LIPID BILAYER FORMATION IN AQUEOUS SOLUTIONS OF IONIC LIQUIDS

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ABSTRACT

The formation of lipid bilayers between ionic liquid droplets is presented as a new means of forming functional bimolecular networks. Ionic liquids are molten salts that have a number of interesting properties, such as the ability to be a liquid at room temperature and exceedingly low vapor pressure. Our research demonstrates that it is possible to consistently and repeatable form lipid bilayers on droplets of ionic liquid solutions. Characterization of the bilayers interfaces shows that the ionic liquids have negligible effects on the stability and electrical properties of the bilayer. It is also shown that the conductance levels in the gating events of Alamethicin peptide are affected by some ionic liquids.

Acknowledgments

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To my family, thank you for the love, support and encouragement through all the years. I never would have made it this far without you all.

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Glossary

Single Interface Tray Substrate (SITS): the substrate used in almost all experiments presented in this thesis.
Droplet Interface Bilayer (DIB): A lipid bilayer formed between two droplets of aqueous droplet solution.
Aqueous Droplet Solution (ADS): All-encompassing term for the solution used to create the aqueous droplets for a particular experiment. Reference to this solution will always include phospholipids, and may include uncured hydrogel and or alamethicin peptide. If ionic liquids are included it will be noted.
Stock Lipid Solution (SLS): A mixture of phospholipids, KCl and MOPS. Used as the base for all other mixtures.
Regulated attachment Method (RAM): a technique for controlling the position of two aqueous droplets in order to for droplet interface bilayers.
Ionic Liquid (IL): A salt with a relatively low melting point, typically below 100 °C. The ionic liquids used in this study had melting points below room temperature and thus could be tested in liquid state.
Ionic Solution: a liquid containing both charged and uncharged molecules.
Transmembrane Ion Transport: The movement of charged molecules through the bilayer via a pore formed by a protein or peptide.
Bilayer Quality: Defines the bilayers capacitive nature, its resistance value and the amount of current allowed to "leak" through the bilayer (shown as spikes in current), ie a high quality bilayer has very little leak, produces a square wave with straight horizontal peaks and has very electrical resistance (1-10 GΩ).
Bilayer Stability: Defines a bilayers tendency to remain at a constant size and resistance value over time and under different applied voltage potentials.

Chapter 1: Introduction and Background

1.1 **Introduction**

Ever since man learned to walk upright he has been working to improve the tools he uses to manipulate his environment. Today we enjoy the luxury of engineered technology that was only a figment of our science fiction imagination a short time ago. Invariably the aim of engineered technology is coveted because it increases our productivity - no different from elite Olympic athlete pushing their bodies in athletic competition to test the boundaries of their physical abilities. As we celebrate and cheer their achievements with pride so too are the less fortunate hearing impaired quietly rooting for the next advancement in biologically engineered hearing aids.

Biologically inspired technology is a large, rapidly growing field that has been attracting a lot of attention lately. Taking advantage of evolutionary solutions to

the problems faced by designers and engineers today is logically the best path to take. Bio-mimetic sensors in particular have been gaining ground in fields like robotics, medicine and agriculture. The goal of the Leo group is to create a sensor that imitated the mechanotransduction processes found to transpire in the hair cells inside the inner ear of mammals which facilitate auditory sensing.

1.1.1 Motivation

The goal of the work presented in this thesis was to investigate the effects ionic liquids have on the properties and behaviors of lipid bilayers and how they may be used to enhance the biological sensor. The alterable properties of some ionic liquids make them useful for acquiring desirable behaviors from the lipid bilayers.

Work was also done to create a new substrate for testing single droplet interface bilayers (DIB). The substrate still uses the Regulated Attachment Method (RAM) [1], but gave more precise control over the size and position the DIBs and thus better repeatability of experiments. This heightened control made the data on the effects of the ionic liquids more reliable.

In this thesis, aqueous droplet solution is used as an all-encompassing term for the solution used to create the aqueous droplets for a particular experiment. All references to this solution will always include phospholipids, salts and the buffer solution, and may include uncured hydrogel and or alamethicin peptide. If ionic liquids are included it will be noted.

1.2 **Background**

1.2.1 Cell Biology

Animal cells have many components called organelles that work together to allow the cell to perform the processes for which it is designed. One of the most important components is the cell membrane. At only 5-7 nm thick, it surrounds the cell and acts as a semipermeable barrier between the cell and it environment by selectively allowing molecules to pass through [2, 3]. This discriminatory transportation facilitates many of cell functions including the electrical impulses that move your body[4, 5].

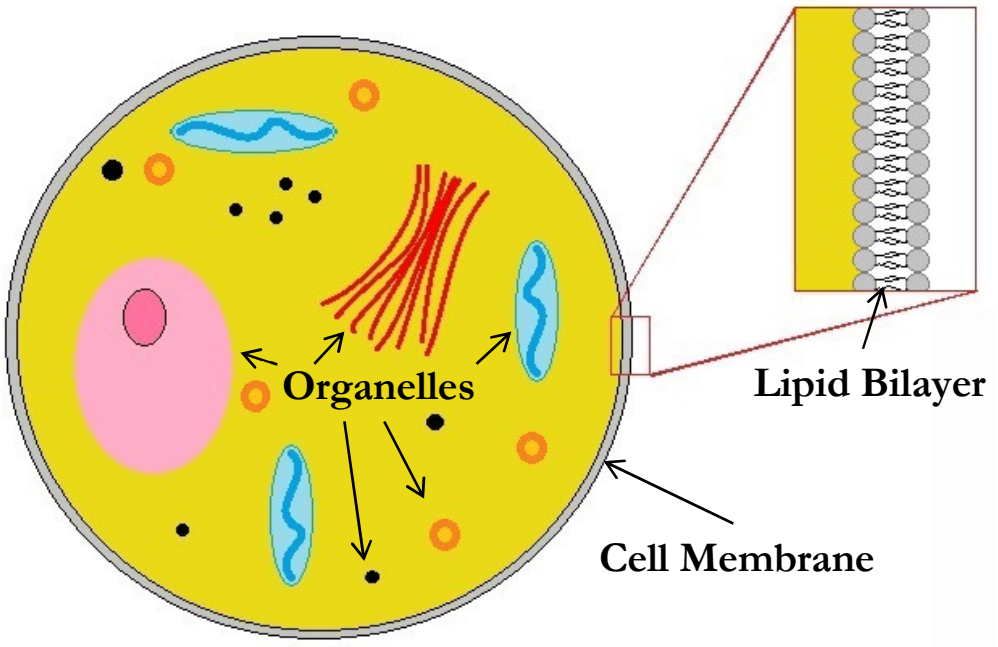


Figure 1.1: Diagram of an animal cell showing the cell membrane and organelles.

The cell membrane, depicted in Figure 1.1, is constructed of biological molecules called phospholipids in the form of a lipid bilayer. The lipid bilayer is a fluid structure allowing the lipids to shift and move relative to each other[3, 6]. It can be permeated by many uncharged molecules such as oxygen and water which the cell needs to function[7]. However the cell also needs other molecules such as sodium and potassium which carry a charge and cannot directly pass through the bilayer. There a number of different transmembrane proteins and peptides that allow the cell to control the passage of these charged molecules through the bilayer[8]. Many of these polymers are able to "pump" molecules against equilibrium concentrations to form concentration gradients and resting voltages, which can be expressed by the Nernst equation [2, 9, 10]. The work presented in this thesis uses the peptide alamethicin to study ion transport across the bilayer.

1.2.2 Lipids

The main focus of the work done in the Leo Group for the last few years has been the study of lipids and the membranes they form. Lipids are amphiphilic, meaning that for each molecule one part is hydrophobic and the other part is hydrophilic. Lipids can also be characterized as surfactants because they lower the interfacial tension at the interface between two dissimilar liquids [8, 11, 12]. This nature causes the lipid molecules to spontaneously self-assemble into structures that maximize water contact for the hydrophilic heads and minimize contact for the hydrophobic tails (portrayed in Figure 1.2) [13]. In polar environments such as the inside of a cell or a water droplet, the lipids form liposomes (shown in Figure 1.1) or vesicles which are spherical in shape and where the hydrophilic heads shield the

hydrophobic tails from the polar surroundings [8, 14]. The opposite happens in non-polar environments such as oil droplets. This behavior is called the Hydrophobic Effect and occurs whenever there is a high enough concentration of amphiphilic molecules in the solution[15-17]. The behavior is primarily attributed to the repulsive forces associated with the hydrophilic regions of the lipids and the surrounding water as well as the attraction of like molecules [18, 19].

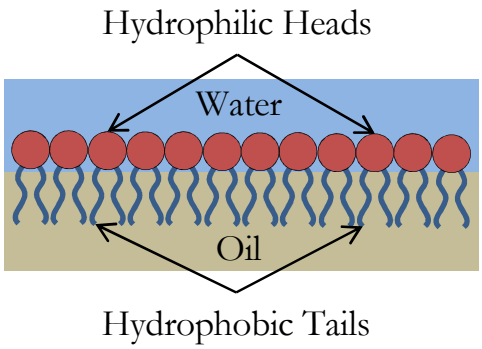


Figure 1.2: Amphiphilic phospholipids aligned at an oil water interface forming a monolayer. The hydrophilic head groups are attached to the water while the hydrophilic tails repel the water.

The size, shape and chemical composition of the lipids dictate the type of structure formed from the self-assembly process[16, 20]. By controlling the lipid and solvent properties one can create micelles, inverse micelles, vesicles and bilayers. This work specifically focuses on dual-tailed phospholipids that form lipid bilayers.

1.2.3 Lipid Bilayers

Lipid bilayers were first discovered by Isaac Newton and Robert Hooke when they observed that bimolecular films formed from lipid molecules did not reflect

transmitted light. This gave them a black appearance and lead to them being called "black lipid membranes" [21]. They were able to provide evidence that the molecules were able to self-assemble into ordered structures at the molecular level. Since then there has been a great deal of work done to characterize and document the properties and behaviors of lipid bilayers. There are many publications that give in depth descriptions and analysis of the chemical and physical properties of lipid bilayers [22-25]. The discussion below aims to give a basic description of lipid bilayers properties and formation techniques to facilitate understanding throughout the document.

Lipid bilayers are composed of two phospholipid monolayers which spontaneously self-assemble into a single molecule thick membrane on an oil water interface as described in the previous section. There are numerous methods for bringing these monolayers together [20, 26-32] many of which involve the use of microfluidic devices, pores in hydrophobic membranes or lipid painting. These methods can be separated into two categories; supported and unsupported. Supported methods involve the use of a structure to directly support the lipid bilayer and to facilitate bilayer formation. The unsupported methods were developed relatively recently and involve using the interface between touching droplets of aqueous liquid to form bilayers.

Supported Bilayer Formation

The majority of bilayer formation techniques utilize synthetic support structures which inhibit the movement of the lipid molecules and result in fragile, unstable bilayers. The surface properties, geometry and material properties of the support

structure have an effect on how and the speed at which the bilayer assembles, as well as the procedures that can be used to form the membrane [8].

One commonly used technique is the Langmuir-Blodgett (LB) method in which a glass slide is dipped into a water bath, upon which a monolayer of lipid molecules has been deposited. The hydrophilic regions of the molecules adhere to the glass at the air water interface forming a monolayer on the glass; a second dip linked the hydrophobic regions forming a bilayer film on the glass[33]. This technique produces very repeatable bilayers but they are not as consistent as other techniques [34, 35]. It is also difficult to incorporate membrane proteins resulting in diminishing use of this method [36].

The Montal-Mueller (MM) or lipid folding technique is another frequently used bilayer formation method. It utilized a sheet of nonconductive hydrophobic material such as Teflon or silicon, with an aperture formed in it across which the bilayer is formed. The sheet initially separates two baths of electrolyte solution with the aperture above the surface of the liquid. A solution of phospholipid in an organic solvent is spread across the electrolyte surface forming a monolayer at the air/water interface. The level of the electrolyte solution is then raised at the same rate on each side of the sheet and when it gets to the level of the aperture the monolayers fold together to form a lipid bilayer. This technique allows for multiple bilayers to be formed simultaneously and proteins are easily incorporated into the bilayer[37, 38]. However it also requires very precise control of the fluid levels which can be difficult[39].

Lipid painting is yet another bilayer formation technique in which an aqueous solution of phospholipids and organic solvent is physically brushed onto a hydrophilic substrate. The solvent keeps the lipids fluid during application and then is allowed to evaporate leaving a film of lipids on the substrate surface. The substrate is then immersed in an aqueous medium and the self-assembly process goes to work forming a bilayer with one side of head groups oriented towards the substrate and the other away from it shielding the tails[8, 40]. This method is very simple, but residual solvent can cause serious bilayer stability problems and denaturization of proteins[41, 42].

Unsupported Bilayer Formation

In an attempt to solve the fragility problem associated with supported bilayer formation a new method was developed that did not have a ridged support structure, which was dubbed droplet interface bilayers DIB. In this method the bilayer is formed at the interface between two contacting aqueous droplets submerged in an organic solvent. Initially the phospholipids were contained within the solvent and the heads assembled on the surface of the aqueous droplets [8, 27]. It was later shown that monolayers formed faster when the phospholipids were mixed into the aqueous phase which is how it is depicted in Figure 1.3A [8]. When the surfaces of the aqueous droplets are brought into contact, a small amount of oil is typically trapped between the droplets (Figure 1.3B). Electrostatic and hydrostatic forces slowly push this oil out bringing the monolayers into direct contact [8, 43, 44]. This causes the hydrophilic tails of the lipid molecules to “zip” together and form a lipid bilayer (Figure 1.3C).Proteins for membrane insertion can

also be added into the aqueous droplets [8, 45]. Initial experiments used microfluidic devices to control the size and position of the droplets, but Holden showed that quality bilayer could be formed in open wells of oil [46].

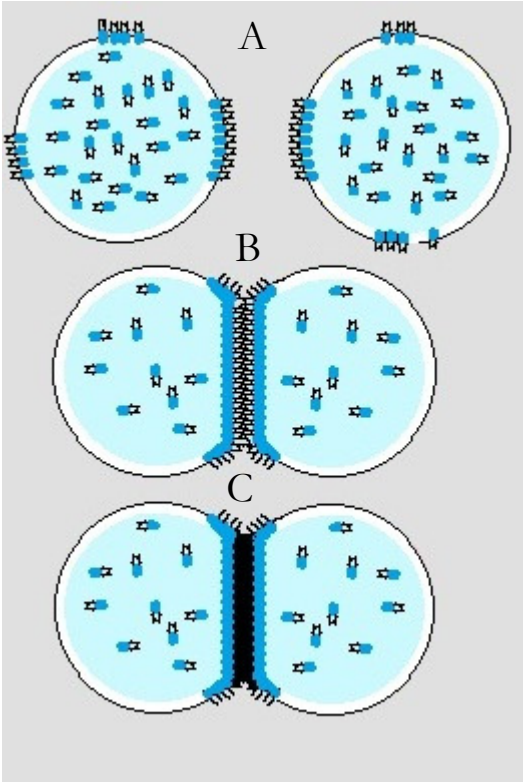


Figure 1.3: Diagram of the formation of droplet interface bilayer. (A) Phospholipids in suspension in the aqueous phase self-assemble at the oil water interface. (B) The droplets are brought into contact aligning lipid tails and trapping oil between the monolayers (C) The oil is forced out and the bilayer forms.

Bilayers formed in this way were shown to last for multiple days[46, 47] and were more tolerant to vibration than those formed using other methods. Holden was also able to make long chains of droplets connected by bilayers [46]. This method was not without its drawbacks. In order to take electrical measurements, electrodes had to be inserted into droplet which was made difficult by the small size of the droplets and the surface tension. Some experimenters used high precision equipment to position wire electrodes accurately.

The work presented in this thesis was conducted using a method that is a hybrid of the supported and unsupported techniques. The method uses a flexible substrate to support and control the aqueous droplets used to make droplet interface bilayers. This is called the regulated attachment method (RAM) and is used in one form or another in all current experiments performed by The Leo Group [1, 48-50]. The

specifics of the substrate used in this work will be discussed in greater detail in the Chapter 2.

1.2.4 Ionic Liquids

The term ionic liquid (IL) refers to a broad class of semi-organic salts or salt mixtures, composed entirely of ions and ion pairs, that have a relatively low melting point. It is generally accepted that the salt must melt at 100°C or below to be considered an ionic liquid [51-53]. Ionic liquids have recently gained popularity as environmentally friendly alternatives for many chemicals used in industrial processes. Ionic liquids can replace volatile organic solvents [54] and catalytic components [55]. The ability to tailor their properties allows for the creation of custom solvents that will meet the specific requirements for a particular reaction[51].

Due to the complexity of the chemical names for ionic liquids a variety of abbreviation methods have developed in literature [56]. One of the most common styles found in literature and the style used in this thesis denote both the cation and anion within sets of brackets. For example, one of the ionic liquids used in this thesis, 1-ethyl-3-methylimidazolium ethylsulfate, is abbreviated as [EMIm] [$EtSO_4$] where [EMIm] is the cation and [$EtSO_4$] is the anion.

1.3 **Research Goals**

This thesis details the procedure for testing and characterizing the incorporation of ionic liquids into a lipid bilayer system. The ionic liquids would be used to favorably alter the properties of the bilayer and any imbedded proteins. The use of ionic liquids to alter the properties of lipid bilayers systems has never before been attempted. A new substrate using the RAM technique was developed for the purpose of testing and is described in Chapter 2. Then the ability to form lipid bilayers in the presence of ionic liquids was tested and confirmed. The bilayers were tested for any changes to the physical or electrical properties caused by the ionic liquids. Finally, the ion transport behavior of the peptide alamethicin was tested with the ionic liquids.

Chapter 2: Experimental Methodology

This chapter presents a discussion of the substrate used in the experiments in this thesis. The construction procedure and materials for the substrate are outlined first, followed by a description of the operating method. The discussion then moves to testing the substrates ability to form, control and measure bilayer properties and ensuring there are no negative effects on the bilayer. The ion transport capabilities of alamethicin are also tested for consistency. This outlines the basic experimental procedure for bilayer tests for this thesis.

2.1 **Experimental Substrate**

2.1.1 Single Interface Tray Substrate

The data presented in this thesis was all acquired using the single interface tray substrate (SITS), which was developed for this research. The SITS is a modified version of the array substrate previously developed by the Leo Group [49]. A 3D

model of the substrate was created using computer-aided design (CAD) software. The substrate consists of three main parts, the tray and two inserts, each of which can be seen in the diagram in Figure 2.1. The tray forms the structure of the substrate, contains the oil and holds the inserts in place. The two inserts support and suspend the aqueous droplets using a hydrogel pad formed in a hemispherical well on each insert. The inserts also hold the electrodes in the wells so that they remain in the droplets. The inserts are designed to flex to allow the droplets to come into contact and form a bilayer when a force is applied to the side of the substrate.

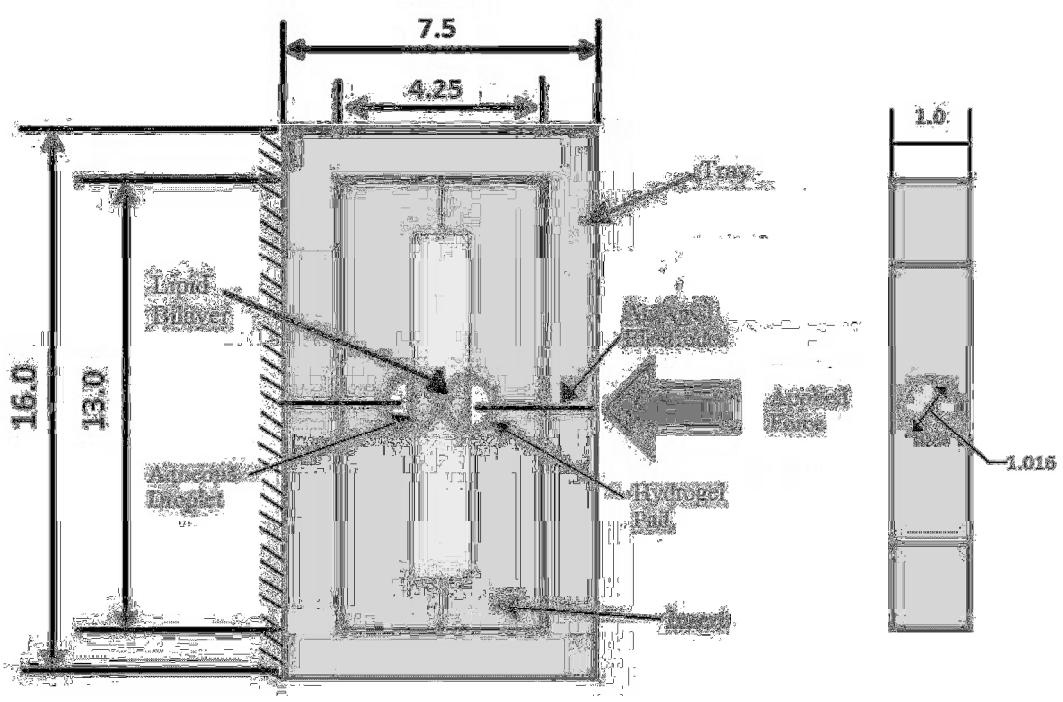


Figure 2.1: Top down view of the Single Interface Tray Substrate. Note: Dimensions are in mm. Drawing is not to scale.

Molds for the different pieces of the substrate were created from the CAD model and were machined into a sheet of acrylic using a computer numerical control (CNC) milling machine. The molds were filled with Clearflex 50 (Smooth-on, Inc.) polyurethane (PU) and degassed in a vacuum chamber at approximately 27 inHg until air bubbles no longer came out of solution. The molds were then cured at 80°C on a hot plate to form the pieces of the substrate. A biopsy punch (300 μm diameter) was used to manually punch a hole in the bottom of each divot through which the electrodes were fed.

The electrodes used in this substrate were made from 125 μm diameter silver wire coated with a layer of silver chloride, creating a silver-silver chloride (Ag/AgCl) to prevent electrical charge from building up on their surface [57, 58]. The electrodes were ball ended so that they would catch and hold in the divots in the inserts. The balling was achieved using a blowtorch to melt the end of the silver wire and allowing the molten metal collect at the end. The chloride layer was then deposited by soaking the balled electrodes in chlorine bleach for at least one hour [48].

Hydrogel pads on the inserts are used as anchors for the aqueous droplets and electrodes. Two different UV curable hydrogels, Poly(N-isopropylacrylamide) (NIPAAm) and polyethylene glycol (PEG), were tested to determine how well they would attach to the PU wells and to the aqueous droplets [59]. The testing procedure consisted of placing the uncured gel mixtures into the wells of the inserts with the electrodes in place. Each gel was then polymerized into its respective well using a handheld UV light. DI water was added to the pads to rehydrate them, the substrates were assembled and hexadecane oil was added to

the tray. Force was applied to actuate the substrate simulating the standard experimental motion. The tests showed that the PEG gel did not attach to the PU substrate as well as the NIPPAm and thus NIPPAm was used in all future experiments.

Once the inserts have electrodes and hydrogel pads, they are inserted into the tray as shown in Figure 2.1. 400 nL of the aqueous droplet solution (ADS) being tested is then pipetted onto each hydrogel pad, which forms two opposing aqueous lenses held in place by the hydrogel pads. Once the monolayer has formed, a force is applied to the side of the substrate to bring the lenses into contact and a lipid bilayer forms at the interface.

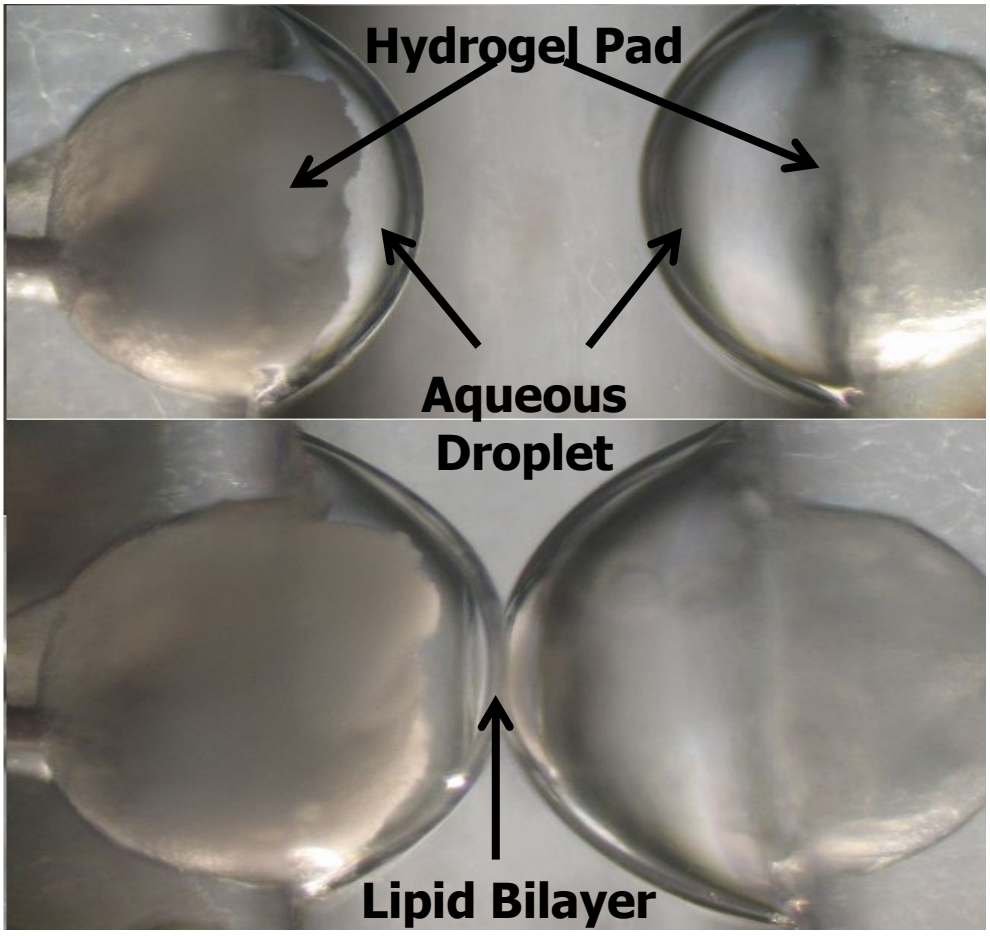


Figure 2.2: (A) Two aqueous droplets attached to hydrogel pads, with a monolayer formed at the oil water interface. (B) The aqueous droplets are brought together and a lipid bilayer forms at the point of contact.

Aqueous Droplet Solution

As previously described, bilayer formation requires an aqueous phase and a solvent phase. The aqueous phase consists of a suspension of phospholipid vesicles, biological buffering agents and salts in ultrapure deionized water [49]. The solvent phase consists of hexadecane oil (99%, Sigma Aldrich, St. Louis, MO).

The aqueous droplet mixture is prepared by combining 1,2- diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) vesicles (Avanti Polar Lipids, Inc., Alabaster, AL) with 500 mM potassium chloride (KCl, Sigma Aldrich, St. Louis, MO), and 10 mM 3-(N-morpholino) propanesulfonic acid (MOPS, Sigma Aldrich, St. Louis, MO), with the final mixture having a pH of 7. The solution is put through six freeze-thaw cycles to allow vesicles to form. After the final thaw cycle the solution is filtered using a polypropylene in-line holder (Sterlitech, Kent, WA) with a 0.1 μm Isopore membrane filter (Millipore, Billerica, MA). The filter is changed every third filtering and the solution is filtered six times. The final solution is at a concentration of 2 mg/ml. The lipid-buffer solution could be stored for up to three months in a refrigerator at approximately 4°C or up to ten months in a freezer at -20°C.

The peptide alamethicin (A.G. Scientific, San Diego, CA) used in the experiments came as a powder and is mixed in ethanol (Sigma Aldrich, St. Louis, MO) to a 0.1% (w/v) concentration and stored at -20°C. Ethanol can destabilize and break down lipid bilayers so the alamethicin ethanol solution had to be diluted with the DPhPC-lipid vesicle solution to a final concentration of between 100 ng/ml to 10 ng/ml in order to be viable [1, 49, 60].

2.2 Bilayer Formation

Bilayer formation is the most basic aspect of the research presented in this thesis. This section describes the formation of bilayers in the SITS substrate and outlines the test procedure used for all experiments in this thesis.

2.2.1 Monolayer Formation

A series of experiments were conducted to determine the ideal amount of time the monolayer needed for form in order to produce a stable bilayer. The experiments involved pipetting 400 nl of ADS onto the hydrogel pads and leaving the droplets separated for varying amount of time; thirty seconds, one, five, ten, thirty and sixty minutes. After the desired time interval the droplet were brought together and the bilayer was allowed to form. The time it took for the bilayer to form and the amount of time the bilayer was stable was recorded and analyzed. The experiments showed that monolayers left for thirty seconds and for one minute produced bilayers that were typically unstable. They also showed that monolayers left for more than ten minutes showed diminishing returns and it was determined that the slight increase in stability was not worth the extra time. Five to ten minutes was determined to be the ideal monolayer formation time.

Once it was determined that a monolayer had formed, force was applied to the side of the substrate bringing the droplets into contact. Initially there are oil molecules trapped between the droplets and therefor between the monolayers. Physical pressure and hydrostatic forces slowly force the oil out allowing the hydrophilic tails of the phospholipids to “zip” together, forming a two molecule thick membrane which is designated a lipid bilayer.

2.2.2 Baseline Formation Tests

Before any tests were conducted using a new ADS, the ability to form a bilayer with the solution was tested. The tests consisted of depositing droplets of the new ADS into a substrate and attempting to form a bilayer while taking a recording of the formation using the Axopatch 200 system. An example of this measurement can be seen in Figure 2.3. These formation tests established baseline measurements for a bilayer formed between droplets of a specific solution. Baselines were established every time a change was made to the ADS including the mixing of new stock lipid solution from dry lipids, the addition of a new protein solution, use of a different hydrogel and use of a new ionic liquid. All baselines were conducted in the SIT substrate; some comparative data from other works was taken in the RAM substrate.

Stock lipid aqueous droplet solution

Baseline measurements of ADSs that consisted of only phospholipids, MOPS and KCL (stock lipid solution) were conducted every time a new batch of solution was mixed. The measurements taken through the Axoscope of the capacitance and resistivity of the formed bilayer were then compared to previous baselines and to data collected from literature. These measurements ensured that the solution had been properly produced and quantitatively confirmed consistency between mixtures made at different times.

An inconsistent baseline test would halt experimental progress allowing for any issues to be identified before time was not wasted on obtaining flawed and

unusable results. When the solution was mixed incorrectly and produced undesirable bilayers, attempts were made to correct any issues with the solution, but were rarely successful. Typically a new solution had to be mixed using fresh lipids.

The SITS substrate allows for very precise control over the size of the bilayers it supports. This allowed the designation of a standard size to which all testing bilayers should conform and would be designated by the amplitude of the current response. The standardizing value was chosen to be 200 pA peak to peak, which corresponds to a bilayer radius of about 230.5 μm and a bilayer capacitance of 1001.5 pF. All tests were conducted with bilayers sized within 20 pA (8.3 μm^2) of this standard.

Experiments were also conducted to test the feasibility of storing ingredients in dehydrated hydrogel pads. This would allow a sensor using this system to only require the addition of DI water in order to form the aqueous droplets. The test consisted of mixing the ADS with each of the uncured hydrogel solutions at a one to one ratio. The new mixture was then pipetted into the insert wells, cured using UV light and allowed to sit out in the open air at room temperature to dehydrate. Once dehydrated, the substrate was assembled and the hexadecane was added. DI water was then pipetted onto the hydrogel pads to form the aqueous droplets. Bilayer formation and protein gating tests were conducted as described above to confirm that the dehydration process had not damaged any of the ingredients. While this process did cause the monolayers to require more time to form, the resulting bilayers were stable and the protein gating activity was unaffected.

Complications in Bilayer Formation

If left in storage for an extended period of time, approximately six months, phospholipids tend to lose the ability to form usable bilayers. There are multiple theories as to the cause of this deterioration including oxidation of the lipids[61, 62], extended exposure to higher than desirable temperatures [63], the formation of large vesicles[64], the introduction of contaminates and the deterioration of the MOPS organic buffer. Re-filtering the solution was most often performed as a corrective action as it breaks up the larger vesicles and remove large contaminates.

The introduction of alamethicin into the ADS brought its own problems to bilayer formation. Alamethicin is stored in ethanol which can destabilize bilayers in high enough concentrations. If the concentration of alamethicin was higher than 1μg/ml, the ethanol would not allow a bilayer to form. Also at such a high concentration of alamethicin, there would be a greater number of peptides in the bilayer, increasing the likelihood of multiple independent pores and making single channel measurements very difficult to obtain. The baseline measurements were used to ensure proper concentrations in these experiments as well.

2.2.3 Electrical Characterization

The two important electrical properties of lipid bilayers are the resistance and the capacitance of the interface. These properties are measured using two different methods. Electrical impedance spectroscopy (EIS) measurements are taken using an Autolab PGSTAT12 with FRA module (Eco Chemie) [48] and current measurements are measured using AxoPatch200B and Digidata 1440A (Molecular

Devices) instruments. Measurements from these systems enabled the confirmation of bilayer formation, characterization of the bilayers and the response of transmembrane proteins to transmembrane voltage potentials and estimates of bilayer size, capacitance and resistance.

Bilayer Formation Confirmation

Bilayer formation is confirmed using current measurements taken using AxoPatch200B and Digidata 1440A (Molecular Devices) measurement devices connected to the Ag/AgCl electrodes. A 10 mV triangular voltage waveform with a frequency of 10 Hz is applied to the electrodes using an external function generator. The resulting current is measured at a sample rate of 10 kHz and is passed through a 1 kHz low pass filter.

The current response is a square waveform due to the capacitive nature of the bilayer. The amplitude of the current response is related to the magnitude of the capacitance of the bilayer according to,

$$i(t) = C\frac{\mathrm{dV}}{\mathrm{dt}} \qquad \textbf{2.1}$$

Where C is the capacitance of the system, dV/dt is the time rate of change of the applied potential and i(t) is the peak to peak amplitude of the current response. The time rate change of the voltage can be expressed as:

$$\frac{\mathrm{dV}}{\mathrm{dt}} = \frac{A}{\frac{T}{2}} = \frac{2A}{T} = 2\mathrm{Af} \qquad \textbf{2.2}$$

Where A is the peak to peak amplitude of the applied voltage wave, T is the period and f is the frequency of the input voltage signal. The Axoscope measuring device applies 20 mV to the bilayer for every 1V input from the signal generator meaning that A=0.02 $\times$ the peak-to-peak amplitude set on the signal generator. Plugging Eq. 2.2 into Eq. 2.3 gives

$$i(t) = C(2\mathrm{Af}) \text{ or } C = \frac{i(t)}{2\mathrm{Af}} \qquad \textbf{2.3}$$

Since the bilayer can be modeled as a plate capacitor and thus the area of the bilayer can be calculated by dividing the capacitance by the normalized capacitance,

$$\mathrm{A} = \frac{C}{C_{normalized}} \qquad \textbf{2.4}$$

The effective area and effective radius are respectfully defined as:

$$\mathrm{A}_{effective} = \frac{\frac{i}{(2 * A * f)}}{C_{normalized}}\left(\frac{\mathrm{pA}}{\frac{\mathrm{mV} * \mathrm{Hz}}{\mu\mathrm{F}}{\mathrm{cm}^2}}\right) = \frac{\frac{i}{(2 * A * f)}\left(\frac{10^{-12}}{10^{-3}}\right)}{0.6 * 10^{-6}} = 1.67 * 10^5 \mu m^2 * \frac{i}{(2 * A * f)} \qquad \textbf{2.5}$$

$$\mathrm{r}_{effective} = \sqrt{\frac{\mathrm{AreaConstant}}{\pi}} = 230.5\ \mu m * \sqrt{\frac{i}{(2 * A * f)}} \qquad \textbf{2.6}$$

As the oil is forced out from between the monolayers and the bilayer begins to form the capacitance of the system increases. By monitoring the current response for this gradual increase in amplitude, the formation of the bilayer can be confirmed. Figure 2.3 shows an example of the current response seen during the formation of a lipid bilayer in which the bilayer reached a steady state current

response of 240 pA peak to peak. The peak to peak amplitude of the applied voltage waveform was 10 mV, resulting in a capacitance of 1201.7 pF and a bilayer radius of 252.5 µm. The estimated area of the bilayer is also shown on the left axis in Figure 2.3 [57, 65].

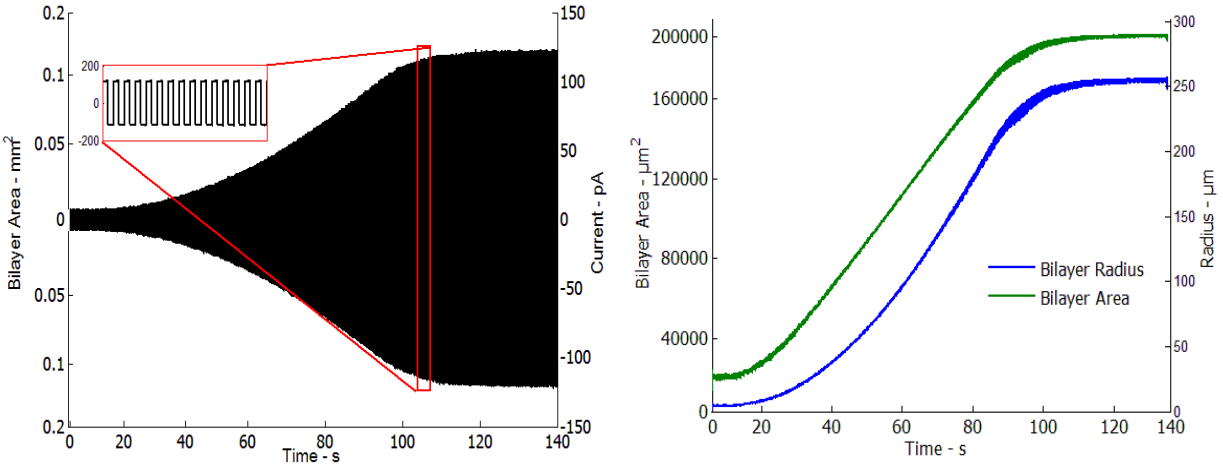


Figure 2.3: (**Left**) Current response during the formation of a lipid bilayer. The increase in the amplitude of the square wave response is caused by the increasing capacitance magnitude as the bilayer forms. The left axis shows the calculated area of the bilayer and the right shows the measured current magnitude. (**Right**) The calculated bilayer area (green) and radius (blue). The plot shows the growth of the dimensions as the bilayer forms.

All data taken in this thesis has a corresponding bilayer property measurement which consists of a recording of the square wave current response produced by the bilayer when subjected to a 10 mV peak to peak, 10 Hz triangle wave voltage potential. From this recording, the size, quality and stability of the bilayer being tested can be deduced and used as a reference.

Electrical Impedance Spectroscopy

Electrical Impedance Spectroscopy (EIS) is defined as the study of how the opposition to the flow of electrical current (electrical impedance) through a system changes with respect to frequency. Complex electrical impedance, $Z(\frac{j}{\omega})$, is mathematically define as the ratio of the complex voltage, $V(\omega)$ to the complex current, $I(\omega)$,

$$Z\left(\frac{j}{\omega}\right) = \frac{V(\omega)}{I(\omega)} \quad \textbf{(2.1)}$$

where ω is the excitation frequency with units of radians per second (rad/s), giving electrical impedance units of Ohms (Ω). In a purely resistive system, the impedance does not vary with respect to frequency, thus Equation (2.1) simplifies to Ohms Law. However, a lipid bilayer is not a purely resistive system, it does oppose the flow of charge but it also stores charge at the interface. Therefore, electrical measurements result in a voltage-current relationship that mimics that of a resistor and capacitor in parallel. The electrical impedance of a pure capacitor is dependent upon frequency, according to the expression,

$$Z\left(\frac{j}{\omega}\right) = \frac{1}{j\omega C} \quad \textbf{(2.2)}$$

where j is the imaginary number, ω is the excitation frequency and C is the capacitance. From this we write the electrical impedance of a interfacial bilayer as,

$$Z\left(\frac{j}{\omega}\right) = \frac{R_B}{1 + j\omega R_B C_B} + R_s \quad \textbf{(2.3)}$$

where R_B is the resistance and C_B is the capacitance of the bilayer and R_S is the resistance of the rest of the system including the electrodes and ADS in the droplets. R_S is assumed to be orders of magnitude less than R_B; on the order of 1-10kΩ compared to the bilayers 1-10 GΩ [1, 8, 47, 57].

The experimental impedance measurements presented in this thesis were taken by applying a sinusoidal voltage potential to the lipid bilayer and recording the current response. The Autolab measured the current response and calculated the real and imaginary components of the impedance across the specified frequency range (typically 10 mHz to 10 kHz). Figure 2.4 depicts the bode plot for the experimentally acquired impedance measurement of a lipid bilayer. For reference, the impedance measurement of a RC circuit with a resistance of 10 GΩ and a capacitance of 2 nF is included in the figure as a dashed line.

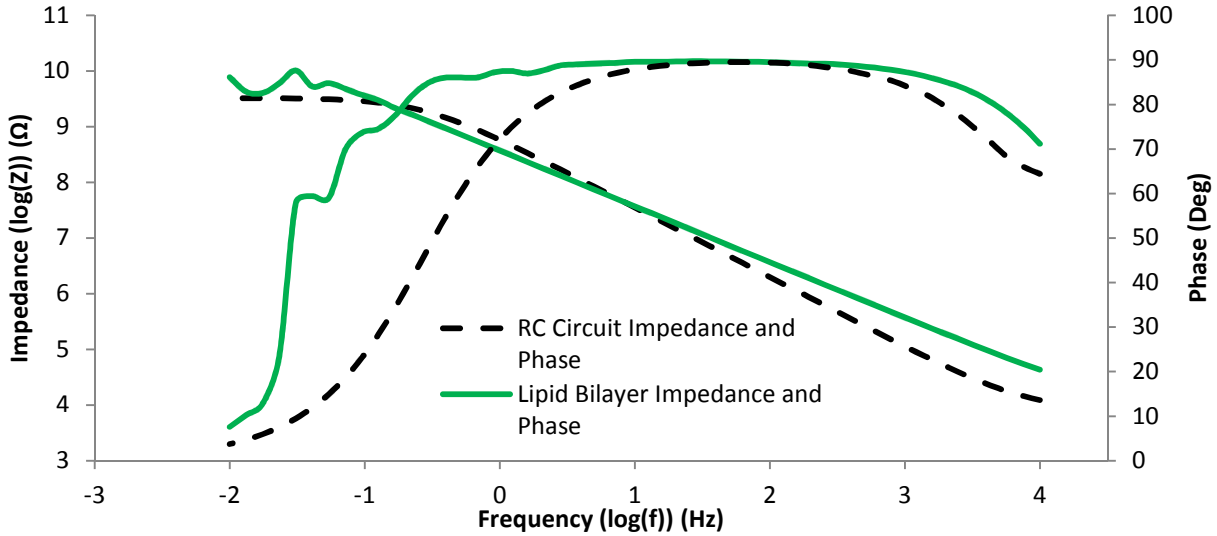


Figure 2.4: The magnitude and phase plots of the Bode diagram of the electrical impedance data for a manufactured RC circuit and a lipid bilayer.

2.2.4 Transmembrane Ion Transport

The ability to measure the transport of ions through the bilayer was tested using the peptide alamethicin. As stated above alamethicin was incorporated into the ADS at a concentration of 100 ng/ml. Under the influence of an applied potential, alamethicin self-inserts into the bilayer and form conductive ion channels [8, 45, 59, 65, 66]. These channels are only open for fractions of a second, thus in order to capture these events data is taken at a sample rate of at least 100 kHz [48, 67].

The opening of the conductance channels, called gating events, creates an immediate stepped increase in current, which is recorded as shown in Figure 2.5. In order to quantify the gating events the conductance of the system at the time of

the event is calculated. At any one point, conductance (G) is calculated by dividing the magnitude of the measured current by the applied voltage potential.

$$G = \frac{I}{V} \tag{2.4}$$

In Figure 2.5, adjusting for the baseline noise measurement, the highest current peak averaged 147 pA and was under an applied potential of 150 mV. Using equation 2.4, the capacitance is calculated to be 0.98 nS. The histogram in Figure 2.5 shows the dispersion of the conductance measured at every data point. It nicely depicts the grouping of the conductance magnitudes into three distinct conductance levels and the ground state. The average conductance magnitude for each level is labeled and aligned with it's respective current level.

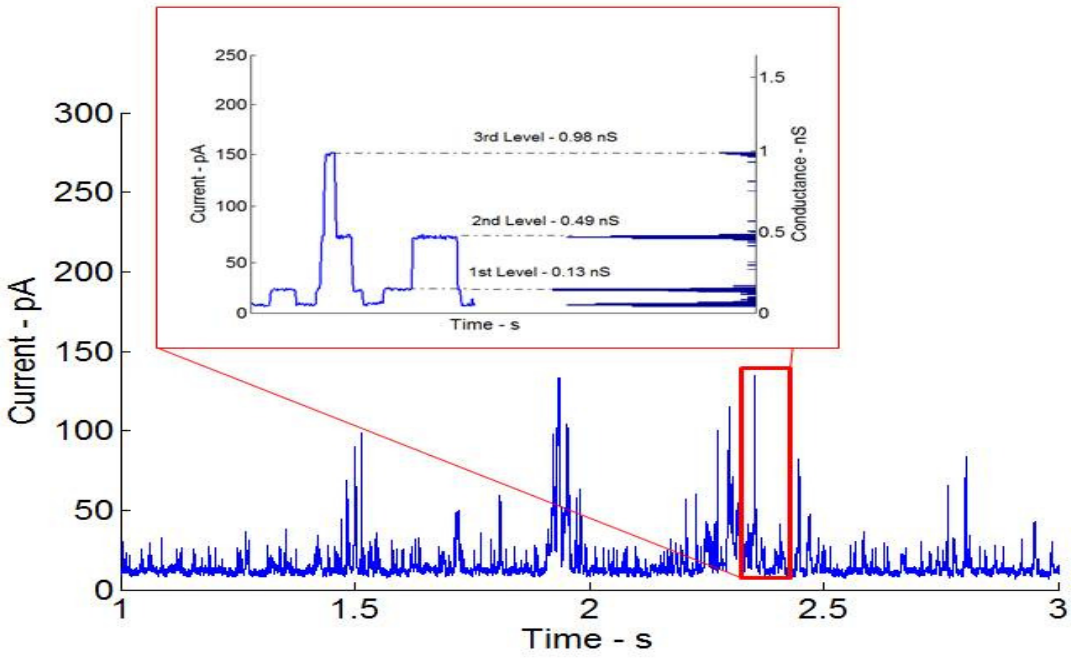


Figure 2.5: Single channel current recording of alamethicin gating events and corresponding conductance histogram. The first three conductance levels are shown and labeled and depict the consistency of the levels magnitudes.

The magnitude of the current increases are believed to be related to the type and size of channel that has opened as well as the number of proteins involved [8, 45, 65]. As the proteins insert into the bilayer, they tend to clump together and open together creating larger pores. Pores having different numbers of peptides create what is called conductance levels, which can be seen in Figure 2.5. Each consecutive conductance level is of an increasing magnitude that is consistent for that level for all measurements [68-70]. Through experimentation the magnitudes of the first four conductance levels have been found to be approximately 0.14, 0.50, 0.95, and 1.45 nS, respectively, which is consistent with values found in literature [71-73].

2.3 Chapter 2 Conclusions

The Single Interface Tray Substrate developed for this work allows precise control over the position of lipid filled aqueous droplets which allows for the repeatable formation of lipid bilayers. The multi part construction allows for single parts to be replaced in the event of damage, malformation or contamination rather than requiring the entire substrate to be replaced. The CNCed molds allow for rapid production and easy modification of each individual part as the direction of the work shifts.

The substrate was put through a series of experiments to ensure that it would be able to produce bilayers comparable to those used in past works. It was shown that this design could form quality bilayers and did not alter any of the bilayer properties, ion transport capabilities or interfere with any electrical measurements.

The substrate increased control and ease of repeatability compared to other RAM based substrates.

Chapter 3: Incorporation of Ionic Liquids

The second component of this work involved the incorporation of ionic liquids into the aqueous droplet solution. The goal was to show that lipid bilayers could be formed in the presence of ionic liquids and to characterize any bilayer property changes caused by the ionic liquids.

3.1 **Ionic Liquids**

Three different ionic liquids (IL) were provided by The Long Research Group at Virginia Tech for investigation in this work; 1-ethyl-3-methylimidazolium ethylsulfate ([EMIm] [$EtSO_4$]), 1-ethyl-3-methylimidazolium triflate ([EMIm] [TfO]) and 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm][BF_4]). These three were chosen because they have melting points below room temperature and thus could be tested in liquid form, have the same cation but differing anions and are miserable in water which is the base for the lipid solution.

3.1.1 Ionic Liquid Material Properties

Table 3.1 contains a number of properties of the ionic liquids used in this work. The solubility parameter (δ), shown in the second column of Table 3.1, is a quantitative indication of the solubility of a solvent. This value is the square root of the cohesive energy density of the material and it thus related to the interaction between the molecules of the material. Materials with similar solubility parameter values are likely to be miscible. It can be seen from the values in Table 3.1 that the ionic liquids should all be miscible with water and that the [EMIm][$EtSO_4$] is the most similar while the [EMIm][TfO] is the least similar.

Table 3.1: Properties of the ionic liquids used in testing taken from literature.

Ionic Liquids	Solubility Parameter (δ) ($MPa^{1/2}$)	Melting Point (°C)	Density (g/cm^3)	Water Content (ppm)
Water	48.0 ± 0.8	0	0.998 ± 0.001	N/A
[EMIm][$EtSO_4$]	28.5 ± 1.0	-30	1.24 ± 0.01	11.7 ± 2.4
[EMIm][BF_4]	24.4 ± 0.2	15	1.29 ± 0.03	11.8 ± 1.7
[EMIm][TfO]	22.3 ± 0.3	-9	1.38 ± 0.01	18.9 ± 1.0

Also provided in Table 3.1 is a measurement of the baseline water content of each of the ILs [74-78]. ILs absorb water from the surrounding environment and the baseline would allow for any discrepancies to be documented. Premeasuring the water content at the conclusion of experimentation showed that there were no significant changes in the water content of the ILs used in the experiments.

3.1.2 Bilayer Formation in the Presence of Ionic Liquids

Initial tests with ionic liquids set out to confirm that it was possible to form a lipid bilayer in an ionic liquid solution. The initial solutions were mixed at a ratio of 1 to 1 ionic liquid to stock lipid solution. Examples of the resulting bilayers are shown in Figure 3.1.

It can be seen in Figure 3.1 that the solutions containing [EMIm][$EtSO_4$] and [EMIm][TfO] were able to form stable bilayers, but the [EMIm][Bf_4] caused the bilayer to rupture before it came to steady state. At this concentration, the bilayers formed between the [EMIm][$EtSO_4$] and [EMIm][TfO] were not stable enough to allow extended experimentation. For this reason, a study was conducted to determine the ideal ionic liquid to stock lipid solution mixture ratio that would produce stable, testable bilayers.

A series of experiments were conducted in which the concentration of ionic liquids was incrementally increased. The tests were conducted using all three ionic liquids in an attempt to find a concentration at which the [EMIm][Bf_4] did not catastrophically destabilize the forming lipid bilayer.

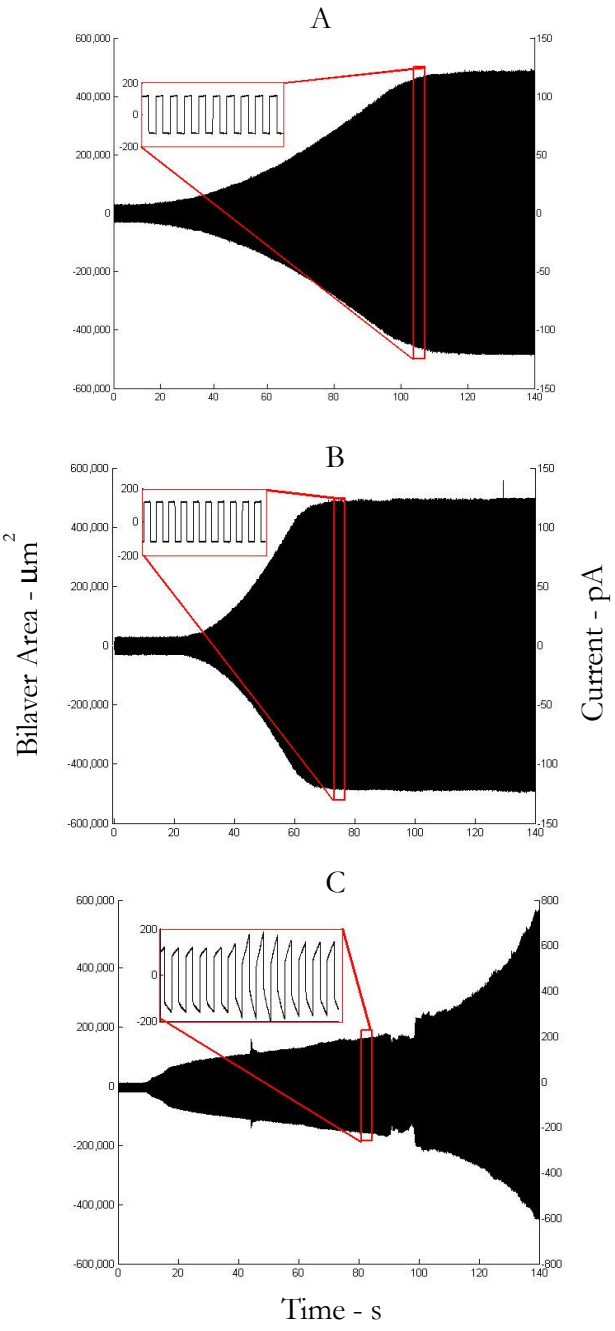


Figure 3.1: Bilayer formation with solutions of the three ionic liquids used in this thesis. (a) [EMIm] [$EtSO_4$] (b) [EMIm] [TfO] and (c) [EMIm] [BF_4]

Ionic Liquid/Stock Lipid Solution (IL/SLS) mixtures were made at ratios from 1/5 to 5/1 (ml IL/ml SLS) in 1ml increments. Each solution was pipetted into a substrate, given ten minutes to allow a monolayer to form and brought into contact. Measurements of the amount of time it took for the bilayer to form, the peak current measured at steady state and the amount of time it lasted were taken; a table of the results can be seen in Appendix A:. Each one was also empirically analyzed for stability and quality. It was found that a solution mixed at a ratio of 2 to 1 SLS to either [EMIm][$EtSO_4$] or [EMIm][TfO] resulted in stable bilayers. [EMIm][Bf4] was found to only allow viable bilayers at mix ratios of 5 to 1 (SLS to IL) or less. Such a low concentration was judged to be too low to be able to provide any desired effects and thus [EMIm][Bf4] was

dropped from the study. Any IL concentration above a 1 to 1 ratio (IL/SLS) resulted in an inability to form a bilayer at the interface.

All experiments with ILs from this point on were conducted using the 2:1 (vol:vol) ratio of aqueous droplet solution to ionic liquid. It should be noted that if the ADS for a particular experiment included alamethicin peptides or uncured hydrogel, the ADS was mixed before the ILs were added.

3.1.3 Bilayer Comparison

The next set of tests aimed to determine the effects the ionic liquids had on the physical and electrical properties of lipid bilayers. This was accomplished using three tests; bilayer formation time, voltage endurance and impedance measurements. Mixtures that are a combination of stock lipid solution and an ionic liquid will be referred to as ionic solutions

Bilayer Formation Times

The first round of experiments looked at the amount of time it took for a stable bilayer to form for each ionic solution. For each trial the droplets were allowed to sit for ten minutes to allow the monolayer to form, they were brought into contact and a timer was started. The times at which the bilayer began to form and when it stopped growing were marked. The bilayer was then left for another ten minutes to confirm that it was stable. Any data from bilayers that ruptured before coming to a steady state was discarded and the trial was repeated. Bilayers lasting longer than ten minutes were ruptured and considered to be very stable. A plot of the resulting data is shown in Figure 3.2.

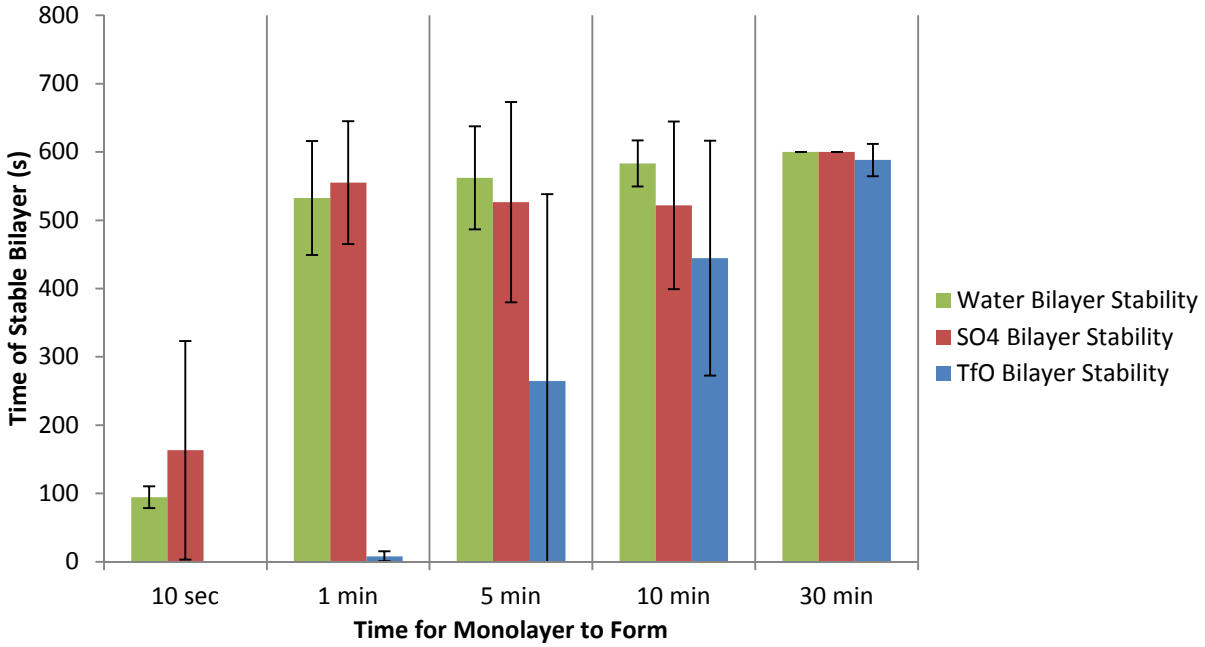


Figure 3.2: Plot showing the amount of time a bilayer remained stable with respect to the amount of time the monolayer was given to form. As expected the bilayer becomes more stable the more time the monolayer is given. The error bars in this plot show the maximum and minimum time values for each set of trials. Trials lasting more than ten minutes were ended and considered "very stable".

This experiment illustrates the large amount of uncertainty involved in bilayer formation. The error bars in the Figure 3.2 show the minimum and maximum varies in each set of trials and show the extensive deviation of the measurements from their averaged values. The magnitude of these variations diminish the longer the monolayer is given to for which is why many of the experiments in this thesis have long standardized time allowances for formation and stabilization of bilayers.

3.1.4 Electrical Characterization

Electrical impedance spectroscopy (EIS) was used to identify modifications to the electrical properties caused by the ionic liquids. It was hypothesized that the ILs

would be most likely to affect the electrical properties of the lipid bilayers. A baseline was established using stock lipid solution and then fifteen trials were conducted for each ionic solution. The tests were conducted over a frequency range of 10 mHz to 10 kHz on bilayers that were 41 $\mu m^2 \pm 8\ \mu m^2$. Figure 3.3 shows a Bode diagram of the measurements.

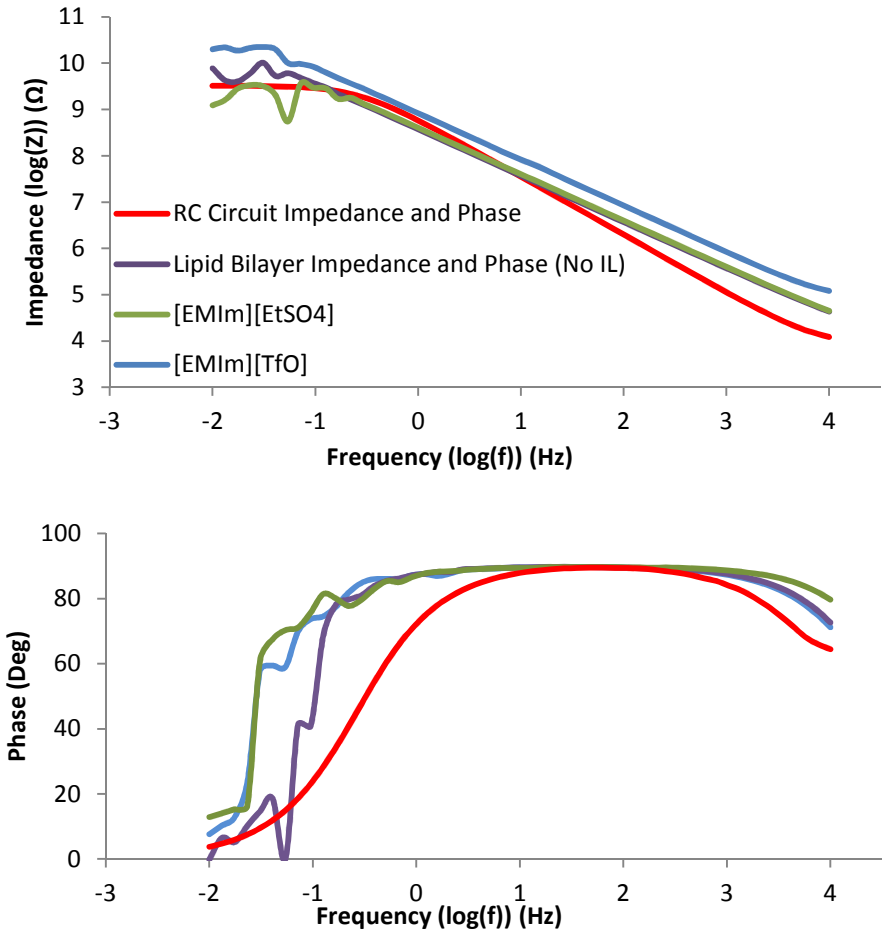


Figure 3.3: The magnitude (a) and phase (b) plots of the Bode diagram for the electrical impedance data collected on four different DIB cases; stock lipid solution (no ILs), $EtSO_4$, TfO and a mathematically ideal resistor and capacitor wired in parallel.

The low frequency impedance of all the bilayers was measured to be in the 1-10 GΩ range, which is in agreement with values from literature. The phase change is also consistent between all trials and literature. This confirms that, at this concentration, neither of the ILs are negatively affecting the lipids ability to assemble on the oil water interface nor are they affecting the ability of the monolayers to link into a bilayer. It also means that the ionic liquids do not have a significant effect on the electrical properties of the lipid bilayers. This is contrary to expectation considering the ILs are composed of ions and this should create a more conductive aqueous droplet solution.

The significance of this result lies in the physical properties they represent. The resistance of the bilayer signifies the bilayers ability to prevent ions from passing through the interface. A higher resistance means the membrane is very complete and consistent in its composition and thus provides a better seal. Since ILs are composed of ions, and a good seal is imperative for the bilayer to function as a sensor, that fact that the impedance measurements are not affect by the ILs is a very positive result.

Voltage Endurance

The final set of experiments focused on the magnitude of applied voltage potential a bilayer could sustain before rupturing while in the presence of ILs. This test was done in preparation for alamethicin gating which requires the bilayer to endure applied potentials of 70 mV to 150 mV. The test was structured such that the monolayers were given the standard ten minutes to form and then the droplets were brought together. Once the bilayer was confirmed to have formed and come

to a steady state, a voltage potential was applied and incrementally increased from zero until the bilayer ruptured; the rupture voltage was recorded. Each ionic solution was run through ten trials and the averaged data is shown in Figure 3.4.

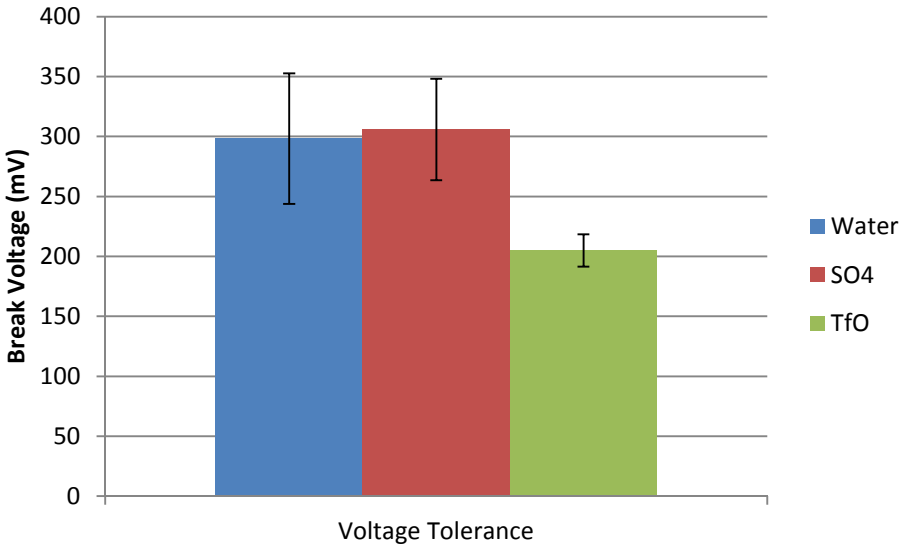


Figure 3.4: A plot showing the average potential a bilayer could endure when formed in the presence of each ionic liquid. This shows that the bilayers in both cases can endure the 150 mV necessary for alamethicin gating.

This set of test gave qualitative confirmation that the bilayers formed between droplets of the ionic solutions could survive the magnitude of voltage potential necessary for alamethicin gating to occur. It was also found that for all solutions, the bilayers would begin to leak current starting at approximately 150 mV in the [EMIm][TfO] trials and at approximately 180 mV in both the [EMIm][$EtSO_4$] trials and in the trials with no ionic liquids.

The data also shows that [EMIm][$EtSO_4$] did not affect the bilayer's rupture voltage. The [EMIm][TfO] on the other hand, caused a 80 mV reduction in the break voltage.

This may be related to a result presented in the next section involving a change in the magnitude of conductance levels caused by the presence of [EMIm][TfO].

3.1.5 Ion Transport

The final comparison test preformed with the ionic liquids tested the behavior of trans-membrane protein ion transport using alamethicin. A change in the alamethicin's gating behavior was expected due to the presence of the ionic liquid. Experiments were conducted to identify and characterize these changes.

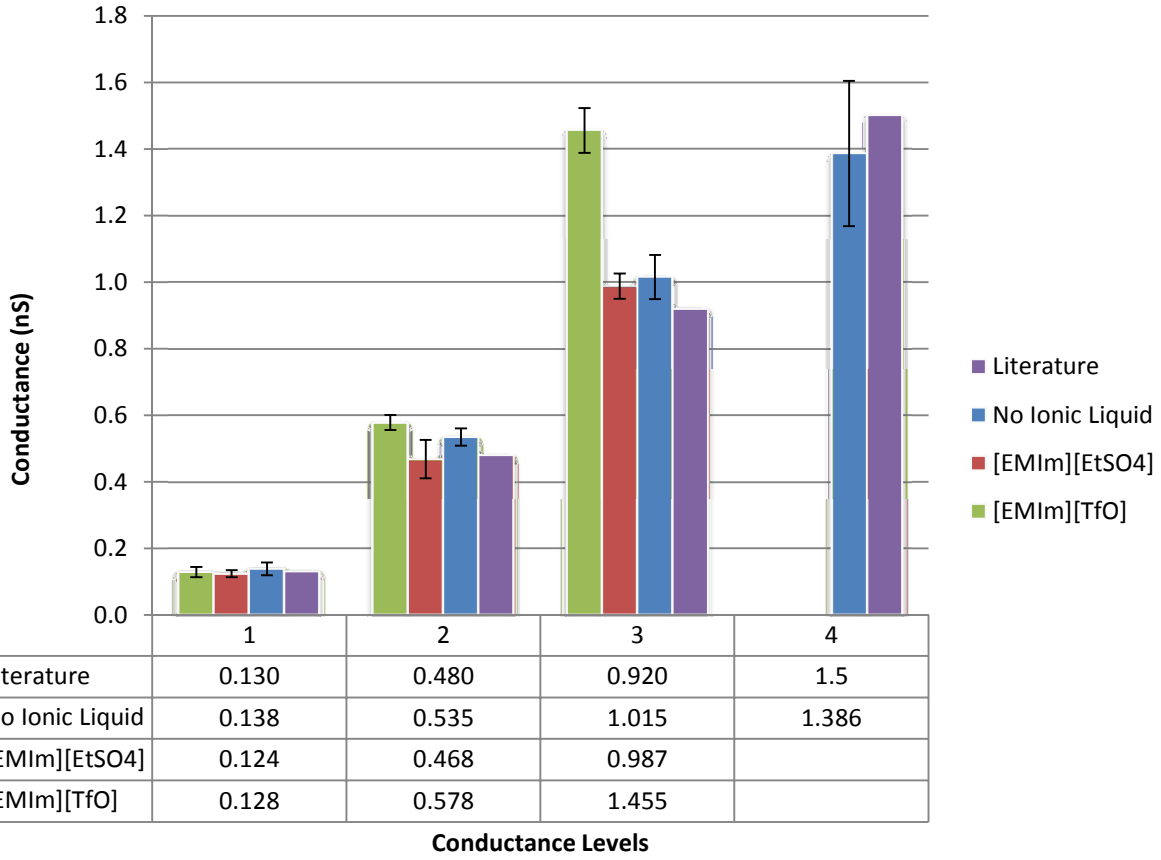
Experimental Procedure

An initial lipid alamethicin solution was mixed to a concentration of 100 ng/ml (Alm/SLS). This solution was then mixed with each ionic liquid at a 2 to 1 ratio, ionic liquid to Alm/SLS. A new solution was mixed weekly to eliminate the possibility that degradation of the solution could impact results.

Initial feasibility tests were conducted in a similar fashion to those done with water when testing the new substrate, presented in the last chapter. A stable bilayer was formed between two droplets of the ionic Alm/SLS solution. Once steady state was achieved, a voltage potential was applied to the electrodes, causing the alamethicin to insert into the bilayer. The potential was incrementally increased until the alamethicin began gating. In tests with both ionic liquids, the alamethicin began gating between 70 mV and 80 mV, which is in agreement with results in literature [45, 70, 73].

Conductance Levels

During the feasibility tests it was noticed in the [EMIm] [TfO] trials that the third conductance level was producing a larger current spike than the other solutions. Further experiments were conducted to study the conductance levels magnitudes for all the ionic solutions and the stock solution for use as a baseline. The experiments were conducted by forming a bilayer, applying a constant potential between 70 mV and 150 mV, recording the resulting gating activity for 60 seconds and then returning the applied potential to zero. The results were then analyzed to determine the magnitude of each conductance level at the different voltage levels. A comparison of the conductance level magnitudes is shown in the plot in Figure 3.5. The data shown in the plot is the average of 30 different trials. The trials were conducted at six different voltage levels between 80 mV and 130 mV, in increments of ten. This range was used rather than the total tested range because gating events typically begin reliably occurring at 80 mV and the bilayers tended to start to leak above 130 mV making analysis of the entire recorded file inaccurate. Plots of the conductance level magnitudes at each individual voltage setting can be found in the appendix.



	1	2	3	4
Literature	0.130	0.480	0.920	1.5
No Ionic Liquid	0.138	0.535	1.015	1.386
[EMIm][EtSO4]	0.124	0.468	0.987	
[EMIm][TfO]	0.128	0.578	1.455	

Conductance Levels

Figure 3.5: Plot of the magnitudes of the conductance levels for each ionic solution. Magnitudes for stock alamethicin solution and the values from literature are shown for reference. The magnitude of the third conductance level for [EMIm][TfO] is significantly higher than the accepted values and is close to the values designated for the fourth conductance level.

Upon analyzing the data presented in Figure 3.5, it was found that the magnitude of the [EMIm] [TfO] alamethicin solution's third conductance level was closer to the established values for alamethicin's fourth conductance level. This shows that the [EMIm] [TfO] anion is causing alterations in the alamethicin's ion channels.

Time Spent Gating

Experiments were conducted to measure the amount of time the alamethicin spent gating and how that changed with respect to the applied voltage. For these tests, once a bilayer was formed the voltage potential was set to a designated value between 70 mV and 110 mV, incremented by ten. Gating activity was recorded for 60 seconds for each of the 5 predefined voltages. Five trials were taken for each voltage setting and were averaged together. The results from the trials performed at 110 mV are shown in Figure 3.6.

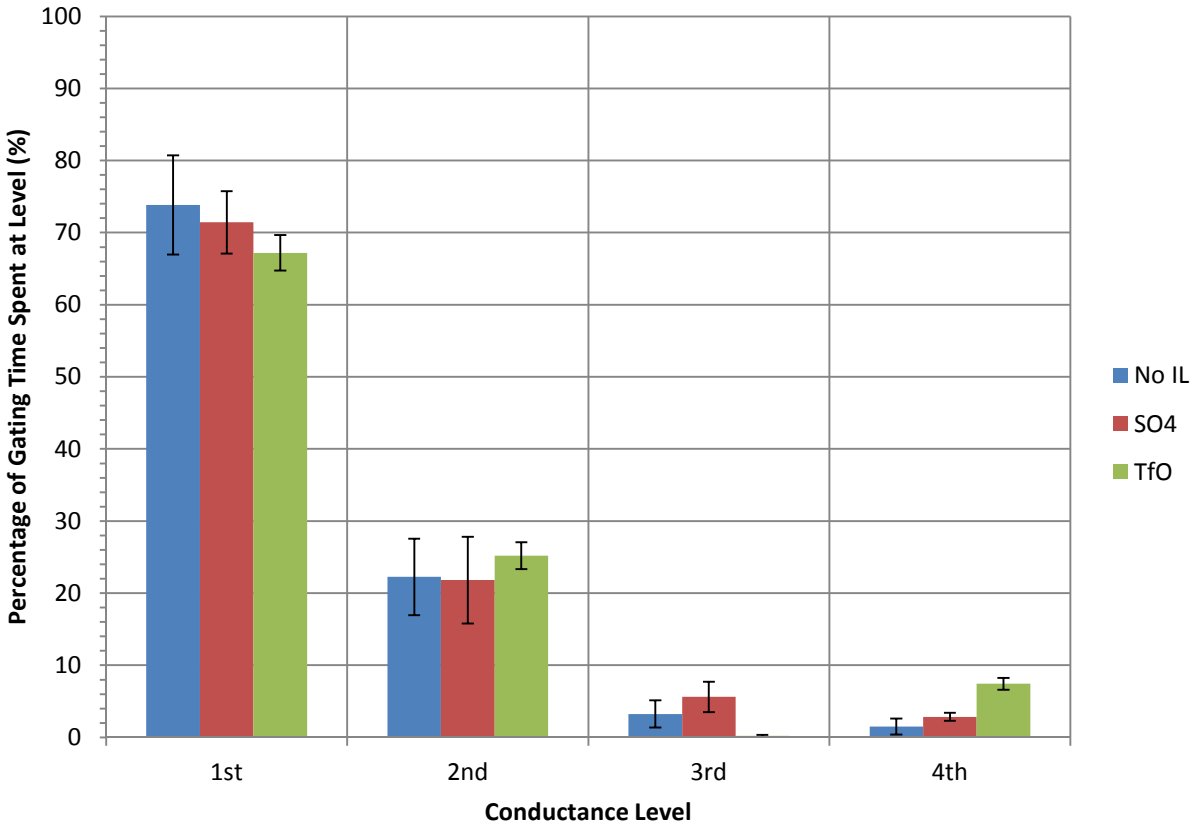


Figure 3.6: Plot of the time spent at each conductance level as a percentage of the total time spent gating with a 110 mV potential applied to the bilayer. It shows that the most time is spent at the first conductance level and time spent reduces for each increasing conductance level.

The results were organized to show the amount of time spent in each conductance level as a percentage of the total time spent gating. The tests confirmed that at low voltages (70 mV - 80 mV) all gating activity is limited to the first conductance level. As the voltage increases the time spent at the different conductance levels became more evenly distributed. At the high voltage level, Figure 3.6 shows a relatively even distribution of the time among the conductance levels. It can also be seen that the [EMIm][TfO] trials do not have any time spent in the third conductance level. This is due to the conductance level designation being based on a range of magnitude values in the plotting program and the third conductance level being above the upper threshold for the standard third level.

3.1.6 Chapter 3 Conclusions

The goal of this work was to study any effects ionic liquids have on the properties of lipid bilayers formed in their presence. The property changes were identified and characterized in an attempt to incorporate them into lipid bilayer based sensor systems.

Including [EMIm][TfO] into the aqueous droplet solution resulted in less stable lipid bilayers that didn't last as long and had a lower voltage tolerance. It also altered the ion transport properties of alamethicin peptides embedded in the bilayer. These alterations mean that the [TfO] anion is not ideal for use in future sensors.

[EMIm][EtSO4] was shown to have a negligible effect on the bilayer properties tested in this thesis. Its anion had an insignificant effect on the ion channels formed

by alamethicin peptide. This inertness makes it an ideal candidate for further testing and possible property impregnation for future lipid bilayer based sensors.

Chapter 4: Conclusions and Future Work

4.1 **Conclusion**

The goal of this project was to identify and characterize the effects ionic liquids have on lipid bilayers. The process for achieving this goal was broken into three main parts; a new substrate design, bilayer formation and protein transport testing.

The redesign of the substrate brought about a mechanism that allowed for more accurate control over bilayer size and position. The SIT substrate utilizes the RAM technique to form droplet interface bilayers and uses NiPAM hydrogel pads to hold the aqueous droplets in place. This substrate made it possible to repeatedly make bilayers of consistent dimensions which are needed when testing both bilayer formation and trans-membrane ion transport.

This design has a few advantages over the previously used substrates. One advantage is that monolayer formation occurs in the relaxed state, thus the

substrate can be transported without concern for the formed bilayer. Another advantage results from the aqueous droplets being suspended above the bottom of the substrate; particulate contaminants such as dust have less of an effect on the bilayer formation than in previous substrates. The substrate does have shortcomings, including its increased sensitivity to vibration which caused long life bilayers more difficult. The substrate also has multiple moving parts, which add to the complexity and increase the number of things that can go wrong.

Once the design for the substrate was finalized, work moved to bilayer formation and characterization. Initially, it had to be determined whether or not a bilayer could be formed in the presence of ionic liquids. Once that was confirmed, identification of any changes in bilayer properties could begin.

Initially, three ionic liquids were tested for in bilayer formation potential; [EMIm] [$EtSO_4$], [EMIm] [TfO] and [EMIm] [BF_4]. It was found that bilayers could only be formed on [EMIm] [$EtSO_4$] and [EMIm] [TfO]. The physical and electrical properties of bilayers in the presence of these ionic liquids were tested and analyzed for discrepancies. The [EMIm] [$EtSO_4$] was shown to have no significant effect on any of the bilayer properties. This was somewhat contrary to expectations due to the slightly hydrophobic nature of [EMIm] [$EtSO_4$]. The presence of [EMIm] [TfO] in the aqueous droplets caused a reduction in the stability and voltage endurance of bilayers formed between them. While this made further testing more difficult, the reduction was not significant enough to make result unreliable.

The final set of tests focused on the transport of ions through the membrane via alamethicin peptide. Since ionic liquids have ions that are dissimilar to the ions in the KCl in the aqueous droplet solution, the results were difficult to predict. [EMIm] [Tfo] produced a magnitude change in the conductance levels of the peptide. Specifically the magnitude of the third conductance level increased to a level equivalent to the fourth conductance level of other bilayers. It is unclear what causes for this behavior and is a topic for future study. [EMIm] [$EtSO_4$] did not have a discernible effect on the peptides ability to transport ions through the membrane. It has proven to be effectively inert with respect to lipid bilayer function. This makes [EMIm] [$EtSO_4$] is the ideal ionic liquid for future sensor integration.

4.2 **Future Work**

There are multiple directions in which this work could be taken. The purpose for this study was to determine the viability of utilizing ionic liquids to introduce desired traits into the bilayer sensor. Therefor it would have to be determined what ionic liquid properties are desired for the sensor. Since [EMIm] [$EtSO_4$] does not have a significant effect on the physical and electrical properties of the bilayer, it would be a good candidate for future testing if the desired traits can be infused into its chemical makeup.

Investigation into additional ionic liquids with differing properties could also be conducted to find one with more idea properties. All bilayer formation and

property tests would have to be conducted using each new ionic liquid to ensure compatibility.

The work with hydrogel pads could be expanded upon to assist droplet placement when work moves to arrays of bilayers. The development of an automated dispensing system would remove much of the placement error caused by the human factor. The pads could be placed in the desired locations and a stream of aqueous droplet solution could be passed over them, leave a thin droplet attached to the pad. This would facilitate fabrication of large array systems where individually adding droplets would be impractical. This would also assist fabrication as the arrays are scaled down to the microscopic scale making manual droplet pipetting no longer possible.

Appendix A: Ionic Bilayer Formation Table

No Ionic Liquid

Time allowed for monolayer to form		10 sec	1 min	5 min	10 min	30 min
Time to form bilayer	Trial 1	100	70	54	33	41
	Trial 2	165	175	47	40	29
	Trial 3	70	110	52	32	36
	Trial 4	124	142	139	73	32
	Trial 5	30	73	48	128	37
Level off value	Trial 1	59	63	65	70	167
	Trial 2	110	55	60	62	172
	Trial 3	68	62	64	68	163
	Trial 4	70	83	92	85	169
	Trial 5	82	97	76	87	160
Rupture time	Trial 1	200	380	600	175	600
	Trial 2	246	600	600	600	237
	Trial 3	175	600	463	212	370
	Trial 4	600	163	216	600	600
	Trial 5	145	600	600	600	600
Time of stable bilayer	Trial 1	100	310	546	142	559
	Trial 2	81	425	553	560	208
	Trial 3	105	490	411	180	334
	Trial 4	476	21	77	527	568
	Trial 5	115	527	552	472	563

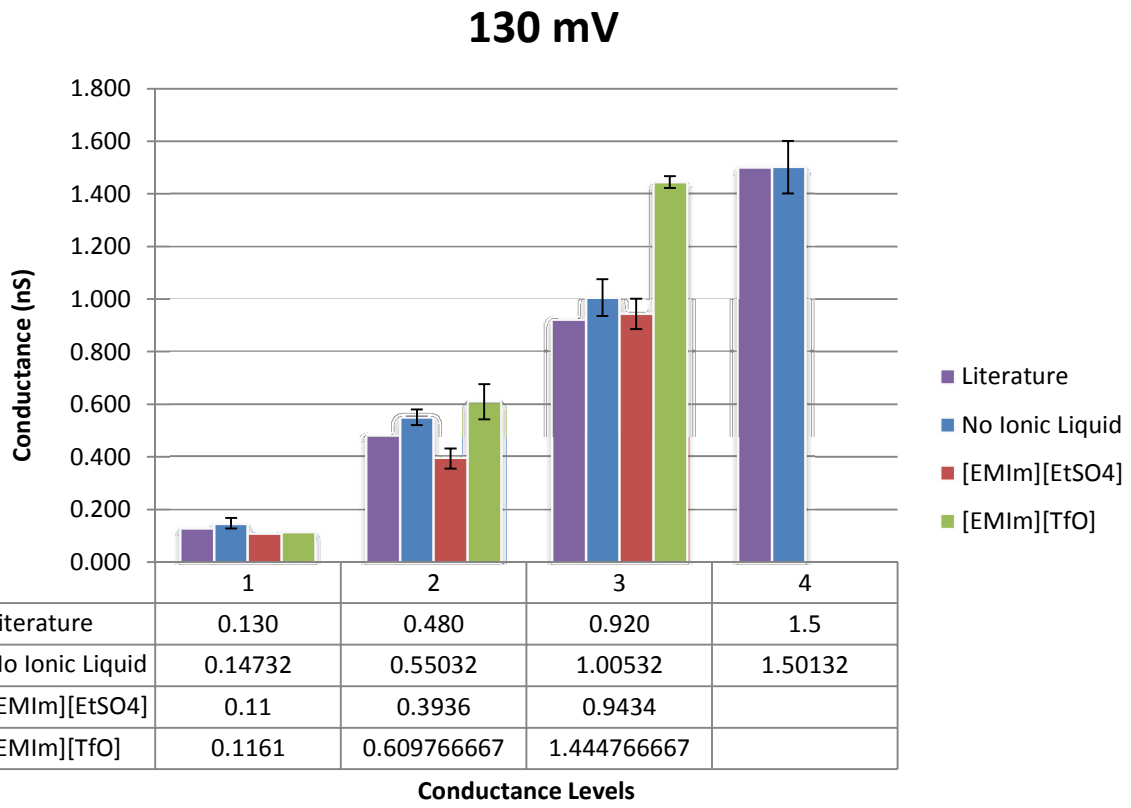
[EMIm] [$EtSO_4$]

Time allowed for monolayer to form		10 sec	1 min	5 min	10 min	30 min
Time to form bilayer	Trial 1	142	95	101.82	85	94
	Trial 2	115	72	99	89	112
	Trial 3	93	71	193.02	94	104
	Trial 4	89	82	139	78	99
	Trial 5	131	73	48	95	108
Level off value	Trial 1	120	100	150	89	62
	Trial 2	94	100	100	95	69
	Trial 3	109	150	100	102	74
	Trial 4	142	62	92	86	69
	Trial 5	87	97	76	84	52
Rupture time	Trial 1	231	1206	600	368	809
	Trial 2	372	712.2	332	782	742
	Trial 3	179	1195.8	1143	643	849
	Trial 4	274	157	216	205	1626
	Trial 5	241	600	600	893	928
Time of stable bilayer	Trial 1	89	1111	600	283	715
	Trial 2	257	640.2	233	693	630
	Trial 3	86	1124.8	949.98	549	745
	Trial 4	185	75	77	127	1527
	Trial 5	110	527	552	798	820

[EMIm] [TfO]

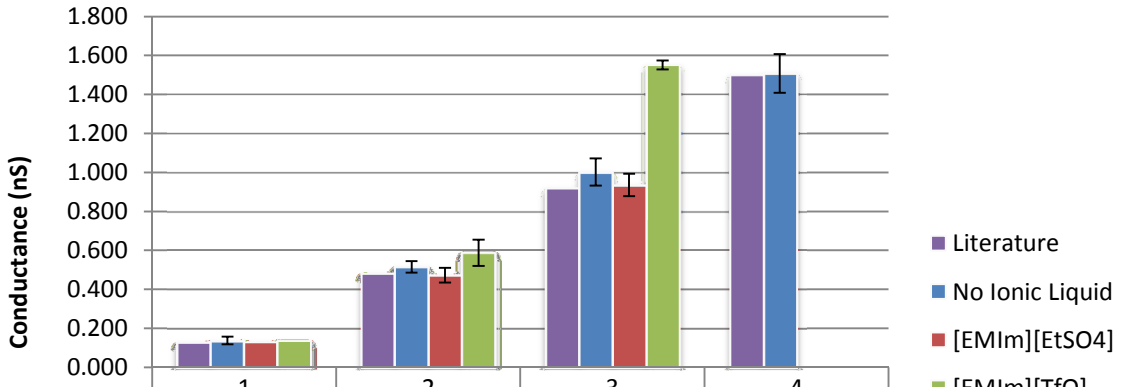
Time allowed for monolayer to form		10 sec	1 min	5 min	10 min	30 min
Time to form bilayer	Trial 1	0	98	89	138	134
	Trial 2	0	104	123	135	153
	Trial 3	0	0	123	97	142
	Trial 4	0	43	63.6	138	132
	Trial 5	0	0	116	127	134
Level off value	Trial 1	0	0	40	46	54
	Trial 2	0	0	53	52	52
	Trial 3	0	0	52	41	63
	Trial 4	0	0	48	74	58
	Trial 5	0	0	55	58	52
Rupture time	Trial 1	0	107	126	662	932
	Trial 2	0	116	167	182	783
	Trial 3	0	0	720	653	692
	Trial 4	0	62	780	267	673
	Trial 5	0	0	159	573	749
Time of stable bilayer	Trial 1	0	9	37	524	798
	Trial 2	0	12	44	47	630
	Trial 3	0	0	597	556	550
	Trial 4	0	19	716.4	129	541
	Trial 5	0	0	43	446	615

Appendix B: Ionic Bilayer Conductance Level Magnitude Plots



	1	2	3	4
Literature	0.130	0.480	0.920	1.5
No Ionic Liquid	0.14732	0.55032	1.00532	1.50132
[EMIm][EtSO4]	0.11	0.3936	0.9434	
[EMIm][TfO]	0.1161	0.609766667	1.444766667	

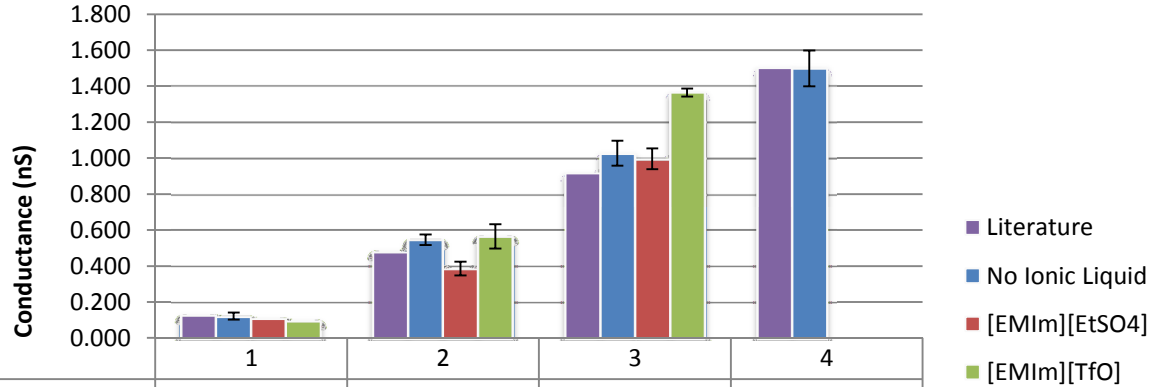
120 mV



	1	2	3	4
Literature	0.130	0.480	0.920	1.5
No Ionic Liquid	0.13688	0.51488	1.00188	1.50788
[EMIm][EtSO4]	0.13316	0.47216	0.93616	
[EMIm][TfO]	0.139533333	0.587866667	1.5512	

Conductance Levels

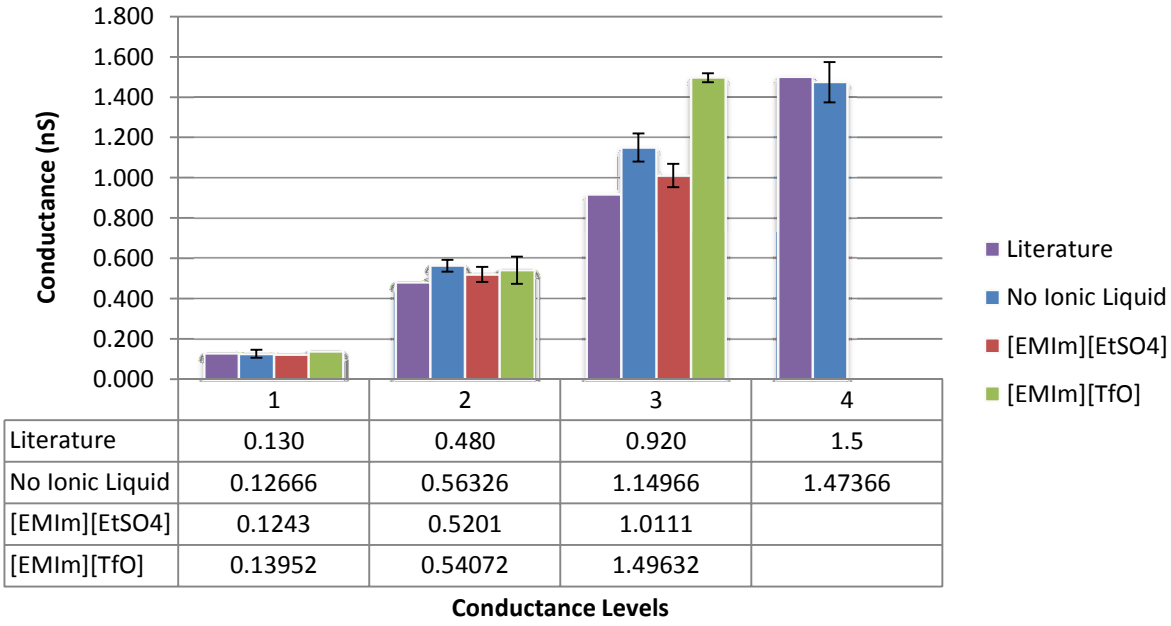
110 mV



	1	2	3	4
Literature	0.130	0.480	0.920	1.5
No Ionic Liquid	0.12212	0.54632	1.02712	1.49912
[EMIm][EtSO4]	0.1108	0.3862	0.9962	
[EMIm][TfO]	0.099133333	0.5648	1.3648	

Conductance Levels

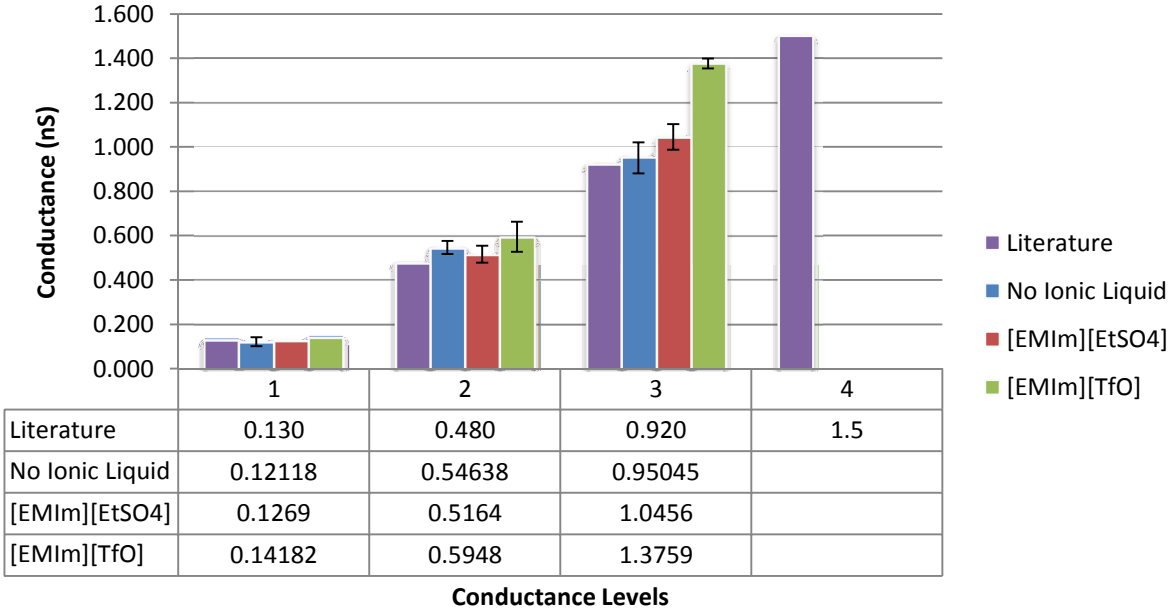
100 mV



	1	2	3	4
Literature	0.130	0.480	0.920	1.5
No Ionic Liquid	0.12666	0.56326	1.14966	1.47366
[EMIm][EtSO4]	0.1243	0.5201	1.0111	
[EMIm][TfO]	0.13952	0.54072	1.49632	

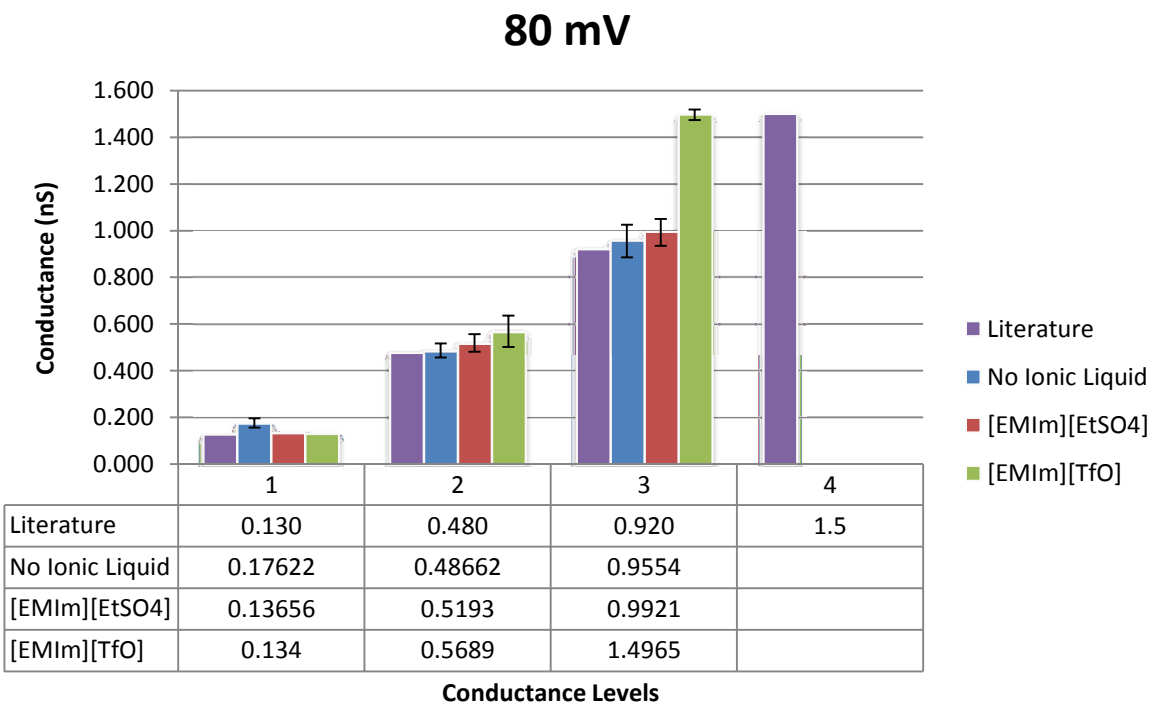
Conductance Levels

90 mV



	1	2	3	4
Literature	0.130	0.480	0.920	1.5
No Ionic Liquid	0.12118	0.54638	0.95045	
[EMIm][EtSO4]	0.1269	0.5164	1.0456	
[EMIm][TfO]	0.14182	0.5948	1.3759	

Conductance Levels



	1	2	3	4
Literature	0.130	0.480	0.920	1.5
No Ionic Liquid	0.17622	0.48662	0.9554	
[EMIm][EtSO4]	0.13656	0.5193	0.9921	
[EMIm][TfO]	0.134	0.5689	1.4965	

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