Preparation and Characterization of N-alkylated Chitosan Derivatives

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ABSTRACT: N-alkylated chitosan derivatives were synthesized in water containing acetic acid by the Michael addition reaction of chitosan with various acryl reagents. The chemical structures of the chitosan derivatives were characterized with Fourier transform infrared and ^{1}H-NMR techniques. The ^{1}H-NMR results indicated that the degree of substitution of the chitosan derivatives ranged from 0.13 to 0.39. The chitosan derivatives exhibited different solubilities in distilled water. X-ray diffraction analysis showed that the chitosan derivatives were amorphous. The chitosan derivatives still had antimicrobial ability. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 1093–1098, 2008

Key words: biological applications of polymers; biomaterials; chitosan

INTRODUCTION

Chitosan is an abundant natural polysaccharide that is a deacetylated derivative of chitin and a linear copolymer of β-(1,4)-2-amino-2-deoxy-D-glucopyranose (GlcN), including some *N*-acetyl-D-glucosamine residues. The biocompatibility,[1,2] biodegradation,[3] and nontoxic properties of chitosan have resulted in a number of applications in fields such as drug delivery systems,[4,5] wound healing,[6,7] and tissue engineering.[8] Although chitosan is an attractive biopolymer whose abundance is second only to that of cellulose in nature, it is not soluble in aqueous solutions above pH 7; however, it is soluble in acidic water solutions because of its rigid crystalline structure and deacetylation, and this limits its applications. The presence of chitosan amino groups is interesting, and many efforts have been made to improve its solubility in water. Various kinds of modifications have been made to chitosan in recent years, including acylation,[9,10] akylation,[11,12] carboxymethylation,[13,14] and quaternarization.[15] The research has focused on the solubility of chitosan derivatives,[9] and these methods[10,12,16] are complex and luxurious. The Michael addition reaction is a well-known process for adding various amines to unsaturated carbonyl compounds, and many chitosan derivatives have been prepared successfully with this method. In particular, many efforts to prepare functional derivatives by Michael addition reaction chemical modifications to increase solubility in water have been reported,[17–19] and this can occur under moderate conditions. However, these studies have not reported the antimicrobial ability of the chitosan derivatives; moreover, the modification of chitosan with *N*,*N*-dimethylacrylamide (DMMA), *N*-isopropylacrylamide (NIPAM), and *N*-methyloacylamide (MAA) has not been reported.

The objective of this work was to synthesize N-alkylated chitosan derivatives by the Michael addition reaction of chitosan with various acryl reagents such as DMMA, NIPAM, acrylic acid (AA), MAA, and hydroxyethyl acrylate (HEA) under moderate reaction conditions, these productions were defined as DMMA-CS, NIPAM-CS, AA-CS, MAA-CS, and HEA-CS, and their structure and properties were characterized in detail. Antimicrobial tests of MAA–CS, DMMA–CS, NIPAM–CS, AA–CS, and HEA–CS were carried out as well.

EXPERIMENTAL

Materials

Chitosan from crab shells was purchased from Yuhuan Ocean Biochemical Co., Ltd. (Zhejiang, China), and used as received. Its molecular weight was 200 kDa, and its degree of deacetylation was about 88.0%. MAA was purchased from Tokyo Kogyo Co, Ltd. (Tokyo, Japan); DMMA was pro-

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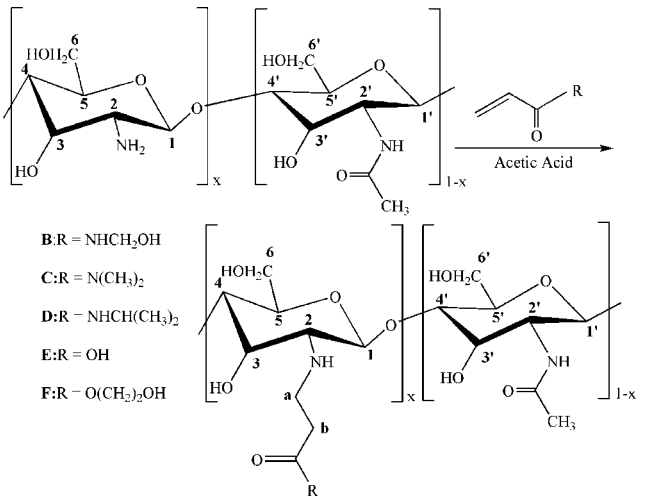

Scheme 1 Michael reaction of chitosan with MAA, DMMA, NIPAM, AA, and HEA.

vided by Johnson Matthey Co. (USA); and NIPAM was supplied by Shanghai Wujing Chemical Co., Ltd. (Shanghai, China). All other reagents and solvents were purchased from Beijing Chemical Reagent Co. (Beijing, China) and used without further purification.

Synthesis of the chitosan derivatives

Chitosan (2.0 g, NH_2 = 0.010 mol) was added to a 1 wt % acetic acid aqueous solution (w/w), and an acryl reagent (4 equiv/NH_2) was added to the solution, which was stirred (Scheme 1). This mixture was reacted at 50°C for 48 h and then filtered. The solution was poured into acetone to remove the unreacted reactant. The precipitate was washed three times with acetone and dried *in vacuo* at 30°C overnight to obtain the chitosan derivatives.

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectrum was recorded on a Nicolet 5700 instrument (Nicolet Instrument, Thermo Co., Madison, WI). Samples were prepared on KBr pellets and scanned against a blank KBr pellet background in the wave-number range of 4000–600 cm^{-1} with a resolution of 4.0 cm^{-1}.

^{1}H-NMR spectroscopy

^{1}H-NMR spectra were taken with a Bruker (Rneinstetten, Germany) AV 600-MHz instrument. Chitosan and the chitosan derivatives were dissolved in a mixed solvent of D_3CCOOD and D_2O. The degree of substitution (DS) was calculated from the peak area at the $—CH_{2b}—$ proton against that of the NHAc proton.

Solubility test

The solubility of the chitosan derivatives was evaluated in distilled water at the concentration of 5 mg/mL under 40°C for 6 h.

X-ray diffraction (XRD)

XRD patterns of the sheet samples were recorded on a X-ray diffractometer (D/Max 2500VB2+/Pc, Rigaku, Tokyo, Japan) with an area detector operating at a voltage of 40 kV and a current of 50 mA with Cu Kα radiation (wavelength = 0.154 nm). The scanning rate was 1°/min, and the scanning range of 2θ was 5–50° at room temperature.

Antimicrobial test

The antibacterial activity of chitosan and chitosan derivatives against *Escherichia coli* was evaluated as follows. *E. coli* (ATCC 25922) was obtained from the American Type Culture Collection (Manassas, VA). First, a nutrient liquid medium was prepared that consisted of 2.00 g of peptone, 1.80 g of broth, and 100.00 g of distilled water. A representative bacterial colony was picked off, inoculated into it, and incubated in an air bath shaker (37°C, 130 rpm) for 12 h. Then, the obtained fresh microbe suspension in which the bacterial cells grew luxuriantly was ready for the antibacterial test. Lastly, 1.00 g of chitosan derivatives was dissolved in a 100-mL acetic acid solution (0.5 wt %). A 0.25-mL solution of the microbe suspension and a 0.25-mL solution of the chitosan derivatives were orderly added to Petri dishes, and both were mixed and spread in the shaker uniformly. All the Petri dishes were incubated at 37°C for 24 h and taken out for photographs.

RESULTS AND DISCUSSION

FTIR analysis

The FTIR spectra of chitosan and chitosan derivatives are shown in Figure 1. A broad band around 3443 cm^{-1} was attributed to —NH and —OH stretching vibrations as well as intermolecular and extramolecular hydrogen bonding of chitosan molecules. A weak band at 2926 cm^{-1} was from —CH stretching. The characteristic peaks at 1654, 1597, and 1323 cm^{-1} were assigned to the amide I, amine $—NH_2$, and amide III absorption bands of chitosan, respectively. The absorption band at 1153 cm^{-1} was assigned to the asymmetric stretching of the C—O—C bridge. The peaks at 1075 and 1033 cm^{-1} were assigned to the skeletal vibrations of C—O stretching. Meanwhile, the peak at 1597 cm^{-1} for primary amine $—NH_2$ bending and the peak around 3443 cm^{-1} decreased in the chitosan derivatives because of the

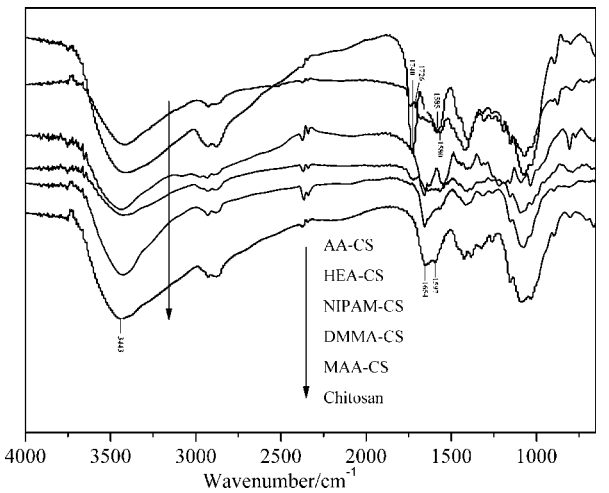

Figure 1 FTIR spectra of chitosan and chitosan derivatives.

Michael addition reaction of MAA, DMMA, NIPAM, AA, and HEA with chitosan. In particular, HEA–CS showed new absorption peaks at 1726, 1580, and 1405 cm^{-1} due to the asymmetrical and symmetrical stretching of the —COO— group, and the strong absorption peaks at 1740 and 1585 cm^{-1} were assigned to the COOH group in AA–CS.

^{1}H-NMR analysis

The ^{1}H-NMR spectra of chitosan, DMMA–CS, MAA–CS, NIPAM–CS, AA–CS, and HEA–CS in D_3CCOOD/D_2O are shown in Figure 2. Two small peaks at about 2.00 and 4.87 ppm existed because of the presence of $—CH_3$ of the N-alkylated GlcN residue and H_1 of GlcN and N-alkylated GlcN. A singlet at 3.10 ppm was assigned to H_2 of GlcN and N-alkylated GlcN, and the multiplets from 3.5 to 3.8 ppm were attributed to H_3, H_4, H_5, and H_6 of GlcN and N-alkylated GlcN [Fig. 2(A)]. Two new small peaks at 2.18 and 2.23 ppm were attributed to $—CH_{2c}OH$ and $—CH_{2b}$ because of the MAA reaction with chitosan, and the multiplets from 3.50 to 3.88 ppm were attributed to $N—CH_{2a}—$ of the group, H_3, H_4, H_5, and H_6 of GlcN and N-alkylated GlcN [Fig. 2(B)]. Two new small peaks at 2.20 and 2.95 ppm were ascribed to $—N(CH_3)_2$ and $—CH_{2b}$ because of the side of DMMA, and the multiplets from 3.50 to 3.89 ppm were attributed to $N—CH_{2a}—$ of the group, H_3, H_4, H_5, and H_6 of GlcN and N-alkylated GlcN [Fig. 2(C)]. Two new small peaks at 2.19 and 1.12 ppm were ascribed to $—CH_{2b}$ and $—C(CH_3)_2—CH_{2b}$ because of the introduction of the NIPAM side, and the multiplets from 3.50 to 3.86 ppm were attributed to $N—CH_{2a}—$ and $—CH_c$ of the group, H_3, H_4, H_5, and H_6 of GlcN and N-alkylated GlcN [Fig. 2(D)]. A newly formed peak at 2.69 ppm was assigned to the $—CH_{2b}—COO$ group because of the introduction of the AA side, and multiplets from 3.5 to 3.9 ppm were attributed to $N—CH_{2a}—$ of the *N*-alkyl group, H_3, H_4, H_5, and H_6 of GlcN and N-alkylated GlcN [Fig. 2(E)]. Two newly formed peaks at 2.87 and

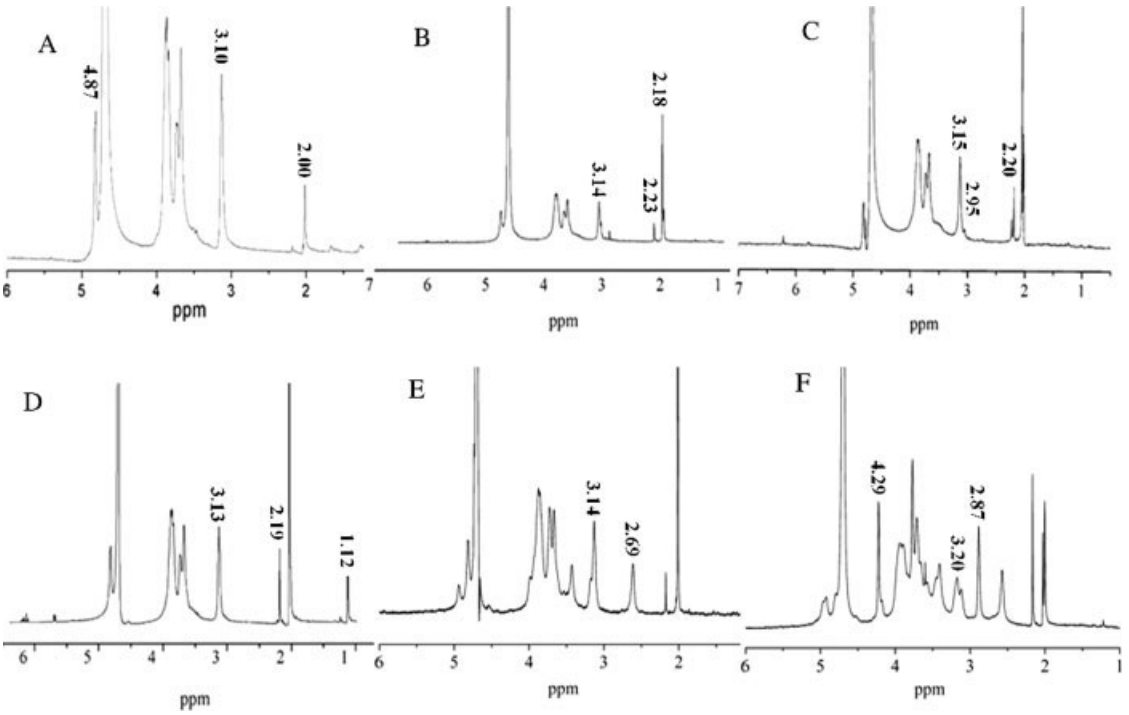

Figure 2 ^{1}H-NMR spectra of chitosan and chitosan derivatives: (A) chitosan, (B) MAA–CS, (C) DMMA–CS, (D) NIPAM–CS, (E) AA–CS, and (F) HEA–CS.

TABLE I
DS Values and Solubility of Chitosan Derivatives at 40°C

	Sample				
	MAA–CS	DMMA–CS	NIPAM–CS	AA–CS	HEA–CS
DS	0.17	0.13	0.18	0.36	0.39
Solubility	±	−	−	+	+

The reactions took place at 50°C for 48 h. The chitosan $-NH_2$/reagent ratio was 1:4. + = soluble; ± = partially soluble or swelling; − = insoluble.

4.29 ppm were due to the appearance of the two groups of $-CH_{2b}-COO-$ and $-CH_{2d}$ adjacent to the 2-amino group of GlcN, and multiplets from 3.5 to 3.8 ppm were attributed to $N-CH_{2a}-$ and $-CH_{2c}-$ of the *N*-alkyl group, H_3, H_4, H_5, and H_6 of GlcN and N-alkylated GlcN [Fig. 2(F)]. The H_2 proton signals of MAA–CS, DMMA–CS, NIPAM–CS, AA–CS, and HEA–CS were shifted from 3.10 to 3.14, 3.15, 3.13, 3.14, and 3.20 ppm, respectively, in comparison with chitosan. The DS values based on $-CH_{2b}$ were determined from the relative intensities of these signals and were calculated from the peak area of the $CH_{2b}-$ proton against that of the NHAc proton;[20] the DS values of MAA–CS, DMMA–CS, NIPAM–CS, and HEA-CS were 0.17, 0.13, 0.18, 0.36, and 0.39, respectively.

DS values and solubility of the chitosan derivatives

Table I lists the DS values and solubility of the chitosan derivatives in distilled water. Different solubilities for the chitosan derivatives and different DS values were obtained under the same reaction conditions. The results showed that the DS values of AA–CS and HEA–CS were higher than those of MAA–CS, DMMA–CS, and NIPAM–CS. AA–CS and HEA–CS could dissolve in distilled water, but the other three did not. It could be concluded that the sides of the chitosan derivatives disturbed the formation of hydrogen bonding among the amino groups of chitosan. The higher DS of the amine group ($-NH_2$) would be an advantage for the solubility of the chitosan derivatives. Furthermore, the presence of AA and HEA sides, which were hydrophilic groups, could change the solubility of chitosan.

XRD

Figure 3 illustrates the XRD patterns of the chitosan and chitosan derivatives. The chitosan showed three characteristic peaks around 2θ values of 10.3, 15.9, and 20.1°, which indicated a high degree of crystallinity of chitosan in agreement with previous reports.[21,22] The reflection fall at 2θ = 10.3° was assigned to crystal form I, and the strongest reflection appeared at 2θ = 20.1°, corresponding to crystal form II, but the XRD patterns of the chitosan derivatives were significantly different from the crystalline structure of chitosan, and the chitosan derivatives had only one broad peak of 2θ ≈ 20°. The lower crystallinity of the chitosan derivatives was ascribed to the presence of reacted residues such as MAA, DMMA, NIPAM, AA, and HEA, which might have hindered the formation of intermolecular and extramolecular hydrogen bonds after chemical modification, so MAA–CS, DMMA–CS, NIPAM–CS, AA–CS, and HEA–CS could be amorphous. The results also indicated that there was no connection between the crystallinity of the chitosan derivatives and the kinds of reacted residues, and they also showed that there was no relationship between the crystallinity of the chitosan derivatives and their DS values.

Antimicrobial analysis

Figure 4 shows the antimicrobial activity of the chitosan and chitosan derivatives. The *E. coli* bacteria were selected as test cells because they are the most frequent bacteria in wound infections and are representative Gram-negative bacteria. As shown in Figure 4, there were nearly no bacterial cells in the Petri dish shown in Figure 4(1); there were few bacterial cells in the Petri dishes shown in Figure 4(2–4),

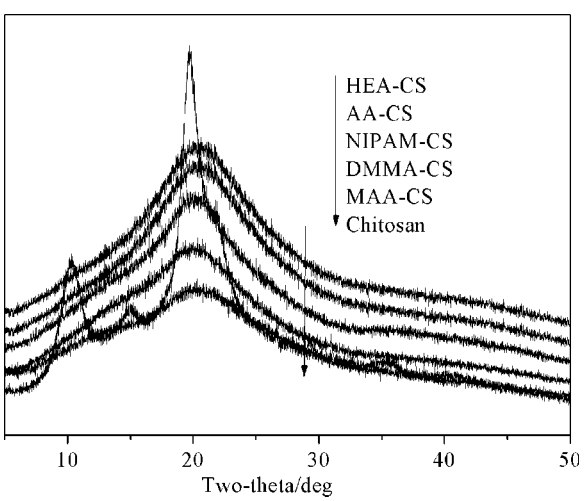

Figure 3 XRD patterns of chitosan and chitosan derivatives.

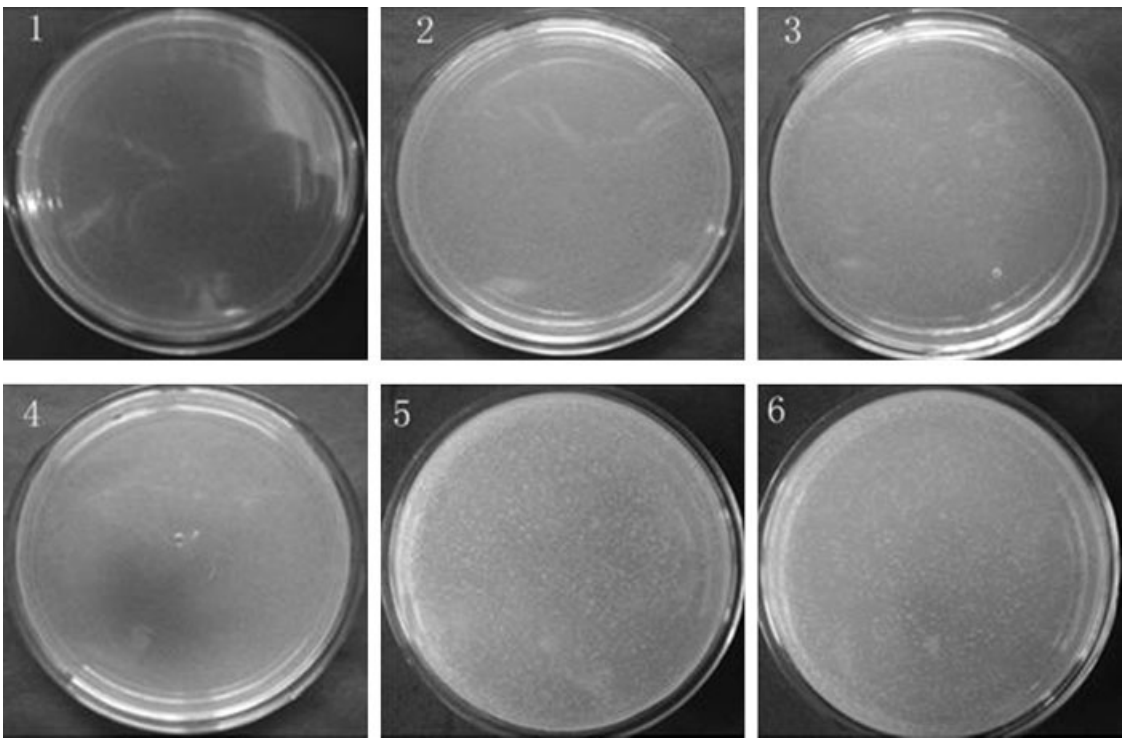

Figure 4 Images of antimicrobial activity to *E. coli*: (1) 1% chitosan and 0.5% acetic acid, (2) 1% MAA–CS and 0.5% acetic acid, (3) 1% DMMA–CS and 0.5% acetic acid, (4) 1% NIPAm–CS and 0.5% acetic acid, (5) 1% AA–CS and 0.5% acetic acid, and (6) 1% HEA–CS and 0.5% acetic acid.

and all of them had nearly the same number of bacterial cells. With the DS values of the chitosan derivatives increasing, there was definite spreading of the bacterial cells in the Petri dishes shown in Figure 4(5,6) versus those shown in Figure 4(2–4), but the undersides of the Petri dishes were not completely covered by the bacterial cells. The results showed that the chitosan derivatives still had the ability to kill bacterial cells; there was significant antimicrobial activity to *E. coli*, and the antimicrobial activity of chitosan was appreciably higher than that of the chitosan derivatives. The MAA–CS, DMMA–CS, and NIPAM–CS chitosan derivatives had almost the same antimicrobial activity, but AA–CS and HEA–CS had lower antimicrobial ability. Chitosan was electropositive because of its amino groups at the C_2 position, and it adsorbed the electronegative substance in the cells, which could agglomerate them. On the other hand, it also formed a polymer membrane, which could prevent the transport of essential nutrients entering the cell.[23–25] Therefore, the physiological activities of the bacteria were destroyed, and this resulted in severe leakage of cell constituents and eventually cell death. The concentration of amino groups at the C_2 position of the chitosan derivatives was reduced after modification, so the antimicrobial activity of the chitosan derivatives was slightly weaker than that of chitosan. The antimicrobial activity of the chitosan derivatives decreased with the increasing DS values of the chitosan derivatives.

CONCLUSIONS

MAA–CS, DMMA–CS, NIPAM–CS, AA–CS, and HEA–CS were successfully synthesized, and their chemical structures were defined with FTIR, ^{1}H-NMR, and XRD techniques in detail. The chitosan derivatives with MAA, DMMA, NIPAM, AA, and HEA groups showed different solubilities in distilled water. The N-alkylated chitosan derivatives still had antimicrobial activity. This procedure will be useful for novel chemical modifications used to prepare water-soluble, biodegradable, and antimicrobial chitosan derivatives.

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