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Kinetics and mechanism of degradation of chitosan by combining sonolysis with H_2O_2/ascorbic acid

Tiantian Wu,† Chunhua Wu,† Yingchun Xiang, Jiaqi Huang, Lanlan Luan, Shiguo Chen and Yaqin Hu*

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This study demonstrated the combined use of sonolysis with the H_2O_2/ascorbic acid (Vc) redox reaction to degrade chitosan (CS). The influence of operating parameters, such as the initial CS concentration (1–15 mg mL^{-1}), the input power level (15–45% of the total input power), the H_2O_2 and Vc concentration (10–70 mM) and the pH (3.0–5.0) of the reaction solution, on the molecular weight and degradation kinetics of CS were investigated and optimized. Based on the degradation kinetics, a synergetic effect of sonolysis with H_2O_2 and Vc on the degradation of CS was observed. Structural analysis by FT-IR and ^1H and ^{13}C NMR indicated no significant difference between the chemical structure of chitosan before and after degradation. Moreover, intermolecular hydrogen bonds were broken, and the crystallinity of degraded chitosan decreased. Using the above analysis, CS degradation by the combination of sonolysis with the H_2O_2/Vc redox reaction was proposed to be due to mechanical effects along with $HO^{\bullet}$ attack on the β-1,4-glucoside linkages of glucosamine units. These results suggest that the sonolysis/H_2O_2/Vc combined technique is promisingly suitable for large-scale manufacture of chitooligosaccharide.

1. Introduction

Chitosan (CS) is a cationic polysaccharide obtained by partial deacetylation of chitin.[1] It is a polymer composed of β-(1,4)-linked glucosamine (GlcN) and *N*-acetylglucosamine (GlcNAc) units in a random sequence.[2] Due to its many desirable properties, such as biocompatibility, biodegradability, nontoxicity, antioxidant activity, and antibacterial activity, CS has received much attention as a functional biopolymer for diverse applications in biomedical, environmental, and agricultural domains.[3,4] However, CS always has a high molecular weight and low solubility in aqueous solvents, which limit its applications, especially in the medical, cosmetic, and food industries.[5] Additionally, several studies have shown that water-soluble CS with low molecular weight has some special properties, such as fat-binding and hypocholesterolemic and anti-tumor activities.[6] Thus, to improve its solubility and properties, the preparation of low molecular weight CS has become a hot topic for researchers.[7,8]

A variety of techniques, including acid-[4] and enzyme-catalyzed hydrolysis,[9,10] microwave,[11] hydrodynamic cavitation,[5] and γ irradiation,[12] as well as sonolysis,[8,13,14] have been reported to prepare water-soluble chitosans with low molecular weights. Among these methods, sonolysis is considered to be the most gentle in its preservation of chemical structure and elimination of side reactions.[14] Moreover, the separation and further purification that would be necessary in the case of chemical treatment methods could be avoided.[15] Over the last several decades, most studies have focused on the use of low-frequency sonolysis for the degradation of CS.[8,13,14] The mechanism involved in the degradation of CS is generally accepted to be due to the mechanical effects of shear force caused during the collapse of cavitation bubbles.[13] However, owing to the specific structure of chitosan, its degradation efficiency by sonolysis alone is still relatively low. From this starting point, a simple free-radical degradation method was introduced to the sonolysis system in this current study to produce lower molecular weight CS.

Free radical degradation is also used frequently to obtain low molecular weight polysaccharides.[16,17] For instance, the hydroxyl radical ($OH^{\bullet}$) is a short-lived but powerful oxidant.[18] It can react with all sugars exceedingly fast.[19] Generally, $OH^{\bullet}$ can be generated by the decomposition of hydrogen peroxide (H_2O_2) in atmospheric air[20] or *via* the Fenton reaction, which is catalyzed by reduced transition metals, *e.g.*, Fe^{2+} and Cu^{2+}.[16,21] Additionally, $OH^{\bullet}$ can be formed in the presence of ascorbic acid (Vc) without the need for transition metals because Vc facilitates the formation of H_2O_2 from oxygen.[22] Compared with other $OH^{\bullet}$ formation methods, the combination of H_2O_2 and Vc in a redox reaction is a novel, eco-friendly procedure.[23] Because Vc can reduce H_2O_2 to $OH^{\bullet}$ in the absence of trace metal,[24] further separation and purification processes are simplified. To date, the combination of H_2O_2 and Vc has been used for the

College of Biosystems Engineering and Food Science, Fuli Institute of Food Science, Zhejiang Key Laboratory for Agro-Food Processing, Zhejiang R & D Center for Food Technology and Equipment, Zhejiang University, Hangzhou, 310058, China. E-mail: yqhu@zju.edu.cn

† These authors contributed equally to this work.

degradation of hyaluronic acid,[25] porphyran,[26] and polysaccharides from *Athyrium multidentatum* (Doll.) Ching[27] and *Enteromorpha linza*.[24] The degradation efficiency of these polysaccharides was significantly enhanced in comparison to their degradation by H_2O_2 or Vc individually at similar concentrations. However, to the best of our knowledge, no report has been published on the application of this system for CS degradation. Although many researchers have studied the coupling of the $OH^{\cdot}$ reaction with sonolysis for the efficient degradation of hazardous materials,[28] there have been few reports on its application to organic materials, including water-soluble polysaccharides.[29] Furthermore, there has been no study on the combination of sonolysis with the H_2O_2 and Vc redox system for the degradation of CS.

Therefore, given the widespread use of these methods, the combination of sonolysis with H_2O_2 and Vc was used to degrade CS in the present study. The influence of the initial CS, H_2O_2, and Vc concentrations, the sonolysis intensities, and the solution pH, as well as the synergetic effect of sonolysis with H_2O_2 and Vc, on the molecular weight and degradation kinetics of CS were investigated. The structural properties of the degraded chitosan were characterized by FT-IR, ^{13}C NMR, and XRD spectral analyses. The mechanism of degraded CS is also proposed. The current investigation may provide encouragement for further exploration of the degradation of natural polysaccharides.

2. Material and methods

2.1 Materials

Chitosan was purchased from Qingdao Yunzhou Biochemistry Co. Ltd. (Qingdao, China). The degree of deacetylation (DD) and the molecular weight of the commercial chitosan used in this study were determined experimentally and were found to be 92.21% and 209.45 kDa, respectively. Acetic acid (CH_3COOH), sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), Vc, 5,5-dimethylpyrroline-*N*-oxide (DMPO) and other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Experimental facility and batch experiments for the demonstration of the synergetic effect of sonolysis-assisted H_2O_2/Vc degradation CS

CS was completely dissolved in 1% acetic acid solution before degradation. Sonication was performed with an ultrasonic generator (Scientz-IID, Ningbo Scientz Biotechnology Co., Ningbo, Zhejiang, China) equipped with a 10 mm horn microtip. The sample was added to 20 mL of nanopure water in a glass tube (diameter = 3 cm, height = 10 cm), which was immersed in a water bath (DC-1006, Safe Corp., Ningbo, Zhejiang, China) to maintain a temperature of 25 ± 1 °C. The ultrasonic probe was submerged to a depth of 1 cm from the surface of the liquid layer.

The effects of the following factors on the degradation of CS were investigated: the initial CS concentration (1, 2, 5, 10, and 15 mg mL^{-1}), the input power level (15, 25, 35, and 45% of the total input power), the H_2O_2 concentration (0, 10, 30, 50 and 70 mM), and the Vc concentration (0, 10, 30, 50 and 70 mM) and the pH (3.0, 3.5, 4.0, 4.5, and 5.0) of the reaction solution.

The intensity of the ultrasonic power that dissipated from the microtip with a radius of 10 mm was calculated according to the report of our previously study.[30] The input power levels were set at 15, 25, 35, and 45% of the total input power, which corresponded to ultrasonic powers of 182, 303, 424, and 545 W cm^{-2}, respectively.

After degraded, the treated chitosan solution was neutralized with 5% NH_4OH solution to pH 7.5, then precipitated with absolute ethanol. The chitosan precipitate was filtered, washed with ethanol several times and lyophilization. The dried chitosan samples were collected for further GPC, FT-IR, XRD and NMR investigations.

2.3 Determination of the molecular weight of degraded CS

The average molecular weight (average M_w) and polydispersity index (PDI) were determined by gel permeation chromatography (GPC) according to our previously studies, with some modifications.[30] The determination of average M_w was carried out with a Waters 1525 HPLC system (Waters, Milford, MA, USA) with a Waters Ultrahydrogel 250 and 2000 column. Twenty microliters of the sample solution was injected and eluted with 0.2 M ammonium acetate (pH 4.5) at 40 °C for 25 min at a flow rate of 0.5 mL min^{-1}. The eluent was monitored with a Waters 2414 refractive index detector. The results were analyzed by the GPC software. Dextran standards (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) with different molecular weights (from 0.5 to 670 kDa) were used to obtain calibration curves each time.

2.4 Degradation kinetics models

The degradation behavior of CS was expressed according to the model of first-order reaction kinetics,[7,31] and the degradation rate constant (k) was determined by the following formula:

$$\frac{1}{M_t} = \frac{1}{M_0} + \frac{kt}{m} = \frac{1}{M_0} + k't \quad (1)$$

where M_t and M_0 are the weight-average molecular weights of CS samples at time t and time 0 (kDa), respectively. k (min^{-1}) and k' (mol g^{-1} min^{-1}) represent the rate constants; m is the molecular weight of CS monosaccharides [m = 161 + 42(1 − DD)], and t (min) is the reaction time.

2.5 Characterization of chitosan degradation products

The degree of deacetylation was determined by an acid–base titration method from the literature.[8] Chitosan samples (0.1 g) were dissolved in a known excess of 0.1 M HCl (10 mL). By titrating this solution with a 0.1 M NaOH solution, a curve with two inflection points was obtained. The amount of the acid consumed between these two points was considered to correspond to the amount of free amino groups in the solution. The titration was performed using a pH meter (Mettler, Delta 340).

The Fourier-transform infrared (FT-IR) spectra of the initial and degraded chitosan were recorded in powder form on KBr discs over the range of 400 to 4000 cm^{-1} using an AVATAR 370

spectrophotometer (Thermo Nicolet Corporation, Madison, USA).

The crystallographic structures of the initial and degraded chitosan were determined by a Bruker AXS D8 Advance X-ray diffractometer (Bruker Inc., Germany) using Ni-filtered Cu Kα radiation at 35 kV and 24 mA. The X-ray diffraction (XRD) patterns of samples were recorded at a scanning rate of 10 min^{-1} in the 2θ range from 5 to 60°.

The ^{1}H and ^{13}C NMR spectra were recorded at 25 °C with samples dissolved in CD_3COOD/D_2O (1% acetic acid) using a 600 MHz NMR spectrometer (Bruner, Switzerland).

Electron paramagnetic resonance (EPR) measurements were conducted on a Bruker A300 EPR spectrometer (Bruner, Switzerland), operating in 9.75 GHz microwave frequency with a field modulator frequency of 100 kHz. The magnetic field was scanned from 3300 G to 3500 G and the microwave power was 20 mW.

3. Results and discussion

3.1 Effects of reaction parameters on chitosan degradation

3.1.1. Effect of initial chitosan concentration. The effect of solution concentration on the degradation of treated chitosan was investigated, as shown in Fig. 1a and b. The kinetic rate constants k and the determination coefficient of formula (1) R^2 are presented in Table 1. The degradation efficiency decreased as the concentration rose from 0.1 to 1.5 mg mL^{-1}, with lower initial concentrations causing increased CS degradation. This is consistent with published studies of CS degradation.[5,7,13,14] The kinetics study also revealed that k decreased with increasing CS concentration (Fig. 1b; Table 1). This may be because the increased polymer concentration led to a corresponding increase in the solution viscosity, thereby reducing the extent of the cavitation activity and hence the polymer scission rate. In addition, as the concentration decreased, the entanglement between the polysaccharide chains weakened.[30] Thus, the random coiled structure of the molecule became more extended and therefore more vulnerable to attack from the shear force and the hydroxyl radical, which improved the degradation.[7] Therefore, lower solution concentrations improve CS degradation.

3.1.2. Effect of ultrasonic intensity. The influence of ultrasound intensity on the degradation of CS is shown in Fig. 1c and d. The kinetic rate constants k and the determination coefficient of formula (1) R^2 are presented in Table 1. As expected, the extent of degradation increased with an increase in ultrasonic intensity. As the ultrasonic intensity increased from 182 to 545 W cm^{-2}, the reaction rate constants (k) for CS increased from 4.494×10^{-7} to 1.245×10^{-6} mol g^{-1} min^{-1}. This finding could be attributed to the enhancement of cavitation energy.[32] Similar observations were made by others for the degradation of CS,[13] sea cucumber fucoidan,[30] dextran,[33] and a polysaccharide from *Phellinus linteus*.[32] Higher ultrasonic intensity can effectively increase the number of cavitation events and result in more opportunities for enhanced degradation.[14] In addition, increased ultrasonic intensity accelerated the generation of $OH^{\cdot}$ radicals from H_2O_2 or H_2O_2/Vc,[34] which

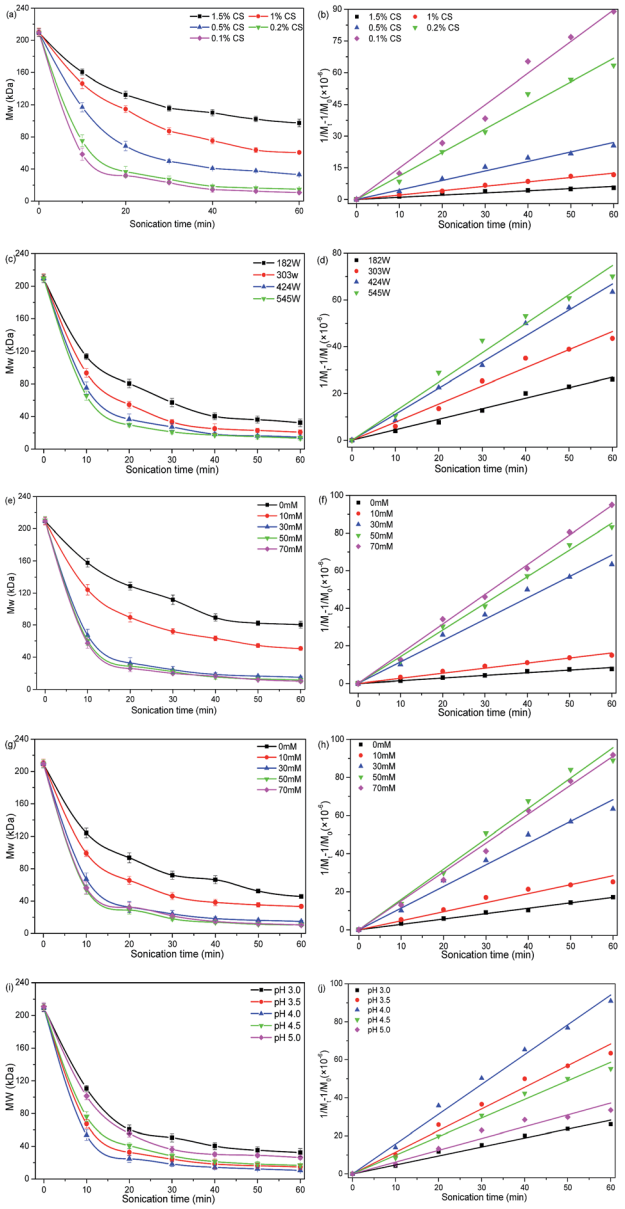

Fig. 1 Effects of factors on CS degradation: (a) solution concentration (concentration, 1, 2, 5, 10 or 15 mg mL^{-1}; ultrasonic intensity, 424 W cm^{-2}; H_2O_2 concentration, 50 mM; Vc concentration, 30 mM; pH 3.5). (b) Kinetics of CS degradation at different solution concentration; (c) ultrasonic intensity (ultrasonic intensity, 182, 303, 424, or 545 W cm^{-2}; H_2O_2 concentration, 50 mM; Vc concentration, 30 mM; pH 3.5; and CS concentration, 2 mg mL^{-1}); (d) kinetics of CS degradation at different ultrasonic intensity; (e) H_2O_2 concentration (concentration, 0, 10, 30, 50 or 70 mg mL^{-1}; ultrasonic intensity, 424 W cm^{-2}; CS concentration, 2 mg mL^{-1}; Vc concentration, 30 mM; pH 3.5); (f) kinetics of CS degradation at different H_2O_2 concentration; (g) Vc concentration (concentration, 0, 10, 30, 50 or 70 mg mL^{-1}; ultrasonic intensity, 424 W cm^{-2}; CS concentration, 2 mg mL^{-1}; H_2O_2 concentration, 50 mM; pH 3.5); (h) kinetics of CS degradation at different Vc concentration; (i) pH value (3.0, 3.5, 4.0, 4.5 or 5; ultrasonic intensity, 424 W cm^{-2}; CS concentration, 2 mg mL^{-1}; H_2O_2 concentration, 50 mM; Vc concentration, 30 mM; pH 3.5); (j) kinetics of CS degradation at different pH value.

Table 1 Degradation rate coefficients and determination coefficient of formula (1) R^2 of chitosan degradation

Process		k' (mol g^{-1} min^{-1})	R^2
Solution concentration (mg mL^{-1})	1	1.333×10^{-6}	0.977
	2	1.138×10^{-6}	0.983
	5	4.380×10^{-7}	0.994
	10	1.435×10^{-7}	0.934
	15	8.260×10^{-8}	0.977
Ultrasonic intensity (W cm^{-2})	182	4.494×10^{-7}	0.988
	303	7.741×10^{-7}	0.996
	424	1.138×10^{-6}	0.986
	545	1.245×10^{-6}	0.974
H_2O_2 concentration (mM)	0	1.141×10^{-7}	0.973
	10	2.696×10^{-7}	0.985
	30	1.138×10^{-6}	0.986
	50	1.442×10^{-6}	0.990
	70	1.560×10^{-6}	0.960
Vc concentration (mM)	0	2.835×10^{-7}	0.996
	10	4.747×10^{-7}	0.985
	30	1.138×10^{-6}	0.986
	50	1.593×10^{-6}	0.982
	70	1.517×10^{-6}	0.969
pH	3.0	4.705×10^{-7}	0.992
	3.5	1.138×10^{-6}	0.986
	4	1.568×10^{-6}	0.997
	4.5	9.784×10^{-7}	0.994
	5	6.202×10^{-7}	0.983
Sonolysis		3.172×10^{-7}	0.953
H_2O_2/Vc		9.261×10^{-7}	0.975
Sonolysis assisted H_2O_2/Vc		2.126×10^{-6}	0.989

also improved the degradation efficiency of CS. Consequently, the degradation efficiency increased with the ultrasonic intensity. It is worth noting that the M_w of CS dropped rapidly in the initial 30–40 min and then decreased slowly and slightly over the remaining 20–30 min of ultrasonic treatment, regardless of ultrasonic intensity. This may be attributed to the preferential breaking of longer polymer chains over shorter chains by shear force and the OH$^\bullet$ radical.[13,18,24,30]

3.1.3. Effect of H_2O_2 concentration. As Fig. 1e illustrates, the degradation rate of CS was enhanced with the increased H_2O_2 concentration. The kinetic rate constants k increased obviously with H_2O_2 concentration from 10 to 50 mM; however, at higher H_2O_2 concentrations, the change in k was insignificant (Fig. 1f and Table 1). This indicates that when a certain amount of H_2O_2 was added, a reasonable amount of Vc could increase the production of OH$^\bullet$, thus contributing to the degradation of CS. This result was in accordance with previous studies on the degradation of others polymer by OH$^\bullet$.[17,24,27] Additionally, as discussed above, small molecules with shorter chains are not sensitive to OH$^\bullet$ radical attack, which decreases the efficiency of degradation. It should be noted that the decrease in the M_w of CS was strongly dependent on the concentration of H_2O_2 when $c(H_2O_2)/c(Vc) < 1$. Nevertheless, higher concentrations of H_2O_2 (>50 mM) were not effective on the reaction, as measured by degradation efficiency (Fig. 1f), resulting in a waste of resources. According to these results, it can be concluded that the optimal concentration of H_2O_2 for degradation experiments is 50 mM.

3.1.4. Effect of Vc concentration. The effect of the Vc concentration on CS degradation is shown in Fig. 1g and h. A degradation trend similar to that seen for H_2O_2 was observed for Vc. It can be seen that addition of Vc (10–50 mM) can greatly reduce the required dosage of H_2O_2 and increase the degradation efficiency. In addition, excess Vc provides no advantage for CS degradation. From the kinetic study (Table 1), it can be seen that the most degradation efficiency was found at $c(H_2O_2)/c(Vc) = 1$. Based on these results, it was concluded that the molar ratios of the two reagents should be kept at 1 to maximize efficiency in obtaining lower M_w CS.

3.1.5. Effect of initial pH. The pH of the solution plays an important role in CS degradation with H_2O_2/Vc redox reactions. The pH value of the solution was adjusted by 1 M acetic acid or 1 M sodium hydroxide. As Fig. 1i and j show, the highest CS degradation efficiencies were found at pH 4.0. A similar trend was found for the reaction rate constants (Table 1). This phenomenon might be related to the stability of H_2O_2 and the protonation of the CS molecular chain at various pH values.[20,25,35] Studies have shown that the stability of H_2O_2 decreases with increasing pH and that it quickly decomposes in alkaline conditions.[25] Thus, its ability to generate free radicals declined, and the greater the pH, the poorer the degradation effect. On the other hand, the protonation of NH^{3+} groups in the CS chain under acidic condition generates electrostatic interactions between molecules and makes the chain easy to stretch and break, which is convenient for degradation. However, it is also noted that, when increasing the concentration of H^+, the concentration of anions in the acid will increase as well, making more salt linkage in CS molecules and thus increasing steric hindrance to glycosidic bond cleavage and reducing the rate of degradation of the molecules.[7,36] Therefore, the optimum pH in the experiment was found to be 4.0.

3.1.6. Comparison of sonolysis-assisted H_2O_2/Vc degradation, sonolysis alone and H_2O_2/Vc alone. To investigate the synergetic effects of sonolysis with H_2O_2/Vc on the degradation of chitosan, 50 mL CS solution (2 mg mL^{-1}) was treated with sonolysis alone, H_2O_2/Vc alone, and H_2O_2/Vc in the presence of sonolysis at 25 ± 1 °C for 60 min. The resulting M_w was plotted as a function of time. The kinetic rate constants (k) and the determination coefficient of formula (1) R^2 are presented in Table 1. As illustrates in Fig. 2a and b, the degradation efficiency of CS using a combination of sonolysis with H_2O_2/Vc was significantly higher than that for sonolysis or H_2O_2/Vc alone. The degradation efficiency of CS by H_2O_2/Vc system is

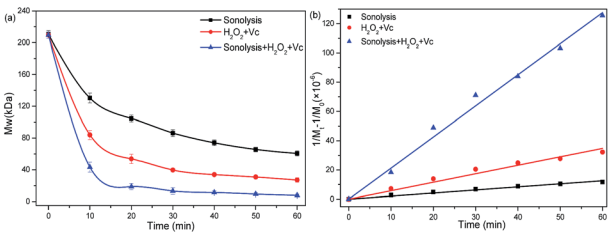

Fig. 2 Effect of the degraded methods on the degradation efficiency of CS.

significant higher than that of sonolysis alone. Interestingly, sonolysis and H_2O_2 appear to have a synergistic effect in CS degradation, because the degradation rate constants of the combined process ($k_{sonolysis+H_2O_2+Vc}$) are greater than the sum of the rate constants of the individual processes ($k_{sonolysis}$ + $k_{H_2O_2+Vc}$). Compared with other degradation methods, sonolysis-assisted H_2O_2/Vc degradation showed remarkable advantages, such as lower required concentration of H_2O_2, shorter reaction time and lower molecular weight obtained. To confirm the properties and the mechanism of degraded CS, these three samples were selected for further characterizations.

3.2 Properties of the degradation products

3.2.1. DD and molecular distribution of degraded CS. The DD and molecular distribution of the native and degraded chitosan samples are listed in Table 2. The DD of degraded chitosan samples did not appear to change with decreasing molecular weight, suggesting that the *N*-acetyl-glucosamine is stable enough during degradation and that glycosidic bonds might be randomly cleaved. Table 2 also suggests that the oligomerized CS samples have low polydispersity (M_w/M_n), with a similar molecular weight distribution as the initial CS.

3.2.2. FT-IR spectra of degraded CS. The structures of the degraded CS as well as the initial CS were investigated by FT-IR spectrometry, and the results are shown in Fig. 3. The characteristic absorption peaks of untreated CS are assigned as follows: major absorption bands approximately 3380 cm^{-1} were attributed to O–H stretching; the signal at 2870 cm^{-1} was due to C–H stretching of CH_2 groups; the feature absorption peaks at 1640 cm^{-1} and 1560 cm^{-1} were attributed to the bending vibration of amide I and amide II groups, respectively; the signal at 1153 cm^{-1} and 1075 cm^{-1} corresponded to the deforming vibrations of C–O–C and C–O groups; and the band at 898 cm^{-1} was attributed to the β-(1,4)-glycosidic bond in the CS chain.[2,35] As can be seen, the FT-IR spectra of all the degraded CS are similar to that of native CS though some differences exist, indicating that the main chain structure of degraded CS still remained during degradation process. Compared to sonolysis degraded CS, the intensity of the amide I and amide II groups H_2O_2/Vc treated CS (c and d) showed a lower than that of native CS, suggesting the degradation of CS by H_2O_2/Vc system were more acutely than sonolysis alone. This was in agreement with the molecular weight data of degraded CS samples, as discussed above. Additionally, no new band

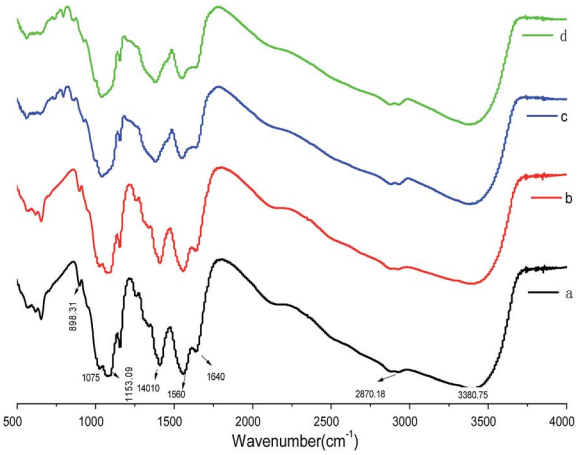

Fig. 3 FT-IR spectra of the degraded CS as well as initial CS. (a) Initial chitosan; (b) sonolysis alone; (c) only with H_2O_2/Vc; (d) sonolysis in the presence of H_2O_2/Vc.

Table 2 Degree of deacetylation (DD) and molecular distribution of the native and degraded CS[a]

CS samples	DD (%)	M_w (kDa)	M_n (kDa)	M_w/M_n
a	92.13 ± 1.64	209.49 ± 4.13	120.40 ± 2.37	1.74 ± 0.12
b	93.12 ± 2.25	60.62 ± 3.81	42.70 ± 3.46	1.42 ± 0.10
c	91.42 ± 1.81	27.25 ± 2.94	18.41 ± 2.59	1.48 ± 0.16
d	90.67 ± 2.23	7.67 ± 1.37	5.28 ± 2.25	1.45 ± 0.14

[a] a: Native chitosan; b: sonolysis alone; c: only H_2O_2/Vc with; d: sonolysis in the presence of H_2O_2/Vc.

approximately 1730–1740 cm^{-1}, assigned to –COOH groups, was seen, indicating that the degradation mainly involved cleavage of β-glycosidic linkages and that no –COOH was formed. Moreover, there was no obvious change in the characteristic peaks at 1640 cm^{-1} caused by amide I group stretching vibrations, indicating no obvious deacetylation during the degradation process. Similar results have been found after the degradation of CS by ozone,[8] sonolysis,[8] microwave,[11] and hydrodynamic cavitation.[5]

3.2.3. NMR spectra of degraded CS. The detailed structure of CS before and after degradation was further investigated by ^1H and ^{13}C NMR, and the results are shown in Fig. 4. As shown in Fig. 4, native CS exhibited a typical ^1H NMR spectra that a single peak at δ 1.95 ppm (the *N*-acetyl protons), at δ 2.9 ppm (H-2) and multiple peaks at δ 3.3–3.7 ppm (H-3, H-4, H-5 and H-6).[37,38] As similar to FT-IR analysis, the ^1H NMR spectrum of degraded CS has similar to that of the original CS, which coincided with the reported data.[38] For ^{13}C NMR of CS samples, there was also almost no change in the CS spectrum before and after degradation, except that the absorption peak of the C-1 carbon downshifted. This strongly suggested that, after degradation, the electron density around the C-1 and C-4 carbon changed;[39] therefore, the breakage and formation of chemical bonds most likely occurred at or near the C-1 or C-4 carbon. According to previous reports on the degradation of CS,[4,39,40] it is reasonable to believe that the degradation of CS in the current study also occurred *via* attack on the C-1 or C-4 carbon and then breakage of the adjacent C–O–C glycosidic bond in the main chain. Moreover, no new signals were obvious at approximately 175 ppm, assigned to the –COOH group, which proved that no –COOH group had formed. These data further confirmed that the main backbone structure of CS did not change during the degradation process.

3.2.4. X-ray diffraction patterns of degraded CS. Fig. 5 shows the X-ray diffraction patterns of the original CS and the degraded CS. The original chitosan exhibited two characteristic peaks at 2θ = 11.31 and 20.18°, in agreement with the findings

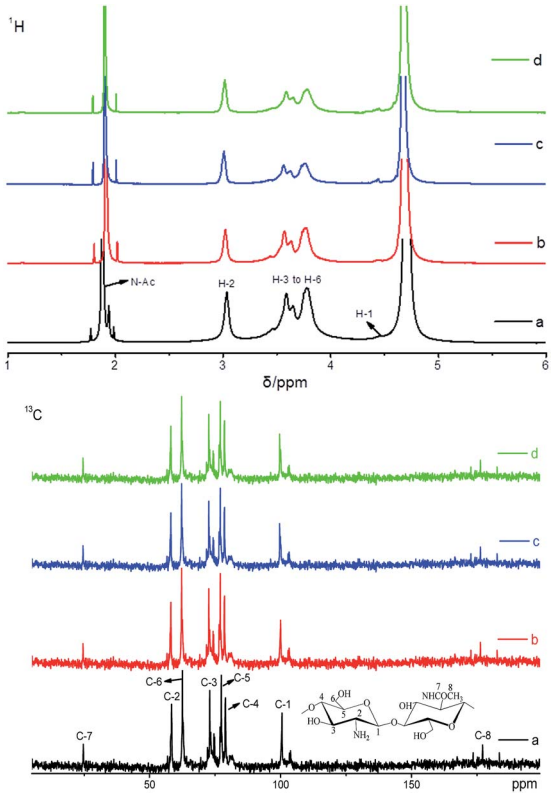

Fig. 4 ^{1}H and ^{13}C NMR spectra of the degraded CS as well as initial CS. (a) Initial chitosan; (b) sonolysis alone; (c) only with H_2O_2/Vc; (d) sonolysis in the presence of H_2O_2/Vc.

from previously studies.[41] Compared with the original chitosan, the degraded CS had reduced peak intensities in its X-ray diffraction patterns. The crystallinity of products formed from sonolysis combined with H_2O_2/Vc had the lowest intensity compared with the other two samples. These results demonstrated that the CS degraded by synergetic sonolysis with H_2O_2/Vc caused a significant loss of the crystal structure. Therefore, the solubility of degraded CS also increased substantially. This decrease in crystallinity is also seen in CS degraded by other methods.[5,7,38,40,41]

3.3 Mechanisms of the synergetic effects of sonolysis-assisted H_2O_2/Vc for the degradation of chitosan

Sonolysis-assisted H_2O_2/Vc is a mild, effective, and environmentally friendly method to degrade polysaccharides. It is widely acknowledged that the mechanism of low frequency ultrasonic degradation of CS has been attributed mainly to the action of shear forces (mechanical effect) caused during the collapse of cavitation bubbles.[13,14] The mechanical effect combined with the cavitation field of sonolysis not only breaks the β-1,4-glucoside linkage of the CS chain, resulting in a lower M_w, but also destroys hydrogen bonds among native CS, loosening its tight structure and providing access for easy further degradation.

The mechanism of degradation by H_2O_2 and Vc appears to be *via* OH$^\bullet$ because Vc is easily oxidized by H_2O_2 and generates HO$^\bullet$ according to the following equation:[23,42]

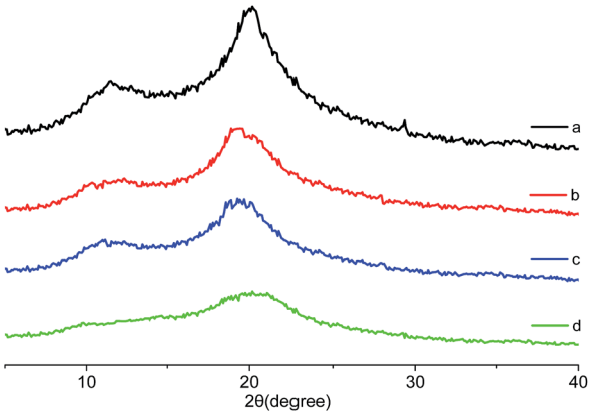

Fig. 5 X-ray diffraction patterns of the degraded CS as well as initial CS. (a) Initial chitosan; (b) sonolysis alone; (c) only with H_2O_2/Vc; (d) sonolysis in the presence of H_2O_2/Vc.

$$AH_2 + H_2O_2 \rightarrow HO^\bullet + H_2O + DKG$$

where AH_2 is Vc and DKG represents 2,3-diketogu-lonic acid.

To confirm the generation routes of OH$^\bullet$ and it role in degradation process, the electron paramagnetic resonance (EPR) were introduced to detect free radicals and DMPO was used as the spin trap. As shown in Fig. 6, the H_2O_2 + Vc + DMPO system show a strong EPR signal, and the presence of CS in reduced the EPR signal, however, no EPR signal were observed for the CS + DMPO and Vc + DMPO only. These data suggested that the mixing of H_2O_2 and Vc leads to a production of $^\bullet$OH and furthermore to the degradation of CS, resulting in a decrease of the molecular weight of CS. Similar phenomenon were reported by others.[43,44]

The OH$^\bullet$ radical possess an unpaired electron and is a very powerful oxidant. Its reactions are thought to be non-selective.[20] According to previous free-radical degradation studies, OH$^\bullet$ can quickly abstract the H atom from C-1, C-2, C-3, C-4, or C-5 of pyranose rings in a (1 → 4)-linked CS chain[39,45] and form CS macro radicals ((GlcN$^\bullet$)m − (GlcN)n, R$^\bullet$):

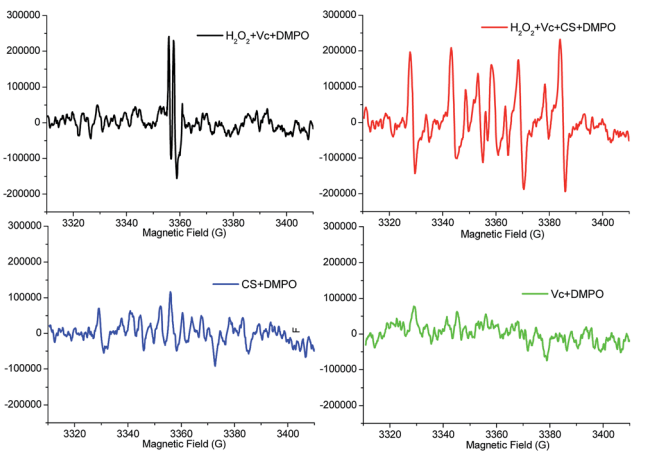

Fig. 6 ESR spectrum of DMPO–OH adduct formed compounds.

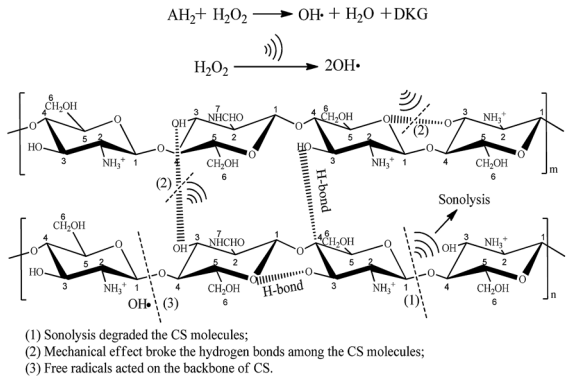

Fig. 7 Possible mechanism of sonolysis assisted H_2O_2/Vc action on the CS chain.

$$(\mathrm{GlcN})m - (\mathrm{GlcN})n + \mathrm{HO}^{\bullet} \rightarrow (\mathrm{GlcN}^{\bullet})m - (\mathrm{GlcN})n + \mathrm{H_2O}$$

where m and n are the numbers of glucosamine residues, and GlcN represents the glucosamine residue in CS chain. These R$^{\bullet}$ then undergo further reactions and break down CS molecules to form smaller molecules by the scission of the glycosidic bond:

$$(\mathrm{GlcN}^{\bullet})m - (\mathrm{GlcN})n + \mathrm{H_2O} \rightarrow (\mathrm{GlcN})m + (\mathrm{GlcN})n$$

Thus, the deamination and partial ring-opening oxidation would also happen[20] during degradation process. However, the FT-IR and NMR spectra of the original and degraded CS exhibited no distinct change in chemical structure during depolymerization, which indicated the scission of the glycosidic bond of CS in the current study was predominantly from H-abstraction at C-1 and C-4.

As discussion above, the CS chain decomposed very rapidly to short oligomers under the combined methods of sonolysis with H_2O_2/Vc. The mechanical and cavitation effect of sonolysis made the CS chain short and more vulnerable to attack by HO$^{\bullet}$ generated by H_2O_2/Vc. In addition, the presence of sonolysis was found to significantly enhance the production of HO$^{\bullet}$ from the decomposition of H_2O_2 or H_2O_2/Vc in the vicinity of cavitation bubbles.[34] Thus, there were more opportunities for HO$^{\bullet}$ to attack the β-1,4-glucoside linkages of CS and break the linkages, which made the M_w of CS decrease in a short time. Combining results from our data and related literature reports,[14,38,39] a possible sonolysis-assisted H_2O_2/Vc degradation mechanism for CS is proposed in Fig. 7. It is reasonable to conclude that sonolysis and H_2O_2/Vc in combination synergistically cause the degradation of CS.

4. Conclusion

A combination of sonolysis and the H_2O_2/Vc redox reaction can be efficiently used for the degradation of CS. The effects of initial CS concentration, ultrasonic intensity, H_2O_2 concentration, Vc concentration, and pH of the reaction solution on the molecular weight and degradation kinetics of CS were investigated and optimized. Based on the degradation kinetics, a synergetic effect on the degradation efficiency of CS was observed for sonolysis in combination with H_2O_2 and Vc. The results of the FT-IR and ^{13}C NMR spectra indicated that the chemical structure of the resulting degraded CS was unchanged, indicating that the degradation mainly involved the cleavage of β-glycosidic linkages without –COOH formation. Moreover, intermolecular hydrogen bonds were broken, and the crystallinity of degraded chitosan decreased. According to the above analysis, the synergistic degradation of CS by sonolysis and the H_2O_2/Vc redox reaction was attributed to mechanical effects and HO$^{\bullet}$ attack on the β-1,4-glucoside linkages of glucosamine units. These results suggest that a combined sonolysis/H_2O_2/Vc technique is promisingly suitable for large-scale manufacture of chitooligosaccharides. The current investigation will also provide encouragement for further exploration into the degradation of natural polysaccharides.

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