CHAPTER II

EXPERIMENTAL

Materials: All reagents were purchased from Sigma-Aldrich, Fluka®(Switzerland), Merck® (Germany). *N*-(2-aminoethyl) pentacosa-10,12-diynamide (AEPCDA), *N,N'*-ethylenebispentacosa-10,12-diynamide (EBPCDA), were prepared according to the literature.[51] . The diacetylenic monomer 10,12-pentacosadiynoic acid(97%) was purchased from Fluka. Linear poly(ethylenimine)(PEI) was purchased from Aldrich. Chitosan was purchased from Seafresh Chitosan (Lab) Company, Thailand. Polyvinylalcohol was commercial grade. The resin base screen ink GPV0906 was purchased from Chaiyaboon brothers Co., LTD. Other analytical grade solvents were used as received without further distillation. Solvent such as methylene chloride, hexane, ethyl acetate and methanol used for extraction and chromatography were commercial grade and distilled before use. Column chromatography was performed using Merck silica gel 60 (70-230 mesh). The extraction solvent except diethyl ether and chloroform was reagent grade. Deionized water was used in all experiment.

Analytical instruments: ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 400 MHz NMR spectrometer (Varian, USA) and AC Bruker 200, 250 and 500 MHz NMR spectrometer (Bruker, USA) using the residual solvent proton resonance of $CDCl_3$ at 7.26 ppm as the reference. Infrared spectra were measured from KBr pellets on a Nicolet Impact 410 FT-IR spectrophotometer (Thermo Nicolet, USA). UV/vis spectra were recorded on Varian Cary 100 Bio UV-Visible spectrophotometer (Varian, USA). Raman spectra were performed on a Perkin-Elmer 1760 FT-IR spectrophotometer (Thermo Fisher Scientific company, USA) with an Nd:YAG laser source and a Raman sample compartment attached to the FT-IR instrument. TEM images were recorded on a JEOL JEM-2100 transmission electron microscope (JEOL, Japan). AFM images were taken on a SPA 400 atomic force microscope (Seiko, Japan). Mass analysis was conducted with a Microflex MALDI-TOF MS (Bruker Daltonics, Germany). The particle size measurements were made by a dynamic light scattering using a Nanosizer (Malvern instruments, England). UV-irradiation was performed by UV light source (TUV 15W/G15 T18 lamp; Philips, Holland). Sonication was performed by ultrasonicator Transinic T570/S, Elma, Germany. pH was measured from pH meter (Twin pHB 212, Japan).

2.1 Vesicle preparation and polymerization

Preparation of 10,12-pentacosadiynoic acid stock solution

The diacetylene monomer 10,12-pentacosadiynoic acid (PCDA) was dissolved in diethyl ether and filtered to remove polymerized material. The filtrate was dried under a rotary evaporator to give a white solid. A stock solution of PCDA was prepared by dissolving a controlled amount of PCDA in chloroform, which was kept in refrigerator.

General method to prepare polydiacetylene vesicles

A known amount of PCDA stock solution was pipetted into the flask followed by rotary evaporation to remove solvent. A volume of Milli-Q water was added and the suspension was heated to 75-85 °C, followed by sonication in an ultrasonic bath for 30 min when a semitransparent or transparent vesicle solution was obtained. The solution was kept in refrigerator 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-colore poly(PCDA) vesicle solution.

Effect of chilling time on crystallization

A poly(PCDA) vesicles solution (0.5 mM, 30 mL) was prepared according to general procedure. After sonication and cooling to room temperature, the resulting solution was kept in an ice bath. The sample was sampling to irradiate with UV light. After polymerization the visible adsorption spectrum of polydiacetylene vesicle was observed at 630 nm (vesicle 0.1 mL, total volume 2 mL)

Effect of UV irradiated time on the photo polymerization

A poly(PCDA) vesicles solution (0.5 mM, 40 mL) was prepared according to general procedure. After sonication and cooling in a refrigerator overnight, the transparent solution was irradiated by UV light. The resulting solution was pipette along the irradiation. Visible spectroscopy was employed to observe the vesicles polymerization.

2.2 Characterization of polydiacetylene vesicles

UV-Vis 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 700 to 500 nm with the zero absorbance set at 700 nm. The λ_{max} of the blue and red phases of each sample was determined at 25 and 90 °C.

Transmission Electron Microscopy (TEM)

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

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 Mechanism of chromic transition

Solvatochromic study 0.3 mL of 1 mM vesicle solution was added into 2.7 mL of ethanol solution which have 0.1 mM of poly(PCDA) in final concentration. The resulting solution was incubated for ten minutes before collecting the data.

Alkalinochromic study 4 mL of 0.1 mM vesicle solution was titrated with 1N NaOH solution. The resulting solution was incubated for ten minutes before corrected data.

Thermochromic study 3 mL of 0.1mM vesicle solution was gently heated from 20°C to 90°C in steps of 1°C per minute by a temperature control led UV-vis spectrophotometer. The temperature at each step was maintained for 10 minutes before the data was collected. In the reversible process, the temperature of UV cell was decreased to 20°C and record the data for 10 minutes before heating up to the another temperature.

2.4 Preparation of the layer by layers deposition of polydiacetylene vesicles

2.4.1 Study of deposition condition on polydiacetylene vesicle

Preparation of polydiacetylene vesicles

An appropiate amount of PCDA stock solution was pipetted into the flask which followed by streaming of N_2 to remove solvent. A volume of Milli-Q water was added and the suspension was heated to 75-85 °C, followed by sonication in an ultrasonic bath 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.

Substrate preparation

Glass slides (4 x 1 x 0.2 cm) was cleaned by sonicated them in the piranha solution (70% H_2SO_4, 30% of 30% H_2O_2 v/v) for 30 minutes. Then washed the glass slides by deionized water.

Chitosan solution preparation

Weight chitosan powder (0.5 g, Mw $8x10^5$, 84% degree of deacetylation) and 40 mL of water into 200 mL beaker followed by adding 0.8 mL of glacial acetic into the suspension. The mixture was stirred overnight before diluted to 0.1% w/w (6.1 mM of chitosan monomer unit). The pH of the chitosan solution was adjusted to pH 3 to 5 by 2 M HCl solution. The chitosan solution was filtered and stored in a refrigerator.

Layer by layers deposition

The glass substrate was dipped into the polycationic solution followed by three rinsing steps in deionized water or rinsing solution which depend on a condition. The substrate was then immersed in poly(PCDA) vesicles solution then rinsed by the rinsing solution. The deposition was carried out by repeating these adsorption cycles as many times as needed. UV-Vis spectrophotometry was used to confirm the layer by layer deposition process by mornitoring the increase in absorbance as a function of the number of deposit layers.

Atomic Force Microscopy (AFM)

The AFM images 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 poly(PCDA) vesicles prepared from a drop of diluted solution on freshly cleaned mica. For the imaging of the PEM film containing

vesicles, a freshly prepared film sample on a clean glass substrate was air-dried overnight prior to the measurement.

Study of poly(PCDA) vesicles concentration on the film absorption

The glass substrate wad immersed in 0.1% w/w solution at pH 4.0 which was adjusted by acetic acid then rinsing by 10 mM acetate buffer pH 5.0. The concentration of poly(PCDA) vesicles were 0.1 to 0.8 mM of monomer in 10 mM acetate buffer pH 5.0. The dipping time is 1 minute and the rinsing time is 20 seconds. The 20 layers of chitosan/vesicles film were dipped in ethanol to change the blue color vesicles to red vesicles before record the adsorption spectra at 539 nm.

Study of dipping time on the film absorption

0.1% w/w Chitosan solution and 1mM of poly(PCDA) vesicles (monomer unit) were introduced to study the dipping time – adsorption relation. The rinsing solution is 10 mM acetate buffer pH 5.0. The dipping time was 1, 5, 10, 20 and 60 minutes. The 10 layers of chitosan/vesicles film were dipped in ethanol to change the blue vesicles to red vesicles before record the adsorption spectra at 540 nm.

Study of the adsorption time of the vesicles absorption

0.1% w/w Chitosan solution and 1 mM of poly(PCDA) vesicles (monomer unit) were introduced to study the saturated vesicles adsorption time. The prior 5 supporting layers of chitosan-vesicles films were prepared by dipping the glass substrate 2.5 cycles at 1 minute dipping time and 20 seconds rinsing time in each layer. The adsorption time of vesicle adsorption on the six layers were varied at 1, 5, 10, 20 and 60 minutes. The 6 layers of chitosan/vesicles film were dipped in ethanol to change the blue vesicles to red vesicles before record the adsorption spectra at 540 nm.

Condition optimization for PEI/poly(PCDA) vesicles deposition

The 1 mM and 10 mM of PEI solution (monomer unit) were introduced to prepare the multilayer films by varied concentration and pH condition. The resulting films were observed by naked eyes.

2.4.2 Chromic properties of polydiacetylene films

Solvatochromism study

The solvatochromic properties of 20 layers chitosan/vesicles film, PEI/vesicles film and 0.1 mM poly(PCDA) vesicles solution were studied by UV-Vis spectrophotometer. The samples were immersed in various concentration of ethanol solution in a UV-cuvett for five minutes before recording data.

Affinochromism study

The affinochromic properties of 12 layers of chitosan/vesicles film, 20 layers of PEI/vesicles film and 0.1 mM poly(PCDA) vesicles solution were studied by UV-Vis spectrophotometer. The samples were immersed in various pH of 100 mM phosphate buffer in a UV-cuvett for five minutes before recording data.

Thermochromism study

The thermochromic properties of 20 layers chitosan/vesicles film, PEI/vesicles film and 0.1 mM poly(PCDA) vesicles solution were studied by temperature controlled UV-Vis spectrophotometer. The samples were immersed in Milli-Q water in a UV-cuvett. The temperature was heated from 20-90°C. The data were collected after the temperature reached to each setting temperature and waited for 10 minutes.

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 $\%CR = (PB_0\text{-}PB)/PB_0*100$. Where $PB = A_{blue}/(A_{blue}+A_{red})$, A_{blue} and A_{red} are the absorbance of the blue and the red phase at 639 and 540 nm, respectively. PB_0 is the initial percent blue of the vesicle solution and film before given stimulant. The %CR correspond only the color shade of vesicle during the color changing from blue to red which the concentration of polydiacetylene vesicle should not interfere as in equation.

From $$A = \varepsilon bC$$

Then $$PB = \varepsilon_{639}bC/(\varepsilon_{639}bC+\varepsilon_{540}bC)$$

$$PB = \varepsilon_{639}/(\varepsilon_{639}+\varepsilon_{540})$$

Then $$\%CR = 100 \times \frac{((\varepsilon_{639}/(\varepsilon_{639}+\varepsilon_{540}))_0 - (\varepsilon_{639}/(\varepsilon_{639}+\varepsilon_{540}))}{(\varepsilon_{639}/(\varepsilon_{639}+\varepsilon_{540}))_0}$$

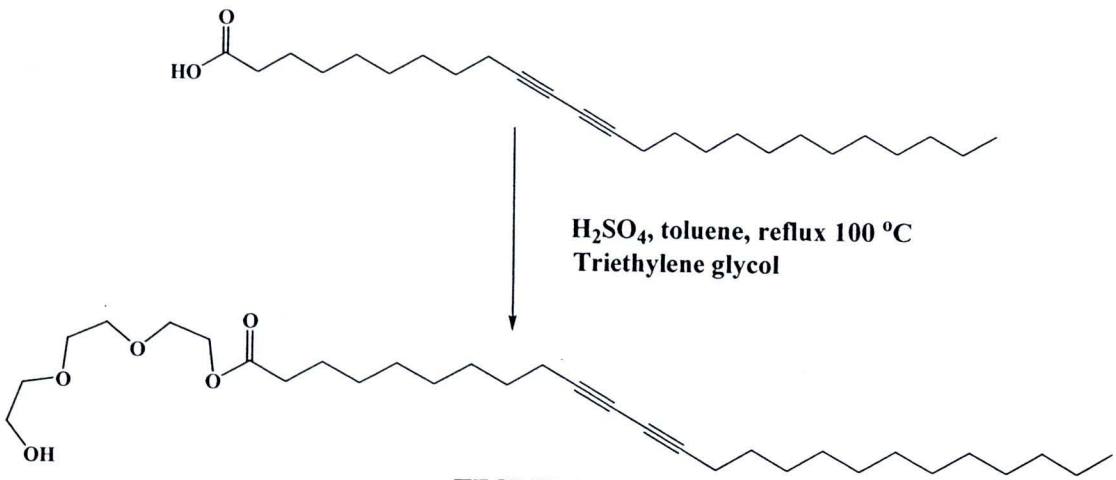
However the absorption of baseline produced an error on the %CR by increasing of absorbance from baseline which $A_{observe} = A_{vesicle} + A_{baseline}$. A zero baseline correction technique was used to provide the resulting data.

2.5 Development of metal ion sensor from polydiacetylene vesicle

2.5.1 Responses of poly(PCDA) sol to transition metal ions

The 1.0 mM poly(PCDA) vesicle was prepared by controlling the pH of solution with 1 M NaOH. The vesicles were tested the colorimetric response by adding Hg^{2+}, Pb^{2+}or Zn^{2+} ion solution. The final concentrations were 0.5 mM for poly(PCDA) vesicle and 50 mM for metal ion.

2.5.2 Synthesis of triethylene glycol ester of 10, 12-pentacosadiynoic acid (TEGPCDA)



TEGPCDA

In a 150 mL, round bottom flask with a magnetic stirrer, A solution of 10,12 pentacosadiynoic acid (0.6967 g, 1.86 mM), triethylene glycol 2 mL and sulfuric acid (cat.) in toluene was refluxed for 5 hours at 100°C. The resulting mixture was evaporated under reduced pressure and extracted by CH_2Cl_2 (50mL) and 2N HCl (2X50 mL). The organic phase was collected and drying over anhydrous Na_2SO_4 and the solvent was removed. The product was further purified by column chromatography using ethyl acetate in hexane as eluent yielding the white solid as a desire product (0.6960 g, 73% yield). TLC R_f 0.62 EtOAC

^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.197 (t, 2H, CH_2OC=O, J =4.4Hz), 3.677(m, 8H, CH_2O), 3.569 (t, 2H, CH_2O, J = 4.4 Hz), 2.290 (t, 2H, CH_2C=O, J = 7.6 Hz) 2.194 (t, 4H, CH_2alkyne, J = 6.8 Hz), 1.573 (m, 2H, CH_2), 1.446 (m, 4H, CH_2), 1.214 (m, 28H, CH2), 0.836 (t, 3H, CH_3, J = 6 Hz)

^{13}C-NMR (400 MHz, $CDCl_3$) δ 173.704 (C=O), 77.423 (C≡C), 77.263(C≡C), 72.413 (CH_2O), 70.414 (CH_2O), 70.175 (CH_2O), 69.06 (CH_2O), 65.185 (C≡C), 65.105 (C≡C), 63.086 (CH_2O), 61.574 (CH_2O), 33.999 (CH_2), 31.797 (CH_2), 29.529 (CH_2), 29.511 (CH_2), 29.491 (CH_2), 29.363 (CH_2), 29.232 (CH_2), 28.978 (2C, CH_2), 28.924 (CH_2), 28.784 (CH_2), 28.734 (CH_2), 28.637 (CH_2), 28.228 (CH_2), 28.182 (CH_2), 24.710 (CH_2), 22.574 (CH_2), 19.069 (CH_2), 19.052 (CH_2), 14.017 (CH_3)

2.5.3 Molecular assembly of triethylene glycol ester of TEGPCDA mixed with PCDA

Molecular assembly of poly TEGPCDA/PCDA was prepared by sampling a stock solution of PCDA and TEGPCDA into glass tube. After removing organic solvent, MiliQ water was added. The lipid suspension was sonicated for 30 minutes to obtain a clear suspension. After chilling suspension products in a refrigerator, the resulting suspension was polymerized by UV light for 5 minutes.

2.5.4 Study of colorimetric response of TEGPCDA/PCDA vesicle induced by metal ions

The colorimetric response with metal ion of 20% TEGPCDA/PCDA vesicle was studied by addition metal ion solution (100 mM of $CoCl_2$, $CuSO_4$, $FeSO_4$, $Hg(OAc)_2$, $NiSO_4$, $Pb(NO_3)_2$, and $Zn(OAc)_2$) into the vesicle solution (The final concentration of metal ion and mixd vesicle were 5 mM and 0.1 mM, respectively. The colorimetric response was observed by necked eye.

Study of colorimetric response of TEGPCDA/PCDA vesicle with Pb^{2+} ion

0.5 mM TEGPCDA/PCDA vesicle was prepared by various percent mole of TEGPCDA at 0, 10, 20 and 30%. The colorimetric response was studied by addition Pb^{2+} ion into vesicle solution. The total volume of sample solution was 3 mL which was 300 μl of 0.5 mM vesicle solution (50 μM of TEGPCDA/PCDA vesicle at final

concentration), 100 mM acetate buffer pH 6.0 300 μL (10 mM at final concentration), or MilliQ water. The resulting solution was detected by UV-vis spectrophotometer.

Kinetic study of colorimetric response between TEGPCDA/PCDA vesicle and Pb^{2+} ion

The colorimetric response was studied by addition 1mM Pb^{2+} ion into vesicle solution (50 μM for vesicle at final concentration). The absorption spectra were started to record after addition Pb^{2+} ion for 1 minute.

2.6 Study of themochromism of TEGPCDA/PCDA vesicle

Study of themochromism of TEGPCDA/PCDA vesicle

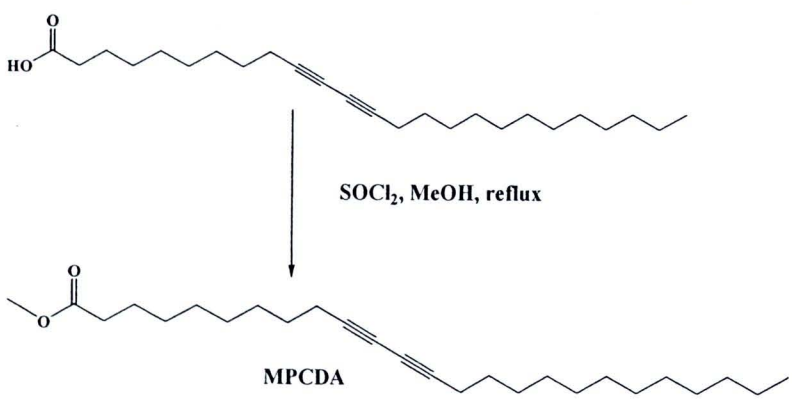
0.5 mM TEGPCDA/PCDA vesicle was prepared by various percent mole of TEGPCDA at 0, 10, 20 and 30%. The thermochromic response was studied by temperature controled UV-vis spectrophotometer at 540 nm and heating rate of 1°C/ minute.

Study of turbidity of TEGPCDA suspension and 10,12 pentacosadiynoic acid vesicle

0.5 mM of TEGPCDA suspension and 0.5 mM of 10,12 pentacosadiynoic acid vesicle were studied a temperature-turbidity dependence. The temperature control UV-vis spectrophotometer was used to record absorption spectra at 540 nm.

2.7 Fabrications of polydiacetylenes as temperature sensitive labels

Synthesis of methyl 10,12 pentacosadiynoate (MPCDA)



In a 150 mL, round bottom flask with a magnetic stirrer, a solution of 10,12 pentacosadiynoic acid (1,5552 g, 4.2 mM), thionyl chloride (0.9 mL, 12.3 mM) in methanol (50 mL) was refluxed for 4 hours at 100°C. The resulting mixture was

evaporated under reduced pressure and extracted by hexane (3x50 mL) and 10% $NaHCO_3$ (2X50 mL). The organic phase was collected and dried over anhydrous Na_2SO_4 and the solvent was removed. A desired product was collected as colorless liquid (1.6060 g, 98% yield). TLC R_f 0.85 hexane

^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.659 (s, 23H, $CH_3OC=O$), 2.293 (t, 2H, $CH_2C=O$, J = 7.6 Hz) 2.231 (t, 4H, CH_2alkyne, J = 6.8 Hz), 1.500 (m, 2H, CH_2), 1.357 (m, 4H, CH_2), 1.278 (m, 28H, CH2), 0.870 (t, 3H, CH_3, J = 6 Hz)

Printing of polydiacetylene vesicle

Vesicle solution of AEPCDA and EBPCDA were synthesized according to the procedure in reference.[51] Poly(EBPCDA) sol (1 mM) was filled into a empty black ink container of a ink-jet printer (C58 series, Epson). The printer was connected to a personal computer and a graphic image was printed on an A4 paper.

Preparation of PVA films containing polydiacetylene vesicles

Each tested polydiacetylene vesicle solution (1.0 mM, 75 mL) was mixed with an aqueous PVA (Mw = 146,000-186,000) solution (10% (w/v), 75 mL) under continuous stirring. A 150 mL volume of mixture was then poured into a tray (30 × 35 cm^2) 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 tray, was obtained.

Preparation of PVC-based screening ink

Diacetylene monomer and screen ink base resin (GPV0906, Chaiyaboon brothers Co.,LTD) was mixed into 10 mL vial. The diacetylene monomer used was 5% w/w for PCDA, EBPCDA, AEPCDA and 10% w/w for MPCDA compare with the resin weight.

Screening and polymerization

Screen ink was screened into commercial PVC sticker by using a stencil. After screening, the PVC label was kept in a refrigerator for 2-4 hr. The label was polymerized by UV irradiation at room temperature except MPCDA. The MPCDA was polymerization in an ice bath at the temperature below 10°C.