Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 73 (2008) 173–177
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Short communication

Synthesis and hydroxyl radicals scavenging activity of quaternized carboxymethyl chitosan

Zhanyong Guo [a,b], Ronge Xing [b], Song Liu [b], Zhimei Zhong [b], Pengcheng Li [b,*]

[a] *Yantai Institute of Coastal Zone Research for Sustainable Development, Chinese Academy of Sciences, Yantai 264003, China*
[b] *Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China*

Received 16 July 2007; received in revised form 22 October 2007; accepted 29 October 2007
Available online 6 November 2007

Abstract

In order to determine the effect of the forms of the amido groups of chitosan on antioxidant activity, quaternized carboxymethyl chitosan (QCMC) derivatives were prepared with a degree of quaternization ranging from 34.3% to 59.5%. The antioxidant activity of QCMCs against hydroxyl radicals was assessed. The results indicated that QCMCs have better hydroxyl radicals scavenging activity than that of carboxymethyl chitosan, as a result of the positive charge of the quaternized chitosan.

Keywords: Quaternized carboxymethyl chitosan; Synthesis; Antioxidant activity

1. Introduction

Chitosan, the deacetylated derivative of chitin, is the second most abundant polysaccharide found on the earth next to cellulose. As one of the natural renewable resources, chitosan has attracted people's attention for its physicochemical characteristics and bioactivities (Avadi et al., 2004; EI Ghaouth, Arul, Wilson, & Benhamou, 1997; Guo et al., 2005; Lim & Hudson, 2004; Zhang, Liu, Han, & Wang, 2007). However, because of its poor solubility above pH 6.5 chitosan shows its bioactivities only in acidic medium. In order to improve its aqueous solubility, many derivatives of chitosan have been synthesized, and carboxymethyl chitosan (CMC) is the most important one. Because of its solubility the use of CMC enlarges the scope of the application of chitosan (Muzzarelli, 1988; Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982). Generally, CMC is obtained by the reaction of chitosan and chloroacetic acid in 2-propanol in the presence of NaOH. Unfortunately, there have been few further studies on the modification of CMC. In the CMC molecule, the degree of substitution (DS) of NH_2 groups 0.1–0.2 (Chen & Park, 2003; Chen et al., 2004), So there are enough NH_2 groups to take part in other reactions for CMC that is obtained from highly deacetylated chitosan.

The quaternization of chitosan gives rise to an important derivative of chitosan and it's bioactivities have been reported (Domard, Rinaudo, & Terrassin, 1986; Muzzarelli & Tanfani, 1985). In this paper, quaternized carboxymethyl chitosan (QCMC), another novel kind of derivative of chitosan was synthesized by the quaternization of the active NH_2 groups of CMC. And the antioxidant activity against hydroxyl radicals was assessed in order to determine the effect of the forms of the amido groups of chitosan on antioxidant activity.

2. Experimental

Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China). Its degree of deacetylation was

---

Abbreviations: TCMC, *N*,*N*,*N*-trimethyl carboxymethyl chitosan; EDCMC, *N*-ethyl-*N*,*N*-dimethyl carboxymethyl chitosan; PDCMC, *N*-propyl-*N*,*N*-dimethyl carboxymethyl chitosan; BDCMC, *N*-butyl-*N*,*N*-dimethyl carboxymethyl chitosan; IBDCMC, *N*-isobutyl-*N*,*N*-dimethyl carboxymethyl chitosan.

[*] Corresponding author. Tel.: +86 532 82898707; fax: +86 532 829689561.

E-mail addresses: qdioqdio@yahoo.com.cn (Z. Guo), pcli@ms.qdio.ac.cn (P. Li).

0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.10.021

97%, and the viscosity-average molecular weight was 2.0×10^5. Formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, sodium iodide (anhyd NaI), sodium borohydride ($NaBH_4$), and iodomethane (CH_3I) were purchased from the Sigma–Aldrich Chemical Co. The IR spectra were measured on a Nicolet Magne-Avatar 360 with KBr disks. ^1H NMR spectra were recorded on a Bruker Apx 500 (500 MHz) NMR spectrometer in Dimethyl Sulphoxide (DMSO) solvent. The other reagents were all analytical grade and were used without further purification.

CMC was prepared according to Chen et al. (2004), QCMCs were synthesized as follows (Guo, Liu, Chen, Ji, & Li, 2006; Jia, Shen, & Xu, 2001): 3 g CMC was dissolved in 100 mL of H_2O, and various aldehydes (3-fold excess to added CMC) were added, respectively, with stirring at room temperature. After 2 h, 10% $NaBH_4$ (1.5-fold excess relative to aldehyde) was added, and the solutions were reacted for 2 h. The mixture was precipitated in acetone and filtered. The *N*-substituted CMCs were obtained after drying at 60 °C for 24 h. *N*-substituted CMC (1 g) was dispersed in 50 mL of *N*-methyl-2-pyrrolidone (NMP) for 12 h at rt. Then, 0.5 mL NaOH (1 M), 1 g NaI and 4 mL CH_3I were added. Each reaction was carried out with stirring at 50 °C for 20 h. The product was obtained by precipitation with excess acetone, and the QCMCs were obtained by drying at 60 °C for 24 h (see Scheme 1).

The antioxidant activity was carried out according to Wang (Wang et al., 1994). The reaction mixture, total volume 4.5 mL, containing the samples of chitosan derivatives (CMC, TCMC, EDCMC, PDCMC, BDCMC, and IBDCMC), was incubated with EDTA–Fe^{2+} (220 μM), safranine O (0.23 μM), H_2O_2 (60 μM) in potassium phosphate buffer (150 mM, pH 7.4) for 30 min at 37 °C. The absorbance of the mixture was measured at 520 nm. Hydroxyl radicals bleached the safranine O, so increased absorbance of the reaction mixture indicated decreased hydroxyl radicals scavenging ability and the capability of scavenging hydroxyl radicals was calculated using the follow equation:

$$\text{Scavenging effect } (\%) = [(A_{\text{sample 520nm}} - A_{\text{blank 520nm}}) / (A_{\text{control 520nm}} - A_{\text{blank 520nm}})] \times 100$$

where $A_{\text{blank 520nm}}$ was the absorbance of the blank (distilled water instead of the samples), $A_{\text{control 520nm}}$ was the absorbance of the control (distilled water instead of H_2O_2).

All data were expressed as means ± SD and analyzed by an analysis of variance ($P < 0.05$) and the means separated

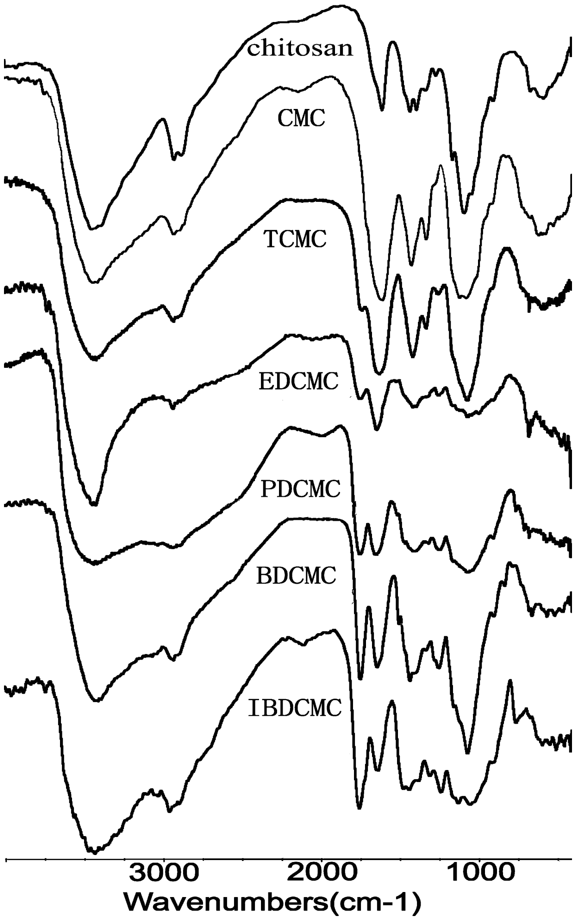
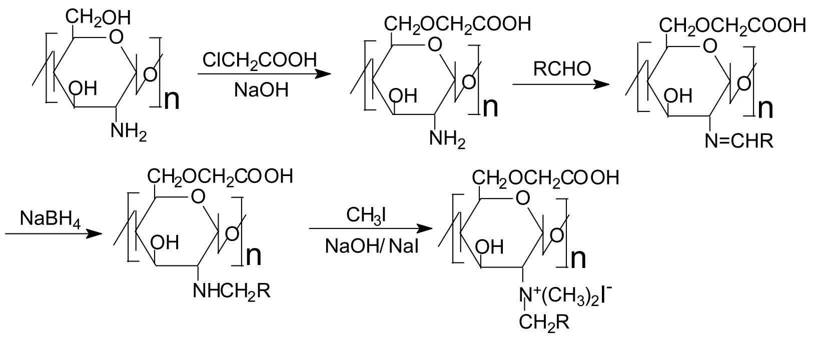


Fig. 1. IR spectra data of chitosan, CMC, and QCMC.



TCMC: R=H; EDCMC: R=CH_3; PDCMC: R=CH_3CH_2; BDCMC: R=$CH_3CH_2CH_2$; IBDCMC: R=$(CH_3)_2CH$

Scheme 1. Synthetic pathway for QCMCs.

by Duncan's multiple range test. The results were processed by computer programme: Excel and Statistical software (1999).

3. Results and discussion

3.1. The structure of QCMC

The IR spectra of chitosan, CMC and QCMC are shown in Fig. 1, and the ^{1}H NMR spectra are shown in Fig. 2. As shown in Fig. 1 the IR spectrum of chitosan shows peaks assigned to the saccharide structure at 895 and 1155 cm^{-1} and a characteristic amino peak at around 1600 cm^{-1}. CMC is confirmed by a strong absorption band in the 1600 region [$\gamma_{sym}(CO_2)$] and 1416 cm^{-1} [$\gamma_{as}(CO_2)$] (Sun, Xie, & Xu, 2004). All the QCMC compounds have strong peaks at about 1730 cm^{-1}, which were assigned to the carboxylic acid groups (as the reaction progresses, H^+ is released and the —COO$^-$ groups were protonated to —COOH groups). Characteristic peaks of amine (N—H) vibration deformation appeared at 1600 cm^{-1} for chitosan. After quaternization, new peaks appear at high wave numbers, which are assigned to the quaternary ammonium salt. There are peaks at about 1415–1430 cm^{-1}, which are assigned to the characteristic absorption of N—CH$_3$ (Jia et al., 2001). The ^{1}H NMR (in DMSO) spectra data shows signals at about δ 2.85 (H-9) indicated that the —CH$_3$ was grafted in all the QCMC compounds. The signals at δ from 1.8 to 2.2 are the N—H groups which not reacted, and the signals at about 2.6 are the *N*-alkyl groups which not quaternized. And it also indicates the degree of substitution and quaternization is incomplete. And the —COOH groups of QCMC compounds are confirmed by the δ at about 12.8. The above-mentioned results demonstrate that the QCMC is obtained.

The degree of the quaternization of the chitosan derivatives was determined by Domard's method (1986). Potentiometric titration of the halide form was carried out with aqueous silver nitrate, using a calomel electrode as the reference, and a silver electrode for the measurement (Jia et al.,

Table 1
The yields and the degree of quaternization of QCMC

Compounds	Yields (%)[a]	Degree of quaternization (%)
TCMC	33.1	34.3
EDCMC	39.2	38.6
PDCMC	49.3	48.6
BDCMC	51.5	53.2
IBDCMC	58.3	59.5

[a] Yield is the ratio of QCMC and added CMC.

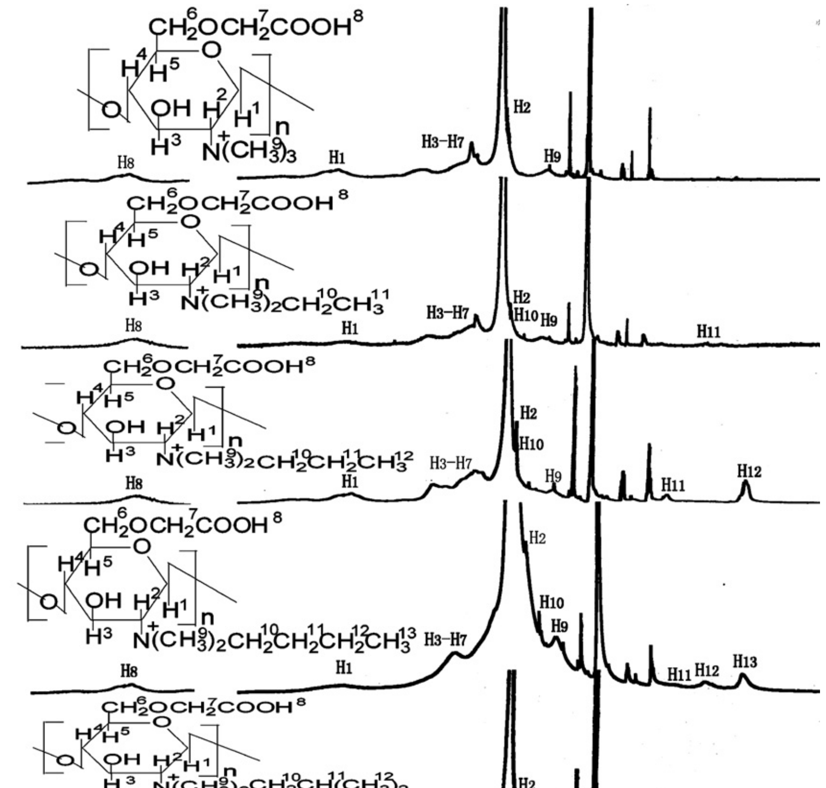


Fig. 2. ^{1}H NMR spectra data of QCMCs.

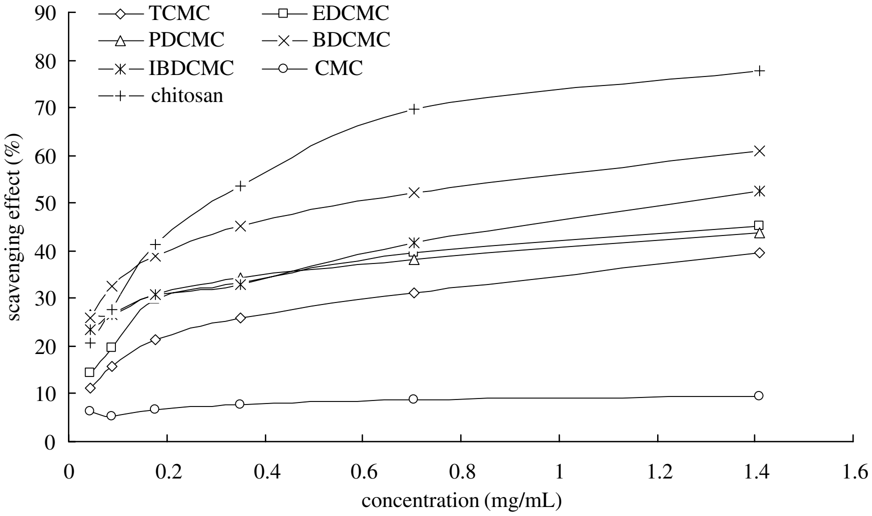


Fig. 3. Scavenging effect of chitosan, CMC, and QCMCs on hydroxyl radicals. Each value is expressed as mean ± SD ($n = 3$).

2001). As shown in Table 1, the yields and the degree of quaternization varied with the different aldehydes, and they are increased from TCMC to IBDCMC. These may be explained as follows: There are intramolecular hydrogen bonds in the molecules of chitosan which affect the reactivity of chitosan. After modification, some of the intramolecular hydrogen bonds of chitosan are destroyed and the reactivity is improved. The degree of destruction of these bonds may be affected by the size of the groups grafted to chitosan, with (large groups generally improving the reactivity of chitosan.

3.2. The hydroxyl radicals scavenging activity of chitosan, CMC, and QCMC

As shown in Fig. 3, chitosan has the best scavenging ability against hydroxyl radicals, and the IC_{50} is 0.32 mg/mL. Compared with chitosan, CMC has slight scavenging activity against hydroxyl radicals, with a scavenging index of 9.3% at a concentration of 1.4 mg/mL. Polysaccharides with a scavenging effect on the hydroxyl radical have the same structural feature in that all of them have one or more alcohol or phenolic hydroxyl groups. The scavenging ability is related to the number of active hydroxyl groups in the molecule. The scavenging activity of chitosan on hydroxyl radicals is also due to the active hydroxyl groups. Part of the active hydroxyl groups are substituted in the molecule of carboxymethyl chitosan, and it causes a decrease of the scavenging activity against hydroxyl radicals (Guo et al., 2006).

Both hydroxyl groups and amido groups affect the bioactivity of chitosan, and our previous work has investigated the effect of the forms of the amido groups of chitosan on antioxidant activity. Quaternized chitosan has better scavenging ability on hydroxyl radical than chitosan, Schiff bases of chitosan and *N*-substituted chitosan, which caused by the positive charge of quaternized chitosan (Guo et al., 2006). From our work in this paper, all QCMC derivatives show better scavenging activity against hydroxyl radicals than CMC. Thus, the scavenging activity of positive charge on hydroxyl radicals scavenging activity is proved again. Moreover, as shown in Fig. 3, among all the QCMC derivatives, the scavenging activity is increased from TCMC to IBDCMC, according with the change of the degree of quaternization. The results show the influence of the positive charge on the scavenging activity against hydroxyl radicals.

4. Conclusion

The quaternization of CMC gives rise to a novel kind of derivative of chitosan–QCMC, and it's scavenging activity is better than CMC, in agreement with a previous report. The positive charge of quaternized chitosan and QCMC should be the key of the better activity of these derivatives of chitosan, and should form the basis for further research.

References

Avadi, M. R., Sadeghi, A. M. M., Tahzibi, A., Bayati, K., Pouladzadeh, M., Zohuriaan-Mehr, M. J., et al. (2004). Diethylmethyl chitosan as an antimicrobial agent: Synthesis, characterization and antibacterial effects. *European Polymer Journal, 40*(7), 1355–1361.

Chen, X. G., & Park, H. J. (2003). Chemical characteristics of *O*-carboxymethyl chitosans related to the preparation conditions. *Carbohydrate Polymers, 53*(4), 355–359.

Chen, S. C., Wu, Y. Y., Mi, F. L., Lin, Y. H. L., Yu, C., & Sung, H. S. . *Journal of Controlled Release, 96*(2), 285–300.

Domard, A., Rinaudo, M., & Terrassin, C. (1986). New method for the quaternization of chitosan. *International Journal of Biological Macromolecules, 8*(2), 105–107.

EI Ghaouth, A., Arul, J., Wilson, C., & Benhamou, N. (1997). Biochemical and cytochemical aspects of the interactions of chitosan and *Botrytis cinerea* in bell pepper fruit. *Postharvest Biology and Technology, 12*(2), 183–194.

Guo, Z. Y., Liu, H. Y., Chen, X. L., Ji, X., & Li, P. C. (2006). Hydroxyl radicals scavenging activity of *N*-substituted chitosan and quaternized chitosan. *Biorganic & Medical Chemistry Letters, 16*(24), 6348–6350.

Guo, Z. Y., Xing, R. E., Liu, S., Yu, H. H., Wang, P. B., Li, C. P., et al. (2005). The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl. *Chitosan Biorganic & Medicinal Chemistry Letter, 15*(24), 4600–4603.

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

Lim, S.-H., & Hudson, S. M. (2004). Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. *Carbohydrate Research, 339*(2), 313–319.

Muzzarelli, R. A. A. (1988). Carboxymethylated chitins and chitosans. *Carbohydrate Polymers, 8*(1), 1–21.

Muzzarelli, R. A. A., & Tanfani, F. (1985). The *N*-permethylation of chitosan and the preparation of *N*-trimethyl chitosan iodide. *Carbohydrate Polymers, 5*(4), 297–307.

Muzzarelli, R. A. A., Tanfani, F., Emanuelli, M., & Mariotti, S. (1982). *N*-(carboxymethylidene) chitosans and *N*-(carboxymethyl) chitosans: Novel chelating polyampholytes obtained from chitosan glyoxylate. *Carbohydrate Research, 107*(16), 199–214.

Sun, T., Xie, W. M., & Xu, P. X. (2004). Superoxide anion scavenging activity of graft chitosan derivatives. *Carbohydrate Polymers, 58*(4), 379–382.

Wang, J. C., Xing, G. S., Hu, W. D., Zhu, T. L., Wang, Q., & Zhao, H. (1994). Effects of Ge-132 on oxygen free radicals and the lipid peroxidation induced by hydroxyl free radical in vitro. *Chinese Pharmaceutical Journal, 29*, 23–25.

Zhang, L., Liu, W. S., Han, B. Q., & Wang, D. F. (2007). Synthesis and antitumor activity of arginine–glucosamine conjugate. *Carbohydrate Polymers, 69*(4), 644–650.