

## CHAPTER III

### EXPERIMENTAL SECTION

#### 3.1 Materials

Chitosan with a degree of deacetylation of 0.85 was provided by Prof. Suwalee Chankrachang, Asia Institute of Technology.

Toluene-4-sulfonyl chloride and N,N-Dimethylacetamide (AR grade) were purchased from Fluka Chemica Company. Stearic acid was supplied from Carlo Erba. Sodium hydroxide (>99.0%) and N,N-Dimethylformamide were purchased from Ajax Chemicals Company and methyl alcohol (AR grade) and chloroform (AR grade) from J.T. Baker Company. All chemicals were used without further purification.

#### 3.2 Instruments and Equipment

##### 3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

Qualitative FTIR spectra were obtained from FT-45 A Bio-Rad Spectrometer and quantitative data was obtained from VECTOR 3.0 BRUKER Spectrometer with 16 scans at a resolution of 8 cm<sup>-1</sup>. A frequency range of 4000-400 cm<sup>-1</sup> was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D\*, of 1x10<sup>9</sup> cm.Hz<sup>1/2</sup>W<sup>-1</sup>.

### 3.2.2 Thermal Gravimetric Analysis (TGA)

NETZSCH TG 209 Thermogravimetric analyzer was used for TGA studies. Samples (approximately 10-12 mg) were loaded in an alumina crucible and heated under a N<sub>2</sub> flowing rate of 20 mL/min. The normal heating rate was 10°C/min and the heating range was from room temperature to 700°C.

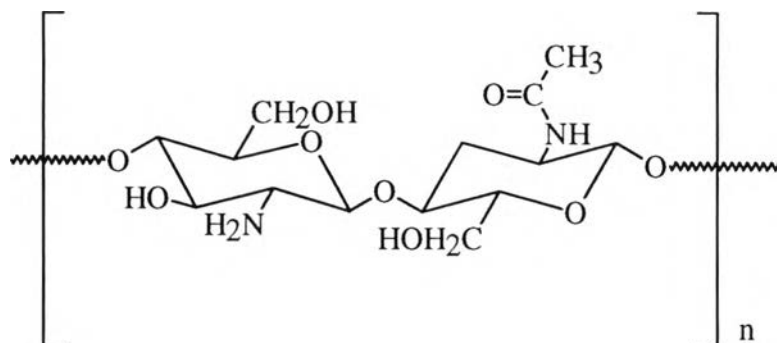
### 3.2.3 Elemental Analysis (EA)

The percent elements were obtained from PE 2400 Series II CHNS/O Analyzer with combustion temperature 975°C, reduction temperature 500°C and vial receptacle 1000 runs. The sample was put in tin foil and weighed approximately for 1-2 mg in tin foil excluding foil weight and analyzed under air atmosphere (flowing rate 60 psi) with O<sub>2</sub> as a combustion gas (flowing rate 15 psi) and He gas as a carrier gas (flowing rate 20 psi).

## 3.3 Experimental Procedure

Chitosan was obtained as a powder with a degree of deacetylation 0.85, according to the determination by acid hydrolysis-HPLC technique (Chankrachang, 1996).

The chemical structure of chitin-chitosan copolymer is represented as Figure 3.1



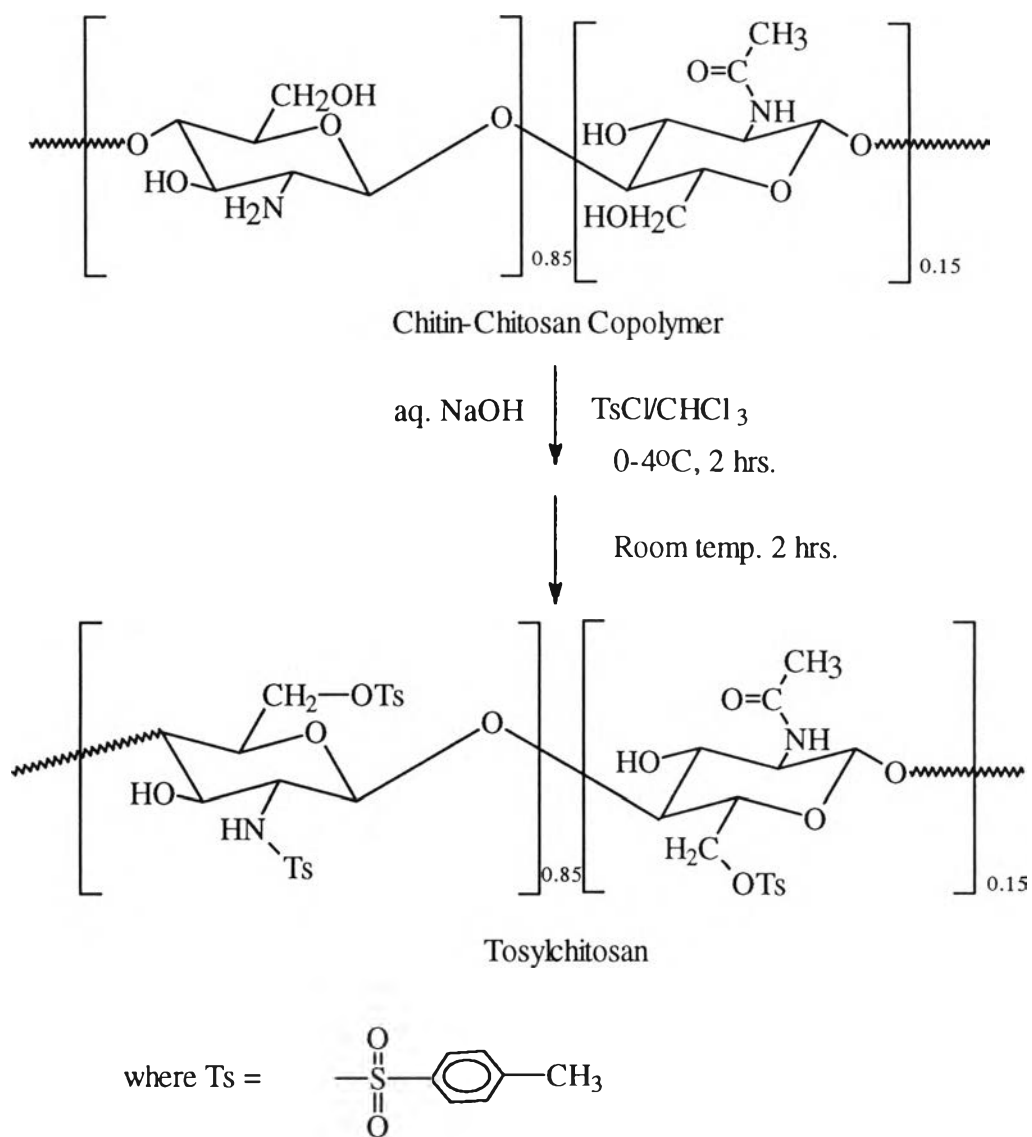
**Figure 3.1** Chemical Structure of chitin-chitosan copolymer.

### 3.3.1 Preparation of Chitosan Acetate

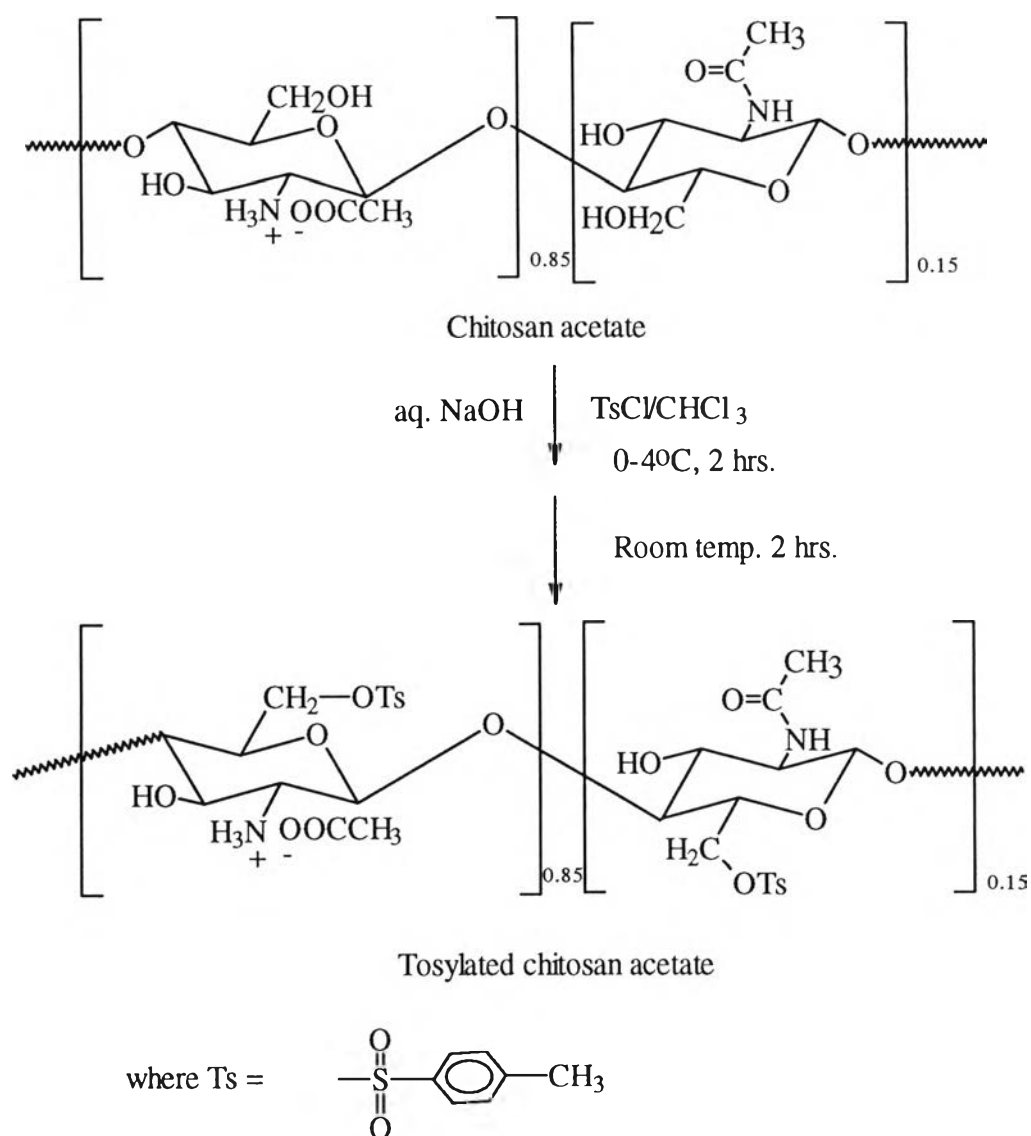
A solution of chitosan (3 g) with a degree of deacetylation of 0.85 was prepared by dissolving chitosan in 10% aqueous acetic acid. The obtained yellowish liquid was poured into acetone for reprecipitation. The precipitate was filtered off, followed by washing thoroughly with acetone, methanol, and acetone. The fine white, fibrous product was then dried *in vacuo* (2.8 g).

### 3.3.2 Tosylation of Chitosan and Chitosan Acetate

Tosylation was operated for 2 types of starting material, chitosan and chitosan acetate with the same procedure. A mixture of 0.5 g chitosan or chitosan acetate and 10 mL of 42% aqueous sodium hydroxide was left in a reduced pressure for 3 hours. 25 g of crushed ice made from deionized water was added to the mixture, following by stirring until the ice vanished. It was cooled in an ice bath, and 15 equiv mol to pyranose rings of tosylchloride dissolved in 20 mL of chloroform was added with vigorous stirring for 2 hours. Then, the ice bath was replaced by a water bath of temperature around 20°C, and the mixture was stirred for an additional 2 hours. The resulting white powder was then washed with methanol and dried in a vacuum oven.



**Figure 3.2** Schematic diagram of the tosylation of chitosan.

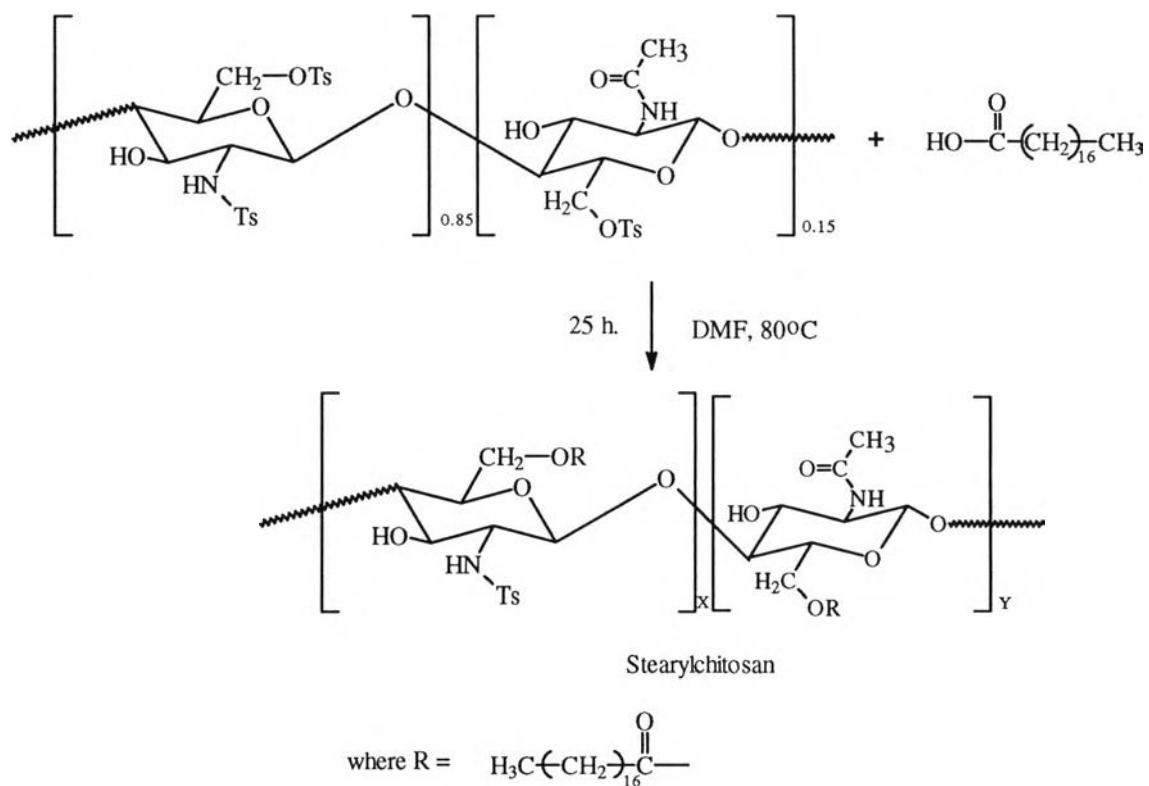


**Figure 3.3** Schematic diagram of the tosylation of chitosan acetate.

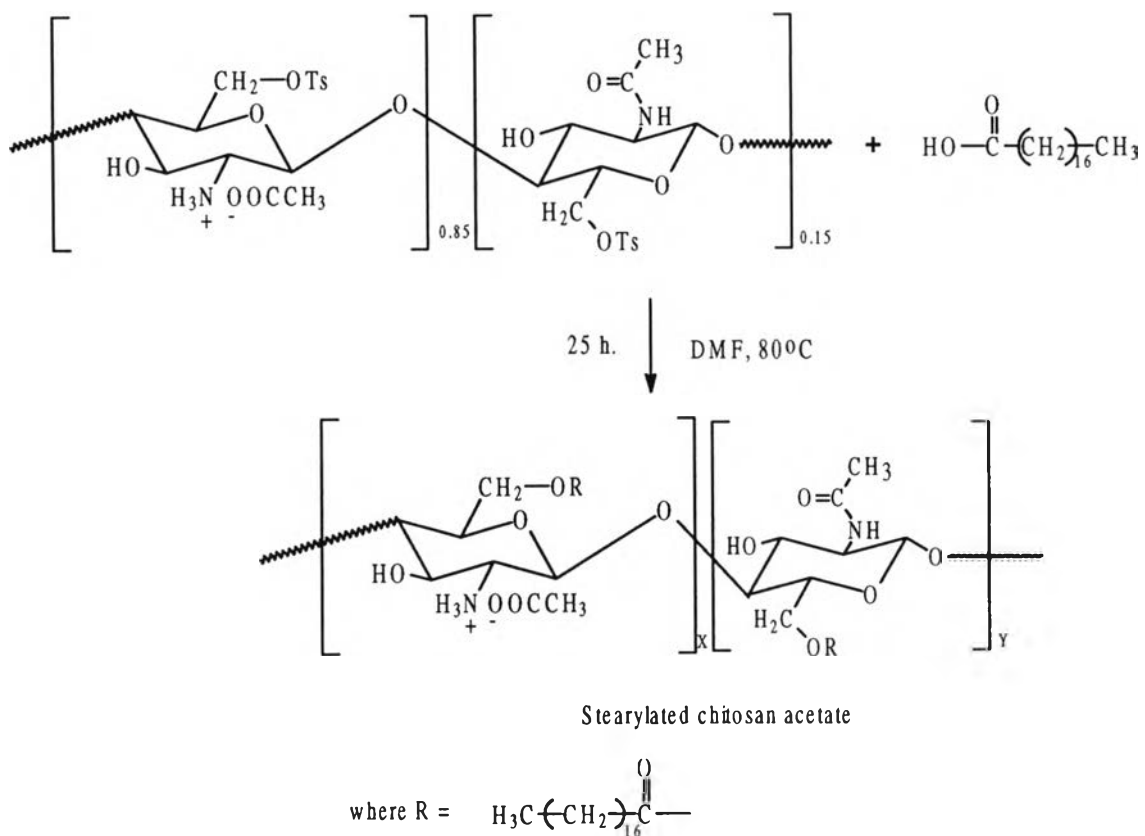
### 3.3.3 Stearylation of Tosylated Chitosan and Tosylated Chitosan Acetate

Similarly, acylation was done for both the starting material of tosylated chitosan and tosylated chitosan acetate. A dispersion of 0.5 g tosylated chitosan or tosylated chitosan acetate was stirred well in 15 mL of dimethylformamide. The solution of 10 equivalent mole stearic acid in dimethylformamide was gradually dropped to the system. The mixture was

reacted at 80 °C for 25 hours. The obtained product was filtered off, following by washing with chloroform and dried in vacuum oven to obtain a white powder product. The product was analyzed by FTIR, EA and TGA.



**Figure 3.4** Schematic diagram of the acylation of tosylated chitosan.



**Figure 3.5** Schematic diagram of the acylation of tosylated chitosan acetate.