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# Synthesis and antibacterial activities of quaternary ammonium salt of chitosan

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**Abstract**

Chitosan derivatives with quaternary ammonium salt, such as *N*,*N*,*N*-trimethyl chitosan, *N*–*N*-propyl-*N*,*N*-dimethyl chitosan and *N*-furfuryl-*N*,*N*-dimethyl chitosan were prepared using different 96% deacetylated chitosan of $M_v$ $2.14 \times 10^5$, $1.9 \times 10^4$, $7.8 \times 10^3$. Amino groups on chitosan react with aldehydes to from a Schiff base intermediate. Quaternized chitosan were obtained by reaction of a Schiff base with methyl iodide. The yields, degree of quaternization and water-solubility of quaternized chitosan were influenced by the molecular weight of the chitosan sample. The antibacterial activities of quaternized chitosan against *Escherichia coli* were explored by calculation of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in water, 0.25 and 0.50% acetic acid medium. Results show the antibacterial activities of quaternized chitosan against *E. coli* is related to its molecular weight. Antibacterial activities of quaternized chitosan in acetic acid medium is stronger than that in water. Their antibacterial activities is increased as the concentration of acetic acid is increased. It was also found that the antibacterial activity of quaternized chitosan against *E. coli* is stronger than that of chitosan. 

*Keywords:* Quaternary ammonium salt; Chitosan; Antibacterial activity; Synthesis

## 1. Introduction

Chitin is a natural polysaccharide usually obtained from the exoskeletons of shellfish and insects. Although chitin is naturally abundant, it has a limited application because of its poor solubility and reactivity. Deacetylation of chitin readily affords chitosan, poly-β-(1 → 4)-D-glucosamine. Chitosan is soluble in acetic acid and other organic solvents and has found a wide variety of applications in both industrial and medical fields.[1,2] Some antibacterial and anti-fungal activities have been described with chitosan and modified chitosan derivatives.[3–6] However, chitosan shows its biological activity only in acidic medium because of its poor solubility above pH 6.5. Thus, water soluble chitosan derivatives which are soluble in both acid and basic physiologic circumstances might be good candidates for a polycationic biocide.

To increase the solubility, the quaternization of chitosan was investigated. Muzzarelli and Tanfani[7] reported the formation of *N*-dimethyl chitosan and the preparation of *N*-trimethyl chitosan iodide with formaldehyde and sodium borohydride. Trimethyl chitosan ammonium iodide was also obtained by reaction of a low acetyl content chitosan with

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methyl iodide and sodium hydroxide under controlled conditions.[8] Kim et al. reported that *N*-alkyl chitosan were prepared by introducing an alkyl group into the amine groups of chitosan ($M_v$ $7.25 \times 10^5$) via Schiff's base intermediates.[9] *N*-Alkyl chitosan derivatives were reacted with methyl iodide to produce water soluble quaternary ammonium salt of chitosan. Their antibacterial activities increased with increase in the chain length of the alkyl substitute.

The effect of molecular weight on some antibacterial and anti-fungal activities has been explored: Chen noticed that chitosan with molecular weight ranging from 10,000 to 100,000 would be helpful in restraining the growth of bacteria.[10] Simojoh and Eukushima discovered that the chitosan from squid pen with molecular weight of 220,000 was most active in antibacterial activity.[11] Tokura et al. reported chitosan with an average-molecular weight of 9300 to be effective in restraining *Escherichia coli*. while that with a molecular weight of 2200 accelerated growth.[12]

In order to research the relationship between antibacterial activities of quaternary ammonium salt of chitosan and its molecular weight we have used three different molecular weight chitosan samples to prepare a series of water soluble quaternary ammonium salts of chitosan and determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of quaternary ammonium salt of chitosan against *E. coli*. The effect of the molecular weight of chitosan on the yield, water solubility and antibacterial activities against *E. coli* of quaternary ammonium salts are discussed.

## 2. Experimental

*Material.*—Chitosan(I) was purchased from Zhoushan Xinxing medical chemistry factory (China). It originated from shrimp and its degree of deacetylation was determined to be 96% by a potential titration method.[15] The viscosity average-molecular weight was determined to be $2.14 \times 10^5$ by the method described below. In order to obtain low-molecular-weight chitosan, two methods were used as follows: Chitosan(I) was hydrolyzed using 85% phosphoric acid by a modified method based on that of Hasegawa et al.[13] to afford chitosan(II). Chitosan(I) was dissolved in 1% AcOH and then ultrasonically degraded (SCQ-50, China) for 8 h at an energy levels of 150 watts at 80 °C. The degraded solutions were neutralized with 0.1 mol/L NaOH to precipitate the degraded chitosan. They were collected and washed with water until neutral, then dried to get low-molecular-weight chitosan(III).[14] The viscosity average-molecular weight and degree of deacetylation of three chitosan sample are summarized in Table 1.

Table 1
Properties of chitosan

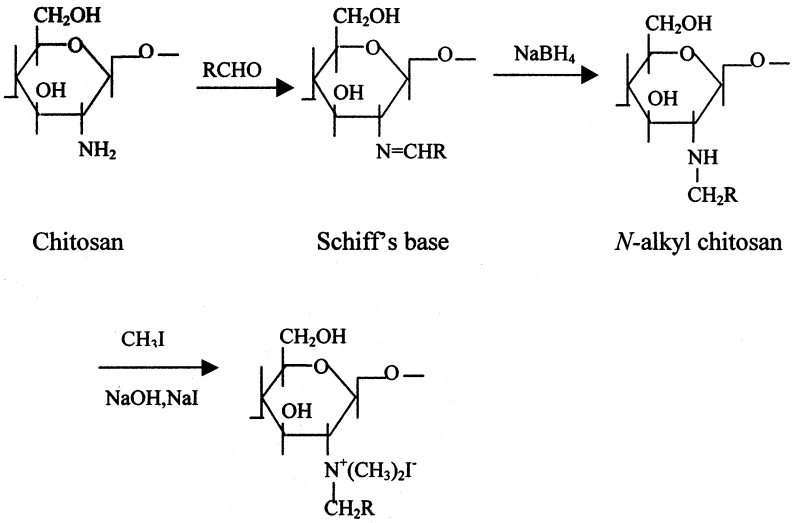
| Sample | Degree of deacetylated (%) | $M_v$ |
|---|---|---|
| Chitosan(I) | 96 | $2.14 \times 10^5$ |
| Chitosan(II) | 96 | $1.9 \times 10^4$ |
| Chitosan(III) | 96 | $7.8 \times 10^3$ |

Formaldehyde, propylaldehyde, furfurol, *N*-methyl-2-yrrolidone, ethanol, ether, acetone, $CH_3I$, NaI and $NaBH_4$ were purchased from Shanghai Chemical Co, China. *E. coli* (ATCC25925) and nutrient broth were obtained from Hangzhou Sanitation and Antiepidemic Station.

*Molecular weight determination.*—Dried chitosan (0.5 g) were accurately weighed and dissolved in 0.1 mol/L $CH_3COONa$–0.2 mol/L $CH_3COOH$ solution. Five concentrations of chitosan solution were prepared. The relative viscosity was measured with a viscosity meter at $30 \pm 0.5$ °C in a constant temperature bath (Jianshu Changzhou Science Instrument Factory). Intrinsic viscosity, defined as $[\eta] = C(\eta_{red}) \rightarrow 0$, was obtained by extrapolating the reduced viscosity versus concentration data to zero concentration, the intercept on the ordinate is the intrinsic viscosity. The viscosity molecular weight was calculated based on the Mark Houwaink equation as follows[16]:

$$[\eta] = KM^a$$

where $K = 1.64 \times 10^{-30} \times DD$[14]; $a = -1.02 \times 10^{-2} \times DD + 1.82$; DD, the degree of deacetylation of chitosan.




Scheme 1. The synthesis of quaternized *N*-alkyl chitosan.

*Estimation of water solubility.*—Dried chitosan derivatives (0.4 g) were accurately weighed and dissolved in 2 mL of distilled water. If the solution was not clear, 2 mL of distilled water was added slowly until the sample was dissolved completely. The water-solubility was expressed as percent composition of chitosan solution.

*Determination of degree of substitution.*—The degree of the quaternization of the chitosan derivatives was determined by the potentiomerty.[17] Potentiometric titration of the halide form was carried out with the aq silver nitrate, using a calomel electrode as the reference, and a silver electrode for the measurement.

*Synthesis of quaternized* N-*alkyl chitosan derivatives.*—Quaternized chitosan derivatives were prepared by a modified method based on that of Kim et al.[9] Scheme 1 shows a schematic representation of the preparation of quaternized chitosan derivatives. The different molecular chitosan solutions were prepared by dissolving 7 g of chitosan into 1% AcOH. Various aldehydes were added to the chitosan solution at rt. After 1 h of stirring, the pH of the solution was adjusted to 4.5 by adding 1 mol/L NaOH solution. To this solution, 10% $NaBH_4$ solution (1.5-fold excess to added aldehyde) was added, and the solution stirred for 1.5 h. The precipitants of *N*-alkyl chitosan derivatives were obtained by adjusting the pH of the solution to 10. These precipitants were washed with the distilled water to neutrality and the unreacted aldehyde and the inorganic products were soxhlet-extracted with EtOH and ether for 2 days. Chitosan derivatives (5 g) were dispersed in 250 mL of NMP for 12 h at rt. To each dispersion, 1 mol/L NaOH and $CH_3I$ (fivefold excess to amine of chitosan) were added. NaI was added to adjust the concentration in the reaction medium to 0.2 mol/L. Each reaction was carried out with stirring for 12 h at 50 °C. The solution was collected by precipitation with acetone, which was dried to obtain the quaternized *N*-alkyl chitosan derivatives.

*Infrared spectra.*—Fourier transform infrared (FT-IR) spectra were obtain with a Shimadzu 470 Infrared spectrometer.

*Determination of MIC.*—The MIC were determined by a method based on that of Li et al.[18]

*Determination of MBC.*—The MBC were determined by a method based on that of Li et al.[18]

## 3. Result and discussion

In order to obtain quaternized chitosan derivatives in good yields several conditions

Table 2
Yield and degree of substitution of quaternized chitosan prepared from various chitosan

| Quaternized chitosan | Yield (%) [a] | | | Degree of quaternization | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| *N*,*N*,*N*-Trimethyl chitosan | 75.8 | 61.5 | 79.6 | 90.5 | 89.2 | 90.7 |
| *N*-*N*-Propyl-*N*,*N*-dimethyl chitosan | 84.5 | 86.7 | 51.6 | 91.8 | 89.2 | 90.7 |
| *N*-Furfuryl-*N*,*N*-dimethyl chitosan | 38.4 | 36.1 | 28.9 | 84.1 | 81.6 | 89.8 |

[a] Yield is the ratio of quaternized chitosan and added chitosan.
I. Quaternized chitosan prepared by chitosan with $M_v$ $2.14\times10^5$. II. Quaternized chitosan prepared by chitosan with $M_v$ $1.90\times10^4$. III. Quaternized chitosan prepared by chitosan with $M_v$ $7.80\times10^3$.

were tried. It was found that the rate of the reaction between chitosan and aldehyde depended on the concentration of aldehyde and $NaBH_4$, reaction time and size of chitosan. Under optimal conditions, nine quaternized chitosans were obtained by the reaction between three different molecular weight chitosans and formaldehyde, propylaldehyde, furfurol. Table 2 lists the yields of quaternized chiotsan and the degree of quaternization. The yields varied with different aldehydes. Sodium hydroxide was added to avoid the protonation of the $-NHCH_2R$ groups and fix the iodohydric acid liberated. In the absence of base, a low degree of quaternization was achieved despite the use of a large excess of $CH_3I$.

Fig. 1 shows the FT-IR spectra of chitosan and chitosan derivatives. There are two characteristic peaks of chitosan at 3455 and 1093 $cm^{-1}$.[12] Characteristic peaks of amine NH vibration deformation appeared at 1595 $cm^{-1}$ for chitosan. Note that this peak disappears in chitosan derivatives, caused by formation of quaternary ammonium salt at C-2 in the chitosan[17] and a new peak appears at a high wave number. Characteristic peaks of alcohol and second alcohol between 1160 and 1030 $cm^{-1}$ were not changed confirming the lack of the introduction of an alkyl group at C-3 and C-6 in the chitosan. *N*-Furfuryl-*N*,*N*-dimethyl chitosan absorbs at about 3060 $cm^{-1}$ demonstrating a furfuran group.

Table 3 shows the water solubility of quaternized chitosan. *N*-Furfuryl-*N*,*N*-dimethyl chitosan with $M_v$ $7.80\times10^3$ shows the highest water-solubility. Experiment results indicated that the water-solubility of chitosan was improved by quaternization and lowering the molecular weight.

After quaternization, the chitosan became a water-soluble polyelectrolyte with a high charge density. Cationic antibacterial agents have been widely used. The target site of the cation is the negatively charged cell surface of bacteria.[19] Polycationic biocides including chitosan can interact and form polyelectrolyte complexes with acidic polymers produced at the bacterial cell surface.[20] Some antibacterial activities have been described with quaternized chitosan.[9,21–23] Table 4 shows the MIC and MBC of quaternized chitosan against *E. coli* in water medium. The MIC and MBC of *N*–*N*-propyl-*N*,*N*-dimethyl chitosan have the lowest antibacterial activity. The MIC and MBC of quaternized chitosans with different molecular weights are different. The antibacterial activity against *E. coli* of quaternized chitosan with $M_v$ $2.14\times10^5$ is higher than that of

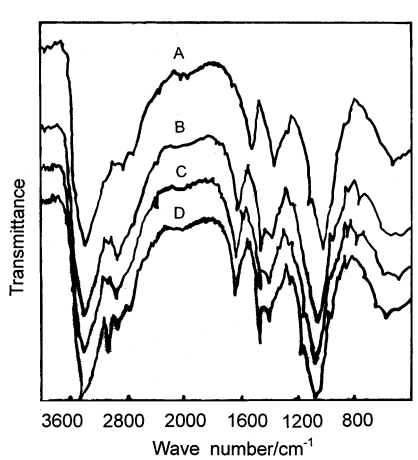



Fig. 1. FT-IR spectra of chitosan and quaternized chitosan. A, chitosan; B, *N*,*N*,*N*-trimethyl chitosan; C, *N*–*N*-propyl-*N*,*N*-dimethyl chitosan; D, *N*-furfuryl-*N*,*N*-dimethyl chitosan.

Table 3
Water solubility of quaternized chitosan

| Quaternized chitosan | Solubility (%) | | | Dissolution time | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| *N*,*N*,*N*-Trimethyl chitosan | 10 | 10 | 10 | 2 h | 1 h | 30 min |
| *N*-*N*-Propyl-*N*,*N*-dimethyl chitosan | 5 | 10 | 10 | 3 h | 1 h | 20 min |
| *N*-Furfuryl-*N*,*N*-dimethyl chitosan | 10 | 15 | 15 | 15 min | 20 min | 10 min |

I. Quaternized chitosan prepared by chitosan with $M_v$ $2.14\times10^5$. II. Quaternized chitosan prepared by chitosan with $M_v$ $1.90\times10^4$. III. Quaternized chitosan prepared by chitosan with $M_v$ $7.80\times10^3$.

Table 4
MIC and MBC of quaternized chitosan against *E. coli* in water medium

| Quaternized chitosan | MIC (μg/mL) | | | MBC (μg/mL) | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| *N*,*N*,*N*-Trimethyl chitosan | 1 | 1 | 2 | 2 | 2 | 4 |
| *N*-*N*-Propyl-*N*,*N*-dimethyl chitosan | 0.5 | 1 | 1 | 1 | 2 | 2 |
| *N*-Furfuryl-*N*,*N*-dimethyl chitosan | 1 | 1 | 1 | 2 | 2 | 2 |

I. Quaternized chitosan prepared by chitosan with $M_v$ $2.14\times10^5$. II. Quaternized chitosan prepared by chitosan with $M_v$ $1.90\times10^4$. III. Quaternized chitosan prepared by chitosan with $M_v$ $7.80\times10^3$.

Table 5
MIC and MBC of quaternized chitosan and chitosan against *E. coli* in 0.25% acetic acid

| Quaternized chitosan | MIC (μg/mL) | | | MBC (μg/mL) | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| *N*,*N*,*N*-Trimethyl chitosan | 0.5 | 0.5 | 0.5 | 1 | 1 | 1 |
| *N*-*N*-Propyl-*N*,*N*-dimethyl chitosan | 0.25 | 0.25 | 0.5 | 1 | 1 | 1 |
| *N*-Furfuryl-*N*,*N*-dimethyl chitosan | 0.25 | 0.25 | 0.25 | 1 | 1 | 1 |
| Chitosan | | 2.50 | 2.50 | | | |

I. Quaternized chitosan prepared by chitosan with $M_v$ $2.14\times10^5$. II. Quaternized chitosan prepared by chitosan with $M_v$ $1.90\times10^4$. III. Quaternized chitosan prepared by chitosan with $M_v$ $7.80\times10^3$.

others. The antibacterial activity against *E. coli* of *N*–*N*-propyl-*N*,*N*-dimethyl chitosan is higher than that of *N*,*N*,*N*-trimethyl chitosan demonstrating that the alkyl chain length strongly affects the antibacterial activity of the chitosan derivatives in good agreement with the previous studies by Kim et al.[9]

Tables 5 and 6 show the MIC and MBC of quaternized chitosan and chitosan against *E. coli* in 0.25 and 0.50% acetic acid, respectively. One can see that the antibacterial activity of quaternized chitosan is higher than that of chitosan. The antibacterial activity of *N*–*N*-propyl-*N*,*N*-dimethyl chitosan against *E. coli* is 20 times that of chitosan indicating that the chitosan derivatives with the cationic charge of ammonium salt exhibit particularly high activity.

The antibacterial activity of chitosan against *E. coli* was affected by pH.[24] This result can be explained in terms of the protonation of $-NH_2$. When the pH is low, the $-NH_2$ groups at $C_2$ on the glucosamine residues will be protonated, which favors interaction with negative residues at bacterial cell surface. Acidic conditions can also in-

Table 6
MIC and MBC of quaternized chitosan and chitosan against *E. coli* in 0.50% acetic acid

| Quaternized chitosan | MIC (μg/mL) | | | MBC (μg/mL) | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| *N*,*N*,*N*-Trimethyl chitosan | 0.25 | 0.25 | 0.5 | 0.5 | 0.5 | 1 |
| *N*-*N*-Propyl-*N*,*N*-dimethyl chitosan | 0.125 | 0.25 | 0.25 | 0.5 | 0.5 | 0.5 |
| *N*-Furfuryl-*N*,*N*-dimethyl chitosan | 0.25 | 0.25 | 0.25 | 0.5 | 0.5 | 1 |
| Chitosan | 2.50 | 2.50 | 2.50 | 5 | 5 | 5 |

I. Quaternized chitosan prepared by chitosan with $M_v$ $2.14\times10^5$. II. Quaternized chitosan prepared by chitosan with $M_v$ $1.90\times10^4$. III. Quaternized chitosan prepared by chitosan with $M_v$ $7.80\times10^3$.

crease the antibacterial activity of quaternized chitosan against *E. coli*. The MIC of *N*–*N*-propyl-*N*,*N*-dimethyl chitosan decreased to 0.25 and 0.125 μg/mL in 0.25 and 0.50% acetic acid solution, respectively. The MIC of *N*,*N*,*N*-trimethyl chitosan decreased to 0.50 and 0.25 g/mL in 0.25 and 0.50% acetic acid solution, respectively. The antibacterial activity of quaternized chitosan increased when the concentration of acetic acid was increased indicating that the acidic condition can enhance formation of the iodhydric acid. It should be mentioned that acetic acid also has antibacterial activity suggesting a cooperative effect of acetic acid in the antibacterial activity of quaternized chitosan.

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