

CHAPTER III EXPERIMENTAL

3.1 Materials

3.1.1 Silk Cocoons

Silk cocoons used in this study was Polyvoltine x Bivoltine waste cocoon, Dok Bua.

3.1.2 Chemicals

Polyacrylamide (PAM) 50%wt in water (MW: 10000) were purchased from Aldrich. The three chemical reagents used in this work were ethylenediamine [Panreac Sintesis], trimellitic anhydride [Fluka], and sulfuric acid [Carlo Erba]. The solvents used in this work were *N,N*-dimethylformamide [DMF; Carlo Erba], and ethanol [J.T. Baker], 99% formic acid [Carlo Erba].

3.2 Equipments

3.2.1 Autoclave

The autoclave machine (KT-40D) worked under pressure gauge 1.3 kg/cm² at 110°C for 1 h.

3.2.2 Freeze Drying Machine

The freeze drying machine (HETO FD8) worked under pressure 0.5 MPa and vacuum condition. The temperature of condenser and shelf was -40°C and 40°C, respectively.

3.2.3 High Voltage Power Supply

A Gamma High Voltage Research (UC5-30 N) power supply was used to charge the spinning solution by connecting the emitting electrode of negative polarity to the needle and the grounding electrode to the collector screen.

3.2.4 Fourier-Transform Infrared Spectrometry (FTIR)

FTIR spectra were recorded on the EQUINOX55 BRUKER spectrometer with a resolution of 3.5 cm^{-1} in the frequency range from 4000 to 400 cm^{-1} . Spectra grade KBr (Carlo Erba) was used as a background.

3.2.5 Proton Nuclear Magnetic Resonance ($^1\text{H-NMR}$)

$^1\text{H-NMR}$ spectra were recorded on the BRUKER BIOSPIN AG Switzerland (DPX-300). It was run at 300.13 MHz of the frequency and 16 number of scan. The 99.9% of D_2O was used as a solvent.

3.2.6 Thermogravimetric Analyzer

Thermogravimetric analyzer (Pyris Diamond TG/DTA) was used to study the thermal properties of polymer samples. It was run between 40°C and 700°C at a heating rate of 10°C under nitrogen purge with purge rate 200 ml/min.

3.2.7 Gel Permeation Chromatography (GPC)

All of polymer samples were characterized by Waters Gel Permeation Chromatography model 150-CV. Deionized water was used as a mobile phase at flow rate 1.0 ml/min. Polymer solutions were manual injected with a volume of $100\ \mu\text{l}$ at temperature 40°C . The column set of used was PLgel 10 μl mixed B 2 columns (Church Stretton, UK). Pollulan standards were used for calibration.

3.2.8 Scanning Electron Microscope (SEM)

JEOL Scanning Electron Microscope (JSM-6400) with operating at an acceleration voltage of 15 kV was used to study the morphology of nanoparticles

and nanofibers. The sample was placed on the brass-stub using carbon tape. The prepared samples were coated with gold using an Ion Sputter under vacuum for 4 min and placed into the specimen stage inside the microscope chamber.

3.3 Methodology

3.3.1 Preparation of Sericin

Sericin (SS) was extracted from cocoon pieces by autoclave at 110°C for 1 h. The sericin solution was purified by centrifugation at 10,000 rpm for 20 min, adjusted to pH 8-9, and dialysed for 2 days before freeze drying.

3.3.2 Modified Sericin by Solution Blending (SS/PAM)

The concentration of sericin and polyacrylamide in water was 10 and 50 wt% in water, respectively. The SS/PAM blend solution with different mixing ratios (0/100, 10/90, 20/80, 30/70, 40/60, 50/50, and 100/0 SS/PAM, w/w) were mixed overnight before electrospinning. The SS/PAM blend solution was poured into a 9-cm diameter of polyethylene plate at room temperature for 24 h and then placed in a vacuum oven for 24 h. The obtained films were used for FTIR, ¹H-NMR, and thermal gravimetry characterization.

3.3.3 Electrospinning of Modified Sericin (SS/PAM)

The SS/PAM blend solutions with different mixing ratios (0/100, 10/90, 20/80, 30/70, 40/60, 50/50, and 100/0 SS/PAM, w/w) were contained in syringe with ID 0.9 mm of a syringe needle. The electrospun SS/PAM was collected on a target which was placed at a distance of 15 cm from the syringe needle. A voltage at 15, 20 and 25 kV was applied to the syringe needle by a high voltage power supply.

3.3.4 Modified Sericin by Chemical Reaction (SS-PAM)

3.3.4.1 *Preparation of PAM Derivative a*

Ethylenediamine 20 ml was added in 25 ml of PAM. The reaction mixture was stirred at 100°C for 5 h. The mixture was isolated in ethanol,

filtered, washed with ethanol, and dried under vacuum at 70°C. The reaction scheme was outlined in scheme 4.2a.

3.3.4.2 *Preparation of PAM Derivative b*

The 0.01 mol/l of PAM derivative **a** was dissolved in water. Then trimellitic anhydride (TMA) 0.5 mol/l in DMF was added, stirred at 65-70°C for 15 h. This product was finally isolated by adding the reaction mixture to excess of ethanol, filtered, washed with ethanol, and dried under vacuum at 70°C. The reaction scheme was outlined in scheme 4.2b.

3.3.4.3 *Synthesis of Modified Sericin (SS-PAM)*

The SS powder 0.5 g was dissolved in DMF 20 ml and PAM derivative **b** 0.5 g were added in this solution under acid condition. The mixture was reacted at 70°C for 4 h. The modified sericin was isolated in cold ethanol, filtered, washed with ethanol, and dried under vacuum at 50°C. The reaction scheme was outlined in scheme 4.3. The different ratios of SS and PAM derivative **b** (1:1, 1:5, and 1:10 w/w) were prepared.

3.3.5 Electrospinning of Modified Sericin (SS-PAM)

The modified sericin (SS-PAM) solutions with different mixing ratios, 1:1; 1:5; 1:10 wt/wt of SS and PAM derivative, were contained in syringe with ID 0.9 mm of a syringe needle. The electrospun SS-PAM was collected on a target which was placed at a distance of 15 cm from the syringe needle. A voltage at 15, 20 and 25 kV was applied to the syringe needle by a high voltage power supply.