Assessment of the Use of Low Molecular Weight Diblock Copolymers for the Formation of Stable, Tunable Droplet Interface Bilayers

Joseph Tawfik
University of Tennessee, Knoxville, jtawfik1@vols.utk.edu

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Assessment of the Use of Low Molecular Weight Diblock Copolymers for the Formation of Stable, Tunable Droplet Interface Bilayers

A Thesis Presented for the

Master of Science

Degree

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Joseph Tawfik

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ABSTRACT

This thesis presents the use of diblock copolymers, poly(butadiene)-b-poly(ethylene oxide) (PBₘPEOₙ) and poly(isoprene)-b-poly(ethylene oxide) (PIₙPEOₘ), as amphiphilic molecular building blocks for the formation of synthetic polymer bilayer membranes using the droplet interface bilayer (DIB) technique. The DIB technique makes use of the self-assembly of amphiphilic macromolecules along oil-water droplet interfaces that can then be physically connected for the construction of liquid supported macromolecular bilayers at the droplet interface. These bilayer membranes are capable of hosting both naturally occurring and synthetic protein channels. This technique has been used to form synthetic bilayer membranes using various combinations of macromolecules. Much success has been had with a variety of lipids as the primary surfactant in the formation of DIBs, but questions remain regarding the use of diblock copolymers as the building blocks of DIBs.

A diblock copolymer is a combination of two separate polymer blocks, in this case a hydrophobic block (polybutadiene) and a hydrophilic block (polyethylene oxide). Block copolymers (BCPs) exhibit a high level of tunability, with previous studies showing the possibility of varying the types of polymers used in either block, the chain length of either block and effective bilayer thickness, and/or terminal functional groups of the blocks, effectively changing the BCP’s functionality. BCP structures have been shown to have a higher stability and greater longevity than lipid structures due to their higher molecular weight. BCPs could allow for a new dimension of customization at the interface with a greater potential for testing a variety of applications.

Previous attempts at using BCPs in the formation of DIBs were successful in forming bilayers with applied voltage, but the interfaces proved to be too thick for the successful incorporation of protein channels. The goals of this study are to show that a BCP with a lower molecular weight, PB₁₂PEO₈ or PI₁₇PEO₁₇, can successfully form a DIB, and then to quantify the effects of BCP presence in DIBs. With BCP bilayer DIBs realized, a wealth of potential applications could arise, ranging from drug delivery and protein characterization to neural networks and biomimetic computation.
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Chapter 1
INTRODUCTION
The bilayer membrane as it is found in a living cell is an extremely complex and robust barrier that selects what can and cannot cross the membrane. The cell membrane is composed of on the order of thousands of different types of molecules\textsuperscript{[1]} from three of the four main types of macromolecules: lipids, carbohydrates, and proteins. Scientists have been intrigued by the robustness and effectiveness of the cell membrane, pursuing the ability to harness this barrier selectivity for other applications: cell-cell communication studies\textsuperscript{[2]}, isolation and characterization of integral proteins\textsuperscript{[3]}, observation of lipid organization during various processes\textsuperscript{[4, 5]}, electrical measurements of ion channels\textsuperscript{[6]}, and many more. Figure 1-1 shows a handful of experimental methods, collectively referred to as model membranes, that mimic the cell membrane.

A great variety of model membranes have arisen as scientists attempt to delve more deeply into the wealth of molecular complexity that is found in cell membranes. One of the newer and more versatile model membrane techniques is known as the droplet interface bilayer (DIB) technique.

1.1 The Droplet Interface Bilayer
The droplet interface bilayer (DIB) technique is a method that allows for the formation of a synthetic bilayer membrane made up of amphiphilic macromolecules\textsuperscript{[8]}. The current technique utilizes silver-silver chloride wire electrodes to suspend water droplets in an oil reservoir. Either the water droplets or the oil may contain phospholipids or another type of amphiphilic molecule that behaves as a surfactant, provided these molecules will self-assemble along the oil-water interface along each droplet’s surface. These amphiphiles should assemble in such a way that the hydrophilic portion of the molecule is anchored in the water droplet while the hydrophobic portion rests in the oil phase. When enough of these molecules have coated the droplet surfaces, the water droplets are brought into contact, which results in the exclusion of the nonpolar phase (typically oil) and allows the opposing monolayers of the droplets to become thin and form a two-molecule thick structure, a DIB (Figure 1-2).

There are multiple benefits to using the DIB technique. The first is that there is no need to use a complex substrate. Other model membranes require substrates that have been processed extensively to make them suitable for model membrane formation and characterization, but the
Figure 1-1 - Multiple model membrane systems and simulations. (a) Giant unilamellar vesicles (GUV’s), (b) GUV networks connected by lipid microtubules, (c) solid-supported GUV’s, (d) membrane nanodiscs hosting transmembrane proteins, (e) supported lipid bilayers simulated using software, (f) cell membranes ruptured onto solid supports, (g) bilayers connected to a solid support hosting ion channels, (h) vesicles tethered to a supported bilayer, (i) multi-scale simulations represented visually.[7]

Figure 1-2 - A simplified image showing adsorption of lipid molecules at the oil-water interface.[8]
DIB technique only requires a well to contain the oil used in the experiment. Also, since this model membrane simply requires the suspension of water droplets oil for lipid self-assembly at the oil-water interface, the setup can be performed on a microscope equipped with a camera for simple imaging from beneath. This allows for the calculation of bilayer area using image processing. Asymmetric cases can be tested with this technique since individual droplets are pipetted onto either electrode, offering another layer of customizability to the model membrane. Finally, since the water droplets are suspended on silver electrodes, the electrodes can be used for mechanical manipulation of the droplets, allowing the user to maneuver the droplets into contact once monolayer formation is complete to form a bilayer. Once a DIB is formed, the user can also manipulate the bilayer to change the area and contact angle of the DIB. Also, droplets are intentionally suspended in the oil using a conductive material to make electrical interrogation of the DIB possible.

Both water droplets in a DIB are solutions of a common salt like potassium chloride (KCl) along with the amphiphile being used to form the bilayer. This allows electrical signal to be propagated through the DIB, as the dissolved salt provides the vehicle for electrons to be carried through the circuit without fear of signal loss to the surroundings since hexadecane and silicone oils are non-conductive organic solvents. Due to this simplification, the only contributions to the circuit that need to be considered are the resistance and capacitance at each electrode-buffer interface\(^9\), the resistance of each buffer solution\(^10\), and the resistance and capacitance of the bilayer membrane. The bilayer membrane is both resistive and capacitive due to its ability to separate charges without transferring them as well as its finitely resistive nature, so recording the bilayer response to electrical signals provides anecdotal evidence regarding the completeness of the bilayer seal.

Since the electrodes being used are composed of silver-silver chloride and chloride-based salt buffers are used, the interface between the electrodes and the water droplets have negligible resistance and capacitance values. Also, the resistances of the aqueous buffers can be added since they are in series, giving an equivalent resistance. This reduces the characteristic DIB circuit to a resistor and capacitor in parallel at the bilayer in series with the resistance of the electrolyte solution, as shown in Figure 1-3 below.

This means that DIBs can be accurately characterized optically and electrically without intensive computations, allowing for the calculation of bilayer area, resistance and capacitance, surface
Figure 1-3 – Simplified characteristic circuit showing DIB modeled with electrical components.
tension of the droplets of bilayer membranes.

With the capability to form bilayers using the self-assembling properties of these macromolecules, a wealth of studies has been conducted varying the molecular components of DIBs to suit various applications from molecular characterization\cite{8, 11, 12} to biomimetic computation\cite{13-15}. The DIB platform has been used to probe multiple research questions, and the use of yet another class of amphiphilic molecules could lead to novel applications with this tried and true research technique.

1.2 Literature Review

The DIB technique as showcased in the previous section is an effective method for forming bilayer structures between water droplets in oil and characterizing different biological molecules. The synthetic bilayer of a DIB formed with traditional lipids like DPhPC and DOPC is a biomimetic, 5-7nm thick membrane which offers an appropriate environment for the incorporation of transmembrane proteins\cite{16-19}. The following literature review investigates the physical and chemical nature of block copolymers (BCPs), structures formed with BCPs and combinations of BCPs and lipids, and applications of BCP structures to date. This information is given to motivate the use of BCPs for potential modification to current compositions used in the DIB technique, answering questions regarding bilayer stability and customizability. BCPs could provide a unique alternative to traditional phospholipid DIBs, utilizing the same robust characterization methods available with the DIB technique while affording greater stability and customizability than synthetic phospholipid membranes.

1.2.1 Block Copolymers

Block copolymers (BCPs) are linear chains of molecules made up of two or more blocks of distinct polymer types\cite{20}. The polymer chains found in BCPs are bonded covalently\cite{21}, uniting two unique chains of molecules. Since BCPs are synthesized combinations of polymers, there is a great deal of control over the composition of BCPs. Changing the types of polymers and the degrees of polymerization of either of the blocks affects the resultant mechanical properties of the BCP. This in turn affects the mechanical properties of aggregate structures formed by BCPs.

Varying the types of polymers included in a BCP is one of the most fundamental ways to change a BCP’s structure and function. The ability to handpick which types of polymers to bond to one
another\textsuperscript{[22]} in the synthesis phase offers the potential for combining two sets of desirable properties for a select outcome in the overall BCP. As previously discussed, amphiphilicity is a key feature of membrane molecules which is a characteristic of molecules containing both hydrophobic and hydrophilic regions. BCPs can be synthesized with a hydrophobic and hydrophilic chain to mimic the structure and function of membrane molecules. Another parameter that significantly affects the structure and function of BCPs is the effective interaction energy between the monomers in the blocks\textsuperscript{[21]}. Higher interaction energies reduce the monomer interaction within a BCP, resulting in more stable BCP aggregate structures. Reducing the interaction energy will result in more amphiphile exchange between aggregates and more fluid aggregates. These are just a few examples of important parameters that can affect the structure and function of BCPs and BCP structures, allowing for the tailoring of BCPs to fit a desired application.

Another potential for variation in the molecular makeup of BCPs is the ability to control the length of the polymer chain of one or both blocks in a BCP\textsuperscript{[21, 22]}. Varying the polymerization in a BCP refers to a change in the number of monomers that make up either polymer in a BCP. Multiple properties of BCPs and their composite structures depend on the level of polymerization of both blocks of the BCP, both as stand-alone values and in relation to one another.

Increasing the length of one or both blocks most obviously affects the molecular weight of the BCP. More massive molecules move less freely and fluidly through solution, meaning they will interact less frequently with other molecules in solution, and when they do, they will be less likely to make the mechanical and conformational changes required to undergo a change in structure or function. BCPs with higher molecular weights, therefore, have a higher stability\textsuperscript{[21]}. Changes in the degree of polymerization also affect the chain length of the BCP. In PB_mPEO_n, the thickness of a vesicle membrane can increase up to \( \sim 20\text{nm} \)\textsuperscript{[23]} by increasing the polymer chain length. If the degree of polymerization of one block changes with respect to that of other blocks in a BCP, the resultant structural motifs could also be affected, as is shown in Figure 1-4 below\textsuperscript{[24]}.

Finally, BCPs are very easily functionalized and otherwise chemically modified both during synthesis and \textit{in situ}. A wide variety of methods for functionalizing BCPs exists, including but not limited to click chemistry\textsuperscript{[25, 26]}, inverse electron-demand Diels-Alder\textsuperscript{[27]}, oxime chemistry\textsuperscript{[28]}, and thiol-ene chemistry\textsuperscript{[29]}. These methods allow for a single BCP type to be tuned chemically and mechanically for a variety of applications using common functionalization techniques. More
recently, a technique allowing for functionalization *in situ* directly before BCP self-assembly into aggregate structures was developed, yielding functionally active polymersomes with customizable functional handles[^30]. On top of this, other *in situ* chemical modifications such as cross-linking between adjacent polymers in BCP structures like micelles and vesicles[^31] allow for further modification in BCP structures and even greater control over BCP and BCP aggregate characteristics.

Many scientists have attempted using BCPs for the formation of model membranes. In 2016, the triblock copolymer poly(methyloxazoline)-b-poly(dimethyl siloxane)-b-poly(methyloxazoline) (PMOXA-b-PDMS-b-PMOXA) was used to form DIBs[^32]. The triblock copolymer used has a molar mass of ~7300 g/mol, which is nearly 9 times heavier than DPhPC (846.3g/mol), a typical lipid used in DIB formation. The triblock copolymer formed DIBs with applied voltage to drive the water droplets into an adhered state, but the resultant bilayer between the droplets was about 120Å thick (DIBs made of pure lipids are usually in the range of 20-30Å thick); much too thick for the incorporation of typical transmembrane proteins. Nonetheless, this study took the first step in showing that it is possible to form DIBs using molecules other than phospholipids. Figure 1-5 shows data collected on droplets containing a typical lipid (a) and droplets containing BCPs (b).

Wolfgang Meier has also studied BCPs’ ability to form a variety of model membranes from asymmetric planar free-standing membranes for improved stability, customizability, and surface area[^33] to simple vesicle structures for the formation of light-activated nanoreactors[^34]. He has qualified many different model membrane types. Figure 1-6 shows some 2-D schematics of a few different types of model membranes studied by Meier[^35].

With this range of tunable parameters, a variety of studies have been conducted using BCPs customized to the specifications of the respective application. One study showed that bacterial OmpF protein channels, which are routinely incorporated into lipid vesicles, could insert into hybrid vesicles composed of DPhPC lipid and different sized PI-PEO polymers[^36]. More recently, a study was conducted that used different size molecules of PBₙPEOₙ to form vesicles with the intention of assembling peptide-appended pillar[5]arene (PAP) protein channels into the vesicles[^37]. This protein had already been characterized in lipid bilayers, but had not yet been characterized in BCPs. The study found that by using a relatively short length of the BCP molecule, PB₂₃PEO₁₆, PAP channels could be incorporated into the bilayer. Interestingly, fewer PAP
Figure 1-4 - Morphology diagram showing the relationship between degree of polymerization of the hydrophobic block ($N_{PB}$) and weight percent of the hydrophilic block ($w_{PEO}$). Regions of the plot are demarcated and labelled with a letter designating the resultant morphology of the molecular composition: B is a bilayer vesicle, Y is a y-junction, C is a cylinder, and S is a monolayer sphere. The cryo-TEM images above the diagram show vesicles, cylinders, and spheres in A, B, and C respectively, with scale bars of 100nm.[24]
Figure 1-5 – a) DIB formation using DPhPC lipid in hexadecane oil. The current inset is characteristic of a stable DIB with tight molecular packing at the interface. b) DIB formation as a result of induced voltage bias in droplets containing triblock copolymer. While the current inset is different here than in a lipid case, it is still indicative of DIB formation.\textsuperscript{[32]}

Figure 1-6 – 2-D diagrams of various polymer membranes. a) monolayer at air-water interface, b) free-standing membrane, c) solid-supported membrane, d) nanoporous solid-supported membrane, and d) planar substrate immobilized vesicles\textsuperscript{[35]}. 
channels inserted into the polymer vesicles than into the lipid vesicles. Figure 1-7 shows simulations of polymersome walls with varied hydrophobic thickness containing the PAP protein. Biomimetic structures composed of combinations of lipids and BCPs have been produced and characterized in many different studies[21, 24, 32, 38, 39]. These structures offer a suitable and unique environment for hosting transmembrane proteins and other common biomolecules[3, 8, 11, 36, 40]. With this capability comes a vast potential for testing and tuning compositions to meet different requirements depending on the application at hand.

From varying synthesis conditions to leveraging the chemical versatility of individual BCP molecules and BCP structures, BCPs represent an attractive class of amphiphilic to use due to their potential to be tuned both mechanically and chemically. This level of control over a molecule’s properties allows for the selection of multiple other parameters such as molecular geometry, weight, polarity, and volatility to react with other chemicals in solution. Knowledge of how changing these parameters affects BCP aggregate morphologies and functions can be leveraged to select specific synthesis and functionalization techniques to fit the desired application. BCPs have already been used in a wide variety of applications including drug delivery[38, 39, 41, 42], nanoscale reactions[40, 43], and for nanoscale self-propelled motion[44] to name a few. With the available knowledge about BCPs, it could be possible to synthesize a class of BCPs with ideal characteristics for DIB formation.

1.2.2 A Comparison of Diblock Copolymers and Phospholipids

Phospholipids and tuned diblock copolymers can be similar in their amphiphilicity. The structures formed when solvated in polar and nonpolar solvents are also similar because of this amphiphilicity, although there are some key distinctions between the two types of amphiphiles. The main distinctions are their chemical structure and makeup, and their capacity to be modified. Phospholipids are composed of a hydrophilic head group containing phosphate, and a hydrophobic tail typically consisting of one or two fatty acid chains. The fatty acid chain length of a commonly used lipid, 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC), is 16 carbons. The molecular weight of the molecule is 846.3 g/mol. A chemical structure of DPhPC is shown in Figure 1-8 below. On the other hand, diblock copolymers are composites of two different polymer chains covalently bonded to one another to make a larger chain. This means it is more appropriate to think of BCP molecules as polar and nonpolar chains instead of a polar head and a nonpolar tail. Also,
Figure 1-7 – a) Interior view of a PB₁₂PEO₉ polymersome wall containing an inserted PAP channel. b) Interior view of a PB₂₃PEO₁₆ polymersome wall with the same protein inserted. The juxtaosition of a) and b) demonstrates the finding that these polymer vesicles exhibit an adaptive hydrophobic thickness, with an inherently thicker bilayer of the same polymer still effectively incorporating the PAP protein. [37]

Figure 1-7 - Chemical structure of DPhPC lipid.
the molecule is composed of two sets of repeating monomers. The diblock copolymers being studied here, PB$_{12}$PEO$_8$ and PI$_{17}$PEO$_{17}$, are composed of a block of 12 butadiene monomers and 17 isoprene monomers, respectively, covalently bonded to a block of 8 and 17 ethylene oxide monomers, respectively. In Figures 1-9, 1-10 and 1-11 below, chemical structures of monomers and their polymers are shown side by side. Figures 1-12 and 1-13 show the chemical structures of the PB$_{12}$PEO$_8$ and PI$_{17}$PEO$_{17}$ molecules.

Despite these differences in chemical structure, both types of amphiphiles are known to form vesicle type structures when hydrated with water. The other major difference between diblock copolymers and lipids is the degree of customization and functionalization that is possible with either type of molecule. The types of customization that are possible with BCPs has already been detailed in Section 1.2 of this introduction. Lipids, on the other hand, are most commonly functionalized through the addition of polyethylene glycol (PEG). Many studies have shown that the addition of PEG to lipids improves the stability of liposomes$^{[45]}$. Newer studies are being conducted on the potential for the functionalization of lipids with unsaturated fatty acid tails$^{[46]}$. Nonetheless, the potential for customization of BCPs far outmatches that of lipid molecules.

1.3 Document Overview

This chapter provided information about the current state-of-the-art droplet interface bilayer techniques as well as the current limitations on experiments resulting from the mechanical and chemical limits of phospholipids. This background information motivates the study of a novel class of macromolecules, block copolymers, for assembly into biomimetic membranes using the droplet interface bilayer method. Successful integration of BCPs into DIBs offers the promise shown regarding this class’ suitable physical and chemical properties: increased stability and customizable polymer types, hydrophobic thicknesses, and terminal functional groups. The rest of this document offers methods for integrating techniques that have been established and mastered using phospholipids with a new building block molecule in BCPs to further unleash the experimental possibilities behind the droplet interface bilayer modality. The research methods provided herein are experimental and are presented in a way that reflects the chronological progress made using BCPs.
Chapter 2 details the materials and methods used in this research, offering detail regarding the

Figure 1-8 - Chemical structure of butadiene (a) monomer and (b) polymer.

Figure 1-9 - Chemical structure of isoprene (a) monomer and (b) polymer.

Figure 1-10 - Chemical structure of ethylene oxide (a) monomer and (b) polymer.
Figure 1-11 - Chemical structure of ethylene oxide (a) monomer and (b) polymer.

Figure 1-12 – Chemical structure of PI17PEO17 (1900g/mol).
procedure for solution preparation, anecdotal evidence of the efficacy of BCPs as found in pendant drop experiments, and characterization techniques used to study droplet interface bilayer properties. Chapter 3 expands on the results of each of the experimental techniques described in Chapter 2 with an emphasis on comparing the properties of BCP droplet interface bilayers with those of traditional phospholipid droplet interface bilayers. Finally, Chapter 4 offers conclusions concerning the efficacy of BCPs in forming droplet interface bilayers as well as future directions in the further use of BCPs for making more tunable and stable droplet interface bilayers.
Chapter 2
MATERIALS AND METHODS

2.1 Research Question- Minimal stability and tunability in lipid DIBs
This research is motivated by the potential for combining the robustness of the DIB technique with the longevity and customizability offered by BCPs. The seal of the droplet interface bilayer is frequently compromised in lipid bilayers due to the low chemical and mechanical stability of lipid bilayers. One approach to improve this is to change to a more chemically and mechanically stable amphiphile, namely, PB$_{12}$PEO$_8$ and PI$_{17}$PEO$_{17}$.

Bilayer membrane stability can be measured on a more macroscopic level by whether the DIB forms successfully without droplet coalescence, and anecdotal evidence can be offered with regard to stability on the molecular level by observing the electrical current through the DIB. The focus of this work is to show that DIBs can be formed using diblock copolymers with relatively low molecular weights, and to characterize the properties of the DIBs that are formed. Multiple types of experiments will be conducted to verify the success of the proposed composition including DLS measurements, pendant drop experiments, and DIB experiments. The aim is to realize the potential to form BCP DIBs, thus laying the foundation for future tuning of DIBs by modifying the BCP building blocks.

2.2 Solution Preparation

2.2.1 Film Rehydration to Form Polymersomes
The diblock copolymer PB$_{12}$PEO$_8$ is received from collaborators in a gel phase. To prepare a solution of polymersomes, the gel must first be solvated in chloroform (CHCl$_3$) at a concentration of 10mg/mL. Then, the desired volume is drawn out and the chloroform is evaporated at first using nitrogen stream, and then using a high vacuum treatment. Once the solvent is fully evaporated, a polymer film is all that remains in the vial. At this point, the film can be rehydrated with the desired volume of 500mM KCl 10mM MOPS buffer. The solution must either be extruded through a filter with a mesh size of at most 0.2 microns at least 11 times or be sonicated for at least 10 minutes to obtain a solution of monodisperse and unilamellar vesicles. The apparatus for extrusion is shown in Figure 2-1 below. The resultant solution is kept refrigerated at 4 degrees C to prevent degradation from heat and light exposure.

2 Diblock copolymers PB$_{12}$PEO$_8$ and PI$_{17}$PEO$_{17}$ were obtained from Dr. Manish Kumar at UTexas Austin.
Figure 2-1 - Top view of extrusion apparatus. The starting syringe (left) contains the unextruded, multilamellar vesicle solution. Passing the solution through the hex-nut apparatus (middle) containing the extrusion filter is considered one pass. This must be done at least 11 times (odd number to ensure that the final solution is not in the starting syringe) to produce a solution containing unilamellar vesicles.
2.3 Monolayer Formation

2.3.1 Interfacial Tension as an Indicator of Molecular Self-Assembly

The pendant drop goniometer is an instrument that can be used for studying interfaces between two media. This is done by filling a cuvette with at least 4mL of one of the media and filling a syringe with at least 50µL of the other medium. The tip of the syringe is submerged in the medium contained in the cuvette and a small volume is dispensed from the syringe such that a droplet is suspended from the syringe in the medium. The droplet must be small enough that it does not shear from the tip of the needle during the experiment as the interfacial tension between the two phases drops. Images are taken of the droplet interface with the medium in the cuvette over the course of the experiment. The interfacial tension is calculated at each frame, and these calculations are used to observe the change in interfacial tension over time. Figure 2-2a shows an image of the pendant drop goniometer apparatus, and Figure 2-2b shows the first and last frame of pendant drop experiments on pure water in hexadecane oil, and 500mM KCl 10mM MOPS containing 2mg/mL DPhPC in hexadecane oil.

Interfacial tension can be used as an indicator of monolayer formation because as amphiphilic molecules self-assemble at the oil-water interface, the tension between the two media is reduced. A plot of the interfacial tension of pure water and 500mM KCl 10mM MOPS buffer containing 2mg/mL DPhPC (a commonly used lipid) are shown in Figure 2-3 below to elucidate the effects of molecular self-assembly at the oil-water interface.

2.4 Droplet Interface Bilayer Measurements

2.4.1 Bilayer Formation

The DIB technique allows for the connection of two 200-300nL droplets (~0.5mm in diameter) to form a bilayer. This modality also allows for easy imaging from beneath the suspended DIB. As a result, calculating the contact area of a DIB becomes simple. An image of the droplets can be taken from beneath, and the visible region in contact becomes the diameter of the circle of contact between the droplets. The bilayer area is then simply calculated as the area of the circle. Bilayer formation can be observed optically and is confirmed electrically.

As mentioned previously, the bilayer membrane can be modeled as a resistor and capacitor in parallel. Using the definition of capacitance and the relationship between capacitance and current,
Figure 2-2 – a) Pendant drop goniometer apparatus, showing a 500 microliter Hamilton syringe loaded above a cuvette. The apparatus also contains a camera for imaging. b) First and final frames of a pendant drop goniometer experiment showing visible change in interfacial tension.

Figure 2-3 – Interfacial tension over time of pure water and 2mg/mL DPhPC in 500mM KCl buffer in hexadecane oil for comparison. The lipid solution shows the effects of lipid self-assembly at the oil-water interface, dropping the final interfacial tension of the droplet to about 1.02mN/m. The droplet of pure water undergoes a minimal change in interfacial tension, with a final interfacial tension around 38.5 mN/m.
the formation of the bilayer can be monitored. Equation 1 below demonstrates how capacitance is calculated, where $C$ is capacitance, $A$ is area, $\varepsilon$ is the permittivity of the bilayer, and $d$ is the bilayer thickness. Equation 2 demonstrates the relationship between current and capacitance, where $I$ is current, $V$ is voltage, and $t$ is time.

With this knowledge, a 10mV, 10Hz triangle wave is applied to the droplets while the current through the bilayer is measured. If no bilayer is present between the droplets, minimal current is recorded. However, as the bilayer begins to form, the oil is excluded from between the droplets and the bilayer thins. This is demonstrated electrically as a growing capacitance and a proportional growth in current. Since a triangle wave voltage is being applied to the droplets, a square wave current is measured as an output. If the bilayer is unstable, the measured current will appear less square and more triangular, revealing the ohmic nature of the contact between incomplete portions of the bilayer. The slope of the crests of the induced current are inversely proportional to the resistance of the bilayer, so the more square-shaped the current, the higher the resistance. Higher membrane resistance is also related to tighter molecular packing. A bilayer is considered stable if the measured current between the droplets is square and capacitive and the droplets are visibly adhered for 3 minutes. Electrical and optical data that is indicative of bilayer formation, as well as the sloped region of the square wave current used to calculate resistance are shown in Figure 2-4.

2.4.2 Bilayer Characterization Techniques
Two main experiments are used here to characterize the bilayer: an experiment relating the bilayer capacitance to the bilayer area, and an experiment quantifying the electrowetting response of the bilayer to increasing steps of dc voltage. These experiments are expounded upon in Taylor’s work in developing this technique for in situ measurement specific capacitance, monolayer, and bilayer tensions of a DIB\cite{47}. Figure 2-5 shows both experiments and their outputs.
Figure 2-4– a) shows the current induced by a 10Hz 10mV triangle wave on a DIB. Using the knowledge that capacitance, and therefore current, should rise as the bilayer thins, DIB formation can be identified electrically by observing the induced current response of two droplets are in contact. b) shows the visible change at the interface of the droplets as the DIB forms. c) shows a slight triangular peak at the crest of a capacitive square wave. The slope of this peak is used to extract membrane resistance, as the membrane resistance is inversely proportional to the slope of these peaks.
The first experiment involves the calculation of the bilayer capacitance at different bilayer area values. The bilayer area is changed in this experiment by moving the electrodes farther apart at each measurement. This experiment utilizes the assumption that the bilayer thickness and permittivity from Equation 1 are constant. This means that the relationship between bilayer capacitance and bilayer area must also be a constant proportion. This proportion is known as membrane capacitance, $C_m$, and is measured in units of capacitance per area. As previously mentioned, bilayer area is calculated using images taken from beneath the bilayer, and the current of the bilayer is constantly being measured. Using Equation 2, the capacitance can be calculated since both the change in voltage with respect to time and the current response are known. Therefore, the capacitance can be extracted from the data at multiple different bilayer areas, and a trend between the two can be obtained. Figure 2-6 shows the varied current response at different bilayer areas.

The second experiment begins with the bilayer at a relatively low initial area. The electrodes are no longer used to manipulate the bilayer area. The bilayer area is recorded, and then the applied dc voltage is ramped up 20mV. About 20 seconds are allowed for the bilayer to reach its new equilibrium area and current, and then an image of the bilayer is taken and the current response is noted. The voltage is then ramped up another 20mV. This process is continued until sufficient electrowetting data has been collected. The raw outputs of this experiment are the induced current and the images taken at each step. Figure 2-7 shows the change in current response with increasing applied voltage.

This experiment utilizes the knowledge that the application of an electric field to the droplets increases the external contact angle between the droplets, in turn increasing the bilayer area. The relationship between the change in contact angle and the membrane capacitance and monolayer tension is illustrated by equation 3 below\(^{[47]}\), where $\theta_0$ is the contact angle with no voltage applied, $\theta_V$ is the contact angle at the applied voltage, and $\gamma_M$ is the monolayer tension. Bilayer tension can also be calculated, as it is the reaction force of the bilayer to the components of the monolayer tensions in the plane of the bilayer\(^{[47]}\). This is shown in Equation 4. Figure 2-8 shows a diagram of a DIB with these forces drawn in.
The experiment diagramed in 2 above shows the mechanical manipulation of the electrodes to decrease the bilayer area in steps, recording the current response at each step. The data taken from this experiment is shown in B above; a trend between bilayer capacitance and bilayer area can be observed and specific capacitance (CM) can be extracted from the relationship. 3 above shows that the electrodes are fixed, and images of the DIB are taken before and after applying voltage. The contact angle is measured with this experiment and the data can be interpreted using C^{[47]}.

Figure 2-6 – Current response of a DIB with decreasing contact areas.
Figure 2-7 – Current response of electrowetting experiment. Increasing applied voltage causes growth in current at each step.

Figure 2-8 – DIB showing monolayer and bilayer tensions.
\[
\cos(\theta_0) - \cos(\theta_V) = \frac{C_M}{4\gamma_M} V^2
\]
Eq 2-3

\[
\gamma_b = 2\gamma_M \cos \theta
\]
Eq 2-4

Another telling piece of data that can be calculated from the monolayer tensions and contact angles is the free energy of formation of a DIB, \(\Delta F\). The free energy of formation is calculated as shown in equation 5. Using Equation 4, Equation 5 can be rewritten as Equation 6 below.

\[
\Delta F = 2\gamma_M (1 - \cos \theta)
\]
Eq 2-5

\[
\Delta F = 2\gamma_M - \gamma_b
\]
Eq 2-6

Equation 6 shows that the free energy of formation of a DIB can be thought of as the difference between the energies of the monolayer tensions and the energy of the bilayer. When this value is maximized, the DIB is highly favorable as it reduces the overall energy of the system.

The calculated values from this experiment are the relationship between the change in bilayer area and applied voltage, the change in bilayer capacitance and applied voltage, the monolayer tension and bilayer tensions, and finally the free energy of formation. The monolayer tension calculated from this experiment can be compared with values obtained from the previously mentioned pendant drop experiments for verification.
3.1. PB\textsubscript{12}PEO\textsubscript{8} and PIPEO Dynamic Self-Assembly Kinetics and Pseudo-Equilibrium at the Oil-Water Interface

Before studying the self-assembly properties of PB\textsubscript{12}PEO\textsubscript{8}, some control cases were tested. The first control case was a varied KCl concentration in pure hexadecane oil. The cases tested were pure water, 100mM KCl 10mM MOPS buffer, and 500mM KCl 10mM MOPS buffer. The results are shown below in Figure 3-1.

The next control that was tested was constant salt buffer concentration in varied oil composition. The 500mM KCl 10mM MOPS was used due to its minimal interfacial tension shown in the first control case. The two oil compositions that were tested are pure hexadecane oil and a 1:1 ratio of hexadecane oil to AR20 silicone oil. The results of this experiment are shown in Figure 3-2 below. AR20 silicone oil is an inherently low surface tension oil, so increasing this oil content reduces the interfacial tension between the water and oil phases dramatically.

Once controls had been studied, the self-assembly properties of PBPEO and PIPEO were examined by placing both in buffer solutions and comparing their self-assembly to that of DPhPC. The results are shown in Figure 3-3.

While both solutions showed drops in interfacial tension characteristic of amphiphile containing solutions, neither reached the minimum interfacial tension that can be seen in lipid-containing solutions. Also, the speed of self-assembly at the interface of both diblock copolymers is lower, reaching within 2% of the final interfacial tension in 4.4 minutes for PIPEO and 6.7 minutes for PBPEO. For comparison, DPhPC reaches steady state in about 4 minutes.

The next test performed was to compare the self-assembly properties of PBPEO when dissolved in oil compared with PBPEO dissolved in water. The concentration of the PBPEO in water is 5mg/mL, and the concentration of the PBPEO in hexadecane oil is 0.5mg/mL. The results of this test are shown in Figure 3-4.

Moving PBPEO to the oil phase had a clear effect on the self-assembly kinetics of the polymer. This is most likely due to the change in the structures formed in water versus in oil. In water, the hydrophobic regions of the molecule are shielded by forming vesicle structures to reach the most energetically favorable state. In oil, however, the hydrophilic regions are shielded. Since PBPEO
Figure 3-1 – Salt concentration in pure water is varied to determine how it affects interfacial tension between water and oil phases. This figure shows that increasing salt concentration lowers interfacial tension.

Figure 3-2 – Interfacial tension over time of 500mM KCl 10mM MOPS in pure hexadecane oil and in 1:1 hexadecane to AR20 silicone oil.
Figure 3-3 – Interfacial tension of PBPEO and PIPEO compared with interfacial tension of DPhPC. PBPEO reaches 5.5mN/m, while PIPEO only reaches 12.5mN/m. PIPEO concentration could not be increased due to its poor solubility in water.

Figure 3-4 – The self-assembly properties of PBPEO dissolved in and out of the water droplet are shown here. PBPEO out shows elevated self-assembly kinetics compared with PBPEO in, reaching 0.34 mN/m in ~1 minute.
is ~65% hydrophobic by weight, the resulting structure in oil is an inverted micelle. These structures supply the oil-water interface with molecules more readily than vesicles do, so the accelerated self-assembly kinetics are to be expected when PBPEO is dissolved in oil. Next, 5mg/mL PBPEO solutions with varying salt concentration were tested to observe whether the salt concentrations influenced the self-assembly kinetics of PBPEO. The results of this experiment are seen in Figure 3-5.

Increasing salt concentration reduces the interfacial tension of PBPEO solutions at constant concentration. The reason for this is likely that since the oil-water interface is inherently negatively charged\cite{48}, and the hydrophilic regions of PBPEO molecules are also negatively charged, there is a natural repulsion between the polymers and the oil-water interface. The addition of ions to the solution helps to screen the charges of both the interface and the polymers, reducing the effects of the inherent repulsion between the two. The more ions that are present in solution, the more molecules can reach the interface, the more tightly the molecules pack at the interface, and the more the interfacial tension is reduced.

The effects of oil composition of 5mg/mL PBPEO solutions was also examined to show whether AR20 silicone oil was more favorable for monolayer packing than pure hexadecane was. The results of this study are shown in Figure 3-6 below.

While increasing AR20 silicone oil content reduces the interfacial tension of 5mg/mL PBPEO solutions, it is unclear whether this is the result of enhanced monolayer packing due to improved interaction between the diblock copolymers and the interface or whether it is simply the result of reduced interfacial tension between the water and lower surface tension oil. Next, the effects of increasing PBPEO concentration in water were observed. The first concentration tested was 3mg/mL to match concentration used in literature\cite{37}, and the concentrations were increased to 5mg/mL and 8mg/mL to observe the change in self-assembly kinetics. The results of these tests are shown in Figure 3-7.

Increasing PBPEO concentration enhanced self-assembly kinetics, reducing the steady-state interfacial tension considerably. The more PBPEO in solution, the more molecules that are available to supply the oil-water interface with. This results in tighter molecular packing, and lower interfacial tension. Due to the minimal change between 5mg/mL and 8mg/mL concentrations,
Figure 3-5 – The effect of salt concentration on PBPEO self-assembly kinetics is shown here. Increased salt concentration decreases the interfacial tension between the water and oil phases.

Figure 3-6 – The effects of increasing AR20 silicone oil content on 5mg/mL PBPEO water droplets are shown here. Increasing silicone oil content reduces the interfacial tension.
Figure 3-7 – Interfacial tension over time of various concentrations of PBPEO in 500mM KCl 10mM MOPS buffer in 1:1 hexadecane to AR20 silicone oil.
5mg/mL was primarily tested for the remainder of these experiments.

Finally, the self-assembly kinetics of different combinations of DPhPC lipids and PBPEO polymer were studied to observe how additions of PBPEO polymer affected the self-assembly of a common lipid. The cases tested were pure DPhPC and 5%, 10%, and 15% molar ratios of PBPEO added to DPhPC lipid. The concentration of lipid was kept constant at 2mg/mL to match previously found threshold concentration for forming DIBs in literature\cite{49}, while the polymer concentration was varied with respect to this concentration. Figure 3-8 shows the results of this study.

The pseudo-steady state behavior of polymer containing mixtures seems to be due to unintended changes in volume. These compositions needed to be studied at particularly small volumes to prevent the droplet falling from the needle tip as low interfacial tensions were reached. These volumes may be near the boundary of what our pendant drop goniometer can maintain. The data that can be collected from these pseudo-steady states is that the minimum interfacial tension values decrease as mole percent of added PBPEO increases. Also, self-assembly kinetics of higher PBPEO concentrations were slowed, taking 8, 12, and 20 minutes to reach within 2% of their steady state values compared with 4 minutes in the pure lipid case. This all suggests that while self-assembly kinetics may be slowed, the ability of combinations of PBPEO and DPhPC to pack the oil-water interface with molecules is greater than the ability of either molecule on its own. Table 3-1 presents the results of each experimental case, highlighting cases with optimal self-assembly properties for DIB formation.

3.2 Bilayer Formation Studies

DIB formation was attempted with multiple different cases and yielded a variety of interesting results. The first set of cases showed a growth in current which at first glance could look like a DIB beginning to form, but upon closer examination, the current is triangular, indicating ohmic contact between the droplets. Cases that resulted in this type of electrical response were 3, 8, and 16mg/mL PBPEO all in 500mM KCl buffer. The tests were all conducted in hexadecane oil. Each attempt allowed between 10 and 20 minutes for monolayer formation at each oil-water interface, as the self-assembly kinetics from the previous studies indicated that it took about twice as long for PBPEO to reach a steady state interfacial tension value compared with pure DPhPC compositions. Results from the 8 and 16mg/mL cases can be seen in Figure 3-9 below.
Figure 3-8 – Interfacial tension over time of 2mg/mL DPhPC in 500mM KCl 10mM MOPS buffer with varying mole percentages of PBPEO added. Increasing PBPEO concentration slows molecular self-assembly kinetics, but minimum interfacial tension values decrease, indicating tighter monolayer packing. Indicates minimum IFT.

Table 3-1 – All compositions studied using the pendant drop goniometer technique are shown here. The top row is the control lipid case. The yellow rows show studies varying polymer concentration, the orange rows show studies varying oil composition, the gray row shows polymer in oil, and the green rows show combinations of polymer and lipid. Highlighted cases are carried over to the next experiments. *Minimum IFT is reported instead of steady state.
Figure 3-9 – Current response of 8mg/mL and 16mg/mL PBPEO before coalescence of droplets. Both show induced currents that indicate bilayer thinning, but the current is purely ohmic indicating insufficient packing for stable DIB formation.
While these current responses appear to indicate thinning of the bilayer, there was no visual evidence that a bilayer had formed, and these cases resulted in coalescence of the droplets shortly after this growth in current. An interesting detail to note is that droplets remained stable and current grew for a longer period with increasing concentration of polymer, indicating slightly improved stability. The droplets did not appear any more adhered in these cases.

Other attempts were made with the polymer in the oil phase due to the low steady state interfacial tensions and fast monolayer formation shown in the self-assembly kinetics studies, but induced current did not increase and there was no visible adhesion between the droplets before coalescence. This indicates that while monolayers must have formed since the droplets did not coalesce immediately upon contact, there was no thinning to form a bilayer between the droplets. This could be due to a remaining layer of BCP inverse micelles in the oil phase acting as a buffer between the droplets, preventing bilayer formation. For this reason, it was concluded that it is not possible to form a stable DIB with a low molecular weight PBPEO molecule as the only surfactant in solution.

After this conclusion had been made, the effects of PBPEO concentration on DIB formation of the common lipid DPhPC were qualified. The same analyses were performed, allowing monolayer formation for between 5 and 10 minutes as was indicated by the self-assembly results of the lipid-polymer mixtures. DIBs were successfully formed with each composition, although the percentage of success decreased as the mole percent of polymer added increased. Results of the 0% and 15% PBPEO added cases are shown in Figure 3-10 and 3-11 below.

While all four compositions could form DIBs, a comparison of the case with no PBPEO added and the case with the most PBPEO added offers some insight into the qualities of the bilayers. Both compositions showed highly resistive bilayers, with almost no visible slope at the crests of the square-wave induced current. However, the peak induced current at steady state after bilayer formation in the pure lipid case is around 250pA, while the highest induced current in the 15% PBPEO added case is just around 60pA.

To verify the meaning of the electrical data, imaging data of bilayer formation needed to be analyzed. When images of DIBs at steady state after formation were studied, it could be seen that initial bilayer areas and contact angles were consistently lower in cases containing 15% PBPEO added. These initial results from DIB qualifying data imply that while DIBs can be formed with
Figure 3-10 – Bilayer formation of pure DPhPC.

Figure 3-11 – Bilayer formation of 2mg/mL DPhPC with 15% mole ratio PBPEO.
compositions containing up to 15% molar ratio of PBPEO, the DIBs that are formed with PBPEO have a less adhered state. Images of DIBs at steady state after bilayer formation are shown in Figure 3-12. Table 3-2 below shows qualifications of bilayer formation of each composition. More detailed bilayer property quantification can be seen in the next section.

3.3 Characterization of Bilayers Containing PB_{12}PEO_{8}

For each of the DIB compositions formed in the above section, capacitance measurements and electrowetting experiments were conducted with n greater than or equal to 3 trials. This data was then compiled and compared between each composition to quantify the effects of PBPEO concentration on DIB characteristics. The results of this section are statistically tested using pairwise comparisons of the means and variances of the data. If the variance intervals of two groups are disjoint, the groups are considered significantly different. If any overlapping between intervals occurs, there is no statistical difference between the groups.

The first variables that were compared were membrane capacitance ($C_M$) and membrane resistance ($R_M$). While no clear trend was apparent with increasing mole percent of polymer added to the composition, it can at least be said that membrane capacitance either remains the same or grows based on these trials. This result indicates that if PBPEO is present in the DIB, it is not thickening the bilayer, but thinning it. This range of membrane capacitance values corresponds to a membrane thickness range of 22 to 29.5Å. For comparison, naturally occurring cell membranes have typically have a hydrophobic thickness ~30Å\cite{50}, as do pure lipid DIBs. DIBs made purely of the 7300g/mol triblock copolymer in Tamaddoni’s work were 100-200Å thick\cite{32}. A plot showing mean $C_m$ values is shown in Figure 3-13.

There is a similar lack of clarity in the relationship between PBPEO concentration and $R_M$. Once again, it can at least be said that the membrane resistances either remained about the same or increased with increasing PBPEO content. Higher resistance implies a greater obstruction to the flow of ions between the droplets, which is anecdotal evidence for tighter molecular packing at the interface. This result is supported by the self-assembly kinetics data showing that compositions containing greater mole percentages of PBPEO had lower minimum interfacial tension values. Figure 3-14 below shows the relationship between PBPEO concentration and mean membrane resistance.
Figure 3-12 – Adhered state of a) pure DPhPC DIBs and b) 15% PBPEO DIBs. Pure lipid DIBs show greater contact areas and angles at steady state after DIB formation than 15% PBPEO cases do.

Table 3-2 – DIB formation results are shown below. Pure PBPEO solutions could not form a bilayer. Mixtures of PBPEO and DPhPC could form DIBs, although the success rate declined with increasing PBPEO content.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Monolayer Formation Time (min)</th>
<th>Formation %</th>
<th>Mean Amplitude (pA)</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>3mg/mL PBPEO</td>
<td>15-20</td>
<td>0/5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>8mg/mL PBPEO</td>
<td>15-20</td>
<td>0/10</td>
<td>N/A</td>
<td>Super Leaky</td>
</tr>
<tr>
<td>16mg/mL PBPEO</td>
<td>15-20</td>
<td>0/2</td>
<td>N/A</td>
<td>Super Leaky</td>
</tr>
<tr>
<td>0.5mg/mL PBPEO in oil</td>
<td>0-0.5</td>
<td>0/4</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>2mg/mL DPhPC</td>
<td>~5</td>
<td>3/3</td>
<td>~250</td>
<td>Square</td>
</tr>
<tr>
<td>2mg/mL DPhPC 5% PBPEO</td>
<td>~5</td>
<td>3/3</td>
<td>~200</td>
<td>Square</td>
</tr>
<tr>
<td>2mg/mL DPhPC 10% PBPEO</td>
<td>~6</td>
<td>3/4</td>
<td>~150</td>
<td>Square</td>
</tr>
<tr>
<td>2mg/mL DPhPC 15% PBPEO</td>
<td>~8</td>
<td>3/7</td>
<td>~60</td>
<td>Square</td>
</tr>
</tbody>
</table>
Figure 3-13 - Mean membrane capacitance with increasing mole percent of PBPEO added to 2mg/mL DPhPC DIBs. There is no apparent trend in this data, although the membrane capacitance appears to remain the same or increase.

Figure 3-14 – Mean membrane resistance with increasing mole percent of PBPEO added to 2mg/mL DPhPC DIBs.
Data regarding the monolayer tensions of the droplets in the bilayer could be calculated from the electrowetting data and compared with the data collected from the pendant drop experiments. The calculated monolayer tensions were close to the expected values shown by the pendant drop experiments, which are also shown below. The change in monolayer tension versus PBPEO concentration can be seen in Figure 3-15 below.

While there is no statistically significant change in monolayer tension among the cases, it can be said that the monolayer tension either remains the same or decreases. This would agree with the trend found in the self-assembly experiments showing decreasing minimum interfacial tensions. Bilayer tensions could also be calculated from the monolayer tensions and contact angles. Mean bilayer tensions are shown in Figure 3-16.

The mean bilayer tensions of the 10% and 15% PBPEO cases are significantly different than the pure lipid and 5% cases. Bilayer tension can be indicative of adhesion between the droplets. Bilayer tension value with respect to monolayer tension also has a clearer implication on the energetic favorability of maintaining the bilayer. More sense can be made of this result when taken together with the mean contact angle at 0mV applied. Figure 3-17 shows the mean contact angle with respect to PBPEO concentration.

A clear trend is present that with increasing PBPEO content, the mean initial contact angle of DIBs in electrowetting experiments decreases. Recalling Equation 4, the bilayer tension is calculated as the product of the cosine of the contact angle and two times the monolayer tension. Therefore, as the contact angle between the droplets falls, the bilayer tension comes closer and closer to the full magnitude of double the monolayer tension. Contact angle is a direct indicator of adhesion between droplets in a DIB. Increasing contact angle implies more oil exclusion and stronger adhesion between droplets in a DIB, while the opposite implies low levels of adhesion. This data suggests decreasing adhesion with increasing PBPEO content, and an analysis of the free energy of formation of DIBs in each case helps to shed more light on this trend. Figure 3-18 below shows change in free energy of formation with respect to PBPEO concentration.

There is a statistical difference between mean free energy of formation of the 5% and 15% PBPEO cases using pairwise comparison testing. Recall that high free energies of formation are indicative of favorable bilayers. This difference suggests that beyond a certain PBPEO concentration, DIB
Figure 3-15  – Relationship between monolayer tension and PBPEO concentration.

Figure 3-16  – Relationship between bilayer tension and PBPEO concentration. Brackets show a statistical difference between cases.
Figure 3-17 – Mean contact angle at 0mV applied with respect to PBPEO concentration. Brackets show a statistical difference between cases.

Figure 3-18 – Mean free energy of formation with respect to PBPEO concentration. Brackets show statistical difference between cases.
formation becomes significantly less favorable. This data agrees with the trend of decreasing contact angle, as the free energy of formation is taken from the difference between the bilayer tension and twice the monolayer tension. With decreasing contact angle, this difference shrinks, meaning that the droplets in a DIB become less and less adhered to one another. These results all have implications on the electrowetting response of each composition.

To characterize a DIB’s tendency to adhere more tightly with applied voltage, the relationships between normalized capacitance and voltage, and normalized area and voltage must be examined. Figure 3-19 below shows an example of these relationships as they are determined for a pure lipid case.

This trend shows that with increasing voltage applied, the bilayer area and capacitance increase. The relationship between area and applied voltage is simple and direct, but the same cannot be said for membrane capacitance and voltage. Recall the capacitance is dependent both on the area of the bilayer and on the thickness of the bilayer. To clarify which bilayer characteristics are changing, the change in area and capacitance must be taken together. Figure 3-20 shows the mean relationships between normalized area and voltage and normalized current and voltage with respect to increasing polymer concentration.

The first thing to notice about this trend is that area and capacitance exhibit similar changes in magnitude with changing concentration. If the change in capacitance and change in area are almost equal, then the implication is that the change in capacitance due to bilayer thinning must be negligible. In other words, electrocompression is not a factor with increasing polymer concentration.

There is a significant difference between the electrowetting response of pure lipid cases and the electrowetting response of 15% PBPEO cases. This difference can most easily be attributed to the decreasing adhesion of DIBs with increasing polymer content. It is known that lower initial contact angles typically have a greater change in nominal contact angle\[^{[47]}\]. Along with this, it is known that for droplets of fixed volume, growth in contact angle are directly related to growth in area. Since DIBs show decreasing initial contact angles with increasing PBPEO content, the corresponding growth in electrowetting response is only natural.
Figure 3-19 – Normalized capacitance and area relationships with applied voltage. These relationships describe the tendency of droplets in a DIB to wet to one another with applied voltage.

Figure 3-20 – Relationships between the change in area and voltage, as well as the change in capacitance and voltage, with respect to PBPEO concentration.
Chapter 4
CONCLUSIONS AND RECOMMENDATIONS

Studies on self-assembly kinetics of PBPEO and PIPEO showed higher net reductions in interfacial tension with PBPEO solutions than in PIPEO solutions. PIPEO had a relatively low solubility limit of 3mg/mL compared to PBPEO’s ability to dissolve up to 16mg/mL in water, so PBPEO became a favorite candidate for its high solubility in both water and oil. Self-assembly kinetics studies on PBPEO solutions showed that pure PBPEO is more surface active when dissolved in oil, both in speed to reach the oil-water interface and in net reduction of interfacial tension. This implies that PBPEO inverted micelles form monolayers faster and more effectively than PBPEO polymersomes can. The self-assembly kinetics results also indicate that when mixing PBPEO and DPhPC, a lower interfacial tension is reached than either molecule type can reach on its own, implying tighter packing of the monolayer at the oil-water interface.

Bilayer formation results show that DIBs cannot be formed using PB\textsubscript{12}PEO\textsubscript{8} molecules alone due to poor monolayer packing. They show that DIB formation is possible with low percentages of PBPEO added to a mainly DPhPC DIB, but the probability of success decreases with increased PBPEO content. Increasing PBPEO content also seems to have result in decreased contact area and contact angle between the droplets of a DIB at steady state following DIB formation.

Finally, DIB characterization experiments do not show clear trends between membrane capacitance or membrane resistance with PBPEO content. There was also no clear trend between monolayer tension and PBPEO content. There was a trend of decreasing bilayer tension with increasing PBPEO content, as well as a trend in decreasing initial contact angle with increasing PBPEO content. These trends were confirmed by a trend in decreasing free energy of formation of DIBs containing more PBPEO. These trends all explain the increased electrowetting response of DIBs with elevated PBPEO concentrations; lower initial contact angles allow for greater changes in area with respect to applied voltage. At low free energies of formation, the system has more residual energy to dissipate, resulting in more exaggerated electrowetting responses.

This study has shown that it is possible to form DIBs that contain mixtures of DPhPC and the low molecular weight diblock copolymer PB\textsubscript{12}PEO\textsubscript{8}. Including varied percentages of the polymer allows for the tuning of a DIBs electrowetting response. With a higher proclivity for the bilayer to change in area with applied voltage, smaller changes in voltage should elicit the same response.
that pure lipid bilayers show. This characteristic of diblock copolymer containing DIBs could be leveraged for higher resolution responses to voltage signals in biomimetic computing.

Now that DIBs containing PBPEO have been formed, one next step is to determine whether peptides like alamethicin could insert into a DIB containing PBPEO, and then to characterize the insertion of alamethicin with increasing PBPEO content.

Literature indicates that when PBPEO is terminally functionalized with a carboxyl group, the resulting structures in an aqueous environment are a mixture of vesicles and micelles\textsuperscript{[37]}. However, if the polymer is terminally functionalized with a hydroxide group, the structures formed by PBPEO are primarily micelles. It would be interesting to observe the change in self-assembly kinetics when this functional group is changed, as micelles are simpler structures to destroy, which could potentially accelerate self-assembly kinetics at the oil-water interface.

Another potential variable to test is the oil type. Only two types of oils were tested, and plenty more remain. An oil with a smaller molecule size (like decane) could yield improved results for DIB stability. While less oil will be excluded from the bilayer and the bilayer will be thicker, there could be a lower risk of droplet coalescence with more oil between the droplets, increasing the likelihood of forming DIBs with pure polymer compositions.

Literature also showed that greater length diblock copolymers than the ones used here could still incorporate protein channels into vesicles\textsuperscript{[36, 37]}, so attempting to form DIBs with higher molecular weight diblocks could be another avenue for research. If DIB formation is successful using larger diblocks, it would be interesting to extract thickness data and compare with the DIBs formed in this work.

Vibrational sum frequency generation\textsuperscript{[51]} could be used to shed light on the location of PBPEO during self-assembly at the monolayer. The capacitance results in this study do not show a discernible change in bilayer thickness, which would indicate that it is favorable for the polymer to be excluded from the bilayer, but studies elucidating the whereabouts of the polymer during monolayer assembly in pendant drop experiments are crucial to test this initial hypothesis.

Finally, dye leakage experiments have been used in literature to characterize membrane permeability\textsuperscript{[52]}. A suite of dye leakage experiments could be useful for confirming increased...
packing of molecules in DIBs with increased PBPEO concentration. If one droplet contains a dye like carboxyfluorescein, and the other does not, the time for leakage of the dye across bilayers of each composition could confirm that molecules are packed more tightly at the interface.
REFERENCES


VITA

Joseph Tawfik earned his Bachelor of Science in Biomedical Engineering from the University of Tennessee, Knoxville in 2018. During his undergraduate studies, he took an interest in cellular level transport and designing materials that mimic the selective transport found in cell membranes. As a result, he studied Biotransport Processes, a graduate level course, with Dr Andy Sarles where he learned about principles of diffusion and was introduced to the experimental platforms and techniques used in Dr Sarles’ lab.

His primary research interest is to combine the ingenuity and versatility of the droplet interface bilayer experimental platform with the vast world of molecular complexity to reveal the many intricate ways that these droplet interface bilayers can be tuned to different purposes and applications. In the past, he has worked with the Center for Nanophase Materials Sciences (CNMS) at the Oak Ridge National Laboratory to microfluidic devices sputtered with intricate electrode designs to connect arrays of droplet interface bilayers for biomimetic computation. This method requires bilayers with high durability and longevity properties for prolonged experiments. He is currently working with the Sarles Lab on forming BCP droplet interface bilayers repeatably for their enhanced stability and tunability with the goal of unleashing a new class of methods for tuning droplet interface bilayers via molecular conformational changes in situ.