# การเตรียมและสัณฐานวิทยาของพอลิไดอะเซทิลีนจากการจัดเรียงตัวเองของไดอะเซทิลีนลิปิด

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ปีการศึกษา 2550

# PREPARATION AND MORPHOLOGY OF POLYDIACETYLENE FROM SELF-ASSEMBLY OF DIACETYLENE LIPIDS

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

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|---|---|
| Thesis Title | PREPARATION AND MORPHOLOGY OF POLYDIACETYLENE FROM SELF-ASSEMBLY OF DIACETYLENE LIPIDS |
| By | Miss Sansanee Boonchit |
| Field of Study | Petrochemistry and Polymer Science |
| Thesis Advisor | Anawat Ajavakom, Ph.D. |
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Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

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สรรสนีย์ บุญชิต : การเตรียมและสัณฐานวิทยาของพอลิไดอะเซทิลีนจากการจัดเรียงตัวเองของไดอะเซทิลีนลิปิด. (PREPARATION AND MORPHOLOGY OF POLYDIACETYLENE FROM SELF-ASSEMBLY OF DIACETYLENE LIPIDS) อ.ที่ปรึกษา: อ.ดร.อนวัช อาชวาคม, อ.ที่ปรึกษาร่วม: รศ.ดร.มงคล สุขวัฒนาสินิทธิ์, 75 หน้า.

ได้ศึกษาสมบัติการจัดเรียงตัวได้เองของพอลิไดอะเซทิลีนเวสิเคิลที่เตรียมจาก 10,12-เพนตะโคซะไดไอโนอิกแอซิด (PCDA) และอนุพันธ์ของไดอะเซทิลีนที่มีหมู่เอไมด์ ซึ่งวิเคราะห์การจัดเรียงตัวได้เองจากเทคนิค dynamic light scattering (DLS), atomic force microscopy (AFM) and transmission electron microscopy (TEM) ที่ได้จากปฏิกิริยา ควบแน่นระหว่าง PCDA กับ เอทิลีนไดเอมีน 5 และ 0.5 อิควิวาแลนท์ เกิดเป็นอนุพันธ์ไมโนเอไมด์ (AEPCDA) และ ไดเอไมด์ (EBPCDA) โดยมีวัตถุประสงค์ที่จะศึกษาภาวะที่เหมาะสมต่างๆในการเตรียมได้แก่ pH การเติมโซเดียมคลอไรด์ที่ความเข้มข้นต่างๆกัน และความเข้มข้นของลิปิดในน้ำของ poly(PCDA) และอนุพันธ์ สำหรับ pH ที่สภาวะเริ่มต้นที่เหมาะสมต่อการเตรียม poly(PCDA) นาโนเวสิเคิล คือ pH 5-7 และ pH 3 สำหรับ poly(AEPCDA) ผลของการเติมเกลือโซเดียมคลอไรด์ที่ความเข้มข้นแตกต่างกันจะไม่มีผลต่อ poly(PCDA) และอนุพันธ์ เพียงแต่สามารถรายงานค่าความเข้มข้นสูงสุดเพื่อใช้สำหรับการเตรียมเมมเบรนทางชีวภาพ สำหรับการศึกษาปัจจัยต่างๆของ poly(EBPCDA) จะไม่ส่งผลต่อการเตรียมเนื่องจากมีโครงสร้างที่เสถียรและจัดเรียงตัวแน่นชิดติดกัน ซึ่งยากต่อการรบกวนจากสิ่งเร้าภายนอก หลังจากนั้นได้ทำการศึกษาคุณสมบัติการเปลี่ยนสีที่อุณหภูมิต่างๆด้วยเทคนิคยูวีวิสิเบิลสเปคโตรสโคปีที่ควบคุมอุณหภูมิ พบว่าขนาดของอนุภาคสัมพันธ์กับอุณหภูมิการเปลี่ยนสี โดย poly(PCDA) ที่มีขนาดอนุภาคนาโนเวสิเคิลที่ pH เริ่มต้น 5-7 จะมีช่วงอุณหภูมิการเปลี่ยนสีต่ำกว่า pH อื่นๆ ในทางกลับกัน poly(AEPCDA) pH 3 ที่มีขนาดอนุภาคนาโนเวสิเคิล จะมีช่วงอุณหภูมิการเปลี่ยนสีที่สูงกว่า pH อื่นๆ เนื่องจากผลของประจุที่หมู่หัวได้รับโปรตอนหรือสูญเสียโปรตอนเมื่อสิ่งแวดล้อมแตกต่างกันในแต่ละโมโนเมอร์ ยิ่งไปกว่านั้นการเปลี่ยนสีของ poly(EBPCDA) จะแสดงสมบัติการผันกลับได้ดีเยี่ยมที่ pH ภาวะเริ่มต้น 5-7 ขณะที่ poly(PCDA) และ poly(AEPCDA) จะไม่สามารถแสดงสมบัติการผันกลับได้ จากการศึกษาภาวะที่เหมาะสมต่างๆ จะสามารถนำไปประยุกต์ใช้สำหรับการตรวจจับสารชีวโมเลกุลและการเปลี่ยนสีตามอุณหภูมิ

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต.....
ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

## 4872498623: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEY WORD: POLYDIACETYLENE/ SELF-ASSEMBLY/ NANOVESICLES

SANSANEE BOONCHIT: PREPARATION AND MORPHOLOGY OF POLYDIACETYLENE FROM SELF-ASSEMBLY OF DIACETYLENE LIPIDS. THESIS ADVISOR: ANAWAT AJAVAKOM, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. MONGKOL SUKWATTANASINITT, Ph.D., 75 pp.

Self–assembly of polydiacetylenes derived from vesicles of 10,12-pentacosadiynoic acid (PCDA) and its amide derivatives were investigated by using dynamic light scattering (DLS), atomic force microscopy (AFM) and transmission electron microscopy (TEM). The condensation reaction of PCDA with 5 and 0.5 equivalents, ethylenediamine yielded the corresponding monoamide (AEPCDA) and diamide (EBPCDA). The purpose of this investigation was to find the optimum conditions by varying conditions such as pH, ionic strength (NaCl) and concentration of lipids in Milli Q water. The optimum pH for preparing the nanovesicles (<200 nm) was pH 5-7 for poly(PCDA) and pH 3 for poly(AEPCDA). For ionic strength, there was no significant difference of effect of various NaCl concentration in poly(PCDA) and its amide derivatives, so the required NaCl concentration for biomembrane preparation would be in a range of 0.1 mM – 1 mM NaCl. The various conditions for poly(EBPCDA) were ineffective because the stable and closed pack structure of poly(EBPCDA) to the external perturbations was tolerant. The study of thermochromism properties were also investigated by colorimetric method using a temperature controlled UV-Vis spectrometer. Accordingly the results indicate that the particle size had a close relation to the transition temperature. In poly(PCDA) at initial pH 5-7 nanovesicles had lower transition temperature than others. In contrast, poly(AEPCDA) at pH 3 nanovesicle had higher transition temperature than other, probably due to the effect of charged at headgroup that was protonated or deprotonated in difference environment of each monomer. Moreover, the color transition of poly(EBPCDA) exhibited excellent reversibility at initial pH 5-7 while those of poly(PCDA) and poly(AEPCDA) were irreversible. Those optimum conditions would become the useful applicational source for biosensors and thermochromic devices.

Field of Study: Petrochemistry and Polymer Science Student's Signature: Sansanee Boonchit
Academic Year: 2007 Advisor's Signature: A. Ajavakom
Co-Advisor's Signature: Mongkol Sukwattanasinitt

# ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Anawat Ajavakom, Ph.D and my Co-Advisor Associate Professor Mongkol Sukwattanasinitt, Ph.D. for his invaluable guidance, excellent and kind supervision, and helpful suggestion throughout this research. This thesis research would not be completed without his advice and guidance.

My appreciation is also given to Professor Pattarapan Prasassarakich, Ph.D., and Associate Professor Wimonrat Trakarnpruk, Ph.D., thesis defense committee, for their kind attention, valuable suggestion and recommendations. I also would like to thank Associate Professor Tirayut Vilaivan, Ph.D. for permission to use UV-Vis spectrophotometer throughout this research.

I would like to thank Gamolwan Tumcharern, Ph.D., thesis defense committee from National Nanotechnology Center (NANOTEC) for the nanosizer (DLS) and AFM data and suggestions.

Furthermore, I gratefully thank to my friends at dormitory for their genuine, help and friendship and everyone in MS-group, especially Anupat, Chantana and Jasuma for their educated, suggestion and big help in everything. Moreover, I express my appreciation to Theodtoon, Sulaleewan, Suriyakamol, Nakorn, Thirawat, Warathip and Chaiwat for spirit, smile, good wish and their helps in everything.

Finally, I would like to express thankfulness to my family for their love, care, encouragement and support throughout my study.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| PDA | Polydiacetylene |
| PCDA | 10,12-pentacosadiynoic acid |
| AEPCDA | *N*-(2-aminoethyl)pentacosa-10,12-diynamide |
| EBPCDA | *N,N'*-ethylenebispentacosa-10,12-diynamide |
| CTT | Color transition temperature |
| CR | Colorimetric response |
| NMR | Nuclear magnetic resonance spectroscopy |
| AFM | Atomic force microscopy |
| TEM | Transmission electron microscopy |
| DLS | Dynamic light scattering |
| °C | Degree celsius |
| g | Gram |
| mL | Millilitre |
| mM | Millimolar |
| nm | Nanometre |
| min | Minute |
| % | Percent |
| rpm | Round per minute |

# CHAPTER I

# INTRODUCTION AND THEORY

## 1.1 Overview

The developments of conjugated polymers as sensing materials have recently gained much attention, especially for polydiacetylenes (PDAs) due to its unique chromic properties. PDA is an ene-yne conjugated polymer resulted from topopolymerization of DA monomers *via* 1,4-addition reaction initiated by heat, UV or γ-irradiation. The apparent color and optical absorption of PDAs associate with a π-to-$\pi^*$ transition of electrons within the π-conjugated polymer backbone. Several PDAs used as sensing materials change color drastically from blue to red under external perturbation such as temperature, pH, solvent, mechanical stress and ligand-receptor interactions [1-8]. This color transition of PDAs can be either reversible or irreversible and is usually measured experimentally as a shift of the absorption band from low to high energy in the visible spectrum [9-11].

Topopolymerization of DA monomers requires the molecules of monomer to pack in a very ordered state such as crystals, self assembled films and lipid bilayer vesicles. Diacetylene lipids have recently emerged as one of the most studied class of monomers because they can form nano-structured vesicles uniformly dispersed in an aqueous medium. One of the most commonly studied DA is 10,12-pentacosadiynoic acid (PCDA) and its derivertives. PCDA is amphiphilic containing hydrophilic carboxylic head group and long hydrophobic hydrocarbon tail. In acidic aqueous solution, PCDA molecules can self assemble in the form of lipid bilayer vesicles that can be readily polymerized efficiently by UV-irradiation. The resulting nanospherical vesicles or liposomes of poly(PCDA) homogeneously disperse in aqueous media appeared as a blue solution that allow itself into several successful development of colorimetric biosensors [12-15]. Although the applications of poly(PCDA) have been extensively studied, there are only limited information available on the relationships of the preparation conditions and the physicochemical properties of the resulting vesicles.

In self-assembly, the individual components contain enough information in themselves to build a template for a structure composed of multiple units. The construction of a monolayer, in which a single layer of closely-packed molecules sticks to a surface in an orderly and closely-packed fashion, is an example of it so often called self-assembly monolayer (SAM). Self-assembly should not be confused with positional assembly, a technique that has been suggested as a means to build objects, devices and systems on a molecular scale using automated processes in which the components that carry out the construction process would follow programmed paths [16].

The self-assembly of organic molecules with the assistance of noncovalent forces, such as hydrogen bonding, electrostatic interactions and π-stacking, provides and efficient method for creating nanoscopic and mesoscopic structures. The self-assembly of a conjugated polymer for forming different morphology and particle size is currently attracting considerable attention, and controlled morphology of conjugated polymers is of particular interest for potential applications, such as biosensor, chemical sensors, electron transfer, solar energy conversion and molecular devices. The development of a simple method is based on the associated effect that is self-polymerization and self-assembly of noncovalent intermolecular interaction of π-π stacking and hydrogen bonding interaction for construction of polymer nanoscale supramolecular materials. Two key conditions are required for the direct formation of polymer aggregate nanostructures by the mentioned associated effect: (I) the monomer is able to be self-polymerized by UV light. (II) the self-polymerization polymer could be self-assembled by noncovalent forces [17].

In this work, the experiments were designed to present the effects of preparation conditions, *i.e.* pH and ionic strength of the media and the concentration of the lipids, on the morphology and size of assembly of PCDA and its derivatives. The modification of the carboxylic head group of PCDA should cause significant change in the orientation and strength of the hydrogen bonds between the head groups. Two amide derivatives *N*-(2-aminoethyl)pentacosa-10,12-diynamide (AEPCDA) and *N*,*N*′-(ethan-1,2-diyl)bispentacosa-10,12-diynamide (EBPCDA) are derived from the condensation of ethylenediamine with one and two equivalents of PCDA, respectively. These amide diacetylenes may be polymerized to form PDA vesicles possessing. Thermochromic responses of the prepared PDAs will also be investigated to gain insight the relationship between the assembly parameters and the transition temperature.

## 1.2 Theory

### 1.2.1 Self-assembly of lipids

Spontaneous assembly of lipids is mainly driven by the hydrophobic effect organizing amphiphilic lipid molecules so as to minimize entropically unfavorable interactions between hydrophobic acyl chains with surrounding water and is further fine-tuned by various intermolecular forces such as electrostatic interactions, hydrogen bonding, as well as van der Waals and dispersion forces. Lipid packing into small scale 3-D structures is governed by effective shape of the lipid that is determined by van der Waals volume and geometry of the molecule as well as hydration shell, conformation, and intermolecular forces acting on a molecule. This relationship is generally described by packing parameter $p$ defined as

$$p = v/al$$

where $v$ is the volume of hydrocarbon chain(s), $a$ is the area occupied by headgroup, and $l$ is the maximum length of hydrocarbon chains. Accordingly, conventional spherical micelles are formed when molecule preferentially adopts conical shape, i.e. $p < 1/3$, and non-spherical (e.g. rod-like or discoidal) micelles form when molecular geometry resembles truncated cone with $1/3 < p < 1/2$ (Figure 1.1). Alternatively, very large headgroup area compared to area occupied by acyl chains can drive bilayer into chain interdigitated phase as the energetic penalty of exposing acyl chains to aqueous phase is counterbalanced by increased separation of bulky headgroups. Biologically most important mode of lipid packing, lipid bilayer, requires nearly cylindrical molecular shape with $1/2 < p < 1$. If $p > 1$, inverted structures with negative spontaneous curvature (e.g. inverted micelles or inverted hexagonal phase) can be formed [18].

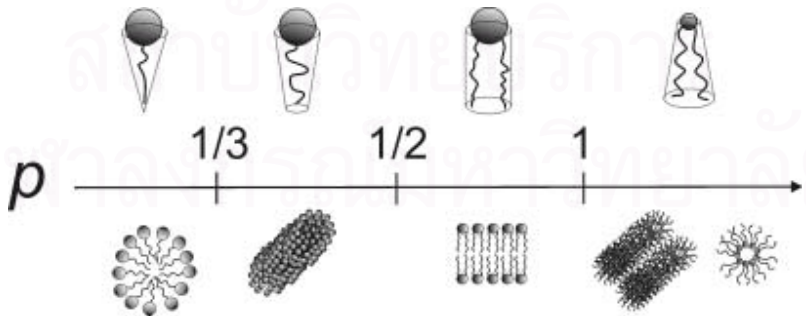



**Figure 1.1** Schematic illustration of the impact of packing parameter ($p$) on lipid assemblies formed in aqueous solution.

### 1.2.2 Polydiacetylene self-assembly

Diacetylene polymerization occurs only when it is in a highly ordered state (i.e. topochemical or solid-state polymerization). It requires an optimal packing of the diacetylene units to allow propagation of the linear chain through the organized phase. When the system is extremely well-ordered and extent of polymerization is high, the potential for application is enhanced because polymerization does not alter the morphology. The packing of monomers are determined by the side groups attached to the diacetylene units, thus it can be tailored by choosing the appropriate substituents or head groups. The key structural factors for a diacetylene lipid system include the total chain length, the position of the diacetylene unit within the chain and the chemical nature of the head group. Interactions between neighboring molecules *via* head groups are especially important for the propagation of induced with one-dimensional ordered nanostructures. When diacetylene polymerization occurs in the middle of an alkyl chain, highly arranged self-assembled systems in which the diacetylene groups are aligned as a band form. Unlike polydiacetylene itself, these materials are rather tractable, especially if a linear superstructure is required. At various one-dimensional ordered nanostructures have been constructed from the polymerization of diacetylene in the past decade such as nanorods, nanowires, nanoplanes, helical ribbons and vesicles. The general explanations suggested for the driving force of the self-assembly and the mechanisms of the aggregation process are hydrogen bonding, hydrophobic interactions and π-π stacking [19].

### 1.2.3 Polydiacetylene vesicles

Polydiacetylene (PDA) forms a unique class of polymeric material that couple highly aligned conjugated backbone with tailorable pendent side groups and terminal functionalities. PDA is conjugated polymers resulted from topopolymerization of diacetylene monomers *via* 1,4-addition reaction to form alternating ene-yne polymer chains (Figure 1.2) upon heat, irradiation with light or γ-irradiation [20].

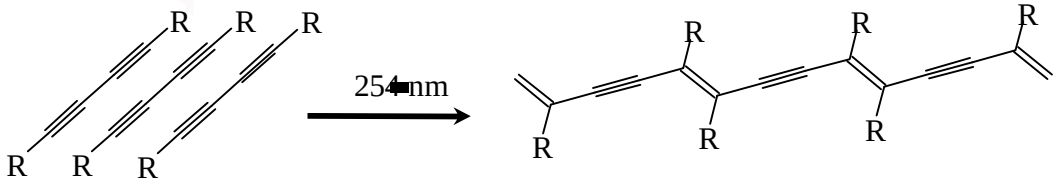



**Figure 1.2** Polymerization of diacetylene monomers by irradiation with UV light.

The topopolymerized diacetylene crystals are nearly perfectly ordered crystals which can not be occurred by solution polymerization or recrystallization of a preformed polymer from solution or melt [10, 21]. The resulting PDA, if generated under optimized conditions appears as an intense blue-colored PDA. PDA can change color from blue to red, having the maximum absorption peak at 630 nm and 540 nm in the blue and red form, respectively, under external purtubations such as temperature, pH, solvent, mechanical stress and ligand-receptor interactions [1-8] due to the reduction of effective conjugation length resulted from strain and torsion imposed onto the backbone induced by order-disorder transitions in the side chains [22-23]. Owing to these color changing properties, PDA-based sensors have been prepared in a wide range of organized structures such as single crystals, thin films on solid supports using Langmuir-Blodgett or Langmuir-Schaefer techniques, self-assembled monolayers, liposome or vesicles in water.

Diacetylene lipid acids are known to spontaneously arrange into vesicle structure in aqueous media which can be further photopolymerized by UV light to provide spherical nanostructure of polydiacetylene vesicles. One of the most commonly used lipid monomer for preparation of vesicles is 10,12-pentacosadiynoic acid (PCDA). PCDA monomers have carboxylic group that can dissociate in water and make these monomers hydrophilic but long hydrocarbon chain make these monomers hydrophobic. PCDA monomer can thus assemble in the form of lipid bilayer vesicles in water and can be polymerized by irradiation with UV light (Figure 1.3).

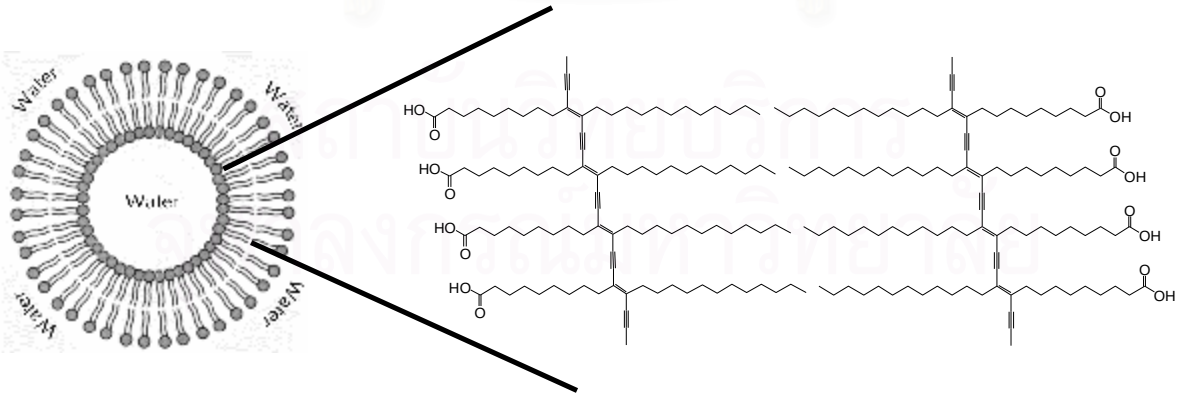



**Figure 1.3** Illustration of poly(PCDA) vesicle.

#### 1.2.3.1 Electronic transition of polydiacetylene

Optical absorption in polydiacetylene occurs *via* $\pi \rightarrow \pi^*$ absorption within the linear π-conjugated polymer backbone. Upon polymerization, frequently the first chromogenically interesting state of PDA appears blue in color. The exposure of PDA to environmental perturbations such as heat (thermochromism), mechanical stress (mechanochromism), or solvent (solvatochromism) involve a significant shift in absorption from low to high energy bands of the visible spectrum, so the polydiacetylene transforms from blue to red color that resulted from molecular conformational changes such as side chain packing, ordering, and orientation, impart stresses to the polymer backbone that alter its conformation, thus changing the electronic states and the corresponding optical absorption [24].

The color transition of the polymerized vesicles was monitored by measuring the absorbance differences between the vesicles before and after stimulation by an interesting parameter. This information is often converted to a percentage, termed the Colorimetric Response (CR) [25].

#### 1.2.3.2 Colorimetric response (CR)

A quantitative value for the extent of blue-to-red color transition is given by the colorimetric response (%CR) which is defined as

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

where $PB = A_{blue}/(A_{blue}+A_{red})$, $A_{blue}$ and $A_{red}$ are the absorbance of the blue and the red phase at 630 and 540 nm, respectively. The visible absorbance was measured by a temperature controlled UV-Vis spectrometer. $PB_0$ is the initial percent blue of the vesicle solution and film before heated. All blue-colored PDA vesicle solution and film samples were heated from 25 to 90 °C.

### 1.2.4 Thermochromism of polydiacetylene vesicles

Thermochromism, the color transition upon the rise of temperature, is one of the interesting chromic properties of polydiacetylenes both for its applications and fundamental understanding. Thermochromism in polydiacetylenes arises from the conformational changes of the conjugated backbone from planar to non-planar due to movement of the side chains. The color transition is thus resulted from the increase of energy gap between the HOMO and LUMO

level. The color transition of polydiacetylenes is driven by the relief of mechanical strain in their structures [26].

For polydiacetylene vesicles, hydrogen bonding between the head groups of the lipid monomers is usually responsible for the planarity of the conjugated backbone. Thermal energy can break or weaken the hydrogen bonding between the head groups resulting in random movement of the side chains and lower the planarity of the backbone. As a consequence, the average conjugation length of $\pi$ electrons along the polymer backbone becomes shorter and changes the color change from blue to red [27].

## 1.3 Literature survey

In 1994, Batchelder *et al.* [28] reported the formation of self-assembled dialkyl disulfide-functionalized monolayers containing diacetylenes. The diacetylene derivative was used as $[S(CH_2)_2COO(CH_2)_9C{\equiv}CC{\equiv}C(CH_2)_{13}CH_3]_2$ and observed to form well-ordered monolayers when adsorbed on gold in THF. The monomer was readily converted to the polymeric form by a short exposure to UV radiation. A "blue" polymer once converted was stable against further irradiation; that is, it did not become "red**".** During the polymerization the alkyl side chains displayed some reorganization.

In 1997, Saremi *et al.* [29] studied types of polydiacetylene multilayer. The first type was based on electrostatic self–organization of diacetylene bolaamphiphiles and polyelectrolytes on a charged substrate followed by subsequent ultraviolet (UV) polymerization. The second type was prepared by direct adsorption of water-soluble polydiacetylene and a polyelectrolyte in alternating sequence. The monomeric diacetylenes were general formula of $X\text{-}(CH_2)_9\text{-}C{\equiv}CC{\equiv}C(CH_2)_9\text{-}X$, with X being sulfate, phosphate or pyridium head group. It was found that the multilayer from sulfate and phosphate head group of monomers could be self- assembled intomultilayer and polymerized on the substrate.

In 1998, Cheng. *et al.* [30] investigated the role of headgroup in colorimetric transition. In order to do so, a series of amino acid-derivatized PCDA lipids have been synthesized. The UV-polymerized liposomes underwent an irreversible color change from blue to red in response to a change of specific solution pH. In this system, the color changes because of the charge-induced headgroup rearrangement which perturbs the conjugated backbone assembly. Considering the reduced degree of freedom for lipid molecules in polymeric liposomes,

mechanism for the colorimetric transition could be proposed due to a rigid, staggered conformation change in polydiacetylene conjugation (Figure 1.4).

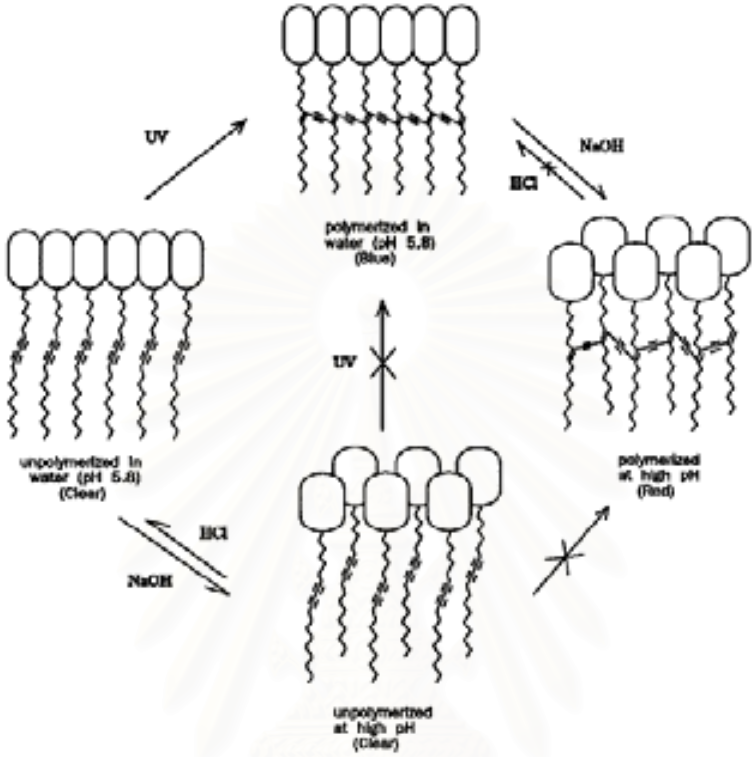



**Figure 1.4** Schematic diagrams of amino acid-derivatized polydiacetylene liposomes in chromatic transition.

In 1999, Jonas *et al.* [10] studied a novel system based on hydrazide derivatives of single–chain diacetylene lipids which are 10,12-Tricosadiynohydrazide (THY) and 10,12-Pentacosadiynohydrazide (PHY). These materials showed an unusual aggregation and polymerization behavior in organic solution, in contrast to the parent carboxylic acids. In addition, these hydrazide lipids undergo an unprecedented reversible color change (blue/red) in polymerized vesicles when the pH of the surrounding aqueous medium is cycled between acidic and basic conditions. This unusual behavior is attributed to the unique hydrogen-bonding pattern of the hydrazide headgroup (Figure 1.5).

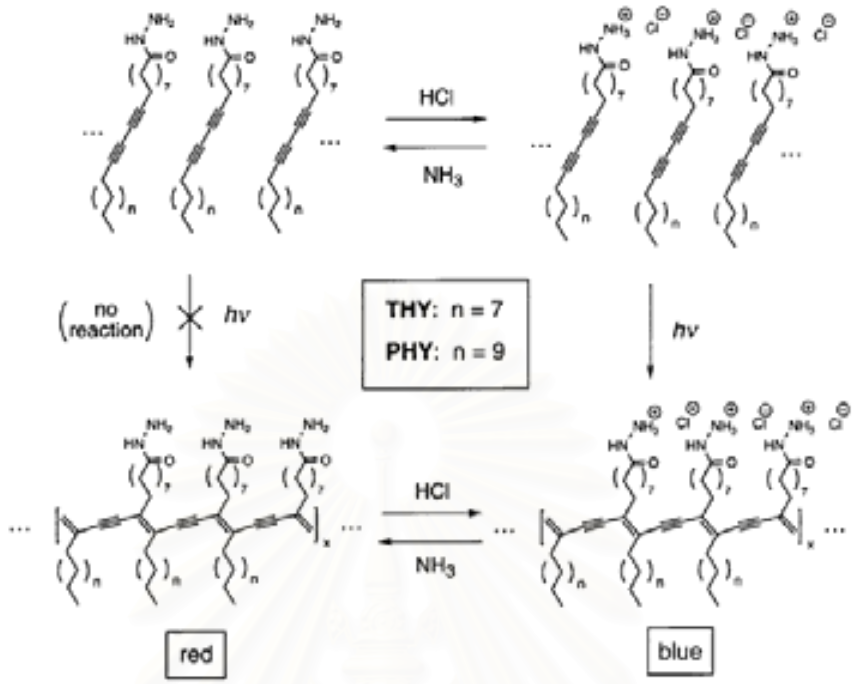



**Figure 1.5** Schematic representations of the polymerization behavior and colorimetric changes of the diacetylene hydrazides PHY and THY in the presence of HCl or $NH_3$.

In 1999, Huo ***et al.*** [3] attempted to reveal the effect of hydrogen bonding network on the photopolymerization and chromatic properties. A polymerizable diacetylene in which two hydrophobic 10,12-pentacosadiynoic acid chains were attached to a triaminotriazine (TAZ) polar headgroup through ethylenediamine linkers, was synthesized. With the continuous increase of the length of the polymeric chainor increase of irradiation time from 200s to 1000s, the original linear polyenyne backbone started to “self-fold” to a “zigzag” structure due to the free rotation of single bonds within the polymer chain of PCDA. In general, it seemed PCDA and PCDATAZ showed the same propensity in their chromatic behavior, although it took much longer for PCDATAZ to shift from blue to red form than PDA does. The efficient π-electron delocalization along the polyenyne backbone was interrupted by this process, leading to a chromatic change from blue to red form of polydiacetylenes as shown in figure 1.6. On the other hand, the process “self-folding” for PCDATAZ on pure water subphase had strong linear noncomplementary hydrogen bonding network between TAZ headgroups, the hydrogen bonding was still stronger than between the carboxylic groups from PCDA.

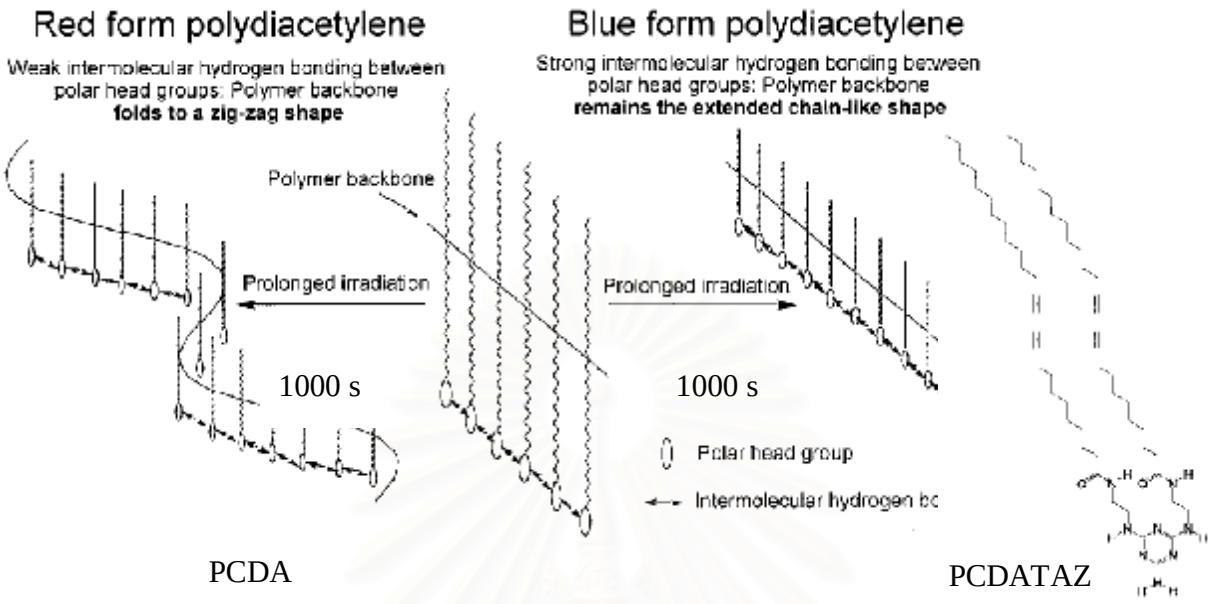



**Figure 1.6** "Self-folding" model of the polymer backbone to explain the chromatic properties of polydiacetylene films upon prolonged UV irradiation.

In 2002, Zheng. *et. al.* [32] investigated salt and pH induced reversible aggregation of vesicles composition of a nonionic synthetic glycolipid, 1,3-di-*o*-phytanyl-2-*o*-(β-maltotriosyl) glycerol. The aggregation of the vesicles appears reversible with respect to change in pH value of the medium as from the reversible change in the turbidity. It was found that in an acidic region (pH 4-6), the size of the aggregated vesicles were above 1,000 nm. But in an alkaline region (pH 8-10), the sizes were 110-130 nm, close to their original size of 100-110 nm, which strongly suggested the reversible disaggregation and confirmed the lack of vesicle fusion. The ζ-potentials of vesicles were measured in the presence of NaCl with pH changes of the vesicle suspension. The surface charges of vesicles arised from two independent mechanisms; one was excess 'adsorption' of $OH^-$ ions at the vesicle-water interfaces and the other was dissociation of hydroxyl groups in a high pH region (pH>11). The changes of the surface charges were thought to be the major factor which induced the aggregation and disaggregation of this nonionic glycolipid vesicle.

In 2001, the bisfunctional polydiacetylenes were designed and synthesized by Song ***et al.*** [33]. These bisfunctional PDAs tend to form well-defined microstructures under mild conditions and have high biological relevance as mimics of natural transmembrane lipids. The double functionalities of these lipids make them good candidates for the fabrication of materials with asymmetric interfacial properties. They synthesized an asymmetric bolaamphiphilic lipid by using L-glutamic acid head group of diacetylene unit (Figure 1.7). From Transmission Electron Microscopy (TEM) results (Figure 1.8), increasing solution pH caused the fraying of helical ribbons into nanofibers suggesting the loss of chirality in packing accompanied by blue-to-red chromatic transition that useful in the design of sensor and other "smart" nanomaterials requiring defined molecular templates.

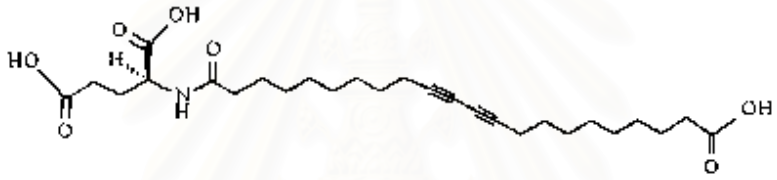



**Figure 1.7** Molecular structure of L-Glu-Bis-3.

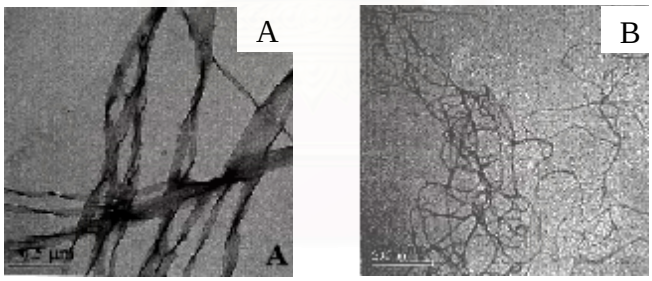
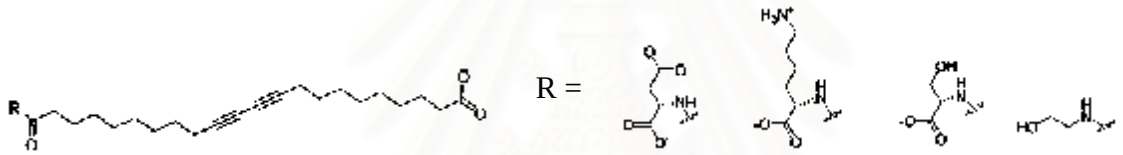



**Figure 1.8** TEM micrographs of **Poly-*L*-Glu-Bis-3** the domain edges of wide flat ribbons (A) then change to coiled nanofibers when treated with pH 7.5 Tris buffer (B).

In 2002, Song ***et al.*** [34] continued their study about the morphological transformations of bolaamamphiphilic PDA (L-Glu-Bis-3) lipid assemblies from helical ribbons to vesicles and flat sheet by doping with cell surface receptor $G_{M1}$ ganglioside and cholesterol. From TEM images, PDA(L-Glu-Bis-3) doping with 5% $G_{M1}$ ganglioside and 5% cholesterol gave the interesting results wide flat ribbons (Figure 1.8A) then increase to 10% cholesterol revealed that

with increased cholesterol content more vesicles were formed with continued coexistence of the ribbon structures. Moreover, it changed to coiled nanofibers when treated with pH 7.5 Tris buffer (Figure 1.8B).

In 2004, Song *et al.* [35] designed, preparation and characterization of self-assembling functional bolaamamphiphilic polydiacetylene by placing polar head groups on both ends of the diacetylene lipids (Figure 1.9) and by altering the chemical nature of the polar surface residues the conjugated polymers could be engineered to display a range of radiation, thermal and pH induced colorimetric responses. Increasing solution pH change morphologies from helical ribbons to nanofibers accompanying charge-induced chromatic transitions suggested the both side-chain disordering and main-chain rearrangement in altering the effective conjugation lengths of poly(ene-yne). These results can be utilized for the development of BPDA-based colorimetric sensors.




BPDA L-aspartic acid L-lysine L-serine ethanolamine

**Figure 1.9** Bolaamphiphilic diacetylene lipids with various 4 headgroups modifications.

The secondary amine HBr salt as the head groups of single chain diacetylene lipids was synthesized by Lee *et al.* in 2004 [36]. This molecule could form a number of different self-assembled nanostructures in aqueous or organic solvents. The nanotube structures were investigated by Scanning Electron Microscopy (SEM) and TEM, a hollow inner core and were found to be a wall consisting of five lipid bilayers (Figure 1.10.) The versatility of this new material as a platform for nanotubes design and synthesis was enhanced by its biocidal activity and the development of a range of applications from biosensors to artificial ratinas.

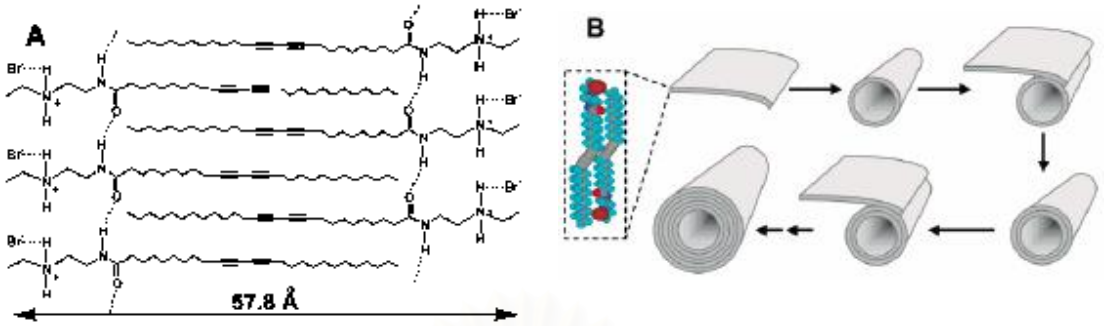



**Figure 1.10** (A) Schematic structure of a bilayer of nanotube. (B) Schematic of the assembly of a nanotube.

In 2006, Jahnke *et al.* [37] studied the topochemical diacetylene polymerization of self-assembled supramolecular polymers consisting of β-sheet-forming oligopeptides as scaffolds. The solution contained tetra (L-alanine) amino acids head group, which forms hydrogen bonding networks to obtain a well-defined supramolecular polymer with a double-helical topology and NHAc end group which is necessary for the formation of the required parallel β-sheet structures. These properties may be useful for optoelectronic applications at the surface with biosciences.

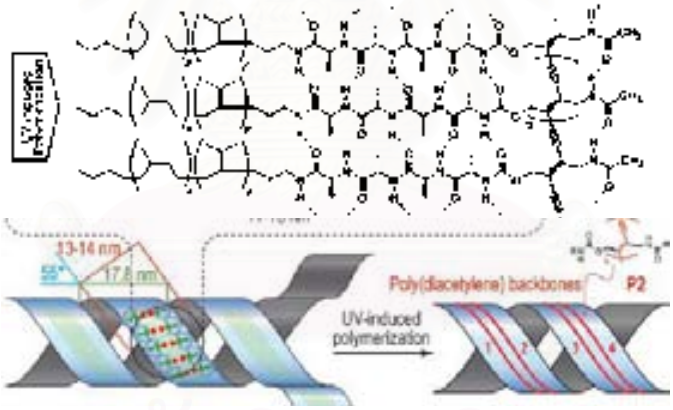



**Figure 1.11** Self-assembly and topochemical polymerization of diacetylenes functionalized with a β-sheet-forming oligopeptide.

In 2006, Simon *et. al* [38] investigated the chromic response to pH changes of 10,12-Tricosadiynoic acid (TRCDA) vesicle assemblies fabricated in milli Q water (0.50 mM solutions). It showed an irreversible blue-red transition when the pH was increased by adding NaOH, consistent with the chromic transitions known to be triggered by pH changes and proposed to be based on surface ionization. In comparison, acidification to pH < 4 had no effect on the colorimetric properties of the vesicles. The irreversible nature of the color transition for

pH > 4 indicated in the red-phase was thermodynamically stable with respect to the blue-phase. The acid-base properties of TRCDA vesicles were confirmed to be that expected for spatially confined interfacial carboxy groups, exhibiting attenuated acidity and pKa shifts over several orders of magnitude compared to soluble carboxy groups. Furthermore, the blue-red transition was quantitatively correlated with the extent of deprotonation of the interfacial carboxy groups, which accounted for the high pH range at which the basification triggered blue-red response occurs. From TEM it could be seen that the pH-chromictransition was also accompanied by a significant morphological change, which may explain why the chromic transition was irreversible as shown in figure 1.12

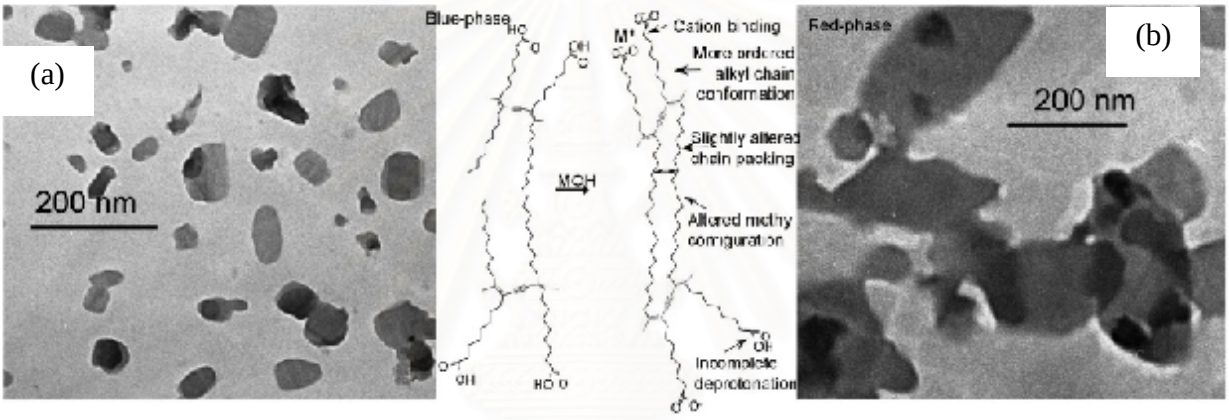



**Figure 1.12** TEM images of dehydrated 0.50 mM TRCDA blue-phase vesicles before (a) and after (b) basification to form the red-phase by the addition of 1 molar equivalent 0.1 N NaOH. Scheme for the proposed structural transition that accompanies the blue - red colorimetric process also shown.

In 2007, Ma. *et. al* [39] reported the influence of hydrocarbon chain lengths in the single-chain 2,4-alkyl-diynoic acids $CH_3-(CH_2)_n-C\equiv C-C\equiv C-COOH$ (n= 9, PDDA; n= 15, HCDA; n= 17, TCDA) on the ability to form polydiacetylene vesicles had been studied by TEM. The experimental results showed that 2,4-tricosadiynioc acid (TCDA), 2,4-heneicosadiynioc acid (HCDA) except 2,4-pentadecadiynoic acid (PDDA) could form blue polydiacetylene vesicles, suggesting that the alkyl chain lengths of the 2,4-alkyl-diynoic acids play crucial role in the polymer vesicle formation. These polydiacetylene vesicles were stable for over a period of 6 months (Figure 1.13) and were not destroyed by 0.2 M NaCl aqueous solution (Figure 1.14).

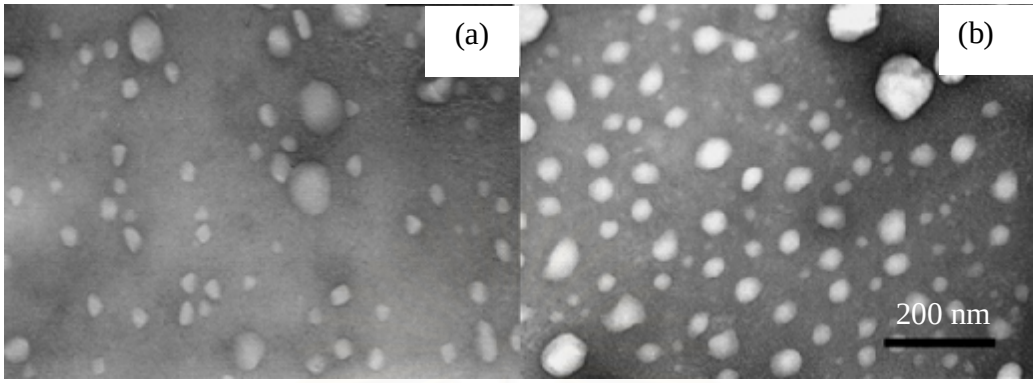



**Figure 1.13** TEM images of polymer TCDA (a) and HCDA (b) vesicles after 6 months.

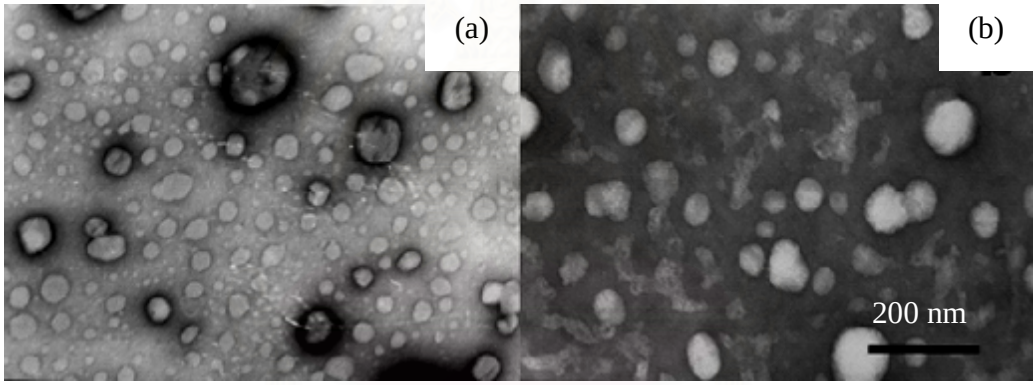



**Figure 1.14** TEM images of polymer TCDA (a) and HCDA (b) vesicles in 0.2M sodium chloride aqueous solution.

## 1.4 Objective and scope of the research

The objective of this thesis is to prepare polydiacetylene vesicles at various effects of preparation conditions, *i.e.* pH and ionic strength of the media and the concentration of the lipids, on the morphology and size of assembly of PCDA and its derivatives. To achieve the objective, the work scope includes 1) synthesis of amide derivatives of 10,12-pentacosadiynoic acid (PCDA), 2) preparation of polydiacetylene nanovesicles from PCDA and its derivatives, at various optimum conditions, 3) characterization of the prepared vesicles by AFM, TEM and dynamic light scattering for morphology and particle sizes, 4) study of thermochromic properties of the prepared vesicle solutions.

# CHAPTER II

# EXPERIMENTAL

## 2.1 Chemicals

1. 10,12-pentacosadiynoic acid (PCDA), Fluka, USA
2. *N,N'*-dicyclohexylcarbodiimide (DCC), Fluka, Switzerland
3. Ethylenediamine, Carlo Erba, France
4. Diethyl ether, reagent grade, Lab-Scan, Ireland
5. 1,1,1-Trichloromethane (chloroform), AR grade, Lab-Scan, Ireland
6. Dichloromethane (methylene chloride), commercial grade TSL Chemicals, Thailand
7. Hexane, commercial grade, TSL Chemicals, Thailand
8. Ethylacetate, commercial grade, TSL Chemicals, Thailand
9. Propan-1-ol (methanol), commercial grade, TSL Chemicals, Thailand
10. Sodium chloride, Merck, Germany
11. Sodium hydroxide, Merck, Germany
12. Sodium carbonate anhydrous, Fisher Scientific, UK
13. Sodium sulfate anhydrous, Riedel-deHaën®, Germany
14. Silica gel 60, Merck, Germany

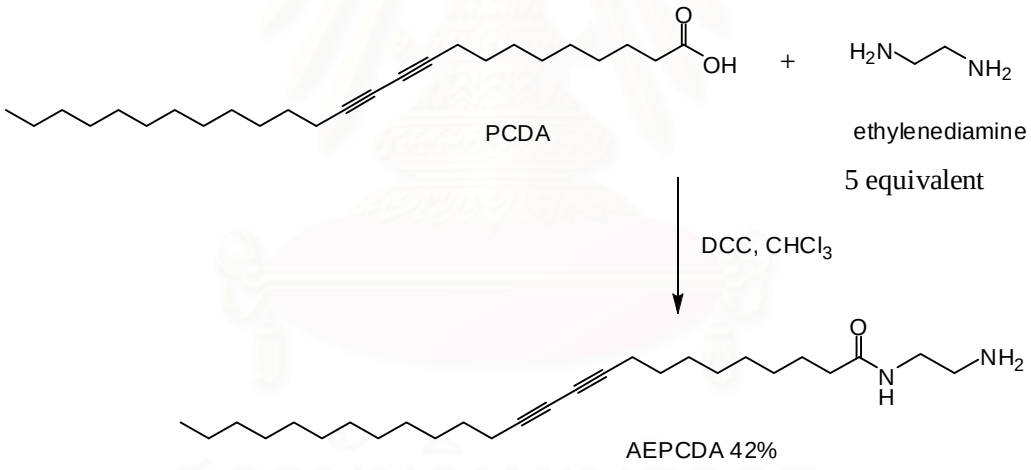
## 2.2 Apparatus and equipments

1. Rotary evaporator, R200, Buchi, Switzerland.
2. Ultrasonicator, Transsonic T570/H, Elma, Germany
3. Magnetic stirrer, Fisher Scientific, USA
4. Hot plate magnetic stirrer, Corning, USA
5. pH meter, Twin pH B 212, Japan
6. pH meter, Bench pH/mV/Temperature Meter, CyberScan pH510, EUTECH, Singapore
7. Pipette man (P20, P200 and P5000), Gilson, France

8. Pipette man (Le100 and Le1000), Nichiryo, Japan
9. Ultracentrifuge, MR 23i, Jouan, USA
10. Differential Scanning Calorimetry (DSC), DSC 823e/400, Toledo, USA
11. Nuclear Magnetic resonance spectrometer (NMR) 400 MHz, Mercury 400, Varian, USA
12. Mass spectrometer, Quattro micromass, Waters, France
13. UV-*vis* spectrophotometer, Cary 100 Bio, Varian, Australia
14. Dynamic light scattering spectrometer (DLS), Nanosizer, Malvern Instrument, England
15. Atomic force microscopy (AFM), Seiko SPA 400, Japan
16. Transmission electron microscope (TEM), JEOL TEM-2100, Japan

## 2.3 Procedures for synthesis of diacetylene lipid monomers

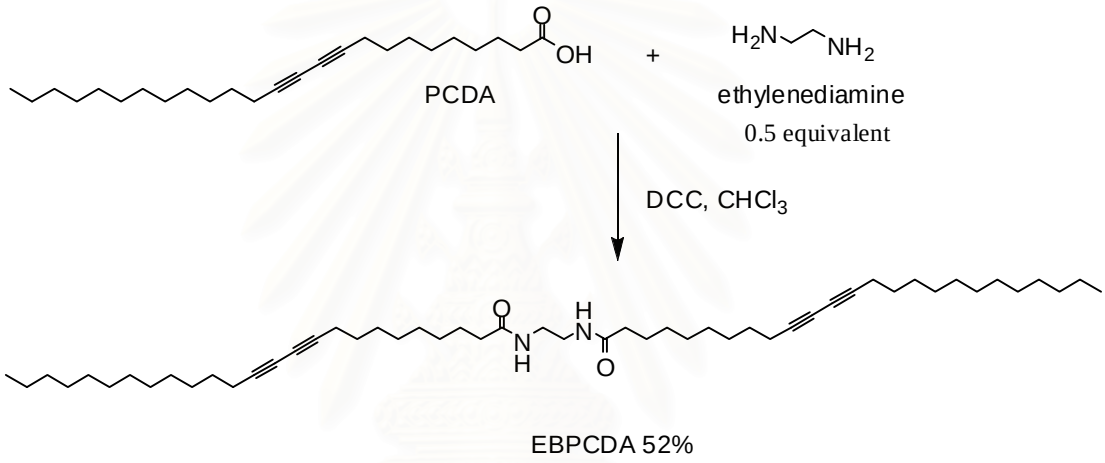
### 2.3.1. N-(2-aminoethyl)pentacosa-10,12-diynamide (AEPCDA)




*N,N'*-dicyclohexylcarbodiimine (495.2 mg, 2.4 mmol) in chloroform (2 mL) was added dropwise into a solution of 10,12-pentacosadiynoic acid (749.2 mg, 2.0 mmol) in chloroform (2 mL). The mixture was stirred for 1 hour at room temperature and was then added dropwise into ethylenediamine (669.6 μL, 10.0 mmol). The reaction mixture was stirred for an hour when the white precipitate was clearly observed. The reaction mixture was poured into dichloromethane (5 mL) and then extracted with saturated sodium carbonate solution (20 mL) followed by distilled water ($2 \times 10$ mL). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to yield the crude product as a yellow oil.

Purification was accomplished by colmn chromatography on silica gel (35.50 g) eluting with EtOAc:MeOH (70:30) to give *N*-(2-aminoethyl)pentacosa-10,12-diynamide (352.4 mg, 42%) as a white solid. Characterization data for AEPCDA: mp. 70-75 °C. $^1$H NMR (400 MHz, $CDCl_3$) *d* 0.86 (3H,t, *J* = 8.0 Hz, $CH_3$), 1.24-1.51 (32H, m, $CH_2$), 2.17 (2H, t, *J* = 5.6 Hz, $CH_2$), 2.22 (4H, t, *J* = 6.9 Hz, $CH_2$), 2.24 (2H, br, $NH_2$,), 2.84 (2H, t, *J* = 5.6 Hz, $CH_2$), 3.32 (2H, q, *J* = 5.7 Hz, $CH_2$), 6.17 (1H, br, NHC=O).

### 2.3.2. N,N'-ethylenebispentacosa-10,12-diynamide (EBPCDA)




*N,N'*-dicyclohexylcarbodiimide (247.6 mg, 1.2 mmol) in chloroform (2 mL) was added dropwise into PCDA (374.6 mg, 1.0 mmol) in chloroform (2 mL). After stirring for an hour ethylenediamine (33.4 μL, 0.5 mmol) was then added dropwise and continued stirring for another hour. The white precipitate formed in the reaction mixture was collected by filtration and redispersed in methanol under stirring. The precipitate was collected again by filtration and characterized as *N,N'*-ethylenebispentacosa-10,12-diynamide (197.9 mg, 52%). Characterization data for EBPCDA: mp 120-125 °C. $^1$H NMR (400 MHz, $CDCl_3$); *d* 0.88 (6H, t, *J* = 6.8 Hz, $CH_3$), 1.25-1.51 (64H, m, $CH_2$), 2.10 (4H, t, *J* = 7.5 Hz, $CH_2$), 2.17 (8H, t, *J* = 6.9 Hz, $CH_2$ ), 3.39 (4H, br, $CH_2$), 6.10 (2H, br, NH=CO).

## 2.4 Procedures for preparation of polydiacetylene vesicles

### 2.4.1 Poly(10,12-pentacosadiynoic acid) vesicles

10,12-pentacosadiynoic acid (PCDA, 11.2 mg) as a white solid was dissolved in chloroform (2 mL) in a test tube and the solvent was removed under reduced pressure. A volume of milli-Q water was added to provide the lipid concentration of 1.0 mM. The suspensions were heated to 75-85ºC, followed by sonication in an ultrasonicating bath for 20 minutes when a semitransparent or transparent vesicle solution was obtained. The solution was kept at 4 ºC overnight. The vesicle solution was irradiated with UV light (254 nm) for 5 minutes and filtered through a filter paper (No.1) to give a clear intense blue-colored poly(PCDA) vesicle solution. The solution showed maximum visible absorption at wavelength ($\lambda_{max}$) of 630 nm.

### 2.4.2 Poly(N-(2-aminoethyl)pentacosa-10,12-diynamide) vesicles

The preparation of poly(*N*-(2-aminoethyl)pentacosa-10,12-diynamide), poly(AEPCDA), vesicle solution applied a similar procedure to that of poly(PCDA) except for that the UV irradiation time for 2 minutes was conducted in an ice bath. Poly(AEPCDA) vesicle solution was obtained in a clear intense blue color with $\lambda_{max}$ = 630 nm. Without an ice bath, the solution turned into red.

### 2.4.3 Poly(N,N'ethylenebispentacosa-10,12-diynamide) vesicles

The preparation of poly(*N,N'*-(ethan-1,2-diyl)dipentacosa-10,12-diynamide), poly(EBPCDA), vesicle solution applied a similar procedure to that of poly(PCDA) except for that the lipid suspension was heated to 120-130°C into glycerol bath prior to the sonication and the temperature was kept under sonication at the temperature above 80°C for 60 minutes The temperature of the sonication should be kept higher than 80°C to avoid the formation of big aggregated solid suspension. The resulting cloudy vesicle solution was obtained and kept at 4ºC overnight. After UV irradiation at room temperature, poly(EBPCDA) vesicle solution was obtained in a clear deep blue color. The vesicle solution was irradiated with $\lambda_{max}$ = 636 nm.

## 2.5 Preparation of polydiacetylene vesicles at various conditions

The analysis of sample should be maintained in an aqueous environment; the following procedure was found to be useful and may be treated as exemplary. For the optimum condition of vesicle was prepared by adjust into Milli Q water before added into thin film or lipid bilayer. pH effect was adjusted by adding dropwise of 0.1 M HCl for acidic lipid (pH 3 and 5) and 0.1 M NaOH for basic lipid (pH 7 and 9) until pH reached as a initial pH study. In the study of effect from ionic strength at various concentrations at 0.1, 1 and 10 mM NaCl After the adjustment of Milli Q water was followed by sonication in an ultrasonicating bath.

## 2.6 Analysis of vesicles

### 2.6.1 Differential scanning calorimetry (DSC)

The thermal stability of vesicle measured the melting point of prepolymerized monomers. Monomer was added 2 – 3 mg sealed in aluminum pans and heated at a rate of 20 °C/min. The time and temperature scales of the calorimeter were calibrated against the enthalpies of melting temperatures range. (temperature onset to peak)

### 2.6.2 UV-Visible spectroscopy

The visible absorption of vesicle solutions was taken in a quartz cuvette with 1 cm. optical path length on a temperature controlled UV-Vis spectrometer. The spectra were collected from 750 to 400 nm with the zero absorbance set at 750 nm. The $\lambda_{max}$ of the blue and red phases of each sample was determined at 25 and 90°C. The correlation between the absorbance at the $\lambda_{max}$ and adjust the initial absorbance in the range of 0.4 – 0.5

### 2.6.3 Transmission electron microscopy (TEM)

TEM images were completed using a JEOL TEM-2100 electron microscope equipped with a CCD camera. The accelerating voltage was 200 KV. The vesicle solution were deposited onto Formvar coated copper grids (200 mesh), and stained with 2% uranyl acetate solution for 5 min and dried at room temperature in desiccator.

### 2.6.4 Atomic force microscopy (AFM)

Vesicles were deposited on a freshly cleaved mica plate and dried at room temperature in desiccator for 4 hours. Seiko SPA 400 (SII Nanotechnology Inc.) operating in non contact mode using a SI-DF20 cantilever was used to observe the morphology and particle size of the deposited vesicles. The image of the vesicles was

measured on an air-dried sample of vesicles were prepared from a drop of solution on a freshy cleaved on mica plate.

#### 2.6.5 Dynamic light scattering (DLS)

The mean size of vesicles and the size distribution were determined by nanosizer (zeta sizer Nano ZS Malvern Instruments). The samples were sonicated for 1 min before measurement and vesicle solutions was taken in a plastic cuvette with 2 cm..Each measurement was repeated 3 times in order to acquire an average data. Analyze and report by using intensity statistics mode to plot range of particle size graph.

#### 2.6.6 Zeta – potential

Vesicles dispersions were diluted with water and were taken in green cuvette with 1 ml depending on the experiment and their electrophoretic mobility was measured at 25°C by photon correlation spectroscopy (zeta sizer Nano ZS Malvern Instruments). The zeta potentials of the dispersions were calculated according to the Helmoholtz – Smoluchowski equation.

### 2.7 Separation of particle size from vesicle solution

Vesicles solution was taken in centrifuge tube with 8 ml and was centrifuged at 3,000 and 12,000 RPM for 60 minutes and temperature was controlled at 4°C (Jouan centrifuge MR23i). After the centrifugation, the centrifuge tube was visibly inspected. The concentrated dark blue solution at the bottom (inside) of the tube was taken using syringe. The supernatant was carefully removed and characterized particle size or size distribution by Dynamic Light Scattering.

### 2.8 Thermochromism study

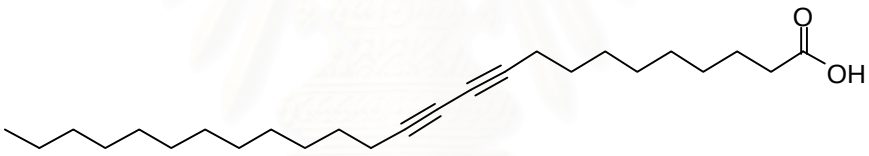
#### 2.8.1 Colorimetric response (%CR)

A quantitative value for the extent of blue-to-red color transition is given by the colorimetric response (%CR) which is defined as $\%CR = (PB_0-PB)/PB_0*100$. Where $PB = A_{blue}/(A_{blue}+A_{red})$, $A_{blue}$ and $A_{red}$ are the absorbance of the blue and the red phase at 630 and 540 nm, respectively. The visible absorbance was measured by a temperature controlled UV-*vis* spectrometer. $PB_0$ is the initial percent blue of the vesicle solution and film before heated. All blue-colored PDA vesicle solution and film samples were heated from 25 to 90°C.

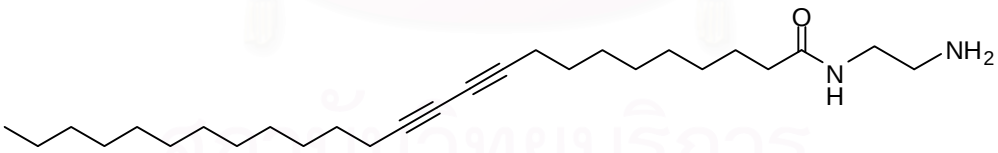
# CHAPTER III

# RESULTS AND DISCUSSION

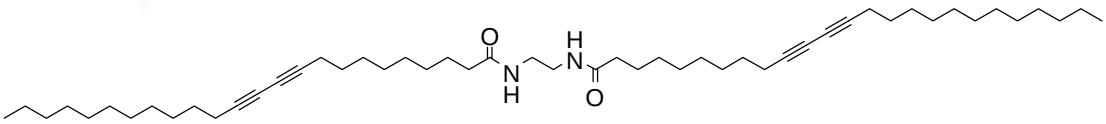
Although the applications of polydiacetylene (PDA) have been extensively studied, there is only limited information available on the relationships of the preparation conditions and the physicochemical properties of the resulting vesicles. In wfh0bthis work, three types of diacetylene monomers, PCDA and its two amide derivatives. PCDA is a commercially available diacetylene lipid containing a carboxylic head group and long aliphatic chain (25 carbons including the carboxylic head group). Two amide derivatives of PCDA, *N*-(2-aminoethyl) pentacosa-10,12-diynamide (AEPCDA) and *N,N´*-ethylenebispentacosa-10,12-diynamide (EBPCDA), were synthesized from the condensation of ethylenediamine with one and two equivalents of PCDA, respectively (Figure 3.1).




10,12-pentacosadiynoic acid (PCDA)




*N*-(2-aminoethyl) pentacosa-10,12-diynamide (AEPCDA)




*N,N´*-ethylenebispentacosa-10,12-diynamide (EBPCDA)

**Figure 3.1** Chemical structures of PCDA, AEPCDA and EBPCDA.

The assemblies of these three types of diacetylene lipids were studied using varying conditions, *i.e.* pH and ionic strength of the media and the concentration of the lipids. The morphology and size of the assemblies were analyzed and conmpared. Thermochromic responses of the prepared PDA assemblies were investigated to gain insight the relationship between the assembly attributes and the thermochromism behaviors. The results of these studies will be elaborated and discussed in this chapter of thesis.

### 3.1 Synthesis of diacetylene lipid monomers

Synthesis of AEPCDA was acheived by dropping a solution of PCDA and DCC in chloroform into a large excess amount of ethylenediamine (5 equivalents). The reaction yielded AEPCDA as the major product (42% isolated yield) and a trace amount of EBPCDA from the double substitution of PCDA on ethylendiamine (Figure 3.2). The synthesis of EBPCDA was performed by inversed addition of the reactants, dropping a solution of 0.50 equivalent of ethylenediamine into a solution of PCDA and DCC in chloroform, allowing greater formation of EBPCDA (52% isolated yield).

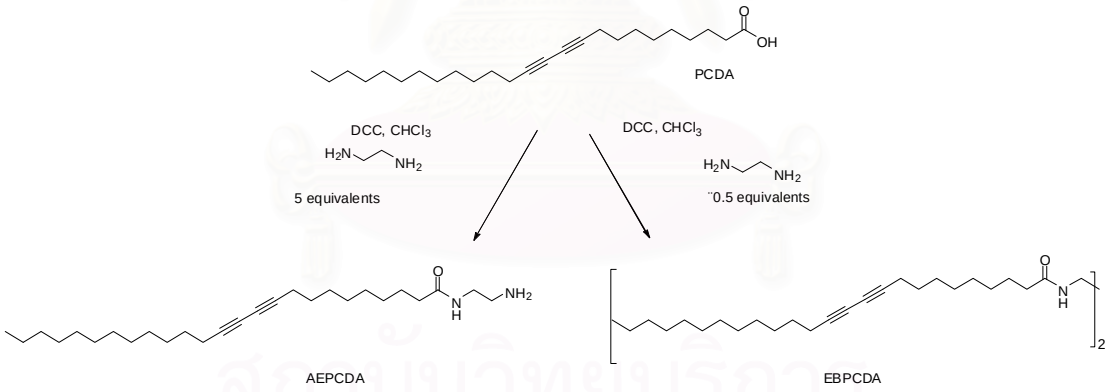



**Figure 3.2** Synthesis of AEPCDA and EBPCDA lipids.

The $^{1}$H NMR signals of AEPCDA and EBPCDA were quite similar to those of PCDA monomer (Figure 3.3). The most indicative signal for the formation of the amide products appeared at 3.4 ppm corresponding to the methylene protons next to the amido nitrogen (v). This signal was absent in the spectrum of PCDA. The less distinctive signal was a broad signal at 6.2 ppm belonging to the amido N-H protons (u). The signal of the methylene proton connected to the carbonyl group (t) also shifted slightly from 2.4 to 2.2 ppm upon the conversion from the carboxylic to the

amido group. For AEPCDA, another characteristic signal was also observed at 2.8 ppm belonging to the methylene protons (w) next to the amino group. Most of the protons in the aliphatic chain in all three products give the signals in the range of 2.2-0.9 ppm.

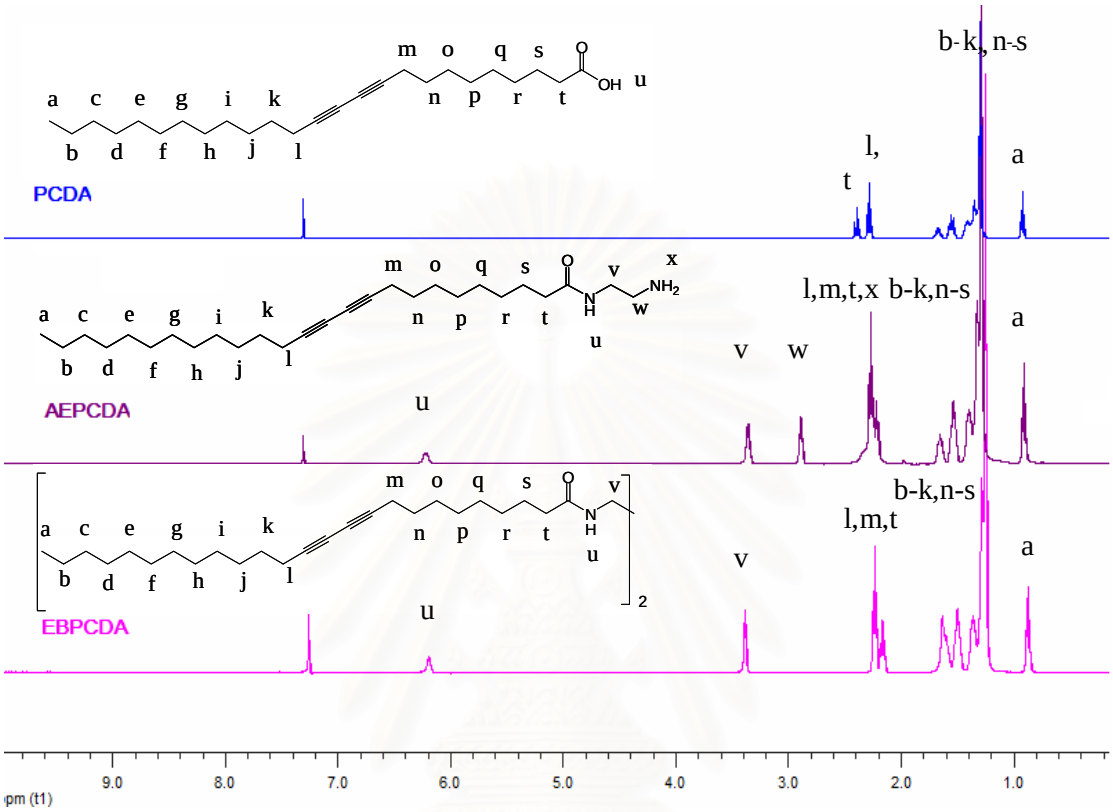
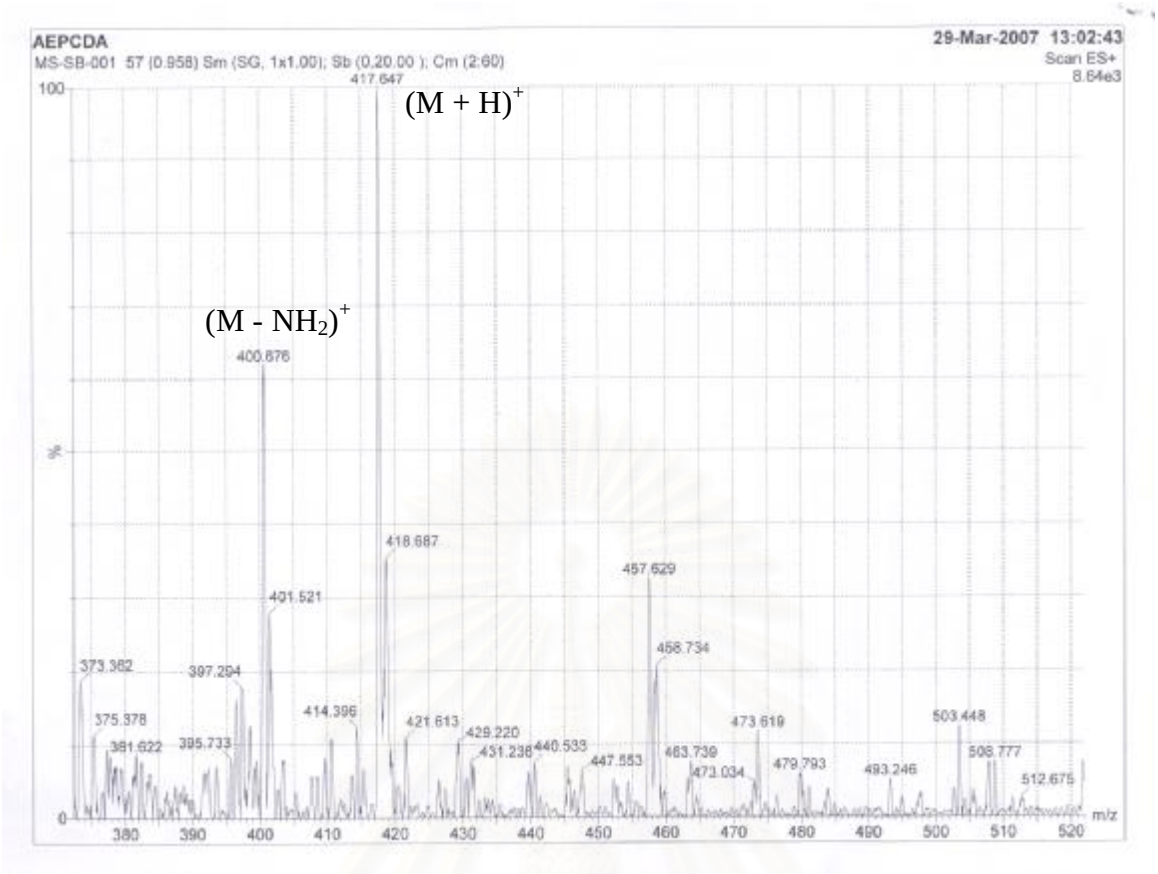
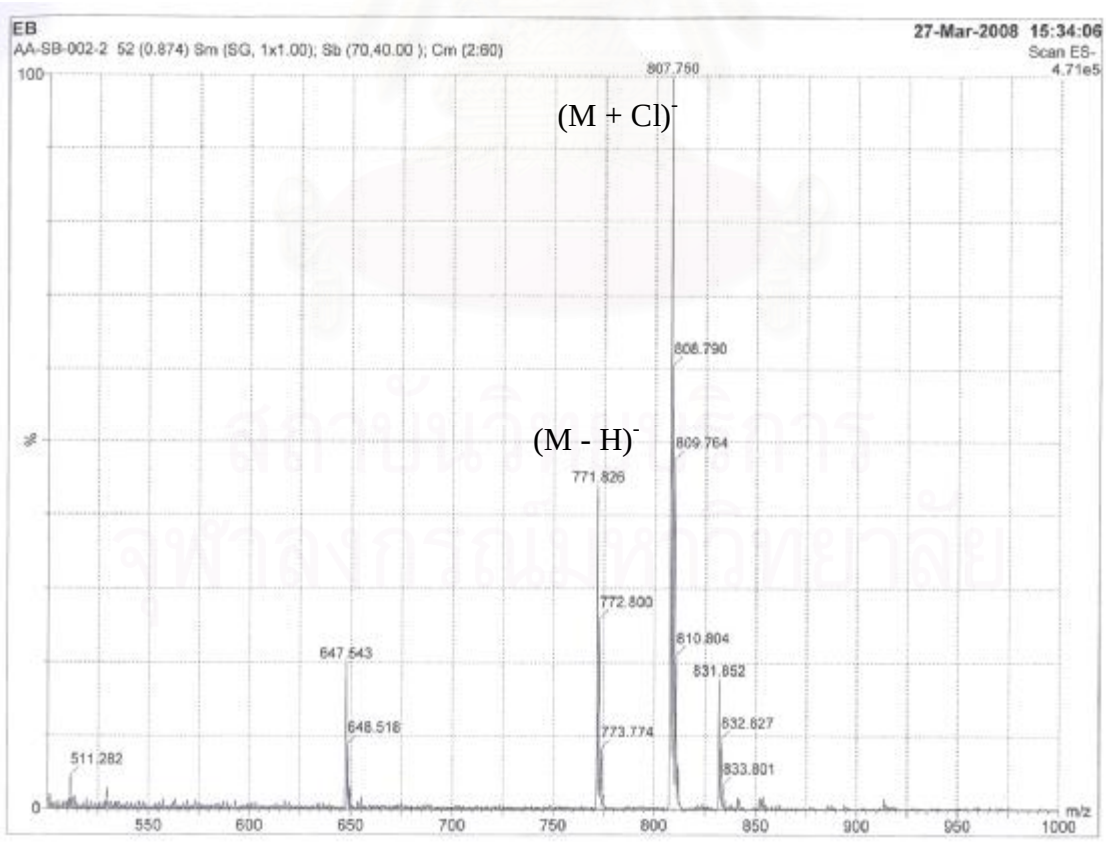



**Figure 3.3** $^{1}$H NMR spectra of PCDA, AEPCDA and EBPCDA lipids in $CDCl_3$.

ESI-Mass spectrometry was used to determine the molecular weight of the synthesized lipids. The MS spectrum of AEPCDA showed signals at *m/z* = 417.6 and 400.6 corresponding to the $(M + H)^+$ molecular ion and the $(M - NH_2)^+$ fragment, respectively (Figure 3.4a). The MS spectrum of EBPCDA, on the other hand, showed signals at *m/z* = 772.7 and 771.8 corresponding to the $(M - H)^-$ molecular ion and $(M + Cl)^-$ adduct, respectively (Figure 3.4b).




a)




b)

**Figure 3.4** ESI mass spectra of a) AEPCDA and b) EBPCDA

### 3.2 Poly(PCDA) assembly in various preparative conditions

Amphiphilic compounds such as fatty acid can form different types and sizes of supramolecular assemblies in aqueous media, depending on the ionization degree of the carboxyl group.

The assembly of PDA supramolecules has been used advantageously for the creation of well-defined nano- and microscale structures. Several examples include helical tubular inorganic architectures that are prepared by using a chiral diacetylene monomer as a template molecule. PDA-silica nanocomposites possess hexagonal, cubic, and lamellar structures and hybrid sol-gel matrices with encapsulated antibody-containing PDAs for biosensor applications [40-41]. PDA-based sensors have been prepared in a wide range of organized structures such as single crystals, thin films on solid supports using Langmuir-Blodgett or Langmuir-Schaefer techniques, self-assembled monolayers, liposomes or vesicles in water. The PDA vesicles could be deposited in a layer-by-layer fashion on the solid substrates.

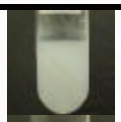
The poly(PCDA) self-assembled in form of liposomes or vesicles homogeneously disperses in aqueous media appeared as a blue solution that allow itself into several successful development of colorimetric biosensors [12-15]. Although the applications of poly(PCDA) have been extensively studied, there are only limited information available on the relationships of the preparation conditions and the physicochemical properties of the resulting vesicles. This experiment were designed to present the effects of preparation conditions, *i.e.* pH, ionic strength of the media was adjusted into milli Q water before sonication and the concentration of the lipids, on the morphology and size of assembly of PCDA. The morphology and size of the assemblies were analyzed by DLS, AFM and TEM technique and conmpared between the assembly attributes and the thermochromism behaviors.

#### 3.2.1 pH variation

The pH used in this experiment was adjusted by 0.1 M HCl for pH 3,5 and 0.1 M NaOH for pH 7,9 into milli Q water before sonication to produce PCDA vesicles. For the initial mixture, pH values were measured again after sonication and UV-irradiation (Table 3.1). After sonication, the pH of lipid suspension with high initial of 7 and 9 dropped significantly lower to 5.5 and 6.0 indicating the greater dispersion of the acidic PCDA molecules in water. As PCDA is a carboxylic acid with $pK_a \sim 4$ - 5, its dispersion did not affect the pH of the more acidic media (pH 3 and 5). Interestingly, the photopolymerization of the PCDA assembly brought about the pH

increase to the range of 6.0-6.8 indicating that the polymerization reduced the acidity of the PCDA assembly. The polymerization probably caused the hydrogen bonding between the carboxylic head group of the lipid chains became stronger and less likely to ionized. However, no pH change was observed for the mixture at pH 3.0, in which the carboxylic head group is hardly ionized and the pH of the media is hence unaffected by the change of the acidity of this head group.

**Table 3.1** Appearance and pH at 25°C of 1 mM poly(PCDA) dispersed in water at various states sonication time 20 min and irradiation time 5 min

| Initial pH | pH after sonication | | pH after UV-irradiation | |
|---|---|---|---|---|
| 9±0.1 | 6.0±0.1 |  | 6.8±0.2 |  |
| 7±0.1 | 5.5±0.1 |  | 6.5±0.2 |  |
| 5±0.1 | 5.0±0.1 |  | 6.0±0.1 |  |
| 3±0.1 | 3.0±0.1 |  | 3.0±0.1 |  |

Conventionally, soft nanoparticles formed from organic materials are defined by the size of 200 nm or smaller [43]. The sizes of poly(PCDA) vesicles obtained from DLS were grouped into three ranges *i.e.* nano (diameter ≤ 200 nm), submicro (200 < diameter ≤ 1 μm) and micro (diameter > 1 μm) (Figure 3.5). This size grouping can also reduce inferior size fluctuation in the DLS measurement that display clearer distinction of the vesicle sizes prepared at various pH. The vesicles prepared at pH 5 and 7 were relatively smaller than those obtained at pH 3 and 9. The vesicles prepared at pH 3 and 9 also appeared to be less stable upon storage as they seriously aggregated within a month while the vesicles prepared at pH 5 and 7 could be stored for a few months in refrigerator. It is also important to state here that the preparation of PCDA vesicles at the pH lower and higher than this range resulted in severe lipid aggregation. The optimum pH range for preparation of poly(PCDA) vesicles is hence in the range of 5 to 7. The more acidic or basic conditions may cause

charge imbalance at the carboxyl groups due to the protonation and deprotonation processes.

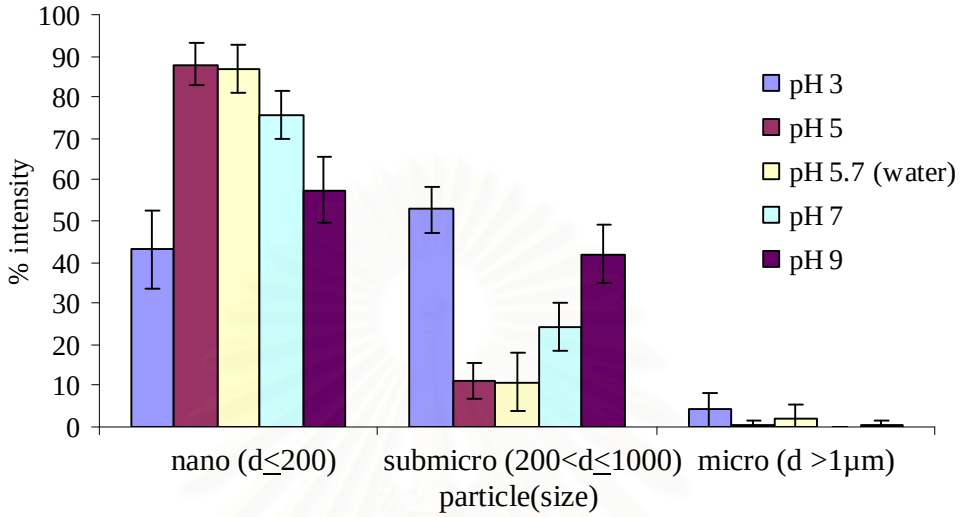
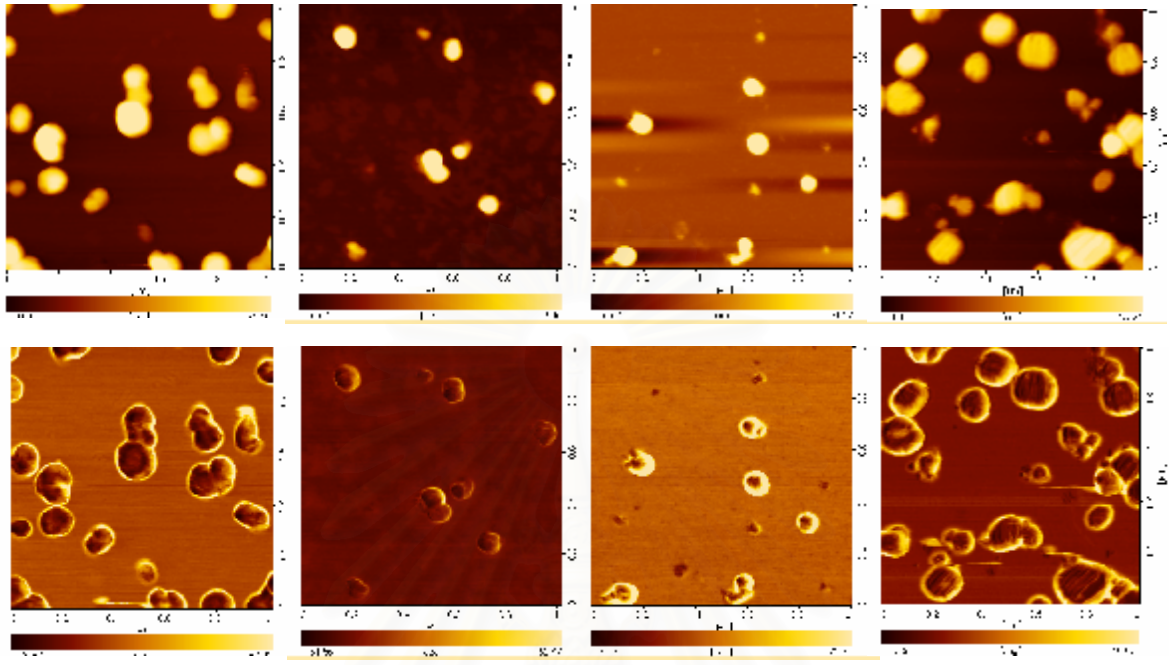



**Figure 3.5** Size distribution of 1 mM poly(PCDA) prepared at various pH determined by DLS.

Atomic force microscopy (AFM) was utilized to observe the shape and size of the air-dried poly(PCDA) vesicles. The AFM images showed spherical structures of poly(PCDA) vesicles (Figure 3.6). The AFM results were in good agreement with the DLS results. The vesicles prepared at pH 5 and 7 appeared to be smaller than those prepared at pH 3 and 9 because poly(PCDA) vesicles content with carboxylic nearly equal carboxylate anion can imply that pH ≈ pKa. The vesicles prepared at pH 3 and 9 also appeared to aggregate to greater extent.

With the acidification of this aqueous solution, to pH 3 the poly(PCDA) lipid would be assembled into a bilayer (vesicle) where almost all of the molecules were protonated. In strong alkaline solution, pH 9, almost all of carboxyl groups of the poly(PCDA) vesicles were deprotonated and strong electrostatic force made molecule repel each other. The carboxylate coordinated with $Na^+$ of hydroxyl groups become induced neutral charged, which can reduce the electrostatic repulsion of headgroup area. Obviously the area of polar group ($A_0$) greatly reduced and packing parameter (p) will fall into the range ½ -1, which will lead to the formation of larger sized of

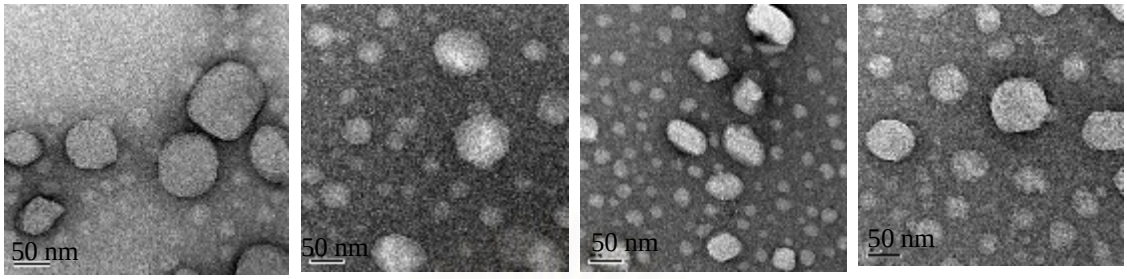
vesicles structures. A similar phenomenon had been reported in previous research [44].



pH 3 pH 5 pH 7 pH 9

**Figure 3.6** Morphology (top row) and phase (bottom row) AFM images of dry poly(PCDA) vesicles prepared at various pH.

The transmission electron microscopy (TEM) images of poly(PCDA) vesicles also confirmed the results obtained from DLS and AFM. Prepared at pH 5 and 7, the vesicles were smaller than those prepared at pH 3 and 9 (Figure 3.7). In the TEM image, there was no significant aggregation of the vesicles observed in all pH used. Some discrepancies in sizes and aggregation observed by different techniques (DLS, AFM and TEM) are conceivable for soft nanomaterials, such as lipid vesicles, given that the conditions of sample preparation can affect the individual particle size and aggregation state. As the theory for DLS technique assumes that the particles measure have spherical shape, the use of DLS results should be used with some caution.




pH 3 pH 5 pH 7 pH 9

**Figure 3.7** TEM images of dry poly(PCDA) vesicles prepared at various pH.

### 3.2.2 Variation of ionic strength

Increase of ionic strength of the aqueous medium is known to reduce the critical micellar concentration of amphiphilic compounds. In this work, poly(PCDA) vesicles were prepared in various concentration of NaCl (0.1-10 mM) to observe the effect of ionic strength on the lipid assembly. As the concentration of NaCl increased, the poly(PCDA) sol became more turbid. The DLS analysis showed that increasing the ionic strength resulted in an increase of submicron size vesicles with an expense of nano-sized vesicles (Figure 3.8). The TEM images also showed that there were more of the larger sized vesicles when the preparation was done in 10 mM NaCl. However, increasing the concentration of ionic strength would induce the form in carboxylate anion aqueous media and therefore reduce the influence of the electrostatic repulsion. As a consequence, sodium chloride cation would neutralize carboxylate anion effect on and allow the lipids to be closer to each other then aggregate large particles or fuse of vesicles. (Figure 3.9 b) [40].

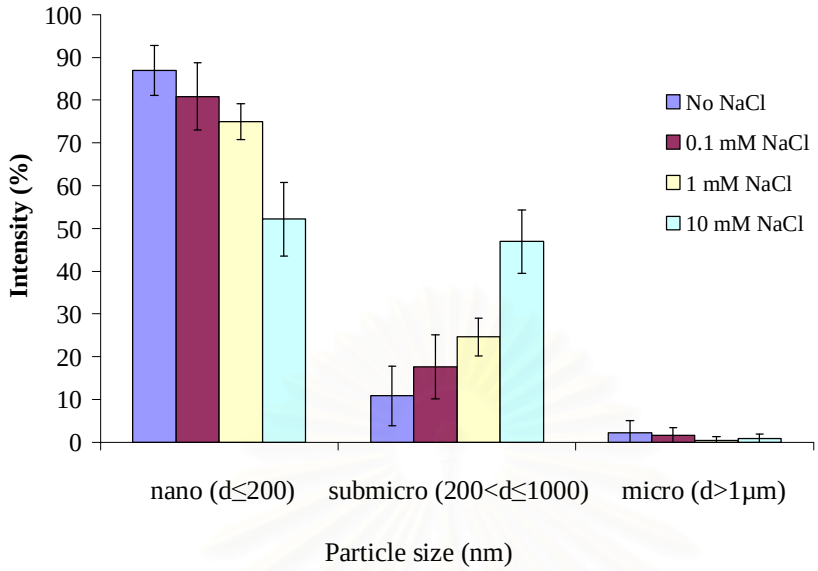



**Figure 3.8** Size distribution of 1 mM poly(PCDA) vesicles prepared in the presence of various concentration of NaCl.

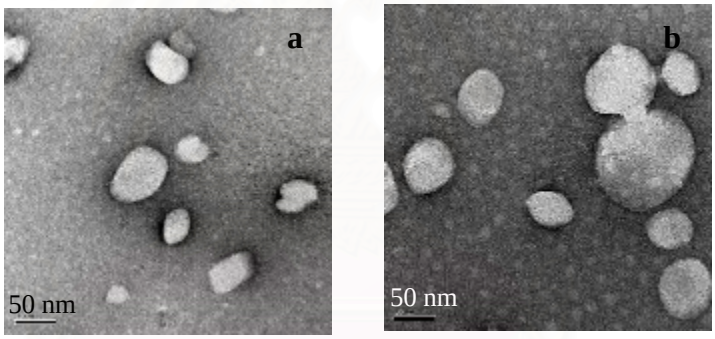



**Figure 3.9** TEM images of dry poly(PCDA) vesicles prepared in the presence of various concentration of NaCl at (a) 0.1 mM NaCl and (b) 10 mM NaCl.

### 3.2.3 Thermochromic response to pH variation

Since all PCDA vesicle solutions prepared in this work were adjusted by various pH before sonication, the electronic absorption spectra of these solutions were recorded in the visible range 400-750 nm. For the sake of simplicity in comparison of the spectra, the absorbance of all solutions was arbitrarily set to zero at 750 nm. At 25°C, all the vesicle solutions appeared blue and showed absorption band in the range of 500-700 nm (Figure 3.10). When the temperature was raised from 25 to 90°C, the color of the vesicle solutions change from blue to red and the absorption bands shift to 400-600 nm range. Although the detail features of the absorption band of each pH of

PCDA vesicles are quite similar, they have quite similar $\lambda_{max}$, ~ 640 nm and ~ 540 nm for blue and red phase PDCA at various pH. The conclusion in this section implied that in various preparing conditions the optical absorption still provided the same $\lambda_{max}$ blue and red phase [9-11,41].

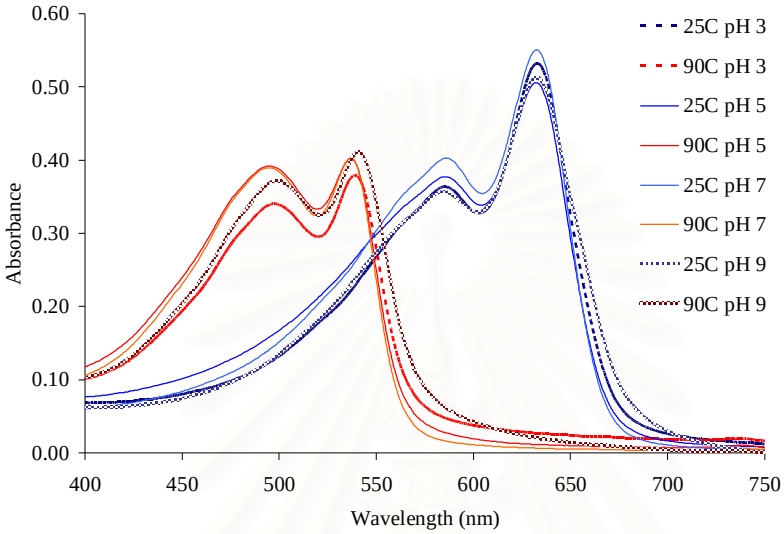



**Figure 3.10** Optical absorption spectra of 1 mM poly(PCDA) blue-phase at 25°C ($I_{max}$= 640 nm) red-phase at 90°C ($I_{max}$= 540 nm) at various pH.

In this section, the thermochromic responses of the prepared poly(PCDA) assemblies in various pH 3,5,7 and 9 were investigated to gain insight the relationship between the pH variation attributes and the thermochromism behaviors. The color transition temperatures (CTT) of 1 mM poly(PCDA) at various pH observed were in the following order: pH 5, 7 < pH 3 < pH 9, suggesting the same order for the strength of the electrostatic repulsion amongst the carboxylic headgroups of poly(PCDA) vesicle. pH 5-7 with less electrostatic repulsions can be successively used to prevent the aggregation of vesicle. The CTT of poly(PCDA) vesicles at pH 5 and 7 were not significantly different from pure poly(PCDA) vesicle. The derivative plot between absorbance blue and temperature obtained from spectrometer with transition temperature range of poly(PCDA) vesicle solution at various pH were shown in figure 3.11. The result indicated that the CTT poly(PCDA) at pH 5,7 however lowered the CTT of poly(PCDA) vesicle at pH 3 and 9 by about 10°C.

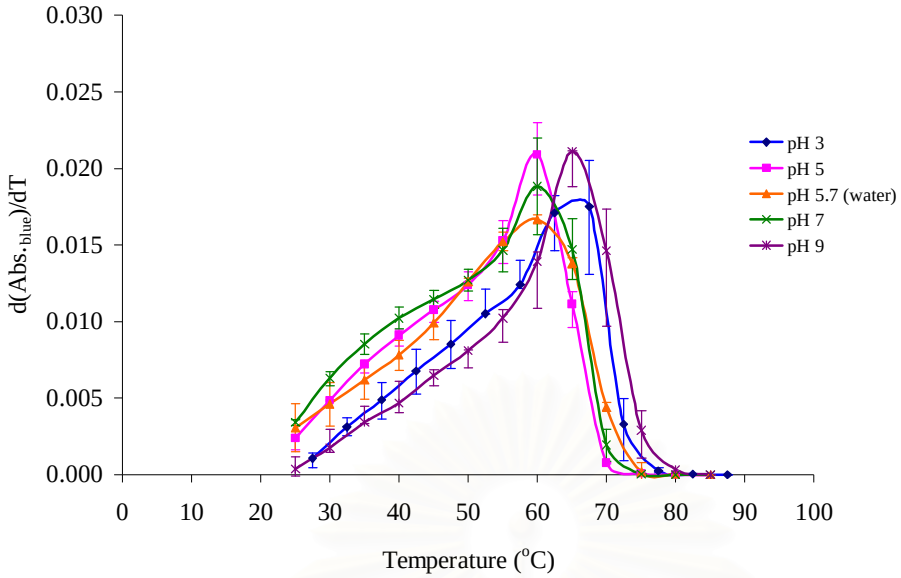



**Figure 3.11** Derivative plots of 1 mM poly (PCDA) vesicle prepared at various pH.

### 3.2.4 Influence of particle sizes to sensitivity of thermochromism

The pH effect towards the particle sizes were demonstrated herein. The particle sizes in range of sub-micro at pH 3,9 provide CTT higher than pH 5-7 which extremely gives particle size in range of nanosize. Therefore, the aim of this experiment was to prove the hypothesis that the influence of particle sizes to sensitivity of thermochromism. These results indicated that the separation of particles sizes by using ultracentrifuge at various centrifugal speeds of 3,000 and 12,000 rpm for an hour effect to the sensitivity of thermochromism (figure 3.12). From DLS data, Z-average size of 3,000 rpm = 69.67±1.30, PDI = 0.51±0.03 and Z-average size of 12,000 rpm = 41.23±0.55, PDI = 0.60±0.05. The particles sizes from DLS correspond with AFM images as shown in figure 3.13. The size distribution after high centrifugal speed should be narrower than low speed. Moreover, the PDI values also indicate the slope of %CR, for example, if PDI lower than 0.5 shows high slope of % CR, it means that self-asssembly into a complete uniform nanostructure (figure 3.14).

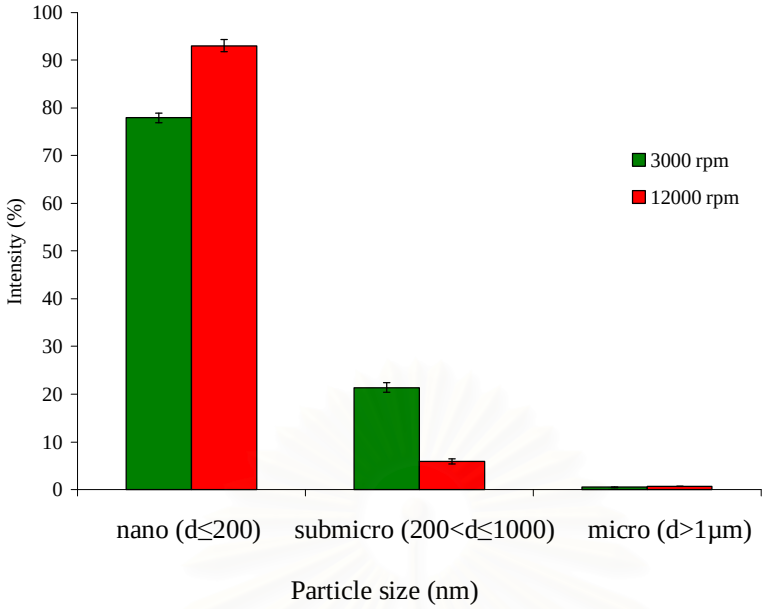
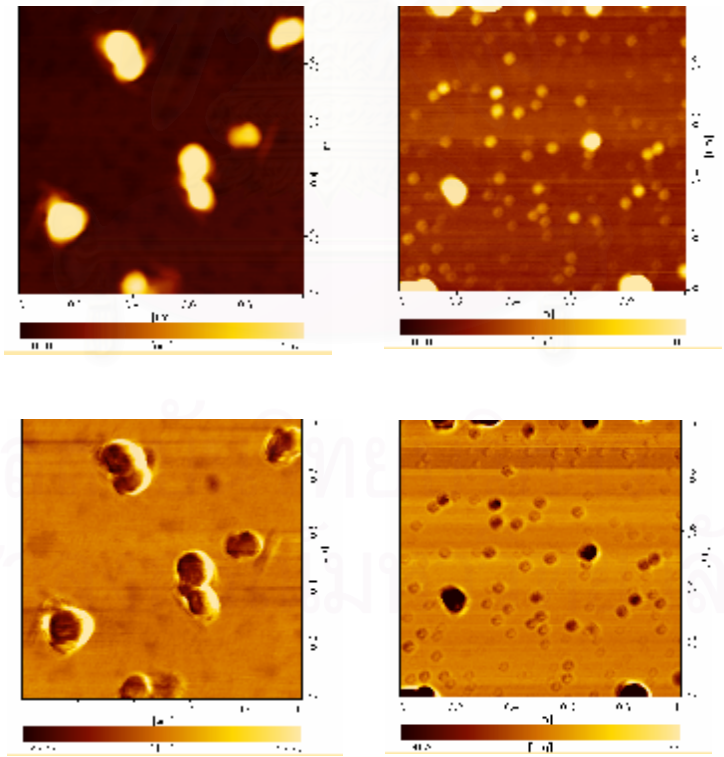



**Figure 3.12** Size distribution of 1 mM poly(PCDA) at various centrifugal speed of 3,000 and 12,000 rpm.



3,000 rpm 12,000 rpm

**Figure 3.13** Morphology (top row) and phase (bottom row) AFM images of dry poly(PCDA) vesicles at various centrifugal speed of 3,000 and 12,000 rpm.

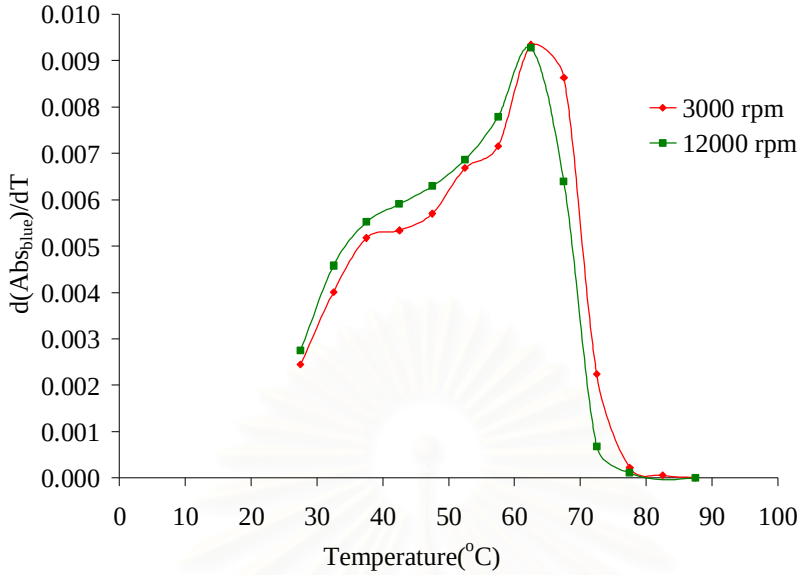



**Figure 3.14** The derivative plots between d(Abs$_{blue}$)/dT and temperature of 1 mM poly(PCDA) at various centrifugal of speed 3000 and 12000 rpm for separate particle size effect to thermochromism.

It was thought that probably due to particle sizes effect to thermochromism, the vesicles with smaller sizes were noted to have slightly higher sensitivity because it turned to red at the temperature lower than 65°C. It is important to control the size distribution of polydiacetylene vesicles when applying in thermal sensing and sharper responses to obtaine vesicles having narrower size distribution [41].

### 3.3 Poly(AEPCDA) assembly in various preparative conditions

Unfortunately, the complexity of these molecules makes it difficult to understand whether headgroup size and charge, chain terminus, or chirality dominates in the nanostructure self-assembly process. The molecular structure of poly(AEPCDA) could determine both the ability to form stable vesicles and the polymerization and chromatic behavior. Most remarkably, UV cross-linked diacetylene display an irreversible blue/red color change dependending on nanovesicle particle size, pH and ionic strength of surrounding aqueous solution. Specific hydrogen-bonding of polydiacetylene modified by ethylenediamine groups in balance with hydrophobic interactions in the alkyl tails was found to be responsible

for these unusual properties, implying that secondary forces rather than chirality can induce the formation of diverse nanovesicles.

It also would be interesting; to synthesize a cationic lipid with a long chain diacetylene tail and a quaternary ammonium salt headgroup in order to provide a molecule capable of acting as both an antimicrobial and a biosensor. As in the research of Lee ***et al.***, synthesis of the quarternary ammonium salt was attempted by directing quaternization to the diacetylene amine unit. The reaction product was found to be a remarkably versatile nanovesicles-forming moiety that when self-assembled could kill cells and induce a visible color change [36].

### 3.3.1 pH variation

The experiment was accordingly proceeded as in 3.2.1. The pH value of initial mixture was measured after the sonication and UV-irradiation (Table 3.2). After sonication, the pH of lipid suspension with high initial value of 7 and 9 dropped significantly to 4.8 and 6.8 indicating the greater dispersion of weak acid AEPCDA molecules in water. As AEPCDA is an ammonium with $pK_a \sim 9$, its dispersion did not affect the pH of the more acidic media (pH 3 and 5). Interestingly, the photopolymerization of the AEPCDA assembly could increase the pH upto 5.8-7.2 indicating that the polymerization reduced the acidity of the AEPCDA assembly. The polymerization probably tightened the hydrogen bonding between the ammonium head group of the lipid chains to become stronger and less likely to ionize. However, no pH change was observed for the mixture at pH 3.0. At this pH, the ammonium head group is hardly ionized and the pH of the media is hence unaffected by the change of acidity of the head group.

**Table 3.2** Appearance and pH at 5°C of 1 mM poly(AEPCDA) dispersed in water at various states sonication time 20 min and irradiation time 5 min in ice bath

| Initial pH | Sonicated pH | Polymerized pH |
|---|---|---|
| 9±0.1 | 6.8±0.2 | 7.2±0.2 |
| 7±0.1 | 4.8±0.2 | 5.8±0.2 |
| 5±0.1 | 4.1±0.1 | 4.7±0.1 |
| 3±0.1 | 3.0±0.1 | 3.5±0.1 |

The sizes of poly(AEPCDA) vesicles that were prepared at various pH from DLS were group into three ranges *i.e.* nano (diameter ≤ 200 nm), submicro (200 < diameter ≤ 1 μm) and micro (diameter > 1 μm) (Figure 3.15). The vesicles prepared at pH 3 were relatively smaller than those obtained at pH 5, 7 and 9. The optimum pH range for preparation nanovesicles of poly(AEPCDA) was hence at pH 3. For the comparison of DLS the trend of poly(AEPCDA) vesicles size were opposite to the results of poly(PCDA) vesicles size. The more acidic or basic conditions may cause charge imbalance at the ammonium groups due to the protonation and deprotonation processes.

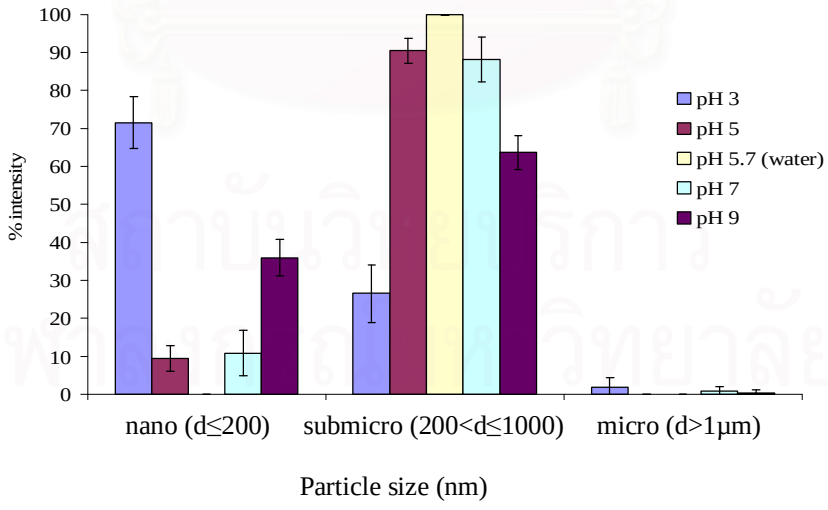


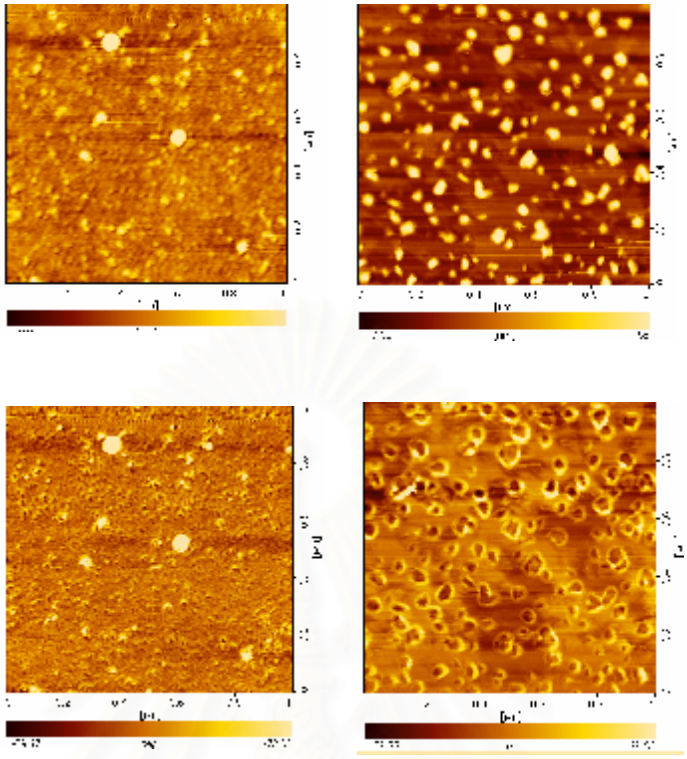

**Figure 3.15** Size distribution of 1 mM poly(AEPCDA) prepared at various pH determined by DLS.

With the acidification of this aqueous solution, pH 3, to the poly(AEPCDA) lipid would be rigidly assembled in very closed packing structure, and by having a

intensed charge repulsion of ammonium groups obtained from the protonation of the $NH_2$ groups on the vesicle surface to form Gaussian curvature. In basicidic aqueous media, pH 9, almost all the ammonium groups of the poly(AEPCDA) vesicles were deprotonated back into amine groups (neutral charged) in which lone pair of amine groups would coordinate with $Na^+$ of NaOH. Increasing in size of the headgroups can reduce the packing parameter and also the size of vesicles. A similar phenomenon had been reported in previous research [44].

Actually there have been many reports on week acids, few of vesicular structures can be formed at other pH (5, 7, and 9) values with the submicron particle sizes (<1000 nm). Like carboxylic acids, the monomers synthesized from ethylenediamine lipids were capable of forming vesicles. In contrast to other diacetylene lipids with acid or amide headgroups, diacetylene lipids with amine assemble in the form of nanotubes. [36,42].

Atomic force microscopy (AFM) was consumed to observe the shape and size of the air-dried poly(AEPCDA) vesicles. The comparison of AFM images between poly(AEPCDA) vesicles at pH 3 and pure poly(PCDA) (Figure 3.16). The AFM results were in slight agreement with the DLS results. The vesicles prepared at pH 3 appeared to be smaller than those prepared at pure poly(AEPCDA) vesicles. The vesicles prepared with no adjustment also appeared to extensively aggregate to greater size.



pH 3 pure vesicle

**Figure 3.16** Morphology (top row) and phase (bottom row) AFM images of dry poly(AEPCDA) vesicles at pH 3 and pure vesicle

### 3.3.2 Variation of ionic strength

The poly(AEPCDA) vesicles with ionic strength have good solubility and stability in the presence of high concentrations of salt. Vesicle dispersions were prepared with 0.1, 1, 10 and 100 mM NaCl to observe the effect of ionic strength on the lipid assembly. As the concentration of NaCl increased, the poly(PCDA) solution became more turbid. From DLS analysis, increasing the ionic strength of the medium contributed to the formation of the larger sized vesicles. As addition of 100 mM NaCl resulted in an increase of submicro size vesicles with an expense of nano-sized vesicles (Figure 3.17). The results could be similarly explained as the consequence of the charge of NaCl concentration as in PCDA case. When the NaCl concentration was increased, less nanovesicles were formed.

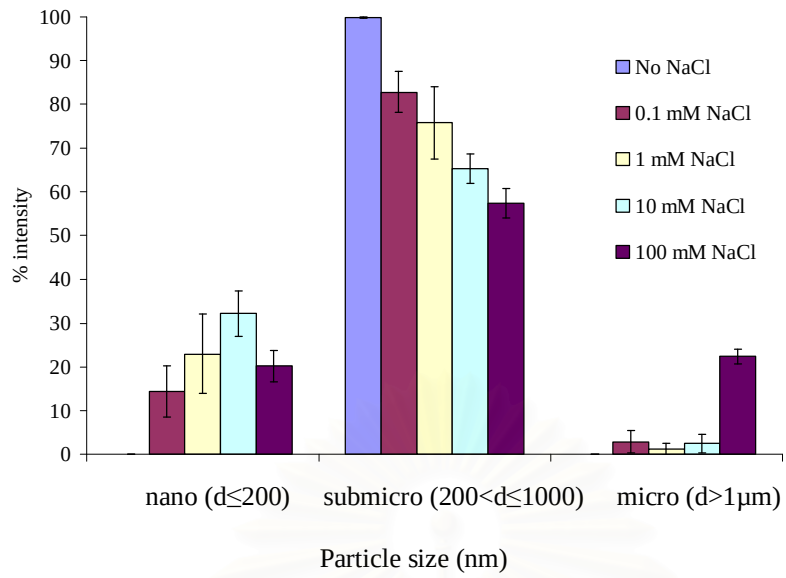



**Figure 3.17** Size distribution of 1 mM poly(AEPCDA) prepared in presence of various concentration of NaCl from DLS data.

However, the effects of salt on the ammonium headgroup good solubility in water and on this characteristic property of surfactant-containing vesicles have not been studied as a function of salt concentration. The turbidity of the vesicle dispersion was increased as additioning amount of NaCl was increased. Based on these results, the fact that NaCl-induced size growth in vesicles solution was limited in media of low ionic strength, the maximal hydrodynamic diameter increased in range of nanoparticle (≤ 200 nm), when the NaCl concentration was raised from zero to 10 mM NaCl. However further increase of the ionic strength up to 100 mM NaCl resulted in relatively smaller effects, forming more micro-sized particle (>1 μm) as indicated by increasing % intensity.

### 3.3.3 pH-ionic strength variation

In order to apply this work to the preparation of biomembrane in biology or use as biosensor for bacteria detection; regarding to our experimental results, the vesicle formation in the preparation of biomembrane should be nicely achieved in the presence of NaCl salt under the pH adjusted conditions. For poly(AEPCDA) was adjusted at pH 3 provided the nanoparticle sizes. Therefore, the preparation of membrane in NaCl salt solution maybe able to control particle size, nanovesicle, and various NaCl concentrations for the optimum condition. The DLS data results were shown in figure 3.18.

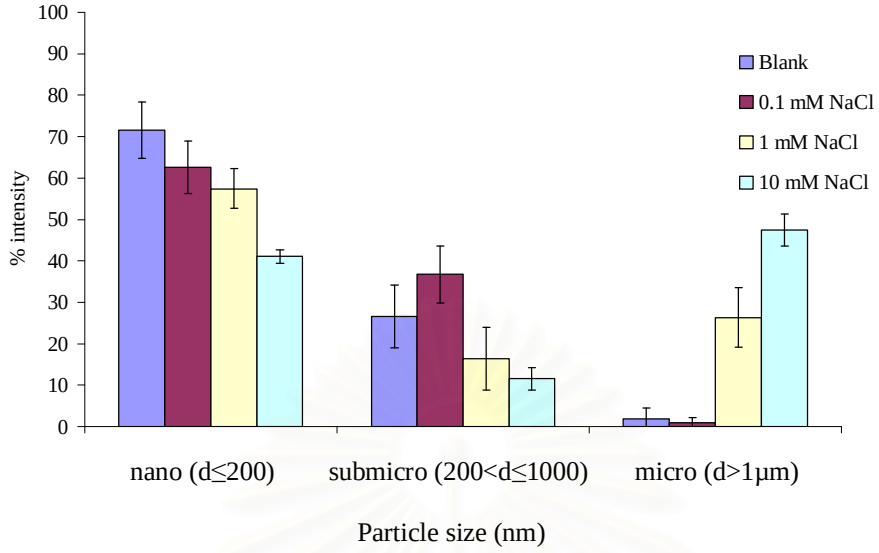
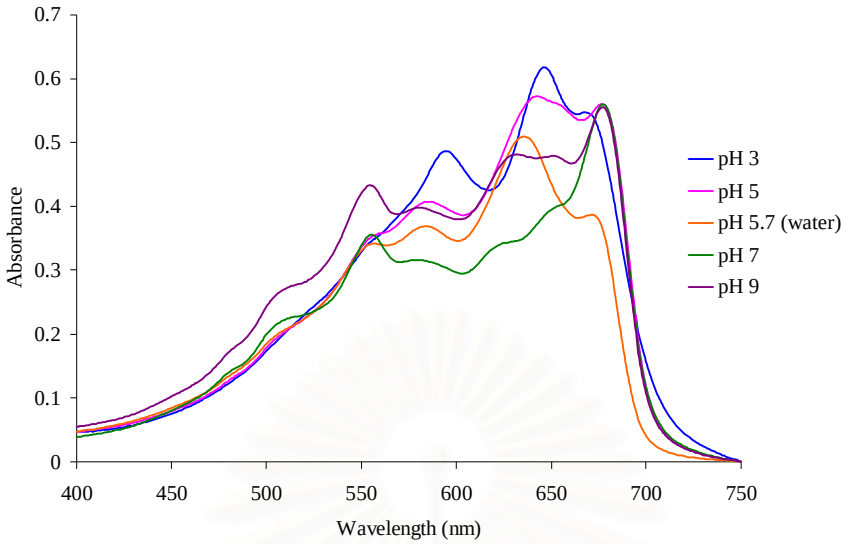



**Figure 3.18** Statistic graph of 1 mM poly(AEPCDA) pH 3 at various concentration of NaCl from DLS data.

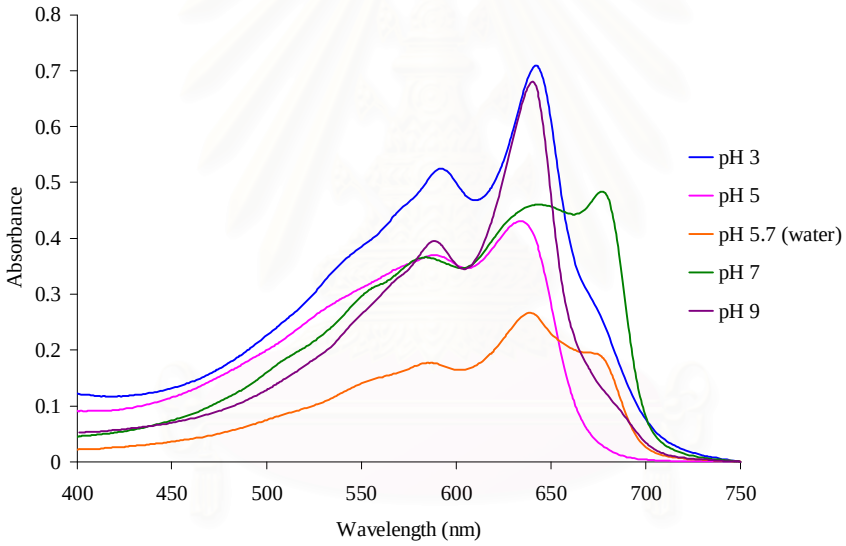
From DLS results, increase concentration of NaCl salt up to 10 mM NaCl showed a slight decrease in nanoparticle size. At 0.1 mM NaCl it was found that although the micro-sized aggregates were less formed, more sub-micro particles were generated. However no experiments could provide a clear reason for size decrease in control.

### 3.3.4 Thermochromic response to pH variation

Since all poly(AEPCDA) vesicle solutions prepared in this work are highly colored, the electronic absorption spectra of these solutions were recorded in the visible range 400-750 nm. For the sake of simplicity in comparison of the spectra, the absorbance of all solutions was arbitrarily set to zero at 750 nm. At 25 °C, all the vesicle solutions appeared blue and showed absorption band in the range of 500-700 nm. The absorption spectrum was not reproducible depending on the preparation conditions and the unstability of poly(AEPCDA) vesicle solution (Figure 3.19 a,b). Although the detail features of the absorption band of each poly(AEPCDA) vesicles are different, having similar $\lambda_{max}$, ~ 640 nm and ~ 540 nm for blue and red phase. The absorbance at each $\lambda_{max}$ was used for the calculation of derivative plots of $d(A_{blue})/dT$.




(a)




(b)

**Figure 3.19** Visible absorption spectra of poly(AEPCDA) at various pH 25°C vesicle solution at (a) first batch and (b) second batch.

The change of the pH the water subphase caused the ionization or deionization of the ethylenediamine headgroups. As a result of repulsive Coulombic interactions, the heaadgroups rearrange themselves to a nonpolar packing mode results in a zigzag polymer backbone. The polymerized liposomes undergo a color change from blue to red under these conditions. The thermochromic behaviors of poly(AEPCDA) show that the red from absorption band started to appear at transition temperature as low as 40°C, it is very unlikely that the side chains would change to a disordered

conformation. The derivative plots blue absorbance and temperature obtained from spectrometer with transition temperature range of poly(AEPCDA) vesicle solution at various pH was shown in figure 3.20. It was found that derivative plot of pH 3 was slower than others pH because pH 3 was nanovesicle size and also close packed more stable difficult to external perturbation that effect to high transition temperature around 40°C.

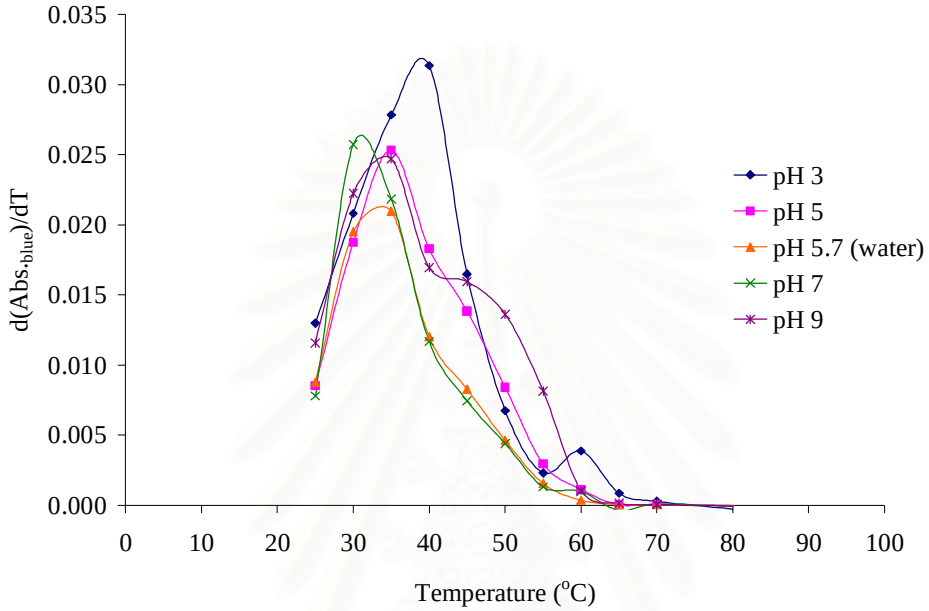



**Figure 3.20**. Derivative plots between $d(Abs_{blue})/dT$ and temperature of poly(AEPCDA) solution at various pH.

### 3.3.5 Chromic response to pH–ionic strength

In this study, it was found that the chromic response (%CR) of poly(AEPCDA) pH 3 in nanoparticle size at various ionic strength concentration [NaCl] had no significant difference between adding and non-adding NaCl. The vesicles formed in the presence of 1 mM NaCl changed faster around 40°C at %CR = 70, however the transition temperatures in the case of non adding, 0.1 and 10 mM NaCl were still higher than 45°C (figure 3.21). However, the effects of salt on the phase behavior and on the characteristic property of surfactant-containing vesicles have not been systematically studied as a function of salt concentration.

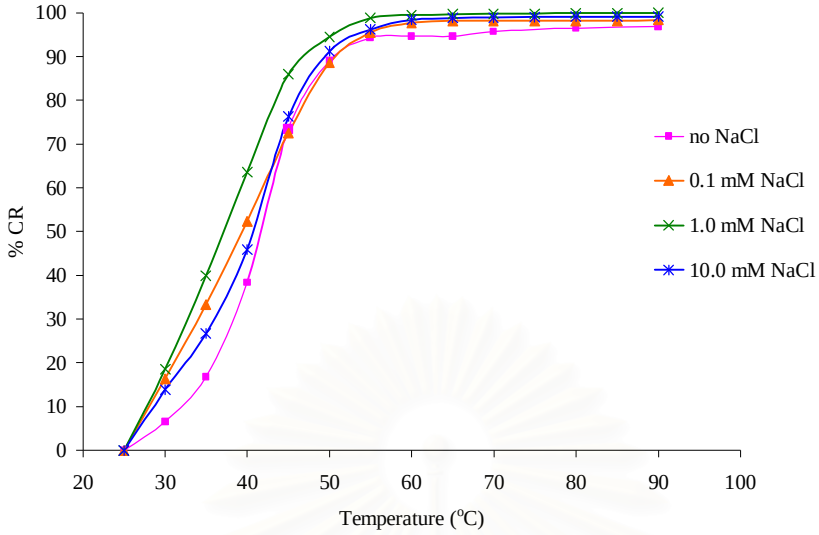



**Figure 3.21** %CR of poly(AEPCDA) pH 3 at various ionic strength concentration [NaCl].

## 3.4 Poly(EBPCDA) assembly in various preparative conditions

EBPCDA was synthesized from inverse addition of the reactants or using lower excess of ethylenediamine resulted in greater formation. Indicate that either the two amide groups or two polymerizable diacetylene units of EBPCDA involve in keeping the polymeric side chain in position and more close-packed, rigid on vesicle. Those imply that the double hydrogen bonds between the diamide groups are responsible for the reversibility of the thermochromism it is not immediately clear why the observed thermochromism for vesicles behaves inversely compare to morphology of other lipids.

### 3.4.1 pH variation

The effect of pH in aqueous medium, conventional fatty acids will form different types of aggregates, depending on the ionization degree of two amide group that can form strongly interacting hydrogen bonds. From the study, the vesicles were found at different sizes at only pH ranges 5,7 and 9 that was no significant differences between pH media and vesicle solution. DLS was used for measuring the average particle sizes and size distribution of the PDA vesicles at various pH dispersed in the solution as shown in figure 3.22. The pH data here in this experiment was adjusted by initial pH and thoughtless polymerized pH (Table 3).

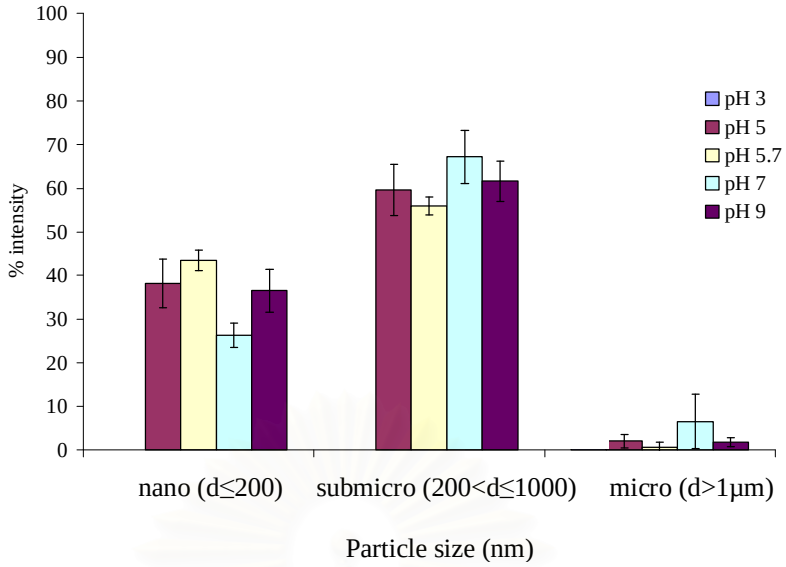



**Figure 3.22** Statistic graph of 1 mM poly(EBPCDA) at various pH from DLS data.

**Table 3.3** Appearance and pH at 25°C of 1 mM poly(EBPCDA) dispersed in water at various states. Sonication time an hour and irradiation time 5 min

| Initial pH | pH after sonication | pH after UV -irradiation |
|---|---|---|
| 9±0.1 | 6.9±0.1 | 7.1±0.1 |
| 7±0.1 | 6.2±0.1 | 6.8±0.2 |
| 5±0.1 | 5.7±0.1 | 6.5±0.2 |
| 3±0.1 | 3.1±0.1 | precipitate |

The appearance between the initial pH and polymerization efficiency that report in the form of three ranges as shown in Table 3.3. These results demonstrated that, after sonication more lipid molecules can be disperse in water and thus their amide headgroups less ionize stability to give protons that make the solution became more acidic (lower pH). Interestingly, the polymerization brought the pH of solution higher because the polymerization was occurred bonding between lipid chains that effect to amide chain ends have less movement can be promoted hydrogen bonding. After polymerization, the vesicle was behaved like buffer in solution owing to the reduction of polymerized pH at high pH lower than initial pH (not reach to the purpose) was occurred.

### 3.4.2 Ionic strength of variation

Increasing the ionic strength of the medium, for any given lipid and NaCl concentrations, resulted in a decrease at 30 % intensity of nanoparticle size from DLS data at 1 mM NaCl as shown in Figure 3.23.

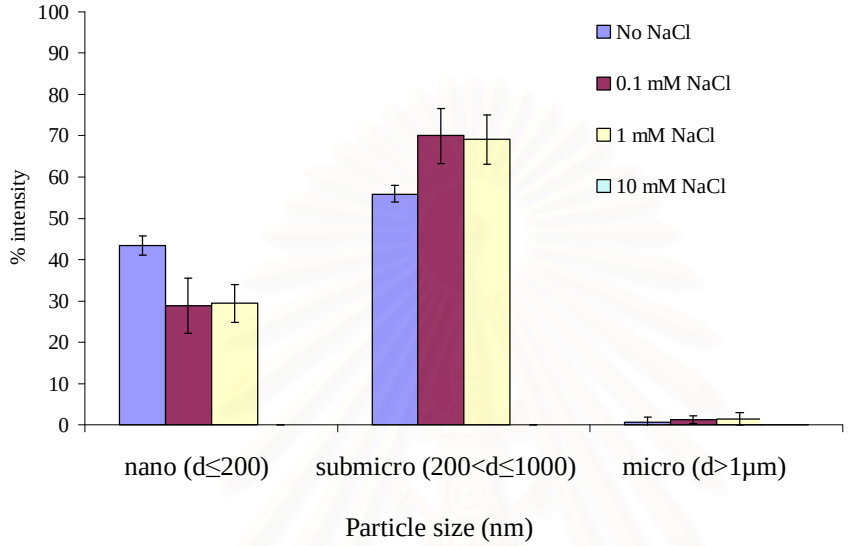



**Figure 3.23** Statistic graph of 1 mM poly(EBPCDA) at various concentration of ionic strength [NaCl] from DLS data.

From the data obtained, it was no significant difference the effect of adding ionic strength concentration an into vesicle solution. Because poly(EBPCDA) have two amide and then strongly interacting hydrogen bonds giving more closed packed and stable that difficult to external perturbations.

### 3.4.3 Thermochromic response to pH variation

The reversible color transition was most likely caused by the slight folding of the polymeric backbone. The side chains remained in an ordered conformation. If strong intermolecular interactions exist between the polar headgroups of the polymer, one can expect the irreversible color change to occur at than in the case of polymers lacking such interactions. Indeed, the observation of the color transition reversibility for poly(EBPCDA) but not for poly(PCDA) and poly(AEPCDA) suggests that either the two amide groups or two polymerizable diacetylene units of EBPCDA involve in keeping the polymeric side chain in position which can allow the return of their original packing.

The hydrogen bonding network of poly(EBPCDA) might be temporarily disrupted during the heating; however, after cooling, the network structure was restored swiftly, leading to the original linear chain like poly(EBPCDA) backbone.

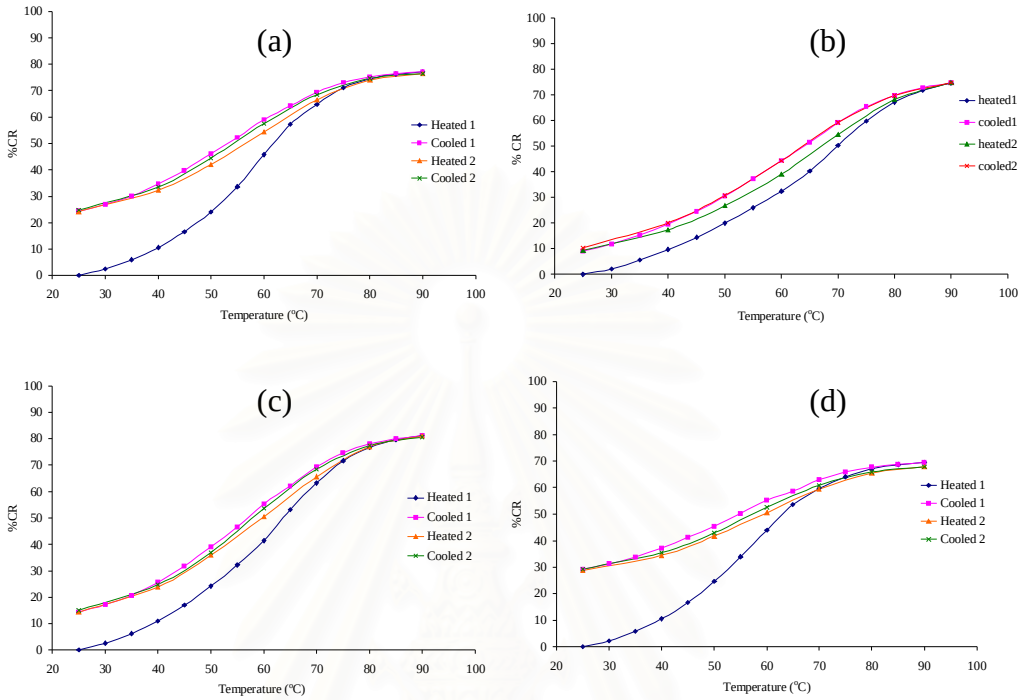



**Figure 3.24** The reversibility of the colorimetric responses of poly(EBPCDA) at various pH vesicle solution subjected to two heating-cooling cycles (a) pH 5, (b) pH 5.7 (water), (c) pH 7 and (d) pH 9.

Only the reversibility of the thermochromism of poly(EBPCDA) vesicle solution pH 5.7 (water) and pH 7 will be described here some thermochromic in the first heating-cooling cycle. The second cycles however showed complete reversibility of the %CR (Figure 3.24). The results imply that the double hydrogen bonds between the diamide group are responsible for the reversibility of the thermochromism of both pH 5.7 (water) and pH 7 vesicles while the polymerization of an extra diacetylene unit in EBPCDA is responsible for the lower solvent memory that means of complete thermal hysteresis. On the other hand, pH 3 and pH 9 do not revert completely to the initial state (blue phase).

## 3.5 Comparison of preparation of differnce type of monomers

### 3.5.1 Preparation of polydiacetylene vesicles

All diacetylene monomers (PCDA, AEPCDA and EBPCDA) were prepared in the form of vesicles in water by using sonicator bath (230 watt) at 75-85 °C. Only PCDA and AEPCDA but not EBPCDA dispersed well in water to form semitransparent or transparent colloids without discernible lipid suspension at this temperature range. Poorer dispersion of EBPCDA is probably due to its high melting point (120-125°C). Dispersion of EBPCDA could be improved by preheating its suspension in boiling water prior to the sonication. After keeping the colloids of the lipids at 4 °C overnight and irradiated with UV light (254 nm) for 5 min at room temperature and filtered through filter paper (No.1) or cotton filter to give blue solutions indicating that the vesicle lipid readily undergoes photopolymerization to form ene-yne alternated polymer chains. The blue color of PCDA and AEPCDA were deeper than that of EBPCDA suggesting higher polymerized vesicle concentration of PCDA and AEPCDA comparing to EBPCDA. The blue color of the vesicle solution of AEPCDA changed to purple or red during 2 min of the irradiation period. Therefore, the UV irradiation of AEPCDA vesicle solution was conducted in an ice bath that provided a clear intense blue solution of poly(AEPCDA) vesicles.

To study the efficiency of poly(PCDA) nanovesicle formation at various lipid initial concentrations. After photopolymerization, the blue poly(PCDA) vesicle solution was filtered to remove undesired lipid aggregate. The experiment was performed in duplicate at each concentration. As the initial concentration of the monomeric lipid increased from 0.5 and 1.0 mM, the lower efficiency of the nanovesicle formation was obtained due to higher tendency of aggregation at higher lipid concentration. At more than 1.0 mM, 2.0, 4.0, 8.0 and 16.0 mM, it would be sub-micro nanoparticle vesicles according to the dynamic light scattering data, % intensity statistic graph, as shown in Figure 3.25. The results also show that the total transformation of PCDA into to poly(PCDA) can be obtained at 1.0 mM. Therefore, the lipid concentration of 1.0 mM was used and the quantitative conversion was assumed in all subsequent experiments unless specified.

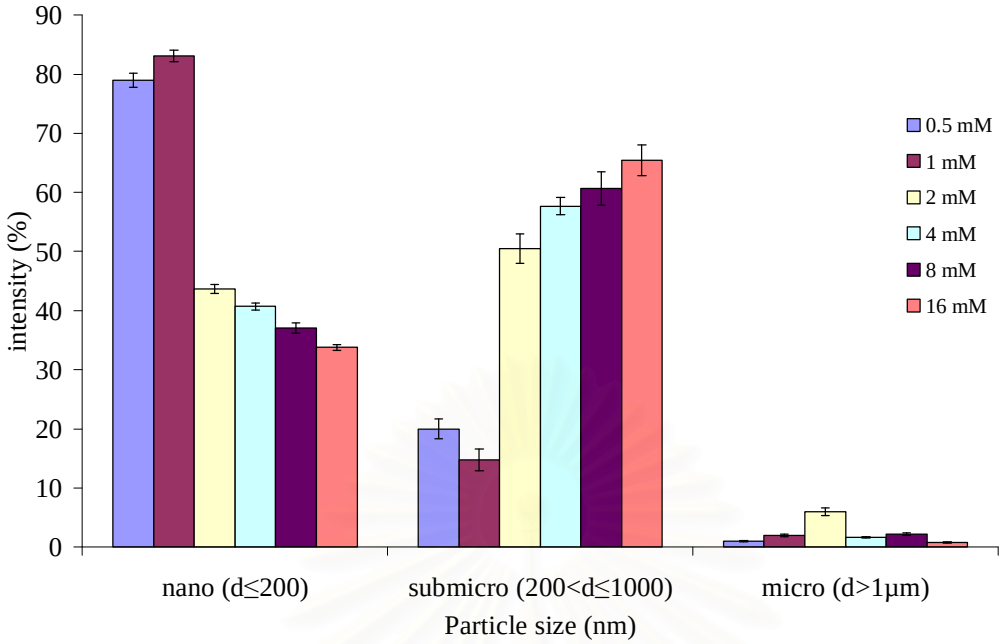



**Figure 3.25** Statistic graph of poly(PCDA) at various concentration from DLS data.

For the poorer dispersion of EBPCDA could be improved by preheating its suspension in boiling water prior to the sonication. Photopolymerization gave light blue poly(EBPCDA) vesicle suggesting lower polymerized vesicle concentration of EBPCDA comparing to PCDA and AEPCDA. To study the efficiency of poly(EBPCDA) vesicle formation at various lipid initial concentrations. After photopolymerization, the blue poly(EBPCDA) vesicle solution was filtered to remove undesired lipid aggregate. The experiment was performed in duplicate at each concentration. As the initial concentration of the monomeric lipid increased from 0.2, 0.5 and 1.0mM, the lower efficiency of the nanovesicle formation (≤200nm) was obtained due to higher tendency of aggregation at higher lipid concentration. At more than 0.5mM, it would be sub-micro nanoparticle vesicles (200<diameter≤1000 nm) according to the dynamic light scattering data, % intensity statistic graph, as shown in Figure 3.26. The results also showed that the total transformation of EBPCDA into to poly(EBPCDA) can be obtained at 0.5 mM. The lipid concentration of 1.0 mM was used and the quantitative conversion was assumed in all subsequent experiments unless specified.

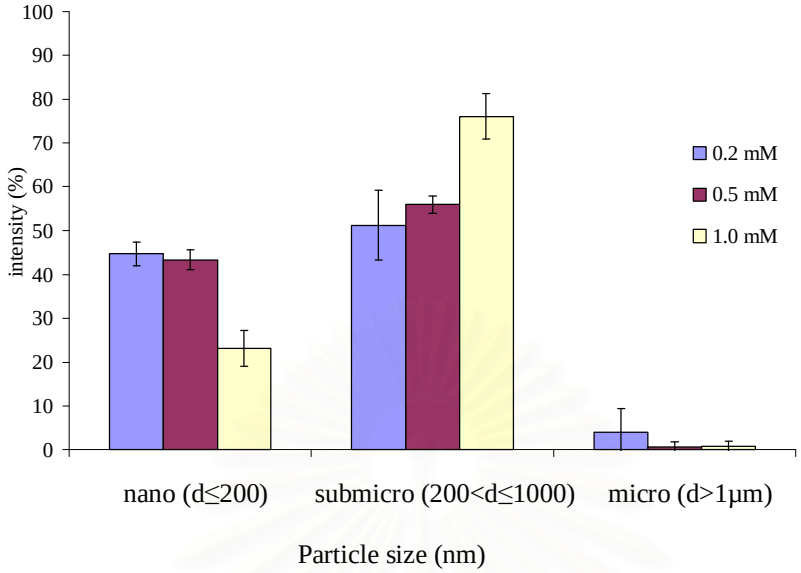



**Figure 3.26** Statistic graph of poly(EBPCDA) at various concentration from DLS Data.

### 3.5.2 Comparison pH-sensitive aggregation behavior

Zeta potential is one of the main forces that mediate interparticle interactions. Particles with a high zeta potential of the same charge sign, either positive or negative, will repel each other. Conventionally a high zeta potential can be high in a positive or negative sense, i.e. <-30mV and >+30mV would both be considered as high zeta potentials. For molecules and particles that are small enough, and of low enough density to remain in suspension, a high zeta potential will confer stability, i.e. the solution or dispersion will resist aggregation.

The aqueous stability at various pH and solubility of vesicles were related to the surface charge density of particles, which can be evaluated by zeta potential measurements as shown (Figure 3.27) After polymerization, the vesicle was behaved like buffer in solution owing to the reduction of polymerized pH at high pH lower than initial pH (not reach to the purpose) was occurred. In case of titration by 0.1 mM NaOH into vesicle solution after polymerization started with pH 3 until pH 9 was found that poly(AEPCDA), the positive charge on surface decrease but for ploy(PCDA) the negative charge on surface increase and the vesicle solution change from blue to purple color but not precipitate (figure 3.27 (b)).

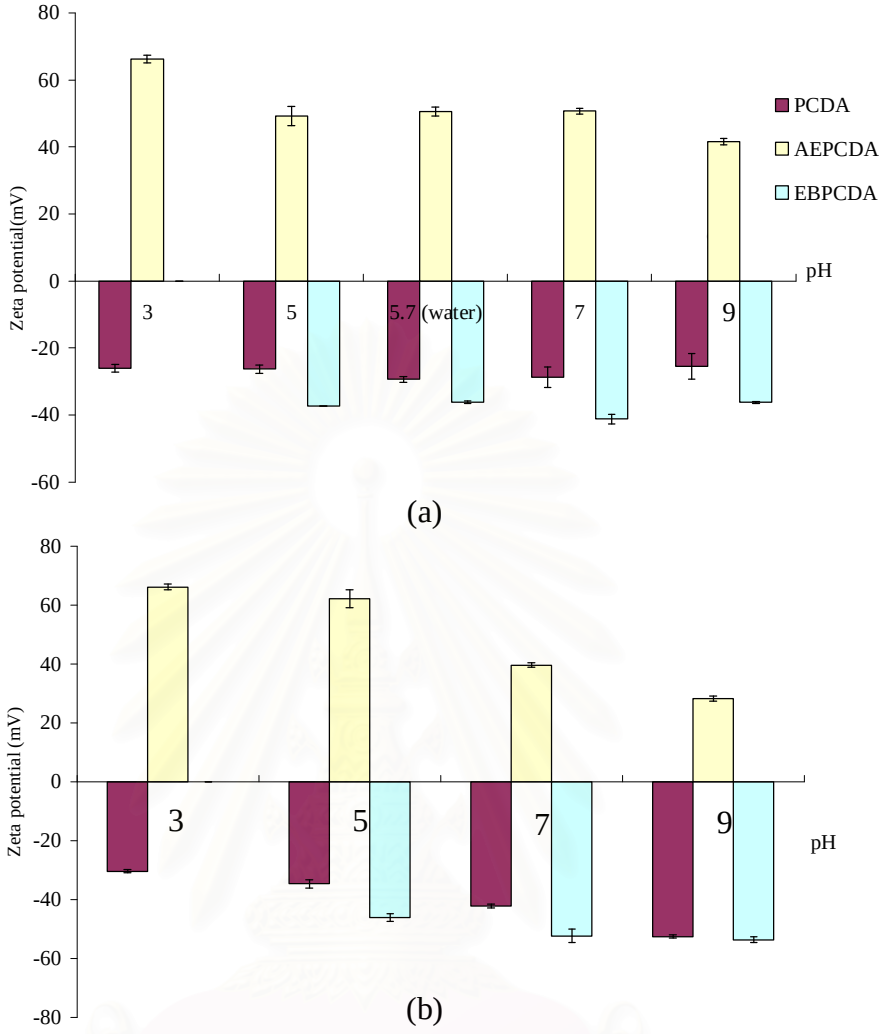



**Figure 3.27** Zeta potential of polydiacetylene vesicles as functions of pH at 25°C (a) adjust pH before sonicate and (b) titrate by 0.1 M NaOH after Polymerized.

For poly(AEPCDA) have positive charged on surface electrical properties were investigated at various pHs. Strong interaction between amine headgroup caused a positively charged vesicle solution. The zeta potential decreased significant at pH 9 as shown in Figure 3.26. The pH sensitivity of poly(AEPCDA) vesicle under basic condition by adjusted 0.1 M NaOH was originated from deprotonation of amine headgroup which lead to partial collapse of vesicle structure unfavorable contact with water.

Both poly(PCDA) and poly(EBPCDA) also have negative charged on vesicles surface. In general, a more negative zeta potential was observed at higher pH due to the deprotonation of COOH groups on vesicles. The trend of zeta potential and

particle stability did not differ significantly at various pH. For poly(PCDA) have negative zeta potential lower than poly(EBPCDA) because poly(PCDA) have only one carboxylic headgroup but poly(EBPCDA) have two amide headgroups or two polymerizable diacetylene units. Poly(EBPCDA) is stable structure which can increase repulsion between vesicles and its surroundings have been shown to compact vesicles when pH increase. Most significally, spontaneous poly(EBPCDA) self-assembly and aggregation process have been shown to result from the confinement of large amounts of vesicles at pH 3.

### 3.5.3 Effect of physical properties to thermochromism behavior

Differential scanning calorimetry (DSC) is used to measure the main phase transition ($T_m$). The existence of a phase transition also provides additional evidence for the presence of ordered structures. The $T_m$ represents the transition between a phase with closely packed (all trans) conformation of the lipid chains to a more disordered phase with the lipid chains rotating out of the all-trans conformation. The diacetylenic lipids that formed vesicles exhibited a $T_m$ in poly(PCDA) in the range of 62-64 °C, poly(AEPCDA) in the range of 65-72 °C and poly(EBPCDA) in the range of 120-123 °C. After heating the lipid suspension above $T_m$, the vesicle solution must be cooled to allow the formation of the solid-analogous phase and subsequent polymerization. DSC diagrams as shown in appendixes A.

# CHAPTER IV

# CONCLUSION AND SUGGESTION

## 4.1 Conclusion

Two derivatives of 10,12-pentacosadiynoic acid (PCDA), i.e. N-(2-aminoethyl)pentacosa-10,12-diynamide (AEPCDA) and N,N'-ethylenebispentacosa-10,12-diynamide (EBPCDA) were selectively synthesized from PCDA. Polydiacetylene nanovesicles can be successfully prepared, according to the optimum conditions, from the suspension of these diacetylene lipids in water and photopolymerized to form blue solutions of poly(PCDA), poly(AEPCDA) and poly(EBPCDA) vesicles. The prepared polydiacetylene vesicles were in the range of nanovesicles (<200 nm) by various pH; pH 5-7 for poly(PCDA), and pH 3 for poly(AEPCDA). For ionic strength, there was no significant difference of effect of various NaCl concentration in poly(PCDA) and its amide derivatives, so the required NaCl concentration for biomembrane preparation would be in a range of 0.1 mM-1 mM NaCl. The various conditions for poly(EBPCDA) were ineffective because the stable and closed pack structure of poly(EBPCDA) to the external perturbations was tolerant. The study of thermochromism properties were also investigated by colorimetric method using a temperature controlled UV-Vis spectrometer. Accordingly the results indicate that the particle size had a close relation to the transition temperature. In poly(PCDA) at initial pH 5-7 nanovesicles had lower transition temperature than others. In contrast, poly(AEPCDA) at pH 3 nanovesicle had higher transition temperature than other, probably due to the effect of charged at headgroup that was protonated or deprotonated in difference environment for each monomer. Moreover, the color transition of poly(EBPCDA) exhibited excellent reversibility at initial pH 5-7 while those of poly(PCDA) and poly(AEPCDA) were irreversible. These optimum conditions would become the useful applicational source for biosensors and thermochromic sensing device.

## 4.2 Suggestion for future work

The results from this thesis provide some insight for logical design of self – assembly for other monomers. For examples, varying length of alkyl chain or lipid chain length or changing functional head groups e.g. alcohol, amine and aromatics compounds, which directly effect to the packing parameter. Moreover, the extensive study of diacetylene self-assembly may include bolaamphiphilic which can form or pack differently from amphiphilic lipid molecules. Also the study of the optimum conditions for preparing of these molecules would be needed.

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# APPENDICES

**Appendix A: DSC diagram of synthesized monomers**

**Figure A1:** DSC diagram of PCDA monomers

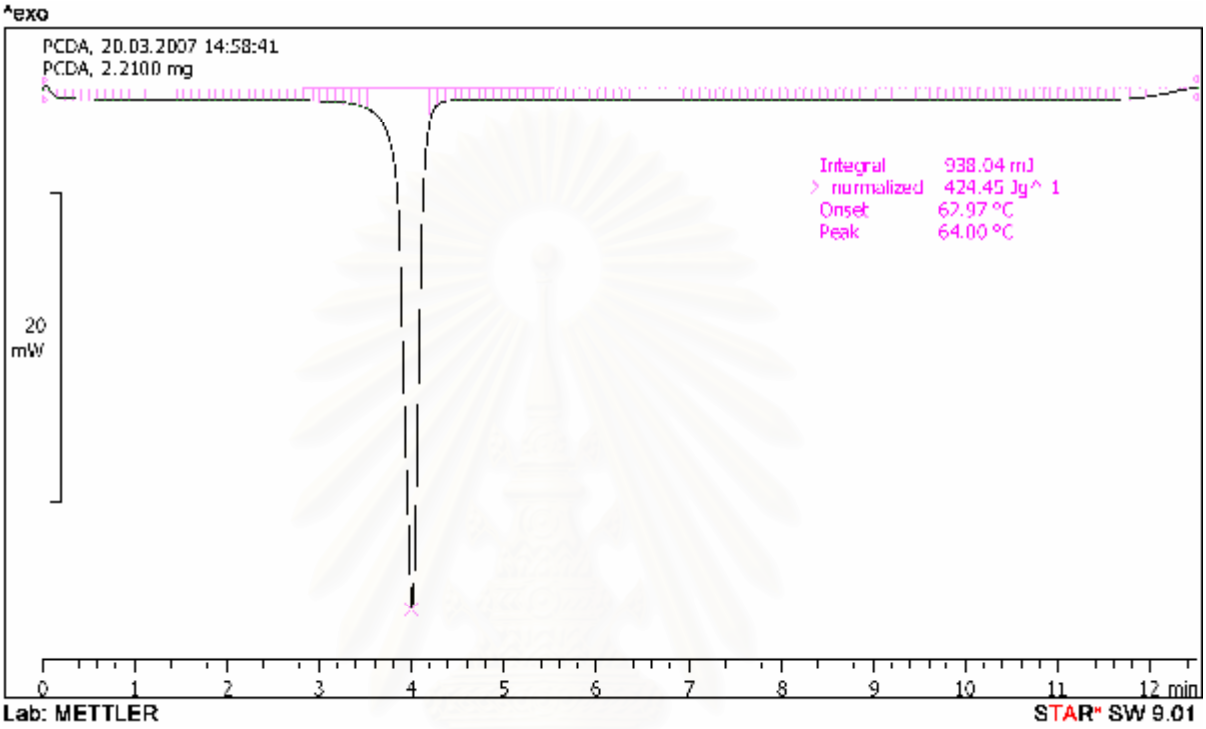


**Figure A2:** DSC diagram of AEPCDA monomers

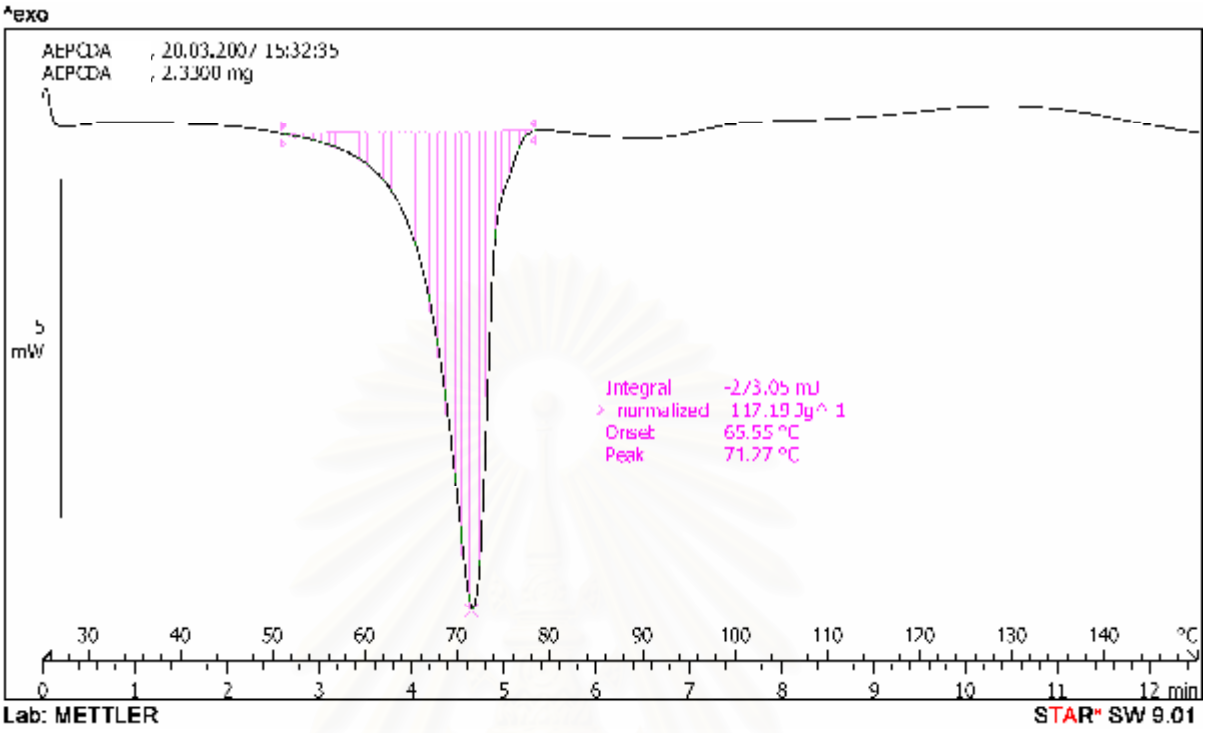
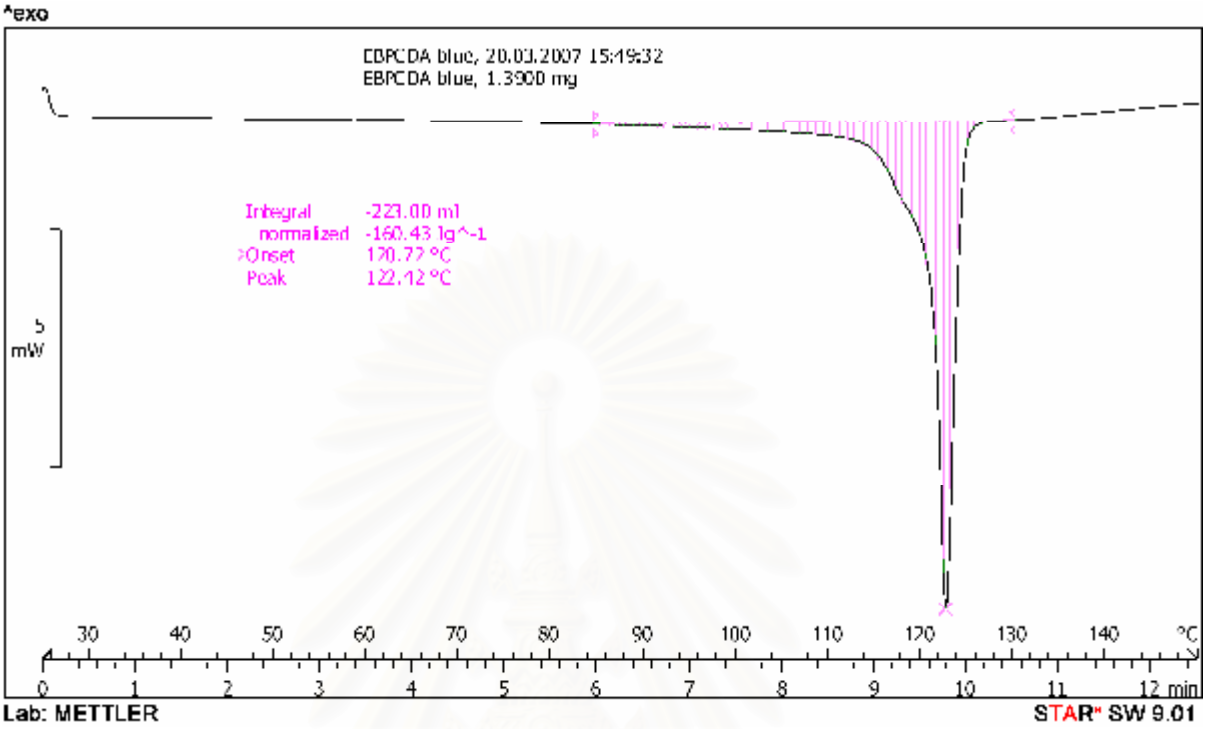
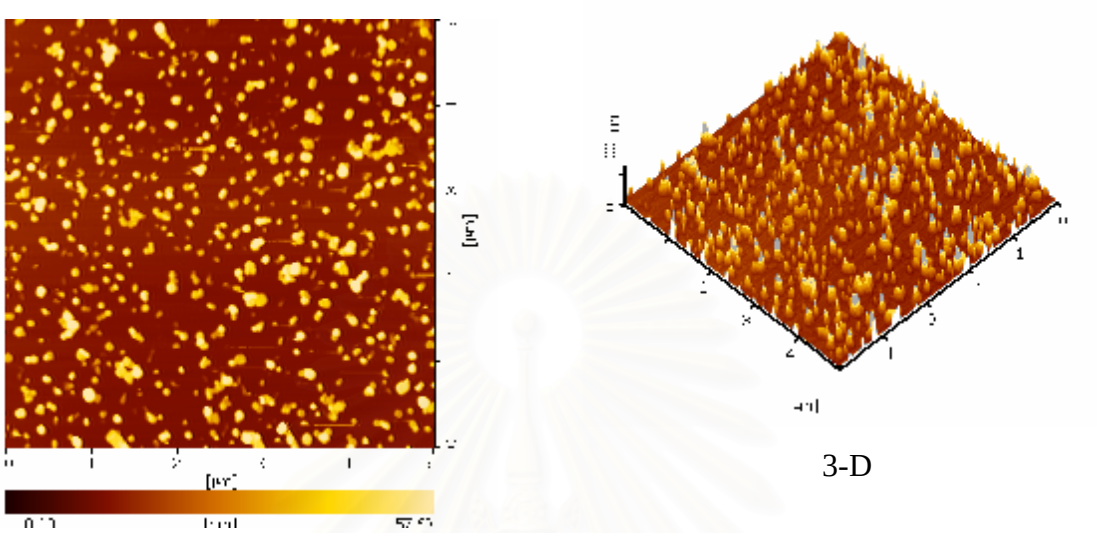


**Figure A3:** DSC diagram of EBPCDA monomers



## Appendix B: AFM images of poly(PCDA) at various pH

**Figure B1:** AFM images of poly(PCDA) at pH 3



3-D

Scan area at 5 µm

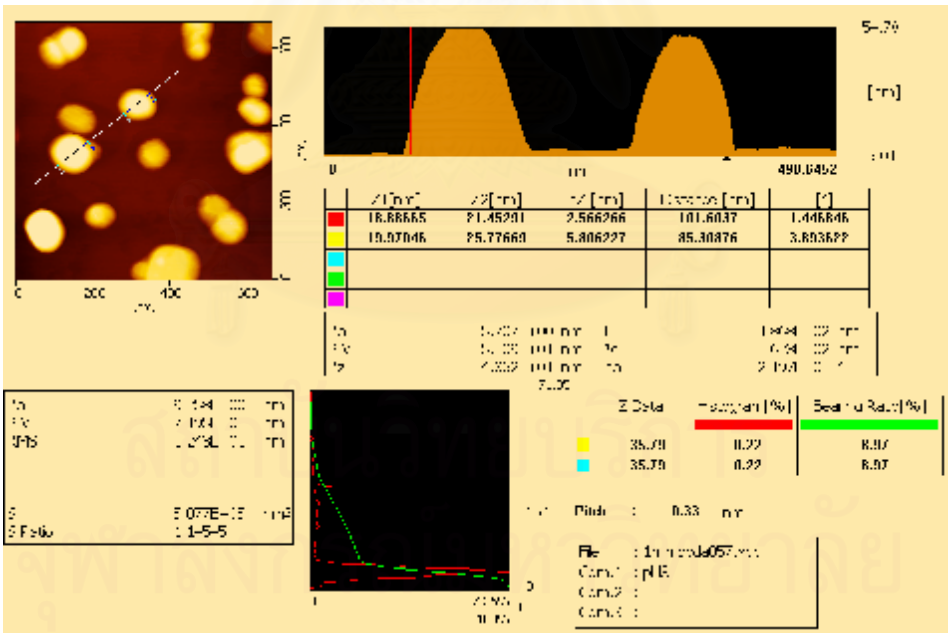
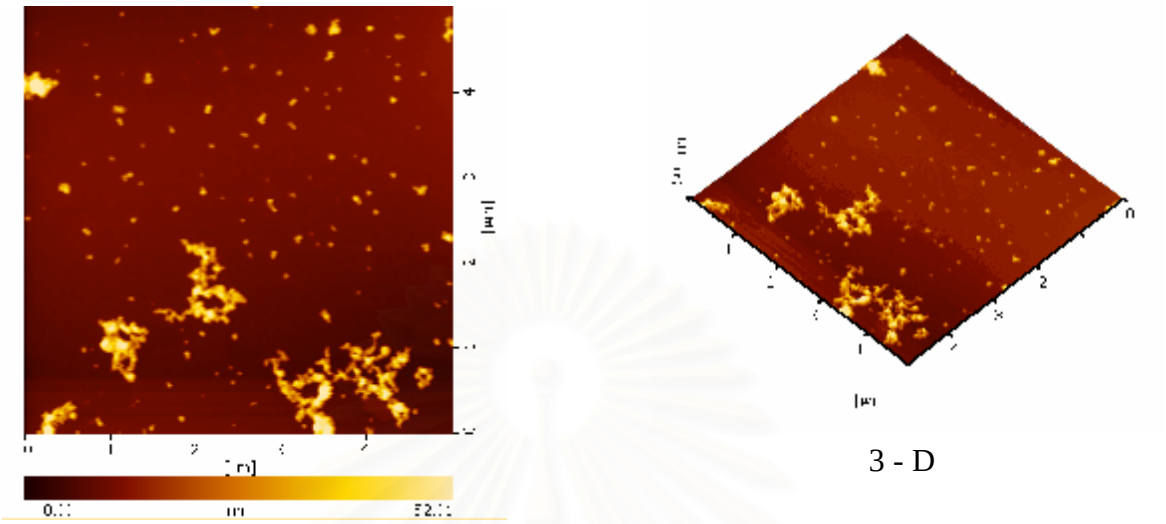


**Figure B2:** AFM images of poly(PCDA) at pH 5



3 - D

Scan area at 5 μm

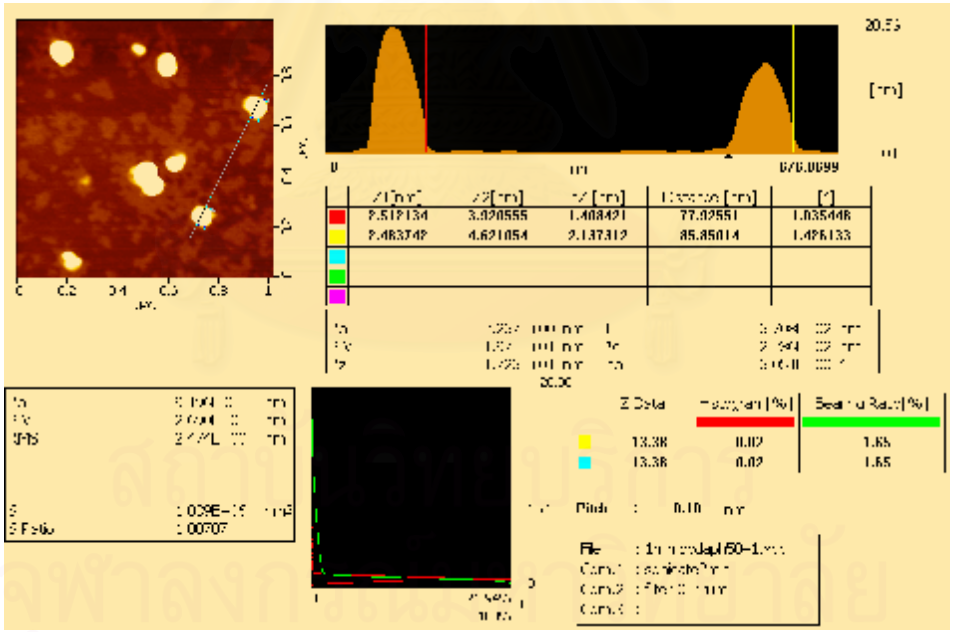
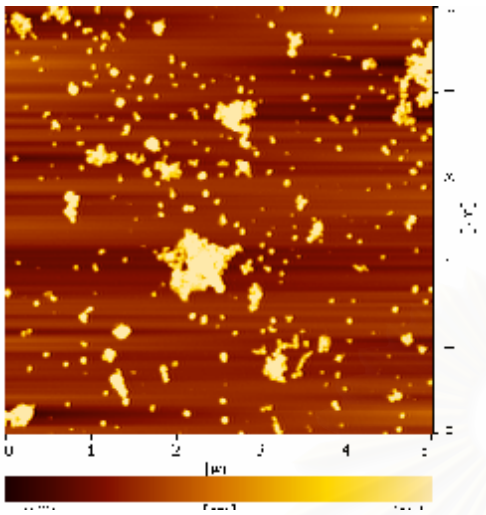
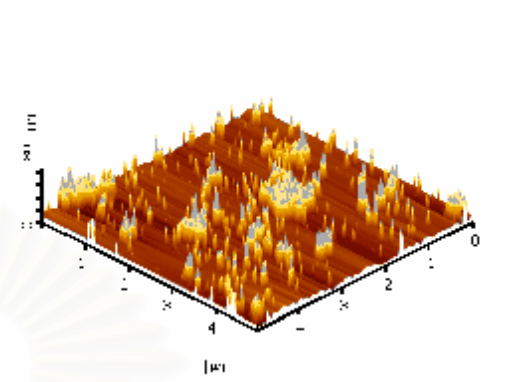


**Figure B3:** AFM images of poly(PCDA) at pH 7





3 - D

Scan area at 5 µm

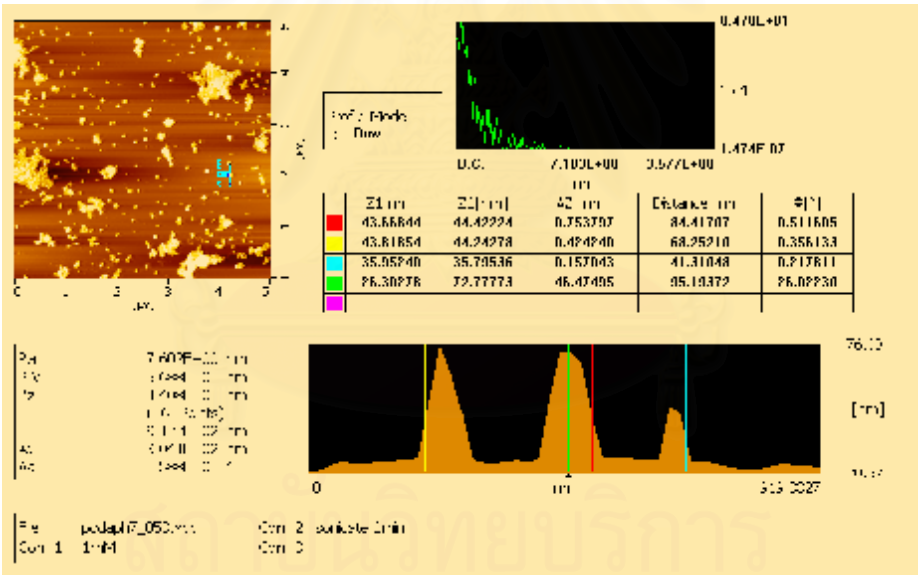
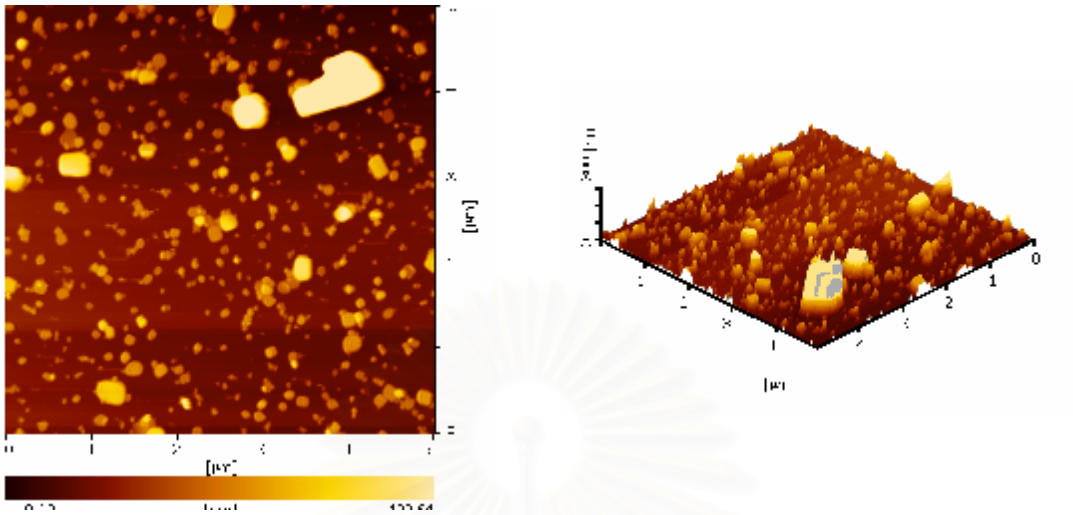
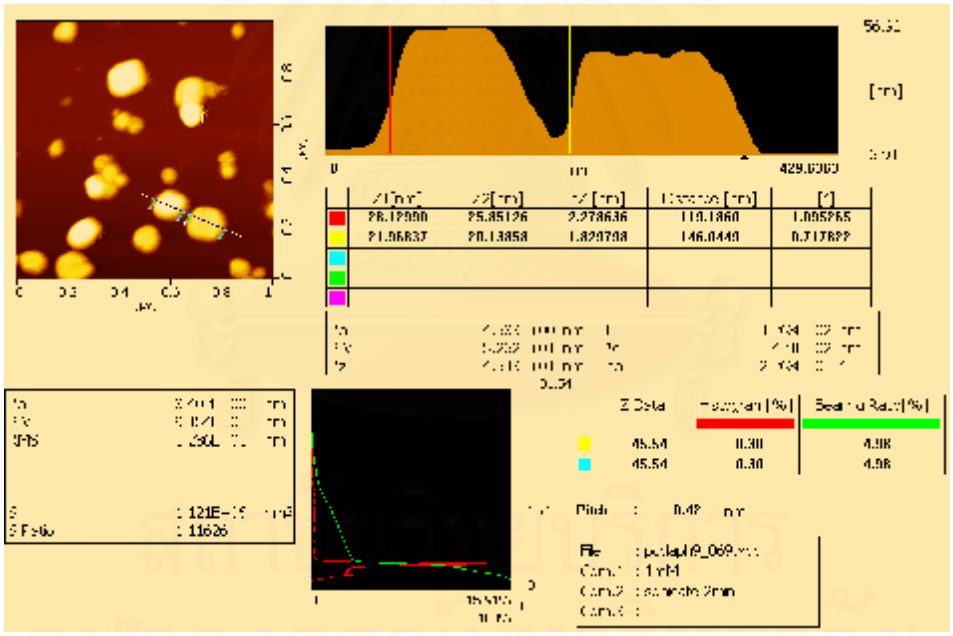


**Figure B4:** AFM images of poly(PCDA) at pH 9

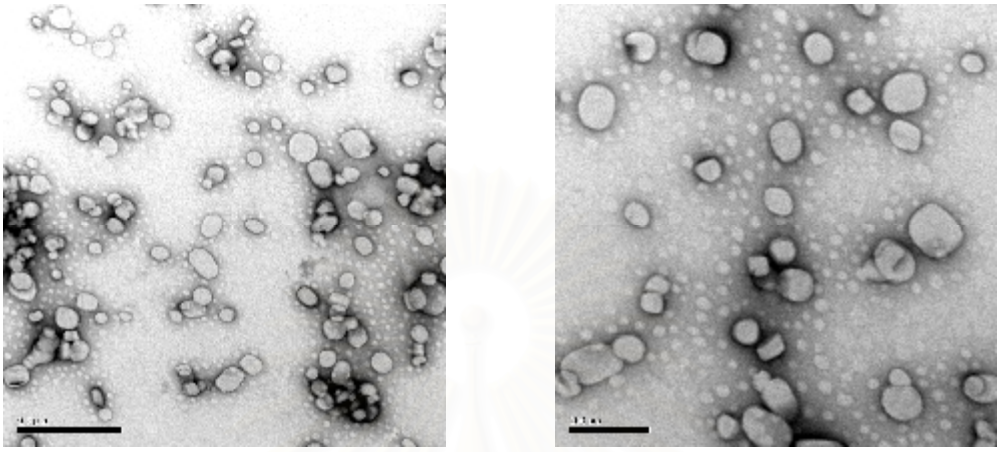


Scan area at 5 µm



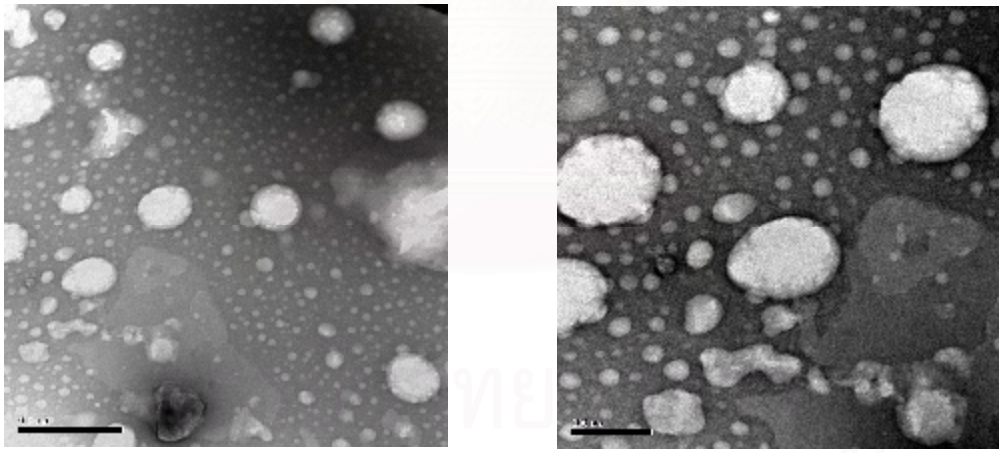
## Appendix C: TEM images of poly(PCDA) at various pH

**Figure C1:** TEM images of poly(PCDA) at pH 3



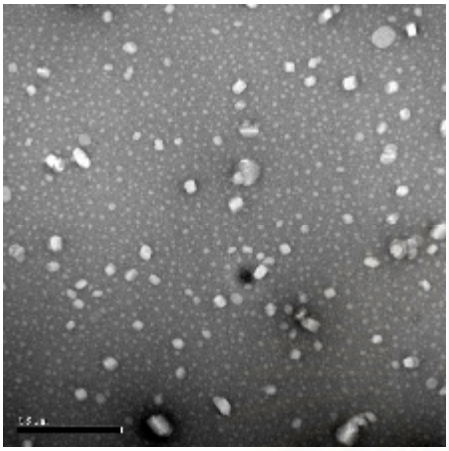
Scale bar 0.5 μm Scale bar 200 nm

**Figure C2:** TEM images of poly(PCDA) at pH 5

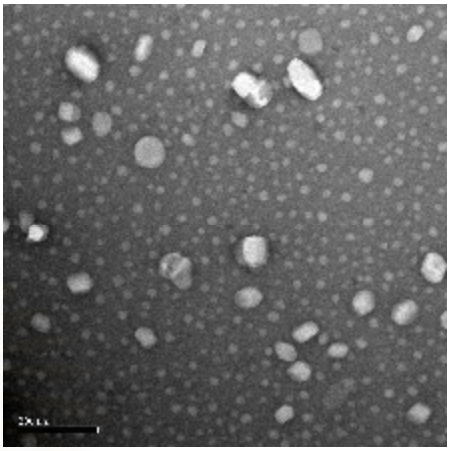


Scale bar 0.5 μm Scale bar 200 nm

**Figure C3:** TEM images of poly(PCDA) at pH 7

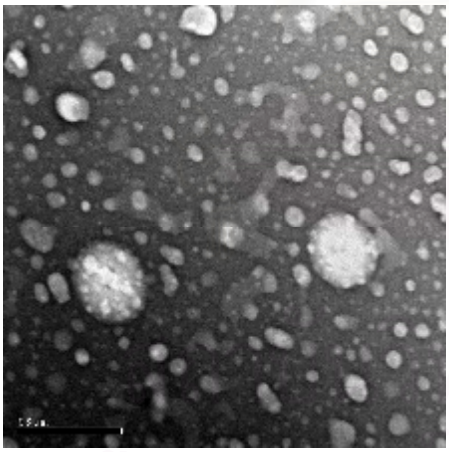


Scale bar 0.5 μm

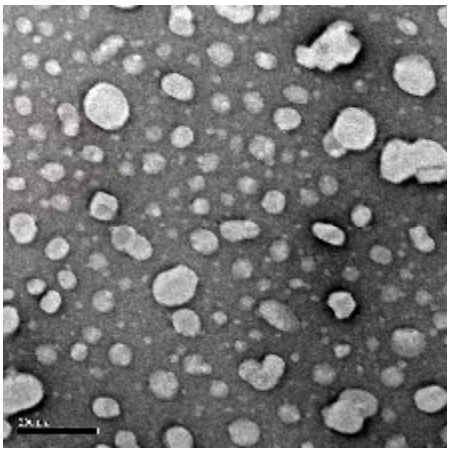


Scale bar 200 nm

**Figure C4:** TEM images of poly(PCDA) at pH 9



Scale bar 0.5 μm



Scale bar 200 nm

**Appendix D: Visible absorption spectra of poly(PCDA) vesicle solution at various pH upon heating process**

**Figure D1:** Visible absorption spectra of poly(PCDA) vesicle solution at pH 3

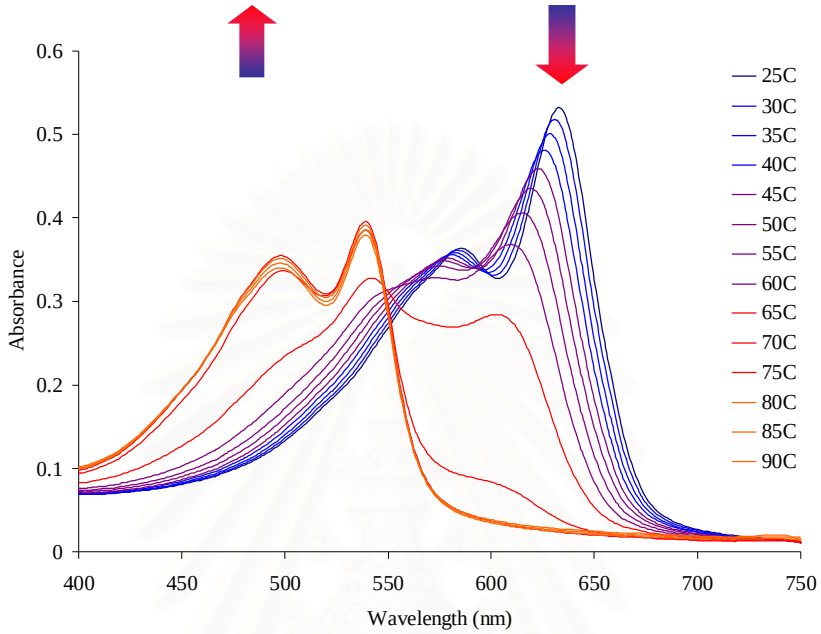



**Figure D2:** Visible absorption spectra of poly(PCDA) vesicle solution at pH 5

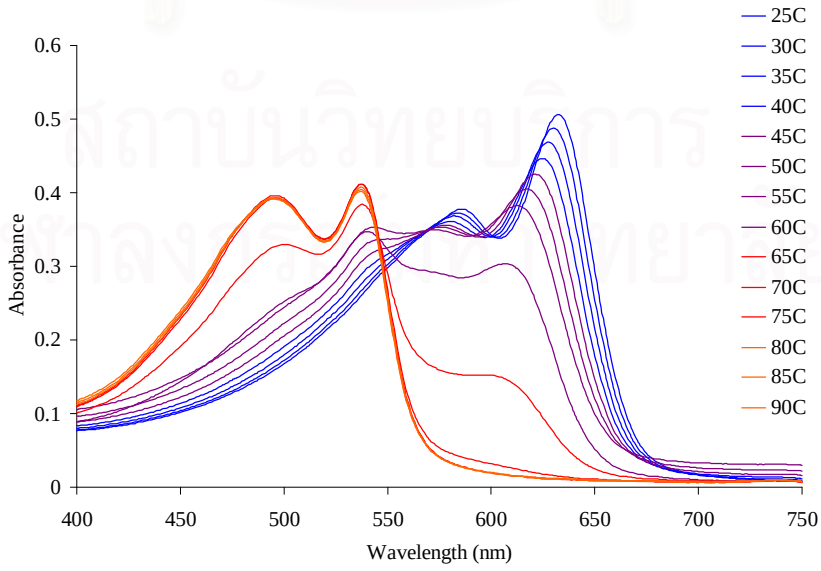


**Figure D3:** Visible absorption spectra of poly(PCDA) vesicle solution at pH 7

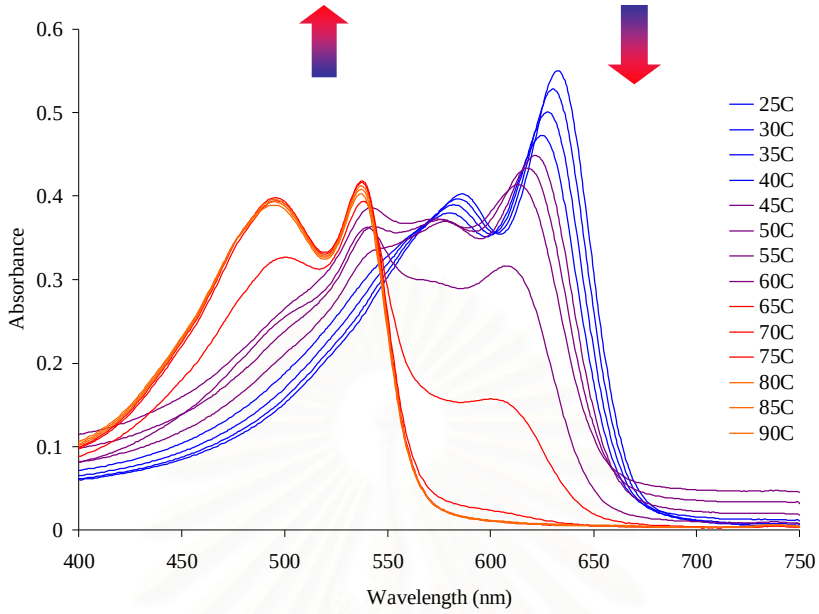
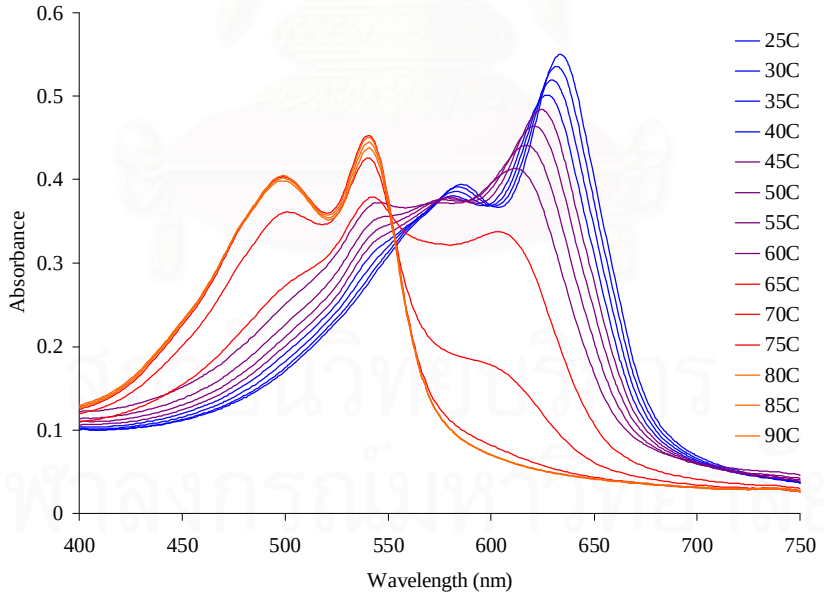



**Figure D4:** Visible absorption spectra of poly(PCDA) vesicle solution at pH 9



## Appendix E: Visible absorption spectra of poly(AEPCDA) vesicle solution at various pH upon heating process

**Figure E1:** Visible absorption spectra of poly(AEPCDA) vesicle solution at pH 3

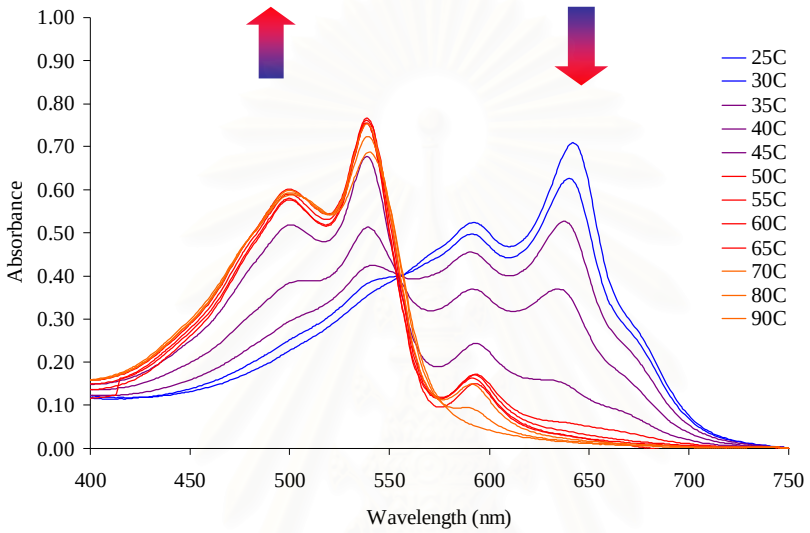



**Figure E2:** Visible absorption spectra of poly(AEPCDA) vesicle solution at pH 5

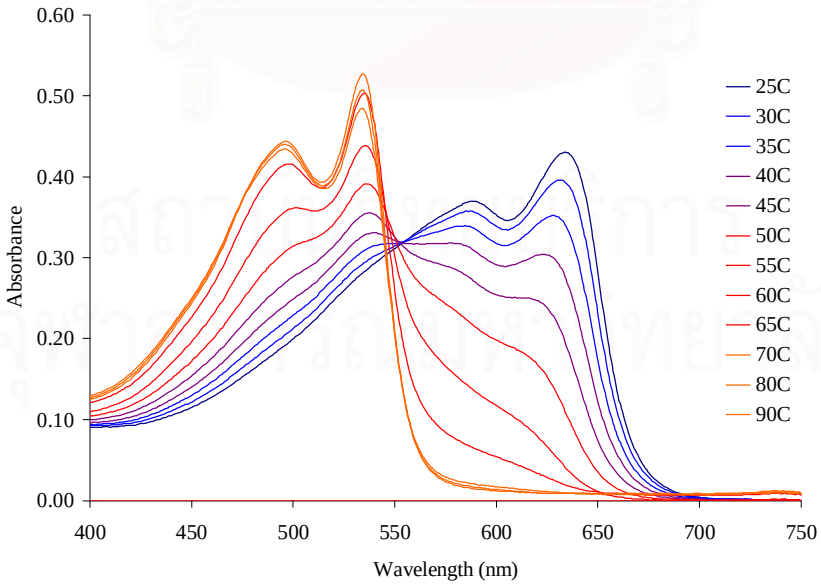


**Figure E3:** Visible absorption spectra of poly(AEPCDA) vesicle solution at pH 7

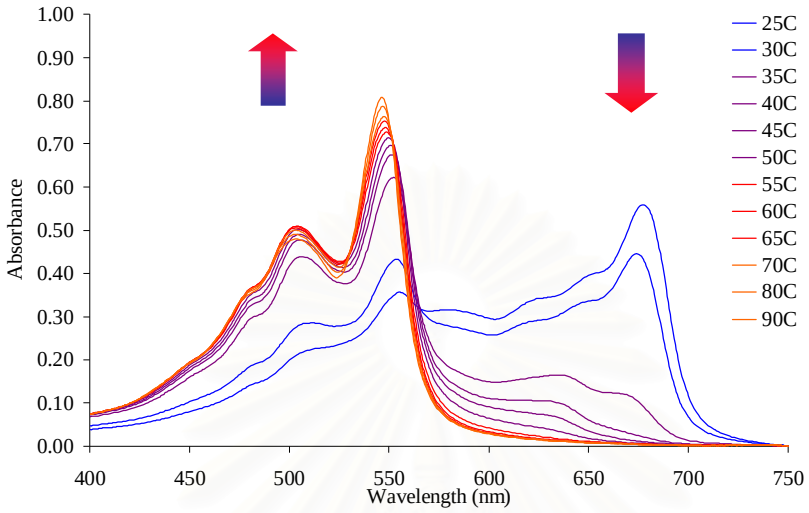
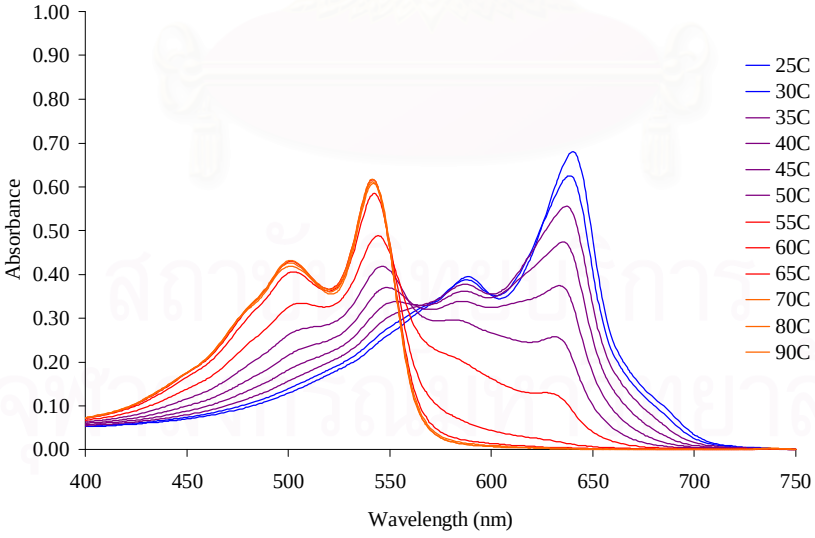



**Figure E4:** Visible absorption spectra of poly(AEPCDA) vesicle solution at pH 9



# VITAE

Miss Sansanee Boonchit was born on February 21st, 1983 in Bangkok, Thailand. She received a Bachelor's Degree of Engineering, majoring in Petrochemicals and Polymeric Materials from Faculty of Engineering and Industrial Technology, Silpakorn University in 2004. Since 2005, she has been a graduate student studying Petrochemistry and Polymer Science as her major course from Faculty of Science, Chulalongkorn University and completed the program in 2007.

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