



CHAPTER III EXPERIMENTAL

3.1 Materials

Chitosan with percent deacetylation (%DD) 95% and molecular weight of 227,000 were locally supplied from Seafresh Chitosan (Lab) Company Limited, Bangkok Thailand. 1-Hydroxybenzotriazole monohydrate (HOBt·H₂O) and water-soluble carbodiimide hydrochloride (WSC·HCl) were purchased from Wako Pure Chemical Industries Co. Ltd., Japan. L-asparagine monohydrate and L(-)-tryptophan were obtained from Fluka Chemika, Switzerland. Sodium hydroxide and hydrochloric acid were purchased from Lab-Scan, Ireland. Na⁺-montmorillonite clay was supplied from Southern Clay Product, Inc., Texas. Poly(ethylene glycol) (PEG, *M_n* 1450 Da) were purchased from Sigma-Aldrich, Inc., USA. Succinic anhydride was provided from Fluka Chemika, Switzerland. All chemicals were used without further purification.

3.2 Instruments and Equipment

3.2.1 Structural Analysis

Qualitative analysis by Fourier transform infrared spectrophotometer (FTIR) was obtained from a Thermo Nicolet Nexus 670 with 32 scans at a resolution of 2 cm⁻¹ in a frequency range of 4000-400 cm⁻¹ using a deuterated triglycinesulfate detector (DTGS). Proton nuclear magnetic resonance (¹H NMR) spectra were obtained from a 400 MHz JEOL JNM-GSX spectrometer.

3.2.2 Thermal Analysis

A Dupont thermal gravimetric analyzer was applied using a Perkin Elmer Pyris Diamond with N₂ flowing rate of 20 mL/min and a heating rate of 10°C/min from 50°C to 500°C.

3.2.3 Morphology Observation

The morphology was investigated by using a JEOL JSM-5200 scanning electron microscopy (SEM) at 15 kV.

3.3 Methodology

3.3.1 Preparation of Chitin Whisker (Scheme 1)

Chitin flakes (1.00 g), were treated in 3 N hydrochloric acid (HCl) (100 mL) and stirred at reflux for 3 h before centrifugation. The treatment with 3 N HCl was repeated three times. Finally, the residues were collected and dialyzed in distilled water until neutral to obtain chitin whiskers.

3.3.2 Preparation of Chitosan Whisker (Scheme 1)

Chitin whisker (20 mL) was stirred in NaOH aq. (40% w/v 100 mL) at reflux for 7 h before leaving at room temperature overnight. The treatment with 40% NaOH aq. was repeated three times. The crude product was dialyzed until neutral in distilled water to obtain chitosan whisker.

3.3.3 Synthesis of Chitosan-L-Asparagine (Scheme 1)

Chitosan whisker (0.1 g, 0.61 mmol) was vigorously stirred with HOBt·H₂O (0.09 g, 0.61 mmol) to that of chitosan in deionized water 8 mL at ambient temperature until the clear solution was obtained. Chitosan whisker-HOBt aqueous solution was mixed with L-asparagine (0.29 g, 0.61 mmol) followed by adding WSC·HCl (0.37 g, 0.61 mmol). The reaction was carried out at room temperature for overnight. The crude product was dialyzed and lyophilized.

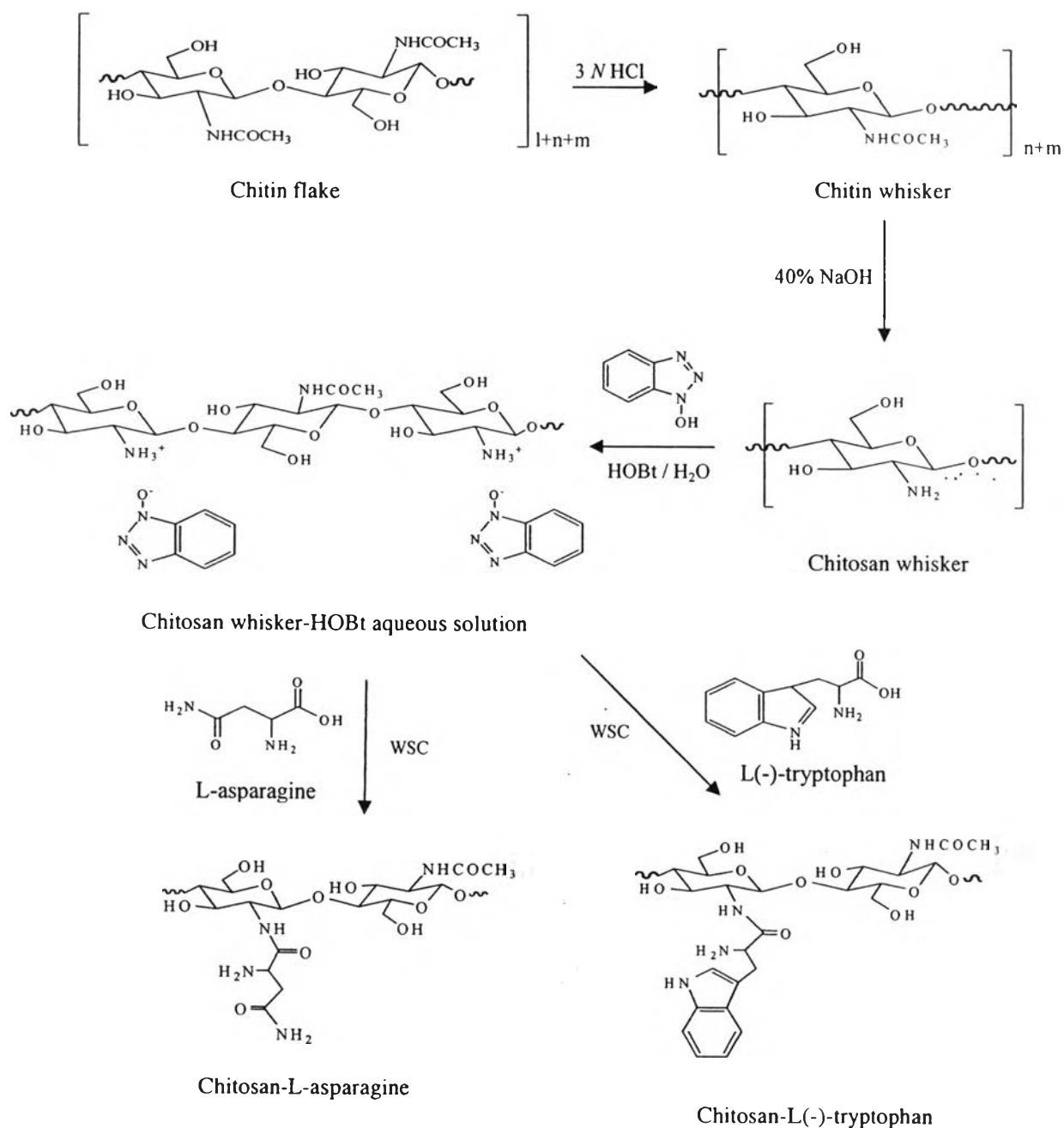
FT-IR (KBr, cm⁻¹): 1641 cm⁻¹ (amide I). ¹H NMR (δ, ppm): 2.74 (-CH₂-), 2.03 (NHAc), 3.01 (H₂ of GluN unit in chitosan), and 3.16-3.75 (H₃-H₆ of pyranose ring).

3.3.4 Synthesis of Chitosan-L(-)-Tryptophan (Scheme 1)

Chitosan-L(-)-tryptophan was prepared similar to chitosan-L-asparagine but using L(-)-tryptophan.

FT-IR (KBr, cm⁻¹): 1640 cm⁻¹ (amide I), 749 cm⁻¹ (benzene ring). ¹H NMR (δ, ppm): 1.90 (NHAc), 3.00 (H₂ of GluN unit in chitosan), 3.18-3.29 (-CH₂-), 2.90-3.74

(-NH-CH₂- and H3-H6 of pyranose ring), and 7.43-7.72 (-CH- of benzene ring in tryptophan).



Scheme 1. Synthesis of chitosan-L-asparagine and L(-)-tryptophan.

3.3.5 Chitosan-HOBt Aqueous Solution (Scheme 2)

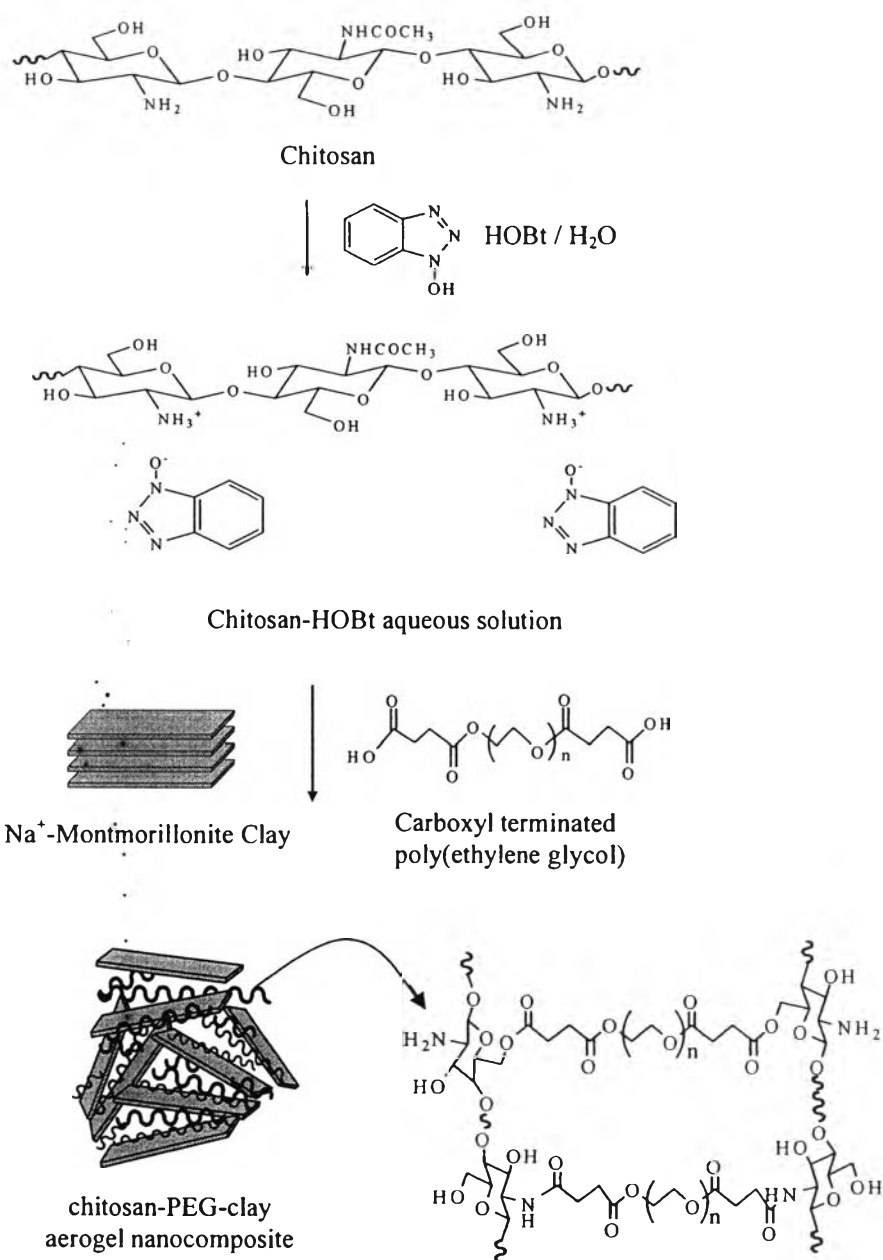
Chitosan (0.5 g, 3.06 mmol) was vigorously stirred with HOBt·H₂O (0.47 g, 3.06 mmol) in deionized water 40 mL at ambient temperature until the clear solution was obtained.

3.3.6 Synthesis of Chitosan-PEG-clay Aerogel Nanocomposite (Scheme 2)

Na⁺-montmorillonite clay 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.07 g was suspended in 10 mL deionized water. PEG (M_n 1450, 20.00 g, 13.8 mmol) was reacted with succinic anhydride (2.76 g, 2.76 mmol) at 65°C for 24 hours. The crude product was purified by reprecipitating in diethyl ether, washing for several times and drying in vacuum to obtain carboxyl terminated poly(ethylene glycol) (COOH-PEG-COOH).

To the solution of chitosan-HOBt (40 mL), carboxyl terminated poly(ethylene glycol) (COOH-PEG-COOH) (1.70 g, 1.53 mmol), WSC (0.59 g, 3.06 mmol), and Na⁺-montmorillonite clay suspension were added and stirred at room temperature for 24 hours. Carboxyl terminated poly(ethylene glycol) (COOH-PEG-COOH) was varied in 0.34, 0.68, 1.02, 1.36 and 1.70 g. The product was lyophilized to obtain aerogel.

FTIR (KBr, cm⁻¹): 2874 (CH stretching), 1732 (C=O ester), 1650 (amide I), and 1565 cm⁻¹(amide II).



Scheme 2. Synthesis of chitosan-PEG-clay aerogel nanocomposite.