CHAPTER II

EXPERIMENTAL

2.1 Chemicals

1. 10,12-pentacosadiynoic acid (PCDA), Fluka, USA
2. Hexanoic acid (caproic acid, C6), Fluka, Switzerland
3. Tetradecanoic acid (myristic acid,C14), Fluka, Switzerland
4. Octadecanoic acid (steric acid, C18), Fluka, Switzerland
5. cis-9-octadecenoic acid (oleic acid), Fluka, Switzerland
6. N,N'-dicyclohexylcarbodiimide (DCC), Fluka, Switzerland
7. Octadecanoyl chloride (Stearoyl chloride), Fluka, Switzerland
8. Oxalyl chloride, Fluka, Switzerland
9. Pyridine, Fluka, Switzerland
10. Ethylenediamine, Carlo Erba, France
11. Diethylether, reagent grade, Lab-Scan, Ireland
12. 1,1,1-Trichloromethane (chloroform), AR grade, Lab-Scan, Ireland
13. Dichloromethane (methylene chloride), commercial grade TSL Chemicals, Thailand
14. Hexane, commercial grade, TSL Chemicals, Thailand
15. Ethylacetate, commercial grade, TSL Chemicals, Thailand
16. N,N-Dimethylformamide, Fluka, Switzerland
17. propan-1-ol (methanol), commercial grade, TSL Chemicals, Thailand
18. Sodium chloride, Merck, Germany
19. Sodium hydroxide, Merck, Germany
20. Sodium carbonate anhydrous, Fisher Scientific, UK
21. Sodium sulfate anhydrous, Riedel-deHaën®, Germany
22. Poly(vinyl alcohol), Sigma-Aldrich, Germany
23. Silica gel 60, Merck, Germany

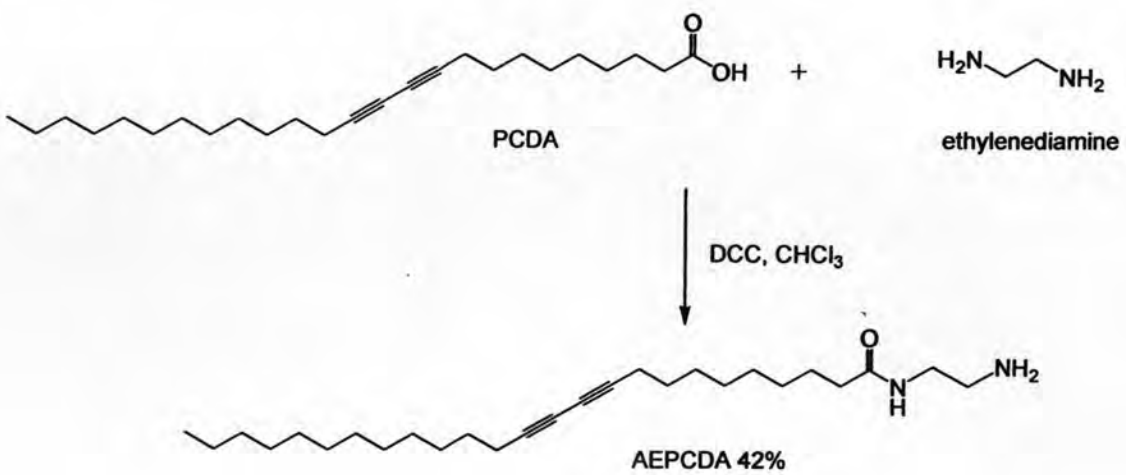
2.2 Apparatus and equipments

1. Rotary evaporator, R200, Buchi, Switzerland.
2. Ultrasonicator, Elma, Germany
3. Magnetic stirrer, Fisher Scientific, USA
4. Hot plated magnetic stirrer, Corning, USA
5. pH meter, Twin pH B 212, Japan
6. Pipette man (P20, P200 and P5000), Gilson, France
7. Pipette man (Le100 and Le1000), Nichiryo, Japan
8. Freeze-dryer, Freezone 77520, Benchtop, Labconco, USA
9. Electrothermal 9100, Fisher Scientific, USA
10. Nuclear Magnetic resonance spectrometer (NMR) 400 MHz, Mercury 400, Varian, USA
11. Mass spectrometer, Quattro micromass, Waters, France
12. Fourier transform infrared spectrometer (FTIR), Impact 410, Nicolet, USA
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

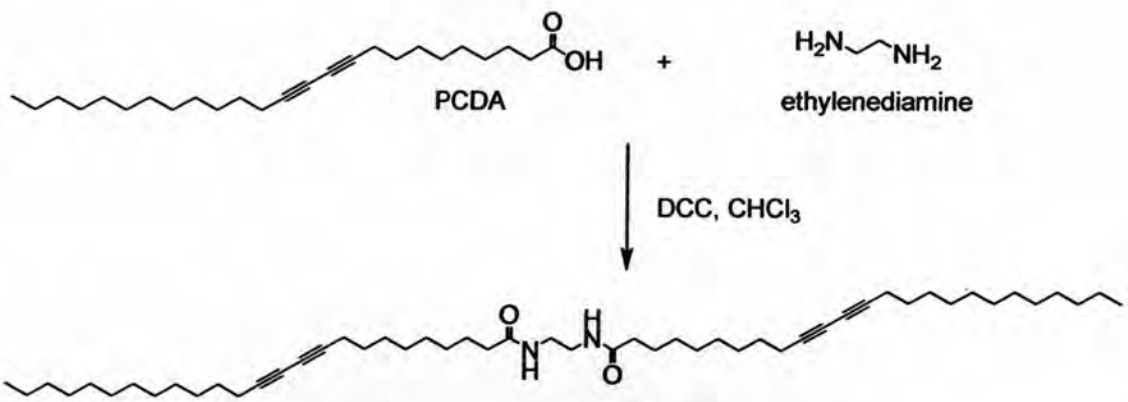
2.3.1 Synthesis of diacetylene lipid monomers

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



N,N'-dicyclohexylcarbodiimine (0.4952 g, 2.4 mmol) in chloroform (2 mL) was added dropwise into a solution of 10,12-pentacosadiynoic acid (0.7492 g, 2.0 mmol) in chloroform (2 mL). The mixture was stirred for 1 hour at room temperature and was then added dropwise into ethylenendiamine (0.6696 mL, 10.0 mmol). The reaction mixture was stirred for 1 hour when the white precipitate was clearly observed. The reaction mixture was poured into dichloromethane (5 mL) and was extracted with saturated sodium carbonate solution (20 mL) followed by distilled water (2 × 10 mL). The organic extract was dried over anhydrous Na_2SO_4. After removal of solvent, the crude product was eluted through a silica gel column by ethyl acetate:methanol (70:30). The major product was collected and the solvent was removed to give *N*-(2-aminoethyl)pentacosa-10,12-diynamide (0.3524 g, 42%) as a white solid. Charecterization data for AEPCDA: mp 103-110 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (t, CH_3, 3H, J = 8.0 Hz), 1.24-1.51 (m, CH_2, 32H), 2.17 (t, CH_2, 2H, J = 5.6 Hz), 2.22 (t, CH_2, 4H, J = 6.9 Hz), 2.24 (brs, NH_2, 2H), 2.84 (t, CH_2, 2H, J = 5.6 Hz), 3.32 (q, CH_2, 2H, J = 5.7 Hz), 6.17 (brs, NHC=O, 1H).

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

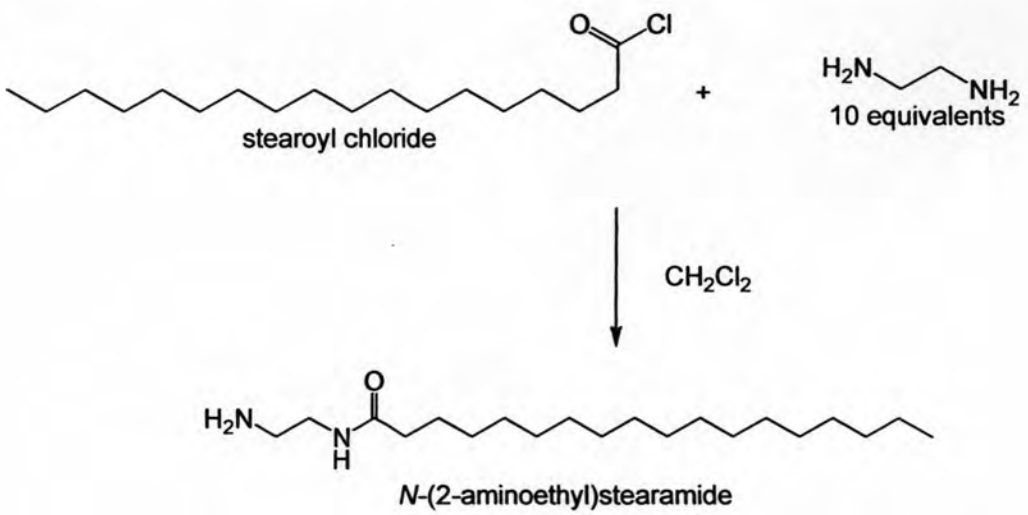


EBPCDA 52%

N,N'-dicyclohexylcarbodiimide (0.2476 g, 1.2 mmol) in chloroform (2 mL) was added dropwise into PCDA (0.3746 g, 1.0 mmol) in chloroform (2 mL) and stirred for 1 hour. Ethylenediamine (0.0334 mL, 0.5 mmol) was then added dropwise into the reaction mixture and stirred for 1 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 (0.1979 g, 52%). Characterization data for

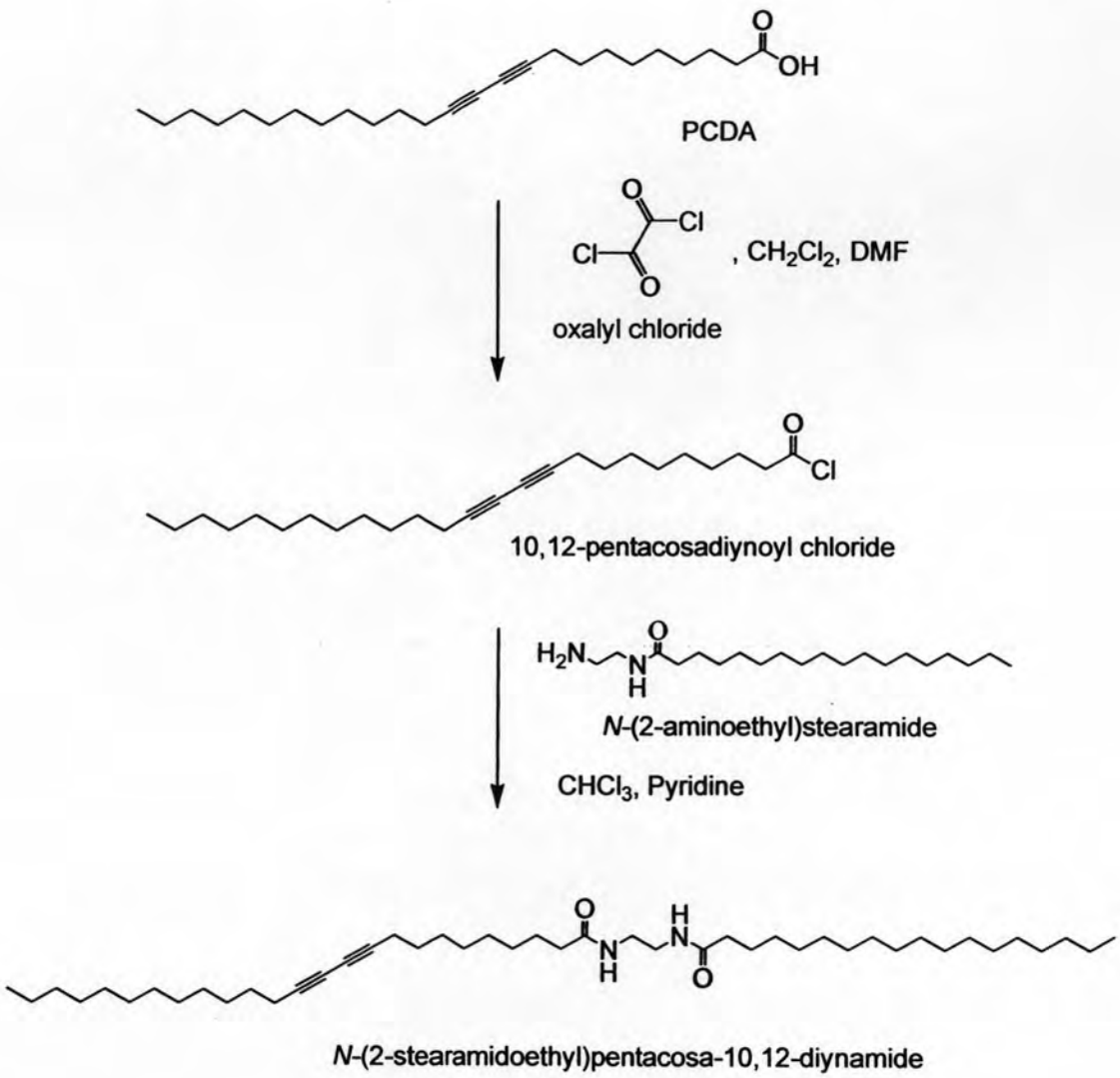
EBPCDA: mp 125-130 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, CH_3, 6H, J = 6.8 Hz), 1.25-1.51 (m, CH_2, 64H), 2.10 (t, CH_2, 4H, J = 7.5 Hz), 2.17 (t, CH_2, 8H, J = 6.9 Hz), 3.39 (brs, CH_2, 4H), 6.10 (brs, NH=CO, 2H).

***N*-(2-aminoethyl)stearamide**



Octadecanoyl chloride (stearoyl chloride 0.7 mL, 2.1 mmol) in dichloromethane (20 mL) was added dropwise into ethylenediamine (1.4 mL, 20.0 mmol) and stirred to give colorless solution containing white precipitate. The reaction mixture was stirred overnight at room temperature and was extracted with 0.1N NaOH followed by dichloromethane. Purify by stirring in hexane. The white solid was collected by filtration and characterized as *N*-(2-amonoethyl)stearamide (0.3634 g, 54%).

N-(2-stearaminoethyl)pentacosa-10,12-diynamide (SEPCDA)



Oxalyl chloride (0.2 mL) was added dropwise into PCDA (0.3807g, 1.02 mmol) in dry dichloromethane 3 mL and stirred for 30 min. A drop of *N,N*-dimethylformamide (DMF) was added into the reaction mixture and stirred for 30 min. After removal of solvent and oxalyl chloride, 10,12-pentacosadiynoyl chloride was obtained. The mixture of *N*-(2-amonoethyl)stearamide in chloroform (10 mL, 1.11 mmol) and pyridine 0.4 mL were added dropwised into 10,12-pentacosadiynoyl chloride and stirred overnight. The reaction mixture was extracted with 1N HCl (50 mL) followed by distilled water (3 × 50 mL). The organic extract was dried over anhydrous Na_2SO_4. Purifiy by stirring in dichloromethane and hexane. The white solid was collected by filtration and characterized as *N*-(2-stearaminoethyl)pentacosa-10,12-diynamide (0.3006 g, 43%). Characterization data for SEPCDA: mp 124-132

°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, CH_3, 6H, J = 6.8 Hz), 1.25-1.38 (m, CH_2, 62H), 2.17 (t, CH_2, 4H, J = 7.7 Hz), 2.24 (t, CH_2, 4H, J = 6.9 Hz), 3.38-3.39 (brs, CH_2, 4H), 6.17 (brs, NH=CO, 2H).

2.3.2 Preparation of polydiacetylene vesicles

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 by rotating evaporator. A volume of milli-Q water was added to provide the lipid concentration of 1.0 mM. The suspensions were heated to 75-85 °C, followed by sonication in an ultrasonicating bath for 20 min when a semitransparent or transparent vesicle solution was obtained. The solution was kept at 4 °C overnight. The vesicle solution was irradiated with UV light (254 nm) for 5 min and filtered through a filter paper no.1 to give clear intense blue-colored poly(PCDA) vesicle solution. The solution showed maximum visible absorption at wavelength (λ_{max}) of 630 nm. Poly(SEPCDA) vesicles were also prepared by the same procedure and showed maximum visible absorption at wavelength (λ_{max}) of 638 nm.

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 was conducted in an ice bath. Poly(AEPCDA) vesicle solution was obtained in clear intense blue color with λ_{max} = 630 nm. Without an ice bath, the solution turned into red.

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 90-100 °C prior to the sonication and the temperature was kept under sonication at the temperature above 80 °C for 40 min. 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 were obtained and was kept at 4 °C 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 λ_{max} = 636 nm.

Mixed lipid vesicles

Vesicle solution of mixed lipids between PCDA and various long chain fatty acid (hexanoic, tetradecanoic, octadecanoic and oleic) were prepared by mixing stock solutions of the lipids at the designated mole fraction (0.1, 0.2, 0.3, 0.5, 0.7, 0.8 and 0.9) prior to the evaporation step. The same procedure and conditions as those of poly(PCDA) vesicle solution were applied in the sonication and photopolymerization steps.

2.3.3 Preparation of films containing polydiacetylene vesicles.

Preparation of polyacrylic latex films containing polydiacetylene vesicles.

Polyacrylic latex was obtained from Hexion specialty chemicals Samutsakorn Ltd., Thailand. The latex was polymerized from two main monomers, methacrylic acid (MAA) and ethyl acrylate (EA), and a mixture of crosslinking monomers, glycidyl methacrylate (GMA), n-metylol acrylamide (nMA) and ethylene glycol methacrylate (EGDMA) with a total non-volatile content of 35-36% . The latex had viscosity of 8-25 cP and pH = 2.4-2.8. Each tested polydiacetylene vesicle solution (1.0 mM) was mixed with the polyacrylic latex solution at the ratio of 2:1 (0.2% w/w of solid) under continuous stirring, and then hand cast on glass slide. The liquid was allowed for an air dry at room temperature for 1 day. A blue transparent brittle film was obtained.

Preparation of PVA films containing polydiacetylene vesicles

Each tested polydiacetylene vesicle solution (1.0 mM, 10 mL) was mixed with an aqueous PVA (Mw = 146,000-186,000) solution (5% (w/v), 10 mL) under continuous stirring. A 20 mL volume of mixture was then poured into a Petri dish (diameter: 9.0 cm) and allowed for an air dry at room temperature for 2 days. A transparent blue-colored soft film, which could be peeled off from the dish, was obtained.

2.3.4 Analysis of vesicles

UV-Vis spectroscopy

The visible absorption of vesicle solutions and films were 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 λ_{max} of the blue and red phases of each sample were

determined at 25 and 90 °C. The correlation between the absorbance at the λ_{max} (A) and the concentration of the lipid vesicles was carried out by using poly(PCDA) vesicles prepared from the monomer lipid concentration range of 0.2-1.0 mM as a representative. Vacuum dried weight of the filtered vesicle solution was used to determine the concentration of poly(PCDA).

Fourier transform infrared spectrometer (FTIR)

An FTIR spectrometer (Nicolet Infrared Spectrometer, model Impact 410) were used to examine the nature of the hydrogen-bonding among the head groups of the diacetylene monomers in the vesicles of poly(PCDA), poly(AEPCDA), poly(EBPCDA) and poly(SEPCDA). All samples were ground with dried KBr powder. The KBr disc was dried again, pressed and subjected to the FTIR spectrometer.

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 desicator.

Atomic Force Microscopy (AFM)

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

Dynamic light scattering (DLS)

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

2.3.5 Thermochromism study

Visual observation

The blue-colored PDA vesicle solution and PVA film containing PDA vesicles were heated from 25 to 90 °C in hot water for 6 and 10 min, respectively at each

temperature. The change of color of these samples was observed by naked eyes and photographed.

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.