Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 68 (2007) 761–765
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Study on the heterogeneous degradation of chitosan with hydrogen peroxide under the catalysis of phosphotungstic acid

Qun Zeng Huang [a,*], Shi Ming Wang [b], Jin Feng Huang [b], Li Hong Zhuo [a], Ying Chen Guo [a]

[a] College of Chemistry and Pharmacy Engineering, Nanyang Normal University, Nanyang, China
[b] College of Chemistry and Materials Science, Fujian Normal University, Fuzhou, China

Received 9 July 2006; received in revised form 2 August 2006; accepted 21 August 2006
Available online 7 November 2006

Abstract

The oxidative degradation of chitosan with H_2O_2 aqueous solution was carried out under the catalysis of phosphotungstic acid in heterogeneous phase. The optimal conditions of degradation were determined by orthogonal tests. The structure of the degraded product was characterized by Fourier-transform infrared spectra (FTIR), diffuse reflectance spectra (DRS) and X-ray diffraction (XRD) analysis. The mechanism of the degradation was correlated with cleavage of the glycosidic bond. The experimental results showed that chitosan can be effectively degraded with H_2O_2 under the catalysis of phosphotungstic acid.

Keywords: Chitosan; Phosphotungstic acid; H_2O_2; Catalysis; Degradation

1. Introduction

Chitosan, (1 → 4)-2-amino-2-deoxy-β-D-glucan, is a natural polymer generally obtained by full or partial deacetylation of chitin. Due to many unique properties such as biocompatibility, biodegradability, bioactivity and non-toxicity (Carmen & Roland, 1997; Cheng et al., 2003), chitosan has broad range of actual and potential applications. Much attention is focused on the use of chitosan in many areas such as biotechnology, pharmaceuticals, wastewater treatment, cosmetics, agriculture, food science and textiles, etc. (Li, Dunn, Grandmaison, & Goosen, 1997).

However, chitosan has a high molecular weight resulting in its low solubility in most solvents, which limits its wide applications especially in medicine and the food industry. To improve its solubility and biological, chemical and physical properties, several methods have been tried to prepare a water-soluble low molecular weight chitosan (LWCS) without altering its chemical structure, some of which show advantages, but also disadvantages (Rege & Block, 1999; Tanioka et al., 1996; Terbojevich, Cosani, & Muzzarelli, 1996; Vårum, Ottøy, & Smidsrød, 2001; Wang, Huang, & Wang, 2005; Zhang & Neau, 2002).

H_2O_2 has long been used in the treatment of chitosan because it is easy to handle, easily available and environmentally friendly (Chang, Tai, & Cheng, 2001; Qin, Du, & Xiao, 2002; Shao, Yang, & Zhong, 2003). This technique is based on the formation of free radicals, which can attack the β-D-(1 → 4) glucosidic linkages of chitosan. However, the formation of radical groups is inefficient when H_2O_2 is used alone. Recently, to improve the efficiency, other degradation patterns of chitosan with H_2O_2 have been reported (Shao et al., 2003; Wang et al., 2005). But to date there have been few reports about the degradation with H_2O_2 under the catalysis of heteropoly acids in heterogeneous phase.

Heteropoly acids with Keggin anion structures have received considerable attention due to many advantages (Okuhara, 2002; Okuhara, Mizuno, & Misono, 2001), such as simple preparation, high reactivity, non-corrosive, non-pollutive and excellent stability. Specifically, phosphotungstic acid is among the most extensively studied (Dias, Caliman, Dias, Paulo, & de Souza, 2003; Hu & Xu, 2004;

* Corresponding author. Tel./fax: +86 377 63513540.
E-mail address: qz_huangny@126.com (Q.Z. Huang).

0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.08.015

Kozhevnikova & Kozhevnikov, 2004) since it possesses high acidic strength and relatively high thermal stability (Devassy et al., 2005), which can be used as acid, oxidative and bifunctional catalysts in homogeneous or heterogeneous phase.

Usually the oxidative degradation of chitosan with H_2O_2 occurs in homogeneous phase, such as in acetic acid solution. However, in this paper, the degradation in heterogeneous phase was studied, which avoided using acetic acid, furthermore, made the precipitation process of LWCS convenient. The effect of volume of H_2O_2, dosage of phosphotungstic acid, reaction temperature and time on the degradation was discussed by orthogonal tests. The degradation mechanism was also discussed by FTIR, DRS and XRD analysis.

2. Experimental details

2.1. Materials

Original chitosan, obtained from Yuhuan Biology Engineering (Zhejiang, China), whose degrees of acetylation is 95.54%, its viscosity-average molecular weight (Mv) is about 700,000, determined based on viscosity measurements (Wang et al., 2005). Phosphotungstic acid, phosphomolybdic acid, tungstosilicic acid, hydrogen peroxide and other reagents used, supplied by Fuzhou Chemical Agent Corporation (Fujian, China), were utilized without further purification.

2.2. Heterogeneous degradation of chitosan

Chitosan 1.5000 grams was put into 50 mL conical flask, then phosphotungstic acid, H_2O, 30% (wt%) H_2O_2 aqueous solutions were also added. The volume of mixing solution was 20 mL. The solution was stirred and reacted at the desired temperature for different duration. After the reaction, the solution was filtrated, the collected solid was washed with distilled water until reaching pH 7, and then dried at 50 °C in vacuum. LWCS was precipitated by adding ethanol to the filtrate and collected after drying in vacuum.

2.3. Characterization techniques

The degradation ratio of chitosan was calculated according to the following equation:

$$R(\%) = \frac{M_0 - M_x}{M_0} \times 100 \quad (1)$$

where R refers to degradation ratio, M_0 refers to the quantity of original chitosan, M_x refers to the quantity of collected solid chitosan without degraded at different condition, respectively.

The IR spectra of original chitosan and LWCS were obtained using a Nicolet Nexus 670 FTIR spectrometer using KBr pellets, respectively.

The DRS of original chitosan and LWCS were recorded on a Cary-500 Scan UV–vis-NIR spectrophotometer equipped with a HARRICK diffuse reflectance accessory.

X-ray diffraction patterns of original chitosan and LWCS were carried out on a XPERT PRO diffractometer and used a CuKα target at 40 kV and 50 mA.

3. Results and discussion

3.1. Oxidative degradation of chitosan under the catalysis of different heteropoly acids

In order to reveal the contrast of catalysis between phosphotungstic acid and other heteropoly acids, four experiments on the degradation of chitosan were designed. In these experiments, catalyst was tungstosilicic acid, phosphotungstic acid and phosphomolybdic acid in experiment 1, 2 and 3, respectively, the quantity of each catalyst was 0.02 g. In experiment 4, catalyst was not used. The other reaction conditions were determined as follows: chitosan 1.5000 g, 30% (wt%) H_2O_2 3 mL, H_2O 17 mL, reaction temperature 70 °C, reaction time 30 min. The degradation ratio of chitosan was confirmed according to Eq. (1). The contrast of degradation ratio under different condition is depicted in Fig. 1.

As can be seen from Fig. 1, the degradation ratio is only 43% without catalyst, which indicated that the degradation is inefficient when H_2O_2 was used alone. But in the presence of tungstosilicic acid, phosphotungstic acid and phosphomolybdic acid, the degradation ratio increased, which added up to 64,100 and 79%, respectively. Therefore, it can be predicated that phosphotungstic acid is the best catalyst for oxidative degradation of chitosan with H_2O_2 in heterogeneous phase.

3.2. Orthogonal test

The optimal condition for degrading chitosan was studied by an orthogonal test. Four controllable variables, the

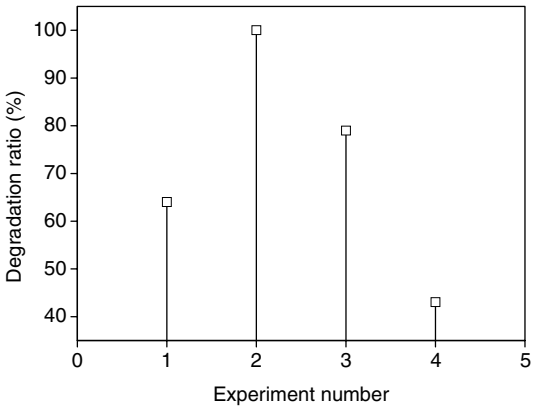

Fig. 1. The contrast of degradation ratio under different conditions.

volume of H_2O_2, reaction temperature and time, dosage of catalyst were selected, each at three levels. The variables were investigated and their test levels were listed in Table 1.

Reference to the experimental design theory, the orthogonal array L_9 (3^4) was selected to arrange the test program. The test results were listed in Table 2.

Obviously, the order of influence of each variable is C > D > B > A. For each variance, level 3 is the best according to the theory of orthogonal test, so the optimum level of each variable is $A_3B_3C_3D_3$. However, for dosage of phosphotungstic acid, the value of K_2 is very close to that of K_3, that is to say, 0.02 g of phosphotungstic acid is sufficient for the reaction system, therefor, level 2 is chosen for the sake of the saving of catalyst. Similarly, for reaction temperature, level 2 is chosen in order to make reaction condition moderate. Thus, the optimum level of each variable is $A_2B_3C_3D_2$, the optimum reaction conditions were determined as follows: 30% (wt%) H_2O_2 3 mL, dosage of phosphotungstic acid 0.02 g, reaction temperature 70 °C, reaction time 30 min.

Chitosan was degraded in parallel for three times under the optimum reaction conditions, each degradation ratio is 100%. LWCS was precipitated by adding ethanol and its *M*v is about 4700 analyzed by viscometry measurement (Wang et al., 2005).

3.3. FTIR spectral analyses

FT-IR spectroscopy has been shown to be a powerful tool for the study of the physicochemical properties of polysaccharides. Curves a and b in Fig. 2 show the IR spectra of initial chitosan and LWCS produced under the optimum reaction conditions.

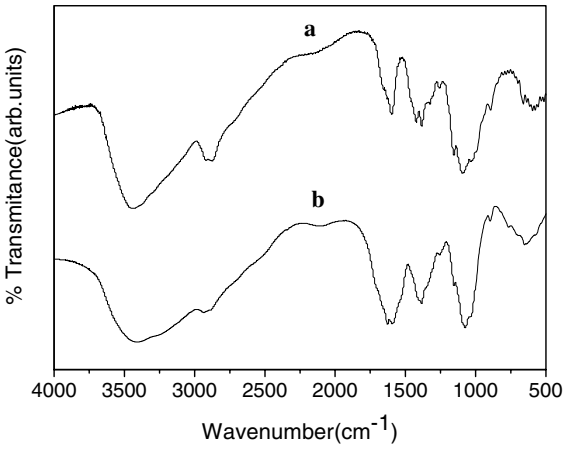

Fig. 2. FTIR spectra of (a) original chitosan and (b) LWCS.

The main bands in the spectrum of original chitosan are as follows: Both the N–H stretching and O–H stretching vibrations can be characterized by a strong, broad band centered at 3440 cm^{-1}. The peak at 2875 and 2920 cm^{-1} is due to C–H stretching. The symmetrical deformation peak of $–CH_3$ and $–CH_2$ was observed at 1422 cm^{-1}. The bands at 1602 and 599 cm^{-1} correspond to the binding vibrations of the amido groups, the bands in the range 1158–895 cm^{-1} are assigned to the characteristics of its polysaccharide structure (Peniche et al., 1999).

In comparison with the FTIR spectrum of chitosan, that of LWCS shows a new peak at 1634 cm^{-1}, which is assigned to absorbance of C=O, which indicates that the carboxyl or carbonyl groups do exist (Shao et al., 2003).

3.4. DRS spectral analyses

Curves a and b in Fig. 3 show the DRS of original chitosan and LWCS produced under the optimum reaction conditions.

Table 1
The variables investigated and their levels

Variables investigated	Levels of each variable		
	1	2	3
A: reaction temperature (°C)	60	70	80
B: reaction time (min)	10	20	30
C: volume of H_2O_2 (mL)	1	2	3
D: dosage of phosphotungstic acid (g)	0	0.02	0.04

Table 2
Experimental arrangement and test results

Experiment number	A	B	C	D	Degradation ratio (%)
1	1	1	3	2	56
2	2	1	1	1	2
3	3	1	2	3	57
4	1	2	2	1	15
5	2	2	3	3	100
6	3	2	1	2	56
7	1	3	1	3	40
8	2	3	2	2	79
9	3	3	3	1	69
K_1	111	115	98	86	
K_2	181	171	151	191	
K_3	182	188	225	197	
Variance	71	73	127	111	

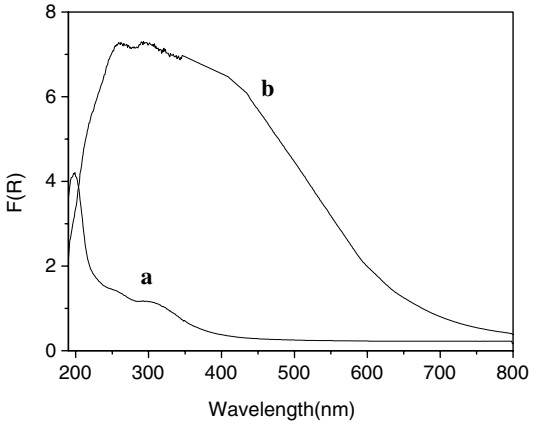

Fig. 3. DRS patterns of (a) original chitosan and (b) LWCS.

As can be seen, there is an absorption band at 200 nm in curve a, which was caused by the $n \rightarrow \sigma^*$ transition of amido groups in chitosan. For LWCS, two strong absorption bands were exhibited, as shown in curve b, one band at 260 nm corresponds to the $n \rightarrow \sigma^*$ transition of amido groups, the other absorption band at 300 nm is assigned to the $n \rightarrow \pi^*$ transition of carbonyl or carboxyl groups (Wang et al., 2005). It is most likely that the new side group is formed during the degradation of chitosan.

3.5. X-ray analysis

The X-ray diffractograms of original chitosan and LWCS are shown in Fig. 4. The pattern of original chitosan shows the characteristic peak at $2\theta = 10.09°$ and 20.38°, which are assigned to (020) and (100) reflection (Kim & Lee, 1993; Tian, Liu, Hu, & Zhao, 2003), respectively.

For LWCS, the peak at $2\theta = 10.09°$ disappeared and the characteristic peak at $2\theta = 20.38°$ decreased obviously. It can be thought that the crystalline structure of LWCS was destroyed and the crystallinity decreased in a certain extent, indicating that it was considerably more amorphous than original chitosan. Hence, the degradation reaction took place preferentially in the amorphous region and then proceeded very moderately from the edge to the inside of the crystalline region. With deep degradation, the crystalline structure will be destroyed thoroughly and the crystallinity disappeared completely.

3.6. Effect of reaction time on Mv of chitosan

Chitosan was degraded under the optimum reaction conditions for 30, 60, 90 and 120 min. After reaction, the resulting LWCS was obtained. Then the *M*v of chitosan is plotted as a function of the reaction time. As shown in Fig. 5, the *M*v of chitosan dropped sharply from 700,000 to 4700 within 30 min. It indicated that H_2O_2 was decomposed effectively and a great number of free radicals were produced within 30 min under the catalysis of phosphotungstic acid. These free radicals attacked the β-D-(1 → 4) glucosidic linkages of chitosan, which made the *M*v of chitosan decrease in a short time. However, when chitosan was degraded continuously after 30 min, the *M*v decreased slowly, finally reached 4300 after120 min. It was obvious that the decreasing value of *M*v was only 400 from 30 to 120 min, which indicated that the effect of phosphotungstic acid on the decomposing of H_2O_2 was very slight after 30 min.

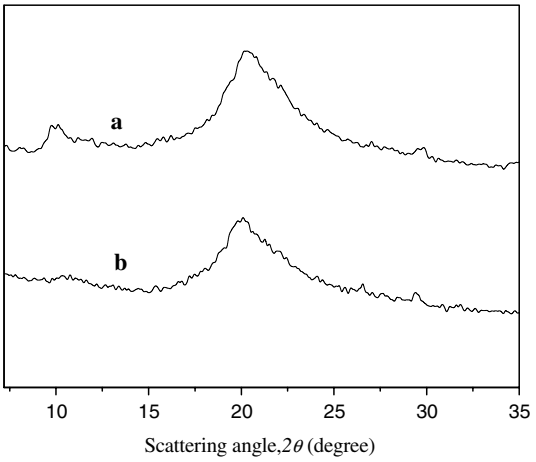

Fig. 4. X-ray diffraction patterns of (a) original chitosan and (b) LWCS.

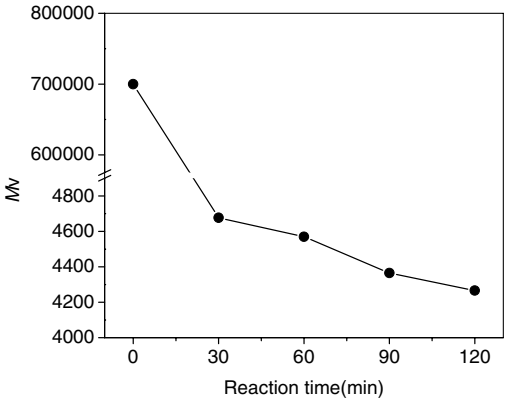

Fig. 5. Effect of reaction time on *M*v of LWCS.

3.7. Mechanistic discussion

The studies of Chang et al. (Chang et al., 2001) and Tian et al. (Tian et al., 2003) suggest that the homogenous degradation system of chitosan with H_2O_2 is predominantly caused by radical reactions. They thought that H_2O_2 is decomposed to HO·, which is a very powerful oxidant, attacked the β-D-(1 → 4) glycosidic bonds of chitosan and subsequently broke the glycosidic linkages.

In fact, the homogenous degradation with H_2O_2 mostly occurred in dilute acetic acid aqueous solutions. In acid systems, the degradation reaction was affected by the concentration of H^+. If $[H^+]$ is low, The repellency affected by protonated amine groups with low concentration makes chitosan stretched, which causes the decrease of steric shield of amino groups. So the degradation reaction readily occurred. When $[H^+]$ is high, the steric shield of the amino groups will increase, which makes the degradation be difficult (Tian et al., 2003). However, the heterogeneous degradation avoided the disadvantage, because in the system chitosan was not necessarily dissolved in dilute acid solution. In this work, a free radical degradation mechanism of chitosan with H_2O_2 under the catalysis of phosphotungstic acid was proposed in the following.

Phosphotungstic acid, which is a solid super acid, it was completely decompose to H^+ and $[PW_{12}O_{40}]^{3-}$ in aqueous solution, then these protons will bond with the amine groups of chitosan molecules to form the electron-absent $R–NH_3^+$, the repellency of protonated amine groups made chitosan stretched, which causes the destroying of ordered

structure and decrease of crystallinity of chitosan, as described in the X-ray diffraction patterns. Thus the corresponding reactions will be readily carried out. Subsequently, the heteropoly anions, $[PW_{12}O_{40}]^{3-}$, will be pulled by the protonated amine groups of chitosan molecules via electrostatic attraction. These restricted heteropoly anions reacts with H_2O_2 and produces another heteropoly anion, $\{PO_4[WO(O_2)_2]_4\}^{3-}$, as shown as below:

$$[PW_{12}O_{40}]^{3-} + 8H_2O_2 = \{PO_4[WO(O_2)_2]_4\}^{3-} + 8H_2WO_4 \quad (2)$$

$\{PO_4[WO(O_2)_2]_4\}^{3-}$, which has strong reactive activity at high temperature, will react with H_2O_2 continually and lead to the rapid formation of hydroxyl radical (HO·) and hydroperoxide radical (HO_2·) (Neumann & de la Vega, 1993), as shown in the following equations:

$$\{PO_4[WO(O_2)_2]_4\}^{3-} + H_2O_2 = H\{PO_4[WO(O_2)_2]_4\}^{2-} + HO_2\cdot \quad (3)$$

$$H\{PO_4[WO(O_2)_2]_4\}^{2-} + H_2O_2 = \{PO_4[WO(O_2)_2]_4\}^{3-} + HO\cdot + H_2O \quad (4)$$

HO· and HO_2· are much more powerful oxidant, can quickly attack the stretching chitosan main chain which is composed of pyranose rings linked by β-(1,4) glycosidic bond. Consequently, it is reasonable to believe that free radicals would attack the C-1 or C-4 carbon and the adjacent C–O–C glycosidic bond is degraded into two shorter chains, one chain should end with a carbonyl group in the terminal ring (Hsu, Don, & Chiu, 2002), concluded by the FT-IR and DRS spectral analyses of LWCS.

4. Conclusions

In heterogeneous phase, chitosan was effectively degraded with H_2O_2 under the catalysis of phosphotungstic acid. The optimum reaction conditions determined by orthogonal tests were as follows: 30% (wt%) H_2O_2 3 mL, amount of phosphotungstic acid 0.02 g, reaction temperature 70 °C, reaction time 30 min.

By FTIR, DRS and XRD analysis, it was presumed that the mechanism of degradation be attributed to free radical formed by the reaction of phosphotungstic acid and H_2O_2, which result in the rupture of glycosidic linkages and the formation of carboxyl groups.

Compared with the oxidative degradation occurred in homogeneous phase, the method not only avoided using acetic acid, but also made the precipitation process of LWCS convenient, which may have potential applications.

References

Carmen, R. L., & Roland, B. (1997). Mechanical, water uptake and permeability properties of crosslinked chitosan glutamate and alginate films. *Journal of Controlled Release, 44*, 215–225.

Chang, K. L. B., Tai, M. C., & Cheng, F. H. (2001). Kinetics and products of the degradation of chitosan by hydrogen peroxide. *Journal of Agricultural and Food Chemistry, 49*, 4845–4851.

Cheng, M. Y., Deng, J. G., Yang, F., Gong, Y. D., Zhao, N. M., & Zhang, X. F. (2003). Study on physical properties and nerve cell affinity of composite films from chitosan and gelatin solutions. *Biomaterials, 24*, 2871–2880.

Devassy, B. M., Shanbhag, G. V., Lefebvre, F., Böhringer, W., Fletcher, J., & Halligudi, S. B. (2005). Zirconia-supported phosphotungstic acid as catalyst for alkylation of phenol with benzyl alcohol. *Journal of Molecular Catalysis A: Chemical, 230*, 113–119.

Dias, J. A., Caliman, E., Dias, S. C. L., Paulo, M., & de Souza, A. T. C. P. (2003). Preparation and characterization of supported $H_3PW_{12}O_{40}$ on silica gel: a potential catalyst for green chemistry processes. *Catalysis Today, 85*, 39–48.

Hsu, S. C., Don, T. M., & Chiu, W. Y. (2002). Free radical degradation of chitosan with potassium persulfate. *Polymer Degradation and Stability, 75*, 73–83.

Hu, M. Q., & Xu, Y. M. (2004). Photocatalytic degradation of textile dye X_3B by heteropolyoxometalate acids. *Chemosphere, 54*, 431–434.

Kim, J. H., & Lee, Y. M. (1993). Synthesis and properties of diethylaminoethyl chitosan. *Polymer, 34*, 1952–1957.

Kozhevnikova, E. F., & Kozhevnikov, I. V. (2004). A calorimetric study of the acidity of bulk and silica-supported heteropoly acid $H_3PW_{12}O_{40}$. *Journal of Catalysis, 224*, 164–169.

Li, Q., Dunn, E. T., Grandmaison, E. W., & Goosen, M. F. (1997). Application and properties of chitosan. In M. F. A. Goosen (Ed.), *Applications of chitin and chitosan* (pp. 3–29). Lancaster: Technomic Publishing.

Neumann, R., & de la Vega, M. (1993). Oxidation of alkylaromatic compounds with hydrogen peroxide catalyzed by mixed addenda Keggin heteropolyanions. *Journal of Molecular Catalysis, 84*, 93–108.

Okuhara, T. (2002). New catalytic functions of heteropoly compounds as solid acids. *Catalysis Today, 73*, 167–176.

Okuhara, T., Mizuno, N., & Misono, M. (2001). Catalysis by heteropoly compounds – recent developments. *Applied Catalysis A: General, 222*, 63–77.

Peniche, C., Waldo, A. M., Davidenko, N., Sastre, . R., Gallardo, A., & Roman, J. S. (1999). Self-curing membranes of chitosan/PAA IPNs obtained by radical polymerization: preparation, characterization and interpolymer complexation. *Biomaterials, 20*, 1869–1878.

Qin, C. Q., Du, Y. M., & Xiao, L. (2002). Effect of hydrogen peroxide treatment on the molecular weight and structure of chitosan. *Polymer Degradation and Stability, 76*, 211–218.

Rege, P. R., & Block, L. H. (1999). Chitosan processing: influence of process parameters during acidic and alkaline hydrolysis and effect of the processing sequence on the resultant chitosan's properties. *Carbohydrate Research, 321*, 235–245.

Shao, J., Yang, Y. M., & Zhong, Q. Q. (2003). Studies on preparation of oligoglucosamine by oxidative degradation under microwave irradiation. *Polymer Degradation and Stability, 82*, 395–398.

Tanioka, S., Matsui, Y., Irie, T., Tanigawa, T., Tanaka, Y., Shibata, H., et al. (1996). Oxidative depolymerisation of chitosan by hydroxyl radical. *Bioscience, Biotechnology, and Biochemistry, 60*, 2001–2004.

Terbojevich, M., Cosani, A., & Muzzarelli, R. A. A. (1996). Molecular parameters of chitosans depolymerized with the aid of papain. *Carbohydrate Polymers, 29*, 63–68.

Tian, F., Liu, Y., Hu, K., & Zhao, B. Y. (2003). The depolymerization mechanism of chitosan by hydrogen peroxide. *Journal of Materials Science, 38*, 4709–4712.

Vårum, K. M., Ottøy, M. H., & Smidsrød, O. (2001). Acid hydrolysis of chitosans. *Carbohydrate Polymers, 46*, 89–98.

Wang, S. M., Huang, Q. Z., & Wang, Q. S. (2005). Study on the synergetic degradation of chitosan with ultraviolet light and hydrogen peroxide. *Carbohydrate Research, 340*, 1143–1147.

Zhang, H., & Neau, S. H. (2002). In vitro degradation of chitosan by bacterial enzymes from rat cecal and colonic contents. *Biomaterials, 23*, 2761–2766.