Surface Thermodynamics of Decanoic Acid

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Abstract

While modern cell membranes are composed of phospholipids, primitive membranes were probably made from simpler fatty acids. Due to the prebiotic availability of fatty acids, fatty acid vesicles are a popular model for proto-cell membranes in origin-of-life studies. Vesicles of one such fatty acid, decanoic acid, are often used to model early membranes, but the mechanism by which decanoic acid forms vesicles is not clear. Monolayer studies of the stability of decanoic acid on water surfaces may help illuminate the key molecular interactions that result in vesicle formation. To this end, consistent amounts of decanoic acid were deposited on the surface of aqueous salt and buffer solutions, and a variety of isotherms were collected with a Langmuir trough and Wilhelmy balance. Time versus surface pressure isotherms were analyzed and it was found that there was no statistical difference in the isotherms collected on 50 mM and 500 mM NaCl, indicating that the effects of salt on the phase behavior of decanoic acid might plateau at a lower concentration. However, the isotherms run on a Tris buffer substrate were statistically different from the isotherms at both salt concentrations. These preliminary results suggest that pH and buffer type are influential on decanoic acid surface partitioning and stability, and that the speed of equilibration increases at higher bulk ionic strength.

Introduction

Fatty acids exhibit complex phase behavior, forming a variety of three-dimensional structures, including vesicles. The type of structure formed has a strong dependence on the conditions of the aqueous solution in which it was assembled. While the stability of these structures under an array of conditions has been well characterized, the understanding of the phases of fatty acids, especially shorter fatty acids such as decanoic acid is incomplete and purely empirical. To gain insight into the intermolecular interactions between monomers of decanoic acid in these vesicles, simple, one-dimensional monolayer films of fatty acids on an aqueous substrate were observed. The surface partitioning and thermodynamics of these monolayers were studied as the bulk aqueous phase composition was varied, with the goal of comparing the results to the stability of vesicles of similar composition, in order to better quantify the molecular interactions which allow for self-assembly of vesicles the context of prebiotic chemistry.

Prebiotic chemistry examines how simple biomolecules are able to form favorable environments and networks of reactions in the absence of biological machinery that result in an increase in chemical complexity, and eventually the evolution of life. Modern life is incredibly complicated, and consists of myriad and interconnecting biochemical pathways. Studies of the evolution of life, therefore, focus on ways to develop several key aspects of life as we know it today, including self-replication and metabolism.[1] Another vital piece in the overall evolution of

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[1] Cleland, C. E., 2013. Conceptual Challenges for Contemporary Theories of the Origin(s) of Life. Current Organic Chemistry 17, 1704–1709.

life is the development of primitive enclosures and membranes.[2] Regardless of how life arose, it is thought that membranes played an important role.[1]

In modern cells, the cell membrane is responsible for maintaining a microenvironment within the cell conducive to biological reactions. For a primitive cell, a membrane would have served similar functions: it would separate the cell from its possibly hostile outside environment and allow for the capture of energy and concentration of molecules necessary to build a metabolism or reproductive system.[3]

Modern cell membranes are composed of double-tailed phospholipids (Figure 1, right). These molecules form bilayers which are stable under a wide range of conditions. However, phospholipids lack a satisfactory abiotic synthesis pathway, and are created in modern life by complex biotic synthesis with enzymatic assistance.[3,4,5] Consequently, prebiotically plausible models of primitive enclosures use simpler such as fatty acids (Figure 1, left) that were likely available on the early Earth.
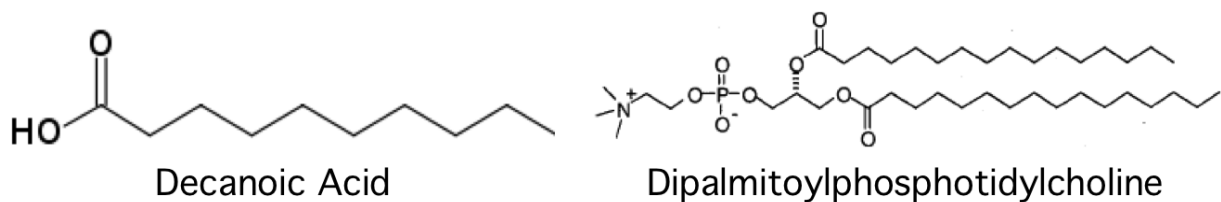

Figure 1: A fatty acid (left) and phospholipid (right). Phospholipids form more stable membranes than fatty acids tend to, but were most likely unavailable prebiotically.

Fatty acids, like phospholipids, preferentially partition to the water surface, a consequence of their amphiphilic behavior. These molecules have hydrophilic "head" groups and hydrophobic "tail" groups (Figure 2, left). The water surface can then align, concentrate, and orient amphiphilic molecules (Figure 2, right). The amphiphilic nature of surfactants is also relevant to prebiotic chemistry,[3] as it facilitates aggregation resulting in three-dimensional structures, such as those that make up cell membranes. Above a certain critical concentration where it becomes thermodynamically unfavorable for surfactants to be dissolved as monomers in solution, the surfactants begin to aggregate. This aggregation occurs because of the hydrophobic effect. Water, due to the network of hydrogen bonds it forms with itself, is more strongly attracted to itself than to other molecules. Oily compounds tend to nucleate at interfaces such as that between water and air, which has an incomplete hydrogen-bond network.[6] At higher

[2] Hargreaves, W.R.; S.J. Mulvihill; D.W. Deamer, *Nature* **1977,** *266*(5597), 78-80.

[3] Monnard, P.-A., Deamer, D.W., 2002. Membrane self-assembly processes: Steps toward the first cellular life. Anat. Rec. 268, 196–207. doi:10.1002/ar.10154

[4] Apel, C.L., Deamer, D.W., 2005. The Formation Of Glycerol Monodecanoate By A Dehydration Condensation Reaction: Increasing The Chemical Complexity Of Amphiphiles On The Early Earth. Orig Life Evol Biosph 35, 323–332. doi:10.1007/s11084-005-2046-8

[5] Furuuchi, R., Imai, E.-I., Honda, H., Hatori, K., Matsuno, K., 2005. Evolving lipid vesicles in prebiotic hydrothermal environments. Orig Life Evol Biosph 35, 333–343. doi:10.1007/s11084-005-2039-7

[6] Chandler, D., 2002. Two Faces of Water. Nature 417, 491.

concentrations, when these interfacial sites are mostly occupied and become unavailable, it becomes energetically favorable for surfactants to form three-dimensional structures that are either soluble in water or phase separate from water, as the entropic cost of self-assembly is outweighed by the minimization of the enthalpy of solvation.[7]
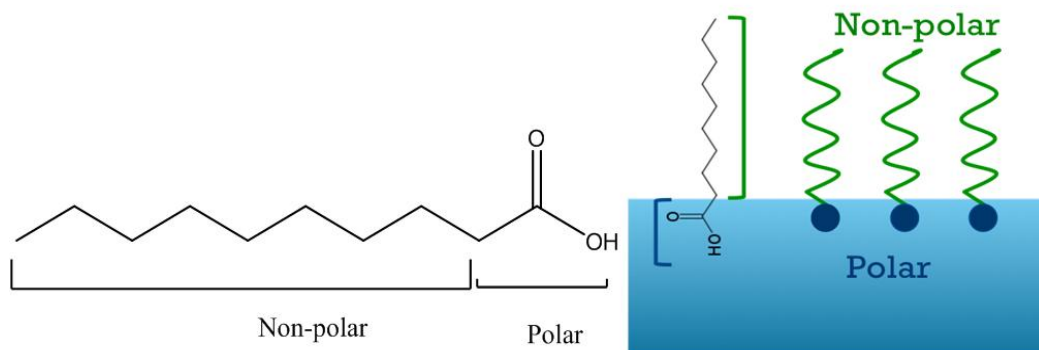

Figure 2: Left: A general fatty acid structure, with a polar carboxylic acid head group and non-polar alkyl chain tail. Right: Due to the hydrophobicity of the non-polar tails, and hydrophilicity of the polar head groups, fatty acids tend to align on water surfaces as shown.

Surfactants in aqueous environments can form vesicles, micelles, or oil drops (Figure 3). Micelles are an aggregate with the head groups facing outward and no water encapsulated in the middle (Figure 3, left); vesicles (Figure 3, middle) are formed from a membranous bilayer that encapsulates water. In both cases, hydrophobicity drives the orientation of the molecules; the hydrophilic head groups face toward bulk or encapsulated water, protecting the hydrophilic tails. Oil droplets (Figure 3, right) are sometimes referred to as "reverse micelles," as the head groups point inwards. Unlike vesicles and micelles, which are soluble in water, the outward orientation of the tail groups in oil droplets causes phase separation. A certain concentration of surfactant must exist in solution for these three-dimensional structures to form; e.g., the critical vesicle concentration (CVC) describes how much surfactant must be present to form vesicles in solution, and the critical micelle concentration (CMC) must be present in order to form micelles.

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[7] Chandler, D., 2005. Interfaces and the driving force of hydrophobic assembly. Nature 437.
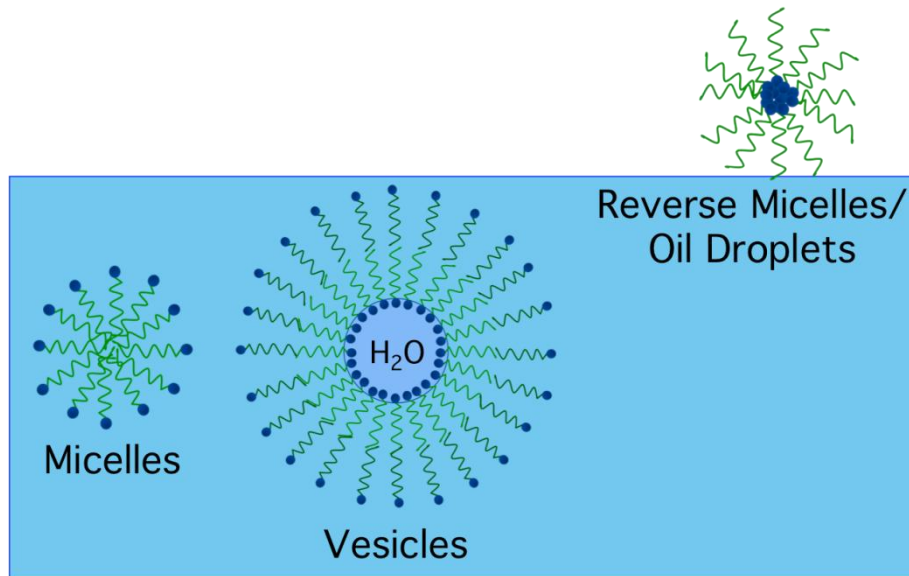
Figure 3: Three-dimensional structures formed by fatty acids. Micelles and vesicles, because the hydrophilic head groups face outwards, are water-soluble; oil droplets are not.

Models of early membranes, enclosures, and protocells focus on vesicles because of their ability to encapsulate material. Phospholipids readily form robust vesicles,[8] but because they are not prebiotically relevant, studies on primitive enclosures use simpler molecules that can be synthesized prebiotically, such as fatty acids, carboxylic acids with long carbon chain tails. Fatty acids were likely available on the early Earth, as they have been found in carbonaceous meteorites[9,10] and could have been produced abiotically by Fischer-Tropsch synthesis.[11,12] Decanoic acid, a fatty acid with ten carbons, is perhaps the most commonly used model species for these studies.[13] Decanoic acid is the fatty acid with shortest alkyl tail that readily forms stable vesicles, and shorter-tailed species are generally considered more prebiotically plausible, as abiotic syntheses, such as Fischer-Tropsch, have highest yields for lipids consisting of 7 to 9 carbons. Decanoic acid is also synthesized in these reactions.[11] Decanoic acid has also been

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[8] Bangham, A.D., Standish, M.M., Weissmann, G., 1965. The action of steroids and streptolysin S on the permeability of phospholipid structures to cations. Journal of Molecular Biology 13, 253–IN28. doi:10.1016/S0022-2836(65)80094-8

[9] Cronin, J.R., Pizzarello, S., 1990. Aliphatic hydrocarbons of the Murchison meteorite. Geochimica et Cosmochimica Acta 54, 2859–2868. doi:10.1016/0016-7037(90)90020-L

[10] Naraoka, H., Shimoyama, A., Harada, K., 1999. Molecular Distribution of Monocarboxylic Acids in Asuka Carbonaceous Chondrites from Antarctica. Orig Life Evol Biosph 29, 187–201. doi:10.1023/A:1006547127028

[11] McCollom, T.M., Ritter, G., Simoneit, B.R., 1999. Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions. Orig Life Evol Biosph 29, 153–166.

[12] Rushdi, A.I., Simoneit, B.R.T., 2001. Lipid Formation by Aqueous Fischer-Tropsch-Type Synthesis over a Temperature Range of 100 to 400 °C. Orig Life Evol Biosph 31, 103–118. doi:10.1023/A:1006702503954

[13] Morigaki, K., Walde, P., Misran, M., Robinson, B.H., 2003. Thermodynamic and kinetic stability. Properties of micelles and vesicles formed by the decanoic acid/decanoate system. Colloids and Surfaces A: Physicochemical and Engineering Aspects 213, 37–44. doi:10.1016/S0927-7757(02)00336-9

specifically identified as a component in meteorites,[10] meaning it might also have been synthesized exogenously and transported to Earth

The prebiotic relevance of fatty acids makes them a good model system, but they have much more complicated phase behavior than phospholipids. Decanoic acid, for instance, because of its relatively short alkyl tail, only forms vesicles at relatively high concentrations and under a narrow range of pH conditions. These vesicles also tend to be more sensitive to salt concentrations than phospholipid vesicles.[3] The sensitivity towards salt poses potential difficulties when using fatty acid vesicles to model primitive cells, as it is thought that the early ocean had a higher salt concentration than today.[14]

The three-dimensional structures decanoic acid forms have been well-characterized for a variety of solution conditions.[15] As a result, the origin-of-life community often assumes that decanoic acid's phase behavior is well understood. A schematic of the accepted rationale for the assembly of fatty acids into different three-dimensional structures as a function of solution pH is presented below in Figure 4.[3] These structures are formed when the concentration of fatty acid is above a critical aggregation concentration (CAC), labeled here specifically as the critical vesicle concentration (CVC).Unlike phospholipids, which form kinetically-trapped vesicles, it is generally accepted aggregates of fatty acids are equilibrium structures, meaning that even above the CAC there will be equilibrium between monomers and aggregates in solution. This implies that the type of aggregate that will be stable and favored under specific solution conditions will be the lowest energy structure.

As shown in Figure 4, the structures formed have a strong dependence on the solution pH, which is due mainly to the change in ionization state of the head group. At high pH, the head groups are largely deprotonated, favoring the formation of micelles since their structure minimizes the repulsion between carboxylate ions by maximizing the distance between head groups while maintaining favorable Van der Waals interactions between the alkyl tails. On the other hand, oil droplets form at low pH when the head groups are protonated and do not experience repulsion. Fatty acid vesicles are thought to form when approximately half the head groups are deprotonated and half are protonated; this occurs when the pH of the solution is equivalent to the so-called pK_a of the aggregate.[3] This pK_a of aggregation, however, is significantly higher than the pK_a of the monomeric carboxylic acids and is reported as approximately 7, compared to the normal pK_a of decanoic acid, 4.9. This difference suggests that the ionization state of fatty acids in aggregates likely differs from that in the bulk, and that the intermolecular interactions causing self-assembly are likely more complicated than this simple model. The stability of vesicles under changing pH conditions has been empirically studied, but the relationship between the head group interactions and membrane stability has not been quantified. Prior study of fatty acid behavior at varying pH has found that the fatty acid pK_a

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[14] Knauth, L.P., 1998. Salinity history of the Earth's early ocean [letter]. Nature 395, 554–555. doi:10.1038/26879

[15] Namani, T., Walde, P., 2005. From Decanoate Micelles to Decanoic Acid/Dodecylbenzenesulfonate Vesicles. Langmuir 21, 6210–6219. doi:10.1021/la047028z

changes with chain length, and that on the surface, the bulk pH changes the fatty acid ionization state and its ability to form films.[16]
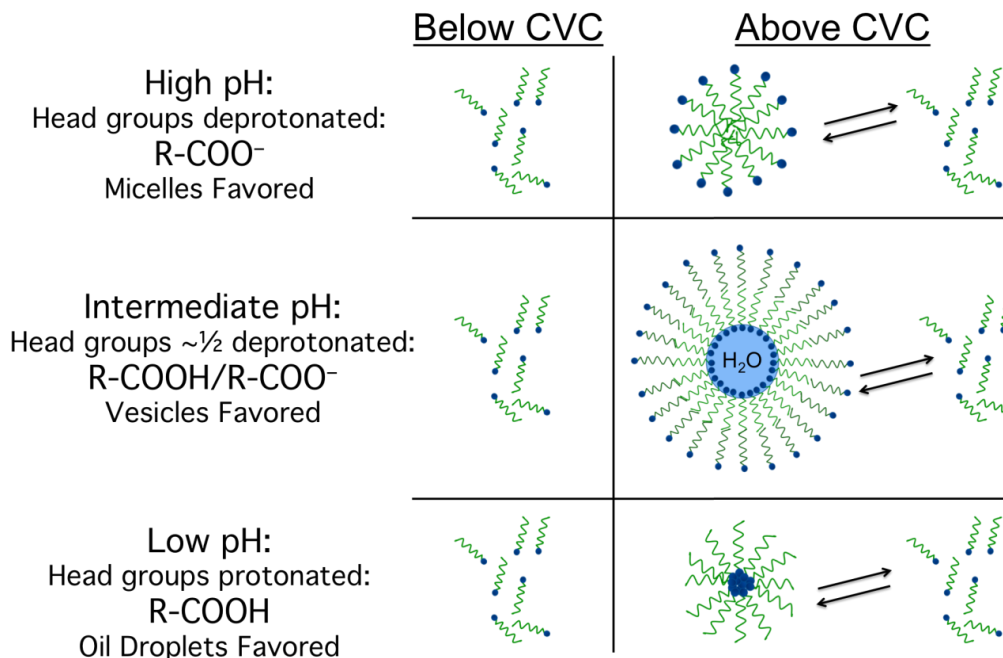

Figure 4: This phase diagram depicts the current understanding of fatty acid behavior in solution, where depending on the pH and concentration of fatty acid, oil droplets, vesicles, or micelles may be formed. These structures are all thought to be in equilibrium with monomers in solution. (Based off of a figure in Monnard and Deamer's 2002 paper[3])

A thorough review of the literature on decanoic acid shows that the phase behavior depicted above is not as well understood as is often assumed. The reported critical aggregation concentrations vary significantly, ranging from 10 to 40 mM in the recent literature and summarized in Figure 5. Vesicles in these experimental systems are always made in buffered solutions that contain additional counter-ions with millimolar concentrations of salt, usually NaCl. However, little systematic work has been done to understand the effect of either salt or buffer on the observed CAC, and how changes in these may affect the pH conditions under which vesicles are stable. An additional subtle aspect to decanoic acid's phase behavior is that the CAC is not necessarily equivalent to the CVC. Indeed, it is clear that aggregation begins to occur at much lower concentrations than those required for the formation of vesicles,[17,18,19] but

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[16] Kanicky, J.R., Poniatowski, A.F., Mehta, N.R., Shah, D.O., 1999. Cooperativity among Molecules at Interfaces in Relation to Various Technological Processes: Effect of Chain Length on the pKa of Fatty Acid Salt Solutions. Langmuir 16, 172–177. doi:10.1021/la990719o

[17] Cape, J.L., Monnard, P.-A., Boncella, J.M., 2011. Prebiotically relevant mixed fatty acid vesicles support anionic solute encapsulation and photochemically catalyzed trans-membrane charge transport. Chem. Sci. 2, 661–671. doi:10.1039/c0sc00575d

[18] Apel, C.L., Deamer, D.W., Mautner, M.N., 2002. Self-assembled Vesicles of Monocarboxylic Acids and Alcohols: Conditions for Stability and for the Encapsulation of Biopolymers. Biochimica et Biophysica Acta 1559, 1–9.

because the origin-of-life community is largely focused solely on vesicular structures this point is often ignored in the literature. The mechanism by which these three-dimensional structures form is also not agreed upon although a number of mechanisms of aggregate formation have been proposed.[20,21]

Figure 5 highlights some the discrepancies in the literature, while also attempting to represent the actual behavior of decanoic acid with a more complete phase diagram representation. This is a schematic representation based on literature values, and therefore shows only the likely behavior of decanoic acid with dashed lines indicating phase transitions for which there is little evidence in the literature and are therefore only hypothetical. While not directly represented in Figure 5, it is important to also recognize that these aggregates are also in equilibrium with monomers. The roles of buffer and salt have also not been included, but are likely to contribute to some of the variation of reported CVC in the literature.
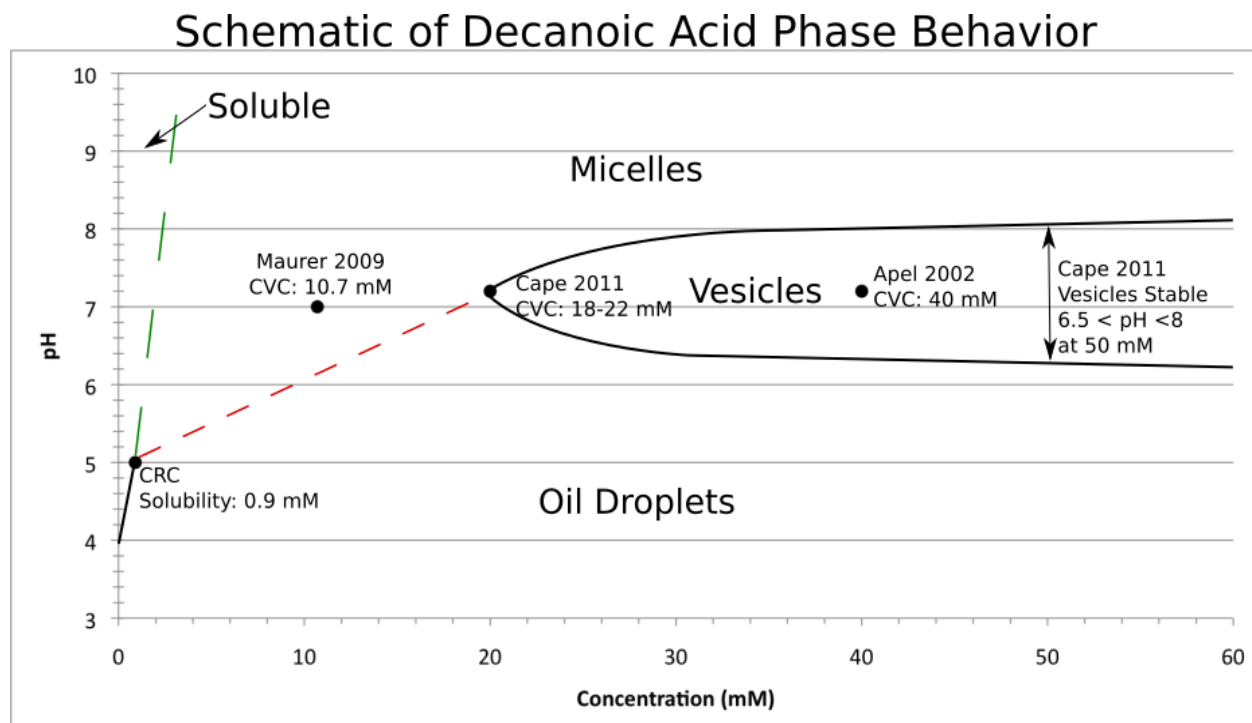

Figure 5: A schematic outlining the likely phase behavior of decanoic acid as a function of pH and concentration, based off the CVC reported by Cape et al.[17] The literature studying the formation of vesicles and micelles from decanoic acid reports a variety of CVCs, some of which are presented here.[17,18,19] The CRC reports the solubility of decanoic acid as 0.9 mM.[22] Taken together, it is clear that the phase behavior of decanoic acid is more complicated than is usually assumed. The red and green dashed lines represent hypothetical phase transitions. (Rebecca Rapf, personal communication, March 2015)

[19] Maurer, S.E., Deamer, D.W., Boncella, J.M., Monnard, P.-A., 2009. Chemical Evolution of Amphiphiles: Glycerol Monoacyl Derivatives Stabilize Plausible Prebiotic Membranes. Astrobiology 9, 979–987.

[20] Lasic, D., 1988. The Mechanism of Vesicle Formation. Biochem. J. 256, 1–11.

[21] Lipowsky, R., 1991. The conformation of membranes. Nature 349, 475–481. doi:10.1038/349475a0

[22] CRC Handbook of Chemistry and Physics. 95th ed. CRC Press: Boca Raton, FL, 2014-2015; 5-164.

Some of the discrepancy in the literature may be due to the difficulties inherent in bulk analysis of vesicles in solution. Simplifying the system could remove some of the challenges of characterizing vesicle behavior. For surfactants, one such simpler system is a monolayer on a bulk aqueous solution. Analysis of surfactants in a monolayer at the water surface can provide information about molecular interactions in aggregates, establishing monolayers as useful models for more complicated physical systems;[23] they have been used before to study surface ionization[24] and aggregate behavior.[25] Monolayers can more easily be manipulated and observed than bilayers in a bulk solution. The approach chosen here is to study the surface partitioning and thermodynamics of monolayers of fatty acids and to compare the results to the stability of vesicles of similar composition.
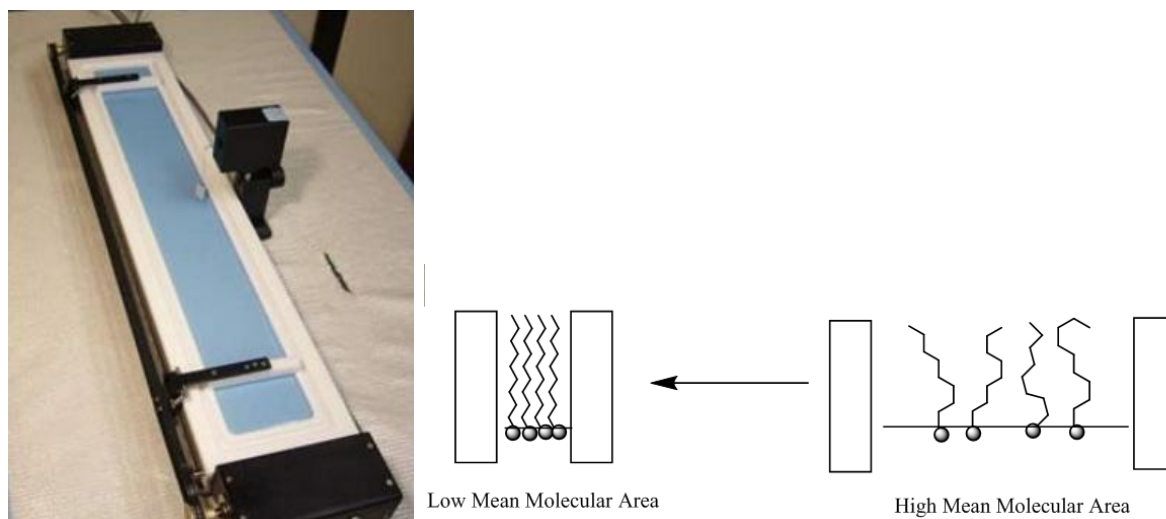

Low Mean Molecular Area

High Mean Molecular Area

Figure 6: Left, a Langmuir trough with a Wilhelmy balance and two moveable barriers, which compress monolayers as shown on the right.

A Langmuir trough equipped with a Wilhelmy balance (Figure 6) can be used to study the surface partitioning and thermodynamics of monolayers; the barriers on the trough sit on the liquid surface and can be used to induce packing changes in the monolayer while surface tension is monitored, as water slips under the barriers as they are closed, but molecules at the surface in general do not and are instead compressed. Surface tension is the primary thermodynamic quality that can be monitored by a Langmuir trough; it is the proportionality constant γ for the work needed to change the surface area (σ) by an infinitesimal amount, is described by Equation 1.[26] This can be related to the Helmholtz energy A (Equation 2) for surface formation taking place at

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[23] Kaganer, V.M., Mohwald, H., Dutta, P., 1999. Structure and phase transitions in Langmuir monolayers. Rev. Mod. Phys. 71, 779–819. doi:10.1103/RevModPhys.71.779

[24] Griffith, E.C., Vaida, V., 2013. Ionization state of L-Phenylalanine at the Air-Water Interface. J. Am. Chem. Soc. 135, 710–716. doi:10.1021/ja308089n

[25] Fedotenko, I.A., Stefaniu, C., Brezesinski, G., Zumbuehl, A., 2013. Monolayer Properties of 1,3-Diamidophospholipids. Langmuir 29, 9428–9435. doi:10.1021/la401692e

[26] Atkins, P., de Paula, J., 2010. Physical Chemistry, 9th ed. W. H. Freeman and Company.

constant volume and temperature, or to the Gibbs energy G for surface formation at constant pressure (Equation 3).

$$dw = \gamma d\sigma$$

<div align="right">**Equation 1**</div>

$$dA = \gamma d\sigma$$

<div align="right">**Equation 2**</div>

$$dG = \gamma d\sigma$$

<div align="right">**Equation 3**</div>

When a surfactant monolayer is present, the surface tension of the system changes. The difference between the surface tension of the surfactant-covered system, γ_0, and that of the pure solvent, γ, is called surface pressure, π (Equation 4).[23]

$$\pi = \gamma_0 - \gamma$$

<div align="right">**Equation 4**</div>

Surface pressure versus area and time can be monitored with a microbalance, which operates with a plate attached to the balance in contact with the solution. The surface tension of a solution is related to the force exerted on the plate by the solution after accounting for the contact angle between the plate and the solution, θ, and the perimeter of the plate, l, as seen in Equation 5. The contact angle is often assumed to be $0°$ for filter paper plates,[27] meaning that equation for surface tension simplifies further. The surface pressure is then reported as the deviation of this measured surface tension from the surface tension of pure water.

$$\gamma = \frac{F}{l\cos\theta}$$

<div align="right">**Equation 5**</div>

Because of the controllable surface area on a Langmuir trough, if the amount of a surfactant that has been deposited is known, it is possible to calculate the surface concentration of an insoluble surfactant. The mean molecular area (MMA), or inverse surface concentration, of a surfactant can also be calculated by dividing the surface area between the barriers by the number of surfactant molecules. MMA is a way of reporting the average amount of space that each surfactant molecule can explore. Additional information that can be obtained from compression isotherms, includes the compressibility of the film by analysis of the steepness of the slope of isotherms. The stability of the monolayer can also be determined by looking at the collapse pressure of a compression isotherm; weaker films will have lower collapse pressures.[26]

An idealized isotherm from the compression of a generic surfactant is presented below (Figure 7). Such isotherms are collected by depositing a surfactant in a volatile solvent onto an aqueous substrate, allowing the solvent to evaporate, and then compressing the surfactant layer between the trough's barriers.

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[27] Butt, H.-J., Graf, K., Kappl, M., 2003. Physics and Chemistry of Interfaces. Wiley-VCH, Weinheim.
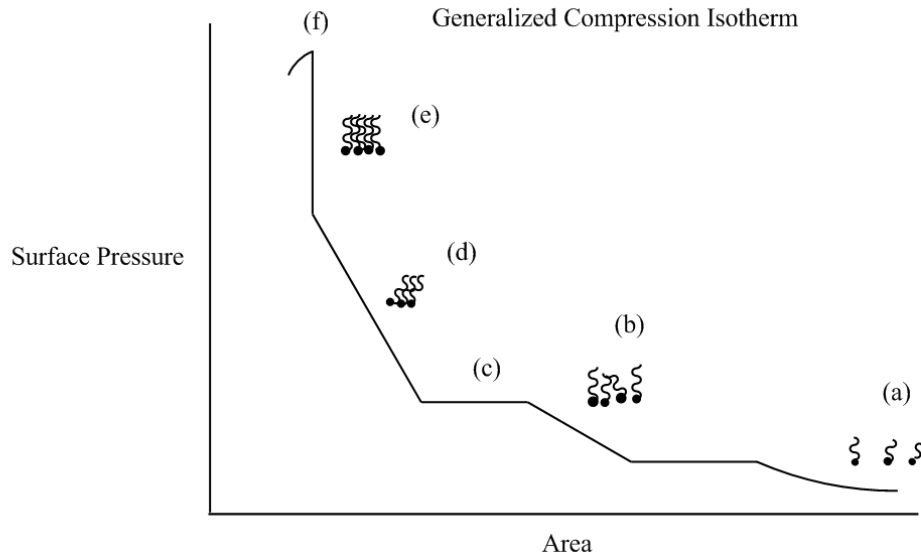
Figure 7: A general compression isotherm. Not all surfactants experience the same phase transitions when compressed. a) Initially, the molecules are in a two-dimensional gaseous phase, as they are too far apart to interact strongly; b) further compression leads to a liquid condensed phase where the tails are still disorganized but the surface pressure increases; c) the flat sections of the isotherm indicate where two phases coexist, in this case the liquid condensed and tilted condensed phases; d) the dominance of the tilted condensed phase is marked by a steeper isotherm slope; e) another change in slope marks the transition to the untilted condensed phase; f) finally, the film is compressed far enough to collapse the monolayer and create multi-layer aggregates. This figure is based off of the theoretical isotherm presented in Kaganer et al 1999.[23]

Different surfactant species undergo different sets of phase transitions; the transitions presented here are may not all be observed for a given surfactant monolayer, but most compression isotherms will include at least two. In general, the surfactant molecules are initially in a two-dimensional "gaseous," or "liquid expanded," phase, where they are too spread out to interact strongly (Figure 7, a). Further compression leads to a "liquid condensed" phase where the molecules are still disordered with respect to one another, but the surface pressure has increased (Figure 7, b). This is followed by a more horizontal section where the liquid condensed and a more tightly packed and ordered "tilted condensed" phase coexist (c); the slope of the isotherm then steepens as the tilted condensed phase takes over (d), and then steepens again for the untilted condensed phase where the surfactant molecules pack even more tightly (e).[23] Collapse, when the monolayer breaks up into multi-layer aggregates, occurs after even more compression (f).

In the event that the surfactant is too soluble for compression isotherms to provide meaningful information, adsorption isotherms (Figure 8) can be made instead. As opposed to π-area compression isotherms, a dilute bulk solution of the molecule in question is deposited on the trough, and the surface pressure at constant area is monitored as it adsorbs to the surface. The Gibbs adsorption isotherm (Equation 6) relates the surface excess concentration of a surfactant (Γ) to the change in surface tension with surfactant concentration ($d\gamma/d(\ln c)$).

Example Adsorption Isotherm
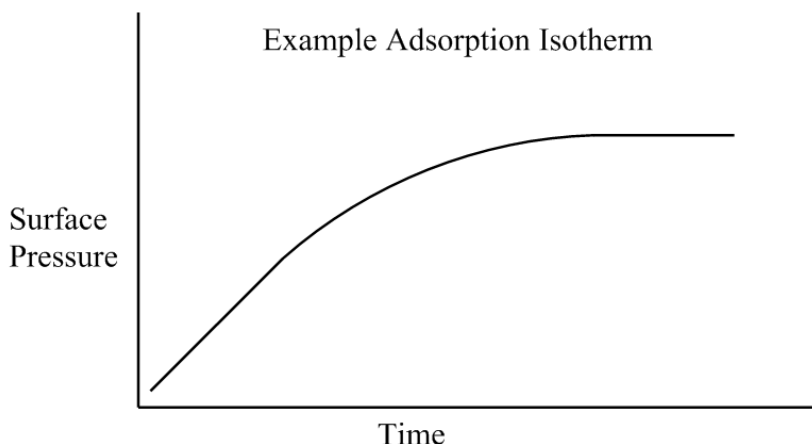
Surface
Pressure

Time

Figure 8: Adsorption isotherms monitor the rising surface pressure as monomers adsorb to the water surface from a dilute bulk solution. These isotherms level off at a maximum surface pressure.

$$\Gamma = -\frac{1}{RT} * \frac{d\gamma}{d(\ln(c))}$$

Equation 6

The information provided by Langmuir trough isotherms allows for studies of the molecular interactions governing decanoic acid monolayer stability. As noted above, it is known that decanoic acid's aggregation behavior changes at different pH's; micelles are present at high pH when the head groups are entirely deprotonated and repel each other, whereas vesicles appear to form at lower pH's where the head groups are half-protonated and half-deprotonated, and oil droplets are present when all the head groups are protonated and experience little repulsion. Previously, these aggregates have only been described with empirical techniques such as light scattering, and as noted in Figure 5, are associated with a number of different CAC's in the literature. Collecting Langmuir isotherms of decanoic acid monolayers on a variety of substrates at different pH's and salt concentrations should provide a better picture of the molecular interactions and phase transitions that lead to vesicle formation. This type of study has been carried out before to investigate the stability of stearic acid films on sea water.[28] Compression isotherms taken at low, neutral, and high pH's were expected to follow pattern presented in Figure 9. The changes in lift-off, slope, and collapse in these isotherms are expected to be small, however, so successfully observing them would require precise and highly reproducible results.

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[28] Brzozowska, A.M., Duits, M.H.G., Mugele, F., 2010. Stability of Stearic Acid Monolayers on Artificial Sea Water. Colloids and Surfaces A: Physicochemical and Engineering Aspects.
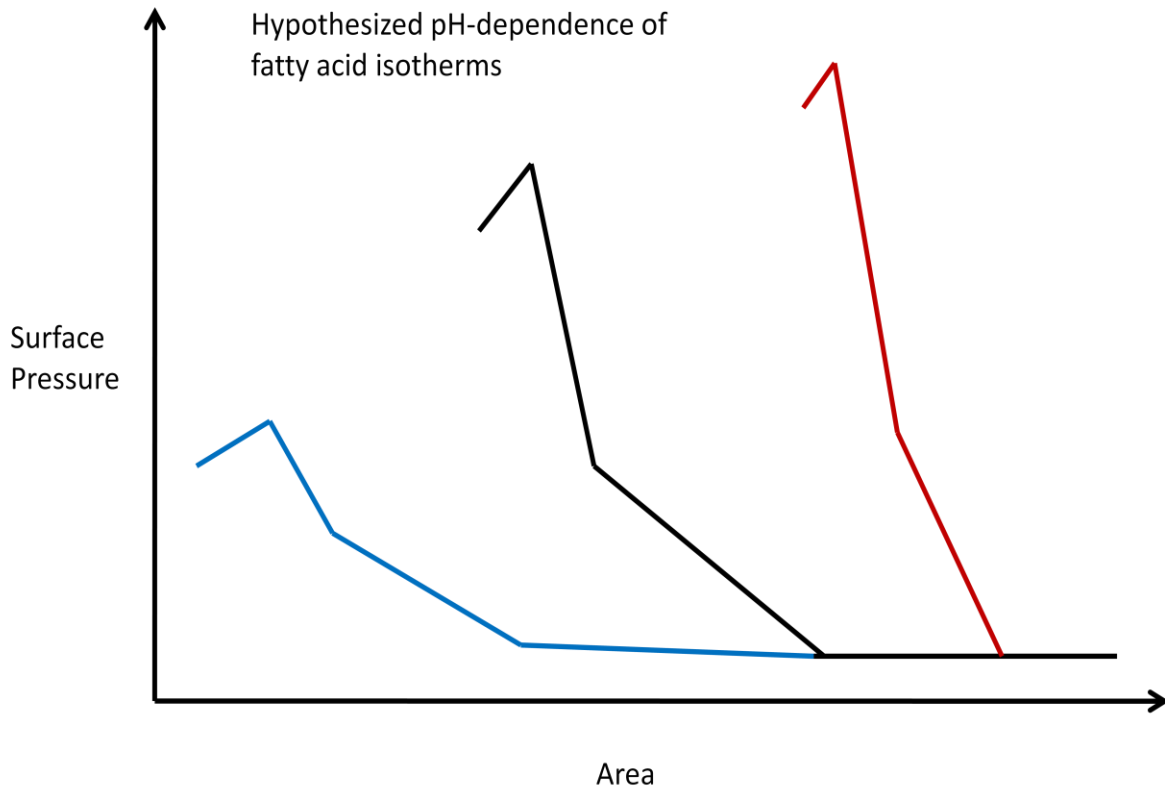
Figure 9: Hypothetical compression isotherms for decanoic acid deposited on different pH solutions. Red indicates a hypothetical isotherm at low pH, black at neutral pH, and blue at high pH.

Another component of the solutions used to make vesicles is salt, usually sodium chloride. While the pH of vesicle solutions is usually reported in the literature, the exact salt concentration and buffer conditions often are not, though it is thought that vesicles cannot form without salt in the solution. The effect of salt on vesicles is less understood than that of pH, but salt and buffer ions could associate with the fatty acid head groups and alter the head-group interactions thought to hold vesicles together. Figure 10 presents a set of hypothetical compression isotherms of decanoic acid on varying salt concentrations. Better knowledge of how salt alters fatty acid interactions could help clarify the range of CACs in the literature.
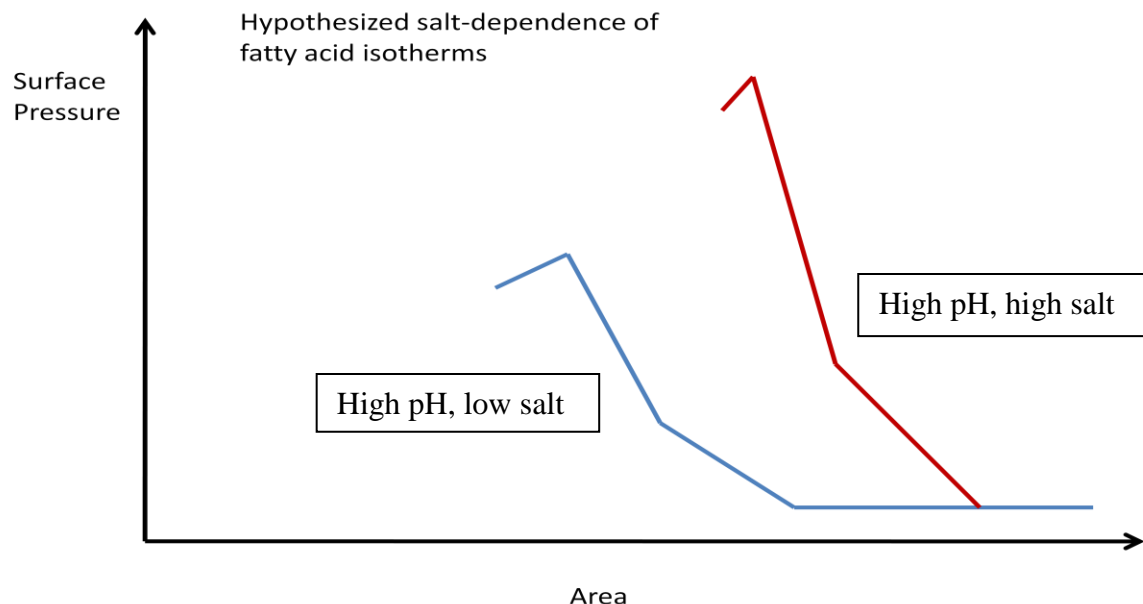
Figure 10: Hypothetical compression isotherms for decanoic acid deposited on different monovalent salt solutions at high pH. Red indicates a higher salt concentration, and blue indicates a low salt concentration where ion screening does not reduce the destabilizing intermolecular forces in the fatty acid film. The difference between these two isotherms is expected to be less than the differences between the isotherms at different pHs.

High salt concentrations should screen deprotonated head groups to the point where repulsive fatty acid interactions are weakened, mitigating the delayed lift-off and lower pressure collapse of the isotherm. For isotherms under high pH conditions, the presence of a high salt concentration will shift the isotherm towards a higher collapse point resembling that of an isotherm at a lower pH. Under low pH conditions, where there are fewer deprotonated fatty acids, the screening effect of salt should be minimal. It is also likely that the salt's effects on fatty acids will plateau at some concentration (where screening is maximized), and that the salt effects will be smaller than that of pH. Therefore, it is critical to isolate the action of salt, as pH could hide its effects.

These experiments could help distinguish the roles of pH and salt in vesicle formation; they are currently entangled in the bulk studies of vesicle behavior. Compression isotherms of decanoic acid on salt solutions alone and buffer solutions alone should offer insight into the respective molecular actions of pH and salt. The initial results of these studies are reported below.

Methods

Decanoic acid, >98.0% purity, was obtained from Sigma Aldrich. Stearic acid at >98.0% purity was also acquired from Sigma Aldrich.

For all experiments, a $52 \times 7 \times 0.5$ cm PTFE Langmuir trough with two computer-controlled mechanical PTFE barriers and Wilhelmy balance was used; the software and computer interface were from NIMA (NIMA Technology Ltd., UK). The barrier positions

ranged from 300 cm^2 between the open barriers, to a minimum of 30 cm^2; for compression isotherms, the barriers were closed at a constant speed of 75 cm^2/min. Isotherms with information on surface pressure, area, and time were collected with this equipment.

MilliQ-filtered water (18.2 MΩ, 3 ppm total organic carbon) was used to make all solutions and for all experiments.

Preparation of salt and buffer solutions:

Salt solutions were prepared by weighing out the appropriate amount of sodium chloride (Fisher Scientific, >99.0% purity) on a balance, then dissolving in one liter of water. NaCl concentrations were chosen to match the salt concentration used when making vesicles (50 mM) and to mimic ocean water (500 mM).[14]

A solution of 200 mM Tris buffer (Fisher Scientific), 50 mM NaCl, and MilliQ water was made to match conditions at which decanoic acid vesicles are stable, and filtered through a 0.2 μm surfactant-free cellulose acetate Nalgene filter. This solution had a pH of 7.51, adjusted with 37% HCl (Mallinckrodt Baker, Inc.). This solution composition was chosen because decanoic vesicles are known to be stable in these conditions. This was confirmed by the generation and isolation of dye-encapsulated decanoic vesicles in the Vaida lab using this buffer solution.

Stearic acid pressure-area (π-A) isotherms on water surface:

The trough was first cleaned by scrubbing with isopropanol three to four times, rinsing with MilliQ water three to four times, removing the water through aspiration. The cleanliness of the trough was confirmed by obtaining a pressure-area isotherm for pure water that did not show an increase in surface pressure. Isotherms were considered clean if the surface pressure at the minimum surface area was 0.1 mN/m or lower. Stearic acid films were made by the deposition of 25-30 μL of 4 mM stearic acid in chloroform, placed dropwise on the water surface by syringe. The stearic acid was then allowed to sit undisturbed for twenty minutes to allow the solvent to evaporate. After this, the barriers were compressed at 75 cm^2/minute and a (π-A) isotherm was collected on the NIMA Trough software.

Decanoic acid pressure-area (π-A) isotherms on water surface:

The trough was cleaned in the same manner described above. Experimental surfactant films of decanoic acid were created by deposition of twenty to thirty microliters of 80 mM decanoic acid in chloroform, placed dropwise on the water surface by syringe. The decanoic acid was then allowed to sit undisturbed for twenty minutes to allow the solvent to evaporate. After this, the barriers were compressed at 75 cm^2/minute and an isotherm was collected on the NIMA Trough software.

Decanoic acid time-pressure (π-t) absorption isotherms:

After cleaning the trough, 25.0 μL of 80 mM decanoic acid in chloroform was also deposited on buffer/salt and salt solution substrates. Time-pressure isotherms were recorded

during deposition and until the surface pressure decreased to 0.1 or lower and had stabilized, indicating equilibration within the solution. Compression isotherms were also run following this equilibration.

Results and Discussion

Stearic acid is often used as a model surfactant for surface pressure studies and has been extensively characterized on the Langmuir Trough, as it is extremely well-behaved and reproducible. Compression isotherms of stearic acid show several phase transitions. The discontinuities in the slope of the isotherm below (Figure 11) represent changes in surface tension that indicate the packing of molecules on the water surface has been altered by compression. The liquid expanded and two liquid condensed phases can be seen in the stearic isotherm. Stearic acid, like decanoic acid, is a fatty acid, but it has 18 carbons instead of 10. The additional carbons in its alkyl tail reduce its solubility and make it very insoluble in water, which helps ensure reproducible compression isotherms.
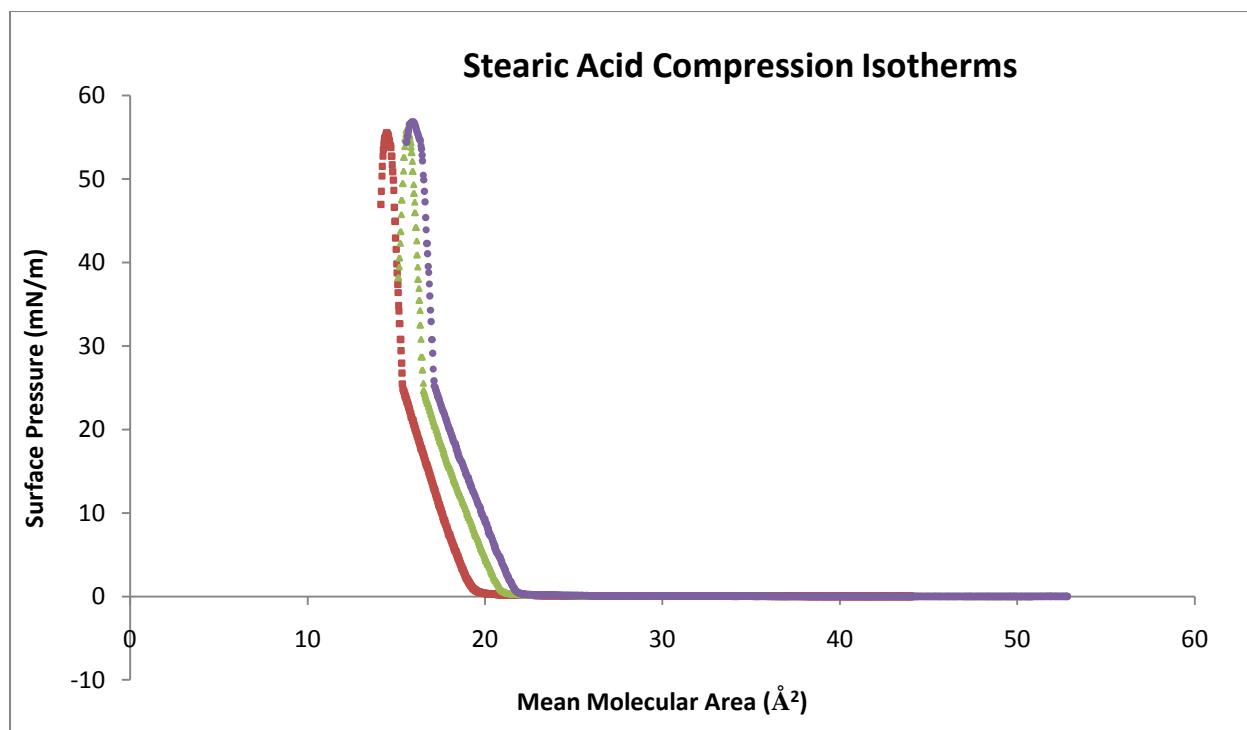

Figure 11: Stearic acid isotherms. The collapse of the film at ~55 mN/m is easily seen here, as is the kink in the isotherm indicating the phase transition from the tilted condensed phase to the untilted condensed phase. Molecular footprints for the tilted condensed phase were found by extrapolating the line back to the x-axis, and were found on average to be 20.8 Å^2 with a standard deviation of 1.2 Å^2.

For this reason, a number of stearic acid isotherms were prepared for familiarization with the trough (Figure 11). Because the proposed decanoic acid experiments are attempting to probe small effects in the surface stability due to salt and pH, highly reproducible technique in deposition is required. Because stearic acid is so well-behaved, comparison of multiple

compression isotherms gives a good measure of the reproducibility of the deposition technique. The stearic acid π-A isotherms obtained had an average molecular footprint of the tilted condensed phase of 20.8 Å^2, with a standard deviation of 1.2 Å^2; this is near the 20 Å^2 footprint expected from the literature,[29] and the standard deviation can be taken as the a benchmark for changes in isotherms due to small differences in how surfactant was deposited on the surface, and as a limit of detection for seeing differences in decanoic acid isotherms on different bulk solutions. Since reproducible isotherms were obtained, stearic acid was also used as a further indicator of trough cleanliness.

Once consistent deposition technique was ensured, the decanoic acid system was investigated, beginning with π-A compression isotherms of decanoic acid deposited on a pure MilliQ water substrate. Isotherms created by depositing 25.0 µL of 80 mM decanoic acid in chloroform on the water surface and waiting twenty minutes before compression are shown in Figure 12. Compression occurred twenty minutes after deposition to allow for solvent evaporation. Considerably more decanoic acid had to be deposited onto the surface than stearic acid to observe changes in surface pressure during compression. The resultant surface concentration for decanoic acid, with fully open barriers, gives a MMA of 2.5 Å^2 if it is assumed all the decanoic acid remains on the surface. Compared to the 50 Å^2 MMA for the stearic acid with fully opened barriers, and the 20 Å^2 compressed-phase stearic acid footprint, this is unfeasible. Additionally, unlike the highly reproducible stearic acid isotherms, the shape of these isotherms was not consistent.
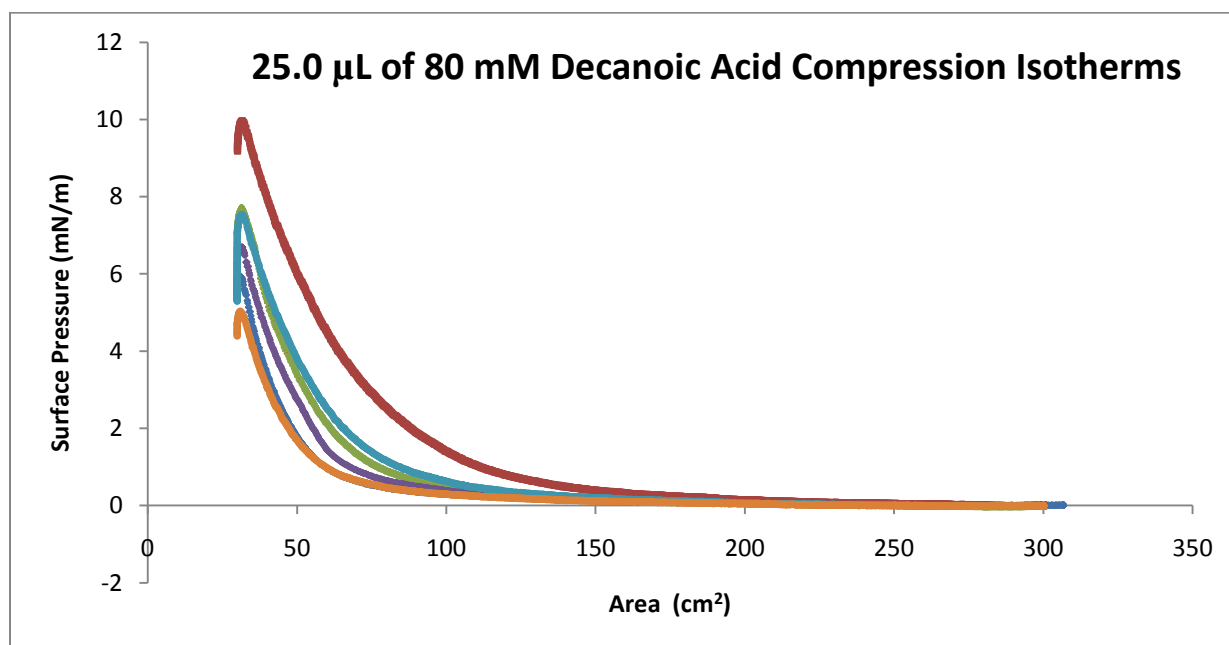

Figure 12: 80 mM, 25.0 µL decanoic acid deposition on a water surface. The decanoic acid was compressed after sitting on the trough for 20 minutes.

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[29] Roberts, G., 1990. Langmuir-Blodgett Films. Plenum Press, New York.

While these issues limit the applicability and interpretation of these results, some statistical analysis was conducted. The average maximum surface pressure reached upon compression was found to be 8.0 mN/m with a 95% confidence interval of ± 1.9 mN/m. None of the compression isotherms could be rejected as an outlier with a 95% confidence level Grubbs test on the peak surface pressure. This peak surface pressure indicates the maximum compression of the decanoic acid monolayer when the trough barriers were at their minimum area. Unlike the stearic acid isotherms, only one phase transition between a less condensed phase and more condensed phase is evident, and no collapse of the film was observed before the barriers have reached the minimum area. Compression isotherms were also collected on a substrate of 5 mM NaCl, with similar variability as seen in the isotherms above.

The variance of the maximum surface pressure might be due to small inconsistencies in the amount of decanoic acid deposited on the trough, although based on the stearic acid controls, deposition was generally reproducible. Changes in deposition amount also do not account for the shape of the isotherm. Short differences in the time allowed for chloroform evaporation after deposition before compression are a possible explanation for the variance seen above. For a well-behaved system, such as stearic acid, changes in evaporation time have a minimum effect on the observed isotherms because surfactants with long alkyl chains (18 carbons in the case of stearic acid) are quite insoluble in water. However, for a shorter surfactant this might not be the case; if the molecule is somewhat soluble, it may be partitioning into the bulk phase from the surface and therefore the amount of equilibration time allowed before beginning compression will affect the resultant isotherm. This possibility is further supported by the unrealistically high MMA obtained when all molecules were assumed to stay at the surface.

In order to account for these discrepancies, the starting surface pressure recorded before zeroing the instrument was taken into account, as it should indicate the extent to which the decanoic acid had left the surface and absorbed into the bulk. The surface pressure recorded by the Wilhelmy microbalance is usually re-zeroed immediately before compression after the period of solvent evaporation as a matter of experimental protocol to account for incidental contact and movement of the balance during deposition. In general, the change in re-zeroing is on the order of a few tenths of a mN/m. Differences larger than this may indicate surface coverage of surfactant greater than a monolayer. All the isotherms plotted in Figure 12 above were at a non-zero surface pressure prior to zeroing, ranging from 0.6 to 1.2 mN/m. The non-zero surface pressure could indicate that more than a monolayer was present on the surface, and the isotherms resulted from having enough decanoic acid left on the surface after twenty minutes to cause an appreciable rise in surface pressure when compressed. It was noticed that for isotherms which had similar surface pressures prior to zeroing had more consistent shapes.

Taking all of this into account, it seems clear that decanoic acid is not acting as an insoluble surfactant; rather, it is solubilizing into the bulk aqueous phase. The surface activity seen after twenty minutes of equilibration time is due to the remaining excess on the surface, but the lack of reproducibility indicates this equilibration is not complete. Changing the aqueous

substrate is expected to generate small changes in the isotherms, so the low reproducibility of the compression isotherms makes them appear to be an ineffective method for detecting changes in decanoic acid phase behavior.

The surface activity of soluble molecules can be monitored by observing the adsorption of these molecules from the aqueous bulk to the surface. As discussed in the Introduction, adsorption isotherms are collected by filling the trough with a bulk solution of aqueous decanoic acid and monitoring the surface pressure as decanoic acid adsorbs to the surface. Given decanoic acid's apparent solubility, adsorption isotherms may be a more useful measure of its phase behavior than the compression isotherms above. Initially, a 0.8 mM aqueous decanoic acid solution was made just under the CRC solubility limit of 0.9 mM.[22] While it initially appeared soluble, it was observed to phase separate overnight, as a crystalline appearing solid was formed (Figure 13). This indicates that the literature solubility value was either inaccurate or for a different pH. The pH of the CRC solubility value was not reported and was assumed to be unadjusted, pointing further to the inconsistencies in the decanoic acid literature. A more dilute solution, 0.09 mM, appeared to be soluble and an adsorption isotherm was run. This adsorption isotherm showed an increase in surface activity after deposition indicating that decanoic acid was partitioning to the surface. However, the solubility of decanoic acid is difficult to evaluate visually, since it is known to create aggregates in the bulk that might not be large enough to scatter visible light.

The solubility range of decanoic acid is problematic for compression or adsorption isotherms. In the case of compression isotherms, knowing the surface concentration of decanoic acid is necessary in order to quantify its phase transitions, and it is too soluble to accurately determine the surface concentration as it is compressed; the acid could be going into solution during compression, removing the quantitative power of the π-A isotherms. Adsorption isotherms were complicated by the possibility that the dilute decanoic acid solutions prepared might be above some unknown CAC, instead of being simply monomers in solution. Without knowing if the aqueous solutions of decanoic acid used contain aggregates, it is difficult to justify the use of adsorption isotherms. Even when light scattering is used to detect micelles in solution, a certain bulk concentration of aggregates must be present in order to be detected. Decanoic acid's solubility is an intermediate regime that makes it too soluble for compression isotherms, but too insoluble for the confident preparation of the dilute solutions used to make adsorption isotherms.
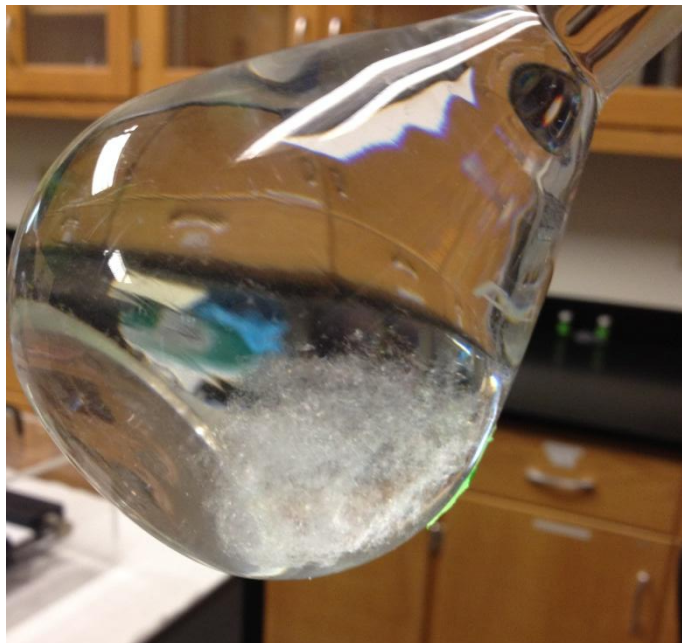
Figure 13: A 0.8 mM aqueous solution of decanoic acid produced this aggregate overnight.

Given these issues, a new approach was designed to study decanoic acid. "Absorption" isotherms were obtained by monitoring surface pressure over time (π-t) during and after decanoic acid is deposited on a bulk solution, until the deposited acid had equilibrated with the bulk solution. Absorption isotherms potentially may be used to bracket solubility regimes and acquire meaningful information about how decanoic acid's phase behavior changes on different bulk solutions.

Absorption isotherms measuring surface pressure over time (π-t) were collected by monitoring the surface pressure as 80 mM decanoic acid in chloroform was deposited on water, salt, and buffer substrates, and equilibrated with the bulk solution. A typical absorption isotherm may be seen in Figure 14. The surface pressure increased at the beginning of the isotherm (<50 s) as decanoic acid was deposited, then decreased over time as the decanoic acid was absorbed into the bulk solution. The pressure increase during deposition was much more dramatic on the salt and pure water substrates, reaching 25-32 mN/m at the peak.
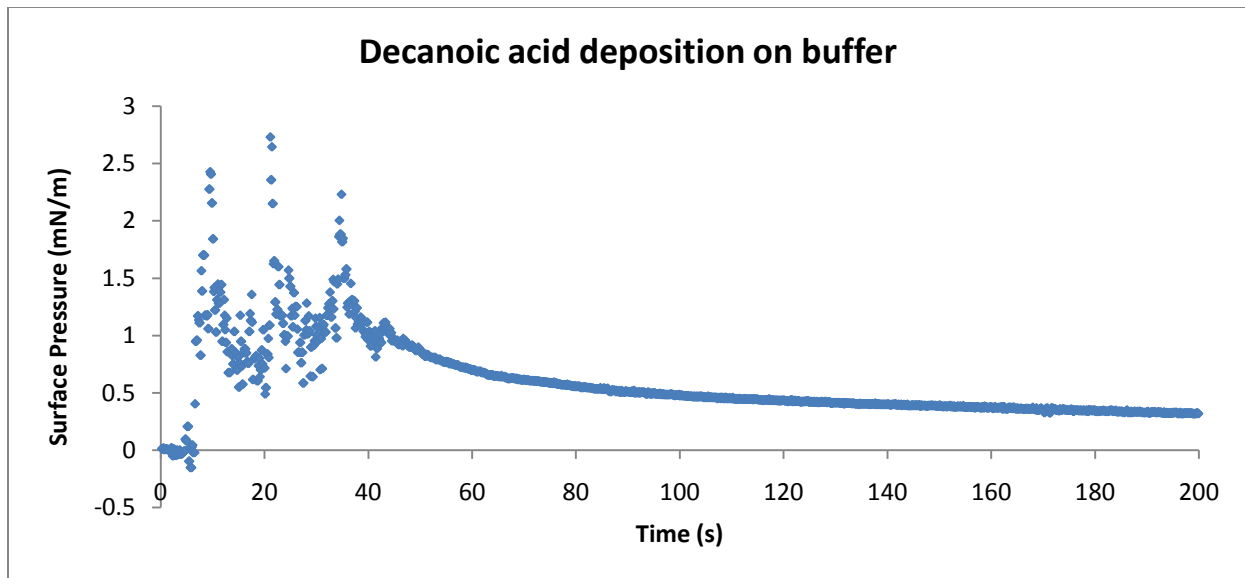
Figure 14: A typical time-pressure isotherm. The jagged peaks before 40 s represent the deposition of decanoic acid onto the surface, with the solid curve following indicating the equilibration of decanoic acid with the solution. Decanoic acid took much less time to equilibrate on buffer than on the salt solutions.

Three absorption isotherms were collected on a substrate of the 200 mM Tris buffer and 50 mM salt solution used to prepare decanoic acid vesicles, as well as on two salt substrates, 50 mM NaCl and 500 mM NaCl in water. Prior to depositing decanoic acid on the substrates, compression isotherms of just the substrate were collected; no surface activity was seen in the salt solutions without decanoic acid, but the buffer had noticeable surface activity (Figure 15, left). This could be due to the Tris buffer (Figure 15, right), or a contaminant introduced during filtration of the buffer. Further study is needed to determine the surface activity of the Tris buffer alone.
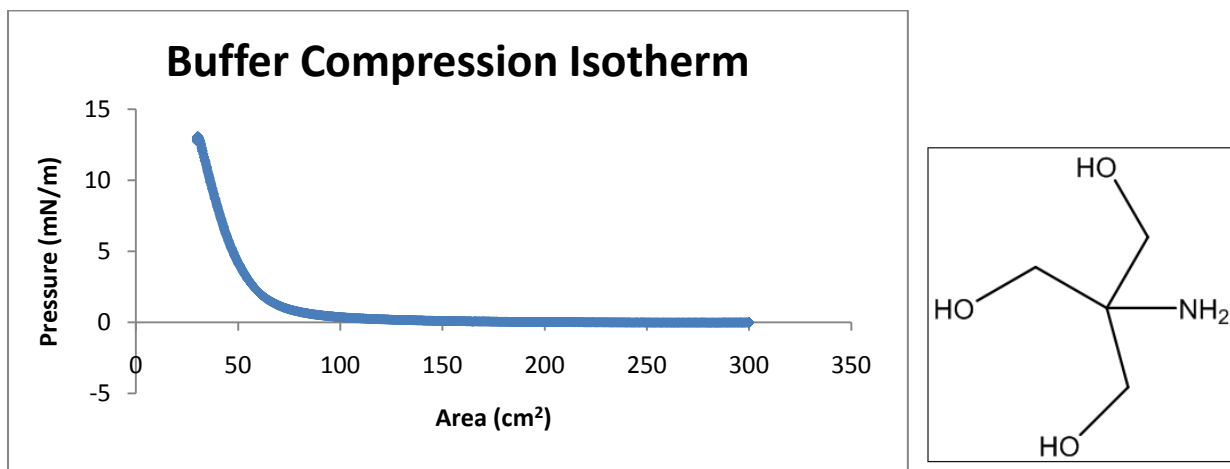

Figure 15: Buffer compression isotherm. The height indicates surface activity in the buffer, from the tris molecule (right) or an unknown contaminant.

Statistical analysis was carried out on these time-pressure absorption isotherms. In these cases, the equilibration time required to reach a pressure of 1.0 mN/m after the peak pressure occurred were compared. The variation among isotherms is recorded below in Table 1. A more reliable indicator of isotherm similarity might be the time between 1.0 and 0.1 mN/m, which may reduce some of the variability due to the speed at which decanoic acid was deposited on the trough.

Substrate	Time to reach 1.0 mN/m after peak pressure reached (s)	Standard deviation in time (s)
50 mM NaCl	700	400
500 mM NaCl	489	18
Buffer	19	6
MilliQ Water	1211	N/A

Table 1: Comparison of time-pressure absorption isotherms for 25.0 μL of 80 mM decanoic acid deposited on different substrates. Only one isotherm was collected on pure water.

Time versus pressure isotherms were collected for each substrate, and the time required for the isotherm to decline from its peak height to a pressure of 1.0 mN/m was recorded for each. It was found that these equilibration times were not statistically different for the 50 and 500 mM salt solutions. The equilibration time was, however, statistically different between the buffer and the salt solutions, with the decanoic acid reaching equilibrium much faster on the buffer solution. It also appears that equilibration occurs more slowly on water than on the other solutions tested, as may be expected from prior research.[28] Compression isotherms were also run after equilibration; while the amount of decanoic acid deposited for these experiments was too low to produce compression isotherms that were different than the blank, this may offer a way to collect data on slightly-soluble surfactants.

The absorption times presented above are not particularly reproducible. This may be due in part to small sample size, but it may also indicate further complexities in decanoic acid's behavior in addition to the solubility and uncertain phase transitions already seen. Nonetheless, a trend in equilibration speed can be seen in the data above; as ionic strength of the bulk increases, the equilibration speed also increases. Introducing ions into solution tends to disrupt the network of hydrogen bonding present in pure water, so it may be that faster equilibration indicates that these small disruptions in the network reduce the energetic cost of absorption for decanoic acid. Given the anticipated lack of effect of salt concentration on low-pH solutions, it is interesting that the 50 mM salt solution has a faster equilibration time than the pure water; this indicates that perhaps screening is not the only interaction experienced between decanoic acid and salt solutions. That the 50 mM salt and buffer solutions had very similar ionic strengths and such different equilibration times indicates the importance of pH in decanoic acid's surface behavior.

The preliminary results presented here indicate that the salt concentration present in a given solution may reach its maximum screening ability at a very low concentration, since the 50 and 500 mM isotherms behaved in statistically not different ways. With more reproducible data, it might be possible to pick out statistically significant differences between the two isotherms; as

it is, only large statistical differences can be ruled out. It is also apparent that decanoic acid equilibrates much faster with a buffer solution than with salt solutions.

These are very preliminary results. While the applicability of these results are limited, the emergence of a trend in the equilibration times with substrate composition indicates that absorption isotherms may be a useful technique for probing differences in intermolecular interactions under different conditions. The collection of decanoic acid isotherms on a variety of bulk pH's and salt concentrations would therefore be helpful in shedding light on aggregate behavior, especially given the apparent lack of influence of salt at the concentrations tested. It is possible that the maximum screening of fatty acid from salt occurred below the 50 mM used here. Additionally, more of these absorption isotherms need to be carried out in the future in order to have a larger sample size. With only a few runs on each substrate, it is difficult to draw conclusions about the behavior of decanoic acid, though these results tentatively indicate that the effects of salt plateau at some lower concentration, as no statistically significant difference was seen here; more experiments on 50 and 500 mM salt could also more firmly show if a trend in faster equilibration times at high ionic strength exists. It seems that the pH (or even the presence of a particular buffer) is influential on decanoic acid's surface partitioning behavior.

To this end, further decanoic acid absorption isotherms should be conducted on the following substrates: 1) water, to provide a "blank" for comparison to other solutions, 2) <50 mM NaCl concentration in water, to better determine how salt affects decanoic acid by screening; in particular, to determine where the salt screening effect saturates, 3) acidic and basic pH water, with and without buffer present, to determine if buffer or pH alone changes monolayer stability, 4) if possible, several different buffers to determine if the buffer type affects fatty acid behavior, 5) varying concentrations of divalent salts, which have been shown to particularly affect vesicle stability.[4] Though ionic strength is a bulk property and may not correlate to surface chemistry, correlating phase behaviors with ionic strength, rather than just pH, in a phase diagram of decanoic acid aggregates could better account for the effects of salt often added in to vesicle 'recipes.'

Adsorption isotherms should also be collected at a variety of decanoic acid concentrations in order to determine the surface excess concentration (see Equation 6), though the possibility of low, unknown CACs must be kept in mind. Determination of surface excesses would permit the quantitative correlation of surface concentrations to the concentration of fatty acid molecules in lipid membranes. The determination of a surface concentration which correlates to the concentration of fatty acids in a membrane would allow a more specific phase diagram to be produced, and the quantification of the film's stability via trough studies.

Given the variety of environments in which life may have formed, it would also be worth designing further experiments for conditions mimicking hydrothermal vent systems[30] or

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[30] Baross, J.A., Hoffman, S.E., 1985. Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life. Origins Life Evol Biosphere 15, 327–345. doi:10.1007/BF01808177

terrestrial travertine springs[31] in order to determine fatty acid behavior in other early earth environments. Monolayers on bulk solutions to match these environments, as well as mixtures of fatty acids on the same solutions, could be investigated to match empirical vesicle stability studies.[32]

Conclusions

The π-A compression isotherms of decanoic acid on water showed one phase transition as the molecules at the surface changed packing during compression from a less-condensed to more-condensed phase, but these isotherms were not reproducible enough to show the small changes in shape from altering the salinity and pH of the bulk solution. The solubility of decanoic acid in water was then shown to be different than the literature value; this solubility would not permit the quantitative application of π-A compression isotherms, though decanoic acid is still insoluble enough to complicate the use of adsorption isotherms due to the possibility of aggregate formation at low concentrations. This required a different approach to be used. Consequently, π-t absorption isotherms were carried out. These isotherms, while not reproducible enough to differentiate small changes associated with different bulk solutions, showed that the larger effects of 50 mM and 500 mM salt solutions on decanoic acid were not statistically different. On the other hand, when deposited on the buffer/salt solution used to make vesicles, decanoic acid was shown to equilibrate with the bulk faster than it did for either salt solution. Additionally, a trend can tentatively be seen in the equilibration times of all solutions tested: as the ionic strength of the bulk increases, the equilibration speed increases. At similar ionic strengths, it appears that high-pH solutions cause decanoic acid to equilibrate faster.

Further experiments of both adsorption and absorption isotherms would need to be carried out in order to accomplish the goal of quantifying decanoic acid's phase behavior. Adsorption isotherms could be used to determine the surface excess concentration of decanoic acid, while absorption isotherms could be carried out on a variety of bulk solutions in order to determine how phase behavior might change in different solutions. Both pH and salt warrant further investigation.

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[31] Sleep, N.H., Bird, D.K., Pope, E.C., 2011. Serpentinite and the dawn of life. Philos. Trans. R. Soc. B-Biol. Sci. 366, 2857–2869. doi:10.1098/rstb.2011.0129

[32] Namani, T., Deamer, D.W., 2008. Stability of Model Membranes in Extreme Environments. Orig Life Evol Biosph 38, 329–341. doi:10.1007/s11084-008-9131-8