Measurements and Modeling of the Failure Pressure of Bilayer Lipid Membranes

by

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Abstract

Bilayer lipid membranes (BLMs) are formed from phospholipid molecules which self-assemble into a lipid bilayer with 4 to 9 nm thickness when submerged in an aqueous solution. This is due to their amphiphilic nature, meaning that one part of the molecule is hydrophilic, or attracted to water, and one part is hydrophobic, or repelled by water. They are the primary structural component of cell membranes in living organisms and therefore are useful for modeling the properties of cell membranes since they share many of the same chemical and physical properties.

The objective of this dissertation is to investigate the maximum pressure that can be withstood by a BLM formed over a porous substrate, which will be referred to as the failure pressure. This work represents the first time that this quantity has been measured and reported. The failure pressure is investigated in several complementary ways, including mechanical, electrical, and optical measurements and modeling. The phospholipids that are tested include 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphatidylcholine (SOPC) and mixtures of SOPC and cholesterol (CHOL), which was chosen because cholesterol is known to increase the strength of SOPC BLMs.

A new test methodology was developed to measure the failure pressure of BLMs. A custom test fixture was used to pressurize BLMs while measuring the applied pressure with a high degree of precision and repeatability. The BLMs were tested in an electrolyte solution over substrates that contained a single pore and also substrates that contained an array of many pores. SOPC BLMs were tested over single pore substrates with pore sizes ranging from 5 to 20 µm, and this resulted in failure pressures from 67 to 19 kPa, respectively. For single pore tests, the addition of 50 mol% cholesterol to SOPC resulted in a 56% higher
failure pressure on average than SOPC alone. For multi pore substrates, SOPC BLMs were tested using pore sizes between 0.05 and 10 µm, which yielded bulk failure pressures of 380 to 1.5 kPa, respectively. For multi pore tests, SOPC/CHOL-50 mol% resulted in a 47% higher bulk failure pressure on average.

A model was developed to predict the pressurization curve of BLMs and was applied to both the single and multi pore tests. It was found that the failure pressure of BLMs follows a distribution which was well modeled by a Weibull distribution with a positive skew. Parameters such as the Weibull shape parameter were determined by fitting the model to the experimental pressurization curves and it was found that the shape of the Weibull distribution was nearly the same for every pore size. Using the pressurization model it was estimated that the percentage of failed BLMs that were pressurized over a multi pore substrate ranged from 4% to 33%. The model also coupled the bulk failure pressure of BLMs formed over multiple pores to the failure pressure of a single BLM, showing that the bulk failure pressure of multiple BLMs is smaller than the failure pressure of a single BLM because it represents the failure of only the weakest BLMs in a group.

Electrical impedance was measured before and after pressurization of the BLMs, and these measurements were modeled by assuming that the BLMs act as a resistor and a capacitor configured in parallel. In general, the impedance magnitude dropped by two to three orders of magnitude after BLM pressurization, which was a result BLMs failing and opening conductive pathways through the subsequently empty pores. It was found that normalized conductance values for SOPC BLMs were between \( G_{BLM}/A = 4 \times 10^{-12} \) and \( 2 \times 10^{-8} \) S/cm\(^2\), and normalized capacitance values varied between \( C_{BLM}/A = 3 \times 10^{-14} \) and \( 1 \times 10^{-10} \) F/cm\(^2\). In the literature these values ranged from \( G_{BLM}/A = 10^{-1} \) to \( 10^{-9} \) S/cm\(^2\) and \( C_{BLM}/A = 10^{-6} \) to \( 10^{-8} \) F/cm\(^2\), having a wide range of values due to the many variations of experimental test procedures.

Visual images of BLMs were produced using fluorescence microscopy. Images were recorded before and after pressurization of SOPC BLMs formed over a multi pore substrate. As predicted by the pressurization model, it was found that some but not all BLMs fail after pressurization. It was also found that BLMs fail over the center of a pore, and leave remnants around the perimeter of the pore.
To my two best friends, Leslie and Sully, for all of the walks that we have shared in Blacksburg and in life, and for all of the walks that are still to come.
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Chapter 1

Introduction

1.1 Motivation for the study of bilayer lipid membranes formed over porous substrates

Bilayer lipid membranes (BLMs) are formed from phospholipid molecules which self-assemble into a lipid bilayer with 4 to 9 nm thickness when submerged in an aqueous solution (Cevc and Marsh, 1987; Hamley, 2005; Tien and Ottova, 2003). This is due to their amphiphilic nature, meaning that one part of the molecule is hydrophilic, or attracted to water, and one part is hydrophobic, or repelled by water. They are the primary structural component of cell membranes in living organisms and therefore are useful for modeling the properties of cell membranes since they share many of the same chemical and physical properties.

One possible engineering application of BLMs includes biomimetic actuation devices that are inspired by the motion and growth of plant cells. Movement in plants such as that in leaves or the opening and closing of stomatal pores is caused by fluid transport across cell membranes (Hill and Findlay, 1981). Changes in turgor pressure in response to chemical stimuli that occur in specific plant cells, the motor cells, produce changes in volume at the cellular level that lead to bulk deformation of the plant. Thus, according to the engineering definition, a plant can be considered an actuator since it converts biochemical fuels such as adenosine triphosphate (ATP) into mechanical energy.

The cellular processes that activate plant deformation have inspired the development of an actuator that can be used in engineering applications (Leo, 2005). A prototype of
this biologically-inspired actuator concept has been developed by researchers at Virginia Tech and is shown schematically in Figure 1.1. The actuator uses ion transporter proteins that were harvested from plant cells to pump fluid across a planar BLM from a reservoir into an enclosed expansion chamber when stimulated by a pH gradient. The fluid exerts pressure on the enclosed chamber causing its volumetric expansion (Sundaresan and Leo, 2006). This prototype actuator has been able to achieve displacements as large as 60 µm.

The performance of the biomimetic actuator is limited by the maximum pressure that can be maintained inside of the expansion chamber before the bilayer lipid membrane fails. In this design a porous polycarbonate substrate coated with a BLM is used to separate the fluid reservoir from the enclosed expansion chamber (See Figure 1.1). Pressure inside of the chamber increases as fluid is pumped into the expansion chamber. At some critical pressure the BLM will rupture and cause a loss of pressure within the actuator. The ability to characterize this critical pressure and increase the strength of the BLM may lead to a better design for the actuator with a greater displacement output. For this reason the present study focuses on determining the maximum pressure that can be withstood by BLMs that are formed over porous substrates.

Testing the mechanical properties of BLMs is a developing field in the biological sciences. The literature contains methods for measuring a variety of different properties and many researchers have used porous substrates as a test base for studying BLMs. However no work has been done to measure and analyze the failure due to pressurization of BLMs formed over porous substrates. This research not only introduces a new mechanical testing method, but also explores a property of BLMs that has missed the attention of the scientific community. This work should be useful for scientists and engineers whose work requires an understanding of the stability of bilayer lipid membranesacting under pressure, and may
open possibilities for other applications that use BLMs as structural components.

1.2 Technical objectives

The primary objective of this research is to perform a thorough investigation of the maximum pressure that can be withstood by a BLM formed over a porous substrate, which will subsequently be referred to as the failure pressure. This objective is divided into four components. First, a new test fixture and test methodology is developed to measure the failure pressure of BLMs with a high degree of precision and repeatability. A variety of different phospholipid combinations are tested in order to observe differences in BLM failure pressure based on the chemical composition of the phospholipids. Bilayer lipid membranes are pressurized over a range of pore sizes to determine the dependence of failure pressure on pore size.

Second, a model is developed to provide a physical interpretation of the failure characteristics of BLMs formed over porous substrates. The model is used to predict pressurization curves for BLMs formed over single pore and multi pore substrates. This is particularly useful for understanding the multi pore substrate behavior, since the failure of an array of BLMs that are pressurized in parallel is more complex than for a single BLM.

Third, BLM failure is investigated by measuring and modeling the electrical impedance of the BLM before and after it is pressurized. Electrical impedance measurements are used as an independent measurement for confirming the formation of a BLM over a pore before pressurization, and for confirming the failure of a BLM after pressurization. Since BLMs are known to have the electrical properties of a resistor and capacitor in parallel, these parameters are measured and reported.

Fourth, fluorescence microscopy is used to observe the failure of BLMs optically. A test setup is developed that has the ability to view a BLM using a fluorescence microscope while simultaneously applying pressure to the BLM. Images are recorded of BLMs before and after failure. Results from these measurements are correlated with other methods of measuring BLM failure.
1.3 History of BLM research

The study of lipid films began in the same era as the invention of the optical microscope, which is generally credited to the Dutch eyeglass makers Zaccharias and Hans Janssen in 1590. This invention formed the groundwork for an improved design of the microscope that was built by Robert Hooke. Among engineering circles Hooke is best known for his mathematical relationship describing the elastic deformation of materials, Hooke’s law, but he is also credited with many other scientific discoveries. Most significantly he discovered that all living things are made of cells. He coined the term ‘cell’ in his book *Micrographia*, published in 1665, to describe an array of pores in a slice of cork that he observed using his microscope (Hooke, 1665). The history of lipid film research includes a long list of scientists who were not generally considered biologists but who experimented with soap bubbles. Indeed Hooke was among them when he used his microscope to observe ‘black holes’ in soap films.

Shortly after the first observations of lipid films researchers attempted to measure their thickness. Isaac Newton developed a method for measuring the thickness of thin films according to the wavelength of light that is refracted off of their surface. In 1692 he wrote the book *Opticks* in which he estimated the thickness of black soap films to be 9 nm (Newton, 1704). Current measurements confirm Newton’s original measurement, placing the thickness of a soap film between 4 and 9 nm depending on the type of soap solution (Tien and Ottova, 2003).

Another famous experiment in the study of lipid film thicknesses was reportedly performed by Benjamin Franklin in 1774. Franklin poured a teaspoon of oil into a pond to determine how far it could spread and observed that it calmed approximately a half-acre surface area of water (Tien and Ottova, 1999). Over a century later in 1891 Agnes Pockels reported in Nature on an apparatus that she developed in her kitchen to measure the surface area of thin films (Tien and Ottova, 2003). This apparatus was later adapted by Irving Langmuir into a device known as Langmuir’s film balance which he used to determine the dimensions of fatty acid molecules of a lipid monolayer (Langmuir, 1917). Gorter and Grendel used this equipment to make the groundbreaking discovery that lipids could also form a bilayer (Gorter, 1925). In their experiment they were able to extract the lipids from a red blood cell and they found that the surface area of those lipids was approximately
twice the surface area of the blood cell itself. From this they deduced that the red blood cell was covered by a lipid layer that was two molecules thick. Later attempts to reproduce this experiment showed that Gorter and Grendel’s measurements were inaccurate, but by chance several of their errors canceled each other out and led them to the correct conclusion (Sadava, 1993). Gorter and Grendel’s observations eventually led to our current model of the cell membrane which was proposed by the biochemists Singer and Nicholson in 1972. Known as the fluid mosaic model, it consists of a bilayer lipid membrane that contains protein channels that span the bilayer and float freely within it (Singer and Nicholson, 1972).

The first account of a bilayer lipid membrane formed in vitro came in 1962 from Donald Rudin and his research group at the Pennsylvania Psychiatric Institute (Mueller et al., 1963). Rudin was studying soap bubbles using equipment purchased from a local toy store when he was able to form a black lipid soap bubble underwater, and later form a BLM made from bovine brain extracts underwater (Tien and Ottova, 2003). Shortly thereafter Alec Bangham made an equally important discovery when he observed egg lecithin phospholipids in an aqueous solution using an electron microscope and found that they spontaneously form into closed spherical membranes that he referred to as liposomes (Bangham et al., 1965). This spurred later research for the use of liposomes as models of cell membranes and also for pharmaceutical use as drug delivery mechanisms (Gregoriadis, 1984).

1.4 Structure of BLMs

The primary functions of bilayer lipid membranes in a cell membrane are to provide rigidity and structure to the cell and its organelles, to serve as a mechanical, electrical, and chemical barrier between the inside and outside of the cell, to serve as a conduit for transport via protein ion pumps and channels, and to communicate with the environment using chemical and electrical signal receptors (Tien and Ottova, 2003). An animal cell membrane is composed of fatty lipids which give the membrane its form and structure, steroids such as cholesterol which strengthen the membrane, and proteins which transport ions across the membrane (Kunneke et al., 2004). Plant cell membranes also have layers of cellulose and chitin that provide additional rigidity.

Lipids, like oils, are fatty acid hydrocarbon chains that are insoluble in water but
soluble in organic solvents. Phospholipid molecules are composed of a hydrophilic, polar phosphate head group and two hydrophobic, nonpolar hydrocarbon tail groups (Cevc and Marsh, 1987), as illustrated in Figure 1.2. Cell membranes are largely composed of a specific class of phospholipids known as phosphatidylcholines (PC) or lecithin (these terms are used interchangeably). The unique amphiphilic nature of this molecule causes it to self assemble into a double-monomer chain known as a lipid bilayer when it is immersed in an aqueous solution. It forms such that the hydrophilic heads of each of the two monolayer groups faces outward toward the aqueous solution and the hydrophobic tails face inward toward each other in order to avoid contact with the solution, as illustrated in Figure 1.3. This behavior is known as the hydrophobic effect. Hydrogen bonding, van der Waals forces, and the hydrophobic effect hold the two monomer groups together in the bilayer configuration (Cevc and Marsh, 1987; Needham and Zhelev, 2000). The bilayer is structured as a smectic liquid crystal, which is a layered phase (Hamley, 2005). The molecules have orientational order, but only one degree of long range positional order, which is normal to the layers. There is no translational order for molecules within the layers, and like a liquid they float freely. The bilayer is characterized by a high electrical resistance and a low permeability to water which is essential to the protection of the internal cell components (Needham and Zhelev, 2000).

At high concentrations amphiphiles will aggregate into one of several possible geometries. The primary factor determining the aggregate geometry is the shape of the individual molecules, which is quantified by an aspect ratio known as the packing parameter, $P = V/Al$ (Hamley, 2005). Here $V$ is the total volume of the molecule, $A$ is the cross sectional area of the head group, and $l$ is the length of the hydrocarbon chain. Amphiphilic molecules with the lowest packing parameters, $P < 1/3$ will form micelles, which are spherical balls
in which the hydrophobic tails of the amphiphile point inward. A slightly larger packing parameter, $1/3 < P < 1/2$, yields a cylindrical shaped micelle. Next, for $1/2 < P < 1$, is a vesicle, which is a spherical shell of amphiphilic molecules. A packing parameter of $P \approx 1$ yields a planar bilayer, or lamella. Finally, a packing parameter $P > 1$ yields an inverse micelle in which the hydrophobic tails point outward.

Phospholipid molecules are most likely to form into either vesicles or planar lamellae due to their double hydrocarbon chain shape. A vesicle is a spherical shell composed of a bilayer with an interior volume that is of the aqueous medium. A vesicle that is composed of a single bilayer of amphiphilic molecules is referred to as a unilamellar vesicle, while one composed of several bilayers is referred to as a multi layer vesicle. Vesicles that are formed from lipid amphiphiles are also known as liposomes. Liposomes are of interest to researchers because they are simplified models of living cells.

In an aqueous solution some phospholipid molecules will spontaneously form into a liposome structure, which is an energetically favorable geometry because it is a closed shape with no edges (Needham and Zhelev, 1996). This process can be accelerated and better controlled using mechanical and electrical perturbations such as extrusion through porous polycarbonate membranes (Hunter and Frisken, 1998), ultrasonication (Pereira-Lachataignerais et al., 2006), and electroformation (Angelova and Dimitrov, 1986). In the extrusion method, phospholipids are forced through a porous filter containing pores on the order of 0.1 to 1 µm in diameter. A pressurization system such as compressed nitrogen gas or a mechanical syringe and plunger is used to extrude the phospholipids through a pore, much like blowing a soap bubble through a ring, to form vesicles on the order of or slightly larger than the pore size. Ultrasonication is a technique in which vesicles are formed either by simple dispersion in aqueous solution or by extrusion and then are further perturbated by
vibration in an ultrasonicator. Higher power and longer sonication times result in smaller vesicle diameters. Electroformation is a technique pioneered by Miglena Angelova in the 1980s for stimulating liposome formation using electrical disturbances. To do this, two platinum wires are fixed parallel to one another and immersed in fluid. One or both of the platinum electrodes are coated with a phospholipid solution and a sinusoidal current is passed between them. The sinusoidal current causes a periodic electro-osmotic mechanical disturbance in the fluid which is the driving force for the generation of liposomes. The fluid motion is directed perpendicular to the electrode surface, pulling the lipid coating from the electrode and causing discrete sections of lamellae to separate into a mushroom shape which will eventually close into a spherical liposome (Angelova, 2000). A microscope image of an electroformed liposome that was created in the Center for Intelligent Material Systems and Structures (CIMSS) laboratory at Virginia Tech is shown in Figure 1.4.

Phospholipids can also form into a planar bilayer. This behavior is encouraged by the deposition of the BLM onto a planar substrate, such as glass, silicon, or mica (Tien, 1985). One well known method for depositing a bilayer or multilayer onto a substrate is the Langmuir-Blodgett technique (Hamley, 2005). In this method a trough known as a Langmuir trough is filled with water and a monolayer film of amphiphiles is condensed along the surface. Amphiphiles prefer to collect at an air-water interface and consequently lower the interfacial surface tension. A movable barrier is used to control the density of the monolayer on the surface. A hydrophilic substrate is then dipped into the trough and removed, causing a BLM to deposit on its surface, as illustrated in Figure 1.5.
1.5 Contributions

This work represents the first investigation of the failure characteristics of BLMs formed over porous substrates. The following contributions have been made to the field:

- A new test methodology was developed for pressurizing BLMs formed over porous substrates and measuring failure pressure.

- Failure pressures were reported for the first time for SOPC and SOPC/CHOL-50 mol% BLMs formed over single pore substrates with pore sizes ranging from 5 to 20 \( \mu \text{m} \) diameter and multi pore substrates with pore sizes ranging from 0.05 to 10 \( \mu \text{m} \) diameter.

- A new model was developed to predict the pressurization curve of a BLM formed over a single pore, which was based on the pressurization and flow of fluid through a porous medium.

- The model was expanded to further describe the pressurization of BLMs formed over a multi pore substrate, which assumed that the failure of an array of many BLMs follows a statistical distribution. This was found to be well fit by a Weibull distribution with a positive skew. The result of this model was to quantify the distribution parameters for BLMs, and to quantify the fraction of BLMs that fail after pressurization.
• It was further found that BLMs of all pore sizes had nearly the same Weibull shape parameter. Because of this the model could be generalized such that the distribution of BLM failure for any pore size could be predicted based on the distribution of one nominal pore size.

• Electrical impedance measurements were used to independently verify the failure of BLMs as a result of pressurization. The impedance measurements were modeled to show that before pressurization BLMs were formed over porous substrates, and after pressurization BLMs failed, leaving an open pore in their place.

• Another new test methodology was developed to pressurize BLMs formed over porous substrates while simultaneously recording optical images of the BLMs using fluorescence microscopy. Photographs and video footage were recorded of SOPC BLMs before, during, and after pressurization to directly prove that BLMs failed as a result of pressurization.

1.6 Document overview

The purpose of this research is to study the failure pressure of bilayer lipid membranes formed over porous substrates. This introductory chapter is intended to provide a basic explanation of what a BLM is, and the history of BLM research. An engineering application in which BLMs are used in a biomimetic micro-hydraulic actuator is also described. Chapter 2 reviews experimental methods that are used to characterize a BLM. Some methods are common in the literature and some are unique to this project. This chapter is broken into two sections; one section describes methods for testing mechanical properties of BLMs and the other section describes methods for testing electrical properties. Chapters 3 and 4 report on the testing and results of BLMs formed over a single pore substrate and a multi pore substrate, respectively. They are arranged as complete and independent papers that are to be submitted for publication, and therefore contain some amount of redundant information. Each includes an introduction, materials and methods, modeling, results, discussion, and conclusion. Chapter 5 summarizes the work and draws conclusions. Major contributions are reviewed and suggestion for future work are presented.
Chapter 2

Experimental Methods for Mechanical and Electrical Measurements of BLMs

2.1 Mechanical measurements of BLMs

Characterizing the mechanical properties of bilayer lipid membranes (BLMs) presents a unique challenge because BLMs are difficult to image, only exist in an aqueous solution, have a thickness on the order of nanometers, and are very soft. These characteristics preclude the use of any conventional mechanical tests such as a tensile test or hardness indenter. To date there is no standard for testing the mechanical properties of a BLM, and most methods found in the literature require expensive, customized equipment. Despite these difficulties, investigators in biomechanics have developed many creative ways to evaluate the mechanical behavior of bilayer lipid membranes.

Two methods for measuring mechanical properties of BLMs that stand out in the literature are micropipet aspiration and atomic force microscopy. The micropipet aspiration technique was pioneered by Mitchison and Swann (Mitchison and Swann, 1954). It has been developed extensively by Evans and Needham (Evans and Needham, 1987) and is among the most established methods for measuring the mechanical properties of vesicles. For the micropipet aspiration technique a single vesicle is suctioned into a micropipet and its deformation is observed. In atomic force microscopy a stylus is used to probe the surface.
topography and structural properties of a BLM (Hoh and Schoenenberger, 1994). Both of these will be reviewed in more extensive detail. Another method called laser tweezers uses a laser to manipulate a particle that is embedded within a cell membrane or vesicle. The particle deforms the BLM and the applied force can be measured (Lim et al., 2004; Henon et al., 1999). Wobschall developed a test fixture for measuring the change in film tension in a BLM by pressurizing the BLM with a diaphragm connected to an acoustical driver (Wobschall, 1971). The film tension was modeled as a function of the change in electrical capacitance across the bilayer, and this was in turn used to estimate the Young’s modulus of the BLM. Kummrow and Helfrich used an alternating electric field to controllably deform a single circular vesicle into an ellipsoid (Kummrow and Helfrich, 1991). They calculated the lateral tension of the vesicle membrane based on its eccentricity and subsequently were able to determine the bending rigidity of the BLM. Other methods for testing BLMs include magnetic twisting cytometry (Wang et al., 1993), cytoindentation (Shin and Athanasiou, 1999), microplate manipulation (Thoumine and Ott, 1997), tensile testing (Miyazaki et al., 2000; Nagayama et al., 2006), phase contrast microscopy (Faucon et al., 1989), and others (Lim et al., 2006).

Several methods for characterizing the mechanical properties of bilayer lipid membranes have been reviewed here. Some representative material properties that have been measured by various methods and for various types of lipid materials are presented in Table 2.1. Two material properties that have not been discussed in this literature review but appear in Table 2.1 are the membrane shear modulus $\mu$ and the membrane bending modulus $k_c$. A more extensive discussion of these properties appears in works by Evans and Needham (Evans and Skalak, 1980; Evans and Needham, 1987; Needham and Zhelev, 1996).

2.1.1 Micropipet aspiration measurements of BLMs

In the last three decades, micropipet aspiration has been the most widely used method for measuring mechanical properties of bilayer lipid membranes. In this method a single vesicle (10 - 200 $\mu$m diameter) is suctioned into a micropipet (1 - 15 $\mu$m diameter) causing it to deform, as illustrated in Figure 2.1. The resulting deformation can be used to quantify several properties of the lipid bilayer including the elastic modulus, strength, phase transition temperatures, shear viscosity, and permeability (Evans and Needham, 1987).
Table 2.1: Summary of BLM material properties measured by various methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Lipid</th>
<th>$E$ (MPa)</th>
<th>$K_a$ (mN/m)</th>
<th>$\tau_u$ (mN/m)</th>
<th>$\mu$ (µN/m)</th>
<th>$k_c$ ($\times 10^{-20}$ J)</th>
<th>Ref$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM</td>
<td>Egg yolk PC</td>
<td>1.97 ± 0.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>AFM</td>
<td>Ray synapse</td>
<td>0.2 – 1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>AFM</td>
<td>POPS</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>AFM</td>
<td>DOPG-Na</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D</td>
</tr>
<tr>
<td>MA</td>
<td>SOPC</td>
<td>39</td>
<td>193 ± 20</td>
<td>5.7 ± 0.2</td>
<td>9.0 ± 0.6</td>
<td></td>
<td>E,F</td>
</tr>
<tr>
<td>MA</td>
<td>SOPC/Chol50%</td>
<td>130 – 160</td>
<td>640 – 781</td>
<td>19.7 ± 3.2</td>
<td>24.6 ± 3.9</td>
<td></td>
<td>E,F</td>
</tr>
<tr>
<td>MA</td>
<td>RBC/Chol40%</td>
<td>85</td>
<td>423 ± 41</td>
<td></td>
<td></td>
<td></td>
<td>E</td>
</tr>
<tr>
<td>MA</td>
<td>Human RBC</td>
<td></td>
<td>4 – 10</td>
<td></td>
<td></td>
<td></td>
<td>G,H</td>
</tr>
<tr>
<td>LT</td>
<td>Human RBC</td>
<td></td>
<td>2.5 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>LT</td>
<td>Human RBC</td>
<td></td>
<td>11 – 18</td>
<td></td>
<td></td>
<td></td>
<td>J</td>
</tr>
<tr>
<td>AFM</td>
<td>Egg yolk PC</td>
<td></td>
<td></td>
<td>2.1 ± 0.8</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>PCM</td>
<td>Egg yolk PC</td>
<td></td>
<td></td>
<td>4 – 5</td>
<td></td>
<td></td>
<td>K,L</td>
</tr>
<tr>
<td>ACEF</td>
<td>Egg yolk PC</td>
<td></td>
<td></td>
<td>2.47</td>
<td></td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>ACEF</td>
<td>Egg yolk PC</td>
<td></td>
<td></td>
<td>6.6 ± 0.6</td>
<td></td>
<td></td>
<td>N</td>
</tr>
</tbody>
</table>

1. AFM = atomic force microscopy; MA = micropipet aspiration; LT = laser tweezers; PCM = phase contrast microscopy; ACEF = alternating current electric field

2. PC = phosphatidylcholine; POPS = 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[Phospho-L-Serine]; DOPG-Na = 1,2-Dioleoyl-sn-Glycero-3-[phospho-rac-1-Glycerol-Na]; SOPC = 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphatidylcholine; Chol50% = 50 mol% cholesterol; Chol40% = 40 mol% cholesterol; RBC = red blood cell

3. A = (Liang and Guangzhao Mao, 2004); B = (Laney et al., 1997); C = (Kunneke et al., 2004); D = (Jing and Run-Guang, 2002); E = (Needham and Nunn, 1990); F = (Evans and Rawicz, 1990); G = (Evans, 1973); H = (Hochmuth and Waugh, 1987); I = (Henon et al., 1999); J = (Lim et al., 2004); K = (Faucon et al., 1989); L = (Engelhardt et al., 1985); M = (Kummrow and Helfrich, 1991); N = (Angelova et al., 1992)

Figure 2.1: Illustration of the micropipet aspiration method in which a single vesicle is deformed by being suctioned into a micropipet.
Mechanical properties measured by micropipet aspiration

In micropipet aspiration a vesicle is formed by electroformation or another suitable method and is suctioned into a micropipet (see Figure 2.1). In the simplest of experiments a single vesicle is pressurized and released in incrementally larger pressures until the vesicle bursts. This experiment is used to generate a $\tau \sim \alpha$ stress-strain plot, where $\tau$ is the membrane stress and $\alpha$ is the area strain. Four material properties can be determined from this plot: (1) the area expansion modulus $K_a$, which is the slope of the stress-strain curve; (2) the ultimate tensile strength at vesicle failure $\tau_u$; (3) the critical areal strain at vesicle failure $\alpha_c$; and (4) the strain energy at failure $E_s$ which is the area under the stress-strain curve. The deformation of the lipid bilayer in this experiment is elastic. Each time the vesicle is pressurized and released it is observed to recover to its original undeformed state until the pressure is eventually large enough to cause failure. Typical values for these properties for an SOPC/cholesterol-40 mol% vesicle is an area expansion modulus of 360 mN/m (Needham and Nunn, 1990). This can be converted to a bulk Young’s modulus of 72 MPa by dividing by the bilayer thickness of approximately 5 nm. The same vesicle has an area ultimate tensile strength of 17.2 mN/m, which similarly can be converted to a bulk strength of 3 MPa.

The isotropic membrane stress $\tau$ is a ratio of the applied pressure, the micropipet radius, and the vesicle radius,

$$\tau = \frac{\Delta P R_p}{2 - 2 R_p/R_v},$$

(2.1)

where $P$ is the suction pressure, $R_p$ is the inside diameter of the micropipet, and $R_v$ is the radius of the outer spherical portion of the vesicle (Evans and Needham, 1987).

The area strain $\alpha$ is $\Delta A/A$,

$$\frac{\Delta A}{A} = \frac{2\pi R_p \Delta L (1 - R_p/R_v)}{4\pi R_v^2 - \pi R_p^2 + 2\pi R_p L},$$

(2.2)

where $L$ is the projection length of the vesicle inside the micropipet (Evans and Needham, 1987; Zhou and Raphael, 2005). This assumes that the vesicle has constant volume since BLM permeability is very low in an aqueous solution.

The area expansion modulus $K_a$ is analogous to Young’s modulus and the relationship between area expansion modulus, stress, and strain is analogous to Hooke’s law (Evans and Skalak, 1980),

$$\tau = K_a \alpha.$$  

(2.3)
Area expansion modulus is also the slope of the stress-area strain curve.

**Thermal properties measured by micropipet aspiration**

BLMs exist in two primary thermal states: an anisotropic liquid crystalline state $L_\alpha$ with fluid hydrocarbon tails and in a gel state $L_{\beta'}$ with crystalline hydrocarbon chains (Cevc and Marsh, 1987; Evans and Needham, 1987). Some phospholipids also experience an intermediate phase $P_{\beta'}$ in which the bilayer is rippled. X-ray diffraction has revealed that in the liquid crystalline phase $L_\alpha$ the hydrocarbon chains are normal to the membrane surface and in the gel phases $P_{\beta'}$ and $L_{\beta'}$ the hydrocarbon chains are tilted with respect to the membrane surface, as denoted by the prime symbol. The transition temperature $T_c$ between the liquid crystalline state and the gel state varies widely according to the composition of the hydrocarbon chain, typically ranging between 0 °C and 70 °C.

Evans and Needham have performed extensive studies on the influence of temperature on properties of phospholipid vesicles including area change, mechanical stiffness and strength, and hydrocarbon chain tilt angle (Evans and Needham, 1987; Needham and Evans, 1988; Needham et al., 1988; Needham and Zhelev, 1996). A plot of area strain versus temperature is used to determine the transition temperature $T_c$ and the areal coefficient of thermal expansion (Evans and Needham, 1987). The micropipet aspiration method can be used to determine stiffness and strength as a function of temperature.

Shear rigidity and shear viscosity are measurable for BLMs in the gel phase but not in the liquid phase. Recall that in its liquid crystalline state a BLM does not have any rigidity in-plane, and molecules are free to flow amongst each other. These values are obtained from an analysis of the threshold suction pressure required to initially deform a gel phase vesicle into a micropipet and from the subsequent flow rate of the vesicle into the micropipet (Needham and Zhelev, 2000). A typical value for the shear yield stress of a lipid bilayer is 5 kPa and the shear viscosity is 2 MPa s, which are comparable to the properties of polyethylene at room temperature.

**Effects of adding cholesterol to phospholipid BLMs**

The most effective way to increase the stiffness and decrease the water permeability of a lipid bilayer, even more than freezing it to its gel phase, is by the addition of cholesterol (Needham and Zhelev, 1996). In a saturated lipid compound such as 1-Stearoyl-
2-Oleoyl-sn-Glycero-3-Phosphatidylcholine (SOPC) it has been shown that an increase in the concentration of cholesterol will increase the stiffness $K_a$ of the bilayer lipid membrane in an exponential fashion up to approximately 60 mol%. The maximum area modulus of a SOPC/cholesterol-58 mol% BLM is 1207 mN/m (Needham and Nunn, 1990). When divided by the bilayer thickness of approximately 5 nm this converts to a Young’s modulus of 240 MPa, which is comparable to that of polyethylene (Needham and Nunn, 1990). Cholesterol stiffened BLMs also have a trend for increasing failure strength $\tau_u$, decreasing failure strain $\alpha_c$, and decreasing strain energy $E_s$ (Needham and Nunn, 1990).

Hydrocarbons consist of a chain of carbon atoms whose extra bonding sites are filled by hydrogen atoms. When all carbon atoms in a chain are joined by single bonds the hydrocarbon is referred to as saturated. If the chain has one double bond then it is mono-unsaturated, two double bonds make it di-unsaturated, and many double bonds make it poly-unsaturated. Cholesterol is highly effective at stiffening and strengthening phospholipid bilayers with hydrocarbon chains that are saturated, mono-unsaturated or di-unsaturated. It is much less effective for phospholipids with hydrocarbon chains that are poly-unsaturated (Needham and Nunn, 1990). By contrast, phospholipid bilayers without cholesterol show very little dependence of the degree of saturation on the area expansion modulus $K_a$ (Rawicz et al., 2000).

2.1.2 Atomic force microscopy measurements of BLMs

The atomic force microscope (AFM) was developed in 1986 by Binnig, Quate, and Gerber as a collaboration between IBM and Stanford University (Bennig et al., 1986). Atomic force microscopy is a subclassification of scanning probe microscopy (SPM) in which a sharp probe is run across the specimen surface and information is acquired as a result of interactions between the probe and the surface. The advent of the AFM was made possible by advances in piezoelectric actuators for ultra precise positioning and the ability to manufacture probes with an atomically sharp tip (Albrecht et al., 1990; Bennig et al., 1987).

Only a short time after the invention of the AFM, Egger et al. first used an AFM to generate a topographic image of a planar BLM and were able to resolve the molecular structure of the headgroups (Egger et al., 1990; Weisenhorn et al., 1991). Since then it has quickly gained popularity among researchers as a way to image and characterize the
mechanical properties of BLMs. Other methods for determining the mechanical properties of lipid bilayers such as micropipet aspiration require highly specialized equipment, but atomic force microscopes have many uses and are becoming commonplace in universities and research laboratories. It also has unique technical advantages over other techniques such as the ability to image the specimen’s nanostructure in an aqueous solution in real time, the ability to directly measure localized mechanical properties via force curves, and the ability to perturb the specimen and observe the response (Dufrene and Lee, 2000).

**Surface imaging of specimens using atomic force microscopy**

The AFM probe consists of a cantilevered beam that is made from either silicon or silicon nitride. A tip with a radius of approximately 5 nm protrudes from the end of the cantilever. The tip is swept across the surface of the specimen by a piezoelectric actuator that can control motion in the $X$, $Y$, and $Z$ directions. A laser is reflected off of the back of the cantilever onto a photodiode array which monitors its deflection. The cantilever deflects as it encounters surface features in the specimen. This is detected by the photodiode array and a controller signals the piezoelectric actuator to adjust the $Z$ position of the cantilever so that a constant force is maintained between the cantilever and the specimen. The $Z$ position of the cantilever is recorded at each $X$ − $Y$ location to create a topographic image of the specimen (Veeco, 1997). This setup is illustrated in Figure 2.2.

The two most common methods for scanning a specimen are contact mode and tapping mode. In contact mode the tip is dragged across the surface of the specimen such that it is always touching the specimen. A constant cantilever deflection is maintained by the controller. Contact mode works well for hard specimens and for rough surfaces that have extreme changes in vertical topography. For soft specimens, such as biological specimens, contact mode can cause damage from the tip scraping the surface. In tapping mode the cantilever is oscillated in the vertical direction at or near its resonant frequency as it is scanned over the specimen surface. A constant oscillation amplitude is maintained by the controller. The amplitude of oscillation has to be large enough to prevent the tip from sticking in an absorbed fluid layer that is present on all surfaces in ambient air (Veeco, 1997). No lateral forces are applied to the specimen in tapping mode, making it less damaging to soft specimens. It has been shown that when performing atomic force microscopy on bilayer lipid membranes, contact mode can cause significant damage to the bilayer while tapping
Figure 2.2: Schematic of the feedback loop of an atomic force microscope.

Several papers and literature surveys have been published regarding the interpretation of AFM force curves for the material properties of BLMs and other materials (Bonnell, 1993; Burnham and Colton, 1989; Burnham et al., 1993; Butt et al., 1995; Cappella and Dietler, 1999; Dufrene and Lee, 2000; Weisenhorn et al., 1993). To generate a force curve the AFM tip is held fixed in the $X$ and $Y$ directions while the specimen is moved up and down in the $Z$ direction, causing the cantilever to deflect. The cantilever deflection $\Delta z_c$ is monitored by the photodiode array and the piezoelectric stack position $\Delta z_p$ is monitored by the controller. The cantilever deflection $\Delta z_c$ can be converted into force according to Hooke’s law $F = k\Delta z_c$ where $k$ is the spring constant of the cantilever. The piezoelectric stack position $\Delta z_p$ can be converted into the tip-sample separation distance $D$ using the geometric relationship $D = \Delta z_p - \Delta z_c$ (Butt et al., 1995; Dufrene and Lee, 2000). An example of a typical force $F$ versus distance $D$ curve is shown in Figure 2.3.

Three regions of the force curve provide information about the physical and chemical properties of the specimen (Burnham et al., 1993). The approach portion of the curve gives
Figure 2.3: Example of an AFM force curve generated at Virginia Tech for a steel specimen showing (A) the tip extending, not yet in contact with the specimen, (B) the tip being pulled down by attractive forces near the surface, (C) the cantilever bending upwards as the tip indents into the surface, (D) the tip retracting, (E) surface adhesion holding the tip as it retracts, (F) the tip breaking free of the surface, (G) the tip fully retracted, no longer in contact with the specimen.

information on the surface force between the specimen and the tip, such as van der Waals and electrostatic attractive forces (Dufrene and Lee, 2000). Since the cantilever tip is very small these surface forces are stronger than the gravitational force acting on the tip, and at a certain critical distance will abruptly pull the tip towards the specimen (Butt et al., 1995). The contact portion of the curve can be used to quantify mechanical properties of the specimen such as the local Young’s modulus. The retraction portion of the curve reveals information about adhesive forces that cause the tip to remain stuck to the specimen as it is pulled away. This portion of the curve can be used to estimate the specimen’s surface energy (Dufrene and Lee, 2000).

The Young’s modulus can not be determined directly from the force versus distance curve, but instead a contact mechanics model must be used. The most commonly used model was solved by Hertz for a spherical indenter (Hertz, 1881). Hertz’s model assumes linear elasticity and an infinite sample thickness, but these assumptions are usually violated when probing a BLM. Commercially available AFM tips are too sharp, causing large strains that violate the linear elasticity requirement. Spherical tips have been shown to improve estimates of mechanical properties, but also reduce the resolution of the measurement because of an increased radius of curvature (Dimitriadis et al., 2002). Also, BLMs are extremely
thin (4 to 9 nm) and properties measured using the AFM can be influenced by the supporting substrate, which is usually mica or silicon (Kunneke et al., 2004). Dimitriadis et al. have suggested modifications to the Hertz model to correct for these problems (Dimitriadis et al., 2002). Another difficulty in using the Hertz model is determining when contact occurs between the cantilever tip and the specimen. Although there is a clearly defined kink in the force versus distance curve for a hard material (See Figure 2.3), soft materials tend to have a smooth curve, making the point of contact more ambiguous. Therefore the point of contact is generally used as a curve fitting parameter (Jing and Run-Guang, 2002), and Dimitriadis et al. have performed an error analysis for this parameter (Dimitriadis et al., 2002).

Another non-trivial aspect of implementing an atomic force curve is determining the spring constant of the cantilever. The spring constant $k$ is defined as the ratio of applied normal force from the cantilever tip to the normal deflection of the cantilever at the tip. The spring constant can be determined in a number of ways, including analytical and experimental methods. Clifford and Seah wrote an extensive literature review of analytical methods for calculating spring constant (Clifford and Seah, 2005). Some methods consider the cantilever to be a simple beam (Albrecht et al., 1990), while others consider more complex geometries (Sader, 1995). In these methods significant errors can be induced by uncertainty in the material properties and geometries of the cantilevers. Geometrical parameters are sometimes reported by the manufacturer but are limited by the precision with which the cantilever thickness and tip radius of curvature can be controlled (Dimitriadis et al., 2002; Serry, 2005). For the best accuracy these parameter must be measured by scanning electron microscopy or other suitable methods.

Experimental methods generally produce a more accurate spring constant than analytical methods in exchange for being more time consuming and risking damage to the cantilever. In one method, tungsten spheres of a known mass are attached to the cantilever and the spring constant is proportional to the deflection caused by gravity (Senden and Ducker, 1994). In another method, tungsten spheres are attached to the cantilever and the spring constant is calculated based on the change in resonant frequency (Cleveland et al., 1993). In both of these methods the uncertainty in the added mass can be large (Clifford and Seah, 2005). In a third method, the free vibration of the cantilever due to thermal fluctuations in air is used to determine the spring constant, which has the advantage of
being non-destructive (Hutter and Bechhoefer, 1993; Serry, 2005).

2.1.3 Pressurization of BLMs formed over porous substrates

For this project a new experimental methodology and model has been developed for obtaining the maximum pressure, or failure pressure, that can be withstood by a BLM spanning a circular aperture. An understanding of this behavior has many possible applications in engineering and the science including predicting the maximum stress and strain that can be developed by the biomimetic actuator that has inspired this study. None of the experimental methods in the literature can be used to directly measure this quantity. A short overview of the test setup and some sample results will be presented here and then discussed more thoroughly in subsequent chapters.

A stepper motor is used to move a piston in a linear fashion in order to pressurize a fluid chamber. An opening in the fluid chamber is covered with a porous substrate, and a BLM is formed across that substrate (Hopkinson and Leo, 2007). The porous substrate is clamped in place, sealing the fluid chamber. A custom Labview program was used to operate the stepper motor, and also to record the internal pressure of the fluid chamber via a pressure transducer. Photographs of the test setup are shown in Figure 2.4. Two silver/silver chloride (Ag/AgCl) electrodes are mounted on either side of the porous substrate in order to measure the electrical impedance of the BLM. A photograph of a Ag/AgCl electrode is shown in Figure 2.5, where it has been set in epoxy into a custom threaded fixture so that it can be mounted into the high pressure fluid chamber.

In one series of tests, which are described in Chapter 3, a BLM is formed over a polycarbonate substrate that contains a single micron size pore and is then pressurized. An idealized curve of pressure versus piston displacement for a BLM formed over a single pore is shown in Figure 2.6. The piston pressurizes the BLM by moving at a constant velocity for the duration of the test. Initially the fluid chamber is sealed by the BLM and therefore the pressure increases. At some critical pressure, the BLM fails and allows fluid to escape the chamber, causing pressure to drop almost to zero.

In another series of tests, which are described in Chapter 4, a BLM is formed and then pressurized over a polycarbonate substrate that contains thousands of micron or sub-micron size pores. This multi pore arrangement can be thought of as pressurizing thousands of micro-BLMs simultaneously, which can be useful for applications in which a BLM must
Figure 2.4: Photograph of the stepper motor test fixture, (a) assembled, and (b) disassembled. Photographed by author.

Figure 2.5: Photograph of an Ag/AgCl electrode mounted in a custom threaded fixture. Photographed by author.
cover a large surface area with more stability than can be achieved by a single large pore. An idealized pressure curve for a BLM formed over a multi pore substrate is shown in Figure 2.7. As with the previous series of tests, pressure initially rises when the pores are sealed by micro-BLMs. At a critical peak pressure, enough BLMs fail that the pressure begins to drop. BLM failure pressure follows a statistical distribution, however, and the peak pressure is not high enough to cause all micro-BLMs to fail. Because of this, the pressure does not drop to zero, but instead reaches some steady-state equilibrium value as the piston continues to move.

Fluorescence microscopy has been used to image the BLMs before and after pressurization. For these experiments a fluorescent dye was added to the phospholipid solution. The dye that was chosen illuminates under fluorescent lighting when it is in the presence of phospholipid molecules. A fluorescence microscope image of a BLM that was formed over a multi pore polycarbonate substrate is shown in Figure 2.8. The red circles are micro-BLMs that span a pore.

2.2 Electrical measurements of BLMs

Electric conductors can be classified as either first or second class (Koryta et al., 1993). In a first class conductor electric current is carried by electrons, whereas in a second class conductor it is carried by ions. When an ionic solid, e.g. salt, is dissolved in a solvent,
Figure 2.7: Idealized pressurization curve of multiple BLMs formed over an array of pores.

Figure 2.8: Fluorescence microscope image of a BLM formed over a multi pore substrate. Photographed by author.
e.g. water, it dissociates into a conductive solution of positively and negatively charged ions (Hibbert, 1993). The study of the flow of electric charge through ionic conductors is the basis of electrochemistry. This is highly relevant to the study of BLMs considering that electrolytic solutions are the natural environment of living cells, and that an important function of a cell membrane is to either resist or conduct the motion of ions. Therefore one way to characterize a BLM is to observe its electrical properties by measuring the electrical impedance across the membrane.

In a direct current (DC) electric circuit, voltage $V$ is proportional to current $I$ according to Ohm’s law, $V = IR$. The resistance $R$ is a measure of how much the circuit opposes the flow of electric charge. The inverse of resistance is conductance $G$, which measures how well electric charge can flow through a circuit. Electrical impedance extends this concept to alternating current (AC) circuits in which the applied potential is harmonic. Like resistance, impedance measures the opposition of the flow of electric charge. However, whereas resistance is a scalar value, impedance is a vector consisting of both magnitude $Z$ and phase $\theta$, and is dependent on the frequency of alternating current. The inverse of impedance is admittance, which is also composed of a magnitude and phase. A resistor, which is a device that resists the flow of electric charge, has an impedance of $Z_R = R$ (Dorf and Svoboda, 2006). A capacitor, which is a device that stores electric charge, has an impedance of $Z_C = 1/j\omega C$, where $j = (-1)^{1/2}$, $\omega$ is frequency, and $C$ is capacitance. An inductor, which is a device that generates a magnetic field when charge flows through it, has an impedance of $Z_L = j\omega L$, where $L$ is inductance.

Often times a BLM is modeled as a resistor and a capacitor configured in parallel (Bordi et al., 2002; Naumowicz et al., 2005). Therefore when researchers measure the electrical properties of BLMs, they often report both a resistance ($R_{BLM}$, with units of $\Omega$) and a capacitance ($C_{BLM}$, with units of $F$), or a conductance ($G_{BLM}$, with units of $S$) and capacitance. Since conductance and capacitance are dependent on the area covered by the BLM, it is typical to normalize these values by dividing by area ($G_{BLM}/A$, with units of $S/cm^2$ and $C_{BLM}/A$, with units of $F/cm^2$).

In the literature, quantitative measurements of electrical impedance of a BLM vary widely. This is due to factors such as differences in measurement equipment, frequency range, type and concentration of electrolyte, type of phospholipid, and method of BLM formation. The magnitude of impedance that constitutes a well formed BLM is a subject
that is open to interpretation. Some results that have been reported in the past are reviewed below, and summarized in Table 2.2.

Yoshikawa et al. formed BLMs from soybean lecithin (recall that the terms lecithin and phosphotidylcholine are used interchangeably) using the Langmuir-Blodgett technique on porous filter paper with pore sizes ranging from 0.22 to 10 µm (Yoshikawa et al., 1987). They measured a DC electrical conductance of $G_{BLM}/A = 1.8 \times 10^{-9}$ S/cm$^2$ across the BLM and noted that the conductance increased by an order of 10$^5$ when the BLM was broken by mechanical shock.

In a study with similar experimental parameters, Mountz and Tien also formed BLMs from phosphotidylcholine (PC) on porous filter paper with pore sizes ranging from 0.05 to 8 µm in an electrolyte of 2 mM FeCl$_3$ (Mountz and Tien, 1978). In a test with a pore size of 5 µm, a DC conductance of $G_{BLM}/A = 2.6 \times 10^{-6}$ S/cm$^2$ was measured.

Kobatake et al. formed BLMs using various quantities of dioleyl phosphate (DOPH) on porous filter paper with various pore sizes, using a KCl electrolyte with various concentrations, and with various distances between square platinum electrodes (Kobatake et al., 1970). Using a frequency range of 20 to $3 \times 10^6$ Hz, 2 mM KCl, 1.5 mg/cm$^2$ of DOPH, 2.4 cm of electrode separation, and 5 µm pores, a conductance of $G_{BLM}/A = 5.7 \times 10^{-5}$ S/cm$^2$ and capacitance of $C_{BLM}/A = 8.5 \times 10^{-6}$ F/cm$^2$ was measured. Other impedance measurements varied according to other test parameters.

Naumowicz et al. measured the electrical impedance across an unsupported BLM formed from a mixture of egg lecithin and cholesterol (CHOL) with varying proportions of cholesterol in an electrolyte of 100 mM KCl using Ag/AgCl electrodes (Naumowicz et al., 2005). With a frequency range of $10^{-1}$ to $10^5$ Hz, they measured BLM conductances and capacitances of $G_{BLM}/A = 4.4 \times 10^{-6}$ S/cm$^2$ and $C_{BLM}/A = 6.2 \times 10^{-7}$ F/cm$^2$ for pure lecithin, $G_{BLM}/A = 4.7 \times 10^{-7}$ S/cm$^2$ and $C_{BLM}/A = 8.8 \times 10^{-7}$ F/cm$^2$ for pure cholesterol, and $G_{BLM}/A = 1.0 \times 10^{-6}$ S/cm$^2$ and $C_{BLM}/A = 7.9 \times 10^{-7}$ F/cm$^2$ for a mixture of lecithin and 50 mol% cholesterol.

Bordi et al. measured the electrical impedance of dipalmitoylphosphatidic acid (DPPA), 1,2-dipalmitoyl-snglycero-3-Phosphatidylcholine (DPPC), and linoleic acid (LIN) BLMs which were formed directly on the surface of a freshly cleaved silver electrode (Bordi et al., 2002). Using a frequency range of $10^3$ to $10^6$ Hz with electrolytes of 1 mM KCl, 10 mM KCl, 0.6 mM CaCl$_2$, 0.6 mM MgCl$_2$, and 0.6 mM ZnCl$_2$, they measured a range of
Table 2.2: Summary of normalized conductance and capacitance values for BLMs found in the literature.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Substrate</th>
<th>Electrolyte</th>
<th>Frequency range (Hz)</th>
<th>$G_{BLM}/A$ (S/cm$^2$)</th>
<th>$C_{BLM}/A$ (F/cm$^2$)</th>
<th>Ref$^i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>filter paper</td>
<td>DC</td>
<td></td>
<td>$1.8 \times 10^{-9}$</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>PC</td>
<td>filter paper</td>
<td>2 mM FeCl$_3$</td>
<td>DC</td>
<td>$2.6 \times 10^{-6}$</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>DOPH</td>
<td>filter paper</td>
<td>2 mM KCl</td>
<td>20 to $3 \times 10^6$</td>
<td>$5.7 \times 10^{-5}$</td>
<td>$8.5 \times 10^{-6}$</td>
<td>C</td>
</tr>
<tr>
<td>PC</td>
<td>teflon</td>
<td>100 mM KCl</td>
<td>$10^{-1}$ to $10^5$</td>
<td>$4.4 \times 10^{-6}$</td>
<td>$6.2 \times 10^{-7}$</td>
<td>D</td>
</tr>
<tr>
<td>CHOL</td>
<td>teflon</td>
<td>100 mM KCl</td>
<td>$10^{-1}$ to $10^5$</td>
<td>$4.7 \times 10^{-7}$</td>
<td>$8.8 \times 10^{-7}$</td>
<td>D</td>
</tr>
<tr>
<td>PC/CHOL-50 mol%</td>
<td>teflon</td>
<td>100 mM KCl</td>
<td>$10^{-1}$ to $10^5$</td>
<td>$1.0 \times 10^{-6}$</td>
<td>$7.9 \times 10^{-7}$</td>
<td>D</td>
</tr>
<tr>
<td>DPPA</td>
<td>silver</td>
<td>1 mM KCl</td>
<td>$10^3$ to $10^6$</td>
<td>$1.0 \times 10^{-1}$</td>
<td>$9.2 \times 10^{-7}$</td>
<td>E</td>
</tr>
<tr>
<td>DPPC</td>
<td>silver</td>
<td>1 mM KCl</td>
<td>$10^3$ to $10^6$</td>
<td>$3.7 \times 10^{-2}$</td>
<td>$9.0 \times 10^{-8}$</td>
<td>E</td>
</tr>
<tr>
<td>LIN</td>
<td>silver</td>
<td>10 mM KCl</td>
<td>$10^3$ to $10^6$</td>
<td>$4.2 \times 10^{-2}$</td>
<td>$4.6 \times 10^{-7}$</td>
<td>E</td>
</tr>
</tbody>
</table>

1. A = (Yoshikawa et al., 1987); B = (Mountz and Tien, 1978); C = (Kobatake et al., 1970); D = (Naumowicz et al., 2005); E = (Bordi et al., 2002)

BLM conductances from $G_{BLM}/A = 2.0 \times 10^{-2}$ to $1.0 \times 10^{-1}$ S/cm$^2$ and capacitances from $C_{BLM}/A = 5.0 \times 10^{-8}$ to $9.2 \times 10^{-7}$ F/cm$^2$. The authors note that conductances for BLMs that are supported on a substrate are known to be larger than that of unsupported BLMs, which are typically on the order of $10^{-8}$ to $10^{-7}$ S/cm$^2$. They believe that this is due to a greater occurrence of defects in supported BLMs.

2.2.1 Equivalent circuit of a BLM system

An impedance measurement of a BLM normally has several components other than the BLM itself. Other components that contribute to the impedance include the electrode/electrolyte interface, the electrolyte, and the supporting substrate. Figure 2.9 shows one representation for how these components can contribute to the total equivalent circuit.

The surface of an electrode in solution is surrounded by a double layer of ions (Hibbert, 1993; Rieger, 1994). In order to maintain charge neutrality, any charged surface that is submerged in an electrolyte solution will attract ions of the opposite charge and repel ions of the same charge. The charge on the surface and the oppositely charged ions that collect on the surface form two parallel layers known as the double layer. This is analogous to a capacitor which has two plates of separated charge. The interface between the electrode and electrolyte is often represented by a resistor $R_i$ in parallel with a capacitor $C_i$ (Bordi et al., 2002). This model, however, does not account for diffusion and mixing of ions at the
interface, and therefore the Warburg impedance was developed to better model these mass transfer phenomena (Macdonald, 2005). The electrode/electrolyte interface is sometimes modeled as a resistor $R_i$ in series with a Warburg circuit element $Y_i$, which are in parallel with a capacitor $C_i$.

The resistance of the electrolyte $R_e$ is analogous to that of a solid conductor, $R = \rho L/A$, where $\rho$ is resistivity, $L$ is length, and $A$ is cross sectional area of the conductive medium. Conductivity $\kappa$ is the inverse of resistivity, $\kappa = 1/\rho$, and because the conductivity of an electrolyte is dependent on the concentration of ions $c$, the molar conductivity $\Lambda$ is defined as $\Lambda = \kappa/c$. Conductance increases as ion concentration increases. Therefore the overall resistance of an electrolyte as a function of molar conductivity is,

$$R_e = \frac{L}{\Lambda c A}. \quad (2.4)$$

Although researchers have developed empirical models to predict the molar conductivity of electrolytes (most notably the Onsager equation), there is also tabulated experimental data that is widely available for the conductivity of most electrolytes (Hibbert, 1993; Rieger, 1994).

If an electrolyte solution is divided by a porous partition, then the partition will increase the electrical impedance of the solution. The resistance associated with a pore can
be divided into two parts. One is the convergence resistance $R_c$, which is the result of a funneling effect as the ions are restricted from a large cross sectional area of motion to a smaller one (Hall, 1975). Hall has estimated the convergence resistance as,

$$R_c = \frac{\rho}{4a},$$

(2.5)

where $a$ is the pore radius. The other part is the resistance within the pore $R_p$. Since $R = \rho L/A$, the resistance within the pore is,

$$R_p = \frac{l \rho}{\pi a^2},$$

(2.6)

where $l$ is the length of the pore (Wonderlin et al., 1990). These two resistances together are referred to as the access resistance $R_a = 2R_c + R_p$. The convergence resistance $R_c$ is multiplied by a factor of two to account for the convergence of ions on either side of an open pore.

The impedance of the BLM is commonly represented by a resistor $R_{BLM}$ in parallel with a capacitor $C_{BLM}$ (Bordi et al., 2002; Naumowicz et al., 2005). The BLM is a physical barrier to ion motion which gives it resistance. It also separates and stores charge which gives it capacitance.

### 2.2.2 Sample impedance measurements of components of a BLM system

The following control experiments were used to measure the impedance contributions of the individual electrical components that were outlined in Section 2.2.1. The first experiment was to measure the impedance of an electrolyte and electrode interface. Next the impedance of an electrolyte with a single pore partition was measured. Finally impedance was measured for a multi porous partition. After these three control experiments are presented, two other experiments will be discussed in which impedance is measured for a BLM. In one case the BLM is formed over a single pore substrate and in the other case the BLM is formed over a multi pore substrate.

A custom test fixture was designed to measure the impedance of an electrolyte solution with or without a porous partition. Shown in Figure 2.10, the test fixture consists of two pieces of polycarbonate tube (9.5 mm inner diameter and 12.7 mm outer diameter) with square polycarbonate base plates attached to both ends. The base plates bolt together, joining the two tubes and allowing a porous partition to be clamped between the two tubes.
Rubber gaskets are used to seal the partition from leaking. Three holes have been drilled into the top of each tube to allow silver/silver chloride (Ag/AgCl) electrodes (model EP2, World Precision Instruments, Inc., Sarasota, FL) to be inserted into the test fixture at various spacings. When assembled, the spacing between the Ag/AgCl electrodes is 30 mm, 60 mm, or 90 mm. The test fixture was filled with 0.1 M NaCl (100.0% purity, Mallinckrodt Baker, Inc., Paris, KY) in dionized water solution. An Autolab PGSTAT 12 impedance analyzer with FRA 2 module (Eco Chemie B.V., The Netherlands) was used to measure the impedance between the two electrodes. A frequency range of $10^{-1}$ to $10^5$ Hz was used to record 30 data points with logarithmic spacing at an amplitude of 50 mV.

**Impedance measurements of an electrolyte**

In the first set of control experiments the impedance of the electrolyte (0.1 M NaCl) and electrode interface was measured. Ag/AgCl electrodes were spaced at 30 mm, 60 mm, and 90 mm with no partition between them. The electrode/electrolyte interface is modeled using a resistor and Warburg circuit element in parallel with a capacitor. The electrolyte is modeled as a resistor. This model of an electrode interface combined with the electrolyte resistance make up the Randle equivalent circuit, shown in Figure 2.11 (Macdonald, 2006). The impedance for a resistor is $Z_R = R$, for a capacitor is $Z_C = 1/j\omega C$, and for a Warburg
element is \( Z_W = 1/(j\omega)^{1/2}Y \), where \( Y \) is a constant for the Warburg circuit element. The total impedance of the circuit is the combination of the interface impedance \( Z_i \) and the electrode impedance \( Z_e \),

\[
Z_{tot} = 2Z_i + Z_e. \tag{2.7}
\]

Substituting components and reducing gives a total impedance of,

\[
Z_{tot} = 2 \left( \frac{1 + R_iY_i(j\omega)^{1/2}}{Y_i(j\omega)^{1/2} + C_i(j\omega) + R_iY_iC_i(j\omega)^{3/2}} + R_e \right). \tag{2.8}
\]

At low frequencies both the electrode interface \( (Z_i) \) and the electrolyte \( (Z_e) \) contribute to the total impedance of the system. At high frequencies, however, the impedance of the electrode interface approaches zero, and only the electrolyte resistance remains.

Representative impedance measurements are shown in Figure 2.12. From the impedance data at high frequency, the resistance of the electrolyte for 30 mm electrode spacing is \( R_e = 478 \ \Omega \). A simple calculation using Equation 2.4 produces a reasonable prediction of this experimental result. For a length of \( L = 30 \) mm, a molar conductivity for NaCl of \( \Lambda = 107 \ \text{cm}^2/\Omega \text{mol} \) (Rieger, 1994), an electrolyte concentration of \( c = 0.1 \text{ M} \), and a cross sectional area of the tube of \( A = 71.3 \ \text{mm}^2 \), the predicted electrolyte resistance is \( R_e = 393 \ \Omega \). At electrode spacings of 60 mm and 90 mm (2 and 3 times the 30 mm spacing), the electrolyte resistance was experimentally measured as 912 \( \Omega \) and 1310 \( \Omega \) respectively (1.9 and 2.7 times the resistance at 30 mm spacing). This confirms that the electrolyte resistance is approximately proportional to the distance between electrodes.

The electrode/electrolyte interface also contributes to the impedance response of this system, particularly at low and middle frequencies. The model in Figure 2.12 provides a reasonable fit of the data, although it does not predict phase as accurately as impedance magnitude. \( R_i, Y_i, C_i, \) and \( R_e \) were curve-fitted to the data and are summarized in Table 2.3.
Figure 2.12: Impedance measurement of 0.1 M NaCl with 30, 60, and 90 mm Ag/AgCl electrode separation.

Table 2.3: Summary of curve-fitted electrolyte and electrode interface impedance parameters arranged by electrode separation distance.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>30 mm</th>
<th>60 mm</th>
<th>90 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_e$ ($\Omega$)</td>
<td>478</td>
<td>912</td>
<td>1310</td>
</tr>
<tr>
<td>$R_i$ ($\Omega$)</td>
<td>600</td>
<td>700</td>
<td>950</td>
</tr>
<tr>
<td>$Y_i$ ($S \times 10^{-3}$)</td>
<td>2.7</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>$C_i$ ($\mu F$)</td>
<td>35</td>
<td>25</td>
<td>20</td>
</tr>
</tbody>
</table>
Impedance measurements of an electrolyte with a single pore partition

In the second set of control experiments impedance was measured with a single pore partition between the electrodes. The partition was a sheet of 0.79 mm thick polytetrafluoroethylene (teflon) with a single circular hole drilled through its center. The hole size was varied for different trials of the experiment, and included 5.0 mm, 3.1 mm, 1.6 mm, 0.95 mm, and 0.50 mm diameters. Electrodes were spaced 30 mm apart and the electrolyte was 0.1 M NaCl. For comparison, one impedance measurement with no partition is shown for an electrode spacing of 30 mm (data was used from the previous section). The partition acts like a resistor in series with the electrode interface and electrolyte, shown schematically as the circuit in Figure 2.13. The total impedance of the circuit is,

$$Z_{\text{tot}} = 2Z_i + Z_e + Z_a,$$

where the access impedance is defined as $Z_a = 2Z_c + Z_p$. Substituting components and reducing gives a total impedance of,

$$Z_{\text{tot}} = \frac{1 + R_iY_i(j\omega)^{1/2}}{Y_i(j\omega)^{1/2} + C_i(j\omega) + R_iY_iC_i(j\omega)^{3/2}} + R_e + 2R_c + R_p. \quad (2.10)$$

The impedance measurements from these trials are shown in Figure 2.14. As would be expected, a smaller pore size causes greater restriction of ion motion and raises the impedance magnitude. Equations 2.5 and 2.6 were used to model the resistance of the partition. Again, molar conductivity for 0.1 M NaCl is $\Lambda = 107 \text{ cm}^2/\Omega \text{ mol}$, which using the relationship $\rho = 1/\Lambda c$ gives a resistivity of $\rho = 0.936 \Omega \text{ m}$. For this range of pore sizes, the model for the access resistance of a pore is quite accurate.

Impedance measurements of an electrolyte with a multi pore partition

In the third set of control experiments impedance was measured with a multi porous partition between the electrodes. The partition was an Isopore track-etched polycarbonate
Figure 2.14: Impedance measurement of 0.1 M NaCl separated by a partition with various diameter pores.

Table 2.4: Geometric parameters for Isopore polycarbonate filters.

<table>
<thead>
<tr>
<th>Pore diameter (µm)</th>
<th>Pore density (pores/cm²)</th>
<th>Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>3 × 10⁷</td>
<td>9</td>
</tr>
<tr>
<td>1.2</td>
<td>2 × 10⁶</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>2 × 10⁷</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>4 × 10⁵</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>1 × 10⁵</td>
<td>10</td>
</tr>
</tbody>
</table>

filter (Millipore, Bedford, MA) with various pore sizes including 0.6, 1.2, 2, 5, and 10 µm diameter. Geometric parameters for each pore size that were used in the model were obtained from the manufacturer and are given in Table 2.4. Electrode spacing was 30 mm and the electrolyte was 0.1 M NaCl. Again, for comparison one trial is shown for an electrolyte with no partition, where the electrode spacing was also 30 mm.

In this case the partition contains many pores, but the access resistance of each pore is the same as that of a single pore which was modeled by Equations 2.5 and 2.6. The resistance of the pores combines in parallel, shown schematically in Figure 2.15, where \( n \) is the total number of pores in the partition. In this case the total impedance of the system is,

\[
Z_{tot} = 2Z_i + Z_e + \frac{Z_a}{n}.
\] (2.11)
Figure 2.15: Equivalent circuit for the impedance of an electrolyte with a multi pore partition.

Substituting components and reducing gives a total impedance of,

$$Z_{\text{tot}} = 2 \left( 1 + R_i Y_i (j\omega)^{1/2} \right) \frac{1}{2} Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2} + R_e + \frac{2R_e + R_p}{n}. \quad (2.12)$$

Impedance measurements for these trials are shown in Figure 2.16. Because the pores are arranged in parallel, a higher number of pores $n$ reduces the electrical resistance of the partition. The number of pores is very large for these partitions, varying from $n = 7 \times 10^4$ for the $10 \mu$m pore size to $n = 2 \times 10^8$ for the $0.6 \mu$m pore size (the total area of the filter is that of a circle with a 9.5 mm diameter). Because of this the multi porous filter adds a negligible amount of resistance to the overall impedance of the system, as can be seen in Figure 2.16. The model confirms this, and shows only a slight increase of 1 to 3 $\Omega$ in resistance when a multi pore partition is present. The increase in resistance is so small that all model lines appear to fall directly on top of each other.

Sample impedance measurements of a BLM formed over a single pore

For this study, BLMs were formed over porous substrates and then pressurized until failure using the test setup that was described in Section 2.1.3. Impedance was measured before and after failure. The following are samples of some generic impedance measurements that were made using the pressurization test fixture from Figure 2.4. This test fixture consists of an aluminum lower chamber and a polycarbonate upper chamber, with the BLM and substrate clamped horizontally between them. Two Ag/AgCl electrodes are spaced 18.4 mm apart in a chamber with a 3.2 mm diameter bore. In this sample experiment a BLM was formed over a single micron size pore and pressurized until failure. These results will be discussed in a general fashion with more detailed explanation in Chapter 3.
In Figure 2.17, the total impedance before BLM failure is represented schematically as the combination of the electrode interface, the electrolyte, the substrate, and the BLM. For impedance measurements of a BLM formed over a single pore substrate, the impedance of the substrate itself was found to make a significant contribution to the total impedance of the system. Both the BLM and the substrate are modeled as a resistor and capacitor in parallel. The total impedance of the system is,

$$Z_{\text{tot}} = 2Z_i + Z_e + Z_{\text{BLM}} Z_{\text{sub}}.$$

Substituting components and reducing gives a total impedance of,

$$Z_{\text{tot}} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2}} + R_e + R_{\text{BLM}} R_{\text{sub}}$$

$$+ R_{\text{BLM}} (1 + j\omega C_{\text{sub}} R_{\text{sub}}) + R_{\text{sub}} (1 + j\omega C_{\text{BLM}} R_{\text{BLM}}).$$

Figure 2.18 (a) shows the experimental data and model before BLM failure. This model represents the experimental data quite well across the entire frequency range. The resistance $R_{\text{sub}}$ and capacitance $C_{\text{sub}}$ of the substrate are determined by control experiments that will be described in subsequent chapters, and the resistance $R_{\text{BLM}}$ and the capacitance $C_{\text{BLM}}$ of the BLM are used as fitting parameters.

After the BLM has failed, the partition has effectively become an open pore. The access impedance of the pore and the impedance of the substrate combine in parallel, as is
Figure 2.17: Equivalent circuit for the impedance of a BLM formed over a single pore.

Figure 2.18: Impedance of a BLM formed over a single micron size pore (a) before failure, and (b) after failure.
Figure 2.19: Equivalent circuit for the impedance of a BLM formed over a single pore after failure.

represented schematically by Figure 2.19, and mathematically by,

\[ Z_{\text{tot}} = 2Z_i + Z_e + \frac{Z_aZ_{\text{sub}}}{Z_a + Z_{\text{sub}}} \]  

(2.15)

Substituting components and reducing gives a total impedance of,

\[
Z_{\text{tot}} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2}} + R_e + \frac{R_a R_{\text{sub}}}{R_a (1 + j\omega C_{\text{sub}} R_{\text{sub}}) + R_{\text{sub}}} \]  

(2.16)

Impedance data of a single micron size pore after BLM failure is shown in Figure 2.18 (b). The access resistance is calculated using Equations 2.5 and 2.6. The model fits the data for an open pore of micron size reasonably well. The measurements of impedance before and after pressurization make for a useful check of whether or not the BLM is formed across the pore. The before failure measurement has an impedance signature that is similar to a solid substrate with no pores. This indicates that before pressurization the single pore is well covered by the BLM. The after failure measurement has an impedance with a reduction in magnitude of approximately three orders of magnitude, and the corresponding model shows that it has the impedance signature of a single micron size pore. This indicates that after pressurization the BLM has failed, leaving the pore open. Again, further discussion is in Chapter 3.

**Sample impedance measurements of a BLM formed over multiple pores**

BLMs were also formed over multi pore substrates and pressurized until failure using the same test setup. Impedance was again measured before and after failure. In this generic sample experiment, a BLM was formed over multiple micron size pores and pressurized until failure.
Before failure the BLMs act in parallel, shown schematically in Figure 2.20. For a multi pore arrangement, the substrate itself does not make a significant contribution to the impedance of the system, as was shown by the control data in Figure 2.16 and therefore it is omitted from the model. Mathematically the model is represented as,

$$Z_{tot} = 2Z_i + Z_e + \frac{Z_{BLM}}{n}. \quad (2.17)$$

Substituting components and reducing gives a total impedance of,

$$Z_{tot} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2}} + R_e + \frac{R_{BLM}}{n (1 + j\omega C_{BLM} R_{BLM})}. \quad (2.18)$$

This model was fitted to the before failure experimental data as shown in Figure 2.21 (a). The number of pores can be calculated based on the known pore density of the substrate, which is specified by the manufacturer. The resistance and capacitance of the micro-BLMs are used as fitting parameters to fit the model to the data.

After the bulk failure point some micro-BLMs burst and leave an open pore, while others remain intact. Data that will be presented in Chapter 4 indicates that only a small proportion of micro-BLMs actually fail during these tests. Therefore the total impedance of this system after failure is a combination of BLM impedances and pore access resistances acting in parallel, shown schematically in Figure 2.22. The proportion $\chi_{tot}$ of micro-BLMs
Figure 2.21: Impedance of a BLM formed over multiple micron size pores (a) before failure, and (b) after failure. The dash-dot line is a model that assumes all BLMs fail after pressurization. The dotted line is a model that assumes only proportion $\chi_{tot}$ of BLMs fail after pressurization.

that have failed can be used as a fitting parameter. This is represented mathematically as,

$$Z_{tot} = 2Z_i + Z_e + \frac{Z_{BLM}Z_a}{n(\chi_{tot}Z_{BLM} + (1 - \chi_{tot})Z_a)}.$$  \hspace{0.5cm} (2.19)

Substituting components and reducing gives a total impedance of,

$$Z_{tot} = 2 \frac{1 + R_iY_i(j\omega)^{1/2}}{Y_i(j\omega)^{1/2} + C_i(j\omega) + R_iY_iC_i(j\omega)^{3/2}} + R_e + \frac{RaR_{BLM}}{R_a(1 + j\omega C_{BLM}R_{BLM})n(1 - \chi_{tot}) + R_{BLM}n\chi_{tot}}.$$  \hspace{0.5cm} (2.20)

Figure 2.21 (b) shows the experimental data and model for the multi pore BLMs after failure. The values for $R_{BLM}$ and $C_{BLM}$ used in the model are from the curve fitting analysis of the impedance before failure. Two model lines are shown. For the dash-dot model line it is assumed that all micro-BLMs have failed during pressurization ($\chi_{tot} = 1$). In reality, not all micro-BLMs fail, so in this case the model under-predicts the impedance of the system. For the dotted model line the proportion of failed micro-BLMs has been fitted to the experimental data and it is found that $\chi_{tot} << 1$, meaning that only a fraction of the total number of BLMs have failed during this test. As with the single pore test, the magnitude of impedance was found to drop significantly (by about two orders of magnitude) after pressurization. This is a good indicator that BLMs have failed, opening up conductive pathways for ion flow. These measurements will be analyzed in more detail in Chapter 4.
Figure 2.22: Equivalent circuit for the impedance of a BLM formed over multiple pores after failure.
2.3 Chapter summary

In this chapter methods have been reviewed from the literature for measuring the mechanical and electrical properties of bilayer lipid membranes, and the methods that will be used in this research have been introduced. Mechanical tests from the literature include micropipet aspiration, atomic force microscopy, and numerous others, which were used to measure mechanical properties such as the Young’s modulus, the area modulus, ultimate tensile strength, shear modulus, bending modulus, and others. The values of these properties varied widely based on experimental methodology. For example, the Young’s modulus for various lipid compounds was estimated to be between 0.2 and 160 MPa for the methods that were surveyed. Also a new mechanical test method that is used for measuring the maximum pressure that a BLM can withstand over a porous substrate has been introduced. Currently there are no methods in the literature for measuring this quantity. The results from this test are presented in the subsequent chapters.

A literature survey was also presented for measurements of the electrical properties of BLMs. A BLM is typically represented as a resistor and capacitor combined in parallel. Again, the results in the literature varied due to many differences in test methodology. Normalized conductance values ranged from $G_{BLM}/A = 10^{-1}$ to $10^{-9}$ S/cm$^2$ and normalized capacitance values were from $C_{BLM}/A = 10^{-6}$ to $10^{-8}$ F/cm$^2$. In subsequent chapters the electrical impedance is measured for a BLM before and after failure due to pressurization. These measurements have many components, though, including the electrode/electrolyte interface, the electrolyte, the porous substrate, and the BLM. Therefore control trials were presented in which the impedance of each component has been analyzed using sample measurements. Table 2.5 is a summary of the different impedance measurements that were made, including the circuit schematics and equations.
<table>
<thead>
<tr>
<th>Diagram of measurement</th>
<th>Electrical schematic</th>
<th>Equation</th>
</tr>
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<tbody>
<tr>
<td>Electrode interface and electrolyte</td>
<td><img src="image" alt="Electrode Interface Diagram" /></td>
<td>$Z_{sw} = 2Z_r + Z_e$</td>
</tr>
<tr>
<td>Single pore partition</td>
<td><img src="image" alt="Single Pore Diagram" /></td>
<td>$Z_{sw} = 2Z_r + Z_e + Z_s$</td>
</tr>
<tr>
<td>Multi pore partition</td>
<td><img src="image" alt="Multi Pore Diagram" /></td>
<td>$Z_{sw} = 2Z_r + Z_e + \frac{Z_{ion}Z_{tot}}{Z_{ion} + Z_{tot}}$</td>
</tr>
<tr>
<td>BLM over a single pore</td>
<td><img src="image" alt="BLM Single Pore Diagram" /></td>
<td>$Z_{sw} = 2Z_r + Z_e + \frac{Z_{n}Z_{tot}}{Z_{n} + Z_{tot}}$</td>
</tr>
<tr>
<td>Failed BLM over a single pore</td>
<td><img src="image" alt="Failed BLM Single Pore Diagram" /></td>
<td>$Z_{sw} = 2Z_r + Z_e + \frac{Z_{n}Z_{tot}}{Z_{n} + Z_{tot}}$</td>
</tr>
<tr>
<td>BLM over multi pores</td>
<td><img src="image" alt="BLM Multi Pore Diagram" /></td>
<td>$Z_{sw} = 2Z_r + Z_e + \frac{Z_{BLM}}{Z_{BLM} + Z_{tot}}$</td>
</tr>
<tr>
<td>Failed BLM over multi pores</td>
<td><img src="image" alt="Failed BLM Multi Pore Diagram" /></td>
<td>$Z_{sw} = 2Z_r + Z_e + \frac{Z_{BLM}Z_{tot}}{Z_{BLM} + (0 - Z_{n})}$</td>
</tr>
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</table>
Chapter 3

Pressurization of BLMs Formed Over a Single Pore Substrate

3.1 Introduction

In this chapter bilayer lipid membranes (BLMs) are formed over a substrate containing a single pore, ranging in size from 5 µm to 20 µm diameter, and the BLMs are pressurized until failure. BLMs are pressurized using a custom test fixture that uses a stepper motor and piston assembly to apply pressure in very fine increments. When the piston moves it pressurizes a fluid chamber inside of the test fixture, which subsequently pressurizes the BLM. Pressure is recorded for the duration of each test, and electrical impedance is recorded before and after failure.

Lipids that are tested include 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphatidylcholine (SOPC) and mixtures of SOPC and cholesterol (CHOL). SOPC is used because phosphatidylcholine (PC) based phospholipids are a major component of BLMs in cell membranes, and also because of its common use for mechanical testing in the literature (Needham and Zhelev, 1996). Mixtures of SOPC/CHOL are used because studies have shown that cholesterol increases the strength of BLMs (Needham and Nunn, 1990). They have further concluded that cholesterol concentrations of 50 to 60 mol% will maximize the strength of an SOPC/CHOL BLM, which was the basis for the concentration chosen for this study.

The data from these experiments is used to plot pressure as a function of piston displacement. This characteristic pressure curve of a BLM is modeled according to the
pressurization and flow of fluid through the porous substrate. Pressure initially increases as the piston moved until it reaches the maximum pressure that can be withstood by the BLM, at which time the BLM fails and the pressure drops as fluid escapes through the porous substrate (Hopkinson and Leo, 2007).

The stepper motor test fixture has silver/silver chloride (Ag/AgCl) electrodes mounted on either side of the BLM. For each test electrical impedance is measured between the two electrodes before and after BLM failure. Impedance is observed to drop by nearly three orders of magnitude after BLM failure. An equivalent electrical circuit is developed for these two cases, before failure and after failure, and this is used to model these impedance measurements.

3.2 Materials and methods

3.2.1 Phospholipid preparation

SOPC and cholesterol were purchased in powder form from Avanti Polar Lipids (Alabaster, AL). SOPC powder was dissolved in n-decane (99% purity, Alfa Aesar, Ward Hill, MA) at a concentration of 40 mg/mL and mixed for 30 minutes using a sonicator (model 50, VWR, West Chester, PA). For some experiments 50 mol% cholesterol was mixed with SOPC to create stronger BLMs. An aqueous salt solution was prepared with 0.1 M NaCl (100.0% purity, Mallinckrodt Baker, Inc., Paris, KY) and deionized water. The BLMs were reconstituted in the aqueous salt solution over modified track etched polycarbonate membrane filters acquired from Sterlitech (Kent, WA). The porous polycarbonate substrates were treated by the manufacturer with a hydrophilic polyvinylpyrrolidone (PVP) coating which facilitates the formation of a BLM on its surface. Tests were performed for pore diameters of 5 µm, 8 µm, 10 µm, 12 µm, 14 µm, and 20 µm. These porous substrates contained an array of many pores, but they were modified in order to isolate a single pore. A needle was used to puncture a small hole with approximately a 50 µm diameter into a piece of clear tape (model 3710, 3M, St. Paul, MN). The tape was then firmly pressed onto a porous substrate and imaged with a microscope, an example of which is shown in Figure 3.1. All pores that were covered by the tape were blocked from use, but any pores that were positioned over the hole remained open and clear. Substrates in which a single pore was isolated were used for testing. For modeling purposes it was also necessary to know the
Figure 3.1: Microscope image of a multi pore substrate that has been masked to isolate a single pore with (a) 100 times magnification and (b) 400 times magnification. Photographed by author.

Table 3.1: Thickness specifications for Sterlitech porous polycarbonate substrates.

<table>
<thead>
<tr>
<th>Pore Dia. ($\mu$m)</th>
<th>Thickness ($\mu$m)</th>
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<tbody>
<tr>
<td>5</td>
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<td>20</td>
<td>3</td>
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</tbody>
</table>

substrate thickness, which was provided by the manufacturer and is reported in Table 3.1.

3.2.2 Pressurization test procedure

The test fixture was composed of a stepper motor (model L1MGJ-M200XX060, EAD Motors, Dover, NH) used to drive a lead screw in a linear motion (see Figures 3.2 and 3.3). A stainless steel piston with an outside diameter of 12.2 mm was attached to the end of the lead screw, which had a thread pitch of 1.57 threads/mm. For each step the motor turns $0.9^\circ$, which translates into a linear piston motion of $1.59 \, \mu$m. The piston was used to pressurize a lower fluid chamber inside of the aluminum body of the test fixture, which had an internal volume of 5.0 mL. The lower fluid chamber was connected to a polycarbonate upper chamber via a 3.2 mm diameter hole. The lower and upper chambers were sealed using rubber gaskets. The porous substrate was clamped between the lower and upper fluid chambers so that the lower fluid chamber was under pressure and the upper chamber was open to the atmosphere. A pressure relief valve was connected to the lower fluid chamber and a polycarbonate fluid reservoir. The pressure relief valve remained open.
while the porous substrate was clamped into place and was then closed before the test was started. This procedure was necessary to prevent an initial pressure buildup during assembly. Pressure was measured using a pressure transducer with a range of 0 to 690 kPa (model PX181B-100G5V, Omega Engineering, Inc., Stamford, CT), which was reported by the manufacturer to have an accuracy of 0.3% of full scale. Pressure was recorded using a multifunction data acquisition module (model USB-6009, National Instruments, Austin, TX) and Labview software (National Instruments, Austin, TX).

For each trial a porous substrate was coated with 5 $\mu$L of lipid/n-decane mixture by using a pipettor (model Calibra 822, Socorex, Switzerland) and was then clamped between the lower and upper fluid chambers. Pressure was slowly applied by running the stepper motor at a fixed frequency of 1 Hz for the entire duration of the test. The failure pressure of the BLM was determined at the point when pressure began to decrease. Ten trials were performed for each combination of lipid mixture and substrate pore size. Control trials were also performed for each pore size by pressurizing a porous substrate with no BLM. The stepper motor was again run at a frequency of 1 Hz and pressure was recorded.

### 3.2.3 Electrical impedance test procedure

An Ag/AgCl electrode (model EP2, World Precision Instruments, Inc., Sarasota, FL) was mounted in the stepper motor test fixture on either side of the porous substrate so that it was submerged in the salt solution. The two electrodes were spaced 18.4 mm apart in a chamber with a 3.2 mm diameter bore with the porous substrate halfway between
Figure 3.3: Photograph of the stepper motor test fixture (a) assembled and (b) disassembled. Photographed by author.

The electrical impedance was measured across the BLM using an impedance analyzer (Autolab PGSTAT 12 with FRA 2 module, Eco Chemie B.V., The Netherlands) (Tien and Ottova, 1999). The impedance was recorded over a frequency range of $10^{-1}$ to $10^5$ Hz with a resolution of 30 data points taken with logarithmic spacing. Measurements were taken once at the start of the test before the BLM was pressurized and once at the end of the test after the BLM had failed.

Three control trials were performed for the electrical impedance measurements. In the first control trial, impedance was measured across the electrodes with no partition between them. A model was used to determine the impedance parameters for the electrolyte and electrode/electrolyte interface. In the second control trial, impedance was measured across a solid partition that contained no pores. This substrate was fabricated by using a porous polycarbonate substrate and using tape to mask over all pores. A model was used to determine the impedance parameters for the substrate. For the third control trial, impedance was measured across a partition with a single $8 \mu m$ pore and no BLM. The impedance contribution of the open pore was predicted using a model.
3.3 Modeling

3.3.1 Modeling of BLM pressure curves

The pressurization curve of the BLMs in this study can be modeled according to the pressurization of fluid and the flow of fluid through the porous substrate. It has been experimentally determined that after all visible air bubbles have been bled from the lower chamber, approximately 1% by volume of the fluid is composed of dissolved air. Therefore it is a basic premise of this model that the change in pressure inside the lower chamber is a result of the contraction and expansion of this volume of air according to Boyle’s law, \( PV = k \), where \( P \) is absolute pressure, \( V \) is volume, and \( k \) is a constant of proportionality.

The constant \( k \) can be calculated according to the initial test conditions as,

\[
k = \frac{P_{\text{atm}} V_{i,\text{air}}}{V_{i,\text{air}}}.
\]

(3.1)

where the initial pressure \( P_{\text{atm}} \) is one atmosphere, and the initial volume of air \( V_{i,\text{air}} \) is 1% of the internal volume of the lower test chamber.

It is assumed that at the outset of each trial a BLM covers the substrate pores, effectively sealing the lower chamber of the test fixture. As the stepper motor pushes the piston forward, it reduces the internal volume of the lower chamber by the amount \( \Delta V_{\text{piston}} \). This change in volume compresses the air that is dissolved in the aqueous fluid, causing the pressure inside the lower chamber to increase. When the BLM fails, fluid is free to flow out through the porous substrate. For each volume increment of fluid that flows out of the lower chamber, the air within the lower chamber is free to expand by that amount, \( \Delta V_{\text{pores}} \), thereby decreasing the pressure inside the chamber. This relationship can be summarized as,

\[
P = \frac{k}{V_{i,\text{air}} - \Delta V_{\text{piston}} + \Delta V_{\text{pores}}}.
\]

(3.2)

In these experiments the stepper motor was operated at a fixed frequency, \( f_{\text{stepper}} \), of 1 Hz, creating a linear piston motion, \( \Delta x_{\text{stepper}} \), of 1.59 \( \mu \text{m} \) per step. Therefore the volume that has been displaced by the motion of the piston can be calculated by,

\[
\Delta V_{\text{piston}} = \Delta t A_{\text{piston}} \Delta x_{\text{stepper}} f_{\text{stepper}},
\]

(3.3)

where \( A_{\text{piston}} \) is the cross-sectional area of the piston and \( \Delta t \) is the amount of time that has elapsed since the start of the experiment.
When the BLM fails, pressure inside of the lower test chamber forces fluid to flow through the porous substrate to the upper chamber of the test fixture, which is open to the atmosphere. The flow rate of fluid exiting the pores can be approximated using Darcy’s law for fluid flow through porous media (Darcy, 1856; Bear, 1972),

\[ Q = \frac{kA}{\mu l} \Delta P, \quad (3.4) \]

where \( Q \) is flow rate, \( k \) is the permeability of the medium (which is usually an experimentally determined value), \( A \) is the cross sectional area to flow, \( \mu \) is the viscosity of the fluid, \( l \) is the length of flow through the medium, and \( \Delta P \) is the pressure drop. Flow rate \( Q \) can be equivalently written as the change in volume with respect to time, \( Q = \Delta V_{pores}/\Delta t \).

For this model, \( k \) will be considered the permeability of the porous substrate and \( k' \) will be considered the combined permeability of the porous substrate and the BLM. The cross sectional area is the area of the substrate that is under pressure, \( A_{substrate} \), which is based on the 3.2 mm diameter of the orifice that connects the lower and upper fluid chambers. The pressure drop \( \Delta P \) is simply the difference between the pressure inside the lower test chamber and the atmosphere, \( \Delta P = P - P_{atm} \). Therefore Equation (3.4) can be rewritten as,

\[ \Delta V_{pores} = \Delta t \frac{k' A_{substrate}}{\mu l} (P - P_{atm}). \quad (3.5) \]

The combined permeability of the porous substrate and the BLMs is quite simple for a single pore scenario. At the outset of the test when the pore is covered by a BLM, the permeability is zero, \( k' = 0 \) for \( P < P_f \), where \( P_f \) is the failure pressure. When the BLM fails under pressure, the combined permeability becomes that of the single pore substrate alone, \( k' = k \) for \( P \geq P_f \).

### 3.3.2 Modeling of BLM failure pressure based on pore size

While the model described in the previous section can be used to predict the pressurization curve of a BLM by curve fitting the failure pressure, it is also desirable to be able to predict the value of failure pressure based on pore size. It is known empirically from a plot of failure pressure versus pore size that these two values are related by an inverse power law. The general form for an inverse power law is,

\[ P = \frac{1}{bd^{m}}, \quad (3.6) \]
where $P$ is pressure, $b$ is a constant, $d$ is pore diameter, and the exponent $m$ is another constant. It is sometimes convenient to linearize this equation by taking the logarithm of both sides,

$$\log P = -m \log d - \log b. \quad (3.7)$$

A log-log plot of failure pressure versus pore diameter should fall approximately on a straight line. Linear regression analysis can then be used to determine the slope, $-m$, and the intercept, $-\log b$.

If the failure pressure $P_1$ is known for pore size $d_1$, then the failure pressure $P_2$ can be predicted for any other pore diameter $d_2$ using the inverse power law in Equation 3.6. This is done by writing the equations for the two pore sizes, $P_1 = 1/bd_1^m$ and $P_2 = 1/bd_2^m$, solving for constant $b$, and then setting the two equations equal to each other. The resulting prediction of failure pressure $P_2$ is,

$$P_2 = P_1 \left( \frac{d_1}{d_2} \right)^m. \quad (3.8)$$

Here $m$ is a measure of the magnitude of the size effect.

As a first estimate for the failure pressure of a BLM based on its mechanical properties, the BLM might be considered an isotropic linear elastic solid plate. In reality this is not true, because BLMs are known to act like smectic liquid crystals, which are rigid through the thickness but are liquid-like in-plane (Hamley, 2005). For the sake of qualitatively comparing the results to a well established model, though, this inconsistency will be temporarily ignored. Because the BLMs occupy circular holes and are subjected to fluid pressure during the experiments, they can be idealized as simply supported circular plates under uniform load as depicted in Figure 3.4.

First, assume that the deflections of the plate are small deflections. Under this assumption the deflection of the plate is considered small compared to its thickness, there is no deformation of the midplane during bending, normals to the midplane remain normal after bending, and stresses that are transverse to the plate are ignored. Then the relationship between the maximum stress, $\sigma_{\text{max}}$, and the radius of the plate, $a$, takes the form (Timoshenko and Woinowsky-Krieger, 1959),

$$\sigma_{\text{max}} = \frac{3(3 + \nu)qa^2}{8h^2}, \quad (3.9)$$

where $q$ represents the uniform load, $\nu$ is the Poisson’s ratio, and $h$ is the thickness of the plate. The maximum stress occurs at the center of the plate. From Equation (3.9) it readily
Figure 3.4: Schematic of a BLM covering a circular pore that is idealized as a circular plate subjected to uniform load.

follows that,

\[ q = \left( \frac{h}{a} \right)^2 \frac{8\sigma_{\text{max}}^2}{3(3 + \nu)} , \]  

where \( \sigma_{\text{max}} \) is taken to be the ultimate strength of the material. By taking the common logarithm of both sides of Equation 3.10 one obtains,

\[ \log q = -2 \log a + \log \left[ \frac{8h^2\sigma_{\text{max}}}{3(3 + \nu)} \right] . \]  

On a logarithmic plot of \( q \) versus \( a \), Equation (3.11) becomes a straight line with a negative slope of \( m = 2 \) and a y-intercept that is equal to the second term of the right hand side.

Second, assume that the deflections of the plate are large deflections. This implies that the deflection of the plate is no longer small compared to its thickness but is still small compared to its radius. Deformation of the midplane is considered significant and is included in the analysis. In this case the relationship between the maximum stress and the radius of the plate becomes (Timoshenko and Woinowsky-Krieger, 1959),

\[ \sigma_{\text{max}} = CE \frac{w_o^2}{a^2} , \]  

where \( C \) is a constant depending on the boundary conditions and Poisson’s ratio, \( E \) is the Young’s modulus, and \( w_o \) is the deflection at the center of the plate given by,

\[ \frac{w_o}{h} + A \left( \frac{w_o}{h} \right)^3 = B \frac{q}{E} \left( \frac{a}{h} \right)^4 , \]  

with \( A \) and \( B \) as constants depending on the boundary conditions and the Poisson’s ratio. Again, the maximum stress occurs at the center of the plate. By combining Equations (3.12) and (3.13) it follows that the uniform load is,

\[ q = \frac{1}{B} \left( \frac{h}{a} \right)^3 \left( \frac{E\sigma_{\text{max}}}{C} \right)^{\frac{1}{2}} + \frac{A}{B} \left( \frac{h}{a} \right) \left( \frac{\sigma_{\text{max}}^3}{C^3E} \right)^{\frac{1}{2}} . \]
By taking the common logarithm, Equation (3.14) reduces to

\[
\log q = \log \left[ \frac{1}{B} \left( \frac{h}{a} \right)^3 \left( \frac{E \sigma_{\text{max}}}{C} \right)^{\frac{1}{2}} + \frac{A}{B} \left( \frac{h}{a} \right) \left( \frac{\sigma_{\text{max}}^3}{C^3 E} \right)^{\frac{1}{2}} \right]. \tag{3.15}
\]

When \( h \ll a \) the first term of the logarithm argument, which appears on the right hand side of Equation (3.15), becomes negligible. (This also depends on the values of the other constants.) Then Equation (3.15) can be approximated by,

\[
\log q = -\log a + \log \left[ \frac{A}{B} \left( \frac{h^2 \sigma_{\text{max}}^3}{C^3 E} \right)^{\frac{1}{2}} \right]. \tag{3.16}
\]

On a logarithmic plot of \( q \) versus \( a \), Equation (3.16) becomes a straight line with a negative slope of \( m = 1 \) and a \( y \)-intercept that is equal to the second term of the right hand side. Values for \( A \), \( B \), and \( C \) can be derived based on boundary conditions or found in tables (Timoshenko and Woinowsky-Krieger, 1959). Material properties including the Young’s modulus and the ultimate strength have been estimated for BLMs composed of various lipids, including SOPC and SOPC/CHOL (Needham and Nunn, 1990).

3.3.3 Modeling of BLM electrical impedance

Electrical impedance models for the control trials

In the first control trial the impedance of the electrolyte and electrode/electrolyte interface is measured with no partition between the electrodes. The impedance of the electrode/electrolyte interface is due to the double layer of ions that surround a charged electrode in an electrolyte solution (Hibbert, 1993; Rieger, 1994). This is commonly modeled as a resistor \( R_i \) and Warburg circuit element \( Y_i \) in parallel with a capacitor \( C_i \). The capacitor accounts for charge separation at the double layer while the Warburg circuit element accounts for diffusion and mixing of ions. The electrolyte solution is modeled as an ideal resistor \( R_e \). Like a solid conductor, \( R_e = \rho L/A \), where \( \rho \) is the resistivity of the ionic solution, \( L \) is the distance between electrodes, and \( A \) is cross sectional area. Together, the electrode interface and the electrolyte make up the Randle equivalent circuit, shown schematically in Figure 3.5 (Macdonald, 2006). The total impedance of the circuit is the combination of the interface impedance \( Z_i \) for two electrodes and the electrolyte impedance \( Z_e \),

\[
Z_{\text{tot}} = 2Z_i + Z_e. \tag{3.17}
\]
The impedance for a resistor is $Z_R = R$, for a capacitor is $Z_C = 1/j\omega C$, and for a Warburg element is $Z_W = 1/(j\omega)^{1/2}Y$. Substituting components and reducing gives a total impedance of,

$$Z_{tot} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2} + R_e} + R_e. \quad (3.18)$$

At low frequencies both the electrode interface ($Z_i$) and the electrolyte ($Z_e$) contribute to the total impedance of the system. At high frequencies, however, the impedance of the electrode interface approaches zero, and only the electrolyte resistance remains.

For the second control trial, the impedance of a solid substrate with no pores was measured. The impedance of the solid substrate was well modeled by a resistor $R_{sub}$ and a capacitor $C_{sub}$ combined in parallel. The total impedance of this system is the combination of the interface impedance $Z_i$, the electrode impedance $Z_e$, and the substrate impedance $Z_{sub}$, which is shown schematically in Figure 3.6 and is represented mathematically as,

$$Z_{tot} = 2Z_i + Z_e + Z_{sub}. \quad (3.19)$$

Substituting components and reducing gives a total impedance of,

$$Z_{tot} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2} + R_e} + \frac{R_{sub}}{1 + j\omega C_{sub} R_{sub}}. \quad (3.20)$$

For the third control trial, the impedance of a substrate with a single pore was measured (no BLM was formed over this single pore substrate). This should result in a similar impedance measurement to a single pore with a BLM after the BLM has been
pressurized and has failed. This scenario will be further described by the model in Equations 3.25 and 3.26.

**Electrical impedance model for a BLM before failure**

Electrical impedance has been recorded before BLM failure and after BLM failure. Before failure, the electrical impedance of the system has four components: the electrode/electrolyte interface, the electrolyte, the substrate, and the BLM. BLMs are typically modeled as a resistor $R_{BLM}$ in parallel with a capacitor $C_{BLM}$ (Bordi et al., 2002; Naumowicz et al., 2005). The BLM and the substrate combine in parallel as shown schematically in Figure 3.7. The total impedance of the system is,

$$Z_{tot} = 2Z_i + Z_c + \frac{Z_{BLM}Z_{sub}}{Z_{BLM} + Z_{sub}}.$$  \hfill (3.21)

Substituting components and reducing gives a total impedance of,

$$Z_{tot} = 2\frac{1 + R_iY_i(j\omega)^{1/2}}{Y_i(j\omega)^{1/2} + C_i(j\omega) + R_iY_iC_i(j\omega)^{3/2} + R_c} + \frac{R_{BLM}R_{sub}}{R_{BLM}(1 + j\omega C_{sub}R_{sub}) + R_{sub}(1 + j\omega C_{BLM}R_{BLM})}. \hfill (3.22)$$

**Electrical impedance model for a BLM after failure**

After the BLM fails the resistance of the partition becomes that of an open pore. The impedance of an open pore is modeled as a resistor, and this resistance can be divided into two parts. One is the convergence resistance $R_c$, which is the result of a funneling effect.
Figure 3.8: Equivalent circuit for the impedance of a BLM formed over a single pore after failure.

as the ions are restricted from a large cross sectional area of motion to a smaller one (Hall, 1975). Hall has estimated the convergence resistance as,

$$R_c = \frac{\rho}{4a}, \quad (3.23)$$

where $a$ is the pore radius. The other part is the resistance within the pore $R_p$. Since $R = \rho L/A$, the resistance within the pore is (Wonderlin et al., 1990),

$$R_p = \frac{l\rho}{\pi a^2}, \quad (3.24)$$

where $l$ is the length of the pore. These two resistances together are referred to as the access resistance, $R_a = 2R_c + R_p$. The convergence resistance $R_c$ is multiplied by a factor of two to account for the convergence of ions on either side of an open pore.

Components of the impedance of the system after BLM failure include the electrode/electrolyte interface, the electrolyte, and the partition. After BLM failure the partition is a combination of the substrate and the access resistance of an open pore acting in parallel. This is shown schematically in Figure 3.8 and mathematically as,

$$Z_{tot} = 2Z_i + Z_e + \frac{Z_a Z_{sub}}{Z_a + Z_{sub}}, \quad (3.25)$$

where the access impedance is $Z_a = 2Z_c + Z_p$. Substituting components and reducing gives a total impedance of,

$$Z_{tot} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2}} + R_e + \frac{R_a R_{sub}}{R_a (1 + j\omega C_{sub} R_{sub}) + R_{sub}}. \quad (3.26)$$
3.4 Results

3.4.1 Pressurization results

Results for pressurization control trials without a BLM

Figure 3.9 is a plot of pressure versus piston displacement for the control trials, in which single pore substrates were pressurized without a BLM formed over them. For 10, 14, and 20 µm pore sizes, no significant pressure buildup was observed, indicating that fluid was free to flow through the porous substrate in the absence of a BLM. For 5, 8, and 12 µm pore sizes, some back pressure was generated, but this back pressure was still small when compared to pressurization of a BLM formed over the same substrate and had a significantly different pressure curve shape. The back pressure in these cases may be due to clogging of the pore by salt ions and other debris or simply because of the low permeability of a very small pore.

Results for pressurization trials with a BLM

Figure 3.10 (a) is a plot of pressure versus piston displacement for ten pressurization trials under the same test conditions of SOPC BLMs formed over substrates with an 8 µm diameter pore. Figure 3.10 (b) is the same plot for substrates with a 14 µm diameter pore. Overall trends in the pressure curves were repeatable, although there was some random
Figure 3.10: Pressurization curves for ten trials of an SOPC BLM formed on a substrate with a single (a) 8 µm diameter pore, and (b) 14 µm diameter pore.

variation in the peak failure pressures. Initially, the porous substrate is sealed by a BLM and the pressure rises as the piston compresses the internal fluid chamber of the test fixture. At some critical pressure the BLM fails and pressure drops rapidly, almost to zero, as fluid escapes through the pore. Due to the large amount of data that has been collected, only these two sample plots are shown.

For 8 and 14 µm pore sizes, the average of ten pressurization trials of SOPC BLMs is shown with the 95% confidence interval for a t distribution with nine degrees of freedom in Figures 3.11 (a), and (b), respectively. Also, modeled pressure curves were calculated according to Section 3.3.1. For the model, the failure pressure $P_f$ was determined by the peak in the experimental pressure curves and the permeability $\kappa$ was a curve fitting parameter, which are reported in Table 3.2. Again, because of the large number of pore sizes that have been tested, the model and average data has only been shown for 8 and 14 µm pore sizes.

A logarithmic plot of failure pressure versus pore size for SOPC and SOPC/CHOL-50 mol% BLMs is shown in Figure 3.12. Data points represent the average failure pressure of ten trials, and errorbars represent the standard error $\sigma_f/\sqrt{n}$. The experimental results establish a clear trend between the failure pressure of planar BLMs and the pore size of their supporting substrate, demonstrating that failure pressure increases as pore size decreases. Also, failure pressure was on average 56% higher for mixtures of SOPC and cholesterol than for pure SOPC.
Figure 3.11: Pressurization of SOPC BLMs where the solid line is the experimental average of ten trials, the dash-dash lines are the 95% confidence interval of the experimental data, and the dotted line is the model for (a) an 8 µm pore and (b) a 14 µm pore.

Table 3.2: Parameters for the pressurization model of SOPC BLMs including the pore diameter \( D \), failure pressure \( P_f \) and the substrate permeability \( \kappa \).

<table>
<thead>
<tr>
<th>( D ) (µm)</th>
<th>( P_f ) (kPa)</th>
<th>( \kappa ) (m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>66.8</td>
<td>( 17 \times 10^{-14} )</td>
</tr>
<tr>
<td>8</td>
<td>44.7</td>
<td>( 3.7 \times 10^{-14} )</td>
</tr>
<tr>
<td>10</td>
<td>35.6</td>
<td>( 4.3 \times 10^{-14} )</td>
</tr>
<tr>
<td>12</td>
<td>26.4</td>
<td>( 9.5 \times 10^{-14} )</td>
</tr>
<tr>
<td>14</td>
<td>23.5</td>
<td>( 8.6 \times 10^{-14} )</td>
</tr>
<tr>
<td>20</td>
<td>18.7</td>
<td>( 2.6 \times 10^{-14} )</td>
</tr>
</tbody>
</table>
Figure 3.12: Failure pressure versus pore size where squares are SOPC BLMs, circles are SOPC/CHOL-50 mol% BLMs, vertical solid lines are errorbars, and dotted lines are regression lines.

Also shown in Figure 3.12 are regression lines for each data set where the slopes have been indicated. Using Equation 3.8, the failure pressure for any pore size can be predicted based on the failure pressure of one nominal pore size. If the smallest pore size that was tested is chosen as the nominal pore size, \(d_1 = 5 \mu m\), then the nominal failure pressure for SOPC BLMs from Table 3.2 would be \(P_1 = 66.8 \text{ kPa}\). The size exponent \(m\) is equal to the negative slope of the regression line, \(m = 0.95\). If the model for the pressurization curve is again applied to the 8 and 14 \(\mu m\) pore sizes but instead uses the predicted values for failure pressure based on a nominal pore size of 5 \(\mu m\), then the resulting model curves are shown in Figure 3.13. In this case the predicted failure point is slightly off from the experimental data, but overall it is quite accurate.

3.4.2 Electrical impedance results

Results for electrical impedance control trials with no BLM

First a control trial was performed in which the electrical impedance was measured with no partition between the electrodes. Using the model from Equation 3.18, the impedance parameters for the electrode/electrolyte interface and the electrolyte were curve fitted to the experimental data, shown in Figure 3.14 (a). From this model, it was found that \(R_e = 1.1 \text{ k}\Omega\), \(R_i = 16 \text{ k}\Omega\), \(Y_i = 4.0 \times 10^{-4} \text{ S } s^{1/2}\), and \(C_i = 80 \mu F\).
Figure 3.13: Pressurization of SOPC BLMs where the failure pressure for the model has been predicted based on a nominal pore size of 5 µm for (a) an 8 µm pore and (b) a 14 µm pore.

The second control trial measured the electrical impedance of a solid substrate with no pores. The model from Equation 3.20 was fitted to the data as shown in Figure 3.14 (b) in order to determine the impedance parameters for the substrate. It was found that the resistance of the substrate was \( R_{sub} = 5.0 \times 10^7 \) Ω and the capacitance was \( C_{sub} = 1.0 \times 10^{-10} \) F. This relatively low impedance value may indicate a parasitic pathway for ion travel around the substrate.

In the third control trial, impedance was measured for a substrate with a single pore and no BLM. The model from Equation 3.26 was a good fit to the experimental data, as shown in Figure 3.14 (c). The access resistance of the pore, which was \( R_a = 2.5 \times 10^5 \) Ω, and the resistance of the substrate, \( R_{sub} = 5.0 \times 10^7 \) Ω, combined in parallel, so the smaller resistance \( R_a \) dominated the system.

**Results for electrical impedance trials with a BLM**

For SOPC BLMs formed on 8 µm pore substrates, Figure 3.15 (a) is a plot of impedance and phase versus frequency for ten trials before pressurization, and Figure 3.15 (b) is impedance after the BLM failed. Figures 3.15 (c) and (d) are the same measurements for 14 µm pore substrates. In both cases, there is a reduction of nearly three orders of magnitude in the peak impedance between the before failure case (\( \approx 10^8 \) Ω) and after failure (\( \approx 10^5 \) Ω). This drop in impedance is attributed to the opening of a conductive...
Figure 3.14: Electrical impedance control trials for (a) the electrode interface and electrolyte alone, (b) a solid substrate with no pores, and (c) a substrate with a single 8 µm pore and no BLM.
Figure 3.15: Impedance measurements for an SOPC BLM formed on a substrate with a single (a) 8 µm diameter pore before pressurization, (b) 8 µm diameter pore after pressurization, (c) 14 µm diameter pores before pressurization, (d) 14 µm diameter pores after pressurization.

pathway through the pore after the BLM fails. Overall these impedance measurements were very repeatable, although there is some random noise in a few trials, such as in the phase plots for both Figures 3.15 (a) and (c). As with the pressure data, impedance plots are shown only for select pore sizes because of the large amount of data that was recorded.

For each pore size an average impedance was calculated as well as a 95% confidence interval using a t distribution with nine degrees of freedom. The models that were described in Section 3.3.3 were applied to the before and after failure cases. The average curves, 95% confidence intervals, and models are shown in Figure 3.16 for the 8 and 14 µm pore sizes. In the before failure cases (see Figures 3.16 (a) and (c)), the resistance and capacitance of the BLM, $R_{BLM}$ and $C_{BLM}$, can not be determined because the response of the system is
dominated by the substrate. If a typical value for the normalized conductance of a BLM is $G_{BLM}/A = 10^{-7} \text{ S/cm}^2$ (see Table 2.2) and the area of an 8 µm pore is $A = 5 \times 10^{-7} \text{ cm}^2$, then the resistance of a BLM formed over an 8 µm pore should be about $10^{13} \Omega$. So while the resistance of the substrate is $R_{sub} = 5 \times 10^7 \Omega$, the resistance of the BLM is expected to be much higher at about $R_{BLM} = 10^{13} \Omega$. When these two resistances combine in parallel, the contribution of the larger resistance becomes negligible, and a similar effect occurs with capacitance.

For the after failure cases (see Figures 3.16 (b) and (d)), the impedance signature was very similar to the third control trial, in which impedance was measured for a single pore substrate with no BLM. This result showed that after BLM failure the substrate returns to an open pore state. In this case the substrate impedance combined in parallel with the access resistance of an open pore. Since the access resistance of the pore was much smaller than the substrate, the contribution of the substrate was negligible for the after failure impedance response.

### 3.5 Discussion

The test methodology that was presented here provides a unique way to investigate the mechanical stability of a bilayer lipid membrane formed across a pore. The method is somewhat analogous to a tensile test of a conventional solid material in which a specimen is loaded in slowly increasing amounts until it reaches its failure, producing a characteristic stress versus strain curve for the material. In this case the specimen is a liquid crystalline material that is loaded using increasing fluid pressure until failure, producing a characteristic curve of pressure versus piston displacement. This curve was quite repeatable, and consisted first of a rise in pressure when the pore was sealed by a BLM, and then a sharp drop in pressure after the BLM failed and allowed fluid to escape through the pore. The small equilibrium pressure after BLM failure reflects that the pore has returned to an open state since it is of approximately the same magnitude as the equilibrium pressure in the control trials from Figure 3.9. The equilibrium pressure is slightly above zero because of the resistance to fluid flow caused by the small pore size. The average pressurization curves were fitted to a model, and the average failure pressures for each pore size were reported. The model was further generalized by allowing it to predict the failure pressure of any pore.
Figure 3.16: Average impedance, 95% confidence interval, and model for an SOPC BLM formed on a substrate with (a) an 8 µm diameter pore before pressurization, (b) an 8 µm diameter pore after pressurization, (c) a 14 µm diameter pore before pressurization, and (d) a 14 µm diameter pore after pressurization.
size based on the failure pressure of one nominal pore size. This is possible because BLM failure pressure follows an inverse power law relationship with pore diameter. When this generalized model was applied to the 8 and 14 µm pore sizes in Figure 3.13, it was found to be quite accurate.

In a logarithmic plot of BLM failure pressure versus pore size (see Figure 3.12), regression lines have been shown for each data set and the slope has been indicated. This was done for comparison to the solid plate models of failure pressure that were presented in Section 3.3.2. Two models for a circular plate under uniform load were given, one assuming that the plate undergoes small deflections and one assuming large deflections. The small deflection model resulted in a linear relationship between the logarithm of failure pressure and the logarithm of pore diameter with a slope of -2, and the large deflection model resulted a slope of -1. From Figure 3.12, the slope of the SOPC data was -0.95 and the slope of the SOPC/CHOL-50 mol% data was -0.67. Although there is clearly some experimental variability, this failure pressure data seems to roughly align with a slope of -1 as in the large deflection model, showing that failure pressure varies with the inverse of pore size ($P_f \propto 1/d$). Unfortunately the magnitude of both the small and large deflection plate models seem to have very little relationship to this experimental data, and further investigation will be needed to accurately model the physics that dictate the failure of a BLM (Hopkinson et al., 2006).

The electrical impedance was investigated by taking measurements before and after failure of the BLM. Before pressurization, the impedance was modeled with the BLM and the substrate acting in parallel. By measuring the impedance of a solid substrate alone, it was found that the impedance of the substrate was much smaller than the expected impedance of the BLM. This may have been due to a parasitic conductive pathway that existed through the substrate which would have the effect of lowering the substrate resistance. Since the substrate and the BLM act in parallel, the response of the substrate dominated the system. Therefore the impedance contribution of the BLM could not be determined, although this did not eliminate the usefulness of the impedance test. After BLM failure the impedance response was successfully modeled as an open pore. Therefore the impedance measurements showed that before pressurization the pore was sealed by a well formed BLM, and after pressurization the BLM had failed, allowing the pore to reopen.
3.6 Conclusions

The primary purpose of this study was to measure the failure pressure of BLMs formed over substrates with a single pore. Using a custom test fixture, this was accomplished for six pore sizes from 5 to 20 $\mu$m, and it was found that average failure pressures for SOPC BLMs were between 67 and 19 kPa, respectively. Failure pressures were also measured for a mixture of SOPC and 50 mol% cholesterol and these were found to be on average 56% higher than SOPC alone. A model was able to accurately reproduce the pressure versus piston displacement curve of a BLM by using failure pressure and substrate permeability as curve fitting parameters. Failure pressure and pore size were found to be related by an inverse power law, and using this relationship the failure pressure of any pore could be predicted based on the failure pressure for one nominal pore size. Using the inverse power law, it was further found that failure pressure varies roughly with the inverse of pore diameter, $P_f \propto 1/d$. Electrical impedance was measured before and after BLM pressurization, and it was found that impedance dropped by nearly three orders of magnitude after BLM failure. Models of these measurements showed that before pressurization the BLM completely sealed the pore it was formed over, and after pressurization the pore reopened, confirming that the BLM had failed.
Chapter 4

Pressurization of BLMs Formed Over a Multi Pore Substrate

4.1 Introduction

In this chapter bilayer lipid membranes (BLMs) are formed over a substrate containing an array of many pores, ranging in size from 0.05 μm to 10 μm, and the BLMs are pressurized until failure. Using a porous substrate as a support structure for the formation of a BLM over a large surface area is a technique that researchers use to enhance its stability (Mountz and Tien, 1978; Kobatake et al., 1970; Yoshikawa et al., 1987; Thompson et al., 1982). For analysis purposes, each pore that is covered by a BLM can be considered an independent entity that will be referred to as a micro-BLM.

Several types of lipids are tested including asolectin, which is a mixture of soybean phospholipids, 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphoethanolamine (POPE), 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphatidylcholine (SOPC), mixtures of POPE and cholesterol (CHOL), and mixtures of SOPC and cholesterol. Mixtures of SOPC/CHOL are used because studies have shown that cholesterol increases the strength of BLMs (Needham and Nunn, 1990; Needham and Zhelev, 1996). They have further concluded that cholesterol concentrations of 50 to 60 mol% will maximize the strength of an SOPC/CHOL BLM, which is the basis for the concentration chosen for this study.

Two different test fixtures are used to apply pressure to the micro-BLMs and results from the two methods are compared. One test fixture consists of a hollow upright tube
that is mounted above the porous substrate and BLM. An aqueous salt solution is slowly added to the upright tube, which increases the hydrostatic pressure that is being applied to the BLM. At low pressures the BLM is able to support the water column, but at some critical pressure the BLM fails and the salt solution is observed to flow through the porous substrate, causing the height of the water column to drop. This critical pressure will be referred to as the bulk failure pressure of the BLM (Hopkinson et al., 2006).

Another test fixture consists of a stepper motor that is used to move a piston in a linear fashion. When the piston moves it pressurizes a fluid chamber inside of the test fixture. A BLM supported by a porous substrate is clamped into the fluid chamber and pressure is recorded using a pressure transducer. Pressure initially increases as the piston moves, until it reaches the maximum pressure that can be withstood by the BLM, at which time the BLM fails and the pressure drops (Hopkinson and Leo, 2007). This test setup had the advantages over the water column test setup of being able to achieve higher pressures with greater accuracy and more automation.

The data from these experiments is used to plot pressure as a function of piston displacement. This characteristic pressure curve of a BLM is modeled according to the pressurization and flow of fluid through the porous substrate. Pressure initially increases as the piston moves until it reaches the maximum pressure that can be withstood by the BLM, at which time the BLM fails and the pressure drops as fluid escapes through the porous substrate (Hopkinson and Leo, 2007). All micro-BLMs do not fail at precisely the same pressure, but rather the failure pressure follows a statistical distribution. In this case the distribution is assumed to be a Weibull distribution.

The Weibull distribution is highly versatile. It consists of three parameters for scale, shape, and location. When the shape parameter is 1 the Weibull distribution reduces to the exponential distribution, a shape parameter of 2 reduces to the Rayleigh distribution, and a shape parameter of 3.4 closely mimics the normal distribution as this is where its skewness is minimized. The Weibull distribution is well known for its use in reliability engineering in which it can be used to predict the lifetime of mechanisms or components based on the value of the shape parameter (Hall, 1975). It is also commonly used to model the strength of materials such as ceramics and carbon fibers (Wachtman, 1996; Mallick, 1993). For these cases the Weibull distribution is referred to as a weakest link theory because it assumes that the strength of the material is limited by the most severe flaw that is contained within the
material (Weibull, 1951). A larger sized material (usually measured by volume for ceramics or length for fibers) will contain more flaws and will have a correspondingly lower strength. This behavior is also observed for BLMs.

The stepper motor test fixture has silver/silver chloride (Ag/AgCl) electrodes mounted on either side of the BLM. For each test electrical impedance is measured between the two electrodes before and after BLM failure. Impedance is observed to drop by about two orders of magnitude after BLM failure. An equivalent electrical circuit is developed for these two cases, before failure and after failure, and this is used to model these impedance measurements.

Finally, micro-BLMs are imaged using fluorescence microscopy. A fluorescent dye that is sensitive to the presence of phospholipid molecules is added to the phospholipid solution. When the dye is illuminated by a fluorescent light source, the micro-BLMs can be seen with an optical microscope. Another test fixture was developed so that the micro-BLMs can be pressurized using a hand operated syringe while they are viewed with the microscope and pictures are taken using a digital camera. Pictures are taken before and after pressurization of the micro-BLMs, and failure is observed.

4.2 Materials and methods

4.2.1 Phospholipid preparation

Asolectin was purchased in powder form from Sigma Aldrich (St. Louis, MO) and SOPC, POPE, and cholesterol were purchased in powder form from Avanti Polar Lipids (Alabaster, AL). The lipid powders were dissolved in n-decane (99% purity, Alfa Aesar, Ward Hill, MA) at a concentration of 40 mg/mL and mixed for 30 minutes using a sonicator (model 50, VWR, West Chester, PA). For some experiments cholesterol was mixed with POPE or SOPC to create stronger BLMs. In particular, 30 mol% and 50 mol% cholesterol were added to POPE and 50 mol% cholesterol was added to SOPC. The BLMs were reconstituted in an aqueous salt solution over Isopore polycarbonate membrane filters acquired from Millipore (Bedford, MA). The aqueous salt solution was prepared with 0.1 M NaCl (100.0% purity, Mallinckrodt Baker, Inc., Paris, KY) and deionized water. The porous polycarbonate substrates were treated by the manufacturer with a hydrophilic polyvinylpyrrolidone (PVP) coating which facilitates the formation of a BLM on its surface. Tests were
Table 4.1: Specifications for Isopore porous polycarbonate substrates.

<table>
<thead>
<tr>
<th>Pore Dia. (µm)</th>
<th>Thickness (µm)</th>
<th>Pore Density (pores/cm²)</th>
<th>Permeability (m²)</th>
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<td>$4.2 \times 10^{-15}$</td>
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<td>10</td>
<td>$1 \times 10^5$</td>
<td>$1.2 \times 10^{-14}$</td>
</tr>
</tbody>
</table>

performed for pore diameters of 0.05 µm, 0.1 µm, 0.2 µm, 0.4 µm, 0.8 µm, 2 µm, 5 µm, and 10 µm. For modeling purposes it was also necessary to know the substrate thickness and pore density, which was provided by the manufacturer, and the permeability, which was determined experimentally. These values are reported in Table 4.1. Measuring permeability is further explained in Section 4.4.2.

4.2.2 Water column pressurization test procedure

Preliminary testing of the bulk failure pressure of BLMs was performed by applying hydrostatic fluid pressure using an upright water column. The test fixture that was used in this procedure is shown in Figure 4.1. It consisted of a polycarbonate hollow cylindrical tube with 3.2 mm inner radius and 4.8 mm outer radius that was fitted inside a polycarbonate base structure. The height of the tube was marked in millimeter divisions. A removable aluminum bottom plate with a center hole of radius 3.2 mm was used to clamp the porous substrate into the base structure. A porous polycarbonate substrate was coated with the lipid/n-decane mixture by using a brush and was then clamped between the bottom plate and the base. Rubber gaskets were placed on either side of the substrate to seal it from fluid leaks. The base was then submerged in a reservoir filled with the 0.1 M NaCl salt solution. Additional salt solution was slowly poured into the upright cylindrical tube in small increments until the fluid level was observed to drop. The maximum fluid height that could be supported by the BLM was considered the bulk failure pressure of the BLM. Pressure was calculated using the relationship $P = \rho gh$, where $P$ is pressure, $\rho$ is fluid density, $g$ is acceleration due to gravity, and $h$ is fluid height. Lipids that were
tested include asolectin, SOPC, SOPC/CHOL-50 mol%, POPE, POPE/CHOL-30 mol%, and POPE/CHOL-50 mol%. Six trials were performed for each combination of lipid and pore size.

4.2.3 Stepper motor pressurization test procedure

The data measured using the water column test fixture was limited to small pressures (< 12 kPa) and therefore substrates with relatively large pore sizes. A new experimental test fixture was developed in an effort to increase the range of pore sizes that could be tested while also increasing the accuracy of the experiments. This test fixture is able to produce pressures of > 690 kPa. It was composed of a stepper motor (model L1MGJ-M200XX060, EAD Motors, Dover, NH) used to drive a lead screw in a linear motion (see Figures 4.2 and 4.3). A stainless steel piston with an outside diameter of 12.2 mm was attached to the end of the lead screw, which had a thread pitch of 1.57 threads/mm. For each step the motor turns 0.9°, which translates into a linear piston motion of 1.59 µm. The piston was used to pressurize a lower fluid chamber inside of the aluminum body of the test fixture, which had an internal volume of 5.0 mL. The lower fluid chamber was connected to a polycarbonate upper chamber via a 3.2 mm diameter hole. The lower and upper chambers were sealed using rubber gaskets. The porous substrate was clamped between the lower and upper fluid
chambers so that the lower fluid chamber was under pressure and the upper chamber was
open to the atmosphere. A pressure relief valve was connected to the lower fluid chamber
and a polycarbonate fluid reservoir. The pressure relief valve remained open while the
porous substrate was clamped into place and was then closed before the test was started.
This procedure was necessary to prevent an initial pressure buildup during assembly.

Pressure was measured by two different pressure transducers that had different
ranges. A pressure transducer with a range of 0 to 690 kPa (model PX181B-100G5V,
Omega Engineering, Inc., Stamford, CT) was used for pore sizes from 0.05 to 0.2 µm and
a pressure transducer with a range of 0 to 41 kPa (model PX181B-006G5V, Omega En-
gineering, Inc., Stamford, CT) was used for pore sizes from 0.4 to 10 µm. Both pressure
transducers are reported by the manufacturer to have an accuracy of 0.3% of full scale.
Pressure was recorded using a multifunction data acquisition module (model USB-6009,
National Instruments, Austin, TX) and Labview software (National Instruments, Austin,
TX).

For each trial a porous substrate was coated with 20 µL of lipid/n-decane mixture by
using a pipettor (model Calibra 822, Socorex, Switzerland) and was then clamped between
the lower and upper fluid chambers. Pressure was slowly applied by running the stepper
motor at a fixed frequency of 1 Hz for the entire duration of the test. The bulk failure
pressure of the BLM was determined at the point when pressure began to decrease in the
lower chamber of the test fixture. Lipids that were tested include SOPC and SOPC/CHOL-
50 mol%. Ten trials were performed for each combination of lipid mixture and substrate
pore size. Control trials were also performed for each pore size by pressurizing a porous
substrate with no BLM. The stepper motor was again run at a frequency of 1 Hz and pressure was recorded.

4.2.4 Electrical impedance test procedure

A silver/silver chloride (Ag/AgCl) electrode (model EP2, World Precision Instruments, Inc., Sarasota, FL) was mounted in the stepper motor test fixture on either side of the porous substrate so that it was submerged in the salt solution. The two electrodes were spaced 18.4 mm apart in a chamber with a 3.2 mm diameter bore with the porous substrate halfway between them. The electrical impedance was measured across the BLM using an impedance analyzer (model 4192A, Hewlett Packard, Palo Alto, CA) (Tien and Ottova, 1999). The impedance was recorded over a frequency range of $10^1$ to $10^6$ Hz with a resolution of 50 data points taken with logarithmic spacing. Measurements were taken once at the start of the test before the BLM was pressurized and once at the end of the test after the BLM had failed.

Two control trials were performed for the electrical impedance measurements. In the
first control trial impedance was measured across the electrodes with no partition between them. A model was then used to determine the impedance parameters for the electrolyte and electrode/electrolyte interface. In the second control trial impedance was measured for a porous substrate with 2 \( \mu \text{m} \) pores and no BLM. The impedance contribution of the open pores was predicted using a model.

### 4.2.5 Fluorescence microscopy test procedure

Another custom test fixture was developed in order to image a BLM using fluorescence microscopy while simultaneously applying pressure to it. The test fixture, shown in Figures 4.4 and 4.5, consists of a polycarbonate base plate that attaches to a mechanical microscope stage. A porous substrate was coated with a BLM and mounted on top of a flat O-shaped rubber gasket with a 12.7 mm outer diameter, a 3.2 mm inner diameter, and a 1.6 mm thickness. A similar rubber gasket was mounted over the top of the porous substrate, and a top plate was used to clamp the assembly in place. A hand operated syringe and needle was used to pierce the lower rubber gasket and the 0.1 M NaCl salt solution was injected below the porous substrate to pressurize the BLM. The BLM was imaged from underneath the test fixture through a 1.0 mm thick clear polycarbonate plate using an inverted microscope (model Axiovert 40 CFL, Carl Zeiss Microimaging, Inc., Peabody, MA) with 400 times magnification, equipped with a fluorescent light source and fluorescent filter (model F25, Zeiss, Peabody, MA). A digital camera (model Powershot G6, Canon, Lake Success, NY) was used to record images of the BLMs before and after pressurization of the BLM. A stage micrometer was used to calibrate the image scale.

A pipettor (model Calibra 822, Socorex, Switzerland) was used to deposit 5 \( \mu \text{L} \) of SOPC solution onto a porous substrate, followed by 3 \( \mu \text{L} \) of di-8-ANEPPS (Sigma Aldrich, St. Louis, MO) fluorescent dye. In the presence of phospholipid molecules, di-8-ANEPPS dye absorbs light in the green wavelengths and emits red. The substrate was a black porous polycarbonate filter with 8 \( \mu \text{m} \) diameter pores (Sterlitech Corp., Kent, WA).

Several control trials were performed to show that fluorescent images were only visible in the presence of both phospholipid molecules and fluorescent dye. First, a BLM was formed without any fluorescent dye and images were recorded. Second, fluorescent dye was imaged without any phospholipid solution.
Figure 4.4: Photograph of the fluorescent pressurization test fixture (a) assembled and (b) disassembled. Photographed by author.

Figure 4.5: Photograph of the fluorescent pressurization test fixture attached to an inverted optical microscope. Photographed by author.
4.3 Modeling

4.3.1 Modeling of BLM pressure curves

Fluid pressurization

The pressurization curve of the BLMs in this study can be modeled according to the pressurization of fluid and the flow of fluid through the porous substrate. It has been experimentally determined that after all visible air bubbles have been bled from the lower chamber, approximately 1% by volume of the fluid is composed of dissolved air. Therefore it is a basic premise of this model that the change in pressure inside the lower chamber is a result of the contraction and expansion of this volume of air according to Boyle’s law, \( PV = k \), where \( P \) is absolute pressure, \( V \) is volume, and \( k \) is a constant of proportionality. The constant \( k \) can be calculated according to the initial test conditions as,

\[
k = P_{atm}V_{i,\text{air}},
\]

where the initial pressure \( P_{atm} \) is one atmosphere, and the initial volume of air \( V_{i,\text{air}} \) is 1% of the internal volume of the lower test chamber.

It is assumed that at the outset of each trial a BLM covers the substrate pores, effectively sealing the lower chamber of the test fixture. As the stepper motor pushes the piston forward, it reduces the internal volume of the lower chamber by the amount \( \Delta V_{\text{piston}} \). This change in volume compresses the air that is dissolved in the aqueous fluid, causing the pressure inside the lower chamber to increase. When the BLM fails, fluid is free to flow out through the porous substrate. For each volume increment of fluid that flows out of the lower chamber, the air within the lower chamber is free to expand by that amount, \( \Delta V_{\text{pores}} \), thereby decreasing the pressure inside the chamber. This relationship can be summarized as,

\[
P = \frac{k}{V_{i,\text{air}} - \Delta V_{\text{piston}} + \Delta V_{\text{pores}}},
\]

In these experiments the stepper motor was operated at a fixed frequency, \( f_{\text{stepper}} \), of 1 Hz, creating a linear piston motion, \( \Delta x_{\text{stepper}} \), of 1.59 \( \mu \text{m} \) per step. Therefore the volume that has been displaced by the motion of the piston can be calculated by,

\[
\Delta V_{\text{piston}} = \Delta t A_{\text{piston}} \Delta x_{\text{stepper}} f_{\text{stepper}},
\]
where \( A_{\text{piston}} \) is the cross sectional area of the piston and \( \Delta t \) is the amount of time that has elapsed since the start of the experiment.

When the BLM fails, pressure inside of the lower test chamber forces fluid to flow through the porous substrate to the upper chamber of the test fixture, which is open to the atmosphere. The flow rate of fluid exiting the pores can be approximated using Darcy’s law for fluid flow through porous media (Darcy, 1856; Bear, 1972),

\[
Q = \frac{kA}{\mu l} \Delta P,
\]

(4.4)

where \( Q \) is flow rate, \( k \) is the permeability of the medium (which is usually an experimentally determined value), \( A \) is the cross sectional area to flow, \( \mu \) is the viscosity of the fluid, \( l \) is the length of flow through the medium, and \( \Delta P \) is the pressure drop. Flow rate \( Q \) can be equivalently written as change of volume with respect to time, \( Q = \Delta V_{\text{pores}}/\Delta t \). For this model, \( k \) will be considered the permeability of the porous substrate and \( k' \) will be considered the combined permeability of the porous substrate and the BLM. The cross sectional area is the area of the substrate that is under pressure, \( A_{\text{substrate}} \), which is based on the 3.2 mm diameter of the orifice that connects the lower and upper fluid chambers. The pressure drop \( \Delta P \) is simply the difference between the pressure inside the lower test chamber and the atmosphere, \( \Delta P = P - P_{\text{atm}} \). Therefore Equation (4.4) can be rewritten as,

\[
\Delta V_{\text{pores}} = \Delta t \frac{k' A_{\text{substrate}}}{\mu l} (P - P_{\text{atm}}).
\]

(4.5)

**Combined permeability of the substrate and BLMs**

The combined permeability of the porous substrate and the BLMs changes as the BLMs begin to fail. When all pores are covered by a BLM, the permeability is zero. If all of the BLMs were to fail, then the combined permeability would be that of the porous substrate alone. Between these two cases, the combined permeability varies proportionally with the fraction of BLMs that have failed. This has been proven by considering the porous substrate to be an array of capillary tubes (Matteson and Orr, 1987). The Hagen-Poiseuille equation is a solution of the Navier Stokes equation for flow through capillary tubes,

\[
Q = \frac{n \pi d^4}{128 \mu} \frac{\Delta P}{l},
\]

(4.6)
where \( n \) is the number of capillaries and \( d \) is the capillary diameter. If a unit area \( A = 1 \) is assumed for Darcy’s law, then,

\[
Q = \frac{\kappa \Delta P}{\mu l}.
\]  

(4.7)

Combining the Hagen-Poiseuille equation with Darcy’s law (Equation 4.4) and solving for permeability, \( \kappa \), gives the following relationship,

\[
\kappa = \frac{n\pi d^2}{128}.
\]  

(4.8)

Porosity, \( \epsilon \), for an array of circular pores is defined as,

\[
\epsilon = \frac{n\pi d^2}{4A}.
\]  

(4.9)

Assuming a unit area \( A = 1 \) again and combining Equation 4.9 with Equation 4.8 gives the relationship,

\[
\kappa = \frac{\epsilon D^2}{32},
\]  

(4.10)

which shows that permeability \( \kappa \) is proportional to porosity \( \epsilon \). The constant \( 1/32 \), however, is usually not representative of experimental observations and therefore it is often determined using additional modeling or by experimental methods (Scheidegger, 1963).

At the outset of the test a BLM usually does not fully form over every pore. Due to random variability in the formation of the lipid bilayer, some pores remain open. The small fraction of micro-BLMs that do not initially form over a pore, \( \chi_i \), is used as a curve-fitting parameter. The fraction of micro-BLMs that subsequently fail after the start of the test due to pressurization is \( \chi_f \). Therefore the overall combined permeability of the porous substrate and the BLM is,

\[
\kappa' = (\chi_i + \chi_f) \kappa.
\]  

(4.11)

\( \chi_f \) can be equivalently written as,

\[
\chi_f = (1 - \chi_i) \Phi(P),
\]  

(4.12)

where \( \Phi(P) \) is the fraction of BLMs that have failed for a given pressure \( P \). The failure pressure of the BLMs follows a distribution, which was found to be well modeled by a Weibull distribution. In this case \( \Phi(P) \) represents the cumulative distribution function of a Weibull distribution.
Use of the Weibull distribution to model BLM failure

These experiments investigate a BLM that is coated over a substrate with many pores. This setup can also be thought of as an array of individual micro-BLMs each covering a pore. In this scenario, all BLMs do not fail at the same pressure, but rather they follow a statistical distribution. The mean failure pressure, $P_\mu$, is the average failure pressure of a single BLM (this quantity was measured for various pore sizes in Chapter 3). When multiple BLMs are pressurized simultaneously, the point at which enough BLMs have failed to cause pressure loss is the bulk failure pressure, $P_b$.

It is assumed here that the failure pressure of the BLMs follows a Weibull distribution with a positive skew. The Weibull distribution has three parameters: the scale parameter $P_0$ (this will also be referred to as the characteristic pressure), the shape parameter $\beta$ (sometimes referred to as the Weibull modulus), and the location parameter $P_{\text{min}}$. In statistics, a probability density function (PDF) gives the frequency at which an event occurs.

The PDF of a three parameter Weibull distribution as a function of pressure $P$ is (Weibull, 1951; Devore, 2000),

$$
\phi (P) = \frac{\beta}{P_0} \left( \frac{P - P_{\text{min}}}{P_0} \right)^{\beta-1} \exp \left[ - \left( \frac{P - P_{\text{min}}}{P_0} \right)^\beta \right], \quad (4.13)
$$

where $P > P_{\text{min}}$.

An example of a positively skewed Weibull PDF is shown in Figure 4.6. In this case the location parameter was chosen as $P_{\text{min}} = 50$ kPa, which offsets the distribution so that no BLMs can fail below that value. The characteristic pressure $P_0$ was chosen so that the mean of the distribution would fall at $P_\mu = 150$ kPa. The shape parameter was arbitrarily chosen as $\beta = 1.1$ to show an example of a distribution with a positively skewed shape. The mode or peak of the PDF will later be shown to be related to the bulk failure pressure of BLMs, $P_b$, and therefore it will be assumed that $P_{\text{min}} = P_b$.

The cumulative distribution function (CDF) predicts the probability of an event occurring, and is found by integrating the PDF. The CDF for a three parameter Weibull distribution is (Devore, 2000),

$$
\Phi (P) = 1 - \exp \left[ - \left( \frac{P - P_{\text{min}}}{P_0} \right)^\beta \right], \quad (4.14)
$$

where $P > P_{\text{min}}$. At pressure $P$ the probability of a BLM having reached its failure pressure is $\Pr (P_{\text{failure}} \leq P) = \Phi (P)$. If there are $n$ multiple BLMs being pressurized simultaneously,
then the number of BLMs that have failed at pressure $P$ is $\Phi(P) \times n$.

The general form for the mean or expected value $\mu$ of a distribution is found by integrating (Devore, 2000),

$$\mu = \int_{-\infty}^{\infty} x \cdot f(x) \, dx,$$

which for a Weibull distribution is,

$$P_\mu = P_{\min} + P_0 \Gamma \left(1 + \frac{1}{\beta}\right),$$

where $\Gamma(x)$ is the gamma function. The gamma function $\Gamma(x)$ is defined by,

$$\Gamma(x) = \int_{0}^{\infty} y^{x-1}e^{-y} dy.$$  

Weibull incorporated the concept of the weakest link theory into his distribution, using the analogy that the probability of failure of a chain is limited by the probability of failure of the weakest link in the chain (Weibull, 1951). In terms of materials strength, this can be interpreted to mean that a larger size material will have a greater probability of failure under load because it will have more ‘links’ and therefore contain more defects. The strength of a carbon fiber, for example, is highly dependent on its length (Wisnom, 1999). A long carbon fiber will have a lower strength than a short carbon fiber because of the increase in the number of defects present in the fiber. By testing carbon fibers of different lengths, the degree by which strength changes as a function of length can be determined. In modeling for carbon fibers and ceramics, the failure distribution is often expressed as
a function of the size of the material (Mallick, 1993; Wachtman, 1996). Using weak link scaling analysis, the failure strength of any size material can be predicted by knowing the failure strength of any other size material (Pickering and Murray, 1999).

Experimentally the failure pressure of BLMs follows an inverse power law relationship with pore diameter,

\[
P = \frac{1}{bd^m},
\]

(4.18)

where \(P\) is pressure, \(b\) is a constant, \(d\) is pore diameter, and the exponent \(m\) is another constant. It is sometimes convenient to linearize this equation by taking the logarithm of both sides,

\[
\log P = -m \log d - \log b.
\]

(4.19)

A log-log plot of failure pressure versus pore diameter should fall approximately on a straight line. Linear regression analysis can then be used to determine the slope, \(-m\), and the intercept, \(-\log b\).

If the failure pressure \(P_1\) is known for pore size \(d_1\), then the failure pressure \(P_2\) can be predicted for any other pore diameter \(d_2\) using the inverse power law in Equation 4.18. This is done by writing the equations for the two pore sizes, \(P_1 = 1/bd_1^m\) and \(P_2 = 1/bd_2^m\), solving for constant \(b\), and then setting the two equations equal to each other. The resulting prediction of failure pressure \(P_2\) is,

\[
P_2 = P_1 \left(\frac{d_1}{d_2}\right)^m.
\]

(4.20)

Here \(m\) is a measure of the magnitude of the size effect. In the analysis of carbon fibers, it is often found that there is a relationship between the Weibull modulus \(\beta\) for the strength distribution and the size effect exponent \(m\) (Wisnom, 1999). Specifically, it is normally found that \(m = 1/\beta\). The Weibull modulus \(\beta\) is a measure of the amount of variability in fiber strength for a given length. A low value of \(\beta\) indicates a high degree of variability and vice versa. This means that a high variability in fiber strength results in a larger size effect (Laurencin et al., 2007). Some authors, however, have found that this relationship does not accurately reflect experimental data and therefore introduced another parameter, the correlation parameter \(\alpha\), so that \(m = \alpha/\beta\) (Watson and Smith, 1985). The correlation parameter \(\alpha\) allows a more precise way to specify the relationship between strength variability and the size effect. For \(0 \leq \alpha \leq 1\), a value of \(\alpha = 0\) would indicate that there is no
dependence of material strength on size, and a value of $\alpha = 1$ would indicate that the size effect is directly correlated to the variability in strength.

As it relates to the pressurization of BLMs, two parameters scale according to pore size in the Weibull CDF from Equation 4.14, the characteristic pressure $P_0$ and the location parameter $P_{\text{min}}$. Substituting the scaling relationship in Equation 4.20 into the Weibull CDF in Equation 4.21 for $P_0$ and $P_{\text{min}}$ gives a more generalized form of the Weibull CDF,

$$
\Phi(P) = 1 - \exp \left[ - \left( \frac{P}{P_{0,1} (d_1/d_2)^{\alpha/\beta}} - \frac{P_{\text{min},1}}{P_{0,1}} \right)^\beta \right],
$$

(4.21)

where $P_{0,1}$, $P_{\text{min},1}$, and $d_1$ are nominal values of the characteristic pressure, minimum pressure, and pore diameter, respectively. Pore diameter $d_2$ is the current pore size.

**Calculation of the fraction of failed BLMs after bulk failure**

When many BLMs are pressurized simultaneously it is observed that not all BLMs fail at the end of a test, resulting in a residual back pressure after bulk failure of the BLMs. The pressurization model can be used to determine the fraction of BLMs that have failed based on the value of the back pressure, or equilibrium pressure $P_{\text{eq}}$. Pressure reaches an equilibrium when the flow rate of fluid that is moved by the piston, $Q_{\text{piston}}$, is equal to the flow rate of fluid exiting through the pores, $Q_{\text{pores}}$. By setting Equations 4.3 and 4.5 equal to each other and solving for the combined permeability $\kappa'$ it is found that,

$$
\kappa' = A_{\text{piston}} \Delta x_{\text{stepper}} f_{\text{stepper}} \frac{\mu l}{A_{\text{substrate}} (P_{\text{eq}} - P_{\text{atm}})},
$$

(4.22)

Substituting Equation 4.11 for $\kappa'$ and also relabeling the total fraction of failed BLMs after pressurization as $\chi_{\text{tot}} = \chi_i + \chi_f$ produces the result,

$$
\chi_{\text{tot}} = A_{\text{piston}} \Delta x_{\text{stepper}} f_{\text{stepper}} \frac{\mu l}{\kappa A_{\text{substrate}} (P_{\text{eq}} - P_{\text{atm}})}.
$$

(4.23)

**4.3.2 Modeling of BLM electrical impedance**

**Electrical impedance models for the control trials without BLMs**

In the first control trial, the impedance of the electrode/electrolyte interface and electrolyte was measured with no partition between the electrodes. The impedance of the electrode/electrolyte interface is due to the double layer of ions that surround a charged electrode in an electrolyte solution (Hibbert, 1993; Rieger, 1994). This is commonly modeled
as a resistor $R_i$ and Warburg circuit element $Y_i$ in parallel with a capacitor $C_i$. The capacitor accounts for charge separation at the double layer while the Warburg circuit element accounts for diffusion and mixing of ions. The electrolyte solution is modeled as a simple resistor $R_e$. Like a solid conductor, $R_e = \rho L / A$, where $\rho$ is the resistivity of the ionic solution, $L$ is the distance between electrodes, and $A$ is cross sectional area. Together, the electrode interface and the electrolyte make up the Randle equivalent circuit, shown schematically in Figure 4.7 (Macdonald, 2006). The total impedance of the circuit is the combination of the interface impedance $Z_i$ for two electrodes and the electrolyte impedance $Z_e$,

$$Z_{\text{tot}} = 2Z_i + Z_e.$$  (4.24)

The impedance for a resistor is $Z_R = R$, for a capacitor is $Z_C = 1/(j\omega C)$, and for a Warburg element is $Z_W = 1/(j\omega)^{1/2}Y$. Substituting components and reducing gives a total impedance of,

$$Z_{\text{tot}} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2} + C_i (j\omega)^{3/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega)} + R_e.$$  (4.25)

At low frequencies both the electrode interface ($Z_i$) and the electrolyte ($Z_e$) contribute to the total impedance of the system. At high frequencies, however, the impedance of the electrode interface approaches zero, and only the electrolyte resistance remains.

For the second control trial, impedance was measured for a porous substrate without a BLM formed over it. The impedance of an open pore is modeled as a resistor, and this resistance can be divided into two parts. One is the convergence resistance $R_c$, which is the result of a funneling effect as the ions are restricted from a large cross sectional area of motion to a smaller one (Hall, 1975). Hall has estimated the convergence resistance as,

$$R_c = \frac{\rho}{4a},$$  (4.26)

where $a$ is the pore radius. The other part is the resistance within the pore $R_p$. Since
Figure 4.8: Equivalent circuit for the impedance of an electrolyte with a multi pore partition.

\[ R = \rho L / A, \] the resistance within the pore is (Wonderlin et al., 1990),

\[ R_p = \frac{l\rho}{\pi a^2}, \tag{4.27} \]

where \( l \) is the length of the pore. These two resistances together are referred to as the access resistance, \( R_a = 2R_c + R_p \). The convergence resistance \( R_c \) is multiplied by a factor of two to account for the convergence of ions on either side of an open pore.

In the case of a multi pore substrate there are many pores whose resistances combine in parallel, shown schematically in Figure 4.8, where \( n \) is the total number of pores in the partition. In this case the total impedance of the system is,

\[ Z_{tot} = 2Z_i + Z_e + \frac{Z_a}{n}. \tag{4.28} \]

Substituting components and reducing gives a total impedance of,

\[ Z_{tot} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2}} + R_e + \frac{2R_c + R_p}{n}. \tag{4.29} \]

**Electrical impedance model for BLMs before failure**

Electrical impedance has been recorded before BLM failure and after BLM failure. Before failure, the electrical impedance of the system is the sum of three components; the electrode/electrolyte interface, the electrolyte, and the BLM. BLMs are typically modeled as a resistor \( R_{BLM} \) in parallel with a capacitor \( C_{BLM} \) (Bordi et al., 2002; Naumowicz et al., 2005). The BLM is a physical barrier to ion motion which gives it resistance and it separates charge which gives it capacitance.
This experimental setup is not just one BLM formed over one pore, but rather an array of many micro-BLMs formed over many pores. The array of micro-BLMs combine in parallel, as shown schematically in Figure 4.9. Because they combine in parallel each individual micro-BLM has a higher impedance than the total group. The total impedance of the circuit is,

\[ Z_{\text{tot}} = 2Z_i + Z_e + \frac{Z_{\text{BLM}}}{n}, \quad (4.30) \]

where \( n \) is the total number of micro-BLMs. Substituting components and reducing gives a total impedance of,

\[ Z_{\text{tot}} = 2 \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2}} + R_e + \frac{R_{\text{BLM}}}{n (1 + j\omega C_{\text{BLM}} R_{\text{BLM}})}. \quad (4.31) \]

### Electrical impedance model for BLMs after failure

Only a small percentage of BLMs actually fail before pressure begins to drop inside of the stepper motor test fixture. Some pores become open while other continue to be blocked by a BLM. Components of the impedance of the system after failure include the electrode/electrolyte interface, the electrolyte, and a combination of BLMs and open pores acting in parallel, as shown in Figure 4.10. This is represented mathematically as,

\[ Z_{\text{tot}} = 2Z_i + Z_e + \frac{Z_{\text{BLM}} Z_a}{n (\chi_{\text{tot}} Z_{\text{BLM}} + (1 - \chi_{\text{tot}}) Z_a)}. \quad (4.32) \]
where $\chi_{tot}$ is the fraction of BLMs that have failed. Substituting components and reducing gives a total impedance of,

$$Z_{tot} = \frac{1 + R_i Y_i (j\omega)^{1/2}}{Y_i (j\omega)^{1/2} + C_i (j\omega) + R_i Y_i C_i (j\omega)^{3/2} + R_e} + \frac{R_a R_{BLM}}{R_a (1 + j\omega C_{BLM} R_{BLM}) n (1 - \chi_{tot}) + R_{BLM} n \chi_{tot}}.$$  

(4.33)

### 4.4 Results

#### 4.4.1 Water column pressurization results

Failure pressure was measured as a function of pore size for six different lipid combinations using the water column test fixture. For ease of viewing they have been split into two different plots, Figure 4.11 (a) and (b). Figure 4.11 (a) is bulk failure pressure versus pore size for asolectin, SOPC, and SOPC/CHOL-50 mol%. Errorbars indicate the standard error $\sigma/\sqrt{n}$ for the six trials of each pore size. For this group of lipids, asolectin had the
Figure 4.11: Failure pressure versus pore size measured using the water column test fixture. (a) BLMs formed from asolectin, SOPC, and SOPC/CHOL-50 mol%. (b) BLMs formed from POPE, POPE/CHOL-30 mol%, and POPE/CHOL-50 mol%.

lowest bulk failure pressure, which is expected because it is a mixture of different phospholipids that contains many impurities. The bulk failure pressure of SOPC was on average 43% higher than asolectin. It was also to be expected that the mixture of SOPC/CHOL-50 mol% had a higher bulk failure pressure than pure SOPC (of 24% on average) since it is known that cholesterol increases the strength of some phospholipids.

Figure 4.11 (b) is bulk failure pressure versus pore size for POPE, POPE/CHOL-30 mol%, and POPE/CHOL-50 mol%. Here POPE and POPE/CHOL-30 mol% had close to the same average failure pressure, with POPE/CHOL-30 mol% actually having a 2% lower failure pressure than POPE on average. This is somewhat surprising, although Needham and Nunn have found that cholesterol does not significantly increase the failure pressure of phospholipids for concentrations less than 50 mol% (Needham and Nunn, 1990). POPE/CHOL-50 mol% gave better results, having a 34% higher failure pressure than POPE alone. Finally, in a comparison of POPE with SOPC, POPE had a 28% higher bulk failure pressure than SOPC on average.
4.4.2 Stepper motor pressurization results

Results for pressurization control trials without BLMs

Figure 4.12 is a plot of pressure versus piston displacement for the control trials, in which porous substrates were pressurized without a BLM formed over them. For most pore sizes, no significant pressure buildup was observed, indicating that fluid was free to flow through the porous substrate in the absence of a BLM. For the 0.05 µm pore size, a sizable back pressure was generated, but this back pressure was still small when compared to pressurization of a BLM formed over the same substrate and had a significantly different slope.

The permeability of each porous substrate was determined experimentally. To do this, a porous substrate without a BLM was pressurized using the stepper motor test fixture. The stepper motor was turned on at time $t_0$ and run at a high frequency (5 to 10 Hz) to generate back pressure inside the lower chamber. Then the stepper motor was turned off at time $t_1$ and the back pressure was allowed to decrease as fluid exited through the pores. The model described in Section 4.3.1 was used to curve-fit the permeability. The model was modified such that $\Delta V_{\text{piston}}$ was held as a constant value after time $t_1$ when the piston stopped moving, and the permeability was held as a constant value for the entire simulation. Figure 4.13 is an example in which a substrate with 0.2 µm diameter pores was pressurized by running the stepper motor at a frequency of 5 Hz for approximately 35 s. At
time $t_1 = 35$ s, the stepper motor was turned off and the back pressure decreased to zero. Ideally the model should provide a good fit of the experimental pressure curve both when stepper motor is turned on and when it is turned off. However, often times two different permeability values are needed to fit these two different regimes. Before $t_1$ the modeling of the permeability is complicated by the fact that pressure is increasing due to the piston motion while simultaneously decreasing due to fluid flow through the pores. The model is simplified after $t_1$ since the piston is stopped. The experimental response in this regime also has less noise and is more repeatable. Therefore a permeability value was chosen that provided the best curve-fit after time $t_1$, when pressure was decreasing. In the example shown in Figure 4.13 the permeability was estimated as $8.0 \times 10^{-17}$ m$^2$.

**Results for pressurization trials with BLMs**

For tests that used the stepper motor test fixture, Figure 4.14 (a) is a plot of pressure versus piston displacement for ten pressurization trials under the same test conditions of SOPC BLMs formed over substrates with 0.2 $\mu$m diameter pores. Figure 4.14 (b) is the same plot for substrates with 2 $\mu$m diameter pores. Pressure curves that were measured using the stepper motor test fixture were very repeatable. Initially, the porous substrate is

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**Figure 4.13:** A trial in which a 0.2 $\mu$m porous substrate without a BLM was pressurized by running the stepper motor at 5 Hz starting at time $t_0$, and then turning it off at time $t_1$ to allow pressure to decrease. The experimentally measured curve is marked by a solid line and the model based on a curve-fitted permeability is marked by a dotted line.
sealed by BLMs and the pressure rises as the piston compresses the internal fluid chamber of the test fixture. At some critical pressure many of the BLMs fail and allow fluid to escape the internal chamber, causing a pressure drop and giving the pressure curve a characteristic peak. This peak pressure is considered the bulk failure pressure of the BLMs. Not all BLMs fail, however, and so the pressure does not drop to zero but instead reaches a steady state equilibrium pressure. The piston continues to pump fluid through the porous substrate and the BLMs that remain intact resist the fluid motion. Due to the large amount of data that has been collected, only these two sample plots are shown.

A logarithmic plot of failure pressure versus pore size is shown in Figure 4.15, where each point represents the mean bulk failure pressure of all trials for that pore size. In this plot data is shown for SOPC and SOPC/CHOL-50 mol%, as measured using the water column test fixture and the stepper motor test fixture. Data points from the water column test fixture represent the average of six trials while data points from the stepper motor test fixture are the average of ten trials. Errorbars were calculated by the standard error $\sigma_f/\sqrt{n}$ and are represented by vertical lines but appear to be very small because the range of pressure is very large on a logarithmic scale.

The experimental results establish a clear trend between the failure pressure of BLMs and the pore size of their supporting substrate, demonstrating that failure pressure increases as pore size decreases. On the logarithmic plot from Figure 4.15 this relationship was nearly
Figure 4.15: Failure pressure versus pore size. Squares denote SOPC BLMs measured by the stepper motor test fixture; diamonds denote SOPC BLMs measured by the water column test fixture; plus marks denote SOPC/CHOL-50 mol% BLMs measured by the stepper motor test fixture; X marks denote SOPC/CHOL-50 mol% BLMs measured by the water column test fixture; vertical lines denote errorbars.

linear. Data taken with the water column test fixture is in good agreement with data taken using the stepper motor test fixture. Also, bulk failure pressure was on average 47% higher for mixtures of SOPC and cholesterol than for pure SOPC as measured by the stepper motor test fixture.

In the previous chapter, failure pressures were measured for BLMs formed over a single pore, but only over a range of pore diameters from 5 to 20 µm. The average of these failure pressures represent the mean of the distribution, $P_\mu$. Failure pressure could not be measured for a single BLM with a pore size smaller than 5 µm because it was difficult to isolate a single pore of these sizes and because the very large failure pressures would eventually exceed the capacity of the test fixture. The mean failure pressures for single BLMs between 0.05 and 2 µm were therefore estimated by fitting a line to the 5 to 20 µm data and extrapolating to smaller pore sizes, as shown in Figure 4.16. Interestingly the slopes of the failure pressure for multi and single BLMs were very similar (-0.95 and -1.07 respectively), although the magnitude of failure pressure was approximately 17 times higher for single BLMs.

For each pore size the average of ten pressurization trials of SOPC BLMs is shown with the 95% confidence interval for a t distribution with nine degrees of freedom in Figures
Figure 4.16: Extrapolation of SOPC single BLMs to smaller pore sizes. Squares denote failure pressure of SOPC BLMs on multiple pores; triangles denote failure pressure of SOPC BLMs on single pores; diamonds denote extrapolated values of single pore failure pressure for SOPC BLMs; the dotted line is the regression line.

4.17 (a), (b), and (c). The data has been separated into three plots for ease of viewing. Also, modeled pressure curves were calculated according to Section 4.3.1. For the model the three Weibull parameters, $P_0$, $\beta$, and $P_{min}$, are needed as well as the fraction of initially failed BLMs, $\chi_i$. The characteristic pressure $P_0$ was determined using Equation 4.16, where the mean pressure $P_\mu$ was the average failure pressure of a single BLM. The location parameter $P_{min}$ was set equal to the bulk failure pressure $P_b$ for BLMs formed over multiple pores. This had the effect of offsetting the Weibull distribution so that a large number of BLMs failed simultaneously at the bulk failure pressure $P_b$. When a large number of BLMs fail at once, fluid rapidly exits the test fixture through newly opened pores causing pressure to drop. When this happens the pressure curve exhibits a peak, as in Figures 4.14 (a) and (b). The shape parameter $\beta$ and the fraction of initially failed BLMs $\chi_i$ were determined purely by curve fitting. All of these parameters are reported in Table 4.2, as well as the total fraction of failed BLMs at the end of the test, $\chi_{tot}$, which was calculated according to Equation 4.23.

Using the generalized Weibull CDF in Equation 4.21, any pressurization curve can be predicted based on the failure characteristics of one nominal pore size. If the smallest pore size that was tested, 0.05 $\mu$m, is chosen as the nominal pore size, then the nominal pore
Figure 4.17: For the pressurization of SOPC, the solid line is the experimental average of ten trials, the dash-dash lines are the 95% confidence interval of the experimental data, and the dotted line is the model. (a) 0.05 μm, and 0.1 μm diameter pores. (b) 0.2 μm, 0.4 μm, and 0.8 μm diameter pores. (c) 2 μm, 5 μm, and 10 μm diameter pores.
Table 4.2: Parameters for the modeling of SOPC pressurization curves: nominal pore diameter $D$, mean failure pressure $P_\mu$ of a single BLM, the Weibull characteristic pressure $P_0$ (the scale parameter), the Weibull shape parameter $\beta$, the bulk failure pressure of multiple BLMs which was used as the Weibull location parameter $P_{\text{min}} = P_b$, the fraction of BLMs that are initially failed $\chi_i$, and the calculated total fraction of failed BLMs at the end of the test $\chi_{\text{tot}}$.

<table>
<thead>
<tr>
<th>$D$ ((\mu)m)</th>
<th>$P_\mu$ (kPa)</th>
<th>$P_0$ (kPa)</th>
<th>$\beta$</th>
<th>$P_{\text{min}} = P_b$ (kPa)</th>
<th>$\chi_i$</th>
<th>$\chi_{\text{tot}}$</th>
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</table>

diameter is $d_1 = 0.05 \ \mu\text{m}$, the nominal characteristic pressure is $P_{0,1} = 1060 \ \text{kPa}$, and the nominal minimum pressure is $P_{\text{min,1}} = 350 \ \text{kPa}$. The shape parameter $\beta$ is nearly the same for every pore size, and therefore it can be defined as the average value of $\beta$, which is $\beta_{\text{avg}} = 0.42$. The correlation parameter $\alpha$ is a measure of the dependency of failure pressure on pore size. It can determined by plotting $P_0$ and $P_{\text{min}}$ versus pore diameter on a logarithmic scale and calculating the slope of the regression line, where slope = $-\alpha/\beta_{\text{avg}}$. This plot is shown in Figure 4.18. Based on this plot, values of $\alpha$ were determined to be $\alpha = 0.39$ for $P_0$ and $\alpha = 0.46$ for $P_{\text{min}}$. However, it is believed that the slopes of the $P_0$ and the $P_{\text{min}}$ lines are coupled to each other, and the differences in slope only reflect experimental variation. Therefore only one value of $\alpha$ will be used, which will be determined as the average for $P_0$ and $P_{\text{min}}$. This value is $\alpha_{\text{avg}} = 0.43$.

In Figure 4.19 the average pressurize curves and modeled pressure curves have again been plotted for each pore size. For this model, though, the $P_0$ and $P_{\text{min}}$ parameters have been predicted for each pore size based on a nominal pore size of 0.05 \(\mu\text{m}\) and average values for $\alpha$ and $\beta$. The generalized model is a less accurate fit to the experimental pressure curves than it was when the parameters were fitted to each individual pore size. For many pore sizes, though, this model is still a reasonable representation of the experimental data.
Figure 4.18: Plot of $P_0$ and $P_{\text{min}}$ versus pore size that is used to determine the correlation parameter $\alpha$ based on the slope of the regression line, where slope $= -\alpha/\beta_{\text{avg}}$.

### 4.4.3 Electrical impedance results

**Results for electrical impedance control trials with no BLMs**

First a control trial was performed in which the electrical impedance was measured with no partition between the electrodes. Using the model from Equation 4.25, the impedance parameters for the electrode/electrolyte interface and the electrolyte were curve fitted to the experimental data, shown in Figure 4.20 (a). From this model, it was found that $R_e = 1.1 \text{k}\Omega$, $R_i = 16 \text{k}\Omega$, $Y_i = 4.0 \times 10^{-4} \text{S}\text{s}^{1/2}$, and $C_i = 80 \mu\text{F}$.

The second control trial measured the impedance of a multi pore substrate with 2 $\mu$m pores without a BLM, shown in Figure 4.20 (b). The contribution of the multi pore substrate to the total impedance of the system was predicted using Equation 4.29. Because the pores are arranged in parallel, a higher number of pores $n$ reduces the electrical resistance of the partition. The number of pores is very large for these partitions, and in this case for the 2 $\mu$m pore substrate there are $n = 1.6 \times 10^5$ pores. Because of this the multi porous filter adds a negligible amount of resistance to the overall impedance of the system, as can be seen in Figure 4.20 (b). The model confirms this, and shows only a slight increase of 18 $\Omega$ in resistance when this multi pore partition is present.
Figure 4.19: Model for the pressurization of SOPC where the model parameters have been predicted based on the nominal parameters for a 0.05 \( \mu \)m pore size. (a) 0.05 \( \mu \)m, and 0.1 \( \mu \)m diameter pores. (b) 0.2 \( \mu \)m, 0.4 \( \mu \)m, and 0.8 \( \mu \)m diameter pores. (c) 2 \( \mu \)m, 5 \( \mu \)m, and 10 \( \mu \)m diameter pores.

Figure 4.20: Electrical impedance control trials for (a) the electrode interface and electrolyte alone, and (b) a porous substrate with 2 \( \mu \)m pores and no BLM.
Results for electrical impedance trials with BLMs

For SOPC BLMs formed on 0.2 µm pore substrates, Figure 4.21 (a) is a plot of impedance and phase versus frequency for ten trials before pressurization, and Figure 4.21 (b) is impedance after bulk BLM failure. Figures 4.21 (c) and 4.21 (d) are the same measurements for 2 µm pore substrates. In both cases, there is a clear reduction by approximately two orders of magnitude in the peak impedance between the before failure case (≈ 10^6 Ω) and the after failure case (≈ 10^4 Ω). This drop in impedance is attributed to the opening of conductive pathways through the pores as the BLMs fail. Overall these impedance measurements were very repeatable. As with the pressure data, impedance plots are shown only for select pore sizes because of the large amount of data that was recorded.

For each pore size an average impedance was calculated as well as a 95% confidence interval using a t distribution with nine degrees of freedom. The models that were described in Section 4.3.2 were applied to the before and after failure cases. The average curves, confidence intervals, and models are shown in Figure 4.22 for the 0.2 and 2 µm pore sizes. Several curve fitting parameters were used to fit the model to the experimental data. For the before failure case the curve fitting parameters were the resistance and capacitance of the BLM, $R_{BLM}$ and $C_{BLM}$. A normalized value for conductance, which is the inverse of resistance, and capacitance were also calculated by dividing by the surface area $A$ covered by the BLM. For the after failure case the fraction of failed micro-BLMs $\chi_{tot}$ was used as a fitting parameter. Values for these fitting parameters are summarized in Table 4.3. However, the values of $\chi_{tot}$ that were found by the electrical impedance method were much smaller than the values that were found by the pressurization model in Table 4.2. One possible explanation for this is that when a micro-BLM fails it leaves some remnants around the perimeter of the pore. This effectively leaves the open pore with a smaller diameter than its nominal size. Another approach for modeling this impedance would be to use the fraction of failed BLMs that was calculated from the pressurization model in Table 4.2 and to fit the access resistance of the open pores to the experimental data. Then using Equations 4.26 and 4.27, the effective diameter of the pore after BLM failure, $D_f$, can be calculated. Since the open pores are partially blocked by BLM remnants these diameters should be smaller than the nominal pore diameter. These diameters $D_f$ are shown in Table 4.3.
Figure 4.21: Impedance measurements for an SOPC BLM formed on a substrate with (a) 0.2 µm diameter pores before pressurization, (b) 0.2 µm diameter pores after pressurization, (c) 2 µm diameter pores before pressurization, and (d) 2 µm diameter pores after pressurization.

Table 4.3: Parameters for the modeling of SOPC impedance curves: nominal pore diameter $D$, BLM resistance $R_{BLM}$, BLM capacitance $C_{BLM}$, normalized BLM conductance $G_{BLM}/A$, normalized BLM capacitance $C_{BLM}/A$, fraction of failed BLMs $\chi_{tot}$, and effective pore diameter after BLM failure $D_f$.

<table>
<thead>
<tr>
<th>$D$ (µm)</th>
<th>$R_{BLM}$ (Ω)</th>
<th>$C_{BLM}$ (F)</th>
<th>$G_{BLM}/A$ (S/cm²)</th>
<th>$C_{BLM}/A$ (F/cm²)</th>
<th>$\chi_{tot}$</th>
<th>$D_f$ (µm)</th>
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Figure 4.22: Average impedance, 95% confidence interval, and model for an SOPC BLM formed on a substrate with (a) 0.2 \( \mu \)m diameter pores before pressurization, (b) 0.2 \( \mu \)m diameter pores after pressurization, (c) 2 \( \mu \)m diameter pores before pressurization, and (d) 2 \( \mu \)m diameter pores after pressurization.
4.4.4 Results for fluorescence imaging of BLMs

Results for fluorescence control trials

Images were recorded of SOPC BLMs formed over porous substrates with 8 μm pores using a fluorescent dye and a fluorescence equipped microscope. Areas in which the dye has attached to the SOPC molecules appears red or orange when exposed to fluorescent lighting. First, several control trials were performed. In Figure 4.23 (a) a BLM was formed over a porous substrate but no dye was added. In Figure 4.23 (b), dye was added to a porous substrate without any lipids. In both cases, the image was black with only trace amounts of fluorescent red spots, which were probably due to contamination in the test setup. This indicates firstly that a BLM without a dye is not visible under fluorescent lighting, and secondly that the dye alone does not fluoresce in the absence of phospholipid molecules. Only the combination of the two provides a visible image.

Results for BLM fluorescence trials

For other trials a BLM was formed over a porous substrate, dye was added, and then it was pressurized using a hand operated syringe. An image was recorded before and after pressurization. Before pressurization BLMs were observed to form across most pores, appearing as red or orange circles (see Figure 4.24 (a)). In many cases, pressurization did not cause any BLMs to fail in the recorded image area, such as Figure 4.24 (b). This
is not surprising, though, considering that mechanical and electrical modeling predicted that only a small fraction of BLMs fail when pressurized. Some BLMs do fail in every trial, but the microscope can only view a small area of the porous substrate at once, and therefore the selected viewing area will not always capture a BLM failure. In some instances, however, BLM failures were recorded, such as Figures 4.25, 4.26, and 4.27. White circles were added to the images to mark failed BLMs. In another trial, a freshly cleaved mica substrate was punctured using the tip of a needle, producing a single pore with a diameter of approximately 40 $\mu$m. An SOPC BLM was formed over the substrate and pressurized until failure, as shown in Figure 4.28. For most trials that were performed using this mica substrate a BLM was not successfully formed over the large diameter pore. In the trial shown, a BLM was formed but easily failed when it was pressurized.

### 4.5 Discussion

Failure pressure was measured for BLMs formed over multi pore substrates using two methods, by pressurization using a water column and by pressurization using a piston actuated by a stepper motor. Results from the water column test fixture in Figure 4.11 established a clear trend between increasing failure pressure with decreasing pore size. This may be due to an increasing likelihood of defects occurring in larger BLMs, which is the most widely accepted reason for such behavior in other materials such as carbon fibers (Chi and Chou, 1983). Several different types of lipids were tested. It was found that asolectin had
Figure 4.25: Fluorescent image in which some BLMs were observed to fail, (a) before pressurization, and (b) after pressurization. Photographed by author.

Figure 4.26: Fluorescent image in which some BLMs were observed to fail, (a) before pressurization, and (b) after pressurization. Photographed by author.
Figure 4.27: Fluorescent image in which some BLMs were observed to fail, (a) before pressurization, and (b) after pressurization. Photographed by author.

Figure 4.28: Fluorescent image in which a BLM was formed on a mica substrate with a single pore, (a) before pressurization, and (b) after pressurization. Photographed by author.
the lowest failure pressure, SOPC had a higher failure pressure, and POPE had the highest
failure pressure. The addition of cholesterol was found to increase the strength of SOPC and
POPE BLMs at a concentration of 50 mol%. Test results for SOPC and SOPC/cholesterol
were expanded to smaller pore sizes in Figure 4.15 using the more advanced stepper motor
test fixture. Failure pressures that were measured using the water column were in good
agreement with those measured using the stepper motor.

The pressurization model presented in Section 4.3.1 and Figure 4.17 provided some
physical interpretation of the pressurization and failure of BLMs using the stepper motor
test fixture. Most of the parameters that were needed for the model were known geometrical
or other physical properties of the test fixture and porous substrates. However, the Weibull
parameters and the fraction of initially failed BLMs were used as fitting parameters. Several
interesting insights were gained through the fitting of this pressure model using a Weibull
distribution.

The experimental pressure curves normally consisted of a pressure rise, a peak (which
occurs at the bulk failure pressure of the BLMs), and then a drop in pressure. Through
fitting of the model it was found that the pressure peak can only occur if a large number
of BLMs fail simultaneously. When this happens a large amount of fluid is able to flow
through the open pores and cause a sudden decrease in pressure. To model this the PDF
of the distribution must have a sharp increase in BLM failures at the bulk failure pressure.
A normal distribution can not accurately reproduce this pressure peak. This is because the
mean failure pressure in a normal distribution is always the location of the mode if the mean
pressure of the distribution is assumed to be the mean failure pressure of a single pore. In
order to create the desired pressure peak, the mode of the distribution must occur below
the mean failure pressure at the location of the bulk failure pressure. Therefore the PDF
for this distribution is better modeled as having a positive skew. The Weibull distribution
can have a positive skew with a mode at the bulk failure pressure by setting the location
parameter $P_{min}$ equal to the bulk failure pressure $P_b$. By doing this no BLMs can fail below
$P_b$, but when the pressure reaches $P_b$ a large number of BLMs fail at once.

The model made a clear connection between the failure pressure $P_\mu$ that was mea-
sured for a single BLM and the bulk failure pressure $P_b$ that was measured for multiple
BLMs. The failure pressure of a single BLM represented the mean of the Weibull distribu-
tion, while the bulk failure pressure of multiple BLMs represented the minimum failure

105
pressure of the distribution. Figure 4.16 showed that the bulk failure pressure for any pore size was approximately 17 times smaller than the average failure pressure of a single BLM. This indicates that in a multi pore configuration, not all BLMs fail at the same pressure, and the weakest BLMs fail first. The fact that the slope of failure pressure versus pore size is nearly the same for a single BLM or for multiple BLMs is evidence that there is a coupling between the failure pressure of these two configurations.

It was found that the shape of the distribution for BLM failure pressure was independent of the pore size. The shape parameter $\beta$ varied over a very narrow range of 0.36 to 0.50, which corresponds to a positively skewed distribution. This indicates that each pore size follows roughly the same distribution. This is also true for distributions of other materials such as fibers and ceramics. These material are commonly modeled as having only one distribution, but the distribution is scaled according to the size of the material using weak link scaling. Weak link scaling has been applied to the failure pressure distribution of BLMs to create a generalized model where the characteristic pressure $P_0$ and the location parameter $P_{\text{min}}$ have been estimated based on one nominal pore size. When this model was used to predict pressurization curves in Figure 4.19 it provided a reasonable fit to the experimental pressure curves, but with more error than a curve fit to each individual pore size as in Figure 4.17. So while this model is very powerful in that it can predict the behavior of a BLM at pore sizes that have not been measured, it is also limited by the variation that is inevitably found in experimental data.

The model also provided a way to determine the fraction of BLMs that did not form initially, $\chi_i$, and the total fraction of BLMs that failed at the end of the test, $\chi_{\text{tot}}$. It was known that some BLMs did not fully form over pores at the outset of a test because pressure increased at a more shallow slope than would otherwise have been predicted by the model. Matching the slope of the initial pressure rise revealed that the fraction of failed BLMs was between 1% and 12%. Also, the final equilibrium pressure was nonzero, which indicated that some portion of BLMs had not failed at the end of a test. If all BLMs were to fail during the test then the pressure would fall to zero. Instead, the BLMs that do not fail continue to block the substrate pores creating a back pressure in the test chamber. Using Equation 4.23 the final fraction of failed BLMs was calculated to be between 4% and 33%.

In most cases, the experimental pressure curve consisted of a pressure rise, a peak, and a steady decay in pressure to some equilibrium value. These aspects of the experimental
pressure curves were fitted by the model quite well. However, in some instances the pressure did not decay to a constant equilibrium value but instead had a steadily increasing pressure with large fluctuations (see, for example, the 0.4 \( \mu \text{m} \) and 2 \( \mu \text{m} \) pressure curves in Figure 4.17). This phenomenon could be due to several factors including failed BLMs reforming to block previously open pores, salt from the NaCl solution clogging open pores, and random local pressure fluctuations near the pressure transducer which appear more dramatic for the smaller pressure measurements associated with larger pore sizes. The model can not account for these random phenomena because it assumes that the permeability of the substrate is a constant and that BLMs do not reform after they have failed.

Electrical impedance was measured before and after bulk BLM failure and a model was applied to the average impedance curve for each pore size. The model was used to curve fit the resistance and capacitance of the BLM for each pore size, and also to determine the number of BLMs that failed after pressurization. From Table 4.3, the resistance and capacitance of BLMs varied according to pore size, but a better way to compare the resistance of the BLMs is to normalize the conductance by dividing by the BLM area (recall that conductance is the inverse of resistance). If \( G_{BLM}/A \) is compared among different pore sizes in Table 4.3, it has a fairly consistent value between \( 10^{-12} \) and \( 10^{-11} \) S/cm\(^2\) for sub-micron pore sizes, and between \( 10^{-8} \) and \( 10^{-9} \) S/cm\(^2\) for pore sizes that are greater than one micron. The difference between the normalized resistance of a sub-micron and micron size BLM may be in the number of defects in the structure of the BLM. A larger BLM is expected to have more defects and this may lead to a lesser ability to impede the flow of ions. In the literature these values ranged from \( G_{BLM}/A = 10^{-1} \) to \( 10^{-9} \) S/cm\(^2\) and \( C_{BLM}/A = 10^{-6} \) to \( 10^{-8} \) F/cm\(^2\), having a wide range of values due to the many variations of experimental test procedures (Yoshikawa et al., 1987; Mountz and Tien, 1978; Kobatake et al., 1970; Naumowicz et al., 2005; Bordi et al., 2002).

After BLM failure the electrical impedance dropped by approximately two orders of magnitude. The experimental impedance after failure was higher than would be predicted by a model that assumes that all BLMs fail during a pressurization test. However, since it is known that not all BLMs fail during a test, the model in Equation 4.32 was used to curve fit the fraction of failed BLMs, which was found to be between 0.1% and 1%. This is significantly lower than the percentage of failed BLMs that was predicted using the pressurization model, which was between 4% and 33%. One possible explanation for this is
that some BLMs only partially fail. The fluorescence images showed that in many cases a failed BLM leaves lipid remnants around the perimeter of a pore. Another approach that was used for modeling the electrical impedance after failure was to use the fraction of failed BLMs that was calculated from the pressurization model and to vary the diameter of the pore to determine how much of the pore was blocked by BLM remnants. In this case it was found that the effective pore diameter after BLM failure varied between 0.012 and 2.0 µm for nominal pore sizes of 0.05 to 10 µm, respectively. The true proportion of failed BLMs and the true effective diameter most likely falls between these bounding cases. The fraction of failed BLMs is between the values that were estimated using electrical impedance and the values that were estimated using the pressurization model, and the effective diameter is between the values that were estimated using electrical impedance and the nominal values.

A fluorescence microscope was used to image BLMs before and after pressurization. These images provided visual confirmation that not all BLMs fully form over a pore before pressurization, and that some but not all BLMs fail after pressurization. Videos were made of the porous substrates during pressurization, and fluid could be seen passing through pores in which BLMs failed, which provided further confirmation that some BLMs had indeed failed. BLMs failed in the center of the pore, and some remnants of the BLM were left in varying amounts around the perimeter of the pore after failure (see Figure 4.25 for examples of this). Also, some BLMs that failed after pressurization were not fully formed to begin with, indicating that weaker BLMs may fail before stronger BLMs (again, see Figure 4.25).

4.6 Conclusions

The failure of BLMs formed over multi pore substrates was investigated in three ways: by mechanical pressure measurements, by electrical impedance measurements, and by optical imagery. BLMs formed from various lipids were pressurized using a water column test fixture and a stepper motor test fixture, and failure pressures from both methods were in agreement with each other. An inverse power law relationship of increasing failure pressure with decreasing pore size was established. Pore sizes between 0.05 and 10 µm were tested using the stepper motor test fixture, and for SOPC BLMs bulk failure pressures were between 380 and 1.5 kPa, respectively. The addition of 50 mol% cholesterol increased the bulk failure pressure of SOPC by 47%.
Using a model of the pressurization curves it was found that the failure of multiple SOPC BLMs pressurized in parallel was well fit by a Weibull distribution with a positive skew. Weibull parameters were curve fitted to the experimental data and it was found that the shape parameter $\beta$ was nearly the same for all pore sizes, ranging from 0.36 to 0.50. Based on this the model was generalized so that the distribution of any pore size could be predicted from one nominal pore size. This generalized model was applied to each pore size and was able to provide a reasonable fit of the experimental pressure curves.

The pressurization model was also used to determine the fraction of BLMs that did not form over a pore at the outset of a test, which was between 1% and 12%, and to calculate the fraction of failed BLMs at the end of pressurization tests, which was between 4% and 33%. The electrical impedance model was also used to estimate the fraction of failed BLMs, which was between 0.1% and 1%. This value may have been skewed by BLMs that left remnants around the pore after failure, and the true fraction of failed BLMs is likely to fall between the bounding values that were found by pressurization and electrical impedance.

Electrical impedance was measured before and after BLM pressurization, and it was found that impedance dropped by approximately two orders of magnitude after BLM failure. A model was used to predict the resistance (or equivalently, the conductance) and capacitance of BLMs, and also the proportion of failed BLMs after pressurization. Normalized conductance values varied between $G_{BLM}/A = 4 \times 10^{-12}$ and $2 \times 10^{-8}$ S/cm$^2$, and normalized capacitance values varied between $C_{BLM}/A = 3 \times 10^{-14}$ and $1 \times 10^{-10}$ F/cm$^2$. 


Chapter 5

Summary and Conclusions

5.1 Summary and conclusions

In this work the failure of bilayer lipid membranes (BLMs) has been observed in several ways: mechanically, electrically, and optically. BLMs were formed in an electrolyte solution over substrates with a single pore and substrates with an array of multiple pores, and were pressurized until failure. A custom test fixture was designed and fabricated so that BLMs could be pressurized in very precise increments while measuring the applied pressure and the electrical impedance across the BLM. The application of pressure and recording of data was controlled using a custom Labview computer interface. Using this test setup, the maximum pressure that could be withstood by a BLM spanning various size pores was measured. In both single and multi pore tests, the test fixture applied pressure to the BLMs and pressure was observed to increase until a critical failure pressure was reached, after which the pressure dropped sharply. A similar phenomenon was observed using electrical impedance measurements. For each pressurization test, electrical impedance was measured across the BLM before pressurization and again after pressurization of the BLM. The electrical impedance was observed to drop by several orders of magnitude after pressurization. Before pressurization pores in the substrate were covered by the BLM, which has a high resistance to ionic motion, and therefore a high impedance was measured. After pressurization the BLM had failed, leaving pores open and allowing ions to move freely, resulting in a low impedance measurement. Finally, using a different custom test fixture, BLMs were pressurized and observed optically using fluorescence microscopy. Microscope images showed cases in which BLMs were fully formed across a pore before pressurization,
1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphatidylcholine (SOPC) BLMs were pressurized over single pore substrates with pore sizes ranging from 5 to 20 \( \mu m \). It was found that average failure pressures at these pore sizes were between 67 and 19 kPa, respectively. The addition of 50 mol% cholesterol (CHOL) to SOPC caused an overall average increase in failure pressure of 56%. A trend of increasing failure pressure with decreasing pore size was established, and this relationship was well modeled by an inverse power law.

SOPC BLMs were also pressurized over multi pore substrates with pore sizes ranging from 0.05 to 10 \( \mu m \), and with average bulk failure pressures ranging from 380 to 1.5 kPa, respectively. The addition of 50 mol% cholesterol to SOPC caused an average increase in failure pressure of 47%, which was comparable to the single pore case. For the same pore size, BLMs that were formed over a multi pore substrate had a 17 times average smaller bulk failure pressure than the failure pressure of a BLM formed over a single pore. This is because in a multi pore arrangement the failure of BLMs follows a statistical distribution where the weakest BLMs fail first, and not all BLMs fail during a test. This causes the bulk failure pressure to appear to be relatively small.

A model was developed to predict the pressurization curve of the BLMs. The model was applied to both the single and multi pore cases, but differed in the multi pore case by assuming that BLMs fail following a Weibull distribution. The model included several curve fitting parameters, which in the multi pore case included parameters for the Weibull distribution and the fraction of BLMs that did not fully form at the outset of a test. By fitting this model to the experimental pressure curves, it was found that between 1% and 12% of BLMs were failed initially. The pressurization model also provided a way to calculate the fraction of BLMs that had failed at the end of the test, which ranged from 4% to 33%, with no apparent relationship to pore size. One key result of using the Weibull distribution was that the failure of a BLM formed over a multiple pores was coupled to the failure of a BLM formed over a single pore. It was found that the distribution had a positive skew, and that the failure pressure of a single BLM represented the mean of the distribution while the bulk failure pressure of multiple BLMs represented the minimum failure pressure of the distribution. This behavior could not be simulated using a normal distribution.

The Weibull modulus was nearly the same for all pore sizes, with a value of approximately \( \beta = 0.4 \). Because of this it was possible to generalize the pressurization model so that
the distribution for any pore size could be predicted based on parameters for one nominal pore size. The pressure models that were predicted based on a nominal pore size provided a reasonable fit to experimental data, and any error was primarily due to experimental variability.

Electrical impedance was measured before and after BLM failure for the single and multi pore cases. An equivalent electrical circuit was developed for each measurement and these were used to model the impedance measurements. BLMs were modeled as a resistor and capacitor in parallel. In the single pore case, the impedance of the supporting substrate was found to mask the impedance of the BLM, and therefore the resistance and capacitance values for the BLM could not be determined. However, the before and after measurements were still useful for confirming the failure of the BLM, as they clearly demonstrated that a single pore the size of those tested opened up after the BLM was pressurized, causing a drop in peak impedance of approximately three orders of magnitude.

In the multi pore case, resistance and capacitance values were determined for SOPC BLMs. Normalized conductance values varied between $G_{BLM}/A = 4 \times 10^{-12}$ and $2 \times 10^{-8}$ S/cm$^2$, and normalized capacitance values varied between $C_{BLM}/A = 3 \times 10^{-14}$ and $1 \times 10^{-10}$ F/cm$^2$, both of which are within a reasonable range of values that have been reported in the literature. For multi pore substrates, impedance measurements that were taken after the pressurization of BLMs showed that some BLMs had failed, as the peak impedance values dropped by approximately two orders of magnitude from the before pressurization measurement. The electrical impedance was also used to estimate the fraction of failed BLMs after bulk failure, which was between 0.1% and 1%, although these values may have been skewed by BLMs that only partially fail after pressurization. The true fraction of failed BLMs is believed to fall between the value that was estimated by pressurization and the value estimated by electrical impedance.

Fluorescence microscopy was used to image SOPC BLMs formed over a multi pore substrate before, during, and after pressurization. These images visually confirmed that a BLM formed over most but not all pores at the outset of a test. After pressurization it was found that only a small fraction of BLMs had failed, which had been predicted previously using the pressure curve model. BLMs appeared to fail over the center of pores, and typically BLM remnants were left around the perimeter of the pore after failure.
5.2 Contributions

This work represents the first investigation of the failure characteristics of BLMs formed over porous substrates. The following contributions have been made to the field:

- A new test methodology was developed for pressurizing BLMs formed over porous substrates and measuring failure pressure.

- Failure pressures were reported for the first time for SOPC and SOPC/CHOL-50 mol% BLMs formed over single pore substrates with pore sizes ranging from 5 to 20 \( \mu \text{m} \) diameter and multi pore substrates with pore sizes ranging from 0.05 to 10 \( \mu \text{m} \) diameter.

- A new model was developed to predict the pressurization curve of a BLM formed over a single pore, which was based on the pressurization and flow of fluid through a porous medium.

- The model was expanded to further describe the pressurization of BLMs formed over a multi pore substrate, which assumed that the failure of an array of many BLMs follows a statistical distribution. This was found to be well fit by a Weibull distribution with a positive skew. The result of this model was to quantify the distribution parameters for BLMs, and to quantify the fraction of BLMs that fail after pressurization.

- It was further found that BLMs of all pore sizes had nearly the same Weibull shape parameter. Because of this the model could be generalized such that the distribution of BLM failure for any pore size could be predicted based on the distribution of one nominal pore size.

- Electrical impedance measurements were used to independently verify the failure of BLMs as a result of pressurization. The impedance measurements were modeled to show that before pressurization BLMs were formed over porous substrates, and after pressurization BLMs failed, leaving an open pore in their place.

- Another new test methodology was developed to pressurize BLMs formed over porous substrates while simultaneously recording optical images of the BLMs using fluorescence microscopy. Photographs and video footage were recorded of SOPC BLMs.
before, during, and after pressurization to directly prove that BLMs failed as a result of pressurization.

5.3 Future work

The following are recommendations for possible areas of continuing research:

- Expand the range of variables that are tested in the failure pressure of BLMs to include BLMs that have different cellular structures embedded in them, such as transporter proteins. Also test the failure pressure of BLMs formed over substrates with different surface treatments, both hydrophobic and hydrophilic.

- Measure the electrical impedance at a fixed frequency and as a function of time during BLM pressurization. Use this data to confirm that the failure of the BLM occurs simultaneously by mechanical and electrical measurements.

- Modify the fluorescence microscopy test fixture in order to quantifiably measure pressure and electrical impedance to be studied synchronously with video images of BLM failure.

- Develop a model for predicting the failure pressure of a BLM based on material properties, such as the Young’s modulus and Poisson ratio.

- Measure the failure pressure for a large number (> 50) of BLMs formed over single pores to more accurately determine the probability density function of failure.

- Further investigate the possibility that BLMs have the ability to spontaneously reform after failure.
Bibliography


Vita

David Hopkinson was born to Jane and Phil Hopkinson on February 4, 1979 in Fort Wayne, Indiana. He grew up in Charlotte, North Carolina, where he graduated from Providence High School in 1997. That year David enrolled in the Mechanical Engineering program at Georgia Tech. At Georgia Tech he was a member of the marching band and also participated in the cooperative work program in which he alternated semesters of study and work at the Michelin tire company. He graduated with highest honor in 2002 with his Bachelor's of Science degree in Mechanical Engineering. Following graduation, David remained at Georgia Tech as a member of the five year BS/MS program where he researched piezoelectric composite unimorph actuators under the direction of Dr. Chris Lynch. He graduated with his Master of Science degree in Mechanical Engineering in 2003, as the first graduate of the five year BS/MS program in Mechanical Engineering at Georgia Tech. David entered the graduate program at Virginia Tech in 2004, where he studied the mechanical stability of bilayer lipid membranes formed over porous substrates under the direction of Dr. Don Leo. During this time he was the recipient of the Virginia Space Grant Consortium fellowship. David defended and completed his Doctor of Philosophy degree in Mechanical Engineering in the Fall of 2007.