

CHAPTER III EXPERIMENTAL

3.1 Materials

Silk cocoon (*Bombyx mori*) from four species; Nang Noi, Nang Lai, Dok Bua, and Luang Pairote were purchased from local sericulture in Thailand. Sodium-bentonite was supported from Thai Nippon Co.,Ltd, Thailand. The bentonite is a commercial sodium activated bentonite (Mac-Gel© (GRADE SAC)) with cationic exchange capacity (CEC) of 49.74 meq/100 g clay and used without extra modification. L-lactide monomer (CAS No. 4511-42-6) (99.5% purity) was purchased from Shenzhen Brightchina Industrial Co., Ltd. Stannous (II) octoate (CAS No. 301-10-0) used as catalyst was purchased from Sigma Aldrich Corp., Japan. Acrylic acid (CAS No. 79-10-7) used as cross-linked agent was purchased from Sigma Aldrich Corp., USA with molecular weight 72.06 g/mol.

3.2 Experimental Procedures

3.2.1 Extraction of Silk Sericin

The method was prepared according to the method of Aramwit *et al.* (2010). Silk cocoons were rinse with water to eliminate contaminated matter. 20 g of silk cocoons were cut into small pieces about $5 \times 5 \text{ mm}^2$ and mixed with 300 ml of purified water. Silk cocoons were autoclaved under pressure of 0.8-0.9 atm at 120 °C for 60 min. The silk fiber (fibroin) was filtered out to obtain the sericin solution. Silk sericin solution was frozen in the glass shells at -40 °C for 12 hr. After freezing, the glass shells were put into a freeze-dryer maintained at -110 °C for 48 hr under vacuum. Then, freeze-dried silk sericin was grinded into powder.

3.2.2 Preparation of Sericin-g-PLA

Stannous (II) octoate ($\text{Sn}(\text{Oct})_2$) (0.2 wt%) was added into sericin powder. Then, they were mixed with lactide monomers in glass reactor and stirred at 400 rpm, 140 °C for 10 hr as shown in Figure 3.1. After 10 hr of mixing, the mixture was

cooled down to the room temperature to get the sericin-g-PLA. Then, sericin-g-PLA was grinded into powder.

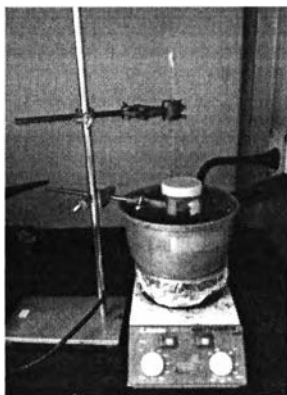


Figure 3.1 Preparation of sericin-g-PLA.

3.2.3 Preparation of Cross-linked Sericin-g-PLA with Acrylic Acid

Sericin-g-PLA powder (5 wt%) were dispersed in purified water of 50 mL and mixed with 4 mL acrylic acid for 1 hr. After that the mixtures were treated by plasma in air for 15, 30, 60, and 120 s and stirred for 15 min. Then, the mixtue was immediately frozen in petri dishes at $-40\text{ }^{\circ}\text{C}$ for 12 hr and attached to a freeze-dryer maintained at $-110\text{ }^{\circ}\text{C}$ for 48 hr to sublime the ice out.

3.2.4 Preparation of PLA

Stannous (II) octoate ($\text{Sn}(\text{Oct})_2$) (0.2 wt%) was added into lactide monomers and stirred at 400 rpm, $140\text{ }^{\circ}\text{C}$ for 10 hr. After 10 hr of synthetic, the synthesized PLA was cooled down to the room temperature. Then, PLA was grinded into powder.

3.2.5 Preparation of Sericin-g-PLA Clay Aerogel with Acrylic Acid

Sericin-g-PLA powder (5 wt%) was dispersed in purified water of 50 mL and mixed with acrylic acid for 1 hr. The mixture was added into the dispersed Na-bentonite under constant stirring followed by continuous stirring for 2 hr. After that the gel was treated by plasma in air and stirred for 15 min. Then, the gel was immediately frozen in cylindrical glass shells at $-40\text{ }^{\circ}\text{C}$ for 12 hr and attached to a freeze-dryer maintained at $-110\text{ }^{\circ}\text{C}$ for 48 hr to sublime the ice out.

3.3 Characterizations

3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups of freeze-dried silk sericin and sericin-g-PLA were analyzed by Thermo Nicolet Nexus 670 FTIR spectrometer. About 1-2 mg of sample powder was ground with KBr and was pelletized into the pellet with the thickness less than 0.5 mm. The spectra were recorded over the wavenumber range from 400-4,000 cm^{-1} with the resolution of 4 cm^{-1} and the number of scan at 64.

3.3.2 Soxhlet Extractor

The grafting percentage was determined by soxhlet extractor (VELP SCIENTIFICA, SER 148) with paper thimble. Chloroform and water were used to extract the crude copolymer at 180 °C for 3 hr. After that the remaining yield was dried in vacuum oven at 60°C and weighed. This method was repeated at less 3 times for each sample. The grafting percentage, homo PLA and free sericin were calculated as shown in Eqs. 3.1-3.3, respectively.

$$\text{grafting percentage} = \frac{M_3}{M_1} \times 100 \quad (\text{Eq. 3.1})$$

$$\text{homo PLA} = \frac{M_1 - M_2}{M_1} \times 100 \quad (\text{Eq. 3.2})$$

$$\text{free sericin} = \frac{M_2 - M_3}{M_1} \times 100 \quad (\text{Eq. 3.3})$$

where M_1 was the sample weight before Soxhlet extraction, M_2 was the sample weight after Soxhlet extraction with chloroform and M_3 was the sample weight after Soxhlet extraction with chloroform and water.

3.3.3 Gel Permeation Chromatography (GPC)

The molecular weight (MW) and molecular weight distribution index (MWD) were determined by gel permeation chromatography (GPC). GPC was

carried out in SHIMADZU GPC instrument using Tetrahydrofuran (THF) solvent as the mobile phase using a Water Styragel HR 6E 0F 7.8mmID × 300 MM column packed. The conditions of this machine were temperature of 40 °C, flow rate of 1 ml/min, and runtime of 15 min. The samples were dissolved in THF solvent and filtered by syringe filter. Then the sample solutions were injected into the instrument with injection volume of 60 μL. The molecular weight was calibrated according to polystyrene standards.

3.3.4 Solubility

The solubility in water and chloroform solvent were obtained. A dried sample powder (~0.1 g) was immersed in 40 mL of solvent. After 24 hr of immersion at 25 °C, the remnant was dried at 60 °C for another 24 hr to determine the weight of dry matter not dissolved in solvent (W_2). The weight of the dry matter dissolved in solvent was calculated by subtracting the remaining dry matter from the initial dry matter (W_1). The amount of the solubility percentage was calculated as shown in Eq. 3.4.

$$\text{solubility percentage} = \frac{W_1 - W_2}{W_1} \times 100 \quad (\text{Eq. 3.4})$$

3.3.5 Swelling Behavior

The swelling behavior of cross-linked sericin-g-PLA with acrylic acid was studied by using conventional gravimetric procedure. The sample was dried in an oven at 60 °C over night and weighed to obtain weight of dry sample (W_1). The dry sample was immersed in water at 25 °C for 24 hr. The swollen sample was withdrawn, wiped to remove the excess water out, and reweighed (W_2). The swelling percentage was calculated as shown in Eq. 3.5.

$$\text{swelling percentage} = \frac{W_2 - W_1}{W_1} \times 100 \quad (\text{Eq. 3.5})$$

3.3.6 Density Measurement

The density of the aerogels was determined by mass and dimension measurement according to an equation:

$$\rho = \frac{M}{V} \quad (\text{Eq. 3.6})$$

where ρ is mass density (g/cm^3), M is mass of sample (g) and V is volume of sample (cm^3). Mass and volume was measured using Sartorius BS 224 S analytical balance and digital vernier caliper.

3.3.7 Thermogravimetric-Differential Thermal Analyser (TG-DTA)

Thermal stability of sericin-g-PLA clay aerogel with acrylic acid was examined by PERKIN-ELMER Pyris Daimond thermogravimetric analysis. The weight of sample was in the range of 5-7 mg and heated at the heating rate of 10 $^{\circ}\text{C}/\text{min}$ from 50-800 $^{\circ}\text{C}$ in nitrogen atmosphere with 20 ml/min of nitrogen flow rate.

3.3.8 Universal Testing Machine

The Young's modulus of sericin-g-PLA clay aerogel with acrylic acid was investigated by LLOYD Lrx Universal Testing Machine in compression mode with 500 N load cell at constant crosshead speed of 1 mm/min. The aerogels were prepared in the cylindrical shape with ~20 mm in diameter and height. Five samples of each composition were tested for reproducibility. The compressive modulus was calculated from the slope of the linear portion of the stress-strain curve.

3.3.9 Dynamic Mechanical Analysis (DMA)

DMA analyze was carried out by using a dynamic-mechanical analyzer GABO EPLEXOR QC 25 instrument. The testing temperature was set from -50 to 150 $^{\circ}\text{C}$, heating rate 2 $^{\circ}\text{C}/\text{min}$, 1 Hz for frequency. The aerogels were prepared in the cylindrical shape with ~20 mm in diameter and height. The compression mode was used.

3.3.10 Scanning Electron Microscopy (SEM)

The morphology of sericin-g-PLA clay aerogel with acrylic acid was observed by using TM 3000 Scanning Electron Microscope. The samples were fixed

on stubs with carbon tape and coated with platinum under vacuum. SEM micrographs were taken with low magnification at 40 and high magnification at 40.0 k using an accelerator voltage of 15 kV.

3.3.11 Gas Chromatography (GC)

The ethylene adsorption ability of the aerogels was investigated by using BUCK Scientific MODEL 910 GAS CHROMATOGRAPH (GC) with FID detector and Porapak N column. The aerogels were prepared in the cylindrical shape with ~20 mm in diameter and ~30 mm height. A 1 mL calibration ethylene gas spanning the concentration range from 1000 to 6000 g mL⁻¹ was injected into the GC. A standard curve was then made by plotting the peak area versus the concentration injected. The samples were weighed and collected in the gas collecting bottle with closed by rubber stopper. Then the ethylene gas was injected into the bottle with the concentration of 5000 g mL⁻¹. The ethylene concentration of the analyte was calculated according to the standard curve.

3.3.12 Surface Area Analyzer

The surface area of the aerogels was analyzed by Quantachrome (Autosorb-1) instrument. The samples were weighed about 0.07-0.08 g into the sample tube and degassed at 150 °C overnight under vacuum and nitrogen trap. The sample was analyzed at 150 °C and nitrogen was used as an adsorbent gas.