

CHAPTER II

EXPERIMENTAL



2.1 Materials

All reagents are analytical grade and used without further purification.

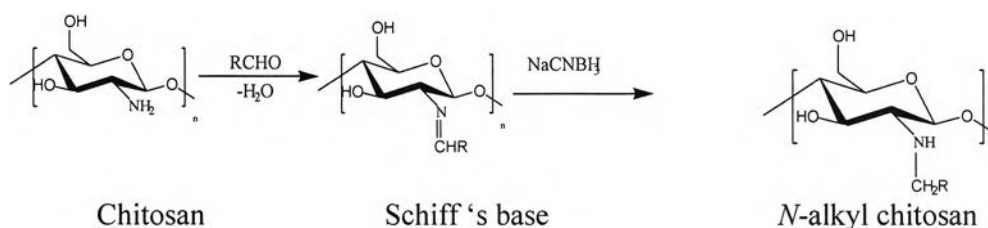
1. Chitosan, Mw = 100,000 ; 95%DD : Seafresh Chitosan (Lab) Co., Ltd.
2. N-methyl-2-pyrrolidone : Fluka
3. Sodium Chloride : Merck
4. Sodium iodide : Riedel-dettaen
5. Methyl iodide : Riedel-dettaen
6. Acetic acid : Merck
7. Ethanol : Merck
8. Sodium cyanoborohydride : Fluka
9. Hydrochloric acid : Merck
10. Anionic exchange resin : Comex 50wx 2-100, Aldrich.
11. Benzaldehyde : Merck
12. Butyraldehyde : Merck
13. Dialysis tube : Aldrich

2.2 Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra of chitosan were obtained from 1% chitosan dissolved in 1% CF_3COOH in D_2O using 400 MHz (^1H) on Varian mercury 400 spectrometer. Chemical shifts are in ppm.

2.3 Synthesis of *N*-alkyl chitosan derivatives

1 g chitosan was dissolved in 90 ml of 1% acetic acid (v/v). When dissolution was complete, 5 ml ethanol was added. This solution was stirred at room temperature for 4 h. Then the solution of aldehyde (butyraldehyde or benzaldehyde at 0.5, 1 or 2 eq/ NH_2) in ethanol was added into the chitosan solution and stirred for 1 h. The pH of this mixture was adjusted to 4 with 1.4 M NaOH. In this solution, sodium cyanoborohydride (0.0174 mmol; 3 eq/ NH_2) was added and stirred for 6 h. The precipitants of the *N*-alkyl chitosan derivatives were obtained from adjusting the pH of the solution to 10 with 1.4 M NaOH. These precipitants were washed with 30% ethanol (v/v) and increasing water content to 100%. The unreacted aldehyde and inorganic products were removed from the precipitant by refluxing with ethanol at 40°C for 4 h and changed ethanol solution every 2 h. The precipitants were washed again by DI water twice. The product was characterized by NMR spectroscopy.



Scheme 2.1 Synthesis of *N*-alkyl chitosan

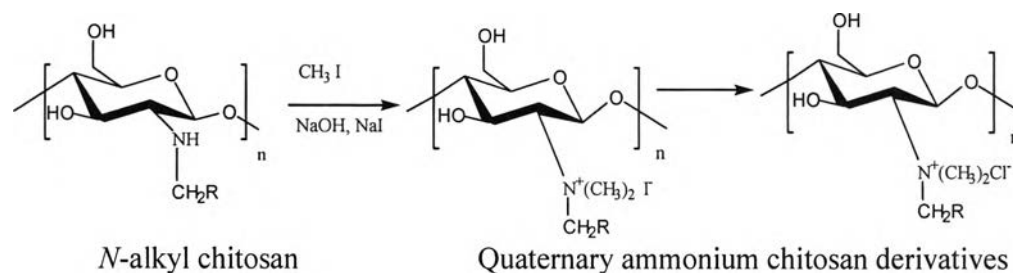
2.4 Quaternization *N*-alkyl chitosan

0.3 g *N*-alkyl chitosan were dispersed in 12 ml NMP for 12 h at room temperature. To each dispersion, 1.2 ml of 15% NaOH were added and stirred for 15

min. CH_3I and NaI (12 eq and 2.5 eq of alkyl chitosan group) were added into these mixture substances and carried out with 50°C for 24 h. The solution was collected by precipitation with acetone. These precipitates were dissolved in water for $\text{I}^- - \text{Cl}^-$ exchanged using chloride ion resin. Then the solution was further dialyzed in deionized water for 1 week. The solution was freeze-dried to remove water. White solid was obtained.

Methyl iodide was added to the reaction mixture in one, two and three steps as follows:

1. Twelve equivalents of CH_3I were added at one time at the beginning of the reaction period.
2. CH_3I was added in 2 portions; 8 equivalents at the beginning and 4 equivalents after 6 h.
3. CH_3I was added in 3 portions; 8 equivalents at the beginning, 2 equivalents after 6 h and another 2 equivalents after 12 h.



Scheme 2.2 Synthesis of quaternized *N*-alkyl chitosan

2.5 Estimation of solubility of QAC in water

Freeze-dried chitosan derivatives (1 mg) were accurately weighed in a test tube. 1 ml distilled water was added to the chitosan derivative. The test tube was stirred until a clear solution was obtained.

2.6 Determination of degree of methyl substitution

2.6.1 *N*-alkyl chitosan derivatives

The degree of *N*-alkylation at the amino group of chitosan was determined by ¹H-NMR spectroscopy. The sample was dissolved in D₂O-CF₃COOF. The degree of *N*-alkylation was calculated by using Eq. (1) for the *N*-butyl chitosan (δCH₂ of butyl group at 1.6 ppm) VS (δC-2 of chitosan at 3.2 ppm) and Eq. (2) for the *N*-benzyl chitosan. (δCH₂ at 4.3 ppm from *N*-benzyl) VS (δC-2 of chitosan at 3.2 ppm)

$$\%DS = 100 \times \frac{(\text{peak area of N-CH}_2\text{-CH}_2\text{R})}{2 \times (\text{peak area of H-2 of chitosan})} \dots\dots\dots (\text{Eq1})$$

$$\%DS = 100 \times \frac{(\text{peak area of N-CH}_2\text{-Ar})}{2 \times (\text{peak area of H-2 of chitosan})} \dots\dots\dots (\text{Eq2})$$

2.6.2 Quaternization of *N*-alkyl chitosan.

The degree of quaternization of the *N*-alkyl chitosan derivatives was determined by NMR spectroscopy. The sample was dissolved in D₂O. The degree of quaternization was calculated by using Eq. (3) for the *N*-butyl chitosan (δ N⁺-(CH₃)₂R of quaternized *N*-butyl group at 3.2 ppm and N-CH₂-CH₂-CH₂-CH₃ at 1.6 ppm) and Eq. (4) for the *N*-benzyl chitosan (δN⁺-(CH₃)₂R of quaternized *N*-benzyl group at 3.2 ppm and N-CH₂-Ar at 4.3 ppm).

$$\%DQ = 100 \times \frac{2 \times (\text{peak area of N}^+\text{-(CH}_3\text{)}_2\text{-R))}}{6 \times (\text{peak area of N-CH}_2\text{-CH}_2\text{-R)}} \dots\dots\dots (\text{Eq3})$$

$$\%DQ = 100 \times \frac{2 \times (\text{peak area of N}^+\text{-(CH}_3\text{)}_2\text{-Ar))}}{6 \times (\text{peak area of N-CH}_2\text{-Ar)}} \dots\dots\dots (\text{Eq4})$$

2.7 Fruit coating study

Banana was selected for fruit coating study of chitosan and its quaternary ammonium derivatives. Shape, color, and size of all banana samples were controlled to be uniform. For each compound four replicates were carried out. All test solution were brushed on banana peel and placed in a basket at ambient atmosphere. The methods of fruit coating are as follows:

1. Green bananas were cleaned with tap water twice and left at room temperature till dry.
2. The test solution was applied on the peel of each banana. All banana samples were left in a basket at room temperature for 1 h.
3. Weight and appearance of banana at first time point was recorded.
4. Weight and appearance of banana samples were monitored every 2 days for up to 10 days

Six experimental sets were designed to include two quaternized *N*-butyl chitosan and *N*-benzyl chitosan as follows:

- Banana without any coating.
- Banana coated with 0.5% acetic acid.
- Banana coated with 20% of chitosan in 0.5% acetic acid.
- Banana coated with 20% of quaternized *N*-butyl chitosan in 0.5% acetic acid.
- Banana coated with 20% of quaternized *N*-benzyl chitosan in 0.5% acetic acid.
- Banana coated with 20% of quaternized *N*-butyl chitosan in DI water.