Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Polymers 53 (2003) 355–359
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Chemical characteristics of *O*-carboxymethyl chitosans related to the preparation conditions[☆]

Xi-Guang Chen[a,b], Hyun-Jin Park[b,*]

[a] *The Life Science College, Ocean University of Qingdao, Qingdao 266003, People's Republic of China*
[b] *The Graduate School of Biotechnology, Korea University, 1, 5-Ka, Anam-Dong, Sungbuk-Ku, Seoul 136-701, South Korea*

Abstract

Carboxymethyl chitosan (CM-chitosan) was prepared by chemical reaction with monochloroacetic acid under various conditions, and the chemical structure was analyzed by IR and NMR. The water solubility of the CM-chitosans had close relationships to the modifying conditions and the degree of carboxymethylation. The CM-chitosans, prepared at temperatures of 0–10 °C were soluble in water. But the CM-chitosan prepared between 20 and 60 °C were insoluble in the water at near-neutral pH. The water insolubility of CM-chitosans at various pHs varied with the degree of carboxymethylation. The increase in reaction temperature increased the fraction of carboxymethylation and increased the insolubility at lower pHs; the increase of the ratio of water/isopropanol in the reaction solvent decreased the fraction of carboxymethylation and increased the insolubility at higher pHs.

Keywords: Chitosan; Carboxymethyl-chitosan; Water solubility; Chemical nature; Carboxymethylation

1. Introduction

To improve the solubility of chitosan, generally, a partial N-acetylation or chemical modification is required. Partially acetylated chitosans, which have a degree of deacetylation around 50%, are able to dissolve in pure water (Kubota, Tatsumoto, Sano, & Toya, 2000). Various studies were conducted to make water-soluble derivatives of chitosan by chemical modification techniques, such as PEG-grafting (Ouchi, Nishizawa, & Ohya, 1998), sulfonation (Holme & Perlin, 1997), quaternarization (Jia, Shen, & Xu, 2001), N- and O-hydroxylation (Machida, Nagai, Abe, & Sannan, 1986) and carboxymethylation chitosan (CM-chitosan) (Krause, Goldsmith, Ebner, Zazanis, & McKinnon, 1998).

Among the water-soluble chitosan derivatives, CM-chitosan is an amphiprotic ether derivative, contains –COOH groups and $-NH_2$ groups in the molecule. The structure, antibacterial activity, toxicity and membrane properties and *N*- and *O*-carboxymethyl-chitosans have previously been reported by Liu, Guan, Yang, Li, and Yao (2001), Muzzarelli, Ilari, and Petrarulo (1994), Rinaudo, Le Dung, Gey, and Milas (1992) and Zhang, Guo, Zhou, Yang, and Du (2000), respectively. The water solubility of CM-chitosan is important but has not been reported. The solubility depends on the molecular structure and the solvent.

In this paper, various reaction treatments for the preparation of carboxymethyl chitosan, and the water solubility properties at various pHs were investigated.

2. Materials and methods

2.1. Materials

Chitosan, 100 mesh, degree of deacetylation 90%, molecular weight 400 KDa, was obtained from Biotch. Co., Ltd., Korea. Monochloroacetic acid was supplied by the Sigma Co. St Louis, USA.

2.2. CM-chitosan preparation

Carboxymethyl chitosan was prepared by the method of Liu et al. (2001). Chitosan (10 g), sodium hydroxide (13.5 g), and solvent (100 ml) were added into a flask (500 ml) to swell and alkalize at a given temperature for 1 h. The temperature was maintained in a water bath (Thermo-controller, Coma-biotech. Co., Korea). The monochloroacetic acid (15 g) was

---

[☆] This work is conducted in the Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea.

[*] Corresponding author. Tel.: +82-2-3290-3450; fax: +82-2-927-9028.
E-mail address: hjpark@korea.ac.kr (H.-J. Park).

0144-8617/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0144-8617(03)00051-1

Table 1
CM-chitosan samples (Na-form) prepared from different reaction temperatures and reaction solvents and the yields

Samples	Water/isopropanol (v/v)	Temperature (°C)	Yield (%)[a]	Ratio (%)[b]
CM-chitosan1	1/9	0	1.2	100.5
CM-chitosan2	1/9	10	12.8	100.3
CM-chitosan3	1/9	20	66.7	113.2
CM-chitosan4	1/9	30	72.7	137.6
CM-chitosan5	1/9	40	71.8	128.0
CM-chitosan6	1/9	50	81.0	151.5
CM-chitosan7	1/9	60	82.3	134.7
CM-chitosan8	0/10	50	49.6	114.3
CM-chitosan9	2/8	50	99.8	139.6
CM-chitosan10	5/5	50	98.6	118.4
CM-chitosan11	8/2	50	13.6	105.1
CM-chitosan12	10/0	50	2.4	103.1

[a] Yield = Water soluble CM-chitosan (g)/Raw product (g) × 100%.
[b] Ratio = Raw CM-chitosan product (g)/chitosan (g) × 100%.

dissolved in isopropanol (20 ml), and added into the reaction mixture dropwise for 30 min and reacted for 4 h at the same temperature, then stopped by adding 70% ethyl alcohol (200 ml). The solid was filtered and rinsed in 70–90% ethyl alcohol to desalt and dewater, and vacuum dried at room temperature. The products were Na salt CM-chitosans. The CM-chitosan samples and the preparation conditions are shown in Table 1.

Na salt CM-chitosan (1 g) was suspended in 80% ethyl alcohol aqueous solution (100 ml), hydrochloric acid (10 ml, 37%) was added and stirred for 30 min. The solid was filtered and rinsed in 70–90% ethyl alcohol to neutral, vacuum dried. The products were the H-form of CM-chitosans (Sakairi et al., 1998).

2.3. FT/IR spectroscopy

The IR spectrum of CM-chitosan was recorded on an FT/IR-430 Fourier Transform Infrared Spectrometer (Jasco Co. Tokyo, Japan) at room temperature based on the method of Shigemasa, Matsuura, Sashiwa, and Saimoto (1996). A pellet was formed from 2 mg CM-chitosan and 100 mg of KBr. Data analysis was carried out using Jwstda-32 (Windows 95/NT).

2.4. ^{1}H NMR spectroscopy

^{1}H NMR spectrum of CM-chitosan was recorded on a Bruker ARX 300 spectrometer in D_2O at 25 °C. The sample was dissolved in a 5 mm diameter tube at a concentration of 20 mg/ml. For water-insoluble samples, CD_3OOD (99.9%) was added (1%, v/v). The measurement conditions were as follow: a spectral window of 500 MHz, 32k data points, a pulse angle of 30°, an acquisition time of 2.03 s, and 32 scans with a delay of 1 s between scan (Rinaudo et al., 1992; Sashiwa & Shigemasa, 1999).

2.5. Water solubility

Water solubility of the CM-chitosan was evaluated from the turbidity based on the method of Sashiwa & Shigemasa (1994). The sample was dissolved in deionized water. The transmittance of the solution recorded on a DU-650 Spectrophotometer (Beckman Co. USA) using a quartz cell with an optical path length of 1 cm at 600 nm. The pH dependence of the water solubility of the CM-chitosan was also estimated from measurement of transmittance of the solution. The sample concentration was 0.2 mg/ml. The various pH solutions of the CM-chitosan were prepared by adjusting with 0.5% aqueous HCI and NaOH.

3. Results and discussion

3.1. Preparation and product characterization

Since chitosan was in the solid state during carboxymethylation, swelling of the particles was required before the chemical reaction could take place. In Table 1, the CM-chitosan 1–7 samples were prepared at different reaction temperatures. The yields (CM-chitosan 1–2) were very low at 0–10 °C, but as the temperature increased above 20 °C, the yields were much higher (CM-chitosan 3–7). The higher temperature in the preparation had benefit of increasing the CM-chitosan yields. The CM-chitosan 8–12 samples were prepared in different solvents. In 100% isopropanol (CM-chitosan 8), the yield was only 49.6%. The reason maybe that it was not alkalized in the non-aqueous solvent. And in water alone (CM-chitosan 12), the yield was 2.4%. The reason was that the previously formed CM-chitosan easily swelled in water to form jelly. The jelly coated the outside of the chitosan particle and inhibited the reaction. Yields of close to 100% were obtained when the ratios of water/isopropanol were 1:4 and 1:1. That means the ratio of water/isopropanol is an important elements in the reaction of CM-chitosan preparation.

3.2. Structure and chemical composition

3.2.1. FT/IR data analysis

The IR spectrums of CM-chitosan 6 and chitosan are shown in Fig. 1. Fig. 1A shows the basic characteristics of chitosan at: 3455 cm^{-1} (O–H stretch), 2867 cm^{-1} (C–H stretch), 1598 cm^{-1} (N–H bend), 1154 cm^{-1} (bridge-O-stretch), and 1094 cm^{-1} (C–O stretch) (Brugnerotto et al., 2001; Shigemasa et al., 1996). And in the H-form CM-chitosan 6 (Fig. 1C), the peaks at 1741 cm^{-1} (–COOH), 1070–1136 cm^{-1} (–C–O–) and 1624 and 1506 cm^{-1} ($-NH_3^+$) were the characteristics of *O*-CM-chitosan (Liu et al., 2001; Zhang et al., 2000; Zhao, Kato, Fukumoto, & Nakamae, 2001). Fig. 1B was the IR spectrum of CM-chitosan 6 in Na salt. The peak of –COOH was changed to –COONa (1598 cm^{-1}), the $-NH_3^+$ changed to $-NH_2$

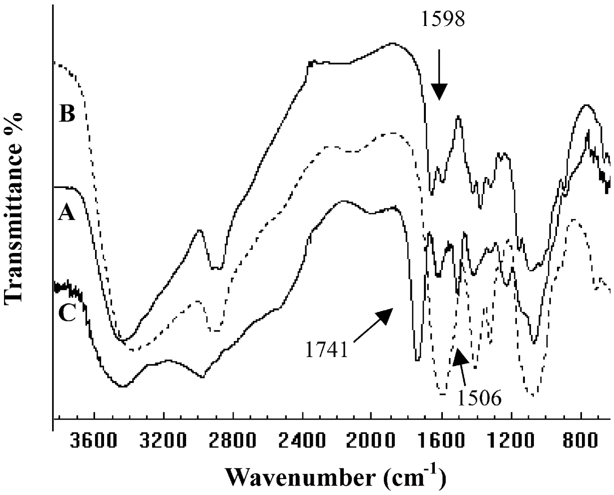


Fig. 1. FT/IR spectrum of chitosan, H-form CM-chitosan and Na-form CM-chitosan. (A) Chitosan, (B) Na-form CM-chitosan 6, (C) H-form CM-chitosan 6.

(1592 cm^{-1}). The IR spectrum of the H-form and Na salt CM-chitosan showed a high fraction of $-COOH$ and $-NH_2$ groups, and indicated the carboxymethyl groups to be on the $-OH$ position.

The CM-chitosans, which were prepared under different modifying conditions, had a similar IR spectrum (Figs. 2 and 3). All of the samples had large $-COOH$ group (1741 cm^{-}) and $-NH_3^+$ group (1506 cm^{-1}) peaks. From these results, the CM-chitosan samples had similar chemical compositions to each other.

3.2.2. NMR data analysis

The 500 MHz ^{1}H NMR spectrum of CM-chitosan 10 in D_2O is shown in Fig. 3. The basic assignment of the chitosan resonance is that: a is the resonance of H–1D (4.8 ppm), b is H–1A (4.65 ppm), h is the resonance of 3 acetyl-protons (2.0 ppm), e is H3–6 protons (3.6–3.9 ppm), g is H–2D proton resonance (3.1 ppm). These resonances can be found in the ^{1}H NMR spectrum of chitosan described by Kubota and Eguchi (1997) and Shigemasa et al. (1996). In the region between 4.05 and 4.55 ppm, the resonances are the protons of 3- and 6-substituted carboxmethyl ($-O-CH_2-COOD$) of CM-chitosan, d is the resonance of 3 protons from H-6′ (2 protons) and H-3′ (1 proton), c is the resonance of 1 proton from H-3′ (Ragnhild et al., 1997). The resonance signal of the protons from $N-CH_2-COOD$ group can be found at f (3.25 ppm). And the signal can be found at the ^{1}H NMR spectrum of *N*-carboxymethyl-chitosan (Muzzarelli, Ilari, & Petrarulo, 1994), also described by Ragnhild et al. (1997), the resonance between 3 and 3.5 ppm, different from the H–2D signal at 3.15 ppm. The result shows that the amino groups were partly carboxymethylated along with the hydroxyl groups.

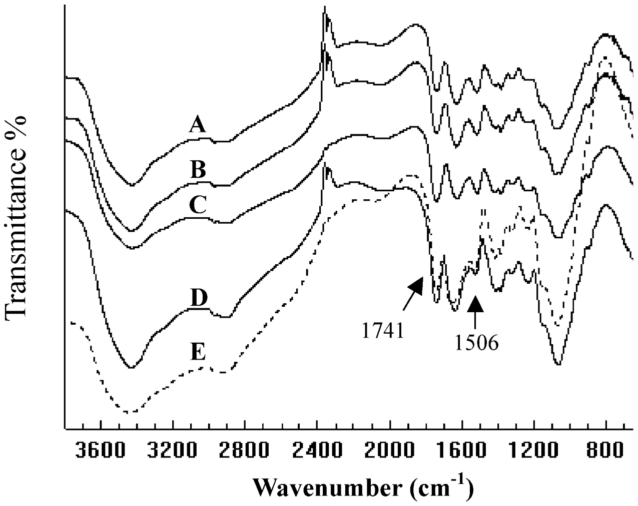


Fig. 2. FT/IR spectrum of CM-chitosan in H-form, each CM-chitosan was prepared from different solvents (water/isopropanol ratio = W:I). (A) W:I = 1:0, (B) W:I = 4:1, (C) W:I = 1:1, (D) W:I = 1:4, (E) W:I = 0:1.

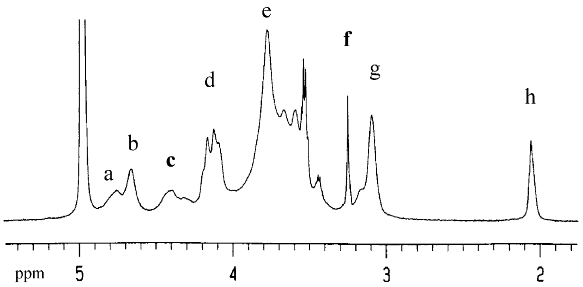


Fig. 3. ^{1}H NMR spectrum of CM-chitosan 10 in D_2O at 25 °C (500 MHz; 300 K). (a) H–1D, (b) H–1A, (c) H-3′, ($O-CH_2COOD$), (d) H-6′ and H-3′, ($O-CH_2COOD$), (e) H3–6, (f) H-2′, ($N-CH_2COOD$), (g) H–2D, (h) H-2′, (CH_3CO-).

3.3. Determination of the degree of substitution

The substitution of carboxymethyl groups on 6-O-, 3-O- and 2-N- can be determined from the ^{1}H NMR spectra using

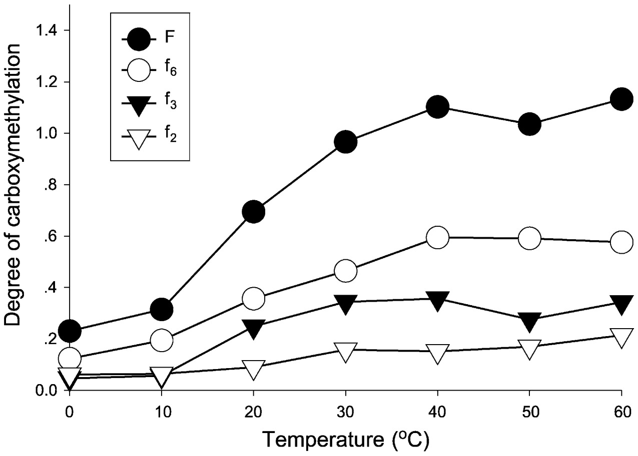


Fig. 4. The distribution of the fraction of carboxymethylation on CM-chitosan for various preparation temperatures. (*F*) The total fraction, (f_6) 6-O-position fraction, (f_3) 3-O-position fraction, (f_2) 2-N-position fraction.

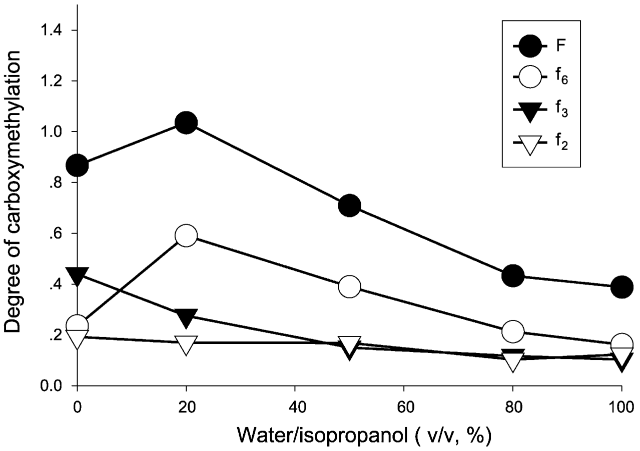


Fig. 5. The distribution of the fraction of carboxymethylation of CM-chitosan for various preparation solvents. (*F*) The total fraction, (f_6) 6-O-position fraction, (f_3) 3-O-fraction position, (f_2) 2-N-position fraction.

the method of Ragnhild et al. (1997). The calculated equations are as follows

$$f_6 = (1/2)(I_d - I_c)/(I_b + I_g) \tag{1}$$

$$f_3 = I_c/(I_b + I_g) \tag{2}$$

$$f_2 = (1/2)I_f/(I_b + I_g) \tag{3}$$

$$F = f_6 + f_3 + f_2 \tag{4}$$

Where f_6, f_3 and f_2 are the fractions of carboxymethylation at the position 6-O-, 3-O- and 2-N-, respectively. F is the total fraction of carboxymethylation. The total value of H-1 can be obtained by summing $(I_a + I_b)$ or $(I_b + I_g)$. The distribution of each fraction of carboxymethylation on the CM-chitosan and the relation to the preparation conditions are shown in Figs. 4 and 5.

Fig. 4 shows that the fraction of carboxymethylation depend on the reaction temperature. The total fraction of carboxymethylation (F) and the fraction at position 6 (f_6), position 3 (f_3) and position 2 (f_2) of chitosan were increased as the reaction temperature increased. The fraction of carboxymethylation at position 6 was always larger than position 3 and 2. At temperatures of 0–10 °C, f_3 and f_2 had no marked difference in values. When the reaction temperature was above 20 °C, the increase in the value of f_6 and f_3 is greater than f_2, the order being $f_6 > f_3 > f_2$. That means in the solvent mixture the higher reaction temperature favoured the substitution of the carboxymethyl on the –OH groups.

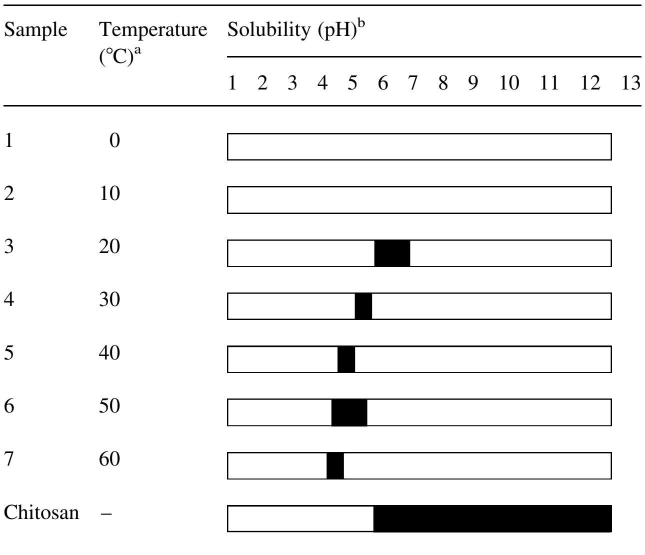
In isopropanol alone, the values of f_6 and f_2 were close each to other, and f_3 had the largest fraction (Fig. 5). In the water-containing solvents, the value of f_6 was the largest. The largest total fraction of carboxymethylation (F) was when the solvent contained 20% water. With increasing water content in the solvent, the value of f_6 decreased. In water only solvent, the fraction of carboxymethylation at each position of 6, 3 and 2 was very similar, and the total fraction of carboxymethylation was at the lowest value. That means in the water solvent at 50 °C, the substitution reaction had no selectivity between –OH and $-NH_2$ groups.

3.4. Solubility

The solubility of CM-chitosan 1–7 samples in aqueous solution of various pHs were shown in Table 2. Samples 1 and 2 were soluble over the pH range 1–13. As the reaction temperatures was increased above 20 °C, each CM-chitosan sample had water insoluble region, close to pH 7. The insoluble region may be due either to aggregation of highly acetylated chain segments or to amide formation subsequent to thermal drying. The region moved slightly to lower pH area with the increasing reaction temperature. According to Fig. 4, the increase in reaction temperature resulted in an increase in the degree of carboxymethylation. Therefore, higher substitutions result in the insoluble-region moving to lower pHs.

The same phenomena of CM-chitosan 8–12 samples, which were prepared from different reaction solvent, could be seen in Table 3. The CM-chitosan 9 had the highest degree of carboxymethylation (Fig. 5), and the water-insoluble region was in the lowest pH region. The results were that in the preparation of CM-chitosan the higher temperature and the lower ratio of water/isopropanol increased the fraction of carboxymethylation, and increased the fraction of

Table 2
Solubility of CM-chitosan in water of various pHs and preparation temperatures



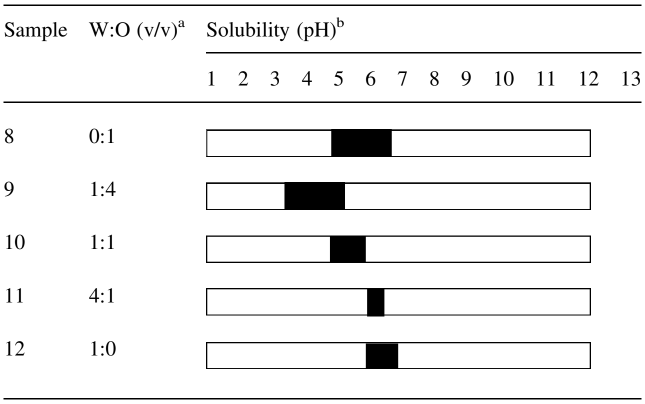
Sample	Temperature (°C)[a]	Solubility (pH)[b] 1 2 3 4 5 6 7 8 9 10 11 12 13
1	0	
2	10	
3	20	
4	30	
5	40	
6	50	
7	60	
Chitosan	–	

Solid sample (10 mg) was dispersed in H_2O (50 ml) on the base of method of Sashiwa et al. (1999).

[a] Preparation temperature of CM-chitosan.

[b] White bar: solution; black bar: insoluble.

Table 3
Solubility of CM-chitosan in water of various pHs, and in relation to the preparation solvents



Solid sample (10 mg) was dispersed in H_2O (50 ml) on the base of method of Sashiwa et al. (1999).

[a] Ratio of H_2O:$CH_3CH(OH)CH_3$ in solvent.

[b] White bar: solution; black bar: insoluble.

carboxymethylation resulting in the insoluble region of CM-chitosan moving to lower pHs.

4. Conclusion

The yields of CM-chitosan prepared in the mixed solvents were higher than in water alone or in isopropanol alone. The highest yields were close to 100% at water/-isopropanol ratios between 1/4 and 1/1 at 50 °C. The carboxymethyl groups were mostly substituted on the –OH groups, with a small amount on the $-NH_2$ groups. The 6-OH group had the highest degree of substitution. When the reaction temperatures were at 0 and 10 °C, the CM-chitosans had good water solubility, and at higher temperatures, the CM-chitosans were insoluble at near-neutral pHs. The pH for insolubility depended on the conditions used for modification and the reaction temperatures increased, the insolubilities were at the lower pHs; and as the ratios of water/isopropanol increased, the insolubilities were at the higher pHs.

Acknowledgements

Thus research was supported by the Korea Science and Engineering Foundation (Project number 1999-2-220-009-4).

References

Brugnerotto, J., Lizardi, J., Goycoolea, F. M., Arguelles-Monal, W., Desbrieres, J., & Rinaudo, M. (2001). An infrared investigation in relation with chitin and chitosan characterization. *Polymer*, *42*, 3569–3580.

Holme, K. R., & Perlin, A. S. (1997). Chitosan *N*-sulfate. A water-soluble polyelectrolyte. *Carbohydrate Research*, *302*, 7–12.

Jia, Z., Shen, D., & Xu, W. (2001). Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. *Carbohydrate Research*, *333*, 1–6.

Krause, T. J., Goldsmith, N. K., Ebner, S., Zazanis, G. A., & McKinnon, R. D. (1998). An inhibitor of cell proliferation associated with adhesion formation is suppressed by *N*,*O*-carboxymethyl chitosan. *Journal of Investigative Surgery: the Official Journal of the Academy of Surgical Research*, *11*, 105–113.

Kubota, N., & Eguchi, Y. (1997). Facile preparation of water-soluble N-acetylated chitosan and molecular weight dependence of its water-solubility. *Polymer Journal*, *29*(2), 123–127.

Kubota, N., Tatsumoto, N., Sano, T., & Toya, K. (2000). A simple preparation of half N-acetylated chitosan highly soluble in water and aqueous organic solvents. *Carbohydrate Research*, *324*, 268–274.

Liu, X. F., Guan, Y. L., Yang, D. Z., Li, Z., & Yao, K. D. (2001). Antibacterial action of chitosan and carboxymethylated chitosan. *Journal of Applied polymer Science*, *79*, 1324–1335.

Machida, Y., Nagai, T., Abe, M., & Sannan, T. (1986). Use of chitosan and hydroxypropylchitosan in drug formulations to effect sustained release. *Drug Design and Delivery*, *1*, 119–130.

Muzzarelli, R. A. A., Ilari, P., & Petrarulo, M. (1994). Solubility and structure of *N*-carboxymethylchitosan. *International Journal of Biological Macromolecules*, *16*, 177–180.

Ouchi, T., Nishizawa, H., & Ohya, Y. (1998). Aggregation phenomenon of PEG-grafted chitosan in aqueous solution. *Polymer*, *39*(21), 5171–5175.

Ragnhild, J., Hjerde, N., Varum, K. M., Grasdalen, H., Tokura, S., & Smidsrod, O. (1997). Chemical composition of *O*-(carboxymethyl)-chitins in relation to lysozyme degradation rates. *Carbohydrate Polymers*, *34*, 131–139.

Rinaudo, M., Le Dung, P., Gey, C., & Milas, M. (1992). Substituent distribution on *O*,*N*-carboxymethylchitosans by ^{1}H and ^{13}C NMR. *International Journal of Biological Macromolecules*, *14*, 122–128.

Sakairi, N., Suzuki, S., Ueno, K., Han, S. M., Nishi, N., & Tokura, S. (1998). Biosynthesis of hetero-polysaccharides by *Acetobacter xylinum*—Synthesis and characterization of metal–ion adsorptive properties of partially carboxmethylated cellulose. *Carbohydrate Polymers*, *37*, 409–414.

Sashiwa, H., & Shigemasa, Y. (1999). Chemical modification of chitin and chitosan 2: Preparation and water-soluble property of N-acetylated or N-alkylated partially deacetylated chitins. *Carbohydrate Polymers*, *39*, 127–138.

Shigemasa, Y., Matsuura, H., Sashiwa, H., & Saimoto, H. (1996). Evaluation of different absorbance ratios from infrared spectroscopy for analyzing the degree of deacetylation in chitin. *International Journal of Biological Macromolecules*, *18*, 237–242.

Zhang, L., Guo, J., Zhou, J., Yang, G., & Du, Y. (2000). Blend membranes from carboxymethylated chitosan/alginate in aqueous solution. *Journal of Applied Polymer Science*, *77*, 610–616.

Zhao, X., Kato, K., Fukumoto, Y., & Nakamae, K. (2001). Synthesis of bioadhesive hydrogels from chitin derivatives. *International Journal of Adhesion and Adhesives*, *21*, 227–232.