MIMICKING BIOLOGICAL MEMBRANES

WITH LAYER-BY-LAYER ASSEMBLED

THIN FILMS

By

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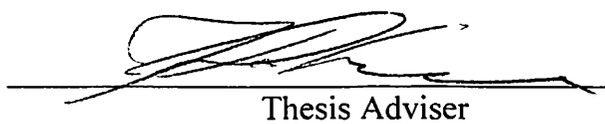

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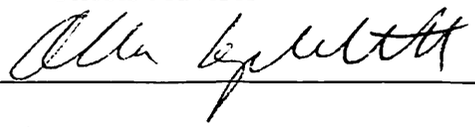

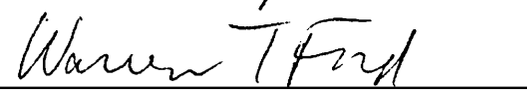

Dean of the Graduate College

Dedicated To,

My Family

And

Dr. Nicholas Kotov

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a	Radius of macromolecule
AFM	Atomic force microscopy
CA	Cellulose acetate
FITC	Fluorescein isothoicyanate
FW	Formula weight
IR	Infrared
kDa	Kilo daltons
LBL	Layer-by-Layer
Mco	Molar mass for particle with radius equal to pore radius
M	Molar mass for particle with radius a
MWCO	Molecular weight cutoff
N	Mass flux
PAA	Poly(acrylic acid)
PAH	Poly(allylamine hydrochloride)
PBS	Phosphate buffer solution
PDDA	Poly(diallyldimethylammonium chloride)
PEI	Poly(ethyleneimine)
PET	Poly(ethyleneterephthalate)
PMAA	Poly(methacrylic acid)
PSS	Poly(styrenesulfonate) sodium salt
PVA	Poly(vinyl alcohol)

R	Fractional retention
r	Pore radius
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
UV	Ultraviolet
Vis.	Visible
ρ	Density of macromolecule
λ	Reduced molecular weight

INTRODUCTION

Bio-artificial materials

Bio-artificial polymeric materials represent a new class of polymeric materials based on the blend of synthetic and natural polymers. They serve the purpose of producing new materials with enhanced properties with respect to the single components. The success of synthetic polymers as biomaterials relies mainly on their wide range of mechanical properties, transformation processes that allow a variety of different shapes to be easily obtained, and low production costs. Biological polymers present good bio-compatibility but their mechanical properties are often poor. The necessity of preserving biological properties complicates their processability and their production or recovery costs are very high. The basic aim of the bio-artificial polymeric materials is to smooth the interactions between synthetic and living systems by creating a two component material. As a result of the interactions between the synthetic and biological components, changes occur at molecular level inside the film before coming into contact with the living tissue. Such films with pre-established molecular interactions should have better biological response than a fully synthetic material.

Bio-artificial polymeric materials based on blends of synthetic and biological polymers have been designed with the purpose of producing new processable polymeric materials that possess both good mechanical properties and biocompatibility [1]. Bio-artificial polymeric materials have been prepared using poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), ethylene/vinyl alcohol

copolymers and polyurethanes as synthetic components, collagen, gelatin, hyaluronic acid, starch and dextran as biological components. The feasibility of using these films will depend mostly on their permeability of molecules or ions through them. This is very interesting since the polymer network may entrap large molecules but allow penetration of smaller ones.

The purpose of this project is

a) To prepare free standing bio-artificial thin films with poly(acrylic acid) and collagen by layer-by-layer (LBL) adsorption
b) To study the permeability of these films and their potential applications.

Films made with poly(acrylic acid) and collagen by layer-by-layer assembly are expected to have applications in tissue engineering such as artificial human skin and substitutes for kidney membranes.

Kidney failure

Kidney disease is a major problem, affecting about 5 percent of the population and accounting for over 60,000 deaths per year in the United States. Each year over 260,000 Americans suffer from chronic kidney failure, requiring hemodialysis or kidney transplant to survive. Maintenance on dialysis is estimated to be successful for 85% in their first year (that is 15% death rate is expected). In the subsequent 9 years about 10% of the previous year's population is expected to die each year. At any point, a patient may

undergo transplantation, but this process is constrained by the limited availability of donor kidneys.

In order for blood to perform its essential functions of bringing nutrients and oxygen to the cells of the body and carrying waste materials away from those cells, the chemical composition of the blood must be carefully controlled. Blood contains particles of many different sizes and types, including cells, proteins, dissolved ions, and organic waste products. Some of these particles such as proteins like hemoglobin are essential for the body. Other compounds such as urea (a waste product from protein metabolism) must be removed from the blood or they will accumulate and interfere with normal metabolic processes. The largest responsibility for maintaining the chemistry of the blood falls to the kidneys. The kidneys meet these challenges through a remarkably elegant system. Essentially, kidneys act like dialysis units for blood, making use of the different sizes of the particles and specially-maintained concentration gradients. Blood passes through the membrane-lined tubules of the kidney, which are analogous to the dialysis membranes. Particles that can pass through the membrane pass out of the tubules by diffusion, thus separating the particles that remain in the blood from those that will be removed from the blood and excreted.

When the kidneys are severely damaged, the membranes inside kidneys loose their ability to separate waste materials from blood. This leads to fatal illness unless an artificial system is employed to perform the essential work of the kidneys. Although the available artificial systems are life sustaining, it is expensive, time consuming and is uncomfortable as the patient feel fatigued. The sequence in Figure 1 illustrates the consequence of kidney failure.

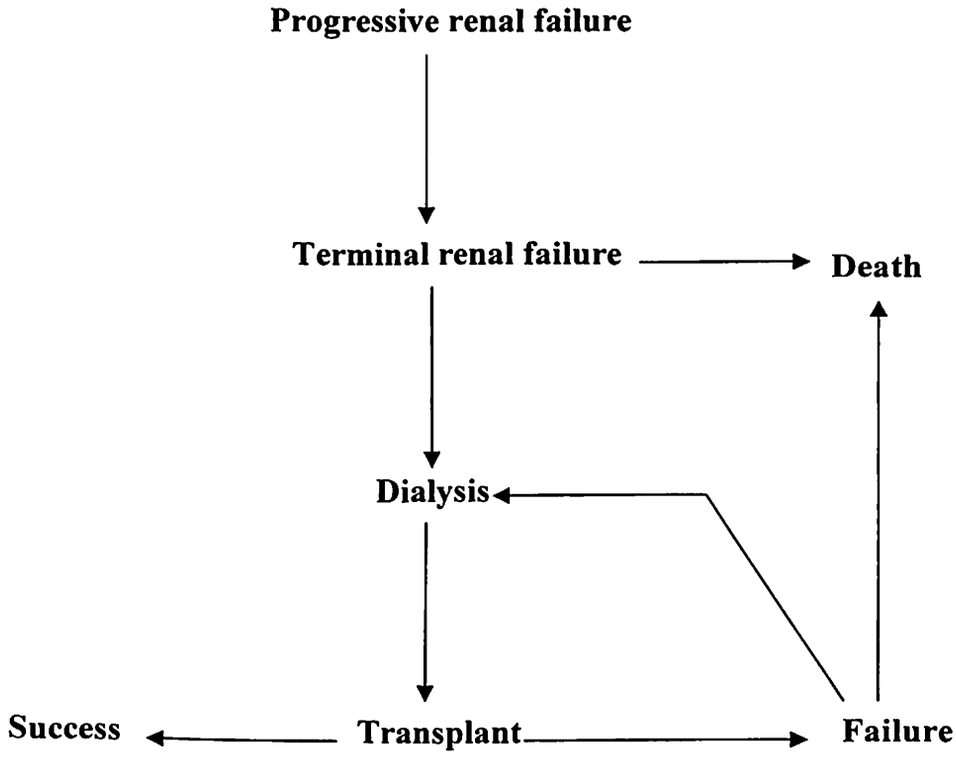


Figure 1. The sequence of events which may occur to a patient who develops terminal renal failure

Nanotechnology combined with tissue engineering is a rapidly growing area that seeks to create, repair or replace tissues and organs by using combinations of cells, biomaterials, or biologically active molecules. It is an interdisciplinary field that integrates aspects of engineering and other quantitative sciences with biology and medicine. This combined field with biocompatible thin films may bring the opportunity for various biomedical applications such as tissue replacement and membrane replacement for chronic diseases such as liver failure, chronic heart failure, chronic kidney failure, and severe burns. Laboratory-made biological membranes serve the purpose of a supporting matrix. These membranes can be shaped as needed, seeded with living cells and bathed with growth factors. When cells multiply, they fill the scaffold and grow into three dimensional tissues and, once implanted in the body, the cells recreate their intended functions. Blood vessels attach themselves to the newly formed tissues and lead to the blending of newly grown tissues with its surroundings. There still a lot of research to be done before this process is applicable to human trials or actual patients.

Challenges in tissue engineering for kidney failure

Every year millions of people suffer from end stage renal failure. Although the currently available therapies such as transplanting organs or using mechanical devices have saved and improved lives, they remain imperfect solutions. Major damage to a tissue or an organ can neither be repaired nor long term recovery be produced using these methods. The available therapies do not provide active transport, metabolic and endocrinologic functions of the kidney which are located predominantly in the tubular elements of the kidney [2]. Substitution of these tubular functions cannot be achieved

with inanimate artificial membrane devices but requires the use of naturally evolved biological membranes [3].

Tissue engineering applies the principles of engineering and life sciences towards the development of materials that can restore and improve tissue function [4-6]. Even though many advances have been made in the regeneration of skin, bones, and cartilage, significant challenges yet remain in the regeneration of the complex organs that could exhibit full metabolic functions and substitute at least some functions of the human organs. The performance of tissue engineered kidney, engineered living tissues and cell based therapies are inherently tied to the structural and functional analysis of material components at molecular, cellular and tissue length scales. Researchers believe that many problems could be overcome by the careful scaffold design by using materials which can provide the structural requirements and increase the activity of cells.

The biggest challenge in tissue engineering is the development of uniform surface that can control the cells shape and function. The process of forming tissues from cells on degradable scaffolds is a highly orchestrated set of events that occurs over time scales ranging from seconds to weeks and with dimensions ranging from .0001 cm to 10 cm [7]. In many biological functions, it is often desired that the newly placed system have improved biocompatibility and have extended stability periods to perform a desired function. The discovery of molecules that can regulate cell behavior along with the development of materials that contain the information about amino acid sequences represent a major area of future research direction. The distribution of functional groups on the biomaterials surface leads to the macromolecular structure which is a key factor in determining the interactions with the surrounding medium.

Tissue engineering and replication of the kidney is a formidable task because a mature kidney is a remarkably complex structure [8]. Formation of the implanted tissue on the biological membrane is greatly influenced by the architecture, composition and three dimensional environment of the scaffold and also by the biocompatibility of the biomaterials [9, 10]. Moreover, material porosity, pore size distribution and continuity largely influence the attachment of the specific cell types and interactions of the biomaterials with the host [11]. Many implants need to be made of composite materials with tailored properties. The methods of manufacturing such reproducible implants are crucial to the success of tissue engineered bio-artificial organs.

The creation of the controlled release systems that are capable of delivering molecules for long time periods is also an important factor for bio-artificial membranes [12]. Regulation of extracellular matrix, pattern formation, and morphogenesis in tissue engineering remains largely unexplored. Tissue engineered membranes for kidney failure provide the basic extracellular supporting system made up of polymer scaffolds. These scaffolds should have large surface area to volume ratios which can support large cell numbers in the implantation of genetically engineered cells to patients. Thus, a significant activity involves the basic understanding of biological and physical processes underlying the tissue formation to design a new generation materials that can promote various biomedical applications.

The versatility of the tissue engineered scaffolds and organs depend on our ability to

(a) synthesize or deliver novel materials

(b) fabricate or assemble into appropriate two or three dimensional form

(c) control material related physical and biological properties to achieve a desired clinical response.

Notably, the development of innovative materials and scaffolds that can modulate the cellular responses should be achieved. Clarification of structure/function relationships of tissues and natural matrices is required to serve as a starting point and reference base for material design [13]. Growing tissues and organs require a variety of different cell types assembled into a specific architecture. Cells in tissues are under constant stimulation by mechanical forces. These forces influence cell shape which in turn affects how a cell responds. Recently, the importance of special signals in three dimensional cultures that can play crucial roles in tissue engineering have been recognized. Thus, tissue engineering faces the need to switch from two dimensional to three dimensional cultures and employment of designing principles.

Molecular biologists and physical chemists are designing new materials with controlled properties including molecular architecture and bio-stability through a variety of approaches. Recent advances in nanotechnology, material science and engineering, and cell biology offer new insights into the molecular scale assembly of composite biomaterials to achieve such goals. Long term research for designing scaffolds from nanostructured materials should be compatible with the three dimensional device fabrication methods which will allow simultaneous structural control at molecular scale to facilitate cell growth and organized growth of multiple cell types [14-17].

Layer-by-layer assembly

Sequential adsorption of oppositely charged macromolecular species by layer-by-layer (LBL) deposition is a powerful thin film preparation that can be applied to the design of many biological materials, with well controlled interfacial, mechanical, and biological functions. This control over the structure of thin film is not apparent by other thin film deposition methods such as Langmuir-Blodgett, sputtering or spin coating.

The LBL technique was first proposed by Iler in 1966 for the purpose of making uniform thin films by alternative adsorption of positively and negatively charged colloidal particles [18]. After many other attempts to develop the process [19, 20], Decher and his coworkers demonstrated a simple and universal method for the layer buildup with polymers [21-25]. Preparation of ultrathin films is based on the electrostatic attraction of oppositely-charged polyions. Alternating adsorption of anionic and cationic polyelectrolytes leads to the formation of regular multilayer assemblies and the films are remarkably uniform.

Figure 2 shows a simplified molecular concept of first two deposition cycles starting with a negatively charged substrate. The substrate is immersed in a solution which contains cationic polyelectrolyte, and a layer of polycation is adsorbed. Since the adsorption is carried out at relatively high concentrations of the polyelectrolyte, a number of ionic groups remain exposed to the solution, and thus the surface charge is effectively reversed. The surface charge reversal is important with each adsorption step and is the basis of multilayer build up. The substrate is rinsed with deionized water to remove superficial molecules and dried if necessary. The substrate is then immersed in a solution of an anionic polyelectrolyte and finally the substrate is washed with deionized water.

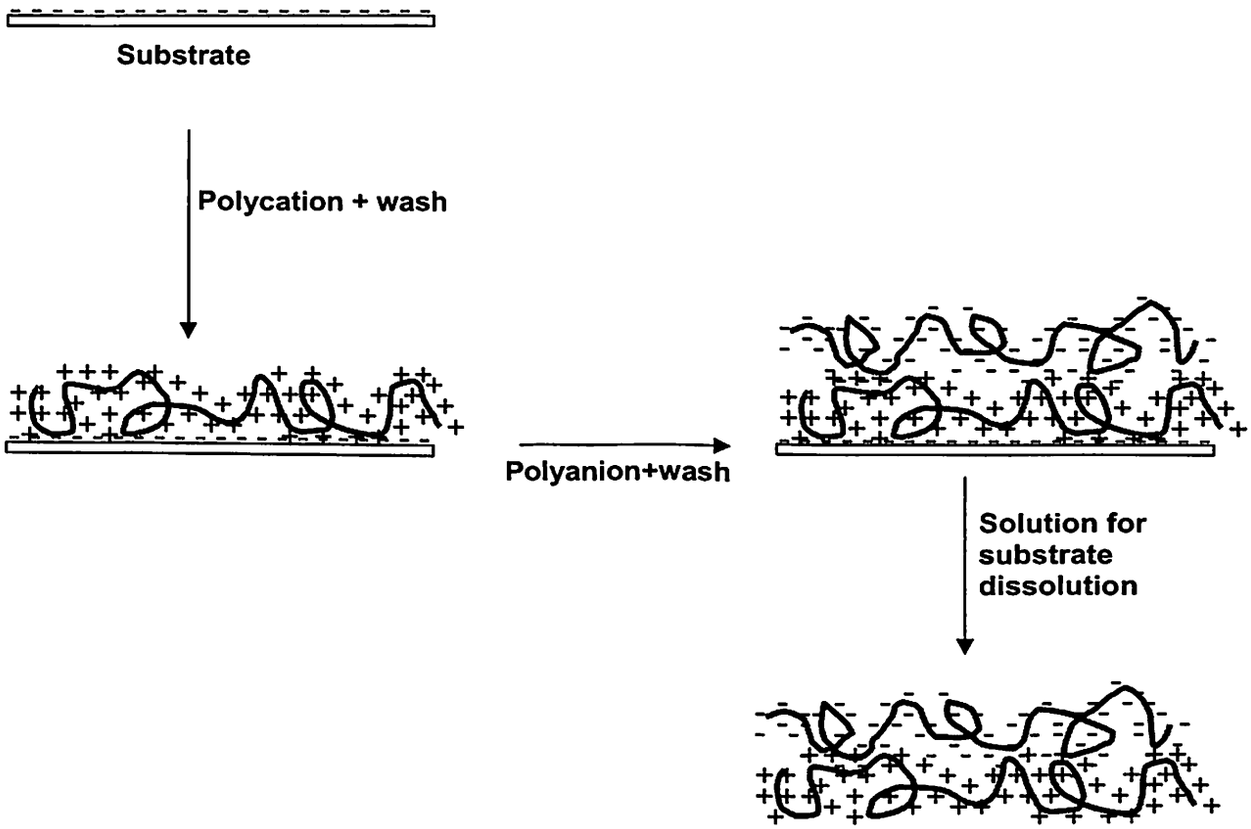
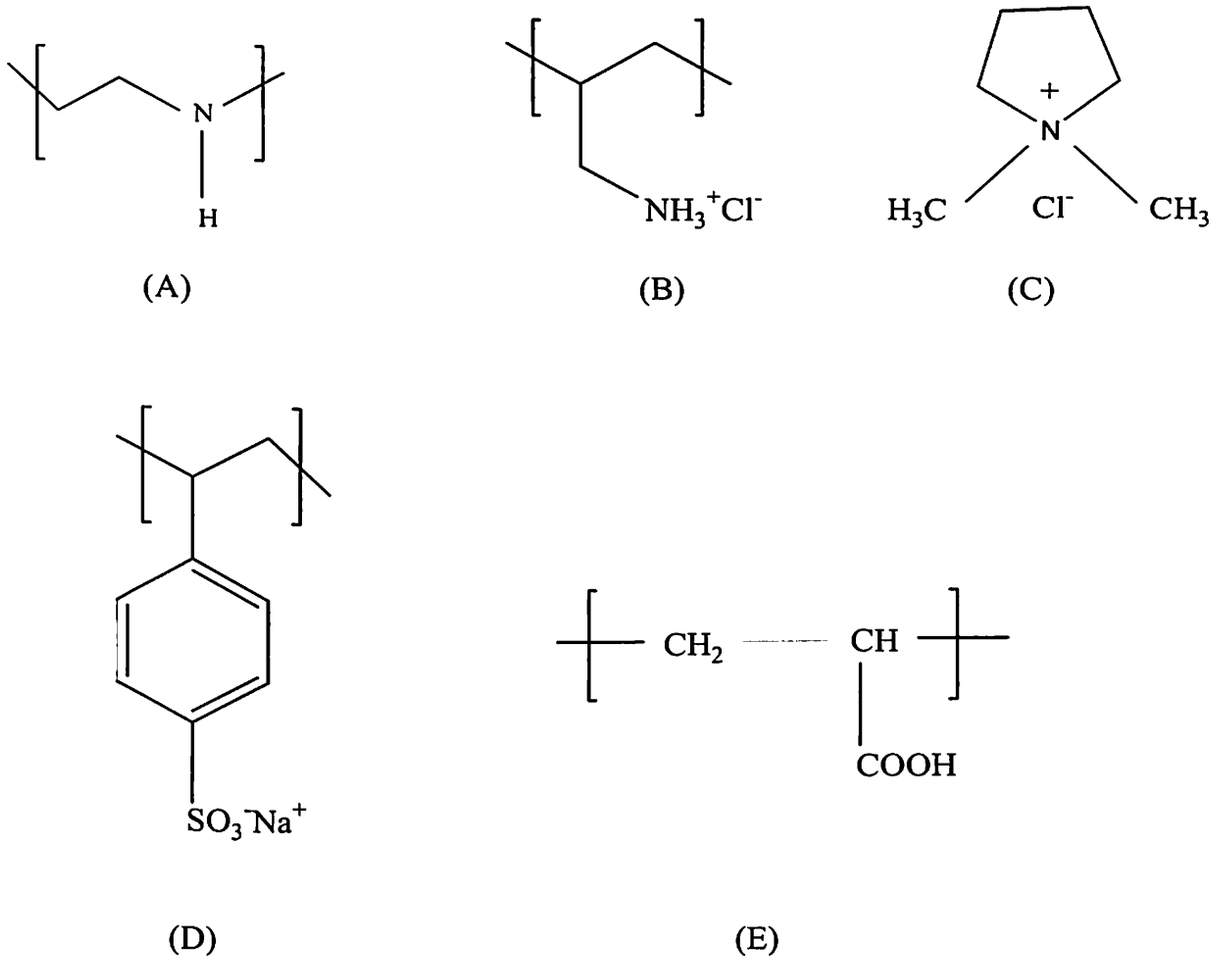


Figure 2. Schematic representation of the film deposition process

A new polymer layer is formed, but now the original surface charge is restored. Alternating multilayer assemblies are obtained by repeating these steps. Layer thickness can therefore be increased by increasing the number of deposition cycles. To achieve freestanding thin films, the substrate is dissolved in suitable solvent. Cellulose acetate was used as a substrate in the present work and it was dissolved in acetone to obtain self standing thin films.

The unique aspect LBL deposition in comparison with the other film deposition techniques is the broad range of materials that are available for incorporation into polyelectrolyte multilayers. This method was successfully applied to proteins [26], D.N.A [27], metal and semiconductor nanoparticles [28-37], and dyes [38-41]. Some of the commonly used polyelectrolytes are shown in Figure 3. Due to the high degree of ionization, poly(diallyldimethylammoniumchloride) (PDDA) and poly(sodium styrenesulfonate) (PSS) are strong electrolytes. For poly(acrylic acid) (PAA), the linear charge densities are tunable with changes in pH and thus it is weak electrolyte. In the present work we worked with weak polyelectrolyte Poly(acrylic acid), where the degree of ionization in the solution and thus the multilayer properties can be adjusted by the solution pH value.

The multilayers are characterized by a sequence of layers in which each layer has its individual structure and properties. More often the arrangement of the layers is highly ordered, the property of each layer can be described as an average over a certain area. The thickness of the each layer is dependent on the nature of the underlying surface and on the deposition conditions. With respect to the underlying surface, nature and density of the charged groups, their local mobility and the surface roughness are the important



(A) (B) (C)

(D) (E)

Figure 3. Commonly used polyelectrolytes in LBL assembly.

(A) Poly(ethyleneimine)

(B) Poly (allylamine hydrochloride)

(C) Poly(diallyldimethylammonium chloride)

(D) Poly(styrenesulfonate) sodium salt

(E) Poly(acrylic acid)

parameters. The film deposition is mainly dependent on pH of the solutions, concentration of adsorbing species, adsorption time, temperature, nature, solvent, concentration of added salt, rinsing time, dipping speed etc

. One should keep constant deposition conditions in order to achieve highly reproducible films. The conditions under which the measurements are taken are also important. For example, relative humidity and temperature can influence deposition. The thickness of the film decreases with increase in temperature and increases with the increase in relative humidity.

Monitoring multilayer build up

Ellipsometry is used to monitor the growth of the layers. We can also use UV/Visible spectroscopy to monitor the film thickness. These two methods are simple and sufficient to get some preliminary idea on the deposition behavior. Other methods include surface plasmon spectroscopy [42-44], optical waveguide lightmode spectroscopy [45], scanning angle reflectometry [46], in-situ atomic force microscopy [45, 47], surface force measurements [48], X-ray and neutron reflectometry [49] and quartz crystal microbalance [50].

Factors controlling layer thickness

A basic understanding of some of the fundamental issues needs to be considered in order to control the structure, molecular organization, thickness, and properties of the multilayer thin films. Most of the work has been carried out with fully charged polyelectrolytes such as poly(sodium styrenesulfonate) and poly(allylamine). Molecular

organization with these polymers can easily be controlled by changing the ionic strength of the dipping solutions [51]. This approach is limited to a certain extent by the poor solubility of high molecular weight polyelectrolytes in solutions of high ionic strength.

To overcome this limitation Dongsik Yoo and his coworkers used weak polyelectrolytes and achieved a higher level of versatility in the layer-by-layer sequential adsorption process [52]. In the past 4 years, progressive work has been done on the effects of pH on assembly of weak polyions [52, 53]. They found that the important characteristics of the basic bilayer building block of sequentially adsorbed layers of weak polyelectrolytes can be systematically varied over a wide range by simply controlling the pH of the dipping solutions. Michael Rubner has showed that the layer thickness can be precisely controlled by the pH in case of weak polyelectrolytes [54].

In the case of weak polyelectrolytes such as poly(acrylic acid), it is generally understood that the polymer chains tend to adsorb as thin layers with flat chain conformations when they are highly charged (high pH) and thicker, more loopy type structures when they are less charged (low pH). In addition, the amount of polyelectrolyte adsorbed to a surface increases with increasing surface charge density since more material is needed to balance and overcompensate the higher charge density of the oppositely charged surface. Since the degree of ionization of a weak polyelectrolyte can be controllably varied via pH changes, it is possible to control both the linear charge density of an adsorbing polymer chain and the surface charge density. The pH of the polyanion dipping solution determines the linear charge density of the polyacid and therefore the amount of polyacid adsorbed and the pH of the polycation solution determine the charge density of the previously adsorbed outermost polyacid layer.

Many researchers mentioned the effects of non-specific, hydrophobic or other interactions on different charged substrates or different types of counter polyions. N.A. Kotov has demonstrated that without the added effects of hydrophobic interactions at the surface, typical polyion systems would not adsorb at all [55]. Rubner showed that the surface wettability, the level of layer interpenetration, the thickness, and the surface roughness of the films are sensitive to the charge density of the polyelectrolytes, which was varied by changing the pH of the dipping solutions [52, 56]. However, in practice it is very difficult to control the degree of the ionization as it is dependent on the local concentration of protons, which may vary with distance from substrate.

Drying of the multilayer film during each deposition cycle is said to increase the layer thickness. The surface layer collapses into a denser layer when water is removed from it. That means the loops and tails that extend from the surface in the dipping solution collapse onto the surface after drying. This will lead to the deposition of the thick film on the top of a thin previously adsorbed layer which will produce a surface that is enriched in segments from the outermost adsorbed layer.

Frank Caruso group has examined the influence of environmental salt concentration on the structure of multilayer films made of poly(acrylic acid) and poly(allylamine) [57]. Monovalent salts (e.g., sodium chloride) are added to polyelectrolyte solutions to promote the adsorption process. The layer thickness increased with the addition of salt. The main effect of the salt is to screen the electrostatic charges. The repulsion of charges of the same sign on the polymer chain is reduced by screening and thus the polyelectrolyte in solution changes from a stretched to coiled conformation. It also causes a decrease in the repulsion between polyelectrolytes of the same species.

The observed larger thicknesses of polyelectrolyte multilayers assembled in the presence of salt can be explained by both of these effects [58]. These findings were also supported by the studies of Dubas and Schlenoff [59]. They observed dependence of thickness for PSS/PDDA system with the concentration of the added salt. The increase in thickness is almost linear with respect to the salt concentration. It is also evident that the maximum adsorption occurs during first 5-8 minutes and then reaches saturation after a particular time in the presence of 1.0 M NaCl. These findings have also been supported by Yuri Lvov with PSS/PAH system [60].

The rinsing step during each deposition of the polymer enables higher adsorption [61]. The rinsing removes some polymer molecules that are only loosely attached to the preadsorbed polymer layer. Presumably during the rinsing step, some rearrangements occur in the adsorbed layer that makes the layer more stable.

Transport through LBL assembled multilayer thin films

The permeability measurements of LBL assembled multilayer have been highlighted by Bruening's [62, 63] group. They alternatively deposited LBL films with varying numbers of layers onto the surface of porous alumina substrates and observed the permeability of different kinds of anions in the solution as well as gas molecules. From their work, it is evident that the permeability of anions through the PSS/PAH system on porous alumina substrate decreases as the number of layers of PSS/PAH increases [63]. Also data shows less selectivity and also less permeability from 0-10 layers. N.A. Kotov's group also reported gas permeation properties of montmorillonite/PDDA self assembled multilayer systems on a hydrophobic poly(ethyleneterephthalate) (PET)

support by means of layer-by-layer deposition [64]. Results indicated the decreased permeability of gas molecules with increase in the number of layers.

An overall understanding of the permeability of the thin films is difficult to achieve since it not only depends on the pH, ionic strength, and time of adsorption but also depends on the degree of cross linking and pore formation. Addition of salt to the deposition solutions changes the structure and the thickness of the polyelectrolyte films [65]. The films also change conformations from flat to coil upon salt addition. Polyelectrolytes without the addition of salt assume flat structure due to large amount of charge-charge repulsions between the monomer units. However, the addition of salt screens the ionic charges and allows the changes in the structure of the films. This ultimately changes the permeability of the thin films.

At higher pH, films swell and begin to delaminate. Due to this swelling, the films exhibit higher permeabilities at higher pH values. Studies on the transport properties of polyelectrolyte multilayer membranes were investigated by Tieke and others [66]. Their studies show that the transport of small molecules and ions through the membrane is strongly the function of the density of the network formed by the complex formation of the complex between the oppositely charged polyelectrolytes. High separation capability and low flux are due to the high charge density. High density of ions pairs are formed when highly charged polyelectrolytes are adsorbed. These, in turn, favor the transport of highly polar liquids such as water but retard the transport of more hydrophobic alcohols and reject divalent ions of high charge density more strongly than the monovalent ions.

Regine V. Klitzing worked on a realistic diffusion model for ultrathin polyelectrolyte films [67]. Their results show that the alternating sequences of

polyelectrolytes with positive and negative excess charge have no influence on the permeability of molecules. However, the internal structure has an influence on the permeability. They found that the transport of rhodamine molecule is about 3 times faster through the flat polyelectrolyte molecules than through the loopy structures. This indicates films in flat structure are less tightly packed than those composed of loops due to higher electrostatic repulsion between the equally charged polyelectrolytes of one layer. It was observed that the rhodamine permeability near the film surface is higher than in the film bulk. This implies the outer layers are more loosely packed than the layers far away from the surface in the film bulk.

Diffusion characteristics of collagen films were studied by Ming-Thau [68]. They made collagen films by treating collagen gel solutions with different concentrations of glutaraldehyde. From their work, it is possible to modify diffusion characteristics of the collagen films by crosslinking with different concentrations of glutaraldehyde.

Interactions between proteins and polyelectrolytes

Protein interactions with polyelectrolyte multilayers have been studied by Pierre Schaaf [69]. They found that albumin adsorbs both on multilayers terminating with PSS or PAH. After the formation of the protein film they observed that when the albumin solution is replaced by a pure buffer solution of the same ionic strength as the adsorption solution, no desorption takes place. A significant amount of adsorbed proteins is released, when a buffer solution of higher ionic strength is brought in contact with the albumin film. They also found for albumin solutions of given concentration, the adsorbed amount depends on the ionic strength of the adsorption solution.

Interactions between collagen and poly(acrylic acid) are primarily due to the hydrogen bonding [70-72]. Films made with blends of collagen and poly(acrylic acid) showed improved mechanical properties than that of single components. However some of the studies showed the possibility of existing strong ionic interactions between these two polymers [73]. It was reported that poly(acrylic acid) interact with either synthetic or biological polybases to form polyelectrolyte complexes [74-76]. With collagen/PAA layers, ionic interactions takes place between to macromolecules and this is responsible for the formation of the poly complex. The isoelectric point of collagen is 5.5. Below the pH 5.5 most of the amino groups are in NH_3^+ form and the acid groups are mainly in –COOH form. This is the favorable condition for the formation of the multilayers with poly(acrylic acid). Barbani showed that the interactions between collagen and PAA result from a complex of the two polymers that is formed in aqueous solution within a narrow pH range, through electrostatic interactions involving the NH_3^+ groups of collagen and the COO^- groups of PAA [77-79].

Free standing thin films

Although the LBL films exhibit promising insight about permeability applications, still many problems need to be resolved. Firstly, there is requirement for the preparation of the free standing films. Most of the studies conducted with LBL multilayers are based on solid supports. While considering the permeability of the film, we should also be aware of the effect of permeability through the substrate. It is obvious that these substrates cover most of the surface of the multilayer film which eventually reduces the overall permeability. Therefore, films without the substrate enable direct and

actual experimental values of many physical properties. This is highly desirable in the application of bio-artificial films due to the incompatibility of biological systems with artificial substrate or skin substitutes [80]. Secondly, the separation capabilities of these films should be high for various applications such as gas separation membranes, sensors, and dialysis membranes. Thirdly, the most important parameter for the feasibility of the multilayer films in biological applications is its biocompatibility.

After considering these points, it would be appropriate to prepare biocompatible free standing films and determine their feasibility. The basic idea for the preparation of the free standing film is that LBL assembly was first carried out on a substrate and after attainment of fixed number of layers, the substrate is removed from the film either by means of dissolving substrate in suitable solvent or by breaking bonds between the film and the substrate. The main criterion for the substrate is that the organic solvents should not damage the film and the substrate must be hydrophilic to achieve deposition by LBL assembly.

Previously our coworkers have reported biocompatibility and controlled thickness of the collagen thin films [81]. They deposited collagen films with poly(sodium styrenesulfonate) on the bottom of a Petri dish, which served the purpose of LBL substrate. They demonstrated the attachment of the C2C12 myoblast cultured cell and its growth on the collagen/PSS multilayers. The growth of the cells is uniform over entire collagen/PSS multilayers surface (Figure 4).

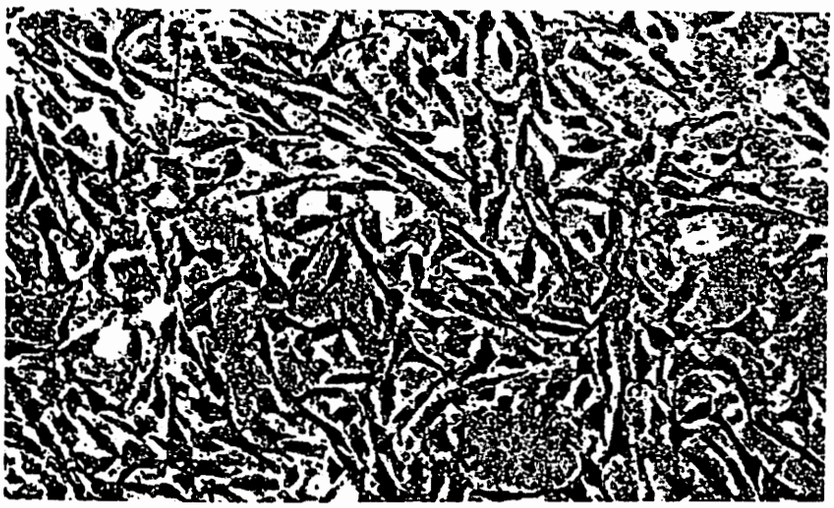


Figure 4. Light micrograph (original magnification 100x) of seeding muscle myoblast cells (C2C12) on substrates coated by LBL film of PDDA(PSS/collagen)$_2$

Substrates for LBL assembly

A number of substrates can be used for building multilayer thin films. Common substrates are glass, silicon and quartz. Pretreatment of the substrate either by heating in oxidizing medium or by grafting an organic molecule on the surface was performed according to many previously reported procedures for the layer-by-layer self-assembly of polyelectrolytes [82, 83], proteins [84, 85], and nanoparticles [86, 87].

All the substrates are cleaned well and the surface is charged (if necessary) before subjecting to LBL deposition. More often, a precursor film is deposited to adsorb the polyelectrolyte for the further growth of the layers. Polyelectrolyte thin films can also be built on polymer substrates [88, 89]. However, choices of the substrates are limited for obtaining free standing films. One must ensure that the films were separated from the substrate without much difficulty. For our work cellulose acetate is more suitable substrate as it enables the easier separation of the film without much mechanical damage from the substrate.

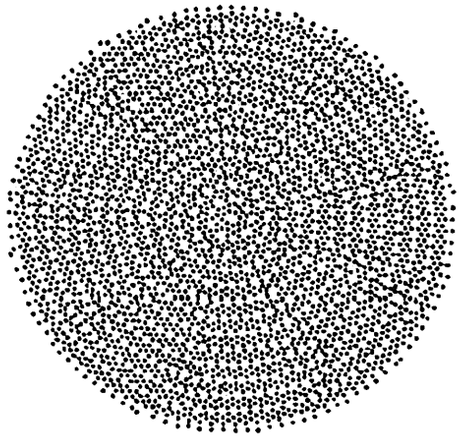
Role of Collagen

Collagen is the most abundant form of protein found in the body and is a natural protein that provides structural support. The structure of collagen is complex, but very similar in humans and higher animals including cattle and pigs. Chains of amino acids make up collagen. These chains form collagen molecules which in turn, form fibrils [Figure 5(A]. These fibrils produce fibers that when bundled lead to body tissue formation.

There are 18 different types of Collagen. Type 1, 3, and 5 are specific for skin. Collagen molecules are triple helices with a length of approximately 300 nm [Figure 5(B)]. Two chains in this triplet (called α_1) are associated with a third chain (of type α_2), which is similar but not identical. A special amino acid sequence makes the tight collagen triple helix particularly stable. Every third amino acid is a glycine, and many of the remaining amino acids are proline or hydroxyproline. At the bottom, the helix is less regular, because many different amino acids are placed between the equally-spaced glycines.

Collagen is the main protein in the extracellular matrix, a support system necessary for the survival of every cell in the body [92]. Collagen is frequently used as a biomaterial due to its ability to persist in the body long enough to carry out its specific role without developing a foreign body response that could lead to the premature rejection or overall failure of the biomaterial. The degree to which a device avoids the foreign body response is a measure of its biocompatibility. Because of its ability to involve in many important biological functions such as tissue regeneration and cell attachment, as well as its general compatibility with living tissues, collagen is widely held to be an ideal biomaterial [93, 94]. Collagen has the ability to self assemble into rigid gels and fibers [95-97]. Indeed many devices made of less biocompatible materials are routinely coated with collagen in an effort to make them biocompatible.

Most of the biological responses are governed by the control of the surface structure. It is well known that biomaterials are highly organized from molecular to the nano, micro and macro scales often in a hierarchical manner with intricate nanoarchitectures. In many biocomposites, the micro and nano structures are organized in



(A)

(B)

Figure 5. (A) Model for the cross-section through a collagen fibril [90, 91].

Each dot corresponds to a collagen molecule

(B) Basic helix model of collagen

layered architectures leading to the control of shape and function [98, 99]. Nanofabrication, nanotechnology and nanoscience represent growth areas in research and development. Nature has been using nanofabrication ideas since the beginning of biological evolution. Advances in surface science and nanotechnology should allow the synthesis of such materials with desirable properties.

EXPERIMENTAL PROCEDURE

Materials

The positively charged poly(diallyldimethylammonium chloride) (PDDA, M_W = 400000-500000; 20 wt% solution in water) and the negatively charged poly(acrylic acid) (PAA, M_W = 250000; 35 wt% solution in water) were obtained from Aldrich (Milwaukee, WI). Collagen Type III (acid soluble, from calf skin), fluorescein sodium salt ($C_{20}H_{10}O_5Na_2$; FW 376.3), fluorescein isothiocyanate-dextran (FITC-dextran), with different molecular weights 4 kDa, 10 kDa, 20 kDa, 40 kDa and 250 kDa, glutaraldehyde ($C_5H_8O_2$; FW 100.1, 25% aqueous solution) were bought from Sigma-Aldrich (St.Louis, MO). Cellulose acetate (CA) films were purchased from EM Science (Gibbstown, NJ). 99% acetone was bought from Pharmco (Brookfield, CT) and phosphate buffer solution (PBS; 0.2M Potassium phosphate, 1.5M NaCl, pH = 7.2) was purchased from Rockland, PA.

The pH of the solutions was adjusted either with dilute HCl or with dilute NaOH. All the chemicals were used without any further purification. Ultra pure deionized water (18 MΩ/cm) was used for all experiments and rinsing procedures.

Methods

(a) Layer-by-layer build up

All the experiments were performed at room temperature unless specified and LBL build up was done manually. 20% aqueous solution of PDDA was diluted 40 times

to obtain 0.5% solution and pH was adjusted to 3.0. Similarly 35% solution of PAA was diluted approximately 70 times to form 0.5 % solution and pH is changed to 4.0. Solution of 0.5% of collagen was prepared and pH is adjusted to 4.2 to 4.3. All solutions were sufficiently stirred to obtain uniform solutions. LBL deposition of collagen films was carried out on cellulose acetate substrate which has negative charge on its surface due to partial oxidation.

Cellulose acetate film was first washed in soap solution for 5 minutes and then thoroughly rinsed with pure water. One side of the CA film was covered with scotch tape and the other side was used for film deposition. To improve hydrophilicity, the CA film was treated with 0.1M NaOH solution for 5 minutes and then rinsed with deionized water several times. The substrate was then immersed in 0.5% solution of PDDA for 10 minutes. PDDA acts as a precursor film which improves surface charge and enable uniform deposition of negatively charged PAA. After the substrate was washed for two times, 30 seconds for each wash, it was exposed to PAA solution for 10 minutes causing the surface charge to become negative. The same washing procedure was repeated and the substrate was dipped in positively charged collagen solution for 20 minutes and rinsed with water again. This method enables deposition of PDDA/PAA/collagen layer.

Further adsorption of the PAA/collagen layers was carried out by immersing the substrate repeatedly in the solutions of PAA, washing with water and immersing in a solution of collagen and rinsing. Intermediate drying of the film was carried out for each cycle before immersing the substrate into collagen solution. After formation of the required number of layers, the film was dried and the scotch tape was removed carefully.

This yielded a LBL assembly one side of the film. To increase the mechanical

strength, the film was crosslinked with the vapors of 8% aqueous solution of glutaraldehyde. The film with LBL assembly was placed on a plastic Petri dish and allowed to float over the surface of 50 ml of 8% aqueous glutaraldehyde solution. The whole system was then placed in a water bath and heated to 37 degree centigrade for 16 hours. After completion of crosslinking, the film was thoroughly washed repeated number of times with water and then dried. To obtain the free standing films, the LBL assembly with CA substrate was placed in acetone for 24 hours. After complete removal of cellulose acetate, the film was transferred to a glass Petri dish containing water and then made acetone free by treating it with 100 ml of fresh water 2-3 times.

(b) Set up for the permeability measurements

Phosphate buffer solution (PBS) was diluted 20 times with pure water and the pH was adjusted to 7.4. For fluorescence measurements, FITC-dextran with different molecular weights (4 kDa, 10 kDa, 20 kDa, 40 kDa, and 250 kDa) was used. 0.1 mg of FITC-dextran from each of the five different molecular weight compounds was weighed and dissolved in 10 ml of the PBS to make five different solutions of FITC-dextran. Fluorescein solution with similar absorbance as FITC-dextran was prepared by comparing the absorbance with UV/Visible spectrometer.

A sample holder was made in our laboratory for the purpose of measuring permeability. Permeability through an area of the circle of 0.5 cm diameter of the collagen films was measured. The sample holder was made with teflon and assembled as shown in the Figure 6. Two columns made with plastic were used for filling two different solutions. The LBL thin film was placed on the surface of the small circular ring and

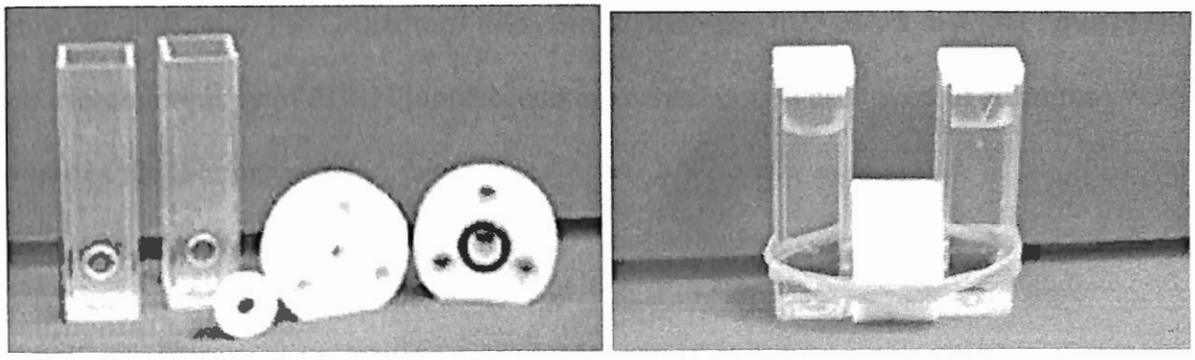


Figure 6. Set up for permeability measurements

placed in the groove of the holder and the assembly was fixed with screws and the two columns were attached to the holder and tied firmly as shown in the Figure 6.

One side of the column was filled with 3 ml of PBS and the other column was filled with 3 ml of FITC-dextran. Small magnetic stirrers were placed inside each column and were stirred at 700 r.p.m. FITC-dextran start diffuse into the PBS and the relative amount of diffused FITC-dextran into the PBS was measured by the increase in fluorescence intensity in PBS. Fluorescence measurements were taken at time intervals of 30 minutes for 2 hours.

Instrumentation

Different magnifications of SEM images were performed on a JEOL JXM 6400 scanning electron microscope in the electron microscopy lab at OSU. Atomic force microscopy (AFM) images were obtained in tapping mode with standard silicon tips. Ellipsometric measurements were taken using an AutoEL MS ellipsometer from Rudolph Research Corp, (Flanders, NJ). The DaflBM program supplied by Rudolph Technologies was used for the thickness measurements. Fluorescence measurements were made on a Fluoromax-3 from Jobin Yvon Horiba. TEM images were obtained on a JEOL JSM 100 CX II transmission electron microscope with a tungsten filament.

RESULTS AND DISCUSSION

Thin film build up profiles

Before carrying out LBL deposition on the cellulose acetate surface, layer build up was monitored by ellipsometry on silicon substrate. This procedure was used to determine the adsorption behavior. The substrate silicon wafer was subjected to extensive cleaning in a freshly prepared piranha solution (2:1 concentrated 98% H_2S0_4 and 30% H_2O_2) for 5 minutes. Then it was thoroughly rinsed with water and finally dried under a stream of nitrogen. Alternative layers of PAA/collagen were adsorbed as described in the experimental procedure. After the adsorption of each cycle, the silicon wafer was dried with nitrogen gas and the thickness of adsorbed layer was measured. Figure 7 shows the thickness increment with the number of deposition cycles. The average thickness for each layer was found to be around 60 nm. These measurements were also confirmed by TEM images of these films. Experiments on these multilayer films have shown that the newly incorporated layer interpenetrates the previous layer strongly and sometimes even the one before that with partial interpenetration. However, there is not complete mixing between the layers and each layer keeps its identity [100].

Polymer adsorption is an important topic which is highly relevant in biology and medicine, since many biological macromolecules function at natural interfaces such as cell membranes, and adsorb on artificial interfaces such as implanted membranes in tissue engineering. The layer properties strongly depend on the experimental procedure.

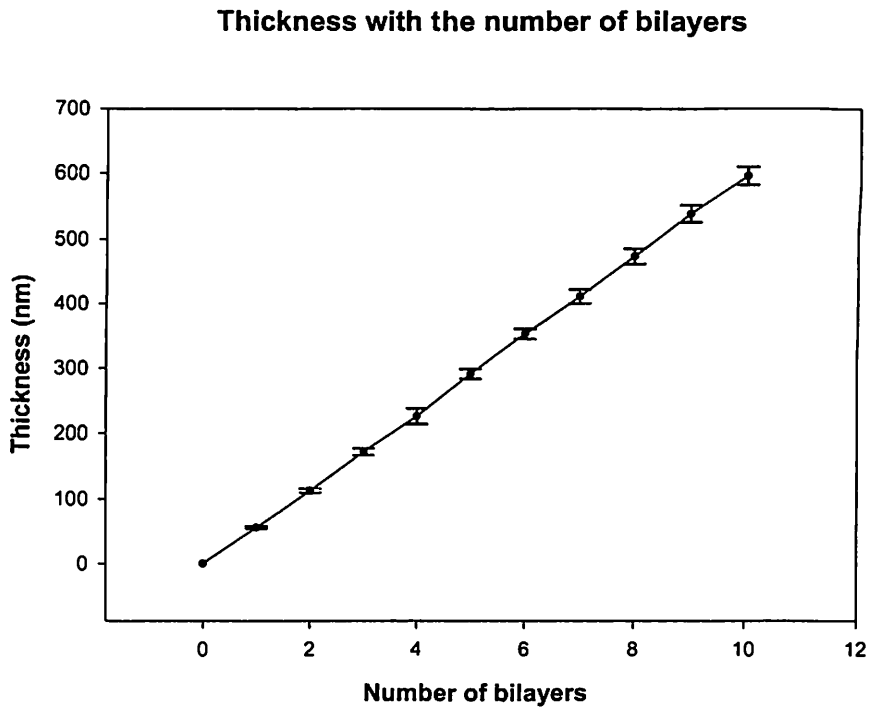


Figure 7. Increase in layer thickness with the deposition cycles for 10 bilayers of PAA/collagen film.

Extreme care was taken in the preparation of the PAA/collagen films to achieve consistent results. It was observed that each and every parameter plays a role in the structural properties of these thin films. Especially, in the case of biological films made with collagen and PAA, the effect of external and internal reaction conditions plays significant roles in preserving the uniformity and integrity of the assembled layers. The resulted uniform PAA/collagen thin films were found to be highly systematic and required very narrow ranges of the reaction conditions such as pH and concentrations when compared to the multilayer thin films that are made with the blends of synthetic polymers.

Three different types of multilayer thin films containing 10 layers of PAA and collagen were deposited at a PAA/collagen solution pH 4.0/4.0, 4.0/4.2 and 4.0/4.5. The isoelectric point of collagen is 5.5 and below pH 5.5, amino groups of collagen were in NH_3^+ form [101]. At pH 4.0, carboxylic acid groups of PAA were partially ionized [102]. It was observed that a 0.1 unit change in pH of collagen solution can ultimately dictate the formation and stability of the PAA/collagen thin films. The variation of thickness with the change in solution pH for PAA/collagen thin films was shown in the Figure 8. At lower pH levels of collagen solution (pH = 4.0), films formed were very thin and fragile. This complicates the process of isolation of the thin films when the substrate is dissolved in acetone. The films disintegrated when added to the acetone even with slight shaking. When the pH of collagen solution was increased from 4.0 to 4.2-4.3, keeping pH of PAA solution at 4.0, very uniform thin films were achieved with good mechanical strength. Isolation of free standing thin films at this pH is easier compared to that of lower collagen solution pH. At higher collagen solution pH (pH = 4.5), much thicker

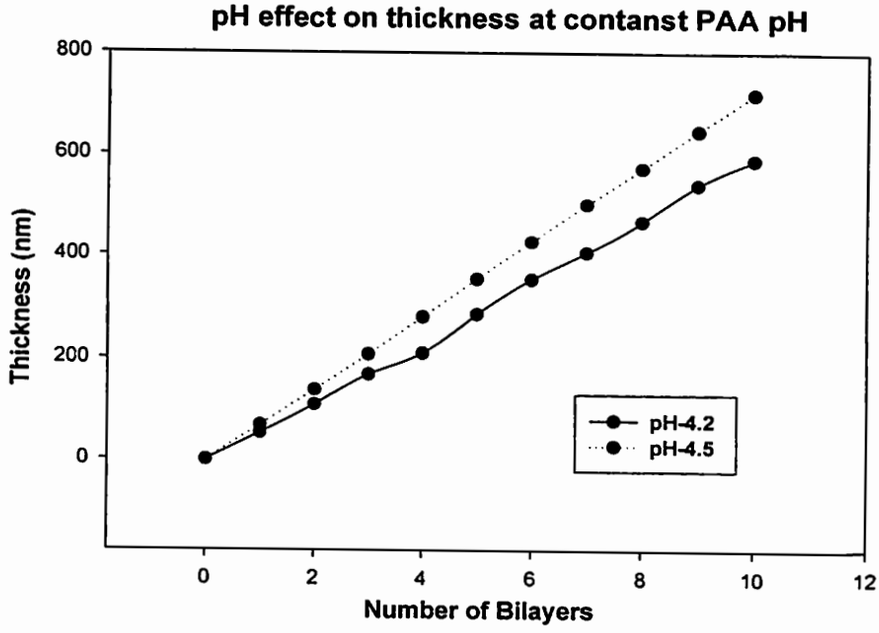


Figure 8. Effect of collagen solution pH on the thickness for 10 bilayers of the film.

films were formed with more surface roughness.

Morphology of collagen/PAA thin films

AFM images were obtained using a Nanoscope IIIa multimode digital instrument operating in tapping mode using AFM cantilevers with their attached silicon tips. Images were obtained for

a) one layer of PDDA/PAA/collagen on silicon

b) three bilayers of PAA/collagen on silicon substrate.

The substrate was cleaned thoroughly with pure water, presoaked in acetone for 30 minutes and washed with water for 20 minutes. This cycle was repeated two times and the substrate was dried in air. The immersion time for clean silicon substrate in PDDA is 10 minutes, in PAA is 10 minutes and in collagen is 20 minutes. Each immersion is followed by washing in three successive baths of pure water.

Since the polymers used are weak polyelectrolytes with incomplete charge, alternative adsorption of multilayers with high percentage of segments comprising loops and tails were formed under pH driven conditions. AFM images of one bilayer of PAA/collagen and three layers of PAA/collagen on silicon substrate were shown in the Figures 9 and 10. From the images we can observe the complex entanglement of bundles of polymer chains in random fashion. As the number of layers increased, complexity of this entanglement of polymer chains also increased suggesting the formation of multilayers with complex internal structure. These multilayers are not strictly stratified due to the fact that the formation of complexes may lead to inter- digitations between

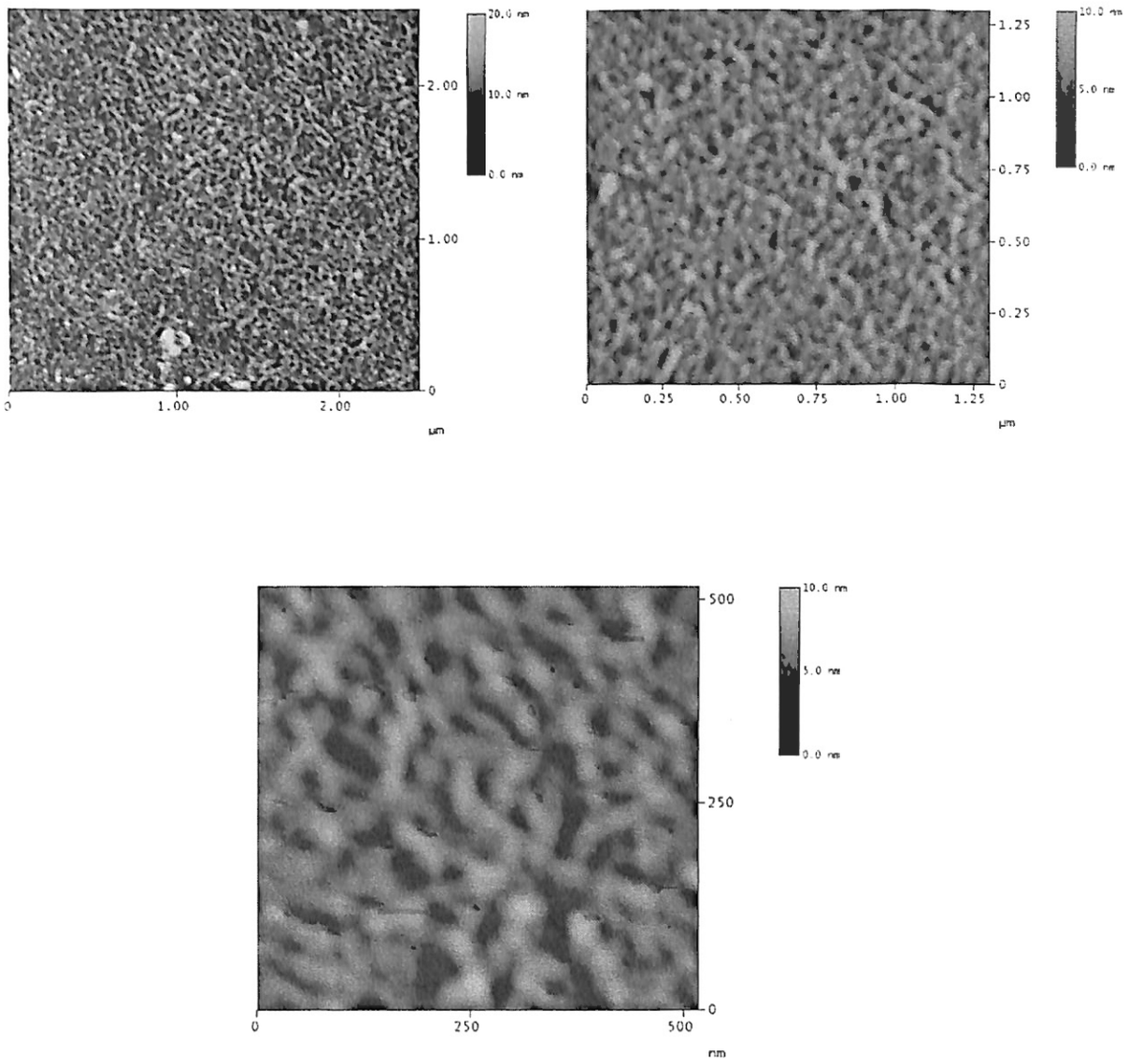


Figure 9. AFM images of 1 bilayer of PAA/collagen

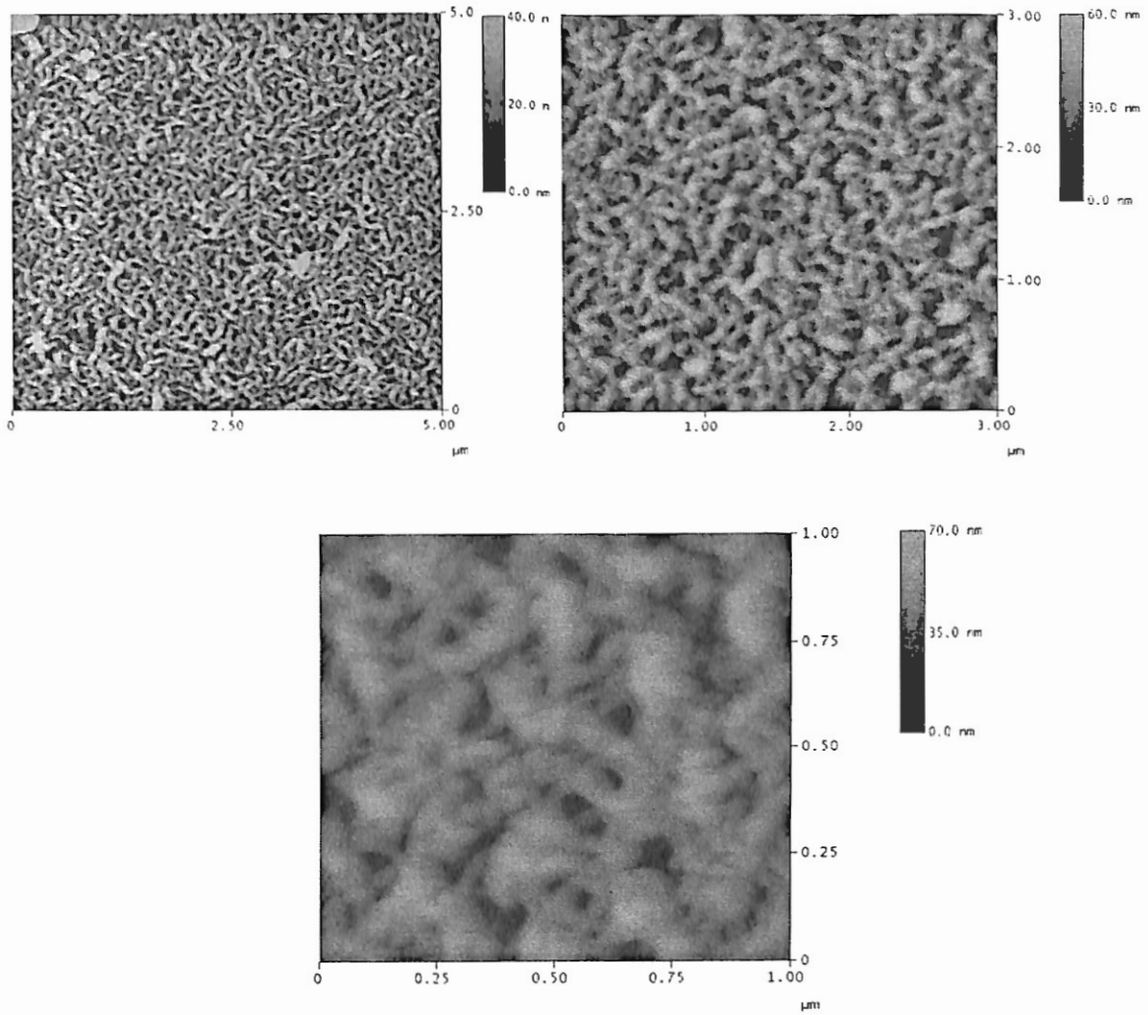


Figure 10. AFM images of 3 bilayers of PAA/collagen

adjacent polyelectrolyte layers.

Adsorbed polymers are able to change their conformation during adsorption with an associated rearrangement of polymer chains at the surface. Depending on their contact with the surface, the adsorbed chain can be divided into trains, loops and tails (Figure 11). All types of segments that are adsorbed at the surface are called trains. A loop connects two trains and its segments are not adsorbed at the interface. The tails are free ends of a polymer chain and extend outwards of the polymer chain. As can be seen from the AFM images (Figures 9 and 10), different loops and tails are entangled in random fashion. Exposing these films to acetone for 2 hours did not change the polymer bundles structure or integrity (Figure 12). It was assumed that acetone may dehydrate the film but the changes would not be significant and would not damage the polymer film or affect the permeability. It was also evident that the entanglement doesn't change even after exposure to acetone solution.

It was found that the permeability could be controlled even after the films were dehydrated with acetone. This clearly shows that the pore size, size distribution of defects in the multilayers and the overall architecture are not much affected by acetone. However, more experimental evidence for the effect of acetone on the internal molecular structure and rearrangements within polymer chains may be necessary.

SEM images of these films were obtained for 10 bilayer film. The film was first coated with gold/palladium particles at low vacuum in an effort to make the film conductive. These images revealed the interconnectivity of these layers leading to the formation of bundles of fibers (Figure 13). The cross sectional thickness of the film was confirmed by TEM images (Figure 14). Thin sections of PAA/collagen film were cut

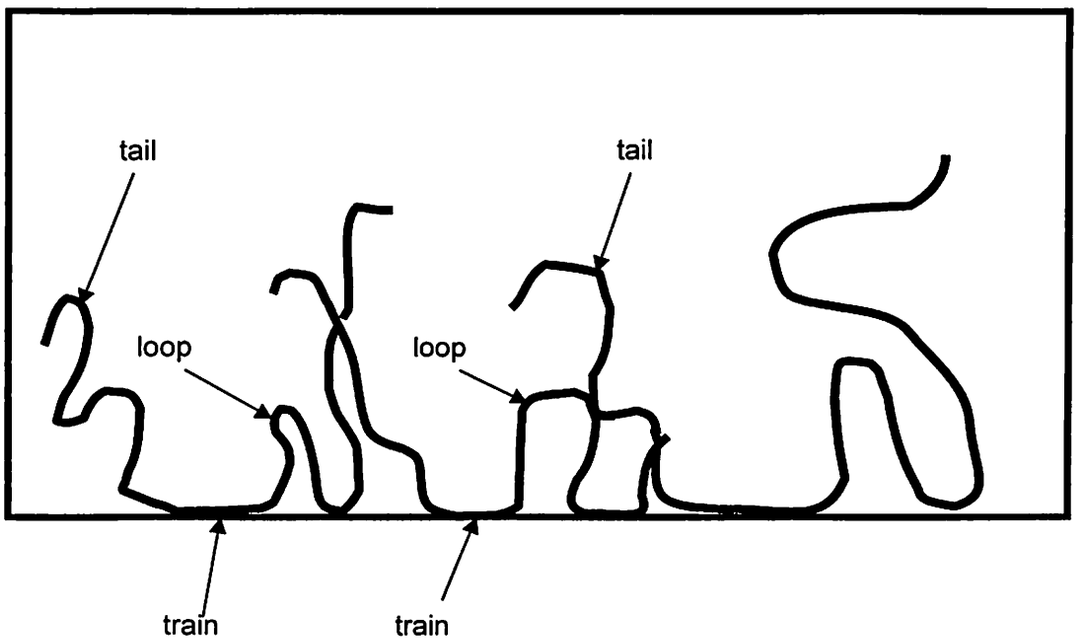


Figure 11. Pictorial representation of an adsorbed polymer layer

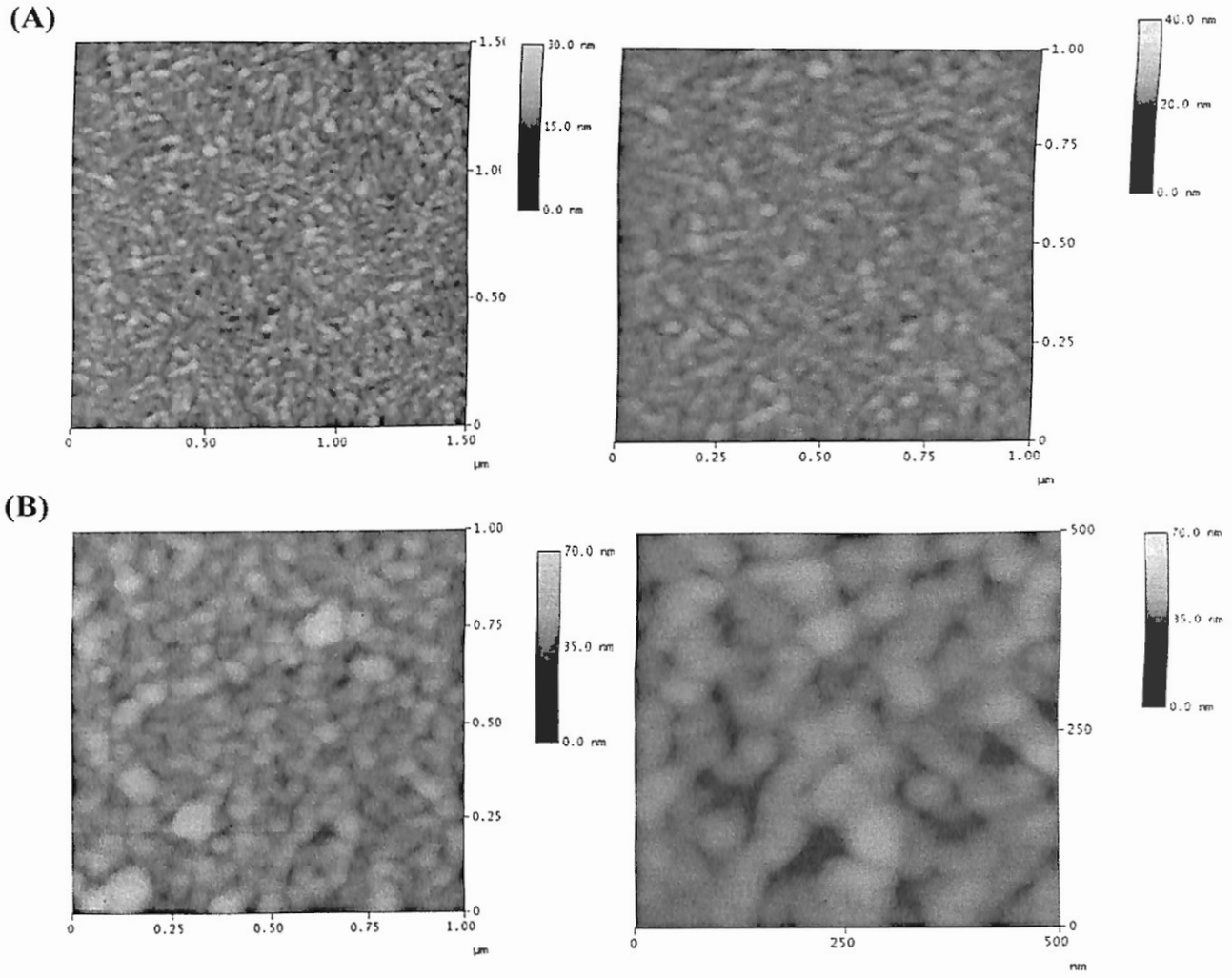


Figure 12. (A) AFM image of 1 bilayer of PAA/collagen after treating with acetone solution

(B) AFM images of 3 bilayers of PAA/collagen after treatment with acetone

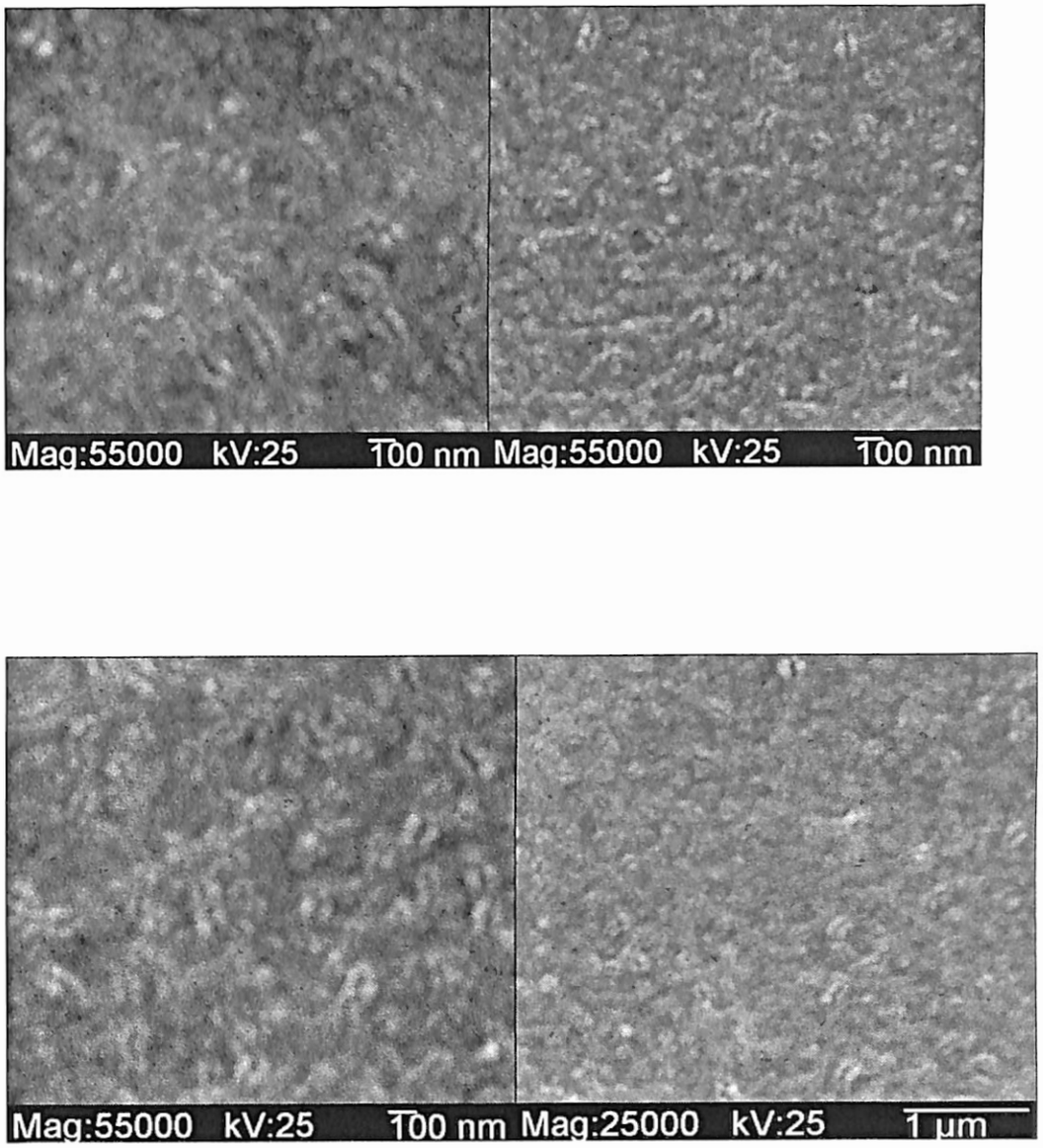


Figure 13. SEM images of 10 layers of PAA/collagen film

with a Diatome diamond knife at 70 nanometer thickness with a Sorvall MT5000 ultramicrotome. The sections were placed on the grid by picking the grid with the forceps and setting them on top of the sections in the water boat and then wicking off the excess water with filter paper. The grids with thin sections were stained with 2.5% aqueous solution of uranyl acetate. After air drying in air, the grid was placed on the sample holder and observed in transmission electron microscope. The thickness was found to be 550-650 nm for 10 layers of the film.

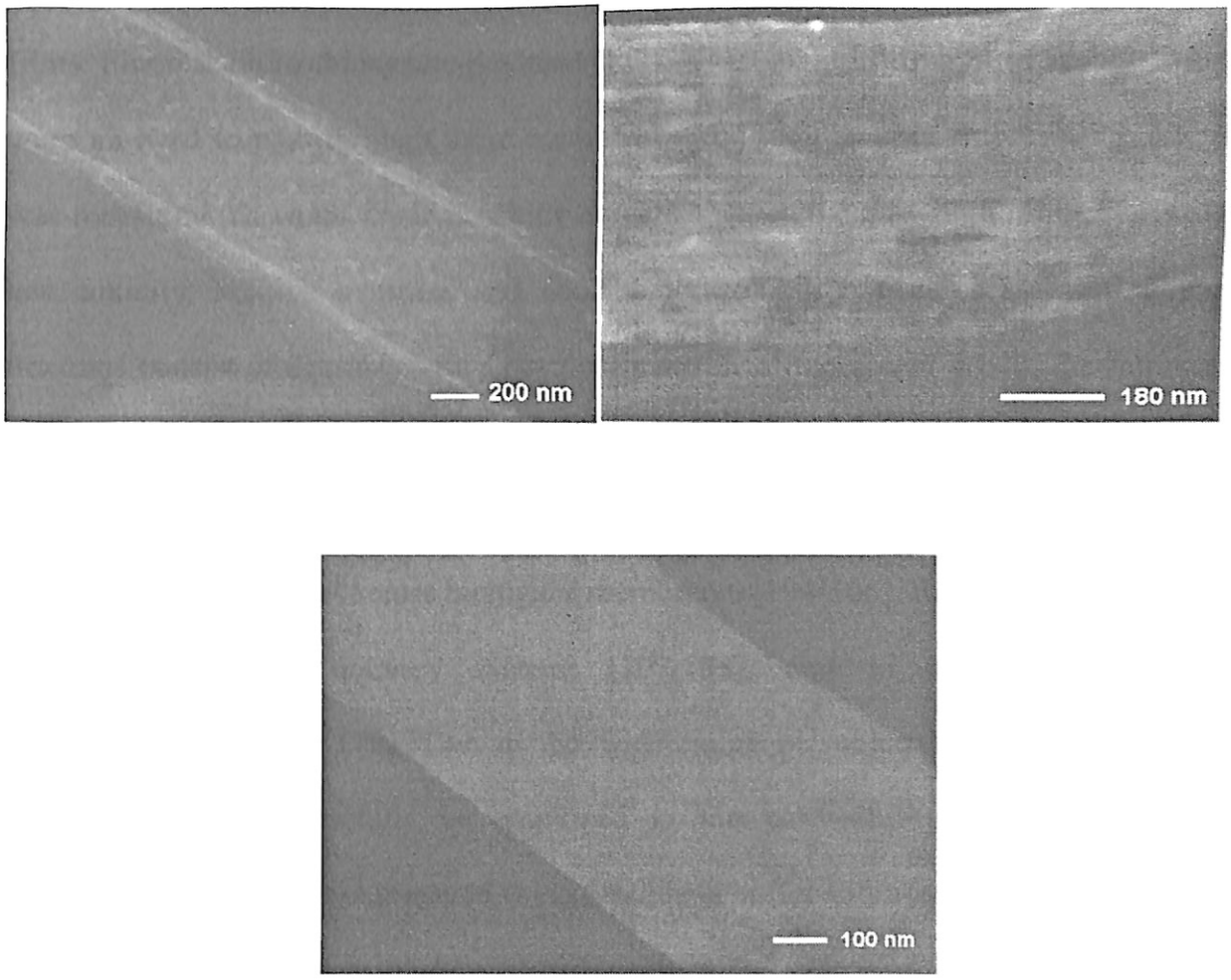


Figure 14. TEM images of 10 layers of PAA/collagen film

Transport results for fluorescein and FITC-dextran

Experiments were conducted to examine the permeability of the PAA/collagen films. Fluorescein isothiocynate-dextrans (FITC-dextrans) of different molecular weights were allowed to pass through these membranes and the resulting fluorescence intensity was measured. Dextrans are hydrophilic and are characterized by high molecular weight, low toxicity, relative inertness and good water solubility. Fluorescein isothiocynate-dextrans consist of dextrans with a covalently attached fluorescent group, the fluorescein isothiocyanate [103]. FITC-dextans are widely used in variety of applications due to their properties. The most important of applications are their adoption for the characterization of polar solute transport across biological membranes [104-106], for the kinetic studies on drug release from delivery systems [107-109], and to investigate membrane permeabilization [110-118]. Due to the wide range of molecular masses, the FITC-dextrans could successfully be employed in the permeability studies. Fluorescein compound solutions were prepared using phosphate buffer solution (PBS) at pH 7.4.

Experiments were performed to determine the effect of film thickness and the molecular weight of FITC-dextrans on the transport behavior. Selectivity for particular molecular weights based on the difference in molecular size would be particularly significant for kidney dialysis membranes. Further experiments were needed to determine quantitatively the exact amount of these materials that could be transported. In the case of LBL collagen based thin films the controlled release of FITC-dextrans over a period of time was observed. When fluorescein and FITC-dextrans were allowed to pass through the membrane with 10 layers, selective permeability was found for these molecules depending on their molecular size (Figure 15). Fluorescein and FITC-dextrans with

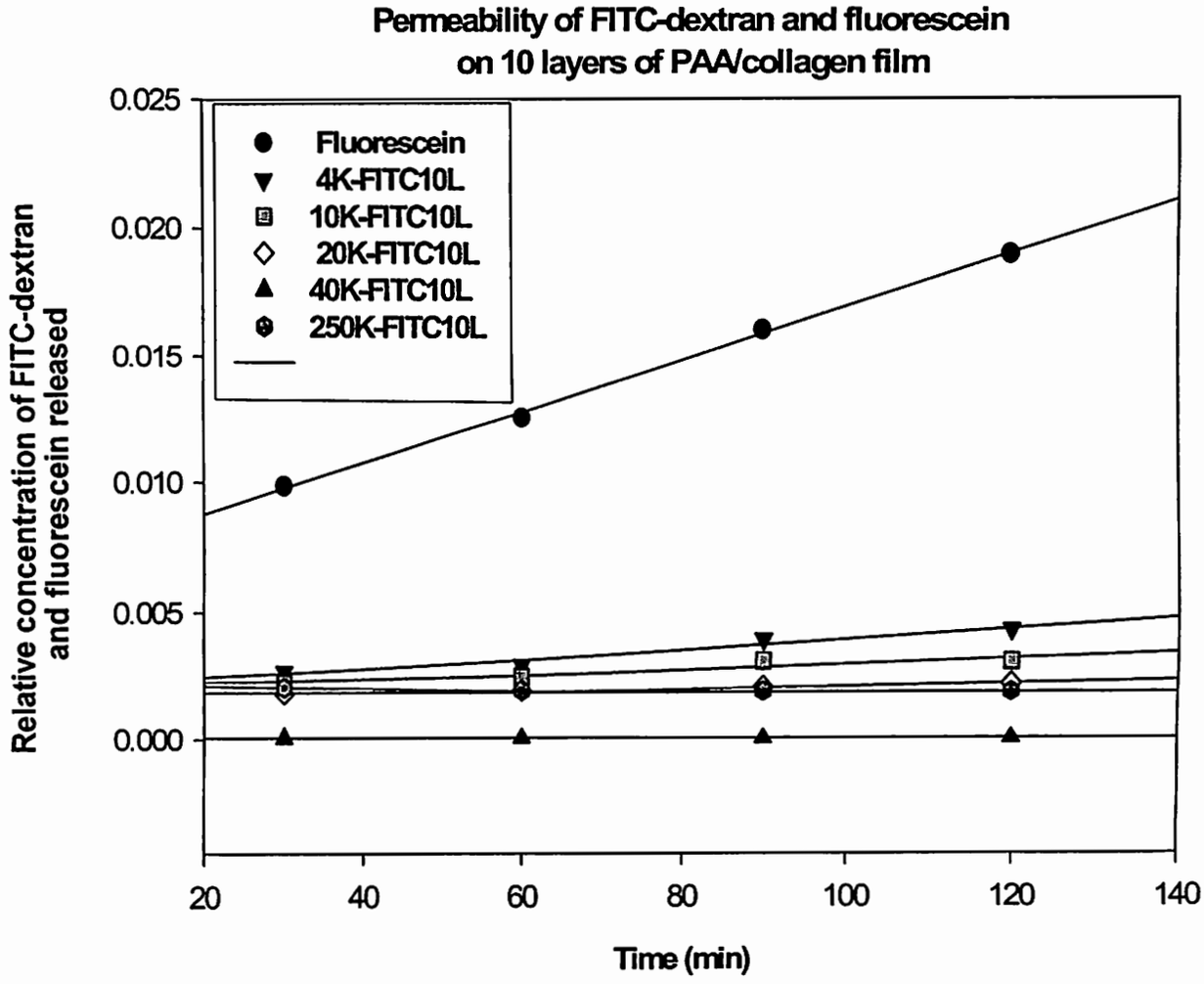


Figure 15. Plot of increase in relative concentration of FITC-dextran and fluorescein on the permeate side of the membrane with respect to time for 10 bilayers of PAA/collagen film.

molecular weights 4 kDa, 10 kDa and 20 kDa were permitted and higher molecular weight FITC-dextrans were retained by the membrane. No increase in the luminescence with respect to time was observed for higher molecular weight FITC-dextrans predominantly above 20 kDa. These results suggest that the molecular weight cutoff for these membranes is 20 kDa. Transport of molecules decreases gradually with the increase in the molecular weight. A plot of relative concentration per time with the molecular weights of fluorescein and FITC-dextrans is given in the Figure 16. The dependence of rate of permeation of FITC-dextrans with their molecular weights is observed indicating that the transport through these LBL films is size selective.

Similar experiments were repeated for 15 and 20 layer films. The results were plotted for the transport of FITC-dextrans and fluoroscein (Figures 17-20). The transport of these molecules is found to be the function of number of layers. Permeability decreased as the number of layers increased as shown in the Figure 21. Although the permeability of FITC-dextrans and fluorescein was decreased some extent with the increase in the number of layers of the film, there is no blocking for either fluorescein or for low molecular weight FITC-dextrans. This results from the fact that the path length of solute particle increases inside the pore with the increase in the thickness of the film and possibly tortuosity also increases.

Comparing transport of these molecules with the number of layers, transport of FITC-dextrans and fluorescein is a function of both molecular weight of the compounds and also function of the number of deposited layers. (i.e. thickness of the film). Transport is reduced with the increase in the number of layers and with the increase in the molecular weights of FITC-dextrans. It is also evident from the above results that the

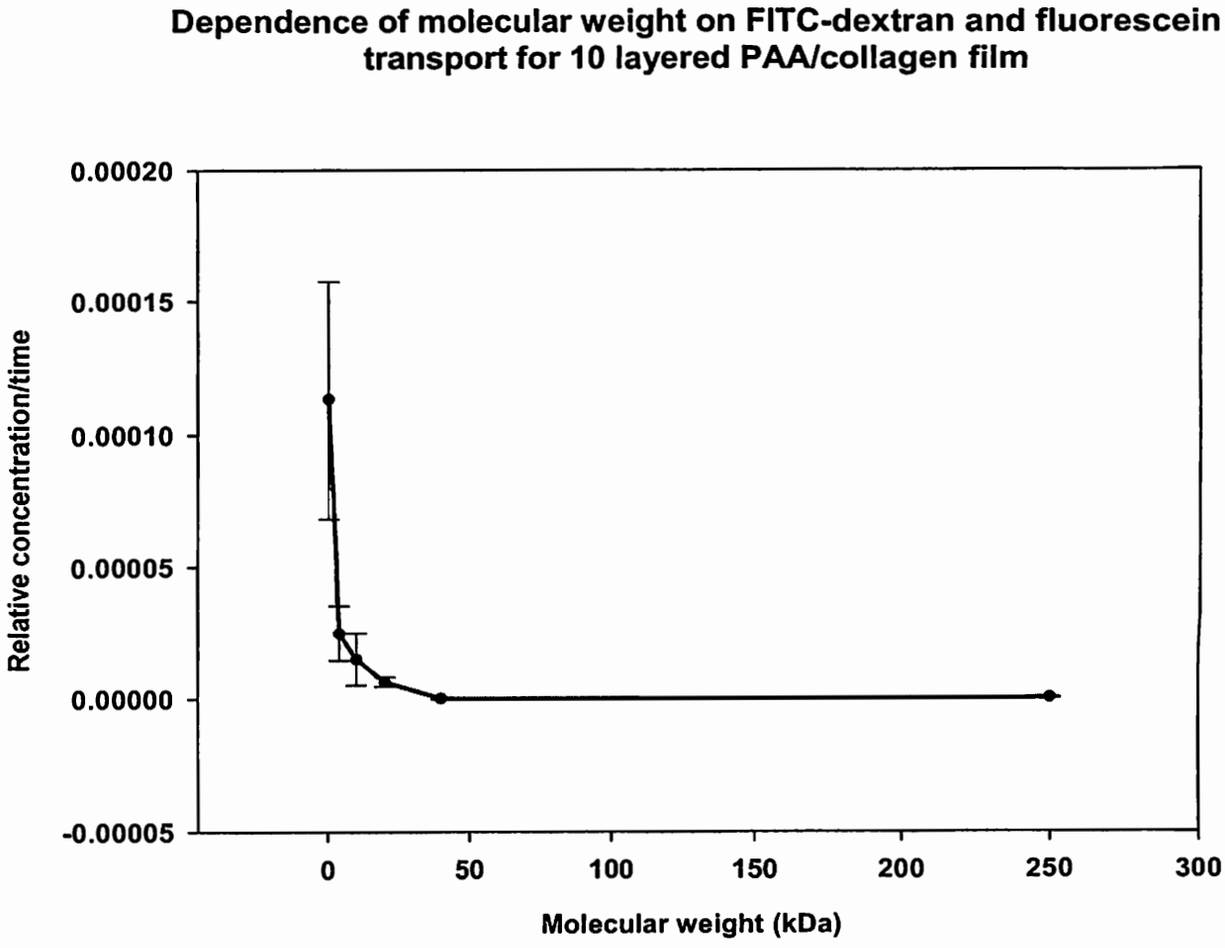


Figure 16. Plot of molecular weights of FITC-dextran and fluorescein against the relative concentration/time for 10 bilayers of PAA/collagen film.

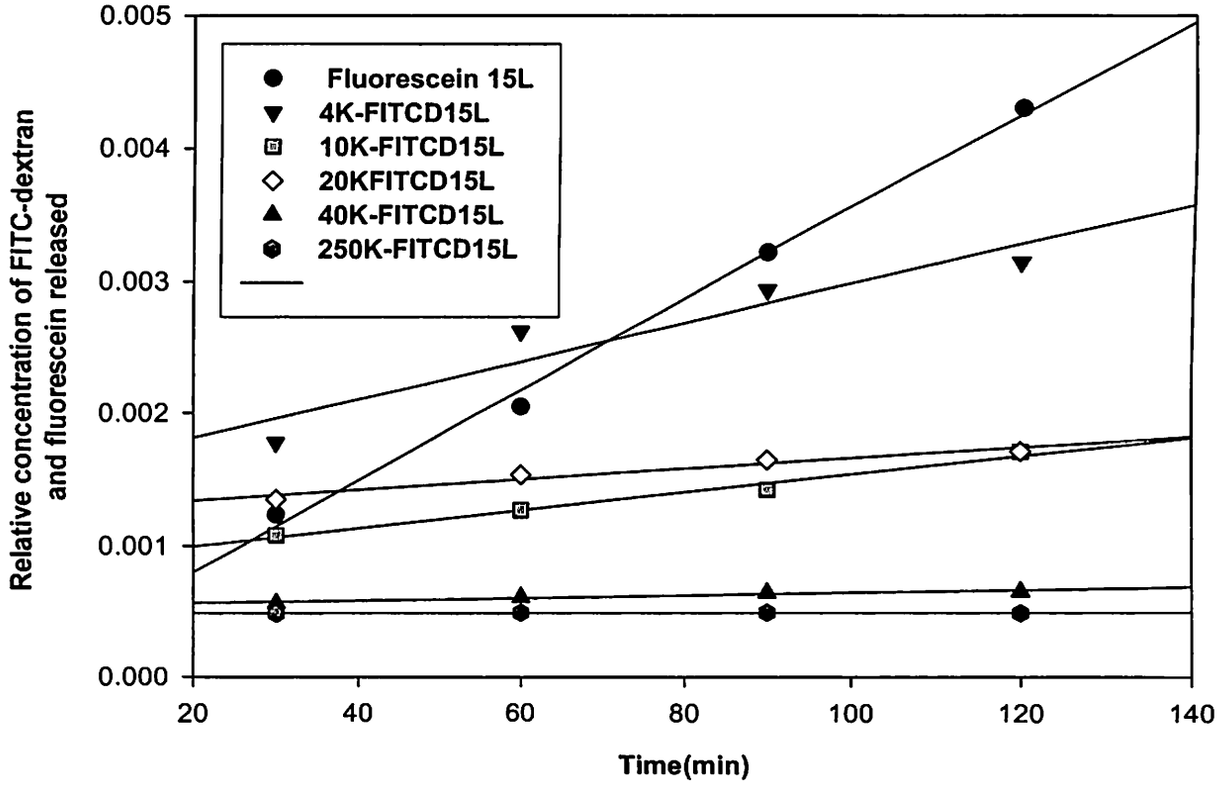


Figure 17. Plot of increase in relative concentration of FITC-dextran and fluorescein on the permeate side of the membrane with respect to time for 15 bilayers of PAA/collagen film.

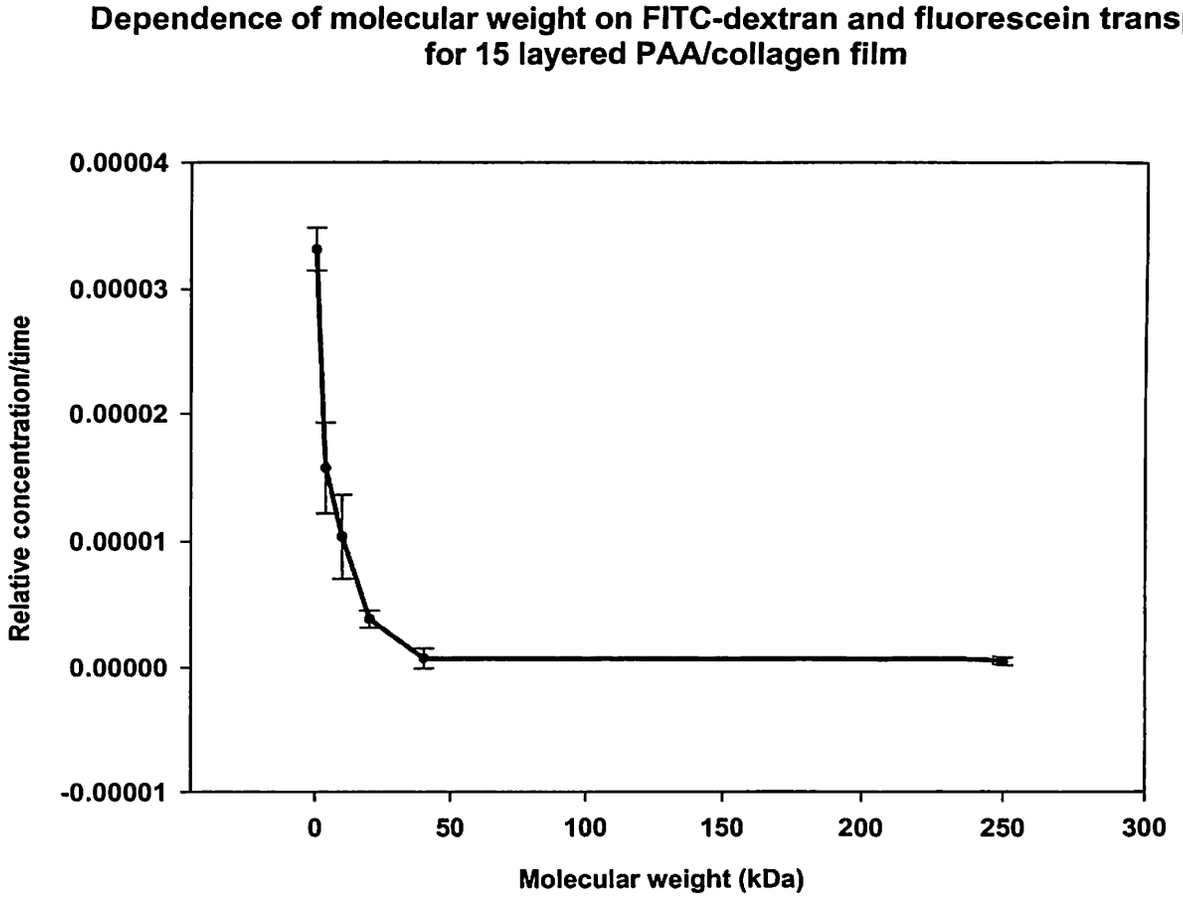


Figure 18. Plot of molecular weights of FITC-dextran and fluorescein against relative concentration/time for 15 bilayers of PAA/collagen film.

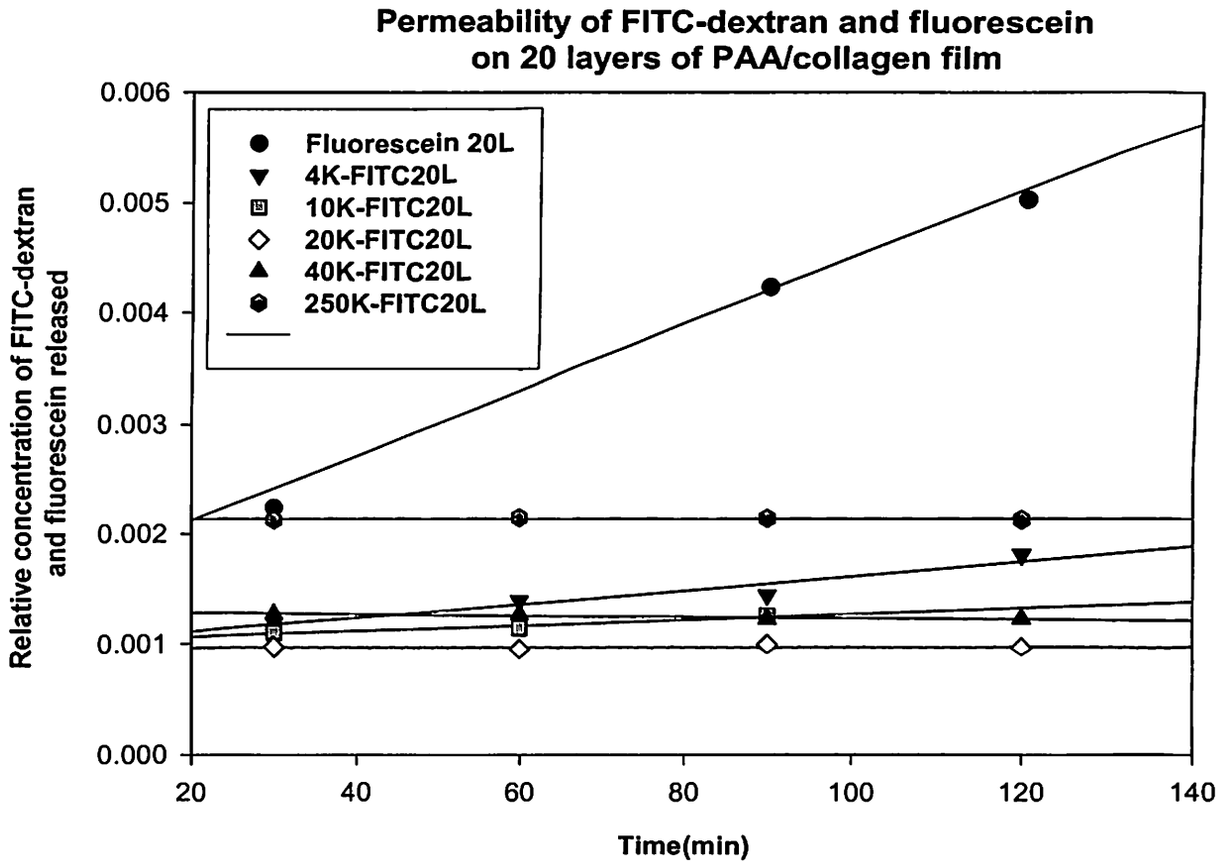


Figure 19. Plot of increase in relative concentration of FITC-dextran and fluorescein on the permeate side of the membrane with respect to time for 20 bilayers of PAA/collagen film.

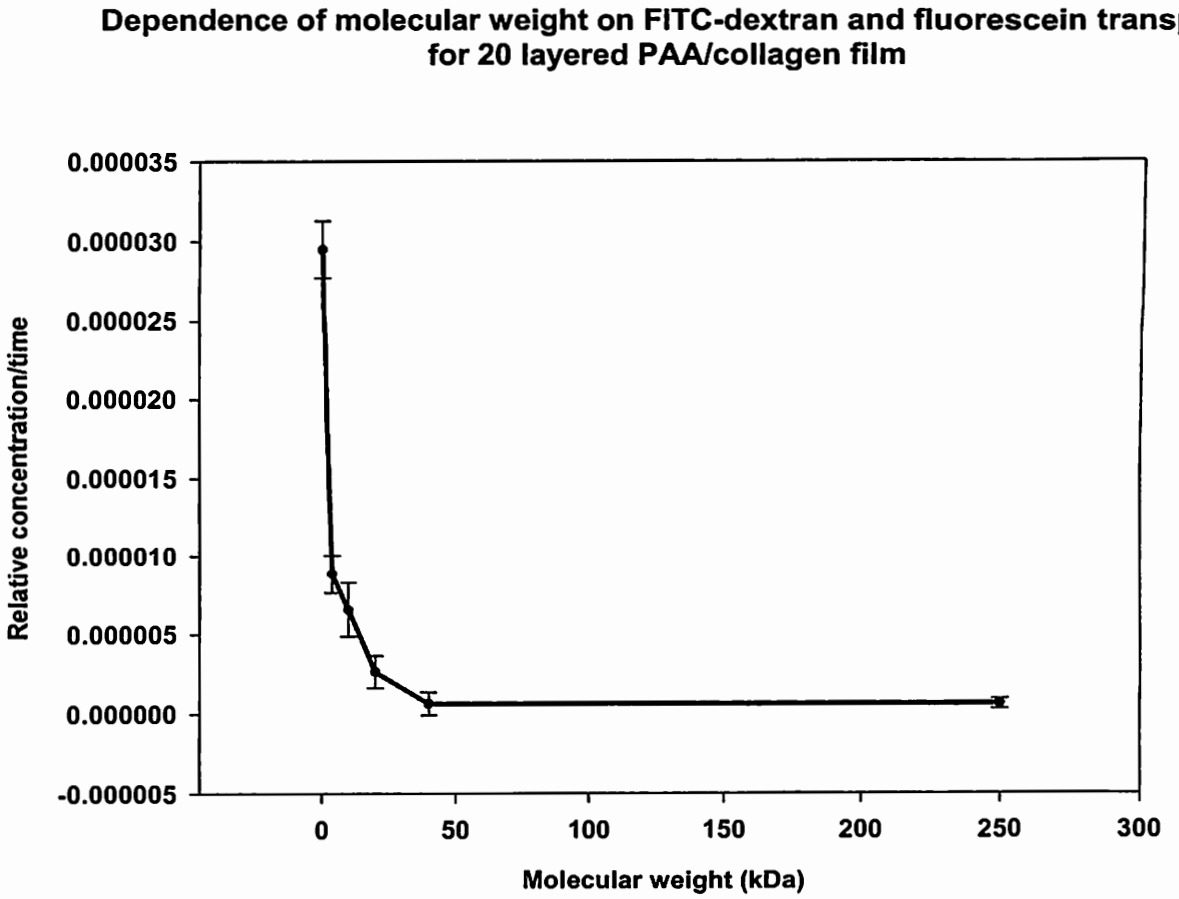


Figure 20. Plot of molecular weights of FITC-dextran and fluorescein against relative concentration/time for 20 bilayers of PAA/collagen film.

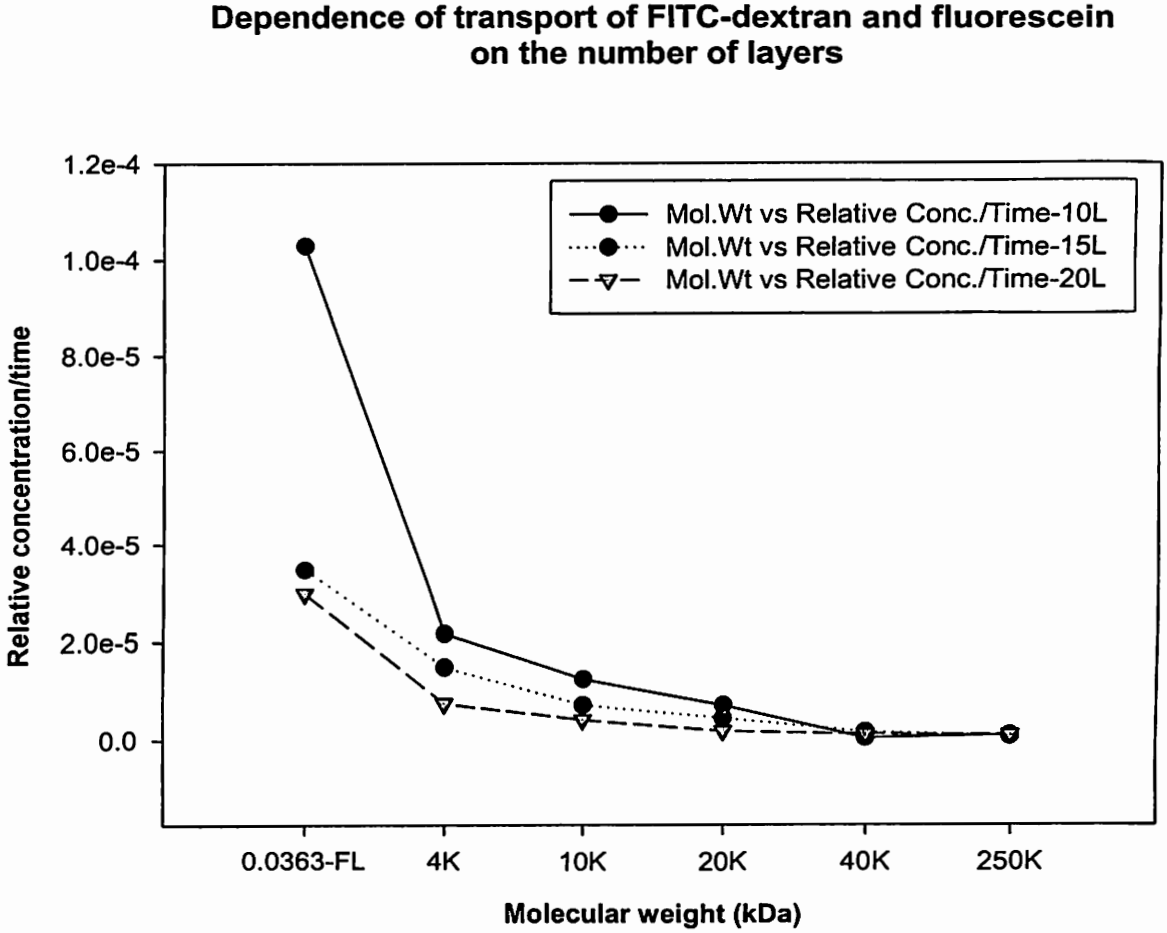
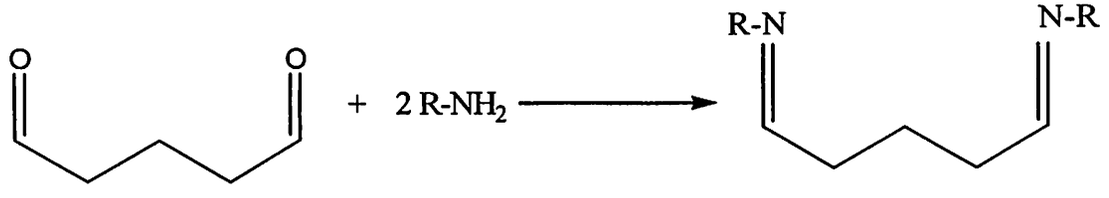


Figure 21. Plot of relative concentration/time with respect to the number of deposition bilayers.

transport of molecules is almost negligible with the molecular weights of FITC-dextran above 20 kDa.

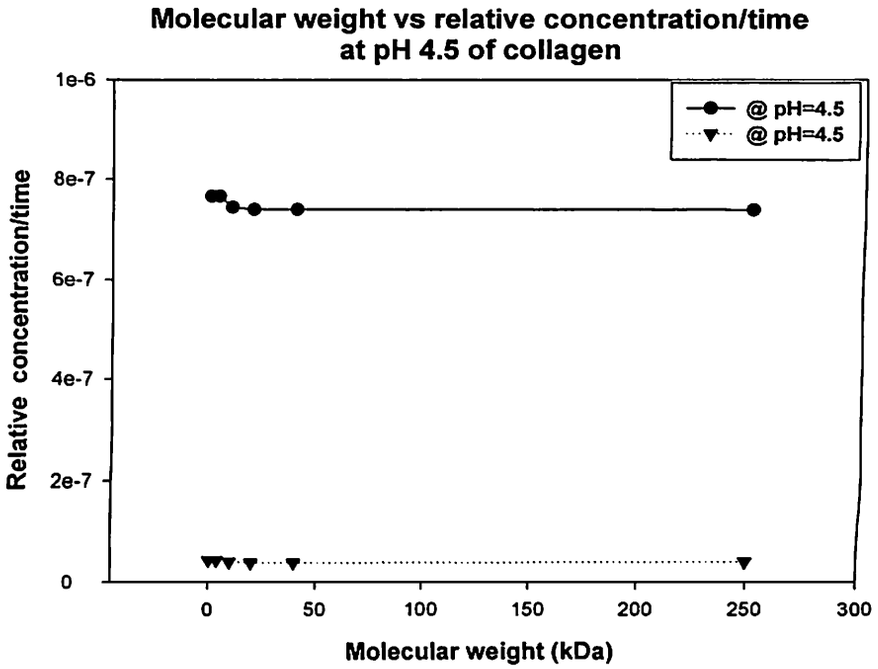
When observing the transport of FITC-dextrans and fluorescein through these films at 30 minute intervals, the release of FITC-dextrans between two successive intervals may differ slightly or sometimes significantly from the previous amount. However, the average release rate of FITC-dextrans is almost fixed after four 30 minute intervals. This behavior can be attributed to the polymer swelling in wet conditions causing alternation of transport of FITC-dextrans. In addition to the composition, the transport is also controlled by the extent of cross-linking between the polymeric chains, which tends to reduce molecular mobility. The exact nature and the extent of cross linking in collagen based LBL thin films is not available in literature. Collagen consists of a sequence of amino acids and cross linking with glutaraldehyde (Equation 1) may occur between different amino acids in a random manner.



(1)

During optimization of the LBL process, experiments were performed under different conditions. The permeability measurements for the films prepared from a PAA solution at pH 4.0 but with varying collagen solution pH at 4.5 and 4.1 gave very different permeability results [Figure 22 (A) and 22 (B)]. Much lower or negligible permeability was observed at higher collagen pH 4.5 and the results obtained were

(A)



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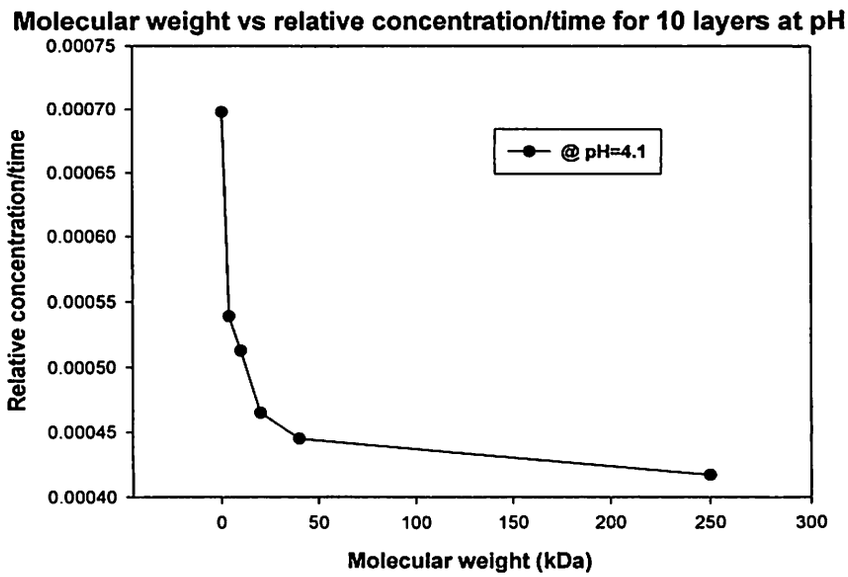

Figure 22. (A) Effect of molecular weight on the permeability at the pH 4.5 of collagen solution.

(B) Effect of molecular weight on the permeability at the pH 4.1 of collagen solution.

inconsistent. This behavior might be due to the presence of irregular and dense film structure at higher pH and increased pore size at low pH. It may be concluded that at higher pH of collagen solution, there is no control on the film morphology.

Although transport of these molecules at lower pH of collagen is hindered to an extent with the increase in molecular weight of FITC-dextran, there is significant permeability for higher molecular weights above 20 kDa. This increased permeability might be due to the increased pore size at lower pH. Membrane selectivity must be high in filtration processes at the molecular scale. Therefore extreme precaution is necessary in developing these films in order to produce optimum results. With slight changes in pH, transport of the molecules changes drastically.

The films were crosslinked by exposing the vapors of 8% of glutaraldehyde to increase their mechanical strength. However, glutaraldehyde is a toxic compound and exposure to reduced concentrations of glutaraldehyde is preferable in dealing with biological membranes during crosslinking. Permeability through the films that were crosslinked (as described in the experimental section) with 50 ml of 8% and 1% glutaraldehyde was measured. Figure 23 shows the effect of permeability with glutaraldehyde concentration. From the results, permeability was found to be almost same for both the films suggesting the fact that maximum crosslinking occurred even with 1% glutaraldehyde solution. These results are in agreement with the experimental findings on the crosslinked collagen films [119].

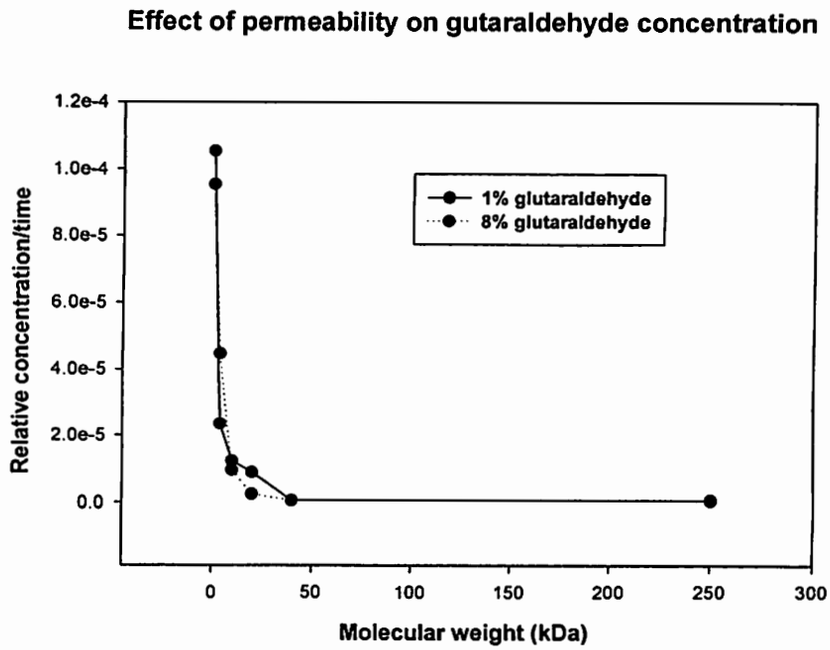

Figure 23. Effect of permeability on glutaraldehyde concentration.

DISCUSSION

The performance of membrane systems is determined by their transport processes. These influence the three independent stages of transport that involve: (a) convective and diffusive flows on the feed side of the membrane; (b) permeation of the material through these membranes; (c) transfer of material into the permeate stream. The first two stages are important because the resistance associated with transfer into the permeate stream is negligible. The separation achieved by these membrane techniques is solvent-solute separations (concentration and purification). It is an important step consisting of concentration of solutions or of purification by removing salts and small molecules (diafiltration).

The main objective is to retain particles, cells or proteins and to let smaller molecules through. Since molecular weight cutoff values relate the pore dimensions of the membrane to the size of the macromolecular solutes in the solution, some factors need to be considered are a) the deformation of polymer chains induced by shear forces, b) concentration polarization, and c) molecular weight distribution. The process selectivity depends upon the porous film structure, on the chemical and physical properties of the membrane materials, which in turn control membrane-macromolecule interactions.

Most of the mathematical models that represent the membrane transport assume the pores to be straight, all of the same diameter and length. (homoporous membrane). It was reported that diffusion causes convection too. Convective flow can have many

causes. It may occur because of pressure gradient or temperature differences. Even in isothermal and isobaric systems, diffusion will always produce convection [120]. This was clearly stated by Maxwell more than 100 years ago: "Mass transfer is due partly to the motion of translation and partly to that of agitation" [121].

The description of transport of macromolecules through these thin films was considered by both diffusive and convective transport. This combination of convection and diffusion complicates the analysis of mass transport in the LBL thin films. A precise determination of this flow would be extremely difficult since the flow depends on the internal structure, size and shape of the pores, path of flow (mostly tortuous) which is not well defined and also on the type of solvent-solute systems employed. Most flow experiments assume that the pores of these membranes were straight and not interfered by other external forces which are not true in multilayer thin films by LBL assembly. The configuration of the pores (cylindrical) used in simple model description deviates from the actual morphology. Nevertheless, a combination of well defined characterization techniques may give information about membrane morphology and thus explain the transport behavior with more details.

When a molecule travels through a pore, non specific interactions are most probable together with Van der Waal's forces. If the interactions between membrane and macromolecules are attractive, macromolecules adsorb on the pore walls and remain attached to the pore walls. This may lead to narrowing pore size. For this reason, attractive forces can provide selective way of separating molecules only until the interacting sites are saturated. On the other hand, repulsive interactions play a role when both the walls and the macromolecules surface bear same charge [122]. In practice,

proteins and other macromolecules may adsorb on the surface of the membrane, even *if* their global charges are opposite because other forces than electrostatic attractions lead to adsorption, such as hydrogen bonding and hydrophobic interactions.

Due to the nature of LBL films, each layer has its own intrinsic properties which depend on charge, so that internal pores would have alternating positive and negative charge or randomly varying charge. This means the entering macromolecules may undergo different types of interactions when passing through these films.

(i) Retention behavior of macromolecules

In daily applications, polymer chemists or biochemists use the term molar mass (molecular weight) when talking about the size of the macromolecules. In our transport mechanism of macromolecules we consider that the macromolecules have a spherical symmetry [122]. Therefore their volume is proportional to the cube of their characteristic size (equivalent radius), noted a. Assuming that the molecules from a single family such as dextrans have same density ρ, one can write an expression for the molar mass M (gram/mol or Dalton):

$$M = \rho N (4/3) \pi a^3 \qquad (1)$$

Where N is mass flux (diffusive or convective) of molecules through a pore (Kg/m^3).
The exponent 3 is a theoretical one. In practice, Granath and Kwist obtained the following empirical relationships for dextrans [123];

$$M = \rho N (4/3) \pi a^{2.17} \quad (2)$$

For the sake of simplicity, we keep exponent of 3 since it is close to the one found for proteins [124].

The molar mass of the spherical molecule having the radius r, that is the same as the average pore radius of the membrane is,

$$M_{CO} = \rho N (4/3) \pi r^3 \quad (3)$$

M_{CO} represents theoretical molecular weight cutoff (MWCO) of the membrane.

Since the thin film act as a separating device, it is expected that some materials will be rejected by the membrane and other materials transmitted. Assuming that the flow through pores is not disturbed by the presence of particles, a simplified formula for the fractional retention of the molecules R is given by,

$$R = [1-(1-\lambda)^2]^2 \quad (4)$$

λ is the reduced molecule radius which is equal to the molecule radius divided by the pore radius (a/r).

Equation (4), can also be expressed as,

$$R = [1-\{1-(a/r)^2\}]^2 \quad (5)$$

Substituting for r and a in equation (5), we obtain fractional retention of the molecules as,

$$R = [1-\{1-(M/M_{CO})^{1/3}\}^2]^2 \qquad (6)$$

The above retention equation would be the most suitable way of describing transport of macromolecules through multilayers in terms of their molecular size or molar mass (weight) [122]. The molecular weight cutoff for the membrane is 20 kDa. The retention coefficients of the membrane for fluorescein and FITC-dextrans below 20 kDa are calculated by using equation (6) and are tabulated (Table 1). As for example, from the Table 1, a 4 kDa FITC-dextran should be retained at 68% by 20 kDa MWCO membrane.

(ii) Applicability of results in kidney dialysis and tissue engineering

One of the important applications of these films is developing them as platforms for basement membranes of kidney and also as dialysis membranes in artificial dialyzers. The experimental results indicate that these membranes could be used in modeling artificial organs in Tissue engineering. As mentioned before, Blood contains particles of many different sizes and types, including cells, proteins, dissolved ions, and organic waste products. Some of these particles, such as proteins like hemoglobin, are essential for the body. Others, such as urea (a waste product from protein metabolism), must be removed from the blood or they will accumulate and interfere with normal metabolic processes.

Compound	Molecular Weight (Daltons)	Retention Coefficient (R)
Fluorescein	376.3	0.2127
FITC-dextran	4,000	0.6849
FITC-dextran	10,000	0.9166
FITC-dextran	20,000	1.0

Table 1. Values of retention coefficients for PAA/collagen membrane according to size exclusion (Equation 6).

Urea and other waste materials have molecular weights less than 20 kDa and need to be filtered out [123]. Proteins in our blood have molecular weights predominantly above 50 kDa. Table 2 shows a list of the main proteins in the blood with their molecular weights [124]. All the above proteins in blood should be retained by the membranes during excretion of waste materials. The main disadvantage of artificially kidney model is that it can perform only excretion of waste materials by filtration but it can not maintain other metabolic and endocrinologic functions of the kidney. These functions cannot be replaced by an inanimate artificial kidney but requires the use of naturally evolved biological membranes [125]. It was reported that collagen membranes made by LBL assembly are biocompatible and allow the cells grow uniformly [126]. Experiments were performed to mimic these natural biological membranes with LBL assembled thin films. All of the permeability experiments were done with PBS as blank at the pH of 7.4, which is has similar pH to that of blood. In future, these membranes could be successfully employed as building materials for

a) Basement membranes in kidney

b) Diffusion membranes for dialysis

c) Supporting materials for tissue growth in modeling artificial organs such as liver, skin, kidney and heart.

With more research, it could be possible to shape these materials, seed with living cells. When cells grow, they multiply and fill the gaps in scaffold and grow into three dimensional structures. Once implanted into the body, the cells may become compatible with the cells inside body and perform their intended functions.

Protein	Molecular weight
Albumin	69,000
α_1 –Globulin	60,000
α_2 – Globulin	200,000
β – Globulin	100,000
γ – Globulin	150,000
Fibrinogen	390,000

Table 2. List of main proteins with their molecular weights in the blood.

CONCLUSIONS

Layer-by-layer assembled thin films with collagen and PAA are biocompatible and are mechanically stronger compared to those of films made with only natural polymers. The layer thickness and the transport of macromolecules can be tuned by changing the pH of the polymer solutions. Film growth is characteristic for a selected pair of polyelectrolytes. It depends on the matching between concentrations and pH and also on the other conditions of adsorption. The use of cellulose acetate as a substrate has avoided complicated slicing procedures and enabled the formation of free standing films with minimum mechanical damage. The challenges in the preparation of uniform biocompatible surfaces could be overcome by incorporating LBL assembled thin films.

The pore size, pore distribution and the effect of all types of interactions need to be well defined. For this reason, complete understanding of flow behavior inside these membranes still remains elusive. Unfortunately, biological materials tend to have batch to batch variations. This is an important limitation for these films when talking about reproducibility. In the case of charged solutes such as sodium chloride, transport properties depend on charge interactions between LBL membrane and the entering molecule with the other forces.

These films could be used as platforms for basic supporting membranes in modeling artificial organs by tissue engineering. Scientists must still Figure out the best materials for the scaffolds that shape the organs, determine exactly the right growth factors, and pick the right cells. Transport results through these films suggest that they

may have similar transport behavior as kidney membranes (basement membranes) and may also work as diffusion dialysis membranes.

It is worthy to note that all of these transport process are highly 'dictated' by the dexterity in making uniform LBL films with great accuracy in their architecture. Permeation of FITC-dextrans through collagen films can be regulated. Much of the interest in this work is related more with the applicability of these films rather than with the theoretical details of each process that is associated with these films. Layer-by-layer assembled biological thin films offer promising direction for the revolutionary future research in medicine and bio-related research.

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VITA /

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