CHAPTER III
EXPERIMENTAL SECTION

3.1 Materials

Chitosan with 75.8 % deacetylation was provided by Prof. Suwalee Chandrkrachang (The Asian Institute of Technology) and ground in Retsch S-1 Centrifugal Ball Mill at 50 rpm for 18 hours. Chloramphenicol (AR grade) and potassium dihydrogenphosphate were purchased from Fluka Chemika, Switzerland. Methylene chloride, chloroform, methanol, ethanol and hydrochloric acid were purchased from J.T. Baker, USA. Acetic acid , *N,N*-dimethylformamide and sodium hydroxide were supplied from UNIVAR, Australia. *N,N'*-carbonyl diimidazole was produced from TCI, Japan. Sodium tetraborate decahydrate or Borax was obtained from Carlo Erba, France. All these chemicals were used without further purification.

3.2 Instruments

3.2.1 Fourier transform infrared spectrophotometer (FTIR)

FTIR spectra were obtained on a FT-45A Bio-Rad Spectrometer with 16 scans at a resolution of 8 cm^{-1}. A frequency range of 4000-400 cm^{-1} was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^*, of $1 \bullet 10^9$ $cm \bullet Hz^{1/2} \bullet W^{-1}$.

3.2.2 Ultraviolet-Visible Spectrophotometer (UV-VIS)

A Lambda-16 UV-VIS spectrophotometer from Perkin-Elmer was used for qualitative and quantitative analysis. The concentration of chloramphenicol in aqueous media in the release study was measured at 278 nm.

3.2.3 Nuclear Magnetic Resonance Spectrometer (NMR)

^{1}H and ^{13}C-NMR spectra were analyzed by a DPX-300 Avance 300 MHz Digital NMR Spectrometer of Bruker, Switzerland by courtesy of the National Metal and Material Technology Center (MTEC), Bangkok, Thailand.

3.3 Methodology

3.3.1 Preparation of chitosan beads containing chloramphenicol

Chloramphenicol was chosen as a model drug and prepared to have a concentration of 2% w/v in absolute ethanol. This solution was then mixed with 5% w/v of chitosan solution in 1% v/v acetic acid aqueous solution. The alkali-ethanol solution was prepared by adding 2.5 N sodium hydroxide into 1:1 (v/v) absolute ethanol/water. The mixed solution of chloramphenicol and chitosan was gradually dropped into the prepared alkali-ethanol solution. This process led to the precipitation of polymer-rich beads with a diameter of approximately 1 mm. The beads were washed thoroughly in 80% v/v ethanol and dried at room temperature. The total amount of chloramphenicol in the beads was determined by grinding followed by extraction with distilled water. The supernatant was collected and the concentration was determined by UV-VIS spectrophotometer at the characteristic peak of chloramphenicol 278 nm.

3.3.2 Study on release of chloramphenicol from chitosan beads

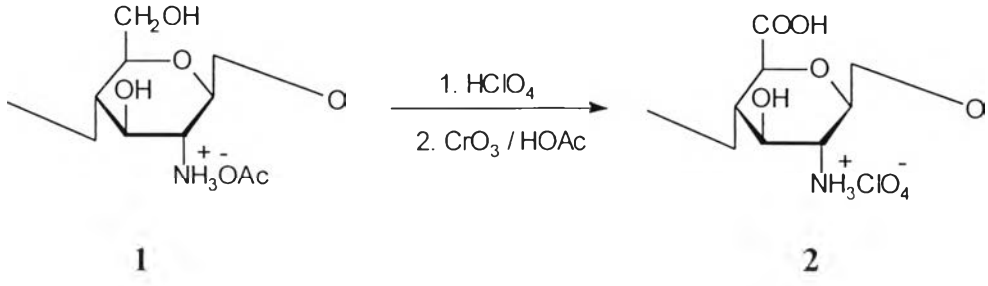
Kolthoff's buffer pH 6, 7, 9 and 0.1N HCl solution (pH 1) were used as modulated media. The beads (0.5 g) were suspended in 20 ml of medium at room temperature. The release of chloramphenicol was quantitatively analyzed by the UV-VIS spectrophotometer at 278 nm every 10 minutes until the concentration of chloramphenicol reached equilibrium (usually 3 hours). The sampling solution was collected back into the solution in order to maintain the total amount of chloramphenicol in the system. The drug release was studied as a function of time.

3.3.3 Preparation of chitosan acetate

Chitosan was dissolved in 10 % v/v aqueous acetic acid and reprecipitated in acetone. The white fine powder was achieved as chitosan acetate. The obtained product was further used in the synthesis of chitosan precursor.

3.3.4 Synthesis of chitosan precursors

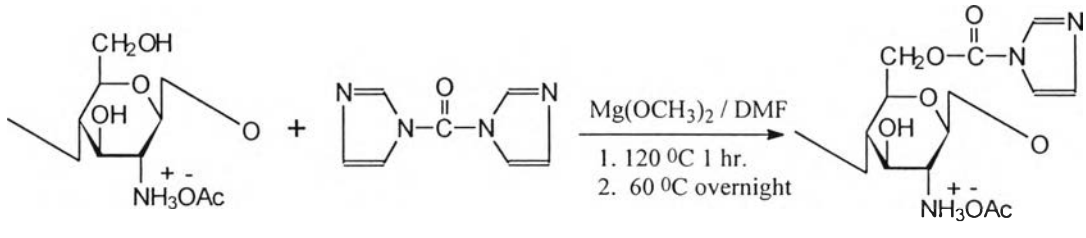
3.3.4.1 Synthesis of β-(1→4)-2-amino-2-deoxy-β-D-glucopyranuronan perchlorate. A small amount of distilled water was gradually added into the dispersed mixture of chitosan acetate from 3.3.3 in glacial acetic acid until all the chitosan acetate was dissolved. Acetic acid was then added to make the final volume approximately 100 ml. Finally, 1.2 ml of 60% aqueous perchloric acid 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.



1 2

Chromium (VI) oxide (0.3g) was dissolved in 3 ml acetic acid and 0.3 ml of water to prepare a chromium (VI) oxide solution. This solution was added to the rapidly stirred suspension. After 30 minutes, the same amount of chromium (VI) oxide solution was added repeatedly and the reaction was allowed to proceed for a further 30 minutes. Another chromium (VI) oxide solution was prepared by dissolving 0.2 g CrO_3 in 7.5 ml of water and added to the suspension. The solution was stirred continuously for 1 hour and methanol was added to decompose the excess oxidant. After stirring for 30 minutes, the mixture was allowed to settle. The precipitate was then filtered and washed with methanol until both precipitate and supernatant were colorless. The product **2** was further washed by ether and dried under vacuum at room temperature. The products were characterized by FTIR.

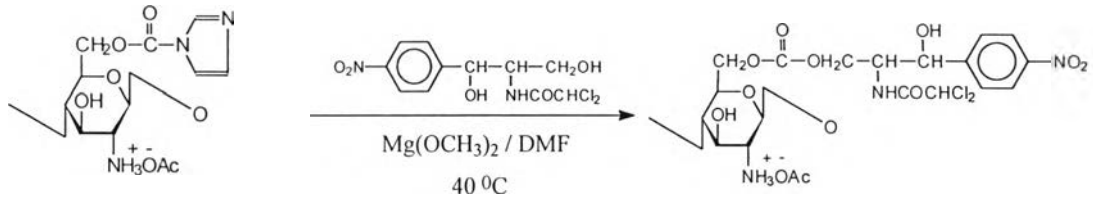
3.3.4.2 Synthesis of chitosan carbonyl imidazolide.



Chitosan acetate was dispersed in DMF under vacuum conditions and heated to 120°C. A catalytic amount of magnesium methoxide was added to this suspension. After stirring, *N,N'*-carbonyl diimidazole was added and reacted for 1 hour. Then, the temperature was reduced to 60°C and stirring was continued overnight. The functional groups of obtained product

were qualitatively confirmed by FTIR after washing thoroughly with water and chloroform.

3.3.5 Synthesis of chitosan containing chloramphenicol



The achieved product from 3.3.4.2 was reacted overnight with chloramphenicol at 40°C with a catalytic amount of magnesium methoxide. The product was filtered off , washed with chloroform and *iso*-propanol, then dried in vacuo and characterized by FTIR.

3.4 Release Studies from chitosan-chloramphenicol conjugate

0.1 g of chitosan-chloramphenicol conjugate was immersed in 100 ml of 0.1N HCl solution (pH1) at 25°C. The procedure of release studies was followed as mentioned in 3.3.2. These same procedures were done for studies at pH 7 and 9.