

CHAPTER III EXPERIMENTAL

3.1 Materials

Chitosan with a degree of deacetylation of 74.0% was provided from the Asian Institute of Technology (AIT), Bangkok. *N,N'*-carbonyldiimidazole was produced by TCI, Japan. Methanol, hydrochloric acid, and chromium trioxide were purchased from J.T. Baker, USA. Acetic acid, *N,N*-dimethylformamide, and sodium hydroxide were supplied from UNIVAR, Australia. Sodium hydride, and phthalic anhydride were purchased from Fluka Chemmika, Switzerland. Ethanol, and acetone were produced from BDH Laboratory Supplies, England. Perchloric acid was purchased from Mallinckrodt Baker, Inc., USA. Sodium acetate was produced by Wako Pure Chemical Industries, Ltd., Japan. All these chemicals were used without further purification.

3.2 Instruments and Equipment

3.2.1 Fourier Transform Infrared Spectroscopy (FT-IR)

Qualitative and quantitative FT-IR spectra were obtained from VECTOR 3.0 BRUKER Spectrometer with 64 scans at a resolution of 4 cm^{-1} . A frequency range of $4000\text{-}400\text{ cm}^{-1}$ was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^* , of $1 \times 10^9\text{ cm.Hz}^{1/2}\text{w}^{-1}$.

3.2.2 Ultraviolet-Visible Spectrophotometer (UV-VIS)

A Lamda-10 UV-VIS Spectrophotometer from Perkin-Elmer was used for qualitative and quantitative analysis. The amount of carbonyl and carboxyl groups in chitosan chain after γ -irradiation were quantitatively measured at λ_{\max} 247 and 290 nm, respectively.

3.2.3 Thermal Gravimetric Analysis (TGA)

DuPont Thermogravimetric analyzer was used for TGA study. Samples (approximately 5-7 mg) were loaded into a platinum pan and heated under a N₂ flowing rate of 20 mL/min. The heating rate was 20°C/min from 30°C to 600°C.

3.2.4 X-ray Diffraction (XRD)

X-ray diffraction patterns were obtained from a RIGAKU RINT 2000. CuK α ($\lambda = 0.154$ nm) was used as X-ray source and operated at 40 kV, 30 mA with Ni filter. Sample (0.1-0.2 g) was spread on a glass slide specimen holder to examine at 2θ of 5-50°.

3.2.5 Elemental Analysis (EA)

The percent elements were obtained from YANAKO CHN CORDER MT-3, MT-5 Analyzer with combustion temperature at 950°C. The sample was put in tin foil for 1-2 mg and analyzed under air with O₂ as a combustion gas (flowing rate 20 mL/min) and He as a carrier (flowing rate 200 mL/min).

3.2.6 Nuclear Magnetic Resonance Spectrometer (NMR)

Solid state ¹³C-NMR spectra were analyzed by a DPX-300 Advance 300 MHz Digital NMR Spectrometer of Bruker, Switzerland, by a courtesy of the National Metal and Materials Technology Center (MTEC), Ministry of Science and Technology, Thailand.

^1H and ^{13}C - NMR measurements were carried out by a courtesy of Kagoshima University, Department of Applied Chemistry and Chemical Engineering with a JEOL GSX-400 (400 MHz) spectrometer in acetic acid- d_4 , and dimethyl- d_6 sulfoxide for chitosan and modified chitosan, respectively.

3.3 Experimental

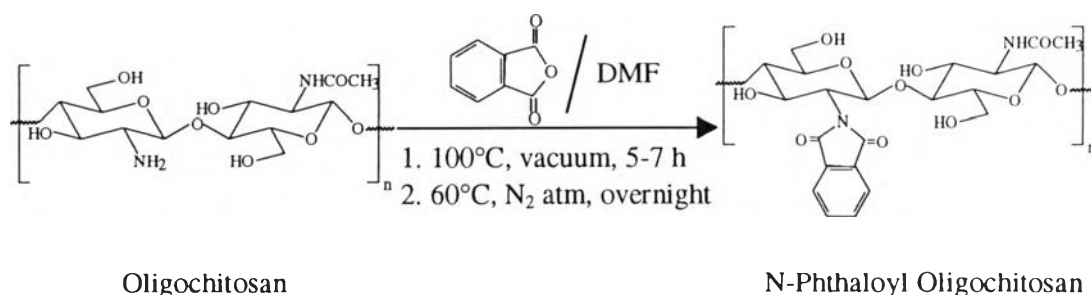
3.3.1 Preparation of Oligochitosan

The γ -irradiation of chitosan samples was carried out in a γ -cell (Co-60) by a courtesy of Office of Atomic Energy for Peace, Ministry of Science and Technology, Thailand. Samples were irradiated with various doses of up to 96.23 kGy at a dose rate of 6.88 kGy/h.

3.3.2 Preparation of N-phthaloyl Oligochitosan

Oligochitosan (1 g, 31.0 mmol) was stirred in *N,N*-dimethylformamide (30 mL) under vacuum condition and heated to 100°C. Phthalic anhydride (2.76 g, 186.2 mmol) was added to the solution. After 5-7 h, the mixture became a viscous solution and the temperature of the system was adjusted to 60°C. The system was left for the reaction under nitrogen atmosphere for overnight (Scheme 3.1). The obtained solution was concentrated and reprecipitated in ice water. The precipitate was collected and washed completely by ethanol for three times, and dried in vacuo to give white precipitate N-phthaloyl oligochitosan. The obtained product was characterized by FT-IR, EA, UV, TGA, NMR and XRD.

Scheme 3.1 Preparation of N-phthaloyl oligochitosan

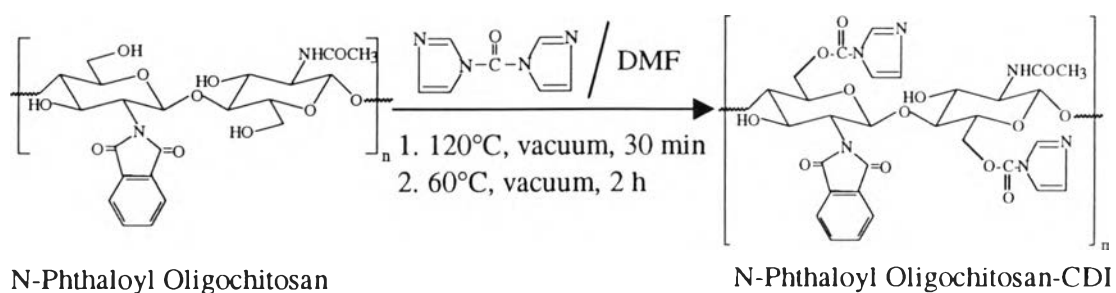


3.3.3 Preparation of Chitosan Precursors

3.3.3.1 Synthesis of N-Phthaloyl Oligochitosan- Carbonyl Imidazolide

N-Phthaloyl oligochitosan (1.00 g) was dispersed in *N,N*-dimethylformamide (30 mL) and heated to 100°C in vacuo. After 30 min, *N,N'*-carbonyldiimidazole (3.0 g, 4 moles equivalent to pyranose rings) was added and reacted at 100°C for 30 min. The temperature was then reduced to 60°C and stirring was continued for 2 h (Scheme 3.2). After the reaction, the mixture was concentrated and reprecipitated in methanol. The precipitate was collected and washed thoroughly with methanol followed by drying in vacuo to give a pale yellow powder. The product was characterized by FT-IR, EA, TGA, and XRD.

Scheme 3.2 Preparation of N-phthaloyl oligochitosan-carbonyl imidazolide



3.3.3.2 Synthesis of Carboxyl N-Phthaloyl Oligochitosan

A small amount of distilled water (2.5 mL) was gradually added into the dispersed mixture of N-phthaloyl oligochitosan (0.6583 g, 45.2 mmol based on 2-amino-2-deoxy-D-glucose phthalimido residues) in glacial acetic acid (10 mL) until all the N-phthaloyl oligochitosan was dissolved. The solution was evaporated to a viscous syrup. Acetic acid (10 mL) was added and the solution was evaporated; this process was repeated several times to remove water until further addition of acetic acid resulted in incomplete dissolution. Acetic acid was then added to make the final volume approximately 100 mL. Finally, 70% aqueous perchloric acid (0.5 mL) was gradually added with vigorous stirring resulting in the formation of a white precipitate in the solution. This suspension was used directly in the next step.

Chromium trioxide (0.15 g) was dissolved in acetic acid (1.5 mL) and water (0.15 mL) to prepare a chromium trioxide solution. This solution was added to the rapidly stirred suspension. After 30 min, the same amount of chromium trioxide solution was added repeatedly and the reaction was allowed to proceed for a further 30 min. Another chromium trioxide solution was prepared by dissolving chromium trioxide (0.1 g) in water (3.75 mL) and added to the suspension. The solution was stirred continuously for an hour and methanol (1.25 mL) was added to decompose the excess oxidant. After stirring for 30 min, the mixture was allowed to settle (Scheme 3.3). The precipitate was then filtered and washed with 0.5N hydrochloric acid and methanol until both precipitate and supernatant were colorless. The obtained product was dried under vacuum. The product was characterized by FT-IR, TGA, and XRD.

Scheme 3.3 Preparation of carboxyl N-phthaloyl oligochitosan