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Study of the depolymerization behavior of chitosan by hydrogen peroxide

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Abstract

The IR and ^{1}H NMR study verify the breakage of 1,4-β-D-glucoside bonds of chitosan leads to the depolymerization. X-ray quantitative analysis shows the crystallinity degree of chitosan was changed in the depolymerization. In crystal region the chitosan is depolymerized by peeling-off process while the amorphous portion is depolymerized by penetrating pattern. Moreover, the depolymerization rate of chitosan depends on the deacetylation degree of chitosan, the concentration of hydrogen peroxide and the reaction temperature.

Keywords: Chitosan; Hydrogen peroxide; Depolymerization

1. Introduction

Water-soluble chitosan with low molecular weight (LWCS) has some special hysiological functions, differently from the ordinary chitosan, such as binding lipid, affecting the mitogenic respons and restraining the growth of tumors. Moreover, its solubility broadens its applications in medicine.

LWCS are sometimes obtained by the enzymatic depolymerization of chitosan with hydrolytic enzymes (Nagasawa, Tohica, Inoue, & Tanoura, 1971) or by the acidic depolymerization with different acids, such as hydrochloric acid and sulfuric acid (Kabal'nova et al., 2001; Varum, Anthonsen, Grasdalen, & Smidsrod, 1991). In addition, LWCS can also be obtained by the oxidative depolymerization with oxidants, such as O_3, $NaNO_2$ and H_2O_2 (Allan & Peyron, 1995; Chang, Tai, & Cheng, 2001; Tanioka et al., 1996) Thus, there have been few reports on the depolymerization mechanism of chitosan by H_2O_2. The studies of Chang et al. (2001) and Tanioka et al. (1996) suggest that the depolymerization was predominantly caused by radical reactions. The metal ions induced the depolymerization of H_2O_2, which caused chitosan degradation. Thus in the study of Chang et al. (2001), the content of Fe in chitosan was trace (14 ppm), however even when free radical scavengers and metal chelator were added, the depolymerization still took place. Tanioka et al. (1996) suggested chitosan was depolymerized by hydroxyl radical generated through Cu (II)- ascorbate- H_2O_2 in ultraviolet light in room temperature. Nevertheless they did not explain how $HO^{\cdot}$ generates in the heat system and the feasibility of producing LWCS by hydrogen peroxide. In this paper we found the crystallinity degree of chitosan was changed in the depolymerization by X-ray diffraction quantatively and explained how the depolymerization of chitosan by htdrogen peroxide occurs. Factors affecting the reaction such as the degree of deacetylation of chitosan, the concentration of H_2O_2, and the reaction temperature are also studied.

2. Expermential

2.1. Materials

Chitin, 100 meshes, was purchased from Yuhuan Biochemical Co. (Zhejiang, PRC). The following chemicals were all obtained and used as reagent grade: sodium hydroxide, hydrogen peroxide, hydrochloric acid and ethanol.

2.2. Deacetylation of chitin

Deacetylations were carried out by heating solutions of chitin in aqueous 50% NaOH solution, at 100 °C, under a nitrogen atmosphere. Aliquots of the reaction mixture were

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Table 1
DD and Mv of the chitosan samples with different reaction time

Sample	Time (h)	Elemental composition (%)				DD (%)	η (l/g)	$M_v \times 10^{-4}$
		C	H	N	O			
A	0.5	43.65	6.81	7.36	42.18	54	17.44	1378
B	1	44.37	6.95	7.71	4097	64	16.91	1327
C	2	44.19	8.13	7.98	39.70	77	16.21	1258
D	4	44.44	8.09	8.39	39.08	91	15.51	1196

collected periodically at 0.5, 1, 2, 4 h intervals. The reaction products were subsequently washed in hot distilled water until reaching a neutral pH and then dried in a 60 °C hot vacuum oven for 48 h. The deacetylation degree of the dried powder (noted in Table 1) was analyzed by Elemental Analysis (EA). The molecular weight was obtained by viscometery measurements.

2.3. Depolymerization of chitosan

2 g chitosan was completely dissolved in 100 ml 0.5% hydrochloric acid solution, then 5 ml H_2O_2 aqueous solution (the concentration was 0.5, 1.0, 1.5, 2.0 M, respectively) was added. The solution was stirred and reacted at the desired temperature (25, 40, 50, 70 °C) for different durations, 1, 2, 3 h, respectively. After the reaction, the solution was filtrated. The collected solid was washed with distilled water until reaching pH = 7, and then dried in vaccum. The obtained product was the water-insoluble chitosan. The filtrate was adjusted to pH 7.0 with NaOH solution and a precipitate was obtained by adding ethanol. LWCS was collected after dried the precipitate in vaccum.

2.4. Determination of deacetylation degree

Samples were absolutely dried in a 60 °C hot air oven for 48 h prior to submission of samples for EA. All elemental analyses were performed by Perkin Elmer 2400-II. The degree of deacetylation determined by EA was calculated using the following equations (Xu, McCarthy, & Gross, 1996):

$$\mathrm{DD} = \left[1 - \frac{\mathrm{C/N} - 5.14}{1.72}\right]100\% \tag{1}$$

Where C/N is the ratio (w/w) of carbon to nitrogen.

2.5. Determination of M_v viscometry measurements

In the present paper, viscometry measurement is used to determine the molecular weight of chitosan. The viscosities of chitosan samples were measured in a solvent of 0.25 M CH_3COOH/0.25 M CH_3COONa at 25 °C using an Ubbleohde capillary viscometer. The efflux times were recorded in triplicate. The viscosity average molecular weights of chitosan were determined using the classical Mark-Houwink equation (Knaul, Kasaai, Bui, & Creber, 1998):

$$[\eta] = 1.40 \times 10^{-4} M_v^{0.83} \tag{2}$$

2.6. Infrared spectrometry

Samples were ground with i.r.-grade potassium bromide in an agate mortar. Spectra were recorded with a Perkin-Elmer infrared spectrometer Model Equinox 55.

2.7. ^1H NMR

Samples were dissolved in D_2O, and transferred to 5 mm NMR tubes. The measurements were performedon a Dp × 400 Bruker Avance spectrometer at 400 MHz, 25 °C. All chemical shifts were determined relative to internal standard tetramethyl silane (TMS) (Table 2).

2.8. X-ray diffraction

X-ray diffraction measurements were carried out on a D/max-3A diffractomer at the Cu Kα ray ($\lambda = 1.54$ Å) in the range 5–40° 2θ at a scan rate of 2° 2θ per minute. The spectra were recorded at 40 kV, 25 mA.

The X-ray diffraction profiles were deconvoluted with the Levenberg-Marquardt algorithm of the nonlinear least squares fitting method. A fifth-degree polynomial function was used for the background function, and a pseudo-Voigt (pV) function was used to fit the crystalline reflections (Wada & Saito, 1997). The pV function $P(2\theta)_{\mathrm{pV}}$ was

$$pV(2\theta) = \eta P(2\theta)_L + (1 - \eta)P(2\theta)_G \tag{3}$$

Where η is a parameter varying from 0 to 1 and $P(2\theta)G$, and $P(2\theta)L$ are the Gaussian and Lorentzian functions, respectively, represented by the following equations:

$$pV(2\theta)_G = \frac{2}{H}\left(\frac{\ln 2}{\pi}\right)^{1/2} \exp\left[-4\ln 2\left(\frac{2\theta - 2B}{H}\right)^2\right] \tag{4}$$

$$P(2\theta)_L \frac{2}{\pi H}\left(1 + 4\left(\frac{2\theta - 2B}{H}\right)^2\right)^{-1} \tag{5}$$

Where B is the Bragg angle and H is the full width at half-maximum (FWHM).

Table 2
^1H NMR chemical shifts (ppm) for chitosan and LWCS in D_2O solution

	H-1	H-2	H-3	H-4	H-5	H-6	H-7
Chitosan	4.87	3.18	3.78	3.83	3.74	3.87	2.07
Water-soluble chitosan	4.71	3.00	3.46	3.65	3.39	3.76	1.94

3. Results and discussion

3.1. FT-IR analysis

The FT-IR spectra of chitosan (DD = 91%), LWCS and water-insoluble chitosan (both obtained from chitosan DD = 91%) are shown in Fig. 1a–c, respectively. In Fig. 1a, the characteristic absorptions of the original chitosan at 1597 cm^{-1} ($-NH_2$), 1648 cm^{-1} (amide I band) and 1555 cm^{-1} (amide II band) while no absorption at 1480 cm^{-1} (C=O of $COCH_3$) are similar to those of high deacetylation degree chitosan that has been reported in literature (Pearson, Marchessault, & Liang, 1960). The peaks at 3200–3500 cm^{-1} (O–H, N–H), 1423 cm^{-1} (symmetrical deformation of $-CH_3$ and $-CH_2$), 1254 cm^{-1} (twisting vibration of O–H), 1089 cm^{-1} (strenching vibration of hydroxyl), 1028 cm^{-1} (strenching vibration of the C–O–C in glucose circle), 1153 cm^{-1} and 895 cm^{-1} (the special absorb peaks of β (1 → 4) glucoside bond in chitosan) are both shown in Fig. 1a and b. These data shows the structures of the main chain of chitosan and LWCS are the same.

In comparison with the FT-IR spectrum of chitosan, that of LWCS shows a new peak at 1623 cm^{-1}, which is assigned to the absorbance of C=O. It might be the new side group of LWCS.

In the FT-IR spectrum of water-soluble chitosan (Fig. 1c), the peak at 777.5 cm^{-1} is assigned to the out-of-plane deformation of the N=CHCOO$^-$ group, which is a consequence of the N=C double bond (Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982). This suggests that Maillard reaction between –CHO of 2, 5-anhydro-D-mannose end group and $-NH_2$ of chitosan was occurred.

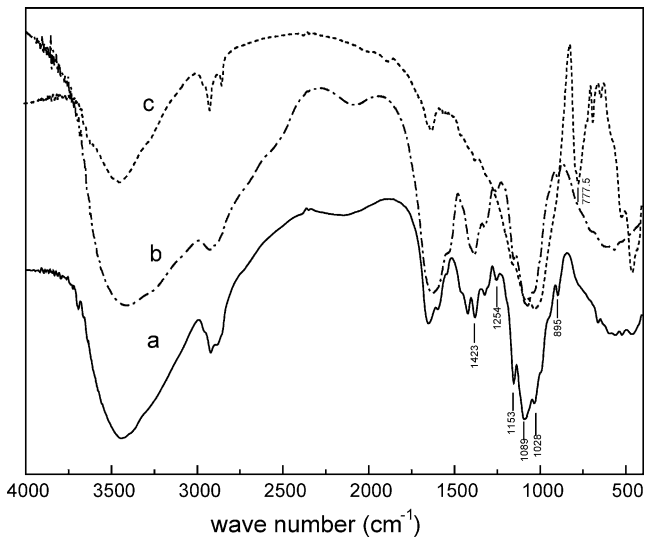
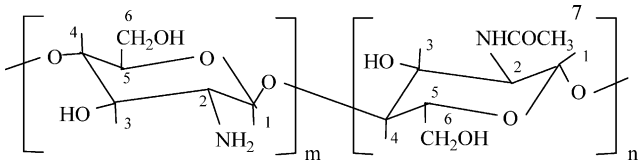


Fig. 1. IR spectra of (a) chitosan (b) LWCS and (c) water-insoluble chitosan.

3.2. ^1H NMR analysis

The ^1H NMR spectrum of LWCS is shown in Fig. 2. A singlet at 3.0 ppm (H2) and multiplets at 3.4–3.8 ppm (H3, H4, H5, H6) corresponding to the ring methane protons together with a singlet at 1.94 ppm (H7), which is due to the *N*-acetyl glucosamine units having survived the saponification chitin, and a small signal at 4.7 ppm (H1). Compared with the ^1H NMR spectrum of chitosan, the shift of each ^1H of water-soluble chitosan is corresponding to the shift of chitosan, so the significant changes in characteristic shifts of water-soluble chitosan do not take place in comparison to original chitosan in the spectra. The structure of the product keeps the same as the chitosan.



3.3. X-ray diffraction analysis

So far, the following six polymorphs have been proposed for chitosan: 'tendon chitosan', 'annealed', '1–2', 'L-2', 'form-I' and 'form-II' (Samuels, 1981). The form I crystal is orthorhombic with a unit cell of $a = 7.76$, $b = 10.91$, and $c = 10.30$ Å. The strongest reflection appears at $2\theta = 11.4°$, which is assigned to (100) reflection. The form II crystal is also orthorhombic with a unit cell of $a = 4.4$, $b = 10.0$, and $c = 10.3$ Å (fiber axis). The strongest reflection appears at $2\theta = 20.1°$, which also corresponds to the (100) reflection. As shown in Fig. 3, the original chitosan shows the strongest reflection at $2\theta = 19.84°$, which is coincided with the pattern of the form II crystal.

In the unit of chitosan, there are 11 H atoms, 6 C atoms, 1N atom, and 4 O atoms. For calculating the crystallinity conveniently, we consider the degree of deacetylation of chitosan is 100%, we have

$$f^2 = 11f_H^2 + 6f_C^2 + 4f_O^2 + f_N^2$$

In this way, we can calculate the crystallinity degree of samples.

LWCS-A was the water soluble product of chitosan (DD = 91%) depolymerized with H_2O_2 solution at 60 °C for 1 h. As shown in Fig. 3a and b, the peak intensities of LWCS-A were significantly lower than those of chitosan at $2\theta = 10.38°$, $2\theta = 19.84°$ and $2\theta = 22.46°$, whose crystallinity degree is 20.06% decreased from 26.1%of initial chitosan. When the reaction time reached 2 h, the characteristic reflection of the water-soluble depolymerized product LWCS-B (shown in Fig. 3c) at $2\theta = 10.38°$ and $2\theta = 19.84°$ decreased further and the peak is broaden, its crystallinity degree only 8.1%. Therefore, LWCS-A, LWCS-B are still including crystal

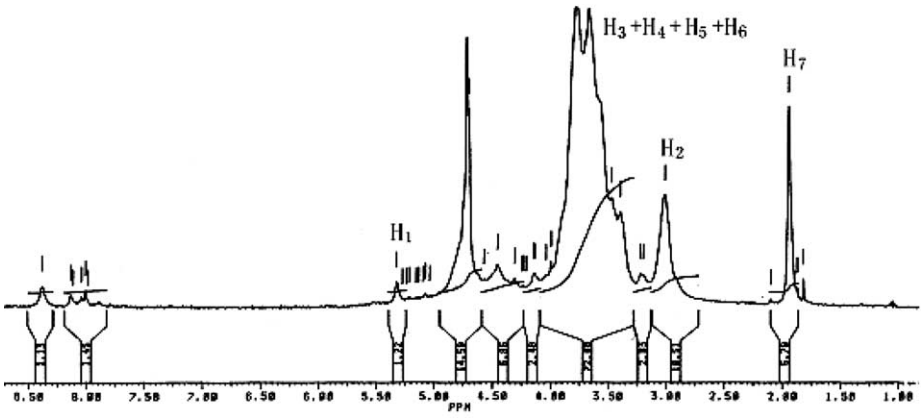


Fig. 2. ^{1}H NMR spectrum of LWCS.

portion. During the depolymerization, the change in peaks was caused by that the amorphous part of chitosan was preferentially degraded and the crystal part was temporally maintained. The main chains of chitosan are not all coplanar and have difficulties of rotation relative to each other, so the main chains are constrained under hydrogen bonds in chitosan structures. The disordered packing of the side chains having rotational freedom prevents the occurrence of well-defined reflections for main chains. Consequently, in the beginning of the depolymerization some crystal part was temporally maintained. In the same reaction system, prolong the reaction time until chitosan was further depolymerized to amorphous LWCS, which was shown in Fig. 2d, only a decreased wide peak at $2\theta = 22.32°$, whose crystallinity degree can not be calculated.

Schweiger (1973) proposed a peeling-off pattern to explain the high degree of the substitution in the sulfation of cellulose fiber. This pattern appears to be applicable to our results according to the above X-ray analysis. Thus, it was assumed in the crystal portion, the reaction occurs at the surface of the chitosan powder. As soon as the outer layer is sufficiently depolymerized to become soluble in the reaction medium, it peels off. The depolymerization of the chitosan can proceed in the same manner while the amorphous portion presumably is penetrated quite rapidly by the reaction medium, and becomes soluble without difficulty (Fig. 4).

3.4. Effect of the deacetylation degree of chitosan

It can be seen from Fig. 5. that the chitosan with high deacetylation degree is easily depolymerized, which is related to the mechanism of the chitosan depolymerization by H_2O_2. In acidic reaction system, more amino groups became protonated group, which improves the solubility of chitosan and helps to increase the pH of solution. This suggests that the more free amino groups in the polysaccharide chains there are, the more easily $-NH_2$ react with H_2O_2 to break down the chitosan chain. This result is confirmed to that of Hawkins and Davies (1996). They indicated that the amino groups on C-2 of chitosan facilitated a site-specific fragmentation of the glycosidic linkages while the *N*-acetyl group slowed the rearrangement of radicals during β-cleavage.

3.5. Effects of temperature

It can be seen from Fig. 6. that the rise of the temperature increases the initial rate of the destruction. In our experimental, the LWCS products obtained below 40 °C could be directly precipitated by ethanol and the final product was white. However, when the temperature was

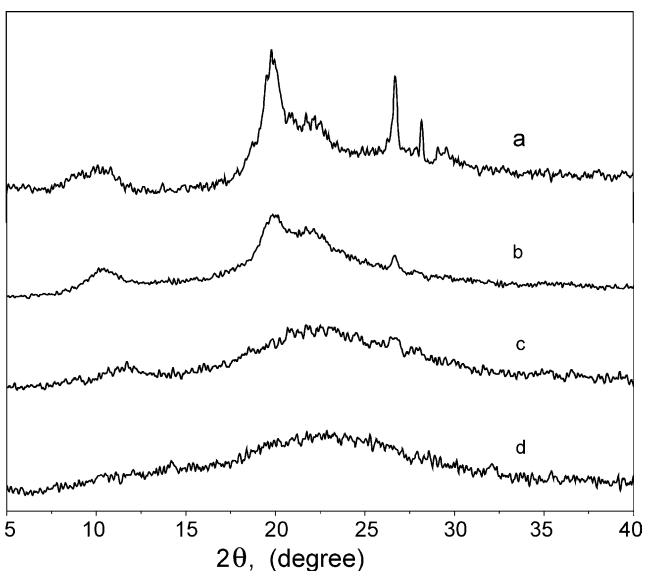


Fig. 3. X-ray diffraction patterns of (a) initial chitosan (DD = 91%) (b) LWCS-A (reacted at 60 °C for 1 h),(c) LWCS-B (reacted at 60 °C for 2 h) (c) LWCS-C (reacted at 60 °C for 3 h).

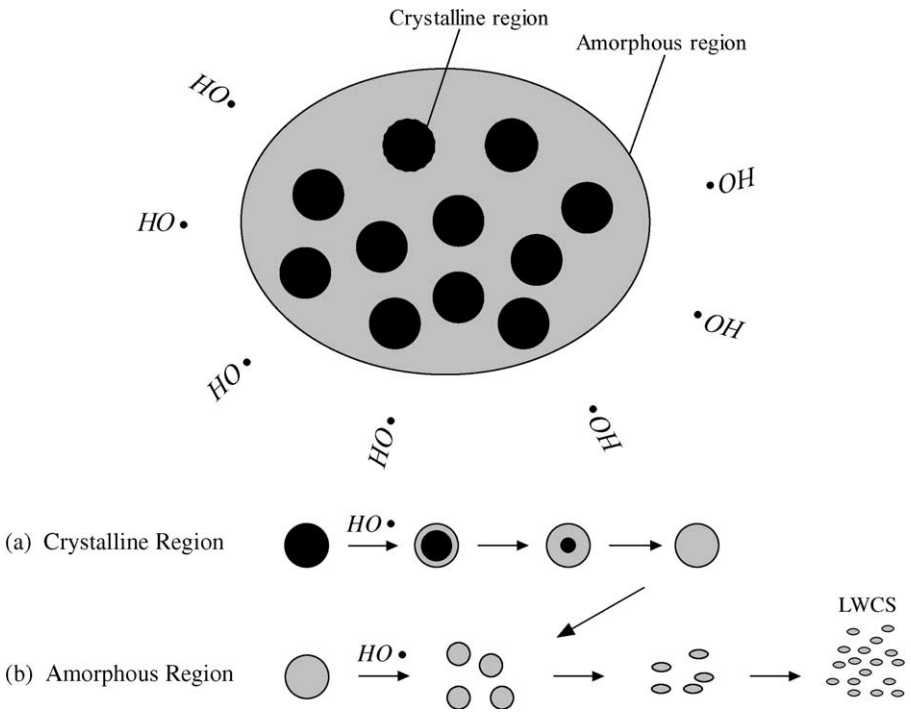


Fig. 4. The illustration of the depolymerization of chitosan (a) the peeling process in crystalline portion (b) the penetrating process in amorphous potion.

over 70 °C, the color of the product became dark when kept in the air. The IR spectrum of the product is complex (not shown here). It may be because when the reaction temperature is too high, the depolymerization of chitosan became rapid and severe. Some chitosan was depolymerized to the oligosaccharide with aldehyde side groups. This oligosaccharide may be react with unreacted chitosan, ethanol to produce more complicated by-product. So the reaction temperature should not be too high during the depolymerization. According to the experimental results, the most suitable depolyermization temperature was about 40–60 °C.

3.6. Effect of the concentration of H_2O_2 on the depolymerization of chitason

Table 3 shows that the molecular weight of the products gradually decreased with the increase of the concentration of H_2O_2 in the reaction. Small quantity of hydrogen peroxide can lead to the decrease of chitosan molecular weight. When other reaction conditions keep the same, the higher the concentration of H_2O_2 is, the smaller the product molecular weight is. When the concentration of H_2O_2 is more than 0.5 M, the main part of the products is LWCS. These results indicated that the concentration of H_2O_2 affects the depolymerization rate and the yield of products.

3.7. Discussion of the depolymerization mechanism

In the depolymerization system of chitosan with H_2O_2, the balances exist as shown in Eqs. (6) and (7), and the total reaction is shown in Eq. (8).

$$R{-}NH_2 + H^+ = R{-}NH_3^+ \quad (6)$$

$$H_2O_2 = H^+ + HOO^- \quad (7)$$

$$H_2O_2 + R - NH_2 + H^+ = R - NH_3^+ + HOO^- + H^+ \quad (8)$$

The hydroperoxide anion is very instable and easily decomposed to high reactive hydroxyl radical ($HO^{\cdot}$) (Fang, Sun, Salisbury, Fowler, & Tomkinson, 1999),

$$HOO^- \rightarrow OH^- + O^{\cdot} \quad (9)$$

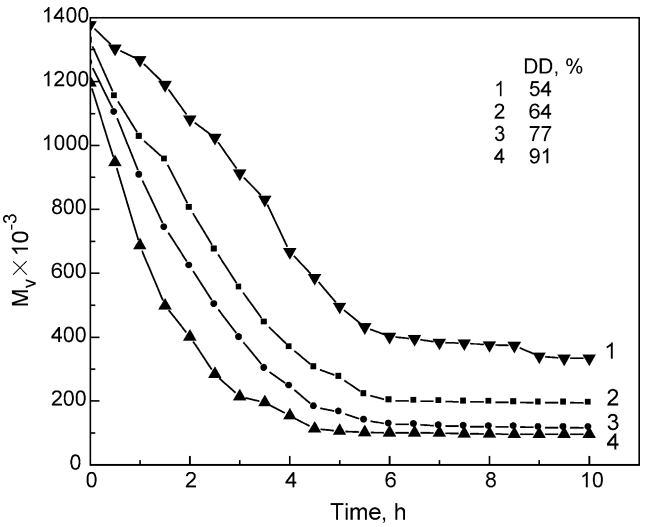


Fig. 5. Effect of the deacetylation degree of chitosan on the depolymerization.

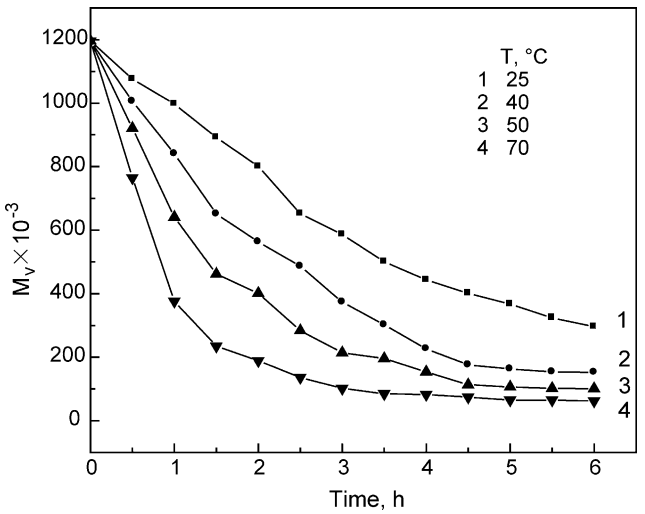


Fig. 6. Effect of the reaction temperature on the depolymerization.

$$H_2O_2 + HOO^- \rightarrow HO\cdot + O_2\cdot^- + H_2O \quad (10)$$

The hydroxyl radical is a very powerful oxidant. The main chemical action of $HO^{\cdot}$ with polysaccharide has been demonstrated to be hydrogen abstraction (Kawakishi, Kito, & Namiki, 1977). It reacts with carbohydrates very quickly and the reaction is shown in Eqs. (11) and (12). $HO^{\cdot}$ pulls off a hydrogen atom and combines with it to form water.

$$(GlcN)_m - (GlcN)_n + HO \rightarrow (GlcN)_m - (GlcN)_n + H_2O \quad (11)$$

$$(GlcN)_m - (GlcN)_n + H_2O \rightarrow (GlcN)_m + (GlcN)_n \quad (12)$$

During the treatment, the $R-NH_2$ preferentially reacts with H^+ to produce $R-NH_3^+$, which causes the decrease of $[H^+]$ and the increase of pH. In addition, HOO^- is rapidly decomposed to $HO^{\cdot}$, which means that H_2O_2 is continually decomposed as shown in Eq. (8). These radicals undergo further reactions rapidly to form water-soluble oxidation products with low molecular weight.

In this work, when chitosan was depolymerized by 2.0 M H_2O_2 at 60 °C for 4 h, the M_v of LWCS is 1.1×10^4. The reduction of M_v indicates the decrease of chitosan polysaccharide chain. However, the FT-IR spectrum of the LWCS and the original chitosan shows no distinct difference. It indicates that no obvious change takes place in the structures of chitosan chain during the depolymerization. The results obtained verify the rupture of 1, 4-β-D-glucoside bonds in macromolecule to be the basic process during amino groups of chitosan protection by acids.

Table 3
Effect of the concentration of H_2O_2 on the depolymerization of chitosan (DD = 91%)

$[H_2O_2]$ (M)	Water-insoluble chitosan		Water-soluble chitosan	
	$M_v \times 10^{-4}$	Yield (%)	$M_v \times 10^{-4}$	Yield (%)
0	21.7	97	–	–
0.1	11.4	87	–	–
0.5	4.6	61	3.2	13
1.0	2.2	24	1.9	58
1.5	–	–	1.1	67
2.0	–	–	0.9	64

4. Conclusions

In the depolymerization of chitosan by hydrogen peroxide, the breakage of 1,4-β-D-glucoside bonds in polysaccharide chain leads to the decrease of chitosan molecular weight. It is verified by IR and ^1H NMR spectrum. X-ray diffraction quantative analysis shows the crystallinity degree of chitosan is changed in the depolymerization. It is proposed that in the crystal regions of chitosan, the depolymerization occurred by a peeling-off pattern while in the amorphous regions, it is occurred by penetrating pattern. In addition, the increase of the deacetylation degree of chitosan and the concentration of H_2O_2 in the reaction system led to a more rapid decrease in the chitosan polymerization degree. The temperature rise of the reaction also accelerated the process.

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