CONTROLED LOADING AND RELEASE OF MODEL DRUGS FROM POLYELECTROLYTE MULTILAYER THIN FILMS

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ปวีณ์นุช กิตติธีรนันท์ : การควบคุมการกักเก็บและการปลดปล่อยของยาตัวอย่างจากฟิลม์บางหลายชั้นของสารพอลิอิเลคโตรไลท์ (Controled Loading and Release of Model Drugs from Polyelectrolyte Multilayer Thin films) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ดร. สเตฟาน เทียรี่ ดูบาส, 180 หน้า.

เทคนิคการสร้างฟิล์มบางหลายชั้นของพอลิอิเล็กโทรไลต์มีความสำคัญต่อเคลือบผิววัสดุทางชีวภาพและระบบการนำส่งยา สารพอลิอิเล็กโทรไลต์ประจุบวกได้แก่ พีดีเอดีเอ็มเอซี (poly(diallydimethyl ammonium chloride), PDADMAC) หรือไคโตซาน และสารพอลิอิเล็กโทรไลต์ประจุลบได้แก่ พีเอสเอส (poly(sodium 4-styrene sulfonate, PSS) หรือพีเอสเอสเอ็มเอ (poly(diallyldimethylammonium chloride), PDADMAC) ถูกสร้างขึ้นเพื่อเป็นพอลิเมอร์แม่แบบและเคลือบเพื่อควบคุมการปลดปล่อยยาชนิดต่างๆ ลักษณะโครงสร้าง, ความหนา, ความขรุขระและความเสถียรของฟิลม์บางหลายชั้นตรวจสอบโดยเครื่องยูวีสเปคโตโฟโตมิเตอร์และการทดสอบเอเอฟเอ็ม ยาตัวอย่าง(ขมิ้นชัน, ไดโคลฟีแนคโซเดียม และเจนเชียนไวโอเลต) ถูกรวมเข้าไปในพอลิเมอร์แม่แบบ พฤติกรรมการกักเก็บและการปลดปล่อยของยาสามารถบอกปริมาณโดยเครื่องยูวีสเปคโตโฟโตมิเตอร์โดยขึ้นอยู่กับความสัมพันธ์ของสารละลายตัวกลางภายนอก การกักเก็บของยาตัวอย่างบรรลุผลโดยแรงไฮโดรโฟบิคหรือแรงอิเลคโทรสเตติคและควบคุมโดยลักษณะของสารละลายตัวกลาง เช่น ส่วนประกอบของตัวทำละลาย, ค่าพีเอชของสารละลายยา, ค่าความแรงในการแตกตัวเป็นไอออน และยังขึ้นอยู่กับเวลาในการกักเก็บและจำนวนชั้นของฟิล์ม โครงสร้างพอลิเมอร์แม่แบบที่แตกต่างกันพบว่ามีอิทธิพลต่อพฤติกรรมการกักเก็บและการปลดปล่อยผ่านใต้สภาวะอื่นที่คงที่ ผลการทดลองชี้ให้เห็นถึงการปลดปล่อยของขมิ้นชันเป็นผลมาจากส่วนประกอบของตัวทำละลายระหว่างน้ำและเอทานอลและค่าความแรงในการแตกตัวเป็นไอออน ในขณะที่การปลดปล่อยของไดโคลฟีแนคโซเดียมและเจนเชียนไวโอเลตเป็นผลส่วนใหญ่จากค่าพีเอชของสารละลายในการปลดปล่อยและค่าความแรงในการแตกตัวเป็นไอออน ดังนั้นพอลิเมอร์แม่แบบจาก พีดีเอดีเอ็มเอซี/พีเอสเอส, พีดีเอดีเอ็มเอซี/พีเอสเอสเอ็มเอ และ ไคโตซาน/พีเอสเอส สามารถใช้งานเป็นภาชนะในการควบคุมการเก็บกักรวมถึงการปลดปล่อยโมเลกุลต่างๆ นอกจากนี้ชั้นของฟิล์มกีดขวางถูกสร้างบนชั้นของพอลิเมอร์แม่แบบเพื่อยืดระยะเวลาการปลดปล่อยขมิ้นชันจากชั้นของพอลิเมอร์แม่แบบภายใต้สภาวะทางชีวภาพ ฟิล์มกีดขวางสร้างขึ้นโดยฟิลม์บางหลายชั้นของพอลิอิเล็กโทรไลต์ประจุบวกได้แก่ไคโตซานและพอลิอิเล็กโทรไลต์ประจุบวกลบได้แก่อัลจิเนต หรือพีเอเอ (poly acrylic acid, PAA) ฟิล์มกีดขวางทั้งสองชนิดลดระยะเวลาในการปลดปล่อยขมิ้นชันเมื่อเปรียบเทียบกับฟิล์มกีดขวางที่ทำจาก พีดีเอดีเอ็มเอซี/พีเอสเอส โดยพบฟิล์มกีดขวางที่ทำจาก ไคโตซาน/พีเอเอ มีประสิทธิภาพดีที่สุด สุดท้ายฟิลม์บางหลายชั้นที่กักเก็บยาถูกประยุกต์ใช้งานโดยเคลือบบนผลไม้และกักเก็บขมิ้นชัน โดยมีการคำนวนปริมาณของขมิ้นชันที่กักเก็บบนผิวของผลไม้นั้น

สาขาวิชา วิทยาศาสตร์นาโนและเทคโนโลยี ลายมือชื่อนิสิต .....

ปีการศึกษา 2554 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

5087774920 : MAJOR NANOSCIENCE AND TECHNOLOGY
KEYWORDS: DRUG LOADING / POLYELECTROLYTE / LAYER BY LAYER THIN FILM

PAVEENUCH KITTITHEERANUN: CONTROLED LOADING AND RELEASE OF MODEL DRUGS FROM POLYELECTROLYTE MULTILAYER THIN FILMS. ADVISOR: STEPHAN THIERRY DUBAS, Ph.D. 180 pp.

The Layer-by-Layer (LbL) self assembly technique of polyelectrolyte multilayers (PEM) has shown great potential in bio-coating material as well as local drug delivery systems. Herein, combination of positively charged polyelectrolyte of poly(diallyldimethylammonium chloride) (PDADMAC) or chitosan (Chi) and negatively charged polyelectrolyte of poly(sodium 4-styrenesulfonate) (PSS) or poly(4-styrene sulfonic acid-co-maleic acid, sodium salt) (PSSMA) were constructed and used as polymer matrix and coatings for control release of various drugs. Structure, thickness, roughness and stability of the PEM films were characterized by uv-vis spectrophotometer and AFM measurement. Model drugs (curcumin, diclofenac sodium and gentian violet) were incorporated in the polymer matrix and their loading and release behavior was quantified by uv-vis spectrophotometer as a function of the external media. The loading of the model drugs was achieve through hydrophobic interaction or electrostatic interaction and controlled by solution media characteristics such as solvent composition, pH, ionic strength but also loading time and number of constructed layers. Differences in polymer matrix structures have been found to influence the loading and release behavior under otherwise fixed conditions. Results indicated that the release of curcumin was affected by changing the solvent composition of water/ethanol and ionic strength while the release of diclofenac sodium and gentian violet was most affected by the pH and ionic strength of the loading medium. The polymer matrix of PDADMAC/PSS, PDADMAC/PSSMA and Chi/PSS can therefore be used as drug reservoir for the controlled loading of functional molecules and their release. In addition, PEM blocking layer deposited on top of polymer matrix were used to prolong the slow release of curcumin from PEM matrix substrates when exposed to physiological buffer. The blocking films was constructed by the LbL self assembly of positively charged of chitosan (Chi) and negatively charged of alginate (Alg) or poly acrylic acid (PAA). Both blocking films delayed the release of curcumin when compared to a PDADMAC/PSS blocking film with the Chi/Alg film being the most efficient. Finally, in an attempt to explore an innovative application of drug loaded PEM films, fruit were coated with PEM films and loaded with curcumin was used to quantify the amount of curcumin loaded at the surface of the fruit.

Field of Study: Nanoscience and Technology Student's Signature……

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LIST OF ABBREVIATIONS

PEM	:	Polyelectrolyte Multilayer Thin Films
LbL	:	Layer by Layer technique
PDADMAC	:	Poly(diallyldimethylammonium chloride)
PSS	:	Poly(sodium 4-styrene sulfonate)
PAA	:	poly(acrylic) acid sodium salt
PSSMA	:	Poly(4-styrenesulfonic acid-co-maleic acid) sodium salt
Chi	:	Chitosan
DS	:	Diclofenac sodium salt
GV	:	Gentian violet
mM	:	milli Molar
nm	:	nanometer
et al.	:	*et alii*

CHAPTER I
INTRODUCTION

Over the past several years, the field of drug delivery has advanced considerably, resulting a new controlled and sustained loading and release system. The main objective of drug delivery system is to achieve an effective therapeutic system administration via sustained drug release over an extended period of time.[1] Loading of drugs and realizing their sustained release are not only technically challenging but also of practically importance because drugs release from the substrate can promote/support the cell viability, proliferation and differentiation.[2] Controlled drug delivery system is already used in various applications, such as surgical implants, sutures and wound dressing with pharmaceutical agents.[3] Various approaches, including embedding of drugs into biodegradable polymers,[4] nanoencapsulating of drug particles,[5] chemical grafting of drugs to natural or polymeric matrix[6] are useful techniques for applying to control drug loading and release behavior. Ruiz and their co-worker[7] prepared the surface of polypropylene (PP) films for improving the loaded and the sustained of vancomycin. The PP films were first grafting with acrylic acid (AAc), and then cross-linking of poly(N-isopropyl acrylamide) (PNIPAAm) in order to achieve the maximum loading of vancomycin. In the same way, some novel controlled loading and release carrier have been developed. Agarwal et al.[8] prepared nanocolloids of poorly insoluble drug (tamoxifen and pacliaxel) by nanoencapsulating technique. The release of drug in their work can be controlled by increasing the shell density and thickness. In addition, various sustained delivery system of microstructure of hydrogels with high porous were also decribed. [9] Although the loading and release of drug was achieved via various approaches, a rapid release of drug during the process, especially an initial bust release was still investigated. Diller et al.[10] found that the model antibiotic drug was completely release in the initial 30 minutes with approximately 10% of release from poly-L-lactic acid electrospun fiber. Such a faster and lower release could be due to the small indentations of size that might not reach deeply into the fiber interior. One possibility

for reducing the release rate and suppressing the initial burst is preparing the drug loading within the LbL self assembly thin films.

The Layer-by-Layer (LbL) self assembly technique becomes more attractive and interesting in many applications due to its large potential. [11] This technique was discovery by Decher and co-workers in the 1990s. [12] The alternatively absorption is well known as a fundamental process for fabricating polyelectrolyte assembled on a given substrates. The LbL self assembly technique can be summarized as the alternatively dipping of a substrates in a solution of both cationic and anionic polyelectrolyte followed by a rinse step, leading to the multilayer thin film growth. The diving forces of LbL assembly comprise of intermolecular forces, including electrostatic interaction,[13] hydrogen bonding,[14] hydrophobic interaction, [15] charges transfer interaction and sterecomplex formation. [16] The multilayer thin films offer excellent characteristics, such as fine films tuning in terms of thickness, mechanics, chemistry and stability.

Recently, the LbL self assembly thin films are applied to develop the drug delivery system. It is used to sustain and control the loading and release of biomolecules. A variety of biomolecules, including, drug, lipid, protein, enzymes, DNA and even large polysaccharides, have been successfully loaded into multilayer thin films. [17] Normally, the loading and release behavior of drugs through multilayer thin films was depended on the permeability, the disassembly, erosion of the multilayers structure and other experimental variables.[18] One of the most important issues to consider for developing the multilayer thin films is how to increase the loading capacity of these thin films. Jiang et al.[19] assembled weak polyelectrolyte of poly(allylamine hydrochloride) (PAH) and DNA to construct the multilayer thin films by the LBL technique. By varying the pH environmental, many character of weak multilayer films, such as layer of thickness, surface wettability and degree of interpenetration could be greatly affected. By selecting the correct pH conditions, drug delivery of molecules could be successfully loaded in the multilayer thin films.

Base on LbL self assembly technique, there are generally two methods to load drug into the multilayer thin films. The first one is directly alternative deposition of drug with an opposite charge species to construct multilayer films. By using this

method, the degradable multilayer films and multilayer layer thin films could be capable of releasing drugs under changed environmental conditions. In the second method, the drug is loaded by first constructing multilayer matrix films and then loading of drug by a post diffusion/absorption step.[20] Drugs containing few charges cannot be used to produce the multilayer thin films as in the first method. Thus, the second approach is more suitable for these drugs.

Generally, the release rate is controlled by the internal factor, such as the multilayer film thickness and the outer coating layers, which is usually adjusted by changing the number of layers and type of polyelectrolyte. In addition, the release condition, such as pH of buffer solution,[21] temperature[22] and ionic strength[23] can also affects the release as external factors. Although the use of multilayer films in controlled release application has received considerable attention, only the few studies have focus on the using of small charged drug molecule for control release. One such study by Ren et.al[24] involved the use of DNA as an anionic polyelectrolyte and poly-L-lysine (PLL) to construct PLL/DNA thin films. It is interesting that the films is erasable upon changing in ionic strength after incubate into solution medium, which might serve as another method to controlled release of DNA. They reported that the increase ionic strength of salt concentration in the solution medium can induced the deconstruction system of (PLL/DNA) films, resulting the release of DNA. In a related study, Jian and Li[25] demonstrated control over the release rate of charged drug molecule from polypeptide nanofilms of poly-L-lysine and poly-L-glutamic acid (PLL/PLGA) using effect of pH buffer solution. The loading and release of both charged drug (cefazolin, gentamicin and methylene blue) can be controlled by several variables, including deposition layer, pH of films preparation and post-preparation treatment. By changing the interaction between drug molecules and multilayer films components, the films-drug interaction can be tuned the release rate. Therefore, it is still challenging to consider the developed multilayer thin films with active small drug molecules, which can be controlled over loading and releasing at the definite time. Several hydrophobic and hydrophilic drugs have been used to study for their ability of the mechanism action and their active sites. Curcumin is an active ingredient of turmeric isolated from the rhizome of Curcuma Longa Linn. It has been used to

relieve the pain and wound healing in traditional medicine.[26]Curcumin possesses not only chemo preventive property but also exhibits anti-oxidative, anti-cancer, and anti-inflammatory properties.[27] Diclofenac sodium is a well-known non-steroidal anti-inflammatory drug (NSAID) used for treatment of different diseases, such as rheumatoid arthritis and osteoarthritis.[28] Gentian violet is also known as crystal violet dry, which is used as active ingredient in gram's stain to classify bacteria and an acid–base indicator. It has ability to antifungal, antibacterial and used as an external skin disinfectant in humans and animals. [29]

The objective of this dissertation is to construct polyelectrolyte multilayer thin films and exploit their potential used as a biocoating material for over loaded and released model drugs. In this studies, multilayer thin films was first constructed by strong and weak polyelectrolyte base on LBL self assembly technique. The growth of multilayer thin films was characterized physical and chemical properties such as thickness, roughness, stability and decomposition by uv-vis spectroscopy and AFM measurement. The obtainable multilayer thin films were used as polymer matrix for control loaded and sustained release of model drugs. Hydrophobic drug (curcumin), anionic drug (diclofenac sodium) and cationic drug (gentian violet) were loaded into multilayer thin films. These model drugs can be easily traced visually as well as assayed spectrophotometrically due to their characteristic absorbance spectra. By changing the solvent composition, pH of drugs solution, loading time as well as number of layers, the loaded of model drugs into multilayer thin films were controlled. Solvent composition and buffer pH solution was used as a release medium in order to realize the release characteristic of model drugs through polymer matrix. The release rate of model drugs from each multilayer thin films condition during period time was determined by uv-vis spectroscopy. The effect of buffer pH solution and ionic strength of release medium was also investigated. Furthermore, suppression of the initial burst and prolonging the duration of release of hydrophobic drug molecule from polyacrylic substrate and polymer matrix were achieved by controlling the blocking film to hinder release. Finally, multilayer thin films were applied to coat on surface of fruit. The result can prove that multilayer films-based coating could be

used effectively as carrier of hydrophobic drug. This is a new method that might have potential used of bio-coating material in biotechnology application.

CHAPTER II
THEORY AND LITERATURE REVIEW

2.1 Nanotechnology on drug delivery system

Nanotechnology is the manufacturing and engineering of materials at the atomic and molecular scale. In its definition, nanotechnology refers to structures in the rang 1-100 nm size regime in at least one dimension.[30] Despite this size restriction, nanotechnology commonly refers to structures that are up to several hundred nanometers in size and that are developed by bottom-up or top-down engineering of individual components. Nanotechnology is expected to have an impact on all industries including semiconductors, manufacturing, and biotechnology.[31] One of the most promising societal impacts of nanotechnology is in the area of drug delivery.

The application of nanotechnology to drug delivery is widely expected to change the area of biotechnology industries, pharmaceutical application and medical application. The Personalized health care, rational drug design, and targeted drug delivery are some of the benefits of a nanotechnology-based approach to therapy.[32] The advantage of nanotechnology for drug delivery can summarized as following:

1. Improved delivery of poorly water-soluble drugs;
2. Targeted delivery of drugs in a cell and specific tissue area;
3. Delivery of large macromolecule drugs to intracellular sites of action;
4. Co-delivery of two or more drugs or therapeutic modality for combination therapy;
5. Transcytosis of drugs across tight epithelial and endothelial barriers;
6. Real-time read on the *in vivo* efficacy of a therapeutic agent.
7. Visualization of sites of drug delivery by combining therapeutic agents with imaging modalities

Additionally, the manufacturing complexity of nanotechnology therapeutics may also create a significant hurdle for generic drug companies to develop equivalent

therapeutics readily. Therefore, nanotechnology was mainly enormous impact that promises for drug delivery.

2.2 Drug delivery system

Drug delivery systems are an effective way to control over the concentration of therapeutic agents and to improve their bioavailability. The blood level of a drug is known to rapidly increase after administration and gradually decreases below the lowest level required for therapeutic action. [33] Consequently, repeated administration of a drug is often required for patients to maintain the blood level of the drug within the range of effective therapeutic action. However, drug molecules are embedded in any kind of substrate that can control the release rate of the drug, which results in sustained drug release. In addition, systems for stimuli-sensitive drug release can be constructed if the polymer carrier is sensitivity to specific stimuli. The external stimuli such as light, temperature, electric and magnetic fields, are often used as the stimuli by which drug release is controlled.[34]Moreover, the internal stimuli including pH, ionic strength, biological ions and molecules can also influence.[35] Therefore, stimuli-sensitive drug delivery, required amounts of the drug can be released at the site of drug action in response to stimuli.

Targeted drug has also been attracting attention due to its therapeutic advantages in improving bioavailability, lower toxicity and minimizing systemic side effects. [36] Drug-embedded microparticles can be targeted to specific cells or tissues by modifying the particle surface with a ligand.[37] Differently shaped nano carriers made from a wide range of molecules architectures and composed of various monomer units can lead to use as drug-carrier matrix as shown in Figure 2.1. Nowadays, it is interesting to look at the structures of polymers that are currently in clinical trials as drug delivery carriers. Therefore, different types of natural and synthetic materials are widely employed for the construction of devices for controlled drug delivery.

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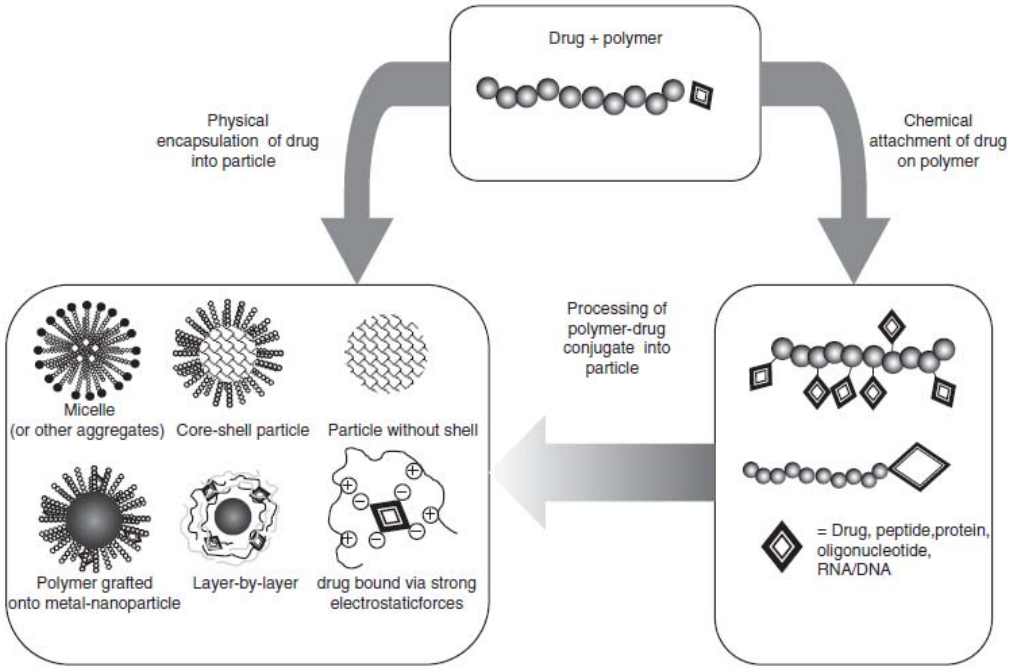


Figure 2.1 Common types of nanocarrier drug delivery systems for combination therapy.

2.2.1 Mechanism of drug delivery

Mechanisms that control the rate of drug release from the polymeric drug delivery systems can be classify as being controlled by diffusion controlled, swelling controlled, erosion controlled and by an external stimulus controlled. [38]

Firstly, diffusion-based delivery systems release drug down a concentration gradient. Despite alterations to the polymer or the geometry to manipulate drug release, Fick's laws of diffusion, was limited release, resulting in nonlinear release profiles.[39]

Secondly, swelling-controlled systems release based on water swelling of the delivery system. The Swell behavior can increases polymer chain flexibility and makes larger pores, resulting in better drug mobility. Therefore, drug release profiles may be linear release for short periods, resulting in dissolution of drug from interface at the definite time.[40]

Thirdly, Erosion-controlled systems have gained popularity with the development of biodegradable polymers. This release behavior was used the loss chemical or physical polymer or material to control drug delivery. Drug release from these systems can be complicated.[41] However, erosion is generally a combination of mass transport and chemical reaction, which can involve drug dissolution, porosity creation, polymer degradation, diffusion in polymer matrix, micro-environmental changes in pH, and autocatalytic effects. Release from erosion controlled systems can be product multiphase combination depending on the level of polymer degradation in the system.

Finally, stimulus-controlled systems release therapeutic factors upon activation by a stimulus, leading to a chemical or physical change in the delivery environmental. The change in the polymer allows for increased side chains motility, and thus release of the active molecules. Common stimuli include physical changes in temperature, pH, or chemical composition. [42]The summarize of drug release control represent in Figure 2.2.

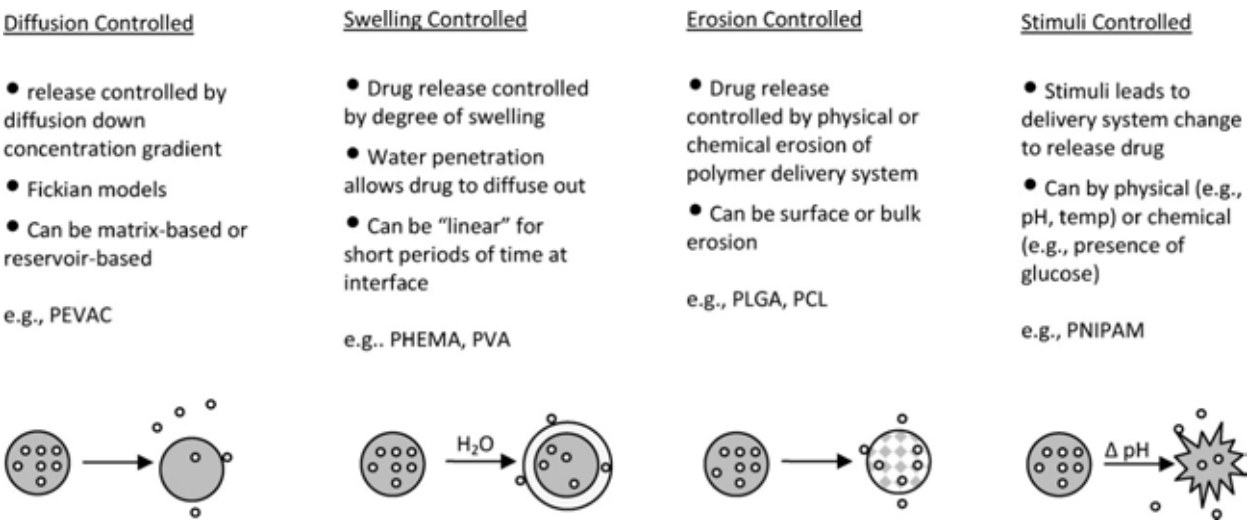


Figure 2.2 Summary of four categories of drug delivery systems based on mechanism of drug release.

2.2.2 Polymer matrix for drug delivery

One of the most common approaches to get controlled release is to embed a drug in a hydrophobic or hydrophilic matrix to speed up or to reduce drug release kinetics. Although wax, polyethylene, polypropylene, and ethylcellulose usually constitute hydrophobic matrices, hydrophilic matrices are generally made up by hydroxypropylcellulose, methylcellulose, and polysaccharides.[43] Matrix-based delivery systems find a great variety of applications in the pharmaceutical field especially in oral administration. Matrices can be prepared by mixing the drug, in the form of a thin powder, with the prepolymer. Then, the whole mixture is poured into the polymerization reactor.[44] Alternatively, matrix can be structured in advance and then put in contact with a highly concentrated drug solution able to swell the matrix (solvent swelling technique). Another approach relies on the mechanical energy supplied to the drug–carrier (usually a polymer constituting a matrix network) couple by cogrinding. In this manner, it is possible to load a drug into a matrix network avoiding the use of solvents, whose elimination from the final formulation can represent a very expensive and delicate step.[45] In addition, it can represent a profitable tool to achieve drug loading inside matrices. Indeed, if on one hand supercritical fluids show a density approaching that of liquids (this usually implies a good solubility with respect to drug or solvents used in the solvent swelling technique), then on the other hand they are characterized by low viscosity, typical of gases. Consequently, they easily and efficiently swell the matrix (bringing the drug inside the matrix network or extracting solvents) and they can be then removed by a simple pressure decrease. [46] Indeed, as a pressure decrease provokes the transition from the supercritical condition to the gas one, the matrix can be easily devoided by the supercritical solvent without dragging out the drug.

2.3. Layer-by-Layer self assembly technique

There is an increasing interest in developing new coating to prepare biomaterial matrix surface that can be control over loading and release in drug delivery area. Being the focus of many researches, the most important step toward this end concerns the improvement at the nano-scale and micro-scale of materials which surface properties can be turned to thickness or roughness. Many kinds of technique have been used for the fabrication of films coating on substrate such as leaching of glass, sol-gel deposition, chemical vapor deposition (CDV), sputtering, grafting and Layer-by-Layer (LbL) self assemble method.

Since polyelectrolyte multilayers were first introduced by Decher et al.[47], the amount of interest attention to these ultra-thin polymer films has grown exponentially. The major reasons for this interest are their ease of processing, variety of materials which can be incorporated into assembly system, and the versatility of the substrate. Compare to other strategies, LbL self assemble technique approach offers several applications such as electrochromic devices,[48] nonlinear optical devices, optical sensors, [49] and biomedical application.[50] The multilayers can be applied using any method that allows the polyelectrolyte solution to come into contact with the substrate, with the most common methods including dip-coating, deposition on colloids, or spin-coating. Besides the variety of materials that can be incorporated into the films, a wide range of substrates can be employed including polymers, metals, fiber, glass and particle. Therefore, The LbL process is relatively easy to perform and it should be possible to apply it to materials of virtually any size and shape, which for most applications makes it superior compared to other coating techniques.

2.3.1 Definition and general description of polyelectrolyte

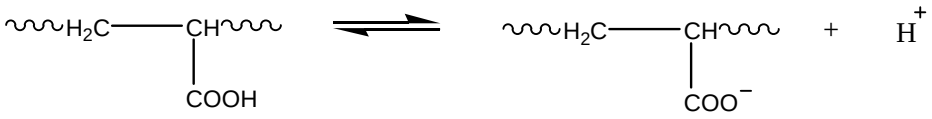
The term “polyelectrolyte” is employed for polymer systems consisting of a “macroion,” i.e., a macromolecule carrying covalently bound anionic or cationic groups. Examples of anionic and cationic polyelectrolyte (PEL) are presented in Figure 2.3.

(a) (b)

Figure 2.3 Structure of (A) PSS as an anionic polyelectrolyte and (B) PDADMAC as an cationic polyelectrolyte.

Both polystyrene sulfonate and poly (diallyldimethylammonium chloride) are dissociated into macroion and counterions in aqueous solution in the total pH range between 0 and 14.[51] Also polymers like poly(acrylic acid) or poly(ethylene imine) are usually classified as polyelectrolytes, in spite of the fact that they form a polyion-counterion system only in a limited pH range, and remain as an undissociated polyacid in the acid range or an undissociated polybasic in the alkaline range as shown in Figure 2.4 This is a behavior typical for weak polyelectrolytes and quite analogous to weak low molecular electrolytes. [52]

On the other hand, a polymer like cellulose capable of dissociating partially into cellulosed anions and counterions at extremely alkaline conditions (pH > 14) can not be classified as a polyelectrolyte, as in the conventional pH range of dilute aqueous systems the OH groups of polymer are not ionized.



(A)

(B)

Figure 2.4 Dissociation equilibrium of the weak polyelectrolytes. (A) Poly (acrylic acid) and (B) Poly (ethylene imine).

A special case of polyelectrolytes, the “polyampholytes” carrying both anionic and cationic groups covalently bound to the macromolecule, are represented in nature by an abundant number of proteins but can also be obtained by various synthetic routes. An example is presented in Figure 2.5 as a typical polyampholyte, this copolymer carries cationic charges in an acid and anionic charges in an alkaline medium, while at the so-called “isoelectric point,” in the example pH 4, no free net charge exists in the macromolecule. [53]

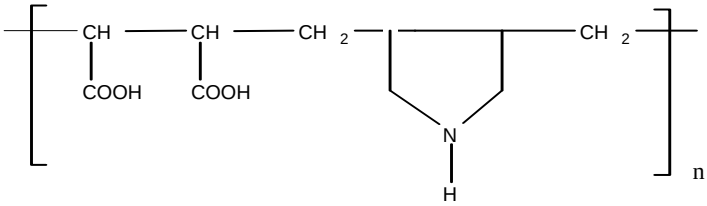


Figure 2.5 Chemical structure of a maleic acid-diallylamine copolymer

In principle, any macromolecular chemical structure can be transformed into a polyelectrolyte structure by covalently attaching a reasonable number of ionic groups to the polymer backbone, with linear or branched macromolecules at a compound soluble in an aqueous medium of appropriate pH after a sufficient number of ionic groups. [54] While in the case of a cross linked polymer its swell ability in aqueous media is enhanced by transferring into a polyelectrolyte. Limiting our further considerations to linear and

branched structures, a vast number of polyelectrolyte classes are known today, a section of which is listed in Table 2.1

Table 2.1 Selected classes of polyelectrolytes.

Anionic and cationic polysaccharides and polysaccharidic derivatives
Nucleic acids
Gelatin
Lignosulfonic acids
Polyacrylic and polymethacrylic acid and its copolymers
Maleic acid anhydride copolymers
Polystyrene sulfonic acid
Polyethylene imine
Polyamines and polyamidamines
Ionenes
Poly(diallyldimethylammonium chloride)
Homo-and copolymers of cationic acrylic acid esters

Today's commercial polyelectrolytes are predominantly obtained by a polymerization, polycondensation, or polyaddition process. [55] Also numerous important polyelectrolyte also originate from nature, such as gelatin, as a representative of the widespread class of proteins or pectin belonging to the group of anionic polysaccharides. Furthermore, some polyelectrolytes of practical importance result from a chemical modification of nonionic natural polymers such as cellulose or starch.[56] From these relevant articles from polyelectrolyte multilayers were successfully constructed and then some published articles were studied about the parameters controlling the growth of PEM as follow.

Dubas and Schlenoff[57] studied the factors controlling the growth of polyelectrolyte multilayers and the dependence of polyelectrolyte multilayer thickness on

salt concentration, salt type, solvent quality, deposition time, and polymer concentration. Polymers are deposited on spinning silicon wafers. For the strong polycation/polyanion pair studied, film thickness is approximately proportional to the number of layers and the salt concentration. The irreversibility of overall molecule adsorption is indicated by the lack of exchange of surface (radiolabeled) of solution polymer. The hydrophobic nature of the driving force for polymer sorption is illustrated by the choice of salt counterion or solvent. Salt interact with polyelectrolyte segments can permit localized rearrangements. In the mechanism proposed, excess polymer is accommodated within several layers, rather than in one layer of loops and tails. Steric barriers coupled with slow conformational changes are responsible for long-term polymer adsorption. Considering the disorder and interpenetration, multilayer buildup has much in common with solution phase or coprecipitated polyelectrolyte complexes. Surface hydrophobicity can be enhanced using fluorinated surfactants as counterions.

Dubas and Schlenoff [58] then studied polyelectrolyte multilayer containing a weak polyacids. They found that the growth of multilayer made from a combination of a weak polyacid and a strongly polycation is a function of salt concentration and molecular weight. Film thickness reaches a maximum at around 0.3 M of NaCl and then decreases quickly. The multilayer thin films are shown to decompose rapidly when exposed to aqueous solutions of NaCl more than 0.6 M. The apparent dissociation of multilayer polyelectrolyte complexes is due to competition for polymer/polymer ion pairs by external salt ions.

2.3.2 Formation of polyelectrolyte multilayer thin films

Polyelectrolyte multilayer films created via Layer-by-Layer (LbL) deposition are currently used to modify the surface properties of materials. These polyelectrolyte based films are capable of self-organization. The self-organization process of polyelectrolyte films, also referred to as electrostatic self-assembly, has been well documented over the past ten years. [59]

The LbL process is based on the alternating adsorption of cationic polyelectrolye and anionic polyelectrolyte as show in Figure 2.6. The process begins by properly charging a substrate. The charged substrate is then immersed by adsorbing a layer of a positively charged polyelectrolyte to that imparted to the substrate. Once the surface of substrate is positively charged, it is then immersed into a solution of negatively charged polyelectrolyte. A rinse step is included between the two adsorption processes to remove excess as well as to prevent cross-contamination of the polyelectrolyte solutions. These simple steps complete the LbL deposition of the nanolayers. Multiple layers can be created by simply dipping the substrate in alternating cationic and anionic baths.

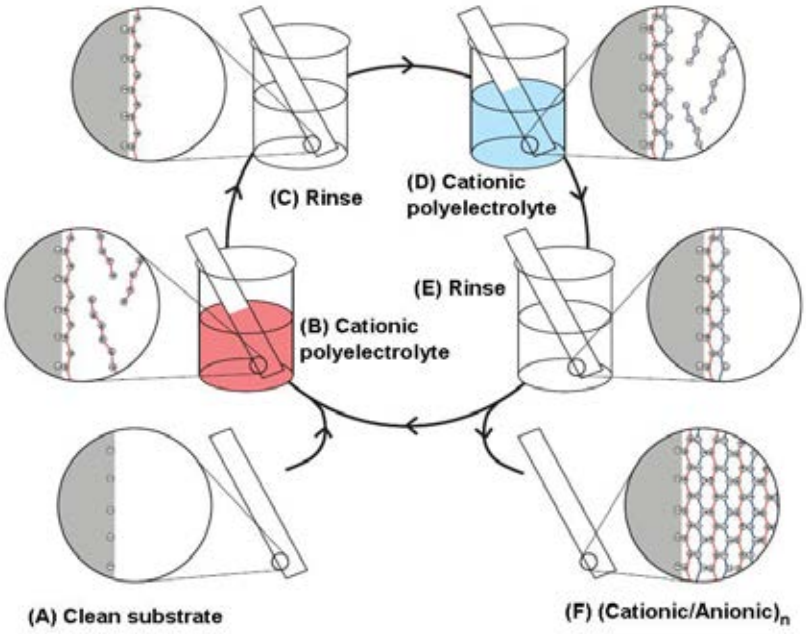


Figure 2.6 Buildup of LbL multilayer structures. (A) A clean substrate is (B) immersed in a positively charged polyelectrolyte solution (C) rinsed, (D) consecutively submerged in a negatively charged polyelectrolyte solution and (E) rinsed. The steps B–E are then repeated “n” times, until (F) the desired number of layers pairs are deposited.

2.3.3 Experimental parameters and LbL absorption

Given the large set of materials which are easily incorporated into multilayer films, LbL deposition is a rather general approach for the fabrication of complex surface coatings. It combines several parameters that control the films build-up as shown in Table 2.2 It is possible to coat almost any solvent-accessible surface starting with sub-micron objects up to the inside of tubing or even objects with a surface of several square meters. [60] Like a chemical reaction, the precise structure of each layer depends on a set of control parameters such as, salt concentration, number of layers, pH, or polyelectrolyte concentration but in general the processing window is rather broad.

Table 2.2 Parameters controlling the growth of PEM.

Major parameters	Minor parameter
NaCl concentration	Polyelectrolyte concentration
Number of layers	Deposition time
pH	Surface roughness
	Surface charge

2.3.3.1 Effect of NaCl concentration

In the case LbL thin film, electrostatic interactions between the positively charged and negatively charged result in film formation. Therefore, alterations in the electrostatic charge via ionic strength were affecting the build-up of polyelectrolyte interaction. Multilayer film thickness can increase linearly or exponentially with each step, when the polyelectrolyte in the solution interacts exclusively with the outer layer of the multilayer film.[61] Generally, an increase in ionic strength of NaCl concentration results in an increase in film thickness due to polyelectrolyte charge compensation resulting in more globular rather than extended polyelectrolyte structure. With diffusion of the adsorbing polyelectrolyte into the interior takes place, the film thickness increases exponentially.

NaCl ions affected the electrostatic charge between layers and consequently on layer thickness many types of films such as PDADMAC/PSS.[62] However, an increase in NaCl concentration compensated all charge and the polyelectrolyte formed turbid, resulted in dispersions. No adhesion or successful multilayer thin films could be constructed under these conditions. It is clear that an increase in the NaCl concentration in the polyelectrolyte solution will force the adsorption step to slow down due to competitive binding between $Na^{+}Cl^{-}$ and polyelectrolyte charged. Therefore, exceeding the critical salt concentration the adsorbed polyelectrolyte on a surface may even be supported the growth of films.

2.3.3.2 Effect of number of layer

Once of the basis method for the control of multilayer thin film growth is to vary the number of layers. In the case, the main parameters which are pH of solution, NaCl concentration, the concentration of polyelectrolyte and dipping time are constant. The increase of number of deposition layer can increase thickness of multilayer thin films.[63] The exponential growth is observed in higher number of deposition layers. Therefore this simple parameter can vary the obtainable thickness in LbL deposition process.

2.3.3.3 Effect of pH of medium

A change in the pH of the solutions will alter the swelling and disassembly of the polyelectrolytes and ions, which will alter the successive adsorption steps. Similarly to NaCl concentration, a change in pH range also resulted in a linear or exponential growth of film layers for pH-dependence polyelectrolyte upon a charge density mismatch under defined pH environmental.[64] Moreover, a critical charge density was elaborated for selected polyelectrolyte absorbed in media containing a varying NaCl concentration. Below the unexpected pH value, no growth in the multilayer construct was observed. [65]

Multilayer layer thickness of strong polyelectrolytes is amendable by adjusting the salt concentration in the respective solutions, whereas layers constructed from weak polyelectrolyte are more sensitive to the variation in the pH solution medium. Thus the film thickness can be controlled by adjusting both of ionic strength and/or the pH of a specific polyelectrolyte solution.

2.3.3.4 Effect of polyelectrolyte concentration

Concentrated of polyelectrolyte solutions are required for successful adsorption and to prevent colloid depletion during LbL process and to exceed the minimum concentration for absorption that can reverse charge polarity for each adsorbed layer. The optimum polyelectrolyte concentration is primarily dependent on solubility and charge density of the polyelectrolyte chain.[66] Previous studied showed the optimum concentration of PADMAC and PSS is 10 mM that lead to thickness films.[67] Above that, concentration was irrelevant to adsorption, however, resulted in an exponential increase in the thickness of the monolayer.

2.3.4 pH tunable weak polyelectrolytes

Early studied of LbL self assembly systems focused on use of strong polyelectrolytes, where the charges density is effective constant over a broad pH rang. More recently, weak polyelectrolytes are increasingly being used in multilayer thin films because of the greater controlled over film properties. Polyelectrolyte multilayers made from weak polyelectrolytes have the advantage that their properties can be tuned by simple pH adjustments. For this reason, the pH of weak polyelectrolyte solutions is an extremely important parameter when controlling the films.

Generally, pH-sensitive polymers have weak acids or bases with pKa values between 3 and 10. [68] Primary or tertiary amino, carboxylic and sulfonate groups exhibit a change in ionization state as a function of pH, polymer conformation ,transitions in

solubility, and swelling arise due to changes in ionization, where specific polymer groups switch between a neutral and charged state. When weak polyelectrolyte is constructed as constituents of multilayer, their sensitiveness to the pH of the polyelectrolyte dipping solutions may induce dramatic changes in the thickness of the sequentially adsorbed polyelectrolyte layer.[69] Using this approach, controlling the thickness and reversible pH-induced swelling with the deconstruction and desorption of polyelectrolyte multilayers thin films have been observed. [70]

One system that has been wildly studied is that of poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH).[71] This weak polyelectrolyte pairs is promising as dramatic changes in film properties that can be achieved by variations in pH. This enables greater control over not only layer thickness, but also film morphology and internal composition of the polymer film as changes in pH alter the charge density and conformation of weak polyelectrolytes. Poly(acrylic acid) (PAA) is a weak polyanion, well-known for its biocompatibility, and is widely used as polyelectrolyte in different drug delivery applications.[72] Moreover, the presence of carboxylic groups in PAA allows it to be functionalized with bioactive molecules such as growth factors, proteins peptides.

Another studied for obtaining the weak polyelectrolyte system is the used of poly (4-styrene sulfonic acid-co-maleic acid) copolymer (PSSMA), which composes of styrene sulfonated groups (SS) and maleic acid groups (MA). In this system, the carboxylate of maleic acid are either protonated or deprotonated depending on the solution pH, while the sulfonated groups remain charged across most of the pH rang. In another example of poly (4-styrene sulfonic acid-co-maleic acid) copolymer (PSSMA) layers with poly(allylamine hydrochloride) (PAH),[73] the prepared films exhibited greater responsiveness to pH and ionic strength compared with the traditional PSS/PAH films.[74]

Most of natural polyelectrolytes that have been used are polysaccharides, such as, chondroitin sulfate, dextran derivatives, hyaluronan, and chitosan (Chi).[75] Chitosan is a biodegradable polysaccharide derived by partial deacetylation of chitin. Chitosan has been widely used in biomedical and pharmaceutical areas, due to its favorable biological

properties such as, biocompatibility, biodegradability, low toxicity, bacteriostatic, hemostatic, and anticancerogen. [76] The deacetylated chitosan backbone of glucosamine units has a high density of amine groups, permitting strong electrostatic interactions with biomolecules which carried overall negatively charged at neutral pH conditions. Chitosan has been the subject of interest for its use as a polymer matrix for drug carrier in dosage form design due to its biodegradability, low toxicity and relatively low production cost from abundant natural sources. However, one drawback of using this natural polysaccharide in modified release dosage forms for oral administration is its fast dissolution rate in the stomach. [77] Since chitosan is positively charged at low pH values (below its pKa value), it spontaneously associates with negatively charged polyelectrolyte to form polyelectrolyte complexes and polyelectrolyte multilayer thin films.

Alginate (Alg) is an anionic polyelectrolyte that composed of a naturally occurring block copolymer of two monosaccharide units obtained from marine brown algae.[78] The negatively charged carboxylic acid groups of alginate interact with the positively charged amino groups of chitosan by electrostatic reaction to form polyelectrolyte multilayer thin films. In addition, alginate is one of the most negatively charged polyelectrolyte to form polyelectrolyte complex with chitosan because they are still biodegradable and biocompatible but mechanically stronger at lower pH values where chitosan dissolves. [79] It was shown that ionic strength, pH, charge ratio and molecular weight that influence the particle size, particle surface charge and stability of Chi/Alg polyelectrolyte complexes. [80]

In a study wherein chitosan and alginate were reacted in their completely ionized states by maintaining the pH values of each polyelectrolyte solution at a specific value (pH 2 for the chitosan solution and pH 6.5 for the alginate solution) polyelectrolyte complex beads were formed that showed improved stability of the entrapped α-amylase.[81]The selected pH values can cause an increased charge density on each polymer and led to intense cross-linking during polyelectrolyte complex formation and consequently beads with small micropores were formed.

Yuan and co-workers[82] constructed Chi/Alg multilayer thin films via layer-by-layer self-assembly. They demonstrate that the Chi/Alg surface composition of the self-assembled multilayer film can be controlled over pH during the assembly process. With decreasing of assembly pH of alginate, less positively charged chitosan can interpenetrate to the outermost layer of alginate, resulting in an increase of negative charge density from alginate for more antibody immobilization. As result, the loading capacity of the antibody on the multilayered film and the binding activity of the antigen to the immobilized antibody could be changing by pH control. This work can provide the scientific insight in the interaction between antibody and Chi/Alg polymer matrix that render a novel simple approach to build high-performance biointerfaces by pH control.

2.3.5 Polyelectrolyte multilayer as biofilms

One of the main challenges in bionanotechnology, pharmaceutical and biomedicine is to develop a system able to provide a controlled release of bioactive molecule. This is attractive in view of the obvious advantages of controlled release, such as high sustain loading efficiency and lower toxicity. LbL films containing bioactive molecules offer the ability to not only the amount of the molecules but also to control the release and thus enable control “on demand”. Therefore, LbL films can act as reservoirs and release carriers. Bioactive films made by the LbL technique have been extensively studied by many research groups.[83] The films can host not only bioactive molecules introduced as constituents of the film, but also carriers with encapsulated various biomolecules. Many biomolecules that can incorporate into LBL films are biomacromolecules, carriers, drug and particles as illustrated in Figure 2.7.

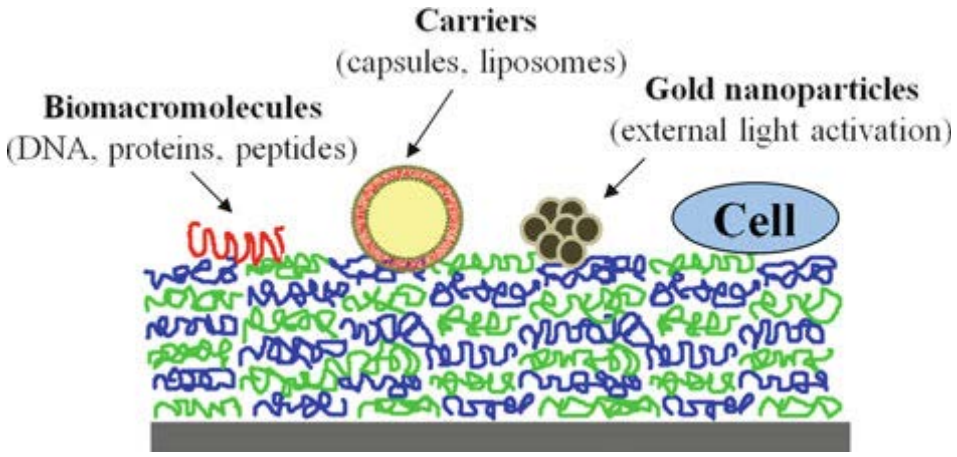


Figure 2.7 LbL assembled polymeric films, which can host different species molecule.

Bioactive macromolecules like peptides, proteins, and nucleic acids have been successfully embedded in LbL films. One of the main challenges in the field of biocoating engineering by the LbL method is to increase the load of biomolecule in the films within refinish time. Taori et al.[84] construct ultrathin multilayer assembly of cationic poly(L-tartaramidopentaethylenetetramine) (T4) and anionic pDNA for in vitro controlled release of plasmid pDNA. The results indicated that the increase loading capacity of pDNA with respect to an increase in the number of T4/pDNA bilayers deposited. An increase in the characteristic absorbance at 260 nm for double stranded pDNA implied the increased incorporation of pDNA with the increase in the number of T4/pDNA bilayers. For these reasons, they hypothesized that T4 was an excellent candidate to create stable and ultrathin multilayer films via LBL deposition of polycation and polyanion on various substrates.

The combination of the advantages of liposome with of LbL assembly technique was also investigated. Haidar and team work[85] prepared core shell nanoparticle via the LbL self-assembly of chitosan and alginate for delivery of biomacromolecules such as, bovine serum albumin, (BSA). They characterized nanoparticle and loaded BSA to evaluate its encapsulation efficiency. This result obtained small and stable of cationic particles with average size of 383 nm. This system demonstrated a good capacity for the

encapsulation and loading of BSA than 80%. The in vitro release profile suggested that BSA has been entrapped in both, the aqueous liposomal core and within the polyelectrolyte shell.

Artemisinin (ART) and its derivatives are widely used throughout the world. ART is an endoperoxide-containing sesquiterpene developed from an ancient Chinese herbal. ART has been of special biological interest for many years because of its strong cytotoxic activity and antitumor activity. Chen. Et al.[86] studied the encapsulate ART with chitosan, gelatin, and alginate for the purpose of controlled release. ART crystals became hydro-philic after encapsulation, and the crystals were dissolved from PEM shells in a mild solution. Additionally, it has been proven that parameters of assembly conditions, such as the type of polyelectrolyte, the number of layers, and NaCl or ethanol concentration in polyelectrolyte solution are able to effectively control release properties of ART nanocapsule. The results of this work suggest that optimization of the capsule wall thickness and composition is expected to yield new-type nanometer capsules of natural drugs for con- trolled drug release.

Insulin delivery systems are in great demand to regulate the amount of insulin released according to the change of blood glucose level. Chen et al.[87] demonstrated glucose-sensitive LbL multilayer film based on a kind of 21-arm star polymer. The LbL multilayer film was consisted of positively charged poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) star polymer and negatively charged insulin and glucose oxidase (GOD). It is found that the unique structure of star PDMAEMA and inter diffusion of charged insulin are the main factors to control the on-off status of the film. GOD can convert glucose into gluconic acid thus change the pH of the microenvironment within the film. Then, the stretchable conformation of ionized star PDMAEMA and inter diffusion of charged insulin will cause a pH responsive phase. Due to its well-controlled structure in nanoscale, the novel multilayer film based on 21-armstar polymer has a great potential as a self-regulated insulin delivery system. The multilayer film could also continuously release enough insulin after being subcutaneously implanted.

2.3.6 Application of polyelectrolyte multilayer in drug delivery

A major challenge in the development of advanced drug delivery is the elaboration of delivery systems capable of controlling sustained loading and release of bioactive molecule. Controlled and release mechanisms via LbL deposition technique offer greater effectiveness such as lower toxicity and improved patient convenience over conventional method.[88] The advantage of layer by layer deposition technique in drug delivery, including 1) the ability to control the order and location of multiple component layers with nanometer-scale precision and 2) the ability to define the concentrations of incorporated materials simply by varying the number of multilayer. The release behavior would be dependent on the permeability or breakdown of the polyelectrolyte multilayer structure. The release stimulated of bioactive molecule, drug, dye and another material can cause by many parameters, such as pH-stimulated release, ionic strength-stimulated release, electrically stimulated release, thermo-stimulated release and photo-stimulated Release.[89]

Similar to the deconstruct mechanism of multilayer thin films using pH as the stimulus, changing the ionic strength of a solution has also been utilized to control the electrostatic interactions between polyelectrolytes or macromolecules and drrug, leading to improvements in stability or degradation of thin films depending on the chemical compositions within the composites1. Dubas et al. have shown that PAA/ PDDA multilayered films were removed at NaCl concentrations more than 0.6 M because the polyelectrolytes within multilayers were dissociated by ion-exchange competition of $Na^{+}Cl^{-}$ ions for polyelectrolyte ion pairs. Ren et al. [90] demonstrated the construction of PLL/DNA multilayered films contained 0, 0.05, 0.1, 0.5, or 1M of NaCl. They found that with the increase of the NaCl concentration in deposition solution, there was an increase incorporation of DNA into the multilayer thin films. The build up the films was sustained in NaCl concentrations up to 0.5 M. Then the further increase of the salt concentration decreased the incorporation of DNA. The salt induced change of the incorporation of DNA and PLL is mainly reason for the increase thickness of polyelectrolytes. NaCl-

induced the deconstruction of $(PLL/DNA)_{10}$ films was carried out to study the relationship between the ionic strength and the deconstruction of the films. They found that the multilayer thin films were deconstructed in relatively high NaCl concentration. With the increase of NaCl concentration, the extent of deconstruction of the films was increased.

The study of pH-dependent loading and release behavior in polyelectrolyte multilayer films has drawn similar conclusions: incorporation and release of materials from such films depends on the degree of film swelling, the ability of the guest molecules and bioactive molecules to diffuse in the film, and the attractive and repulsive interactions occurring between the bioactive molecules and the acidic/basic functional groups in the film. Jiang and coworkers[91] investigated the loading and release of a typical cationic dye, 5, 10, 15, 20-tetrakis(4-*N*-methylpyridyl)porphine-tetra-(*p*-toluenesulfonate) (TMPyP), in the DNA/PAH multilayer thin films. Stimulated by the pH change of the dye solution, the dye can be easily loaded into into the DNA/PAH multilayer thin films. They was expected that the protonation and deprotonation of PAH, TMPyP, and DNA in the film play important role in realizing these properties. In an alkaline condition, the dye could be rapidly loaded into the DNA/PAH film under room temperature, while in an acidic condition, the dye could be rapidly released. Based on this concept, Zhao et al.[92] synthesized calcium carbonate (CaCO3) particles with chondroitin sulfate (CS) through a reaction between calcium nitrate tetrahydrate solutions and sodium carbonate suspended with CS macromolecules. The CS-integrated microcapsules were loaded with bovine serum albumin (BSA) using as a model drug, and it was shown that pH was an effective in term of controlling the loading and release of BSA. Substantially more BSA was loaded at pH 3.8 than at pH 5.0 (i.e., pI of BSA. Moreover, pH can also be used to tune the release of encapsulated BSA from CS-integrated microcapsules. The electrostatic attraction of BSA and CS decreases as pH increases from pH 1 to 7.4. This is because BSA has net negative charges above its pI (pH 5.0) and net positive charges below its pI, and CS is negatively charged. As a result,

BSA and CS repel each other at a high pH and interaction each other at a low pH. This leads to a faster release of BSA at a higher pH.

As an alternative to the examples discussed above, novel thermo-responsive thin-film systems have been produced using the LBL method that show thermal tunability in control release of molecule. Among all the studied systems, microcapsule has been the most extensively studied as a vehicle for controlled drug release. Ye et al.[93] investigated the controlled release of indomethacin (IDM) encapsulated with natural chitosan (CHI) and alginate (ALG) as deposited materials using layer by layer deposition technique. Increasing deposition temperature can efficiently prolong the release rate by increasing the multilayer thickness and producing a more perfect multilayer structure. There are two possibilities for the above significant change in the release rate when deposited at higher temperature: Firstly, the ALG/CHI multilayer film on the IDM microcrystal becomes thicker. Secondly, the film becomes more compact through conformation rearrangement because ALG and CHI have the similar polysaccharide backbone structure and same distance between charged groups. Therefore, increasing deposition temperature can enhance the thickness and perfectness of the ALG and CHI multilayer film deposited on the IDM microcrystal. Therefore, the thermo-stimulated release provides an efficient method to control the release rate.

Another approach to realize film degradation without stimulated-release but rather by introduction of self-biodegradable products into the LbL process is also possible. Macdonald et al.[94] constructed polyelectrolyte multilayer films that incorporate synthetic, hydrolytically degradable polymer capable of controlled release and model protein. They investigated the sustained release of significant protein quantities from micron scale conformal thin films over periods of several days to weeks at body temperature. Protein rate and the kinetic of release from the film are tunable by choosing the anionic polyelectrolyte, the properties of the degradable polymer, as well as the number of layers that are deposited upon the surface of interest. By combining hydrolytic degradability in a polyelectrolyte directly with the protein of choice in LbL assembly, it

is possible to protect and retain protein for long periods of time while sustaining the ability for extended release at biologically conditions.

2.4 Model drugs

2.4.1 Curcumin

Curcumin 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6- heptadiene-2,5-dione (Figure 2.8) is a yellow colored pigment obtained from powdered rhizome of *Curcuma longa* Linn. It has been used to relieve the pain and wound healing in traditional medicine.[95] Curcumin was not only possesses chemo preventive property but also found to exhibit anti-oxidative, anti-cancer, and anti-inflammatory properties. Nowadays, Curcumin is becoming more and more popular medical herb due to its profound effect on human health. The pure curcumin on the maket consists of a mixing of three antrully curcuminoids : curcumin, demethoxy- and bisdemethoxycurcumin, which curcumin is the mail constituent. [96]

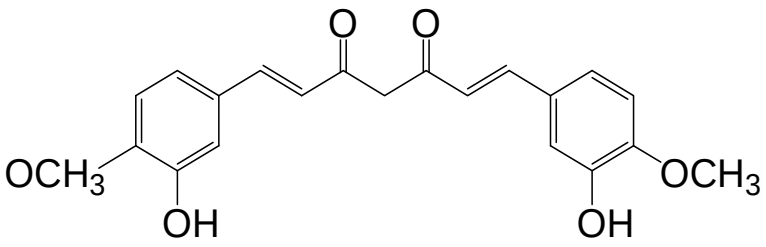


Figure 2.8 Molecular structure of curcumin.

Curcumin with polyphenol structure is water insoluble and scarcely dissolved in organic phase. Previously, absorption and metabolism of curcumin after previous time showed its poor stability properties. [97] Moreover, curcumin is unstable at neutral and basic pH, and serum-free medium caused it is degraded to various produces. A major challenge to manipulate curcumin for drug loading and release characteristics is the poor aqueous solubility, which significantly limits its availability in biological systems.

Curcumin is not water soluble, but it is soluble in organic solvent such as ethanol, methanol or dimethylsulfoxide. [98] The aqueous solubility of curcumin can be improved by increasing the organic solvent and prepared the water-soluble curcumin by incorporation into various surfactant systems (e.g. sodium dodecyl sulfate, cetylpyridinium bromide, gelatine, polysaccharides, polyethylenglycol, cyclodextrins).[99] However, this approach leads to an undesirable outcome such as a high rate of degradation by alkaline hydrolysis and their stability. Many attempts have been made to prepare through encapsulation in polymeric micelles, liposomes, polymeric nanoparticles, lipid-based nanoparticles, and hydrogels to increase its aqueous solubility and bioavailability.[100] Dubas and co-workers investigated loading of curcumin into PDAD/PSS polyelectrolyte multilayer films. [101] The loading of curcumin was driven by its partitioning in the PEM film, and its partitioning coefficient between the 80/20 solvent and the PEM thin film was found to have a value of $2.07x10^5$. They showed that the loading of curcumin into multilayer thin films films increased with the number of deposited layers, implying that almost of curcumin absorb into the bulk of the polymer matrix. Their results suggest that LbL thin films are a promising matrix for incorporating curcumin, especially for hydrophilic drug delivery applications.

2.4.2 Diclofenac sodium salt

Diclofenac sodium (Ds) is a nonsteroidal anti-inflammatory drug (NSAID) designated as 2-[(2,6-dichlorophenyl) amino] benzeneacetic acid, sodium salt (Figure 2.9). DS has shown anti-inflammatory, analgesic and antipyretic properties. It is used for the treatment of degenerative joint diseases such as osteoarteritis, rheumatoid arthritis and ankylosing spondilitis.[102] DS was dissolution in the low pH of gastric fluid but allows a rapid release of drug in the higher pH environment of the duodenum.

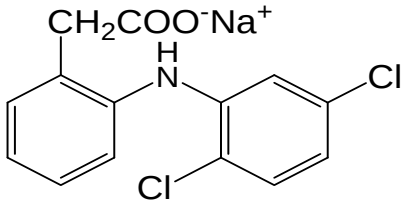


Figure 2.9 Molecular structure of diclofenac sodium salt.

In several investigations the feasibility of development of a sustained release form for diclofenac sodium was studied.[103] Agnihotri et al. [104] prepare nanoparticle from poly (lactide-co-glycolide) and poly (lactide-co-glycolide-leucine) loaded with diclofenac sodium (DS), with the aim of improving the ocular availability of the drug. The in vitro release profiles reported the burst effect that could be attributed to the escape of drug from the surface of the polymeric system. DS present in the polymeric matrix diffuses to the release medium through the pores and channels of the polymeric nanoparticle. Another reason for the rapid release of DS from such systems could be that the drug diffuses easily out of the polymeric systems when colloidal systems are placed in release medium at pH 7.4. Therefore, DS was ionized and distribution of the ionized molecules in the release medium to a faster by changing pH condition.

Schneider et al.[105] prepare cross-linked polyelectrolyte multilayer films (CL PEM) of chitosan/hyaluronan (CHI/HA) and poly(L-lysine)/hyaluronan (PLL/HA) for loading diclofenac sodium (DS) bysmple diffusion of the drug. DS loading can be modulated by varying the number of layers in the film. They observed that the amount of DS incorporated in the film is directly related to the number of layers in the film. Moreover, DS was released from the film in about 10 h. These results prove that it is possible to design multifunctional multilayer films that have bioactivity properties for loading bioactive molecule.

2.4.3 Gentian violet

Gentian violet (or Crystal violet) is a triarylmethane dye as shownthe structure in Figure 2.10. Gentian violet has antibacterial, antifungal, and anthelmintic properties and was formerly important as a topical antiseptic.[106] Gentian violet is used in many applications, including dye in textile and paper, pH indicator and using as active ingredient in gram's stain. The colour of the dye depends on the acidity of the solution. At a pH of 1.0 the dye is green with absorption maxima at 420 nm and 620 nm while in a strongly acidic solution (pH of -1), the dye is yellow with an absorption maximum at 420 nm. The different colour is a result of the different charged states of the dye molecule.[107] In the yellow form all three nitrogen atoms carry a positive charge, of which two are protonated, while the green colour corresponds to a form of the dye with two of the nitrogen atoms positively charged. At neutral pH both extra protons are lost to the solution leaving only one of the nitrogen atoms positive charged.[108]

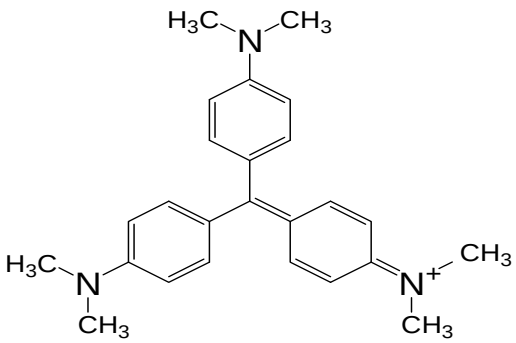


Figure 2.10 Molecular structure of gentian violet.

2.5. Edible film for fruit coating

The use of edible coatings represents one of the important methods being used for prolong fruit quality. Edible coatings have been traditionally used to improve fruit appearance and maintain quality because they are considered environmentally friendly.[109] Coating films can act as barriers to oxygen and moisture during handling,

processing and storage. Moreover, they can retard fruit deterioration by inhibiting the growth of microorganisms, due to their natural property or to the incorporation of antifungal compounds. Normally, edible films are made of polysaccharides or proteins that can also help to maintain moisture, thereby improving shelf life.[110] However, the hydrophilic nature of these compounds limits their ability to provide desired edible film functions. Current approaches to extend its functional and mechanical properties of these films composed of following:

1. Incorporation of hydrophobic compounds such as lipids to improve their resistance to water.

2. Optimization of the interaction between polymers (protein-protein interactions, charge–charge electrostatic complexes between proteins or polysaccharides).

3. Cross-linking or fictionalization through chemical, physical or enzymatic treatments.

CHAPTER III
EXPERIMENTAL

3.1 Research methodology

This research can be divided into three main parts as summarized in Figure 3.1-3.3. The first part is to construct and characterize polyelectrolyte multilayer thin films. The structure of polyelectrolyte multilayer thin films were used as a polymer matrix for absorb hydrophobic and anionic and cationic drugs. Parameters that control loading and released of model drug were demonstrated. The second part is to construct and characterize blocking film. The ability of blocking films barrier for prolong the release of hydrophobic drugs were represented. Effect of solvent release and blocking films type were also determined. The final part is to development polyelectrolyte multilayer thin films based-coated fruit. The hydrophilic/hydrophobic behavior of the polyelectrolyte multilayer thin films based-coated fruit was confirmed by a contact angle. Finally, multilayer thin films-based coating containing curcumin were then calculated curcumin content.

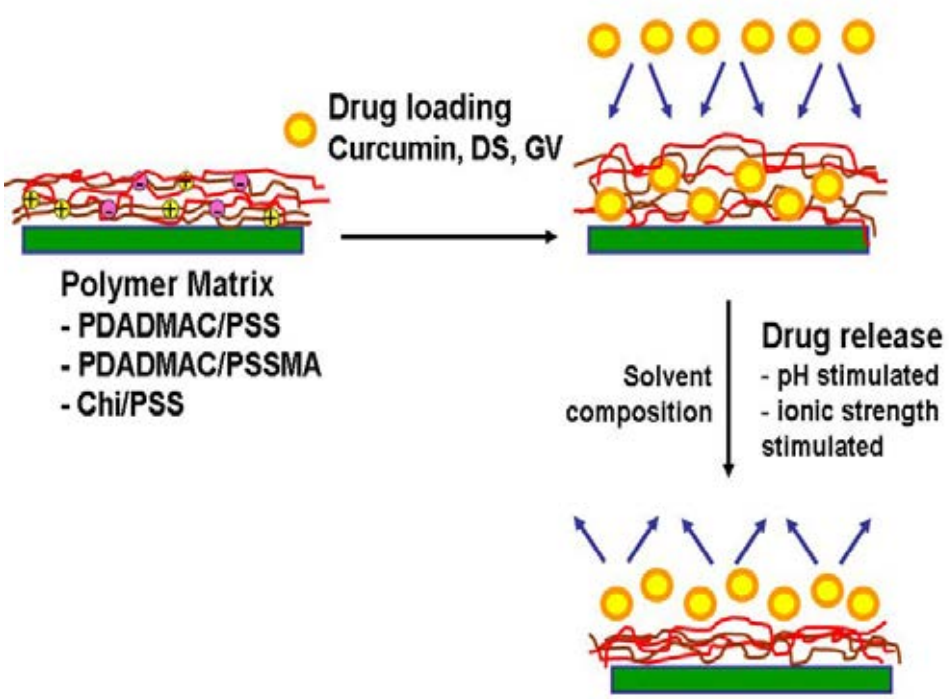


Figure 3.1 Schematic representation of experimental part I.

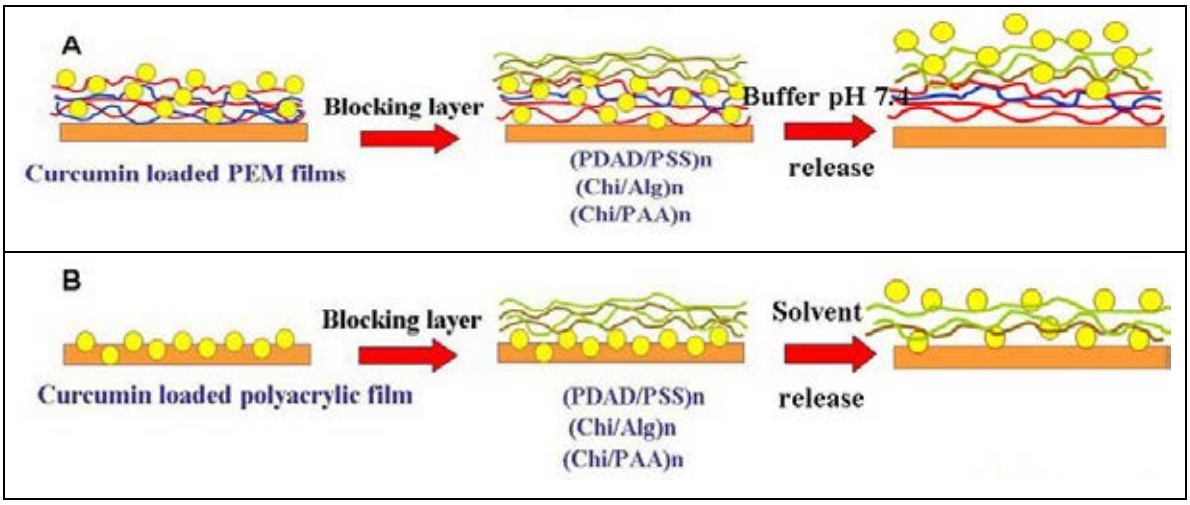


Figure 3.2 Schematic representation of experimental part II.

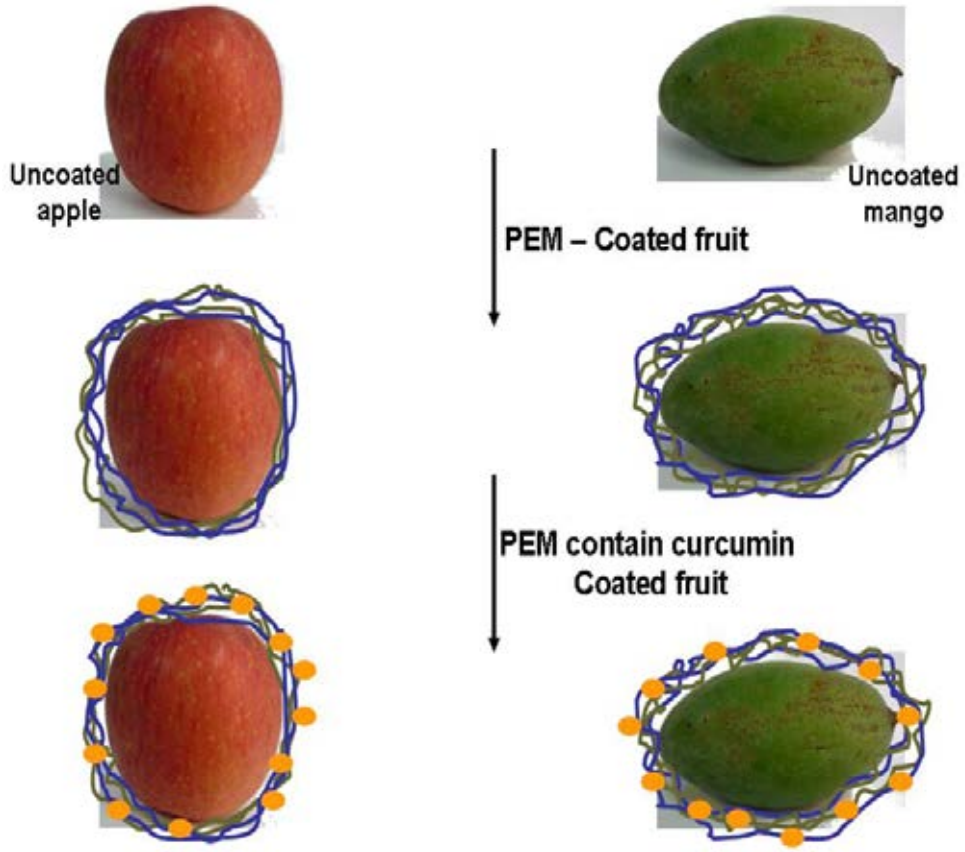


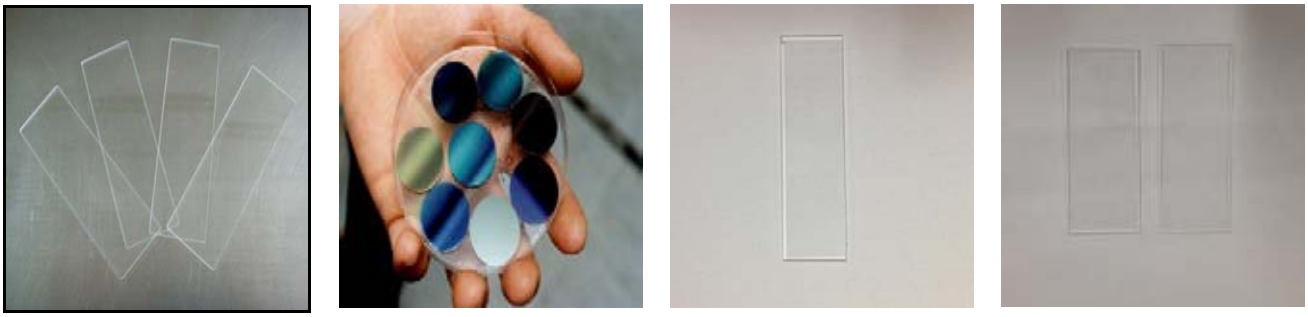
Figure 3.3 Schematic representation of experimental part III.

3.2 Materials and chemicals

3.2.1 Substrates

Substrates used in this research are showed in Figure 3.4.

1. Glass slides (78.2 x 25.4 mm. Sail Brand, Bangkok, Thailand)
2. Silicon wafer (Nove electronic materials, Carrollton, TX)
3. Quartz slides (50x 25 mm. Becthai, Thailand)
4. Polyacrylic films (50x 25 mm. Becthai, Thailand)



(A) (B) (C) (D)

Figure 3.4 (A) Glass slide (B) Silicon wafer (C) Quartz slides and (D) Polyacrylic films.

3.2.2 Chemicals

Chemicals used in this research are listed in Table 3.1. All chemicals were used as received without further purification. Double distilled water was used in all experiments.

Table 3.1 Chemicals used in this research

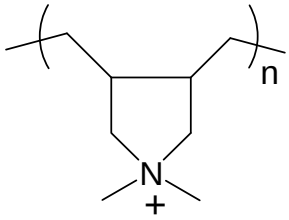
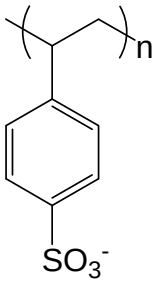
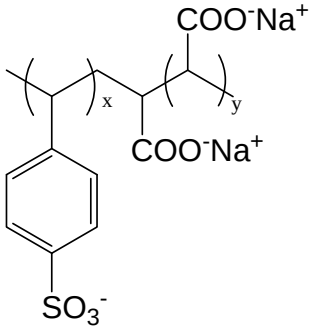
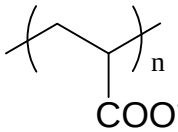
Chemicals	Chemical structure	Properties	Made
Poly (diallydimethyl ammonium chloride) (PDADMAC)		- Typical M_w 200,000-350,000 - bp 100°C - mp -2.8- 0 °C - density 1.04 g/ml	Sigma-Aldrich St.Louis MO
Poly(sodium 4-styrene sulfonate) (PSS)		- Typical M_w 70,000 - Density 0.801g/ml - mp 460 °C	Sigma St.Louis MO
Poly(4-styrene sulfonic acid-co-maleic acid, sodium salt) (PSSMA)		- Typical M_w 20,000 - Density 0.94g/ml	Sigma St.Louis MO
Poly(acrylic, sodium salt) PAA		- Typical M_w 5,00	Sigma St.Louis MO

Table 3.1 (cont.) Chemical used in this research

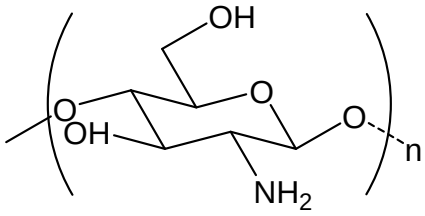
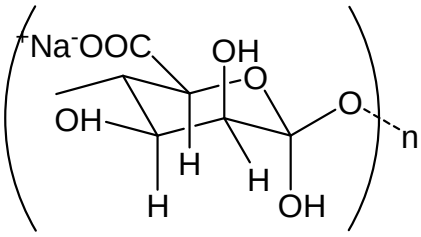
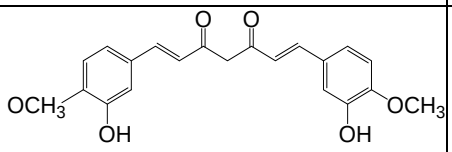
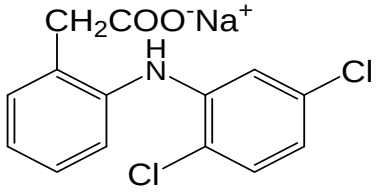
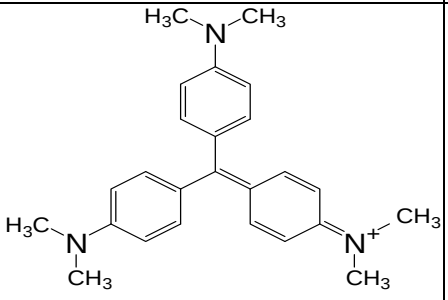
Chemicals	Chemical structure	Properties	Made
Chitosan (Chi)		- Mw 8 x 10^5 - 84% deacetylation - vicosity 200 cps	Sigma St.LouisMO
Alginate (Alg)		- viscosity 20-50 cps	Sigma St.Louis MO
Curcumin			Sigma St.Louis MO
Diclofenac, sodium salt (DS)		- mp 275-277 °C	Sigma St.Louis MO
Gentian violet (GV)		- mp 137 °C	Fluka Steinheim Germany
Sodium hydroxide	NaOH	- FW 40.0 - bp 1390 ^{0}C	Carlo erba, Italy

Table 3.1 (cont.) Chemical used in this research

Chemicals	**Chemical structure**	**Properties**	**Made**
Sodium chloride	NaCl	- FW 58443	Lab-Scan Bangkok Thailand
Acetic acid glacial	CH_3COOH	- bp 118 ^{0}C - density 1.05	Lab-Scan Bangkok Thailand
Di sodium hydrogen phosphate anhydrous	Na_2HPO_4	- FW 141.96	Lab-Scan Bangkok Thailand
Sulphuric acid	H_2SO_4	- FW 98.078 - density 1.835	Carlo erba, Italy
Ethanol	C_2H_5OH	- bp 78.3 ^{0}C - density 0.79-0.793	Carlo erba, Italy

3.3 Instruments

Instruments used in this research are listed in Table 3.2

Table 3.2 Instruments used in this research

Instrument	Brand/Model	Made
Automatic dipping machine	Self-made	-
pH/ion meter	Precisa, pH 900	Qingdao,China
Stirr	VELP Scientfica, Code 4001099	China
Atomic force microscope	Digital Instrument Nanoscope IIIa	Santa Barbara, CA
UV-spectrophotometer	SPECORD S 100, Analytikjena,	Tokyo, Japan

3.4 Experimental procedures

3.4.1 Layer by layer deposition of polyelectrolyte multilayer thin films for loading and release of model drugs

3.4.1.1 Preparation of coated substrates

Glass slides, quartz slides and silicon wafers were cleaned in 70% H_2SO_4 (concentrated) / 30% $H_2O_{2\ (aq)}$ (This solution is called “piranha” which is a strong oxidizer and should not be stored in closed containers). Then, the cleaned substrates were dipped in hot 30% H_2O_2/ammonia/water, 1:1:5 v/v in order to increase the electrostatic charge density. Subsequently, the substrates were rinsed in water and dried at ambient temperature, thus the cleaned substrate ready to deposition.

3.4.1.2 Fabrication of PEM films

Multilayer thin films were deposited on substrate using by layer-by-layer self assembly technique. Multilayer thin films of cationic polyelectrolyte and anionic polyelectrolyte were formed by repeating the following steps on the each substrate; (1) immerse the substrate in positively charged polyelectrolyte solution for 2 minute; (2) wash the substrate three times with distilled water or buffer solution for 20 sec to remove excess polyelectrolyte (3) immerse the substrate in negatively charged polyelectrolyte for 2 minute. (4) remove excess polyelectrolyte solution in the same procedure as in (2). Figure 3.7 shows the Layer-by-Layer deposition technique.

The immersing and washing procedure described above was repeated until the constructed multilayer thin films were obtained. By repeating the deposition cycle, (cationic polyelectrolyte /anionic polyelectrolyte)$_n$ were denoted where n is the number of deposition cycles or bilayers.

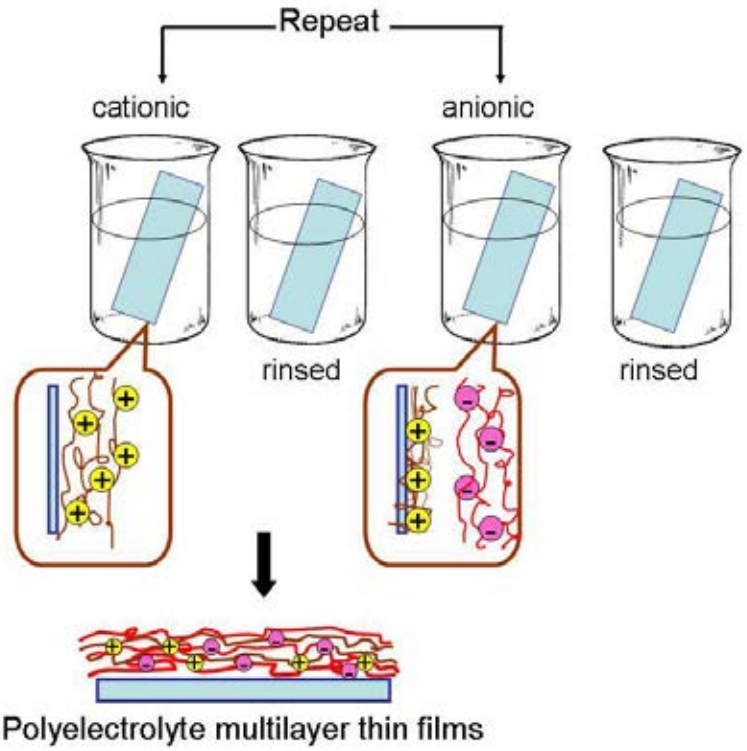


Figure 3.5 Schematic of the layer-by-layer self assembly technique.

Table 3.3 displays experimental conditions for constructing polymer matrix in Part I. The buildup of polyelectrolyte multilayer thin films was used both of strong and weak polyelectrolyte, including poly(diallyldimethylammonium chloride) (PDADMAC), poly (sodium 4-styrenesulfonate), Poly(4-styrene sulfonic acid-co-maleic acid, sodium salt) (PSSMA) and Chitosan (Chi). PDADMAC and PSS were used without pH adjusting. The pH of the PDADMAC/PSSMA solutions as well as the distilled water was adjusted to pH of 3, 4, 5, and 6 with 10 mM of acetic acid and 10 mM of sodium acetate as necessary, where as adjusted pH 8 and 10 by 10 mM of Na_2HPO_4. The pH of the Chitosan solutions as well as the pH buffer was adjusted to pH 3-6 by 10 mM of acetic acid, 10 mM of sodium acetate and/or 10 mM of NaOH. In both positively charged and negatively charged polyelectrolyte solutions sodium chloride salt was added, and then stirred in order to dissolve. After added the salt, pH of solutions might be adjusted if needed. The build up of multilayer thin films were characterized by UV- vis spectroscopy. The surface topographic feature of films and film thickness was monitored by atomic force microscope (AFM). The root mean square (RMS) roughness values were obtained from the AFM software. All measurements were carried out under room temperature.

Table 3.3 Experimental condition used in the preparation of PEM films.

Condition	**Concentration (mM)**		**NaCl Concentration (M)**	**Dipping time (minute)**	**pH**	**No. of layers**
	Cationic polyelectrolyte	**Anionic polyelectrolyte**				
PDADMAC/ PSS	PDADMAC	PSS	1	2	-	10 - 30
	10 mM	10 mM				
PDADMAC /PSSMA	PDADMAC	PSS	0.15, 0.3, 0.5, 1	2	3, 4, 5, 6, 8, 10	10 - 30
	10 mM	10 mM				
Chi/PSS	Chi	PSS	1	2	3, 4, 5, 6	2-20
	6.6 mM	10 mM				

3.4.1.3 Determination of the stability/decomposition of PEM film

In order to investigate the stability of multilayers thin films before loading model drugs, $(PDADMAC/PSS)_7$ thin films on quartz slides were immersed in 0.1M of HCl, 0.1 M of NaOH, 100% ethanol and PBS buffer solution pH 7.4. $(PDAD/PSSMA)_{10}$ thin films were immersed in buffer pH solutions 7.4 with varies ionic strength (0, 0.15, 0.3, 0.5, 1 M). In addition, $(Chi/PSS)_{10}$ thin films were immersed in buffer pH solutions 3 and 7.4 with varies ionic strength (0, 0.15, 0.3, 0.5, 1 M). The experiment conditions for the stability decomposition of polymer matrix are summarized in Table. 3.4. During time periods of 30-720 minutes, the samples were rinsed with distilled water and dried under room temperature. The formation of PDADMAC/PSS and PDADMAC/PSSMA multilayer thin films on quartz slide and glass slide were monitored their stability/decomposition properties using UV-vis spectrophotometer at wave length 190-800 nm.

Table 3.4 Parameters of the stability/decomposition study of PEM film.

Condition	**Strong acidic condition (pH 1)**	**Strong basic condition (pH 13)**	**Pure Ethanol**	**Acidic condition (pH 3)**					**Neutral condition (pH 7.4)**				
				NaCl concentration (M)					**NaCl concentration (M)**				
				0 M	**0.15 M**	**0.3 M**	**0.5 M**	**1 M**	**0M**	**0.15 M**	**0.3 M**	**0.5 M**	**1 M**
PDADMAC/PSS	✓	✓	✓						✓				
PDADMAC/PSSMA									✓	✓	✓	✓	✓
Chi/PSS				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

3.4.1.4 Loading and release characteristics of hydrophobic drug into PEM films

3.4.1.4.1 Loading of curcumin into PEM films

The loading characteristics of curcumin in polymer matrix was studied by varying parameters, including solvent composition, surface charged of outer layer, number of layers and loading time as summarized in Table 3.5. Curcumin powder (1 g.) was dissolved in 100 ml of absolute ethanol (100%) and the solution was stirred constantly until curcumin completely dissolved. In order to study effect of solvent composition, mixed solvent composition of water/ethanol (%), v/v was used to dissolve curcumin solution. The mixed solvent of water/ethanol (%), v/v was varied from 100/0-0/100. Multilayer thin films, which were prepared in the previous section, were immersed in curcumin solution for 120 minutes. To determine the loading of curcumin, 0.01% (w/v) of curcumin was dissolved in 80/20 of water/ethanol. Multilayer thin films were loading by immersion in curcumin solution form 5-540 minutes. Then, Multilayer thin films were rinsed with same ratio of solvent composition for 10 minutes to remove excess curcumin and dried under room temperature prior to use. The loading of curcumin into multilayer thin films was determined by UV-vis spectrophotometer at wave length 433 nm.

Table 3.5 Parameters control curcumin loading PEM films.

Parameters	**PDADMAC/PSS**	**PDADMAC/PSSMA**	**Chi/PSS**
Solvent composition water/ethanol (%), v/v	100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 20/80, 0/100	80/20	80/20
Surface charged of outer layer	10,11,14,15	-	-
Number of layers	10 - 60	10-20	10-20
Loading time (minutes)	30 - 540	240	240

- Determination of loading efficiency and actual curcumin content

As prepared standard solution, a stock solution of curcumin was prepared by dissolving 0.1 g of curcumin in 100 ml of absolute ethanol (100%). Then, solution was diluted to 0.25 PPM – 3 PPM in 20/80 water/ethanol (%), (v/v) and determined from its absorption at 433 nm using UV-vis spectrophotometer. From this calibration curve, the amount of curcumin in the PDADMAC/PSS thin films was calculated and discussed.

To determine the amount of curcumin loading into $(PDADMAC/PSS)_n$ multilayer thin films, each films was first dissolve in 40 ml of mixed solvent of 20/80 water/ethanol (%), (v/v). At 3 hours, 3 ml of sample solution was withdrawn and an equal amount of fresh mixed solvent was refilled. The amount of curcumin in sample solution was determined using the UV-vis spectrophotometer at wavelength 433 nm. The obtained data was calculated to determine the amount curcumin content in PDADMAC/PSS multilayer thin films at each condition. The loading efficiency of curcumin was calculated and reported in microgram/centimeter square ($\mu g/cm^2$).

- Determination of light stability

The stock solution of curcumin was diluted to 0.01% (w/v) in mixed solvent composition of water/ethanol (%), v/v in order to prepare the aqueous solution for test the light stability. The obtainable $(PDADMAC/PSS)_7$ multilayer thin films were immersed in 0.01% of curcumin solution for 60 minute and the rinse, and let film dried under room temperature. All curcumin solution and curcumin loading $(PDADMAC/PSS)_7$ multilayer thin films were exposed to neon light in ambient temperature. Moreover, the effect of light to PDADMAC/PSS multilayer thin films was also determined by exposing in the sun light compare to the dark condition. The duration time of light exposure to substrates was measured every day with a total of 7 days and detected the remaining of curcumin by UV-vis spectrophotometer at wavelength 433 nm.

3.4.1.4.2 *In vitro* release of curcumin

The release of curcumin was used both of solvent and buffer solution pH 3 ad 7.4 as a release medium as illustrated in Table 3.6. To study the release characteristics of curcumin in solvent, curcumin loading $(PDADMAC/PSS)_7$ multilayer thin film were release in 40 ml solvent by varying ratios of water/ethanol (%), v/v from 100/0, 50/50, 20/80 and 0/100. In addition, curcumin loading $(PDADMAC/PSS)_7$ multilayer thin film were release in buffer pH 7.4 as prepared by 10 mM of Na_2HPO_4 and 10 mM of acetic acid. The ionic strength of the buffer pH solution was varied from 0.15 M to 1 M using NaCl. The release of curcumin from PDAD/PSSMA deposited at pH 3 and 5 were placed in buffer pH 7.4 containing 0.15 M of NaCl. Chi/PSS multilayer thin films deposited at pH 6 was chosen to study release behavior. To study the effect of buffer pH solution, Chi/PSS multilayer thin films deposited at pH 6 were immersed in buffer pH 3 and 7.4. Chi/PSS multilayer thin films deposited at pH 6 were removed at the interval time, dried, and the absorbance was measured. Experiment was recorded in triplicates (n=3). Data in the Figure represent ± average standard deviation.

All measurements were carried out at room temperature. The release of models drug from multilayer thin films was analyzed using UV-vis spectrophotometer. These data were calculated to determine the cumulative release of drugs from multilayer thin films at each a specific time period as showed in the formula follows.

$$\text{Cumulative release of drugs (\%)} = \frac{(Abs_0 - Abs_t)}{Abs_0} \times 100$$

Where Abs_0 and Abs_t are absorbance of drugs loading multilayer thin films at initial time and at time *t*, respectively. The cumulative of drugs release (%) were carried out in triplicate and the results were reported as the average percentage.

Table 3.6 Parameters control curcumin release from PEM films.

Condition	Release medium	
	Solvent	**pH buffer**
PDADMAC/PSS	100/0 - 0/100 of water/ethanol	pH 7.4 (0-1 M of NaCl)
PDADMAC/PSSMA	-	pH 7.4 (0.15 M of NaCl)
Chi/PSS	-	pH 3 and 7.4

3.4.1.5 Loading and release characteristics of anionic drug into PEM films

3.4.1.5.1 Loading of diclofenac sodium (DS) into PEM films

Negatively charged of DS was selected as anionic model drug. The loading characteristics of DS in multilayer thin films was investigated by varying parameters, including pH of drug solution, surface charged of outer layer, number of layers and loading time as summarized in Table 3.7

DS (1 gram) was dissolved in 100 ml of distilled water in order to prepare the stock solution. The DS stock solution was stirred thoroughly to ensure complete mixing of drug before loading. The dilution of 0.05% of DS was then loading into multilayer thin films which were prepared in the previous section, then rinsed and dried as described for film assembly. The absorption of DS loading multilayer thin films were demonstrated by UV-spectrophotometer at wavelength 288 nm.

Table 3.7 Parameters control DS loading PEM films.

Parameters	PDADMAC/PSS	PDADMAC/PSSMA	Chi/PSS
pH of drug solution	5, 5.5, 6, 7	3, 5	3, 6
Surface charged of outer layer	10,11,14,15	-	-
Number of layers	9-25	9-25	9-25
Loading time (minutes)	30 - 540	60	60

- Determination of loading efficiency and actual DS content

DS solution with different concentration (0.1-80 PPM) was immersed in buffer pH 7 in order to prepare the standard solution. DS loading $(PDADMAC/PSS)_n$ multilayer thin films were dissolved in 40 ml of buffer pH 7. The absorbance of the DS solution was measured using UV-spectrophotometer. Values obtained from absorbance were converted into DS content in microgram/centimeter square ($\mu g/cm^2$).

3.4.1.5.2 *In vitro* release of DS

The release of DS was used buffer solution as a release medium as represented in Table 3.8. DS loading $(PDADMAC/PSS)_{10.5}$ multilayer thin films were immersed into 40 ml of pH buffer solution (pH 3, 4, 5, 6 and 7) in ambient temperature. To investigate the effect of ionic strength, DS was release in buffer pH 7.4 with varied NaCl concentration from 0.005M – 0.1 M. PDADMAC/PSSMA deposited at pH 5 were released in buffer pH 7.4 containing 0.15 M of NaCl. It can be noted that no release of Chi/PSS was investigated due to it un-loading films.

All multilayer thin films were analyzed the absorbance by UV-spectrophotometer at wave length 288 nm for each a specific time period and calculated the cumulative

release as repotted in section 3.4.1.3.4. Experiment was recorded in triplicates (n=3). Data in the Figure represent ± average standard deviation.

Table 3.8 Parameters control DS release from PEM films.

Condition	Release medium
	pH buffer
PDADMAC/PSS	pH 3, 4, 5, 6, 7 (0-1 M of NaCl)
PDADMAC/PSSMA	pH 7.4 (0.15 M of NaCl)
Chi/PSS	-

3.4.1.6 Loading and release characteristics of cationic drug into PEM films

3.4.1.6.1 Loading of Gentian violet (GV) into PEM films

Positively charged of GV was selected as cationic model drug. The loading characteristics of GV in multilayer thin films were investigated by study parameters including, pH of drug solution, surface charged of outer layer, number of layers and loading time as summarized in Table 3.9

GV (0.5%, w/v) was dissolve in 100 ml of distilled water followed by stirring. Multilayer thin films that prepared in previous part were immersed to the dilution of GV solution ($5x10^{-4}$%, w/v). After a given time of immersion, films were rinsed into distilled water or pH buffer to remove the excess GV molecules and dried under ambient temperature. The pH of the GV solution as well as buffer pH solution was adjusted to pH of 3-6 by 10 mM of acetic acid and 10 mM of sodium acetate, where as 10 mM of Na_2HPO_4 was used to adjust pH 7 and 8 as necessary. Then, GV loading multilayer thin films were measured their loading absorbance by UV-spectrophotometer at wavelength 550 nm.

Table 3.9 Parameters control GV loading PEM films.

Parameters	PDADMAC/PSS	PDADMAC/PSSMA	Chi/PSS
pH of drug solution	4, 5, 6, 7	3, 5, 8	3, 6
Surface charged of outer layer	10,11,14,15	10,11,14,15	-
Number of layers	2-22	8-28	10-20
Loading time (minutes)	0 - 540	0 - 540	0 - 540

- Determination of loading efficiency and actual GV content

The amount of GV loading loading PDADMAC/PSS and PDADMAC/PSSMA multilayer thin films was calculated by measuring the absorbance of GV solution using as standard solution from 0.1 PPM to 5 PPM. GV loading $(PDADMAC/PSS)_n$ and $(PDADMAC/PSSMA)_n$ multilayer thin films were dissolved in 40 ml of buffer pH 3. The total among of drug content in the withdrawn solution suspension was calculated with reference to a calibration curve. Values obtained from absorbance were converted into GV content in microgram/centimeter square ($\mu g/cm^2$).

3.4.1.6.2 *In vitro* release of GV

GV release from multilayer thin films was investigated by immersed films in pH buffer solution at room temperature as shown in Table 3.10. Buffer pH solution were used 10 mM of acetic acid and 10 mM sodium acetate in order to adjust pH to 3 and 5 whereas 10 mM of acetic acid and 10 mM of Na_2HPO_4 were used to reach pH 7. To determine the effect of ionic strength, 0-1 M of NaCl concentration was added into the solution of release medium. All multilayer thin films were analyzed the absorbance by

UV-spectrophotometer at each a specific time period and calculated the cumulative release as repotted in section 3.4.1.3.4. Experiment was recorded in triplicates (n=3). Data in the Figure represent ± average standard deviation.

Table 3.10 Parameters control GV release from PEM films.

Condition	Release medium
	pH buffer
PDADMAC/PSS	pH 3, 5, 7 (0-1 M of NaCl)
PDADMAC/PSSMA	pH 3, 5, 7 (0-0.5 M of NaCl)
Chi/PSS	pH 7.4 (0.15 M 0f NaCl)

3.4.2 Construction of blocking film by Layer-by-layer deposition for prolong release of hydrophobic drug

3.4.2.1 Fabrication of blocking films

The glass substrates and quartz substrate were cleaned in hot piranha solution as described detail in part 3.4.1.1. Table 3.11 summarized experimental conditions for constructing blocking films in Part II.

As described previously, polyelectrolyte multilayer films were constructed using layer-by-layer technique. Substrate was fist immersed to Chi solution using as positively charged polyelectrolyte. In this way, the substrate was covered with a chitosan layer. After rinsing with pH buffer, the resulting substrate was transferred into a solution of P, Alg or PAA using as negatively charged polyelectrolyte. The dipping time was 2 minutes in each step. By repeating the above two steps in a cyclic, the multilayer film was fabricated. The pH of the polyelectrolyte solutions as well as the pH buffer was adjusted to pH 5.5 with 10 mM of acetic acid and 10 mM of sodium acetate. The build up of multilayer thin films were characterized by UV-spectroscopy. The surface topographic feature of films and film thickness was monitored by atomic force microscope (AFM). The root mean square (RMS) roughness values were obtained from the AFM software. All measurements were carried out under room temperature.

Table 3.11 Experimental condition used in the preparation of blocking films.

Condition	Concentration (mM)		NaCl Concentration (M)	Dipping time (minute)	pH	No. of layers
	Cationic polyelectrolyte	Anionic polyelectrolyte				
Chi/Alg	Chi 6.6 mM	Alg 10 mM	1	2	5.5	2-20
Chi/PAA	Chi 6.6 mM	PAA 10 mM	0.2	2	5.5	2-20

3.4.2.2 Determination of the stability/decomposition of blocking films

Stability and decomposition studies of multilayer thin films were carried out in acidic condition (pH 3) and neutral condition (pH 7.4) using as a solution medium. Multilayer thin films were immersed in buffer pH solution. NaCl induced decomposition studies were also carried out through adding NaCl into the incubation solution medium at varying NaCl concentration. The experiment conditions for the stability decomposition of blocking films are summarized in Table. 3.12. Then, multilayer thin films were then removed from the buffer pH solution, washed and dried under room temperature. The remaining of chitosan absorbance was report as the normalized absorbance at wave length 198 nm by UV-spectroscopy at the interval time. The surface topographic feature of films and film thickness was monitored by atomic force microscope (AFM). The root mean square (RMS) roughness values were obtained from the AFM software. All measurements were carried out under room temperature.

Table 3.12 Parameters of the stability/decomposition study of blocking films.

Condition	Acidic condition (pH 3)					Neutral condition (pH 7.4)				
	NaCl concentration (M)					NaCl concentration (M)				
	0 M	0.15 M	0.3 M	0.5 M	1 M	0M	0.15 M	0.3 M	0.5 M	1 M
Chi/Alg	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Chi/PAA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

3.4.2.3 Prolonged release of hydrophobic drug from PEM films

- Preparation of curcumin loading polyacrylic substrates and polymer matrix

Polyacrylic films were cut into the diameter 50 x 25 mm using as substrates in this experiment. 0.01% of curcumin in absolutely ethanol solution was directly loading to the surface of polyacrylic films. To ensure the maximum loading of curcumin, the loading time was 3 hour. Then, polyacrylic films were pulled out, rinsed the excess curcumin molecule 3 times and dried under room temperature. The obtained of curcumin load polyacrylic films were stored in dark condition prior to coat blocking films. PDADMAC/PSS polyelectrolyte multilayer thin films that prepared in previous section were also used as a polymer matrix to load curcumin.

- Deposition of blocking layer

The layer by layer deposition of blocking films was constructed on top of curcumin loading polyacrylic films and polymer matrix as summarized condition in Table 3.13. Curcumin loading polyacrylic films and polymer matrix were alternatively immersed in the positively charged polyelectrolyte, then rinse in distilled water or buffer pH for 2 minutes. The surface of substrate was converted to the negatively charged by immersed in the solution of anionic, rinsed and dried. The blocking films was fabricated by the repeating this steps until receive the desired layers.

Table 3.13 Experimental condition used in the deposition of blocking films coated polyacrylic substrate.

Condition	Concentration (mM)		NaCl Concentration (M)	Dipping time (minute)	pH	No. of layers
	Cationic polyelectrolyte	Anionic polyelectrolyte				
PDAD/PSS	PDAD 10 mM	PSS 10 mM	1	2	-	4, 8, 12, 16, 20
Chi/Alg	Chi 6.6 mM	AlG 10 mM	1	2	5.5	4, 8, 12, 16, 20
Chi/PAA	Chi 6.6 mM	PAA 10 mM	0.2	2	5.5	4, 8, 12, 16, 20

- Determination of solvent and buffer release

To investigate the effect of solvent on the prolong release, curcumin loading uncoated polyacrylic were placed in varied solvent composition of water/ethanol (%), v/v (60/40, 40/60, 20/80, 10/90, 0/100). The release of curcumin from uncoated polyacrylic was performed in the dark condition at room temperature and recorded the absorbance at interval time from minute to hour by UV- spectrophotometer at wave length 433 nm. To study the effect of blocking film types on the prolong release, uncoated polyacrylic and blocking films coated polyacrylic were immersed in 20/80 water/ethanol (%) and buffer pH 7.4 as a release solvent. The amount released of curcumin was determined by UV-spectrophotometer at each time point and divided by the total amount to obtain cumulative release value. Experiment was recorded in triplicates (n=3). Data in the figure represent ± average standard deviation.

3.4.3 Development of Layer-by-Layer thin films–based coated fruit

3.4.3.1 Preparation of fruit samples

Fresh Apple and mango were obtained from local market, Bangkok, Thailand. Apple and mango were carefully selected to ensure the uniformity in sized, weight, and physical appearance. The average weigh of apple and mango were 210.55 gram, 240.31 gram, respectively. Before measurement, all fruit were washed, dried, and maintained at low temperature (about $10\,^{0}$C).

3.4.3.2 Preparation of PEM-based coated fruit

The coating solutions used for apple and mango were PDADMAC and PSS as the positively charged polyelectrolyte and negatively charged polyelectrolyte. Table 3.14 shows experimental conditions for multilayer thin films coated fruit. The fresh fruit were immersed carefully into the PDADMAC solution, and rinse with distilled water for 5 minute as ensure the un-excess polyelectrolyte. The fruit were alternatively immersed in of PSS solution and rinse as the previous procedure. This cycle was repeated until the desired number of $(PDADMAC/PSS)_n$ had been deposited. All fruits were dried at ambient temperature. Fruit without coated were used as controls and were stored under same conditions as those for coated fruit.

Table 3.14 Experimental condition used in the preparation of PEM-based coated fruit.

Condition	Concentration (mM)		NaCl Concentration (M)	Dipping time (minute)	pH	No. of layers
	Cationic polyelectrolyte	Anionic polyelectrolyte				
PDADMAC/ PSS	PDADMAC 10 mM	PSS 10 mM	1	5	-	1 - 20

- Investigation of hydrophilic/hydrophobic property

Contact angles of multilayer thin films coated fruit were measured in order to investigate the hydrophilic/hydrophobic property. A camera was used to recorded images of the water spreading on the sample. The images were analyzed using computer

program. Four different measurements were made for each sample and the average was calculated. All measurements were performed with water pH of 7.4 at ambient temperature.

3.4.3.3 Preparation of PEM containing curcumin-based coated fruit

To prepare curcumin solution, stock solutions of 0.1% of curcumin were diluted the concentration to 0.01% in mixed solvent composition of 80/20 water/ethanol (%), v/v. The obtained multilayer thin films coated fruit in previous section were immersed in curcumin solution. The loading time was 3 hour and maintained curcumin coated fruit without the light. Then, curcumin coated fruit were rinsed in 80/20 of water/ethanol (%), v/v as carefully, and dried under ambient temperature.

- Determination of loading efficiency of curcumin coated fruit

Curcumin content was extract from fruit by immersion in mixed solvent of 20/80 water/ethanol (%), v/v for 3 hour. At the definite time, 3ml of curcumin solution was taken and analyzed by UV-spectrophotometer at wavelength 433 nm. The obtained value were calculated by the calibration curve of known curcumin concentration and reported in $\mu g.g^{-1}$.

3.4.4 Characterization Techniques

3.4.4.1 UV-vis spectroscopy analysis

All of model drugs loading and release experiment were confirmed by UV-vis spectrophotometer (SPECORD S 100, Analytikjena, Japan). The loading of curcumin, diclofenac sodium and gentian violet were occurred the absorbance peak at wavelength 430, 288 and 550 nm respectively. All of values are given as mean ± standard derivative at least n=3. The absorbance values were translated to concentration values by comparing

to the standard solution of known concentration that was used to plot the calibration curve. This calibration curve followed the Beer-Lambert law: $A= \varepsilon .b.C$

Where A is the absorbance, ε is the molar absorptivity (L mol^{-1} cm^{-1}), b is the absorbance medium pathlength (cm) and C is the concentration (mol L^{-1}).

3.4.4.2 Atomic force microscope (AFM)

Film thickness and surface roughness of polyelectrolyte multilayer thin films was measured by an atomic force microscope (AFM).This technique is based on using shap tip that is scanned over the surface at the constant force (for height in formation) or height (for force information)using a feed-back loop mechanism. Tips are typically made from silicon or silicon nitride and extend down to the end of the reflective cantilever. A diode laser is focused onto the back of this reflective cantilever. As the tips scan the surface of the sample, moving up and down with the contour of the surface, the laser beam is deflected off the attracted cantilever into a dual element photodiode. The photodetector measures the different in light intensity between the upper and lower photodetector, and then converts to the voltage. Feedback from photodiode difference signal, throught software control from the computer, enables the tip to maintain either a constant force or constant height above the sample.

A scanning probe microscope in tapping mode was operated at ambient temperature. The surface thickness was recorded with a standard silicon tip on a cantilever beam. The spring constant of the cantilever was between 20 and 100 n/m and the length was 125 μm with a resonant frequency of 298 kHz.

CHAPTER IV
RESULTS AND DISCUSSION

PEM thin films have been tested as a matrix for drug loading and as a blocking layer for drug release. These two aspects are presented in the first two parts of this chapter. The last segment of this chapter is dedicated to the application of these films as fruit coatings loading with curcumin as potent anti-fungi. In the first part, polymer matrix based both of strong and weak polyelectrolyte (PDADMAC/PSS, PDADMAC/PSSMA and Chi/PSS) were prepared characterized and used for drug loading. PDADMAC/PSS is used as a reference since they are non pH dependant and widely used in PEM assembly while PSSMA has a weak acid maleic part and chitosan a weak base. Model drugs either neutral, anionic or cationic were used namely curcumin (hydrophobic drug), diclofenac sodium (anionic drug) and gentian violet (cationic drug). The effect of the pH, ionic strength and number of layers on the loading and release of the selected drugs is presented. Similar parameters where investigated in the second part while using the PEM coating as a blocking barrier. Chitosan based PEM coating was deposited with Alg or PAA. Interesting results on solvent permeability was presented. Finally, the coating of fruit with decomposable PEM film pre-loading with curcumin is detailed.

4.1 Layer by layer deposition of polyelectrolyte multilayer thin films for loading and release of model drugs

4.1.1 Fabrication and characterization of PEM films

4.1.1.1 Formation of PDADMAC/PSS multilayer thin films

In the present study, multilayer thin films were constructed based on electrostatic layer by layer self assembly on quartz substrate. The multilayer build-up of PDADMAC and PSS was studied by monitoring its absorbance to the surface of quartz using UV-vis spectroscopy as shown in Figure 4.1. PSS had a characteristic UV absorption band at

around 227 nm, while PDADMAC showed no absorption band in this region. From the UV-vis spectra, the absorbance at 227 nm for PSS peaks appeared, indicating the assembly of PDADMAC/PSS multilayer thin films. The growth of multilayer film was regular absorption and desorption trend because the PDADMAC spectra band at the odd number layers could not be detected by UV-vis spectroscopy. Additionally, the PSS spectra band trended to saturated and limit the absorption when increase the number of layer than that of 14 layers. With a sufficient number of layers, thickness of 14 layers of PDADMAC/PSS multilayer thin films was reached 223.25 nm.

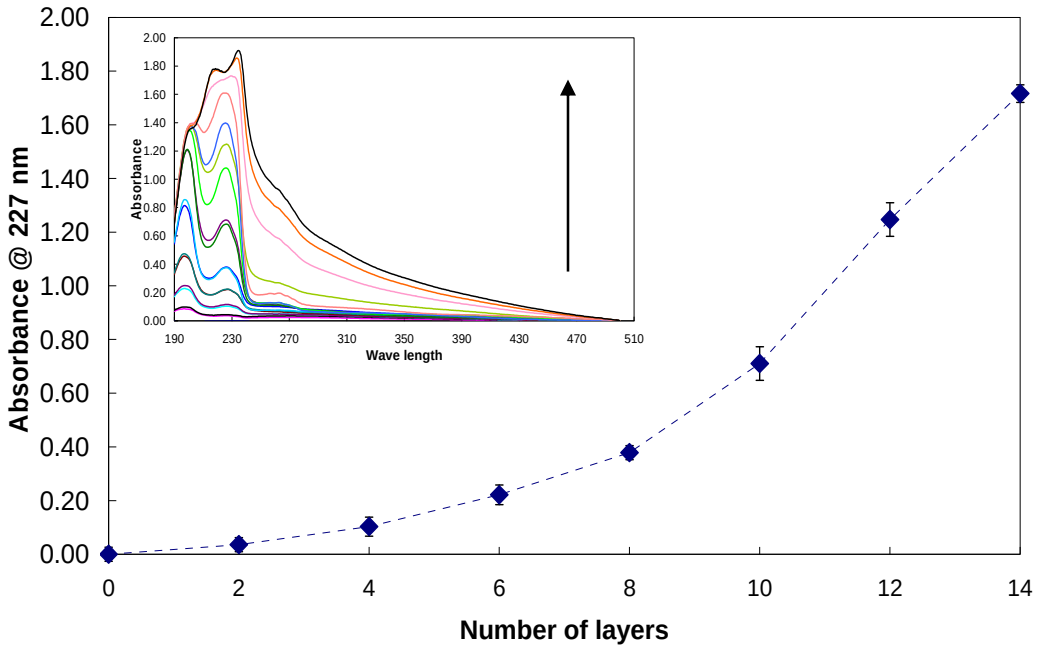


Figure 4.1 Absorbance of PDADMAC/PSS multilayers as a function of the number of layers deposition cycles. The inserted figure shows multilayer thin film growth of PDADMAC/PSS on quartz substrate.

4.1.1.2 Formation of PDADMAC/PSSMA multilayer thin films

Multilayer thin films composed of strong and weak polyelectrolytes generated significant interest because they are pH responsive. In addition, it is well known that the ionic strength can significantly effect the conformation and charge density of the

polyelectrolytes and thereby the growth and properties of multilayers.[111] In this study, a copolymer of sulfonated and maleic acid (poly-(4-styrenesulfonic acid-co-maleic acid), PSSMA) was assembled in order to construction multilayer thin films with PDADMAC.

- Effect of NaCl on the formation of PDADMAC/PSSMA multilayer thin films

The absorbance values sulfonated group from UV-vis spectroscopy was significantly lower when no salt was employed in polyelectrolyte solutions as shown in Figure 4.2. The result suggested that the ionic strength of deposition solution has a significant influence on the formation of PDADMAC/PSSMA multilayer thin films except for PDADMAC/PSSMA deposition at pH 3. Interestingly, PDADMAC/PSSMA deposited at pH 5, 6, 8 and 10 reached the maximum absorbance at 0.5 M of NaCl. When no NaCl was added, polyelectrolyte chains became more extended conformation with lower loops and tails because charges on the chain were less screened. Consequently, the polyelectrolyte can absorbed as thinner layer. As NaCl concentration increased to 0.1- 0.5 M, such repulsions would be active by salt ions. Basically, salt promotes interpenetration of absorbing polyelectrolytes into multilayers, resulting in enabling more effective charge compensation and enhancing the stability of multilayer thin films by inducting more entanglements of the polyelectrolyte chains.[112] In addition, the effect of salt was due to the screening of the electrostatic repulsion during absorption onto a substrate, which induces a higher absorbance rate of polyelectrolytes onto substrate. However, as the ionic strength of polyelectrolyte solutions was higher, such as 1 M, salt ions in the solution could effectively shield the carboxylic groups of PSSMA from the quaternary amine groups of PDADMAC. As the result, the electrostatic attraction which is important to self assembled process is no longer operative. Therefore, the formation of polyelectrolytes and the attraction between PDADMAC and PSSMA could be tuned by the ionic strength, which is one of parameters that affect the thickness of multilayer thin films. Dubas and Schlenoff have reported the layer by layer assembled prepared from PDADMAC and

polyacrylic acid (PAA). The growth of multilayer thin films was increased when the NaCl concentration reached the maximum at 0.3 M and then decreased. However, PDADMAC/PSSMA deposited at pH 4 showed the maximum absorbance at 1 M of NaCl. This unexpected resulted might be because the $Na^{+}Cl^{-}$ and polyelectrolyte chain have stronger the electrostatic repulsion during absorption onto a substrate. Therefore, this observation indicated that, when PDADMAC and PSSMA absorbed at all conditions, salt is necessary to promote regular multilayer growth.

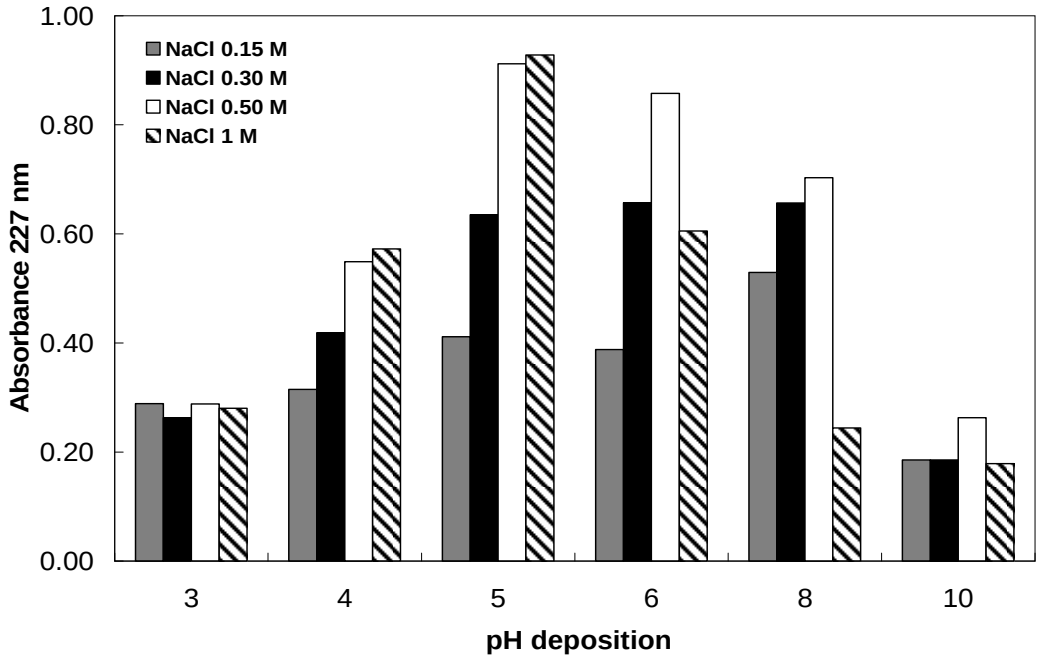


Figure 4.2 Absorbance of $(PDADMAC/PSSMA)_{10}$ multilayer thin films deposited at pH 3,4,5,6,8 and 11. Multilayer thin films constructed with varying the NaCl concentration during assembly process.

- Effect of pH on the formation of PDADMAC/PSSMA multilayer thin films.

The growth of PDADMAC/PSSMA of multilayer thin films deposition pH 3, 4, 5, 6, 8 and 10 was monitored by using UV-vis spectrophotoscopy (Figure 4.3). UV-vis

spectrophotometer provided information on the amount of PSSMA incorporated into the films, as styrene sulfonated groups absorbed at wavelength 227 nm. Multilayer thin films deposition at different pH solutions of PSSMA showed different growth behavior. In all experiment, deposition pH of multilayer thin films was varied at fixed NaCl concentration of 0.5 M. All of growth curve show the increase of absorbance, especially for PDADMAC/PSSMA deposition pH 5, 6 and pH 8. In this case, thickness and roughness of these films was greater comparing with all multilayer films observed. The study shows that the absorption spectra of PSSMA decreased when pH of the PSSMA solution increased (from pH 5 to pH 10). At high pH values, a greater proportion of carboxylic group in PSSMA was ionized, leading to the increase in the effective charge density of the polymer. When the charge density of the polyelectrolytes increases, the repulsive force between the charged groups also increases, causing the polyelectrolytes adopt a flatter conformation. Therefore, the multilayer thin films can adsorbed as a thinner layers, especially at pH 10. As PDADMAC/PSSMA deposition at pH 5, an initially proportion of the carboxylic acid groups is protonated, leading to the decrease of a charged density (pK_a of 3:1 PSSMA in solution are 2.8 and 8.3).[113] This pH deposition can causes the polyelectrolytes to adopt more coil conformation which contain both of neutralized sulfonated groups and carboxylic acid groups. Furthermore, greater amounts of charge density polyelectrolyte are required to reverse the charge on the surface of the multilayer. One can see that, decreasing the charge density of PSSMA would cause it to adopt more coil conformation. [114] Hence, it is generally observed that lowering the charge density of polyelectrolyte results in the formation of thicker films. Conversely, decreasing the charged density of PSSMA to pH 3 and would cause it to deionized, resulting in lower charge density along the polymer chains. Additionally, the lower charge density of PSSMA means that less PDADMAC chains are required to compensate and reverse the surface charged of preceding PSSMA layer. Therefore, the multilayer thin films become thinner during adsorption process.

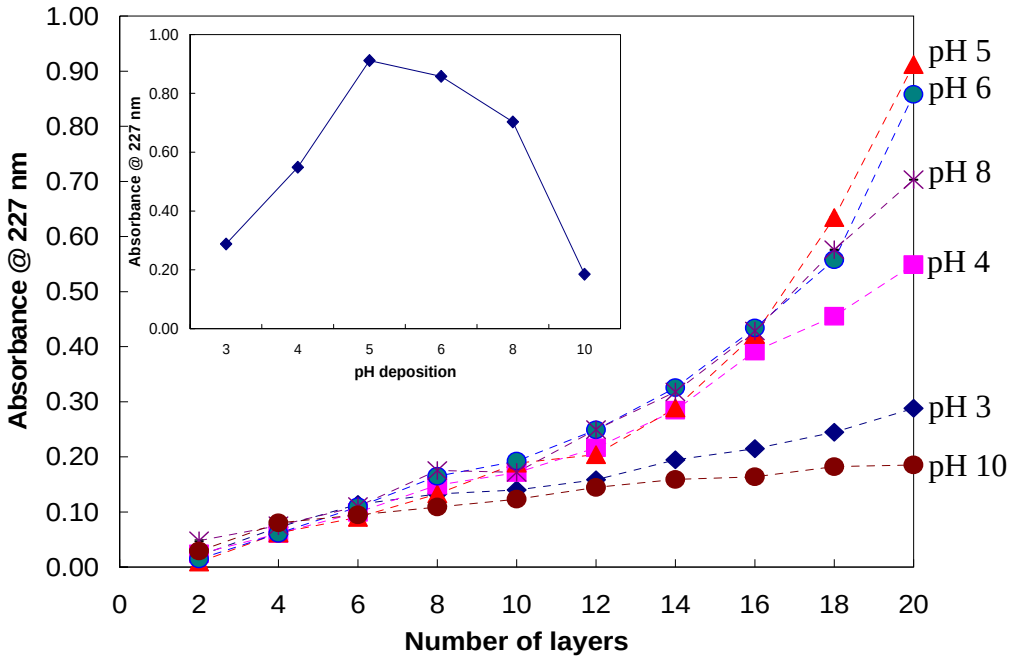


Figure 4.3 Absorbance of PDADMAC/PSSMA multilayers thin films deposition at different pH. Odd and even deposition number of layers corresponded to PDADMAC and PSSMA respectively. The inserted figure shows the absorbance of $(PDADMAC/PSSMA)_{10}$ layers on quartz substrate.

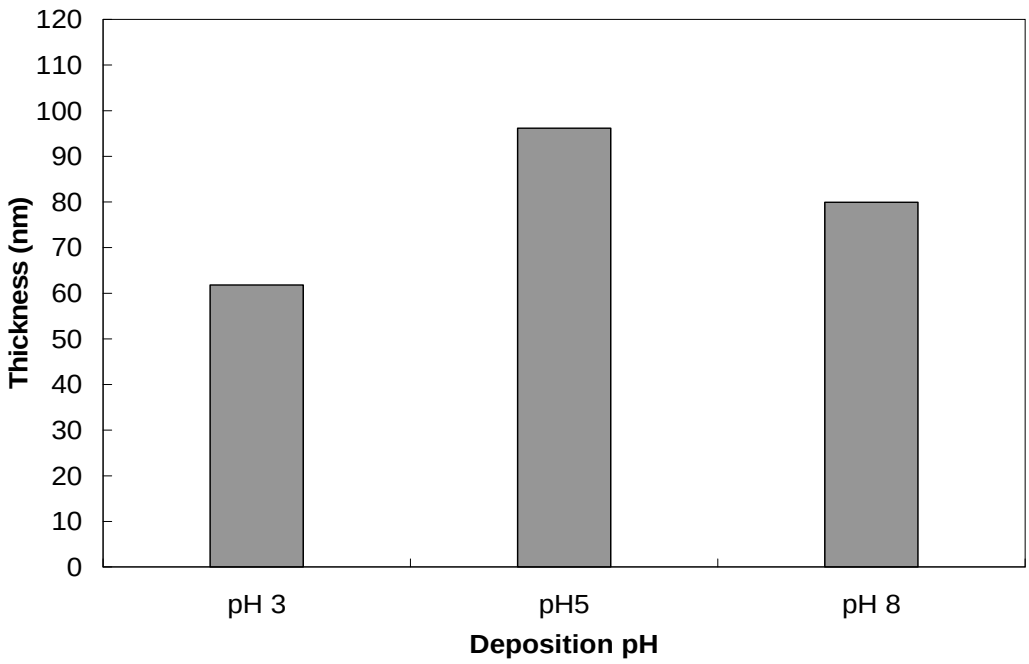
- Thickness of PDADMAC/PSSMA multilayer thin films

The final films thickness under various pH depositions was also investigated by using AFM measurement as presented in Figure 4.4 A. The multilayer films thickness deposition at pH 3, 5 and 8 were 61.79 nm, 96.16 nm and 79.94 respectively. As indicated by AFM data, multilayer films thicknesses showed the same trend as the UV-Vis data. PSSMA molecules have less stretched conformation at pH 3 than that pH 5 because of the lower degree of ionization of carboxylic groups at lower pH. Multilayer films deposition at pH 5 have greater thickness comparing with those films deposition at all pH conditions. However, the thinner films deposition at pH 8 are likely to be the result of more carboxylic acid groups are ionized at high pH. Thus, the polymer chains adopt

the extended conformation and increase the effective charge density of PSSMA under this pH condition.

It should be noted that the styrene sulfonic acid and maleic acid **(**SS:MA) ratio in the PSSMA solution was 3:1. The higher charge density of PSSMA 3:1 results in more PDADMACMAC adsorbing on the surface of PSSMA to neutralize and reverse the surface charges. [115] Remarkably, high SS moieties in PSSMA 3:1 lead to the forming of strong complex in each polyelectrolyte chain which is likely to provide strong electrostatic force as schematic represented in Figure 44 B. Overall, it could be concluded that the pH value of the polyelectrolyte deposition has significant effect on the conformation and charge density of polyelectrolytes and thereby the growth and thickness of the multilayers.

(A)



(B)

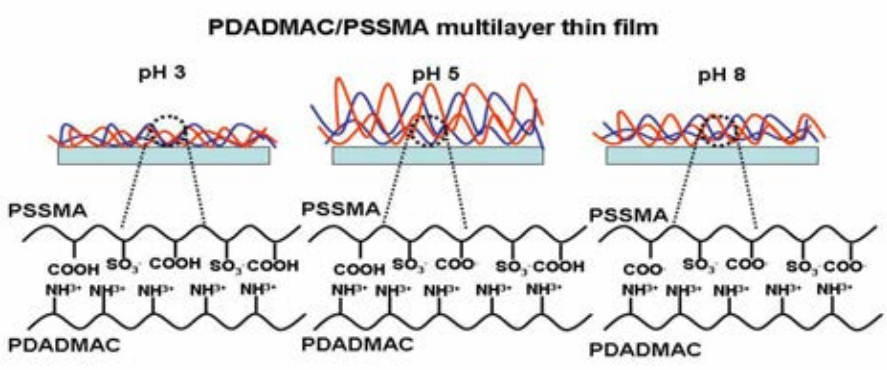


Figure 4.4 (A) Thickness of $(PDADMAC/PSSMA)_{10}$ layers as determined using AFM measurement. (B) Schematic represents PDADMAC/PSSMA multilayer thin films.

4.1.1.3 Formation of Chi/PSS multilayer thin films

Chitosan (Chi) has been well known for its use as a matrix for drug delivery application due to its biocompatibility, biodegradation, and low production cost from natural source. Since chitosan is positively charged at low pH associating with negatively charged of PSS to form polyelectrolyte multilayer thin films via electrostatic interaction.

- Effect of pH on the formation of Chi/PSS multilayer thin films

To verify the formation of Chi/PSS multilayer thin films based on uv-spectrophotometer, Chi and PSS were deposited alternately on the quartz slide. Figure 4.5 compares the built-up of Chi/PSS deposition at different pH from 3 to pH 6. Linear growth of multilayer thin films was investigated by monitoring the peak at 227 nm, corresponding with the absorbance of PSS. It can see that the pH of deposition has influence the growth curve of Chi/PSS multilayer thin films. A relatively high absorbance spectrum was observed at the deposition of pH 6. On the other hand, relatively absorbance spectrum of Chi/PSS multilayer thin films became lower at the end of deposition process at pH 3. Since polyelectrolyte deposition is governed by electrostatic interaction, the degree of ionization of charged molecule is important. According to a

strong polyelectrolyte of PSS, its net charge density was fixed at all pH range. Therefore, the fomation of sequentially adsorbed layer is sensitive to the pH of solution since the charge density and conformation of chitosan is influenced by the change of pH. It is expected that the increase of chitosan charge density at pH 3 due to the protonation of amine groups, enhanced electrostatic interaction with sulfonated groups of PSS, resulting in lower thickness of multilayer thin films. On the other hand, at pH of 6, chitosan contained both of amine groups and ammonium groups, which have non-protonated form. Charges overcompensation is the key rule for the formation of polyelectrolyte multilayer. When the charges density of chitosan (at pH 7) decreases, more polyelectrolyte chains are need to overcompensate and inverts the surface charges, explaining the higher thickness. Another explanation can suggested from the result is at pH 6, chitosan bear less charge groups to interact with PSS, therefore, the film structure is more roughness. Thickness and morphology of Chi/PSS multilayer thin films will be discussed in the next section.

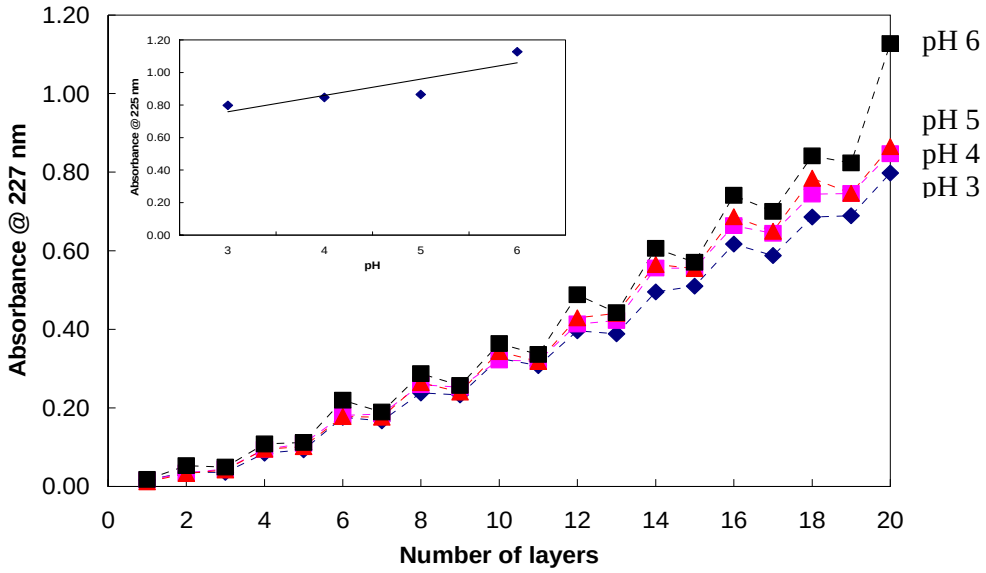


Figure 4.5 Absorbance of Chi/PSS multilayers thin films at different pH deposition. Odd and even deposition number of layers corresponded to Chi and PSS respectively. The insert figure shows the absorbance of $(Chi/PSS)_{10}$ layers on quartz substrate.

- Thickness and roughness of Chi/PSS multilayer thin films

AFM thickness and AFM surface morphology of Chi/PSS multilayer thin films deposition in various pH conditions are depicted in Figure 4.6 and 4.7, respectively. It obtains values of 172.88 nm, 189.22 nm, 196.95 nm and 288.78 nm for the final thickness of Chi/PSS multilayer thin films deposition at pH 3, 4, 5 and 6, respectively. This result was in agreement with the last investigation that showed the higher thickness of Chi/PSS film at pH 6. Considering the surface morphology, Chi/PSS deposition at pH 3 revealed more compact and smooth behavior with the average roughness 2.65 nm. In the other hand, an increasing of the deposition pH of Chi/PSS multilayer thin films can cause the loose and rough morphology. The average roughness of Chi/PSS multilayer thin films deposition at pH 4, pH 5, and pH 6 were 2.79 nm, 4.04 nm and 6.06 nm, respectively. Therefore, this result can confirm that the increasing pH of deposition Chi/PSS multilayer thin films leads to chitosan charged decrease, resulting in more coiled conformation structure.

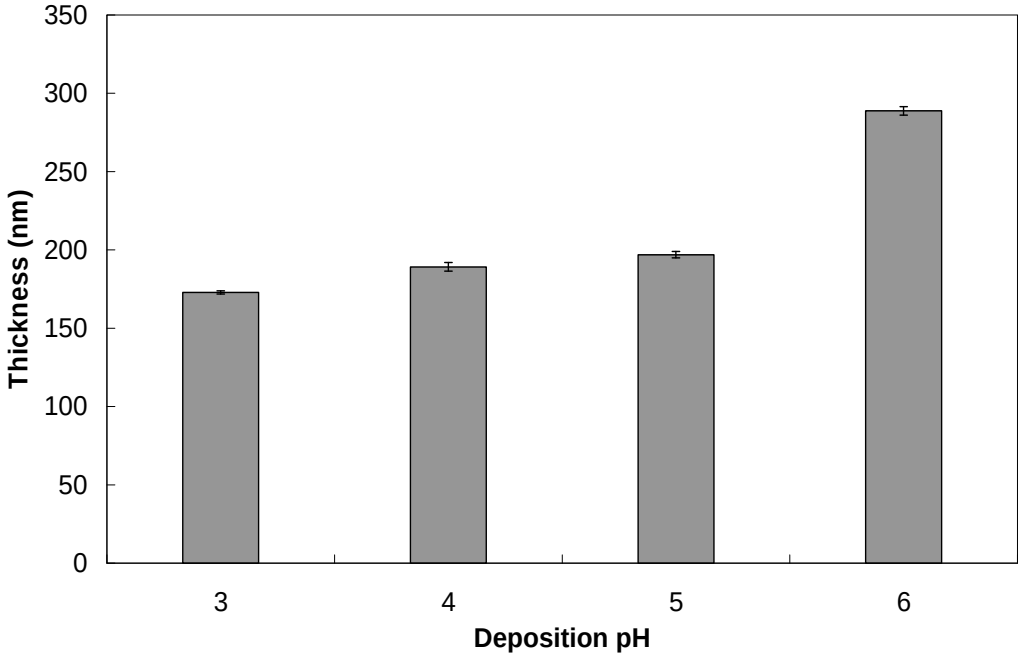


Figure 4.6 Thickness of $(Chi/PSS)_{10}$ layers as determined by AFM measurement. Chi/PSS Multilayer thin films were deposition at different pH conditions.

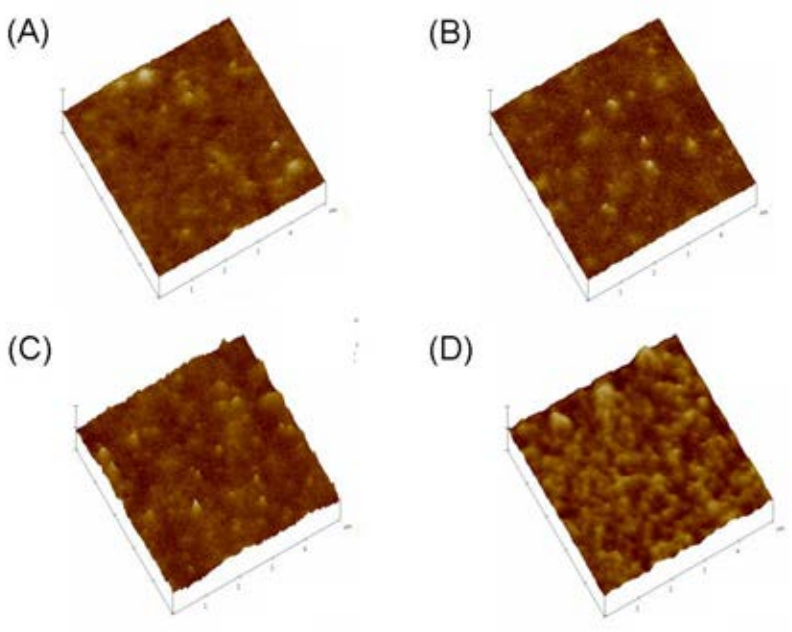


Figure 4.7 Surface roughness of $(Chi/PSS)_{10}$ multilayer thin film on silicon wafer with different pH deposition. (A) pH 3 (B) pH 4 (C) pH 5 and (D) pH 6.

4.1.2 Effect of pH and NaCl on the stability of PEM films

One concern in developing the polyelectrolyte multilayer films is their stability. In several cases, intact LbL constructed system is required to remain stable in order to control the release of substrates. The disassembly can be affected by the completed deconstruction of matrix or controlling a sustain erosion of various layers of the construct film. Moreover, electrostatic screening with NaCl is studied as an intermediate disassembly trigger for polyelectrolyte multilayer thin films. This section demonstrates the effect of NaCl and pH condition which rearrange the formation of matrix.

4.1.2.1 Stability/Decomposition of PDADMAC/PSS multilayer thin films

The stability of PDADMAC/PSS multilayer thin films was determined by immersing films in 0.1 M of HCl, 0.1 M of NaOH, ethanol and PBS buffer as shown in Figure 4.8 Our stability study of multilayer thin films in aqueous solutions displayed no changes in absorbance in the wave length 227 nm during 720 minutes. Another previous experiment also shows that the multilayer thin films with PSS as the outermost layer are very stable in aqueous media and resist high shear stress during streaming potential measurements and rinsing without destruction of the films.[116] This means that PDADMAC/PSS multilayer thin films are stable and have possibility to act as the stable matrix for the loading of model drugs.

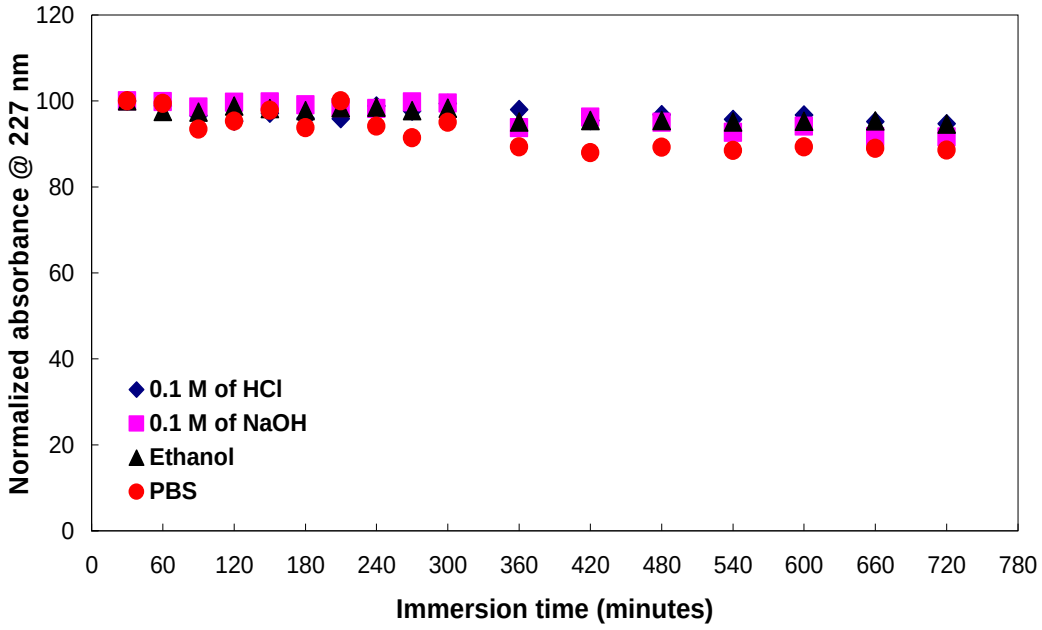


Figure 4.8 Stability of PDADMAC/PSS multilayer thin films in aqueous solutions.

4.1.2.2 Stability/Decomposition of PDADMAC/PSSMA multilayer thin films

The charge density in the conformation of polymer chains in weak polyelectrolyte multilayer thin films has not only profound effects on their overall thickness in the dry state, but it also has large variation in the degree of swelling when exposed to solutions of

different pH values. In this work, PDADMAC/PSSMA multilayer thin films were immersed into buffer pH 7.4 in order to investigate their stability of the films. These multilayer thin films were characterized the normalized absorbance of PSSMA by UV-vis spectroscopy. In all case, relatively ionic strength was also employed in order to determine the effect of NaCl concentration.

In Figure 4.9, multilayer thin films seem to stable after the 240 minutes of exposure process. The loss of materials in all pH buffers without NaCl was quite small except for the PDADMAC/PSSMA films deposition at pH 3 and 10. For instance, the PDADMAC/PSSMA deposition at pH 4, 5, 6 and 8 remained the percentage of PSSMA: 94.25, 95.07, 94.19 and 95.47 respectively. In all pH buffers without NaCl, it was evidence that the multilayer thin films deposition at pH 10 were largely destroyed while those deposition at pH 5 lose were about 4-5% of PSSMA percentage. For weak PEM films, pH not only influenced their bulk properties, but also tuned their surface characteristics as well. Schlenoff and co-workers[117] have reported that, at different pH regimes, chains of weak polyelectrolyte can exist with a mixture of topologies: ladder and scrambled salt. The Ladder–like act as the architecture with cooperatively stitched chain segments whereas many chains of polyelectrolytes adopt more statistical distribution of ion pairs to form the scrambled salt. When the weak polyelectrolytes are fully charged, chains are linked together with the large fraction of stitched chain as a ladder–like form. Conversely, at lower charge density, chains of polyelectrolytes present more loop structure as the scrambled salt and the ion pairs of segment are random. However, a mixture of ladder and scrambled salt are expected under this condition.

Multilayer thin films deposition at pH 5 and 6 show no obvious gradual change in PSSMA percentage. This result indicates that these multilayer thin films were still stable and did not exhibit the large swelling along polymer back bone chains during 120 minutes of exposure time. Conversely, multilayer thin films deposition at pH 3 was slightly disassembly. This is because the un-ionized carboxylic groups in multilayer chain became ionization, resulting in an excess of negatively charged with in multilayer thin films and subsequent release of PSSMA into the solution.

Interestingly, multilayer thin films deposition at pH 10 was disassembled after exposed to buffer pH 7.4. As films deposition pH 10, the polyelectrolyte was assumed to be more ionization, as such, a reduction of carboxylic groups (COO- ionic links) was sufficient to allow large segments of polymer chains to release into the lower pH condition. Moreover, the increase in the amount of negative charged in multilayer thin films leaded to a charged imbalance, which caused the release of polymer chains into the buffer pH 7.4 condition. This can explain the different behaviors of multilayer thin films assembled upon exposure within buffer pH solution. Another observation supporting this study is the fact that multilayer thin films deposition at pH 5 is quite stable when exposes to buffer pH 7.4. In all cases, we noted that the stability of multilayer thin films in buffer pH solution was first discussed without the effect of NaCl.

To further investigation of the effect of ionic strength, multilayer thin films were immersed in buffer pH 7.4 by varying the NaCl concentration. It is shown that the amount of PSSMA remaining in the multilayer thin films decreased when the concentration of NaCl increased. In all cases, the electrostatic interaction between the carboxylate groups of PSSMA and the quaternary amine groups of PDADMAC were formed during the construction of multilayer thin films. However, exposure to extreme solution conditions, such as high salt concentration, leads to deconstruction of the films and breaking the interaction of PDADMAC and PSSMA which hold the layers together. Therefore, polyelectrolyte segments with weak interaction have large permeability and they are prone to disassociation

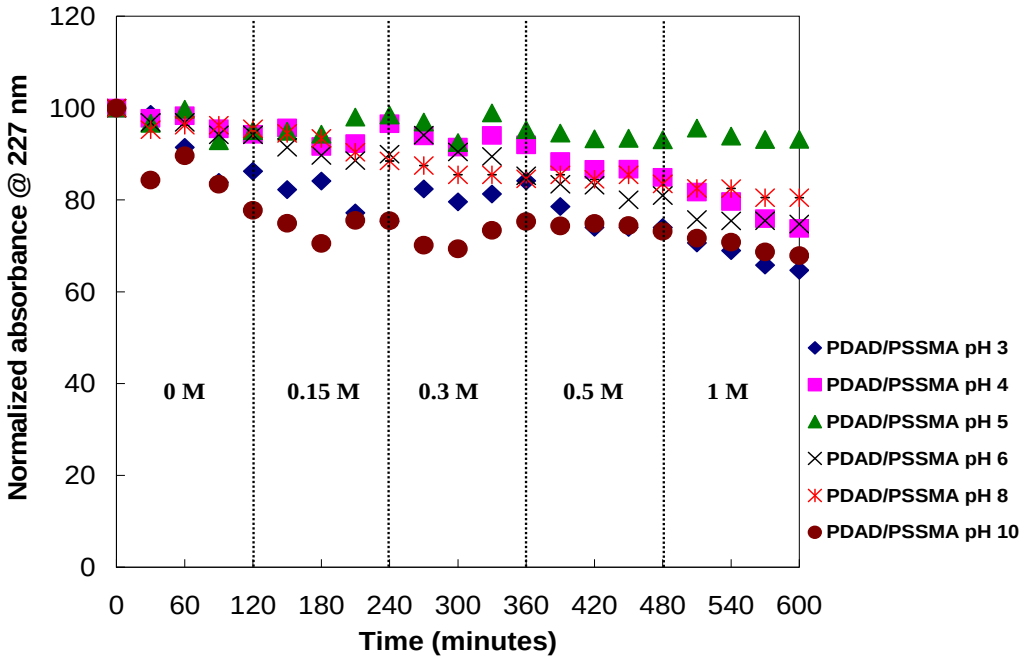


Figure 4.9 Stability of PDADMAC/PSSMA multilayer thin films in buffer pH 7.4 with varying the ionic strength.

4.1.2.3 Stability/Decomposition of Chi/PSS multilayer thin films

In order to investigate the effect of buffer pH on the stable of Chi/PSS multilayer thin films, series of experiment were carried out. The percentage of PSS remaining in Chi/PSS multilayer thin films in buffer pH 3 and pH 7.4 is determined in Table 4.1. As expected, Chi/PSS multilayer thin films demonstrated that pH is sensitive not only in acidic condition but also in physiological condition pH 7.4. As pH changed after administration, the charged balance inside films and degree of electrostatic interaction between polyelectrolyte chains decreased, leading to the dissociation of polyelectrolyte chains.

Focusing on Chi/PSS multilayer thin films deposition at pH 3, the ammonium groups of chitosan remain constant (95.51%) after expose to acidic condition. However, the ammonium groups of chitosan lost their charge density after expose to neutral condition, resulting in the decomposition of Chi/PSS multilayer thin films. Oppositely,

Chi/PSS multilayer thin film deposition at pH 6, which contains either amine group or ammonium group exhibited swelling of multilayer side chains in acidic condition. Moreover, it also decomposed in neutral condition, but less than another conditions.

Table 4.1 Percentage of PSS remaining in $(Chi/PSS)_{10}$ multilayer thin films after exposed to buffer pH solution.

Deposition pH	% PSS remaining		
	pH 3	pH 7.4	pH 7.4 + 0.15 NaCl
3	95.51 ± 0.83	28.39 ± 0.22	24.67 ± 0.23
4	91.89 ± 0.18	30.61 ± 0.15	26.76 ± 0.13
5	97.00 ± 0.23	36.94 ± 0.22	32.67 ± 0.66
6	88.92 ± 0.21	54.69 ± 0.80	50.36 ± 0.34

The schematic concerning the decomposition of Chi/PSS multilayer thin films after exposured at acidic condition and neutral condition is represented in Figure 4.10. It demonstrates that some parameters, such as ionic strength in the buffer pH also have the effect of decomposition of Chitosan/PSS multilayer thin films. As swelling of Chi/PSS multilayer thin films is influenced by many factors, fine modulation of drug release is possible.

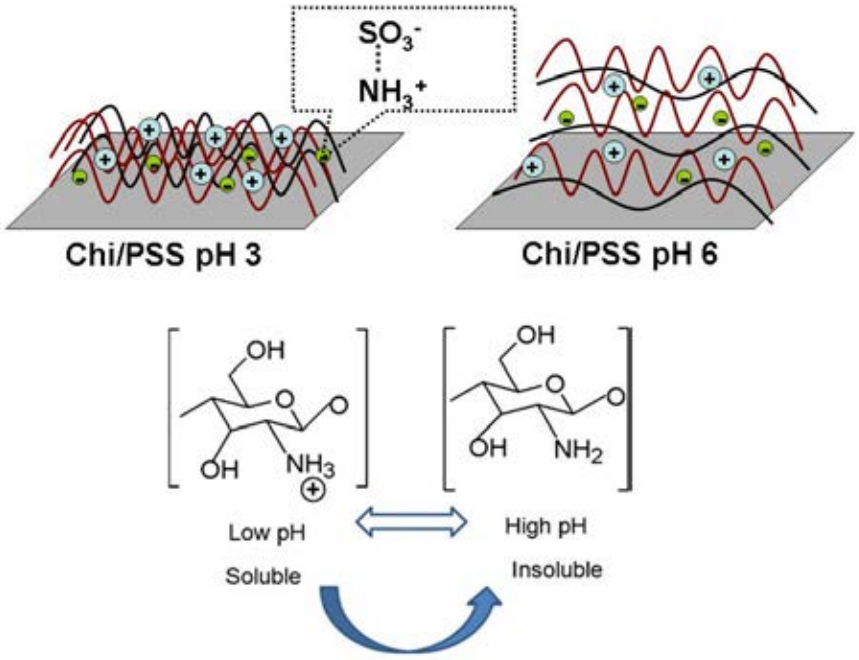


Figure 4.10 Schematic illustrations of Chi/PSS multilayer thin films. At low pH(less than about 6), the ammonium group of chitosan are protonated. At high pH, (above pH 6.5), the amine group of chitosan are deprotonated and reactive.

4.1.3 Loading and release characteristics of hydrophobic drug into PEM films

4.1.3.1 Loading of curcumin into PEM films

- Effect of solvent composition

The loading of small molecule into polyelectrolyte multilayer thin films and other types of pH sensitive polymer films are effectively governed by a number of properties of systems such as swelling, charge density, hydrophilic/hydrophobic balance as well as solvent solubility. In this experiment, PDADMAC/PSS was used as strong polyelectrolytes. PDADMAC and PSS had a high charge density and stable all of pH rang range. Another factor that must be considered as the loading efficiency which is able

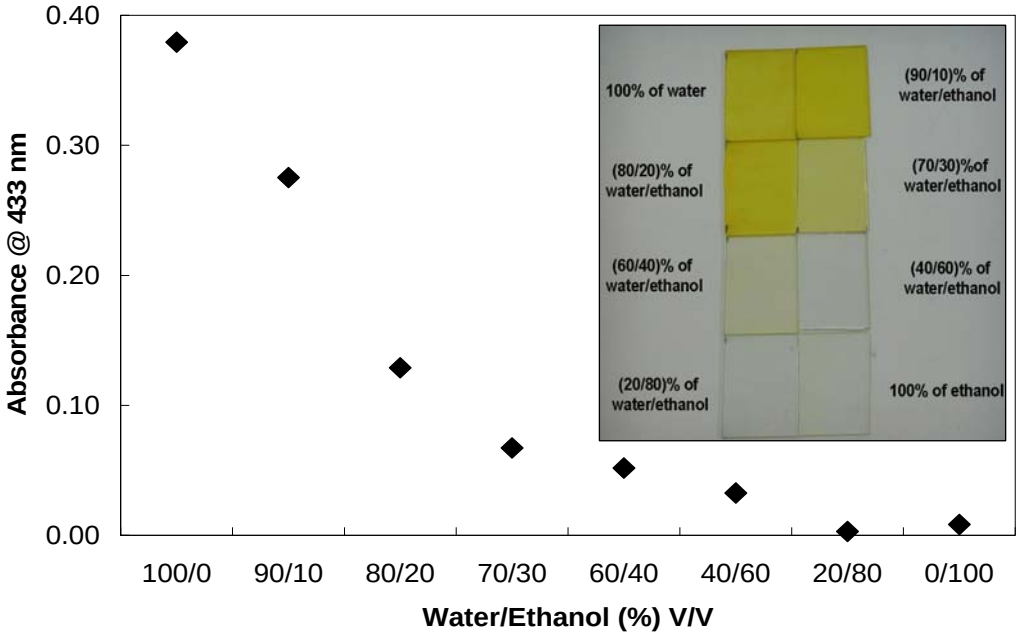
to turn the absorbance of curcumin into PDADMAC/PSS multilayer thin film is the potential of solvent composition.

Normally, curcumin is soluble in organic solvents like methanol, ethanol, DMF, DMSO, chloroform and acetonitrile. Moreover, it is moderately soluble in hexane, cyclohexane, carbon tetrachloride tetrahydrofuran, dioxane and terta-butanol.[118] Since ethanol is a good solvent for dissolving curcumin, it expressed strong absorption in the UV–vis region with absorption maximum in the range from 427 to 435 nm[119] Figure 4.11A shows the absorbance of curcumin loading PDADMAC/PSS multilayer thin films which were varying the ratio of mixed solvent in order to dissolve curcumin during 120 minutes. When curcumin was dissolved in pure ethanol, no load in the multilayer thin films was observed because of its good solubility in this solvent. Nevertheless, when the volume fraction of water in ethanol was increased to above 50%, the partitioning of curcumin in PDADMAC/PSS multilayer thin films film was visible as the film turned bright yellow. As a results, the modified surface via electrostatic self-assembly can enhance the hydrophobic characteristics of multilayer thin films. This enhancement in hydrophobic behavior of multilayer thin films could be applied to absorb in several drug molecules and molecular biology. According to insolubility of curcumin in water which has both hydrophobic and hydrophilic parts along the chain therefore, the loading of curcumin in the PDADMAC/PSS multilayer thin films are mainly from the hydrophobic nature of multilayer thin films. Other attractive interactions can also contribute, such as hydrogen bonding, electrostatic interactions between polyion - curcumin molecules.

Figure 4.11B represents the total curcumin solutions in various ratio of water/ethanol (%), v/v. The increase of ethanol volume fraction more than 30% may cause the excessiveness of curcumin solubility in the miscible system, leading to the decrease of curcumin loading into PDADMAC/PSS multilayer thin films. In further, the observation which was found out during this experimental was the unstable of curcumin solution, which was precipitated over 24 hour period. Although, the unstable of curcumin solution was reached after 24 hour, the load of curcumin into PDADMAC/PSS multilayer

thin films can be employed with the optimum loading time within 2 hours. This observation will be discussed in the next section.

(A)



(B)

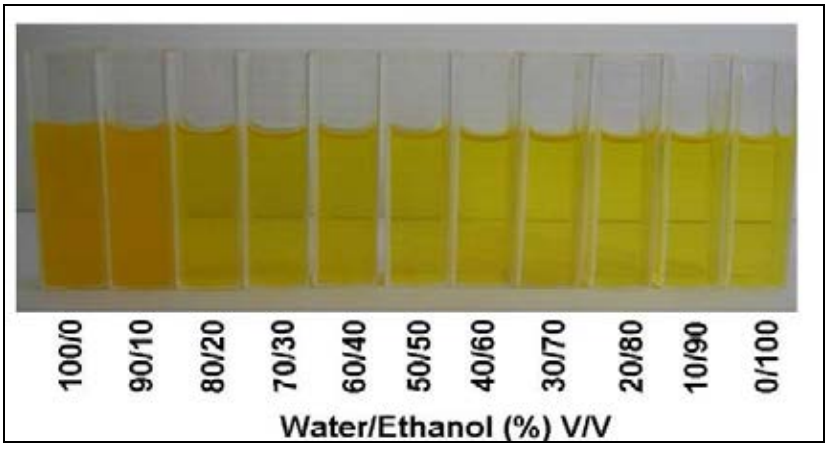


Figure 4.11 (A) Loading of curcumin into PDADMAC/PSS multilayer thin films as a function of solvent composition. The inserted picture show glass slides of curcumin

loading PDADMAC/PSS multilayer thin films at 120 minutes. (B) Images of curcumin solutions dissolve in different ratio of water/ethanol (%), v/v.

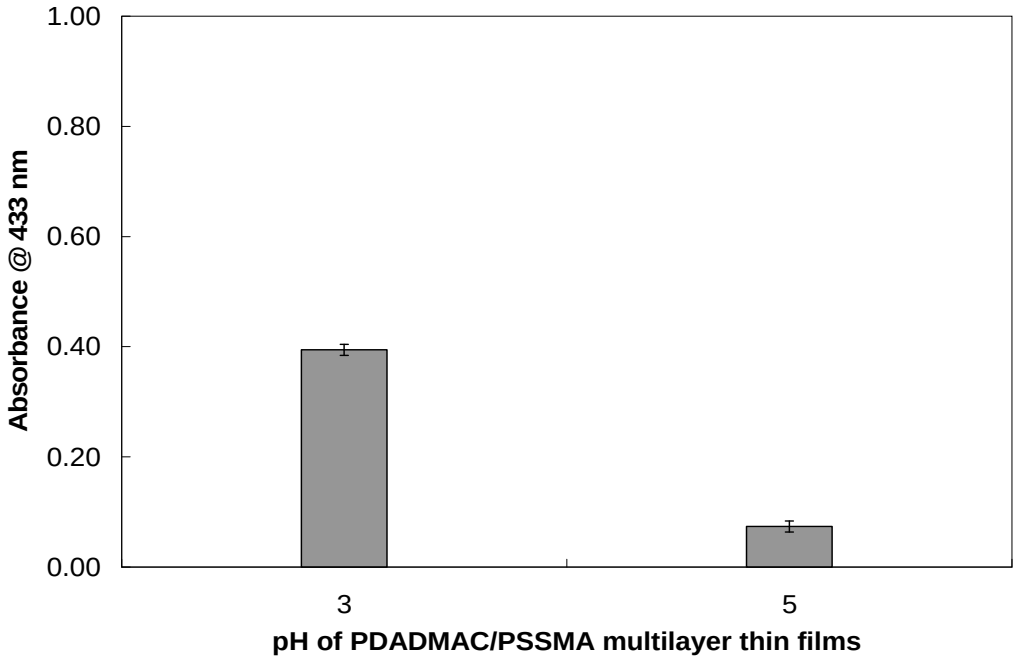
- Effect of pH deposition of multilayer thin films

Curcumin is poorly soluble in water in acidic or neutral pH condition with the macroscopic undissolved flakes visible in the solution. In basic condition, the dissociation of compound underwent hydrolytic degradation rapidly. In addition, the stability of curcumin molecule in the pH range from1 to 11 was also considered as one of parameters that effect release rate. When pH is <1, curcumin has a red colour, which indicates the protonated form (H_4A^+). Curcumin in solution 1-7 has a yellow colour. In this pH range, the majority of curcumin molecule is in the neutral form (H_3A). At pH > 8, the colour change to arrange red.[120] To obtain optimum stability of preparation, the pH should be maintained below pH 7.

Water miscibility of ethanol and curcumin behavior in aqueous phase are two important factors causing successful loading of hydrophobic curcumin to multilayer thin films. This study fixed the ratio composition at 80/20 of water/ethanol (%), v/v as the maximum ratio in order to load of curcumin into the matrix.

Figure 4.12 shows the effect of pH deposition on the load of curcumin into PDADMAC/PSSMA and Chi/PSS multilayer thin films. Both multilayer thin films have the saturation of curcumin loading time at 2 hours (data not show). The absorbance of curcumin into PDADMAC/PSSMA deposition at pH 3 and 5 were 0.39 and 0.07, respectively as presenting in Figure 4.12A. The higher absorbance of PDADMAC/PSSMA deposition at pH 3 is because the protonated COOH group of PSSMA at pH 3 has more hydrophobic region which can interact with the aromatic phenol of curcumin molecules to form intermolecular complex, resulting in high curcumin loading content.

(A)



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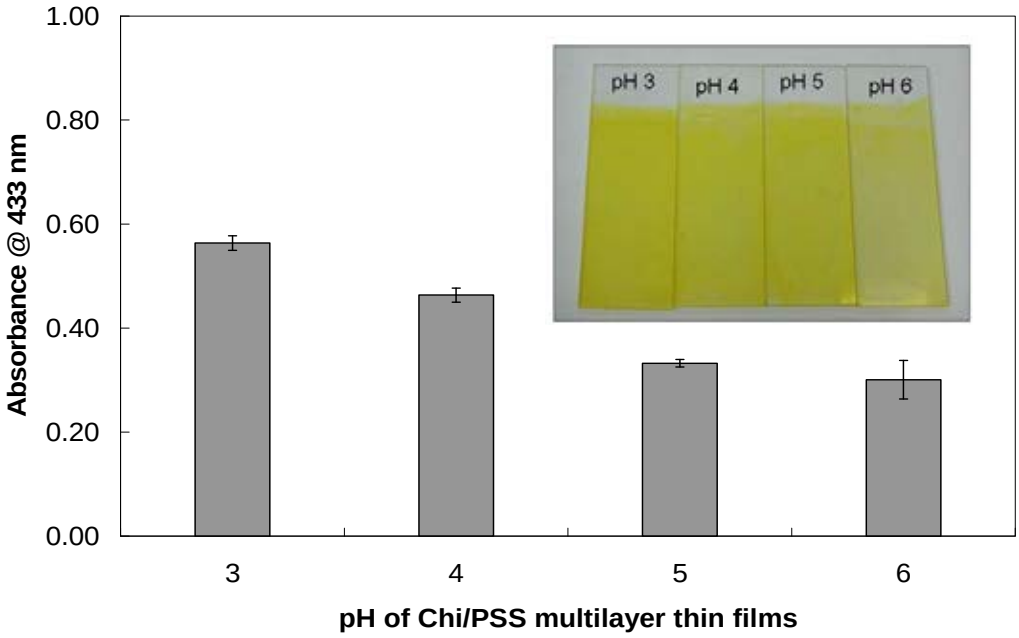


Figure 4.12 Loading of curcumin into (A) PDADMAC/PSSMA and (B) Chi/PSS as a function of pH deposition of multilayer thin films. The inserted picture show images of curcumin loading Chi/PSS multilayer thin films on glass slide substrates.

Similar result was observed in the case of Chi/PSS multilayer thin films as shown in Figure 4.12B. The absorbance value of curcumin loading Chi/PSS deposition at pH 3, 4, 5 and 6 were 0.56, 0.46, 0.33 and 0.30, respectively. As a result, curcumin showed the highest absorbance in Chi/PSS multilayer thin films deposition at pH 3. This result demonstrates the pronounced hydrophobic effect, especially at lower pH deposition, at which the loading of curcumin was preferred to absorb. It may be predicted that the decrease of pH increases the OH group of phenolic rings and forms the hydrogen bonding of multilayer-curcumin interaction. Therefore, the attractive interaction of PDADMAC/PSSMA and Chi/PSS multilayer thin films and curcumin attribute to the hydrophobic interaction and hydrogen bonding. Curcumin loading Chi/PSS multilayer thin films showed homogeneous absorption with its hue derivation from natural color of curcumin as presenting the inserted picture in Figure 4.12B.

- Effect of surface charged of outer layer

One of the advantages of electrostatic self assembly is to adjustment the charged coverage surface of multilayers. To investigate the effect of surface charge on the loading of curcumin, the built-up of positively and negatively charge on the outer of the multilayer films was formed. It can be observed that, curcumin which was no obvious charged can be loading in both of positively and negatively charge of outer multilayer film as show in Figure 4.13. As mention before, the load of curcumin in the multilayer thin films was mainly based on the hydrophobic nature of their structure and hydrophobic surface of multilayer thin films. Additionally, the load of curcumin was significantly in creased with increase in terms of the thickness from 10 to11 layers and 14 to 15 layers and demonstrated the similar result of effect of surface charge. Therefore, the load of curcumin could be absorption/diffusion into multilayer thin films without any effects of surface charge.

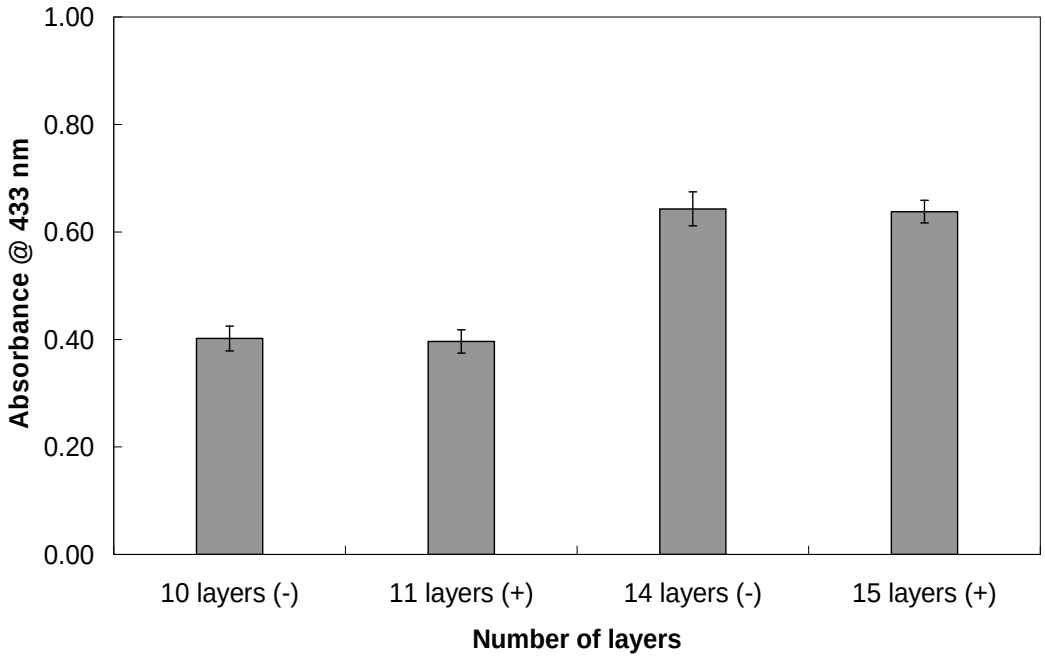


Figure 4.13 Loading of curcumin into PDADMAC/PSS multilayer thin films as a function of surface charge of outer layer.

- Kinetic loading of curcumin

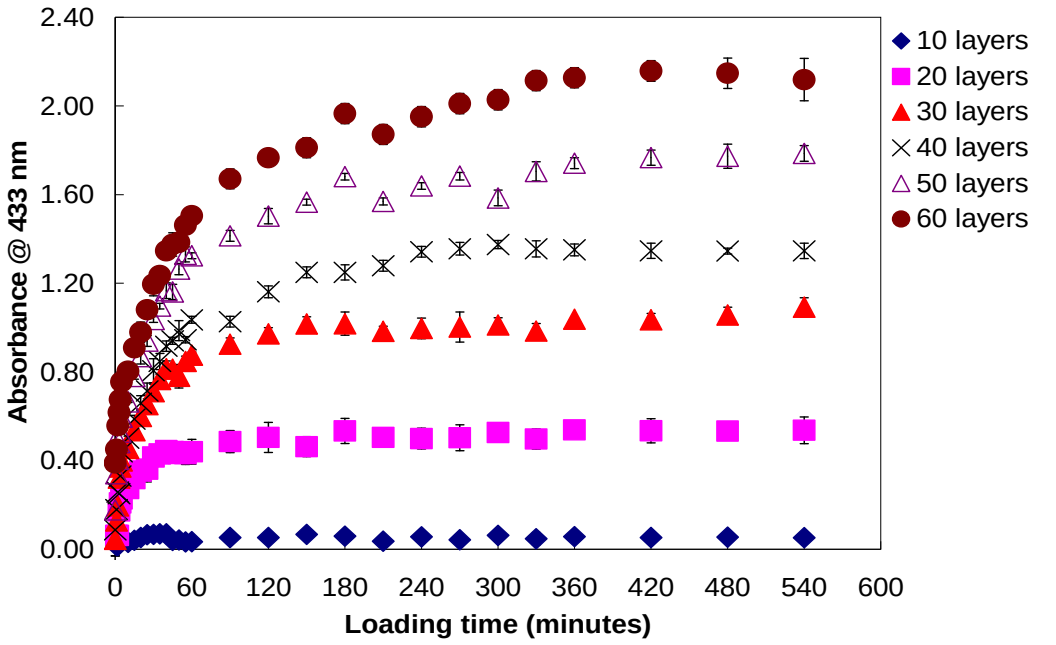
To investigate this model drug which could be used advantageously to diffuse into multilayer thin films, the effect of loading time was reported. In the initial loading time of curcumin in Figure 4.14A, PDADMAC/PSS multilayer thin films with various numbers of layers were rapidly increased in absorbance at 433 nm during 100 minutes. 10 layers of curcumin loading PDADMAC/PSS multilayer thin films were slightly absorbed and limited of loading efficiency after 60 minutes. On the other hand, the absorption kinetic of curcumin loading 60 layers of PDADMAC/PSS multilayer thin film was increased significantly, suggesting that most of curcumin was still incorporated into thin films of substrate.

Normally, the absorption of model drugs loading multilayer thin films has two mains adsorption phenomena including bulk and surface absorption. The bulk absorption

property is the diffusion of drug molecules pass through multilayer thin films. The increasing in number of layers significantly induce drug molecules absorb/or diffuse inside the matrix of the film. Conversely, the surface absorption property is the adsorption of drug molecules on the surface of thin films. Therefore, the increase of number of layers has no effect on the load of drug molecules into multilayer thin films.

We then examined the absorption of curcumin in PDADMAC/PSS multilayer thin films by varying number of layers. Figure 4.14B represent the absorbance of curcumin loading multilayer thin films in 540 minutes loading times. As a result, the amount of curcumin incorporated in multilayer thin films is directly related to the number of layers as a bulk characteristic. The bulk absorption of model drugs are qualitatively similar to the results which was obtained by Wang et al.[121]They showed that the total amount of drug increases when the number of layer pairs increases. This agreement of result confirms that the model drugs in this study are diffused through the whole matrix and the matrix behaves as a drug reservoir.

(A)



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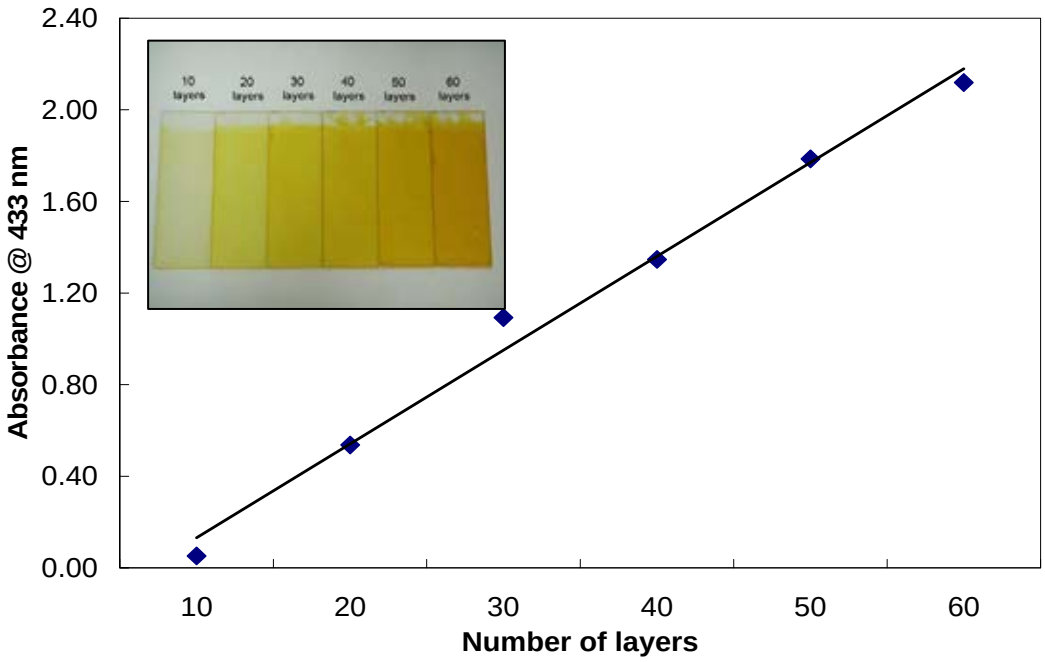
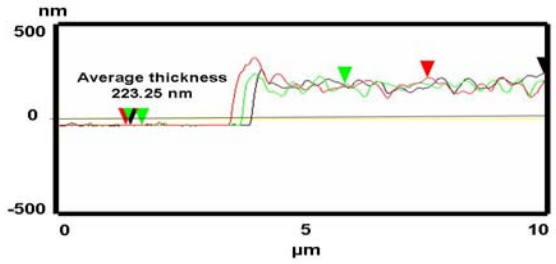


Figure 4.14 (A) Kinetic loading of curcumin into PDADMAC/PSS multilayer thin films as a function of time at 433 nm. (B) Absorption property of curcumin as a function of number of layers at 540 minutes of loading time. The inserted picture show image of curcumin loading PDADMAC/PSS multilayer thin films on glass slides.

AFM thickness of $(PDADMAC/PSS)_7$ multilayer thin films before and after loading curcumin at 2 hour of loading time are depicted in Figure 4.15. The original of $(PDADMAC/PSS)_7$ multilayer thin films appears smoothly when the average thickness was about 223.25 nm. $(PDADMAC/PSS)_7$ multilayer thin films became rougher when the average thickness about 249.17 nm after loading in curcumin solution. Thus the change in films thickness reflects the gradual diffusion of curcumin into multilayer thin films.

(A)



(B)

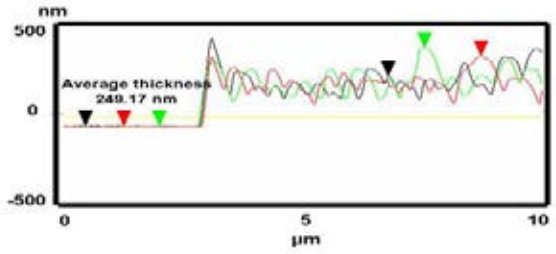


Figure 4.15 AFM thicknesses of PDADMAC/PSS multilayer thin films (A) before and (B) after loading curcumin.

- Loading efficiency of curcumin

To evaluate the loading efficiency of curcumin into PDADMAC/PSS multilayer thin film, the effect of curcumin in various solvent compositions was studied by detecting the absorbance at 433 nm. The absorbance was translated to concentration by comparing the standard solutions as shown in Figure 4.16. The mixed solvent of 20/80 of water/ethanol, % was used as a standard solution due to its good release solvent. Thus the final curcumin solution after release out from multilayer thin films was calculated in terms of the amount of curcumin content using the calibration curve.

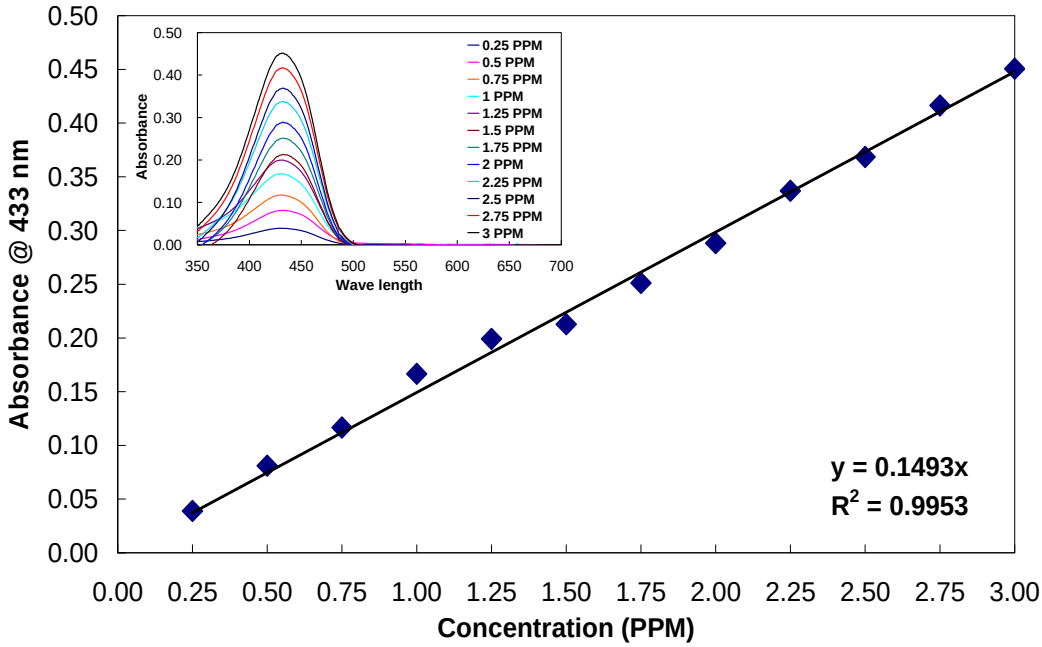


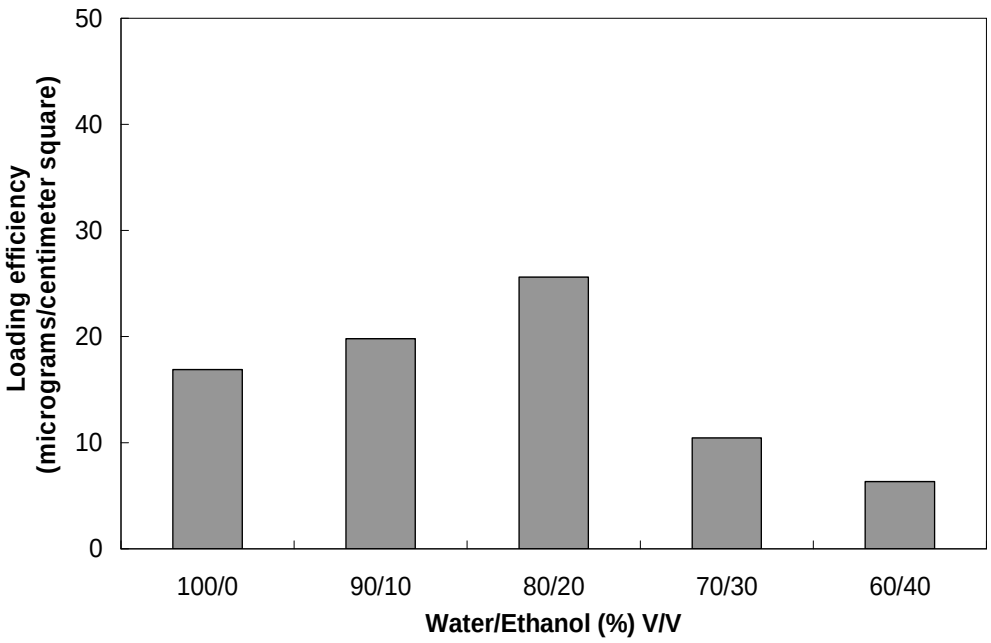
Figure 4.16 Calibration curve of curcumin 0.25 - 3 PPM.

Figure 4.17 (A) demonstrates the loading efficiency of curcumin in PDADMAC/PSS multilayer thin films in the range of 6-30 μg/cm^2. In this result, curcumin loading into multilayer thin films was affected by the solvent composition of water/ethanol in the system. The loading efficiency of curcumin increased when the percentage of ethanol to 20% increased. The loading efficiency of curcumin dissolution in 100/0, 90/10 and 80/20 of water/ethanol were 16.88, 19.80 and 25.59 μg/cm^2

respectively. However, curcumin dissolution in 70/30 of water/ethanol, (%) tended to decrease loading efficiency. As a result, the miscible of water and ethanol system and the hydrophobic nature of multilayer thin films are the two important factors causing successful loading of curcumin into PDADMAC/PSS thin films. Therefore, curcumin dissolution in 80/20, (%) of water/ethanol, was obtained as a solvent composition in order to prepare the loading solution in further studies.

Next step, we examined some factors which affect the loading efficiency of curcumin into PDADMAC/PSS multilayer thin films. The loading time was fixed at 2 hour as it was enough to attain equilibrium loading system. The relationship between the numbers of multilayer and the loading efficiency of curcumin is demonstrated in Figure 4.17 B. The load of curcumin efficiency increased initially from 6.05 $\mu g/cm^2$ and reached saturation value of 58.74 $\mu g/cm^2$. Based on this loading efficiency trend, linear growth was observed when there was diffusive component which absorbed into the bulk growing of multilayer thin films, and subsequent deposition cycle leaded to increments of curcumin absorption behavior. This result confirms that the load of curcumin through multilayer thin films is dependent from the thickness of films, which can be further controlled by the number of deposition layers. One implication of this behavior is that the expectation of loading curcumin was greatest in the diffusion zone of multilayer thin films (i.e. matrix zone) which contributes to the release behavior followed by a more controlled release.

(A)



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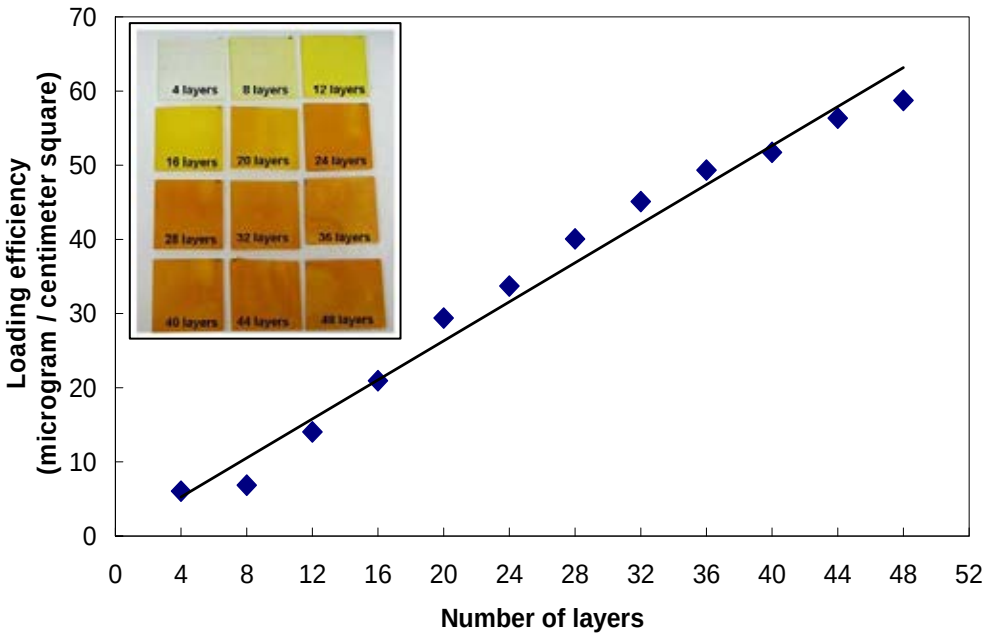


Figure 4.17 (A) Loading efficiency of curcumin into PDADMAC/PSS multilayer thin films as a function of solvent composition after 120 minutes of loading time. (B) Loading efficiency of curcumin into PDADMAC/PSS multilayer thin films as a function of number of layers. The inserted picture shows image of curcumin loading PDADMAC/PSS multilayer thin films.

- Light stability of curcumin

To investigate the effect of light on the stability of curcumin, curcumin solutions were exposed to the neon light under room comparing with the dark control. Curcumin was dissolved with a final concentration of 0.01% w/w by varying solvent composition of water/ethanol, (%). At the period of time from 1- 5 days, 3 ml of curcumin solution was taken out and curcumin in mixed solvent composition was determined. Figure 4.18 shows the bar graph of the curcumin solutions, reporting as the remaining percentage of curcumin in various solvent compositions at definition of 5 day. The remaining percentage of curcumin soluble in 100/0 and 90/10 of water/ethanol in the present of light were 8.3% and 9.4% respectively. On the other hand, the remaining percentage of curcumin soluble in 80/20, 70/30, 60/40, 50/50 and 0/100 of water/ethanol in the present of light were 75.25%, 55.11%, 71.25%, 68.48% and 84.15% respectively. In the dark control, the remaining percentage of curcumin dissolution in solvent composition (80/20, 70/30, 60/40, 50/50 and 0/100) closed to its beginning value during 5 days.

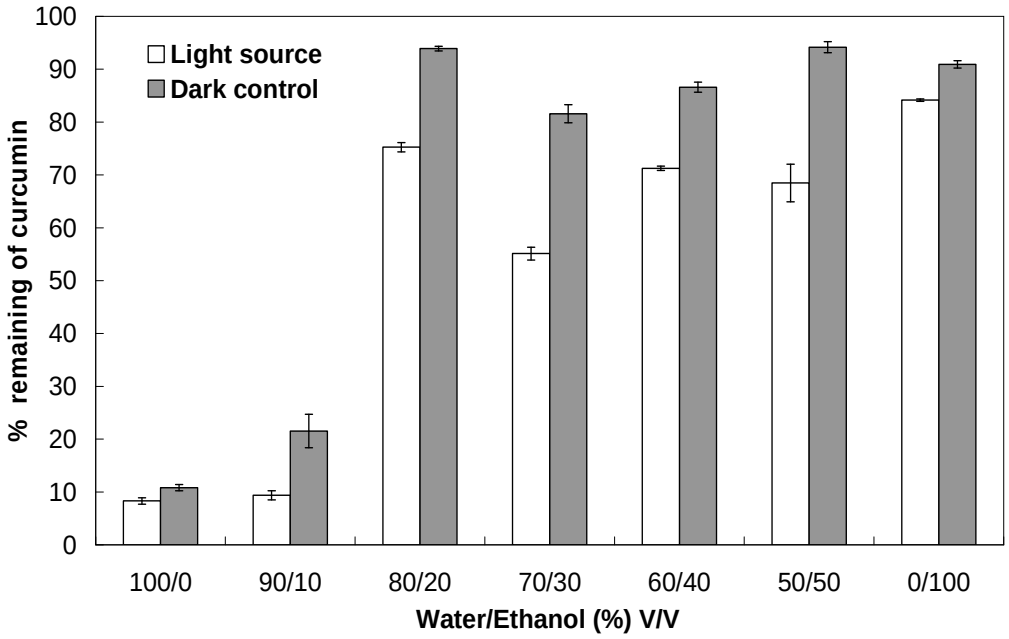


Figure 4.18 Light stability of curcumin solution which varies the solvent composition in the present of light and dark control after 5 days.

In previous studies, they reported that curcumin is unstable under light exposure which can decompose into many products.[122]The main decomposition products have been identified as feruloymethane, ferulic acid and vanillin as showed in Figure 4.19. Therefore, the use of curcumin is limited due to low water soluble, high decomposition rate in alkaline media as well as photo degradation. There are many studies preparing water soluble of curcumin by complex formation or interaction with other molecules.[123] Moreover, the improvement of light stability of curcumin depends on either the presence of an organic solvent or the absent of water content. According to poor dissolution of curcumin in pure water and instability under light exposure, our study suggests that the light stability of curcumin in solvent composition (80/20, 70/30, 60/40, 50/50 and 0/100 water/ethanol, (%)) is possible if curcumin solution was stored and maintained in the dark condition.

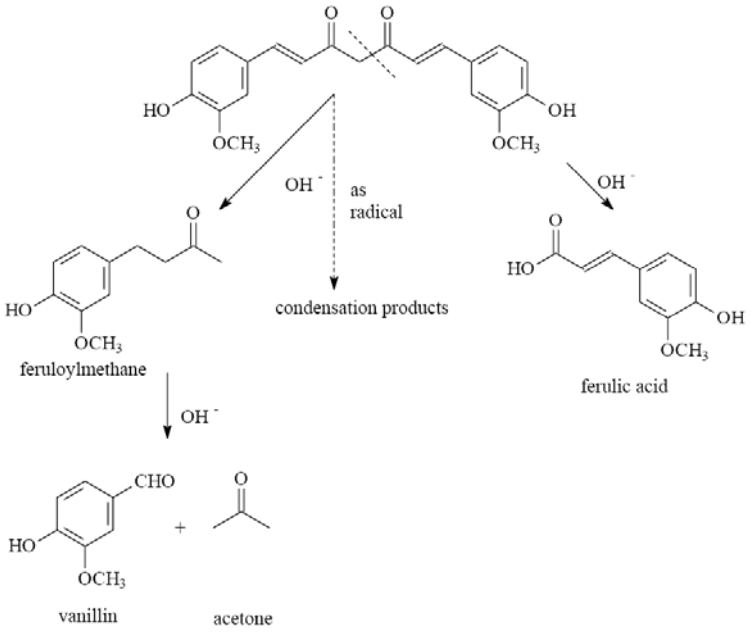


Figure 4.19 Photochemical degradation of curcumin.

In addition, the light stability of curcumin solution and curcumin loading PDADMAC/PSS multilayer thin films were investigated as shown in Figure 4.20. Curcumin solution and curcumin loading PDADMAC/PSS multilayer thin films were kept in the absence and presence of neon light in order to investigate the light stability of

curcumin. The results revealed that after storage in the absence of light for 7 days, the remaining percentages of curcumin solution, and curcumin loading PDADMAC/PSS multilayer thin films were 75.47 and 83.74 respectively. In the presence of light, the percentages of the remaining curcumin solution, and curcumin loading PDADMAC/PSS multilayer thin films were 48.58 and 69.37 respectively. Interestingly, the remaining percentages of curcumin loading PDADMAC/PSS multilayer thin films was higher than that curcumin solution's after exposed to the light, suggesting the present of polyelectrolyte multilayer thin films can delay the degradation of curcumin molecule. In addition, the growth of multilayer thin films can decrease the water content in multilayer thin films. These findings are in agreement with the results of other researchers, suggesting that the formation of polyelectrolyte multilayer thin films can improve the stability of light and oxygen sensitive substances.[124]

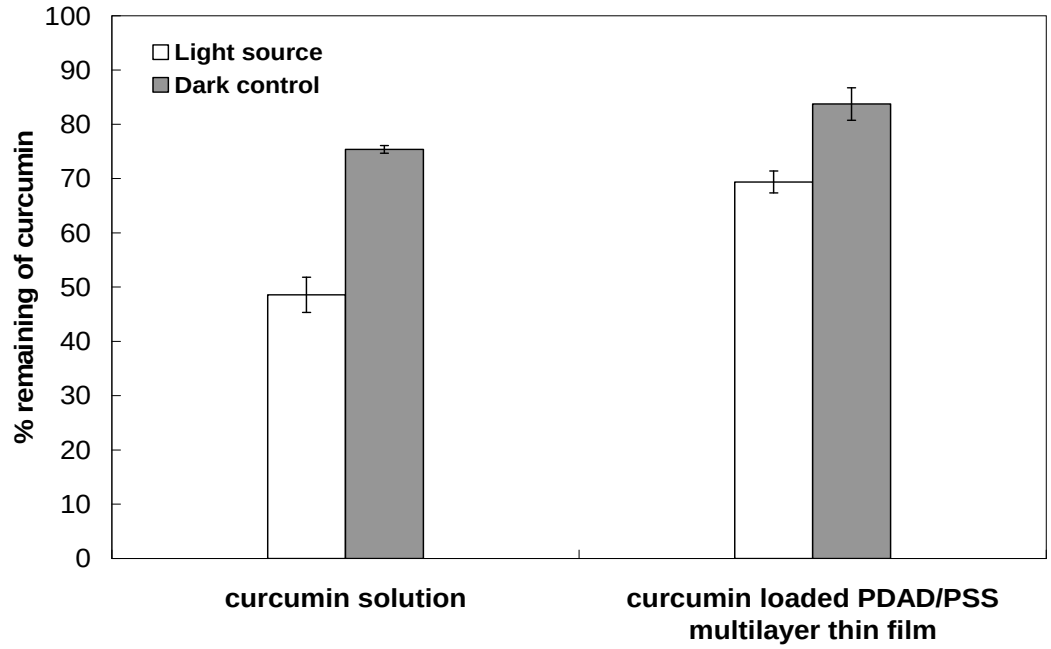


Figure 4.20 Light stability of curcumin solution and curcumin loading PDADMAC/PSS multilayer thin films after exposed to the light and dark room control after of 7 days.

The Image of the glass slide loading curcumin into PDADMAC/PSS multilayer thin films showed a dark yellow colour with uniform surface (Figure 4.21A). Glass slide stored in dark room control has less yellow colour (Figure 4.21B). On the other hand, glass slide explored in neon light showed almost degradation of curcumin. From this resulted, it is important to maintain their stability of commercial curcumin in the absence of light under room temperature.

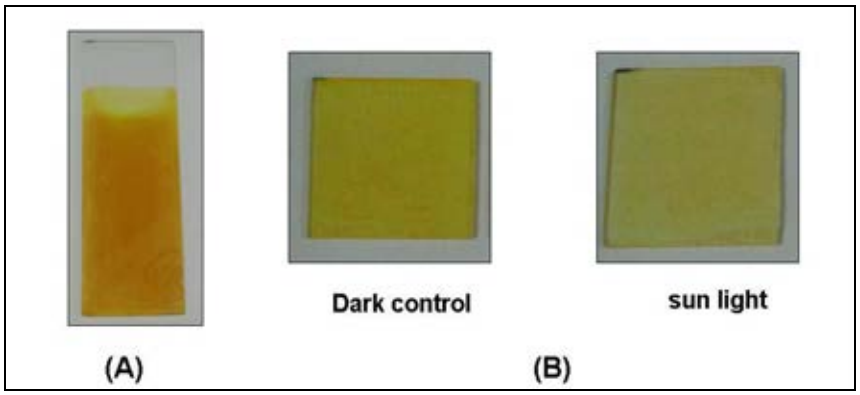


Figure 4.21 Images of glass slide substrate of curcumin loading PDADMAC/PSS thin films before and after exposed to the light. (A) The first preparing of glass slide before exposing. (B) Curcumin loading PDADMAC/PSS thin films after exposed to the dark control and neon light at final of 7 days.

4.1.3.2 Release of curcumin from PEM films

- Effect of solvent composition

Curcumin is a high hydrophobic molecule with very low solubility in water. Moreover, curcumin is unstable under alkaline solution and decompose into several products. The main decomposition products are identified as feruloymethane, ferulic acid and vanillin. Therefore, the release characteristic of curcumin loading PDADMAC/PSS multilayer thin films was investigated by immersion multilayer thin films in mixed solvent composition of water/ethanol as shown in Figure 4.22. This result suggests that

curcumin was released approximately 90% from PDAD/PSS multilayer thin films within 24 hours by pure ethanol, while only 9 % of curcumin was released by pure water during 45 hours. Interestingly, the release characteristic of curcumin in the mixed solvent of 20/80 of water/ethanol has a faster release profile when compares to that of pure ethanol. Approximately, 95% of the total curcumin loading PDADMAC/PSS multilayer thin film was released in the mixed solvent of 20/80 of water/ethanol within 9 hours. The faster curcumin release profile is due to the presence of water molecule which enhance molecule of ethanol to diffuse inside the hydrophobicity part of multilayer thin films. It can be noted that, the increase in volume of water more than 30% can cause the poor solvent in the release system, resulting in a decrease of curcumin released from multilayer thin films.

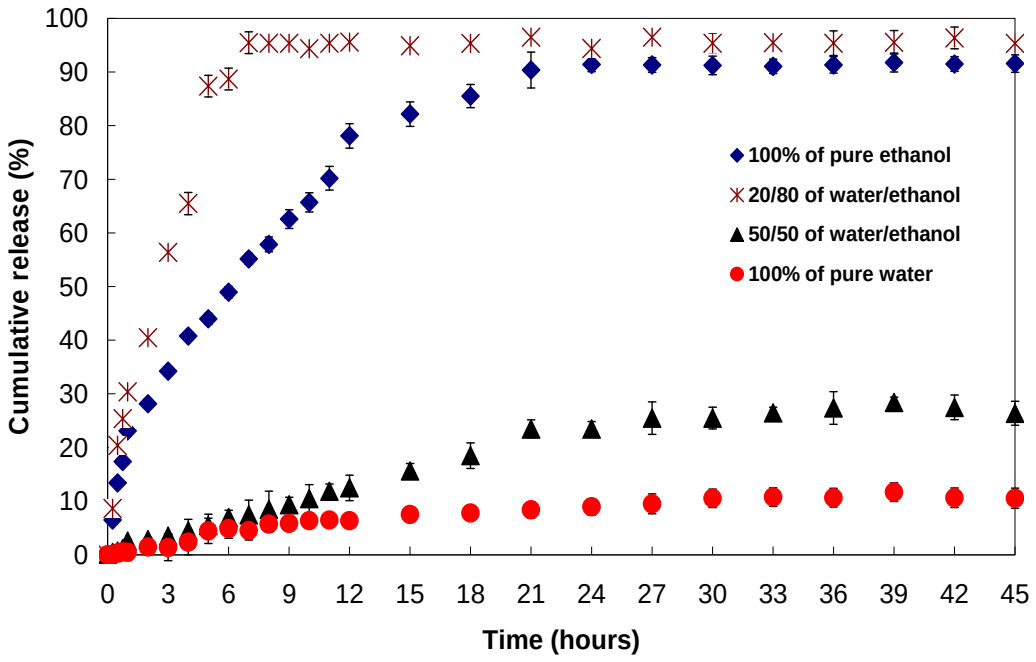


Figure 4.22 Profile release of curcumin from PDADMAC/PSS multilayer thin films in mixed solvent of water/ethanol (%), v/v.

- Effect of buffer pH solution

A major challenge in drug delivery is to produce controlled release system for small encapsulated drug molecules. The pH variation along the gastrointestinal (GI) tract is well known and should be taken into consideration when develops drug administrations. The gastrointestinal tract has a dynamic range of environment, especially with respect to the pH of aqueous fluids. Therefore the release of curcumin from Chi/PSS multilayer thin films was determined at pH 3 and pH 7.4, corresponding of the pH of gastric chamber to the neutral intestine lumen. To avoid the evident of the degradation of curcumin from long time exposure in water, curcumin loading Chi/PSS multilayer thin films was stored in the dark condition. The release kinetic of curcumin from Chi/PSS multilayer thin films was pH dependent as represented in Figure 4.23. The increase of pH significantly speeded up the release of curcumin. For example, only 21% of curcumin was released in buffer pH 3, while 40% of total of curcumin was released in buffer pH 7.4.

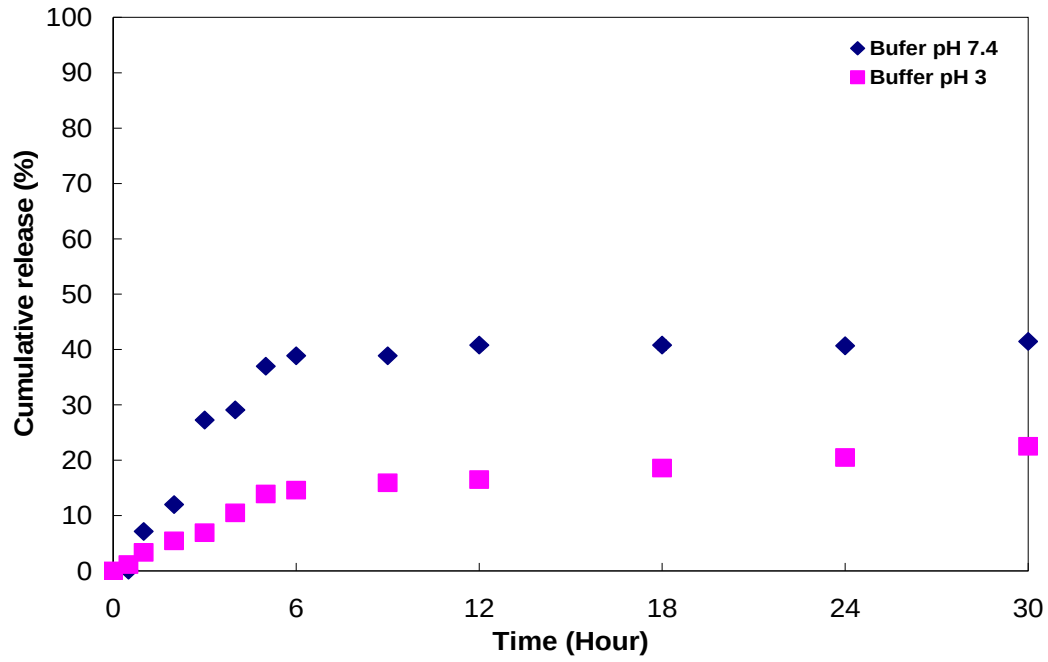


Figure 4.23 Profile release of curcumin from Chi/PSS multilayer thin films deposition at pH 6 in buffer pH 3 and 7.4.

To explain the pH sensitivity of drug release behavior, the swelling behavior of multilayer thin films must be considered. As discussed above, the interaction of Chi and PSS is known as pH dependent, while PSS chains remain constant sulfonated groups at all pH value. As mentioned before, the pKa of chitosan is about 6.5 depending on the degree of deacetylation and its molecular weight. In the current study of Chi/PSS at pH 6, most amino groups of chitosan were protonated, thus, the electrostatic reaction between (NH_3^+---SO_3^-) is strong. At buffer pH 3, the amino groups of chitosan were protonated, which behaved as the un-swells. As the results, curcumin release from Chi/PSS multilayer thin films is strongly retarded, leading to the slow release rate. As the buffer pH increased to 7.4, almost of amino groups of chitosan were deprotonated. Thus, the electrostatic interaction between Chi and PSS is weak, leading to curcumin diffuse from multilayer thin films.

Basically, once of drug load films are immersed in a solution, there are two main processes which lead to the release of its content: *Diffusion release* and *decomposition release*. Figure 4.24 shows a schematic illustration of drug release from polymer matrix. Drug can be loading in multilayer thin film and released upon change in the pH through enhanced permeability of the film or decomposition of the film entity. The enhanced permeability of the film is result in the accelerated diffusion of drugs out of the film. A similar mechanism is often employed in pH-sensitive drug release from films. In this release mechanism, it is possible to construct pulsated release systems where drug release is accelerated and suppressed alternately in an on-off fashion in response to pH changes. The diffusion behavior also depends on drug-solubility, pH and the hydrophobic/hydrophilic properties of drug-matrix interaction. On the other hand, decomposition of the film entity is more straightforward to trigger the release of drugs. Decomposition behavior is the rapid fragmentation of multilayer thin films and, therefore, the burst release.

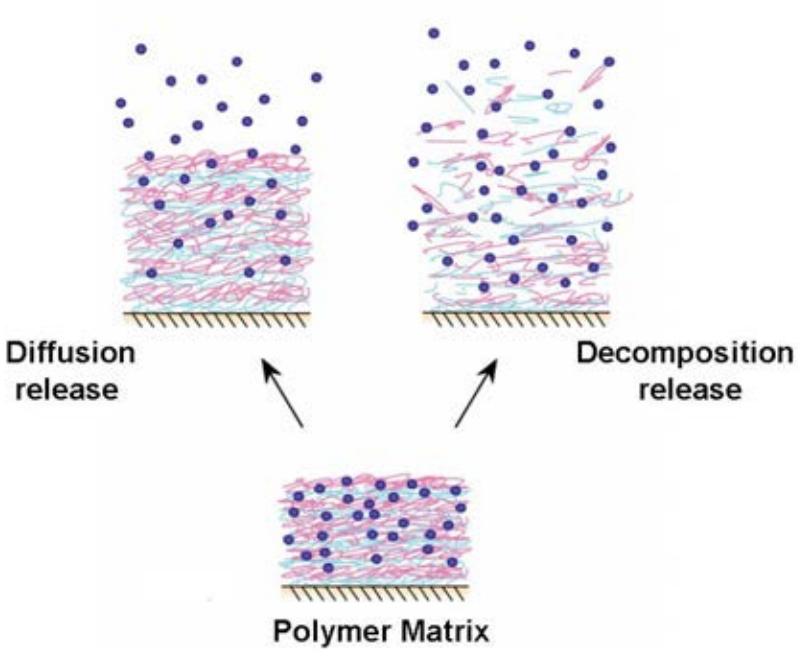


Figure 4.24 Possible routes for pH-sensitive release of drugs from polymer matrix.

- Effect of ionic strength

Another parameter that can influence the polymer matrix structure is the ionic strength. These structural rearrangements happen in both case of increase and decrease of the ionic strength. Figure 4.25 shows the release kinetics of curcumin from PDADMAC/PSS multilayer thin films as a function of NaCl concentration. To avoid the evident degradation of curcumin from the long time exposure in water, the release kinetics was studied at physiological condition without the light. As a result, the release kinetics of curcumin from multilayer thin films depends on the NaCl concentration. The increase of NaCl concentration significantly speeds up the release of curcumin. For example, an initial release of 40.1% of curcumin from multilayer thin films was observed in 6 hours, followed by sustained release about 44% for NaCl 0 M after a period of 30 hours. When concentration of NaCl was increased to 0.15 M, 0.5 M and 1 M, the amount of curcumin release from PDADMAC/PSS multilayer thin films reached 55.33%, 63.75% and 70.87% respectively, during the same time period of 30 hours. Basically, the

release profile of curcumin from strong polyelectrolyte, such as PDADMAC/PSS, is controlled by diffusion mechanism as a result of partitioning between polymer matrix and surrounded aqueous phase. Moreover, the release profile of hydrophobic drug from the polymer matrix thought diffusion mechanism is also determined by the swelling of polymer matrix.[125]The higher swelling, the lager surface area of multilayer surface would be exposed to the diffusing medium at the certain time. This hypothesis is supported by the effect of ionic strength on the release profile of curcumin from PDADMAC/PSS multilayer thin film. The $Na^{+}Cl^{-}$ has the property to disrupt the structure of polyelectrolyte by breaking down the electrostatic interaction, leading to enhancing the release of curcumin molecule. While this experiment confirms the diffusion controlled release of hydrophobic drugs in aqueous medium, it also suggests that the addition of salt can be used to control the release of hydrophobic drug from multilayer thin films.

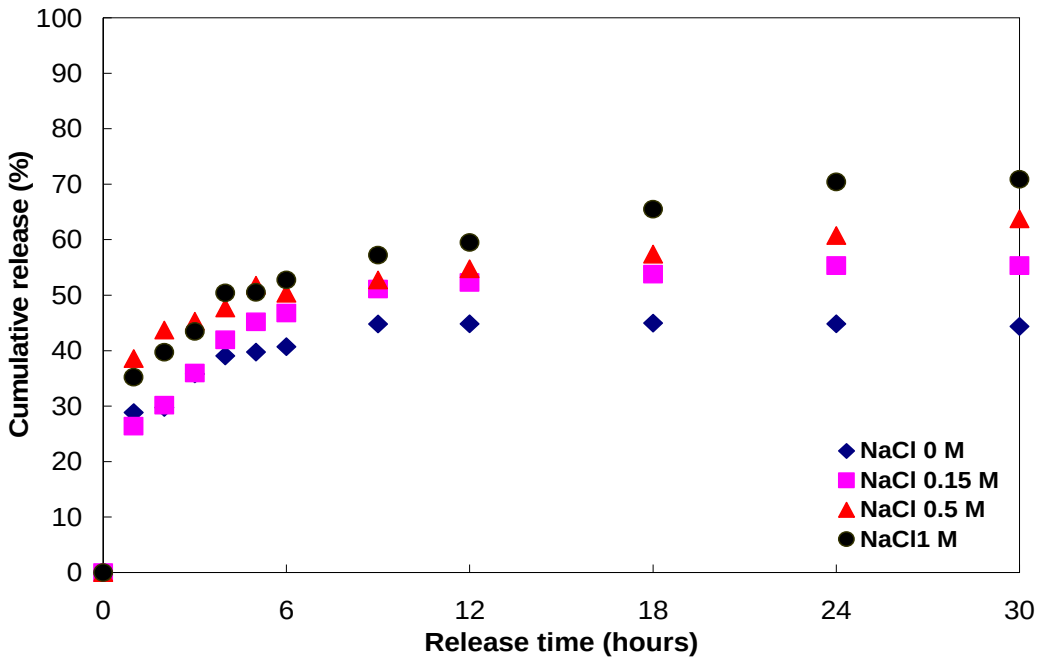


Figure 4.25 Profile release of curcumin from PDADMAC/PSS multilayer thin films in buffer solution pH 7.4 with various ionic strength.

Each polyelectrolyte pair which is employed as layer component has unique properties depending on the properties of each individual component formation. The films built-up leads to the formation of stoichiometic interpolyelectrolyte chains which can be broken upon any shift from the absorption conditions. Therefore, the most important parameters leading to the shift are pH and ionic strength. Figure 4.26 demonstrates the release of curcumin from PDADMAC/PSSMA multilayer thin films in buffer pH 7.4 added 0.15 M of NaCl. Curcumin was release higher form of PDADMAC/PSSMA multilayer thin films deposition at pH 3 than that of deposition at pH 5. As determine in Figure 4.9, multilayer thin films deposition at pH 3 was disassembly of films in buffer pH 7.4. This is because the un-ionized carboxylic groups in PSSMA multilayer chain became ionization, resulting in an excess of negative charge within multilayer thin films and subsequent decomposition of films. By contrast, the profile release of curcumin from PDAD/PSSMA deposition at pH 5 showed a slower rate within 10 hours of release time. This finding is in consistent with our observation (Figure 4.8) where it shows that the film deposition at pH 5 was stable in buffer pH 7.4. Therefore, this result demonstrates the decomposition release behavior of curcumin from PDADMAC/PSSMA polymer matrix whereas the matrix is pH-sensitive control.

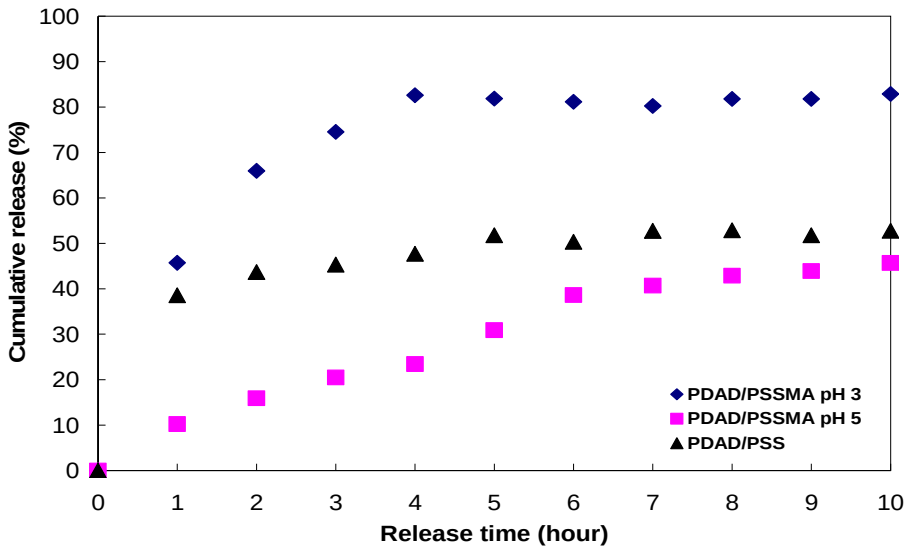


Figure 4.26 Profile release of curcumin from PDADMAC/PSSMA multilayer thin films in buffer solution pH 7.4 containing 0.15 M of NaCl.

4.1.4 Loading and release characteristics of anionic drug into PEM films

4.1.4.1 Loading of Diclofenac sodium (DS) into PEM films

- Effect of pH of drug solution

The influence of drug solution pH on drug loading multilayer thin films was investigated as shown in Figure 4.27. DS was dissolved in a pH range from 3 to 10. It was found that when the microenvironment pH increased, the rate of drug solubility increased which might increase drug loading as well. This result indicated that the solubility of DS was pH dependent. Diclofenac was a weak acid with pKa = 4.18 and it was almost insoluble at low pH.[126] Moreover, it formed an opaque solution at lower pH condition.

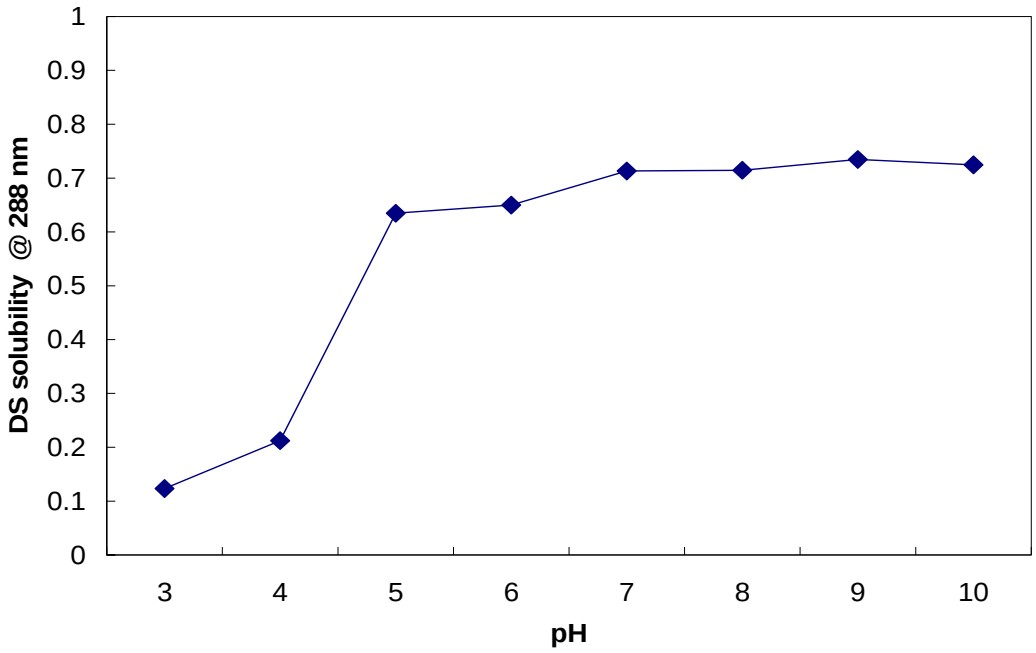


Figure 4.27 The solubility of DS as a function of pH.

Figure 4.28 show DS loading into PDADMAC/PSS polyelectrolyte multilayer thin films as a function of drug solution pH. As a result, the loading of DS which is dissolved in buffer pH 5, 5.5, 6 and 7 has absorbance spectra 0.80, 0.76, 0.58, 0.26 respectively. As a result, DS contains carboxylic group in its structure, the increasing in

pH could improves the solubility of its formulation. At pH 1-3, there was no swelling of DS based on its structure and tended to precipitation. During pH 5-6 which was closed to pH of distilled water, DS showed free solubility and higher absorb into multilayer thin films. However, our study found that since limitation of DS was lower at acid pH, DS was also practically dissolution at pH above 7. It have been reported in several studies that at pH 7 which is higher than the pKa of drugs, the ionization of diclofenac can be extremely promoted. [127] This result is in contrast with our study. It is probably because the over free carboxylic groups always place and distribute in the ionized solution rather than diffuse/absorb into hydrophobic surface of multilayer thin films. Thus, the solution pH of DS was fixed at pH 5 in order to study drug loading and releasing profile of PDADMAC/PSS multilayer thin films.

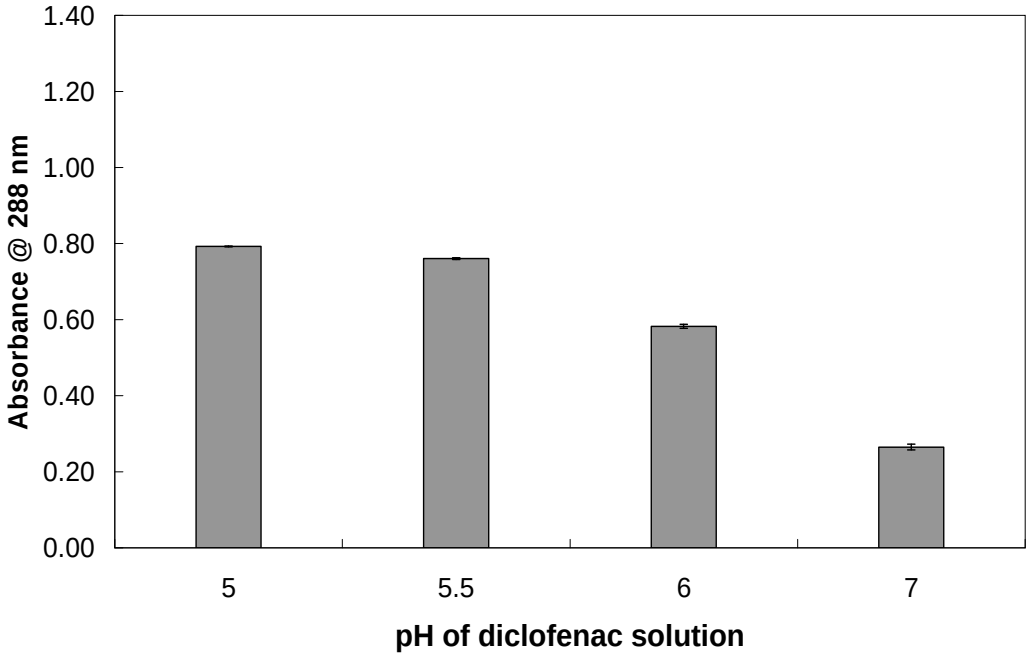


Figure 4.28 Loading of DS into PDADMAC/PSS polyelectrolyte multilayer thin films as a function of drug solution pH.

Figure 4.29A shows the load of DS into PDADMAC/PSSMA multilayer thin films. DS cannot be loading into the formation of PDADMAC/PSSMA deposition at pH 3 due to its insoluble in this pH condition. As the increase of pH condition to pH 5, DS

had ability to load at pH 5 due to its free dissociation. By contrast, no loading of DS was observed at all deposition of Chi/PSS multilayer thin films as demonstrated in Figure 4.29B. The similar un-loading DS in Chi/PSS deposition at pH 3 and 6 could be explained by the basic ionization of chitosan molecule at different pH condition. At pH 3 the amine group of chitosan was protonated to NH_3^+ group and the protonated group of COOH of DS caused the repulsion charge of each other, resulting in un-loading ability. In contrast, at higher pH, deprotonation of NH_2 groups of chitosan occurred, whereas carboxyl groups of DS was ionized to produce COO^- groups and generate weaker electrostatic interaction between polyelectrolyte chains and DS molecule.

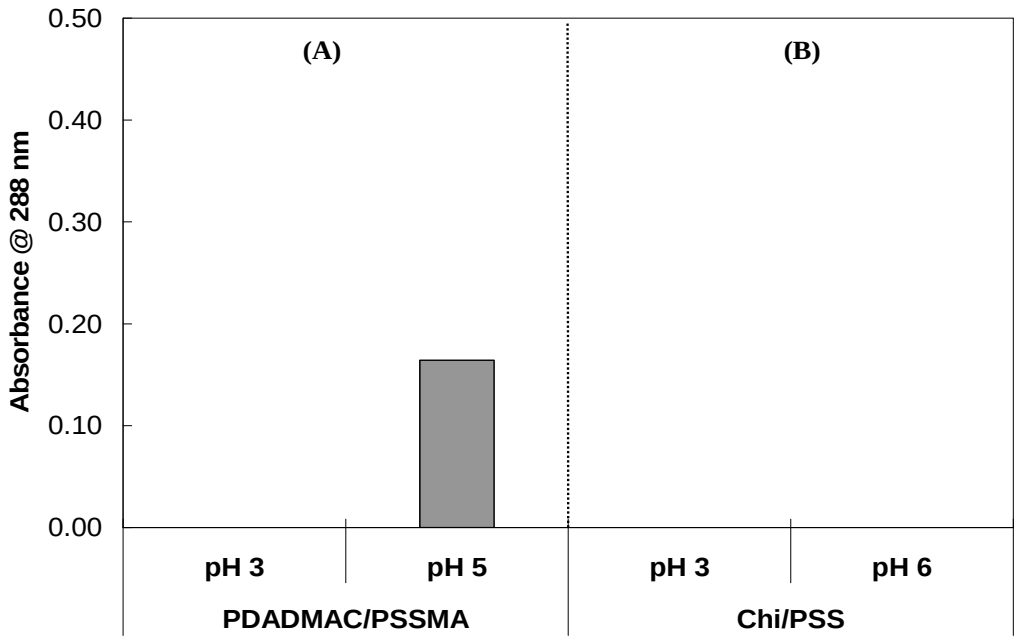


Figure 4.29 Loading of DS into (A) PDADMAC/PSSMA multilayer thin films deposition at pH 3 and 5 and (B) Chi/PSS deposition at pH 3 and 6 as a function of drug solution pH.

- Effect of surface charge of outer layer

The effect of outer layer of PDADMAC/PSS multilayer thin films on the load of DS was observed as show in Figure 4.30. There are interesting differences between the

positive charge and negative charge of multilayer outer surface. Basically, DS has the negative charge in aqueous medium and unfavorable interaction with exist the negative charge polyelectrolyte. Therefore, it associates with positive charge of multilayer thin films based on electrostatic interaction, leading to high drug absorption. This clear result is consistent with Schneider et al.[128] who prepared cross-link multilayer thin films (CL) to diffusion molecule of DS. Schneider and his team's work suggested that not only the electrostatic interaction can absorb the DS into CL films but the hydrophobic interaction can also support the free carboxylic groups to interact into CL films.

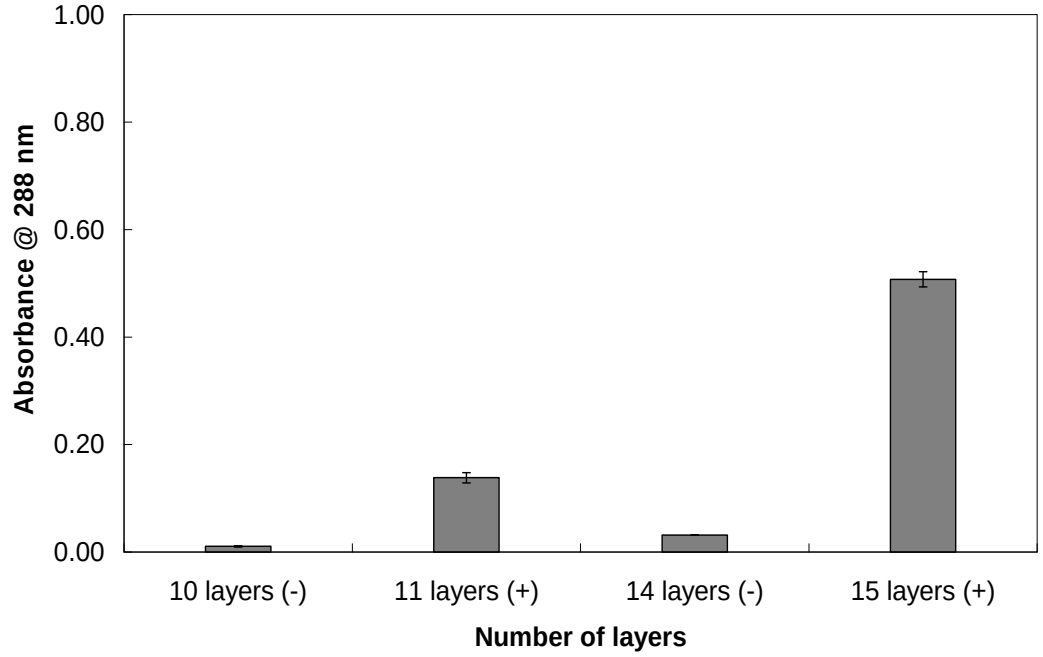


Figure 4.30 Loading of DS into PDADMAC/PSS multilayer thin films as a function of surface charge of outer layer.

- Kinetic loading of DS

In the kinetic of DS loading, the faster absorbance increases suddenly followed by stable in absorbance during the short loading time as shown in Figure 4.31A. One can see that the loading of DS in multilayer thin films was faster process, and required just few minutes to obtain stable loading absorption. The most efficient time of DS loading was

30 minutes, while major of drug reached steady state equilibrium. The amounts of DS can be loading rapidly is because of the electrostatic interaction and small sized of DS which is able to sustain effectively into multilayer thin films. In addition, it is reasonable to assume that, kinetic load of DS also depends on the thickness of multilayer thin films. The load of DS into PDADMAC/PSS multilayer thin films as a function of number of layer was plotted in Figure 4.31B. This experiment was attempted to maintain a constant DS concentration and fixed the definition time at 540 minutes. By increasing the number of layers, the absorbance spectra were directly increased as a linear trend. This observation is supported by the fact that multilayer thin films behave as a drug reservoir matrix. On the basic of this concept, PDADMAC/PSS provides a basic for designing the load of drugs into the whole surface matrix, which can be controlled by the thickness of multilayer thin films.

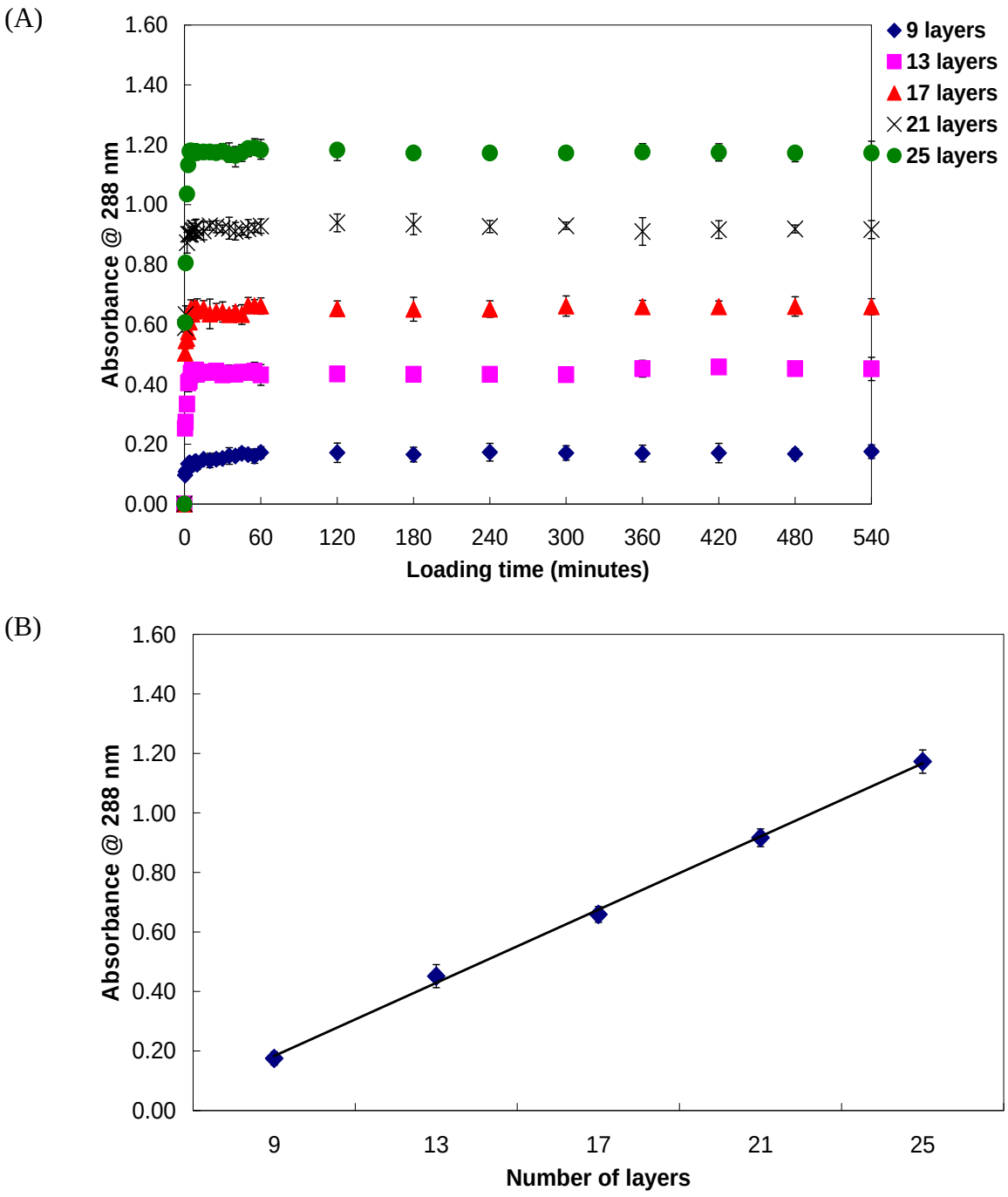


Figure 4.31 (A) Kinetic loading of DS into PDADMAC/PSS multilayer thin films as a function of time at 288 nm. (B) Absorption property of DS as a function of number of layers at 540 minutes of loading time.

- Loading efficiency of DS

DS loading PDADMAC/PSS multilayer thin films were immersed into buffer pH 7 and their loading efficiency was quantified. The calibration curve of DS standard is linear in rang of 0 – 80 PPM as shown in Figure 4.32. The absorbance spectrum reached the peak at λ_{max} 288 nm, which absorbance values increased when increased the DS concentration. However, a small shift in the λ_{max} was observed after diclofenac was loading into PDADMAC/PSS multilayer thin films. This finding attributes to the association carboxylic group of DS and quaternaries amine group of PDADMAC.

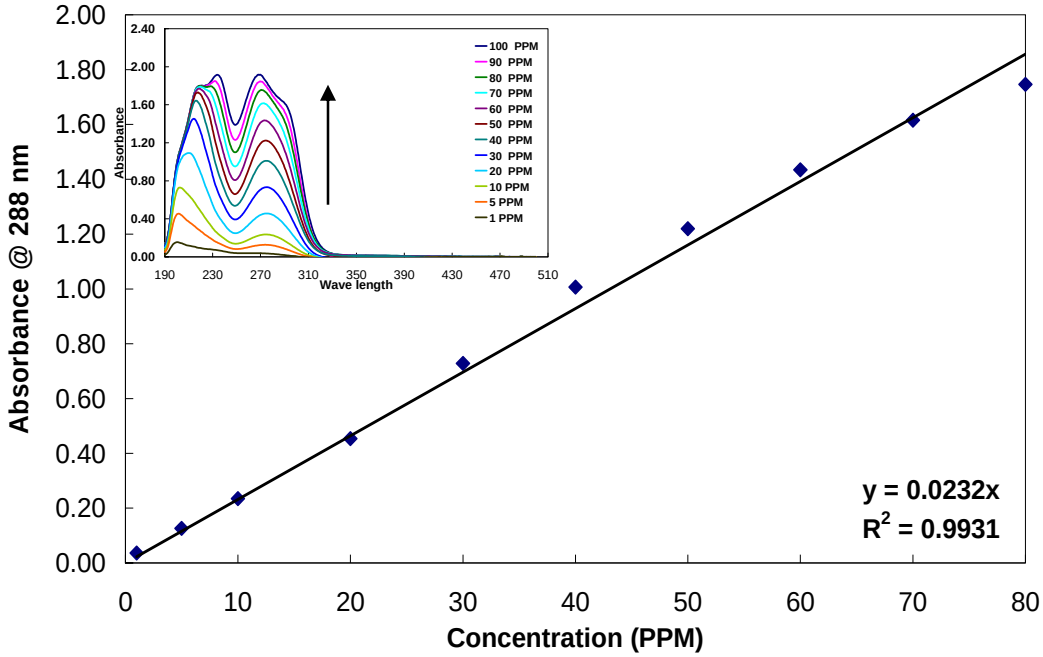


Figure 4.32 The calibration curve of DS 0.1-80 PPM.

The thickness influence on loading efficiency of DS was examined by varying the number of layers from 9 layers to 25 layers (Figure 4.33). The results are in line with the calibration curve law and the loading efficiencies were calculated for 9 layers, 13 layers, 17 layers, 21 layers, and 25 layers with the result of 232.99 μg/cm^2, 524.47 μg/cm^2, 684.45 μg/cm^2, 975.12 μg/cm^2 and 1157.31 μg/cm^2, respectively.According to previous discussion, the loading efficiency of DS is improved by increasing the thickness of

multilayer thin films. This phenomenon was also observed in other polymeric matrix that high thickness can cause the increase of loading efficiency.[129] It was found that there was significant increase of loading efficiency as a linear trend, which attributed to the electrostatic interaction between drug and multilayer thin films.

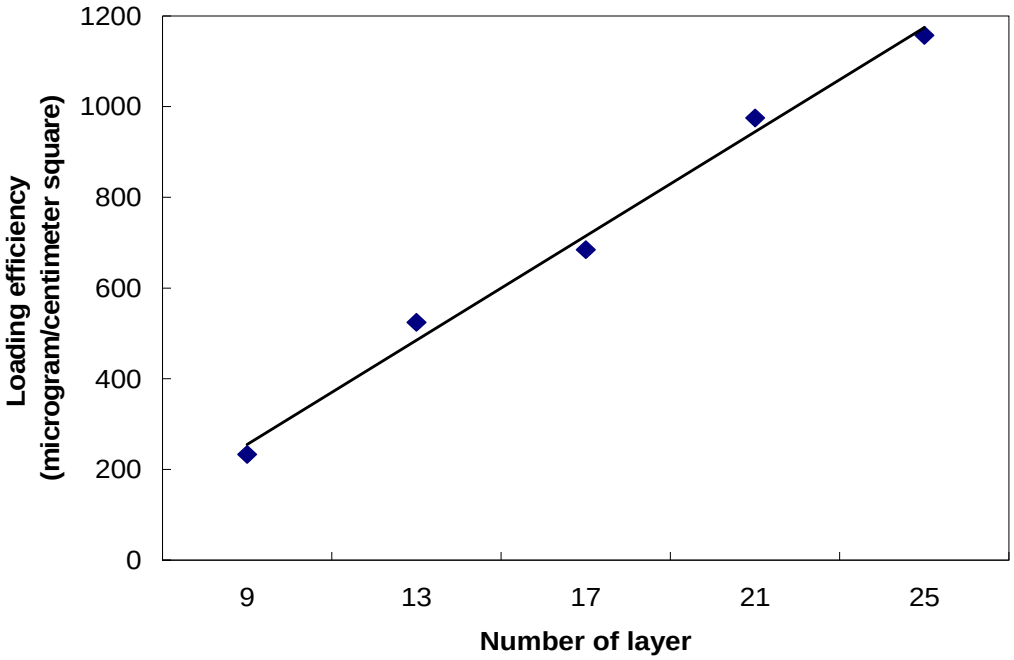


Figure 4.33 Loading efficiency of DS into PDADMAC/PSS multilayer thin films as a function of number of layers.

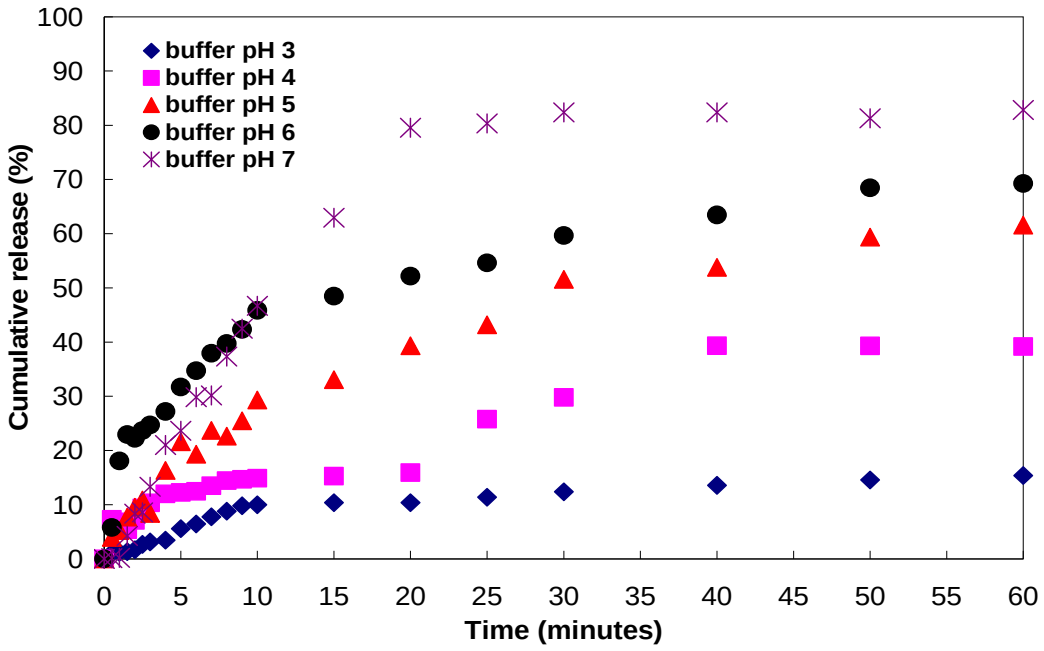
4.1.4.2. Release of Diclofenac sodium (DS) from PEM films

- Effect of buffer pH solution.

Diclofenac release from PDADMAC/PSS multilayer thin films was assessed in vitro and the experiment parameters were set in order to reproduce the physiological conditions. It is known that the pH environment of the gastrointestinal tract varies from acidic in the stomach to slightly increase pH to alkaline in the intestine. Thus, the effect of pH on the release profile of DS from PDADMAC/PSS multilayer thin films is determined. As can see in Figure 4.34, when pH of medium is 3, the cumulative release

of DS is lower than 16% at the end of experiment (60 minutes). Base on drug chemistry, the relative low release of DS at low pH was related to low solubility of DS in acidic condition. In addition, DS was converted into its ionized form, which is well known as insolubility in the stomach condition. At pH of medium is 7, the cumulative release of DS is 80% after 20 minutes, whereas almost loading drug is released within 60 minutes. This result is expected that DS presents a monovalent molecule like salt. Therefore, drug reconverts into soluble salt when expose in the intestine condition. Overall, the release profile of DS is attributed to the increasing of drug diffusion driving force of PDADMAC/PSS multilayer thin films.

(A)



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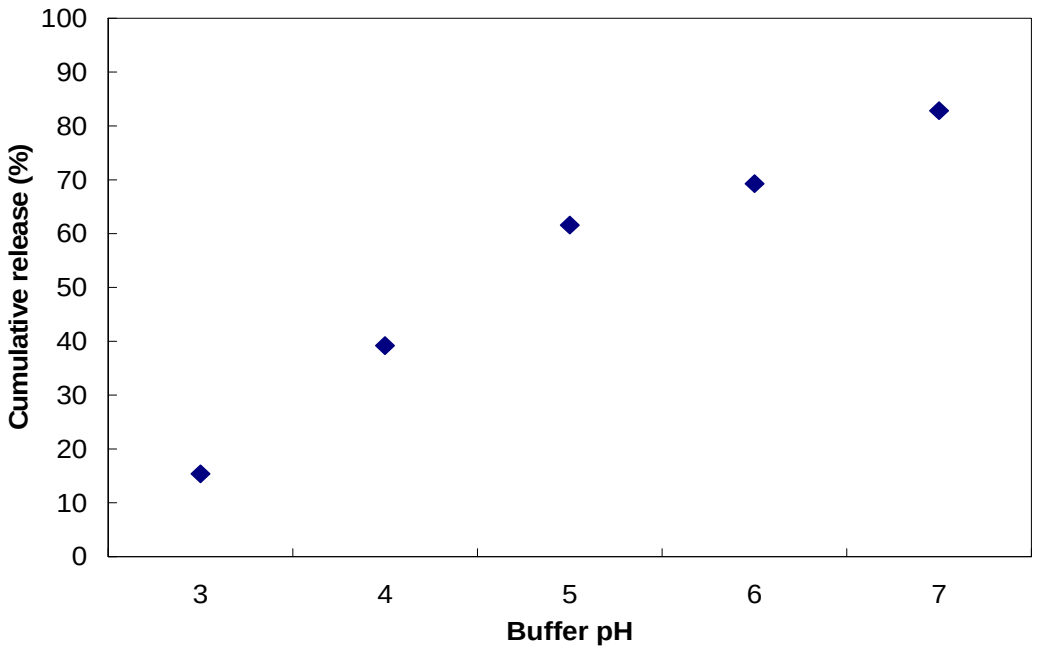


Figure 4.34 (A) Profile releases of DS from PDADMAC/PSSMA multilayer thin films in buffer pH solutions. (B) The releases of DS from PDADMAC/PSSMA multilayer thin films in buffer pH solution at 60 minutes of release time.

- Effect of ionic strength

The release of DS from multilayer thin films depends on the pH of buffer medium. Since DS has limited solubility in acetic condition, the release kinetic in buffer pH 7.4 was evaluated as the body physiological condition. The rapid release of DS from multilayer thin films in Figure 4.35 was almost complete 95% of drug release within 20 minutes. Considering without effect of ionic strength, rapid release was attributed to the diffusion of drug from multilayer thin films. At pH 7.4, carboxylate group of DS molecules was free ionization and great dissolution which is able to disperse drug molecule out from multilayer thin films. A rapid drug release can also associate with high solubility and diffusion of the drug in polymer matrix. This resulted is consistent with many previous studies, indicating that the rapid release of DS was faster at high pH buffer medium.[130] In addition, the DS behaving like a monovalent charge can be loading and released immediately. Another reason for the rapid release from such system can be explained by the structure of matrix. According to more hydrophilic of PDADMAC/PSS multilayer thin films comparing with another polyelectrolyte, the release of DS is due to the amorphous nature of multilayer thin films without the decomposition under pH environment.

To recognize the effect of ionic strength on the release profile, NaCl was added to buffer pH medium. The release rate of DS clearly depends on the ionic strength of buffer medium. The DS release became burst release profile as soon as the NaCl concentration increases. As the influence of NaCl, small $Na^{+}CL^{-}$ ions are known as weaken electrostatic bond between the polyelectrolytes to induce the swelling of multilayer thin films. NaCl can be effective by changing not only the polymer conformation but also affecting the motility of electric charge. The increase in ionic strength can decrease or break the electrostatic barrier between ionized groups of drug and polymer chain of thin film. It is reasonable to assume that the drugs and multilayer films with weaken interaction have larger permeability and are prone to dissociation. Similar effects of ionic strength on the diffusion rates of drug and dyes have been reported.[131]It can be inferred that the release

profile of drugs with high ionic strength can enhance the permeability of drugs through multilayer thin films.

The similar result was observed through DS release from PDADMAC/PSSMA multilayer thin films deposition at pH 5 as shown in Figure 3.46. With all DS loading films was immediate burst release within 5 minutes in buffer pH 7.4 containing 0.15 M of NaCl. Base on the above discussion, this data provided interesting information of drug in terms of controlling the release rate by pH and ionic strength and a tool for improving the design of drug release profile from polymer matrix.

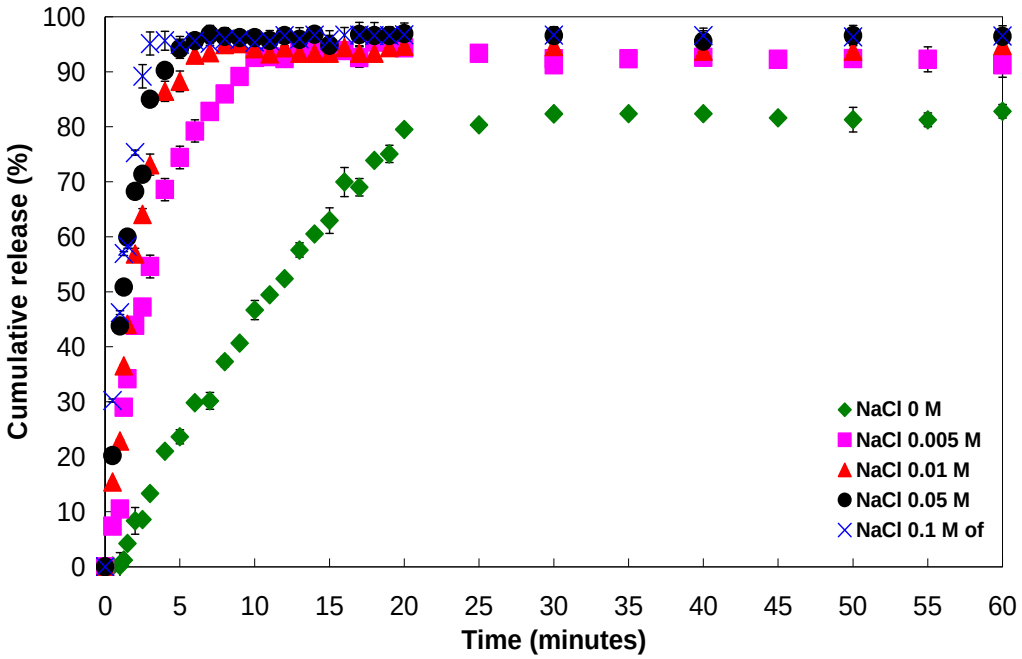


Figure 4.35 Profile release of DS from PDADMAC/PSS multilayer thin films in buffer pH 7.4 with varying the ionic strength.

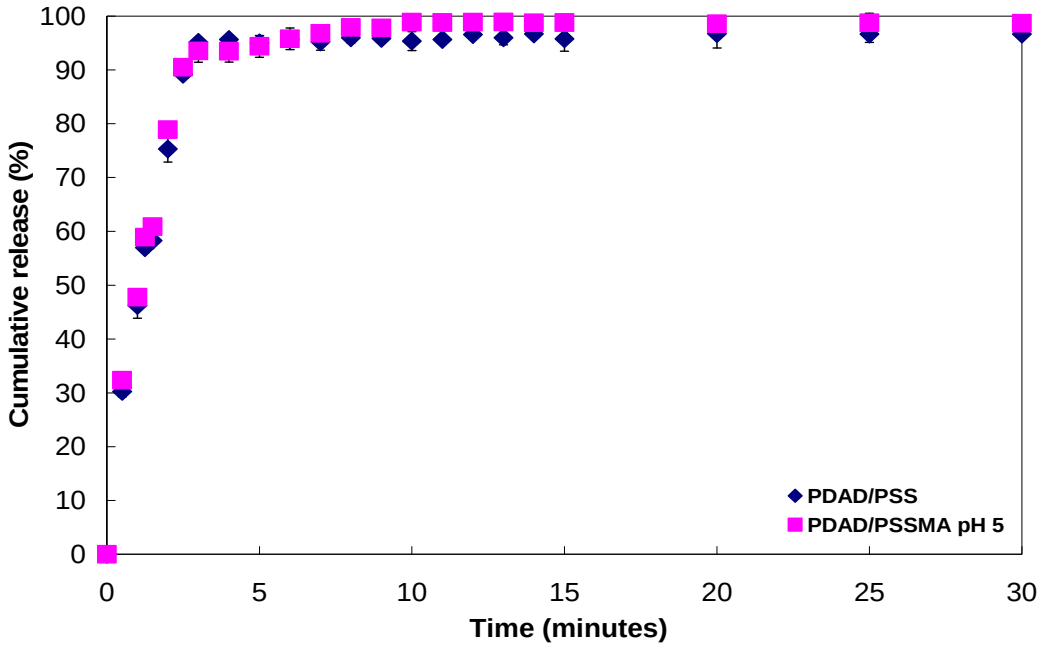


Figure 4.36 Profile release of DS from PDADMAC/PSSMA multilayer thin films deposition at pH 5 in buffer pH 7.4 containing 0.15 M of NaCl.

4.1.5 Loading and release characteristics of cationic drug PEM films

4.1.5.1 Loading of Gentian violet (GV) into PEM films

- Effect of pH of drug solution

In this case, GV was dissolved in buffer pH ranging from 4 to 7. The drug solution pH did not significantly affected the absorption of GV loading PDADMAC/PSS multilayer thin films as shown in Figure 4.37. The loading of GV in PDADMAC/PSS multilayer thin films had absorbance spectra at around 1.05. GV is cationic drug in aqueous solution whose absorbance spectra remains invariant in the pH range 4–11. At pH 4, the GV was deprotonated[132] and all three amine groups were blocked by the H^+. As the pH increased from 4 to 7, the GV was no longer protonated and the sites were available for absorption to multilayer thin films because of the electrostatic attraction. A similar observation is reported by Li et al.,[133] that the optimum pH of GV absorption is 6.4 which is better than neutral and alkaline medium solution. Meanwhile, without the

shifted in maximum wavelength and no changed in color of GV is observed. In addition, our studied found that the pH increasing to pH 11 can cause the dye become colourless because of degradation and decomposition of its structure (data not shown).

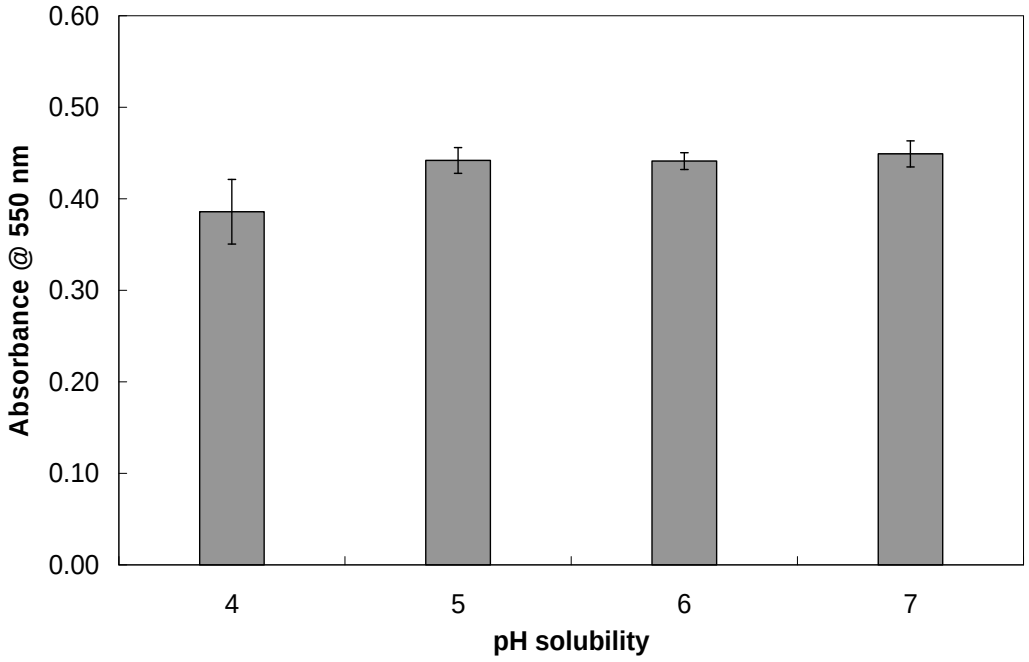


Figure 4.37 Loading of GV into PDADMAC/PSS multilayer thin films as a function of drug solution pH.

On the basis of drug loading property of polyelectrolyte multilayer thin films with different polyanionic, we further investigated curcumin loading PDADMAC/PSSMA multilayer thin films. In the case of PDADMAC/PSSMA multilayer thin films, the pH value of initial solution is an important parameter in the loading process. In order to investigate the effect of pH on the load of GV, PDADMAC/PSSMA multilayer thin films were deposited at pH 3, 5 and 7. As such, GV solutions were adjusted to pH 3, 5 and 7. As shown in Figure 4.38, the solution pH affects the chemistry of GV and the loading ability. GV loading into PDADMAC/PSSMA multilayer thin films became lower at pH 7. This behavior can be explained on the basis of changes in the surface thickness of multilayer thin films. As the pH of GV system increases, triphenylmethane groups ($=N^{+}(CH_3)_2$) becomes positive which is able to support the formation of negative charge of multilayer thin films. Sine greater thickness of PDADMAC/PSSMA multilayer films

is obtained at pH 5. Significant high electrostatic attraction occurred between negative charge of carboxylic groups and positive charge of GV. Base on the result, GV loading PDADMAC/PSSMA multilayer thin film deposition at pH 5 was considered as the optimum pH value for the loading system.

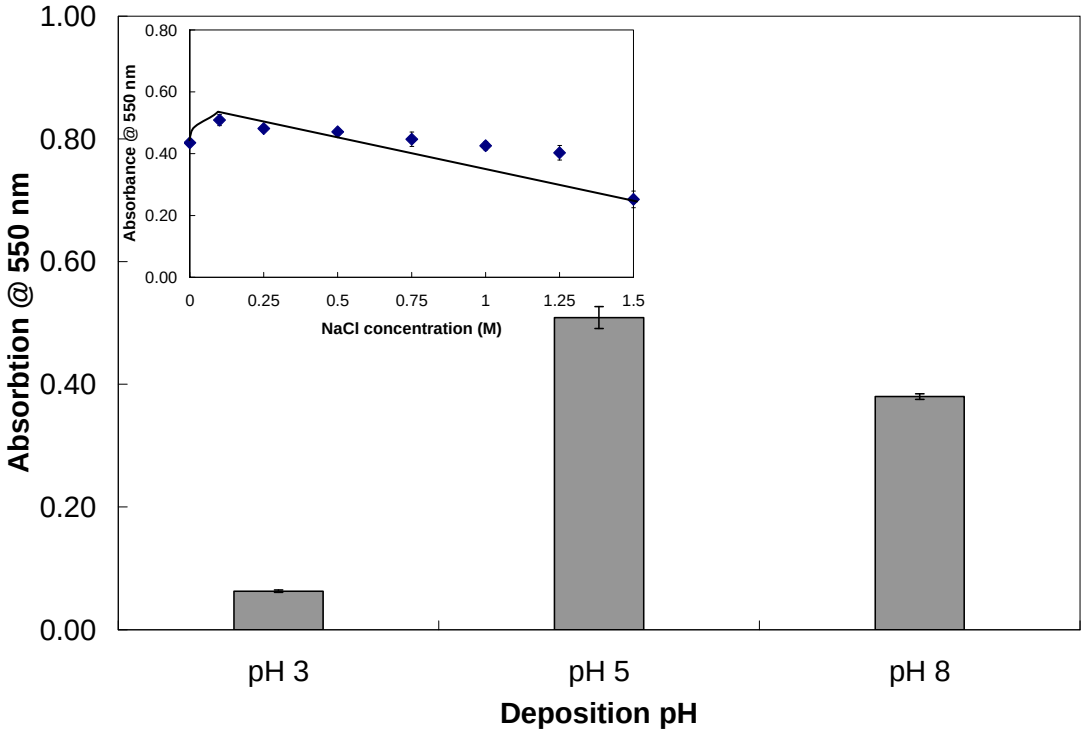


Figure 4.38 Loading of GV into PDADMAC/PSSMA multilayer thin films as a function of drug solution pH. In each case, multilayer thin films were measured as deposition at pH 3, 5 and 8. The inserted figure showed the effect of ionic strength on the loading of GV into PDADMAC/PSSMA deposited at pH 5.

The ionic strength of the solution is one of the parameters that control the electrostatic attraction between drug molecule and multilayer thin films. In this case, the effect of ionic strength was investigated by adding different amounts of NaCl concentration to the initial GV solutions pH 5. As seen in the inserted Figure 4.38, increasing the ionic strength of GV up to 0.1 M causes the increase in the load of GV into multilayer thin films. Meanwhile, the GV solution was completely dissolved when the

NaCl concentration less than 0.1 M. However, the increasing of ionic strength more than 0.25 M can cause the reduction of the loading ability due to precipitation of GV solution. As a result, GV solution was completely precipitated when the NaCl concentration was higher than 1.5 M.

Figure 4.39 demonstrates the loading of GV into Chi/PSS multilayer thin films deposited at pH 3 and pH 6. Chi/PSS multilayer thin films deposition at pH 6 represented the higher load of GV rather than pH 3. As the increase pH of GV to pH 3, caused the protonated groups of ($=N^{+}(CH_3)_2$) interact with the SO_3^- of PSS via the strongly electrostatic interaction..

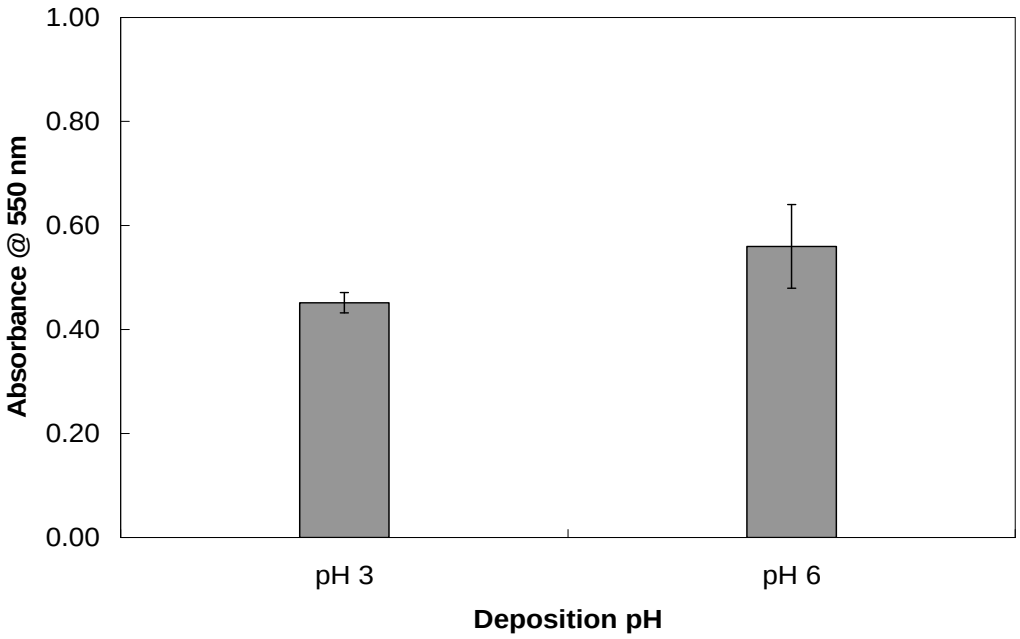


Figure 4.39 Loading of GV into Chi/PSS multilayer thin films as a function of drug solution pH. In each case, multilayer thin films were measured as deposition at pH 3 and pH 6.

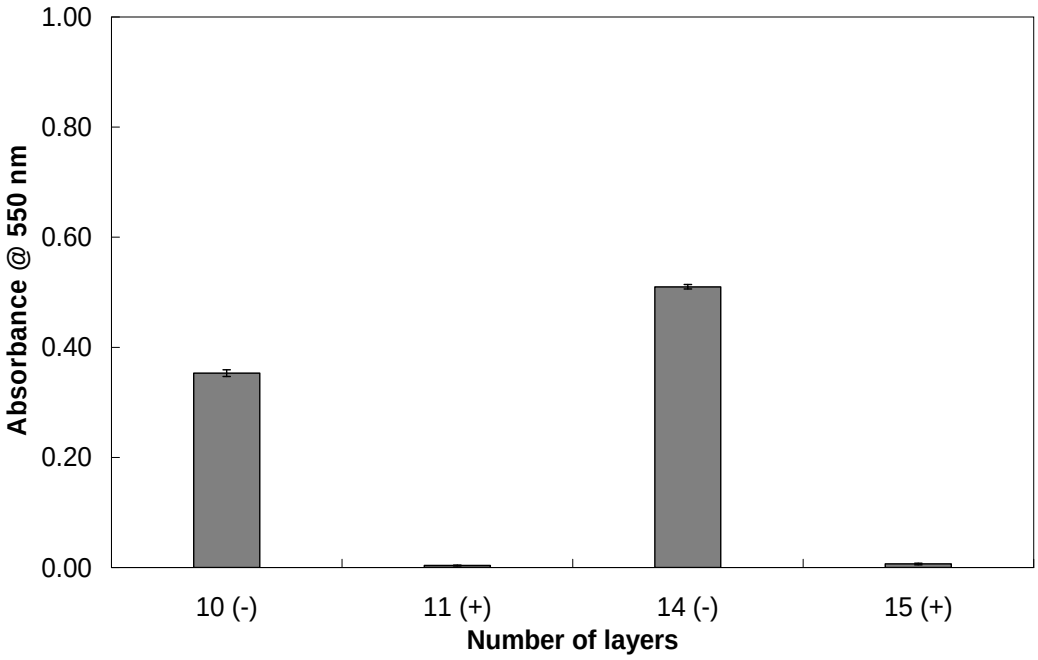
- Effect of surface charge of outer layer

To better understand about the surface charge, positive and negative GV loading outer layer of thin films was investigated. As shown in Figure 4.40A and 4.40B, the load of GV on positive and negative multilayer surface was different based on its molecular structure and interaction force. At negative charge of outer layers (10 layers and 14 layers), GV was absorbed dramatically in multilayer thin films. In contrast, no load of GV was observed for the positive charge of outer layers (11 layers and 15 layers).

It is must be pointed out that, GV is a cationic drug which have amine groups in its structure. In the case of PDADMAC/PSS multilayer thin films, it was found that the triphenylmethane groups ($=N^{+}(CH_3)_2$) were interacted with the sulfonated groups of PSS via strong electrostatic interaction. Beside the interaction mentioned above, our study suggested that the interaction of GV and multilayer thin films were also associated with the weak interaction such as van der Waals interaction.[134]

In the case of PDADMAC/PSSMA multilayer thin films, the addition of NaCl screens the repulsive electrostatic forces between the ($=N^{+}(CH_3)_2$) group and negative charge of PSSMA to adopt more favored conformation. Although electrostatic forces have major influence on controlling the load of GV molecule into PDADMAC/PSSMA multilayers thin films, other interactions also play important roles in the mechanism.[135]

(A)



(B)

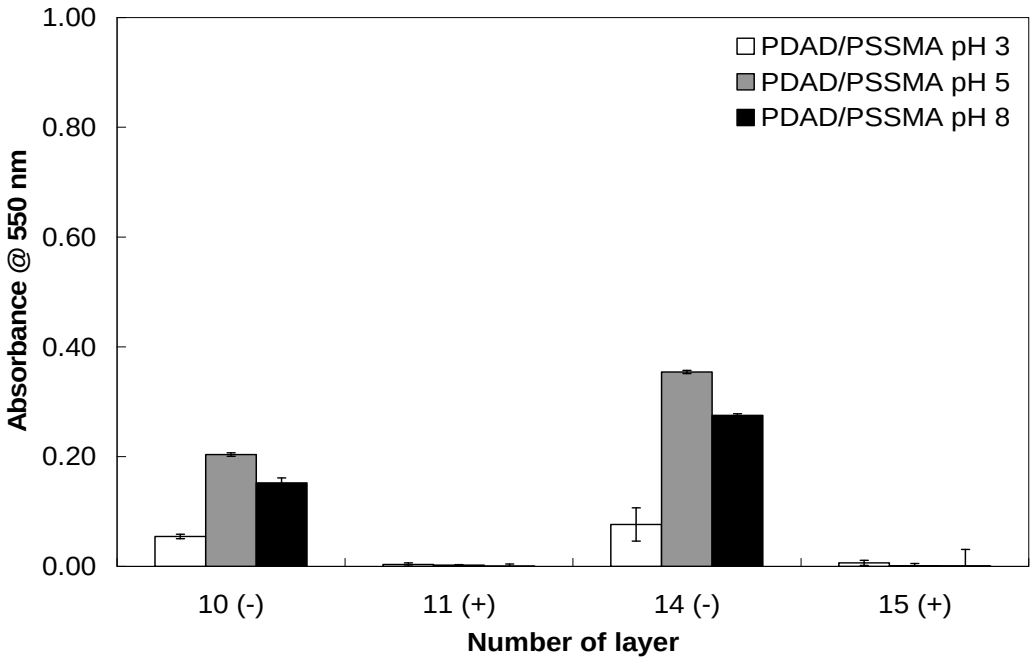


Figure 4.40 Loading of GV into polyelectrolyte multilayer thin films as a function of surface charge of outer layer. (A) PDADMAC/PSS multilayer thin films. (B) PDADMAC/PSSMA multilayer thin films. In PDADMAC/PSSMA case, multilayer thin films were measured as deposition at pH 3, 5 and 8.

Based on the structure of GV, the loading mechanism can be assumed to involved (i) hydrogen bonding interaction between the nitrogen containing amine groups of GV and carboxylic groups of PSSMA (ii) hydrophobic - hydrophobic interaction between the hydrophobic parts of GV and multilayer thin films matrix (iii) weak interaction such as the van der Waals forces. Thus, the proposed mechanism loading of GV into PDADMAC/PSSMA is shown in Figure 4.41. Overall, the structure of drug is an important factor to permit molecule of drug interact in surface of multilayer thin films. Additionally, the surface charge of outer multilayer thin films and charge of small drug molecules can cause the strong/weak interaction that effecting drug loading and releasing behavior. Therefore, this result suggests that surface charge plays an important role for load of GV into the polymer matrix.

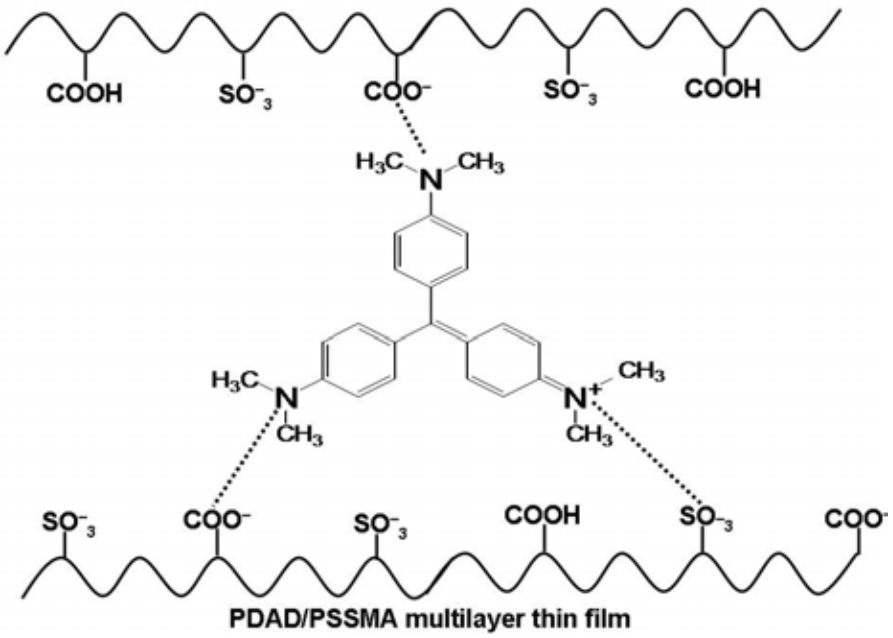


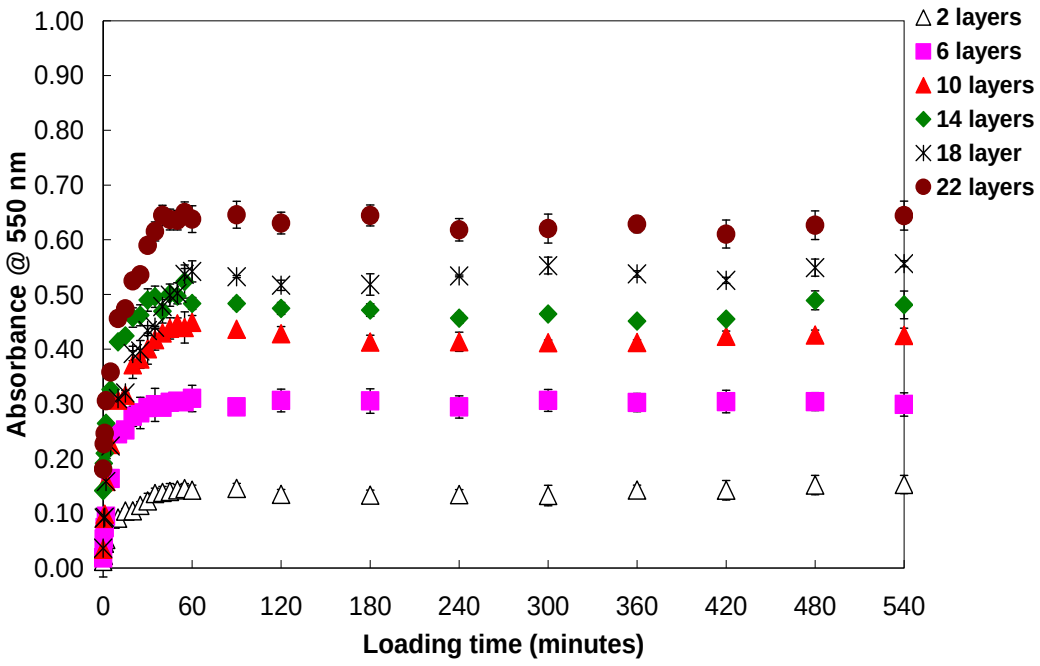
Figure 4.41 Proposed mechanism of the load of GV into PDADMAC/PSSMA multilayer thin films.

- Kinetic loading of GV

Figure 4.42A illustrates the kinetic loading of GV in PDADMAC/PSS multilayer thin films as a function of time. The load of GV also increases when increases the loading time. The increase in absorbance of GV is because the side chain of triphenylmethane groups could induce molecule of drug to absorb inside surface of multilayer thin films. Interestingly, the kinetic loading of curcumin and GV was saturated after 180 minutes and tended to be stable until 9 hours. The absorption property of GV loading PDADMAC/PSS multilayer thin films showed the liner trend when number of layers increases as shown in Figure 4.42B. The insert picture in Figure 4.42B can confirm that GV can be loading homogeneously thought the polymer matrix of PDADMAC/PSS multilayer thin films.

The loading of drug into polyelectrolytes multilayer thin films and other types of pH-sensitivity of multilayer thin films are effectively controlled by various properties of the system such as surface charge, charge density, pore size of thin films, hydrophilic/liphophilic balance. Moreover, the loading ability can be controlled by the properties of drugs molecule such as, charge, and size.[136] Since GV has the strong positive charge at pH 5 and they can effectively bind to negative charge surface. For this reason, PDADMAC/PSSMA multilayer thin films deposition at pH 5 was used in studying the kinetic loading by varying the number of layers.

(A)



(B)

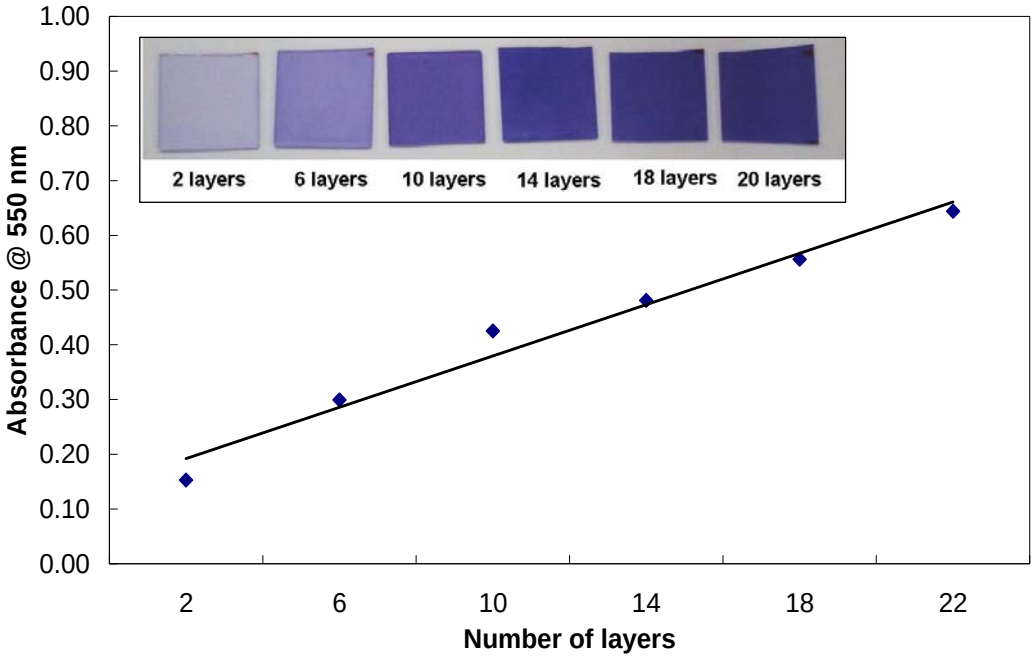
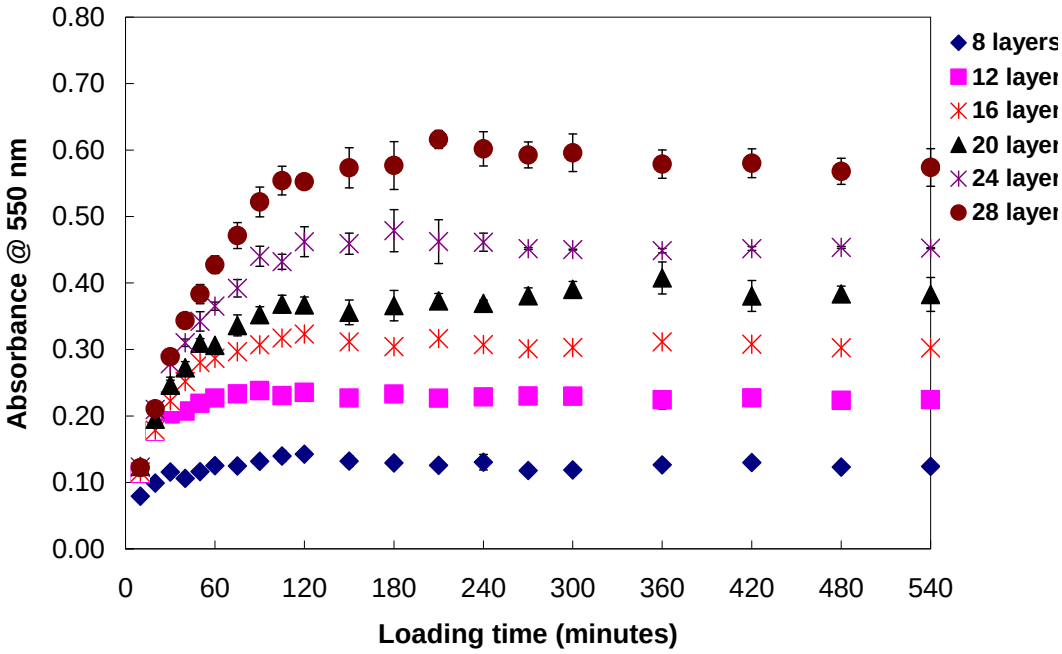


Figure 4.42 (A) Kinetic loading of GV into PDADMAC/PSS multilayer thin films as a function of time at 550 nm. (B) Absorption property of GV as a function of number of layers at 540 minutes of loading time. The inserted pictures show image of GV loading PDADMAC/PSS multilayer thin films on glass slide substrates.

This similar result can be observed from the kinetic of GV loading PDADMAC/PSSMA multilayer thin films as shown in Figure 4.43A. The loading of GV in PDADMAC/PSSMA multilayer thin films increases with increasing the number of layers. From this plot, it could be seen that after the great increase in absorbance at the beginning, slow increase was observed after 180 minutes. After about 180 minutes, no further change in loading profile was investigated, suggesting the saturation of load. Since the loading absorbance of GV in multilayer thin films increased linearly, the loading process can be measured by plotting the absorbance of GV as a function of number of multilayers as shown in Figure 4.43B. This implies that the GV was able to diffuse throughout the bulk matrix of the PDADMAC/PSSMA multilayer thin films.

(A)



(B)

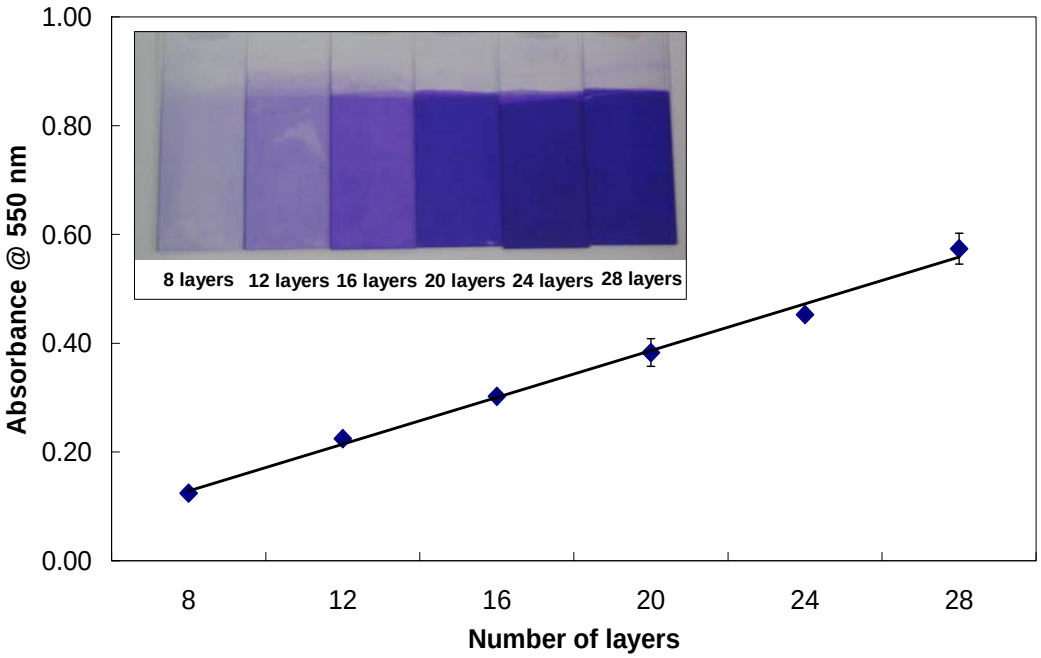


Figure 4.43 (A) Kinetic loading of GV into PDADMAC/PSSMA multilayer thin films deposition at pH 5 at 550 nm. (B) The absorption property of GV as a function of number of layers at 540 minutes of loading time. The inserted picture show image of GV loading PDADMAC/PSSMA multilayer thin films on glass slide substrates.

- Loading efficiency of GV

Loading efficiency of GV from PDADMAC/PSS and PDADMAC/PSSMA multilayer thin film were calculated based on the calibration curve on the data in Figure 4.44. GV concentration range from 0.1 PPM to 5 PPM was dissolved in buffer pH 3 in order to transform absorbance determinations into concentrations.

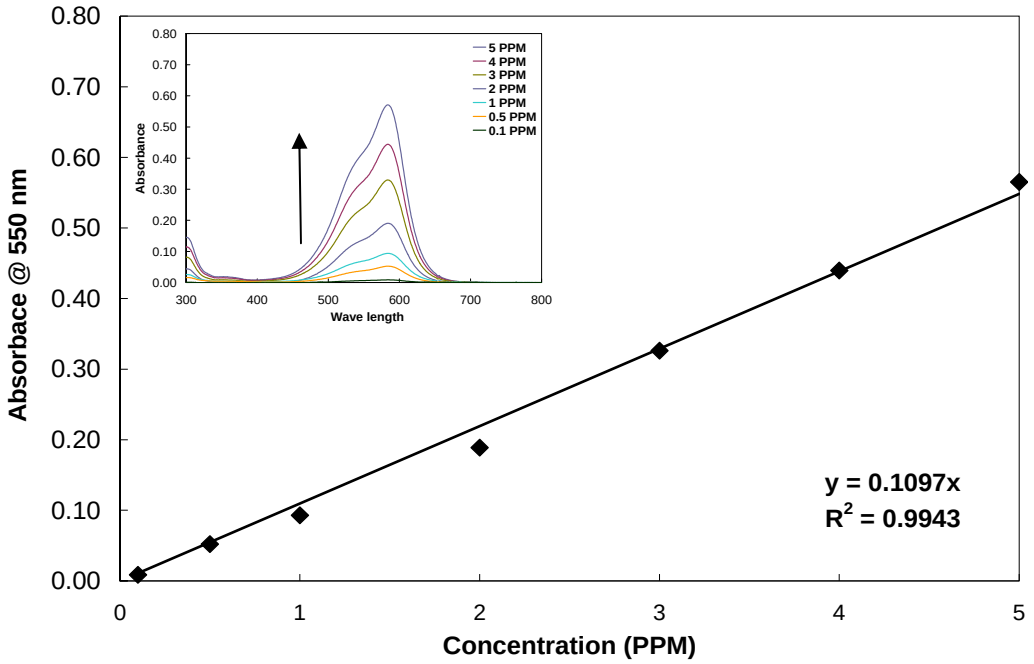
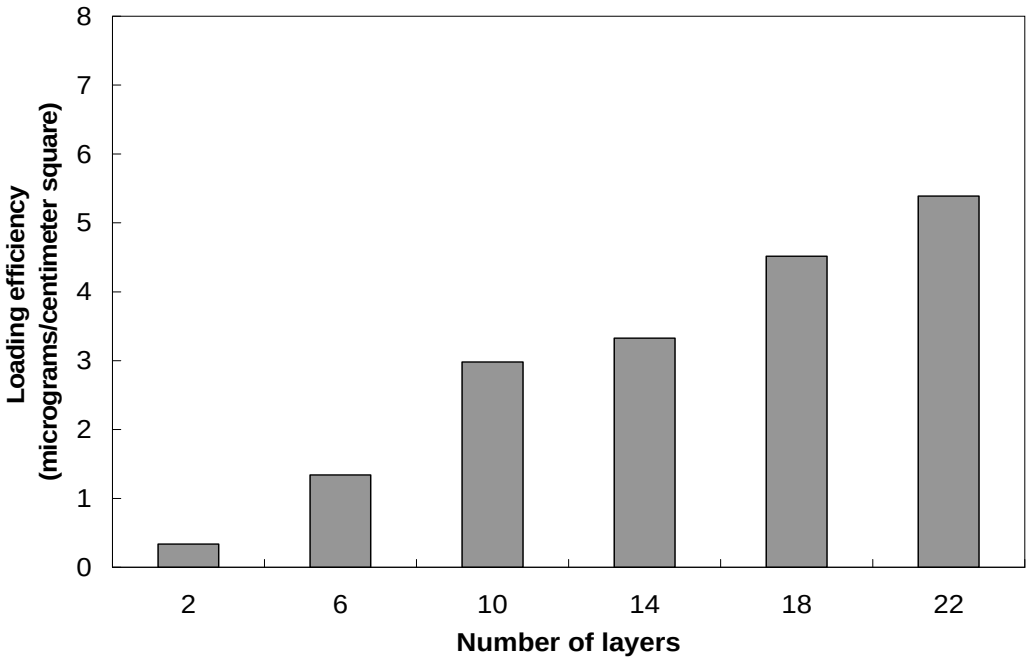


Figure 4.44 The calibration curve of GV 0.1 – 5 PPM.

Figure 4.45 shows the loading efficiency of GV in multilayer thin films as a function of number of layers. The amounts of GV loading PDADMAC/PSS multilayer thin films were 0.34 μg/cm^2, 1.34 μg/cm^2, 2.98 μg/cm^2, 3.33 μg/cm^2, 4.51 μg/cm^2 and 5.39 μg/cm^2 for 2 layers, 6 layers, 10 layers, 14 layers, 18 layers, and 22 layers , respectively. In addition, the amounts of GV loading PDADMAC/PSSMA multilayer thin films were 0.06 μg/cm^2, 0.10 μg/cm^2, 1.17 μg/cm^2, 2.09 μg/cm^2, 2.71 μg/cm^2, 5.36 μg/cm^2 and 9.29 μg/cm^2 for 8 layers, 12 layers, 16 layers, 20 layers, 24 layers, 28 layers and 32 layers, respectively. As expected, loading efficiency increased when the number of multilayer increased and therefore thicker films contained higher amount of GV.

Consequently, the observed loading efficiency of GV on PDADMAC/PSS multilayer thin films is higher than the expected for PDADMAC/PSSMA multilayer thin films in each layer. The higher loading of GV in each layer is also attributed to the higher thickness of each films. This is an advantage of layer by layer self assembly thin films because fine tuning of drug loading multilayer thin films by controlling parameters such as thickness.

(A)



(B)

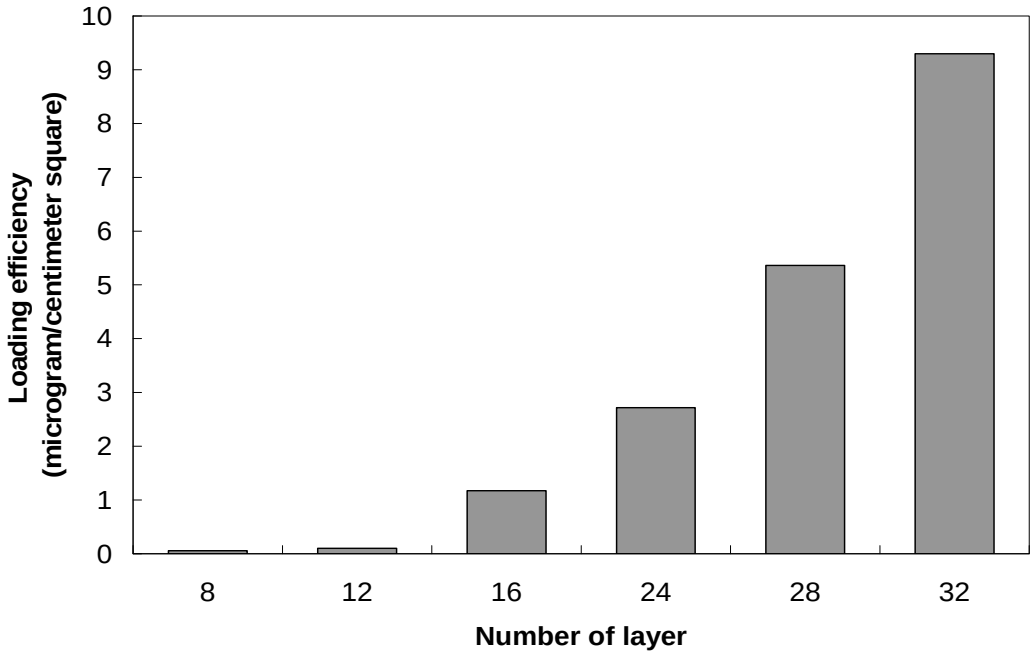


Figure 4.45 Loading efficiency of GV into (A) PDADMAC/PSS multilayer thin film and (B) PDADMAC/PSSMA multilayer thin film deposition at pH 5.

4.1.5.2 Release of GV from PEM films

- Effect of buffer pH solution

The release characteristic of GV from multilayer thin films was investigated in buffer pH 3, 5 and 7 using as a release medium. Figure 4.46 presented the kinetic release of GV from PDADMAC/PSS multilayer thin films, which was found to be pH independent. It is possible to note that the release rate of GV in buffer pH 3 was higher than those obtained in the other two release medium, which are similar.

As can be seen, the release rate of GV in buffer pH 5 and pH 7 is only 15.64% and 12.23% respectively. While in buffer pH 3, the release rate is much higher into 33.36 % during 720 minutes due to the deprotonated of triphenylmethane groups. Since PDADMAC/PSS multilayer thin films was stable at all pH rang, the release GV can be caused by the diffusion release of drug molecule form polymer matrix.

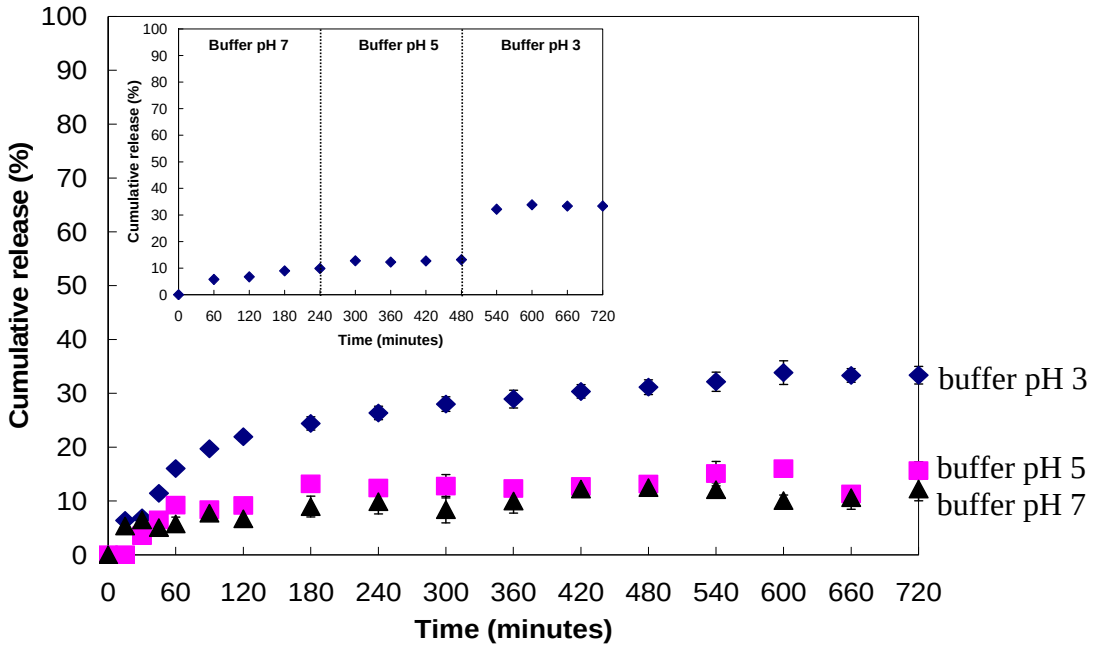


Figure 4.46 Profile release of GV from PDADMAC/PSS multilayer thin films in buffer pH solution. The inserted picture shows the release of GV during continuous exposure to changing pH environmental.

It is also supposed that the strong electrostatic interaction between drug and PDADMAC/PSS multilayer thin films may be formed during the drug loading process, which lowers the exchange probability between drug and release medium, leading to the slower release.

One of the key issues in developing pH-sensitive multilayer thin films is to design appropriate pH-sensitive polymeric materials and use suitable combinations for Layer-by-Layer deposition. Since PSSMA is a weak polyelectrolyte, and its degree of ionization depends on the pH of the solution, the release characteristic of GV from PDADMAC/PSSMA could be controlled by changing the solution pH. For this study, PDADMAC/PSSMA multilayer thin films were deposition at pH 5 because this pH condition results in maximum drug loading. Figure 4.47 shows the time-dependent release of GV from multilayer thin films in buffer solutions with different pH value. Interestingly, the release of GV is dependent on the buffer pH solution. Almost of GV molecules were released from multilayer thin at buffer pH 3, while only 35% and 15% were release at buffer pH 5 and buffer pH 7, respectively. The release profile of GV was slower in neutral solution (buffer pH 7) due to the positive charged of GV remaining bound with the negative charged of PSSMA to prevent GV from releasing. As the buffer pH solution becomes more acid, the electrostatic attractive forces between multilayer thin film and GV become dissociate due to triphenylmethane groups ($=N^{+}(CH_3)_2$) of GV begin to lose their charge from positive to negative. Furthermore, PSSMA is a weak polyanionic and the charge density along the polymer chain can be change by adjust the pH value. In buffer pH 3, the repulsion forces created by the increasing charge density of carboxylate groups of the PSSMA chains also promote the release of the GV molecules until it reach the equilibrium value. This result is consistent with the study on loading and releasing cationic molecules in polyelectrolyte multilayer thin films where the rate of release of drug from the multilayer increased with decreasing pH condition.[137]

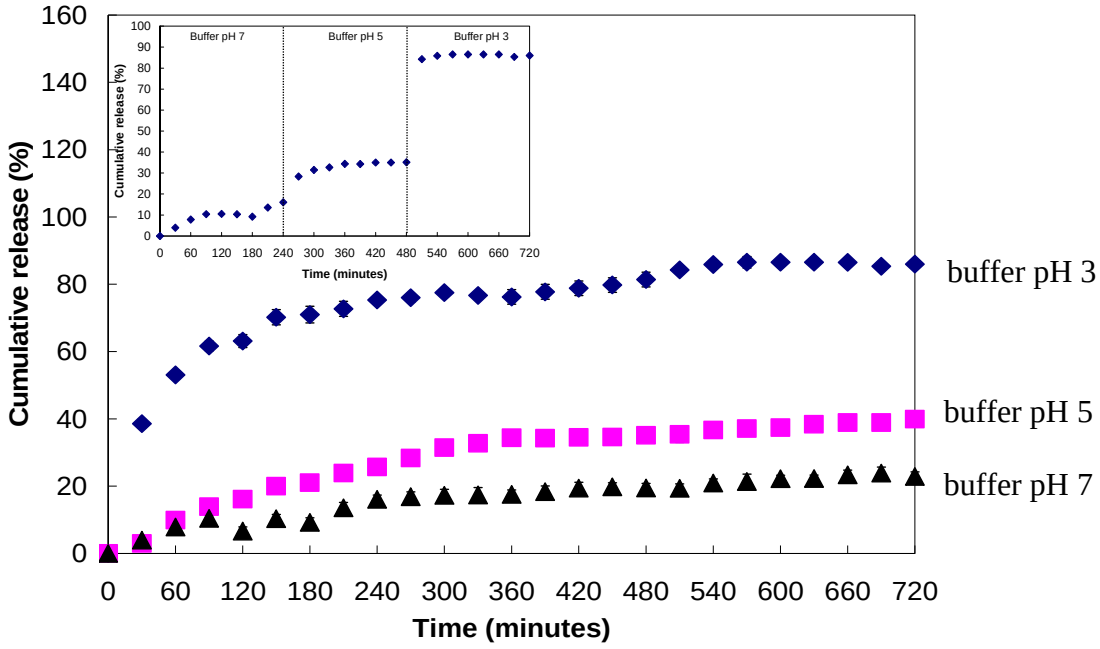


Figure 4.47 Profile release of GV from PDADMAC/PSSMA multilayer thin films in buffer pH solution. The inserted picture shows the release of GV during continuous exposure to changing pH environmental.

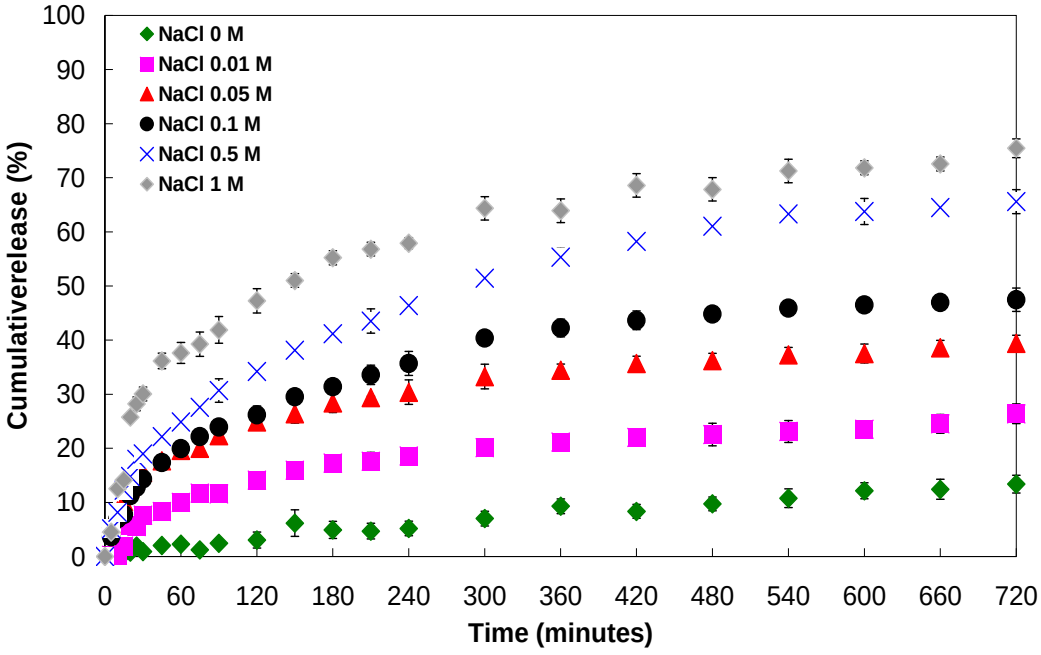
Although electrostatic forces have a major influence on controlling the release of GV molecules, the swelling/decomposition of weak polyelectrolyte also play a role in the release mechanism. PDADMAC/PSSMA multilayer thin films which deposition in pH 5 could be useful for turning the release of multilayer thin films. As mentioned previously, the multilayer thin films composed of PDADMAC and PSSMA were found to be unstable and decomposition when considering without effect of NaCl. These decomposition of PDADMAC/PSSMA multilayer thin films were also enhanced the release of the GV molecules in all of pH buffer solutions. The loading GV was released completely on in buffer pH 3 whereas certain amount of GV remains in multilayer with higher pH solution. Therefore, the used of weak polyelectrolyte whose net charged can be shifted from positive to negative by changing the environmental pH, can be highly effective for turning the pH threshold for the decomposition of multilayer thin films.

These studies clearly demonstrate that PDADMAC/PSSMA multilayer thin films are effective for protecting drug release in a neutral environment (i.e., the small intestine) while drug can be release in weakly acidic (pH 5) and strongly acidic medium (i.e., the stomach). Overall, the results suggest the potential used of electrostatic bond based Layer-by-Layer self assembly thin films can be control the release of pH-sensitive drug delivery.

- Effect of ionic strength

To study the effect of ionic strength on the release of GV, NaCl concentration was added to the physiological pH 7.4 from 0 M to 1 M. The release rate of GV was clearly depending on the effect of the ionic strength in buffer medium as plotted in Figure 4.48. In this case, the release profile of GV from PDADMAC/PSS multilayer thin films can be described as two steps process, i.e. an initial release effect followed by subsequent slower release. The slower release of GV from multilayer thin film may be ascribed to their structure. Considering the structure of GV, it is seen that the drug containing triphenylmethane are not easily release at pH 7.4. However, the release rate of GV was increases in proportion to the NaCl concentration. For instance, GV was release 13.37%, 26.39%, 39.37%, 47.46%, 65.58% and 75.46%, when increase the ionic strength from 0-1 M within 720 minutes. As discussed previously, the present of salt can caused the dissociation between drug molecule and polyelectrolyte multilayer chain, resulting in diffusion of drugs out of multilayer thin film. Therefore, the control of electrostatic dissociation between PDADMAC/PSS and GV through ionic strength variations let to the relative release of GV.

(A)



(B)

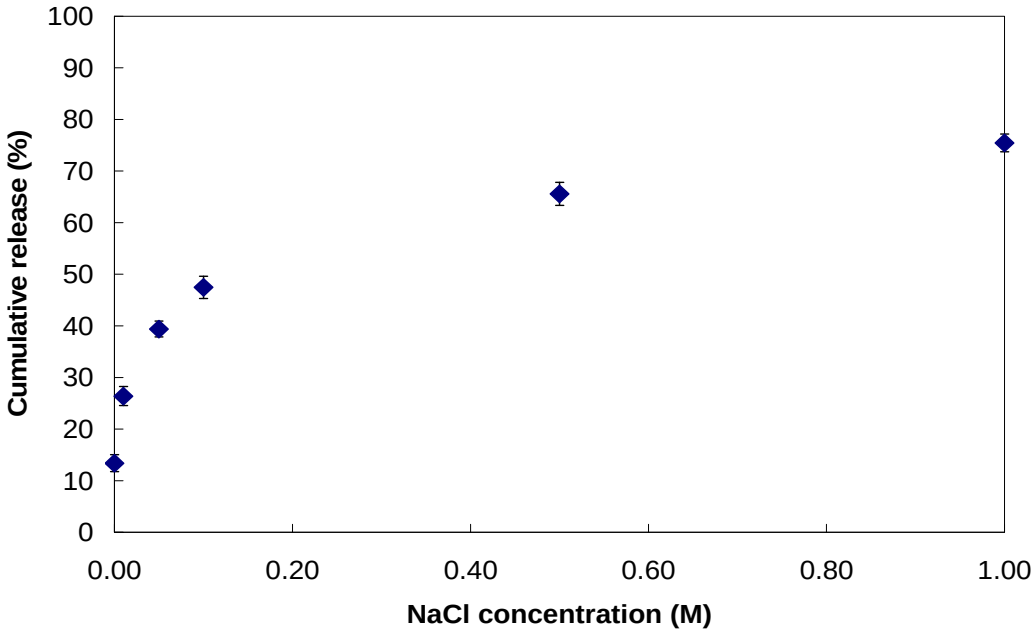
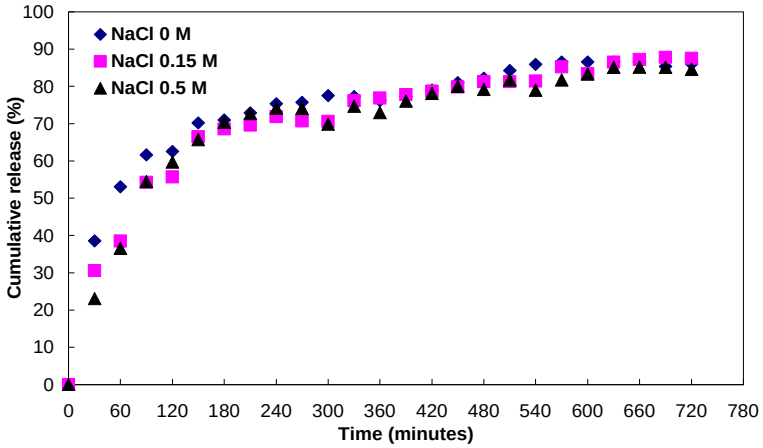


Figure 4.48 (A) Profile release of GV from PDADMAC/PSS multilayer thin films in buffer pH 7.4 with varying the ionic strength. (B) The releases of GV from PDADMAC/PSS multilayer thin films at 720 minutes of release time.

Another interesting characteristic of the release behavior of GV from PDADMAC/PSSMA multilayer thin films is that the release is also strongly dependent on the ionic strength of pH buffer solution. The effect of the ionic strength on the release characteristic was observed as showed in Figure 4.49. In acidic solution with lower pH value, large amount of H^+ is very easily to penetrate into multilayer thin films and the protonation of PSSMA occurred rapidly. As expected, the released of GV from multilayer thin films was fastest in buffer pH 3, whereas the release characteristic was independent on ionic strength of solutions. However, the release characteristic of GV in buffer pH 5 and 7 significantly increase in the presence of NaCl in pH buffer solutions as demonstrated in Figure 4.49B and 4.49C. The observed effects of ionic strength can be rationalized based on the charge shielding by high concentration of NaCl in solution environmental. When multilayer thin films were immersed in pH buffer solution containing NaCl, the Na^+Cl^- ion would attack the pairs of polyelectrolyte. This attack will weaken or break the interaction between PDADMAC and PSSMA chains. Then, multilayer thin films were deconstructed, leading to the rapidly release of GV out of multilayer thin films. One can see that the release rate of GV can be adjusted by varying the ionic strength of the solution.

(A)



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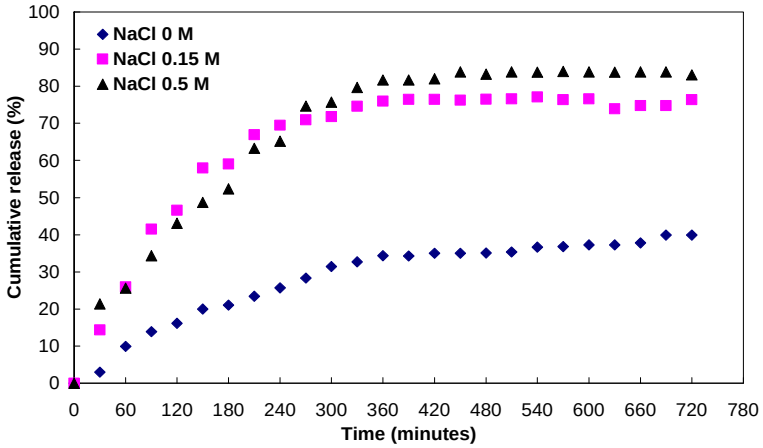


(C)

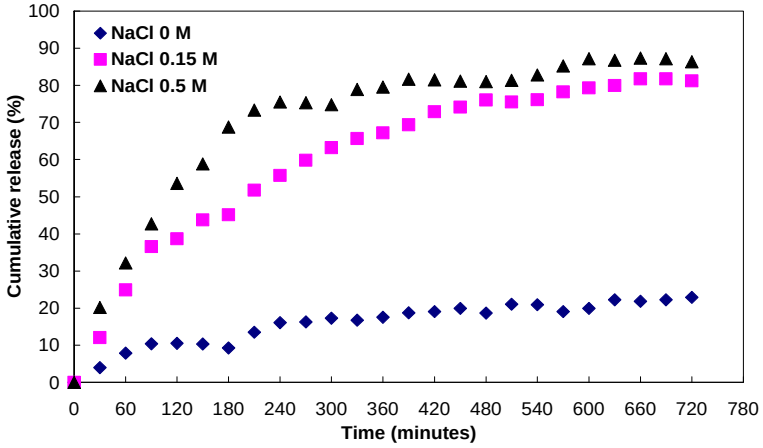


Figure 4.49 Profile release of GV from PDADMAC/PSSMA multilayer thin films in buffer pH solutions with varying the ionic strength. (A) buffer pH 3 (B) buffer pH 5 and (C) buffer pH 7. In all case, PDADMAC/PSSMA multilayer thin films were deposition at pH 5.

Figure 4.50 represented the release of GV from Chi/PSS multilayer thin films in buffer pH 7.4 containing 0.15 M of NaCl. The release rate of GV from Chi/PSS deposition at pH 3 showed the higher rate than that of pH 6. This higher release behavior is due to the fact that Chi/PSS deposition at pH 3 was unstable. Chi/PSS multilayer thin films deposited at pH 3, curcumin showed a higher release, and the whole release quantity show a sustained release about 71.90% in the end of 10 hour. In contrary, curcumin loading Chi/PSS multilayer thin films deposition at pH 6 exhibit lower release rate (66.75% for 10 hour). It seems that the effect of deposition pH of Chi/PSS multilayer thin films was mainly effect the release rate of GV from multilayer thin films. This can be also due to the swelling rate of Chi/PSS side chains. This result data was consistent with the result obtain from the decomposition as shown in Table 4.1. At Chi/PSS pH 3, the amino groups of chitosan become lose their charge density after expose to neutral pH condition, resulting in the decomposition of Chi/PSS multilayer thin films. In the case of Chi/PSS multilayer thin film deposition at pH 6, which contain either of amine groups and ammonium groups can exhibit swelling in neutral pH condition, but less than that Chi/PSS deposition at pH 3.

Base on this reason, it is reasonable to assume that the released of GV from weak multilayer thin films can be influenced by the changing of pH solution and the controlling of ionic strength of solution environment. These studies serve as a basis for understanding how to effectively use polyelectrolyte multilayer thin films in controlled over loading and release applications.

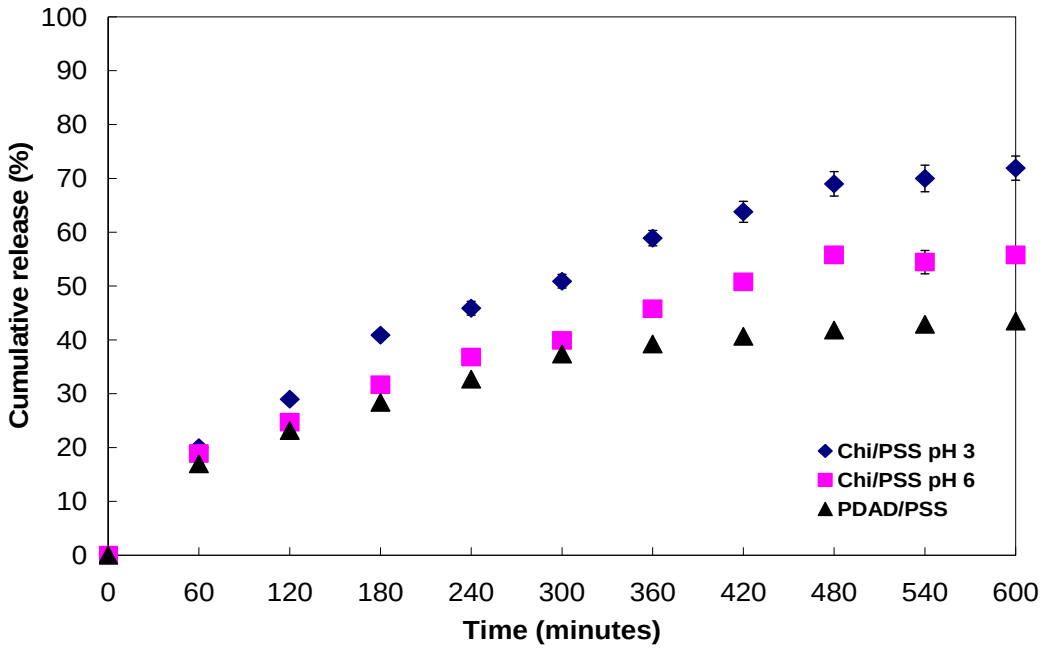


Figure 4.50 Profile release of GV from Chi/PSS multilayer thin films in buffer pH 7.4 containing 0.15 M of NaCl.

4.2 Construction of blocking film by Layer-by-layer deposition for prolong release of hydrophobic drug

To determined the prolong release of hydrophobic drug from multilayer thin films, the blocking films (PDADMAC/PSS, Chi/Alg and Chi/PAA) were fabricated in order to extension the release of curcumin. Curcumin were released from polymer matrix and polyacrylic substrates in the releasing medium, which using both of buffer pH 7.4 and the composition of water/ethanol. The effect type of blocking films and number of blocking layer and on the prolong release was studied as following.

4.2.1 Fabrication and characterization of blocking films

4.2.1.1 Formation of Chi/Alg multilayer thin films

Chitosan (Chi) and alginate (Alg) are two oppressively charged natural polyelectrolyte and very sensitive toward changes in external factor such as pH. The absorbance spectra of Chi/Alg multilayer thin films show the N-acetylglucosamine at λ_{max} 198 nm. Therefore, the formation of Chi/Alg was prepared and their UV absorbance at λ_{max} at 198 nm was measured as represent in Figure 4.51. One can see that the absorbance spectra of Chi/Alg deposited at pH 5 was increased slowly as a function of number of layers. As we know, the pKa of Chi is 6.5 and the pKa of Alg is 4. At pH 5.5, the amino groups of Chi mainly exist in the form of NH_3^+ and the carboxylate groups of Alg mainly exist in the form of COO^-.[138] In this case, the present of both NH_3^+ and COO^- along polyelectrolyte backbone could enhance the electrostatic interaction of the multilayer thin films. Considering low pH condition, such as pH 2, the carboxylate group of Alg does not carry the electrostatic charged, resulting in weaken electrostic interaction between Chi/Alg. Moreover, at pH 2, multilayer chain of Chi/Alg exhibit high swelling due to hydration of uncharged Alg segment, with probably causes the weaken electrostatic interaction. Oppositely, at high pH condition, such as pH 8, the amino groups of chitosan become ionized, therefore, there are exist a small quantity of NH_3^+ and

excess COO^-. This characteristic of charged density in side chains could also weaken electrostatic interaction in polyelectrolyte multilayer thin films. As a result, it should be pointed out that in subsequent experiments the optimum pH value of formation film was chosen as pH 5.5.

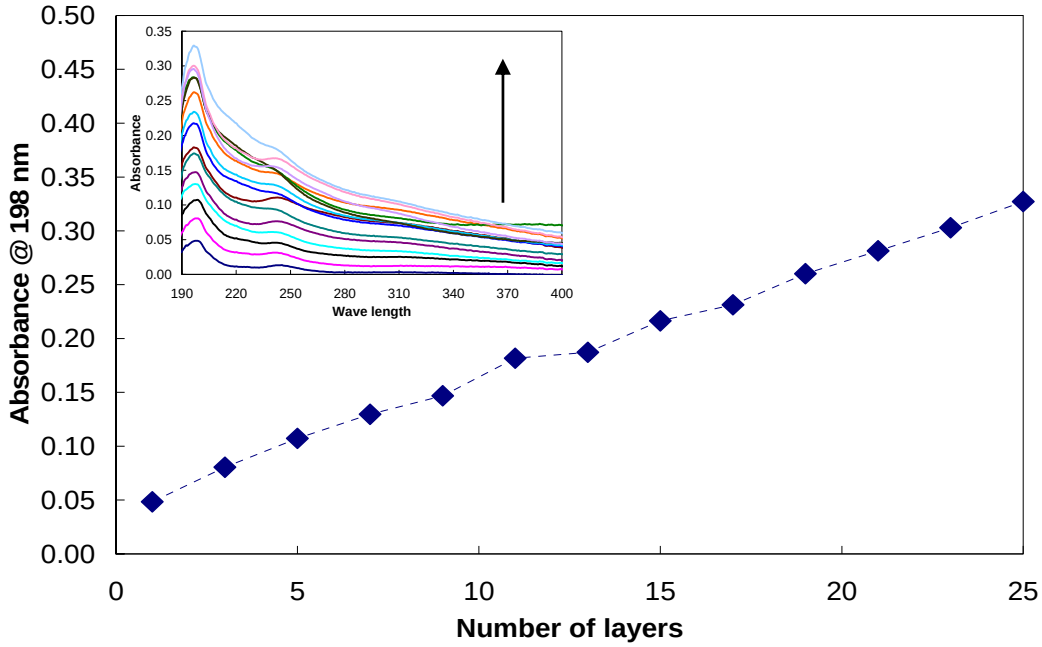


Figure 4.51 Absorbance of Chi/Alg multilayer thin films deposited at pH 5.5 as a function of the number of layers deposition cycles. The insert shows multilayer thin film growth of Chi/Alg on quartz substrate.

- Thickness and roughness of Chi/Alg multilayer thin films

The AFM Thickness of Chi/Alg multilayer thin films deposited at pH 5.5 is increase proportional to the increase of number of layers as shown in Figure 4.52. The AFM thickness of 8, 12, 16 and 20 layers was 29.99 nm, 31.86 nm, 43.56 nm, and 54.31 nm, respectively. The observed different in thickness of the multilayer thin films are suggested to arise from different in degree interpenetration between chitosan and alginate during deposition process.

The surface morphology of Chi/Alg multilayer thin films at pH 5.5 were also characterized via AFM (Figure 4.53).Interestingly, surface morphology shows a regular evolution with the average roughness 9.02 nm. There are small globules around multilayer thin films, which are composed of Chi/Alg excess complex.

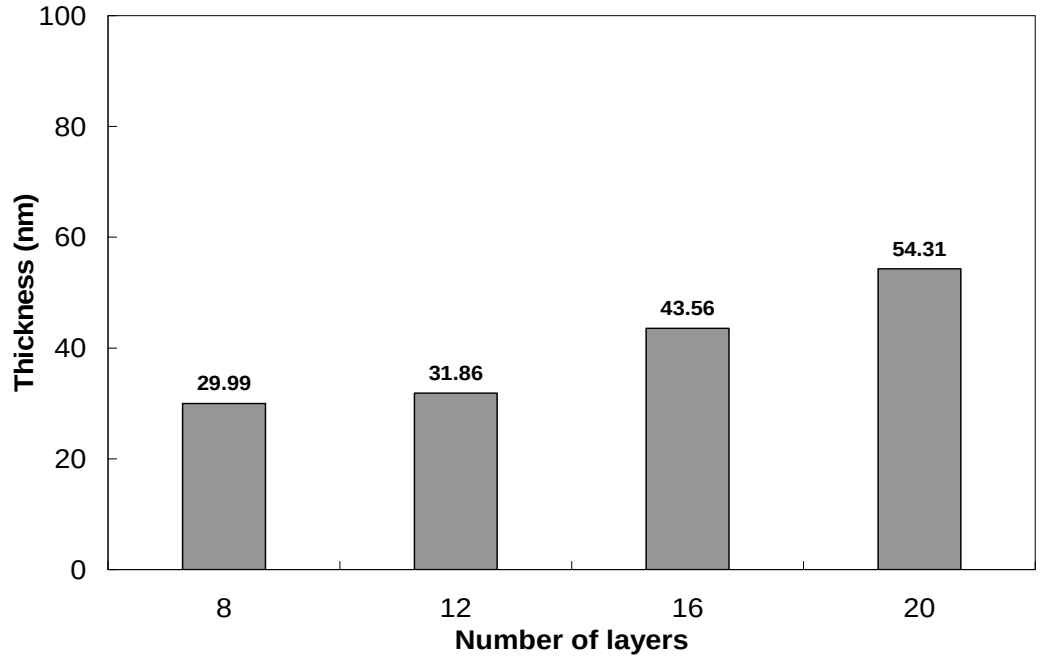


Figure 4.52 Thickness of Chi/Alg multilayer layer thin films deposited at pH 5.5 determined using AFM measurement.

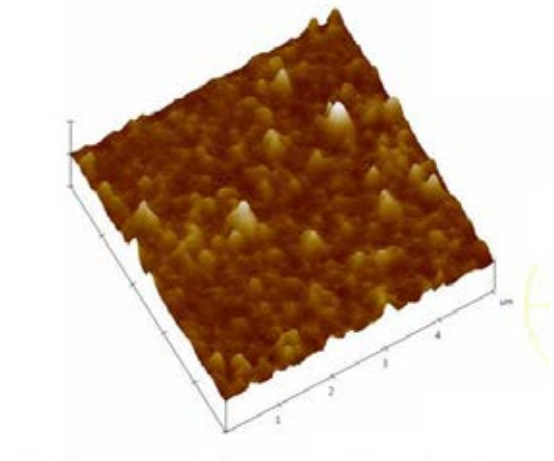


Figure 4.53 Surface roughness of Chi/Alg multilayer thin film on silicon.

4.2.1.2 Formation of Chi/PAA multilayer thin films

Poly acrylic acid (PAA) is a weak polyanionic, well known for its biocompatibility and its widely uses as polyelectrolyte in biomedical application.[139] PAA has the ionic groups located regular on the molecule backbone, with high ability to compensate with the positively charged of Chitosan. The formation of Chi/PAA deposited at pH 5.5 was first monitored as shown in Figure 4.54. Chi/PAA exhibits initial growth curve phenomena, followed by exponential growth beyond 9 layers. These results may be described to the lower flexibility of chitosan (which has the semi-rigid chain, unlike the synthetic polycation which is flexible), which influenced the intrinsic charged compensation between the multilayer chains. Therefore, the formation of Chi/PAA multilayer thin films could be attributed to the electrostatic interaction between COO^- groups of PAA and NH_3^+ groups of chitosan.

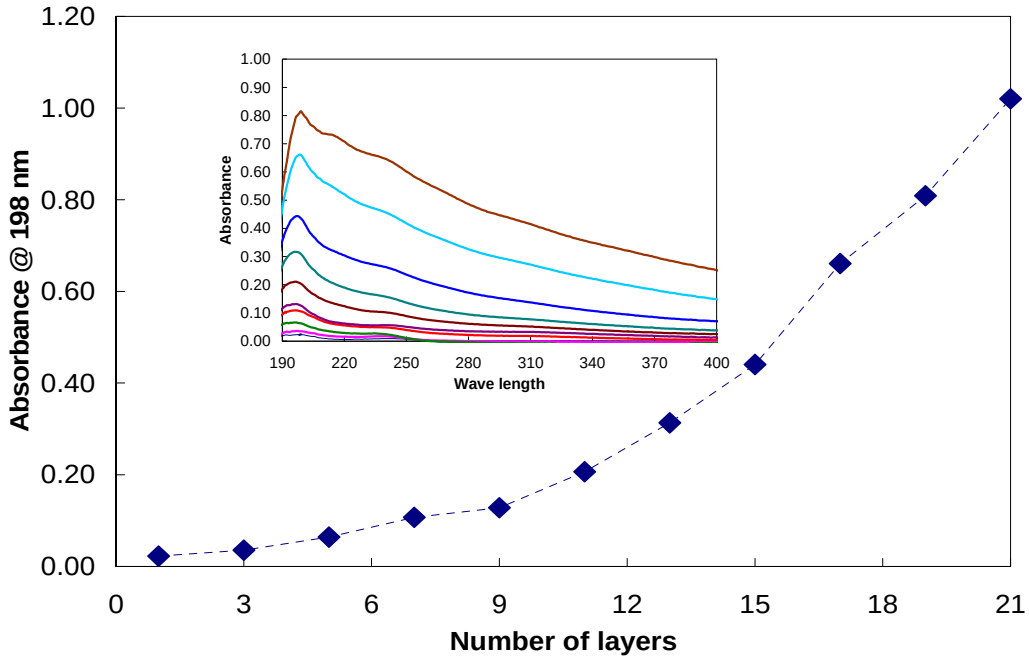


Figure 4.54 Absorbance of Chi/PAA multilayer thin films as a function of the number of layers deposition cycles. The insert shows multilayer thin film growth of Chi/PAA on quartz substrate.

It is known that the charge density of chitosan and PAA are mainly controlled by the pH. The pH value at which the ionization curve (Figure 4.55) of chitosan intercepts the ionization curve of PAA was considered as the ideal pH for preparation of multilayer thin films. The maximum degree of swelling in polyelectrolyte multilayer chains is determined by the balance between repulsion and interaction force.[140] If there is a high degree of swelling, polyelectrolyte can be dissolved. Therefore, Chi/PAA multilayer thin films were chosen the optimum pH value at 5.5 in order to construction multilayer thin films. With the pH 5.5, it is possible to preparation of multilayer thin films in order to determine drug loading and release in further.

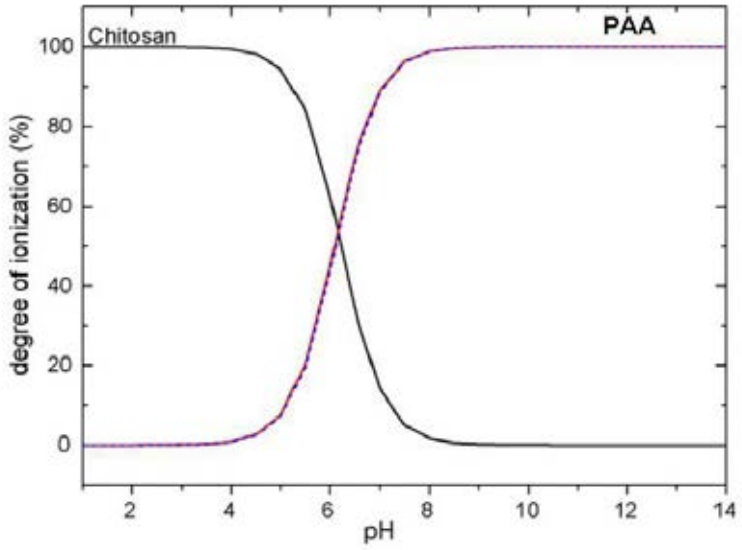


Figure 4.55 Degree of ionization of chitosan and PAA according the pH.[141]

- Thickness and roughness of Chi/PAA multilayer thin films

The AFM thickness of Chi/PAA multilayer thin films as a function of number of layers was investigated as shown in Figure 4.56. As can see, the average thickness value was depended on the increase of number layers. It can be noted that, the thickness of multilayer assembled which contain 50% of charged density is known to be quite thick films. As can see from Figure 4.56, chitosan and PAA contain about 50% of charge

ionization at pH 5.5, can cause the dramatically increased of thickness, especially at 20 layers. The dramatically of thicker films at 20 layers could be due to the partially charged of PAA that lead to high thickness of films and loopy conformation. This expected data can be confirmed by the roughness of surface thin films. The surface topography of Chi/PAA multilayer thin films was investigated by AFM as shown in Figure 4.57. The uninformed surface was also observed at 20 layers with the average surface roughness 32.14 nm. Normally, a surface comprised of a significant population of Loops and Tails, upon drying produce a rough surface. In addition, a surface dominated by Flat and Tail-like segments produce more smooth surface. As a result, the surface roughness increases significantly due to the establishment of a more loopy conformation arrangement. Chen and co-worker[142] confirmed that weak polyelectrolyte, such as PAA, exhibit behavior that is unique to their ability to adjust their density in response to local environmental variations. The charge adjusting behavior of PAA makes it possible for the polyelectrolyte chins to adopt charge distribution that promotes maximum thickness and roughness multilayer thin films. Therefore, a transition from thin flat layers to adopt more loop absorbed layers occurred when the charge density of Chi/PAA decreased from their fully charged state to 50%-70% charged unit.

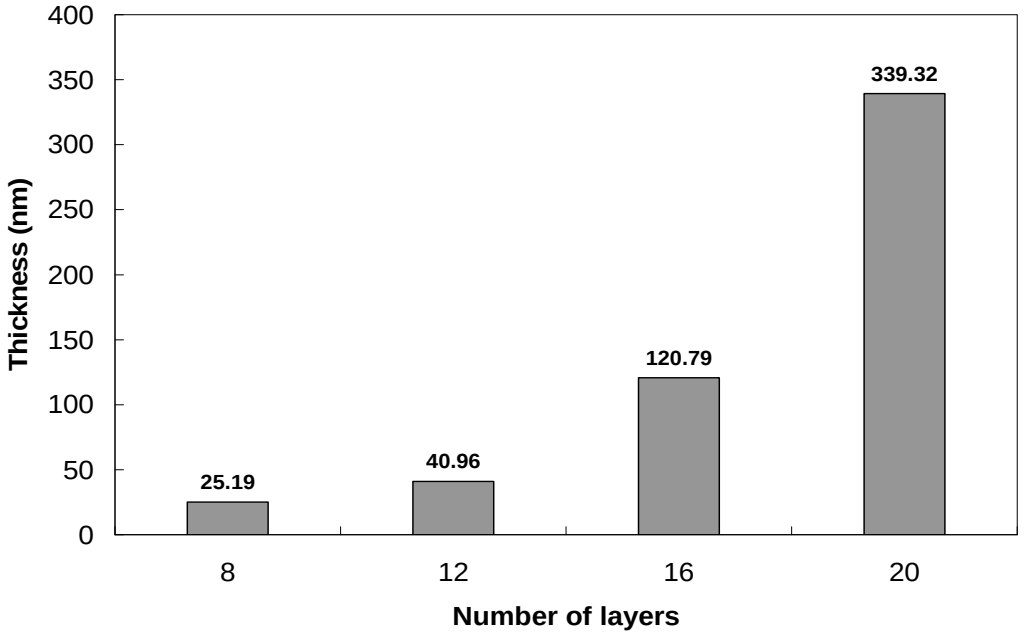


Figure 4.56 Thickness of Chi/PAA multilayer layers as determined using AFM measurement.

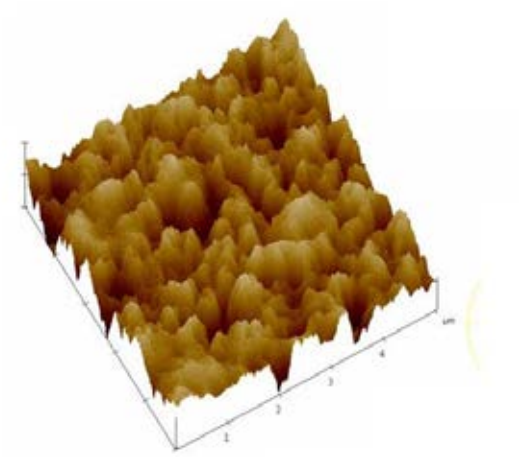


Figure 4.57 Surface roughness of $(Chi/PAA)_{10}$ multilayer thin film on silicon.

4.2.2 Effect of pH and NaCl on the stability of blocking layer

4.2.2.1 Stability/Decomposition of Chi/Alg multilayer thin films

The stability/decomposition studies were conduced in low pH condition and neutral pH condition via UV-spectrophotometer. The decomposition of Chi/Alg multilayer thin film in function of time is given in Figure 4.58. At buffer pH 3, multilayer thin films decomposed slightly within 600 minutes, while at pH 7, much fast release is observed. The significant difference decomposition of multilayer thin films with the change in solution pH could be referring to the swelling of chitosan and alginate chins. The swelling behavior can be explained by the fewer water molecules capable of incorporating the chitosan chain due to the entanglement of the chain. At acidic solution as buffer pH 3, the carboxylic of alginate side chain in multilayer thin films trend to protonated. Theoretically, alginate shrinks at low pH and converts into alginic acid skin, which insoluble in water. However, chitosan dissolve easily at low pH that can causes it to form protonation of amine groups. This protonation leads to chin repulsion and diffusion the proton and counter ion with water inside, therefore dissociation of multilayer chains. Once passed into the higher pH of release medium, such as pH 7, the alginic acid skin is converted to a soluble viscous layer. This pH dependent behavior of alginate can be exploited to release medium whereas chitosan act as insoluble in release medium. It was also report that charge ratio, molecular weight and ionic strength influence the stability of multilayer thin films. In this case, Chi/PSS multilayers were found to increase the swelling when the increasing of salt concentration. While Chi/Alg multilayer thin films display a swelling behavior, salt can reduced electrostatic interaction between polymer side chains, allowing for a decrease of thickness and thus, dissociated multilayer thin films. Since the rate of decomposition may be regulated by changing pH solution and ionic strength, it indicates that Chi/Alg multilayer thin films have a high potential for using as a matrix for control release of drug delivery system.

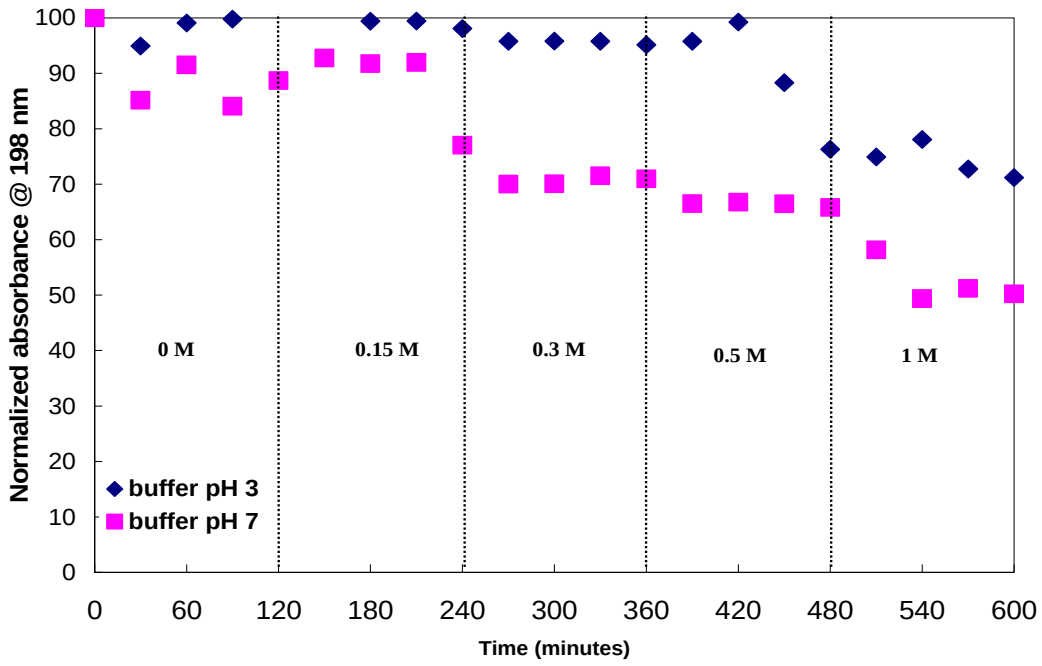


Figure 4.58 Stability of Chi/Alg multilayer thin films in buffer pH 3 and 7 with varying the ionic strength.

4.2.2.1 Stability/Decomposition of Chi/PAA multilayer thin films

One of the most important properties of weak polyelectrolyte multilayer thin films is their swelling behavior. Figure 4.59 represented the effect of environmental pH on the stability/or decomposition of Chi/PAA multilayer thin films. The decomposition rate of Chi/PAA multilayer thin films in buffer pH 3 has initially larger than that the decomposition rate in buffer pH 7. It is known that the pKa value of PAA is about 4. As Chi/PAA deposited at pH 5.5, the electrostatic interaction between side chins of polyelectrolyte has been associated. Moreover, the other interaction, such as hydrophobic interaction as well as the hydrogen bonding (>NH---O=C<) also participated in multilayer system. At lower pH 3, the carboxylic groups remaining the unionized (COOH) form. As a result, PAA chains remain collapsed and tightly bond. Thus, the PAA chains are unstable to interaction with amino groups of chitosan, leading to a significant fraction of carboxylic groups as nonbonding. By the way, it is evident that the

variation in carboxylic groups of PAA and amino groups of chitosan does not change much beyond 7. As the pH of medium increase to pH 7, chitosan was loosed their charged and rearrange into NH_2, while PAA have full ionized charged of COO^-. It was report that PAA has some potential of interactions with oppositely charged polyelectrolyte through the carboxylic group in its structure.[143] These groups should remain the compact conformation that can protect the dissolution polyelectrolyte during exposure in pH 7. The effect of ionic strength on the decomposition was also affected the swelling of Chi/PAA multilayer thin films. At buffer pH 3, the decomposition rate was significant decrease with the increasing NaCl concentration. While the high ionic strength, the charged of polyelectrolyte chain are neutralized by Na^+Cl^-, which support the dissociation of electrostatic interaction between Chi/PAA multilayer thin films.

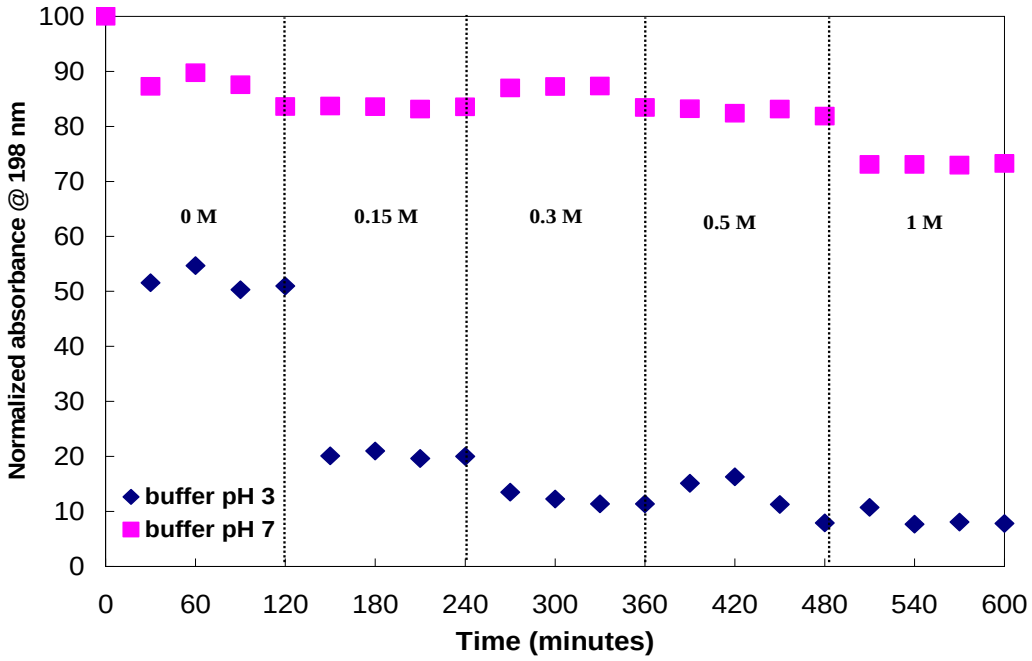


Figure 4.59 Stability of Chi/PAA multilayer thin films in buffer pH 3 and 7 with varying the ionic strength.

4.2.3 Prolonged release of hydrophobic drug from PEM films

4.2.3.1 Design concept and structures of the polyacrylic substrate and polymer matrix

The structure of the polyacrylic film and curcumin loading polymer matrix are illustrated in Figure 4.60. The designed films consist of two layers: main drug layer and blocking layer. The structure of drug was used curcumin as drug model, whereas the structure of blocking layers consisted of PDADMAC/PSS, Chi/Alg and Chi/PAA. The surface of blocking layer is designed to control the drug release in two respects: The first one is to extension drug release from substrate towards the release environmental. It functions as a release controlling layers to protect the burst release of drug from the polyacrylic substrate and polymer matrix. The second is to provides additional supporting force for the drug loading substrates, compared with which that having only drug-loading layer cannot offer strong supporting force with a relatively high drug loading. The release solvent was selected buffer pH 7.4 and the solvent composition of water/ethanol (%), v/v.

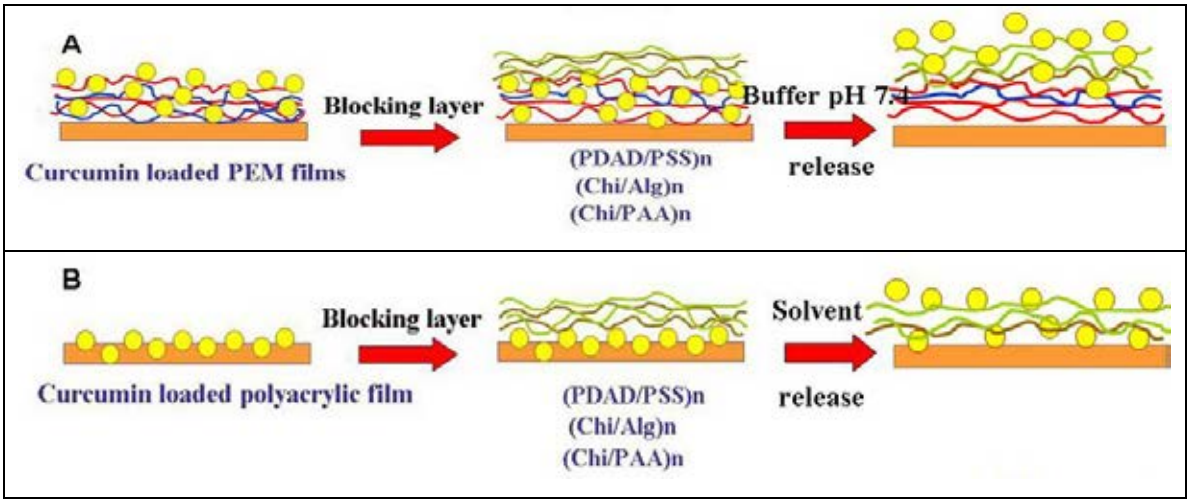


Figure 4.60 Schematic representation of curcumin release through blocking layer in (A) buffer pH 7.4 and (B) solvent composition of water/ethanol.

4.2.3.2 Effect of solvent composition

The effect of solvent composition of water/ethanol (%), v/v was studied in order to obtain the highest percentage of release solvent. Curcumin loading polyacrylic film, which uncoated any blocking films, were immersed in the variation ratio of solvent composition. Figure 4.61 shows the release profile of curcumin loading pure polyacrylic films in mixtures of water and ethanol with different compositions. The release profile is dramatically accelerated with addition some water content into the release solvent. Normally, curcumin is completely soluble in organic solvent, like ethanol. The solubility of curcumin in the adding of 20% of water is much higher that pure ethanol, leading to increase the release rate. This is due to 20% of water can adjust the system of release environment to become hydrophilic that support the hydrophilic parts of curcumin prefer to escape. However, the increasing of water more than 30 % has no ability the release of curcumin due to the unstable of release environment. Therefore, this result confirm that the 20/80 of water/ethanol using as a release solvent, has efficient to wash out all curcumin content from substrate in the end of definite time. This ratio of composition was used as release solvent in the further experiment.

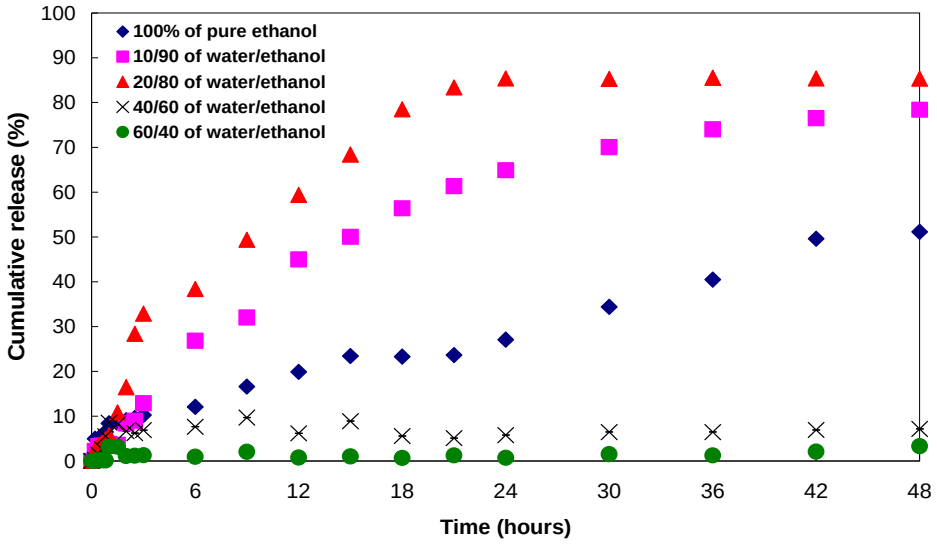


Figure 4.61 Profile release of curcumin loading uncoated polyacrylic substrates in mixed composition solvent of water/ethanol (%), v/v.

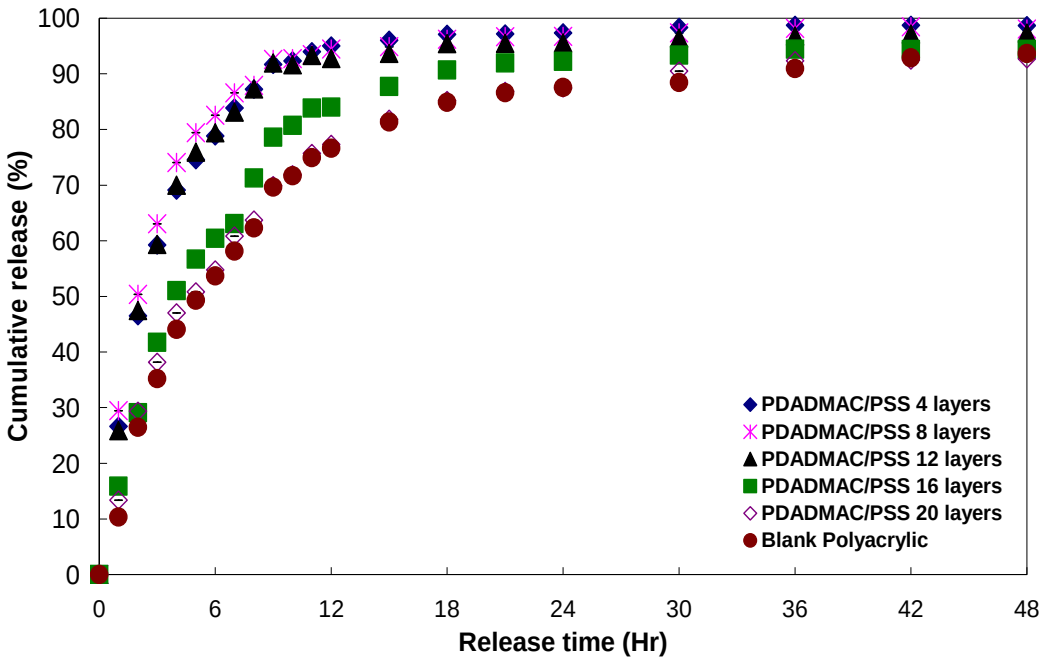
4.2.3.3 Effect of blocking films types

To find out whatever the blocking films could realize its function of extent drug release, the release solvent were performed. Curcumin loading polyacrylic substrate were coated the blocking films with different types (PDADMAC/PSS, Chi/Alg and Chi/PAA) to investigate the effect on prolong rate of release. Considering PDADMAC/PSS blocking films, the process of curcumin molecule release from polyacrylic substrates was rapid during 12 hour and trend to complete release rate in the end of 48 hour. Moreover, the percentage of curcumin release from uncoated and coated PDADMAC/PSS blocking films for 4, 8, 12, 16, 20 layers were 98.67%, 98.05%, 97.32%, 94.41%, 92.7% and 93.66%, respectively. As can see from Figure 4.62A, the effect of number of blocking layer show no significant different of prolong release of curcumin. This result is due to the face that PDADMAC and PSS chain exhibits high hydrophobic region that can not prevent the interaction of ethanol solvent. Therefore PDADMAC/PSS blocking films have no ability to prolong the release of curcumin.

Figure 4.62B shows the prolong release of curcumin from uncoated polyacrylic and Chi/Alg blocking films. Comparison to the uncoated polyacrylic with 93.66% release, the release amount of curcumin from Chi/Alg blocking films for 16 layers and 20 layers is less than 50% within 48 hour. Moreover, the initial rapid release was significant suppressed and the duration of completion release was considerable prolonged by coated Chi/Alg blocking films. The percentage of curcumin release from Chi/Alg blocking films for 4, 8, 12, 16, 20 layers were 93.04%, 86.08%, 84.39% 48.49% and 26.78%, respectively. As a result, the prolong release depends on the number of blocking layers, which decrease rate when increase the number of blocking layers. The further decrease in the release rate was attributed to the hydrophilic part of ammonium groups of chitosan and carboxylic groups of alginate that acts as a barrier hindrance by coating more Chi/Alg layers.[144] These results demonstrate that the added Chi/Alg blocking films show the release and preserved the incorporated curcumin, and thus a longer drug release period can be expected.

Similar prolong release behavior was observed from curcumin coated Chi/PAA blocking films with varies number of layers (Figure 4.62C). For all Chi/PAA blocking layer, the initial rapid release in the first 24 hour was much lower (<60%) than that of uncoated polyacrylic. This result was attributed to the hydrophilic part of ammonium groups and carboxylic groups that can prevent the hydrophobic part of curcumin to release into solvent. We also found that the thickness of blocking films is also significant influent on the prolong release of curcumin.[145]Comparison of 20 layers of blocking film, Chi/PAA blocking films have average thickness 342 nm whereas Chi/Alg blocking films have average thickness 52.41 nm. Therefore, the effect of thickness was also greater influent the prolong release, indicated that the permeability of curcumin from the lower thickness is more sensitive to the higher thickness films.

(A)



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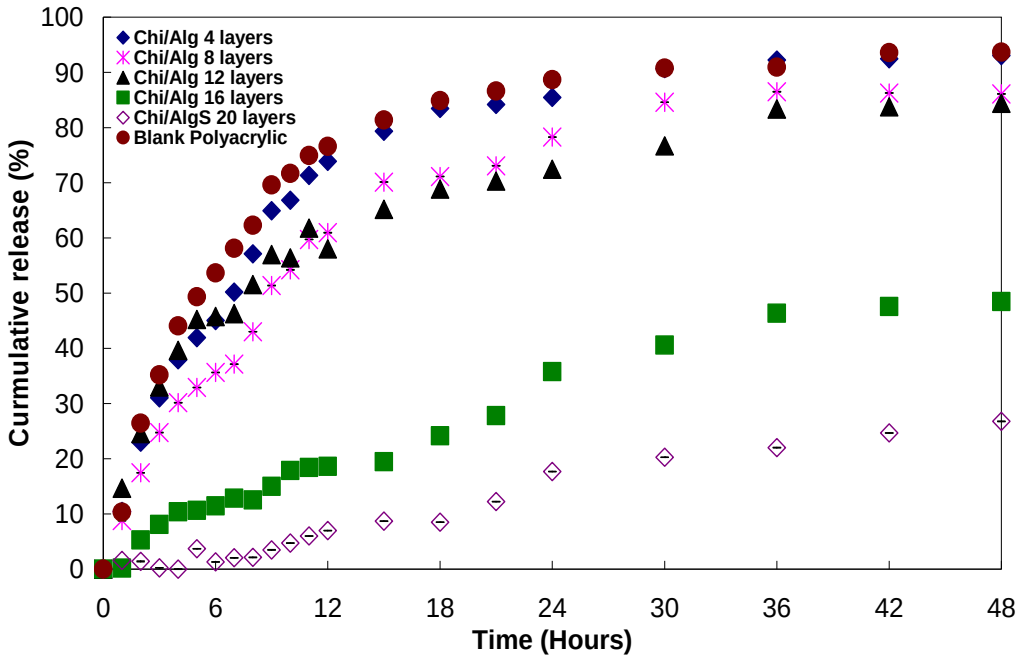


(C)

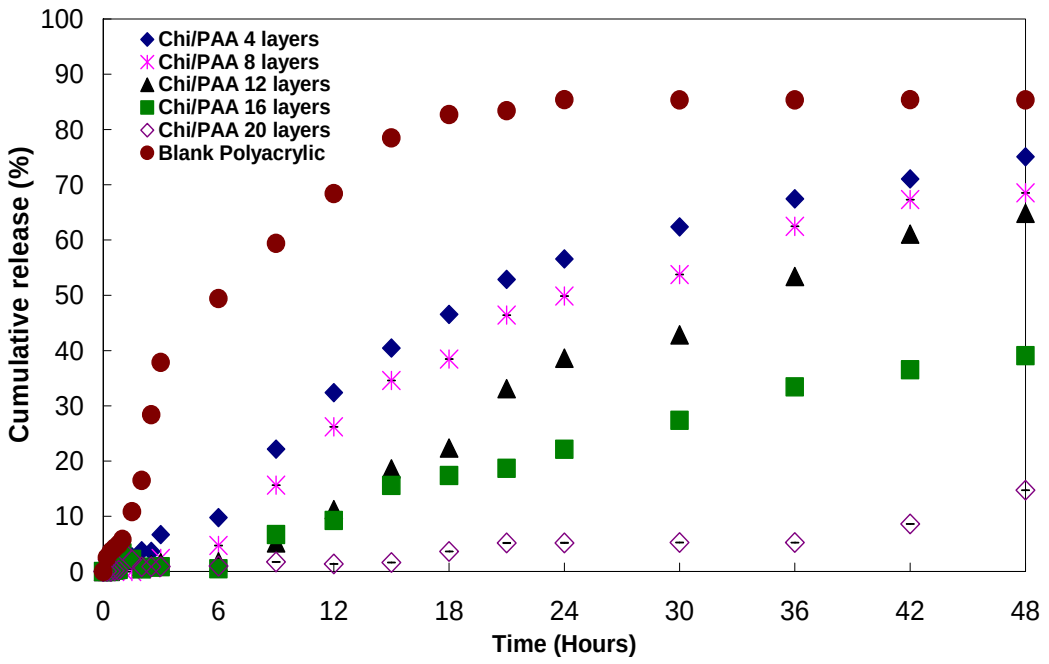


Figure 4.62 Extension release of curcumin from blocking films coated polyacrylic substrates in 20/80 of water/ethanol. (A) PDADMAC/PSS (B) Chi/Alg and (C) Chi/PAA.

The summary of the effect of blocking films on the prolong release of curcumin from polyacrylic substrate after 24 hour were demonstrated in Figure 4.63. At 20 layer of

blocking films, the normalized of curcumin release per films thickness of Chi/PAA and Chi/Alg were 4.09 and 4.80 respectively which mean that the Chi/PAA characteristic have higher ability to act as a barrier layer in solvent. With this advantage, the blocking films were shown to be an attractive barrier to enhance the slow release characteristic of drug in a controlled and localized manner.

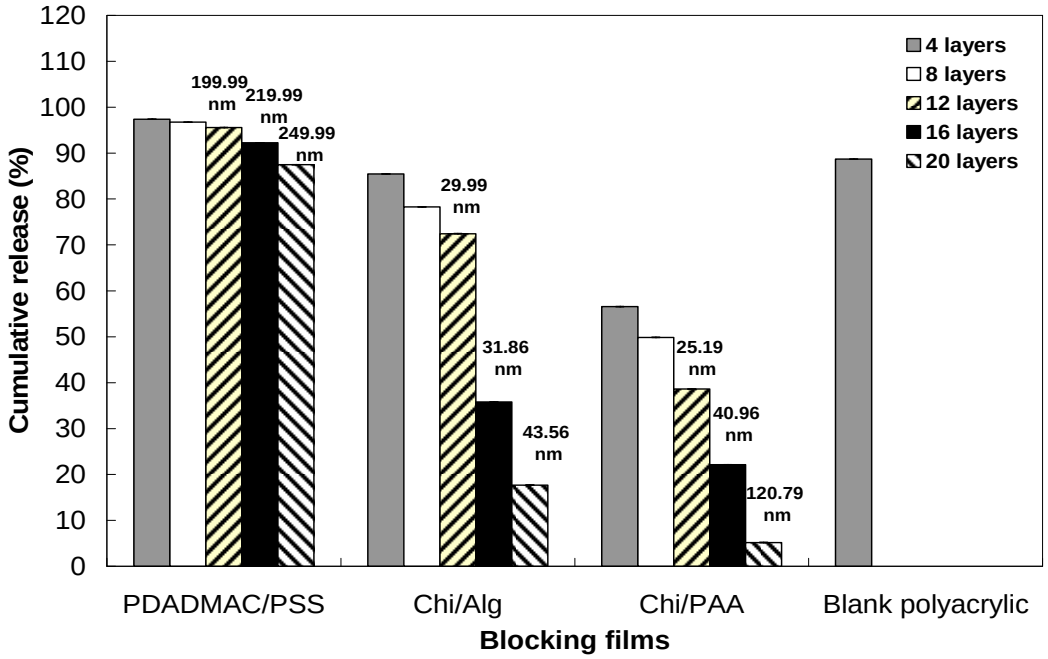


Figure 4.63 Comparison of blocking films on the extension release of curcumin from polyacrylic substrates at 24 hours.

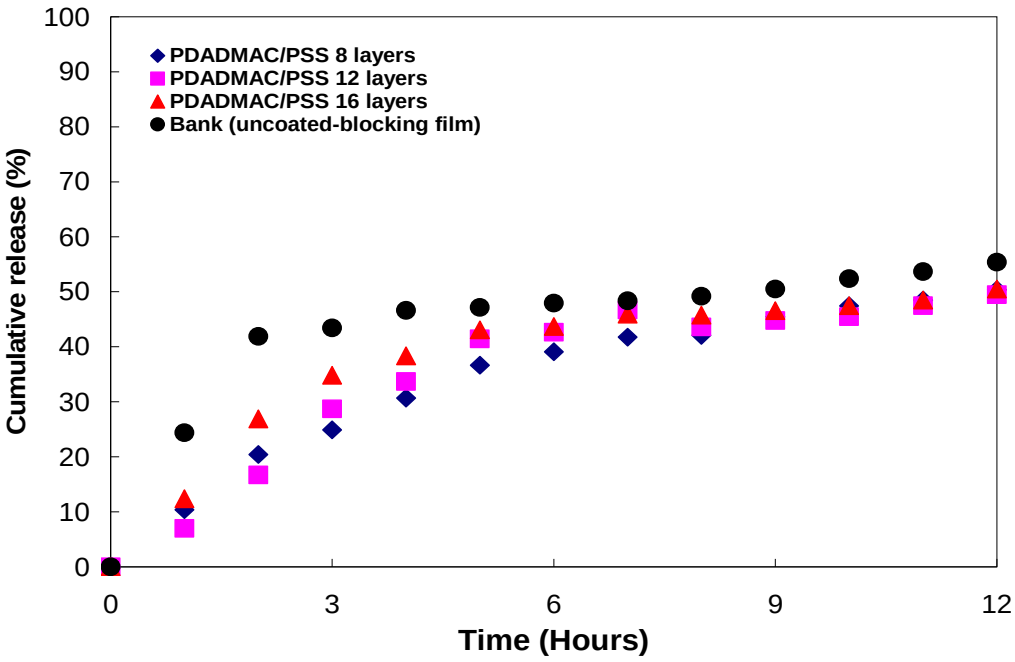
The amount of curcumin release from polymer matrix with different blocking types was also quantified in buffer pH 7.4. This data was determined as a function of time as shown in Figure 4.64. For PDADMAC/PSS blocking films, the percentage release of curcumin in buffer medium behaved similarly to the bank of uncoated blocking films (approximately 55.38%). The total percentage release of curcumin from PDADMAC/PSS 8, 12 and 16 layers were 50.37%, 49.47% and 50.48%, respectively. In addition, PDADMAC/PSS blocking films with varies the number of layers did not demonstrate a significant to delay release characteristic. This result can confirm that the hydrophobicity

properties of PDADMAC/PSS have no ability to prolong the release of curcumin from polymer matrix films.

As Chi/Alg blocking films, the percentage release of curcumin were 47.54%, 45.48% and 42.28% for 8, 12 and 16 layers, respectively. In addition, the percentage releases of curcumin from Chi/PAA blocking films were 44.99%, 37.38% and 34.48% for 8, 12 and 16 layers, respectively. The amount of curcumin release from both of Chi/Alg and Chi/PAA blocking films 8 layers was much less than release from blocking films 16 layers, which mean that the amount of curcumin release from polymer matrix could be controlled by adjusting the number of blocking layers. It have been reported that the extension release kinetic is influenced by molecular weight of polymer, solubility of drug and the stability/decomposition of polymer films. An important aspect of the current study is the use of chitosan as a blocking layer. Chitosan is a weak polyelectrolyte with the pKa 6.5, therefore it have positively charge at pH 5.5 in order to form the electrostatic interaction with the negatively charge of alginate or PAA. Moreover, Chi/Alg and Chi/PAA have less decomposed in buffer pH 7.4 as report in previous resulted (see Figure 4.58 and 4.59). The higher stability of Chi/Alg and Chi/PAA multilayer thin films should be attributed to the differences in their architecture. Meanwhile the natures of the functional groups and their respective placement along the polymer backbone are also important. Basically, the Chi/Alg multilayer thin films are obtained stepwise by the electrostatic interaction of the amino groups of Chi and the carboxylic groups of Alg chains. Since the pKa of a weak polyelectrolyte can be a complex function of the degree of ionization, the term pKa in this section indicates the pH at which approximately 50% of the polymer's functional groups are ionized. It has been report that the pKa of PEM thin films/ and PEM complex could be shift and higher than that each pKa of its individual polymer solution. As a result, Chi/Alg and Chi/PAA polyelectrolyte multilayer thin films show stability which less change of films decomposition after exposed in buffer pH 7.4. In addition, Chi/Alg multilayer thin films can prolong the release of curcumin due to their similarly structural characteristics. Therefore, the formation of

Chi/Alg and Chi/PAA has ability to delay the release of curcumin into buffer pH 7.4 which were mainly from the stability of their structure.

(A)



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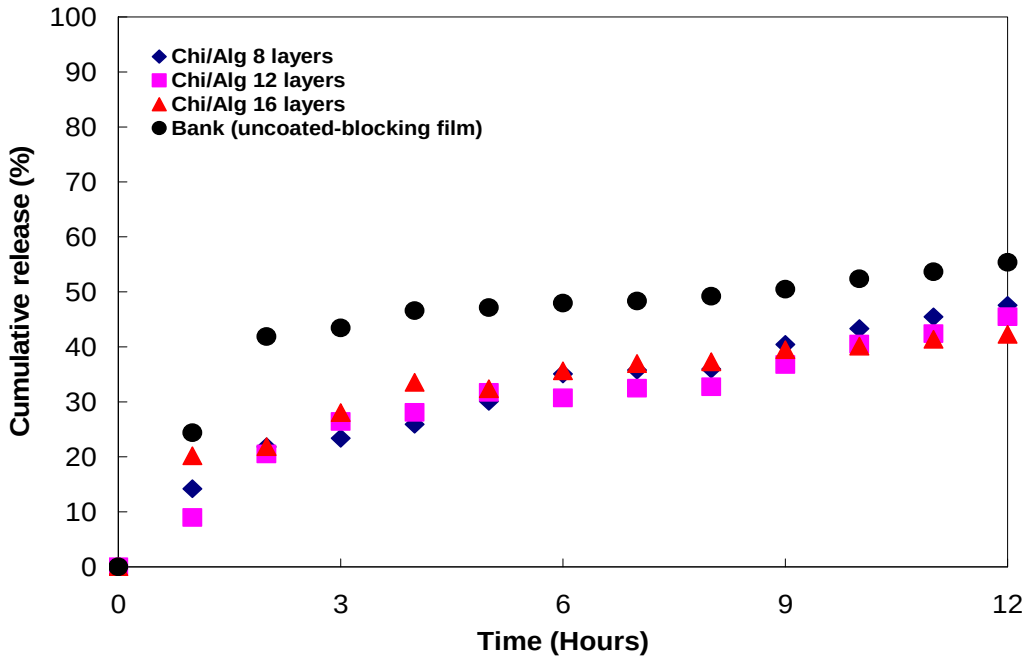

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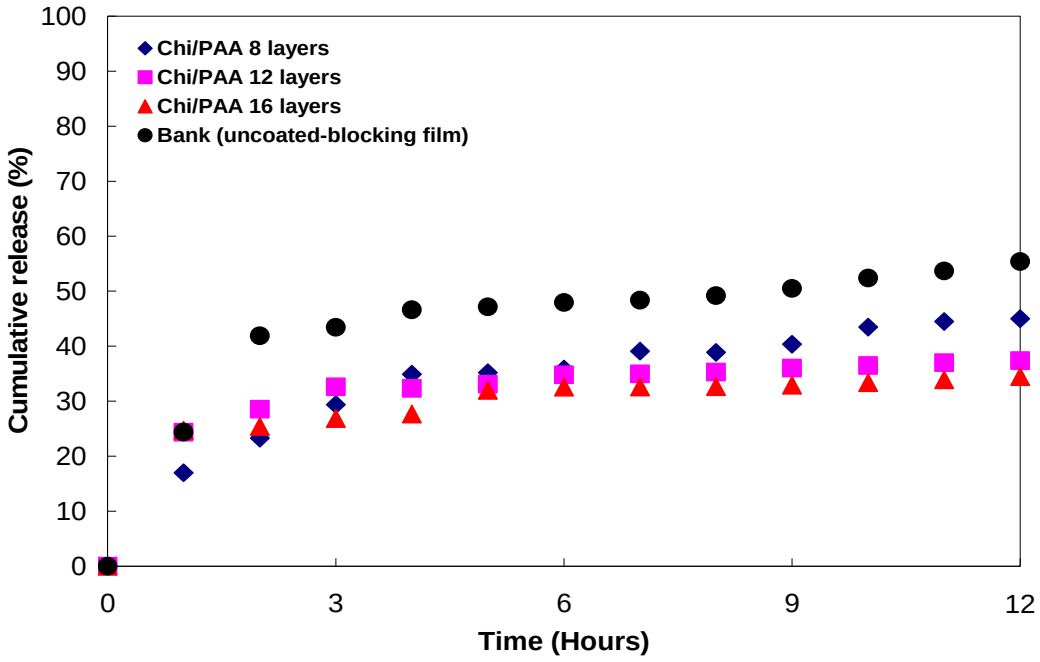


Figure 4.64 Extension release of curcumin from blocking films coated polymer matrix in buffer pH 7.4. (A) PDADMAC/PSS (B) Chi/Alg and (C) Chi/PAA.

The summary of the effect of blocking films on the prolong release of curcumin from polymer matrix after 12 hour were demonstrated in Figure 4.65. We also found that the thickness of blocking films is also significant parameter on the prolong release of curcumin from polyacrylic substrate and polymer matric.[145]Comparison of 16 layers of blocking film, Chi/PAA blocking films have average thickness 120.79 nm whereas Chi/Alg blocking films have average thickness 43.56 nm. Clearly, the normalized of curcumin release per films thickness of Chi/PAA and Chi/Alg were 20.69% and 9.21% respectively which mean that the Chi/Alg characteristic have higher ability to act as a barrier layer. This is also attributed to the similar structure of chitosan and alginate that can cause the polymer chain become dense structure of thin films. As a result, the effect of thickness was also greater influent the prolong release, indicated that the permeability of curcumin from Chi/Alg blocking films is more efficient rather than Chi/PAA blocking films.

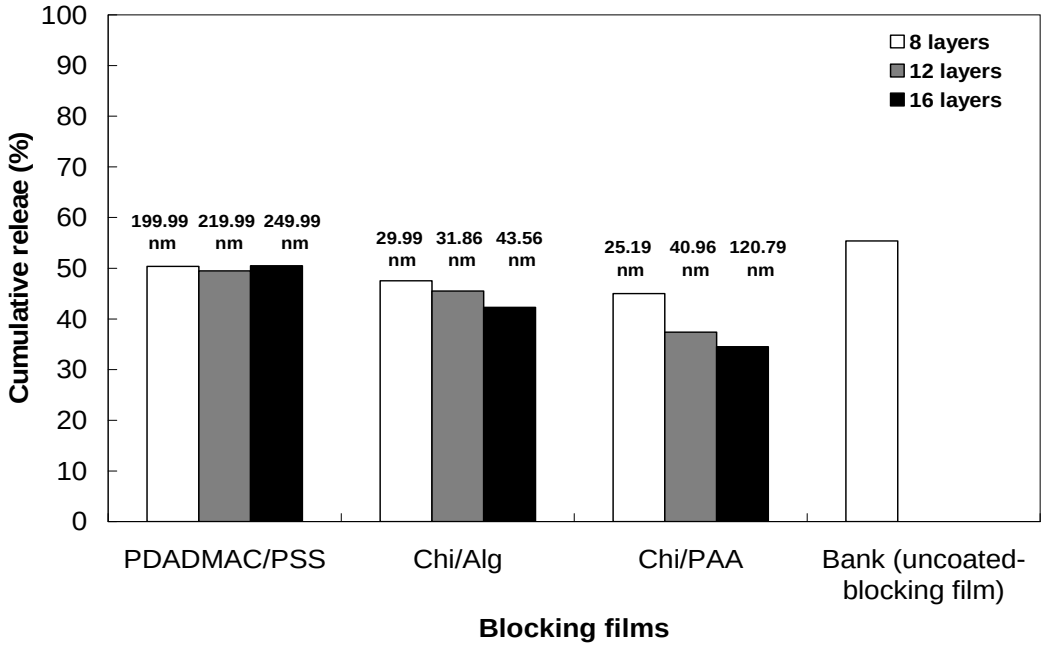


Figure 4.65 Comparison of blocking films on the extension release of curcumin from polymer matrix at 12 hours.

4.3 Development of Layer-by-Layer thin films-based coated fruit

Edible films and coatings are non polluting methods that have developed to extend product shelf life. Furthermore, it can load to such coating in order to prevent microorganism growth. The immobilization of curcumin compound can maintain high concentration of the active compound on the surface of fruit in order to achieve longer storage time. Although several method have been report to prepare the edible films coating, there is limit information focus on the advantage of the layer by layer self assembly technique to development fruit coating. Therefore, this part showed the potential of PDADMAC/PSS multilayer thin films as edible films containing curcumin – based coated fruit. The effect of hydrophobic/hydrophilic of surface of fruit and drug content was presented.

4.3.1 Effect of PEM-based coated fruit

4.3.1.1 Hydrophobic/hydrophilic properties

Water contact angle measurement is a surface selective technique and can indicate the outermost layer properties. Meanwhile, it is the interaction between the forces of cohesion and the forces of adhesion which determines whether or not wetting, the spreading of a liquid over a surface occurs. On extremely hydrophilic surfaces, a water droplet will completely spread (an effective contact angle of 0°). This occurs for surfaces that have a large affinity for water (including materials that absorb water). On many hydrophilic surfaces, water droplets will exhibit contact angles of 10° to 30°. On highly hydrophobic surfaces, which are incompatible with water, one observes a large contact angle (70° to 90°). Some surfaces have water contact angles as high as 150° or even nearly 180°. On these surfaces, water droplets simply rest on the surface, without actually wetting to any significant extent.

The hydrophobic/hydrophilic property of apples and mangoes coated with $(PDADMAC/PSS)_{10}$ were investigated. Figure 4.66 show the picture of apples and

mangoes before and after alternate deposition sequence of PDADMAC/PSS covering. Apparently, the surface of apples and mangoes show the hydrophilic properties with the fully spread of water droplet.

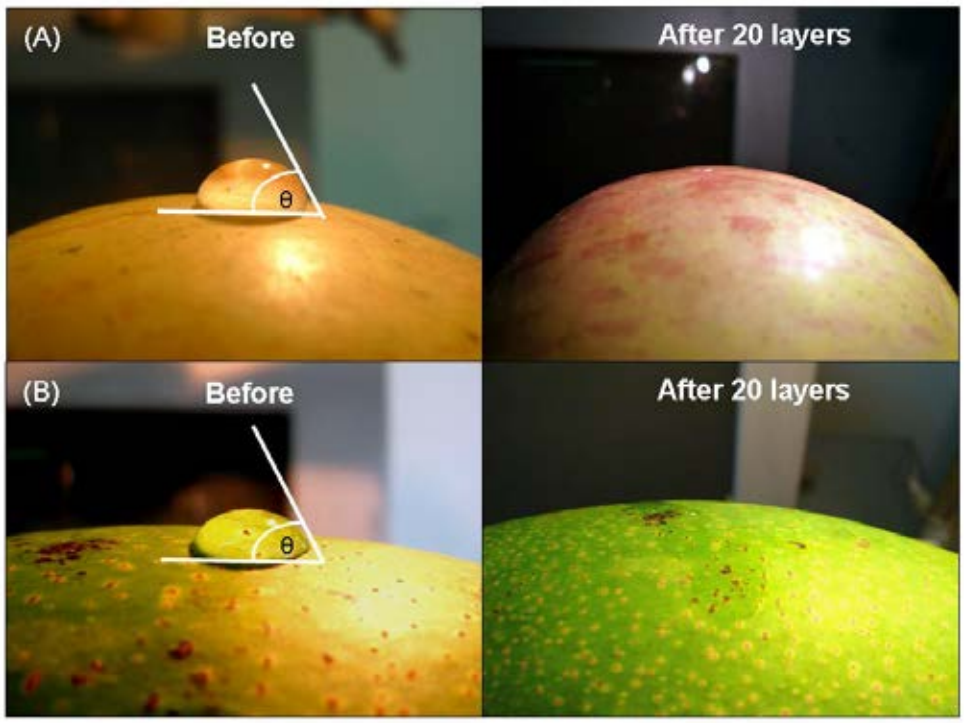


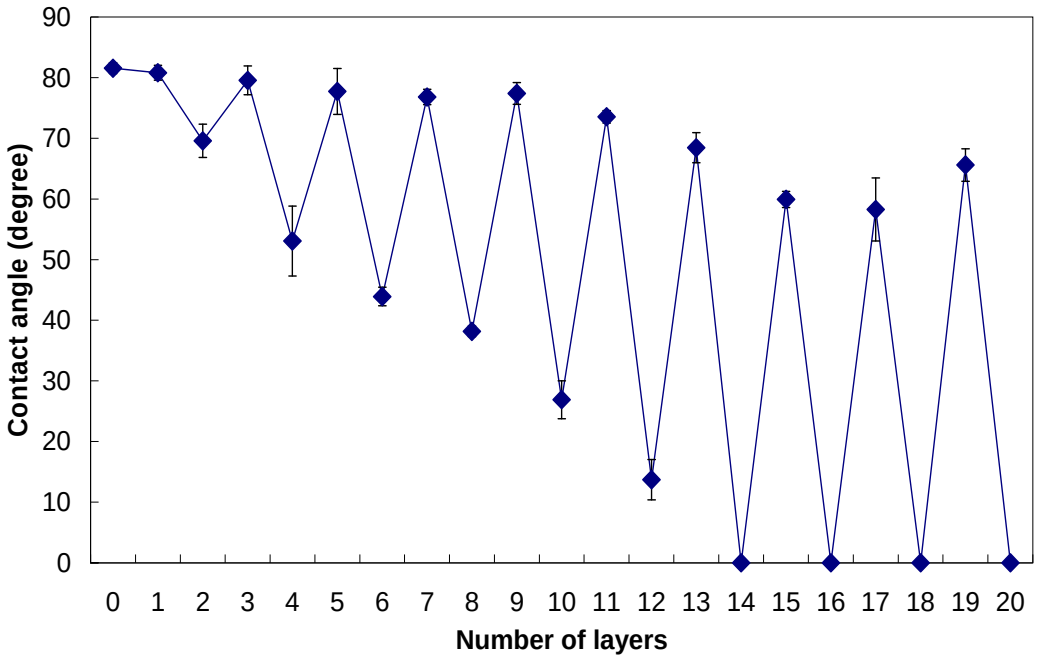
Figure 4.66 Contact angle of water liquid drop on (A) apple and (B) mango before and after coated 20 layers of PDADMAC/PSS multilayer thin films.

The virgin apples and mangoes surface exhibit a value of contact angle 81.55 and 72.66 respectively, which exhibit a hydrophobic surface. The water contact angle of both fruit was similar as demonstrated in Figure 4.67. At the odd layers, PDADMAC is the outermost layer while at the even layers, PSS is the outermost layer. With further increasing in the deposition cycle, the alternately changes of contact angle was occurred. The contact angle jumped alternatively between 75^0 and 30 in the first coating multilayer thin films 10 layers and showed a completely spread at the end of 20 layers. The results indicated that, PSS as the outer layer was significant hydrophilic property rather than

PDADMAC. This is due to negative charged of PSS which contain its sulfonated groups along back bone chains, exhibits more hydrophilicity properties. However, employing PDADMAC as the outer layer, the contact angle could again be increased. Our data in agreement with the result contact angle of Schonhoff et al.[146] who reported that the odd–even effect is not only controlled by the hydration properties of the outer layer itself, but that is also the sign of the surface potential which induces changes of the water mobility.

It was suggested that the different contact angle behavior may be caused by electrostatic interaction, or its molecular conformations upon adsorption. Despite the hydrophilic character of the surface of fruit, it could be the high water absorption capacity due to the present of film coating, which might led to the absorption of water under certain environmental condition. Therefore, this result reflects the hydrophilicity property of fruit that can improve by the coating of PDAD/PSS multilayer thin films. The hydrophilicity property of fruit may also influence the permeability of water/ or gas that has effect the apparent and shelf life of fruit.

(A)



(B)

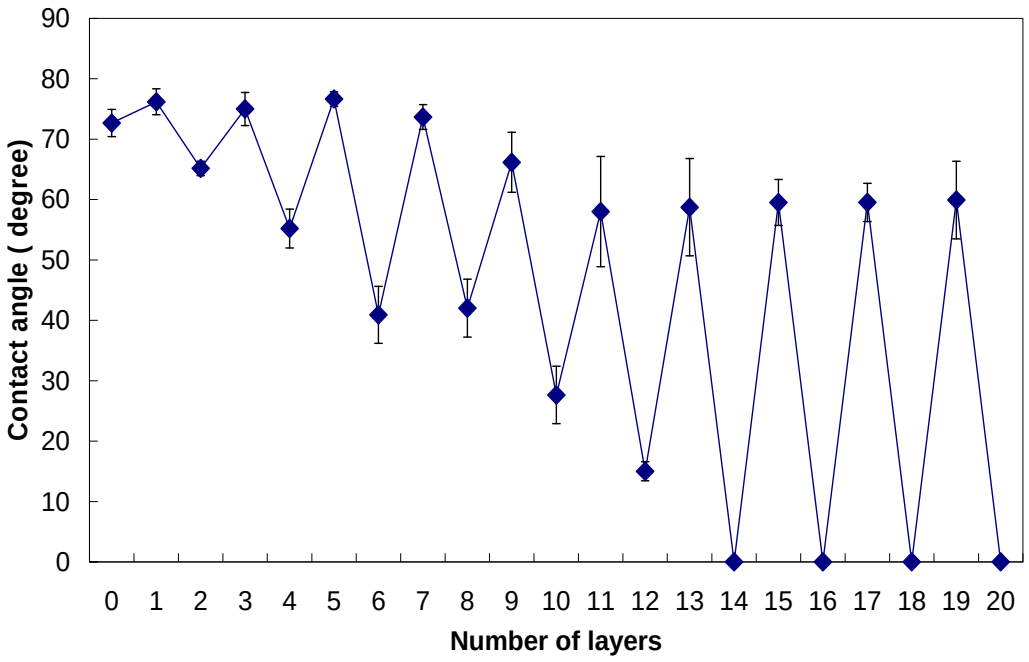


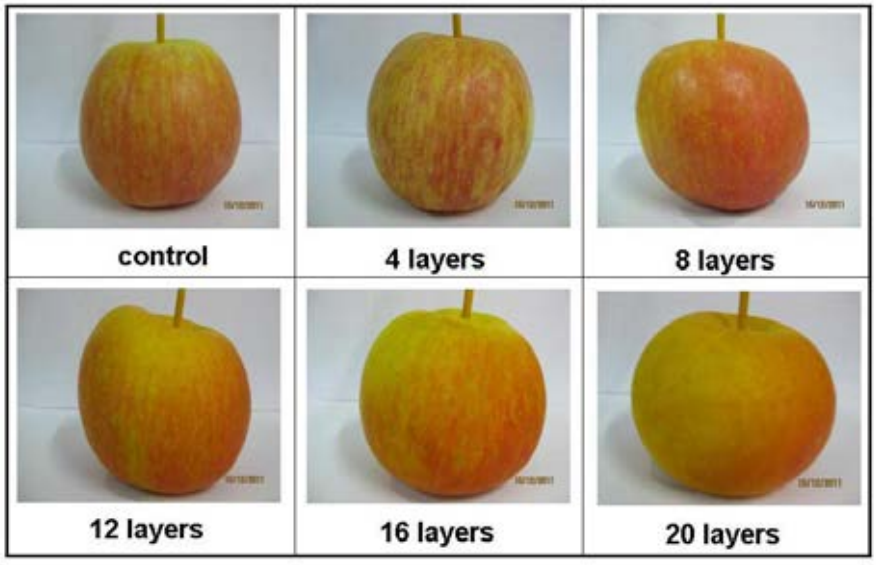
Figure 4.67 Change in contact angle of (A) apples and (B) mangoes coated with PDADMAC/PSS multilayer thin films. Odd and even deposition number of layers corresponded to PDADMAC and PSSMA, respectively.

4.3.2 Evaluation PEM containing curcumin coated fruit

4.3.2.1 Curcumin content on fruit

PDADMAC/PSS multilayer thin films containing curcumin were coated onto the surface of apples and mangoes as show in Figure 4.68. Apparently, curcumin coated fruit show a yellowness colour and increasing color proportion to the number of PDAD/PSS multilayer, as attributed to the thickness.

(A)



(B)

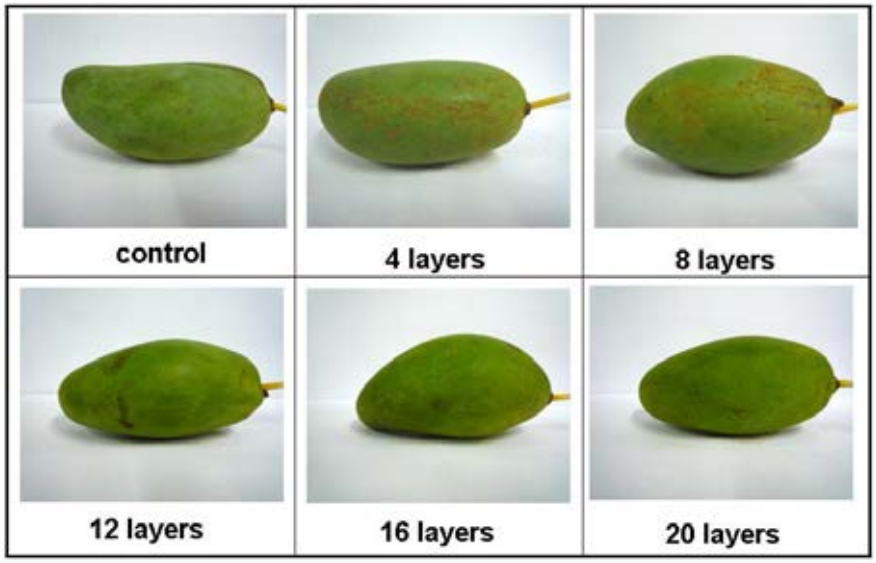
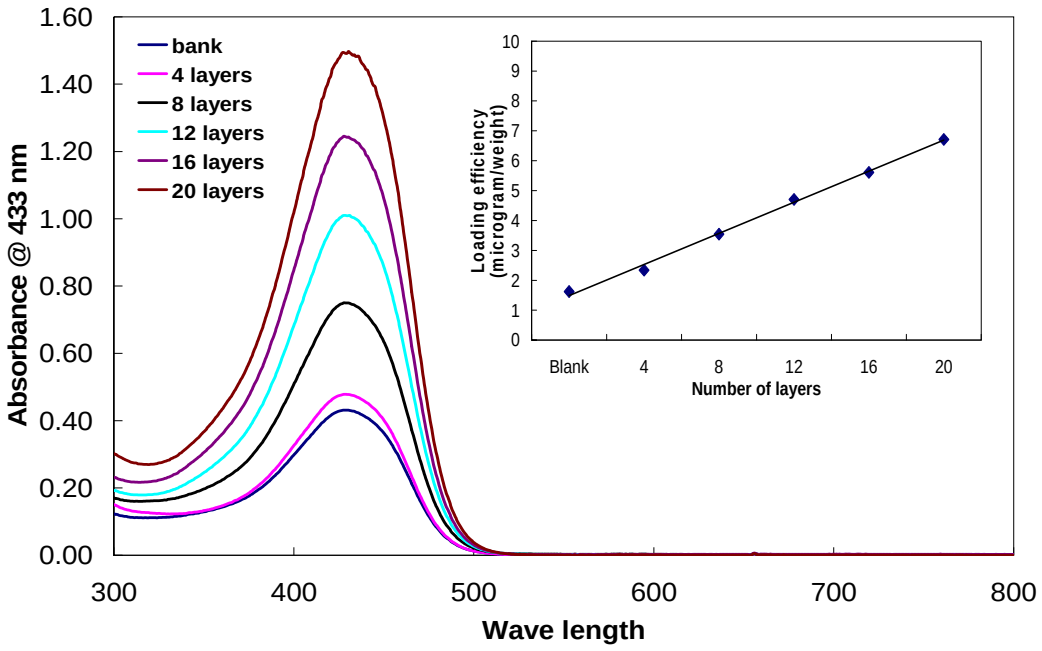


Figure 4.68 Appearance of (A) apples and (B) mangoes coated by PDADMAC/PSS multilayer thin films containing curcumin.

Although the above result was show the possibility of coating films on fruit, detailed studies are still required to quantify the loading efficiency in respond to drug feeding content. The loading time of curcumin on fruit was fixed at 3 hour that enough for the loading equilibrium as known from previous experiments. Figure 4.69 is the absorbance of the ethanol solutions after releasing curcumin from different fruit coated with PDADMAC/PSS composed of 4, 8, 12, 16 and 20 layers. By recording the UV-Vis absorbance spectra of the ethanol solution after dipping the fruit, it is possible to quantify the amount of curcumin present at the fruit surface.

(A)



(B)

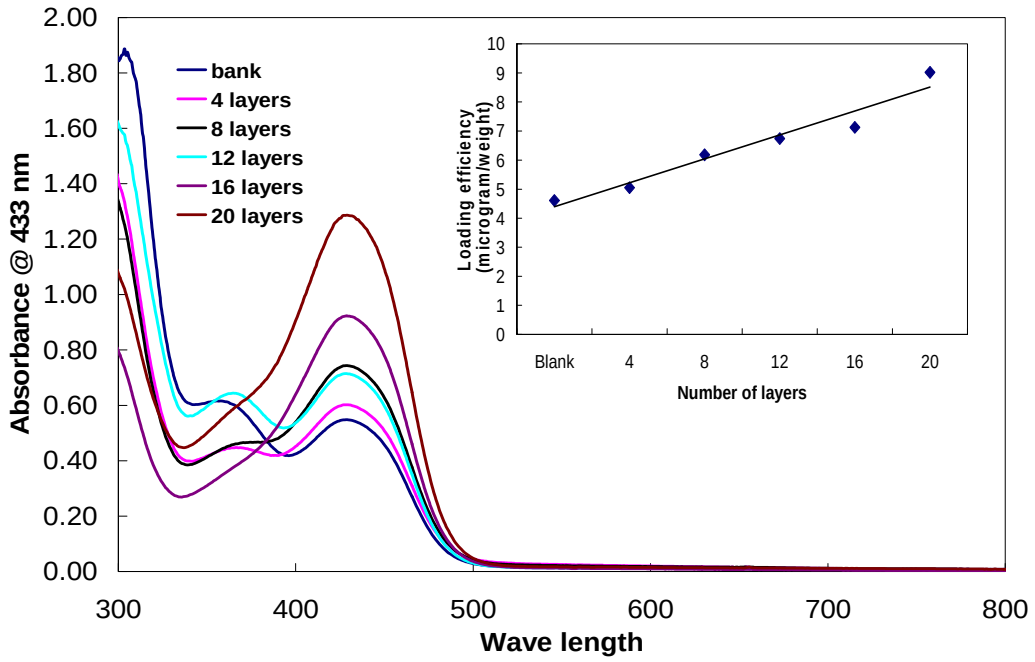


Figure 4.69 UV-Vis absorbance of ethanol solutions after releasing curcumin from PDADMAC/PSS coated (A) apples and (B) mangoes. The inserted figure showed the loading efficiency of curcumin as a function of number of PEM layers.

Table 4.2 demonstrated the curcumin content on apples and mangoes after coated by PDADMAC/PSS multilayer thin films. With increasing the number of coating layers from 4layer to 20 layers, the loading efficiency increase and have the highest dose 6.71 $\mu g.g^{-1}$and 9.02 $\mu g.g^{-1}$for apple and mangoes, respectively. The higher curcumin content in mangoes is attributed to its large surface area to volume ratio, thus carrying more curcumin than that in apples. However, the uncoated of apples and mangoes also have some curcumin content. This unexpected data could be come from the original hydrophobic surface of uncoated fruit that can also attraction with the hydrophobic part of curcumin. The obtained result was in agreement with previous discussion that confirms the ability of curcumin loading into multilayer thin films that mainly by the hydrophobic interaction.

It has been report that the incorporate multilayer thin films – based coated fruit show no effect on the increasing theirs shelf life as compare to control. It is possible caused from water permeability that occurred between surface of fruit and polyelectrolyte chain during the preparation. Further research on this problem need to be done to find out the optimization experiment. Overall, this result can prove that multilayer films-based coating could be used effectively as carrier of curcumin. This finding provides important information on properties of PDAD/PSS-based coating films in the view of their use by the fruit storage conditions.

Table 4.2 Curcumin content in PDADMAC/PSS multilayer thin films coated apples and mango with at various number of film layers. Control: uncoated polyelectrolyte multilayer thin films.

Fruit	Curcumin content ($\mu g.g^{-1}$)					
	Number of (PDADMAC/PSS) layers					
	Control	4	8	12	16	20
Apples	1.63 ± 0.22	2.34 ± 0.32	3.54 ± 0.87	4.71 ± 0.32	5.61 ± 0.23	6.71 ± 0.38
Mangoes	1.61 ± 0.76	5.05 ± 0.36	6.18 ± 0.38	6.74 ± 0.39	7.13 ± 0.71	9.02 ± 0.97

CHAPTER V
CONCLUSIONS

LbL assembly technique was applied to construct multilayer thin films that can effectively load and release of models drugs. The multilayer thin films consisting of both strong polyelectrolyte and weak polyelectrolyte (PDADMAC, PSS, PSSMA, and Chi) were fabricated at different pH deposition. The structure of multilayer thin films and their stability could be flexibly tuned by varying the ionic strength and pH condition during the deposition process. The results show PDADMAC/PSS multilayer thin films were stable at all pH range while PDADMAC/PSSMA and Chi/PSS multilayer thin films exhibit a novel pH-dependent polymer matrix. By controlling parameters, multilayer thin film could be used as polymer matrix in order to use as films reservoir for loading and released model drugs. To render the films bioactive, model drugs (curcumin, diclofenac and gentian violet) were loaded into multilayer thin films by different interaction behaviors. The amount of drugs loading could be controlled by changing the number of layers as well as the loading time in the film. Our study showed that the loading of drugs in multilayer thin films was different depending on of solvent solubility, pH of drug solution and the surfaced charged of drug-outer layer of multilayer thin films. One of the most interesting in this study is that it is possible to tune the release of model drugs from multilayer thin films by controlling the polymer matrix disassembly and changing release medium (pH and ionic strength) under physiological condition. The release of model drugs from PDADMAC/PSS multilayer thin films is mainly due to the diffusion controlled. Oppositely, drugs release from PDADMAC/PSSMA and Chi/PSS is attributed to both of diffusion controlled and decomposition controlled, depending on the pH of deposition. On the basis of these results, our approach could provide a fundamental for designing the controlled drug delivery coating on a variety of substrates with the attributes of LbL self assembly method.

The multilayer thin film was designed to prolong release of hydrophobic drug from polyacrylic substrates and polymer matrix. Chi/Alg and Chi/PAA were construction via LbL assembly technique in order to use as blocking films

comparison to the traditional PDADMAC/PSS multilayer thin films. Thickness, roughness and stability/decomposition of blocking films were measured by uv-vis spectroscopy and AFM measurements. Curcumin, as hydrophobic drug, was loading into uncoated polyacrylic substrate displayed the different release behavior depending upon the solvent composition medium. It was found that the ratio of 20/80 of water/ethanol reached the maximum solvent composition that can release curcumin out of polyacrylic substrate within short time. Suppression of the initial burst release and prolonging the duration of release of the curcumin was controlled by changing the blocking films types and number layers to hinder release. The results demonstrated the applicability of obtaining hydrophobic domains of blocking films for extend the release of solvent medium. This could potentially be applied as a bio-film coating to prevent organic solvent release of bioactive molecule in term of biomedical devices and other biomedical implants. Additionally, there is a significant reduction in the release of curcumin in buffer pH 7.4, which it is reasonable to assume the present of blocking films. The designed Chi/Alg blocking films was found to be the best efficient in order to prolong release of curcumin from polymer matrix due to their high stability of their structure in buffer pH 7.4.

LBL assembly technique was also developed to edible fruit coating application. PDADMAC/PSS multilayer thin films-based coated fruit shown significant improved the hydrophilicity of outer surface of apples and mangoes. In addition, multilayer thin films-based coatings demonstrated their capabilities to carry high concentration of curcumin as a function of number of layers. The results from this study could be used as a foundation for the future study of multilayer thin films containing bioactive molecule for fruit-coating. However, future studies to fully understand the properties such as moisture and gas barrier of such coating at different polyelectrolyte condition are necessary.

In summary, it is reasonable to prove that the LbL self assembly technique can applied to use as bio-coating material in bionanotechnology areas such as drug delivery, biomedical device and edible film coating.

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APPENDIX

A. SCHOLARSHIP

2008 – 2010	The Thailand Graduate Institute of Science and Technology (TG-55-09-51-034D), National Science and Technology Development Agency
2010-2011	Chulalongkorn Graduate school Thesis Grant Graduate school, Chulalongkorn University
2010	Conference Grant for Ph.D. student Graduate school, Chulalongkorn University

B. CONFERENCES AND MEETINGS

Poster Presentation;

Kittitheeranun P, Dubas S T, Sanchavanakit N. The Improvement of Cell adhesion *In Vitro* Study on Substrate Via Layer by-Layer Nanoassembly Technique. The 1st Surface Engineering Exhibition (King Mongkut's University of Technology Thonburi, Bangkok, Thailand, February 8, 2008), p 31 (*Poster, 3rd Award).*

Kittitheeranun P, Dubas S T, Sanchavanakit N, Sajomsang W. Layer-by-Layer Assembly Multilayers Thin Films Containing Model Drugs: Loading and Release Behavior. Chulalongkorn-Inha Joint Symposium on Advanced Polymeric Nanomaterials (Pathumwan Princess Hotel, Bangkok, Thailand, July 10, 2010)

P. Kittitheeranun, S.T. Dubas, N. Sanchavanakit and W. Sajomsang. Loading and Release of Lipophilic Drug from Self Assembly Multilayer Thin Films**.** The 10th International Conference on Nanostructured Materials. (La Spienza University, Rome, Italy, September 13-17, 2010)

Oral Presentation;

Kittitheeranun P, Dubas S T, and Potiyaraj P. Preparation of Gelatin Thin Films for Cell Adhesion Improvement. The 2nd mathematics and physical sciences graduate congress (MPSGC), (National University of Singapore, Singapore, December 11-15, 2006), p 65.

C. LIST OF PUBLICATIONS

1. **Kittitheeranun, P**., Potiyaraj, P., Bunaprasert , T., Sanchavanakit, N., Dubas, S. T. Improved L-929 Cell Growth from self assembled PDADMAC/Gelatin thin films. *Journal of Metals, Materials and Minerals*, 18 (1), 2007, 39-45.
2. Dubas, S. T., **Kittitheeranun, P**., Rangkupan , R., Sanchavanakit, S., Potiyaraj, P. Coating of Polyelectrolyte Multilayer Thin Films on Nanofibrous Scaffold to Improve Cell Adhesion. *Journal of Applied Polymer Science*, 114, 2009, 1574-579.
3. **Kittitheeranun, P**., Sanchavanakit, N., Sajomsang, W., Dubas, S. T. Loading of Curcumin in Polyelectrolyte Multilayers. *Langmuir*, 26 (10), 2010, 6869-873.

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PUBLICATIONS

1. **Kittitheeranun, P**, Potiyaraj, P., Bunaprasert , T., Sanchavanakit, N., Dubas, S. T. Improved L-929 Cell Growth from self assembled PDADMAC/Gelatin thin films. *Journal of Metals, Materials and Minerals*, 18 (1), 2007, 39-45.
2. Dubas, S. T., **Kittitheeranun, P**., Rangkupan , R., Sanchavanakit, S., Potiyaraj, P. Coating of Polyelectrolyte Multilayer Thin Films on Nanofibrous Scaffold to Improve Cell Adhesion. *Journal of Applied Polymer Science*, 114, 2009, 1574–1579.
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