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Effects of ultrasound on molecular properties, structure, chain conformation and degradation kinetics of carboxylic curdlan

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ABSTRACT

In this study, high-intensity ultrasound (20 kHz), a simple, effective and without any additive method, was used to the degradation of carboxylic curdlan (Cc) produced by 4-acetamido-TEMPO-mediated oxidation. The effects of ultrasound on molecular properties, structure and chain conformations of Cc were investigated by viscometry, size-exclusion chromatography with multiangle laser-light scattering (SEC–MALLS) analysis, as well as FTIR and NMR spectroscopies. The results indicated that the intrinsic viscosity [η] and the weight-average molecular weight (M_w) of Cc decreased obviously after ultrasound, and a uniform and narrow distribution of degradation product was obtained. The *z*-average radius of gyrations (R_g) firstly increased and then decreased as the sonication time prolonged. Ultrasound destroyed the hydrogen bonds resulting in the transition from compact random coil conformation to more flexible and even shorter extended chains. Ultrasonic treatment could not alter the primary chemical structure of Cc molecules according to the structural analysis by FTIR and NMR spectroscopies. Degradation kinetics based on Schmid model was applied to estimate the degradation rate constant *k*. It was found that the *k* value of Cc decreased with increasing the polymer concentration from 0.05 to 0.2% (w/v).

1. Introduction

Polysaccharides represent a major class of biomacromolecules. In addition to their well-known functions as structure and storage molecules in the cells of living organisms, polysaccharides play important and specialized roles in living organisms such as by acting as immunomodulators or biological response modifiers (BRMs) of the innate immune responses. Natural polysaccharides from plants and microbial sources have been widely explored for their antitumor and immunomodulatory properties and other health benefits (Zong, Cao, & Wang, 2012). Because of strong antitumor and immumodulatory activities, a variety of the purified β-glucans and polysaccharide–protein and –peptide complexes (PSPs) from mushrooms (fungi) have found clinical applications for immunotherapy and cancer treatment (Giavasis, 2014; Stachowiak & Reguła, 2012; Zhang, Cui, Cheung, & Wang, 2007), such as Lentinans from *Lentinus edodes* (Xu, Yan, Tang, Chen, & Zhang, 2014), polysaccharide D-fraction and MD-fraction from *Grifola frondosa* (Boh & Berovic, 2007), and PSP from *Coriolus versicolor* (Cui, 2003).

In general, biological activities of polysaccharides are closely related to their chemical composition, configuration and chain conformation, as well as their physical properties. Thus, differences in bioactivities can be correlated with solubility in water, molecular weight, branching ratio, chemical structure and chain conformation (Yang & Zhang, 2009). Especially, polysaccharides in an aqueous solution exhibit various forms of chain conformation, most commonly random coil and various helical forms, single helix, double helix and triple helix, which are important to their properties and functions. Previous studies have suggested that the solution property and chain conformation of polysaccharides are important factors for their bioactivities, though the specific bioactive conformation forms varied with the polysaccharide structures and also with the bioassay systems. However, a number of high-molecular weight (high-M_w) polysaccharides exhibit poor solubility, high viscosity and unstable physicochemical properties, which are unfavorable for clinical uses. Moreover, some polysaccharides with high-M_w tend to form aggregates in solution that may mask the behavior of individual macromolecules and influence the bioactivities and functions. It is desirable to improve the solubility and chain conformation and reduce the viscosity of polysaccharides in aqueous solutions by modification of the polysaccharide molecules. Furthermore, most studies have demonstrated that biological activities of polysaccharides are obviously improved by molecular modifications or degradations (Xing et al., 2005).

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Several methods have been applied to degrade polysaccharides, including chemical degradation, enzymatic hydrolysis, and physical depolymerization (Liu, Bao, Du, Zhou, & Kennedy, 2006). Amongst of them, physical treatments, such as various radiation means of γ-ray, ultraviolet light, microwave and power ultrasound (Vodeničarová, Dřímalová, Hromádková, Malovíková, & Ebringerová, 2006), are considered as the most convenient and frequently used technologies that are few or no chemicals need to be added to the polysaccharide solutions and the degraded products are easier to recover and purify. It is interesting and important to point out that ultrasonic treatment is one of the most promising, effective and environmentally friendly approaches for the degradation of polymer in aqueous medium (Suslick & Price, 1999). Up to now, there are a number of polysaccharides and their derivatives, such as dextran (Zou et al., 2012), chitosan (Czechowska, Rokita, Lotfy, Ulanski, & Rosiak, 2005), pullulan (Ohta, Kato, & Kawahara, 1984), fucoidan (Guo et al., 2014), xylogucan (Vodeničarová et al., 2006), carrageenans (Lii, Chen, Yeh, & Lai, 1999), pectin (Zhang et al., 2013) and carboxymethycellulose (Grönroos, Pentti, & Hanna, 2008), have been used for ultrasonic degradation to improve their physical and chemical properties such as solubility, viscosity and molecular weight. Moreover, these studies mainly focus on effects of ultrasonic degradation on viscosity, molecular weight, chemical structure and degradation kinetics. However, to our best knowledge, there have been no or only few efforts have been made to elucidate changes in the chain conformations of polysaccharides before and after ultrasound.

Curdlan is a bacterial polysaccharide produced by *Alcaligenes faecalis* that has been of significant recent interest due to its interesting and valuable rheological properties and its inherent bioactivity (Lehtovaara & Gu, 2011; Zhang & Edgar, 2014). However, curdlan is insoluble in water, which limits its applications in food industry and biomedicine. More recently, our group has successfully prepared carboxylic curdlan (Cc) derivatives bearing the β-1,3-polyglucuronic acid structure by using 4-acetamido-TEMPO/NaClO/$NaClO_2$ system under mild conditions (Yan et al., 2014). The Cc produced by 4-acetamido-TEMPO-mediated oxidation under pH 4.8 and 40 °C for 4 h had the higher M_w (>500 kDa) and the poor solubility exhibited as a compact random coil structure in an aqueous medium. Therefore, the present study aims to investigate and evaluate the effects of ultrasonic degradation on viscosity, molecular weight, structure and chain conformations of Cc at different concentrations. Meanwhile, the degradation kinetics and mechanism were also discussed.

2. Materials and methods

2.1. Materials and chemicals

Commercial curdlan was obtained from Wako Pure Chemical Co., Japan. Carboxylic curdlan (Cc) bearing the β-1,3-polyglucuronic acid structure was prepared with 4-acetamido-TEMPO-mediated oxidation at pH 4.8 and 40 °C for 4 h based on our previous work (Yan et al., 2014). The carboxylate content of Cc was 1.98 mmol/g by electric conductimetric titration (Saito & Isogai, 2004). The weight-average molecular weight (M_w) and molecular weight distribution (M_w/M_n) of Cc were 9.50×10^5 g/mol and 2.30, respectively, as determined by the SEC–MALLS analysis described in Section 2.4. All other chemicals and solvents were of laboratory grade and used without further purification.

2.2. Ultrasonic degradation

Ultrasonic degradation experiments were performed with a Model VCX 750 processor (Sonics & Materials Inc., Newton, USA) of 20 kHz frequency and 750 W maximum output power. 0.05%, 0.1% and 0.2% (w/v) of Cc solutions were prepared with deionized water to investigate the influence of concentration on Cc ultrasonic degradation. The Cc solution (20 mL) was placed in 50-mL plastic centrifuge tube. The ultrasound probe (13 mm diameter) was submerged into the solution at a fixed depth of 2.0 cm, and the output power of ultrasonic setup was fixed at 600 W in all the experiments. The sample tube was placed in an ice bath during the ultrasonic treatment to maintain an average temperature of 40 ± 5 °C over the treatment period. After ultrasonic treatment for selected periods of time (5–90 min), the Cc solutions were centrifuged at 10,000 rpm for 30 min and then freeze dried and stored at 20 °C before use.

2.3. Intrinsic viscosity determination

Intrinsic viscosity [η] was determined by the serial dilution method (Yan et al., 2009) and the viscosity of diluted Cc was measured in 0.1 M NaCl aqueous solution at 30 ± 0.1 °C with an Ubbelohde viscometer (0.5–0.6 mm capillary diameter). The kinetic energy correlation was always negligible. The [η] value of each sample was estimated by the Huggins and Kraemer equations,

$$\frac{\eta_{sp}}{c} = [\eta] + k' \times [\eta]^2 \times c; \quad \ln \frac{\eta_r}{c} = [\eta] - \beta \times [\eta]^2 \times c \tag{1}$$

where k' and β are constants for a given polymer at a given temperature in a given solvent, c the polymer concentration, η_{sp}/c the reduced specific viscosity, $(\ln \eta_r)/c$ the inherent viscosity.

2.4. Size-exclusion chromatography with multiangle laser-light scattering (SEC–MALLS) analysis

The weight-average molecular weight (M_w), number-average molecular weight (M_n), molecular weight distribution (M_w/M_n), z-average radius of gyration (R_g), and chain conformation of Cc samples before and after ultrasonic treatment were determined by using SEC–MALLS (DAWN HELEOS II, λ = 658 nm: Wyatt Technologies Corporation, USA) on an Agilent 1100 system equipped with two SEC columns (OHpak SB-806 M HQ and SB-805 HQ, 8 mm Φ × 30 cm, Shodex, Japan) in 0.1 M NaCl at 25 °C and a refractive index detector. In the typical analysis, 100 μL aliquot of the sample solution (0.1%, w/v) was injected with 0.1 M NaCl as the mobile phase at a flow rate of 0.5 mL/min, with a refractive index increment (dn/dc) of 0.125 mL/g (Shibata, Yanagisawa, Saito, & Isogai, 2006). Data acquisition and analysis were performed using on-line Astra software (Wyatt Technologies, USA). Details of the SEC–MALLS system used in this study and the operation conditions are described in our previous work (Yan et al., 2014).

2.5. FTIR and NMR spectroscopies

The FTIR spectra of Cc samples before and after ultrasonic treatment were determined using a Nexus 670 FTIR spectrometer (Thermo Nicolet Co., USA) in the wavenumber range from 500 cm^{-1} to 4000 cm^{-1} with KBr pellets and referenced against air. For NMR analysis, Cc and its products (30 mg) were dissolved in 0.5 mL of D_2O. The ^{13}C NMR spectra were recorded by a Bruker AVANCEIII 400 MHz spectrometer (Bruker, Rheinstetten, Germany) at 25 °C. All chemical shifts were expressed in reference to Me_4Si.

3. Results and discussion

3.1. Effect of sonication on intrinsic viscosity

Fig. 1 shows the intrinsic viscosity of the Cc solutions at different concentrations by ultrasonic degradation as a function of sonication time from 5 to 90 min. Irrespective of the concentration

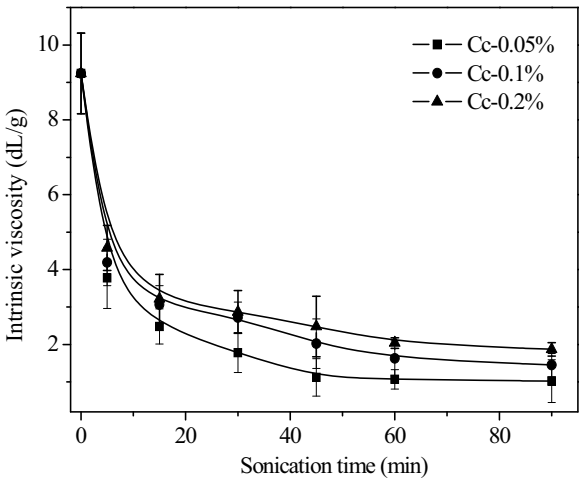

Fig. 1. Effects of sonication on the intrinsic viscosity of carboxylic curdlan at different concentrations. (Error bars for standard deviations at $n = 3$.)

of Cc, the intrinsic viscosity of Cc solution dropped rapidly within the first 15 min but decreased slowly and slightly in the remaining 30–90 min of ultrasonic treatment. The intrinsic viscosity of Cc solution at 0.05% (w/v) decreased more dramatically than that of Cc solutions were remained at 0.1% and 0.2% with increasing the sonication time. After sonication of 60 min, the values of intrinsic viscosity of Cc solutions at 0.05%, 0.1% and 0.2% (w/v) were 1.07, 1.63 and 2.04 dL/g, respectively. When sonication time increased to 90 min, the intrinsic viscosity of Cc at different concentrations almost unchanged. These results indicated that the extent of degradation was more pronounced in dilute solutions, which was similar to the previous reports (Wang, Cheung, Leung, & Wu, 2010; Zhou, Yu, Zhang, He, & Ma, 2012). This might be due to the fact that the probability of de-aggregation and glycosidic bond scission in the degradation stage caused by efficient shearing in the polymer chain is greater in dilute solution.

It is well-known that the intrinsic viscosity is an intrinsic property of the chain of a polymer, which is mainly dependent on the polymer chain length in the solvent (Lapasin & Pricl, 1995). The intrinsic viscosity is very sensitive to the extension of polymer chains in solution. At the initial 15 min, the shear force generated by the cavitation may break-up compact random coils (or aggregates) of Cc molecules in solution. As sonication time increased, the polysaccharide chains or glycosidic linkages may be destroyed instead of disaggregation. Cavitation is regarded as the primary effect that in consequence results in the bond-breaking of Cc molecules in the system. Therefore, ultrasonic treatment generated Cc molecules with shorter molecular size, and leads to decreased viscosity with increasing the period of sonication time (Wang et al., 2010).

3.2. Effect of sonication on molecular weight and polydispersity

In this work, SEC–MALLS analysis was used for the determination of the weight-average molecular weights (M_w), number-average molecular weights (M_n) and the polydispersity (M_w/M_n) of the Cc in aqueous solution before and after ultrasonic treatment as a function of sonication time and the obtained results were summarized in Table 1. The degradation of Cc during ultrasonic treatment under different concentration had similar trends as sonication time. The M_w of Cc decreased rapidly with increasing the sonication time, and then gradually tends to a constant value after 90 min. For example, the M_w of Cc at 0.05% (w/v) decreased from 950×10^3 to 255×10^3 g/mol when sonication time increased from 5 to 30 min. The M_w further reduced from 255×10^3 to 92×10^3 g/mol after exposure to sonication for 90 min. It should be pointed out that the shearing force produced from the rapid collapse of cavitation bubbles could break up the polysaccharide molecules during ultrasonic treatment (Grönroos et al., 2008; Wu, Zivanovic, Hayes, & Weiss, 2008). In general, the polymers with longer chains are easier to be broken by shear force than that of small molecules with shorter chains. Thus, the M_w of the sample decreased obviously in the first 30 min, and then the rate showed as most of the Cc molecules fractured into small particles. In addition, the rate of decrease of M_w is higher in low concentration solution such as 0.05% (w/v) and decreases at higher concentrations. These results suggested that the polymer concentration has an important influence on the rate of decrease of M_w and Cc depolymerized and degraded faster in more diluted solutions. This is consistent with the results described in the previous studies (Taghizadeh & Bahadori, 2014; Zou et al., 2012). It may be due to the fact that the entanglement between polymer chains became less intense with decreasing the solution concentration (Kanwal, Liggat, & Pethrick, 2000). At lower concentrations, the compact random coil structure of Cc extended more freely and the Cc chains are more easily attacked by the shear force produced from the larger cavitation bubbles, the same phenomenon was also observed in dextran (Zou et al., 2012). In the meantime, the velocity gradients around the collapsing bubbles became higher in more dilute solutions, which may be also convenient for the ultrasonic treatment.

It has been reported that ultrasonic degradation could narrow the molecular weight distribution of the polysaccharides in solution (Grönroos, Pirkonen, & Ruppert, 2004; Li, Li, Guo, & Li, 2005). In the present study, the M_w/M_n values of Cc in different solution concentration derived from ultrasonic degradation as a function of sonication time were obtained and also presented in Table 1. The M_w/M_n values of Cc in different concentration obviously decreased with increasing of sonication time. Moreover, the rate of decrease of M_w/M_n was faster in lower concentration solution. For example, the M_w/M_n value of Cc decreased from 2.30 to 1.05 with increasing in sonication times under solution concentration of 0.05% (w/v), but the M_w/M_n value of Cc in 0.2% (w/v) reduced from 2.30 to 1.19 after 90 min of ultrasonic treatment. This result is in agreement with Taghizadeh and Bahadori (2014). The form of changes in M_w/M_n depends on the mechanisms of chain scission (Emsley & Heywood, 1995). Random scission does not change the M_w/M_n, but midpoint scission will decrease the M_w/M_n. The experimental data in Table 1 illustrated that ultrasonic degradation of the water-soluble polymers mainly occurred by the midpoint scission due to the mechanical action. In addition, the changes in chain conformation of Cc in solution before and after ultrasonic degradation were also related to the polydispersity index. In this regard, ultrasonic treatment could be developed as a simple and efficient approach for reducing the polydispersity of a polymer.

3.3. Effect of sonication on chain conformation

As shown in Table 1, the z-average radius of gyration (R_g) of Cc in aqueous solution at different concentrations under ultrasound as a function of sonication time was obtained by SEC–MALLS analysis. As sonication time increased, the R_g increased in the initial 30 min and then decreased after 30 min of sonication irregarless of solution concentration. For example, the R_g value of Cc at 0.05% (w/v) increased from 122.1 to 211.3 nm with increasing of sonication time, but reduced from 213.3 to 133.4 nm after 90 min of ultrasonic degradation. In general, R_g is a measure of the molecular dimensions of a polymer chain, and smaller for polymer chains with a more compact conformation. The change in R_g for Cc before and after ultrasonic treatment could be explained by the fact that the shearing force produced from cavitation bubbles weakened the non-covalent intra- or inter-molecular hydrogen bonds of polymer

Table 1
Molecular characterizations and conformational parameters of carboxylic curdlan under ultrasound treatment at different concentrations in 0.1 M NaCl aqueous solution at 25 °C by SEC–MALLS analysis.

Sample	Sonication time (min)	$M_w \times 10^5$ (g/mol)	$M_n \times 10^5$ (g/mol)	M_w/M_n	R_g (nm)	α[a]
Cc	0	9.50	4.12	2.30	122.1	0.46
Cc-0.05%	5	6.41	3.22	1.99	152.6	0.49
	15	3.14	1.95	1.61	176	0.53
	30	2.55	1.74	1.46	213.3	0.56
	45	1.57	1.22	1.29	197.6	0.58
	60	1.23	1.15	1.07	180.4	0.58
	90	0.92	0.88	1.05	163.4	0.59
Cc-0.1%	5	6.65	3.44	1.93	144.9	0.49
	15	4.38	2.55	1.72	172.3	0.52
	30	3.43	2.09	1.64	206.2	0.54
	45	2.40	1.78	1.35	191.8	0.55
	60	1.83	1.61	1.14	175.4	0.56
	90	1.43	1.29	1.11	157.6	0.56
Cc-0.2%	5	7.87	3.78	2.08	134	0.48
	15	4.85	2.66	1.82	151.8	0.51
	30	3.72	2.28	1.63	194.6	0.53
	45	2.94	1.92	1.53	185.9	0.54
	60	2.36	1.88	1.26	173.2	0.55
	90	1.90	1.60	1.19	154.6	0.55

[a] The exponent of power law functions $R_g = f \times M_w^{\alpha}$.

chains, leading to the disaggregation of polymer clusters. The Cc molecules existed as looser or more flexible random coils instead of compact conformations in aqueous solution after ultrasonic degradation. Therefore, the disaggregated molecules under ultrasound with larger R_g and the original Cc with smaller R_g. When sonication time continued to be increased from 30 to 90 min, the cleavage of loose or flexible polymer chains may be taken place by ultrasound energy instead of disaggregation. As a result, the R_g value was decreased. This was in good agreement with the results of M_w.

The SEC–MALLS chromatogram can produce the function of $R_g = f \times M_w^{\alpha}$. The power law of the former can be estimated from many experimental points in the SEC chromatogram (Wang, Xu, & Zhang, 2008). The plot of R_g versus the logarithm of the M_w can be used to determine the exponent α, which can provide valuable information about the polymer conformation. In general, the α of about 0.33 suggests compact coils in a theta solvent, α values are about 0.6 and 0.5 for flexible random coils in a good solvent and a theta solvent, respectively, and about 0.7 for a relatively stiff rod-like conformation (Kato, Okamoto, Tokuya, & Takahashi, 1982; Sato, Norisuye, & Fujita, 1984). In the present study, the values of α for Cc at different concentrations before and after ultrasound were in the range of 0.46–0.59 (Table 1). The result indicated that the Cc molecules before and after ultrasound existed as random coil conformations in aqueous solution irrespective of sonication time. This suggests that the degradation of Cc is mechanical action not free radical induced. This may be due to the fact that free radical degradation would lead to the occurrence of macromolecular free radicals, and the formation of side chains and a conformational change may be taken place by the recombination of these macromolecular free radicals (Wu et al., 2008). The α value of Cc increased with increasing the sonication time, suggesting that the random coil conformation of Cc become more flexible and expanded in aqueous solution.

3.4. Structure analysis of degradation product

Fig. 2 illustrates the FTIR spectra of the native Cc and its degraded products by ultrasonication. Two characteristic absorptions of polysaccharides were observed; a strong and wide stretching peak at approximately 3400 cm^{-1} for the O—H stretching vibration, and a weak absorption peak of approximately 2924 cm^{-1} for the C—H stretching vibration. The absorption peak at 1634 cm^{-1} was attributed to asymmetrical COO^- stretching vibration (in Cc molecules), whereas that at 1420 cm^{-1} was ascribed to symmetrical COO^- stretching vibration (Delattre et al., 2009). In addition, the absorption peak at about 1040 cm^{-1} was attributed to the C—O—H stretching vibration. After ultrasonic treatment, the main IR peaks at 3400, 2924, 1634, 1420 and 1040 cm^{-1} had remained unchanged. Moreover, there were no obvious changes in the characteristic peaks at 1634 and 1420 cm^{-1} represented the COO^- stretching vibration, but the intensity of absorption peak at 3400 cm^{-1} was attributed to O—H stretching vibration increased with the increase of sonication time. These results indicated that ultrasonic treatment mainly caused the breakage of glycosidic linkages but not significant change of the primary chemical structure of the Cc molecules. The same results were also observed by Guo et al. (2014), Zhang et al. (2013).

The changes in structural features of Cc before and after sonication were further investigated by ^{13}C NMR, and the results were shown in Fig. 3. The assignments of the carbon signals were based on our previous study (Yan et al., 2014). In the ^{13}C NMR spectra, all three samples (Cc, Cc-A, and Cc-D) showed similar spectra: a signal at approximately 175.3 ppm was attributed to COO^- groups at the C-6 position, and a signal at about 60.7 ppm was assigned to the C6-OH; the carbon signals at 102.4, 84.0, 75.6, 73.3, and 68.1 ppm

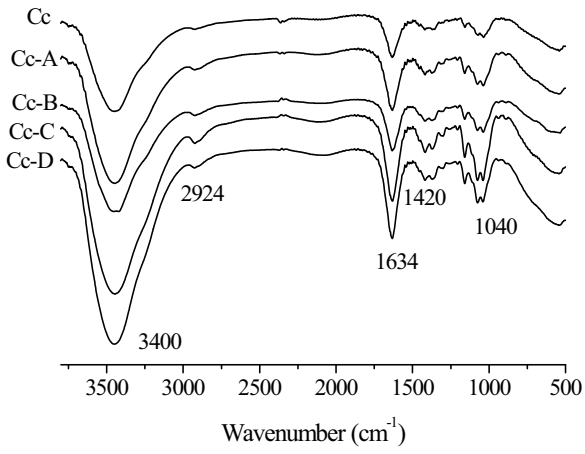

Fig. 2. FTIR spectra of Cc before and after ultrasonic treatment. Cc-A, Cc-B, Cc-C, and Cc-D were assigned to degraded Cc products prepared by ultrasonication for 15, 30, 60, 90 min, respectively, when the concentration of Cc was at 0.05% (w/v).

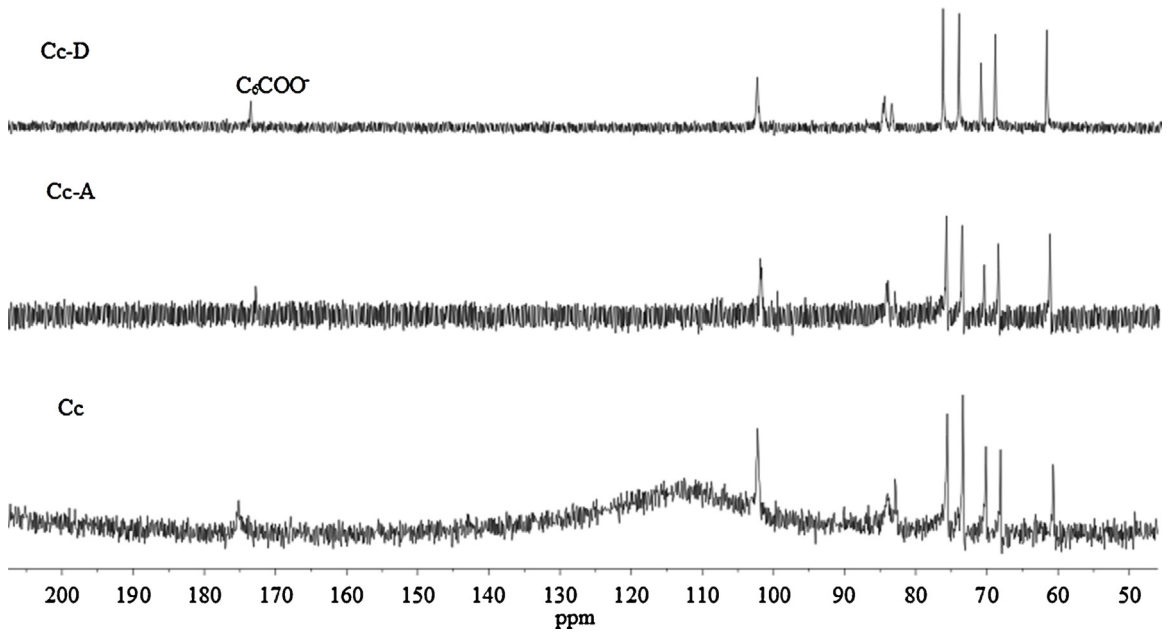

Fig. 3. ^{13}C NMR spectra of Cc before and after ultrasonic treatment. Cc-A and Cc-D were assigned to degraded Cc products prepared by ultrasonication for 15 and 90 min, respectively, when the concentration of Cc was at 0.05% (w/v).

were attributed to C-1, C-3, C-5, C-4, and C-2, respectively. No signal was obviously changed during the ultrasonic process, further indicating that ultrasonic treatment could not alter the main backbone structure of Cc.

3.5. Degradation kinetics and possible mechanism

Up to now, some researchers have proposed some models for ultrasonic degradation of polymer to evaluate the kinetics of the ultrasonic degradation process. All of these models offer the same rough description of the reduction of the average molecular weight during the process. Furthermore, the polymer after ultrasonic treatment degrades rapidly in the initial phase of the process and then the rate of the decrease of the molecular weight slows down and approaches a limiting molecular weight (Madras & Chattopadhyay, 2001; Nguyen & Kausch, 1992). In the present study, the Cc M_w reduction with time during ultrasonic treatment are consistent with the characteristic trend of polymer degradation by high-power ultrasound, which usually follows the first-order kinetics, processing more rapidly with larger polymer molecules and ceasing as the size or M_w reaches a minimum limit, as represented by Schmid kinetic model (Price, 1990; Lorimer, Mason, Cuthbert, & Brookfield, 1995),

$$M_{wt} = (M_{w0} - M_{w\,\mathrm{lim}})\ e^{-k\times t} + M_{w\,\mathrm{lim}} \quad (2)$$

where k is the degradation rate constant, M_{w0} and M_{wt} are the weight-average molecular weight at time 0 and t, respectively, and M_{wlim} is the minimum or limiting weight-average molecular weight. Based on the kinetics model, the degradation kinetic parameters of Cc at different concentrations were derived, and the result was summarized in Table 2. At the same time, the kinetic curves of Cc under ultrasonic treatment at different concentrations as a function of sonication time were shown in Fig. 4. It indicated that the Schmid model Eq. (2) was reasonable for the degradation kinetics model of Cc under ultrasound with $R^2 > 0.97$. It can be seen that the degradation rate constant k of ultrasound on Cc decreases with increasing solution concentration. For example, the k value was much higher at the lower concentration. When solution concentration increased from 0.05 to 0.2% (w/v), the k value decreased from 5.26×10^{-7} to 4.50×10^{-7} mol/g min. Similar results were also reported on chitosan and its derivatives (Kim & Lee, 2002; Taghizadeh & Bahadori, 2014). It has been previously reported that the extent of degradation under ultrasonic treatment mainly depended on the solution concentration. This may be due to the fact that the molecules become more freely in the lower concentration solutions, and the cavitation bubbles are easier to form. Moreover, in the more diluted solutions, the velocity gradients around the collapsing bubbles become larger. Besides, the

Table 2
Degradation kinetic parameters from Schmid model for the reduction of Cc M_w at different concentrations by ultrasonic treatment.

Concentration (%, w/v)	$M_{wlim} \times 10^5$ (g/mol)	k (mol/g min)	R^2
Cc-0.05	0.92	$(5.26 \pm 0.41) \times 10^{-7}$	0.970
Cc-0.1	1.43	$(4.66 \pm 0.28) \times 10^{-7}$	0.982
Cc-0.2	1.90	$(4.50 \pm 0.23) \times 10^{-7}$	0.986

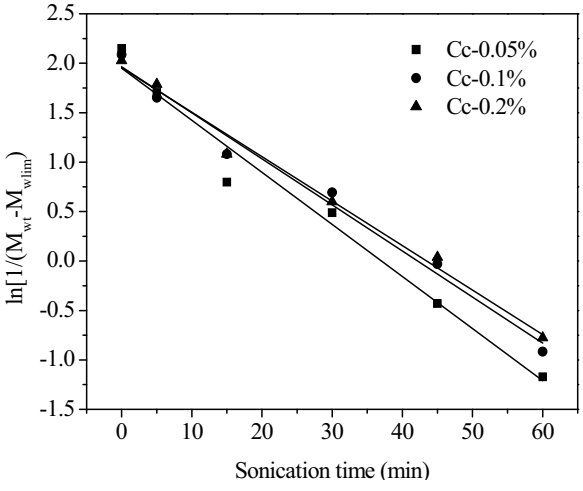

Fig. 4. The degradation kinetics curves (Schmid model) of carboxylic curdlan under ultrasonic treatment at different concentrations as a function of sonication time.

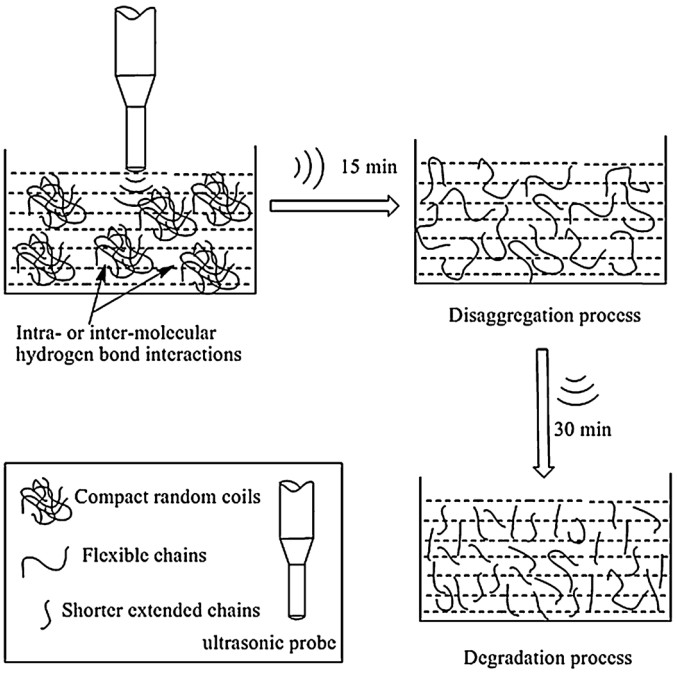

Fig. 5. Scheme diagram of degradation mechanism of aqueous carboxylic curdlan by ultrasonic treatment.

greater number of polymer chains will destroy the solvent flows around the bubble with increasing the solution concentrations. As a consequence, the effect of degradation under ultrasound will be lessen (Desai, Shenoy, & Gogate, 2008).

According to the above discussion, the degradation mechanism of Cc in aqueous solution under ultrasound can be depicted schematically in Fig. 5. In this work, Cc bearing the β-1,3-polyglucuronic acid structure prepared by 4-acetamido-TEMPO-mediated oxidation system existed as a compact random coil conformation or aggregates due to the formation of strong intra- and inter-molecular hydrogen bonds (Yan et al., 2014). Upon ultrasound treatment, the shear force, which produced by the relative motion between solvent and polymer chains during bubble collapse, may be responsible for the disaggregation of polymer clusters by destroying the non-covalent intra- and inter-molecular bonds in the initial sonication time (15 min). As a result, the compact conformation of Cc was gradually dispolymerized to become more flexible or extended random coil chains in aqueous solution. Meanwhile, the M_w value decreased but the R_g value increased. With prolonged ultrasonic treatment, the backbones of Cc molecules in aqueous solution may be broken by cleaving the β-1,3-glycosidic linkage, and the extended or flexible chains were further split into the shorter random coil chains. In the meantime, the relatively high molecular weight fragments were degraded into low molecular weight fragments as the ultrasonic time increased. However, the R_g value, which reflects the mean square of the distance between the segment and the mass center, showed a decreased trend. Zou et al. (2012) reported that the more extended random coil structure of dextran chains are vulnerable to be broken by the action of hydrodynamic forces. Therefore, Cc molecules in extended random coil conformation should be more susceptible to the sonication effect.

4. Conclusions

In summary, the effects of ultrasonic degradation on the intrinsic viscosity, molecular weight, structure, chain conformation and degradation kinetics of carboxylic curdlans (Cc) at different concentrations were investigated. The results showed that the intrinsic viscosity [η] and the weight-average molecular weight (M_w) of Cc decreased obviously after ultrasound treatment. The *z*-average radius of gyrations (R_g) of Cc molecules firstly increased and then decreased as the ultrasonic time prolonged. Cc molecules with a more uniform molecular weight distribution existed as more flexible and extended random coil conformations in aqueous solution after ultrasonic degradation. Moreover, ultrasonic treatment destroyed the glycosidic linkages but no change in the primary chemical structure of Cc molecules according to the result determined by FTIR and ^{13}C NMR analyses. The kinetic model for Cc degradation under ultrasound fitted to Schmid model at the tested polymer concentrations from 0.05 to 0.2% (w/v). The schematic diagram of ultrasonic degradation on Cc was therefore suggested and presented. The results suggested that ultrasonic treatment could be explored as a simple and effective approach to degrade the polysaccharides, especially β-glucans, for improved bioactivities and functions. Further investigations on relationship between changes in molecular properties and chain conformation of Cc after sonication and bioactivities and functions are underway.

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