# CHAPTER III
# EXPERIMENTAL

## 3.1 Materials

### 3.1.1 Chemicals

- Silk cocoons (Chul Thai Silk Co.,Ltd.)
- Sodium carbonate (Riedel-de Haën, AR grade)
- Lithium bromine anhydrous (Sigma-Aldrich, AR grade)
- Carbazole (Merck, AR grade)
- Ammonium persulfate (Sigma-Aldrich, AR grade)
- Glutaraldrhyde solution 50% in water (Fluka, AR grade)
- Sodium dodecyl sulfate (Sigma-Aldrich, AR grade)

### 3.1.2 Solvents

- Dichloromethane (RCI Labscan, AR grade)
- Hydrochloric acid 37 % (RCI Labscan, AR grade)
- Ammonium hydroxide solution 27% (AR grade)
- Deionized (DI) water

## 3.2 Equipments

- Fourier Transform Infrared spectrometer (FTIR)
- Particle Size Analyzer (PSA)
- Thermogravimetric Analyzer (TGA)
- Scanning Electron Microscope (SEM)Melt rheometer
- Two point probe couple with an electrometer
- DC power supply
- Cellulose acetate membrane (molecular weight cutoff, MWCO 3500, Pierce)

**3.3 Methodology**

    3.3.1. <u>Preparation of silk fibroin (SF) solution and formation of SF hydrogels</u>

        According to Kim *et al.* (2004), SF aqueous solution was prepared by boiling silk cocoons for 30 min in 0.02 M of sodium carbonate and then rinsed thoroughly with water to extract sericin and wax. Then the extracted SF was dissolved in 9.3 M of lithium bromine solution at 60 °C for 4 h to yield a 20 %w/vsolution. This solution was dialyzed in water using a cellulose acetate membrane for 2 days. The final concentration of the SF solution was about 7%w/v, which determined by weighing the remaining solid after drying. SF hydrogels were prepared by crosslinking concentrated SF solution with 0.01, 0.05, 0.1, 0.5, and 1 %v/v of glutaraldehyde solution at room temperature.

    3.3.2. <u>Characterizations of SF hydrogels</u>

        *3.3.2.1 Fourier transform infared spectrophotometry (FT-IR)*

        FTIR(Nicolet, Nexus 670)analysis was carried out to assess β-sheet structure for SF hydrogels. This technique was used in transmission mode with 64 scans at a resolution of ±4 cm$^{-1}$, covering a wavelength range of 4000-400 cm$^{-1}$, using deuterated triglycine sulfate as a detector. Optical grade KBr was used as a background material. The sample was ground with KBr and pressed to form pallets.

        *3.3.2.2 Thermogravimetric Analysis*

        Thermal properties of SF hydrogels were characterized by TGA (Thermo, TGA Q50). The sample was weighted of 5-10 mg and placed it in platinum pan, and then heating it under nitrogen flow with the heating rate 10 °C/min from 30-800 °C.

        *3.3.2.3 Scanning Electron Microscopy (SEM)*

        The morphological feature of SF hydrogels was observed by SEM (Hitachi, S4800) after freeze-drying at -80°C. The freeze-dried samples were cut into a small piece and stick on a brass-stub and sputtered with platinum.

    3.3.3. <u>Polymerization of polycarbazole (PCZ)</u>

The polymerization of polycarbazole was performed at room temperature. 1.2 M of ammonium persulfate was dissolved in 50 ml of 0.5 M hydrochloric acid solution and 60 mM of carbazole monomer was dissolved in 50 ml of dichloromethane. The aqueous and non-aqueous solution were mixed and stirred for 6 h to get the green slurry solution. After filtering, the green precipitate was collected and dried in the oven. Dedopedpolycarbazole was performed by stirring the obtained polycarbazole in 0.1 M of ammonium hydroxide solution and doped again with concentrated hydrochloric acid. The mole ratio of hydrochloric acid to carbazole ($N_{HCl}:N_{CZ}$) was adjusted to be 1:1, 10:1, 100:1, 200:1, and 300:1.

### 3.3.4. Characterizations of polycarbazole

#### 3.3.4.1 Fourier transform infared spectrophotometry (FTIR)

FTIR (Nicolet, Nexus 670) was used to identify the functional groups for dedoped and doped polycarbazole. This technique was used in transmission mode with 64 scans at a resolution of $\pm 4$ cm$^{-1}$, covering a wavelength range of 4000-400 cm$^{-1}$, using deuterated triglycine sulfate as a detector. Optical grade KBr was be used as a background material. The sample was ground with KBr and pressed to form pallets.

#### 3.3.4.2 Particle size analyzer

A particle size analyzer (Malvern, Mastersizer) was used to determine the averagesize and particle size distribution of polycarbazole after grinding.

#### 3.3.4.3 Thermogravimetric Analysis

TGA (Thermo, TGA Q50) was used to characterize thermal properties of polycarbazole.The thermal behavior was investigated by weighting a sample of 5-10 mg and placing it in a platinum pan, and then heating it under nitrogen flow at a heating rate of 10 °C/min from 30-900 °C.

#### 3.3.4.4 Electrical conductivity

The electrical conductivity of polycarbazole was determined by pressing into disk pellets using a hydraulic press (diameter of 10 mm and 0.2 mm thick). Electrical conductivity was measured using a custom-built two-point probe meter connected with a voltage supplier, whose voltage was varied and the resultant

current measured. The conductivity measurement was performed under atmospheric pressure, 40-60 %RH and at 25-27 °C. The regime where the resultant current is linearly proportional to the applied voltage is called the linear Ohmic regime. The voltage and the current in the Ohmic regime were converted to the electrical conductivity via Equation 3.1:

$$\sigma = \frac{1}{\rho} = \frac{1}{R \times t} = \frac{I}{R_s \times V \times t} \qquad (3.1)$$

where $\sigma$ is the specific conductivity (S/cm), $\rho$ is the specific resistivity ($\Omega$.cm), $R_s$ is the sheet resistance ($\Omega$/sq), $t$ is the thickness of the sample pellet (cm), $V$ is the applied voltage (V), $I$ is the measured current (A), and $K$ is the geometric correction factor of the two-point probe meter. All sample thicknesses were measured by a thickness gauge (Peacock, model PDN-20).

### 3.3.5. Blending polycarbazole into silk fibroin hydrogels

Polycarbazolewas dispersed into10 ml SF solution filled with 3 %v/v of sodium dodecyl sulfate and glutaraldehyde solution. The concentration of polycarbazole was varied from 0.001, 0.005, 0.01, 0.05, 0.1, to 0.5 %v/v. The solution was poured onto a Petri dish for the composite hydrogel casting at room temperature.

### 3.3.6. Characterizations of silk fibroin/polycarbazole hydrogel composites

*3.3.6.1 Scanning Electron Microscopy (SEM)*

SEM (Hitachi, S4800) was used to examine the morphological structure and the dispersion of polycarbazole in silk fibroin hydrogels. The freeze-dried samples were cut into small pieces and attached to a brass-stub. Prior to observation, samples were platinum sputtered.

*3.3.6.2 Thermogravimetric Analysis*

TGA (Thermo, TGA Q50) was used to characterize thermal properties of the blending of polycarbazole in silk fibroin hydrogel. The thermal behavior was examined by weighing a sample of 4-5 mg and placed it in a platinum pan, and then heating it under nitrogen flow at a heating rate of 10 °C/min from 30-900 °C.

*3.3.6.3 Electromechanical property measurement*

The electromechanical properties were measured by the melt rheometer, fixed with a custom-built copper parallel plate fixture diameter of 25 mm. DC voltage was applied with a DC power supply (Instek, GFG 8216A), which can deliver the electric field up to 4 kV. A digital multimeter (Tektronix, CDM 250) was used to monitor the voltage input. The samples were prepared in the configuration of polyimide-hydrogel-polyimide sandwich to prevent short circuit formation. The polyimide (PI), ®Kapton TH-012 (12 micron) was produced by and obtained from ©2006 Saint Gobain Performance plastic Corporation. The PI film represents an excellent insulator up to its breakdown voltage of 7 kV/mm; with a dielectric constant value of 3.3; and a volume resistivity $> 10^{16}$ S/cm. In these experiments, an oscillatory shear strain was applied and the dynamic modulus (G') was measured as a function of the frequency and electric field strength. Strain sweep tests were first carried out to determine the suitable strains to measure G' in the linear viscoelastic regime. Temporal response experiments were carried out at 300 K. Frequency sweep tests in linear viscoelastic regime were carried out to measure G' of each sample as a function of frequency and electric field strength. The deformation frequency was carried out from 0.1 to 100 rad/s. In each measurement, each SF hydrogel was pre-sheared at a low frequency (0.038 rad/s) and then the electric field was applied for 15 min to ensure a steady state condition before the G' measurement.

The relative dielectric permittivity was measured by an LCR meter (HP, model 4284A) connected to the melt rheometer with a 25 mm parallel plate fixture. The thickness of the sample was 1 mm with the diameter of 25 mm. The top and bottom sides of the specimen were coated with silver adhesive to improve the electrical contacts between the specimen and electrodes. The measurements were carried out at 300 K. The AC voltage applied was varied between 1 and 10 V, depending on the materials. The electric permittivity at a frequency of 20 Hz was divided by the permittivity of free space (8.85 pF/m) to obtain the relative dielectric permittivity or the dielectric constant.

*3.3.6.4 Measurement of the dielectrophoretic force*

  The dielectrophoresis force was determined by measuring the deflection distances of the SF hydrogel in a vertical cantilever fixture under an electric field. The specimens were vertically immersed in silicone oil (viscosity=100 cSt) between parallel copper electrode plates (68 mm of length, 40 mm of width, and 2 mm of thickness). The gap between the pair of the electrodes was 30 mm. A DC voltage was applied with a DC power supply (Goldsun, GPS 3003B) connected to a high voltage power supply (Gamma High Voltage, model UC5-30P and UC5-30N) which can deliver and electric field up to 25 kV. The output voltage from the high voltage power supply was calibrated using a Fluke 40 kV High Voltage Probe. A CCD video camera was used to record the movement during the experiment. Pictures were captured from the video and the deflection distances in x (d) and y axes (l) at the end of the specimen were determined using the SemAfore software (version 5.21). The electric field strength was varied between 0 and 600 V/mm at the room temperature of $300 \pm 1$ K. Both the voltage and the current were monitored. The resisting elastic force of the specimens under the electric field was calculated using the non-linear deflection theory of a cantilever, which can be obtained from the standard curve of $(F_e l_0^2)/(EI)$ plotted versus $d/l_0$ ($l_0$ = initial length of specimens); $F_e$ is the elastic force, d is the deflection distance in the horizontal axis, $l$ is the deflection distance in the vertical axis, $E$ is the Young's modulus which is equal to $2G'(1 +v)$, where G' is the shear storage modulus taken to be G' ($\omega= 1$ rad/s) at various electric field strength and I is the moment of inertia $1/12t^3 w$, where $t$ is the thickness of the sample and w is the width of the sample. The electrophoresis force can be calculated from the static horizontal force balance consisting of the elastic force, the corrective gravity force term (mg(sin θ)), and the buoyancy force, as shown in Equation 3.2:

$$F_d = F_e + mg(\sin \theta) - \rho Vg(\sin \theta) \quad (N), \quad (3.2)$$

where $g = 9.8$ ms$^{-2}$, m = the mass of the specimen, θ is the deflection angle, ρ is the density of the fluid , and V is the volume of displaced fluid.