พอลิอิเล็กโทรไลต์มัลติเลเยอร์ฟิล์มที่มีพอลิไดแอเซทิลีนเวสิเคิลสำหรับตรวจหาสารประกอบแอโรเมติกด้วยตาเปล่า

นายเทอดทูน แจ่มไพบูลย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2550

POLYELECTROLYTE MULTILAYER FILM CONTAINING POLYDIACETYLENE VESICLES FOR NAKED EYE DETECTION OF AROMATIC COMPOUNDS

Mr. Thoedtoon Champaiboon

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2007

Thesis Title	POLYELECTROLYTE MULTILAYER FILM CONTAINING POLYDIACETYLENE VESICLES FOR NAKED EYE DETECTION OF AROMATIC COMPOUNDS
By	Mr. Thoedtoon Champaiboon
Field of Study	Petrochemistry and Polymer Science
Thesis Advisor	Associate Professor Mongkol Sukwattanasinitt, Ph.D.
Thesis Co-Advisor	Gamolwan Tumcharern, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Science
(Professor Supot Hannongbua, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Supawan Tantayanon, Ph.D.)

.....Thesis Advisor
(Associate Professor Mongkol Sukwattanasinitt, Ph.D.)

.....Thesis Co-Advisor
(Gamolwan Tumcharern, Ph.D.)

.....Member
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

.....Member
(Associate Professor Sanong Ekgasit, Ph.D.)

เทอดทูน แจ่มไพบูลย์: พอลิอิเล็กโทรไลต์มัลติเลเยอร์ฟิล์มที่มีพอลิไดแอเซทิลีนเวสิเคิลสำหรับตรวจหาสารประกอบแอโรเมติกด้วยตาเปล่า. (POLYELECTROLYTE MULTILAYER FILM CONTAINING POLYDIACETYLENE VESICLES FOR NAKED EYE DETECTION OF AROMATIC COMPOUNDS) อ. ที่ปรึกษา: รศ.ดร. มงคล สุขวัฒนาสินิทธิ์, อ. ที่ปรึกษาร่วม: ดร. กมลวรรณ ธรรมเจริญ, 75 หน้า.

การวิเคราะห์เบื้องต้นเพื่อหาสารเคมีที่เป็นพิษในสิ่งแวดล้อมจำเป็นต้องมีอุปกรณ์ที่สามารถตรวจวัดสารเหล่านี้ได้โดยง่าย สะดวก และราคาไม่แพง พอลิไดแอเซทิลีนจัดเป็นสารที่มีสมบัติเป็นตัวตรวจวัดที่สามารถเปลี่ยนสีได้เมื่อถูกกระตุ้นด้วยสารเคมีบางชนิด จึงง่ายต่อการสังเกตเห็นได้ด้วยตาเปล่า ในการศึกษาวิทยานิพนธ์นี้ได้ทำการสร้างเซ็นเซอร์จากพอลิไดแอเซทิลีนโดยใช้ 10,12-เพนตะโคซะไดอายโนอิก แอซิด ในรูปของฟิล์มพอลิอิเล็กโทรไลต์หลายชั้นโดยเทคนิกการเตรียมฟิล์มทีละชั้น พบว่าฟิล์มพอลิอิเล็กโทรไลต์หลายชั้นที่เตรียมโดยใช้พอลิไดแอเซทิลีนเป็นพอลิแคตไอออน และใช้ไคโตซานเป็นพอลิแอนไอออนมีคุณภาพฟิล์มที่ดี โดยฟิล์มที่เตรียมขึ้นสามารถเปลี่ยนสีจากสีน้ำเงินเป็นสีแดงได้เมื่อทดสอบกับสารละลายแอลฟาไซโคลเด็กซ์ตริน (α-CD) ที่ความเข้มข้น 10 mM และการเปลี่ยนสีของฟิล์มสามารถถูกยับยั้งได้ด้วยสารประกอบแอโรเมติกบางชนิด เช่น พาราไนโตรฟีนอล, เบนโซอิก แอซิด และ 2,4-ไดคลอโรฟีนอกซีแอซิติก แอซิด (2,4-D) ที่ความเข้มข้นเท่ากันกับ α-CD โดยคาดว่าเกิดจากกระบวนการแข่งขันกันเกิดอินคลูชันคอมเพล็กซ์ ซึ่งค่าความจำเพาะเจาะจงของการยับยั้งการเปลี่ยนสีด้วยสารประกอบแอโรเมติกน่าจะเกี่ยวข้องกับความแรงของการจับกับ α-CD นอกจากนี้ฟิล์มพอลิไดแอเซทิลีนหลายชั้นยังสามารถพัฒนาให้อยู่ในรูปของแผ่นตรวจวัดหลายช่อง โดยใช้ช่องที่ทำจากพอลิไดเมทิลไซล็อกเซน ซึ่งอุปกรณ์ที่เตรียมขึ้นนั้นให้ค่าการเปลี่ยนแปลงของสีที่รวดเร็ว ที่สามารถตรวจวิเคราะห์ได้หลายตัวอย่างพร้อมๆ กันบนแผ่นตรวจวัดเดียวกัน

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต เทอดทูน แจ่มไพบูลย์

ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

4872303923: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEY WORD: POLYDIACETYLENE/ VESICLES/MULTILAYER FILM/LAYER-BY-LAYER DEPOSITION/CHEMOSENSOR

THOEDTOON CHAMPAIBOON: POLYELECTROLYTE MULTILAYER FILM CONTAINING POLYDIACETYLENE VESICLES FOR NAKED EYE DETECTION OF AROMATIC COMPOUNDS. THESIS ADVISOR: ASSOC. PROF. MONGKOL SUKWATTANASINITT, Ph.D., THESIS CO-ADVISOR: GAMOLWAN TUMCHARERN, Ph.D., 75 pp.

For preliminary analytical screening in the field test of toxic chemicals, it is desirable to have a sensing system which is inexpensive and convenient to use. Polydiacetylene is known to possess unique colorimetric sensing properties in which its color changing upon exposure to chemical stimulants can be easily observed by naked eyes. In this thesis study, a sensing system consisting of an active polydiacetylene agent, poly(10,12-pentacosadiynoic acid) (PCDA) vesicle, is constructed in a form of polyelectrolyte multilayer (PEM) film using a layer-by-layer deposition technique. The PEM film using PCDA as a polyanion and chitosan as a polycation has the most satisfactory film quality. The color of poly(PCDA)/chitosan PEM film can be induced from blue to red by aqueous solution of 10 mM α-cyclodextrin (α-CD). The α-CD induced color transition of poly(PCDA) PEM film can be inhibited by equimolar some aromatic compounds such as *p*-nitrophenol, benzoic acid and 2,4-dichlorophenoxyacetic acid (2,4-D) probably by the competitive inclusion complexation. There are some selectivity in the color transition inhibition of various aromatic compounds probably depended on their binding strength with α-CD. The multichannel chip constructed from the poly(PCDA)/chitosan PEM film and the polydimethylsiloxane channel show fast colorimetric response to α-CD that can provide a simultaneous test of multiple samples on the chip.

Field of Study Petrochemistry and Polymer Science Student's Signature…Thoedtoon Champaib
Academic Year 2007 Advisor's Signature…
Co-Advisor's Signature…

ACKNOWLEDGEMENTS

This thesis work is partially funded by the Thailand Graduate Institude of Science and Technology (TGIST), the National Science and Technology Development Agency (NSTDA), Thailand.

He would like to express his sincere gratitude to his advisor, Associate Professor Mongkol Sukwattanasinitt, Ph.D. who not only originated this work, but also gave him helpful suggestions, invaluable guidance, constructive criticism, constant encouragement, inspiration and vital help throughout this research. This thesis research would not be completed without his advice and guidance.

He would like to give special thanks to his co-advisor, Gamolwan Tumcharern, Ph.D. from National Nanotechnology Center (NANOTEC), for the recommendation, strong support and helping complete the work.

He also wishes to express his grateful thanks to his thesis committees, Associate Professor Supawan Tantayanon, Ph.D., Assistant Professor Warinthorn Chavasiri, Ph.D and Associate Professor Sanong Ekgasit, Ph.D., for their kind attention, valuable suggestion and recommendations.

He appreciates all professors for the education at the Petrochemistry and Polymer Science, Chulalongkorn University. He also would like to thank Assistant Professor Orawan Sanguaruang, Ph.D. for permission to use UV-Vis spectrophotometer throughout this research.

He is deeply indebted to Anuput Potisatityuenyong, Ph.D. for his genuine comments, suggestion and big help in everything. Furthermore, he gratefully thank to his friends at dormitory for their honest, help and friendship and everyone in MS-group, especially Jasuma and Sansanee for their good spirit and excellent cooperated in the innovation project. He wishes to thank Nakorn for synthesis some testing chemical throughout this work. Moreover, he expresses his appreciation to Kaewkan for her suggestion in English writing.

Finally, he wishes to express thankfulness to his family for the education, understanding, love, care, encouragement and support throughout his study.

CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

Figure **Page**

Figure **Page**

LIST OF ABBREVIATIONS

PDA	Polydiacetylene
PCDA	10,12-pentacosadiynoic acid
TCDA	10,12-tricosadiynoic acid
PDADMAC	Poly(diallyldimethylammonium chloride)
PDMS	Poly(dimethylsiloxane)
PEG	Poly(ethylene glycol)
PEI	Poly(ethylenimine)
PEM	Polyelectrolyte Multilayer
PVA	Poly(vinyl alcohol)
L-S film	Langmuir-Schaefer film
L-B film	Langmuir-Blodgett film
α-CD	α-cyclodextrin
β-CD	β-cyclodextrin
γ-CD	γ-cyclodextrin
4-NP	4-nitrophenol
DMPC	Dimyristoylphosphocholine
TEOS	tetraethyl orthosilicate
VOCs	Volatile organic compounds
CR	Colorimetric response
AFM	Atomic force microscopy
TEM	Transmission electron microscopy
DLS	Dynamic light scattering
°C	Degree celsius
g	Gram
mL	Millilitre
mM	Millimolar
nm	Nanometre
min	Minute
%	Percent

CHAPTER I

INTRODUCTION AND THEORY

1.1 Overview

Nowadays, aromatic compounds are widely used in many industries and agricultures such as plastic and dye, including pesticide. The contamination of aromatic compounds in environment and food may result in gene mutation or cancer. The up-taken aromatic compounds affect the blood pressure and remain in the bone. The damaged DNA causes the myelogenous and nonlymphocytic leukemias [1-2]. *p*-nitrophenol is an example of benzene derivative released from the factory. This toxic compound can stimulate mutation and damage the embryo of mammals [3]. Several techniques; for examples, spectrophotometry, chromatography and immunoassay, have been used for the quantitative determination of the aromatic derivative in the environment [4-7]. However, the above mentioned techniques are complicated, expensive and time-consuming. For preliminary analytical screening in the field test, it is desirable to have a sensing system which is inexpensive and convenient to use.

In this research, we developed the polyelectrolyte multilayer (PEM) thin film that containing polydiacetylene (PDA) vesicles of 10,12-pentacosadiynoic acid (PCDA) by using a layer-by-layer deposition technique for aromatic detection. PDA is an ene-yne conjugated polymer that changes color from blue to red by the external stimuli. The PEM film was prepared simply by alternate dipping of a substrate into positively charged polyelectrolyte, chitosan, and negatively charged polyelectrolyte, PDA vesicles. The thickness of the film can easily controlled by the number of layers deposited, which is convenient for preparation of colorimetric sensing devices detectable by naked eyes. The color transition of the prepared PEM film was tested with α-CD in the absence and in the presence of 4-nitrophenol (4-NP) and other inclusion complexes. This study aims to invent aromatic compound detector which is user-friendly, inexpensive and rapid.

1.2 Theory

The development of conjugated polymers as sensing materials have gained much attention because changes in their absorption, emission, and redox properties. An advantage of using conjugated polymer-based sensors with small molecules found in their potential for signal amplification [8]. Polydiacetylene (PDA) is an ene-yne conjugated polymer that changes color from blue to red by external stimuli such as thermal activation, pH, mechanical stress, solvents and chemical reagents through the changes in the effective conjugation length of its backbone [9]. PDA has thus been fabricated into various forms for colorimetric indicators of temperature, chemicals and biological agents [10-16].

1.2.1 Polydiacetylene

Polydiacetylene (PDA) is a π-conjugated polymeric prepared from photopolymerization of appropriate diacetylene monomer *via* 1,4-addition reaction to form alternating ene-yne polymer chains upon heat, irradiation with light or γ-irradiation [8]. PDA can be prepared from various kinds of diacetylene monomers especially the diacetylene lipid which consists of hydrophilic carboxylic acid head group and hydrophobic long chain hydrocarbon (Figure 1.1). The resulting PDA, if generated under the optimized conditions, appears as an intense blue-colored PDA.

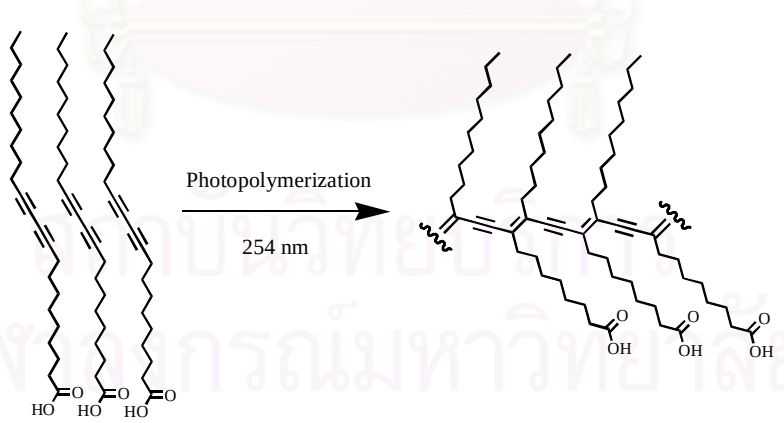


Figure 1.1 Photopolymerization of 10,12-pentacosadiyonic acid (PCDA) by UV-irradiation.

The topopolymerized diacetylene crystals are nearly perfectly ordered crystals which cannot be occurred by solution polymerization or recrystallization of a preformed polymer from solution or melt [17-20]. Reactivities of the monomers as well as the properties of the corresponding PDA are controlled by the monomer crystal packing that is highly influenced by the substituents [21]. PDA can be structured in the form of bulk materials, multilayer and monolayer films, polymerized vesicles, and even incorporated into inorganic host matrices to form nanocomposites. The resulting polymer exhibits the array of spectacular properties, most notably with fantastic colorimetric transitions that can be optically, thermally, chemically, or mechanically activated. Therefore, PDA has been widely purposed as the naked-eye sensors for chemical and bio-molecular. On a more basic level, the color transition property has led to new comprehensive concerning chromatic phenomena in polymers [22].

PDA is a well-organized polymeric material that exists in the form of both single crystal and lipid vesicle. Either solution or thin film of PDA can be used as a colorimetric sensor. The layer of the polymeric material on a solid support is prepared by Langmuir-Blodgett, Langmuir-Schaefer, or self-assembled monolayers (SAMs) techniques [17,23].

10,12-pentacosadiyonic acid (PCDA) is a well-known diacetylene lipid acid that contains both hydrophilic (carboxylic group) and hydrophobic (long hydrocarbon chain) parts. Therefore, PCDA monomers are spontaneously organized to the uniformly vesicles in aqueous media which can be further efficiently photopolymerized by UV light to yield the intense blue-colored of the spherical nanostructure of poly(PCDA) (Figure 1.2) [24].

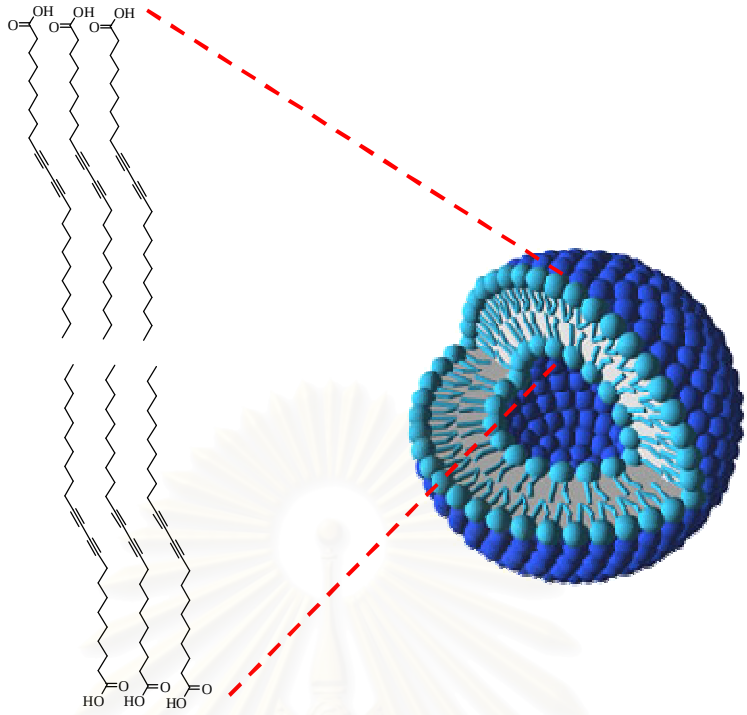


Figure 1.2 Illustration of poly(PCDA) vesicle.

Chromatic transition of polydiacetylene

The blue phase of PDA transfers to the red one when the polymeric material is exposed to the environmental stimuli; for examples, temperature (thermochromism), pH, solvent (solvatochromism) and mechanical stress (mechanochromism) [4]. The external stimuli alter molecular conformation by stressing of the polymer backbone or the change of the packing, ordering, and orientation of the side chain. The conformation change influences in the twisting of the structure and the decreasing in the length of conjugate system which concerns the interaction between side chains of PDA, thus changing the electronic states and the corresponding optical absorption [25-26]. Generally, visible absorption in PDA occurs *via* $\pi \rightarrow \pi^*$ absorption within the linear π-conjugated polymer backbone. A significant shift in the visible absorption spectrum from low to high energy band is a result of the change of light absorbed by π electron of conjugate system in a polymer main chain. In addition, the C-C bond rotation in the polymer backbone may influence the overlap of the PDA-molecular orbitals and consequently the change of the energy level. The red PDA may consist of a non-planar backbone configuration in combination with the alkyl side-chains rotation and distortion (Figure 1.3) [22].

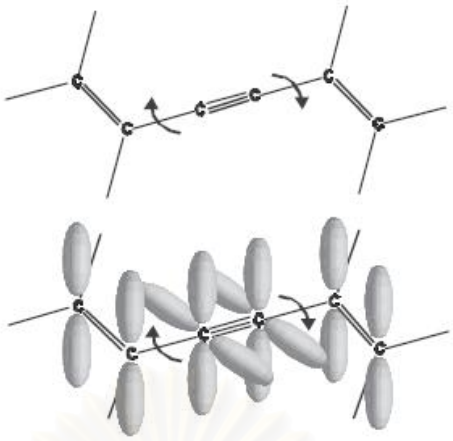


Figure 1.3 Molecular orbitals in the π-conjugated PDA backbone in the planar configuration [22].

Colorimetric Response (CR)

The blue-to-red color transition of the polymerized vesicles was monitored by measuring the absorbance differences between the vesicles before and after stimulation by an interesting parameter. A quantitative value for the extent of blue-to-red color transition is given by the colorimetric response (CR) [25] which is defined as

$$\%CR = \frac{(PB_0 - PB)}{PB_0} \times 100$$

Where PB is the percent blue calculated from $A_{blue}/(A_{blue}+A_{red})$. A_{blue} and A_{red} are the absorbance of the blue and the red phase at 630 and 540 nm, respectively. The visible absorbance was measured by a temperature controlled UV-Vis spectrometer. PB_0 is the initial percent blue of the vesicle solution or film before exposure to the stimulus.

1.2.2 Chemochromism of polydiacetylene vesicle

Chemochromism, the color change of material upon the presence of specific chemicals, is one of the interesting applications of PDA. The color transition of PDA from blue to red in contact with α-cyclodextrin (α-CD) solution was observed for the first time in Langmuir-Schaefer film (L-S film) prepared from PDA containing aniline head group [17]. The inclusion of the carboxylic moiety of poly(PCDA) vesicles into α-CD interrupts the hydrogen bonding between the carboxylic head-groups of the neighboring lipid chain (Figure 1.4) leading to the color change. The blue to red transition of polydiacetylene, in water [27] and in monolayer thin film [17], is inhibited by the penetration of *p*-nitrophenol into α-CD ring. This study indicated that inhibition of changing in color of polydiacetylene vesicle by forming inclusion complex with cyclodextrin can be basic of sensor development.

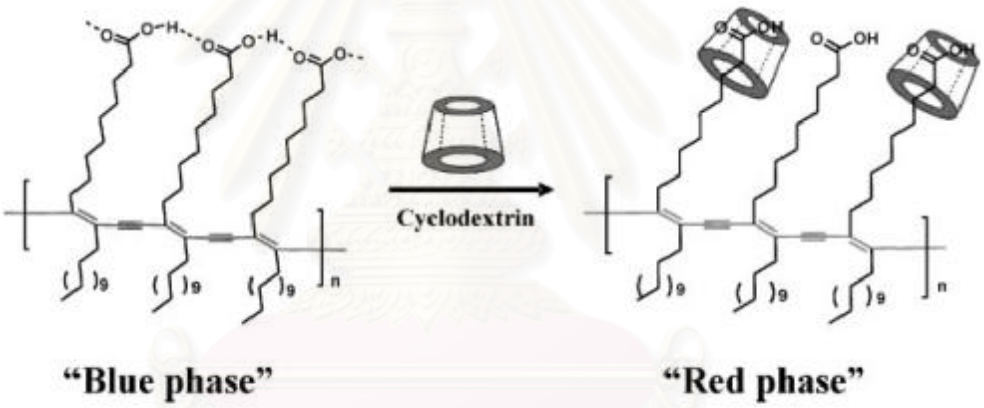


Figure 1.4 Interaction between carboxylic head-group of PDA and α-CD [27].

Cyclodextrin, α-, β-, or γ-CD (Table 1.1), is a family of well-known naturally water-soluble cyclic oligosaccharides consisted of D-(+)-glucopyranosyl units. (Figure 1.5) [28-33]. CD displays a large variety of the inclusion of small organic molecules (Guest, G); such as a diacetylene monomer and an aromatic compound, into the cavity [32]. The *p*-nitrophenol is stabilized inside the ring by hydrophobic, van de Waals, or dipole-dipole interaction.

Table 1.1 Characteristics of the various CDs (Applied from [29-31])

	a-**CD**	b-**CD**	g-**CD**
Empirical formula (anhydrous)	$C_{36}H_{60}O_{30}$	$C_{42}H_{70}O_{35}$	$C_{48}H_{80}O_{40}$
Number of glucose units	6	7	8
Molecular weight	972	1135	1297
Internal cavity diameter (angstroms)	5	6	8
Water solubility (g/100mL: 25 ^{o}C)	14.2	1.85	23.2
pKa (by potentiometry, 25 ^{o}C)	12.33	12.20	12.08
Melting range (^{o}C)	255-260	255-265	240-245
Water of crystallization	10.2	13-15	8-18
Water molecules in cavity	6	11	17

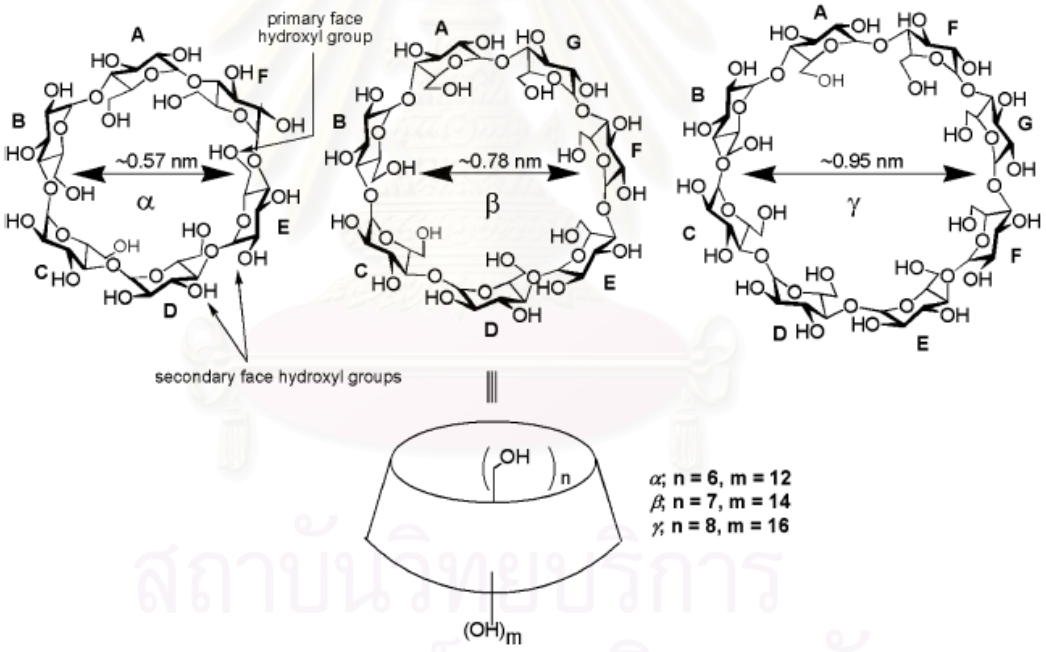


Figure 1.5 A schematic representation of the CDs [32].

Rate of inclusion complexation can determine by the following equation.

$$CD + G \underset{k_D}{\overset{k_R}{\rightleftharpoons}} CD\text{-}G \qquad k_{total\ rate} = k_D / k_R$$

k_D and k_R represent the dissociation and recombination of CD-G, respectively. The CD:G encapsulated ratio of 1:1 is commonly observed. Stability constant (K or log K) of CD:G complex in the equilibrium can determine by the following equation.

$$K_{1:1} = \frac{[CD\text{-}G]}{[CD][G]}$$

The stability of the inclusion complexation depends on the hydrophobic nature of the host-guest interactions. Basically, the neutral guest molecule shows the higher affinity to the CD cavity compared to the corresponding charged species derived from the same original guest molecules. [29].

1.3 Literature Survey

In the past decade, PDA has gained much attention to use as a colorimetric sensor. Many researchers attempt to overcome the drawback and improve the property of PDA.

In 1997, Lio *et al.* [10] prepared thin film of PDA by photo-polymerization of the Langmuir-Blodgett film (L-B film) of diacetylene-glycolipid (PCDA) and sialic acid derivatives of PCDA. The film color can be changed from blue to red in the temperature range of 70-90 °C. The color transition arises from reduction of the effective conjugated length of the polymer eyn-yne backbone influenced by the pendant side groups.

In 1998, Okada *et al.* [25] reported that the color of PDA depended on the type of diacetylene monomer and the semi-quantitative of color change calculated by the colorimetric response (CR). The shorter length between diacetylene group and carboxylic head group leads to not only lower in the temperature but also increase in the sensitivity. However, the lipid chain length does not significantly affect to the temperature response.

In 2000, Kolysheva *et al.* [34] prepared the mixed lipid vesicle from dimyristoylphosphocholine (DMPC), 10, 12-tricosadiynoic acid (TCDA) phospholipid and ionophore. The presence of alkaline cation, e.g. Na^+ and K^+, resulted in the blue to violet or red color change of the vesicle due to the interaction

between metal cation and ionophore. In the next year, Kolusheva and co-worker [35] prepared the mixed lipid vesicle from epitope, phospholipid, and polydiactylene for the detection of N-terminus of epitope connected to antibody.

In 2003, Kim *et al.* [36] prepared thin film of PDA vesicle from an amine-terminated diacetylene monomer (PCDA-NH_2, Figure 1.6). The glass substrate is modified to have aldehyde group on the surface prior to react with amine group of diacetylene lipid. The formation of amide moiety after photopolymerization of the lipid vesicle results in the red film.

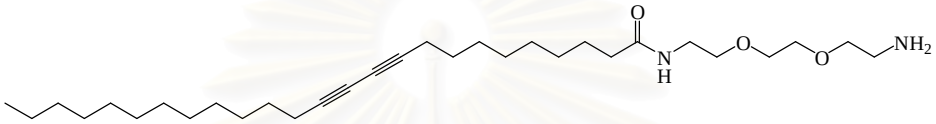


Figure 1.6 The chemical structure of PCDA-NH_2.

In 2004, Shim *et al.* [37] fabricated PDA on a glass plate by reacted diacetylene containing *N*-hydroxy succimide with 3-aminopropyltriethoxysilane (APS) prior to photopolymerization process. The blue film in square, 70×70 μm, or circle, diameter 15-50 μm, was obtained by using the micro-contact-printing (μCP) and observed by using fluorescence microscope. The patterning film will be used for the development of sensor array.

In 2005, Kim *et al.* [23] developed fluorescence sensor micro array chip from two mixed-lipid vesicle, 10,12-pentacosadiynoic acid with 2,2'-(ethylenedioxy)bis-(ethylamine) (PCDA-EDEA), or ethylenediamine (PCDA-EDA), alternatively attached on the aldehyde-coated glass plate (Figure 1.7). The micro array was changed to red after exposure to α-CD and poly(acrylic acid) (PAA).

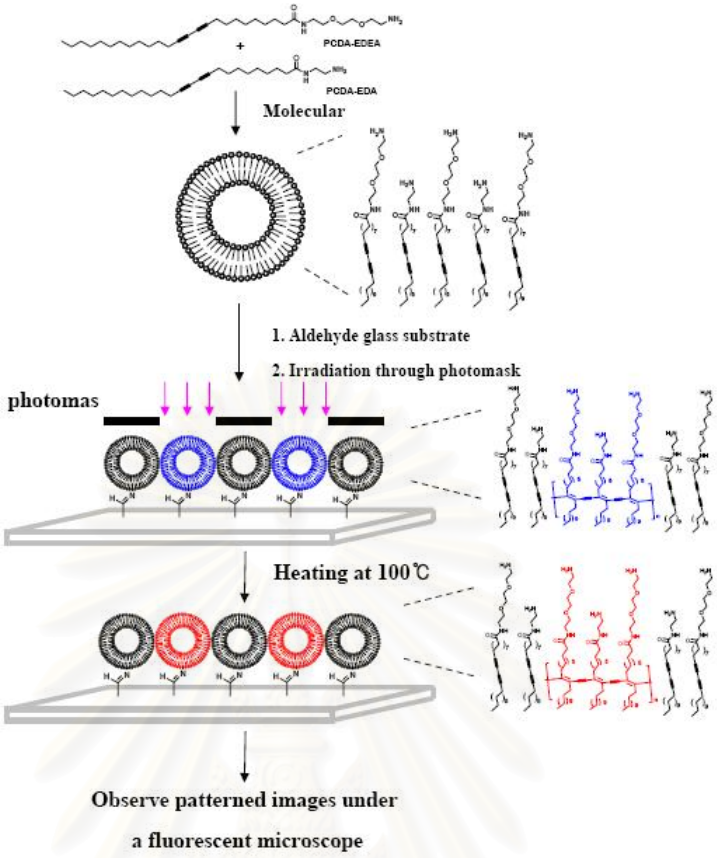


Figure 1.7 Procedures for patterned fluorescent images using the photolithographic method [23].

In 2004, Su *et al.* [38] synthesized the mixed lipid vesicle from dimyristoylphosphatidylcholine (DMPC) and 10,12-tricosadiynoic acid (TCDA) containing antibodies. The blue to red color transition was observed when the specific immunoreaction took place at the vesicle surface. The presence of natural lipid, DMPC, in the vesicle decreased the lower detection limit (down to 1 mg/mL of antigen) of the chromatic immunoassay.

In 2005, Su *et al.* [39] studied interaction between surfactants and lipid vesicles composed of DMPC and polymerized PCDA in aqueous solution by using colorimetric method. The electrostatic interaction and hydrophobic interaction play a key role in the interaction between the mixed lipid vesicles and surfactant that the cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Triton X-100 were selected as a cationic, anionic and nonionic surfactant, respectively. The addition of surfactants into mixed vesicles induced color change from blue to red that resulted from electrostatic interaction between the head groups of the surfactants and

the vesicles promoting the insertion of alkyl chain of surfactants into the hydrophobic domain of the vesicles. The insertion of alkyl chain of the surfactants perturbed the conformation of polymer backbone in mixed vesicles and led to color change of the mixed vesicles. Again, incorporation of DMPC in mixed lipid vesicles interrupted molecular packing of PCDA that facilitated the insertion of surfactants. Moreover, DMPC-polymerized PCDA vesicles functionalized with glycolipid was applied as biosensor for detection of E. coli [40]. In the same year, Su and co-worker [41] also prepared multi-layer film of PDA vesicles on PEI-modified quartz slide by electrostatic layer-by-layer deposition using negatively charged PCDA vesicles and polyelectrolyte polyethylenimine (PEI) or positively charged PCDA-2′-aminiethylamide (PCDA-NH_2) vesicles. The color change of this film from blue to red was observed at 60 °C.

In 2005, Kim *et al.* [27] investigated the effect of CD on the PCDA monomer and polymer (Figure 1.8). Unlike β- and γ-form, α-cyclodextrin interacted with diacetylene monomer, diacetylene vesicle and poly(PCDA) vesicle. The inclusion of diacetylene chain into the cavity of α-CD resulted in the inhibition of diacetylene polymerization and color transition. In the presence of 4-nitrophenol (4-NP), the color change was not observed because the carboxylic head group of PCDA cannot replace 4-NP in the α-CD cavity.

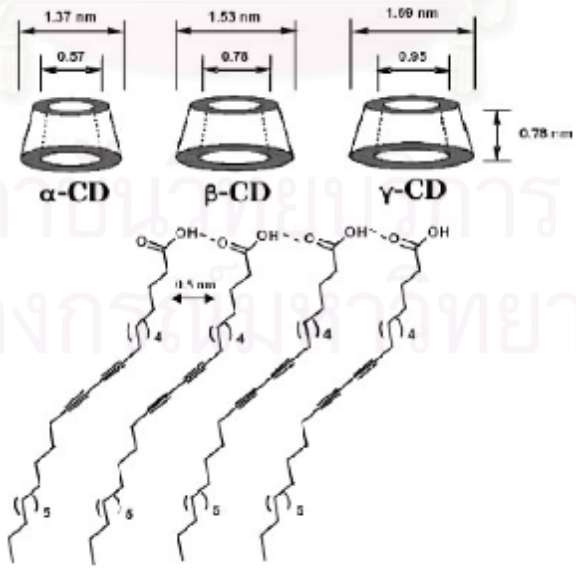


Figure 1.8 A Schematic representation of cyclodextrins and self-assembled PCDA monomer [27].

In 2006, Wang *et al.* [15] prepared PDA vesicle from mixtures of 10,12-tricosadiynoic acid (TCDA), dimyristoylphosphatidylcholine (DMPC), and probe-oligonucleotides (probe-DNA) at different molar ratio. A new PDA-based sensing system was applied for the detection of oligonucleotide by colorimetric method. The presence of DMPC in the PDA vesicle played an important role in the colorimetric response of oligonucleotide. The PDA vesicles composed of TCDA/DMPC/probe-DNA with molar ratio of 0.60:0.39:0.01 provided the highest colorimetric response (CR) upon the addition of cDNA. The change of vesicle morphology was illustrated by TEM.

In 2006, Lee *et al.* [42] invented a micro-multichannel sensor chip using hydrogel of PDA vesicle and poly(ethylene glycol) diacrylate (PEG-DA) as a colorimetric sensing materails. The color transition was observed upon the addition of α-CD. The multi-channel is useful for the detection of various samples simultaneously. However, the color transition takes long detection time (more than 1 hour) and need high α-CD concentration (more than 25 mM).

In 2006, Potisatityuenyong *et al.* [43] prepared polyelectrolyte multilayer (PEM) thin films on glass substrates using layer-by-layer deposition technique. The resulting film was prepared from 10,12-pentacosadiynoic acid (PCDA) vesicles as a polyanion and poly(ethylenimine) (PEI) or chitosan as a polycation. The vesicle particles were shown by AFM image and the color intensity of the films can be controlled through the number of vesicle layers deposited. The PDA multilayer film has excellent storage stability, as no significant change in their appearance and chromic properties occurred over a year. In addition, the films retained all chromic properties *i.e.* solvatochromism, pH sensing, and thermochromism of the vesicles dispersed in water.

In 2006, Kim *et al.* [44] prepared PDA vesicles embedded in PVA film using a mixing-drying process. The PDA-embedded PVA film was stable for several months. The heating process was used to study the thermochromic behavior of thin-film. The blue-colored PVA film at 25 °C gradually changed to purple at 80 °C and eventually changes to red above 100 °C. However, the PDA-embedded PVA film is not suitable to use as a chemosensor for aqueous solution due to the water soluble property of PVA. Therefore, the poly(PCDA)-embedded PVA film with silica gel was prepared.

The colorimetric film containing silica gel is not only more stable but also better sensitivity.

In 2006, Yoon *et al.* [45] prepared the PDA-embedded silica-enforced microfibers from diacetylene (DA) monomer, poly(ethylene oxide) (PEO), and tetraethyl orthosilicate (TEOS). The electrospun microfiber was used as a colorimetric detector for volatile organic compounds (VOCs) by using the electro-spinning technique.

In 2007, Chae *et al.* [46] prepared a single PDA-embedded PMMA electrospun fiber with a pattern that able to observed by visible and fluorescence microscopes. The incubation of PDA-embedded polymer fibers in a solution of α-CD (10 mM) resulted in the red fluorescence emission.

In 2007, Lee *et al.* [47] immobilized PDA vesicles as an array on the surface of ZnO single-crystal substrates by chemisorptions. The PDA array is sensitive to a number of environmental parameters including chemicals such as α-CD.

In 2007, Lee *et al.* [48] found the relation between inclusion complex formation of PDA-CD and reversible/irreversible color transition of polydiacetylene (Figure 1.9). The blue color of PDA which was promoted to purple or red by α-CD showed the irreversible (IR) colorimetric thermochromism behavior. In other hand, PDA which remain blue color upon the addition of α-CD displayed the reversible (R) thermochromism behavior.

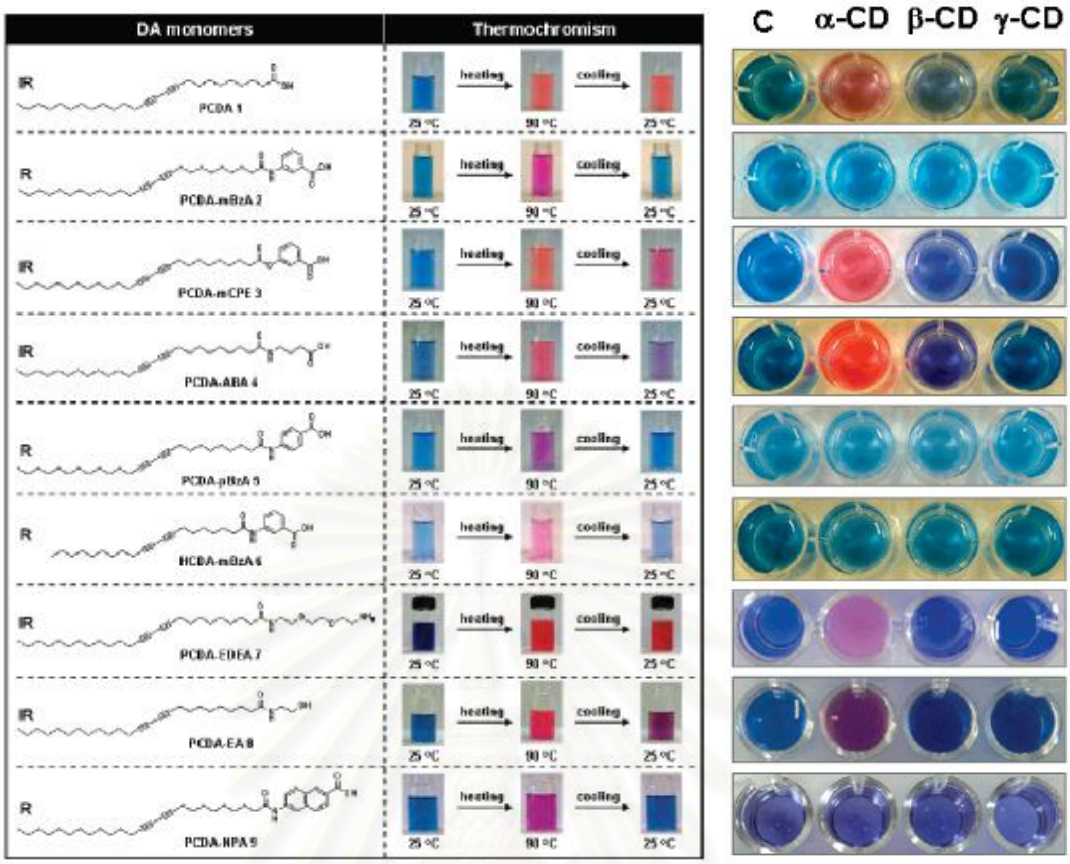


Figure 1.10 Diacetylene monomers and photographs of PDA solutions that display irreversible (IR) or reversible (R) thermochromism and PDA solutions (1 mM) incubate in cyclodextrin (10 mM) at 25 °C for 10 min [48]

In 2008, Lee *et al.* [49] immobilized PDA microarray on a cellulose acetate butyrate (CAB)-coated glass substrate as a PDA-based sensor chip. The microarray emitted red fluorescence under a thermal stress and a specific molecular recognition. The immobilization of PDA on CAB coated plate was successful by terminated the polymeric chain with amine, carboxylic acid or hydroxy moiety.

1.4 Objective and scope of this thesis

By the way, our group successfully synthesized PDA multilayer film. This study, thus, aim to apply the multi-layered PDA with concept of that aromatic compounds can be penetrated into α-CD to invent aromatic compound model detector which can changing color of PDA vesicle in negative mode when detect aromatic compounds in water. Since carboxylic group in PCDA can be carboxylate ion, PCDA of polydiaetylene is used to prepare multilayer with layer-by-layer deposited polycation e.g. chitosan, PEI, PDADMAC. The advantage of multilayer film is film thickness and color intensity can be controlled by the amount of film layer. Thus, this method is suitable for preparation film sensor which can detect by naked eye or colorimetric analysis.

The objective of this research is to study the color-transition inhibition of PDA film by competitive inclusion between PDA and aromatic compound into the cyclodextrin cavity. Chemochromic multichanel system based on layer-by layer deposited PDA film was developed.

To achieve the objective, the scope of this research work includes:

1) Preparation of PDA vesicles from PCDA or PCDA and stearic acid.
2) Preparation of PDA multilayer film by using layer-by-layer deposition technique.
3) Study of the color transition of prepared PDA solution and film with cyclodextrin.
4) Study of factors which influence the sensitivity e.g. detection temperature, mixed lipid vesicles, and number of film layers.
5) Study of the inhibition of color transition of prepared PDA solution and film with aromatic compounds by means of inclusion complexation.
6) Study of particle size and morphology change in response to cyclodextrin by dynamic light scattering (DLS), transmission electron microscopy (TEM), and atomic force spectrometry (AFM).
7) Fabrication of PDA micro-multichannel device.

CHAPTER II

EXPERIMENTAL

2.1 Chemicals

1. 10,12-Pentacosadiynoic acid (PCDA), Fluka, USA
2. Octadecanoic acid (stearic acid, C18), Fluka, Switzerland
3. Poly(dimethylsiloxane) (PDMS) & Curing agent, Sylgard®184 (Silicone elastomer base), Dow Chemical, USA
4. Poly(ethylenimine) (PEI), Aldrich, USA
5. Poly(diallyldimethylammonium chloride) (PDADMAC), Aldrich, USA
6. Chitosan Powder, Seafresh Chitosan (Lab) Company, Thailand
7. Diethylether, reagent grade, Lab-Scan, Thailand
8. 1,1,1-Trichloromethane (chloroform), AR grade, Lab-Scan, Thailand
9. Sodium hydroxide, Merck, Germany
10. Hydrochloric acid fuming 37% (conc. HCl), Merck, Germany
11. Glacial acetic acid, Scharlau, Spain
12. α-cyclodextrin, Fluka, USA
13. β-cyclodextrin, Fluka, USA
14. γ-cyclodextrin, Fluka, USA
15. *o*-nitrophenol, BDH Laboratory reagent, UK
16. *m*-nitrophenol, Fluka, USA
17. *p*-nitrophenol, BDH Laboratory reagent, UK
18. *p*-methoxybenzyl alcohol, Fluka, USA
19. *p*-nitrotoluene, Fluka, USA
20. Benzoic acid, Fluka, USA
21. Phenol, Merck, Germany
22. Indole, Fluka, USA
23. 2,4-Dichlorophenoxyacetic acid (2,4-D), Fluka, USA
24. Dimethyl viologen diiodide (DV^{2+}), Synthesis
25. Methyl viologen iodide (MV^{+}), Synthesis

2.2 Apparatus and equipments

1. Rotary evaporator, R200, Buchi, Switzerland.
2. Ultrasonicator, Elma, Germany
3. Centrifuge, Sanyo Centaur2, Japan
4. Magnetic stirrer, Fisher Scientific, USA
5. Hot plated magnetic stirrer, Corning, USA
6. pH meter, pH 510, Eutech Intruments, Singapore
7. Pipette man (P20, P200, P1000 and P5000), Gilson, France
8. Pipette man (Le100 and Le1000), Nichiryo, Japan
9. UV-Vis spectrophotometer, UV-2550, Shimadzu, Japan
10. Dynamic light scattering spectrometer (DLS), Nanosizer, Malvern Instrument, England
11. Atomic force microscopy (AFM), Seiko SPA 400, Japan
12. Transmission electron microscope (TEM), JEOL TEM-2100, Japan

2.3 Procedures

2.3.1 Preparation of polydiacetylene vesicles

Poly(10,12-pentacosadiynoic acid) vesicles

10,12-Pentacosadiynoic acid (PCDA, 37.5 mg) as a white solid was dissolved in diethyl ether (10 mL) in an Erlenmeyer flask and the solvent was removed by a rotary evaporator for lipid bilayer formation. A volume of milli-Q water (200 mL) was added to provide the lipid concentration of 0.5 mM. The PCDA suspension (0.5 mM) was sonicated by a 230 watt bath sonicator for 20 min at 75-85 °C when a semitransparent or transparent vesicle solution was obtained. The resulting solution was kept at 4 °C overnight. The temperature was then brought up to room temperature and then the solution was polymerized by UV irradiation 254 nm for 5 min to afford a blue solution of poly(PCDA) vesicles. The blue-colored poly(PCDA) vesicle solution was filtered through a paper filter (pore size 1/125 mm) to remove the undesired lipid aggregates. The solution showed the maximum visible absorption at wavelength (λ_{max}) of 635 nm.

Mixed lipid vesicles

Vesicle solution of mixed lipids between PCDA and stearic acid was prepared by mixing the stock solutions of the lipids at the designated mole ratios of PCDA:stearic acid being 70:30 and 50:50 with a fixed concentration of PCDA at fix 0.5 mM. The same procedure and conditions as those of poly(PCDA) vesicle solution were applied in the sonication and photopolymerization steps.

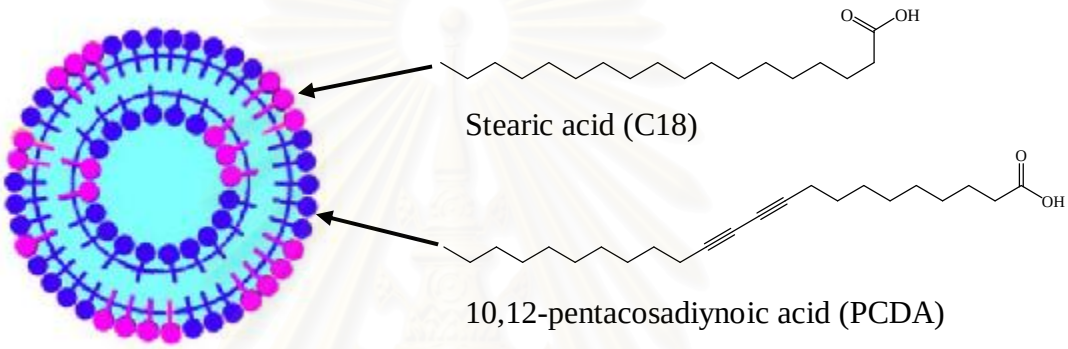


Figure 2.1 Illustration of poly(PCDA) mixed lipid vesicle.

2.3.2 Preparation of polycationic solutions

Chitosan solution

Chitosan flakes (0.5 g) were dissolved in 1% v/v aqueous acetic acid solution. The mixture was allowed for complete dissolution by stirring for 12 hours. The chitosan solution was diluted to 0.5% v/v using deionized water. The insoluble polymer was separated by centrifugation (3,000 rpm, 15 min). The clear solution was collected for further experiment.

Polyethylenimine (PEI) solution

PEI aqueous solution (10 mM) was prepared by the dissolution of pure PEI liquid (0.042 g) in deionized water (100 mL).

Poly(diallyldimethylammonium chloride) (PDADMAC) solution

PDADMAC aqueous solution (10 mM) was prepared by the dilution of 20% w/v PDADMAC solution (0.162 g) in deionized water (100 mL).

2.3.3 Preparation of poly(PCDA) multilayer film

Glass Slide Substrate

Glass slides with dimensions 1.25 × 3.75 cm^2 were used as substrates. The glass slides were prewashed by cleaning Teepol detergent and rinsing with deionized water. The glass substrates were cleansed of organic contaminants by soaking in Piranha solution (3:1 volume ratio of sulfuric acid and hydrogen peroxide) for 10-12 hours. After throughly rinsed with deionized water, the clean substrates were kept in deionized water in a closed container for the further experiment.

Film preparation

A polyelectrolyte multilayer (PEM) film of poly(PCDA) and polycation was prepared according to the literature [42]. Briefly, poly(PCDA) and polycation (0.1% w/w for chitosan, 10 mM for PEI or PDADMAC) solutions at pH 3.0-3.3 adjusted by HCl (0.1 mM) were used as polyelectrolytes. A clean glass slide was dipped into the polycationic solution for 5 min. After rinsing with dilute HCl (0.1 mM) for 3 times, the polycationic coated substrate was immerged into the poly(PCDA) solution for 5 min. The rinsing process was repeated for 3 times. The double layer deposition was carried out at the desired cycles.

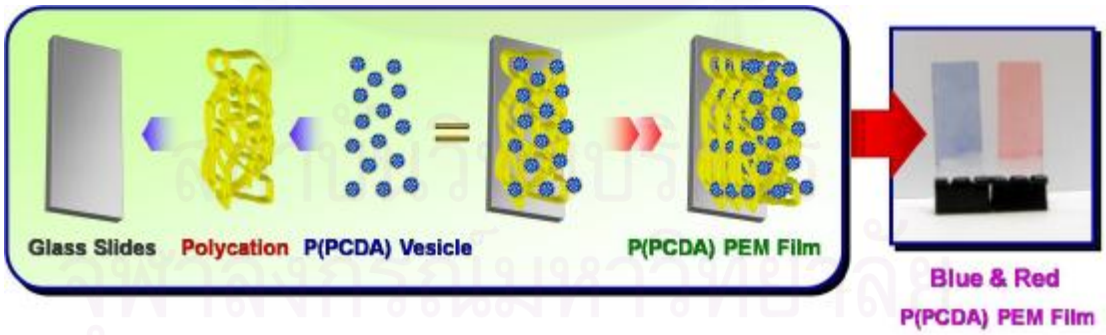


Figure 2.2 Preparation of a poly(PCDA) multilayer film.

Film formation induced by acidic solution

The effect of pH on poly(PCDA)/polycation multilayer films (0-10 layers) was carried out by UV-Vis spectrometer (absorption at 635 nm). Both HCl and acetic acid were used to adjust pH to reach 1, 2 and 3.

2.3.4 Measurement of colorimetric response

UV-Visible spectroscopy

The visible absorption spectra of the vesicle solutions and films were taken in a quartz cuvette with 1 cm optical path length using a temperature controlled UV-Visible spectrophotometer. The spectra were collected from 800 to 400 nm at 25 °C with the zero absorbance set at 800 nm.

Colorimetric measurements

The colorimetric response (%CR) was used to quantitatively evaluate the blue-to-red transition. The %CR can be expressed as $100 \times (PB_0\text{-}PB)/PB_0$, the PB is the percent blue calculated from $A_{blue}/(A_{blue}+A_{red})$ where A_{blue} and A_{red} are the absorbance of the blue (λ_{max} = 635 nm) and red (λ_{max} = 540 nm) phases of poly(PCDA), respectively. The initial percent blue (PB_0) was determined on the samples before exposure to any stimuli.

Study of color transition induced by cyclodextrin solution

A poly(PCDA)/chitosan multilayer film was introduced into a quartz cuvette filled with deionized water (pH = 6.0). The temperature was maintained at 25 °C for 5 min by using a temperature controlled UV-Vis spectrophotometer prior to the spectrum acquisition. The spectra were collected from 800 to 400 nm with the zero absorbance at 800 nm. After repeated the measurement procedure, the deionized water was replaced by cyclodextrin (α-, β-, γ-CD) solutions with the concentrations varied from 0-20 mM. All measurements were carried out using as-prepared poly(PCDA)/chitosan film. For complete complexation, the multilayer films were immerged into each CD solution at 25 °C for 5 min prior to the spectrum acquisition.

The color transition of poly(PCDA) vesicle solution was studied in comparison to the film by mixing a fresh vesicles solution (A = 0.15-0.20 at 635 nm) with each CD solution.

2.3.5 Study of sensitivity dependence

Detection Temperature

A quartz cuvette filled with deionized water was stand inside the temperature-controlled UV-Vis spectrophotometer for 5 min. Then the poly(PCDA) vesicle solution (A = 0.15-0.20 at 635 nm) was added into the cuvette and mixed with deionized water. The temperature was maintained for further 5 min prior the spectrum acquisition. The deionized water was replaced by α-CD solution with the concentrations varied from 0-10 mM. The temperature dependence on colorimetric response was investigated by controlling the environment at 10, 25 and 40 °C, respectively.

Mixed lipid vesicles

The effect of stearic acid on α-CD detection was investigated by replaced poly(PCDA) vesicle solution with mixed lipid vesicle solutions (mole ratio of poly(PCDA):stearic acid being 70:30 and 50:50). The vesicle solutions were mixed with α-CD solution with the concentrations varied from 0-10 mM. All measurements were carried at controlled temperature (25 °C).

Number of layers

The effect of number of poly(PCDA)/chitosan layer (2, 10, 20 layers) on α-CD detection was investigated. The measurement procedures were done in the same manner as described for “Color transition induced by cyclodextrin solution”.

2.3.6 Study of particle size and morphology change in response to α-cyclodextrin

Dynamic Light Scattering (DLS)

The mean size of vesicles and the size distribution were determined by nanosizer (Malvern Instruments). The samples were sonicated for 1 min before measurement. Each measurement was repeated 3 times in order to acquire an average data.

Transmission Electron Microscopy (TEM)

TEM images were completed using a JEOL TEM-2100 electron microscope equipped with a CCD camera. The accelerating voltage was 200 KV. The vesicle solution was dropped onto a formvar coated copper grid (200 meshes), stained with 2% uranyl acetate for 5 min and dried at room temperature in desicator. For completely color transition, the mixed solution between poly(PCDA) vesicle (0.5 mM) and α-CD (10 mM) was kept overnight prior to drop onto the TEM grid.

Atomic Force Microscopy (AFM)

Vesicles were deposited on a freshly cleaved mica plate and dried at room temperature in desicator for 4 hours. Seiko SPA 400 (SII Nanotechnology Inc.) operating in non contact mode was used to observe the morphology and particle size of the deposited vesicles.

2.3.7 Inhibition of color transition by inclusion complexation

Nitrophenol

The sensing capabilities of poly(PCDA)/chitosan multilayer film to nitrophenol (*o*-, *m*-, and *p*-NP) were investigated by the aids of inclusion complexation between α-CD and nitrophenol. The mixture of α-CD (10 mM) and nitrophenol (20 mM) solutions was sonicated for 2 min. The poly(PCDA)/chitosan film was dipped into the inclusion complex solution for 5 min. After rinsing with deionized water for 3 times, the PEM film was immerged into a quartz cuvette filled with deionized water. The color transition was observed by UV-Vis spectrometer (400-800 nm). The colorimetric response of the PEM film towards nitrophenol at lower concentration was investigated to determine detection limit.

Aromatic Compounds

The sensing capabilities of poly(PCDA)/chitosan multilayer film to aromatic compounds (phenol, *p*-nitrobenzene, *p*-methoxyphenol, *p*-nitrotoluene, benzoic acid and indole) were investigated by the aids of inclusion complexation between α-CD and aromatic compound. The experimental procedure was done in the similar manner as described for nitrophenol. All measurements were carried at room temperature for 10 min prior the spectrum acquisition.

The inhibition of the color transition of a solution compared to a film was studied replaced the poly(PCDA)/chitosan multilayer film with the poly(PCDA) vesicle solution (A=0.15-0.20 at 635 nm).

Herbicides

The sensing capabilities of poly(PCDA)/chitosan multilayer film to herbicides (Dimethyl Viologen^{2+}, DV^{2+}, Methyl Viologen^{+}, MV^{+} and 2,4-Dichlorophenoxyacetic acid, 2,4-D) were investigated by the aids of inclusion complexation between α-CD and herbicide. The experimental procedure was done in the same manner as described for aromatic compounds.

2.3.8 Fabrication of micro-multichannel device

Fabrication of glass mold

A glass slide was cut into a 1.25×3.75 cm^2 size and the side of glass slide was scrubbed by whetstone. A PVC sticker was cut into a 7.25×9.75 cm^2 size and perforated with a 1.25×0.5 cm^2 size along the slide (figure 2.3). Another PVC sticker was cut into a 3.25×5.75 cm^2 size.

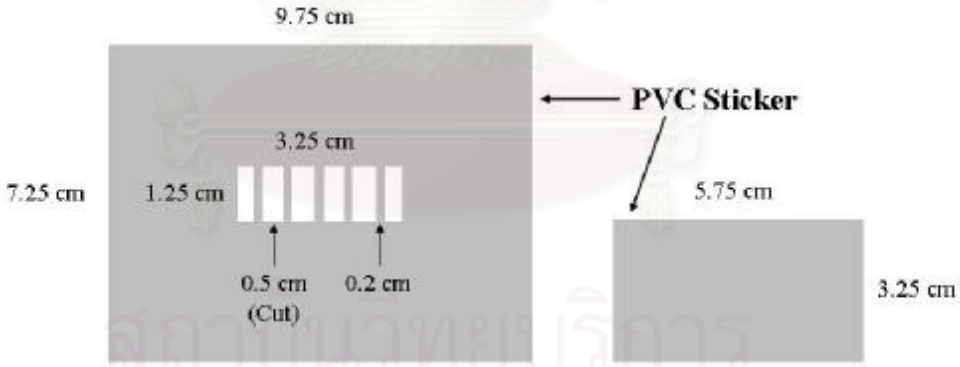


Figure 2.3 Size of PVC sticker.

There was a simple design for fabricated the PDMS mold. To prepare the base of mold, a glass slide was mask channel patterns with PVC sticker. Another PVC sticker (3.25×5.75 cm^2) was attended on back side of the glass slide. The mask-patterning glass slide was incubated in hydrofluoric acid (HF) for 3 hours. HF was mortised the unmark-surface glass ~1.5 mm. After 3 hours, the glass slide was rinsed with deionize water and all PVC masks were removed. The 4 pieces of glass slide

were cut for fabricated the edge of PDMS mold. The glasses were put together and combined by using silicon glue. The glass mold was carried at room temperature for 24 hours before used to prepared PDMS multichannel.

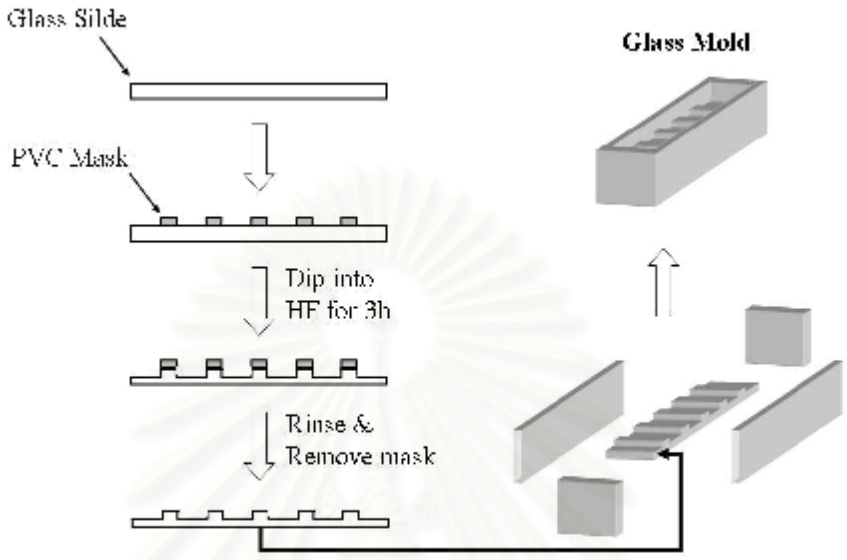


Figure 2.4 Schematic diagram of glass mold design and overall scheme for glass-surface development.

Fabrication of PDMS multichannel

PDMS prepolymer was mixed with a curing agent in a ratio of 10:1 by volume. The mixing solution was contained in vacuum desicator for 30 min to remove gas in the solution. After degas, The PDMS solution was filled into glass mold and perfumed at 60-65 °C for 2 hours. Multichannel was formed by bonding PDMS replica to glass mold. Take the PDMS channel outside the mold and covered with poly(PCDA) PEM film (20 layers), got poly(PCDA) multichannel device.

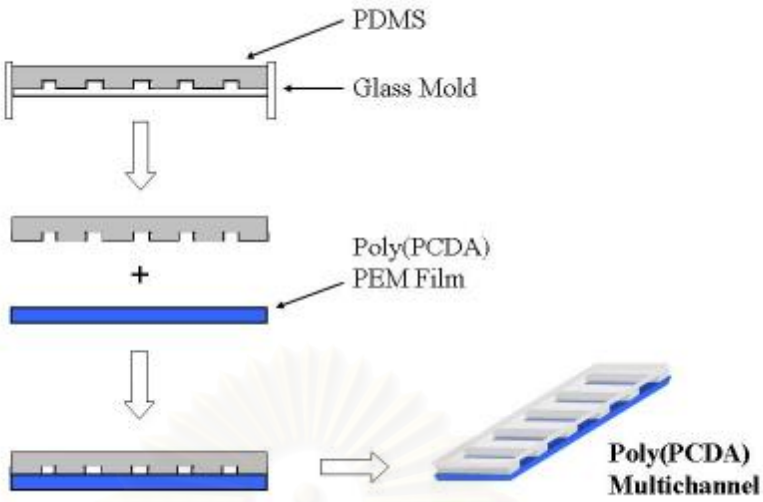


Figure 2.5 Schematic diagram of poly(PCDA) multichannel fabrication.

CHAPTER III

RESULTS AND DISCUSSION

In this work, a layer-by-layer deposition technique was utilized to prepare polyelectrolyte multilayer (PEM) thin film containing poly(PCDA) vesicles of 10,12-pentacosadiynoic acid (PCDA). The PEM film was prepared simply by alternate dipping of a substrate into positively charged polyelectrolyte, polycation, and negatively charged polyelectrolyte, poly(PCDA) vesicles. The thickness as well as the color intensity of the film can be conveniently controlled by the number of the vesicle layers deposited [43]. The application of the PEM film containing poly(PCDA) vesicles for detection of various aromatic compounds in water was investigated in comparison to poly(PCDA) sol. The PEM film showed several advantages over the sol.

3.1 Preparation of polydiacetylene vesicles

10, 12-Pentacosadiynoic acid (PCDA), a diacetylene monomer, was dispersed well in water to form translucent colloidal sol without discernible lipid suspension after sonication at 75-85 °C for 15 minutes and no precipitation formed after keeping the colloids at 4 °C overnight. An intense blue sol containing little blue solid suspension was obtained after irradiation with UV light (254 nm) for 5 minutes at room temperature indicating that the lipid assembly readily undergoes photopolymerization to form ene-yne conjugated polydiacetylene, called poly(PCDA) in this thesis. The poly(PCDA) sol was filtered through a filter paper (pore size 1/125 mm) to give a clear blue sol (Figure 3.1).

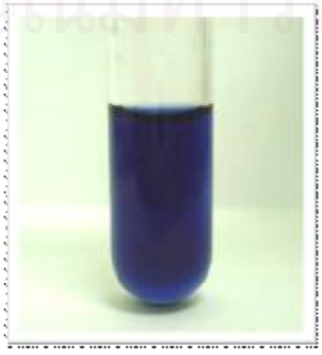


Figure 3.1 Color photograph of poly(PCDA) sol.

It is known that PCDA forms nano vesicles with an average diameter of 50-200 nm depending on the preparation condition [8-9]. Dynamic light scattering (DLS) spectroscopy was thus used for measuring the average particle sizes and size distribution of poly(PCDA) vesicles prepared in this work. From 6 repetitions, the average diameter of poly(PCDA) vesicles was 66-75 nm with PDI = 0.515 ± 0.021 (Figure 3.2, Appendix A1).

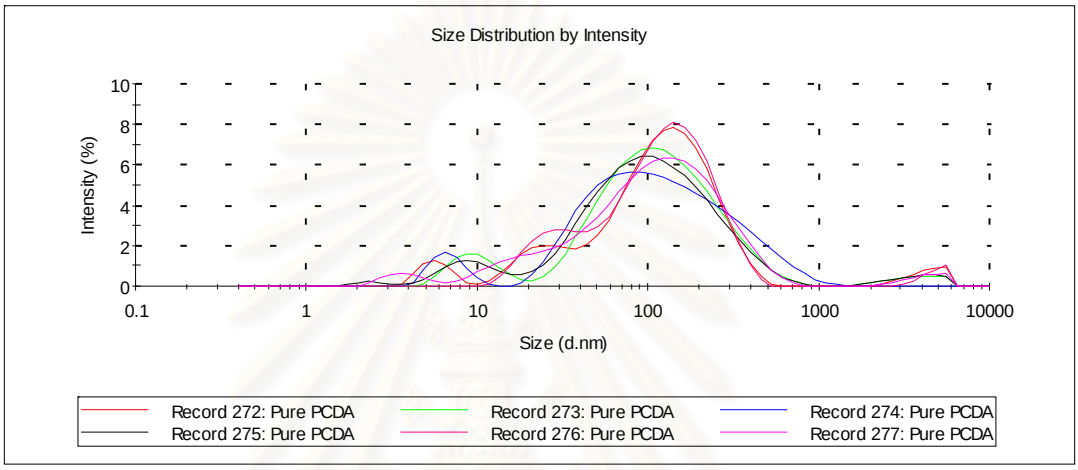


Figure 3.2 Particle size distribution of poly(PCDA) vesicles.

Atomic force microscopy (AFM) and transmission electron microscopy (TEM) were utilized to observe the shape and size of the air-dried poly(PCDA) vesicles. AFM and TEM images of poly(PCDA) showed that the spherical vesicles with observed diameters of 50-120 nm (Figure 3.3). The particle sizes measured by different techniques (DLS, AFM and TEM) were thus in very good agreement for soft nanomaterials such as lipid vesicles.

(a) (b)

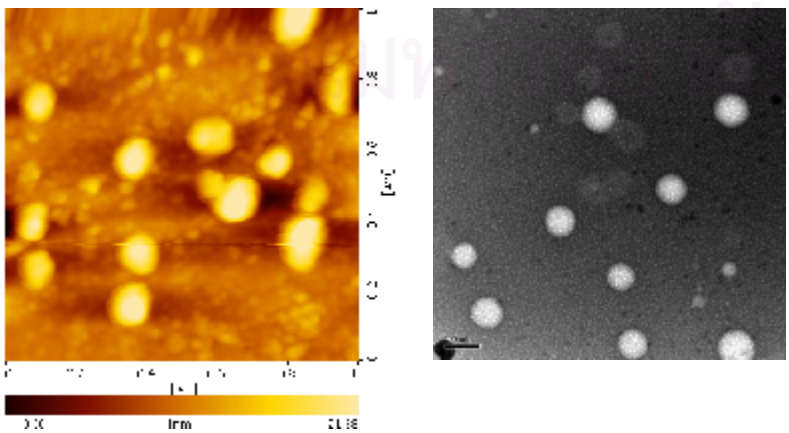


Figure 3.3 AFM images (a) and TEM images (b) of poly(PCDA) vesicles.

3.2 Preparation of poly(PCDA) PEM film

As the structures of the polycationic polymers and the degree of ionization of poly(PCDA) vesicles can appreciably influence the quality and thickness of poly(PCDA) PEM films, various polycationic polymers were tested under different pH adjustment. Since the goal of this work is to develop a simple analytical tool for detection of aromatic compounds, it is also of significance to demonstrate the reproducibility of the film preparation. The results discussed herein will thus focus on these aspects.

3.2.1 Effect of polycation on film quality

Poly(PCDA) multilayer film was prepared on a clean glass substrate by a layer-by-layer deposition technique using poly(PCDA) vesicle as a polyanion and cationic polymer (chitosan, PEI, PDADMAC) as a polycation (Figure 3.4). The increase of the number of layers of poly(PCDA) vesicles adsorbed led to the deeper blue color. Under carefully controlled condition, the uniformly blue poly(PCDA) PEM films (Figure 3.5a) could be obtained with chitosan as a polycationic polymer. The PEM film was constructed by the aids of the electrostatic interaction between a carboxylate moiety on poly(PCDA) vesicle and an ammonium group on chitosan. The use of PEI and PDADMAC in place of chitosan as a polycationic polymer gave PEM films with deeper blue color however with rather less uniformity (Figure 3.5b and 3.5c). Several factors may contribute to the uniformity of poly(PCDA)/chitosan PEM film. The mobilizable charges due to protonation/deprotonation process may allow more flexible interaction between the polycationic chain and the carboxylate head groups of the vesicles. The hydroxyl groups in chitosan may also incorporatively form hydrogen bonding with the carboxylic groups of the vesicles. The lighter color of poly(PCDA)/chitosan PEM film indicating less vesicles deposited in each layer may be attributed to the lower charge density of chitosan comparing to the permanent positive charge on PEI and PDADMAC.

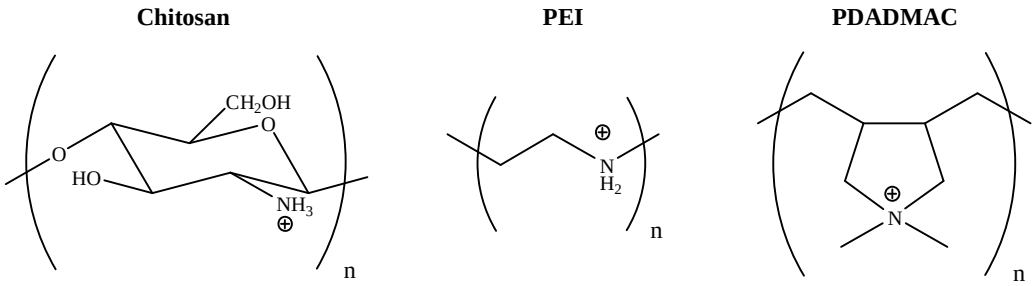


Figure 3.4 The chemical structures of polycationic polymer.

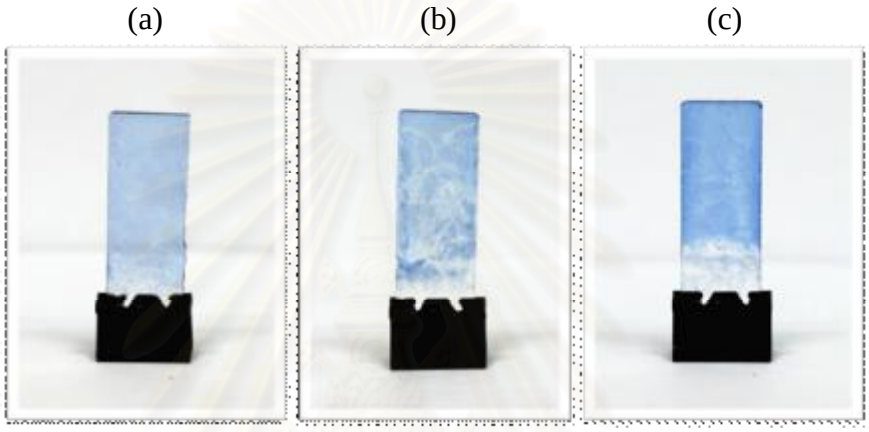


Figure 3.5 Photography of poly(PCDA) multilayer film (20 layers) with various polycation: (a) chitosan, (b) PEI, (c) PDADMAC prepared at pH 3.0-3.3.

Due to a better film quality, poly(PCDA)/chitosan PEM film was studied and used from hereon throughout this thesis.

3.2.2 Effect of pH on the color intensity of the films

To study the effect of pH on the poly(PCDA)/chitosan PEM film quality, the pH of poly(PCDA) sol, chitosan solution and rinsing water was adjusted lower by HCl (0.01 M) and acetic acid (0.01 M). Without the pH adjustment, the electronic absorbance of the PEM films at 635 nm increased linearly with the number of layers of poly(PCDA) vesicles deposited signifying a rather constant thickness of each layer. When the pH was adjusted lower than 2, poly(PCDA) vesicles were appallingly aggregated making them no longer suitable for film preparation. At pH 3, the layer-by-layer deposition however can give uniform poly(PCDA)/chitosan PEM films with linear relationship between the electronic absorbance and the number of poly(PCDA) layers deposited (Figure 3.6). Comparing to at the unadjusted pH, the increase of

electronic absorption at each layer was apparently greater at pH 3, especially when the pH adjustment was done by using HCl, indicating the greater numbers of poly(PCDA) vesicles adsorbed onto each of the chitosan layer. At lower pH, amino groups of chitosan can be more readily protonated and turn into more cationic ammonium groups which may in turn increase the adsorption on the glass substrate and the interaction with the carboxylic head groups on poly(PCDA) vesicles. The interaction between ammonium and carboxylic groups under relatively high acidic condition probably involves an ion exchange process; $RNH_3^+ \cdot X^- + RCO_2H \rightarrow RNH_3^+ \cdot {}^-O_2CR + H^+ + X^-$. It is thus quite reasonable that the pH adjustment with HCl ($X = Cl^-$) gave higher adsorption of poly(PCDA) vesicles than that with CH_3CO_2H ($X = CH_3CO_2^-$). The preparation of poly(PCDA)/chitosan PEM films in the subsequent studies were thus carried out at pH 3 adjusted with HCl.

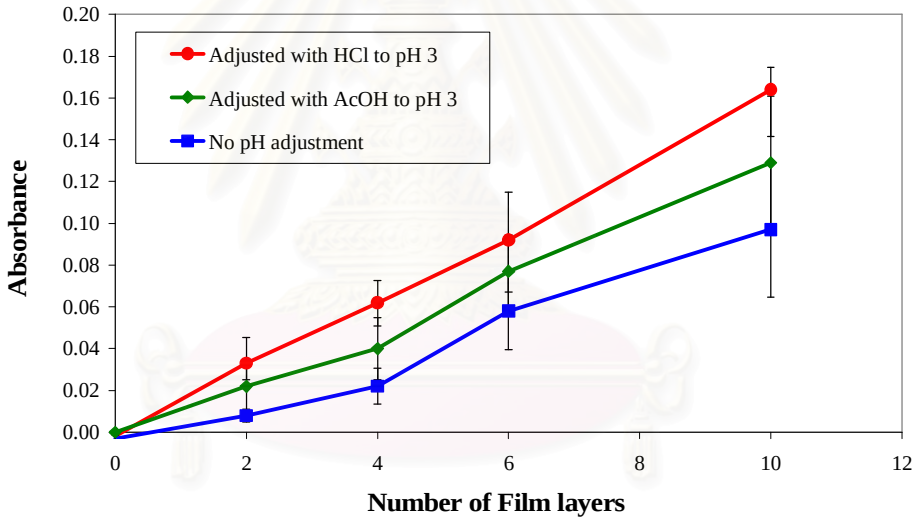


Figure 3.6 Absorbance at 635 nm of poly(PCDA)/chitosan PEM films in relation to the increasing layers of poly(PCDA)/chitosan deposited.

The adjustment of pH to higher pH than the original pH of the involving materials was not suitable due to low solubility of chitosan and the color transition of poly(PCDA) vesicles at higher pH.

3.2.3 Reproducibility of film preparation

To study the reproducibility in the preparation of poly(PCDA)/chitosan PEM films, 10 samples of ten-layered films were prepared from the solutions with pH 3 adjusted with HCl. The absorbance of the films at 635 nm (A_{635}) was in the range of 0.131-0.177 (Table 3.1). The results suggested that the conditions for layer-by-layer deposition technique developed in this work can be used to prepare the films with reproducible absorbance with standard deviation of 10% (A_{635} = 0.150 ± 0.015).

Table 3.1 The absorbance of 10 layers of poly(PCDA)/chitosan PEM films at 635 nm

Film Sample	1	2	3	4	5	6	7	8	9	10
A_{635}	0.133	0.143	0.131	0.159	0.134	0.154	0.149	0.159	0.164	0.177

3.3 Colorimetric response in the α-CD induced color transition of poly(PCDA) sol

The color of poly(PCDA) vesicles associates with the π-π* electronic transition within the ene-yne conjugated backbone that can absorb visible light wavelengths. Poly(PCDA) sol exhibits distinct color change from blue to red by increasing the temperature, changing of solvent or increasing the pH [2-11]. The color transition from blue to red indicates the increases of the energy gap between the ground and excited states of the π-conjugated backbone of poly(PCDA). The increase in the energy gap of poly(PCDA) is widely interpreted as the reduction of the length of the π-conjugation system due to the twisting of the single bonds between the double and triple bonds [22]. However, another theory envisions the increase of the energy gap based on the relief of mechanical strain in the conjugated backbone by lengthening the double and triple bonds to their normal bond length resulting in lowering ground state [25]. Both theories however related to the weakening or lost of the hydrogen bonding between the carboxylic head group of the diacetylene lipid side chain.

The color transition of modified poly(PCDA) has also been reported for applications in naked eye detection of influenza virus [50], cholera toxin [51], *Escherichia coli* [16] and glucose [17]. The color transition of poly(PCDA) sol from blue to red can also be induced by α- cyclodextrin (α-CD) through the formation of an inclusion complex between the carboxylic head group of PCDA and α-CD [27].

The extent of blue-to-red color transition has often been measured quantitatively as the colorimetric response (%CR) which is defined by the following equation [25].

$$\%CR = \frac{(PB_0 - PB)}{PB_0} \times 100$$

In this equation, PB is the percent blue calculated from $A_{blue}/(A_{blue}+A_{red})$. The A_{blue} and A_{red} were the absorbance of the blue and red phases of poly(PCDA) measured at λ_{max} around 635 nm and 540 nm, respectively. PB_0 is the initial percent blue of the vesicle solution and film before exposure to the stimulus. Typically in this thesis, the electronic absorption spectra of poly(PCDA) samples were recorded in the visible range (400-800 nm) with the absorbance at 800 nm was set to zero.

3.3.1 α-CD induced color transition of poly(PCDA) sol

The electronic spectrum of blue poly(PCDA) sol showed the maximum absorption wavelength (λ_{max}) at 635 nm (Figure 3.7). Addition of α-CD (10 mM) induced a color transition of the solution from blue to red. Although there were several changes in the absorption band of the transitioning poly(PCDA) sol, the distinct changes were the increase of absorbance at 540 nm with the expense of the absorbance at 635 nm. The absorbance at these λ_{max} were thus used for the calculation of the colorimetric response (%CR).

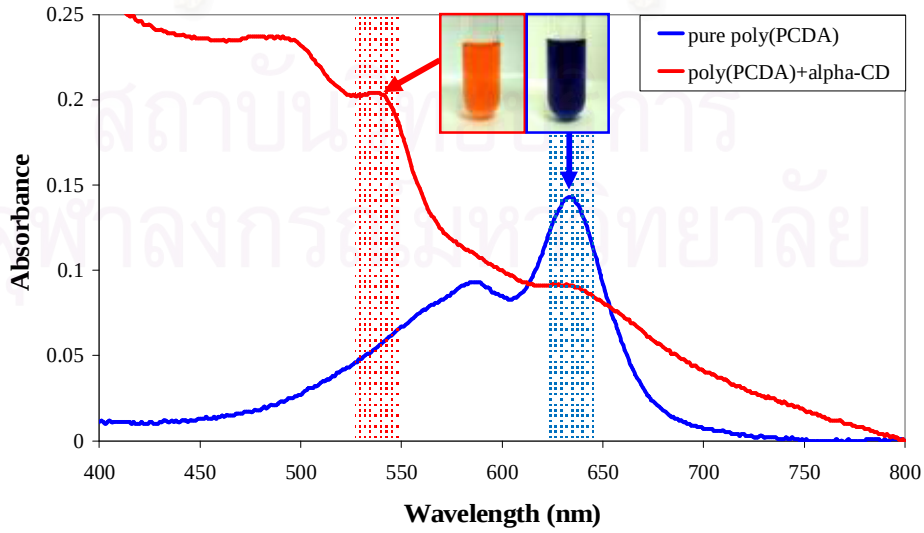


Figure 3.7 Visible spectra of poly(PCDA) sol before (blue line) and 5 min after an addition of α-CD (10 mM) (red line) at 25 °C.

Along with the color change, the poly(PCDA) sol also turned turbid suggesting that α-CD not only induced the color transition but also caused some aggregation of the vesicles. The turbidity of poly(PCDA) sol also caused the increase of the absorption baseline that is more drastically in the range of shorter wavelength.

To study a time-dependent of α-CD induced color transition of poly(PCDA) sol, the colorimetric responses of the sol (~0.1 mM) after an addition of α-CD (5-10 mM) for variable times were recorded. At 5 mM of α-CD, the %CR steeply increased in the first 5 minutes and seemed to reach saturation after 15 minutes (Figure 3.8). The faster responses were observed for the higher concentrations of α-CD, for examples, %CR reached saturation after 10 and 5 minutes at 7 and 10 mM of α-CD, respectively. The response time of less than 15 minutes is quite suitable for further development of a sensor kid that the concentration of α-CD should thus be kept within the range of 5-10 mM. Although the higher concentration of α-CD may give even faster response, it is limited by low solubility and the high cost of α-CD.

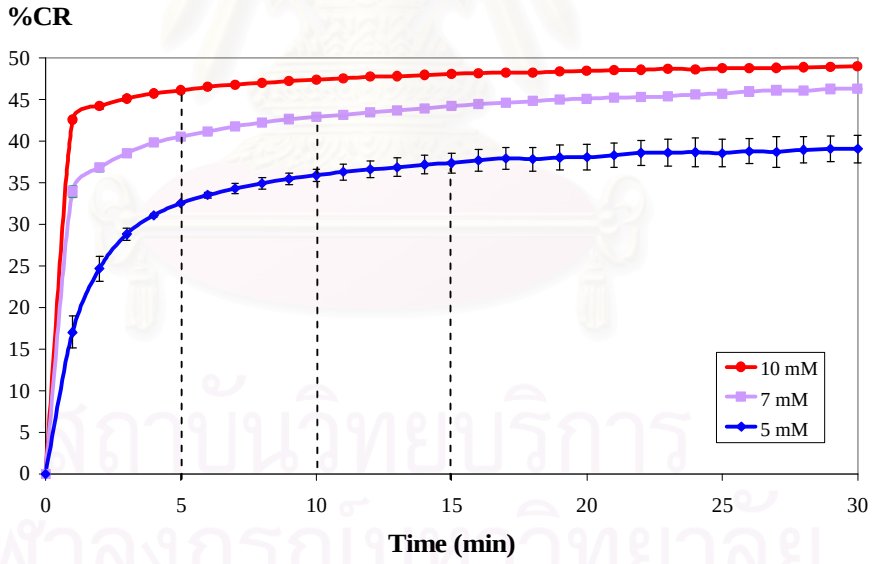


Figure 3.8 Time dependence of the colorimetric responses of poly(PCDA) sol to α-CD solution (5-10 mM) at 25 ^{o}C.

3.3.2 Effect of temperature on the sensitivity of poly(PCDA) sol in the α-CD induced color transition

The colorimetric response in α-CD induced color transition of poly(PCDA) sol was measured at 10, 25, and 40 °C. Poly(PCDA) sol became more sensitive to α-CD at higher temperature showing greater colorimetric response at every α-CD concentration (Figure 3.9). Thermal energy may loosen the hydrogen bond between the carboxylic groups on the surface of poly(PCDA) vesicles leading to acceleration greater accessibility of α-CD to form the inclusion complex. The sensitivity of poly(PCDA) sol increased significantly when the detection temperature increased from 10 to 25 °C but only slightly when the detection temperature increased from 25 to 40 °C. At the temperature higher than 40 °C, the color of poly(PCDA) sol turned purple prior to the addition of α-CD. The subsequent experiments were thus conducted at 25 °C.

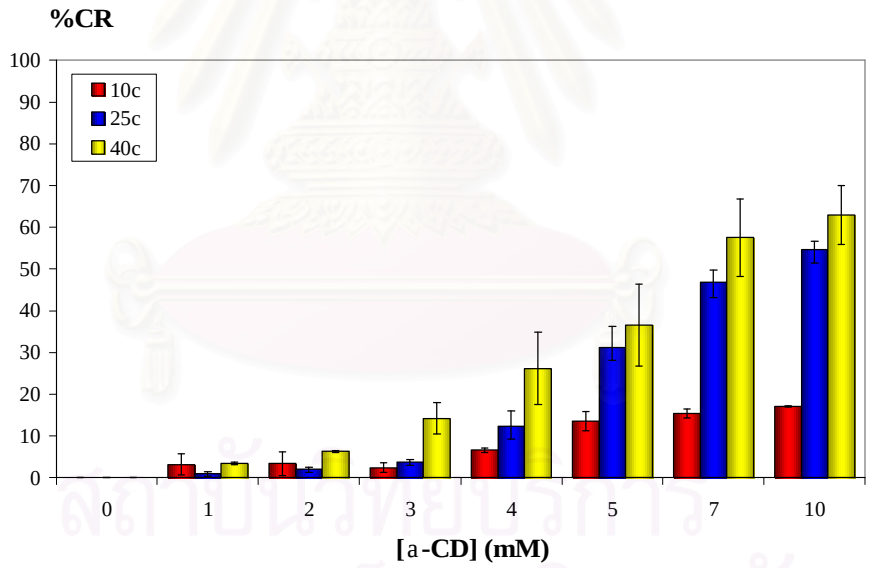


Figure 3.9 Colorimetric response of poly(PCDA) sol upon exposure to α-CD solution for 5 min at various temperatures.

3.3.3 Mixed lipid vesicles

Poly(PCDA) vesicles containing stearic acid were prefigured to be the flexible vesicles that might be more sensitive to the α-CD induced color transition. The observed colorimetric response of the mixed lipid sol with PCDA/steric acid mole ratio of 50:50, 70:30 and 100:0 to α-CD were however not significantly different

from that of pure poly(PCDA) sol (Figure 3.10). Mixed lipid vesicles were thus not pursued in the subsequent experiments.

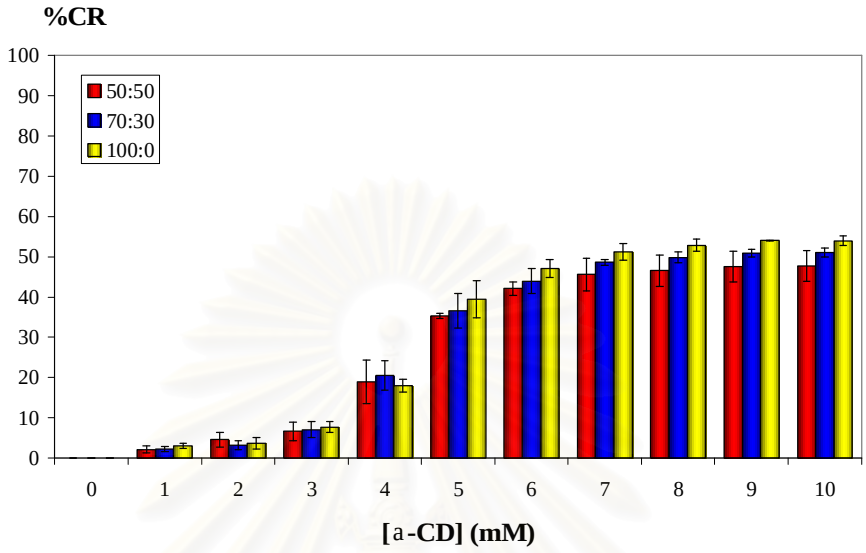


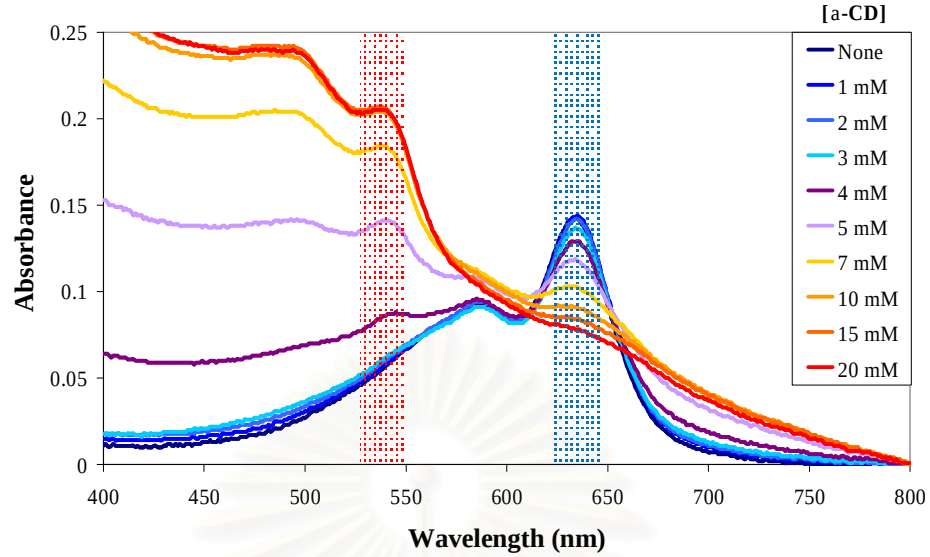
Figure 3.10 Colorimetric response of poly(PCDA)/stearic acid mixed lipid sol having various PCDA/stearic acid mole ratio upon exposure to various concentration of α-CD solution at 25 °C for 5 min.

3.4 Color transition of poly(PCDA)/chitosan PEM film induced by α-CD

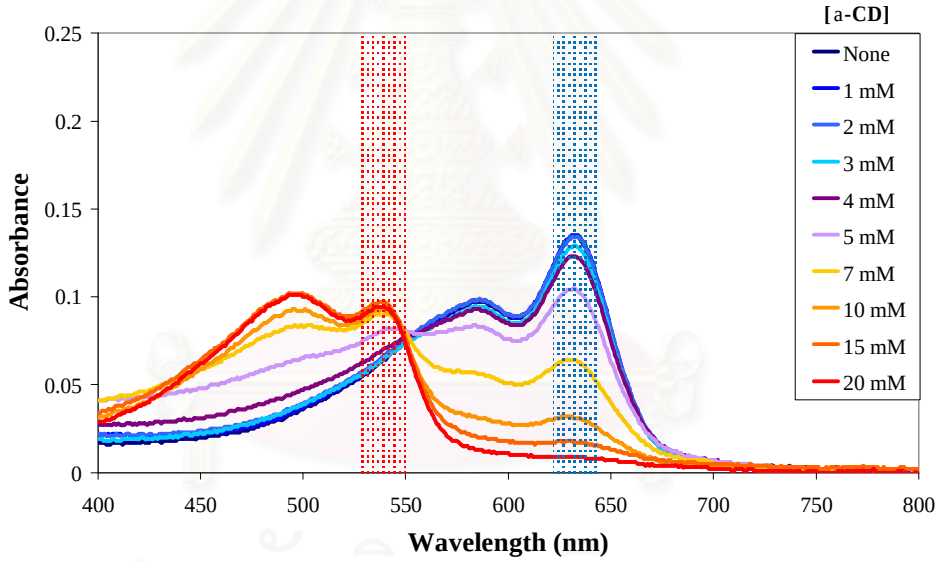
As the main objective in this thesis is to develop poly(PCDA) for simple to use naked eyes detecting devices, it is thus interesting to compare the colorimetric response of α-CD induced color transition of poly(PCDA) PEM film with that of poly(PCDA) sol.

3.4.1 Comparison of poly(PCDA) PEM film with poly(PCDA) sol

In the comparison study, the transitioning visible absorption spectra of poly(PCDA) poly(PCDA)/chitosan PEM film and poly(PCDA) sol tested with varied concentrations of α-CD solutions (0-20 mM) were compared (Figure 3.11). As mentioned in the previous section, the blue to red color transition of poly(PCDA) sol occurred with considerable turbidity evidenced by the increasing baseline absorption (Figure 3.11a). Interestingly, poly(PCDA) film exhibited the color transition without significant change of the baseline absorption (Figure 3.11b).



(a)



(b)

Figure 3.11 UV Spectrum of poly(PCDA) vesicles solution (a) and 10 layers of poly(PCDA)/chitosan PEM film (b) after exposure to 0-20 mM α-CD solution at 25 ^{o}C for 5 min.

The turbidity observed for the poly(PCDA) sol is the result of aggregation of poly(PCDA) vesicles probably due to the lost of their spherical assembly and surface stability upon the interaction with α-CD. The destabilized vesicles thus reduce their surface energy by coalescing or binding to each other as evidenced by the transmission electron microscopy (TEM) images (Figure 3.12). The original poly(PCDA) vesicles were spherical and rather uniform in size (Figure 3.12a) while the vesicles observed after mixing with α-CD were irregular in both size and shape with a good portions of vesicles aggregated or coalesced (Figure 3.12b).

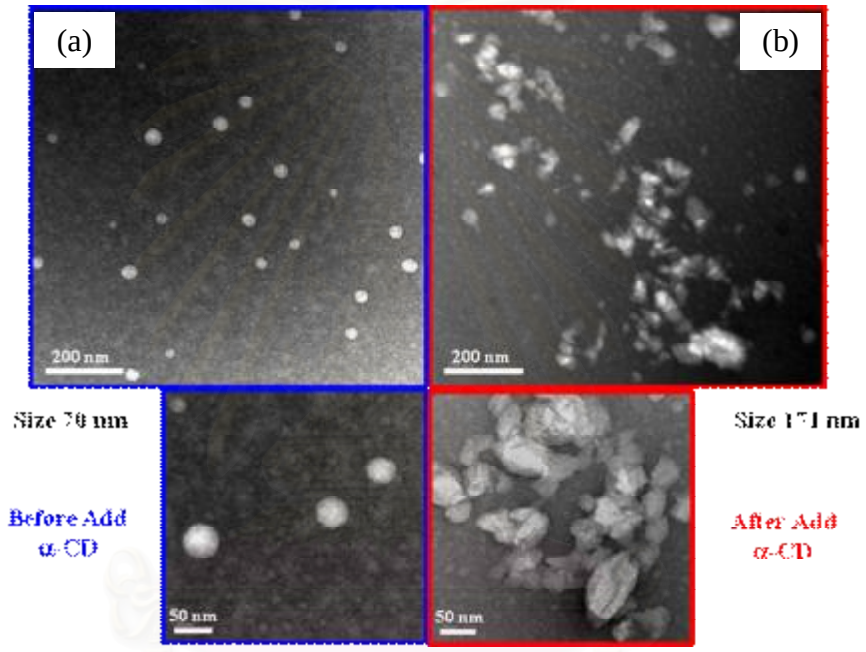


Figure 3.12 TEM images of poly(PCDA) vesicles (Stained with 2% Uranyl Acetate for 5 min): (a) before and (b) after exposure to α-CD (10 mM).

The changes in size and shape of vesicles in poly(PCDA) were also observed by atomic force microscopy (AFM) (Appendix B). Dynamic light scattering (DLS) showed the average diameter of poly(PCDA) vesicles changed from 66-75 nm to 156-204 nm with PDI = 0.427 ± 0.062 (Figure 3.13, Appendix A2).

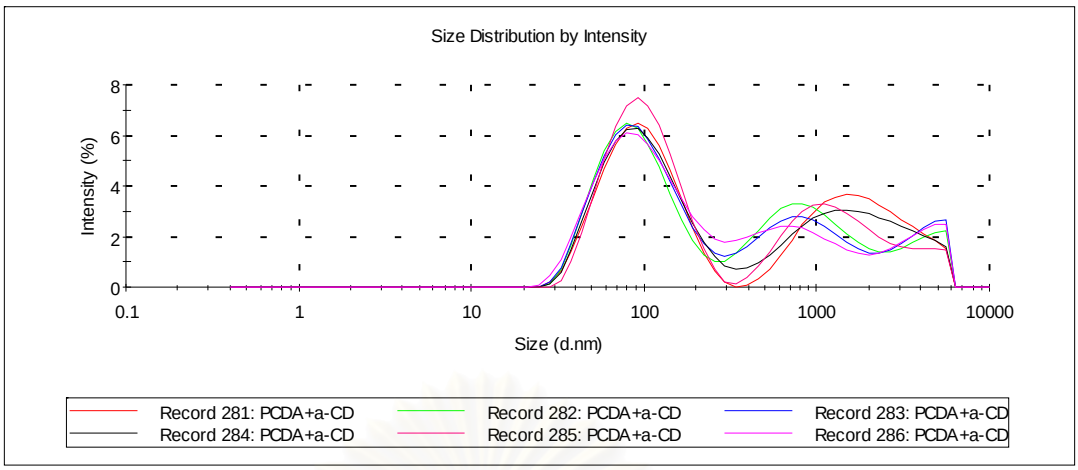


Figure 3.13 Particle size distribution of poly(PCDA) vesicles after exposure to α-CD.

Since the vesicles are immobilized in the solid PEM film, they are less likely to move to contact each other and thus reducing the chance for creating turbidity in the films. Comparing the colorimetric responses of poly(PCDA)/chitosan PEM film to that of poly(PCDA) sol at various concentration of α-CD tested, poly(PCDA)/ chitosan PEM film showed lower %CR at low concentration of α-CD but higher %CR at higher concentration of α-CD (Figure 3.14). The higher response of poly(PCDA) sol is probably faultily contributed by the increase of baseline absorption at the shorter wavelength due to turbidity. At higher concentration of α-CD, poly(PCDA) sol became badly turbid so that the baseline absorption became the major part of the whole absorption (Figure 3.12). Mathematically, %CR becomes insensitive to the real color change when the baseline absorption become the major contributor to the whole absorption and thus %CR of poly(PCDA) sol reached its maximum of around 60%. Thanks to less interference from turbidity, the PEM films showed wider linear colorimetric response range to α-CD (4-20 mM) comparing to that of poly(PCDA) sol (4-10 mM).

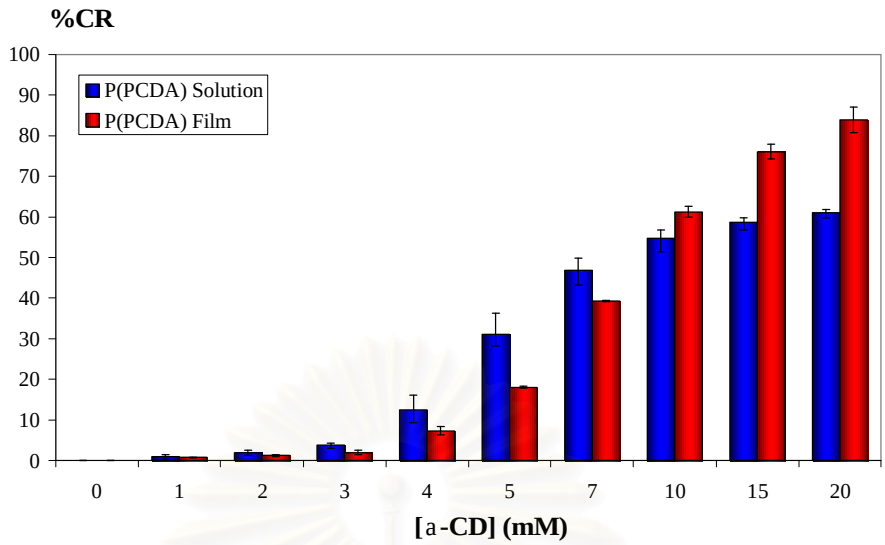


Figure 3.14 Colorimetric responses of poly(PCDA) sol and poly(PCDA)/chitosan PEM films after exposure to α-CD solution at 25 ^{o}C for 5 min.

3.4.2 Effects of the number of deposition layers on the colorimetric responses of poly(PCDA)/chitosan PEM film

The colorimetric responses of poly(PCDA)/chitosan PEM films constituted of 2, 10 and 20 layers induced by α-CD (0-10 mM) were determined. The results indicated that the lower the number of layers of poly(PCDA) deposited, the higher the sensitivity of the PEM film (Figure 3.15) especially for the PEM films constituted of 2 layers (1 layer of poly(PCDA) and 1 layer of chitosan). However, as the absorbance of the two-layered PEM film was so low that it is very close to the detection limit of the spectrometer (Appendix G), the reproducibility of the colorimetric responses were very poor evidenced by wide error bars. Although the PEM film constituted of 10 layers showed lower sensitivity to α-CD, it possessed good reproducibility and its color is good enough to be seen by naked eyes. The twenty-layered PEM film has about the same response as that of the ten-layered films but it is more laborious to be prepared.

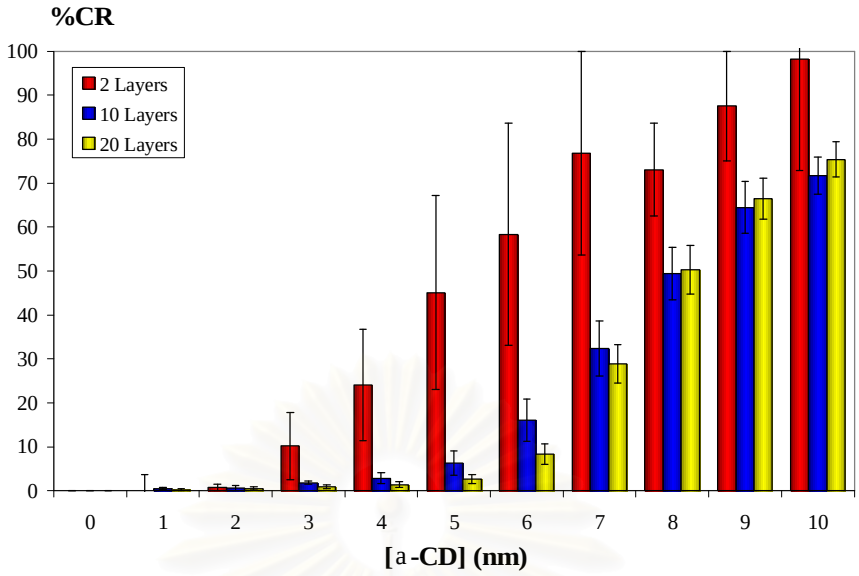


Figure 3.15 Colorimetric responses of poly(PCDA)/chitosan PEM film with various layers (2-20 layers) after exposure to 0-10 mM α-CD solution at 25 °C for 5 min.

3.5 Inhibition of α-CD induced color transition by aromatic compounds

3.5.1 Nitrophenols

Previously, *p*-nitrophenol has been report to inhibit the color transition induced by α-CD [17]. The inhibition process has been suggested to involve the competition between the *p*-nitrophenol and carboxylic head group of the vesicles to form an inclusion complex with α-CD [27]. It is thus of interest to investigate if different isomers of nitrophenols would have different inhibition ability. Three regioisomers of nitrophenols, *o*-, *m*- and *p*-, were investigated for their ability to inhibit the α-CD induced color transition of the poly(PCDA)/chitosan PEM film. In the absence of nitrophenol, an α-CD solution (10 mM) turned the color of poly(PCDA)/chitosan film from blue to red with the colorimetric response of ~60% (Figure 3.16). The colorimetric response became significantly lower in the presence of *m*- and *p*-nitrophenols but not in the presence at *o*-nitrophenol. The complete inhibition of the color transition (%CR = 0) was observed when the film was dipped into the α-CD solution (10 mM) having either 15 or 10 mM of *m*- and *p*-nitrophenols, respectively.

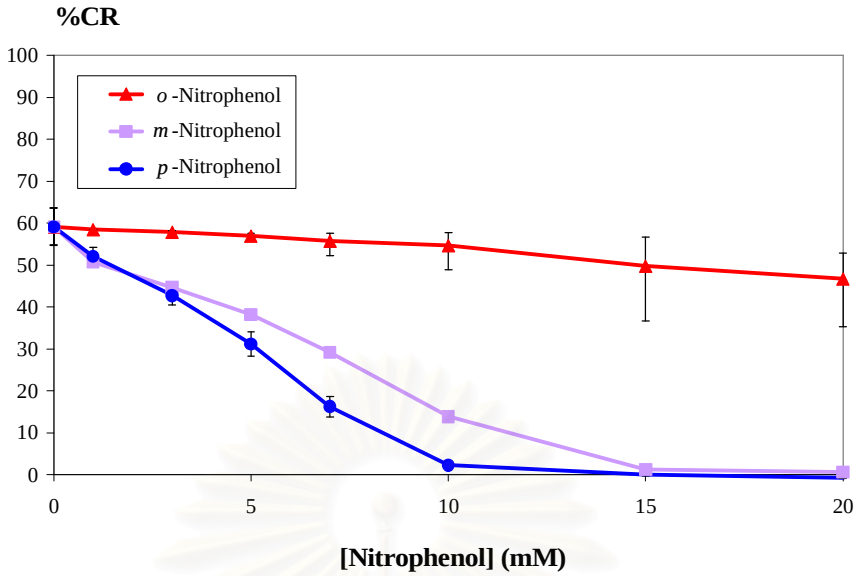


Figure 3.16 Colorimetric responses of poly(PCDA)/chitosan PEM films upon exposure to the mixture of α-CD (10 mM) and various concentrations of nitrophenols at 25 °C for 5 min.

The results suggested a series of binding strength between α-CD host and the nitrophenol guests as follows: *p*-nitrophenol > *m*-nitrophenol >> *o*-nitrophenol. Considering the molecular geometry of nitrophenols (Figure 3.17), the difference in the binding strength in this case should be mainly attributed to the competitive interaction [52-54]. In case of *o*-nitrophenol, the reason of the least stable inclusion complexes may come from the intramolecular hydrogen bonding between hydroxyl and nitro groups. The results also demonstrated that poly(PCDA)/chitosan PEM films can be used for simple distinguishing of the regoisomers of nitrophenols.

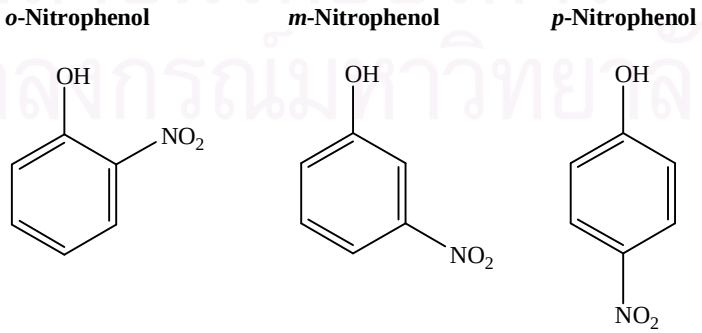


Figure 3.17 The chemical structures of nitrophenols.

3.5.2 Aromatic compounds

As discovered in the previous section that *p*-nitrophenol has good ability in inhibiting the color transition induced by α-CD due to its low steric bulkiness, it is thus of interest to extend the investigation to other *p*-substituted phenols having different substituents. Varying the electronic effect of the substituents, phenol, *p*-nitrophenol and *p*-methoxyphenol were tested for their ability to inhibit the α-CD induced color transition by immersing the PEM film into a mixed solution of a tested phenol and α-CD (10 mM) at various guest/host mole ratios *i.e.* 2:1, 1:1 and 1:2 for 10 minutes and the color changes of the PEM films were recorded by color photographing. Deionized (DI) water was used as a positive inhibition control as the PEM films retained its blue color (Figure 3.18a) and a solution of α-CD (10 mM) was used as a negative inhibition control as the PEM films turned completely red (Figure 3.18h) upon the immersion.

Phenol showed no inhibition of α-CD induced color transition that the PEM films appeared red at all guest/host mole ratio within the range tested. Again, *p*-nitrophenol exhibited good color transition inhibition at the guest/host mole ratio of 2:1 and 1:1 but only partial inhibition at the guest/host mole ratio of 1:2 where the PEM films turned purple. For *p*-methoxyphenol, partial inhibition was observed at the guest/host mole ratio of 2:1 but the inhibition was apparently negative at the lower guest/host mole ratio. The results suggested that a binding strength series as *p*-nitrophenol > *p*-methoxyphenol > phenol which is probably partly depended on the molecular dipole moment of the guest molecules.

Moving away from the phenol series (pK_a in the range of 7.15-10.16), a more acidic compound such as benzoic acid (pK_a = 4.20) was also tested for the color transition inhibition on the PEM film. Benzoic acid showed excellent color transition inhibition even at the guest/host mole ratio of 1:2 (Figure 3.18e) suggesting a stronger binding of benzoic acid to α-CD comparing to the phenolic compounds. The well recognition results from two strong H-bonds between the carboxylic acid group on benzoic acid and the hydroxy moiety on the edge of α-CD cavity. A relatively more hydrophobic compound such as *p*-nitrotoluene, on the other hand, showed no inhibition at all levels of mole ratio tested (Figure 3.18f). The results strongly support the theory that the inclusion complex between guest compounds with α-CD host is not only driven by the hydrophobic interaction within the inner side of the α-CD

cavity but also involved the hydrogen bonding near the rim of α-CD [55-57]. Interestingly, a heterocyclic compound such as indole partially inhibited the color transition of the PEM film at the guest/host mole ratio of 2:1 (Figure 3.18g). The secondary amine group of indole interacted with the hydroxyl moiety on the rim of α-CD [58].

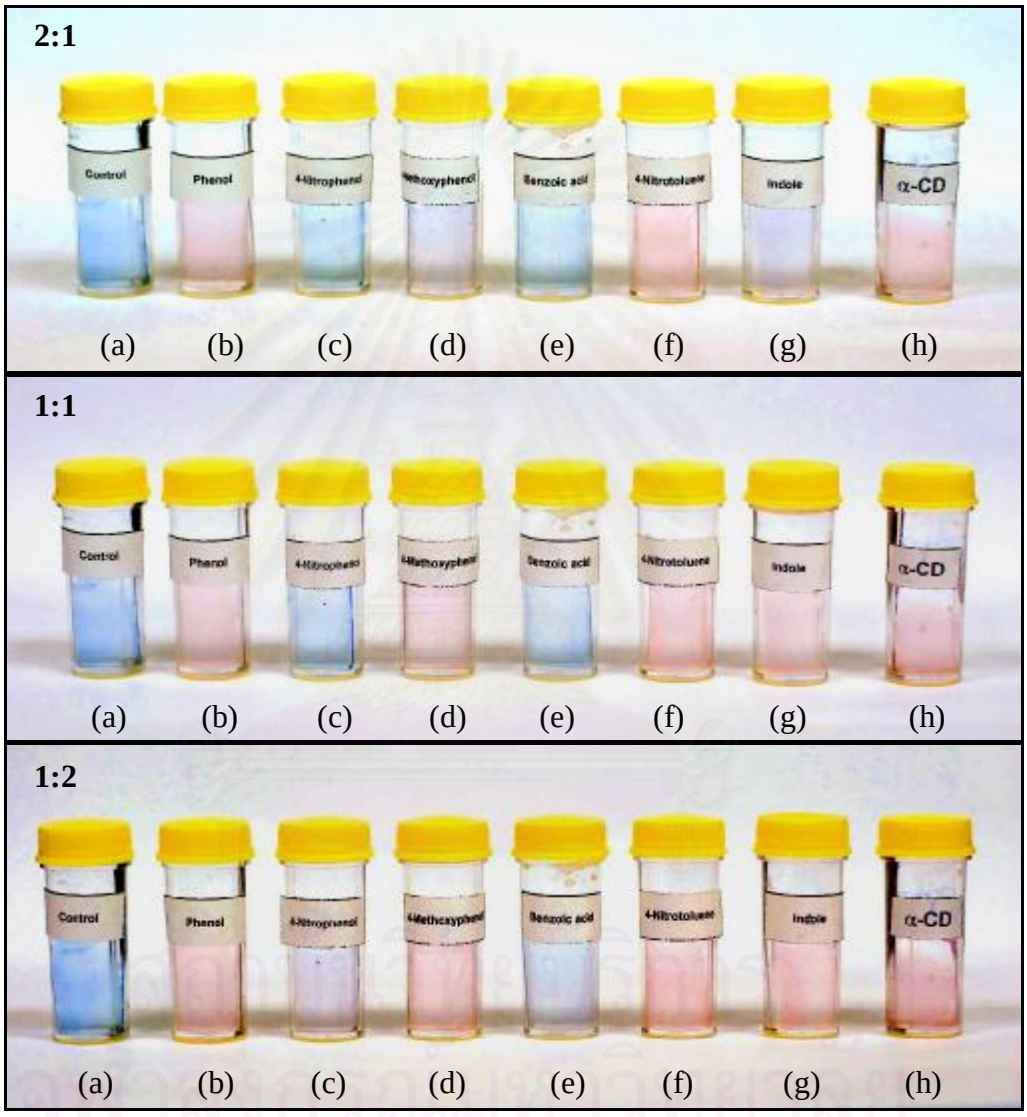


Figure 3.18 Color photographs of poly(PCDA)/chitosan PEM film (10 layers) after 10 min exposure to the mixture of aromatic compounds/α-CD (10 mM) at mole ratio of 1:2, 1:1 and 2:1 at 25 °C. DI water is used as a positive inhibition control (a). The aromatic compounds are phenol (b), *p*-nitrophenol (c), *p*-methoxyphenol (d), benzoic acid (e), *p*-nitrotoluene (f), indole (g). A solution of 10 mM α-CD is used as a negative inhibition control (h).

The ability to inhibit the color transition observed in this work implies a order of binding strength between α-CD host and the aromatic guests as follows: benzoic acid > *p*-nitrophenol > *p*-methoxyphenol ~ indole > phenol ~ *p*-nitrotoluene. Geometrically, the compounds tested in this series (Figure 3.19) can fit into the α-CD host cavity. The difference in the stability of the inclusion complex is probably contributed by subtle combination of electronic effects of the compounds.

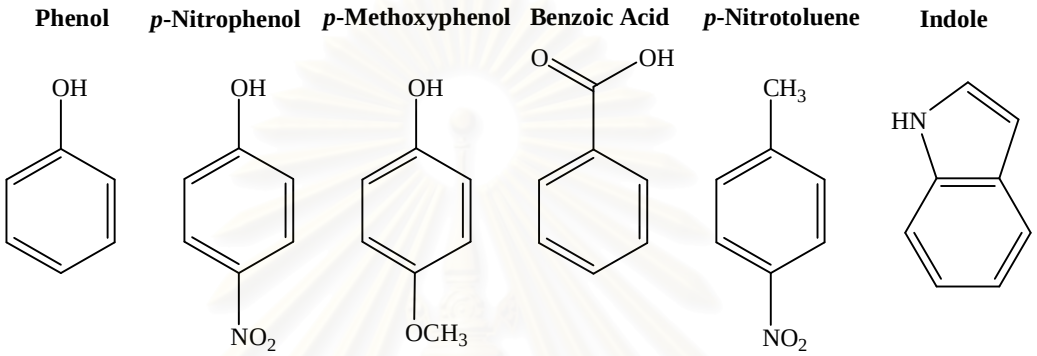
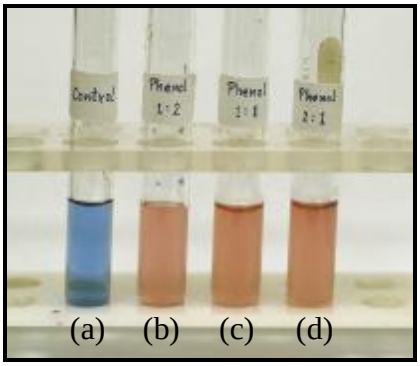


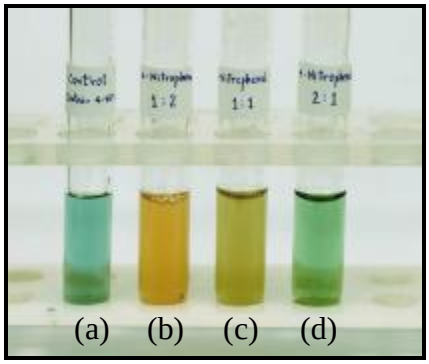
Figure 3.19 The chemical structures of aromatic compounds tested in the inhibition study.

The inhibition of the α-CD induced color transition by the inclusion of an aromatic compound was also tested using poly(PCDA) sol. The visual results (Figure 3.20) were quite similar to those of the PEM films, nevertheless, the PEM films presented a few major advantages including the reduction of interference from the color of the tested compound *e.g. p*-nitrophenol besides the reduction of the interference from the turbidity due to vesicle aggregation as previously mentioned.

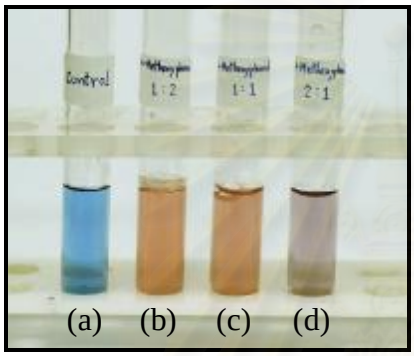
Although it is still impossible to draw an appropriate theory to account for all the inhibition abilities of all the guest compounds tested, it is quite obvious that, with appropriate experimental design, the α-CD induced color transition of poly(PCDA)/chitosan PEM films can be developed as a sensing system to discriminate these compounds either by naked eyes or simple spectrometer.



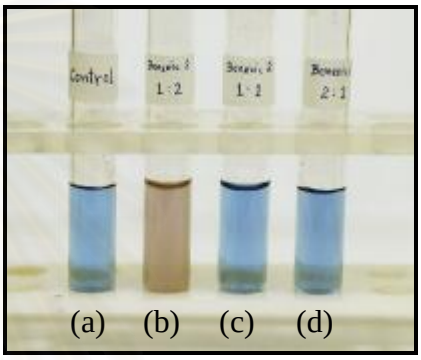
Phenol



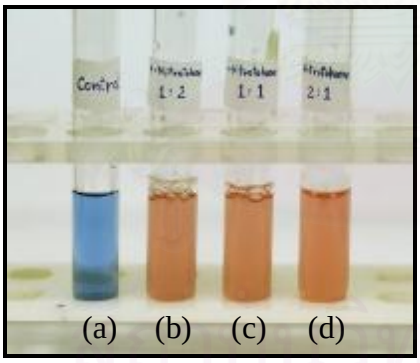
***p*-Nitrophenol**



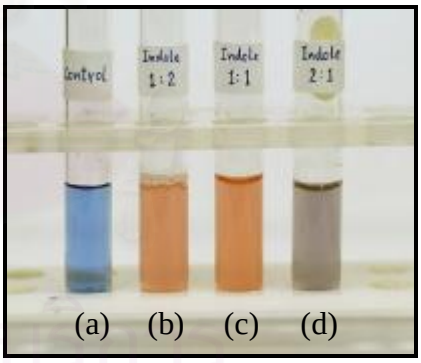
***p*-Methoxyphenol**



Benzoic acid



***p*-Nitrotoluene**



Indole

Figure 3.20 Color photographs of poly(PCDA) solution after 10 min exposure to the mixture of aromatic compounds/α-CD (10 mM) at mole ratio of 1:2 (b), 1:1 (c) and 2:1 (d) at 25 °C. DI water is used as a positive inhibition control (a) except *p*-nitrophenol control condition used DI water and *p*-nitrophenol (20 mM).

3.5.3 Quantitative evaluation of the inhibition using colorimetric responses

The colorimetric responses of poly(PCDA)/chitosan PEM films in the inhibition test of α-CD induced color transition by aromatic guests were determined using the guest/host mole ratios of 2:1, 1:1 and 1:2. The results presented as a 3-D chart (Figure 3.21) agree somewhat with the visual observation. Benzoic acid showed excellent color transition inhibition at all ratios tested while *p*-nitrophenol displayed excellent inhibition at the guest/host mole ratios of 2:1 and 1:1 but only partial inhibition at the 1:2 ratio. For phenol, *p*-methoxyphenol, *p*-nitrotoluene and indole, the degree of inhibition discernibly increased at different gradients within the guest/host ratios tested. Comparing to the results observed by eyes, the profiles of colorimetric responses gave a little more details about the inhibition abilities of the weaker inhibitors such as phenol, *p*-methoxyphenol, *p*-nitrotoluene and indole.

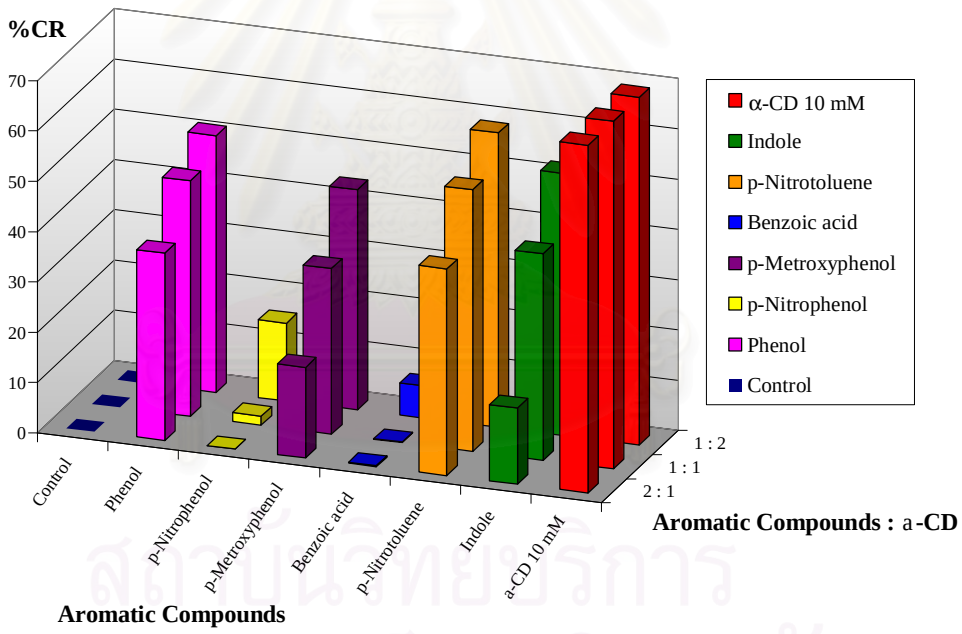


Figure 3.21 Colorimetric responses of poly(PCDA)/chitosan PEM film after 10 min exposure to the mixture of α-CD host (10 mM) in the presence of the aromatic guest at various guest/host mole ratios at 25 °C.

3.5.4 Sensing of 2,4-D and paraquat

Extensive use of agrochemicals has posted serious environmental concern. The first widely used herbicide was 2,4-dichlorophenoxyacetic acid, often abbreviated 2,4-D. It was first commercialized by the Sherwin-Williams Paint Company and saw use in the late 1940s. Another herbicide, paraquat (dimethylviologen) is one of the most widely used herbicides in the world. It is quick-acting, non-selective, and kills green plant tissue on contact. Both 2,4-D and paraquat are toxic to mammals and humans potentially leading to serious acute symptoms and chronic diseases [59-63].

Geometrically, paraquat ion (Dimethyl Viologen^{2+}, DV^{2+}), monoquat ion (Methyl Viologen^{+}, MV^{+}) and 2,4-D have structures (Figure 3.21) that should be able to form inclusion complexes with α-CD and hence inhibiting the α-CD induced color transition of poly(PCDA)/chitosan PEM film. The compounds were thus tested for their ability to inhibit the color transition. The results indicated that 2,4-D had the highest ability in inhibiting the color transition that it can inhibit the color change at all levels of the guest/host mole ratio tested (Figure 3.22). DV^{2+} and MV^{+} showed lower inhibition ability that the complete inhibition could be observed by eyes at the guest/host ratio of 2:1. There is a slight difference in inhibition abilities of DV^{2+} and MV^{+} that may be seen from the color of the PEM films when using the guest/host mole ratio of 1:1, purple for DV^{2+} and red for MV^{+}. The color-changing inhibition observed by order: 2,4-D (blue color) > DV^{2+} (purple color) > MV^{+} (red color).

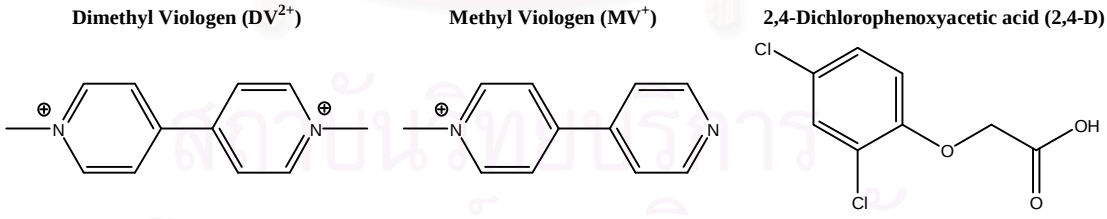


Figure 3.22 The chemical structures of herbicide compounds.

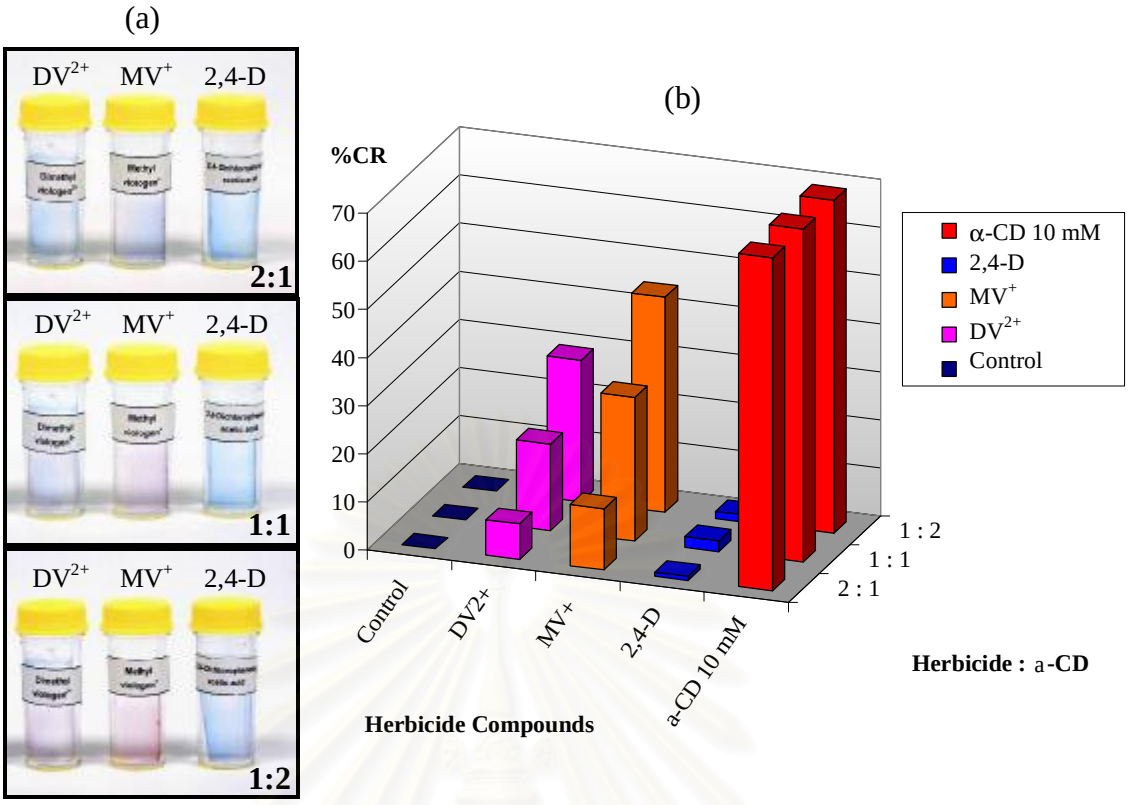


Figure 3.23 Color photographs (a) and colorimetric responses of poly(PCDA)/ chitosan PEM film (b) after 10 min exposure to a mixture of α-CD (10 mM) and a tested compound at various mole ratios at 25 ^{o}C.

3.6 Development of a multichannel device

A sensing chip containing multiple channels for sample introduction can offer simultaneous test of several samples including the control on one chip. Such a device can reduce cost and time per sample that is very suitable for making a disposable sensor kit for preliminary screening in the field test.

Polydimethylsiloxane (PDMS) elastomer is widely used in microfluidic applications to form components such as channels, valves, and diaphragms. It is elastic and can form fluid seals effectively. It can be easily processed by molding and acquired for low costs. The PDMS material also offers other advantages including its optical transparency and biocompatibility [64-65].

A prototype of multichannel device was constructed from two components, the poly(PCDA) PEM film (20 layers) on glass substrate and PDMS channel. The PDMS channel was pressed onto the PEM film and slid against the glass substrate to wipe out part of the PEM film where the PDMS came into direct contact. There were five lines of the PEM film left on the glass substrate which was then covered back

with a cleaned PDMS channel to form five fluid sealed channels with each one having PEM film on the glass surface (Figure 3.24). The PDMS can be attached and detached from the glass substrate as desired that is very convenient for reusing of the PDMS channel.

Figure 3.24 Color photograph of the multichannel device.

To demonstrate the simultaneous test using the multichannel device developed in this work, each channel of the device was injected with water and a solution of cyclodextrin series *i.e.* α-CD, β-CD and γ-CD (10 mM) through a syringe needle. As expected, after 10 minutes, only the channel injected with α-CD solution turned red (Figure 3.25b). It is important to note here that the multichannel device fabricated from the poly(PCDA)/chitosan PEM film in this work displays much faster colorimetric response comparing to the device fabricated from poly(PCDA)/ poly(ethylene glycol) diacrylate hydrogel reported recently in literature (60 minutes response time) [18].

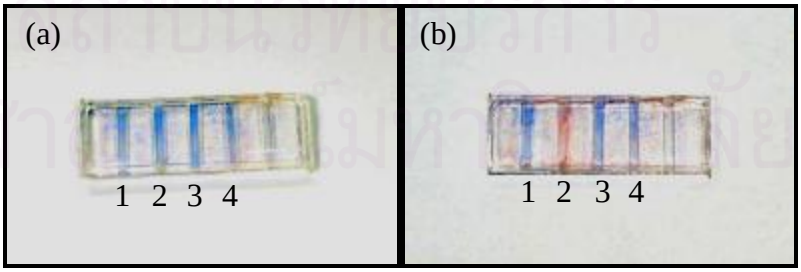


Figure 3.25 Color photographs of multichannel device containing poly(PCDA)/ chitosan PEM film before (a) and 10 minutes after (b) the injection of (1) DI water, (2) α-CD, (3) β-CD and (4) γ-CD solutions.

CHAPTER IV

CONCLUSION AND SUGGESTION

4.1 Conclusion

A chemosensing system based on polyelectrolyte multilayer (PEM) film of poly(10,12-pentacosadiynoic acid) (PCDA) vesicle and chitosan was developed by using a layer-by-layer deposition technique. Using the principle of competitive inclusion complexation with α-cyclodextrin (α-CD) for color transition inhibition, the application of the poly(PCDA)/chitosan PEM film for naked eye detection of some aromatic compounds such as *p*-nitrophenol, benzoic acid and 2,4-dichlorophenoxy acetic acid (2,4-D) was demonstrated. The detection limit was ~10 mM for visual observation but it can be improved to 5 mM by using colorimetric responses measured by UV-Vis spectrometer. Some selectivity in the detection among various aromatic compounds was also observed. The PEM film offers several advantages over the poly(PCDA) sol *i.e.* low interference from turbidity and color of samples, long shelf life, convenient to use and handy. The multichannel chip constructed from the poly(PCDA)/chitosan PEM film and the polydimethylsiloxane channel showed fast colorimetric response. The chip can be used for simultaneous test of multiple samples. Hence, the poly(PCDA)/chitosan PEM film offers several features suitable for further development into applicable sensor kit for field test of some toxic substances.

4.2 Suggestion for future work

- Use mixed solvent such as ethanol/water in the expectation to enhance the sensitivity of the film.
- Synthesize new diacetylene monomers with different head groups to enhance sensitivity and change selectivity of the sensing system.
- Develop a new platform for multiple detection similar to a well plate (Figure 4.1) which can be more convenient to use.

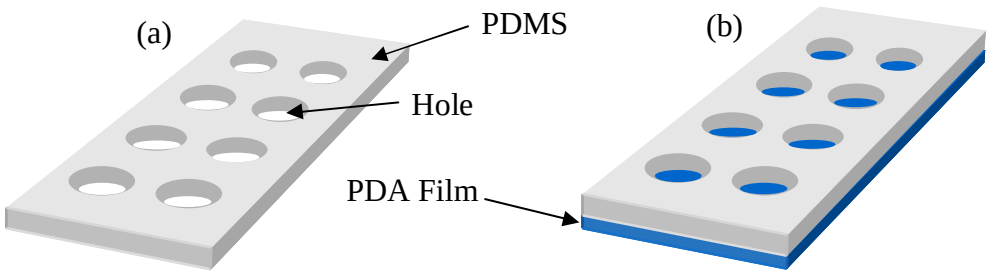


Figure 4.1 Schematic of PDMS (a) and PDA-PDMS (b) well plates.

REFERENCES

[1] Lewis, J. G.; Odom, B.; and Adams, D. O. Toxic Effects of Benzene and Benzene Metabolites on Monomuclear Phagocytes. *Toxicol. Appl. Pharmacol.* 92 (1988): 246-254.

[2] Lindsey, R. H., Jr.; Bender, R. P.; and Osheroff, N. Effects of Benzene Metabolites on DNA Cleavage Mediated by Human Topoisomerase IIα : 1,4-Hydroquinone Is a Topoisomerase II Poison. *Chem. Res. Toxicol.* 18 (2005): 761-770.

[3] Lei, Y.; Mulchandani, P.; Chen, W.; Wang, J.; and Mulchandani, A. A Microbial Biosensor for *p*-Nitrophenol Using *Arthrobacter* Sp. *Electroanalysis*. 15 (2003): 1160-1164.

[4] Pablo, F.-B.; Maria, B. G.-G.; and Agustin, C.-G. Flow Screen-Printed Amperometric Detection of *p*–Nitrophenol in Alkaline Phosphatase-Based Assays. *Anal. Bioanal. Chem*. 385 (2006): 1202-1208.

[5] In-On, P.; Biadul, P.; Hongtrakul, T.; and Suwannabutra, S. A Modified Procedure for Analysis of 4-Nitrophenol and Dimethylphosphate in Human Urine. 31st STT, Suranaree University of Technology, 18-20 Oct 005.

[6] Wrobel, K.; Wrobel, K.; Urbina, E. M. C.; and Romero, J. M. The Determination of 3-Nitrophenol and Some Other Aromatic Impurities in 4-Nitrophenol by Reversed Phase HPLC with Peak Suppression Diode Array Detection. J. Phar. Bio. Anal. 22 (2000) 295-300.

[7] Xiao, W.; Xiao, D.; Yuan, H. A Functionalized Mesoporous Silica Sensor for the Determination of *p*-Nitrophenol or 2,4-Dinitrophenol Based on Fluorescence Quenching. Sen. Lett. 2 (5) (2007) 445-449.

[8] Kim, J.-M.; Lee, J. S.; Choi, H.; Sohn, D.; and Ahn, D. J. Rational Design and in-Situ FTIR Analyses of Colorimetrically Reversible Polydiacetylene Supramolecules. *Macromolecules*. 38 (2005): 9366-9376.

[9] Jung, Y. K.; Park, H. G.; and Kim, J. M. Polydiacetylene (PDA)-Based Colorimetric Detection of Biotin-Streptavidin Interactions. *Biosens. Bioelectron.* 21 (2006): 1536-1544.

[10] Lio, A.; Reichert, A.; Ahn, D. J.; Nagy, J. O.; Salmeron, M.; and Charych, D. H. Molecular Imaging of Thermochromic Carbohydrate-Modified Polydiacetylene Thin Films. *Langmuir*. 13 (1997): 6524-6532.

[11] Cheng, Q.; and Stevens, R. C. Charge-Induced Chromatic Transition of Amino Acid-Derivatized Polydiacetylene Liposomes. *Langmuir*. 14 (1998): 1974-1976.

[12] Cheng, Q.; Yamamoto, M.; and Stevens, R. C. Amino Acid Terminated Polydiacetylene Lipid Microstructures: Morphology and Chromatic Transition. *Langmuir*. 16 (2000): 5333-5342.

[13] Charych, D.; Cheng, Q.; Reichert, A.; Kuziemko, G.; Stroh, M.; Nagy, J. O.; Spevak, W.; and Stevens, R. C. A 'Litmus Test' for Molecular Recognition Using Artificial Membranes. *Chem. Biol.* 2 (3) (1996): 113-120.

[14] Simon, J. K.; and Elizabeth, A. H. H. pH Response of Carboxy-Terminated Colorimetric Polydiacetylene Vesicles. *Anal. Chem.* 78 (2006): 2231-2238.

[15] Wang, C.; Mar, Z.; and Su, Z. Facile Method to Detect Oligonucleotides with Functionalized Polydiacetylene Vesicles. *Sens. Actuators B.* 113 (2006): 510–515.

[16] Scindia, Y.; Silbert, L.; Volinsky, R.; Koluusheva, S.; and Jelinek, R. Colorimetric Detection and Fingerprinting of Bacteria by Glass-Supported Lipid/Polydiacetylene Films. *Langmuir*. 23 (2007): 4682-4687.

[17] Cho, J. T.; Woo, S. M.; Ahn, D. J.; Ahn, K. D.; Lee, H.; and Kim, J.-M. Cyclodextrin-induced Color Changes in Polymerized Diacetylene Langmuir-Schaefer Films. *Chem. Lett.* 3 (32) (2003): 282-283.

[18] Wenzel, M.; and Atkinson, G. H. Chromatic Properties of Polydiacetylene Films. *J. Am. Chem. Soc.* 111 (1989): 6123-6127.

[19] Jonas, U.; Shah, K.; Norvez, S.; and Charych, D. H. Reversible Color Switching and Unusual Solution Polymerization of Hydrazide-Modified Diacetylene Lipids. *J. Am. Chem. Soc.* 121 (1999): 4580-4588.

[20] Morigaki, K.; Buamgart, T.; Jonas, U.; Offenhausser, A.; and Knoll, W. Photopolymerization of Diacetylene Lipid Bilayers and Its Application to the Construction of Micropatterned Biomimetic Membranes. *Langmuir*. 18 (2002): 4082-4089.

[21] Sukwattanasinitt, M.; Lee, D. C.; Kim, M.; Wang, X.; Li, L.; Yang, K.; Kumar, J.; Tripathy, S. K.; and Sandman, D. J. New Processable, Functionalizable Polydiacetylenes. *Macromolecules*. 32 (1999): 7361-7369.

[22] Carpick, R. W.; Sasaki, D. Y.; Marcus, M. S.; Eriksson, M. A.; and Burns, A. R. Polydiacetylene Films: A Review of Recent Investigations into Chromogenic Transitions and Nanomechanical Properties. *J. Phys.: Condens. Matter*. 16 (2004): R679-R697.

[23] Kim, J.-M.; Lee, T. B.; Jang, D. H.; Lee, J. S.; Lee, G. S.; and Ahn, D. J. A Polydiacetylene-Based Fluorescent Sensor Chip. *J. Am. Chem. Soc.* 127 (2005): 17580-17581.

[24] Ji, E. K.; Ahn, D. J.; and Kim, J.-M. The Fluorescent Polydiacetylene Liposome. *Bull. Korean Chem. Soc.* 23 (2003): 667-670.

[25] Okada, S.; Peng, S.; Spevak, W.; and Charych, D., Color and Chromism of Polydiacetylene Vesicles. *Acc. Chem. Res.* 31 (1998): 229-239.

[26] Shibata, M.; Kaneko, F.; Aketagawa, M.; and Kobayashi, S. Reversible Colour Phase Transitions and Annealing Properties of Langmuir-Blodgett Polydiacetylene Films. *Thin solid films*. 179 (1989): 433-437.

[27] Kim, J.-M.; Lee, J. S.; Lee, J. S.; Woo, S. Y.; and Ahn, D. J. Unique Effects of Cyclodextrins on the Formation and Colorimetric Transition of Polydiacetylene Vesicles. *Macromol. Chem. Phys*. 206 (2005): 2299-2306.

[28] Rekharsky, M. V.; and Inoue, Y. Complexation Thermodynamics of Cyclodextrins. *Chem. Rev*. 98 (1998): 1875-1917.

[29] Atwood, J. L.; Davies, J. E. D.; Macnicol, D. D.; and Vogtle, F. Comprehensive Supramolecular Chemistry. Volume 3 Cyclodextrins, Pergamon, Mar (1999): 189-241.

[30] Szejtli, J. Introduction and General Overview of Cyclodextrin Chemistry. *Chem. Rev.* 98 (1998): 1743-1753.

[31] Connors, K. A. The Stability of Cyclodextrin Complexes in Solution. *Chem. Rev.* 97 (1997): 1325-1357.

[32] Engeldinger, E.; Armspach, D.; and Matt, D. Capped Cyclodextrins. *Chem. Rev.* 11 (103) (2003): 4147-4173.

[33] Wenz, G.; Han, B.-H.; and Muller, A. Cyclodextrin Rotaxanes and Polyrotaxanes. *Chem. Rev.* 106 (2006): 782-817.

[34] Kolusheva, S.; Kafri, R.; Katz, M.; Jelinek, R. Rapid Colorimetric Detection of Antibody-Epitope Recognition at a Biomimetic Membrane Interface. *J. Am. Chem. Soc.* 123 (2001): 417-422.

[35] Kolusheva, S.; Shahal, T.; and Jelinek, R. Cation-Selective Color Sensors Composed of Ionophore-Phospholipid-Polydiacetylene Mixed Vesicles. *J. Am. Chem. Soc.* 122 (2000): 776-780.

[36] Kim, J.-M.; Ji, E. K.; Woo, S. M.; Lee, H.; and Ahn, D. J. Immobilized polydiacetylene vesicles on solid substrates for use as chemosensors. *Adv. Mater.* 15 (2003): 1118-1121.

[37] Shim, H. Y.; Lee, S. H.; Ahn, D. J.; Ahn, K. D.; and Kim J. M. Micropatterning of Diacetylenic Liposomes on Glass Surfaces. *Mater Sci. Eng. C.* 24 (2004): 157-161.

[38] Su, Y.-L.; Li, J.-R.; and Jiang, L. Chromatic Immunoassay Based on Polydiacetylene Vesicles. *Colloids Surf. B.* 38 (2004): 29-33.

[39] Su, Y.-L.; Li, J.-R.; and Jiang, L. A Study on the Interactions of Surfactants with Phospholipid/Polydiacetylene Vesicles in Aqueous Solution. *Colloids Surf.* 257-258 (2005): 25-30.

[40] Su, Y.-L.; Li, J.-R.; Jiang, L.; and Cao, J. Biosensor Signal Amplification of Vesicles Functionalized with Glycolipid for Colorimetric Detection of *Escherichia coli*. *J. Colloids Interface Sci.* 284 (2005): 114-119.

[41] Su, Y.-L., Assembly of Polydiacetylene Vesicles on Solid Substrates. *J. Colloids Interface Sci*. 292 (2005): 271-276.

[42] Lee, N. Y.; Jung, Y. K.; and Park, H. G. On-Chip Colorimetric Biosensor Based on Polydiacetylene (PDA) Embedded in Photopolymerized Poly(ethylene glycol) Diacrylate (PEG-DA) Hydrogel. *Biochem. Eng. J.* 29 (2006): 103–108.

[43] Potisatityuenyong, A.; Tumcharern, G.; Dubas, S. T.; and Sukwattanasinitt, M. Layer-by-layer Assembly of Intact Polydiacetylene Vesicles with Retained Chromic Properties. *J. Colloid Interface Sci*. 304 (2006): 45-51.

[44] Kim, J.-M.; Chae, S. K.; Lee, Y. B.; Lee, J. S.; Lee, G. S.; Kim, T. Y.; and Ahn, D. J. Polydiacetylene Supramolecules Embedded in PVA Film for Strip-type Chemosensors. *Chem. Lett.* 35 (2006): 560-561.

[45] Yoon, J.; Chae, S. K.; and Kim J. M. Colorimetric Sensors for Volatile Organic Compounds (VOCs) Based on Conjugated Polymer-Embedded Electrospun Fibers. *J. Am. Chem. Soc.* 129 (2006): 3038-3039.

[46] Chae, S. K.; Park, H.; Yoon, J.; Lee, C. H.; Ahn, D. J.; and Kim J.-M. Polydiacetylene Supramolecules in Electrospun Microfibers: Fabrication, Micropatterning, and Sensor Applications. *Adv. Mater.* 19 (2007): 521-524.

[47] Lee, C. W.; Choi. H.; Oh, M. K.; Ahn, D. J.; Kim, J.-M.; and Ren, F. ZnO-Based Cyclodextrin Sensor Using Immobilized Polydiacetylene Vesicles. *Electrochem. Solid-State Lett.* 1 (10) (2007): J1-J3.

[48] Lee, S.; and Kim, J.-M. α-Cyclodextrin: A Molecule for Testing Colorimetric Reversibility of Polydiacetylene Supramolecules. *Macromolecules.* 26 (40) (2007): 9201-9204.

[49] Lee, C. H.; Oh, E.-H.; Kim, J.-M.; and Ahn, D. J. Immobilization of Polydiacetylene Vesicles on Cellulose Acetate Butyrate(CAB)-Coated Substrates for Self-Assembled Supramolecular Sensor Arrays. *Colloids Surf. A.* 313-314 (2008): 500-503.

[50] Charych, D. H.; Nagy, J. O.; Spevak, W.; and Bednarski, M. D. Direct Colorimetric Detection of a Receptor-Ligand Interaction by a Polymerized Bilayer Assembly. *Science*. 261 (1993): 585-588.

[51] Charych, D. H. and Pan, J. J. Molecular Recognition and Colorimetric Detection of Cholera Toxin by Poly(diacetylene) Liposomes Incorporating G_{m1} Ganglioside. *Langmuir*. 13 (1997): 1365-1367.

[52] Guo, Q.-X.; Liu, L.; Cai W.-S.; Jiang, Y.; and Liu, Y.-C. Driving Force Prediction for Inclusion Complexation of α-Cyclodextrin with Benzene Derivatives by a Wavelet Neural Network. *Chem. Phy. Lett.* 290 (1998): 514-518.

[53] Hamai, S.; and Takahashi, A. Thermodynamic and ^{13}C NMR Studies of the Inclusion Complexes of α- and β-Cyclodextrins with Cyano- and Nitrophenols in Aqueous Solution. *J. Incl. Phenom.* 37 (2000): 197-207.

[54] Bergeron, R. J.; Channing, M. A.; Gibeily, G. J.; and Pillor D. M. Disposition Requirements for Binding in Aqueous Solution of Polar Substrates in the Cyclohexaamylose Cavity. *J. Am. Chem. Soc.* (1977): 5146-5151.

[55] Easton, C. J. and Lincoln, S. F. Modified Cyclodextrins: Scaffolds and Templates for Supramolecular Chemistry. Singapore: Imperial College Press. (1999): 12-18.

[56] Huo, Q.; Russell, K. C.; and Leblanc, R. M. Chromatic Studied of a Polymerizable Diacetylene Hydrogen Bonding Self-Assembly: A "Self-Folding" Process to Explain the Chromatic Changes of Polydiacetylenes. *Langmuir*. 15 (1999): 3972-3980.

[57] Lewis, E. A.; and Hansen, L. D. Thermodynamics of Binding of Guest Molecules to α- and β-Cyclodextrins. *J. Chem. Soc. Perkin Trans.* 2 (1973): 2081–2085.

[58] Cao, J.; Zhao, C.; Huang, L.; Ding, Y.; Wang, L.; and Han, S. Solubilization of substituted indole compounds by β-cyclodextrin in water. *Chemosphere.* 40 (2000) 1411-1416.

[59] Wikipedia. 2, 4-Dichlorophenoxyacetic acid [Online]. Available from: http://en.wikipedia.org/wiki/2,4-D

[60] Walters, J. Environmental Fate of 2,4-Dichlorophenoxyacetic Acid. Environmental Monitoring and Pest Management Department of Pesticide Regulation Sacramento. CA 95814-3510

[61] Wikipedia. Paraquat [Online]. Available from: http://en.wikipedia.org/wiki/Paraquat

[62] Copyscape. Chemical Database: Paraquat (Paraquat dichloride) [Online]. Available from: http://environmentalchemistry.com/yogi/chemicals/cn/Paraquat%A0(Paraquat%A0dichloride).html

[63] Inchem. Paraquat (Pesticide residues in food: 1976 evaluations) [Online]. Available from: http://www.inchem.org/documents/jmpr/jmpmono/v076pr19.htm

[64] Wikipedia. Polydimethylsiloxane [Online]. Available from: http://en.wikipedia.org/wiki/Polydimethylsiloxane

[65] Jo, B.-H.; Lerberghe, L. M. V.; Motsegood, K. M.; and Beebe, D. J. Three-Dimensional Micro-Channel Fabrication in Polydimethylsiloxane (PDMS) Elastomer. *J. Microelectromechanical Syst.* 1 (9) (2000): 76-81.

APPENDICES

Appendix A: Particle size distribution of polydiacetylene vesicles.

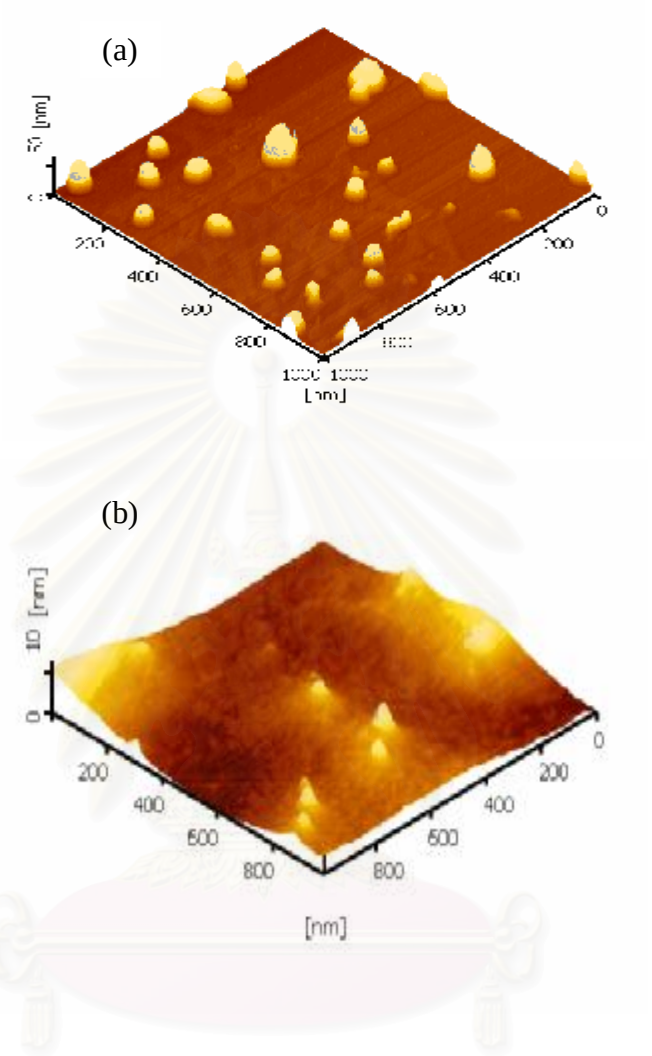
Table A1: Particle size distribution of poly(PCDA) vesicles.

	Particle size (nm)	**Polydispersity Index (PdI)**
1st	75.2	0.497
2nd	70.5	0.532
3rd	68.3	0.518
4th	65.9	0.514
5th	72.0	0.485
6th	69.1	0.542
Mean	**70.17**	**0.515**

Table A2: Particle size distribution of poly(PCDA) vesicles after exposure to α-CD.

	Particle size (nm)	**Polydispersity Index (PdI)**
1st	204	0.367
2nd	156	0.519
3rd	169	0.424
4th	164	0.477
5th	169	0.362
6th	164	0.411
Mean	**171**	**0.427**

Appendix B: AFM images of poly(PCDA) vesicles before (a) and after (b) exposure to a-CD (10 mM).



Appendix C: UV Spectrum of 10 layers of poly(PCDA)/chitosan PEM film after exposure to 0-10 mM with various CD solution at 25 ^{o}C for 5 min.

Figure C1: UV Spectrum of 10 layers of poly(PCDA)/chitosan PEM film after exposure to 0-10 mM β-CD solution at 25 ^{o}C for 5 min.

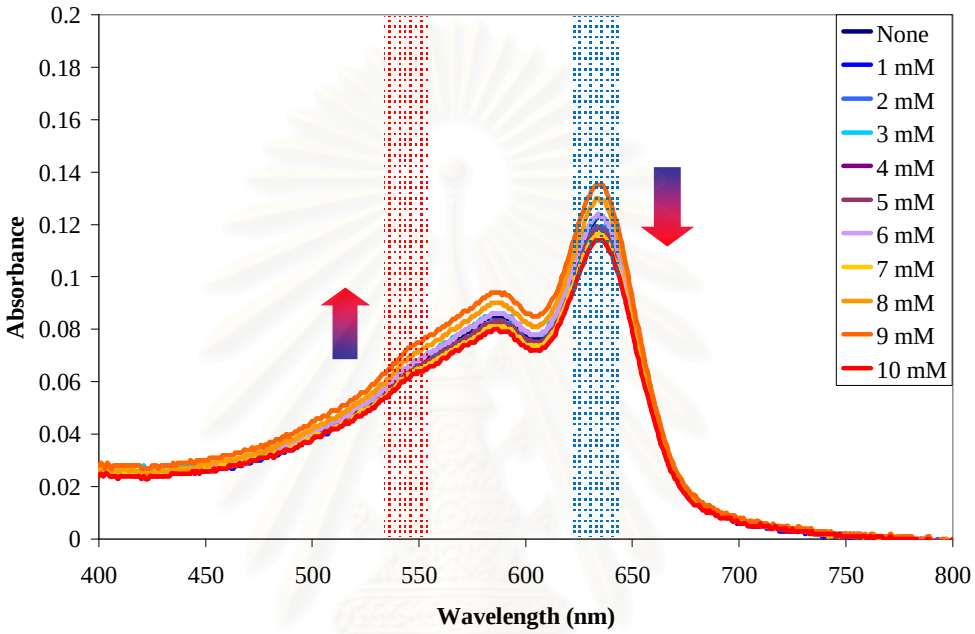
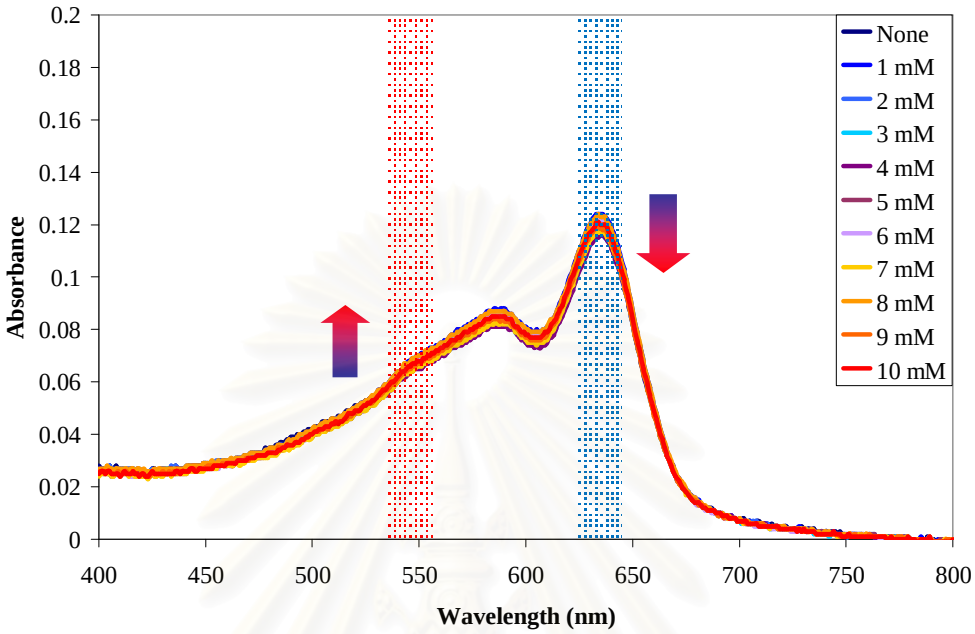
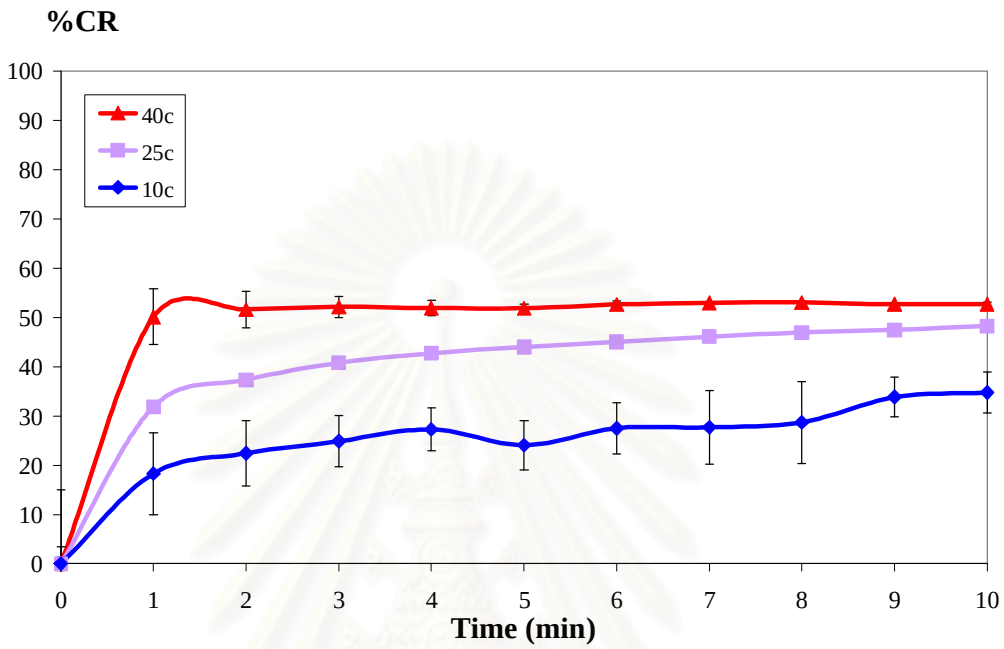


Figure C2: UV Spectrum of 10 layers of poly(PCDA)/chitosan PEM film after exposure to 0-10 mM γ-CD solution at 25 ^{o}C for 5 min.



Appendix D: Time dependence of the colorimetric responses of poly(PCDA) sol to a-CD solution (10 mM) at various detection temperature (10, 25, 40 ^{o}C) for 0-10 min.



Appendix E: UV Spectrum of poly(PCDA) sol upon exposure to a-CD solution at various temperatures for 5 min.

Figure E1: UV Spectrum of poly(PCDA) sol upon exposure to α-CD solution at 10 ^{o}C for 5 min.

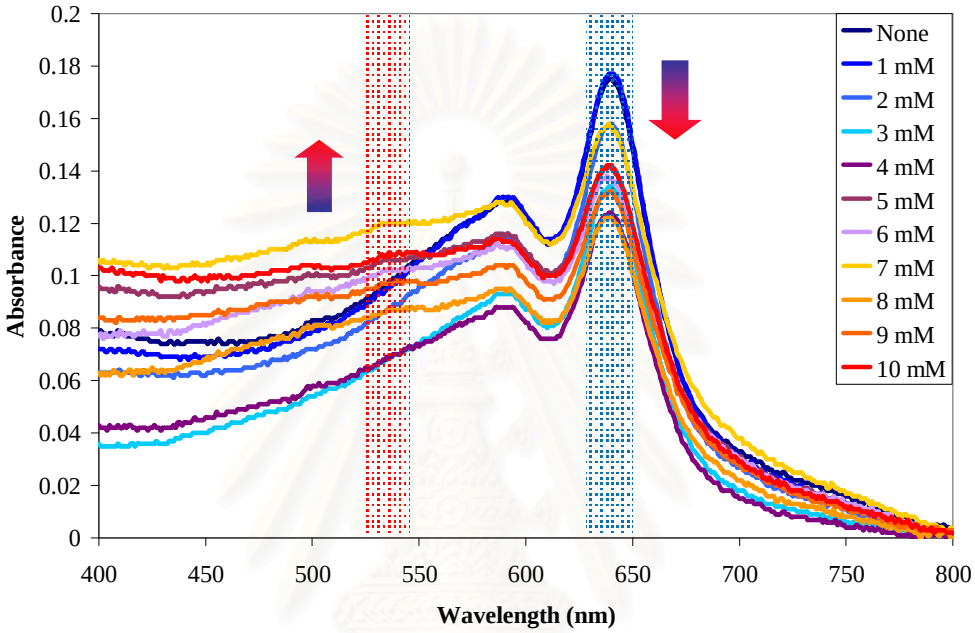
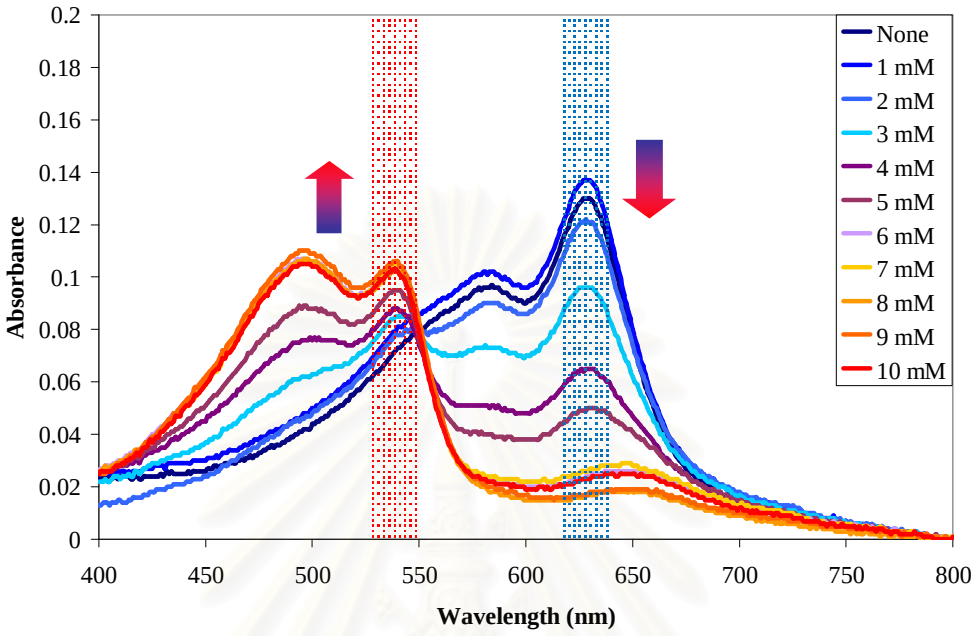


Figure E2: UV Spectrum of poly(PCDA) sol upon exposure to α-CD solution at 40 ^{o}C for 5 min.



Appendix F: UV Spectrum of poly(PCDA)/stearic acid mixed lipid sol having various PCDA/stearic acid mole ratio upon exposure to various concentration of a-CD solution at 25 °C for 5 min.

Figure F1: UV Spectrum of poly(PCDA)/stearic acid mixed lipid sol (PCDA/stearic acid mole ratio 70:30) upon exposure to various concentration of α-CD solution at 25 °C for 5 min.

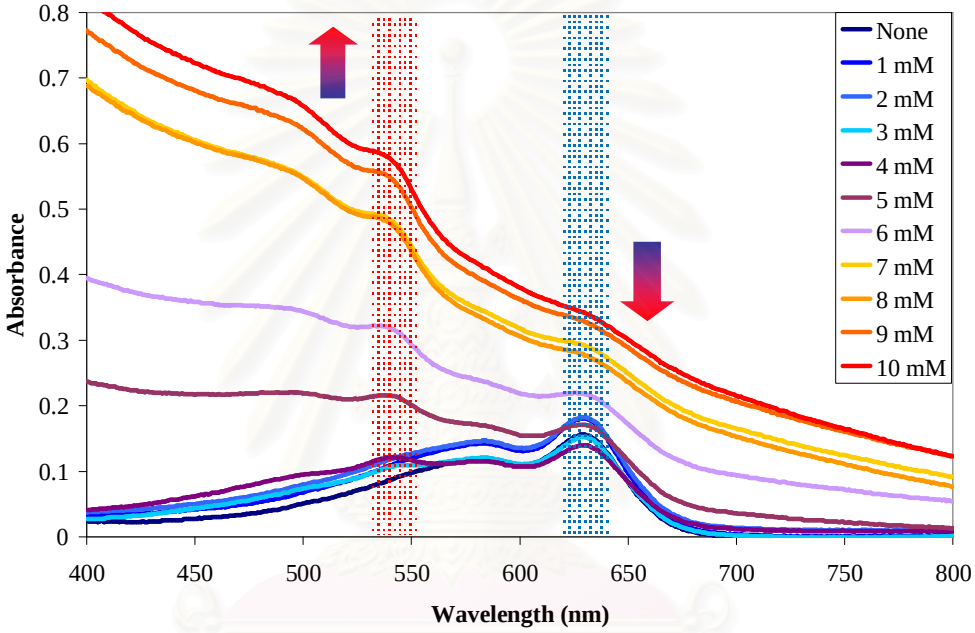
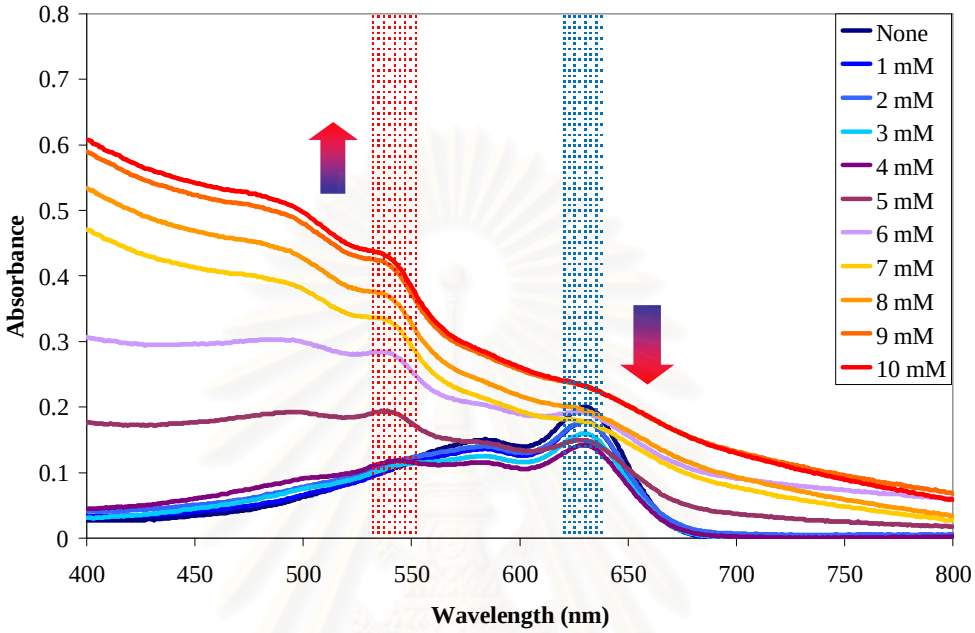


Figure F2: UV Spectrum of poly(PCDA)/stearic acid mixed lipid sol (PCDA/stearic acid mole ratio 50:50) upon exposure to various concentration of α-CD solution at 25 °C for 5 min.



Appendix G: UV Spectrum of poly(PCDA)/chitosan PEM film with various layers (2-20 layers) after exposure to 0-10 mM a-CD solution at 25 °C for 5 min.

Figure G1: UV Spectrum of poly(PCDA)/chitosan PEM film with 2 layers after exposure to 0-10 mM α-CD solution at 25 °C for 5 min.

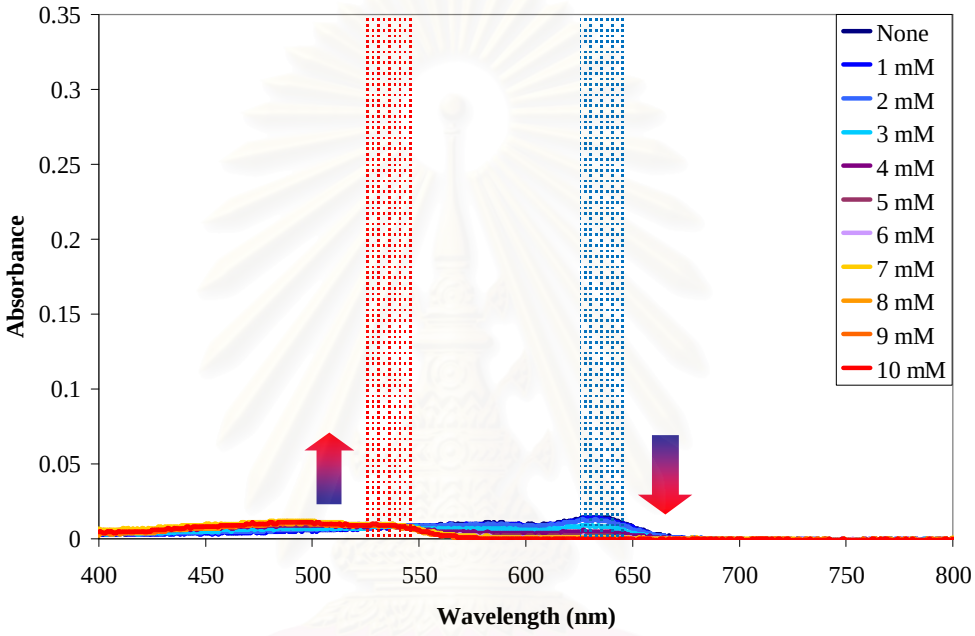
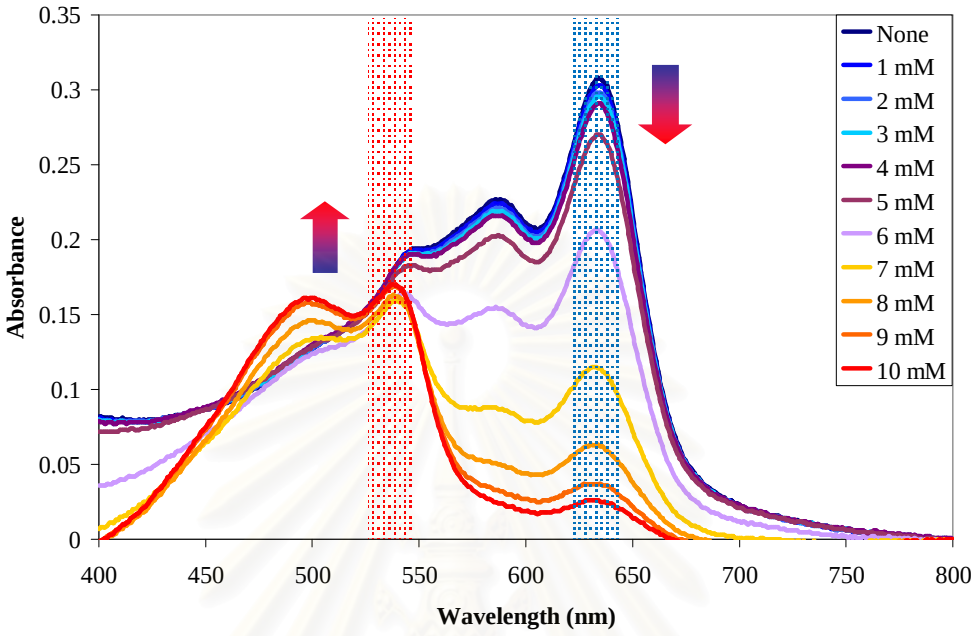


Figure G2: UV Spectrum of poly(PCDA)/chitosan PEM film with 20 layers after exposure to 0-10 mM α-CD solution at 25 °C for 5 min.



Appendix H: UV Spectrum of poly(PCDA)/chitosan PEM films upon exposure to the mixture of a-CD (10 mM) and various concentrations of nitrophenols at 25 °C for 5 min.

Figure H1: UV Spectrum of poly(PCDA)/chitosan PEM film upon exposure to the mixture of α-CD (10 mM) and various concentrations of 2-nitrophenol (0-20 mM) at 25 ^{o}C for 5 min.

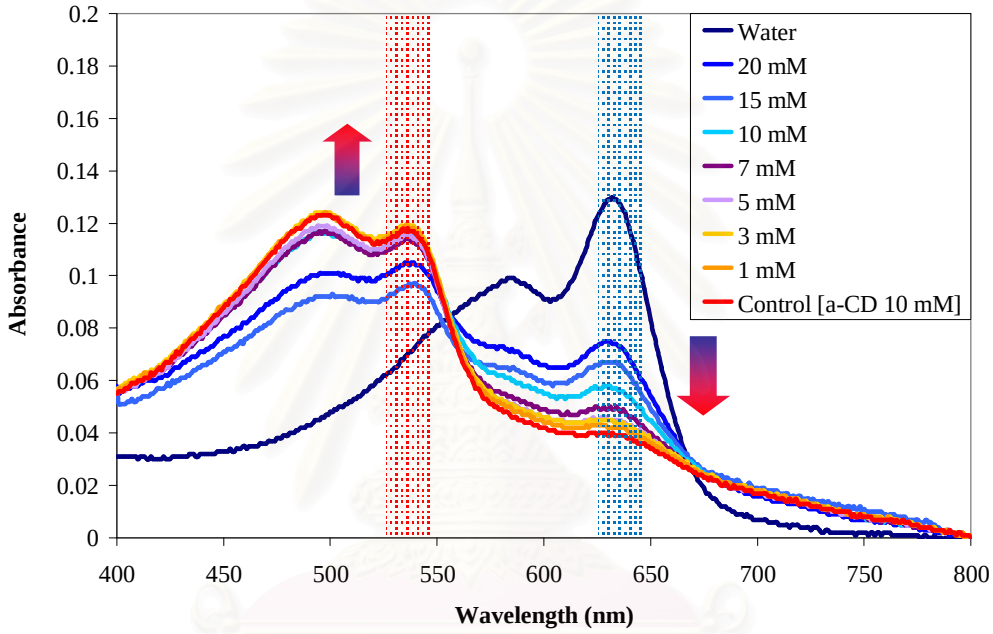


Figure H2: UV Spectrum of poly(PCDA)/chitosan PEM film upon exposure to the mixture of α-CD (10 mM) and various concentrations of 3-nitrophenol (0-20 mM) at 25 ^{o}C for 5 min.

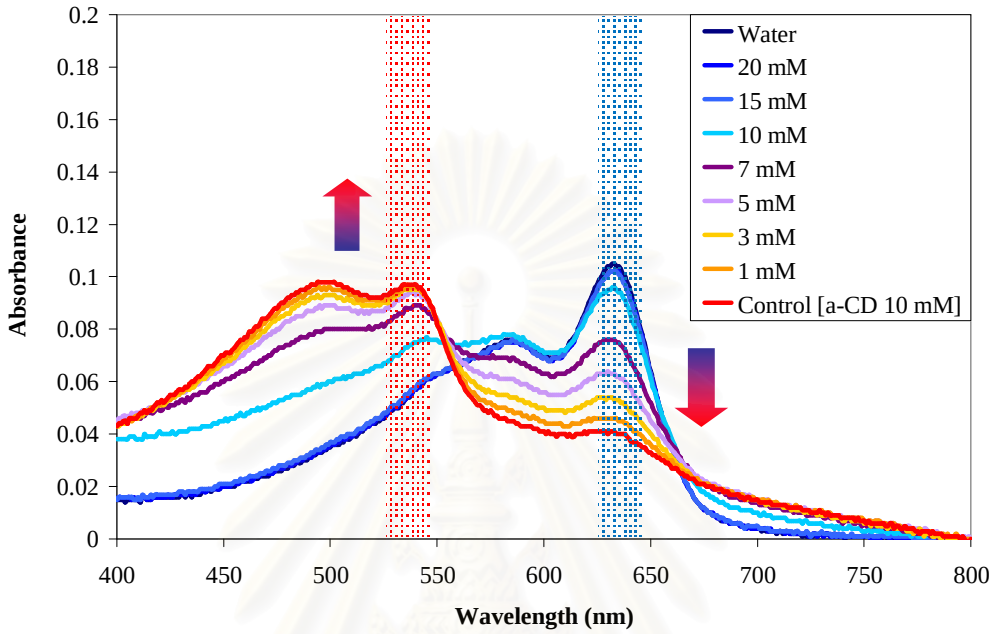
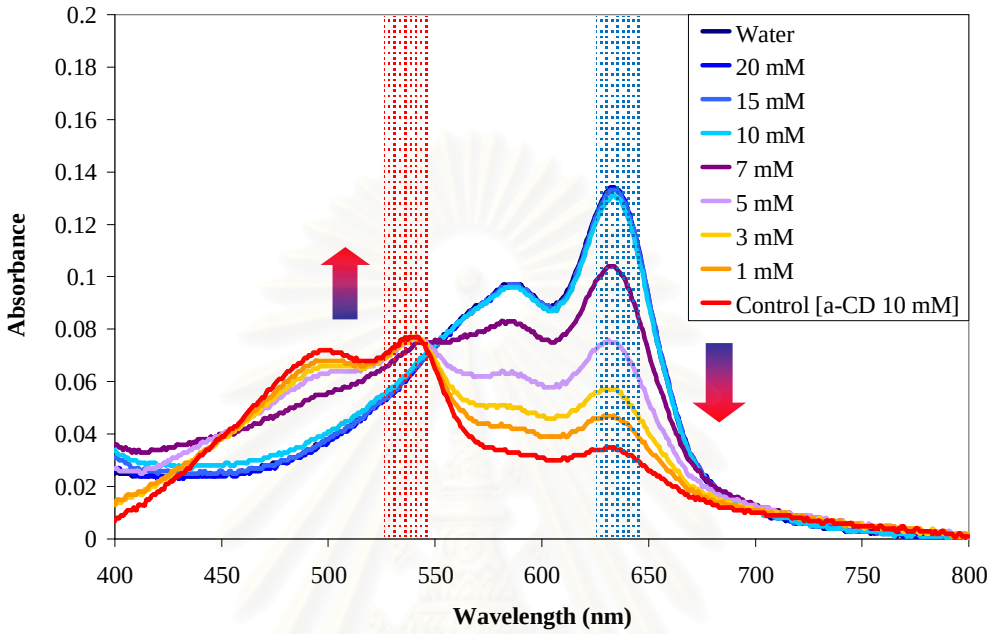
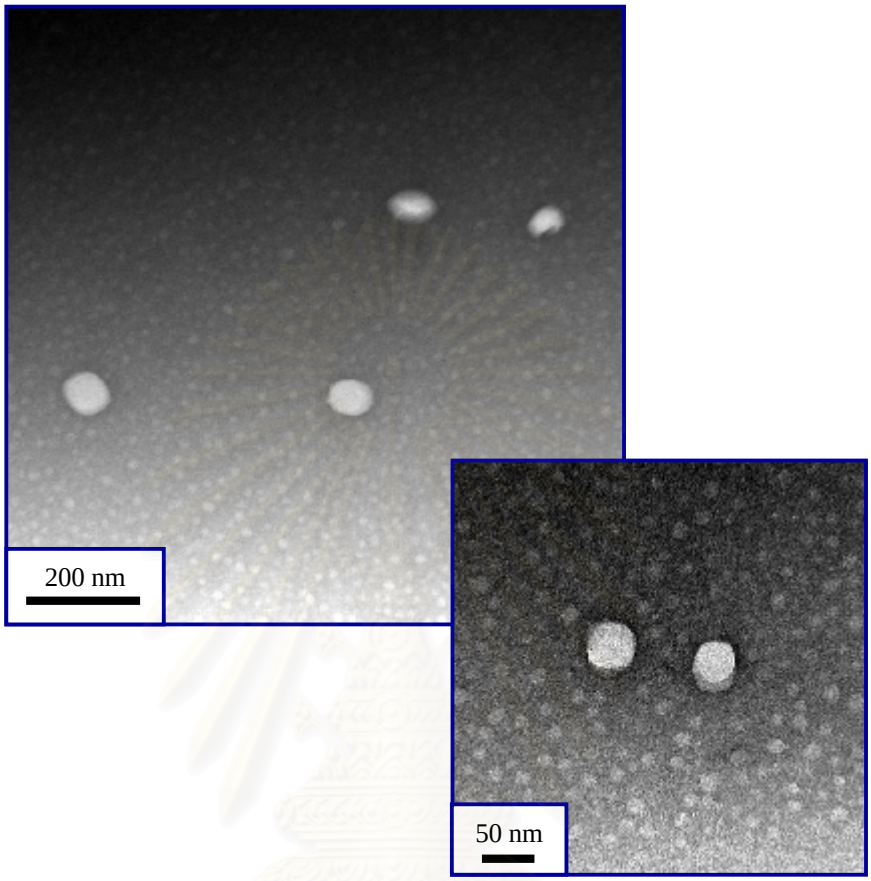


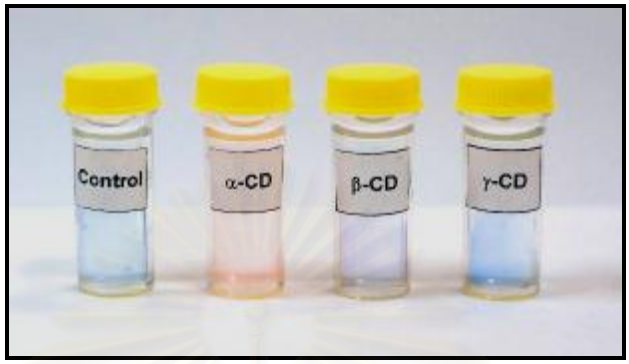
Figure H3: UV Spectrum of poly(PCDA)/chitosan PEM film upon exposure to the mixture of α-CD (10 mM) and various concentrations of 4-nitrophenol (0-20 mM) at 25 ^{o}C for 5 min.



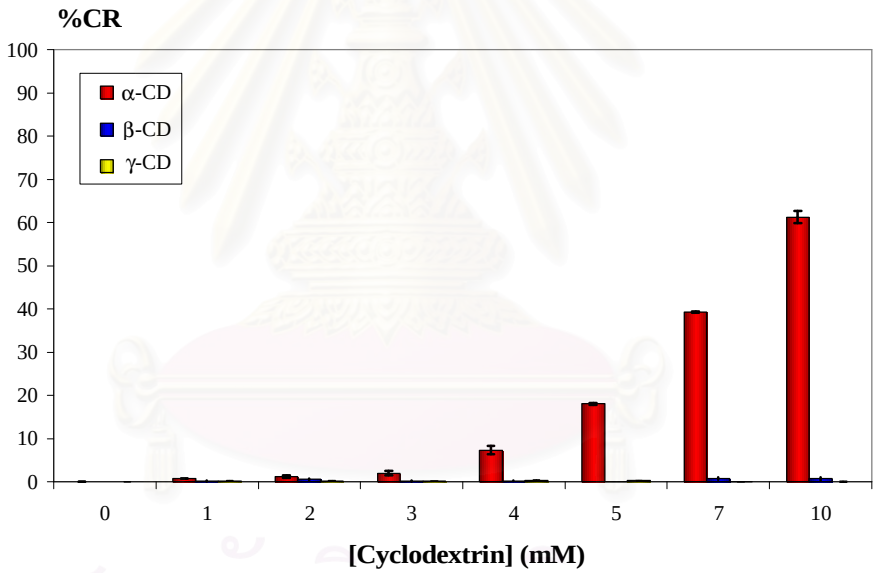
Appendix I: TEM images of poly(PCDA) vesicles after exposure to the mixture of benzoic acid and 10 mM a-CD (mole ratio 2:1).



Appendix J: Color photographs (a) and colorimetric responses of poly(PCDA)/ chitosan PEM film (b) after 10 min exposure to a-CD solution at 25 ^{o}C for 5 min.



(a)



(b)

VITA

Mr. Thoedtoon Champaiboon was born on November 30th, 1982 in Chachoengsao, Thailand. He received a Bachelor's Degree of Science, majoring in Chemistry from Faculty of Science, Thammasat University in 2004. Since 2005, he has been a graduate student studying Petrochemistry and Polymer Science as his major course at Chulalongkorn University and completed the program in 2007.

His present address is 9 Moo 6, Bangkanak, Bangnumpriow, Chachoengsao, Thailand 24150.