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Characterization of Polyelectrolyte Multilayers and Complexes: Towards Bio-Implant Applications

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CHARACTERIZATION OF POLYELECTROLYTE MULTILAYERS
AND COMPLEXES: TOWARDS BIO-IMPLANT APPLICATIONS

By

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ABSTRACT

A self assembled multilayer technique based on alternating deposition of oppositely charged polyelectrolytes onto charged solid substrates is described in this thesis. The main principles and methodologies of this technique are explained in details in an effort to develop new technologies that would be beneficial for making new products or enhancing the quality of existing ones.

In this thesis, fundamental studies to characterize the water content, swelling by different salts, annealing effect, viscoelastic and mechanical properties of polyelectrolyte multilayers and complexes are illustrated and conducted.

Using the attenuated total reflectance Fourier Transform Infrared, ATR-FTIR, spectroscopy it is possible to probe the bulk of the PDADMA/PSS and P4VMP/PSS multilayers thus gaining valuable information about their water and counter-ion content. The multilayer resembles a polymeric network comprising regions of varying intensities of polyion-polyion interactions the extent and distribution of which are sensitive to the type of the polyelectrolyte used and to the type and concentration of the salt in the surrounding medium.

A distinction is afforded in terms of the swelling and doping abilities of 14 different salts based on their hydration state. For both PDADMA/PSS and P4VMP/PSS multilayers, one can cautiously generalize that hydrophobic ions are more effective dopers than hydrophilic ions.

Water content of those two multilayers was studied extensively using a range of different experiments conducted by ATR-FTIR technique. Osmotic pressure experiment is a good approach to characterize the water content inside a PEMU. Polyethylene glycol, PEG, a highly water-soluble polymer, was used as an osmotic stressing agent. Unlike salt solutions, PEG does not diffuse inside the PEMU from solution. It rather creates enough osmotic pressure to pull water out from the PEMU. A good estimate of the number of water molecules inside a multilayer was achieved using PEG.

Ion-pairing and water content are key factors influencing permeability of molecules into polyelectrolyte multilayers. They are also important in controlling the
multilayer mechanical properties which are especially important when considering the use of PEMUs in biomaterials applications.

Layer by layer buildup of polyelectrolyte multilayers on a germanium crystal and ATR-FTIR measurements of ratios of water to sulfonate and azide to sulfonate peaks allowed us to monitor the annealing of PDADMA-PSS multilayers of different thicknesses in different ionic strength solutions. The film loses extrinsic sites as it anneals.

PDADMA/PSS complexes were produced and their mechanical and thermal properties were characterized using different analytical methods. Dynamic mechanical analysis (DMA), thermogravimetric analysis (TGA), and mechanical stretching machines were used to conduct different experiments on those complexes. Swelling of the PDADMA/PSS complexes was investigated. We were able to get preliminary data about both the elastic modulus and the shear modulus of those complexes. Their water content, mechanical properties, thermal properties, and swelling behavior all encourage us to apply them in the biomedical field, in particular as bio-implants to replace intervertebral disc in the spinal cord. We are still investigating the different properties of those complexes and working on enhancing their structure and biocompatibility. We are synthesizing PAA-co-PAEDAPS and PDADMA-co-PAEDAPS for that purpose. The synthesis of those two polyzwetterions PZs is one of many steps taken towards producing biocompatible complexes that can be used as bio-implants.
CHAPTER 1
POLYELECTROLYTES, POLYELECTROLYTE
MULTILAYERS, AND POLYELECTROLYTE COMPLEXES:
DEFINITIONS, PROPERTIES AND APPLICATIONS

1.1 Polyelectrolytes: Definition and Applications

Any macromolecular species that dissociates into a highly charged polymeric molecule upon being placed in any ionizing solvent (e.g. H$_2$O) is termed a polyelectrolyte or polyanion. Smaller oppositely charged counter ions that tend to neutralize the charge on the repeating units of the polyelectrolyte preserving electro neutrality are usually accompanied by such dissociation.\textsuperscript{1-6} Polyelectrolytes are classified according to their origin into natural and synthetic. Proteins and DNA are examples of naturally occurring biopolymers while poly(diallyl dimethyl ammonium chloride), PDADMA, and poly(styrene sulfonic acid), PSS, are examples of synthetic polymers. A polyelectrolyte, in low ionic strength solutions, tends to be in its most extended and uncoiled form.\textsuperscript{2} This is mainly due to the intramolecular repulsion of the unscreened charges on each monomeric unit of the macromolecule. But if the ionic strength of the solution is increased, a polyelectrolyte tends to become thicker and more coiled due to the screening effects of polymer charges by the excessive presence of smaller salt counter-ions in solution as is shown in Figure 1.1.

Polyelectrolytes have found a number of important applications in the major fields of science, such as chemistry, biochemistry, physics, biophysics, biology, and engineering such as chemical, industrial, biomedical and materials engineering. Their applications in chemistry are mainly centered at the interface of polymer, materials, colloids, surface and analytical chemistry. Recently, polyelectrolytes have found extensive use in a variety of projects and technologies both in the academic and the industrial research and development areas. Polyelectrolytes have been used in the health and personal care industry as thickening reagents,\textsuperscript{7} rheology modifiers\textsuperscript{8} and viscosity
enhancers for shampoos, conditioners, deodorants and body lotions.\textsuperscript{9,10} They have also been used in water treatment,\textsuperscript{11} waste treatment,\textsuperscript{12} sludge dewatering\textsuperscript{13} and the pulp and paper industry as retention aids as well as flocculating and coagulating agents for solid-liquid separations. Other polyelectrolytes are used as additives to alter the physical properties of aqueous products. For instance, they are used for water retention, adhesive strength, binding power, film formation and protective colloidal, suspending and emulsifying actions. Recently Shiratori and Sato have designed a thin film coating of polyelectrolytes assembled from chitosan and another polyelectrolyte containing an enzyme which was extracted from bamboo.\textsuperscript{14} The thin film wrap was effective in preventing decomposition and ripening of fresh fruits such as melons by suppressing their emission of ethylene gas, and thus kept these fruits fresh for longer periods of time. In addition to that, assembling these polyelectrolytes into ultra thin film composite membranes is one of the most important applications and has received significant attention and interest recently.\textsuperscript{2,5,9,14-25}

![Add NaCl](image)

**Figure 1.1** Scheme showing the effect of ionic strength on the shape of a polyelectrolyte molecule in solution where + and – signs inside circles correspond to Na and Cl ions respectively.
Figure 1.2 Some applications of PEMUs: (A) PEMUs found their way into contact lenses industry. [Refojo, M. F. *Journal of Applied Polymer Science* **1967**, *11*, 1991-2000].

(B) PEMUs also been used as food wrappers, the coated melon (left) compared to the uncoated melon (right). [Shiratori, S.; Sato, T. *PMSE Preprints* **2003**, *88*, 117-118]. (C) PEMUs been also used in corrosion studies, here two steel wires are shown where one was coated with a multilayer (right) and compared to the bare wire (left). [Farhat, T. R.; Schlenoff, J. B. *Electrochem. Solid-State Lett.* **2002**, *5*, B13-B15].
1.2 Polyelectrolyte Complexes: Introduction and Applications

When two oppositely charged polyelectrolyte solutions are mixed, the polymers have a tendency to form a dense phase and to separate from the solvent. The dense phase is known as a polyelectrolyte complex (PEC) (Figure 1.3).\textsuperscript{26,27} The complex is thermally stable with electrostatic interactions constituting the predominant attractive forces.\textsuperscript{27}

Polyelectrolyte complexes (PECs) have received the attention of scientists since the early 1930s\textsuperscript{2} through the work of Bungenberg de Jong and co-workers who found that some naturally occurring polyelectrolytes could interact in aqueous media to form colloidal complexes which they called complex coacervates. Insoluble PECs have been known since the early 1960s\textsuperscript{26} due to the availability of a number of high linear charge density synthetic polymers. Many properties of such complexes were widely described in the 1960s by Alan Michaels and coworkers,\textsuperscript{26,28} some of which include their swelling and plasticizing by aqueous electrolytes. However, it was only in recent years that industrial interest in such materials started to develop.

The interaction between positively and negatively charged polyelectrolytes appears to be athermal.\textsuperscript{27,29} Thus the favorable association is rather entropic in origin and not enthalpy driven. A net gain in entropy is achieved mainly because this interaction causes the liberation of small salt counter ions (Figure 1.3) and a number of water molecules from the hydration shell around the polymers.\textsuperscript{30} The structure of the PEC does not resemble a ladder or DNA-like configuration, where a polymer is associated with only one other complementary molecule; they rather form amorphous scrambled salts.\textsuperscript{30,31} The electrostatic links are diffuse and take place randomly between the oppositely charged sites.
Figure 1.3 Scheme showing the formation of PEC from two oppositely charged polyelectrolytes (PDADMA and PSS), accompanied with the release of salt counter ions.

PECs have many principle projected uses such as hydrophilic soil binders preventing wind and water erosion, membranes for dialysis, ultrafiltration, battery separators and fuel cell membranes, electrically conductive coatings, medically conductive coatings, contact lenses, and enzyme mimics. The importance of PECs lies in the fact that they constitute the theoretical foundation of polyelectrolyte multilayers PEMUs which, in recent years, have also found their way into commercially available products such as contact lenses and antibacterial food wrappers.

In this thesis, extensive research was done on different PECs studying their formation, properties and behavior in an effort to apply them in certain biomedical fields, in particular as artificial replacement for intervertebral discs (IVDs) as will be discussed in Chapter 5 in details.
1.3 Polyelectrolyte Multilayer Thin Films: An Overview

The concept of ion-pairing which defines the formation of polyelectrolyte complexes was extended further and applied to assemble thin film membranes. A new layer-by-layer (LBL) sequential assembly technique was utilized.\textsuperscript{2,42,43} The LBL assembly technique was first mentioned by Iler in 1966.\textsuperscript{44} In 1991, however, Decher and Hong expanded that technique and brought it to the forefront of materials science and engineering.\textsuperscript{2,45} Since then, research in this field had attracted the attention of scientists, all over the world, across the academic and industrial fields. The technique is based on the idea of alternating the rinses of a substrate or a solid support in two oppositely charged polyelectrolytes along with rinse steps in between to remove any excess material that is loosely bound to the surface.\textsuperscript{2,46} Figure 1.4 is a diagram detailing the layer-by-layer assembly technique. Thus, one can build as many layers as needed, with any desired thickness, depending on the particular type or requirements of the experiment. Ultra thin films could be built offering high flux and selectivity for membrane separations, filtrations and purifications applications.\textsuperscript{17,21,46,47} Thicker membranes could also be built offering hydrophobic coatings,\textsuperscript{48} hydrophilic coatings\textsuperscript{48} or anti-corrosive coatings.\textsuperscript{49} Multilayers reported in the literature had thicknesses that ranged between 10 nm and 10 µm.\textsuperscript{51-53}

This LBL technique is superior, in many respects, to other deposition techniques that are reported in the literature such as vacuum deposition, solvent casting, spin coating or Langmuir-Blodgett (LB) deposition. Vacuum deposition requires specialized expensive instrumentation and specific types of substrates. Solvent casting produces thick and non-uniform films. Solvent casting and spin coating do not follow contours well. The earliest technique used to fabricate multilayer films was invented by Langmuir and Blodgett\textsuperscript{54} to make LB films.
Figure 1.4 A schematic representation of the formation of a polyelectrolyte multilayer on charged surface (upper), and another cartoon showing the ordering of a PEC into a PEMU (Lower). The substrate is dipped in both cationic and anionic solutions for 5 minutes each, while rinsed in pure water for 30 seconds in between.
LB films are highly ordered, have uniform controlled thickness and continuous. However, they have several limitations. The requirements for substrates are stringent: they must be smooth, homogeneous\textsuperscript{14} and have regular shape. Further more LB fabrication requires expensive equipment. LB multilayers have limited stability against solvents and thermal treatments, and defects formed in a given layer are difficult to cover up by subsequent layers.\textsuperscript{55} Also, it is a very slow technique. On the other hand, the LBL assembly technique is a very robust, reproducible, cost effective and environmentally friendly method. It is done at room temperature, so there is no need for any vacuum equipment or special instrumentation. It can be used to assemble various types of materials,\textsuperscript{56} polymers,\textsuperscript{57} composites,\textsuperscript{58} clay,\textsuperscript{59} proteins,\textsuperscript{60} dyes,\textsuperscript{61} carbon nanotubes\textsuperscript{62} or nanoparticles.\textsuperscript{63} This is a limitation of other techniques because they only work for special kinds of materials. Also, it can be coated on various kinds of substrates such as silicon, gold, platinum, plastic, glass, quartz, stainless steel, clay, nanoparticles, blood cells and colloidal particles.\textsuperscript{64} The whole process can be automated making the coating procedure less time consuming and applicable for commercial applications where productivity and labor are major problems. Above all, this technique offers very precise control over the thickness and unprecedented uniformity of the coating or the membrane built, down to the sub nanometer scale.

1.3.1 Ion Pairing in PEMU

Polyelectrolytes in solution self assemble on the charged surface via complexation with the constantly reversing surface charge throughout PEMU construction. The evolving three dimensional structure is stabilized through anchoring points which are provided by ion-pairs created between oppositely charged polyelectrolyte segments.\textsuperscript{65,66} Polymers are solvated before complexation, but the requirements of the associated state set a lower limit on the excluded volume. Some water molecules of hydration are thus freed which enhances the overall entropy gain, which facilitates the athermal ion-pairing mechanism as we mentioned earlier.

Opposite charges are matched either intrinsically or extrinsically. Salt ions such as sodium and chloride for example, are capable of interacting with multilayers during and after build-up.\textsuperscript{67} They tend to enter the bulk of the multilayer due to a difference in
chemical potential and compensate charges on the polymer repeat units, a process which we refer to as extrinsic compensation.\textsuperscript{21} PEMUs differ in the layer to layer charge compensation ratio and there always exist a number of charges that are compensated by counter ions from solution. The sites created by NaCl salt are called extrinsic sites, which play an important role in the swelling, thickness, flexibility, and mechanics of the multilayer; in contrast to intrinsic sites between polyelectrolyte segments which is the dominating conformation in salt-free solutions.\textsuperscript{17,46,68}

Previous experiments showed that extrinsic charge compensation exists at the surface of the multilayer (overcompensation region) while strong intrinsic charge compensation (ion-pairing) exists within the bulk.\textsuperscript{68,69}

1.3.2 Factors Affecting Structure and Buildup of PEMU

Alternating deposition of solutions of oppositely charged polymers on a certain substrate leads to adsorption of reproducible quantity of material and reverses the charge on the surface priming it ready for the next deposition cycle.\textsuperscript{42} The amount of the polyelectrolyte deposited on the surface depends to a large degree on the ionic strength of the solution, the type of the salt used, and the electrolyte species.\textsuperscript{66} All these are factors we investigated in our work and discussed in Chapter 3.

The increase in the ionic strength of the solution leads to compensation of polyelectrolyte charges by the oppositely charged counter ions from the solution.\textsuperscript{66} This will increase the hydrophobicity of the polymer and more polyelectrolyte is needed in the surface charge compensation which results in thicker deposited layers.\textsuperscript{70}

The salt type is another factor affecting the growth process.\textsuperscript{66,71} Salt ions are capable of interacting with multilayers during and after build-up. Different salts have varying ability to swell the multilayer. Since we are considering ion-exchange as the main driving reason for adsorption, we expect that different ions with differing affinity should modulate the amount of adsorption.

Electrolyte species is also one other factor that controls the amount of polyelectrolyte deposited on the surface.\textsuperscript{66} Multilayers built from polymers with higher charge density were found to be more stable with a limited mobility since the
polyelectrolyte molecules will be strongly bound both to the newly adsorbing molecule and to the underlying polymer.

1.3.3 Water Content of PEMUs

The water content\(^{49,68,72-75}\) of a polyelectrolyte multilayer is a very important factor in regulating its build-up and applicability to different fields. Multilayers which are more hydrated and hydrophilic are good inhibitors for biological growth,\(^{76}\) while multilayers with very low water content and hydrophobic properties are an excellent choice for corrosion protection.\(^{49}\)

Water can exist inside the multilayer\(^{68,74,77-80}\) in different configurations. It can closely hydrate electrostatic pairs, either between polyions or between doping ions and polyions. Water hydrating electrostatic pairs can also do so weakly and be further away from the site but still associated with it. Water can also exist in pools trapped inside the more hydrophobic regions of the PEMU.

The content of this thesis project includes studying the swelling behavior of PEMUs, their water content, and hydration contributions to association in both PECs and PEMUs. All these topics will be discussed in details in Chapter 3. Having a good idea about the water content of our PEMU will pave the way for our work to apply them as bio-implants as will be discussed in more details in Chapter 5.

1.4 Thesis Outline

The experimental details of this thesis are described in Chapter 2, where emphasis is on instrumentation, with more experimental details listed in each subsequent chapter.

In Chapter 3, polyelectrolyte multilayer layer by layer buildup and profound differences in their swelling behavior in water were studied comprehensively in various salt combinations using attenuated total reflectance Fourier transform infrared spectroscopy ATR-FTIR. Swelling with water partly due to exchange of different counter ions has been monitored by measuring characteristic significant infrared (IR) signals of water in the PEMU using ATR-IR. Poly(4-styrenesulfonate) (PSS), a negatively charged polymer, poly(diallyldimethylammonium chloride) (PDADMA) and poly(4-
vinylmethylpyridinium) (P4VMP) both positively charged polymers were used in our investigation. Similar methods allowed for exact measurements of extrinsic sites within the PEMU by calculating the ratios of IR active counter ions peaks to polyelectrolyte layer peaks. A good quantitative estimation was reached for the swelling constant ($\Psi_{S\theta}^{H,\varphi}$), doping constant ($k_{dop}$) and the hydration numbers ($H_2O_{hydrat}$) for all ion pairs used. Also, poly(ethylene glycol) (PEG), a high osmotic stressing agent, was used to provide information about water content of the PEMU.

In Chapter 4 annealing of PEMUs was monitored on PDADMA/PSS PEMUs with different number of layers and in various ionic strength solutions using ATR-FTIR. This method provided good, in situ quantitative data about the conformation of the PEMUs bulk with regards to water content and intrinsic/extrinsic compensation by employment of $N_3^-$ ion, an IR active probe used in very low concentration (0.01M).

Chapter 5 deals with producing compact polyelectrolyte complexes (ComPECs) and studying their mechanical properties versus changes in environmental conditions. Our goal is to produce ComPECs which can be used in certain biomedical applications, in particular as artificial intervertebral discs applied in surgical operations performed on spinal cord of the human body.
CHAPTER 2
EXPERIMENTAL TECHNIQUES AND INSTRUMENTATION

2.1 Chemicals and Materials

All chemicals were used as purchased unless otherwise specified. Poly(diallyldimethylammonium chloride) (Aldrich) $M_w = 3.69 \times 10^5$ and $M_w/M_n = 2.09$, poly(styrene sulfonate) (Scientific Polymer Products) $M_w = 5.75 \times 10^4$ and $M_w/M_n = 1.03$, and poly(ethylene glycol) (Sigma) were used as received. Poly(4vinylimethylpyridinium), $M_w = 65,500$ was purchased from Polysciences Inc. 1,3-propane sultone (PS), acrylic acid and diallyl dimethyl ammonium chloride (DADMAC) were obtained from Aldrich. 3-[2-(acrylamido)-ethyl dimethyl amine] (AEDA) was obtained from GFS Chemicals Inc. All salts were purchased from Fisher Scientific: sodium chloride (NaCl), sodium fluoride (NaF), sodium iodide (NaI), sodium bromide (NaBr), sodium azide (NaN₃), sodium thiocyanate (NaSCN), sodium nitrate (NaNO₃), sodium perchlorate (NaClO₄), lithium chloride (LiCl), potassium chloride (KCl), cesium chloride (CsCl), calcium chloride (CaCl₂), magnesium chloride (MgCl₂), and yttrium chloride (YCl₃). Solutions were made with 18 milli-ohm deionized water (Barnstead, E-pure, Milli-Q). Very accurate and precise concentrations of the salt solutions used were achieved using conductivity experiments. Structures of polyelectrolytes used are shown in Figure 2.1.
Figure 2.1: Polyelectrolytes used in this work starting from left side to right side: Poly (styrene sulfonate), poly (4-vinylmethylpyridinium) and poly (diallyldimethylammonium chloride).
2.2 Instrumentation

2.2.1 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

When a beam of light is confined across two interfaces of different refractive indices \(n_1\) and \(n_2\) part of the light is reflected, while the other part is transmitted according to Snell’s law:

\[ n_1 \sin \theta = n_2 \sin \alpha \]  

(2.1)

Where \(\theta\) is the angle of incidence, and \(\alpha\) is the angle of refraction.\(^{81}\)

In ATR-FTIR setup, light propagates from a dense medium of refractive index \(n_1\) to a rare medium (i.e., air or vacuum) of refractive index \(n_2\). The incident light traverses the surface at an angle of incidence \(\theta\), while the reflected portion propagates with perpendicular polarization. When \(\theta\) is bigger than the critical angle \(\theta_c\), light will totally internally reflect in the denser medium. The critical angle \(\theta_c\) is given by:

\[ \theta_c = \sin^{-1} \frac{n_2}{n_1} \]  

(2.2)

\(n_{21}\) being the ratio of the refractive index of the rare medium to the dense medium.

The IR radiation is typically focused by a mirror onto the entry face of the ATR crystal. When \(\theta\) is bigger than the \(\theta_c\), light will totally internally reflect in the denser medium.

An evanescent wave extends beyond the surface of the ATR crystal and probes the coating or the solution in direct contact with the crystal. The depth of penetration \(d_p\) represents the distance at which the evanescent wave drops to \(1/e\) times the intensity at the surface. The depth of penetration can be calculated using the following formula:\(^{82,83}\)

\[ d_p = \frac{\lambda}{2 \pi n_1 (\sin^2 \theta - \frac{n_2^2}{n_1^2})^{1/2}} \]  

(2.3)
Where $\lambda$ is the wavelength of light propagating through the rare medium (i.e., air or film), $n_1$ is the refractive index of the dense medium (i.e., ATR crystal) and $n_{21}$ is the same as in Equation 2.2. Typical values for experiments using Ge crystal ($n_2 \approx 4.0$), $\lambda$ at 1033 cm$^{-1}$ and $\theta=45^\circ$ gave $d_p$ values $\sim 0.60 \, \mu$m. The beam enters the ATR crystal normal to the trapezoidal edge minimizing reflection losses. The internal standard, which all absorbance measurements are matched to, is the sulfonate [SO$_3^-$] stretch at 1033 cm$^{-1}$.

ATR deposition measurements were performed with a Nicolet Nexus 470 fitted with a 0.5 mL capacity flow-through ATR assembly housing a 70 x 10 x 6 mm zinc selenide or germanium crystal (Specac Benchmark) depending on the particular type of experiment conducted. The physical and chemical properties for both crystals are specified in Table 2.1. Multilayers were deposited on the ATR crystal while it was mounted on the flow cell by passing polyelectrolyte and rinse solutions, in an alternating manner, through the cell.

Figure 2.2 This sketch represents a section of ATR-FTIR flow cell and the germanium crystal
Table 2.1 Comparison of the physical properties of the germanium and zinc selenide ATR crystals.

<table>
<thead>
<tr>
<th>Physical Property</th>
<th>ZnSe ATR crystal</th>
<th>Ge ATR Crystal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Useful ATR Range (cm⁻¹)</td>
<td>2000-650</td>
<td>5500-675</td>
</tr>
<tr>
<td>Practical pH range</td>
<td>3-10</td>
<td>1-14</td>
</tr>
<tr>
<td>Refractive Index at 1000 cm⁻¹</td>
<td>2.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Maximum Useful Temperature in Air (°C)</td>
<td>300 °C</td>
<td>270 °C</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>5.27</td>
<td>5.32</td>
</tr>
<tr>
<td>Hardness</td>
<td>137</td>
<td>550</td>
</tr>
</tbody>
</table>

2.2.2 Ellipsometry⁸⁴,⁸⁵

It is an optical technique which utilizes a monochromatic light of known polarization and wavelength to determine the refractive index and thickness of thin films (typically<200nm). It is based on the principle that the state of polarization of a linearly polarized light becomes elliptical after reflection from the surface of the sample. The ellipse of polarization is characterized by an amplitude ratio, Ψ, and a relative phase shift, Δ, between the two wave components of the electric field of the beam. Computer software analyzes and compares Ψ and Δ of the reflected light to that of the incident light, and based on that it calculates the thickness and refractive index of the film.

Thickness was measured with a Gaertner Scientific L116S autogain ellipsometer with 632.8 nm radiation at 70° incident angle. A refractive index of certain value was employed for the multilayers. The thickness measurements were done in two steps. Initially, the ellipsometer was asked to guess the refractive index of the multilayer as well as its thickness. Subsequently, the refractive index was fixed at the value obtained in the first run, and thickness measurements are recorded as an average of 10 different trials on different regions throughout the surface of the coating.
2.2.3 Profilometry\textsuperscript{86,87}

A KLA-Tencor P-15 profiler, having a standard range diamond stylus head (Microhead llsr) with a scan area of 200 x 200 mm. It measures surface profiles, including film thickness, by mechanically tracing the surface with a stylus. Thicknesses greater than 100 Å may be measured by the profilometer.

2.2.4 Fourier Transform Infrared Spectroscopy

Transmission Fourier transform infrared (FTIR) spectra were recorded using 32 scans and at 4 cm resolution using a Nicolet Avatar FTIR on multilayers deposited on double polished silicon wafers (Si 100, undoped, 1” diameter, 0.5 mm thick, purchased from Topsil Inc). FTIR was also done on controlled conditions of constant temperature and humidity to guarantee reproducibility.\textsuperscript{68}

2.2.5 Atomic Force Microscopy\textsuperscript{88}

AFM images were performed in air using Digital Instruments Nanoscope Dimension 3100 unit (Digital Instruments Inc., Santa Barbara, CA) with a type RTESP silicon tip with a 125 µm length and 300 KHz resonance in Tapping Mode. Scanning probe microscope provides morphology/topology at near-atomic resolution.

2.2.6 Dynamic Mechanical Analysis (DMA)\textsuperscript{89,90}

Dynamic mechanical analysis (DMA) is a thermo analytical method by which the mechanical behavior of a sample subjected to a specific temperature program is studied under the effect of a load which changes with time. DMA characterizes the viscoelastic properties of materials. In DMA, a complex modulus $E^*$, elastic modulus $E'$, and an imaginary loss modulus $E''$ are calculated, all three allowing better characterization of the material, because we can examine the ability of the material to return or store energy $E'$ to its ability to lose energy $E''$ and the ratio of these effects which is the damping effect (Figure 2.3).
Figure 2.3 This figure shows the relationship between storage modulus $E'$, loss modulus $E''$, complex modulus $E^*$, and loss angle $\delta$ where damping or $\tan \delta = \frac{E''}{E'}$.

By clamping our complexes into the DMA apparatus and an oscillatory force is applied which causes a sinusoidal stress to be applied to the complex generating a sinusoidal strain. By measuring both the amplitude of the deformation as the peak of the sine wave and the lag between the stress and strain sine waves (Figure 2.4), quantities like the modulus (stiffness) and the damping (energy loss) can be calculated as a function of time and temperature.
Figure 2.4 This figure shows the relationship between stress and strain during a dynamic mechanical test. The continuous curve represents applied stress while the dashed curve represents the measured strain.

2.2.7 Thermo-gravimetric Analysis (TGA) \textsuperscript{91,92}

Thermo-gravimetric Analysis (TGA) is a technique where a sample is heated at constant, usually linear, rate and meanwhile it is continuously weighed. The resulting plot of weight change (mass) (whether loss or gain) versus temperature gives information concerning the thermal stability and composition of the sample, specially the amount of water, organic compounds, and inorganic compounds. By burning the sample, water will evaporate first, followed by organic contents, and finally the inorganic particles. Weight % of all sample components can be determined by TGA. TGA analysis was acquired on
TA instruments SDT-2960. All samples were run in alumina pans under N\textsubscript{2} or O\textsubscript{2} atmospheres at a heating rate of 10 °C/ min.

### 2.2.8 Mechanical Testing

Mechanical testing of the PECs was conducted by SHIMAZU tensile machine (SHIMAZDU INC.) according to the ASTM D 638. This instrument (Figure 2.5) can be used to get certain data such as: tensile strength (at yield and at break), tensile modulus, strain, elongation and % elongation. We used this instrument in the college of engineering in order to find the elastic modulus of our complexes.

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**Figure 2.5** SHIMADZU tensile instrument for mechanical testing experiments [http://www.ptli.com/video/tensile.jpg].
CHAPTER 3
SWELLING OF POLYELECTROLYTE MULTILAYERS WITH DIFFERENT SALTS

3.1 Introduction

When two polyelectrolyte solutions of opposite charges are mixed, the polymers have a tendency to form a dense phase and to separate from the solvent. The dense phase is known as a polyelectrolyte complex (PEC). Polyelectrolyte complexes (PECs) have been known to scientists since the early 1930s. Insoluble PECs have been known since the early 1960s due to the availability of a number of high linear charge density synthetic polymers. Many properties of such complexes were widely described in the 1960s by Alan Michaels and coworkers, some of which include their swelling and plasticizing by aqueous electrolytes. Michaels stated that “moderate or even low concentrations of certain electrolytes will plasticize the PEC and this plasticizing action is manifested as a softening and swelling of the hydrogel in the case of neutral PEC of low water content, and results in network collapse and hydrogel shrinkage in case of non-neutral PEC with low gel-water content.” He compared the effect of different ions on swelling of PECs and found out that different ions vary in their swelling effect, which, according to him, reflect the relative site-binding capacities of these ions to polyions. Salt ions can break the electrostatic bond between two polyelectrolytes and create defects in the PEC where some ionic groups become extrinsically compensated by salt counter ions. The amount of added salt determines the amount of extrinsically compensated segments. The effect of salt on the intrinsically compensated polyelectrolyte complex segments can be explained by pointing out a free energy change. The change in free energy is basically due to an entropy gain caused by release of counterions originally localized nearby polyelectrolyte coils.

PECs have lots of principle projected uses such as hydrophilic soil binders preventing wind and water erosion, membranes for dialysis, ultrafiltration,
battery separators and fuel cell membranes, electrically conductive coatings, medical applications, contact lenses, and enzyme mimics. The importance of PECs lies in the fact that they constitute the theoretical foundation of PEMUs which, in recent years, have also found their way into commercially available products such as contact lenses and food wrappers.

Layer-by-layer build-up of polyelectrolytes has proven to be an excellent procedure of making thin and well-defined organic films. The amount of the polyelectrolyte deposited on the surface depends to a large degree on the ionic strength of the solution, the type of salt used, and the electrolyte species. All these are factors which we investigated in our work.

The increase in the ionic strength of the solution modifies the overall free energy of interaction by competing for polymer charge, a mechanism which is better defined as competitive ion pairing which implies that the main driving force is ion pairing between polyelectrolyte segments, driven by release of counterions and water. The compensation of polyelectrolyte charges by the oppositely charged counterions in the solution usually increases the hydrophobicity of the polymer. This results in thicker deposited layers. The salt type is another factor affecting the growth process. All these effects were found to vary with the counterion almost following the trend of the Hofmeister series. This series was named after its inventor and the Hofmeister effect refers to an ordered sequence of ions, also called lyotropic series and depends on the ability of the salts to precipitate a given macromolecule. The Hofmeister effect of anions was found to be greater than the effect of cations. The anions in the Hofmeister series were divided into two groups according to their hydrophobicity from the least hydrophobic anions to the most hydrophobic ones.

Salt ions such as sodium and chloride for example, are capable of interacting with multilayers during and after build-up. Singly charged counterions can easily diffuse across a PEMU and loosen up and eventually break electrostatic bonds formed between oppositely charged polyelectrolytes, transforming some of the intrinsically compensated segments into extrinsic sites within the bulk of the PEMU. This process can be defined by the following equilibrium:
Pol$^+$ Pol$^-$ + M$_{aq}^+$ + A$_{aq}^-$ $\leftrightarrow$ Pol$^+$ A$_{aq}^-$ + Pol$^-$ M$_{aq}^-$  \hspace{1cm} (3.1)

Where Pol$^+$ and Pol$^-$ refer to positive and negative polyelectrolyte segments in the multilayer, M$^+$ and A$^-$ are salt ions, and $m$ refers to the multilayer phase.

Determination of swelling in situ of certain pairs of electrolytes was investigated using atomic force microscopy (AFM).\cite{70} Salt ions tend to enter the bulk of the multilayer due to a difference in chemical potential and compensate charges on the polymer repeat units. This process is referred to as extrinsic compensation.\cite{42} Sites created by salt are called extrinsic sites in contrast to the intrinsic sites between the polymeric segments which is the dominating conformation in salt-free solutions.

The free energy of ion pairing between polyelectrolyte segments, “interaction energy,” or “energy of association” is the reverse of Equation (3.1). Different pairs of polyelectrolytes and different salt ions are expected to have different interaction strengths.

Osmotic stress experiments\cite{102-106} offer another approach to measure water movement in and out of a film. Poly(ethylene glycol), a neutrally charged but highly hydrated polymer, was used as the osmotic stressing agent. PEG does not diffuse into the PEMU from solution due to its large radius of gyration and creates enough osmotic pressure to pull water out from the PEMU.

3.2 Experimental Section

3.2.1 Instrumentation

ATR measurements were performed with a Nicolet Nexus 470 FTIR fitted with 0.5 ml; flow-through ATR assembly housing a 70 x 10 x 6 mm, 45° germanium crystal (Specac Benchmark). The crystal was cleaned using 50:50 v/v ethanol/H$_2$O in saturated NaCl solution with sonication for 20 minutes. The crystal was then washed with 18 milli-ohm deionized water and dried with a stream of nitrogen gas. PEMU deposition on the Ge crystal was always carried out by hand by flowing polyelectrolyte solutions (10mM polymer concentration (based on the repeat unit) and 1.0M NaCl concentrations) in an alternating manner through the ATR housing assembly with 1.0M NaCl rinse solution in-
between. The film was then annealed with 1.0M NaCl for one week. The deposition time for each layer was five minutes. The time for rinse was one minute.

Salt solutions (made from 14 different salts) were applied on the 2 multilayers with each salt solution prepared and diluted to get a wide range of different concentrations (5-7 different concentrations for each salt used).

The “osmotic stress” experiment exposed PEMU thin films to PEG concentrations ranging from 0 to 30% by weight in solutions prepared with different NaCl contents before and after annealing.

All spectra were recorded with 256 scans and 4 cm\(^{-1}\) resolution. Initial background spectra were recorded on the bare, dry germanium crystal in order to preserve the water and sulfonate peaks in subsequent samples taken. Spectra were also recorded for the LBL buildup and shown below (Figure 3.1) for both the PDADMA-PSS and for P4VMP-PSS multilayers. The mole ratio of water to sulfonate was determined from the area under the respective peaks (range from 3706 - 2979 cm\(^{-1}\) for water and from 1052 – 989 cm\(^{-1}\) for sulfonate). To calibrate instrument response to concentration of sample a 1.0 M solution of poly(styrenesulfonate) with a mole ratio of water to sulfonate of 55.6 was passed over the uncoated crystal. Using the sulfonate signal as a convenient internal standard normalizes out differences in absolute absorbance intensities of water (or propyl groups) that result from changes in refractive indices of the multilayer. The ranges used for the IR active salts are listed below:
Table 3.1 Values of the ranges used for the 4 Infrared active salts.

<table>
<thead>
<tr>
<th>Anion</th>
<th>Range used (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitrate</td>
<td>1434 - 1267</td>
</tr>
<tr>
<td>azide</td>
<td>2123 - 1957</td>
</tr>
<tr>
<td>perchlorate</td>
<td>1116 - 1052</td>
</tr>
<tr>
<td>thiocyanate</td>
<td>2105 - 2017</td>
</tr>
</tbody>
</table>

The results for these salts were also standardized by using 1.0M solution of each salt and passing it over the dry and bare crystal.

Figure 3.1 IR examples of spectra. A – NO\(_3^–\), B – ClO\(_4^–\), C – N\(_3^–\), D – SCN\(^–\), E – PDADMA/PSS water content, F – P4VMP/PSS water content. All spectra were recorded with 256 scans and 4 cm\(^{-1}\) resolution. The mole ratio of water to sulfonate was determined from the area under the respective peaks (range from 3707 - 2980 cm\(^{-1}\) for water and from 1053 – 989.3 cm\(^{-1}\) for sulfonate).
3.3 Results and Discussion

3.3.1 Layer by Layer Build-up

Typical LBL build-up of PDADMA/PSS on ATR crystal, from solutions of 10 mM polymer concentration and 1.0 M NaCl, LiCl and KCl concentration respectively was performed in order to build 30 layers which are thick enough to cross the evanescent wave of the ATR which is 600 nm for our system. The dry thickness for the 30 layers film was measured by profilometer and was found to be ~ 980 nm. The rinsing step between each polymer layer, which removes unadsorbed polymers, was done using 1.0 M concentration of same salt used in polymer solution. Results show that the thickest PEMU is built with KCl, K$^+$ being the least hydrated anion followed by Na$^+$ and then Li$^+$ as shown in Figure 3.2. The trend reveals that the least hydrated ions yield thicker films. Since we are considering ion-exchange as the main driving reason for adsorption, we expect that different ions with differing affinity should modulate the amount of adsorption. As a rule, the ion that swells the exchanger the least, binds more strongly making the PEMU more stable.

The alternating behavior observed in the multilayer LBL build-up in Figure 3.2 can be explained by osmotic swelling and shrinkage of PEMU from negative to positive layer additions. When PDADMA (+) is added it expands the PEMU since it is more hydrophilic. The next PSS (-) depositing layer shrinks the multilayer since PSS is more hydrophobic than PDADMA (+). The decrease in the sulfonate signal when a PDADMA layer is added might be due to the fact that this added layer takes more sulfonate group further from the ATR crystal, where they are sampled more weakly with the evanescent wave. This alternating phenomenon decreases as the evanescent wave in the IR is surpassed in height by the building PEMU. The PEMU becomes thick enough to entirely contain the evanescent wave, and thus no additional signal is observed, though the multilayer is still growing linearly. As the film becomes thicker than 600 nm, which is the thickness of the evanescent wave, the percent change in volume due to the swelling/shrinking by a layer addition becomes negligible.

This fluctuating behavior is also affected by the type of polymers used. The build-up of P4VMP/PSS multilayer in 1.0M NaCl took 330 layers to reach a thickness
comparable 30 layers of PDADMA/PSS 1.0M NaCl built with the same technique as seen in Figure 3.2. This difference in the number of layers needed to cross the evanescent wave gives us an idea about the effect of polymer charge density on the multilayer formation and stability. Multilayers built from polymers with higher charge density (P4VMP/PSS) are more stable and the mobility of the polyelectrolyte molecules will be strongly limited since they are strongly bound both to the newly adsorbing molecule and to the underlying polymer. This strong polyelectrolyte behavior builds a thin, strongly interacting film and it requires many layers in order to get similar thickness to PDADMA/PSS PEMU. At the same time, the alternating behavior is much less noticeable in the P4VMP/PSS film buildup mainly due to the fact that less collapsing of PEMU occurs when either polyelectrolyte is added, maybe because they are both more strongly and equally interacting with each other.

3.3.2 Doping Equilibrium

PDADMA/PSS and P4VMP/PSS multilayers were annealed with 1M NaCl for one week. Annealing eliminates most of the extrinsic sites and provides more interaction between poly-ions on subsequent layers. When a multilayer is annealed, the length of charge penetration is inherently decreased due to poly+/poly- coupling and expulsion of counter ions from the bulk of the multilayer. This leads to a lower concentration of excess charge closer to the surface (while the polyelectrolyte amounts do not change). The extrinsic site concentration is proportional to the concentration of NaCl in solution, and the equilibrium constant for that reaction is written as:

$$K_{dop} = \frac{y^2}{(1 - y)[M^+]_{AQ}[A^-]_{AQ}} = \frac{y^2}{[MA]_{aq}^2} \quad \text{as} \quad y \to 0 \quad (3.2)$$

Where $y$ is the “extrinsic site concentration,” or doping level, caused by the presence of polyelectrolyte ionic groups which are neutralized by small counterions, and $(1-y)$ can be defined as the amount of intrinsically compensated regions when the multilayer is placed in a salt solution.
Figure 3.2 Plot of sulfonate peak area at 1052 nm (y-axis) versus number of layers. Panel A depicts LBL buildup of 30 layers PDADMA/PSS (10mM polymer concentration each) using three different salts (1.0M concentration each) for counterions: Δ represents KCl, □ represents NaCl, and ◊ represents LiCl. Panel B represents buildup of 330 layers of P4VMP/PSS using 1.0M NaCl as a counterion. Panel C represents P4VMP/PSS buildup for the first 30 layers. Observe how much thinner this film is compared to 30 layers of PDADMA/PSS. The odd layers represent additions of PDADMA or P4VMP while the even ones represent additions of PSS.
Even though the introduction of salt ions and the formation of extrinsic sites is a reversible process and occurs as long as the multilayer is stable in salt, polyelectrolyte rearrangement to produce a system with lower total free energy occurs only once, after the multilayer is first exposed to a solution of considerable ionic strength. Polyelectrolyte multilayer annealing causes thickness and surface roughness to decrease. The fact that salt can cause polyelectrolyte rearrangement and polymer charge matching in multilayers is attributed to the fact that entropy is being gained by releasing counter ions from the film to solution.

IR active anions were used for extrapolating the number of extrinsic sites in the annealed PEMU. This value was inferred from the y intercept of a straight line given by \([\text{salt}]/[\text{SO}_3^-]\) or “y” measurements for several salt concentrations. The \([\text{SO}_3^-]\) signal is the sulfonate peak corresponding to one monomer of PSS. The “[salt]” is the signal corresponding to the IR active salt. Both these values were standardized and plotted as molar ratios. In fact, \(y\) represents the average number of ions (one anion per cation) per monomer. Since we assume that there must be one cation (Na\(^+\)) for each anion that we can see in the IR, this number represents exactly the number of extrinsic sites for an annealed PEMU.

Four IR active anions were used for this purpose: azide (N\(_3^-\)), perchlorate (ClO\(_4^-\)), nitrate (NO\(_3^-\)) and thiocyanate (SCN\(^-\)). The results were plotted in Figure 3.3.

The slope of the fitted line at low \(y\) values may be used to determine \(K_{dop}\) which represents the “doping” constant with which the anion/cation will ion-exchange a polyanion and form a new extrinsic site. This “doping” process of the multilayer is reversible. The ions stay in line with the order proposed in the Hoffmeister series or what we will refer to as the “hydrophobic series”. The most hydrophobic ions dope the multilayer the most efficiently.
Figure 3.3 Plot of the mole ratio of salt/sulfonate which represents number of extrinsic sites for each of the IR active ions versus salt concentrations of those salts: ■ represents perchlorate, ● is thiocyanate, ▲ is nitrate and ◆ is azide. Panel A depicts PDADMA/PSS, and panel B represents P4VMP/PSS.
Table 3.2 Depiction of $K_{dop}$ (doping constant) and $y_{residual}$ (residual # of extrinsic sites) determined from Figure 3.3, and $\Delta G_{association}$ or $\Delta G_a$ (in kJ mol$^{-1}$) calculated from the equation $\Delta G_{association} = RT \ln K_{dop}$. (A) represents measurement on PDADMA/PSS and (B) represents measurements on P4VMP/PSS.

<table>
<thead>
<tr>
<th>Salt</th>
<th>$K_{dop,PDADMA/PSS}$</th>
<th>$y_{residual}$</th>
<th>$\Delta G_a$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaClO$_4$</td>
<td>3.8</td>
<td>0.06</td>
<td>3.3</td>
</tr>
<tr>
<td>NaSCN</td>
<td>0.42</td>
<td>0.05</td>
<td>-2.1</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>0.27</td>
<td>0.06</td>
<td>-3.2</td>
</tr>
<tr>
<td>NaN$_3$</td>
<td>0.031</td>
<td>0.06</td>
<td>-8.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salt</th>
<th>$K_{dop,P4VMP/PSS}$</th>
<th>$y_{residual}$</th>
<th>$\Delta G_a$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaClO$_4$</td>
<td>2.9</td>
<td>0.02</td>
<td>2.6</td>
</tr>
<tr>
<td>NaSCN</td>
<td>0.18</td>
<td>0.03</td>
<td>-4.2</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>0.071</td>
<td>0.02</td>
<td>-6.6</td>
</tr>
<tr>
<td>NaN$_3$</td>
<td>0.011</td>
<td>0.02</td>
<td>-11</td>
</tr>
</tbody>
</table>
The intercept for the lines in Figure 3.3 (y_{residual}) represents the number of residual extrinsic sites in the multilayer. On average PDADMA/PSS shows 6.6% residual extrinsic sites in an annealed multilayer. P4VMP/PSS shows 2.4% residual extrinsic sites on average, so about 2.75 times fewer extrinsic sites than what PDADMA/PSS has. This insight into the morphology of the film explains behaviors like different thicknesses, different diffusion properties, roughness, refractive index, and tensile strength. These two types of films have been annealed for the same one week time period and the number of extrinsic sites has been standardized to percent values. A PEMU with a higher percent of extrinsic sites should be thicker and should be more permeable to an ionic diffusing species.

The intercepts are very close to each other with ± 0.01 error, but do not intercept the same exact point. This might be attributed to residual ions from previous runs on the PEMU, even after careful rinsing. All salt runs were conducted on the same PEMU and our results [( y_{residual} )= 0.05 for perchlorate and 0.07 for azide] for the PDADMA/PSS multilayer are not far from to the results obtained in the work done by Farhat et al [( y_{residual} )= 0.03 for perchlorate and 0.02 for azide)].

3.3.3 Water Swelling Equilibrium

The water distribution inside a multilayer can be represented by the following equation:

\[ \text{Pol}^+\text{Pol}^-a\text{H}_2\text{O}_{(m)} + \text{M}^+b\text{H}_2\text{O}_{(aq)} + \text{A}^-c\text{H}_2\text{O}_{(aq)} \leftrightarrow \text{Pol}^+\text{A}^-d\text{H}_2\text{O}_{(m)} + \text{Pol}^-\text{M}^+e\text{H}_2\text{O}_{(m)} + f\text{H}_2\text{O} \quad (3.3) \]

Where \( a \) represents number of water molecules from the undoped multilayer (“intrinsic” water”), \( b \) and \( c \) represent hydration numbers of cations and anions respectively, \( d \) and \( e \) are the numbers of water molecules associated with the ion-polymer pair, and \( f \) represents the balance of the water molecules (and can be negative or positive).
Table 3.3 Values from literature for hydration numbers $b_{\text{H}_2\text{O}}$ and $c_{\text{H}_2\text{O}}$ or $\text{H}_2\text{O}_{\text{hydra}}$ of the different ions used in our experiments. The numbers clearly shows that using different methods for estimating the hydration number of different ions or salts gives different results each time. For the table below three different values of hydration number of ions are reported from three different sources.\textsuperscript{112-114}

<table>
<thead>
<tr>
<th>Ion</th>
<th>Reference \textsuperscript{112}</th>
<th>Reference \textsuperscript{113}</th>
<th>Reference \textsuperscript{114}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>3.5</td>
<td>4.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Li$^+$</td>
<td>5.2</td>
<td>4.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>10.0</td>
<td>10.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>7.2</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>K$^+$</td>
<td>2.6</td>
<td>3.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Cs$^+$</td>
<td>2.1</td>
<td>2.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Y$^{3+}$</td>
<td>14.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>2.0</td>
<td>2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>F$^-$</td>
<td>2.7</td>
<td>4.0</td>
<td>2.2</td>
</tr>
<tr>
<td>I$^-$</td>
<td>1.6</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>1.9</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>N$^3_-$</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN$^-$</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ClO$_4^-$</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
An annealed PEMU was exposed to different concentrations of different salts and plotted the molar ratio of H$_2$O to SO$_3^-$ against these concentrations. Once the standardization is performed, the y-axis becomes the ratio [H$_2$O]/[SO$_3^-$] and it symbolizes counts of H$_2$O molecules per counts of PSS monomers (PSS contains the SO$_3^-$ group). When a line is fitted to these points and its y-intercept is calculated, one can approximate the number of water molecules that hydrates the annealed PEMU i.e. (aH$_2$O). This is also the water that hydrates the mostly intrinsic sites (~95 %) existing in the annealed form. aH$_2$O represents the number of associated water molecules per polyelectrolyte ion-pair at the limit of complete intrinsic compensation.

The slope of the line corresponds to the swelling coefficient (Ψ$_{Salt}^{H_2O}$) due to salt carrying water inside the multilayer (“extrinsic water”), which can be represented as:

$$\Psi_{Salt}^{H_2O} = \frac{rH_2O - a}{[MA]}$$

Where rH$_2$O = [H$_2$O]/[SO$_3^-$] represents number of molecules of water per sulfonate, [MA] is the salt concentration, and a is the number of water molecules from the multilayer.

The doping constant does not always describe the same phenomenon as the swelling constant. If we were to only consider the nature of the ions doping the PEMU, strong dopants like ClO$_4^-$ are poorly hydrated so they should swell poorly. The experimental results however show that strong dopants also swell the PEMU much when compared to weak dopants. These results suggest that even though the strong dopants carry a smaller hydration shell when entering the PEMU, due to a higher rate of ion exchange, more of them settle and form extrinsic sites, thus increasing the overall H$_2$O count when comparing to weaker dopants but richer in H$_2$O ions. The latter ions bring more water inside per ion but much fewer ions settle and form extrinsic sites. Overall, salts formed from hydrophobic ions have lower (Ψ$_{Salt}^{H_2O}$) swelling coefficients.
Figure 3.4 A representative drawing showing a plot of number of water molecules versus salt concentration. It depicts the effect of adding salt to a multilayer, the descending part represents what we call a “polyelectrolyte effect” at low salt concentration and the ascending part occurs when the electrostatic forces overcome the polyelectrolyte effect. The $aH_2O$ represents the number of water molecules present originally in the multilayer.
Figure 3.5 This figure represents a plot that shows the number of water molecules per ion pair at different salt concentrations for 7 different salts used on PDADMA/PSS multilayer (Panel A) and P4VMP/PSS multilayer (Panel B). ■ represents NaClO₄, ● represents NaSCN, ◇ depicts LiCl, △ depicts KCl, □ represents NaCl, ◆ represents NaN₃ and ▲ depicts NaNO₃.
Figure 3.6 This figure represents a plot that shows the number of water molecules per ion pair at different salt concentrations for 4 different salts used on PDADMA/PSS multilayer (upper) and P4VMP/PSS multilayer (lower). ○ represents NaF, Δ represents NaBr, □ represents NaCl, and ◊ represents NaI.
Figure 3.7 This figure represents a plot that shows the number of water molecules per ion pair at different salt concentrations for 4 different salts used on PDADMA/PSS multilayer (upper) and P4VMP/PSS multilayer (lower). ○ represents CsCl, △ represents CaCl$_2$, □ represents MgCl$_2$, and ◇ represents YCl$_3$. Notice the difference in behavior between CsCl and multivalent ions.
The order of the swelling coefficient $\Psi^{H_2O}_{Salt}$ coming from the slopes of the trend lines, which we will refer to as “hydrophobic series”, goes like this: $N_3^- > NO_3^- \approx Br^- > Cl^- > SCN^- > I^- > ClO_4^-$ and it is compared (Table 3.5) to the Hofmeister series. The Hofmeister series for the anions goes from the strongly hydrated to the weakly hydrated as follows: $F^- > Cl^- > Br^- > NO_3^- > I^- > SCN^- > ClO_4^-$. 97,100 and for the cations from the weakly hydrated to strongly hydrated as follows: $Cs^+ < Rb^+ < K^+ < Na^+ < Ca^{2+} < Mg^{2+} < Al^{3+}$. 97 Hydrophobic ions (or weakly hydrated), strong dopants and strong destabilizers like $ClO_4^-$ and $SCN^-$ have the highest $\Psi^{H_2O}_{Salt}$ values when compared with other anions (e.g. $NO_3^-$ and $N_3^-$) pairing with the same cation ($Na^+$) in Table 3.4. Also, when we kept the anion constant at an intermediate anion ($Cl^-$) and compared the cations, they also behave within the Hofmeister series within experimental error (Table 3.4).

The results obtained for $aH_2O$ shows that P4VMP/PSS multilayer contains less water than the PDADMA/PSS one. Polyvalent cations like $Ca^{2+}$, $Mg^{2+}$ and $Y^{3+}$ swell the PEMU in line with the more hydrophobic anions. By having lower surface to charge ratio they carry more $H_2O$ and can counter two ($Mg^{2+}$) or three sites ($Y^{3+}$) simultaneously and form two or three extrinsic sites at the same time. These are properties that offer a high $\Psi^{H_2O}_{Salt}$ values for the multivalent ions.

The results obtained for the swelling coefficient $\Psi^{H_2O}_{Salt}$ coming from the slopes of the trend lines or the “hydrophobic series”, are compared in Table 3.5 with both the Hofmeister series for the anions and the values for the hydration numbers of these anions obtained from literature.
Table 3.4 Depiction of $\Psi_{Salt}^{H_{2}O}$ and $aH_{2}O$ (water hydrating a polyion electrostatic site) calculated from equations of trend lines in Figures 3.5, 3.6, and 3.7. First two columns represent measurements on PDADMA/PSS and the last two columns represent measurements on P4VMP/PSS. The average number of water molecules per polymer ion pair $aH_{2}O$ for all salts used except NaF was $6.9 \pm 1.7$ for PDADMA/PSS multilayer and $2.1 \pm 0.6$ for P4VMP/PSS multilayer.

<table>
<thead>
<tr>
<th>Salt</th>
<th>$\Psi_{Salt}^{H_{2}O}$</th>
<th>$aH_{2}O$</th>
<th>$\Psi_{Salt}^{H_{2}O}$</th>
<th>$aH_{2}O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF</td>
<td>-5.8</td>
<td>19.2</td>
<td>-0.04</td>
<td>4.2</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.0</td>
<td>5.7</td>
<td>0.72</td>
<td>2.2</td>
</tr>
<tr>
<td>NaBr</td>
<td>5.5</td>
<td>7.0</td>
<td>2.3</td>
<td>2.2</td>
</tr>
<tr>
<td>NaI</td>
<td>22.3</td>
<td>5.9</td>
<td>5.7</td>
<td>2.0</td>
</tr>
<tr>
<td>NaN$_3$</td>
<td>5.4</td>
<td>5.4</td>
<td>0.65</td>
<td>2.2</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>5.4</td>
<td>5.4</td>
<td>6.8</td>
<td>1.8</td>
</tr>
<tr>
<td>NaClO$_4$</td>
<td>19.8</td>
<td>5.6</td>
<td>5.9</td>
<td>2.3</td>
</tr>
<tr>
<td>NaSCN</td>
<td>10.5</td>
<td>7.0</td>
<td>0.81</td>
<td>2.7</td>
</tr>
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<td>KCl</td>
<td>3.1</td>
<td>7.4</td>
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<td>2.1</td>
</tr>
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<td>CsCl</td>
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<td>LiCl</td>
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<td>YCl$_3$</td>
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<td>CaCl$_2$</td>
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<td>8.7</td>
<td>7.7</td>
<td>2.2</td>
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<tr>
<td>MgCl$_2$</td>
<td>13.5</td>
<td>8.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.5 The results for swelling coefficient $\Psi_{\text{Salt}}^{H_{2}O}$ were put in order and compared with the order from Hofmeister series of anions and also compared with the order of the hydration numbers for the same anions which we already presented in Table 3.3. The anion series has Na cation in common, while the cation series has Cl anion in common.

<table>
<thead>
<tr>
<th>$\Psi_{\text{Salt}}^{H_{2}O}$</th>
<th>ClO$_4^-$ &gt; I &gt; SCN$^-$ &gt; Cl$^-$ ≈ Br$^-$ ≈ NO$_3^-$ &gt; N$_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hofmeister</td>
<td>ClO$_4^-$ &gt; SCN$^-$ &gt; I &gt; NO$_3^-$ &gt; Br$^-$ &gt; Cl$^-$</td>
</tr>
<tr>
<td></td>
<td>Mg$^{2+}$ &gt; Ca$^{2+}$ &gt; Na$^+$ &gt; K$^+$ &gt; Rb$^+$ &gt; Cs$^+$</td>
</tr>
<tr>
<td>cH$_2$O</td>
<td>ClO$_4^-$ &lt; SCN$^-$ &lt; I &lt; Br$^-$ ≈ N$_3^-$ &lt; NO$_3^-$ ≈ Cl$^-$</td>
</tr>
<tr>
<td>bH$_2$O</td>
<td>Y$^{+3}$ &gt; Mg$^{2+}$ &gt; Ca$^{2+}$ &gt; Li$^+$ &gt; Na$^+$ &gt; K$^+$ &gt; Cs$^+$</td>
</tr>
</tbody>
</table>

3.3.4 “Complete” Analysis

From the above data we were able to derive the average number of “water of hydration” for each of the IR active salts used.

Table 3.6 Values for $(d\text{H}_2\text{O}+e\text{H}_2\text{O})$ of the IR active ions diffusing into the PEMU measured in PDADMA/PSS and P4VMP/PSS

<table>
<thead>
<tr>
<th>Ion-Polymer Pair</th>
<th>PDADMA/PSS</th>
<th>P4VMP/PSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pol$^-$ Na$^+$, Pol$^+$ ClO$_4^-$</td>
<td>9.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Pol$^-$ Na$^+$, Pol$^+$ SCN$^-$</td>
<td>9.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Pol$^-$ Na$^+$, Pol$^+$ NO$_3^-$</td>
<td>10.6</td>
<td>8.5</td>
</tr>
<tr>
<td>Pol$^-$ Na$^+$, Pol$^+$ N$_3^-$</td>
<td>11.6</td>
<td>8.8</td>
</tr>
</tbody>
</table>
3.3.5 Swelling from residual Extrinsic Sites: the “Polyelectrolyte Effect”

The graphs for all the salts used show similar behavior: descending curve at low salt concentrations until it reaches a minimum point, and then ascending curve at higher salt concentrations. The reproducible decrease in swelling at low salt concentrations is interpreted as the drawing out of water molecules unassociated electrostatically with specific ion pairs (“liquid water”) under an increasing external osmotic pressure. This is what we call the “polyelectrolyte effect”. As the number of extrinsic sites inside the film increases the PEMU has enough ionic strength to keep its water and as this number increases even more, ions bring hydration waters into the PEMU as they diffuse inside. Osmosis swells the PEMU as the salt concentration inside the film increases even further. The electrostatic forces now overcome the “polyelectrolyte effect”. This behavior that was shown before in Figures 3.5, 3.6 and 3.7 for the different salts used is shown here for NaCl in Figure 3.8

![Polyelectrolyte effect](image)

**Figure 3.8** This figure represents a plot of number of water molecules per ion pair (y-axis) versus NaCl concentration (x-axis) on a PDADMA/PSS multilayer. The upper curve represents the first run cycle of a freshly prepared multilayer using water peak at 3417 cm⁻¹ depicted by ◊, and using water peak at 1645 cm⁻¹ depicted by x. The lower curve represents a run on a 1 M NaCl annealed multilayer using water peak at 3417 cm⁻¹ depicted by □, and using water peak at 1645 cm⁻¹ depicted by Δ. The dashed line represents an extrapolation to calculate the number of associated water molecules per polyelectrolyte ion-pair at the limit of complete intrinsic compensation. Note the descending behavior of the curve at low salt concentrations which is due to what we called previously “Polyelectrolyte effect”.

42
3.3.6 Osmotic Pressure Effect

In previous experiments using ATR, analysis on the water content of a multilayer made good estimates for water content. The soft probing of ATR is efficient enough to measure the change in the water content of a polyelectrolyte film that is soaked in water. Poly(ethylene glycol) [(PEG (8000)], neutral but highly water-soluble polymer, was used as an osmotic stressing agent. PEG does not diffuse inside the PEMU from solution since its radius of gyration is larger than the average distance between two polymer chains inside the PEM film. This is how it creates enough osmotic pressure to pull water out from the PEMU. Different PEG concentrations were used (see Figure 3.9) ranging from 0%, 10%, 15%, 20%, 25% till 30% in solutions prepared with 10mM and 0.5M NaCl. The results shown in Figure 3.9 show that the number of water molecules per ion pair decreases as the concentration of PEG increases till it reaches a level (~ 7 water molecules) at 30% PEG where the number of water molecules stay constant. This shows the efficiency of using PEG as an osmotic stressing agent and its capability of osmotically removing water out of the PEMU film without diffusing inside the film.

The PEMU film was then annealed with 1.0M NaCl for one week and the same series of several PEG concentrations with 10Mm NaCl was used to see effect of PEG on an annealed film. The results showed almost no change in water content. The number of water molecules per ion pair for the annealed film was almost the same as before annealing when high PEG concentration was used.

The same annealed PEMU was used to run the same series of PEG concentrations but this time with higher salt concentration (0.5M of NaCl) instead of the 10mM used previously. The results observed did not vary much from the previous results with low salt concentration which tells us about the effect of annealing on the free energy of the PEMU. Once the film is annealed, it is irreversibly conformed into its lowest energy form and it will not swell/lose water effectively anymore. However the slight difference between using the 10mM and the 0.5M NaCl in the PEG solutions is due to the higher osmotic pressure exerted by the higher salt concentration absorbing more water from the film which leaves it with less water molecules per ion pair at each PEG concentration.
Figure 3.9 This figure shows a plot of number of water molecules (primary y-axis on the left) versus wt% of PEG (x-axis) which is also plotted versus osmotic pressure $\Pi$ in dynes/cm$^2$ (secondary y-axis on the right side of the chart). The plot represents the effect of using osmotic stressing agent such as PEG on a PDA/PSS multilayer where ■ is 10mM NaCl before annealing with 1 M NaCl for one day, ♦ represents 10mM NaCl after annealing and ▲ depicts 0.5 M NaCl after annealing, and it also shows that osmotic pressure increases with higher PEG concentrations represented by ●.
The results from Figure 3.9 show that when PEG concentration is increased then the osmotic pressure exerted increases too and at the same time the number of water molecules inside the multilayer decreases because of this pressure being applied which pulls the water out of the multilayer.

### 3.4 Conclusion

Layer by layer buildup of polyelectrolyte multilayers on a germanium crystal and ATR-FTIR measurement of water and sulfonate peaks allows us to calculate the swelling due to water and the number of intrinsic and extrinsic sites inside PDADMA-PSS and P4VMP-PSS thin films. Previous work in our lab showed that P4VMP-PSS is less permeable to ions when compared to PDADMA-PSS. This could be due to less extrinsic sites in the multilayer. Our work suggests that less water present in a P4VMP/PSS PEMU of similar thickness to PDADMA-PSS could be attributed to a lower number of extrinsic sites in this multilayer. Also, experiments done with different salts gave us valuable information about the effect of the salt type on the thickness, water content, stability and behavior of multilayers. The PEG experiment proved to be a good method for studying water swelling in an annealed versus non-annealed multilayer. ATR-FTIR proved to be a highly sensitive non-invasive technique that was very useful in studying water structuring inside polymeric films.
CHAPTER 4

ANNEALING OF POLY ELECTROLYTE MULTILAYERS

4.1 Introduction

As was mentioned in the previous chapter, polyelectrolyte multilayer layer by layer build-up can be explained by the concept of overcompensation of charges. As the PEMU is exposed to environments such as high ionic strength solutions, extreme temperature or pH, it begins to anneal and change its conformation in bulk and on its surface. Our experiments suggest the surface becomes smoother and the PEMU becomes more strongly bounded in bulk by an increase in intrinsic compensation and a decrease in extrinsic compensation. Different PEMUs anneal at different rates and they eventually reach a specific annealing extrinsic site concentration (SAESC) where the annealing curves plateau. In our experiments, this plateau depends on the ionic strength of the annealing solution.

Small salt ions can break the electrostatic bond between two polyelectrolytes and create defects in the PEMU where some ionic groups become extrinsically compensated by salt counter ions. The amount of added salt determines the amount of extrinsically compensated segments. The increase in the ionic strength of the solution modifies the overall free energy of interaction by competing for polymer charge, a mechanism which is better defined as competitive ion pairing.

The salt type is another factor affecting the growth process. Residual extrinsic sites are “defects” in the multilayered film. Depending on the nature of the interaction between the building polyelectrolytes (weaker vs. stronger) films will build linearly (strong interaction) or exponentially (weaker interaction). Linear growth of PEMUs is explained by overcompensation of charges occurring mainly at the surface while exponentially growing multilayers overcompensate in the bulk also.

The ionic strength of the solution the PEMU stays in and the amount of time it spends in that solution affect the thin film through a process called annealing. When a PEMU anneals, it becomes smoother on the surface and intrinsic compensation predominates in the bulk. Annealing depends on the concentration of the ions in solution.
and on annealing time. Higher salt concentrations will anneal the multilayer quicker and will create a higher extrinsic to intrinsic site ratio.

4.2 Experimental Section

4.2.1 Materials

All chemicals were used as purchased unless otherwise specified. Poly(diallyldimethylammonium chloride) (Aldrich) $M_w=3.69 \times 10^5$ and $M_w/M_n=2.09$, poly(styrene sulfonate) (Scientific Polymer Products) $M_w = 5.75 \times 10^4$ and $M_w/M_n=1.03$, were used as received. Sodium chloride (NaCl) and sodium azide (NaN$_3$) were purchased from Fisher Scientific. Solutions were made with 18 MOhm deionized water (Barnstead, E-pure, Milli-Q).

4.2.2 Instrumentation

ATR measurements were performed with a Nicolet Nexus 470 FTIR fitted with 0.5 ml; flow-through ATR assembly housing a 70 x 10 x 6 mm, 45° germanium crystal (Specac Benchmark). Annealing of the film in several NaCl concentration solutions was monitored using a 30 minute doping response of the annealing film to 0.1M NaN$_3$. The deposition time of each layer was five minutes. The rinse time was one minute.

Annealing was monitored with an IR active probe (N$_3^-$) by passing it over the PEMU for 30 minutes. Annealing of the PEMU was achieved in 1.0M NaCl and it was only interrupted when 0.01M NaN$_3$ was passed over the multilayer. The low NaN$_3$ concentration does not further anneal the PEMU but it only provides a way of measuring the amount of extrinsic sites present in the PEMU after a certain amount of annealing has occurred. The more the PEMU is annealed, the fewer extrinsic sites are present and less N$_3^-$ binds into the film. After the 30 minutes experiment, the PEMU was further exposed to the higher annealing NaCl concentrations. A total of 125 hours of effective annealing (counted without the 30 minutes monitoring experiments) were completed for each multilayer. The complete experiment took about one week for one trend line, with each
point on the curve coming from IR spectra taken at the end of a 30 min exposure to the probe (N$_3^-$) taken at set intervals throughout the 125 hour total period.

All spectra were recorded with 256 scans and 4 cm$^{-1}$ resolution. Initial background spectra were recorded on bare, dry germanium crystal in order to preserve the water and sulfonate peaks in subsequent samples taken. Spectra were also recorded for the LBL buildup. The mole ratios of water to sulfonate and azide to sulfonate were determined from the area under the respective peaks (3706 – 2979 cm$^{-1}$ for water, 1052 – 989 cm$^{-1}$ for sulfonate and 2123 - 1957 cm$^{-1}$ for azide). To calibrate the instrument response to the concentration of the sample, a 1.0 M solution of poly(styrenesulfonate) and 0.5M sodium azide with a mole ratio of water to sulfonate of 55.6 to 1 was passed over the uncoated crystal. The sulfonate signal is a convenient internal standard which normalizes differences in absolute absorbance intensities of water (or propyl groups) that result from changes in refractive indices of the multilayer.

4.3 Results and Discussion

4.3.1 Layer by Layer Build-up

Typical LBL build-up of PDADMA/PSS on ATR crystal, from solutions of 10 mM polymer concentration and 1.0 M NaCl concentration was performed in order to build odd and even numbered layers which were then monitored while annealing. The rinsing step between each polymer layer, which removes unadsorbed polymers, was done using 1.0 M NaCl.

The alternating behavior observed during the multilayer LBL build-up in Figure 4.1 was explained in details in the previous chapter. The build-up for the films ending with PDADMA on top (i.e. odd number of layers: 11, 19, 25, 31 and 39) is displayed in Figure 4.1. The y-axis is plotted as a ratio of the molar concentration of the sulfonate signal and water (SO$_3^-$/H$_2$O). When more water is present in this ratio (PDADMA is added) the plot reads lower values. The buildup plot levels out when the PEMU reaches a steady state value for the “bulk”. That value is at approximately 6 water molecules per PSS monomer.
Figure 4.1 Layer by layer PEMU buildup for odd and even layered films. All films were built in 1.0M NaCl solution and 10mM polymer concentration, rinsed with 1.0M NaCl. These solutions were flowed through the ATR-FTIR cell and built on the Ge crystal. The zigzag behavior can be explained by expansion and shrinkage of the film as subsequent hydrophobic/hydrophilic layers are added. When the curve goes up, a hydrophobic layer was added (PSS) and when it drops down, a hydrophilic layer was added (PDADMA). All films had PDADMA and PSS as (+) positive and (-) negative polyelectrolyte building blocks. △ represents the buildup of 11 layers, □ represents 19 layers, ◊ represents 25 layers, ○ represents 31 layers and + represents 39 layers. “Odd” layers have PDADMA on top and “even” layers have PSS on top.
4.3.2 Annealing

The annealing of a thin film (PEMU) is the process that occurs to the film as it is exposed to factors such as stronger ionic strengths\(^ {70,115} \), different pHs or temperatures. The effect of high ionic strength (1.0M NaCl) on PEMUs of different thicknesses has been monitored over a period of 125 hours. The behavior of the PEMU has been expressed in terms of a ratio of an IR active ion that fills in extrinsic sites and the sulfonate IR signal given by PSS, one of the building blocks of the PEMU. Extrinsic sites\(^ {47} \) are defined as “defects” in the PEMUs interaction between building blocks. These sites are filled in by small counter ions coming from solution (Scheme 4.1).\(^ {116} \) The more extrinsic sites present the weaker the overall adhesion between polymer layers and the looser the PEMU is bound together. The extrinsic sites’ indicator was chosen as an IR active anion (N\(_3^−\)) with small swelling capability and with absorption bands in an otherwise uneventful spectrum zone. Sodium azide was used in very small concentrations (0.01M).

Scheme 4.1 “Defects” diffuse out of multilayer aided by polymer interdiffusion. Ions in solution assist the process by “lubricating” the polymer chains with extrinsic sites and allowing them to interdiffuse and let “defects” move down the charged polyelectrolyte backbone and eventually out of the film.
Upon buildup in 1.0M NaCl, the thin film was then annealed in 1.0M NaCl solution. Exposure to this high ionic strength solution was interrupted briefly (30 min intervals) and a 0.01M N$_3^-$ solution was rinsed over the PEMU. A spectrum was taken and the ratio of the N$_3^-$ signal to the SO$_3^-$ signal was recorded.

The amount of N$_3^-$ going inside the PEMU and doping it by forming extrinsic sites depends on how much the high ionic strength solution (1.0M NaCl) annealed the PEMU before the interruption and on how much the PEMU changed its conformation in response to this annealing process.

Our data (Figure 4.2) shows a difference in response depending on layer number and more importantly, depending on the type of layer (charge) that was last deposited. When a (+) positive layer tops the thin film (odd number of layers) we observed higher overall N$_3^-$ presence. Azide ion has a negative charge and we attributed this behavior mainly to surface ion exchange. A positive top layer will pair up successfully with oppositely charged small ions in solution. This surface charge compensation attracts more counter ions than the multilayer would otherwise incorporate in their bulk. When a (-) negative layer tops the PEMU (even number of layers) less overall N$_3^-$ ions compensate surface charges. In this case the azide ion will fill in extrinsic sites in the bulk of the film.

When the PEMU is thinner (fewer layers) it will have fewer extrinsic sites. Curves corresponding to films progressively thicker stack up in the correct order (Figures 4.2 and 4.4). As the thickness of the films increases till it entirely contains the evanescent wave of the ATR instrument, curves come closer together as the IR beam will see a similar amount of PEMU, upper layers not being probed by it. Smaller differences are still seen as the film layers interpenetrate to a large extent and the same thickness of film will have different film densities for different PEMU thickness.
Figure 4.2 This figure represents a plot that shows the decrease in extrinsic site doping level as PEMUs anneal in 1.0M NaCl at room temperature. The vertical axis represents \( y \), the “extrinsic site concentration” or the ratio \([N_3^-]/[SO_3^-] \). This ratio was calculated by taking standardized molar peak values from the IR spectra collected during the experiments. Panel A depicts 5 different films with odd number of layers (PDADMA on top), while Panel B depicts 5 films with even layers (PSS on top). \( \Delta \) represents 11 layers.
in panel A and 12 layers in panel B, □ represents 19 and 20 respectively, ◊ represents 25 and 26 respectively, ○ represents 31 and 32 and + represents 39 and 40 respectively.

Odd numbered layers behave differently than even layered ones. The N$_3^-$/SO$_3^-$ ratio is not a true indication for bulk extrinsic sites for “odd” multilayers since most of the compensation observed takes place on top of the PEMU. A thin layer shows more N$_3^-$ present because it is thinner than the height of the instrument’s evanescent wave and the IR beam will probe all the way to the top of the film. As films become thicker and approach (or pass) the height of the evanescent wave, the IR beam will “see” less and less of the surface compensation and eventually will only see bulk ion compensation. Since odd numbered layers have most of their ion compensation occurring on the surface, less N$_3^-$ levels for thicker films can be explained. This also accounts for less difference in N$_3^-$ signal between layers of different thicknesses.

Table 4.1 $y_{\text{residual}}$ values at $t_{\infty}$ annealing time for multilayer thin films of different thicknesses

<table>
<thead>
<tr>
<th>Layer numbers</th>
<th>$y_{\text{residual}}$ at $t_{\infty}$ for odd layers</th>
<th>$y_{\text{residual}}$ at $t_{\infty}$ for even layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>0.166</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td>19</td>
<td>0.146</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>0.098</td>
</tr>
<tr>
<td>25</td>
<td>0.159</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>0.132</td>
</tr>
<tr>
<td>31</td>
<td>0.145</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>39</td>
<td>0.158</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>0.138</td>
</tr>
</tbody>
</table>

Another difference in behavior between PEMU’s ending in oppositely charged layers is the annealing rate. Even layered films reach a stable annealed state much quicker, somewhere between 3 and 15 hours, whereas odd layered films anneal gradually over the whole 125 hour time period. However, as described earlier the graph is not a true representation of bulk annealing in the latter case.
“Defect” content has been expressed in Table 4.1 as the $y_{\text{residual}}$ at $t_{\infty}$, which represents the number of “defects” halfway through the 125 hour annealing process. This data has also been plotted in Figure 4.3, displaying trend lines for “defects” at $t_{\infty}$ for odd and even layer numbers. Odd layers (+) layer on top of the film, experience a decrease in number of “defects” as the film anneals while even layers (-) on top of the film experience an increase in number of “defects”. In the even layered films, when the azide probe does not compensate on the surface but in the bulk of the film, one can see an increase in “defect” content as multilayers become thicker.

Azide signal for the same concentration used in the probing experiment (0.01M) is very small in solution when compared to signal for the same concentration in a PEMU. This is due to the accumulation of $N_3^-$ inside the film as it exchanges in extrinsic sites created by NaCl.

Both odd and even layers anneal the PEMUs by the same type mechanism. The film has fewer extrinsic sites as it anneals. The PEMU restructures under high ionic strength solution (1.0M NaCl) and becomes smoother on the surface (according to AFM pictures) and more interaction between the building polymers is observed in the bulk (according to the current data set). The film loses extrinsic sites as it anneals. This can be explained by a “lubricating” effect of the high NaCl concentration annealing solution. When small ions are present in excess, they provide a way of transport for existing bulk extrinsic sites by allowing hopping from charged monomer to monomer and eventually escaping the PEMU. Thermodynamically the polymer-polymer electrostatic interactions are favored. A fully annealed PDADMA/PSS PEMU will only have approximately 5% extrinsic sites.
Figure 4.3 Plot of $y_{\text{residual}}$ at $t_\infty$ for odd and even layered multilayers where ■ represents odd layers (11,19,25,31, and 39), and ♦ represents even layers (12,20,26,32, and 40). Even layers end in a negative layer and the azide probing ion compensates more in the bulk of the film than on the surface. This accounts for a true representation of bulk “defects” in the film. As the film becomes thicker it accumulates more extrinsic sites (defects) due to a larger overcompensation region as the multilayer grows.
4.3.3 Water Content

Water absorbs well in the IR and this allowed us to get a very good idea of how the PEMU changes its water content as it anneals. Again, the behavior is different from odd to even layered multilayers. The cause of this is in part different doping behaviors for these two types of films but also due to the hydrophilicity of the last layer. Within experimental error, PEMUs ending in the more hydrophilic PDADMA will have more water than similar thickness layers ending in the less hydrophilic PSS. This is also compounded by the fact that the more hydrophilic PDAD is also positively charged and will compensate N$_3^-$ which also is a more hydrophilic ion than Na$^+$, its counter ion in solution. When the PEMU is thinner than the height of the evanescent wave, the IR beam will see a lot of solution water. This explains how thicker films are represented by curves positioned lower on the H$_2$O/SO$_3^-$ axis. As the film reaches the top of the evanescent wave or surpasses it, the IR beam will only see PEMU bulk water.

Even if the position of the water content curves are not true indicators for PEMU water content for thinner films, the shape of the curves does represent the kinetics of the annealing process faithfully. A drop in doping levels inside the PEMU will result in a drop in the water content inside that film and our data displays this event clearly. N$_3^-$ coming out of the film carries out water molecules in its hydration cell, leaving the film dryer than before. The kinetics of the process is in accord with the extrinsic sites data.

When the hydrophilic (+) positively charged PDADMA tops the film we see a similar drop in water levels as the film becomes thicker when compared to when the less hydrophilic (-) negatively charged PSS is on top. In both cases, the only difference between layers of different thicknesses is the water that hydrates the extra N$_3^-$ ions doping the PEMU. Our results suggest a small difference in this water as the films thickness approaches the height of the evanescent wave.

4.3.4 Different Annealing Environments

PEMUs were also annealed in different environments by using 3 different ionic strength solutions (1.0M, 0.5M and 0.25M NaCl). These conditions affected three different films of similar thickness with the same number of layers (32 layers). This ensures the film is thick enough to cross the height of the evanescent wave so that the IR beam will only see the N$_3^-$ forming extrinsic sites inside the bulk of the film and not the
surface ion compensation which occurs above the height of the evanescent wave. Table 4.2 tabulates $y_{residual}$ at $t_{1/2}$ for same thickness multilayers annealed in solutions of progressively lower ionic strength. The intercept of a trend line of a plot of these values (0.043 on the $y$ axis in Figure 4.5) approximates the number of “defects” or extrinsic sites of a fully annealed multilayer bathed in 0M salt concentration.

Figure 4.4 Water content during annealing. These are odd and even layered PEMUs monitored throughout 125 hours of annealing at room temperature in 1.0M NaCl solution. Mostly solution water is seen until the thickness of the PEMU passes the height of the evanescent wave. $r_{H_2O} = [H_2O]/[SO_3^-]$ represents number of molecules of water per PSS monomer. $\Delta$ represents 11 layers in panel A and 12 layers in panel B, $\square$ represents 19 and 20 respectively, $\diamond$ represents 25 and 26 respectively, $\circ$ represents 31 and 32 and + represents 39 and 40 respectively.
Table 4.2 $y_{\text{residual}}$ values at $t_{\infty}$ annealing time for multilayer thin films of the same thickness but annealed in different salt concentration solutions.

<table>
<thead>
<tr>
<th>[NaCl] annealing</th>
<th>$y_{\text{residual at } t_{\infty}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 M</td>
<td>0.123</td>
</tr>
<tr>
<td>0.5 M</td>
<td>0.096</td>
</tr>
<tr>
<td>0.25 M</td>
<td>0.062</td>
</tr>
</tbody>
</table>

The doping annealing curves give kinetic and thermodynamic information for annealing in three NaCl concentrations different enough from each other to show useful information on the process. The highest ionic strength solution (1.0M NaCl) is fastest in annealing the PEMU. Lower ionic strength solutions will also anneal the PEMU and reach a “specific annealing extrinsic site concentration” (SAESC) but they will be kinetically slower in doing so, showing more gradual drops in their curves.

The position of the SAESC on the N$_3^-$/SO$_3^-$ axis represents less extrinsic sites at the end of annealing in a lower ionic strength solution. This might appear counterintuitive but it can be explained by a “lock-in mechanism”. This means that the ionic strength of the solution annealing a PEMU leaves its mark on the conformation of the PEMU which was not erased in the 125 hours of annealing used in our experiments. Higher salt concentration solutions annealing a PEMU will “lock-in” more extrinsic sites than a lower concentration solution but it will reach the SAESC point quicker.

Water swelling during annealing of three films of same thickness was very similar throughout the process and not different enough to suggest any specific mechanism. This is in part due to no change in the upper hydration layer and to small differences in SAESC level for the three annealing concentrations.
Figure 4.5 Plot of $y_{\text{residual at } t_\infty}$ for annealing curves at different NaCl concentrations of a 32 layered film. $Y$–intercept for the trend line of 0.0485 represents the “defects” content of a fully annealed multilayer bathed in 0M salt solution.
Figure 4.6 Annealing a PEMU in three different ionic strength solutions. Film has 32 layers for all curves but annealing solution ionic strength changes from 0.25 to 0.5 to 1.0M NaCl. Higher salt concentrations will anneal the multilayer quicker and will imprint a higher extrinsic to intrinsic site ratio. • represents 1.0M NaCl, □ represents 0.5M NaCl and △ represents 0.25M NaCl.
4.4 Conclusion

Layer by layer buildup of polyelectrolyte multilayers on a germanium crystal and ATR-FTIR measurements of ratios of water to sulfonate and azide to sulfonate peaks allowed us to monitor the annealing of PDADMA-PSS multilayers of different thicknesses in different ionic strength solutions. The film loses extrinsic sites as it anneals. This can be explained by a “lubricating” effect of the high NaCl concentration annealing solution. When small ions are present in excess, they provide a way of transport for existing bulk extrinsic sites by allowing for hopping from charged monomer to monomer and eventually escaping the PEMU. Thermodynamically the polymer-polymer electrostatic interactions are favored. A drop in doping levels inside the PEMU will result in a drop in the water content inside that film and our data displays this event clearly. \( N_3^- \) coming out of the film carries out water molecules in its hydration cell, leaving the film dryer than before. The kinetics of the water migration process is in accord with the extrinsic sites data.

Doping annealing curves also give kinetic and thermodynamic information for annealing in three NaCl concentrations different enough from each other to show useful information on the process. The highest ionic strength solution (1.0M NaCl) is kinetically fastest in annealing the PEMU. Lower ionic strength solutions will also anneal the PEMU and reach a “specific annealing extrinsic site concentration” (SAESC) but they will be kinetically slower in doing so, showing more gradual drops in their curves. Higher salt concentration solutions annealing a PEMU will “lock-in” more extrinsic sites than a lower concentration solution but will reach the SAESC point quicker.
CHAPTER 5
TOWARDS POLYELECTROLYTE COMPLEXES FOR BIOIMPLANTS

5.1 Introduction

5.1.1 Intervertebral Disc Replacement: an Overview

The intervertebral disc is a complex joint that comprises the spinal motion segment. The disc permits limited motion and flexibility to be transmitted, while maintaining stability and acting as the spine shock absorbing system. It consists of an outer annulus which is formed from collagen fibers that serve to stabilize the motion segment, and a nucleus which occupies about 40% of the disc total cross-sectional area and which is made of a mucoid material that contains mainly proteoglycans and very small amount of collagen (Figure 5.1).

Due to these constituents, the nucleus appears as a loose hydrated gel-like matter that contains 70-90% water, has a biomechanical role, and resists compression. The

Figure 5.1 This Figure shows a cross-sectional sketch for intervertebral disc [www.spineuniverse.com].
nucleus acts in almost the same way the air pressure in a tire supports the weight of a car. The disc is avascular, and the structure and function of the disc may be changed by processes such as physiological aging, mechanical factors including trauma and repetitive stress, segmental instability of the spine, and inflammatory and biochemical factors, all these leading to various disc abnormalities (Figure 5.2)\textsuperscript{119}

![Examples of Disc Problems](image)

**Figure 5.2** different disc problems [www.spineuniverse.com].

Extensive research has been done by scientists and surgeons since 1950’s in order to understand the mechanism of action of the intervertebral disc. Hirsch et al\textsuperscript{120} found that degenerated discs can be more easily compressed than healthy discs and are more sensitive to an increasing load. The degree of compression rises more rapidly as the load increases. Johanessen et al\textsuperscript{121} stated that the disc mechanics are restored following cyclic loading and unloaded recovery. Virgin\textsuperscript{122} investigated experimentally the properties of intervertebral discs. He concluded that the disc holds the property of elasticity to a very marked degree and this property depends largely upon the ability of the disc to absorb
and lose fluid. He also studied the hysteresis phenomenon and found out that hysteresis varies with age, where it has largest value in both immature discs (very young ages) and in aged people, while it was least for people in middle decade of life. In one of the hysteresis experiments, where two successive loading tests were done on the discs, he demonstrated that hysteresis during the second test was always less than that during the first test, i.e. the mechanical efficiency of the disc improved with use and the energy lost during recovery became less (Figure 5.3).\textsuperscript{122}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5_3.png}
\caption{This Figure shows the effect of repeated loading and unloading. The continuous line represents first loading and the dashed line represents second loading. One minute was the interval between the two tests. Note that hysteresis resulting from second load (C-D) is less than that from the first (A-B). Also note that during the interval the disc partly regained its thickness. [Virgin, W. J. The Journal of bone and joint surgery. British volume 1951, 33-B, 607-611].}
\end{figure}

The work done on mechanical testing of intervertebral discs has increased in recent years despite the different problems that face the researchers. One of the problems is the difficulty in obtaining accurate values when doing mechanical testing due to the natural formation of the disc. Discs mechanics varies with age, from outer layers to inner
layers, even within the same layer. Ebara et al obtained different stress-strain curves for the same disc when they tested multiple specimens from four regions of a L3-L4 disc\textsuperscript{123} (Figure 5.4).

Figure 5.4 showing stress-strain curves for specimens from four regions on the disc: AO represents anterior outer, AI represents anterior inner, PO represents posterolateral outer, and PI represents posterolateral inner. The tensile elastic modulus (E) was calculated from the above curves (reported in Figure 5.3). [Ebara, S.; Iatridis, J. C.; Setton, L. A.; Foster, R. J.; Mow, V. C.; Weidenbaum, M. Spine 1996, 21, 452-461].

Due to these differences, researchers mainly focused on testing the mechanical properties for annulus fibrosus and nucleus pulposus separately. Mechanical properties for nucleus pulposus where studied extensively\textsuperscript{124} and different experiments, ranging from dynamic shear testing and dynamic frequency sweep to stress relaxation, were done in order to calculate different moduli for the disc.\textsuperscript{124}

Intervertebral disc surgery is one of the operations performed nowadays with surgeons trying to replace the degenerated disc by an artificial one.

The factors that has to be considered in the design and implantation:
material must provide proper intervertebral spacing
- It should allow for motion
- It must provide stability
- Acts as shock absorber
- It must be biocompatible and no corrosion
- Without any inflammatory response
- Immediate and long term fixation to bone

5.1.2 PECs as intervertebral disc replacement

Polyelectrolyte Complexes (PECs) had found their way into biomedical applications, and into commercially available products such as contact lenses and antibacterial food wrappers. In this work we produced and developed PECs in a way that they act as water-bed mattress where they might be used as a material for replacing the intervertebral disc. We studied their properties extensively, and we expect their biocompatibility and mechanical properties to match those of the natural disc.

Special PECs, compact polyelectrolyte complexes (ComPECs), were produced to be used in certain biomedical applications, in particular as artificial intervertebral discs applied in surgical operations performed on spinal cord of the human body. Their mechanical properties were studied versus changes in environmental conditions (i.e. salt, pH, temperature).

The properties we expect for our (ComPECs) might resemble those of the nucleus pulposus more than the annulus fibrosus, so they might be used as a replacement for the nucleus pulposus rather than the whole disc.

5.1.3 Salt-dependent mechanical properties of PEMUs and PECs

Much work has been done recently on the mechanical properties of PEMUs. In a study by Jaber et al., a series of steady state stress-strain experiments were performed on multilayers prepared from poly(4-styrenesulfonate) (PSS), and poly(diallyldimethylammonium) (PDADMA), under different salt concentrations. The results demonstrated the influence of solution ion strength on PEC mechanical properties.
In general, the elastic modulus $E$, which measures the resistance to deformation of a material when stress is applied, is defined by:

$$E = \sigma/e$$  \hspace{1cm} (5.1)

Where $\sigma$ is the stress and $e$ is the strain. $E$ was observed to decrease as the ionic strength increased (Figure 5.5). At 0 M NaCl concentration, $E$ was found to be around 17 Mpa compared to the 0.3 MPa at 1 M NaCl. This increase in the elastic modulus is compared to the elastic modulus of the main two components of the intervertebral disc: annulus fibrosus and nucleus pulposus in both young and old people in Figure 5.5. The elastic modulus of the annulus fibrosus in one study ranged from 5-50 MPa$^{123}$, and for the nucleus pulposus from 4.5-1500 kPa$^{125-127}$.

![Graph showing the elastic modulus of the intervertebral disc](image)

**Figure 5.5** showing a plot for elastic modulus $E$ of a multilayer made from PDADMA/PSS versus salt concentration.$^{19}$ The graph shows the elastic modulus decreases as NaCl increases. This change in elastic modulus is compared to elastic modulus of annulus fibrosus and nucleus polposus of intervertebral disc in both young and old people. The elastic modulus range is 5-50 MPa for annulus and 0.45-1500 kPa for the nucleus. Both ranges increase with age where the disc starts to lose its elasticity.
5.2 Experimental Section

5.2.1 Materials

All chemicals were used as purchased unless otherwise specified. Poly(diallyldimethylammonium chloride) (Aldrich) $M_w = 3.69 \times 10^5$ and $M_w/M_n = 2.09$, poly(styrene sulfonate) (Scientific Polymer Products) $M_w = 5.75 \times 10^4$ and $M_w/M_n = 1.03$, were used as received. Sodium chloride (NaCl) was purchased from Fisher Scientific. Solutions were made with 18 MOhm deionized water (Barnstead, E-pure, Milli-Q).

5.2.2 Instrumentation

The (ComPECs) were prepared using XL-A/I Analytical ultracentrifuge (Beckman-Coulter instruments) with a TL series type rotor 90 Ti at a speed of 55000 rpm.

The polycarbonate tubes, for ultracentrifugation, were purchased from Beckman-Coulter instruments.

The complexes were heated in air using thermogravimetric analysis instrument. By burning the sample, usually water will evaporate first, followed by organic contents, and finally the inorganic particles. In our case the highest temperature used was 600 °C, so the inorganic particles constituted the weight remained at the end of each run. Weight % of all sample components can be determined by TGA.$^{91,92}$

TGA analyses were performed on a TA instrument SDT-2960 simultaneous thermal analysis and differential scanning calorimeter. Samples were loaded into alumina pan of the dual pan system. The temperature was ramped up to 600 °C at a rate of 10 °C / min.

Mechanical testing experiments were performed using SHIMAZU tensile machine (SHIMAZDU INC.) according to the ASTM D 638. The samples were cut into rectangular shaped thin sheets with certain size limitations (25 x 10 x 3 mm) and stretched, the machine records all the data and different tensile properties can be obtained from this instrument. All tensile properties were conducted in accordance with the American Society for Testing and Materials (ASTM) standard test methods.

A dynamic mechanical analysis DMA$^{90}$ analyzer from TA instruments (New Castle, DE) was used to test the thermal mechanical properties of the complexes. All samples were tested using thermal Analysis DMA Analyzer (model: DMA 2980)
equipped with a dual cantilever test fixture. The testing was done in accordance with ASTM standard test methods and the specimens cut into square sheets with the specified size (10 x 10 x 2 mm).

5.3 Results and Discussion

5.3.1 Compact polyelectrolyte complexes (ComPECs)

PDADMA/PSS complexes were prepared by dissolving PDADMA and PSS in water in separate beakers to get 10% concentration solution for each. Sodium chloride (NaCl) salt was added to each solution, after fixing the pH to ~ 7 for each by adding HCL or NaOH, in a 2.5M concentration. After mixing the 2 polymer solutions for 5 minutes using a magnetic stirrer, a complex is formed. The complex was put in a polycarbonate tube and ultracentrifuged inside a rotor (type TL series 90 Ti) under the following conditions: 55K rpm speed, 4 hours time, and at 25 °C temperature. A complex with high water content and soft texture was formed as shown in Figure 5.6.

Thermogravimetric analysis was done for the resulting complexes. After running TGA tests for 25 different portions from the same complex sample, the salt content in those complexes ranged from 16-31 % of their weight and the average salt content was 24%. The 25 portions were taken from different parts of the same complex sample and the test was repeated many times with reproducible results. However when these samples were put in water for few hours, the TGA test after that shows that salt almost completely goes out of the complexes and there was ~ 0% by weight NaCl salt left in the complex after putting them in water. This behavior was also proved by performing conductivity tests on the complexes. A PDADMA/PSS complex was put in 100 mL water under constant stirring and a conductivity cell was used to measure the change in the concentration of the solution with time. The results showed that salt particles leave the complex gradually up to a level where minimal salt amount is left in the complex. A sample TGA graph for a PDADMA/PSS complex that was put in water for few hours is shown in Figure 5.7.
5.3.2 Swelling of Compact polyelectrolyte complexes (ComPECs)

Three PDADMA/PSS samples were prepared using same procedure mentioned before. After centrifuging, one sample was put in deionized water, another one in 1M NaCl, and the third one was put in physiological saline. The increase in weight % was monitored for few days and recorded versus time. The samples were weighed every 20 minutes in the first 300 minutes, then every 60 minutes till 3000 minutes. The results were plotted in Figure 5.8 as increase in weight% versus time, where increase in weight % was calculated as follows:

\[
\text{Increase in weight } \% = \frac{\text{weight after certain time} - \text{dry weight}}{\text{Dry Weight}} \tag{5.2}
\]

Figure 5.6 (top) PECs in Centrifuge tubes; (middle) Fresh PDADMA/PSS complex shaped into a ball after centrifuging; (bottom) Fresh PDADMA/PSS complex cut into rectangular sheets to be used in DMA tests. The samples were prepared by mixing 10%
concentration solutions of PDADMA and PSS and adding 2.5M NaCl concentration. The complexes obtained were centrifuged in polycarbonate tubes for 4 hours at a speed of 55K and 25 °C temperature.

Figure 5.7 This figure shows a typical TGA curve for PDADMA/PSS complex left in different salt concentrations for few hours. The PDADMA/PSS complexes were prepared in 2.5 M NaCl, and 3 different parts were left in 3 different salt concentrations: 0M, 1.0M and 2.5M for few hours before running the TGA tests.
**Figure 5.8** Swelling of PDADMA/PSS complexes in water, 1M NaCl and Saline. ♦ represents swelling in water, ▲ represents swelling in physiological saline, and ■ represents swelling in 1M salt.
5.3.3 Tensile Properties Measurements

Tensile strength measurements were performed on PDADMA/PSS complex samples of same size using SHIMAZU tensile machine (SHIMAZDU INC.) according to the ASTM D 638. The experiments were run at room temperature. The samples were formed by cutting the complexes into rectangular sheets (25 x 10 x 3 mm).

The strain under stress is defined as the change in length relative to the initial length of the specimen (Equation 5.1). The strength and modulus can be calculated on the basis of the initial cross section. A sample plot for a PDADMA/PSS complex is shown in Figure 5.9. The PDADMA/PSS complex was cut into flat rectangular sheets. Each sheet was stretched by the machine that records the force (N) versus stroke (mm). By measuring the original sample dimensions before stretching, we can get the value of the elastic modulus of our complex. The average elastic modulus for our samples ranged from 10-100 KPa. Note here that elastic modulus for a multilayer made from solutions with no salt was 17 MPa, and for a multilayer made from 1M NaCl salt solutions was 0.3 MPa.19

The time taken for the stretched sample to break was 7 minutes, and the stretched length is more than double its original length. For example, the average length for our samples was 30 mm, and it becomes 70-80 mm after stretching. Also, after taking the samples out of the machine, they retain their original length and size in few seconds, even when they were left in the stretched position for about 10 minutes.

5.3.4 Measurement of Stress Relaxation and Elastic Hysteresis

Viscoelastic materials are characterized by a well-documented phenomenon which is stress relaxation. PEMUs were observed to have relatively fast stress relaxation in comparison with polymer networks. A stress relaxation curve for a PDADMA/PSS multilayer is shown in Figure 5.10.19
Figure 5.9 A plot of tensile strength measurement of a PDADMA/PSS complex.
Figure 5.10 Stress relaxation of PDADMA/PSS multilayer at different salt concentrations with a 0.4% strain where a, b, c, d, e and f correspond to salt concentrations of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 M NaCl respectively. [Jaber, J. A.; Schlenoff, J. B. *J. Am. Chem. Soc.* **2006**, *128*, 2940-2947].
Elastic hysteresis is defined as difference between the strain energy required to generate a given stress in a material and elastic energy at that stress.\textsuperscript{19} It is the energy dissipated as heat in a material in one cycle of dynamic testing. The elastic hysteresis measurements for our complexes were performed at same conditions as tensile strength measurements with two cycles for every measurement. Hysteresis can be determined by evaluating the area between the extension and retraction curves. Elastic recovery is defined by the ratio of the strain corresponding to first reaching zero stress during retraction to the fixed maximum strain. Figure 5.11 shows a sample hysteresis curve for a PDADMA/PSS complex.

**Figure 5.11** A plot showing a sample hysteresis curve for a PDADMA/PSS complex.
5.3.5 Dynamic Mechanical Analysis

DMA is a thermoanalytical method by which the mechanical behavior of a sample subjected to a specific temperature program is studied under the effect of a load which changes with time. DMA characterizes the viscoelastic properties of materials. In DMA, a complex modulus $E'$, elastic modulus $E'$, and an imaginary loss modulus $E''$ are calculated, all three allowing better characterization of the material, because we can examine the ability if the material to return or store energy $E'$ to its ability to lose energy $E''$ and the ratio of these effects which is the damping effect. By clamping our complexes into the DMA apparatus and an oscillatory force is applied which causes a sinusoidal stress to be applied to the complex generating a sinusoidal strain. By measuring both the amplitude of the deformation as the peak of the sine wave and the lag between the stress and strain sine waves, quantities like the modulus (stiffness), the viscosity, the damping (energy loss) can be calculated as a function of time and temperature.

DMA measurements were done using instrument model 2980 DMA V1.7B. PDAD/PSS complexes were cut into small square-shaped sheets with 10 mm length for each side, and 1-3 mm thickness. Two sheets were used at a time. The sheets were wrapped to preserve to avoid their drying. The experiment time averages 15 minutes for each sample. A sample graph is in shown in Figure 5.12.

Due to the nature of our samples there were difficulties in obtaining DMA measurements that we can depend on. So we moved forward to use the Rheometer, and the preliminary results that we obtained were consistent with the stretching experiments.
Figure 5.12 Shows a DMA data output for PDADMA/PSS complex. Storage modulus is plotted on the y-axis, loss modulus and tan delta on the secondary y-axis, while frequency (Hz) is plotted on the x-axis. The PDADMA/PSS complexes in graph were wrapped by Teflon, which increased the stiffness of the complex and that explains the higher-than-expected values for both storage modulus and loss modulus.
5.4 Conclusion

In this chapter, the research aimed at producing special polyelectrolyte complexes from different polymers, characterizing them, and enhancing their properties. All of this was done as an attempt to provide direction towards improved complexes that can be applied in the human body as bio-implants, specifically to replace intervertebral discs.
CHAPTER 6
CONCLUSIONS AND REMARKS ON FUTURE STUDIES

In this thesis, fundamental studies to characterize the water content, swelling by different salts, annealing effect, viscoelastic and mechanical properties of polyelectrolyte multilayers and complexes have been illustrated and conducted.

In Chapter 3, using the ATR-FTIR technique, it was possible to probe the bulk of the PDADMA/PSS and P4VMP/PSS multilayers thus gaining valuable information about their water and counter-ion content. The multilayer resembled a polymeric network comprising regions of varying intensities of polyion-polyion interactions the extent and distribution of which are sensitive to the type of the polyelectrolyte used and to the type and concentration of the salt in the surrounding medium.

A distinction was afforded in terms of the swelling and doping abilities of 14 different salts based on their hydration state. For both PDADMA/PSS and P4VMP/PSS multilayers, one can cautiously generalize that hydrophobic ions are more effective dopers than hydrophilic ions.

Water content of the two multilayers was studied extensively using a range of different experiments conducted by ATR-FTIR technique. Osmotic pressure experiment provided a good approach to characterize the water content inside a PEMU. Poly(ethylene glycol), a highly water-soluble polymer, was used as an osmotic stressing agent. Unlike salt solutions, PEG does not diffuse inside the PEMU from solution. It rather creates enough osmotic pressure to pull water out from the PEMU. A good estimate of the number of water molecules inside a multilayer was achieved using PEG.

Ion-pairing and water content are key factors influencing permeability of molecules into polyelectrolyte multilayer. They are also important in controlling the multilayer mechanical properties which are especially important when considering the use of PEMUs in biomaterials applications, for example to spread them on surface and control cell and protein adsorption. It has been reported that stem cells attached to a surface can be induced to differentiate into specific cell lines depending on the stiffness of the underlying interface. Water as a plasticizer and salt as a reversible doper provide
an ample tool to influence the stiffness of polymeric surfaces. Thus, by controlling water content and crosslink density, one may ultimately affect cell fate.

In Chapter 4, Layer by layer buildup of polyelectrolyte multilayers on a germanium crystal and ATR-FTIR measurements of ratios of water to sulfonate and azide to sulfonate peaks allowed us to monitor the annealing of PDADMA-PSS multilayers of different thicknesses in different ionic strength solutions. The film looses extrinsic sites as it anneals.\textsuperscript{13-16} This can be explained by a “lubricating” effect of the high NaCl concentration annealing solution. When small ions are present in excess, they provide a way of transport for existing bulk extrinsic sites by allowing for hopping from charged monomer to monomer and eventually escaping the PEMU. Thermodynamically the polymer-polymer electrostatic interactions are favored. A decrease in doping levels inside the PEMU will result in a decrease in the water content inside that film. The kinetics of the water migration process is in accordance with the extrinsic sites data.

Doping annealing curves also give kinetic and thermodynamic information for annealing in three NaCl concentrations different enough from each other to show useful information on the process. The highest ionic strength solution (1.0M NaCl) is kinetically fastest in annealing the PEMU. Lower ionic strength solutions will also anneal the PEMU and reach a “specific annealing extrinsic site concentration” (SAESC) but they will be kinetically slower in doing so, showing more gradual drops in their curves. Higher salt concentration solutions annealing a PEMU will “lock-in” more extrinsic sites than a lower concentration solution but will reach the SAESC point quicker.

In Chapter 5, polyelectrolyte complexes made from PDADMA and PSS were produced. Their mechanical and thermal properties were characterized using different analytical methods. DMA, TGA and mechanical stretching machines were used to conduct different experiments on those complexes. Swelling of the PDADMA/PSS complexes was investigated. We were able to get preliminary data about both the elastic modulus and the shear modulus of those complexes. Their water content, mechanical properties, thermal properties, and swelling behavior all encourage us to apply them in the biomedical field, in particular as bio-implants to replace intervertebral disc in the
spinal cord. We are still investigating the different properties of those complexes and working on enhancing their structure and biocompatibility.

Protein and cell adsorption control is a very critical factor in any biomedical application. Some applications require materials that induce bio-adsorption. These applications include biosensors, bio-separation methods, templates for tissue engineering, bio-catalysis, and bio-electronic materials. Other applications, such as medical bio-implants (which is our case with the ComPECs) and blood contacting biomedical devices, favor materials that prevent bio-adsorption.

A potential pitfall for using our complexes would be their weak biocompatibility when it comes to using them as implants. Protein and cell adhesion to these complexes will be highly expected. However, since our aim is directed towards producing biocompatible complexes that prevents protein adsorption and cell adhesion, we decided to take another approach in our research. We tried to produce minimally adhesive complexes using polyelectrolytes rich in zwitterionic functionality, thus controlling the complexes surface chemistry. Unlike polyelectrolytes, which exhibit either anionic or cationic charges, polyzwitterions (PZs) are well hydrated polymers with both negatively and positively charges which appear to be closely associated with each other. Incorporation of (PZs) into a complex will significantly reduce protein adsorption due to their neutrality. In a study conducted in our lab, Salloum et al demonstrated that PEMU surfaces influence cell behavior and that polyelectrolyte copolymer surfaces can be generated that are highly resistant to cell and protein interaction by incorporating (PZs) and by varying the % concentration of those (PZs) inside the film. For this purpose, we are working right now on sythesizing PAA-co-PAEDAPS, a hydrophilic zwitterionic copolymer with 75 mol% poly(acrylic acid) and 25 mol% poly((3-[2-(acrylamido) ethyl dimethylammonio]-propane sulfonate)), and PDADMA-co-PAEDAPS, another zwitterionic copolymer with 75 mol% poly(diallyldimethylammonium) and 25 mol% poly((3-[2-(acrylamido) ethyl dimethylammonio]-propane sulfonate)). The structures for those two copolymers are shown in Figure 6.1.
Figure 6.1 Structures of PDADMA-*co*-PAEDAPS and PAA-*co*-PAEDAPS.
Our future research might focus on preparing complexes from combining those two PZs with other polymers. By using those highly hydrophilic PZs in preparing the complexes low salt concentrations should be enough to make them sufficiently hydrated. This is a great advantage for us since we will need to test these complexes in salt concentrations that match physiological saline (e.g. 0.15M NaCl). Using PZs, the complexes will be prepared with almost the same procedure mentioned before for the PDADMA/PSS complexes except for the salt concentration change. Also, we will try to use certain salts that are biocompatible and swell the complex at low concentrations (e.g. calcium citrate).
Chapter 1


76. Olenych, S. G.; Moussallem, M. D.; Salloum, D. S.; Schlenoff, J. B.; Keller, T. C. S. *Biomacromolec.*, ACS ASAP.


**Chapter 2**


**Chapter 3**


**Chapter 4**


Chapter 5


**Chapter 6**


20. Olenych, S. G.; Moussallem, M. D.; Salloum, D. S.; Schlenoff, J. B.; Keller, T. C. S. *Biomacromolec.*, ACS ASAP.
BIOGRAPHICAL SKETCH

Amir H. Rmaile was born in Beirut, Lebanon, in the spring of 1980. In 1997, he attended Beirut Arab University and graduated in June 2003 with a bachelor of dental surgery. He was licensed as an oral and dental surgeon in Lebanon. He spent the next seven months practicing in a private clinic in Beirut. In 2004, he joined the research group of Professor Joseph B. Schlenoff at the Department of Chemistry and Biochemistry at Florida State University where his graduate work was supported by the Center for Materials Research and Technology (MARTECH) in Tallahassee, FL. During this time, he gained extensive experience in analytical methods, biocompatible surfaces, materials science, colloid chemistry and nanotechnology. He was also nominated for OTTA “outstanding teaching assistant award” for three consecutive years: 2005, 2006 and 2007. During that time, he successfully supervised and worked efficiently with some undergraduate and graduate students, including a post-doc. In May 2007, he received his Masters Degree in Analytical Chemistry.
HIGHLIGHTS

- Use of polyelectrolyte complexes as soft materials for bone and tissue regeneration
- Extensive research in using water-soluble polymers, interfacial assembly, biocompatible nanofilm coatings, surface techniques, polymer synthesis and modifications as well as analytical techniques to modify and improve the quality of existing products, materials and technologies
- Outstanding research abilities in the field of analytical, polymer, colloid, surface and materials chemistry as evidenced by presentations and publications
- Surface modifications using various kinds of hydrophilic, hydrophobic, zwitterionic polymers biocompatible materials and ultra thin film coatings
- Extensive knowledge of hydrophilic, hydrophobic and ultrahydrophobic surfaces
- Extensive research in using polyelectrolytes to be applied as bio-implants
- Excellent knowledge of biomaterials (e.g. polymers, composites, adhesives, nanomaterials)
- Licensed Dental and Oral Surgeon in Lebanon

EDUCATION

January 2004 – present  Masters in Analytical Chemistry (Special focus on materials and surface chemistry) The Florida State University and Center for Materials Research and Technology (MARTECH), Tallahassee, FL, 32306

Expected: June 2007

-GPA: 3.5/4.0

Advisor: Professor Joseph B. Schlenoff

1998-2003  Bachelor of Dental Surgery, Beirut A. University, Beirut, Lebanon

RESEARCH AND TEACHING EXPERIENCE

January 2004 – present
Research and Teaching Assistant, The Florida State University
Advisor: Professor J. B. Schlenoff

- Gained extensive experience in surface (SEM, AFM, ellipsometry, profilometry, XRF), spectroscopic (ATR-FTIR, IR) thermal (TGA, DSC, DMA) as well as mechanical properties testing techniques (mechanical stretching, poking, elastic and shear properties of materials) during the course of my research.
- Trained to use various spectroscopic, surface and characterization instruments and techniques while studying the newly synthesized thin film coatings and materials in addition to having a solid background in biomaterials.
- Nominated for OTTA “Outstanding Teaching Assistant Award” for three consecutive years (2005, 2006, 2007).
- Supervised and worked effectively with undergraduate, graduate students and a postdoctoral researcher in our laboratory.
- Led Advanced Analytical Chemistry (Chemical instrumentation) and General Chemistry Laboratories for 4 semesters.
- Excellent written, verbal, communication and interpersonal skills.
- Ability to work efficiently in an interdisciplinary research and teaching environment.
- Demonstrated problem solving, creative thinking, innovation and leadership abilities during the course of my research.
- Very experienced in writing technical research papers as well as progress reports for publications in peer-reviewed journals.

1998-2003

Oral and Dental surgeon, Beirut A. University

- General practitioner: dental and oral surgery clinics, Beirut A. University, Lebanon.
- Dental biomaterials Lab supervisor: Fabrication of dentures from different synthetic polymers.
- Presented different dental research papers.
- Led different dental laboratories: dental biomaterials lab, dental anatomy lab, x-ray lab.

PUBLICATIONS & PREPRINTS

- Have 3 research papers to be submitted.
Rmaile, Amir H.; Bucur, Claudiu B.; Schlenoff, Joseph B. Hydration Contributions to Association in Polyelectrolyte Multilayers and Complexes: Visualizing Hydrophobicity.

Rmaile, Amir H.; Schlenoff, Joseph B. Towards Polyelectrolyte Complexes for Bio-implants.

Bucur, Claudiu B.; Rmaile, Amir H.; Schlenoff, Joseph B. Defect Diffusion in Polyelectrolyte Multilayers.
• Had a preprint for ACS WASHINGTON 2005


PRESENTATIONS


MEMBERSHIPS

• American chemical society member, ACS, 2005-present
• Member of the American Association for the Advancement of Science, AAAS
• Licensed as dental and oral surgeon in Lebanon, 2003-present

CERTIFICATES, SCHOLARSHIPS & ACTIVITIES

• Teaching certificate from “Program of Instructional Excellency” PIE at Florida State University in 2004
• Conference presentation and travel grants from Congress Of Graduate Students (COGS) in March, 2006
• Excellent feedback from students’ “State University System Student Assessment of Instruction” SUSSAI forms
• Volunteer for the SDRC (Student Disability Resource Center) tutors at Florida State University
• Science Judge: Capital Regional Science and Engineering Fair 2006 and 2007
• Teaching Certificate from the Program of Dental Progress, Beirut A. University
• Teaching Certificate from Beirut A. University “Annual Instructional Meeting”
• Teaching Certificate from “Dental Implantology Meeting”
• Awarded an internship at the “Lebanese Dental Association Implant Meeting”
• Attended and helped in the organization of the Lebanese Annual Dental Conference, Lebanese Dental Association, Summer 2003
• 5-year Scholarship from UNRWA (monetary value of ~$1000/year awarded to the top 10% students in their class) (United Nations Relief and Works Agency) during the course of my study for my dental degree in Lebanon