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Radiation-induced degradation of carboxymethylated chitosan in aqueous solution

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Abstract

Aqueous solutions of carboxymethylated chitosan (CM-chitosan) were radiated with γ-ray in various conditions. The degradations of CM-chitosan were faster in the presence of nitrous oxide or hydrogen peroxide, but it was inhibited obviously after adding isopropanol because of the changes of the concentration of hydroxyl radicals in above different conditions. The radiation chemical yields of CM-chitosan degradation were found to decrease at lower pH in which the polymer chains tend to coiled conformation. Intrinsic viscosity of CM-chitosan decreased faster than that of carboxymethylated chitin, indicating that amino groups could enhance the reactivity. FTIR and UV spectra showed that main chain structures of CM-chitosan were remained and some carbonyl/carboxyl groups were formed during the degradation. The data of EA indicated the contents of C/N were increased, which suggested the elimination of partial amino groups during radiation.

Keywords: Carboxymethylated chitosan; Radiation; Degradation mechanism

1. Introduction

Chitosan is a poly-β-(1-4)-D-glucosamine derivative, which is obtained by full or partial deacetylation of chitin. Chitin is a kind of abundant natural polysaccharide and mostly distributed in the shell of crustaceans, the cuticle of insects, and the cell wall of some fungi and microorganisms (Muzzarelli, 1973). Chitosan has many important biological, chemical and physical properties, thus it can be applied in many fields such as photography, ophthalmology and medical materials, cosmetics and food additives. Especially in recent years, some antibacterial and antifungal activities of chitosan and its derivatives have been studied (Majeti & Kumar, 2000; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003).

However, chitosan usually has high molecular weight and strong network of intermolecular or intramolecular hydrogen bonds. Such structure and characteristic of chitosan result in poor water-solubility and limit its further application. Whereas chitosan with low molecular weight ($<10^4$ Da) can be easily dissolved in the water, while biological properties were remained. Therefore, many methods were utilized to enhance the solubility of chitosan, such as oxidation (Chang, Tai, & Cheng, 2001), acid (Belamie, Domard, & Giraud-guille, 1997; Jia & Shen, 2002), supersonic, ultraviolet, γ-ray radiation (Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005), and enzyme (Niederhofer & Müller, 2004) degradation. Radiation by γ-ray is a preferable methods for degradation of chitosan because it could be carried out at room temperature, controlled easily and the products have high purity. Related studies focused mainly on the characteristics and applications of radiation-degraded chitosan as plant growth promoter, protecting plants from the environmental stress and fruit protection (Hai, Diep, Nagasawa, Yoshii, &

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Kume, 2003; Yoksan, Akashi, Miyata, & Chirachanchai, 2004; Zhao, Zhong, Yu, Zhang, & Sun, 1993). Radiation-induced changes in chitosan are attributed to free-radical reactions. Pulse radiolysis technique has been used for investigating radical-induced chain scission reactions in chitosan and the degradation mechanism of chitosan has been discussed (Ulański & Rosiak, 1992; Ulanski & Sonntag, 2000). It was found that hydroxyl radicals (·OH) are the main active species for chain breakage of chitosan molecules. In addition, Park and Kim found that chitooligosaccharides have strong radical scavenging effects on ·OH radicals and superoxide radicals (Park, Je, & Kim, 2003).

The solubility of chitosan can be improved remarkably by introducing carboxymethyl groups to the chitosan molecules. The structure of CM-chitosan is similar to that of amino acids because it has both amino groups and carboxyl groups in the molecules. CM-chitosan is a kind of important derivative due to its good water-solubility, low toxicity and special biological activity. It was reported that intra-articular injection of CM-chitosan may significantly suppress the mRNA expression of matrix metalloproteinase-1,-3 and may have protective effect on articular cartilage of osteoarthritis (Liu, Qiu, Chen, Peng, & Du, 2005). In addition, the antibacterial activities of CM-chitosan depend on the characteristic of degree of deacetylation (DDA), molecular weight and degree of substitution (DS) (Liu, Guan, Yang, Li, & Yao, 2001). Compared with chitosan, studies on radiation effect of γ-ray on CM-chitosan were very few. CM-chitosan undergoes degradation as radiated in solid state and in diluted solution. However, when the concentration of CM-chitosan was more than 10%, crosslinking will be observed. Hydrogels formed by radiation crosslinking of CM-chitosan have antibacterial activity, which is an important feature for biomedical materials (Zhao, Mitomo, Nagasawa, Yoshii, & Kume, 2003). Because CM-chitosan is a water-soluble chitosan derivative both in acidic and basic physiological media, it might be a better candidate than chitosan for studying the mechanism of degradation reaction of chitosan derivatives. Laser photolysis of CM-chitosan in aqueous solution has been investigated in our previous work for elucidating reactive activity of CM-chitosan with different radicals such as ·OH and $SO_4^{\cdot -}$ radicals (Zhai et al., 2004a, 2004b).

In this paper, we used γ-ray radiation to obtain oligosaccharides of CM-chitosan and carboxymethylated chitin (CM-chitin), discussed the influence of additives, such as nitrogen gas (N_2), nitrous oxide (N_2O), hydrogen peroxide (H_2O_2), isopropanol, pH of the CM-chitosan solutions and the substituted groups of CM-chitosan on the radiation degradation of CM-chitosan in aqueous solution. EA, FTIR and UV spectra of radiation-degraded CM-chitosan were also studied to testify the structure of oligosaccharides and discuss the degradation mechanism of CM-chitosan. The knowledge of degradation process is essential to optimize conditions of degradation and extend applications of CM-chitosan.

2. Experimental

2.1. Materials

Both of CM-chitosan (DDA = 84.0%) and CM-chitin (DDA = 31.4%) were purchased from Koyou Chemical Industrial Co. Ltd. Japan, which were in the form of sodium salt and used without further purification. Their characteristic parameters were summarized in Table 1. H_2O_2 and isopropanol were reagent grade and purchased from Beijing Reagent Company. The solutions were made with deionized water and the pH was adjusted by 0.1 mol/L $HClO_4$ or 0.1 mol/L NaOH.

2.2. Radiation degradation of CM-chitosan

1% (w/v) CM-chitosan solutions (conditions a–f) or CM-chitin solution (condition g) were radiated in different conditions with a ^{60}Co source (dose rate was 3 kGy/h) from 0 to 15 kGy. Conditions a–g are described as follows: (a) N_2-saturated, the pH of 1% original CM-chitosan solution was 9.8; (b) N_2O-saturated, pH 9.8; (c) N_2-saturated and the concentration of H_2O_2 was 0.02 mol/L, pH 9.8; (d) N_2-saturated and the concentration of isopropanol was 0.76 mol/L, pH 9.8; (e) N_2-saturated and the pH of 1% CM-chitosan solution was adjusted to 7.9 by 0.1 mol/L $HClO_4$ because perchlorate ions are inactive with ·OH radicals and do not interfere with experimental results; (f) N_2-saturated and the pH of 1% CM-chitosan solution was adjusted to 1.1 by 0.1 mol/L $HClO_4$; (g) N_2-saturated, the pH of CM-chitin solution was adjusted to 9.8 by 0.1 mol/L NaOH.

2.3. Determination of molecular weight of CM-chitosan

The intrinsic viscosities of CM-chitosan and CM-chitin were determined by the viscometric method with an Ubbel-ohde capillary viscometer. The efflux times that solutions passed the capillary were recorded in triplicate. In this experiment, the following experience equation of One-Point Method was used to calculate the intrinsic viscosity (Fan, Chen, Sun, & Hua, 2002):

$$[\eta] = \frac{4\eta_{sp}^{1.02} \ln \eta_r}{C^{1.01}(3\eta_{sp} + \ln \eta_r)} \tag{1}$$

Where $[\eta]$ is intrinsic viscosity, η_r is relative viscosity, η_{sp} is specific viscosity, C is the concentration of CM-chitosan in

Table 1
Characteristic parameters of CM-chitin and CM-chitosan used in this study

Sample	DS[a]	DDA[b] (%)	Intrinsic viscosity (dL/g)	M_w
CM-chitosan	0.91	84.0	2.43	3.1×10^4
CM-chitin	0.83	31.4	2.32	2.9×10^4

[a] Degree of substitution.
[b] Degree of deacetylation.

0.1 mol/L sodium chloride aqueous solution. Validity of this equation has been testified in 30 ± 0.1 °C with the concentration range of CM-chitosan from 2–5 mg/mL. In this method, we can determine intrinsic viscosity more conveniently and timely. The viscosity average molecular weight was calculated by Mark-Houwink equation:

$$[\eta] = KM_\eta^\alpha \tag{2}$$

Where $[\eta]$ is intrinsic viscosity, M_η is viscosity average molecular weight, the constants K and α depend on the characteristic of polymer. For CM-chitosan, $K = 7.92 \times 10^{-5}$, $\alpha = 1.00$ (Zhao, Sun, & Sun, 2002), but no report for CM-chitin yet. The accuracy of Eqs. (1) and (2) have been identified that the deviation can be less than 5% in proper conditions.

2.4. Purification of radiation-degraded CM-chitosan

The pH of radiation-degraded CM-chitosan solution was adjusted to 6.0 approximately with appropriate volumes of 0.1 mol/L $HClO_4$ solutions. The flocculent precipitate was appeared and collected by centrifuge (2000 rpm, 5 min). The solid was washed with ethanol and dried in vacuum to get pure samples and analyzed by FTIR, UV and EA.

2.5. Structure analysis of radiation-degraded CM-chitosan

Infrared Spectra of radiation-degraded CM-chitosan were recorded with a Fourier transform infrared spectrometer (Bruker VECTOR22) by the KBr tablet method at a resolution of 4 cm^{-1} and an accumulation of 32 scans (Chen & Park, 2003). UV spectra of radiation-degraded CM-chitosan solutions were carried out by a Hitachi Model 3010 spectrophotometer. The reference solution was the same solution without radiation. The scanning wave range was from 190 to 450 nm and the interval was 0.5 nm. Compositions of radiation-degraded CM-chitosan were determined by an Elementar Analysensysteme GmbH (German) model vario EL.

3. Results and discussion

3.1. Radiation degradation of CM-chitosan in different conditions

Crosslinking reaction will appear to form gels after irradiation when the concentration of CM-chitosan is more than 10%, and Zhao et al. has studied radiation synthesis and characteristic of CM-chitosan hydrogels (Zhao et al., 2003). What reactions are there under radiation of CM-chitosan dilute solution? In order to elucidate these reactions, 1% CM-chitosan aqueous solutions in different conditions were radiated, and the experimental results were shown in Fig. 1. It was found that the degradation of CM-chitosan took place and its viscosity average molecular weight (M_η) decreased continuously with the increase of dose in all conditions, while the decrease of M_η was different obviously in various conditions. Low concentration of H_2O_2 and N_2O (the concentration of saturated solution is 2.5×10^{-2} mol/L) can greatly accelerate the degradation of CM-chitosan. M_η of CM-chitosan decreased to 3.0×10^3 as the absorbed dose was 4 kGy in condition c. Due to saturation with N_2O in condition b, M_η of CM-chitosan decreased to 3.0×10^3 when the absorbed dose was 5 kGy. Contrarily proper concentration of isopropanol can prevent the degradation of CM-chitosan obviously. When absorbed dose was 14 kGy, M_η of CM-chitosan decreased to 1.8×10^4 merely.

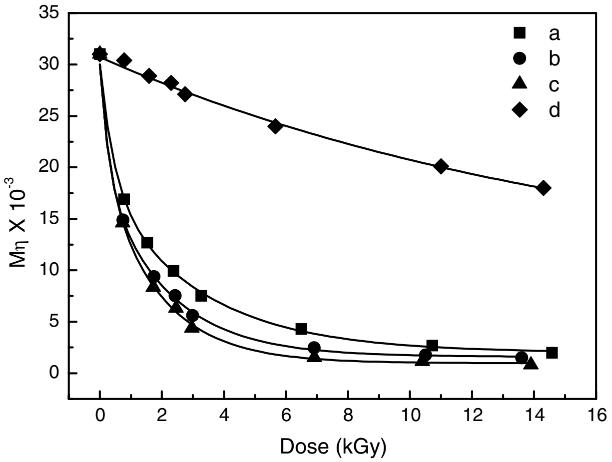


Fig. 1. Influence of absorbed dose on the $M\eta$ of CM-chitosan. (a) N_2-saturated, pH 9.8; (b) N_2O-saturated, pH 9.8; (c) 0.02 mol/L H_2O_2, N_2-saturated, pH 9.8; (d) 0.76 mol/L isopropanol, N_2-saturated, pH 9.8.

3.2. Radiation chemical yield of radiation-induced degradation of CM-chitosan

G_d is defined as radiation chemical yield which represents the number of radiolysis events caused by the absorption of 100 eV of radiation. G_d expresses the degradation susceptibility of the polymer during radiation and can be calculated by the following equation (Nagasawa, Mitomo, Yoshii, & Kume, 2000):

$$\left(\frac{1}{M_n} - \frac{1}{M_{no}}\right) = \frac{G_d \times 6.24 \times 10^{16}}{N_A} \times D \tag{3}$$

Where M_{no}, M_n are number average molecular weights of the polymer before and after degradation respectively, N_A is Avogadro's number, D is absorbed dose. When $\alpha = 1$ (α is the constant in Mark-Houwink equation: $[\eta] = KM_\eta^\alpha$), M_η equate to $2M_n$ (Flory, 1953). The relationship between $1/M_n$ and D gained by Eq. (3) and the results in Fig. 1 were shown in Fig. 2. G_d of radiation-induced degradation of CM-chitosan in different conditions can be obtained by the slope of the straight line in Fig. 2.

G_d of radiation-induced degradation of CM-chitosan in conditions a–g and linear correlation coefficients (r) of the straight line of $1/M_n$ to D were listed in Table 2. In the CM-chitosan solutions which contained 0.02 mol/L H_2O_2

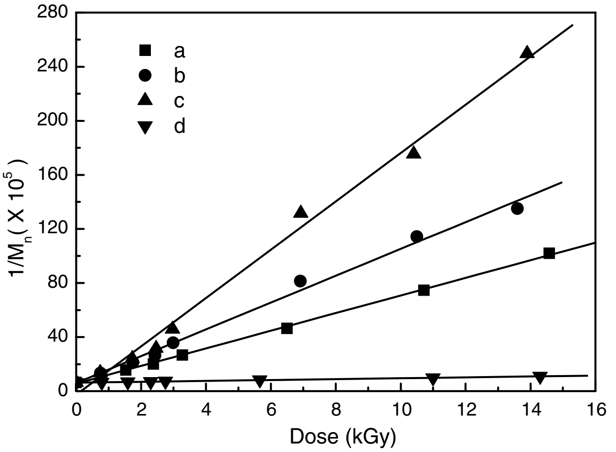


Fig. 2. Relationship between $1/M_n$ and absorbed dose. (a) N_2-saturated, pH 9.8; (b) N_2O-saturated, pH 9.8; (c) 0.02 mol/L H_2O_2, N_2-saturated, pH = 9.8; (d) 0.76 mol/L isopropanol, N_2-saturated, pH 9.8.

or 2.5×10^{-2} mol/L N_2O, G_d were 2.8 and 1.5 times as much as that in condition a. In condition d, because 0.76 mol/L isopropanol was added into the CM-chitosan solution, G_d decreased to 5% as much as that in condition a. Good linear relationships between $1/M_\eta$ and D were shown by the values of r.

3.3. Influence factors of degradation process

3.3.1. Influence of reactive species on CM-chitosan degradation

It is well known that the radiation energy of γ-ray is absorbed mainly by water in dilute CM-chitosan aqueous solutions, and the direct effect of radiation on CM-chitosan can be neglected. The radiolysis of water yields reactive species such as ·OH, hydrated electrons (e_{aq}^-), hydrogen atoms (·H) (reaction (4)) (Hai et al., 2003). Radiation chemical yields of these reactive species are constant in the wide range of pH. I.e. $G(e_{aq}^-) = G(\cdot OH) = 2.8 \times 10^{-7}$ mol/J, $G(\cdot H) = 0.6 \times 10^{-7}$ mol/J (Woods & Pikaev, 1994).

$$H_2O \xrightarrow{\gamma\text{-ray}} \cdot OH, e_{aq}^-, \cdot H, H_2O_2, H_2, H^+, OH^- \qquad (4)$$

In condition a, CM-chitosan aqueous solution was radiated with saturated N_2. The active species resulted in degradation of CM-chitosan were ·OH, ·H, and e_{aq}^-, and the effect of other reactive transients can be neglected. In condition b, e_{aq}^- can be readily converted into ·OH radicals (reaction (5)) and G_d increased to 1.5 times as much as that in condition a.

$$e_{aq}^- + N_2O + H_2O \rightarrow \cdot OH + N_2 + OH^- \qquad (5)$$

Furthermore, radiolysis of H_2O_2 forms ·OH by reaction (6) directly in condition c, and e_{aq}^- can react with H_2O_2 to produce ·OH by reaction (7) too. Low concentration of H_2O_2 (0.02 mol/L) can make G_d increased by 2.8 times.

$$H_2O_2 \rightarrow \cdot OH + \cdot OH \qquad (6)$$

$$e_{aq}^- + H_2O_2 \rightarrow \cdot OH + OH^- \qquad (7)$$

Isopropanol is the scavenger of ·OH and ·H. It can react with ·H or ·OH radicals and form isopropanol radicals, which are inert for polymers (reactions (8) and (9)) (Zhai et al., 2004a, 2004b). In condition d, 0.76 mol/L isopropanol induced G_d decreased to 5%.

$$(CH_3)_2CHOH + \cdot H \rightarrow (CH_3)_2\dot{C}OH + H_2 \qquad (8)$$

$$(CH_3)_2CHOH + \cdot OH \rightarrow (CH_3)_2\dot{C}OH + H_2O \qquad (9)$$

From the above experimental results, we can know that e_{aq}^- can hardly initiate the degradation of CM-chitosan immediately, both ·H and ·OH are main reactive radicals for H-abstraction reactions of CM-chitosan in dilute CM-chitosan aqueous solution.

3.3.2. Influence of pH

CM-chitosan is a polyampholyte, which contains amino, acetamido and carboxyl groups, and the pH of 1% original CM-chitosan aqueous solution is 9.8. With the change of pH, there is a change in the conformation of CM-chitosan macromolecular chain. Following the concept of conformation, one expects a rather strong pH dependence of the rate of reactions of polymer with ·OH radicals. In our previous laser photolysis experiments, it was found that the rate of the reactions of CM-chitosan with ·OH radicals decreased at lower pH when polymers chains tended to coiled conformation (Zhai et al., 2004a, 2004b). In this work it was found that M_η of CM-chitosan decreased more slowly in a lower pH solution (Fig. 3). G_d which was calculated by Eq. (3) decreased to 43% at pH 1.1 (condition f) as much as that in near neutral solution (condition e) as shown in Table 2.

In conditions a, e, and f, the reactive transients were the products of water radiolysis. Their radiation chemical yields may be various in a neutral or alkaline solution,

Table 2
G_d of radiation degradation of CM-chitosan in different conditions

Sample	Condition	G_d	r	Additives and ambience
CM-chitosan	a	62.9	0.999	N_2-saturated, pH 9.8
	b	95.9	0.996	N_2O-saturated, pH 9.8
	c	176	0.997	N_2-saturated, 0.02 mol/L H_2O_2, pH 9.8
	d	3.19	0.999	N_2-saturated,0.76 mol/L isopropanol, pH 9.8
	e	46.3	0.999	N_2-saturated, pH 7.9
	f	20.3	0.986	N_2-saturated, pH 1.1

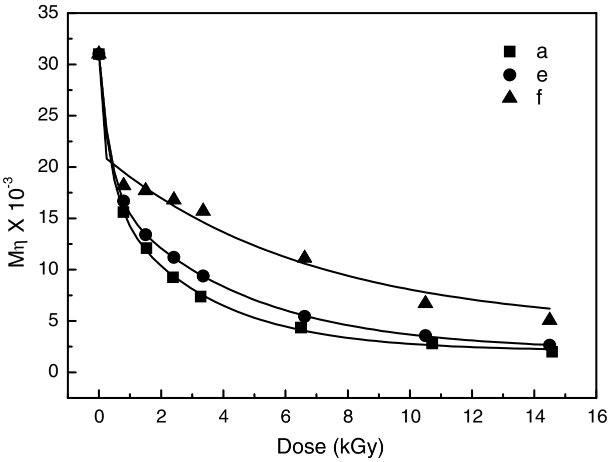


Fig. 3. Influence of absorbed dose on the $M\eta$ of CM-chitosan. (a) N_2-saturated, pH 9.8; (e) N_2-saturated, pH 7.9; (f) N_2-saturated, pH 1.1.

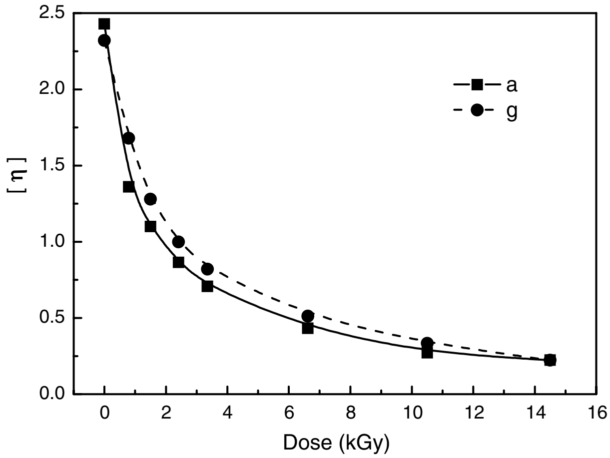


Fig. 4. Influence of absorbed dose on the intrinsic viscosity of CM-chitosan and CM-chitin. (a) CM-chitosan, N_2-saturated, pH 9.8; (g) CM-chitin, N_2-saturated, pH 9.8.

but the changes are often neglected. Therefore radiation chemical yields of these transients such as ·H, ·OH and e_{aq}^- are constant in the wide range of pH (Woods & Pikaev, 1994). However, e_{aq}^- can also convert to ·H quantitatively in acid solution such as in condition f (reaction (10)).

$$e_{aq}^- + H^+ \rightarrow \cdot H \qquad (10)$$

e_{aq}^- converts into ·H can accelerate the degradation of CM-chitosan, which means that in acidic condition, there are more ·H to initiate the degradation of CM-chitosan. However, contrary result was obtained in this work (Fig. 3 and Table 2), which suggested that the change of the conformation of CM-chitosan molecules with pH is a main factor influencing the degradation of CM-chitosan, and the action of ·H can be neglected in acid solution. In fact, radiation-chemical yield of ·H comprises less than 10% (even pH 2, less than 20%) of water radiolysis transients. When compared with ·OH, ·H reacts with polymers at one order of magnitude lower rate constant, and its role for degradation can be neglected (Wach et al., 2004). Therefore, ·OH radicals played the most important role for degradation of CM-chitosan in the radiation system of dilute CM-chitosan aqueous solution (Yoksan et al., 2004; Choi, Ahn, Lee, Byun, & Park, 2002; Tanioka et al., 1996; Tian, Liu, Hu, & Zhao, 2004; Qin, Du, & Xiao, 2002).

3.3.3. Influence of substituted group

According to our previous work, the rate constants of the reactions of ·OH radicals with CM-chitosan are always higher than those of CM-chitin, indicating that the amino groups could enhance the reactivity of CM-chitin derivatives (Zhai et al., 2004a, 2004b). However, no K and α in the Mark-Houwink equation for CM-chitin were reported in former literatures. It is the reason why we use intrinsic viscosity ([η]) to denote the degradation processes of CM-chitosan and CM-chitin. The results in Fig. 4 showed that [η] of CM-chitosan decreased more quickly than that of CM-chitin. Considering that CM-chitosan and CM-chitin have similar DS and molecular weight except DDA of CM-chitosan and CM-chitin were 84.0% and 31.4%, respectively, we concluded that DDA can influence on the degradation of CM-chitosan solution. With the increase of DDA, that is, more amino groups can accelerate the degradation of CM-chitosan in aqueous solution. As it is well know that ·OH is a strong electrophilic reagent and H-abstraction reaction mostly occurred at the position with high density of electron, which is the reason that CM-chitosan with high DDA degraded much faster than CM-chitin for its high density of electron.

3.4. Structure analysis of radiation-degraded CM-chitosan

3.4.1. Element analysis

As we can see from the Table 3, the contents of C, N decreased and the content of H increased during the radiation degradation in conditions a, b and c. Furthermore the whole content of C, N, and H decreased and the molar ratio of C/N increased. It indicated that partial amino

Table 3
Element analysis data for CM-chitosan and radiation-degraded CM-chitosan

Sample	Condition (kGy)	C (%)	N (%)	H (%)	$SUM_{C,H,N}$(%)	N_C/N_N
CM-chitosan	Original, 0	37.56	5.50	5.60	48.7	7.97
	a, 10	33.81	4.66	6.26	44.7	8.46
	b, 10	35.81	4.93	5.94	46.7	8.47
	c, 10	34.34	4.77	5.93	45.0	8.40

groups were got off and oxidation reactions were happened in the degradation of CM-chitosan.

3.4.2. FTIR absorption spectra

The FTIR spectra of CM-chitosan in different degradation conditions are shown in Fig. 5. Curve d is the FTIR spectrum of original CM-chitosan samples. The strong, broad bands in spectrum at 3445 cm^{-1} is assigned to the hydrogen-bonded –OH and –NH bands. The bands at 2923 cm^{-1} and 1598 cm^{-1} correspond to C–H stretching vibration and N–H bend vibration (Chen & Park, 2003), respectively. It was known that the bands at 1410 cm^{-1} and 1385 cm^{-1} which result from the coupling of C–N axial stretching and N–H angular deformation (Britto & Campana-Filho, 2004). Furthermore bands at 1320 cm^{-1} (υ_{C-N} of amide), 1070 cm^{-1} (υ_{C-O} of –COH–) and 886 cm^{-1} (vibration of ring) were confirmed in spectrum. Because carboxyl groups of CM-chitosan were in the form of sodium salt, no bands at 1730 cm^{-1} for –COOH were observed in the spectrum. Curves a and b are the spectra of radiation-degraded CM-chitosan sample with the absorbed dose of 10 kGy in the conditions a and b, respectively. Curve c is the spectrum of radiation-degraded CM-chitosan with 0.02 mol/L H_2O_2 added and the absorbed dose was 13.5 kGy. In curves a and b, the band at 1630 cm^{-1} was appeared and the intensity of absorption peak at 1420 cm^{-1} decreased, but the absorption intensity at 1380 cm^{-1} increased. The same changes also can be observed in curve c, besides the band at 1598 cm^{-1} was disappeared. As we know the band at 1630 cm^{-1} appeared in curves a, b, and c which was assigned to the absorbance of carbonyl groups (Tian et al., 2004). All the results denoted that radiation degradation of CM-chitosan lead to carbonyl groups formed and make the structure of substituted groups of CM-chitosan at C(2) changed during degradation process. Furthermore, the profiles of curves a, b, and c are similar so that we can conclude that the main polysaccharide chain structure was remained during degradation process.

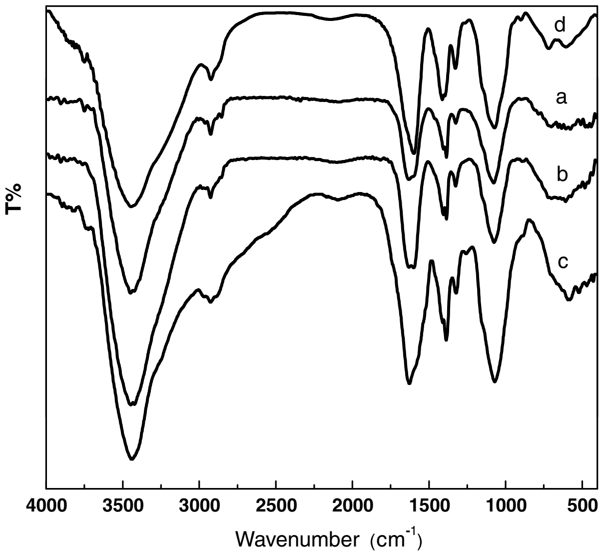


Fig. 5. FTIR spectra of CM-chitosan in different conditions. (a) pH 9.8, N_2-saturated, 10 kGy; (b) pH 9.8, N_2O-saturated, 10 kGy; (c) pH 9.8, 0.02 mol/L H_2O_2, 13.5 kGy; (d) original sample.

3.4.3. UV scanning spectra

Figs. 6(a)–(c) were UV spectra of 1% CM-chitosan solutions after irradiated in N_2-saturated, N_2O-saturated or added 0.02 mol/L H_2O_2, respectively. It was found that the solutions of radiation-degraded CM-chitosan had a peak at 290 nm in conditions a and b (Figs. 6(a) and (b)). But in the presence of H_2O_2, a broad maximum at about 255–269 nm was found (Fig. 6(c)). Furthermore, the absorption peaks tend to high wavelength and increase in intensity with absorbed dose increased. Yoksan et al. found that radiation-degraded chitosan had a peak at about 290 nm belonging to the carbonyl group (Yoksan et al., 2004). Figs. 6(a) and (b) showed that carbonyl and carboxyl groups formed and the number of carbonyl/carboxyl groups increased with increasing absorbed dose, whereas they do not mention of the peak at 260 nm. UV spectra of alginates and celluloses irradiated with various doses in aqueous solution show a new absorption band at 265 nm and peak intensity increases with increasing the dose (Nagasawa et al., 2000; Andrady, Torikai, & Kobatake, 1996). Nagasawa et al. assigned the peak to double bonds of alginate, which formed after main chain scission and/or hydrogen abstraction reaction by radiation. They have confirmed their presumption by introducing ozone in the form of bubbling gas into the alginate solution after radiation. We use ozone to identify whether double bonds were formed by γ-ray radiation, and no similar discolored phenomena were observed for degradation of CM-chitosan. Based on difference structure of these three polymers (hydroxyl groups for alginates and celluloses and amino or acetamido groups for CM-chitosan at C(2) of the ring), we considered that partial amino groups were eliminated at C(2) of ring as H_2O_2 added into CM-chitosan solution during radiation.

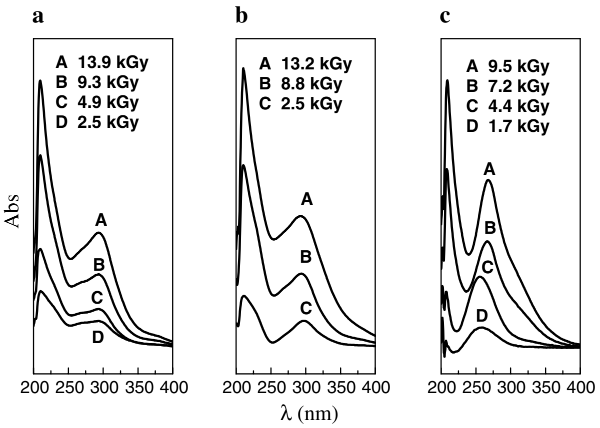
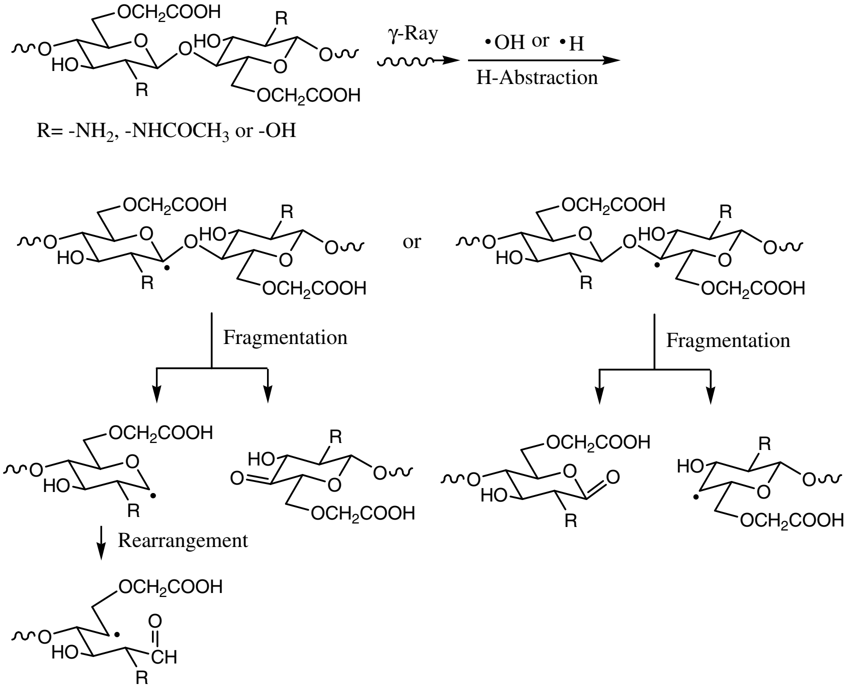


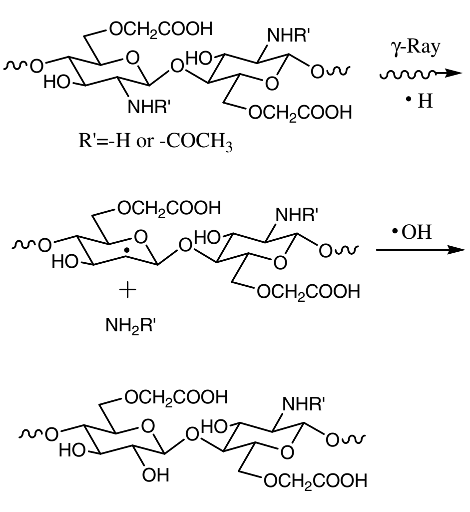
Fig. 6. UV spectra of 1% radiation-degraded CM-chitosan solutions in different conditions. (a) N_2-saturated; (b) N_2O-saturated; (c) 0.02 mol/L H_2O_2, N_2-saturated.



Scheme 1. The possible mechanism of main chain scission of CM-chitosan during radiation.

3.5. *Radiation degradation mechanism of CM-chitosan*

According to above experimental results, the possible reaction pathways of chain breakage of CM-chitosan are expected to follow the general procedure in Schemes 1 and 2. The main mechanisms are H-abstraction reaction, fragmentation and/or rearrangement of radicals at C(1), C(2), C(4), and C(5), which is similar to the degradation of chitosan. As Scheme 1 was shown, in the CM-chitosan aqueous solution the radiolysis of water yields reactive species such as ·OH and ·H, which will cause H-abstraction reaction firstly, and then fragmentation and/or rearrangement of CM-chitosan radicals to result in degradation of CM-chitosan. From Scheme 1, it also can be found that the degradation of CM-chitosan induces the formation of carbonyl groups at the terminal chains of CM-chitosan. Simultaneously the formation of hydroxyl groups at C(2) and partial amino groups will be eliminated as Scheme 2 was shown. The difference between condition c and other conditions is that fragmentation occurred by Scheme 2 more frequently with low concentration of H_2O_2.



Scheme 2. The possible mechanism of amino groups eliminated from CM-chitosan during radiation.

4. Conclusion

In summary, the degradation of CM-chitosan in aqueous solutions will occur with γ-ray radiation, and different additives, such as N_2, N_2O, H_2O_2, isopropanol, pH of the CM-chitosan solutions and the substituted groups of CM-chitosan have obvious influence on the radiation degradation of CM-chitosan in aqueous solution. ·OH radicals are supposed as the main reactive radicals for H-abstraction reactions with CM-chitosan to form CM-chitosan macroradicals, and then fragmentation and/or rearrangement of CM-chitosan macroradicals to result in degradation of CM-chitosan. FTIR and UV spectra showed that main chain structure of CM-chitosan was remained and some carbonyl/carboxyl groups were formed during the degradation. The elimination of partial amino groups during radiation was also identified owing to the increase of C/N contents in EA data.

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