

CHAPTER III EXPERIMENTAL

3.1 Materials

Chitin-chitosan with a degree of deacetylation (%DD) of 70, 80, 85, 90, and 95 were supported by the Seafresh Chitosan (Lab) Company Limited, Bangkok, Thailand. Acetic acid, *N,N*-dimethylformamide, and sodium hydroxide were supplied from UNIVAR, Australia. Ethanol, acetone, and sodium borohydride were produced from BDH Laboratory Supplies, England. Triethylamine was produced from Unilab, Australia. Glutaraldehyde (5.6 M in water) and sodium hydride were purchased from Fluka Chemmika, Switzerland. Dodecylamine and stearylamine were supplied from TCI, Japan. Methanol, hexane, hydrochloric acid were purchased from J.T Baker, USA. Dichloromethane and chloroform were purchased from Lab-Scan, Thailand. Ether was supplied from Scharlau, Spain. All chemicals were used without further purification.

3.2 Instruments and Equipments

3.2.1 Fourier Transform Infrared Spectroscopy (FT-IR)

Qualitative and quantitative FT-IR spectra were obtained from VECTOR 3.0 BRUKER Spectrometer with 64 scans at a resolution of 4 cm⁻¹. A frequency range of 4000-400 cm⁻¹ was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^* , of 1×10^9 cmHz^{1/2}w⁻¹.

3.2.2 Ultraviolet-Visible Spectrophotometer (UV-Vis)

A Lamda-10 UV-Vis Spectrophotometer from Perkin-Elmer was used for qualitative and quantitative analysis. The scan rate was 240 nm/min from 190-500 nm.

3.2.3 Thermal Gravimetric Analysis (TGA)

A DuPont Thermogravimetric analyzer 2950 was used for TGA study. Samples (approximately 4-6 mg) were loaded into a platinum pan and heated under a N₂ flowing rate of 20 mL/min. The heating rate was 20°C/min from 30°C to 600°C.

3.2.4 X-Ray Diffraction (XRD)

X-ray diffraction patterns were obtained from a RIGAKU RINT 2000. CuK_α ($\lambda = 0.154$ nm) was used as X-ray source and operated at 40 kV, 30 mA with Ni filter. Sample (0.1-0.2 g) was spread on a glass slide specimen holder to examine the pattern between 2θ of 5-50°.

3.3 Procedures

3.3.1 Preparation of γ -Ray Irradiated Chitosan

A series of chitosan samples with different %DD were soaked in deionized water overnight before γ -ray irradiation was carried out. The γ -ray irradiation was done in a γ -cell (⁶⁰Co) by a courtesy of Office of Atomic Energy for Peace (OAEP), Ministry of Science and Technology, Thailand, with various amounts of dose at 20, 40, 60, and 80 kGy with a dose rate of 6.06 kGy/h.

3.3.2 Determination of γ -Ray Irradiation Effects on Chitosan Properties

3.3.2.1 *Molecular Weight*

Chitosan samples before and after irradiation with ^{60}Co γ -ray at various doses were determined for the intrinsic viscosity. The molecular weights of chitosan samples were calculated using Mark-Houwink-Sakurada Equation. The K [$1.64 \times 10^{-30} \times \text{DD}^{14.0}$] and a [$(-1.02 \times 10^{-2} \times \text{DD}) + 1.82$] values reported by Wang *et al.* (1989) were used.

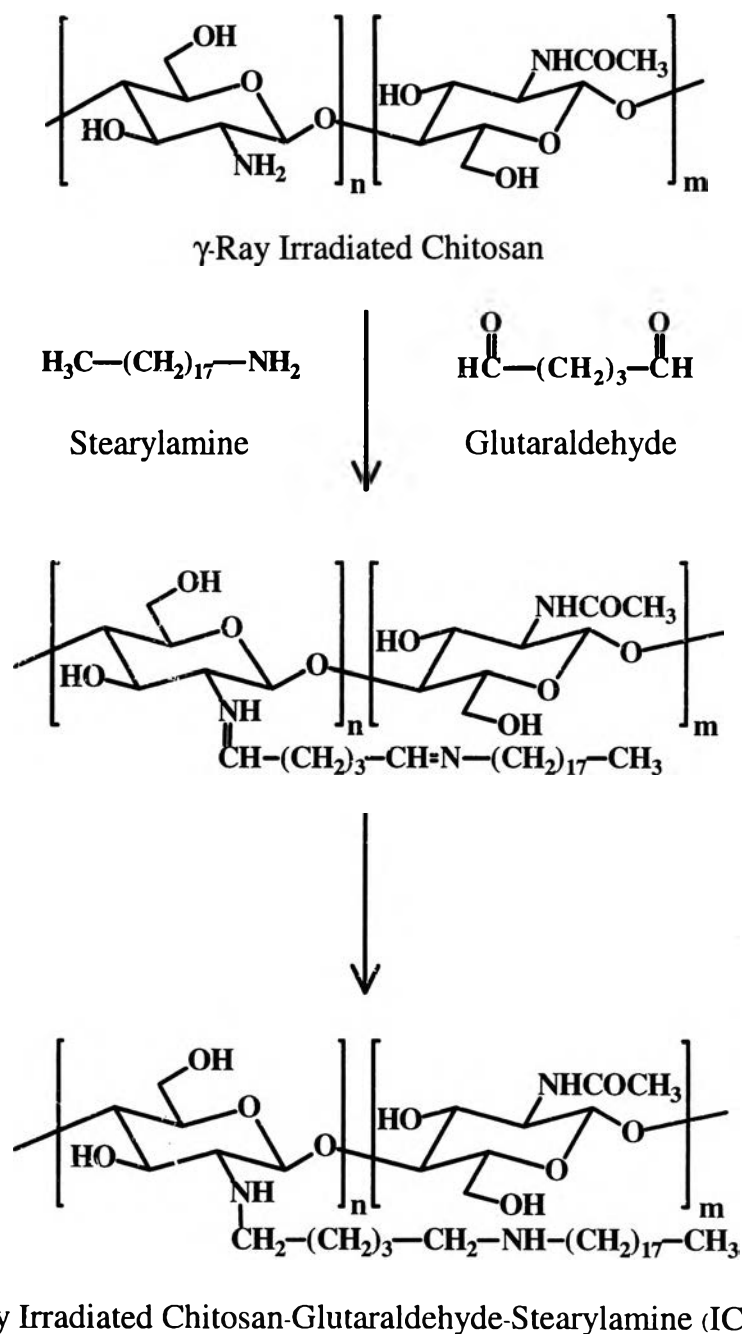
3.3.2.2 *Chemical Structure*

The γ -ray irradiated chitosan products were dissolved in 0.04 M acetic acid solution. The amounts of carbonyl and carboxyl groups in the γ -ray irradiated chitosan chain were quantitatively measured at 247 and 290 nm by UV spectrophotometer, respectively (Ulanski and Rosiak, 1992). The chemical structure of γ -ray irradiated chitosan was also observed by FT-IR technique.

3.3.3 Preparation of γ -Ray Irradiated Chitosan-Glutaraldehyde-Stearylamine (ICGS)

γ -Ray irradiated chitosan 0.5 g (3 mmol) was dissolved in 1% (v/v) acetic acid solution 100 mL. Stearylamine 0.82 g (1 mol equivalent to amino group) dissolved in methanol 20 mL was added into a γ -ray irradiated chitosan in 1% (v/v) acetic acid solution (Scheme 3.1). The mixture was stirred vigorously and heated to 80°C for 15 min. Glutaraldehyde 0.54 mL (1 mol equivalent to amino group) diluted in deionized water 10 mL was added into the mixture dropwisely. The reaction was further stirred for 3 hours and evaporated at 80°C for 3 hours. The viscous solution was reprecipitated in acetone and rinsed with hexane and methanol, respectively. The obtained

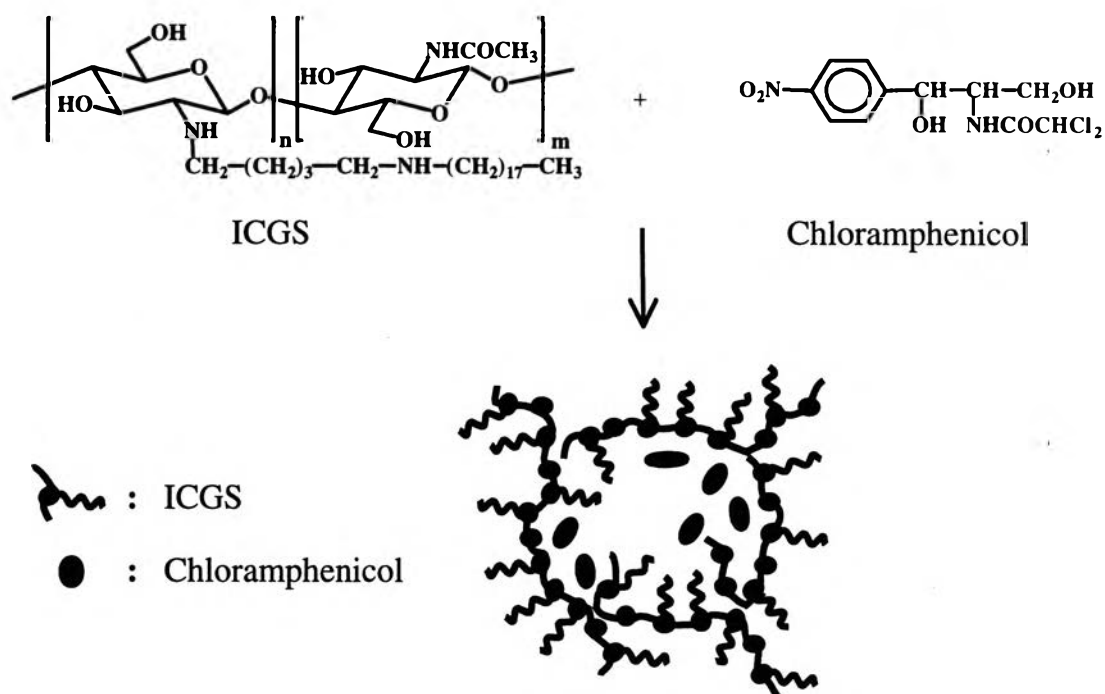
product was dried in vacuum at ambient temperature to give a dark red powder. The characterizations were done by FT-IR, TGA, XRD, and UV-Vis techniques.



Scheme 3.1 Preparation of γ -ray irradiated chitosan-glutaraldehyde-stearylamine (ICGS).

3.3.4 Aggregation Study of γ -Ray Irradiated Chitosan-Glutaraldehyde-Stearylamine (ICGS)

The γ -ray irradiated chitosan derivatives were dissolved in 0.04 M acetic acid solution. Chloramphenicol was dissolved in ethanol and adjusted the concentrations to be 0.8 of UV absorbance value. ICGS and chloramphenicol solutions were mixed together at various concentration ratios of 0:6, 1:5, 2:4, 3:3, 4:2, 5:1, and 6:0. The UV absorbances at 274 nm referred to carbonyl groups of chloramphenicol were observed.



Scheme 3.2 Schematic draw of γ -ray irradiated chitosan-glutaraldehyde-stearylamine (ICGS) aggregated with chloramphenicol.