo-induced decarboxylation of aqueous pyruvic acid was confirmed through infrared spectra taken of the gas produced after two hours of photolysis, as seen in Figure 4.1.

![Infrared spectrum of collected gas over 0.1M aqueous pyruvic acid after photolysis for two hours. The inset shows acetoin features (broad peaks beneath the sharp residual water lines) between 1000 and 2000 cm\(^{-1}\) (black) with pure acetoin gas-phase spectrum (red) overlaid for comparison.](image)

The intense features between 2200 and 2400 cm\(^{-1}\) are due to the CO\(_2\) produced. A closer look at the region between 1000 and 2000 cm\(^{-1}\) reveals features due to an additional compound being released into the gas phase concurrent with decarboxylation (see inset of Fig. 4.1), buried beneath the residual water vapor lines. This second gas phase component is identified as acetoin through comparison with the gas phase spectrum of pure acetoin shown in red, which was

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6 Acetoin was initially identified by its distinctive odor by Barry K. Carpenter.
obtained in this lab. It is important to note that it is difficult to detect acetoin both in gas phase using FTIR and in aqueous solution using NMR due to the interference of water.

The NMR spectra (Figs. 4.2a and 4.2b) clearly demonstrate the presence of both acetoin and lactic acid in solution after photolysis.

![Superimposed spectra of aqueous 500 MHz NMR spectra of Pyruvic Acid after photolysis (red), 0.1M acetoin (green), and 0.1M Lactic Acid (blue). (a) 2D-gradient COSY with WET suppression, (b) 2D-gradient gHMBCad. Circed peak in (b) is assigned as acetic acid.](image)

Figure 4.2: Superimposed spectra of aqueous 500 MHz NMR spectra of Pyruvic Acid after photolysis (red), 0.1M acetoin (green), and 0.1M Lactic Acid (blue). (a) 2D-gradient COSY with WET suppression, (b) 2D-gradient gHMBCad. Circed peak in (b) is assigned as acetic acid.
The $^1$H and $^{13}$C NMR of aqueous pyruvic acid after photolysis yields a complex mixture of products, as shown in Figure 4.2b; however, two-dimensional NMR not only provides the chemical shift information of the $^1$H and $^{13}$C NMR, but also the key through-bond correlations present between coupled protons (two-dimensional gradient-selected homonuclear correlation spectroscopy (gCOSY) in Fig. 4.2a) and between protons and carbons via multiple-bond correlations (gradient-selected heteronuclear multiple bond correlation using adiabatic pulses (gHMBCad) in Fig. 4.2b) for each compound. By acquiring the same two-dimensional NMR data using samples of aqueous acetoin (presented in green in Fig. 4.2), and lactic acid (presented in blue in Fig. 4.2) the direct correspondence of the $^1$H and $^{13}$C chemical shifts as well as the identical through-bond correlations between those resonances with signals present in the photolyzed pyruvic acid mixture, confirms the presence of both of these compounds in the photolyzed product. Acetic acid was also identified in the mixture via the chemical shifts of the methyl group ($^1$H and $^{13}$C, 2.07 and 20.6 ppm, respectively), and the carboxyl carbon shift of 176.8 ppm via direct gHMBC correlation to the methyl resonance at 2.07 ppm, circled in Fig. 4.2b. These shifts correspond to values from known library spectra (ACD Labs NMR prediction software).

Oligomers\textsuperscript{7} of pyruvic acid, observed previously in the work performed by Guzman et al. \cite{13}, were also detected here using both ESI Mass Spectrometry (Fig. 4.3) and DOSY NMR (Fig. 4.4). After two hours of photolysis, the mass spectrum of the photolyzed mixture shows a major product peak at 177 m/z, consistent with Guzman et al.’s “Product A” (dimethyltartaric acid), as well as a minor peak at 175 m/z, the mass of which is consistent with their oligomer “Product B” \cite{13}.

\textsuperscript{7} The authors recognize the formal misuse of the term “oligomer”, but are using this term in congruence with the published literature (reference 13). The oligomer products here are formed in a termination step, through reaction between two radicals.
The DOSY data (Fig. 4.4) confirms the presence of oligomers, primarily consisting of dimers of various forms, which represent a majority of the components of the photolyzed mixture. Using the sample containing the mixture of photolysis products, the calibrated diffusion coefficient (D) for most of the NMR signals between 1-3 PPM is measured to be between 6.5 - 6.9 (x10^{-10} M^2/sec). The well-resolved signals assigned to acetoin (^1H quartet, 4.41 ppm) shows D=10.2x10^{-10} M^2/sec, lactic acid (^1H quartet, 4.36 ppm) gives D=9.7 x10^{-10} M^2/sec, and acetic acid (^1H singlet, 2.07 ppm) gives D=11.2 x10^{-10} M^2/sec. (See DOSY data, Fig. 4.4). For verification of both the assignments of acetoin and lactic acid, and to confirm the diffusion measurements, identical wet-DOSY experiments were performed using separate aqueous samples of 0.1M acetoin and 0.1M lactic acid. These experiments yielded D = 10.0-10.2 x10^{-10} M^2/sec for acetoin, and 9.3-9.6 x10^{-10} M^2/sec for lactic acid, closely matching the values for each compound measured in-situ in the photolysis mixture.
Figure 4.4: DOSY spectrum of photolysis products of pyruvic acid. (a) projection of the diffusion dimension (b) full 2D DOSY plot showing all components.
Comparing the D values for acetoin and lactic acid with the D values for the majority photolysis products with lower diffusion coefficients, the ratio is found to be in the range of 1.4:1 to 1.6:1 ($D_{\text{acetoin/lactic acid}} : D_{\text{majority product}}$ ratio). Doubling the size/mass of the molecule (i.e. either a homo-dimer or hetero-dimer of the small molecules) should lower the measured D value by approximately $\sqrt{2}$, depending upon the molecular geometry, suggesting that the majority of the photolysis products are approximately two times the size of the individual molecules already identified (i.e. acetoin and lactic acid) [45, 46]. These diffusion coefficients indicate that the major products of photolysis of pyruvic acid are small oligomers. Although no structural information was obtained directly in this work, the size and masses of these oligomers are consistent with the two oligomer products first identified by Guzman et al. [13].

Finally, Figure 4.5 presents a UV-visible spectrum of aqueous pyruvic acid after one hour of photolysis (black solid line), along with the underlying components of the spectrum shown in broken lines.

![Figure 4.5](image)

**Figure 4.5:** UV-visible absorption spectrum of aqueous pyruvic acid after one hour of photolysis (black), with scaled underlying components shown in broken lines. Pyruvic acid shown in red, acetoin shown in blue, and remaining absorption due to oligomer product shown in green.
In the spectrum after one hour of photolysis, there is still some amount of unphotolyzed pyruvic acid remaining (scaled experimental spectrum of pyruvic acid shown as the red broken line). Acetoin is also seen as an aqueous phase product (scaled experimental spectrum shown as the blue broken line). The addition of these two components, however, does not account for the entire absorption between 250 and 400 nm shown in Fig. 4.5. The residual absorption is shown in green (broken line). This absorption cannot be due to the oligomer dimethyltartaric acid, as it is lacking in a UV chromophore in the wavelength range of interest. Rather, this residual absorption is consistent with another dimer product containing a UV chromophore. A UV-visible absorption spectrum was also taken after two hours of photolysis (not shown). No new absorbers were apparent in the spectrum, but a further decrease in pyruvic acid contribution is observed as well as an increase in the contribution due to acetoin and the oligomer product, consistent with the progression of the same reaction scheme.

4.4 Discussion

Aldehydes, and to a lesser degree ketones, are known to hydrate in aqueous solution [47-54]. It is known that pyruvic acid (PA) is reversibly hydrated in aqueous solution to its gem-diol, and that the equilibrium is both pH and temperature dependent, with lower pH and lower temperature both favoring the hydrate [55, 56]. In aqueous solution at 298 K, pyruvic acid exists ~35% in its keto form, with the majority existing as its gem-diol [13, 49, 50]. The keto form contains a UV chromophore, which can be excited in the near UV to induce photolysis. UV irradiation of aqueous PA is known to result in phosphorescence from a $^3(n,\pi^*)$ state [57]. This triplet is presumed to be involved in the aqueous phase photochemistry of pyruvic acid. Ab initio calculations [36] have suggested that the $S_1$ state of PA is $^1(n,\pi^*)$, and that this is higher in
energy than both the T₁ and T₂ states, which are found to be \(^3(n, \pi^*)\) and \(^3(\pi, \pi^*)\), respectively. Assuming these calculations to be at least qualitatively correct, it seems likely that the UV photochemistry of PA with light of \(\lambda > 300\) nm proceeds by the sequence \(S_0 \rightarrow S_1 \rightarrow T_2 \rightarrow T_1\). This intersystem crossing to the triplet surface is markedly different than the gas-phase photochemistry, which occurs solely on the singlet surface to generate methylhydroxycarbene [28-30, 58]. However, in the aqueous study presented here, PA is seen to react from its T₁ state to produce acetoin, lactic and acetic acids, and oligomers, as described below and depicted in Scheme 4.1.

Scheme 4.1: Summary of the proposed mechanism for aqueous PA photochemistry.
CBS-QB3 calculations suggest that the T\textsubscript{1} state of PA can abstract a hydrogen atom from the carboxyl group of PA with a classical activation enthalpy of 2.7 kcal/mol. The reaction is found to be endothermic by 0.9 kcal/mol at the same level of theory. The corresponding calculation cannot be carried out for hydrogen atom transfer from the carboxyl of the PA hydrate, because the resulting carboxyl radical is found to be unbound: it loses CO\textsubscript{2} with zero activation barrier. In accord with this finding, the computed transition state structure for H-atom abstraction by PA T\textsubscript{1} from PA hydrate has one very long C–C bond (Figure 4.6).

![Figure 4.6: B3LYP/CBSB7 transition state structure for the reaction of PA T\textsubscript{1} state with ground-state PA hydrate. An IRC calculation reveals that this is the transition state for a concerted H-atom transfer and decarboxylation.](image)

When an intrinsic reaction-coordinate calculation is carried out at the B3LYP/CBSB7 level (which is used for geometry optimization in the CBS-QB3 composite method), it reveals that the reaction is a concerted hydrogen-atom transfer and decarboxylation. Hence, the products would be a triplet radical pair with substantial separation between the spins, as shown in reaction (1) of Scheme 4.1. The overall reaction with the gem-diol has a slightly higher classical barrier (4.3 kcal/mol) than the PA (T\textsubscript{1}) + PA (S\textsubscript{0}) reaction (used in ref. [13]), but unlike that reaction, is exothermic by 22.1 kcal/mol, according to the CBS-QB3 calculations. Thus, although it is conceivable that the photo-excited T\textsubscript{1} PA molecule could abstract a hydrogen atom from either a
ground-state (S₀) PA molecule in its keto or gem-diol form, the reaction with the gem-diol form is utilized here due to its excess in solution as well as its favorable exothermicity and concerted H-abstraction and decarboxylation.

The radical pair shown in reaction (1) of Scheme 4.1 will be generated in a solvent cage, but geminate reactions between the radicals will presumably not occur until the pair has undergone intersystem crossing to a singlet-coupled state. In competition with this, escape of the radicals from the cage is likely. Hence, one must consider several possible radical-radical reactions. Two of these reactions, resulting in products detected in this work are shown in reactions (2) and (3) of Scheme 4.1. The product of reaction (2) is a hydrate of acetolactic acid. Its dehydration should be thermodynamically favorable because, unlike PA hydrate, the product is not an α-keto acid. Decarboxylation of acetolactic acid, known to occur within minutes at 25 °C in aqueous solution [41, 42], would yield acetoin, a principal product of aqueous PA photolysis. This sequence is depicted in reactions (4a) – (4c) of Scheme 4.1. Finally, Reaction (3) of Scheme 4.1 is a disproportionation that generates lactic and acetic acids, both of which are observed products of aqueous PA photolysis here (see NMR results, Fig 4.2). The alternative disproportionation, shown below in reaction (i), would generate ground-state PA and the hydrate of acetaldehyde, which is also a known product of PA photochemistry [39], but was not detected here.

\[ \text{(i)} \]

\[
\begin{align*}
\text{O} & \quad \text{H} \\
\text{H}_3\text{C} - \text{OH} & \quad \text{OH} \\
\text{H}_3\text{C} - \text{OH} & \quad \text{OH} \\
\text{H}_3\text{C} - \text{OH} & \quad \text{OH} \\
\text{H}_3\text{C} - \text{OH} & \quad \text{OH} \\
\end{align*}
\]
In addition, there could be other recombination and disproportionation reactions occurring, like the one shown below in reaction (ii).

\[
\text{H}_3\text{C} - \text{OH} \quad \text{H}_3\text{C} - \text{OH} \quad \rightarrow \quad \text{HO} \quad \text{HO} \quad \text{HO} \\
\text{H}_3\text{C} \quad \text{HO} \quad \text{CH}_3
\]

(ii)

Reaction (ii) would generate a double hydrate of butane-2,3-dione, which has not been reported or detected here as a product of PA photochemistry, and would probably not be stable under the photochemical reaction conditions. In addition to the symmetric recombination reactions shown in reaction (5) of Scheme 4.1 and reaction (ii) shown above, there could presumably be disproportionation reactions, involving hydrogen-atom transfers between the radicals. Such reactions may be kinetically important, but they are not significant in terms of product identification because they generate products already considered in Scheme 4.1 or products that are not observed here, and hence they are not discussed further.

In addition to acetoin, lactic and acetic acids, small oligomers (dimers) were observed both in this work and in the previous work by Guzman et al. [13] in aqueous solution, and by Leermakers and Vesley [39] in organic solvents. Reaction (5) of Scheme 4.1 shows the generation of 2,3-dimethyltartaric acid, presumably in both meso and DL diastereomers, from reaction between two molecules of the prevalent radical intermediate (PA•). This same oligomer has been identified in earlier studies [13, 39], and is detected in the present work as a significant product of aqueous PA photochemistry using Mass Spectrometry (Fig. 4.3). This dimer product is also consistent with the approximate size of oligomers detected using DOSY NMR (Fig. 4.4). Although at least one other dimer product is detected in this work, as described in the results
section, since no structural information is available, it is not included in the mechanism presented here.

The key feature of our mechanism is the concerted hydrogen-atom transfer and decarboxylation shown in reaction (1) of Scheme 4.1. According to our CBS-QB3 calculations, the radical H₃C–C(OH)₂–CO₂• resulting from H-abstraction from the gem-diol of pyruvic acid, is unbound; it decarboxylates with zero barrier. This results in the concerted H-abstraction and decarboxylation proposed here. By contrast, the radical H₃C–CO–CO₂•, resulting from H-abstraction from a pyruvic acid molecule in its keto form, is found to be bound at the same level of theory. This emphasizes the need for water in the solution-phase photodecarboxylation chemistry presented here (reactions (1) – (4) in Scheme 4.1). The concerted H-atom transfer and decarboxylation of reaction (1) of Scheme 4.1 requires the presence of the gem-diol, which is formed only in aqueous environments.

There are a few key points to emphasize concerning this aqueous phase mechanism. First, it is important to note that CO₂ is produced in aqueous solution concurrent with the formation of acetoin, lactic and acetic acids, but is not required in the production of the oligomer dimethyltartaric acid. In our mechanism, the formation of dimethyltartaric acid (reaction (5) of Scheme 4.1) does not require water or necessarily the production of CO₂, merely a good H-atom donor to produce the radical intermediate PA•. Leermakers and Vesley [39] originally proposed this to explain their observation of photoreduction in organic solvents (in contrast to photodecarboxylation), proposing H-abstraction facilitated by reaction of the T₁ state of PA with a solvent molecule, resulting in PA• formation followed by self-reaction to form dimethyltartaric acid. Our results are consistent with this assertion. It is also interesting to note that gas-phase photolysis does not require the presence of water for decarboxylation [39], whereas solution
phase decarboxylation chemistry does. This is likely due to the fact that gas-phase photochemistry of pyruvic acid occurs predominantly on the singlet surface, with pyruvic acid directly decarboxylating in a unimolecular reaction, to form a methylhydroxycarbene, followed by H-atom transfer to form acetaldehyde and vinyl alcohol [28, 58]. In water however, the chemistry occurs on the triplet surface, proceeding bimolecularly through reaction of one molecule of PA in the T1 state with one molecule of PA in its gem-diol form. Water appears to be playing a role in energy flow from the singlet excited state to the triplet surface, in contrast to the favored singlet chemistry in gas phase. Once the photo-excited PA molecule is on the triplet surface, water again plays a role in allowing for bimolecular reaction specifically with the gem-diol form of PA (the predominant form in aqueous environments).

This photochemistry of pyruvic acid in aqueous solution is also known to be pH sensitive, with the protonated form of pyruvic acid decarboxylating with a yield nearly 20 times that of the anionic form (pyruvate) at higher pH values [39], an observation that is consistent with the mechanism presented here. The presence of the acid hydrogen atom is essential to the decarboxylation chemistry, confirming the first abstraction – decarboxylation reaction of Scheme 4.1. It is interesting to note that this pH effect is exactly the opposite of what has been found to occur for reaction with OH [16], a competing removal pathway for pyruvic acid in the troposphere. This indicates that, in aqueous environments, the dominance of either direct photolysis or OH reaction in the removal of pyruvic acid may itself be pH dependent.

Finally, it has been observed previously that UV irradiation (λ > 300 nm) of aqueous PA glass at 77 K results in the formation of triplet-coupled radical pairs with a separation of ≥ 5 Å between the spins, and is accompanied by ultrafast decarboxylation [59]. The explanation offered for the last observation is a long-range electron transfer between the S0 and T1 states of
PA, resulting in a triplet-coupled radical ion pair. The radical cation component of this pair is proposed to be responsible for the decarboxylation [13, 59]. The mechanism presented in this work provides an alternative explanation for this cryogenic aqueous glass photochemistry. During the concerted H-abstraction and decarboxylation of reaction (1) in Scheme 4.1 (transition state structure depicted in Fig. 4.6), the carbons that carry the highest spin density in the products are already separated by 5.5 Å in the transition state structure. It seems that this mechanism could provide an alternative explanation of the EPR spectrum, resulting in similar spin separations, and the ultrafast decarboxylation seen in the aqueous glass photochemistry of PA, in lieu of the radical ion pairs previously proposed [59].

4.5 **Implications for the Modern Atmosphere**

The contrast between the chemistry performed by UV photolysis of gaseous and aqueous phase pyruvic acid highlights the importance of a thorough understanding of chemistry as a function of the molecule’s environment for inclusion in atmospheric models. This work shows that photochemical processes in the aqueous phase of aerosols result in different products than the analogous gas phase chemistry, thus affecting aerosol composition and optical properties differently. The bulk ocean is slightly basic, causing the progression of this reaction scheme near the ocean surface (where UV light still penetrates) to be unlikely due to the presence of pyruvic acid predominantly in its anionic form. Atmospheric aerosols, however, are known to experience a wide range of pH values throughout their atmospheric lifetime, and can reach acidic pH values where this chemistry will become dominant [60]. These aerosol particles have the added advantage of concentrating the reactant species through selective evaporation of water, resulting in high concentrations of reactant monomers [61-65]. Therefore, although this
chemistry is unlikely to be an important contributor to bulk ocean chemistry, it will be important in acidic aqueous atmospheric aerosols. In addition, the production of acetoin, which partitions both to the aqueous phase as well as the gas phase, may contribute to the seeding of new particles [66]. Finally, the direct photolysis of aqueous pyruvic acid presented here as well as the known competing OH reaction pathway result in the formation of oligomers, a unique product to the aqueous phase chemistry (compared with the gas phase chemistry), which have been implicated in SOA formation [1, 5, 12, 14].

### 4.6 References for Chapter 4


44. Frisch, M.J., et al., Gaussian 03, Revision C.02, 2003.


5 Pyruvic Acid: Light-Initiated Chemistry in the Origin of Life

5.1 Introduction

The ability to capture and utilize external energy to drive chemical reactions is a hallmark of life. Thus, finding an environment where necessary chemical reactions for life are coupled with an abundant energy source is an essential part of origin of life research. On early Earth, there were many energy sources available, ranging from geothermal sources such as hydrothermal vents [1, 2] to shock waves from impacts [3, 4] to electrical discharges [5, 6]. However, the most abundant energy source for ancient Earth was sunlight [7], as it is in the contemporary atmosphere [8]. Shapiro [9] has proposed the inclusion of a “driver reaction” coupled to an energy source as essential to the emergence of a primitive metabolic system prior to the more complex enzymatically driven processes seen today. He has specifically proposed pyruvate (or pyruvic acid in its protonated form) as a promising prebiotic molecule to jump-start primitive metabolism, due to its central role in many known metabolic pathways on Earth today, where it serves as an intermediate in both aerobic and anaerobic metabolism. This allows for its potential use in both very primitive life in the early anoxic environment on Earth and after the Great Oxygenation Event as more complex life evolved.

In addition, pyruvic acid was likely available on early Earth through abiotic synthesis under hydrothermal vent conditions [10, 11] as well as exogenous synthesis and delivery by meteorites [12, 13], although its exact concentration is unknown. In this chapter, the photochemistry of pyruvic acid detailed in Chapters 3 and 4 will be discussed in a prebiotic context.

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context with further suggestions of the usefulness of this chemistry in future studies of the origin of life. The emphasis is on how pyruvic acid, coupled to the energy source provided by the early sun, cannot only increase the carbon complexity of the system as a whole, synthesizing four or higher carbon-number molecules from a three carbon molecule, but can also produce metabolites seen in modern bacterial cells in the absence of enzymatic catalysis.

Despite its prevalence on early Earth and importance for modern life in photosynthetic processes, light has seldom been used as an energy source in origin of life research. Cyanobacteria, thought to have existed as long as 3.5 bya [14], are photosynthetic organisms, suggesting the antiquity of light-initiated processes in life. However, photosynthesis is a complex process. Today, photosynthesis is initiated by visible light absorption by the pigment chlorophyll. The resultant light energy is then transformed into usable chemical energy for the cell through the usage of complex biological machinery and networks. Extending this notion to early forms of life has been pointed out to have two problems: first, biological pigment molecules are hard to conceive as prebiologically plausible, and second, even if a primitive pigment could be found, there had to be a complicated electron transport network present to transform the absorbed light energy into chemical energy [7]. Although these concerns are valid for a primitive form of biological photosynthesis, photochemistry is a much more widely applicable phenomenon. Even small molecules can absorb light depending on their chemical functionality, albeit often at much higher energies, and subsequently undergo unique chemical reactions. Some prebiotically relevant light driven reactions have been investigated [15-17], primarily for the production of small organic molecules like cyanide (from UV light and electric discharge through a mixture of gases containing CO₂, CO, and N₂) [7] and formaldehyde (from UV light on a gas phase mixture of CO₂, water, and N₂) [18], or in photoredox chemistry,
necessitating coupling with a metal catalyst, as is the case in the demonstrated abiotic synthesis of sugars [19].

However, the penetration of high-energy UV photons to the surface of early Earth is often thought of as a significant problem for the synthesis of more complex chemical compounds [20, 21]. UV photons have enough energy to break chemical bonds, thus are often thought to break apart complex molecules into simpler ones, contrary to the necessity of synthesizing more complex molecules in the progression towards life’s origins. On Earth today, CO$_2$ and O$_2$ prevent the majority of the highest energy UV light (λ < 195nm) from reaching Earth’s surface, and ozone (O$_3$) blocks wavelengths less than ~290nm. However, oxygen species (i.e. O$_2$ and especially O$_3$) would have been scarce in the anoxic Archean atmosphere, leaving only CO$_2$ to block wavelengths less than ~190 nm from reaching the surface [22]. In the absence of a UV shield such as ozone, significant UV intensity would reach Earth’s surface (as seen in the blackbody radiation curve at 5700K plotted in Figure 1), which could have been detrimental to early life. The young sun experienced by the early Earth is also suggested to have had more UV intensity by a factor of 10-1,000 [23] merely exacerbating the problem of the absence of a UV shield. This has prompted many origin of life researchers to search for a plausible UV shield on early Earth, suggesting fractal aerosols [24], ammonia [25], sulfur [26], and various other organic hazes [27-30]. It is also one of the reasons why deep-sea environments, such as hydrothermal vents, are currently considered advantageous environments for the origin of life [31, 32].

Here, UV photons are utilized as the energetic driver for building more complex molecules via plausible aqueous prebiotic chemistry. Aqueous environments were not only prevalent on the water-rich early Earth, but are the host environment of modern life. In addition,
water provides a very special reaction environment [33-36], even having the ability to markedly change a reaction pathway. This is the case in the system presented here: pyruvic acid photochemistry in the gas phase has traditionally been shown to result in a decrease in chemical complexity, breaking the molecule in half producing a C₂ molecule, acetaldehyde or vinyl alcohol, and CO₂ [37-41]. The presence of water markedly alters the photochemistry of pyruvic acid, as described below, allowing for the generation of complex products.

In modern biology, reactions resulting in increased chemical complexity (one function of metabolism) are often facilitated by enzymatic catalysis, processes not available in the most primitive life-forms. Pyruvic acid, a molecule integral to much of life today through its versatile role in metabolism, absorbs light through an n→π* transition, with an absorption maximum of ~325 nm in aqueous solution, as shown in Figure 5.1.

![Figure 5.1: Electronic absorption spectrum of aqueous pyruvic acid (left axis) and the calculated blackbody radiation of the sun at 5700K (right axis) with area under the curve shaded in yellow.](image)

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9 See Chapter 3 for a discussion of new results illustrating that this chemistry is highly dependent upon reaction environment, resulting in more available reaction products than acetaldehyde and vinyl alcohol.
This absorption has significant overlap with the solar spectrum (yellow shaded section of Fig. 5.1) resulting from a blackbody at 5700K in the absence of any UV absorbers (as shown in Fig. 5.1). Even in an atmosphere with a UV cut-off of ~290nm, as is the case with the ozone shield in Earth’s modern atmosphere, pyruvic acid photochemistry will still occur [42, 43]. The chemistry presented here takes advantage of the likely increased UV intensity that reached the surface of early Earth to provide energy to molecules in an aqueous environment to ultimately build chemical complexity in a non-enzymatic system. The chemistry documented here is contrary to the traditional notion of photo-induced decomposition. The aqueous environment is an important ingredient in this chemistry, since pyruvic acid photochemistry proceeds through a very different pathway in the gas phase [37-41]. In this aqueous environment, the constant energy source provided by the sun potentially serves as a prelude to the complex biological machinery utilized later in biochemical evolution.

5.2 Materials and Methods
Pyruvic acid (98%) was purchased from Sigma-Aldrich, and was distilled under reduced pressure prior to use. Pyruvic acid was then prepared to a final concentration of 0.1M in distilled water, with a pH of 2 (pH remained constant throughout the photolysis). Photolysis was performed, under ambient air and in a glass container covered with a quartz window, for 2 hours using unfiltered light from a 450 W Xe arc lamp (Newport), while the pyruvic acid solution was kept chilled in a 4°C temperature controlled water bath (actual temperature of the reaction solution was likely between 4 – 10°C [44]). Xe arc lamps are often used as solar simulators due to their significant spectral overlap with natural sunlight (wavelength output ~ 200 - 2500 nm, corresponding to a color temperature of ~5800K), thereby exposing the sample of interest to a
wide-range of wavelengths congruent with what it would experience upon exposure to the natural sun. Due to this similarity, the discussion presented here will refer to the energy source for reaction as “sunlight” or as “simulated sunlight” in reference to the experiments themselves. The photolyzed pyruvic acid solution was allowed to return to room temperature before any subsequent analytical techniques were performed. Acetoin (≥92%, natural) was also purchased from Sigma-Aldrich, and was used without further purification. The NMR reference sample (Fig. 5.2) was prepared by dissolving acetoin in distilled water to a final concentration of 0.04M. Lactic acid (racemic, meets USP testing specifications) was purchased from Sigma-Aldrich and was prepared to a final concentration of 0.04M in DI water without further purification.

5.2.1 UV/visible Spectroscopy
UV-vis spectra were obtained using a USB2000 miniature fiber optic UV-vis spectrometer from Ocean Optics.

5.2.2 Nuclear Magnetic Resonance
NMR spectra were acquired using a Varian INOVA 500 MHz NMR spectrometer operating at 499.60 MHz for $^1$H observation. All samples were prepared as 0.1M solutions in pure water, with a volume of 0.8 ml in 5mm NMR Tubes. 0.050 ml of D$_2$O (99.9% deuterated) was added to provide a field-frequency lock, yielding a final solvent composed of approximately 95% H$_2$O, 5% D$_2$O. In order to perform NMR with $^1$H detection in aqueous solution, the WET solvent suppression was used to eliminate > 99% of the H$_2$O signal [45]. Two-dimensional NMR experiments were performed using the WET solvent suppression pulse-sequence prepended to the experimental pulse sequence, as described in Chapter 4.
5.3 Results and Discussion

The absorption of light by pyruvic acid is known to result in efficient decarboxylation in aqueous solution [42-44, 46-48]. Although this effect has been known for many years, the additional products formed in the aqueous-phase UV photochemistry remain controversial as discussed in detail in Chapter 4. In Chapter 4, it is shown [42] that acetoin is present in both the gas phase above the sample after photolysis as well as in solution along with lactic acid, acetic acid, and dimers with the same molecular weight as those detected by Guzman et al. [43]. The detailed mechanism of this aqueous phase photochemistry is described in Chapter 4, a modified version of which is shown here focusing on the products and processes of prebiotic interest (Scheme 5.1), incorporating steps relevant to early metabolism.

Here, we present a portion of the gCOSY (two-dimensional gradient-selected homonuclear correlation spectroscopy) NMR spectra taken of the solution after photolysis in Figure 5.2, to highlight the two products formed from pyruvic acid photolysis most relevant in a prebiotic context: lactic acid and acetoin. Figure 5.2 shows an expansion of the through-bond, cross-correlation NMR signals that are shared by the methyl resonances to the adjacent methyne (CH) resonance within the molecule. The photolysis mixture spectrum (black) is overlaid with pure aqueous NMR spectra of acetoin (red) and lactic acid (blue). The cross peak at (1.36, 4.4) ppm is attributed to acetoin and the neighboring cross peak at (1.40, 4.35) ppm is attributed to lactic acid. The cross-peak presented in Figure 5.2 confirm that both of the signals corresponding to lactic acid and acetoin are present in the photolysis product mixture.
Figure 5.2: Overlay of expanded region of HH-gCOSY NMR of 0.1M pyruvic acid photolysis products (black), lactic acid (blue), and acetoin (red).

A reaction scheme for the light-initiated chemistry of aqueous pyruvic acid is shown in Scheme 5.1, highlighting the products observed in this work as well as a few other pathways shown in the literature and in modern biology.
Scheme 5.1: Simplified reaction scheme of the photochemistry of pyruvic acid increasing carbon complexity through the production of dimers, and producing metabolites in the absence of enzyme catalysis [42]. The products observed in this work are depicted in red. The synthesis of branched-chain amino acids from \(\alpha\)-acetolactic acid has not been demonstrated in prebiotically plausible conditions, but is enzyme-catalyzed in modern biology and is depicted in gray in the reaction scheme. Pyruvic acid can be produced under prebiotically plausible conditions from lactic acid through mineral photoelectrochemistry [11] and is shown here in green.

Once the highly reactive radical intermediate (PA\(\cdot\)) is formed, three different pathways may be pursued. In the first pathway, as observed by Guzman et al. [43] and in this work, dimers are formed. In the second pathway, \(\alpha\)-acetolactic acid can be formed. \(\alpha\)-acetolactic acid is a \(\beta\)-keto
acid that spontaneously decarboxylates thermally to form acetoin [48, 49], an important bacterial metabolite [50]. \(\alpha\)-acetolactic acid is also the intermediate in modern biology for the enzyme-catalyzed synthesis of branched chain amino acids (as shown in gray in Scheme 5.1). Although no abiotic pathway has been found as of yet for the conversion of \(\alpha\)-acetolactic acid to these amino acids, the abiotic production of \(\alpha\)-acetolactic acid suggests the possibility may be worth considering under prebiotic conditions. In the final pathway, lactic acid can be formed from these radical-radical reactions. Guzman and Martin [11] have then shown that pyruvic acid can be produced from lactic acid through mineral photoelectrochemistry (green arrow in Scheme 5.1) in environments such as shallow hydrothermal vents, creating a possible abiotic regeneration pathway for pyruvic acid.

The abiotic formation of acetoin is of particular importance in a prebiotic context. In modern bacterial cells, acetoin serves as a pH regulator, through the prevention of over-acidification of the cell’s interior, but also is used as an energy storage molecule and can be used as a substitute for glucose [50]. Acetoin is formed in modern bacterial metabolism through glucose [50] or citrate fermentation [51], both of which produce pyruvic acid as an intermediate. The progression from pyruvic acid to acetoin then utilizes two primary enzymes, \(\alpha\)-acetolactate synthase (ALS) and \(\alpha\)-acetolactate decarboxylase (ALDC) [50].

\[
\begin{align*}
2 \text{Pyruvate} & \xrightarrow{ALS} \alpha\text{-acetolactate} \xrightarrow{ALDC} \text{Acetoin} \\
& \xrightarrow{CO_2} \xrightarrow{CO_2}
\end{align*}
\]

Scheme 5.2: Enzyme-catalyzed production of acetoin from two pyruvate molecules, as observed in bacterial metabolism [50, 51]
As shown in Scheme 5.2, ALS facilitates the reaction of two pyruvic acid molecules with the loss of CO$_2$ to form α-acetolactate. Then, ALDC catalyzes the decarboxylation of α-acetolactate to acetoin. In this work, light replaces the ALS enzyme, giving the necessary energy to convert pyruvic acid to α-acetolactic acid. Then, α-acetolactic acid can spontaneously decarboxylate to acetoin, albeit more slowly than through enzyme catalysis. It is important to note here that although the decarboxylation of α-acetolactate to acetoin is spontaneous thermally, its rate is dependent upon pH [49]. In the higher pH values present physiologically, α-acetolactic acid exists in its anionic form (as α-acetolactate), and its decarboxylation is slower, thereby requiring the ALDC enzyme for the decarboxylation to occur on a reasonable biological timescale. However, at the low pH values utilized in this experiment (pH ~ 2), α-acetolactate would be produced in its protonated form (as α-acetolactic acid) which has been seen to decarboxylate on the order of minutes, much faster than its anionic counterpart [49]. This low pH environment is relevant to early Earth conditions found in the likely acidic early ocean [52] or atmospheric aerosol particles (which acidify by dissolution of CO$_2$ to form carbonic acid, H$_2$CO$_3$).

One of the functions of modern metabolism is to increase the carbon complexity present in a given system. Acetoin (a C$_4$ molecule) is produced from pyruvic acid (a C$_3$ molecule) through enzyme catalysis, but the exact mechanism of enzymatic catalysis is currently unknown. However, the enzymes allow for non-spontaneous or kinetically hindered reactions to occur. On early Earth, it is often problematic to conceive of plausible reaction schemes where such reactions occur spontaneously and abiotically. Here, it is shown that sunlight can facilitate the same reactions that occur enzymatically in modern biology. Therefore, the abiotic production of acetoin through UV photolysis of pyruvic acid provides a potential route for the beginnings of a
primitive metabolism, by increasing the carbon complexity of the system, prior to the emergence of more complex biological machinery.

The dimers formed through UV photolysis show further complexity, which may be interesting prebiotically. Here, it was also observed that these dimers, when extracted into chloroform, showed increased partitioning to the water surface when compared with the same extract of pyruvic acid before photolysis. Due to this increase in hydrophobicity, it is possible that such dimers could have been utilized as components of primitive membrane structures as has been suggested of those formed in simulated hydrothermal vent conditions [53]. Under such conditions, pyruvic acid was seen to form much larger polymers and even aromatic compounds, subsequently forming primitive compartments similar to those formed from Murchison meteorite extracts [54].

The production of lactic acid (confirmed in solution through NMR, Fig. 5.2) from the UV photolysis of pyruvic acid is also of particular interest. Guzman and Martin have proposed the conditions provided by modern-day shallow-water hydrothermal vents as representative of conditions present on early Earth [11]. In these shallow vents, light from the sun may penetrate and participate in the chemical reactions catalyzed by colloidal semiconductor minerals in these geochemical environments [11, 55, 56]. They have then shown that numerous reactions important in the reductive tricarboxylic acid (rTCA) cycle may be facilitated in these environments through photoelectrochemistry. Amongst the reactions they have demonstrated is the production of pyruvic acid from lactic acid [11]. The inclusion of this reaction into the ones demonstrated in this work (Scheme 5.1) provides a regeneration pathway for pyruvic acid after its removal through photochemistry. In essence, this provides a simple cyclic reaction to facilitate a more long-term use of this reaction network.
Interestingly, lactic acid may also serve as a primitive regulator for the pyruvic acid photochemistry presented here. Lactic acid can regenerate one molecule of pyruvic acid through de-activating a photo-activated pyruvic acid molecule (PA*, see Scheme 5.3).

Scheme 5.3: Deactivation of photo-excited pyruvic acid (PA*) through reaction with lactic acid (LA), regenerating one molecule of lactic acid (LA) and one molecule of deactivated pyruvic acid (PA).

In this reaction PA* abstracts a hydrogen from lactic acid (LA) producing two PA• radical intermediates. Then, these two PA• molecules can undergo a disproportionation reaction to reform lactic acid and a ground-state pyruvic acid molecule. This reaction can be thought of as a regulatory pathway if this photochemistry is occurring in some sort of enclosed environment, such as an atmospheric aerosol particle [57] or other primitive enclosure (i.e. a protocell) near the water surface. As lactic acid accumulates in the enclosure, the likelihood of a photo-activated pyruvic acid (PA*) molecule encountering a lactic acid molecule increases relative to it encountering another reaction partner shown in Scheme 5.1. Since reaction with lactic acid de-activates the PA* molecule, returning it to its unphotolyzed ground-state, it would slow down the production of the other products (acetoin, dimers, etc.). In modern metabolism, regulation is a key component of the functioning of cells, preventing damage to the cellular system due to accumulation of one of the metabolites. In a similar fashion, lactic acid behaves as a primitive regulator for pyruvic acid photochemistry.
It is important to note, however, that the chemistry presented in this work has not yet achieved the label of an autocatalytic reaction network, as commonly discussed theoretically by metabolism-first proponents [9, 58-59]. In reality, there has been no experimental validation of the emergence of such networks spontaneously in the absence of more complex biological machinery. To truly conceive of a plausible primitive metabolism, this step must be demonstrated. This work and others relating to primitive metabolism [11, 55] merely show plausible abiotic syntheses of important metabolic components, and/or abiotic reactions facilitating functions analogous to those performed by enzyme-mediated metabolism today (as in increasing carbon complexity shown here). Therefore, more experimental work is needed to bridge the gap between mimicking certain functions of a metabolic network in plausible prebiotic conditions and actually creating a complete, propagating “proto-metabolism”.

Finally, the photochemistry presented in this work necessitates an aqueous environment on early Earth with access to UV light from the sun. Therefore, plausible environments would include regions of shallow water (the same environment that Guzman and Martin propose), near the ocean surface, or in atmospheric aerosols. Atmospheric aerosols have been proposed previously to be important in prebiotic chemistry due to their provision of a unique reaction environment, and their constant circulation between the feedstock of molecules provided by the bulk ocean and the fluctuating conditions they experience throughout their atmospheric lifetime [36, 57, 60-62]. In the case of the photochemistry presented here, atmospheric aerosols would be an advantageous environment for this chemistry due to their ability to concentrate organic molecules (like the pyruvic acid reactants) through selective evaporation of water, and their allowance for penetration of the UV light from the sun through the entire volume of the particles. Therefore, the potential environments for this chemistry on early Earth are plentiful, and can
easily be coupled with advantageous reaction environments proposed by others, such as hydrothermal vents [10, 56, 63], allowing for combination and exchange of reactants and products.

In addition, as illustrated in the preceding chapters, pyruvic acid photochemistry is highly sensitive to environment. When the aqueous phase photochemistry was performed in the absence of oxygen (under a nitrogen-only atmosphere), the yield of acetoin was greatly increased, causing it to compete as a more major reaction product [64]. This is important as the early Earth is thought to have had an anoxic atmosphere prior to the emergence of life. It is significant that the production of a potentially useful biological product is enhanced in a more prebiotically relevant environment.

Finally, in addition to the photochemistry of pure pyruvic acid in water, this chemistry can be envisioned as useful in other reaction pathways on early Earth. For example, the radical intermediate PA• can be used as a radical initiator for radical chain reactions as in the reaction of PA with methyl vinyl ketone (MVK) shown below in Scheme 5.4. MVK is known to produce long oligomers through reaction with •OH radicals [65]. An analogous reaction scheme can be envisioned using PA• in lieu of •OH, extending the usefulness of this type of chemistry even to the anoxic atmosphere of early Earth.

\[
\begin{align*}
\text{PA•} + \text{MVK} &\rightarrow \text{long oligomer} \\
\end{align*}
\]

Scheme 5.4: Reaction of PA• with MVK initiating a radical chain reaction to produce a long oligomer of n repeating units
Many biomolecules, such as sugars, are formed from identical repeating units. Thus, utilizing the easily generated PA•, radical chain reactions may be promoted to form such molecules. This is especially useful when direct photochemistry or other drivers are unavailable for such molecules (as is the case with MVK).

5.4 Conclusions

Pyruvic acid photochemistry illustrates the advantage of utilizing sunlight as an energy source coupled with an aqueous environment in abiotic chemical reactions on early Earth. Sunlight, as the most abundant energy source, is shown here to facilitate an increase in chemical complexity of the system as a whole, while also producing molecules utilized in modern bacterial metabolism. In addition, the environments for this chemistry relevant to early Earth are abundant, only requiring the presence of water and access to UV photons from the sun. Environments such as the region near the surface of the ocean or aqueous atmospheric aerosols then allowed for mixing of both reactants and products of this chemistry with other known prebiotic sources on early Earth such as hydrothermal vents. Combining the photochemistry of pyruvic acid with chemistry such as mineral photoelectrochemistry from shallow hydrothermal vents allows for the regeneration of pyruvic acid from lactic acid (one of the products of its photochemistry presented here). This allows for a primitive chemical cycle to form, decreasing the depletion of the photochemical reactant and allowing for the maintenance of this chemistry over time. This work illustrates the advantageous role of UV photons in increasing complexity on early Earth, on the road towards the origin of life.

5.5 References for Chapter 5


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6 Photoinitiated Synthesis of Self-Assembled Vesicles

6.1 Introduction

Enclosures are universally considered necessary for life [1]. The search for the emergence of such primitive cellular constructs in the origin of life on Earth requires the existence and/or synthesis of a prebiotically plausible membrane component (a surfactant), followed by self-assembly into a stable enclosed structure [2]. Although much work has been performed studying the properties of vesicles composed of various surfactants [3-6], the synthesis of such self-assembled structures and their components under plausible prebiotic conditions has received much less attention. Here the photochemical synthesis of a double-tailed surfactant is shown from a single-tailed one followed by spontaneous self-assembly into vesicles (pictorially shown in Fig. 6.1 below).

![Figure 6.1: Cartoon illustrating, from left to right, photochemistry of 2-OOA forming OOA-OOA dimer followed by self-assembly into vesicles.](image)

The single-tailed surfactant utilized, 2-oxooctanoic acid (2-OOA, an 8-carbon oxo-acid), is a plausible prebiotic molecule: oxo-acids have been found in meteoritic samples [7], and the short, 8-carbon chain of 2-OOA is among the most prevalent length synthesized in the common Fischer-Tropsch type synthesis of lipids (C7 – C9 are most common) [8, 9]. With no further perturbation of the reaction system, as the photochemistry proceeds, the products spontaneously self-assemble into stable vesicles. These vesicles were found to be monodisperse (200 nm in diameter), to be temporally (over a timescale of months) and thermally stable (between at least 4 - 22°C), and to persist in the presence of MgCl₂ salt. This work provides a potential route for the synthesis of membrane components and a stable primitive enclosure and contributes to the evolution of primitive membrane structures.

6.2 Materials and Methods
2-OOA (≥99%) was obtained from Sigma-Aldrich and was used without further purification. A 6 mM aqueous solution of 2-OOA was prepared in DI water and sonicated until dissolved, followed by filtration through a 0.1 µm syringe filter. The final solution was then photolyzed, unstirred, using a 450-W Xe arclamp (Newport) for 5 hours while being kept at a constant temperature of 4°C in a temperature-controlled water bath. The Xe arc lamp is used here as a solar simulator due to its significant overlap with the solar spectrum (wavelength output ~ 200 - 2500 nm, corresponding to a color temperature of ~5800K).

6.2.1 Mass Spectrometry
The reaction mixtures, both before and after photolysis, were analyzed using ESI-TOF mass spectrometry.
6.2.2 Fluorescence Microscopy
The self-assembled vesicles were stained with 10 µM Rhodamine 6G (BioReagent, Sigma) and visualized by fluorescence microscopy (Nikon A1R confocal microscope).

6.2.3 Dynamic Light Scattering
The size of vesicles in solution was determined by DLS (DynaPro Titan, Wyatt Technology) after 10-fold dilution at 20°C.

6.2.4 Nuclear Magnetic Resonance
High resolution 2D-DOSY NMR was performed on a Varian INOVA 500-MHz NMR spectrometer operating at 499.60 MHz for ¹H observation using WET [10] suppression of the H₂O resonance, combined with the convection and non-uniform-gradient compensated stimulated PFG echo pulse sequence that is available in the VNMRJ 3.2A instrument (Agilent Technologies, Inc.). The data analysis was performed using the VNMRJ software, including non-uniform gradient compensation, and multi-component fitting of the DOSY decay curves [11, 12].

6.2.5 UV/visible spectroscopy
The electronic spectrum of aqueous 2-OOA was obtained using a USB2000 miniature fiber optic UV-visible spectrometer from Ocean Optics.

6.2.6 Langmuir Trough
The solutions before and after photolysis were identically extracted with chloroform to separate out the hydrophobic components. The chloroform solutions then sat overnight to allow for evaporation of the solvent, and were subsequently re-solvated in a small amount of chloroform for deposition on the Langmuir trough. 100µl of the chloroform extract (either before or after photolysis) was deposited dropwise on an aqueous subphase on the Langmuir
trough. After allowing 15 minutes for chloroform evaporation, $\pi - A$ isotherms were recorded (barrier speed constant at 75 cm$^2$/min).

Surface pressure – area ($\pi - A$) isotherms were obtained using a custom built PTFE Langmuir trough (52 x 7 x 0.5 cm) with computer-controlled, PTFE mechanical barriers allowing for control of surface area (A), coupled with a Wilhelmy balance (KSV-NIMA, Biolin Scientific) to measure surface pressure ($\pi$). The $\pi - A$ isotherm yields surface thermodynamic information, and is used here as an indication of surfactant hydrophobicity through a measure of its surface activity.

### 6.3 Results and Discussion

2-OOA first absorbs light through its carbonyl chromophore with an absorption maximum of 320 nm as illustrated in Figure 6.2.

![Figure 6.2: Electronic absorption spectrum of 6 mM 2-OOA in water with inset illustrating absorption of light by the carbonyl chromophore.](image)
Figure 6.3 illustrates the photochemical mechanism resulting in the production of the dimer molecule OOA-OOA (Fig. 6.3A) as well as its detection by mass spectrometry (mass spectrum before photolysis shown in Fig. 6.3B\textsuperscript{11} and after photolysis in Fig. 6.3C).

Figure 6.3: Photochemical synthesis of double-tailed surfactant (OOA-OOA) from single-tailed surfactant (2-OOA) (A) Photochemical reaction mechanism of 2-OOA to produce OOA-OOA dimer, (B) Mass spectrum of 2-OOA solution prior to photolysis, (C) Mass spectrum of photolysis products with peaks due to OOA-OOA dimer (317.2 m/z), PA-OOA dimer (247.1 m/z) and unreacted 2-OOA (157.1 m/z) indicated.

\textsuperscript{11} It is important to note that the peak at 315 m/z is not the OOA-OOA dimer, but is an unreactive contaminant that is present in the mass spectrum both before and after photolysis.
Analogous to the well-known photochemistry of pyruvic acid [13-15] (which has the same reactive functionality as 2-OOA), the photo-excited 2-OOA molecule can react with a ground-state 2-OOA molecule to form the radical intermediate 2-OOA•. Then, two 2-OOA• radicals recombine to form the OOA-OOA dimer. The products of photolysis, when extracted into chloroform, were observed to be more surface active than 2-OOA prior to photolysis using Langmuir trough methods (Fig. 6.4), confirming the greater hydrophobicity of the photolysis products.

![Figure 6.4: π – A isotherms of chloroform extract before photolysis (red) and after 8 hours of photolysis (black) illustrating greater surface activity of photolysis products.](image)

In addition to the double-tailed dimer OOA-OOA, another dimer product was detected at 247.1 m/z in the mass spectrum. This product is formed through a Norrish Type II photochemical reaction mechanism (Fig. 6.5), but remains as a single-tailed surfactant with similar hydrophobicity to 2-OOA. It is, however, interesting to note that in the mechanism for the production of the PA – OOA dimer pyruvic acid is produced. As seen in Chapters 4 and 5, pyruvic acid is a very interesting molecule in a prebiotic context. Despite the Norish Type II
reaction resulting in the splitting of the 2-OOA molecule in its initial steps, the smaller molecule produced can be quite useful in prebiotic chemistry as a prelude to modern metabolism. Thus, although the final product (PA – OOA dimer) is not discussed further here, the production of pyruvic acid in this reaction could be another interesting source of pyruvic acid in the complex prebiotic environment.

Figure 6.5: Norrish type II photochemical reaction of 2-OOA resulting in the production of pyruvic acid (PA) and 1-pentene. Pyruvic acid then photochemically reacts to form the radical intermediate PA•, finally forming the other dimer detected in the mass spectrum at 247.1 m/z labeled PA – OOA dimer.

Prior to exposure to light, the 2-OOA solution is clear and devoid of detectable particles (image on left in Fig. 6.6A). Although the critical bilayer concentration (CBC) of 2-OOA is not known, similar surfactants have CBCs greater than 100 mM [16], and thus vesicles are not expected, and indeed are not observed, at the low concentration of 6 mM used in this work.
Over the course of photolysis, however, the solution becomes opalescent (image on right in Fig. 6.6A) indicating the presence of particulates in solution. These particles were observed microscopically using both phase contrast microscopy (not shown) and fluorescence microscopy after staining with the membrane stain Rhodamine 6G (Fig. 6.6B). Further, they were determined to be monodisperse with a narrow size distribution centered around 200 nm in diameter using dynamic light scattering (DLS, Fig. 6.6C).

![Figure 6.6: Characterization of self-assembled vesicles (A – C) and indication of stability (D, E). (A) Photograph of clear 2-OOA solution before photolysis (left) and opalescent solution after photolysis (right), (B) Fluorescence microscope image of vesicles stained with Rhodamine 6G, (C) Dynamic light scattering of vesicles formed illustrating mean radius of 100 nm, (D) Fluorescence microscope images of stained vesicles after 5 weeks at 4°C and (E) in the presence of 16 mM MgCl₂.](image)

These particles were stable in solution for months (Fig. 6.6D after 5 weeks time), over the temperature range of 4 – 22°C, and in the presence of MgCl₂ salt (Fig. 6.6E). At 200 nm in
diameter, they are too large to be micelles, and their long-term stability in solution as well as their apparent spherical shape suggest that disordered aggregates of monomers are unlikely.

In addition, the DOSY NMR (Fig. 6.7) spectrum detects these particulates as being much larger than any monomers in solution and to be very slowly diffusing (Fig. 6.7A), yielding a diffusion coefficient of $4 \times 10^{-8}$ cm$^2$/s, consistent with the value of $3 \times 10^{-8}$ cm$^2$/s determined by DLS. This is in contrast to the measured diffusion coefficient of the aqueous free monomer (2-OOA), measured by DOSY NMR to be $7 \times 10^{-6}$ cm$^2$/s. When looking at the decay of the NMR signal with gradient strength (Fig. 6.7B), it is also apparent that the signals due to the hydrocarbon chain protons specifically near the ends of the tails exhibit a two-component decay profile: one short decay with sharp signals sitting atop broadened signals with a very long decay. This is indicative of two types of environments present in solution, one of monomers and one of similar molecules with confined motion. Additional information about the confined molecules can be obtained by noting the absence in signal from the protons closest to the polar head-groups at large gradient strength. This is common in lipid vesicles [17], where the NMR signals from the protons on the rigid head groups are broadened due to dipolar relaxation, while the signals from the more flexible tails exhibit less dipolar broadening and are thusly easier to detect.

Therefore, taken together with the size, monodispersity, and stability, these data indicate that the particulates detected in solution are ordered structures consistent with self-assembled vesicles.
Figure 6.7: DOSY NMR illustrating the diffusion coefficients observed in the mixture of photolysis products, including the superposition of decay profiles for rapidly and slowly diffusion components, in the 0.5-1.5 ppm range of the NMR spectrum. (A) Full DOSY plot. (B) Decay of NMR signal with increase in gradient strength.

This work illustrates the advantageous environment provided by the water surface region. 2-OOA, as a soluble surfactant, partitions significantly to the water surface, resulting in an inhomogeneous distribution of 2-OOA molecules in solution. The surface excess concentration of 2-OOA allows for a local concentration that is greater than the nominal bulk concentration of 6mM. This increases the probability of contact between reactive species, thereby increasing the reaction yield. In addition, it allows for orientation of the hydrocarbon tail of 2-OOA, resulting
in a greater propensity to undergo the photochemical reaction mechanism illustrated in Fig. 6.3A forming the OOA-OOA dimer, rather than the Norrish type II reaction (Fig. 6.5) resulting in a single-tailed dimer. The Norrish type II reaction necessitates more motion of the hydrocarbon tail, allowing it to fold over on itself, a situation requiring a more dilute solute environment (less likely in the surface region). Water surfaces, such as those found on oceans, lakes, and atmospheric aerosol particles, have been implicated as unique prebiotic reaction environments previously [18-24]. Here, we add an additional example of the advantageous environment provided by the water surface for chemistry.

Thus far, simple vesicles (enclosures) composed of fatty acids have been used as a model system for primitive cellular constructs; they have the ability to mimic many functions of modern life and their monomers (fatty acids) are plausible components on early Earth [1-4, 6, 8, 25, 26]. However, such protocells are unlikely to have been the first enclosures due to the very specific environmental constraints put on their synthesis and stability (requisite pH, high concentration, intolerance of vesicles to salts) [27, 28]. Modern cells are enclosed in a membrane composed of primarily double-tailed surfactants having none of the constraints put on fatty acid vesicles, however their prebiotic synthesis is often thought to be too difficult due to their chemical complexity [16]. Here we provide an alternative, sunlight-driven route to primitive enclosures in addition to what has been traditionally considered. Beginning with a dilute solution of a short single-chained soluble surfactant, well below its CBC, simulated sunlight prompts synthesis of a double-tailed surfactant followed by self-assembly without external perturbation. No complicated synthetic steps or preparations are required nor are high concentrations of surfactant. In addition, the resultant vesicles are stable over time and persist in the presence of
Mg$^{2+}$ ions, a likely component of the early ocean [29]. In contrast, the more common models of fatty acid vesicles are unstable in the presence of divalent cations [28].

Regardless as to whether or not the first enclosures were composed of single-tailed surfactants, a transition is needed at some point in molecular evolutionary history to membrane constituents with a double-tailed structure, as they are prevalent throughout modern biology. Once mixed membranes composed of both single and double-tailed surfactants are facilitated, even with minimal double-tailed surfactant, unique competition and subsequent evolution has been seen to emerge [30]. This work illustrates a simple, prebiotically plausible transition from single-tailed to double-tailed surfactants using only the energy provided by the early sun. Therefore, the work presented here provides a possible route to the prebiotic synthesis of a simple enclosure as well as a potential contribution to primitive membrane evolution.

6.4 References for Chapter 6


7 Ionization State of L-Phenylalanine at the Air–Water Interface\textsuperscript{12}

7.1 Introduction

Water surfaces are ubiquitous on Earth and have long been identified as unique environments for chemical and physical processes. Surfaces are known to be important contributors to atmospheric chemistry in the modern atmosphere [1-9], and have been proposed as favorable environments for essential reactions in prebiotic chemistry [10-15]. The potential for reactions at water surfaces is influenced by the state of ionization of the reactants, thus prompting the need for a fundamental understanding of not only the ionization state of potential reactants at the water surface, but also of the ionic character of the bare water surface itself. In this work, the surface-active amino acid L-phenylalanine was used as a probe of the environment experienced by ionizable molecules in the surface region. L-phenylalanine has the unique advantage of possessing a hydrophobic group (an aromatic ring) to drive its adsorption to the surface, but also two different hydrophilic groups, a carboxylic acid and an amine base, which can deprotonate and protonate respectively depending on the ionic environment they experience at the water surface.

Numerous studies have been formulated to attempt to understand the air-water interface on a fundamental, molecular level [4, 16-26]. One property of the air-water interface that has been intensely debated is the propensity of ions for the surface. This has subsequently prompted the investigation of the pK\textsubscript{a} of molecules at the surface versus in the bulk as well as the more fundamental problem of the pH of the bare water surface itself [27-29]. Historically, and still in many textbooks, the surface was considered to favor neutral molecules over their ionic form due

to the repulsion of ionic species from the interface by an electrostatic image force [30, 31]. Experimentally, this is seen as an increase in surface tension with the addition of an ionic species to water [31]. In contrast to this traditional view, more sophisticated techniques and theory in recent years have suggested that not only are some ions present at the surface, but some are actually present in excess over the bulk [16, 21, 27, 32-35].

Although most acknowledge there is a shift in the pKₐ of molecules residing at the surface, there is no agreement on the magnitude or direction of the change. Vibrational Sum Frequency Generation (VSFG) studies have shown that long-chain acids at the surface exhibit a significant increase in their pKₐ when compared to bulk solution [36-38]. A similar effect was seen with long-chain phosphates at the air-water interface using changes in surface pressure/area with changes in subphase pH [39]. In contrast, amphiphilic amino acid residues at the air-water interface (looking at the amine group only) showed a significant decrease in their pKₐ compared with the bulk solution as determined by ¹H NMR titration [40]. In this work, L-phenylalanine contains both an acid (carboxylic acid) and base (amine) group to allow for simultaneous analysis of a high and low pKₐ at the air-water interface. If the shift in pKₐ at the surface is only toward the neutral form (as is indicated by the decrease in pKₐ of the amine group of the amphiphilic amino acid residues [40] and the increase in pKₐ of the fatty acid [36] seen in the literature), then more neutral polar group vibrational bands (i.e. more vibrations due to COOH and NH₂) relative to the bulk should be present in the surface IRRAS spectra throughout the pH range.

Further, the question as to the surface pH of water itself is far from being resolved [33]. Although anions in general are considered to have a slight propensity for the surface over cations, many think that hydronium ions (H₃O⁺) are at a higher concentration at the surface than
hydroxide ions (OH\(^-\)), yielding an acidic surface pH [16, 33]. This has been experimentally confirmed by VSFG and Second-Harmonic Generation (SHG) measurements [32, 41], as well as molecular dynamics simulations [42] for the air-water interface. A similar, but less pronounced, effect was seen at the air-ice interface [43]. Investigations performed in the colloid community using zeta potential measurements and titrations, gas collisions with aqueous microjets, as well as other molecular dynamics simulations suggest the opposite: that the water surface is basic [33, 44-48]. Needless to say, the ionic nature of the bare air-water interface is of paramount importance in determining the ionization of adsorbed species. In the work presented here, the surface ionization state of L-phenylalanine, a naturally occurring amino acid used in modern biochemistry, over a wide range of pH values (pH 1-13) is identified using the in situ, surface-sensitive technique of Infrared Reflection-Absorption Spectroscopy (IRRAS) and subsequently compared with the same molecule’s bulk phase ionization state. This allows for a direct gauge of the change in pK\(_a\) of molecules residing at the surface relative to the bulk as well as an indirect indication of the surface pH experienced by the L-phenylalanine molecules.

7.2 Materials and Methods

Sodium hydroxide (NaOH, ACS grade) and hydrochloric acid (HCl, 37%) were purchased from Mallinckrodt Baker, Inc. All chemicals were used without any further purification. Stock solutions with pH values ranging from pH 0 through 14 were prepared by dissolving the appropriate amount of either sodium hydroxide or hydrochloric acid in distilled water. L-phenylalanine (99%) was purchased from Alfa Aesar, and was prepared to a concentration of 0.1M in the stock pH solutions yielding final pH values of 0.61 (0.1M Phenylalanine in 1M HCl), 1.62 (0.1M Phenylalanine in 0.1M HCl), 5.67 (0.1M Phenylalanine in 1M HCl), and...
in distilled water only), 10.56 (0.1M Phenylalanine in 0.1M NaOH), and 13.13 (0.1M Phenylalanine in 1M NaOH). The pH of all final solutions was recorded using a Corning 320 pH meter. For the remainder of this discussion, the bulk pH values will be referred to as their nearest whole number pH value (1, 2, 6, 11, and 13 respectively) for convenience.

7.2.1 Infrared Reflection-Absorption Spectroscopy

IRRAS spectra were taken using the external port of a Bruker Tensor 27 FTIR Spectrometer coupled to a Langmuir trough (NIMA Technology Ltd., UK). The Langmuir trough consists of a PTFE trough equipped with two computer-controlled PTFE barriers to control surface area, as well as a Wilhelmy balance to monitor changes in surface pressure. In the experiments presented here, the barriers were kept at their maximum open position (70 cm$^2$) for the duration of the experiment. The infrared beam exited the external port of the spectrometer and was passed through a CaF$_2$ focusing lens. Two 2in. diameter gold mirrors were positioned over the trough surface to direct the focusing IR beam onto the aqueous surface at an angle of 22° relative to the surface normal, and then direct the reflected light from the surface to a liquid nitrogen cooled MCT detector. This angle of incidence is within the optimum angles found to be ideal for unpolarized light incident on an air-aqueous interface [49]. Also, with this angle of incidence using unpolarized light on an air-aqueous interface, the expected absorption bands will be negative. The spectrum resulting from this experimental set-up is a Reflectance-Absorbance (RA) spectrum, where RA = -log(R/R$_o$) with R being the IR reflectivity of the surface of interest, and R$_o$ being the IR reflectivity of the background surface. These reflectivities are fully described in the literature using the Fresnel equations [50], whose details will not be presented here. In the work presented here, spectra were collected at a 1 cm$^{-1}$ resolution followed by averaging over 200 – 600 scans depending on the solution (200 scans for
pH 6, 600 scans for all other pH values), using the single channel spectrum of the pH solution of interest (in the absence of phenylalanine) as the background ($R_o$). Before computation of the reflectance-absorbance spectrum, the single channel spectra were atmosphere corrected (subtraction of water and CO$_2$ lines) utilizing the OPUS software equipped to the Bruker spectrometer.

The spectra presented here remain as the resulting reflectance-absorbance spectra and are shown in Fig. 7.3 with negative-facing peaks, while in Fig. 7.4(a.1) - 7.4(a.5) the IRRAS peaks are flipped to face upwards for easier comparison with the solid-state infrared spectra. Also, in Fig. 7.3, the baselines are scaled by an additive constant to differentiate between spectra of different pH values. Finally, it is important to note that when analyzing IRRAS spectra there are residual features (appearing as positive, broad peaks) due to small changes in the structure of the water surface throughout the measurement. These features appear in every spectrum (i.e. the large positive peak seen in Fig. 7.3 between 1600 and 1700 cm$^{-1}$) and should not be confused with peaks resulting from surface-residing molecules of interest.

7.2.2 Solid-State Infrared Spectroscopy

Solid-state spectra were obtained using the internal compartment of the Bruker Tensor 27 FTIR Spectrometer. A sample of the phenylalanine solutions at the various pH values were dried out over at least 24 hours, and then were pressed into KBr pellets for solid-state analysis. The spectra were obtained with a 1 cm$^{-1}$ resolution and were averaged over 100 scans. Although the ideal method of analysis of bulk ionization state would be direct IR spectra of the aqueous solution, due to the strong water absorption in the regions of interest solid-state analysis was performed here. It has been shown in the literature that although the infrared bands do shift according to the degree of hydration of the polar group, there is no change in the overall
ionization state of the bulk molecules upon dehydration [51]. A few bound water molecules are preserved on the polar groups throughout the dehydration process, allowing for the maintenance of charge. Therefore, in the study presented here, the ionization state of the dehydrated samples will be used as representative of the bulk ionization state.

7.3 Results and Discussion

Infrared Reflection-Absorption Spectroscopy (IRRAS) was used to identify the changes in the ionization state of L-phenylalanine at the air-water interface with changes in subphase pH. L-phenylalanine is a slightly soluble amino acid that is known to exhibit some surface activity [52]. With its large, non-polar aromatic ring, phenylalanine has one of the highest hydrophobicity ratings of the naturally occurring amino acids [53]. From an aqueous solution, it spontaneously adsorbs to the air-water interface forming an excess surface concentration of phenylalanine molecules. The adsorption isotherms of varying pH solutions of L-phenylalanine were measured and are shown in Figure 7.1 below.

![Figure 7.1: Adsorption isotherm of 0.01M L-Phenylalanine to the air-water interface over time in solutions of varying pH: black is pH 2, red is pH 6, and blue is pH 11.](image)
Although the kinetics of adsorption differs, the maximum surface pressure reached, and hence the surface excess concentration of L-phenylalanine molecules, is the same (within the error of the Langmuir trough surface pressure measurement) across the pH range used in this work. This surface excess is then detectable using the surface-sensitive IRRAS technique. Amino acids have two polar groups, a carboxylic acid and an amine, allowing for their progression through three different ionization states with changes in pH in an aqueous solution: cation at low pH (protonated amine, neutral carboxylic acid), zwitterion at intermediate pH (protonated amine, carboxylate anion), and anion at high pH (neutral amine, carboxylate anion), structures illustrated in Figure 7.2. In bulk aqueous solution, amino acids exist primarily as zwitterions throughout a very broad pH range, encompassing environmentally relevant pH values [54-56].

![Diagram of ionization states of L-phenylalanine](image)

**Figure 7.2**: Change in ionization state of the polar groups of L-phenylalanine with changes in pH

### 7.3.1 Assignment of polar group IRRAS bands

Figure 7.3 shows IRRAS spectra of phenylalanine at the air-water interface at three different subphase pHs progressing from pH 13 in blue (Fig. 7.3a), to pH 6 in red (Fig. 7.3b), and
finally to pH 1 shown in green (Fig. 7.3c). From this pH progression as well as from both IRRAS [57] and solid-state [51, 58-60] infrared literature comparison, the polar group vibrational modes are assigned as presented in Table 7.1.

Figure 7.3: IRRAS spectra of L-phenylalanine at the air-water interface at bulk pH values of: (a) pH 13 (blue), (b) pH 6 (red), and (c) pH 1 (green).
Table 7.1: Summary of polar group assignments from the IRRAS spectra of L-phenylalanine shown in Fig. 7.3 at the indicated bulk pH values

<table>
<thead>
<tr>
<th>pH 1 Cation</th>
<th>pH 6 Zwitterion</th>
<th>pH 13 Anion</th>
<th>Assignments [51, 57-61]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1717</td>
<td>-</td>
<td>-</td>
<td>( \nu (\text{C}=\text{O}) )</td>
</tr>
<tr>
<td>1600</td>
<td>-</td>
<td>-</td>
<td>( \beta_{\text{as}} (\text{NH}_3^+) )</td>
</tr>
<tr>
<td>-</td>
<td>1583</td>
<td>1542 / 1522 (sh)</td>
<td>( \nu_{\text{as}} (\text{COO}^-) )</td>
</tr>
<tr>
<td>1530 (sh) / 1514</td>
<td>1507</td>
<td>-</td>
<td>( \beta_{\text{s}} (\text{NH}_3^+) )</td>
</tr>
<tr>
<td>-</td>
<td>1396</td>
<td>1397</td>
<td>( \nu_{\text{s}} (\text{COO}^-) )</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>1349</td>
<td>( \gamma_{\text{s}} (\text{CH}<em>2) + \beta</em>{\text{s}} (\text{NH}_2) )</td>
</tr>
<tr>
<td>1284</td>
<td>1284</td>
<td>-</td>
<td>( \rho (\text{NH}_3^+) )</td>
</tr>
<tr>
<td>1140</td>
<td>1140</td>
<td>-</td>
<td>( \rho (\text{NH}_3^+) )</td>
</tr>
</tbody>
</table>

Detailed studies have been made of stearic acid (a carboxylic acid with an 18-carbon long hydrocarbon chain) at the air-water interface using the IRRAS technique [57, 61-63], but only limited information is available about primary amines due to experimental difficulties [64, 65]. While absorption bands due to N-H groups in both primary and secondary amides have been reported using IRRAS, none from amines have ever been observed [64]. This absence has been attributed to either the difference in the nature of the bonds themselves [64], or to a simple unfavorable orientation of those modes relative to the surface to be observed using the IRRAS technique [64, 65]. Tilting of the transition moments of vibrations relative to the surface normal could result in either weakening or the complete absence of these bands from the resulting IRRAS spectrum, which may have been the case in the attempted IRRAS spectra of long-chain primary amines at the surface [65]. Therefore, direct comparison of the spectra presented here with literature IRRAS spectra could not be made for identification of the amine state of
ionization, thus assignments were made through comparison with solid-state spectra of phenylalanine [51, 58] and other amino acids [59, 60].

Beginning with the carboxylic acid group assignments, the neutral carboxylic acid C=O stretch is experimentally observed in the pH 1 IRRAS spectrum at 1717 cm$^{-1}$. This assignment was made through comparison with the neutral carboxylic acid C=O stretch of stearic acid at the air-water interface using IRRAS taken and assigned by Gericke and Hühnerfuss [57]. As the pH increases, the carboxylic acid group is deprotonated resulting in two carboxylate stretches. At a subphase pH of 6, $\nu_{as}$(COO$^-$) is observed at 1583 cm$^{-1}$ and the symmetric stretch $\nu_s$ (COO$^-$) is observed at 1396 cm$^{-1}$. At pH 13, the antisymmetric stretch is shifted to lower energy at 1542 cm$^{-1}$ with a shoulder at 1522 cm$^{-1}$, while the symmetric stretch is observed at 1397 cm$^{-1}$. The symmetric stretch frequency agrees well with the literature [57], as do both of the antisymmetric stretch frequencies [57, 66]. The shift in the antisymmetric carbonyl stretch between pH 6 and pH 13 has been attributed to differences in hydration seen experimentally in the splitting of the antisymmetric carbonyl vibration observed in fatty acid studies [66]. Hydrogen bonding is known to shift carbonyl stretches to lower frequency [62, 66], and thus, if a change in hydration of the carbonyl group occurs between these two pH values, such a shift would be expected to occur.

The amine group assignments are more difficult. Only one peak was observed and assigned to the neutral amine group – the combination band arising from the symmetric out-of-plane phenyl ring CH$_2$ bending mode ($\gamma_s$ (CH$_2$)) and the symmetric NH$_2$ bending mode ($\beta_s$ (NH$_2$)) at 1349 cm$^{-1}$ observed only in the pH 13 spectrum. This peak was assigned through comparison with solid-state spectra of anionic L-phenylalanine in the literature [51, 58]. The protonated amine vibrations were more apparent. At pH 1, the antisymmetric NH$_3^+$ bend was
observed weakly at 1600 cm\(^{-1}\), the symmetric bend at 1514 cm\(^{-1}\) with a shoulder at 1530 cm\(^{-1}\), and two NH\(_3^+\) rocks at 1284 and 1140 cm\(^{-1}\) [59, 60]. At pH 6, only the symmetric bend was observed at 1507 cm\(^{-1}\), as well as the two NH\(_3^+\) rocks at 1284 and 1140 cm\(^{-1}\).

The pH 1 IRRAS spectrum also shows a strong enhancement in three peaks centered around 1228 cm\(^{-1}\). These peaks are not due to the amine or the carboxylic acid group on the phenylalanine molecules, and are instead attributed to non-polar moiety vibrations [51, 67]. This enhancement could be due to a change in orientation of the hydrophobic aromatic ring of the phenylalanine molecules residing at the surface due to the transition of the polar groups to a cationic form at this low subphase pH value. IRRAS is a technique which is sensitive to orientation at the surface, and intensity changes have been previously attributed to an orientational change at the interface [68, 69]. As the intensity of IRRAS absorption bands is a complex quantity depending on many different variables, it is difficult to assign this intensity enhancement conclusively.

7.3.2 Comparison of surface vs. bulk ionization state

Using the assignments made from the IRRAS spectra (Fig. 7.3 and Table 7.1), the ionization state of phenylalanine at the air-water interface at other intermediate subphase pHs could be made (Fig. 7.4a.1 – 7.4a.5, tabulated in Table 7.2). IRRAS spectra at various subphase pHs from 1 through 13 are shown on the left-hand side of Fig. 7.4 (a.1 – a.5).
Figure 7.4: Comparison of IRRAS spectra (a.1 – a.5) with peaks flipped for clarity (upward facing peaks) with solid-state infrared spectra (b.1 – b.5) of L-phenylalanine at bulk pH 1 (a.1, b.1), pH 2 (a.2, b.2), pH 6 (a.3, b.3), pH 11 (a.4, b.4), and pH 13 (a.5, b.5).
Table 7.2: Ionization state of surface and dried-from-bulk L-phenylalanine molecules from the IRRAS and solid-state infrared spectra, respectively.

<table>
<thead>
<tr>
<th>pH</th>
<th>IRRAS COOH / COO⁻</th>
<th>IRRAS NH₂ / NH₃⁺</th>
<th>surface ionization state</th>
<th>bulk ionization state</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COOH</td>
<td>NH₃⁺</td>
<td>cation</td>
<td>cation</td>
</tr>
<tr>
<td>2</td>
<td>COOH &amp; COO⁻</td>
<td>NH₃⁺</td>
<td>cation &amp; zwitterion</td>
<td>cation</td>
</tr>
<tr>
<td>6</td>
<td>COO⁻</td>
<td>NH₃⁺</td>
<td>zwitterion</td>
<td>zwitterion</td>
</tr>
<tr>
<td>11</td>
<td>COO⁻</td>
<td>NH₂</td>
<td>anion</td>
<td>zwitterion</td>
</tr>
<tr>
<td>13</td>
<td>COO⁻</td>
<td>NH₂</td>
<td>anion</td>
<td>anion</td>
</tr>
</tbody>
</table>

At pH 1, phenylalanine exists as a cation at the surface. At pH 2, phenylalanine was observed as both a cation and as a zwitterion at the surface. At pH 6, only the zwitterion form of phenylalanine was observed. Finally, at pH 11 and 13, only the anion form of phenylalanine was observed at the surface. The right-hand side of Fig. 7.4 (b.1 – b.5) show solid-state infrared spectra of samples dried from bulk solutions of the same subphase pH as the corresponding IRRAS surface spectra. The ionization state of phenylalanine in these spectra (Table 7.2) was identified through comparison with literature spectra [51, 58]. This allows for comparison between the ionization state of phenylalanine at the water surface with that in the bulk solution. Through this comparison, it becomes apparent that there is, in fact, a difference in the ionization state of phenylalanine at the surface compared with the bulk. The ionization states agree at a bulk pH of 1, 6 and 13, but do not agree at pH 2 or pH 11. At pH 2, the surface shows both the cationic and zwitterionic form of phenylalanine while the bulk spectrum only shows the cationic form. At pH 11, the surface phenylalanine molecules are anions only, whereas the bulk molecules are zwitterions.

This difference in ionization state gives insight into the surface pKₐ of the polar groups of L-phenylalanine relative to the bulk. The polar groups of the molecules at the surface transition
to their deprotonated state (NH₃⁺ to NH₂, and COOH to COO⁻) at a lower bulk pH than the polar groups of the molecules in the bulk. This illustrates a decrease in the pKₐ of the polar groups at the air-water interface relative to the polar groups in the bulk. Others have proposed that this shift in pKₐ is simply towards more neutral molecules [70], but this is not seen here. Since there is a shift to the conjugate base of the respective polar groups at a lower pH than in the bulk, the pKₐ of both the carboxylic acid group and the protonated amine group of L-phenylalanine are lowered at the interface. Although the shift in pKₐ of the protonated amine group does favor the unionized polar group, the carboxylic acid group actually transitions toward more ionic species (more carboxylate anions) at the surface. It is difficult to quantify this change in pKₐ using the IRRAS technique due to the complex composition of the peak intensities, but the qualitative change between the bulk and surface ions is clear.

Indirectly, the transition to the conjugate bases of the polar groups at the surface at lower pH values also indicates that the surface pH is higher than the bulk pH in the presence of L-phenylalanine. Physically, phenylalanine molecules see a higher propensity for OH⁻ ions at the surface compared with H₃O⁺ ions. The ionic nature of the bare water surface is an area of great interest, currently with much controversy [16, 33]. IRRAS allows for the indirect observation of the ion propensity for the surface through the transition between ionization states of ionic species at the air-water interface as seen here with phenylalanine. The presence of ionic species at the surface does disrupt the structure of the water surface itself [4, 71-77]. Therefore, although it is not possible to comment on the pH of a neat water surface using solely the results presented here, this work does show a chemically relevant change in surface pH versus the bulk aqueous solution in the presence of an adsorbed organic molecule.
It is important to note that the IRRAS technique allows for analysis of molecules in the surface region, but is not limited to the region of noncentrosymmetry. In fact, the probe depth of IRRAS has been reported to be as large as 1 – 2 µm [78]. Surface-sensitive techniques such as VSFG and SHG have much smaller probe depths [79], but this depth varies depending upon the molecules residing at the surface: ions present at the surface have been seen to increase the interfacial depth [72, 80]. It is possible that the difference seen in the direction of the shift of the surface pK\textsubscript{a} in this work compared with other studies as well as the implied higher OH\textsuperscript{-} concentration may be attributed to this difference in surface region probed. It has been proposed that since many of the strictly surface-layer spectroscopic techniques (VSFG, SHG) show an increased hydronium (H\textsubscript{3}O\textsuperscript{+}) concentration at the surface, there must then be a complementary hydroxide (OH\textsuperscript{-}) rich sub-surface layer to maintain charge-neutrality [33]. The probe depth of the IRRAS technique is greater than the probe depth of VSFG [78], and could therefore be sampling this under-layer as well as the surface layer, leading to the observation of a higher OH\textsuperscript{-} concentration and decreased pK\textsubscript{a}. Regardless, the surface region sampled using IRRAS of L-phenylalanine molecules in this work is the reactive region of interest, as evidenced by the difference in surface vs. bulk ionization state of phenylalanine observed.

Finally, it is apparent from many studies of the water surface that it is a complicated environment that is not yet fully understood. Some of the controversy in the literature regarding the ionic nature of the surface of water itself as well as the pK\textsubscript{a} of surface-residing species is likely due to a complex combination of the use of different molecular probes, different techniques, as well as different experimental conditions. The environment experienced by molecules residing at the surface is complex and dependent upon many of these interworking factors. For example, the compression state of the monolayer affects the orientation of the polar
groups at the interface, which then can affect the hydration state of the polar groups themselves through the restricted availability of surface water molecules. The hydration state of the polar groups can thus influence the propensity for ion formation. Thus, directly comparing studies of water-soluble molecules such as L-phenylalanine, that merely have a surface excess concentration, with surfactants such as stearic or palmitic acid that reside solely at the interface anchored by a long hydrophobic tail, can be problematic. Each of these molecules orients itself differently and has a different compression state at the surface, and thus its polar groups are exposed to different environments. Further, as discussed earlier, the technique used to probe the water surface can be probing different molecular environments merely by the depth into the bulk solution that they penetrate. Therefore, although the findings in this work do not agree with all of the literature studies performed previously with different techniques and different molecules, it adds a unique probe to the overall understanding of water surfaces. L-phenylalanine, with both an acid and base group, was shown in this work to experience a hydroxide-rich environment in the surface region as probed by the in situ, surface-sensitive technique IRRAS, and to exhibit a lowering of the pKs of both of its polar groups at the surface relative to the bulk.

7.4 Conclusions

In this study, the ionization state of the hydrophobic amino acid L-phenylalanine was identified in situ in the surface region using the surface sensitive technique IRRAS, and was subsequently compared to the ionization state of bulk molecules through a range of bulk pH values (pH 1 – 13). Through the use of an amino acid containing both an acid and base polar group, it was shown that both of the polar groups of the molecules residing in the surface region transitioned to their deprotonated state at a lower pH than the molecules in the bulk. This
indicates directly a decrease in the pK$_a$ of the polar groups at the interface and indirectly a higher propensity of OH$^-$ for the surface region. More broadly, it is apparent from both this study as well as the cited literature that the ionization state at the surface is qualitatively different from the bulk. The increased acidity of polar groups at the interface observed in this work subsequently has consequences on the chemistry these molecules are capable of performing.

7.5 References for Chapter 7


50. Gericke, A., A.V. Michailov, and H. Hühnerfuss, Polarized external infrared reflection-absorption spectrometry at the air/water interface: comparison of experimental and


8 Peptide Bond Formation at the Air – Water Interface

8.1 Introduction

Protein synthesis (condensation of amino acids through sequential peptide bond formation) is a fundamental and ubiquitous reaction in biology. Aqueous media are the required environments in which this chemistry takes place, however protein synthesis is unfavorable in aqueous solution. In modern biology, the condensation reactions necessary in the formation of peptide bonds are facilitated catalytically by the large subunit of the ribosome. The mechanism of this catalyzed reaction originally proposed by Nissen et al. in 2000 [1] involved favorable orientation of peptide precursors, acid-base catalysis and transition state stabilization, and the altered pK$_a$ of the functional groups of the precursors due to the reaction environment provided by the active site, as such pK$_a$ shifts had previously been seen in the active sites of other proteins [2]. Since then, the original mechanism has been contested [3, 4]. The acknowledged mechanistic function of the ribosome’s active site is its ability to bring the precursors in close proximity and orient them for reaction, with further mechanistic details remaining unresolved [4]. Studies of peptide bond formation in the absence of modern biological machinery can give insight into the mechanism employed by the ribosome’s active site, as well as yield important information in the prebiotic route to the first peptides in the origin of life. The formation of a peptide bond (reaction R1 shown below) is a condensation reaction, eliminating a water molecule for each peptide bond formed, and thus faces both thermodynamic and kinetic constraints in bulk aqueous solution [5].

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The equilibrium constant for peptide bond formation in water is extremely small and greatly favors the amino acid monomers [5, 6]. Amino acid monomers have the added kinetic disadvantage of existing primarily as zwitterions at environmentally and physiologically relevant pH values in bulk aqueous solution [5, 7]. Insightful experiments have been performed yielding peptide bonds in anhydrous solvents with amino acid ester starting materials and copper(II) ion catalysis [8, 9]. Transition metal ions are thought to have been components of the early ocean [10] with one source being the heavy meteoritic and cometary bombardment experienced by the early Earth, but the anhydrous solvents used in these studies are neither physiologically relevant nor likely to have been present on early Earth. The same mechanism was attempted in aqueous solution, but no peptide formation was detected [9]. Polymer formation in aqueous environments would most likely have been necessary on early Earth as the liquid ocean would have been the reservoir of amino acid precursors needed for protein synthesis. In this work, the air-water interface is utilized as the auspicious environment for peptide bond formation, coupling the water surface with the bulk water reservoir of monomers. In situ, surface-sensitive techniques are used here to observe the condensation reaction of a model system composed of a small, water-soluble amino acid ester (leucine ethyl ester) through Cu$^{2+}$ coordination.

Air-water interfaces are found now, as on prebiotic Earth, at the surfaces of lakes, oceans, and atmospheric aerosols. The air-water interface generally, and on atmospheric aerosols in particular, has been proposed to be important in prebiotic chemistry previously [11-14] because it provides a unique environment for chemistry through its ability to concentrate and align
biochemical precursors and to alter the state of ionization of surface species (as seen in the case of the amino acid L-Phenylalanine in Chapter 7) [15-19]. Contemporary marine aerosols have been found to contain the amino acid precursors necessary for peptide bond chemistry [20], enabling the possibility for their use in such reactions. Further, the fluctuating conditions experienced by aerosols throughout their atmospheric lifetime, including evaporation of water, coagulation, and possibly re-entry into the ocean, would naturally provide the compression of the surface layer shown in this work to be necessary for Cu\(^{2+}\)-coordination leading to peptide bond chemistry [12].

In addition, the unfavorable equilibrium constant for peptide bond formation in bulk aqueous solution is shifted when the molecules experience a water-restricted reaction environment at the water surface. Although the exact surface pH of water is debated [21-23], the surface is known to alter the pK\(_a\) of surface-active molecules [24, 25], which can aid in the promotion of peptide bond chemistry at the interface by alleviating the kinetic constraint on peptide bond synthesis by reducing zwitterion formation. However, as was seen in Chapter 7, the water surface does not cause the transition purely to neutral polar groups for amino acids. Rather, it promoted a decrease in pK\(_a\) of both polar groups, favoring a shift to the deprotonated form of both groups. Thus, as will be discussed in more detail shortly, a small ethyl ester of the natural amino acid L-leucine was used in this work partly as a protector against ionization of the carboxylic acid group.

The air-water interface has been reported in the literature to have a catalytic role in peptide bond formation [26-29] using synthetic long-chain amino acid esters that are anchored to the surface by the polar groups attached to their 18-carbon long hydrocarbon tails, and thus are forced to reside solely at the surface of the water. Reaction amongst the surface monomers was
promoted in these studies [27-29] through surface compression, and supported by subsequent collection, drying, and analysis of the surface products. In the work presented here, Infrared Reflection-Absorption Spectroscopy (IRRAS) and Langmuir trough methods were used to observe, in situ, complex formation with a metal cation followed by condensation chemistry leading to peptide bond formation occurring at the air-water interface. This is the first time such condensation reactions have been observed in situ at the interface and with such a small activation group (an ethyl ester) on the starting amino acid precursor in an aqueous environment.

8.2 Materials and Methods

All reagents were purchased from Sigma-Aldrich Chemical Co. and used without further purification. A stock pH 8 buffer solution was prepared by dissolving $8.5 \times 10^{-4}$ moles of sodium phosphate monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, ACS grade) and $9.15 \times 10^{-3}$ moles of sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, ACS grade) in 1L deionized water. Then, Copper(II) chloride ($97\%$) was added to an aliquot of the buffer solution immediately before the experiments were performed to yield a final concentration of 1mM Cu$^{2+}$ and a final pH of 7.5. Leucine ethyl ester hydrochloride ($99\%$) was then added to the Cu$^{2+}$/PO$_4^{2-}$ solution to a concentration of 0.06M of leucine ethyl ester. This final solution was then sonicated until a transparent solution resulted.

The prepared solutions were spread on the Langmuir trough and the system was allowed to purge for 30 minutes. Then, the mechanical barriers of the Langmuir trough were compressed to hold the surface at a constant surface pressure of 15 mN/m for at least 1 hour, during which IRRAS spectra were taken every 10 minutes. The surface pressure of 15 mN/m was chosen because it was found to put some strain on the surface molecules and subsequently force them to
orient to allow for complex formation with Cu$^{2+}$ and subsequent condensation reaction, without excessive loss of surface molecules back into the bulk solution. Without compression, the adsorbed molecules were not observed to form complexes with the copper ions or react in any way. Afterwards, the barriers were returned to their fully open position (at a surface area of 70 cm$^2$), and the surface molecules were allowed to react overnight. Then, after the addition of some buffer solution underneath of the surface film to compensate for the decrease in the surface level due to evaporation overnight, another IRRAS spectrum was taken of the surface. Surface crystals that spontaneously formed overnight were then collected, dried out, and analyzed using solid-state FTIR. IRRAS spectra of leucine ethyl ester in the absence of copper(II) ions were also taken for comparison following the same procedures outlined above.

### 8.2.1 Langmuir Trough

The Langmuir trough used in these studies was purchased from NIMA (KSV NIMA, Finland) and consisted of a PTFE trough (145 x 70 x 0.5 mm) coupled to two computer-controlled PTFE barriers. The barriers were limited at their open position to a surface area of 70 cm$^2$. The Langmuir trough was equipped with a Wilhelmy balance which allows for simultaneous measurement of surface pressure during surface area changes induced by the mechanical PTFE barriers. The result is a surface pressure – area ($\pi – A$) isotherm yielding interfacial thermodynamic information.

### 8.2.2 Infrared Reflection-Absorption Spectroscopy

The Langmuir trough described above was coupled with a custom-built IRRAS set-up. The IRRAS spectra were obtained using the external port of a commercial Bruker Tensor 27 FTIR Spectrometer, with all external optics and equipment (including the Langmuir trough) being constantly purged with dry house air. The IR beam exited the spectrometer and was
passed through a CaF$_2$ lens after which it was reflected off of two 2 in. gold mirrors directing it onto the air-aqueous interface and then directing the reflected light to a liquid nitrogen cooled MCT detector. The two gold mirrors were positioned over the Langmuir trough so that the IR beam was incident on the air-aqueous interface at an angle of 22° relative to the surface normal. This angle is within the optimum angles found to be ideal for unpolarized light incident on an air-aqueous interface [30]. Spectra were then collected and averaged over 200 scans with 1 cm$^{-1}$ resolution. A single channel spectrum of the surface of the Cu$^{2+}$/PO$_4^{2-}$ buffer solution (in the absence of leucine ethyl ester) was used as the background spectrum. The IRRAS spectra here are shown as Reflectance-Absorbance (RA) spectra, where RA = $-\log(R/R_o)$ with R being the IR reflectivity of the surface of interest, and $R_o$ being the IR reflectivity of the background surface. The details of such reflectivities can be described by the Fresnel equations which are presented elsewhere [31]. With an angle of incidence of 22° relative to the surface normal and unpolarized light on an air-aqueous interface, the absorption bands will be negative.

It is important to note that the IRRAS technique does not only sample the top layer of molecules at the air-water interface. Rather, it samples the surface region, which for soluble molecules adsorbing to the surface (such as leucine ethyl ester) can be as deep as 1 – 2 µm [32]. Other surface techniques such as vibrational sum-frequency generation arguably have smaller probe depths, but for the work presented here, the IRRAS technique samples the reactive region of interest.

8.2.3 Solid-State Infrared Spectroscopy

Solid-state infrared spectra were obtained using the internal chamber of the Bruker Tensor 27 FTIR Spectrometer being purged with dry house air. Infrared spectra were taken with
the samples pressed between two CaF₂ windows, averaging over 50 scans with a 1 cm⁻¹ resolution.

8.3 Results and Discussion

Leucine ethyl ester is the short ester analog of leucine, one of the naturally occurring hydrophobic amino acids used in modern biochemistry. It is soluble in water, but exhibits a small amount of surface activity (Fig. 8.1), allowing it to be observed using IRRAS, a surface-sensitive IR spectroscopic technique that allows for vibrational characterization of molecules residing in the surface region.

![Figure 8.1: π-A isotherm of leucine ethyl ester illustrating its surface activity](image)

In this work, the “surface” or “interface” discussed is the reactive region of interest at or near the surface sampled by the IRRAS beam [32]. The ester group of leucine ethyl ester serves to protect the carboxyl side of the molecule by eliminating the possibility of ionization to a carboxylate anion, and to activate the molecule as a whole for reaction. The short ester group gives a better leaving group for the reaction (ethanol rather than water, see reaction R2 below),
but also serves to decrease the $pK_a$ of the amine side of the molecule from 9.60 in the natural amino acid to 7.64 in the amino acid ester [5, 33], shifting the favorable form of the amine group toward neutral.

\[
\text{O} - \text{CH}_{2} \text{CH}_{2} - \text{N} - \text{H}_{2} + \text{CH}
\text{O} - \text{CH}_{2} \text{CH}_{2} - \text{N} - \text{H} \rightarrow \text{O} - \text{CH}_{2} \text{CH}_{2} - \text{N} - \text{CH} \text{H}_{2} + \text{CH}
\text{OH} \quad \text{R2}
\]

In this work, there is no disruption of the surface film during analysis, a problem faced in the collection procedures in previous studies of long-chain amino acid esters [27-29]. We thereby demonstrate unambiguously that the chemistry occurred at the air-water interface. The long-chain amino acids used [27-29] were also highly synthetic molecules which were unlikely to have been readily available on early Earth. Here, a two-carbon long amino acid ester is used as a more plausible model for the initial abiotic peptide bond formation reactions, as it contains a much smaller activation group and is also water-soluble. In addition, in these previous studies [27-29], the reactants were insoluble and their concentration is limited by the monolayer which is deposited only at the surface. There is some debate in the literature as to whether the long-chain amino acid esters are capable of forming any peptides more than two monomers long [29] due to the confined nature of the surface film. Here, this problem is eliminated, as there is a constant supply of monomers available for reaction adsorbing to the interface from the bulk solution, analogous to the reservoir of monomers provided by the ocean on early Earth. The chemistry observed in this work involves leucine ethyl ester at the water surface at the environmentally and physiologically relevant $pH$ of 7.5, coordination of the leucine ester with $\text{Cu}^{2+}$ through surface compression, and subsequent peptide bond formation at the air-water interface.
8.3.1 Copper – leucine ethyl ester complex formation at the air-water interface

In Figure 8.2(a), the black trace shows the reflectance-absorbance spectrum of leucine ethyl ester at the air-water interface. The most prominent peak in the spectrum is the ester C=O stretch at 1726 cm⁻¹, which remains prominent throughout the rest of the spectra shown in Fig. 8.2(a). The remainder of Fig. 8.2(a) illustrates the evolution of leucine ethyl ester’s IRRAS spectrum through coordination with Cu²⁺ ions when the surface film is being held at a constant pressure of 15 mN/m using the mechanical barriers of the Langmuir trough. The coordination only occurs with the amine group on the molecule (proposed coordination complex shown in Fig. 8.3) as seen spectroscopically by the enhancements in the IRRAS bands below 1200 cm⁻¹ in Fig. 8.2(a). This is seen more clearly in the subtraction spectrum shown in Fig. 8.2(b) in which the IRRAS spectrum of leucine ethyl ester in the absence of Cu²⁺ ions (black) is subtracted from the Cu²⁺-coordinated IRRAS spectrum shown in green. The subtraction spectrum that results shows only the changes due to the coordination complex formed between leucine ethyl ester and the copper ions.

From this spectrum, it is clear that there is no change at all above 1200 cm⁻¹, but there are four peaks that grow in below 1200 cm⁻¹. These four peaks can all be assigned as associated with the amine side of the molecule through comparison with the literature infrared spectra of ethylenediamine complexes with Cu²⁺ ions [34] as follows: the two strongest peaks occurring at 987 and 1037 cm⁻¹ are assigned to the C-N stretching mode, and the two weaker peaks at 1089 and 1139 cm⁻¹ are assigned to the NH₂ twisting mode. This shows that through constant pressure, the copper ions form a coordination complex with the amine group of the leucine ethyl ester molecules at the surface. These results constitute an unambiguous spectroscopic characterization of the process of peptide bond formation at the water-air interface.
Figure 8.2: IRRAS spectra showing Cu\(^{2+}\) coordinated to the amine group of leucine ethyl ester over time with compression. (a) Black: leucine ethyl ester in the absence of Cu\(^{2+}\) ions, Red: immediately after beginning constant pressure of 15 mN/m, Blue: after 30 minutes of constant pressure, Green: after 60 minutes of constant pressure. (b) Subtraction spectrum (gray) of un-coordinated leucine ethyl ester (black) from the Cu\(^{2+}\)-coordinated leucine ethyl ester IRRAS spectrum (green) resulting in only the peaks enhanced through coordination.

Figure 8.3: Proposed Cu\(^{2+}\) - leucine ethyl ester complex formed at the interface as observed in the IRRAS spectra of Figure 8.2.
The IRRAS spectrum of the copper-complex precursor also gives mechanistic insight into the peptide bond formation reaction at the surface. In anhydrous solutions, peptide bond formation is facilitated between two amino acid ester monomers via the formation of a chelate ring around a central Cu$^{2+}$ ion, with coordination occurring only through the amine side of the molecule [8]. In aqueous solutions, it is well known that if the copper ion directly coordinates with the ester side of the molecule, hydrolysis of the ester bond is kinetically enhanced [35], resulting in the loss of the activation group utilized in the peptide bond formation reaction here. Since there is no enhancement or shift in the ester C=O stretch in Fig. 8.2, there is no copper ion coordination with the ester side of the molecule at the air-water interface [36, 37]. This shows the importance of the environment provided by the water surface. The copper ions only coordinate with the amine group at the surface, thereby simultaneously avoiding hydrolysis of the ester and promoting peptide bond formation.

### 8.3.2 Compression promoted peptide bond formation at the air-water interface

This complex between the amine and the Cu$^{2+}$ ions only forms through compression of the surface layer, and was found to be a necessary precursor to the subsequent chemistry. No change was seen in the IRRAS spectrum in the absence of Cu$^{2+}$ ions (Fig. 8.4) under the same experimental conditions presented here.
It has been proposed in the literature that metal complexes with amines at the air-water interface induced through surface compression can result in an orientational change of the molecules residing at the surface [38]. The orienting effect of the complex at the surface promotes subsequent condensation to form peptide products as observed in our studies. In Fig. 8.5, a comparison is shown between the IRRAS spectrum of the copper complex precursor (Fig. 8.5(a)) and the condensation product formed at the surface (Fig. 8.5(b)).
Figure 8.5: IRRAS spectra of (a) leucine ethyl ester complexed with Cu$^{2+}$ ions and (b) the peptide product formed at the surface with red dotted line indicating new Amide I band of peptide bond formed in surface reaction

In the top spectrum (Fig. 8.5(a)), the most prominent peaks are the ester C=O band at 1726 cm$^{-1}$ and the copper-complex amine peaks below 1200 cm$^{-1}$ as described previously.

Then, after allowing the reaction to proceed at the surface overnight, the spectrum in Fig. 8.5(b) results. In this spectrum, the ester band is still prominent at 1726 cm$^{-1}$, but there is a new prominent peak at 1625 cm$^{-1}$, which is not seen in the leucine ethyl ester spectrum. This peak is assigned as the Amide I band of the peptide product formed by the condensation reaction of two or more leucine ethyl ester molecules. The Amide I band can yield secondary structure
information about the peptide product, as its position is very sensitive to its environment [37, 39, 40]. The position of the Amide I band of the peptide in the IRRAS spectrum reveals that the peptide product is still coordinated to the Cu$^{2+}$ ion which catalyzed its reaction, as the coordinated peptide should have an Amide I band at 1625 cm$^{-1}$, whereas the uncoordinated peptide’s Amide I band would appear around 1675 cm$^{-1}$ [37].

Concurrent with the peptide bond chemistry floating crystals spontaneously formed at the surface, and were subsequently manually collected and then analyzed using solid-state infrared spectroscopy. From the infrared spectra, which are shown in Fig. 8.6, it was determined that the collected material is a mixture of the peptide product formed at the surface and the leucine ethyl ester starting material from the bulk solution.

![Solid-state infrared spectra of collected solid formed at the surface (black) showing amide I (*) and amide II (**) bands of the peptide, and leucine ethyl ester commercial solid (red)](image)

Figure 8.6: Solid-state infrared spectra of collected solid formed at the surface (black) showing amide I (*) and amide II (**) bands of the peptide, and leucine ethyl ester commercial solid (red)

The collected material (black) shows many of the same features as the leucine ethyl ester starting material (red), but exhibits a new peak at 1625 cm$^{-1}$ and a new shoulder at 1520 cm$^{-1}$, being assigned to the Amide I and II bands respectively. This Amide I band assignment (Fig. 8.6)
corresponds directly with the Amide I band observed in the IRRAS surface spectrum (Fig. 8.5b), confirming the identity of the peptide product formed in the surface region.

8.4 Conclusions
Here, we have unambiguously demonstrated peptide bond formation at the air-water interface using small, water-soluble amino acid esters. Condensation reactions that must eliminate water are thermodynamically unfavorable in aqueous bulk, and yet are ubiquitous and essential to life. In addition, peptide bond formation will not occur between two amino acids in their zwitterionic form, the predominant state in a bulk aqueous environment. Water-air interfaces, characteristic of the surface of oceans, lakes and atmospheric aerosols (see Fig. 8.7), provide an auspicious environment for this condensation chemistry, through their provision of a water-restricting environment, alteration of the ionization state of surface species, and their ability to concentrate and align monomers.

Figure 8.7: Illustration of peptide bond formation at environmental water-air interfaces
Through *in situ* spectroscopic measurements, we have identified that the peptide bond forms through the coordination of the amine group of leucine ethyl ester to Cu$^{2+}$ ions at the surface, inducing an orientational change at the surface observed using IRRAS. Then, peptide bond formation occurs spontaneously at the surface of water, facilitated by the formation of the copper complex at the interface. This work gives insight into oligomeric peptide formation *en route* to the emergence of more complex biomolecules on early Earth and reinforces the importance of orientation, alignment, and proximity in the functioning of modern ribosomal peptide bond synthesis.

### 8.5 References for Chapter 8


9 Mixed Aromatic – Aliphatic Films at the Air – Water Interface Case I: Phenylalanine with Stearic Acid

9.1 Introduction

Atmospheric aerosols are globally distributed and are known to play an important role in Earth’s atmosphere [1, 2], although their overall effect on climate is difficult to quantify [3]. Organic compounds are ubiquitous components of atmospheric aerosols, recognized to play an important role in climate [4-6]. Aerosols containing organic complex mixtures are either directly emitted or formed by gas-phase photochemistry and adsorbed onto preexisting particles (secondary organic aerosols). Their morphological and chemical properties remain poorly characterized hampering models of their influence on aerosol properties [7, 8]. Field measurements have established that many organic compounds found on aerosols in the atmosphere are surface active [9-13]. The morphology and structure of such organic aerosol particles affect their optical properties [14, 15], the heterogeneous reaction mechanisms and rates [16, 17], their ability to form cloud droplets [18-20] and to nucleate ice particles [21, 22].

Due to complex mixtures of hydrophobic and hydrophilic phases in these particles, they can adopt very different structures. Aqueous aerosols, both marine and continental, have been proposed to exist with an inverted micelle structure, with an aqueous core surrounded by a layer of organics [19, 23-25]. Recent field observations and laboratory studies find that aerosol particles, in addition to such inverted micelle structures, can adopt other morphologies and contain organic inclusions [5, 26, 27]. Reid et al. have proposed a complex structure where the organics can form surfactant lenses [28]. The presence of such different phases and structures

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[29] give rise to different thermodynamic constraints and hence different morphological, optical and chemical properties. The scattering efficiency of aerosol particles has a strong dependency on size [30], requiring quantitative insight into aerosol size distribution. The composition of the aerosol, especially of the surface, can influence the particle’s size through altering its ability to evaporate or absorb water [8, 20, 31].

It is commonly accepted that surface films composed of long-chain organics impede water transfer [25, 32-36]. This effect is dependent upon extent of surface coverage and the solubility of the surfactant. The nature of the films formed by soluble versus insoluble surfactants varies; soluble surfactants form more expanded, permeable films, whereas many insoluble surfactants form close-packed, more impermeable films [25, 33, 37, 38]. Environmental interfaces are more complicated systems however, as they contain mixtures of surface-active organics. The long-chain fatty acids found on aerosols (like stearic acid) are insoluble saturated surfactants, and therefore should impede water transfer into and out of the particle, thereby affecting aerosol growth. This idea has been tested experimentally on model aerosol particles in the laboratory, but the results are conflicting [20, 39-42]. Moreover, this simplified view relates to one-component monolayers comprised completely of long-chain fully saturated fatty acids. It is likely that both monolayer structure and interfacial water transfer are highly sensitive to both the composition and morphology of the interfacial film. Optical trapping has been used recently to begin to understand some of these complex properties of aerosols (hygroscopicity, mass accommodation, etc.), but it is still a developing field of study [43].

In this study, a Langmuir film is utilized as a laboratory model for the surface of oceans, lakes, and atmospheric aerosol particles. Because of the diversity of the sources from which the atmospheric organics stem, mixed films are more appropriate than homogeneous monolayers, in
order to better represent the complex composition of atmospheric aerosols [1, 9, 44, 45]. Mixed films composed of surfactants with similar hydrophobic groups have been studied previously [44, 46]. In this work, we chose a system composed of a soluble surfactant (L-Phenylalanine) in conjunction with an insoluble surfactant (stearic acid), to monitor surface pressure induced morphological changes at the air-water interface in a mixed film. Here, two very different hydrophobic groups are utilized: L-Phenylalanine has a bulky hydrophobic group composed of an aromatic ring, while stearic acid contains a long hydrocarbon chain as its hydrophobic group. The use of very different hydrophobic groups in a mixed film will add to the knowledge of the surface morphology of complex environmental interfaces like the surface of atmospheric aerosols. Isotherm compression-expansion cycles were then utilized as a model for the complex dynamics at the ocean surface and processes that aerosol particles undergo throughout their atmospheric lifetime. In the atmosphere, the surface coverage of the aerosol particle may be changed through collision and subsequent coagulation of particles, as well as selective evaporation and uptake of water. Manually changing the surface area through the use of the trough’s mechanical barriers mimics the compression and expansion the surface of the aerosol particles undergoes through these processes.

The choice of molecules used in this study is due to their atmospheric relevance and known surface activity. Stearic acid, as mentioned earlier, is one of the major surfactants found in atmospheric aerosols stemming from biogenic sources [42], and forms a floating monolayer (solubility in water of only 0.0029 g/kg [47]) with well-known phase changes through induced surface pressure [33]. L-Phenylalanine was chosen as it has one of the highest hydrophobicity ratings of the naturally occurring amino acids but is still soluble in water with a solubility of 27.9 g/kg, and has been previously seen to exhibit some surface activity [48-50]. Amino acids are
also found in atmospheric aerosols [51], and are known to participate in atmospheric processes [52-54]. The mixed film formed by deposited stearic acid with adsorbed L-phenylalanine at the air-water interface was studied using the Langmuir trough in conjunction with Brewster Angle Microscopy (BAM) and Infrared Reflection-Absorption Spectroscopy (IRRAS).

9.2 Materials and Methods

All solvents and reagents were used without further purification. Stearic acid (octadecanoic acid, 98+%) was purchased from Aldrich Chemical Co., Inc and then dissolved in chloroform (ACS grade, Mallinckrodt Baker, Inc.) to a concentration of 1 mg/ml. L-phenylalanine (99%) was purchased from Alfa Aesar, and was prepared to a concentration of 0.02M in distilled water (solution had a final pH of 6).

For the isotherm cycle experiments, the trough was initially filled with the desired subphase: either distilled water or 0.02M L-phenylalanine in distilled water. 30 µL of stearic acid solution was then deposited drop-wise on the surface with the barriers at the open position. The system was then allowed to equilibrate for 20 minutes (to allow for solvent evaporation and L-phenylalanine adsorption), after which ten isotherm compression/expansion cycles were performed. BAM video of the interface was continuously obtained throughout this process, and still images were later extracted from the video. IRRAS spectra were also taken before and after the isotherm cycles (1 cm\(^{-1}\) resolution, averaged over 200 scans) with the barriers at their fully open position. The pH of the solution was unchanged throughout all experiments, and remained at a pH of 6.

For the adsorption experiment, the Langmuir trough was filled with a 0.02M solution of L-phenylalanine followed by the deposition of stearic acid as described above. The surface
pressure was then monitored over time with the barriers in their fully open position (300 cm$^2$). As with the isotherm cycles, the BAM video of the interface was continuously recorded during the adsorption process.

### 9.2.1 Langmuir Trough

The Langmuir trough for isotherm studies was custom-built, and consists of a PTFE trough (52 x 7 x 0.5 cm) coupled with two computer-controlled, PTFE barriers. The barriers were controlled using a computer interface and software purchased from NIMA (NIMA Technology Ltd., UK). The barriers were limited at their open position to an area of 300 cm$^2$, and at their closed position to an area of 30 cm$^2$ or surface pressure of 45 mN/m (whichever was reached first), with a constant barrier speed of 100 cm$^2$ min$^{-1}$.

The Langmuir trough for isotherm, Brewster Angle Microscope (BAM), and Infrared Reflection-Absorption Spectroscopy (IRRAS) studies was equipped with a Wilhelmy balance which allowed for the measurement of surface pressure as the mechanical barriers were moved across the surface, with the measured area being the area between the moving mechanical barriers. This produces a surface pressure – area ($\pi - A$) isotherm, thereby giving interfacial thermodynamic information. In the experiments presented here, isotherm compression/expansion cycles were performed, during which the mechanical barriers continuously moved from their open to closed positions with constant barrier speed (with the limiting conditions described above).
9.2.2 Brewster Angle Microscope

A similar Langmuir trough as that described above (168 mm x 86 mm, KSV minitrough, Finland) was equipped with a custom-built BAM which further allowed visualization of the air-water interface during the isotherm cycles [55, 56]. The BAM experiments were carried out on a self-assembled symmetric goniometer system. The laser light source emits 5W p-polarized at 543 nm (Research Electro-Optics, Inc.), which is incident at the Brewster angle of the subphase. The incident beam is passed through a Glen Thompson polarizer before reaching the liquid surface. An infinity-corrected 10º Nikon lens together with a tube lens are used to form the image. The BAM image is collected on an Andor charge-coupled device (CCD; Andor DV887) of 512 x 512 pixels. In the BAM images shown here, the darkest (black) regions represent areas with negligible surfactant coverage (a two-dimensional vapor phase), the medium gray areas indicate the presence of a condensed monomolecular film (e.g. of stearic acid), and very bright areas indicate the presence of three-dimensional structures (e.g. hydrophobic aggregates).

9.2.3 Infrared Reflection-Absorption Spectroscopy

IRRAS spectra were obtained using the external port of a Bruker Tensor 27 FTIR Spectrometer. The IR beam exited the FTIR spectrometer and was passed through a CaF$_2$ lens (remaining unpolarized), before being reflected off of two 2 in. gold mirrors positioned over a Langmuir trough, utilized to direct the IR beam onto the air-aqueous interface at an angle of 22° relative to the surface normal. This angle of incidence is within the optimum 0 - 40° found to be ideal for an air-aqueous interface and unpolarized light [57]. The reflected beam was then directed to a liquid nitrogen cooled MCT detector. All external optics and equipment were constantly purged with dry house air. Spectra were collected with a 1cm$^{-1}$ resolution and were
averaged over 200 scans. A single channel spectrum of a bare water surface was used as the background spectrum. The IRRAS spectra presented here remain as Reflectance-Absorbance (RA) spectra, where \( RA = -\log(R/R_o) \) with \( R \) being the IR reflectivity of the surface of interest, and \( R_o \) being the IR reflectivity of the bare water surface background. Such reflectivities are described using the Fresnel equations, whose details are presented elsewhere [58]. With unpolarized light incident at an angle of 22° relative to the surface normal of an air-aqueous interface as used here, the expected absorption bands will be negative.

9.3 Results

In the following, complementary thermodynamic information from \( \pi-A \) isotherms was used in combination with structural information from BAM images and IRRAS spectra to develop a model regarding the behavior of the interfacial layer. All of these techniques are highly sensitive to surface films, and thereby give information about changes in orientation, packing density and morphology of the molecules residing at the air-aqueous interface. Stearic acid forms a very stable film with known thermodynamic features [59]. With negligible solubility [50] and vapor pressure [60], stearic acid is unlikely to partition into the aqueous or gas phases, and remains primarily as a floating monolayer film. These characteristic phases are discussed in detail in Chapter 2, and are described fully in the cited literature [61-69]. Specifically of interest here are two features evident in the \( \pi-A \) isotherm shown in the inset of Figure 9.1, the molecular footprint (\( A_o \)) and the collapse pressure (\( \pi_c \)). For stearic acid, the characteristic molecular footprint, or mean area occupied by a stearic acid molecule at the water surface, is 20 Å²/molecule and the characteristic collapse pressure is 55 mN/m.
In the isotherms shown in Figure 9.1, the film was subjected to a series of compression/expansion cycles, of which only the compressions are shown.

![Diagram]

**Figure 9.1:** Isotherm compression cycle numbers 1, 6, and 10 of (a) stearic acid deposited on an aqueous subphase and (b) stearic acid deposited on an aqueous 0.02M solution of L-phenylalanine. Inset shows a typical stearic acid isotherm on an aqueous subphase past the point of collapse, with the molecular footprint ($A_o$), collapse pressure ($\pi_c$), and maximum pressure for the isotherm cycles (*) indicated.

The film was not allowed to reach collapse, constrained to a maximum surface pressure of 45 mN/m. In Fig. 9.1a, stearic acid alone was deposited on an aqueous subphase, while in Fig. 9.1b, stearic acid was deposited on a subphase composed of an aqueous solution of L-phenylalanine.
Throughout the course of the isotherm cycles in Fig. 9.1a, little loss of stearic acid was seen from the interface, evidenced by the small change in the footprint value (there was a decrease of ca. 1 Å²/molecule throughout ten isotherm cycles). This is reinforced by BAM images of the stearic acid film alone (Figure 9.2), which show that at a surface area of 21 Å²/molecule in each cycle, there is simply a close-packed monolayer film with no evidence of any disruption or collapse structures.

Figure 9.2: BAM images of stearic acid deposited on a bare water surface taken at a surface pressure of 21 Å²/molecule (scale bar represents 50µm). Numbers (1) – (10) represent the isotherm cycle during which the images were extracted.

When an aqueous solution of L-phenylalanine is present prior to the deposition of stearic acid however, the stearic acid film is modified (evidenced by the progressive decrease in the footprint presented in Fig. 9.1b).

Phenylalanine is a soluble amino acid that partitions to some extent to the interface [49]. This surface partitioning is observed here as an increase in surface pressure over time as the molecule adsorbs to the air-water interface, a phenomenon that has been observed and
characterized in other systems [70]. Fig. 9.3 illustrates the change in surface pressure due to the adsorption of L-phenylalanine over time in the presence of a dilute stearic acid film.

![Graph showing surface pressure measured over time of L-phenylalanine solution](image)

**Figure 9.3:** Surface pressure measured over time of 0.02M L-phenylalanine solution after deposition of stearic acid with corresponding BAM images (scale bar representing 50µm). The asterisk indicates the time at which the isotherm cycles were started in the experiments shown in Figures 9.1 and 9.4.

During this experiment, the stearic acid monolayer was present at a molecular area of ~40 Å², conditions under which two-phase coexistence between a two-dimensional gas and liquid condensed phase would be observed in the absence of a soluble surfactant. The asterisk and vertical broken line indicates the time at which the isotherm cycles were started in the experiment shown in Figs. 9.1b and 9.4. The surface pressure ultimately reaches a maximum of 25 mN/m, at which point it remains constant for the duration of the adsorption experiment. The BAM images, indicated at varying points along the adsorption process of L-phenylalanine,
suggest that a two-phase film is present throughout this adsorption process, with grey condensed phase regions appearing along with dark gas phase regions.

Thus, in Fig. 9.1b, the increased surface pressure at large molecular areas can be attributed to the presence of adsorbed L-phenylalanine. However, at molecular areas approaching 20 Å² in the first isotherm cycle portrayed in Fig. 9.1b, the isotherm appears to mirror that of a pure stearic acid monolayer. The footprint obtained through extending the stearic acid feature to zero surface pressure is consistent with the footprint of stearic acid on a bare aqueous subphase (Fig. 9.1a). These observations are consistent with the removal (i.e. “squeezing out”) of phenylalanine from the interface at surface pressures above ~30 mN/m. As the isotherm cycles progress, however, the stearic acid character diminishes evidenced by a progressively smaller footprint. This contrasts sharply with the behavior described earlier of a stearic acid film on a bare water subphase where very little loss in surfactant is seen throughout all ten isotherm cycles. Similar behavior was seen in the presence of Cd²⁺ (data not shown), indicating a persistent change even in the presence of ions known to stabilize fatty acid monolayers [71]. This indicates the modification of the otherwise stable stearic acid film due to the presence of the adsorbed L-phenylalanine. The modification was seen to persist over the course of at least 24 hours, indicating that it was an irreversible change to the surface monolayer.

The disruption of the stearic acid film is visualized in the BAM images in Fig. 9.4. This series of images was taken at an area of 21 Å²/molecule of stearic acid during each compression cycle.
Figure 9.4: BAM images of stearic acid deposited on an aqueous 0.02M solution of L-phenylalanine taken after compression to an area of 21 Å²/molecule of stearic acid. Numbers 1 – 10 correspond to the isotherm cycle during which the image was taken with the scale bar representing 50µm.

At this molecular area, the stearic acid film should be approaching its liquid condensed phase, with a close-packed, interacting monolayer. This area is also well below the collapse of the monolayer. In the image from the first cycle, all that can be seen is a close packed stearic acid film (seen as a fairly uniform gray color). There are no apparent holes or hydrophobic aggregates as is to be expected from the isotherm data. As early as cycle 2, bright spots representing early collapse structures begin to appear. As the cycles progress, the collapse structures are more pronounced, begin to coalesce, and the surrounding monolayer film exhibits two-dimensional phase coexistence of condensed and gaseous regions. These two-dimensional gas phase regions become more prominent in the later cycles seen as large dark voids in the images.

The IRRAS spectra shown in Fig. 9.5 also confirm the processing of the stearic acid film facilitated by phenylalanine.
Figure 9.5: IRRAS spectra of the Phenylalanine – Stearic Acid mixed film before isotherm cycles (black) and after isotherm cycles (red). The full IRRAS spectrum is shown in (a) and only the C-H stretching region is shown in (b).

The spectrum shown in Fig. 9.5a is the full spectrum of the stearic acid – phenylalanine mixed film before (black) and after 10 isotherm cycles (red) taken at a molecular area of 32 Å² of stearic acid. In this spectrum, it is clear that there is in fact a mixed film of stearic acid and phenylalanine at the surface both before and after isotherm cycles, evidenced by the strong C-H stretch peaks around 2900 cm⁻¹ due to the long hydrocarbon tail of stearic acid, as well as the weaker peaks below 1600 cm⁻¹ due to phenylalanine (with the exception of the sharp peak at 1470 cm⁻¹ which is due to the CH₂ scissoring mode of stearic acid). When comparing the C-H stretch region before and after cycles, shown in Fig. 9.5b, there is a decrease in intensity after the
isotherm cycles. This decrease in intensity can be attributed to either fewer absorbing molecules in the illuminated surface area, or an orientational ordering change of the stearic acid molecules at the surface [46, 72]. It is likely that both of these are contributing here, when these data are taken in conjunction with the BAM images shown in Fig. 9.4. There is an orientational order change of the stearic acid hydrocarbon tails, seen in the BAM images by the formation of three-dimensional aggregates throughout the cycles (bright spots in the images). There is also loss of stearic acid from the surface as seen by the large dark voids in the BAM images. Over the course of the isotherm cycles, the BAM images and IRRAS spectra reinforce the information gained from the isotherms themselves – that the presence of phenylalanine causes modification of the stearic acid film from a uniform hydrophobic surface to a heterogeneous surface containing 3D hydrophobic aggregates, regions of condensed monolayer, as well as exposed hydrophilic "holes" (2D gas).

9.4 Discussion
Pure films of insoluble surfactants (like stearic acid) are known to undergo the transition from a two-dimensional film to form more complex three-dimensional structures once a characteristic surface pressure has been reached, and the film collapses [66]. Among the suggested collapse structures formed of single-component films are micelles which partition into the bulk aqueous phase, three-dimensional aggregates that nucleate and grow at the interface, and the formation of bilayers and trilayers at the surface through film fracture followed by folding [65-68]. The formation of these collapse structures is often described as having a nucleation and growth mechanism, and has been visualized using BAM [67, 68, 73]. Mixed monolayers can have even more complex collapse mechanisms, depending on the components’
miscibilities [69, 70, 74-77]. It is evident from the first isotherm cycle of the stearic acid/L-phenylalanine mixture (Figure 9.1b), that stearic acid and adsorbed L-phenylalanine form an immiscible mixed film. In a true miscible mixed film, there will only be one distinct collapse pressure that is usually different than that of either individual component [69, 74]. In this case, there are two distinct collapse pressures, each characteristic of the individual components which points toward their existence at the surface as an immiscible mixed film [69, 74]. This is seen as the “squeezing out” of L-phenylalanine, evidenced by the near-horizontal region of the isotherm at a surface pressure of ca. 25 mN/m, prior to the expected stearic acid liquid condensed phase.

The collapse of a monolayer to form soluble micelles may be envisioned through the tilting of adjacent surfactant molecules due to tail-tail interactions as described by Safran et al. [62, 78]. The heads are assumed to form an ordered array on the surface, while the hydrophobic tails are allowed to rotate. Safran et al. suggest that the mismatch between the size of the heads and tails of the surfactants promote the formation of “micellelike clusters”, a deviation from the normal uniform tilt state. Here, there is either a larger head-head spacing than is preferred by the tail-tail interaction, or there is a larger tail-tail spacing than is preferred by the head-head interaction (resulting in the tails splaying outward from each other). The resultant structures are named “antisolitons” and “solitons” respectively. Safran et al. then concluded that the antisoliton state can be lower in energy than a uniform tilt state [78]. These antisoliton “micellelike clusters”, when subjected to the external pressure supplied by the mechanical trough barriers, could fold on top of one another forming a cluster of surfactants with hydrophilic heads on the outside and hydrophobic tails on the inside.

It is clear from the BAM images shown in Figure 9.4 that the collapse structures observed in this study are not folds resulting in multilayers (which would be seen as long, line-like
structures) [66], nor are they micelles, which are nm-scale objects that would be invisible via BAM: micelles or larger hydrophilic aggregates would be soluble in the subphase, and would not remain at the surface in the plane of view. In fact, the structures that are formed during the compression cycles are consistent with macroscopic hydrophobic aggregates (i.e. particles) that remain at the interface, presumably due to their hydrophobic character [79]. This mechanism is consistent with recent work by Sierra-Hernandez and Allen showing aggregate formation of long chain halides on the surface of stearic and palmitic acid Langmuir films [46]. In order for such a structure to form, however, the tails of the surfactants must splay outward rather than bunch inward (comparable to Safran et al.’s soliton state versus antisoliton state [78]). This is generally considered to be a less favorable state (energetically) than either the state where the tails are bunched inward or a uniform tilt state. However, if a large hydrophobic group (like the phenyl ring from phenylalanine) is inserted between some of these long hydrocarbon tails, it could force them to splay outward and begin the formation of these structures.

The stability and mechanism of the collapse of a monolayer is usually considered to be due to interactions among the head-groups of the surfactant molecules at the interface [68, 80]. Through hydrogen bonding among the head-groups, stable bridging interactions are formed, and prevent the disruption of the ordering of the head-groups through collapse [80, 81]. Hydrophobic interactions, however, may also be important in the formation of hydrophobic collapse structures at the interface. Hydrophobic interactions have been seen to disrupt stable hydrogen-bonded networks (like the network formed by the hydrophilic heads of the surfactant molecules) in previous studies [82-84]. Recently, Zangi et al. have used molecular dynamics simulations to propose a mechanism for the denaturing of proteins by urea, where the hydrophobic interaction between urea and the protein backbone is stronger than the interaction of
water with the protein, thereby forcing its unfolding [82]. Chandler has more broadly explained hydrophobic phenomena which occur at interfaces as essentially water-excluding interactions, where a hydrophobic interaction is facilitated by the exclusion of water from between the hydrophobic regions along with the necessary disruption of hydrogen bonds at the interface [85]. Chen et al. have experimentally shown using BAM and sum-frequency spectroscopy that small soluble molecules such as dimethylsulfoxide compete for the surface and can exclude water from the head-group region, caging and prematurely condensing lipids [84, 86]. In a system such as the one presented here this disruption would be facilitated by the phenyl ring forcing its way in between the hydrocarbon tails and then, through a hydrophobic interaction, forcing the distortion of the monolayer. The hydrophilic head of the surfactant would still be able to form hydrogen bonds to the carboxylic acid and amine group on the amino acid, and the phenyl group would have been suspended above the surface as has been observed using sum-frequency spectroscopy [87, 88], allowing for the necessary hydrophobic interaction with the hydrocarbon tail. The resultant distortion would be manifested by the splaying of the long chains outward, and then through continued external pressure on the film, would force the formation of hydrophobic aggregates. In contrast to the micelles described earlier, these hydrophobic aggregates would remain at the surface, as was observed using BAM and confirmed by the continuing presence of stearic acid C-H stretches observed using IRRAS.

9.5 Conclusions
Surface films on atmospheric aerosols play an important role in chemistry and climate in both the prebiotic and contemporary atmospheres [24, 25, 89, 90]. In the modern atmosphere, aerosols are known to have an effect on climate [3]. The aerosol direct effect (light attenuation)
is significantly influenced by aerosol size, prompting the need for a better understanding of the hygroscopicity of the particles. Surfactants on aerosols have been proposed to impede the particles’ ability to uptake water, thereby impeding its growth, but experiments present conflicting results. The aerosol indirect effect (ability to serve as cloud condensation nuclei) has also been suggested to be dependent upon the surface morphology of the particle through the dependence of Köhler theory on surface tension [91]. In this study we have shown that an otherwise stable surfactant film can be modified due to the presence of a soluble amino acid, exposing hydrophilic holes and forming hydrophobic aggregates at the air-water interface. This hydrophobic collapse suggests that the effect of surfactants on atmospheric aerosols is more complicated than a simple monolayer impeding water transfer. Instead, the composition of the entire aerosol and its atmospheric processing must be taken into consideration because molecules from the aerosol’s interior may have the ability to significantly disrupt the surface morphology, thereby changing the surface tension of the particle (affecting its propensity to serve as a CCN) and possibly opening up hydrophilic holes to allow for increased water uptake and growth.

9.6 References for Chapter 9


10 Mixed Aromatic – Aliphatic Films at the Air – Water Interface Case II: Benzoic Acid / Benzaldehyde with Stearic Acid\(^\text{15}\)

10.1 Introduction

Water surfaces, characteristic of lakes, oceans, and the surface of aqueous atmospheric aerosols, are ubiquitous on Earth and are often rich in organic content [1-4]. These organics stem from a variety of sources, both anthropogenic and biogenic, resulting in a diverse and complex organic surface composition. In this work, the effect of introducing a surfactant with a very different hydrophobic structure (an aromatic ring) on the stability and morphology of a stearic acid film is explored. Experiments using Langmuir trough methods as well as the \textit{in situ} surface-sensitive spectroscopic technique IRRAS (Infrared Reflection-Absorption Spectroscopy) are complemented by molecular dynamics simulations to investigate mixed films of oxidized aromatics (benzoic acid, benzaldehyde) with stearic acid.

Since it is experimentally difficult to probe environmental surfaces directly, two-dimensional model surfaces (such as those provided by the Langmuir trough) are often utilized in laboratory settings. Much work has been performed studying long-chain fatty acid films at the water-air interface, using both pure and multi-component monolayers [5-12]. Among the common long-chain fatty acids studied are stearic, palmitic, and oleic acids, with similar hydrophobic structure composed of long hydrocarbon chains. These three surfactants in particular are also common surfactants found on the surface of atmospheric aerosols in field studies [3, 4, 13, 14], primarily due to biogenic emissions. Stearic acid (the fatty acid used in

this work) is a well-characterized surfactant, known to produce very stable floating monolayers on water [7]. It exhibits characteristic two-dimensional phase changes at the water surface, finally resulting in collapse [15-18] (fracture of the two-dimensional surface film causing the formation of three-dimensional structures) above pressures of 55 mN/m [19]. This behavior allows for control and monitoring of the morphology and phase behavior of the stearic acid film in a laboratory setting.

In addition to biogenic emissions, environmentally catastrophic events such as the Deepwater Horizon Oil Spill in the Gulf of Mexico in 2010 result in further complicated emissions into the atmospheric, with poorly understood consequences. A NOAA mission collected aerosols in a variety of locations around the spill and discovered that not only was there a higher concentration of volatile organics that had directly evaporated from the oil, but also a significant concentration of less-volatile organics [20, 21]. Such anthropogenic emissions are then suggested to not only be present in the atmosphere due to catastrophic events such as oil spills, but may also be a normal component in other polluted regions of the atmosphere [20].

Oil, although vastly diverse in composition, can contain a significant concentration of aromatic species [22]. These aromatics differ significantly in hydrophobic structure from biogenic surfactants (e.g. stearic, palmitic, oleic acids) currently taken into consideration in aerosol models and laboratory studies. Although crude oil contains primarily non-polar (un-oxygenated) molecules, once exposed to the atmosphere they can be readily oxidized [9, 23-27]. It has recently been suggested that current field studies of oil spill emissions may be missing a significant fraction of the crude oil released into the environment due to the limitations of their instrumentation which only detect un-oxygenated components [23]. Once the oil has been “weathered”, a significant fraction contains oxygenated residues, resulting in oxidized
surfactants likely to interact at the water surface differently than their unoxidized counterparts [28]. This has further implications on the interactions of oil components with biogenic surfactants naturally present in the atmosphere at the water surface (e.g. the surface of atmospheric aerosols). In this work, we demonstrate the modification to the aqueous surface containing a common biogenic surfactant (stearic acid) due to the presence of two different oxidized aromatics, benzoic acid and benzaldehyde, suggesting the importance of not only the hydrophobic aromatic group, but also the identity of the polar group in the mechanism by which the surface is modified.

Finally, the surface morphology of atmospheric aerosol particles has wide-ranging effects on the particle’s impact on climate [27, 29-37]. Various morphologies have been observed in laboratory studies and in field observations ranging from an inverted micelle structure with the organic components partitioning to the exterior of the particle in a core-shell structure [6, 27, 38, 39], to more complex morphologies containing organic inclusions [40-42] or even surfactant lenses [43]. Two-dimensional films composed of long-chain surfactants (like stearic acid) are known to impede water transfer [6, 44-47], although the quantitative effect on three-dimensional aerosol particles has thus far yielded conflicting results [10, 13, 34, 48-50], possibly due to these varying surface morphologies. Since aerosol optical properties are dependent upon their size [51], the ability for the uptake or evaporation of water across the particles surface is an important property to understand. Although compressed single-component monolayers composed of long-chain surfactants will impede water transfer, less work has been performed exploring the more environmentally relevant mixed films with varying hydrophobic character. This work utilizes the complexity of a mixed film with differing hydrophobic groups (both aliphatic and aromatic)
to contribute to an understanding of the varying morphologies likely present on aqueous aerosols in the atmosphere.

### 10.2 Materials and Methods

All solvents and reagents were used without further purification. Stearic acid (octadecanoic acid, 98+%) was purchased from Aldrich Chemical Co., Inc and then dissolved in chloroform (ACS grade, Mallinckrodt Baker, Inc.) to a concentration of 1 mg/ml. Benzoic acid (ACS grade, ≥ 99.5%) was purchased from Sigma-Aldrich and was dissolved to a final concentration of 0.01M in distilled water. Benzaldehyde (ReagentPlus, ≥ 99%) was also purchased from Sigma-Aldrich and was prepared to a concentration of 0.01M in distilled water for the isotherm experiments and to a concentration of 0.002M for the IRRAS experiment. The higher concentration of 0.01M could not be used for the IRRAS experiment due to the interference of the gas-phase benzaldehyde signal with the much weaker IRRAS absorbance (vapor pressure of benzaldehyde is approximately 1 mmHg at 25°C compared to benzoic acid’s 0.001 mmHg at the same temperature). The features in the isotherms with the two different concentrations are similar, however the extent of modification to the stearic acid film is exacerbated at lower concentrations. This difference is attributed to fewer benzaldehyde molecules partitioning into the gas phase during the compression of the surface layer.

In the isotherm cycle experiments, aqueous solutions of benzaldehyde or benzoic acid were prepared to the concentrations specified above, and spread on the corresponding Langmuir trough. The stearic acid solution (1 mg/mL in chloroform) was immediately spread on the aqueous interface (10 µL for the IRRAS experiment and 30 µL for the isotherm cycles only) and the solvent was allowed to evaporate for 20 minutes without any disruption. After solvent
evaporation, isotherm compression-expansion cycles were performed (the pressure was zeroed immediately prior to the start of the cycles to result in all surface pressure readings to be relative to the initial surface conditions), with the mechanical trough barriers progressing at a constant speed between their fully open position (280 cm$^2$ for the large trough for isotherm measurements and 70 cm$^2$ for the small trough used in the IRRAS measurements) and a maximum surface pressure of 40 mN/m (remaining well below the collapse pressure of the stearic acid film). IRRAS spectra were taken with the barriers fully open (surface area of 70 cm$^2$) before isotherm cycles for both aromatics, after three isotherm cycles for benzaldehyde, and after four isotherm cycles for benzoic acid.

10.2.1 Langmuir Trough

The Langmuir trough for isotherm studies was custom-built, and consists of a PTFE trough (52 x 7 x 0.5 cm) coupled to two mechanical, computer-controlled, PTFE barriers. These barriers were manipulated using software and a computer interface purchased from KSV-NIMA (Bilion Scientific, Finland). The Langmuir troughs used for both isotherm and IRRAS studies were equipped with a Wilhelmy balance that allowed for the measurement of surface pressure concurrent with the mechanical limitation of the surface area (due to the motion of the barriers across the aqueous surface). The measured surface area is the area between the moving mechanical barriers measured in cm$^2$, and converted to Å$^2$ molecule$^{-1}$ when stearic acid is present. This produces a surface pressure – area ($\pi$ – A) isotherm, thereby giving interfacial thermodynamic information. In the experiments presented here, isotherm compression/expansion cycles were performed, during which the mechanical barriers continuously moved from their open to closed positions with constant forward and backward
barrier speeds (100 cm²/min) with no wait time between cycles. It should be noted that only the compression isotherms are presented in the isotherm cycle figures in this work.

10.2.2 Infrared Reflection-Absorption Spectroscopy

IRRAS spectra were taken with the external port of a Bruker Tensor 27 FTIR Spectrometer coupled to a NIMA PTFE Langmuir trough (14.5 x 7 x 0.5 cm) controlled using software purchased from KSV-NIMA (Biolin Scientific, Finland). The infrared beam (unpolarized) exited the spectrometer and was passed through a CaF₂ lens before being directed onto the aqueous surface of the Langmuir trough. Two 2 in. diameter gold mirrors were used to direct the IR beam onto the surface, with an angle of incidence of 22° relative to the surface normal, and then direct the reflected light to the liquid nitrogen cooled MCT detector. All optics and equipment (including the Langmuir trough) were constantly purged with dry house air during all experiments. Single channel spectra were collected with a 1 cm⁻¹ resolution and were averaged over 200 scans. A single channel spectrum of a bare water surface was used as the background spectrum for the IRRAS spectra collected here. All single channel spectra were atmosphere corrected (compensating for water vapor and CO₂ signals). The resulting reflectance-absorbance (RA) spectrum presented is then obtained using the equation RA = -log(R/R₀), where R is the IR reflectivity of the surface of interest (in this work, the mixed film of stearic acid and either aqueous benzoic acid or benzaldehyde), and R₀ is the IR reflectivity of the bare water surface background. These reflectivities are fully described in the literature using the Fresnel equations, which are presented elsewhere [52, 53]. In this experiment, the expected absorption bands will be negative resulting from the use of unpolarized light incident at an angle of 22° relative to the surface normal of an air-aqueous interface.
10.2.3 Molecular Dynamics Simulations

The MD simulation results presented in this chapter were performed by Andre Pimental and Teobaldo R. C. Guizado at the Pontificia Universidade Católica do Rio de Janeiro. The stearic acid and aromatic compounds interaction parameters were obtained using the automated topology builder (ATB) and repository [54]. Two stearic acid films were initially composed of 196 stearic molecules each, arranged in such a way as to yield an area of 21 Å² molecule⁻¹. The stearic acid molecules were assembled in two rectangular arrays with 14×14 molecules each in the x and y directions respectively, separated by 6 nm in the axis z with the polar heads pointing to the aqueous phase. The space between the two stearic acid films was filled with 20 molecules of aromatic compounds and ~8,000 SPC water molecules [55] at random positions, and the box edge along the z direction was set to 50 nm, resulting in one stearic film on each air-aqueous solution interface. Aqueous solutions of benzaldehyde and benzoic acid were also built inside a box with the same dimension, resulting in two air-aqueous solution interfaces without the stearic acid film. The periodic boundary conditions produce an infinite slab with two air-aqueous solution interfaces perpendicular to the z-axis at a constant volume. 20 molecules of aromatic compounds and ~8,000 SPC water molecules were randomly generated within a slab of 6 nm in the middle of this box of 50 nm in the z direction, yielding approximately 0.15 mol L⁻¹ aqueous solutions of aromatic compounds. The lower concentration of 0.01M could not be used in the MD simulation due to the need of about 10 times more water molecules, which is unfeasible computationally. On the other hand, only two molecules of aromatic compounds with ~8,000 SPC water molecules yield a concentration of 0.01M, but in this system it would be impossible to visualize the aggregates inside the ensemble as we desire to observe in the MD simulation.
These structures were equilibrated using 5000 steps of the steepest descent, followed by L-BFGS minimization [56, 57] to avoid strong repulsive contacts between stearic acid molecules. Then, a molecular dynamics simulation was used to relax the system for 200 ps. Finally, a 50 ns molecular dynamics simulation was performed to estimate the thermodynamic properties of the system using the constant number of particles N, volume V and temperature T (NVT) ensemble. This last step was performed using weak thermal coupling ($\tau_T = 0.1$ ps) at $T = 298$ K [58].

Trajectories were run up to 50 ns with a time step of 2 fs without any geometry constraint. Non-bonded interactions were truncated using a 1.0 and 1.2 nm twin-range cutoff for Lennard-Jones and Coulomb interactions, respectively. Dispersion correction was applied to the energy, and the neighbors list was updated every 10 steps. All analyses were performed using the last 30 ns of the data for mass density and order parameter of the film, using coordinates and energies recorded at 2 ps intervals. All simulations were carried out using the GROMACS 4.5.3 package [59].

Besides characterizing which orientation corresponds to each isotherm, molecular dynamics simulations allow a thorough description of the molecular organization for each film. This is an important issue because the degree of order may be correlated with the order parameter ($S_z$), which is related to the average spatial orientation of molecules and may be defined as,

$$S_z = \frac{1}{2} \langle 3 \cos^2 \theta_z - 1 \rangle$$  \hspace{1cm} (10.1)

where $\theta_z$ is the angle between a reference vector in stearic acid molecules and the $z$ axis and the brackets denote the average over all equivalent atoms and over time. From MD simulations, the
order parameter is calculated for consecutive C-C bonds. As a united atom forcefield is used (without hydrogen or deuterium atoms), the C–D bond vector needs to be reconstructed. To do so, the C(i – 1) – C(i + 1) vector for each i C atom is usually taken to be the z-axis. The x- and y-axis are defined perpendicular to the z-axis and to each other, with the y-axis in the C(i – 1) – C(i) – C(i + 1) plane. Using this definition, $S_z$ can be compared bond-by-bond directly to $^2$H-NMR data. The perpendicular direction emerges as a natural choice to compute order parameters for monolayers spread on the x-y plane. $S_z$ values range from -0.5 to +1.0, meaning an orientation either fully perpendicular or fully parallel to the z axis, respectively. A value of around 0 means an average random orientation of the vector with respect to the z axis. For the aliphatic atoms, the line joining the reference atom and the next heavy atom attached to it will define the vectors.

10.3 Results
In this work, mixed films of oxidized aromatics (benzaldehyde and benzoic acid) with a well-known aliphatic compound (stearic acid) are explored experimentally using Langmuir trough methods and Infrared Reflection-Absorption Spectroscopy (IRRAS), and theoretically using Molecular Dynamics (MD) simulations. The phase changes of surfactant films at the water surface are described in detail elsewhere [60-63], as well as in Chapter 2 of this thesis, but will be described briefly here. Two-dimensional phase changes are indicated in the $\pi$ – A isotherm by sharp slope changes (discontinuities, e.g. kinks or plateaus). In a stearic acid isotherm (see Fig. 2.4 of Chapter 2), the film begins (moving from large to small area, right to left on the area axis) in a disordered two-dimensional gaseous phase where the molecules are highly fluid and disordered on the surface (negligible surface pressure). Then, in stearic acid, the film transitions directly to a tilted liquid condensed phase [64, 65], where the molecules are
ordered but tilted relative to the interface [63]. Finally, the stearic acid molecules transition to an
untilted liquid condensed phase where the molecules are tightly packed and occupy a minimum
area at the surface.

Conventionally, the untilted liquid condensed phase is extrapolated to zero surface
pressure yielding a characteristic area that the molecule occupies at the surface, or its “footprint”
($A_o$). For stearic acid, the footprint is known to be ca. 20 Å$^2$ molecule$^{-1}$ [7]. The footprint of a
surfactant is a useful tool for following modifications to the surface morphology and/or
composition. When isotherm compression-expansion cycles were performed for a pure stearic
acid monolayer (Chapter 9), this footprint changed by less than 1 Å$^2$ molecule$^{-1}$, illustrating the
stability and reproducibility of the phases exhibited by a floating stearic acid film [66].
However, as seen in Chapter 9, when phenylalanine was also present in the surface region,
significant disruption of the stearic acid film was observed in the isotherm cycles, evidenced by
the significant loss of the stearic acid character from the isotherms seen in the decrease of its
footprint over the course of the isotherm cycles [66]. In addition to the decrease in footprint,
there was also a decrease in slope of the untilted liquid condensed phase, indicating a decline in
phase stability.

In this chapter, a similar analysis can be employed utilizing a stearic acid film deposited
on an aqueous solution of benzoic acid or benzaldehyde, two decreasingly oxidized aromatics
(isotherm cycles shown in Figures 10.1(a) and 10.1(b) respectively).
Figure 10.1: Aromatic – stearic acid mixed film $\pi$ – A isotherm cycles. Only compression isotherms from cycles (1), (5) and (10) are shown (as indicated). (a) Benzoic acid (0.01M) – stearic acid mixed film. (b) Benzaldehyde (0.01M) – stearic acid mixed film.

Both benzoic acid and benzaldehyde are surface active, exhibiting an increased surface pressure at small surface areas in both pure aqueous solution isotherms (Fig. 10.2) as well as in MD simulations of the pure aqueous solutions in the absence of stearic acid (Fig. 10.3).
Figure 10.2: $\pi$-A isotherms of 0.01M aqueous solutions of benzoic acid (black) and benzaldehyde (red)

Figure 10.3: Snapshots of the MD simulations of benzaldehyde (A) and benzoic acid (B) solutions only at the water surface after 10 ns. The benzoic acid and benzaldehyde molecules are primarily located at the water/air interface, corroborating with the surface partitioning observed in isotherms.

This is consistent with previous MD simulations performed on aqueous solutions of anionic benzoate as well as other aromatics, which indicated the strong surface propensity of these molecules [67]. In the mixed film of benzoic acid and stearic acid (Fig. 10.1(a)), the isotherm plateaus around 16 mN/m, the same surface pressure the isotherm of pure aqueous benzoic acid changes slope (Fig. 10.2). Therefore, this feature is due to the presence of benzoic acid.
Analogously, in Fig. 10.1(b), the plateau around 12.5 mN/m is due to benzaldehyde, with the same surface pressure exhibiting a phase change in the pure benzaldehyde isotherm (Fig. 10.2). After these plateaus, there is a sharp increase in surface pressure due to the phase change in stearic acid to its untilted liquid condensed phase. Analogously to the pure stearic acid isotherm, this phase may be extrapolated to zero surface pressure to yield the footprint of stearic acid. In both Figs. 10.1(a) and 10.1(b), in isotherm cycle 1 the stearic acid footprint is comparable to its value in its pure monolayer (ca. 20 Å² molecule⁻¹). Then, as the isotherm cycles progress, the benzoic acid – stearic acid mixed film (Fig. 10.1(a)) exhibits a marked decrease in the stearic acid footprint indicating modification to the stearic acid film due to the presence of benzoic acid, while the decrease in the stearic acid footprint in the benzaldehyde – stearic acid mixed film (Fig. 10.1(b)) is less pronounced indicating a lesser modification.

The IRRAS spectra in Figure 10.4 yield further insight into the modification of the stearic acid film due to the aromatic group present on both benzaldehyde and benzoic acid. The most prominent peaks at 2918 cm⁻¹ and 2850 cm⁻¹ are due to the asymmetric and symmetric CH₂ stretches respectively of the stearic acid molecules present at the surface. The C-H stretching region in IRRAS spectra is sensitive to orientational ordering changes at the surface [68, 69]. Here, there is an increase in intensity in the C-H stretch region in both the stearic acid – benzaldehyde and stearic acid – benzoic acid mixed films after the films have been subjected to a few isotherm compression-expansion cycles. As has been noted in phospholipid monolayers, this can be indicative of either more molecules in the illuminated surface area or a change in the orientational ordering of the hydrocarbon tails of the surface molecules [68]. Both of these cases can indicate a prematurely condensed stearic acid film at the surface.
This claim is supported by the calculated order parameters and tilt angles of pure stearic acid versus the mixed aromatic – stearic acid films. In the study of molecular monolayers with large hydrocarbon chains, it is useful to characterize the relative order of these chains with respect to a reference axis. Experimentally, the deuterium order parameter is obtained by NMR, where the hydrocarbon chains are selectively deuterated and the resultant angle between the deuterium and carbon backbone is measured relative to an axis perpendicular to the interface. Figure 10.5 shows the calculated deuterium order parameters for pure stearic acid (red circles), mixed benzoaldehyde/stearic acid (black squares), and mixed benzoic acid/stearic acid (green triangles) films.
Our MD simulations show that mixed benzaldehyde/stearic acid films and mixed benzoic acid/stearic acid films are slightly more ordered than the pure stearic acid film. This result is consistent with all carbon atoms in the hydrocarbon chain. Further, the calculated tilt angle ($\theta$) of the pure stearic acid film relative to the stearic acid film mixed with benzaldehyde/benzoic is around 20°, which is the angular shift caused by the formation of benzaldehyde or benzoic acid aggregates. It is important to note that the stearic acid film mixed with benzaldehyde or benzoic acid aggregates is more perpendicular to the interface than the pure stearic acid film because of the increase of packing caused by the inclusion of benzaldehyde or benzoic acid molecules into the stearic acid film, i.e., less room available among stearic acid molecules. In both the IRRAS spectra (Fig. 10.4) as well as the calculated order parameters (Fig. 10.5) and tilt angles, the presence of benzaldehyde or benzoic acid is shown to reduce the area available for the stearic
acid molecules, which facilitates their alignment with the normal axis with respect to the interface.

Snapshots of the MD simulations performed are shown in Figures 10.6-10.8. Figure 10.6 portrays the benzoic acid/stearic acid system during the first 7 ns of the simulation (total simulation time was 50 ns).

Figure 10.6: Sample snapshots of the first 7 ns of benzoic acid adsorption at the interfacial region. During this time, the benzoic acid molecules are seen to migrate to the interface and into the hydrocarbon chains forming π-stacked aggregates.
At the beginning of the simulation, the benzoic acid molecules are distributed throughout the bulk solution (0 ns), but partition to the interfacial region within the first 7 ns of simulation. Figure 10.6 also illustrates the propensity of these aromatic molecules to form aggregates through π-stacking of the aromatic rings. Benzaldehyde exhibited similar behavior and is therefore not shown explicitly here. The simulations were allowed to proceed for a total of 50 ns, after which snapshots were taken and are presented in Figs. 10.7 (stearic acid only, benzaldehyde/stearic acid mixed film) and 10.8 (benzoic acid/stearic acid mixed film). In Figure 10.7, the resulting mixed film of benzaldehyde and stearic acid is shown in A (front view) and B (view from the bulk toward the interface), with stearic acid only presented in C (front view) and D (view from the bulk toward the interface) for comparison. First, stearic acid remains as an ordered stable film after 50 ns of simulation. Looking from the bulk at the interface (Fig. 10.7D), there is a fairly uniform coverage of stearic acid molecules, in a sense shielding the bulk phase from the air above the film. In the benzaldehyde/stearic acid mixed film however, the benzaldehyde molecules are seen to have intercalated into the stearic acid film (Fig. 10.7A), forming π-stacked aggregates within the hydrocarbon chains of the stearic acid film layer.
Figure 10.7: Mixed benzaldehyde/stearic acid film ((A) and (B)) and a pure stearic acid film ((C) and (D)) at the air-water interface after 50 ns of simulation time with a molecular area of 21 Å² per molecule of stearic acid. (A) and (C) are from a front view. (B) and (D) are from the bulk towards the top layer. The “holes” appear in the mixed film (B), but not in the pure film (C), i.e. the control system.

Figure 10.8 then shows the benzoic acid/stearic acid mixed film after 50 ns, again from both a front view (Fig. 10.8A) and viewed from the bulk towards the interface (Fig. 10.8B).
Figure 10.8: Mixed benzoic acid/stearic acid film at the interface after 50 ns at 21 Å² molecule⁻¹. In the front view (A) aggregation oriented in the direction of the hydrocarbon chains is observed. The view from the bulk towards the top layer is presented in (B), showing “holes” in the film.

As with benzaldehyde in Fig. 10.7, benzoic acid intercalates into the stearic acid film. However, rather than retaining its π-stacked aggregate form, it forms dimers within the stearic acid film through hydrogen bonding of the carboxylic acid head groups following the orientation of the hydrocarbon chains of the stearic acid film. The result of both of these aromatic molecules intercalating into the stearic acid film is a perturbation to the ordered film layer, forming “holes” or regions lacking in surfactant coverage (region circled in Fig. 10.7B and Fig. 10.8B). These “holes” allow for easier access to bulk molecules, regardless of the compressed state of the surfactant film. These aggregates (both the π-stacked aggregates of benzaldehyde and the H-
bonded aggregates of benzoic acid) are also the source of the tilt angle change of the stearic acid film seen in both the calculated value described above and in the IRRAS spectra shown in Fig. 10.4.

10.4 Discussion

This work explores the effect of the interaction of an aromatic soluble surfactant with a stearic acid film. In Chapter 9, L-phenylalanine was seen to interact with the stearic acid monolayer and cause “early hydrophobic collapse” of the aliphatic film [66]. It was then suggested that this collapse was specifically caused by hydrophobic interactions between the aromatic ring of phenylalanine and the hydrocarbon tail of stearic acid. The bulky aromatic ring caused splaying of the otherwise ordered tails of the stearic acid molecules, analogous to the micelle-like clusters named “solitons” by Safran et al. [70], resulting in the formation of hydrophobic aggregates at the surface. These aggregates were evidenced by diminishing stearic acid character in the isotherm cycles, and were directly visualized using Brewster Angle Microscopy (BAM). The work presented in this chapter expands on the work presented in Chapter 9 by exploring the effect of changing the hydrophilic group of the aromatic molecule, while also adding MD simulations to yield a molecular visualization of the stearic acid film disruption.

It is clear from the results due to phenylalanine as well as the aromatic molecules used in this work that the significantly different hydrophobic groups (between stearic acid and the aromatic molecules) present at the surface facilitates the modification to the stearic acid film. However, this work also illustrates the role of the hydrophilic group in this surface modification. The aldehyde group on benzaldehyde, although hydrophilic, is not as oxidized as the carboxylic
acid group on benzoic acid. This results in benzoic acid having a higher propensity for hydrogen bonding (as seen in the dimers formed within the surface film seen in Fig. 10.8A) as well as being much less volatile than other aromatics. Benzaldehyde has a lower propensity for hydrogen bonding, and hence a much higher vapor pressure. Both aromatics do intercalate into the stearic acid film, but with increased surface pressure invoked by the mechanical barriers, they behave differently due to their hydrophilic character. Benzaldehyde, with its higher volatility, is easily squeezed out into the gas phase, resulting in very little enduring modification to the stearic acid film throughout the isotherm cycles (Fig. 10.1b) beyond the initial change in orientation of the hydrocarbon tails (evidenced by IRRAS C-H stretch intensity increase and calculated stearic acid tilt angle and order parameters). In contrast, benzoic acid has a much more pronounced disruption of the stearic acid film with repeated isotherm cycles (Fig. 10.1a). Because of its ability to hydrogen bond much more strongly to itself, as seen in Fig. 10.8A, and to the water molecules or stearic acid molecules in the interfacial region, benzoic acid is much less volatile and is retained in the stearic acid film. Rather than being squeezed out into the gas phase as benzaldehyde does, benzoic acid remains in the interfacial layer and forces further, more long-lasting, modification to the stearic acid film with increased external pressure from the mechanical barriers, evidenced by the more significant decrease in stearic acid footprint with isotherm cycles (Fig. 10.1a). Note, however, that although the footprint of stearic acid decreases, the slope remains consistent. This is in contrast with the complete loss of stability of the stearic acid film evidenced by a significant decrease in slope of the untilted liquid condensed phase seen in the phenylalanine/stearic acid mixed film system [66]. Although both benzoic acid and phenylalanine modify the stearic acid film, their effect is qualitatively different.
The data presented here along with the previous work on the phenylalanine/stearic acid mixed film system [66], yields a more complete picture of the effect of aromatics on aliphatic films. Although the largely different hydrophobic group provided by the aromatic ring does facilitate the adsorption to the interfacial region as well as the initial surface film disruption (orientation and ordering change), the extent of modification with repeated changes in surface pressure (during the isotherm cycles) is greatly affected by the hydrophilic head group. Although a detailed mechanism has yet to be obtained, this work indicates that there is a complex interplay between the hydrophilic headgroup as well as the hydrophobic group in facilitating the modification to the surfactant film. It is clear that the aromatic hydrophobic group drives the surface adsorption, as well as mediates aggregate formation through $\pi$-stacking (at least initially, although the aggregate structure may change at later times as evidenced in the benzoic acid case here). These aggregates initially alter the orientation of the surfactant hydrocarbon chains (see IRRAS spectra in Fig. 10.4 as well as calculated order parameters in Fig. 10.5). Any further modification to the stearic acid film however is determined by the nature of the hydrophilic head group. Without the ability to strongly hydrogen bond, the aromatics may simply be squeezed out into the gas phase without significant alteration of the surface, as seen in the benzaldehyde case here. With the ability to hydrogen bond, however, the initial $\pi$-stacked aggregates may transition to hydrogen-bonded dimers that remain within the surfactant hydrocarbon tails, inducing further surface modification (see isotherm cycles in Fig. 10.1a).

### 10.5 Atmospheric Implications

Understanding the surface morphology of atmospheric aerosol particles is of great importance in determining the particle’s effect on climate, due to the surface’s impact on water
transfer into and out of the particle. Although two-dimensional surface films of long-chain fatty acids are known to impede water transfer, this work illustrates that the presence of a soluble surfactant with a significantly different hydrophobic structure may significantly change the surface morphology, and hence the water transfer properties. Long-chain fatty acids such as stearic acid used here are the primary surfactants found on atmospheric aerosol particles but stem primarily from biogenic sources. Emissions from anthropogenic sources such as oil spills can significantly alter the composition of the aerosol particles, with oil components existing in a variety of oxygenated states after atmospheric weathering. The oxidized aromatic components from these emissions can greatly complicate the surface morphology of aerosol particles containing some portion of a biogenic surfactant coating, forcing regions of the aerosol core to be exposed despite the surfactant coating. This destabilization of the surface film is expected to induce greater permeability of the particle as a whole to water, thereby changing the size of the particle and hence its impact on climate.

10.6 References for Chapter 10


11 Mixed Aromatic – Aliphatic Films at the Air – Water Interface Case III: Phenylalanine with DPPC

11.1 Introduction

Aromatic residues serve many purposes throughout modern biology [1] ranging from playing key roles in membrane channel gating functions [2, 3] to the formation of deleterious amyloid structures [4, 5]. Phenylalanine residues in particular have been identified as a key component in the formation of amyloid structures [6]. In general, phenylalanine is a unique molecule in terms of its propensity for self-assembly. Short peptides solely composed of phenylalanine (e.g. Phe-Phe) are known to self-assemble into ordered nanostructures in water [7]. Further, the monomer amino acid has recently been shown to self-assemble into amyloid-like fibrils resulting in cytotoxicity relevant to Phenylketonuria (PKU) disease [8, 9]. This effect is of particular interest since this is the first example of a single amino acid that has this behavior. Despite the vast knowledge currently being amassed on the many functions of aromatic residues, and even single aromatic amino acids, it is still quite difficult to obtain molecular-level information on the cause and mechanism of these functions on a biological scale.

In this work, the interaction of the single amino acid L-phenylalanine (Phe) with a monolayer composed of a model membrane phospholipid (DPPC) is explored with complementary information obtained through experiment, using Langmuir trough methods and Brewster Angle Microscopy (BAM), and through Molecular Dynamics (MD) simulations.

Here, the simplified system of a monolayer is used rather than the bilayer structure found in real biological systems. Despite being a model system, monolayers have the same lipid – water interface present in bilayer systems and thus still allow for exploration of perturbations to the membrane. In addition, using a monolayer rather than a bilayer has many advantages [10].
Monolayer experimental studies are quite simple and well established, allowing for a wide range of experimental conditions to be controlled and explored in detail. Thus, the monolayer system is used here as a model system to explore the interactions of Phe with a phospholipid membrane, in order to gain molecular-level information not traditionally available to more phenomenological molecular biology studies.

In particular, the work by Adler-Abramovich et al. [8] is intriguing in this context. They demonstrated the self-assembly of Phe monomers into long fibrils that were subsequently observed to be cytotoxic, even resulting in misshapen cell membranes for the remaining viable cells. This effect is very important in understanding the cause of the disease phenylketonuria (PKU), characterized by an inability to process ingested phenylalanine. However, no mechanistic insight into this cytotoxicity has been obtained to date beyond the assertion of the apparent similarity to amyloid structures. Thus, the aim of this work is to gain insight into the molecular-level interactions of Phe with a model phospholipid – water interface, characteristic of cell membranes. This will aid in not only understanding the mechanism of the cytotoxicity of Phe in PKU disease, but also in the interactions of other Phe-rich aggregates found in modern biology with cell membranes.

11.2 Materials and Methods
All materials were purchased and used without further purification. L-phenylalanine was purchased from Alfa Aesar (99%) and was dissolved in DI water to a final concentration of 2.5 mM. The solution was prepared fresh for each experiment. 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, >99%, Sigma-Aldrich) was dissolved in chloroform (ACS grade, Mallinckrodt Baker, Inc.) to a final concentration of 1.6 mg/mL, to be deposited dropwise onto the Langmuir trough in the subsequent experiments.
11.2.1 Langmuir Trough

In this work, Langmuir trough methods were used to monitor and characterize the phase behavior of the mixed film system. The Langmuir trough used was custom-built (52 x 7 x 0.5 cm), consisting of a PTFE trough as well as two PTFE barriers controlled by software purchased from NIMA (KSV-NIMA, Finland). This trough was coupled to a Wilhelmy balance used to measure changes in surface tension, producing a surface pressure – area (π – A) isotherm. This isotherm yields surface thermodynamic information allowing for the monitoring of changes in the phase behavior of the surfactant of interest (DPPC) in the presence of a perturbant (Phe).

11.2.2 Brewster Angle Microscopy

Brewster Angle Microscopy (BAM) is a useful tool to visualize morphological changes on a microscopic scale at the water surface [11, 12]. A similar Langmuir trough to the one described in 11.2.1 was used (168 mm x 86 mm, KSV minitrough, Finland) coupled with a custom-built BAM. The BAM consisted of a self-assembled symmetric goniometer system, using 543 nm, 5W, p-polarized laser light (Research Electro-Optics, Inc.) that is incident on the water-air interface at the Brewster Angle. Before reaching the interface, the laser light was passed through a Glen Thompson polarizer. To form the BAM image from the reflected light, an infinity-corrected 10º Nikon lens coupled with a tube lens was used. Finally, the BAM image is collected on a CCD (Andor DV887, 512 x 512 pixels). In the resultant image, bright spots correspond to regions of significant surfactant coverage, whereas dark regions correspond to areas of minimal surfactant coverage.

11.2.3 Molecular Dynamics

Classical Molecular Dynamics (MD) were used in this work to investigate molecular-level interactions resulting in the morphological and phase behavior effects observed in the
Langmuir trough and BAM studies. Three different systems were used; all were composed of two monolayers of DPPC (64 molecules each) on either side of an aqueous slab, with vacuum on the opposite side of the DPPC monolayers, in a 6.69 x 6.69 x 28 nm box. Periodic boundary conditions were used in all directions. The aqueous slab was composed of water, water containing zwitterionic Phe, or water containing neutral Phe molecules. Although Phe does exist in its zwitterionic form in aqueous solution at physiologically relevant pH values, it could transition to its neutral form in the phospholipid bilayer as has been seen with other membrane-perturbing molecules [13]. Thus, both situations were simulated and subsequently analyzed.

Each system’s initial configuration is described in more detail below.

11.2.3 (a) DPPC only
For the DPPC only simulation two pre-equilibrated monolayers of DPPC (64 molecules each) are solvated with a slab containing 6876 water molecules.

11.2.3 (b) Zwitterionic Phe/DPPC system
For the zwitterionic Phe/DPPC simulation, the initial DPPC configuration (64 molecules on each side of the aqueous slab) was taken from the equilibrated configuration described in 11.2.3(a). The aqueous slab contained 20 zwitterionic Phe molecules distributed randomly and solvated with 5531 water molecules that was then inserted between the pre-equilibrated DPPC monolayers. This corresponds to an initial concentration of Phe of 200 mM. After completion of the 100 ns production run (see below), most Phe molecules had partitioned into the interfacial regions, depleting the bulk aqueous phase. Thus, a second simulation was performed with 15 additional molecules of zwitterionic Phe added to the interior of the aqueous slab to simulate the true experimental conditions of a large supply of monomers from the bulk. Then, an additional 100 ns of simulation was performed with the added Phe molecules.
11.2.3 (c) Neutral Phe/DPPC system
For the neutral Phe/DPPC system, the initial DPPC configuration was again taken from the equilibrated configuration in 11.2.3(a). The aqueous slab was composed of 20 neutral Phe molecules distributed randomly and solvated with 5613 water molecules between the DPPC monolayers. This corresponds to an initial concentration of 198 mM Phe in the simulation system.

For all of the above system configurations, with the exception of the pre-equilibrated DPPC-only system, a short energy minimization was first run followed by a 10ns equilibration period. Then a 100ns production run was completed of which the last 50ns was used for analysis. The SLipids force field [14, 15] was used for the DPPC molecules, while ff03 [16, 17] was used for Phe molecules. The charges were calculated using geometry optimization followed by RESP (restrained electrostatic potential) fitting at the B3LYP/cc-pVTZ level of theory. For zwitterionic Phe, the charge calculation was carried out including the PCM (polarizable continuum model). The TIP3P model was used for water molecules [18]. Bond lengths were constrained using the LINCS algorithm [19]. A particle-mesh Ewald scheme [20] was used for the long-range part of the electrostatic interactions. A cut-off of 1 nm was used for the Lennard-Jones and short-range electrostatic interactions. The simulations were all NVT simulations with no pressure coupling. The temperatures of the simulations were 310K with a Nosé-Hoover thermostat [21] used for temperature control using a time constant of 0.5 ps. Equations of motion were integrated utilizing the leap-frog algorithm with a timestep of 2 fs, with snapshots saved every 10ps. Simulations were run and analyzed using GROMACS version 4.6.3 [22] and were visualized using VMD [23].
In the following, both experimental and MD simulation results are presented for the system composed of L-phenylalanine (Phe) dissolved in water in the presence of a DPPC monomolecular film. Langmuir trough methods and BAM were used to explore the effect of Phe on the phase behavior of DPPC at a molecular area representative of cell membranes (70 Å²/molecule) [24]. Then, MD simulations were used to gain insight into the interactions between the Phe molecules and the DPPC film, as well as the effect of the intercalation of Phe on the DPPC film itself. First, for reference throughout the rest of the analysis, the atom map used in the simulations is shown in Figure 11.1 below.

![Atom map](image)

Figure 11.1: Atom map of (a) L-phenylalanine and (b) DPPC used in the MD simulation and analysis. The carbon atoms in the DPPC molecule are shown with only numbers as their labels and all hydrogen atoms are removed from the atom map for clarity.

### 11.3 Results and Discussion

#### 11.3.1 Influence of L-phenylalanine on surface tension

When DPPC was deposited on a subphase of aqueous Phe (2.5 mM) there was a rise in surface pressure over time (adsorption isotherm shown in inset of Fig. 11.2), indicative of
adsorption of Phe to the interfacial region [25]. Once saturation of the interfacial region was reached (indicated by a constant surface pressure), a pressure-area ($\pi$ – A) compression isotherm was obtained (Fig. 11.2).

![Figure 11.2: $\pi$ – A isotherms of DPPC deposited on bare water surface (blue) and DPPC deposited on a 2.5 mM aqueous solution of Phe (black). Inset shows adsorption of Phe to the interface over time in the region indicated by the gray dotted line, prior to surface compression (black) as well as adsorption of Phe to a bare aqueous interface over time at the same concentration (2.5 mM) for comparison (red).](image)

When compared with a $\pi$ – A isotherm of DPPC deposited on a bare water subphase, it is immediately clear that there is a change in the phase behavior of DPPC due to the presence of Phe. In the pure DPPC film, the film begins in a gas phase at large areas, transitions to a liquid condensed phase around 100 Å$^2$/molecule. Then, the plateau between 80 and 60 Å$^2$/molecule indicates the liquid expanded – liquid condensed (LE – LC) coexistence region, the region representative of the phase experienced by phospholipids in cell membranes [24]. Finally, the
film transitions to a pure liquid condensed phase around 55 Å²/molecule. In the mixed film however, after deposition of DPPC at a mean molecular area of 70 Å²/molecule, there is a clear perturbation to the LE – LC coexistence region, seen by the initial increased surface pressure followed by transition to the LC phase. This increase in surface pressure in the first observed phase in the mixed film is attributed to the presence of Phe in the interfacial region.

The change in surface tension illustrated in Figure 11.2 due to the presence of Phe can have significant consequences on the stability and morphology of a cell membrane. For example, regions of differing surface tension on the cell membrane surface can induce changes in the cell shape and even can cause the membrane to rupture [26, 27]. Combining the results presented here with those of Adler-Abramovich et al. [8], it is likely that this decrease in surface tension induced by the presence of Phe in the interfacial region is contributing to the cytotoxicity as well as the change in cell morphology observed.

11.3.2 Partitioning of L-phenylalanine at the interface

The isotherms indicate the presence of Phe in the interfacial region and their effect on the surface tension, however no information can be gained as to whether the Phe molecules are strictly partitioning to the lipid – water interface or whether they are intercalating into the lipid film. To gain further insight into the partitioning of the Phe molecules, MD simulations were used of the mixed film system, beginning with Phe molecules solvated in the bulk aqueous phase in the presence of an interface coated with DPPC molecules at a mean molecular area of 70 Å²/molecule (LE – LC coexistence region, representative of cell membranes [24]). In the bulk aqueous phase, zwitterionic Phe molecules do form dynamic aggregates, constantly forming and breaking apart throughout the simulation time (examples of aggregate structures formed shown in Figure 11.3).
Figure 11.3: Examples of Phe aggregates formed in the bulk simulation (water has been removed from the images). A: Phe trimer formed through both an ionic interaction from the head groups forming the right-hand dimer and aromatic interactions between all three molecules; B: Ionically associated Phe dimer (ionic-only interactions less common); C & D: larger Phe clusters with both ionic and aromatic interactions

These aggregates are also seen in the interfacial region both in the presence and absence of a DPPC film. No persistent, highly-ordered fibril-like structures were observed here like the ones seen by Adler-Abramovich et al. [8]; however, the simulation conditions between the two studies are quite different. The simulations presented by Adler-Abramovich et al. were performed at what they deem as high pH conditions, corresponding to the anionic form of Phe. Then, their fibril-like structures are formed incorporating counter ions. Ions are known to mediate many different supramolecular self-assembled structures such as G-quadruplexes [28]. Thus, it is unsurprising that similar stable assemblies are not seen in the studies here using the zwitterionic
form of Phe with no free ions present in solution (in neither the experimental studies nor the simulations). In addition, it is unlikely that Phe exists predominantly in its anionic form at physiologically relevant pH values [29, 30]. Rather, it is likely to exist as a zwitterion. Regardless as to the exact structure of the aggregates formed, Phe does exhibit a clear propensity towards aggregation in the simulations performed here as well as in the published literature.

To ascertain the average positioning of the Phe molecules in the interfacial region, density profiles along the direction normal to the DPPC interface were calculated and are shown in Figure 11.4.

![Figure 11.4](image)

**Figure 11.4**: Density profiles illustrating average positioning of Phe (20 molecules) during the last 50 ns of simulation time (100 ns total simulation) in comparison with DPPC density profiles. (a) and (b) are zwitterionic Phe and (c) and (d) are from the neutral Phe simulation. Black/brown traces are density profiles of Phe atoms: solid line in (a) and (c) due to C from carboxyl group and broken line due to N from amine group; brown solid line in (b) and (d) due to CZ atom of Phe. DPPC density profiles are colored as follows: green corresponds to O21; blue solid line corresponds to N from the choline group, blue broken line to P from the phosphate group; red line corresponds in (b) to C24 and in (d) to C26. Water density profile shown as the gray dotted line.
For comparison, both zwitterionic Phe and neutral Phe were simulated, examining both systems in the event that Phe does transition to its neutral state once within the phospholipid monolayer. Figure 11.4(a) and (b) show the density profiles of the simulated zwitterionic Phe/DPPC system, and (c) and (d) show the density profiles of the simulated neutral Phe/DPPC system. In the first column (parts (a) and (c)), the density profiles of the ionic head groups are compared, and in the second column (parts (b) and (d)), the density profile of the terminal ring carbon of Phe (CZ in the atom map shown in Figure 11.1(a)) is matched with the closest density profile of a carbon atom on the hydrocarbon tails of DPPC (shown in red). From these density profiles it can be concluded that zwitterionic Phe is positioned, on average, with its head group sitting between the phosphate group of DPPC (P) and O21 on the sn2 chain.\textsuperscript{16} The aromatic group of zwitterionic Phe does penetrate into the hydrocarbon tails of DPPC with the density profile of the CZ atom of Phe corresponding most closely to the density profile of the C24 atom in DPPC. Neutral Phe penetrates deeper into the DPPC film, with its head group sitting beyond O21 of DPPC, and its CZ atom aligning with the C26 atom of DPPC.

This is an important effect; if Phe does transition to its neutral state upon penetration into the film, it can penetrate deeper into the DPPC membrane. Visual inspection of the trajectory reveals that neutral Phe molecules can change their orientation within the film, dehydrating their polar groups and embedding deeper into the nonpolar phase. Zwitterionic Phe, however, remains with its polar groups hydrated and anchored closer to the water surface. Although this is a monolayer study and membrane crossing events are impossible, the dehydration and deeper penetration of neutral Phe into the membrane region opens the possibility for such crossing.

\textsuperscript{16} Density profiles for corresponding atoms on the two chains are nearly identical and thus only the atoms from the sn2 chain are shown here.
events in a bilayer system. In addition, Adler-Abramovich et al. observed Phe on the interior of surviving cells [8], supporting the assertion of membrane-crossing events.

11.3.3 Morphological and ordering effect on the DPPC film

Beyond understanding the intercalation of Phe into the DPPC monolayer, it is important in a biological context to understand the effect this intercalation has on the DPPC film itself. The domain morphology of a DPPC film is one good way to experimentally gain insight into the effect of additives such as Phe on a phospholipid membrane [31]. BAM images of DPPC deposited on a bare water subphase and deposited on an aqueous Phe subphase (2.5 mM) are shown in Figure 11.5 (A) and (B) respectively. It is immediately apparent that there is a significant perturbation to the domain morphology of DPPC in the presence of Phe. On a bare water subphase (Fig. 11.5(A)) DPPC exhibits the characteristic island structure formed in the LE/LC coexistence region of its isotherm [31]. However, in the presence of Phe (Fig. 11.5(B)), DPPC exhibits extended condensed domains, with ribbons and circular domains of lesser DPPC coverage.

Figure 11.5: BAM images of a DPPC film (70 \(\text{Å}^2\)/molecule) deposited (A) on bare water surface and (B) on a 2.5 mM L-phenylalanine solution.
This experimental effect was supported by the simulation results in which voids were periodically formed in the DPPC film in the presence of zwitterionic Phe coupled with evidence of visually condensed regions. One snapshot showing this effect is presented in Figure 11.6.

![Figure 11.6: Top view snapshot of a defect in the film (defect circled in white). DPPC molecules are colored blue with terminal methyl groups colored yellow. Water molecules are red and white, and Phe molecules can be seen by their green aromatic rings.](image)

The proximity and ordering of the terminal methyl groups of DPPC (colored in yellow in Figure 11.6) can be used as an indicator of condensed domains in the film [32]. In conjunction with the void formed in the monolayer in Fig. 11.6, there are also regions of condensed film (seen as clusters of yellow terminal methyl groups). To quantify this visual condensing effect, deuterium order parameters were calculated and are presented in Figure 11.7.
Figure 11.7: Deuterium order parameters for DPPC on a pure water slab (black), in the presence of 20 molecules of zwitterionic Phe (red), with 15 additional zwitterionic Phe molecules added after initial 20 molecules partitioned into the monolayer (green), and in the presence of 20 molecules of neutral Phe (blue). (a) and (b) are the order parameters calculated for the sn2 and sn1 chains respectively with error bars of one standard deviation. (c) and (d) are the change in order parameter from that of the pure DPPC monolayer of the sn2 and sn1 chains respectively.

In all cases studied, both of zwitterionic and neutral Phe, the presence of Phe increases the order of the hydrocarbon chains of the DPPC molecules. In the presence of 20 molecules of zwitterionic Phe the perturbation to the tails of DPPC is fairly uniform, evidenced by the near linear change in order parameters plotted in Fig. 11.7(c) and (d), with a slightly more pronounced effect closest to the head group of DPPC. To simulate a more realistic situation where there is a near constant supply of Phe monomers in the bulk to replenish those that have already
partitioned into the monolayer, a subsequent simulation was run with an additional 15 molecules of zwitterionic Phe added to the bulk underneath the DPPC film, yielding a total of 35 molecules of Phe in the simulation. After this addition, there is a much more pronounced effect on the order parameters, with now a much more distinct perturbation near the head group of DPPC, but still with significant perturbation to the rest of the tail. Finally, in the presence of 20 neutral Phe molecules, there is also a significant increased ordering of the hydrocarbon tails of DPPC, but now with a maximum effect around carbons 5 and 6, with less of an effect closer to the head group. These results are consistent with the density profiles (Fig. 11.4) that showed neutral Phe partitioning deeper into the DPPC film than zwitterionic Phe. Finally, in general, these results are consistent with the BAM images (Fig. 11.5) portraying the condensing effect on the DPPC film due to the presence of Phe. Such a condensing effect would correspond with the increased ordering of the hydrocarbon tails of DPPC observed in the simulations.

**11.4 Conclusions**

In conclusion, it is shown here that L-phenylalanine does intercalate into a DPPC film at the air-water interface, thereby affecting the surface tension, phase morphology, and ordering of the DPPC film. Using this work as a model for the interactions of Phe with a phospholipid membrane surface, these effects can have great influence on the fluidity and stability of the cell membrane. It is proposed here that the perturbation of Phe inside the DPPC membrane on the surface tension and phase behavior of the membrane itself can cause instability and cell morphology changes resulting in some cases in destruction of the cell, as reported by Adler-Abramovich et al. [8]. Both the experimental Langmuir trough and BAM results illustrating the change in surface tension and phase behavior, as well as the simulation results characterizing the
molecular-level interactions and perturbation to the ordering of the hydrocarbon chains of DPPC support this assertion. In addition, if Phe transitions to its neutral state once within the hydrophobic part of the phospholipid phase, it is shown here to have the ability to penetrate deeper within the hydrocarbon core, enhancing the possibility for membrane crossing events. This provides a potential explanation for the observation of Adler-Abramovich et al. [8] of the presence of Phe on the interior of the surviving cells. Regardless, it is shown here that Phe significantly perturbs the structure and morphology of a two-dimensional film used as a model for a cell membrane composed of DPPC, contributing to an understanding of the cytotoxicity observed in PKU disease due to Phe accumulation.

11.5 References for Chapter 11


12 Conclusions

The origin of life is a universally intriguing problem for scientific research. Prior to the emergence of the first living organism, many chemical and physical phenomena resulting in increased complexity were necessary in a bulk environment favoring simplicity. This thesis has shown the advantageous environment provided by water and its surface in increasing both chemical and physical complexity, relevant to life and its origins. It is my personal opinion that laboratory origin of life research must be innovative yet simple in practice, and must be performed with as little human intervention as possible. This is a hallmark of my PhD work, evidenced particularly in the photochemical synthesis of self-assembled vesicles presented in Chapter 6 and the demonstration of abiotic peptide bond synthesis at the water surface in Chapter 8.

In addition, water is a common theme throughout this thesis. Water provides a unique environment for chemistry, and its surface in particular provides many additional advantages as portrayed throughout my work. Earth is a water-rich planet, a fact that is mirrored in its prevalence in modern life. Water is often considered as the solvent for life. Chapter 3 displayed unique photochemistry of gas phase pyruvic acid in the presence of water vapor and aerosol particles, portraying the sensitivity of chemical reaction pathways to environmental conditions. Chapter 4 explored the photochemistry of pyruvic acid in aqueous solution, providing a detailed photochemical reaction mechanism that was different still than the gas phase studies. The use of photons to drive useful, complexity-building chemical reactions is not often considered in origin of life research. I have shown in Chapter 5 that photons can drive abiotic chemical reactions reminiscent of modern metabolic pathways that are enzymatically driven. In addition, I showed in Chapter 6 a novel abiotic synthesis of membrane-forming surfactants using photons, following
the same chemistry described in detail in Chapters 4 and 5. This work in particular used the near-surface region of water to push the reaction pathway towards this membrane-forming product, and also provided an advantageous increase in concentration to allow for the spontaneous self-assembly into vesicles.

Much of my thesis work has centered around processes unique to the water surface region. In Chapter 7, I showed the change in ionization state of the amino acid L-phenylalanine at the air-water interface compared with its ionization state in bulk aqueous solution using an Infrared Reflection-Absorption Spectrometer (IRRAS) that I constructed in the Vaida lab. Further, using the same technique, I observed in situ abiotic peptide bond formation at the water surface (Chapter 8), eliminating the need for collection processes that may otherwise influence the chemistry occurring. Peptide bond formation does not occur, due to thermodynamic and kinetic constraints, in bulk aqueous solution. However, using the unique environment provided by the water surface, this necessary biological chemistry becomes possible.

Finally, it is interesting to note that much of this work is not strictly relevant to origin of life chemistry, but can rather be more broadly extended to the growing body of knowledge about modern life. For example, the enzymatic mechanism is not known for modern ribosomal peptide bond formation. The conclusions drawn from my work in Chapter 8 regarding the unique conditions provided by the water surface enabling abiotic peptide bond formation, can shed light on the functions of the much more complex biological machinery performing this task in modern life. In Chapter 11, I used surface-sensitive techniques in conjunction with Molecular Dynamics simulations to aid in developing a molecular-level mechanistic understanding of the toxicity of L-phenylalanine in the disease Phenylketonuria. There, the air-water interface coated with a phospholipid is used as a model for the lipid-water interface of cell membranes. Finally, in
Chapters 9 and 10, I used mixed films at the air-water interface to understand the complex structure of real air-aqueous interfaces in the natural environment and the potential effect they may have on climate. Aqueous aerosols are known to have complex compositions, and thus my work contributes to a better understanding of their surface morphologies, and indirectly their optical properties relevant to climate. Thus, my work presented in this thesis shows chemical and physical processes that are unique in the presence of water and at its surface, contributing to a better understanding of life and its origins.
13 Works Cited


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Appendix A: L-leucine – Cu$^{2+}$ Complex: A Supramolecular Assembly at the Air-Water Interface

A.1 Experimental Methods

A pH 8 buffer solution was prepared by dissolving $8.5 \times 10^{-4}$ moles of sodium phosphate monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, ACS grade) and $9.15 \times 10^{-3}$ moles of sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, ACS grade) in 1L deionized water. Then, L-leucine was dissolved in the phosphate buffer to a final concentration of 0.1M. Separately, a concentrated solution of Copper(II) chloride (97%) was prepared in buffer solution for later injection. The L-leucine solution was first spread on the Langmuir trough and allowed to equilibrate during which time IRRAS spectra were recorded. Then, the CuCl$_2$ solution was injected underneath the surface layer to obtain a final Cu$^{2+}$ concentration of 1 mM. Immediately after injection, IRRAS spectra were continuously obtained to monitor changes to the surface over time.

A.2 Results

Immediately after injection of Cu$^{2+}$ ions beneath the surface layer (within the 10 minutes time to obtain the first IRRAS) a robust gel-like substance formed only in the surface region (upon inspection, the underlying layer remained liquid). This gel-like substance was highly reflective when compared with the water background reflectivity, resulting in very intense IRRAS signal. This IRRAS spectrum did evolve slightly with time, as seen in Figure A.1, with peaks increasing in intensity and shifting slightly to lower energy over time. This slight shift could be due to slight structural changes to equilibrate the coordination complex formed in the surface region.
Figure A.1: Evolution of IRRAS spectrum of L-leucine after Cu\textsuperscript{2+} injection over time. Black: immediately after injection, Red: ten minutes after injection, and Blue: two hours after injection.

To further shed light on the structure of the complex formed in the surface region, the IRRAS spectrum obtained was compared with solid-state infrared spectra of the dipeptide Leu-Leu, the commercial solid of L-leucine, and a sample collected from the surface of the experimental system on the trough (containing some of the gel-like surface structure as well as some of the underlying bulk material). Through comparison with the infrared spectrum of the dipeptide, it was apparent that the gel-like substance at the surface was not covalently bound. In addition, when the same experiment was performed using Leu-Leu instead of the bare amino acid, no gel or precipitate was formed in the trough (the entire solution just became blue in color after Cu\textsuperscript{2+} injection). Comparison with the other two substances (the collected sample and the
commercial solid reference spectrum of L-leucine) is shown in Fig. A.2 with a few peaks denoted explicitly.

Figure A.2: Spectra of L-leucine scaled for comparison; Black: solid-state infrared absorption spectrum of L-leucine commercial solid, Red: IRRAS spectrum of L-leucine – Cu$^{2+}$ complex at the water surface (peaks flipped to appear positive for comparison), Blue: solid-state infrared absorption spectrum of collected L-leucine after injection of Cu$^{2+}$ illustrating features from collected surface complex as well as un-coordinated bulk L-leucine molecules

Even from a cursory examination, there are distinct differences between Leu commercial solid and the gel-like substance formed at the surface. A few tentative assignments are given in Table A.1.
Table A.1: Notable vibrational bands in both coordinated and uncoordinated L-leucine spectra with tentative assignments.

<table>
<thead>
<tr>
<th>L-leucine, no complex (cm⁻¹)</th>
<th>L-leucine – Cu²⁺ complex (cm⁻¹)</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1408</td>
<td>-</td>
<td>ν₅ (COO⁻)</td>
</tr>
<tr>
<td>1513</td>
<td>-</td>
<td>NH₃⁺ sym. deformation</td>
</tr>
<tr>
<td></td>
<td>1565</td>
<td>NH₂ scissor</td>
</tr>
<tr>
<td>1581</td>
<td>1618</td>
<td>ν₃ (COO⁻)</td>
</tr>
</tbody>
</table>

From both Fig. A.2 and Table A.1, it is apparent that some of the most distinct changes between Leu commercial solid and the IRRAS spectrum are in the polar region of the leucine molecule. First, the symmetric stretch of the COO⁻ group disappears completely in the IRRAS surface spectrum and the asymmetric stretch shifts to higher energy with significant intensity enhancement. In addition, there appears to be a transition from the protonated amine (NH₃⁺) in the bare leucine molecule to the neutral form (NH₂) in the IRRAS surface spectrum after Cu²⁺ injection. Finally, the collected material exhibits a mixture of features from the IRRAS spectrum and the Leu commercial solid spectrum, indicating some perturbed leucine molecules as well as some remaining unperturbed (a marked difference from the IRRAS spectrum itself which indicates a uniform assembly). This suggests that there is an inhomogeneous distribution of coordinated and uncoordinated L-leucine molecules, with the underlying solution containing unperturbed aqueous L-leucine and the surface region containing Cu²⁺ - coordinated L-leucine molecules.

A.3 Discussion

The spectral evidence suggests that this is not a covalent assembly (i.e. not consistent with peptide bond formation). The alteration to both the carboxylic acid and the amine side of the molecule and the uniformity of the IRRAS spectral features when compared with the collected material (that suggests a mixture of components) suggest that this is a non-covalent,
supramolecular assembly formed specifically in the water surface region. Thus, a coordination structure involving both the amine and carboxylic acid groups of Leu with Cu$^{2+}$ ions is suggested (shown in Fig. A.3).

![Figure A.3: Proposed coordination of both amine and carboxylic acid groups of L-leucine in the surface region with Cu$^{2+}$ ions.](image)

No further characterization was performed of this assembly, but it may be interesting to further explore in the context of origin of life research. Supramolecular assemblies, such as the DNA double helix, are key in modern biology. Thus, it may be interesting to further explore the water surface as a unique environment for the formation of other supramolecular assemblies in addition to other amino acid – metal complexes. Finally, it is interesting that this assembly appears to only form with the monomer amino acid but not with the dipeptide. The selection for monomers suggests that there is some preferable organization achievable by the smaller molecule, but more work is needed to confirm this assertion.

In order to more fully characterize this system, a few steps should be taken. First, the collected surface material should be compared with a bulk sample to confirm that the complex is formed at the surface but not in the bulk. It is possible that NMR may also be able to confirm the structure of the coordinated assembly beyond the evidence provided by the IRRAS spectrum. Finally, the role of phosphate in this assembly needs to be explored to determine whether phosphate ions are or are not participating in the surface assembly.
Appendix B: Some General Remarks on Contemporary Origin of Life Theories

Currently, there are two leading theories of the origin of life on Earth: genes-first (the RNA world) and metabolism-first (small molecule world, henceforth SM world). These two competing theories have caused intense, heated debate amongst origin of life scientists, with the RNA world standing as the current popular contender. The RNA world asserts that the ideal molecule at life’s origins was a self-catalytic RNA molecule that had spontaneously formed on early Earth. This view, historically, stems from the discovery made separately by Thomas Cech and Sidney Altman in the early 1980s (which won them both the Nobel Prize in 1989) that RNA can fold into specific structures that can catalyze chemical reactions. This catalytic activity showed for the first time that RNA could act not only as a genetic carrier but also as an enzyme, a role previously thought to only be filled by proteins. To truly understand the magnitude of this discovery and hence its impact on origin of life theories, we must delve for a moment into molecular biology. Life on Earth today facilitates information flow through the interplay of three essential biopolymers: DNA, RNA, and proteins. These biopolymers work together in what is known as the central dogma of molecular biology, a scheme that was first proposed by Francis Crick [1] and is shown below in its most general form.

\[ \text{DNA} \leftrightarrow \text{RNA} \leftrightarrow \text{protein} \]

In its essence, this scheme says that DNA can make copies of itself, DNA can make RNA (specifically mRNA in transcription), and finally RNA can make protein (translation). In its most general form, DNA is responsible for the genotype of the system and proteins are
responsible for gene expression, or the phenotype of the system. In most of modern biology, this is the scheme that is followed, with all three biopolymers being necessary in the process. But, all three of these biopolymers are highly complex molecules, with specific sequences and associated highly regulated biological machinery. It is unlikely that all three spontaneously emerged on early Earth simultaneously, but all three are essential to each other and to modern life on Earth. And thus, until the work performed by Altman and Cech, origin of life researchers had a “chicken and egg” problem [2]. Evolutionary biology (Darwinian evolution by natural selection) requires a genotype – phenotype distinction that is essentially controlled by the replicator (DNA) and chemical catalyst (protein) respectively on a molecular level. Without all three biopolymers working in concert, no living system could function and participate in natural selection. One major contribution of the Nobel Prize winning work by Altman and Cech was to show that RNA can actually solve the “chicken and egg” problem. Since RNA was then known to essentially possess the properties of both DNA (genetic carrier, replication) and proteins (chemical catalysis), it became the ideal first biopolymer at the core of a theorized simplest “living” system in the origin of life on Earth.

The SM world takes a different approach. Proponents point out the many difficulties in the prebiotic likelihood of producing a complicated, fragile molecule such as RNA, and instead utilize small molecules that are more prebiotically plausible. The focus of small molecule proponents is on self-propagating chemical reaction networks, culminating in a proto-metabolism. They concentrate on increasingly complex chemical reaction networks that essentially maintain their cycles against the natural energy flow, propagating disequilibrium through time. The focus is therefore on catalysis rather than information storage and propagation. Catalysis, by definition, requires a deviation from the energetic equilibrium that
normally drives undirected (in the absence of complex biological machinery) chemistry. Although this is an essential feature of life – the maintenance of disequilibrium – the origin of such a kinetic rather than thermodynamic control of even the simplest chemical systems can be rather difficult [3].

Although catalysis is, mechanistically, the focus of many SM world theories, they also recognize the need for some form of heredity. But, rather than requiring a traditional information-bearing molecule such as RNA or DNA, SM world proponents suggest that a “compositional genome” may be established through small molecule networks alone [4, 5]. In this way, the composition of the metabolic network itself – the presence of certain organic molecules and the absence of others – generates an analogous genome to the one composed of DNA in traditional biological systems today. Further, this compositional genome may evolve through compositional changes in response to environmental stressors. In the eyes of SM world proponents, primitive metabolic systems are not only more probable to have formed spontaneously on early Earth than a complicated biopolymer such as RNA, but these systems can grow and evolve in an analogous way to more advanced life.

B.1 Where’s the “Smoking Gun”? 

The distinction between historical science, such as origin of life science, and traditional experimental science has been addressed previously by Cleland [6], and shall be discussed briefly and applied here. Historical hypotheses are, firstly, not grounded in prediction, as are experimental hypotheses. Second, as an extension of Lakatos’s original theory of Sophisticated Methodological Falsification [7], historical hypotheses may be rejected on the basis of evidence that does not refute them. Rather, historical scientists look for a “smoking gun” that makes one
hypothesis more plausible than the other competing hypotheses. Finally, historical hypotheses are grounded in explanatory power. They are accepted or rejected based on their power to explain puzzling evidence from fieldwork, rather than their predictive power as in experimental science.

This description of historical science is highly accurate when analyzing science such as mass extinction events or the construction of evolutionary trees. In these instances, the driving force for the construction of historical hypotheses is in fact the discovery of evidence through fieldwork, followed by theory formulation to explain the evidence remaining from the past event. An interesting example of the use of historical science methodologies is seen in forensic science. Forensic scientists use gathered evidence from a crime scene, such as fingerprints, fibers, footprints or DNA remnants, analyze this evidence, and then construct a sequence of events (all of which happened in the past) from the evidence gathered. They may initially have a pool of suspects due to multiple persons fitting the available evidence, but crime scene investigators will continue to search for additional evidence (tracking the validity of alibis or comparing DNA samples of the suspects to DNA found at the crime scene) until they find one suspect fits the gathered evidence better than the rest. Some piece of evidence, like a DNA match, is therefore the “smoking gun” (a term first put forth by Cleland in the context of historical science [8]) necessary in choosing one suspect over the rest.

The origin of life theories being analyzed here, however, were formulated a bit differently than the more common examples of historical hypotheses (such as mass extinction events, or forensic science). There are no fossils or evidence to examine from the field, or really any direct evidence of any kind to examine. Rather than finding field evidence of a historical event and then formulating a theory to explain the evidence, origin of life scientists examine
modern life, determine its most essential features differentiating it from non-living matter, and extrapolate backwards in time to construct a theory of its origins. They are formulating theories without any direct evidence of that event in time. In this respect, origin of life theories appear to be distinct from other historical hypotheses. In a sense, they follow a different methodological path to theory construction.

The most obvious culprit for this apparent difference in theory formulation is the time lapse between the event occurring and the time in which the theory is formulated. Forensic scientists typically collect evidence from an event that has occurred in the very recent past, certainly within their lifetime. In contrast, origin of life scientists are formulating theories about events which happened on a billion-year-ago time scale. So, is this difference merely a time-lapse problem? Take the example of the knowledge of the age of the Universe. Certainly the Big Bang occurred prior to the emergence of life on Earth, and thus, one would expect to find the methodological process leading to the determination of the age of the Universe to more closely resemble that of origin of life scientists than forensic scientists. But this is not the case. The discovery of the microwave background radiation prevalent throughout the observable Universe allowed scientists to extrapolate backwards in time to the Big Bang, and hence date the Universe. This key piece of evidence (the background radiation) was directly observable evidence of the event that occurred in the distant past. Thus, it is now apparent that the root cause of the methodological difference in theory formulation of the origin of life scientists is not due to time-lapse, but rather due to the complete lack of observable evidence from that event in time.

This lack of evidence complicates matters when attempting to decide between competing theories such as SM and RNA world hypotheses. Without any direct field evidence whatsoever, how can one expect to find a smoking gun? There is, of course, always the possibility that some
evidence will eventually be found which can be such a smoking gun for these theories. One example of an unexpected field discovery contributing to origin of life research is the carbon-isotope ratio evidence of the earliest life on Earth by Steve Mojzsis et al. [9]. Prior to their work, the earliest known microfossils were 3.5 billion years old but were already structurally highly complex. Therefore, it was inferred that simpler life had arisen prior to 3.5 bya, but there was no fossil evidence to support any sort of time frame. Mojzsis et al. analyzed 3.8 billion year old sedimentary rock samples from Greenland and found anomalous carbon isotope ratios, with an excess of the lighter isotope (\(^{12}\text{C}\)). The only known way to have such excess is through living systems – life prefers the use of the lighter isotope of carbon to the heavier one. From this field evidence, it was concluded that life was present on Earth as far back as 3.8 bya.

It is important to note that a few extra assumptions are interjected when drawing conclusions from indirect evidence such as this. First, it is assumed that the same processes known today to produce such isotopic anomalies were the only ways in which isotopic anomalies could form at that point in history. This has been proven time and again to not be a hard and fast rule. For example, recently scientists discovered that DNA mutation rates in humans are much slower than previously thought [10, 11]. This finding has major implications on evolutionary time estimates. These mutation rates are used to extrapolate backwards to date certain diversions in evolutionary history. For example, under the previously accepted rates, the split between humans and orangutans was thought to be between 13 and 14 million years ago. This time frame fits well with evidence from fossil records, which dated the split between 9 and 13 million years ago. With the newly discovered mutation rates however, the split would have occurred between 34 and 46 million years ago. This is a significant difference from the dates inferred from fossil evidence. Due to this discrepancy, scientists are beginning to question the constancy of mutation
rates throughout history. In its essence, this is a questioning of the uniformity of nature. Further, it is assumed that the knowledge we hold today of the cause of past events, such as the previously discussed isotope anomalies, is truly the only cause possible in all of nature. As in any field of science, there is always the possibility that new discoveries will prompt the retraction of old explanations and theories. This assumption is especially problematic when the only evidence we have of past events is indirect evidence. There are no fossils to examine of the life present 3.8 bya, and thus the only record we have to examine is the indirect impact of that life on the environment around them.

Nevertheless, it is not impossible that field evidence will eventually be found contributing to the RNA vs. SM world debate. But the question remains, what would a smoking gun look like to decide between SM and RNA world hypotheses? It’s hard to imagine. Take the RNA world as an example. The RNA world hypothesis is characterized by a simple, self-replicating RNA molecule. The problem here is that there is little hope that such an RNA molecule would survive billions of years. It is a very fragile molecule in the best of environments, and it is hard to imagine an environment where it could have been preserved. One could imagine remnants of these RNA molecules existing as observable quantities such as an excess of the lighter carbon isotope, but that would not distinguish the RNA world from any other theory of life’s origins. It would only confirm that some primitive form of life had been present at that time. Regardless, this is a question that needs to be answered before any progress can be made on the heated debate between these two theories in the scientific community.
B.2 The Plague of Equilibrium

One overarching problem plaguing both of the leading origin of life theories is the transition from non-living components to an independent, nominally living, system. Stemming from their differing levels of analysis, RNA and SM world theories have different problems in this respect. The RNA world stems from a biological level analysis, originating in the historical discovery of the ribozyme in modern life followed by the extrapolation to the origin of life. In contrast, the SM world stems from a chemical level of analysis and hence concentrates on self-organized and self-propagating chemical systems. In this way, the RNA world is closer to a true transition from non-life to life, since it uses modern life as the starting point, whereas it is more difficult to envision an autocatalytic chemical system propagating through time as an example of early life. But, the RNA world is plagued by equilibrium and the drive toward the lowest energetic (thermodynamic) state. The SM world, through its concentration on catalysis, has focused on this issue. Life is known to be a highly out-of-equilibrium system. Our cells maintain gradients through actively pumping material into and out of themselves, thereby avoiding the tendency to achieve equal concentrations of the material in question on both sides of the barrier, on a regular basis. In these respects, the RNA world and SM world theories face different problems in the transition from non-life to life, which we shall now discuss in more detail.

Let’s begin with an analysis of the RNA world since, as asserted earlier, it has come closest to attempting the transition from non-life to life. We will concentrate on the work done in the laboratory of Jack Szostak on protocells. These protocells couple the RNA world with another necessary component of life as we know it – an enclosure. Both containment and organization are essential to the formation and subsequent evolution of primitive life. Since the
protocells of Szostak and his collaborators are thought of as synthetic attempts at primitive forms of life, it may be considered that work such as Szostak’s could be a “smoking gun” of sorts for origin of life theories. As there is no direct evidence as of yet to decide between origin of life theories found in the field, maybe a plausible recreation of past events may substitute for such direct evidence. However, it is important to note that it is not at all clear that synthesized life will in any way be representative of primitive life on Earth, a point conceded by Szostak in his published work. This does not mean that such work is not without use for origin of life theories. A “cell” stripped of its complex biological machinery and assembled with only its most basic components is instructive as a model for what a primitive cell, or protocell, would consist of and what functions it could perform. It is in this context that much of Szostak’s work is presented, and it is in this context that his group’s work will be analyzed here.

Szostak has done a remarkable amount of work illustrating a number of capabilities of protocells. In essence, the protocells are vesicles composed of a primitive membrane (normally composed of lipids such as fatty acids or alcohols rather than the more complex phospholipids found in modern cell membranes) and are usually formed encapsulating RNA. Then, they were able to demonstrate a variety of abilities analogous to those known to be necessary for function in modern life, like growth and division [12], selective permeability (maintenance of the RNA/DNA on the interior of the protocell; transfer of some ionic species across the membrane but not others) [13, 14], as well as the generation of a pH gradient [15]. His work has been much celebrated, and for good reason. It is remarkable the number of properties these simple protocells seem to possess through merely physical processes, that modern biological cells require complex machinery to perform. But, we need to look deeper to determine exactly what Szostak’s work is contributing to the RNA world theory as a whole.
One key difference between protocells and modern cells are the composition of their membrane. Most modern cells have membranes composed of a bilayer of phospholipids, with a polar headgroup that can interact with water-rich environments, and two non-polar tails that repel water. Phospholipid membranes are very stable and thus very rigid and impermeable. In modern biology, complicated pumps and enzymes facilitate the transport of essential molecules and ions across these membranes. This poses a problem, however, in the design of protocells. Selective transport is an essential property of any living cell, even the most primitive, as communication between the interior and the exterior of the cell is essential. Rather than utilizing phospholipids in protocell design, many researchers have utilized a simpler model membrane composed of fatty acids. Similar to phospholipids, fatty acid membranes are composed of a bilayer of fatty acid molecules, with their polar heads interacting with the water-rich environments and their non-polar tails repelling water. The difference between them is that fatty acids are composed of a polar headgroup but only one non-polar tail. This small difference (two tails in phospholipids vs. one tail in fatty acids) has significant consequences on the stability and permeability of the vesicle formed. Fatty acid vesicles have much more fluid membranes and are therefore less stable than phospholipid membranes, with the individual fatty acid molecules having the ability to transfer in and out of the membrane more easily. This is an advantage in terms of allowing some molecules to more easily diffuse into and out of the protocell. It can be a disadvantage, however, when considering the stability of the vesicle. In order to keep the membrane intact, there must be a significant concentration of fatty acid monomers in solution around the formed vesicle. These monomers can take the place of any monomers transferring out of the vesicle membrane. Without such a concentration of monomers in solution (i.e. if the
solution around the vesicles is too dilute in fatty acid monomers), the membrane will quickly disassemble [16].

This limitation poses a serious problem for the use of such protocells in the origin of life on Earth. In the early ocean, in order for enclosures reminiscent of these synthesized protocells to have formed spontaneously, an equilibrium must exist between the monomers in the bulk ocean and the molecules organized in the membrane itself. Such an equilibrium is only achieved once a very high concentration of fatty acid monomers is reached, namely the critical aggregate concentration [16, 17], a scenario that is highly unlikely in the dilute bulk ocean environment available on early Earth. It has been suggested that the necessary high concentrations for their formation may have been reached in local environments on early Earth, such as in the porous rock found in some hydrothermal vents lined with fatty acid monomers [18, 19]. It is true that these local environments will allow for the formation of the protocells, but once they diffuse into the open ocean they will immediately disassemble to their free fatty acids, destroying the container needed for primitive life [16]. Therefore, although such high concentrations of lipid monomers may be achieved and maintained in the laboratory, without an analogous prebiotically plausible environment, they are not particularly useful in the construction of a more comprehensive theory on the origin of life on Earth.

The Szostak group along with its collaborators have further demonstrated the ability of these protocells to grow and divide using merely physical principles [12]. When micelles (compartments composed of a single-layer of fatty acid monomers) are added to a solution containing vesicles, growth of the vesicles in solution is initiated. The newly added micelles contribute fatty acid monomers to the existing vesicle membranes, forcing them to grow into rod-like structures. Eventually, with further growth of the membrane, the rod-like structure
pinches off into two “daughter” vesicles. It was also shown that the vesicles predominantly retained their RNA content through this growth and division. Model protocellular systems were also shown to maintain a pH gradient across their surface, another essential function of modern cells [15]. Chen and Szostak propose that the energy released during membrane growth may be captured in the formation of a pH gradient across the membrane. Energy capture is essential to create and maintain a gradient of any kind, and thus this is a novel use of the energy produced in membrane growth. Their work showed, however, that although a gradient may be formed in this way, its maintenance is difficult. Chen and Szostak showed that in the absence of any membrane-permeable cations in solution, the pH gradient could be maintained for hours. In the presence of permeable cations (i.e. any alkali metal cations, a known presence in the early ocean) however, the gradient dissolved in seconds. Without the complicated biological machinery present in life today, this disequilibrium is nothing more than transient.

The most important thing to note about the previous discussion is that all of the properties demonstrated by protocells are controlled by the drive toward the lowest energetic state of the system as a whole. All protocell studies thus far are limited by this drive toward equilibrium. Life avoids such a limitation through highly evolved biological machinery, as in the membrane pumps and complicated regulatory enzymes alluded to earlier. Without such machinery, origin of life scientists have thus far been unable to create an artificial primitive cell that can maintain out-of-equilibrium conditions. Transient disequilibrium is established with every perturbation to the system. The addition of micelles to a solution of vesicles pushes the system out of equilibrium. The growth that results is the system’s response to this perturbation – the system as a whole pushes back towards its lowest energy, equilibrium state by adding fatty acid monomers to the pre-existing vesicle membranes, thereby restoring the equilibrium between monomers in
solution and monomers in their aggregate form (vesicles). Then, by merely physical principles, namely the drive toward the lowest energetic state, the strained rod-shaped vesicle pinches off and divides into two smaller, lower energy spherical daughter enclosures. Everything in this process is dominated by the drive toward equilibrium.

The story is the same for the maintenance of the pH gradient across the membrane. Although a transient disequilibrium is established, the drive toward equilibrium, and thereby destruction of the gradient, occurs at best in hours and at worst in seconds. This hardly provides time for any form of natural selection and further evolution of the primitive cell necessary for the development of more complicated life. Thus, although the work that has been done on protocells is a step in the right direction in the search for the constituents of a viable primitive living system in Earth’s history, it is far from accomplishing the transition from a collection of non-living matter to a whole, living system. Disequilibrium is a necessary component of life, and even its most primitive form must possess this quality. Therefore, origin of life scientists must push forward to a system, or an environment containing a system, which can achieve disequilibrium on a longer timescale.

My work illustrating the photo-initiated synthesis of self-assembled vesicles (Chapter 6) could be an example of the transition from an equilibrium system under thermodynamic control, to one that is out of equilibrium or under kinetic control, to use the syntax proposed by Luisi [3]. The initial system is one simply composed of monomers dissolved and distributed throughout an aqueous solution. This is the lowest energy state for this system since the concentrations used are not only below the solubility limit but also below the critical aggregate concentration (no particles were observed in solution and were also not expected under the conditions used). Then, as the photochemistry proceeds, the double-tailed surfactant formed (OOA-OOA dimer)
spontaneously self-assembles into vesicles. It is possible that these vesicles are kinetically trapped structures, and thus are not at equilibrium. In general, it is unclear whether vesicles are at equilibrium or not [20, 21]. However, one possibility is that vesicles composed of fatty acids, where there is constant flux of monomers into and out of the membrane, are at equilibrium, whereas vesicles composed of double-tailed surfactants, where exchange of monomers is limited, are merely kinetically trapped and are thus out of equilibrium. More work is needed to determine whether the vesicles composed of OOA-OOA dimers allow exchange with monomers in solution, however it is an intriguing possibility that chemical reactions driven by photons may promote the formation of out-of-equilibrium structures necessary for life.

The SM world theories have, in a sense, addressed the problem of equilibrium. As alluded to earlier, SM world theories contend that chemical reaction networks self-organized on early Earth and could subsequently propagate and evolve through time. Self-organization requires a decrease in entropy, thereby taking energy from the environment and putting it into the self-organizing system. Thermodynamically, increased entropy is favored. Therefore, through this transfer of energy to facilitate a decrease in entropy, the system is out of thermodynamic equilibrium. If the chemical reaction network can continue over time, constantly taking energy from the environment and utilizing it in its network, then it is capable of maintaining disequilibrium – a property not yet achieved by RNA world researchers. As mentioned earlier, there is little experimental work supporting these claims as of yet, but the ability to achieve and maintain disequilibrium is a necessary component of life, and gives SM world theorists a leg-up over RNA world proponents.

The real problem even with the ability to create and maintain this disequilibrium is whether an autocatalytic reaction network has achieved the transition from non-living matter to a
living entity. Although disequilibrium is a necessary component of life, it is not sufficient. One well-known self-propagating reaction network is the Belousov-Zhabotinsky (BZ) reaction [22]. The BZ reaction is the classical example of a set of reactions not controlled by thermodynamic equilibrium. The BZ reaction is a reduction-oxidation reaction between bromate and malonic acid, which produces a macroscopically observable, colored, oscillation pattern. The reaction not only avoids thermodynamic equilibrium, but also can maintain this disequilibrium over some time. SM world proponents assert that the emergence of autocatalytic reaction networks marked the transition from nonlife to life. If this were the case, then the BZ reaction, being a spontaneous autocatalytic reaction, would represent a transition from nonlife, composed of simply bromate and malonic acid, to a living system. However, few would claim that this is the case. Although the BZ reaction is of interest in the study of spontaneous organization into a far from equilibrium system, there is little correlation between it and known life today. It is clear that a system that only possesses the ability to propagate disequilibrium through time, such as an autocatalytic reaction, is necessary but not sufficient to have made the transition from nonlife to life.

B.3 Conclusions

In conclusion, the origin of life on Earth is a challenging problem both scientifically and philosophically. There are no easy answers. However, it is important that all of these challenges are contemplated and addressed by those performing the research and disseminating the results. Deciding between competing theories is difficult if not impossible. Thus, we must treat our theories as such, always keeping in mind that we are trying to solve a problem that no living thing witnessed and accept that the best we can do is suggest plausible routes to the beautiful complexity we observe daily.
B.4 References for Appendix B


