

CHAPTER III

EXPERIMENTAL

3.1 Materials

Chitin was prepared from shrimp shells by the methods of Shimahara and Takigushi (1978). Chitin was powdered and passed through the sieve to obtain the particle size in the range of 71-75 μm . Cellulose from spruce was purchased from Fluka. N, N-dimethylacetamide (DMAc) (Lab Scan), Lithium chloride (LiCl) (BDH) and Isopropyl alcohol (BDH) were an analytical grade.

3.2 Equipment

3.2.1 Restch seiving machine

The chitin powder with the size of 71 to 75 microns was sieved and collected separately by using Restch Sieving Machine type Vibro.

3.2.2 Capillary viscometer

The viscosity-average molecular weight of chitin was determined by using Cannon Ubbelohde-type number 150 of capillary viscometer.

3.2.3 FTIR spectrophotometer

The FTIR spectrum of chitin/cellulose blend films were recorded with Bruker FTIR Spectrophotometer, model Vector 3.0, with 16 scans at a resolution of 4 cm^{-1} . A frequency of $4000\text{-}400\text{ cm}^{-1}$ was observed by using deuterated triglycinesulfate detector (DTGS) with specific detectivity of $1 \times 10^9\text{ cm.Hz}^{1/2}.\text{w}^{-1}$.

3.2.4 Wide-angle x-ray diffractometer (WAXD)

The wide-angle X-ray diffractometer used in this study was D/MAX-2000 series of Rigaku X-ray Diffractometer system. X-ray of Cu k-30 mA were used as a source. The k-beta filter was used to eliminate interference peak. Divergence slit and scattering slit at 1 deg together with 0.3 mm of receiving slit were set on the instrument. The experiment was performed in the range of 5-30 degree with scan speed 5 deg/min and 0.02 deg of scan step.

3.2.5 Thermogravimetric analyzer (TGA)

The thermogravimetric analyzer (TGA) used to evaluate the thermal stability of the blend films was TGA 5.1 Dupont Instrument model 2950.

3.2.6 Lloyd tensile tester

The strength of the blend films was characterized by Lloyd Instrument LRX series of Lloyd tensile tester with the maximum load of 2500 N.

3.2.7 Gas permeability tester

The Brugger gas permeability tester type GDP/E as shown in Figure 3.1 was used to detect the permeability of oxygen gas through the blend films. The flow rate of oxygen was controlled at 100 cm³/min at ambient temperature.

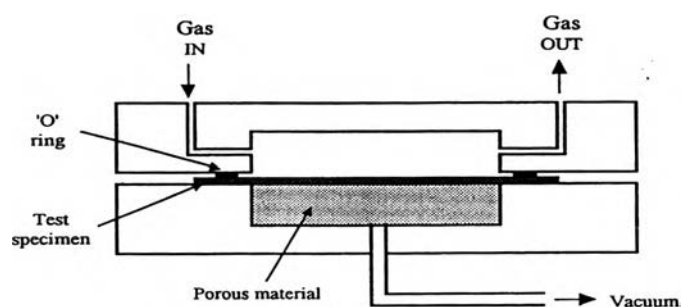


Figure 3.1 Schematic drawing of a Brugger gas permeability tester.

3.1 Methodology

3.3.1 Preparation of chitin

Chitin was prepared from shrimp shell waste. There were two steps to prepare chitin by the method of Shimahara and Takigushi (1988). The shells of shrimp were washed with water, dried under sunlight and crushed into chips about 0.5 cm² in size. The first step was demineralization with hydrochloric acid. The dried shell chips (100g) were immersed in 1 L of 1 N hydrochloric acid. The mixture was kept for 2 days at room temperature with occasional stirring using a glass rod. At the initial stage of the reaction, frequent stirring was required to prevent the floating of the shell chips caused by the generation of carbon dioxide gas. In the middle stage of the treatment, the exhausted hydrochloric acid was changed. The demineralized shell chips were collected and washed with distilled water until neutral to pH paper. The yield of demineralized shell chips is approximately 50%. The second step was deproteination with sodium hydroxide aqueous solution. The demineralized shell chips was added to 1 L of 4% NaOH solution and heated at 70-80 °C for 4 h with occasional stirring. An approximate amount of water should be added as the vaporization of water proceeded. After boiling, chitin chips were collected and washed with distilled water until neutral. The product was dried in oven at 110°C for 24 h. The yield of chitin from shrimp shell was approximately 30% on dry weight basis.

3.3.2 Degree of deacetylation of chitin

The method used to determine the degree of deacetylation of chitin is based on infrared spectroscopic measurement by Sannan *et. al.* (1978). About 3 mg of chitin powder, passed through a 200-mesh sieve, was mechanically mixed with 400 mg of potassium bromide powder to prepare a KBr disk. An infrared spectrum was recorded in a range from 4000 cm⁻¹ to 400 cm⁻¹. The absorbances at 2878 cm⁻¹(the C-H band) and 1550 cm⁻¹ (the

amide II band) were evaluated by the baseline method. The degree of deacetylation (D) was calculated from equation 3.1.

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

When D = Degree of deacetylation (%)

A_{1550} = Absorbance at 1550 cm^{-1} (the C-H band)

A_{2878} = Absorbance at 2878 cm^{-1} (the amide II band)

3.3.3 Viscosity-average molecular weight of chitin

The different concentration solutions (0.00, 0.025, 0.04, 0.05, 0.06, 0.075, 0.085 and 0.1 g/100 mL) of chitin dissolved in 5%LiCl/DMAc were prepared. The Ubbelohde viscometer was filled with 10 mL of sample and then equilibrated in water bath, which maintained the temperature at 30°C . The sample was pass through the capillary once before the running time was measured. Each sample was measured 5 times. The running times of solvent and solutions were used to calculate the relative viscosity, specific viscosity, and reduced viscosity. The reduced viscosity was plotted against the concentration with the intercept being the intrinsic viscosity.

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

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

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

$$\text{Intrinsic viscosity } [\eta] = (\eta_{\text{red}})_{c \rightarrow 0} \quad (3.5)$$

When t is the running time of chitosan solution, t_s is the running time of solvent and C is the concentration in g/100mL.

The viscosity-average molecular weight of chitosan was determined based on Mark-Houwink equation as followed

$$[\eta] = 8.93 \times 10^{-4} M^{0.71} \quad (3.6)$$

When $[\eta]$ = Intrinsic viscosity
 M = Viscosity-average molecular weight

3.3.4. Preparation of chitin / cellulose blend films

3.3.4.1 *LiCl/DMAc solvent preparation*

The 5% LiCl/ DMAc for chitin dissolution was prepared by dissolving LiCl 50 g in 1 L of DMAc. The solution was stirred at room temperature until clear solution was obtained. For the 9% LiCl/DMAc used for cellulose dissolution, 27 g LiCl was added in 300 ml of DMAc in a 500-ml three-necked flask. The solvent was heated and held around 80°C with mechanical stirring until LiCl was completely dissolved. The LiCl/DMAc solvents were prepared immediately prior to use to minimize moisture uptake.

3.3.4.2 *Preparation of chitin solution*

Chitin powder (5g) was added in 1 L of 5% LiCl/DMAc. The suspension was stirred continuously overnight, filtered with sintered glass and centrifuged to remove undissolved particles.

3.3.4.3 *Preparation of cellulose solution*

There were two steps to dissolve cellulose by using the method of McCormick *et al.* (1985). The first step was cellulose pretreatment and the second step was cellulose dissolution. First, cellulose powder (20g) was stirred overnight in 500 ml of distilled water. After that the mixture was vacuum filtered with sintered glass. The cellulose was then added to 500 ml

of methanol, stirred for 1 h, and filtered. Next, the cellulose was added to 500 ml of DMAc and the mixture was stirred for 1 h. The swollen cellulose was separated by vacuum filtered with sintered glass. The cellulose content was calculated by drying several 1.0 g portions of the swollen cellulose in a vacuum oven at 80°C for 48 h. The average cellulose content of swollen cellulose was 0.38 g from drying 1 g of the swollen cellulose.

For the second step, the swollen cellulose (15.79g) was added in 300 ml of 9% LiCl/DMAc . The mixture was heated to 150°C for 15 min and allowed to cool down to room temperature. This process was repeated four times. The solution was cooled down and centrifuged to remove undissolved particles.

3.3.4.4 Preparation of the blend films

Pure and the blend films with different blend ratios were prepared from chitin and cellulose by separately dissolving chitin and cellulose in 5% LiCl/DMAc and 9% LiCl/DMAc, respectively. The blend ratios of chitin to cellulose were 100/0 80/20, 60/40, 40/60, 20/80, and 0/100 by weight. The solutions were poured into glass plates (30 cm x 37.50 cm x 2mm) and the solvent was allowed to evaporate. The solution slowly formed a gel as the solvent was evaporated. After the gel was allowed to set for 4 h, it was immersed in isopropyl alcohol overnight. The gel was washed with methanol and dried.

3.3.5 FTIR spectra of chitin/cellulose blend films

FTIR spectra of chitin/cellulose were recorded on the Bruker Fourier transform infrared spectrophotometer, Model Vector 3.0 with 16 scans at a resolution of 4 cm⁻¹. The samples with the thickness of 10 μm were attached to the sample frames and scanned from frequency of 4000 to 400 cm⁻¹ by using deuterated triglycinesulfate detector (DTGS) with specific detectivity of 1x10⁹ cm.Hz^{1/2}.w⁻¹.

3.3.6 Thermogravimetric analysis

The thermogravimetric analysis (TGA) of the blend films was carried out under N₂ atmosphere at heating rate of 10°C/min from 40°C to 700°C. About 8 mg of sample was used for each measurement.

3.3.7 X-ray diffraction pattern

The wide-angle X-ray diffractograms of the blend films were recorded at room temperature using Rigaku X-ray Diffractometer system, Model D/Max-2000. The X-ray source was Cu k-alpha (40 kV/30 mA). The k-beta filter was used to eliminate interference peak. The dried films with thickness of 25µm were attached the sample holders and scanned from 5 to 30° 2θ at a speed of 5°/min. and 0.02 degree for scan step.

3.3.8 Equilibrium water content (EWC)

To measure the Equilibrium Water Content (EWC), a preweighted dry film was immersed in distilled water. After 30 min, the film, which equilibrated with water, was removed from the water. After excess water at the surface of the film was blotted out with Kimwipes paper, the weight of the swollen film was measured and the procedure was repeated until there was no further weight change. Water content was determined by gravimetric method (Kim *et al.*, 1996) and calculated from the following equation:

$$\text{EWC (\%)} = \frac{W_h - W_d}{W_d} \times 100$$

Where W_h and W_d denote the weight of hydrate and dry film, respectively.

3.3.9 Swelling behavior

The effect of salt type, salt concentration and pH on the degree of swelling of the blend films were studied. Preweighted dry films were immersed in the 0.125M, 0.25M and 0.5M salt solutions, namely NaCl, LiCl, CaCl₂, AlCl₃, and FeCl₃ solutions. The effect of pH on the degree of swelling

of the blend films was studied at pH 3,5,7 and 10. Sodium hydroxide and hydrochloric acid solutions were used to adjust the pH. For each experiment, after excess water at the surface of the films was blotted out with Kimwipes paper, the weight of swollen films were measured and the procedure was repeated until there was no further weight change. The degree of swelling was calculated by using the following equation (Wang *et al.*, 1996):

$$\text{Degree of swelling (\%)} = \frac{W_s - W_d}{W_s} \times 100$$

Where W_s and W_d denote the weight of swollen and dry films, respectively.

3.3.10 Mechanical properties

The tensile strength and elongation at break of the chitin/cellulose blend films were measured by Lloyd Tensile Tester by following the standard ASTM D882 using the gauge length of 50 mm and an extension rate of 20 mm/min at room temperature. Test films were cut in the dimension of 25 mm x 150 mm and the thickness of the films were in the range of 35-45 μm . Then the films were dried at 60°C for 24 h before testing.

3.3.11 Oxygen permeability testing

The Brugger Gas Permeability Tester was used to measure the oxygen permeability rate of the blend films. Permeability measurement were performed at 298 K. The measuring part of the apparatus was outgassed before each run by evacuation until there was no appreciable increase in pressure on a pressure gauge. Subsequently, a penetrated gas was introduced into the upstream side compartment and the pressure increase in the down stream side compartment was measured on a pressure gauge connected to a recorder. Oxygen gas (O_2) of 99.9 % minimum purity was employed as penetrated gas. Samples were cut in the diameter of 11 cm and the thickness of the films were in the range of 10-12 μm . The oxygen permeability rate was calculated by the following equation (Kawakami *et al.*, 1982):

$$\text{Gas permeability rate (G)} = \frac{1.47 \times 10^9}{KN}$$

Where G is gas permeability rate ($\text{cm}^2/\text{m}^2 \text{ d bar}$), K is the temperature (298 K) and N is the inverse of the slope of the plot between scale division (mm) versus the time (s).