ตัวกรองเมมเบรนดัดแปรด้วยพอลิอิเล็กโทรไลต์มัลติเลเยอร์เพื่อเพิ่มความเข้มข้นอัลบูมิน

นางสาวอภิสราณัฐ สังขออง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปีการศึกษา 2556

MEMBRANE FILTER MODIFIED BY POLYELECTROLYTE MULTILAYERS FOR ALBUMIN PRECONCENTRATION

Miss Apisaranut Sungkaong

A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	MEMBRANE FILTER MODIFIED BY POLYELECTROLYTE MULTILAYERS FOR ALBUMIN PRECONCENTRATION
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Field of Study	Petrochemistry and Polymer Science
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อภิสราณัฐ สังขออง : ตัวกรองเมมเบรนดัดแปรด้วยพอลิอิเล็กโทรไลต์มัลติเลเยอร์เพื่อเพิ่มความเข้มข้นอัลบูมิน (MEMBRANE FILTER MODIFIED BY POLYELECTROLYTE MULTILAYERS FOR ALBUMIN PRECONCENTRATION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร.ลักษณา ดูบาส , อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ดร.มนพิชา ศรีสะอาด , 100 หน้า.

เทคนิค layer-by-layer ถูกนำมาใช้ดัดแปรพื้นผิวของเมมเบรนเซลลูโลสอะซีเตต (CA) ด้วยพอลิอิเล็กโทรไลต์มัลติเลเยอร์เพื่อใช้เป็นตัวกรองเมมเบรนสำหรับเพิ่มความเข้มข้นอัลบูมินที่ความเข้มข้นต่ำ ความหนาของมัลติเลเยอร์ ลักษณะทางกายภาพ และเสถียรภาพของฟิล์ม พอลิอิเล็กโทรไลต์มัลติเลเยอร์จะถูกประเมินผลและตรวจสอบโดยควบคุมปัจจัยที่มีผลต่อการสร้างฟิล์มบางพอลิอิเล็กโทรไลต์มัลติเลเยอร์ เช่น ชนิดของพอลิอิเล็กโทรไลต์ พีเอชของสารละลายพอลิอิเล็กโทรไลต์อ่อน ความเข้มข้นของสารละลายเกลือ จำนวนชั้นของมัลติเลเยอร์ พบว่าคู่พอลิอิเล็กโทรไลต์ที่เหมาะสมในการสร้างฟิล์มบาง ได้แก่ พอลิไดแอลิลไดเมทิลแอมโนเนียมคลอไรด์ (PDADMAC) กับพอลิสไตรีนซัลโฟเนต (PSS) เป็นพอลิอิเล็กโทรไลต์ที่การแตกตัวไม่ขึ้นกับค่าพีเอช และคู่พอลิไดแอลิลไดเมทิลแอมโนเนียมคลอไรด์ กับพอลิสไตรีนโคมาเลอิกแอซิด (PSSMA) เป็นพอลิอิเล็กโทรไลต์ที่การแตกตัวขึ้นกับค่าพีเอช ณ พีเอช 5.5 เป็นพีเอชที่ดีที่สุดในการสร้างชั้นฟิล์มบาง PDADMAC/PSSMA ในการติดตามการโตของมัลติเลอร์ฟิล์มบนเมมเบรนใช้เทคนิค UV-Vis, ATR-FTIR, และ SEM จำนวนชั้นที่เหมาะสมสำหรับเคลือบบนตัวกรองเมมเบรนสำหรับระบบ PDADMAC/PSS และ PDADMAC/PSSMA คือ 13 ชั้นซึ่งมี PDADMAC เป็นชั้นนอกสุด เมมเบรนที่ถูกดัดแปรพื้นผิวจะถูกนำไปใช้กรองสารละลาย bovine serum albumin (BSA) ความเข้มข้น 1 ppm ที่พีเอช 7.4 โดยใช้ syringe pump จากผล ATR-FTIR แสดงว่า BSA สามารถดูดซับบนฟิล์มบางได้ทั้ง PDADMAC/PSS และ PDADMAC/PSSMA โดยอาศัยแรงดึงดูดระหว่างประจุ และปริมาณ BSA ที่ดูดซับบนฟิล์มบางจะเพิ่มขึ้นตามจำนวนชั้นของฟิล์มบาง นอกจากนี้ BSA ที่ดูดซับบนเมมเบรน PDADMAC/PSS และ PDADMAC/PSSMA จะถูกปลดปล่อยโดยใช้สารละลายซิตริก-ซิเตรตบัฟเฟอร์พีเอช 3 และในการตรวจวัดเชิงปริมาณโดยใช้ Bicinchoninic acid assay (BCA) และ urine test strip โดยความเข้มข้นของ BSA หลังผ่านกระบวนการกรองด้วย PDADMAC/PSS และ PDADMAC/PSSMA พบว่ามีความเข้มข้นเพิ่มขึ้นจาก 1 ppm เป็น 29.6 ± 1.2 ppm และ 41.6 ± 1.2 ppm ตามลำดับ ประสิทธิภาพของตัวกรองเมมเบรน PDADMAC/PSS ถูกรบกวนจากสารรบกวนน้อยเมื่อเปรียบเทียบกับตัวกรองเมมเบรนที่เคลือบด้วย PDADMAC/PSSMA นอกจากนี้ความสามารถในการทำซ้ำของขั้นตอนการเพิ่มความเข้มข้นนี้ให้ค่าเบี่ยงเบนมาตรฐานเท่ากับ 1.3 ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์เท่ากับ 4.3% และค่าร้อยละของการคืนกลับเท่ากับ 90.8% ดังนั้นตัวกรองเมมเบรนนี้เป็นชุดเพิ่มความเข้มข้นที่ใช้งานง่าย ราคาถูก ซึ่งจะเป็นประโยชน์ในการตรวจสอบเบื้องต้นเพื่อการคัดกรองของผู้ป่วยโรคไตในระยะเริ่มต้น

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2556 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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5372541723: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE

KEYWORDS: POLYELECTROLYTE MULTILAYER/ MEMBRANE FILTER/ PROTEIN ADSORPTION/ ALBUMIN/ PRECONCENTRATION

APISARANUT SUNGKAONG: MEMBRANE FILTER MODIFIED BY POLYELECTROLYTE MULTILAYERS FOR ALBUMIN PRECONCENTRATION. ADVISOR: LUXSANA DUBAS, Ph.D., CO-ADVISOR: MONPICHAR SRISA-ART, Ph.D., 100 pp.

The layer-by-layer deposition technique (LbL) was used to modify the surface of cellulose acetate membrane (CA) as a membrane filter for preconcentration of trace amount of bovine serum albumin (BSA). By controlling the fabrication parameters, such as types of polyelectrolyte, pH of the weak polyelectrolyte, concentration of NaCl and the number of deposition layers, the thickness of multilayer films, morphology multilayer films and stability of multilayer films were evaluated and controlled. The suitable polyelectrolyte pairs are poly(diallyl dimethyl ammonium chloride) (PDADMAC) / poly(sodium 4-styrenesulfonate) (PSS) as a strong polyelectrolyte pair and poly(diallyl dimethyl ammonium chloride) (PDADMAC)/ poly (4-styrene sulfonic acid-co-maleic acid) copolymer (PSSMA) as a weak polyelectrolyte pair. The pH of 5.5 was found to be the best to fabricate PDADMAC/PSSMA. The polyelectrolyte multilayers (PEMs) film growth on the membrane was characterized using UV-Vis, ATR-FTIR and SEM. The optimum number of PEMs layers on the membrane filter of both PDADMAC/PSS and PDADMAC/PSSMA are 13 layers, in which PDADMAC is the outermost layer. The modified membranes were used to filter 1 ppm of BSA solution (pH 7.4). ATR-FTIR results showed that the BSA was adsorbed on the PDADMAC/PSS and PDADMAC/PSSMA via electrostatic interaction and the amount of adsorbed BSA increased with the numbers of coating layers of the thin film. The BSA adsorbed on the PDADMAC/PSS and PDADMAC/PSSMA membrane were then released using citric-citrate buffer solution pH 3.0 and measured quantitatively using the Bicinchoninic acid assay (BCA) and the urine test strip. Albumin concentration through the filtration process with PDADMAC/PSS and PDADMAC/PSSMA was found to be enhanced from 1 ppm to 29.6 ± 1.2 ppm and 41.6 ± 1.2 ppm, respectively. Furthermore, performance of PDADMAC/PSS membrane filter was found to be less interfered from interferences compared to PDADMAC/PSSMA membrane filter. The excellent repeatability of the preconcentration step was observed as evidenced by the standard deviation (SD) of 1.3, % relative standard deviation (%RSD) of 4.3 and % recovery of 90.8. Therefore, the proposed membrane filter would be useful as a simple and cost-effective preconcentration unit for screening test of renal failure at an early stage.

Field of Study : Petrochemistry and Polymer Science Student's Signature .....

Academic Year : 2013 Advisor's Signature .....

Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

PDADMAC	=	Poly(diallyldimethyl ammonium chloride)
PSS	=	Poly(sodium 4-styrenesulfonate)
PSSMA	=	Poly(4-styrene sulfonic acid-co-maleic acid, sodium salt)
PAH	=	Poly(allylamine hydrochloride)
BSA	=	Bovine serum albumin
SAM	=	Self assembled monolayer
PEM	=	Polyelectrolyte multilayer thin films
LBL	=	Layer-by-Layer technique
pI	=	Isoelectric point
pKa	=	Acid dissociation constant
min	=	Minute
mL	=	Milliliter
L	=	Liter
mM	=	Milli Molar
%RSD	=	Relative standard deviation percentage
SD	=	Standard deviation
SEM	=	Scanning electron microscope
AOAC	=	Association of Official Chemists

CHAPTER I

INTRODUCTION

The identification of proteins in biological fluids is important for clinical diagnostics. For example, the presence of albumin in urine (30-300 ppm) is used as a marker for the early stage of renal failure, that can be cured [1]. Therefore, the determination of albumin at low concentrations in urine is essential to prevent towards the end stage of renal failure.

The routinely used methods for determination of albumin are based on immunoassays, including radioimmunoassay [2], enzyme-linked immunosorbent assay (ELISA) [3], immunonephelometry, immunoturbidimetry, fluorescence immunoassay [4] and immunoresonance scattering spectral assay [5]. However, most of these existing methods have significant drawbacks. The radioimmunoassay is notorious for its health hazards, and thus is not widely used [6]. In addition, it is commonly known that most of the immunoassay-based methods consume large amounts of expensive reagents, especially the antibody specific for albumin. A strip test is normally used as a simple semi-quantitative test of albumin in the clinical. The strip test is based on the color change of an indicator in the presence of protein. The detection threshold of the strip test is in the range of 30-150 ppm. Accordingly, low concentrations less than 30 ppm could not be detected using the strip test [7]. In order to overcome this problem, a simple method for albumin preconcentration is required.

In recent years, the Layer-by-Layer (LbL) self assembly technique becomes more attractive and interesting in many applications due to its high potential to be applied for protein adsorption. This technique was discovered by Decher and co-workers in the 1990s [8]. The alternatively adsorption is well known as a fundamental process for fabricating polyelectrolyte assembled on a given substrate. The LbL self assembly technique can be summarized as alternative dipping of a substrate in a solution of both cationic and anionic polyelectrolytes following by a rinsing step at each dipping step, leading to multilayer thin film growth. Driving forces of LbL

assembly comprise of intermolecular forces, including electrostatic interaction [9], hydrogen bonding [10], hydrophobic interaction [11] and charge transfer interaction [12]. Multilayer thin films offer excellent characteristics, such as fine films tuning in terms of thickness, mechanics, chemistry and stability.

Several studies have shown that proteins can be adsorbed on either positively or negatively charged polyelectrolytes via electrostatic or hydrophobic interactions [13-16]. Since the fabrication of polyelectrolyte multilayers (PEMs) films were previously prepared on various substrates for protein adsorption studies, the PEMs could be possibly employed for protein preconcentration. In the light of information, there is no report on PEMs-modified membranes for protein preconcentration applications.

In this present study, we demonstrated that the cellulose acetate (CA) membrane surface modified with PEMs could be used as a membrane filter to preconcentrate trace amount of albumin, so one can use a urine strip test. The poly(diallyldimethyl ammonium chloride (PDADMAC) was chosen as a strong polycation, while poly(sodium 4-styrenesulfonate) (PSS) was selected as a strong polyanion. The studied weak polycations and polyanions were poly(allylamine hydrochloride (PAH) and poly (4-styrene sulfonic acid-co-maleic acid (PSSMA), respectively. The PEMs modified membrane can be used as a novel membrane filter for albumin preconcentration.

1.1 Research Objective

The objective of this work is to modify a cellulose acetate (CA) membrane as a membrane filter to preconcentrate the trace level of albumin.

1.2 Scope of the research

The scope of this research includes:

1) Study of the suitable PEMs film system and characterization. The PEMs films were fabricated on both quartz and PDMS substrates by controlling the fabrication parameters, such as types of polyelectrolyte, pH of the weak polyelectrolyte, concentration of NaCl, the number of deposition layers that

have influenced on stability of PEMs films and protein absorption on PEMs films.

2) Study protein adsorption on modified PEMs membrane filter. Parameters on protein adsorption, such as morphology PEMs films, charges of the outermost PEMs layer, the number of layers of PEMs, breakthrough of PEMs modified membranes and thickness of PEMs films.
3) Preconcentration of protein on modified membrane filter. The preconcentration parameters including time of releasing agent, the pH of albumin solution for protein preconcentration were investigated.
4) To determine the PEMs modified membrane filter performance for protein preconcentration.
5) Repeatability and recovery of modified PEMs membrane filters for protein preconcentration.

1.3 Benefits of Research

This research aimed to obtain a simple step and cost-effective for albumin preconcentration unit.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Layer by layer self assembly technique

There are many technologically important coating techniques on physical interactions between deposited molecules and substrates, such as spray coating, spin coating and dip coating. These processes allow the large amount of polymer solutions and time are required, the constructed film is not an ultrathin one, as well as producing a large area coating film is problematic. The layer-by-layer (LbL) technique is a method to fabricate multilayer sequential adsorption of oppositely charged polyelectrolytes deposited onto a charged substrate. The LbL has been considered to be one of the most efficient and also extremely high precision with regard to the thinness of the coatings and practical methods owing to its simplicity in fabrication steps as well as versatility in the choice of both depositing materials and substrates.

The LbL assembly technique was first introduced by Decher et al [8]. Afterwards, the number of publications dealing with the LbL multilayer films expanded dramatically, ranging from the basic principles addressing LbL deposition mechanism to numerous potential applications for the last two decades [17], as shown in Figure 2.1. This methodology is easy because it requires less equipment and has a low cost benefit as the price of polymer and equipment is low. In addition, the major reasons for this interest are their ease of processing, variety of materials which can be incorporated into the assembly system, and the versatility of substrates. Polyelectrolyte multilayer thin films can be grown on any substrate, such as glass slide, quartz, silicon wafer, poly(dimethylsiloxane) (PDMS) and membrane. Accordingly, polyelectrolyte multilayers based on LbL self assemble technique offer several applications, such as optical devices, optical sensors [18] and biomedical applications [17]. Since fabrication of polyelectrolyte multilayers allows

polyelectrolyte solutions coming into contact with a substrate, most common methods including dip-coating, deposition on colloids, or spin-coating. Besides, a variety of materials, including polymers, metals, fibers, glass and particles, can be used as a substrate for PEMs fabrication. Therefore, polyelectrolyte multilayers are relatively easy to fabricate onto a variety of substrates of virtually any material, size and shape. This makes PEMs superior to be used in various applications compared to other coating techniques.

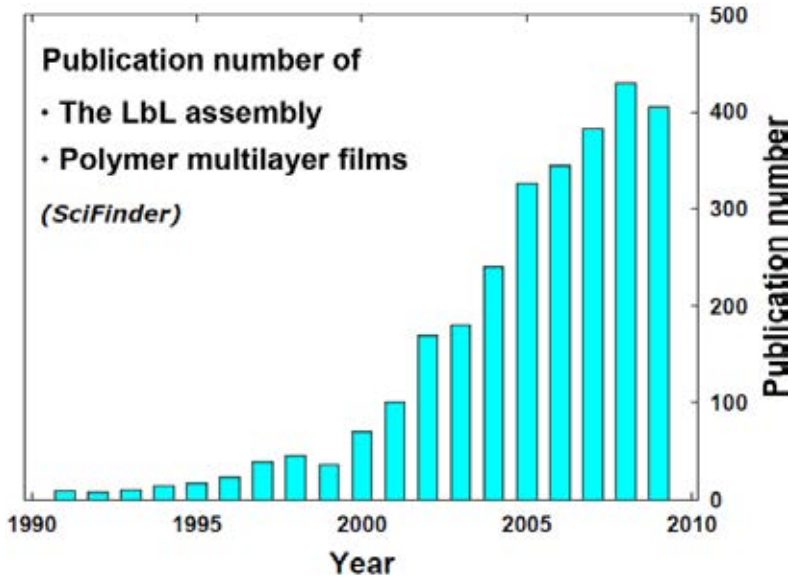


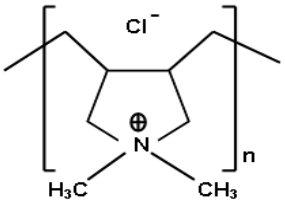
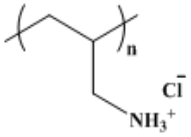
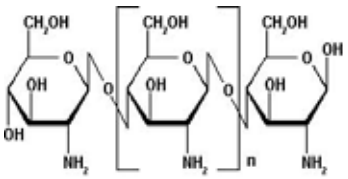
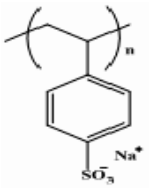
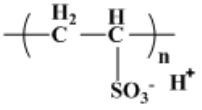
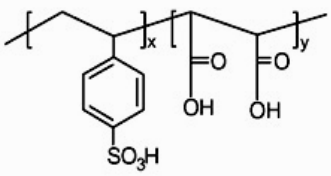
Figure 2.1 The chronological trend in the number of publications under the title of _the LbL assembly‘ and _polymer multilayer films‘ of SCI journals searched by SCI Finder [*November, 2010*].

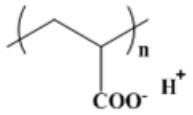
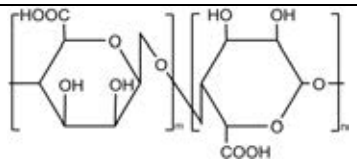
2.1.1 Polyelectrolyte

A polyelectrolyte multilayer thin film is formed by depositing alternately layers of anionic and cationic polyelectrolytes. Polyelectrolytes can be classified into many groups, such as cationic/anionic, synthetic/natural and strong/weak polyelectrolytes. Strong polyelectrolytes are dissociated into macro-ion and counterions in aqueous solutions in a total pH range between 0 and 14 [19]. For instance, poly(diallyldimethyl ammonium chloride) (PDADMAC) and poly(sodium 4-styrenesulfonate) (PSS) are known as strong polyelectrolytes. Weak

polyelectrolytes are dissociated into a polyion-counterion system only in a limited pH range. For example, poly(acrylic acid) is an undissociated polyacid in the acidic range while poly(ethylene imine) is an undissociated polybase in an alkaline range. Table 2.1 shows polyelectrolytes and their chemical structures.

Table 2.1 Polyelectrolytes and their chemical structures.

Chemical structure	Name	Ionic type	Classification
	Poly(diallyldimethyl ammonium chloride)	cationic	strong
	Poly(allylamine hydrochloride)	cationic	weak
	Chitosan	cationic	weak
	Poly(sodium 4-styrene sulfonate)	anionic	strong
	Poly(vinyl sulfonic acid)	anionic	strong
	Poly (4-styrene sulfonic acid-co-maleic acid)	anionic	weak

	Poly(acrylic acid)	anionic	weak
	Alginate	anionic	weak

Moreover, a special class of polyelectrolytes and polyampholytes present both anionic and cationic groups covalently bound to macro molecules, such as proteins and maleic acid–diallylamine copolymers, as shown in Figure 2.2.

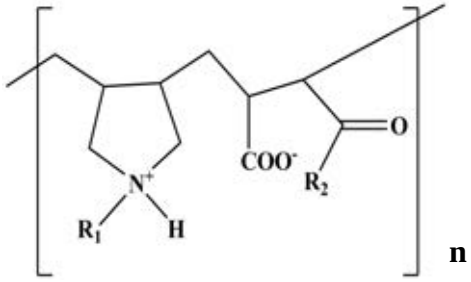


Figure 2.2 Chemical structure of a maleic acid–diallylamine copolymer [20].

2.1.2 Fabrication of polyelectrolyte multilayer (PEMs) thin films

Polyelectrolyte multilayer films fabricated via Layer-by-Layer (LbL) deposition are currently used to modify the surface properties of materials. The LbL process is based on alternating adsorption of a cationic polyelectrolye and an anionic polyelectrolyte, as shown in Figure 2.3. First, a charged substrate is dipped into a polyelectrolyte solution which has an opposite charge to the substrate, is called first layer of PEMs. In few first layer of thin film require a strong adsorption for anchoring the polyelectrolyte on the surface. The various of strong polyelectrolyte can be used as the primer to form a stronger anchor adsorption [21] such as PDADMAC/PEI and PDADMAC/PSS. Second, the substrate is rinsed during the PEMs formation for each

layer in order to remove the excess polyelectrolyte. A rinsing step is very important to PEMs construction because it can affect PEMs surface morphology. Moreover, water or buffer solution can be used as rinsing reagents. Third, the first layer of PEMs on substrate is dipped into a polyelectrolyte solution which has an opposite charge to the outermost layer of PEMs, is called second layer of PEMs and is rinsed again using water or buffer solution. The dipping cycle was repeated until the desired number of layers.

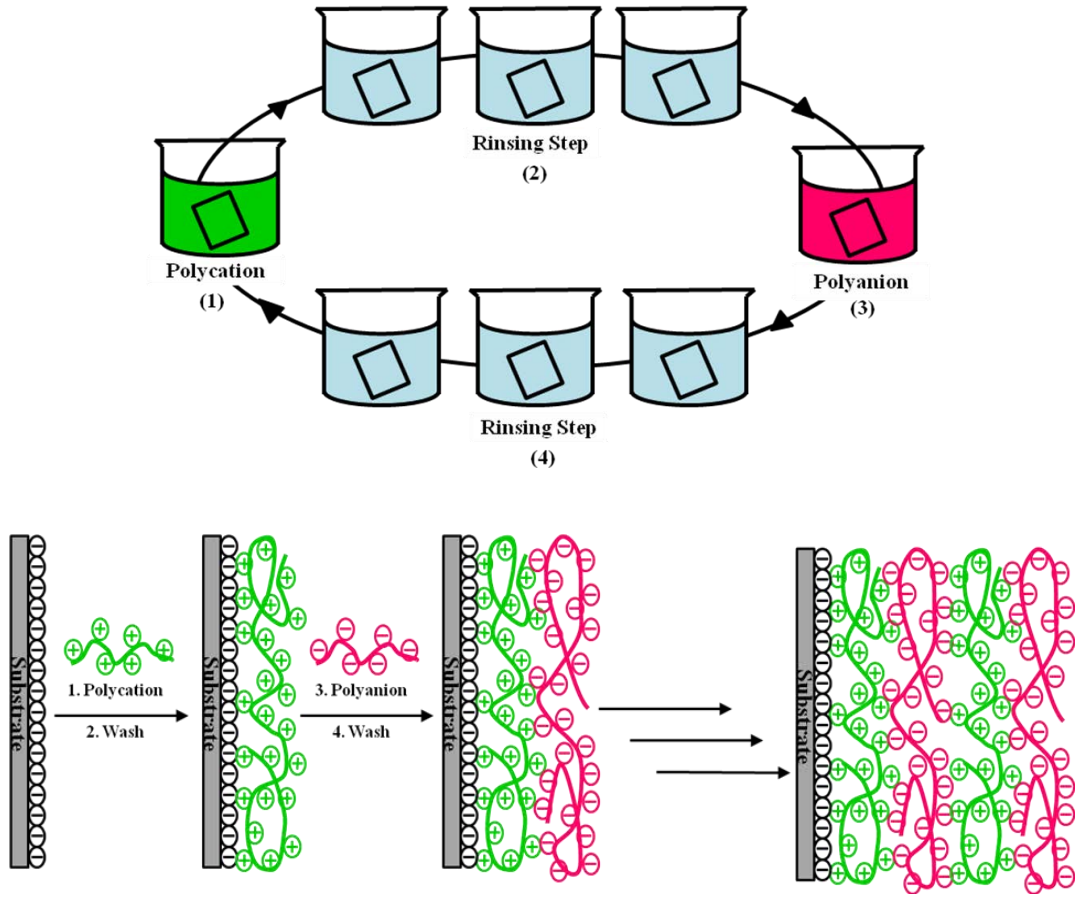


Figure 2.3 Schematic of the electrostatic self-assembly of PEMs.

2.1.3 Parameters affecting the growth of PEMs

Several parameters that control the film build-up to the precise structure of each layer depends on a set of controlled parameters, such as salt concentration,

number of layers, pH, or surface charge of PEMs, which are major parameters. In addition, polyelectrolyte concentration and deposition time affect the growth of PEMs, but the effect is minor.

2.1.3.1 Effect of NaCl concentration

The salt concentration in a polyelectrolyte solution affects the ionic strength of the solution. Different ionic strengths cause the growth of polyelectrolyte multilayer differently. The effect of salt concentration to polyelectrolyte multilayer system is related to an electrostatic theory [22]. When adding salt into the solution, salt ions induce more adsorption of polyelectrolyte on the surface, resulting in a thicker layer. At the same number of layer, a linear relationship between the thickness of PEMs film and salt concentration was observed when using salt concentration ranging from 0.01 M to 2 M (Figure 2.4) [23]. When adding salt into a polyelectrolyte solution, the thickness of each polyelectrolyte layer was increased because polyelectrolytes adsorbed as coils conformation at high salt concentration conditions [24].

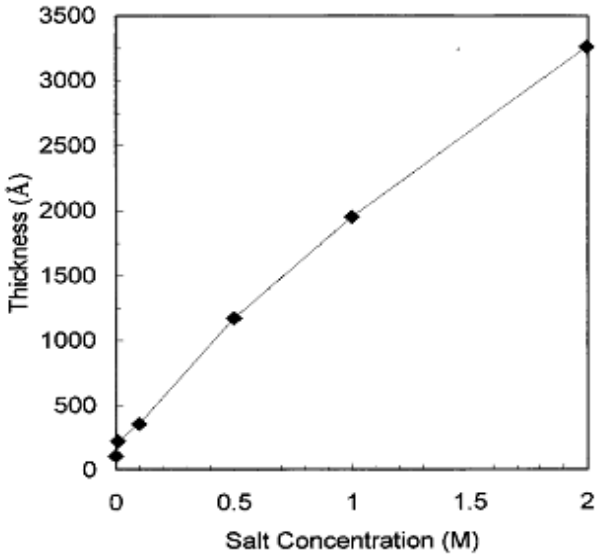


Figure 2.4 The relationship between film thickness of PDADMAC/ PSS at 10 layer pairs and salt concentration [23].

2.1.3.2 Effect of number of layers

The thickness of multilayer film is based on the number of multilayer films on the substrate surface. The thickness of polyelectrolyte multilayer is found to increase with the number of layer. The coating on the first few layers on the substrate is usually thinner than other layers. It is due to the lower electrical charge of the surface in relation to the polyelectrolyte chains. After the first few layers, the fabrication process is stable and the properties of the films are independent of the feature of the substrate surface [25].

2.1.3.3 Effect of pH

The adsorption of strong polyelectrolyte is independent of the pH polyelectrolyte solution because it can dissociate in every pH value. The pH of solution affects the ionization degree of the functional groups on weak polyelectrolytes, resulting in different adsorption behaviors. Each of weak polyelectrolyte has different *p*Ka values. Thus, the structure of weak polyelectrolyte films on substrates can be tuned by controlling pH values [22, 26].

2.1.3.4 Effect of polyelectrolyte concentration

The polymer concentration affected to adsorption only the early stage because it is limited by the number of charges on the former layer. Even through the polymer concentration is increased, it has no effect on adsorption. This occur from the polymer are adsorbed to the surface sufficiently and the like-charged electrostatic barrier can prevent the approach of additional polymer. Previous study reported that the optimum concentration of PADMAC and PSS are 10 mM, resulting in suitable film thickness [27]. The result is shown in Figure 2.5.

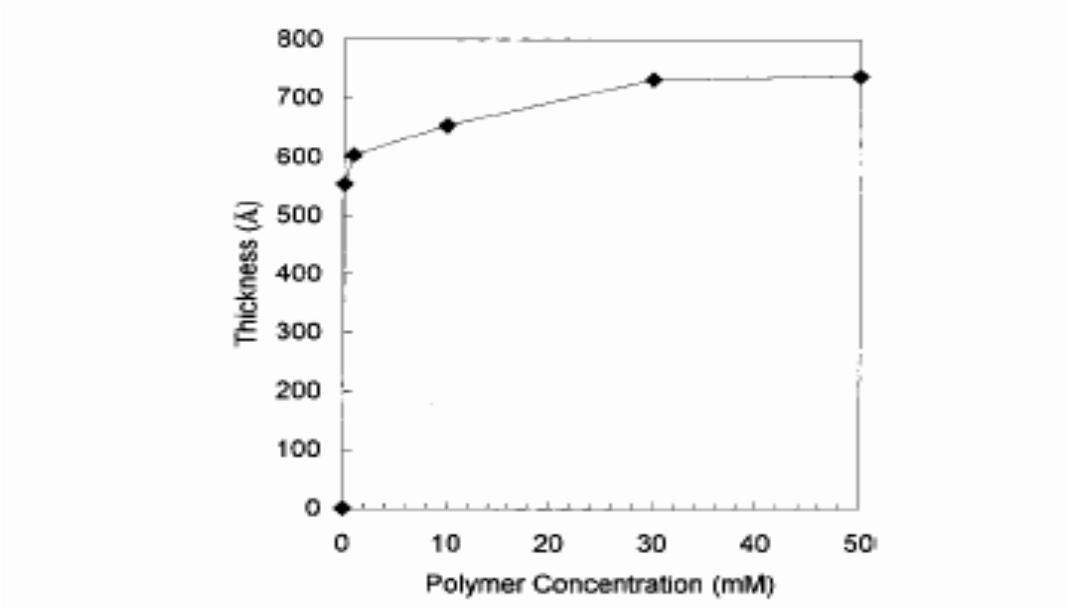


Figure 2.5. The relationship between film thickness of PDADMAC/ PSS at 5 layer pairs and polymer concentration [23].

2.1.3.5 Effect of deposition time

The deposition time per layer is a minor parameter for controlling the growth of PEMs. The mechanism of monolayer formation includes two steps. The first step is diffusion of ions to the substrate surface and the second step is binding of the ions to the surface. The relationship between deposition time as a function of thickness in monolayer adsorption of PSS/PDADMAC as shown in Figure 2.6. The high slope in the early stage was observed at first few minutes, which represent to high flux on the surface. The number of equivalent monolayers is higher in first few minutes, representing the high flux on the surface.

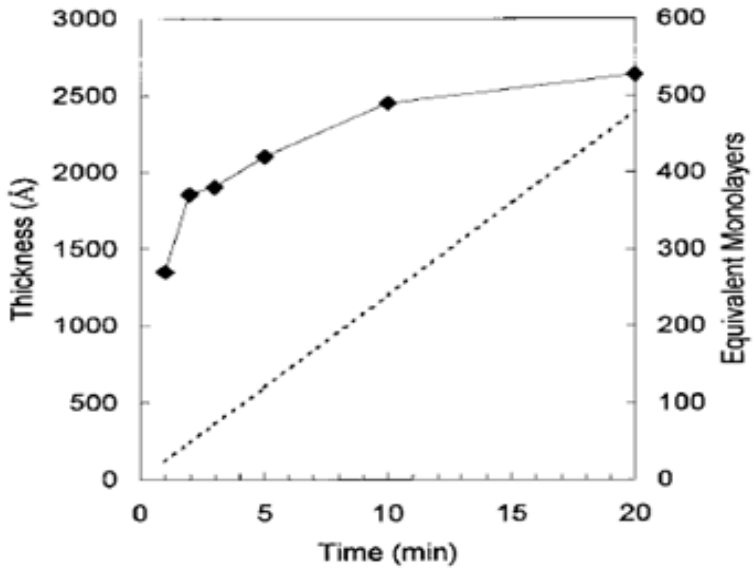


Figure 2.6 The relationship between film thickness of PDADMAC/ PSS at 10 layer pairs and deposition time [23].

2.2 Protein adsorption

Protein adsorption can be defined as adhesion or sticking of proteins on a variety of surfaces. Studied proteins are generally serum proteins, enzymes, antibodies, and foreign antigens [28, 29].

Proteins, one or more linear polypeptide chains of amino acids, contain a substantial number of intramolecular hydrogen bonds. Most hydrogen bonds are formed between amide and carbonyl groups of the backbone. The arrangement of protein structures affects the polarity of side chain amino acids. Structures of protein have four different levels: *Primary structure* is the sequence of amino acid in the peptide. The *secondary structure* is repetitive structure units such as α-helices and β-sheets. The *tertiary structure* is the three-dimensional arrangement of the polypeptide. The *quaternary structure* is the combination of independent tertiary structures in proteins with more than one polypeptide chain [30]. For protein adsorption process, proteins re-arrange their structures on a substrate surface. Changes in conformation of

proteins can occur immediately during adsorption or slowly over time after the protein has attached to the surface [28]. An example of well-known model proteins used for protein assay is bovine serum albumin (BSA). The BSA has several isomeric forms depending on pH values. The conformation of BSA can undergo reversible conformation [31, 32]. At neutral pH, the conformation of BSA is in its common physiological state, referred as a normal form (N-form). At the pH values below 4.3, the conformation is changed from the normal conformation (N-form) to the fast conformation (F-form). In the very acidic solution (pH< 2.7), the conformation is changed from the F-form to the Expanded conformation (E-form). For pH values above 8, the N-form is changed to the Basic conformation (B-form). The conformation was transformed to another aged form (A-form). The conformational transition is shown in Figure 2.7 [31].

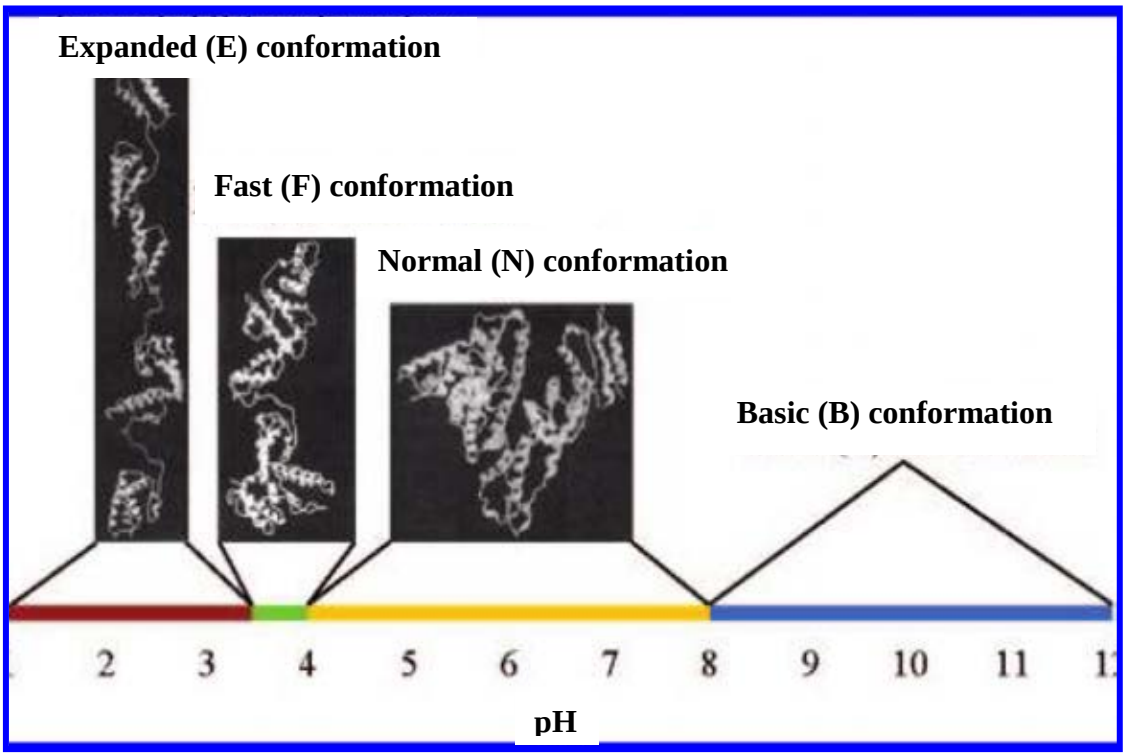


Figure 2.7 BSA conformation at difference pH values [33].

The interaction forces between protein molecules and polymer surface can be classified as follows: 1) Van der Waals interactions, 2) hydrophobic interactions, 3) electrostatic (or ionic) interactions and 4) hydrogen bonding [28, 34].

1) *Van der Waals interactions*: This interaction force is operative over small distances, only when water has been excluded and the two nonpolar groups come close to each other. Lewin's calculation showed that the Van der Waals interaction is negligible compared with the force involving in the entropy increases, i.e., hydrophobic interaction.
2) *Hydrophobic interaction*: It plays a major role in protein adsorption phenomena on hydrophobic surfaces and around of its isoelectric point [24, 35]. Adsorption of proteins due to hydrophobic interaction on hydrophobic surfaces is greater than that on hydrophilic surfaces.
3) *Electrostatic (or ionic) bonding*: The charges of proteins depend on the pH of solution. Therefore, the electrostatic interaction plays an important role when the surfaces possess some charges. Moreover, the electrostatic interaction has a significant influence on the structural stability of the protein molecule. Effect of ionic strength on protein-surface electrostatic interactions has been studied [36]. The ionic strength influences protein-protein, surface-protein and intramolecular interactions. Protein adsorption via electrostatic attractions decreases with the increase of salt because electrostatic affinity between surface and protein molecules is decreased.
4) *Hydrogen bonding*: Protein adsorption can occur through hydrogen bonding. Hydrogen bonding is formed between hydroxyl-carbonyl or amide-carbonyl radicals or amide-hydroxyl bonds are also formed in protein adsorption. Carboxyl radicals are also important in protein adsorption by hydrogen bonding, of low and moderate pHs [36].

2.3 Literature reviews

Polyelectrolyte multilayers (PEMs) have been proposed to modify the surface of materials by sequential adsorption of positively and negatively charge polyelectrolyte on various substrates. Basic method for the preparation of polyelectrolyte multilayers was first demonstrated in 1991 by Decher and Hong [1] and developed continuously since then, the self assembled polyelectrolyte multilayers have proven to be versatile materials. Parameters affecting the coating and characteristics of PEMs film were studied and reported by Dubas et al. [23] The studied parameters were types of polyelectrolyte, concentration of polyelectrolyte, concentration of sodium chloride, pH value of polyelectrolyte, number of layers, and deposition time. Types of polyelectrolyte were found to have a strong influence on the property and morphology of PEMs. Therefore, many studies have been conducted to understand the effect of type of polyelectrolyte on the protein adsorption.

Protein could be adsorbed on the PEMs film via many forces. The electrostatic interaction was found to be a major key on this phenomenon. The charged protein can be adsorbed on the opposite charged of outermost polyelectrolyte multilayer. Therefore, charge of surface and charge of protein molecule can affect the protein adsorption on the multilayer surface. Poly(L-lysine), PLL and poly(L-glutamic acid)-g-poly(ethylene glycol), PGA-g-PEG on silica (SiO_2) were used by Boulmedais et al. to improve the protein adsorption and anti-microbial protection [37]. The outer layer of poly-L-lysine, which is positively charged surface, adsorbed protein much more than the outer layer of poly(L-glutamic acid)-g-poly(ethylene glycol) which is negative charged surface. The same trend of protein adsorption on surface was found by Salloum and Schlenoff [14]. Poly(diallyl dimethyl ammonium chloride) (PDADMAC) and poly(styrene sulfonate) (PSS) were used to fabricate PEMs on silicon wafer in this study. Results shown that negative charged BSA was adsorbed on the positively charged of PDADMAC outermost layer via electrostatic interactions.

In addition, it was found that adsorption of protein on like-charged substrate occurred through nonelectrostatic interaction, but less than electrostatic interactions. Moreover, Salloum and Schlenoff, found that the thickness of multilayer plays an

important role in adsorption process because the PEMs acted as a matrix to load proteins.

Watanabe et al [15]. reported that PDADMAC/PSS film built at high ionic strengths can adsorb BSA better than the film built at low ionic strengths. This is because of the effect of NaCl that changes not only the polymer conformation, but also the charge density. The ionic strength was increase resulting decreases the electrostatic barrier between the polymer chain already adsorbed on the surface and the polymer chain originating from the solution, and this promotes the adsorption of the polyelectrolyte [38].

Shen et al [39]. reported polyelectrolyte multilayers, PDADMAC/PSS assemblies, fabricating on polystyrene (PS) multiwell plates to be used as immunosensor. The PEMs modified plate was then coated with ovalbumin, an excellent blocking reagent, to inhibit nonspecific binding of the target antigen. It was found that this approach showed the highest sensitivity of antigen detection, compared to the conventional PS plate in the ELISA system.

From previous work, several studies have shown that PEMs were successfully prepared on various substrates and proteins can be adsorbed on either positively or negatively charged outermost of polyelectrolyte multilayers film (PEMs) via electrostatic or hydrophobic interactions [13-15]. In addition, the fabrication of PEMs on substrate was applied such as protein adsorption. Therefore, PEMs could be possibly employed to adsorbed protein for protein preconcentration. So far, there is no report of PEMs-modified membranes for such an application.

CHAPTER V

EXPERIMENTAL

3.1 Materials and chemical

3.1.1 Substrates

1. Quartz slides (50 × 25 mm Electron Microscopy Sciences, Hatfield, PA)

2. Silicon wafer (Novel electronic materials, Carrollton, TX)

3. Poly(dimethylsiloxane) (PDMS) (30 x10 mm Dow Corning, USA)

4. Cellulose acetate membrane (25 mm and 47 mm diameter and 0.2 μm pore size, Vertical Chromatography, Thailand)

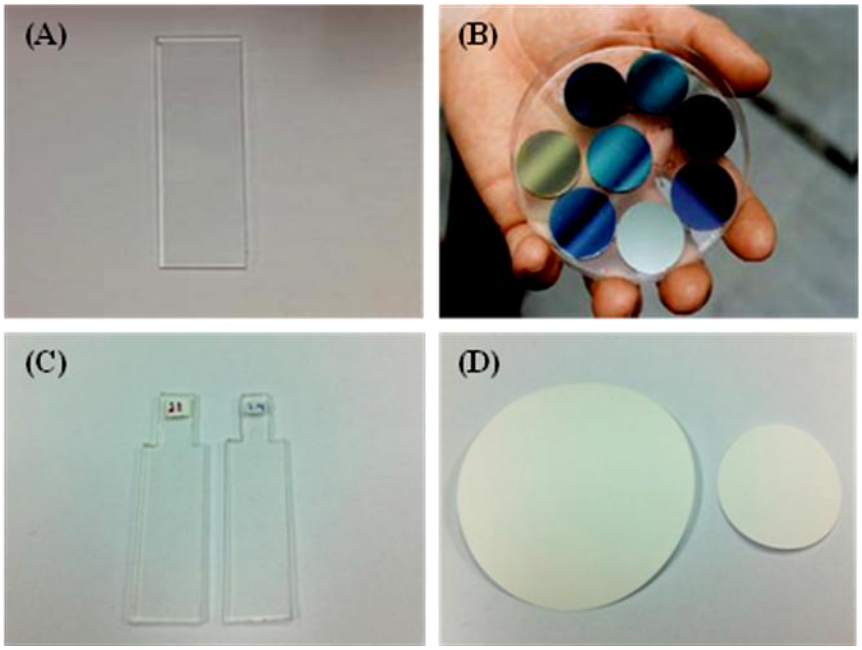


Figure 3.1 (A) quartz slides (B) silicon wafer (C) poly(dimethylsiloxane) films and (D) cellulose acetate membrane**.**

3.1.2 Chemicals

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

Table 3.1 Chemical list

Chemicals	Supplier
1. Poly(diallyldimethyl ammonium chloride) (PDADMAC) medium molecular weight, 20 wt% in water, average MW ~200,000-350,000	Sigma-Aldrich
2. Poly(sodium 4-styrenesulfonate) (PSS), average MW ~70,000	Sigma-Aldrich
3. Poly(4-styrene sulfonic acid-co-maleic acid, sodium salt) (PSSMA), average MW ~20,000	Sigma-Aldrich
4. Poly(allylamine hydrochloride) (PAH), average MWM_w ~100000	Sigma-Aldrich
5. Bovine serum albumin, M_w 66 kDa	Sigma-Aldrich
6. Globulins human blood, M_w 155-160 kDa	Sigma-Aldrich
7. Sodium chloride, A.R. Grade	Carlo Erba Reagent
8. Potassium dihydrogen phosphate, A.R. Grade	Carlo Erba Reagent
9. Di-sodium hydrogen phosphate anhydrous, A.R. Grade	Carlo Erba Reagent
10. Potassium chloride, A.R. Grade	Carlo Erba Reagent
11. Tri-sodium citrate dihydrate	Carlo Erba Reagent
12. Citric acid anhydrous	Carlo Erba Reagent
13. Hydrogen peroxide 30%, for synthesis	Merck

14. Sulfuric acid 95-99%, A.R. Grade	Merck
15. QuantiPro BCA assay kit	Sigma-Aldrich
16. Coomassie Brilliant Blue G-250 (CBBG)	Acros
17. Urine strip test (Microalbumin strip)	Cybow
18. Ammonia solution 25%	Merck
19. Glucose	Sigma-Aldrich
20. DI and Mill-Q water	-

3.2 Apparatus

The apparatuses used in this study are listed in Table 3.2.

Table 3.2 Apparatus list

Apparatus	Company, model
1. UV-visible spectrophotometer	Hewlett Packard 8453
2.Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)	Nicolet 6700
3. Atomic Force Microscope	Nanoscope IV
4. pH meter	Precisa pH 900
5. Syringe pump	QIS, model NE1000
6. Syringe filter holder	Millipore
7. Syringe 50 mL	Nipro

3.3 Preparation of solutions

3.3.1 1 and 10 mM phosphate buffer solutions at pH 5.5, 6.2, 6.8, 7.4 and 11.0

10 mM phosphate buffer saline (PBS) solutions with various pH values were prepared as the stock solutions. To achieve pH of 5.5, 6.2, 6.8, 7.4 and 11.0, various ratios of di-sodium hydrogen phosphate anhydrous and potassium dihydrogen phosphate were weighed according to Table 3.3. Then, 8.00 ± 0.10 g sodium chloride and 0.20 ± 0.01 g potassium chloride were added into 800 mL of Milli-Q for controlling ionic strength. The solution was then adjusted to a desired pH value using either 0.1 M HCl or 0.1 M NaOH. Then, the final volume of 1 L was adjusted using deionized water to achieve a concentration of 10 mM PBS solution. The stock solutions were diluted 10 times with deionized water to achieve the final concentration of 1 mM phosphate buffer solution.

Table 3.1 Weight of di-sodium hydrogen phosphate anhydrous and potassium dihydrogen phosphate for preparing buffer solutions

pH	Weight (grams)	
	di-sodium hydrogen phosphate anhydrous	**potassium dihydrogen phosphate**
5.5	0.030 - 0.039	1.570 - 1.579
6.2	0.130 - 0.139	1.240 - 1.249
6.8	0.410 - 0.419	0.970 - 0.979
7.4	1.440 – 1.449	0.240 - 0.249
11.0	2.130 – 2.139	-

3.3.2 10 mM citric-citrate buffer solutions at pH 2.0 and 3.0

10 mM citric-citrate buffer solutions at pH 2 and 3 were prepared by mixing citric acid and tri-sodium citrate dehydrate with different weight ratios (Table 3.4). The buffer solutions were adjusted to pH 2 and 3 by either 10 mM HCl or 10 mM NaOH.

Table 3.4 Weight of citric acid and tri-sodium citrate dihydrate for preparing buffer solutions

pH	Weight (grams)	
	Citric acid	Tri-sodium citrate dihydrate
2.0	0.520 - 0.529	-
3.0	0.630 - 0.639	0.150 - 0.159

3.3.3. Bovine serum albumin in buffer solution

A 1 ppm bovine serum albumin solution in 1 mM phosphate buffer solutions was prepared by dissolving 100 ± 1 mg of BSA in a phosphate buffer at the desired pH. Then, the final volume of solution was adjusted to 100 mL using 1 mM phosphate buffer.

3.4.3 BSA standard solutions

BSA standard solutions were prepared following the BCA assay kit [40]. First, 1.9 mL of buffer solution was pipetted into a test tube and then 0.1 mL of the 1000 ppm protein standard (obtained from the assay kit) was added into the tube. The BSA concentration of this 2 ml solution is 50 ppm as a stock standard solution. The protein standard solutions were prepared with the concentration in the range of 0–40 ppm using the newly prepared 50 ppm stock protein standard.

3.3.5 Polyelectrolyte solutions

3.3.5.1 100 mM of polyelectrolyte solutions (the stock solution)

A 250 mL of 100 mM of polyelectrolyte solution was prepared as a stock solution for multilayer thin film preparation. The poly(diallyldimethyl ammonium chloride (PDADMAC) was chosen as a strong polycation, while poly(sodium 4-styrenesulfonate) (PSS) was selected as a strong polyanion. The weak polycations and polyanions were poly(allylamine hydrochloride (PAH) and poly (4-

styrene sulfonic acid-co-maleic acid (PSSMA), respectively. The weights of polyelectrolytes were listed in Table 3.5.

Table 3.5 Weight of polyelectrolytes for preparing stock solutions.

Polyelectrolytes	Weight (grams)
PDADMAC	20.19 ± 0. 10
PSS	5.15 ± 0.10
PAH	2.34 ± 0.10
PSSMA	8.60 ± 0.10

3.3.5.2 10 mM of polyelectrolyte solutions in 1 M NaCl solution

All 10 mM polyelectrolytes in 1 M NaCl were prepared in the same manner by pipetting 100 mL of a polyeletrolyte stock solution into a volumetric flask. NaCl (58.44 ± 0.10 g) was added in the solution. Then, the final volume of solution was adjusted to 1000 mL using either DI water or an appropriated buffer solution.

3.4 Fabricating substrates

3.4.1 Quartz slides and silicon wafers

To be able to monitor the growth of polyelectrolyte multilayer film, quartz slides and silicon wafers were used as fabricating substrates. The quartz slides were cleaned in the piranha solution (conc.H_2SO_4: 30% H_2O_2, 3:1) for 30 minutes [23], while the silicon wafers were cleaned in a hot ammonia solution (27% NH_4OH: 30% H_2O_2 : H_2O, 1:1:5) at a temperature of 60 °C [23]. Then, the substrates were rinsed with deionized water several times and dried by an air dryer.

3.4.2 Poly(dimethylsiloxane) (PDMS)

In order to identify a suitable PEMs system for protein absorption, a PDMS was also used as a fabricating substrate. Sylgard 184 PDMS oligomer was mixed with a curing agent at the ratio of 10:1 (w/w) to form a PDMS prepolymer mixture and degassed by a vacuum pump. The 10-12 g of the mixture was weighed and poured into a mold to control the thickness of PDMS. After curing at 65°C for 2 hours [41] and cooling at room temperature, PDMS substrates were peeled off and cut into a rectangular shape (1 cm × 3 cm). All PDMS pieces were immersed into methanol for 5 mins and then cleaned with magic tape. Then, the PDMS piece was immersed into 0.1 M NaOH for 15 mins before further usage.

3.5 Fabrication of Polyelectrolyte Multilayers (PEMs)

Polyelectrolyte multilayer thin film was coated on substrates by using the layer-by-layer electrostatic deposition technique. Cleaned substrates were dipped into a polycationic solution for 5 minutes. After the first dipping, the substrates were rinsed 3 times with DI water or an appropriated buffer solution (rinsing reagent). Accordingly, the surfaces of substrates were coated by a positive layer of polycation as the first layer. Then, the substrates were dipped into a polyanionic solution for 5 minutes and were rinsed 3 times with the rinsing reagent. The substrates with the first positive layer were coated with a negative layer of the polyanions as the second layer. The multilayer thin film of PEMs was dipped alternately until the desired number of layers.

3.5.1 Study the types of polyelectrolyte on PEMs build-up and protein adsorption

In the present study, types of PEMs both strong (PDDMAC/PSS) and weak (PDADMAC/PSSMA, PAH/PSS and PAH/PSSMA) polyelectrolytes were fabricated on quartz and PDMS. The fabricating condition was shown in Table 3.6.

Table 3.6 Experimental conditions used for the preparation of PEM films

Conditions	Concentration (mM)		NaCl Concentration (M)	pH
	Cationic polyelectroyte	Anionic polyelectroyte		
PDADMAC/PSS	PDADMAC 10 mM	PSS 10 mM	1	-
PDADMAC/PSSMA	PDADMAC 10 mM	PSSMA 10 mM	1	5.5
PAH/PSS	PSS 10 mM	PSS 10 mM	1	5.5
PAH/PSSMA	PAH 10 mM	PSSMA 10 mM	1	5.5

The adsorption of BSA on the PEMs modified PDMS occurred due to immersing the PEMs modified PDMS into 4 mL of 1 ppm BSA solution at pH 7.4. Adsorption of the protein was allowed to proceed at room temperature for 2 hours. Then, the studied substrate was washed with 10 mL of phosphate buffer at pH 7.4 to remove excess BSA and dried with a hair dryer.

In this study, the pH effect of the chosen weak polyelectrolyte solution on a fabrication of multilayers thin film was studied. The pH of weak polyelectrolyte was kept at various pH values of 2.0, 5.5 and 11.0 to fabricate PEMs.

3.5.2 Effect of NaCl on the formation of PEMs

In this research, we investigated the effect of ionic strength on preparation of multilayers thin films. Adjusting ionic strength of a solution was performed by adding salt into the polyelectrolyte solution to control the growth of PEMs. The ionic strengths were 0.5 M NaCl and 1 M NaCl. The absorbance value of the 12^{th} layer-PEMs modified quartz slide was collected.

3.6 Stability of PEMs

One concern in developing the polyelectrolyte multilayer films is their stabilities on the working pH. The stability of PEMs multilayers deposited on a substrate was evaluated by dipping into pH 7.4 PBS buffer solution for overnight. The FT-IR spectra of PEMs modified PDMS before and after dipping were compared.

3.7 Protein adsorption on PEMs modified cellulose acetate (CA) membrane

The similar fabrication process as described in section 3.5 was used to fabricate the PEMs on CA membrane.

3.7.1 The filtration of protein on PEMs membrane processes

PEMs modified and unmodified membranes were used to filter BSA solutions using a syringe pump. The flow rate was fixed at 1 mL/min. Experimental setup for the filtration is shown in Figure 3.2.

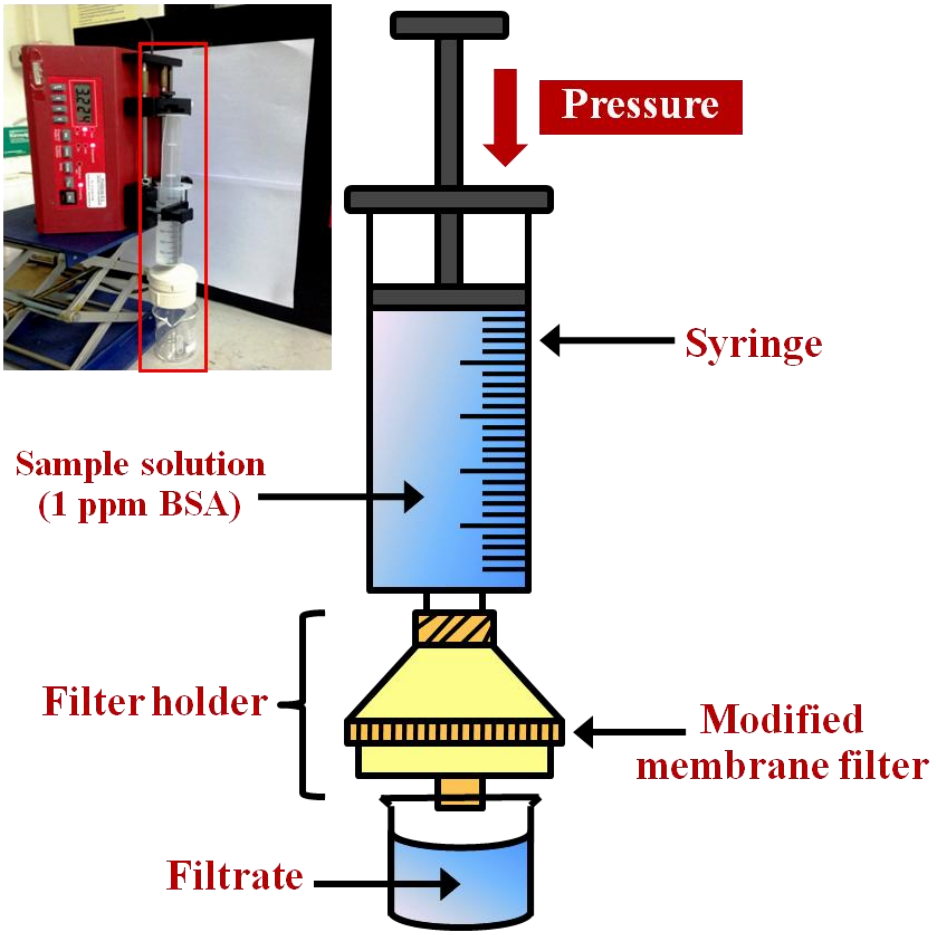


Figure 3.2 Scheme of protein adsorption on membrane filter process

3.7.2 Effect of charge of the outermost layer of PEMs and the number of deposition layers on protein adsorption

The chosen PEMs systems were fabricated on membrane with various numbers of layers. The odd layers are the polycations as the outermost layer, while the even layers are the PEMs film with polyanions as the outermost layer. The fabricated PEMs membranes were used to filter 1 ppm BSA solutions pH 7.4.

3.7.3 Breakthrough of PEMs modified membrane in protein adsorption

After the suitable PEMs system including types, number of layers was identified, the breakthrough or capacity of modified PEMs membranes was studied for adsorption of BSA. The modified membranes were used to filter 1 ppm BSA solution (pH 7.4) with various volumes ranging from 50-250 mL and 20-150 mL for 30 ppm BSA solution.

3.8 Characterization of PEMs thin film

3.8.1 Ultraviolet/Visible spectrophotometer

The build-up of PEMs was studied using UV/Vis spectrophotometer by monitoring the absorbance values at 227 nm.

3.8.2 Attenuated Total Reflectance Fourier transform Infrared Spectrometer (ATR-FTIR)

ATR-FTIR was also used to monitor the building up process and characterize the surface of substrates. For PDMS substrate, the measurement was performed only one position per a substrate. For CA filter membrane, the evaluation was performed at 3 different positions per a substrate on the studied surface. For protein adsorption study, the spectra of adsorbed BSA on the modified PEMs were subtracted with the spectra of the modified PEMs. Then, the total peak areas of amide I (1650 cm^{-1}) and amide II (1540 cm^{-1}) were measured using OMNIC PROGRAM.

3.8.3 Scanning Electron Microscope (SEM).

The surface morphology of PEMs modified and unmodified CA membrane was characterized by SEM using 3000x magnification.

3.8.4 Atomic Force Microscopy (AFM)

The multilayer films thickness was investigated using AFM measurements. The PEMs films were fabricated on silicon wafers using the same condition in the preparation of PEMs on PDMS substrates (in section 3.5) for 13 coating layers. The

thicknesses of PEMs films before and after being immersed into the BSA solution pH 7.4 for 2 hours were performed in air at ambient temperature using a tapping mode.

3.9 Protein preconcentration step

After the filtration process, the substrate was immersed in a releasing solution. In this study, citric-citrate buffer at pH3 was selected. Because at this pH, which is lower than the *p*I of the BSA (*p*I = 4.7), the BSA possessed positively charged. Therefore, the BSA will be desorbed from PEMs layer. After releasing process, the solution was quantitatively measured using BCA assay to quantify the amount of protein as described in section 3.9.1.

3.9.1 Quantitative measurement of protein

The content of BSA was quantified by Bicinchoninic acid assay (BCA) following the BCA assay kit [41]. Briefly, the 1 mL of protein solution was mixed with 1 mL of BCA working reagent and incubated at 60 °C for 1 hour. Then, the absorbance of mixing protein solutions was measured at 562 nm.

The protein standard solutions were prepared using the pH 3 buffer solution with the concentration in the range of 0–40 ppm as described section 3.3.4. A calibration curve of BSA solutions was plotted between net absorbance at 562 nm (Net A_{562}) and concentrations of protein [41]. The net A_{562} is calculated by subtracting the recorded A_{562} values of either the protein standards or unknown samples with the absorbance (A_{562}) of the blank (0 ppm). Then, the protein concentration of each protein sample was determined from the calibration curve.

3.9.2 Study of the suitable releasing time

The suitable soaking times to leach the albumin absorbed on the PEMs modified membranes were evaluated. The sample volumes used in this study were based on the volume found in the section 3.7.3. The studied times were 3, 5, 10, 15 and 20 min using 3 mL of pH 3 buffer solution.

3.9.3 Effect of pH of albumin solution for protein preconcentration

The modified membrane filters, including bare CA were used to filter 1 ppm BSA solution at various pHs (pH 5.5, 6.2, 6.8 and 7.4). The BSA volumes used in this study were based on the volume found in the section 3.7.3

The adsorption of 0.6 ppm immunoglobulin (IgG) solution, one of the interferences, on the PEMs-modified filter was also evaluated at pH 6.2, 6.8 and 7.4 using similar experiment condition as in the BSA study.

3.10 PEMs modified membrane filter performance: enrichment factor and interferences

Based on the results found in the previous section, the suitable PEMs system, the optimum releasing condition, the performance of our PEMs-modified membrane filter was evaluated.

From breakthrough study on PEMs, the optimum volumes of BSA with different concentrations (1 and 30 ppm) were pumped through the PEMs-modified membrane filter using a syringe pump as described in section 3.7.1. The amount of albumin adsorbed on the PEMs membranes was then released using an appropriate buffer and then measured using the BCA assay. The amount of albumin after the preconcentration process was calculated from the calibration curves and tested using the simple semi-quantitative urine test strip. The enrichment factor was calculated using the following equation:

$$\text{Enrichment factor} = \frac{\text{Concentration of BSA after the preconcentration process (ppm)}}{\text{Concentration of BSA before the preconcentration process (ppm)}}$$

The effect of interferences on the BSA preconcentration was also studied in this section. The concentrations of interferences were fixed at 0.6 ppm IgG, 300 ppm NaCl and 250 ppm glucose. The testing solution contained 1 ppm BSA solution and one type of interference at a time. The preconcentration, releasing steps were performed as described in section 3.9.2. The amount of IgG and NaCl was measured using BCA assay as described in section 3.9.1 while the amount of glucose was

measured using Coomassie Brilliant Blue G-250 (CBBG) assay. The 1 mL of 0–40 ppm protein standard solutions (as described section 3.3.4) were mixed with 1 mL of CBBG working reagent and incubated at room temperature for 1 hour. Then, the absorbance of mixing protein solutions were measured at 595 nm. The calibration curve of BSA solutions was plotted between net absorbance at 595 nm and concentrations of protein.

3.11 Repeatability and recovery of PEMs-modified membrane filter for albumin preconcentration

The same-day, repeatability and recovery of nine PEMs-modifed membrane filter and preconcentration process were tested using 1 ppm BSA.

CHAPTER IV

RESULTS AND DISCUSSION

Our goal in this study is to increase the concentration of trace level albumin for potential used in urine samples. The bovine serum albumin (BSA) was used as a solute probe. The layer-by-layer deposition technique (LbL) was used to modify the surface of cellulose acetate membrane (CA) as a membrane filter to adsorb trace level of bovine serum albumin (BSA). The suitable PEMs system was identified by considering the BSA adsorption efficiency on PDMS substrates. In this step, the fabrication parameters including types of polyelectrolyte, pH values of weak polyelectrolyte solutions, concentrations of sodium chloride, and number of layers on the growth of polyelectrolyte multilayer and protein absorption were optimized. Next, the chosen PEMs were fabricated on a CA membrane and BSA preconcentration was studied. Finally, the performance of PEMs modified membranes on the protein preconcentration was evaluated and validated.

4.1 Study of the suitable polyelectrolyte multilayers film system (PEMs)

4.1.1 Effect of types of polyelectrolyte on the PEMs growth

The PEMs films based on an electrostatic self assembly method were fabricated on quartz and polydimethylsiloxane (PDMS) substrates The PDMS was selected as one of the studied material to shorten the selection process to find a suitable PEMs pairs before fabricating on membrane filter. Four types of polyelectrolytes were chosen in this study, including PDADMAC/PSS, PDADMAC/PSSMA, PAH/PSS and PAH/PSSMA. Although all of these polyelectrolytes were successfully fabricated [13, 42-44], there is no reported comparison study on protein preconcentration applications. The multilayer build-up on quartz slides was monitored using UV-vis spectrosphotometer. The growth of PEMs films on PDMS was followed using ATR-FTIR, because the deposition of multilayer thin film on PDMS cannot be investigated by UV-Vis spectroscopy due to the overlay of absorbance spectra between PDMS and polyelectrolytes. In addition, a

fabricating pH for weak polyelectrolytes was fixed at 5.5, which is lower than the *p*Ka of PAH (*p*Ka = 8.8) [45], but higher than the pKa_1 of PSSMA (pKa_1= 2.7 pKa_2= 8.3) [43]. Therefore, PAH possesses positive charges, whereas the charges of PSSMA are negative.

The growth of PDADMAC and PAH as positive layers and PSS and PSSMA as negative layers were shown in Figure 4.1. The odd layers are PDADMAC and PAH is the outermost layers, while the even layers are the PEMs film with PSS and PSSMA is the outermost layers. A characteristic UV absorption band of the styrene group of PSS and PSSMA is approximately 227 nm [13], while PDADMAC and PAH showed no absorption band in this region. From the UV-vis spectra, the absorbance at 227 nm for styrene of PSS and PSSMA peaks increased with the number of deposition layers, indicating the assembly of the all PEMs types (PDADMAC/PSS, PDAMAC/PSSMA,PAH/PSS and PAH/PSSMA). In addition, the absorption spectra of PDADMAC/PSS and PDADMAC/PSSMA films slowly increased within the first eight layers, and then the absorbance increased dramatically. Since the absorbance signals of styrene of PSS and PSSMA were saturated after the number of deposition layers, the PEMs growth on the quartz slide was monitored until the 14^{th} layer as the last layer. While the absorption spectra of PAH/PSS and PAH/PSSMA films were slightly increased, indicating the growth of PDADMAC/PSS and PDADMAC/PSSMA films better than that of PAH/PSS and PAH/PSSMA films.

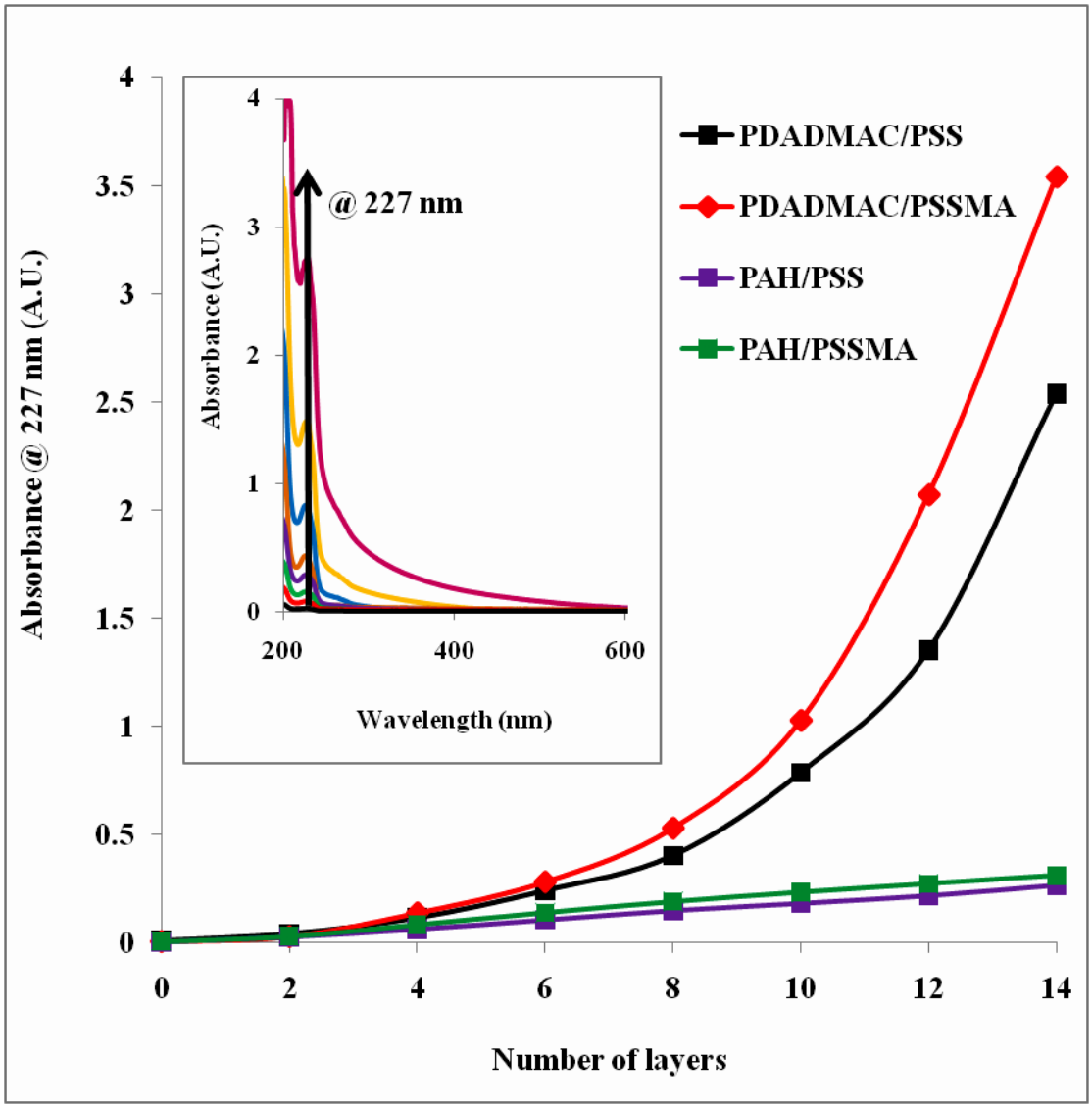


Figure 4.1 Absorption spectra of PDADMAC/PSS, PDADMAC/PSSMA, PAH/PSS and PAH/PSSMA multilayers as a function of the number of layers. The inset shows multilayer thin film growth of PDADMAC/PSS on a quartz substrate.

FTIR spectra of all PEMs types on PDMS were monitored using ATR-FTIR. Figure 4.2 (A) shows a typical FTIR spectrum of PDMS. The strongest double-peak band at ~1072 and ~1027 cm^{-1} is attributed to the stretching vibrations of Si-O-Si. The characteristic peaks of CH_3 were located at ~2962 and ~2906 cm^{-1}. A sharp single peak at ~1260 cm^{-1} is attributed to the vibration of CH_3 in Si-Me_2 group [15, 46]. Figure 4.2 (B-E) shows FTIR spectra of PDADMAC, PAH, PSS and PSSMA, which

the characteristic peaks of sulfonate groups of both PSS and PSSMA (~1000 and ~1100 cm^{-1}) [14] overlay with the Si-O-Si peak of PDMS (1000 - 1100 cm^{-1}). Therefore, in this study, the studied PEMs film was fabricated on PDMS with either PDADMAC or PAH as the outermost layer. In the case of PDADMAC as the outermost layer, the stretching of C–N group at ~1180 cm^{-1} and the stretching of quaternary ammonium ($NR_4^{+)}$ at ~1640 cm^{-1} were monitored [47]. For the PEMs system using PAH as polycations, the characteristic peak of the N-H bending vibrations at ~1635 cm^{-1} was monitored [48].

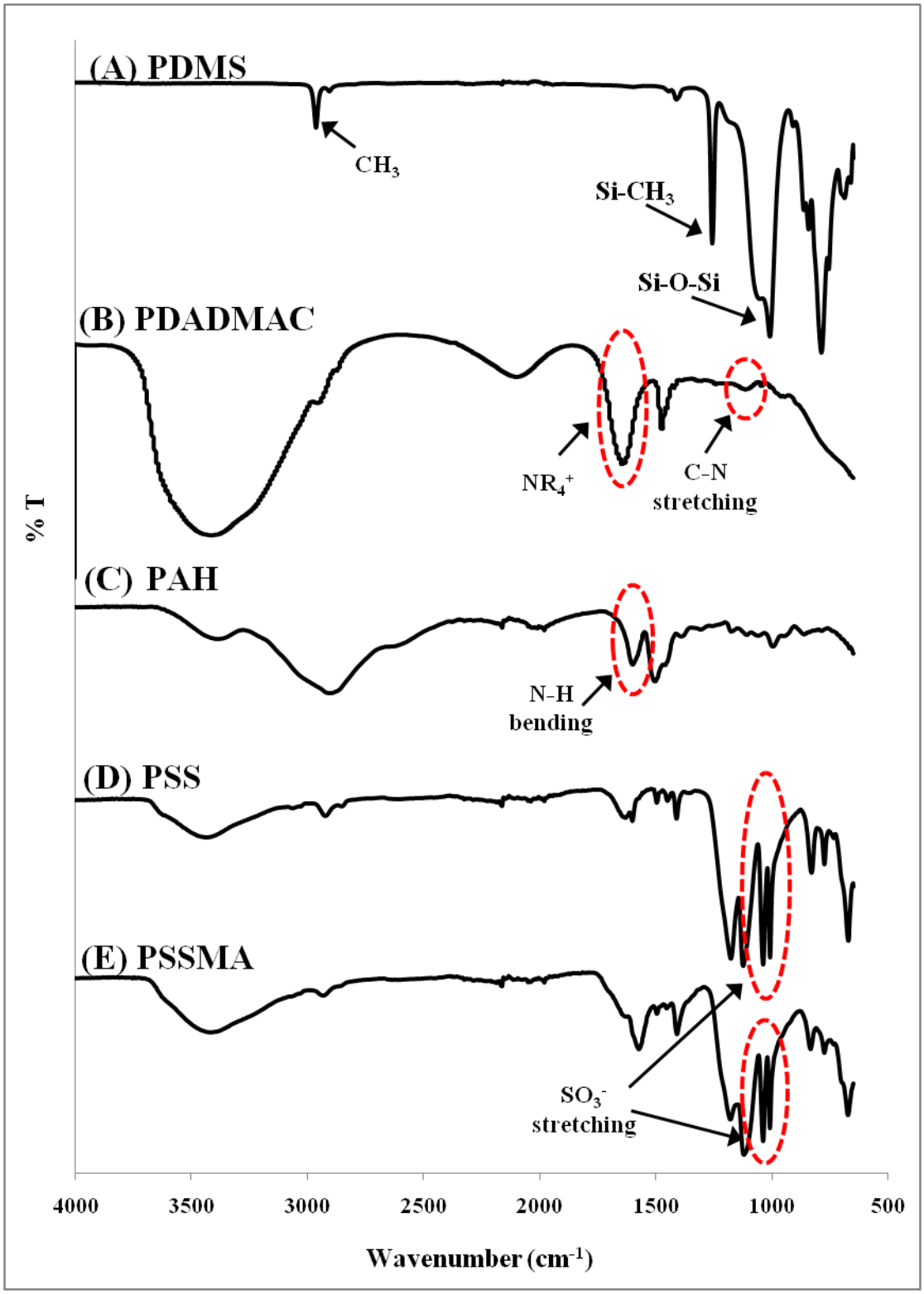


Figure 4.2 ATR-FTIR spectrum of PDMS, PDADMAC, PAH, PSS and PSSMA.

The growth of PDADMAC/PSS and PDADMAC/PSSMA systems was confirmed by the FTIR shown in Figures 4.3-4.4, which the increase in the peak height of C-N and NR_4 of PDADMAC was clearly observed. However, the increase in the peak height of the characteristic peak of PAH (-N-H bending) was slightly increased with the increase in the number of the coating layers (Figures 4.5-4.6).

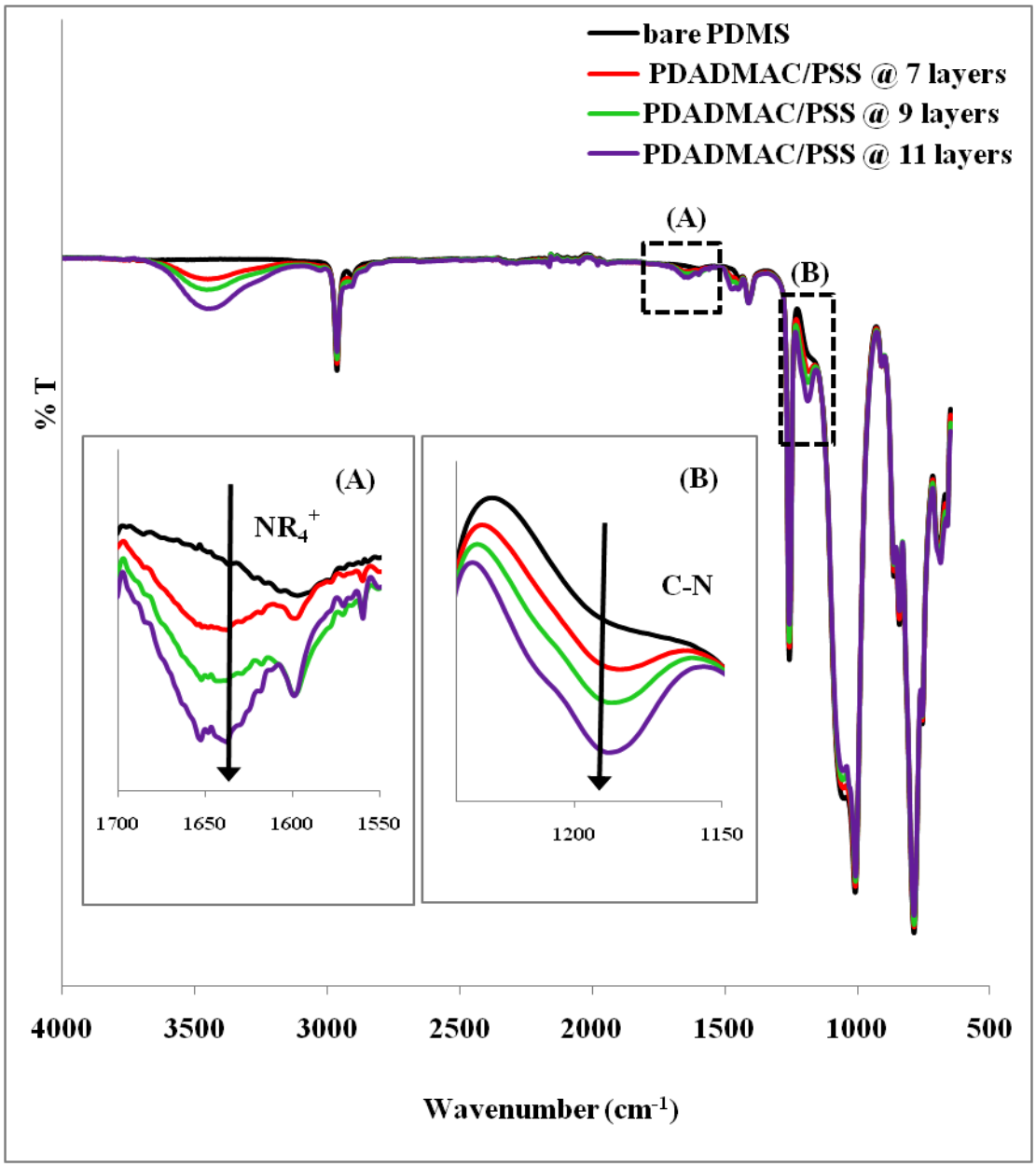


Figure 4.3 ATR-FTIR spectra of unmodified and modified PDMS with PDADMAC/PSS layers (shown only odd layers). Inset shows the increase in (A) NR_4^+ peak at ~1640 cm^{-1} and (B) C-N peak at ~1180 cm^{-1}.

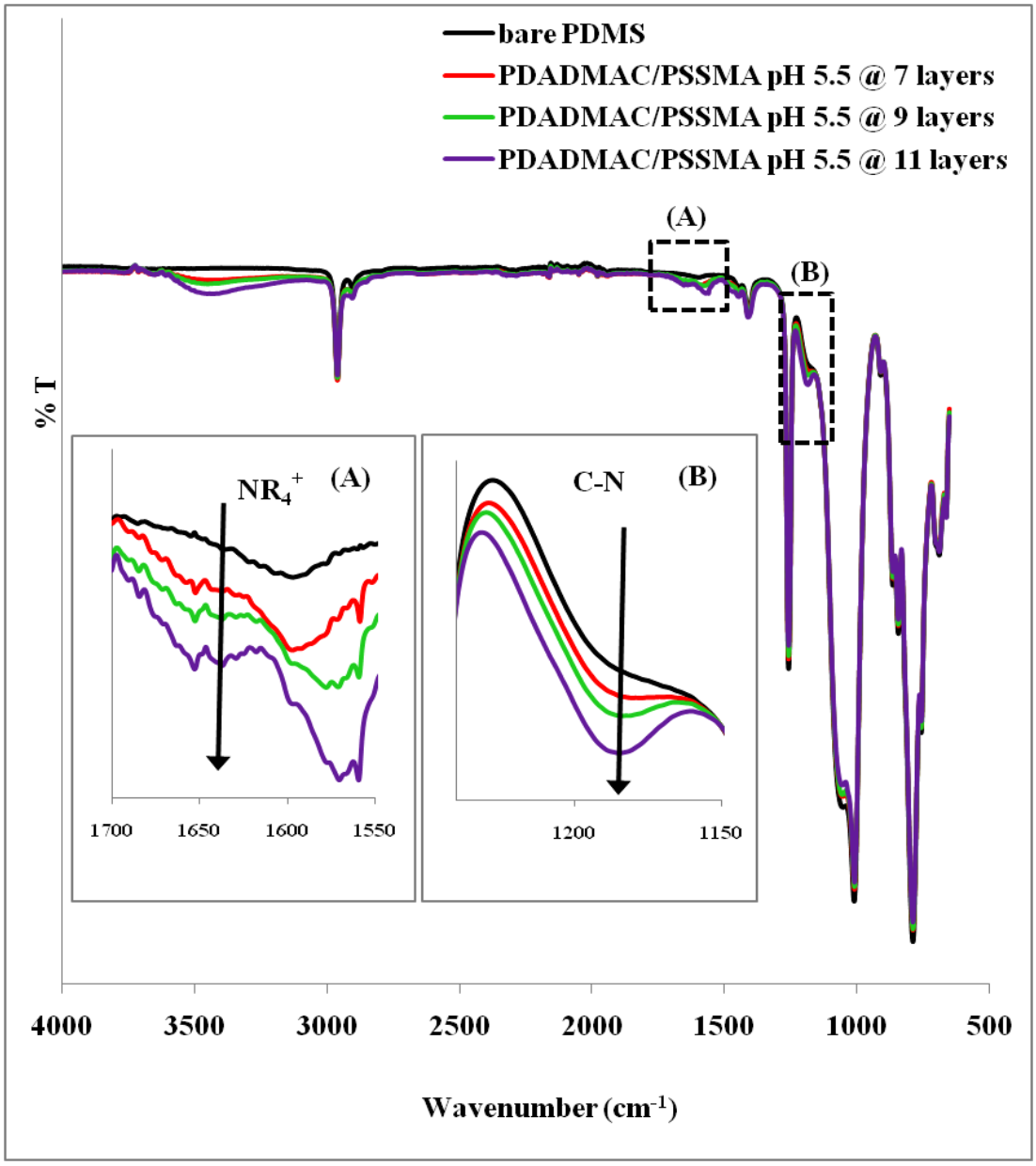


Figure 4.4 ATR-FTIR spectra of unmodified and modified PDMS with PDADMAC/PSSMA layers at pH 5.5 (shown only odd layers). Inset shows the increase in (A) NR_4^+ peak at ~1640 cm^{-1} and (B) C-N peak at ~1180 cm^{-1}.

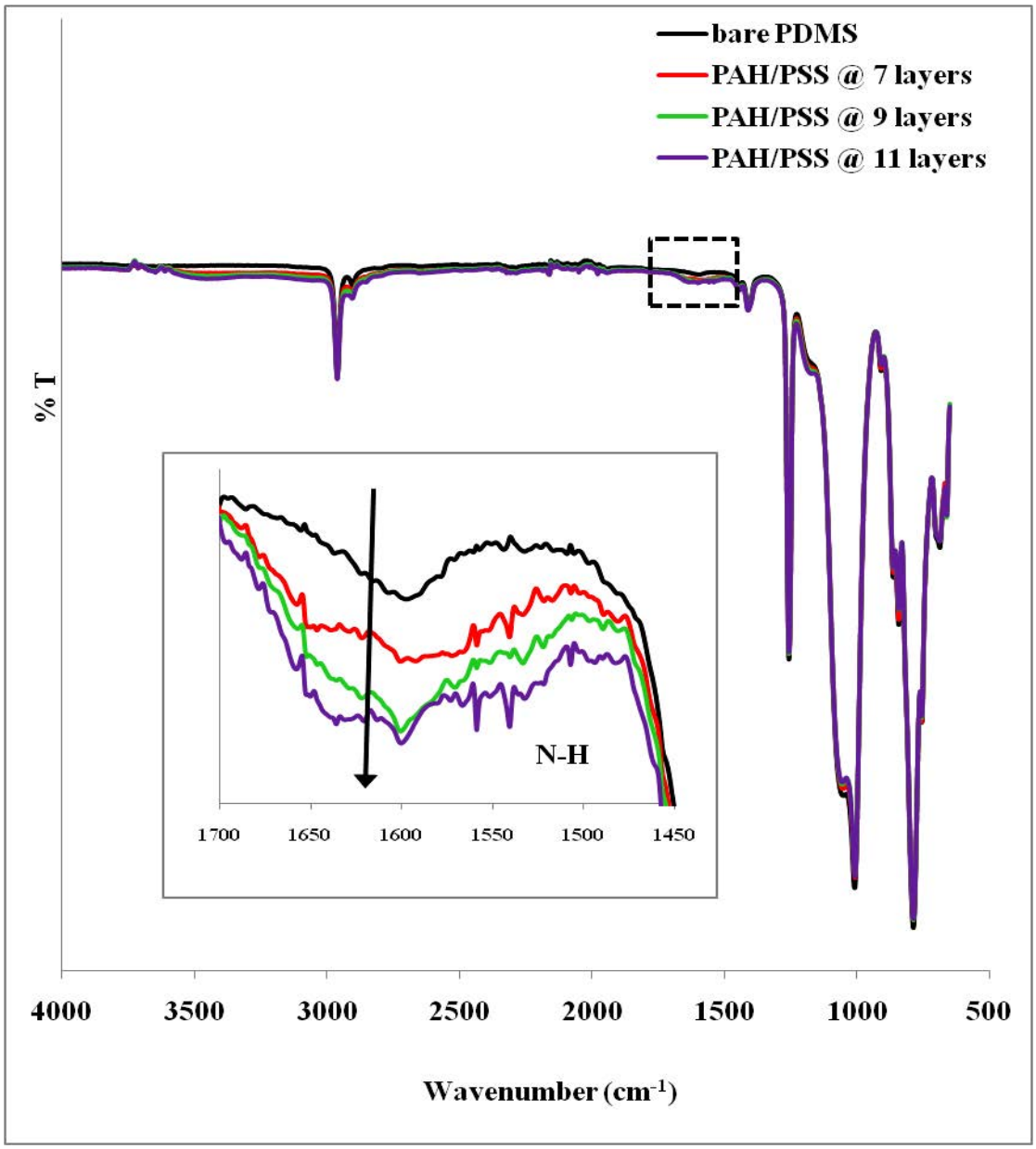


Figure 4.5 ATR-FTIR spectra of unmodified and modified PDMS with PAH/PSS layers at pH 5.5 (shown only odd layers, which has PAH as the outermost layer). Inset shows the increase in N-H peak at ~1635 cm^{-1}.

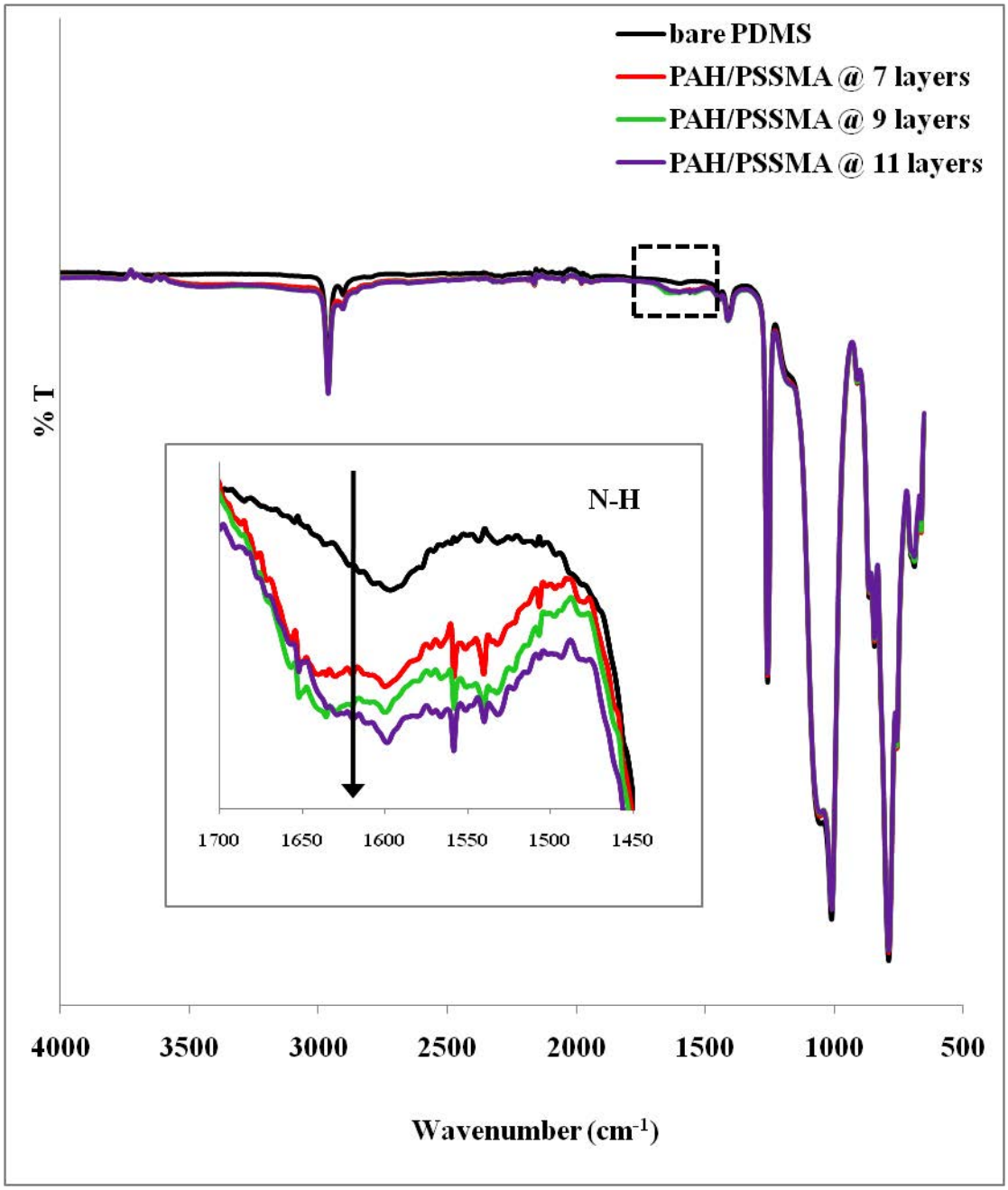


Figure 4.6 ATR-FTIR spectra of unmodified and modified PDMS with PAH/PSSMA layers at pH 5.5 (shown only odd layers). Inset shows the increase in N-H peak at ~1635 cm^{-1}.

Based on the FTIR spectra, the PEMs of all polyelectrolytes could be fabricated in our studied conditions. The peak height of all PEMs increased with the number of coating layers, which corresponds to UV data in section 4.1.1.

For comparison purpose, the UV absorbance values of all PEMs films were plotted against the PEMs system (in Figure 4.7). One can see that the PDADMAC/PSS and PDADMAC/PSSMA deposited at pH 5.5 showed higher depositing content than the PAH/PSS and PAH/PSSMA, implying a thicker film.

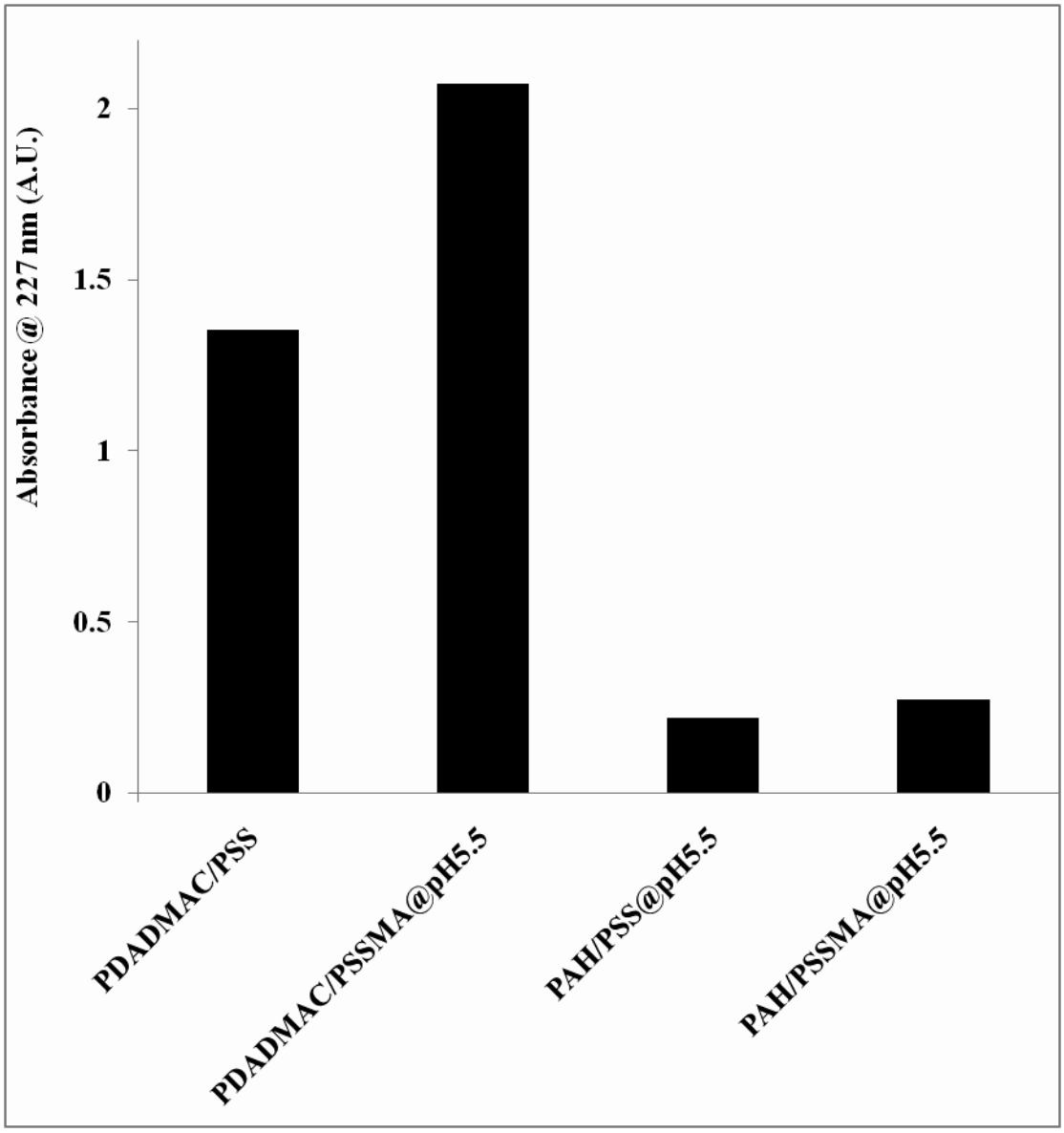


Figure 4.7 Absorbance at 12 layers of PDADMAC/PSS, PDADMAC/PSSMA, PAH/PSS and PAH/PSSMA multilayer thin films

4.1.2 Stability of polyelectrolyte multilayers

One concern in developing the polyelectrolyte multilayer films is their stability because the build-up PEMs mechanism is mostly based on the ionic interaction. The change in pH in the solution should affect the film stability. Therefore, the stability of polyelectrolyte multilayer film in buffer solution was tested. In comparison of ATR-FTIR spectra of PDMS modified PEMs layer before being dipped into the pH 7.4 buffer solution with a PEMs coating on PDMS after the immersion, one can conclude that the all PEMs types were stable after being dipped into the buffer solutions (Figures 4.8-4.11).

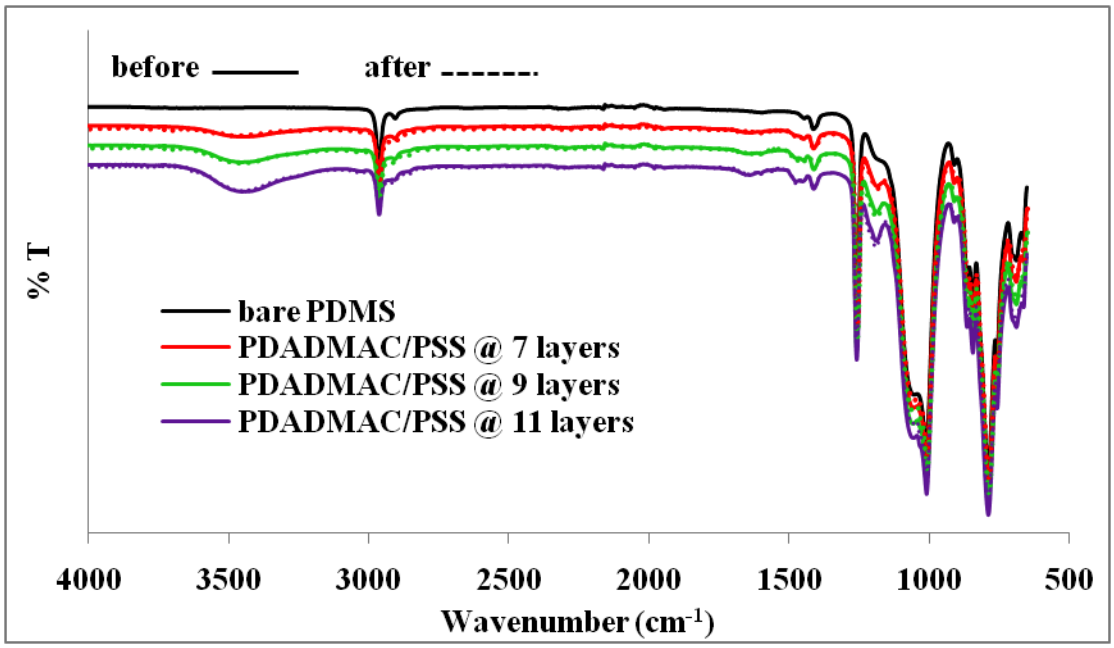


Figure 4.8 Stability of PDADMAC/PSS modified layers on PDMS. Solid lines represent the data of PDMS modified with PEMs before dipping substrate into buffer pH 7.4, whereas the dashed lines represent the data obtaining after dipping.

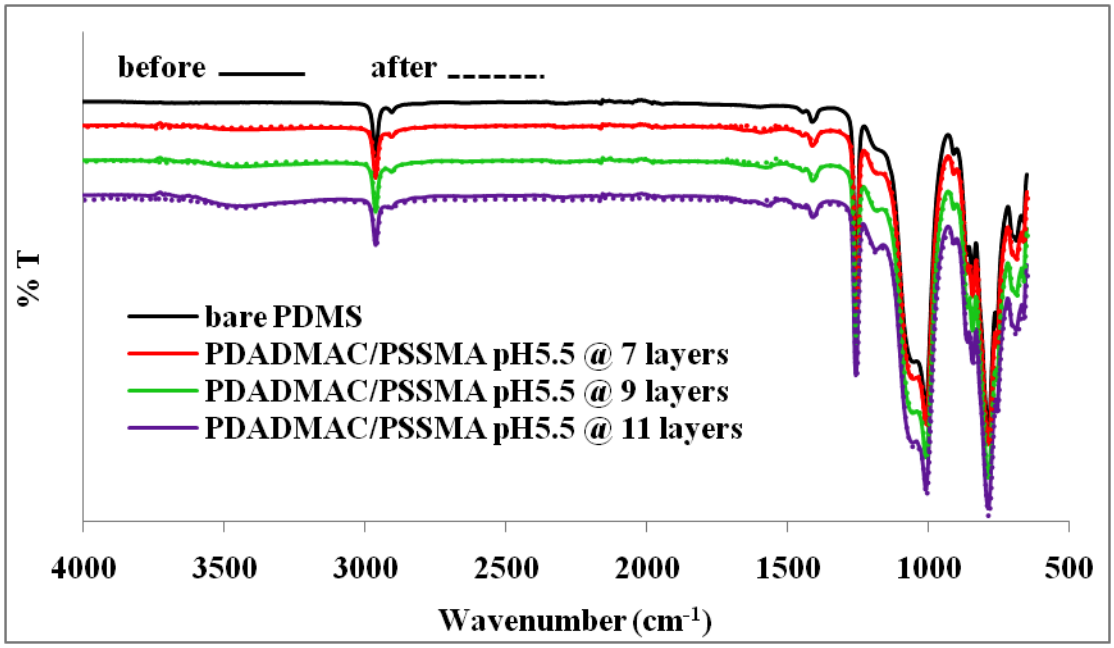


Figure 4.9 Stability of PDADMAC/PSSMA modified layers at pH 5.5 on PDMS. Solid lines represent the data of PDMS modified with PEMs before dipping substrate into buffer pH 7.4, whereas the dashed lines represent the data obtaining after dipping.

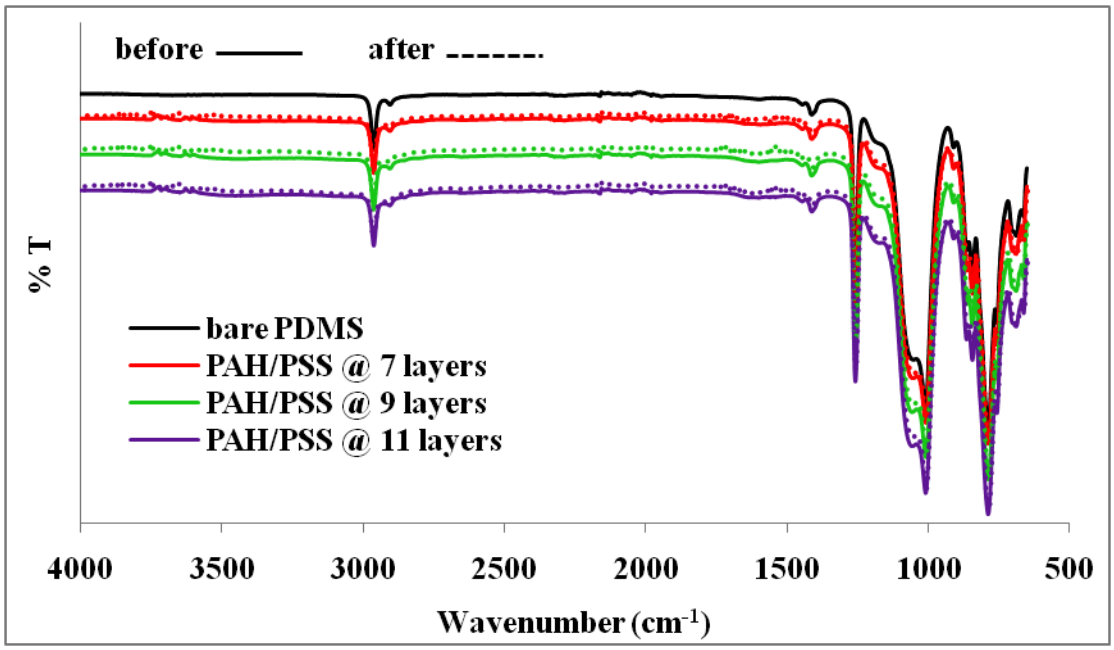


Figure 4.10 Stability of PAH/PSS modified layers on PDMS. Solid lines represent the data of PDMS modified with PEMs before dipping substrate into buffer pH 7.4, whereas the dashed lines represent the data obtaining after dipping.

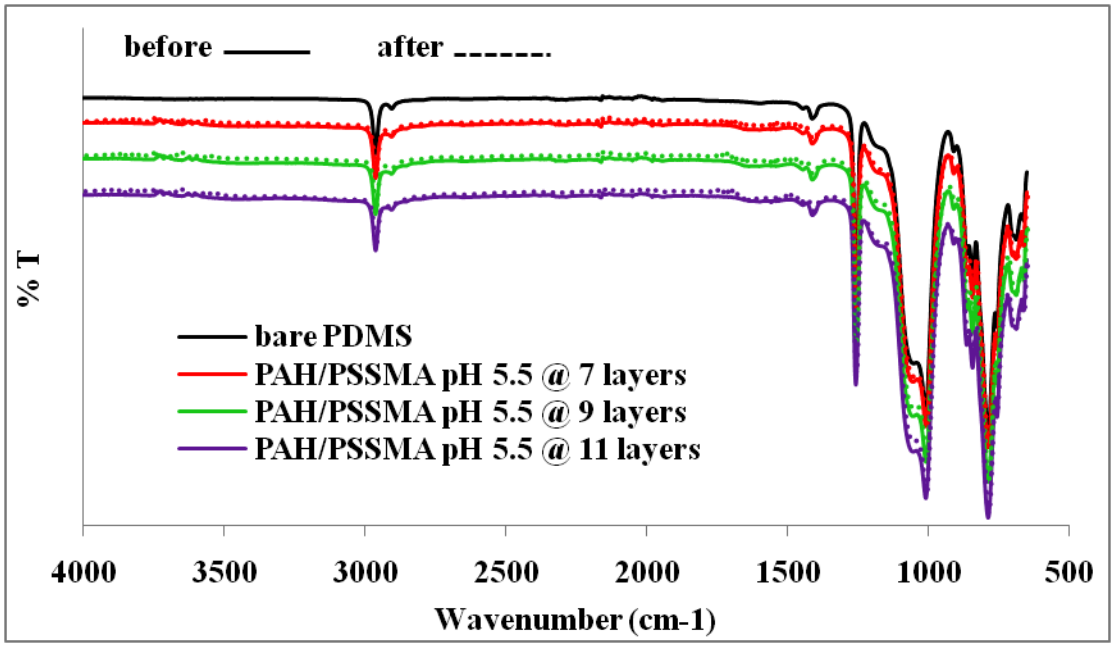


Figure 4.11 Stability of PAH/PSSMA modified layer at pH 5.5 on PDMS. Solid lines represent the data of PDMS modified with PEMs before dipping substrate into buffer pH 7.4, whereas the dashed lines represent the data obtaining after dipping.

4.1.3 Effect of various types of polyelectrolyte on the protein adsorption

Protein molecules can be adsorbed on polyelectrolyte multilayers via various types of interactions, especially electrostatic interaction, which is our strategy in this study. Therefore, tuning the charges of PDMS modified surface and protein molecules is crucial for the protein adsorption on the multilayer surface. A pH of BSA solution was kept at 7.4 because this pH is the normal range for pH of blood of human body [15]. Types of multilayer thin films were varied, but the outermost layer is always positively charged because at pH 7.4, BSA solution possesses the negative charges. The adsorbed amount of protein on various types of PEMs was evaluated using ATR-FTIR.

ATR-FTIR spectra of protein adsorbed on PDADMAC/PSS multilayers were shown in Figure 4.12. The presence of BSA molecules in PDADMAC/PSS multilayers on PDMS is clearly demonstrated by the following characteristic peaks: ~1650 cm^{-1} assigned to amide I (amide I: C=O stretching), and ~1540 cm^{-1} for amide

II (amide II: CN stretching and NH bending) [15, 16]. At pH 7.4, protein possesses negative charges, which could be adsorbed on positively charged surface of PDMS modified with PDADMAC as the topmost layer. The amount of adsorbed protein on PDADMAC increases with the number of the PEMs layers. At seventh coating layer, the amount of adsorbed protein was low. When the numbers of layer were increased to an eleven-layer coating, the amount of protein adsorbed was found to be greatly enhanced, especially in the PDADMAC/PSS system. Accordingly, the amount of adsorbed protein depends on the number of layers and the thickness of polyelectrolyte multilayer film, which is in great agreement with the previous work [49]. When the numbers of layers and the thickness of polyelectrolyte multilayer film were increased, PEMs can act as a "sponge" or a matrix to high loading proteins. Similar result was observed for the PDADMAC/PSSMA that the BSA was adsorbed and the amount of protein adsorbed increased with the number of deposition layers (Figure 4.13).

From the ATR-FTIR spectra in Figures 4.14 and 4.15 the amount of protein adsorbed on PAH/PSS and PAH/PSSMA are relatively low. Considering our result in section 4.1.1, the growth of these systems are poor, which could affect the strength of the ionic interaction between PAH (the outermost layer) and albumin.

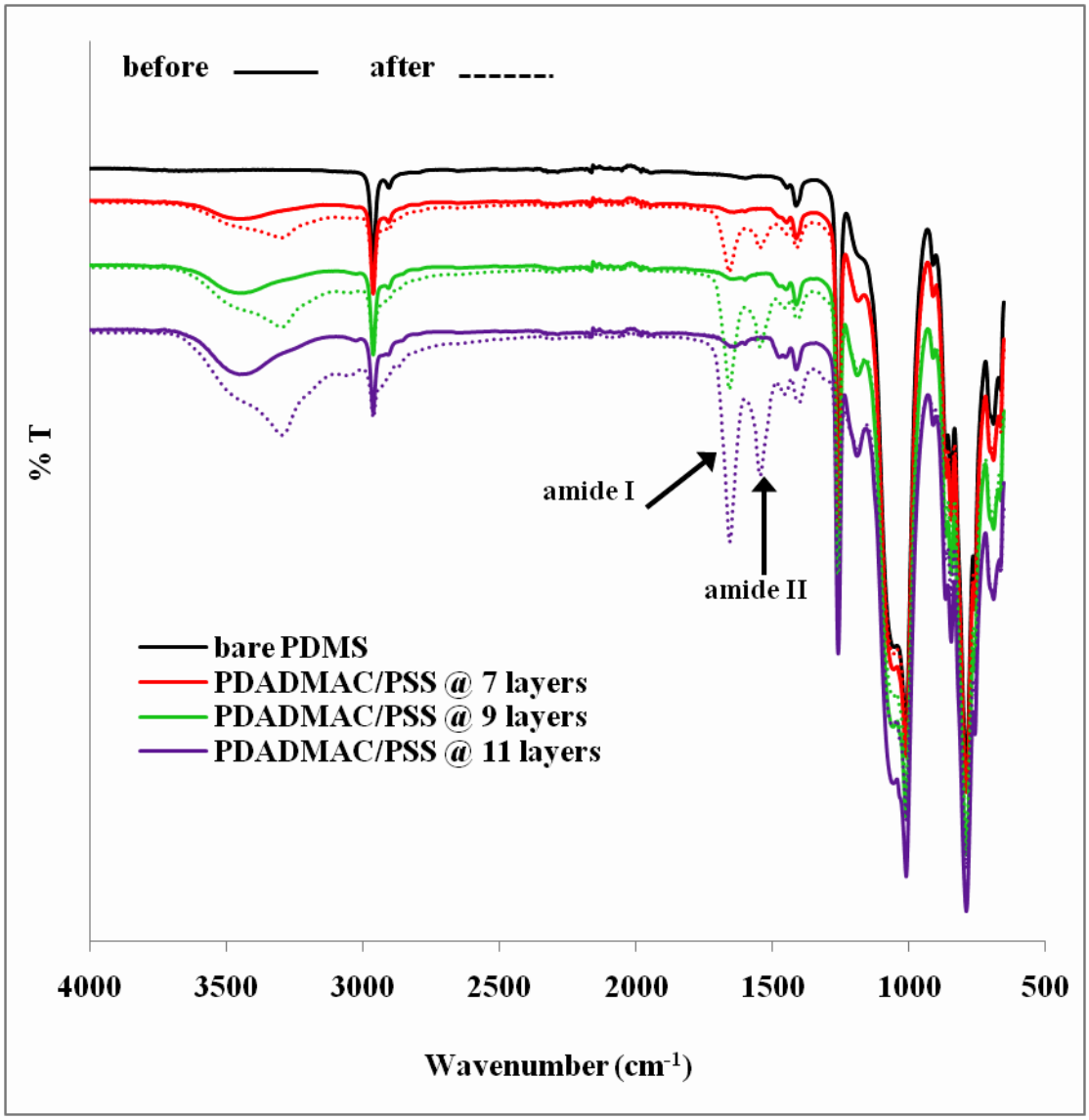


Figure 4.12 ATR-FTIR spectra of protein adsorbed at pH 7.4 on PDMS modified with PDADMAC/PSS multilayers with various numbers of layers. Solid lines represent the data of PDMS modified with PEMs before BSA adsorption, whereas the dashed lines represent the data obtaining after BSA adsorption.

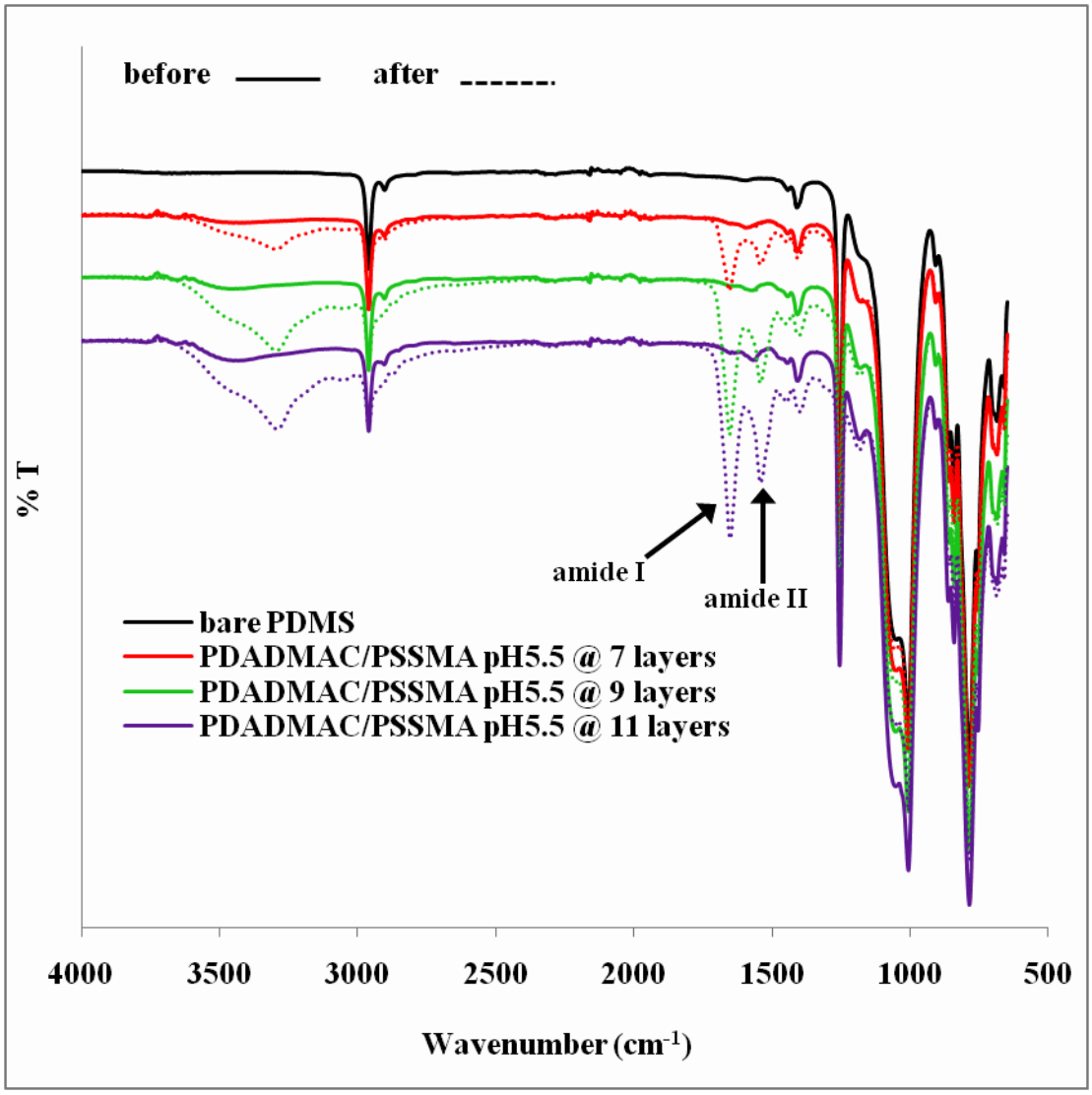


Figure 4.13 ATR-FTIR spectra of protein adsorbed at pH 7.4 on PDMS modified with PDADMAC/PSSMA at pH 5.5 multilayers with various numbers of layers. Solid lines represent the data of PDMS modified with PEMs before BSA adsorption, whereas the dashed lines represent the data obtaining after BSA adsorption.

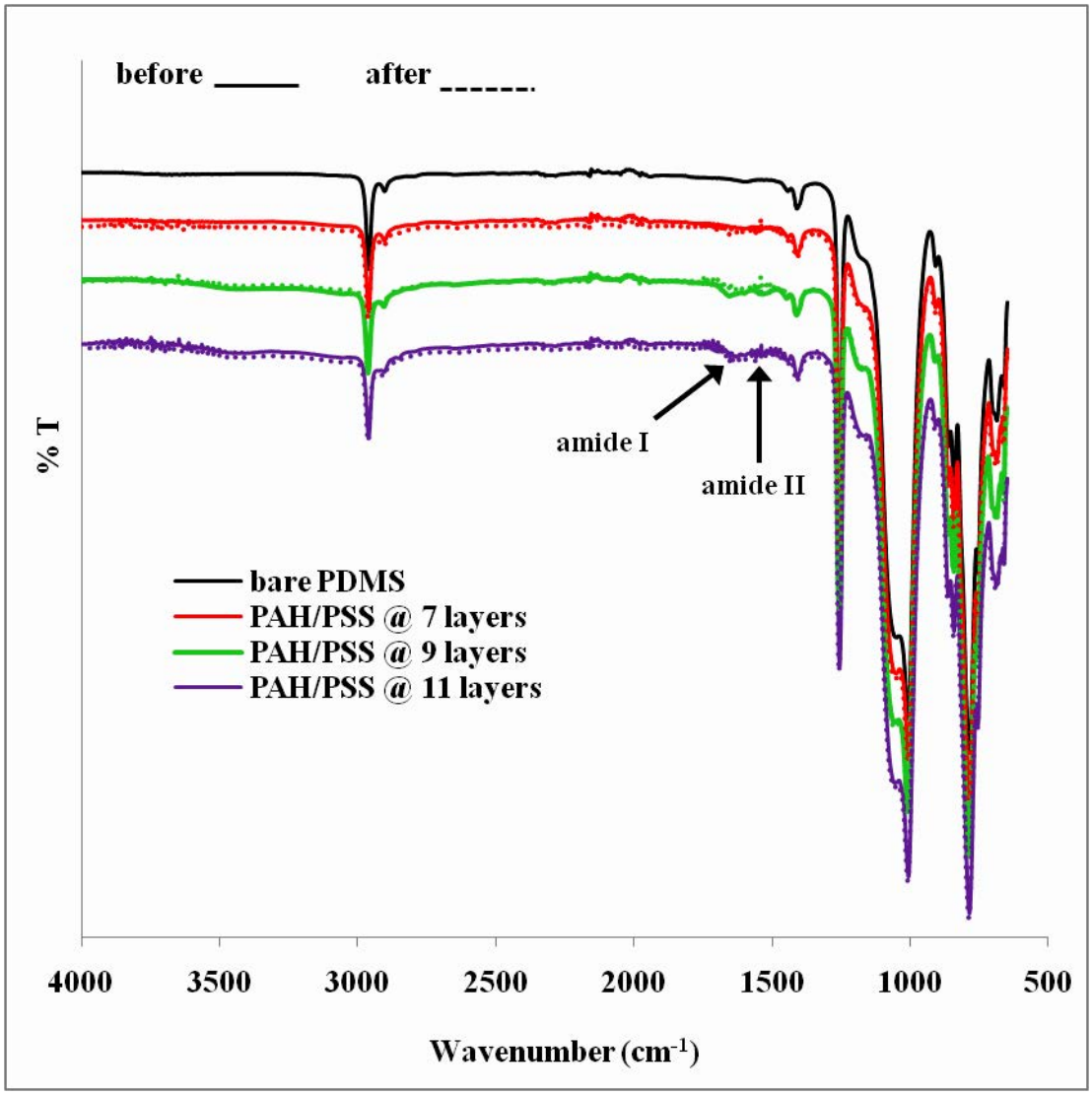


Figure 4.14 ATR-FTIR spectra of protein adsorbed at pH 7.4 on PDMS modified with PAH/PSS multilayers with various numbers of layers. Solid lines represent the data of PDMS modified with PEMs before BSA adsorption, whereas the dashed lines represent the data obtaining after BSA adsorption.

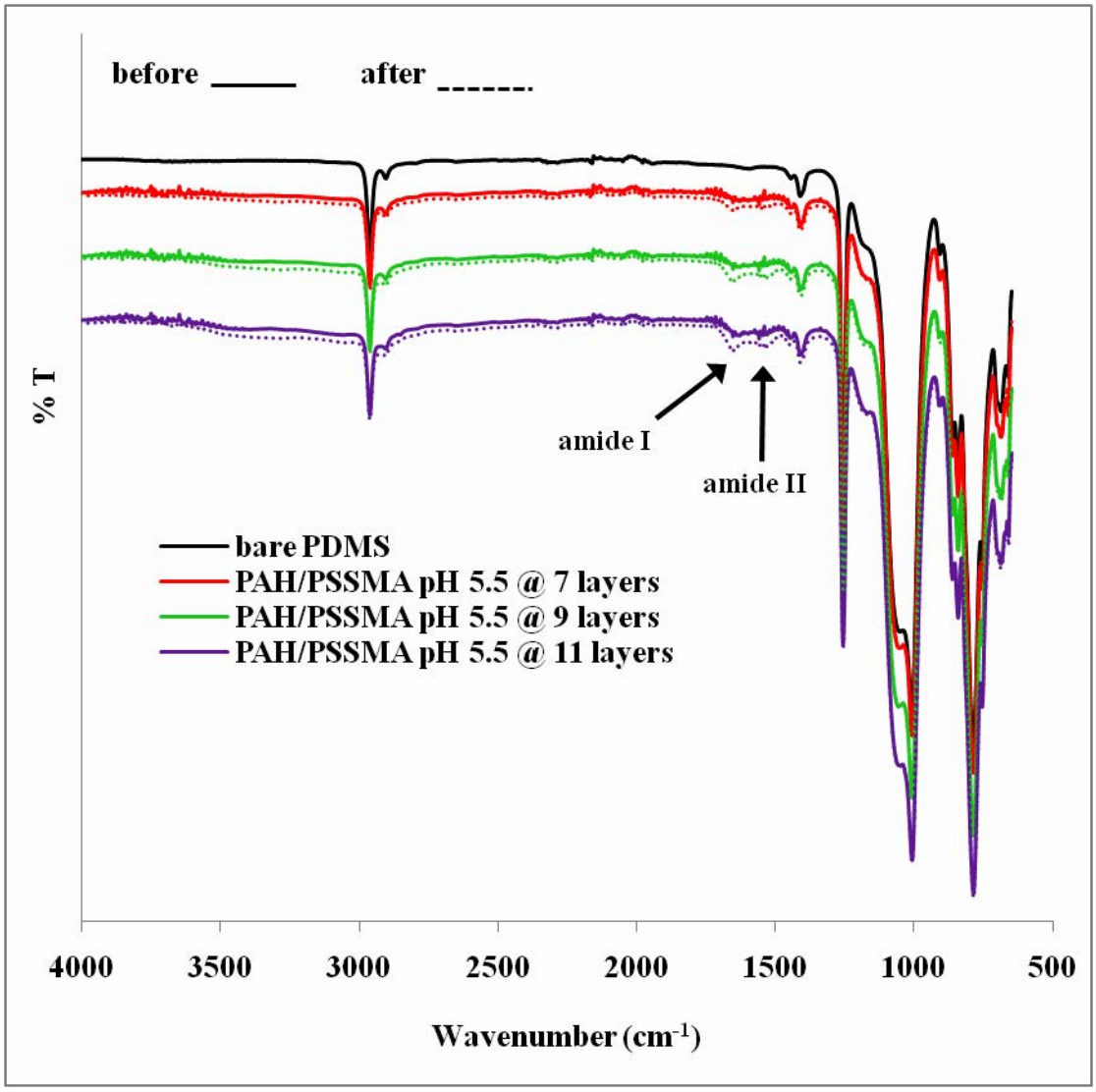


Figure 4.15 ATR-FTIR spectra of protein adsorbed at pH 7.4 on PDMS modified with PAH/PSSMA multilayers with various numbers of layers. Solid lines represent the data of PDMS modified with PEMs before BSA adsorption, whereas the dashed lines represent the data obtaining after BSA adsorption.

The subtracted peak areas of amide I and II (between before and after dipping) were measured using OMNIC PROGRAM as shown in Figure 4.16. It was found that the amount of BSA adsorbed on PDADMAC/PSS and PDADMAC/PSSMA fabricated on PDMS is higher than that on the other PEMs systems. This phenomena could be the result of the higher depositing layer of PDADMAC/PSS and

PDADMAC/PSSMA systems compared to PAH/PSS and PAH/PSSMA at the same number of coating layer.

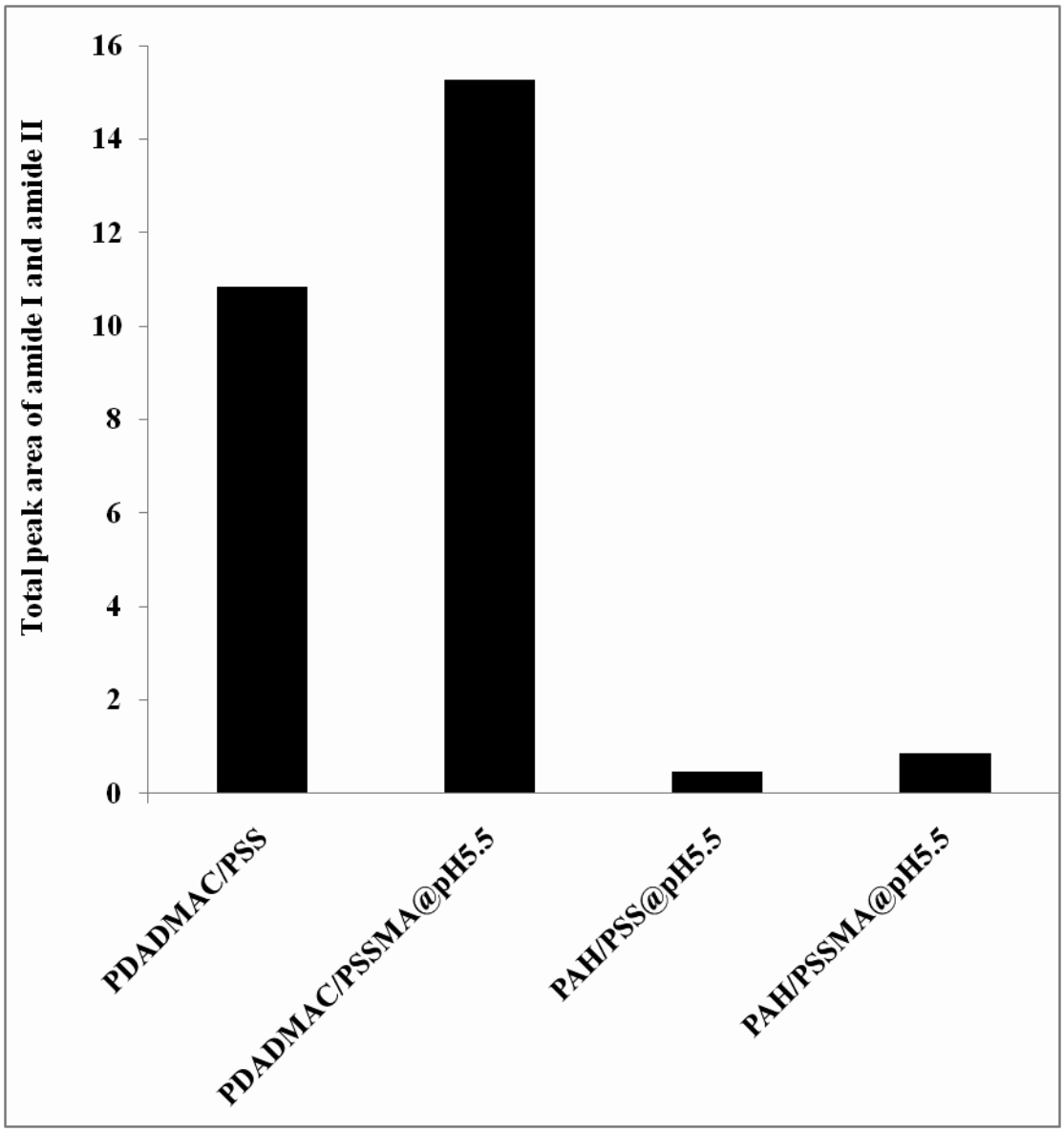


Figure 4.16 Total peak areas of amide I and amide II of PDADMAC/PSS, PDADMAC/PSSMA at 5.5, PAH/PSS and PAH/PSSMA multilayer thin films.

Based on our finding, the PDAMAC/PSS and PDADMAC/PSSMA were chosen to further protein adsorption study.

4.2 Optimization of PEMs build-up conditions

4.2.1 Effect of pH on the growth of PADMAC/PSSMA multilayers and stability

Multilayer thin films composing of strong and weak polyelectrolytes generated significant interest because they are pH responsive. The adsorption of strong polyelectrolyte is independent of pH of the solution because it can ionize in every pH value. The pH of solution affects the ionization degree of the functional groups on weak polyelectrolytes, resulting in different adsorption behaviors. PSSMA is a weak polyelectrolyte, which consists of a strongly charged styrene sulfonate (SS) group and a weakly charged maleic acid (MA) group (pKa_1 and pKa_2 of PSSMA in solution are 2.7 and 8.3) [43]. Effect of pH of the solution on the PDADMAC/PSSMA multilayer growth on a quartz slide and PDMS were investigated.

The growth of PDADMAC/PSSMA multilayer thin films at pH values of 2, 5.5 and 11 was monitored using a UV-vis spectrophotometer. In Figure 4.17, UV spectra provided information on the amount of PSSMA incorporated into the films due to UV absorption of styrene sulfonated groups at a wavelength of 227 nm. Multilayer thin films fabricated at different pH solutions of PSSMA showed different growth behaviors. All of the growth curves show the increase in absorbance, especially for PDADMAC/PSSMA deposited at pH 5.5.

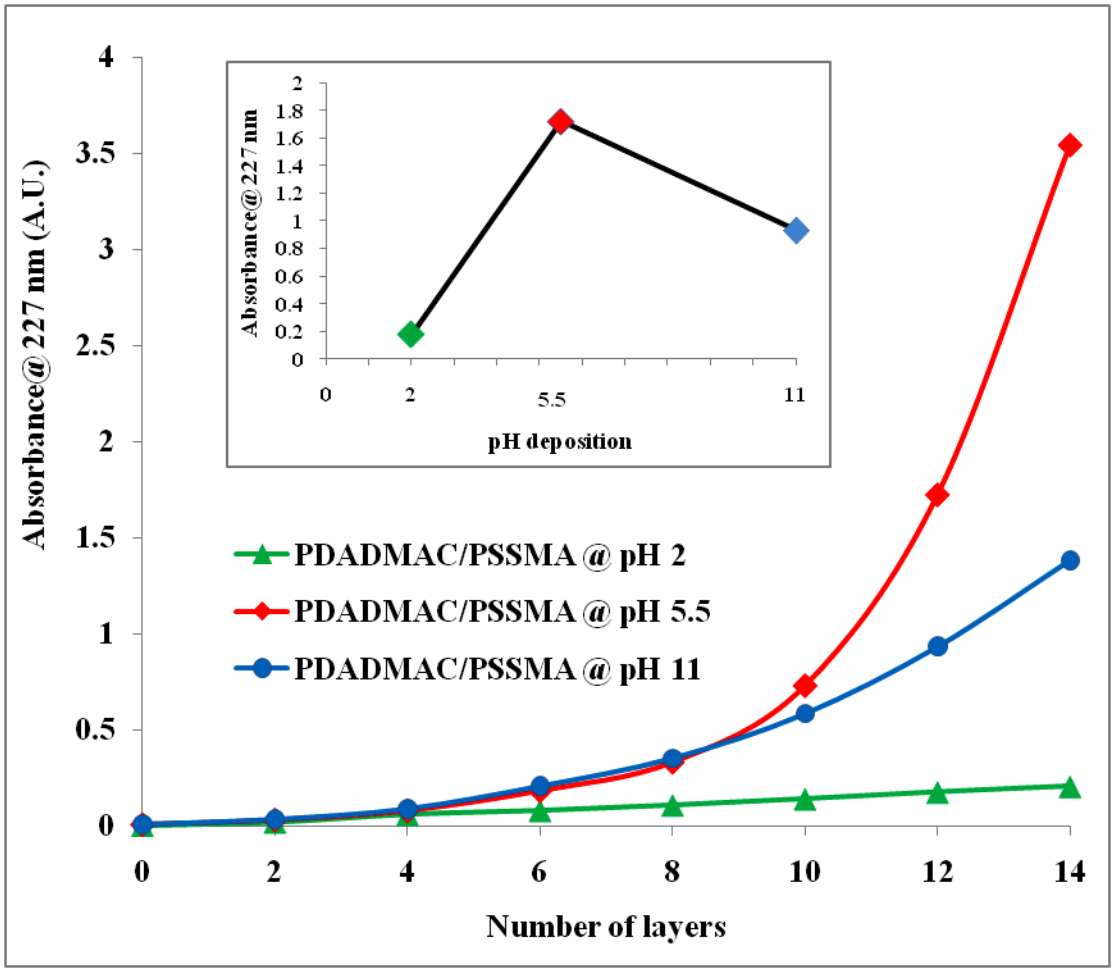


Figure 4.17 Absorbance of PDADMAC/PSSMA multilayers as a function of the number of layers. Fabrication condition: pH 2, 5.5 and 11, 1 M NaCl. Inset shows the absorbance at 227 nm of 12th layer PDADMAC/PSSMA as a function of pH.

The study showed that the absorption spectra of PSSMA decreased when pH of the PSSMA solution was increased from pH 5 to pH 11(*p*Ka of PSSMA in solution are 2.8 and 8.3). At high pH level, 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 polyelectrolyte increases, the repulsive force between the charged groups also increases, causing the polyelectrolytes adopt a flatter conformation [43]. Therefore, the multilayer thin films can be adsorbed as thinner layers. This accounts for the lower amount of deposited polymer observed at higher pH value, especially at pH 11 as previously shown by Caruso [13]. When the

deposition process of PDADMAC/PSSMA was performed at pH 5.5, the negative charges on the polymer decrease because only one carboxylic acid groups of MA could be ionized (pKa1 = 2.8), leading to the decrease of charged density. At this pH, the structure of the polyelectrolytes was changed to a coil conformation because of the lower repulsive interaction on the polyelectrolyte chain inducing thicker film [43]. Conversely, the pH of deposition solution was decreased from 5.5 to 2, causing the polymer to be a non-ionized form. It could result in lower charge density along the polymer chains. Additionally, the lower charge density of PSSMA means that less PDADMAC chains is required to compensate charge of the preceding PSSMA layers. Therefore, the multilayer thin films become thinner and lower deposited mass during the adsorption process. The scheme of fabricated PDADMAC/PSSMA at all pHs were represented in Figure 4.18 [50]. 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.

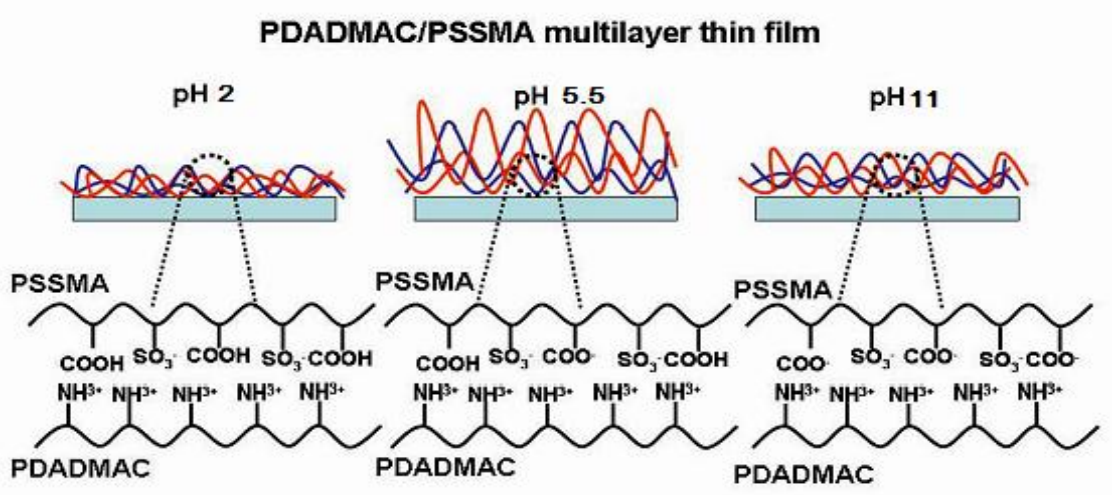


Figure 4.18 Scheme represents the fabrication of PDADMAC/PSSMA multilayer thin films [50].

In addition, the effect of pH on the growth of PDADMAC/PSSMA multilayer thin film at pH 2, 5.5 and 11 on PDMS was also investigated using ATR-FTIR by monitoring characteristic infrared peaks of the stretching of NR_4 and C–N groups of

PDADMAC at ~1640 (Figure 4.19). Similar result was observed that the PDADMAC/PSS film fabricating at pH 5.5 was better than that at other pH values.

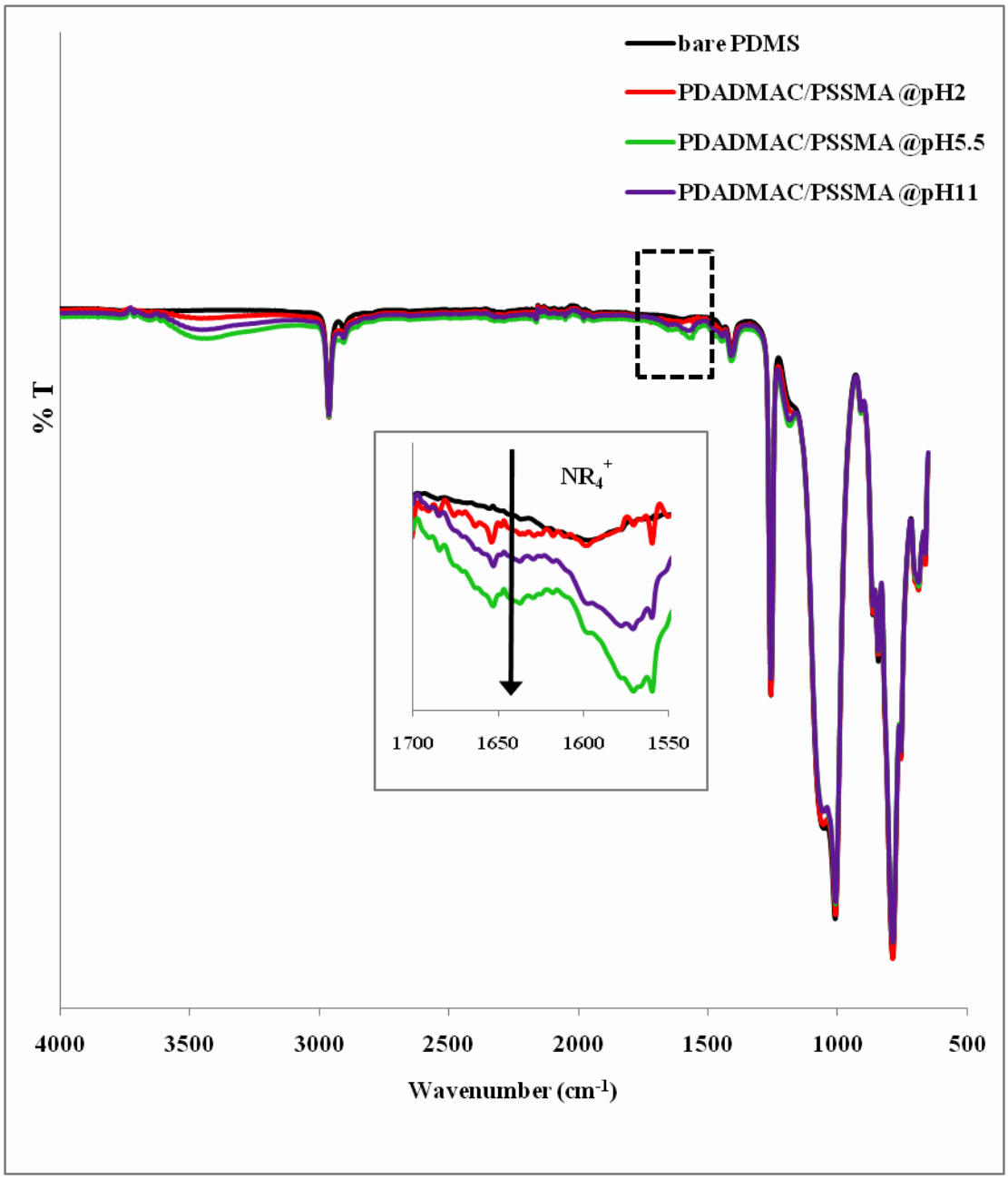


Figure 4.19 ATR-FTIR spectra of unmodified and modified PDMS with PDADMAC/PSSMA with 11 layers deposited at pH 2 (_____), pH 5.5 (_____) and pH 11 (_____) (shown only odd layers of multilayers). Inset shows the increase in NR_4^+ peak at ~1640 cm^{-1}.

The stability of PDADMAC/PSSMA film fabricating at these pH values was also investigated in the same manner as described in section 4.1.2. As seen in Figure 4.20, no change of ATR-FTIR spectra was observed, so we can conclude that these PEMs were stable and can be used for protein adsorption study.

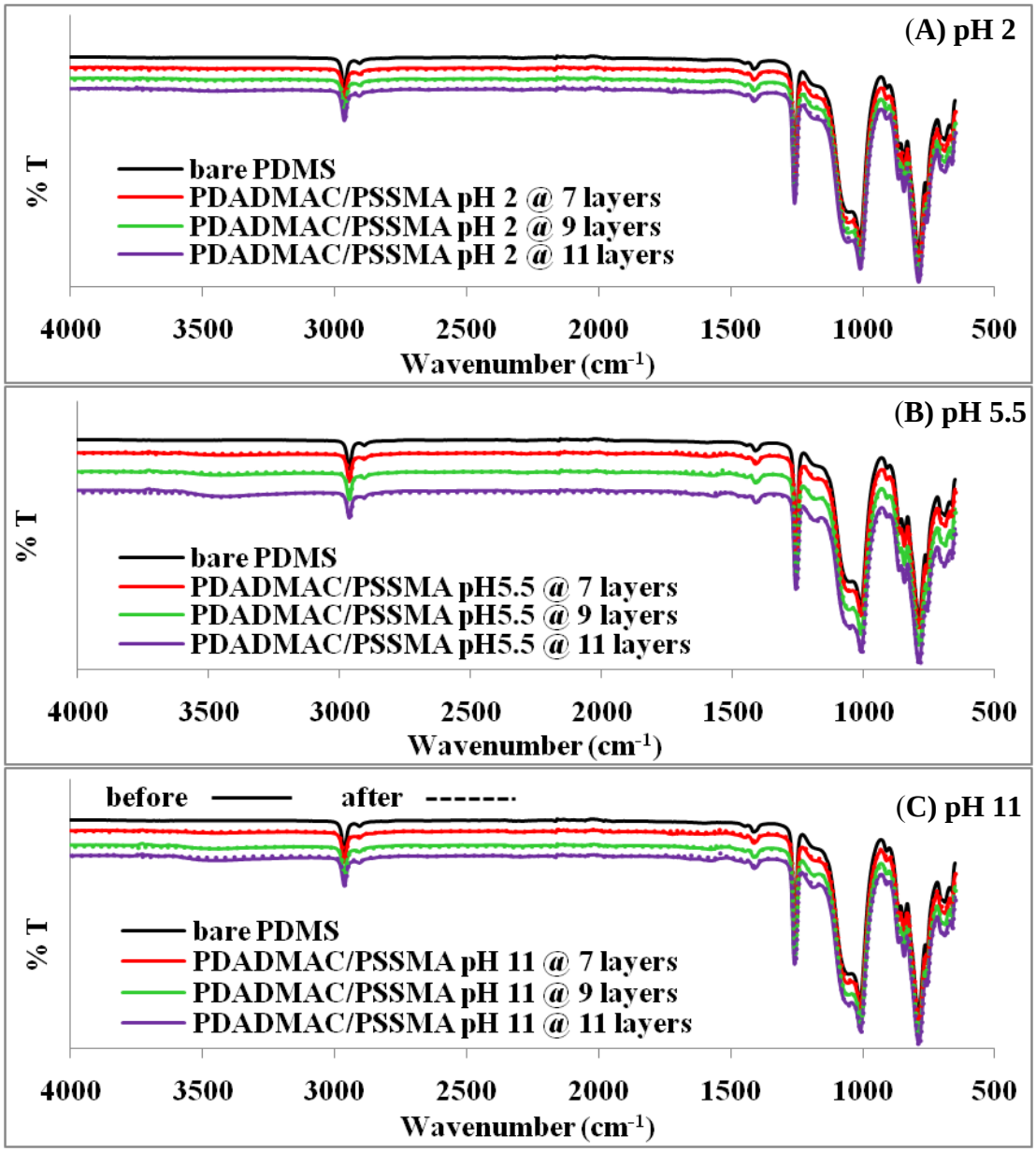


Figure 4.20 Stability of PDADMAC/PSSMA at pH 2 (A), 5.5 (B) and 11 (C) modified on PDMS. Solid lines represent the data of PDMS modified with PEMs before dipping substrate into buffer pH 7.4, whereas the dashed lines represent the data obtained after dipping.

4.2.2 Effect of NaCl on the formation of PEMs

As described in section 2.1.3.1, the concentration of sodium chloride, ionic strength, is one of the major factors controlling the growth of PEMs. Therefore, the effect of ionic strength on PEMs fabrication was also studied by monitoring the UV absorbance at 227 nm, signal of styrene sulfonated group of PSS or PSSMA layer. In this experiment, the studied ionic strength values were 0.5 M and 1 M NaCl. The number of PEMs layers was fixed at the twelfth coating layers.

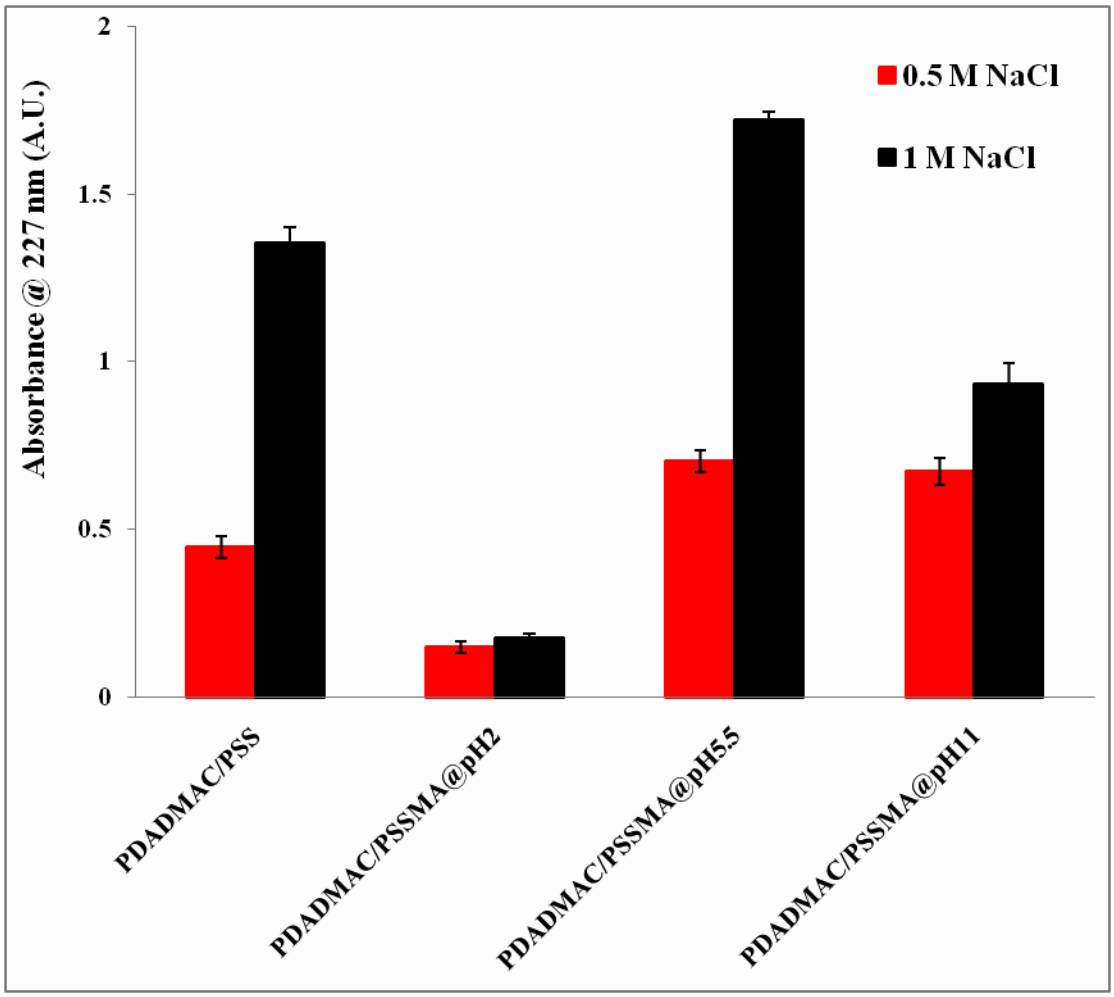


Figure 4.21 Absorbance at 12 layers of PDADMAC/PSS multilayer thin films and PDADMAC/PSSMA multilayer thin films deposited at pH 2, 5.5 and 11. Multilayer thin films were constructed with different NaCl concentrations during the assembly process.

As seen from Figure 4.21, all PEMs types show higher absorbance values when using 1 M NaCl, higher concentration. This might be because the ionic strength affects the conformation and charge density of the polyelectrolytes including the growth of the multilayers. With increasing salt concentration, the polyelectrolyte molecules in solution become more entangled due to the screening effect [51]. For example, dipping the negatively charged surface into the polycations solution, the coiled polyelectrolytes with negative counterions could interdiffuse into negatively charged polyelectrolytes (coiled conformation) previously adsorbed on the substrate. Therefore, a thicker is produced [51]. Schlenoff have reported the layer by layer assembly prepared from PDADMAC and polyacrylic acid (PAA) and found that the thickness of multilayer thin films was increased when the NaCl concentration reached the maximum at 1 M [42], which is in good argeement with our results. Therefore, the 1 M NaCl was used in the performance testing.

4.2.3 The protein adsorption on PDADMAC/PSSMA fabricating at different pHs

Figure 4.22 shows protein adsorption on the PDADMAC/PSSMA multilayer thin films fabricated at pH 2, 5.5 and 11, respectively. Based on our result, the highest amount of BSA absorbed was found to be on the surface of PDADMAC/PSSMA multilayer thin films fabricated at pH 5.5. The total peak areas of amide I and II of protein adsorbed on each PDADMAC/PSSMA system were also measured using the OMNIC program and compared with the total peak areas obtained from PDADMAC/PSS, as shown in Figure 4.23. It was found that the amount of adsorbed BSA on the PDAMAC/PSS and PDADMAC/PSSMA fabricated at pH 5.5 films was higher than that on the other PEMs films.

This is because at this pH the polyelectrolytes adopt more coil conformation, which contains both sulfonated groups and one ionized carboxylic acid groups of MA. This results in the higher amount of deposited polyelectrolyte and thicker film, as reported in section 4.2.1. Therefore, it can interact with more BSA molecules to form

intermolecular complexes, resulting in more amount of adsorbed BSA compared to the PEMs fabricated at pH 11 and pH 2. This resulted in that the PDAMAC/PSS and PDADMAC/PSSMA at pH 5.5 can adsorb more negative charge of BSA compared to the other PEMs. Therefore, PDAMAC/PSS and PDADMAC/PSSMA fabricated at pH 5.5 were chosen to further protein adsorption study. Moreover, ATR-FTIR spectra of protein adsorption for every studied layer were monitored. The amount of absorbed protein was increased with the numbers of PEMs layers.

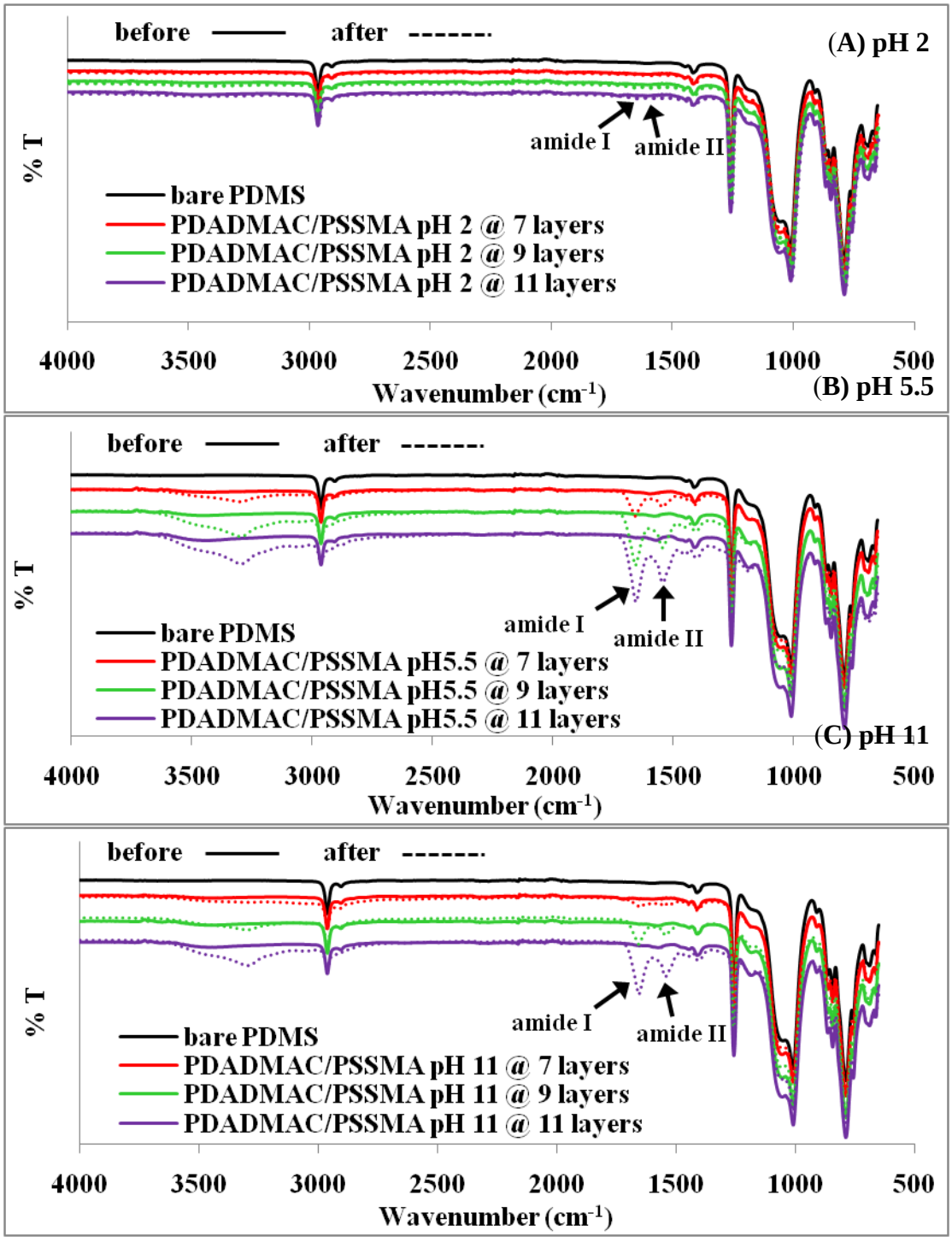


Figure 4.22 ATR-FTIR spectra of protein adsorbed at pH 7.4 on PDMS modified with PDADMAC/PSSMA at pH 2 (A), 5.5 (B) and 11 (C) with various numbers of layers. Solid lines represent the data of PDMS modified with PEMs before BSA adsorption, whereas the dashed lines represent the data obtaining after BSA adsorption.

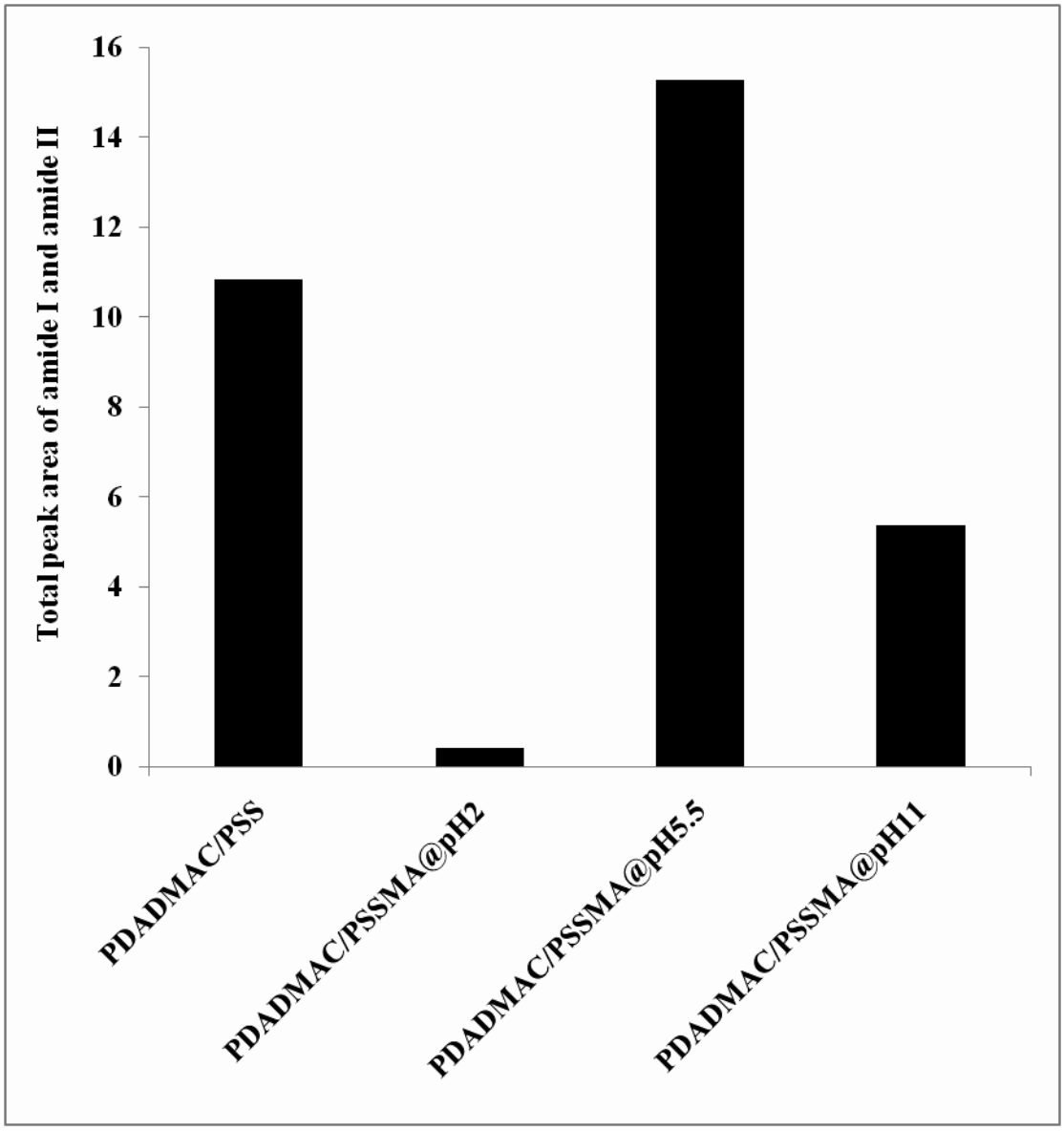


Figure 4.23 Total peak areas of amide I and amide II of PDADMAC/PSS, PDADMAC/PSSMA multilayer thin films at pH 2, 5.5 and 11.

4.3 Protein adsorption on modified PEMs membrane

The multilayer of PDADMAC/PSS and PDADMAC/PSSMA (pH 5.5) were fabricated on membrane using the optimized fabricating condition. The fabricated PEMs membranes were then used to filter 1 ppm BSA solutions pH 7.4. ATR-FTIR was used to monitor the BSA protein adsorption onto the multilayers-modified membrane filter.

4.3.1 Fabrication of PEMs on CA membrane

The multilayer build-up of PDADMAC/PSS and PADMAC/PSSMA on cellulose acetate (CA) membrane was monitored using ATR-FTIR. An ATR-FTIR spectrum of a CA membrane is shown in Figure 4.24. A strong carbonyl stretching band of acetyl groups on the CA chain was observed at 1,746 cm^{-1}. The C–H stretching vibrations of methylene groups between 2,800 and 3,000 cm^{-1}, and symmetric and antisymmetric bending of methylene groups at 1,370 cm^{-1} and 1,435 cm^{-1} were observed. Also, bands corresponding to asymmetric stretching of a carboxylate group and asymmetric C–O–C bond stretching from the pyranose ring were observed at 1,234 cm^{-1} and 1,049 cm^{-1} [52], respectively.

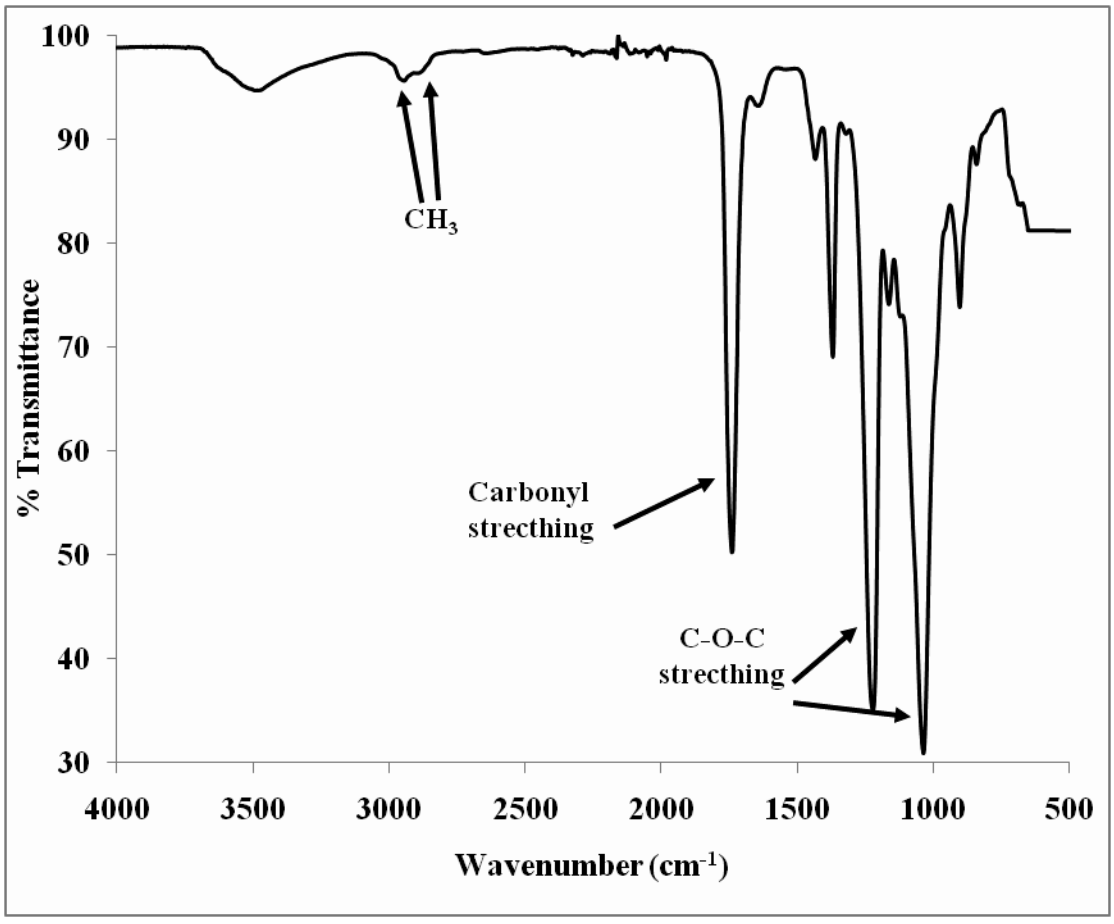


Figure 4.24 An ATR-FTIR spectrum of a bare CA membrane.

4.3.1.1 Formation of PDADMAC/PSS and PDADMAC/PSSMA multilayers

The PDADMAC/PSS and PDADMAC/PSSMA multilayer thin films fabricated on a CA membrane were investigated by ATR-FTIR. In this study, the spectrum of PSS cannot be investigated by ATR-FTIR due to the overlay of the spectrum band at ~ 1000 - 1100 cm^{-1} between CA membrane and PSS. The spectra are shown only for PEMs with the odd layers having PDADMAC as the outermost layers. Figure 4.25 shows characteristic infrared peaks of the stretching of C–N group at ~1125 cm^{-1}, the stretching of NR_4 at ~1640 cm^{-1} and the board peaks of O-H and N-H appeared at the position of ~3300 cm^{-1} [24, 53]. It can be seen that the C-N and NR_4 peak height increased with the number of the coating layers, indicating the growth of PEMs on the membrane surface.

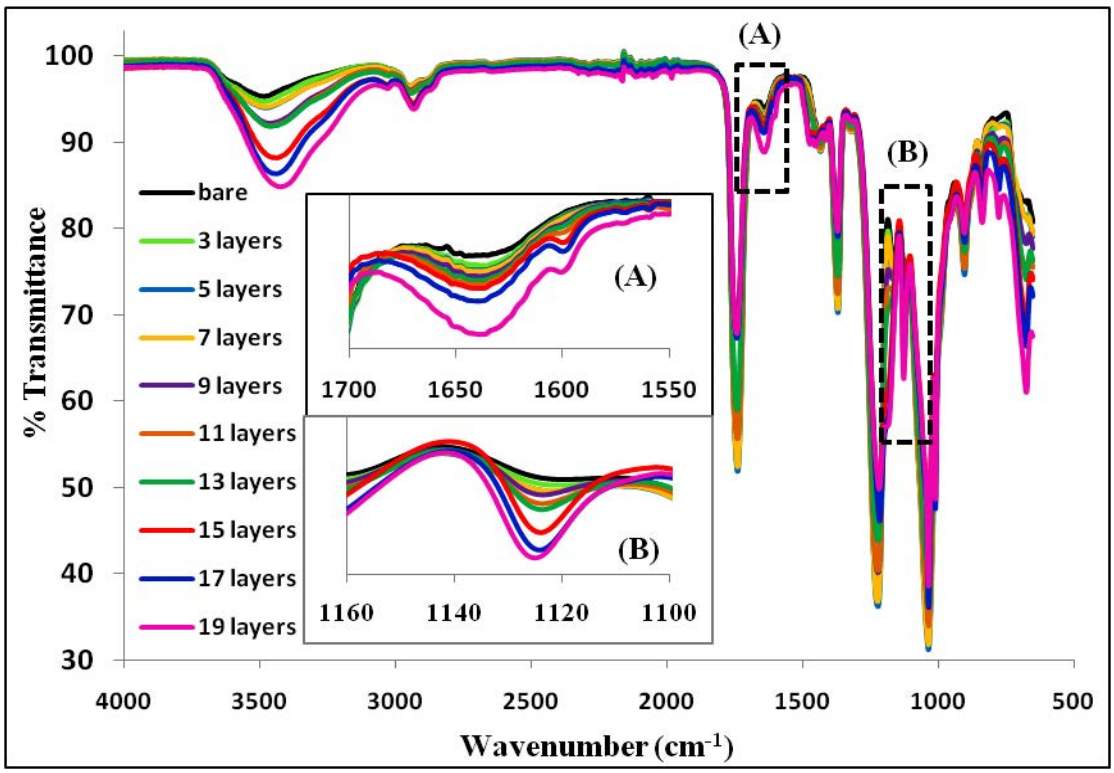


Figure 4.25 ATR-FTIR spectra of unmodified and modified CA membranes with PDADMAC/PSS from the 3rd to the 19th layers (shown only the odd layers of the multilayers). Inset shows the increase in (A) NR_4^+ peak at ~1640 cm^{-1} and (B) C-N peak at ~1125 cm^{-1}.

Based on the result in the section 4.2.3, the PDADMAC/PSSMA fabricated at pH 5.5 was chosen to build on a CA membrane and the growth of the PEMs was investigated using ATR-FTIR. Similarly, the deposition of multilayers thin film on a membrane was studied with the PDADMAC as the outermost layer (Figure 4.26). The increase of C-N and NR_4 peak heights indicated the growth of PEMs on the membrane surface.

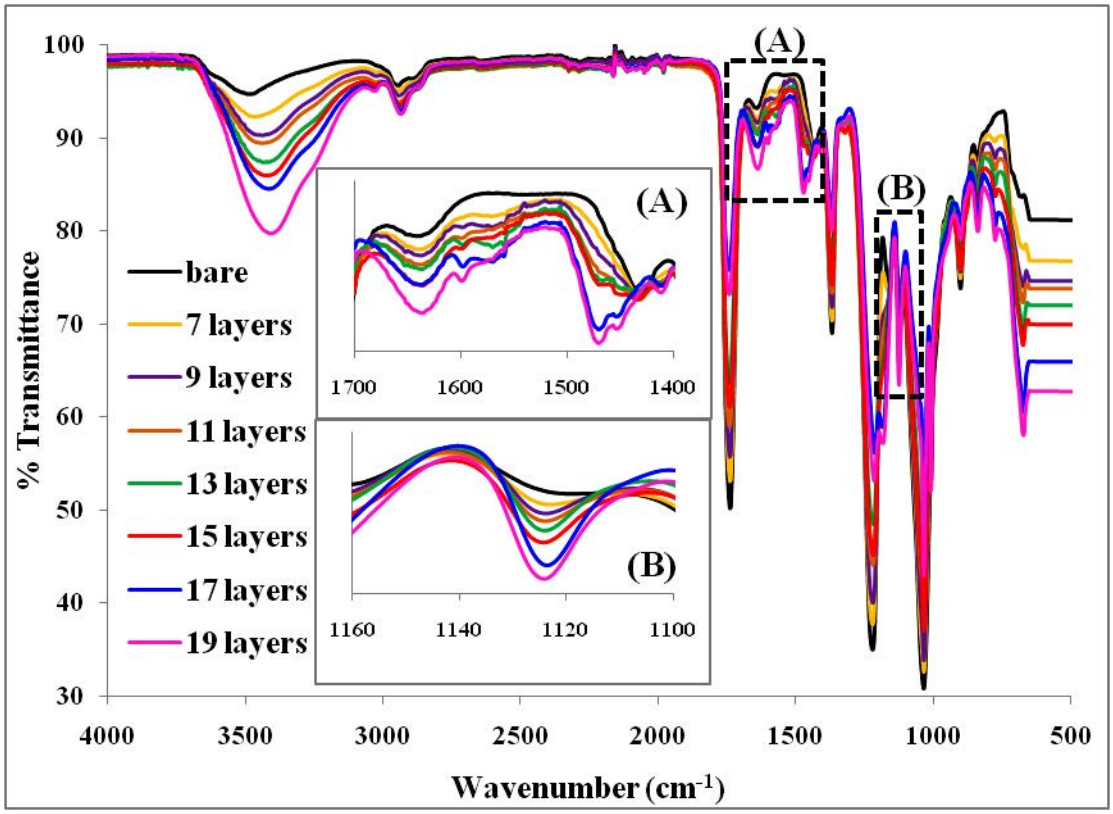


Figure 4.26 ATR-FTIR spectra of unmodified and modified CA membranes with PDADMAC/PSSMA at pH 5.5 from the 7th to the 19th layers (shown only the odd layers of the multilayers). Inset shows the increase in (A) NR_4 peak at ~1640 cm^{-1} and (B) C-N peak at ~1125 cm^{-1}.

4.3.2 The morphology of PEMs on CA membrane

The surface morphology of the unmodified and modified-PEMs membranes, with different numbers and types of polyelectrolyte multilayers, has been examined using SEM. As shown in Figure 4.27, the porosity of the modified membranes (both PDADMAC/PSS and PDADMAC/PSSMA) decreased when increasing the number of coated polyelectrolyte multilayers, which corresponds to the previous work [54]. In addition, the SEM image of the unmodified CA membrane was clearly different from the images obtained from the modified membranes. This confirms the success of the polyelectrolyte modification onto the CA membranes.

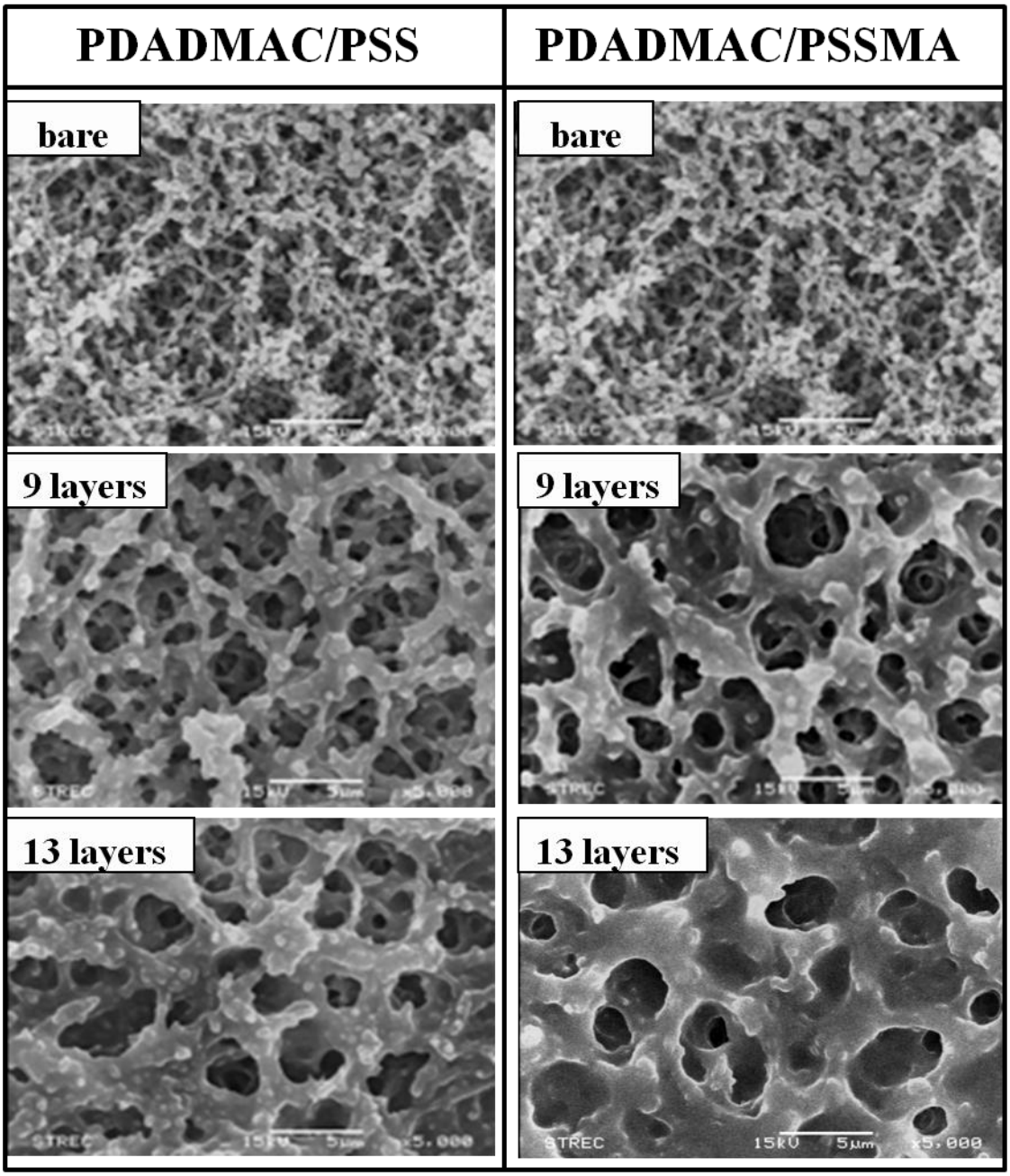


Figure 4.27 SEM images of unmodified CA membrane (bare) and CA membranes modified with PDADMAC/PSS and PDADMAC/PSSMA at 9th and 13th layers.

4.3.3 Effect of charge of the outermost layer of PEMs on protein adsorption

ATR-FTIR was used to monitor the BSA protein adsorption onto the multilayers of the PDADMAC/PSS membrane filter. The odd layers are PDADMAC as the outermost layer, while the even layers are the PEMs film with PSS as the outermost layer.

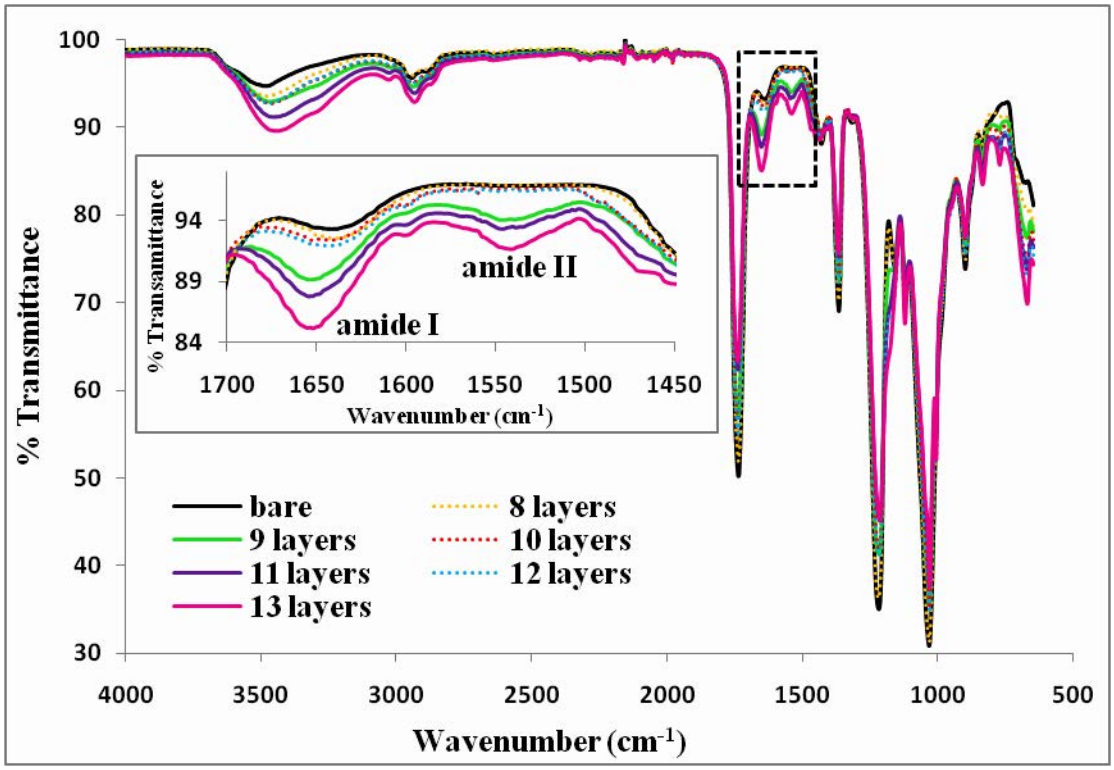


Figure 4.28 ATR-FTIR spectra of protein adsorbed on CA membrane modified with PDADMAC/PSS multilayers having varied number of layers from 8th to the 13th layers. The solid lines represent the protein adsorbed on the odd layers of PDADMAC as the outermost layer, whereas the dashed lines represent the protein adsorbed on the even layers of PSS as the outermost layer. Inset shows the increase in amide I and amide II peaks at ~1650 and ~1540 cm^{-1}, respectively.

Figure 4.28 presents characteristic infrared peaks of BSA adsorbed on the PDADMAC/PSS films. The amide peak intensity of BSA adsorbed onto the PDADMAC, which is positively charged, was much higher than that of BSA

adsorbed onto the negatively charged PSS as the outermost layer [2]. In addition, no amide peak was observed from the unmodified membrane, indicating no BSA adsorption onto the unmodified CA membrane. These results were expected because the adsorption was performed at a pH value above the isoelectric point (pI) of BSA (pI = 4.7) [17], which makes the net charge of the protein to be negatively charged. Through electrostatic interaction, the negatively charged protein is expected to adsorb favorably onto the positively charged PDADMAC. The amount of adsorbed protein on the PSS outermost layer was less, due to electrostatic repulsion between negatively charged BSA and PSS. In addition, when the number of PEMs layers was increased, the amount of protein adsorbed was found to be greatly enhanced, especially in PEMs of PDADMAC as the outermost layer. However, the amount of adsorbed protein on modified membrane with the negatively charged PSS as the outermost layer was slightly increased with the number of coating thin film layers of the thin film. This might be due to the non-electrostatic forces, such as π-π interaction or hydrogen bonding.

4.3.4 Effect of the number of layers of PEMs on protein adsorption

4.3.4.1 Protein adsorption on PDADMAC/PSS membrane filter

ATR-FTIR was used to investigate the BSA protein adsorption onto the multilayers of the PDADMAC/PSS membrane filter. The deposition of multilayers thin film on membrane was shown only the odd layer (between 5-13 layers) of PEMs film with PDADMAC as the outermost layers. Figure 4.29 represents FTIR spectra of PEMs modified membranes at different layers used to filter protein at pH 7.4. FTIR peak substraction between before and after dipping into 1 ppm BSA solution was also demonstrated and shown in Figure 4.30.

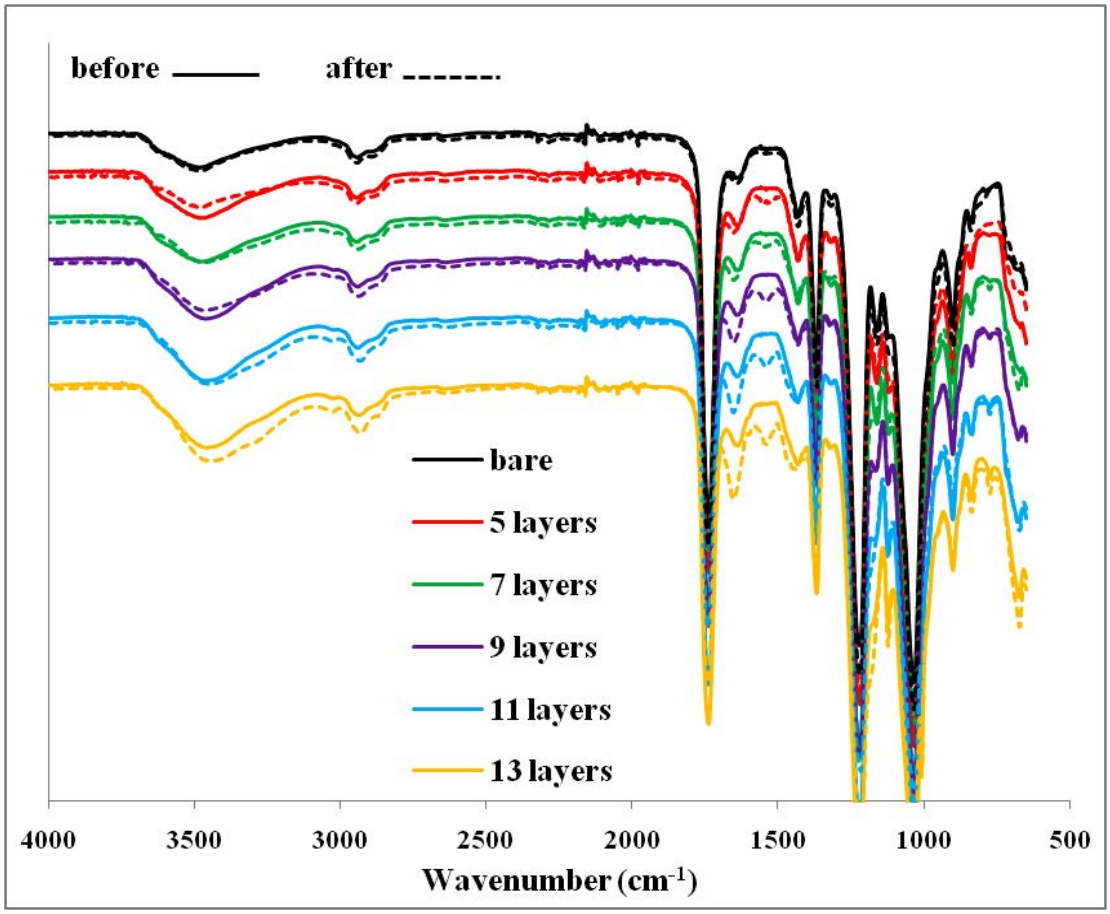


Figure 4.29 ATR-FTIR spectra of protein adsorbed at pH 7.4 on CA membrane modified with PDADMAC/PSS multilayer with various numbers of layers. Solid lines represent the data of CA membrane modified with PEMs before filtration of BSA solution, whereas the dashed lines represent the data obtaining after filtration BSA solution.

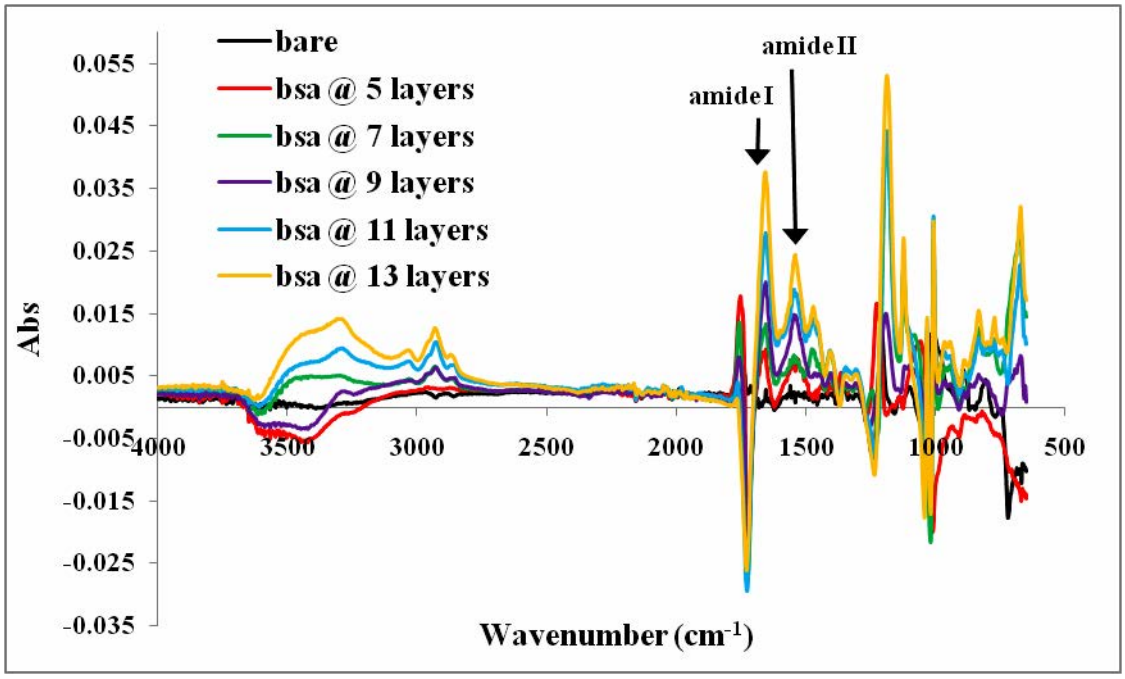


Figure 4.30 FTIR peak substraction between before and after filtration of protein at pH 7.4 on CA membranes modified with PDADMAC/PSS at different layers.

When the number of PEMs layers was increased, the amount of protein adsorbed was found to be greatly enhanced. This is in great agreement with the previous work [49].

The total peak areas of amide I and amide II of BSA at ~1650 cm^{-1} and ~1540 cm^{-1}, relating to the amount of adsorbed BSA, was measured using the OMNIC program. It was found that the amount of absorbed protein was increased with the number of PEMs layers as seen in Figure 4.31. Although the amount of protein adsorption depends on the number of PEMs coating layers [14], the highest number of PDADMAC/PSS layers on the membrane filter was found to be the 13^{th} layer, in which PDADMAC (polycation) is the outermost layer. This is due to the high back pressure leading to the leakage of solution from the filtration unit when membrane modified with numbers of PEMs coating layers is more than 13 layers.

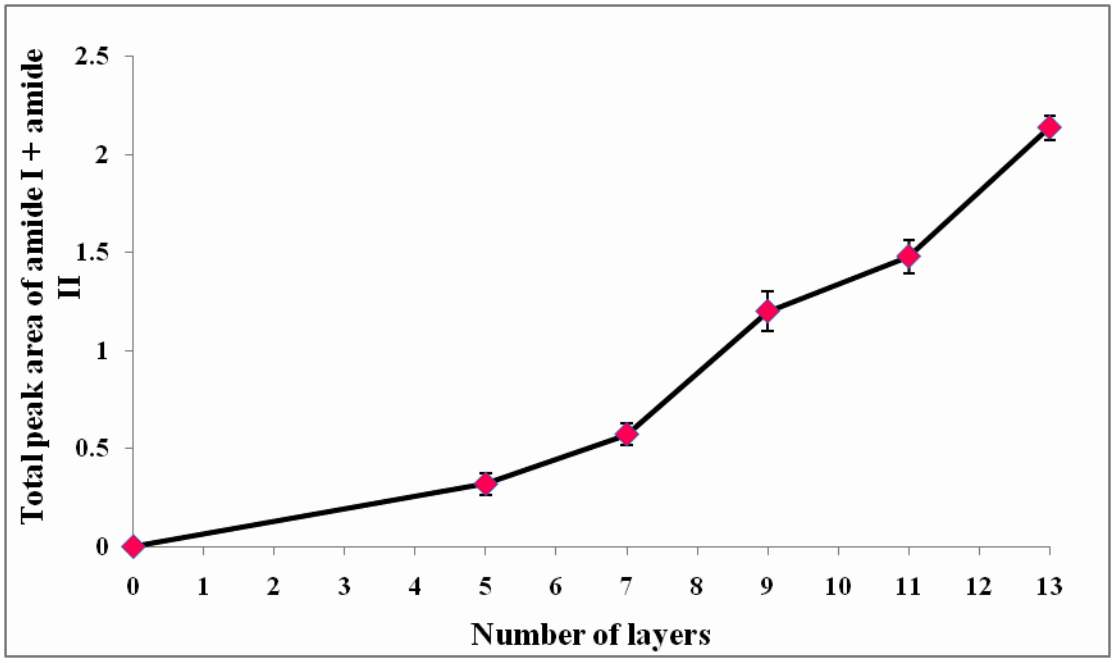


Figure 4.31 The plot of peak area of amide I and amide II of BSA adsorbed on the PDADMAC/PSS membrane versus the number of coating layers.

4.3.4.2 Protein adsorption on PDADMAC/PSSMA membrane

Based on Figure 4.32 to Figure 4.34, the FTIR spectra obtained from PDADMAC/PSSMA was showed. When the number of PEMs layers was increased, the amount of protein adsorbed was found to be greatly enhanced, as seen from the increase in amide I and amide II peak heights. Similarly, the optimum number of PDADMAC/PSSMA coating layers on the membrane filter was found to be 13 layers due to the leakage during the filtration process.

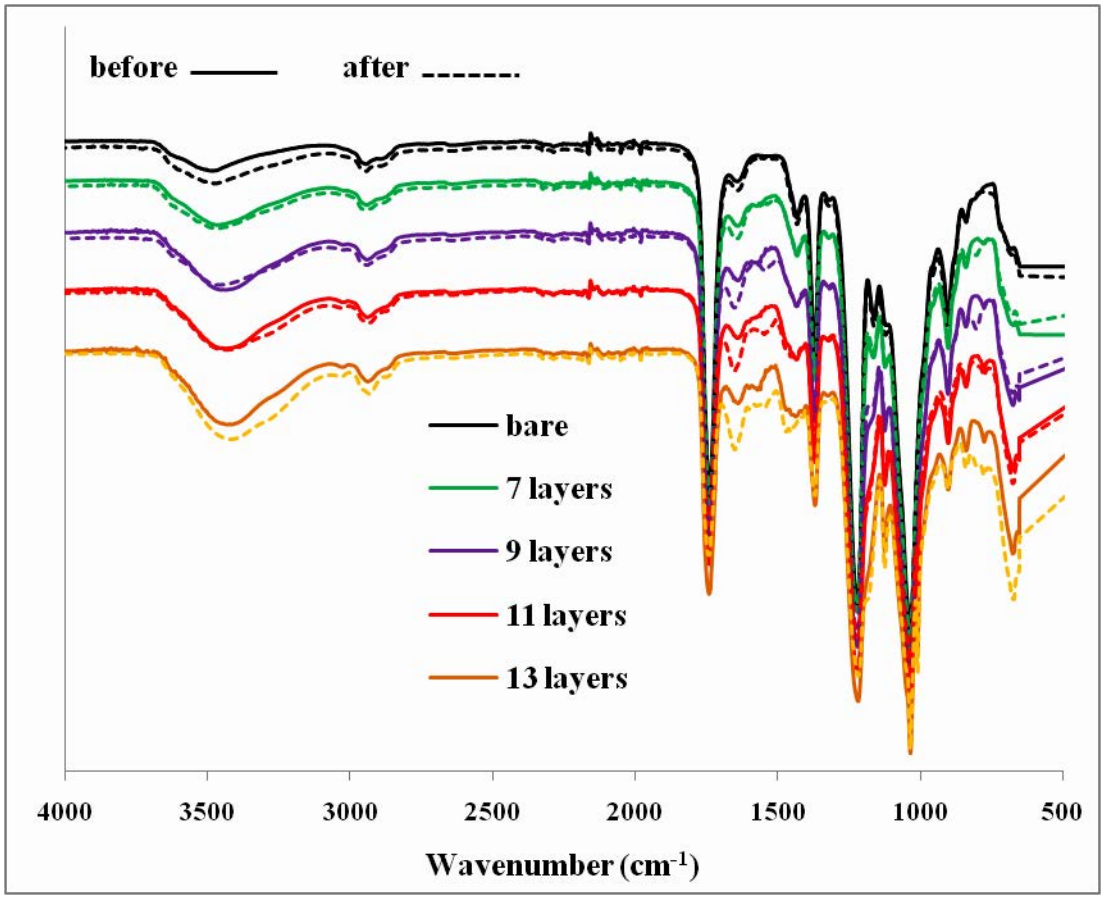


Figure 4.32 ATR-FTIR spectra of protein adsorbed at pH 7.4 on CA membrane modified with PDADMAC/PSSMA multilayer with various numbers of layers. Solid lines represent the data of CA membrane modified with PEMs before filtration of BSA solution, whereas the dashed lines represent the data obtaining after filtration of BSA solution.

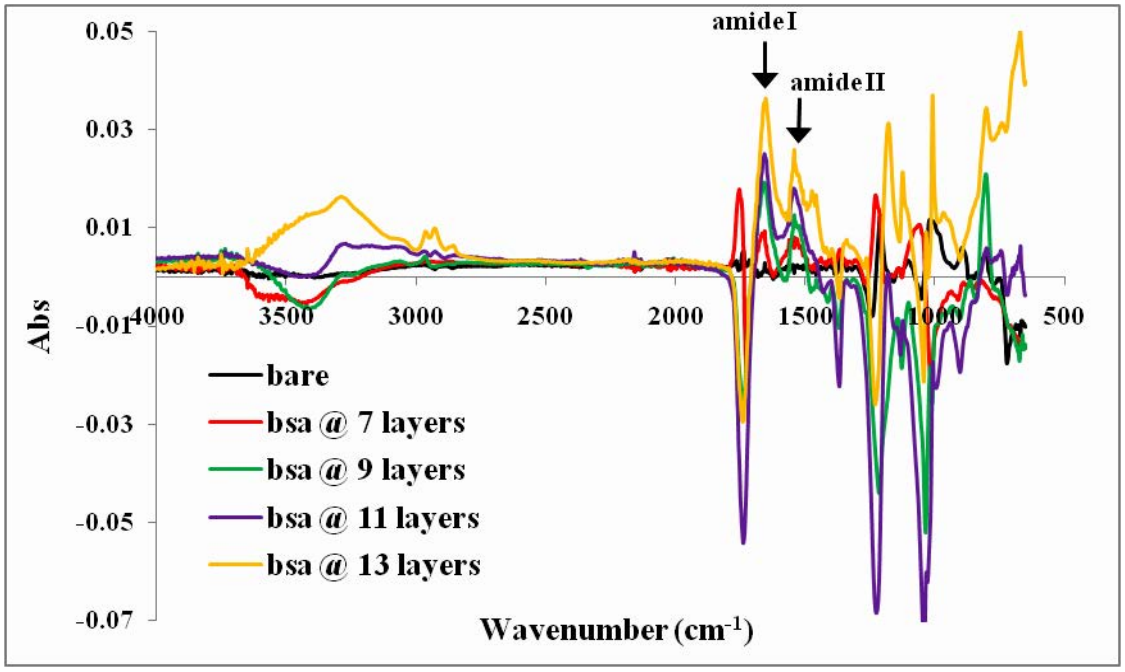


Figure 4.33 The FTIR peak substraction between before and after filtration of protein at pH 7.4 on CA membranes modified with PDADMAC/PSSMA at different layers.

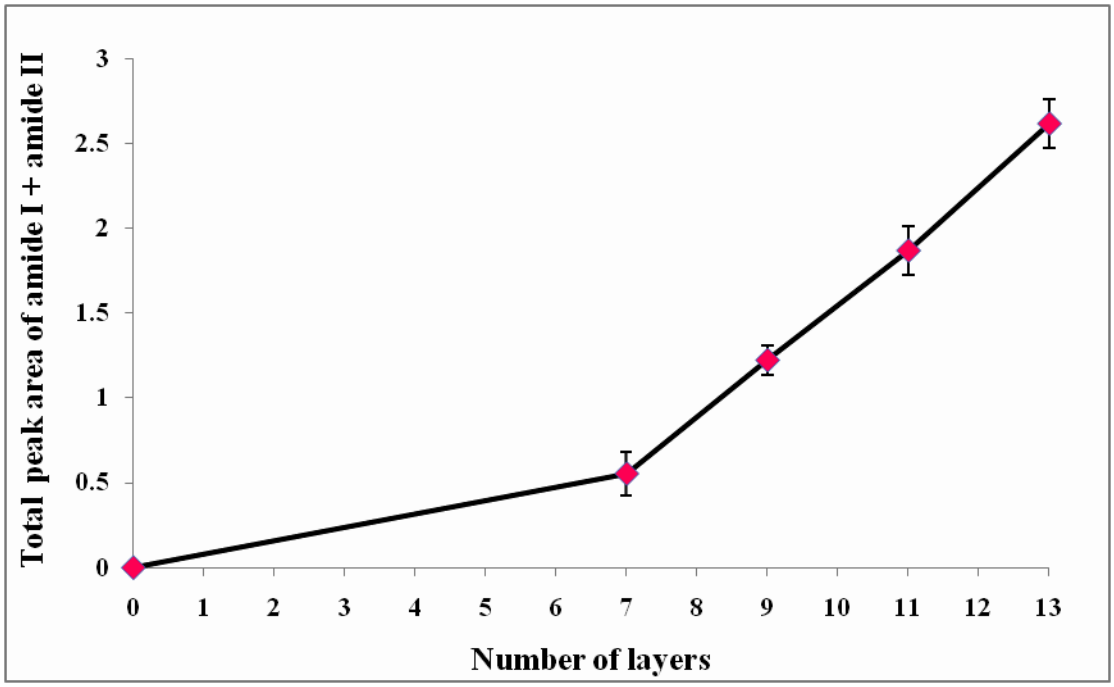


Figure 4.34 The plot of peak area of amide I and amide II of BSA adsorbed on the PDADMAC/PSSMA membrane versus the number of coating layers.

From this study, PDADMAC/PSS and PDADMAC/PSSMA can intake protein. Furthermore, when the number of PEMs coating layers was increased, the amount of protein adsorbed was found to be greatly enhanced. Therefore, the amount of adsorbed protein depends on the charged and the number of coating layers of polyelectrolyte multilayers film. The optimum number of PDADMAC/PSS and PDADMAC/PSSMA coating layers on the membrane filter was found to be 13 coating layers, in which PDADMAC (polycation) is the outermost layer.

4.3.5 Breakthrough of PEMs modified membrane in protein adsorption

Breakthrough curves for BSA adsorption of the modified PEMs membranes with PDADMAC/PSS and PDADMAC/PSSMA were studied. The breakthrough curves would indicate the maximum volume of BSA that can be loaded onto PEMs. The concentrations of 1 ppm and 30 ppm of BSA (pH 7.4) solutions were chosen because this concentration range indicates an early stage of the renal failure. Protein adsorbed at pH 7.4 on the membrane filter was monitored using the total peak area of amide I (~1640 cm^{-1}) and amide II (~1540 cm^{-1}).

4.3.5.1 Breakthrough of BSA on PDADMAC/PSS membrane on BSA protein adsorption

The design of this experiment was based on that the concentration of sample is unknown and the preconcentration of trace level of BSA is essential. The volume required to reach highest adsorbed amount when the concentration of BSA in sample is 1 ppm should be a key. In case of the higher content of albumin presenting in the sample, the volume required to reach the plateau should be lower. Therefore, the breakthrough study of PDADMAC/PSS was studied using 1 ppm BSA.

The membranes modified with PDADMAC/PSS with 13 deposited layers, in which PDADMAC is the outermost layer, were used to filter 1 ppm of BSA solution. From Figure 4.35, it was found that protein adsorbed on the PEMs film rapidly increased when the loading volume increased from 50 to 100 mL as one can

be seen from the increase in the total amide peak area. The absorbed mass was reached the highest value after passing 100 mL of 1 ppm BSA.

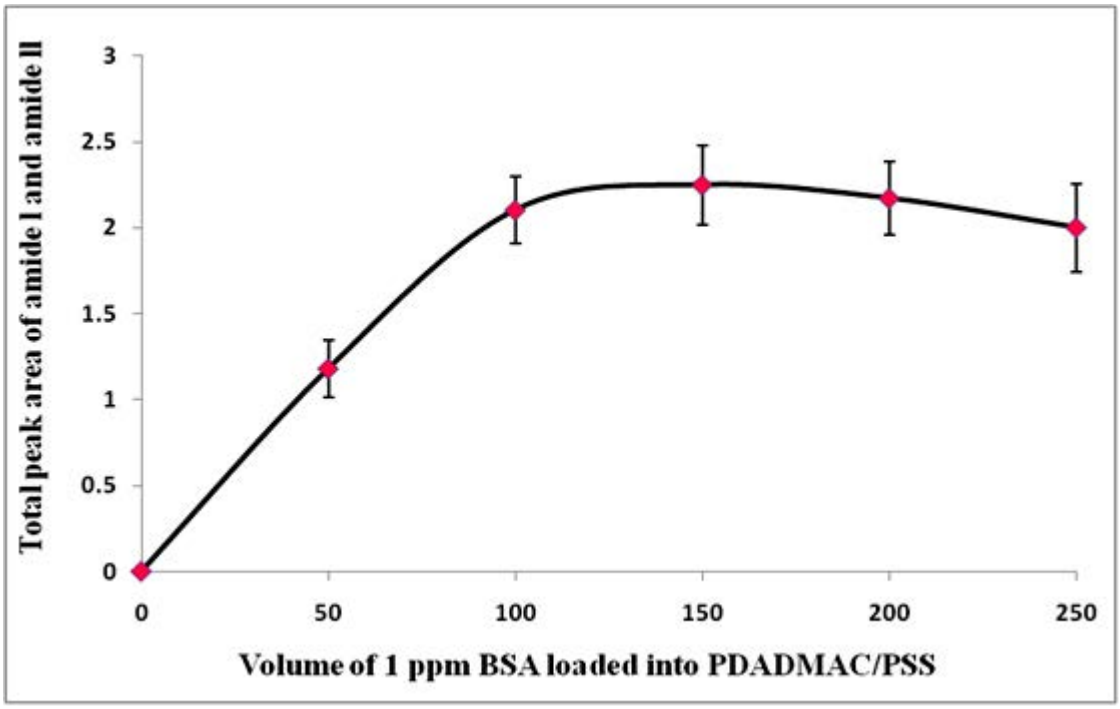


Figure 4.35 A breakthrough curve of the PDADMAC/PSS membrane filter when loading 1 ppm BSA solution at different volumes.

In order to prove our assumption that the volume required to reach the highest adsorbed amount of BSA, when the BSA concentration in sample is higher than 1 ppm, should be lower. The breakthrough curve study using 30 ppm BSA was conducted. The result was shown in Figure 4.37. A similar result was observed that increasing the volume of 30 ppm BSA resulted in the increase in adsorbed BSA content in PDADMAC/PSS film. The intake of protein on the PDADMAC/PSS membrane filter reached the equilibrium after flowing 50 mL of 30 ppm of BSA solution.

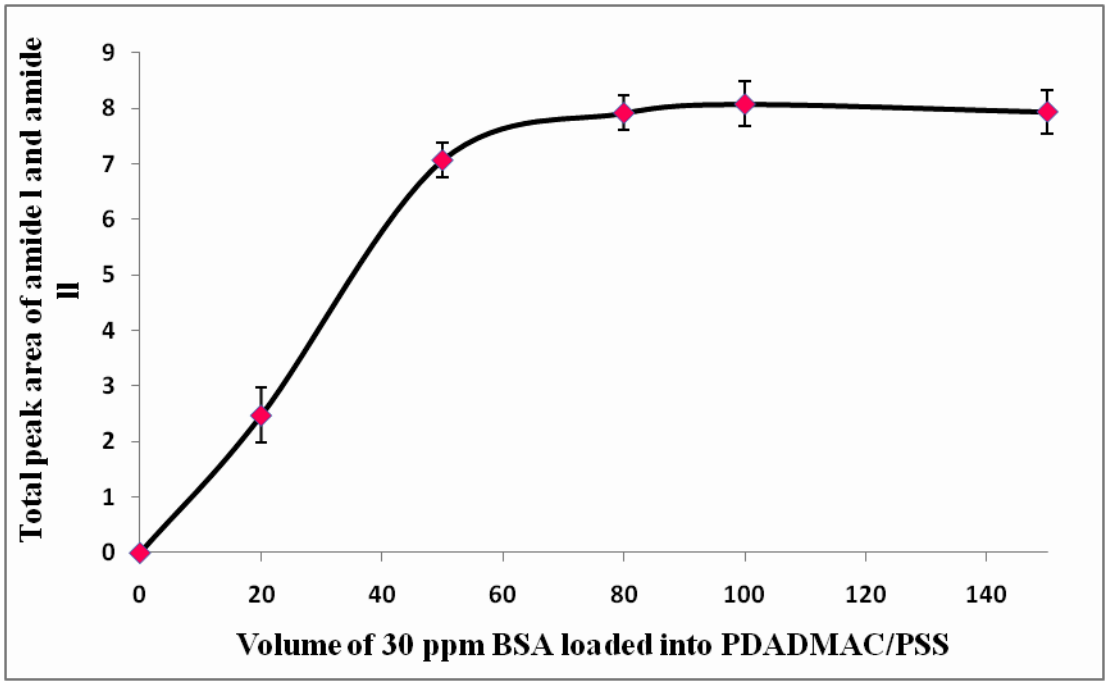


Figure 4.36 A breakthrough curve of the PDADMAC/PSS membrane filter when loading 30 ppm BSA solution at different volumes**.**

4.3.5.2 Breakthrough of BSA on PDADMAC/PSSMA membrane on BSA protein adsorption

In this section, only the breakthrough curve study of 1 ppm was conducted. The maximum loading volume of 1 ppm BSA solution was 150 mL as shown in Figure 4.37.

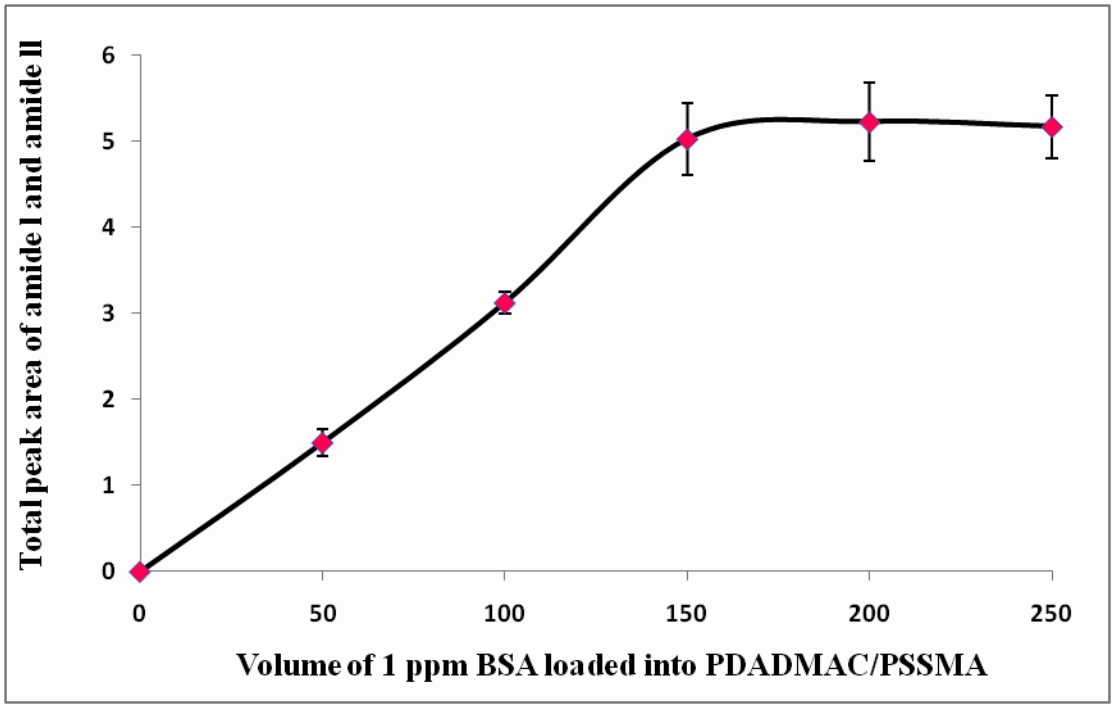


Figure 4.37 A breakthrough curve of the PDADMAC/PSSMA membrane filter when loading 1 ppm BSA solution at different volumes**.**

From Figures 4.35 and 4.37, it can be seen that larger amount of 1 ppm BSA can be loaded into the PDADMAC/PSSMA. This could be because of the thicker film obtained from PADMAC/PSSMA pair compared to the PDADMAC/PSS film. This was confirmed by AFM data as presented in Table 4.1. The PDADMAC/PSS and PDADMAC/PSSMA multilayer films thickness were 129.03 and 241.17 nm, respectively. From the AFM data, multilayer films thicknesses showed the same trend as the UV-Vis data that more polyelectrolyte deposited on CA membrane was found in the system of the PDADMAC/PSSMA compared to the PDADMAC/PSS pair. Therefore, the PDADMAC/PSSMA film could adsorb protein more than PDADMAC/PSS film did.

Figure 4.38 showed an AFM image of protein adsorbed on the PDADMAC/PSS films.

Table 4.1 Thickness of PEMs before and after protein adsorption.

Type of PEMs membrane filter	**Thickness (nm)**	
	Before dipped into protein	**After dipped into protein**
PDADMAC/PSS	129.03 ± 2.99	144.33 ± 4.64
PDADMAC/PSSMA at pH 5.5	241.17 ± 8.79	272.80 ± 5.46

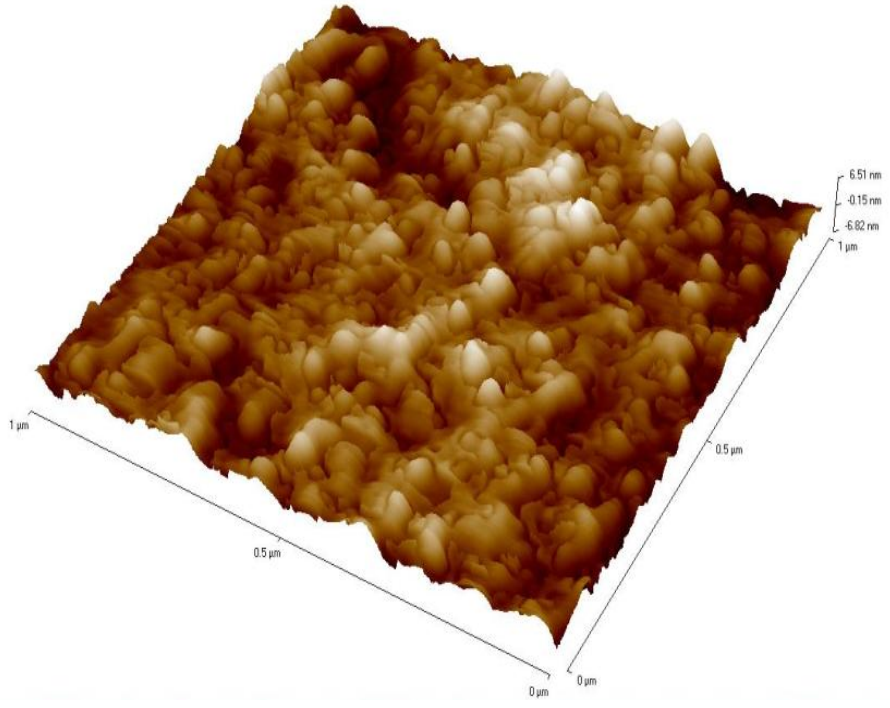


Figure 4.38 An AFM image of BSA adsorbed on $(PDADMAC/PSS)_{13}$ multilayer thin films.

4.4 Preconcentration of Protein

4.4.1 Study of the suitable releasing time

Releasing of protein adsorbed on the modified membrane was performed using pH 3.0 citric-citrate buffer solution, which was previously used as a desorption solvent by Yang, B. et al. [18]. At this pH, the net charge of protein is changed to be positive, resulting in electrostatic repulsion between positively charged protein and PDADMAC film. The citric-citrate buffer was chosen as a releasing agent. The optimum volume of releasing agent was chosen to be 3 mL because this is a minimum volume required to cover the whole diameter of the membrane in order to avoid the dilution effect.

The releasing time of protein adsorped onto PEMs-modified membrane filter was evaluated by passing 1 ppm BSA through the thirteen-coating layer PDADMAC/PSS and PDADMAC/PSSMA membranes. Then, the studied filters were immersed in the citric-citrate buffer solution (pH 3.0) at various times (3, 5, 10, 15 and 20 min). After releasing step, the solution was quantitatively measured using BCA assay to quantify the amount of protein. Based on Figure 4.39, the amount of BSA in the releasing buffer solution was rapidly increased between 0 to 3 minutes, and then the slower releasing rate was found between 3 to 10 minutes. After 10 minutes soaking time, the amount was found to be relatively constant. Therefore, the 10 minutes was selected as a suitable desorption time used in all further experiments.

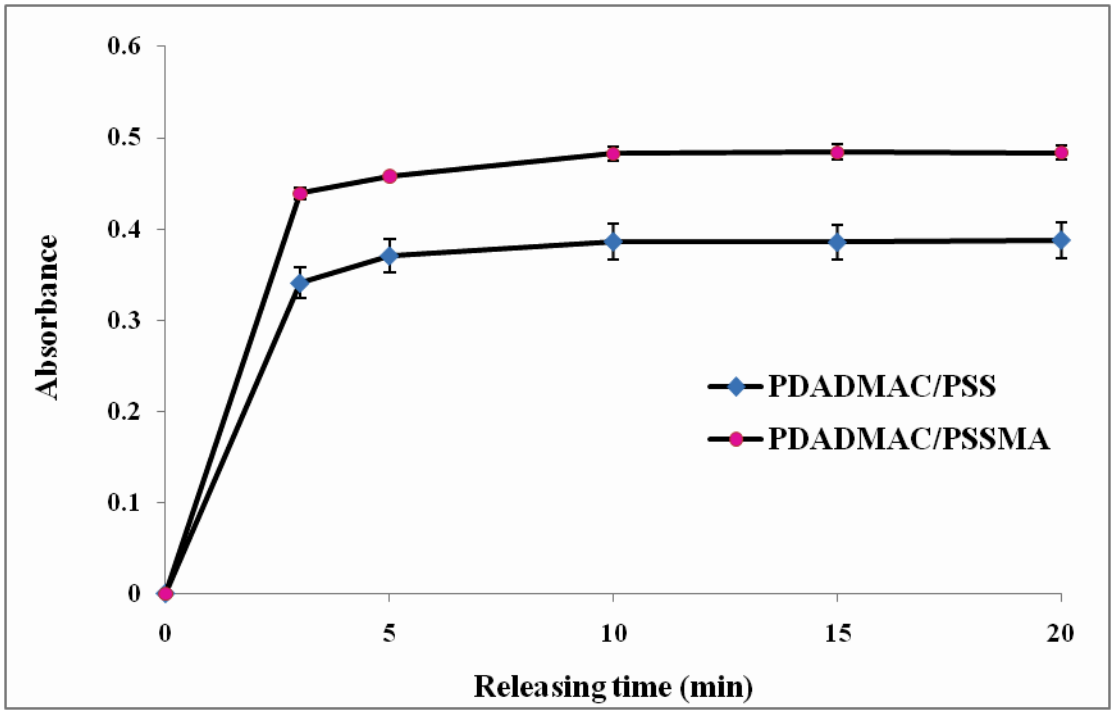


Figure 4.39 Releasing time of BSA adsorbed on the PDADMAC/PSS and PDADMAC/PSSMA membranes. Protein releasing was performed using the citric-citrate buffer and the amount of protein was measured using BCA assay.

After the desorption step, the chemistry of PEMs-modified membranes was studied using ATR-FTIR to confirm that the BSA was desorbed from PEMs-modified membrane completely. As showed in Figure 4.40 and 4.41, there were no peaks of amide I and amide II of BSA, implying that , the success of the desorption step.

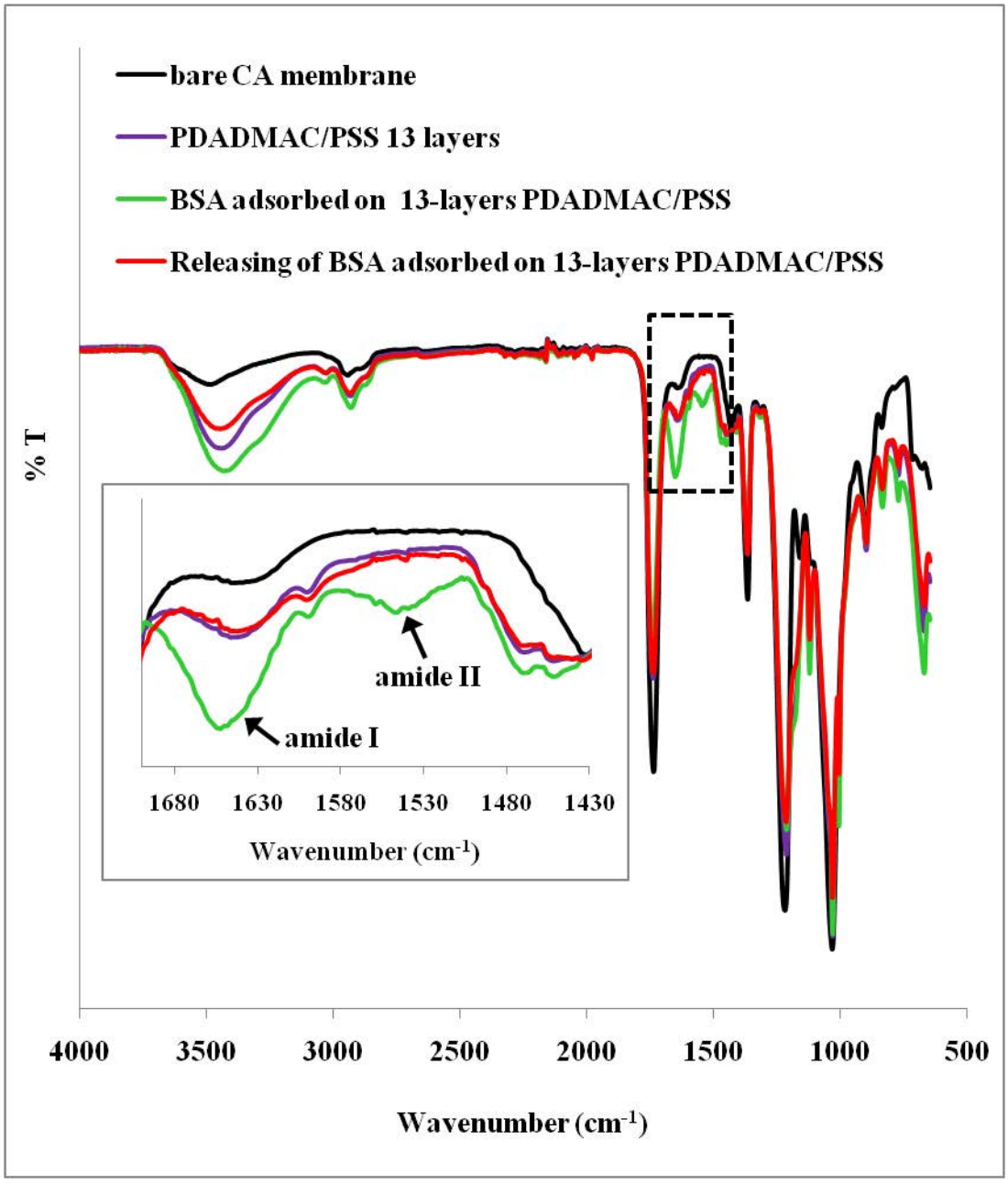


Figure 4.40 ATR-FTIR spectra of CA membrane modified with 13-layers of PDADMAC/PSS multilayers before immersing in BSA solution (_____), after immersion step (_____) and after desorption step (_____). Inset shows the increase in amide I and amide II peaks at ~1650 and ~1540 cm^{-1} when BSA was adsorbed on PEMs membranes and the decrease in amide I and amide II peaks when BSA was desorbed.

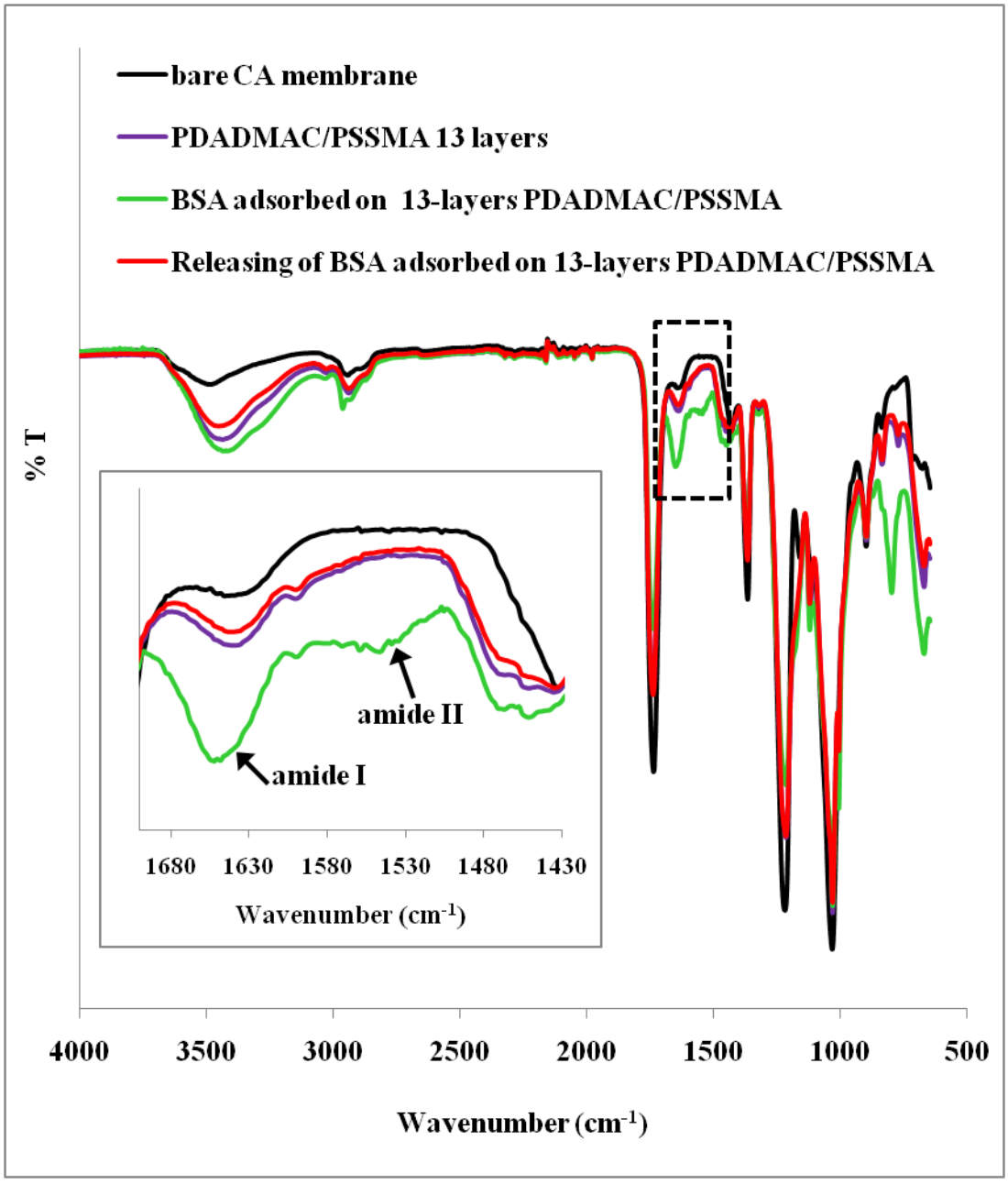


Figure 4.41 ATR-FTIR spectra of CA membrane modified with 13-layers of PDADMAC/PSSMA multilayers before immersing in BSA solution (_____), after immersion step (_____) and after desorption step (_____). Inset shows the increase in amide I and amide II peaks at ~1650 and ~1540 cm^{-1} when BSA was adsorbed on PEMs membranes and the decrease in amide I and amide II peaks when BSA was desorbed.

4.4.2 Effect of pH of albumin solution for protein preconcentration

The effect of pH of BSA solution on protein preconcentration was investigated. Results are shown in Table 4.2.

Table 4.2 The amount of albumin after the preconcentration step of 1 ppm BSA at various pHs

Type of PEMs-	**Average amount of BSA (ppm), (n=3)**			
	pH 5.5	**pH 6.2**	**pH 6.8**	**pH 7.4**
bare	0.02 ± 0.30	1.30 ± 0.06	1.46 ± 0.15	3.55 ± 0.90
PDADMAC/PSS	12.2 ± 0.5	22.3 ± 0.8	26.6 ± 1.7	29.8 ± 0.4
PDADMAC/PSSMA	17.7 ± 1.2	26.0 ± 0.7	32.5 ± 0.5	40.6 ± 1.7

The enrichment factor was increased with increasing pH, especially at pH 7.4. Since the isoelectric point of BSA is 4.7, at the pH 7.4, albumin posses more negative charges than at pHs 5.5 and 6.8, resulting in more content of albumin adsorbed on the PEMs. Moreover, the preconcentration capability of the PDADMAC/PSSMA membrane is better than that of PDADMAC/PSS. This is because of the effect of thicker PEMs layer as shown in Table 4.1 and the higher loading capacity of PADMAC/PSSMA over the PADMAC/PSS (section 4.2.1).

In this study, immunoglobulin (IgG) having pI of 6.85 [19] was chosen as one of the interferences. Because the charges of IgG might interfere with adsorption of BSA on PEMs, the pH effect on the IgG adsorption on the PEMs-modified membrane filter must be evaluated to find the suitable working pH value. The studied pH range was picked according to the result in the section 4.4.2. The membrane filters,

including bare CA, CA membranes modified with either PDADMAC/PSS or PDADMAC/PSSMA, were used to filter 0.6 ppm IgG solution at various pHs of 6.2, 6.8 and 7.4. A pH 5.5 was not used in this study because the amount of albumin after the preconcentration process was low. The content of adsorbed IgG was quantified as described in section 3.9.1. Results are shown in Table 4.3.

Table 4.3 The content of IgG after the preconcentration step at various pHs.

Type of PEMs membrane filter	**Average amount of IgG (ppm), (n=3)**		
	pH 6.2	**pH 6.8**	**pH 7.4**
bare	0.52 ± 0.36	3.93 ± 0.28	10.7 ± 0.5
PDADMAC/PSS	N/A	0.62 ± 0.18	0.95 ± 0.89
PDADMAC/PSSMA	3.80 ± 1.13	10.1 ± 1.1	16.5 ± 0.6

As expected, the amount of adsorbed IgG was increased with increasing pH value. Since the isoelectric point of IgG is 6.8, at pH 7.4, IgG molecules posse negative charges. The higher content of the IgG was found on the positively charged surface, whereas at pH 6.8 (IgG posses a net charge of zero) and pH 6.2 (IgG posses positive charges) showed lower adsorption of IgG. Although, at pHs of 6.2 and 6.8, IgG was less adsorbed onto the PEMs, the preconcentration of albumin was also low. Therefore, pH 7.4 was chosen for albumin preconcentration.

4.5 PEMs modified membrane filter performance

For protein preconcentration, the concentrations of 1 ppm and 30 ppm BSA (pH 7.4) solutions were chosen. A 13-layer PDADMAC/PSS membrane filter was used to filter 100 mL of 1 ppm BSA solution and 50 mL of 30 ppm BSA solution, whereas a PDADMAC/PSSMA membrane filter was used to filter 150 mL of 1 ppm BSA solution.

First, the matrix effect on the response of BCA assay was tested. In this study, the calibration curves were constructed using two types of buffer pH 3 as solvents. The BSA standard solutions were prepared into two sets: freshly prepared citrate-citrate pH 3 buffer solution and citrate-citrate pH 3 buffer solution after soaking with the PEMs-modified filter membranes for 10 minutes. There is no difference in the BCA response (data were not shown here). Therefore, in this study, the calibration curve was constructed using buffer pH 3 as a solvent. The amount of albumin after the preconcentration step was measured and calculated from the calibration curve. Results are shown in Table 4.4.

Table 4.4 The amount of albumin after the preconcentration step using the PEMs-modified membrane filters.

Sample	**Amount of albumin after the preconcentration process (ppm)**		
	PDADMAC/PSSMA	**PDADMAC/PSS**	
	1 ppm BSA	1 ppm BSA	30 ppm BSA
1	41.3	29.2	130.6
2	43.0	31.0	122.6
3	40.7	28.7	118.7
Average (n=3)	41.6 ± 1.2	29.6 ± 1.2	124.0 ± 6.1
Enrichment factor	41.6	29.6	4.1

At 1 ppm BSA, the PDADMAC/PSSMA membrane filter showed higher capability for BSA preconcentration compared to the PDAMAC/PSS membrane filter. This could be due to the thicker film obtained from the PADMAC/PSSMA pair. In addition, the concentration of 30 ppm BSA was enhanced to 124.0 ppm. However, the enrichment factor obtained after preconcentration of 30 ppm of BSA is only 4.1, which is quite low compared to the enrichment factor obtained from the preconcentration study of 1 ppm BSA. This might be because the optimized desorption time is not suitable to desorp this high BSA concentration level. Moreover, the enhanced concentration (124.0 ppm) is still too low to be detected with the protein strip test (300 ppm), which is the cheaper strip test than the microalbumin strip test Therefore, the 30 ppm BSA solution was not chosen for further study due to the time limit.

In addition, the preconcentration efficiency using the modified PDADMAC/PSS and PADMAC/PSSMA membranes filter were tested using the simple semi-quantitative urine test strip. Figure 4.42 shows the test strips after being dipped into the BSA solutions (1 and 30 ppm) both before and after performing preconcentration step. Clearly, the 1 ppm-BSA solution could not be detected by this strip test, while the albumin solution after preconcentration step could be easily detected by the strip test. Based on the Figure 4.42 (a and c), the 1 ppm-albumin solution was increased to be approximately 30 ppm, which is quite close to the found concentration using the BCA assay (29.6 ppm and 41.6 ppm). Based on the color of the strip test in Figure 4.43 (b), the 30 ppm BSA solution was found to be between 80-150 ppm.

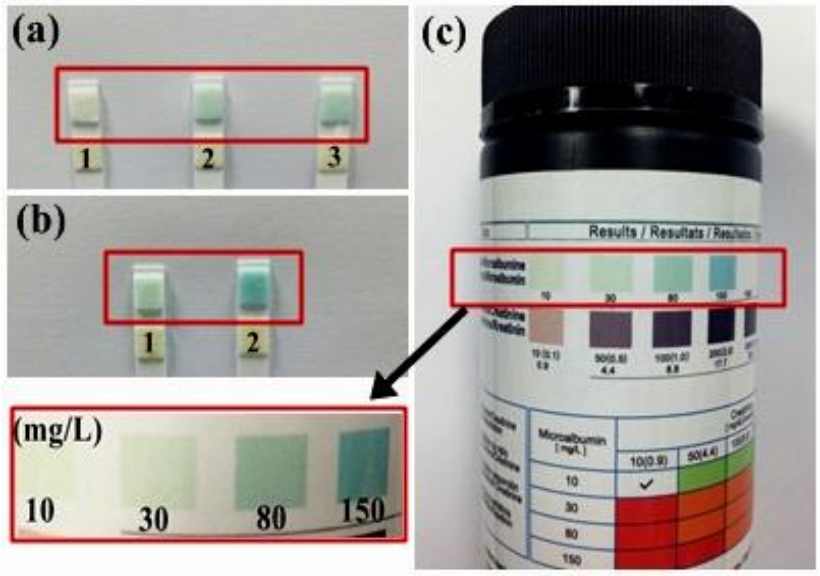


Figure 4.42 The test strips after being dipped into (a.1) 1 ppm of BSA (before preconcentration), (a.2-3) solution obtained from the preconcentration of 1 ppm BSA using PDADMAC/PSS and PDADMAC/PSSMA, respectively, (b.1) 30 ppm of BSA (before preconcentration), (b2) solution obtained from the preconcentration of 30 ppm BSA using PDADMAC/PSS and (c) the color blocks printed on the bottle label with an expanded inset.

Based on our results, one can see that the PEMs-modified membrane filter can be used to enhance the albumin concentration, making it possible to use microalbumin urine strip test to detect the trace amount of protein, such as 1 ppm where the solely urine strip test fail to detect. In the further study, the performance of PEMs-modified membrane filter was tested against 1 ppm BSA solution.

Although the PDADMAC/PSSMA-modified membrane filter delivered better preconcentration factor for 1 ppm BSA, the IgG adsorption is also large compared to the filter modified with PDADMAC/PSS. This will interfere the adsorption of BSA on the PEMs. Therefore, the membrane filter modified with PDADMAC/PSS was chosen for the further study.

4.5.1 Interferences of protein preconcentration performance

To study the effect of interfering substances on the preconcentration of albumin using the PEMs modified membrane, several common interfering substances, such as iImmunoglobulin (IgG), glucose (glu) and NaCl, were added to 1 ppm BSA standard solution. The interference concentrations were prepared according to the previous work [55] by keeping the similar ratio between albumin and the interferences (0.6 ppm IgG, 300 ppm NaCl and 250 ppm glucose).

The content of albumin and interferences (IgG and NaCl) after the preconcentration step with the PDDMAC/PSS membrane was measured using the BCA assay as described earlier. The percentage difference response was calculated using the following equation:

$$\text{Difference response (\%)} = \left[\frac{C_{BSA + Interference} - C_{BSA}}{C_{BSA}} \right] \times 100$$

where $C_{BSA+ Interference}$ is concentration of BSA and interferences after preconcentration process; C_{BSA} is concentration of BSA after preconcentration process.

Table 4.5 The amount of albumin and interferences after the preconcentration process of 1 ppm BSA with the PDDMAC/PSS membrane.

Type of PEMs membrane filter	**Total concentration (ppm), (n=3)**		
	BSA	**BSA + IgG**	**BSA + NaCl**
PDADMAC/PSS	30.0 ± 0.9	33.8 ± 1.4	31.6 ± 0.7
(%) Difference response	-	12.5	5.2

Results from Table 4.5 showed that IgG interfered the performance of the modified membrane (12.5%). However, NaCl showed little impact on the performance. This could be because NaCl can slightly swell PEMs, resulting in more adsorption of BSA on PEMs, but not a significant effect.

Because glucose can reduce Cu^{2+} to Cu^{1+}, interfering the response of BCA assay, glucose was measured using the CBBG assay to quantify the amount of protein. The absorbance of solutions from the assay was recorded at 595 nm. Results are shown in Table 4.6.

Table 4.6 The amount of albumin and glucose measured using the CBBG assay after the preconcentration process step of 1 ppm BSA with the PDDMAC/PSS membrane filter.

Type of PEMs membrane filter	**Total concentration (ppm), (n=3)**	
	BSA	**BSA + Glu**
PDADMAC/PSS	29.7 ± 0.2	29.4 ± 0.6
(%) Difference response	-	1.2

From Table 4.6, glucose showed no interfering effect on the BSA preconcentation when tested with the CBBG assay. This could be because small molecule of glucose cannot be adsorbed on the PEMs membranes.

Although, IgG and NaCl affect on performance of PEMs membrane filter (Table 4.4), interferences does not affect to the strip test as shown in Figure 4.43. Therefore, PEMs modified membrane filter can be used to preconcentrate the 1 ppm albumin solution and the simple urinary strip test can be employed without any interferring of neither IgG, NaCl nor glucose.

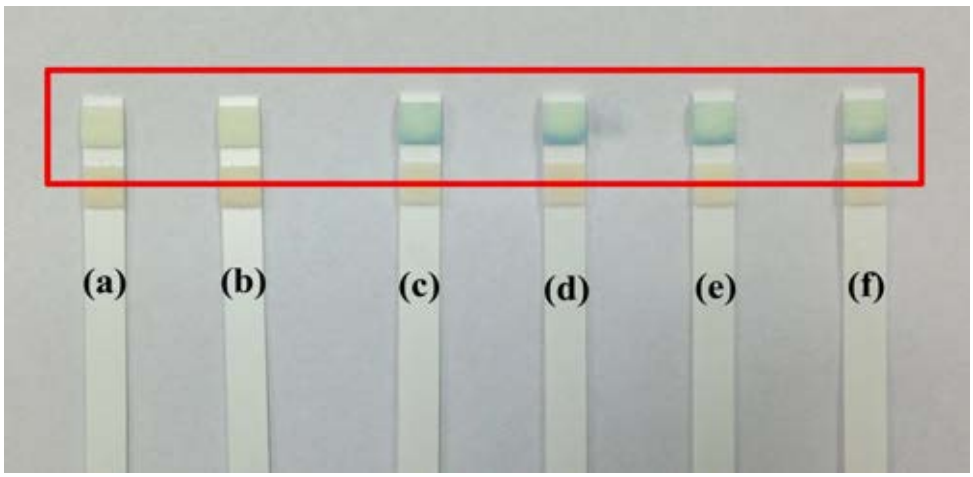


Figure 4.43 The test strips after being dipped into various solutions: a pH 3 buffer as a blank (a), 1 ppm of BSA (before preconcentration) (b), solution obtained after preconcentration step of BSA (c), solution obtained after preconcentration step of BSA + IgG (d), solution obtained after preconcentration step of BSA + NaCl (e) and solution obtained after preconcentration step of BSA + Glu (f).

4.6 Repeatability and recovery of PDADMAC/PSS modified membrane filter for albumin preconcentration

The repeatability and %recovery study of PDADMAC/PSS modified membranes for preconcentration of 1 ppm BSA were evaluated (n = 9). Results indicated that nine PDADMC/PSS modified membranes showed good repeatability on albumin preconcentration with %RSD of 4.33 and %recovery of 90.8, which is accepted for Association of Official Chemists, AOAC (%RSD < 11) and (%recovery 80-110), respectively as shown in Table 4.7.

Table 4.7 Repeatability of PDADMAC/PSS modified membrane filter for preconcentration of 1 ppm BSA at pH 7.4.

Number of modified PDADMAC/PSS membrane filter	**Amount of BSA after the preconcentration process, (ppm)**	**Enrichment factor**	**Recovery %**
1	29.9	29.9	89.7
2	30.5	30.5	91.4
3	30.4	30.4	91.3
4	30.0	30.0	90.0
5	29.2	29.2	87.6
6	33.0	33.0	99.0
7	29.4	29.4	88.2
8	28.1	28.1	84.9
9	30.0	30.0	90.0
Average amount of BSA after the preconcentration process, (ppm)	30.3 ± 1.3 (%RSD = 4.3)	30.3 ± 1.3	90.8 ± 4.0

CHAPTER V

CONCLUSION

A cellulose acetate membrane was modified using the layer-by-layer deposition technique as a membrane filter to adsorb bovine serum albumin (BSA) for albumin preconcentration. The suitable PEMs system was identified by considering the BSA adsorption efficiency on a PDMS substrate. Variables affecting in this step, include types of polyelectrolyte, pH value of weak polyelectrolyte solutions, concentration of sodium chloride, and number of layers on the growth of polyelectrolyte multilayer and protein absorption. The suitable polyelectrolyte pairs are poly(diallyl dimethyl ammonium chloride) (PDADMAC)/poly(sodium 4-styrenesulfonate) (PSS) as a strong polyelectrolyte pair and poly(diallyl dimethyl ammonium chloride) (PDADMAC)/poly (4-styrene sulfonic acid-co-maleic acid) copolymer (PSSMA) as a weak polyelectrolyte pair. In addition, pH 5.5 was found to be the best to fabricate the thin film of the weak polyelectrolyte pair. The optimum number of the PEMs layers fabricated at a NaCl concentration of 1 M on the CA membrane filter both PDADMAC/PSS and PDADMAC/PSSMA is 13 layers, in which PDADMAC is the outermost layer.

The modified membranes were used to filter 1 ppm of BSA solution (pH 7.4) using a syringe pump. ATR-FTIR results showed that the BSA was adsorbed on the PDADMAC/PSS and PDADMAC/PSSMA via electrostatic interaction and the amount of adsorbed BSA increased with the numbers of coating layers of the thin film. In addition, the breakthrough curves of the modified PEMs membranes were studied for BSA adsorption on the PDADMAC/PSS and PDADMAC/PSSMA. The breakthrough volumes, indicating the maximum volume of 1 ppm BSA that can be loaded onto PEMs of PDADMAC/PSS and PDADMAC/PSSMA membranes were found to be 100 mL and 150 mL, respectively. The higher breakthrough volume of PDADMAC/PSSMA could be because the thickness of PADMAC/PSSMA is higher than that of PDADMAC/PSS. The amount of BSA adsorbed on the PDADMAC/PSS

and PDADMAC/PSSMA membranes was then released using a citric-citrate buffer solution pH 3.0 and measured quantitatively using the Bicinchoninic acid assay and the urine test strip. In addition, the effect of pH of BSA solution on protein preconcentration with PEMs modified membrane was investigated and found that the amount of albumin after the preconcentration process increased with pH, especially at pH 7.4. Since the isoelectric point of BSA is 4.7, at pH 7.4, albumin posses more negative charges compared to pH 5.5 and 6.8, resulting in more adsorption of albumin onto the PEMs. The amount of adsorbed BSA onto PDADMAC/PSS and PDADMAC/PSSMA modified membrane were found to enhance from 1 ppm to 29.6 ± 1.2 ppm and 41.6 ± 1.2 ppm, respectively. Furthermore, interferences, including Immunoglobulin (IgG), glucose (glu) and NaCl, were studied and found that the PDADMAC/PSS modified membrane was less interfered from the interference than PDADMAC/PSSMA modified membrane. Therefore, the PDADMAC/PSS membrane was chosen for the preconcentration of BSA. Results showed that glucose did not interfere with the BSA preconcentation when tested using the CBBG assay with only 1.21% of interfering performance. IgG interfered the BSA preconcentration process up to 12.5% and NaCl showed low interfering (5.2%). Although, IgG and NaCl seem to have interfering effect on BSA preconcentation process, this does not affect the strip test. Repeatibility of the fabrication of PDADMAC/PSS modified membrane for the protein preconcentration was evaluated and found that nine PDADMC/PSS modified membranes showed excellent repeatability on albumin preconcentration with %RSD of 4.3 and %recovery of 90.8. This confirms the reproduction of the PEMs fabrication and preconcentration step. From the results obtained, the proposed PEMs-modified filter membrane has shown its ability for trace amount protein preconcentration. This could be useful as a simple and cost-effective preconcentration unit for screening test of renal failure at an early stage where only the microalbumin urine strip test fail.

Suggestion for future research

Suggestion for the further work is to study the performance at 10 ppm BSA using the PDADMAC/PSS membrane filter to cover the concentration where the

urine strip test cannot detect. Additionally, the proposed albumin preconcentration method should be applied for real samples, to guarantee the efficacy of the proposed method for protein preconcentration.

REFERENCES

[1] Peters, T., How Should We Measure the Albumin in Urine? *Clinical Chemistry* **2006**, 52, 555-556.

[2] Woo, J.; Floyd, M.; Cannon, D. C.; Kahan, B., Radioimmunoassay for Urinary Albumin. *Clinical Chemistry* **1978**, 24, (9), 1464-1467.

[3] Wu, T.-L.; Chang, P.-Y.; Li, C.-C.; Tsao, K.-C.; Sun, C.-F.; Wu, J. T., Microplate ELISA for Urine Microalbumin: Reference Values and Results in Patients with Type 2 Diabetes and Cardiovascular Disease. *Annals of Clinical & Laboratory Science* **2005**, 35, (2), 149-154.

[4] Choi, S.; Choi, E. Y.; Kim, H. S.; Oh, S. W., On-Site Quantification of Human Urinary Albumin by a Fluorescence Immunoassay. *Clinical Chemistry* **2004**, 50, (6), 1052-1055.

[5] Liang, A.-H.; Huang, Y.-J.; Jiang, Z.-L., A rapid and sensitive immunoresonance scattering spectral assay for microalbumin. *Clinica Chimica Acta* **2007**, 383, (1-2), 73-77.

[6] Pegoraro, A. A.; Peracha, W.; Hasnain, M.; Ranginwala, N.; Shaykh, M.; Singh, A. K.; Arruda, J. A. L.; Dunea, G., Evaluation of a new fluorescent dye method to measure urinary albumin in lieu of urinary total protein. *American journal of kidney diseases: the official journal of the National Kidney Foundation* **2000**, 35, (4), 739-744.

[7] Penders, J.; Fiers, T.; Delanghe, J. R., Quantitative Evaluation of Urinalysis Test Strips. *Clinical Chemistry* **2002**, 48, (12), 2236–2241.

[8] Decher, G., Fuzzy Nanoassemblies: Toward Layered Polymeric Multicomposites. *Science* **1997**, 277, 1232-1237.

[9] Podgornik, R.; Ličer, M., Polyelectrolyte bridging interactions between charged macromolecules. *Current Opinion in Colloid & Interface Science* **2006**, 11, (5), 273-279.

[10] Bergbreiter, D. E.; Liao, K.-S., Covalent layer-by-layer assembly—an effective, forgiving way to construct functional robust ultrathin films and nanocomposites. *Soft Matte*r **2009**, 5, (1), 23.

[11] Glinel, K.; Déjugnat, C.; Prevot, M.; Schöler, B.; Schönhoff, M.; Klitzing, R. v., Responsive polyelectrolyte multilayers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2007**, 303, (1–2), 3-13.

[12] Dobrynin, A. V.; Rubinstein, M., Theory of polyelectrolytes in solutions and at surfaces. *Progress in Polymer Science* **2005**, 30, (11), 1049-1118.

[13] Tjipto, E.; Quinn, J. F.; Caruso, F., Layer-by-layer assembly of weak-strong copolymer polyelectrolytes: A route to morphological control of thin films. *Journal of Polymer Science Part A: Polymer Chemistry* **2007**, 45, (18), 4341-4351.

[14] Salloum, D. S.; Schlenoff, J. B., Protein Adsorption Modalities on Polyelectrolyte Multilayers. *Biomacromolecules* **2004**, 5, (3), 1089-1096.

[15] Watanabe, J.; Shen, H.; Akashi, M., Polyelectrolyte droplets facilitate versatile layer-by-layer coating for protein loading interface. *Acta biomaterialia* **2008**, 4, (5), 1255-62.

[16] Mathew, J.; Sreedhanya, S.; Baburaj, M. S.; Aravindakumar, C. T.; Aravind, U. K., Fabrication of switchable protein resistant and adhesive multilayer membranes. *Colloids and Surfaces B: Biointerfaces* **2012**, 94, 118-124.

[17] Jang, Y.; Park, S.; Char, K., Functionalization of polymer multilayer thin films for novel biomedical applications. *Korean Journal of Chemical Engineering* **2011**, 28, (5), 1149-1160.

[18] Hoshi, T.; Saiki, H.; Kuwazawa, S.; Tsuchiya, C.; Chen, Q.; Anzai, J.-i., Selective Permeation of Hydrogen Peroxide through Polyelectrolyte Multilayer Films and Its Use for Amperometric Biosensors. *Analytical chemistry* **2001**, 73, (21), 5310-5315.

[19] Sun, Q.; Tong, Z.; Wang, C.; Ren, B.; Liu, X.; Zeng, F., Charge density threshold for LbL self-assembly and small molecule diffusion in polyelectrolyte multilayer films. *Polymer* **2005**, 46, (13), 4958-4966.

[20] Jaeger, W., Regular Polyelectrolytes with Pyrrolidinium Units Novel Precursors for Complexes of Charged Polymers. *Annals of the New York Academy of Sciences* **1997**, 831, (1), 86-94.

[21] Cameron, G. G., Polyelectrolytes: formation, characterization and application. *Polymer International* **1995**, 38, (1), 106-106.

[22] Decher, G., Polyelectrolyte Multilayers, an Overview. In Multilayer Thin Films, Wiley-VCH Verlag GmbH & Co. KGaA **2003**, 1-46.

[23] Dubas, S. T.; Schlenoff, J. B., Factors Controlling the Growth of Polyelectrolyte Multilayers. *Macromolecules* **1999**, 32, (24), 8153-8160.

[24] Aravind, U. K.; Mathew, J.; Aravindakumar, C. T., Transport studies of BSA, lysozyme and ovalbumin through chitosan/polystyrene sulfonate multilayer membrane. *Journal of Membrane Science* **2007**, 299, (1-2), 146-155.

[25] Dubas, S. T.; Farhat, T. R.; Schlenoff, J. B., Multiple Membranes from "True" Polyelectrolyte Multilayers. *Journal of the American Chemical Society* **2001**, 123, (22), 5368-5369.

[26] Shiratori, S. S.; Rubner, M. F., pH-Dependent Thickness Behavior of Sequentially Adsorbed Layers of Weak Polyelectrolytes. *Macromolecules* **2000**, 33, (11), 4213-4219.

[27] Ladhari, N.; Hemmerlé, J.; Haikel, Y.; Voegel, J.-C.; Schaaf, P.; Ball, V., Stability of embossed PEI-(PSS–PDADMAC)20 multilayer films versus storage time and versus a change in ionic strength. *Applied Surface Science* **2008**, 255, 1988-1995.

[28] Wahlgren, M.; Arnebrant, T., Protein adsorption to solid surfaces. *Trends in Biotechnology* **1991**, 9, (1), 201-208.

[29] Absolom, D. R.; Zingg, W.; Neumann, A. W., Protein adsorption to polymer particles: Role of surface properties. *Journal of Biomedical Materials Research* **1987**, 21, (2), 161-171.

[30] Chang, C. W.; Guo, L.; Kao, Y. T.; Li, J.; Tan, C.; Li, T.; Saxena, C.; Liu, Z.; Wang, L.; Sancar, A.; Zhong, D., Ultrafast solvation dynamics at binding and active sites of photolyases. *Proceedings of the National Academy of Sciences* **2010**, 107, (7), 2914-2919.

[31] Qiu, W.; Zhang, L.; Okobiah, O.; Yang, Y.; Wang, L.; Zhong, D.; Zewail, A. H., Ultrafast Solvation Dynamics of Human Serum Albumin: Correlations with Conformational Transitions and Site-Selected Recognition. *The Journal of Physical Chemistry B* **2006**, 110, (21), 10540-10549.

[32] Edri, E.; Regev, O., pH Effects On BSA-Dispersed Carbon Nanotubes Studied by Spectroscopy-Enhanced Composition Evaluation Techniques. *Analytical Chemistry* **2008**, 80, (11), 4049–4054.

[33] Seyrek, E.; Dubin, P. L.; Tribet, C.; Gamble, E. A., Ionic Strength Dependence of Protein-Polyelectrolyte Interactions. *Biomacromolecules* **2003**, 4, (2), 273-282

[34] Kim, J.-H., Protein adsorption on polymer particles. *Encyclopedia of Surface and Colloid Science* **2002**, 4374-4381.

[35] Boributh, S.; Chanachai, A.; Jiraratananon, R., Modification of PVDF membrane by chitosan solution for reducing protein fouling. *Journal of Membrane Science* **2009**, 342, (1–2), 97-104.

[36] Yoon, J.-Y.; Kim, J.-H.; Kim, W.-S., The relationship of interaction forces in the protein adsorption onto polymeric microspheres. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1999**, 153, (1–3), 413-419.

[37] Boulmedais, F.; Frisch, B.; Etienne, O.; Lavalle, P.; Picart, C.; Ogier, J.; Voegel, J. C.; Schaaf, P.; Egles, C., Polyelectrolyte multilayer films

with pegylated polypeptides as a new type of anti-microbial protection for biomaterials. *Biomaterials* **2004**, 25, 2003-2011.

[38] Schoeler, B.; Kumaraswamy, G.; Caruso, F., Investigation of the Influence of Polyelectrolyte Charge Density on the Growth of Multilayer Thin Films Prepared by the Layer-by-Layer Technique. *Macromolecules* **2002**, 35, 889-897.

[39] Shen, H.; Watanabe, J.; Akashi, M., Polyelectrolyte Multilayers-Modified Polystyrene Plate Improves Conventional Immunoassay: Full Covering of the Blocking Reagent. *Analytical Chemistry* **2009**, 81, (16), 6923–6928.

[40] QuantiPro BCA Assay Kit (QPBCA): Technical Bulletin. Sigma-Aldrich. [Online].**2011**. Available from: http://www.sigmaaldrich.com/etc/ medialib/docs/qpbcabul.pdf. [2012, November 9]

[41] Hu, S.; Ren, X.; Bachman, M.; Sims, C. E.; Allbritton, N., Surface Modification of Poly(dimethylsiloxane) Microfluidic Devices by Ultraviolet Polymer Grafting. *Analytical Chemistry* **2002**, 74, (16), 4117-4123.

[42] Schlenoff, J. B.; Dubas, S. T., Mechanism of Polyelectrolyte Multilayer Growth: Charge Overcompensation and Distribution. *Macromolecules* **2001**, 34, (3), 592-598.

[43] Tjipto, E.; Quinn, J. F.; Caruso, F., Assembly of Multilayer Films from Polyelectrolytes Containing Weak and Strong Acid Moieties. *Langmuir* **2005**, 21, 8785-8792.

[44] Su, P.-G.; Cheng, K.-H., Self-assembly of polyelectrolytic multilayer thin films of polyelectrolytes on quartz crystal microbalance for detecting low humidity. *Sensors and Actuators B: Chemical* **2009**, 142, (1), 123-129.

[45] Goicoechea, J.; Arregui, F. J.; Corres, J. M.; Matias, I. R., Study and Optimization of Self-Assembled Polymeric Multilayer Structures with Neutral Red for pH Sensing Applications. *Journal of Sensors* **2008**, 1-8.

[46] Guo, D.-J.; Han, H.-M.; Jing, W.; Xiao, S.-J.; Dai, Z.-D., Surface-hydrophilic and protein-resistant silicone elastomers prepared by hydrosilylation of vinyl poly(ethylene glycol) on hydrosilanes-poly(dimethylsiloxane) surfaces. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2007**, 308, (1–3), 129-135.

[47] Jun Chen, M. L.; Liu, H.; Ma, L.; Gao, C.; Zhu, S.; Zhang, S., Synthesis and properties of thermo- and pH-sensitive poly(diallyldimethylammonium chloride)/poly(N,N-diethylacrylamide) semi-IPN hydrogel. *Chemical Engineering Journal* **2010**, 59, 247–256.

[48] Yang, Y.-J.; Tao, X.; Hou, Q.; Ma, Y.; Chen, X.-L.; Chen, J.-F., Mesoporous silica nanotubes coated with multilayered polyelectrolytes for pH-controlled drug release. *Acta biomaterialia* **2010**, 6, (8), 3092-3100.

[49] Müller, M.; Keßler, B.; Ouyang, W., In-Situ-ATR-FTIR Detection of Protein Sorption at Polyelectrolyte Multilayers: Variation of the Thickness. *Zeitschrift für Physikalische Chemie* **2007**, 221, (1), 127-138.

[50] Kittitheeranun, P., Controled Loading and Release of Model Drugs from Polyelectrolyte Multilayer Thin Films. Doctoral dissertation, Nanoscience and Technology, Chulalongkorn University, **2011**.

[51] Jomaa, H. W.; Schlenoff, J. B., Salt-Induced Polyelectrolyte Interdiffusion in Multilayered Films: A Neutron Reflectivity Study. *Macromolecules* **2005**, 38, (20), 8473-8480.

[52] Zhou, W.; He, J.; Cui, S.; Gao1, W., Studies of Electrospun Cellulose Acetate Nanofibrous Membranes. *The Open Materials Science Journal* **2011**, 5, 51-55.

[53] Watanabe, J.; Shen, H.; Akashi, M., Polyelectrolyte droplets facilitate versatile layer-by-layer coating for protein loading interface. *Acta Biomaterialia* **2008**, 4, (5), 1255-1262.

[54] Magnenet, C.; Jurin, F. E.; Lakard, S.; Buron, C. C.; Lakard, B., Polyelectrolyte modification of ultrafiltration membrane for removal of copper ions.

Colloids and Surfaces A: Physicochemical and Engineering Aspects **2013**, 435, 170-177.

[55] Laiwattanapaisal, W.; Songjaroen, T.; Maturos, T.; Lomas, T.; Sappat, A.; Tuantranont, A., On-Chip Immunoassay for Determination of Urinary Albumin. *Sensors* **2009**, 9, (12), 10066-10079.

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