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Use of atomic force microscopy to study a molecular micelle as an anionic polymer for polyelectrolyte multilayer deposition on polymeric substrates

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USE OF ATOMIC FORCE MICROSCOPY TO STUDY A MOLECULAR MICELLE AS AN ANIONIC POLYMER FOR POLYELECTROLYTE MULTILAYER DEPOSITION ON POLYMERIC SUBSTRATES

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The Department of Chemistry

by

Angela Douglas
B.S., Grambling State University, 2000
December 2006
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<th>Abbreviation</th>
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<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
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<tr>
<td>BGE</td>
<td>Background electrolyte</td>
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<td>CE</td>
<td>Capillary electrophoresis</td>
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<tr>
<td>CEC</td>
<td>Capillary electrochromatography</td>
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<tr>
<td>CGE</td>
<td>Capillary gel electrophoresis</td>
</tr>
<tr>
<td>CIEF</td>
<td>Capillary isoelectric focusing</td>
</tr>
<tr>
<td>CITP</td>
<td>Capillary isotachophoresis</td>
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<tr>
<td>CZE</td>
<td>Capillary zone electrophoresis</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical micelle concentration</td>
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<td>EOF</td>
<td>Electroosmotic Flow</td>
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<tr>
<td>GC</td>
<td>Gas chromatography</td>
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<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<td>LbL</td>
<td>Layer-by-layer</td>
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<tr>
<td>LIF</td>
<td>Laser-induced fluorescence</td>
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<td>MEKC</td>
<td>Micellar electrokinetic chromatography</td>
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<td>µCE</td>
<td>Microchip Electrophoresis</td>
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<tr>
<td>packed-CEC</td>
<td>Packed capillary electrochromatography</td>
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<td>OT-CEC</td>
<td>Open tubular capillary electrochromatography</td>
</tr>
<tr>
<td>PC</td>
<td>Polycarbonate</td>
</tr>
<tr>
<td>PDADMAC</td>
<td>Poly(diallyldimethylammonium chloride)</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PEM(s)</td>
<td>Polyelectrolyte multilayer(s)</td>
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<tr>
<td>Poly SULA</td>
<td>Poly(sodium N-undecylenyl- L-leucyl-alanate)</td>
</tr>
<tr>
<td>Poly SUS</td>
<td>Poly sodium undecenyl sulfate</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methylmethacrylate)</td>
</tr>
<tr>
<td>SPM</td>
<td>Scanning probe microscopy</td>
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<tr>
<td>STM</td>
<td>Scanning tunneling microscopy</td>
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ABSTRACT

Polyelectrolyte multilayers (PEMs) have been used as stationary phases for various chiral and achiral separations in open-tubular electrochromatography (OT-CEC). However, the physical characteristics of PEMs are not well understood. The production of PEM coatings involves flowing alternating rinses of positive and negative charged polyelectrolytes onto a surface. Although PEMs are typically deposited on fused silica capillaries, there is growing interest to use this approach in conjunction with microchip devices to enhance separation characteristics. Since microchips are fabricated using polymeric substrates, the deposition of PEMs on these substrates needs to be examined.

The study reported in this thesis uses atomic force microscopy (AFM) to examine the thickness and integrity of PEMs deposited on polycarbonate (PC), poly(methylmethacrylate) (PMMA), oxidized poly(dimethylsiloxane) (PDMS) and a glass wafer as a control. Poly(diallyldimethylammonium chloride) (PDADMAC) and a polymeric surfactant, poly(sodium N-undecylenyl-L-leucyl-alanate) (poly-L-SULA), were the polyions used in this study. The PEMs flowed through a microfluidic network defined by PDMS channels in contact with the polymer surface. Since salt is used in OT-CEC to increase the surface area in which analytes can interact, the effect of varying the salt concentration of the PDADMAC on the polymeric substrates was investigated, as well as the overall heights and integrity of the PEMs on the various substrates. Inconsistency of the PEM heights within a single system was observed and is most likely the result of roughness or defects within the substrates, leading to incomplete surface coverage. Preliminary data suggest that PDADMAC/SULA coating may prove to be
beneficial in achieving microchip separations NaCl concentration of 0.1M and lower since higher concentrations lead to the collapse of the PEM assembly.
CHAPTER 1

INTRODUCTION

1.1 Capillary Electrophoresis

Electrophoresis is a separation technique that is based on differences in analyte mobilities in an applied electric field. A Swedish scientist, Arne Tiselius, published the first electrophoretic separation by separating serum proteins according to their charge using a u-shaped tube filled with buffer in the 1930s. Tiselius was awarded the Nobel Prize in 1948 for his research efforts [1]. Although the basic concept of electrophoresis was first demonstrated more than 75 years ago, capillary electrophoresis was not explored until 1981, when James Jorgenson and Krynn Lukacs performed the high-efficiency separation of amino acids, dipeptides, amines, and a human urine sample using narrow glass capillaries [2]. Jorgenson and Lukacs extended the vitality of the separation technique by incorporating the use of fused silica capillaries, which in contrast to glass capillaries, are transparent in the ultraviolet region [3].

In recent years, capillary electrophoresis (CE) has become a significant technique for a variety of analytical separations which are both used in academia and industry. The CE method is applicable for the determination of a number of compounds ranging from metal [4, 5] and inorganic ions [6, 7] to complex biomolecules [8], such as nucleic acids [9] and proteins [10]. The CE approach continues to be used extensively in academic and industrial laboratories due to characteristics such as faster separation times, higher efficiencies, and smaller sample and reagent consumption in comparison to its predecessors: gel electrophoresis, high-performance liquid chromatography (HPLC) and gas chromatography (GC) [1, 11]. Due to its similarities to chromatographic techniques, CE is often considered as the merging of the powerful separation
mechanisms of electrophoresis with the instrumentation and automation concepts of chromatography [12].

There are six modes of operation within CE. These modes include 1) capillary zone electrophoresis (CZE), 2) capillary gel electrophoresis (CGE), 3) capillary isoelectric focusing (CIEF), 4) capillary isotachophoresis (CITP), 5) micellar electrokinetic chromatography (MEKC), and 6) capillary electrochromatography (CEC). Both CZE and CEC will be discussed in detail as they are the techniques used for the research presenting in this thesis.

1.2 Capillary Zone Electrophoresis

Capillary zone electrophoresis (CZE) is the most widely used form of CE and serves as the basis for different modes of CE [13-14]. Figure 1.1 illustrates a schematic diagram of a typical CE instrument. It consists of a fused-silica capillary, where the ends are placed in the buffer reservoirs, a UV lamp, a detector, and two electrodes that are connected to a high voltage supply. The method of CZE is done in free solution under an applied electric field created by use of an anode and a cathode. A buffer, commonly referred to as the background electrolyte (BGE), in conjunction with the applied electric field, is used to achieve the migration of analytes through the capillary by use of the process of electrophoresis. Analytes are separated according to their differences in electrophoretic mobilities which are proportional to their charge-to-size ratio as shown in Equation 1.1.

\[ \mu_e = \frac{q}{6\pi \eta r} \]  

The \( q \) is the charge of a particular ion; \( \eta \) is the viscosity of the solution; and \( r \) is the hydrodynamic radius for a spherical ion. This equation illustrates that the hydrodynamic radius and the viscosity of the solution is inversely proportional to the electrophoretic mobility. Therefore, ions with smaller radii migrate faster than ions with larger radii [11, 15].
The electroosmotic flow (EOF) is the underlying force of analyte migration. The inner walls of the fused silica capillaries used in CZE are anionic due to the ionization of silanol groups at pH values greater than two [16]. In solution, the negatively charged surface of the inner capillary walls is counterbalanced by cations in the BGE. The immobilized layer formed by the cations is called the Stern layer. The remaining ions in solution make up the diffuse layer, which extends into the bulk liquid. This arrangement of ion in solution forms the electric double layer. When an electric field is applied across the capillary, the cations of the buffer migrate toward the cathode. Since ions are solvated by water, the bulk solution within the capillary is pulled, thus, generating the EOF [11, 15]. Figure 1.2 is a depiction of the electrical double layer.
1.3 Micellar Electrokinetic Chromatography

The MEKC method is a chromatographic technique in which samples are separated by differential partitioning between an aqueous phase, the running buffer, and a pseudostationary phase which is added to the run buffer. The run buffer and the pseudostationary phase are carried through the capillary by an applied electric field. Micelles are common pseudostationary phases in MEKC and will be discussed in greater detail in the following section. Unlike other electrophoresis modes, MEKC can distinguish between different neutral compounds according to their hydrophobicity [17-19]. The migration times of charged and neutral compounds are dependent on their partitioning with a micelle, illustrated in Figure 1.3. This technique was pioneered by a Japanese scientist, Shigeri Terabe and although it was initially developed for the separation of neutral compounds, it also has the ability to separate ionic species [19, 20-22].
1.4 Surfactants and Micelles

An important characteristic of amphiphilic molecules is the ability to spontaneously aggregate in an aqueous environment. Aggregation is dependent on the amphiphilic species as well as conditions of the system in which they are solubilized. When a certain concentration is exceeded, an abrupt change in many physicochemical properties of surfactants takes place. This change is attributed to the formation of oriented molecular aggregates. The narrow range of concentration for which these changes occur is defined as the critical micelle concentration (CMC). The aggregates that form above the CMC are typically known as micelles. For micelles to aggregate, the surfactant concentration must be above the CMC [23, 25-26].

Above the CMC, surfactant monomers spontaneously aggregate to form organized assemblies. One such assembly is a micelle which is generally considered to be spherical in shape. Micelles are composed of surfactant molecules. Surfactants are amphiphilic molecules consisting of a polar hydrophilic moiety and a nonpolar hydrophobic moiety [23]. Figure 1.4 is a representation of a surfactant monomer and a micelle. The hydrophilic or “water-liking” part of
the molecule is often called the head, while the hydrophobic or “water-hating” portion is called the tail. The hydrophobic moiety is usually an elongated alkyl chain. The charge of surfactants are based on the charge carried by the polar head group and can be anionic, cationic, zwitterionic, or nonionic. Due to the presence of the hydrophilic group, surfactants are fairly soluble in water [18].

![Surfactant molecule](image)

**Figure 1.4** Representation of a surfactant monomer and micelle.

The number of monomers that form a micelle, defined as the aggregation number, N, determines the geometry and size of the micelle. Aggregation numbers for surfactants in aqueous solution normally range between 10 to 100. As with the CMC, aggregation numbers are dependent on the concentration of surfactant, presence of additives, and the temperature. Methods for determining the number of monomers in a micelle include diffusion, light scattering, and nuclear magnetic resonance [27].

While it has been well accepted that amphiphiles spontaneously aggregate when their concentrations exceed the CMC, the structure of these aggregates has been subject of great discussion. Hartley has proposed that micellar aggregates are spherical with the head groups arranged at the micellar surface [28]. McBain suggested that spherical and lamellar forms
coexist [29]. Using X-ray studies, Harkins et al. have suggested a lamellar (sandwich) model [24]. Later, Debye, and Anacker proposed that micelles arrange themselves in a rod-like manner rather than spherical [30]. A cross section of the rod would be circular, with the hydrophilic portion of the micelle lying on the periphery while the hydrophobic tails fill the interior. Hartley’s spherical version of the micelle was largely confirmed by Reich [31] in 1956, and this model is now generally accepted as an approximate structure of a micelle [24]. Figure 1.5 depicts each proposed model for micelles.

![Proposed models for shape of a micelle aggregate.](image)

**Figure 1.5** Proposed models for shape of a micelle aggregate.

### 1.5 Molecular Micelles

Conventional micelles have been successfully employed in MEKC separations; however, several drawbacks exist to this approach. The formation of a micelle is a dynamic process where equilibrium exists between the free monomer and micelle aggregate. This dynamic equilibrium can be disrupted, for example, with a change in temperature or by the addition of additives to the BGE. Thus, the micelle stability can be compromised [32]. Another complication occurs when separating analytes with the same electrophoretic mobility as the micelle. When this happens, organic modifiers or an increase in the surfactant concentration is needed to facilitate separation.
However, the addition of organic modifiers can disrupt the structure of the micelle and increasing the surfactant concentration can cause longer migration times, as well as and Joule heating. All of these attributes may have adverse effects on separations using MEKC [33-35]. The use of molecular micelles (polymeric surfactants), as a pseudostationary phase alleviates these common problems associated with micelles.

Amphiphiles, designed to contain a polymerizable double bond in the hydrophobic tail, can be polymerized at concentrations exceeding the CMC. The resulting polymer, often termed a molecular micelle, is thought to resemble a conventional micelle, and can be used as a pseudostationary phase for MEKC separations. Molecular micelles have several advantages for MEKC as compared to conventional micelles [36-41]. For example, the dynamic equilibrium existing between monomers and conventional micelles is eliminated in molecular micelles due to the presence of covalent bonds between the surfactant monomers formed during the polymerization process. Thus, the stability of the pseudostationary phase is not compromised and analyte resolution is improved. In addition, molecular micelles can be used at concentrations well below the CMC because of the polymerization process; hence, Joule heating, an effect of increasing surfactant concentration, is minimized. In addition, the added stability of molecular micelles allows the use of higher concentration of organic modifiers [42-43]. Figure 1.6 depicts the polymerization process of a molecular micelle which is accomplished by exposing a surfactant monomer (at a concentration above its CMC) to radiation. After radiation exposure, the product is then dialyzed. Figure 1.7 is a representation of a typical molecular micelle synthesized in our laboratory, poly sodium undecenyl sulfate (Poly SUS). It consists of surfactant molecules with sulfate head groups that are covalently bound at the tail.
Figure 1.6 Polymerization of molecular micelles (polymeric surfactants).

Figure 1.7 Representation of a typical molecular micelle, Poly SUS.
1.6 Capillary Electrochromatography

Although CEC was introduced in the early 1970s by Pretorius et al. [44], it was not until the 1990s that an increased interest in the technique was seen [45-51]. CEC integrates the high selectivity of HPLC with the high separation efficiency of CE [52-58]. Separation of charged and neutral analytes species is achieved by the combination of differential analyte partitioning between the stationary and mobile phase as well as differences in electrophoretic mobilities. The combination of these two concepts makes CEC a popular tool for the separation of difficult analytes such as neutral and chiral molecules; both of which can not be separating using traditional CE methods such as CZE.

In CEC as with every chromatographic technique, the column is considered the most important element since it serves as a chamber for the mobile phase as well as the separation channel for analytes [47, 59]. Thus, the preparation of the column, and most importantly the stationary phase is a vital component for CEC separations. CEC is commonly divided into three modes and is categorized according to the stationary phase used. These modes include packed-CEC, monolithic CEC columns, and open-tubular-CEC (OT-CEC) and an illustration of each stationary phase is shown in Figure 1.8.

![Figure 1.8 Illustration of end view various types of CEC stationary phases: (a) packed-CEC (b) Monolithic CEC column and (c) OT-CEC. Adapted from reference [60].](image-url)
In packed-CEC, a fused-silica capillary is packed with a stationary phase that usually consists of octadecyl silica. A slurry of the stationary phase is pumped into the capillary using high pressure, and the packing material is trapped between two frits. There are several limitations to the use of packed-CEC. One problem arises from the need to fabricate the frits in order to retain packing material in the capillary. Bubbles will form near the frits or around the packing material, causing inconsistent elution times, unstable baselines, and current breakdown. Additionally, the packing procedure is can more difficult than for HPLC packing procedures due to the narrow diameter of the capillaries used in CEC [61]. To compensate for these limitations, monolithic and open-tubular columns have been employed as alternatives to packed-CEC.

Monolithic CEC uses a continuous unitary porous structure prepared by either in situ polymerization or sol-gels [62-65]. Porous monoliths have been synthesized by polymerizing of monomers using UV, thermal, or radical initiation [66-67]. Sol-gel preparations involve the hydrolysis and poly-condensation of monomers which produce a porous silica gel network [68]. For in situ polymerization, particle fixed (i.e. immobilized) beds are created using temporary frits to pack the stationary phase. Polymerization of the stationary phases occurs after a mixture of organosilanes or a methyacrylate-based monomer and a porogenic solvent is pumped through the capillary. Monolithic CEC is advantageous over packed CEC due to the elimination of frits and the relatively simple procedure for polymerization.

In open tubular-CEC (OT-CEC), the stationary phase is deposited onto the surface of the capillary using a number of techniques including covalent bonding, adsorption, chemical bonding/etching, sol-gel, molecular imprinting, and porous silica layers [69-79]. OT-CEC yields higher separation efficiencies than packed-CEC due to the elimination of frits and the use of capillaries with smaller internal diameters. Although OT-CEC is a viable alternative to packed-
CEC, its phase ratio and sample capacity is low as a result of the relatively small surface area of the coating. Kapnissi et al. [36, 80] and Kamande et al. [83] have demonstrated the use of a layer-by-layer assembly, employing a molecular micelle, for use in OT-CEC. The assembly, termed a polyelectrolyte multilayer, incorporates a polycation and a polyanion as a separation medium. Polyelectrolyte multilayers will be discussed in greater detail in the following section. A schematic representation of the layer by layer assembly in their work is shown in Figure 1.9.

**Figure 1.9** Schematic representation of a layer-by-layer assembly in OT-CEC.

### 1.7 Polyelectrolyte Multilayers

Although coatings in OT-CEC use adsorption techniques (i.e. physical and dynamic adsorption) that have simple procedures and good reproducibilities, their lifetimes are often short and have limited pH ranges [80-82]. Adsorbed coatings are usually adhered to the surface of the inner capillary wall (i.e. fused silica) via hydrogen bonding and electrostatic interactions, both of which are weaker than covalent bonds. For greater stability and longer lifetimes, a coating using a layer-by-layer (LbL) assembly possessing multiple electrostatic interactions has been developed [80, 81, 83, 85]. This system is defined as a polyelectrolyte multilayer (PEM) and
was first introduced by Katayama et al. [81, 82] in the late 1990s for OT-CEC separations. PEMs are created in situ by alternating rinses of positively and negatively charged polyelectrolytes [85-88], where the negatively charged polymer maybe a molecular micelle [36, 80, 83].

Adsorption of polyelectrolytes (onto an opposite charged surface) is an ion exchange process, where charged moieties replace small salt ions counterbalancing the surface charge as shown in equation 1.2 [89, 90].

\[
\text{Pol}^- \text{M}^+_{(m)} + \text{Pol}^+ \text{A}^-_{(aq)} \leftrightarrow \text{Pol}^- \text{Pol}^+_{(m)} + \text{M}^+_{(aq)} + \text{A}^-_{(aq)}
\] (1.2)

The Pol\(^-\) and Pol\(^+\) are the charged polymer segments; M\(^+\) and A\(^-\) are salt counterions; and \(m\) refers to the area closest to the surface (i.e. multilayer coating). Figure 1.10 shows a schematic representation of a PEM deposited on a negatively charged surface.

![Figure 1.10](image)

**Figure 1.10** Schematic representation of polyelectrolyte multilayer on a negatively charged surface.

The thickness of PEMs occurs as a result of the overcompensation of surface charge and charge reversal after each deposition step [91]. Consecutive adsorption of a new layer onto the previous layer is achievable due to the excessive charge from the preceding layer. The overall charge of the multilayer is derived from the last layer deposited on the surface. Therefore, it is
presumed that an infinite number of layers can be fabricated [92]. However, when PEM coatings are used in a column, the column diameter is the limiting factor for the number of layers.

Although the concept of PEMs is relatively simple, its interior framework is somewhat intricate. Several techniques including neutron reflectometry, infrared spectrometry, and atomic force microscopy have been employed to gain a better understanding of the structure of PEMs. Nevertheless, it is still not very well understood. It has been shown that the polyelectrolyte layers are not arranged in distinct layers but are dispersed and can penetrate through one another [93-94]. Occasionally, a layer could possibly penetrate through three to four layers from its original position [91]. This interpenetration is a result of the inherent structure of the charge compensation with multilayer assembly [90]. Several determinants have been shown to alter the thickness of the PEM coating. Substrate surface charge affects the overall thickness of the PEM where the thickness increases with an increase in surface charge. However, an increase in the thickness of each layer is normally more strongly dependent on the concentration of salt added to the polyelectrolyte solution, and less dependent on polymer concentration, molecular weight, or deposition time [89, 90, 92].

1.8 Microchip Capillary Electrophoresis

In the early 1990s, microchip capillary electrophoresis (µCE) was introduced as a commercial product for application in many chemical, biological, and biochemical assays [95]. Although µCE can follow the same parameters as CE, there are several benefits. These advantages of µCE over traditional electrophoresis techniques include rapid analysis, reduced reagent consumption, and the mass production of devices at low costs [95, 97-99]. Due to the reduced sample volume and the extremely small size of the detection cell, highly sensitive detection methods are essential for capillary electrophoresis in microfabricated devices. Laser-
induced fluorescence (LIF) is by far the most popular detection method for microchip CE due to its sensitivity and instrumental simplicity [100-111].

Early studies of µCE concentrated on microfabrication using glass and quartz as substrates largely due to the fact that micromachining technology was already available for these materials. With the use of glass and quartz, many of the properties of conventional CE were maintained due to their chemical similarity to fused silica. However, there are several drawbacks associated with the use of these materials for fabricating microdevices. Optical-quality glass and quartz are expensive and the cost to develop and maintain fabrication devices may be quite high. Furthermore, the fabrication of glass microchips process produces a permanent seal between the two plates. Thus, if the glass chip breaks or clogs, the device is ruined.

The hardships associated with the use of employing glass microchips in µCE have led to the exploration of alternative substrate materials for the fabrication of microdevices. Polymer substrates are quite favorable because they can be mass-produced, are less fragile than glass, and are relatively inexpensive in comparison to glass. Poly(methylmethacrylate) (PMMA) is a commonly used polymer substrate for µCE devices [113]. Other popular substrates include polydimethylsiloxane (PDMS) and polycarbonate (PC).

As previously stated, the fabrication of polymer microdevices is relatively inexpensive. Fabrication begins with the construction of a molding master that contains the pattern (of channels) to be transferred onto the substrate. The polymer is then molded on top of the molding master, relinquishing the footprint of channels. A second layer of polymer is sealed over the channels, thereby producing a microdevice [113]. Contrary to glass, polymers can be bonded to different materials, including other polymers and glass, and as a result, increases their application in µCE [112].
Although the use of polymers in μCE has gained much popularity over the past fifteen years, the EOF is poorly defined and varies from polymer to polymer. In addition, analyte-wall interaction is a common problem encountered in μCE. Furthermore, the restricted amount of channel length leads to separation efficiencies that are often poorer than those in conventional CE [114]. Since the surface charge of plastics is known to be nonuniform, modifications of the substrate surface are required.

The incorporation of PEMs on the channels of polymer microdevices has shown promise for alleviating the difficulties associated with plastics. Barker et al. [112] and Lui et al. [113] both reported the deposition of PEMs onto the channels within microdevices that facilitated the control and modulation of the EOF. Ro et al. [115] demonstrated the use of PEMs to aid in the separation of coumarins by suppressing the adsorption of neutral analytes to the channel wall. Other applications for PEMs have also been reported. Reyes et al. [116] patterned PEMs on a flat oxidized PDMS surface by employing a microfluidic device and adhering retinal cells to the PEMs. The technique proved to be successful for patterning cells onto microscale features.

1.9 Atomic Force Microscopy

Scanning Probe Microscopy (SPM), is a class of imaging techniques that are capable of providing atomic-level information. SPM is primarily used for measuring the surface topography of samples. The first SPM instrument was developed in 1982 by Binnig and Roher and both were awarded the Nobel Prize in Physics for this achievement. The two most commonly used SPM techniques are scanning tunneling microscopy (STM) and atomic force microscopy (AFM). Both techniques are contingent upon the surface of the sample being scanned in an x/y raster pattern using a very sharp tip that moves up and down along the z axis as the surface topography changes. This movement is then measured and decoded into an image of
the surface topography. SPM instruments are useful in characterizing surface details not only on the lateral $x$ and $y$ axis of a sample but also on the $z$ axis. The resolution in SPM is normally around 20 Å in the $x$ and $y$ directions; however, ideal samples and the more superior instruments yield resolutions as low as 1 Å. In the $z$ direction, the resolution is typically better than 1 Å. In contrast, the resolution of common electron microscopy instruments is about 50 Å.

The STM method was first described by Binnig et al. [117] in 1982 and images features on a conducting solid surface. In STM, the tip remains a constant distance from the sample by a tunneling current between the tip which allows the surface of the sample to be observed and maintained at a constant level. A voltage is applied between the tip and the sample to produce the tunneling current. AFM instruments are advantageous in that they do not require the surface of the sample to conduct electricity, unlike in STM.

The AFM technique was introduced in 1986 by Binnig et al. [118] and in contrast to STM, permits resolution of single atoms on insulating and conducting surfaces. In AFM, a flexible force-sensing cantilever stylus is surveyed in a raster pattern over the sample surface. The force between the cantilever and the surface of the sample produces minute deflections of the cantilever which are optically detected. The motion of the tip, or in some cases the sample, is accomplished with the use of a piezoelectric tube. The force on the tip remains constant by the up-and-down motion of the tip during the scan, providing topographical information. A laser beam is reflected off a spot on the cantilever to a part of the photodiode that is used for detecting the motion of the probe. Output from the photodiode in turn controls the force applied to the tip, thus allowing it to remain constant. Figure 1.11 shows a block diagram of a typical AFM instrument and Figure 1.12 illustrates the laser beam being deflected off the cantilever.
Figure 1.11 Schematic of a typical AFM instrument. Adapted from Reference [119].

Figure 1.12 Illustration of a laser beam being deflected off the cantilever [120].

Physical characteristics of the cantilever and tip are crucial components for the performance of an AFM instrument. Cantilever/tip assemblies are fabricated by etching single chips of silicon, silicon oxide, or silicon nitride. These assemblies are extraordinarily small and
extremely delicate; typical cantilevers and tips are a few tenths of micrometers in length, less than ten micrometers in width, and approximately one micrometer thick.

The most common modes of operation for AFM are contact and tapping mode. The tip is in constant contact with the sample in contact mode, in contrast the tip touches the surface by oscillating the cantilever for a very short period of time, in tapping mode. One of the major disadvantages of contact mode scanning is that the tip is in constant contact with the surface sample. Additionally, the downward force of the tip may not be low enough to prevent damage to the surface and the sample, causing a distortion of the sample’s image. This dilemma is quite troublesome with soft materials, such as biological samples and polymers, and also with very hard samples, such as silicon wafers.

These disadvantages can be avoided by scanning in tapping mode. In this operation, the cantilever is oscillated at a frequency of a few hundred kilohertz. This oscillation is constant and the amplitude is continuously monitored. Positioning of the cantilever is such that the tip only comes into contact with the surface at the bottom of the oscillation cycle. Tapping mode scanning has been successfully used in AFM for a variety of materials that would have otherwise been difficult or inconceivable to image by use of the traditional contact mode [121].

AFM analysis is applicable to several fields of study. For semiconductor techniques, AFM has been used to characterize the silicon surfaces, as well as defects on the surface. The AFM method is also used to image magnetic domains of magnetic materials. In the field of biotechnology, it is often used for imaging DNA and other biological samples [121]. The AFM method is also commonly used to image PEM coatings. Dubas and Schneloff used AFM to image PEMs and to exhibit the change in surface roughness of the PEMs due to the addition of
salt [122]. Reyes et al. [116] employed AFM to image cells patterning on PEMs that were deposited on oxidized PDMS.

1.10 Scope of Thesis

This thesis investigates the deposition of PEMs using an anionic molecular micelle on several common polymeric substrates and comparing their depositions to the PEM deposition of glass. The AFM technique was used to analyze the multilayers that were coated on glass, oxidized poly(dimethylsiloxane) (PDMS), polycarbonate (PC), and poly(methylmethacrylate) (PMMA). The PEMs were deposited in well defined patterns on the surfaces by flowing alternating rinses of positively and negatively charged polyelectrolytes through a microfluidic device. The polyelectrolytes consists of the poly(diallyldimethylammonium chloride) (PDADMAC) and the anionic molecular micelle, poly(sodium N-undecylenyl- L-leucyl-alanate) (poly SULA). Two, four, and eight bilayers of the PEMs were deposited on the four substrates. One bilayer consists of a layer of the cationic polyelectrolyte and a layer of the anionic polyelectrolyte. Three different concentrations of sodium chloride (0, 0.1, 0.5M) were added to PDADMAC and its effect on the PEM heights were investigated as well as the correlation of surface charge (of the polymer substrates) as it relates to the height of the PEMs. Preliminary studies on the use of an anionic molecular micelle as a polyelectrolyte for PEM deposition of polymers microchips show promise for its potential use for µCE separations.
CHAPTER 2

EXPERIMENTAL

2.1 Reagents and Chemicals

PDADMAC, MW (200,000-350,000), was purchased from Sigma (St. Louis, MO). A structural representation of PDADMAC is shown in Figure 2.1. Sodium Chloride was purchased from Mallinckrodt (Phillipsburg, NJ). The N, N-Dicyclohexylcarbodiimine (DCC), N-Hydroxysuccinimide (NHS) and undecylenic acid were used for the synthesis of the poly SULA and were obtained from Sigma. The dipeptide L-leucyl-alaninate was purchased from BaChem Bioscience Inc. (King of Prussia, PA). The PDMS (Sylgard®184) was obtained from Dow Corning (Midland, MI) and synthesized according to product information. The PC sheets were obtained from Lexan GE Co. (Mount Vernon, IN) and PMMA was purchased from Lucite, ICI Acrylics (Memphis, TN). Glass wafers (Pyrex® Corning code 7740) were purchased from Corning (Corning, NY).

![Structural representation of PDADMAC](image)

**Figure 2.1** Structural representation of the cationic polymer, PDADMAC.

2.2 Synthesis of Poly(sodium N-undecylenyl- L-leucyl-alanate)

The surfactant monomer of poly SULA was synthesized from the NHS ester of undecylenic acid according to a previously reported procedure by Wang and Warner [123]. The CMC of the monomer used was found to be approximately 20mM by use of surface tension experiments [123]. Polymerization was accomplished by exposing a 100mM sodium salt
solution of the monomer to $^{60}$Co-γ radiation. A structural representation of the monomer of Poly SULA is provided in Figure 2.2.

![Structural representation of monomer unit of poly SULA.](image)

**Figure 2.2** Structural representation of monomer unit of poly SULA.

### 2.3 Fabrication of the Silicon Master and Polydimethylsiloxane Microfluidic Channels

Fabrication of a silicon master with raised features (parallel lines, 30μm in width) for molding of PDMS microchannels was done using a procedure previously described by Martynova et al. [124]. Molding of the PDMS microfluidic channels was done by pouring the polymer from a beaker by hand over the silicon master and curing at 150°C for 30min.

### 2.4 Preparation of Polymer Substrates

Glass wafers were rinsed with a copious amount of HPLC grade methanol and dried with nitrogen gas before use (gas blown over microdevice until dry). When PDMS was used as a substrate for PEM deposition, the PDMS was poured on a glass microscope slide, cured using the previously mentioned method and then rinsed with methanol and dried with nitrogen gas. Since PDMS is a neutral substrate, it must be oxidized in order to produce PEMs on its surface. Therefore, PDMS was oxidized in an O$_2$ (approximately 2.6 Pa) plasma chamber for one minute and then removed and brought into immediate contact with the microfluidic device by placing the device over the oxidized PDMS surface. The PC and PMMA substrates were cut into squares.
and rinsed with methanol and dried as mentioned above and then bought into contact with the microfluidic device.

2.5 Polyelectrolyte Multilayer Deposition

The polyelectrolyte solutions consisted of 0.5% (w/v) PDADMAC with NaCl concentrations of 0, 0.1, and 0.5M and 0.25% (w/v) poly(L-SULA) without any additives. For the polymer substrate, the microchannels were rinsed with deionized water before deposition of PEMs. The microchannels were then filled using a micropipette with the polycation solution, PDADMAC, and allowed to stand for 5min. The PDADMAC solution was then pumped out using an in-house vacuum system and the microchannels were rinsed with water and dried. The polyanion solution, poly SULA, was then added to the microchannels using a micropipette and the same procedure as for PDADMAC was followed. Alternating layers of the polycation and the polyanion were deposited (rinsing with water between each layer deposition with no resting time in between) until the desired number of bilayers were achieved. The PEMs were allowed to dry overnight after which the microdevice was removed from the substrate, thus exposing the PEMs on the substrate surface. Figure 2.3 is a depiction of the PEM deposition process which is adapted from Reyes et al. [116].

2.6 Atomic Force Microscope Instrumentation

All experiments were performed using an atomic force microscopic instrument (Dimension 5000, Digital Instruments, Santa Barbara, CA). AFM measurements were performed in tapping mode to attain topographical information about the PEM coatings. The height of the PEMs was measured in cross-sectional areas for each number of bilayers. A minimal of four scans for each coating assembly was completed and a $q$ test was performed to
select the appropriate AFM images for this study. Outliers existed in cases where less than four scans are reported in the results.

**Figure 2.3** (a) Microfluidic network is brought into contact with a polymer substrate (PDMS, PC, PMMA, glass). (b) Polyelectrolyte solutions are introduced into the microfluidic network (channels filled with polyelectrolyte solution are shown as darker lines) then rinsed with water after deposition. The cycle is repeated until desired number of layers is obtained. (c) Microfluidic network is removed exposing PEMs (black lines) on the polymer surface. Modified from Reference [116].
CHAPTER 3
RESULTS AND DISCUSSION

3.1 Treatment of Data

When imaging polymeric substrates on an atomic level unevenness of the substrates is seen in some cases. This artifact which can distort the observed PEM thicknesses should be corrected. For all data reported in this thesis, data were first corrected for sloping baselines that may result from an uneven substrate. The arbitrary zero point reported by the instrument was corrected by assuming the smallest value equals zero height. After baseline corrections, the data were plotted in MATLAB to construct a visual image of the surface topography of the PEM coating deposited on each substrate. Rather than determining the average heights with associated standard deviations, histograms and median heights of each PEM coating were calculated. Examinations of the histograms suggested that the distribution of heights were generally not symmetrical. Therefore, the median heights were used to better characterize the thickness of the PEM coatings and the histograms of heights were used to characterize the surface roughness.

As mentioned above, histograms of the heights of the PEM coatings deposited on the substrates were used to demonstrate the roughness of the coatings. Surface roughness of PEMs plays a pivotal role in OT-CEC by increasing the surface area in which analytes can interact and thus may promote the separation of difficult analytes (i.e. neutral or hydrophobic compounds). Selected AFM images, histograms, and median heights of PEMs consisting of two, four, or eight bilayers, where 0, 0.1, and 0.5M NaCl was added to the cationic polymeric solution, for each substrate shown are in the Appendix.
3.2 Thickness in PEM coating vs. Salt Concentration

Under the experimental conditions investigated, the height of the PEMs did not increase linearly with an increase of bilayers. Adsorption (and in some cases desorption) of the PEM was shown to depend primarily on the salt concentration. In the deposition of PEMs on the substrates, polyelectrolytes appear to compete with the salt ions for charged sites on the substrate surface. For adsorption that occurs primarily through electrostatic interactions, at a certain point the concentration of the salt ions becomes sufficiently high such that the PEM coating is displaced from the substrate surface. Such desorption behavior has been theoretically investigated by Muthukumar [125], van de Steeg et al. [126], and Wiegal [127]. Dubas and Schlenoff have also evaluated the effect of several salts, including NaCl, on the thickness and stability of PEMs deposited on silicon wafers [90, 128]. This phenomenon was also observed in the current experiments for each substrate included in the study: glass, oxidized PDMS, PMMA, PC. Previous studies performed in the Warner laboratory have shown that the addition of sodium chloride to the cationic polyelectrolyte solution provided the highest resolution for analytes separated in OT-CEC [121]. Therefore, sodium chloride was chosen as an additive for this study. In most cases, multilayers are clearly deposited on the substrate when up to 0.1M NaCl was incorporated into the cationic polyelectrolyte solution with the exception of glass and PMMA, where in some cases the PEM coating desorbed at higher salt concentrations. The average median heights for the PEM coating deposited on the four substrates were calculated and are shown in Figures 3.1-3.4. The number of scans, $n$, is denoted for each bilayer study on the substrates.
3.2.1 Study of Thickness vs. Salt Concentration of Glass

Glass was chosen as a control in this study due to its comparable characteristics with fused silica, the material for capillaries used in OT-CEC. As seen in Figure 3.1, the median heights range from just under 10 nanometers for two bilayers without addition of salt to nearly 200nm, where two bilayers were deposited with 0.1M NaCl added to PDADMAC. This is a common behavior thought to be the result of the salt ions disrupting the intermolecular forces between individual layers. This disruption causes the multilayers to swell, thereby increasing the film height. However, when 0.5M NaCl was added to PDADMAC during the deposition phase, the film thickness of the two bilayers coating decreased by 28 percent to 143nm. This reduction in height is presumed to be caused by the competition of salt ions with polyelectrolytes for charged sites on the glass surface.

When four bilayers of PDADMAC/Poly SULA were deposited, the PEM height increased from 11 to 103nm as the NaCl concentration increased from 0 to 0.1M and decreased to 67nm with the addition of 0.5M NaCl. The thickness of the four bilayer coating with the addition of 0.5M NaCl is less than the height of the two bilayer coating at the same salt concentration. The decrease in height compared to the two bilayer coating is most likely due to the increase in the number of bilayers of the PEM, which can possibly lead to compression of the coating resulting in a decrease in height. This means that, although a larger amount of polyelectrolyte may be deposited on the surface, layer interpenetration leads to a net reduction in bilayer thickness.

In the study of the eight bilayer system, the coating exhibited unexpected behavior. The coating’s height was relatively small even with the addition of salt. Unlike the two and four bilayers systems, a decrease in coating thickness was observed (from 27 to 10nm) as NaCl was
introduced into the system. However, for 0.5M NaCl, the height of the coating increased to 29nm. These findings may be the result of non-uniform polyelectrolyte deposition or as a result of an artifact due to the relatively few PEM assemblies prepared and images collected. Additional studies must be undertaken in order to confirm the trend observed.

![Figure 3.1](image.png)

**Figure 3.1.** Median heights of PDADMAC/Poly SULA on Glass. Heights at 2, 4 and 8 bilayers were investigated at NaCl concentrations of 0, 0.1, 0.5M for PDADMAC.

### 3.2.2 Study of Thickness vs. Salt Concentration of Oxidized poly(dimethylsiloxane)

Oxidized poly(dimethylsiloxane) (Oxidized PDMS) was selected as a substrate because of its similarities in surface charge to glass and fused silica. Wang et al. [130] reported that oxidized PDMS can produce an EOF comparable to glass, suggesting similar surface charges. Figure 3.2 illustrates the results of PDADMAC/Poly SULA multilayer systems on oxidized PDMS. The coating thickness of two bilayers decreased as the NaCl concentration increased. The observed PEM heights were 307, 111, and 63nm for 0, 0.1 and 0.5M NaCl. This observation was most likely the result of NaCl ions in solution displacing the PEMs from charged sites on the surface of the oxidized PDMS. When four bilayers of PDADMAC/Poly SULA were deposited on the surface, there was a slight increase in the height of the coating from 127 to 151nm as the concentration of NaCl increased from 0 to 0.1M. At 0.5M NaCl, the
thickness of the four bilayer coating decreased by fifty percent to approximately 75 nm. The addition of 0.1M NaCl to the PDADMAC solution resulted in an increase in the thickness of the eight bilayer system which is consistent with trends observed in the four bilayer system. In this case, the initial height of 36nm swelled to 139nm when salt was introduced into the system. A further increase in NaCl resulted in a significant decrease in the coating thickness. Heights observed in this case were only 9nm, indicating an almost complete loss of coating in the areas probed.

Figure 3.2. Median heights of PDADMAC/Poly SULA on Oxidized PDMS. Heights at 2, 4 and 8 bilayers were investigated at NaCl concentrations of 0, 0.1, 0.5M for PDADMAC.

3.2.3 Study of Thickness vs. Salt Concentration of Polycarbonate

Polycarbonate (PC) was included in this study due to its popularity in microchip electrophoresis. Figure 3.3 shows the coating thickness of PDADMAC/Poly SULA bilayers with the PC substrate was affected by the number of bilayers and addition of NaCl. The height of the two and four bilayer coatings showed a large increase as the concentration of NaCl increased from 0 to 0.1M. For the two bilayer PEM assembly, the height increased from 18 to 570nm and for the four bilayer system the bilayer coating increased from 270 to 980nm.
However, when 0.1M NaCl was introduced to the eight bilayer system, the coating thickness decreased by nearly fifty percent. In all cases, 0.5M NaCl resulted in a fairly consistent coating of approximately 20nm in height. It has been established that high salt concentrations will cause desorption of an adsorbed film [128] and therefore, it is possible that the consistent height of approximately 20nm observed for all bilayers at 0.5M NaCl is the height of the substrate in the absence of the PEM coating.

It should be noted that the height of the eight bilayer system deposited in the absence of NaCl, 2000nm, is considerably thicker than any PEM coating reported in the literature to date [90, 116, 128]. PEMs are usually created using linear polyelectrolytes, and to the best of our knowledge, this is the first report describing the topography of a PEM generated using an anionic molecular micelle. The enhanced thickness could be the result of different interactions between the polyelectrolytes employed in this study or hydrophobic interactions between the anionic molecular micelle and the PC substrate. A systematic study of various factors affecting film thickness is required to explain this observation.

![Figure 3.3. Median heights of PDADMAC/Poly SULA on Polycarbonate. Heights at 2, 4 and 8 bilayers were investigated at NaCl concentrations of 0, 0.1, 0.5M for PDADMAC.](image)
3.2.4 Study of Thickness vs. Salt Concentration of Poly(methylmethacrylate)

Poly(methylmethacrylate) (PMMA), is one of the most common polymer substrates used in microchip electrophoresis. Figure 3.4 illustrates trends observed in film thickness as the number of bilayers and NaCl concentration is increased. Although there is a significant increase in coating thickness as the number of bilayers deposited onto PMMA increased from two to four (height increased from 218 to 551nm), the addition of 0.1M NaCl reduced the film thickness to approximately 124nm for both assemblies. Additional salt ions further disrupted the two bilayer system and the coating height decreased to 22nm for a NaCl concentration of 0.5M. The height of the four bilayer coating remained constant as NaCl was increased to 0.5M, possibly indicating the PEM assembly was completely desorbed and the height observed was actually that of PMMA.

In the eight bilayer system deposited on PMMA, a severe reduction in coating thickness was observed. Heights of 693, 47, and eight nanometers were observed for salt concentrations of 0, 0.1, 0.5M respectively. Although bilayer heights on PMMA are not as thick as those shown for PC, the PEM heights for four and eight bilayers in the absence of salt are still higher than those previously reported in the literature, where linear polymers were used to create PEMs [90, 116, 128]. As with PC, the unusual thickness of the multilayer system is likely the result of hydrophobic interactions between PMMA and the hydrophobic core of the molecular micelle. The shape adopted by the molecular micelle during deposition can also affect film thickness. If one assumes that the shape of the molecular micelle is spherical, similar to the shape of conventional micelles, the observed increase in PEM height and surface roughness is not surprising.
Figure 3.4. Median heights of PDADMAC/Poly SULA on PMMA. Heights at 2, 4 and 8 bilayers were investigated at NaCl concentrations of 0, 0.1, 0.5M for PDADMAC.

3.3 Study of the Innermost Section of Coatings on Substrates

AFM images and histograms of representative PEMs are shown in Figures 3.5a-3.8a. Coating heights of approximately 5.5µm from the center of the PEM coating were determined and used to rescale the images in order to better observe the surface roughness of the PEM coatings deposited on the substrates. Figures 3.5b-3.8b shows rescaled images of the representative PEMs.

Figure 3.5a shows four bilayers of PDADMAC/Poly SULA deposited on glass where 0.5M NaCl to PDADMAC. Small aggregates at the center of the PEM coating are faintly observed as well as an accumulation of the polyelectrolytes on either edge of the coating. This behavior could be due to differences in the flow rate of the polyelectrolytes during the deposition process where flow rates may be slower at the edges of microdevices. Also, the removal of the microdevice prior to analysis could have distorted the coating. Using the histogram, the minimum and maximum heights around the center of the coating was obtained. The histogram indicates that PEMs do not coat in a uniform manner and these variations can be clearly seen in the rescaled AFM image in Figure 3.5b.
Figure 3.5 (a) AFM image and histogram of 4 bilayers of PDADMAC/Poly-SULA at 0.5M NaCl deposited on Glass. (b) Image focusing on difference of height near center of coating.

Figure 3.6a shows an AFM image of eight bilayers of PDADMAC with 0.1M NaCl /Poly SULA that has been deposited on oxidized PDMS. A close examination of the image confirms that the PEM coating has not been deposited in a uniform manner. The histogram displays the varying heights observed approximately 5.5µm from the center of the coating. The differences in heights further demonstrate the roughness of the surface. The minimum and maximum heights at the center of the coating were determined and used to rescale the image depicted for Figure 3.6b. By using the heights around the center of the PEM coating, one can focus on the features within the innermost section and clearly demonstrate increased surface area at the center of the multilayer assembly. In addition, the accumulation of polyelectrolytes on both edges of the coating is visible.
Figure 3.6 (a) AFM Image and histogram of 8 bilayers of PDADMAC/Poly-SULA at 0.1M NaCl deposited on Oxidized PDMS. (b) Image focusing on difference in height near center of coating.

Figure 3.7a shows an AFM image of the deposition of two bilayers of PDADMAC/Poly SULA on PMMA where no salt was added to the PDADMAC solution. An accumulation of the coating occurred on the left side which is likely due to hydrophobic interactions between PMMA and Poly SULA, or, is simply an artifact. Small features of the PEMs can be seen vaguely throughout the coating and the histogram provided in Figure 3.6a shows the variation in film height. By using the minimum and maximum heights around the center of the coating as a scale, the smaller features within the system are revealed, thereby showing the increased surface area of the PEM coating (Figure 3.7b).
Figure 3.7 (a) Image and histogram of 2 bilayers of PDADMAC/Poly-SULA at 0M NaCl deposited on PMMA. (b) Image focusing on difference in height near center of coating.

Figure 3.8a depicts four bilayers of PDADMAC/Poly SULA on PMMA with 0.1M of NaCl added to PDADMAC. Small agglomerations that are seen throughout the coating are most likely due to the PEMs not coating uniformly or possibly Poly SULA adhering to the surface of PMMA via hydrophobic interactions. In Figure 3.8a, the histogram shows height variances are indeed present. Figure 3.8b shows a better depiction of the small masses of the PEM assembly and shows the deposition of the coating PMMA surface. As previously stated, the accumulation of the PEM coating at the edges could be the result of flow rate variations that occurred when the polyelectrolytes were being rinsed through the microdevice or perhaps were due to the microdevice removal before the AFM analysis.
3.4 Comparison of PEM Coating in Relationship to Substrate

Although glass and oxidized PDMS are considered similar with respect to surface charge, very different trends in PEM thickness were observed in this study. For example, an increase in the number of bilayers in the absence of salt actually resulted in a decrease in the thickness of the coating deposited on an oxidized PDMS substrate. The AFM images clearly indicate non-uniformity in coating height. The different trends in film thickness for both substrates could be an indication of the degree of interpenetration. Using this argument, PEMs deposited on glass are significantly less penetrating than similar PEMs created on oxidized PDMS and could be due to variations of the surface charge and roughness of oxidized PDMS in this study (during...
oxidation of PDMS in chamber non-uniformity of charge distribution can occur). Of the four substrates investigated, oxidized PDMS is the only substrate to display this behavior. Additional studies are required before this can be fully understood.

The substrates PC and PMMA are both hydrophobic and demonstrated quite different behaviors than glass and oxidized PDMS. Both plastics (PC and PMMA) exhibited thicker PEM coatings than the higher surface charged substrates of glass and oxidized PDMS. The increase in coating height shown for these two substrates (PC and PMMA) was more likely the effect of these hydrophobic compounds interacting with Poly SULA. The PC substrate displayed a greater increase in height than PMMA. This behavior is perhaps the result of their differences in hydrophobicity which in turn could have an affect on the deposition process. Shadpour and et al. [131] reported higher contact angle measurements for PC than for PMMA indicating PC is more hydrophobic than PMMA. Using this argument and the experimental studies shown here, thicker coatings observed for PC could be the result of increased hydrophobic interactions with the hydrophobic core of Poly SULA. However, since plastics obtained from various manufactures have shown to exhibit different properties [131] due to the presence of additives, this observation needs to be further investigated for better understanding.
CHAPTER 4

CONCLUSION AND FUTURE STUDIES

4.1 Conclusion

PDADMAC/Poly SULA multilayer assemblies were deposited on oxidized PDMS, PC, and PMMA, common substrates that used in microchip electrophoresis. A glass substrate was employed as the control. Glass and oxidized PDMS exhibited very different trends in PEM thickness, possibly as a result of the non-uniformity of the surface charge. Thicker coatings for PC and PMMA substrates were likely due to hydrophobic interactions with the plastics and Poly SULA. Inconsistency of the PEM heights within a single system was observed and is most likely the result of roughness or defects with the substrates, leading to incomplete surface coverage. Due to the limited channel length of the microchips, the increased surface area (i.e. surface roughness of PEMs) can be advantageous in separating complex molecules such as proteins or other biological compounds. However, the stability of the PDADMAC/Poly SULA coating deposited on any of the polymer substrates has not been investigated. The PDADMAC/SULA coating could prove to be beneficial in achieving microchip separations as long as the concentration of NaCl is not higher than 0.1M NaCl (higher concentration can lead to collapsing of the PEM assembly).

4.2 Future Studies

The stability of the PDADMAC/Poly SULA coating on the substrates should be investigated in the future. The endurance of the PEMs on the polymeric surfaces can be determined by rinsing the coatings with various buffers under an applied electric field. After which, the coating should be allowed to dry overnight and then examined using AFM. In addition, the durability of the PEM system under various pH values should be explored. Since
pH is known to alter the behavior of PEMs, various buffers with a range of pH values should be introduced to the coating on the polymer surfaces, dried, and then examined using AFM. Lastly, the drying times of this PEM assembly should be investigated as well as the variation of wet and dry coating analysis. After the stability of the coatings has been determined, separations of proteins and other biological compounds can then be studied using microchip electrophoresis.
REFERENCES


APPENDIX: SELECTED IMAGES OF POLYELECTROLYTE MULTILAYERS

Figure 1. AFM image and histogram of two bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~15nm.

Figure 2. AFM image and histogram of four bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~12nm.

Figure 3. AFM image and histogram of eight bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~34nm.
Figure 4. AFM image and histogram of two bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~248nm.

Figure 5. AFM image and histogram of four bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~102nm.

Figure 6. AFM image and histogram of eight bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~11nm.
**Figure 7.** AFM image and histogram of two bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~153nm.

**Figure 8.** AFM image and histogram of four bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~121nm.

**Figure 9.** AFM image and histogram of eight bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on Glass. Median height of PEM coating was ~30nm.
Figure 10. AFM image and histogram of two bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~187nm.

Figure 11. AFM image and histogram of four bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~212nm.

Figure 12. AFM image and histogram of eight bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~52nm.
Figure 13. AFM image and histogram of two bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~102nm.

Figure 14. AFM image and histogram of four bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~191nm.

Figure 15. AFM image and histogram of eight bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~161nm.
**Figure 16.** AFM image and histogram of two bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~53nm.

**Figure 17.** AFM image and histogram of four bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~131nm.

**Figure 18.** AFM image and histogram of eight bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on OxPDMS. Median height of PEM coating was ~14nm.
Figure 19. AFM image and histogram of two bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~31nm.

Figure 20. AFM image and histogram of four bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~251nm.

Figure 21. AFM image and histogram of eight bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~1888nm.
Figure 22. AFM image and histogram of two bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~542nm.

Figure 23. AFM image and histogram of four bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~1223nm.

Figure 24. AFM image and histogram of eight bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~1208nm.
**Figure 25.** AFM image and histogram of two bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~18nm.

**Figure 26.** AFM image and histogram of four bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~18nm.

**Figure 27.** AFM image and histogram of eight bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on PC. Median height of PEM coating was ~21nm.
**Figure 28.** AFM image and histogram of two bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating was ~179nm.

**Figure 29.** AFM image of 4 bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating is ~495nm.

**Figure 30.** AFM image and histogram of eight bilayers of 0M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating was ~645nm.
Figure 31. AFM image and histogram of two bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating was ~177nm.

Figure 32. AFM image and histogram of four bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating was ~157nm.

Figure 33. AFM image and histogram of eight bilayers of 0.1M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating was ~86nm.
Figure 34. AFM image and histogram of two bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating was ~44nm.

Figure 35. AFM image and histogram of four bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating was ~534nm.

Figure 36. AFM image and histogram of eight bilayers of 0.5M NaCl PDADMAC/Poly-SULA deposited on PMMA. Median height of PEM coating was ~17nm.
VITA

Angela Nakeeta Douglas, born August 20, 1978, in Monroe, Louisiana, was the second child born to Sheila and the late Dr. Samuel Douglas. She received her primary education at Alma J. Brown Elementary Laboratory School and attended junior high at Grambling Middle Magnet School, both of which are located in Grambling, Louisiana. In May of 1996, she graduated salutatorian from Grambling State University Laboratory Magnet High School. In the summer of 1996, Angela enrolled at Grambling State University for her baccalaureate degree. During her first two years at Grambling, she participated in programs funded by the Office of Naval Research and the Louisiana Alliance for Minority Participation. For Angela’s final two years of college, she received a fellowship from the Career Opportunities in Research Training and Education Program, which is funded by the National Institute of Mental Health. It was through this fellowship that she was able to attend summer research programs at Louisiana State University and University of Illinois at Urbana-Champaign. She graduated cum laude in May of 2000, with a Bachelor of Science degree in chemistry.

In December of 2006, Angela will receive her Master of Science degree in chemistry from Louisiana State University. While enrolled, she received a fellowship from the Intergrative Graduate Education Research Training Program sponsored by the National Science Foundation. Her research was done under the guidance of Professor Isiah Warner. Angela’s thesis focuses on the analysis of polyelectrolyte multilayers on polymeric substrates. This work was part of collaborative research with Dr. Laurie Locascio and Dr. Sam Forry at the National Institute of Standards and Technology in Gaithersburg, Maryland.