Kinetics and Applications of Polyelectrolyte Membranes and Multilayers

Kristopher D. Kelly
FLORIDA STATE UNIVERSITY
COLLEGE OF ARTS AND SCIENCES

KINETICS AND APPLICATIONS OF POLYEOLECTROLYTE MEMBRANES AND MULTILAYERS

By

KRISTOPHER D. KELLY

A Dissertation submitted to the Department of Chemistry and Biochemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy

2016
Kristopher Kelly defended this dissertation on May 10, 2016.
The members of the supervisory committee were:

Joseph B. Schlenoff
Professor Directing Dissertation

Wu-Min Deng
University Representative

Michael Shatruk
Committee Member

Hedi Mattoussi
Committee Member

The Graduate School has verified and approved the above-named committee members, and certifies that the dissertation has been approved in accordance with university requirements.
This Dissertation is Dedicated
To My Dad Howard Kelly,
Who Has Been a Source of Unwavering
Support and Love My Entire Life.
ACKNOWLEDGMENTS

The success and friendship I’ve enjoyed these past five years could not have been possible without the support and companionship of my fellow graduate students, my advisor, and all of the collaborators I have worked with along the way.

A special thank you goes to my advisor, Dr. Joseph Schlenoff, for all the support, guidance, and incredible patience during my time under his tutelage. I have learned an incredible amount about areas of science I would never have considered and have more importantly learned that failure is just another route to success. Without his mentorship, I would not be able to fully appreciate the opportunities I’ve been granted and put them to good use in my professional life going forward. I also would like to extend my gratitude to the committee members that accompanied me along my journey through this program: Dr. Michael Shatruk, Dr. Hedi Mattoussi, Dr. James Brooks, Dr. Joel Fried, Dr. Thomas Keller, and Dr. Wu-Min Deng.

I would like to thank all of the members of the Schlenoff group over the years that helped me reach my scientific goals: Ramy Ghostine, Ali Lehaf, Zaki Estephan, Ruben Jara Beltran, Carlos Arias, Yara Ghoussoub, Ricky Surmaitis, Jingcheng Fu, José David Delgado, Jessica Martinez, and Hadi Fares. It has been a pleasure working with and learning from all of you.

To all the people I’ve met along the way during my time here in Tallahassee, I want you to know that I could not have completed this program without you. From the moment I set foot in Tallahassee, I sought out new opportunities in the way of activities within the Tallahassee and Florida State communities. Thank you to John Thompson for getting me deeply involved with the Tallahassee Soccer Association, to Nathan Crock and Yuvall Peress for allowing me to co-found the Renegade Boxing Club at FSU, to Melanie Colombo, Scott Chandler, Cody Gilliard, and Megan Mellino for helping me found the Tallahassee chapter of American Outlaws, to Lucky Anguelov for getting me involved with both kickball associations in town and being the best left back a soccer captain could ask for, and finally to my closest friends José Vidal Ros, Alec Morrison, and Nicholas Kramer for being the most fun roommates and support system I could have wanted. It has truly been a pleasure experiencing all the possibilities Tallahassee has to offer. Nothing could ever replace the memories we’ve made together and assuredly will continue to make in the future.
Special appreciation will always be extended to my brothers of Sigma Phi Epsilon who continued to support me despite my separation from our home chapter. Every time I was capable of making the trip home for formals, rituals, weddings, etc., I was welcomed with open arms and it felt as though I’d never left. You will always be family to me and I will never forget the bond that we formed together. Virginia Pi is truly a unique chapter with the strongest brotherhood I can imagine. HFF.

Finally, I would like to thank my father, Howard Kelly. Throughout my entire life, he has been my hero. He set the bar for what a man should be when he demonstrated how to raise two boys as a single father. From when we were old enough to pick up a baseball glove or kick a soccer ball, he coached both of our teams regardless of sport. My brother and I played sports five days of the week, so he took off work early to drive two hours through traffic so that he could be there to support us. Our rock. No matter the circumstance, he always made sure we had what we needed and got the absolute best that we could academically, athletically, and socially. No sacrifice was too great to ensure our success in all of our endeavors. I don’t know if I can ever truly repay you for all that you’ve given me, but I will always strive to do my best to make you proud.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Tables</td>
<td>ix</td>
</tr>
<tr>
<td>List of Figures</td>
<td>x</td>
</tr>
<tr>
<td>Abstract</td>
<td>xvii</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Polyelectrolytes: Definitions and Applications</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Polyelectrolyte Multilayer (PEMU) Thin Films</td>
<td>3</td>
</tr>
<tr>
<td>1.2.1 Layer-by-Layer Assembly Technique</td>
<td>3</td>
</tr>
<tr>
<td>1.2.2 PEMU Growth Mechanism</td>
<td>5</td>
</tr>
<tr>
<td>1.2.3 Effect of Salt on PEMU</td>
<td>8</td>
</tr>
<tr>
<td>1.2.4 Ion Transport in PEMU Thin Films</td>
<td>11</td>
</tr>
<tr>
<td>1.3 Polyelectrolyte Complexes (PECs)</td>
<td>13</td>
</tr>
<tr>
<td>1.3.1 Mechanical Properties of PECs</td>
<td>15</td>
</tr>
<tr>
<td>1.4 Polyelectrolyte Coacervates (PECOVs)</td>
<td>16</td>
</tr>
<tr>
<td>1.4.1 Polyelectrolyte Coacervate Formation</td>
<td>17</td>
</tr>
<tr>
<td>1.4.2 Effect of [KBr] on PECOV Morphology</td>
<td>19</td>
</tr>
<tr>
<td>1.5 Bioapplications of PEMUs</td>
<td>20</td>
</tr>
<tr>
<td>1.5.1 A7r5 and 3T3 Adhesion to PEMUs</td>
<td>21</td>
</tr>
<tr>
<td>1.5.2 Bacterial Adhesion to PEMUs</td>
<td>22</td>
</tr>
<tr>
<td>1.6 Dissertation Outline</td>
<td>23</td>
</tr>
<tr>
<td>2. EXPERIMENT TECHNIQUES</td>
<td>25</td>
</tr>
<tr>
<td>2.1 Materials and Chemicals</td>
<td>25</td>
</tr>
<tr>
<td>2.2 Polyelectrolyte Multilayers</td>
<td>26</td>
</tr>
<tr>
<td>2.2.1 Layer-by-Layer Assembly</td>
<td>26</td>
</tr>
<tr>
<td>2.3 Polyelectrolyte Complexes</td>
<td>28</td>
</tr>
<tr>
<td>2.4 Polyelectrolyte Coacervates</td>
<td>28</td>
</tr>
<tr>
<td>2.5 Spin Coating</td>
<td>29</td>
</tr>
<tr>
<td>2.5.1 Traditional vs. Coacervate Spin Coating</td>
<td>31</td>
</tr>
<tr>
<td>2.6 Surface Characterization Methods</td>
<td>31</td>
</tr>
<tr>
<td>2.6.1 Ellipsometry</td>
<td>31</td>
</tr>
<tr>
<td>2.6.2 Atomic Force Microscopy</td>
<td>32</td>
</tr>
<tr>
<td>2.6.3 Profilometry</td>
<td>34</td>
</tr>
<tr>
<td>2.6.4 Static Contact Angle Measurement</td>
<td>35</td>
</tr>
<tr>
<td>2.7 Spectroscopic Methods</td>
<td>37</td>
</tr>
<tr>
<td>2.7.1 Fourier Transform Infrared Spectroscopy</td>
<td>37</td>
</tr>
<tr>
<td>2.8 Electrochemical Methods</td>
<td>38</td>
</tr>
<tr>
<td>2.8.1 Rotating Disk Electrode (RDE) Voltammetry</td>
<td>38</td>
</tr>
<tr>
<td>2.9 Radiochemical Measurements</td>
<td>41</td>
</tr>
<tr>
<td>2.10 Mechanical Methods</td>
<td>41</td>
</tr>
<tr>
<td>2.10.1 Uniaxial Tensile Testing</td>
<td>41</td>
</tr>
</tbody>
</table>
### 3. SPIN-COATED POLYELECTROLYTE COACERVATE FILMS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Introduction</td>
<td>44</td>
</tr>
<tr>
<td>3.2 Experimental Section</td>
<td>46</td>
</tr>
<tr>
<td>3.2.1 Materials</td>
<td>46</td>
</tr>
<tr>
<td>3.2.2 PEC Preparation</td>
<td>47</td>
</tr>
<tr>
<td>3.2.3 PECOV Preparation</td>
<td>47</td>
</tr>
<tr>
<td>3.2.4 Spin Coating</td>
<td>47</td>
</tr>
<tr>
<td>3.2.5 Profilometry</td>
<td>48</td>
</tr>
<tr>
<td>3.2.6 Radiolabeling</td>
<td>48</td>
</tr>
<tr>
<td>3.2.7 Atomic Force Microscopy</td>
<td>48</td>
</tr>
<tr>
<td>3.2.8 Scanning Electron Microscopy</td>
<td>49</td>
</tr>
<tr>
<td>3.2.9 Mechanical Tests</td>
<td>49</td>
</tr>
<tr>
<td>3.3 Results and Discussion</td>
<td>49</td>
</tr>
<tr>
<td>3.3.1 Dish vs. Dome Morphology</td>
<td>49</td>
</tr>
<tr>
<td>3.3.2 Effect of [KBr] on Morphology of PECOV Spun Films</td>
<td>51</td>
</tr>
<tr>
<td>3.3.3 Free-Standing PECOV Thin Films</td>
<td>58</td>
</tr>
<tr>
<td>3.4 Conclusions</td>
<td>62</td>
</tr>
</tbody>
</table>

### 4. CELL RESISTANT ZWITTERIONIC POLYELECTROLYTE COATING PROMOTES BACTERIAL ATTACHMENT: AN ADHESION CONTRADICTION

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Introduction</td>
<td>63</td>
</tr>
<tr>
<td>4.2 Experimental Section</td>
<td>66</td>
</tr>
<tr>
<td>4.2.1 Materials</td>
<td>66</td>
</tr>
<tr>
<td>4.2.2 Polyelectrolyte Multilayer Preparation and Nomenclature</td>
<td>67</td>
</tr>
<tr>
<td>4.2.3 Polyelectrolyte Multilayer Characterization</td>
<td>67</td>
</tr>
<tr>
<td>4.2.4 Mammalian Cell Culture</td>
<td>68</td>
</tr>
<tr>
<td>4.2.5 <em>Escherichia Coli</em> Culture</td>
<td>68</td>
</tr>
<tr>
<td>4.2.6 Live Cell™ Imaging and Adhesion Analysis</td>
<td>69</td>
</tr>
<tr>
<td>4.2.7 <em>Escherichia Coli</em> Attachment and Retention Analysis</td>
<td>70</td>
</tr>
<tr>
<td>4.3 Results and Discussion</td>
<td>70</td>
</tr>
<tr>
<td>4.3.1 A7r5 and 3T3 Adhesion and Spreading on Zwitterionic Surfaces</td>
<td>73</td>
</tr>
<tr>
<td>4.3.2 <em>Escherichia Coli</em> Adhesion on Zwitterionic Surfaces</td>
<td>75</td>
</tr>
<tr>
<td>4.3.3 Possible Attachment Mechanisms</td>
<td>80</td>
</tr>
<tr>
<td>4.4 Conclusions</td>
<td>85</td>
</tr>
</tbody>
</table>

### 5. INTRINSIC PERFORMANCE OF POLYELECTROLYTE MULTILAYER MEMBRANES: ERASING THE MEMORY OF THE INTERFACE

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Introduction</td>
<td>86</td>
</tr>
<tr>
<td>5.2 Experimental Section</td>
<td>93</td>
</tr>
<tr>
<td>5.2.1 Materials</td>
<td>93</td>
</tr>
<tr>
<td>5.2.2 PEMU Characterization</td>
<td>93</td>
</tr>
<tr>
<td>5.2.3 Rotating Disk Electrode</td>
<td>93</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table 4.1</th>
<th>PEMU Thickness and Contact Angle. ..........................................................81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 5.1</td>
<td>Comparison of Thickness, Permeability, and Membrane Flux for Standard and</td>
</tr>
<tr>
<td></td>
<td>Annealed PDADMA/PSS PEMUs. ...................................................................104</td>
</tr>
</tbody>
</table>

ix
LIST OF FIGURES

**Figure 1.1** Diagram of polyelectrolyte chain conformation in low (extended) and high (collapsed) ionic strength solutions. .................................................................2

**Figure 1.2** Chemical structures of (A) poly(styrene sulfonate) (PSS), (B) poly(diallyldimethylammonium chloride) (PDADMAC), (C) poly(acrylic acid) (PAA), and (D) poly(allylamine hydrichloride) (PAH)...............................................................3

**Figure 1.3** Schematic representation of the solution alternation LbL technique. A charged substrate is immersed in (1) positive polyelectrolyte followed by a (2) 3 minute rinse step to remove excess polymer. After rinsing, the substrate is then immersed in (3) negative polyelectrolyte solution followed by another (4) 3 minute rinse step. This process is repeated “n” times until the desired number of layers is achieved. ........................................................................................................4

**Figure 1.4** Entropically driven electrostatic interactions between positive and negative polyelectrolytes on a substrate. When in close proximity, water and counterions are released into solution, forming ionic crosslinks between the oppositely charged chains..............................................................................................................................6

**Figure 1.5** Three zone diagram of PAH/PSS PEMU. The precursor and outer zones are constant in thickness during buildup, while added polyelectrolytes increase the thickness of the core zone. In the first few layers, or the precursor zone, the film is influenced by the nature of the substrate and overcompensation of one polyelectrolyte by another increases with layer number. The second zone, or the core zone, consists of the bulk of the multilayer where overcompensation is constant with layer number. In the outer zone, the surface of the PEMU, one polyelectrolyte exists in excess, which is overcompensated by the addition of an oppositely charged polyelectrolyte. As more layers get added, the second zone increases due to interdiffusion of polyelectrolytes into the bulk, while the precursor and outer zones retain their properties.................................................................7

**Figure 1.6** Representation of extrinsic and intrinsic compensation in polyelectrolyte multilayers..................................................................................................................8

**Figure 1.7** Representation of doping by NaCl into a PEMU. Salt ions break the intrinsic ion pairing of polyelectrolytes to associate with opposite charges on the polyelectrolyte chains. ...............................................................................................................9

**Figure 1.8** Diagram representing the effect of salt doping on the transport ability of ferricyanide through a PEMU. Salt ions dope into the film, breaking intrinsic ion pair crosslinks between polyelectrolyte chains. The small counterions extrinsically compensate free charges from the polyelectrolytes until ferricyanide dopes into the film, releasing three chloride ions. This process repeats until
ferricyanide reaches the rotating disk electrode, where it is reduced to ferrocyanide. .................................................................13

Figure 1.9  Representation of the three structural conformations of polyelectrolyte complexes (PECs). .................................................................14

Figure 1.10 Cross-section schematic of dried PEC being processed through a laboratory extruder. Polyelectrolyte complex is added to the hopper above a heated rotating screw, which forces the complex through a column to an orifice, which extrudes the complex in accordance to the shape of the chosen header .........................15

Figure 1.11 Representation of the effect of increasing KBr concentration within a PEC. As [KBr] increases, intrinsic compensation is converted to extrinsic compensation, which causes the PEC to swell .................................................................18

Figure 1.12 Schematic representation of the effect of [KBr] on coacervate morphology. ......19

Figure 1.13 Representation of electrostatic cell adhesion to a negatively charged PEMU compared to a zwitterioncoated PEMU. Zwitterions contain both positive and negative charges that create a net neutral charge as well as a steric boundary that prevents cell adhesion ........................................................................21

Figure 2.1  Diagram of PEMU construction technique by alternating polyelectrolyte adsorption .................................................................27

Figure 2.2  Diagram of formation of polyelectrolyte coacervate as [KBr] is increased. ......29

Figure 2.3  Representation of the spin coating process using polyelectrolyte coacervate. ......30

Figure 2.4  Representation of ellipsometry setup for measuring thickness and refractive index of thin films on a reflective surface .................................................................32

Figure 2.5  Schematic representation of an Atomic Force Microscope. ........................33

Figure 2.6  Schematic representation of a contact profilometer ....................................35

Figure 2.7  Static contact angle representation of hydrophilic, hydrophobic, and ultrahydrophobic surfaces. Hydrophilic surfaces exhibit contact angles smaller than 90°, hydrophobic surfaces exhibit contact angles between 90-150°, and ultrahydrophobic surfaces exhibit contact angles greater than 150°. ..........36

Figure 2.8  Schematic of FourierTransform Infrared Spectrometer. An incident beam of infrared light is split into two beams using a half-silvered mirror, which travel to two mirrors, one movable and one stationary, then travel back to the beam splitter to recombine. The recombined beam passes through the sample, which absorbs
certain wavelengths of light and produces an interferogram. This interferogram is then Fourier transformed to produce the sample spectrum.

Figure 2.9  Schematic of three-electrode setup for hydrodynamic rotating disk electrochemistry.

Figure 2.10  Image of the Thümler Gmbh TH2730 tensile testing machine equipped with a 3 kN load cell.

Figure 3.1  Dome versus dish morphology of spun PECOV films. PDADMA/PSS coacervates in 1.7 M KBr were spun on 18 mm glass coverslips at 5000 rpm for (♦) 5 s, (■) 10 s, (▲) 20 s, (▲) 30 s, (◇) 45 s, and (●) 60 s.

Figure 3.2  Viscosity (mPa s) versus [KBr] (M) at room temperature for a coacervate of PSS/PDADMA in 1.25 – 1.78 M KBr (data from reference 131).

Figure 3.3  Thickness comparison of spun films made from (A) 1.7 M KBr coacervate and (B) 1.9 M KBr polyelectrolyte solution as a function of spin speed and time.

Figure 3.4  2-D representation of (A) 1.7 M KBr coacervate, (B) 1.9 M KBr polyelectrolyte solution, (C) theoretical model of 1.7 M KBr coacervate, and (D) 1.9 M KBr polyelectrolyte solution films spun at various spin speeds and times. The model used to determine theoretical thickness does not account for the constantly-changing dynamic viscosity of coacervate, resulting in the discrepancy between result and theory.

Figure 3.5  Optical microscope images of films spun from 1.7-2.1 M KBr at 3000 rpm for 10 seconds as represented by A-E respectively.

Figure 3.6  AFM images of (A) 1.7 M KBr coacervate and (B) 1.9 M KBr polyelectrolyte solution at scales of 20 x 20 nm (left) and 5 x 5 nm (right). The rms roughness for each film was 16 and 4 nm for 1.7 M KBr and 13 and 8 nm for 1.9 M KBr with respect to the two XY ranges.

Figure 3.7  SEM images of PECOV films from (A) 1.7 M KBr coacervate and (B) 1.9 M KBr polyelectrolyte solution at a scale of 20 x 20 µm. As spin times increase from 10-20 seconds, distinct changes are observed in surface texture. The evaporation of water is thought to drive microphase separation, which induces the increased roughness.

Figure 3.8  Comparison of film composition between (A) PDADMA/PSS spun film in 1.9 M KBr and (B) a 40-layer stoichiometric PDADMA/PSS PEMU. The (C) difference spectrum shows no significant variation between the composition of each film.
Figure 3.9  Thickness profile of spun 1.7 M KBr PECOV films on a polished aluminum substrate. AL disks were used in the place of glass for the release of the PECOV films. .................................................................60

Figure 3.10  Released 1” diameter PDADMA/PSS PECOV film spun from 1.7 M KBr coacervate at 1000 rpm. .................................................................60

Figure 3.11  Stress relaxation (top) and stress-strain (bottom) curves of 14 mm x 3 mm x 10 µm 1.7 M KBr spun coacervate films. Stress relaxation showed an equilibrium modulus of the film to be 1050 MPa when strained at 2% of the film length (0.28 mm) and allowed to relax over 10 minutes. The tensile strength for these films was found to be 50 MPa by stretching the film at 7 mm min^{-1} until failure. .................................................................61

Figure 4.1  Illustrative comparison of attachment of mammalian cells to *Escherichia coli* overlaid on a 5 x 5 µm AFM image of an (A) AEDAPS PEMU and (B) AEDAPS-SS PEMU. A 50 µm diameter cell is shown on (C) an AEDAPS-SS PEMU alongside *E coli*. The bacterium was drawn to scale using measurements made on a scanning electron microscope. ..................................................65

Figure 4.2  Structures of polyelectrolytes used for the prevention of cell and bacterial adhesion. .................................................................66

Figure 4.3  FTIR spectra of (A) (PAH/PAABp)2-X-(PAH/PAA-co-AEDAPS)4 PEMU and (B) constituent reference films. Six bilayers of PAH/PAA (red), PAH/PAABp (green), a cast film of PAA-co-AEDAPS (blue), and (PAH/PAABp)2-X-(PAH/PAA-co-AEDAPS)4 (purple) are compared via FTIR spectroscopy. The numbered boxes represent characteristic peaks within each film with a (1) carboxylic acid stretch from the PAH/PAA film, a (2) decomposed diarylketone peak from PAH/PAABp film after crosslinking, and a (3) sulfonate stretch from the cast film of PAA-co-AEDAPS. .................................................................71

Figure 4.4  Diarylketone peak reduction at 1650 cm^{-1} after UV exposure of PAABp-containing PEMUs. FTIR analysis of (PAH/PAABp)2-(PAH/PAA-co-AEDAPS)4 PEMUs (blue) contains a diarylketone peak at 1650 cm^{-1}, which decreases after exposure to UV light between 200-280 nm for 15 min. The PAABp degradation produces free radicals that drive random C-C covalent bond crosslinking within the PEMU and strengthens the film creating the (PAH/PAABp)2-X-(PAH/PAA-co-AEDAPS)4 PEMU denoted as AEDAPS. .................................................................72

Figure 4.5  Zoom in of the behavior of A7r5 rat aortic smooth muscle cells and 3T3 mouse fibroblast cells on uncoated and AEDAPS-coated glass coverslips. .................................................................73

Figure 4.6  A7r5 and 3T3 mammalian cell lines were seeded onto uncoated control glass coverslips and coverslips coated with AEDAPS. The cells were cultured at 37 °C in 40% relative humidity and 5% CO₂. The time of each image capture is
represented at the top of the figure. Both cell lines were able to attach and spread on the control within a short time while were unable to do so on AEDAPS films where they formed free-floating clusters in solution.

Figure 4.7 Cluster-forming behavior of 3T3 mouse fibroblast cells at (A) 24 hours and (B) 48 hours on an AEDAPS coated surface. After 48 hours, the 3T3 cells were replated onto tissue culture plastic represented in (C). DIC microscopy was used for imaging at 24 hours and images B and C were captured using phase contrast microscopy.

Figure 4.8 Images of E. coli attachment to sterile uncoated coverslip control, AEDAPS coated glass coverslip, and AEDAPS-SS coated glass coverslip. The SS represents added zwitterion into the PEMU through increased PEMU soaking time, which displayed increased affinity for bacterial attachment and biofilm formation.

Figure 4.9 AEDAPS PEMU was soaked in PAA-coAEDAPS for various time points up to 16 hours to increase zwitterionic content as measured by (A) FTIR, which produced a loosely-bound layer that could desorb after sufficient time. Observing the sulfonate stretch peak at 1200 cm$^{-1}$ demonstrated a loss in polymer after 16 hours (B).

Figure 4.10 Time dependent surface coverage of Escherichia coli determined by pGLO green fluorescent protein. High bacterial concentrations (2.3 x 10$^7$ CFUs) at 5 and 30 minutes (C and D) were compared to low concentrations (1.5 x 10$^5$ CFUs) at longer exposure times (A and B). All bacterial colonies remain following shaking rinse step, meaning they are tightly bound to the surface. DIC microscopy and fluorescent imaging were combined to produce B and D (Scale bar = 100 µm) and the extent of surface coverage was calculated using ImageJ. Total surface area covered by bacteria was determined over two trials for ten different surface areas of 0.15 mm$^2$. $P$ values of <0.05 compared to average surface coverage percentage on tested surfaces are indicated with * in A and C.

Figure 4.11 AFM images of dried AEDAPS and AEDAPS-SS films on glass substrate. More granular AEDAPS exhibits a roughness of 4 nm, while AEDAPS-SS exhibits a roughness of 6 nm.

Figure 4.12 Diagram of bacterial attachment, biofilm formaion, and release of planktonic bacteria.

Figure 5.1 Illustration of Fe(CN)$_6^{3-}$ transport through a stoichiometric PEMU (left) compared to a positively terminated PEMU (Right). Excess positive charge allows ferricyanide to “hop” through the PEMU by way of counterion release. Three positive extrinsic sites are required to create a vacant site for ferricyanide hopping. Once a negatively charged polyelectrolyte is added to the film, all
excess positive charges become intrinsically compensated, yielding a glassy film that inhibits diffusion of ferricyanide, resulting in decreased membrane current. 88

Figure 5.2 Illustration of the adsorption of PSS to a PDADMAC terminated PEMU at the PEMU/solution interface. Excess positive charges from PDADMAC become intrinsically compensated by negative charges on PSS leaving a glassy, stoichiometric membrane.................................................................89

Figure 5.3 Structures of polyelectrolytes employed: Poly(diallyldimethylammonium) chloride (PDADMAC) and Sodium Poly(styrene) sulfonate (PSS).........................90

Figure 5.4 Bilayer thickness of PDADMAC/PSS PEMU built in 0.25 M NaCl. At seven bilayers, the stoichiometric PEMU was pushed to the top of the film with the rest of the PEMU having an excess of PDADMAC underneath........................................90

Figure 5.5 Schematic showing the boundary through which Fe(CN)$_6^{3-}$ (yellow dots) must diffuse before reaching the RDE. A PEMU was deposited via LbL onto the RDE and acts as the thin membrane that aids in ion transport of ferricyanide (PDADMAC-terminated) or blocks transport via stoichiometric, glassy film (PSS-terminated). On top of the PEMU rests a stagnant layer of solution (blue) of significantly greater thickness than the PEMU (15 µm) that inhibits diffusion of ferricyanide to the RDE.........................................................92

Figure 5.6 Cyclic voltammetry was performed on 1:1 mixtures of ferricyanide and ferrocyanide on a PDADMAC terminated PEMU (17 layers) (blue), PSS terminated PEMU (18 layers) (red), and on a bare electrode (green). When capped with PDADMAC, excess positive extrinsic sites draw negatively charged ferricyanide through the film to the electrode. When PSS terminated the PEMU, the film has no net charge and mobility of ferricyanide was diffusion limited. Both PEMU CV experiments showed complete blocking of ferrocyanide through the film. Removing the PEMU from the electrode surface allows for transport of ferrocyanide to the electrode, yielding the expected hysteretic curve. Experiments were performed in 0.6 M NaCl and 2 mM 1:1 mixture of ferri/ferrocyanide on a platinum electrode with an area of 19.63 mm$^2$ with a rotation rate of 1000 rpm.. 96

Figure 5.7 Limiting current at the rotating disk electrode vs. number of layers for 1 mM ferricyanide, Fe(CN)$_6^{3-}$ in 0.6 M NaCl using a 20 mV/s sweep rate, rotation rate 1000 rpm, at room temperature. Two methods of buildup were performed, standard (red) and annealing (blue). The standard method consists of solution alternation by dipping with intermediate rinsing steps, while the annealing method uses 2 M NaCl following each PSS layer to redistribute charge within the film, yielding a 1:1 stoichiometric film. Due to the stoichiometric nature of the film, polyelectrolyte does not diffuse into the bulk of the membrane, adsorbing only to the surface. This allows the film to grow at a much faster rate than standard buildup procedures. The platinum electrode had an area of 0.1963 cm$^2$. PDADMAC/PSS multilayers were built from 10 mM polymer solutions in 0.25 M
NaCl. Positive extrinsic sites of PDADMAC of \( y_0^+ = 0.21 \) allows for ferricyanide to hop through the membrane, but not actively transported. 

**Figure 5.8**
Extrinsic site labeling by NO\(_3^-\) using FT-IR. The nitrate ion, NO\(_3^-\), was added after each processing step (17 layers, NaCl annealing, and PSS addition) of the PEMU to determine the number of extrinsic sites available in the PEMU. Following the 17\(^{th}\) PDADMAC layer, the addition of NO\(_3^-\) showed an excess of extrinsic sites within the film. Annealing in 1 M NaCl allowed for rearrangement of polymer chains, which redistributed all excess positive extrinsic sites toward the surface. The reduction in positive extrinsic sites was reflected in the reduction of the NO\(_3^-\) at 1350 cm\(^{-1}\). Following the addition of PSS, there were nearly zero positive extrinsic sites available for NO\(_3^-\) to associate with.

**Figure 5.9**
Extrinsic site labeling by NO\(_3^-\) and \(^{35}\)SO\(_4^{2-}\) through FT-IR and radiocounting respectively. The use of \(^{35}\)SO\(_4^{2-}\) confirmed the decrease in extrinsic positive sites observed in Figure 4. Error bars represent the standard deviation of counts.

**Figure 5.10**
Membrane flux of ferricyanide through PEMUs constructed by two different methods. The standard Layer-by-Layer (LbL) PDADMA/PSS PEMU is represented by blue diamonds, which shows greater membrane flux due to inclusion of ferricyanide by positive extrinsic sites on PDADMAC. Annealing the PEMU with 2 M NaCl followed by an extra PSS adsorption step removes the excess positive charge and shows a decrease in membrane flux demonstrated by red squares.

**Figure 5.11**
Plot of PDADMA/PSS PEMU on a rotating disk electrode (RDE) with layer number. Blue diamonds represent Layer-by-Layer (LbL) construction method by solution alternation without any additional processing steps. Red squares represent annealed buildup with immersion in 2 M NaCl followed by additional PSS between each bilayer. Annealing the PEMU in NaCl allows for redistribution of excess PDADMAC within the film, which presents free binding sites at the surface for PSS. Once the PSS is added to the film, excess counterions and their associated waters of hydration are released, producing a stoichiometric, glassy film that acts as a barrier to ferricyanide as demonstrated by a decrease in permeability.

**Figure 5.12**
Six narrow molecular weights of PSS at A) 1 mM and B) 0.1 mM were used to determine the effects of molecular weight on polyelectrolyte adsorption kinetics using rotating disc electrochemistry. As PSS chains bind to the PDADMA terminated PEMU, it becomes more difficult for ferricyanide to diffuse through the film to the electrode, reducing the membrane current. As molecular weight increases, the rate at which PSS diffuses through solution to the electrode-fixed PEMU decreases. This decrease in rate was also attributed to a decreased number of polyelectrolyte chains. PSS addition is noted by a dashed line at 30 s. A ten point average plot was used to reduce noise.
Figure 5.13  Slope of current decrease for a range of narrow molecular weights of PSS to the PDADMAC terminated PEMU at 1 and 0.1 mM. There was a significant reduction in rate from 262k and 615k molecular weight PSS due to the combination of solution diffusion rate and membrane adsorption rate. The slopes were calculated from Chronoamperometry plots in Figure 5.12.
ABSTRACT

Polyelectrolytes have been used in a variety of forms from Layer-by-Layer constructed thin films to spun polyelectrolyte complex as free-standing membranes. The Layer-by-Layer methodology was used to adsorb oppositely charged polyelectrolytes onto a substrate for use across numerous applications depending on their construction and functionalization. This process was achieved by the alternating solution deposition of positively and negatively charged polyelectrolytes with intermediate rinsing steps. Applications of these thin films lie in biocompatible coatings, anti-corrosion surfaces, hydrophilic and hydrophobic coatings, and chromatographic applications among several others. Polyelectrolyte solutions can also be directly mixed to produce polyelectrolyte complexes, which were processed into extruded morphologies or dissolved in salt to form coacervates. These hydrogel-like polyelectrolyte coacervates were then spun into uniformly thick free-standing membranes. Polyelectrolyte complex membranes have applications in ion selectivity and permeability as well as desalination.

In this dissertation, poly(diallyldimethylammonium chloride) (PDADMAC) and poly(styrene sulfonate) (PSS) were used to form polyelectrolyte complex (PEC) by solution mixing. The PEC was then rinsed of salt and dried to produce a tough orange solid, which was ground into a fine powder and dissolved in KBr to produce polyelectrolyte coacervate (PECOV). The coacervate phase exists between [KBr] 1.3-1.8 M, so 1.7 M KBr and 1.9 M KBr concentrations were studied using spin-coating to determine the behavior of a PECOV against a polyelectrolyte solution. Using a specific set of parameters, spun films of PEC were removed from an aluminum substrate for use as free-standing membranes. These films were constructed in a matter of seconds, whereas achieving the same thickness using LbL buildup would take approximately 300 hours assuming 15 minutes per bilayer. Spray alternation of polyelectrolyte solutions could be used, but this process still takes roughly 20 hours to reach a similar thickness. Using LbL or spray alternation would also produce significantly rough films, while spin-coating coacervate produces nanometer roughness. Being able to produce thin films using aqueous solution provides an environmentally friendly alternative to traditional spin-coating processing methods.

A different set of polyelectrolytes was used to study the ability of a PEMU to prevent both mammalian cell and bacterial adhesion to a surface. The polyelectrolytes used were
poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA), with the latter being functionalized with benzophenone (Bp) and 3-[2-(acrylamido)-ethyl dimethylammonio] propane sulfonate (AEDAPS) in different stages during buildup. Benzophenone was used as a photocrosslinking agent to create a tough multilayer on which to build the zwitterion-functionalized layers. Despite the superb ability of these multilayers to repel mammalian cell lines A7r5 and 3T3, the PEMU produced attracted bacteria in an accelerated fashion, creating an interesting contradictory behavior. This result, while not expected, could be useful in the production of biorotors for the remediation of wastewater. Bacteria has previously been grown on the outside of a large hollow drum and rotated in a contaminated water supply often found at industrial complexes. The bacteria digest organic waste, sulfates, phosphates, and other harmful chemicals reducing them to harmless salts, carbon dioxide and nitrogen.

Production of PEMUs by Layer-by-Layer assembly is a widely used, well understood construction technique. The kinetics of polyelectrolyte sorption to an existing membrane has not been comprehensively studied however. Using PDADMAC and PSS, rotating disk electrode chemistry (RDE) was used to measure the rate at which PSS adsorbs to a PDADMAC-terminated PEMU. Six different narrow molecular weights were used at different concentrations to determine the behavior of PSS in solution based on chain length. Low molecular weight PSS was only diffusion limited by the solution and adsorbed to the PEMU quickly, while higher molecular weight PSS chains were hindered in their ability to adsorb based on the availability of extrinsic positive sites from excess PDADMAC as well as the rate in which they diffused through solution. The sorption rates were determined by the decrease in current measured by the reduction of ferricyanide at the rotating platinum electrode. When the PEMU is PDADMAC-terminated, excess positive extrinsic sites allowed negatively charged ferricyanide to “hop” through the membrane. Once PSS diffuses to the PEMU, it created ionic crosslinks with PDADMAC that released small counterions and water into solution, producing a glassy membrane, which can no longer assist in ferricyanide transport. Membranes with the ability to prevent multivalent ion transport are useful in ion selectivity applications.
CHAPTER 1

INTRODUCTION

1.1 Polyelectrolytes: Definitions and Applications

Polymers are large molecules whose structures are composed of many smaller covalently bonded sections called repeat units. Polyelectrolytes, or polymers that contain monomeric positive and negative charges, are known as polycations and polyanions, respectively. There are examples of polyelectrolyte chains that contain both positive and negative charge, which are referred to as polyampholytes,\(^1\) or more recently known as polyzwitterions.\(^2\) These macromolecules have been in use for over a century across a wide array of applications in rubbers,\(^3\) plastics,\(^4\) fibers,\(^5\) and electronics.\(^6-7\) Polyelectrolytes are found commonly in nature and can be synthetically produced for various applications.

Natural polyelectrolytes exist in the form of polypeptides, polysaccharides, proteins, DNA, and RNA, which are responsible for the genetic function of all living organisms. Proteins are made from different combinations of amino acids, which can contain either positive, negative, or zwitterionic groups. Synthetically, several distinct polymerization reactions can be performed: free radical, cationic, anionic, ring opening, and condensation polymerization.\(^8\) Free radical polymerization occurs when an initiator is added to a monomer solution that generates a free radical which propagates throughout the solution, adding monomer units until all excess monomer is consumed.\(^9-10\) Cationic and anionic polymerizations proceed in similar fashion to one another where a cation or anion is generated on a monomer by an ionic initiator. Cationic polymerization requires electron-donating monomers, while anionic polymerization requires electron-accepting monomers and both systems are sensitive to the solvent in which they are performed.\(^11-13\)

Polyelectrolytes can be classified as either “strong” or “weak” based on their degree of ionization when immersed in solution. Strong polyelectrolytes completely ionize at all pH ranges and weak polyelectrolytes only partially ionize depending on the pH of the solution.\(^14\) When polyelectrolytes are dissolved in polar solvents such as water, they obtain high levels of charge along their backbone, which distinguishes them from other polymers.\(^15\) The frequency of charges, or charge density, is the number of charges per repeat unit and is equivalent to the
degree of polymerization or the number of repeat units within the polymer. When a polyelectrolyte is immersed in solution, it is accompanied by a small counterion of opposite charge in order to maintain electroneutrality, which is a phenomenon known as counterion condensation.\textsuperscript{14-15} This behavior is driven by the interplay of electrostatic interactions between oppositely charged chains which decreases entropic gain of counterions within close proximity to the polyelectrolytes.

Depending on the ionic strength of the solution, polymer concentration, and polymer charge, polyelectrolyte chains can exhibit a range of structural conformations from elongated rods to condensed sphere-like shapes.\textsuperscript{16-17} Under low ionic strength, counterions tend to remain free in solution, which causes intrachain repulsion among like charges along the polyelectrolyte. This self chain repulsion forces the polymer into an extended, rod-like conformation.\textsuperscript{18} When ionic strength is increased, counterions are able to screen charges along the polymer, which reduces the intrachain repulsion and allows the polymer to collapse from its rodlike morphology to a flexible, random-coil conformation.\textsuperscript{15-16} This increase in ionic strength also reduces the entropic penalty of the counterions and in turn the Debye length, which is the decay length of electrostatic potential.\textsuperscript{19} The ability of these polymers to undergo such drastic chain conformation changes as shown in Figure 1.1 is specific to charged macromolecules and is known as the “polyelectrolyte effect”.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Diagram of polyelectrolyte chain conformation in low (extended) and high (collapsed) ionic strength solutions.}
\end{figure}
Polyelectrolytes have been used in a wide variety of applications in the form of fuel cells, paper production, lenses, corrosion protection, chromatographic separations, and additives to control the physical properties of aqueous solutions. The use of polyelectrolytes as substrate-bound thin films and free-standing complexes will be discussed.

1.2 Polyelectrolyte Multilayer (PEMU) Thin Films

1.2.1 Layer-by-Layer Assembly Technique

The assembly of polyelectrolyte thin films was first described by Iler in 1966, stating that oppositely charged colloids could be deposited onto glass to form a thin layer. It was not until several decades later in the 1990s that Decher popularized polyelectrolyte multilayer (PEMU) formation by way of alternating solution deposition of positive and negative polyelectrolytes onto a charged substrate, which he called the Layer-by-Layer (LbL) assembly technique. This method was quickly adopted due to its low cost, high reproducibility, environmentally friendly, and reliable method to produce thin films from the nano- to micrometer scale for use in a variety of applications. The most prominent of these applications took hold in fields such as ion separation, drug delivery, medical and electronic devices, corrosion protection, and sensors.

Figure 1.2 Chemical structures of (A) poly(styrene sulfonate) (PSS), (B) poly(diallyldimethylammonium chloride) (PDADMAC), (C) poly(acrylic acid) (PAA), and (D) poly(allylamine hydridchloride) (PAH).
There were two strong polyelectrolytes, poly(diallyldimethylammonium chloride) (PDADMAC) and poly(styrene sulfonate) (PSS), and two weak polyelectrolytes, poly(acrylic acid) (PAA) and poly(allylamine hydrichloride) (PAH) used throughout this work to produce Layer-by-Layer thin films. The two strong polyelectrolytes, PDADMAC and PSS, exhibit low pKa values with PDADMAC being immeasurable and PSS at 2.0,\textsuperscript{38} which means they are fully ionized at all pH levels, while the two weak polyelectrolytes, PAA and PAH, have pKa values of about 5.0 and 9.0 respectively,\textsuperscript{39} meaning that the pH of the solution must be adjusted to fully ionize them. Their structures are represented in figure 1.2.

\begin{center}

**Figure 1.3** Schematic representation of the solution alternation LbL technique. A charged substrate is immersed in (1) positive polyelectrolyte followed by a (2) 3 minute rinse step to remove excess polymer. After rinsing, the substrate is then immersed in (3) negative polyelectrolyte solution followed by another (4) 3 minute rinse step. This process is repeated “n” times until the desired number of layers is achieved.

In order for a polyelectrolyte to efficiently adhere to a substrate, the surface must be charged to adsorb the polymer. In this work, either polished silicon wafers or glass substrates
were used for polyelectrolyte deposition. For silicon wafers, a negative silicon oxide layer was produced that allowed PDADMAC to bind electrostatically to the substrate. This process was mimicked for glass by flame cleaning. Once cleaned, the substrate is alternately dipped in dilute polyelectrolyte solutions accompanied by multiple rinse steps to prevent cross contamination.\textsuperscript{30-31} For the rapid production of multiple PEMUs, an automated system can be used to increase productivity and reproducibility, removing human error during buildup. The substrate is immersed in the polyelectrolyte solutions between 5 and 20 minutes, depending on whether or not stirring is applied to the solution and the types of polyelectrolytes being used.\textsuperscript{40-41} Excess polyelectrolyte that is not directly adsorbed to the substrate or multilayer can be rinsed away in three minutes using deionized water, salt solution, or Tris buffer, if being used to produce biologically compatible films, following the addition of each polyelectrolyte. Any excess polyelectrolyte not bound to the surface may contaminate the opposite polion solution and produce undesirable polyelectrolyte complex (PEC), which will be discussed later. The process of polyelectrolyte dip and subsequent rinsing steps is repeated for the desired number of layers as shown in Figure 1.3.

There are other means by which thin films of polyelectrolytes can be produced such as solution casting, vacuum deposition, and spin coating, but often come with a variety of limitations such as solvent type and volume and polymer chain length.

\subsection{1.2.2 PEMU Growth Mechanism}

Despite the exponential growth in the field of polyelectrolyte multilayers (PEMUs), the exact mechanism of multilayer formation has been widely contested. The ability to adsorb a polyelectrolyte onto a charged substrate can be explained by charge reversal and overcompensation.\textsuperscript{42-45} Formation of the PEMU is entropically driven by the release of water molecules and counterions as shown in Figure 1.4.\textsuperscript{46} Once the chains approach each other, oppositely charged ions along the backbone of the chain are electrostatically attracted to one another forming an ionically crosslinked structure. Numerous characterization techniques were employed to determine the behavior of PEMU growth such as radiolabeled counterions,\textsuperscript{41, 47} attenuated total internal reflectance Fourier transform-infrared spectroscopy,\textsuperscript{48} X-ray and neutron reflectivity,\textsuperscript{49-50} and zeta potential.\textsuperscript{44}
Figure 1.4 Entropically driven ion pairing interactions between positive and negative polyelectrolytes on a substrate. When in close proximity, water and counterions are released into solution, forming ionic crosslinks between the oppositely charged chains.

There are two distinct regions of layer formation during the construction of a polyelectrolyte multilayer that determine the final thickness of the PEMU. During linear buildup, where polyelectrolyte chains are added to the surface, thickness increment is constant for each additional layer. This behavior is observed for strong polyelectrolytes that are fully ionized, where ionic crosslinking is entropically and kinetically favored, which prevents extensive diffusion between both polyelectrolytes throughout the bulk of a thin film. For the exponential regime, at least one polyelectrolytediffuses through the film instead of simply adsorbing to the surface, greatly increasing thickness with each additional layer. This behavior was observed when weak polyelectrolytes such as hyaluronic acid (HA), poly(L-lysine) (PLL), and poly(glutamic acid) (PGA) for all thicknesses and for PDADMA/PSS films once a certain salt concentration was achieved. For most polyelectrolyte systems however, the exponential regime occurs at the beginning of buildup, then becomes linear. Using confocal laser microscopy to study interdiffusion of polyelectrolytes in PEMUs constructed with PGA and PLL, it was found that PGA diffuses throughout the bulk of the multilayer during buildup regardless of film thickness. When PLL is added to the film, any unbound PGA diffuses back to the surface of the PEMU to bind to the PLL.

The growth mechanisms of PEMUs are not strictly limited to either of the two regimes as observed when a PEMU was built using PDADMAC and PSS. Using this combination of polyelectrolyte solutions, a distinct two regime growth was observed depending on thickness of
the PEMU. In order to more comprehensively understand the mechanisms for PEMU growth between linear and exponential regimes, the extent of polyelectrolyte interpenetration, thickness variation with layer number, and distribution of charge from the bulk of the PEMU to the surface were studied. A three-zone growth model was suggested during a study of poly(allylamine hydrochloride) (PAH) and PSS multilayers, which consists of the initial layers (precursor), the bulk of the PEMU (core), and the surface of the multilayer (outer) as illustrated in Figure 1.5.

Figure 1.5 Three zone diagram of PAH/PSS PEMU. The precursor and outer zones are constant in thickness during buildup, while added polyelectrolytes increase the thickness of the core zone. In the first few layers, or the precursor zone, the film is influenced by the nature of the substrate and overcompensation of one polyelectrolyte by another increases with layer number. The second zone, or the core zone, consists of the bulk of the multilayer where overcompensation is constant with layer number. In the outer zone, the surface of the PEMU, one polyelectrolyte exists in excess, which is overcompensated by the addition of an oppositely charged polyelectrolyte. As more layers get added, the second zone increases due to interdiffusion of polyelectrolytes into the bulk, while the precursor and outer zones retain their properties.

Using a polyelectrolyte pair of PDADMA/PSS, Schlenoff et al. suggested that the growth of the PEMU is reliant on two factors: surface charge overcompensation and charge penetration length. Charge overcompensation occurs for both PDADMAC and PSS capped films and is not dependent on salt concentration during buildup. In a later study, it was shown that these multilayers contain up to 10% salt ions compensating polyelectrolyte backbone charges in the PEMU. The inclusion of these counterions within the multilayer varies with the types of polyelectrolyte used as demonstrated through ATR-FTIR by Jaber et al. Using multilayers of PDADMA/PSS and PAH/PSS, the residual counterion content was found to be 3% and 6% respectively.
Ghostine et al.\textsuperscript{41} expanded on the aforementioned ideas to state that PEMUs constructed using PDADMA/PSS grow asymmetrically, producing a zigzag-like growth curve based on ion content. Using scintillation counting of radiolabeled counterions ($^{22}\text{Na}$ and $^{35}\text{S}$) doped into the PEMU, the ion content can be directly measured. When PDADMAC terminates the PEMU, there are excess counterions present in the film determined by the exchange of Cl\textsuperscript{−} ions with radioactive $^{35}\text{SO}_4^{2−}$ ions with an activity of 7.6 Ci mol\textsuperscript{−1}. The presence of counterions in the bulk of the PEMU is due to intrinsic compensation, which means that a charge from the polyelectrolyte chain is paired with a small oppositely charged counterion. When a layer of PSS is added to the film, all extrinsic compensation is converted to intrinsic compensation, where all unbound charges from PDADMAC become bound to opposite charges on PSS. The comparison of extrinsic to intrinsic charge compensation can be seen in Figure 1.6.

1.2.3 Effect of Salt on PEMU

Salt ions have a profound effect on the behavior of polyelectrolyte multilayers in terms of charge compensation and ionic strength of solution. As mentioned earlier, small counterions from the dissolution of salt in solution interact with polyelectrolyte chains by means of extrinsic compensation. The counterpart to extrinsic compensation is called intrinsic compensation, which occurs when the charges from one polyelectrolyte chain electrostatically bind to the opposite charges of another polyelectrolyte chain.\textsuperscript{43}

![Intrinsic Compensation](image)

\textbf{Figure 1.6} Representation of extrinsic and intrinsic compensation in polyelectrolyte multilayers.
Monovalent counterions such as sodium and chloride are able to diffuse through polyelectrolyte multilayers during and after buildup, which allow them to decouple the ion pairings between polyelectrolyte chains. This decoupling reduces the extent of intrinsic compensation and increases extrinsic compensation by small salt counterions. The amount of salt that is able to intercolate into the film is called “doping”, which is represented by the below equilibrium and illustrated in Figure 1.7.

\[
Pol^+Pol^-_m + Cl^-_{aq} + Na^+_{aq} \rightleftharpoons Pol^+Cl^-_{aq} + Pol^-Na^+_{aq} \tag{1.1}
\]

Where \(Pol^+\) and \(Pol^-\) represent the positive and negative polyelectrolytes respectively, “aq” refers to the aqueous phase, and “m” refers to the multilayer phase.

Figure 1.7 Representation of doping by NaCl into a PEMU. Salt ions break the intrinsic ion pairing of polyelectrolytes to associate with opposite charges on the polyelectrolyte chains.

When a significant amount of salt is added, all intrinsic compensation is converted to extrinsic compensation by salt ions, and the multilayer completely dissociates. For multilayers of PDADMA/PSS, PDADMA/PAA, and PAH/PSS, [NaCl] needs to be 2.7 M, 0.4 M, and 5.0 M respectively in order to cause the PEMU to dissociate. Using Equation 1.1, the equilibrium constant for PEMUs can be defined by relationship 1.2:
Where the equilibrium constant, $K$, can be defined as the doping constant $K_{dop}$. In Equation 1.2, $y$ is defined as the fraction of polyelectrolyte charged groups compensated by salt counterions and $1-y$ represents the fraction of intrinsic compensation within the PEMU. Activity is considered to be equivalent to doping ion concentration for these systems.

Doping by salt counterions into a multilayer depends on the identity and concentration of the salt as well as the identity of the polyelectrolytes that comprise the PEMU. Differing the polyelectrolyte pairs shows a change in the free energy of association ($\Delta G_a$) values, which is shown by various doping levels of NaNO$_3$ as determined by multilayer swelling and ATR-FTIR studies. Using the doping coefficient $K_{dop}$ from Equation 1.3, the free energy of association can be determined.

$$\Delta G^0_a = -RTln(K_{dop})^{-1}$$  \hspace{1cm} (1.3)$$

The value $R$ represents the gas constant and $T$ represents the temperature measured in kelvin. The free energy of association ($\Delta G_a$) can be determined for any salt concentrations relative to a reference $\Delta G_a$ at [NaCl] = 1.0 M as seen in Equation 1.5. Increasingly negative values of $\Delta G_a$, meaning stronger polyelectrolyte association, showed increased stability of the PEMU as salt concentration increased and swelling decreased, which made the multilayer more resistant to doping.

$$\Delta G_a = \Delta G^0_a + RTlna^2$$  \hspace{1cm} (1.4)$$

The identity of the salt and its concentration have a strong effect on construction of a PEMU. The addition of salt during buildup alters thickness of the film, the extent of thickness increase per layer, mass of polymer deposited, extent of hydration, permeability of the film, and
A variety of methods were used to measure the mechanical properties of PEMUs including atomic force microscopy (AFM), quartz crystal microbalance (QCM), tensiometry, strain induced buckling, and osmotic swelling. Using a PDADMA/PSS pair, Jaber et al. were able to build a PEMU thick enough to be removed from its substrate for mechanical testing. The elastic modulus of the PEMU decreased as the amount of salt doped into the film increased. Salt ions break the ionic crosslinking of intrinsic pairs of polyelectrolytes, which causes the PEMU to become softer. As salt dopes into the film, it allows waters of hydration to diffuse into the film, causing it to swell. The effect salt has on PEMUs is similar to that of heat on other polymer networks, where increased heat drives a phase change from a glassy state to a rubbery state. Since there is similar behavior in polyelectrolyte networks, but instead using salt, these materials have been termed “saloplastics”.

Jomaa et al. used neutron reflectivity to study polyelectrolyte interdiffusion in PDADMA/PSS PEMUs before and after immersion in high salt concentrations. When a PEMU is introduced to a high salt environment for an extended period of time (18-24 h), it allows the polyelectrolyte chains to move more freely and expel excess salt ions toward the surface in a process called “annealing”. The surface of PDADMA/PSS PEMUs decreases in roughness after being annealed in high salt solution as shown by atomic force microscopy. For this system, a [NaCl] of 0.8 M, PSS is able to diffuse throughout the film at a rate of $2.9 \times 10^{-17}$ cm$^2$ s$^{-1}$, which eliminates bulk extrinsic sites by compensating them intrinsically within the PEMU.

1.2.4 Ion Transport in PEMU Thin Films

Polyelectrolyte multilayers have been proven to be prime candidates for numerous applications in chromatography and nanofiltration since their flux and ion selectivity are controllable. Polyelectrolyte multilayers have been used previously to separate liquids, gases, ions, neutral compounds, and drugs. The ion selectivity of thin films of polyelectrolytes has been studied by a variety of groups. One study done on the permeability of a 200 layer PAH/PSS PEMU on a permeable support film showed that there was a selectivity of 15 for oxygen to nitrogen. Kraseman et al. showed that increasing salt concentration increases the selectivity of a PEMU for monovalent versus multivalent ions during multilayer buildup. Selectivity by the PEMUs comes from a combination of Donnan interactions and the
PDADMA/PSS multilayer behaving as a reluctant exchanger, as suggested by Farhat and Schlenoff.\textsuperscript{58,85}

The permeability and flux of a PEMU is highly dependent on the concentration of salt ions as seen in rotating disk electrode (RDE) studies using mixtures of ferri- and ferrocyanide. Increasing salt concentration allows the \( \text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-} \) pair to dope into the multilayer more efficiently as depicted in Figure 1.8. When the multilayer is charge-balanced, or completely intrinsically compensated, there are no extrinsic sites in which the electroactive species can situate. Upon addition of salt, a sufficient amount of ion pair interactions between polyelectrolyte chains are broken and are then compensated extrinsically by sodium and chloride ions. Ferricyanide molecules are able to “hop” through the film to sites that contain three free positive charges from PDADMA as suggested by Farhat.\textsuperscript{58}

\[
3\text{Pol}^+\text{Cl}^- + \text{Fe(CN)}_6^{3-} \rightleftharpoons \text{Pol}_3^{3+}\text{Fe(CN)}_6^{3-} + 3\text{Cl}^- \quad (1.5)
\]

Equilibrium 1.4 shows three positive extrinsic sites compensated by chloride ions that are replaced by one ferricyanide ion as it hops through the film toward the rotating disk electrode. This process is entropically favorable due to the release of three chloride ions for one trivalent species instead of breaking three ion pairs, which is energetically unfavorable.\textsuperscript{85}

For studies performed on a PEMU-coated rotating disk electrode, several factors come into play for determining the flux of ferricyanide to the RDE: thickness of the PEMU, angular rotation rate of the electrode, concentration of the supporting electrolyte, and concentration of the electroactive species. These factors will be discussed in depth later in Chapter 5. Equation 1.5 represents the membrane flux of ferricyanide to the RDE, which is dependent on the concentration of the analyte and thickness of the PEMU.\textsuperscript{85}

\[
J_m = -\frac{D\tilde{C}}{d} \quad (1.6)
\]

Membrane flux of the electroactive species is represented by \( J_m \), concentration of the ferricyanide within the PEMU is represented by \( \tilde{C} \), the diffusion coefficient of ferricyanide is
represented by $\bar{D}$, and the thickness of the PEMU is represented by $d$. It was shown by Farhat et al. that the diffusion coefficient $\bar{D}$ is independent of salt concentration and remains constant.\textsuperscript{85}

**Figure 1.8** Diagram representing the effect of salt doping on the transport ability of ferricyanide through a PEMU. Salt ions dope into the film, breaking intrinsic ion pair crosslinks between polyelectrolyte chains. The small counterions extrinsically compensate free charges from the polyelectrolytes until ferricyanide dopes into the film, releasing three chloride ions. This process repeats until ferricyanide reaches the rotating disk electrode, where it is reduced to ferrocyanide.

### 1.3 Polyelectrolyte Complexes (PECs)

Polyelectrolyte complexes were first studied by De Jong et al.\textsuperscript{86} as early as the 1930s and was later followed by Fuoss in the late 1940s.\textsuperscript{87} Similar to the mechanism for PEMU formation is the formation of polyelectrolyte complexes (PECs). Instead of using a charged substrate to electrostatically attach a film, oppositely charged polyelectrolyte solutions are added together in solution and thoroughly mixed, forming one of three possible complexes: insoluble polyelectrolyte complex precipitate (PEC), which produces a soft fibrous rubber-like material, quasisoluble polyelectrolyte complex (Q-PEC), and polyelectrolyte coacervate (PECOV), which is a biphasic mixture of PEC that will be discussed later. The chains are attracted in the same manner as PEMUs, where two oppositely charged polyelectrolytes are entropically driven by the interaction of charged monomer groups.\textsuperscript{88-89} Polyelectrolyte complexes can form either in a ladder conformation, where chains crosslink in an ordered fashion with an oppositely charged
chain, randomly crosslink with neighboring chains in a disordered network, or a combination of the two systems as shown in Figure 1.9.

**Figure 1.9** Representation of the three structural conformations of polyelectrolyte complexes (PECs).

Insoluble PECs are a result of mixing strong polyelectrolytes with comparable molecular weights in a 1:1 molar ratio under moderate solution conditions.\(^{88}\) As a result, a completely charge-balanced precipitate is produced. Typically soluble, quasisoluble PECs are constructed using weak polyelectrolytes in a non-stoichiometric fashion with drastically different molecular weights, but their solubility is highly dependent upon pH.\(^{90}\) The solubility of all polyelectrolyte complexes is highly dependent on the identity of the polyelectrolytes used, ionic strength of the solution, temperature, salt concentration, rate and order of mixing, and relative molecular weights.\(^{91-92}\) Combinations of these different parameters produce various morphologies of PEC
from nanoparticles to macroscopic hydrogels. From this range of morphologies comes a similar range of applications in the form of bioapplications, microencapsulation, fuel cells, cartilage mimics, and implant coatings.

1.3.1 Mechanical Properties of PECs

Polyelectrolyte complexes undergo the same phase transitions as PEMUs in that they both exhibit a tough, glassy state and a softer, rubbery state. The glass transition temperature, $T_g$, for polyelectrolyte complexes has been determined previously through the use of dynamic scanning calorimetry (DSC) and dynamic mechanical thermal analysis (DMTA) within a small margin of error. Storage modulus ($G'$) refers to the elastic nature of a material and loss modulus ($G''$) refers to the viscous properties of a material. The glass transition temperature can also be determined at the point where a change in the storage modulus begins to occur. Plotting $G''$ shows an increase in modulus as temperature increases before an inflection point where the material undergoes its glass transition and the modulus decreases again. The peak of the loss modulus curve gives a Tg higher than that of the storage modulus, while the peak of loss modulus tangent is typically used to assign Tg.

Figure 1.10 Cross-section schematic of dried PEC being processed through a laboratory extruder. Polyelectrolyte complex is added to the hopper above a heated rotating screw, which forces the complex through a column to an orifice, which extrudes the complex in accordance to the shape of the chosen header.
The mechanical properties for PDADMA/PSS PECs has been studied previously by Köhler et al.\textsuperscript{103} when they measured a physical transformation at 34 °C using polyelectrolyte complex capsules. The modulus of the PEC capsules fell from 100 MPa to 1 MPa via nanodeformation using atomic force microscopy.\textsuperscript{104} Another group reported a wider range of temperatures, 49-56 °C, for the glass transition of PDADMA/PSS PEMUs using quartz crystal microbalance over a range of ionic strengths.\textsuperscript{105}

These polyelectrolyte complexes can also be dried to a solid, translucent orange precipitate and processed through a heated laboratory extruder, which uses a mechanism that forces the PEC through a screw-filled column and out through an interchangeable orifice called a header, or die, as seen in Figure 1.\textsuperscript{10} The complex is subjected to high shear forces and compression, which binds the PEC together as it reaches the header. As studied previously, PDADMA/PSS PECs can be processed into a variety of morphologies such as tape, fibers, and tubes.\textsuperscript{106}

The mechanical properties of extruded PDADMA/PSS PECs have been measured via stress relaxation using a uniaxial tensiometry setup maintaining a strain of less than 2% to determine the effect of long-term deformations.\textsuperscript{98, 107-108} In order to determine the strain-to-break moduli of ExPECs, each sample was soaked in 0.1, 0.5, and 1.0 M NaCl for 3 hours. The samples were then stretched at a rate of 1 mm min\textsuperscript{-1} with elastic moduli around 160, 475, and 590 kPa respectively.\textsuperscript{98} These tests were bioinspired by the behavior of cartilage, which has a range of elastic moduli from 300-800 kPa.\textsuperscript{109} PECs exhibited equilibrium moduli up to 15 MPa when wet even when extruded up to three times.\textsuperscript{63, 106}

1.4 Polyelectrolyte Coacervates

The term polyelectrolyte complex coacervate, more simply known as polyelectrolyte coacervate (PECOV), first originated from Bungenberg de Jong and Kruyt in 1929 to describe the difference between coacervation of two polyelectrolytes and a polyelectrolyte and a small molecule or colloid.\textsuperscript{110} The earliest theoretical modeling of complex coacervation was first proposed by Michaeli et al.\textsuperscript{111} and proceeded by a number of other groups, who built upon the concept.\textsuperscript{112-114} Coacervation can occur in multiple forms when combining polyelectrolytes with
oppositely charged colloids such as proteins,\textsuperscript{115} micelles,\textsuperscript{116-117} and dendrimers.\textsuperscript{118} These polyelectrolyte-colloid coacervates have found a wide range of uses in the stabilization of foods, cosmetics, and pharmaceuticals.\textsuperscript{119-121} Polyelectrolyte coacervates are the result of polyelectrolyte complexes being dispersed into solutions of salt, forming biphasic mixtures where one phase is highly concentrated in PEC and the other is a dilute equilibrium phase.\textsuperscript{111, 122-123} The concentrated phase is an associative liquid of loosely bound polyelectrolyte chains that behaves similarly to a liquid.\textsuperscript{124-126} Weinbreck et al. suggest that complex coacervates behave rheologically like a viscous particle dispersion rather than a viscoelastic polymer solution.\textsuperscript{127-128}

Interest in PECOVs has recently increased due to recent discoveries that coacervate-based materials are used in nature by organisms such as mussels, tubeworms, and sandcastle worms.\textsuperscript{129-131} The sandcastle worm uses biological coacervate to join bits of shell and sand grains into a cement-like foam with closed pores via covalent crosslinking, although it is relatively weak only having a modulus around 300 kPa.\textsuperscript{130, 132} Using the sandcastle worm as a model, several groups were inspired to produce a synthetic adhesive that was capable of replacing metallic components to repair damaged bone and tissues within the human body.\textsuperscript{132-133} It was found that the strength of the coacervate-bound adhesive was about half of a cyanoacrylate bond (~40 MPa), which was used as a reference point since there are no current bone adhesives currently used in the medical field.\textsuperscript{133}

1.4.1 Polyelectrolyte Coacervate Formation

In order to produce a polyelectrolyte complex coacervate, a stoichiometric ratio of PEC must be produced by mixing polyelectrolyte solutions in molar equivalent ratios. The resultant complex is then rinsed with water for several days to remove any excess salt and dried. Once the PEC has been dried to a translucent tough orange solid, the pieces are ground into a fine powder and placed into a salt solution, in our case KBr. Potassium bromide is used due to its ability to dope more effectively than NaCl as shown by Ghostine et al.\textsuperscript{134} As mentioned earlier, increased salt concentration increases the extent of hydration by PEC through the breaking of intrinsic compensation by ion pair interactions between oppositely charged chains and replacing them with extrinsically compensated charges. The undoped PEC solid contains no counterions and about 38% water by weight, representing approximately ten water molecules per polyelectrolyte
ion pair. When the powdered PEC is dispersed into solutions of KBr, the salt ions diffuse into the complex and soften the material by breaking ionic crosslinks. As this process continues, the association between polyelectrolyte chains decreases, yielding a loose network of polyelectrolyte chains as seen in Figure 1.11.

![Figure 1.11](image)

**Figure 1.11** Representation of the effect of increasing KBr concentration within a PEC. As [KBr] increases, intrinsic compensation is converted to extrinsic compensation, which causes the PEC to swell.

A “backwards” method was applied to disperse PEC into KBr solution in order to increase the rate of coacervate formation. At a diffusion rate of $10^{-6}$ cm$^2$ s$^{-1}$, it would take approximately an hour to reach doping equilibrium for a 1 mm diameter polyelectrolyte complex fiber in less than 1.3 M KBr. To achieve the same results using a dry PEC, it can take up to several weeks to achieve stable coacervate composition. This backwards method consists of dispersing the powdered PEC into a high concentration of KBr then adding water to reach the desired [KBr] once a homogeneous solution phase has been attained. The samples were then heated to 60 °C for several hours then cooled to equilibrium over several days. There was no composition difference observed between using the forward or backward PEC dispersion methods.

As coacervates are formed over a range of KBr concentrations, two distinct phases become apparent. The two phases observed in PECOVs can be broken down into two subsets, macrophase separation and microphase separation. In macrophase separation, there is a distinct barrier between coacervate and dilute solution, whereas microphase separation shows a range of droplet sizes against a continuous background as shown by Wang et al. Microphase
separation is caused by temperature fluctuations away from equilibrium at room temperature, where an increase in temperature produces polyelectrolyte rich droplets in the dilute phase and polyelectrolyte deficient droplets in the concentrated phase. In other words, the dilute phase becomes more dilute and the concentrated phase becomes more concentrated as temperature increases.

1.4.2 Effect of [KBr] on PECOV Morphology

Work done by Wang et al.\textsuperscript{135} shows that there is a gradual continuum for polyelectrolyte coacervate formation over a range of KBr concentrations. PEC in low [KBr] gave the appearance of regular opaque complex with the consistency of loose rubber. As the concentration of KBr increased, the PEC became more translucent and fluid-like, resembling a polyelectrolyte coacervate as shown in Figure 1.12. The ability of salt to produce this coacervate phase separation phenomenon has been previously studied.\textsuperscript{136}

![Figure 1.12 Schematic representation of the effect of [KBr] on coacervate morphology.](image_url)
Below a KBr concentration of ~0.6 M, the free ions in solution are able to penetrate the PEC and gradually begin to break crosslinks between polyelectrolyte chains. After this 0.6 M KBr boundary, counterions and their associated waters of hydration in solution are able to intercalate within the film as well, which begins to swell the material. From [KBr] of 1.33 to 1.69 M, there was a distinct phase separation between coacervate and dilute solution, with the lower concentrations taking weeks longer to equilibrate due to increased viscosity. Once the concentration of KBr reached a threshold concentration of 1.80 M, the biphasic nature of the PECOV ceased to exist and yielded a one-phase component system.

There exists a soft boundary between a polyelectrolyte complex and a polyelectrolyte coacervate as measured by dynamic mechanical testing. At 1.3 M KBr, there is a distinct crossover point between the storage (G’) and loss modulus (G’’) of polyelectrolyte complex, which can be used to define the PEC/PECOV boundary for a PDADMA/PSS system. This inflection point is also confirmed by a drastic decrease in the slope of viscosity.135

### 1.5 Bioapplications of PEMUs

Polyelectrolyte multilayers are capable candidates for use in a wide variety of bioapplications such as cellular adhesion and repulsion, antibacterial applications, and non-fouling thin films.137-138 Their ability to coat an abundant number of surface types, ease of preparation, and tunable mechanical properties make them an inexpensive alternative to current polymeric bioapplications. Protein adsorption onto PEMUs has previously been studied by a variety of different groups.140-142 It was shown that proteins adsorb to the surface of a substrate based on electrostatic interactions, hydrogen bonding, and hydrophobic interactions.143-144 Proteins exhibit a net positive surface charge that can easily bind to negatively charged substrates.145 The use of poly(ethylene oxide) (PEO) to reduce hydrophobicity and surface charge proved to be an effective method at reducing protein adsorption to a substrate.146 Understanding the adhesion mechanisms for proteins is crucial to the understanding of the behavior of mammalian cells onto a PEMU. In this work, PEMUs containing benzophenone, a crosslinking agent, and AEDAPS, a zwitterionic functional group, were used to prevent the adhesion of two mammalian cell types, A7r5 and 3T3, which are rat aortic smooth muscle cells and mouse fibroblast cells, respectively.
1.5.1 A7r5 and 3T3 Adhesion to PEMUs

Due to the ease of manipulation of a polyelectrolyte multilayer and its constituent properties, their bulk and surface composition can be altered to prevent cell adhesion.\textsuperscript{141, 145} The ability to generate an inexpensive surface through green chemistry is important for use in biomedical applications such as stents, where the primary shortcoming is the buildup of A7r5 smooth muscle cells within the stent that can restrict blood flow and cause restenosis.\textsuperscript{147} In order to prevent this cellular attachment, the surface charge and extent of hydrophobicity were controlled, which showed a significant effect on the ability of these cells to adhere to a PEMU.\textsuperscript{140} Due to the surface of the cells being predominantly positively charged, negatively-charged PEMUs showed an increase in cell attachment, whereas positively terminated PEMUs showed improved cell repulsion.

\textbf{Figure 1.13} Representation of electrostatic cell adhesion to a negatively charged PEMU compared to a zwitterion coated PEMU. Zwitterions contain both positive and negative charges that create a net neutral charge as well as a steric boundary that prevents cell adhesion.
Previously, it has been shown that the addition of the zwitterion AEDAPS proved effective at the repulsion of mammalian cell lines due to its net neutral nature and steric boundary to the substrate surface as represented in Figure 1.13. Adhesion response by the cells was shown to only rely on the surface layer of the PEMU and not what was contained within the bulk. In the case of A7r5 smooth muscle cells, their survival is dependent on their ability to adhere to a surface. When these types of cells are unable to adhere to the surface, they will undergo apoptosis and die after a sufficient period of time. On the other hand, mouse fibroblast cells, 3T3, are non-adhesion dependent, spindle-shaped cells that attach to each other and form clusters of cells that can be replated onto more adhesion friendly surfaces where they can attach and proliferate. This process is non-necrotic in nature and does not release excess protein onto the PEMU surface.

1.5.2 Bacterial Adhesion to PEMUs

Similar to the issues that arise due to cellular adhesion onto various surfaces are the negative effects of bacterial attachment, which can cause illness and infection. Current biological implants are prone to bacterial attachment, that causes the patient discomfort which requires removal of the implant through invasive surgery. Due to the constant shear forces experienced by bacteria, they excrete adhesin proteins that behave as anchors to attach them to a surface. These adhesins are site specific in that they are designed to adhere to specific types of surfaces from root tissues in plants to tooth enamel. The use of PEMUs as antibacterial coatings has been studied previously and shown to have positive effect. A combination of the polyelectrolyte chitosan, which is a natural biocompatible cationic polysaccharide, with k-carrageenans demonstrated a significant decrease in bacterial adhesion and growth on a film. Using this as a base, Fu et al. demonstrated the ability of a combination of chitosan with heparin to prevent adhesion of Escherichia coli to a surface by killing the bacteria. While the exact mechanism has yet to be identified, it is theorized that the positively charged chitosan chains interact with negative charges along the cell membrane that causes leakage of proteins and other critical intracellular constituents. The combination of this critical external binding step with the antithrombogenic properties of heparin make this combination highly effective at preventing bacterial adhesion.
1.6 Dissertation Outline

Chapter 2 of this dissertation will discuss the experimental methods used throughout this work. The methodologies used for the construction of polyelectrolyte multilayers (PEMUs), complexes, (PECs), and coacervates (PECOVs) will be presented. This chapter will also discuss the components, specifications, and mechanisms of all analytical techniques used to collect and interpret data, with the exact experimental parameters presented in experimental sections of each subsequent chapter.

Chapter 3 will discuss the formation of polyelectrolyte complexes and coacervates and the means by which they are used to form thin films by spin coating. Polyelectrolyte coacervates produced over a range of [KBr] were used to produce thin polyelectrolyte films with varying morphologies. These films were found to be stoichiometrically equivalent by doping films with NO$_3^-$ to mark positive extrinsic sites on PDADMAC using FT-IR and radiolabeling with $^{125}$I to measure anion content. Cation content was measured using $^{22}$Na$^+$. Samples were produced at thick enough ranges to be removed from the aluminum substrate through heat and chemical release. Cut pieces of these removed films were stressed to breaking by dynamic mechanical tests used previously to measure the strength of extruded PEC.$^{106}$

In Chapter 4, thin polyelectrolyte multilayers were used as substrates to test their effectiveness at preventing the attachment of mammalian cell lines A7r5 and 3T3 and *Escherichia coli* bacteria. These PEMUs contained a benzophenone crosslinking agent, used to construct a firm platform on which to build a zwitterionic surface of AEDAPS with the aim of preventing biological adhesion through charge neutrality and steric boundaries. As was the case with previous surfaces,$^{141}$ the mammalian cell lines A7r5 and 3T3 did not adhere, but the bacteria found the surface desirable to the point of rapid biofilm formation, showing an unusual contradiction. The bacteria excreted extracellular polymeric substances (EPS) in sufficient quantities to bind to the zwitterionic surface even following an increase in zwitterionic content.

Falling back to a more fundamental approach towards polyelectrolyte multilayers, Chapter 5 discusses the rate of adhesion of poly(styrene sulfonate) (PSS) as it was measured by rotating disk electrochemistry (RDE) using electroactive ferricyanide. The protocol for RDE has been used previously to determine the diffusion coefficient of ferri- and ferrocyanide through a PEMU.$^{65, 85}$ Using a range of six narrow molecular weight PSS solutions at varying
concentrations allowed for the determination of membrane flux and permeability of PDADMA/PSS PEMUs with increasing layer number.
CHAPTER 2

EXPERIMENTAL TECHNIQUES

2.1 Materials and Chemicals

Poly(4-styrenesulfonic acid, sodium salt) (PSS; AkzoNobel, VERSA TL130; MW 200000 g mol\(^{-1}\)), and poly(diallyldimethylammonium chloride) (PDADMAC; Ondeo-Nalco, SD 46104; MW 400000 g mol\(^{-1}\)) were used for the production of polyelectrolyte complexes in sodium chloride (NaCl), which were processed, dried, and dispersed into potassium bromide (KBr; Sigma Aldrich) for the formation of polyelectrolyte coacervates. These coacervates were used to produce thin films by spin coating onto glass, aluminum, and poly(ether sulfone) (PES) (PALL Life Sciences; 25 mm diameter Supor-100, 130µm thick 0.1 µm filters) membranes. All solutions were prepared in 18 MΩ deionized water (Barnstead, E-pure).

Poly(allylamine hydrochloride) (PAH; Sigma Aldrich, MW 56000 g mol\(^{-1}\)), poly(acrylic acid) (PAA; Sigma Aldrich, 47.2 wt% in water, MW 100000 g mol\(^{-1}\)), poly(acrylic acid) grafted with 18 mol% benzophenone (PAA\(_n\)BP\(_m\); n = 0.82, m = 0.18) as prepared previously,\(^6\) and poly(acrylic acid) grafted with 25 mol% 3-[2-(acrylamido)-ethyl dimethylammonio] propane sulfonate (AEDAPS) (PAA\(_n\)-co-AEDAPS\(_m\); n = 0.75, m = 0.25) as prepared previously\(^1\) were all used for the preparation and production of photocrosslinked polyelectrolyte multilayers functionalized with the AEDAPS zwitterion for the prevention of cell and bacterial adhesion. Tris(hydroxymethyl)aminomethane (Tris; C\(_4\)H\(_{11}\)NO\(_3\)) was used to control the pH (7.3) of polymer solution during buildup to simulate biological conditions. Sulfuric acid (H\(_2\)SO\(_4\)) and hydrogen peroxide (H\(_2\)O\(_2\)) were used as received from the manufacturer to produce a strong oxidizing agent (Caro’s acid) (70:30 H\(_2\)SO\(_4\):H\(_2\)O\(_2\); caution: strong acid and oxidizer) to clean double-side-polished silicon [100] wafers from Silicon, Inc for FT-IR composition studies. Microscope glass coverslips (Fisher Brand Scientific; no. 1; 22 x 22 x 0.17 mm) were used as a substrate for cell and bacterial adhesion studies. All solutions were prepared in 18 MΩ deionized water (Barnstead, E-pure). Rat aortic smooth muscle cells (A7r5; ATCC CRL-1444) and mouse fibroblast cells (3T3; ATCC CRL-2752) were cultured in high glucose Dulbecco’s Modified Eagle’s Medium (DMEM; Sigma Aldrich; D5648), and Escherichia coli (ATCC;
were used for the determination of biological fouling of PAH/PAA-functionalized PEMUs.

Poly(styrenesulfonate, sodium salt) (PSS; Scientific Polymer Products, narrow MWs 57500, 122400, 262000, 8011000, and 2260000 g mol\(^{-1}\)), poly(styrene sulfonate) (PSS; narrow MW 615000 g mol\(^{-1}\)) as prepared from poly(styrene sulfonate) (PS; MW 311000 g mol\(^{-1}\)) using previous methods, and poly(diallyldimethylammonium chloride) (PDADMAC; Sigma Aldrich, MW 400000-500000 g mol\(^{-1}\)) were used for the construction of PEMUs on a rotating disk electrode (RDE). Potassium ferricyanide (K\(_3\)Fe(CN)\(_6\); Mallinkrodt Inc.), potassium ferrocyanide (K\(_4\)Fe(CN)\(_6\)·3 H\(_2\)O), and sodium chloride (NaCl) were used as received from the manufacturer for the determination of membrane current, membrane flux, and permeability of PEMUs. \(^{125}\)I (Na\(^{125}\)I; PerkinElmer, \(t_{0.5} = 60\) days, \(\gamma\)-ray, \(E_{\text{max}} = 35\) KeV) was supplied as 1 mCi in \(10^{-5}\) M NaOH with a specific activity of 17 Ci mg\(^{-1}\), which was diluted in 0.5 mL deionized water, which was used to determine extent of extrinsic sites within the PDADMA/PSS PEMU. Sodium nitrate (NaNO\(_3\); Sigma Aldrich) was also used as a doping salt for the determination of extrinsic sites in a PDADMA/PSS PEMU.

2.2 Polyelectrolyte Multilayers

2.2.1 Layer-by-Layer Assembly

Polyelectrolyte multilayers are constructed by the alternating deposition of oppositely charged polyelectrolyte chains. This process was performed both manually and with the aid of a robot. The use of a StratoSequence V (nanoStrata Inc.) automated buildup technique was employed to increase production of PEMUs through a repeating sequence of 5-1-1-1 minute polymer-water-water-water dipping technique with spinning. The silicon wafers were cleaned in a 70/30 mix of sulfuric acid and hydrogen peroxide (Piranha, caution: strong acid and oxidizer), which behaves as a strong acid and oxidizing agent to generate a negatively charged silicon oxide surface layer to which the positively charged PDADMAC can adsorb. The cleaned Si wafer is then rinsed with copious amounts of 18 M\(\Omega\) deionized water and dried with a stream of N\(_2\). A 1 inch diameter piranha-cleaned double-sided-silicon wafer was attached to the head of a rotating chuck using Parafilm. The speed of the rotating chuck on which the Si wafer is
mounted can be set to a desired rotation rate. The StratoSequence automation consists of an 8-well plate that holds two beakers of polyelectrolyte (PDADMAC and PSS) and six water rinse beakers. The duration of wafer immersion is controlled by computer software.

The chuck holding the cleaned Si wafer rotates continuously throughout all eight dipping steps to ensure a uniform buildup of PEMU starting with PDADMAC. The Si wafer is then removed from the PDADMAC solution after five minutes and is dipped sequentially into three water beakers for one minute apiece to remove any excess polyelectrolyte. This process is repeated for PSS and its corresponding rinse solutions until the desired number of layers is reached as illustrated in figure 2.1.

**Figure 2.1** Diagram of PEMU construction technique by alternating polyelectrolyte adsorption.
Manual multilayering was performed for PEMUs built on a platinum rotating disk electrode at 1000 rpm to increase the ease of electrochemical measurements between each adsorbed layer.

The resultant multilayers are labeled as \((\text{PE}_1/\text{PE}_2)_n\), where \(\text{PE}_1\) denotes the initial polyelectrolyte layer and \(\text{PE}_2\) denotes its oppositely charged counterpart. All PEMUs were built in 10 mM by repeat unit polyelectrolyte solutions at varying \([\text{NaCl}]\) depending on application as noted in each subsequent chapter’s experimental section.

### 2.3 Polyelectrolyte Complexes

Polyelectrolyte complexes were formed by the same principle as PEMUs with primary differences in the lack of a substrate and solution alternation. To create polyelectrolyte complex, 1 L of 10 mM solutions of PDADMAC and PSS in 0.125 M NaCl were prepared and mixed simultaneously into a 3 L beaker while stirring. The direct combination of polyelectrolytes eliminates the overcompensation seen in PEMUs and creates a stoichiometrically equivalent precipitate with the consistency of a soft rubber with a fibrous nature. After mixing for 30 minutes, the polyelectrolyte-poor supernatant is drained off and the PEC is rinsed with copious amounts of deionized water. To aid in the removal of all excess NaCl from the PEC, the complex is broken into small chunks and placed back into a large beaker full of DI water. This water is changed twice daily for 3 days to ensure removal of all salt ions. The final product is a tough, white solid that can then be dried in an oven to produce a tough, brittle translucent orange solid that can be processed into tapes, fibers, and assorted tubes.\(^\text{106}\)

### 2.4 Polyelectrolyte Coacervates

Once PEC was prepared and dried, it was ground into a fine powder for dispersal into KBr solution. A “backwards” method of preparation was used to create polyelectrolyte coacervates in order to increase the rate at which they form. The standard way of preparing materials like these is to add the desired amount of PEC to a KBr solution of choice and wait for the coacervate to form beneath a polyelectrolyte-poor solution. This process can take up to several weeks as observed by Wang et al.\(^\text{135}\) In order to reduce this waiting period, 1.5 g of PEC
is added to 2.5 M KBr solution and allowed to equilibrate, which takes only a few days. From this point, DI water is added to create the dilution of KBr desired, which allows more rapid formation of the desired coacervate concentration.

Below 0.6 M KBr, the ionic crosslinks of PEC cannot be broken by potassium and bromide counterions and cannot enter the complex along with their associated waters of hydration. After this point, ionic crosslinks begin to break down and the PEC begins to swell. Once a [KBr] of ~1.3 M is reached, the PEC begins to behave like a viscous ionic fluid, which is the beginning of the coacervate regime. The coacervate regime exists between 1.3 and 1.8 M KBr, where increasing salt concentration increases the volume of the coacervate layer as shown in Figure 2.2.

![Diagram of formation of polyelectrolyte coacervate as [KBr] is increased.](image)

**Figure 2.2** Diagram of formation of polyelectrolyte coacervate as [KBr] is increased.

### 2.5 Spin Coating

Spin coating is a technique used to disperse solution across a flat surface to generate a thin film. It usually involves a volatile solvent that evaporates quickly, leaving behind a thin, uniform coating. A typical process of spin coating uses an excess volume of polymer solution placed in the center of a disk, which is rapidly spread outward via centripetal acceleration once the substrate begins to rotate. A majority of the solution is rapidly thrown from the surface of the disk and is not deposited on the substrate. The process of spin coating occurs in three distinct
stages: deposition, initial spreading, and evaporation as shown in Figure 2.3. Small changes to rotation rate, solution volume, and solution viscosity can drastically alter the morphology of the film. The earliest mathematical analysis of spin coating was performed by Emslie et al.\textsuperscript{154} in 1958, which described the thinning of a non-volatile Newtonian fluid on an infinitely large rotating plate using a one dimensional model. This model assumes that there is no radial dependence on solvent content and film thickness due to symmetric flow.

![Figure 2.3 Representation of the spin coating process using polyelectrolyte coacervate.](image)

Meyerhofer\textsuperscript{155} improved upon this model by taking into account a constant evaporation rate based on a uniform solvent distribution in the out-of-plane axis which represents film thickness during spreading. Using this approach, Meyerhofer demonstrated that spin coating occurs in a two step process, which consists of a rapid radial convection outflow that thins the film and a solvent mass transfer phase that depends on solvent diffusion in the film and solvent partial pressure. Due to the rapid nature of spin coating, the majority of its dynamic calculations are merely theoretical with the Meyerhofer model fitting well to thinning rate of organic solvents.
using an interferometric technique. Spin coating has proven to be a useful technique for the production of thin films for a variety of applications such as antireflective coatings, microcircuit fabrication, television screens, and compact disk manufacturing.

2.5.1 Traditional vs. Coacervate Spin Coating

Traditional spin coating techniques are well-known and modeled for typical aqueous and volatile solutions, which shows a decrease in viscosity as solvent evaporates during spinning. This causes the spread of solution to slow and form a stable film with decreasing solvent content. For a system using polyelectrolyte coacervate, the opposite effect is observed due to the polyelectrolyte effect, which allows the movement of polymer chains to increase as salt content increases. This means that as water is removed from the solution during the radial outflow of solution, salt concentration increases, which plasticizes the film.

Polyelectrolyte coacervate of a given concentration was deposited upon a static substrate before initializing the spin coating process. As the solution begins to spread across the surface of the substrate, excess solution is ejected from the surface leaving a thin layer of PECOV film. The remaining coacervate was exposed to air allowing rapid evaporation of water, which drastically increases salt content within the film. Due to the increase in salt, any ionic crosslinks within the coacervate are then broken down by excess counterions decreasing viscosity. Through this process, coacervate spreads more quickly toward the edge of the substrate forming a “dish” morphology, more commonly known as the coffee ring effect. To prevent this feature when producing spun coacervate films, spin times and rates were adjusted to prevent excess solvent evaporation, which will be discussed in detail in Chapter 3.

2.6 Surface Characterization Methods

2.6.1 Ellipsometry

Ellipsometry is an optical characterization technique used to determine the refractive index and thickness of thin and ultrathin films on a reflective surface. The thicknesses of these measured films range anywhere between 1 and 600 nm, which is determined using a polarized
laser light angled toward the surface of the sample that reflects back into a detector. The incident laser light interacts with the dielectric properties of the film and returns an elliptically polarized light, which gives thickness and refractive index data. The polarization of this reflected beam can be described using two parameters: $\Psi$, the amplitude ratio of the incident versus reflected light, and $\Delta$, which is the phase shift between the two beams. A Gaertner Scientific Model L116S autogain variable angle Stokes ellipsometer equipped with a He-Ne laser that used a wavelength of 632.8 nm fixed at a 70° incident angle was used for the determination of thickness for all prepared PEMUs. The instrument is connected to a desktop computer with LGEMP software that processed the electrical signals and translated them into thickness and refractive index. The refractive index for PDADMA/PSS PEMUs was fixed at a value of 1.55.

![Figure 2.4](image)

**Figure 2.4** Representation of ellipsometry setup for measuring thickness and refractive index of thin films on a reflective surface.

### 2.6.2 Atomic Force Microscopy

Atomic Force Microscopy (AFM) is used for the determination of surface topography, thickness, and mechanical properties of a variety of samples from thin films to biological matter such as cells and bacteria. AFM is a high-resolution microscopy technique that uses a sharp tip (5-10 nm radius) made of silicon or silicon nitride attached to a flexible cantilever controlled by a piezoelectric tube that is used to probe the surface of the sample. The piezoelectric tube is controlled by a feedback loop. As the tip scans across the surface, a laser focused on the top of
the cantilever generates a three-dimensional image on an atomic scale as it is reflected back to a position sensitive photodiode detector. AFM can be performed in two primary modes: dynamic (AC) or tapping mode, and static or contact mode. For the dynamic measurement technique, the cantilever oscillates with an external frequency of about 10% the resonant frequency with amplitudes between 20 and 100 nm. This operating frequency can be described by the following equation

\[ f_0 = \frac{1}{2\pi} \sqrt{\frac{k}{m_0}} \]  

(2.1)

Where \( f_0 \) is the frequency, \( k \) is the spring constant of the cantilever, and \( m_0 \) is the mass load that the spring experiences.

![Schematic representation of an Atomic Force Microscope](image)

**Figure 2.5** Schematic representation of an Atomic Force Microscope
In static mode, the tip is placed in close proximity to the surface where it is deflected by surface interaction forces such as Van der Waals, electrostatics, and mechanical contact. The tip is then dragged across the surface at a constant distance from the surface as controlled by the piezoelectric device as determined by Hooke’s Law.

\[ F = kd \]  

Where \( F \) is the force applied by the piezoelectric tube, \( k \) is the spring constant of the cantilever, and \( d \) is the distance the cantilever is displaced during scanning.

A MFP-3D Asylum Research AFM was used for the determination of surface roughness and film thickness of PEMUs constructed for bioadhesion studies in Chapter 4 and polyelectrolyte coacervate thin film studies in Chapter 3.

### 2.6.3 Profilometry

Similar to AFM, profilometry measures thickness and roughness of samples by laterally scanning the surface topography. There are two types of profilometers: contact and non-contact. Contact profilometers use a diamond stylus that directly interacts with the surface of the sample, where a non-contact method uses white-light optical measurements to generate an image of the sample. Non-contact methods are able to scan the surface of a sample more quickly than those using a stylus, but any contamination on the surface is recorded as part of the sample. Using contact profilometry significantly increases data collection time, but the physical contact of the stylus removes any artifacts not bound to the surface of the sample.

In the surface characterization of PECOV spun films, a Tencor Alpha Step 200 Profilometer was used, which used a diamond stylus with a 12.5 micron radius that was placed in contact with a sample having dimensions up to 16.5 mm in thickness and 162 mm in length. The stage can be adjusted in the x and y planes to accommodate samples larger than this range. The stylus is dragged laterally across the surface for a specific distance at a set contact force. Contact force is adjusted based on the sample properties. Soft samples measured under high force can be damaged and may cause fouling of the stylus. Too hard of a sample can cause jamming of the stylus, which will cause the entire sample to be displaced along the stage. As the stylus traverses
the sample, ridges and valleys displace it vertically, which produce an analog signal that is converted into a digital signal, which is then analyzed and imaged via computer. An etch in the sample can be created to determine the thickness of the sample to within 5 nm.

![Schematic representation of a contact profilometer.](image)

**Figure 2.6** Schematic representation of a contact profilometer.

### 2.6.4 Static Contact Angle Measurement

Static contact angle measurements are used to determine the hydrophobicity and hydrophilicity of a surface. A droplet of water is gently placed on the surface of a thin film and the angle measured between the solid-liquid interface of the drop determines the contact angle. If the angle is less than 90°, the surface is considered hydrophilic and the droplet wets the surface, whereas all greater angles mean that the surface of the sample is hydrophobic. Increasing the contact angle beyond 150° means that the surface of the sample is ultrahydrophobic and water droplets are completely unable to wet the surface. An ultrahydrophobic surface causes water to bead and roll off the surface at even the slightest of inclines.
Figure 2.7 Static contact angle representation of hydrophilic, hydrophobic, and ultrahydrophobic surfaces. Hydrophilic surfaces exhibit contact angles smaller than 90°, hydrophobic surfaces exhibit contact angles between 90-150°, and ultrahydrophobic surfaces exhibit contact angles greater than 150°.

Hydrophobicity of PEMU surfaces were measured using a DI water droplet volume of 10 µL and a CCD camera with CAM200 software.
2.7 Spectroscopic Methods

2.7.1 Fourier Transform Infrared Spectroscopy

Infrared spectroscopy is commonly used for the identification of functional groups within a sample that absorb electromagnetic energy within the infrared region. Infrared energy is used to match a natural vibrational frequency of a molecule, which increases the amplitude of this specific frequency and produces a signal. This process involves the excitation of an electron to a higher vibrational level than its ground state, which is measured by the corresponding radiation frequency. The principle of infrared spectroscopy is based on a Michelson interferometer, which splits the incident IR beam into two parts: one focused toward a fixed mirror and the other toward a movable mirror.

![Figure 2.8 Schematic of Fourier Transform Infrared Spectrometer. An incident beam of infrared light is split into two beams using a half-silvered mirror, which travel to two mirrors, one movable and one stationary, then travel back to the beam splitter to recombine. The recombinated beam passes through the sample, which absorbs certain wavelengths of light and produces an interferogram. This interferogram is then Fourier transformed to produce the sample spectrum.](image_url)
These two beams are reflected back to a half-silvered mirror that acts as a beam splitter and recombines them into the beam that passes through the sample and into the detector, generating an interferogram as shown in Figure 2.8. This interferogram is then processed by Fourier transform Omnic software to produce a waveform that is used to identify various functional groups within the molecule. A Thermo Nicolet Avatar 360 spectrometer equipped with a DTGS-KBr detector was used for data collection on double-side-polished silicon wafers offset at a 75° incident angle to reduce noise by scattering.

The vibrational frequency of bonds within the sample can be predicted through the use of the harmonic oscillator equation below.

\[ \nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \]  

Where \( \nu \) is the wavenumber in cm\(^{-1} \), \( k \) is the spring constant, and \( \mu \) is the reduced mass of the atoms forming the bond as described by

\[ \mu = \frac{m_1 m_2}{m_1 + m_2} \]  

Where \( m_1 \) and \( m_2 \) represent the masses of the atoms forming the bond. Using these equations, it is suggested that higher energy radiation is absorbed by bonds formed between lighter atoms or bonds with higher strength.

2.8 Electrochemical Methods

2.8.1 Rotating Disk Electrode (RDE) Voltammetry\(^{173} \)

Rotating disk electrochemistry (RDE) is a hydrodynamic method of investigating reduction-oxidation chemical reactions. The setup for these experiments requires a working
electrode, counter electrode, and reference electrode. The working electrode is the electrode that rotates in solution and is the site at which the redox reaction occurs. The counter electrode acts as the electron accepting electrode to complete the electrical circuit and is larger in surface area than the working electrode. The reference electrode, which is typically a saturated calomel electrode (SCE) or silver chloride electrode, is used as the other half-cell that allows the potential at the working electrode to be determined. This three electrode system is shown in Figure 2.9.

![Figure 2.9 Schematic of three-electrode setup for hydrodynamic rotating disk electrochemistry.](image)

The working electrode is a teflon-housed 5 mm platinum disk, the counter electrode is a platinum wire with a round platinum plate, and the reference electrode is a KCL saturated calomel electrode (KCl-SCE), all placed in a solution of ferricyanide and NaCl. The ferricyanide acts as the electroactive species which is reduced at the PEMU-coated working electrode used to
determine membrane current. Sodium chloride acts as a supporting electrolyte, which serves to eliminate the contribution of migration to mass transfer of ferricyanide and reduces solution resistance between the working and reference electrode. The working electrode is mounted to a Pine AFMSRCE rotator which can be rotated up to 10,000 rpm with 1% precision. During spinning, it is assumed that convection maintains a steady state condition of analyte at the electrode surface. Without rotation a “duck-shaped” cyclic voltammogram is produced while an “S-shaped” voltammogram is produced for a rotating electrode. A Pine AFTP1 WaveNow potentiostat was used to produce voltammograms generated by each experiment with the aid of Aftermath software.

Two types of electrochemical measurements were used throughout this work: cyclic voltammetry (CV) and chronoamperometry, which is also known as controlled potential coulometry. Cyclic voltammetry ramps electric potential linearly versus time recording current as it sweeps until it reaches a vertex potential and proceeds back toward the starting potential. Sweeping from positive to negative potentials drives a reduction from ferricyanide to ferrocyanide and the opposite direction oxidizes the ferrocyanide back to ferricyanide. Oxidation of ferrocyanide to ferricyanide will also occur spontaneously as the 3+ oxidation state is energetically favored. In chronoamperometry, a constant electric potential is applied to the solution, which measures current over time. Eventually, chronoamperometry will drive the complete oxidation or reduction of an electroactive species if performed for a sufficient amount of time. Due to this behavior, solutions should be freshly made every few measurements to prevent a constant decrease in observed current.

The working electrode was polished with 0.03 µm alumina (Buehler), sonicated for 10 seconds, rinsed with 18 MΩ water, then dried under a stream of nitrogen. Once cleaned, the platinum electrode was coated Layer-by-Layer with PDADMA/PSS to a desired thickness and membrane current is determined using the electroactive species ferricyanide, $\text{Fe}_3(\text{CN})_6^{3-}$. PEMUs were built by manual alternation of the working electrode at 1000 rpm into each polyelectrolyte and water rinse solutions. Prior to any electrochemical measurements, the ferricyanide solution was purged of oxygen using argon for 15 minutes using a small tube. Following this period, the argon straw was removed from solution and placed in the gap between the solution surface and the teflon cap to maintain an argon blanket to prevent oxygen from dissolving back into solution.
2.9 Radiochemical Measurements

For determining the existence of extrinsic sites within a PEMU or PEC, radioactive isotopes, or radionuclides, can be used to dope the material and exchange with unlabeled counterions. Following a sufficient doping period, the polyelectrolyte multilayer or complex can be placed onto scintillation plastic, which emits photons of light when excited by ionizing radiation. This scintillation plastic is placed on a scintillation counter, which detects and measures ionizing radiation as measured by a photomultiplier tube that converts light into electrons. Radiation exists in numerous forms: alpha emission ($\alpha$), beta emission ($\beta^-$), positron emission ($\beta^+$), electron capture (EC), and gamma radiation ($\gamma$). For our purposes, radiolabeled iodide ($^{125}\text{I}$) ($t_{0.5} = 59.43$ days, electron capture) and sodium ($^{22}\text{Na}^+$) ($t_{0.5} = 2.6$ years, electron capture with $\beta^+$ and gamma emission) were used to probe spun coacervate films and polyelectrolyte multilayers used for electrochemical studies. Films were soaked in radiolabeled counterions to determine the extent of charge overcompensation by PDADMAC or PSS within the bulk. The details of these studies will be discussed in Chapters 3 and 5.

2.10 Mechanical Methods

2.10.1 Uniaxial Tensile Testing\(^{174}\)

Polyelectrolyte coacervate thin films were cut into thin strips for uniaxial tensile strength measurements on a Thümler Gmbh TH2730 tensile testing machine as shown in Figure 2.10. The ends of the film strips were clasped into homemade plastic grips of the uniaxial tensile machine and stretched to measure its mechanical properties. A glass water jacket could be placed around the entire apparatus for mechanical testing in aqueous conditions. The base of the instrument was a static clasp and the partner clasp was mounted on a load cell that operated in a vertical axis which could be used for tension or compression measurements. Plastic screws were used to tighten the grip of the plastic holders around the sample. For testing coacervate films, the machine was equipped with a 3 kN load cell and strained to 2% coacervate film length at a rate of 7 mm min\(^{-1}\). A strain of 2% was chosen for the PDADMA/PSS film to ensure a viscoelastic response that would avoid irreparable viscoplastic deformation. Films were made
using 1.7 M KBr as they produced sufficiently thick films for removal from their substrate for mechanical testing. Two types of tests were performed on films cut at dimensions of 14 mm x 3 mm x 10 µm: stress relaxation and stress-strain.

**Figure 2.10** Image of the Thümler Gmbh TH2730 tensile testing machine equipped with a 3 kN load cell.

A stress relaxation test was performed to determine the equilibrium modulus of the film by instantaneously stretching to a defined final length where it was held in place while a decrease in force over time was recorded due to molecular relaxations. The other measurement, a stress-
strain test, was performed on another cut film, where the sample was stretched at a constant rate until the point of film failure.

\[ \sigma = \frac{F}{A_0} \quad (2.5) \]

\[ \varepsilon = \frac{L-L_0}{L_0} \quad (2.6) \]

\[ E = \frac{\sigma}{\varepsilon} \quad (2.7) \]

Stress, \( \sigma \), is defined as the applied force, \( F \), per initial cross sectional area, \( A_0 \), and strain, \( \varepsilon \), is the change in length, \( L \), divided by the original sample length, \( L_0 \). The elastic or Young’s modulus, \( E \), is stress divided by strain. Young’s modulus is an important parameter when determining the mechanical properties of a material because it describes the resistance of a material to deform under an applied stress. This property is also referred to as the equilibrium modulus as determined by stress relaxation experiments.

In the event that deformation does not elicit a significant change in sample area, true stress, \( \sigma' \), and true strain, \( \varepsilon' \), should be used to produce a stress strain curve, which accounts for the small change in instantaneous area, \( A \), during deformation as shown in the below equations.

\[ \sigma' = \frac{F}{A} = (1 + \varepsilon) \times \sigma \quad (2.8) \]

\[ \varepsilon' = \ln \left( \frac{L}{L_0} \right) \quad (2.9) \]
CHAPTER 3

SPIN-COATED POLYELECTROLYTE COACERVATE FILMS

3.1 Introduction

Polyelectrolyte complexes (PECs) in the form of a Layer-by-Layer assembly such as polyelectrolyte multilayers (PEMUs) or as a stochiometric bulk complex have been used for a wide variety of materials such as cartilage mimics,\textsuperscript{98} antifouling coatings,\textsuperscript{146, 175} extruded fibers and tapes,\textsuperscript{106} lenses,\textsuperscript{23} and tissue engineering.\textsuperscript{176} Bulk complex, which is driven by the direct mixing of positive, $Pol^+$, and negative, $Pol^-$, polyelectrolytes precipitates as an opaque white solid with fibrous properties. These oppositely charged chains are electrostatically attracted, but their complexation is entropically driven by the release of small counterions and waters of hydration. This complexation process can be reversed through the addition of salt molecules, which can intercalate throughout the bulk, breaking ion pair crosslinks and dissociating the solid. This property of forming and breaking of ionic crosslinks between chains can be defined as intrinsic or extrinsic compensation as described by Equilibrium 3.1.\textsuperscript{58, 134} When the charge of one polyelectrolyte chain is associated with an opposite charge on a different polyelectrolyte chain, intrinsic compensation takes place. When the charge on the polyelectrolyte is associated with a small counterion of opposite charge, this is called extrinsic compensation.

\begin{equation}
Pol^+Pol_m^- + Cl_{aq}^- + Na_{aq}^+ \leftrightarrow Pol^+Cl_{aq}^- + Pol^-Na_{aq}^+ \tag{3.1}
\end{equation}

There exists a vast array of polyelectrolytes,\textsuperscript{177} both natural and synthetic, that can exhibit positive or negative charges, varying levels of hydrophobicity, behave differently based on pH,\textsuperscript{178-180} and either allow biological attachment or prevent it.\textsuperscript{142, 181-182} With this variety comes seemingly innumerable applications in the fields of membranes,\textsuperscript{75, 80, 183-188} nanocomposites,\textsuperscript{189-191} and biological applications.\textsuperscript{74, 98, 149} When prepared by the LbL method, PECs in the form of PEMUs produce uniformly thin films through polyelectrolyte solution alternation and rinsing steps,\textsuperscript{31, 61} although there have been “fuzzy” multilayers observed previously.\textsuperscript{30} It was determined by Salloum et al.\textsuperscript{140} that the surface properties of PEMUs determine the nature of
biological attachment rather than the bulk of the material. The combination of surface properties and mechanical behavior determine how a PEMU or PEC with interact with its nearby environment.

Production of polyelectrolyte thin films occurs over a wide range of times depending on the concentration and types of polyelectrolytes used, pH, and salt concentration.\textsuperscript{66, 178, 192-193} There are two distinct buildup regimes for LbL constructed PEMUs: linear and exponential. The nature of these buildup regimes depends on the ability of polyelectrolytes to penetrate past the surface of the film and into the bulk, which occurs more quickly for weak polyelectrolytes than strong polyelectrolytes.\textsuperscript{41} PEMUs grown entirely in the exponential regime can produce films of micrometer thickness after a few bilayers. Other combinations of polyelectrolytes can only grow within the linear regime because of their stiff conformation due to them being used below their glass transition temperature, T\textsubscript{g}.\textsuperscript{194}

In order to increase layer-by-layer production of PEMUs, several methods were adopted such as hydrodynamic LbL,\textsuperscript{195-196} spin coating,\textsuperscript{197} alternate solution spraying,\textsuperscript{192, 198-200} and combinations of spraying and spinning.\textsuperscript{201} All of these methods were found to produce high-quality PEMUs with shorter processing times than traditional LbL.\textsuperscript{195, 197, 202-204} Simultaneous spraying of polyelectrolytes has been previously demonstrated, but must be carefully controlled to yield a stoichiometric film.\textsuperscript{199, 205} Maintaining a stoichiometrically equivalent film of polyelectrolytes was assumed to be controlled by the self-assembling nature of alternating absorption, but has been shown to fail after a certain number of layers.\textsuperscript{30} Due to the difference in mobility between polyanions and polycations,\textsuperscript{52} an overcompensation by one of the polyelectrolytes creates a layer of excess charge within the bulk of the film.\textsuperscript{134} This overcompensation within the film has been shown to produce inhomogeneities within the film that affect membrane transport and mechanical properties.

Michaels et al.\textsuperscript{88, 206} demonstrated produced methods to dissolve polyelectrolyte complexes in strong ternary mixtures of water, salt, and organic solvents. Using standard polymer film casting techniques, the decoupled chains of polyelectrolytes could be cast onto thin plates to produce thin films. Despite the film-forming process being slow in nature, it was proven to be a successful method in developing films with thicknesses greater than a micron, which proved useful for applications that required free-standing membranes. These PEC membranes were used in the desalination of water by reverse osmosis.\textsuperscript{207}
Bungenberg de Jong and Kruyt\textsuperscript{110} in 1929 described the behavior of complex coacervation during their investigation of biocolloidal systems.\textsuperscript{129} This work sparked interest into these materials; In turn, this motivated our studies into polyelectrolyte coacervates (PECOVs) which bridge the gap between PECs and polyelectrolyte solutions that are composed of a concentrated phase of polyelectrolytes and a dilute phase of polyelectrolytes.\textsuperscript{208-211} The polyelectrolyte-rich phase contains weakly-bound polyelectrolyte chains which are hydrated to the point that they behave as fluids, while retaining enough chain interactions to exhibit an elastic nature.\textsuperscript{211} The remaining physical interactions within the coacervate phase are a combination of ionic pair crosslinks and classical polymer entanglements.\textsuperscript{135} The properties of our PECOVs can be controlled by the concentration of KBr in accordance to Equation 3.1. PDADMAC and PSS were chosen as the constituent polyelectrolytes for these studies as their properties and behavior are well understood.\textsuperscript{98, 106, 212} As KBr is added to PEC, the material transitions from a tough, opaque white solid to a translucent orange hydrogel, which eventually dissolves completely into a solution of polyelectrolytes.\textsuperscript{135} There is only a narrow range of KBr concentrations (1.4-1.8 M), where the coacervate phase exists.

The objective of this work is to use the liquid-like behavior of polyelectrolyte coacervate to produce thin films of PDADMA/PSS on a substrate via spin-coating. The use of spin-coating for the production of thin films with >1 micron thickness is widely known.\textsuperscript{213} Production of semiconducting materials in chip processing requires spin-coating of polymers. This work demonstrates the ability of PECOVs to produce thin films, albeit accompanied by some counterintuitive properties, by spin-coating that were able to be removed from their substrate as free-standing membranes. We found that using PECOVs to spin thin films was able to produce thick (>10 µm) stoichiometric films at a significantly faster rate than previous strategies such as LbL, spray deposition, and spin-assisted multilayering.

3.2 Experimental Section

3.2.1 Materials

Poly(diallyldimethylammonium chloride) (PDADMAC; Ondeo-Nalco, SD 46104; MW 400000 g mol\textsuperscript{-1}), poly(4-styrenesulfonic acid, sodium salt) (PSS; AkzoNobel, VERSA TL130;
MW 200000 g mol$^{-1}$), and potassium bromide (KBr; Sigma-Aldrich) were used as received from the manufacturer. Poly(ethersulfone) (PES) membranes (25-mm-diameter Supor-100 0.1 μm filters, 130 μm thick) were used as received from PALL Life Sciences. All salt solutions were prepared using 18 MΩ deionized water (Barnstead E-pure).

3.2.2 PEC Preparation

PECs were produced by the simultaneous mixing of 1.0 L aqueous solutions of 0.125 M PDADMAC and PSS each in 0.25 M NaCl with stirring for 30 min.$^{106}$ After the white precipitate was formed, the excess salt/water mixture was decanted and the precipitate hand-squeezed to remove as much retained liquid as possible. The remaining fibrous white aggregate was broken into pieces of ~1 cm across and washed with copious amounts of water for 3 days to remove all remaining NaCl. The PEC was then dried in an oven at 120 °C overnight. The dried complex was a tough translucent orange solid, which was then ground to a fine powder in a coffee grinder.

3.2.3 PECOV Preparation

Following an earlier protocol,$^{135}$ 1.5 g of a dry PEC powder was added to a 25 mL scintillation vial with a desired amount of 2.5 M KBr depending on the final KBr concentration. After a few days when the complex has dissolved, 18 MΩ water was added to a total volume of 15 mL to re-form the coacervate. This “backwards” method was preferred to adding PEC to the desired [KBr], which takes significantly longer to produce coacervate. KBr was chosen over NaCl as the salt to make coacervates because of its ability to dope the complex more efficiently. Mixtures containing [KBr] ≥ 1.8 M were in the solution phase, while 1.4–1.8 M KBr provided the coacervate phase.$^{135}$ All experiments were performed at room temperature.

3.2.4 Spin Coating

Thin films of PECOVs were prepared using a Chemat KW-4A spin coater. Aliquots of 250 μL were dispensed onto 18-mm-diameter glass coverslips prior to acceleration to 1000–6000
rpm for 5–60 s for spreading to create a range of films with varying thickness and roughness profiles. For the production of removable intact films, coacervates were spun onto 18-mm-diameter mirror-polished aluminum disks treated with 0.1 M NaOH for 5 s to render them more wettable. Each substrate-bound film was then removed from the spin coater and rinsed in 18 MΩ water three times for 1 min each to extract KBr.

3.2.5 Profilometry

A Tencor Alpha-Step 200 profilometer was used to measure the thickness of the spun films. A 5 μm stylus scanned across the surface of the film at a rate of 10 μm s\(^{-1}\) for 40 s, yielding a line profile of the sample with a z-resolution of 5 nm. To measure the film thickness, the film was etched down to the substrate using a single-sided razor blade. Thickness measurements were collected every 2 mm from the edge of an 18-mm glass disk, with a scratch exactly in the center for a total of five thickness measurements per sample using this step-edge method.

3.2.6 Radiolabeling

A released PECOV film made from 1.7 M KBr was soaked in 0.2 M NaCl for 18 h and then washed and stored in 18 MΩ water for 2 h. The film was then dried and placed in a \(^{125}\text{I}^-\) solution (1.25 Ci mol\(^{-1}\), 10\(^{-3}\) M) to measure the anion content or \(^{22}\text{Na}^+\) (4.5 Ci mol\(^{-1}\), 10\(^{-4}\) M) to measure the sodium (Na\(^+\)) content, for 2 h. After rinsing and drying, scintillation counting was performed using a plastic scintillator and a photomultiplier tube as described previously.\(^{41}\)

3.2.7 Atomic Force Microscopy

The surface topography of spun PEC films was acquired using a MFP-3D atomic force microscope (Asylum Research Inc., Santa Barbara, CA), equipped with an ARC2 controller, IgorPro software, and silicon AC240-TS probes (Olympus; radius = 9 ± 2 nm, height = 14 ± 2 μm on aluminum-coated cantilevers with a spring constant of 2 N m\(^{-1}\)). The alternating-current mode was employed to determine the topography of PEC films made with a range of spin
parameters. The cantilever was tuned to 10% below its resonance frequency with scan sizes of 5 × 5, 10 × 10, and 20 × 20 μm at a scan rate of 1.0 Hz. The root-mean-square (rms) roughness of the surface was collected on four 5 × 5 μm areas on each sample and averaged.

3.2.8 Scanning Electron Microscopy

A JSM-7401f ultra high resolution (resolution 1.5 nm at 1 kV) field-emission scanning electron microscope equipped with a strongly excited low-aberration conical lens and cold-field tungsten single-crystal emitter was used to image the surface of PEC films spun from 1.7 and 1.9 M KBr at 3000 rpm for 10, 15, and 20 s.

3.2.9 Mechanical Tests

Coacervate from 1.7 M KBr was spun onto 1-in.-diameter aluminum-foil-covered silica disks at 3000 rpm. These larger samples were removed with a brief soak in 0.1 M NaOH. Rectangles of dimensions 14 mm × 3 mm × 10 μm were cut and mounted on a Thümler TH2730 tensile testing machine equipped with a 3 kN load cell and strained to 2% at a rate of 7 mm min⁻¹.

3.3 Results and Discussion

3.3.1 Dish vs. Dome Morphology

Spin-coating is commonly used for the production of thin films on a substrate.²¹³⁻²¹⁴ For our work, water is used in place of organic solvent, which provides a “green” processing method in the fabrication of these membranes. There are four distinct steps during the spin-coating process that define the formation of thin films: dispensing, where an aliquot of coacervate is added to a static substrate, “spin up”, where the substrate is accelerated to operational velocity, “spin out”, where coacervate is spread across the substrate surface and any excess material is thrown off the substrate, and evaporation, where any residual solvent left within the film dries out.²¹³⁻²¹⁴ Evaporation occurs across all stages of spin-coating but is primarily defined as the
final step. Emslie et al.\textsuperscript{215} approximated the thickness of spun films in the event of no evaporation at distance $r_0$ from the center of the disk as seen in Equation \ref{eq:3.2}.

$$h = \frac{h_0}{(1 + 4K h_0^2 t)^{0.5}}$$

\begin{equation}
\text{(3.2)}
\end{equation}

Where $h$ is the thickness, $h_0$ is the thickness of the initial fluid layer, $t$ is the spin time, $\omega$ is the angular velocity, and $\eta$ is the viscosity. The initial thickness $h_0$ is independent of $r_0$. This model shows that thicker films thin much more quickly than do films that are already thin in nature, yielding uniform properties. Thickness of the film increases with increasing viscosity and decreases as spin time increases. For a polymer solution that has an ever-changing viscosity during spinning and thinning, including evaporation requires significantly more complex prediction models.

Obtaining high-quality films was difficult using standard spin-coating techniques for polymers in volatile solvent due to the unique behavior of polyelectrolyte coacervate. For example, using poly(methylmethacrylate) (PMMA), more commonly known as Plexiglas, in toluene can produce thin, uniform films when spun at 3000 rpm for 60 seconds.\textsuperscript{214} Using these same conditions, a drastically uneven coacervate film was produced. Due to the saloplastic nature of PECOVs, there exists a specific set of spin times and speeds to achieve uniformly thick films depending on the coacervate viscosity. Any time shorter than required produced a “dome” morphology, which had a thick central area and thin edges, whereas longer spin times produced “dish” morphologies where the middle became significantly thinner than the edges as seen in Figure 3.1. These properties were also seen when maintaining spin time but raising or lowering the spin speed, which showed a dish at high speeds and a dome at low speeds.

The most effective way to reach uniform thickness for PECOV films was to rapidly increase spin speed to the desired rate and abruptly halt without spin down, which could immediately by glassified upon immersion in water to remove any residual salt. In accordance with Equation 3.2, thickness did not decrease significantly with increased spin time. Thicker
films showed the dome morphology, meaning that thinning time was insufficient to produce a uniform film. Increasing thinning time too much resulted in the dish morphology.

![Graph showing dome versus dish morphology of spun PECOV films.](image)

**Figure 3.1** Dome versus dish morphology of spun PECOV films. PDADMA/PSS coacervates in 1.7 M KBr were spun on 18 mm glass coverslips at 5000 rpm for (●) 5 s, (■) 10 s, (▲) 20 s, (△) 30 s, (◇) 45 s, and (〇) 60 s.

After the film was produced, all excess KBr was rinsed from the film, which transformed it into a glassy PEC.\textsuperscript{135} Using the diffusion coefficient of $8 \times 10^{-7}$ cm$^2$ s$^{-1}$ obtained from NaBr in PDADMA/PSS using $\Delta = (2Dt)^{0.5}$, it was determined that a film with a thickness of 10 µm would lose all of its salt ions within 0.6 s.\textsuperscript{134}

### 3.3.2 Effect of [KBr] on Morphology of PECOV Spun Films

As stated earlier, [KBr] plays a major role in the physical properties of PECOVs. The coacervate region of PECs exists between 1.4 and 1.8 M KBr.\textsuperscript{135} Although many different polyelectrolytes could be combined to produce coacervate, the identity of the salt and its concentration must be tailored to each specific pair. For PECOVs of PDADMA/PSS, once the [KBr] is increased past 1.80 M, the coacervate breaks down and a solution of dissolved
polyelectrolyte chains is produced. Due to the critical overlap concentration property of polymers in solution, the viscosity (50-100 cP) is still significantly higher than that of pure water (1 cP).

\[ \eta \ (cP) = \frac{1.5 \times 10^9}{[KBr]^{28}} \]  

(3.3)

The viscosity, \( \eta \), of polyelectrolyte coacervates is highly dependent on salt concentration, where a low concentration displays “sticky” ion pair interactions between polyelectrolyte chains which increases viscosity represented by Equation 3.3. Small increases in [KBr] drive significant decreases in viscosity, meaning the coacervate becomes less viscous as salt plays a larger role during water evaporation. For PDADMA/PSS PECOVs at room temperature, the viscosity is a strong nonlinear function of [KBr] from 1.3-1.8 M as seen in Figure 3.2, which represents data adapted from reference 131.

Figure 3.2 Viscosity (mPa s) versus [KBr] (M) at room temperature for a coacervate of PSS/PDADMA in 1.25 – 1.78 M KBr (data from reference 131).
As water evaporates from the film, PECOV spreads more freely, which increased the rate of formation for the dish morphology.

The unique behavior of PECOVs during spinning is further described by Figure 3.3, where a direct comparison of the behavior between 1.7 and 1.9 M KBr films is illustrated. As stated earlier, once the concentration of KBr surpasses the 1.80 M boundary, all polyelectrolyte chains become fully dissolved and create a freely-flowing solution.

Figure 3.3 Thickness comparison of spun films made from (A) 1.7 M KBr coacervate and (B) 1.9 M KBr polyelectrolyte solution as a function of spin speed and time.
Thickness of 1.7 M KBr films shows little dependence on time with regards to thickness within the same spin speed, but drops quickly once rpm is drastically increased. Viscosity of 1.7 M KBr (300 cP)\textsuperscript{135} plays an important role in this behavior, showing that ion pair interactions between oppositely charged polyelectrolyte chains are still abundant and prevent rapid spreading of material. Once the [KBr] is increased to 1.9 M, which has a significantly lower viscosity at 70 cP,\textsuperscript{135} polyelectrolyte chains were allowed to freely flow past one another yielding film thickness values below 1 µm. Figure 3.4 represents the data from figure 3.3 in a two-dimensional format and compares them with theoretical values, which further demonstrates the confounding behavior of spun PECOV films.

![Graphs showing film thickness vs. spin speed](image)

**Figure 3.4** 2-D Representation of (A) 1.7 M KBr coacervate, (B) 1.9 M KBr polyelectrolyte solution, (C) theoretical model of 1.7 M KBr coacervate, and (D) 1.9 M KBr polyelectrolyte solution films spun at various spin speeds and times. The model used to determine theoretical thickness does not account for the constantly-changing dynamic viscosity of coacervate, resulting in the discrepancy between result and theory.
Most films deposited by the LbL technique display surface roughness, which wouldn’t significantly impact membrane permeability, but could have negative implications for applications in preventing biofouling and could also prevent their use in pattern formation, which requires smooth surfaces. For PDADMA/PSS films built via solution alternation, roughness is directly proportional to thickness with a rms roughness on the order of 20% of the film’s thickness. Using the concept behind surface topography for swollen gels, it can be argued that roughness is a direct result from large volume changes caused by increased inclusion of water within the film, which is dependent on the terminal layer. Due to the high extent of hydration within PDADMA/PSS PECOVs (>60% by weight), it is assumed that significant volume change would take place as salt and water are removed from the film. Using optical microscopy, there is no significant change in surface topography for films spun from 1.7-2.1 M KBr as seen in Figure 3.5, suggesting that this behavior is not observed for spun PECOV films.

**Figure 3.5** Optical microscope images of films spun from 1.7-2.1 M KBr at 3000 rpm for 10 seconds as represented by A-E respectively.

In order to confirm this topological behavior more quantitatively, atomic force microscopy (AFM) was employed to measure surface roughness. Rinsed and dried films of 1.7
and 1.9 M KBr spun films produced at 3000 rpm for 10 seconds were analyzed via AFM on scales of 20 x 20 nm and 5 x 5 nm as displayed in Figure 3.6. For 1.7 M KBr spun films, the surface roughness was found to be 16 and 4 nm respectively. The increased salt content of 1.9 M KBr films showed similar roughness at the 20 x 20 nm scale, but a rougher surface at 5 x 5 nm with values of 13 and 8 nm respectively.

Figure 3.6 AFM images of (A) 1.7 M KBr coacervate and (B) 1.9 M KBr polyelectrolyte solution at scales of 20 x 20 nm (left) and 5 x 5 nm (right). The rms roughness for each film was 16 and 4 nm for 1.7 M KBr and 13 and 8 nm for 1.9 M KBr with respect to the two XY ranges.

While unexpected, these films displayed roughness values less than 0.1% of their thickness, meaning that the spin-coating method produced exceptionally smooth films. This behavior was surprising given previous studies performed on thick LbL assembled PDADMA/PSS PEMUs. One theory behind the unusually smooth nature of PECOV spun films is that KBr is able to continually plasticize the film upon immersion in water to remove excess salt.

In order to increase our understanding of the surface topography of these spun films, 1.7 M and 1.9 M KBr films were spun under the same conditions for analysis via scanning electron microscopy (SEM) as seen in figure 3.7. Interestingly, as spin time was increased, roughness
also increased. This process is counterintuitive to the fact that centripetal acceleration is usually the driving factor behind film smoothing.

**Figure 3.7** SEM images of PECOV films from (A) 1.7 M KBr coacervate and (B) 1.9 M KBr polyelectrolyte solution at a scale of 20 x 20 µm. As spin times increase from 10-20 seconds, distinct changes are observed in surface texture. The evaporation of water is thought to drive microphase separation, which induces the increased roughness.

The texturing of spun films as spin time increases is consistent with previous observations of microphase separation in coacervate systems. For our system, small variations in salt concentration or temperature from the equilibrium conditions of the coacervate can cause microphase separation as the material adjusts to new conditions within the phase diagram.

Fourier Transform Infrared Spectroscopy was used to confirm the composition of PECOV films spun on double-side-polished silicon wafers. Figure 3.8 shows the comparison between a 570 nm 40-layer stoichiometric PDADMA/PSS film produced by alternate adsorption of polyelectrolytes and 690 nm spun film of 1.9 M KBr polyelectrolyte solution.
Figure 3.8 Comparison of film composition between (A) PDADMA/PSS spun film in 1.9 M KBr and (B) a 40-layer stoichiometric PDADMA/PSS PEMU. The (C) difference spectrum shows no significant variation between the composition of each film.

Subtraction of the spectra, corrected for small variation in thickness, from the spun film and the PEMU shows no residual signal from PSS (1008 and 1033 cm\(^{-1}\)) and PDADMA (1475 cm\(^{-1}\)) meaning that there is an equivalent amount of each polyelectrolyte for both methods of production. Films made from 1.7 M KBr were too thick for FT-IR measurement and produced absorbance values outside the linear response of absorbance to thickness in accordance with Beer’s Law.

3.3.3 Free-Standing PECOV Thin Films

While the spectra in Figure 3.8 seem to represent stoichiometrically equivalent films, this method is inaccurate within 5% and cannot completely exclude the presence of small counterions within the film. The presence of these ions may affect the ability of these films to be used as ion

58
selective membranes due to fixed extrinsic sites that can negatively impact selectivity and permeability of the membrane. To quantitatively determine the extent of these extrinsic sites, spun PEC was radiolabeled using $^{125}$I and $^{22}$Na$^+$ in free-standing spun films made using 1.7 M KBr coacervate.

Mirror-polished 18 mm aluminum disks were used as the substrates to produce removable thin films of polyelectrolyte coacervates. Due to the ultra-smooth nature of the aluminum disks (alloy 6061), 0.1 M NaOH was used to etch the surface of the aluminum to allow better wettability of the surface. This brief processing step allowed PECOV to adhere to the surface more substantially and provided ideal conditions for the production of thin films. Once spun, the PECOV-coated aluminum disks were rinsed in DI water, allowed to dry, then immersed in 0.1 M NaOH to aid in the removal of the spun films. The etching of aluminum by NaOH produces bubbles of hydrogen that release the film by floating it to the surface of solution. Once removed, the aluminum disk is quickly immersed in DI water to prevent any further surface damage. NaOH had no effect on the released film.

Once released, the 3.5 mg membranes were rinsed in either $^{125}$I or $^{22}$Na$^+$ to determine ion content through exchange of unlabeled counterions with their radiolabeled counterparts. Using the mass and density (1.27 g cm$^{-3}$) of the film, the amount of PDADMA and PSS groups were estimated to be 11.4µmol. Scintillation counting using $^{125}$I to probe positive extrinsic sites on PDADMA showed 1.26 x $10^{-3}$ µmol of excess PDADMA(Cl$^-$) and $^{22}$Na$^+$ showed 6.54 µmol excess of PSS(Na$^+$). These values translate into an anion content of 0.0011 mol % and a cation content of 0.57 mol % within the spun PECOV film. This further translates into a PDADMA to PSS ratio of 1.0057:1.0000, which is close to 1:1 in nature. The slight excess of PSS is consistent with the observation that PDADMA/PSS coacervate exhibits excess PSS as well.

The coacervate phase of PEC was chosen for use as free-standing this films due to its thicker nature and ease of handling. For reasons not yet understood, PECOV produced under identical conditions to Figure 3.3A on glass produced significantly thicker films on aluminum as seen in Figure 3.9. This difference in thickness may be a result of surface wettability and elastic properties of the coacervate. The thickness of the films was measured by two methods: a digital micrometer and weighing the films, calculating its thickness based on area and density. It was found that these methods produced results within 10% of each other.
Figure 3.9 Thickness profile of spun 1.7 M KBr PECOV films on a polished aluminum substrate. Al disks were used in the place of glass for the release of the PECOV films.

Figure 3.10 Released 1” diameter PDADMA/PSS PECOV film spun from 1.7 M KBr coacervate at 1000 rpm.
Figure 3.11 Stress relaxation (top) and stress-strain (bottom) curves of 14 mm x 3mm x 10 µm 1.7 M KBr spun coacervate films. Stress relaxation showed an equilibrium modulus of the film to be 1050 MPa when strained at 2% of the film length (0.28 mm) and allowed to relax over 10 minutes. The tensile strength for these films was found to be 50 MPa by stretching the film at 7 mm min\(^{-1}\) until failure.

Released PECOV films spun from 1.7 M KBr coacervate were optically transparent in nature as seen in Figure 3.10, were thick enough for use as a membrane supported by a wire mesh, and were flexible and rugged enough for handling. An example of these spun films was demonstrated upon spinning 1.7 and 1.9 M KBr samples onto porous PES, which yielded
thicknesses of 11 and 2 µm respectively. These films were cut into strips (14 mm x 3 mm x 10 µm) that were analyzed via a Thümler TH2730 tensile testing machine and found to have an equilibrium modulus of 1050 MPa and tensile strength of 50 MPa under ambient conditions (23 °C and 44% relative humidity).

In order to produce PDADMA/PSS films of this nature using traditional LbL methods in 1 M NaCl at 5 min per layer and 30 s rinse steps (~15 nm per bilayer), it would take roughly 300 hours. Using the faster spray alternation method at 30 seconds per layer would still take around 20 hours. There is no distinct structure to be expected within a spun PECOV film like may be observed in “fuzzy” assembly during multilayering.

3.4 Conclusions

Using a novel method for the production of thin films of PEC of appropriate thickness for supported membranes for separations, many previously discussed membrane applications in literature may be performed more easily. It should also be readily possible to produce defect-free PECOV films on porous and nonporous substrates by spin-coating with these coacervates. Coacervates exhibit a chain density sufficiently high enough to overcome the critical chain overlap concentration, which allows the PEC to form a thin sheet of continuous polymer that won’t break down into particles when immersed in rinsewater as salt exits the film. Coacervate formation is facilitated by the preparation of PEC by solution mixing with salt. The physical and mechanical properties depend on the types of polyelectrolytes used and identity of salt as described by the Hofmeister series. All processing and production of PECOV thin films is done in aqueous solution using small solution volumes, providing a simple and environmentally friendly route to polymer film formation.
Clinical complications can arise from the fouling of implanted devices by biomaterials, which is often driven by the surface properties of the implant. Surfaces that are improperly designed can promote the adhesion and proliferation of immune cells and macrophages that trigger an acute inflammatory response to foreign bodies. This inflammation has the ability to persist for the duration of the implant’s lifetime and cause extensive damage to the implant mechanically, which will reduce its medical effectiveness and shorten its lifespan. Along with a negative cellular response to these implants can come bacterial adhesion onto implanted prosthetics. The formation of biofilms, which are bacterial colonies protected by a tough polymeric matrix, occurs when bacteria are able to irreversibly attach to biotic or abiotic surfaces. Due to the nature of this tough polymeric coating, many bacterial colonies become highly resistant to antimicrobial agents such as antibiotics, which can cause severe complications that require direct treatment of the implant. This process requires that the patient undergo surgery to have the implant removed and creates potential for further medical complications, not to mention increased cost of care. The formations of these biofilms are primarily caused by three strains of bacteria: Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis, which are known culprits in the infections of metallic implants and intravascular catheters.

In order to decrease the likelihood of these complications, polymeric and metallic components have been subjected to a variety of surface modifications to improve surface chemistry and biocompatibility. Current FDA and ISO approved coatings include the use of plasma spraying hydroxyapatite onto biomaterials, which is a biocompatible coating that is widely used in orthopaedics and dentistry due to its ability to bond directly to bone and increase healing rate. In order to decrease cost and promote more environmentally friendly processing procedures, the use of polyelectrolyte coatings functionalized with proteins or
antibacterial components in the form of complexes and multilayers have been recently studied for use on medical devices.\(^{150, 234-237}\)

Constructing films of oppositely charged polyelectrolytes using alternate adsorption via a Layer-by-Layer (LbL) method are capable of producing biocompatible polyelectrolyte multilayers (PEMUs), which are formed by ionic crosslinks between the polyelectrolyte chains.\(^{141-142, 227}\) The formation of these PEMUs is inexpensive and ideal for coating objects that are irregularly shaped due to the alternate solution dipping method used to build the films. The nature of these PEMUs can be closely controlled through the use of different polyelectrolytes, altering ionic strength of the solution, and raising or lowering the pH, which all affect the surface behavior of the film.\(^{221}\) In order to affect the mechanical properties of the film, various functional groups can be added that allow for random chain crosslinking, which can be performed thermally, chemically, or using ultraviolet light.\(^{137, 238-240}\) The extent of crosslinking defines how much tougher the final PEMU will be based on equilibrium modulus.

One of the functional groups at our disposal for the prevention of biological fouling are zwitterions, which are hydrophilic, well hydrated molecules that exhibit both positive and negative groups resulting in a net neutral charge. Zwitterions have been shown to effectively prevent the adhesion of proteins and eukaryotic cells to surfaces,\(^{137, 241-243}\) attenuate immune responses,\(^{244-246}\) and occasionally prevent bacterial attachment and biofilm formation.\(^{247}\) Zwitterions prevent adhesion of proteins to a surface by eliminating electrostatic attraction between the protein and the substrate by releasing counterions into solution.\(^{248}\) Zwitterions also extend beyond the backbone of the PEMU, which acts as a steric boundary to adhesion. This work focuses on the use of 3-[2-(acrylamido)-ethylidimethyl ammonio] propane sulfonate (AEDAPS) as our zwitterionic group, which has previously shown to be resistant to protein and mammalian cell adhesion.\(^{140, 243}\) These studies along with others on the antifouling properties of zwitterions were the driving force behind the idea that they could be useful as antibacterial coatings.\(^{249}\)

In this work, AEDAPS functionalized films were used to determine zwitterionic effectiveness at preventing the adhesion of A7r5 rat aortic smooth muscle cells (associated with arterial stent fouling and failure)\(^{250-251}\) and 3T3 mouse fibroblast cells (associated with fibroblast encapsulation of implants)\(^{223-224, 252}\). The behavior of these cell lines was directly compared to that of Gram-negative \textit{Escherichia coli} enterobacteria, which is associated with the formation of
biofilms. There is a distinct difference in size between the mammalian cell lines and an *E. coli* bacterium as seen in Figure 4.1, which can play a large part in their adhesion mechanisms.

![Figure 4.1](image)

**Figure 4.1** Illustrative comparison of attachment of mammalian cells to *Escherichia coli* overlaid on a 5 x 5 µm AFM image of an (A) AEDAPS PEMU and (B) AEDAPS-SS PEMU. A 50 µm diameter cell is shown on (C) an AEDAPS-SS PEMU alongside *E. coli*. The bacterium was drawn to scale using measurements made on a scanning electron microscope. Scale bars = 1 µm.

All three studies were performed on PEMUs that comprised poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA), which was functionalized with benzophenone for increasing film strength and stability and AEDAPS for adhesion studies. The benzophenone group was chosen to add much needed stability to the PEMUs by random crosslinking since previous studies showed that films containing increasing amounts of zwitterion were more loosely attached. As will be discussed later, the AEDAPS-containing PEMUs performed as expected for mammalian cell adhesion studies, but proved to be a strong attractant for bacteria, forming biofilms in a rapid manner, showing an interesting contradiction. The reasons for this confounding behavior will be discussed.
4.2 Experimental Section

4.2.1 Materials

Poly(allylamine hydrochloride) (MW 56000 g mol\(^{-1}\)), poly(acrylic acid) (MW 100000 g mol\(^{-1}\), 47.2 wt% in water), sodium chloride (NaCl, 99.5%), sulfuric acid (H\(_2\)SO\(_4\)), tris(hydroxymethyl)aminomethane (Tris, C\(_4\)H\(_{11}\)NO\(_3\) ≥99%), and hydrogen peroxide (H\(_2\)O\(_2\)) were used as received from Sigma Aldrich. PAA grafted with benzophenone (PAABp, benzophenone, Bp, comprises 18 mol% of the polymer, \(n = 0.82, m = 0.18\)), PAA grafted with zwitterionic AEDAPS (PAA-co-AEDAPS, AEDAPS comprises 25 mol% of polymer, \(n = 0.75, m = 0.25\)) were synthesized as described previously.\(^{68, 140}\) All solutions were prepared using 18 MΩ deionized water (Barnstead, E-pure). Structures of polymers are shown in Figure 4.2. Double-side-polished silicon [100] wafers (100 mm) from Silicon, Inc. were cut into 22 × 22 mm squares, cleaned in “piranha” solution (70 : 30 H\(_2\)SO\(_4\) : H\(_2\)O\(_2\), caution: strong acid and oxidizer) for 15 min, rinsed thoroughly with water, then dried under a stream of N\(_2\). Microscope glass coverslips 22 × 22 × 0.17 mm (Fisherbrand Scientific cover slips no. 1) were flame cleaned for 1–2 seconds in three separate intervals.

![Structures of polyelectrolytes used for the prevention of cell and bacterial adhesion.](image)

Figure 4.2 Structures of polyelectrolytes used for the prevention of cell and bacterial adhesion.
4.2.2 Polyelectrolyte Multilayer Preparation and Nomenclature

LbL buildup of PEMUs was done as follows: Si wafers and glass coverslips were mounted onto glass microscope slides using Parafilm™ to hold the edges of the coverslips while exposing one side to solution during multilayer buildup. PEMUs were built using 10 mM (with respect to the repeat unit) PE solutions in 150 mM NaCl, 25 mM Tris, pH 7.3, with the aid of a robot (StratoSequence V, nanoStrata Inc.), which sequentially dipped the Si wafers and glass coverslips for 5 min into 50 mL of PE solutions and rinsed them for 1 min in 50 mL of 25 mM Tris, pH 7.3. After the final rinse, each PEMU was dried under N₂. No antimicrobial agents were used during the buildup of PEMUs. PEMUs were covered during and after buildup to prevent air particulate contamination. Personnel in direct contact with PEMUs used sterile laboratory practices and contamination during buildup and handling of solutions or PEMUs was extremely rare. All coated surfaces were stored dry in sterile petri dishes sealed closed with Parafilm™ to prevent contamination (storage time ranged from a few days to a month at room temperature). In the PEMU nomenclature used here, (A/B)ₙ-(A/B-co-C)ₘ indicates a multilayer containing “n” bilayers of polycation A and polyanion B, and “m” bilayers of A and copolymer B-co-C. Photocrosslinked PEMUs are denoted as (A/B)ₙ-X-(A/B-co-C)ₘ. The PEMUs used for this investigation were: (PAH/PAABp)₂-X-(PAH/PAA-co-AEDAPS)₄PAH (AEDAPS-PAH); (PAH/PAABp)₂-X-(PAH/PAA-co-AEDAPS)₄PAABp (AEDAPS-PAABp); (PAH/PAABp)₂-X-(PAH/PAA-co-AEDAPS)₄ (AEDAPS); (PAH/PAABp)₂-X-(PAH/PAA-co-AEDAPS)₄ PEMU ‘supplemental soaked’ in PAA-co-AEDAPS for an additional 4 h (AEDAPS-SS). The PEMUs containing the base layers (PAH/PAABp)₂ were photocrosslinked in a UVP CL-1000 Ultraviolet Crosslinker as described previously⁶⁸ for 15 min at 200–280 nm before addition of the terminating layer. After buildup, with or without ‘supplemental soaking’, all PEMUs were rinsed with water and dried under a N₂ stream.

4.2.3 Polyelectrolyte Multilayer Characterization

PEMUs were characterized by ellipsometry, static contact angle measurements, and Fourier transform infrared spectroscopy (FTIR). Dry thicknesses of PEMUs were determined using a Gaertner Scientific L116S Autogain ellipsometer with 632.8 nm radiation at 70° incident
angle assuming a film refractive index of 1.55. FTIR spectra of PEMUs were obtained at a resolution of 4 cm\(^{-1}\) with 100 scans using a Thermo Avatar 360 equipped with a DTGS detector. The background was determined using an uncoated (bare) Si wafer. All multilayer buildup and treatments were conducted at room temperature (23 ± 2 °C). The dry surface roughness and thickness of PEMUs were determined using an MFP-3D atomic force microscope (AFM) (Asylum Research Inc., Santa Barbara, CA) equipped with an ARC2 controller. NCHV probes from Veeco (tip radius = 10 nm, spring constant 20–80 N m\(^{-1}\)) were used at a scan rate of 0.5 Hz. Images of 20 × 20 μm and 5 × 5 μm scan ranges were collected and then analyzed using Igor Pro software. Roughness was obtained from 5 × 5 μm regions (at different positions of 20 × 20 μm images). PEMU thickness was determined by scanning the surface across a scratch made in the films and measuring the step height.

4.2.4 Mammalian Cell Culture

Rat aortic smooth muscle A7r5 cells and mouse fibroblast 3T3 cells (originally ATCC CRL-1444 and ATCC CRL-2752 respectively, both maintained through numerous passages and stored frozen in the lab over several years) were cultured on tissue culture plastic (TCP) in high glucose Dulbecco’s Modified Eagle’s Medium (DMEM) (D5648, Sigma-Aldrich) prepared from powder with sterile double distilled H2O and supplemented with 1.5 g L\(^{-1}\) NaHCO\(_3\), 10% fetal bovine serum (HyClone Standard Bovine Serum, Thermo Scientific), 10 μg mL\(^{-1}\) gentamicin (Gibco Gentamicin Reagent Solution, Invitrogen), and an antibiotic-antimycotic supplement providing final concentrations of 100 units mL\(^{-1}\) penicillin G, 100 μg mL\(^{-1}\) streptomycin, 0.25 μg mL\(^{-1}\) amphotericin (Gibco Antibiotic-Antimycotic, Invitrogen). The cells were cultured at 37 °C and 5% CO\(_2\), refed every 3 days, and subcultured when populations were 70% confluent. For adhesion analysis, cells were trypsinized off TCP plates and plated onto uncoated and PEMU-coated glass coverslips.

4.2.5 Escherichia coli Culture

The ATCC-8739 E. coli strain of biofilm-forming Gram-negative enterobacteria (NCBI taxonomy ID 481805) used for this investigation was originally purchased from American Tissue
Culture Collection (ATCC), cultured in Luria Broth (LB) media in the lab, and stored as 40% glycerol stocks at −80 °C and on LB agar plates stored at 4 °C, which were replated on a monthly basis. *E. coli* used in fluorescent adhesion analyses were transformed with pGLO plasmid (Bio-Rad Laboratories). Concentrated culture suspensions were prepared from cultures inoculated with *E. coli* from a single colony into 1 mL of LB media in 1.5 mL microcentrifuge tubes kept at 37 °C with constant shaking (200 rpm) in a Thermo Scientific MaxQ 5000 Incubated/Refrigerated Floor Shaker for 12 h. *E. coli* concentrations in cultures were determined by measuring 600 nm light scattering using a ND-1000 NanoDrop Spectrophotometer (Thermo Scientific) assuming $1 \times 10^8$ *E. coli* CFUs mL$^{-1}$/0.1 OD600 value.$^{253}$

### 4.2.6 Live Cell™ Imaging and Adhesion Analysis

To facilitate DIC imaging of live mammalian cells and bacteria on uncoated and PEMU coated coverslips, sterile 35 mm tissue culture dishes were ‘windowed’ by drilling a hole with a variable speed bench drill press fitted with a 3/4” smooth-finish wood bit. The drilled culture dishes were sterilized with 70% ethanol, washed extensively with sterile PBS, and dried before gluing a PEMU coated or uncoated coverslip over the hole. The glue was allowed to cure for at least 24 h, after which the ‘windowed’ culture dishes were washed extensively with sterile PBS to remove any particulates. Alternatively, 35 mm glass bottom dish with a 20 mm micro-well sealed with a coverslip (#1 from In Vitro Scientific) were used. Live cell imaging was conducted in a microscope-mounted LiveCell™ Chamber (Pathology Devices, Westminster, MD). During live cell recordings of mammalian cells, the chamber was maintained at 37 °C with 5% CO$_2$ input and 40% relative humidity. For live cell imaging of *E. coli* biofilm maturation, the chamber was maintained at 37 °C and 40% relative humidity, but no CO$_2$ was added into the chamber. Differences in reversible and irreversible attachment of *E. coli* under ‘near static’ conditions (some convection was caused by the influx of humidified air continuously pumped into the chamber) were recorded to analyze attachment of planktonic *E. coli* and subsequent biofilm formation during various periods after initial inoculation. Mammalian cells and *E. coli* were imaged using a Nikon TS100 microscope equipped with a Nikon Digital Sight DS-Ri1 digital camera for Phase Contrast imaging and a Nikon Ti-E inverted microscope equipped with a Nikon Intensilight C-HGFI illuminator and a Photometrics
Cool Snap HQ2 camera (Photometrics) for Differential Interference Contrast (DIC) and for Fluorescence imaging using Texas Red, DAPI, and GFP filters (Chroma Technologies Corp, EX: 560 nm, BS: 595 nm, EM: 645 nm; EX: 350 nm, BS: 400 nm, EM: 460 nm; EX: 470 nm, BS: 495 nm, EM: 525 nm). Images were analyzed and processed using NIS-Elements Advanced Research (Nikon), ImageJ (NIH), and Adobe Photoshop.

4.2.7 *Escherichia coli* Attachment and Retention Analysis

For adhesion assays, substrates were washed three times in 1 mL of phosphate buffered saline (PBS, pH 7.4) and soaked in 3 mL of PBS for 30 min at room temp before inoculating with 3 mL bacteria in LB media containing $5 \times 10^4$ *E. coli* CFUs mL$^{-1}$ or $7.7 \times 10^6$ *E. coli* CFUs mL$^{-1}$. Cultures were maintained at 37 °C under static conditions for up to 48 h to allow formation of biofilms. Surface coverage of tightly adherent *E. coli* after various times of incubation was analyzed by washing the surface with five rapid successive swirls in 1 mL PBS three times to remove non-adherent bacteria. Adherent bacteria were fixed and stained for 15 min with filtered 0.01% crystal violet prepared in PBS containing 20% methanol, destained with five rapid successive swirls in 1 mL sterile deionized H2O, and mounted with sterile gelvatol (13% v/v 1.5 M Tris, pH 8.8; 21% v/v glycerol; 10.5% w/v polyvinyl alcohol; 0.02% w/v of sodium azide, NaN3 prepared in deionized H2O and stirred on low heat for 4 h). Prepared slides were imaged with the Nikon Ti-E and analyzed for relative coverage compared to uncoated coverslips with ImageJ (NIH).

4.3 Results and Discussion

A wave of new strategies aimed at using zwitterion-functionalized synthetic polymers arose following the findings that reported the effectiveness of zwitterions at reducing nonspecific adhesion and fouling of natural surfaces. One of these these strategies involved the use of brushes grown from surfaces using controlled living radical polymerization. For example, Zhang et al. showed that poly(sulfo betaine methacrylate) brushes grafted onto surfaces were effective at preventing bacterial adhesion and biofilm formation. The use of AEDAPS as our zwitterionic group within a polyelectrolyte multilayer was done in an effort to create an
alternative method for immobilized suflobetaine groups. It has been previously demonstrated by our group that these AEDAPS-functionalized PEMUs are capable at preventing the adhesion of proteins and mammalian cell lines. One drawback to the introduction of zwitterion within the PEMU is a decrease in charged repeat units in the buildup of the film, which caused loss of material and decreased stability. In order to counteract this behavior, a photocrosslinker, benzophenone (Bp), was introduced as a copolymer in the construction of the PEMU.

**Figure 4.3** FTIR spectra of (A) (PAH/PAABp)$_2$-X-(PAH/PAA-co-AEDAPS)$_4$ PEMU and (B) constituent reference films. Six bilayers of PAH/PAA (red), PAH/PAABp (green), a cast film of PAA-co-AEDAPS (blue), and (PAH/PAABp)$_2$-X-(PAH/PAA-co-AEDAPS)$_4$ (purple) are compared via FTIR spectroscopy. The numbered boxes represent characteristic peaks within each film with a (1) carboxylic acid stretch from the PAH/PAA film, a (2) decomposed diarylketone peak from PAH/PAABp film after crosslinking, and a (3) sulfonate stretch from the cast film of PAA-co-AEDAPS.

The initial layers of the PEMU comprise PAH and PAABp, which act as the foundation for later layers containing AEDAPS. These initial layers are constructed in the absence of light to prevent premature crosslinking. Following crosslinking, the AEDAPS-functionalized PEMU is constructed. PAA grafted benzophenone, which was previously synthesized, contains 18 mol % crosslinker, which is able to create an extensive network of random attachments throughout the initial layers of the PEMU. It was not used in the remainder of the buildup due to the ability of PEMUs to interdiffuse through 3-4 bilayers as demonstrated by PEMUs comprising PAH and PSS. The final multilayer takes the form of (PAH/PAABp)$_2$-X-(PAH/PAA-co-
AEDAPS$_4$ with “X” representing the crosslinking step. The eight layer termination layer is sufficiently thick enough to ensure there is no hydrophobic benzophenone present at the surface that could interact with cells or bacteria.

Figure 4.4 Diarylketone peak reduction at 1650 cm$^{-1}$ after UV exposure of PAABp-containing PEMUs. FTIR analysis of (PAH/PAABp)$_2$-(PAH/PAA-co-AEDAPS)$_4$ PEMUs (blue) contains a diarylketone peak at 1650 cm$^{-1}$, which decreases after exposure to UV light between 200-280 nm for 15 min. The PAABp degradation produces free radicals that drive random C-C covalent bond crosslinking within the PEMU and strengthens the film creating the (PAH/PAABp)$_2$-X-(PAH/PAA-co-AEDAPS)$_4$ PEMU denoted as AEDAPS.

Using FTIR of the completed PEMU in comparison to films of its constituent components shows how each polyelectrolyte and functional group contribute to the final film as seen in Figure 4.3. When exposed to ultraviolet light, the benzophenone group produces a free radical that drives random C-C crosslinking within the PEMU. Figure 4.4 highlights this effect by demonstrating the decomposition of the diarylketone peak at 1650 cm$^{-1}$ between uncrosslinked and crosslinked PEMUs, which amounts to roughly 50% conversion of benzophenone units.
4.3.1 A7r5 and 3T3 Adhesion and Spreading on Zwitterionic Surfaces

AEDAPS functionalized PEMUs, namely PAA-co-AEDAPS (25 mol % AEDAPS), have shown that they are effective at resisting the adhesion of both extracellular matrix (ECM) protein fibronectin as well as A7r5 rat aortic smooth muscle cells (SMCs). In order to confirm these findings, 3T3 mouse fibroblast cells were used to represent a second clinically important cell line, which are also known causes of medical implant fouling. A7r5 and 3T3 cells were separately seeded onto uncoated control coverslips as well as AEDAPS coated coverslips, which are imaged in Figure 4.6. It was found that AEDAPS-directed resistance during the initial spreading and attachment stages of cell adhesion persisted for longer incubation times. Both cell types were successful in attaching to the control coverslips within 3 h of exposure to the surface and were able to increase the level of attachment and spread easily for the duration of the observation period. For AEDAPS-coated coverslips, neither the A7r5 or 3T3 cells could successfully bind to the surface and instead formed isolated, free-floating clusters in solution. Higher focus behavior of cell lines can be seen in Figure 4.5.

Figure 4.5 Zoom in of the behavior of A7r5 rat aortic smooth muscle cells and 3T3 mouse fibroblast cells on uncoated and AEDAPS-coated glass coverslips. Scale bars = 50 µm.
Figure 4.6 A7r5 and 3T3 mammalian cell lines were seeded onto uncoated control glass coverslips and coverslips coated with AEDAPS. The cells were cultured at 37 °C in 40% relative humidity and 5% CO₂. The time of each image capture is represented at the top of the figure. Both cell lines were able to attach and spread on the control within a short time while were unable to do so on AEDAPS films where they formed free-floating clusters in solution. Scale bar = 100 µm.
The AEDAPS-coated coverslips did not exhibit direct cytotoxic behavior,\textsuperscript{141} which is mirrored by the behavior of other zwitterionic functionalities.\textsuperscript{140} It was found that after 24 hour and 48 hour exposure, of A7r5 and 3T3 cell lines respectively, to the AEDAPS-coated surface that both were able to attach to the surface of tissue culture plastic (TCP) and decluster. Figure 4.7 represents this behavior for the mouse fibroblast cell line where $1 \times 10^4$ 3T3 cells were seeded and cultured for several days under normal tissue culture conditions on an AEDAPS surface then replated onto tissue culture plastic. This behavior by each mammalian cell line is consistent with previous studies that display the effect of zwitterionic coatings on animal cells.\textsuperscript{140, 242-243, 246, 258} Despite the non-cytotoxic nature of the AEDAPS coatings, A7r5 cells underwent apoptosis after prolonged exposure to the zwiterionic surface, which is due to the highly adhesion dependent nature of that specific cell line.\textsuperscript{250, 259-260}

\textbf{Figure 4.7} Cluster-forming behavior of 3T3 mouse fibroblast cells at (A) 24 hours and (B) 48 hours on an AEDAPS coated surface. After 48 hours, the 3T3 cells were replated onto tissue culture plastic represented in (C). DIC microscopy was used for imaging at 24 hours and images B and C were captured using phase contrast microscopy. Scale bar = 100 µm.

\textbf{4.3.2 Escherichia coli Adhesion on Zwitterionic Surfaces}

Adhesion of biofilm-forming bacteria onto a substrate depends on several different variables such as the amount and composition of extracellular polymeric substances (EPS) that surround the bacterium, which is dependent on growth conditions.\textsuperscript{253, 261-262}
Figure 4.8 Images of *E. coli* attachment to sterile uncoated coverslip control, AEDAPS coated glass coverslip, and AEDAPS-SS coated glass coverslip. The SS represents added zwitterion into the PEMU through increased PEMU soaking time, which displayed increased affinity for bacterial attachment and biofilm formation. The white arrows indicate initial attachment points of bacteria. Scale bar = 50 µm.
Prior to inoculation of bacteria onto test surfaces, *E. coli* was grown to a stationary phase where the extent and composition of EPS production was sufficient for attachment and biofilm formation. This culturing step was necessary to reproduce conditions in which bacteria irreversibly attach to form biofilms on medical devices with serious clinical consequences.

In order to determine if our AEDAPS surfaces were effective at preventing the formation of *E. coli* based biofilms, sterile uncoated glass coverslip controls and AEDAPS PEMUs were inoculated with $5 \times 10^4$ colony forming units per milliliter (CFUs mL$^{-1}$). In static conditions, or conditions where there was no solution flow, *E. coli* grew as clusters and strands, which was recorded by DIC microscopy for 15 h represented in Figure 4.8. Between 2-5.5 h, images of the bacteria were collected every 15 minutes, where the first image was colored blue, red represented 15 minutes later, and green for a time passage of 30 minutes, which were later merged together to show immobilization. An overlap of blue and red time points is presented as magenta, which describes bacterial attachment within the first 15 minutes. Yellow coloring represents attachment of bacteria after the next 15 minutes (overlap of red and green) and white represents immobilization of *E. coli* for the entire 30 minute time period. The nature of this attachment wasn’t observed even after 5.5 hours on the glass coverslip control demonstrating the bacteria attractant nature of AEDAPS.

Attachment of *E. coli* occurred irreversibly for the AEDAPS PEMU, which served as motivation to increase zwitterionic content for the PEMU. The FTIR absorption bands at 1050 and 1200 cm$^{-1}$ demonstrate the increases in zwitterionic content with time up to 16 hours as extra AEDAPS was added to the film by soaking (AEDAPS-SS) as displayed in Figure 4.9. Interestingly, the attachment was expedited by 2 hours for the AEDAPS-SS multilayer, which can be observed in Figure 4.8. The extent of AEDAPS that could be added to the PEMU reached a plateau, which tapered off and showed a decrease at 16 hours, suggesting that the added polyelectrolyte was loosely bound to the film. Adding in extra AEDAPS produced another consequence in the form of added thickness and noticeable changes in dry surface topography, but had no significant effect on the wettability of the surface as determined by static contact angle measurements as shown in Table 4.1. Increasing the amount of bacteria in solution showed little difference in the rate of adhesion between AEDAPS and AEDAPS-SS PEMUs up to 30 min, but once the bacterial content was decreased, far more colonies adhered sooner and more prolifically to the supplementary soaked PEMU. The loosely-bound nature of
supplementarily soaked AEDAPS-SS PEMUs showed less bacterial adhesion after 24 hours, which can be described by a bacteria “trapping” nature of the terminating layer. It is believed that this layer was able to delaminate from the film taking bacterial colonies with it, which is reflected by loss of polymer at long soaking times as shown in Figure 4.9.

Figure 4.9 AEDAPS PEMU was soaked in PAA-co-AEDAPS for various time points up to 16 hours to increase zwitterionic content as measured by (A) FTIR, which produced a loosely-bound layer that could desorb after sufficient time. Observing the sulfonate stretch peak at 1200 cm\(^{-1}\) demonstrated a loss in polymer after 16 hours (B).

Bacterial adhesion and biofilm formation occur at a variety of exposure conditions as seen in previous work. Usually, these adhesion behaviors are only observed during initial attachment stages by short seeding times followed by a wash step. From a clinical standpoint, however, this is not sufficient to study biofilm formation and propagation, which is the leading cause of implant failures and infections.
Figure 4.10 Time dependent surface coverage of *Escherichia coli* determined by pGLO green fluorescent protein. High bacterial concentrations (2.3 x 10⁷ CFUs) at 5 and 30 minutes (C and D) were compared to low concentrations (1.5 x 10⁵ CFUs) at longer exposure times (A and B). All bacterial colonies remain following shaking rinse step, meaning they are tightly bound to the surface. DIC microscopy and fluorescent imaging were combined to produce B and D (Scale bar = 100 µm) and the extent of surface coverage was calculated using ImageJ. Total surface area covered by bacteria was determined over two trials for ten different surface areas of 0.15 mm². *P* values of <0.05 compared to average surface coverage percentage on tested surfaces are indicated with * in A and C.

In this study, we used a flame-cleaned coverslip control, an AEDAPS PEMU, and its soaked counterpart PEMU of AEDAPS-SS to investigate short and long term adhesion properties of bacteria on a zwitterion functionalized polymer film. Once prepared, these surfaces were exposed to two concentrations of bacteria, 1.5 x 10⁵ CFUs (low) and 2.3 x 10⁷ CFUs (high), and...
cultured for 30 minutes (short) up to 48 hours (long) under static conditions. For imaging purposes, the \textit{E. coli} used were transformed to express pGLO green fluorescent protein. Wash solution swirling was administered prior to imaging to remove any loosely bound bacteria. Surface coverage percentage by \textit{E. coli} can be seen in Figure 4.10.

As expected, increasing exposure time led to increased bacterial adhesion for all surfaces. The behavior of the zwitterion functionalized polymer film is contradictory to our hypothesis in that it acts as an attractant for \textit{E. coli} instead of a repellant. This behavior was only further enhanced by the addition of more AEDAPS during supplemental soaking, showing twice as much coverage after 4 hours and ten times as much coverage after 8 hours. While bacterial adhesion continued to increase for the coverslip control and AEDAPS films, the AEDAPS-SS film showed a marked decrease in bacterial adhesion after 8 hours. After 48 hours, coverage was equivalent for AEDAPS-SS films and coverslips, which was roughly half the coverage observed for the AEDAPS surfaces. High inoculation concentrations for 5 and 30 minutes displayed significantly higher contrast between the glass control surface and zwitterionic PEMUs. These time points are short to prevent bacterial reproduction, which reflects the planktonic behavior of bacterial interactions with themselves and the surfaces.

The performance of both zwitterion films after 24 hours showed higher bacterial adhesion than films comprising only PAH and PAABp. PEMUs with zwitterions in the bulk terminated by positive (PAH) or negative surface charge (PAABp) showed no measurable difference in suspended \textit{E. coli}.

### 4.3.3 Possible Attachment Mechanisms

The behavior of zwitterionic films is based on their net neutral surface charge which prevents ion pair interactions, and a strongly hydrophilic surface that offers no enthalpic gain for interactions with hydrophobic elements. The combination of these two properties should be sufficient in preventing the nonspecific adhesion and attachment of bacteria, which was recently highlighted in a review of zwitterion antifouling.\textsuperscript{248} Prior work suggested that surfaces that showed strong resistance to protein adsorption could potentially resist bacterial adhesion as well, but was shown to be only partially accurate.\textsuperscript{175, 267} This behavior was observed for a thin layer of polyethylene glycol, which showed good protein repellancy, but allowed bacterial attachment.\textsuperscript{268}
Initial attachment of bacteria to a surface depends on a variety of factors including substrate hydrophobicity, nanomorphology, chemical composition, and modulus.\textsuperscript{269-273} There are two major phases that take place in bacterial attachment; phase one is the reversible attachment of planktonic bacteria and phase two is the beginning of biofilm formation.\textsuperscript{274} The adhesion of bacteria was shown to also be zwitterion dependent by Mi et al. when zwitterions were incorporated into a brush structure with varied polysaccharide intermediates.\textsuperscript{275} This variation in zwitterion effectiveness was demonstrated by two other groups where one showed reduced adhesion and the other did not.\textsuperscript{267, 276}

In order to determine a difference in bacterial attachment modes between glass coverslips and our surfaces, the hydrophilicity of each substrate was measured. Static contact angle measurements showed significantly lower contact angles for AEDAPS and AEDAPS-SS when compared to hydrophobic PAH/PAABp films, but slightly higher angles than observed for glass coverslips as seen in Figure 4.11. The hydrophilic properties of the zwitterionic film were not altered upon supplemental soaking of AEDAPS despite an increase in film thickness.

**Table 4.1 PEMU Thickness and Contact Angle**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Thickness (Å)</th>
<th>$\Theta_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coverslip*</td>
<td>N/A</td>
<td>-0°</td>
</tr>
<tr>
<td>AEDAPS</td>
<td>1056±6</td>
<td>10±2°</td>
</tr>
<tr>
<td>AEDAPS-SS</td>
<td>1173±16</td>
<td>10±2°</td>
</tr>
<tr>
<td>(PAH/PAABp)$_2$</td>
<td>207±2</td>
<td>66±3°</td>
</tr>
</tbody>
</table>

* $\Theta_c$ too low to measure

The hydrophilic nature of both the control coverslips and the zwitterionic films suggests that the attachment of bacteria is not based on hydrophobicity, but instead on mechanical and topological mechanisms.

Substrate modulus has been shown to play a role in the attachment behavior of eukaryotic cells, demonstrating that stiffer surfaces promote better adhesion.\textsuperscript{277} This mode of attachment
was then also observed for hydrogels and polyelectrolyte multilayers. While eukaryotic cells have the ability to sense surface stiffness properties, prokaryotic bacteria are not so well equipped in their cytoskeletal machinery. As such, stiffness cues don’t translate into cellular response with regard to adhesion. When looking at surface nanomorphology, however, there is a strong correlation between bacterial adhesion and surface features smaller than a bacterium.

When looking at AEDAPS and AEDAPS-SS surfaces using AFM, both seem to have similar roughness profiles, but AEDAPS-SS boasts added nanomorphology as shown in Figure 4.11. While both films exhibit low roughness (few nm), they are still rougher than that of a clean glass coverslip (0.3 nm).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{AFM_images}
\caption{AFM images of dried AEDAPS and AEDAPS-SS films on glass substrate. More granular AEDAPS exhibits a roughness of 4 nm, while AEDAPS-SS exhibits a roughness of 6 nm.}
\end{figure}
The topography of these zwitterionic PEMUs is finer than that of an \textit{E. coli} bacterium with features comparable to the spacing of their fimbria. Unlike the bulk of the PEMU, which has been photocrosslinked using benzophenone, the zwitterionic surface is more likely softer, which in combination of nanoroughness could provide an explanation for bacterial adhesion. This behavior is only further enhanced by the addition of more AEDAPS during soaking, which creates a more loosely bound film.

Surfaces that demonstrated large variation in adhesion properties of eukaryotic cells and prokaryotic bacteria emphasize the differences in their attachment mechanisms. Mammalian cell lines, such as A7r5 and 3T3, require strong adsorption of extracellular matrix (ECM) proteins such as collagen and fibronectin to a substrate. In early stages of cell adhesion, integrin, one of the transmembrane adhesion proteins, forms specific binding interactions with ECM. These initial cell to ECM binding interactions increases and stimulates formation of focal adhesion complexes, which are established by the activation of signaling G-proteins.\textsuperscript{281} Cells are also capable of forming nonspecific attachments to surfaces through van der Waals interactions of cell surface glycoproteins and oligosaccharides with amino acids, sugars, and peptides deposited on a substrate.\textsuperscript{282}

Bacterial adhesion is not quite as well understood, showing reliance on several interactions like electrostatics, which drives the irreversible attachment to a surface using surface charges from lipopolysaccharides on the surface of the bacterium.\textsuperscript{265,283-285} Biofilm formation by \textit{E. coli} occurs due to nonspecific physical interactions based on roughness,\textsuperscript{286} hydrophobicity,\textsuperscript{287} shear forces,\textsuperscript{288} and ionic strength.\textsuperscript{289} \textit{E. coli}, which is stationary, also adheres much more tightly than mid-log-phase bacteria attributed to a higher degree of local charge heterogeneity.\textsuperscript{262} Using pili and fimbria, which are surface adhesion appendages, bacteria can also exhibit specific interactions with host cells, other bacteria, or a substrate.\textsuperscript{290} These fimbriae contain adhesins that interact with specific molecules and amino acids which promote attachment demonstrated by FimH adhesin that selectively binds D-mannose.\textsuperscript{290} Once bacteria become irreversibly attached to a surface, they excrete extracellular polymeric substances (EPS) in the form of sugars, DNA, and proteins to encase themselves in a protective shell.\textsuperscript{264} The bacterial colonies are able to grow into larger macrocolonies that restart the adhesion cycle by releasing planktonic bacteria back into solution or onto the substrate as seen in Figure 4.12.\textsuperscript{263,283,291}
Comparative studies using Gram-negative and Gram-positive bacteria could more accurately define adhesion mechanisms of *E. coli* on AEDAPS PEMUs.

To date, the most effective bacteria-repelling zwitterion functionalized PEMUs have been brushes that were grown from a substrate using living radical polymerization. These brushes are well extended and prevent adhesion of bacteria through their dense nature, which prevents the penetration of fimbria to a solid surface. The thickness of these PEMUs is on the same scale as the films prepared in this study, but contain a much denser network of zwitterions and lack permanently charged co-units like the carboxylate group paired with AEDAPS. The proximity of this charge could allow for electrostatic attractions with bacteria, which would increase attachment.

Due to the contradictory behavior of our surfaces, they would not be suitable coatings for medical devices and implants, but have potential applications as bioreactors and wound dressings in the form of ‘smart bandages’. The smart bandage works by preventing tissue growth around the bandage and drawing out bacteria from the wound. Purification and filtration applications are also of interest for AEDAPS PEMUs in that they could improve commercially available cell filters that purify cell samples for clinical and experimental studies. The construction of biofilms has also shown to be beneficial in biofuel production, waste management, and designing advanced coatings to test bactericidal and bacteriostatic agents in the case of metal nanoparticle incorporation or for development of ‘living materials’.
4.4 Conclusions

Despite the assumption that a surface which prevents protein and cell adhesion will also prevent bacterial adhesion, the use of AEDAPS proved otherwise. Other work has shown that zwitterions can be effective at reducing irreversible bacterial attachment through the use of neutral, densely-packed grafted brushes. These structures are not found in vivo since they would isolate cells from one another, which is detrimental to living systems. For the primary challenge of producing a dense zwitterionic coating that prevents biofouling, comb or dendrimer polymer structures could be used.
CHAPTER 5

INTRINSIC PERFORMANCE OF POLYELECTROLYTE MULTILAYER MEMBRANES: ERASING THE MEMORY OF THE INTERFACE

5.1 Introduction

Polyelectrolyte multilayers (PEMUs) are prepared by solution alternation by dipping to deposit oppositely charged polymers onto a substrate.\textsuperscript{30, 61, 84, 297} The extent of polymer deposition relies on a number of factors from salt identity and concentration,\textsuperscript{42, 192} pH,\textsuperscript{186, 298} polymer concentration and type,\textsuperscript{32} and the temperature during film construction.\textsuperscript{193, 299-301} Intrinsic compensation between polyelectrolytes in the PEMU results from extensive interactions between oppositely charged repeat units and generates a stable thin film.\textsuperscript{43} Extrinsic compensation occurs when the PEMU is immersed in salt solution and counterions participate in charge compensation throughout the film through the dissociation of ionic crosslinks between polyelectrolytes.\textsuperscript{43} As these counterions associate with charges within the PEMU, solvent molecules are able to permeate through the film more easily and cause it to swell.

Thin films of PEMUs can be used as membranes with many desirable properties such as controlled thickness, high ionic fluxes, variable hydration, and good ion selectivity for numerous applications in gas and liquid separations, biosensing and antifouling, as well as production of free standing membranes.\textsuperscript{81, 83, 184, 302-310} PEMUs are also the subject of recent work done by Schmidt et al.\textsuperscript{311} in biosensing applications using gold nanoparticles to determine the presence of H\textsubscript{2}O\textsubscript{2}. Using Cu\textsuperscript{2+} as a templating agent with PAA allowed for the creation of free ion exchange sites within a film of PAH and PAA, which was useful for anion transport through the PEMU depending on pH.\textsuperscript{312} Recent studies performed by Krasemann et al.\textsuperscript{79} showed that self-assembled PEMU membranes processed under the right conditions were effective at separations of ethanol and water mixtures under pervaporation conditions. Lu et al. demonstrated that polyelectrolyte membranes could be used to separate monovalent (Na\textsuperscript{+}) and divalent (Mg\textsuperscript{2+}) cations to achieve a 95\% rejection of Mg\textsuperscript{2+} with a Na\textsuperscript{+}/Mg\textsuperscript{2+} selectivity of 22.\textsuperscript{303} Stair et al. also reported the use of
PSS/PAH and PAA/PAH PEMUs to separate Cl⁻/SO₄²⁻ and Cl⁻/Fe(CN)₆³⁻ with selectivities of 150 and 3000 respectively.\(^ {313}\)

The extent of doping in PEMU membranes was influenced by the identity and concentration of salt in solution.\(^ {134}\) As the amount of salt increases, so does the amount of doping that can occur in the membrane. This property was a result of forcibly adding ion exchange sites into the PEMU under external chemical potential of the salt, resulting in a film recognized as a “reluctant exchanger”\(^ {85}\). Conversion between intrinsic compensation and extrinsic compensation within a PEMU exists in equilibrium in the presence of salt given by the following relation:

\[
Pol^+Pol^- (s) + Na^+ (aq) + Cl^- (aq) \leftrightarrow Pol^+Cl^- (aq) + Pol^-Na^+ (aq)
\]  

(5.1)

where \(Pol^+Pol^-\) was the intrinsically compensated ion pair and \(Pol^+Cl^-\) and \(Pol^-Na^+\) represent extrinsic compensation by small counterions sodium and chloride.\(^ {43}\) While it’s been well understood the mechanism by which oppositely charged polyelectrolytes pair to form films and membranes, not much was understood about the rate at which this process occurs. Using a rotating disk electrode (RDE), the rate at which PSS binds to a PDADMA-terminated PEMU can be determined using 2 mM ferricyanide as the electroactive species. This behavior can be explained by the inclusion of ferricyanide into the membrane by PDADMAC as explained in Equilibrium 5.2. For ferricyanide, Fe(CN)₆³⁻, which was the redox active ion used to measure the rate of adsorption of PSS, the following equilibrium applies:

\[
3Pol^+Pol^- (s) + Fe(CN)_6^{3-} (aq) + 3Na^+ (aq) \leftrightarrow Pol_{3}^{3+}Fe(CN)_6^{3-} (s) + 3Pol^-Na^+ (s)
\]  

(5.2)

Equilibrium 2 is illustrated by Figure 5.1 demonstrating the “hopping” ability of ferricyanide to free positive extrinsic sites caused by positively terminated PEMUs. In order to
facilitate this process, ferricyanide must “hop” to areas within the PEMU that contain three positive extrinsic sites. This inclusion of negatively oppositely charged species into a PEMU has been previously observed by Chen et al.\textsuperscript{314} when measuring the amount of urate and ascorbate by polycations within the membrane. Upon interaction of ferricyanide with these extrinsic sites, smaller counterions are released, which is entropically favorable.\textsuperscript{85}

Figure 5.1 Illustration of Fe(CN)$_6^{3-}$ transport through a stoichiometric PEMU (left) compared to a positively terminated PEMU (Right). Excess positive charge allows ferricyanide to “hop” through the PEMU by way of counterion release. Three positive extrinsic sites are required to create a vacant site for ferricyanide hopping. Once a negatively charged polyelectrolyte is added to the film, all excess positive charges become intrinsically compensated, yielding a glassy film that inhibits diffusion of ferricyanide, resulting in decreased membrane current.
Doping provides extrinsic sites that accelerate diffusion into the membrane in a strongly nonlinear manner and depends on the PEMU and type of salt used.\textsuperscript{60} Equation 5.3 uses NaCl as an example for the salt.

\[ K_{dop} = \frac{y^2}{(1-y)a_{NaCl}^2} \]  \hspace{1cm} (5.3)

The doping level, or the fraction of $Pol^+Pol^-$ converted to $Pol^+Cl^-$ and $PolNa^+$, was represented by \( y \). As \( K_{dop} \) increases, swelling within the PEMU increases as salt and their associated waters of hydration diffuse into the film. At the dilute doping limit (small values of \( y \)), doping level was proportional to salt concentration. Mass transport of ions through the PEMU was strictly diffusion limited. Extrinsic sites within the film generated by the supporting electrolyte exist in excess with regards to the electroactive ion.\textsuperscript{58, 187}

\textbf{Figure 5.2} Illustration of the adsorption of PSS to a PDADMAC terminated PEMU at the PEMU/solution interface. Excess positive charges from PDADMAC become intrinsically compensated by negative charges on PSS leaving a glassy, stoichiometric membrane.
Figure 5.3 Structures of polyelectrolytes employed: Poly(diallyldimethylammonium) chloride (PDADMAC) and Sodium Poly(styrene) sulfonate (PSS).

Figure 5.4 Bilayer thickness of PDADMAC/PSS PEMU built in 0.25 M NaCl. At seven bilayers, the stoichiometric PEMU was pushed to the top of the film with the rest of the PEMU having an excess of PDADMAC underneath.

Multivalent ferricyanide was not as mobile as the supporting monovalent salt ions, which suppress the electric field within the PEMU and minimize field-driven migration of the electroactive species.\textsuperscript{173} Despite the highly ionic nature of the polyelectrolyte backbones within
the PEMU, their size and position within the membrane renders them immobile with respect to smaller ions and are incapable of charge transport.

Polyelectrolyte thin film membranes of PDADMA/PSS were constructed using alternating solution dipping with rinsing steps between polyelectrolyte adsorption steps. This polyanion pair has been used for nanofiltration membranes,315-316 anticorrosion coatings,317 and as polyelectrolyte complexes for a range of applications318 among other uses.

Construction of PEMUs using PDADMA and PSS occurs through two distinct buildup regions, linear and exponential.41 During linear buildup, polyelectrolyte is added directly to the surface of the film and an incremental thickness is observed. For the exponential buildup region, polyelectrolytes, specifically PDADMA in this study, are able to penetrate beyond the surface of the film and diffuse into the bulk, yielding charge overcompensation, which has been observed previously for weak polyelectrolytes such as PGA and PAH.192,319 This overcompensation by polyelectrolyte significantly increases the thickness of each added layer as illustrated in Figure 5.4.

For this study, PEMUs of PDADMA/PSS were built on a rotating disk electrode at 1000 rpm using solution alternation by dipping. Once a desired number of layers was reached, the coated electrode was immersed into an electroactive solution of 2 mM ferricyanide in 0.6 M NaCl, which acted as the supporting electrolyte. In order to produce an electrical current, the electrode is rotated at 1000 rpm to draw ferricyanide to the membrane-coated platinum disk by convection as illustrated by Figure 5.5. The speed of rotation determines the thickness of stagnant solution layer produced on top of the PEMU, which is of significantly greater thickness.58,85 As rotation rate increases, the depth of the stagnant layer decreases. Once the ferricyanide diffuses through both the stagnant layer of solution and PEMU, it is reduced to ferrocyanide, Fe(CN)$_6^{4-}$, producing an electrical current measured against a saturated calomel reference electrode (SCE).

The inclusion of negatively charged ferricyanide within the film enhances the concentration gradient throughout the film, although this gradient was highly dependent on the rate at which the ferricyanide can diffuse through the stagnant layer of the film.320 Three currents will be measured in this study: Levich current, limiting current, and membrane current. The relationship among these three parameters has been previously determined by Helfferich321 to determine the flux of an electroactive species through a membrane.
Figure 5.5 Schematic showing the boundary through which Fe(CN)$_6^{3-}$ (yellow dots) must diffuse before reaching the RDE. A PEMU was deposited via LbL onto the RDE and acts as the thin membrane that aids in ion transport of ferricyanide (PDADMAC-terminated) or blocks transport via stoichiometric, glassy film (PSS-terminated). On top of the PEMU rests a stagnant layer of solution (blue) of significantly greater thickness than the PEMU (15 µm) that inhibits diffusion of ferricyanide to the RDE.
5.2 Experimental Section

5.2.1 Materials

(PDADMAC) Poly(diallyldimethylammonium) chloride (molecular weight 400000-500000 g mol\(^{-1}\), 21.3 wt% in water) was used as received from Aldrich. (PSS) Sodium poly(styrene) sulfonate (molecular weights 57500, 122400, 262000, 801100, and 2260000 g mol\(^{-1}\)) were all used as received from Scientific Polymer Products. Poly(styrene) sulfonate (molecular weight 615000 g mol\(^{-1}\)) was prepared. Potassium ferricyanide (K\(_3\)Fe(CN)\(_6\)) was used as received from Mallinkrodt, Inc. Potassium ferrocyanide trihydrate (K\(_4\)Fe(CN)\(_6\)·3 H\(_2\)O) and sodium chloride (NaCl) were used as received from manufacturer. All solutions were made in 18 MΩ deionized water (Barnstead, E-pure). Structures of polymers are shown in Scheme 3.

\(^{35}\text{SO}_4^{2-}\) (received as Na\(_2^{35}\text{SO}_4\), half-life 87.4 days, beta emitter, E\(_{\text{max}}\) = 167 KeV) was purchased from PerkinElmer. It was supplied as 1 mCi in 1 mL of water with a specific activity of 1494 Ci mmol\(^{-1}\) and used as a stock solution.

5.2.2 PEMU Characterization

PEMUs were characterized by ellipsometry, radioactive doping, and Fourier transform infrared spectroscopy (FT-IR). Dry thicknesses of PEMUs were determined using a Gaertner Scientific L116S Autogain ellipsometer with 632.8 nm radiation at 70° incident angle and a refractive index of 1.55. FTIR spectra of PEMUs were obtained at a resolution of 4 cm\(^{-1}\) with 100 scans using a Thermo Avatar 360 equipped with a DTGS detector. The background was determined using an uncoated (bare) Si wafer. All multilayer buildup and treatments were conducted at room temperature (23 ± 2 °C).

5.2.3 Rotating Disk Electrode (RDE)

A 100 mL electrochemical cell was equipped with a water jacket controlled over the temperature range of 10-60 °C (±0.1 °C), a platinum wire counter electrode, and a KCl-saturated calomel reference electrode (SCE), against which all potentials were measured. The working electrode was a rotating platinum disk (RDE, Pine Instruments Model AFE3T050PT), with a 5 mm diameter, mounted in a Pine AFMSRCE rotator and speed controller. A Pine AFTP1
WaveNow potentiostat was used to generate potential ramps, and the resulting voltammograms were recorded using Aftermath software. The platinum electrode was polished with 0.3 µm alumina (Buehler), rinsed with 18 MΩ DI water, and dried under a stream of N₂. Adsorption of polyelectrolytes to the electrode was done using the Pine AFMSRCE rotator at 1000 rpm. The electrode was dipped in polyelectrolyte for 5 minutes followed by three 1 minute DI water rinse steps. This process was alternated between positive and negative polyelectrolyte up to the desired number of layers. The polyelectrolyte deposition solutions were PDADMAC and PSS (10 mM) in 0.25 M NaCl with volumes of approximately 15 mL each. The thickness of the PEMUs was determined by a separate experiment using a 1 in diameter single-side polished Si wafer and measured using a Gaertner Scientific L116S Autogain ellipsometer with 632.8 nm radiation at 70° incident angle.

Cyclic voltammograms (CVs) were collected in solutions of 2 mM ferricyanide in 0.6 M NaCl operated at a scan rate of 20 mV s⁻¹ in the range of 300 to -300 mV vs. SCE at 1000 rpm at 20 ºC. Chronoamperometry was performed over a range of 10-60 ºC at a potential of -300 mV for 3000 seconds. The 0.6 M NaCl solution was purged using Ar for 10 min to remove dissolved oxygen and a blanket of Argon was maintained throughout the duration of the experiments. Following construction of the PDADMAC-terminated PEMU, the film was annealed in 1 M NaCl for 15 minutes to redistribute any excess positive charge toward the surface of the film. After the annealing step, the film was placed into the electrokinetic cell and spun at 1000 rpm in 0.6 M NaCl to equilibrate to cell conditions. After 5 minutes, 2 mM ferricyanide was added to the cell. Once the current was steady, PSS was added and the current was continuously measured until the current drops to a consistent level. The result was a sigmoidal curve representing the blocking of ferricyanide by PSS. All experiments performed under the aforementioned conditions were found to be reproducible.

5.2.4 Radiolabeling

Na₂³⁵SO₄ was used to quantify the total amount of positive extrinsic sites. ³⁵Sulfate solution (20 mL) was prepared by diluting 30 µL of the “hot” Na₂³⁵SO₄ in 19.97 mL “cold” 10⁻³ M Na₂SO₄. The resulting solution had a specific activity of 1 Ci mol⁻¹. The specific activity
determines the accuracy and precision of the counting while the solution concentration ensures an excess of radiolabel in the solution.

For counting, a photomultiplier tube (PMT, RCA 8850) placed in a dark box was used. A 3.8 cm plastic scintillator disk was cut from 3 mm thick large sheets (SCSN-81, Kuraray America). It was placed on the 5 cm diameter window of the PMT with a drop of immersion oil under it to ensure good optical contact between the scintillator and the PMT. A Bertran 313B high-voltage supply fixed at 2300 V was connected to the PMT. To record the counts, a frequency counter (Philips PM6654C) interfaced with a computer running LabView software was used. The software facilitates the manipulation and the storage of the data. The pulse threshold was fixed at –20 mV and the gate time was 10 s. Iodide had a counting efficiency of around 29%. The counting efficiency was obtained by dividing the number of counts per second by the disintegrations per second. For each data point, the total number of counts registered ranged from 9 700 to 30 000 with respective counting errors of 0.6% and 1%.

After building the PEMU to 17 layers and triple rinsing it in water, it was dried under a stream of N₂ and soaked in the radiolabeled $^{35}\text{SO}_4^{2-}$ solution for 30 minutes. It was then dried using nitrogen without rinsing and placed, face down, on the plastic scintillator. The counting was performed for 15 min after which the film was placed in $10^{-2}$ M “cold” NaCl solution to exchange out the “hot” sulfate for around 15 minutes. The film was then rinsed quickly in water and dried. The same labeling/cleaning procedure was used for the next 2 steps: 15 min in 1 M NaCl and 5 min in 10 mM PSS in 1 M NaCl. A calibration curve was built by dispensing $1-5\mu$L aliquots of the radiolabel solution covered with a bare Si wafer for good spreading on top of the scintillator. The curve (counts vs number of moles) were used to obtain the number moles for each time point. The surface area of the film was measured and used to obtain moles m⁻².

5.3 Results and Discussion

5.3.1 Limiting and Membrane Currents of PEMU

Membranes are swelled when terminated by PDADMAC,⁴¹ 322-323 which is a result of excess counterions near the surface (when there are 12 layers or fewer) or in the bulk (for more than 14 layers). This excess of counterions increases osmotic pressure that draws more water
into the membrane, causing the film to swell. Once the film was capped with PSS, the membrane becomes a glassy, stoichiometric PEMU that acts as a tough barrier for ferricyanide to penetrate. This glassy barrier greatly reduces flux of ferricyanide and results in lower current for each PSS-terminated layer, which is reflected by a decrease in current measured as seen in Figure 5.6 for a mixture of ferri/ferrocyanide in a 1:1 molar ratio.

![Cyclic voltammetry graph](image)

**Figure 5.6** Cyclic voltammetry was performed on 1:1 mixtures of ferricyanide and ferrocyanide on a PDADMAC terminated PEMU (17 layers) (blue), PSS terminated PEMU (18 layers) (red), and on a bare electrode (green). When capped with PDADMAC, excess positive extrinsic sites draw negatively charged ferricyanide through the film to the electrode. When PSS terminated the PEMU, the film has no net charge and mobility of ferricyanide was diffusion limited. Both PEMU CV experiments showed complete blocking of ferrocyanide through the film. Removing the PEMU from the electrode surface allows for transport of ferrocyanide to the electrode, yielding the expected hysteretic curve. Experiments were performed in 0.6 M NaCl and 2 mM 1:1 mixture of ferri/ferrocyanide on a platinum electrode with an area of 19.63 mm² with a rotation rate of 1000 rpm.

Cyclic voltammetry (CV) was used to determine the limiting current of a PEMU capped with PDADMAC and PSS as well as at a bare RDE for an equimolar mixture of electroactive ferricyanide, Fe(CN)₆³⁻, and ferrocyanide, Fe(CN)₆⁴⁻. Classic S-shaped voltammograms with
plateaus at the convection—diffusion-limited currents were observed. Reduction (positive) currents are attributed to the flux of ferricyanide while negative (oxidation) currents are associated with the flux of ferrocyanide. At 17 layers, excess PDADMAC increased the extent of hydration for the membrane, which coupled with Donnan inclusion of ferricyanide allowed for rapid diffusion through the film.

This inclusion of anions by excess polycation during membrane transport and selectivity has been observed previously. Kharlampieva and Sukhishvili used this principle for the inclusion and release of both cationic and anionic dyes in PEMUs containing weak polyelectrolytes. Upon addition of polyelectrolyte, the dye was released from the PEMU, meaning that this method could be useful for the controlled release of certain molecules. This behavior was also observed by Hübsch et al. for the release of ferrocyanide from a PEMU composed of poly(glutamic acid) (PGA) and poly(allylamine hydrochloride) (PAH). The multivalent ion was not released from the membrane when placed in a Tris-NaCl buffer solution, but immediately diffused out upon immersion in a polyelectrolyte solution. Once PSS was added to the PDADMAC-terminated film, the glassy nature of the PEMU greatly reduced ferricyanide transport and the limiting current decreased accordingly.

Both 17 and 18 layers showed a complete blocking of ferrocyanide, which was consistent with previous work. Once the PEMU was removed from the electrode with 2.5 M KBr, the limiting current showed the expected hysteresis curve associated with an equimolar ferricyanide/ferrocyanide mixture.

For a bare electrode, the limiting current, \(i_{lim}\), is represented by the plateau of the curve, for an electrode area \(A\) rotating at a constant angular velocity is described by the Levich equation:

\[
i_{lev} = 0.620nFAD_{aq}^{2/3} \omega^{1/2} \nu^{-1/6} C_{aq}
\]

(5.4)

where \(n\) represents the number of electrons (1 for ferri/ferro couple), \(F\) represents the Faraday constant (96485 C mol\(^{-1}\)), \(D_{aq}\) represents the solution diffusion coefficient of the electroactive species, \(\nu\) represents kinematic viscosity of electrolyte solution (~0.01 cm\(^2\) s\(^{-1}\)), and \(C_{aq}\) represents the concentration of the solution. Once a membrane is added to the surface of the
The electrode, the Levich current and limiting current are no longer equivalent since limiting current will continue to decrease as the thickness of PEMU increases. The diffusion coefficient for ferricyanide to a bare electrode and through the PEMU at 20 °C was studied by Ghostine et al.\(^6\)

The permeation of electroactive solutes through ultrathin films of PEMUs was also studied by Ikeda et al.\(^3\) in the early 1980s based on theories proposed earlier by Gough and Leypoldt.\(^3\) The effect of a PEMU film on limiting current is due to a resistance to electron transfer at the electrode/PEMU interface and mass transport through the film during reduction of ferricyanide to ferrocyanide, which is described by the following equation

\[
\frac{1}{i_{lim}} = \frac{1}{i_{mem}} + \frac{1}{i_{lev}}
\]  

(5.5)

where \(i_{lev}\) is the current measured at an uncoated electrode through a stagnant layer of solution, \(i_{lim}\) is the current measured for both the membrane and stagnant layer of solution, and \(i_{mem}\) is the current measured within the polyelectrolyte membrane alone. Using the limiting current for bare electrodes, the contribution to the resistance to mass transfer by the stagnant layer can be eliminated from the overall mass transfer resistance to yield membrane current.\(^8\), \(^3\)

Two buildup methods were tested to determine the effect annealing had on limiting current. The first method used was traditional LbL buildup in accordance with previous protocols,\(^4\) while the annealing method adds a 2 M NaCl soak step following each PSS layer. This 2 M NaCl step allows for the rearrangement of polymer chains to make the PEMU stoichiometric in nature. Annealing in NaCl breaks ionic crosslinks within the PEMU and allows for migration of PDADMAC to the surface of the film, producing a stoichiometric bulk membrane with a PDADMAC terminated surface. Following the NaCl annealing step, the PEMU was immersed in PSS to compensate PDADMA charges at the surface of the PEMU. The addition of PSS completes the process of intrinsic compensation which removes any residual counterions and their associated waters of hydration that swell the PEMU that allow easier permeation of ferricyanide. Figure 5.7 demonstrates where ferricyanide was blocked much more rapidly with each increasing annealed layer compared to the traditional build-up method. At 19 layers, thickness becomes the primary inhibitor for ferricyanide flux as shown by decreasing limiting current with each added layer. As demonstrated by Figure 5.6, the limiting current is
reduced with the addition of PDADMA and PSS PEMUs. Previous work showed that the limiting current for membrane coated PEMUs can be increased through addition of supporting electrolyte NaCl, which demonstrates that mass transport is the limiting factor for ferricyanide diffusion.\textsuperscript{85, 187}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure57.png}
\caption{Limiting current at the rotating disk electrode vs. number of layers for 1 mM ferricyanide, Fe(CN)$_6^{3-}$ in 0.6 M NaCl using a 20 mV/s sweep rate, rotation rate 1000 rpm, at room temperature. Two methods of buildup were performed, standard (red) and annealing (blue). The standard method consists of solution alternation by dipping with intermediate rinsing steps, while the annealing method uses 2 M NaCl following each PSS layer to redistribute charge within the film, yielding a 1:1 stoichiometric film. Due to the stoichiometric nature of the film, polyelectrolyte does not diffuse into the bulk of the membrane, adsorbing only to the surface. This allows the film to grow at a much faster rate than standard buildup procedures. The platinum electrode had an area of 0.1963 cm$^2$. PDADMAC/PSS multilayers were built from 10 mM polymer solutions in 0.25 M NaCl. Positive extrinsic sites of PDADMAC of $y_\infty^+ = 0.21$ allows for ferricyanide to hop through the membrane, but not actively transported.}
\end{figure}
5.3.2 Determination of Membrane Flux and Permeability for PDADMAC and PSS Terminated Membranes

Since the flux of ferricyanide was electrostatically and hydrodynamically accelerated, there was little difference between limiting currents recorded at a bare electrode (-97.7 µA) and at 17 layers (-91.8 µA) as seen in Figure 3, which can be attributed to Donnan interactions.

After 17 layers, the PDADMAC-capped PEMU showed significant NO$_3^-$ inclusion by extrinsic sites as seen by the aliphatic NO$_2$ stretch at 1350 cm$^{-1}$. The next stage of PEMU processing was a 15 minute soak in 1 M NaCl to anneal the film and allow charge redistribution to decrease extrinsic compensation by counterions. Any excess positive sites are redistributed to the surface of the PEMU, leaving a 1:1 stoichiometric bulk layer.

**Figure 5.8** Extrinsic site labeling by NO$_3^-$ using FT-IR. The nitrate ion, NO$_3^-$, was added after each processing step (17 layers, NaCl annealing, and PSS addition) of the PEMU to determine the number of extrinsic sites available in the PEMU. Following the 17$^{th}$ PDADMAC layer, the addition of NO$_3^-$ showed an excess of extrinsic sites within the film. Annealing in 1 M NaCl allowed for rearrangement of polymer chains, which redistributed all excess positive extrinsic sites toward the surface. The reduction in positive extrinsic sites was reflected in the reduction of the NO$_3^-$ at 1350 cm$^{-1}$. Following the addition of PSS, there were nearly zero positive extrinsic sites available for NO$_3^-$ to associate with.
In this process, some PDADMAC was also etched from the film, which also yielded fewer extrinsic sites. A significant decrease in NO$_3^-$ was observed following the 1 M NaCl step. The final processing step of the PEMU was the addition of PSS to cap the film. Once PSS was added, the remaining positive charge was intrinsically compensated to yield a 1:1 charge-balanced stoichiometric film. This was determined by the near zero absorbance from NO$_3^-$ in the final FT-IR. Using radiolabeling of extrinsic sites by $^{125}$I during this same process produced similar results.

![Figure 5.9 Extrinsic site labeling by NO$_3^-$ and $^{35}$SO$_4^{2-}$ through FT-IR and radiocounting respectively. The use of $^{35}$SO$_4^{2-}$ confirmed the decrease in extrinsic positive sites observed in Figure 4. Error bars represent the standard deviation of counts.](image)

The current measured through only the adsorbed membrane can be converted to membrane flux, $J_{\text{mem}}$, using the following equation$^{321}$

$$J_{\text{mem}} = \frac{i_{\text{mem}}}{nFA} \quad (5.6)$$

If the concentration of ferricyanide and thickness of the PEMU are known, the diffusion coefficient of the ferricyanide can be determined. The probe ion concentration within the
membrane is roughly equivalent to that in solution, and the thickness of the PEMU was shown to be 40 nm for an 18 layer PEMU.

![Figure 5.10](image)

**Figure 5.10** Membrane flux of ferricyanide through PEMUs constructed by two different methods. The standard Layer-by-Layer (LbL) PDADMA/PSS PEMU is represented by blue diamonds, which shows greater membrane flux due to inclusion of ferricyanide by positive extrinsic sites on PDADMAC. Annealing the PEMU with 2 M NaCl followed by an extra PSS adsorption step removes the excess positive charge and shows a decrease in membrane flux demonstrated by red squares.

From this understanding of flux through PDADMA/PSS PEMUs, the permeability of these membranes can be determined by the following equation

\[ P = \frac{J_{mem}d}{C_s} \]  

(5.7)

where \( C_s \) is the solution concentration and \( d \) is the membrane thickness. The permeability of polyelectrolyte multilayers has been studied previously several groups. This work was furthered by studies performed on ions with different valences, which showed that selectivity for monovalent ions over multivalent ions could be improved with the addition of salt. The
reasoning behind this was Donnan inclusion where increased salt generated more surface charge, which in turn increased selectivity.

**Figure 5.11** Plot of PDADMA/PSS PEMU on a rotating disk electrode (RDE) with layer number. Blue diamonds represent Layer-by-Layer (LbL) construction method by solution alternation without any additional processing steps. Red squares represent annealed buildup with immersion in 2 M NaCl followed by additional PSS between each bilayer. Annealing the PEMU in NaCl allows for redistribution of excess PDADMAC within the film, which presents free binding sites at the surface for PSS. Once the PSS is added to the film, excess counterions and their associated waters of hydration are released, producing a stoichiometric, glassy film that acts as a barrier to ferricyanide as demonstrated by a decrease in permeability.

Using the largest decrease in current ($\Delta = 42 \, \mu A$) between the 17th PDADMAC and 18th PSS layers as motivation from Figure 3, the solution kinetics of PSS was studied using a platinum rotating disk electrode (RDE). For 18 layers, the PEMU had a thickness of 40 nm, where the PEMU was constructed manually at 1000 rpm. At 17 layers, PDADMAC exists in excess throughout the film, yielding a net positive charge in the PEMU membrane, which allows ferricyanide to “hop” through the film to the electrode due to Donnan inclusion as illustrated in Figure 5.1.\textsuperscript{85}
Table 5.1 Comparison of Thickness, Permeability, and Membrane Flux for Standard and Annealed PDADMA/PSS PEMUs.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Standard</th>
<th></th>
<th></th>
<th>Annealed</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thickness (nm)</td>
<td>Membrane Flux (x10^7 mol cm^2 s^-1)</td>
<td>Permeability (x10^11 cm^2 s^-1)</td>
<td>Thickness (nm)</td>
<td>Membrane Flux (x10^7 mol cm^2 s^-1)</td>
<td>Permeability (x10^11 cm^2 s^-1)</td>
</tr>
<tr>
<td>1</td>
<td>2.11</td>
<td>4.5007</td>
<td>4.7403</td>
<td>3.59</td>
<td>2.2287</td>
<td>1.9994</td>
</tr>
<tr>
<td>2</td>
<td>2.34</td>
<td>2.8859</td>
<td>3.3772</td>
<td>3.59</td>
<td>0.6462</td>
<td>1.1594</td>
</tr>
<tr>
<td>3</td>
<td>3.65</td>
<td>3.7091</td>
<td>6.7715</td>
<td>8.24</td>
<td>0.9328</td>
<td>3.8431</td>
</tr>
<tr>
<td>4</td>
<td>4.06</td>
<td>1.5770</td>
<td>3.1989</td>
<td>12.89</td>
<td>0.3179</td>
<td>2.0488</td>
</tr>
<tr>
<td>5</td>
<td>6.05</td>
<td>2.8750</td>
<td>8.6946</td>
<td>16.10</td>
<td>0.7335</td>
<td>5.9047</td>
</tr>
<tr>
<td>6</td>
<td>6.72</td>
<td>1.1333</td>
<td>3.8081</td>
<td>19.31</td>
<td>0.1748</td>
<td>1.6873</td>
</tr>
<tr>
<td>7</td>
<td>10.23</td>
<td>2.9774</td>
<td>15.2250</td>
<td>23.66</td>
<td>0.6048</td>
<td>7.1559</td>
</tr>
<tr>
<td>8</td>
<td>10.77</td>
<td>0.7253</td>
<td>3.9042</td>
<td>28.02</td>
<td>0.0983</td>
<td>1.3767</td>
</tr>
<tr>
<td>9</td>
<td>14.67</td>
<td>2.0467</td>
<td>15.0166</td>
<td>34.69</td>
<td>0.4262</td>
<td>7.3932</td>
</tr>
<tr>
<td>10</td>
<td>15.45</td>
<td>0.4616</td>
<td>3.5649</td>
<td>41.37</td>
<td>0.0403</td>
<td>0.8345</td>
</tr>
<tr>
<td>11</td>
<td>20.20</td>
<td>1.6012</td>
<td>16.1745</td>
<td>44.93</td>
<td>0.2197</td>
<td>4.9360</td>
</tr>
<tr>
<td>12</td>
<td>21.27</td>
<td>0.2493</td>
<td>2.6512</td>
<td>48.48</td>
<td>0.0403</td>
<td>0.9779</td>
</tr>
<tr>
<td>13</td>
<td>26.14</td>
<td>1.3690</td>
<td>17.8954</td>
<td>51.95</td>
<td>0.0887</td>
<td>2.3043</td>
</tr>
<tr>
<td>14</td>
<td>27.52</td>
<td>0.1287</td>
<td>1.7710</td>
<td>55.41</td>
<td>0.0263</td>
<td>0.7288</td>
</tr>
<tr>
<td>15</td>
<td>32.91</td>
<td>0.9634</td>
<td>15.8499</td>
<td>59.46</td>
<td>0.0500</td>
<td>1.4852</td>
</tr>
<tr>
<td>16</td>
<td>34.64</td>
<td>0.0733</td>
<td>1.2687</td>
<td>63.51</td>
<td>0.0222</td>
<td>0.7056</td>
</tr>
<tr>
<td>17</td>
<td>39.62</td>
<td>0.8029</td>
<td>15.9066</td>
<td>71.16</td>
<td>0.0256</td>
<td>0.9123</td>
</tr>
<tr>
<td>18</td>
<td>41.71</td>
<td>0.0532</td>
<td>1.1102</td>
<td>78.81</td>
<td>0.0243</td>
<td>0.9586</td>
</tr>
<tr>
<td>19</td>
<td>47.49</td>
<td>0.2808</td>
<td>6.6682</td>
<td>84.99</td>
<td>0.0204</td>
<td>0.8685</td>
</tr>
<tr>
<td>20</td>
<td>49.99</td>
<td>0.0432</td>
<td>1.0798</td>
<td>91.17</td>
<td>0.0179</td>
<td>0.8153</td>
</tr>
<tr>
<td>21</td>
<td>55.22</td>
<td>0.2435</td>
<td>6.7234</td>
<td>96.91</td>
<td>0.0152</td>
<td>0.7357</td>
</tr>
<tr>
<td>22</td>
<td>57.52</td>
<td>0.0433</td>
<td>1.2450</td>
<td>102.65</td>
<td>0.0121</td>
<td>0.6221</td>
</tr>
<tr>
<td>23</td>
<td>62.47</td>
<td>0.1472</td>
<td>4.5969</td>
<td>111.49</td>
<td>0.0119</td>
<td>0.6656</td>
</tr>
<tr>
<td>24</td>
<td>65.76</td>
<td>0.0394</td>
<td>1.2969</td>
<td>120.33</td>
<td>0.0097</td>
<td>0.5835</td>
</tr>
<tr>
<td>25</td>
<td>69.73</td>
<td>0.1194</td>
<td>4.1641</td>
<td>126.58</td>
<td>0.0102</td>
<td>0.6486</td>
</tr>
<tr>
<td>26</td>
<td>73.40</td>
<td>0.0349</td>
<td>1.2819</td>
<td>132.84</td>
<td>0.0077</td>
<td>0.5104</td>
</tr>
<tr>
<td>27</td>
<td>80.61</td>
<td>0.1087</td>
<td>4.3827</td>
<td>138.99</td>
<td>0.0078</td>
<td>0.5432</td>
</tr>
<tr>
<td>28</td>
<td>84.86</td>
<td>0.0325</td>
<td>1.3786</td>
<td>145.14</td>
<td>0.0060</td>
<td>0.4320</td>
</tr>
<tr>
<td>29</td>
<td>90.45</td>
<td>0.1160</td>
<td>5.2450</td>
<td>149.85</td>
<td>0.0060</td>
<td>0.4504</td>
</tr>
<tr>
<td>30</td>
<td>95.21</td>
<td>0.0335</td>
<td>1.5937</td>
<td>154.56</td>
<td>0.0050</td>
<td>0.3854</td>
</tr>
</tbody>
</table>
5.3.3 Effect of PSS Concentration and Molecular Weight on Sorption Kinetics

Six narrow molecular weight PSS standards were used to determine the effect of concentration and molecular weight on polyelectrolyte transport in solution to a rotating disk electrode using chronoamperometry.

**Figure 5.12** Six narrow molecular weights of PSS at A) 1 mM and B) 0.1 mM were used to determine the effects of molecular weight on polyelectrolyte adsorption kinetics using rotating disc electrochemistry. As PSS chains bind to the PDADMA terminated PEMU, it becomes more difficult for ferricyanide to diffuse through the film to the electrode, reducing the membrane current. As molecular weight increases, the rate at which PSS diffuses through solution to the electrode-fixed PEMU decreases. This decrease in rate was also attributed to a decreased number of polyelectrolyte chains. PSS addition is noted by a dashed line at 30 s. A ten point average plot was used to reduce noise.
Unlike CV, which measures the change in current with electric potential sweep, chronoamperometry measures the change in current over time under a constant electric potential. Following buildup and annealing via 1 M NaCl for 15 minutes, the PEMU-coated RDE was immersed in 0.6 M NaCl along with a platinum wire counter electrode and a saturated calomel reference electrode (SCE). The 0.6 M NaCl solution was purged of oxygen using a continuous stream of argon for 10 minutes. The flow of argon was kept between the top of the solution and the Teflon cell cap to maintain a constant blanket to prevent dissolution of oxygen back into solution. Following 5 minutes of spinning at 1000 rpm in 0.6 M NaCl to equilibrate the film, 2 mM ferricyanide was injected into the solution and allowed to mix for 5 minutes. After this second equilibration period, the desired concentration and molecular weight of PSS was injected into the solution through a small hole in the Teflon cap using a syringe. This PSS adsorption step is illustrated in Figure 5.2.

![Figure 5.13](image_url)

**Figure 5.13** Slope of current decrease for a range of narrow molecular weights of PSS to the PDADMAC terminated PEMU at 1 and 0.1 mM. There was a significant reduction in rate from 262k and 615k molecular weight PSS due to the combination of solution diffusion rate and membrane adsorption rate. The slopes were calculated from Chronoamperometry plots in Figure 5.12.
Two boundary conditions exist for well-stirred systems, such as the one in use for this study, where the boundaries exist at the electrode/PEMU interface and the PEMU/solution interface. At the electrode/PEMU interface, the concentration of the electroactive species was zero since all material was consumed at the diffusion-limited rate, while the membrane concentration of the electroactive species was constant at the PEMU/solution interface as seen by Chen et al.\textsuperscript{329}

At the PEMU/solution interface was a stagnant layer of solution that was much thicker (~15 µm) than the membrane itself.\textsuperscript{58} Depending on these parameters, membrane current decreased at varying rates. For low molecular weight PSS at 1 mM, membrane current dropped quickly since the smaller chains have higher mobility in solution and reach the RDE almost instantaneously after injection. As molecular weight increases or concentration decreases, the rate at which membrane current drops decreases due to fewer polyelectrolyte chains in solution as demonstrated by 2.26M molecular weight PSS at 0.1 mM being the slowest to coat the RDE.

There was a significant drop in rate between 262k and 615k PSS at 1 mM due to a decrease in the number of polymer chains in solution with increasing polymer chain length as well as decreased diffusion rate by large molecules as seen in Figure 5.12. This drop becomes far less exaggerated once the concentration was reduced to 0.1 mM due to a decrease in the number of polymer chains in solution. At lower molecular weights, diffusion of PSS is limited by the solution, whereas at higher molecular weights it is limited by both the adsorption of PSS to the membrane as well as solution limited diffusion. The availability of excess positive charge at the surface needs to match in the amount of incoming negative charge from PSS. For larger chains, there is a higher amount of charge that needs to be compensated, so locating matching sites becomes more difficult, thus decreasing the rate of adsorption to the PEMU.

5.4 Conclusions

A rotating disk electrode was used to determine the kinetics of polyelectrolyte adsorption to an oppositely charged PEMU surface using chronoamperometry. It was found that increasing polymer chain size had a profound effect on chain mobility and concurrently the rate at which membrane current of the PEMU decreased with time. Reducing the concentration of
polyelectrolyte by a factor of ten produced similar effects due to a decrease in the number of polymer chains available to adsorb to the surface of the PEMU. The reduction of membrane current was a result of extrinsic sites within the PEMU being converted to intrinsic sites upon annealing with 1 M NaCl and the addition of PSS. The addition of PSS releases excess counterions within the film, which in turn reduces the extent of hydration of the film, causing it to become more glassy and resistant to ferricyanide diffusion.
CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

This dissertation presents a summary of work performed using polyelectrolytes for both fundamental studies and useful applications in biomaterials and free standing membranes. Depending on the nature of these polyelectrolytes, they can be assembled via a Layer-by-Layer technique to yield uniform thin films or mixed directly in solution to yield tough precipitated complexes. These complexes can be rinsed of salt, dried, and redispersed into KBr to form polyelectrolyte coacervates, which can be used to spin coat thin films for use in membranous applications.

First, polyelectrolyte coacervates (PECOVs) were produced by the dispersal of polyelectrolyte complex (PEC) in powder form into solutions of KBr. KBr served to break ionic crosslinks within PEC to generate a loosely-bound network of polyelectrolyte chains that behaved as a liquid with viscoelastic properties. The extent of association of polyelectrolyte chains depended on the extent of KBr in the coacervate. Below 0.6 M KBr, the PEC did not allow sufficient diffusion of KBr to break ionic crosslinks and swell the material. Above this threshold, the PEC began to swell and produce a translucent orange solid. Over the range of 1.4-1.8 M KBr, a two-phase system was produced where a coacervate phase was produced that contained an excess of polyelectrolyte and a solution phase which was dilute in polyelectrolytes. The dense PECOV phase was used to produce thin films on aluminum substrates for the production of thin films, which could be released using NaOH. Once dried, the films could be handled and used for free standing membrane studies which exhibited an equilibrium modulus of 950 MPa.

Next, polyelectrolytes poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) were used to produce thin films by solution alternation for antibiofouling studies. The initial bilayers of the PEMU contained benzophenone bound to PAA that was used as a crosslinking agent to create a tough substrate for added layers containing a zwitterion. Our zwitterion, AEDAPS, was bound to PAA in 25 mol % to give the PEMU a net neutral surface charge to prevent mammalian cell adhesion to a surface. This work was motivated by the need to decrease the amount of medical complications caused by the fouling of implants, catheters,
and stents by cell lines such as A7r5 and 3T3 as well as biofilm formation by *E. coli*. The findings for this work supported previous studies, showing a significant ability to prevent cell adhesion outright, but produced a confounding contradiction when bacteria rapidly and irreversibly attached to the zwitterion functionalized PEMU. The attachment of bacteria to these films is assumed to be driven by nanoroughness and loosely-bound terminal layers of PAA-co-AEDAPS. It was found that additional AEDAPS added to a PAA-co-AEDAPS PEMU produced “floppy” layers that could physically trap bacteria as well as providing surface features on a scale similar to that of fimbria of bacterium. Despite this contradictory nature, surfaces that are capable of rapidly producing biofilms have proven to be useful in waste remediation and biofuel applications.

Finally, PEMUs of poly(diallyldimethylammonium chloride) (PDADMAC) and poly(styrene sulfonate) (PSS) were used in fundamental adsorption studies using a rotating disk electrode (RDE). Determining the limiting current of membranes built at each layer up to 30 layers allowed us to choose a point that demonstrated the greatest loss of current when PSS was added to the membrane. After 17 layers, which is terminated by PDADMAC, an excess of positive charge existed within the bulk and on the surface of the PEMU. This excess charge incorporated small counterions and their associated waters of hydration yielding an open framework for diffusion of electroactive ferricyanide. Trivalent ferricyanide was able to “hop” through the PDADMAC-terminated film with ease due to exchange with small counterions associated with three positive sites within the PEMU. This expedited diffusion yielded limiting currents similar to those measured on a bare electrode until the membrane reached a sufficient thickness to slow ion diffusion. Once PSS was added to the membrane, extrinsic compensation by counterions was converted to intrinsic compensation by ionic crosslinking, releasing counterions into solution along with their waters of hydration. This conversion caused the PEMU to become glassy, which acted as a tough barrier for ferricyanide diffusion shown as a strong reduction in current. This behavior was determined through the use of a three electrode system in a temperature controlled cell using a platinum rotating working electrode, a saturated calomel reference electrode (SCE), and a platinum wire counter electrode. Cyclic voltammetry and chronoamperometry were used in conjunction for the determination of the sorption kinetics of PSS across a range of narrow molecular weights and concentrations.
From fundamental PEMU formation kinetics to thin film membrane applications, this dissertation addressed a variety of topics using polyelectrolytes. Future work should be directed at the improvement of our zwitterated PEMUs for the prevention of bacterial adhesion or their use in biofuel production and pollution control. The use of polyelectrolyte coacervate to produce free standing membranes demonstrates a method of producing uniformly thick membranes with low surface roughness that can be applied in ion selectivity and desalination applications. Finally, the demonstration of sorption kinetics by RDE should be implemented for other polyelectrolyte pairs to fully understand their behavior during Layer-by-Layer buildup, which is a commonly used PEMU construction technique.
APPENDIX A

LIST OF EXPERIMENTAL TERMS

AEDAPS 3-[2-(acrylamido)-ethyl(dimethyl ammonio)] propane sulfonate
AEDAPS-SS Supplementary Soaked AEDAPS
AFM Atomic Force Microscopy
ATCC American Tissue Culture Collection
Bp Benzophenone
CFU Colony Forming Unit
CV Cyclic Voltammetry
DIC Differential Interference Contrast
DMEM Dulbecco’s Modified Eagle’s Medium
DMFCs Direct Methanol Fuel Cells
DMTA Dynamic Mechanical Thermal Analysis
DSC Differential Scanning Calorimetry
EC Electron Capture
ECM Extracellular Matrix
EPS Extracellular Polymeric Substances
ExPEC Extruded Polyelectrolyte Complex
FTIR Fourier Transform Infrared Spectroscopy
GFP Green Fluorescent Protein
H₂O₂ Hydrogen Peroxide
H₂SO₄ Sulfuric Acid
HA Hyaluronic Acid
KBr Potassium Bromide
LB Luria Broth
LbL Layer-by-Layer
NaCl Sodium Chloride
PAA Poly(acrylic acid)
PAAAbp Poly(acrylic acid)-co-benzophenone
PAH Poly(allylamine hydrochloride)
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PDADMAC</td>
<td>Poly(diallyldimethylammonium chloride)</td>
</tr>
<tr>
<td>PDDA</td>
<td>Poly(diallyldimethylammonium chloride)</td>
</tr>
<tr>
<td>PE</td>
<td>Polyelectrolyte</td>
</tr>
<tr>
<td>PEC</td>
<td>Polyelectrolyte Complex</td>
</tr>
<tr>
<td>PECOV</td>
<td>Polyelectrolyte Coacervate</td>
</tr>
<tr>
<td>PEMU</td>
<td>Polyelectrolyte Multilayer</td>
</tr>
<tr>
<td>PES</td>
<td>Poly(ether sulfone)</td>
</tr>
<tr>
<td>PGA</td>
<td>Poly(glutamic acid)</td>
</tr>
<tr>
<td>PLL</td>
<td>Poly(L-lysine)</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methylmethacrylate)</td>
</tr>
<tr>
<td>PSS</td>
<td>Poly(styrene sulfonate)</td>
</tr>
<tr>
<td>QCM</td>
<td>Quartz Crystal Microbalance</td>
</tr>
<tr>
<td>Q-PEC</td>
<td>Quasisoluble Polyelectrolyte Complex</td>
</tr>
<tr>
<td>RDE</td>
<td>Rotating Disk Electrode/Electrochemistry</td>
</tr>
<tr>
<td>SCE</td>
<td>Saturated Calomel Electrode</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth Muscle Cell</td>
</tr>
<tr>
<td>SPAEK</td>
<td>Sulfonated Poly(Arylene Ether Ketone)</td>
</tr>
<tr>
<td>SPAEK-C</td>
<td>Sulfonated Poly(Arylene Ether Ketone) with Carboxylate Groups</td>
</tr>
<tr>
<td>SPEEK</td>
<td>Sulfonated Poly(Ether Ether Ketone)</td>
</tr>
<tr>
<td>TCP</td>
<td>Tissue Culture Plastic</td>
</tr>
</tbody>
</table>
APPENDIX B

MANUSCRIPT PERMISSIONS


REFERENCES


100. Rieger, J. The glass transition temperature T-g of polymers - Comparison of the values from differential thermal analysis (DTA, DSC) and dynamic mechanical measurements (torsion pendulum). *Polym Test* 2001, 20 (2), 199-204.


Lewandowski, Z. Biofilms in Water and Wastewater Treatment. 2011, pp 529-570.


BIOGRAPHICAL SKETCH

Kristopher D. Kelly was born and raised in Yorktown, VA on August 1\textsuperscript{st}, 1989. He attended Christopher Newport University in Newport News, VA where he attained his Bachelor of Science in Chemistry on May 14\textsuperscript{th}, 2011. Following his undergraduate career, Kristopher was admitted to the graduate program in chemistry at The Florida State University. Within a few months, he joined the Leo Mandelkern Lab of Polymer Chemistry under the guidance of Dr. Joseph Schlenoff in November of 2011. During his time at Florida State, he published three papers and presented research at numerous regional and national conferences. He defended his dissertation on May 10\textsuperscript{th}, 2016, where he was granted his Doctorate of Philosophy in Materials Chemistry.

EDUCATION

• 2011-2016: Ph.D., Materials, Polymer Science, and Analytical Chemistry, Florida State University, Tallahassee, FL. Dissertation title: “Kinetics and Applications of Polyelectrolyte Membranes and Multilayers”
  o Major Professor: Dr. Joseph Schlenoff, Leo Mandelkern Laboratory for Polymer Science
• Presentations
  o 250th ACS national meeting. “Spin Coating Polyelectrolyte Coacervate Thin Films”, Boston, MA, United States, August 16-20, 2015.
• 2007-2011: Bachelor of Science, Chemistry, Christopher Newport University, Newport News, VA
• Presentations
  o Sigma Xi Tidewater Chapter annual meeting 2010. “Aggregation of Pyrene as a Thin Film on an Indium Tin Oxide Substrate”, Newport News, VA, United States, November 19, 2010.
SKILLS

- 5 years of experience in polymer science, polyelectrolyte multilayers (Layer-by-Layer technique), polyelectrolyte complexes, and polyelectrolyte coacervates.
- 2 years of experience in zeolitic removal of heavy metals in an electrokinetic cell.
- 1 year of experience in fluorescent organic molecules in thin films.
- Developed novel free-standing polyelectrolyte thin films via spin coating.
- Developed polyelectrolyte thin films that prevent cell adhesion with bacteria attractive qualities.
- Experienced in FT-IR, ATR-FT-IR, UV-Vis, AFM, Profilometry, Ellipsometry, RDE, Tensiometry, and other analytical techniques.

EXPERIENCE

Graduate Teaching and Research Assistant, Department of Chemistry and Biochemistry, Florida State University, August 2011-August 2016.

- Developed new method for determining PEMU thin film conductivity using a mercury probe.
- Developed and optimized new method for producing multiple uniformly thin films of polyelectrolytes by manual buildup.
- Built lab made spin coater with highly tunable rotation rate and removable chuck for variety of substrate shapes and sizes.
- Developed new method for spin coating polyelectrolyte coacervate into uniformly thick and smooth PEC films with tunable thickness.
- Developed and optimized two new methods for the removal of PEC films from aluminum substrates for use as free standing or supported membranes.
- Built a thermally monitored electrochemical cell for in situ addition of polyelectrolyte solutions onto a rotating disk electrode.
• Experienced in characterization techniques:
  o Spectroscopy: UV-Vis Spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR), Attenuated Total Reflectance (ATR) FT-IR Spectroscopy
  o Surface Characterization Techniques: Ellipsometry, Profilometry, Atomic Force Microscopy (AFM), Static Contact Angle, Plasma Cleaning
  o Electrochemistry: Cyclic Voltammetry (CV), Chronoamperometry
  o Mechanical Characterization: Tensile testing

PUBLICATIONS


AFFILIATIONS

• American Chemical Society (ACS) (August 2011 – Present)
• American Outlaws (AO) (September 2011 – Present)
• Sigma Xi Scientific Research Society (October 2011 – Present)
• Sigma Phi Epsilon (February 2008 – Present)
ACCOMPLISHMENTS

- Awarded an associate membership in Sigma Xi for research presented at the undergraduate and graduate levels at the 2010 National Meeting of the Tidewater Chapter of Sigma Xi.
- Awarded Outstanding General Chemistry Teaching Assistant within first year of graduate program.

HOBBIES AND INTERESTS

- Founded Tallahassee Chapter of American Outlaws United States Soccer Supporters Group.
- Co-Founded Renegade Boxing Club at Florida State University.
- Sigma Phi Epsilon Fraternity: 4 consecutive terms on Standards Board, member coordinator for Sigma and Phi challenges, and planned events for entire undergraduate career.
- Class Council Treasurer: Managed events and financial transactions for Class of 2011 as well as organized all Homecoming events and class gift.
- DJ for WCNU Campus Radio.