การตรวจวัดสารกลุ่มฟีนอลด้วยวิธีคัลเลอริเมตรีโดยใช้พอลิไดอะเซทิลีนเวสิเคิลกับแอลฟาไซโคลเดกซ์ทริน

นางสาวพิมพ์พิมล อเนกธีรกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

COLORIMETRIC DETERMINATION OF PHENOLS USING POLYDIACETYLENE VESICLES WITH α-CYCLODEXTRIN

Miss Pimpimon Anekthirakun

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2008

Thesis Title	COLORIMETRIC DETERMINATION OF PHENOLS USING POLYDIACETYLENE VESICLES WITH α-CYCLODEXTRIN
By	Miss Pimpimon Anekthirakun
Field of Study	Chemistry
Advisor	Assistant Professor Apichat Imyim, Ph.D.
Co-Advisor	Associate Professor Mongkol Sukwattanasinitt, Ph.D.

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

S. Hannongbua
..... Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

Sirirat Kokpol
..... Chairman
(Associate Professor Sirirat Kokpol, Ph.D.)

..... Advisor
(Assistant Professor Apichat Imyim, Ph.D.)

..... Co-Advisor
(Associate Professor Mongkol Sukwattanasinitt, Ph.D.)

W. Aeungmaitrepirom
..... Examiner
(Assistant Professor Wanlapa Aeungmaitrepirom, Ph.D.)

Pornsri Suthanaruk
..... External Examiner
(Pornsri Suthanaruk, Ph.D.)

พิมพ์พิมล อเนกธีรกุล : การตรวจวัดสารกลุ่มฟีนอลด้วยวิธีคัลเลอริเมตรีโดยใช้พอลิไดอะเซทิลีนเวสิเคิลกับแอลฟาไซโคลเดกซ์ทริน. (COLORIMETRIC DETERMINATION OF PHENOLS USING POLYDIACETYLENE VESICLES WITH α-CYCLODEXTRIN) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. อภิชาติ อิ่มยิ้ม, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. มงคล สุขวัฒนาสินิทธิ์, 79 หน้า.

พัฒนาวิธีการตรวจวัดสารกลุ่มฟีนอลเบื้องต้นอย่างง่ายด้วยการใช้พอลิไดอะเซทิลีนร่วมกับแอลฟาไซโคลเดกซ์ทริน ซึ่งพอลิไดอะเซทิลีนจัดเป็นสารที่มีคุณสมบัติในการเปลี่ยนสีได้เมื่อมีการถูกกระตุ้นจากสิ่งเร้าและสารเคมีบางชนิด จึงง่ายต่อการสังเกตการเปลี่ยนแปลงสีด้วยตาเปล่า ทำการสังเคราะห์พอลิไดอะเซทิลีนเวสิเคิล (PPCDA vesicles) ที่มีค่าการดูดกลืนแสงในช่วง 0.4 ถึง 0.5 จาก 10,12-เพนตะโคซะไดอายโนอิก แอซิด ได้สารละลายสีน้ำเงินเข้มและสามารถเปลี่ยนเป็นสีแดงเมื่อเกิดสารเชิงซ้อนอินคลูชันกับแอลฟาไซโคลเดกซ์ทริน (α-CD) ที่มีความเข้มข้นมากกว่า 3 มิลลิโมลาร์ ศึกษาการยับยั้งการเปลี่ยนสีด้วยสารกลุ่มฟีนอล 9 ชนิด ได้แก่ ออร์โธไนโตรฟีนอล เมตาไนโตรฟีนอล พาราไนโตรฟีนอล พาราโบรโมฟีนอล พาราคลอโรฟีนอล พาราเมทิลฟีนอล พาราไฮโดรควิโนน 2,4-ไดไนโตรฟีนอล และฟีนอล พบว่าความเข้มข้นของสารกลุ่มฟีนอลมีผลต่อการเปลี่ยนสี โดยพาราไนโตรฟีนอล และพาราโบรโมฟีนอลที่ความเข้มข้น 3 มิลลิโมลาร์ และพาราคลอโรฟีนอล และเมตาไนโตรฟีนอลที่ความเข้มข้น 5 มิลลิโมลาร์ สามารถยับยั้งการเปลี่ยนสีได้ในขณะที่สารกลุ่มฟีนอลชนิดอื่น ได้แก่ ออร์โธไนโตรฟีนอล พาราเมทิลฟีนอล พาราไฮโดรควิโนน 2,4-ไดไนโตรฟีนอล และฟีนอล ไม่สามารถยับยั้งการเกิดสารเชิงซ้อนอินคลูชันได้ โดยการยับยั้งนี้ เกิดจากการแข่งขันระหว่างสารกลุ่มฟีนอลและ PPCDA vesicles โดยสารกลุ่มฟีนอลดังกล่าวสามารถเกิดสารเชิงซ้อนอินคลูชันกับ α-CD ได้ดีกว่าและเสถียรกว่าเวสิเคิล ทำการตรวจสอบความใช้ได้ของวิธีตรวจวัดสารพาราไนโตรฟีนอล พาราโบรโมฟีนอล พาราคลอโรฟีนอล และเมตาไนโตรฟีนอลด้วยวิธียูวีวิซิเบิลสเปกโทรเมตรี พบว่าเมตาไนโตรฟีนอล มีค่าความเป็นเส้นตรงที่ช่วงความเข้มข้น 1.0–2.0 มิลลิโมลาร์ พาราไนโตรฟีนอล และพาราโบรโมฟีนอลที่ความเข้มข้น 0.50–1.75 มิลลิโมลาร์ และพาราคลอโรฟีนอล ที่ความเข้มข้น 0.75–2.00 มิลลิโมลาร์ โดยพาราไนโตรฟีนอล และพาราคลอโรฟีนอลมีความแม่นสูง และเมื่อทำการทดลองซ้ำ 10 ครั้ง พบว่าความเที่ยงของพาราไนโตรฟีนอล และพาราคลอโรฟีนอล ซึ่งรายงานเป็น %RSD มีค่าต่ำกว่า 2.0%

ภาควิชา	เคมี	ลายมือชื่อนิสิต	พิมพ์พิมล อเนกธีรกุล
สาขาวิชา	เคมี	ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก	
ปีการศึกษา	2551	ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม	

497 24191 23 : MAJOR CHEMISTRY

KEYWORDS : POLYDIACETYLENE VESICLES / CYCLODEXTRIN / INCLUSION COMPLEX / INHIBITION

PIMPIMON ANEKTHIRAKUN: COLORIMETRIC DETERMINATION OF PHENOLS USING POLYDIACETYLENE VESICLES WITH α-CYCLODEXTRIN. ADVISOR: ASST.PROF. APICHAT IMYIM, Ph.D., CO-ADVISER: ASSOC.PROF. MONGKOL SUKWATTANASINITT, Ph.D., 79 pp.

The preliminary determination of phenolic compounds using polydiacetylene (PCDA) with α-cyclodextrin (α-CD) was developed. PCDA is known as remarkable unique color changeable material upon environmental stimuli and chemicals stimulants. It is easily to observe by naked eyes. The PPCDA vesicles solution with an optimal absorbance in the range of 0.4-0.5 was photopolymerized to form an intense blue solution by using 10,12-pentacosadiynoic acid. The color transition from blue-to-red can be induced by inclusion complex of α-CD at the concentration higher than 3 mM. The inhibition of the color change was investigated using 9 phenolic compounds *i.e. o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, *p*-chlorophenol, *p*-methylphenol, *p*-hydroquinone, 2,4-dinitrophenol, and phenol. The concentrations of phenolic compounds played a critical role to inhibit the color change which observed in the presence of 3 mM of *p*-nitrophenol and *p*-bromophenol and 5 mM of *p*-chlorophenol and *m*-nitrophenol. Meanwhile, the effect of other phenolic compounds was not observed. This inhibition ability was due to the competition between phenolic compounds and PPCDA vesicles. The phenolic compounds have greater ability stability than that of PPCDA vesicles to form inclusion complex with α-CD. The validation data for the determination of *m*-nitrophenol showed the linear range of 1.0-2.0 mM, 0.5-1.75 mM of *p*-nitrophenol and *p*-bromophenol, and 0.75-2.0 mM of *p*-bromophenol. This method exhibited a good accuracy for *p*-nitrophenol and *p*-chlorophenol. The precision of *p*-nitrophenol and *p*-chlorophenol which calculated as relative standard deviation (%RSD) is less than 2.0% (n=10).

Department :	Chemistry	Student's Signature	Pimpimon Anekthirakun
Field of Study :	Chemistry	Advisor's Signature	
Academic Year :	2008	Co-Advisor's Signature	

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who made the completion of this thesis possible. First of all, I would like to convey my gratitude to my advisor, Assistant Professor Apichat Imyim, Ph.D., for his suggestions, assistance, constructive criticism, inspiration, encouragement, and strong support throughout the duration of my thesis. I am deeply indebted to his guidance, patience and all the help he rendered to me during times of research as well as the write up for this thesis. I would also like to thank my co-advisor, Associate Professor Mongkol Sukwattanasinitt, Ph.D., for all the valuable suggestions, assistance, strong support which helped in the completion of this work. In addition, I would like to extend my appreciation to the members of my thesis committee; thanks to Associate Professor Sirisat Kokpol, Ph.D., Assistant Professor Wanlapa Aeungmaitrepirom, Ph.D., and Pornsri Suthanaruk, Ph.D., for all their valuable hints, corrections, and offered suggestions for improvement.

This work could not be completed without kindness and assistance of many people. I am particularly grateful to Assistant Professor Fuanfa Unob, Ph.D., Wittaya Ngeontae, Ph.D., and Wanwisa Janrungroatsakul, for their suggestions to solve problems that I encountered during my research. Furthermore, I would like to thank all my colleagues in the Environmental Analysis Research Unit (EARU). I treasure their friendship and their encouragement is what kept me going. I also wish to thank Anupat Potisatiyeunyong, Ph.D. and Mr. Thoedtoon Champaiboon for their valuable instructions in the synthesis of some materials which were used throughout this thesis. The success of this research is attributed to the Environmental Analysis Research Unit (EARU) which is financially supported by the Grant for Research Unit (GRU51-017-23-008).

Finally, I would like to send a heartfelt acknowledgement to my family for the education, understanding, love, care, support, and especially for the encouragement they provided me throughout my study.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **page**

LIST OF ABBREVIATIONS

PDA	Polydiacetylene
PCDA	10,12-pentacosadiynoic acid
PPCDA	Poly(10,12-pentacosadiynoic acid)
CD	Cyclodextrin
α-CD	α-Cyclodextrin
β-CD	β-Cyclodextrin
γ-CD	γ-Cyclodextrin
p-NP	*p*-Nitrophenol
o-NP	*o*-Nitrophenol
PB	The blue phase percentage
PR	The red phase percentage
CR	Colorimetric response
CR′	Colorimetric response
%	Percent
M	Molar
mM	Millimolar
mL	Milliliter
L	Liter
mg	Milligram
μg	Microgram
mg/L	Milligram per liter
μg/L	Microgram per liter
cm	Centimeter
nm	Nanometer
min	Minute

sec	Second
mp	Melting point
bp	Boiling point
°C	Degree celsius
Å	Angstrom
g	Gram
ΔG^0	Gibbs energy
ΔH^0	Enthalpy
kJ	Kilojoule
kcal	Kilocalorie
MDL	Method detection limit
RSD	Relative standard deviation
SD	Standard deviation
UV-Vis	Ultraviolet-visible spectrophotometry
LLE	Liquid-liquid extraction
GC	Gas chromatography
ECD	Electron capture detector
FID	Flame ionization detector
MS	Mass spectrometer
k_R	The recombination rate constant

CHAPTER I

INTRODUCTION

1.1 Statement of the problem

Phenolic compounds are presented in wastewaters arising from a variety of industries. They are widely used for commercial production, which are used in phenolic resins in the construction materials for automobiles and appliances, pesticides, herbicides, petroleum refining, pharmaceutical, and plastic industries. As it is known, phenolic compounds are considered as priority pollutants since they are harmful to human organisms, even when presented at low concentrations [1]. All routes readily absorb phenols are dermal, inhalation, and oral. Phenols are strong eye and respiratory irritant. It is corrosive to skin and eyes upon direct contact [2]. Furthermore, they also affect the taste and odour of water. Many of these compounds have been classified as hazardous pollutants because of their potential harmful to human health. The US Environmental Protection Agency (EPA) regulated for the lowering phenols contents in wastewaters as well as water quality standards of Thailand which also regulated the lowering phenols contents in industrial effluent under the notification of the Pollution Control Department, Ministry of Natural Resources and Environment to less than 1 mg/L [3-4]. Therefore, it is necessary to determine the phenolic compounds contaminated from industrial effluent before discharging into the water stream.

Current official analytical methods for phenolic compounds determination are spectroscopy and chromatography. For spectroscopy, it is widely used as direct photometric method using 4-aminoantipyrine at pH 7.9 $\pm$ 0.1 as a colored antipyrine dye and the absorbance is measured at 500 nm. However, this method does not determine *para*-substituted and aldehyde groups. And the recently method is liquid-

liquid extraction (LLE), used the derivatization and clean up followed by gas chromatography using several detection devices such as electron capture detector (GC/ECD), flame ionization detector (GC/FID), and mass spectrometer (GC/MS) [5-6]. However, these mentioned methods are complicated in sample preparation, high cost, time-consuming, and also use toxic organic solvents for the extraction of analytes. Therefore, economical and simple-to-use analytical devices are essential for onsite detection of phenolic compounds.

Although there are several studies have been undertaken to investigate phenolic compounds in wastewaters, but there is a need to further examine on this study to find a new simple method. Interestingly, there is a sensing material, polydiacetylene vesicles, which can be indicated that whether there is any *p*-nitrophenol present by using the inhibition ability of *p*-nitrophenol to the complexation between polydiacetylene vesicles with α-cyclodextrin (α-CD), it is desirable to have a simple sensing system for phenolic compounds detection. For this reason, polydiacetylene vesicles and α-cyclodextrin (α-CD) were attractive materials for the determination of phenolic compounds which was a simple preliminary study in order to reduce some complicate processes of the former standard methods.

The inhibition of colorimetric transition of photopolymerized 10,12-pentacosadiynoic acid vesicles (PPCDA) induced by α-CD upon addition of aqueous samples of various phenolic compounds is investigated. In this work, the colorimetric sensors of polydiacetylenes (10,12-pentacosadiynoic acid, PCDA) are interesting for phenolic compounds determination. They exhibit color change from blue-to-red upon various stimuli from their surrounding. This color transition is observed in contact with α-cyclodextrin (α-CD) and called inclusion complex. Therefore, if there are any phenolic compounds present, the color change reaction can be inhibited. Various parameters affecting the colorimetric response, *i.e.* colorimetric response time and concentration of reagent and analyte solutions are determined. The application of this

colorimetric method for the phenolic compounds determination in surface water is also described.

1.2 Objective and scope of this research

The objective of this research is to study the effect of phenolic compounds inhibiting inclusion complex between PPCDA and α-CD.

The scope of this research includes:

1) Preparation of PPCDA vesicles solution,
2) Study of pH response of color transition of PPCDA vesicles solution,
3) Study of color transition of PPCDA vesicles solution with α-CD,
4) Study of inhibition of color transition of PPCDA vesicles solution with phenolic compounds,
5) Application of the proposed method to real water samples.

1.3 The benefits of this research

A new indirect colorimetric method of PPCDA vesicles for onsite detection and UV-Vis spectrometric determination of phenolic compounds in water is proposed.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Polydiacetylene

Polydiactylenes (PDAs) are prepared by using photopolymerization of self-assembled diacetylene monomers (Figure 2.1). The proper order and close packing undergo polymerization *via* 1,4-addition reaction of diacetylene monomers in aqueous solution to form the conjugated ene-eye photopolymerized diacetylene upon irradiation with UV light (254 nm) [7].

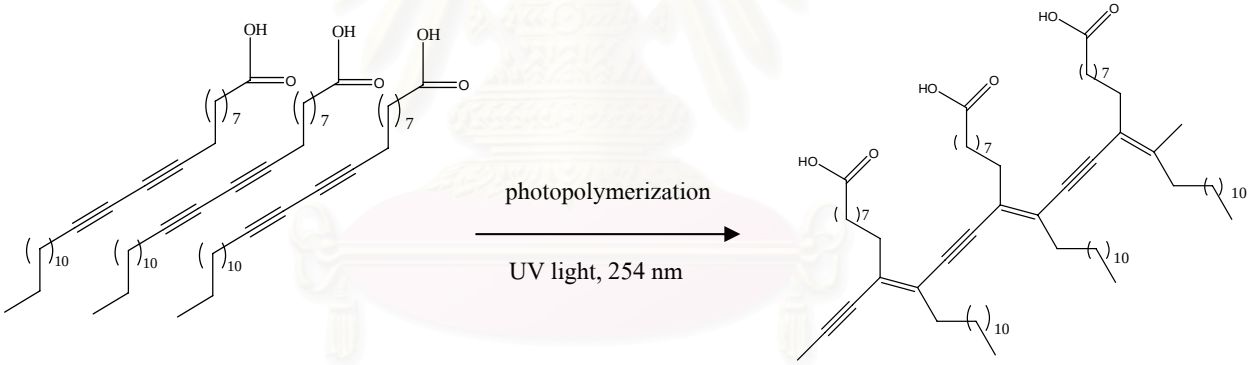


Figure 2.1 Schematic representation of 10,12-pentacosadiynoic acid (PCDA) by UV irradiation.

PDA can be prepared from various kinds of diacetylene monomers. Among diacetylene monomers, diacetylene lipid which consists of hydrophilic carboxylic head group and hydrophobic long chain hydrocarbon is interested. The polymerization of diacetylene is a topochemical reaction, therefore the molecules must be regularly packed in specific arrangement (Figure 2.2) [8]. The topopolymerization can occur when (a) the different distance between the monomer (d_1) and polymer (d_2) approaches

zero, (b) s_1 is smaller than 4.0 Å (lower limit of 3.4 Å), and (c) the angle between diacetylene monomer and translational vector is nearly 45° (±5°). The resulting PDA appears as the intense blue-color to the naked eye, due to electronic delocalization within the conjugated framework, giving rise absorption at 635 nm in the visible region of the electromagnetic spectrum [9]. The structural features of PDA result in the formation of bulk materials, multilayer, multilayer films, polymerized vesicles, and incorporated into inorganic host to form nanocomposites [10].

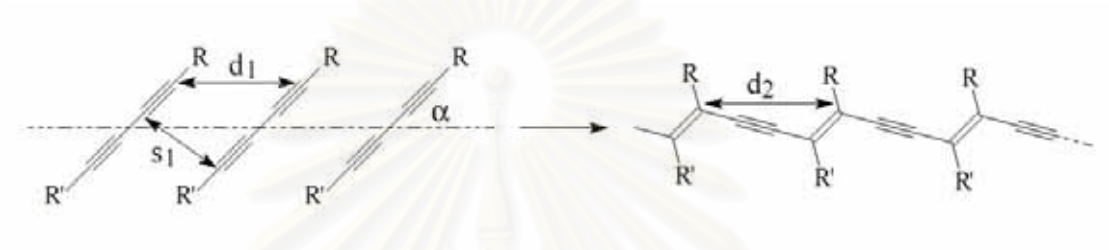


Figure 2.2 Schematic representation of topopolymerization of diacetylene monomers [8].

One of the most commonly used lipid monomer is 10,12-pentacosadiynoic acid (PCDA). PCDA lipid monomer can be spontaneously assembled in aqueous media in the form of spherical nanostructure as nanovesicles after UV irradiation (Figure 2.3) [7].

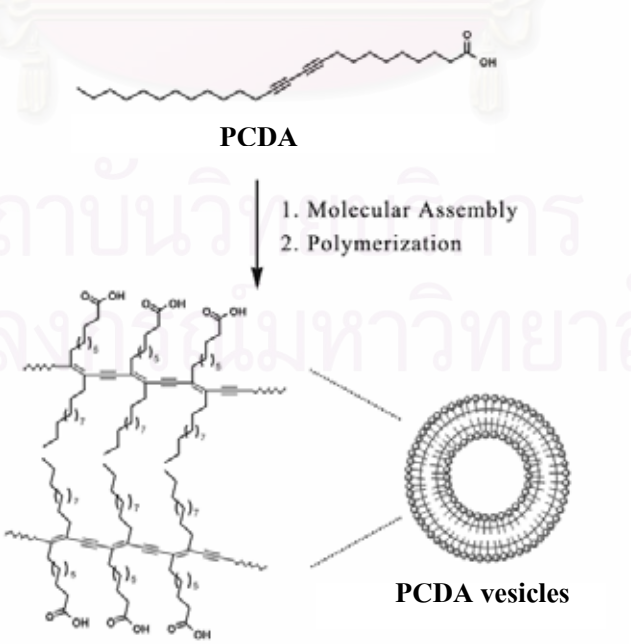


Figure 2.3 Schematic representation of photopolymerization (PCDA) vesicles [7].

2.1.1 Color transition of polydiacetylene

PDA is a remarkable conjugated polymeric system which exhibits unique chromatic properties. Interestingly, PDA can exhibit a color change from blue-to-red upon various environmental stimuli, such as light (photochromism) [11], heat (thermochromism) [12-15], mechanical stress (mechanochromism) [16], solvents (solvatochromism) [17], and binding of specific biological agents (biochromism) [18]. They have two spectroscopically distinct phases absorption at 635 nm and 540 nm, termed in blue and red phase (Figure 2.4).

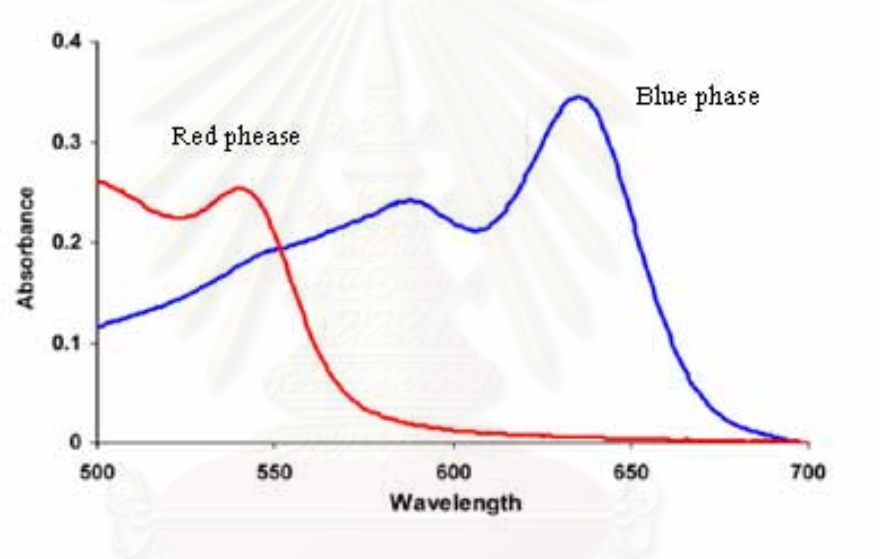


Figure 2.4 Absorption spectra of PCDA vesicles.

The mechanism of the color transition has widely attributed to molecular conformation change, such as side chain packing, orientation, and ordering, impart stresses to PDA conjugated backbone. Thus, changing occurs in the electronic states and the corresponding optical absorption [19-21]. Accordingly, the PDA in blue phase has extended conjugation of *p*-orbital in the main chain of polymers. The conjugated *p*-orbital undergoes distortion by various environmental perturbation, leading to twist of *p*-orbital. Thus, the intense blue-colored PDA gradually shifts to the red-colored PDA (Figure 2.5) [7].

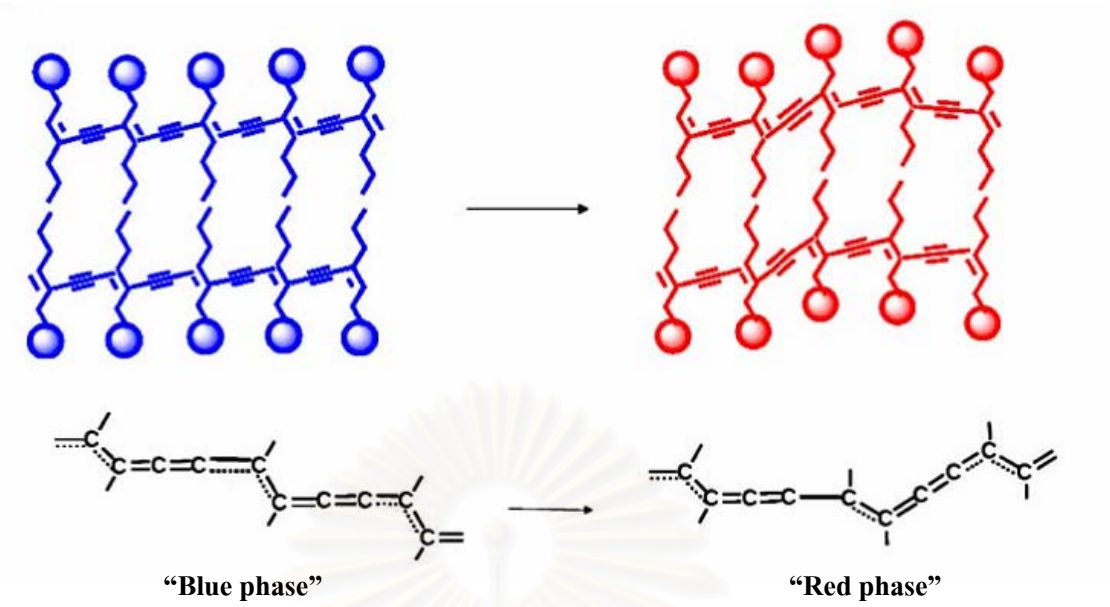


Figure 2.5 Structural features of PDA induction of blue-to-red color transition [9].

2.1.2 Colorimetric response (CR)

To evaluate the color changes, the colorimetric response (CR) is defined to semiquantify blue-to-red transition of PDA vesicles. The CR can be defined as

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

Where PB is the blue phase percentage, defined as

$$PB = \frac{A_{blue}}{A_{blue} + A_{red}}$$

A_{blue} and A_{red} are the absorbances of the blue phase at 635 nm and the red phase at 540 nm in the UV-Vis spectrum, respectively. The initial blue phase percentage, PB_0, is determined before exposure to any stimuli.

2.2 Cyclodextrin

Cyclodextrins (CDs) are multiple (α, D1-4) linked glucopyronose units that display amphoteric properties of a lipophilic central cavity and hydrophilic outer surface. CDs are crystalline, homogeneous and non-hygroscopic substance, which are tours-like macro ring shape [22-23].

The hydrophilic exterior surface of CD molecules is naturally water-soluble, but the hydrophobic cavity provides a microenvironment for appropriately sized non-polar molecules (Figure 2.6) [24].

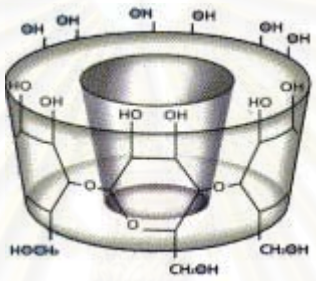


Figure 2.6 Schematic representation of hydrophilic outer surface and hydrophobic cavity of cyclodextrin [25].

Typical CDs are divided into three types, which consist of different number of glucose monomers ranging from six to eight units in a ring as shown in Figure 2.7. Thus:

- α-CD : six membered ring molecule
- β-CD : seven membered ring molecule
- γ-CD : eight membered ring molecule

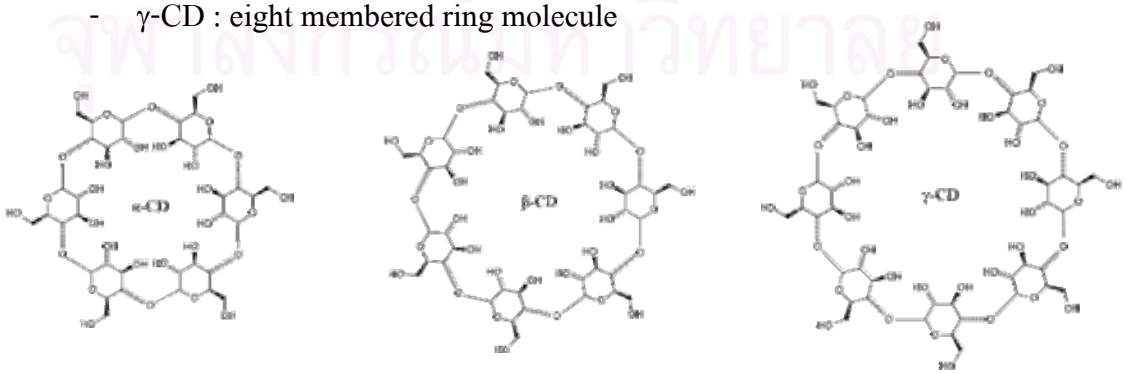


Figure 2.7 Chemical structure of α, β, and γ cyclodextrin [26].

2.2.1 Physical properties of cyclodextrins

Some physical properties of natural cyclodextrins are presented in Table 2.1.

Table 2.1 Some physical properties of natural cyclodextrins [22]

Characteristic	α-CD	β-CD	γ-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
Melting range (°C)	255-260	255-265	240-245
Solubility in water (g/100mL)	14.5	1.85	23.2
Cavity diameter (Å)	4.7–5.3	6-6.5	7.5-8.5
Volume of cavity (approx) (Å)	174	262	472
Crystal forms (from water)	Hexagonal plate	Monoclinic parallelogram	Quadratic prism
Crystal water (%)	10.2	13.2-14.5	8.13-17.7
pKa (by potentiometric) at 25° C	12.3312	12.202	12.081

2.2.2 Chemical properties of cyclodextrins

2.2.2.1 Chemical reactivity

Cyclodextrin has no reducing end groups. No formaldehyde group or formic acid is formed in the periodate oxidation of α, β, and γ CD, providing these molecules do not contain free end groups [22].

2.2.2.2 Acid hydrolysis

Partial acid hydrolysis yields glucose and series of acyclic maltosacharides. In the hydrolysis of oligopolysaccharides, the glycosidic bond of terminal glucose unit is cleaved faster than bond between non-terminal members [22].

2.3 Inclusion complex

An inclusion complex is a complex in which one component of the host form as a cavity containing space in the shape of channel in which molecular entities of a second chemical species as the guest are located. There is no covalent bonding between host and guest, the attraction is generally due to *Van de Waals* forces [27].

2.3.1 Cyclodextrin complexation

In aqueous solution, the slightly apolar CD cavity is occupied by water molecules that are energetically unfavored (polar-apolar interaction), and therefore can be readily substituted by appropriate "guest molecule", which are less polar than water. Inclusion complexation with CD is like host-guest interaction between two molecules. In this reaction CD acts as host molecule, meanwhile another molecule acts as guest molecule to be entrapped in CD host cavity (Figure 2.8). For the formation with cyclodextrin, variety of non-covalent forces like *Van de Waals* forces, hydrophobic interaction, and dipole movement are responsible. In the most cases only a single guest molecule is entrapped in the cavity [22-23, 28-29].

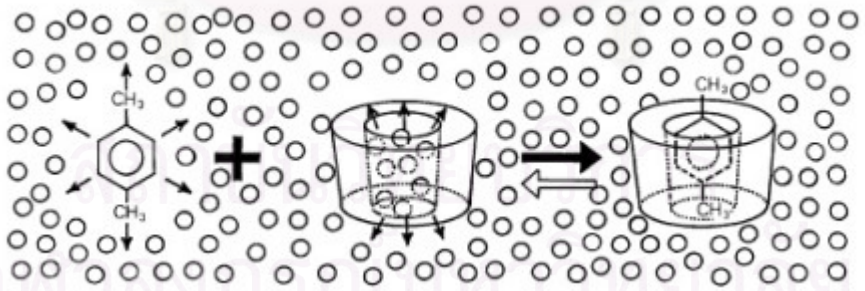


Figure 2.8 Schematic representation of host (CD) and guest (*p*-xylene) interaction [30].

2.3.2 Requirements of the complex formation

Cyclodextrins are able to interact with a great variety of ionic and molecular species and the resulting of inclusion complex belongs to the type of "host-guest" complexes. For the realization of the host-guest relationship, several requirements must be complementary for the binding sites of host and guest molecules. The characteristic features of the host molecule which the binding sites are oriented into the same spatial direction, whereas in the guest molecule the binding sites are diverging in the complex. The association of converging and diverging binding sites by which a complex of host-guest type is formed.

The included molecules are as a rule oriented in the CD in such a position as to achieve the maximum contact between the hydrophobic which is part of the guest and the apolar of CD cavity. The hydrophilic part of the guest molecule remains, as far as possible, at the outer face of the complex to ensure maximum contact with both the solvent and the hydroxyl groups of CD [22].

2.3.2.1 Steric requirements

Cyclodextrins are capable of forming inclusion complexes with compounds having a size compatible with the dimensions of the cavity. The extent of the complex formation depends also on the polarity of the guest molecule. The complexation with molecules significantly larger than cavity may also be possible in such a way that only certain groups or side chain penetrate into CD channel [22].

2.3.2.2 Energetics of complex formation

The energy of covalent chemical bonding is of the magnitude of 10^2 kcal/mole (~ 400 kJ/mole). The energy of hydrogen bonding is about 10^1 and the weaker *Van der Waals* force represent only about 10^0 kcal/mole (~ 40 and 4 kJ/mole) magnitudes of bond energy. The latter are too weak to establish stable molecular species. However, if owing to some species spatial arrangement of two or more

molecules is in a position to establish several of these weak interactions, the species may achieve a stability which is suitable to form covalent bonding. This is the situation in the case of inclusion complexes. *Van der Waals* forces generally originate from dipole-dipole interactions and they are quite weak. Their energy is proposional to molecular polarizability which is proposional to the molecular refraction.

Though the above facts indicate the role of dispersion forces in complex formation, they are not the most important in the stabilization of a complex. Hydrogen bonding alone cannot be crucial either because the sum total of the energies of hydrogen bonding between water and the two separate components does not differ significantly from the energy of hydrogen bonding in the complex. Moreover, stable complexes are formed with the guest molecules which are unable to form hydrogen bonding. The addition of the less polar solvent to water should increase solute-solute interaction, but this is not the case and actually the stability of the inclusion complex is decreased. For example, the dissociation constant of the α-CD-*m*-t-butylphenyl acetate inclusion compound increased from 1.3×10^{-4} to 2.3×10^{-3} after raising the acetonitrile content from 0.5 % to 20.5 % Thus, inclusion complex formation proceeds by an energetically favored interaction of a relatively non-polar guest molecule with an imperfectly solvated hydrophobic cavity [22].

2.3.2.3 The driving force of complex formation

The driving force of the complex formation is either a decrease of the ring strain resulting from complex formation, or the removal of water molecules from the cavity [22].

2.3.3 Rate of inclusion complexation

Rate of inclusion complexation can be determined by the following equation.

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

Where G is guest molecule, k_D and k_R represent the dissociation and recombination of CD-G. The formation ratio of CD-G is commonly observed of 1:1. Stability constant (K or log K) of CD-G complex in equilibrium can evaluate by the following equation [31].

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

2.3.4 Inclusion complex of PDA vesicles with CD

It is known that the carboxylic head group of PDA can interact with another one by hydrogen bonding. Thus, if there are any molecules disrupting hydrogen bonds, the effective conjugated polymer PDA would be altered. CDs were widely investigated because they form inclusion complex with variety of substances. CDs were carried out to interact with PDA by the formation of inclusion complex (Figure 2.9), α-CD was found to be greater to β-CD or γ-CD. α-CD can disrupt the densely packed and self-assembled structure of PDA vesicles providing a rapid color change from blue-to-red [32].

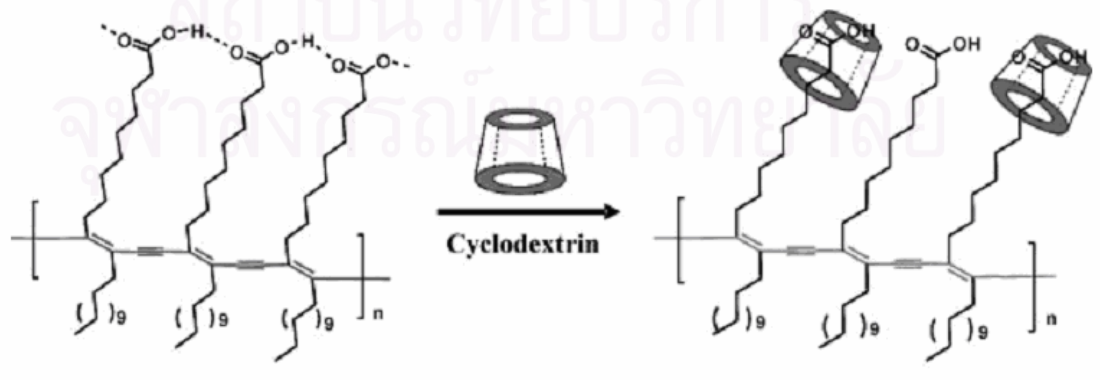


Figure 2.9 Schematic representation of interaction between PDA and α-CD [32].

2.4 Phenolic compounds

Phenolic compounds are defined as hydroxy derivatives directly attached to benzene ring and its condensed nuclei, appear in domestic and industrial waste waters, and potable water supplies [6]. The major sources of phenolic compounds are from paint, pesticide, herbicide, coal, polymeric resin, petroleum refining, and petrochemical industries. These compounds are considered as priority pollutants since they have a detrimental effect on human health even when presented at low concentration. Phenolic compounds can remain in water and atmosphere for longer period of time if a large amount of them are released [33]. Some physical properties of some phenolic compounds are listed in Table 2.2. A number of phenolic compounds are listed in the European Community (EC) Directive 76/464/EEC concerning dangerous substances discharged into the aquatic environment and in the US-EPA list of priority pollutants due to their toxicity and persistence in the environment are shown in Table 2.3.

Table 2.2 Physical properties of some phenolic compounds [34-35]

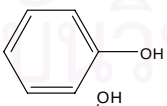
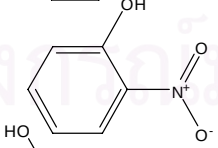
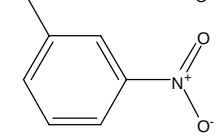
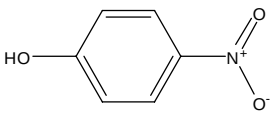
Name	Structure	mp (°C)	bp (°C)	Water solubility (g/100 mL of H_2O)
Phenol		43	181.75	9.3
2-Nitrophenol		43-45	214	0.2
3-Nitrophenol		89-95	-	1.4
4-Nitrophenol		109-114	279	1.7

Table 2.2 (cont.)

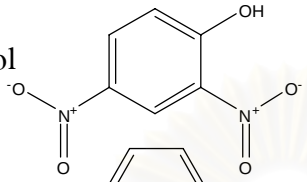
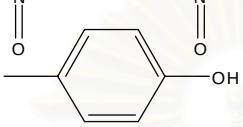
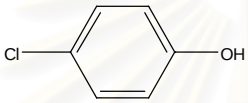
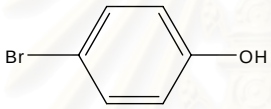
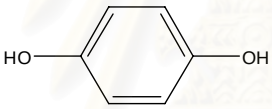
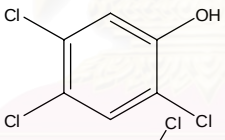
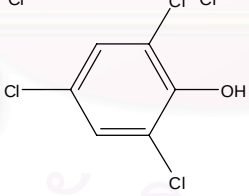
Name	Structure	mp (°C)	bp (°C)	Water solubility (g/100 mL of H_2O)
2,4-Dinitrophenol		108-112	113	2.6
4-Methylphenol		32-35	202	-
4-Chlorophenol		43-45	220	2.7
4-Bromophenol		61-65	235-236	-
Hydroquinone		54-56	287	5.9
2,4,5-Trichlorophenol		64-67	253	-
2,4,6-Trichlorophenol		65-68	246	-

Table 2.3 Phenolic compounds included in priority pollutants list of EC and the US-EPA (method 604 and 8041) [5]

Commission of the European Communities Directive 76/464/EC		
4-Amino-4-chlorophenol	2-Chlorophenol	Trichlorophenol
4-Chloro-3-methylphenol	3-Chlorophenol	
Pentachlorophenol	4-Chlorophenol	

The US-Environmental Protection Agency (EPA 8041)		
Phenol	4-Chloro-3-methylphenol	2,4-Dimethylphenol
2-Methylphenol	2,4-Dichlorophenol	2-Chlorophenol
3-Methylphenol	2,6-Dichlorophenol	2,4,5-Trichlorophenol
4-Methylphenol	Pentachlorophenol	2,4,6-Trichlorophenol
2-Nitrophenol	2,4-Dinitrophenol	Dinoseb (DNMP)
2-Cyclohexyl-4,6-dinitrophenol		2,3,4,6-Tetrachlorophenol
		2,3,5,6-Tetrachlorophenol

2.5 Phenolic compound determination

Phenolic compounds are special concerned owing to the potential increment of these compounds through the environment via leaching which comes from the industries [36]. Standard analytical techniques used in the determination of phenolic compounds are direct photometric method by using 4-aminoantipyrine colorimetric method, chloroform extraction method, and liquid-liquid extraction (LLE), followed by gas chromatography (GC) using several detection methods [6, 36].

2.5.1 Direct photometric method

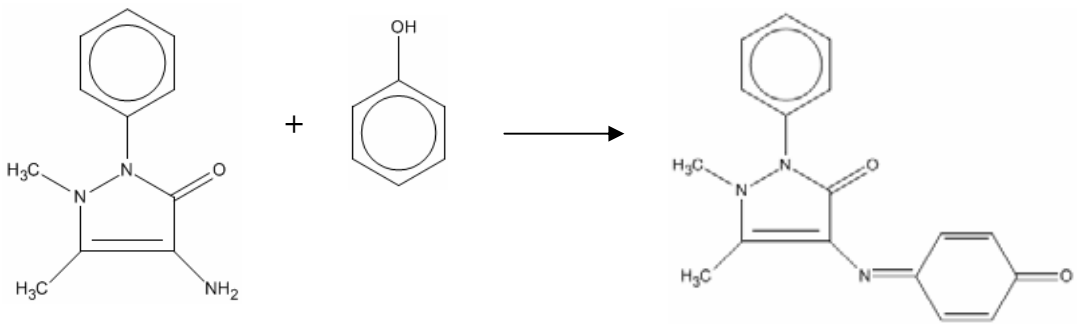
The analytical procedures use the 4-aminoantipyrine to determine phenol, *ortho*-, *meta*-substituted, and *para*-substituted phenols in which the substitution is a carboxyl, halogen, methoxyl, or sulfonic acid group under proper pH conditions. Steam distillable phenolic compounds are reacted with 4-aminoantipyrine at pH 7.9 $\pm$ 0.1 in the presence of potassium ferricyanide [$K_3Fe(CN)_6^-$] to form a colored antipyrine dye. This dye is measured at 500 nm. This method has less sensitivity than chloroform extraction method. The 4-aminoantipyrine method does not determine *para*-substituted phenols where the substitution is an alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde group. A typical example of the latter groups is *p*-cresol (or

p-methylphenol), which may be present in certain industrial wastewaters and in polluted surface waters. The minimum detectable quantity is 10 μg phenol when a 5-cm cell and 100 mL of ditilled sample solution are used.

Two 4-aminoantipyrine methods ; direct photometric and chloroform extraction methods, had been conducted. The latter method has an extreme sensitivity, adaptable for water samples containing phenol less than 1 mg/L. The color is very intense in a non-aqueous solution. Meanwhile, using a direct photometric method, the color is developed in an aqueous solution. Since the relative amounts of various phenolic compounds in a given sample are unpredictable, it is not possible to provide any mixed standard solution containing phenols. For this reason, phenol (C_6H_5OH) itself has been selected as a representative standard for these colorimetric procedures even though other phenolic compounds can produce the same colored compound as phenol. But this method has a drawback that any substituted phenolic compounds give low response, thus this method is recommended only for unsubstituted phenolic compounds or total phenols [6].

2.5.2 Chloroform extraction method

Steam distillable phenolic compounds are reacted with 4-aminoantipyrine at pH 7.9 $\pm$ 0.1 in the presence of potassium ferricyanide [$K_3Fe(CN)_6^-$] to form a colored antipyrine dye. This dye is extracted from aqueous solution with $CHCl_3$ and spectrophotometrically measured at 460 nm. The minimum detectable quantity for clean samples containing no interferences is 0.5 μg phenol when a 25 mL $CHCl_3$ extraction with a 5 cm-cell or 50 mL $CHCl_3$ eaxtraction with a 10 cm-cell is used in photometric measurement. This quantity is equal to 1 μg phenol/L in 500 mL of distillate [6].



4-aminoantipyrine phenol

Figure 2.10 Chemical reaction for 4-aminaoantipyrine method [37].

Phenols and all substituted phenols (except those with *para* substitution), are determined by adding 4-aminoantipyrine to produce a yellow or ambercolored complex in the presence of ferricyanide ion (Figure 2.10). The color is intensified through extraction of the complex into chloroform. Phenol concentrations in samples are quantitatively determined by external calibration method [37].

2.5.3 Gas chromatography

Liquid-liquid extraction (LLE) followed by gas chromatography (GC) in conjunction with various detection devices, mainly FID, ECD or MS, is applicable to the determination of phenol and certain substituted phenols in effluents from industrial manufacturers. This approach has the advantage of high sensitivity and selectivity, but in general derivatization is required prior to analysis. The derivatization, cleanup, and electron capture detector gas chromatography (GC/ECD) are alternatively used to confirm measurements made by the flame ionization detector (GC/FID) procedure. These mentioned methods are appropriate to determine a wide variety of phenols at relatively low concentrations. In addition, gas chromatography/mass spectrometer (GC/MS) can be used to determine the phenols at slightly higher concentrations. Various derivatization reagents have been reported, e.g. pentafluorobenzoyl bromide, acetic anhydride, heptafluorobutyric anhydride or diazomethane, but fail for

nitrophenols and dinitrophenols when using ECD. However, it was pointed out that the US-EPA official method for phenols may often lead to incorrect results because derivatization of phenols, especially nitrophenols, is not straightforward. Recently, the US-EPA has reported a new protocol which recommends the derivatization to methylated phenols instead of to pentaflurobenzoyl ether derivatives. However, this method requires the use of diazomethane which has potential hazards associated with its use (carcinogen and explosive).

The method detection limit (MDL) is measured and reported with the 99% confidence that the value is above zero. The MDL concentrations listed in Table 2.4 was obtained by using reagent water [5-6].

Table 2.4 Chromatographic conditions and method detection limits [6]

Compound	Retention time (min)	Method dectection limit (μg/L)
2-Chlorophenol	1.70	0.31
4-Chloro-3-methylphenol	7.50	0.36
2,4-Dichlorophenol	4.30	0.39
2,4-Dimethylphenol	4.03	0.32
2,4-Dinitrophenol	10.00	13.00
2-Methyl-4,6-dinitrophenol	10.24	16.00
2-Nitrophenol	2.00	0.45
4-Nitrophenol	24.25	2.80
Pentachlorophenol	12.42	7.40
Phenol	3.01	0.14
2,4,6-Trichlorophenol	6.05	0.64

2.6 Literature review

The unique colorimetric property of polydiacetylenes have made candidate in the development of chemosensors. Kim et al. [32] studied the effect of CDs on the color changes of 10,12-pentacosadiynoic acid (PCDA) (Figure 2.11). The result showed that among the CDs investigated, α-CD had most effect on the perturbation of the structure of PCDA vesicles.

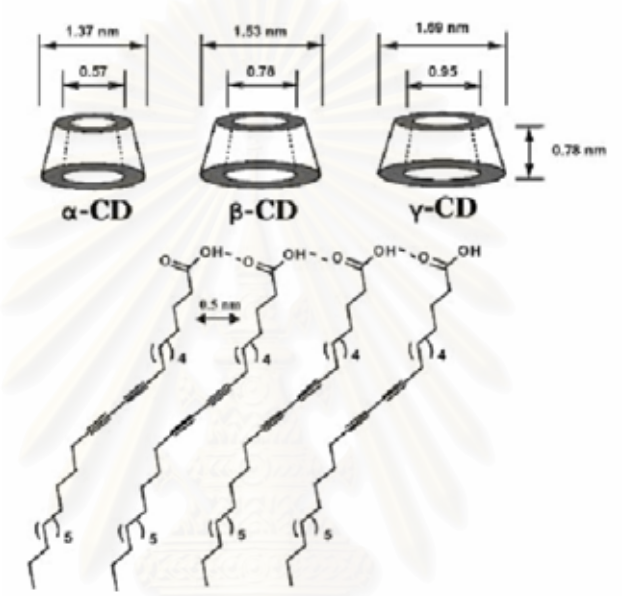


Figure 2.11 Schematic representation of CDs and PCDA monomers [32].

In addition, the observed color change between PCDA vesicles and α-CD was inhibited by using *p*-nitrophenol (*p*-NP). Matsue et al. [38] considered the determination of *o*-NP in the presence of *p*-isomer by cyclic voltammetry, using the specific properties of α-CD. The dissociation constants for *o*-NP-α-CD and *p*-NP-α-CD were measured by polarographic method. Although α-CD can bind both *o*-NP and *p*-NP, its binding abilities for the two compounds are greatly different, in which the reduction peak of the para isomer is shifted toward more negative potentials. Furthermore, the peak current of the para isomer becomes lower since the effective diffusion coefficient of the para isomer decreases due to the formation of an inclusion complex with α-CD molecule. Thus, α-CD can bind *p*-NP more effectively in the cavity than that of *o*-NP. Eftink et al. [39] studied the thermodynamic of the binding of

p-NP to both α-CD and β-CD using flow microcalorimetric technique. The preferential binding of the anionic form of *p*-NP to CDs has been observed, ΔG^0 and ΔH^0 are more negative for the binding to α-CD than to β-CD. Therefore, the interaction involving *p*-nitrophenolate possibly plays a role in the binding of certain α-CD. Nuwer et al. [40] described kinetics and thermodynamics of inclusion complex of *p*-nitrophenolate with α-CD by means of pulse voltammetry and also proposed the host-guest reaction model of α-CD with *p*-nitrophenolate anion (*p*-NP^-) (Figure 2.12).

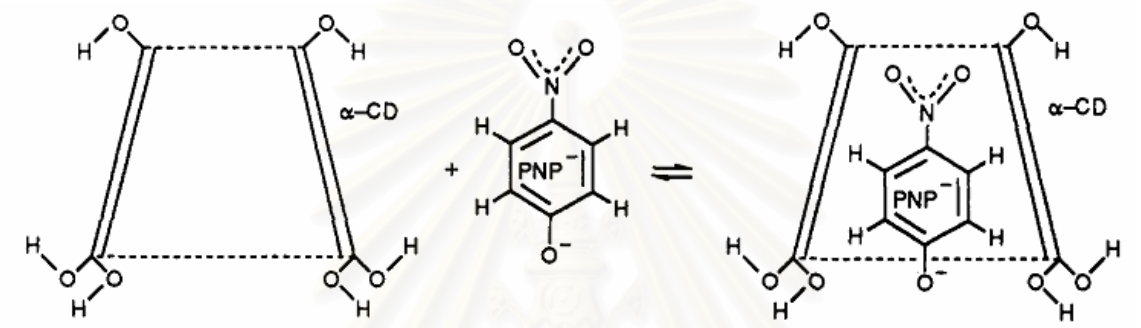


Figure 2.12 Schematic representation of inclusion complex of α-CD with *p*-NP^- [40].

The normal pulse voltammetry of *p*-NP^- illustrated the wave shifts to more negative potentials as the concentration of α-CD was increased. This shift in position was accompanied by decrease of the wave due to the smaller diffusivity of the complex, which is the diffusing species. Therefore, α-CD can truly form an inclusion complex with *p*-NP^-.

As mentioned, there is a certain research to strongly support the inclusion complex study between PCDA vesicles and α-CD as well as *p*-nitrophenol has great ability to include with α-CD. However, if there is *p*-nitrophenol presented simultaneously with the PCDA/α-CD complex, *p*-nitrophenol can perform a great inhibition to PCDA/α-CD complex. Nevertheless, this inhibition study has not been investigated to any other phenolic compounds. Therefore, in this research, the inhibition study of inclusion complex between PCDA vesicles with α-CD in the

presence of various phenolic compounds was investigated in order to invent a new detection method of phenolic compounds in wastewater. Eleven phenolic compounds *i.e. o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol, *p*-methylphenol, *p*-chlorophenol, *p*-bromophenol, hydroquinone, 2,4-dinitrophenol, phenol, 2,4,5-trichlorophenol, and 2,4,6-trichlorophenol were chosen because they have been classified as hazardous pollutants which were considered in the Pollution Control Department, Ministry of Natural Resources and Environment of Thailand and the US Environmental Protection Agency (EPA) regulation.

In this study, the colors changeability of PCDA vesicles is the main interest to observe. They exhibit a color change from blue-to-red when α-CD is presented. Therefore, if there are any phenolic compounds presented, this color change can be inhibited due to the competition between PCDA vesicles and phenolic compounds to form inclusion complex with α-CD.

CHAPTER III

EXPERIMENTAL

3.1 Chemicals

Chemicals	Supplier
p-Bromophenol	Fluka, USA
p-Chlorophenol	Fluka, USA
α-Cyclodextrin	Fluka, USA
Diethyl ether, reagent grade	Lab-Scan, Thailand
2,4-Dinitrophenol	Fluka, USA
Hydrochloric acid, 37%	Merk, Germany
Hydroquinone	Fluka, USA
p-Methylphenol	Fluka, USA
o-Nitrophenol	Fluka, USA
m-Nitrophenol	Fluka, USA
p-Nitrophenol	Fluka, USA
10,12-Pentacosadiynoic acid (PCDA)	Fluka, USA
Phenol	Merk, Germany
Sodium acetate	Carlo erba, Italy
Sodium hydroxide	Merk, Germany
2,4,5-Trichlorophenol	Fluka, USA
2,4,6- Trichlorophenol	Fluka, USA

3.2 Instruments

1. Ultrasonicator, Elma, Germany
2. pH meter, Orion2Star, Thermoelectron corporation, Taiwan
3. Magnetic stirrer, GEM, Thailand
4. Pipette, Brand transferpette, Germany
5. UV-Vis spectrophotometry, Hewlett Packard model HP 8453, Germany

3.3 Preparation of polydiacetylene vesicles

Polydiacetylene vesicles were synthesized according to the preparation of PPCDA vesicles of Potisatiyuenyong et al. [41]. The diacetylene monomer 10,12-pentacosadiynoic acid (11.2 mg) was dissolved in diethyl ether (10 mL) in a test tube and the solvent was gradually evaporated by N_2 purging until lipid bilayer was formed, which can be observed to the alignment of white powder around test tube. Deionized water (30 mL) was added to provide the concentration of 1 mM PCDA vesicles. The PCDA suspended solid (1 mM) was sonicated by a 230 Watt sonicator bath for 30 min at 70-80 °C. The resulting solution was kept at 4°C overnight. The solution was brought to room temperature and then this solution was irradiated by UV radiation at 254 nm for 5 min to yield intense blue-colored poly(10,12-pentacosadiynoic acid) (PPCDA) vesicle solution, shown in Figure 3.1. The blue-colored vesicle solution was filtered through a paper filter to remove any undesired lipid aggregates. The filtrate of PPCDA vesicle showed the maximum visible absorption at 635 nm.

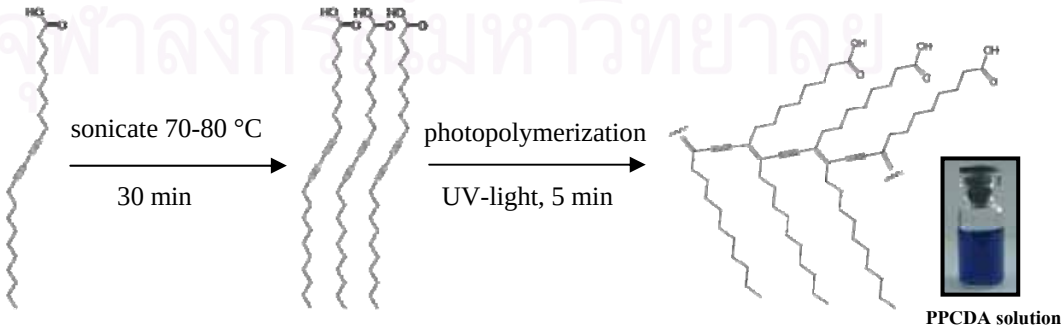


Figure 3.1 Schematic representation of PPCDA vesicle preparation.

3.4 Preparation of α-cyclodextrin solution

α-CD stock solution (10 mM) was prepared by the dissolution of α-CD (486.4 mg) in sodium acetate buffer solution (20 mM, pH 5.8) using a 50 mL volumetric flask.

3.5 Preparation of phenolic compounds solution

To obtain phenolic compounds stock solutions (50 mM), *o*-, *m*-, and *p*-nitrophenol (695.6 mg each kind), *p*-methylphenol (540.7 mg), *p*-chlorophenol (642.8 mg), *p*-bromophenol (865.1 mg), 2,4-dinitrophenol (920.6 mg), 2,4,5-, 2,4,6-trichlorophenol (987.3 mg each kind) were dissolved in DI water by adding 4 M NaOH for complete dissolution, while hydroquinone (620.7 mg) and phenol (470.6 mg) were dissolved in DI water by adding 1 M NaOH for complete dissolution. Each phenolic compound stock solution was prepared by using a 100 mL volumetric flask.

The phenolic compounds stock solutions (50 mM) were diluted with sodium acetate buffer solution (20 mM, pH 5.8) in a 50 mL volumetric flask. The stock solutions were adjusted by using 1 M NaOH and 1 M HCl solution to obtain pH~5.8 and then made up to volume with sodium acetate buffer solution. The final concentration of phenolic compounds solutions was equal to 10 mM, which used in all experiments as stock solution.

3.6 Study of colorimetric response

3.6.1 UV-Vis spectroscopy

All measurements were performed using 0.1 mM PPCDA vesicles solution (the exact concentration of PPCDA was fine-tuned to obtain an optimal absorbance in the range of 0.4-0.5), which obtained from the dilution step of initial concentration of PPCDA stock solution (1 mM). The visible spectra of PPCDA vesicles solution were taken in a quartz cuvette with 1 cm optical path length. The spectra were obtained at wavelengths between 500 and 900 nm at room temperature by UV-Vis spectrometer (Hewlett Packard model HP 8453, Germany).

3.6.2 Colorimetric measurements

The parameter commonly used for quantifying the colorimetric transition was denoted as CR(%), percentage colorimetric response. The CR(%) can be defined as

$$\mathrm{CR}(\%) = \frac{(\mathrm{PB}_0 - \mathrm{PB})}{\mathrm{PB}_0} \times 100$$

Where PB is the blue phase percentage, defined as

$$\mathrm{PB} = \frac{\mathrm{A}_{\mathrm{blue}}}{\mathrm{A}_{\mathrm{blue}} + \mathrm{A}_{\mathrm{red}}}$$

A_{blue} and A_{red} are the absorbances of the blue phase at 635 nm and the red phase at 540 nm in the UV-Vis spectrum, respectively. The initial blue phase percentage, PB_0, is determined before exposure to any stimuli. Typically in this study, the absorption spectra of PPCDA samples were recorded in the visible range (500-850 nm) with the absorbance at 850 nm were set to zero.

3.6.3 Color transition of PPCDA vesicles against pH

To investigate the pH effect on the color transition of PPCDA vesicles, they were studied under pH range from 1.0–14.0 at room temperature. The PPCDA vesicles stock solution (1 mM) was diluted with sodium acetate buffer solution (20 mM, pH 5.8) in a 25 mL volumetric flask. The pH of vesicles solution was adjusted by using 1 M NaOH and 1 M HCl solution to desired pH and made up to volume with sodium acetate buffer solution. The color change was observed by UV-Vis spectrometry. The spectra were collected from 500 to 850 nm with the zero absorbance at 850 nm.

3.6.4 Study of color transition of PPCDA vesicles with α-cyclodextrin

Inclusion complex study between α-CD and PPCDA vesicles was investigated by adding different concentrations of α-CD. The α-CD stock solution (10 mM) was prepared in sodium acetate buffer solution (20 mM, pH 5.8). The absorbance of PPCDA vesicles solution was fixed in the range of 0.4-0.5 and α-CD solutions with the final concentration varied from 0.5–5.0 mM was added. All mixtures were prepared in a test tube to obtain final volume of 3 mL by made up to volume with sodium acetate buffer solution. The mixture was standed at room temperature for 5 min prior to measure by UV-Vis spectrometry. Moreover, time response of inclusion complex was studied by varying time from 5 to 180 min at room temperature. The spectra were collected from 500 to 850 nm with the zero absorbance at 850 nm.

3.6.5 Inhibition study of color transition by phenolic compounds

Phenolic compounds determination by using their inhibition ability to the inclusion complex between α-CD and PPCDA vesicles were studied on 11 phenolic compounds *i.e. o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol, *p*-methylphenol, *p*-chlorophenol, *p*-bromophenol, hydroquinone, 2,4-dinitrophenol, phenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol. Each phenolic compound solution pH was

adjusted to 5.8 by using 1 M NaOH and 1 M HCl solution. Initially, time-dependent of phenolic compounds inhibiting α-CD with PPCDA vesicles were studied and also tested of the addition sequence of α-CD, PPCDA, and phenolic compounds. In this study, the procedure of addition sequence can be divided into two types: (a) pre-equilibrated 3 mM α-CD (pH 5.8) with 3 mM phenolic compounds (pH 5.8) by varying time form 5 to 180 min prior to mix with PPCDA (pH 5.8, absorbance range of 0.4-0.5) to obtain final volume of 3 mL in a test tube by made up to volume with sodium acetate buffer (20 mM, pH 5.8) and standed at room temperature for 120 min, (b) pre-equilibrated 3 mM α-CD (pH 5.8) with PPCDA (pH 5.8, absorbance range of 0.4-0.5) for 120 min prior to mix with 3 mM phenolic compounds (pH 5.8) to obtain final volume of 3 mL in a test tube by made up to volume with sodium acetate buffer (20 mM, pH 5.8) by varying time from 5 to 180 min. After study of time-dependent, the optimum time condition was chosen to use in the next experiment.

When obtained the optimum time condition and optimum addition sequence of three compounds in previous study, the competition study between phenolic compounds and PPCDA with α-CD was investigated by varying phenolic compounds concentrations from 0.5–5.0 mM. In this study, 3 mM α-CD (pH 5.8) and PPCDA (pH 5.8, absorbance range of 0.4-0.5) were pre-equilibrated for 120 min at room temperature followed by adding phenolic compounds (pH 5.8) at various concentration from 0.5–5.0 mM to obtain final volume of 3 mL in a test tube by made up to volume with sodium acetate buffer (20 mM, pH 5.8) and standed at room temperature for 120 min prior to measure by UV-Vis spectrometry. The spectrum was collected from 500 to 850 nm with zero absorbance at 850 nm.

3.7 Method validation

Validation of analytical method is the measurement of performance characteristics such as accuracy, precision, limit of detection, limit of quantitation, method detection limit, linearity, and range. This method was validated by using the spiked water sample with phenolic compounds solution and repeating the same experiments.

Initially, the calibration curve of each four phenolic compound was constructed by varying phenolic compounds concentration from 0.1 to 3.0 mM. In this study, 3 mM α-CD (pH 5.8) and PPCDA (pH 5.8, absorbance range of 0.4-0.5) were pre-equilibrated for 120 min at room temperature followed by adding phenolic compounds (pH 5.8) at various concentration from 0.1–3.0 mM to obtain final volume of 3 mL in a test tube by made up to volume with sodium acetate buffer (20 mM, pH 5.8) and standed at room temperature for 120 min prior to measure by UV-Vis spectrometer. The spectrum was collected from 500 to 850 nm with zero absorbance at 850 nm.

Then, the method validation was performed by using spiked water sample with standard *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol at two concentration levels (1.5 and 1.75 mM) chosen from the concentration within the linear range of calibration curve. Each experiment was repeated 10 times. The sample solution (3.0 mL) containing sodium acetate buffer (20mM, pH 5.8), 3 mM of α-CD, 1.5 and 1.75 mM of each phenolic compound (*m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol) were both individually spiked and allowed to room temperature for 120 min prior to add PPCDA solution (absorbance range of 0.4-0.5) into the mixture solutions and standed room temperature for 120 min prior to measure by UV-Vis spectrometer. Moreover, simultaneous determination of four phenolic compounds was also studied. In this case, the sample solution (3 mL) containing sodium acetate buffer (20 mM, pH 5.8) and 3 mM of α-CD was mixed with 0.375 mM of each phenolic compound to obtain final concentration of 1.5 mM. This solution was standed at room temperature for 120 min prior to add PPCDA

(absorbance range of 0.4-0.5), the final solution also standed at room temperature for 120 min prior to measure by UV-Vis spectrometry. The UV-Vis spectra were collected from 500 to 850 nm with the zero absorbance at 850 nm. The accuracy, describes the closeness between the measured value and the true value, calculated as relative error (%). The precision was evaluated through repeatability and reproducibility, expressed as relative standard deviation (%). The linear range was specified as the concentration range over which the analytical curve is linear. Method detection limit was obtained from the measurement ability of method to determine an analyte in a sample matrix.

3.8 Application to real sample

Real water sample employed in this work was surface water from the pond of Chulalongkorn University, collected in October, 2008. This sample was collected by sampling water at the edge of the pond and contained in a bottle (1 L). Before the application for experiments, the surface water was filtered through a paper filter followed by a milipore membrane filter and the pH of the sample solution was measured.

The method of inhibition study between PPCDA/α-CD was applied to surface water sample which were both individual and quaternary mixtures of four phenolic compounds standard (*m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol). In the case of individual study, the samples were spiked with single standard of each phenolic compound to obtain the final concentration of 1.5 and 1.75 mM. Whereas, simultaneous of phenolic compounds determination was applied to the samples by quaternary mixtures of four phenolic compounds with the concentration of 0.375 mM of each phenolic compounds to obtain the final concentration of 1.5 mM. The experiment procedures were done in the same manner as described for method validation. Each experiment was repeated 10 times.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preparation of polydiacetylene vesicles

A diacetylene monomer, namely 10,12-pentacosadiynoic acid, was dispersed in water. The visual appearance of the result solution was a suspended white solution after sonication at 70-80°C for 30 min and keeping the colloid solution at 4°C overnight. An intense blue-colored solution (Figure 4.1) was obtained after irradiating with UV-radiation for 5 min at room temperature and filtrated through a filter paper. The diacetylene monomer self-assembled underwent complete polymerization to form alternating ene-eye conjugated polymer, called PPCDA. It has spectroscopically distinct blue phase which showed the maximum absorption at wavelength of 635 nm (Figure 4.2). The resulting intensely blue to the eye of the PPCDA polymer was due to the electronic delocalization within the conjugated framework, giving rise to absorption at around 635 nm in the visible region of the electromagnetic spectrum [42].

Figure 4.1 Photograph of PPCDA solution.

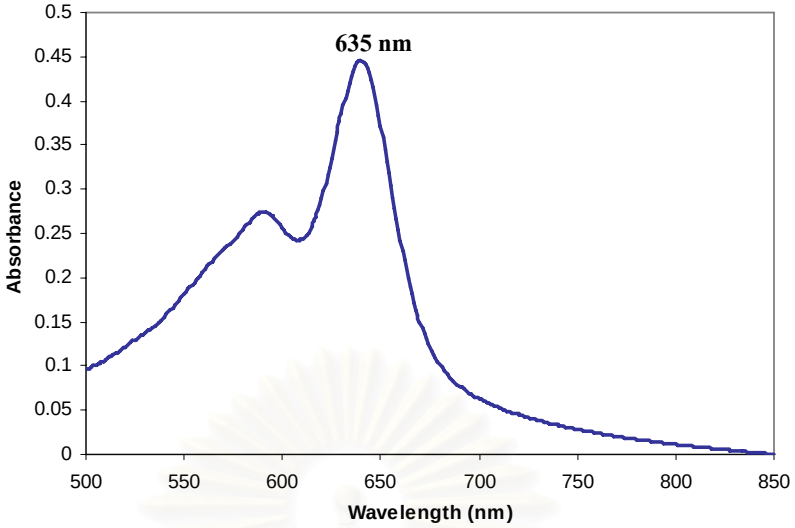


Figure 4.2 Visible spectrum of PPCDA vesicles.

This section attempted to find an optimum concentration of PPCDA for colorimetric analysis by UV-Vis spectrometry and visual observation of the solution color. The initial concentration of PPCDA stock solution obtained from the preparation step was 1 mM. This solution was stepwise diluted with DI water to desired concentrations varied from 0.010 to 0.125 mM and their absorbance was measured at 635 nm. The plot of the absorbance against the concentration was shown in Figure 4.3. The plot in Figure 4.3 showed a very good linearity with R^2 equal to 0.9999. The very light blue color of the solution containing 0.025 mM was observed and the observed blue color intensity increased with the concentration. The solution approximately contained 0.1 mM PPCDA (pH 6.8±0.2) was chosen for further experiments due to its optimal absorbance in the range of 0.4-0.5 and its readily observed blue color.

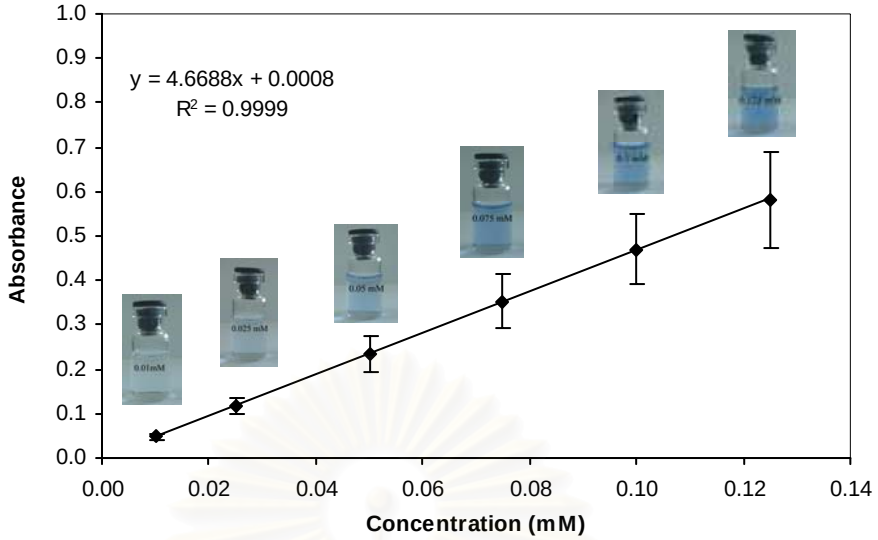


Figure 4.3 Concentration range of PPCDA vesicles at room temperature.

4.2 pH response of polydiacetylene vesicles

Initially, PPCDA vesicles stock solution (1 mM) was diluted by DI water to obtain an approximate final concentration of 0.1 mM (pH 6.8±0.2) with optimal absorbance in the range of 0.4-0.5 and then the pH of diluted solution was adjusted by using 1 M NaOH and 1 M HCl. In the acidic pH range of 1-3 and basic pH range of 13-14, the PPCDA aggregated and precipitated. In the pH range of 4-6, the CR(%) was zero and while the CR(%) increased in the pH range of 7-12. Moreover, the pH of the solution was unsteady after the pH adjustment. Thus, the sodium acetate buffer solution was chosen for the next experiments in order to solve this problem. In this section, sodium acetate buffer solution (20 mM) of pH 5.8 was chosen to avoid the risk of aggregated and precipitated of PPCDA at lower pH and also avoid color change of PPCDA at higher pH. The PPCDA stock solution (1 mM) was diluted by sodium acetate buffer solution (20 mM, pH 5.8) to obtain final concentration of 0.1 mM (pH 5.8±0.1) and this diluted solution was used to compare with other pH range. The pH of PPCDA vesicles solution was adjusted by using 1 M NaOH and 1 M HCl. The PPCDA solutions in Figure 4.4 showed pH induced color transition, which obviously observed by visual observation. The PPCDA solution was blue in the pH range of 4-6

and became light blue in pH 7. The blue solution changed to violet in the pH range of 8-9. When the pH was higher than 9, PPCDA solutions changed to red. However, in the acidic pH range of 1-3 and basic pH range of 13-14, the PPCDA aggregated and precipitated. Figure 4.5 showed the spectra of PPCDA by varying pH from 4 to 12. As pH increases, the absorbance at 635 nm (blue phase) diminishs, while the absorbance at 540 nm (red phase) increases. In this study, the CR(%) of the pH range of 5-6 was held around zero (Figure 4.6), which indicates that there was no any perturbation from the pH adjustment step by using NaOH and HCl. Thus, the buffer solution (pH 5.8) was chosen to control the pH range of PPCDA solution after diluting from the initial concentration of PPCDA stock solution. The color transition was quantified by colorimetric response in Figure 4.6, which showed the plot of CR(%) against pH. The CR(%) is defined as

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

Where PB is the blue phase percentage, defined as $PB = A_{blue}/(A_{blue}+A_{red})$. A_{blue} and A_{red} are the absorbances of the blue phase at 635 nm and the red phase at 540 nm in the UV-Vis spectrum, respectively. The initial blue phase percentage, PB_0, is determined before exposure to any stimuli.

In this study, PB_0 is the approximate initial blue phase percentage of 0.1 mM PPCDA solution (pH 5.8, absorbance range of 0.4-0.5) which determined before exposure to pH adjustment. Whereas, PB is the blue phase percentage which determined after adjusting the pH range of 4-12 of PPCDA solution.

Figure 4.4 Photograph of pH induced color transition of PPCDA vesicles at room temperature.

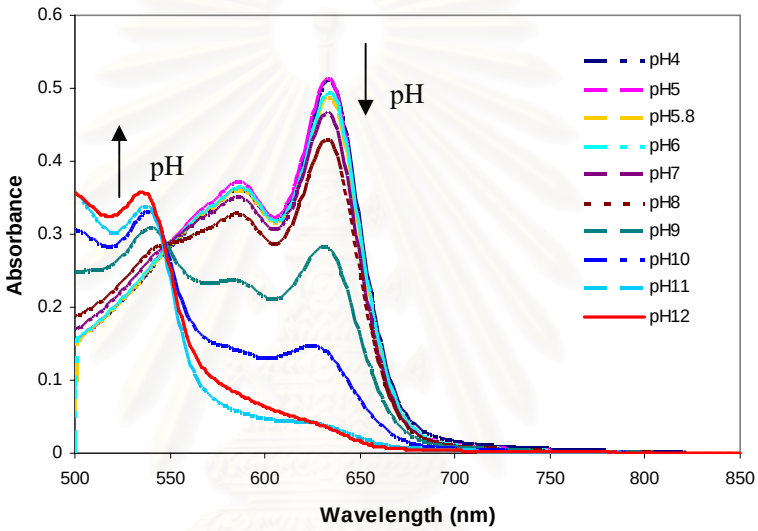


Figure 4.5 Visible spectra of pH induced color transition of PPCDA vesicles at room temperature.

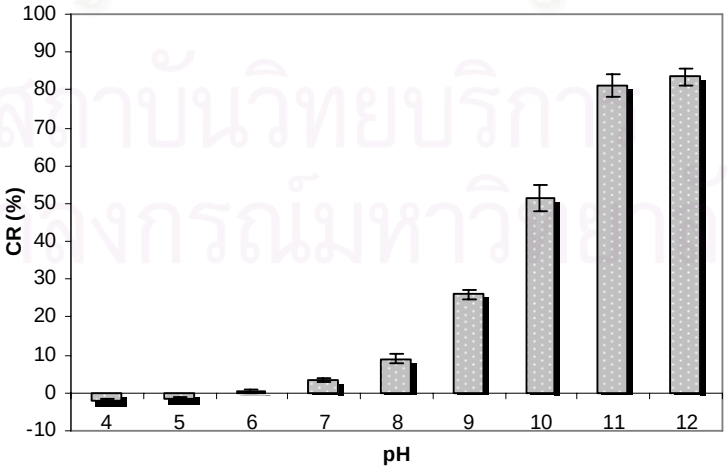


Figure 4.6 Colorimetric response of pH induced color transition of PPCDA vesicles at room temperature.

The PPCDA vesicles in blue form have conjugated of *p*-orbitals in the main chain of polymer. The molecular mechanism corresponding to the color change was believed to be an irreversible stress-induced structural transition of the conjugated backbone of the polymer [42]. Basicification by adding NaOH induced the color transition of PPCDA vesicles solution from blue-to-red by deprotonation of the carboxylic head group of the PPCDA main chain. Considering the reaction equilibria of carboxylic head group (-COOH) of PPCDA vesicles [43], the dissociation equilibrium is as follow;

$$-COOH + H_2O \rightleftharpoons -COO^- + H_3O^+$$

This hypothesis indicates that sodium ion (Na^+), which favors approach to negatively charged carboxylate group ($-COO^-$) of vesicle, critically perturbates the structure of vesicles, leading to partial twist of the *p*-orbitals.

4.3 Color transition of PPCDA vesicles with α-cyclodextrin

PPCDA/α-CD complex was studied at various concentration of α-CD in order to determine desirable concentrations of α-CD, which was the minimum concentration level affecting to color transition of PPCDA solution by compromising relation between good responses to UV-Vis spectrometry and also observe the color change by visual observation. In this section, colorimetric response of PPCDA was studied by varying α-CD concentrations from 0.5 to 5.0 mM. The colorimetric transition from blue-to-red of PPCDA vesicles (absorbance range of 0.4-0.5) can be induced by inclusion complex of α-CD concentration higher than 3 mM starting at 5 min and reaching the equilibrium within 120 min according to the study of time dependence of color transition of PPCDA/α-CD in section 4.3.1, observed by naked eyes (Figure 4.7). The visual observation of PPCDA solutions within 5 min showed blue color in the concentrations range of 0.5-2 mM α-CD and became light purple at 3 and 4 mM of α-CD. At 5 mM of α-CD, the solutions were extremely changed to intense purple. To observe the color change obviously, the equilibration time according to section 4.3.1

was considered to observe the difference of color change in the series of α-CD concentrations. Accordingly, the mixture solution was standed at room temperature for 120 min as previously observes. The color of PPCDA was blue in the concentration range of 0.5-2 mM of α-CD and became purple and pink at 3 mM α-CD. At 4 mM of α-CD, the solutions were extremely changed to intense purple and pink and obviously changed to red at 5 mM α-CD. The distinct changes of the absorbance at 540 nm (red phase) increases with the expense of the absorbance at 635 nm (blue phase), presented in Figure 4.8.

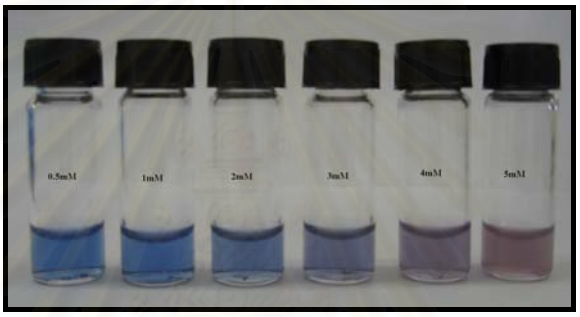


Figure 4.7 Photograph of the color transition of PPCDA vesicles with α-CD (0.5-5 mM) at room temperature.

It is known that the carboxylic head groups of the polydiacetylenes interact with one another by hydrogen bonding. Thus, if there are any cyclodextrins which disturb and stress the hydrogen bonds on the polymer backbone, the effective conjugation length of the polymer would be altered [32]. The α-CD can be able to perturb the ordered structure of the PPCDA liposomes by forming inclusion complex. The conjugated *p*-orbitals undergo distortion by α-CD, leading to partial twist of the *p*-orbitals. Therefore, the intense blue color of the solution gradually shifts to red color.

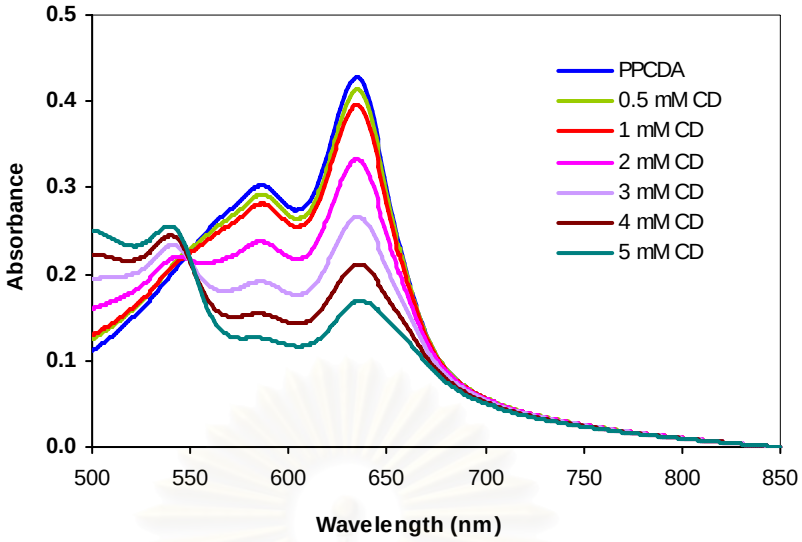


Figure 4.8 Visible spectra of the color transition of PPCDA vesicles with α-CD at room temperature.

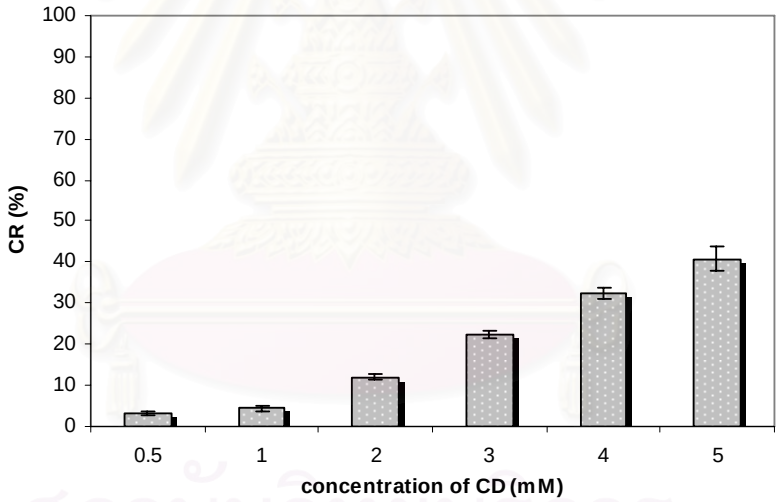


Figure 4.9 Colorimetric response of the color transition of PPCDA vesicles with α-CD at room temperature, response time = 120 min.

In Figure 4.9, the CR(%) values increase with an increase of α-CD concentration. The color transition from blue-to-red started at 2 mM α-CD. The concentration of α-CD more than 5 mM was not suitable for measuring with UV-Vis spectrometry, because the red-colored solution was turbid and also caused the increase of the absorption baseline. Actually, 4 and 5 mM α-CD provided good responses to

color transition of PPCDA, but if higher concentration levels of α-CD were chosen, it might be used higher concentration levels of phenolic compounds to form inclusion complex with α-CD, it was not appropriated for phenolic compounds determination using method of inhibiting PPCDA/α-CD which attempted to find lowest concentration levels that still respond to this method. For the next experiments of inhibition study, 3 mM α-CD was chosen because the minimum concentration level of 3 mM α-CD could be affected to the color transition of PPCDA solution observed obviously by visual observation.

4.3.1 Time dependence of color transition of PPCDA vesicles with α-cyclodextrin

To study the time dependence of the color transition of PPCDA vesicles with α-CD, the CR(%) values were recorded after an addition of α-CD from 5 to 180 min. In this section, 3 mM α-CD was chosen according to the selection of minimum concentration level in section 4.3 in order to study time dependence of the color transition of PPCDA solution. Accordingly, this study might be provided the optimum time response which can be used throughout this work. In this section, the mixture solutions between PPCDA (absorbance range of 0.4-0.5) with 3 mM α-CD was allowed at room temperature. The color of the solution changed from blue to violet starting at 5 min and became very intense purple and pink after 120 min. The absorbances of the solutions were recorded after 5, 15, 30, 60, 90, 120, and 180 min. Figure 4.10 demonstrated the response time of the color change, the CR(%) increased and reached the saturation within 120 min. The solution became turbid after 120 min. The turbidity caused the increase of the absorption baseline (Figure 4.11) and it was not suitable for UV-Vis spectrometric measurement. Thus, the optimum time of 120 min was chosen to equilibrate the mixture solution for the next experiments.

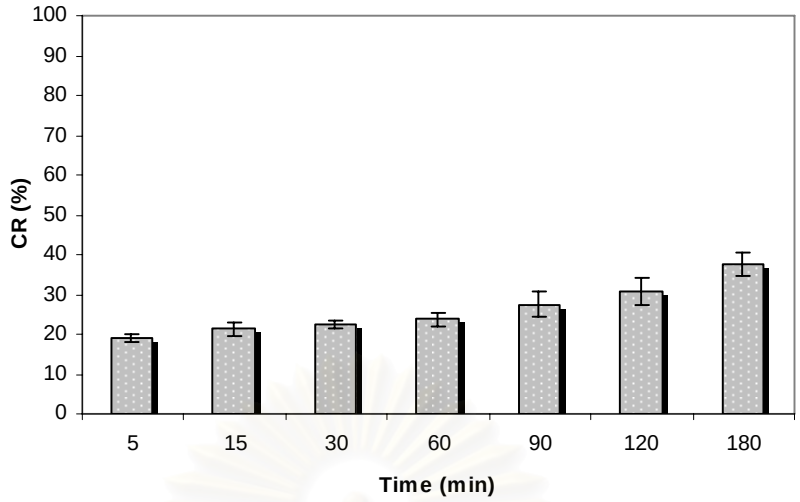


Figure 4.10 Time dependence of the color transition of PPCDA vesicles with 3 mM α-CD at room temperature.

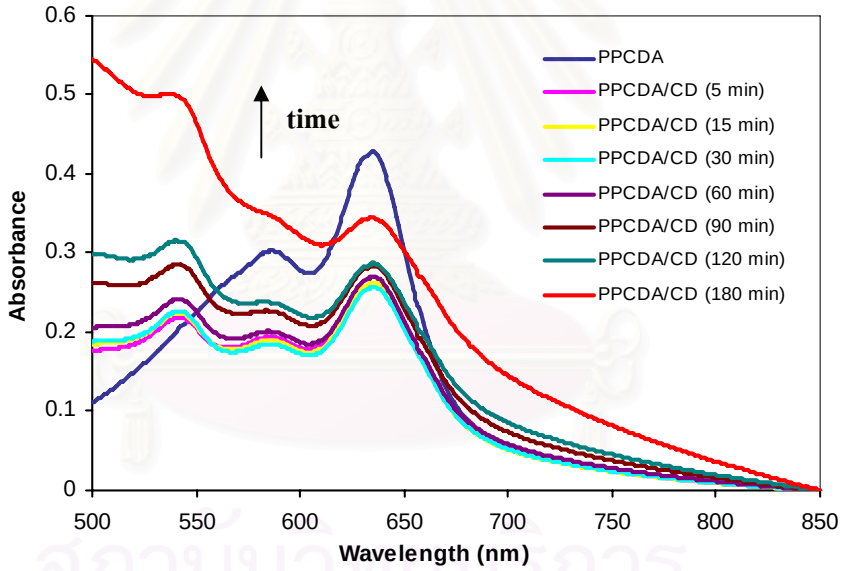


Figure 4.11 Visible spectra of time dependence of the color transition of PPCDA vesicles with α-CD at room temperature.

4.4 Inhibition study

4.4.1 Time dependence of phenolic compounds inhibiting α-cyclodextrin with PPCDA vesicles

The binding ability of α-CD with *p*-nitrophenol was studied by using spectrophotometric measurement, which concluded that α-CD form 1:1 adduct with nitrophenol at acidic and alkaline pH [44]. Thus, nitrophenol and α-CD form a 1:1 complex was supposed to study time dependence of inhibition ability of various phenolic compounds (*i.e. o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol, *p*-methylphenol, *p*-chlorophenol, *p*-bromophenol, hydroquinone, 2,4-dinitrophenol, phenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol), which expected to obtain an optimal time condition for further experiments. In this section, each phenolic compound solution pH was adjusted to acidic pH of 5.8 in order to eliminate the color transition of PPCDA which may occur in the basic pH range according to the previous section. The precipitation of 2,4,5-trichlorophenol and 2,4,6-trichlorophenol solution was observed when the pH was less than 7.0. Therefore, these 2 phenolic compounds would not suitable for the next experiments at pH 5.8. Thus, 9 phenolic compounds were investigated for this study.

In this section, the addition sequence of ternary mixture of α-CD, PPCDA and phenolic compounds was studied, which can be separated into two procedures: (a) pre-equilibrated 3 mM α-CD with 3 mM phenolic compounds by varying time form 5 to 180 min prior to mix with PPCDA (absorbance range of 0.4-0.5) and standed at room temperature for 120 min, (b) pre-equilibrated 3 mM α-CD with PPCDA (absorbance range of 0.4-0.5) for 120 min prior to mix with 3 mM phenolic compounds by varying time from 5 to 180 min. The visual observation after both of two processes were studied and found that procedure (a) showed the difference of color change in every phenolic compound comparing with the color of PPCDA/α-CD and each color solution was also not significantly different by naked eyed detection at different time.

Color of the solution was yellow in the precence of *o*-nitrophenol and 2,4-dinitrophenol, blue of *m*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol, while *p*-methylphenol, hydroquinone, and phenol were purple and pink (Figure 4.13). Time-dependent of phenolic compounds inhibiting PPCDA/α-CD according to procedure (a) was also determined by using UV-Vis spectrometry (Figure 4.15). In contrast, the mixed solution using procedure (b) showed pink color in every phenolic compounds by naked eyes which similar to the color of PPCDA/α-CD, excepted the solution containing *o*-nitrophenol, *p*-nitrophenol, and 2,4-dinitrophenol showed orange (Figure 4.14). Phenolic compounds followed by procedure (b) could not extremely indicated the different of color transition of both detection by naked eyes and UV-Vis spectrometry, which the latter detection found that the colorimertic response, CR(%), of every phenolic compounds were closed to the CR(%) of PPCDA/α-CD.

The results by using procedure (b) strongly support the theory that the majority of PPCDA-based chemosensors were an irreversible fashion. Accordingly, the blue-to-red color change that took place when an external stimulus was applied was not reversed when the external stimulus was removed [45]. The irreversible of PPCDA was probably due to α-CD disrupted the order structures of PPCDA derived from 10,12-pentacosadiynoic acid by forming inclusion complex that directly damaged the hydrogen bond between the carboxylic head groups on PPCDA vesicles surface (Figure 4.12). This phenomenon may causes the occurrences of undesired morphology of PPCDA, which may not recover to the structure as formerly were.

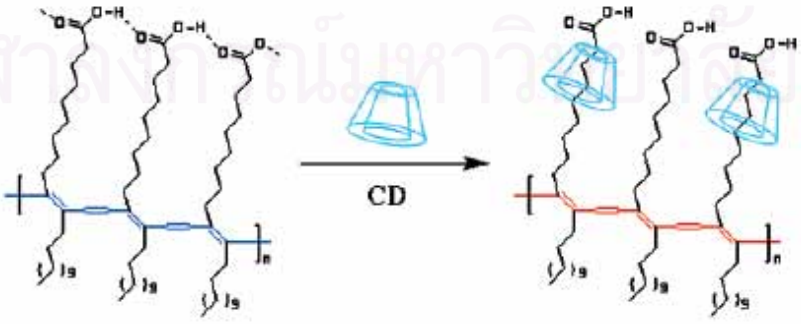


Figure 4.12 A schematic representation of the interaction between PCDA and α-CD [45].

The use of the CR(%) monitoring the color change of PPCDA was unreliable in this case, because PPCDA became totally inefficient chemosensor due to the effect of α-CD disruption. Therefore, procedure (b) was not appropriated to determine the phenolic compounds inhibiting PPCDA/α-CD complex, whereas procedure (a) was greater appropriated to use for phenolic compounds determination. Accordingly, the inhibition study based-procedure (a) was chosen for the next section and throughout this work.

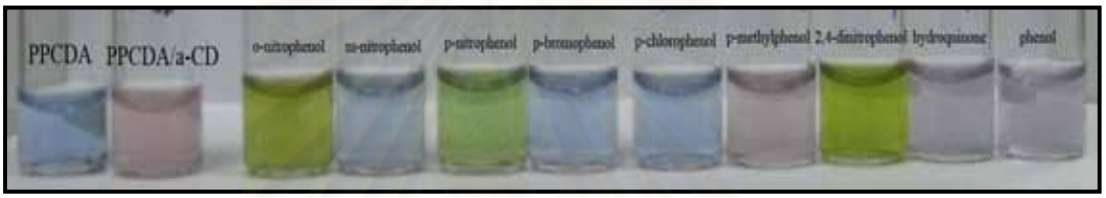


Figure 4.13 Photograph of the color transition of phenolic compounds inhibiting inclusion complex of PPCDA/α-CD at room temperature, procedure (a).

Figure 4.14 Photograph of the color transition of phenolic compounds inhibiting inclusion complex of PPCDA/α-CD at room temperature, procedure (b).

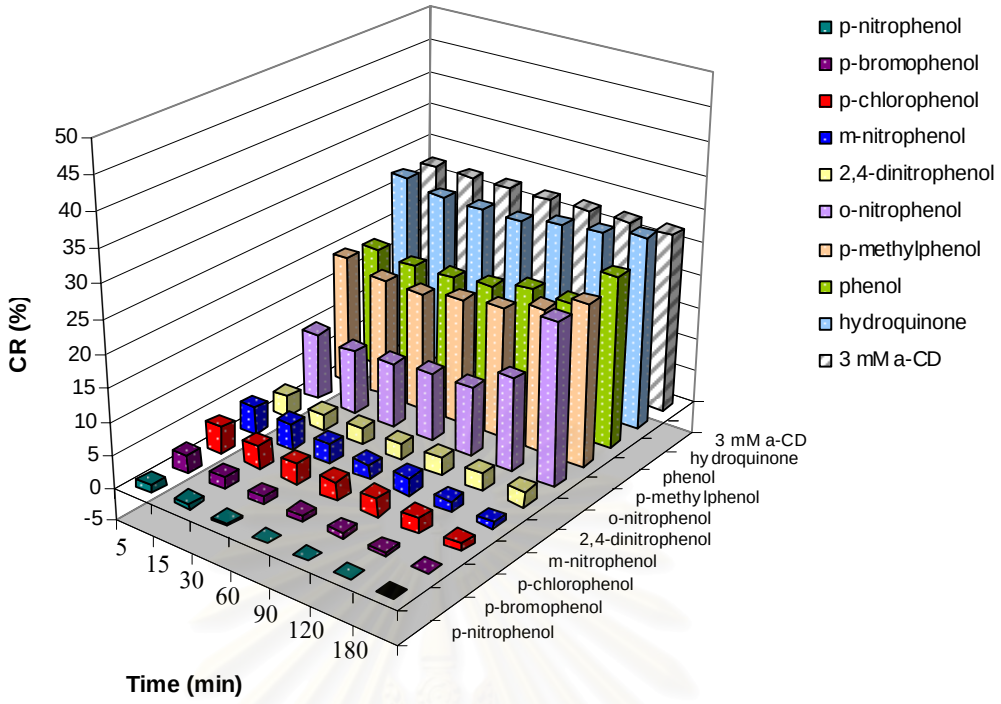


Figure 4.15 Time dependence of phenolic compounds inhibiting α-CD with PPCDA vesicles.

Phenolic compounds determination using their ability to inhibit the α-CD induced color transition by considering the CR(%) should be showed a tendency of decreasing in the series of phenolic compounds concentration, and decreasing of the CR(%) will occur when phenolic compounds have greater inhibition ability to PPCDA/α-CD complexation. Figure 4.15 illustrated the colorimetric response, CR(%), of PPCDA after exposure to various phenolic compounds was compared with CR(%) of PPCDA/α-CD complexation. The complete inhibition of the color transition would occur when the CR(%) was equal to zero. Various phenolic compounds showed different inhibition ability to PPCDA/α-CD complex at different times. For 2,4-dinitrophenol, *o*-nitrophenol, *p*-methylphenol, phenol, and hydroquinone showed no inhibition at all times studied. Only four compounds showed the inhibition ability the α-CD induced color transition *i.e. p*-nitrophenol, *p*-bromophenol, *p*-chlorophenol, and *m*-nitrophenol. In the case of *p*-nitrophenol, it showed excellent inhibition ability to PPCDA/α-CD complex within 15 min by observing the CR(%) closed to zero. This

result was also strongly supports the study of the formation of *p*-nitrophenol-α-CD at low pH by using kinetic measurement, which obtained the recombination rate constant $k_R \geq 4 \times 10^7\ M^{-1}\ sec^{-1}$ [44]. According to the kinetic study, *p*-nitrophenol should exhibit a very fast binding ability to form inclusion complex with α-CD, which related to results in Figure 4.15. The inhibition ability of *p*-bromophenol and *m*-nitrophenol were also observed at 60 and 180 min, respectively. For *p*-chlorophenol, it seemed to exhibit good inhibition ability at higher time response, which showed gradually decreased of the CR(%) when time increased. For these results, the inhibition ability seemed to involve the competition between phenolic compounds and PPCDA vesicles which form inclusion complex with α-CD. In this section, only nitrophenol-α-CD form a 1:1 complex was supposed to study, therefore, the saturation times were partly affected to inhibition ability. Besides the time-dependent of phenolic compounds inhibiting PPCDA/α-CD complex, the effect of phenolic compounds concentration was also considered to monitor inhibition ability at various concentrations. A saturation time of 120 min was expected that most of phenolic compounds would show ability to inhibit the α-CD induced color transition, and saturation time of 120 min was supposed to use to optimize with concentration study in the next section. Thus, saturation time of 120 min for pre-equilibrated of α-CD and phenolic compounds was chosen to study the competition between phenolic compounds and PPCDA vesicles which form inclusion complex with α-CD at various phenolic compounds concentrations.

4.4.2 Effect of phenolic compounds concentrations

The inhibition study of phenolic compounds was investigated after studying of time dependence of phenolic compounds inhibiting α-CD with PPCDA vesicles in previous section. In this study, the procedure of pre-equilibrated α-CD and phenolic compounds prior to mix with PPCDA was considered, the solution of binary mixture

between 3 mM α-CD and various concentrations from 0.5 to 5.0 mM phenolic compounds (*o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol, *p*-methylphenol, *p*-chlorophenol, *p*-bromophenol, hydroquinone, 2,4-dinitrophenol, and phenol) were pre-equilibrated at room temperature for 120 min, according to the chosen time in previous section of time-dependent, prior to add PPCDA (absorbance range of 0.4-0.5) and standed at room temperature for 120 min. After that this ternary mixture was measured by UV-Vis spectrometry.

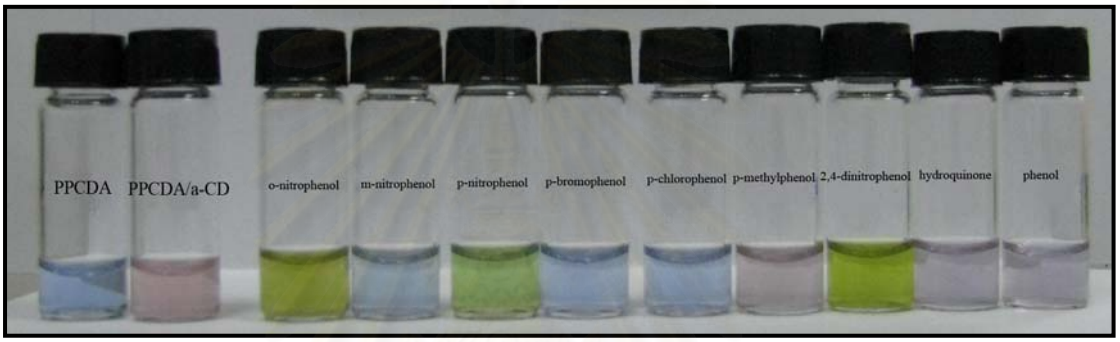
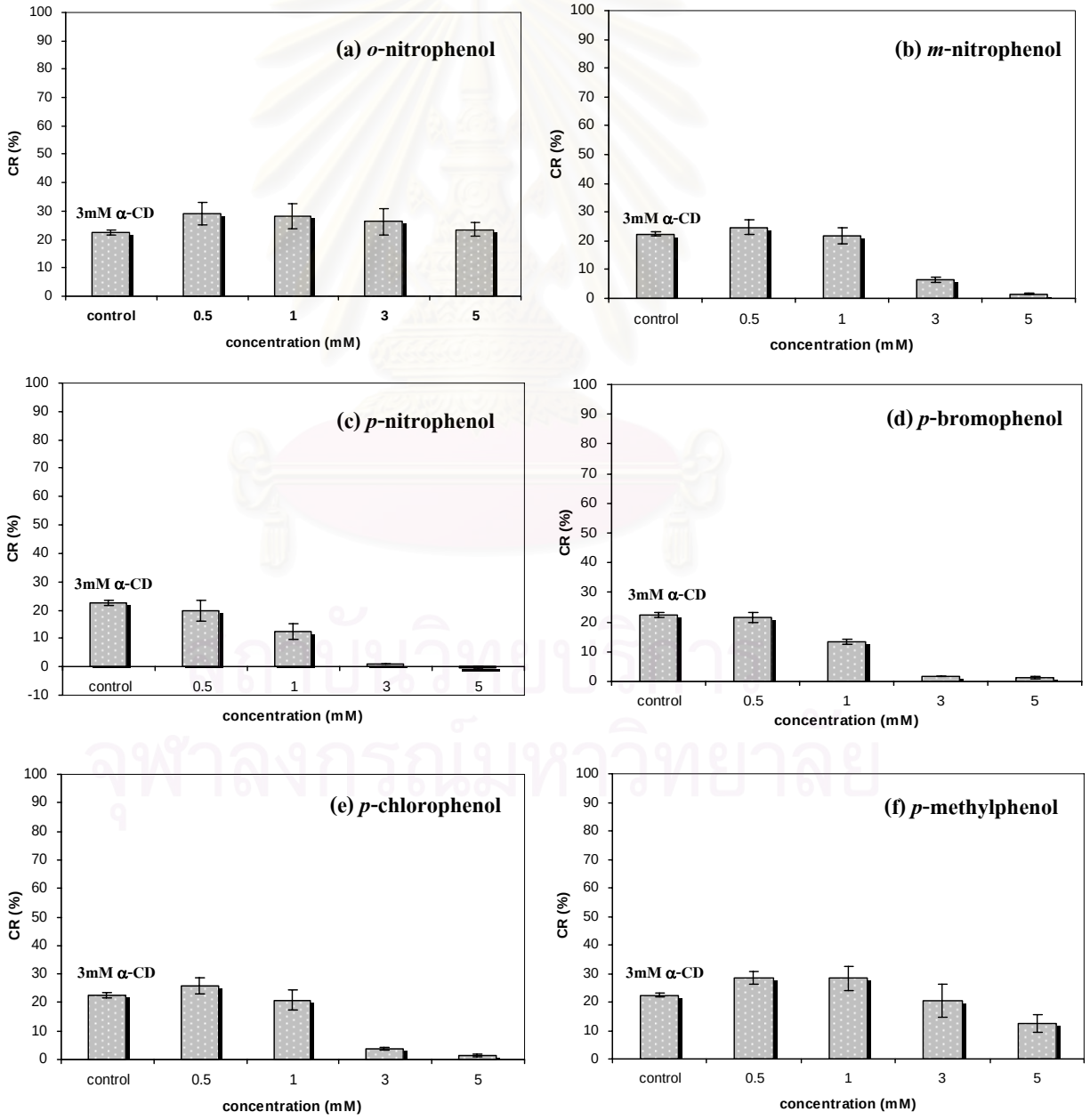


Figure 4.16 Photograph of the color transition of phenolic compounds inhibiting inclusion complex of PPCDA/α-CD at room temperature.

Figure 4.16 demonstrated the color change of PPCDA vesicles solution after adding phenolic compounds at various concentrations, this color change could be observed by naked eyes detection. The color of mixed solution obviously changed in the presence of *o*-nitrophenol, *p*-nitrophenol, 2,4-dinitrophenol, *p*-methylphenol, hydroquinone, and phenol in every concentration ranges of phenolic compounds compared with blue solution of PPCDA, whereas the mixed solution containing *m*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol, showed relatively changed at lower concentration of phenolic compounds, while their color obviously changed at higher concentration of phenolic compounds.

The solution containing 0.5 mM of *o*-nitrophenol, *p*-nitrophenol, and 2,4-dinitrophenol can be clearly observed the color change, which showed brown of

m-nitrophenol, green of *p*-nitrophenol, and yellow of 2,4-dinitrophenol. Interestingly, at the concentration of 3 mM phenolic compounds, the color of *m*-nitrophenol, *p*-bromophenol, *p*-methylphenol, *p*-chlorophenol, hydroquinone, and phenol were significantly different. The color of *p*-methylphenol, hydroquinone, and phenols changed to purple and pink which were similar to the color of PPCDA/α-CD complex. Meanwhile, the color of *m*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol showed no color change and remained in blue solution form which similar to the color of PPCDA.



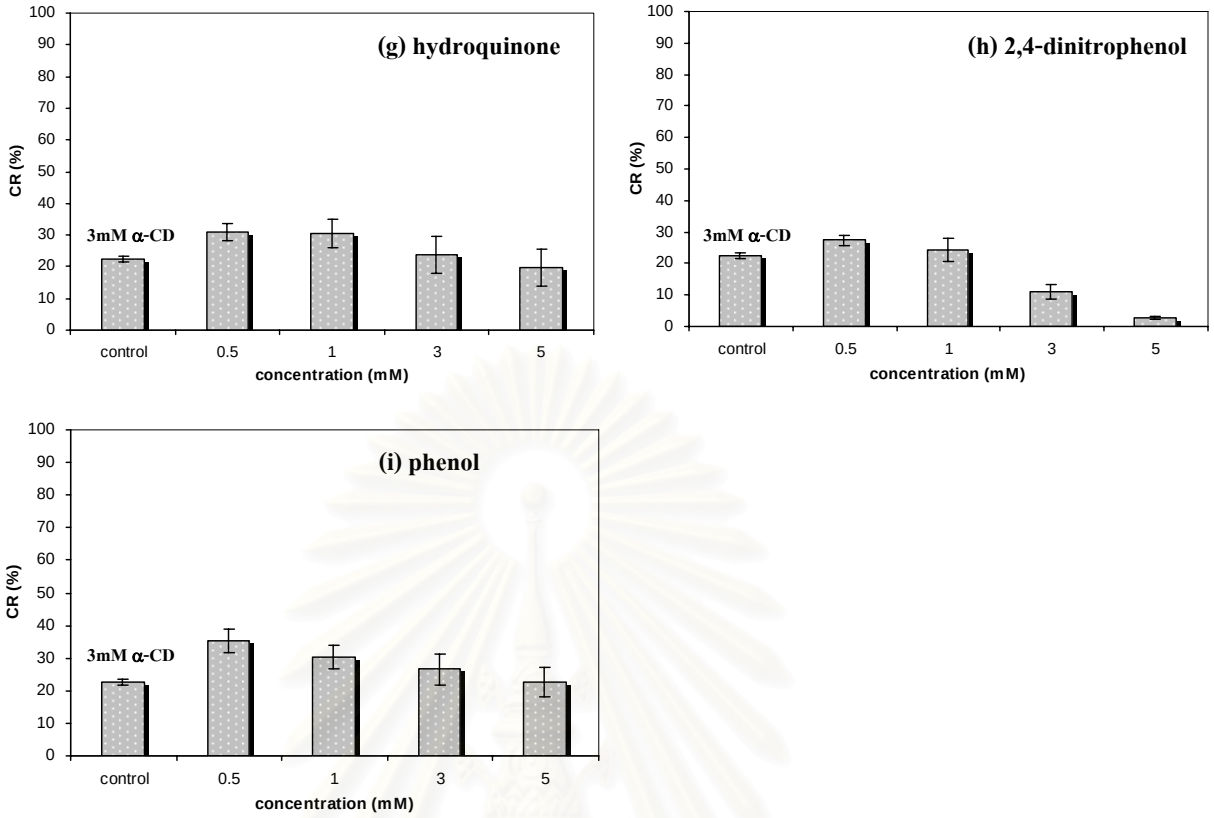


Figure 4.17 The colorimetric response, CR(%), of PPCDA vesicles as a function of different concentrations of phenolic compounds (a) *o*-nitrophenol (b) *m*-nitrophenol (c) *p*-nitrophenol (d) *p*-bromophenol (e) *p*-chlorophenol (f) *p*-methylphenol (g) hydroquinone (h) 2,4-dinitrophenol (i) phenol.

Figure 4.17 showed the colorimetric response, CR(%), of PPCDA after mixing with the phenolic compounds at various concentrations. It was found that *o*-nitrophenol, *p*-methylphenol, hydroquinone, phenol, and 2,4-dinitrophenol showed no inhibition of α-CD induced color transition, indicated by higher value of the CR(%) in every concentration level. Even 2,4-dinitrophenol showed relatively decrease of CR(%) in the series of concentration range, but the complete inhibition ability (CR(%)=0) was not observed and it seemed to exhibit good inhibition at the concentration level higher than 5 mM. However, the complete inhibition of α-CD

induced color transition (CR(%)=0) was observed at different minimum concentrations *i.e.* 3 mM of *p*-nitrophenol and *p*-bromophenol and 5 mM of *p*-chlorophenol and *m*-nitrophenol. Interestingly, for every phenolic compound study, except *p*-nitrophenol, the CR(%) was higher than that of PPCDA/α-CD complex, probably due to the fact that these phenolic compounds could not tightly inserted in α-CD, therefore α-CD remaining in the solution could form inclusion complex with PPCDA by disrupting hydrogen bonding of carboxylic head groups of PPCDA and drastically affected the vesicles deformation. While the sodium ion (Na^+) may be another cause of this turbidity, because Na^+ showed a favorite approach to negatively charged of carboxylic head group of vesicles, critically perturbed the structure of vesicles and leading to partial twist of the *p*-orbital. Thus, probability causes as mentioned leading to aggregation of PPCDA vesicles due to the fact that the lost of surface stability and the occurrence of turbidity was mainly cause of the absorption base line increase and leading to higher value of the CR(%) than usual.

As previously, *p*-nitrophenol, *p*-bromophenol, *p*-chlorophenol and *m*-nitrophenol showed complete inhibition ability to the color transition induced by α-CD. This inhibition process might involve the geometry of phenolic compounds and the binding ability of PPCDA and phenolic compounds with α-CD. In Figure 4.18, the geometry of these compounds was considered, they are suitable for being trapped into the α-CD host cavity. The inhibition ability of the color transition was observed due to the competition between the carboxylic head groups of vesicles and phenolic compounds [32], which are driven by hydrophobic interaction within the inner side of α-CD cavity [46-47]. The results of this inhibition also strongly support the steric of their structure. Thus, the effect of phenolic compounds inhibiting the color transition induced by α-CD due to their low steric bulkiness. Moreover, the hydroxyl groups at the edge of α-CD cavity and molecular dipole moment seemed involved with this inhibition study. In the case of unstable inclusion complex of *o*-nitrophenol (Figure

4.18), may cause from the intramolecular hydrogen bonding between hydroxyl and nitro groups [10].

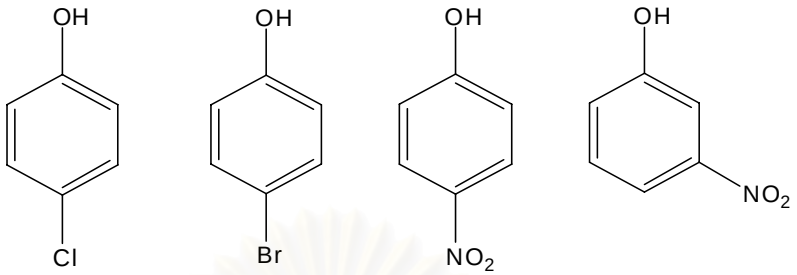


Figure 4.18 The chemical structure of phenolic compounds (a) *p*-chlorophenol (b) *p*-bromophenol (c) *p*-nitrophenol (d) *m*-nitrophenol.

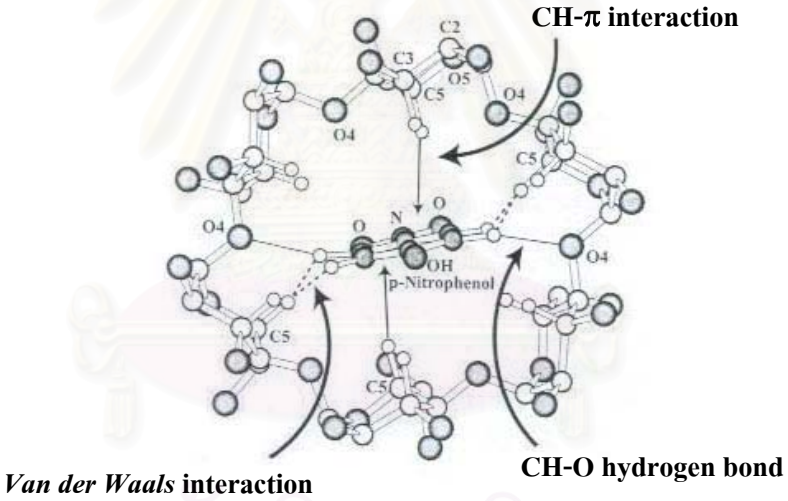


Figure 4.19 Host-guest interaction observed in the α-cyclodextrin complex with *p*-nitrophenol [48].

In the case of *p*-nitrophenol, the inhibition was strongly supported by the short-range reaction within the structure of the crystal of α-CD complex with *p*-nitrophenol, showed in Figure 4.19. The nitrophenyl group is well fitted to α-CD cavity, which is rather elliptical in shape for the accommodation of the planar group. The benzene ring is sandwiched by the two pyranose rings and two C-H bonds point to glycosidic

oxygen atoms to form C-H-O hydrogen bonds. These hydrogen atoms are also in *Van der Waals* contact with hydrogen atoms bonded to C-3 and C-5 of α-CD. The C-H bonds of methine groups, C-3H and C-5H, of the two pyranose rings factoring the benzene ring are perpendicular to the benzene plane, indicating stabilization by C-H-π interaction [48]. Moreover, this well fit of *p*-nitrophenol with α-CD may cause from the intermolecular hydrogen bonding between nitro groups of *p*-nitrophenol and hydroxyl groups at the rim of α-CD. Thus, the inclusion complex of α-CD/*p*-nitrophenol was extremely stable.

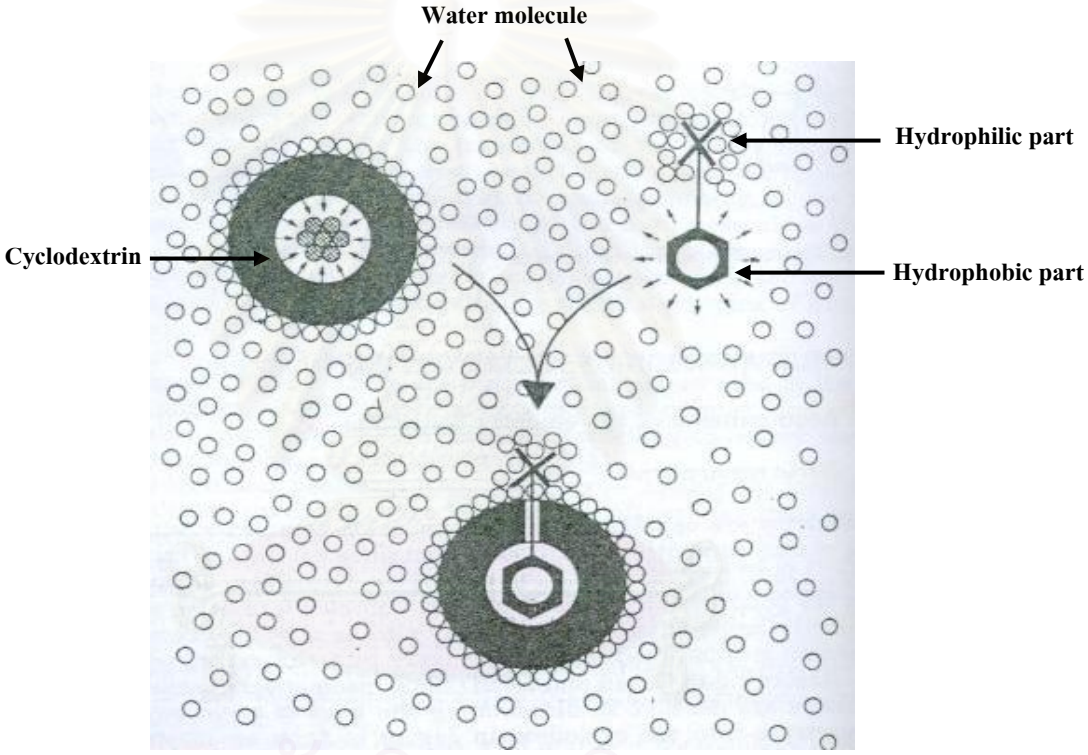


Figure 4.20 Schematic representation of the formation of cyclodextrin inclusion complexes [48].

As mentioned that *p*-methylphenol, hydroquinone, and phenol were not exhibit inhibition ability to PPCDA/α-CD complex, may probably explain by the model in Figure 4.20. As showed in Figure 4.20, the small circle represent water molecules, the large ones is CD ring. The outer surface of CD ring is hydrated, but the water molecules in the cavity are in energetically unfavorable position because of the non-polar surface of the cavity. The hydrophilic part of the guest molecule is highly

hydrated, while the non-polar aromatic rings repulse the water molecules. Thus, the result of the complex formation is that the non-polar part of the guest molecule penetrates into the non-polar cavity of CD. The favorable of this formation is non-polar-non-polar interaction, while the hydrophilic part of the guest molecule outside retains its hydrated shell. In the case of hydroquinone (Figure 4.21), hydroxyl groups of both side of the molecule were probably hydrated and there was no any hydrophobic part interacting with the α-CD cavity as well as phenol (Figure 4.21) molecule which is hydrated at the part of hydroxyl group. *p*-methylphenol (Figure 4.21) was also not performed inhibition ability, probably due to the same situation with *p*-hydroquinone and phenol which a part of hydroxyl group was hydrated. Even another part of *p*-methylphenol was methyl groups which should demonstrate better inhibition ability due to their non-polar part, but they exhibited unstable inhibition ability to PPCDA/α-CD. This behavior seemed to involve dipole-dipole interaction in which *p*-methylphenol has weaker than *p*-nitrophenol, *p*-bromophenol, *p*-chlorophenol, and *m*-nitrophenol, thus *p*-methylphenol did not exhibited a good penetration into α-CD cavity and showed unstable inclusion complex with α-CD. In the case of 2,4-dinitrophenol (Figure 4.21), even one of the position was *para* which seemed to well fit with hydroxyl at the edge of α-CD but if considered another side of *ortho* position, the unstability of this molecule occurred due to the intramolecular hydrogen bonding between hydroxyl and nitro groups.

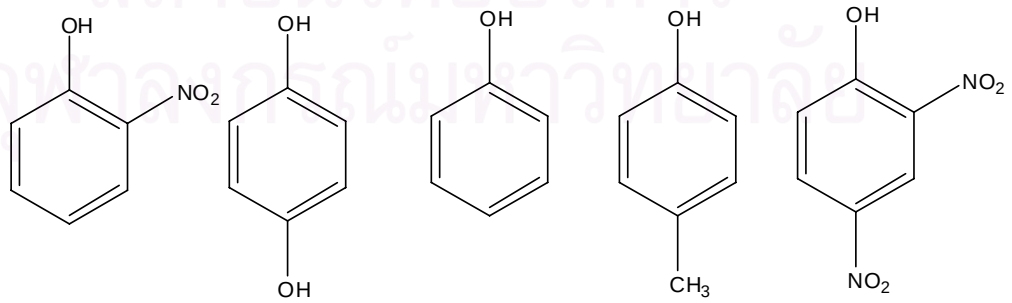


Figure 4.21 The chemical structure of phenolic compounds (a) *o*-nitrophenol (b) hydroquinone (c) phenol (d) *p*-methylphenol (d) 2,4-dinitrophenol.

4.5 Optimization of the plot of phenolic compounds determination

The absorbance of the α-CD induced blue-to-red color transition depends on the increase of the absorbance at 540 nm (red phase) with the expense of the absorbance at 635 nm (blue phase), calculated from the well-defined equation of colorimetric response (CR). However, in this work, CR(%) was not suitable to construct a calibration curve of phenolic compounds determination and also did not provided good relation between CR(%) and the series of phenolic compounds concentrations. Accordingly, the colorimetric response (CR′) was used to quantify remained blue phase which did not form inclusion complex with α-CD. The CR′ would increase when phenolic compounds present and they have good ability in inhibiting the color transition induced by α-CD. The CR′ is expressed as:

$$CR'(\%) = \left(\frac{PR_0 - PR}{PR_0} \right) \times 100 \quad (4.1)$$

Where PR is the red phase percentage, defined as

$$PR = \left(\frac{A_{540}}{A_{635} + A_{540}} \right) \quad (4.2)$$

A_{540} and A_{635} are the absorbances of the red phase at 540 nm and the blue phase at 635 nm in the UV-Vis spectrum, respectively. The initial red phase percentage, PR_0, is determined after exposure to α-CD. And the red phase percentage, PR, is determined in the presence of phenolic compounds.

4.6 Method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. The results from method validation can be used to judge the quality and reliability of analytical results. The validation data for method validation for compound evaluation were linear range, limit of detection, limit of quantitation, accuracy, precision. Their definitions are as follows;

***Linear range*:** The concentration range over which the intensity of the signal obtained is directly proportional to the concentration of the species producing the signal.

***Method detection limit (MDL)*:** The measurement ability of method to determine an analyte in a sample matrix.

***Accuracy*:** The measurement of the exactness of an analytical method, or the closeness of agreement between the measured value and the true value. In this study, relative error (%) and recovery (%) were calculated by equation (4.3) and (4.4), respectively.

$$\text{Error}(\%) = \left[\frac{C - C_{std}}{C_{std}}\right] \times 100 \qquad (4.3)$$

where C = the measured value of phenolic compounds (mM)

C_{std} = the standard value of phenolic compounds (mM)

$$\text{recovery}(\%) = \frac{C}{C_{std}} \times 100 \qquad (4.4)$$

where C = the measured value of phenolic compounds (mM)

C_{std} = the standard value of phenolic compounds (mM)

***Precision*:** The evaluation through repeatability and reproducibility of repetitive measurements of equivalent analyte solution was calculated as relative standard deviation (%) by equation (4.5).

$$RSD(\%) = \frac{SD}{\overline{X}} \times 100 \qquad (4.5)$$

where SD = the standard deviation

$\overline{X}$ = the mean value

The acceptable analyte recovery and RSD at different concentrations are shown in Table 4.1

Table 4.1 Analyte recovery and precision at different concentration [49]

Analyte %	Analyte ratio	Unit	Mean recovery %	RSD %
100	1	100 %	98-102	1.3
10	10^{-1}	10 %	98-102	2.8
1	10^{-2}	1 %	97-103	2.7
0.1	10^{-3}	0.1 %	95-105	3.7
0.01	10^{-4}	100 ppm	90-107	5.3
0.001	10^{-5}	10 ppm	80-110	7.3
0.0001	10^{-6}	1 ppm	80-110	11
0.00001	10^{-7}	100 ppb	80-110	15
0.000001	10^{-8}	10 ppb	60-115	21
0.0000001	10^{-9}	1 ppb	40-120	30

4.6.1 Phenolic compounds determination

In order to determine the concentration of phenolic compounds in water, it was necessary to consider the hypothesis of using phenolic compounds inhibiting PPCDA/α-CD complex in the previous study. This section attempted to construct a calibration curve of individual phenolic compound *i.e. m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol, which exhibited complete inhibition to α-CD induced color transition of PPCDA vesicles.

Phenolic compounds determinations were established with standard solution (pH 5.8) of eleven concentration levels between 0.1 and 3.0 mM. Each concentration level of each phenolic compound was pre-equilibrated with 3 mM α-CD for 120 min prior to mix with PPCDA solution (pH 5.8, absorbance range of 0.4-0.5). The final mixed solutions were standed at room temperature for 120 min prior to measure by UV-Vis spectrometry. The results obtained by applying the plots of the colorimetric response, CR′(%), against phenolic compounds concentration regarding to section 4.5 using absorbance at 540 and 635 nm, are shown in Figure 4.22.

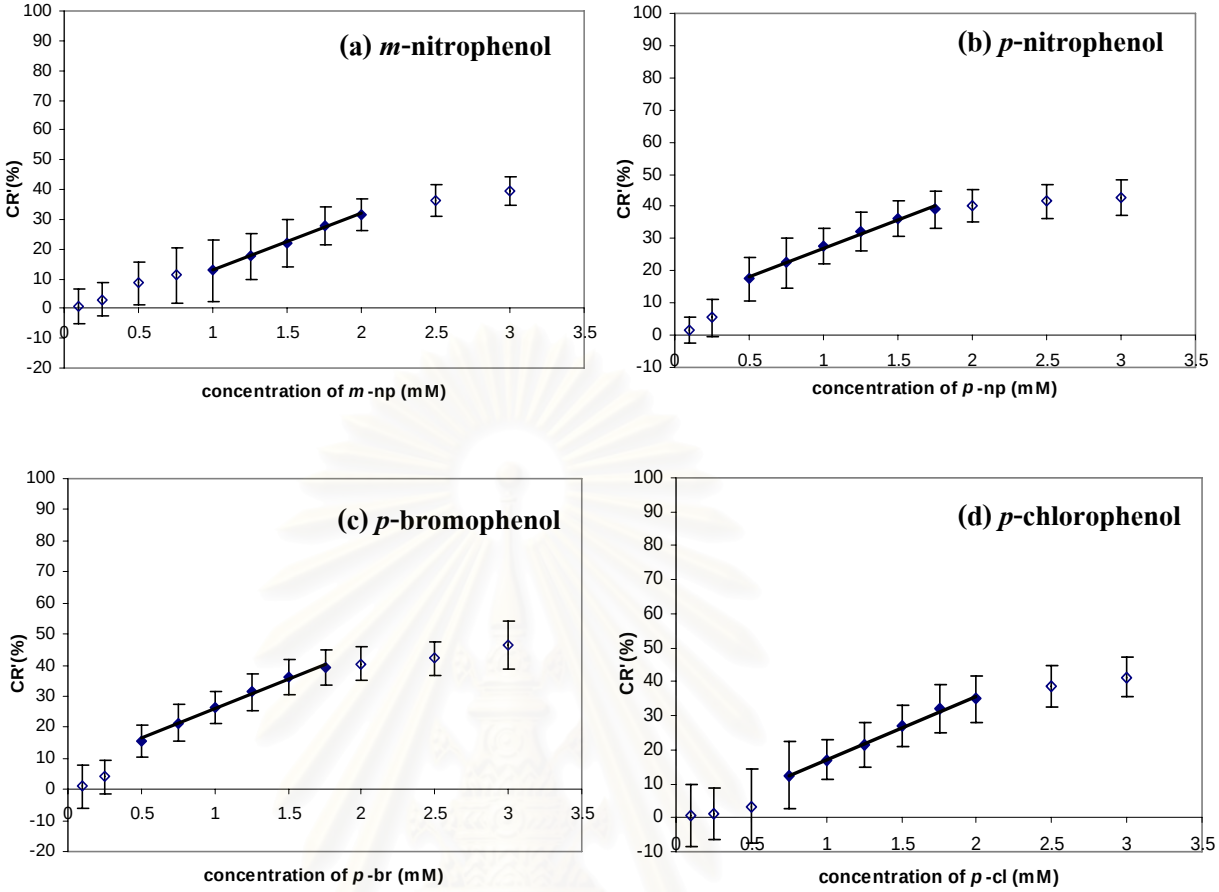


Figure 4.22 Plots of CR′(%) versus the concentration of phenolic compounds (mM). (a) *m*-nitrophenol, (b) *p*-nitrophenol, (c) *p*-bromophenol, (d) *p*-chlorophenol.

Linear ranges of phenolic compounds were observed by inhibiting PPCDA/α-CD complex are shown in Table 4.2.

Table 4.2 Linear ranges of phenolic compounds determination

Phenolic compound	***Equation***	R^2	**Linear range (mM)**
m-nitrophenol	y = 19.242x – 6.5366	0.9970	1.00 – 2.00
p-nitrophenol	y = 17.482x + 9.4878	0.9902	0.50 – 1.75
p-bromophenol	y = 19.125x + 6.8136	0.9913	0.50 – 1.75
p-chlorophenol	y = 18.558x – 1.3763	0.9950	0.75 – 2.00

Linear ranges of *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol showed a very good linearity with R^2 equal to 0.9970, 0.9902, 0.9913, and 0.9950, respectively. Thus, using the plots of colorimetric response, CR′(%), against phenolic compound concentration gave a very good linear relationship.

4.6.2 Method detection limit

Method detection limit of phenolic compounds determination by using inhibiting PPCDA/α-CD complex are shown in Table 4.3.

Table 4.3 Method detection of phenolic compounds determination

Phenolic compound	***mM***	***MDL (mg/L)****
m-nitrophenol	1.00	139
p-nitrophenol	0.50	69
p-bromophenol	0.50	86
p-chlorophenol	0.75	96

*Mean value (n=10)

The results in Table 4.3 showed the lowest concentrations of four phenolic compounds that can be detected by using the method of phenolic compounds inhibiting PPCDA/α-CD complex and measured by UV-Vis spectrometry. The method detection limit of phenolic compounds determination was obtained from the lowest concentration level within linear range (Table 4.2) of calibration curve of each phenolic compound. The method detection limit obtained from Table 4.2 for *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol were 1.0, 0.5, 0.5, and 0.75 mM, respectively. The results in Table 4.3 were calculated by using molecular weight of each phenolic compound to multiply by method detection limit (mM) obtained from Table 4.2.

The method detection limit of *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol were higher than the regulation concentrations for the lowering phenols contents in wastewater when comparing with the US Environmental Protection Agency (EPA) or the Pollution Control Department, Ministry of Natural Resources and Environment of Thailand regulation which is less than 1 mg/L.

4.6.3 Accuracy and precision

The accuracy and precision of phenolic compounds were observed by inhibiting PPCDA/α-CD complex are shown in Table 4.4.

Table 4.4 Accuracy and precision of the inhibition study of phenolic compounds

Sample	***Spiked concentration (mM)***	***Error* (%)***	***RSD* (%)***
m-nitrophenol	1.50	6.35	9.86
	1.75	4.36	5.46
p-nitrophenol	1.50	2.00	1.23
	1.75	0.61	0.66
p-bromophenol	1.50	4.85	2.17
	1.75	7.35	5.51
p-chlorophenol	1.50	1.36	1.77
	1.75	1.14	1.40

*Mean value (n=10)

In this section, accuracy and precision of phenolic compounds determination were performed by spiking standard *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol and *p*-chlorophenol at two concentrations (1.5 and 1.75 mM) in DI water. Each experiment was repeated 10 times. The sample solution (3.0 mL) containing sodium acetate buffer (20 mM, pH 5.8), 3 mM of α-CD, 1.5 and 1.75 mM of each phenolic compound were individually mixed and standed at room temperature for 120 min, and then PPCDA solution (absorbance range of 0.4-0.5) was added into the mixture solutions and allowed at room temperature for 120 min prior to measure by UV-Vis spectrometry. The UV-Vis spectra were collected from 500 to 850 nm with the zero absorbance at

850 nm. The relative error (%) was calculated according to equation (4.3) and the relative standard deviation (%) was calculated according to equation (4.5).

The results in Table 4.4 showed that the unacceptable RSD at both concentration levels of *m*-nitrophenol were obtained and *p*-bromophenol was also found the unacceptable RSD at concentration of 1.75 mM, RSD of this study was compared with acceptable RSD in Table 4.1. However, the relative error values of *p*-bromophenol and *m*-nitrophenol were higher at both concentration levels, probably due to the unstable of inclusion complex which affected to the colorimetric response of *p*-bromophenol and *m*-nitrophenol. Moreover, the turbidity of the solution from aggregation of vesicles due to the lost of their surface stability upon the interaction with α-CD, probably caused the increase of the absorption baseline and affected significantly the colorimetric response value.

4.6.4 Simultaneous determination of phenolic compounds

In this section, simultaneous determination of four phenolic compounds was studied. The solution containing 0.375 mM of *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol was chosen with total phenolic compounds concentration equal to 1.5 mM in order to observe the inhibition ability of quaternary mixture of phenolic compounds compared with 1.5 mM of each phenolic compound which was studied in the previous section of phenolic compounds determination.

In this section, 3 mM of α-CD was mixed with 0.375 mM of each phenolic compound to obtain total final concentration of 1.5 mM. This solution was standed at room temperature for 120 min prior to the addition of PPCDA (absorbance range of 0.4-0.5). The final solution was standed at room temperature for 120 min prior to measure by UV-Vis spectrometry.

Simultaneous determination of four phenolic compounds and *t*-value at 95% confidence are shown in Table 4.5.

Table 4.5 Simultaneous determination of phenolic compounds and *t*-value at 95% confidence

Calibration equation	***Found concentration**** ***(mM)***	***t-value***
m-nitrophenol (y = 19.242x – 6.5366)	2.46 ± 0.19	16.41
p-nitrophenol (y = 17.482x + 9.4878)	1.50 ± 0.11	0.01
p-bromophenol (y = 19.125x + 6.8136)	1.50 ± 0.11	0.04
p-chlorophenol (y = 18.558x – 1.3763)	2.01 ± 0.15	10.60

*Mean value (n=10)

Comparing the *t* statistic to the critical value from the tables (Appendix A) was evaluated to know whether the found concentration was different than 1.5 mM. The critical value of *t* for 9 degree of freedom and 95% confidence is 2.26. If $|t| > t_{crit}$, the null hypothesis will be reject. While the alternative hypothesis will be accept. The results in Table 4.5 showed *t*-value of quaternary mixtures when compared with each phenolic compound. Since *t*-value of *m*-nitrophenol, and *p*-chlorophenol were higher in magnitude than the t_{crit}, thus this null hypothesis was rejected due to the mean of found concentration values were different significantly from 1.5 mM. Whereas *t*-value of using *p*-nitrophenol and *p*-bromophenol equations were lower than the t_{crit}, thus this hypothesis was accepted. Therefore, calibration curve of *p*-nitrophenol and

p-bromophenol were suitable for the determination of total four phenolic compounds in water.

4.7 Real sample

The interested real sample for this work was surface water of the pond of Chulalongkorn University.

The accuracy and precision are shown in Table 4.6.

Table 4.6 Accuracy and precision of the inhibition study of phenolic compounds for determination of phenolic compounds in surface water (n=10)

Spiked analyte	***Added (mM)***	***Found* (mM)***	***Recovery* (%)***	***RSD* (%)***
-	0	nd	-	-
m-nitrophenol	1.5	1.20 ± 0.12	79.94	10.27
p-nitrophenol	1.5	1.42 ± 0.02	94.86	1.26
p-bromophenol	1.5	1.36 ± 0.05	90.43	3.86
p-chlorophenol	1.5	1.36 ± 0.06	90.61	4.30

*Mean value (n=10)

nd = not detectable

The results in Table 4.6 showed the acceptable RSD of three phenolic compounds *i.e. p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol, while *m*-nitrophenol showed both unacceptable RSD and low recovery. And it was found that the CR′(%) of studying in real sample solution was extremely lower than the CR′(%) of standard studying in water, indicated that the blue phase percentage of

PPCDA in real sample decreased, probably due to the interfering ions in surface water which could not eliminated by paper filter and milipore membrane filter and might affect to reduce binding ability of phenolic compounds with α-CD or they might include in α-CD cavity and blocked cavity space for phenolic compounds. In addition, probably due to some interfering ions can disrupt hydrogen bond of carboxylic head group of PPCDA affecting to conformation change which is the most important of color transition of PPCDA, Therefore, aggregation of PPCDA due to the loss of their surface stability gave the lost of blue phase percentage detection and also gave turbidity of the solution to provide lower found concentrations. In contrast, only *p*-nitrophenol showed higher found concentrations, probably due to it has a greater ability and also faster kinetic to form inclusion with α-CD [42]. Thus, it can inhibit α-CD induced color transition of PPCDA and showed higher remaining of blue phase percentage of PPCDA which closed to the CR´(%) of standard studying in water. In order to remove interfering ions in real sample as proposed, it is necessary to use ion chromatography or other ion separation techniques prior to use.

For the next experiments to observe inhibition ability of simultaneous phenolic compounds in surface water, the solution containing 0.0375 mM of *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol was chosen with total phenolic compounds concentration equal to 1.5 mM. The inhibition ability of the mixture was evaluated by comparing with 1.5 mM of each phenolic compounds in previous studied of phenolic compounds determination.

Simultaneous determination of phenolic compounds in surface water and *t*-value at 95% confidence are shown in Table 4.7.

Table 4.7 Simultaneous determination of phenolic compounds in surface water and *t*-value at 95% confidence

Calibration equation	***Found concentration**** ***(mM)***	***t-value***
m-nitrophenol (y = 19.242x – 6.5366)	2.34 ± 0.23	11.37
p-nitrophenol (y = 17.482x + 9.4878)	1.43 ± 0.14	1.61
p-bromophenol (y = 19.125x + 6.8136)	1.43 ± 0.14	1.63
p-chlorophenol (y = 18.558x – 1.3763)	1.91 ± 0.19	6.78

*Mean value (n=10)

According to *t*-value in Table 4.7, $|t|$ of each phenolic compound in surface water were equal to 11.37, 1.61, 1.63, and 6.78 of *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol, respectively. The *t*-value of *m*-nitrophenol and *p*-chlorophenol were higher than 2.26 of t_{crit}. Whereas *t*-value of *p*-nitrophenol and *p*-bromophenol were less than t_{crit}, thus, calibration curve of *p*-nitrophenol and *p*-bromophenol were suitable to the determination of total four phenolic compounds in water.

Previously, the simultaneous determination of phenolic compounds could be done by using the calibration curves of either *p*-nitrophenol or *p*-bromophenol, but they allowed only the determination of total of four phenolic compounds. Accordingly, the simultaneous determination of phenolic compounds in the same sample has a problem of none selectivity. Unfortunately, the proposed method was not appropriate for the determination of individual phenolic compound in a sample containing more than two of those four phenolic compounds.

CHAPTER V

CONCLUSION AND SUGGESTION

5.1 Conclusion

A chemosensor based PPCDA vesicles for phenolic compound detection was investigated by using the principle of competitive inclusion complex between PPCDA vesicles and phenolic compounds with α-cyclodextrin (α-CD) for color transition inhibition. It is thus of interest to extend the investigation to various phenolic compounds *i.e. o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol, *p*-methylphenol, *p*-chlorophenol, *p*-bromophenol, hydroquinone, 2,4-dinitrophenol, and phenol to observe their ability to inhibit the α-CD induced color transition by using both visual observation and colorimetric responses measured by UV-Vis spectrometry. The acidic pH range of 5.8 was chosen to apply the optimum pH condition to the solution throughout this study in order to avoid color transition of PPCDA at basic pH range and precipitated at lower pH range. And the concentration of 3 mM α-CD was chosen to use for phenolic compounds determination. Some complete inhibitions in the detection among various concentrations of phenolic compounds were observed at different minimum concentrations of 3 mM for *p*-nitrophenol and *p*-bromophenol and 5 mM for *p*-chlorophenol and *m*-nitrophenol. Meanwhile, *o*-nitrophenol, *p*-methylphenol, hydroquinone, 2,4-dinitrophenol, and phenol showed no inhibition of α-CD induced color transition. The validation data for determination of *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol showed the linear range of 1.00-2.00 mM for *m*-nitrophenol, 0.50-1.75 mM for *p*-nitrophenol and *p*-bromophenol, and 0.75-2.00 mM for *p*-chlorophenol. This method exhibited a good accuracy for

p-nitrophenol and *p*-chlorophenol. The precision of *p*-nitrophenol and *p*-chlorophenol which calculated as relative standard deviation (%RSD) is less than 2.0% (n=10). The method detection limit was 139, 69, 86, and 96 mg/L for *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol, respectively, using colorimetric responses measured by UV-Vis spectrometry. The proposed method of phenolic compound inhibiting the color transition of PPCDA vesicles induced by α-CD showed a very good potential for the estimation of single *para* substituted phenolic compound in water sample, while there are no reports on this topic that individually identify phenolic compounds using spectrophotometric method. In addition, this proposed method can determine phenolic compounds in water in the visible region of the electromagnetic spectrum to avoid disturbances from absorption band of other aromatic compounds in the ultraviolet region. For this reason, this work beneficially provides a new method to determine single phenolic compounds in water whereas the study of simultaneous determination of phenolic compounds showed a poor selectivity in a sample containing various phenolic compounds, which may also less selectivity when comparing with chromatographic methods.

Although the simultaneous study was not suitable to measure two or more phenolic compounds in the same sample, a spectrophotometric method for resolving ternary mixtures was proposed according to Nevado et al. [50]. Derivative spectrophotometry is an analytical technique of great ability and offers greater selectivity than normal spectrophotometry. This method is based on the simultaneous use of the first derivative of spectra ratio and measurements of zero-crossing wavelengths. The ratio spectrum is obtained by dividing the amplitudes of the absorption spectrum of the mixtures by a standard spectrum of one of the components giving the first derivative of the ratio spectrum. The concentrations of the other components is then determined from their respective calibration graphs established by measuring the ratio derivative analytical signal at the selected zero-crossing points. According to the theory of Nevado et al. [50], it might be used to solve the problem of

none selective in this study in the simultaneous determination of phenolic compounds. The quaternary mixture of *m*-nitrophenol, *p*-nitrophenol, *p*-bromophenol, and *p*-chlorophenol might be determined by using the first derivative of ratio spectra and measurements of zero-crossing wavelengths of one compound, the ratio spectrum is calculated as mentioned earlier. The concentrations of the other three components is then determined from their respective calibration graphs established by measuring the ratio derivative analytical signal at the selected zero-crossing points. However, the zero-crossing of spectrophotometric method seems complicated and be time consuming.

Considering the phenols contents regulation of the US Environmental Protection Agency (EPA) and the Pollution Control Department, Ministry of Natural Resources and Environment of Thailand, they generally reported these contaminants as total phenolic compounds contents. And this simultaneous study which used PPCDA vesicles and α-CD also showed good selectivity for four phenolic compounds as mentioned. Therefore, the simultaneous determination of four phenolic compounds in this work may provide a useful for a preliminary screening test whether there are total of these four phenolic compounds, which are considered as hazardous pollutants, in water by using calibration curve of either *p*-nitrophenol or *p*-bromophenol.

5.2 Suggestion for future work

- This proposed method should be studied further for the matrix compositions of surface water sample and other kinds of waste water.
- Using preconcentration technique such as solid phase extraction or liquid-liquid extraction of phenolic compounds in water prior to determine by PPCDA vesicles with α-CD is recommended.
- A new device for preliminary detection by naked eyes such as the fabrication of polydiacetylene vesicles-embedded thin film in order to reduce a problem of turbidity of the solution at higher concentration level shall be developed.
- This proposed method should be investigated in the trial design of future prospective studies for environmental analysis such as design a new simple test kit for onsite detection of phenolic compounds contaminated in waste water.
- These initial study of phenolic compounds determination using PPCDA vesicles with α-CD point out to a need for further study on more various phenolic compounds which are notified in the regulation of phenols contents of the Pollution Control Department, Ministry of Natural Resources and Environment of Thailand and the US Environmental Protection Agency (EPA).

REFERENCES

[1] Canizares, P.; Carmona, M.; Baraza, O.; Delgado, A.; Rodrigo, M. A. Adsorption equilibrium of phenol onto chemically modified activated carbon F400. Journal of Hazardous Materials 131 (2006): 243-248.

[2] Vasu, A. E. Removal of phenol and *o*-cresol by adsorption onto activated carbon. E-Journal of Chemistry 5 (2008): 224-232.

[3] Banat, F. A.; Al-Bashir, B. Adsorption of phenol by bentonite. Environmental Pollution 107 (2000): 391-398.

[4] Pollution Control Department, Ministry of Natural Resources and Environment, Thailand.
Available from: http://www.pcd.go.th/info_serv/en_reg_std_water04.html#s1 [2009, April 17]

[5] Puig, D.; Barcelo, D. Determination of phenolic compounds in water and waste water. Trends in Analytical Chemistry 15 (8) (1996): 362-375.

[6] Clesceri, L. S.; Greenberg, A. E.; Eaton, A. D. Standard methods for the examination of water and wastewater. American Public Health Association, Washington D.C., 2005.

[7] Ji, E. K.; Ahn, D. J.; Kim, J. M. The fluorescent polydiacetylene liposome. Bulletin of the Korean Chemical Society 24 (2003): 667-670.

[8] Wegner, G. Solid state polymerization mechanisms. Pure and Applied Chemistry 49 (1977): 443-454.

[9] Jelinek, R.; Kolusheva, S. Biomolecular sensing with colorimetric vesicles. Topic in Current Chemistry 277 (2007): 155-180.

[10] Champaiboon, T. Polyelectrolyte multilayer film containing polydiacetylene vesicles for naked eye detection of aromatic compounds. Master's thesis of Science Program in Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University, 2007.

[11] Carpick, R.W.; Sasaki, D. Y.; Burns, A. R. First observation of mechanochromism at the nanometer scale. Langmuir 16 (2000): 1270-1278.

[12] Wenzel, M.; Atkinson, G. H. Chromatic properties of polydiacetylene films. Journal of the American Chemical Society 111 (1989): 6123-6127.

[13] Beckham, H. W.; Rubner, M. F. Structural characterization of the cross-polymerization of a diacetylene-functionalized polyamide. Macromolecules 26 (1993): 5192-5197.

[14] Lio, A.; Reichert, A.; Ahn, D. J.; Nagy, J. O.; Salmeron, M.; Charych, D. H. Molecular imaging of thermochromic carbohydrate-modified polydiacetylene thin films. Langmuir 13 (1997): 6524-6532.

[15] Lee, D. C.; Sahoo, S. K.; Cholli, A. L.; Sandman, D. J. Structural aspects of the thermochromic transition in urethane-substituted polydiacetylenes. Macromolecules 35 (2002): 4347-4355.

[16] Nallicheri, R. A.; Rubner, M. F. Investigations of the mechanochromic behavior of poly(urethane-diacetylene) segmented copolymers. Macromolecules 24 (1991): 517-525.

[17] Jonas, U.; Shah, K.; Norvez, S.; Charych, D. H. Reversible color switching and unusual solution polymerization of hydrazide-modified diacetylene lipids. Journal of the American Chemical Society 121 (1999): 4580-4588.

[18] Reichert, A.; Nagy, J. O.; Spevak, W.; Charych, W. Polydiacetylene liposomes functionalized with sialic acid bind and colorimetrically detect influenza virus. Journal of the American Chemical Society 117 (1995): 829-830.

[19] Song, J.; Cheng, Q.; Kopta, S.; Stevens, R. C. Modulating artificial membrane morphology: pH-induced chromatic transition and nanostructural transformation of a bolaamphiphilic conjugated polymer from blue helical ribbons to red nanofibers. Journal of the American Chemical Society 123 (2001): 3205-3213.

[20] Cheng, Q.; Stevens, R. C. Charge-induced chromatic transition of amino acid-derivatized polydiacetylene liposomes. Langmuir 14 (1998): 1974-1976.

[21] Huo, Q.; Russell, K. C.; Leblanc, R. M. Chromatic studies of a polymerizable diacetylene hydrogen bonding self-assembly: a “self-folding” process to explain the chromatic changes of polydiacetylenes. Langmuir 15 (1999): 3972-3980.

[22] Szejtli, J. Cyclodextrin and their inclusion complexes. Budapest., Hungary., Akademiai kiado budapest., 1982.

[23] Szejtli, J. Past, present, and future of cyclodextrin research. Pure and Applied Chemistry 76 (2004): 1825-1845.

[24] Available from: http://www.lsbu.ac.uk/water/cyclodextrin.htm [2008,October 8]

[25] Dukes, M. D.; Evans, R. O. Impact of agriculture on water quality in the North Carolina Middle Coastal Plain. Journal of Irrigation and Drainage Engineering 132 (3) (2006): 250-262.

[26] Available from: http://www.en.wikipedia.org/wiki/Cyclodextrin [2008, October 8]

[27] Mcnaught, A. D.; Wilkinson, A. Inclusion compound IUPAC Compendium of Chemical Terminology 2nd ed. (the "GoldBook")., Oxford., Blackwell Scientific Publications., 1997
Available from: http://www.iupac.org/goldbook/I02998.html

[28] Baboota, S.; Khanna, R.; Agarwal, S. P. Cyclodextrin in drug delivery systems. Available from: http://www.pharmainfo.net/reviews/cyclodextrins-drug-delivery-systems-update [2008,October 8]

[29] Larrucea, E.; Arellano, A.; Santoyo, S.; Ygartua, P. Study of the complexation behavior of tenoxicam with cyclodextrins insolution: improves solubility and permeability. Drug Development and Industrial Pharmacy 27 (2000): 245-252.

[30] Cyclodextrins in pharmaceuticals: An Overview/ pharmainfo.net
Available from: http://www.pharmainfo.net/pharma-student -magazine/cyclodextrins-pharmaceuticals-overview [2008, October 8]

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

[32] Kim, J. M.; Lee, J. S.; Lee, J. S.; Woo, S. Y.; Ahn, D. J. Unique effects of cyclodextrins on the formation and colorimetric transition of polydiacetylene vesicles. Macromolecular Chemistry and Physics 206 (2005): 2299-2306.

[33] Ahmaruzzaman, M. Adsorption of phenolic compounds on low-cost adsorbents. Advances in Colloid and Interface Science 143 (2008) 48-67.

[34] Solomons, T. W. G.; Fryhle, C. B. Organic Chemistry 7th ed. NewYork., USA., John Wiley and Sons, Inc., 2002.

[35] MATERIAL SAFTY DATA SHEET
Available from: http://msds.chem.ox.ac.uk [2008, October 23]

[36] Nezio, M. S. D.; Pistonesi, M. F.; Fragoso, W. D.; Pontes, M. J. C.; Goicoechea, H. C.; Araujo, M. C. U.; Fernandez, B. S. Successive projections algorithm improving the multivariate simultaneous direct spectrophotpmetric determination of five phenolic compounds in sea water. Microchemical Journal 85 (2007): 194-200.

[37] Available from: https://www.hach.com/fmmimghach?/CODE%3AEX_PHENOLS15824%7C1 [2009, April 6]

[38] Matsue, T.; Fujihira, M.; Osa, T. Cyclic voltammetric determination of *o*-nitrophenol in the presence of *p*-nitrophenol by addition of α-cyclodextrin. Analytical Chemistry 53 (1981): 722-723.

[39] Eftink, M. R.; Harrison, J. C. Calorimetric studies of *p*-nitrophenol binding to α- and β-cyclodextrin. Bioorganic Chemistry 10 (1981): 388-398.

[40] Nuwer, M. J.; O'Dea, J. J.; Osteryoung, J. G. Kinetics and thermodynamics of inclusion of *p*-nitrophenolate with α-cyclodextrin measured with pulse voltammetry. Journal of Physical Chemistry 95 (1991): 10070-10076.

[41] Potisatiyuenyong, A.; Tumcharern, G.; Dubas, S. T.; Sukwattanasinitt, M. Layer-by-layer assembly of intact polydiacetylene vesicles with retained chromic properties. Journal of Colloid and Interface Science 304 (2006): 45-51.

[42] Jelinek, R.; Kolusheva, S. Biomolecular sensing with colorimetric vesicles. Bulletin of the Israel Chemical Society 23 (2008): 6-11.

[43] Kew, S. J.; Hall, E. A. H. pH response of carboxy-terminated colorimertic polydiacetylene vesicles. Analytical Chemistry 78 (2006): 2231-2238.

[44] Cramer, F.; Saenger, W.; Spatz, H. C. Inclusion complex. XIX. The formation of inclusion compounds of α-cyclodextrin in aqueous solutions. Thermodynamics and kinetics. Journal of the American Chemical Society 89 (1967): 14-20.

[45] Lee, S.; Kim, J. M. α-cyclodextrin: A molecule for testing colorimetric reversibility of polydiacetylene supramolecules. Macromolecules 40 (2007): 9201-9204.

[46] Huo, Q.; Russell, K. C.; 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.

[47] Lewis, E. A.; Hansen, L. D. Thermodynamics of binding of guest molecules to α- and β-cyclodextrins. Journal of Chemical Society, Perkin Transactions 2 (1973): 2081-2085.

[48] Dodziuk, H. Cyclodextrin and their inclusion complexes. Germany., WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim., 2006.

[49] Huber, L. Validation and qualification in analytical laboratories. Illinois, Interpharm Press, 1999.

[50] Nevado, J. J. B.; Cabanillas, C. G. Spectrophotometric resolution of ternary mixtures of salicylaldehyde, 3-hydroxybenzaldehyde and 4-hydroxybenzaldehyde by the derivative ratio spectrum-zero crossing method. Talanta 39 (1992): 547-553.

APPENDIX

Appendix A: Significance testing

Table A1: Critical value for the student *t*-tests

Degree of freedom	*t*-value for confidence limit (%)					
	50	80	90	95	99	99.8
1	1.00	3.08	6.31	12.7	63.7	318.0
2	0.82	1.89	2.92	4.30	9.92	22.3
3	0.76	1.64	2.35	3.18	5.84	10.2
4	0.74	1.53	2.13	2.78	4.60	7.17
5	0.73	1.48	2.02	2.57	4.03	5.89
6	0.72	1.44	1.94	2.45	3.71	5.21
7	0.71	1.42	1.90	2.36	3.50	4.78
8	0.71	1.40	1.86	2.31	3.36	4.50
9	0.70	1.38	1.83	2.26	3.25	4.30
10	0.70	1.37	1.81	2.23	3.17	4.14
12	0.70	1.36	1.78	2.18	3.06	3.93
15	0.69	1.34	1.75	2.13	2.95	3.73
20	0.69	1.32	1.72	2.09	2.84	3.55
30	0.68	1.31	1.70	2.04	2.75	3.38
60	0.68	1.30	1.67	2.00	2.66	3.23
α	0.67	1.29	1.64	1.96	2.58	3.09

VITA

Miss Pimpimon Anekthirakun was born on July 18, 1983 in Khonkaen, Thailand. She received a Bachelor's degree of Science in Chemistry from Mahidol University in 2006. Subsequently, she has been a graduate student at the Department of Chemistry Chulalongkorn University and a member of Environmental Analysis Research Unit (EARU). She finished her postgraduate study with a Master's degree of Science in Chemistry in 2009. Her present address is 451 Srisawatdumnern Road, Talad Sub-district, Muang District, Mahasarakham, Thailand, 44000. Contact number is 084-0995209. Email: pimpimon_a@hotmail.com.