CHAPTER III
EXPERIMENTAL

3.1 Materials

Chitosan was purchased from Seafresh Chitosan (Lab) Co., Ltd., Thailand, in the form of pale yellow flakes. It was reported that the material had a degree of deacetylation of 95% and molecular weight of about 1 x 10^5. Glacial acetic acid and NaOH of analytical grade were purchased from Labscan Asia Co., Ltd., Thailand. Calcium chloride dihydrate stated as edible grade was supplied from Asia Drug & Chemical Co., Ltd., Thailand. Methanol was a commercial grade and used without further purification. Anhydrous citric acid (Riedel-de Haën) and 50% aqueous glutaraldehyde solution (Fluka Co., Ltd., Switzerland) were both analytical grade. The other chemicals of reagent grade were used as supplied.

3.2 Equipment

3.2.1 Capillary Viscometer

Cannon Ubbelodhe-type number 75 capillary viscometer was used for the determination of the viscosity-average molecular weight of chitosan.

3.2.2 Digital Rheometer

The apparent viscosities of the chitosan solutions were measured using a Brookfield DV III digital rheometer with a small sample adapter and a #21 spindle.

3.2.3 Fourier Transform Infrared (FTIR) Spectrophotometer

The FTIR spectrum of chitosan was recorded with Vector 3.0 Bruker FTIR Spectrophotometer with 32 scans at a resolution of 4 cm^{-1}. A frequency of 4000–400 cm^{-1} was observed by using deuterated triglycinesulfate detector (DTGS) with specific detectivity of 1 x 10^9 $cm \cdot Hz^{½} \cdot w^{-1}$.

3.2.4 Atomic Absorption Spectrophotometer (AAS)

The atomic absorption spectrophotometer used in this study was Variance SpectrAA 300P. The measurement was performed at the wavelength of 422.7 nm to determine calcium (Ca) concentration and nitrous oxide–acetylene flame was used.

3.2.5 Scanning Electron Microscope (SEM)

SEM micrographs of fibers were taken on a JEOL JSM-5200 scanning electron microscope operating at 10 kV.

3.2.6 Universal Testing Machine

The mechanical properties of the fibers were measured according to ISO 2062:1993(E) using Lloyd LR 100K apparatus.

3.3 Methodology

3.3.1 Characterization of Chitosan

3.3.1.1 Degree of Deacetylation

The degree of deacetylation of chitosan was determined by means of infrared spectroscopic measurement. Chitosan flakes were pulverized to achieve the powders with the size of less than 75 μm, and dried at 60°C overnight. One mg of the sample powder and 60 mg of potassium bromide (KBr) powder were mixed and ground together. The mixture was then pressed to give a KBr disc. FTIR spectrum of the sample was recorded in a range between 4000 to 400 cm^{-1}. The degree of deacetylation of chitosan was evaluated based on the baseline method using Equation 3.1 (Sannan *et al.*, 1978).

$$DD = 98.03 - 34.68(A_{1550}/A_{2878}) \quad (3.1)$$

where:

A_{1550} = absorbance at 1550 cm^{-1} (the amide II band)

A_{2878} = absorbance at 2878 cm^{-1} (the C—H stretching).

3.3.1.2 Molecular Weight

The molecular weight of chitosan was determined based on the intrinsic viscosity method using a mixture of 0.2 *M* acetic acid/ 0.1 *M* sodium acetate as a solvent. Firstly, an appropriate volume of the solvent was added to a weighted amount of chitosan. When chitosan flakes were dissolved completely, the solution was adjusted to obtain a concentration of 0.10 g/dL. After being mixed thoroughly, the chitosan solution was then diluted further into various concentrations: 0.01, 0.02, 0.03, and 0.05 g/dL. The measurement was performed immediately after the sample solutions were prepared and stirred overnight.

About 15–20 mL of filtered solvent was filled into the viscometer which was then mounted vertically in a water bath regulated at 30 ± 0.1°C and left to equilibrate for 15–20 minutes. The solvent was then drawn up the central capillary tube until it was above the upper mark. The flow time of the solvent between the two marks was determined and repeated at least 5 times (± 0.2 s). Next, the solvent was drained out. The viscometer was rinsed twice with a filtered sample solution to be measured. The flow time for the solution was determined in the same manner as for the solvent. The order of the solutions measured was 0.01, 0.02, 0.03, 0.05, and 0.10 g/dL, respectively.

Based on Equation 3.2–3.6,

$$\text{Relative viscosity } (\eta_{rel}) = t/t_s \quad (3.2)$$

$$\text{Specific viscosity } (\eta_{sp}) = (t/t_s) - 1 \quad (3.3)$$

$$\text{Reduced viscosity } (\eta_{red}) = \eta_{sp}/c \quad (3.4)$$

$$\text{Inherent viscosity } (\eta_{inh}) = (\ln \eta_r)/c \quad (3.5)$$

$$\text{Intrinsic viscosity } ([\eta]) = (\eta_{red})_{c \to 0} \quad (3.6)$$

where:

t = flow time of chitosan solution

t_s = flow time of solvent

c = concentration of chitosan solution in g/dL,

the values of reduced viscosity and inherent viscosity were plotted against the concentration. Then, the value of intrinsic viscosity was obtained from the intercept of the plot, multiplied by 100 to change the dimensions into mL/g, and followed by

the calculation of the molecular weight based on the Mark–Houwink–Sakurada equation (shown as Equation 3.7):

$$[\eta] = KM^a \tag{3.7}$$

where $[\eta]$ is intrinsic viscosity, M is viscosity-average molecular weight, K and a are constants determined based on the degree of deacetylation according to Wang *et al.* (1991) as mentioned in Equation 3.8 and 3.9.

$$K = 1.64 \times 10^{-30} \times DD^{14.0} \tag{3.8}$$

$$a = -1.02 \times 10^{-2} \times DD + 1.82 \tag{3.9}$$

where DD is degree of deacetylation.

3.3.2 Solution Viscosity Measurement

Six chitosan solutions with the concentrations of 5, 6, 7, 8, 9, and 10% in 4% (v/v) aqueous acetic acid were prepared and aged overnight (about 17 h) at room temperature. Then, the viscosities of the solutions were measured using a digital rheometer, Brookfield DV III, at stirring speed that gave the highest value of torque.

3.3.3 Fiber Spinning

3.3.3.1 Preparation of Spinning Solution

An appropriate amount of chitosan flakes was gradually added to a certain quantity of 4% (v/v) aqueous acetic acid with vigorous stirring to obtain a solution with the viscosity of about 100 poises. The mixture was aged for about an hour to ensure complete dissolution. Next, the chitosan solution was filtered through double layers of clothes under an applied N_2 pressure of about 0.5 kg/cm^2 to remove insoluble material and then directly poured into the glass column of the spinning apparatus. After that, the solution was left to stand at room temperature overnight to remove any trapped air bubbles.

3.3.3.2 Spinning Process

The spinning apparatus is shown in Figure 3.1.

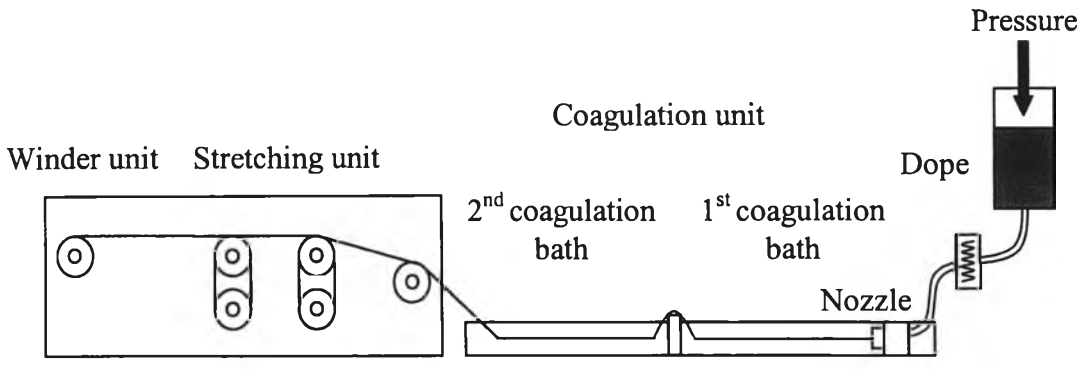

Figure 3.1 Spinning apparatus.

A nitrogen pressure of about 0.5 kg/cm^2 was applied to push the spinning solution through a 50-hole (0.1-mm diameter) viscose-type spinneret into the coagulation bath (50 cm in length, 900 mL in volume) in which the spinneret was submerged. Following the coagulation, the coagulated filaments were subsequently passed through another 1-m long bath containing 50% (v/v) aqueous methanol. Next, the filaments were brought forward to a set of two rollers and then to a winder. After winding up the filament on a bobbin, the fiber was washed several times with methanol and dried in air at room temperature. After drying, the filaments were cut perpendicular to their alignment to remove from the spool, and stored in a sealed plastic bag.

3.3.4 Fiber Analysis

3.3.4.1 Calcium Content

An amount of fiber of exactly known weight was digested in 5 mL HNO_3 with heating until the fiber was dissolved completely. After the solution cooled down to room temperature, it was diluted in a 50-mL volumetric flask with distilled water together with the addition of 5 mL of 4% (w/v) aqueous potassium chloride. Next, the sample solution was filtered through a filter paper (Whatman no. 42) and kept in a plastic bottle at room temperature. The filtered solution was analyzed for the amount of Ca using AAS with a calibration curve created from 1, 2, and 3 ppm standard Ca solutions.

3.3.4.2 SEM Micrographs

A small segment of fiber sample was attached to stub with the help of a piece of adhesive tape and then sputter coated with gold. SEM micrographs were taken on the scanning electron microscope operating at 10 kV.

3.3.4.3 Linear Density

A specimen of yarn was taken from the package, cut into 15-cm length and weighed using a digital electronic balance with four-decimal point. The linear density of the yarn was expressed in tex—the mass, in grams, of one kilometer of yarn. The value quoted for each sample was the average value of 50 specimens.

3.3.4.4 Mechanical Properties

The tensile strength and the elongation at break of the fibers were measured in the form of yarn according to ISO 2062:1993(E). After the specimens of yarns were taken from the package and measured for their linear density as mentioned in Section *3.3.4.3*, they were brought to moisture equilibrium under the conditioned atmosphere overnight before testing. The atmosphere for preconditioning, conditioning, and testing were as specified in ISO 139:1973(E)—a relative humidity of 65 ± 2% and a temperature of 27 ± 2°C. The load cell used was 100 N. Gauge length was set at 50 mm with a rate of displacement of 50 mm/min. The value quoted for each sample was the average value of 20 specimens.