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Particle Size and Frequency Dependent Folding in Langmuir Monolayers

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Particle-Size and Frequency Dependent Folding Reversibility in Langmuir Monolayers

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY
in Physics

by

Jeremy Martin Eaton

Dissertation Committee:
Professor Michael Dennin, Chair
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2016
DEDICATION

To my parents, John and Joyce Eaton who have encouraged and supported me through the entirety of my academic endeavors.
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ABSTRACT OF THE DISSERTATION

Particle-Size and Frequency Dependent Folding Reversibility in Langmuir Monolayers

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Professor Michael Dennin, Chair

Langmuir monolayers, single molecule-thick rafts of surfactant molecules at the air-water interface, are of great interest due to their similarity to the surfactant system present in the lung. These monolayers are compressed and expanded between two Teflon barriers in order to change the surface pressure of the system. The effects of reduced subphase depth, particle size, and compression frequency on the folding dynamics of Survanta and SDS-DODAB monolayers are observed. A monolayer of Survanta, a bovine lung surfactant is deposited on the surface of an aqueous buffer solution to test the effects of depth on the system. The depth is altered by placing a polydimethylsiloxane substrate at the bottom of the monolayer trough. The presence of this substrate is found to shift surface pressure-area isotherms toward regions of lower area by an average value of 8.9 mN/m. Fluorescent polystyrene micro- and nanoparticles are used to test the effects of particle size on SDS-DODAB monolayers. A phase diagram was generated showing the reversibility behavior of the monolayer under various conditions of compression speed and particle size. Particle diameters of 100 and 500 nm were generally observed to result in irreversible folding behavior at sufficiently low barrier speeds. At barrier speeds of 90 cm$^2$/min, all particle sizes were observed to transition to reversible folding.
Chapter 1

Introduction and Theory

1.1 The Alveoli and Lung Surfactant

Biology often finds very clever solutions to challenging engineering problems. One example of this can be found at the air-water interface in the alveoli of the lung. This interface must allow for the exchange of gases and needs to be able to repeatedly expand and contract in a consistent manner to maintain its function. As an engineering problem, this dynamically changing system must find a way to overcome the high surface tension present at the air-water interface. A key component of the solution to this problem is the behavior of a monolayer of organic material at the interface. To better understand the specific role and functionality of this monolayer, we first consider the general mechanism by which respiration in occurs.

Respiration in mammals involves the exchange of oxygen and carbon dioxide gases in the lungs. Inhaled air first passes down into the trachea, then into the branching paths of the bronchioles and finally ends up in organs known as alveoli. These sac-like structures act as the surface through which gases are exchanged to and from the bloodstream. When the alveoli become inflated with air, they expand and increase the available surface area through
which gases can diffuse.

Alveoli are composed of two types of cells: type I and type II cells. Type I cells are smaller in size and mainly play a structural role, making up most of the organ. Type II cells, however, have a more specialized role in making pulmonary surfactant. These cells secrete a mixture of lipids and proteins which forms a Langmuir monolayer at the interface between the hollow air cavity within the alveolus and the aqueous fluid in the epithelial tissue below. This surfactant layer lowers of surface tension of the aqueous fluid and thus reduces the total work required to expand and contract the lung during respiration.[15, 34]

The ease of breathing due to the reduced energy cost supplied by the surfactant monolayer in the lungs is heavily dependent on how completely it covers the surface of the alveoli. Reduced surfactant coverage is a major symptom of acute respiratory distress syndrome in adults and its more common analogue in infants and young children.[19] The alveolar surfactant has been observed to collapse into giant folds as the lung contracts during exhalation and therefore reduces coverage. However, these folds typically undo themselves during re-expansion, returning the coverage to its original state. This sort of process is deemed reversible. On the other hand, if an outside factor interferes and prevents these folds from coming apart, the folding is then irreversible and the net surfactant coverage is reduced. It is thus of interest

Figure 1.1: Cross-sectional schematic diagram of a single alveolus.
to study these folding and unfolding processes in greater detail in order to better understand the physical causes of surfactant deficiencies and how they could be treated.

The key goal this thesis will be to more deeply understand the dynamics of the alveolar surfactant system. In particular, we seek to address the question: what criteria are required to transition a surfactant monolayer from folding reversibly to folding irreversibly? Tiny aerosol particles may be inhaled during respiration and adsorb onto the surface area provided by the alveolar surfactant. As the alveoli inflate and deflate, these particles can be encompassed in folds that form in the monolayer as the surface pressure changes. Here, the particles can affect the properties of these folds and potentially damage the surfactant layer. By studying the interactions between a thin membrane of soft material and particulates, we not only explore the fundamental behavior of this system, but also gain insight on the potential impact of airborne pollutants on respiratory health.

1.2 Soft Condensed Matter

Soft matter is a term used to describe materials that are neither purely solid nor purely liquid. These materials have relevance in many technologies and also occur in a wide range of biological systems. The surfactant monolayer in the lung is just one example from this broad class of materials. Another example of soft matter includes polymer solutions which have been heated beyond their melting point.[38] These polymer melts exist as a single fluid phase in contrast to other types of soft matter which are composed as a mixture of several phases. These multi-phase materials include foams (liquid and gas), many emulsions (liquid and liquid), and granular materials such as sand (discrete solids in gas). Due to the general ability for soft materials to maintain structure while also offering insulation and flexibility, these materials have numerous applications in packaging, culinary techniques and adhesives.[12] Furthermore, soft materials that respond strongly in the presence of electromagnetic fields
such as liquid crystals have found use in the production of television, computer and other electronic screen displays.[22]

Soft condensed matter physics is a branch of materials science which studies the thermal, electrical and mechanical response of soft matter. Due to their unique mechanical properties and important applications, there is significant interest in studying the fundamental behavior of these materials. There is a long history of characterizing these materials - especially in engineering applications - but almost all of the known relationships are purely empirical. A unified description of the mechanical behavior of soft matter is still an open question. A basic issue in any mechanical description is the relationship between stress, strain and rate of strain. We will consider each of these quantities and their role in classifying these materials.

Stress is a measure of the force per unit area that can be applied perpendicular to the surface of a material or in a shear fashion along the plane of the surface. The change in shape due to a stress can be quantified as a strain, which is the amount of deformation some parcel of material has undergone relative to the material’s original configuration.[10] The rate of strain is defined as the rate at which a material changes deforms under stress with respect to time. Whereas conventional solids exhibit shear stress proportional to the strain, and ideal fluids to the rate at which that strain changes over time, materials classified as soft matter display a much wider range of responses and behaviors. A number of interesting stress-strain relations have been used to categorize complex fluids, or soft matter systems. These systems possess mechanical responses that differ from that of the classical Newtonian fluids which possess a linear relationship between applied stress and rate of strain.[20] We will now briefly survey some of the important classifications of complex fluid materials.

The first class we will consider is known as a yield stress material. These materials possess elastic solid-like behavior until acted one by some threshold stress above which they will begin to flow.[4] A second major category is the “power law fluid”. These materials act as ideal fluids under all conditions, but are considered complex fluids because they are
Figure 1.2: Pictorial representations of stress (a) and strain (b). In (a), a force is shown acting perpendicular to the top surface of a block of material. The cross-sectional area this force acts over is highlighted in light blue. In (b), the resulting stress from the force has deformed the material, causing it to elongate in the z-direction. The red portion of the material stretches a length $\Delta L$ above the original height of the block (shown in blue), $L$. The ratio of $\Delta L$ to $L$ gives the strain in the material.
non-Newtonian. For power-law fluids, the stress is a function of the rate of strain to some non-linear power.[31] This effectively results in the material having a non-constant viscosity. Interestingly, many materials can be classified as both a yield-stress material and a power-law fluid and are known as Herschel-Bulkley fluids.[14] Another very broad class of materials are referred to as viscoelastic. This term can be given to any material that demonstrates both viscous and elastic responses under deformation. There are many different ways that this property can be realized. The responses can dependent on frequency, strain, rate of strain or can even be time-dependent.[6] The most common way to represent these materials is a time-dependent relationship between stress and strain or an equivalent frequency-dependent relationship. If the materials have a linear response, the equivalency between these representations is straightforward. In these materials, it is very common to describe their behavior in terms of a response function. While these designations are useful in categorizing the properties of soft materials, they are not mutually exclusive. For example, a yield strain material may display properties more similar to viscoelastic fluids once it begins to flow.[30]

As mentioned earlier, many of the relationships between applied stress and rate of strain for these materials have been determined empirically and thus there exists no one overarching theory to explain this wide variety of behavior. Models focusing on the microscopic scale of these fluids have had success explaining some of these behaviors. For example, the rearrangements of adjacent bubbles (known as T1 events) and the existence of unstable ‘weak zones’ are posited as some of the driving phenomena that govern a foam’s response to applied stress and strain.[18] The mechanisms behind complex fluid behavior for other materials remain open questions. For instance, the questions concerning the existence of a yield stress or why the shear stress depends on a particular power of the rate of strain remain largely unanswered.

This thesis will focus on a special class of soft materials which have a negligible thickness and can therefore be approximated as being two dimensional. Materials with this property
Figure 1.3: Graph demonstrating example relationships between shear stress and the rate of strain for three kinds of fluids. In red, is an ideal Newtonian fluid which has a rate of strain that increases linearly with shear stress. The green curve shows the rate of strain for a power law fluid which increases nonlinearly as the shear stress increases. Lastly, in blue, is the yield stress fluid which has zero rate of strain until it begins to flow like a Newtonian fluid above a threshold stress.
such as the alveolar surfactant layer tend to display viscoelastic properties.\cite{11, 27, 21, 39} These materials display many of the same behaviors seen at the microscopic level in complex bulk fluids and thus their study provides a useful direction in studying the dynamics of the lung surfactant system. These quasi-2D fluids also have the experimental advantage of being easy to image by nature of their single plane geometry. Due to these qualities as well as their interesting role in biological systems, the mechanical properties of these viscoelastic quasi-2D materials, known as Langmuir monolayers, will be the focus of this dissertation.

1.3 Langmuir Monolayers

Langmuir monolayers are structures that form when non-soluble amphiphilic molecules are deposited on the surface of an aqueous liquid. The resulting single-molecule think structure is named after Irving Langmuir, whose 1932 Nobel Prize in chemistry was awarded in part for his work in studying these systems. The molecules that form these structures can generally be describe as being composed of a hydrophilic “head” group and one or more long hydrophobic “tails”. These molecules will align such that their heads are immersed in the supporting fluid while the tails are oriented so as to reduce their contact with the subphase.

The orientation of surfactant tails in monolayers can change depending on the surface pressure at the interface in order to minimize the free energy profile of the system. \cite{9} At low pressures, there is a larger amount of area available to each molecule and the tails of each individual molecule can orient themselves freely with little to no correlation with others. This is the ‘gas phase’ of Langmuir monolayers. As the pressure increases and the area per molecule is reduced, the molecules begin to interact with one another and allow the system to transition into more highly ordered phases. Monolayers can first enter the liquid expanded phase in which individual tails of the molecules start to align with some short-range order. As the pressure reaches even higher levels, the liquid condensed phase begins
Figure 1.4: Diagrams showing the phase behavior of Langmuir monolayers under the effects of decreasing area and therefore increasing surface pressure. As the area reduces, the individual molecules become more crowded and begin to display long range alignment of the hydrophobic tails.

to dominate. This phase is characterized by the long-range ordering of tails at some angle above the subphase.[32, 35]

Langmuir monolayers are great interest to study due to their applications in both soft condensed matter and biophysics. Their unique structure and ease to assemble make them great model systems for experimentally studying phase transitions, symmetry breaking and complex fluid flow in low dimensional conditions.[36] In addition, there are many biological systems that are or are similar to Langmuir monolayers. For example, the cell membrane is a lipid bilayer that can be modeled as two weakly coupled monolayers.[29] As discussed earlier, the alveoli in the mammalian lung also are coated in a lipid and protein monolayer which plays a vital role in its proper function. Therefore, monolayers provide an excellent experimental candidate for their potential applications in physiology and biomechanics. Due to their ease of experimental control and their strong resemblance to biological membranes, we will focus on model Langmuir monolayer systems as a proxy for the dynamics present in the alveoli.
There are two key issues that monolayers allow us to explore: membrane folding and the transport of particulate matter. However, for both of these issues, we ultimately need information about the mechanical response of the material and this is the realm of rheology, the study of fluid flow. We will first examine both membrane folding and potential particle effects on the alveolar surfactant before ending this chapter with a discussion of rheology and its role in studying these issues.

1.4 Thin Membrane Folding

In addition to their rich phase behavior, monolayers also display a variety of collapse phenomena when subjected to higher surface pressure. The conditions for inducing collapse depend on both the properties of the material and the rate of compression. One type of collapse occurs when small regions of the monolayer surface are squeezed out of plane into vesicles. This mechanism for collapse is especially prevalent in biological surfactant where surface pressures can be locally enhanced between membrane proteins. Monolayers are also able to undergo buckling and giant folding [33, 2, 43] The latter mechanism involves the extrusion of the monolayer out of the plane of the interface and deep into the subphase. This process is analogous to Griffith cracking in solid materials under compression [25] In Griffith cracking, fractures form in response to a critical stress level. These fractures allow for the release of strain energy by forming two new surfaces, increasing the overall surface area and thus surface energy of the system. Monolayer folding occurs in a similar fashion: the free energy of the system increases as the average area per molecule decreases, and so the system will eventually release this strain energy by folding into the third dimension, allowing new surfaces to form.

A Langmuir monolayer can be modeled as a thin, self-adhering, elastic sheet which lies atop a fluid substrate with density $\rho$. The system can be described using the Stokes equation for
Figure 1.5: Schematic representation of collapse phenomena of a Langmuir monolayer. The left (a) shows the formation of a small vesicle or buckle in a squeeze-out event and the right (b) shows a large-scale giant fold.

an incompressible fluid ($\nabla \cdot \mathbf{v} = 0$):

$$\mu \nabla^2 \mathbf{v} - \nabla p + \rho g = 0$$ (1.1)

where $\mu$, $\mathbf{v}$ and $p$ are the dynamic viscosity, velocity and pressure of the subphase fluid and $g$ is the acceleration due to gravity. These equations are solved by imposing a no-slip boundary condition at the membrane and also the condition that the force exerted on the membrane by the fluid is equal to the fluid stress dotted with the unit normal. When compressed symmetrically at a slow rate, these equations give an exact solution for the shape of the folded membrane:[8]

$$\phi(s) = 4 \arctan\left[\frac{\kappa \sin[ks]}{\kappa \cosh[ks]}\right]$$ (1.2)
Where $\phi(s)$ the angle to the membrane from the horizontal at arclength $s$. The parameters $k$ and $\kappa$ depend on the surface pressure in the membrane, $P$, and are equal to $\frac{1}{2}\sqrt{2 + P}$ and $\frac{1}{2}\sqrt{2 - P}$ respectively.

Figure 1.6: Graphs showing the monolayer fold profile following compressions at low (left) and high (right) speeds. A z-coordinate of zero corresponds to the height of the air-water interface. The lower compression rate shows a deep fold that makes contact with itself at the center. The higher compression rate does not plunge as deep into the subphase and instead bunches up above the interface on either side of the center fold.

As the compression speed increases above a threshold of about 1 mm/s, viscous forces begin to dominate over the force of gravity. As the profile of the newly forming fold is spherical, it experiences Stokes drag which ultimately prevents the fold from sinking further into the subphase. It then follows that higher compression rates will actually decrease the maximum depth reached by the fold and can reduce the degree of self-adhesion between the two walls of the fold. Therefore, this theory predicts that folds formed at higher compression rates will likely be less stable and more likely to undo themselves during monolayer expansion than those formed at slower rates.
1.5 Lung Dynamics and Atmospheric Particles

Aerosols and particulate matter are prevalent in the atmosphere from both anthropogenic and biogenic sources. These particulates range in size from pollen grains tens of microns in diameter to ultrafine smoke and smog particles on the order of 10 nanometers. Particulates larger than about 10 microns do not generally impact the alveolar surfactant due to the major airways being lined with cilia that work to push these large particles out of the respiratory tract. Particles with sizes smaller than that limit can, however, breach this primary defense and are able to make contact with and embed themselves into the surfactant layer. Therefore, it is of interest to better understand the effects that different particle sizes may have on monolayer dynamics. Although the surface chemistry of these particles are presumed to have some effect, first examining the physical size effects alone provides a strong baseline criterion for assessing the potential harm airborne particulates may have on our health. While the effect of particulates on pulmonary surfactant has been previously studied, those experiments were performed with static monolayers or in living organisms where it is difficult to experimentally control the system.\[40, 13\]

In the lung, the alveolar surfactant is separated from the cell lining below by a layer of aqueous fluid about 0.1 $\mu$m in thickness.\[37\] As large collapse structures can reach dimensions approaching 1mm, it is expected that these folds and vesicles interact with the cell lining beneath them. It is then of interest to study the impact of a substrate within close proximity to the air-water interface on the collapse dynamics of a Langmuir monolayer. The distance of this substrate from the interface is an important factor to test to compare the dynamics of traditional monolayer experiments (which effectively have an infinite depth) to those present in the confined conditions in the alveoli. Additionally, understanding how the surface properties of this substrate affect the folds may shed light on the mechanisms by which particulate matter transgresses the surfactant barrier and ultimately find its way into the bloodstream.
Another parameter of interest is the rate at which the lung expands and contracts. The respiratory rate in humans is known to vary from about 40 breaths per minute in infants to about 15 breaths per minute in adults.[17] Furthermore, the rate of breathing can reach frequencies of up to 50 breaths per minute following periods of intense exercise. This wide spectrum of human breathing rates means that it is important to determine the role of compression frequency on the dynamics of giant folds and the particles within them in order to better make predictions about respiratory health. Through understanding this role, we can gain insight on whether people of certain ages or activity levels are at higher risk for surfactant-related respiratory disorders.

1.6 Rheology

Properties of fluids and soft materials can be determined using techniques from the field of rheology. These methods are able to determine properties of materials using their response to external stresses. Typical rheometers apply shear stress or strain to the target material and measure the resulting displacements or stresses within that material. A common quantity measured in rheology is the complex shear modulus. In a typical elastic solid, the shear modulus is the ratio of the shear stress exerted on a material to the resulting shear strain and essentially measures the ‘stiffness’ of the material. Similarly, for a Newtonian fluid, the viscosity relates the responding rate of strain to a given shear stress and thus describes the fluid’s resistance to flow.[12] For a viscoelastic material – which as described earlier, exhibits both an elastic and viscous response – the complex shear modulus captures both kinds of responses. Rheological measurements on viscoelastic materials are often focused on linear response. In this context, the frequency-dependent shear modulus is sufficient to determine the whole of a material’s behavior.[24]

Frequency dependent measurements are done in practice by operating the rheometer in an
oscillatory mode and measuring the response of the material at that frequency. A perfectly elastic material will move purely in phase with the driving mechanism, whereas a perfectly viscous fluid will have a response that is completely out of phase. From this, one can correctly intuit that the response of a viscoelastic material should lie somewhere in between these extremes. If a material is driven at an angular frequency $\omega$, the strain on that material is then:

$$\gamma = \gamma_0 \sin[\omega t]$$  \hspace{1cm} (1.3)

The stress oscillates at the same driving frequency, but may exhibit a response that is out of phase with that of the strain by some amount $\phi$:

$$\sigma = \sigma_0 \sin[\omega t + \phi]$$  \hspace{1cm} (1.4)

Using phasor analysis, we find that the strain and stress are the respective imaginary parts of $\gamma_0 e^{i\omega t}$ and $\gamma_0 e^{i\omega t}$. The shear modulus $G$ is defined as the ratio between stress and strain and using the above definitions, we are able to write:

$$G = \frac{\sigma_0}{\gamma_0} \cos[\phi] + i \frac{\sigma_0}{\gamma_0} \sin[\phi] = G' + i G''$$  \hspace{1cm} (1.5)

The real part $G'$ is the storage modulus, which describes the elastic behavior of the material and is analogous to the shear modulus of a perfect elastic solid. The imaginary part $G''$ is
called the loss modulus and describes the viscous response. A viscosity of the fluid can then be extracted from the loss modulus. Given that we have an oscillatory shear, $\gamma_0 e^{i\omega t}$, its time derivative is then $\dot{\gamma} = i\omega \gamma$. Using the relationship between stress and the rate of strain, we can thus write:

$$\sigma = \gamma G = \gamma G' + iG'' = \gamma G' + i\omega \gamma \eta$$

From this relation, we ultimately deduce that $G''$ is directly proportional to the material’s viscosity by $G'' = \eta \omega$.

The above results are valid when the oscillatory shear is applied with small amplitude. Larger amplitude measurements such as in the case of steady shear can apply strain which takes the material from a linear to a non-linear regime. In steady shear experiments, the fluid is subjected to a series of constant strain rates. The result is that the system experiences nonlinear behavior as the rate of strain increases steadily over the duration of the entire experiment.[7, 42] This non-linear change in response is similar to the yield stress materials which behave elastically under small applied stress, but begin to flow as a fluid under sufficiently large stresses. This sort of transition may be important ultimately for studying Langmuir monolayer dynamics as the distortions that occur during folding are quite large and may therefore be best explained using the non-linear viscoelastic regime.

Modern advancements in microscopy and computing power have allowed for the development of new rheological techniques that utilize the motion of microscopic tracer particles in order to measure the response of complex fluids on very small scales. These techniques constitute the field of microrheology which has many advantages over typical fluid flow techniques.[5, 28] For one, microrheological techniques are able to discern inhomogeneities within a fluid ma-
material at the scale of the particle size used. In addition, the smaller probes used allow for rheological measurements to be performed on smaller sample volumes. Lastly, microrheology is able to be performed on fragile materials such as biological tissues and lipid monolayers without greatly disturbing their structure. It is due to these advantages that microrheological techniques are ideal for studying and better understanding the properties of thin film materials.

The remainder of this thesis details experiments that were designed and performed in order to answer the above questions concerning membrane folding in the context of the alveolar surfactant. In Chapter 2, we describe a number of important materials and experimental techniques involved in carrying out these experiments. Chapter 3 outlines procedures for performing experiments to test the effect of reduced depth on monolayer dynamics and presents data showing the potential impacts of this geometry. Chapter 4 presents data and results showing the effects of particle size and compression frequency on the reversibility of monolayer folds. Chapter 5 discusses plans to use laser tweezers microrheology to further study monolayer folding and unfolding dynamics. It also discusses changes made to our lab’s tweezer apparatus and as well as procedures relevant to the new system. Lastly, Chapter 6 details conclusions and a final summary of the observed results.
Chapter 2

Experimental Techniques

In order to determine how particle size and frequency effects play a role in the folding dynamics of Langmuir monolayers, several specialized techniques and materials were used. A typical monolayer experiment first involves a thorough cleaning of a monolayer trough. The trough is then filled with an aqueous fluid which serves as the subphase on which the monolayer will rest. The monolayer solution is deposited at the air-water interface between two barriers which enclose the surface. These barriers can expand and contract in order to change the area available to the monolayer. The surface pressure of the system is constantly monitored and images of the surface are captured. This section will describe in more detail the technical specifications of the materials used in this study as well as the techniques utilized to carry out these experiments.

2.1 SDS-DODAB Monolayer

Monolayers are formed from an equimolar solution of sodium dodecyl sulfate (SDS) and dioctadecyldimethylammonium bromide (DODAB). Both types of surfactant are ionic; the
former has a negatively charged head group while the latter possesses a net positive charge. Together, this combination makes for an overall electrically neutral monolayer that is known for its robust folding at higher surface pressures. To prepare solutions of these lipids, the SDS is dissolved in ethanol in order to obtain a molarity of 1.2 mM, and the DODAB is dissolved in chloroform at the same concentration. Equal volumes of each solution are mixed and then further diluted with chloroform to reach a final molarity of 0.12mM for each molecule. Monolayers of the surfactant are formed by depositing 150 µL of the prepared bi-component solution at the surface of an air-water interface using a syringe. The drops of the SDS-DODAB monolayer are placed evenly over the whole surface to ensure a homogeneous distribution between two Teflon barriers. The system then rests for roughly 15 minutes to allow the monolayer to reach equilibrium before any compressions are started.

2.2 Fluorescent Particles

To model the effects of particulate matter on folding surfactant membranes, spherical fluorescent polystyrene particles are deposited onto the monolayer surface. These beads, purchased from Invitrogen, are modified with a fluorescent carboxylate group most efficiently absorb light with wavelengths near 580 nm and emit at 605 nm. The particles range in diameter from 20 nm to our largest size at 1 micron. All stock bead solutions are diluted in ultrapure water at a ratio of 100:1 except for the 20 nm particles. These smallest particles have a tendency to oversaturate images taken by the CCD camera and therefore were diluted further to a concentration of 150:1. Before experimental use, the particle solutions are sonicated for about 15 minutes to prevent large aggregations of particles from being introduced into the samples. A sample consisting of 50 µL of the sonicated solution is evenly deposited over the monolayer interface dropwise using a syringe in order to maintain a uniform distribution of particles over the entire surface.
2.3 Langmuir Trough

Experiments are performed in a Teflon basin known as a Langmuir-Blodgett trough. This trough is filled with 60 mL of ultrapure water with a resistivity of 18.2 MΩ·cm such that the fluid has a slightly positive curvature above the lip of the basin. The trough is further equipped with two motorized barriers also made of Teflon which serve to compress or expand the system to a minimum area of 22 cm² and a maximum area of 78 cm². The barriers move such that they can change the area of the surface at constant rates between 5 cm²/min and 90 cm²/min during both expansion and compression.

Figure 2.1: Birds-eye view of the monolayer trough apparatus. The yellow region is the area which is occupied by the surfactant monolayer and can be compressed and expanded between two motorized Teflon barriers. The grey region is the location of a force transducer attached to a Wilhelmy plate that will measure the surface pressure of the system.

The barriers and the trough itself are thoroughly cleaned before each experiment using a five step cleaning procedure. First, the barriers are separated and manually expanded to the far ends of the trough. The trough is then filled with ultrapure water dispensed from a Millipore Synergy filtration device. Once the trough is full, the barriers are brought together...
to confine any floating debris or surfactant and then the surface and bulk fluid are aspirated away. When the water has dried, a small amount of ethanol is poured into the trough and a Kimwipe held in place with forceps is used to scrub the surfaces of the barriers and trough. Extra care should be given to clean the lens at the bottom of the trough and the undersides of the barriers as contaminants can more readily accumulate in those spots. The alcohol dries after about 5 minutes and this process is repeated with chloroform. As chloroform is especially volatile, there is no need to wait between the next swabbing step which is again performed with alcohol. After the second alcohol step, a final swab is performed again using the Millipore water.

This five-step cleaning cycle should be repeated multiple times if the trough is suspected to be contaminated with an excess amount of oil or surfactant. If surface pressure measurements of pure water in the trough reveal an increase in surface pressure of more than 0.5 mN/m after multiple clean cycles, then the trough should be scrubbed thoroughly with detergent under warm running tap water for 15 minutes. Then, this procedure should be repeated using de-ionized water. After being sufficiently scrubbed and rinsed with the detergent, the five-step cycle should be performed and then trough should be suitable for use.

2.4 Tensiometry

In order to probe the surface pressure of the monolayer system, a Wilhelmy plate apparatus is employed.[26] This device is simply a small paper card with a width of 1 centimeter. The card is hung from the end of a force transducer and its other end is allowed to be submerged in the subphase just below the air-water interface.

The forces on the card are the downward force of the card’s weight, the buoyant force of the subphase pushing up on the card, and the force of surface tension at the air-water interface.
Using the zero function of the force transducer after the card has become completely soaked with the subphase eliminates the effects of buoyancy and weight, and thus the transducer can be prepped to measure only the surface tension. The buoyant force does change slightly as water evaporates from the trough and the card becomes less submerged. However, the time scale of this process is much longer than that of the experiment duration and therefore this effect is negligible. The total measured force on the card is then:

\[ F = 2(w + t)\gamma \cos[\theta] \]  

Where \( w \) is the width of the card, \( t \) is the card’s thickness, \( \gamma \) is the surface tension and \( \theta \) is the contact angle of that the fluid makes with respect to the plate. The paper card is able to soak up the subphase which sets the contact angle to zero. In the limit where the thickness of the card is much smaller than the card’s width, the measured surface tension is thus approximately:

\[ \gamma = \frac{F}{2w} \]  

The measured surface tension can be finally converted to the surface pressure using the following definition:

\[ \Pi = \gamma_0 - \gamma \]
Here $\gamma_0$ is the surface tension of pure water (72 mN/m at room temperature) and $\gamma$ is the surface tension of the monolayer system. The surface pressure is the correct thermodynamic pressure in two dimensions and therefore is a key parameter for characterizing phase transitions. By monitoring the surface pressure, we are able to generate isotherms that show how this pressure changes as a function of the trough area. These isotherms are useful in discerning the pressures under which phase transitions or collapse phenomena occur as well as keeping track of the general status of the monolayer.

Figure 2.2: Sample surface pressure-area isotherm for an SDS-DODAB monolayer with no embedded particles over the course of several cycles. The curves on the top correspond to the pressures during compression, whereas the curves on the bottom represent expansion pressures. The plateau at the very top of the compression signifies the presence of a collapse event.
2.5 Fluorescence Microscopy

An Olympus BX60M microscope is used to closely and directly observe the monolayer surface and provide insight to the nature of the folding dynamics. The microscope is interfaced with an ORCA-05G digital CCD camera manufactured by Hamamatsu which captures images and video of the surfactant system from above. The camera has a pixel resolution of 1344x1024 and can capture nine frames per second while taking video. The microscope is fitted with variable objective lenses enabling magnification of 4x, 10x, 20 or 50x in addition to the 10x eyepiece.

The microscope is furthermore fitted with two filters: a wide-band filter at 450-480 nm and a green-yellow filter (535 nm). These filters are used to induce emission from the fluorescent components of the particles or tagged monolayer respectively. This differential fluorescence allows for the distinct identification of the positions and orientations of both the particles and the surfactant in captured images and videos. Images and video of the folding monolayer are taken near the center of the through to avoid capturing features that arise due to interactions with the barriers.
Figure 2.3: Schematic diagram showing the components and structure of the fluorescence microscope system fitted with the Langmuir trough.
Chapter 3

The Effects of Depth on Monolayer Folding

Experiments on Langmuir monolayers are typically performed in a basin where the depth of the subphase is far larger than any of the relevant length scales seen in monolayer collapse phenomena. Experiments of this type can effectively be treated as if the monolayer were deposited atop a subphase that extends an “infinite depth” below the air-water interface. This is in contrast to the environments found in the surfactant systems in the alveoli where the depth of the subphase is less than 1 micron. It is expected that giant folds may interact with a substrate in two main ways. The first of these interactions is direct: the fold may physically touch the surface below resulting in its rupture or contortion due to this contact. Chemical properties of the substrate and/or the monolayer itself may further allow the fold to bind to the surface below. The second kind of action is more indirect and arises due to the new slip conditions enforced by the presence of the surface. This boundary may create new flow patterns within the subphase which in turn may exert different stresses on the monolayer that it would not experience in the large depth limit.
3.1 Depth Effects – Preliminary Tests

To test the effects of a reduced depth, small platforms were first crafted from polydimethylsiloxane (PDMS) in order to effectively decrease the distance between the air-water interface and the bottom of the trough. Polydimethyl siloxane (PDMS) is a clear, flexible polymer material that is used in these experiments due to its ability to be readily molded into desired forms as well as for its chemical inertness. PDMS is formed by mixing a silicone elastomer base (purchased from Sylgard) with its curing agent at 1:10 mass ratio. These reagents are mixed in a Petri dish of known area so that a desired thickness can be achieved. As the elastomer base has a density of 1.11 g/cm$^2$ and the curing agent has a density of 1.03 g/cm$^2$, the amount of elastomer base needed for a specific thickness $t$ of PDMS is as follows:

$$M_{base} = 1.002At$$ \hspace{1cm} (3.1)

where $A$ is the area of the Petri dish. The two reagents are then thoroughly stirred and the mixture is allowed to sit for about 30 minutes so that any air bubbles that formed during mixing can escape. Once the PDMS has sufficiently degassed, it is placed in a 60°C oven for 24 hours so that it can cure. The cured sample can then be cut into a desired shape using a knife and removed from the Petri dish for later use. The dimensions of these platforms are slightly smaller than the dimensions of the trough when the barriers are closed to their minimum area. This allows for the highest probability of the substrate interacting with a fold while simultaneously preventing any interference with the moving barriers.

Preliminary tests were performed by molding a PDMS substrate with a thickness of 4 mm. A glass coverslip was carefully placed on the surface of the PDMS before curing. This glass slide was treated in a solution of Nochromix and sulfuric acid in order to increase the overall
wetting of the slide, making it less hydrophobic. The substrate is thoroughly cleaned using the same procedure used to prepare the Teflon monolayer trough before each experiment. When the substrate is placed in the trough and filled with water, there is a gap between the glass surface and air-water interface of less than 1 mm. A monolayer of Survanta, a bovine lung surfactant is deposited onto the surface of the water and the barriers were compressed to an area of 50 cm². The system is then allowed to sit for one hour and the surface pressure is constantly measured using the Wilhelmy plate. The experiment is then repeated for the case in which no substrate is present in the trough.

Figure 3.1: Graphs showing the surface pressure over a roughly 1 hour time period for a Survanta system allowed to sit at an area of 50 cm². The left plot shows an increase in surface pressure with no substrate present while the plot at right shows a decrease over the same time period when a PDMS substrate is placed in the trough.

The experiment without a PDMS substrate shows results consistent with water evaporating from the trough over time. As water leaves the trough, the depth of the system decreases and the curvature of the surface changes from positive to negative. This causes the Survanta monolayer to pool more in the center of the trough near the Wilhelmy plate, increasing its average density and therefore causing an increase in surface pressure with respect to time. Conversely, the system with the PDMS substrate showed an average decrease in surface pressure over the same time period. At the low pressures tested, it is unlikely that the monolayer has collapsed and thus there should be no folds or other deep-reaching features interacting directly with the substrate. It is possible that the hydrophobic surface properties
of the PDMS work to push the subphase away from the center of the trough and thus causes the monolayer at the surface to be directed more towards the barriers. This would decrease the average density near the Wilhelmy plate and thus explain the observed increase in surface pressure. These results support the idea that the reduced depth of the system may indirectly affect the monolayer system.

An experiment designed to test the effect of a more dynamic monolayer system was also done using the same PDMS substrate. Here, a Survanta monolayer was deposited at the air-water interface and six full compression-expansion cycles were performed using a barrier speed of 20 cm$^2$/min. These experiments were again repeated for the same system but without the presence of the substrate.

![Figure 3.2: Plots showing the surface pressure-area isotherms over the course of six expansion-compression cycles. The isotherms with a PDMS substrate present (right) show a significant shift toward lower area in comparison to the isotherms with a substrate (left). The circled areas highlight the plateaus corresponding to the squeeze-out of surfactant material during compression.](image)

In typical large-depth isotherms with Survanta, two prominent plateaus are observed: one corresponding to squeeze-out and a second one at higher surface pressures corresponding to collapse into giant folds. The isotherms for the reduced depth experiment shows the first plateau shifted toward a region of lower area and the second plateau is completely absent. Such a shift can arise due to the loss of surfactant material due to an interaction
with the substrate below. Furthermore, the lower maximum surface pressure reached in the low depth experiments is consistent with this material loss. However, structures that form during squeeze-out are usually not of the lengths scales required to make close contact with the substrate, so the precise reason for this shift is currently unknown.

This shift was measured qualitatively using a program in MATLAB which separates each compression and expansion stroke of the isotherm cycle into individual datasets. These datasets were then imported into Mathematica where a second program would measure the local slope around each point, giving the derivative of each isotherm cycle. The portions of these new plots with zero slope clearly mark the plateau regions of the isotherm and therefore the lower area at which these regions begin can be easily extracted. This method determined that the system with the PDMS substrate experienced an average shift in the squeeze-out plateau of 8.9 mN/m toward lower area than the system with no substrate.

![Image](No Substrate Present) ![Image](Substrate Present)

Figure 3.3: Plots showing the change in surface pressure as a function of trough area. The pronounced depression in each graph corresponds to the low rate of change characteristic of the squeeze-out plateau in the surface pressure-area isotherms.

### 3.2 Exploring the Depth Transition

The experiments described in the previous section provide evidence that reducing the depth of the subphase can affect the properties of the monolayer system. As those experiments only
explored the effects of a single depth, it is of further interest to understand the transition between the “infinite depth” limit and the reduced depth limit already tested. To accomplish this, three small blocks were crafted from Teflon of the same width and length dimensions as the PDMS substrate. Teflon was used in this set of experiments over the previous PDMS in order to match the material composition of the trough. These blocks had heights of 2, 3 and 3.5 mm in order to vary the depth of the system.

Experiments involved placing each of these blocks at the center of the trough and then filling the system with an amount of water equal to 60 milliliters minus the volume of each of the three Teflon samples. Care must be taken to ensure that the water is able to totally cover the Teflon substrate as its high hydrophobicity will tend to have any fluid spill off into the surrounding fluid. It is often useful to overfill the trough with a known amount of fluid and then use a pipet to remove water from outside of the barriers until the desired amount is reached. An SDS-DODAB monolayer is then deposited onto the resulting air-water interface and several isotherm cycles are run. This process is repeated for each of the three Teflon blocks and for the case with no block present.

These experiments using the Teflon blocks originally revealed a feature present in the compression isotherm that was not previously observed in earlier experiments using SDS-DODAB. Typically, the SDS-DODAB compression isotherm features a rapid rise in surface pressure followed by a large plateau as the monolayer begins to collapse. When the Teflon blocks were present in the trough, a new slope regime was observed between these two signature features. Furthermore, the experiments involving the Teflon blocks were characterized by a distinctive kink near the beginning of the expansion cycle. These new features suggest that the block’s presence alters the surface pressure of the system.

However, further experiments showed that these results were not reproducible and even manifested when no substrate was present suggesting that the change in depth was not responsible for this effect. It is possible that these Teflon blocks introduced a number of
contaminants into the trough and that their presence was responsible for showing this effect. These contaminants may have persisted even after thorough cleaning procedures and could therefore even produce this isotherm feature in cases with no reduced depth. This experiment could be improved by developing Teflon inserts that fill the entirety of the trough rather than just a small region at the center. In this revised setup, the fluid would fill the trough from the bottom up and the added obstacle of keeping the water atop a hydrophobic pedestal would be eliminated.

3.3 Surface Effects – Cell Culturing

The Teflon and glass slip provide a hard, rigid surface with which folds may potentially interact. However, it is more relevant to test these effects in conditions that more closely parallel those found within the alveoli. In addition, it is also of interest to study the effects that surfaces with different mechanical and chemical properties. To accomplish both of these goals, a procedure for culturing a layer of alveolar cells for use in a monolayer trough was developed.
Though the cells have not been extensively tested in our monolayer trough apparatus, the following section details this procedure for future culturing and experimentation.

These cells come from the A549 line, an alveolar cell derived from carcinoma tissue. These particular cells are used due to their high survival rate and for their actual proximity to the alveolar surfactant system in vivo. In order to increase the frequency at which these experiments can be performed, it is useful to culture and stockpile a large number of cells which can be stored indefinitely. To accomplish this, one must first thaw a frozen seed solution in a 37°C water bath. Once thawed, the solution is placed into a T75 plastic culturing flash with 10 mL of complete media, a solution containing all of the necessary nutrients for the cells to grow. The flask is next placed into an incubator set to 37°C where it rests for about 48 hours. After this time, the media is removed from the flask and the resulting culture can be gently rinsed with 3 mL of Delbecco’s Phosphate Buffer Solution (DPBS). This rinse should be repeated three times to ensure that all of the media has been removed from the flask. Once sufficiently rinsed, the cells are now ready to be removed from the surface of flask for further use; a process known as lifting.

To lift the cells from their surface, 3 mL of the digestive enzyme, trypsin are added to the flask. Once the trypsin solution is evenly distributed over the cell surface, the flask is placed back into the incubator where it rests for three minutes. It is important not to overexpose the cells to trypsin as this enzyme may damage the cell membrane over longer periods of time. After this brief incubation period, 3 mL of media are added to the flask in order to neutralize the trypsin. The cells should now free-floating in the solution and more can be dislodged from the growth surface by gently tapping the flask onto a hard surface. This cell solution is then transferred to a small vial and then centrifuged at 1000 rpm for 5 minutes. The fluid above the small pellet of cells at the bottom of the vial is aspirated away and 1 mL of media is then added. The cells can be more rapidly resuspended into this solution by repeatedly aerating it using a micropipettor. Once this solution is sufficiently mixed, it can
be directly used in an experiment or stored in a container for freezing for further use.

In order to freeze cells for further use, the number of healthy cells in the solution needs to first be determined. Ten µL of the cell solution are extracted using a micropipettor and then mixed with an equal volume of the dye, Trypan Blue. Ten µL of the dyed solution is transferred to a special slide and then inserted into the Cell Countess counting device. The total number of cells is then the readout number on the Countess multiplied by the total volume of suspension solution to be stored. The cell suspension is frozen in specialized cryovials designed to each hold 1 million cells. The cell solution forms roughly 80 percent of the total volume of solution to be added to these vials. The remaining 20 percent is half dimethyl sulfoxide (DMSO) and half fetal bovine serum (FBS). DMSO is a compound that helps to prevent the cell membranes from rupturing due to the formation of ice crystals. FBS is a mixture of vital proteins and sugars needed to ensure the health of the cells. Once the cryoprotectant solution is well-mixed, the vials are placed in an ethanol bath and frozen down to -80 C for about six hours. After this time, the cells are ready to be placed into a liquid nitrogen container where they can be stored indefinitely for future use.

Cells used for the monolayer trough experiments are not grown in a flask, but are instead cultured on a specially treated glass slide affixed to a small block of PDMS. This glass slide is coated in 250 µL of solution composed of a 4:1 ratio of DPBS and fibronectin protein. This protein helps to provide a rough surface to which the cells can bind, similar in texture to that found on the growth surface of the special culturing flasks. The protein mixture is then allowed to incubate for about 45 minutes at room temperature. The coated PDMS substrate is placed into a recess in a Teflon block which acts as a caddy for the sample. This setup ensures that the added cell solution will only cover the desired growth region of the coverslip. Frozen cells are taken from storage and thawed using the procedure above. However, once the cells are mixed with complete media, they are deposited directly onto the glass coverslip and then the Teflon caddy is added to a Petri dish which is finally placed
into the incubator. After this incubation process, the cells are transported to the lab and excess media is removed. The PDMS substrate can then placed directly in the center of the monolayer trough. The trough can be filled with water as normal, but is warmed to constant temperature using a Fischer Scientific Isotemp 1016S device in order to minimize cold shock of the cells.
Chapter 4

Reversibility of Monolayer Folds

4.1 Reversibility Experiments

In addition to examining the effects that surfaces have on the dynamics of a folding surfactant monolayer, it is also of great interest to understand how these folds interact with particulate matter of various sizes. Experiments were designed and performed to study this particle size effect while also examining the role played by the rate of barrier expansion and compression. The primary goal of these tests is to determine what values of particle size and barrier rate result in the formation of folds that are reversible after completely expanding the system to maximum area. This analysis will provide insight to the conditions that may result in reduced surfactant coverage in the lung and thus potentially elucidate the factors which cause some chronic respiratory disorders.

First, the trough is filled with water and a Wilhelmy plate is allowed to soak in the subphase in order to measure the surface pressure of the system at all times. Next, an SDS-DODAB monolayer is deposited onto the surface of the air-water interface in the monolayer trough. After the monolayer has come to equilibrium, 50 µL of solution containing the fluorescent...
particles was added to the surface using a syringe. Particle solutions were monodisperse and particle diameters of 20, 100, 500 and 1000 nm were used. After about 15 minutes, the particles will have sufficiently diffused throughout the monolayer surface and the monolayer is compressed to minimum area. When it reaches this limit, the motion of the barriers is stopped so that the location of a fold is found using the microscope. Folds should be located near the centermost regions of the surface to eliminate features that would be formed due to interactions with the boundaries of the system. Often a fold can be seen with the naked eye as a bright line at the surface of the air-water interface. However, if these features cannot be located, once should carefully adjust the microscope stage near the center of the trough to scan for folds. A fold under the microscope should appear as a bright red vertical line that spans at least the entire field of view.

Figure 4.1: Image of an SDS-DODAB monolayer surface embedded with 100nm particles showing two folds. The bright stripes are regions where fluorescent particles have pushed below the surface within giant folds in high density.
Once a fold is located and its image is focused in the CCD camera software, the isotherm process is initiated. Several expansion-compression cycles are performed at the same barrier speed as the initial compression. Experiments were done using barrier speeds that changed the area of the trough at rates of 5, 20, 40, 60 and 90 \( cm^2/\text{min} \). This translates into a linear speed for each barrier of 0.06, 0.24, 0.48, 0.72, and 1.08 mm/sec respectively. Video is captured during the entire isotherm cycle process in order to observe the formation and potential reversal of giant folds.

We have deemed an expansion cycle as being “irreversible” when upon fully expanded the surface, there exists one or more fold remnants larger than about 8 microns in any dimension. This size was chosen as it represents the average size of a 1 \( \mu \text{m} \) diameter bead undergoing fluorescence in the view of the CCD camera. This helps to visually distinguish the fold remnants as being distinct from the largest features from single particles that we can observe. A “reversible” folding event is conversely defined as having no fold remnants larger than 8 microns upon a complete expansion.

### 4.2 Surface Images

From the videos taken in each trial, we were able to characterize each set of parameters as being indicative of either reversible or irreversible folding. As the barriers expanded, the folds would begin to come apart either completely returning the surface to an unfolded state or leaving many visible fold remnants in the form of vesicles or other collapse structures. By capturing images from the videos at times during maximum compression and expansion we can determine the degree of reversibility after each cycle. The images below give a broad survey of the phase space tested and compare a folded region of the monolayer surface just before expansion and immediately before the next compression stroke.
Figure 4.2: Images showing the folded monolayer surface interspersed with 1 micron sized particles at minimum and maximum area. Folds were seen to be reversed when expanded at all speeds tested.

Figure 4.3: Images showing the folded monolayer surface interspersed with 500 nm sized particles at minimum and maximum area. The red circles for the surface at 80 cm$^2$ highlight the location of irreversible fold remnants.
These images reveal a rich phase space of reversibility for the test parameters. The 1 micron-sized particles, the largest tested, displayed robust reversible folding for all barrier speeds tested. This reversibility transitioned into a regime of irreversible folding as the particles became smaller. The 500 nm particles resulted in irreversible folding at all speeds save for the very highest speed tested at 90 cm²/min. A similar trend was observed for the 100 nm particles which showed irreversible folding for the three lowest speeds and then gave way to reversible folding once the barrier speed increased to higher rates. The smallest particles with diameters of 20nm, however, showed a preference for reversible folding. Folds were observed to be reversible with these particles at all barrier speeds except at 20 cm²/min. These results are summarized in the phase diagram presented in Figure 4-5 - here ‘X’ marks the occurrence of irreversible folding events whereas ‘O’ marks the occurrence of reversible folding.
Figure 4.5: Phase diagram showing the reversibility of monolayer folding over the parameter space of particle radius and barrier speed. The green and red regions show the approximate phase space for reversible and irreversible folding respectively. The red vertical line marks the predicted critical barrier speed at which the fold no longer makes self-contact with itself.
4.3 Surface Pressure-Area Isotherms

In addition to the video and images captured of the monolayer surface, surface pressure data was continuously collected throughout the duration of each experiment. The resulting isotherms reveal robust differences in the shapes of these curves for different combinations of barrier speed and particle size.

Figure 4.6: Surface pressure-area isotherms over the course of five compression-expansion cycles of a SDS-DODAB monolayer at 20 cm²/min (left) and 90 cm²/min (right). These isotherms also highlight changes due to effects of particle diameters of (a) 1000 nm and (b) 500 nm. The green highlighted graphs show parameters that correspond to reversible folding while the red highlighted graphs correspond to irreversible folding.

While all of the experiments displayed a similar compression curve: a rapid increase in surface pressure followed by a long plateau characteristic of monolayer collapse, they differ most strongly along the expansion curves. The major differing feature in these curves is
Figure 4.7: Surface pressure-area isotherms over the course of five compression-expansion cycles of a SDS-DODAB monolayer at 20 cm$^2$/min (left) and 90 cm$^2$/min (right). These isotherms also highlight changes due to effects of particle diameters of (c) 100 nm and (d) 20 nm. The green highlighted graphs show parameters that correspond to reversible folding while the red highlighted graphs correspond to irreversible folding.
the size of a plateau that appears early on in the expansion. For parameters that produced reversible folding, this plateau is elongated and persists for much of the whole expansion stroke. For parameters that produced irreversible folding, the general trend involves a great reduction in the width of this plateau or its complete absence. This suggests that surface-pressure isotherms which show large-scale behavior of the monolayer may be an indicator of the folding behavior.

4.4 Slow Speeds – Wait Time Experiments

At speeds far below those used to produce the above phase diagram, it is expected that the longer timescales experienced during compression allow for the diffusion of particles into fold structures. It is thus expected that at slower speeds, the reversibility of the folds will be impacted by the potential accumulation of particles into the fold. However, due to the mechanical limitations of our barriers being unable to reach speeds below about 0.036 mm/sec, a different approach was developed in order to experimentally probe this diffusion-dominated regime.

In order to first examine the role diffusion of particles across the monolayer interface or into folds may play, an experiment was developed to test whether the particle diffusion was relevant over the lifetime of the monolayer; about 40-45 minutes. To calibrate the time evolution of the particle fluorescence, 50 µL of bead solution was deposited onto a glass coverslip and allowed to dry. Then, the slide was placed under the microscope and images of the beads were captured every two minutes for forty minutes. This experiment revealed that there was no significant change in the intensity of the beads over this time. Therefore, the brightness of the beads themselves is not expected to decay over the relevant experimental timescales. Thus any significant change in image intensity can be attributed to the movement of the particles out of the field of view, into the subphase or into fold structures.
Figure 4.8: Plot showing the average intensity of images of 20 nm particles deposited onto a glass coverslip. Here, the intensity remains roughly constant over time suggesting that the bead fluorescence does decrease with time.
This experiment was repeated for the case of 20 nm particles deposited into the surface of an SDS-DODAB monolayer. The system is compressed to minimum area and several images of the surface in proximity to a giant fold are taken over the course of 40 minutes. This study revealed a significant drop intensity in these images over the course of the experiment, with a particularly strong decrease after about 20 minutes before leveling out. This suggests that particles may be diffusing through the membrane into the subphase or into the saturated region of the image occupied by the fold. If particles are treated as linkers which increase the attraction between the walls of a fold, then this latter effect can be experimentally supported if a transition toward irreversible folding is observed after waiting a significant amount of time before expansion.

Figure 4.9: Plot showing the intensity of images of the monolayer surface in near proximity to a fold over a period of about 45 minutes. The plot shows a sharp decrease in brightness after about 20 minutes. The error bars represent the standard deviation of the intensity values for the beads on the glass cover slip.
In order to effectively simulate these very slow compression times, we compress an SDS-DODAB monolayer laden with 20 nm particles at a speed of 5 cm²/min and then stop the barriers once the point of minimum area is released. Then, the system is allowed to rest for a prescribed amount of time. At the end of this time, a single expansion cycle is initiated and an image of the surface at the location of the observed fold is captured. Folds were deemed to be reversible or irreversible using the same criteria as described above. Wait times of 15, 20 and 25 minutes were used before expanding the barriers. In addition, the expansion speed was also varied in different experiments and the values of 5, 15, 20, 25, 30, and 40 cm²/min.

Reversible folding was observed for all wait time durations when the expansion speed was greater than 25 cm²/min. A stark window of irreversible folding was found for tested expansion speeds of 25 cm²/min and less when the wait time was 20 minutes. At the other wait times for speeds from 15 to 25 cm²/min, the various degrees of reversibility were seen between different trials. These data trend toward having a higher probability of observing reversible folding as the barrier speed increases. Lastly, we find that for the shortest and longest wait time, reversible folding was always observed for the slowest expansion speed of 5 cm²/min.
Figure 4.10: Phase diagram showing the probability of observing reversible folding after waiting various amounts of time before expanding. The barriers expanded at several speeds and a transition to reversible folding was observed as speed increased for all wait times. A narrow window of irreversibility exists at a wait time of 20 minutes.
Chapter 5

Laser Tweezers Microrheology

The results from the reversibility experiments above reveal a rich phase space with non-monotonic trends along parameters of both increasing particle size and increasing barrier speed. However, despite the insight these results provide into monolayer behavior, these data remain primarily qualitative. To provide a more complete picture of monolayer folding, a stronger, more quantitative characterization of the forces experienced in the membrane is still needed. This chapter discusses laser tweezers microrheology, an experimental technique that can be used to more directly probe the forces involved during folding while also outlining several improvements and procedures of our laboratory’s laser tweezer apparatus.

5.1 Principles and Theory

Laser tweezers microrheology uses tracer particles embedded within a fluid which are manipulated by electromagnetic forces in order to probe the forces within that fluid. The ultimate goal in using this technique would be to manipulate a particle entrapped within a fold structure and then use this probe to better understand the forces exerted within the membrane.
during the unfolding process and the forces exerted on the particle by the monolayer itself. It will also be of interest to determine how the properties of the collapsed monolayer differ from adjacent regions in the plane of the air-water interface. The trap in a laser tweezer apparatus is generated by focusing a laser through an objective lens of high numerical aperture. The narrowest region of the focused beam results in a very strong electric field gradient which is able to induce charge in nearby dielectric materials, such as spherical plastic nanoparticles. These charged particles are consequently attracted toward the center of the beam where they can then be manipulated by moving the location of the beam. [1] The thermal fluctuations of the trapped particle can be tracked and transformed into their power spectral density (PSD) which can be directly related to the imaginary part of the complex response function via the fluctuation-dissipation theorem:[23]

\[ \chi''(f) = \frac{\pi f \langle |x_f|^2 \rangle}{k_B T} \]  

(5.1)

Where \( \chi'' \) is the imaginary part of the response function, \( f \) is the frequency conjugate variable of time, \( \langle |x_f|^2 \rangle \) is the PSD of the particle’s fluctuations and \( T \) is the temperature of the system. The remaining real part of the response function can be determined by evaluating the integral as per the Kramers-Kronig relations. However, previous experiments done using our laser tweezer apparatus show that within uncertainty, the real part of the response function is zero.[3] Therefore, we can in practice focus only on the imaginary part shown above. It is useful to non-dimensionalize this response function by dividing it by the expected result \( \chi_0 \) for a spherical particle of radius \( a \) suspended within an aqueous bulk subphase.
of viscosity $\eta$.

$$\chi_0 = \frac{i}{12\pi^2 a \eta f}$$ \hfill (5.2)

### 5.2 Laser Tweezers Apparatus

The optical trap is produced using a Nd:YVO$_4$ laser from Spectra-Physics that produces a beam at wavelength of 1064 nm. The beam initially passes through a beam sink which helps to prevent backscattered laser light from reflecting back and potentially damaging the source. Next, the laser passes into a beam expander which increases the width of the beam. It is important to maximize the size of this width so that it completely fills the back end of the objective lens and therefore increases the overall power of the tweezers trap. The desired beam width is next maintained using a pair of steering lenses, which also aid in serving as a translational degree of freedom when aligning and focusing the beam.

From the steering lenses, a mirror directs the beam upwards where it finally reaches the back of the objective lens where the beam is focused to create the optical trap. The objective is a water immersion lens which is interfaced with a custom monolayer trough. The lens enters the trough through an opening in the bottom and is able to image the monolayer surface from below. The lens is mounted on a vertical translation stage and so the trap can be focused at variable distances below the air-water interface. Particles caught in the trap scatter laser light back into the objective and this light is directed to a CCD camera for direct imaging of the trapped particle and its surroundings. The backscattered light is also directed to a quadrant photodiode (QPD) which allows for direct imaging of the trapped particle and its surroundings. The QPD uses this incident light to generate two-dimensional position data of
the trapped particle at a sampling rate of 66 kHz. Custom Labview software transforms this position data into the power spectrum density from which we can then extract the response function. The tweezers apparatus is generally used with particles of diameter 10 μm or larger due to their ease in tracking and maintaining within the optical trap.

5.3 Redesign and Refurbishment to Tweezers System

In order to accommodate a replacement to a damaged objective lens, several modifications to the optical tweezer apparatus were made. The original lens was an Olympus LUMPlanF 100x water immersion lens with a numerical aperture (NA) of 1.5. The closest replacement had a magnification of only 60x, but had the same NA which is the important factor for creating the optical trap.

The major issue concerning the new lens was that its physical dimensions were larger than
the previously used one and therefore it was not able to be immediately introduced into the apparatus. A new stainless steel collar fit with an O-ring was designed to fit around the tip of the lens and affix it to a flexible Teflon sheath. This sheath allows the lens to easily translate through the opening in the bottom of the trough while also forming a waterproof seal. The larger of size of the lens had further issues with displacing the Teflon sheath out of the cavity and directly into the subphase. This caused the sheath to intrude into the subphase on the order of the trough depth and thus led to potential problems as it would directly interfere with the monolayer at the air-water interface.

![Exploded view showing the construction of the connecting seal between the objective lens and the Langmuir trough.](image)

To combat this problem, a new kind of sheath made from nitrile was created. The more elastic nature of nitrile allows it to stretch as the lens moves up toward the air-water interface instead of bunching up. The nitrile sheath also has the great advantage over the original Teflon design in that it is less likely to wrinkle and thus more easily forms a long-lasting waterproof seal when fixed to the O-ring. The connection is also much easier to assemble
as the sheath’s elastically allows it to be more readily stretched over the pieces that connect it to the lens and the trough. Nitrile is however very soluble in chloroform and thus the lens cannot be attached to the trough during the cleaning procedure. The trough must be cleaned without the nitrile sheath first using the procedure described in Chapter 2. Then, the sheath is affixed to the trough with the lens and the system is then briefly rinsed with water and ethanol. Due to this, the sheath needs to be replaced for each experiment.

5.4 Alignment Procedures

The system was also directly modified to allow for easier alignment. A new mount for the laser was developed in order to allow it to be horizontally translated in the direction perpendicular to that of the beam. The original design was simply a static plate attached to a post on the optical table. That design could be adjusted by loosening the screws used to fix it to the post and rotating it, but it lacks a purely translational component. The new design offers the advantage of being able to adjust the position of the beam without potentially altering the angle with respect to the rest of the apparatus or changing the height of the beam.

There are several techniques and tools that will aid in making the alignment process more efficient. One useful technique is to back trace the beam path using a second laser. A helium-neon (HeNe) laser is mounted vertically at the location where the objective is to be placed such that its beam is pointing downwards toward the optical table. Care should be taken to support this laser such that the downward beam is perfectly perpendicular to the table. It is useful to align the beam with the screw hole directly below the translation stage. Once the position of the HeNe is secured, the beam should be traced back through the optical components leading to the beam source of the Nd:YAG laser. A thin piece of cardboard or other flat opaque surface should be placed in front of Nd:YAG beam opening to prevent
Figure 5.3: Picture showing the new mounting plate for the laser. The bottom left is a knob that allows the laser to be translated horizontally for the purposes of alignment.

damaging the laser. Once the visible HeNe laser spot can be observed at the other laser, the alignment of the path in the return direction should be tested. The path of the Nd:YAG laser should be traced back to the translation stage where the HeNe laser was mounted. As the Nd:YAG laser does not produce a beam at a visible wavelength, the position of the beam can be located using a phosphorescent card from ThorLabs or the Infrared Conversion Viewer from Newport. To ensure that the beam direction is completely vertical at the translation stage, a series of apertures can be mounted to the translation stage in a tower-like fashion. If the beam can be observed at the last aperture, then this suggests that the beam is in good alignment.

The combination of these alignment strategies and refurbishments to the optical tweezers apparatus have allowed us to successfully repair the system. With the new objective lens, we have demonstrated that we are able to trap and manipulate particles for future use in microrheological experiments.
Chapter 6

Discussion and Conclusions

The results presented in this thesis provide insight to the behavior of Langmuir monolayers due to interactions with both surfaces and particulates. The first experiments performed involved altering the effective depth of the trough by placing a small platform at the center of the system. Here, it was expected that as the depth decreased, the folds would interact with the surface potentially altering the dynamics of the system. It was observed that when the platform possessed a hard, glass surface, that experiments performed with a Survanta monolayer had their surface pressure isotherms shifted toward lower area in comparison to those without a substrate. Similarly, when the same experiments were performed using a Teflon substrate and an SDS-DODAB monolayer, new features in the isotherm became apparent as the depth decreased. Though these latter results were ultimately irreproducible, the former results do suggest that reducing the depth does have some effect on the system. It is yet to be determined whether this effect is due to direct interactions with the surface or due to new flows produced by the confined geometry. Future experiments that employ techniques such as Brewster angle and confocal microscopy may be useful in directly imaging the folds below the subphase and help to determine how closely they actually approach the surfaces below. The Teflon substrate experiments further suggest that the cleaning procedure
is especially critical for reduced depth geometries as contaminants may be more readily transported to the air-water interface. Future work in assessing depth effects should take extra care to maintain the cleanliness of both the trough and the substrates.

Procedures were also developed in order to culture and introduce a layer of alveolar cells onto the surface of these substrates. Future work with these cells will allow experiments to compare the effects of a soft, organic boundary to those of the solid, hard boundaries already tested on fold dynamics. The surface proteins and lipids of the cells themselves may furthermore allow the monolayer to more readily bind to the cells, thus introducing new stresses on the monolayer as it unfolds. It is of additional interest to consider particles that have been deposited on the cell surface after a number of expansion and compression cycles. By imaging the surface using fluorescence microscopy, the rate at which particles are diffused into the interior of the cells could be measured which could be used to understand how particulates could be transported from the alveolar surfactant and into the circulatory system.

Another set of experiments were performed to deduce the effects of expansion and compression rate on the reversibility of monolayer folds. As the barriers expanded at the highest speeds, these folds were always observed to undo themselves regardless of the size of the particles embedded within them. This observation supports the theory that rapid compressions at around 1 µm/sec create large viscous forces which alter the shape of the fold, limiting the amount of self-interaction between the fold, and thus decreasing the amount of force required to pull the fold apart.

The effect of particulate matter size on monolayer folding was also tested. The giant folds were seen to be reversible at all speeds when laden with particles 1 micron in diameter. Moderate-sized particles of 100 and 500 µm on the contrary, produced mainly irreversible folding except at the highest speeds tested. These diameters are consistent with the mean size of particulates produced respectively by burning biomass and urban smog.[16, 41] This
suggests that in addition to any toxic chemical effects these particles may have, the physical size of these agents may also fundamentally play a role in causing chronic respiratory disorders. For the smallest particles tested at 20 nm, we see a non-monotonic trend in reversibility where the slowest and highest speeds produce reversible folding, but irreversible folding is observed when the barriers move at the intermediate speed of 40 cm$^2$/min. This transition toward increased reversibility as particle size decreases may be explained as a balance between the self-adhesion strength and bending energy in the fold. As particles become entrapped within a fold, the monolayer warps around them which forces the monolayer to take a corrugated profile. Smaller particles allow for more of the membrane to make self-contact and thus the overall attraction forces between the walls of the monolayer increase. However, there exists a critical size below which bending the membrane in this way to maintain the corrugated shape no longer becomes favorable. Therefore, the force required to break apart the folds decreases and thus the observed transition towards reversible folding is expected.

Experiments were also done to provide insight to the reversibility of monolayer folding at longer timescales where diffusion of particles is expected to play a major role. It was found that brightness of images surrounding the monolayer fold decreased over a time period of 40 minutes. Since the particles themselves do not decay, this suggests that particles are being lost either into the subphase or into the saturated regions of the image occupied by folds. We found that after compressing at the slowest speed and then waiting for 20 minutes, there was a transition toward irreversible folding. This suggests that linker particles may accumulate into the fold via diffusion during this time, increasing the strength of the bond between the fold walls. We also generally observe a transition toward reversible folding as the barrier speed during expansion increases for all wait times. This further supports the earlier findings that sufficiently high expansion rates are able to overcome the self-adhesion forces between the fold walls in systems that displayed irreversible folding at lower speeds.

These findings paint a rich and detailed picture of the dynamics of Langmuir monolayers and
Figure 6.1: Plot showing the binding energy between fold walls due to a balance between self-adhesion and the bending energy of the monolayer. Decreasing particle size allows for more self-adhesion, but as the particles become smaller than about 100 nm in radius, more work must be done to maintain a corrugated profile around the beads and thus the bending energy dominates over self-adhesion, leading to a decrease in binding energy.
particulates. However, in order to better understand these systems quantitatively, further exploration is needed. We plan to utilize a refurbished and newly working optical tweezers system to probe folded regions of the monolayer using microrheological techniques. These methods will help to discern the complex fluid properties of folded monolayer in comparison to that in the interfacial surfactant. They will also help to characterize the forces required to free a particle from the fold or to pull apart the fold itself. This will allow us to more directly compare our experimental results with theory and build a more complete model for monolayer folding.
Bibliography


