การสังเคราะห์และการประยุกต์พอลิไดแอเซทิลีนที่มีหมู่อะมิโนสำหรับการรับรู้สารลดแรงตึงผิวชนิดประจุลบ

นางสาววันวิสาข์ ทองมาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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SYNTHESIS AND APPLICATION OF POLYDIACETYLENES CONTAINING AMINO GROUP FOR SENSING ANIONIC SURFACTANTS

Miss Wanwisa Thongmalai

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
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Thesis Title	SYNTHESIS AND APPLICATION OF POLYDIACETYLENES CONTAINING AMINO GROUP FOR SENSING ANIONIC SURFACTANTS
By	Miss Wanwisa Thongmalai
Field of Study	Petrochemistry and Polymer Science
Thesis Advisor	Assistant Professor Sumrit Wacharasindhu, Ph.D.
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วันวิสาข์ ทองมาลัย : การสังเคราะห์และการประยุกต์พอลิไดแอเซทิลีนที่มีหมู่อะมิโนสำหรับการรับรู้สารลดแรงตึงผิวชนิดประจุลบ (SYNTHESIS AND APPLICATION OF POLYDIACETYLENES CONTAINING AMINO GROUP FOR SENSING ANIONIC SURFACTANTS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. สัมฤทธิ์ วัชรสินธุ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.ดร.มงคล สุขวัฒนาสินิทธิ์, 57 หน้า.

ในผลงานนี้ พอลิไดแอเซทิลีนได้ถูกเตรียมขึ้นเพื่อใช้เป็นตัวตรวจวัดการเปลี่ยนสีต่อสารลดแรงตึงผิวชนิดประจุลบ พอลิไดแอเซทิลีนที่มีกลุ่มอะมิโนเป็นหมู่ปลาย ได้ถูกเตรียมขึ้นจากไอแอเซทิลีนมอโนเมอร์ ซึ่งผ่านกระบวนการเชื่อมต่อให้เป็นพอลิเมอร์โดยใช้รังสียูวี 254 นาโนเมตร ได้เป็นพอลิไดแอเซทิลีนโซลที่มีกลุ่มอะมิโนเป็นหมู่ปลาย เช่น เอมีนขั้นปฐมภูมิ เอมีขั้นทุติยภูมิ หรือ แอมโมเนียมขั้นจตุรภูมิ จากนั้นได้ศึกษาการเปลี่ยนแปลงสีของพอลิไดแอเซทิลีนต่อสารลดแรงตึงผิวต่างๆ พบว่าเมื่อเติมสารลดแรงตึงผิวที่มีประจุลบ เช่น โซเดียมโดเดคคาโนเอต (SDC), โซเดียมโดเดคซิลซัลเฟต(SDS), หรือ โซเดียมโดเดคซิลเบนซีนซัลโฟเนต (SDBS) พอลิไดแอเซทิลีนมีการเปลี่ยนแปลงสีจากสีน้ำเงินไปเป็นสีแดงณ ความเข้มข้นระดับไมโครโมรลาร์ และสามารถติดตามด้วยยูวีวิสิเบิล-สเปกโตรสโกปีซึ่งให้ผลสอดคล้องกันกับการบันทึกภาพถ่าย แต่ไม่พบการเปลี่ยนแปลงสีกับสารลดแรงตึงผิวชนิดประจุบวก เช่น โดเดคซิลเมทิลแอมโมเนียมโบรไมด์ (DTAB), เตตระโดเดคซิลเมทิลแอมโมเนียมโบรไมด์ (TTAB), และ เฮกซะเดคซิลไตรเมทิลแอมโมเนียมโบรไมด์ (HTAB) แต่พบการเปลี่ยนแปลงสีเพียงเล็กน้อยกับสารลดแรงตึงผิวชนิดไม่มีประจ เช่น ทวีน20 (Tween20), บริจ58พี (Brij@58P) และ ไตรตันเอ็กซ์100(TritonX-100) การจำแนกสารลดแรงตึงผิวชนิดประจุลบทั่วๆไปสามารถทำได้ขึ้นอยู่กับการรวมกันของรูปแบบสีของพอลิไดแอเซทิลีนที่มีความหลากหลายโดยใช้เทคนิคการวิเคราะห์ปรินซิปัลคอมโพแนนท์แอนาไลซิส, PCA นอกจากนี้ พอลิไดแอเซทิลีนยังสามารถขึ้นรูปบนกระดาษกรองได้สำเร็จ และการตอบสนองการเปลี่ยนแปลงสีของพอลิไดแอเซทิลีนบนกระดาษกรองถูกทดสอบกับสารละลายของสารลดแรงตึงผิว และผลิตภัณฑ์สารทำความสะอาดในเชิงพานิชย์ที่มีความเข้มข้นต่างๆ

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต.....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5273416023: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORDS: POLYDIACETYLENE/ ANIONIC SURFACTANTS/ CHEMICAL SENSOR

WANWISA THONGMALAI: SYNTHESIS AND APPLICATION OF POLYDIACETYLENES CONTAINING AMINO GROUP FOR SENSING ANIONIC SURFACTANTS. ADVISOR: ASSIST. PROF SUMRIT WACHARASINDHU, Ph.D., CO-ADVISOR: ASSOC. PROF. MONGKOL SUKWATTANASINITT, Ph.D., 57 pp.

In this contribution, the colorimetric sensor array for anionic surfactants detection based on polydiacetylene (PDA) was prepared. PDA sols containing amino group such as primary amine, secondary amine, or quaternary ammonium as the polar head group were prepared from diacetylene lipid monomers upon 254 nm UV irradiation. The addition of anionic surfactants such as sodium dodecanoate (SDC), sodium dodecyl sulfate (SDS), or sodium dodecyl benzene sulfonate (SDBS) into the PDA sols induced color transition from blue to red at micro molar level concentration. The colorimetric changes of PDA sols were also monitored by UV-visible spectrophotometer as well as photograph recorder. The addition of cationic surfactants such as dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB), and hexadecyltrimethylammonium bromide (HTAB), however did not show any colorimetric change while nonionic surfactants such as tween20, brij@58P, and tritonX-100 induced small color change. An identification of common anionic surfactants can be accomplished based on a combination of colorimetric pattern of structurally diverse PDAs using principal component analysis technique. Moreover, PDA was successfully fabricated on filter paper and the colorimetric response of PDA coated on filter paper was tested with solution of surfactants and commercial cleaning agent products at various concentrations. These allowed the direct colorimetric detection of anionic surfactants using the paper-based PDA sensor.

Field of Study: Petrochemistry and Polymer Science Student's Signature: ……
Academic Year: 2011 Advisor's Signature: ……
Co-advisor's Signature: ……..

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PDA	Polydiacetylene
PCDA	10, 12-Pentacosadiynoic acid
AEPCDA	*N*-(2-Aminoethyl) pentacosa-10, 12-diynamide
Et-AEPCDA	*N*-(2-(Ethylamino) ethyl) pentacosa-10, 12-diynamide
Et-Br-AEPCDA	*N*-Ethyl-2-pentacosa-10, 12-diynamidoethanaminium bromide
CR	Colorimetric response
NMR	Nuclear magnetic resonance spectroscopy
AFM	Atomic force microscopy
DLS	Dynamic light scattering
PCA	Principal component analysis
°C	Degree celsius
g	Gram
mL	Millilitre
µL	Microlitre
mM	Millimolar
µM	Micromolar
nm	Nanometre
µm	Micrometre
min	Minute
sec	Second
%	Percent

CHAPTER I

INTRODUCTION

1.1 Overview

Anionic surfactants are widely used in many household products and industrial applications such as cleaning agents and emulsifiers in cosmetics, pharmaceutical products and chemical reaction processes. Detection and monitoring of these compounds in environmental aquatic system is necessary because they were classified as water pollutants by EU-EPA. Contamination causes disturbance and damage to fresh water living organisms [1]. Furthermore, ability to fast identify the type of surfactants should prevent the industry from use of incorrect or ingenious emulsifying agents. Reported chemical and biological detection platform for anionic surfactants monitoring include two phase volumetric titration [2], potentiometry [3], capillary electrophoresis [4], fiber optic biosensor and optode membrane [5]. These multi-steps assays are difficult to apply and require expensive instruments. Consequently, there exists a need for fast and inexpensive method with simple mode of detection such as by naked eye. Recently, conjugated polymers are utilized and developed as senor element for an optical detector because it minimize the need for extensive signal transduction hardware. Among conjugate polymers, polydiacetylenes (PDAs) are conjugated polymers that spur immense interests in the field of chemical and biological sensors [6]. They can be conveniently prepared from polymerization of self-assembled diacetylene monomers by UV-irradiation to generate blue sols of ene-yne conjugated polymers. Various forms of momomeric assemblies such as bulk crystals, self-assembled films and nano-structures such as vesicles, tubes and ribbons have been used for preparation of PDAs [7]. The most interesting features of PDAs are their unique chromic properties displaying dramatic blue to red color changes with various external stimuli such as light (photochromism) [8], heat (thermochromism) [9], mechanical stress (mechanochromism) [10], solvents (solvatochromism) [11], and binding of specific chemical or biological agent (affinochromism/biochromism) [12].

Diacetylene lipid is one of the most useful monomers for preparation of PDA sensors because it can efficiently form nano vesicles homogenously dispersed in aqueous media suitable for biological and environmental sensing application. The application of PDAs for sensing surfactants was first realized by Kim demonstrating that PDA containing containing m-hydroxy- benzaldehyde head group displayed a blue-red colorimetric response selectively to cationic ammonium surfactants [13]. The color transition was presumably initiated by the interaction between the hydroxybenzaldehyde head group with the cationic ammo- nium group of the surfactants. According to these pioneering works, it was envisaged that the PDAs containing cationic side chains could be used as colorimetric transducers in a sensor array for identification of anionic surfactants.

1.2 Theory

1.2.1 Polydiacetylene vesicles (PDA)

Polydiacetylenes (PDAs) are 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 1.1) upon heat, irradiation with light or γ-irradiation [6,14].

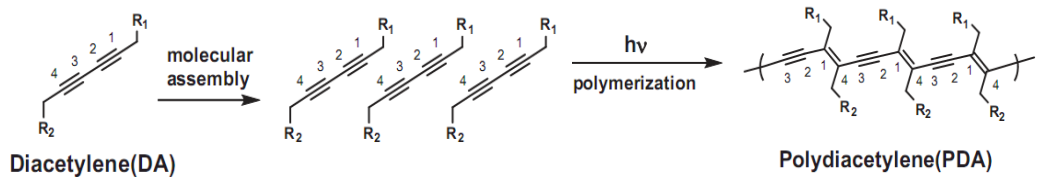

Figure 1.1 Polymerization of diacetylene monomers by irradiation with UV light.

The topopolymerized diacetylene crystals are nearly perfectly ordered crystals which cannot be occurred by solution polymerization or recrystallization of a preformed polymer from solution or melt. 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 perturbation such as temperature, pH, solvent, mechanical stress and ligand-receptor interactions due to reduction of the effective conjugation length resulted from strain and torsion imposed onto the backbone induced by order-disorder transitions in the side chains. 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, PDA-embedded polymer matrix films, self-assembled monolayers, liposomes or vesicles in water.

Diacetylene lipid acids are known to spontaneously organize 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 1.2).

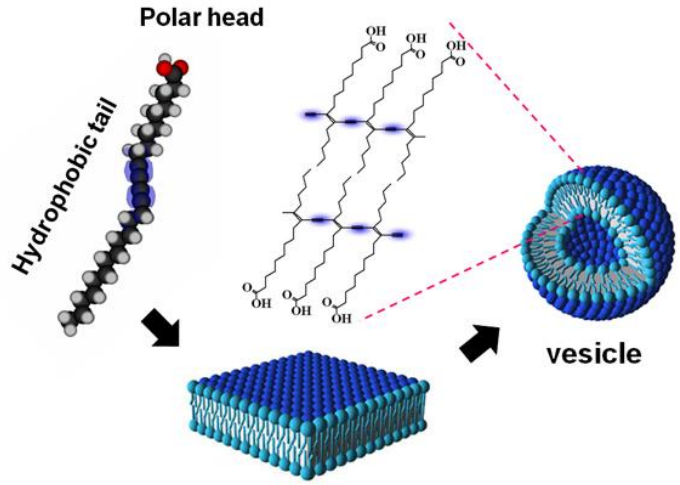

Figure 1.2 Structure and formation of a PDA lipid vesicle [15].

1.2.2 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 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.

1.2.3 Chromisms of polydiacetylene

One of the most fascinating properties of PDA is its ability to change color upon its exposure to an external stimulant such as heat (thermochromism), organic solvents (solvatochromism), mechanical stress (mechanochromism), and ligand-receptor interactions (affinochromism). The color changes of polydiacetylenes involve a shift of the wavelength 640 nm to a band around 540 nm, resulting in a transformation of the material from blue to red.

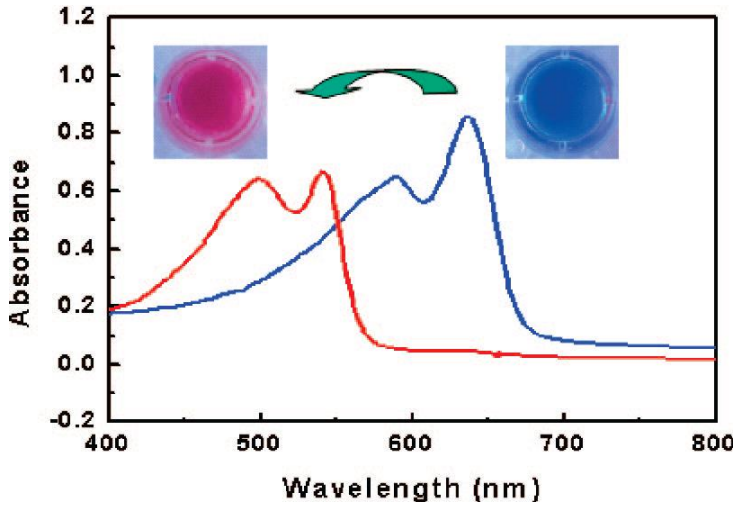

Figure 1.3 Colorimetric transitions of PDA sols [11]

Thermochromism

Thermochromism is one of the initial reported chromatic properties of polydiacetylene [16]. It can show either reversible or irreversible depending on the interaction between the side chain substituent and side chain head groups, and enhancement of hydrogen-bonding interactions among the head groups. For example, in 2010, Pollookin and co-worker [16] have reported the novel class of diacetylene lipid monomer A series of bisdiynamide lipids containing various lengths of methylene spacer between the diynes and the diamide head group and number of

methylene units in their hydrophobic tails were synthesized (Figure 1.3a). The authors have suggested that tuning of color transition temperature of thermochromically reversible bisdiynamide series of polydiacetylenes (PDAs) can be achieved by systematic variation of the length of methylene spacer (m) between the diyne and the diamide head group as well as the number of methylene units (n) in the hydrophobic tail (Figure 1.3b)

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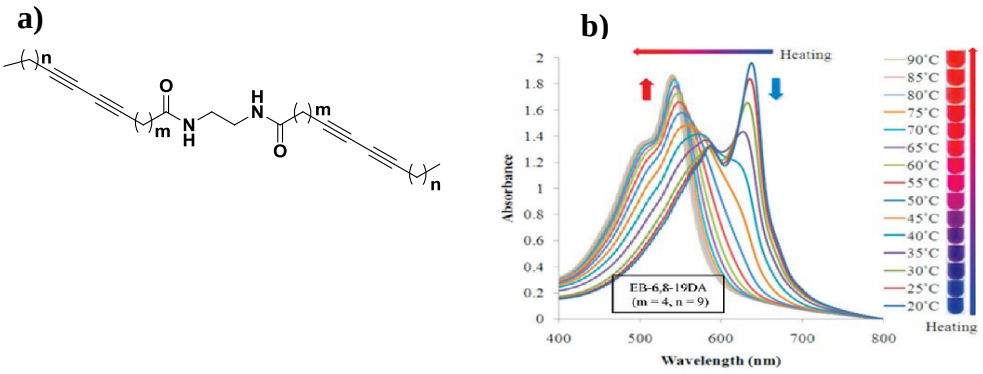


Figure 1.4 a) Chemical structure of bisdiynamide series of diacetylene lipids b) absorption spectra of bisdiynaminde PDA sols (EB-6,8-19DA) upon stepwise heating from 20 to 90 °C

Mechanochromism

Mechanochromism is the phenomenon of color changes induced by mechanical force. An irreversible chromic transition of PDA single crystal which induced by mechanical stress was observed by Muller and Eckhardt [17]. Nallicheri and Rubner have incorporated diacetylenes in polyurethane segmented copolymers. In these materials, the optical properties of diacetylenes were linked with the mechanical properties of thermoplastic elastomers. The resulting of polyurethane elastomers containing polydiacetylene undergoes color changes that are coupled to elastomeric strain (mechanochromism) [18].

Solvatochromism

Solvatochromism is one of the chromic properties of polydiacetylene that were induced by the solvation. It is believed that solvation of polymer side chain in the presence of organic solvent caused a side chain disorder which affected the

conjugation of polydiacetylene backbone. For example, in 2010, S. Wu and colleagues [19] investigated the effects of solvents on structure of micelle-like assemblies of an azo chromophore-functionalized polydiacetylene (polyAzoDA) and polymerized tricosa-10,12-diynoic acid (polyTDA). They used the mixtures of water with glycol, DMSO, ethanol and THF to observe blue-to-red color changes of polydiacetylenes. They found that the colors of polyTDA and polyAzoDA changed at certain contents of organic solvents. The results in figure 1.4 exhibited the strength of the polyTDA-solvent interaction in the following order: THF>ethanol> DMSO>glycol. According to the results, THF was a solvent which can induce blue-to-red color transitions of polyAzoDA in THF/water mixtures. However, they do not observe color transitions in the other solvents/water mixtures. The authors have proposed that the stability to several solvents of polyAzoDA caused by the H- and J-like aggregates of azo chromophores in polyAzoDA.

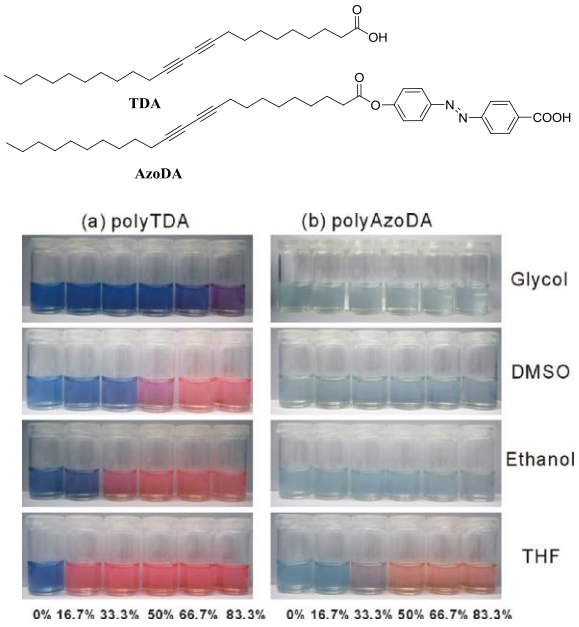


Figure 1.5 Photographs of micelle dispersions in different water-solvent mixtures. At increasing solvent content, the color of (a) polyTDA and (b) polyAzoDA changes from blue to purple/red depending on type of solvent and its relative content

Affinochromism

The most attractive feature of polydiaceylene to be discovered in recent investigations concerns the new chromic changes promoted by interactions with biologically, environmentally or chemically interesting target molecules. Generally, PDAs sensor derived from modification of head group of 10,12-pentacosadiynoic acid (PCDA) are able to underwent blue to red color change upon exposure to the target molecules., The DA monomer were modified to carry a receptor in the head group, matching with the target molecules. After addition of the target molecule , showed the colorimetric transition induced by interaction of a PDAs with target molecules. For example, Kim and co-worker [20] prepared two types of cyanuric acid-carrying PDA monomers; PCDA-CA and PCDA-EG-CA) for melamine detection (Figure 1.5). The vesicle solutions of poly PCDA-EG-CA/PCDA (9/1 mole ratio) displayed the color transition from blue to red upon addition of various concentrations of melamine from 1 to 40 ppm. Such an excellent sensitivity is suitable to detect melamine at the world regulation level.

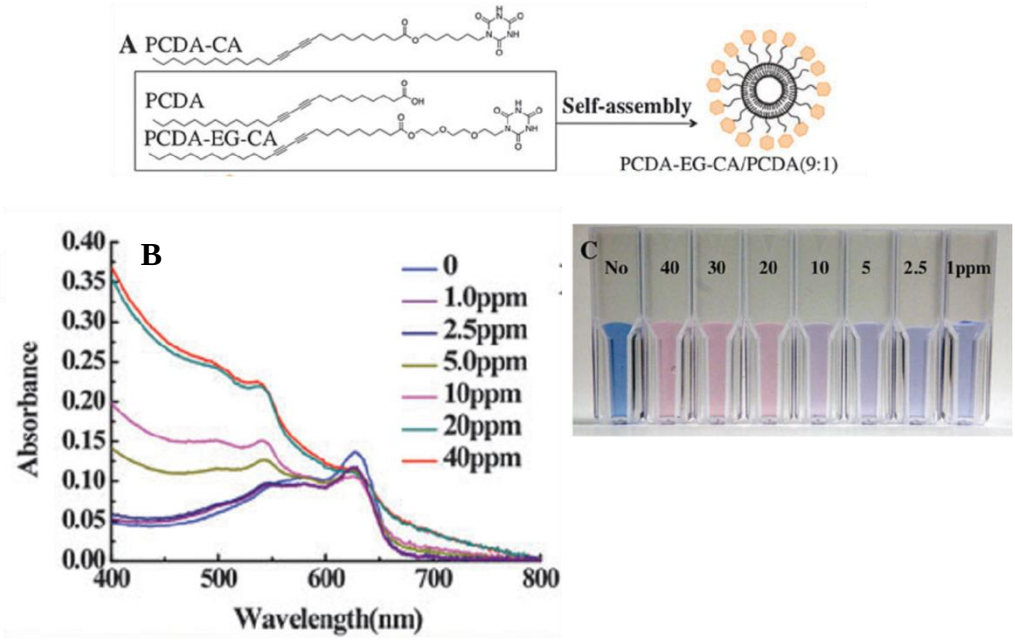


Figure 1.5 A) Structure of diactylene monomers, B) UV-visible spectra and C) colorimetric transition of PDA detection with melamine.

1.2.4 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

$$\%CR = (FB_0\text{-}FB)/FB_0 \times 100$$

Where $FB = A_{blue}/(A_{blue}+A_{red})$, 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. FB_0 is the initial percent blue of the vesicle

1.2 Surfactants

Surfactants are materials that useful in the house because they are compounds that lower the surface tension of liquid. They can form micelle so they always are in the ingredient of the detergents, cleaning agent, shampoo or dishing water. The surfactants are amphiphilic in the water. Head of surfactants are hydrophilic part that soluble in the water and the tail of surfactants are hydrophobic part that dislike the water. So they will form to be micelle. The surfactants can be classified by the presence of formally changed in the head group (Figure 1.6) such as the non-ionic surfactant has no charge groups in the head group, If the charge is negative it is called the anionic surfactant, the cationic also has positive charge in the head group. The zwitterionic surfactant contains a head with two oppositely charged groups. The example of 4 type surfactants were showed in Table 1.1

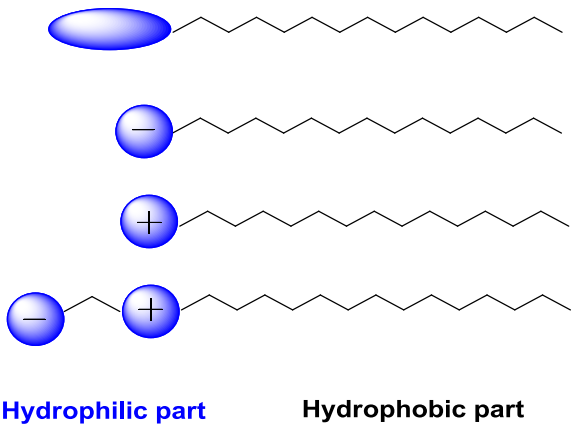
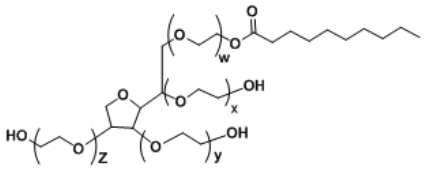
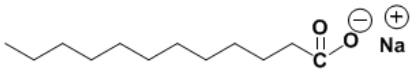
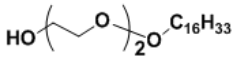
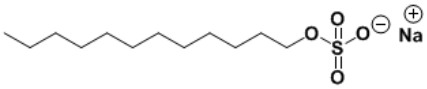
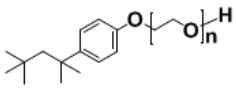
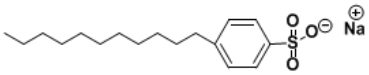
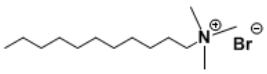
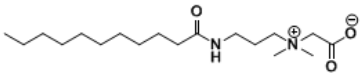
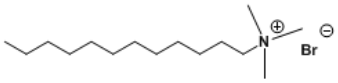
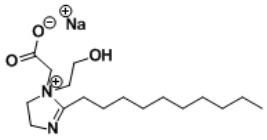
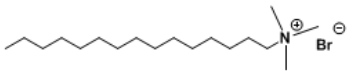
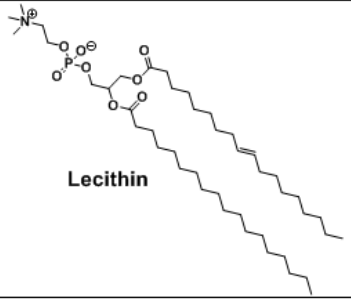


Figure 1.6 Model of type of surfactants

Table 1.1 The example of common nonionic, anionic, cationic, and zwitterionic surfactants

Nonionic surfactants	Anionic surfactants
 Tween 20 W+X+Y+Z = 20	 Sodium dodecanoate (SDC)
 Brij@58P	 Sodium dodecyl sulfate (SDS)
 Triton X-100	 Sodium dodecyl benzene sulfonate (SDBS)

Cationic surfactants	Zwitterionic surfactants
 Tetradecyltrimethylammonium bromide (TTAB)	 Cocamidopropyl betaine (CAPB)
 Dodecyltrimethylammonium bromide (DTAB)	 Sodium lauroamphoacetate
 Hexadecyltrimethylammonium bromide (HTAB)	 Lecithin

1.2.1 Health risks from anionic surfactants

Surfactants use widely the cleaning agents, the house products almost have surfactant as ingredients that they help mix water with oil and dirt so that they can be rinsed into the groundwater. The surfactants are chemicals that have a toxic with environmental. For example, anionic surfactants are using in cleaning agent because they cheap and good strength than others. It may cause irritation to the skin, such as sodium lauryl sulfate (SLS). In the aqueous disposal from washing water or dishwashing liquid concentrate, they usually contain surfactant components into the rivers. They cause a huge bubble make the aesthetics of the watercourse decrease. Also it affects the microorganisms in the water. And it may take longer to process natural bacteria or microbial degradation of surfactants will be exhausted. Moreover, limitation of surfactant allowed by US. EPA in the drinking water is 0.5 mg/L [21].

1.3 Literature reviews on polydiaceylene for the detection of metal ions

In 2009, König and co-worker. [22] prepared DA monomers embedded with metal complex as receptor for biological phosphate ion recognition (Figure 1.7a). Blue polydiacetylene vesicles were obtained upon UV irradiation from mono- and dinuclear zince(II)- cyclen (cyclen = 1,4,7,10-tetraazacyclododecane) and iminodiacetato cupper [Cu^{II} -IDA] (IDA= iminodiacetato) modified diaceylene monomers (LP-4-Zn, LP-8-Zn and LP-11-Cu) as shown in figure 1.7b. Addition of ATP (Adenosine triphosphate) and PPi (Pyrophosphate) to PDA vesicular with embedded only zince (II)- cyclen complex solution induceed a colorimetric change (blue to red), while iminodiacetato cupper [Cu^{II} -IDA] complex were selectively only PPi. Moreover, the PDA vesicles were successfully fabricated on paper and were able to use as phosphate detector (figure 1.7 c)

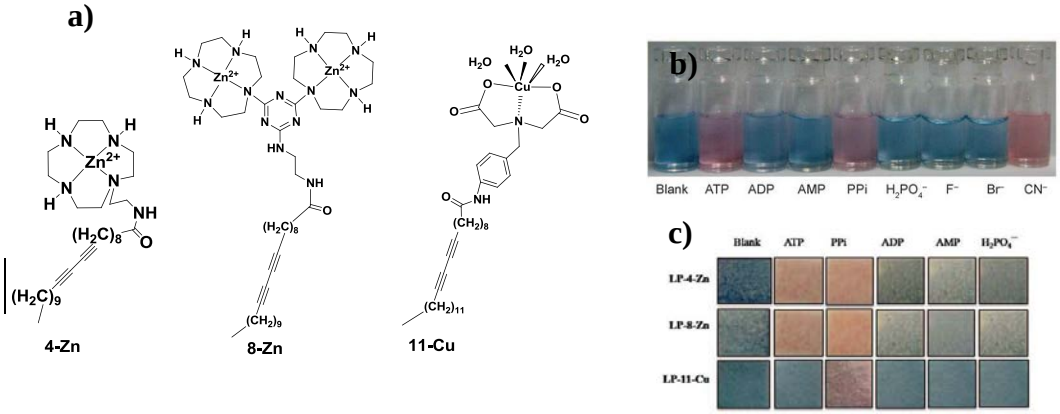


Figure 1.7 a) Structure of diacelylene with embedded metal complex monomers and colorimetric transition of b) PDA sols and c) PDA coated paper in the presence of phosphate ions.

In 2010, König and Jone [23] prepared PDA sols from diacetylene monmers (LS-Terpy, LS-DPA, LS-DP and LS-DEA) carrying N-heterocyclic chelating sites monomers as shown in figure 1.8a. The addition of zinc, manganese, cadmium, mercury or silver salts to solutions of the vesicles indices a color change from blue to red, while the addition of other metal salts failed to show any change (Figure 1.8b). Moreover, the PDA vesicles were embedded on papers and were able to detect the metal ion (Figure 1.8c).

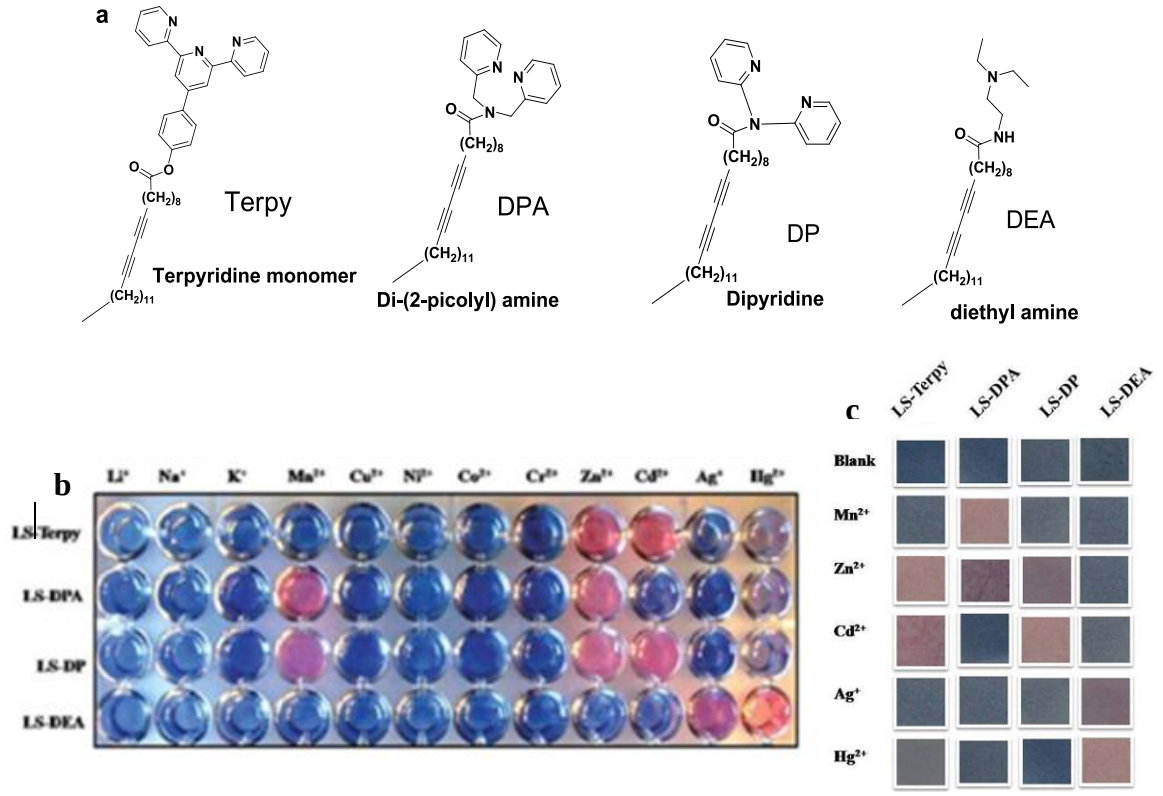


Figure 1.8 a) Structure of diacelylene with embedded N-Heterocyclic ligand monomers and colorimetric transition of b) PDA sols and c) PDA coated paper in the presence of metal ions.

In 2011 Sukwattanasinitt and co-worker [24] prepared the mixed lipid polydiacetylene based blue vesicles from (ethylene glycol) ester monomer as embedded receptor sites and 10,12- pentacosadiynoic acid (DA) (Figure 1.9a).

30mol% of penta (ethylene glycol) ester (5EG-PCDA) polydiaceylene sol induced color change from blue to red selectively to Pb^{2+} at the 100 μM(Figure 1.9 b and c)

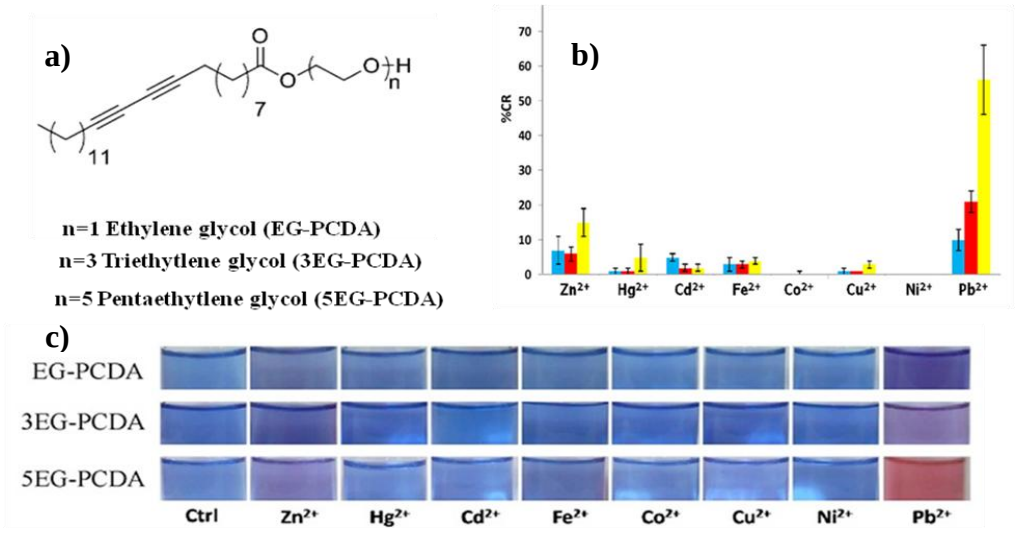


Figure 1.9 a) structure of amphiphilic diacetylene monomer , and b) %CR c) color changes of polydiacetylene with PCDA composite vesicles.

In 2011, Zuo and co-worker [25] prepared the mixed lipid polydiacetylene based blue vesicles from copolymerized vesicles of amphiphilic benzo-15-crown-5-substituted diacetylene monomer (BCDA) and 10,12- pentacosadiynoic acid (DA) as embedded receptor sites specifically for Pb^{2+} (Figure 1.10a). The alkali, alkaline earth and transition metal cations such as Na^{+}, K^{+}, Mg^{2+} , Ca^{2+} , Fe^{2+} ,Co^{2+} , Cu^{2+} , Mn^{2+} , Ba^{2+} , Cd^{2+} , Ag^{+} , Pb^{2+} and Zn^{2+} ions were tested with poly(PBCDA-PDA) vesicles and fund that only Pb^{2+} ion could induce a color change from blue to red (Figure 1.9 b to d). On the other hand, other cations such as Na^{+}, K^{+}, Mg^{2+} , Ca^{2+} , Fe^{2+} ,Co^{2+} , Cu^{2+} , Mn^{2+} , Ba^{2+} , Cd^{2+} , Ag^{+} ,and Zn^{2+} ions did not show any color change as shown in figure 1.10

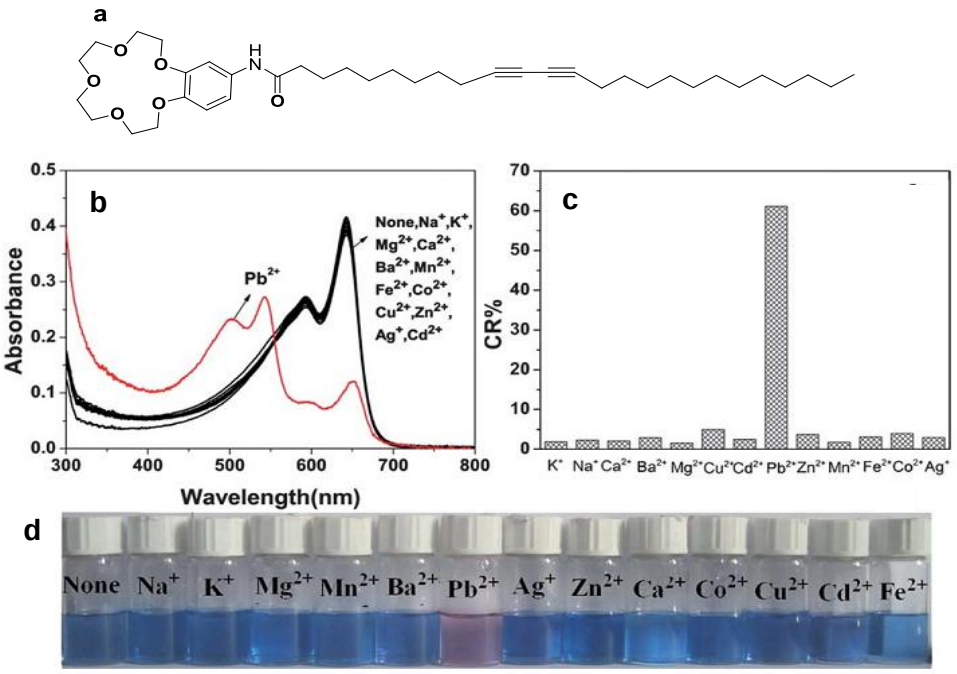


Figure 1.10 a) structure of amphiphilic diacetylene monomer (BCDA), and b) UV-vis spectra, c) %CR d) color changes of PBCDA-PDA composite vesicles.

1.4 Literature reviews on polydiaceylene for detection of surfactants

Recently, ligand-receptor or affinochromism of polydiacetylene is recently were utilized as a concept for the designing and developing of the PDA as surfactant sensor. All the works were created by embedded the opposite charge of the surfactants analyte into the PDA head group in order to induce the color change via the columbic interaction.

For example, in 2010, Kim and co-worker [13] prepared the polydiacetylene (PDA) derived from a hydroxybenzaldehyde substituted diacetylene (PCDA-HBA1) monomer for detection of cationic surfactants as shown in the Figure1.11. Blue vesicles of PCDA-HBA1 were induced a color change (blue to red) upon the addition of CTAC (cetyltrimethylammoniumchloride) and DTAB (dodecyltrimethylammonium bromide) solutions. On the other hand, the other cation ions and anionic surfactants were failed to show any color changes.

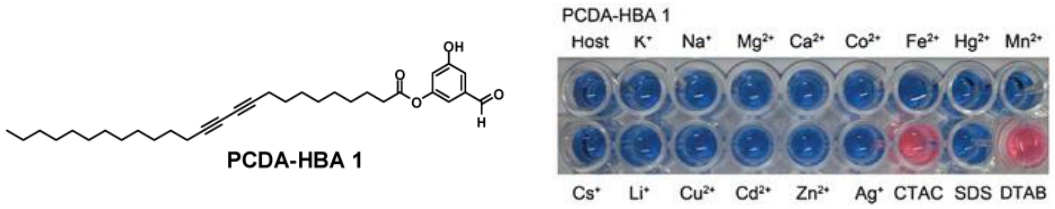


Figure 1.11 a) Structure of diacelylene monomers and colorimetric transition of PDA and b) color changes of PCDA-HBA 1 composite vesicles.

In 2010, Yoon and co-worker [26] prepared the polydiacetylene (PDA) vesicles containing imidazodium group (Figure 1.12) as positive charge head group for specific sensing anionic surfactants. These PDAs responsed specifically to anionic surfactants such as SDS (sodium dodecyl sulfate), SDC (sodium decanoate), SDP (sodium dodecyl phosphate), and SDBS (sodium dodecyl benzene sulfate) solutions as seen in figure 1.9b. They showed the color change from blue to yellow or pink with anionic surfactants, while other anions and cationic surfactants did not show any color changes.

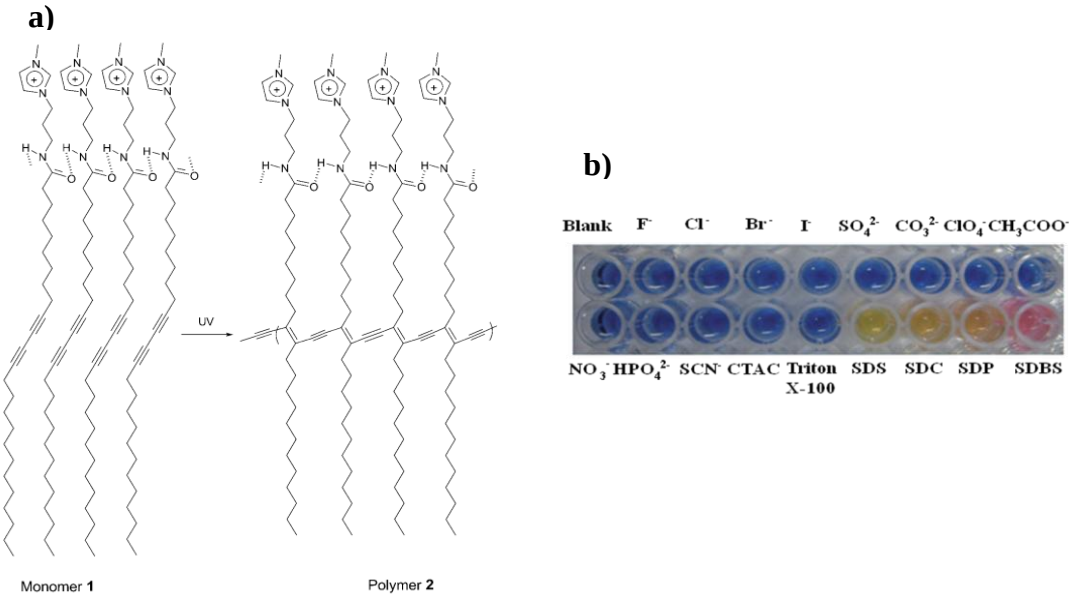


Figure 1.12 a) Structure of diacelylene monomers and b) colorimetric transition of PDA.

1.5 Background of paper-based sensor array

Paper-based sensor arrays are very common in medical applications. The basic elements of these systems comprise patterned paper and a portable device that acquires images and transmits digital information (e.g. a digital camera, a camera-equipped cellular phone and scanner) [27, 28].

In 2008, Whitesides and co-worker described a prototype system for quantifying bioassays [27]. The system used paper-based microfluidic devices for running multiple assays simultaneously, camera phones or portable scanners for digitizing the intensity of color associated with each colorimetric assay. The microfluidic devices were fabricated in paper using photolithography and were functionalized with reagents for colorimetric assays. This system gave accurate and quantitative results when detecting glucose and protein in urine which using small volumes of sample ($\leq 5\ \mu L$). The combination of patterned paper, a portable method for obtaining digital images and a method for exchanging results of the assays with off-site diagnosticians offers new opportunities for inexpensive monitoring of health, especially in situations that require physicians to travel to patients to obtain diagnostic information that might be obtained more effective by less valuable personnel

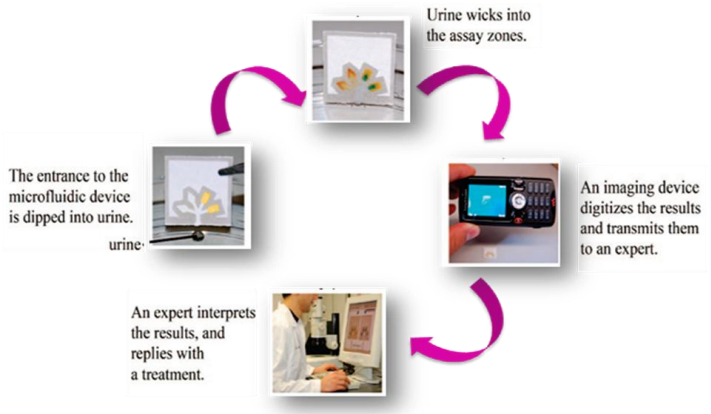


Figure 1.13 The evaluation system of paper-based microfluidic devices for colorimetric assay

In 2009, Whitesides and co-worker continued to report 96- and 384-microzone plates fabricated in paper as alternatives to conventional multiwall plates fabricated in molded polymers as shown in Figure 1.14 [28]. The paper-based plates were

fabricated by patterning sheets of paper by using photolithography. These plates were compatible with conventional microplate readers for quantitative absorbance and fluorescence measurements. The common biological stains such as Coomassie Brilliant Ble, Amaranth and bovine serum albumin (BSA) were tested. The demonstration of quantitative colorimetric correlation uses a scanner or camera to image the zones and to measure the intensity of color to make it possible to conduct assays without a microplate reader.

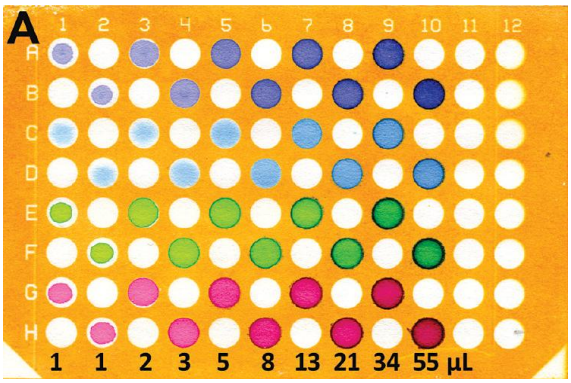


Figure 1.14 Paper plates for multizone assays produced using photolithography.

From the above reviews and our previous works on amido PDAs, [26] we planned to utilize our amido PDA series for specific naked eye detection of anionic surfactants. Also, analysis of the colorimetric response pattern of the prepared PDA sensing array with PCA (principal component analysis) should allow us for the identification of common anionic surfactants. Furthermore, the monomer will be used for the fabrication of a paper based PDA sensor.

1.5 Objectives and scope of the thesis

The objectives of this thesis were to study affinochromism of polydiacetylene containing amido group to the anionic surfactants. To achieve the objectives, the work scope includes

1. Preparation of three PDAs derived from three amphiphilic diacetylene monomers having the amido moiety (**1**-**3**).

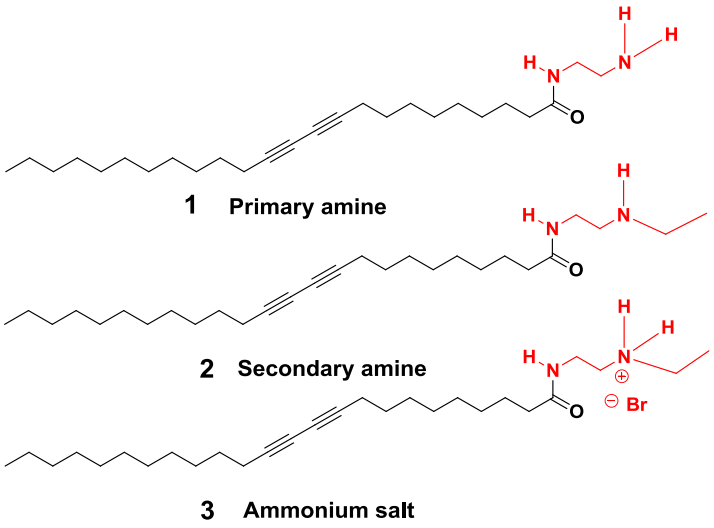


Figure 1.15 Structures of diaceylene monomer

2. Study the affinochromic property of prepared PDAs in both sols and solid state forms in the presence of common cationic surfactants, nonionic surfactants, and anionic surfactants.

CHAPTER II

EXPERIMENTAL

2.1 Materials

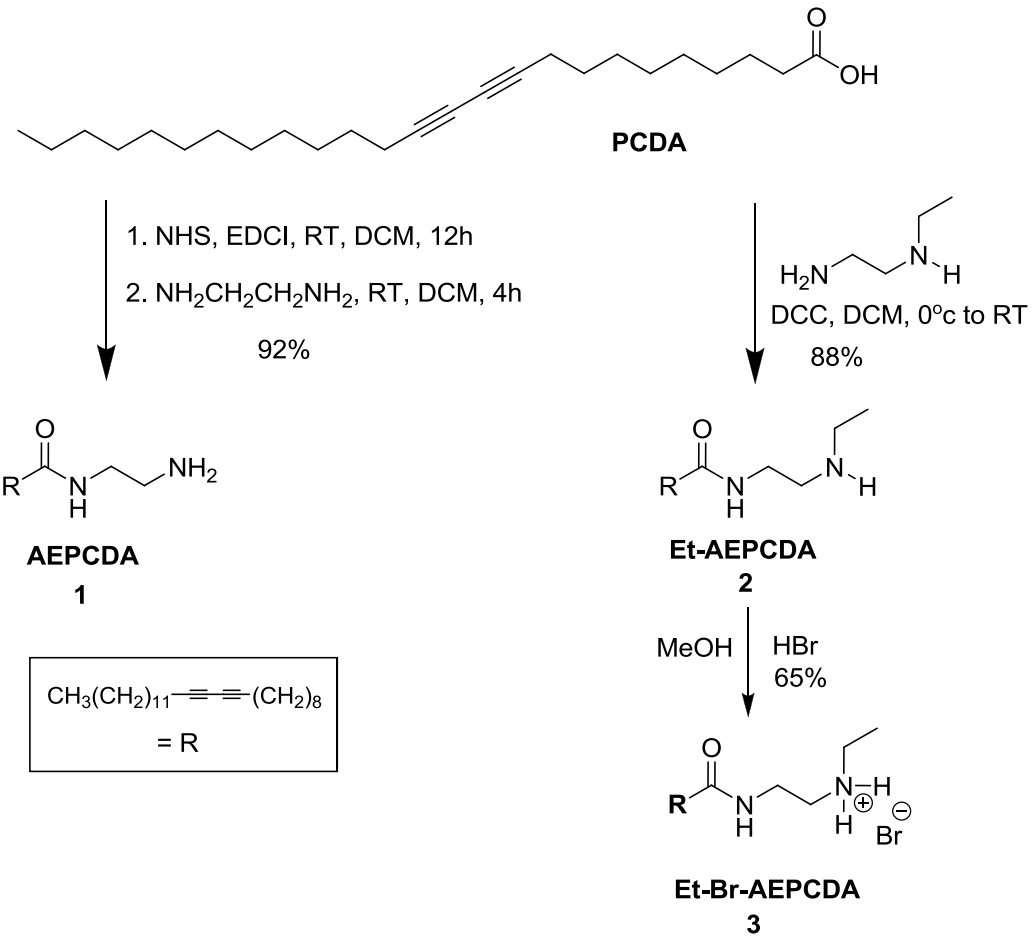
10,12-pentacosanoic acid (PCDA) was purchased from GFS Chemicals (USA). *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3′-dimethylamino) carbodiimide HCl salt (EDCI) and *N,N*′-dicyclohexylcarbodiimide (DCC) were purchased from Fluka (Switzerland). Ethyldiamine, *N*′-ethylethylenediamine was purchased from Aldrich (USA). Milli-Q water with a resistance of 18.1 MΩ was used in all experiments. Analytical grade solvents such as chloroform and methylene chloride were used without further purification. All organic solvents for monomer synthesis and purification were purchased from TSL Chemicals (Thailand). The diacetylene monomers were purified by filtration to remove the polymerized lipid before used. For extraction and chromatography, solvents were commercial grade and distilled before use. Column chromatography was performed on Merck silica gel 60 (230-400 mesh; Merck) stationary phase. Analytical Thin layer chromatography (TLC) was carried out using Merck 60 F254 plates with a thickness of 0.25 mm.

2.2 Analytical instruments

The ^{1}H spectra were acquired by Varian Mercury 400 MHz NMR spectrometer (Varian, USA) and ^{13}C spectra were acquired by Bruker Mercury 400 MHz NMR spectrometer (Bruker, German) using the residual solvent proton resonance of $CHCl_3$ at 7.26 ppm and as the reference. The molecular weights were obtained from a low-resolution quadruple mass analyzer (Quattro Micro API 2000, Micromass) using the electrosprin ionization (ESI) technique. The electronic absorption spectra were recorded on a temperature variable UV-Vis spectrophotometer (Carry 100Bio, Varian). Sonication was carried out in a ultrasonicating bath (Transinic T570/S, Elma, Germany). The AFM images were

taken on a SPA 400 atomic force microscope (Seiko, Japan). The particle size measurements were performed on a dynamic light scattering using a Nanosizer (Malvern instruments, England). The optical microscopy images were taken using Olympus DP72 equipped with 4X objective (Olympus, Japan). UV-irradiation was conducted using UV light source (TUV 15W/G15 T18 lamp; Philips, Holland). The photographic images were recorded by Canon digital camera.

2.3 Synthesis of amphiphilic diacetylene monomers (1-3)



Scheme 2.1 Synthesis of amphiphilic diacetylene monomers.

2.3.1 *N*-(2-aminoethyl) pentacosa-10, 12-diynamide (AEPCDA, 1):

A solution of 1-ethyl-3-(3′-dimethylamino) carbodiimide HCl salt (EDCI) (246.14 mg, 1.28 mmol) in methylene chloride (2 mL) was added dropwise into a solution of 10,12-pentacosadiynoic acid (PCDA) (400.00 mg, 1.07 mmol) in methylene chloride (5 mL). The mixture was stirred for 1 h at room temperature and was then added dropwise into a solution of *N*-hydroxysuccinimide (NHS) (147.77 mg, 1.28 mmol) in methylene chloride (2 mL) at room temperature. The reaction mixture was stirred at room temperature overnight. After that, water (20 mL) was added and the mixture was extracted with methylene chloride (25 x 3 mL). The organic phase was dried with sodium sulfate and rotary evaporated to yield the crude product as a white powder. Then the crude product was dissolved in methylene choloride (10 mL) and added dropwise in to a solution of ethylenediamine in methylene chloride (2 mL). After that, the mixture was kept stirred for 4 h at room temperature. Then the reaction was extracted with methylene chloride (25 x 3 mL). The organic phase was dried with sodium sulfate and rotary evaporated to yield the crude product as a white powder. The crude product was purified by column chromatography on silica gel eluted with a mixture of ethyl acetate and methanol (70:30) to give *N*-(2-aminoethyl) pentacosa-10,12-diynamide(AEPCDA, 1): (325 mg, 92% yield) as a white powder: mp 111-114 °C. ^{1}H NMR (400 MHz,CDCl3): δ (ppm): 0.87 (t, -CH_3 , 3H, J = 6.8 Hz), 1.42 (m, 16-CH_2- , 32H), 2.18 (t,-CH2-, 2H, J = 7.6 Hz), 2.23 (t, -CH_2-, 4H, J = 6.8 Hz), 2.84 (t, -CH_2-,2H, J = 5.7 Hz), 3.31 (q, -CH_2-, 2H, J = 5.8 Hz), 5.94 (brs, NHC=O, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm): 173.6, 77.6, 77.5, 65.3, 65.3, 41.6, 41.6, 41.3, 36.8, 31.9, 29.6, 29.6, 29.5, 29.3, 29.2, 29.2, 29.1, 28.9, 28.9, 28.7, 28.3, 25.7, 22.7, 19.2, 19.2, 14.1. MS (ESI+): m/z = 416.54, 438.47 and 439.34 corresponding to [M]+, [M-H+Na]+ and [M+Na]+, respectively (calcd. for $C_{27}H_{48}N_2O$ 416.68).

2.3.2 *N*-(2-(ethylamino) ethyl) pentacosa-10, 12-diynamide (Et-AEPCDA, 2):

N, N′- dicylohexylcarbodiimide (DCC) (264.18 mg, 1.28 mmol) in methylene chloride (4 mL) was added dropwise into a solution of 10,12-pentacosadiynoic acid (PCDA) (400.00 mg,1.07 mmol) in methylene chloride (7 mL). The mixture was stirred at 0 °C for 1 h. *N*-ethylethylenediamine (146 µL, 1.38 mmol) was added dropwise into the reaction mixture at room temperature. After that, the mixture was

kept stirred at room temperature for overnight. The mixture was extracted with methylene chloride (25 x 3 mL). The organic phase was dried with sodium sulfate and rotary evaporated to yield the crude product as a white powder. The crude product was purified by column chromatography on silica gel eluted with a mixture of ethyl acetate and methanol (70:30) to give *N*-(2-(ethylamino) ethyl) pentacosa-10,12-diynamide (Et-AEPCDA, 2): (417 mg, 88% yield) as a white powder: mp 64-66 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 0.86 (t, -CH_3 , 3H, *J* = 6.8 Hz), 1.09 (t, -CH_3, 3H, *J* = 7.1 Hz), 1.33 (m, 16-CH_2- , 32H), 2.15 (t,-CH_2-, 2H, *J* = 7.6 Hz), 2.21 (t, -CH_2-, 4H, *J* = 6.9 Hz), 2.64 (q, -CH_2-,2H, *J* = 7.2 Hz), 2.74 (t, -CH_2-, 2H, *J* = 7.6 Hz), 3.329 (q, -CH2-, 2H, *J* = 5.7 Hz), 4.75 (brs, -NH-, 1H), 6.11, (brs, NHC=O, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm): 173.5, 77.6, 77.5, 65.3, 65.2, 48.4, 43.7, 38.7, 31.9, 29.6, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 29.1, 29.2, 29.1, 28.9, 28.9, 28.8, 28.4, 28.3, 25.7, 22.7, 19.2. MS (ESI+): m/z = 444.58 and 445.48 corresponding to [M]+ and [M+H]+, respectively. (calcd. for $C_{29}H_{52}N_2O$ 444.41).

2.3.3 *N*-ethyl-2-pentacosa-10, 12-diynamidoethanaminium bromide (Et-Br-AEPCDA, 3):

N-(2-(ethylamino) ethyl) pentacosa-10,12-diynamide (400.00 mg, 0.90 mmol) was dissolved in chloroform and hydrobromic acid (HBr) (97.00 µL, 1.80 mmol). The mixture was stirred at room temperature for 1 h. The mixture was extracted with chloroform and water. The organic phase was dried with sodium sulfate and rotary evaporated to yield the crude product as a white powder and was dissolved in small amount of chloroform followed by slow addition of cool hexane at 0 °C and was dried in a vacuum oven at room temperature to give *N*-ethyl-2-pentacosa-10,12-diynamidoethanaminium bromide (Et-Br-AEPCDA, 3): (307 mg, 65% yield) as a white powder: mp 115-118 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 0.88 (t, -CH_3 , 3H, *J*= 6.8 Hz), 1.40, (m, 16-CH_2- , 32H), 1.62 (t, -CH_3, 3H, *J* = 7.2 Hz), 2.25 (m, 3-CH_2-, 6H), 3.12 (m, -CH_2-, 4H), 3.68 (q, -CH_2-,2H, *J* = 4.4 Hz), 7.65 (brs, NHC=O, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm): 175.5, 77.7, 77.4, 65.3, 65.2, 47.9, 43.5, 36.5, 36.3, 31.9, 29.7, 29.6, 29.7, 29.5, 29.4, 29.2, 29.2, 29.1, 28.9, 28.9, 28.9, 28.3, 28.4, 25.6, 22.7, 19.2, 19.2, 14.1, 11.3. MS (ESI+): m/z = 445.49 (calcd. for $C_{29}H_{53}BrN_2O$ 445.41)

2.4 Preparation of polydiacetylene sols

The diacetylene monomer was dissolved in diethyl ether then filtered to remove polymerized material. The filtrate was dried under a rotary evaporator to give a white solid. A stock of diacetylene monomers were kept in refrigerator. A diacetylene monomer 1, 2 and 3/PCDA (4:1) were dissolved in chloroform and solvent were evaporated by flowing the nitrogen gas. Mill-Q water was added to provide a 0.5 mM aqueous lipid suspension. The suspension was sonicated with sonicator bath at 75-80 °C for 40 min. The suspension was allowed to cool down to room temperature and then kept at 4 °C overnight. The vesicle suspension was irradiated by UV irradiation (256 nm, 15 Watt) for 5 minutes to generate the transparent deep blue solution 0.5 mM in final concentration.

2.5 Affinochromic study of polydiacetylene vesicles

2.5.1 Colorimetric detection of surfactants

The three anionic surfactants (SDC, SDS, SDBS), tree cationic surfactants (TTAB, DTAB, HTAB), and tree nonionic surfactants (Tween20, Brij@58P, TritonX-100) were used in this work. Solutions of surfactant (0, 10, 20, 30, 40 and 50 μM) were added into PDA solution 0.1mM in final concentration. The resulting solutions were stirred for 5 minutes before monitoring by digital camera and UV-Vis spectrometry. The spectra were collected from 800 to 400 nm with the zero absorbance set at 800 nm. The λ_{max} of the blue and the red phase of each sample were determined at blank (0μM) and 50μM of surfactant concentration.

2.5.2 Colorimetric response

The electronic absorption spectra were recorded by the UV-visible spectrophotometer. The quantitative evaluation of colorimetric response (%CR) was determined as percentage of the change of the change of the blue color faction (FB_0 – FB) against the initial blue color fraction (FB_0) according to the following equation

$$\%CR = 100 \times (FB_0 - FB)/FB_0$$

FB is the blue fraction calculated from $A_{blue} / (A_{blue} + A_{red})$ where A_{blue} and A_{red} are the absorbance at the λmax of the blue and the red forms of polydiacetylenes respectively

2.6 Characterization of PDA sols

2.6.1 Dynamic light scattering (DLS)

The mean size of vesicles and the size distribution were determined by nanosizer (Malvern Instrument). The samples were sonicated for 5 min before measurement. Each measurement was repeated 3 times in order to acquire an average data.

2.6.2 Atomic forced microscopy (AFM)

The AFM image were obtained by Multimode SPA400 (Seiko, Japan) in semicontact mode (dynamic mode) using a SI-DF20 cantilever. The image of vesicles was measured on an air-dried sample of polydiacetylene vesicles prepared from a drop of solution on freshly cleaned mica.

2.7 Principal component analysis (PCA)

PCA was performed with Unscrambler 9.7 software version, using the %CR of PDA/surfactant mixtures at 50 μM of surfactants concentration as input. For the anionic surfactants discrimination, multidimensional data set (3 PDAs × 9 surfactants × 9 replicates) was further statistically analyzed by principal component analysis

2.8 Colorimetric response of paper-based PDA sensor arrays

2.8.1 Fabrication of PDAs coated paper

Diacetylene monomer 1 was first dissolved in chloroform, (1% w/v) and 2 μL of the solution was dropped on a piece of filter paper (Whatman No.1 chromatography paper) using auto pipette. The paper was allowed to dry in the air. These colorless papers were stored in the dark and kept 4 °C for 1 hour. Polymerization was conducted using a hand-held UV lamp (500 $\mu W/cm^2$) operating at a wavelength of 254 nm. The lamp were hung 10 cm above the white dots on filter

paper. The samples were then irradiated for 1 min to provide multiple blue PDA spots on the paper.

2.8.2 Detection surfactants sensing on the PDAs paper

The 3 anionic surfactants (SDC,SDS, SDBS), 3 cationic surfactants (TTAB, DTAB, HTAB) and 3nonionic surfactants (Tween20, Brij@58P, TritonX-100) were dissolved in millQ-water to provide a 1mM. PDA test papers were soaked in various concentration of surfactants solution (100,500, and 1000 µM) for 30, 60, 90 and 120 seconds. The color changes were recorded at 5, 10, and 20 minutes using canon digital camera as present in Figure 2.1

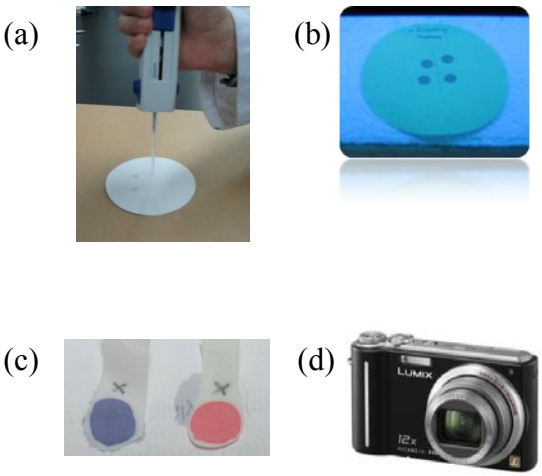


Figure 2.1 Process of colorimetric response of paper-based PDA sensor arrays for anionic surfactants sensing. (a) Drop and dry in the air (b) UV irradiation for 1 min (254 nm, 500 µW/cm2) (c) anionic surfactants sensing at 30 °C for 30, 60, 90, 120 seconds, and 5, 10, 20 minutes (d) recorded with digital camera

2.9 Optical microscopic images

The appearance of before and after dip PDA test papers, in surfactants solution, were recorded using Olympus DP72 equipped with 4X objective lens (Olympus, Japan).

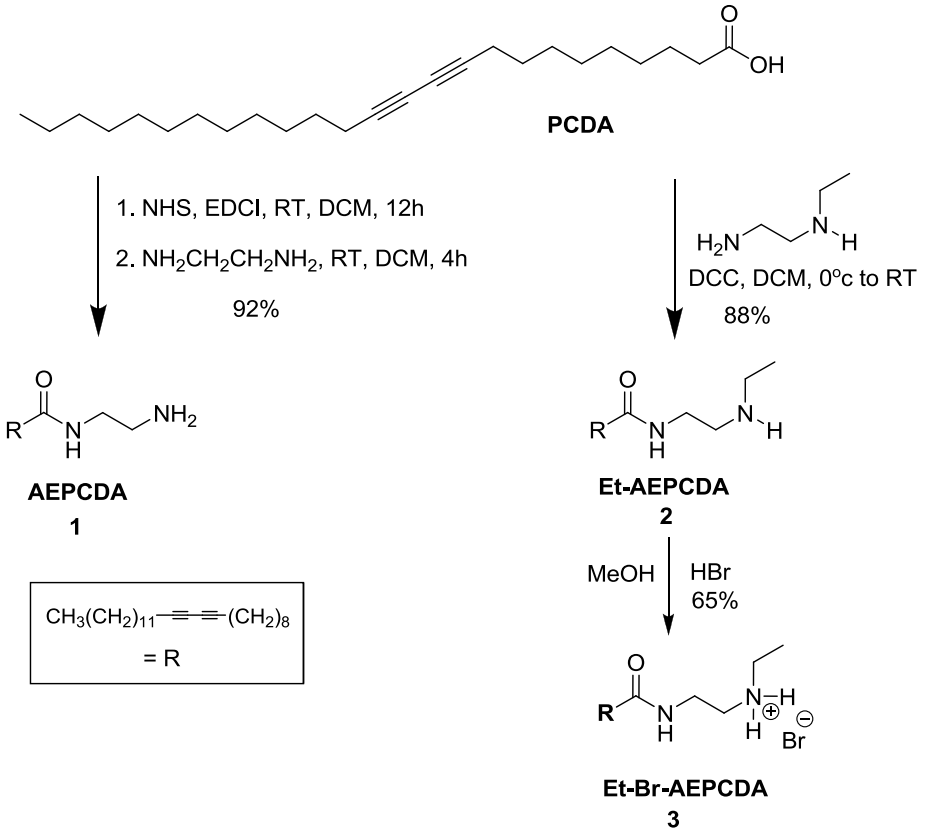
CHAPTER III

RESULTS AND DISCUSSION

3.1 Synthesis of diacetylene lipid monomers

3.1.1 Synthesis of amphiphilic diacetylene monomers

Three amido diacetylene lipids **1-3** were prepared from PCDA. Treatment of ethylene diamine in the presence of coupling agent EDCI (1-Ethyl-3-(3′-dimethylamino) carbodiimide HCl salt) gave the compound **1** in 92 % yield. Similarly, coupling reaction of PCDA with the *N*-ethyl-ethylenediamine in the presence of DCC (*N*,*N*′- dicylohexylcarbodiimide) gave the desired diacetylene monomers **2** in 88% yield. Finally, diacetylene monomers **3** were synthesized upon the addition of HBr to solution of **2** in methanol, generating **3** in 65%.



Scheme 3.1 Synthesis of amphiphilic diacetylene monomers (**1-3**).

3.2 Characterization of diacetylene lipid monomers

^{1}H and ^{13}C NMR and Mass spectra techniques were acquired to characterize all prepared diacetylene monomers **1**, **2** and **3** (for the ^{1}H NMR ^{13}C NMR and mass spectra please see the appendix section).

3.2.1 Characterization of amphiphilic diacetylene monomers

The ^{1}H NMR spectra of the amphiphilic diacetylene monomers (**1-3**) are shown in Figure 3.2. ^{1}H NMR spectra of AEPCDA (**1**) and Et-AEPCDA (**2**) displayed the signals of -NH protons (u) in the chemical shift range of 6.0-6.2 ppm confirming the formation of C-N bond. The signal of –NH protons (u) were deshilded showing a chemical shift at 7.4 ppm in case of Et-Br-AEPCDA (**3**) due to ammonium protons were stabilizated from oxygen from amine group. These data also confirm the formation of ammonium salt. Moreover, two methylene protons next to the amido nitrogen (v, w) of Et-AEPCDA (**2**) appeared at 3.4 and 2.8 ppm respectively while that of Et-Br-AEPCDA **3** slightly shifted to 3.6 and 3.2 ppm respectively due to the high electronegativity of ammonium moiety. Besides, the signals of the methylene protons next to the carbonyl group of amide (t) of **1-3** are displayed similarly around 2.2 ppm. Most of the protons in the aliphatic chain (b-k, n-s) in all three monomers gave the similar pattern showing the signals in the range of 1.8 - 0.9 ppm.

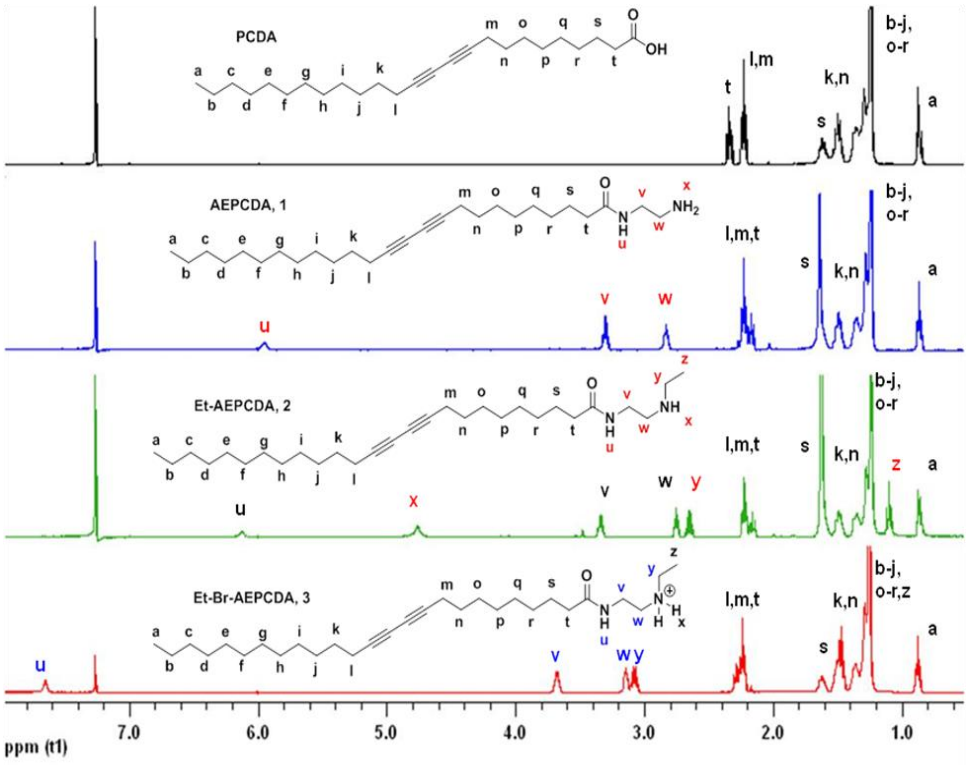


Figure 3.2 ^{1}H NMR spectra of the amphiphilic diacetylene lipids PCDA, 1, 2 and 3

3.3 Preparation of polydiacetylene sols

3.3.1 Polymerization of diacetylene monomers

To study the affinochromism of desired PDAs, all prepared diacetylene monomers were first converted to polydiacetylene sols. Initially, all synthesized monomers were dissolved in chloroform (0.5 mL), then the solvent were evaporated using N_2 gas followed by dispersion in milli-Q water as described in chapter II. Then aqueous sols were then irradiated with UV light (254nm) for 5 minutes at 0 °C to give a blue-color solution. These results indicated the formation of ene-yne conjugated polydiacetylenes. The ability to be hydrated and the color of polymerized diacetylenes were presented in table 3.1. Upon UV light (254nm) for 5 minutes at 0 °C, monomers **1**, and **2** transformed into blue sols while poly **3** showed unstable pale blue sols. This might be caused by poor polymerization due to the steric repulsion of ammonium head group of monomer. To solve this problem, we mixed PCDA into monomer **3** in the ratio of 1: 4 V/V to reduce the charge repulsion between head groups. Pleasingly,

polymeization of **3**/PCDA gave the blue sols. The blue sol of poly **1**, poly **2** and poly **3**/PCDA displayed absorption maximum (λ_{max}) near 640nm.

Table 3.1 Polydiacetylene vesicle before (left) and after (right) polymerization

PDA monomers	**Color before polymerized**	**Color after polymerized**	**λ_{max} (nm)**
1			636
2			640
3/PCDA			638

3.3.2 The colorimetric response of PDAs with surfactants

As mentioned above, the polydiacetylene sols form monomers **1** and **2** appeared as blue solution as shown in Figure 3.3 (blank) while the mixed lipid **3**/PCDA gave the purple PDA sols. To test the sensing ability of these PDA with surfactants, the panels each type of surfactants (50 μM) was added to all prepared PDA sols including poly (PCDA) and the results are presented in Figure 3.3. The blue PDA sol prepared from PCDA did not exhibit any color change upon mixing with anionic (SDC, SDS, and SDBS) and nonionic (Tween20, Brij@58P, and TritonX-100) surfactants. For the cationic surfactants, DTAB and HTAB caused the sol to form blue precipitation whereas HTAB induced unusual color change from blue to yellowish green without any precipitation [29]. Importantly, PDA sols obtained from **1**, **2** and **3**/PCDA mixture exhibited the by design blue to red color transition selectively to the anionic surfactant. No color change was observed upon the addition of the cationic but it displayed small colorimetric response with a nonionic surfactant, Brij@58P.

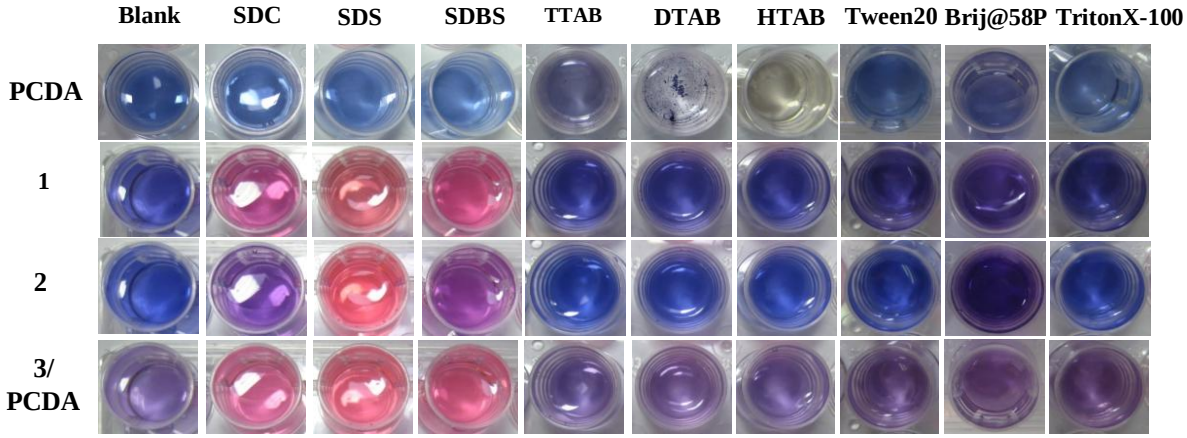


Figure 3.3 Appearance of PDA sols (0.1 mM) in the absence (Blank) and presence of SDC, SDS, SDBS, TTAB, DTAB, HTAB, Tween20, Brij@58P, and Triton X-100 (50 μM)

Moreover, the colorimetric change of PDA sols was also monitored by UV-visible spectrophotometer. The addition of SDC, SDS, and SDBS to prepared PDA sols resulted in the decrease of the absorption at 640 nm with simultaneous increase of the absorption at 540 nm as well as from photograph recorder as shown in Figure 3.4. The colorimetric transition of each anionic surfactant showed difference among PDAs. For SDS, PDA sols derived from 1, 2, and 3/PCDA were able to induce the vivid color change at the concentration below 10 μM while SDC and SDBS caused color change around 20-40 μM. As the sols were acidic with constant pH of ca. 5.0 throughout the experiment, the color transition observed here is likely to be initiated by the columbic interaction between the ammonium head groups of **1**, **2,** and **3** and the anionic head groups of the surfactant molecules.

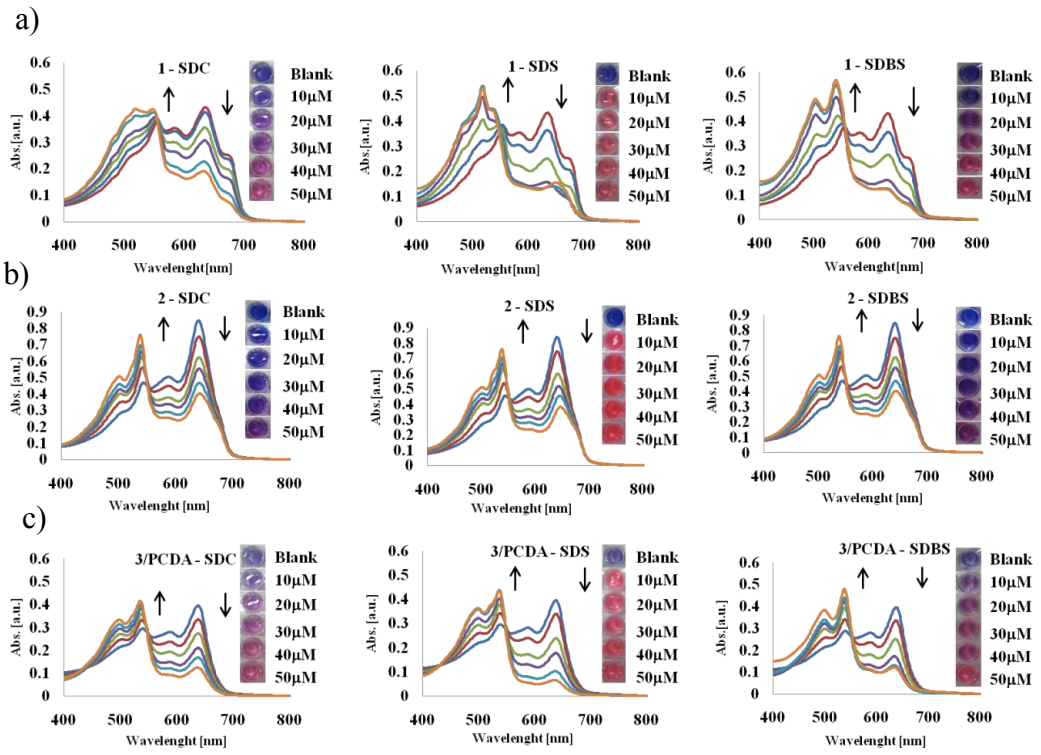


Figure 3.4 UV/Vis spectra and photographs of PDA sols (0.1 mM) derived from a) **1**, b) **2** and c) **3**/PCDA mixture in the presence of various amounts of SDC, SDS, and SDBS at room temperature showing their diverse colorimetric transitions.

On the other hand, the addition of cationic surfactants such as TTAB, DTAB, HTAB and TritonX-100 to PDA of 1, 2, and 3/PCDA were performed in order to see the selectivity of our prepared PDAs. The color changes were monitored by UV-visible spectrophotometer (Figure 3.5). We found that all PDA did not show any significant discretion of the absorption at 640 nm. These results are in the good agreement with the observation by eyes indicating no color changes in these PDAs with all tested cationic surfactants. However, Tween20, and Brij@58P showed a minor change of UV-visible spectrum (Figure 3.6). Presumably, the structures of Tween20 and Brij@58P have a lot of hydroxyls in the head group. They are high electron donating so they could ionic interact with our PDA.

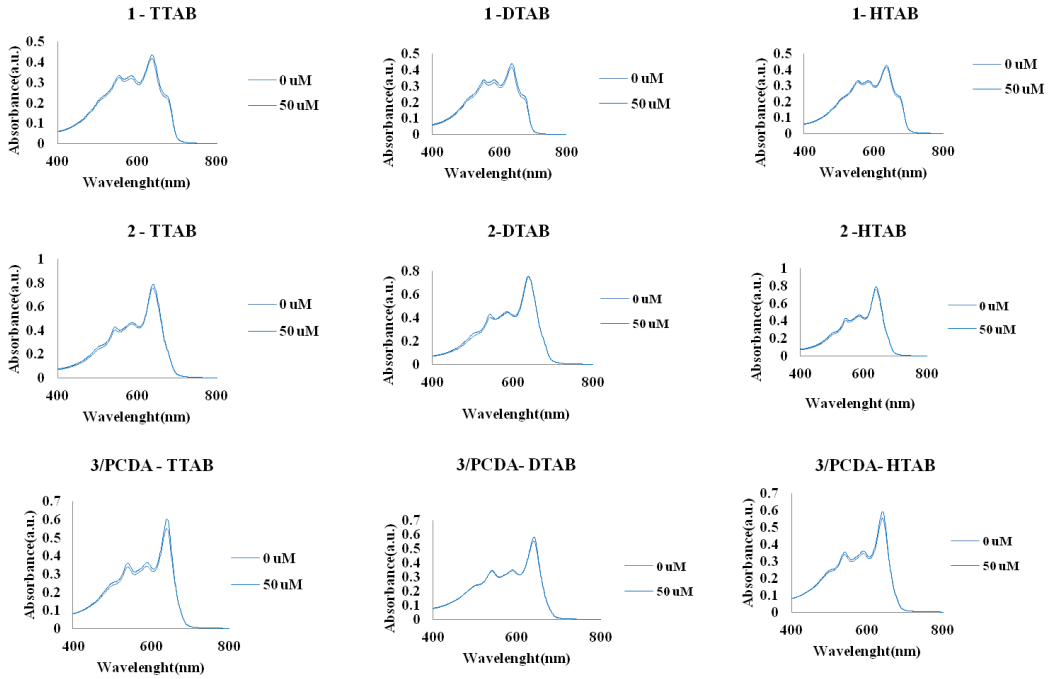


Figure 3.5 UV-visible spectrums of **1**, **2**, and **3**/PCDA with TTAB, DTAB, and HTAB

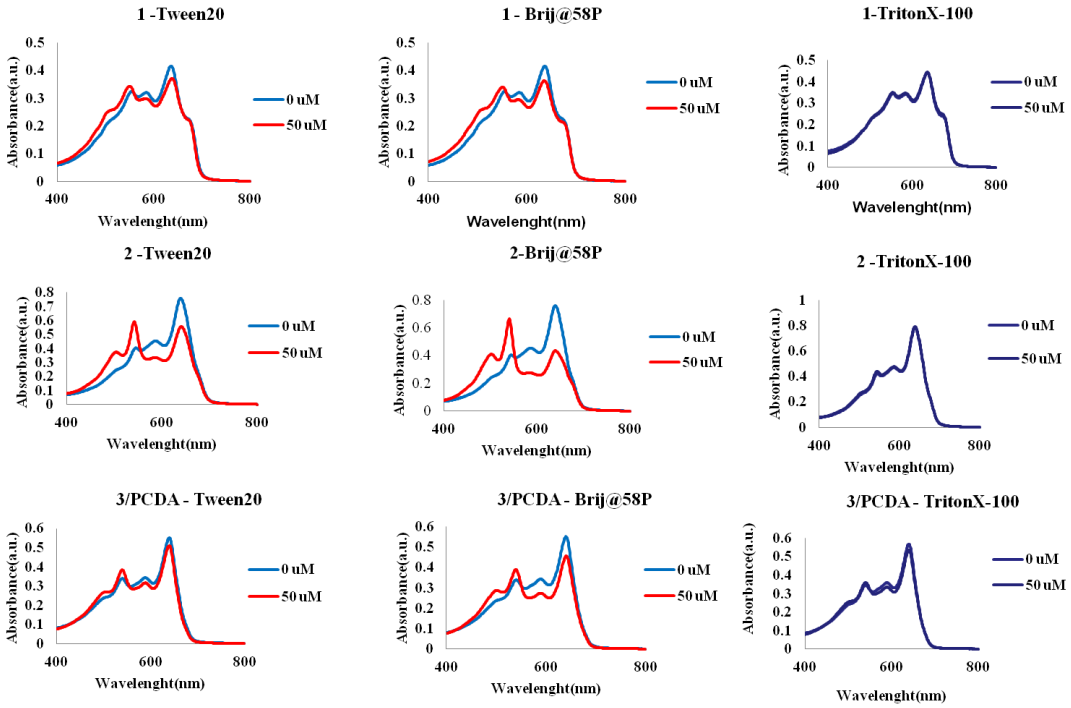


Figure 3.6 UV-visible spectrums of **1**, **2**, and **3**/PCDA with Tween20, Brij@58P, and TritonX-100

3.4 Particle size and Morphology of polydiacetylene sols

3.4.1 Dynamic light scattering (DLS)

The particle size of PDA sol before and after the addition of surfactant is investigated in order to gain the mechanistic information. According to the proposed mechanisms it was assumed that the anionic surfactants inserted into the PDA particles. The size of the PDA colloidal particles measured by dynamic light scattering (DLS) technique revealed that the average particle size of PDA **1** was 53 nm and it was increased to 63 and 81 nm upon the addition of 10 and 50 μM SDBS, respectively (Figure. 3.7).

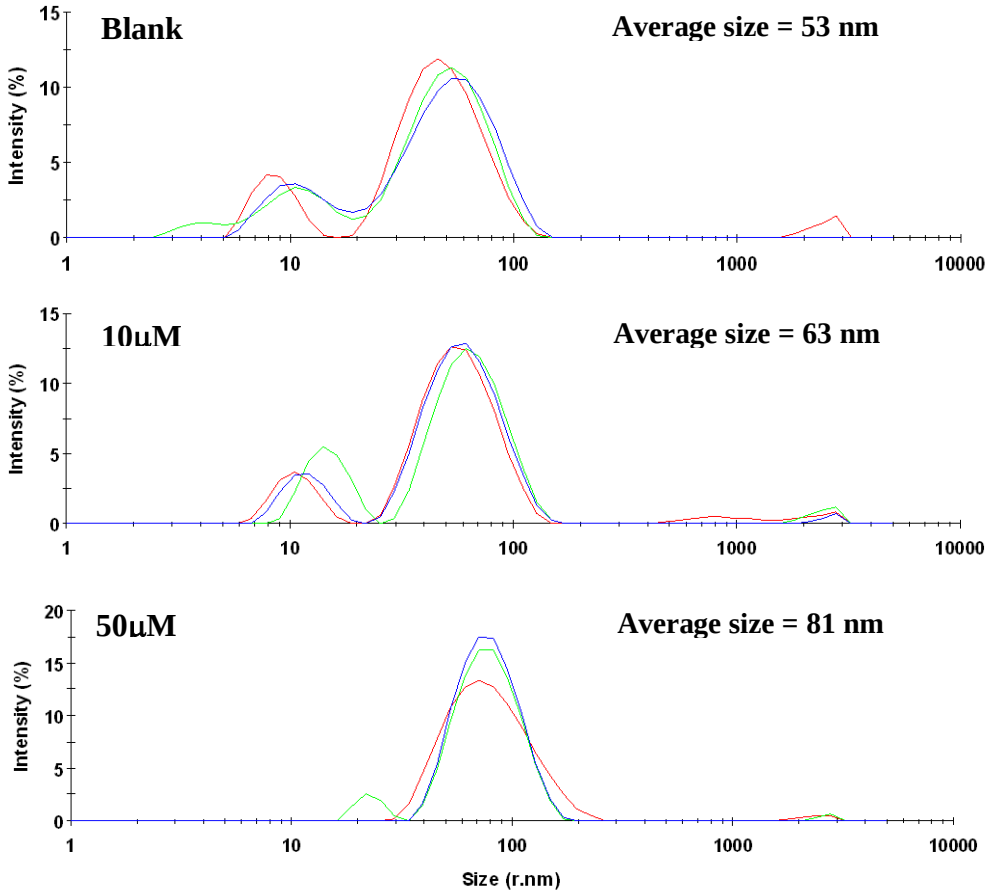


Figure 3.7 Size distributions obtained from dynamic light scattering of PDA derived from **1**, before and after addition of SDBS at 10 and 50 μM

PDA **2** also showed similar trend of which the size increased from 64 to 70 and 100 nm upon the addition of 10 and 50 μM SDBS, respectively (Figure 3.8).

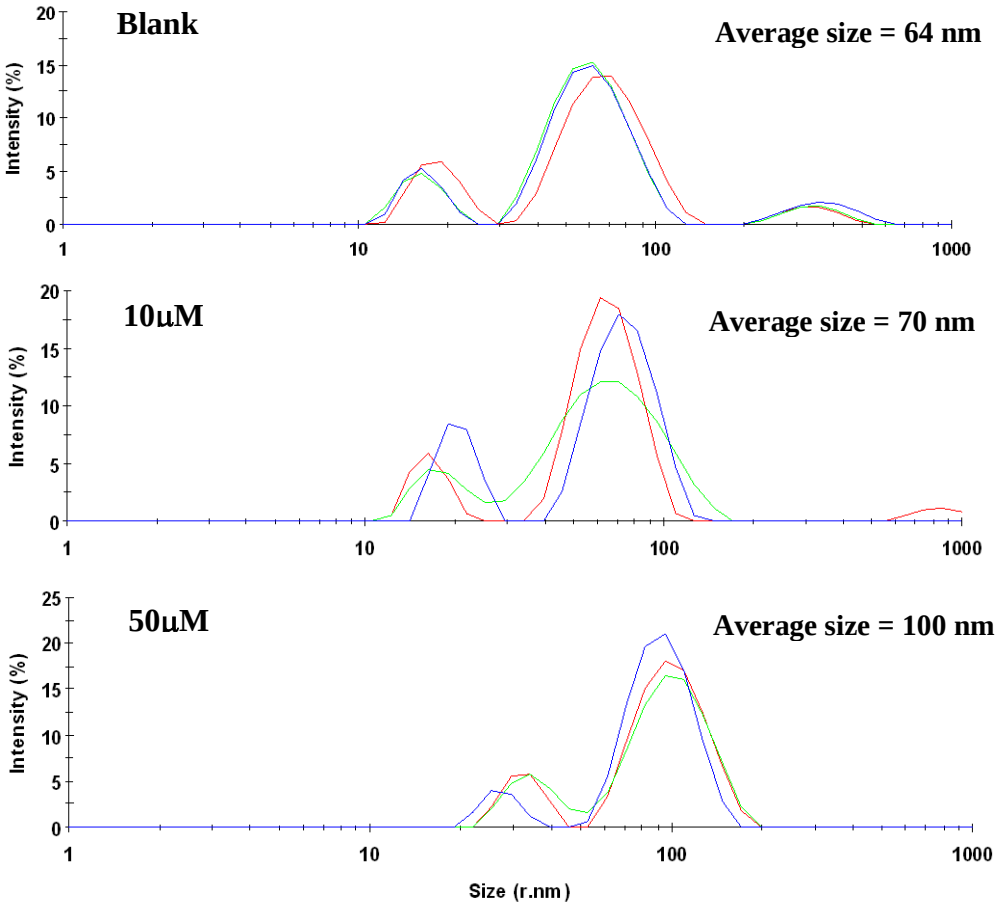


Figure 3.8 Size distribution obtained from dynamic light scattering of PDA derived from **2**, before and after addition of SDBS at 10 and 50 μM

On the other hand, the particle sizes of PDA derived from **3**/PCDA mixed monomers obtained from DLS (78, 82, and 85 nm) did not significantly change upon the addition of 10 and 50 μM SDBS, respectively (Figure 3.9). For results of poly **1** and poly **2**, it was assumed that the anionic surfactants inserted into polydiaceylene sols so they showed particle size increase upon the addition of anionic surfactants while poly 3/PCDA have a some part of particles were broken upon the insertion of SDBS due to we expect the mixed lipid vesicle are relatively weak

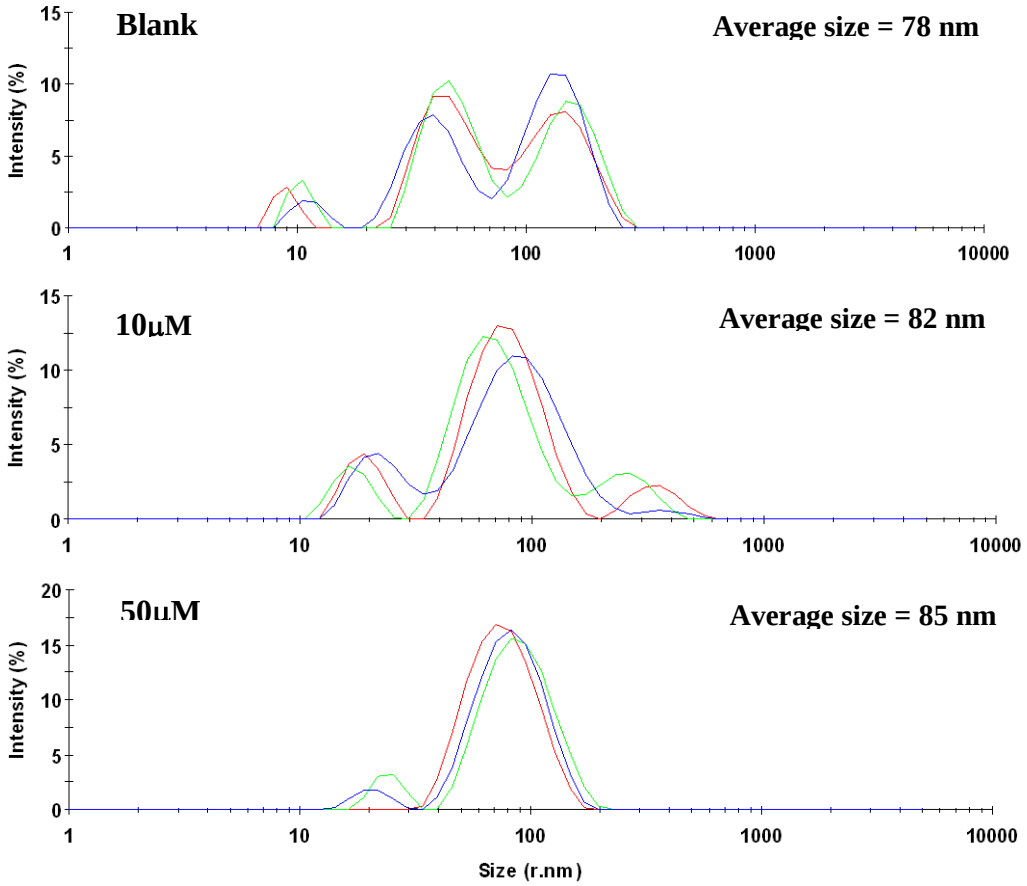


Figure 3.9 Size distribution obtained from dynamic light scattering of PDA derived from **3**/PCDA, before and after addition of SDBS at 10 and 50 μM.

3.4.2 Atomic force microscopy (AFM)

AMF was utilized to observe the shape and size of the air-dried PDA sols. The AFM image showed spherical structure of all PDA sols. PDA of **1** and **2** appeared as spherical-shaped particles before the addition of the surfactant confirming the vesicle formation shown in Figure 3.10a and 3.10b. Upon the addition of SDBS, significant aggregation along with particle size enlargement was observed. The AFM images of PDA **3**/PCDA also did not show any aggregation but some broken particles.

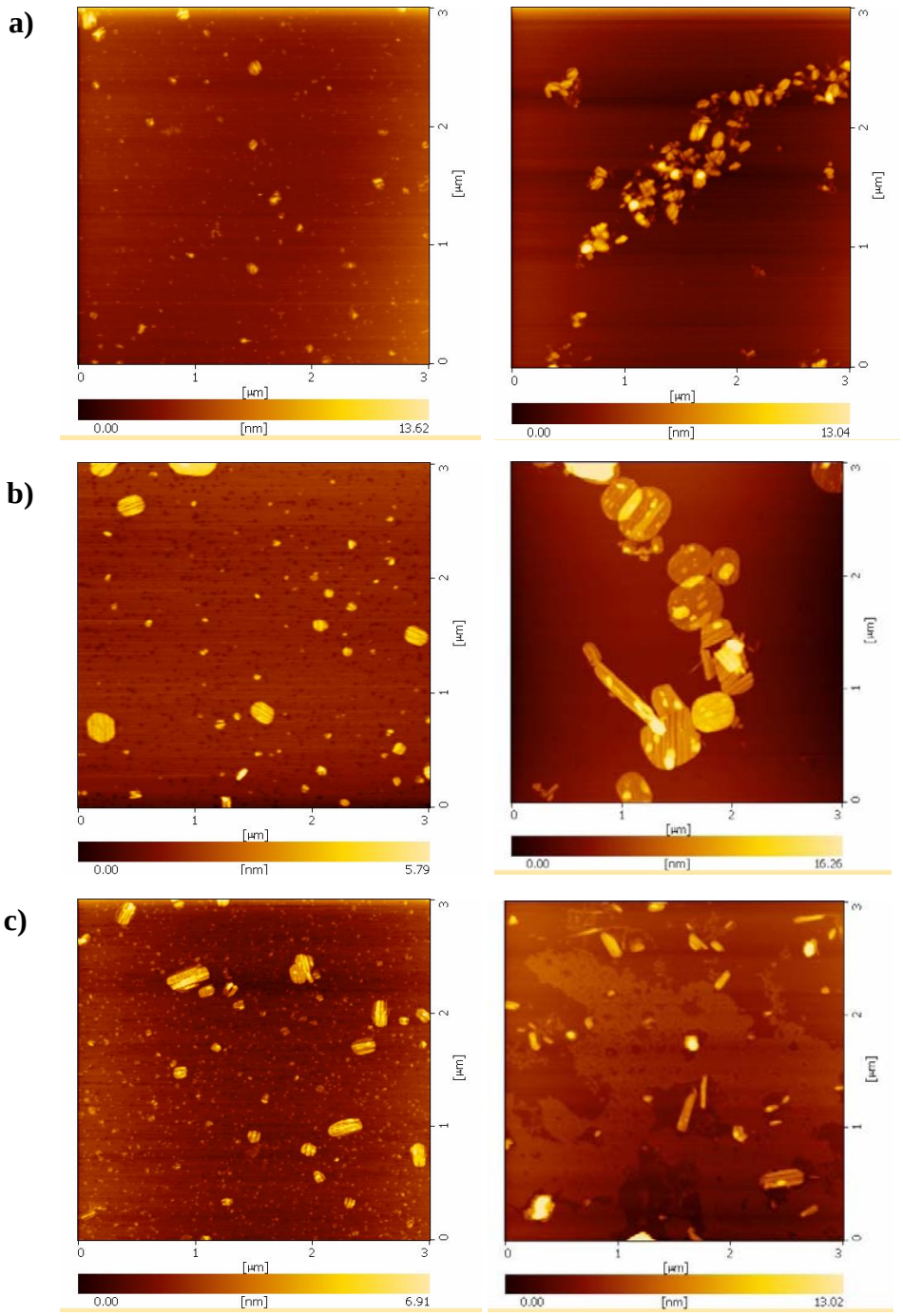


Figure 3.10 AFM images of a) PDA **1** b) PDA **2** c) PDA **3**/PCDA. Left = before and Right = after addition of SDBS at 50 µM

3.5 Proposed mechanism

Based on above described, these DLS results are in good correspondence with the AFM results, we thus proposed the mechanism of these color transition as seen in Figure 3.11. The single lipid vesicles such as PDA of **1** and **2** appear as spherical-shaped particles before the addition of the surfactant confirming the vesicle formation. Upon the addition of SDBS, significant aggregation along with particle size enlargement was observed (Figure 3.11 left). The mixed lipid vesicle, **3**/PCDA, on the other hands did not displayed particle size increasing based on DLS and the AFM images also did not show any aggregation but some broken particles upon the presence of SDBS. It is therefore assumed that the vesicle of 3/PCDA is relatively week and upon the addition of SDBS, the insertion of surfactant occurs causing the particle deformation as proposed in Figure 3.11 (right). The results of DLS and AFM have provides insightful evidences for the surfactant insertion induced color transition of the PDAs.

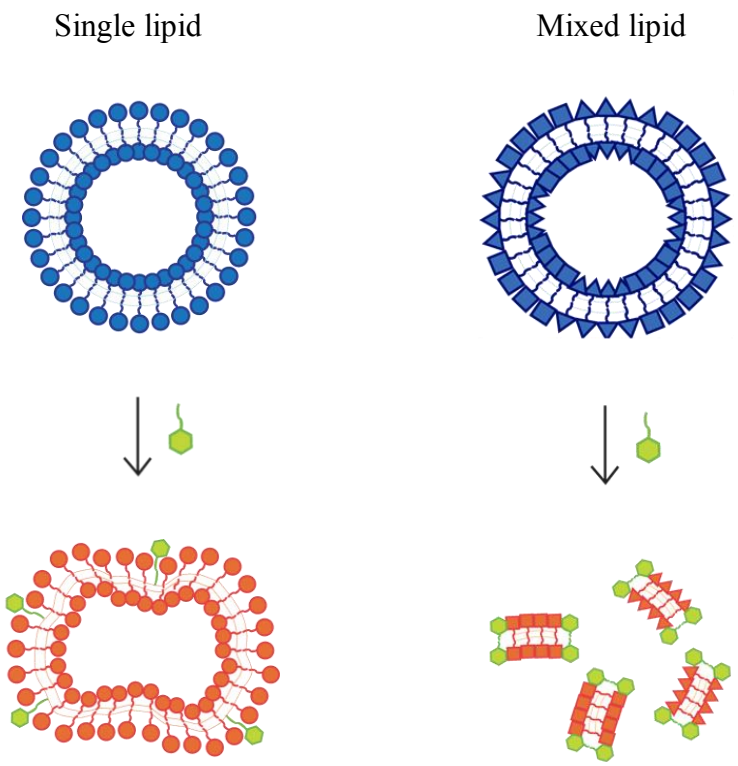


Figure 3.11 Drawings show the morphology changes and proposed interaction of SDBS molecules ( ) with PDA vesicles from single lipid and mixed lipids.

3.6 Identification of surfactants using PDA sensor array

3.6.1 Colorimetric response (%CR)

In order to quantify the degree of color change of PDA in the presence of various surfactant, data from PDAs absorbance spectrums at 50 μM of surfactants were converted to colorimetric response (% CR), the conversion percentage of the blue to red phase absorption and the histogram of the average % CR determined from nine replicates (3 samples × 3 measurements) of the PDA/surfactant mixtures were presented in Figure 3.12. For example, anionic surfactants (SDC, SDS, and SDBS) induced color transition of PDAs **1**, **2,** and **3**/PCDA were higher than 50% CR indicating strong color induction ability which is supported the results from naked eye observation. Moreover, the % CR of PDA/surfactant mixtures showed differentiable patterns of the responses for three anionic surfactants suggesting a possibility of surfactant identification. On the other hand, PDAs gave low responses and similar patterns with the cationic species such as TTAB, DTAB, and HTAB (%CR < 5, for UV-Vis spectrum see in Figure 3.5). As previous discussed, small color transition were observed when PDAs were mixed with nonionic surfactants such as Tween20 and Brij@58P (Figure 3.6). As shown in Figure 3.12, the %CR of PDAs in the presence of Tween20 and Brij@58P were found in the range between 10-50% while showing nearly zero percent CR with the TritonX-100 (see UV-Vis spectrum in Figure 3.6)

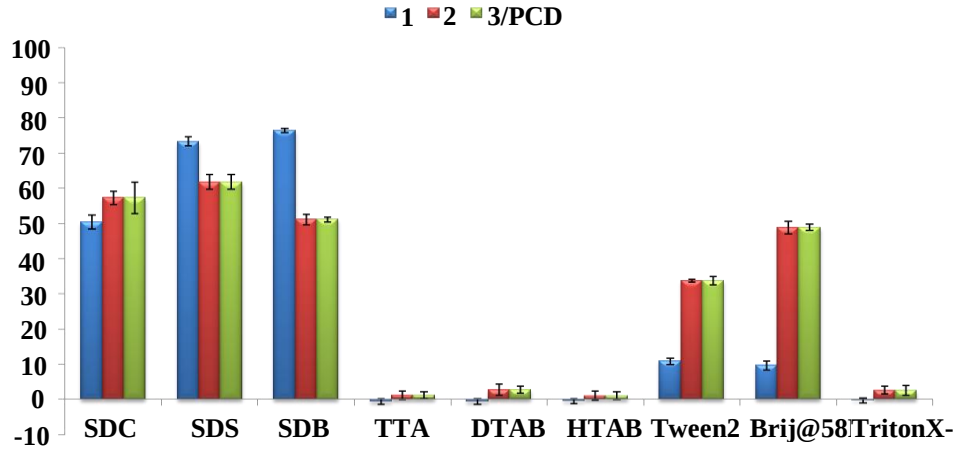


Figure 3.12 Histogram of average %CR determined from nine mixtures of **1**, **2** and **3**/PCDA (0.10 mM) and SDC, SDS, SDBS, TTAB, DTAB, HTAB, Tween20, Brij@58P, and TritonX-100 (50 μM).

3.6.2 Principal component analysis (PCA)

Although, the histogram plot showed the differentiate color pattern for anionic surfactants sensing, the relationship of multidimensional data set (3 PDAs × 3 surfactants × 9 replicates) was further statistically analyzed by principal component analysis (PCA) to transform the % CR values into PC scores in which PC1 and PC2 contributed 94.2 and 5.3%, respectively (Figure 3.13). The PCA score plot clearly showed five clusters of data points demonstrating complete discrimination of SDC, SDS, SDBS, Tween20, and Brij@58P. However, the plot of the results upon mixing cationic surfactants or TritonX-100 with PDA produced a poor separation. This observation is in good agreement with the low colorimetric response and similar colorimetric pattern of analytes with our PDAs array. The results up to this point already suggested that structurally diversed amino containing PDAs were useful for the detection and identification of the common anionic surfactants and some nonionic surfactants. Moreover, the fact that PCA are not able to separate the cationic surfactants indicating the specificity of the synthesized PDA array toward the anionic surfactants but not to the cationic surfactants

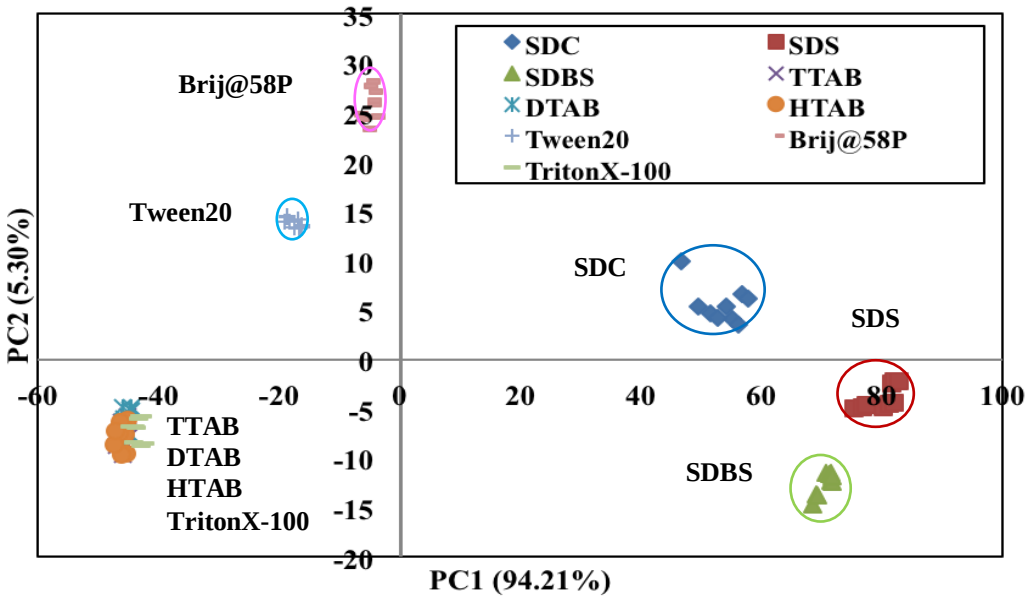


Figure 3.13 a) Histogram and b) PCA score plot of % CR obtained from nine replicated measurements of nine mixtures of 1, 2 and 3/PCDA (0.10 mM) and SDC, SDS, SDBS, TTAB, DTAB, HTAB, Tween20, Brij@58P and Triton X-100 (50 µM).

3.7 Colorimetric response of paper-based PDA sensor arrays

From the above results, the PDA derived from **1** was considered to be the highest potential sensor among the others. PDA of **1** not only produced the best response to all studied anionic surfactants, giving % CR above 50 with SDC, SDS, and SDBS at 50 μM but it is also demonstrated the high selectivity among surfactant species giving CR lower than 10% with nonionic and cationic surfactants. Therefore, the next focus was to develop PDA **1** monomer into a solid-state sensor which should be more practical for onsite application. Although the colloidal liquid sol of PDAs is very suitable for spectroscopic study at the initial developing stage due to its homogeneity and essentially quantitative polymerization, this form of PDAs may not be particularly convenient for sensing applications, in which portability and long shelf life are also very important. In order to make PDAs sensor affordable, user friendly, and equipment free, we decided to fabricate **1** on filter paper which is an inexpensive and environmentally friendly material. The white color of filter paper would make it as an excellent choice of substrate for fabricating a colorimetric sensing agent [27, 28] Monomer **1** coated filter paper was prepared by dropping a dichloromethane solution of **1** onto a piece of filter paper followed by UV irradiation to afford blue colored paper indicating a successful polymerization of **1** into the corresponding PDA. The blue color of the PDA on the papers is stable indefinitely upon storage in a refrigerator and for several days at 25 °C. Initially, blue PDA coated paper **1** was immersed in a solution of anionic surfactants (SDC, SDS, and SDBS) at various concentrations such as 100 , 500, and 1000 μM from 30 seconds to 10 minutes and the color changes were recorded using digital camera (Figure 3.14a to f). In general, anionic surfactants at 100 μM concentrations didn't induce any color change while at 500μM concentrations, small color change were appeared at all kind of surfactants. This indicated that the detection limit of PDA coated paper was between 500-1000 μM of SDBS.

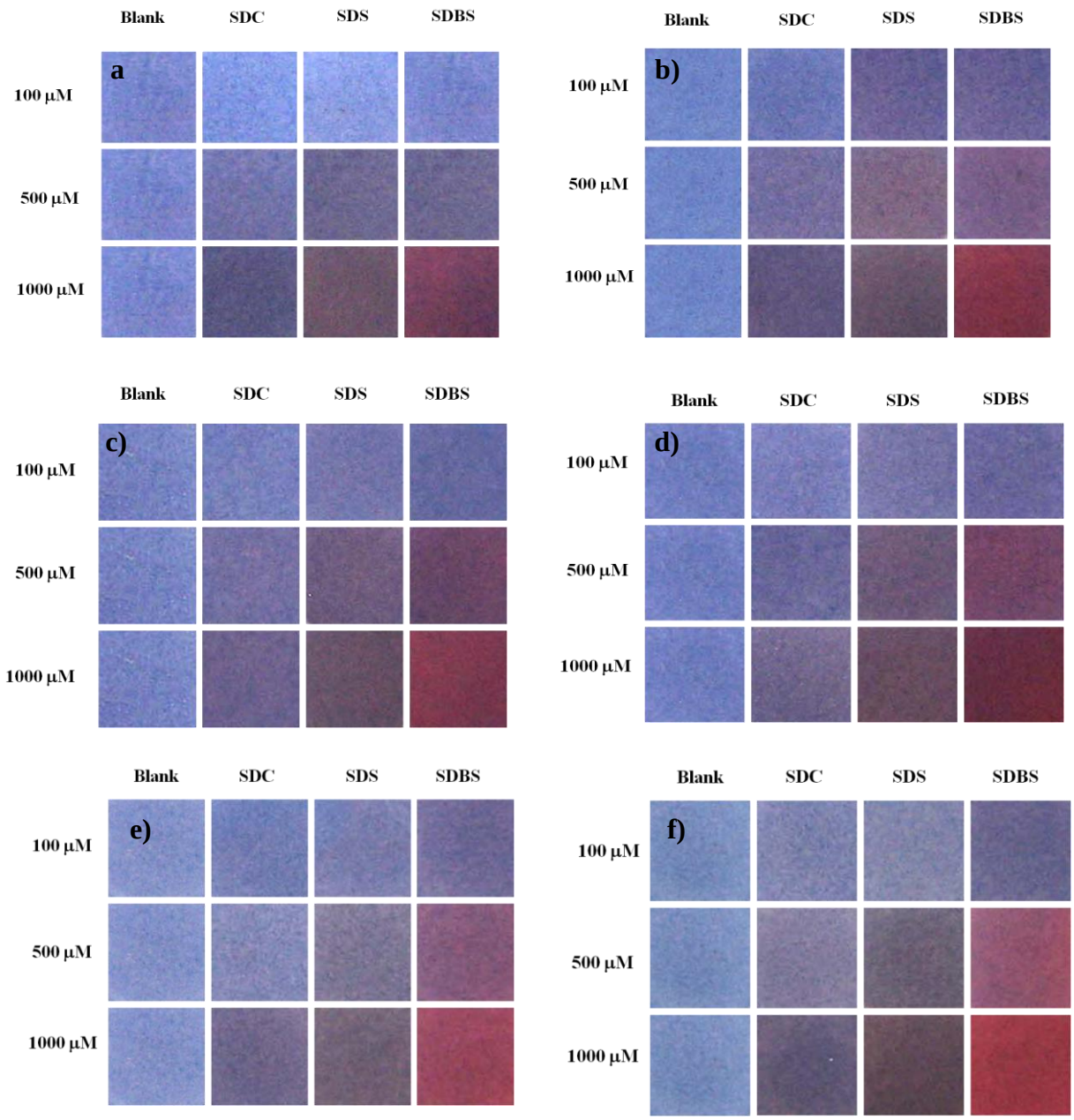


Figure 3.14 Photograph of PDA 1 coated paper (1% w/v) after dipped in solution of SDC, SDS, and SDBS (100, 500, and 1000 µM) for a) 30 sec, b) 60 sec, c) 90 sec, d) 120 sec, e) 5 minutes f) 10 minutes. Blank refers to PDA **1** dipped in Mill-Q water

In order to see the specificity of our prepared PDA coated paper toward the anionic surfactants, PDA paper of **1** was immersed in a solution cationic and nonionic surfactants at 500 μM for 20 minutes in comparison with anionic surfactants and the color changes were recorded using digital camera (Figure 3.15). PDA papers of **1** were showed color changes within anionic surfactants as well as previously described. In term of cationic surfactants, however, it did not induce any color change even at the concentration of 5 mM and only minor color changes were observed for nonionic surfactants such as Tween20 and Brij@58P but not TritonX-100. This indicated that PDA coated paper of **1** was specific toward anionic surfactants as shown in Figure 3.15.

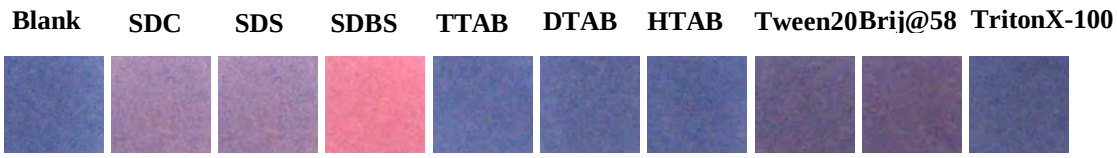


Figure 3.15 Photograph of PDA 1 coated paper (1% w/v) after dipped in solution of SDC, SDS, SDBS, TTAB, DTAB, HTAB, Tween20, Brij@58P, and Triton X-100 (500 μM). Blank refers to PDA of **1** dipped in Mill-Q water

3.8 Optical microscopic images

To investigate the distribution of PDA on filter paper in micrometer scale, the images of the PDA coated paper under optical microscope were taken. The images revealed that the blue pigments of PDA adhere to the surface of all cellulose fibers without penetrating into the fibers (Figure 3.16). This surface coating phenomenon is considered to be desirable for colorimetric sensing application as less sensing material is required and the sensing process involving only at surface is usually more rapid than within the bulk of fiber. The blue to red color transition of the PDA pigments on the fibers were also clearly observed under the optical microscope. The technique for preparation of this paper based sensor is very convenient and offers a very economical platform for solid state PDA sensor.

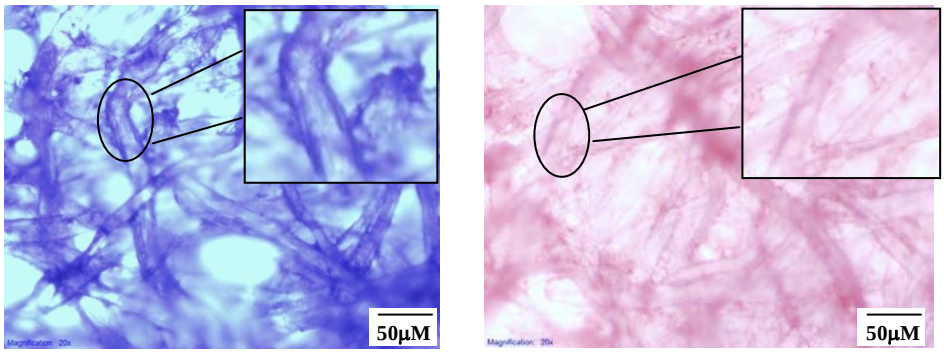


Figure 3.16 Optical microscopic images of PDA paper of 1 dipped in Mill-Q water (left) and in the solution of SDS (right) at 500 µM for 20 min.

3.9 Application with PDA coated papers to sense the commercial products

Surfactants are widely used in many house hold products such as cleaning agent, dishwashing liquid and shampoo etc. For dishwashing liquid (Cle-O clean), it is composed of only anionic surfactants such as sodium alkyl benzene sulfonate 14.4 %W/W (LAS) and sodium lauryl ether sulfate 3.6 %W/W (SLES) while cleaning agent (Magic clean) is made of cationic surfactant (benzalkonium chloride 1.00 %W/W) mixed with nonionic surfactants (ethoxylated and propoxylated alcohols 2.00 %W/W) as an ingredient (Figure 3.17). From difference composition of both products. We believed that our PDA paper can distinguish between the dishwashing liquid and the cleaning agent due to our specificity of our PDA sensor.

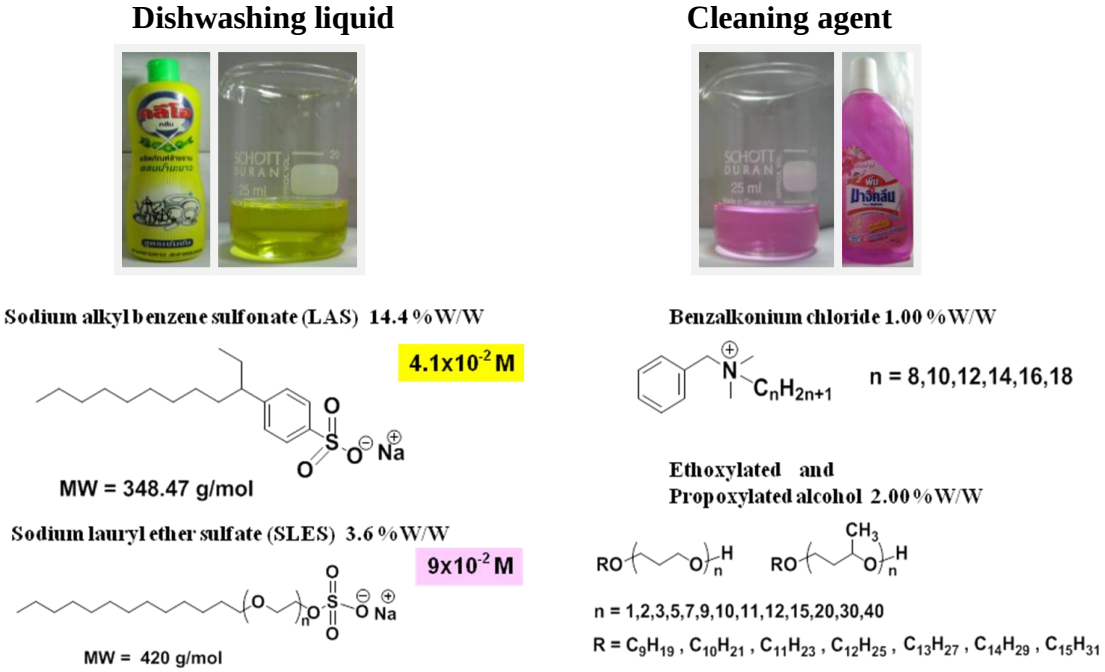


Figure 3.17 Structure anionic surfactants in dishwashing liquid (left) and cationic, nonionic surfactants in cleaning agent (right).

To begin with, the PDA papers of **1** were dipped into dishwashing liquid and cleaning agent products that were prior diluted with milli-Q water at 1:10, 1:100, 1:1000 and 1:1000 ratios for 5 and 20 min. For the cleaning agents, very little color changes were observed at all concentration when our PDA coated papers were immersed into the solution for 5 and 20 minute. On the other hand, the dishwashing liquid induced the strong blue to red color change with solution of 1:10 and 1:100 ratio while at the ratio of 1:1000 and 1:1000, the purple color transition of PDA coated paper were observed suggesting that the detection limit of our sensor is in between 1: 100 to 1: 1000 dilution ratio. These results are in the good agreement with the previous section. The concentration of surfactants in the tested washing products were calculates and presented in the Table 3.2. In order to see the strong color changes in the dishwashing liquid with PDA coated paper, the concentration of SLES must be in between 90-900 mM while LAS are in the range of 40-400 mM concentration which are in the same ranges with the SDS, SDBS and SDC (Figure 3.15). The reason that the PDA coated paper gave the lower sensitivity than the aqueous form in remained unknown to us but it was hypothesized that some of the head group of PDA were coated on the paper and the alky chains were free in the air. And the color change was occurred at the head group but the addition of anionic surfactant can't insert into the PDA head group. While the PDA vesicle, the head group of the anionic surfactants can insert into PDA sols and occurred the charge interaction between the head group of both. And other reaction, the long alky chain of both were repulsed each other. That is why the PDA coated paper gave the lower sensitivity than vesicles.

Table 3.2 The PDA coated paper derived from 1 (1% w/v) after dipped in solution of commercial products for a) 5 and b) 20 minutes.

a) Dilution ratio	Blank	Dishwashing liquid	Cleaning agent	Anionic surfactants concentration (M)	
				SLES	LAS
1:10				9.0×10^{-3}	4.1×10^{-3}
1:100				9.0×10^{-4}	4.1×10^{-4}
1:1000				9.0×10^{-5}	4.1×10^{-5}
1:10000				9.0×10^{-6}	4.1×10^{-6}

b) Dilution ratio	Blank	Dishwashing liquid	Cleaning agent	Anionic surfactants concentration (M)	
				SLES	LAS
1:10				9.0×10^{-3}	4.1×10^{-3}
1:100				9.0×10^{-4}	4.1×10^{-4}
1:1000				9.0×10^{-5}	4.1×10^{-5}
1:10000				9.0×10^{-6}	4.1×10^{-6}

From the above results, The PDA paper of **1** was selective to only anionic surfactants after 20 minutes waiting time and they showed highly sensitivity to sodium lauryl ether sulfate (SLES) 900 mM and sodium alkyl benzene sulfonate (LAS) 410 mM.

CHAPTER IV

CONCLUSION

4.1 Conclusion

In conclusion, highly selective and sensitive colorimetric PDA-based sensors for common anionic surfactants from amphiphilic diacetylenes containing amino groups such as primary amine, secondary amine and ammonium salt were prepared. Specific naked eye detections of anionic surfactant (SDC, SDS, and SDBS) at micro molar concentration level (50 μM) of were achievable by these sensors. No color changes were observed with cationic surfactant (DTAB, TTAB, and HTAB) while small color transition appeared upon the addition with nonionic surfactant (Tween20, Brij@58P, and TritonX-100) at such concentration. , The mechanism of colorimetric transition was investigated using DLS and AFM, suggesting the insertion of surfactant into PDA particles resulting in the swell and aggregation of PDA vesicle. Moreover, assemble prepared PDAs into an array, discrimination of common anionic surfactants using principal component analysis (PCA) were achievable. The fabrication of PDA on filter paper was successful providing the blue PDA coated paper. They showed sensitivity toward SDC, SDC, SDBS, Tween20 and Brij@58P (500 μM), providing the paper based sensor for anionic surfactants potentially useful for onsite detection.. Therefore, the strategy presented here should be valuable for analytical task in the detection and identification of anionic surfactants.

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APPENDICES

Appendix A

Appendix A: ^{1}H NMR and ^{13}C NMR spectra of synthesized diacetylene monomers

Figure A1: ^{1}H NMR; AEPCDA, **1**

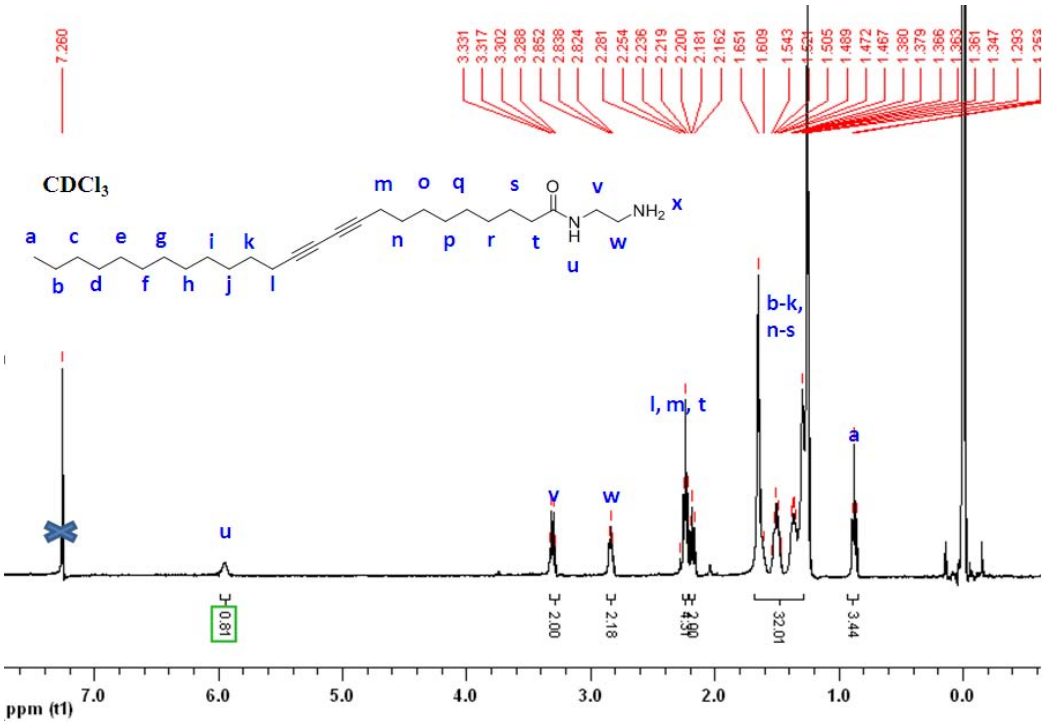


Figure A2: ^{1}H NMR; Et-AEPCDA, **2**

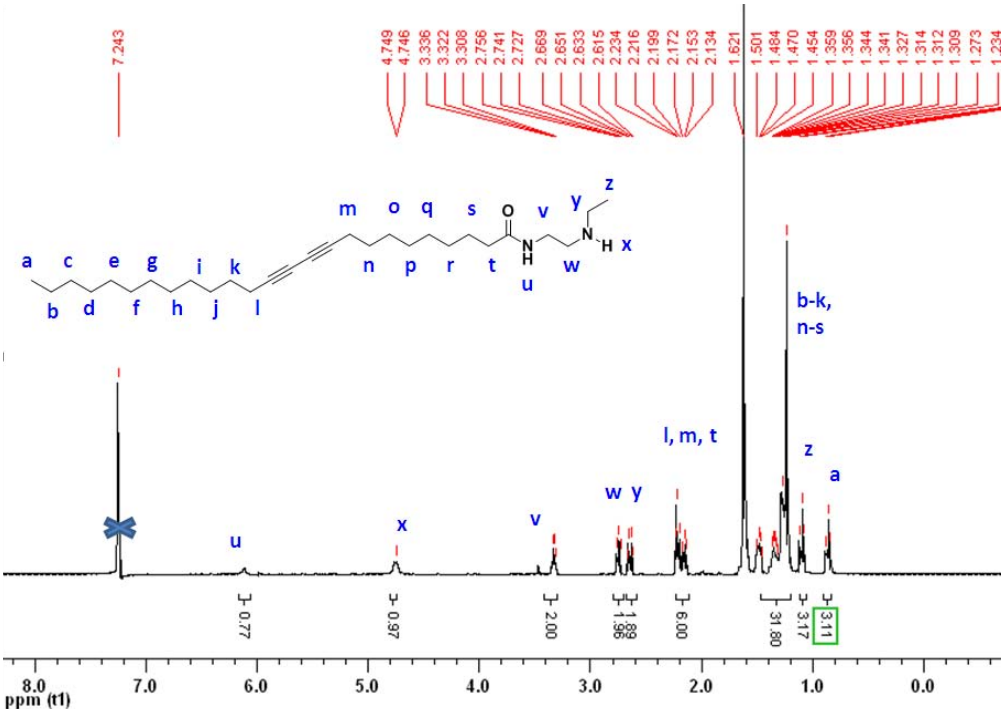


Figure A3: ^{1}H NMR; Et-Br-AEPCDA, **3**

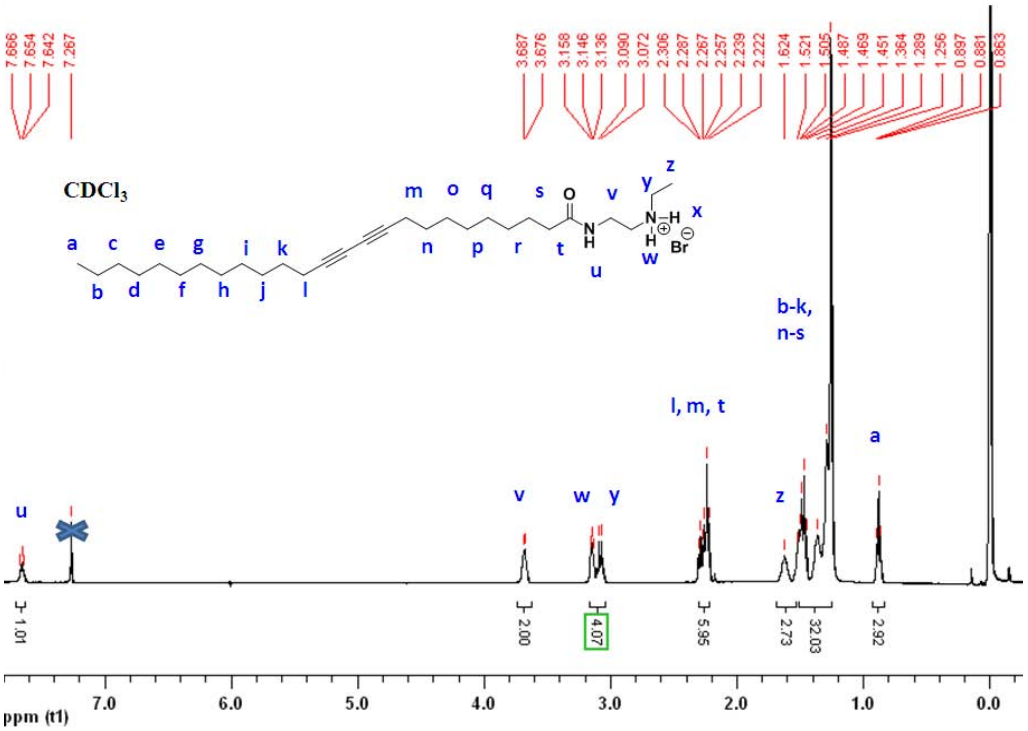


Figure A4: ^{13}C NMR; AEPCDA, **1**

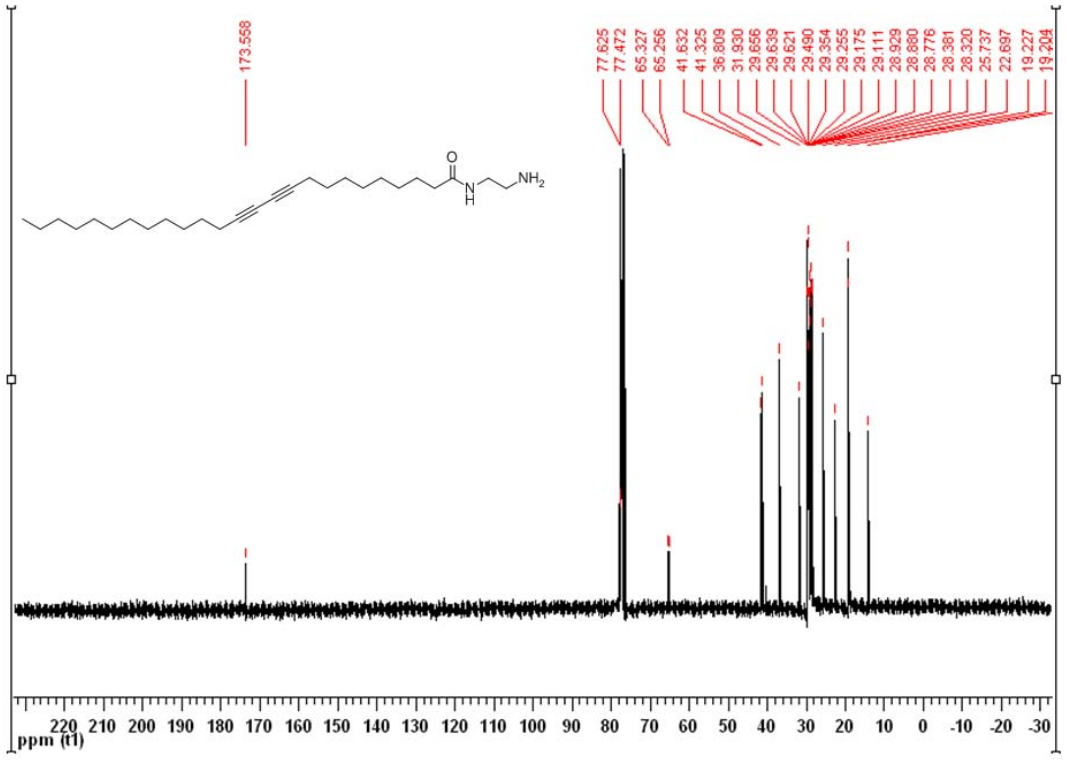


Figure A5: ^{13}C NMR; Et-AEPCDA, **2**

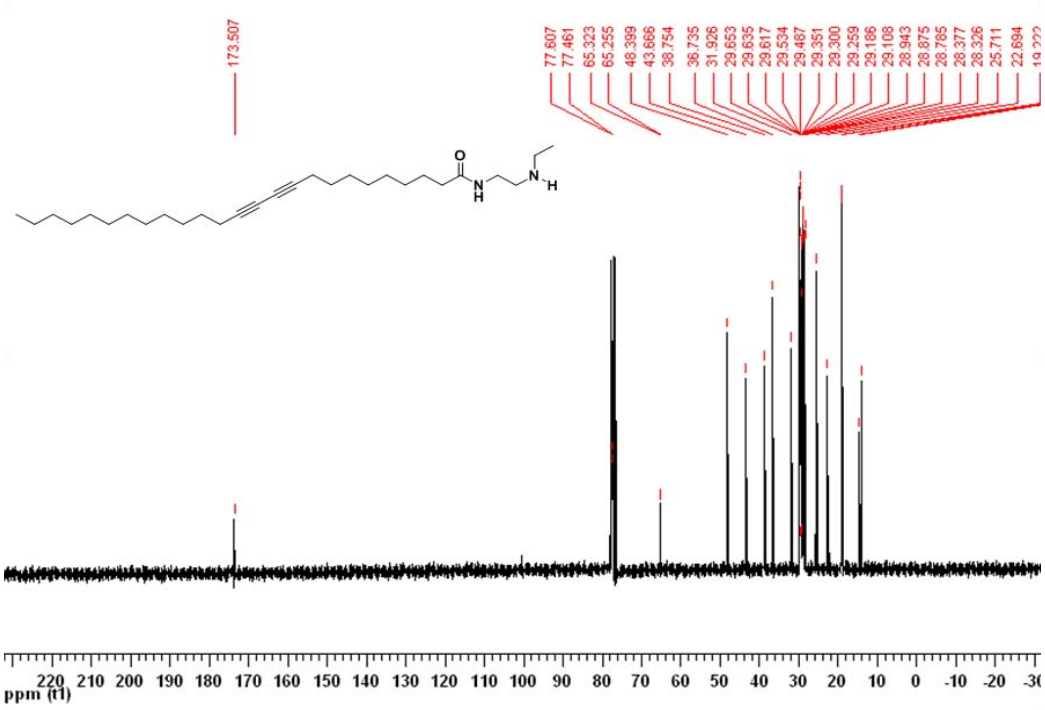
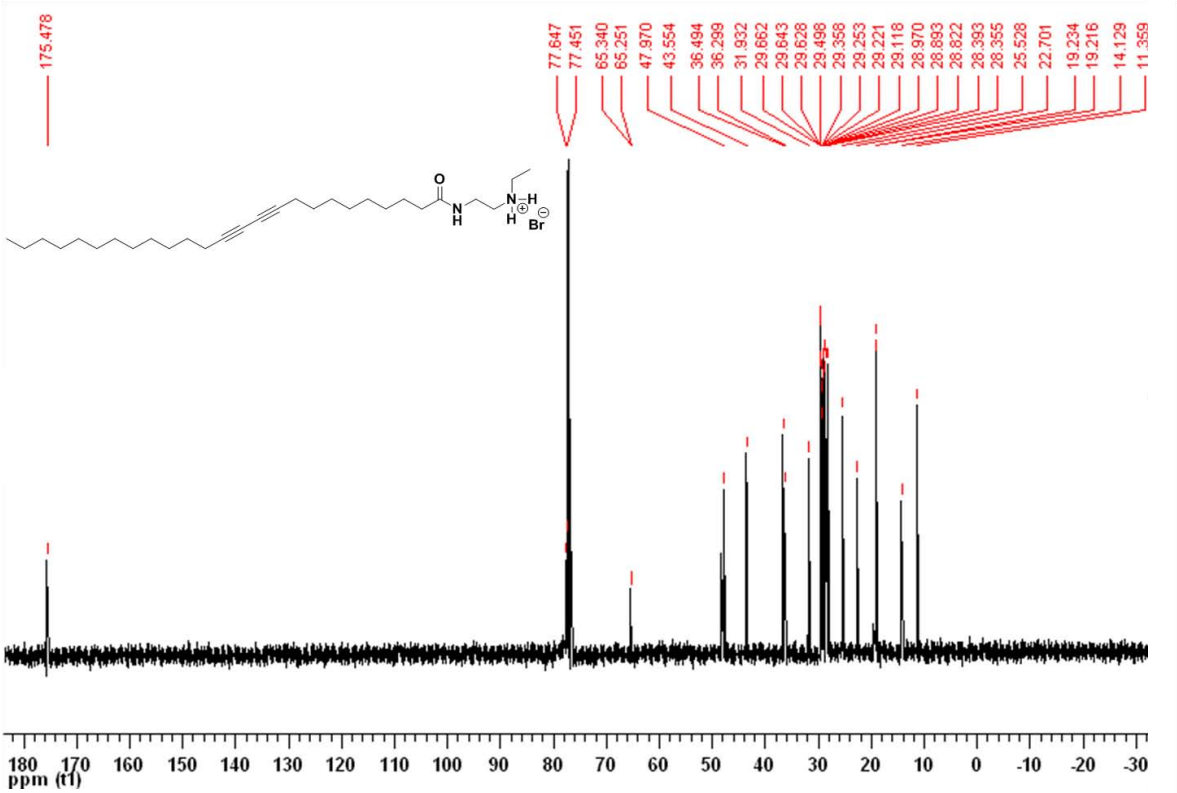


Figure A6: ^{13}C NMR; Et-Br-AEPCDA, **3**



Appendix B

Appendix B: ESI mass spectra of synthesized diacetylene monomers

Figure B1 : ESI mass spectra of AEPCDA, **1**

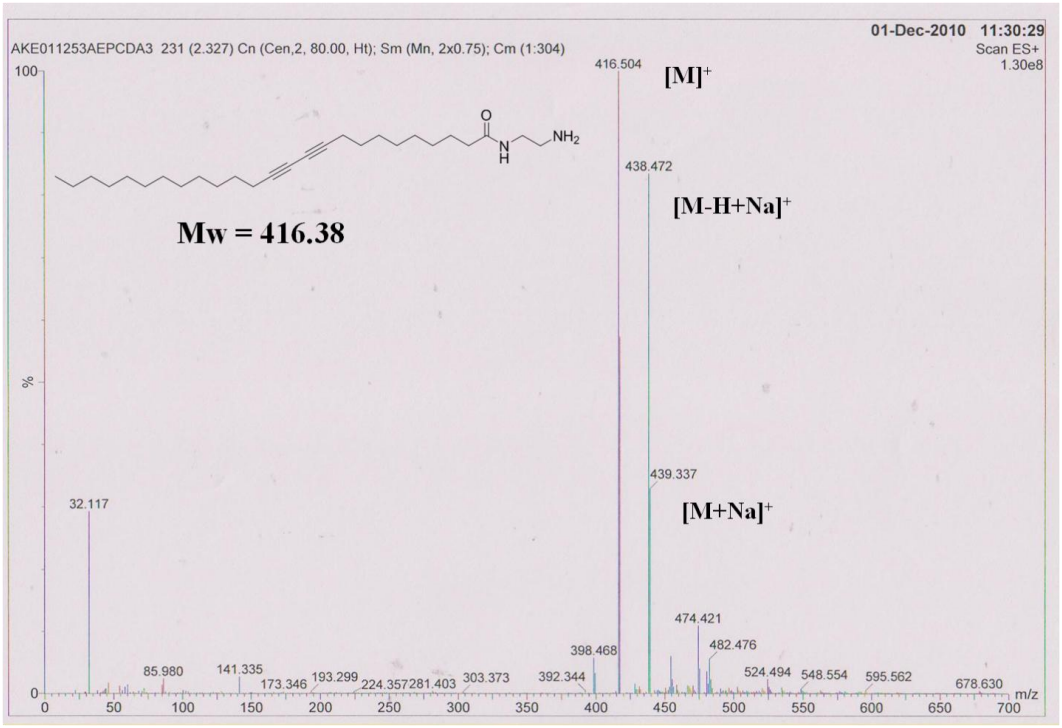


Figure B2 : ESI mass spectra of Et-AEPCDA, **2**

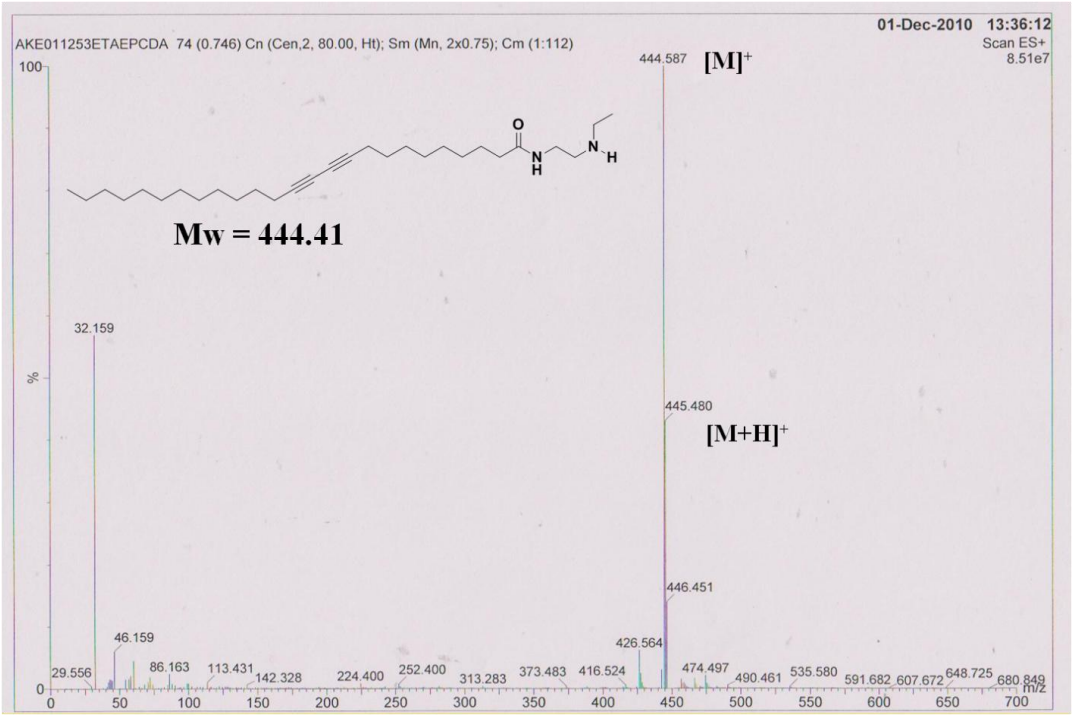
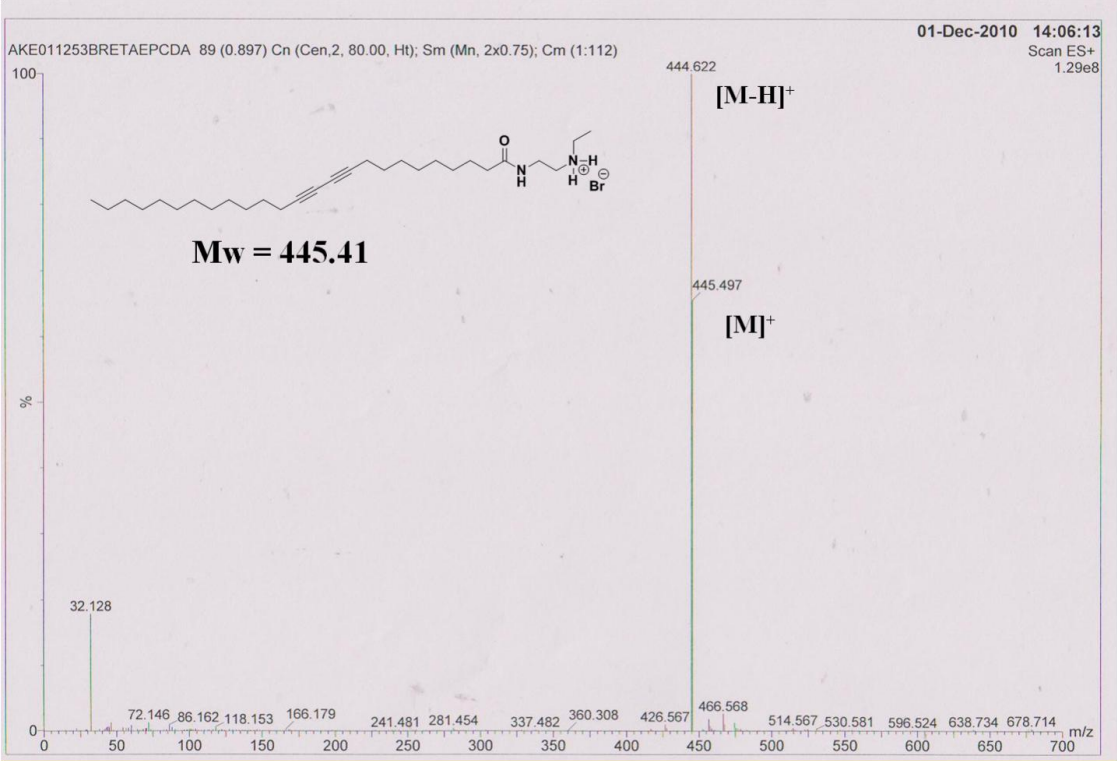


Figure B3 : ESI mass spectra of Et-Br-AEPCDA, **3**



VITAE

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