CHAPTER III
EXPERIMENTAL

3.1 Materials

Chitin was prepared from shrimp shell that was kindly supplied from Surapol Food Company by the method of Shimahara and Takigushi (1988). Chitin was grinded to powder with the size 71-75 μm before use. Poly(vinyl alcohol) was purchased from Fluka. The degree of polymerization of PVA was 1600 and the degree of hydrolysis was 97.75 – 99.5%. Glutaraldehyde was purchased from Fluka. Monochloroacetic acid and sodium hydroxide 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 seived and collected separately by using Restch Seiving Machine type Vibro.

3.2.2 Capillary Viscometer

The viscosity-average molecular weight of CM-chitin was determined by using Cannon Ubbelodhe-type number 75 of capillary viscometer.

3.2.3 Elemental Analysis

The degree of substitution was estimated by elemental analysis using PERKIN ELMER Series II CHNS/O Analyzer 2400.

3.2.4 FTIR Spectrophotometer

The FTIR spectrum of PVA/CM-chitin blend films were recorded with a Bruker FTIR Spectrophotometer, model Vector 3.0, with 32 scans at a resolution of 4 cm^{-1}. The samples with the thickness of 10 μm were attached to the sample frames. A frequency of 2000-400 cm^{-1} was observed by using deuterated triglycinesulfate detector (DTGS) with specific detectivity of 1×10^9 cm.Hz$^{1/2}$.w^{-1}.

3.2.5 Wide-angle X-ray Diffractometer (WAXD)

The wide-angle X-ray diffractometer used in this study was a D/MAX-2000 series of Rigaku X-ray Diffractometer system. X-ray of Cu k-alpha 40 kV/30 mA were used as a source. A Nickel 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 dried films with thickness of 25 μm were attached the sample holders and scanned from 5 to 30 degree 2θ at a speed of 5 degree/min. and 0.02 degree for scan step.

3.2.6 Differential Scanning Calorimeter (DSC)

The differential scanning calorimeter (DSC) used to evaluate the thermal properties of the blend films was a NETZCH model TASC 414/3. The sample of 8-15 mg was placed in an Al pan. The differential scanning calorimeter (DSC) of the blend films was carried out under N$_2$ atmosphere at heating rate of 10°C/min from room temperature to 250°C.

3.2.7 Thermogravimetric Analyzer (TGA)

The thermogravimetric analyzer (TGA) used to evaluate the thermal stability of the blend films was a TGA 5.1 Dupont Instrument model 2950. The sample of 8-15 mg was placed in a Pt pan. The thermogravimetric

analysis (TGA) of the blend films was carried out under N_2 atmosphere at heating rate of 10°C/min from room temperature to 700°C.

3.2.8 Lloyd Tensile Tester

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

3.2.9 Gas Permeability Tester

The Brugger gas permeability tester type GDP/E was used to detect the permeability of oxygen gas through the blend films. The flow rate of oxygen was controlled at 100 cm^3/min at ambient temperature.

3.3 Methodology

3.3.1 Preparation of Chitin

The shells of shrimp were washed with water, dried under sunlight and crushed into chips about 0.5 cm^2 in size. 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. The demineralized shell chips were collected and washed with distilled water until neutral. The second step was deproteination with sodium hydroxide aqueous solution. The demineralized shell chips was added to 1 L of 4% NaOH solution and boiled at 70-80°C for 4 h with occasional stirring. An approximate amount of water was 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.

3.3.2 Preparation of CM-chitin

Alkaline chitin was prepared by suspending powdered chitin (4 g) in 42% NaOH solution. After the suspension was allowed in desiccator for 30 min under reduced pressure, crush ice (160 g) was added and the mixture was mechanically stirred for 30 min in an ice bath. Monochloroacetic acid solution was prepared by dissolving in 14% NaOH in an ice bath and was added dropwise into the alkaline chitin solution with stirring over 30 min. After standing overnight at room temperature, the mixture was neutralized with acetic acid under cooling in an ice bath and dialyzed against running water for 2 days, followed by dialysis against distilled water for 1 day. The dialysate was centrifuged at 5000 rpm for 20 min, in order to remove insoluble material, and 3 volumes acetone was added to the supernatant. After standing overnight, the precipitate was collected by centrifugation and washed with acetone. The product was resuspended in ethanol and collected by filtration. After drying at room temperature, CM-chitin Na salt was obtained (Tokura *et al.*, 1983a).

3.3.3 Degree of Deacetylation of Chitin

The method used to determine the degree of deacetylation of chitin is based on quantitative infrared spectroscopic technique (Sannan *et al.*, 1978). About 3 mg of chitin powder, passed through a 200-mesh seive, 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 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 (DD) was calculated from the following equation:

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

When DD   = Degree of deacetylation (%)

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

A_{2878} = Absorbance at 2878 cm^{-1} (the C-H band)

3.3.4 Viscosity-average Molecular Weight of Chitin

The different concentrations (0.01, 0.02, 0.03, 0.04 and 0.05 g/100 ml) of chitin solutions dissolved in 5% LiCl/DMAc were prepared. The Ubbelohde viscometer was filled with 10 ml of sample solution and then equilibrated in water bath, which was maintained the temperature at 30°C. The sample solution was passed 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.

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

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

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

$$\text{Intrinsic viscosity } [\eta] \quad = (\eta_{sp})_{C\rightarrow0} \quad\quad (3.5)$$

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

The viscosity-average molecular weight of chitin was determined based on Mark-Houwink equation. The K and a values were according to Lee (1974).

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

When [η] = Intrinsic viscosity

M = Viscosity-average molecular weight

3.3.5 Degree of Substitution of CM-chitin

The degree of substitution was estimated by elemental analysis with combustion at 950°C. The sample (1-2 mg) was filled in tin foil analyzed under air with oxygen as a combustion gas (flowing rate of 20 ml/min) and He as a carrier gas (flowing rate of 200 ml/min).

3.3.6 Viscosity-average Molecular Weight of CM-chitin

The different concentrations (0.01, 0.02, 0.03,0.04 and 0.05 g/100 ml) of CM-chitin solutions of dissolved in 0.1 M NaCl were prepared. The Ubbelohde viscometer was filled with 10 ml of sample solution and then equilibrated in water bath, which was maintained the temperature at 25°C. The sample was passed 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 viscosity-average molecular weight of CM-chitin was determined based on Mark-Houwink equation. The K and a values were according to Kaneko $et\ al.$ (1982).

$$[\eta] \quad = \quad 7.92 \times 10^{-5} M^{1} \tag{3.7}$$

When [η] = Intrinsic viscosity

M = Viscosity-average molecular weight

3.3.7 Preparation of PVA/CM-chitin Blend Films

3.3.7.1 Preparation of PVA Solution

A known amount of PVA was dissolved in deionized water at 90°C to prepare PVA solution with concentration of 1% by weight. The solution was stirred until it was clear.

3.3.7.2 Preparation of CM-chitin Solution

CM-chitin solution was prepared by dissolution of CM-chitin in deionized water to obtain the concentration of 1% by weight. The solution was stirred continuously overnight.

3.3.7.3 Preparation of Blend Films

The blend films of PVA and CM-chitin were prepared by mixing various ratios of 1% by weight of PVA solution and 1% by weight of CM-chitin solution. The blend solution was stirred continuously overnight. For crosslinked CM-chitin/PVA blend films, glutaraldehyde was added into the solution to achieve the concentration of 0.005%, 0.01% and 0.05% before casting the solution onto a clean dry plastic plate. The films were allowed to dry at 40°C in a convective oven for 24 h.

3.3.8 Equilibrium Water Content (EWC)

The blend films were cut into the disk form with diameter of 16 mm and 25-30 µm in thickness. A preweighted dry film was immersed in distilled water for 24 h. When the film was removed from the water, excess water at the surface of the film was blotted out with Kimwipes paper and the weight of the swollen film was measured. 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:

$$\text{EWC (\%)} \quad = \quad \frac{W_h - W_d}{W_d} \times 100 \qquad (3.8)$$

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

3.3.9 Swelling Behavior

The blend films were cut into the disk form with diameter of 16 mm and 25-30 µm in thickness. The weights of the completely dried samples were measured, and the samples were dipped into a vial filled with different pH buffer solutions and different salt solutions, i.e., LiCl, NaCl, $CaCl_2$ and $FeCl_3$, with the concentration of 0.25 M at room temperature. The degrees of swelling of these samples were calculated from the following equation (Wang et al., 1996):

$$\text{Degree of swelling (\%)} \quad = \quad \frac{W_s - W_d}{W_d} \times 100 \qquad (3.9)$$

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

3.3.10 Mechanical Properties

The tensile strength and elongation at break of the CM-chitin/PVA 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 was in the range 35-45 µm. Then the films were dried at 60°C for 24 h before testing.

3.3.11 Oxygen Permeability Testing

The blend films were cut into the circular form with the diameter of 110 mm and about 10 µm in thickness. The films were sealed completely with grease on the top of the porous material and two halves of the

permeability cell were clamped together. The 'O' ring ensured an air-tight seal between two halves. The oxygen gas was circulated through the top half of the permeation cell and vacuum applied below the specimen until all the air had been removes from the specimen. Vacuum was then turned off and the rate of oxygen gas permeation through the films was recorded with times. The oxygen permeability rate could be calculated from the following equation.

$$\text{Gas permeability rate (G)} \quad = \quad \frac{1.47 \times 10^9}{KN} \qquad (3.10)$$

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