

Synthesis of Chitosan Quaternary Ammonium Salts

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Abstract: A series of N-alkyl or N-aryl chitosan quaternary ammonium salts were prepared *via* Schiff's base intermediates. Quaternization of N-substituted chitosan derivatives was carried out using methyl iodide to produce water-soluble cationic chitosan quaternary ammonium salt. The products were characterized by IR, ¹HNMR and elemental analysis. The degree of substitution of chitosan quaternary ammonium salt was calculated by elemental analysis.

Keywords: Synthesis, quaternization, chitosan quaternary ammonium salts.

Chitosan has a wide scale of applications¹⁻³. Interestingly, some antibacterial and antifungal activities have been described with chitosan and modified chitosan derivatives⁴⁻⁶. In polycationic biocides, generally, it is reasonably assumed⁷ that the charge density of the polyelectrolyte increases with increase in the molecular weight of its single coil, which leads to the enhanced adsorption of polycations onto the negatively charged cell surface. This is also favorable for the binding of the polycation to the cytoplasmic membrane of the bacterial cells. Therefore, polycationic biocides including chitosan quaternary ammonium salt might be a good candidate as the polycation biocide. They possess higher antibacterial activity and lower toxicity toward mammalian cells⁷.

In previous articles^{8,9}, it was demonstrated that chitosan from a deacetylation product-chitin and some chitosan derivatives quaternary ammonium salt have stronger antibacterial activity than chitin itself, whose activity was strongly dependent upon the molecular weight and the cationic charge of the substituent.

Literature reported the synthesis of the quaternized chitosan derivatives with a long alkyl chain (only 4 compounds)⁸. However, the obtained products had poor water solubility and a low degree of substitution. Especially the aryl by-products were removed difficult from these products. In this article, we reported a method of synthesis of the water-soluble chitosan quaternary ammonium salts. They were proved to have antibacterial activity against *S.aureus* and *E.coli*.

Materials and Methods

IR spectra were taken with a Nicolet-470 FT-IR spectrometer. ¹HNMR spectra (in DMSO) were recorded on an Avance DMX 500. Elemental analysis was performed with the CE instrument Eager 200. Chitosan was purchased from Ausin's chitin Co. (China).

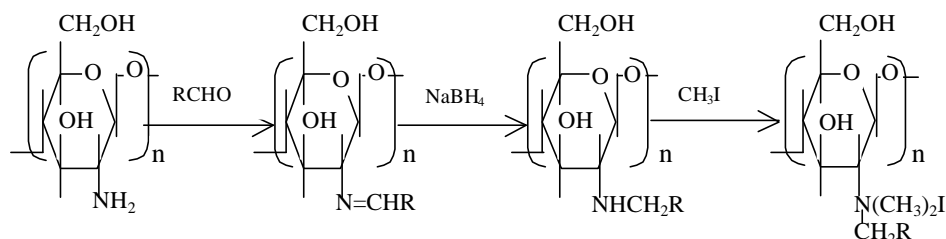
The degree of deacetylation of chitosan is 92.4%. The viscosity measurements were performed using a Ubbelohae capillary viscometer at 25°C. The solvent mixture was 0.25 mol/L acetic acid, 0.2 mol/L sodium acetate and the average viscometric molecular weight (M_v) of the chitosan was calculated from the viscosity law^{10,11} $[\eta] = KM^a$. $M_v = 2.1 \times 10^5$. The degree of substitution of chitosan quaternary ammonium salt was calculated from the C/N value of elemental analysis on the basis of the formula:

$$C_1/N_1 (1-X) + C_2/N_2 X = C_3/N_3 \quad 92.4\%$$

C_1/N_1 is a value calculated from the formula of the nonsubstituted chitosan and C_2/N_2 from the quaternized chitosan. C_3/N_3 is a found value of sample by elemental analysis. X is a substitution degree of quaternization. 92.4% is the deacetylation degree of chitosan.

The chitosan solution was prepared by dissolving 7 g (corresponding to 42 mmole of amine) of chitosan into 1% aq. acetic acid (500 mL). 420 mmol of the various aq. aldehyde solutions were added to the chitosan solution at 35°C. After 30 min of stirring, the pH of the solution was adjusted to 4.5 with 1 mol/L NaOH. To this solution, 15% aq. solution of NaBH_4 was added. The precipitate of the N-alkyl chitosan derivatives were obtained by adjusting the pH of the solution to 8-9. These precipitate were washed with distilled water to neutrality, and then ethanol and diethyl ether. The obtained derivatives were dried for 2 days under vacuum at 50°C. Each 5 g of N-alkyl (or N-aryl) chitosan derivatives were dispersed in 250 mL N-methyl-2-pyrrolidone (NMP) to swell over night. To each dispersion, 1.4 mol/L NaOH, 0.1 mol CH_3I , 0.5 g NaI were added. The reaction was carried out with stirring over night at 50°C. The product was collected by precipitation with acetone, which was infused into acetone (20 fold excess) for 3 times to remove the organic products, and then the obtained products dialyzed through dialyzing bag with water for 12 hr. Then the precipitate was dried to obtain the quaternized N-alkyl (N-aryl) chitosan derivatives.

Scheme 1



Results and Discussion

Reaction of chitosan with aldehydes resulted in nucleophilic addition. The most favorable nucleophilic center of chitosan was the amino group. The resulting N-alkyl (or N-aryl) chitosan derivatives (**Scheme 1**) were quaternized using methyl iodide. In the

reaction conditions alkyl groups were easier introduced into the amine of chitosan *via* Schiff base intermediates than aryl groups (See yield in **Table 1**). **Table 1** showed the data of IR spectra of chitosan and its N-alkyl (or N-aryl) quaternary ammonium salts. There are some characteristic peaks of derivatives in IR spectrum. The intensities of the peaks in range of 2889~2970 cm^{-1} and 1460 cm^{-1} were increased due to the presence of methyl or methylene groups. Formation of the N-aryl derivatives was also confirmed by the presence of characteristic peaks of phenyl or furfuryl group. Specially the peak around 1593 cm^{-1} (N-H) disappeared, it was confirmed that the N-alkyl (or N-aryl) chitosan derivatives were formed.

The examination of ^1H NMR spectra provided the evidence for the structure of the obtained derivatives. The assignments for proton signal in **Table 1** were similar with the data of Domard¹² and Eugene¹³. The data of IR and ^1H NMR spectra of the quaternized chitosan were approximately the same as glucosamine.

Table 1 Data of IR and ^1H NMR for the chitosan quaternary ammonium salts

Entry	N-Substituents	IR (KBr, cm^{-1}) ^a	^1H NMR (δ ppm) ^b	Yield (%) ^c
1	trimethyl	2937, 1460	2.9 (s, 1H, C ₂ -H), 3.4 (s, 9H, N(CH ₃) ₃), 3.6-3.8 (broad signal, 3H, C _{3,5} -H), 3.9 (broad signal, 2H, C ₆ -H), 4.2 (s, 1H, C ₁ -H)	80.8
2	ethyl	2970, 1468	0.9 (s, 3H, CH ₃), 3.0 (s, 2H, CH ₂)	82.5
3	propyl	2970, 1467	0.9 (s, 3H, CH ₃), 1.7 (s, 2H, CH ₂), 3.0 (s, 2H, CH ₂)	85.3
4	benzyl	2926, 1464, 1433, 747	3.3 (s, 2H, ph-CH ₂), 7.1-7.5 (m, 5H, ph)	97.2
5	2-hydroxy-benzyl	2925, 1457, 792	3.3 (s, 2H, ph-CH ₂), 7.1-7.5 (m, 4H, ph), 9.1 (s, 1H, ph-OH)	60.5
6	2-phenyl-ethyl	2918, 1465, 1360, 930, 747, 689	3.0 (m, 2H, CH ₂), 3.3 (t, 2H, CH ₂), 6.9-7.5 (m, 5H, ph)	63.7
7	furfuryl	2889, 051, 1464, 1238, 947, 841, 758	3.2 (s, 2H, CH ₂), 5.1-5.7 (m, 3H, furfuryl-H)	43.6
8	chitosan	3429, 2874, 1645, 1593, 1422, 1375, 1090		
9	glucosamine	3291, 2928, 1616, 1583, 1415, 1387, 1036	2.7 (s, 1H, C ₂ -H), 3.5 (s, 1H, C ₃ -H), 3.3-4.1 (broad signal 4H, C _{4,6} -H), 4.5 (s, 1H, C ₁ -H) ^d	

a. Characteristic peaks of marked differences were given. b. Characteristic chemical shifts of quaternized derivatives were given, not including the chemical shift of the hydrogen of chitosan. in entry 2-7. c. Calculated on the basis of the added chitosan weight. d. In D₂O.

The degree of substitution (DS) was calculated from the C/N value of elemental analysis. As summarized in **Table 2**, the DS values of alkyl aldehydes were higher than that of aryl aldehydes. It was thought that the decrease of the DS was attributed to the steric effect in the course of the reaction of chitosan with aryl aldehydes. The macromolecular backbone chain of chitosan obstructs aryl aldehydes to attack on chitosan from the amino group. It was also supported the yields of N-aryl quaternary ammonium salt were lower than that of N-alkyl.

Table 2 Degree of substitution of the chitosan quaternary ammonium salts from elemental analysis^a

Entry	N-Substituents	Elemental analysis found			
		C ₃ (%)	N ₃ (%)	C ₃ /N ₃ ratio	DS
1	trimethyl	30.73	3.76	8.17	0.93
2	ethyl	31.62	3.62	8.75	0.70
3	propyl	33.02	3.34	9.89	0.93
4	benzyl	32.95	3.78	8.72	0.38
5	2-hydroxybenzyl	35.91	2.96	12.13	0.79
6	2-phenylethyl	30.25	3.39	8.92	0.36
7	furfuryl	30.64	3.16	9.70	0.64

a. C₃ and N₃ are the found value of sample by elemental analysis.

All of synthesized chitosan quaternary ammonium salts had antibacterial activity against *S.aureus* and *E.coli*. The quaternized chitosan with N-aryl group showed the antibacterial capability higher than those with N-alkyl group⁹.

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