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Application of polyelectrolyte layer-by-layer nano-assembly for surface modification, encapsulation and controlled release

Nikhil Anil Pargaonkar

NOTE TO USERS

APPLICATION OF POLYELECTROLYTE LAYER-BY-LAYER NANO-ASSEMBLY FOR SURFACE MODIFICATION, ENCAPSULATION AND CONTROLLED RELEASE

by

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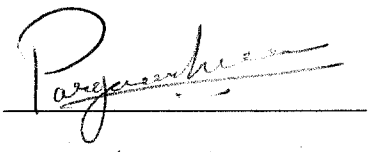
Author 

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ABSTRACT

In this study, we applied the traditional Electrostatic layer-by-layer (ELBL) assembly procedure to fabricate nanothin films over flat surfaces, and modify particle surfaces to influence the drug particle size, and drug release. The ELBL assembly has previously been applied to fabricate multilayer nano-scale thin films, but its ability to instantaneously influencing particle size is unique. Other unique observations such as influence on drug release as a result of polymer complexation, and thermal changes occurring during layer fabrication are recorded.

The ELBL self-assembly process was applied to produce dexamethasone particles layered with various polyelectrolyte layer combinations. These combinations were further applied to modify insulin (PROMAXX®) particles. The protein based PROMAXX® particles were primarily modified to impart stealth and controlled release properties. The nanothin shells were characterized by quartz crystal microbalance measurements for layer assembly and thickness, microelectrophoresis for surface charge, microcalorimetry for thermal activity of assembly process, confocal microscopy, and scanning electron microscopy for visual conformation of layer assembly.

In-vitro release profiles of dexamethasone nanocapsules suspended in water or carboxymethylcellulose gels were measured using vertical Franz-type diffusion cells in conjunction with U-V Spectrophotometer.

Sonication of a suspension of dexamethasone microcrystals in a solution of PDDA not only reduced aggregation but also influenced particle size. Assembly of multiple polyelectrolyte layers around these monodispersed cores produced a polyelectrolyte multilayer shell around the drug microcrystals allowing controlled release depending on the composition and the number of layers. Thus, direct surface modification of dexamethasone microcrystals via the ELBL process produced submicron particles with diffusion controlled sustained drug release through the polyelectrolyte multilayer shell.

An interesting observation was made with the assembly of polyelectrolyte layers around the insulin particles (PROMAXX®). Through the process of complexation each alternate fabricated layer strengthened or weakened the layer interactions with the drug surface leading to slower or faster release rates.

Preliminary testing of a new approach for layer and particle assembly on flat solid substrates using an electrohydrodynamic atomizer was successfully demonstrated. Pre-labeled sub micron drug particles appeared to be assembled over the flat substrate modified by alternate layers of polyelectrolytes.

This dissertation is dedicated to my sister, Meenal Pargaonkar

and my parents Anil Pargaonkar, and

Madhavi Pargaonkar

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CHAPTER 1

INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Introduction

In this research we explore the application of Electrostatic Layer by Layer (ELBL) assembly for modification of drug nanoparticles and other surfaces. This study is geared towards impacting classical drug modification and delivery systems routinely used in the pharmaceutical industry. The system under development in this project achieves three distinct goals. One is to modify the drug particle surfaces to stabilize the drug particles in solution. Secondly, influence the drug particle size and thirdly to achieve time delineated release of the drug. The results obtained will then be applied to other flat surfaces of interest.

ELBL assembly is a sequential adsorption of oppositely charged moieties to form self assembled monolayers on charged templates. It is one of the most powerful techniques to assemble polyelectrolyte multilayer thin films over an array of template materials [1-3]. In a seminal paper published in 1966 [3] Iler described the adsorption of colloids. Self assembly was rediscovered later and was adapted by various researchers for fabrication of thin films using charged polyions [4], dyes [5], nanoparticles, clay nanotemplates [6-8], and proteins [9, 10]. Over the years thin film technology has found substantial application in wide areas of research ranging from

disciplines such as chemical and electrical engineering to health care and pharmaceuticals. Application of ELBL for drug formulations is studied here.

The main areas of importance for any drug are bioavailability, viability and ability to reach its target site without undergoing undue modifications within the human organism prior to its action. Bioavailability of any drug is directly associated with the solubility of the drug in aqueous solutions. In spite of the fact that many hydrophobic drugs are highly effective therapeutic agents in vitro, they fail to prove effective in vivo systems simply due to there inability to dissolve. Furthermore, most hydrophobic drugs are limited to oral route of delivery. Another unique challenge faced by the pharmaceutical industry is that of patent expiration. From the business point of view, in 2004 alone the top 20 cancer drugs generated revenues to the tune of $27 billion US in markets around the world [38]. But once off patent, the revenues will be diluted by increased competition in a tight market share. Prodrugs seem to be the answer to obtain new patents on slightly altered drugs that have been pre-approved by FDA for human use.

Prodrugs are variations of the main drug compound that facilitates, or embarks new functionality to the old drug molecule. Prodrugs are said to maintain the therapeutic actions of its counterpart, yet are easily soluble in aqueous solutions. A prodrug could be a completely new molecule of drug produced by specific alterations done to the chemical structure such as Dexamethasone sodium phosphate [Figure 1.1b] or simple combination of two or more compounds such as chremophor solubalized Paclitaxel. However, chemical modifications of drugs more than

frequently result in complete loss or substantial decrease in the therapeutic activity of the drug. Therefore other alternatives that can modify the solubility and size of these drug particles without chemical alteration are much more attractive

Hydrophobic drugs modified with nano-coatings may be classified as prodrugs. However, there is a distinct difference between classical prodrugs and ELBL coated prodrugs. Drug particles layered by ELBL polymeric nano-coatings do not chemically alter the drug molecule. ELBL assembly simply exploits the electrostatic surface charge over the particle for adhesion of polymeric layers or in some cases forms a complex with the core. The interaction is almost instantaneous and the process seems to be perfectly scalable.

(a) (b)

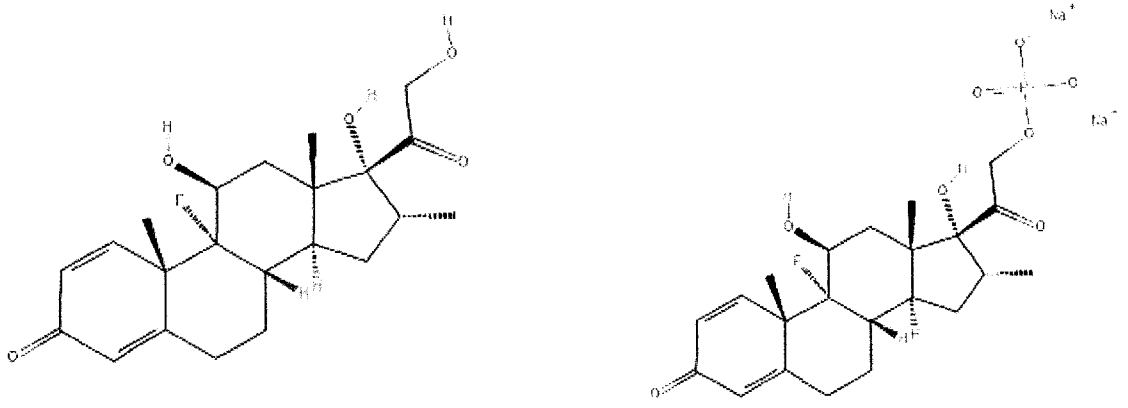


Figure 1.1 Structural formula of pure Dexamethasone (a), Prodrug Dexamethasone Sodium Phosphate (b)

Another direct application of electrostatic layer-by-layer assembly is controlled release (sustained and Quick). The effectiveness of any pharmaceutical treatment largely depends upon the drug reaching the disease site, remaining active until the condition is cured and then exiting the body with minimal side effects. Many drugs fail to reach the end user market for various reasons. The major reason however is the inability of the drug to reach a site specific target. Furthermore, uncontrolled dispersion of the drug in vivo requires relatively higher doses for effectiveness of therapy. Thus, controlled and targeted drug release are few of the major issues in taking a therapeutic agent from cell culture environment and applying it successfully in a human environment. This requires a sufficiently capable delivery system. It has become evident that nanobiotechnology offers promising solutions in overcoming common drug delivery failures. A wide array of techniques are utilized to improve drug delivery, but nanoparticles delivery systems are inarguably the most exiting form of nanobiotechnology currently on the market and in development. Multi-layer thin film fabrication is the first step in developing such a novel nano-capsular delivery system.

Thus ELBL offers a simplistic yet elegant answer to lot of problems faced by the pharmaceutical industry.

1.2 Research Objectives

1.2.1 Polymeric Layer Fabrication on Flat and Particle Surface Using LBL Process

A] Aim: Apply Electrostatic Layer by layer assembly technique to fabricate layers over flat and particle surface.

Task: It has been demonstrated through numerous research publication that ELBL assembly can be successfully applied to flat as well as spherical surfaces. Even though this is true, there are virtually thousands of combinations of various polymers one can test to achieve enhanced functionality of the fabricated structures. We demonstrate here the ability of the combination of selected polymers to form self assembled thin films over flat and spherical surfaces. We have two directional approach. One is to fabricate the layers over drug particle surface to influence release and second is to fabricate the layers over flat surfaces and complex the drug nanoparticles, using novel assembly techniques.

1.2.2 Influence of Various Polyelectrolytes on Drug Particle Size and Creating Viable Nanoparticle Substrates

A] Aim: Apply surface modification techniques to directly influence the drug particle size.

Tasks: Dexamethasone serves as a cell differentiation factor. The drug is hydrophobic in nature and sparingly soluble in aqueous solutions. Thus in its pure form the bioavailability of this drug is very low. Surface modification technique by ELBL self assembly over particle surface not only influences the particle size but

also the colloidal stability and bioavailability of the drug. Surface modification serves as a one step process to produce drug crystal nanotemplates that can be further modified for added functionalities.

B] Aim: To create drug nanoparticles – TIO_2 nanoparticulate complex to protect various light sensitive drugs from direct exposure to light.

Task: Many drugs are sensitive to light degradation and meticulous storage conditions have to be maintained to preserve therapeutic activity. Such intricate storage conditions are impossible in field operations. Nano-protective coatings may be the solution. We demonstrate that the drug nanoparticles created by direct surface modification technique can be further complexsated with TIO_2 nanoparticles to create a light protective shield without modifying the drug properties.

C] Aim: Characterize drug nanoparticle templates using various characterization techniques.

Task: The drug nanoparticulate templates formed by direct surface modification techniques need to be characterized for there bio physical and chemical properties. Specific techniques such as SEM (Scanning Electron Microscopy), Confocal Microscopy with Photoluminescence and Electroluminescence for visualization of nanoparticulate template systems, Zeta potential to measure alteration of surface charge and UV-Vis Spectrophotometry to quantify and characterize drug release were implemented.

1.2.3 Applying Ideal Combinations of Polyelectrolytes to Insulin Particles (PROMAXX®)

A] Aim: Applying ideal combinations of polyelectrolytes to improve structural integrity (impart stealth properties) and improve release rates of the Insulin microparticles (PROMAXX®) obtained from Baxter. Assembly conditions under low temperature and low pH were tested out during this study.

Task: The polyelectrolyte combinations tested and characterized using various techniques described above, were applied to modify surface properties of the insulin particles (PROMAXX®).

1.3 Dissertation Overview

The second chapter starts with understanding interlinked dissolution and diffusion process, properties such as solubility, hydrophobicity and hydrophylicity and explores the possibilities of drug complexation and its effects on the rate of diffusion of the drug. We also explore the possibility of controlling the physical, chemical, biological properties and handling the biocompatibility issue of the drug nano capsules using ELBL assembly. Then we discuss the fundamentals of Electrostatic Layer by Layer assembly and characterization of thin films over flat and spherical surfaces. The techniques used in this study are, Zeta-plus photon correlation spectroscopy and microelectrophoresis for micro-particle surface charge, confocal microscopy, fluorescence microscopy, and Electron microscopy for visual conformation and U-V visible spectroscopy for quantitative analysis. We discuss the

theory of transmittance and absorbance, apply the Beer-Lambert law and describe the standard Franz-type Diffusion cells study for drug release characterization.

The third chapter describes the modified ELBL method that uses polymeric surface modification of drug particles to control particle size, surface characteristics, and drug release. The materials used in this method followed by the actual methods used for assembly and characterization of polymeric thin films.

The fourth chapter summarizes the results obtained from Quartz Crystal Microbalance to assess and optimize the polyelectrolyte ELBL assembly with chosen combinations and derive average monolayer thickness. Microcalorimetric measurement results obtained that help further our understanding of inter layer interactions. Surface modification studies for (PROMAXX) drug particles and Dexamethasone drug particles, characterized by zeta potential measurements to assess the surface charge and microscopic analysis to visualize the influence on drug particle size.

CHAPTER 2

LITERATURE REVIEW

2.1 Understanding Solubility, Hydrophobicity, and Hydrophylicity

2.1.1 Interlinked Dissolution and Diffusion Processes

The release of encapsulated dexamethasone involves two main processes. One, the bulk solution diffuses into the capsule to dissolve the drug crystals and two, the dissolved drug molecules diffuse out of the capsules. Since the encapsulated drug is hydrophobic in nature one can easily speculate that the dissolution of the crystal cores would proceed from the surface towards the center. Also, smaller crystals will dissolve faster than the larger ones.

Within the capsule are numerous unquoted dexamethasone particles. These appear to release faster within the capsule causing high concentration (close to saturation) of dexamethasone in the microenvironment. This leads to an increase in the osmotic pressure within the capsule and a large concentration gradient across the thin layer wall. The concentration gradient seems to be maintained until all the crystals within the capsule are completely exhausted. Microscopic evaluation of nano-shells around the crystals reveals swelling of layers that contain gelatin. This appears to be due to the increase in osmotic pressure within the shells accompanying

the dissolution of the core material. One can easily note that during this phase the capsular wall plays a role in drug permeability.

The core formation process seems to work differently in case of protein based cores. Proteins have three dimensional conformations known as secondary and tertiary structures. Unlike other rigid core substances proteins have the capability to bind with polymers within its three dimensional space rather than just over the surface. This brings us to the discussion of a new concept of complexation of nanoparticles in microcapsules. One could theorize that instead of encapsulation of one solid globular core, there could be complexes of drug particles and polyelectrolytes together forming the core. The illustration in Figure 4.18 on page 73 will help to explain the concept more clearly. It is believed that the release rate is dependent on the number of layers surrounding the particle and the composition of the layers that affects the diffusion coefficient. However, in case of the insulin particles (PROMAXX®) alternate layers increase and decrease the release rates. In case of dexamethasone particles a single monolayer of PDDA substantially increases the release than the bare drug itself or drug particles layered with additional layers. These observations can be explained by the concept of complexation.

The hydrophilic polymers surrounding and transversing through the middle of the core could serve as fast channels or network through which water molecules can freely traverse. Thus the very idea that the core dissolution starts from the surface towards the center may be challenged. Instead the core dissolution may briefly be localized on the surface until the water molecules find hydrophilic channels within

the core. Once water molecules find the channels the dissolution process may take place from outside and within. The results are discussed at length in chapter four.

2.1.2 Solubility and Rate of Dissolution

In order for a solvent to be able to dissolve in a solute, the solvent particles need to be able to separate the solute particles and occupy the intervening spaces. In case of molecular solids such as sugar the week bonds between the individual sucrose molecules need to be broken down and individual $C_{12}H_{22}O_{11}$ molecules released into solution. In case of ionic solids such as common salt ($NaCl_2$), the positive and negative ions are held together with strong force of attraction with particles of opposite charge. When one of these solids dissolves in a polar solvent such as water, individual ions are released in solution and become associated with polar solvent molecules. It takes energy to break the molecular bonds in solvents such as hydrogen bonds in water in order to insert the solute molecules in between. The energy required is provided by the splitting molecules of the solute. Without this energy solid substances cannot dissolve in liquids. Polar solvent molecules can effectively separate the molecules of other polar substances only and non–polar solvents can effectively separate the molecules of non-polar substances. Positive end of a solvent molecule approaches the negative end of a solute molecule and a force of attraction develops between the two molecules. The non polar molecules have no attraction for polar molecules and exert no force that can separate them. The solute molecule is pulled into solution when the force overcomes the attractive force between the solute molecule and its neighboring solute molecule.

In our experiments the dissolution data suggested that diffusion through the single layer of PDDA is five times faster than through PDDA / Gelatin B multilayer or if the uncoated drug crystals themselves. The results are discussed in details in section 4.4.1. Theoretical explanation can be derived from Fick's first law of diffusion.

According to Fick's first law of diffusion

Rate of dissolution = $\frac{D \cdot A \cdot (Cs - Cb)}{h}$

where D is the diffusion coefficient,

A the surface area,

Cs the solubility of the drug,

Cb the concentration of drug in the bulk solution,

and h the thickness of the stagnant layer.

Now, if the Cb is very high then the condition is referred to as sink condition and the equation can be reduced to:

Rate of dissolution = $\frac{D \cdot A \cdot Cs}{h}$

One can see that surface area and concentration of drug in bulk solution are directly proportional to the rate of solution and the thickness is inversely proportional. Single layer of PDDA applied by ELBL is approximately 1 nanometer thick layer. Thus, the combination of factors such as increased concentration of drug in bulk solution within the capsule, increased surface area, increased diffusion coefficient due to presence of water molecules within the layers, and reduced layer thickness may very well be the reason for increased release through the capsular wall.

2.1.3 Hydrophobicity and Hydrophylicity

Hydrophobic materials have no tendency to adsorb water and water tends to bead on the surface. The primary reason why hydrophobic substances are immiscible in water is because they lack the ability to form hydrogen bonds with water. On the other hand *hydrophilic* materials posses the ability to form hydrogen bonds with water and therefore are readily dissolve in polar solvents such as water.

This brings us back to the discussion about hydrophobic drug substances. Since majority of therapeutic chemicals are hydrophobic in nature, a method needs to be designed to increase bioavailability of these agents. One way to achieve this is to make salts forms of these drugs also called prodrugs as described earlier. Salts of weak acids and weak bases generally have much higher aqueous solubility than the free acid or base. Therefore, if the drug can be given as a salt the solubility can be increased and we should have improved dissolution. One example has been discussed before that is of Dexamethasone Sodium Phosphate. Even though some of the prodrugs have high dissolution rates and thus better bioavailability, there therapeutic actions may not be as potent as there non altered counterparts. Thus, this may not be the most desirable path to take. Furthermore, hydrophobic drug particles form large aggregates with each other to lower the surface tension in aqueous solution. This greatly limits the routes of drug administration and reduces the bioavailability of the drug. Surface modification of hydrophobic drug particles reduces the surface tension and improves dispersion in aqueous solutions without chemical alteration and is therefore more appealing process.

2.2 Altering Physical, Chemical, and Biological Properties

2.2.1 Physical Properties

Most hydrophobic drugs are administered in the form of oral tablets that are absorbed in the stomach or intestines via the lipid bilayer of cells and then routed through the lymphatic or the circulatory system to target sight. Subcutaneous depot injections made from reconstituted powdered drug is also a popular mode of drug administration. But intravenous administration of the drug is the most effective way for rapid drug dispersion. This mode cannot be used for hydrophobic drug administration. Human erythrocytes are around 7 μm in diameter; however erythrocytes are highly elastic and flexible and literally squeeze through some of the capillaries. Soon after administration insoluble drug particles more that 3-5 μm in size will simply obstruct the microcirculation leading to catastrophic effects. As mentioned earliear, hydrophobic drug particles tend to aggregate to form colloidal particles because this lowers the interfacial free energy (Surface Tension) and particle sizes can reach up to 40 to 50 microns in diameter sufficient to block even large arterioles and smaller arteries, adding the risk of localized ischemia in various regions and organs. Thus the main physical barrier to the use of hydrophobic drugs in IV mode is the particle size.

Modern milling equipment routinely used in the pharmaceutical industry can produce micron scale drug crystal powders. Further reduction in particle size has proven to be a challenge. NanoCrystal™, a technology developed by Elan Drug Delivery Inc. uses a technique called wet milling [Figure 2.1]. They claim to have

successfully milled hydrophobic drug to particles with average diameter size of 1000 nm (1 micron). Thus neither conventional milling, nor the non conventional wet milling has proven effective against breaking the sub-micron particle size barrier. Direct Surface modification process using polyelectrolyte LBL assembly seems to overcome this barrier. The average drug particle size obtained as a result of this method was less than 500 nm (0.5 micron) after 15 min of sonication in presence of oppositely charged polyelectrolyte.

As illustrated in Figure 2.1, there is a substantial increase in the surface area of drug particles as there size is reduced. According to Fick's first law of diffusion, rate of dissolution of a drug is directly proportional to the surface area, diffusion coefficient, and solubility of the drug or concentration of the drug in bulk solution. Thus one can easily speculate that twice the reduction in the average size of the drug particles from conventional or enhanced milling would at least double the rate of dissolution of these drugs.

Another aspect of physical properties that needs consideration here is the particle aggregation. Bare hydrophobic drug particles form large aggregates (average 40 to 50 microns in diameter). The surface modification technique not only helps reduce the particle size but also masks the hydrophobic surface of drug particles thus making it impossible for coated particles to aggregate. Furthermore, the charged surface prevents particles from settling down in suspensions making it relatively easy to reconstruct solutions in the field.

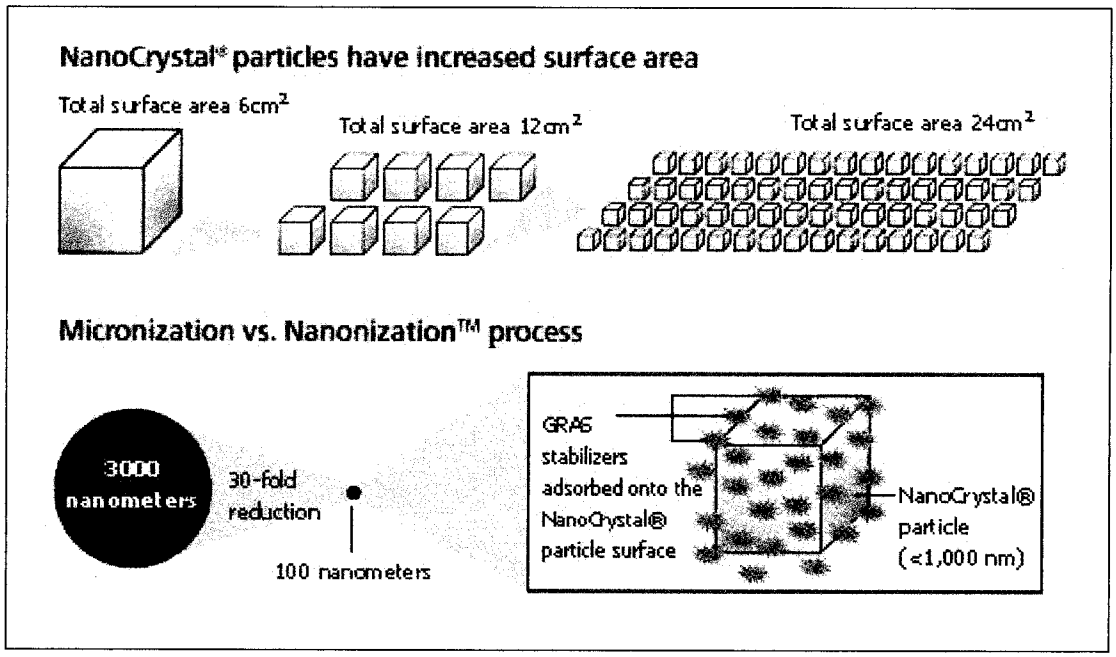


Figure 2.1 Schematic illustration showing increase in surface area as a result of decrease in particle size

2.2.2 Chemical Properties

Chemical modification of the drug may result in alteration of its action, loss of therapeutic properties or additional side effects. Furthermore, the altered drug might require lengthy FDA approvals and increase the development time and expense. An ideal Prodrug will have the original chemical structure of the mother chemical yet will have enhanced therapeutic functions with reduced side effects. We believe that direct surface modification helps to achieve these goals. Another important consideration in drug development is its shelf life. Many drugs are light sensitive and easily convert into inactive forms when exposed to light. Special light protective engineered nano-films over drug particles will allow these drugs to be stored in normal storage containers and increase the threshold of these drugs to handle light

exposure. One such example is drug nano-particles encapsulated by polyelectrolytes alternated with Tin Oxide nano-particles. Tin oxide nano particles are engineered to assemble between nanothin layers. These particles absorb all the exposed light and help protect the drug core. Furthermore, TiO_2 particles are inert and regularly used in many products that have been approved by the FDA as safe for human use. Initial step towards incorporation special nanoparticles in thin film coatings has been successfully demonstrated in this study [Figure 4.6 on page 57].

2.2.3 Biological Properties and Biocompatibility Issues

Hydrophobic drug delivery poses a peculiar problem in terms of biocompatibility. Approximately 40% of newly developed chemical drugs are poorly soluble in water and therefore are difficult or impossible to formulate. Furthermore, present delivery systems add to the side effects of drugs. Some hydrophobic drugs are formulated in cremophor. Cremophor is a mixture of castor oil and ethanol. Drugs such as Paclitaxel are soluble in cremophor. Once in a dissolved state and after cellular uptake these agents can efficiently perform their therapeutic action. Though bioavailability of Paclitaxel is increased due to its solubility in cremophor, it is extremely irritating to blood vessels and requires surgical placement of large catheter for administration. Cremophor may also cause hypersensitivity reactions. Thus, there is a dire need to develop alternate ways to deliver drugs such as Paclitaxel. Direct surface modification method comes to rescue.

Many of the substances that come under the FDA GRAS (Generally Recognized as Safe) list are polymers. Incorporation of charged polymers for Layer-by-Layer self assembly on drug surfaces is simple yet elegant process. Hydrophobic drug particles such as Paclitaxel drug particles carry a surface charge. Our approach then brings two distinct benefits to delivery of hydrophobic drug particles. One by eliminating the need of adjuvant substances such as cremophor and second by applying biocompatible polymers to assemble nano-layers on drug surface, masking the drug surface from immunological identification and attack. Steps have been taken to identify biocompatible polymer pairs that are best suited for this purpose. Self assembly method is an integrative approach that not only solves the drug particle size regulation and dissolution problem but also provides a simple yet elegant solution to drug delivery problem.

2.3 Fundamentals of ELBL Assembly

Electrostatic Layer by Layer assembly is a sequential adsorption of oppositely charged moieties to form self assembled monolayers on charged templates. Thin films from 5–500 nm thickness can be assembled using various linear or branched polyions and nanoparticles with step size of about 1 nm for each layer [23]. ELBL can be used to assemble layers virtually over any surface. The presence of charge is the most important prerequisite for ELBL assembly. The general procedure for assembly over flat surfaces begins with immersion of the clean substrate in a solution of cationic polyelectrolyte for a time optimized duration followed by rinsing and

drying. The surface is then repeatedly immersed sequentially in oppositely charged anionic and cationic polyelectrolyte solutions along with the intermediate rinsing and drying steps. Procedure is repeated until the desired numbers of layers are formed [Figure 2.2].

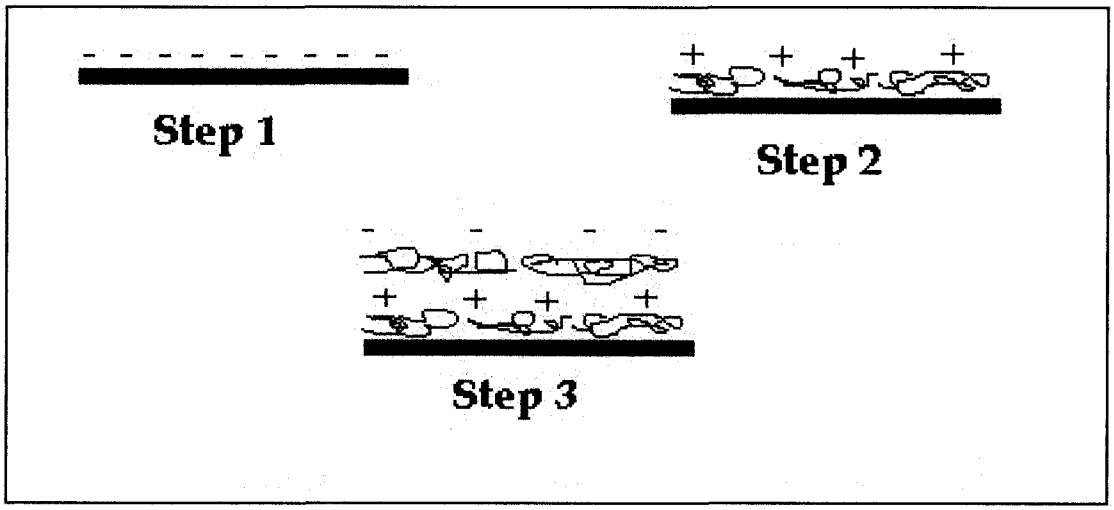


Figure 2.2 Schematic representation of the general procedure for the ELBL Assembly

A slightly modified technique for assembly over flat substrates was tested using an *ultrasonic nebulizer* (UN). The UN generates an aerosol from samples contained within the nebulizing chamber. The principal of aerosol generation using an UN is based on the sample being passed over a quartz plate of the piezo-electric transducer supplied by electrical energy of 1- 2 MHz frequency that causes the transducer to vibrate at a high frequency. The vibrations disperse the sample in to fine droplet aerosol that can be then transported by passing air through the aerosol. The aerosol generated from solutions containing the polyelectrolytes and drug

nanoparticles is sequentially passed over solid glass substrates and tested for presence of particles and layers on the surface.

The procedure for ELBL assembly over flat surfaces is slightly modified for assembly over spherical (particle) surfaces. Similar to the flat surface assembly, presence of charged surface is a prerequisite for particle assembly too. The charge over the spherical or particulate template particles is fist determined using a Zeta-plus photon correlation spectroscopy and microelectrophoresis instrument. The particles are then suspended in an oppositely charged polyelectrolyte solution and incubated for a time optimized period. Ultracentrifugation is used to separate the particles from the solution and the particles are rinsed using buffer solution to remove excess unassembled polyelectrolyte from the solution. The sequential incubation in oppositely charged polymeric solution followed by centrifugation and rinsing is repeated until the desired number of layers is formed over the template particle. After every adsorption, the surface charge is determined using the Zeta potential instrument. Sequential alteration of surface charge is an indication of successful assembly.

2.4 Thin Films Characterization Over Flat Surface

Any charged linear or branched polyelectrolyte and charged nanoparticles can be used for sequential assembly using ELBL procedure. The *QCM (Quartz Crystal Microbalance)* technique is virtually indispensable in establishing and optimizing proper assembly conditions.

The QCM is a well established method to measure small changes in mass and is based on the correlation of change of crystal mass due to the materials firmly attached to it and the oscillator frequency of the crystal [30]. An electronic circuit that produces a specific frequency output signal is called an oscillator. The amplifier within the oscillator receives back a part of its output via a feedback loop. To achieve oscillation, the signal passing from input to output and back to the input via the feedback loop should arrive at the input with no change in amplitude or phase. Frequency determining elements in the oscillators are the piezoelectric quartz crystals that couple mechanical movement to an electrical signal. The resonance frequency is determined by the mass of the crystal and the material constants of the quartz.

Thus if the crystal is loaded with mass that is external to the crystal, it is possible to determining the mass change by monitoring the frequency change of the crystal. Sauerbery equation gives the relationship between the changes in mass per unit area (Δm) and frequency (Δf) [Figure 2.3]. One has to know that this equation assumes that the combination of the adsorbed mass and the crystal is a rigid mass.

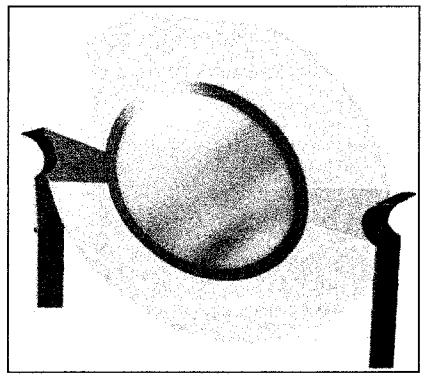


Figure 2.3 QCM Crystal

Sauerbrey equation: $\Delta f = -\Delta m d f^2/\rho q v q$
where:

Δf = change in frequency
Δmd = change in mass density (md, mass per surface area)
f^2 = frequency squared [9 MHZ]
ρq = density of quartz = 2650 kg m-3
vq = propagation of sound in quartz = 3340 m s-1
Diameter of the electrode used = 4 mm

Deriving from the above equation one can easily relate the increase in mass to a decrease in frequency. Thus the change in frequency is directly proportional to the mass change.

2.5 Thin Films Characterization over Spherical Surface

Polyelectrolyte layers can be characterized using QCM and then applied to spherical solid supports. These layers need to be further characterized to determine there presence over the solid spherical templates. One or more of the characterization techniques are used together to determine the presence of the layers.

2.5.1 Zeta-plus Photon Correlation Spectroscopy and Microelectrophoresis

Surface charge is an essential element of ELBL assembly. Particulate and macroscopic materials in liquid solutions acquire an electronic charge. Determination of surface charge of particles is possible using a *Zeta Potential Analyzer* [31]. It is vital to investigate if the results obtained from QCM study are reproducible on a non linear surface (spherical surface). Alteration of charge after each monolayer assembly indicates successful layer fabrication.

Surface charge is a useful indicator to predict the stability of a colloidal solution. The greater the surface charge, the more likely it is that the particles would repel one another and prevents aggregation. This is one of the most important characteristic that we exploit in this research. Hydrophobic drugs in aqueous solutions tend to form aggregates in order to reduce the interfacial free energy (Surface Tension) and particle sizes can reach up to 40 to 50 microns. Simple alteration of the surface with highly charged polyelectrolytes not only creates higher surface charges but also helps in normalizing the charge over extended areas of the surface.

2.5.2 Confocal Microscopy and Use of Fluorescence Techniques

Visual characterization of colloidal particles and the polyelectrolyte layers can be achieved using various microscopy techniques. *Confocal microscopy* [32] comes handy in this situation. Photoluminescence is the emission of light of a particular wavelength when stimulated by a different wavelength of light. This occurs when the incident light causes electrons contained in the specimen to enter into higher energy (exited states). When these electrons revert to their original energy states (ground state), the excess energy is released in the form of light. Fluorescence is characterized by the instantaneous cessation of light emission when the excitation light emission is interrupted. The special characteristics of a fluorescent material are that they should be able to absorb light to emit fluorescence, the wavelength of emission should be longer than that or the excitation wavelength, and the intensity of the fluorescence

should be far lower than that of the excitation light (10^{-3} to 10^{-5}). One of the main drawbacks of fluorescence is photo bleaching. Photo bleaching is fading of fluorescence that occurs over time. Samples need to be shielded from unnecessary exposure to light in order to preserve there fluorescence. The confocal microscope is unique, in that it has fluorescence and bright field functionalities built into one. Of course, a confocal microscope has added advantages over conventional fluorescence microscopy. The incident light in confocal microscope passes through a pinhole that is conjugate to the focal point of the lens thus illuminating only a small portion of the sample at a time. This not only reduces the background noise but also prevents sample in the rest of the field from photo-bleaching. Additional hardware such as an array of dichroic mirrors to filter lights of various wavelengths and special Acoustic Optical Deflectors (AOD) that can speed up sample scanning, existing powerful image capture and sample analysis software components, and very high resolution digital image capture devices make confocal microscope is a phenomenal imaging tool in research. Figure 2.4 is a schematic representation of working confocal microscope. The particles are layered with a fluorophore using partially labeled polyelectrolyte as the last layer. Partially labeled polyelectrolyte with FITC (fluorescein isothiocyanate) is one such example. The fluorophore emits visible light when exited with a particular wavelength (in this case 488 nm for FITC). Images are digitally captured and analyzed for size and shape.

Confocal microscopy technique is widely used in our study for characterization of particle and flat surfaces. The results from the study are discussed in detail in chapter four.

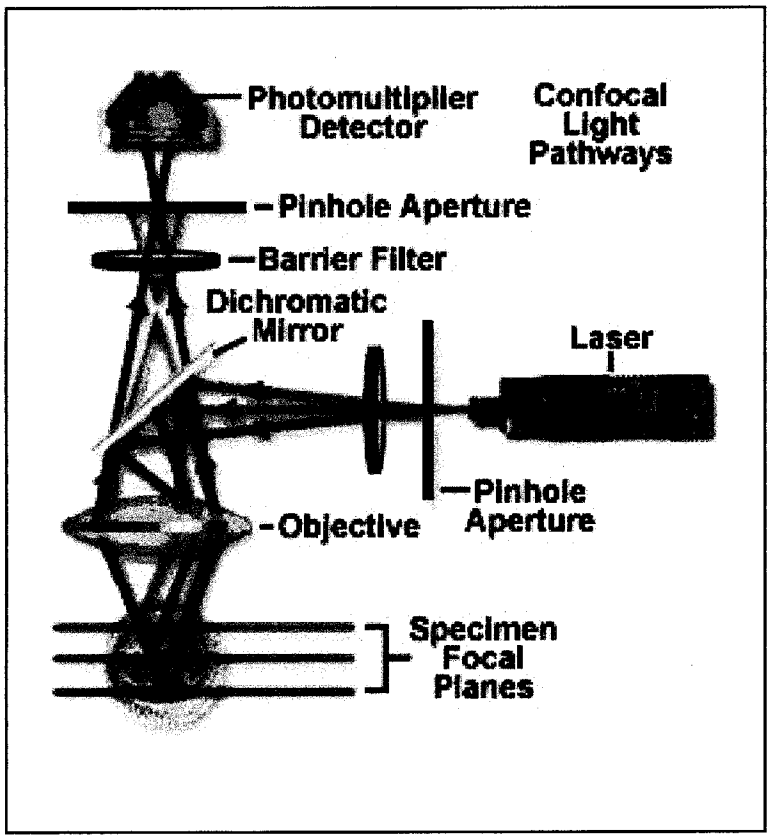


Figure 2.4 Schematic of confocal microscope [33]

2.5.3 Electron Microscopy

One step further in characterization of nanoparticles is the use of electron microscopy. Electron microscope has an ability to capture high resolution pictures in nano scale. Drug nano-crystal images captured at high resolution aid in confirming the reduction in size and successful production of nano-cores. Figure 4.5 indicates

reduction in particle size as a direct result of presence of appositely charged polyelectrolyte and sonication. Two main techniques are available at our disposal.

A) Scanning electron microscopy [SEM]

B) Transmission electron microscopy [TEM]

Transmission electron microscope [36] [Figure 2.5] is made up of an illuminating system consisting of electron gun with condenser lenses that generate and control the amount of radiation striking the specimen. A specimen manipulation system comprising of a specimen stage, holders and related hardware needed to orient the thin specimen outside and inside of the microscope. The imaging system that includes the objective, intermediate, and projector lenses required to focus on, magnify and form an image on the viewing screen or a camera. SEM vacuum system is required to prevent impediment to the flow of high energy electrons. Unlike in SEM the images formed by TEM are from the transmitted electrons and not from the deflected electrons. Due to this very reason the samples for TEM have to be very thin slices that can be easily penetrated by the electron beam. TEM is therefore primarily used for biological samples.

Scanning electron microscope [36] [Figure 2.6] comprises of a lens system that produces a small, focused spot of electrons that is further rastered over the specimen by scan deflection system. A specimen stage serves as a support to hold the specimen relative to the incident electron beam. Secondary electron detector [Everhart – Thornley secondary electron detector] collects secondary electrons emitting out of the samples and the signal generated by these electrons is processed

and displayed on viewing monitors or recorded by recording devices. A vacuum system is required for EM to work since air molecules will impede the passage of high energy electrons. In contrast to TEM that requires thin slices of the sample, SEM can be used to study three dimensional features of individual particles within the sample. Some SEM's permit sample insertions ranging from 3 to 12 inches and allow sample viewing with depth of several millimeters. Our selection of an SEM to view samples was obvious.

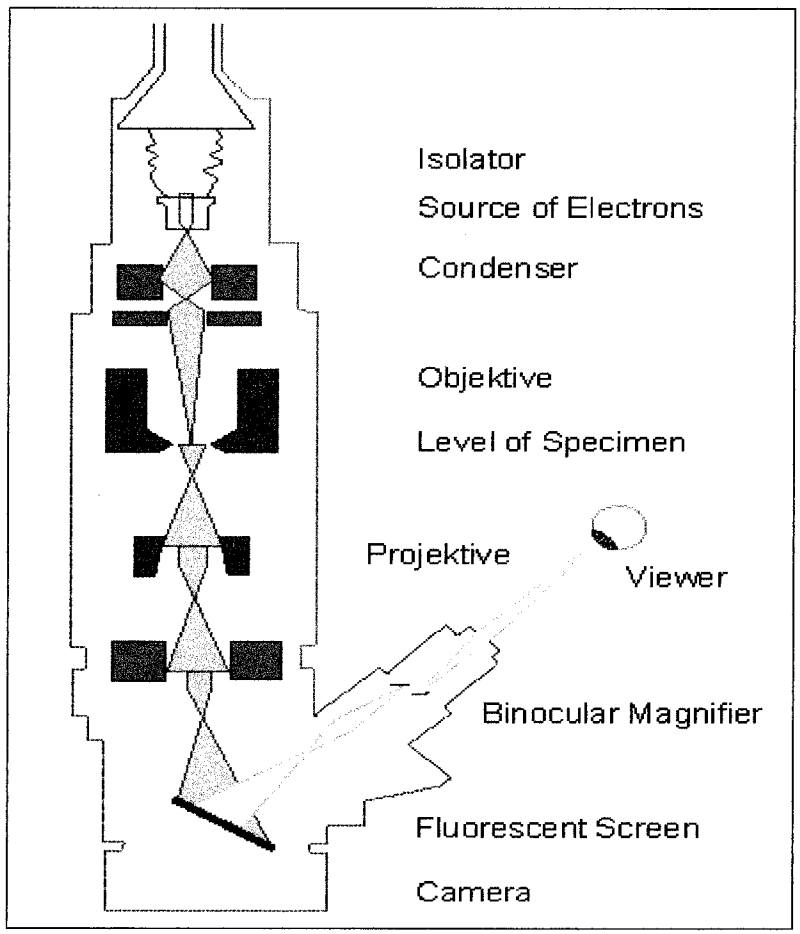


Figure 2.5 Schematic of transmission electron microscope [34]

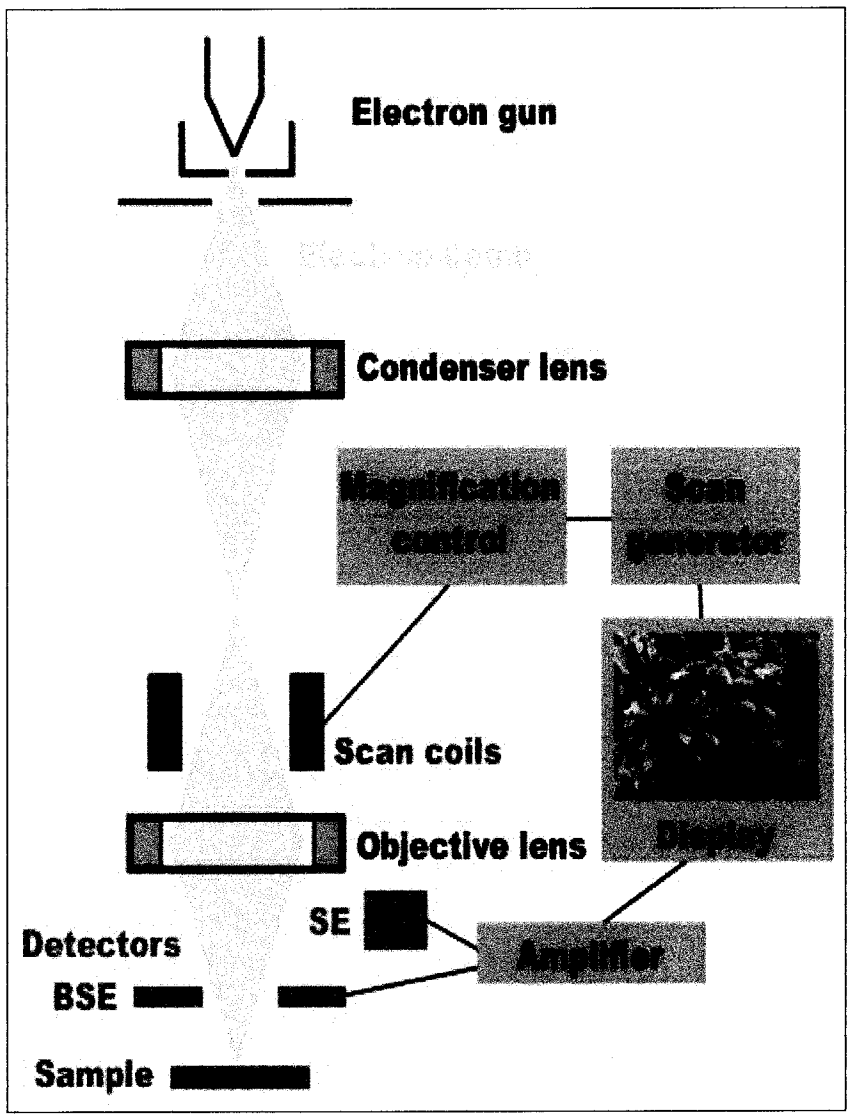


Figure 2.6 Schematic of scanning electron microscope [35]

2.6 Characterization of Drug Release

One of the objectives of this work was to influence the drug release from the modified drug particles. Quantification of drug release with respect to time is vital. This can be achieved by implementing the following methods.

2.6.1 U-V Visible Spectroscopy

Spectrophotometry measures the amount of radiant *energy* a substance absorbs as a function of wavelength. Pigments such as chlorophyll and other colored materials (i.e., dyes) absorb energy in the visible light range (380-760 nm) while other substances absorb at shorter wavelengths (i.e., ultraviolet = UV) or at longer wavelengths (infrared = IR). By measuring the absorption spectrum of a substance, i.e., all the wavelengths at which it absorbs, it is possible to identify it or at least place it in a particular class of compounds [Figure 2.7]. The absorption max or the peak absorption occurs at a particular wavelength. This is very useful when trying to identify an unknown substance. By creating and measuring a series of standards, it is possible to quantify the amount or concentration of a substance in a sample. We measured absorbance for our samples to measure the quantity of dissolved drug in solution as a function of time. This analysis allowed us to determine the effectiveness of thin films applied for quick and sustained release. The amount of dexamethasone in solution was determined by measuring the ultraviolet absorbance at 239 nm with a Multispec-1510 spectrophotometer (Shimadzu, Kyoto, Japan).

Predetermined wavelength is selected by adjusting a prism within the instrument to allow only a narrow range of wavelengths to pass through the sample.

Furthermore, a variety of light sources are available that emit light only in the blue range, or red and so on. For general-purpose work, a *broad range* bulb is used to measure absorbance over the entire visible light range. We will now discuss the *The theory of transmittance and absorbance:* Transmittance (T) = I / Io where T is the fraction of incident light that passes through the sample and Io equals the intensity of light that strikes the sample and I is the intensity of light passing out from the sample.

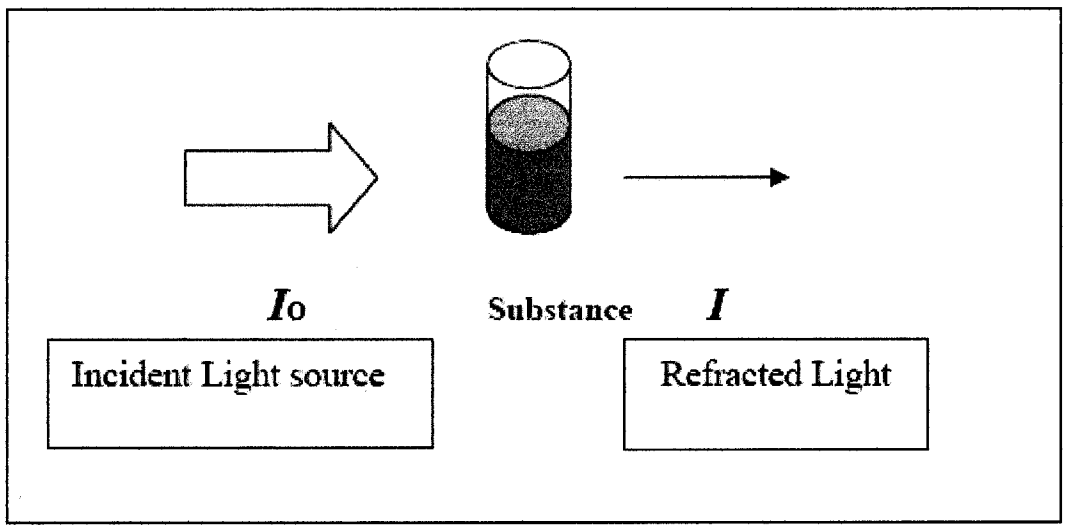


Figure 2.7 Transmittance and absorbance of light through a substance

Transmittance is usually expressed as a percentage:

$$\%T = (I/Io) \times 100$$

Absorbance (A), or optical density is a logarithmic function of T and is expressed as:

$$A = \log10\ (1/T) = \log10\ (Io/I)$$

Where, A= Absorbance

The Beer-Lambert law describes an important relationship between absorbance (A) and two sample parameters - solute concentration (c) and length of the light path (l) and is represented by the following equation:

$$A = \epsilon cl$$

From the above equation one can derive that absorbance, A, is directly proportional to c and l, where, ϵ is a constant, the absorbance coefficient, is a constant that depends on wavelength. In biological research, c is often expressed on a mass/volume basis, e.g., μg / mL, l in centimeters (usually 1 =1 cm), and ϵ in corresponding units. For practical purposes, the light path (l) is the interior length of the cuvette and is the same for all samples. Therefore, a plot of absorbance vs. concentration yields a straight line with slope μ. Such a curve using known concentrations of a pure substance is the standard curve for that sample. Figure 2.8 shows a simple schematic of a typical U/V Spectrometer. A standard curve is then useful for determining the concentration of the same substance in solutions of unknown concentration. By algebraic rearrangement of the above equation, it is clear that concentration can be determined from absorbance alone.

$$C = A / \epsilon l$$

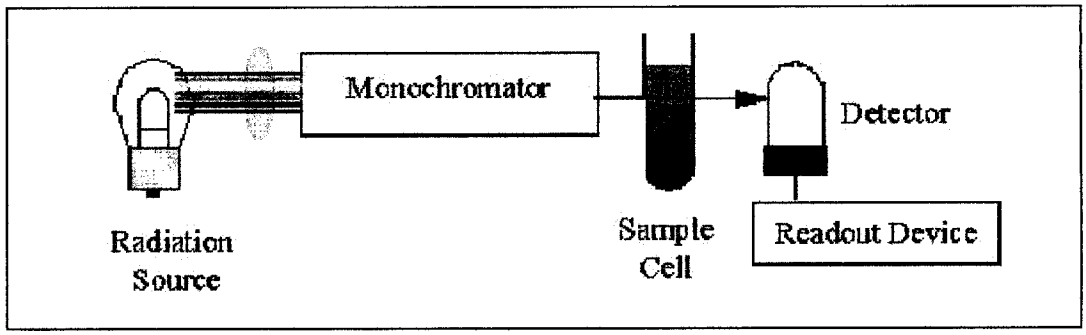


Figure 2.8 U/V Spectrometer schematic diagram

2.6.2 Franz-Type Diffusion Cells Study

In this study the release of dexamethasone from suspensions and gels (1 % carboxymethylcellulose aqueous gel) was measured using vertical Franz-type diffusion cells (Permegear Inc., Bethlehem, PA, USA) [Figure 2.9]. The system used in this study consisted of six cells each with a polyethylene sample ring with a 1 cm diameter hole at the centre, the same size as the opening in the vertical receptor cells, which is placed on top of the 0.2 μm cellulose acetate membranes (Osmonics Inc., USA) and then filled with the suspension or semisolid. The membrane with the sample was placed on top of the vertical receptor cell and clamped tightly into place. The receptor cells were filled with the dissolution medium (phosphate buffer pH 7.2) and a small magnetic stirrer placed in each cell was used for mixing. Samples were removed from the receptor cell at predetermined times, filtered, and suitably diluted and were prepared for U–V Spectrophotometric analysis.

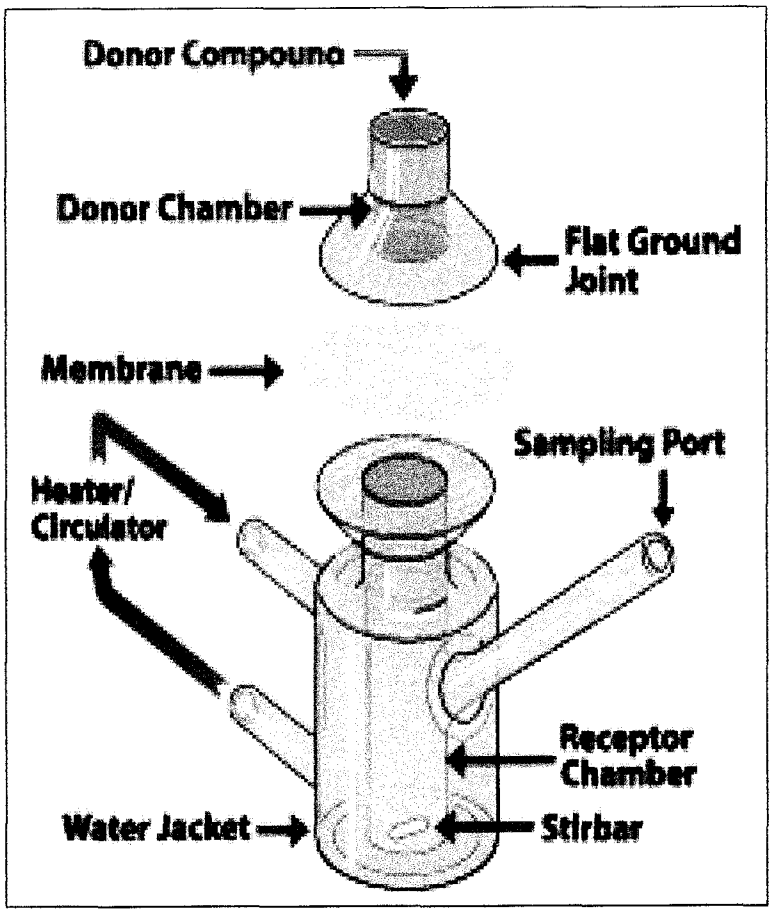


Figure 2.9 Schematic of Franz-Type diffusion cell

CHAPTER 3

MATERIALS AND METHODS FOR POLYMERIC SURFACE MODIFICATION

3.1 Introduction

We describe a new method to synthesize pure drug nano-cores and apply ELBL capability to assemble ultra-thin films over charged hydrophobic drug particles in order to influence the drug particle size and to alter drug release. We also extend our research findings to fabricate nanothin films over proprietary commercial insulin particles (PROMAXX®) with average particle size of 2 μm obtained from Baxter corporation to enhance there stability and release profiles.

In recent years ELBL self assembly has been explored for its ability to assemble ultra-thin films over template surfaces. Inorganic and organic colloidal particles can be coated using ELBL and then the core be decomposed to form hollow capsules with a fixed size and dimension [12]. The empty shells are then typically "loaded" using a consecration diffusion gradient process by simply incubating the empty capsules in supersaturated solutions. Loading thus becomes a very crucial process in the creation of these micro capsules. The approach is impractical due to various shortcomings. The dissolution of the core itself is a challenge since the thin films coated over the template seems to protect the template itself. Furthermore, it is

difficult to move the denatured core template particles out of the capsules through the multilayer thin walls. The loading process is pore size dependent and thus higher molecular weight compounds cannot be efficiently loaded into the capsules. The empty shells have no more solid support and tend to implode. The surface tension within the capsular wall is difficult to overcome and many capsules simply fail to load. It is relatively difficult to purify collapsed capsules. Therefore, developing a different approach for encapsulation was crucial and direct surface modification method helps overcome the shortcomings.

In spite of remarkable reduction of the release rate of the hydrophobic drugs as compared to uncoated drug particles, with some combinations of polyelectrolyte layers, one may still argue that the hydrophobic drug substances by virtue of there physical properties itself may disperse slowly in aqueous solutions. Therefore, reduction in release rates is an interesting find. We believe that quick release and faster dissolution of hydrophobic drugs in aqueous solutions would have substantial applications. Since solubility of the drugs is controlled by the size of the particles, the number of layers over the particles and the composition of the layers, we investigate each of these factors in this study.

Dexamethasone was chosen as an ideal drug substance. Dexamethasone is white, odorless, crystalline powder. It is stable in air and practically insoluble in aqueous solutions. Its insolubility is the main physical characteristic that makes it ideal for ELBL application. The molecular weight is 392.47.

It is designated chemically as 9-fluoro-11(beta), 17, 21-trihydroxy-16(alpha)-methylpregna-1, 4-diene-3, 20-Dione. The empirical formula is $C_{22}H_{29}FO_5$ and the structural formula is shown in Figure 3.1. Dexamethasone is a glucocorticoid that is clinically used for its anti-inflammatory and immunosuppressive effects and was chosen as a model drug because of its potential use in specialized delivery systems in treating peritumoral edema associated with brain tumors and for treating and preventing retinal diseases. A number of side effects, such as hypertension, hydroelectrolytic disorders, hyperglycemia, peptic ulcers, and glucosuria, which restricts the use of dexamethasone in conventional prolonged delivery systems, could be addressed by an alternative delivery system [20]. In addition, the advantageous properties of gelatin were further explored; the use of sonication in polyelectrolyte solution to produce monodispersed microcapsules is shown; and the use of heat of adsorption measurements to follow the ELBL assembling process is reported.

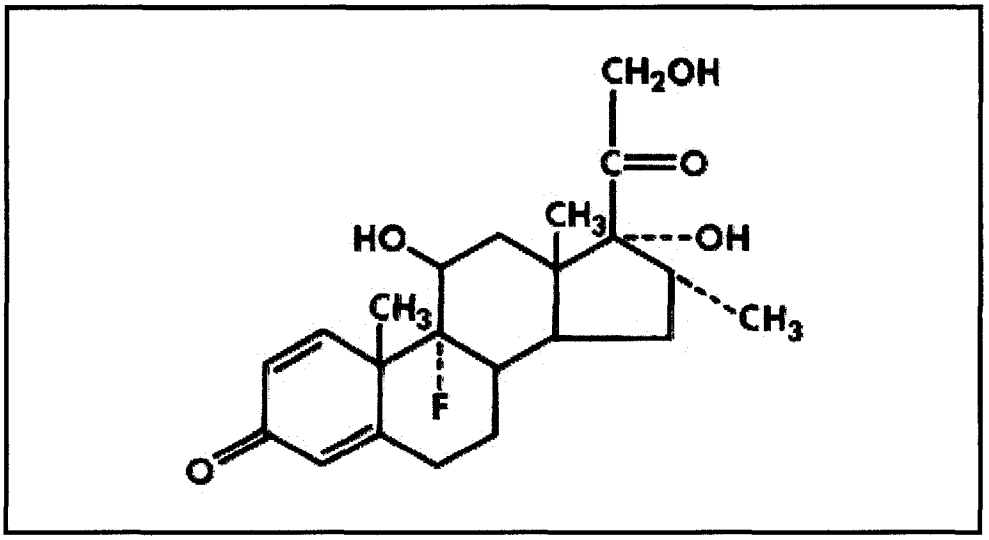


Figure 3.1 Structural formula of Dexamethasone

3.2 Materials

Samples of micronized dexamethasone USP (MW: 392.47) were obtained from Spectrum Chemicals (Gardena, CA, USA). Cationic poly(dimethyldiallyl ammonium chloride) (PDDA;MW 400,000; Sigma Aldrich, St. Louis, MO, USA), anionic sodium poly(styrenesulfonate) (PSS; MW 70,000), and the charged polypeptides, gelatin type A (acid pretreated or porcine gelatin; Bloom strength 300, MW 50,000 -100,000) and gelatin type B (alkali processed or bovine gelatin; Bloom strength 225, MW 50,000 - 100,000), were obtained from Sigma Aldrich. The solvents and dispersion mediums used in this study were 10 mM phosphate buffer pH 5.8 (PBS; Sigma Aldrich), purified deionized water, and 0.2M PBS buffer 7.2 [26]. The solvents and dispersion mediums in case of insulin particle (PROMAXX®) study was 16% PEG buffer. The polyelectrolytes used for this study were negatively charged polyelectrolytes: poly(styrene sulfonate) (PSS), poly(acrylic acid) (PAA), poly-*l*-glutamic acid, poly-*l*-aspartic acid, dextran sulfate, chondroitin sulfate, alginic acid positively charged polyelectrolytes: poly(allylamine hydrochloride) (PAH), polydimethyl diallylamonium chloride (PDDA), poly-*l*-lysine, poly-*l*-arginine. Enzymes: Protamine sulfate (positively charged). (Sigma Aldrich, St. Louis, MO, USA).

Fluorescein- 5-isothiocyanate (FITC; Sigma Aldrich) was used for labeling both uncoated and encapsulated dexamethasone microcrystals.

3.3 Methods Used

3.3.1 Method: QCM Study of LBL Assembly

The coating procedure was elaborated over QCM prior to polyelectrolyte multilayer formation on the dexamethasone microcrystals. Gold electrode resonators of 9-MHz quartz crystal microbalance (QCM; USI-Systems Inc., Kyoto, Japan) were used. The resonators were immersed in polyelectrolyte solution for a period of 15 min for each polyelectrolyte assembly, removed, and dried with air. The added mass and the coating thickness (ΔL) can be calculated from the frequency shift (ΔF), according to the Sauerbrey equation discussed earlier and using a special scaling [15]. For the instrument used in this study, the calibration was ΔL (nm) = 0.017 ΔF (Hz). This calibration is experimentally obtained. In case of insulin particles (PROMAXX®) all natural polyelectrolyte solutions at 1 mg/mL concentration in 16% PEG. The time of deposition was 1 hour. The temperature of assembly was maintained at +2 ^{0}C. Intermediate washings were done with DI water. The films were dried in the stream of nitrogen. Frequency of each resonator was measured after drying Reduction of frequency was observed in all the combination of polyelectrolytes. The combinations of polyelectrolytes that yielded the most stable step growth were chosen for assembly over the micro particles. All conditions of assembly were optimized and used for ELBL shell assembly on microcrystals. Two different series of precursor layers were used for QCM experiments. $(PSS/PAH)_2$/PSS layer and (PAH/PSS)n, (n=3,4) layers. Precursor layers are required

since at the beginning of the assembly process one sees non linear film growth [22-24]. Precursor layers seem to even out the surface charge therefore aiding in further layer assembly.

3.3.2 Method: ELBL Assembly on Dexamethasone and Insulin (PROMAXX®) Particles

The primary objective of applying ELBL to micronized dexamethasone was to influence the particle size, colloidal stability and the release profile of the drug in aqueous solutions. Determination of surface charge is crucial for any solid template intended for use in ELBL. A dexamethasone suspension was prepared in PBS buffer pH 5.8 with a concentration of 2 mg/ml and the charge measured. Surface charge of Dexamethasone was highly negative at pH 5.8 in PBS buffer. Based on the negative charge on the suspended drug particles, 5–10 mg of micronized dexamethasone was suspended in 5 ml of PDDA solution (2 mg/ml in PBS buffer pH 5.8). PDDA carries a net positive charge at pH 5.8. The isoelectric point of PDDA is 12 [20]. This polyelectrolyte and drug mixture was then subjected to ultrasonication for a period of 15 min [Figure 3.2]. The sonication along with oppositely charged polyelectrolytes seems to have an influence on the resulting drug particle size. The suspension was transferred to 1.5 ml centrifuge tubes since 20 ml centrifugation tubes have a limit of 5000 RPM. At lower RPM's most of the nanonized drug particles remain in suspension and cannot be separated out from the solution. This is a good gross indicator of successful reduction in particle size. Using the 1.5 ml centrifuge tubes the suspension was subject centrifugation at 10,000 RPM for 5 min (model 5804R,

Eppendorf, Westbury, NY, USA). The separated drug particles were washed three times with PBS buffer.

The washing step is necessary to remove any unbound polyelectrolyte from the reaction mixture. It has been documented that 1-2 min of intermediate washing between the adsorption cycles aids in removal of approximately 10% of weekly attached unbound polyelectrolytes [21]. Unbound polyelectrolytes may interfere in further assembly by competing for the adhesion to the oppositely charged polyelectrolytes added later in the process to fabricate additional layers. This completes the first layer of polymer on the drug particles. Zeta potential measurements indicated reversal to positive charge due to PDDA masking the negative drug particle surface charge. The particles were then re-suspended in 5 ml of PSS solution (2 mg/ml at pH 5.8), stirred for 20 min to ensure coating, centrifuged, and then washed three times. At this pH, PSS carries a net negative charge because the isoelectric point of PSS is below 1.0. This completes the assembly of the first bi-layer. Gelatin layers seem to delay drug release [15]. Gelatin A and B were both used as layers for the sustained release samples. Gelatin was dissolved in the PBS pH 5.8 at a concentration of 2 mg/ml. Gelatin A has a very weak positive charge at pH 5.8 because the isoelectric point of gelatin A is approximately 7–9 while gelatin B has a net negative charge because the isoelectric point is approximately 4–5. Gelatin A was therefore coupled with negatively charged PSS and Gelatin B with PDDA. The sequential adsorption of charged polyelectrolytes over the drug surface was achieved by repeating the above assembly procedure. There was approximately 20–30% loss

of material during the entire procedure [Figure 3.2]. The loss of materials was approximated from measuring the amount of material released in solution during the release study discussed later. Thus the composition of layers studied were dexamethasone core/(PDDA/PSS)4/PDDA; dexamethasone core/(PDDA/ gelatin A)4/PDDA, dexamethasone core/(PDDA/gelatin B)4/ PDDA, and dexamethasone core/PDDA/(PSS/gelatin A)4/ (PSS/PDDA)1.

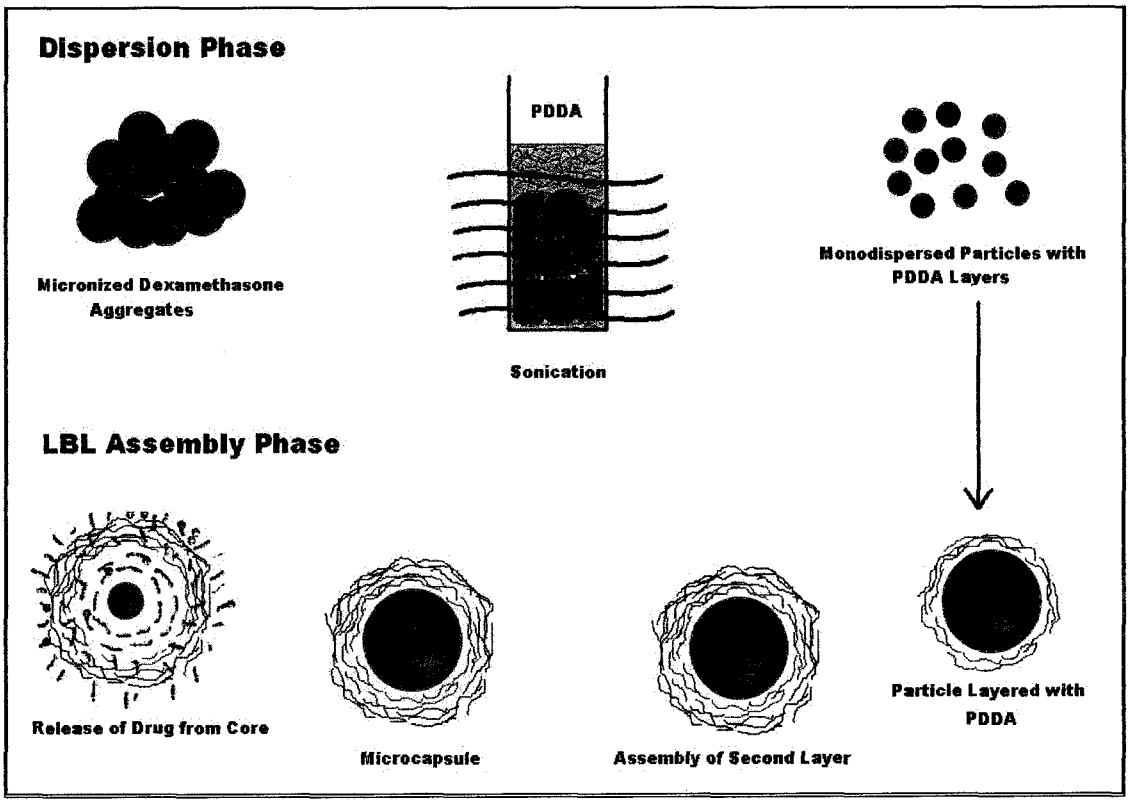


Figure 3.2 Schematic representation of direct surface modification using ELBL assembly technique (Developed at IfM within Dr. Lvov's laboratory)

The primary objective for applying ELBL to insulin particles (PROMAXX®) was to influence the stability of the particles and release of the drug in aqueous

solutions. Charge over insulin particles (PROMAXX®) particles in 16%PEG buffer was determined to be positive. Based on this evidence the polyelectrolyte used for the first layer would be a negatively charged polyelectrolyte. 0.2 mL of 3 mg/ml solution of the polyelectrolytes in 16% PEG was added to 2.0 ml of the insulin particles (PROMAXX®)

3.3.3 Method: Nanoparticle and Polyelectrolyte Layer Assembly Using Ultrasonic Nebulizer

The ultrasonic nebulizer was utilized to produce polyelectrolyte and nanoparticle aerosol. The flat substrates were brought in contact to the aerosol at 90^0 and parallel to the flow. The substrate was kept in continuous contact for a period of less than 1 min for each deposition. There were no intermediate washing steps. The nebulizing chamber was loaded with the appropriate polyelectrolyte and nanoparticle solutions for every alternate deposition. Prior to deposition, the nanoparticles were obtained by direct surface modification technique and were labeled FITC. Confocal micrograph of the solid glass substrate confirms the deposition of the nanometer ranged particles over the substrate. The microscopy results from this study are discussed at length in Chapter 4.

3.3.4 Method: Zeta Potential Measurements to Assess Particle Surface Charge

The reversal of surface charge is indicative of successful layer assembly. The zeta-potential of the suspended particles were measured and the reported results represent the mean of 10 measurements determined with a Zeta-plus photon

correlation spectroscopy and microelectrophoresis instrument (Brookhaven Instruments, Holtsville, NY, USA). 40 μL of Dexa drug sample was added to 1.5 ml of buffer solutions for immediate measurements. In case of insulin particles PROMAXX 40 μL of the particle solution was added to 2.0 ml of 16% PEG buffer for immediate measurements. The electrophoretic movement of the particles is related to the ζ- potential using the Smoluchowski equation [31].

3.3.5 Method: Particle Sizes and Morphology

Micronized Dexamethasone samples were suspended in PBS buffer at pH 7.4 containing (2 mg/ml) cationic polyelectrolyte PDDA. Another sample was prepared Acetate buffer at pH 4.0 with anionic PSS (2 mg/ml) and sonicated from 0.5 to 8 min (model 1510, 40 KHz; Branson Ultrasonics, Danbury, CT, USA). The second sample was to verify if the process would work either ways. Samples from these suspensions were added to a small volume stirred cell to obtain a desired obscuration, and then the geometric particle size was measured by Malvern laser light scattering (Malvern Mastersizer X; Malvern, UK) using a 100-mm Fourier transform lens. As an estimate for the size of the PDDA-coated dexamethasone particles filtered through a 1.2 μm filter, the intensity weighted mean diameter determined by photon correlation spectroscopy (PCS; Malvern Zetasizer 4, He-Ne-laser λ 633 nm) at 25°C under an angle of 90 degrees was measured. All samples were diluted with DI (deionized) particle-free water to an adequate scattering intensity prior to the measurement. A Philips XL 30 scanning electron microscope (Philips, Eindhoven, The Netherlands)

was used to obtain photomicrographs of the sonicated dexamethasone micronized particles. Samples were mounted on a metal stub with an adhesive and coated under vacuum with carbon (Emscope TB500 sputter-coater; Emscope Laboratories, Ashford, UK) before being coated with a thin gold-palladium film (Eiko Engineering Ion Coater IB-2; EIKO Engineering, Ibaraki, Japan). Confocal laser scanning microscopy (model DMI RE2; Leica, Allendale, NJ, USA) and fluorescent spectrometry (Photon Technology International, Lawrenceville, NJ, USA) were used to analyze optical properties and structures of encapsulated dexamethasone particles. For fluorescence, the particles were re-suspended in deionized water and 1 μl of FITC added; FITC attaches to PDDA. The fabricated particles were incubated overnight with FITC.

3.3.6 Method: Microcalorimetry

The heat involved in the formation of the layers at 25°C was measured with a micro differential scanning calorimeter (Micro DSC III; Setaram, Caluire, France) in isothermal mode using 1-ml batch mixing vessels. The suspended drug core particles were placed in the bottom of the mixing vessel while the polyelectrolyte solution was added to the top reservoir. Once the instrument was equilibrated at 25°C, the plunger was pushed down allowing the electrolyte solution to come in contact with the drug suspension. The heat measured, once corrected for the heat involved in mixing and the addition of solvent without electrolyte represents the heat involved in the attachment of the polyelectrolyte molecules to the core particle surface.

3.3.7 Method: Testing In Vitro Release

In this study, the release of dexamethasone from suspensions and gels (1% carboxymethylcellulose aqueous gel) was measured using vertical Franz-type diffusion cells (Permegear Inc., Bethlehem, PA, USA). The system used in this study consisted of six cells each with a polyethylene sample ring with a 1-cm-diameter hole at the center, the same size as the opening in the vertical receptor cells, which is placed on top of the 0.2-_m cellulose acetate membranes (Osmonics Inc., Minetonka, MN, USA) and then filled with the suspension or semisolid. The membrane with the sample was placed on top of the vertical receptor cell and clamped tightly into place. The receptor cells were filled with the dissolution medium (phosphate buffer pH 7.2), and a small magnetic stirrer placed in each cell was used for mixing. Samples were removed from the receptor cell at predetermined times, filtered, and suitably diluted. The amount of dexamethasone in solution was determined by measuring the ultraviolet absorbance at 239 nm with a Multispec-1510 spectrophotometer (Shimadzu, Kyoto, Japan). The UV-method was calibrated and complied with generally accepted specifications for linearity and precision.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 QCM ELBL Characterization

The QCM method was discusses earlier in Section 3.3.1. We summarize the QCM results here.

4.1.1 QCM Studies to Characterize ELBL Monolayer Thickness

It is relatively easy to estimate the average layer thickness using average frequency shift from QCM measurements [13, 18, and 27]. The first layer of positively charged polyelectrolyte PDDA was 147 Hz, and corresponds to a thickness (ΔL) of 2–3 nm. Table 4.1 below, lists the layer thickness calculated for some other ELBL assemblies implemented in this study. The PDDA/PSS layers were 4 times thinner than the PDDA/Gelatin layers. The PDDA/gelatin layer assembled with gelatin B was about 1.7 times as thick as gelatin B layers. PSS / gelatin-A layers were the thickest at 50–60 nm [13]. The measured shell thickness that was seen under the confocal microscope for the hydrated nano-shells indicated that the gelatin layers swelled significantly when suspended in water. Refer to Figure 4.2 and 4.10 on pages 61 and 62. In addition, previous studies have found that layer thickness estimated

from QCM measurements is half of the thickness on micro templates such as drug crystals [13, 18, and 27].

Table 4.1 Film thicknesses for layer-by-layer assembly on dexamethasone core surface calculated from frequency shifts measured with quartz crystal microbalance [ΔL (nm) = 0.017 ΔF (Hz)]

Layer	**Thickness (nm)**
PDDA	2-3
PDDA/PSS	4-6
PDDA/ Gelatin A	6-8
PDDA/ Gelatin B	11-12
PSS/ Gelatin A	10-15
$(PDDA/ PSS)_4$	10-14
$(PDDA/Gelatin A)_4$	25-35
$(PDDA/Gelatin B)_4$	45-55
$(PSS/ Gelatin A)_4$	50-60

4.1.2 Microcalorimetric Measurement to Characterize ELBL Assembly

Another interesting method utilized for characterization of ELBL assembly was the microcalorimetric measurement of heat of assembly. Dexamethasone particles were negatively charged and the interaction between dexamethasone particle surface and positively charged PDDA was an endothermic reaction requiring 21.4 mJ of energy. When layered with PSS as second layer the resulting reaction was an exothermic one with heat release of 5.5 mJ. However when simply added to the PDDA solution, PSS interaction produced only 3.1 mJ of heat. Additional layers of PDDA/ PSS layered onto the first bilayer produced consistent endothermic (PDDA, 2.7 mJ) and exothermic (PSS, 2.6 mJ) heats of adsorption. The heat of adsorption with the use of gelatin A and B as negative polymers instead of PSS produced 1.5 mJ and 1.9 mJ of heat far less as compared to that of PSS 5.5 mJ produced when layered with PDDA.

The heat produced by directly mixing gelatin and PSS solutions was 0.9 mJ for gelatin-A and 0.4 mJ for gelatin-B. Thus, heat of adsorption was higher for the layering process on the surface as compared to solution mixing and gelatin-A produced more heat than gelatin-B during solution mixing. This demonstrated that the positively charged gelatin-A more strongly interacted with PSS than gelatin-B, which is negatively charged at pH 5.8. However, alternating PSS with gelatin-A at pH 5.8 during the assembling process produced such small heat changes that it was not possible to measure it accurately with the microcalorimeter. Still constant and

repeatable changes in the heat of adsorption measurements were also strong evidence of the layering process. Figure 4.1 indicates the results obtained from the calorimetric measurements.

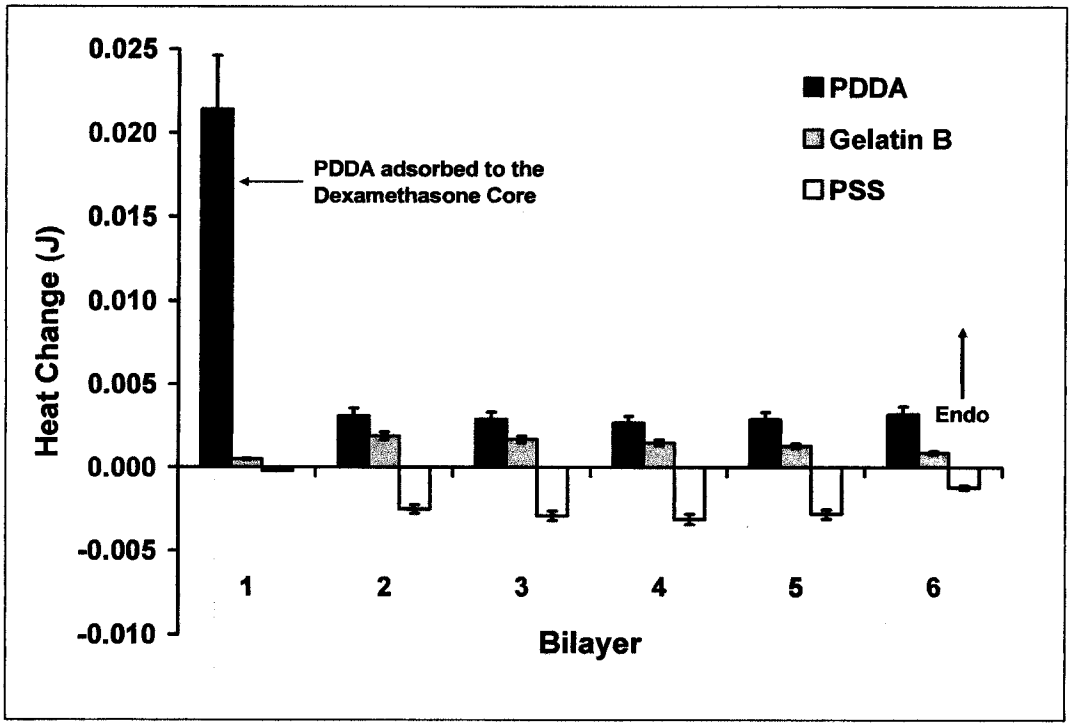


Figure 4.1 Changes in the heat of adsorption for the assembling of the polyelectrolyte bilayers. For each bilayer, the heat of adsorption for both the positive (PDDA) and negative ions (gelatin B and PSS) are shown. Mean ± SD, n = 4

4.1.3 Preliminary QCM Studies to Optimize Layer Conditions for (PROMAXX®) Particles

Preliminary QCM experiments were carried out to optimize the assembly conditions for insulin particles (PROMAXX®). Stabilization of the surface charge is essential for stable layer assembly. Therefore all the QCM resonators were coated

with several layers of PSS/ PAH as precursor bilayers [Figure 4.2]. Prior to our study, no attempt was made to assemble charged polyelectrolytes in presence of other inert polymers such as PEG (Poly Ethylene Glycol). Insulin particles (PROMAXX®) require 16% PEG concentration in buffer for stability. Therefore, 16% PEG – 0.7% NaCl buffer at pH 5.8, high polyelectrolyte concentration of 1mg/mL was used as assembly conditions for QCM studies. Various polyelectrolytes were tested with the above conditions [Figure 4.2]. The data shows steady growth of film with different polyelectrolyte combinations. One can clearly observe that the thickness of the layers is between 2 – 11 nm per bilayer, considerably more than layer deposited in DI water. Presence of PEG in buffer solution may be responsible for complexation of polyelectrolytes thus influencing deposition and layer thickness.

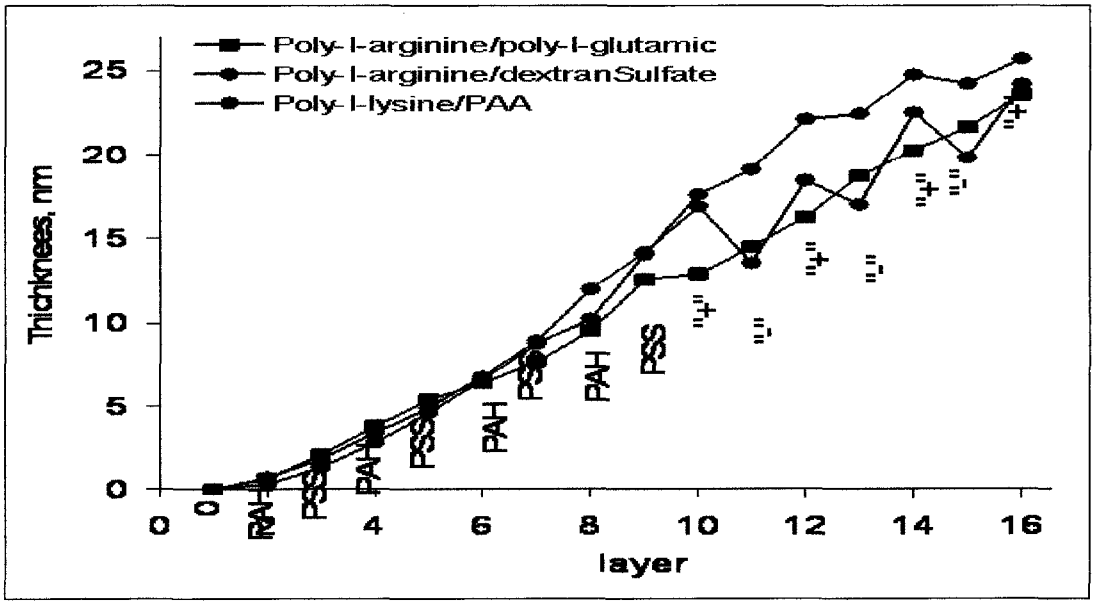


Figure 4.2 Step wise increase in layer thickness as a result of successful polyelectrolyte assembly over QCM

4.2 Application of ELBL for Surface Modification

ELBL was applied to drug particles for surface modification. The surface modification using ELBL was explored to achieve desirable surface properties, modulated release and influence particle size in some cases. The QCM results for ELBL were used as a model to apply polymeric multilayer assembly to drug particles. Successful assembly over insulin microparticles (PROMAXX®) was demonstrated with zeta potential analysis and confocal studies were conducted using partially labeled polyelectrolytes with FITC. The results were further expanded to modify dexamethasone drug particle surface. Here, application of ELBL was extended to influence the drug particle size. Various microscopy techniques were used along with the conventional biophysical analysis of thin film.

4.2.1 Surface Modification Studies for (PROMAXX®) Drug Particles

Insulin particles (PROMAXX®) have a high dissolution rate in aqueous mediums without the presence of 16% PEG buffer or lower temperatures. So one of the primary goals here was to make insulin particles (PROMAXX®) stable at lower temperatures and sustain release the drug. Figure 4.3 indicates the reversal of surface charge after deposition of a single layer of polyelectrolyte at 2°C. The surface charge over the Insulin particles (PROMAXX®) was determined to be positive at pH 5.8. We assembled negative polyelectrolytes PSS, PAA, ChS, DexS, Alginic acid, Poly-Glutamic acid, Poly- Aspartic acid as first layers.

Reversal of surface charge after deposition of the first layer was indicative of successful layer assembly.

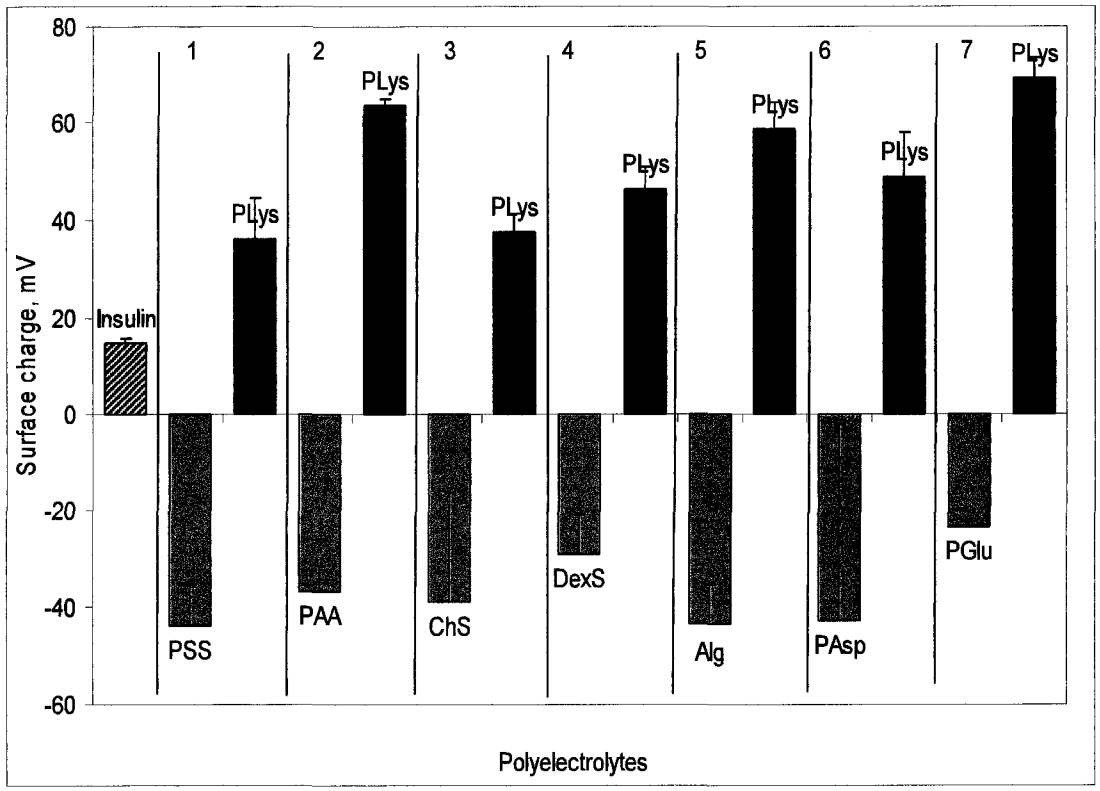


Figure 4.3 Surface charge of microparticles after deposition of different bilayers at pH 5.8

The second layer would naturally be a polycation. We assembled Poly-*l*-Lysine as the second layer. It is interesting to note that the surface charge over Insulin particles (PROMAXX®) coated with different positive polyelectrolytes show variable surface charge for the same negative polyelectrolyte, Poly-*l*-Lysine. One can thus speculate that previously deposited layers greatly influence proceeding layer. Polymeric materials form three dimensional conformations on surfaces. Different

polymers exhibit different conformations and thus the influence on assembly varies. Furthermore, the amount of the preceding polyelectrolyte deposited dictates the available charged ions for further assembly. To explore surface interactions further a calorimetric study for layer interaction was conducted. The results for that study are elaborated in another section of this text.

4.2.2 Surface Modification Studies for Dexamethasone Drug particles

The surface electrical potential (zeta potential) for the dexamethasone nano/microcrystals was measured for every step of the layering process [Figure. 4.4]. ζ-potential of −32 mV was recorded for uncoated dexamethasone drug particles suspended in PBS buffer. The first PDDA layer reversed the charge to +30 mV. The first PSS coating changed the charge to −50 mV. The reversal in change followed the same trend with additional layers and the magnitude of the change remained constant up to 3 complete bilayers [Figure. 4.4]. The surface ζ-potential of the fourth PSS layer declined from −50 mV at the first to −30 mV at the fourth layer.

Probably, at some areas PSS (MW = 70000) did not completely overcome the more bulky PDDA layer (MW = 400 000). This decline could limit total thickness of the shell after 4 bilayers of PDDA/PSS. When PDDA (+) was altered with the polyampholyte gelatin, the gelatin layers tended to have smaller ζ-potentials, averaging −15 mV for gelatin A and −22 mV for gelatin B. When gelatin A, predominantly positively charged at pH 5.8, was layered with PSS (−) the average zeta potential of the layers was +10 mV. The reversal in change and magnitude of the

change remained constant with additional layers up to 4 bilayers of PDDA/gelatins or gelatin A/PSS [Figure. 4.4]. The alternating surface charge of coated drug crystals is strong evidence that the layer-by-layer assembly of the oppositely charged components was successful. In this study, PDDA was always added as the final layer [Figure. 4.4] because sonication studies [Figure. 4.5 and 4.14] indicated that the dexamethasone particles coated with this polyelectrolyte reduced aggregation of the dexamethasone in suspensions and ensured the production of monodispersed microcapsules. In addition, the results demonstrated that the amphoteric polyelectrolyte gelatin A with theoretically a net positive charge at pH 5.8 can have local charge imbalances allowing it to be layered with the strong positively charged PDDA or the negatively charged PSS.

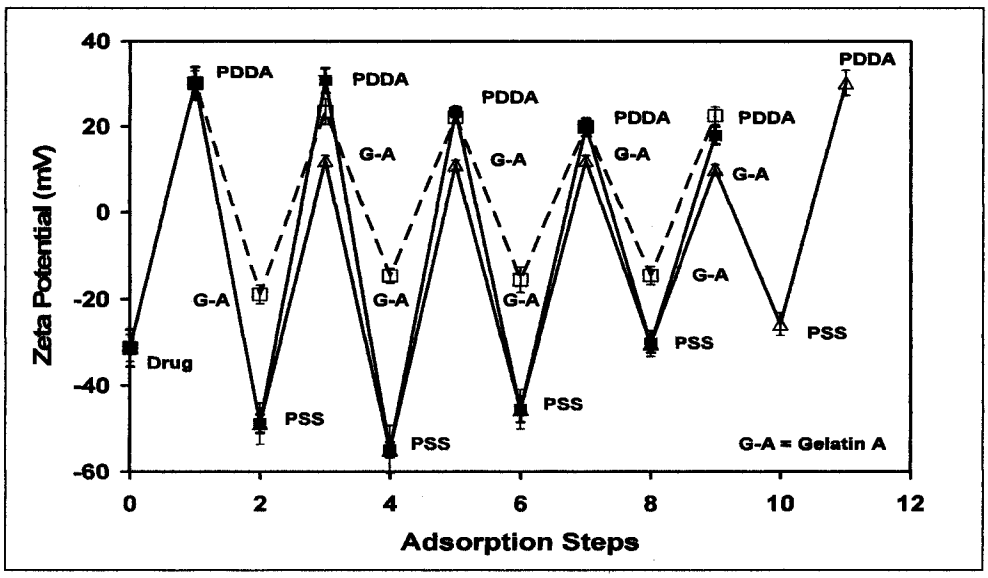


Figure 4.4 Alteration of surface charge as a function of polyelectrolytes – pH 5.8

4.3 Surface Modification Characterization and Influence on Drug Particle Size

4.3.1 Electron Microscopy Results for Dexamethasone Drug Particles

Scanning electron photomicrograph of the bare sample powder [Figure 4.5A] indicates aggregation of the micronized drug particles. The drug particles (0.5% w/v) were then subject to sonication for 10 min in pure buffer solution. The sample shows less aggregation but is still cohesive [Figure 4.5B]. Initial drug surface charge of bare drug particles in buffer solution was estimated to be negative. When suspended in 2mg/ml anionic polyelectrolyte PSS and sonicated for 10 min, aggregation was significantly reduced, and close inspection of the particles [Figure. 4.5D] show larger individual particles, 10–20 μm, coated by smaller particles. However, sonication of a suspension prepared in a 2 mg/ml solution of the cationic polyelectrolyte PDDA not only reduced aggregation but also reduced the size of the individual particles [Figure. 4.5F]. In this sample, a large number of fine particles, <1 μm, was observed. This change can very well be attributed to two factors. One is normalization of the surface charge by polymer assembly on particle surface and charge repulsion between the coated particles.

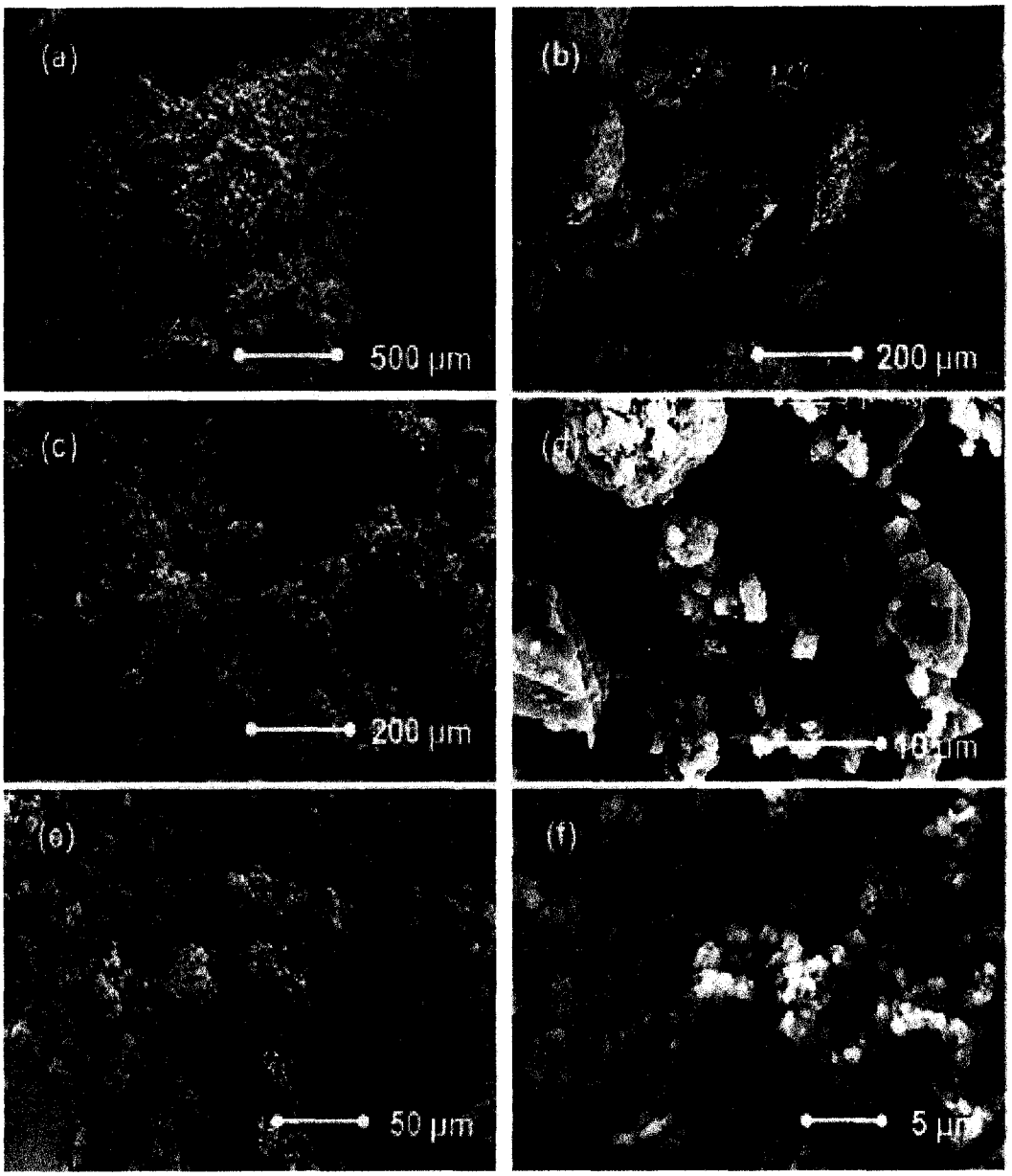


Figure 4.5 SEM photomicrographs of (a) micronized dexamethasone powder and powder recovered from (b) an aqueous suspension sonicated for 10 min; (c) an aqueous suspension containing 2 mg/ml PSS sonicated for 10 min; (d) close-up of sample c; (e) an aqueous suspension containing 2 mg/ml PDDA sonicated for 10 min; (f) close-up of sample e.

The ability of smaller particles to form coating over larger particles [Figure 4.5D] was further elaborated with the use of TIO_2 (Titanium di Oxide) particles. TIO_2 particles are extensively used in various applications as whiteners and for protection against light exposure. We incorporated these to assemble them over drug particles. Figure 4.6 is a SEM picture of a large dexamethasone drug particle layered with TIO_2 nanoparticles. Such hybrid polymer – nanoparticle assembly over drug particles could serve as a protective barrier for light sensitive drugs. Thus, the suggested approach could be further explored and characterized for various drugs.

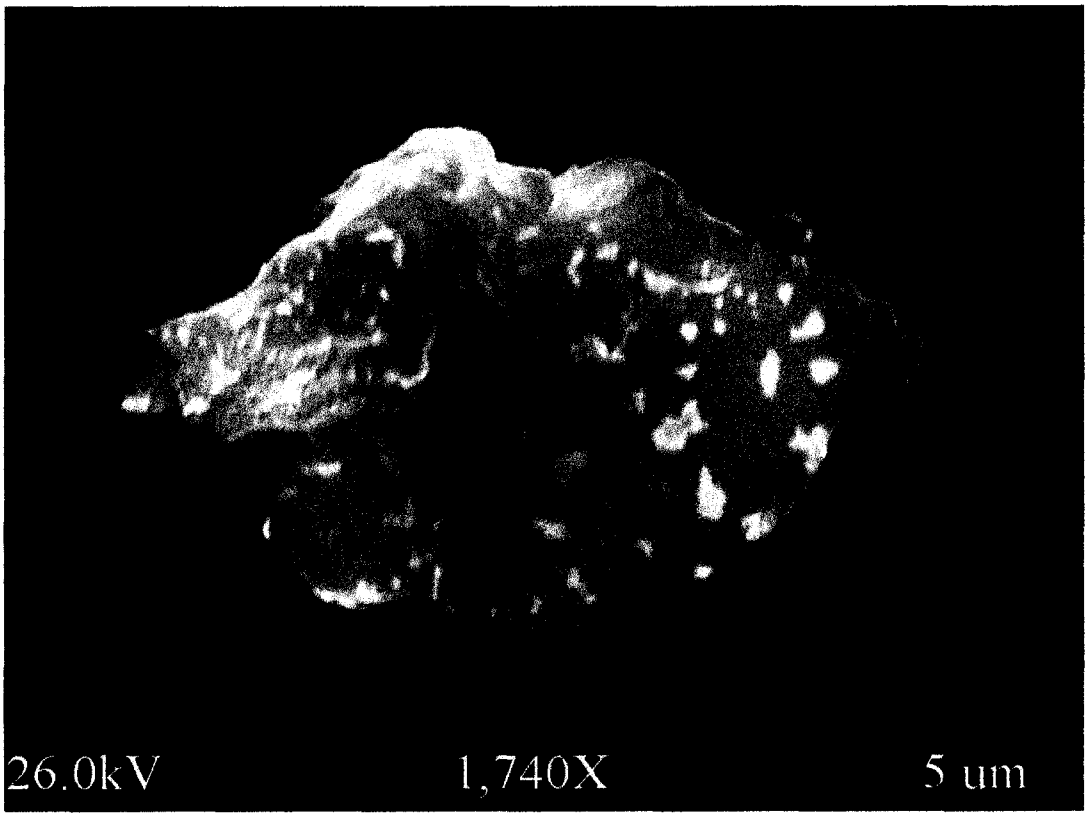


Figure 4.6 SEM picture of an un-cleaved dexamethasone drug particle with TIO_2 particles attached to the surface

4.3.2 Confocal Microscopy Results for Dexamethasone Drug Particles

In the absence of PDDA, small dexamethasone particles adhere together and form large aggregates [Figure 4.7]. On the other hand confocal micrographs of samples that were sonicated in presence of FITC labeled PDDA show no aggregation [Figure 4.8]. Figure 4.8A is a confocal photomicrograph in fluorescence mode and Figure 4.8B is a bright field image. The computer software generates an image overlap that is show in Figure 4.8C. The image overlap helps determine the location of the fluorescence intensity with respect to the rest of the solid support. One can clearly identify the presence of partially labeled PDDA on the periphery of the drug microcapsule. Computer generated 3D images [Figure. 4.9] of the coated particles shows the fluorescence intensity of the labeled polymer shell. This represents the thickness of the polymer shell around the individual dexamethasone core crystals. Figure 4.9 shows a single dexamethasone particle with a diameter of 2.5 μm, coated with multiple layers of PDDA and gelatin. The cross sectional fluorescence intensity profile generated by the computer software not only helps to estimate peak to peak particle diameter but also the layer thickness and location. In solution, the addition of the polyelectrolyte layers seems to almost double the diameter of the particle. The measured shell thickness that was seen under the confocal microscope for the hydrated nano-shells [Figure. 4.8, 4.9 and 4.10] indicates that the gelatin layers swell significantly when suspended in water. In addition, previous studies have found that

layer thickness estimated from QCM measurements is half of the thickness on microtemplates such as drug crystals [13, 18, and 27].

Figure 4.7 Aggregation of dexamethasone drug particles

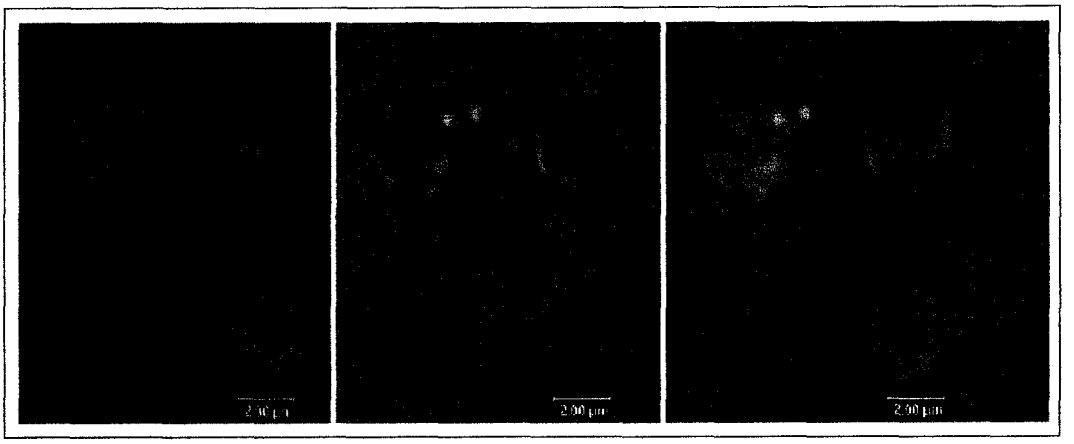


Figure 4.8 A) Indicates presence of FITC attached to the fabricated thin film capsules. B) Indicates bright-field mode image of the same capsules. C) Indicates computer generated overlap of images obtained by bright-field mode and fluorescence mode

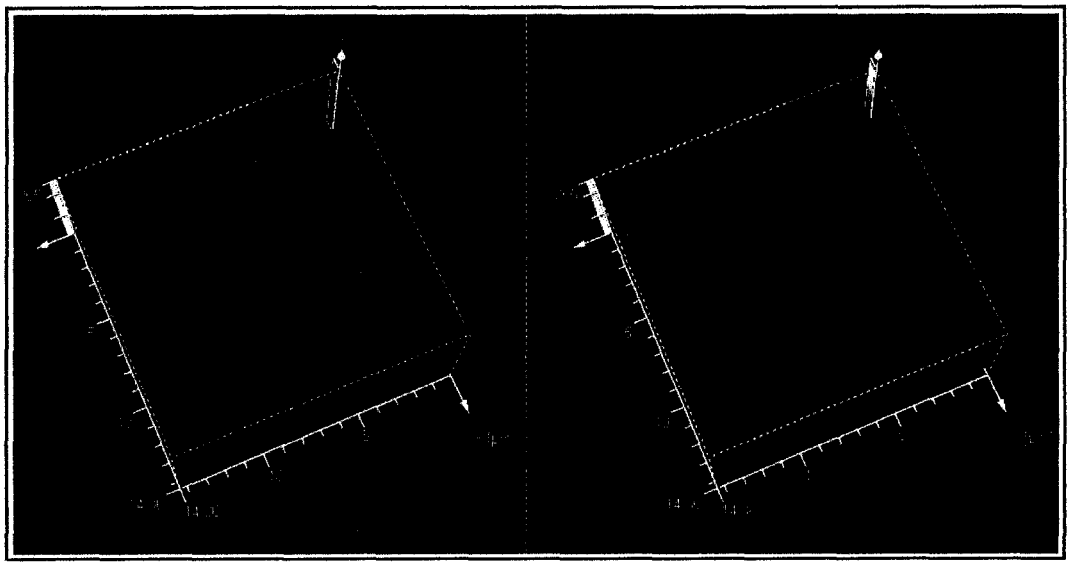


Figure 4.9 3-D computer generated image indicating the presence of FITC fluorescence on the outer surface with relatively less fluorescence towards the center of the capsule

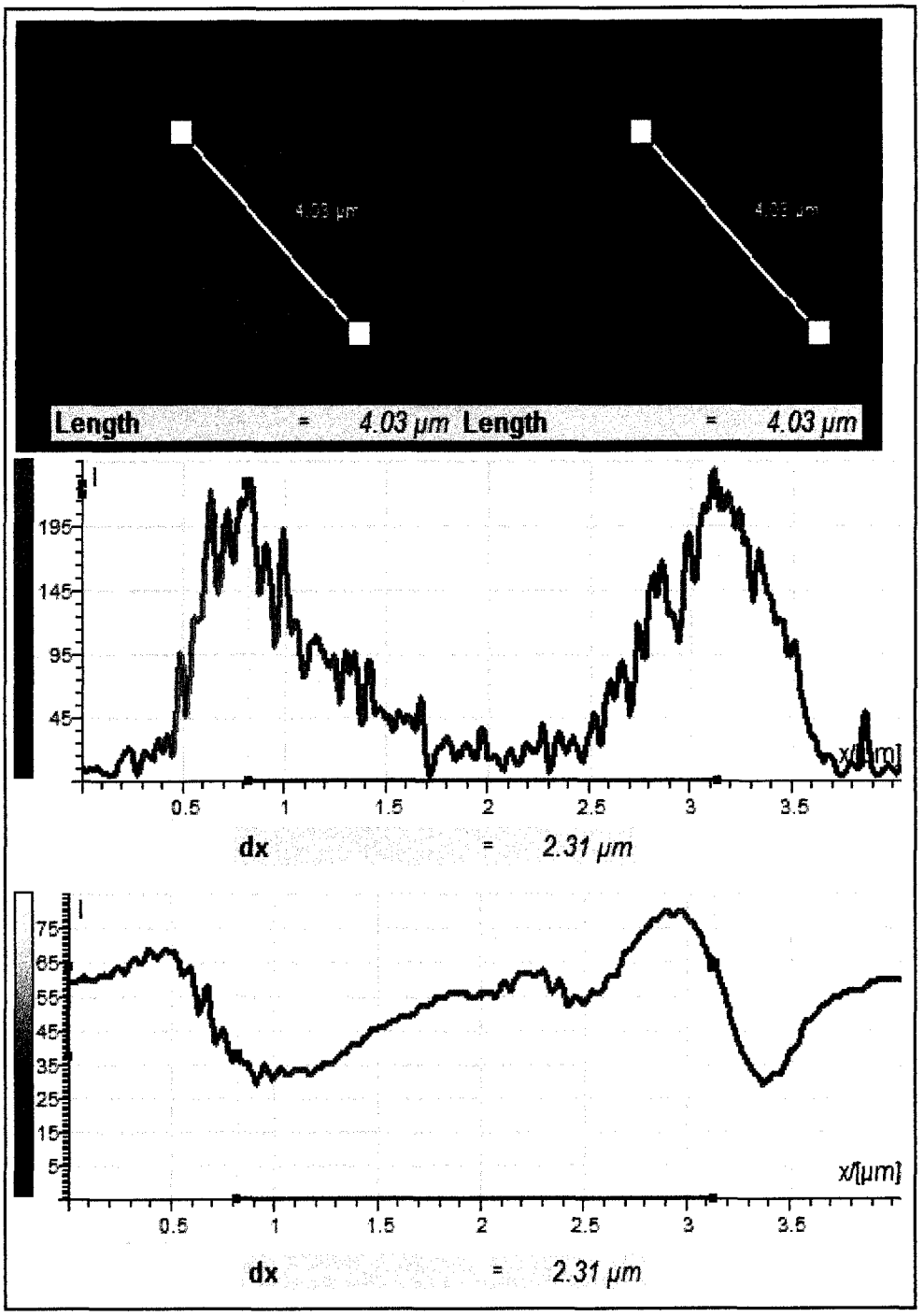
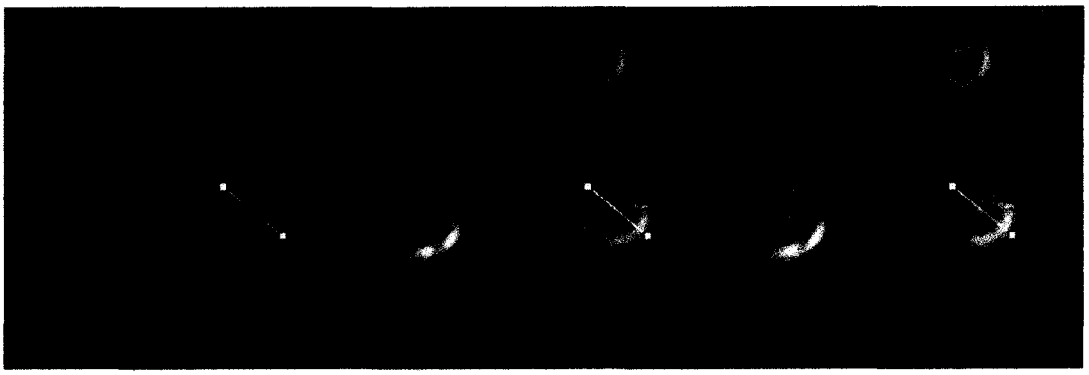


Figure 4.10 Cross sectional study of one dexamethasone micro particle layered with PDDA and gelatin

A new approach for instantaneous polyelectrolyte and nano-particle coating was tested using a standard ultrasonic nebulizer. Using polyelectrolyte and drug nanoparticle solutions, layers were fabricated onto a solid flat glass substrate. Polyelectrolyte gelatin-A was sequentially altered with FITC labeled dexamethasone particle to adsorb (gelatin A / FITC-Dexa)$_2$ bilayers. Figure 4.11A shows presence of labeled FITC dexamethasone particles assembled on glass substrate. Figure 4.11B is a bright field image of the same particles. Figure 4.5C is a computer generated overlap between the fluorescence mode and the bright field more. The concentric ring seen in the bright field mode is an optical artifact generated by the lens system of the microscope. The FITIC intensity inside the concentric ring helps delineate the particle boundaries. Computer generated 3D image and the cross sectional analysis [Figure 4.12] indicates the particle diameter is > 2 μm in diameter. The background roughness on the surface in the bright field mode may be due to the presence of polyelectrolyte layer.

This process will be further explored in future work. Presence of drug particles over the surface is an indicator that drug particles may be trapped in polyelectrolyte layers over the glass substrate. This could be a promising method for rapid application of ELBL assembly and may have the potential to be applied in large scale production of products with unique surface properties.



A B C

Figure 4.11 A) Indicates presence of FITC labeled dexa particles on glass substrate. B) Indicates bright-field mode image of the same capsules. C) Indicates computer generated overlap of images obtained by bright-field mode and fluorescence mode

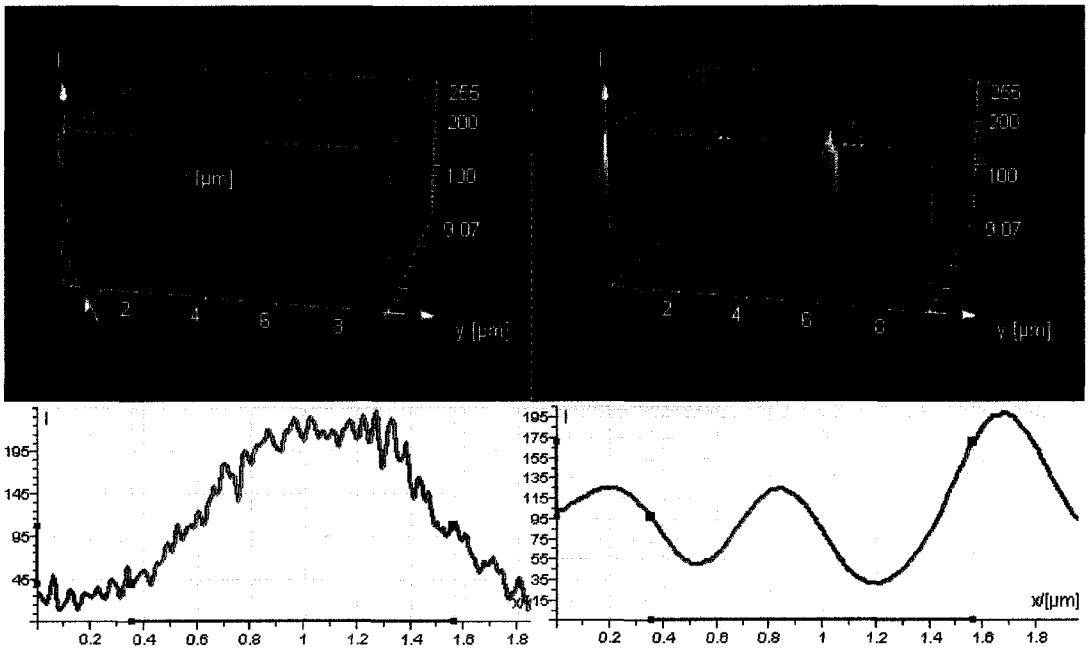


Figure 4.12 Computer generated 3-D image indicating the presence of Figure 4.11 Bottom part is cross sectional analysis of one of the layered particle

4.3.3 Confocal Microscopy Results for Surface Modification of Insulin (PROMAXX®) Drug Particles

A partially labeled polyelectrolyte poly-*l*-lysine with fluorescein FITC (P*l*L-FITC) was utilized in this study. The outermost layer of P*l*L-FITC was deposited over the primary PSS / poly-*l*-lysine layers following the same procedure used to layer polyelectrolyte layers over the insulin particles (PROMAXX®). The confocal images [Figure 4.13] were captured in moments after preparation by applying a drop of suspension on glass slide. Fluorescence mode (upper left) and transmission mode (upper right) were used simultaneously and an overlay image was obtained using the confocal microscope software package (lower left). Fluorescence was observed on the surface of insulin microparticles. On the other hand no background fluorescence was observed confirming deposition of polyelectrolytes over the microparticle surface and resistance to being washed out during the multiple washing cycles that follow deposition.

FITC-intensity profiles taken along the capsular cross section Figure 4.13 (lower right) shows P*l*L-FITC concentrated mainly around the microparticle surfaces. Some level of fluorescence appears to be emitted from microcapsule interiors. This could very well be the surface of the microcapsule facing the lens or could be artifacts from the instruments optical system.

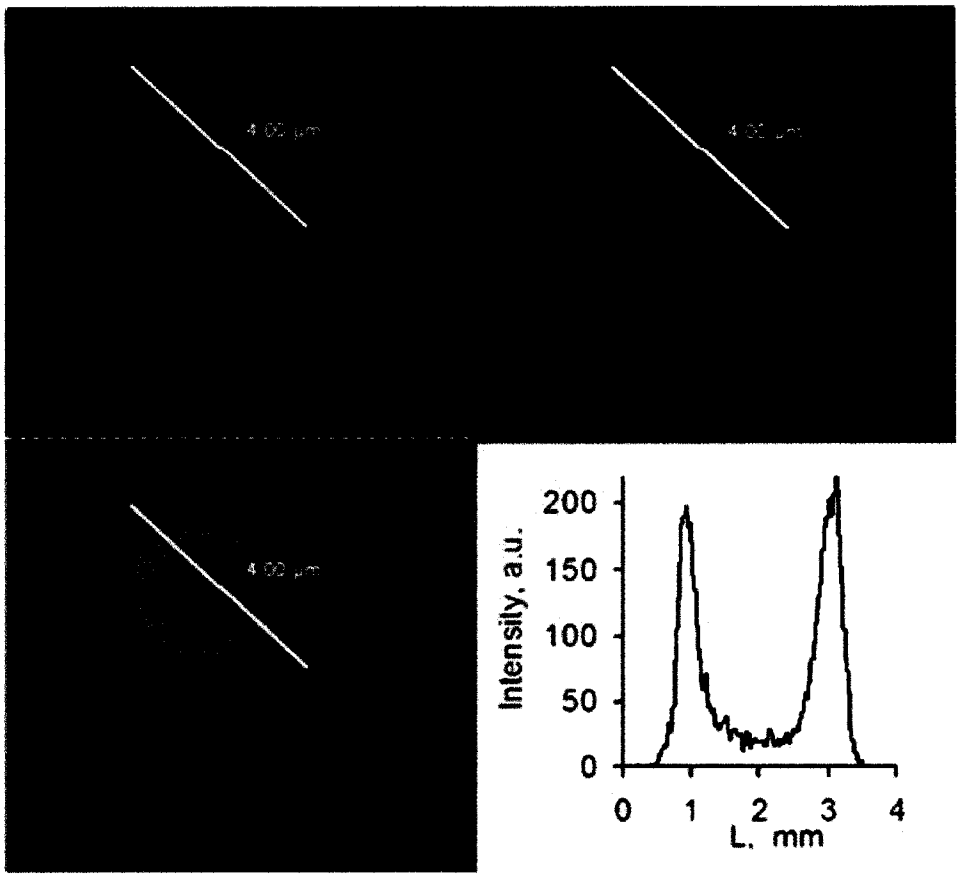


Figure 4.13 Confocal images obtained for insulin microspheres covered with a PSS / P*l*–Lysine FITC bilayer

4.3.4 Particle Size Analysis Results for Dexamethasone Drug Particles

The particle size analysis for various samples was done by dispersing the sample in deionized water within the sample cell of the particle analysis instrument. A magnetic stirring bar was used to keep the sample evenly suspended. All the measurements were done under identical conditions. The mean volume particle size of the micronized drug powder was 11.23 μm with a normal unimodal particle size distribution [Figure 4.14]. The mean volume particle size of the powder sonicated in

the PSS solution was 9.37 μm, nearly similar to that of the suspension in DI water. However, sonication in presence of 2 mg/ml concentration of PDDA, reduced the mean size of the particles to 2.54 μm. UV analysis of the filtered solutions after sonication confirmed that the drug did not dissolve in the suspension mediums. Figure 4.15A shows the effect of an increase in sonication time on the mean volume particle size of the dexamethasone powder in the PDDA solution. The mean size decreased according to a first-order decay process (r^2 = 0.993), and a maximum decrease in the particle size was reached after 8 min. Sonication up to 30 min did not lead to a further decrease in particle size.

Because microscopic evaluation showed that the suspension in a PDDA solution prepared by sonication led to a significant reduction in the size of the dexamethasone particles as shown in Figure 4.5F; this suspension was filtered and the particle size distribution of the filtered solution measured. The PCS measured size distribution of the dexamethasone suspension in 2 mg/ml PDDA solution sonicated for 10 min and filtered through a 1.2-μm filter is shown in Figure 4.15B. The number mean size of the particles was 150 nm, and the mean volume diameter was 420 nm. The filtered sub-micrometer particles were polydispersed with a wide size distribution (distribution and SEM picture shown in Figure. 4.15B).

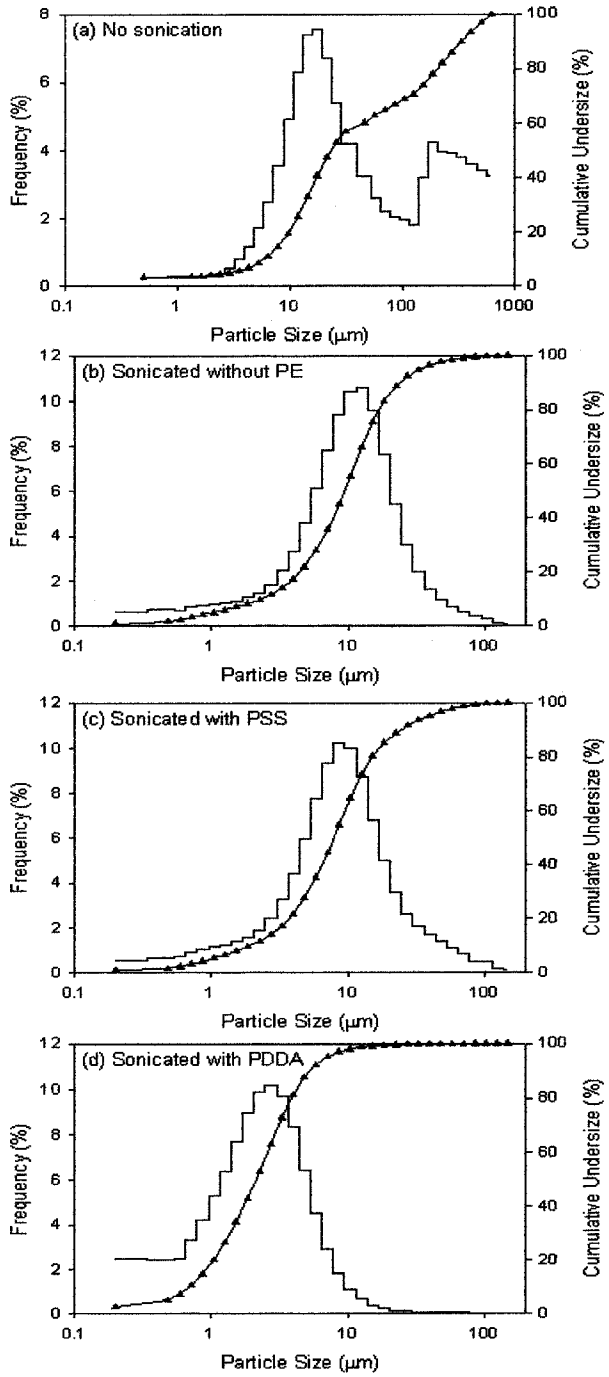


Figure 4.14 Particle size distribution profiles for micronized dexamethasone measured in deionized purified water with and without polyelectrolytes or sonication

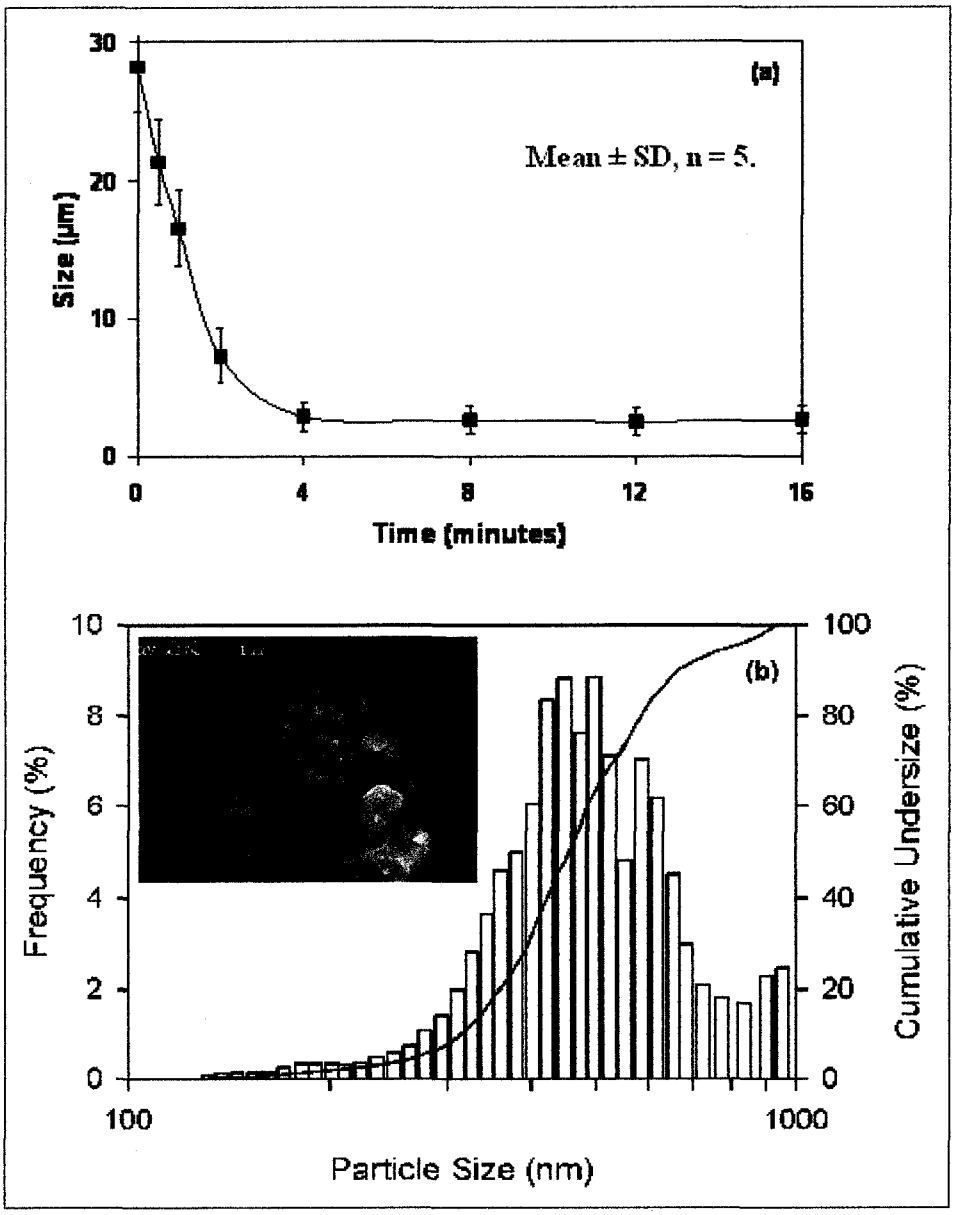


Figure 4.15 (a) The change in mean volume particle size of dexamethasone when sonicated in a solution of the cationic polyelectrolyte PDDA.
(b) Particle size distribution (mean size <420 nm) process.
Insert is a SEM picture of the nano-size particles; the bar represents 1 µm

4.4 Drug Release

4.4.1 Surface Modification Influence on Dexamethasone Drug Particle Release

To obtain the nanoparticles, drug suspension was filtered using a 1.2 μm filter. The collected nanoparticles were then tested for release. Release profiles of different dexamethasone layered products are displayed in Figure 4.16. It is very interesting to note that drug particles layered with one layer of polyelectrolyte PDDA released much faster than bare drug sample in aqueous solution [Figure 4.16A and B]. Compared to the unfiltered sample [Figure 4.16B], the filtered sample [Figure 4.16A] exhibited faster release. ELBL coating of the dexamethasone particles with $(PDDA/PSS)_4/PDDA$ [Figure 4.16C] slowed down the release compared to the PDDA sonicated particles, but the release from these particles is very similar to the suspension prepared without PDDA [Figure. 4.16D]. Furthermore, ELBL assembly with gelatin of $(PDDA/gelatin\text{-}A)_4$/ PDDA [Figure 4.12e], $(PDDA/gelatin\text{-}B)_4/PDDA$ [Figure 4.16F] or $PDDA/(PSS/gelatin\text{-}A)_4/(PSS/PDDA)_1$ [Figure 4.16G] significantly decreased dexamethasone release from the suspended microcapsules. Sustained drug release from the microcapsules reached 80% for PDDA/gelatin-A, 60% for PDDA/gelatin-B, and 60% for gelatin-A/PSS coated particles after 2 h [Figure. 4.16]. The release profiles were best described by a model that represents systems where drug diffusion occurs through a polymeric structure or network,

$M_t/M_\infty = ktn$ ($r^2 > 0.990$),

Where, M_t/M_∞ is the fractional release of the drug,

t is the release time,

k is a constant incorporating structural and geometric characteristics of the controlled release device, and

n is the release constant, indicative of the mechanism of drug release [28, 29].

Amphoteric gelatin-A and B slowed down the release rate considerably when paired with the oppositely charged polyelectrolytes at a specific pH instead of being paired with similarly charged PDDA [Figure 4.16]. A solution of dexamethasone in ethanol and water mixture (1:1 v/v) almost instantaneously diffused throughout the receptor cell (>90% dissolved in 2 min).

The release results from gels containing 0.1% w/v of the dexamethasone microcrystals or microcapsules suspended in 1% sodium carboxymethylcellulose are shown in Figure 4.13. These release profiles agree to the prior release profiles in aqueous solutions. PDDA coated particles (0.163 μg cm^{-2} min^{-1}) [Figure 4.17A] > $(PDDA/PSS)_4$/PDDA coated particles (0.076 μg cm^{-2} min^{-1}) [Figure 4.17B] ≥ uncoated particles (0.071 μg cm^{-2} min^{-1}) [Figure 4.17C] > PDDA/$(PSS/gelatin\ A)_4$/$(PSS/PDDA)_1$ coated particles (0.043 μg cm^{-2} min^{-1}) [Figure 4.17E] > $(PDDA/gelatin\text{-}B)_4$/PDDA coated particles (0.033 μg cm^{-2} min^{-1}) [Figure 4.17D].

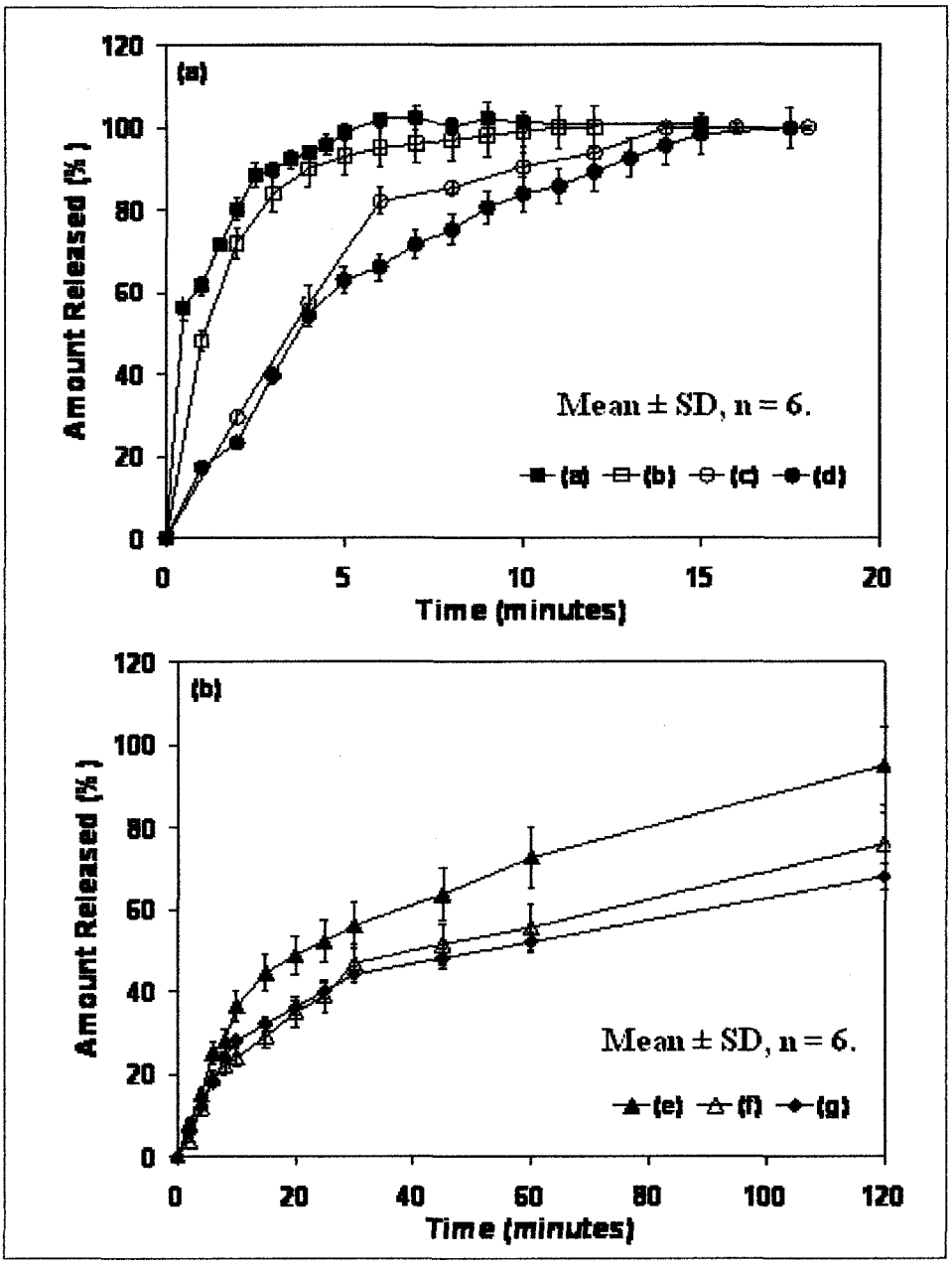


Figure 4.16 Release profiles of dexamethasone particles in PBS buffer pH 7.2

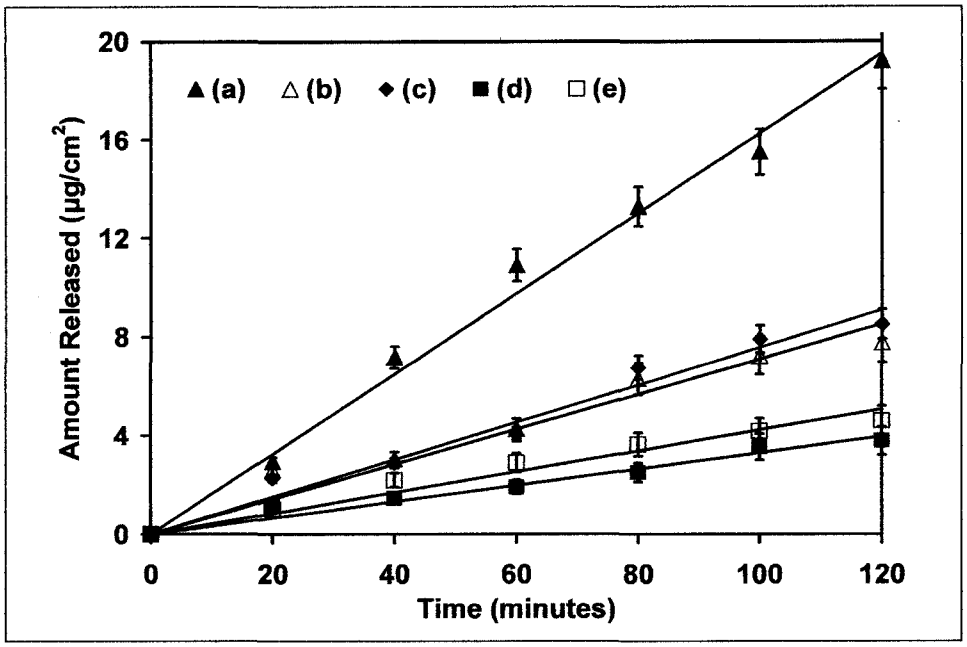


Figure 4.17 Release of dexamethasone from the microcrystals and microcapsules suspended in a 1% w/w carboxymethylcellulose gel for microcrystals

4.4.2 Surface Modification Influence on (PROMAXX®) Drug Particles Release

The insulin particles (PROMAXX®) obtained from the Baxter Corporation were created using controlled phase separation of macromolecules with the help of water soluble polymers. The template particles comprise of protein (human or animal insulin). As a result of ELBL assembly modifications, these protein based cores underwent complexation. Figure 4.18 is a schematic to explain the process of complexation. Protein cores may not behave as one solid core. Instead there might be polymeric penetration within the core leading to protein – polyelectrolyte complex formation. The protein polyelectrolyte interaction is purely electrostatic in nature. The polyelectrolyte layer adhesion and packing is a charge dependent process. The

presence of charged species on the surface dictates the strength of the interaction. Therefore, during adhesion of the first layer, most of the charged species may be interacting with the particle surface resulting in tight packing and firm adhesion. However, presence of a single layer in the core complex may result in formation of nano-channels within the core that facilitate free flow of solvent molecules to traverse through the core resulting in increased dissolution rate. This has already been discussed previously in Chapter two in context with Fick's first law of diffusion. The fabrication of second layer however may increase the adhesion between the particles within the complex thus this interaction improves packing and adhesion, naturally improving release rates. However, addition of further layers sequentially decreases and increases release. The evidence of such an occurrence is seen in our release results [Figure 4.19].

Protein core modified with one layer of polyelectrolyte released relatively faster to all other samples. This result seems to agree with what we saw for dexamethasone drug particles [Figure 4.16A]. Addition of the second layer dramatically reduced the release [CMC/ Protamine Sulfate sample cure Figure 4.19]. Further addition of layers sequentially increased and decreased the release rate.

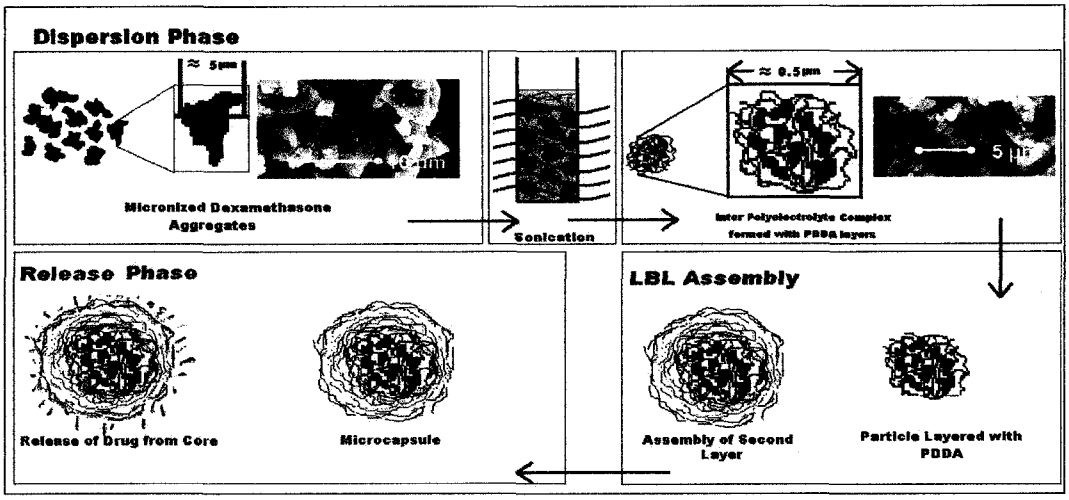


Figure 4.18 Figure representing complexation process

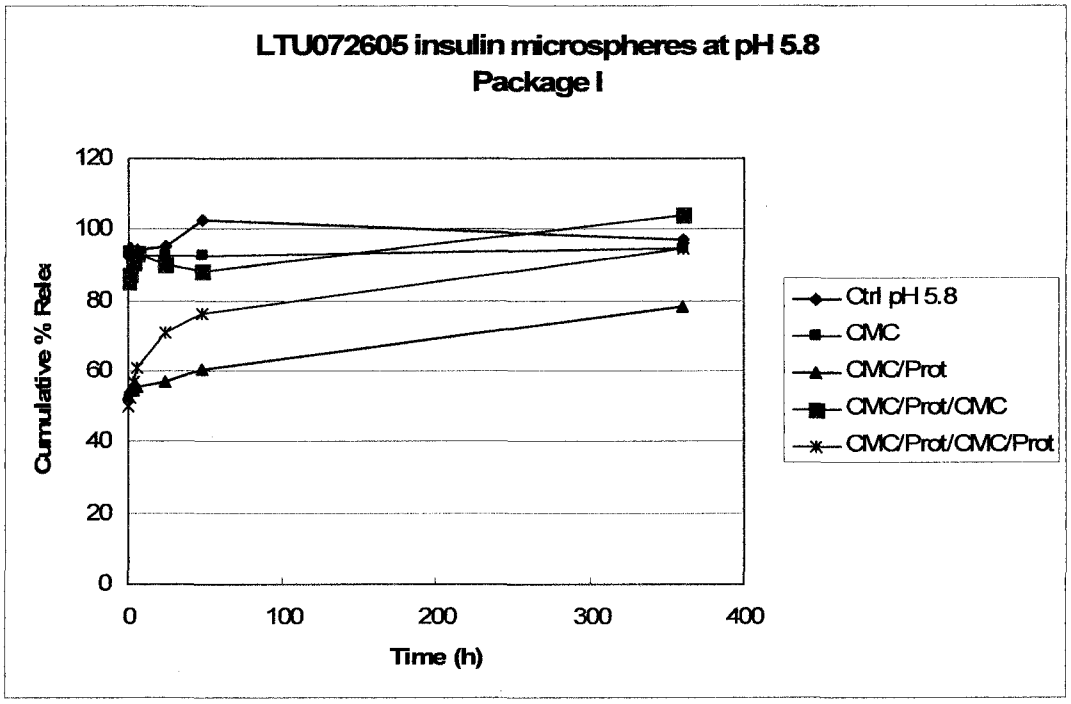


Figure 4.19 Release from insulin particles (PROMAXX®) showing variable release as a result of complexation process

CHAPTER 5

CONCLUSIONS AND RECOMENDATIONS

5.1 Conclusions

Traditional micro-encapsulation involved separate fabrication of a hollow capsule followed by loading of substance of interest. Fabrication required the presence of a template particle for the thin film assembly round the template. Further, in order to encapsulate the substance of interest, the core template particle had to be disintegrated using chemical dissolution. The mobilization of disintegrated core remnants through the fabricated layers out of the capsules and loading of substance of interest into the capsule posed unique problems.

Fabricated layers have intermittent defects termed as pores. The dissolution, removal and loading of substances within the capsule are a pore size dependent process. Thus remnants of core more than often appear as artifacts within the capsule also taking up space within the capsule otherwise required for substance of interest. High molecular weight substances are difficult to load for the very same reason that they cannot pass through the pores. The empty capsules were loaded using concentration diffusion gradient. This process was neither efficient nor cost effective. Furthermore, the amounts of material lost made encapsulation process impractical.

In this study we introduced a new method for ELBL drug encapsulation. Direct surface modification technique is geared towards applying ELBL self assembly technique to modify drug and other surface properties. No template particle is necessary thus eliminating time consuming and inefficient steps. The results from this study suggest that the combination of sonication and ELBL techniques proved useful in reducing drug particle size to nano-scale. Such simultaneous micronization of crystalline drug particles and polyion coating during sonication was the unique feature of this study. These results impact classical drug particle preparation methods such as micronization by air-jet milling routinely used in the pharmaceutical industry. The rapid release of hydrophobic drugs modified with the surface modification technique can address the issue of bioavailability of drugs. Furthermore, the results also indicate that various polyelectrolyte combinations prove useful in controlling release of the drug. The drug release process was believed to be simply diffusion controlled release through the polyelectrolyte multilayer thin films assembled over the drug particles. However, this study proves that the sequential adsorption of polyelectrolyte layers result in complexation, thus affecting the release of drug.

The capability to modify surface properties of drug crystals with simple one step scalable process has far reaching consequences. Addition of nanoparticulate material to the layers can impart additional functionality to the particles. For example, simple addition of TIO_2 particles in layers coating photosensitive drug crystals may protect these drugs from exposure to light. Our study indicates that such coating is possible [Figure 4.5, and 4.6]. The combination of biocompatible

electrolytes tested with the insulin particles (PROMAXX®) can be easily applied to other drug particles. Thus, this method in future could even address issue of biocompatibility.

Formulation of prodrugs is highly sought by pharmaceutical industry. Simple ELBL surface modification of drugs pre approved by FDA with the use of polymers that are pre approved by FDA seems to be the most logical thing to do. The system that can bridge the two is ELBL assembly method. This study is just a beginning for the direction that ELBL assembly technique has taken.

The application of ELBL technique to modify surfaces of hydrophobic drug particles saw tremendous success. We were able to use the charged hydrophobic drugs as template particles and use the surface charge to assembly various polyelectrolyte layers. The layers seem to normalize the charge over drug particle surfaces and reduce aggregation. This is particularly important for drug reconstitution in field and parenteral administration of the drug. Many of the cancer drugs are hydrophobic in nature and many more are dissolved in chremophor for easy administration. Hydrophobic drugs tend to aggregate and hence cannot be directly used and chremophor causes sever anaphylactic reactions. Hence, either way, administration of hydrophobic drugs other than gastrointestinal administration is a problem. This study takes the first steps towards providing some sustainable solutions to the problem.

When used in conjunction to sonication, the ELBL technique seems to influence the size of hydrophobic drug particles. This is another giant step towards

making formulations from hydrophobic drugs that can be parenteraly administered. We found that surface modified drug particles can be easily reconstituted and remain in suspension for relatively longer periods of time without the need of mixing or re-suspending.

Traditional ELBL technique is conducted simply in buffer solutions without the presence of another inert polymer. We explored possibility of ELBL assembly in presence of PEG in buffer solutions. This was unique direction to further applications of ELBL assembly. The insulin particles (PROMAXX®) used in this study is significantly unstable in solutions without the presence of PEG. It was important to prove that ELBL assembly of various polyelectrolytes was possible even in presence of such inert polymers. The study successfully shows increase in insulin particle release time as a result of ELBL surface modification. Strong electrostatic interactions between the first layer over drug particles and the drug particle surface itself can be balanced effectively by fabricating another layer using oppositely charged polyelectrolyte (polyanion /polycation for positive PROMAXX core at pH 5.8). This allows regulation of complexation process and in turn regulation of encapsulated protein release.

To summarize our achievements:

1. Method of nano-encapsulation for low soluble drug was elaborated
2. New method for submicronizing particles via powerful sonication combination with first stage of ELBL was explored
3. ELBL modification was applied for control release from drug particles

4. An approach indicating immobilization of drug nanoparticles over flat surfaces was demonstrated
5. An approach indicating nano-protective coating was demonstrated
6. The ELBL fabrication was applied to encapsulation of soft insulin micro-aggregates.
7. Phenomenon of inter-polyelectrolyte complexation was discovered and its effects on drug release were tested
8. Influence of complexation was seen on both the dexamethasone drug particles and the soft insulin cores.

5.2 Recommendations for Future Work

The present work is just a precursor work to a whole new direction for the ELBL assembly process. The applications of ELBL assembly are vast and the variations that it can offer are phenomenal. It would be prudent to say that new layer combinations can be unearthed by relentless search for new polymers that can impart new and improved functionalities to the assembled layers as well as the encapsulated material.

We applied ELBL along with encapsulated drug nanoparticles to flat surfaces. These modified surfaces need to be tested for sequential release of drug as well as entrapped growth factors. The sequential release would be a very important step in constructing a single system for cell growth, sustenance and cell differentiation. Such

an artificial three dimensional construct has been a dream for scientist for a long time.

We found that ELBL assembly process has a thermal component to it. One component of this study came from our calorimetric study of polymer interactions with one another. Another indication came from the variable time and mass deposition seen in low temperature experiments conducted with insulin particles (PROMAXX®). This could mean that the layer interactions be more than just electrostatic. Further investigation into this area could unravel new insights into layer interactions and would help in optimizing ELBL assembly conditions.

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