CHAPTER III
EXPERIMENTAL

3.1 Materials

Shrimp shell was kindly supplied by Surapon Foods Public Co., Ltd., Thailand. Poly(ε-caprolactone) (PCL) was obtained from Daiseru Chemical Co., Ltd., Japan. Sodium hydroxide solution 50% (w/w) was kindly supplied by KPT Coorporation, Thailand. Hexanoyl chloride was purchased from Fluka Co., Ltd. Methanol was purchased from Labscan Co., Ltd. Pyridine and chloroform obtained from Aldrich Co., Ltd. were distilled and dried over molecular sieve prior to use. The other chemicals were analytical grade and were used without further purification.

3.2 Equipment

3.2.1 Fourier Transform Infrared Spectroscopy

FT-IR spectra of chitosan, H-chitosan, PCL, and H-chitosan/PCL blend films were obtained by using a Vector 3.0 Bruker Spectrophotometer with a resolution of 4 cm^{-1}. The films were prepared with the thickness of 10-20 μm and attached to the sample holder. A frequency ranging from 4000 to 400 cm^{-1} was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^*, of $1x10^9$ $cmHz^{1/2}w^{-1}$ and scanned with a repetition of 32 scans.

3.2.2 Nuclear Magnetic Resonance Spectrometry

^{1}H-NMR and ^{13}C-NMR spectra were recorded by using FT-NMR 500 MHz. spectrometer (JEOL, JNM-A500). H-chitosan was dissolved in $CDCl_3$ and used tetramethylsilane (TMS) as reference for chemical shift measurement.

3.2.3 Elemental Analysis

Elemental analysis results were obtained from a CHNS/O analyzer (Perkin Elmer PE2400 Series II: option CHN) with combustion temperature at 950°C. The sample (1-2 mg) was filled in tin foil and analyzed under air with oxygen as a combustion gas (flow rate of 20 ml/min) and He as a carrier gas (flow rate of 200 ml/min). Degree of substitution of H-chitosan after repeated reaction for one, two, and three times was determined based on the C/N ratio of H-chitosan from elemental analysis. The C/N ratio value obtained from elemental analysis was compared with the calculated value. For calculated values, assuming H-chitosan prepared repeating the reaction for three times was fully substituted by hexanoyl groups to calculate C%, H%, and N% per each repeating unit.

3.2.4 Differential Scanning Calorimeter

A Netzch TASC 414/3 Differential Scanning Calorimeter (DSC) was used to determine the miscibility of blend films. Thermograms of blend films were recorded under N_2 gas with flow rate of 20°C/min and heating rate of 10°C/min from -100°C to 100°C.

3.2.5 Scanning Electron Microscopy

Scanning electron microscopy (SEM) was investigated using a scanning electron microscope (JEOL model, JSM 5000) at a magnification of x1500. The surface morphology of blend films was studied by using SEM. Before measurement, the surface of blend films was etched by cyclohexane in order to remove H-chitosan or acetic acid in order to remove PCL at room temperature for 2 minutes and coated with gold.

3.2.6 Wide-angle X-ray Diffractometer

Wide-angle X-ray diffractograms (WAXD) of blend films were recorded by a Rigaku Rint 2000 diffractometer. High intensity monochromatic Ni-Filtered CuKα radiation was generated at 40 kv and 30 mA. The dried film with thickness of 25 μm was attached to the sample holder. The analysis was performed

at room temperature in the range of 5-50 degree 2θ with scan speed of 5 degree/min and 0.02 degree of scan step.

3.2.7 Thermogravimetric Analysis

Thermal stability of blend films was evaluated using a TGA 5.1 DuPont Instrument model 2950. The film sample (8-15 mg) was placed in the Pt pan and analysis was carried out from 30°C to 650°C at heating rate of 10°C/min under nitrogen atmosphere.

3.2.8 Tensile Tester

Mechanical properties of H-chitosan/PCL blend films were evaluated on a Lloyd Instrument LRX series of Lloyd tensile tester. The film specimens with dimension of 25x150 mm and the thickness 20-30 μm were tested. The test was carried out by using the gauge length of 50 mm and crosshead speed of 500 mm/min according to ASTM D882.

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 blend films were cut into the diameter of 110 mm and had about 27-30 μm in thickness. The flow rate of oxygen was controlled at 100 cm^3/min at room temperature. The oxygen permeability (P) was calculated by the following equation:

$$P = G \times \text{film thickness} \quad (1)$$

$$G = \frac{1.49 \times 10^7}{TN} \quad (2)$$

where P = oxygen permeability ($cm^3 \mu m/m^2.sec.bar$),

G = gas transmission rate ($cm^3/m^2.sec.bar$),

T = temperature (K), and

N = reciprocal of slope from the plot of the change of the vacuum pressure versus time.

3.3 Methodology

3.3.1 Preparation of Chitin

Chitin was prepared by using method adopted from Shimahara and Takigusshi (1988). Shrimp shell was cleaned and dried before grinding into smaller pieces. Demineralization was performed by immersing shrimp shell in 1 N HCl solution for 2 days with occasional stirring. The demineralized product was neutralized by washing with deionized water and protein removal was performed in 4% (w/w) of NaOH solution by boiling at 80-90°C for 4 h. The deproteinized portion was washed with deionized water until neutral. Chitin obtained was dried at 60°C for 24 h.

3.3.2 Preparation of Chitosan

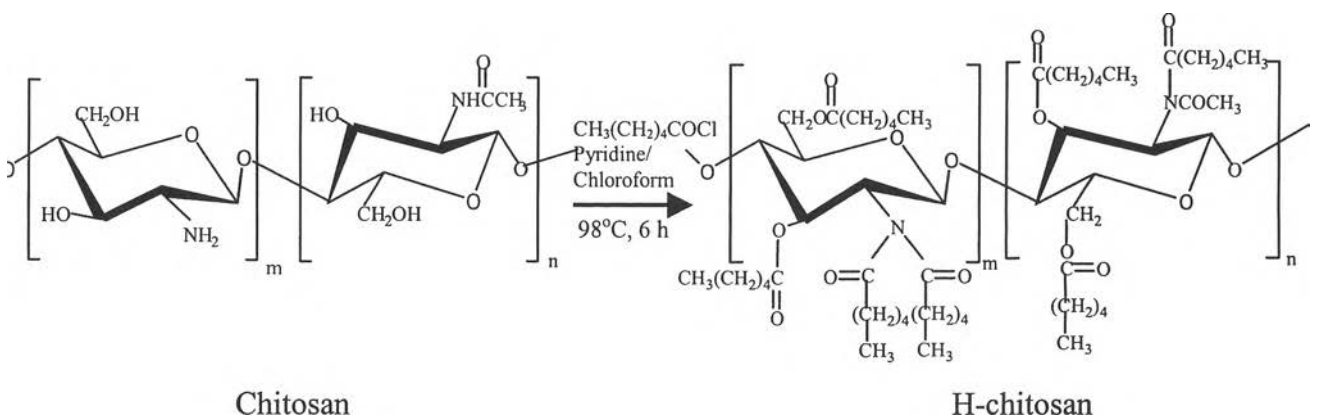
Chitosan was obtained from deacetylation of chitin by heating chitin flakes in 50% (w/w) NaOH solution. $NaBH_4$ 0.5% (w/w) was added based on the weight of chitin to prevent depolymerization. The mixture was heated in an autoclave at 110°C for 1 h. The deacetylated product was washed thoroughly with deionized water until neutral. The resulting chitosan flakes were dried in an oven at 60°C for 24 h. This procedure was repeated three times to obtain the high degree of deacetylation of chitosan. Chitosan powder was sieved using Restch Sieving Machine type Vibro and the portion with the size of 70-75 μm was collected.

3.3.3 Preparation of Hexanoyl Chitosan (H-chitosan)

Chitosan (3.20 g, 19.13 mmol) was soaked in pyridine for one week and filtered off before further soaking in the mixture of pyridine (90 ml) and chloroform (45 ml) for one day. The mixture was cooled to -10°C in an ice-salt bath and then hexanoyl chloride (21.18 ml, 160.67 mmol) dissolved in chloroform (15 ml) was added dropwise to the mixture within 2 h. The mixture was then stirred for 2 h at room temperature and further refluxed for 6 h at 98°C. The resultant mixture was poured into methanol (300 ml). The precipitate was filtered off, dissolved in chloroform, and then reprecipitated into methanol. The precipitate was filtered off,

extracted in a Soxhlet extractor with methanol for 8 h before drying in a vacuum oven at 40°C for 24 h. The sticky yellowish product was obtained.

The dried hexanoyl chitosan, fresh pyridine and chloroform were placed in a flask in the amount described above. This procedure was repeated three times to obtain hexanoyl chitosan that completely substituted with hexanoyl groups.



Scheme 3.1 Synthesis reaction of H-chitosan.

3.3.4 Preparation of H-Chitosan and PCL Blend Films

The H-chitosan and PCL solution were prepared by separately dissolved 1% (w/w) of H-chitosan and 1% (w/w) of PCL in chloroform. The two polymer solutions were mixed to give required blend compositions, and stirred mechanically at room temperature for 4 h. The blend films were prepared by solution casting into petri dish which coated with teflon. The solvent was evaporated at room temperature for 24 h. The thickness of films obtained was varied from 20-30 μm.

3.3.5 Viscosity-average Molecular Weight of Chitosan

The solutions with difference concentrations of chitosan in a mixture solvent containing 0.2 M acetic acid, 0.1 M NaCl and 0.4 M urea were prepared and measured using capillary viscometer at 25°C. The viscosity-average molecular weight of chitosan was determined based on the Mark-Houwink equation:

$$[\eta] = kM^a \qquad (3)$$

$$[\eta] = 8.93x10^{-2}M^{0.71} \quad (4)$$

where $[\eta]$ = the intrinsic viscosity (ml/g),

M = viscosity-average molecular weight,

k = Mark-Houwink constant ($8.93x10^{-2}$ ml/g), and

a = Mark-Houwink constant (0.71) (Lee, 1974).

3.3.6 Degree of Deacetylation of Chitosan

The degree of deacetylation of chitosan was determined based on infrared spectroscopic measurement. Absorbances of peaks at wave number of 2878 cm^{-1} (the C-H stretching) and 1550 cm^{-1} (the amide II band) were evaluated by the based line method (Sannan, 1978). The degree of deacetylation was calculated from the following equation:

$$DD = 98.03\text{-}34.68\ (A_{1550}/A_{2878}) \quad (5)$$

where DD = degree of deacetylation (%),

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

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