

CHAPTER III

EXPERIMENTAL

3.1 Materials

Chitosan with a degree of deacetylation (%DD) of 70 was locally supplied from the SEAFRESH (Lab) Company Limited, Bangkok, Thailand. Acetic acid, hydrochloric acid, calcium chloride, disodium hydrogenphosphate, methanol and tris(hydroxymethyl)aminomethane (Tris) were purchased from Carlo Erba Reagenti, Italy. Sodium acetate, *N,N*-dimethylformamide (DMF), isopropanol and potassium hydroxide were obtained from Univar, Australia. Phthalic anhydride was obtained from Fluka Chemika, Switzerland. Epichlorohydrin was purchased from Acros Organics, Belgium. Hydrazine monohydrate was purchased from Nacalai Tesque., Inc., Japan. All Chemicals were used without further purification.

3.2 Equipment

Qualitative and quantitative Fourier transform infrared spectra were obtained from a Bruker Equinox 55/S with 32 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 $1 \times 10^9\text{ cm.Hz}^{1/2}\text{ w}^{-1}$. A Dupont thermal gravimetric analyzer was applied using a N_2 flowing rate of 20 mL/min with a heating rate of 20°C/min from 30°C to 600°C . X-ray diffraction patterns were obtained from a RIGAKU RINT 2000, using $\text{CuK}\alpha$ ($\lambda=0.154\text{ nm}$) as an X-ray source with 2θ of $5\text{-}50^\circ$ operating at 40 kV , 30 mA with Ni filter. Differential scanning calorimetry was performed on a Perkin-Elmer DSC7. The sample was sealed in a closed aluminum sample liquid pan and heated from 25°C to 120°C at a heating rate of 20°C/min . Intrinsic viscosity $[\eta]$ was measured with a calibrated viscometer Cannon-Ubbelohde (No.2, A149) in 0.1 M sodium acetate/ 0.2 M acetic acid aqueous solution at $30 \pm 0.05^\circ\text{C}$. Molecular weight was

calculated using the Mark-Houwink equation with $K=1.64 \times 10^{-30} \times DD^{14}$ and $a=(-1.02 \times 10^{-2} \times DD)+1.82$ (Wang *et al.*, 1991).

3.3 Methodology

3.3.1 Preparation of *N*-Phthaloyl-Chitosan

N-Phthaloyl-Chitosan was prepared by the reaction as reported elsewhere (Kurita *et al.*, 1991). Chitosan (1.00 g) was reacted with phthalic anhydride (4.27 g, 5 mol equivalent to pyranose rings) in DMF (20 mL) at 100°C under nitrogen for 6 h. The temperature was reduced to 60°C, and the mixture was left overnight. The solution was concentrated to obtain yellowish viscous product. The crude product was reprecipitated in ice water. The precipitate was collected, washed with methanol several times, and dried *in vacuo* to give *N*-Phthaloyl-Chitosan. The product obtained was characterized by FTIR, XRD, and TGA.

3.3.2 Preparation of Epoxy-*N*-Phthaloyl-Chitosan

N-Phthaloyl-Chitosan (1.00 g) was dissolved in DMF (20 mL) and heated to 60°C *in vacuo*. After 30 min, a catalytic amount of potassium hydroxide-isopropanol solution and epichlorohydrin (3.50 g, 10 mol equivalent to pyranose rings) were added. The reaction was carried out at 60°C for 5 h. The solution was concentrated and reprecipitated in cold water. The precipitate was collected and washed with methanol several times, followed by drying *in vacuo*. The product obtained was characterized by FTIR, XRD, and TGA.

3.3.3 Preparation of Epoxy-Chitosan

The deprotection of amino group was carried out as reported by Kurita *et al.*, 1991. Epoxy-*N*-Phthaloyl-Chitosan (1.00 g) and hydrazine monohydrate (3.12 g, 20 mol equivalent to pyranose rings) were mixed in water (20 mL) and heated to 60°C under atmosphere. After 15 hours, the product was concentrated, washed thoroughly with methanol several times, and dried *in vacuo*. In this step, hydrazine will not only function for deprotection of amino group but also

initiation the oxirane ring opening reaction to crosslink with other epoxy-chitosan chains. The curing reaction was confirmed by qualitative FTIR curve fitting and DSC. The product obtained was characterized by FTIR, XRD, and TGA.

3.3.4 Preparation of Epoxy-Chitosan/Hydroxyapatite (HA) Composite

The chitosan/HA composite was obtained by an alternate soaking process as reported by Tachaboonyakiat *et al.*, 2001. Epoxy-Chitosan (1.00 g) was immersed in CaCl₂ (200mM)/ Tris-HCl (pH 7.4) aqueous solution (20 mL) at 37°C for 2 hours, followed by rinsing with distilled water. The product was immersed in Na₂HPO₄ (120 mM) aqueous solution (20 mL) at 37°C for 2 hours and washed thoroughly with water. The soaking was repeated alternatively several times, followed by drying *in vacuo*. The product obtained was characterized by FTIR, XRD, and TGA.