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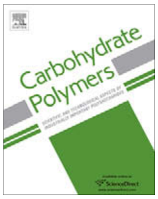

Ultraviolet radiation-induced accelerated degradation of chitosan by ozone treatment

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ABSTRACT

Combined with ultraviolet radiation as an accessory factor, ozone can result in accelerated degradation of chitosan. The ultraviolet radiation-induced accelerated degradation of chitosan was demonstrated by means of determination of viscosity-average molecular weight (Mv). In addition, the structure of the degraded chitosan obtained by ozone treatment combined with ultraviolet radiation was characterized by FT-IR, ^{13}C NMR, and X-ray diffraction spectral analysis. The chemical structure of the degraded chitosan sample was not obviously modified. There was no significant change of the total degree of deacetylation (DD) of degraded chitosan compared with the initial chitosan. The technique of the combination of ozone and ultraviolet radiation is promisingly suitable for scale-up industrial manufacture of low-molecular-weight chitosan.

1. Introduction

Chitosan is a well-known natural biopolymer generally obtained by extensive deacetylation of chitin isolated from crustacean shells. Owing to its biocompatibility, biodegradability, and biological activities, chitosan as a functional biopolymer has won acceptance in many diverse fields, such as food, pharmaceutics, biomedicine, pharmacology, biotechnology, biomaterials, medicine and cosmetics (George & Abraham, 2006; Khor & Lim, 2003; No, Meyers, Prinyawiwatkul, & Xu, 2007; Rinaudo, 2006; Sashiwa, Saimoto, Shigemasa, Ogawa, & Tokura, 1990; Shahidi, Arachchi, & Jeon, 1999; Sugano, Fujikawa, Hiratsuji, Nakashima, & Hasagaea, 1980; Suh & Matthew, 2000). Generally, chitosan obtained from the deacetylation of chitin has a high molecular weight and low solubility in aqueous solvent, which limit its applications. With the progresses in intensive study of chitosan, it has been found that low molecular weight chitosans have significantly different biological activities compared with high molecular weight chitosans, such as antifungal activity, antibacterial activity, and antitumor activity (Chien, Sheu, & Lin, 2007; Liu et al., 2006; Qin, Du, Xiao, Li, & Gao, 2002; Qin et al., 2004; Qin et al., 2006; Tarsi, Corbin, Pruzzo, & Muzzarelli, 1998). Thus the development of an emerging technology for degradation of high molecular weight chitosan to low molecular weight chitosan, without modification of its chemical structure, is of great interest. It was also found that chitosan can be degraded by ozone treatment (Kabal'nova et al., 2001; Seo, King, & Prinyawiwatkul, 2007). However, the degradation of chitosan is inefficient when ozone is used alone.

In our lab, it was found that chitosan was effectively and acceleratedly degraded by ozone treatment combined with ultraviolet radiation as an accessory factor. The ultraviolet radiation-induced accelerated degradation of chitosan by ozone treatment was demonstrated by means of determination of Mv of the degraded chitosan. The technique of the combination of ozone and ultraviolet irradiation for promising and potential production of low-molecular-weight chitosan, which is a rather new method and has never been reported in literature, is based on the enhanced formation of excessive hydroxyl radical due to the accelerated disassociation of ozone in the presence of ultraviolet radiation. Ultraviolet radiation as an accessory factor has been found to be an effective and efficient means for the accelerated degradation of chitosan treated with ozone. Chitosan degradation by ozone treatment combined with ultraviolet radiation is a very promising technique that can be applied successfully in scale-up industrial production of low-molecular-weight chitosan. In addition, the structure of the degraded chitosan was characterized by FT-IR, ^{13}C NMR, and X-ray diffraction spectral analysis, respectively.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Jinke Biochemical Ltd. (Zhejiang, China). The DD and the Mv of the commercial chitosan used in this study ware as follows:

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DD was 88.2 ± 3.2%; Mv was 829 KDa.

Oxygen with a purity of above 99% was purchased from NanNing Gas Station. All other chemicals were of reagent grade.

Ultraviolet lamp (emitting 253.7 nm ultraviolet radiation) was purchased from Nanjing ultraviolet electronic appliance Ltd. (Nanjing, China).

2.2. Characterization of degraded chitosan

Mv was determined by a viscometric method reported in literature (Chen & Hwa, 1996). The relative viscosity, η_{rel}, of chitosan samples was measured using an Ubbelohde capillary viscometer at 25 ± 0.5 °C. Mv was calculated based on the Mark–Houwink equation: $[\eta] = 1.64 \times 10^{-30} \times \mathrm{DD}^{14} \times (\mathrm{Mv})^{-0.0102 \times \mathrm{DD} + 1.82}$

FT-IR spectra were recorded in powder form in KBr discs in the range of 4000–400 cm^{-1} on a Nicolet 5DXB FT-IR spectrophotometer.

^{13}C NMR spectra were recorded on BRUKER AVANCE-500NMR SPECTROMETER. The degraded chitosan as well as initial chitosan were dissolved in $\mathrm{HCl/D_2O}$.

X-ray diffraction patterns of the degraded chitosan samples were measured by a D/max 2500 V diffractometer and used a Cu Kα target at 40 KV and 50 mA at 20 °C.

The DD was determined using the universally valid method reported by Tolaimate et al. (2000).

2.3. Batch experiments for the degradation of chitosan by ozone treatment combined with ultraviolet radiation

The purified chitosan (16 g) was completely dissolved in 800 ml of 0.25 M acetic acid solution. The degradation of chitosan was carried out in a 1200 ml reaction vessel in which the 800 ml chitosan solution was introduced. The ozone was generated from compressed oxygen by a laboratory-scale corona discharge generator and continuously bubbled from the bottom of reaction vessel into the chitosan solution through a bubble diffuser. A stirrer with a mixing speed of about 1100 rpm was used to provide sufficient contact between the chitosan solution and ozone. An ultraviolet water purification lamp with "U" shaped quartz tube (20 W) was used, emitting 253.7 nm ultraviolet radiation. The "U" shaped quartz tube which is impervious to water was perpendicularly installed inside the chitosan solution (The "U" shaped quartz tube was 195 mm in height. The height of the "U" shaped quartz tube immersed in the chitosan solution was 165 mm.). The reaction vessel was immersed into a water bath to keep the temperature around 10 ± 2 °C. The corresponding samples were taken out at 10, 20, 30, 40, and 50 min, respectively. The samples were processed in the same way as that described in the reported literature (Mao et al., 2004). The intrinsic viscosities of the samples were determined and the Mv was calculated according to the Mark–Houwink equation.

The degradation of chitosan by individual ozone treatment (without ultraviolet radiation) and ultraviolet radiation alone (the application rate of ozone was 0 mg/min) were also investigated using the similar method mentioned above, respectively.

3. Results and discussion

3.1. Effect of ultraviolet radiation alone, individual ozone, and ozone combined with ultraviolet radiation on degradation of chitosan

To investigate the effect of diverse reaction conditions on degradation of chitosan, 800 ml chitosan solution (0.02 g/ml) were treated with ultraviolet radiation alone, individual ozone, and ozone combined with ultraviolet radiation at 10 ± 2 °C for 50 min, respectively. The corresponding Mv was plotted as a function of the reaction conditions. The results are illustrated in Fig. 1.

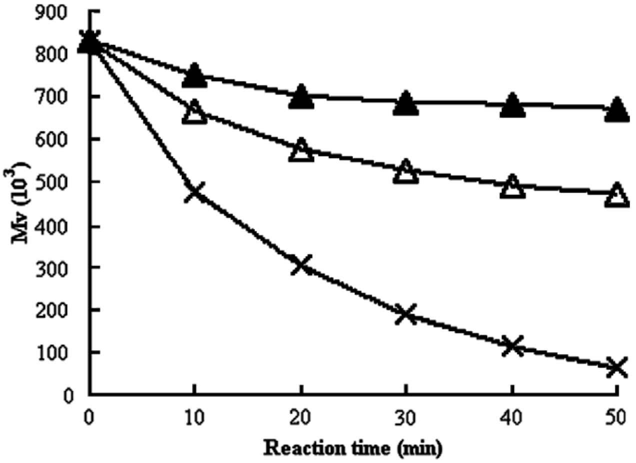

Fig. 1. Effect of diverse reaction conditions on Mv of degraded chitosan. Reaction temperature was kept at 10 ± 2 °C. (▲): ultraviolet radiation alone; (△): individual ozone treatment (46 ± 5 mg/min); (×): ozone (46 ± 5 mg/min) combined with ultraviolet radiation.

When the initial chitosan solution was exposed to ultraviolet radiation alone (marked with symbol ▲), the Mv of chitosan was decreased from 829 to 669 KD for 50 min. The rate of decrease in Mv was 19.30%.

When the initial chitosan solution was treated with individual ozone ($\mathrm{O_3}$ = 46 ± 5 mg/min marked with symbol △), the corresponding Mv of chitosan was decreased from 829 to 473 KD for 50 min. The corresponding rate of decrease in Mv was 42.94%.

When the initial chitosan solution was treated with ozone (46 ± 5 mg/min) combined with ultraviolet radiation (marked with symbol ×), the corresponding Mv of chitosan was decreased from 829 to 64 KD for 50 min. The corresponding rate of decrease in Mv increased to 92.28%. In the combined $\mathrm{O_3}$/ultraviolet radiation reaction system, the corresponding rate of decrease in Mv is much higher than the summation of those numerical values obtained by the ultraviolet radiation alone and the individual ozone treatment, respectively 92.28% > 19.30% + 42.94%.

Conclusion, based on the experimental results, can be drawn that the ultraviolet radiation-induced accelerated degradation of chitosan by ozone treatment can be carried out. It was also found that the individual ozone treatment was more effective than ultraviolet radiation alone in degrading chitosan.

Ultraviolet radiation was found to significantly enhance the radiolysis of ozone (Mishchuk, Goncharuk, & Vakulenko, 2008; Qu, Zhu, & Schinke, 2003; Tezcanli-Guyer & Ince, 2004; Zhu, Qu, Tashiro, & Schinke, 2004). The enhanced radiolysis of ozone in the chitosan solution can result in excessive hydroxyl radicals. The excessive hydroxyl radicals, which are powerful oxidizing species and can attack the β-D-(1,4) glucosidic linkages of chitosan and make the linkages break, may be play a significant role in the accelerated degradation of chitosan treated with ozone combined with ultraviolet radiation.

3.2. FT-IR spectra of degraded chitosan

The FT-IR spectra (Fig. 2) of the degraded chitosan were similar to that of the initial chitosan. The absorption band at 1598 cm^{-1} was characteristic of the amino deformation mode, the peak at 3452 cm^{-1} was $-\mathrm{NH_2}$ bond stretching, and the absorption bands at 1649, 1324 cm^{-1} were referenced as amide I and III bands, respectively. The amide II band had disappeared in the FT-IR

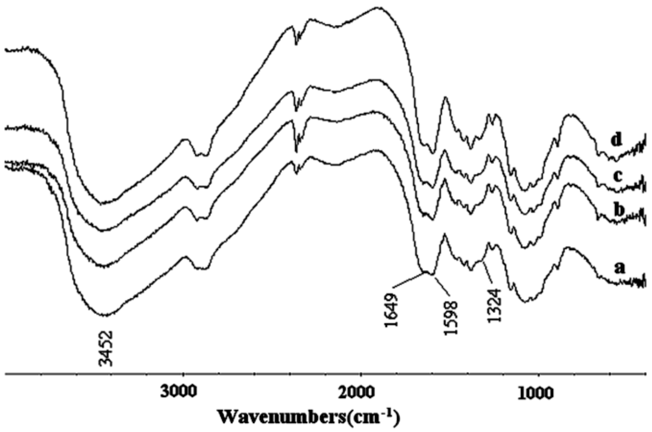

Fig. 2. FT-IR spectra of the degraded chitosan as well as initial chitosan. The degraded chitosan samples obtained after reaction at 10 ± 2 °C for 50 min. (a): initial chitosan; (b): ultraviolet radiation alone; (c): individual ozone treatment (46 ± 5 mg/min); (d): ozone (46 ± 5 mg/min) combined with ultraviolet radiation.

spectra mainly because it overlapped with the band of amino deformation vibratin. The assignments of the peaks were based on the data reported in the published literature (Feng, Du, Li, Hu, & Kennedy, 2008; Hu et al., 2007; Wang et al., 2008; Zhang, Ping, Zhang, & Shen, 2003). The IR spectra suggested that there was no obvious modification of chemical structure of degraded chitosan. No new band at about 1735 cm^{-1} assigned to the carboxylic group was obviously seen. The results also give us the important information that when the reaction temperature was kept at 10 ± 2 °C, the reaction was mainly the cleavage of β-glycosidic linkages and no carboxylic group was formed.

3.3. ^{13}C-NMR spectra of degraded chitosan

In the ^{13}C NMR spectra (Fig. 3), it can be seen that degraded chitosan had the almost same signals as initial chitosan at about 55, 60, 70, 74, 76, and 97 ppm, which are attributed to C-2, C-6, C-3, C-5, C-4, and C-1, respectively. The assignments of signals are based on the data reported in the published literature (Ding, Huang, Li, & Liu, 2007; Hu et al., 2007; Wang et al., 2008; Zhang et al., 2003). No new signals at about 173 assigned to the carboxylic group was obviously seen. This proved that no carboxylic group was formed. This result was in agreement with that experimental result reported by Kabal'nova et al. (2001). The results may give us the important information that the chemical monomeric structure of degraded chitosan was not apparently modified, compared with the initial chitosan.

3.4. X-ray diffraction analysis

For the degraded chitosan sample resulted from ultraviolet radiation alone, individual ozone treatment, and ozone treatment combined with ultraviolet radiation, the intensity of the characteristic peak decreased sharply compared with that of initial chitosan (Fig. 4), indicating that the disordered structure in the degraded chitosan samples had been formed and increased with decreasing molecular weight. The degraded chitosan sample prepared by ozone treatment combined with ultraviolet radiation had only one major peak and became amorphous. The crystalline structure can be destroyed with deeper degradation. Judging from the crystalline structure destroyed (or Judging from the decrease in the intensity of the characteristic peak), the order of degrading ability is "ozone treatment combined with ultraviolet radiation" > "individual ozone treatment" > "ultraviolet radiation alone".

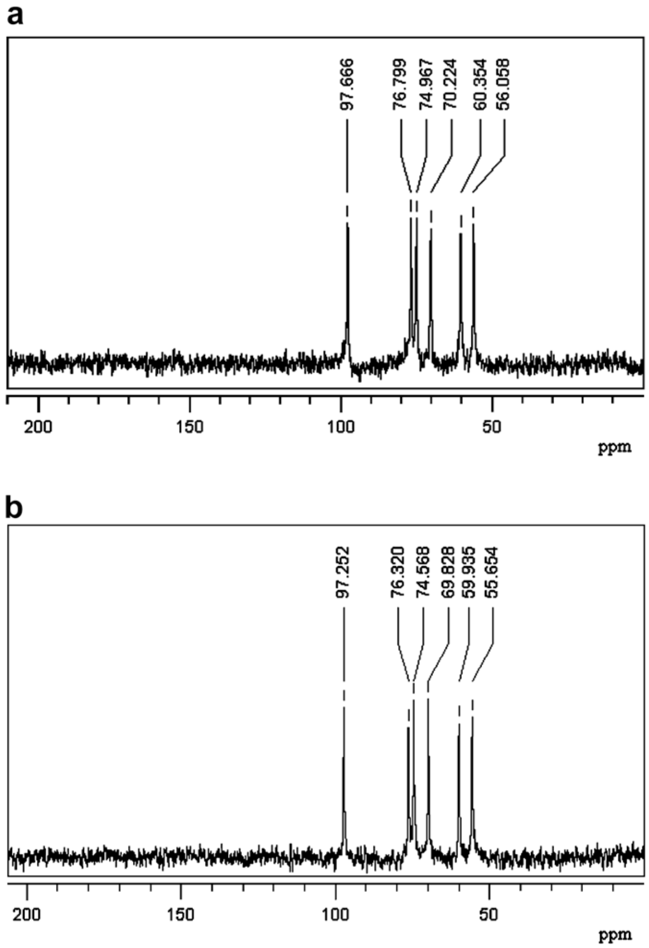

Fig. 3. ^{13}C NMR spectra of the degraded chitosan as well as initial chitosan. The degraded chitosan sample obtained by ozone (46 ± 5 mg/min) treatment combined with ultraviolet radiation at 10 ± 2 °C for 50 min. (A): initial chitosan; (B): degraded chitosan sample.

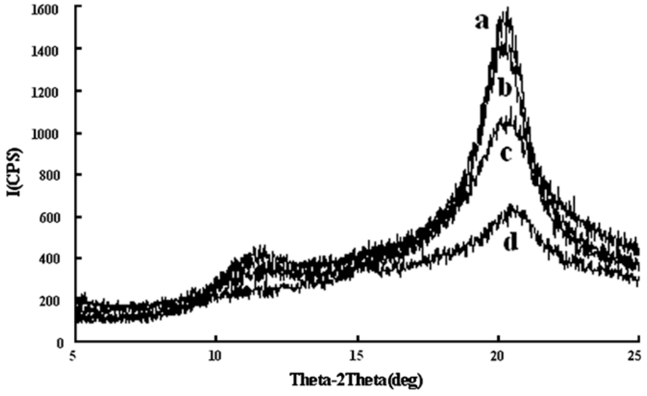

Fig. 4. X-ray diffraction analysis. The degraded chitosan samples obtained after reaction at 10 ± 2 °C for 50 min. (a): initial chitosan; (b): ultraviolet radiation alone; (c): individual ozone (46 ± 5 mg/min) treatment; (d): ozone (46 ± 5 mg/min) combined with ultraviolet radiation.

3.5. The DD of degraded chitosan obtained by ozone treatment combined with ultraviolet radiation

The DD of degraded chitosan samples are listed in Table 1. The DD of degraded chitosan samples did not apparently change with decreasing molecular weight, suggesting that the protonated

Table 1
Mv and DD of the degraded chitosan.

Reaction time (min)	Mv ($\times 10^3$)	DD (%)
0	829	88.2 ± 3.2[a]
10	475	87.9 ± 2.1
20	301	87.6 ± 2.4
30	188	88.6 ± 1.4
40	112	88.1 ± 1.6
50	64	88.1 ± 1.4

[a] Mean ± standard derivation of three independent experiments. Degraded chitosan samples obtained by ozone treatment (46 ± 5 mg/min) combined with ultraviolet irradiation at 10 ± 2 °C.

amino group are stable enough to the attack by hydroxyl radicals formed upon radiolysis of ozone by ultraviolet radiation.

4. Conclusions

Chitosan can be acceleratedly degraded by ozone combined with ultraviolet radiation as an accessory factor. The accelerated degradation of chitosan is probably due to the excessive production of hydroxyl radical generated by the radiolysis of ozone in the presence of ultraviolet irradiation. Results of the FT-IR and ^{13}C NMR spectra indicated that the chemical structure of the resulting degraded chitosan was not modified and that no carboxylic group was formed at reaction temperature of 10 ± 2 °C. Degradation of chitosan by ozone treatment combined with ultraviolet radiation as an accessory factor is a very promising technique that can be applied successfully in scale-up industrial production of low-molecular-weight chitosan.

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References

Chen, R. H., & Hwa, H. D. (1996). Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane. *Carbohydrate Polymers, 29*, 353–358.

Chien, P. J., Sheu, F., & Lin, H. R. (2007). Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life. *Food Chemistry, 100*, 1160–1164.

Ding, P., Huang, K. L., Li, G. Y., & Liu, Y. F. (2007). Preparation and properties of modified chitosan as potential matrix materials for drug sustained-release beads. *International Journal of Biological Macromolecules, 41*, 125–131.

Feng, T., Du, Y., Li, J., Hu, Y., & Kennedy, J. F. (2008). Enhancement of antioxidant activity of chitosan by irradiation. *Carbohydrate Polymers, 73*, 126–132.

George, M., & Abraham, T. E. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan – a review. *Journal of Controlled Release, 114*, 1–14.

Hu, Y., Du, Y., Yang, J., Kennedy, J. F., Wang, X., & Wang, L. (2007). Synthesis, characterization and antibacterial activity of guanidinylated chitosan. *Carbohydrate Polymers, 67*, 66–72.

Kabal'nova, N. N., Murinov, K. Y. U., Mullagaliev, I. R., Krasnogorskaya, N. N., Shereshovets, V. V., Monakov, Y. U. B., et al. (2001). Oxidative destruction of chitosan under the effect of ozone and hydrogen peroxide. *Journal of Applied Polymer Science, 81*, 875–881.

Khor, E., & Lim, L. Y. (2003). Implantable applications of chitin and chitosan. *Biomaterials, 24*, 2339–2349.

Liu, N., Chen, X. G., Park, H. J., Liu, C. G., Liu, C. S., Meng, X. H., et al. (2006). Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli*. *Carbohydrate Polymers, 64*, 60–65.

Mao, S., Shuai, X., Unger, F., Simon, M., Bi, D., & Kissel, T. (2004). The depolymerization of chitosan: Effects on physicochemical and biological properties. *International Journal of Pharmaceutics, 281*, 45–54.

Mishchuk, N. A., Goncharuk, V. V., & Vakulenko, V. F. (2008). Theoretical analysis of physicochemical processes occurring during water treatment by ozone and ultraviolet radiation. *Advances in Colloid and Interface Science, 139*, 62–73.

No, H. K., Meyers, S. P., Prinyawiwatkul, W., & Xu, Z. (2007). Applications of chitosan for improvement of quality and shelf life of foods: A review. *Journal of Food Science, 72*, 87–100.

Qin, C., Zhou, B., Zeng, L., Zhang, Z., Liu, Y., Du, Y., et al. (2004). The physicochemical properties and antitumor activity of cellulase-treated chitosan. *Food Chemistry, 84*, 107–115.

Qin, C., Du, Y., Xiao, L., Li, Z., & Gao, X. (2002). Enzymic preparation of water-soluble chitosan and their antitumor activity. *International Journal of Biological Macromolecules, 31*, 111–117.

Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J., & Du, Y. (2006). Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate Polymers, 63*, 367–374.

Qu, Z. W., Zhu, H., & Schinke, R. (2003). The ultra-violet photodissociation of ozone revisited. *Chemical Physics Letters, 377*, 359–366.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science, 31*, 603–632.

Sashiwa, H., Saimoto, H., Shigemasa, Y., Ogawa, R., & Tokura, S. (1990). Ysozymesusceptibility of partially deacetylated chitin. *International journal of biological macromolecules, 12*, 295–296.

Seo, S., King, J. M., & Prinyawiwatkul, W. (2007). Simultaneous depolymerization and decolorization of chitosan by ozone treatment. *Journal of Food Science, 72*, 522–526.

Shahidi, F., Arachchi, J. K. V., & Jeon, Y. J. (1999). Food applications of chitin and chitosans. *Trends in Food Science & Technology, 10*, 37–51.

Sugano, M., Fujikawa, T., Hiratsuji, Y., Nakashima, K., & Hasagaea, Y. (1980). A novel use of chitosan as a hypocholesterolemic agent in rats. *American Journal of Clinical Nutrition, 33*, 787–793.

Suh, J. K. F., & Matthew, H. W. T. (2000). Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: A review. *Biomaterials, 21*, 2589–2598.

Tarsi, R., Corbin, B., Pruzzo, C., & Muzzarelli, R. A. A. (1998). Effect of low-molecular-weight chitosans on the adhesive properties of oral streptococci. *Oral Microbiology and Immunology, 13*, 217–224.

Tezcanli-Guyer, G., & Ince, N. H. (2004). Individual and combined effects of ultrasound, ozone and UV irradiation: A case study with textile dyes. *Ultrasonics, 42*, 603–609.

Tolaimate, A., Desbrieres, J., Rhazi, M., Alagui, A., Vincendon, M., & Vottero, P. (2000). On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. *Polymer, 41*, 2463–2469.

Wang, W., Du, Y., Qiu, Y., Wang, X., Hu, Y., Yang, J., et al. (2008). A new green technology for direct production of low molecular weight chitosan. *Carbohydrate Polymers, 74*, 127–132.

Zhang, C., Ping, Q., Zhang, H., & Shen, J. (2003). Synthesis and characterization of water-soluble *O*-succinyl-chitosan. *European Polymer Journal, 39*, 1629–1634.

Zhu, H., Qu, Z. W., Tashiro, M., & Schinke, R. (2004). On spin-forbidden processes in the ultra-violet photodissociation of ozone. *Chemical Physics Letters, 384*, 45–51.