



CHAPTER III EXPERIMENTAL

3.1 Materials

Aniline, purchased from Merck, and pyrrole, purchased from Aldrich, were purified by distillation under reduced pressure prior to use. AR grade ammoniumperoxodisulfate (APS), used as an oxidant, was purchased from Merck.

Commercial grade chlorophyllin ($C_{34}H_{31}CuN_4O_6 \cdot 3Na$; $M_w = 724.1$) was purchased from Aldrich.

Chitin, with a degree of deacetylation (%DD) equal to 20%, measured by the method of Baxter *et al.* (Baxter *et al.* 1992), was prepared from shrimp shell (*Penaeus merguensis*), kindly supplied by Surapon Food Co. Ltd, Thailand.

Sodium hydroxide 50% w/w solution was kindly supplied by KPT Cooperation (Thailand). Glacial acetic acid 99.9% w/w purchased from J.T. Baker was analytical grade. Glutaraldehyde 50 % w/w was purchased from Fluka.

AR grade hydrochloric acid, sodium hydroxide, monochloroacetic acid, ethanol, methanol, toluene, and acetone were purchased from Labscan and used as received.

3.2 Equipments

3.2.1 FTIR Spectrophotometer

The FTIR spectra of the samples were recorded with Thermo Nicolet Nexus 670 FTIR Spectroscopy with 16 scans at a resolution of 4 cm^{-1} . A frequency range of $4000\text{-}400 \text{ cm}^{-1}$ was observed by using a deuterated triglycerinesulfate detector (DTGS) with specific detectivity of $1 \times 10^9 \text{ cm}\cdot\text{Hz}^{1/2}\cdot\text{w}^{-1}$.

3.2.2 UV/Visible Spectrophotometer

UV-Visible spectra of the synthesized PANI were determined using a Shimadzu UV-VIS spectrometer, model 2550, in the wavelength range of 250–900 nm. A 1.5 M HCl solution and *N*-methyl-2-pyrrolidone (NMP) were used as solvents

to prepare the emeraldine salt form, or doped state of PANI (PANI ES), and the emeraldine base form, or undoped state of PANI (PANI EB), respectively, at a concentration of 0.3 g/l.

3.2.3 Thermogravimetric Analyzer (TGA)

Thermogravimetric analysis (TGA) used to evaluate the thermal stability and determined the decomposition temperature of all samples was a Dupont Instrument TGA 5.1 model 2950. The temperature range studied was 30-800°C at a heating rate of 10°C/min under a nitrogen gas atmosphere.

3.2.4 Differential Scanning Calorimeter (DSC)

Differential scanning calorimetry (DSC) of all samples was carried out in the temperature range of 30-600°C using Mettler-Toledo DSC 822 at a heating rate of 10°C/min under a nitrogen gas atmosphere.

3.2.5 Scanning Electron Microscope (SEM)

A scanning electron microscope (JOEL model JSM-5800LV) was used to investigate the morphology of the synthesized PANI nanoparticles as well as the surface and cross-sectional morphologies of composite films.

To investigate the morphology of the synthesized PANI nanoparticles, samples were prepared by the dispersion of polyaniline in distilled water, and placing a drop onto a brass stub before gold sputtering (using a JFC-1100E ion sputtering device). The morphology of the synthesized PANI nanoparticles is obtained by using an acceleration voltage of 15 kV with a magnification of 20,000 times.

In case of the investigation of surface morphology of the composite films, the composite films were cut into small pieces and adhered on a brass-stub by using an adhesive tape before gold sputtering. The surface morphology of the composite films is obtained by using an acceleration voltage of 15 kV with a magnification of 2,000 times. Meanwhile, the cross-sectional fracture surfaces of the composite films were achieved by cooling in liquid nitrogen before cracking, subsequently adhering on a brass-stub and sputtering with gold. The magnification of

cross-sectional morphology of composite films was varying from 3,500, 10,000, and 20,000 times.

3.2.6 X-ray Diffractometer (XRD)

The X-ray diffraction (Rigaku, model D/MAX-2000) was used to characterize the crystalline structure of the synthesized PANI nanoparticles. The measurements were carried out in the continuous mode with a scan speed of 5°/min covering the angles 2θ between 5 and 50°. Cu $K\alpha_1$ was used as the X-ray source.

3.2.7 Electrometer

The electrical conductivity of the synthesized PANI powder was measured at ambient temperature and approximately 50% relative humidity using a conventional two-point probe technique equipped with 6517A Electrometer/High Resistance Meter (Keithley, model 7517A).

3.2.8 Rheometer

Steady state and oscillatory shear measurements were performed on a strain controlled TA Instruments AR 2000ex Rheometer. A cone-and-plate geometry with a diameter of 40 mm and a cone angle of 2° was used for all determinations. The instrument was equipped with a solvent trap to prevent water evaporation. All rheological measurements were conducted at a temperature of 25 °C. Steady state rheological measurement was determined in the shear rate range from 0.1 to 100 S⁻¹, while the oscillatory state measurement was done in the angular frequency range from 0.1 to 100 rad/s under the controlled strain 0.2%.

3.2.9 Dynamic Light Scattering Instrument

Dynamic light scattering was used to determine hydrodynamic diameter of the samples at a detection angle of 90° with a Brookhaven instrument, equipped with a 15 mW He - Ne laser emitting vertically polarized light at wavelength 632.8 nm. The measurements were carried out at 25°C. The average diameter was computed from the autocorrelation function data by using the cumulant analysis method.

3.3 Methodology

3.3.1 Synthesis of Polyaniline Nanofibrils by using Chlorophyllin as an In-situ Seed

PANI nanofibrils were synthesized under acidic conditions by the oxidative polymerization of aniline using APS as an oxidizing agent, in the presence of chlorophyllin acting as an *in-situ* seed. The synthesis procedure is described as follows: Chlorophyllin solutions (50 g) having four different concentrations were prepared by dissolving specific amounts of chlorophyllin — 0.05 g (0.07 mmole), 0.5 g (0.7 mmole), 1.0 g (1.4 mmole), and 2.5 g (3.5 mmole) — in distilled water. To each of the chlorophyllin solutions (50 g), 8 g (0.086 mole) of aniline monomer was added, corresponding to mole ratios of chlorophyllin to aniline monomer equal to 8.14×10^{-4} , 8.14×10^{-3} , 1.63×10^{-2} , and 4.07×10^{-2} , respectively. The mixtures were then cooled to 0°C with mechanical stirring at 300 rpm for 1 h. Next, 100 ml of 1.5 M HCl was added dropwise into each of the mixtures over a period of 30 min followed by stirring with a mechanical stirrer for 30 min. A pre-cooled solution, held at a temperature below 5°C, containing 10 g of APS (0.048 mole) in 100 ml of 1.5 M HCl was added dropwise within 30 min, after which the aniline concentration based on the total volume of the reaction mixture was 0.344 M. The reaction was stirred at 0°C for 4 h to complete the polymerization. The resulting suspension was centrifuged at 11,000 rpm for 10 min, and the obtained precipitate was re-dispersed in distilled water and dialyzed against different washing solvents including acetone, 0.5 M NaOH, and distilled water for 3 days, in order to completely remove the chlorophyllin seed. The resulting precipitate was filtered, dried under reduced pressure for 2 days, and kept in a desiccator prior to use.

3.3.2 Preparation of Chitin

Chitin was prepared from shrimp shell by decalcification and deproteinization to remove calcium carbonate and protein, respectively. The shrimp shells were cleaned and dried under sunlight before grinding into small pieces. Shrimp shell chips were treated by immersion in 1-M HCl solution for 2 days with occasional stirring. The decalcified product was washed with distilled water until

neutral. Deproteinization was followed by boiling in 4 % w/w of NaOH solution at 80-90°C for 4 h. After NaOH solution was decanted, the chips were washed with deionized water until neutral. The product obtained was dried at 60°C in a convective oven for 24 h.

3.3.3 Measurement of Degree of Deacetylation of Chitin

The degree of deacetylation of chitin was determined, based on an IR spectroscopic method reported by Baxter *et al.* (Baxter, Dillon, Taylor 1992). About 3 mg of chitin powder, passed through a 200-mesh sieve, was mechanically mixed with 400 mg of potassium bromide to prepare a KBr disk. An infrared spectrum was recorded in a range from 4000 to 400 cm^{-1} . The absorbances at 3,450 cm^{-1} (the O-H band) and 1650 cm^{-1} (the amide I band) were used to quantitate the degree of deacetylation. The degree of deacetylation was calculated from the equation 3.1.

$$DD = 100 - (A_{1650} / A_{3450}) \times 115 \quad (3.1)$$

where DD = degree of deacetylation (%)
 A_{1650} = absorbance at 1650 cm^{-1}
 A_{3450} = absorbance at 3450 cm^{-1}

3.3.4 Preparation of Carboxymethyl Chitin (CM-chitin)

CM-chitin with degree of substitution (DS) equal to 0.43, were prepared by reaction of chitin powder with monochloroacetic acid under basic condition, according to the method described by Wongpanit *et al.* (Wongpanit, Sanchavanakit, Pavasant, Supaphol, Tokura, Rujiravanit 2005). In a typical procedure, CM-chitin was prepared by suspending 5 g of chitin powder in 100 g of 42 %w/w NaOH. The suspension was stored under reduced pressure for 30 min. Then 160 g of crushed ice was added to the suspension and the mixture was stirred at below 5°C for 30 min. A pre-cooled solution at a temperature below 5°C, containing 27 g monochloroacetic acid in 70 ml of 14% w/w NaOH, was slowly added to the mixture with vigorous stirring. The reaction was maintained at 0-5°C for 30 min. After settling at room temperature overnight, the mixture was neutralized with

glacial acetic acid, and subsequently dialyzed in running water, followed by dialysis with distilled water for 1 day. The dialysate was centrifuged at 10,000 rpm for 10 min to remove insoluble material. A white solid was recovered from the supernatant by adding it drop-wise into acetone. The product was washed with ethanol, filtered, and dried in a vacuum at room temperature.

3.3.5 Synthesis of Dendritic Polyaniline Nanoparticles using CM-chitin Template

Dendritic PANI nanoparticles were synthesized in aqueous solution by the oxidative polymerization of aniline using ammoniumperoxodisulfate (APS), used as an oxidant, in the presence of CM-chitin acting as a template. The synthesis procedure was described as followed. 8 g (0.086 mol) of aniline was poured into 50 g of various CM-chitin solutions (0.5 wt%, 1 wt%, and 2 wt%) and the mixture was cooled to 0°C with mechanical stirring at 300 rpm for 1 h. 100 ml of 1.5-M HCl was added drop-wise into the mixture solution within 30 min and the mixture was maintained with mechanical stirring for 30 min. The pre-cooled solution, at a temperature below 5°C, containing 10 g (0.048 mole) $\text{NH}_4\text{S}_2\text{O}_8$ in 100 ml of 1.5-M HCl was added drop-wise within 30 min and the reaction was stirred at 0°C for 4 h to complete the polymerization. The suspension was centrifuged at 11000 rpm for 20 min and the precipitate product was subjected to dialysis with excessive amount of distilled water, until becoming neutral. The precipitated product was filtered, washed with distilled water, dried under reduced pressure for 2 day, and kept in desiccators prior to use.

3.3.6 Synthesis of Polyaniline Nanoparticles with Controlled Size by using Cross-linked CM-chitin as a Template

PANI nanoparticles were synthesized in aqueous solution by the oxidative polymerization of aniline using APS as an oxidizing agent, in the presence of the cross-linked CM-chitin acting as a hydrogel template. The synthesis procedure is described as follows: CM-chitin powder (0.5 g) was added to each of four solutions of glutaraldehyde, prepared by dissolving various amounts of glutaraldehyde (0 μmole , 3 μmole , 9 μmole , 18 μmole) in 49.5 g of distilled water, in

order to achieve 1 wt% of distinct glutaraldehyde-added CM-chitin solution. The mixtures were magnetically stirred overnight to complete the dissolution and cross-linking reaction of CM-chitin. 8 g of aniline (0.086 mole) was then poured into the CM-chitin solution and the mixture was cooled to 0°C with mechanical stirring at 300 rpm for 1 h. Next, 100 ml of 1.5-M HCl was added drop-wise into the suspension in a period of 30 min and the suspension was maintained with mechanical stirring for 30 min. A pre-cooled solution, at a temperature below 5°C, containing 10 g APS (0.048 mole) in 100 ml of 1.5-M HCl, was added drop-wise within 30 min and the suspension was stirred at 0°C for 4 h to complete the polymerization. The resulting suspension was centrifuged at 11,000 rpm for 20 min and the precipitate was subjected to dialysis with an excess amount of distilled water until becoming neutral. The precipitated product was filtered, washed with distilled water to remove the cross-linked CM-chitin template, dried under reduced pressure for 2 days, and kept in desiccators prior to use.

3.3.7 Conductivity Measurement

Two-point probe technique was used to measure the sheet resistivity that measures of the ability of a layer to resist the conduction of electrical carriers within the thin film with can be converted into the specific conductivity. The schematic of two probe used in this work is shown in the Scheme 3.1. Probe tips made from silver were press against the surface of the film. The specific conductivity was obtained by introducing a voltage (V) to the tips and determined the current (I) across two tips. The specific conductivity was calculated from equation 3.2.

$$\sigma = \frac{1}{\rho} = \frac{I}{K \times V \times t} \quad (3.2)$$

where σ is the specific conductivity (S/cm), ρ is the specific resistivity ($\Omega \cdot \text{cm}$), V is the applied voltage (V) to the tips, I is the measured current (A) across two tips, t is thickness of the films (cm), and K is the geometric correction factor

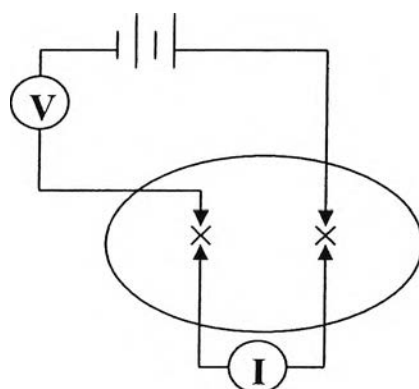
which was obtained from the standard material whose specific conductivity are known from the Resistivity Test Figure (Keithley 8009).

$$K = \rho_{\text{ref}} / R_s \times t \quad (3.3)$$

where ρ_{ref} = Known resistivity ($\Omega \cdot \text{cm.}$)

R_s = sheet resistivity (Ω)

t = film thickness (cm.)



Scheme 3.1 Schematic of two-point probe.

3.3.8 Rheological Measurement

Steady state and oscillatory shear measurements of neat alginate and the PPY/alginate suspensions were performed on a strain controlled TA Instruments AR 2000ex Rheometer. A cone-and-plate geometry with a diameter of 40 mm and a cone angle of 2° was used for all determinations. The instrument was equipped with a solvent trap to prevent water evaporation. All rheological measurements were conducted at a temperature of 25°C and within two days after the preparation of suspension in order to avoid the appearance of any time dependent transitions in the suspensions. Prior to the test, an oscillating pre-sheared at angular frequency of 30 rad/s with the controlled strain of 500% was performed for 10 min followed by equilibrating at angular frequency of 6.283 rad/s with the controlled strain of 0.2% for 10 min. Such pre-conditionings allowed us to erase previous shear history on the

sample. Steady state rheological measurements were performed in the shear rate range from 0.1 to 100 S^{-1} , while the oscillatory state measurements were done in the angular frequency range from 0.1 to 100 rad/s under the controlled strain 0.2%. Both shear viscosity and moduli (G' , G'') measurements were repeated duplicated or triplicate to confirm reproducibility.