CHAPTER III
EXPERIMENTAL

3.1 Materials

Low molecular weight chitosan ($\overline{M}_v$ = 85 000) with 95% deacetylation was provided by Seafresh Chitosan (Lab) Company Limited, Bangkok, Thailand. *N'-N* carbonyldiimidazole (CDI) was obtained from TCI, Japan. Benzaldehyde, acetic acid, acetone, chloroform, ethanol, and methanol were purchased from Carlo Erba Reagenti, Italy. Sodium hydroxide and hydrochloric acid were the products from Lab-Scan, Ireland. Triethylamine was obtained from Unilab, Australia. Sodium acetate was purchased from Univar, Australia. Polyethyleneglycol 1100 monomethylether was supplied from Fluka Chemika, Switzerland. Poly(ethylene glycol) methylethers; M_w 350, 550, 2000, and 5000 were purchased from Aldrich Chemical Company, Inc., USA. Potassium hydroxide, and picric acid were the products of Ajax chemicals (Australia). All chemicals were analytical grade and used without further purification.

3.2 Measurements

Qualitative and quantitative Fourier transform infrared spectra were obtained from a Bruker Equinox 55/S with 32 Scans at a resolution of 2 cm^{-1}. A frequency range of 4000-400 cm^{-1} was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D*, of 1 x 10^9 cm $Hz^{½}$ w^{-1}. Powder X-ray diffraction (XRD) patterns were recorded over a 2θ range of 2-60° by a RIGAKU RINT 2000 using CuKα as an X-ray source and operating at 40 kV and 30 mA with Ni filter. A Dupont thermogravimetric analyzer applied using a N_2 flow rate of 20 mL/min with a heating rate of 20 °C/min from 30 to 600°C. Intrinsic viscosity [η] of low molecular weight chitosan was measured with a calibrated viscometer Cannon-Ubbelohde (No. 2, A149) in 0.2 M CH_3COOH/0.1 M CH_3COONa aqueous solution at 30 ± 0.05°C. Molecular weight was calculated

using the Mark-Houwink equation with $K = 1.64 \times 10^{-30} \times DD^{14}$ and $a = (-1.02 \times 10^{-2} \times DD) + 1.82$ as proposed by Wang *et al.* Potassium picrate concentration in aqueous phase was determined by a Perkin Elmer Lambda-10 UV-VIS spectrophotometer.

3.3 Methodology

The work involves with the details as follows molecular design, synthesis characterization, and study on ion extraction property of chitosan-PEG. This includes the following plan as shown in Scheme II (page 25).

3.3.1 Preparation of *N*-Benzylidene Chitosan (CTB)

In this work, the modification of low molecular weight chitosan at only hydroxyl groups are considered. *N*-benzylidene chitosan was prepared according to Yang *et al.* (1999) in the first step. The product obtained was structural characterized by FT-IR, TGA, and XRD.

3.3.2 Preparation of *N*-Benzylidene Chitosan-Carbonyldiimidazole (CTB-CDI)

Carbonyldiimidazole was chosen as a coupling agent due to its high reactivity with alcohols, carboxylic acids, and amines, which are important functional groups in carbohydrate polymers. The low molecular weight chitosan precursor was carried out according to the method proposed by Yoksan *et al.* (2001). The product obtained was structural characterized by FT-IR, TGA, and XRD.

3.3.3 Preparation of *N* - Benzylidene Chitosan – Polyethyleneglycol Monomethylether (CTB-PEG)

In order to obtain a chitosan chain with pseudocyclic ether, polyethyleneglycol monomethylether was applied. The reaction of polyethyleneglycol monomethylether with the chitosan precursor was expected to proceed at reactive ester. The product obtained was structural characterized by FT-IR, TGA, and XRD.

3.3.4 Preparation of Chitosan - Polyethyleneglycol Monomethylether (Chitosan-PEG)

Chitosan-PEG was prepared by treating *N*-benzylidene chitosan-polyethyleneglycol monomethylether in diluted ethanolic hydrochloride solution. In order to maintain the unique aminosaccharide of chitosan. The product obtained was structural characterized by FT-IR, TGA, and XRD.

3.3.5 Study on Ion Extraction Property of Chitosan and Chitosan-PEG

Each of low molecular weight chitosan and chitosan-PEG was studied on ion extraction property by using potassium picrate salt as a model ion. The change in concentration of potassium picrates was determined by a UV-VIS spectrophotometry at λ 354 nm.