Preparation and Properties of Hydrophilic–Hydrophobic Chitosan Derivatives

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ABSTRACT: Hydrophilic–hydrophobic chitosan derivatives were obtained through the attachment of lactose and alkyl groups to the amino group of chitosan with potassium borohydride. The carboxymethylation of the chitosan derivatives was completed. They had excellent solubility in water. When they were prepared as hydrogels, the hydrogels had adjustable hydrophobicity and excellent hydrophilicity according to swelling measurements. All of them showed potential for applications in medical fields. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 1625–1632, 2004

Key words: hydrophilic polymers; hydrogels; swelling; biomaterials

INTRODUCTION

Chitosan is soluble in aqueous acid solutions and a few organic solvents. Because of its nontoxicity and biocompatibility, it has been widely applied for biomaterials.[1–4] As chitosan is insoluble in neutral water, various soluble chitosan derivatives have been prepared.[5–8] With deeper study, carbohydrates with hydrophilic–hydrophobic structures could remarkably enhance drug stability and biocompatibility in blood.[9–12]

As a drug carrier, chitosan can be prepared in various forms such as hydrogels, particles, and membranes. Hydrogels have been applied to the controlled release of drugs because drugs can be easily dispersed in the matrix, and their controlled release is achieved through the selection of various polymer networks. Our attention has been directed toward chitosan attached to hydrophobic alkyl groups and hydrophilic lactose. Because galactose residues are recognized by liver cells,[13–15] chitosan derivatives should have hepatic targeting. To obtain water-soluble materials, we reacted chitosan derivatives with chloroacetic acid. Hydrogels were prepared from all of these chitosan derivatives.

EXPERIMENTAL

Materials

Chitosan was purchased from the Yuhuan County Chemical Plant (Zhejiang, China). Its degree of deacetylation was 90%, as determined by elemental analysis. *n*-Hexaldehyde, *n*-decylaldehyde, and glutaraldehyde were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Propylaldehyde, potassium borohydride, and chloroacetic acid came from Shanghai Chemical Regents Co. (Shanghai, China).

Measurements

Chitosan derivatives were determined with a Nicolet 200SXV Fourier transform infrared (FTIR) instrument. ^{1}H-NMR and ^{13}C-NMR were conducted with a Varian Unity Inova 400 (D_2O/CH_3COOD). Elemental analyses were performed with a 1106 elemental analyzer (Carlo Erba Corp.).

Synthesis of the chitosan derivatives

A chitosan solution of (1% w/v) was prepared through the dissolution of chitosan in an aqueous 1% acetic acid solution. The lactose was added to the chitosan solution at 25°C. Three kinds of dehydes (propylaldehyde, *n*-hexaldehyde, and *n*-decylaldehyde) with various molar ratios were added to the solution with stirring for 2 h. The pH value of the solution was adjusted to 5. KBH_4 (1.5-fold) of lactose and various aldehydes was added. After 2 days, the chitosan derivatives (G1–G7) were obtained by the adjustment of the pH to 7. The precipitants were soaked in a water/methanol mixture (1/1 v/v) for 4 days. The chitosan derivatives were filtered out and dried at room temperature. *N*-Alkyl chitosan derivatives (G8 and G9) were prepared by the same process (shown in Scheme 1).

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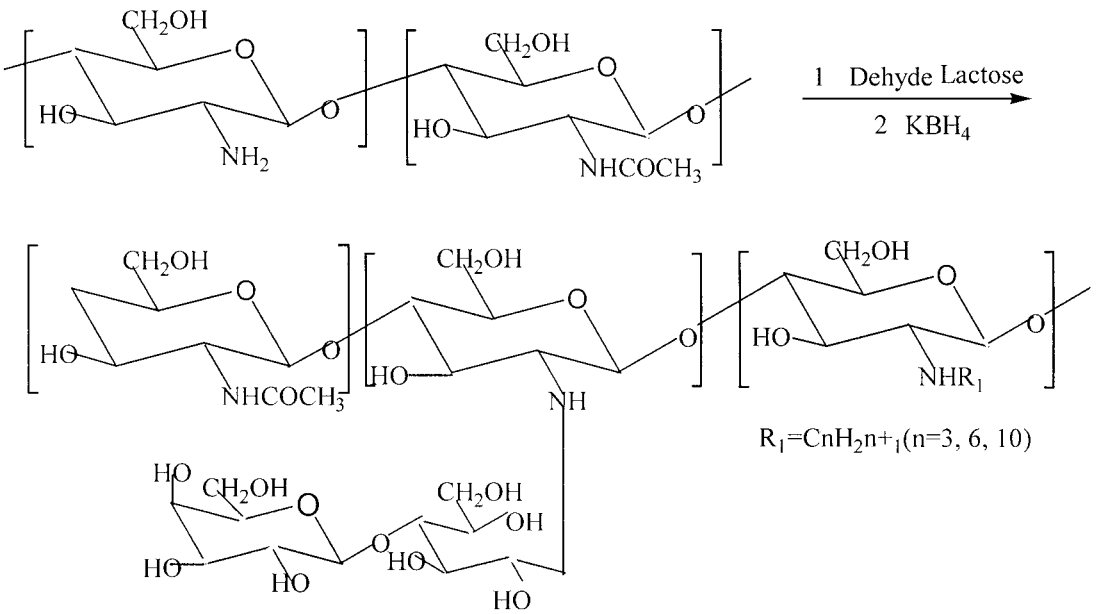


Scheme 1 Synthesis of the chitosan derivatives.

Carboxymethylation of the chitosan derivatives

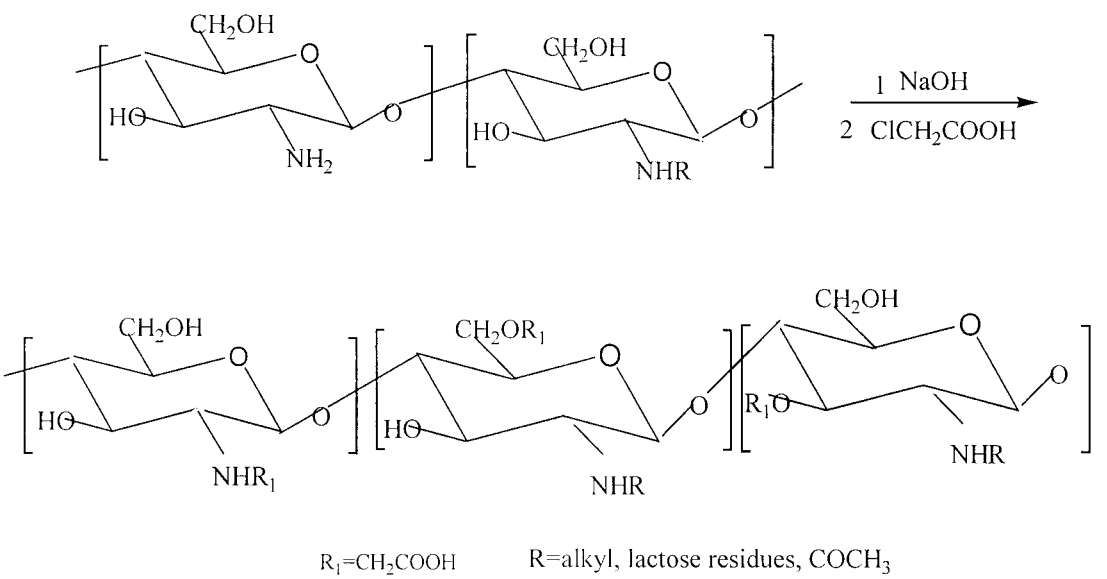
The chitosan derivatives, including G4, G7, G8, and G9, and chitosan were added and slurry-stirred in a 55% NaOH solution (including 0.08% dodecylsulfate sodium salt) at 0°C for 8 h. Chloroacetic acid was added to the solution up to pH 7 with vigorous stirring. The reaction mixture was filtered and washed with 70% (v/v) methanol/water. The washed product was dried at room temperature. The samples were named W4, W7, W8, W9, and carboxymethyl chitosan (CM-chitosan; Scheme 2), respectively.

Synthesis of the hydrogels

Chitosan derivative solutions (3% w/w) were prepared in 1% (w/w) dilute acid. Then, 2% $K_2S_2O_8$ and 0.2% (w/w) glutaraldehyde were added to the solution at 37°C. After 24 h, the hydrogels were obtained, and they were washed with a 1% NaOH solution. The hydrogels were dried at room temperature.

Swelling kinetics

The swelling was determined with the following procedure. Briefly, preweighed dry hydrogels were im-



Scheme 2 Carboxymethylation of the chitosan derivatives.

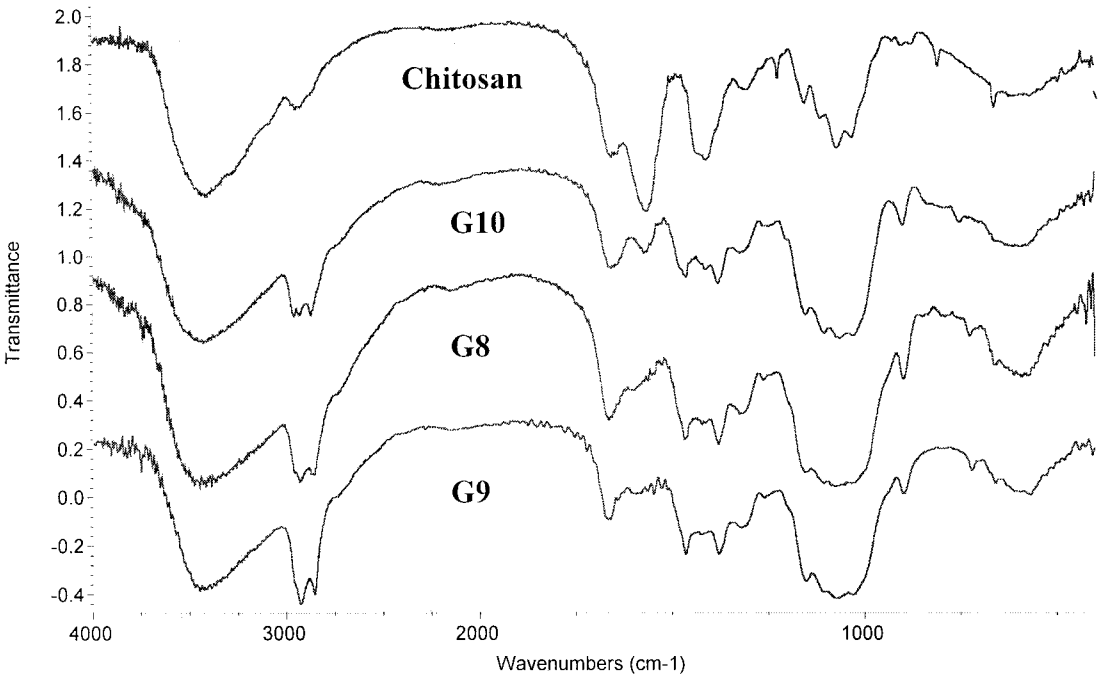


Figure 1 FTIR spectra of *N*-alkyl chitosan (G8 = *N*-hexal chitosan, G9 = *N*-decyl chitosan, and G10 = *N*-propyl chitosan).

mersed in solutions with pH values of 1.0, 3.0, 7.4, 9.2, or 12. At predetermined time intervals, the swelling hydrogels were removed, and then excess water was blotted from the surface. The following equation was used to determine the swelling ratio: $Q_s = W_w/W_d$, where W_w and W_d are the sample weights at time t in the wet and dry states, respectively.

RESULTS AND DISCUSSION

Synthesis of the chitosan derivatives

Alkyl groups were more easily introduced into the amine of chitosan than lactose groups. Figure 1 shows the FTIR spectra of chitosan and its *N*-alkyl derivatives. There existed characteristic peaks of chitosan at

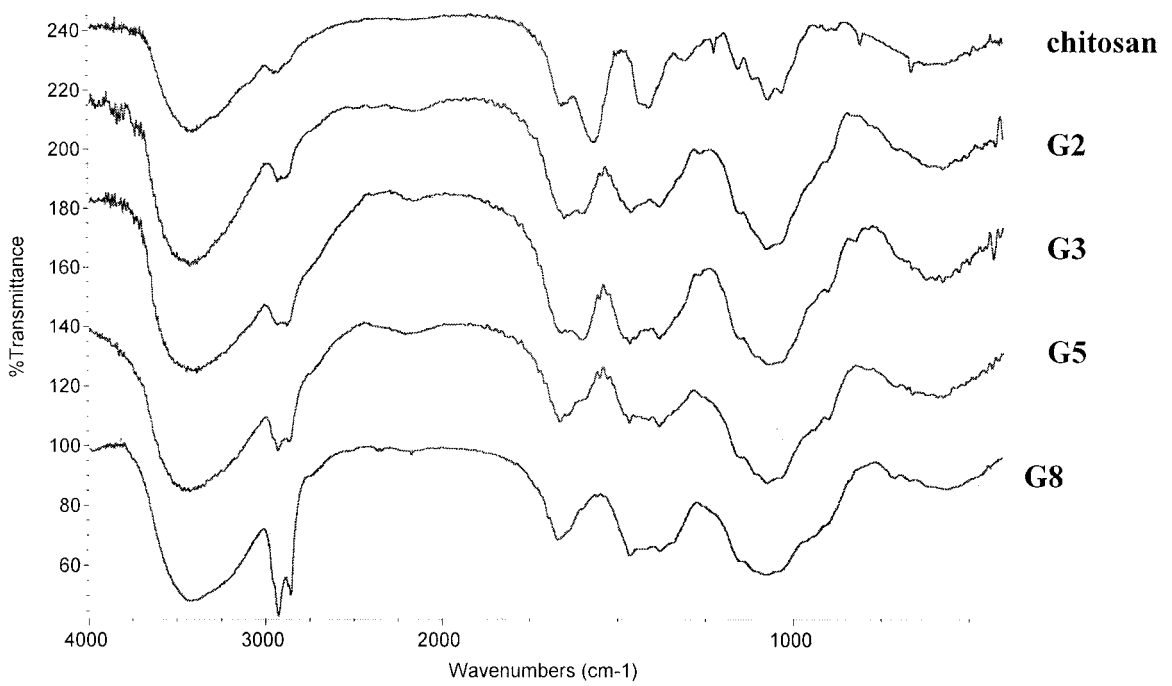


Figure 2 FTIR spectra of the chitosan derivatives with lactose.

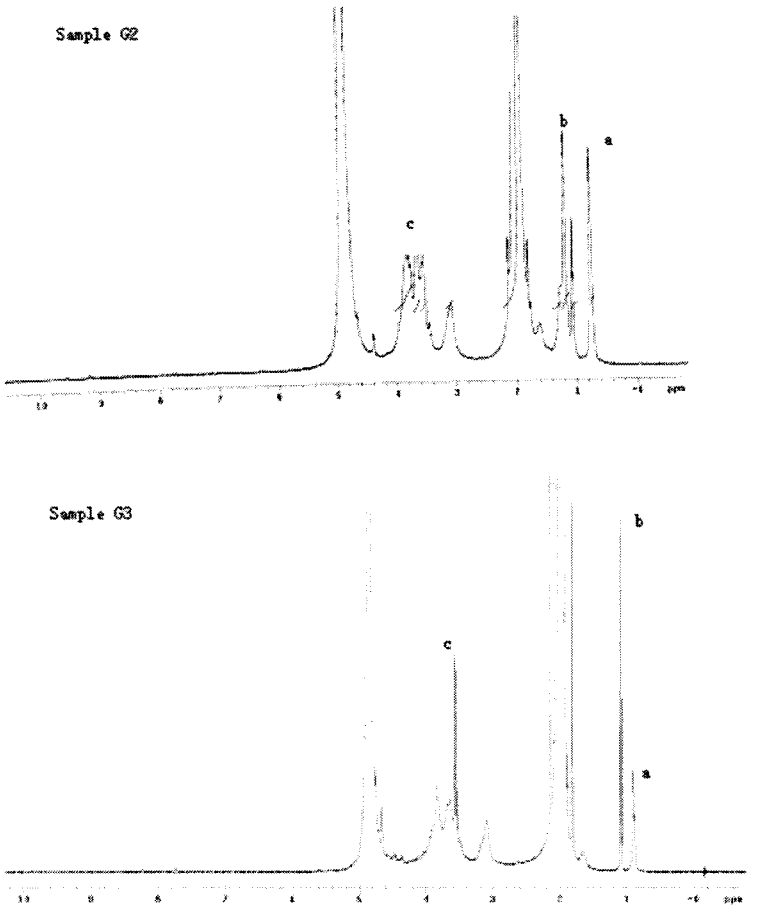


Figure 3 ^{1}H NMR spectra of the chitosan derivatives with lactose [G2 = $(C_8H_{13}NO_5)_{0.1}(C_6H_{11}NO_4)_{0.32}(C_9H_{17}NO_4)_{0.49}(C_{18}H_{33}NO_{14})_{0.09}0.2H_2O$, G3 = $(C_8H_{13}NO_5)_{0.1}(C_6H_{11}NO_4)_{0.32}(C_{12}H_{23}NO_4)_{0.38}(C_{18}H_{33}NO_{14})_{0.20}0.6H_2O$].

2940 (—CH_3, —CH_2), 1640 (C═O stretch vibration), 1570 (secondary amide), 1410 (primary amide), and 1070 cm^{-1} (C—O—C). With an alkyl chain attached to chitosan, new peaks at 1465 (—CH_2, vs), 1379 (C—CH_3, s), and 720 cm^{-1} (CH_2 rocking in the methylene chain) appeared. The signal intensities of peaks at 2940 and 1070 cm^{-1} increased with an increase in the number of alkyl groups. When lactose and alkyl groups were added to chitosan (Fig. 2), the FTIR spectra were similar to those of *N*-alkyl chitosan. To further confirm the formation of the chitosan derivatives, we measured the ^{1}H-NMR and ^{13}C-NMR spectra. The ^{1}H-NMR spectra of the chitosan derivatives (G2 and G3) are shown in Figure 3. The important signals at 0.9 and 1.1 ppm were assigned to the protons of C—H and CH_3 of the alkyl groups. The other signals at 3.1–4.4 ppm were assigned to the interaction of chitosan and lactityl residues. The ^{13}C-NMR spectra of sample G2 are shown in Figure 4. There existed characteristic peaks of chitosan at 21, 62, 72, 75, 79, and 100 ppm. Compared with the peak of chitosan, new peaks at 12 (alkyl group) and 51.5 ppm (NH—CH_2) appeared. The chemical shifts were displaced upfield because of the existence of propyl group and lactityl residues. These results indicated that the chitosan derivatives could be formed.

Table I shows the degree of substitution (DS) of chitosan. The DS of the alkyl groups ranged from 0.34 to 0.65 because of the different amounts and kinds of aldehydes. The DS of the alkyl groups decreased as the alkyl chain length increased and the amount of aldehyde decreased. The DS of lactose, ranging from 0.09 to 0.26, was due to the same reason. These results clearly demonstrated that the DS of the products was strongly controlled by the activity of the aldehyde.

To distinguish CM-chitosan and CM-chitosan derivatives, we took FTIR spectra of CM-chitosan and sample W8 (Fig. 5). There existed characteristic peaks at 1605 and 1420 cm^{-1} assigned to carboxyl groups, which indicated the formation of CM-chitosan derivatives.

Solubility of the chitosan derivatives

Because alkyl groups reduce the solubility of chitosan, *N*-alkyl chitosan (G8 and G9) was insoluble in acetic acid and formic acid (5% w/w). However, when hydrophilic lactose was attached to the amide of chi-

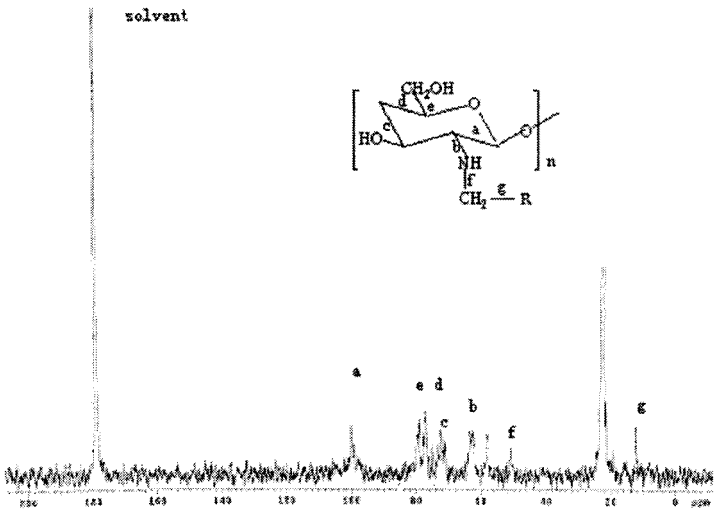


Figure 4 ^{13}C NMR spectrum of the chitosan derivatives (G2) with lactose.

tosan, G3–G5 were soluble in acetic acid, and G6 and G7 were soluble in formic acid. G2 was soluble in neutral water at room temperature, and this agreed with a report by Yalpani.[16] The CM-chitosan derivatives were not only soluble in slightly acetic acid solutions at room temperature but were also soluble in neutral water at higher temperatures (>40°C). Interestingly, the length of the alkyl group sometimes promoted the solubility of the chitosan derivatives. For example, W7 linked with a 10-carbon-atom chain was more easily dissolved than W4 linked with a 6-carbon-atom chain with similar substitution. The structure of the CM-chitosan derivatives was more complicated. Because the C-6 position of chitosan had stronger reactivity than the other positions, the substitution mainly happened here; however, the amide groups and C-3 position could be carboxymethylated. To avoid the hydrolysis and carboxymethylation of the lactose residue, we kept the reactive conditions 0°C and 8 h.

Hydrogel swelling of the chitosan derivatives

To research the properties of chitosan derivatives, we prepared hydrogels with fixed degrees of crosslinking (hydrogels with different crosslinking degrees will be discussed in another article concerning the application

TABLE I
DS of Chitosan Derivatives

Sample	Monomer composition	Calculated formula	Analysis (%)		
			C	H	N
G1	Chitosan/lactose = 1/1	$(C_8H_{13}NO_5)_{0.1}$ $(C_6H_{11}NO_4)_{0.64}$ $(C_{18}H_{33}NO_{14})_{0.26}$ $0.78H_2O$	42.37	7.08	5.65
G2	Chitosan/lactose/propyladehyde = 1/1/1.5	$(C_8H_{13}NO_5)_{0.1}(C_6H_{11}NO_4)_{0.32}$ $(C_9H_{17}NO_4)_{0.49}$ $(C_{18}H_{33}NO_{14})_{0.09}$ $0.2H_2O$	46.68	7.34	6.37
G8	Chitosan/*n*-hexaldehyde = 1/3	$(C_8H_{13}NO_5)_{0.1}(C_6H_{11}NO_4)_{0.25}$ $(C_{12}H_{23}NO_4)_{0.65}1.1H_2O$	50.64	8.85	5.64
G3	Chitosan/lactose/*n*-hexaldehyde = 1/1/1	$(C_8H_{13}NO_5)_{0.1}(C_6H_{11}NO_4)_{0.32}$ $(C_{12}H_{23}NO_4)_{0.38}(C_{18}H_{33}NO_{14})_{0.20}0.6H_2O$	47.8	7.8	5.12
G4	Chitosan/lactose/u-hexaldehyde = 1/1/3	$(C_8H_{13}NO_5)_{0.1}(C_6H_{11}NO_4)_{0.28}(C_{12}H_{23}NO_4)_{0.44}$ $(C_{18}H_{33}NO_{14})_{0.18}0.82H_2O$	46.83	8.37	5.02
G5	Chitosan/lactose/*n*-hexaldehyde = 1/1/6	$(C_8H_{13}NO_5)_{0.1}$ $(C_6H_{11}NO_4)_{0.25}$ $(C_{12}H_{23}NO_4)_{0.52}$ $(C_{18}H_{33}NO_{14})_{0.13}1.53H_2O$	46.83	8.37	5.02
G9	Chitosan/*n*-decylaldehyde = 1/3	$(C_8H_{13}NO_5)_{0.1}(C_6H_{11}NO_4)_{0.55}$ $(C_{16}H_{31}NO_4)_{0.35}$ $0.25H_2O$	53.28	8.55	5.84
G6	Chitosan/lactose/*n*-decylaldehyde = 1/1/1	$(C_8H_{13}NO_5)_{0.1}$ $(C_6H_{11}NO_4)_{0.34}$ $(C_{16}H_{31}NO_4)_{0.34}$ $(C_{18}H_{33}NO_{14})_{0.23}0.72H_2O$	41.2	8.12	4.68
G7	Chitosan/lactose/*n*-decylaldehyde = 1/1/6	$(C_8H_{13}NO_5)_{0.1}$ $(C_6H_{11}NO_4)_{0.27}$ $(C_{16}H_{31}NO_4)_{0.43}(C_{18}H_{33}NO_{14})_{0.20}0.87H_2$	40.44	8.47	4.57

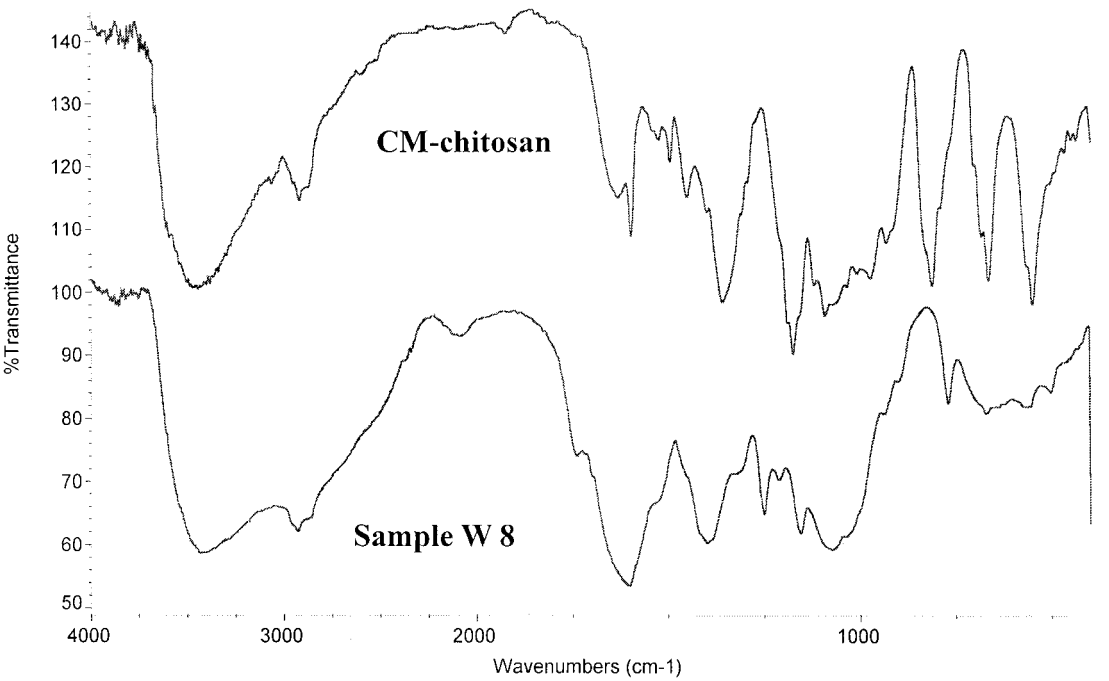


Figure 5 FTIR spectra of the CM-chitosan derivatives (W8 = *O,N*-carboxymethyl-*N*-hexal chitosan).

of chitosan derivatives to the controlled release of drugs). The chitosan derivatives still contained amine groups, which were ionized by protonation in acid solutions. The hydrogels were pH-sensitive because of the ionization. To erase the influence of the ionic strength, we kept the solution concentration constant (0.2*M*). As shown in Figure 6, the hydrogels had a higher swelling ratio under acidic conditions. When more amide groups of the hydrogels were substituted, the swelling ratios were reduced more. At a high pH, because the protonation of the amino groups reached their maximum, the swelling ratio remained constant when the pH was greater than 7.

The CM-chitosan derivatives were not sensitive to the pH because of carboxymethylation. The difference in the swelling ratios was dependent on the amount of

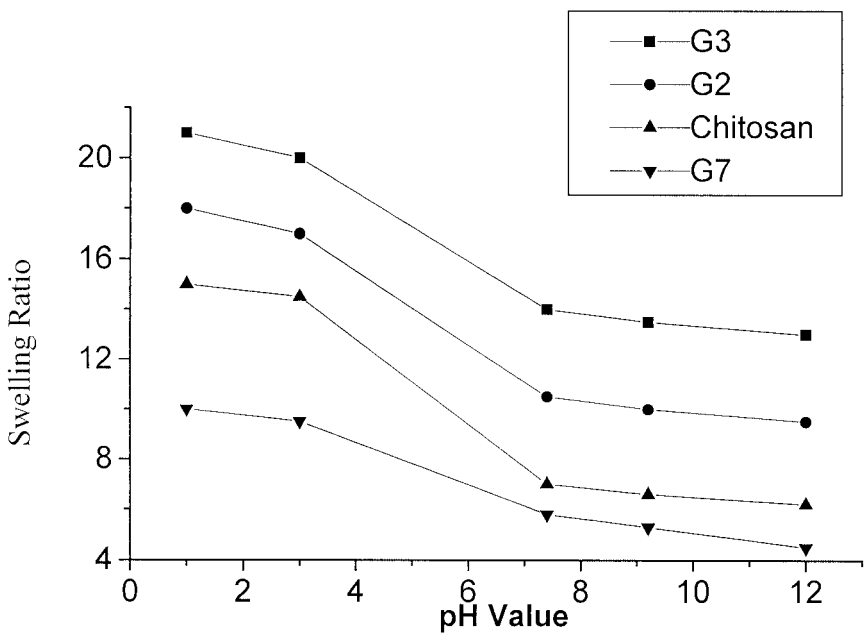


Figure 6 Equilibrium swelling ratio of the chitosan derivatives at various pH values.

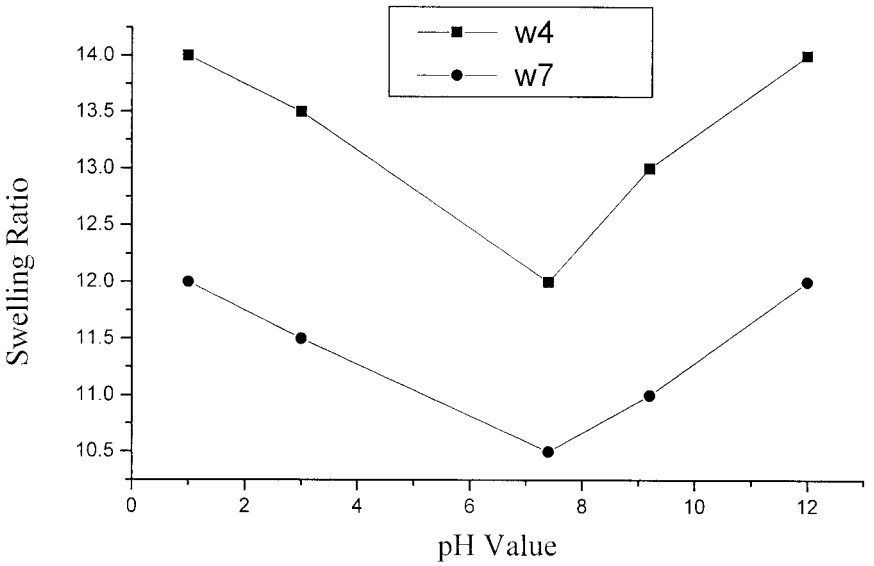


Figure 7 Equilibrium swelling ratio of the CM-chitosan derivatives at various pH values (W4 and W7 came from samples G4 and G7, respectively, by carboxymethylation).

hydrophilic lactose and hydrophobic alkyl groups. As shown in Figure 7, the swelling ratio of the W4 sample was higher than that of the W7 sample. The reason may be due to the differences in the alkyl groups.

Swelling kinetic study

Figure 8 presents the swelling kinetics of the chitosan derivatives. A chitosan hydrogel easily reached the swelling equilibrium in comparison with G3. In the buffer (pH 1), G3 swelled more quickly during the first 40 min, and then the value increased slowly until it reached equilibrium. In comparison with the pH 1 buffer, the swelling process was fast in the pH 7.4 buffer, despite its lower swelling ratio. The CM-chitosan derivatives, as shown in Figure 9, were independent of pH. With the number of carbon atoms increasing, the speed of swelling became slow.

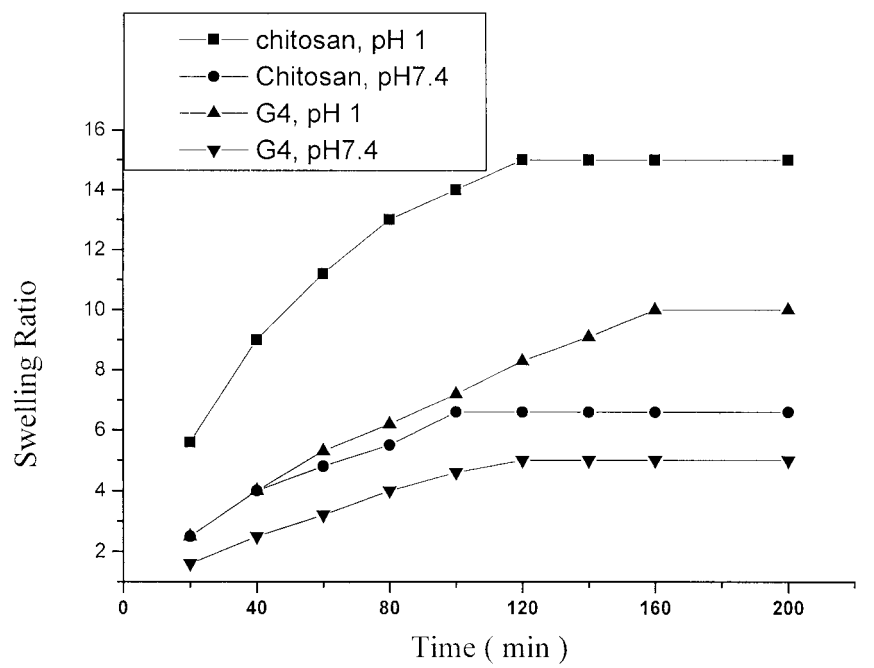


Figure 8 Swelling kinetics of the chitosan derivatives at pH 1 and pH 7.4 [G4 = $(C_8H_{13}NO_5)_{0.1}(C_6H_{11}NO_4)_{0.28}(C_{12}H_{23}NO_4)_{0.44}(C_{18}H_{33}NO_{14})_{0.18}0.82H_2O$].

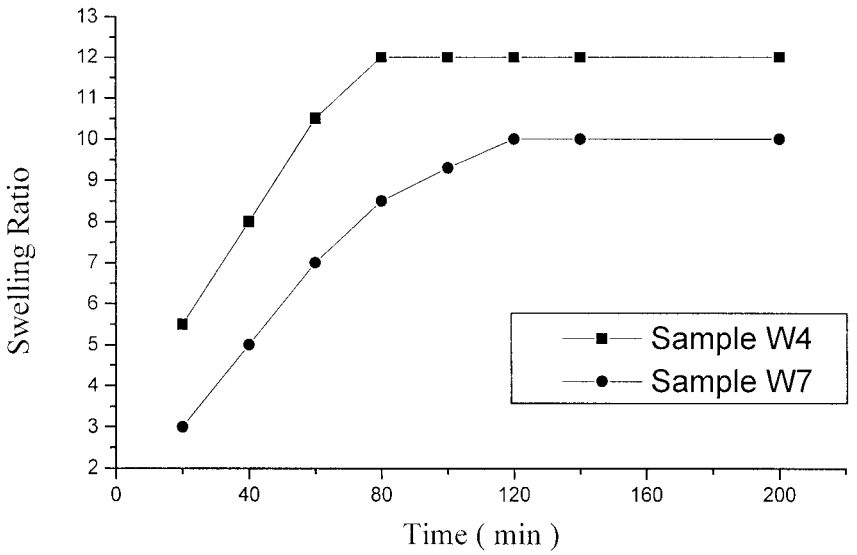


Figure 9 Swelling kinetics of the CM-chitosan derivatives at pH 7.4.

CONCLUSIONS

We have demonstrated a method for transforming chitosan into water-soluble derivatives. This method is suitable for the transformation of chitosan into hydrophilic–hydrophobic derivatives. Chitosan derivatives with straight-chain branches of various lengths with 3–10 carbon atoms and lactose branches have been obtained. Branched, comblike chitosan derivatives can be achieved through the control of the side-chain length and DS. Because of their excellent solubility in water and slightly acidic solvents and their adjustable hydrophobicity, the chitosan derivatives are expected to be applied to drug-delivery systems.

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