

RADIATION DEGRADATION OF CHITOSAN IN THE PRESENCE OF H₂O₂

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Abstract In order to obtain the chitosan oligomers, chitosan was irradiated in the solid state with and without H₂O₂ as a radiation degradation sensitizer, respectively. At room temperature, the viscosity average-molecular weight (M_v) of chitosan was decreased from 1.6×10^6 to 2.2×10^5 at an absorbed dose of 72 kGy without H₂O₂, and decreased to 2.7×10^4 at 2 kGy in the presence of an appropriate H₂O₂ content. In addition, the radiation degradation rate of chitosan containing 38.2wt% H₂O₂ is 59 times higher than that in the solid state without H₂O₂. FT-IR analysis suggests that there is no obvious change in the chemical structure of irradiated chitosan with and without H₂O₂ at a dose below 20 kGy, compared with unirradiated chitosan. On the other hand, the degree of deacetylation (DD) of irradiated chitosan in the studied dose range changed slightly, while DD of irradiated chitosan with H₂O₂ increased significantly. The XRD pattern indicates that the irradiated chitosan with H₂O₂ has more perfect crystalline structure than unirradiated chitosan. Therefore, it could be expected that irradiation of chitosan using H₂O₂ as a sensitizer would be a very effective method to prepare low molecular weight chitosan, because of its feasibility and benignancy to environment.

Keywords Chitosan, Radiation degradation, H₂O₂, Structure

INTRODUCTION

Chitosan, poly- β -(1 $\rightarrow$ 4)-D-glucosamine, can be obtained from chitin by deacetylation with alkali. It is soluble in dilute acidic medium due to the presence of amino groups. The use of chitosan in many areas, such as food processing, biochemistry, pharmaceuticals, medicine, and agriculture has been developed over the past decades^[1, 2].

In recent years, it has been reported that many properties of chitosan depend on the molecular weight^[3]. The chitosan oligomers possess better functional properties such as antimicrobial activity^[4], blood coagulation activity^[1] than chitosan with high molecular weight. Especially, the chitosan with low molecular weight has better solubility in water, which has wide applications in medicine. Many attempts have been made to degrade the β -(1 $\rightarrow$ 4)-linkage of chitosan for the preparation of chitosan oligomers^[5–7]. Recently, H₂O₂ has been used in the degradation of chitosan because it is easily available and environmentally benign^[8–10]. However, the high reaction temperature used in H₂O₂ treatment of chitosan often results in a color change of final chitosan products. Moreover, the chemical structure of chitosan is changed during reduction of the molecular weight of chitosan. In contrast, gamma irradiation^[11–13] could degrade chitosan without producing obvious change in chemical structure of chitosan; moreover, it could usually be carried out at room temperature, and controlled easily. The resulting product could have high purity. On the other hand, the radiation sterilization leads to even better improvement of the biocompatibility of chitosan^[14]. Therefore, the study of degradation of chitosan by irradiation has raised the interest of scientists. However, the problem encountered at present is that the irradiation of chitosan in the solid state results in an initial depolymerization, and its molecular weight changes slowly beyond 100 kGy of dose. Further irradiation causes severe radiolytic oxidation, which results in the browning of the final product. In order to resolve the above problems and obtain chitosan with low molecular weight at low absorbed dose, we combined

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the two methods, that is, the chitosan was irradiated together with H_2O_2 as a radiation degradation sensitizer. In this paper, we proposed a new method to prepare chitosan oligomer effectively and studied primarily the effects of absorbed dose and H_2O_2 content on the viscosity average-molecular weight (M_η); meanwhile, the chemical structure and crystalline state of chitosan irradiated under different conditions were investigated.

EXPERIMENTAL

Materials

The Chitosan 9B was purchased from Katokichi Co. Ltd. Japan. Its M_η was 1.6×10^6 determined by viscosity measurement. All the other chemicals were of reagent grade and used as received.

Irradiation of chitosan

Chitosan was infiltrated uniformly by 30% aqueous H_2O_2 solution under stirring, and then the paste was irradiated by ^{60}Co gamma rays (installed in Institute of Applied Chemistry, College of Chemistry, Peking University) with an absorbed dose range of 0–30 kGy. For comparison, the control samples of chitosan were irradiated directly in the solid state with an absorbed dose range of 0–80 kGy.

Characterization

The viscosity of unirradiated and irradiated chitosan with and without H_2O_2 as a sensitizer was measured at $(25 \pm 0.1)^\circ\text{C}$ in 0.1 mol/L CH_3COOH /0.2 mol/L NaCl buffer solution with a capillary viscometer. The intrinsic viscosity $[\eta]$ was calculated according to an empirical equation^[15]:

$$[\eta] = (\eta_{\text{sp}} + 3\ln\eta_r)/4C \quad (1)$$

where η_{sp} is the specific viscosity, η_r is the relative viscosity, and C is the concentration of chitosan solution (g/mL). Then, the M_η was calculated using the Mark-Houwink equations as follows^[16]:

$$[\eta] = KM_\eta^\alpha \quad (2)$$

where $K = 1.81 \times 10^{-3}$, and $\alpha = 0.93$.

Micro-Fourier transform infrared spectra of powder samples were recorded on a Nicolet Magna FT-IR 750 spectrometer with a resolution of 4 cm^{-1} .

Wide-angle X-ray diffraction (WXR) measurements were carried out at room temperature by using a Rigaku diffractometer with a $\text{CuK}\alpha$ target at 40 kV and 50 mA. The diffraction patterns were determined over a range of diffraction angle 2θ from 5° to 40° at scanning speed of $8^\circ/\text{min}$.

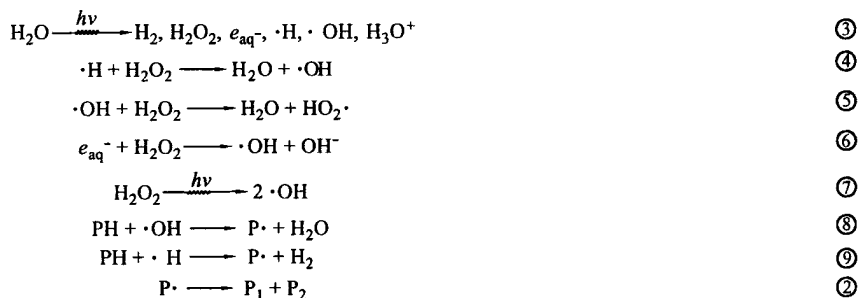
RESULTS AND DISCUSSION

Radiation-Induced Degradation of Chitosan

Figure 1 illustrates the relationship between M_η of chitosan and absorbed dose. For irradiated chitosan without H_2O_2 as sensitizer, its M_η decreases gradually with the absorbed dose, and drops to 2.2×10^5 at 72 kGy. At a concentration of 38.2 wt% H_2O_2 , a rapid drop of M_η is observed at 0.4 kGy, and then it slows down gradually up to 2 kGy where it is reduced to 2.7×10^4 . At low H_2O_2 concentration, the relationship between M_η and dose is similar to that at high H_2O_2 concentration; however, the dose which is needed to reduce significantly the average molecular weight increased to 1.5 kGy. To our knowledge, chitosan in the solid state could be degraded by gamma irradiation; however, 100 kGy of dose should usually be needed to obtain low molecular weight chitosan^[17]. This may be ascribed to the direct action of radiation on the chitosan chains:



As shown in Fig. 1(b), the direct irradiation of chitosan in the presence of H_2O_2 could reduce its M_η very effectively at low absorbed dose. Under gamma irradiation in the presence of aqueous H_2O_2 solution, besides the above reactions ① and ②, the primary reactions might occur as follows^[18]:



Thus, the chitosan molecular chains could be degraded by direct action of radiation; furthermore, it could react with the $\cdot OH$ radicals which was derived from the radiolysis of water and H_2O_2 , in which the reduction species $\cdot H$ and e_{aq}^- are transformed into $\cdot OH$. Since the concentration of H_2O_2 was high in the present irradiation system, consequently, the β -(1 $\rightarrow$ 4) bond of chitosan was attacked and broken, and the molecular weight was reduced significantly. With the exhaustion of H_2O_2 , the oxidized free radicals decreased and the variation of molecular weight of chitosan became smaller.

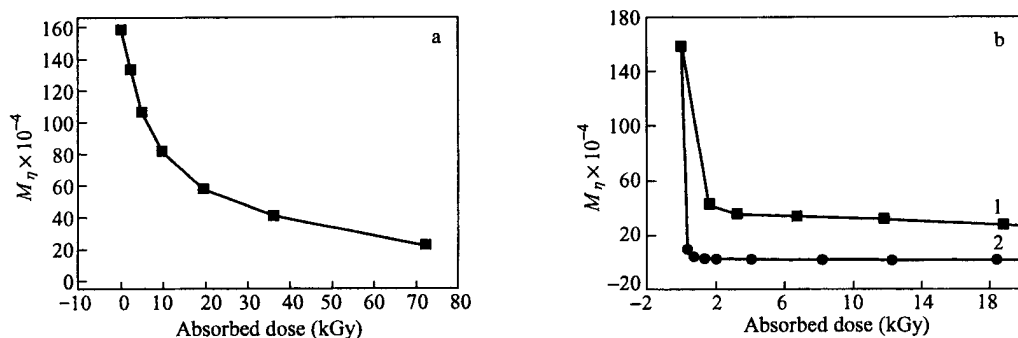


Fig. 1 Effect of absorbed dose on the M_n of chitosan
a) Without H_2O_2 , b) With H_2O_2 (1 wt%–5.7 wt%, 2 wt%–38.2 wt%)

The reduction in M_n could be described by the following Eq.^[17]

$$\frac{1}{M_t} = \frac{1}{M_0} + kD \quad (3)$$

where k mol $\cdot$ g $^{-1}$ $\cdot$ kGy $^{-1}$ represents the rate constant of radiation degradation, D (kGy) is the absorbed dose. Figure 2 shows that a linear relationship exists between the reciprocal of M_n and absorbed dose for irradiated chitosan with and without H_2O_2 . This indicates that a random scission of chitosan chains occurs. Compared with the radiation degradation of chitosan without H_2O_2 , the degradation rate constant k of chitosan irradiated with 38.2 wt% H_2O_2 is 59 times higher than that of irradiated chitosan sample without H_2O_2 , and during the radiation degradation of chitosan with 5.7 wt% H_2O_2 , the rate constant k is about 20 times higher than that of irradiated chitosan sample without H_2O_2 . These data suggest that the addition of a small amount of H_2O_2 could increase greatly the degradation of chitosan under gamma radiation. However, due to the complicated reaction during irradiation of chitosan with H_2O_2 as a sensitizer, such a relationship does not apply very well over the entire range of absorbed dose ($r = 0.88$, $r = 0.84$).

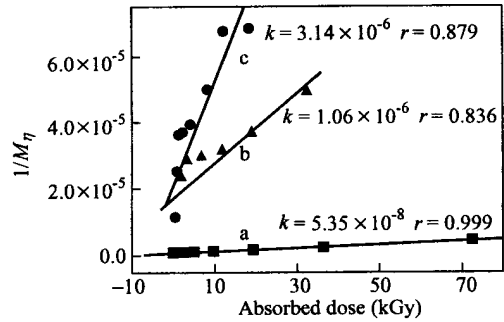


Fig. 2 Relationship between $1/M_\eta$ and absorbed dose, a) without H_2O_2 , b) with 5.7 wt% H_2O_2 , c) with 38.2 wt% H_2O_2

The relationship between H_2O_2 content and M_η of chitosan is demonstrated in Fig. 3. On increasing the H_2O_2 content, M_η decreases significantly in the range of 0 wt%–15 wt%, then reaches a constant value up to 30 wt%, finally M_η is reduced to 6.3×10^4 . A slight change in M_η was found when the weight percent of H_2O_2 was increased further. A similar result was obtained in chemical degradation of chitosan on using the H_2O_2 treatment method^[10]. This suggests that at the initial stage of degradation, the 1,4-glycosidic bonds of chitosan could be broken by radiation induced scissions and oxidation of $\cdot OH$ radicals through the decomposition of H_2O_2 and water, causing an obvious reduction in the M_η of polymer. When the degradation reaches a certain extent, the short molecular chain and few free amino groups will cause some difficulty in further degradation, and consequently, M_η of polymer changes slowly.

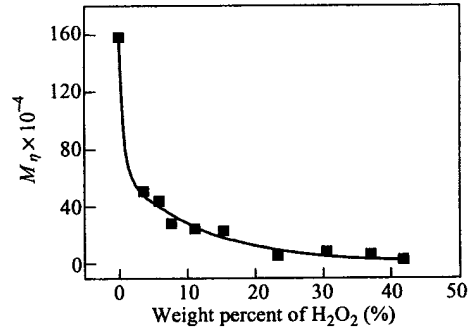


Fig. 3 Effect of H_2O_2 content on M_η of irradiated chitosan (Absorbed dose is 2 kGy)

FT-IR Analysis

Infrared spectroscopy has been widely used to characterize the structure of chitosan because of its simplicity. Some literature reported the application of infrared spectroscopy for degree of acetylation (DA) determination^[19–23]. Figure 4 gives the FT-IR spectra of chitosan powder, and the values of DA and DD are presented in Table 1. DA of chitosan was calculated based on the equation described in the literature^[24] as follows:

$$\frac{A_{1320}}{A_{1420}} = 0.3822 + 0.03133DA \quad r = 0.99 \tag{4}$$

Thus,

$$DD = (1 - DA) \times 100\% \tag{5}$$

As shown in Fig. 4, no obvious change was observed in the spectra of unirradiated and irradiated chitosan without H_2O_2 . The relative absorption ratio between two bonds at 1644 cm^{-1} ($\nu_{C=O}$) and 1590 cm^{-1} (δ_{NH})

decreases with the increase of absorbed dose for irradiated chitosan with H₂O₂. In addition, the height of the peak at 3366 cm⁻¹ (ν_{NH_2} , ν_{OH}) increases. This indicates that the -NH₂ group content increases after irradiation in the presence of H₂O₂. Furthermore, the calculated DD value shown in Table 1 shows that it increases for irradiated chitosan with H₂O₂ at a dose of 18.4 kGy, and the DD value is about 96%. On the other hand, no new peak appeared for the irradiated chitosan with and without H₂O₂, this is different from the FT-IR spectrum of chitosan treated by H₂O₂. In the latter spectrum, carboxyl group could often be detected^[10]. The possible reason is due to the low dose we used in this work.

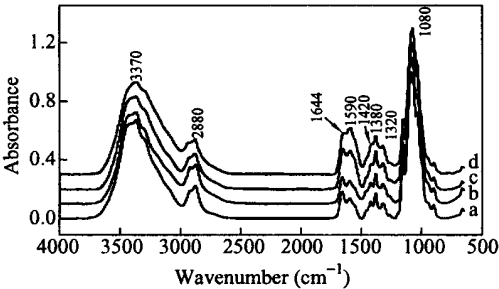


Fig. 4 FT-IR spectra of chitosan powder, (a) unirradiated chitosan (b) 19.3 kGy, without H₂O₂ (c) 2 kGy, 38.2 wt% H₂O₂ (d) 18.4 kGy, 38.2 wt% H₂O₂

Table 1. Relationship between DA and DD with irradiation conditions

Conditions	$\frac{A_{1320}}{A_{1420}}$	DA (%)	DD (%)
Unirradiated chitosan	1.143	24.3	75.7
19.3 kGy, 0 wt% H ₂ O ₂	1.143	24.3	75.7
2 kGy, 38.2 wt% H ₂ O ₂	0.615	7.4	92.6
18.4 kGy, 38.2 wt% H ₂ O ₂	0.500	3.8	96.2

* 1320 cm⁻¹ = $\nu_{\text{C-N}}$, 1420 cm⁻¹ = δ_{CH_2}

Crystallization Behavior

The crystalline state of chitosan and radiation-degraded chitosan was investigated by XRD diffractometry. Figure 5 shows the X-ray diffraction patterns of chitosan irradiated under different conditions. Chitosan has two peaks, $2\theta = 10.8^\circ$ and 20.1° , which is similar to the patterns of chitosan film^[25]. The irradiated chitosan without H₂O₂ has a similar crystalline state to that of unirradiated sample, while the irradiated sample in the presence of H₂O₂ has three peaks (Fig. 5c), a peak at 20.1° was shifted to 19.8° , and a peak at $2\theta = 10.2^\circ$ was increased significantly, in addition, a new peak appeared at $2\theta = 21.6^\circ$. This indicates that the degradation product of irradiated chitosan accelerated by H₂O₂ has more perfect crystalline structure than unirradiated chitosan.

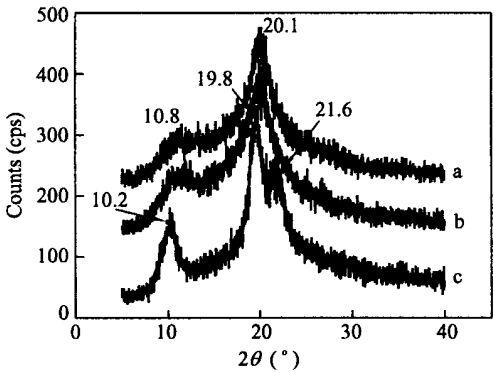


Fig. 5 XRD diagram of unirradiated chitosan a) and irradiated chitosan with and without H₂O₂ (c, b), the content of H₂O₂ is 38.2 wt%

This may be due to the fact that chitosan chains with low molecular weight have higher mobility than chitosan with high molecular weight, and thus they are easy to array regularly and present a more perfect crystalline structure. In addition, the increase of DD could improve the crystalline degree^[26]. On the other hand, the XRD result indicates that the degradation of chitosan by irradiation with H₂O₂ as a sensitizer is achieved favorably in the amorphous and interfacial region between amorphous and crystalline chitosan. The XRD diagram also agrees with the results of FT-IR analysis.

CONCLUSION

By using gamma irradiation, M_n of chitosan could be decreased from 1.6×10^6 to 2.2×10^5 at 72 kGy absorbed dose, whereas direct irradiation in the presence of H₂O₂ could effectively accelerate the degradation rate of chitosan, and the absorbed dose could be reduced by increasing H₂O₂ content; finally, the M_n of chitosan could be reduced to 2.7×10^4 at 2 kGy absorbed dose. Therefore, H₂O₂ is a very effective sensitizer for the radiation degradation of chitosan. In addition, the radiation-degraded chitosan possesses the higher degree of deacetylation and more perfect crystalline structure. No obvious change was observed in the color of the final chitosan product.

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