Self-Assembled Switchable Antimicrobial Polymer Thin Film Coating

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CERTIFICATE OF APPROVAL

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We hereby recommend that the dissertation prepared under our supervision by: **FARIA HASSAN** (**NUST201362102MSMME62413F**) Titled: **"Self Assembled Switchable Antimicrobial Polymer Thin Film Coating"** be accepted in partial fulfilment of the requirements for the award of MS degree with grade___.

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I hereby declare that this research study has been done for partial fulfilment of requirements for the degree of Master of Science in Biomedical Sciences. The intellectual content of this thesis is a product of my own work and no portion of the work referred to in this thesis has been submitted in any other degree or other institute of learning. I also certify that the thesis has been written by me. The help I received during my research work and preparation of the thesis, itself has been acknowledged. Moreover, I certify that all sources and literature used have been indicated in the thesis.

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DEDICATION

Dedicated to my parents, to whom I owe everything!

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All praises to Almighty Allah, the authority of knowledge and creator of resources, skills and opportunities.

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Faria Hassan

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List of Abbreviations

BL	Bilayers
LbL	Layer by Layer
S. aureus	*Staphylococcus aureus*
E.coli	*Escherichia coli*
°C	Degree centigrade
Rpm	Rotations per minute
OM	Optical Microscopy
OP	Optical Profilometry
SEM	Scanning Electron Microscopy
AFM	Atomic Force Microscopy
µm	Micrometre
mV	Millvolts
mM	Milimolar
Mg	Milligram
SAMu	Self-assembled multilayers
+ve	Positive
-ve	Negative
PAA	Poly (acrylic acid)
PAH	Poly (allylamine hydrochloride)
APTMS	3-aminopropyltrimethoxysilane toluene
PDADMAC	Poly (diallyldimethyl ammonium chloride)

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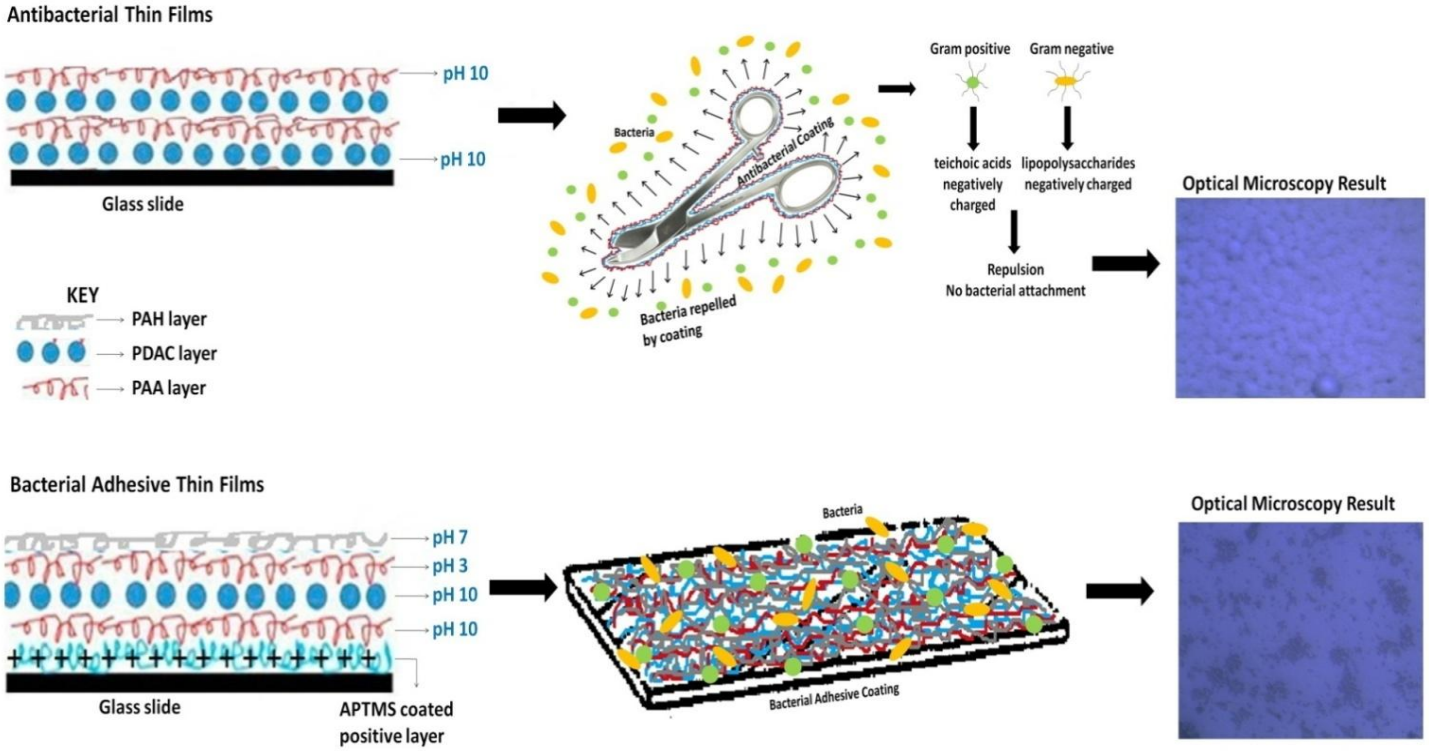
ABSTRACT

Present project aims a molecular fabrication approach to study the behavior of fouling organisms on planar surfaces at specifically controlled pH for the polymeric thin films coatings fabricated by electrostatic layer-by-layer (LbL) assembly methods. Poly(acrylic acid) (PAA, a weak polyanion) and poly(diallyldimethylammonium chloride) (PDADMAC, a strong polycation) were used to fabricate the bulk films. The weak Poly(allylamine hydrochloride) (PAH) was used as a top layer in bacterial adhesive thin films. Surface charge tuning was accomplished by regulating the level of ionization of the weak polyelectrolytes at different pH values and subsequent manipulation of the amount of polyelectrolyte deposited in the one preceding the last and last layers, respectively. The prepared films were investigated for their antimicrobial and bacterial adhesive surface characteristics. The fouling behavior of bacteria on the LbL films with almost comparable hydrophilicity and roughness but varying surface charge densities was studied. Antimicrobial activity of coated glass slides was evaluated against *Escherichia coli* (E. coli, ATCC# 8739) and *Staphylococcus aureus* (S. aureus, ATTC# 6538). The switchable thin film coatings developed allows achieving optimal microbial growth both in terms of repelling and adhesion performances at the precise pH values of the environment. The surface characteristics of the foulants as well as the bacterial adhesive thin films can be used to switchably attach or repel and detect control concentration of bacterial strains.

Keywords: Polymeric thin films, Electrostatic layer-by-layer (LbL) assembly method, Polyelectrolytes, Surface charge tuning, Bacterial adhesive thin films, Antimicrobial coatings, Hydrophilicity.

GRAPHICAL ABSTRACT



CHAPTER 1

INTRODUCTION

Chapter 1

Introduction

1.1 Background

The growth of organic matter and spreading out of plants, microbes or flora and fauna on surfaces is called bio fouling [1]. This process may takes place on any surface engrossed in any marine or biological ecosystem and economical or healthcare problems are usually related with the synthetic surfaces.

Fouling is a known challenge for biomedical usage, water purification processes and for the marine business [2][3][4]. The adsorption of protein adversely affects biomedical implants by not only diminishing the efficiency of the device but also results in adverse side reactions like thrombosis [5][6]. Protein adsorption may result in the development of a conditioning layer on the medical devices that increase the growth of microbes and results in swelling [1].

The growth of biofilm by the attachment of bacteria causes contamination and greater infection risk [1][2]. The organic substances that block the membrane pores often result in membrane fouling that results in an increased functioning pressure and thus diminishing the infuse fluctuation in the filtration systems. The damage is typically everlasting and irreparable and requires membrane replacement that adds to the price of the application [3].

Microbial defilement and infection threat associated with it is a major complication in agricultural industries, medical units and in the society [7][8][9]. The adhesion of microorganisms to various surfaces causes successive colonization that leads to the development of a thin resistant layer of microorganisms known as biofilm [7][10][11]. This bacterial attachment to a surface is mediated via a variety of connections that can either be precise for instance by formation of a protein film on the surface or via nonspecific adsorption for example by hydrophobic interaction [11][12].

Once the bacteria gets attached it can develop on the surface of the membrane and produce insoluble exopolysaccharides (EPS), a three dimensional matrix then enclose

the adhered bacteria. The EPS build-up and bacterial reproduction results in a mature biofilm on the membrane surface that cannot be simply isolated [13].

Biofilm development on biological devices such as catheters, restorative implants and disposable lenses causes contamination [14]. Common management procedures for biofilm exhibiting contamination of medical implants include surgical substitution of the infected devices as well as long term antibiotic treatment that causes extra treatment expenses [8]. These therapies involve long periods of hospitalization, serious functional injury and greater mortality rate [15]. Contamination as a result of biological matter is of great significance when it comes to severe microbial illness that are resulted by bacterial attachment mainly as a result of antibiotic-resistant strains [8][9][16]. Amongst a variety of infections produced by disease causing microorganisms the infections transmitted by *Staphylococcus aureus* that is resistant to methicillin has been of immense apprehension [17][18][19].

In summary, microbial contamination is a major issue that require better apprehension and management. A variety of procedures have been suggested to fight microbial contamination.

Thorough analysis of new antibacterial materials are underway since there is an increase in infections induced by microorganisms [20][21]. The antibacterial materials are used in domestic, business and communal service products including dyes, toys, devices and household appliances plus educational and medical equipment. Natural antimicrobial substances are mostly used but these agents typically have low melting and boiling points consequently they have an affinity to volatile or decay and lose their toxic potential. Whereas inert antiseptic substances are typically present in the form of hybrids as compared to natural antibacterial substances the inorganic–organic hybrids bear some potential side effects on the person's body [22].

On the other hand the most commonly used methods to prevent or destroy fouling organisms are via inert materials [23]. However bactericides are normally poisonous not just to the intended microbes but also to other species or cells in the surrounding area. Furthermore numerous bactericides are not properly biodegradable and as a consequence everlasting contamination of the atmosphere results [1][4].

An ecological safe choice in fouling administration can be brought by substances showing low adherence hence hindering the adhesion of foulants. This approach can be applied through manipulating the interactions among the adhering substances and

the surface that is confined at various level of the foulant adherence procedure. Significantly using low bonding methods is helpful not only inhibiting microbial adhesion on the other hand to avoid the attachment of bio macromolecules like proteins as well. By fine-tuning the surface characteristics like control of microtopography or architecture, crudeness, wettability and charge, nonadhesive materials can be fabricated [24][25][26][27].

As majority of the foulants including microorganisms are charged organic substances therefore electrostatic connections plays a vital role in bioadhesion, mainly in the preliminary level of fouling [28][29]. Electrostatic interactions are generally registered as a basic requirement in order to prepare low-fouling materials [30].

Many studies discussing the impact of surface charge on fouling characteristics are underway to manage and regulate the net charge present on a surface. Treating surfaces in environmental settings by elevated energy irradiation or via using strong oxidants causes them to get enclosed by ionic functional groups [31][32]. As the process of charging in these situations is generally linked to radical oxidation as a result zeta potential values gets negative thus causing the modification of charge complicated. In a different perspective self-assembled monolayers (SAMs) were prepared by combining positive or negative functional groups in order to terminate alkanethiolates with different character [25][30][33]. Similar surface charge regulated self assembled monolayers were also studied to find out either barnacle cyprids of Amphibalanus amphitrite favour particular charges present on the surface. The results showed on the negatively charged self-assembled monolayers more cyprids settled as compared to positively charged and neutral SAMs [25]. For this purpose the addition of various chemical substances on the substrate such as acids and bases is a drawback as electrostatic assistance to surface connections are difficult to separate from other chemically induced effects such as van der Waals interactions etc.

For the control of electrostatic charge distributions polymers were greatly used. For instance polymerizing mixtures of positively charged and negatively charged monomers can be used to adjust the charge of polymer brushes [29][34][35]. In polymeric matrices to create charged hydrogels for controlling adsorption of proteins various positively and negatively charged molecules have also been created [36].

Electrostatic LbL assembly is a convenient, inexpensive environmental friendly, robust, and quick method to prepare tuneable polymer films of desirable properties or microcapsules [37][38]. The films are formed by depositing alternating layers of

oppositely charged materials with wash steps in between or by spraying the subsequent solutions on a surface [39].

There are a wide variety of materials that can be deposited by LbL including: polyions, metals, ceramics, nanoparticles, and biological molecules.

Antimicrobial materials can be prepared by LBL films. Surface properties of the thin films can be simply tuned with the selection of the substances utilized and through specifications of the assembly process [40][41]. The physical characteristics of the thin films like density, mechanized feature and charge can be controlled through altering ionic strength and pH of the polymeric fluid [42]. Through altering the extent of ionization of the subsequent radicals by adjusting the pH of the weak polyelectrolyte solution it is possible to control the thickness of LBL polyelectrolyte films [43][44]. Poly (allylamine hydrochloride)/ Poly (acrylic acid) thin films prepared at alkaline pH have been stated to draw greatly adherent marine fibroblast NR6WT cells. Whereas thick PAH/PAA thin films prepared at acidic pH values swell significantly during physical states to produce greatly hydrated surfaces [45].

Bulk layer by layer films are normally charge adjusted the upper most layer of the thin films is charge compensator so therefore block or boost adsorption of protein via electrostatic connections [46]. It is additionally very much archived that positively charged surfaces may eliminate microscopic organisms [47].

In order to build a surface to kill airborne microorganisms on contact Poly (4-vinyl-*N*-alkylpyridinium bromide) was covalently attached to glass slides [48]. LBL assembly method was utilized to immobilize antimicrobial silver nanoparticles on nylon and silk strands [49]. With a specific end goal to make a multilayer framework to eliminate microbes poly (allylamine hydrochloride) and poly (sodium 4styrenesulfonate) were gathered at alkaline pH to join not charged amine groups into the layer by layer thin films [50]. To decrease the adhesion of cyprids, thin films terminated by positively charged polymers have been well documented [51].

Importantly, present strategy allows achieving optimal antimicrobial performance of a given material taking into account specific pH values of the environment and the surface characteristics of the fouler as behavior of foulants on planar LbL film surfaces at a specifically defined working pH has not yet been exhibited in detail.

CHAPTER 2

LITERATURE REVIEW

Chapter 2

Literature Review

2.1 Layer-by-Layer Self Assembly

LBL self assembly is a deposition method used for the growth of an ultrathin film on solid surfaces by alternating dipping or flip-flop in cationic and anionic species with instant deposition of the oppositely charged particles [52].

This system is extensively utilized for advancement of multilayer models with controllable thickness since the revelation of Langmuir-Blodgett (LB) phenomena for adsorption of various charged species via thin films. On the basis of the kind of natural material utilized this procedure produces multilayers with highly ordered nanoscale features [53][54]. As a result alternative assemblies of oppositely charged colloids on glass support and in sequence arranged layered substrates with oppositely charged metal ions bearing polycrystalline coatings were developed [55][56].

In addition to being easy and vigorous these strategies need simply advanced technology. Through the use of specific stoichiometry, they can easily and without depending on complex chemical reactions deposit consecutive layers.

Lately the layer-by-layer self-assembly approach has emerged as a real alternative to the Langmuir-Blodgett technique. Electrostatic forces are the major driving forces for layer-bilayer self-assembly but sometimes hydrogen-bond interaction is involved as well. Layer-by-layer self-assembly is a rising control of nanotechnology in which items, gadgets and different frameworks with fluctuating structures are shaped without externally applied prodding.

Layer-bilayer self-assembly is chiefly a thin-film fabrication technique that includes deposition of opposite charges having polyions for the development of alternating layers with simultaneous washing steps in between [52].

Multilayers of materials can be assembled on two-dimensional (2-D) supports of any area such as slides, silicon wafers, and plastic surfaces and on three-dimensional (3-D) micro/nanotemplates such as colloidal particles including latex or cells (Fig. 2.1). Charged materials, including linear polyelectrolytes, enzymes, antibodies, viruses and inorganic nanoparticles have been used in 2-D and 3-D nanoassembly processes [57][58]. The architecture of the resulting film can be designed with nanometer precision to meet different requirements such as thickness, biocompatibility, controlled permeability, targeting, and optical or magnetic properties [58].

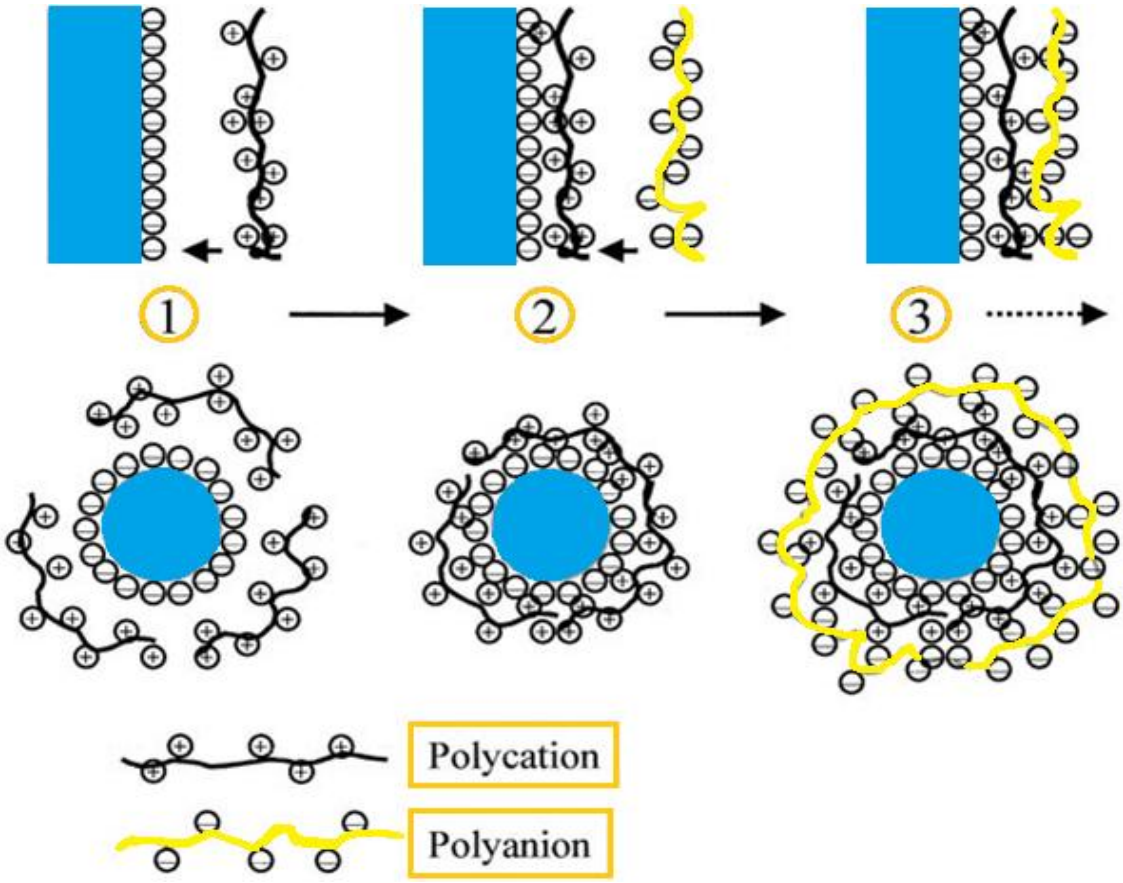


Figure 2.1: The mechanism of electrostatic LBL self-assembly on 2-D substrates and 3-D nanotemplates.

2.1.1 Substrates for Layer-by-Layer Self-Assembly

The basic requirement for layer-by-layer self-assembly is an appropriate substrate that can hold as well as support the assembly that is going to be organized on it. Numerous substrates are used to create assemblies including glass, quartz, silicon wafers, mica and various polymers (Fig. 2.2). Layer-by-layer self-assembly can be assembled on a variety of substrates.

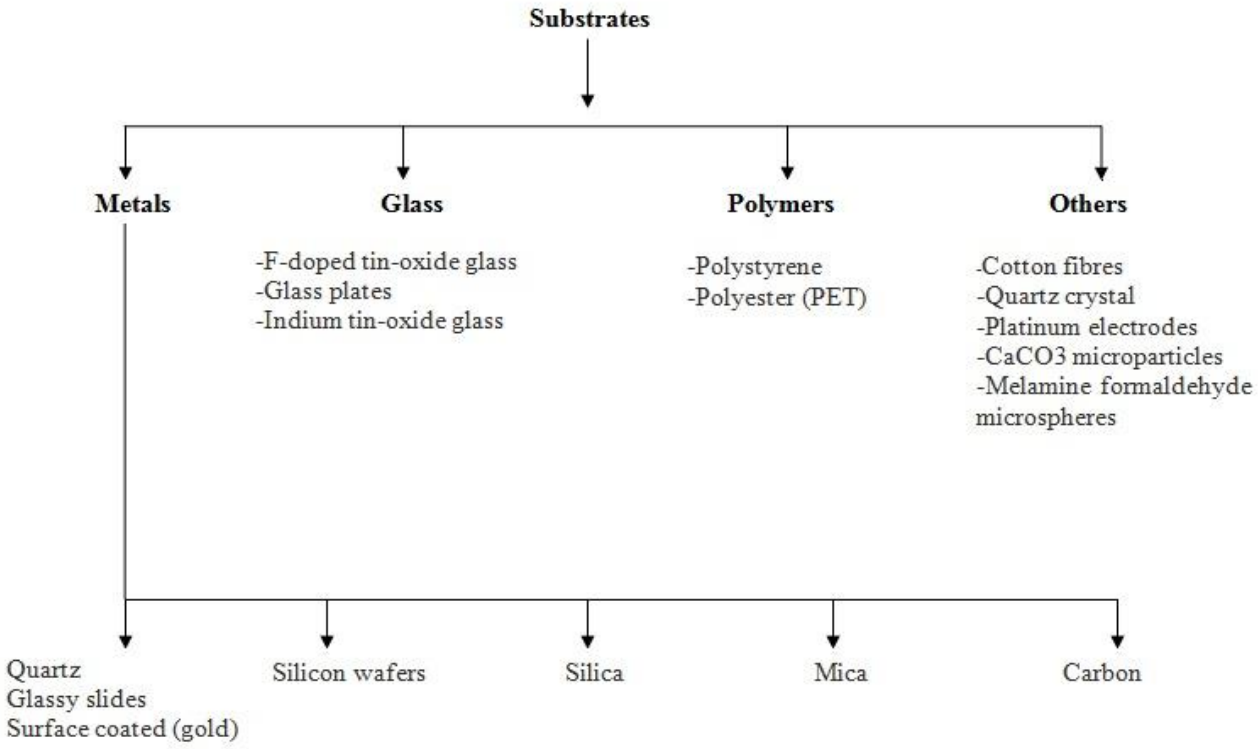


Figure 2.2: Classification of substrates used in fabrication of layer-by-layer self-assembly.

2.1.2 Thin Film Properties

The film thickness and stability of LbL assembly is affected by ionic strength, pH and concentration of the polyion solution. The pH of the polyelectrolyte solutions should be certain to keep a high degree of polyion ionization for the LbL process. The pendant sulfonate groups with pKa = 1 or carbonate groups with pKa around 4 to 5 are normally used to attain the negative charge of the polyanions. Whereas cationic properties of polymers are usually controlled by ionization of amino- and imino-groups that have isoelectric points around pH 8 and 11. So in order to maintain the

charge of several polyanions and polycations including polysaccharides the phosphate-buffered saline (PBS) at pH 7.4 is appropriate [59][60].

PBS also provides physiological ionic strength that is essential for protein or enzyme assembly. The pH value of the coating solution should not be very close to the isoelectric point (PI) of the polyions used as in that case the charge would not be sufficient to support LbL assembly (at least 10% of pendant groups have to be ionized) [59].

By changing the ionic strength of the solution the thickness of each layer in the LbL film can be finely adjusted that in turn induces polymer coil formation. Thicker films are produced at higher ionic strength [59].

The polyion films are usually insoluble in water as well as in many organic solvents and are stable up to at least 250°C [59]. Hydrophilic films formed by LbL self-assembly remained stable after 1-mo incubation in a 90°C oven, whereas hydrophilic surface property of plasma treated polymer surface is vanished after some days [60].

2.1.3 Biomedical applications of layer-by-layer self-assembly

The ability to create thin films on a wide range of surfaces has several biomedical applications. Coatings on medical devices can enhance biocompatibility, lessen the immunological response and make possible to deliver a drug locally. It is thus helpful to find out a method to coat thin films with required properties on a wide range of surfaces [61].

For example, a thin (only few nanometer) film coating on Petri dish can promote cell adhesion and growth in vitro [61].

At present the existing thin film methods comprises of spin coating and solution casting, thermal deposition, polyion LBL assembly, chemical self-assembly, the Langmuir-Blodgett technique, and free-standing films [61].

A major advantage of the LbL self-assembly technique is its ability to coat thin films with ordered structure and nanometer thickness on supports of various shapes and sizes [61][62].

Medical Implants

The majority of medical devices usually require a biointerface between the implant and the neighbouring tissue. As a result local nonspecific adsorption of protein, swelling and infectivity can hamper with prolonged use. For the surface adjustment of biomaterials the fundamental physical characteristics should be retained whilst adjusting only the outermost surface to affect bio interaction [63].

To prolong the lifetime of the product typically a hydrophilic coating with lubricious property on implants is favourable. Before implantation the nonspecific adsorption of proteins should be reduced and useful molecules should become selectively adsorbed onto biomaterials [64]. In order to perform such functions the LbL self-assembly method can be used to deposit thin films on implants.

The LbL self-assembly technique allows to coat ultrathin ordered films in nanometer range and with specific knowledge of their molecular composition [65]. At the beginning of the alternating assembly process a nonlinear film growth commonly occurs [66][67]. A smaller amount of adsorbed polyion is usually present in the first two to three layers. Through the number of adsorption cycles the mass of the film and thickness of subsequent layers increase linearly [67].

The required film thickness depends upon the coating material, surface roughness of the implant and the biological environment of the implant. Films of sufficient thickness can change mechanical properties and surface morphology, whereas very thin films do not offer the desired durability and strength [68].

In polymer film assembly natural polymers are of great interest because of their distinctive characteristics. They are naturally available, nontoxic and biocompatible [68].

In LbL assembly proteins and protein-based polymers such as albumin, collagen, and gelatin, polypeptides such as polylysine, poly (α,L-glutamic acid), poly(aspartic acid) and polysaccharides including hyaluronic acid, dextran, heparin, chondroitin and chitosan can be used. Synthetic polymers are also used in the assembly procedure. Commonly used polyions include polycations, such as poly (ethyleneimine) (PEI), poly (dimethyldiallyl ammonium chloride) (PDDA) and poly (allylamine) (PAH), and polyanions such as poly (styrenesulfonate) (PSS), poly (vinylsulfate) and poly (acrylic acid) (PAA) (Fig. 2.3).

It is vital to use linear or branched polyion interlayers for the successful assembly of protein multilayers. Flexible linear polyions penetrate between protein globules and act as electrostatic glue. The idea of "electrostatic polyion glue," which keeps together neighboring arrays of proteins is essential to protein and nanoparticle assembly [68][69].

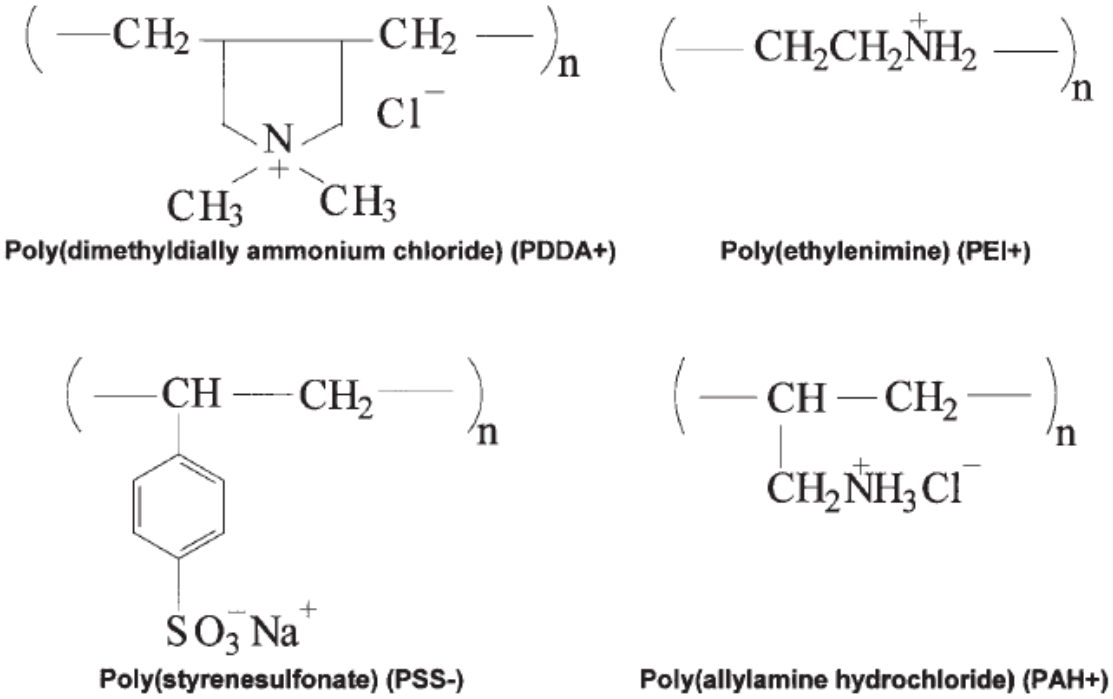


Figure 2.3: Chemical structures of some common synthetic polymers [69].

Hydrophilic materials are more bacterial resistant than hydrophobic materials [70]. Albumin adsorbed on material surfaces has shown inhibitory effects on bacterial adhesion to polymer, ceramic, and metal surfaces [71].

In a rabbit model implants with crosslinked albumin coatings had a much lower prosthetic infection rate than uncoated implants [72]. For biocompatible coatings an albumin/heparin multilayer assembly was developed by the LbL technique with the albumin adsorption density of 0.15 mg/cm2 at physiological pH 7.4 [73]. Hence to diminish bacterial adhesion a coating can be achieved on medical implants through the LbL technique.

To explore further clinical applications in vivo biocompatibility testing of the LbL assembled thin films is important. Films based on humic acids (HAs) have been coated by LbL assembly on implantable glucose sensors to enhance biocompatibility and control glucose permeability [74].

2.1.4 Benefits of LbL Self-Assembled Multilayers

Layer by layer self assembly presents a number of uses compared to other methods of encapsulation and fixation of substances:

1. Adjustable thickness.
2. Tunable properties (roughness, design, surface vitality, erosion resistance and so forth).
3. Several sorts of engineered and natural colloids are accessible for LbL.
4. The location and pattern of the layers can be controlled.
5. Surface labelling with targeting molecules is possible.
6. Stabilization of submicron particles is achievable [75].
7. LbL keeps away from the utilization of thermodynamically unsteady mechanically-micronized particles [76].
8. Multicomposites films [76][77].
9. No restriction on substrate size, geometry and topography.
10. Clean, cheap and easy to handle.

2.2 Polyelectrolytes

Polyelectrolytes are polymers containing separate ionic groups. Their special properties dominated by strong long range electrostatic connections have been studied in the course of recent decades. Significant hypothetical and exploratory endeavours have been made for instance to comprehend the origin of slow domains or loose clusters in semi dilute solutions of highly charged polyelectrolytes. This sort of attractive communication between macroions is not steady with the standard hypothesis in view of the overlap of the electrical twofold layers between charged level surfaces. Charge fluctuation forces between a few polyions because of sharing of their counterions or attraction by development of the condensed layers between charged poles have been recommended to describe the presence of these

arrangements. Specific attention has additionally been set on polyion connections with counterions since their build-up on the polyion surface is a standout amongst the most trademark properties of the polyelectrolytes. The interaction of polyions with other charged or nonpartisan species specifically the adsorption of ionisable polymers at interfaces is the second part of the physical science of polyelectrolytes that has been broadly studied because of the principal significance of this phenomenon and to its vital part in various modern purposes [78].

2.2.1 Definition

A polyelectrolyte by definition is a macromolecular species that after being set in water or other ionizing solvent separates into an exceedingly charged polymeric molecule. Such separation is normally joined by little oppositely charged counter particles that have a tendency to neutralize the charge on the repeating units of the macromolecule safeguarding electroneutrality [79][80]. A polyelectrolyte in low ionic strength solutions has a tendency to be in its most stretched out and uncoiled shape because of the intramolecular repulsion of the unscreened charges on each monomeric unit of the macromolecule. On the other hand when the ionic strength of the solution is increased a polyelectrolyte tends to become thicker and more coiled due to the screening impact of polymer charges by the greater presence of smaller salt counter-ions in solution [80].

2.2.2 Classification of Polyelectrolytes

Acids are classified as either weak or strong (and bases similarly maybe either weak or strong). Similarly polyelectrolytes can be divided into weak and strong types.

Strong Polyelectrolytes

A strong polyelectrolyte is one which dissociates completely in solution for most reasonable pH values.

Weak Polyelectrolytes

A weak polyelectrolyte by contrast has a dissociation constant (pKa or pKb) in the range of 2 to 10, meaning that it will be partially dissociated at intermediate pH. Thus

weak polyelectrolytes are not fully charged in solution and moreover their fractional charge can be modified by changing the solution pH, counterion concentration or ionic strength.

2.2.3 Properties of Polyelectrolytes

The level of charge screening of the polyelectrolyte will be a vital factor for tuning the thickness [81], consistency [82], dependability [83], stability [84], swelling [85], porousness [86] and other critical factors when studying artificially changed and in addition naturally occurring polyelectrolytes and their correspoding layer-by-layer ultrathin film composites or assemblies [87].

2.2.4 pH Sensitive Behavior of Poly (acrylic acid) (PAA) and Poly (allylamine hydrochloride) (PAH)

PAA is a weak Polyanion. It has a pka of 6.5; it is fully ionized at high pH values (pH 9.5 or 10) and almost completely deionized at pH 3. With increasing pH the thickness of an adsorbed layer of PAA decreases. These changes in thickness are associated with the increase in charge density of the PAA chains that results because of an increase in pH. With increasing pH the PAA layer thickness decreases because of the decreasing segmental population of loops and tails that happens as the PAA chains become more highly charged. The pH-dependent adsorption behavior is like what has been seen in single layer adsorption investigations of weak polyelectrolytes onto oppositely charged surfaces [88].

The thickness of an adsorbed layer of PAH and PAA is reliant on the charge density of the adsorbing polymer and the surface and is not dependent upon the thickness, conformation (segmental population of loops, tails and trains) or free ionic binding sites of the previously adsorbed polymer layer. The degree of ionization of PAA at pH 3 is 10%, 60% at pH 7 and almost 100% at pH 10 [89].

PAH is a weak polycation. It has a pka of 8.8; it is fully ionized at lower pH values (pH 6) and completely deionized at pH 12.

The net result in the layer-by-layer deposition process when fully charged chains of PAA and PAH are deposited very thin adsorbed layers are produced that are highly interpenetrated and lying essentially flat within the multilayer.

In support of this detail it has been found that the thickness of an adsorbed polymer layer deposited when both macromolecules are completely charged for example when PAH and PAA are assembled at a pH of 6.5 the thickness of adsorbed layer is free of the sub-atomic weight of the adsorbing polymer over a range of no less than 3000-106 g/mol. So without increasing the thickness of the adsorbed layer larger molecular weight chains simply spread out and occupies more surface area [90].

On the other hand when a fully ionized chain is alternately deposited with a nearly fully ionized chain the two-way zipping process is disturbed to some extent and the chains cannot spread out flat on the surface but instead obtain a conformational arrangement of dense loops that extend away from the surface as a result much thicker layers are deposited. The layers inside the film are still exceptionally interpenetrated however sorted out in an alternate mold. As expected for a loopy conformational plan [90].

As the pH of the PAA solution builds a greater amount of the chain fragments will get to be distinctly charged and the normal size of the loop/tail segments will diminish. Assuming that the conformation of the previously adsorbed PAH layer does not impact this circumstance one would expect the PAA layer thickness to diminish with increasing pH and stay about the same at steady pH and shifting PAH pH [91].The structure of a multilayer surface can be explored indirectly by the measurements of the surface roughness of dried films (i.e., whether it is dominated by loop and tail segments or train segments). Upon drying a molecularly rough surface is produced by a solvated surface comprised of a significant population of loops and tails whereas a more molecularly smooth surface is produced by a surface dominated by flat, train like segments [91].

It has been found in the previous studies that in the pH range of 6-7.5 the multilayer films display low surface roughness (<10 Å) using two completely charged polyions to gather multilayers (no additional salt) [92]. This low surface roughness is the after effect of the flat, surface bound adaptations received by the polymer chains during multilayer assembly. Whereas the surface roughness increases impressively when the films are manufactured at lower or higher pH because of the development of a more loopy conformational arrangement [91].

2.3 Antimicrobial Coatings

Bacterial attachment to a surface takes place through various mechanisms by hydrophobic and electrostatic interactions [93][94]. Microbial attachment might result in biofilm formation and infection when attached to biological implants [94]. The kind of interaction differs starting with one sort of microorganisms then onto the next and even changes inside a particular kind of microscopic organism because of transformations therefore adding to the unpredictability of the issue. Researchers have found that by controlling hydrophobicity, surface roughness, electrostatic interactions and surface compliance bacterial attachment can be reduced significantly [95][96]. It is additionally very well documented that bacterial adsorption on a substrate can happen through a layer of adsorbed protein and therefore surfaces oppose adsorption of protein should also likewise oppose adsorption of microbes [94]. Such surfaces are important since they battle the major issues of biofilm formation and microbial pollution. Since PEG has been known to confer protein resistance to a substrate so many researchers have attempted to fabricate substrates that oppose bacterial adhesion utilizing PEG [94][96]. PEG chains are adaptable and display extensive steric repulsive forces that may hinder the approach of microscopic organisms towards the surface.

Park et al. [97] reported the preparation of PEG-modified polyurethane substrates. PEG molecules exhibiting terminal hydroxyl, amino and sulfonate groups were tested against *Escherichia coli* (*E. coli*) and *Staphylococcus epidermidis* (*S. epidermidis*). They tested the adhesion of bacteria in various media such as tryptic soy broth and human plasma-containing media. The bacterial connection was observed to be subject to the media, the functionalization and the atomic weight of PEG. Generally higher molecular weight PEGs exhibited greater resistance to bacterial attachment than the lower molecular weight ones. Surfaces with terminal sulfonate groups were most effective in reducing microbial attachment.

Norde and co-workers [98] reported the impact of chain length of PEG brushes on the adhesion of various sorts of microbes and yeast. The interaction of proteins with polymer brushes has been studied widely but bacteria and yeasts are larger in size and present a more complicated system. Two bacteria, *S. epidermidis* and *Pseudomonas aeruginosa* (*P. aeruginosa*) and two different types of yeasts, *Candida tropicalis* (*C. tropicalis*) and *Candida albicans* (*C. albicans*) were used. It was seen that the

higher molecular weight PEG and longer brushes opposed the bacterial adhesion more strongly. It was also observed that relatively hydrophobic microbes (*P. Aeruginosa* and *C. tropicalis*) adhered more strongly than the hydrophilic ones (*S . epidermidis* and *C. albicans*) which suggests that hydrophobic interactions favor the attachment of the microbes to the surface. The microbes that adhered to the PEG brushes could be more readily removed by passage of an air bubble than the microbes that adhered to the bare substrate, indicating that the attachment force is weaker on the PEG-modified surface.

As discussed previously Ostuni et al. [99] designed SAMs presenting a number of functional groups that were comparable to SAMs presenting oligo (ethylene glycol) groups in their ability to resist the nonspecific adsorption of proteins. The authors additionally explored the relationship between the protein resistance of SAMs and their capacity to oppose bacterial attachment. They fabricated SAMs of alkanethiolates presenting different groups and compared the adhesion of protein to that of bacteria. It was found that for a given surface the extent of bacterial resistance did not correlate linearly with the protein resistance. Thus it is clear that the parameters required to design a surface that resists the adsorption of proteins are not sufficient to render a surface bacteria-resistant. In spite of the fact that connection of PEG to a surface is widely explored amongst the most generally investigated approaches in creating protein resistant surfaces it is not as viable in lessening bacterial colonization. This incapability might be because of the intricacy of the components through which microscopic organisms join to a surface, a hefty portion of which are still not well understood [100].

Lichter et al. [101] used polyelectrolyte multilayers of poly (allylamine hydrochloride) (PAH) and poly (acrylic acid) (PAA) and studied bacterial adhesion as an element of the mechanical firmness of the multilayers. The stiffness of the multilayers could be effortlessly tuned by changing the pH at which the multilayers were gathered. The authors found that the degree of bacterial connection on a substrate relies on upon the mechanical firmness of the substrate and an expansion in the stiffness of the substrate brought about an increment in the quantity of microbes attached.

CHAPTER 3

MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS

3.1 Materials

Polyelectrolytes including Poly (diallyldimethylammonium chloride) or PDADMC (Mw,: <100 000; 20 wt% solution in H2O) was used as a Polycation, Poly acrylic acid or PAA (Mw, ~ 450 000) was used as a Polyanion and Poly (allylamine hydrochloride) or PAH (Mw= 70 000) was used as a Polycation and were purchased from Sigma Aldrich, they were dissolved in Ultrapure water (Conductivity=0.0055µSiemens, Total dissolved solutes (TDS) =0) obtained from Smart2Pure™ Water Purification System (Thermoscientifc) which was used as a solvent in all experiments and for washing purposes. APTMS (3-aminopropyltrimethoxysilane toluene solution, 10mM) was used for the impartment of positive charge on microscopic glass slides. *Escherichia coli* DH5 (ATCC# 8739) and *Staphylococcus aureus* (ATTC# 6538) were taken from SMME, National University of Sciences and Technology. All chemicals and solvents were utilized as received without further refinement or treatment. The chemical structure of polymers used is shown in Fig. 3.1.

Conc. Sulphuric acid (Sigma-Aldrich, 95-98% purity) and Potassium dichromate (Scharlau, reagent grade) were used to prepare chromerge solution for cleaning of microscopic glass slides (25 x 75 x 1 mm, Globe Scientific Inc., US).

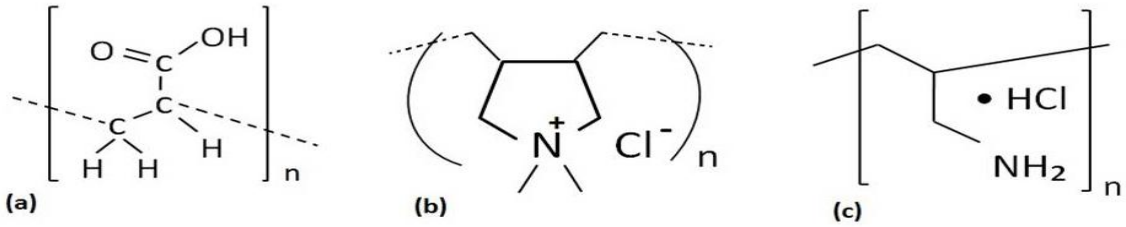


Figure 3.1: (a) PAA (weak Polyanion) (b) PDAC (strong Polycation) (c) PAH (weak Polycation)

3.2 Methods

3.2.1 Cleaning of Glass Slides

For cleaning of microscopic glass slides they were dipped overnight in chromic-sulphuric acid solution that was prepared by mixing 50% sulphuric acid and 50% potassium dichromate. Potassium dichromate was added drop by drop in conc. Sulphuric acid with continuous stirring. The glass slides were then rinsed with tap water followed by rinsing with ultrapure water. The slides were then left to dry at room temperature.

Impartment of Positively Charged Amine Groups

In order to impart positive charge amine groups on the slides they were dipped in 3-aminopropyltrimethoxysilane solution (APTMS) (10mM) for 5 hours that was prepared by adding 0.17929g of APTMS in 100 ml of ultrapure water. The schematic is shown in Figure 3.2.

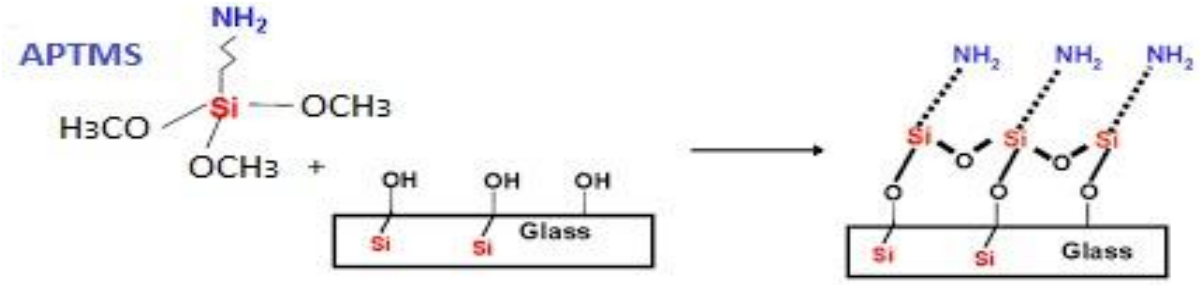


Figure 3.2: Impartment of positive charge amine groups on glass slides.

Assembly of the LbL Films

Fabrication of LBL-SAMu by manual dipping of the freshly cleaned glass slides into the desired solutions for a predetermined time. The pre-treated glass substrates were soaked into aqueous Polyanion solutions (1mg/mL) and Polycation solutions (1mg/mL) for 10 min with wash steps in between. The same cycle was repeated until the required number of bilayer was achieved. The pH of the polyelectrolyte solutions was well controlled. The prepared LbL films were kept in a desiccator before further use. The schematic illustration for the layer-by-layer deposition setup is shown in Figure 3.3.

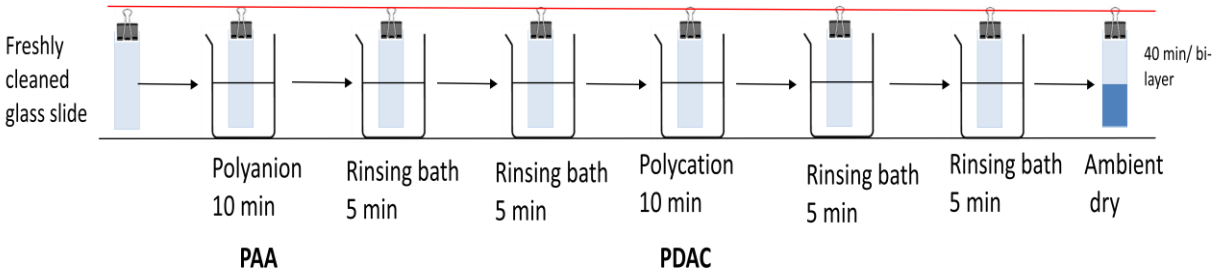


Figure 3.3: Schematic illustration for formation of one bilayer by layer-by-layer deposition setup.

3.2.2 Characterization of Thin Films

Atomic Force Microscopy

Surface morphology of the prepared LbL films was measured by a JSPM-5200, scanning probe microscope (SPM) in AC mode AFM shown in Figure 3.4. AFM images were taken on dried LBL films over scan areas of 2 × 2 μm for observing the morphology.

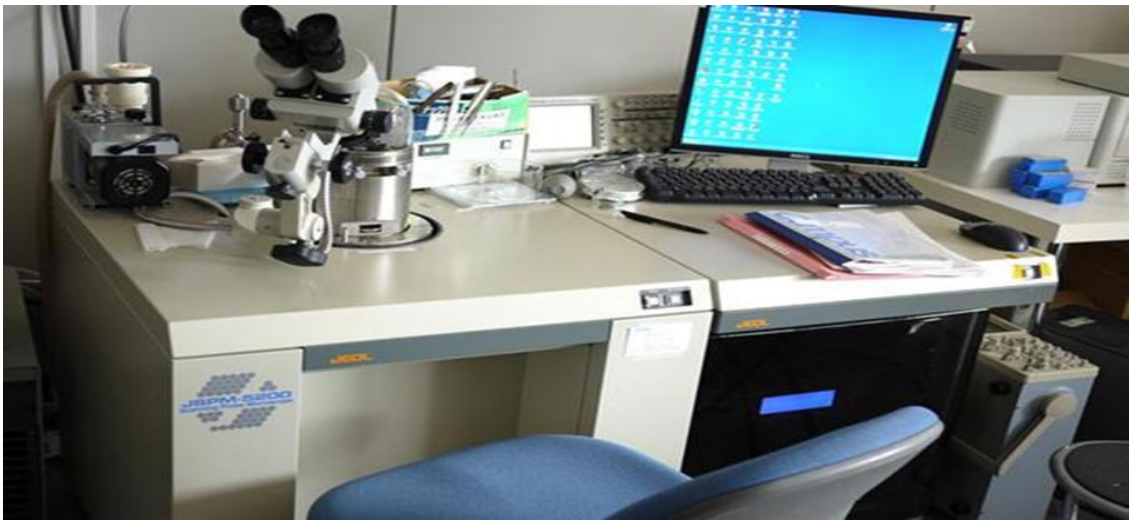


Figure 3.4: The atomic force microscopy JSPM-5200 used to study the surface morphology of LbL films.

Optical Profilometry

The film roughness was measured by a Nanovea PS 50 optical profilometry shown in Figure 3.5. The mean value measured among height differences was considered as the film roughness value.

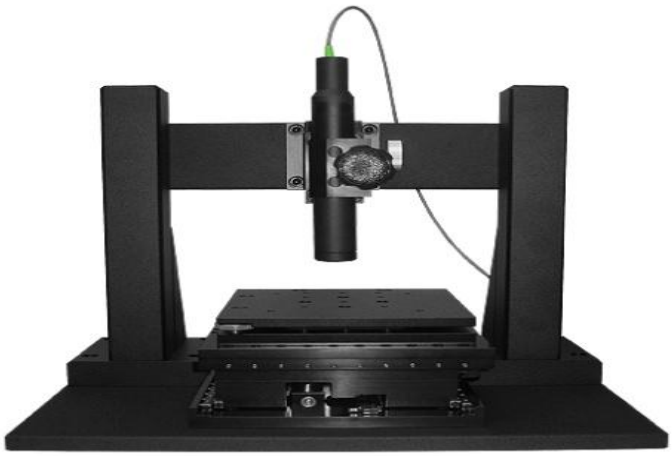


Figure 3.5: The optical profilometer Nanovea PS 50 used to study the film roughness.

Contact Angle Measurement

The wetting characteristics of the assembled LbL films were estimated by custom made water contact angle measurement setup as shown in Figure 3.6. The glass slides with thin films were placed on flat holders. Contact angle of the LbL thin films was measured by placing a 10-20µL drop of ultrapure water on the surface of glass substrate by a syringe or micropipette. Picture of the drop lit up by a light source was caught utilizing an advanced mobile phone camera set in accordance with the light source. For each sample, 10 measurements of water contact angles at different locations on the LbL film surface were made, and the average value of the measurements was used as the representative water contact angle of the film.The angle between the liquid/vapor interface and the solid/liquid interface (i.e. contact angle) was measured using LB-ADSA plug-in in Image J.

Figure 3.6: Custom made water contact angle measurement setup used for measuring contact angle of the LbL films.

3.2.3 Antibacterial Testing

Two microbial strains were used for the antimicrobial tests. *Escherichia coli* (E. coli, ATCC# 8739) and *Staphylococcus aureus* (S. aureus, ATTC# 6538) clinical strains obtained from SMME, National University of Sciences and Technology. These two bacterial strains were cultivated in LB broth. LB broth was prepared by mixing 10 grams of tryptone, 5grams of yeast extract and 10 grams of sodium chloride in 1 litre of ultra pure water and was further autoclaved. The microorganisms were cultivated for about 16 h at 37°C prior to harvest.

The broth containing bacteria was centrifuged at 3000 rpm for 10 minutes and then supernatant was removed, the cells were washed twice and resuspended with phosphate-buffered saline (PBS, pH 7.4) solution. After being incubated with bacterial suspension for 60 minutes the samples were washed three times with PBS before fixing with 3% glutaraldehyde for 5 h at 4 °C. After fixing the substrates were washed with DI water to get rid of the excessive glutaraldehyde and then dried at 60 °C in the oven for 24 h.

The samples after drying were imaged with Optical Microscopy (Optika B-600 MET) at different magnifications to examine any differences in the bacterial attachment

found between uncoated and coated samples and between the samples having different number of bilayers and pH values. The surface coverage of bacteria was evaluated by Optical microscopy micrographs. The bacteria coverage for each sample was visualized on the basis of 10 images taken at various locations. 3 samples were examined for each type of surfaces to examine the average microbial attachment. Cleaned glass slides using chromerge solution were used as a control for bacterial attachment testing.

CHAPTER 4

RESULTS AND DISCUSSION

Chapter 4

Results and Discussion

4.1 Tuning of Surface Charge by Varying Polymer Amount Deposited by Modifying Its Degree of Ionization

In this study the charge and thickness of the thin films was modified by altering the pH of assembled materials across all layers. This technique was derived from previously reported studies relating the degree of ionization of the polyelectrolytes to the film thickness [91]. Three samples of ten bilayers consisting of PAA and PDADMAC were assembled at pH values 3, 7 and 10 to construct the thin films with PDADMAC as the top layer. Three samples were prepared using PDADMAC and PAA at pH 3, 7 and 10 with PAA as the top layer respectively. As shown in table 4.1. On the other hand four different samples of 1, 5, 10 and 15 bilayers were prepared using PAA and PDADMAC, the top layer of the films was made-up by PAH at pH 7. As shown in table 4.2.

Similar techniques has been accounted to fabricate polyelectrolyte multilayers on colloidal silica to modify charge on the surfaces [91][92].

Table 4.1: Polyelectrolyte solution to prepare LBL films at various pH values.

Sample	No. of bilayers	Polycation	Polyanion	pH of solution	Polyelectrolyte and pH of the top layer
1	10	PDADMAC	PAA	pH 3	PDADMAC,3
2	10	PDADMAC	PAA	pH 7	PDADMAC,7
3	10	PDADMAC	PAA	pH 10	PDADMAC,10
4	10	PDADMAC	PAA	pH 3	PAA,3
5	10	PDADMAC	PAA	pH 7	PAA,7
6	10	PDADMAC	PAA	pH 10	PAA,10

Table 4. 2: Polyelectrolyte solutions to prepare LBL films by varying number of bilayers.

Sample	No. of bilayers	Polycation	Polyanion	pH of the polyelectrolyte solution	Polyanion and pH of the penultimate layer	Polycation and pH of the top layer
1	1	PDADMAC	PAA	pH 10	PAA,3	PAH,10
2	5	PDADMAC	PAA	pH 10	PAA,3	PAH,10
3	10	PDADMAC	PAA	pH 10	PAA,3	PAH,10
4	15	PDADMAC	PAA	pH 10	PAA,3	PAH,10

4.2 pH Responsive Behavior of PAA

In this study at pH 3 average roughness value was greater due to accumulation of PAA in loop rich conformations as compared to pH 7 where slightly uniform thin layers were achieved and at pH 10 smooth thin layers were formed. As shown in the figure 4.1 and 4.2.

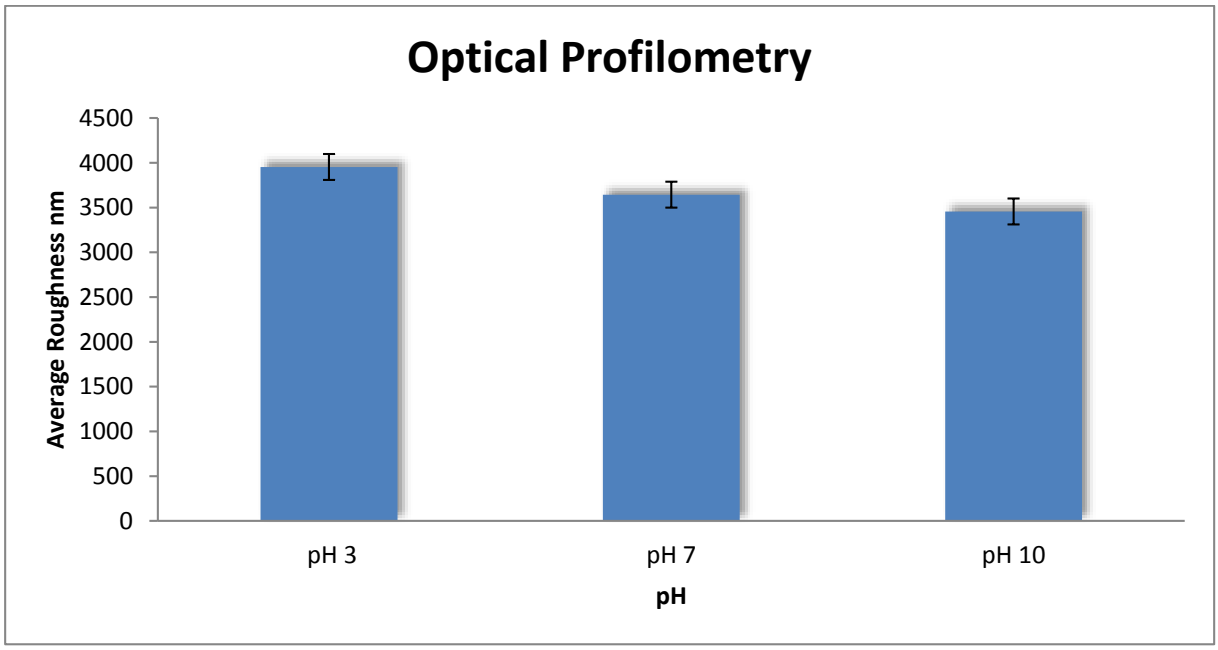


Figure 4.1: Average roughness of samples with PDADMAC as top layer.

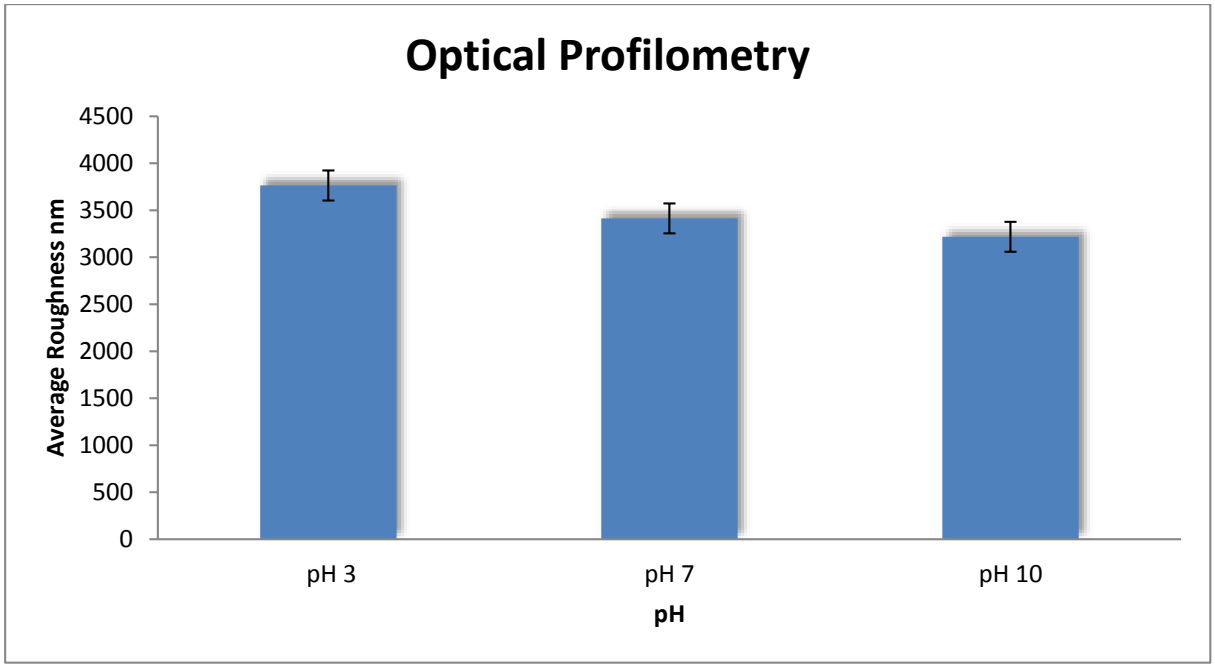


Figure 4.2: Average roughness of samples with PAA as top layer.

As PDADMAC is a strong Polycation where as PAA is a weak Polyanion. PAA has a pka of 6.5; it is fully ionized at high pH values (pH 9.5 or 10) and almost completely deionized at pH 3. Its degree of ionization at pH 3 is 10%, 60% at pH 7 and almost 100% at pH 10 [89].

PAA deposits in loop rich conformations at low pH where as at high pH the fully charged molecule form thin flat layers [88][89]. PAA when deposited at low pH the partially deionized molecule adsorb in loop rich conformations thus giving greater roughness where as when it is deposited at pH greater than 7 the fully charged molecule make thin flat layers [91]. As shown in the figure 4.3.

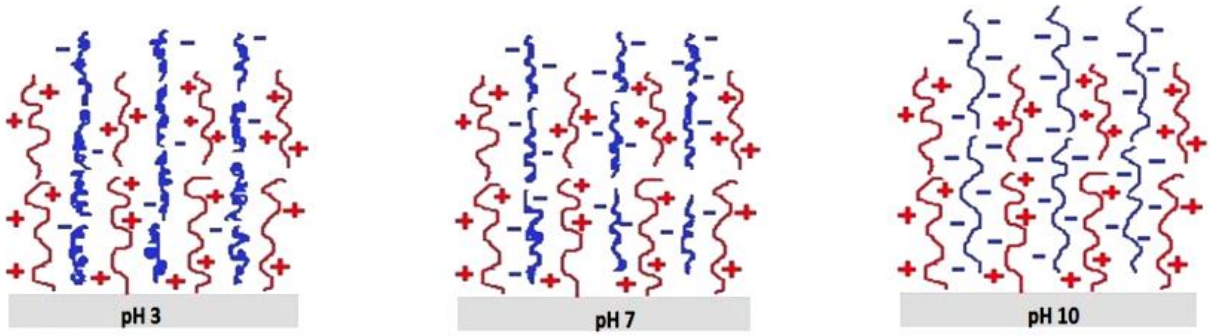


Figure 4.3: pH sensitive behavior of PAA.

When both polymers are fully ionized thinner films are achieved on the other hand thicker films are produced when one of the polyelectrolyte is not completely ionized while other is fully ionized [90].

The positively charged polyelectrolyte PDADMAC bears everlasting charged quaternary ammonium groups and its degree of ionization is not responsive to pH. Whereas PAA that is a weak polyacid with ionization constant of 6.5 incorporate carboxylic groups and its level of ionization is evaluated by the pH. While PAA was assembled at pH 3 most of its carboxyl groups were not ionized so greater amount of PAA was deposited to balance the positive charges from the underlying Polycation layer. Thus it results in greater film roughness due to the greater accumulation of loop like conformation of PAA. As on the basis of the above arguments, the ionization degree of PAA increases with pH as a consequence less amount of PAA is deposited to balance the underlying positive charges at high assembly pH. So as a result more uniform thin films are achieved at pH 10.

PAH is a weak polycation. It has a pka of 8.8; it is fully ionized at lower pH values (pH 6) and completely deionized at pH 12. In this approach for making bacterial adhesive thin films the pH of PAA in the second last layer was decreased to 3 and the pH of the PAH was maintained at pH 7.

The amount of PAH deposited in the top layer that indicate the surface charge of the LbL film can be fine-tuned by adjusting the assembly pH of PAH for the top layer and by tuning the amount of deposited PAA for the second last layer. Low assembly pH utilized for the assembly of PAA in the penultimate layer brought about a greater polymer load. This consequently draws in extra PAH during the last PAH deposition step.

As the number of bilayer increases the average roughness increases due to greater accumulation of PAA with each bilayer [91]. As shown in figure 4.4.

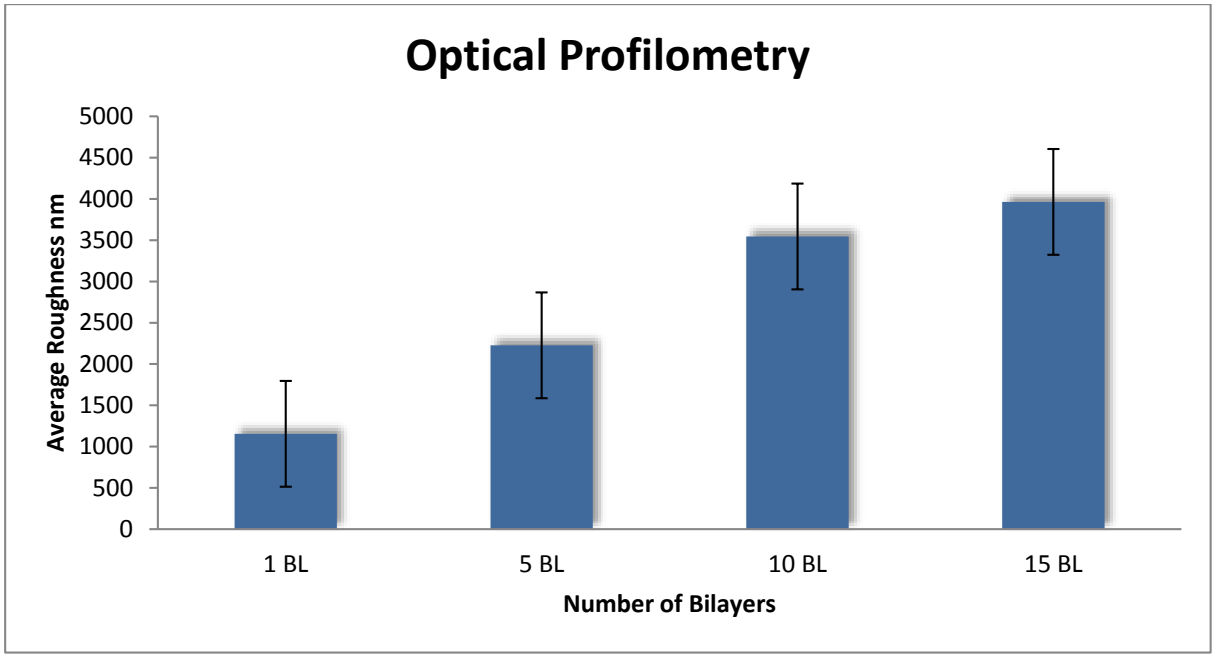
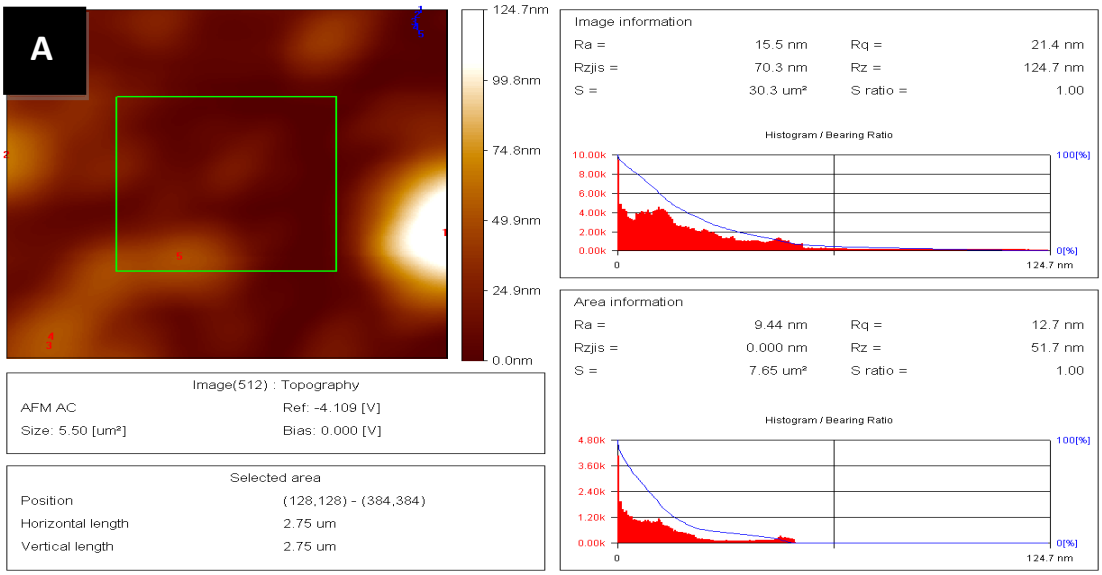


Figure4.4: Average roughness of the samples with PAH as the top layer.

4.3 AFM Studies of LbL Thin Films

Atomic force microscopy (AFM) of the samples surface morphology and roughness was obtained in air by using a JEOL model JSPM-5200. The instrument is operated in tapping mode; JEOL AC-AFM was used for all the AFM images. All samples were measured inside a suspension chamber to minimize ambient disturbance.



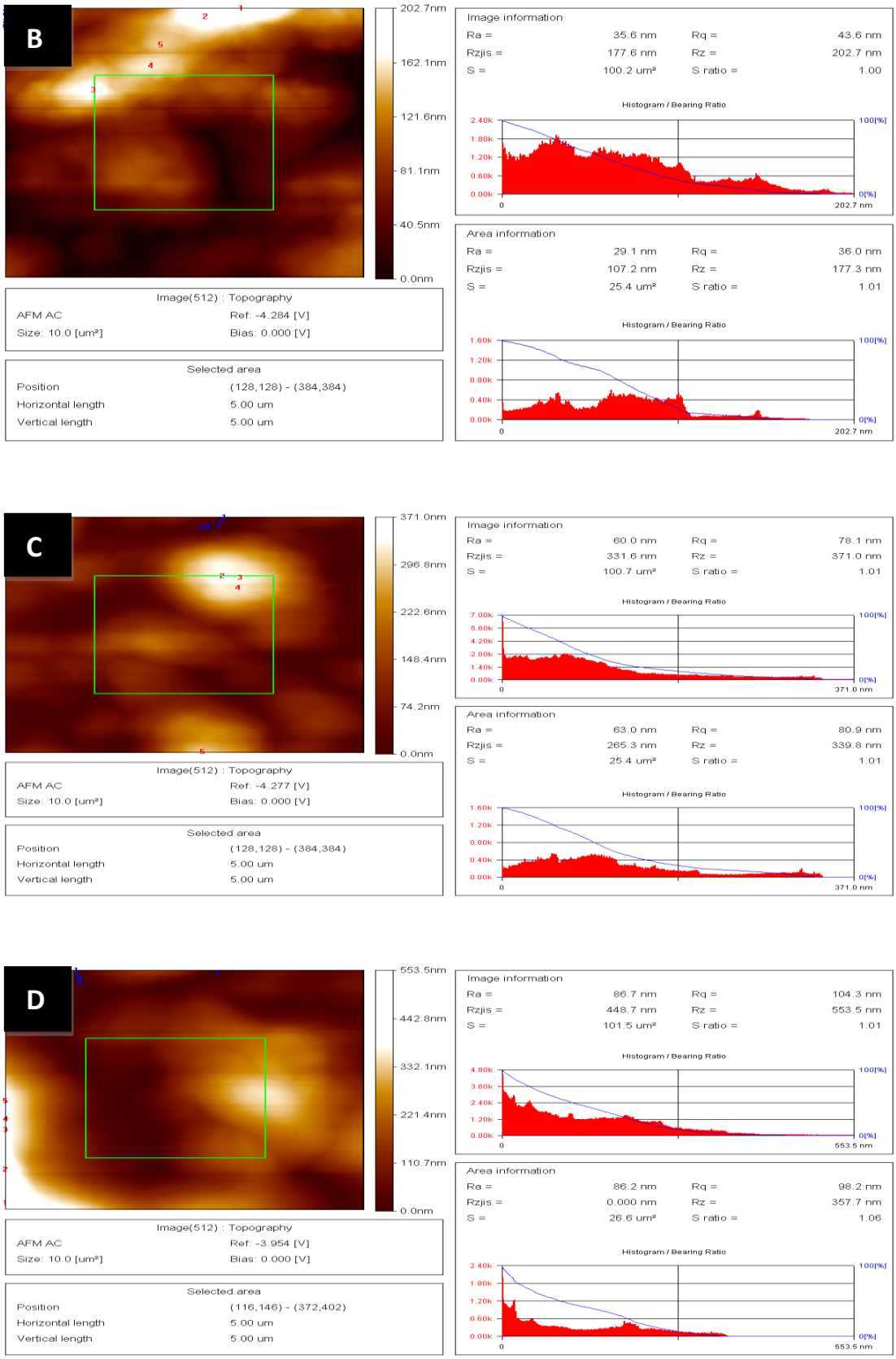


Figure 4.5: 2D image of the samples coated with PAH as the top layer, (A, B, C, D) shows 2D image of 1, 5, 10 and 15 bilayers respectively.

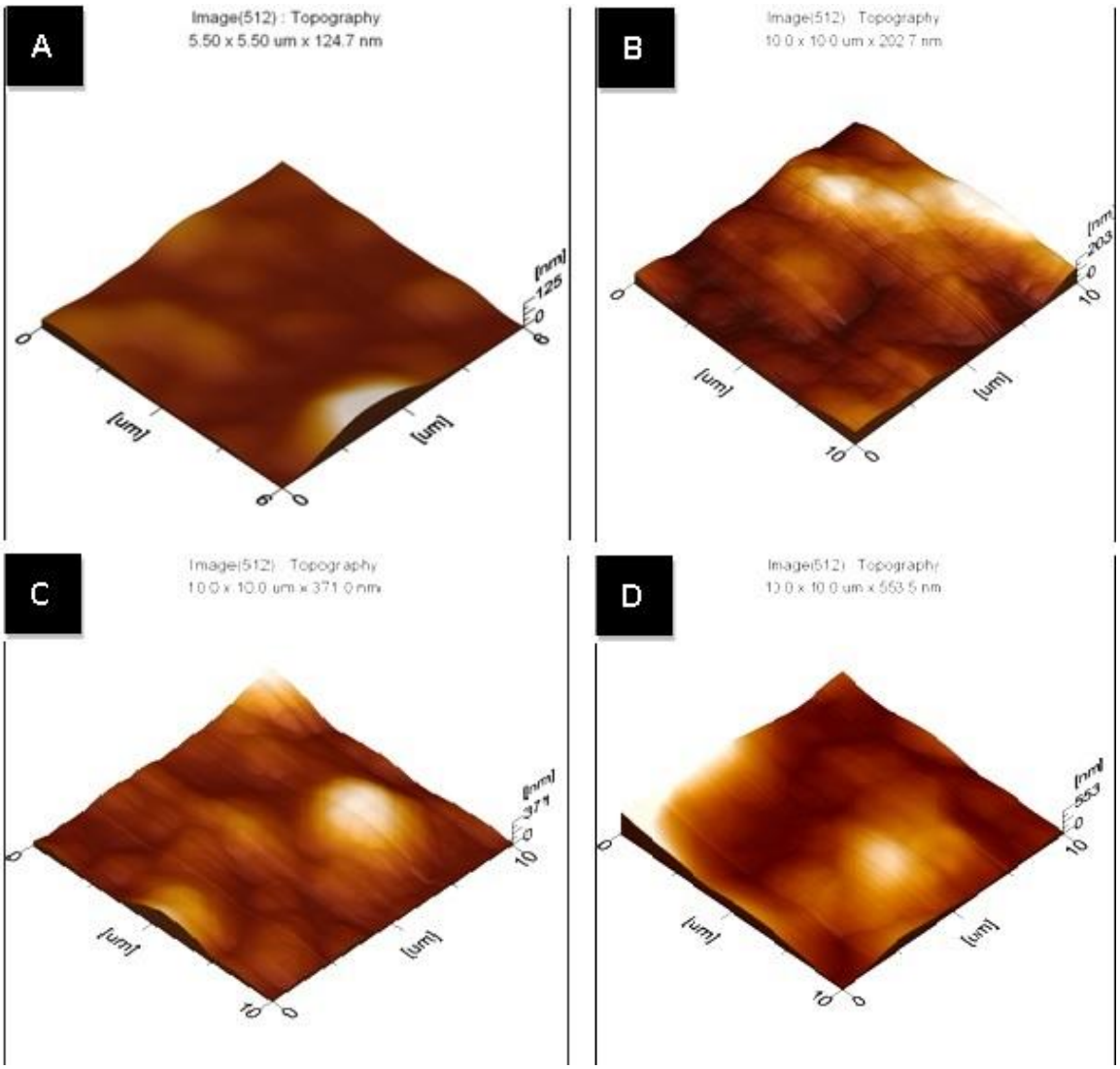


Figure 4.6: 3D image of the samples coated with PAH as the top layer, (A, B, C, D) shows 3D image of 1, 5, 10 and 15 bilayers respectively.

The surface roughness of 1, 5, 10 and 15 bilayer samples with PAH deposited in the last layer that exhibit the surface charge of the layer by layer thin film was observed by AFM. Clearly the surface topography shows an increase in the surface roughness as the number of bilayers increases from 1-bilayer to 15-bilayer.

The low assembly pH used for the deposition of PAA in the penultimate layer resulted in a high polymer load. This consequently draws extra PAH during the final PAH deposition step. As the number of bilayer increases the average roughness increases due to greater accumulation of PAA with each bilayer [91].

4.4 Bacteria Adhesion Testing

As bacterial fouling is chiefly related to biomedical applications, in this study two common microbes were used living in the physiological environment that is E. coli (Gram negative) and S. aureus (Gram positive) negatively charged and positively charged films in PBS (artificial physiological environment) at pH 7.4.
Fouling tests of the above mentioned microbes were carried out using a fixed settlement protocol and were estimated by Optical microscopy.
The bacterial cell surfaces due to the presence of ionized phosphoryl and carboxylate substituent's on their outer cell envelope have net negative electrostatic charge [102].

4.4.1 Bacterial Repellent Thin Films on the basis of Surface Charge and Ionization Degree

In this study the bacterial repellent thin films were prepared by using PAA and PDADMAC. PAA that is a weak Polyanion and is sensitive to pH was used as the top layer.

At pH 10 PAA is fully ionized so greater amount of negative charge is exposed on the surface thus the surface become highly bacteria repellent and no bacterial attachment was found on the surface of glass slides as compared to pH 7 where PAA is not fully ionized and expose less negative charge, resulted in less bacterial attachment on the glass slide and at pH 3 where PAA is almost fully deionized and do not repel bacteria. As a result large amount of bacterial attachment was found. As shown in figure 4.7.

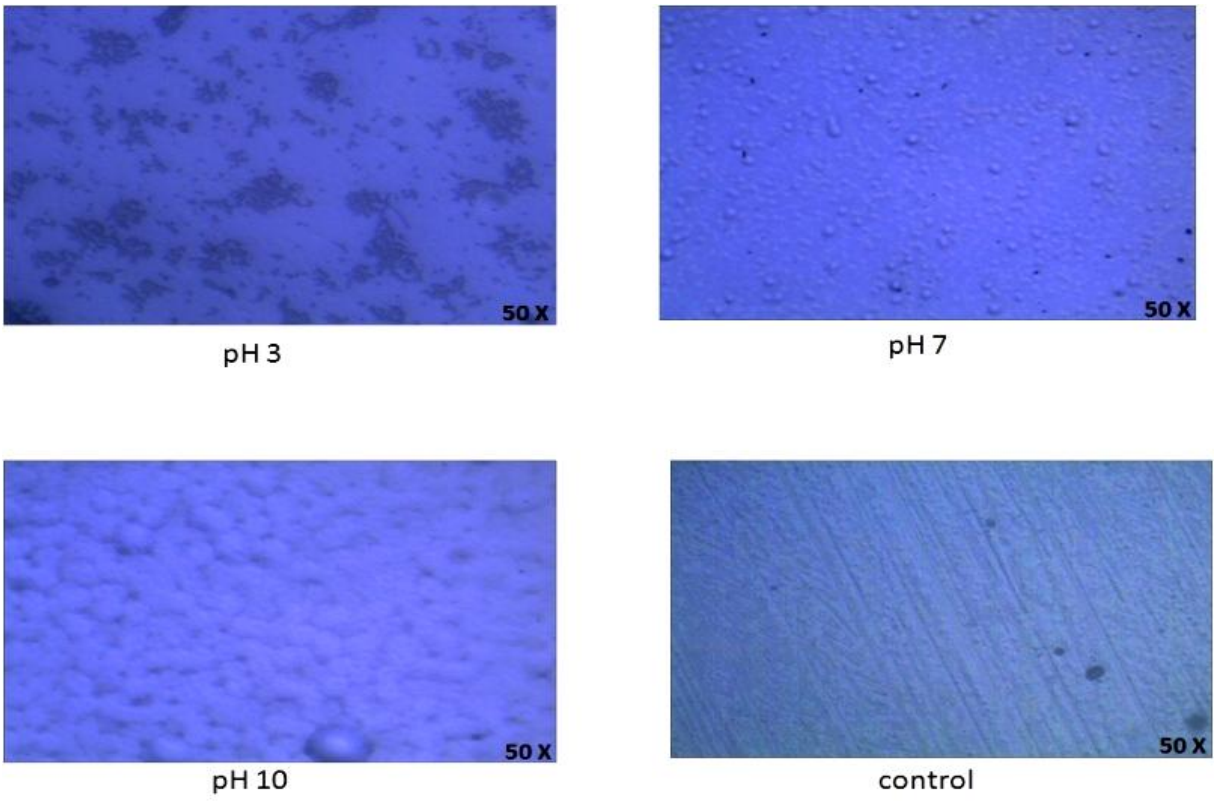


Figure 4.7: Optical microscopy images of the LBL films where PAA was used as the top layer.

4.4.2 Bacterial Adhesive Thin Films

The bacterial adhesive thin films were prepared using PAA and PDADMAC, where PDADMAC that is a strong Polycation was used as the top layer. At pH 3 large amount of bacterial attachment was found due to the greater load of deionized PAA that as a result attracted large amount of PDADMAC in order to compensate the underlying charge. On the other hand less bacterial attachment was found at pH 7 and little bacterial attachment at pH 10 due to fully ionized PAA in the underlying layer. As shown in the figure 4.8.

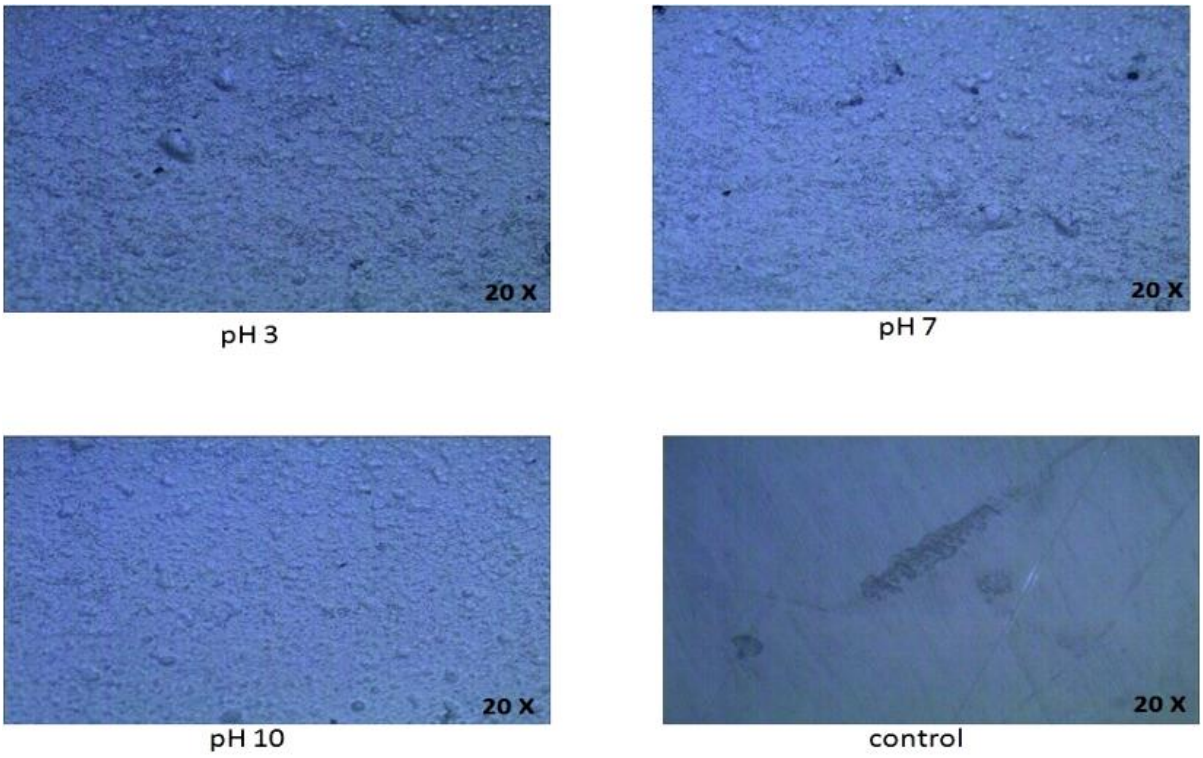


Figure 4.8: Optical microscopy images of the LBL films where PDAMAC was used as the top layer.

All films investigated were hydrophilic as shown in Figure 4.9 and 4.10 showed almost similar water contact angles at around 25°. As shown in Table 4.3.

Table 4.3: Water contact angle of 10-bilayer samples with PAA and PDADMAC as the top layer at different pH values.

Sample	LBL top layer polyelectrolyte and its pH	Water contact angle
1	PAA,3	28.0±1
2	PAA,7	24.8±1
3	PAA,10	22.7±1
4	PDADMAC,3	28.5±1
5	PDADMAC,7	25.9±0
6	PDADMAC,10	23.0±1

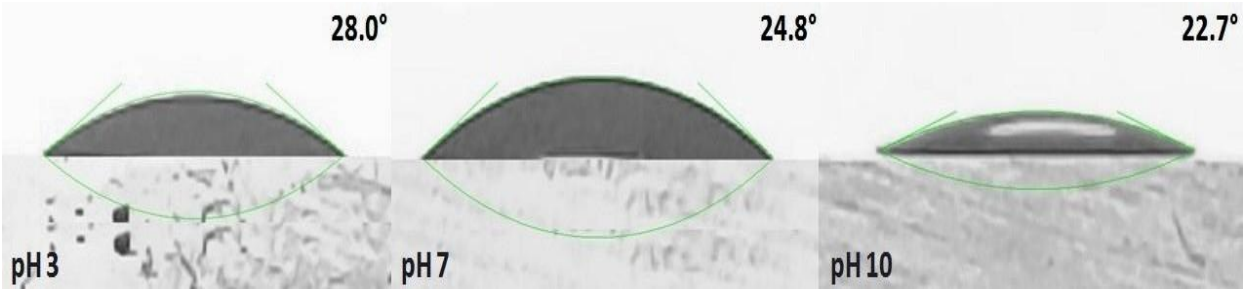


Figure 4.9: Contact angle images of samples with PAA as the top layer.

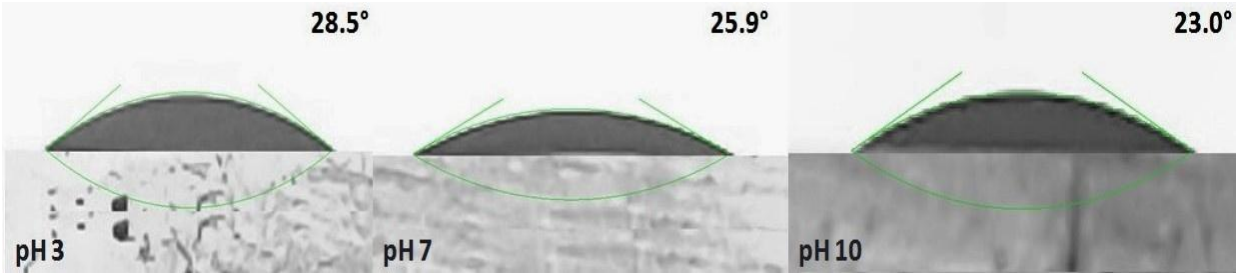


Figure 4.10: Contact angle images of samples with PDADMAC as the top layer.

Lowest value of contact angle was observed at pH 10 because of greater number of surface charges as compared to pH 3, due to the presence of fully ionized PAA at pH 10.

Figure 4.11 and 4.12 shows the trend observed for water contact angle at different pH values of the samples with PAA and PDADMAC as the top layer. Contact angle results were statistically analysed via Correlation in 'GraphPad Prism' software. P value of ≤ 0.05 was considered significant. No significant difference was observed. Table 4.3 and 4.4 shows the correlation p values.

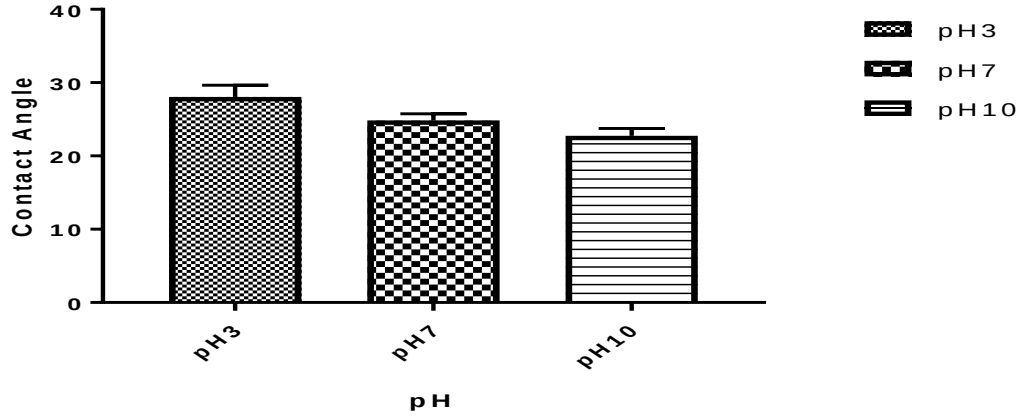


Figure 4.11: Graph showing trend observed for contact angle values of the samples with PAA as the top layer.

Table 4.4: Correlation results for contact angle analysis by changing pH of samples with PAA as top layer.

Correlation P values	pH 3	pH 7	pH 10
pH 3	1	0.465	0.144
pH 7	0.465	1	0.353
pH 10	0.144	0.353	1

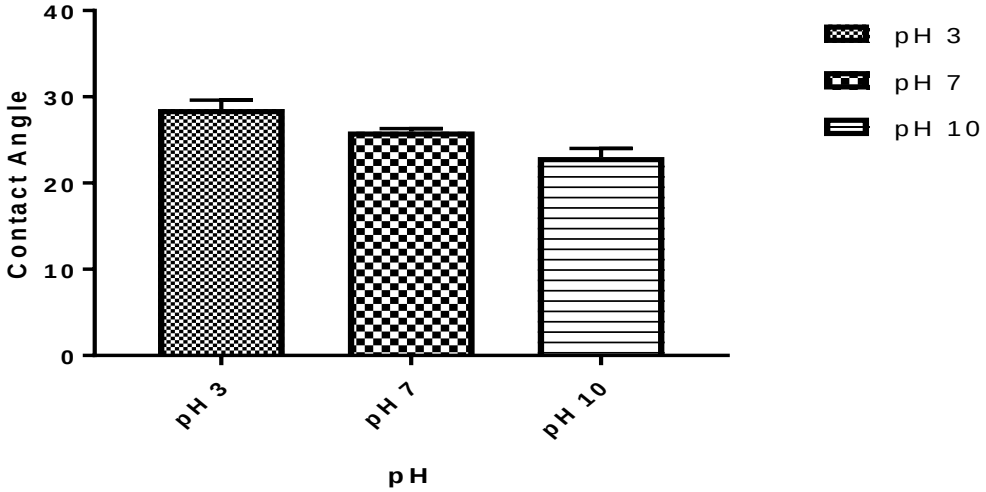


Figure 4.12: Graph showing trend observed for contact angle values of the samples with PDADMAC as the top layer.

Table 4.5: Correlation results for contact angle analysis by changing pH of samples with PDADMAC as the top layer.

Correlation P values	pH 3	pH 7	pH 10
pH 3	1	0.900	0.713
pH 7	0.900	1	0.061
pH 10	0.713	0.061	1

It was found that no significant difference is present between the samples as all the p values were found greater than ≤ 0.05.

In order to make more bacterial adhesive thin films PAH was used as the top layer as it is a weak Polycation and becomes fully ionized at lower pH (pH 6). Bulk films were produced by using PAA and PDADMAC at pH 10 where as the pH of PAA in the penultimate layer was decreased to pH 3 as PAA adsorbs more at pH 3 and attract more PAH ions thus results in greater accumulation of PAH molecules and exposing more positive charges. This resulted in greater bacterial attachment. On the other hand as the number of bilayer was increased from 1 to 15 a significant increase in the bacterial attachment was observed. As shown in the figure 4.13. SEM analysis of the 15 bilayer sample was performed and large amount of bacterial attachment was observed at different magnifications (figure 4.14).

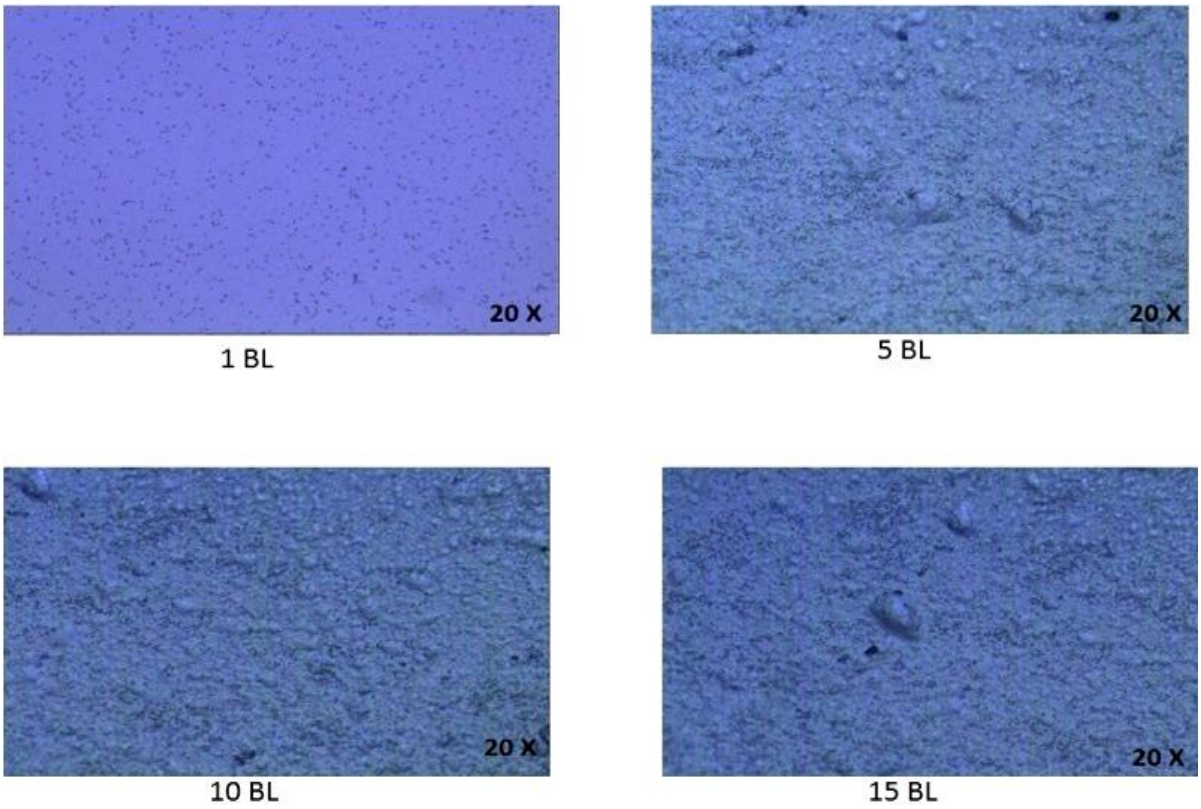


Figure 4.13: Optical microscopy images of the LBL films where PAH was used as the top layer.

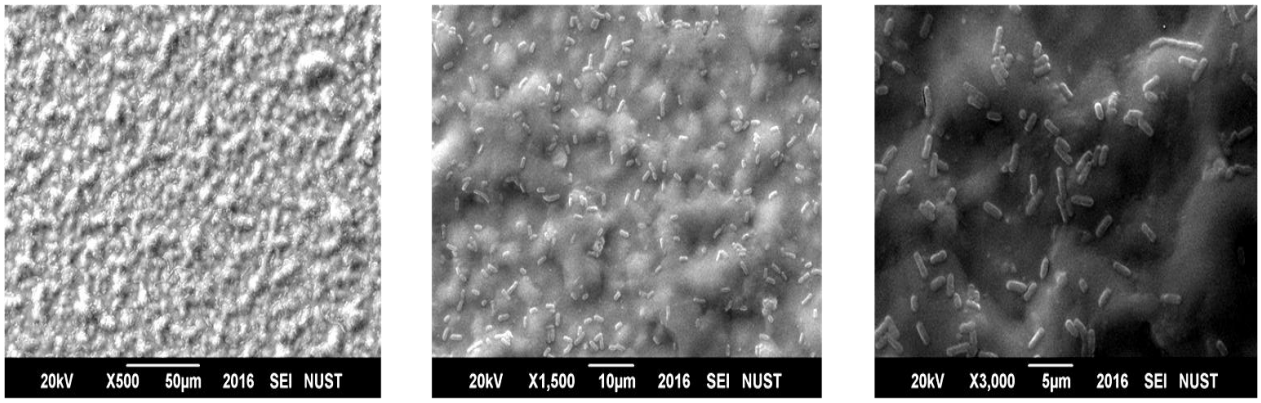


Figure 4.14: SEM images of 15 LbL coated sample at 500X, 1,500X and 3,000X.

A general decreasing trend in contact angle was observed with an increase in number of bi-layers (figure 4.15) which means that more water molecules interact with the surface due to accumulation of a greater number of surface charges. As shown in the table 4.5.

Table 4.6: Water contact angle of 1, 5, 10, and 15-bilayer samples with PAH as the top layer.

Sample	No. of bilayers	LBL top layer polyelectrolyte and its pH	Water contact angle
1	1	PAH,10	85.7±4
2	5	PAH,10	52.1±2
3	10	PAH,10	46.1±1
4	15	PAH,10	36.3±0

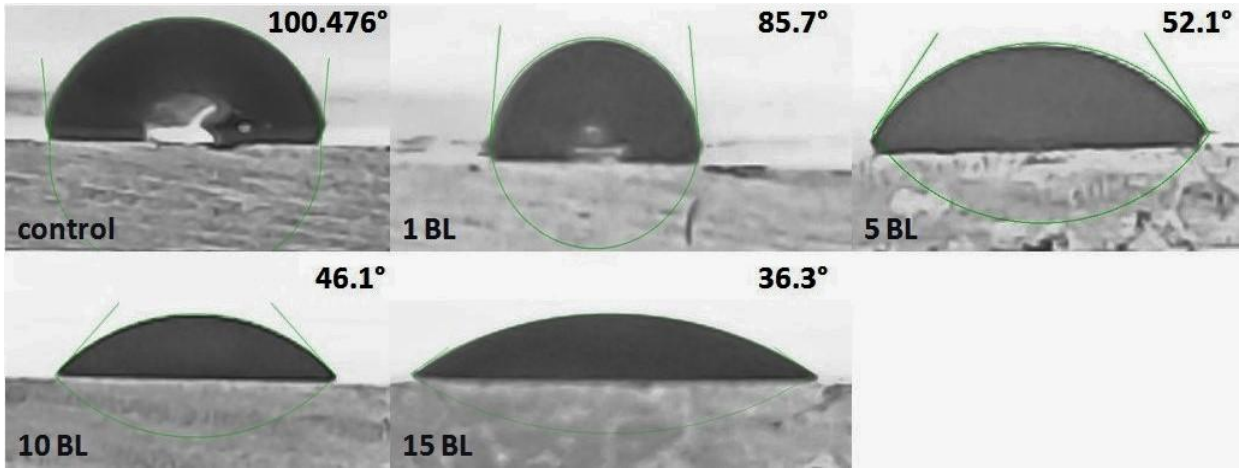


Figure 4.15: Water contact angle images of 1, 5, 10, and 15-bilayer samples with PAH as the top layer.

Figure 4.16 shows the trend observed for water contact angle of different bilayers samples. Contact angle results were statistically analysed via Pearson r in 'GraphPad Prism' software. The negative value of Pearson r showed that there is a negative trend in the contact angle values. P value of ≤ 0.05 was considered significant. Table 4.6 shows the Pearson r and p values.

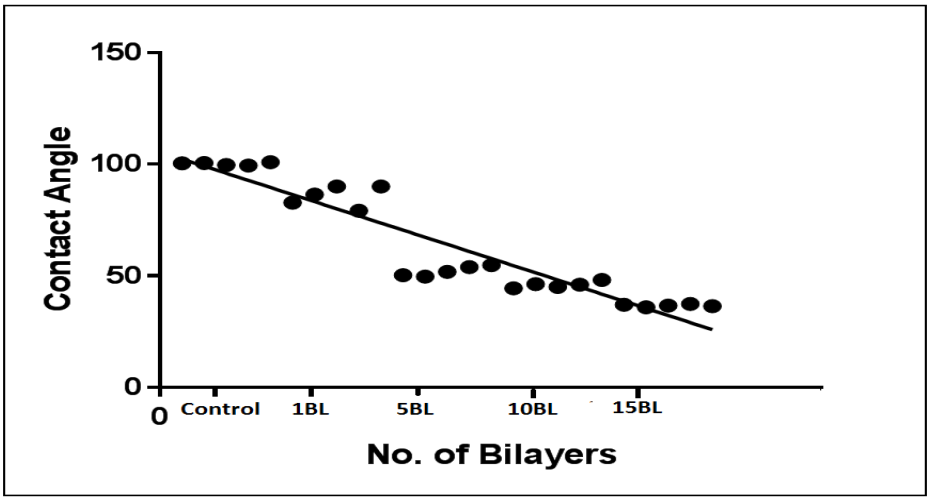


Figure 4.16: Graph showing trend observed for contact angle values for different number of bilayers.

Table 4.7: Pearson r results for contact angle analysis by increasing number of bilayers.

Pearson r		
r	-0.9351	
95% confidence interval	-0.9713 to -0.8565	
R squared	0.8745	
P value		
P	<0.0001	
P value summary	****	
Significant? (alpha = 0.05)	Yes	
Number of XY Pairs	25	

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

5.1 Conclusions

In order to establish fine control of LbL film surface charge, method employing tuning of surface charge, by variation of the polymer amount deposited through adjusting its ionization degree was used for the fabrication of polyelectrolyte films.

In the LbL frameworks of this research PAA as a weak polyelectrolyte and PDADMAC as a strong polyelectrolyte were utilized to create the bulk films and PAH as a weak polyelectrolyte was applied as top layer for few films. The tuning technique of surface charge was based on changing the pH value of PAA and PDADMAC to create bulk films due to the pH responsive behavior of PAA.

The distinctive aspect of this study is that it made possible to attain optimal bacterial adhesive and bacterial repellent performance of a given material by taking into account specific pH values of the environment and the surface characteristics of the fouler.

In bacteria adhesion tests high adhesion was observed on positively charged surface and low on negatively charged surface by using *E. coli* (Gram negative), and *S. aureus* (Gram positive). This was associated to the negative charge bearing bacterial cell walls.

This fabrication technique is not the utmost antifouling solution but instead it provides some guiding principles for bacterial repellent and bacterial adhesive LbL films. Additional steps such as cross linking should be applied to make LbL layers completely work for marine or biomedical applications. Similar studies are underway.

5.2 Future Recommendations

A variety of applications can be envisioned by using this technology that includes specific adsorption and refinement of proteins, purification of waste water by the elimination of metal ions, development of synthetically charged microbial cell walls and the bacterial adhesive thin films to attach and detect desired bacterial strains.

In future these antibacterial and bacterial adhesive coatings can be applied onto other materials such as fabrics like cotton, wool etc. Also they can be modified and employed for coating onto other surfaces and biomedical textile etc. using layer-by-layer coating method.

CHAPTER 6

REFERENCES

Chapter 6

REFERENCES

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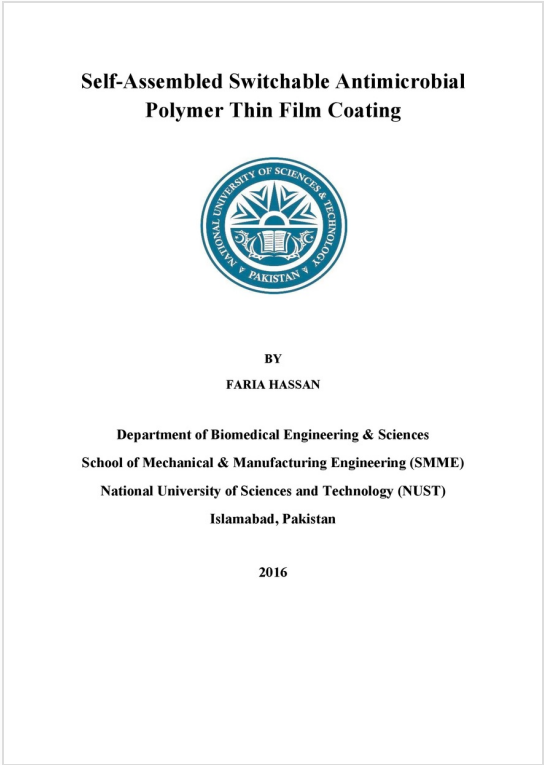

Self-Assembled Switchable Antimicrobial Polymer Thin Film Coating

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2016

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