Effect of Sonolysis on Kinetics and Physicochemical Properties of Treated Chitosan

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ABSTRACT: Low molecular weight chitosan with weight-average molecular weight from 161 to 22,000Da were obtained by sonolysis. Optimal conditions for sonolysis were described. The influence of sonolysis condition and the molecular parameters of initial chitosan on the degradation rate and degradation rate constant were investigated in detail. Weight-average molecular weight (M_w) and molecular weight dispersion (M_w/M_n) of samples were measured by gel permeation chromatography. The structure of degraded chitosan were characterized by Fourier transform infrared, X-ray diffraction, and electrospray ionization mass spectrometry. For a given sonolysis time, the decrease in molecular weight has been found to be greatest at lowest reaction temperature and lowest chitosan concentration. Molecular weight of samples decreased exponentially with increasing sonication time at early stages. The action mode of ultrasound on the splitting of molecular chain of chitosan has been discussed. The degree of deacetylation of the main hydrolysis products almost unchanged compared with the initial chitosan. The decrease of molecular weight led to transformation of crystal structure but the chemical structures of residues were not modified. Ultrasonic treatment on chitosan is an alternative, safe method to prepare chitosan having different molecular weights, which are more suitable for biomedical and food applications. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 2417–2425, 2008

Key words: polysaccharides; degradation; kinetic; ultrasound; characterization

INTRODUCTION

Chitosan is the deacetylated derivative of chitin. The presence of amino groups in chitosan causes the molecule to be polycationic and ultimately gives rise to its unique functional properties. There are many increasing evidences that a particular pharmaceutical and biological application and the effectiveness in exerting a specific action, such as fat-binding, antithrombotic activity, antitumor activity, antimicrobial activity or stimulating plant growth, depends on the molecular weight of chitosan.[1–4] US Food and Drug Administration has approved chitosan for fruit juice clarification, protein recovery from food process waste, edible coatings, and as an additive for animal feed.[5] For all of these applications specific molecular weight of chitosan is required to meet the present day demands.[6–9]

Chitosans with different molecular weights are usually prepared by chemical hydrolysis, enzymatic hydrolysis and physical methods. Chemical treatments, for example acid hydrolysis and oxidation hydrolysis, often involve use of chemicals, generate waste, and modify the glucose ring.[10] Enzymatic processing is a relatively complex procedure[11–13] and using enzymatically hydrolyzed chitosan preparations for pharmaceutical and food purposes is limited due to an undesirable level of chitosan pyrogenicity resulted in the presence of protein of the enzyme admixtures.[14] Because of the presence of additives used to initiate reactions and formation of side products, further purification is necessary for the products obtained using the above methods.

Therefore, the process of physical degradation by means of just providing added energy needed to break the (1→4)-β-linkage was considered as a simpler, more environmentally friendly method and effective than conventional ones[15] where undesired side reactions or the separation of by-product can be avoided. Irradiation and sonication are the two alternative chitosan physical degradation methods. Irradiation is the simplest way to degrade chitosan in any physical form (solid, suspension, paste, solution, etc.). The process is very efficient and can be easily controlled by choosing a proper irradiation dose. Besides, at appropriate conditions sterilization may be accomplished in parallel to the reduction in molecular weight. A disadvantage that may limit the applicability of solid-state irradiation is the possibility of some radicals being trapped in

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TABLE I
The Molecular Parameter of Initial Chitosan

Initial chitosan	DD (%)	M_w ($\times 10^{-4}$)	$k_{n,n+1}$ ($\times 10^5$) (min^{-1})				$k^+_{0,30}$ (min^{-1})	$R_{n,n+1}$ (%/min)		
			$k_{0,10}$	$k_{10,20}$	$k_{20,30}$	$k_{0,30}$		$R_{0,10}$	$R_{10,20}$	$R_{20,30}$
CS 1	67.2	56.8	2.32	2.11	2.10	2.19	0.39	4.30	2.81	2.19
CS 2	75.9	40.0	1.71	1.30	0.95	1.43	0.36	2.85	1.78	1.15
CS 3	81.2	38.6	1.39	1.13	1.04	1.21	0.31	2.41	1.64	1.31
CS 4	91.7	28.6	0.83	0.83	0.83	0.82	0.29	1.26	1.12	0.99
CS 5	95.3	23.5	0.58	0.56	0.55	0.55	0.23	0.77	0.69	0.64

crystalline regions of chitosan, resulting in further degradation (''posteffect''), which is slow but difficult to control.[16] Another factor that may discourage from the application of this technique is that suitable radiation sources or radiation-processing plants are not always readily available. Ultrasound provides a viable alternative[17,18] to produce lower molecular weight chitosan from higher ones just as the conventional methods which are either to mill the polymer or to subject the melt to a hydrodynamic shear and are very time consuming and energy intensive. The ultrasonic method has the merits of high efficiency, equipments to be used are easy to obtain, almost no effects on the degree of deacetylation (DD) of treated chitosan, no by-products etc.

The ultrasound method has been used in the food industry to destroy microorganisms, deactivate enzymes, and homogenize emulsions, tenderized meat and used as an aid in filtration and drying processes. Application of ultrasound to hydrolyze and cleave the molecular chain of chitosan, which is due to the intense mechanical and chemical effects associated with cavitation[18,19] may be the safest method to prepare different molecular weight chitosan for food applications.

In this study, the relationship between the experimental conditions (such as chitosan solution concentration, reaction temperature, and the molecular parameters of initial chitosan) and the degradation rate constant of ultrasonic treatment was shown. The products with different molecular weights prepared by ultrasonication were characterized by gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), X-ray diffraction (XRD), and electrospray ionization mass spectrometry (ESI-MS). The relation between the molecular weight and the physicochemical properties of degraded products is investigated in detail. An understanding of physicochemical properties is essential for better application.

EXPERIMENTAL

Materials

Chitosan was obtained from Yuhuan Ocean Biochemical Co. (Zhejiang, China). The molecular parameters of investigated chitosan were listed in Table I. CS4 was used in experiments, unless otherwise described. All other chemicals were of reagent grade.

Characterizations

Weight-average molecular weight (M_w) and molecular weight dispersion (M_w/M_n) of samples were measured by gel permeation chromatography (GPC). The GPC equipment consisted of TSK G5000-PW, TSP P100 pump, and RI 150 refractive index detector. The eluent was 0.2*M* CH_3COOH/0.1*M* CH_3COONa. Eluent and chitosan sample solutions were filtered through 0.45 μm Millipore filters. The flow rate was maintained at 1.0 mL/min. The temperature of the column was maintained at 30°C. The sample concentration was 0.4 mg/mL. Pullulan standards (Shodex Standard P-82, Showa Denko K.K., Japan) were used for a calibration curve. All data provided by the GPC system were collected and analyzed using the Jiangshen Workstation software package.

FT-IR spectra were recorded with KBr pellets on a Nicolet FT-IR 5700 spectrophotometer. Sixteen scans at a resolution of 4 cm^{-1} were averaged and referenced against air.

X-ray diffraction patterns of the degraded chitosan fractions were measured by a Shimadzu Lab XRD-6000 diffractometer and used a Cu*K*α target at 40 kV and 50 mA at 20°C. The relative intensity was recorded in the scattering range (2θ) of 8–40°. The crystallinity was calculated by the method of Klug[20]:

$$\chi_c = A_c/(A_a + A_c) \times 100\%$$

where A_c is the area of the crystal region and A_a is the area of the amorphous region.

Electrospray ionization mass spectrometry (ESI-MS) measurements were performed on an Agilent MSD 1100 series. The operation parameters were as following: the spray needle voltage was set at 4.0 kV and the spray was stabilized with nitrogen sheath gas (4 L/min) at 325°C. Spectra were obtained by direct injection of the sample (the low molecular weight chitosan solution at concentration of 100 mg/L). The sample was infused into ESI chamber at a

flow rate of 4 μL/min by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company). Spectra were obtained in positive mode in the mass range 250-1100 Da.

Ultrasound treatment

Chitosan was completely dissolved in acetic acid and the solution was stirred overnight to ensure complete solubilization of the chitosan molecules. Then the solution was passed through filter paper to remove the insoluble materials. 150 mL of filtrate in a glass vessel was placed in a water bath at a preset temperature. Sonication was carried out in an ultrasound generator (JY92-II D, Ningbo Scieniz Biotechnology Co., LTD, China) being characterized by applied frequency of 25 kHz, and the sonic intensity of 3.0, 6.0, 9.0 W/cm^2 (from the ratio between the output power and the area of the reactor bottom). Ultrasound radiation was produced using pulsed ultrasound with a 1 s ''on'' and 1 s ''off'' period for 30 min counting the on period only. Details of the reaction conditions are given in the text. After treatment, 0.5 mL of the reaction mixture was taken out to analyze the molecular weight by GPC.

Calculation of the degradation rate (R) and degradation rate constant (k)

The degradation rate (R) was defined as the percentage decrease in molecular weight per minute during sonolysis. It can be expressed by eq. (1).[21,22]

$$R = [1 - (M_t/M_0)] \times 100\%/t \quad (1)$$

Here, R is degradation rate of the chitosan molecular weight, M_0 is the molecular weight of chitosan at time zero, M_t is the molecular weight of the resulting chitosan after the reaction for t min. To calculate the degradation rate during a certain period of time, eq. (2) was used:

$$R_{n,n+1} = [1 - (M_{n+1}/M_n)] \times 100\%/t \quad (2)$$

Within a short sonication time, the degradation reaction by ultrasonic treatment is a first-order reaction. Its degradation rate constant (k) can be obtained from the kinetic eq. (3).[23]

$$1/M_t = 1/M_0 + k\ t/m = 1/M_0 + k't \quad (3)$$

where k is the degradation rate constant (min^{-1}) of molecular weight degradation during sonolysis, k' is in mol g^{-1} min^{-1}, t is the sonolysis time, M_0 and M_t are weigh-average molecular weights of the chitosans before and after the treatment for t min, respectively, and m is the molecular weight of the chitosan monomer unit, m = 161 + 42(1-DD). To calculate degradation rate constant during certain periods of time, eq. (4) was used:

$$1/M_{n+1} = 1/M_n + k_{n,n+1}t/m = 1/M_n + k'_{n,n+1}t \quad (4)$$

Preparation of samples by sonolysis

Chitosan (0.375 g) was completely dissolved in 150 mL 1% (v/v) acetic acid. The chitosan solution in a glass vessel was placed in an ice bath and kept the reaction temperature at 10°C. After sonolysis using pulsed ultrasound with a 1 s ''on'' and 1 s ''off'' for 10, 90, 240, 480 min counting the on period only, the solution were concentrated to about one-twentieth with a rotary evaporator under reduced pressure. With the addition of 1M KOH solution, the pH of the mixture was maintained at 9.0. The precipitate was collected by filtration, and dialyzed for 24 h against deionized water. After dialysis, the precipitate was washed thoroughly with anhydrous ethanol and then collected after drying over phosphorus pentoxide in vacuum to get samples DCs1, DCs2, DCs3, and DCs4.

Potentiometric determination of the DD

The chitosan (0.1 g) was dissolved in a known excess of 0.1M HCl acid (10 mL). Form the titration of this solution with a 0.1M 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 the free amino groups in the solution.[24] The titration was performed with a DELTA-320-S pH meter.

RESULTS AND DISCUSSION

Effects of sonolysis condition

Orthogonal test

The best condition for degradation of chitosan by ultrasound was studied by the orthogonal test. Four controllable variables, concentration of chitosan (%), reaction temperature (°C), sonic intensity (W/cm^2), and concentration of acetic acid (%) were selected, each at three levels. The investigated variables and their test levels are listed in Table II. Reference to the experimental design theory, the orthogonal array $L_9(3^4)$ was selected to arrange the test program. As results indicated, in our study range, the order of influence of each variables on ultrasonication is solution concentration of chitosan (%) > reaction temperature (°C) > concentration of acetic acid (%) > sonic intensity (W/cm^2). The reaction temperature and solution concentration of chitosan were the most two important factors. The concentration of solvent and

TABLE II
Conditions of Ultrasound Treatment and Results of Orthogonal Test

Sample	Chitosan concentration (%)	Reaction temperature (°C)	Sonic intensity (W cm^{-2})	HAc concentration (%)	M_w ($\times 10^{-4}$)	M_w/M_n
1	0.25	10	3.0	1	13.3	3.12
2	0.25	30	6.0	3	15.5	3.29
3	0.25	50	9.0	5	13.8	3.12
4	0.50	10	6.0	5	16.4	3.41
5	0.50	30	9.0	1	17.1	3.65
6	0.50	50	3.0	3	18.1	3.35
7	1.0	10	9.0	3	17.2	3.48
8	1.0	30	3.0	5	19.1	3.98
9	1.0	50	6.0	1	16.6	3.42
k_1	42.6	46.9	50.5	47.0		
k_2	51.6	51.7	48.5	50.8		
k_3	52.9	48.5	48.1	49.3		
Variance	10.3	4.8	2.4	3.8		

the sonic intensity, in our investigated range, did not significantly affect the degradation of chitosan by ultrasonic treatment. As shown in our results the optimum reaction condition was concentration of chitosan solutions 0.25% (w/v), reaction temperature 10°C, concentration of acetic acid 1% (v/v), and sonic intensity 9.0 W/cm^2. The experiment showed that appropriate reaction condition was necessary to prepare low molecular weight chitosan by ultrasound treatment and optimize the ultrasonic condition can improve the degradation efficiency.

Effect of sonolysis time on the molecular weight of degraded chitosan

Figure 1 illustrated GPC curves of CS4 and its degraded products. The peak of the elution curve shifted toward higher elution volumes. Obviously prolonging the duration increased the extent of degradation. As shown in the GPC curve of DCs1, the bimodal shape of molecular weight distribution appeared. The bimodal distribution of molecular weight at early stages of sonolysis of chitosan is related to the specific character of the process, which clearly shows that chitosan molecular undergo nonrandom process of chain scission, with the most probable site of breakage at the mid-point of the chain.[25]

Figure 2 shows the molecular weight of five kind of commercial chitosan treated by ultrasound during different time. As shown in Figure 2, the molecular weight decreased exponentially as the sonication time increased from 0 to 30 min and decreased very quickly during the initial 15 min of treatment, then slowed gradually and approached an asymptote at about 60 min. The data also indicated that with

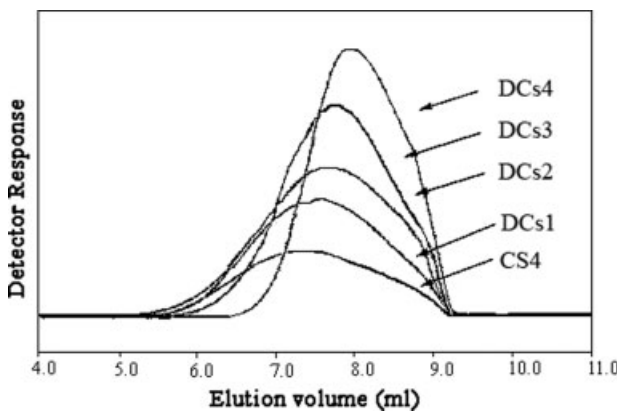

Figure 1 GPC profiles of products of CS4 prepared by ultrasonication at different reaction periods. Ultrasonic treatment condition: concentration of CS4 0.25% (w/v), reaction temperature 50°C, concentration of HAc 1% (v/v), and sonic intensity 9.0 W/cm^2.

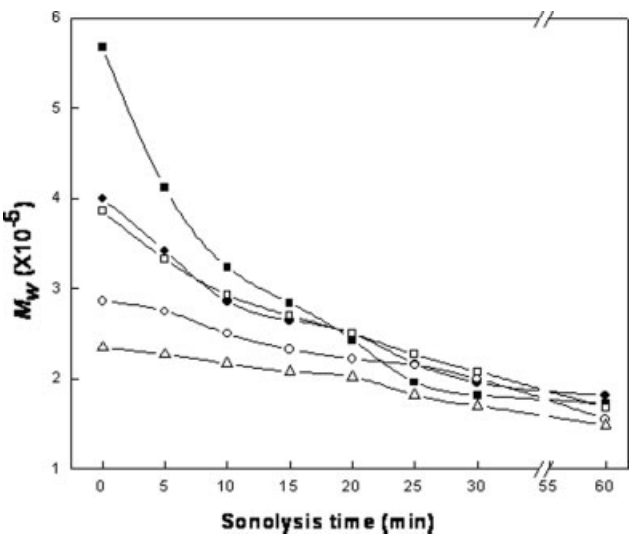

Figure 2 Effect of sonolysis time on the molecular weight of five kinds of initial chitosan during sonolysis process. The parameters of investigated chitosan were listed in Table I, concentration of chitosan solution 0.25% (w/v), reaction temperature 10°C, concentration of HAc 1% (v/v) and sonic intensity 9.0 W/cm^2. (■) CS1, (●) CS2, (□) CS3, (○) CS4, (Δ) CS5.

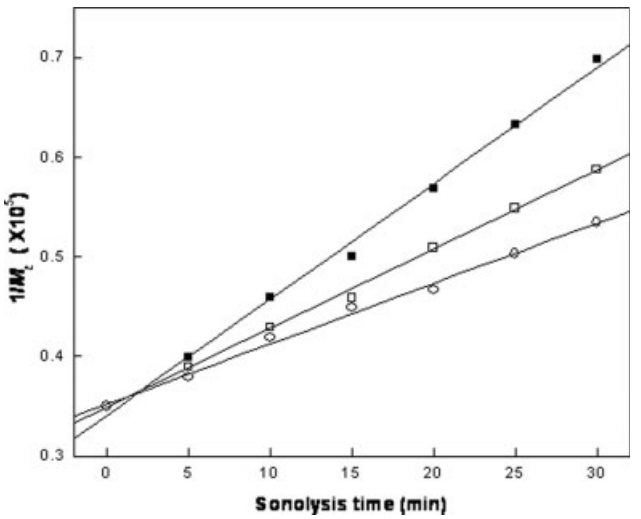

Figure 3 Plot of the reciprocal of molecular weight of degraded chitosan during ultrasonic treatment using 0.25% (w/v) chitosan concentration at different reaction temperature against sonolysis time. (■) 10°C, (□) 30°C, (○) 50°C.

increasing sonication time, the molecular weight of sonicated chitosan approaches a limiting final value. The presence of a limiting final molecular weight is typical for the degradation of large molecules by ultrasound.[17,26] In fact, the presence of a final molecular weight suggested that the decrease in the reduction of molecular weight with increasing sonication time was not due to the production of a molecule that can no longer be degraded but that instead with increasing disruption of intramolecular bonds on the macromolecules the number of total molecule in the dispersion increased. If simultaneously the number of bonds that can be broken within a given time interval remains constant but the number of available molecules increases it would consequently lead to a decrease in molecular weight as well because less bonds can be broken per available molecule. In our experiments, using the chitosan with an initial molecular weight of 286 kDa and degree of deacetylation 91.7% found that after 480 min of sonciation the molecular weight approached a final molecular weight of 22 kDa.

Degradation rate constant (k)

Effect of reaction temperature

As shown in Figure 3, in the investigated range, when concentration of chitosan was 0.25% (w/v), degradation rates constant at lower temperatures were higher than those at higher temperatures. This may be due to an elevated temperature facilitating the loss of cavitation energy and the cavitation bubble generated by the ultrasonic wave more easily escaping out from higher temperature solutions than from lower ones. The shearing effect as the cavitation bubble collapses is considered to be the major force degrading the chitosan during ultrasonic treatment. Therefore, elevating solution temperatures during ultrasonic treatment will retard the depolymerization effect.

Effect of chitosan concentration

As shown in Figure 4, in the investigated range, for a given temperature (10°C) and sonic intensity (9.0 W/cm^2), the degradation rate constant appeared to be concentration dependent, with the k value decreasing with increasing solution concentration of chitosan. This may be due to the fact that at the same cavitation energy level, the fraction of energy reacting to a single molecule is less for the higher concentration solutions than for dilute ones. In higher concentration solutions, viscous fluids hinder the actions of vibrational wave energy, shear stress at the cavitation interphases, and local high pressure and temperature yielded by cavitation formation. In the lower concentration solution, small molecular weight species have lower absorption coefficients or shorter relaxation times to more easily alleviate the sonication stress. Lower concentration of chitosan solution is accompanied with lower number of entanglements and lower solution viscosity, what facilitates the cavitation and makes degradation more effective. The plot of $1/M_t$ *versus* time showed a good linear relationship in Figure 4. This may have been due to the breakage of the β-1,4 glycoside linkages being randomly interrupted under the conditions used in this study.[27,28]

Figure 5 showed the effect of reaction temperature and solution concentration of chitosan on the degra-

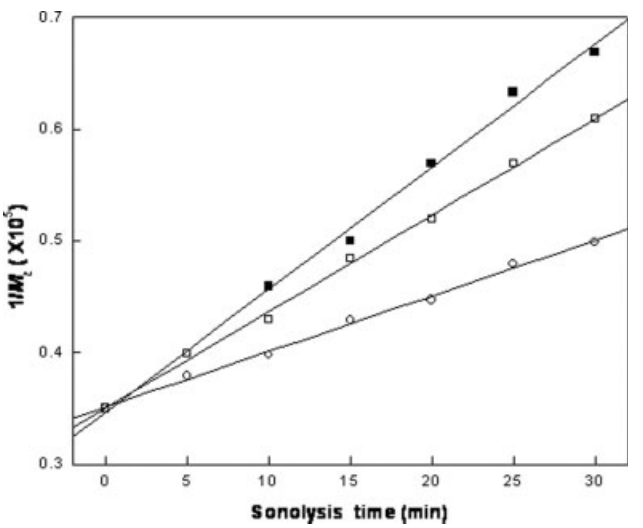

Figure 4 Plot of the reciprocal of molecular weight of degraded chitosan during ultrasonic treatment using different chitosan concentration at 10°C against sonolysis time. (■) 0.25%, (□) 0.50%, (○) 1.00%.

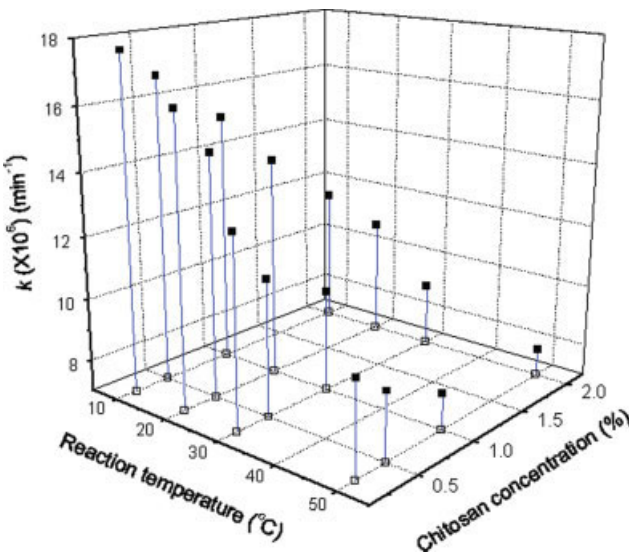

Figure 5 Effect of reaction temperature and concentration of chitosan solutions on the degradation rate constant. Concentration of HAc 1% (v/v) and sonic intensity 9.0W/cm^2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

dation rate constants. The intensity of cavitation, which is the main reason of chain rupture,[29] is more effective at lower temperature due to lower vapor pressure of the solvent and better solubility of gases. At the same time, the decrease of temperature causes the increase of chitosan solution viscosity, what means higher cohesive forces, and lower effectiveness of cavitation. Therefore, the decrease of temperature introduces two features acting in opposite directions and the molecular weight is a resultant of both these effects. As shown in Figure 5, at a lower reaction temperature ($\leq$30°C) and lower solution concentration of chitosan ($\leq$1%), the degradation rate constants increased with decrease of reaction temperature and solution concentration of chitosan. When solution concentration of chitosan was higher (2%), the degradation rate constants of chitosan increased with the increase of reaction temperature (from 20 to 50°C), which is partly due to the decrease of solution viscosity and facilitates the cavitation and makes degradation more effective. When reaction temperature was higher (50°C), decrease of solution concentration of chitosan cannot increase the degradation rate constants of chitosan with lower ($\leq$1%) solution concentration. Our results mean expansion of chitosan molecular with decreasing temperature and, as a consequence, the effect of solution viscosity is compensated, at least partly, by the increase of the intensity of cavitation.

Effect of molecular parameters of initial chitosan

The commercial chitosans obtained from *N*-deacetylation by alkali of chitin result in incomplete *N*-deacetylation and also result in depolymerization to varying extent. Therefore, chitosans are commercially available with a wide range of molecular weights and degrees of deacetylation. At the same time, the physicochemical properties of chitosans, such as chain flexibility in solution, the rheological properties, crystal size and crystalinity of chitosans depends on intrinsic factors such as the degree of deacetylation, distribution of those acetyl groups, molecular weight, and its distribution. Therefore, the effect of molecular weight and DD on the sonolysis of chitosan should be discussed in detail. The influence of molecular weight and degree of deacetylation on time evolution was shown in Table I.

The values of k and R both increased with decreasing the degree of deacetylation and increasing the molecular weight at the early stages of sonolysis. And for all kinds of investigated initial chitosan, degradation rate, and degradation rate constant decreased with an increasing sonolysis time. Those results indicated that chitosan with lower degree of deacetylation was relatively more asymmetric and the degradation by ultrasound could be more efficient. And the hydrodynamic forces are of primary importance and that ultrasonic degradation occurs with the longer molecules being preferentially degraded. The molecular weight and degree of deacetylation of initial chitosan simultaneously affect the degradation rate and degradation rate constant. Separation of the two effects on degradation rate constant was done by dividing k value by M_w. Calculated k^+ values which are independent on initial molecular weight were listed in Table I. Data in Table I show that the decrease in the value of the degree of deacetylation just causes the inconspicuous increase of k^+ in the applied experimental conditions, which indicated that the molecular weight is the more important factor than degree of deacetylation to affect the ultrasonic treatment.

As shown in Table I, the decrease of the k and R values during every 10 min of ultrasonication is higher for initial chitosan with higher molecular weight, while initial chitosan with lower molecular weight remains its degradation rate constant during ultrasonication. The chain scission of chitosan treated by ultrasound undergo at the mid-point of the chain at the early stages which resulted in chitosan with higher molecular weight has a higher initial k value. But with the reduction of molecular weight of initial chitosan with higher molecular weight by increasing sonication time, the number of total molecule in the dispersion increased dramatically and within a given reaction condition the degradation effective decreased. The initial chitosan with lower molecular weight has lower k values compared to the ones with higher molecular weight at the same reaction condition, but the k and R values remained almost

TABLE III
Properties of Degraded Chitosan

Sample	M_w ($\times 10^{-4}$)	M_w/M_n	DD (%)	Yield (%)
CS4	28.6	5.40	91.7	–
DCs 1	16.1	4.87	91.6	97.3
DCs 2	10.5	4.10	91.5	95.9
DCs 3	5.8	3.08	92.1	93.8
DCs 4	2.2	1.97	92.8	89.7

unchanged during ultrasonication. That is to say, the number of bonds that can be broken within a given time interval remains constant during ultrasonication, but with increasing sonication time, the increase in available molecules of initial chitosan with higher molecular weight higher than ones with lower molecular weight, which consequently lead to a decrease in degradation rate and degradation rate constant.

The structural properties of different molecular weight chitosan

The degree of deacetylation of degraded chitosan

The DD of degraded chitosan were listed in Table III. The DD of the main hydrolysate increased a little with the decreasing of molecular weight. During the experiments, the sonicated chitosan was precipitated from solution with alkali, dialyzed against deionized water to remove excess of potassium hydroxide and potassium acetate, and then dried over phosphorus pentoxide *in vaccum*. And during this process, a part of low molecular weight chitosan may be dissolved in dialyzate and cannot be collected after being dialyzed.

FT-IR spectra

FT-IR spectroscopy has been shown to be a powerful tool for the study of the physicochemical properties of polysaccharides. Curves a and b in Figure 5 show the IR spectra of initial chitosan CS4 and degraded chitosan DCs4. The absorption bands at 1655.1, 1600.7, 1324.2 cm^{-1} in initial chitosan are attributed to the amide **I**, N—H bending mode of $—NH_2$ and amide **III** band, respectively.[30] The spectrum of DCs4 is similar to that of initial chitosan, but the amide **I** band shifts to low wave number, this suggested that carbonyl groups had more opportunity to form stronger hydrogen bonds in that the scission of polymer chains led to the increasing mobility of molecule. As can be seen, there is no significant difference between the amide **III** of initial chitosan and DCs4, which indicated that with the decrease of the molecular weight of chitosan, the degree of deacetylation of ultrasonic treated chitosan almost unchanged. The IR spectra suggested that there was no significant difference between the residues of chitosan before and after the ultrasound treatment. All these data coincided well with the data of potentiometric determination of DD.

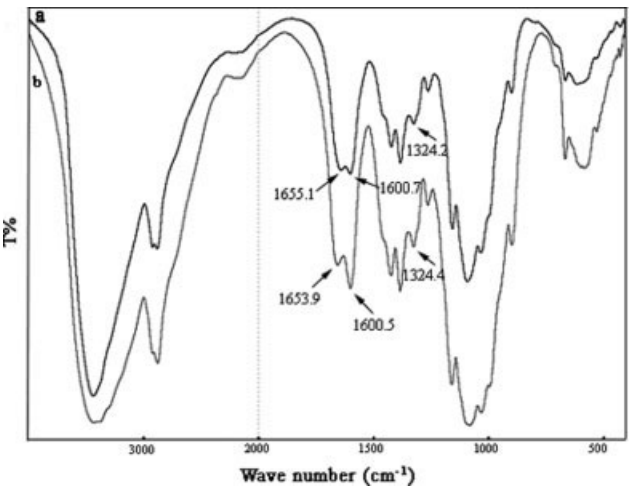

Figure 6 FT-IR spectra of initial chitosan CS4(a) and degraded chitosan DCs4 (b).

X-ray analysis

Figure 6 shows the X-ray diffraction patterns of chitosan and low molecular weight chitosan prepared by sonolysis. The wide-angle X-ray diffraction (WAXD) pattern of initial chitosan CS4 shows its characteristic peaks at $2\theta = 10.4^\circ$ and 19.8°, which coincided with the pattern of the ''L-2 polymorph'' of chitosan reported previously.[31] Comparing with initial chitosan, the peak at $2\theta = 10.4^\circ$ of DCs1 and DCs3 disappeared. The characteristic peak at small

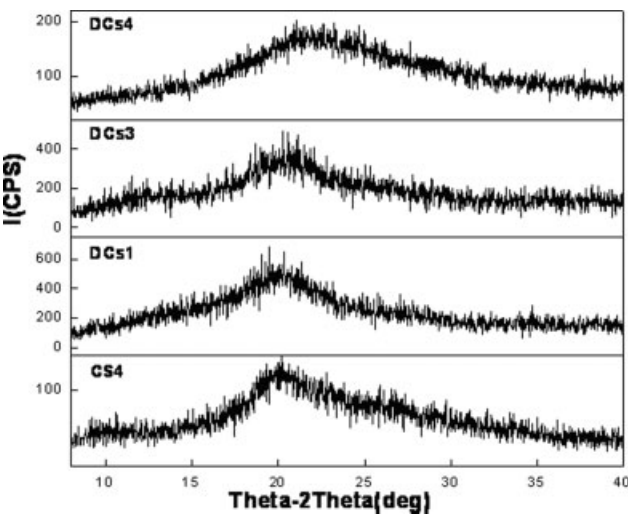

Figure 7 X-ray diffraction patterns of initial chitosan CS4 and degraded chitosans.

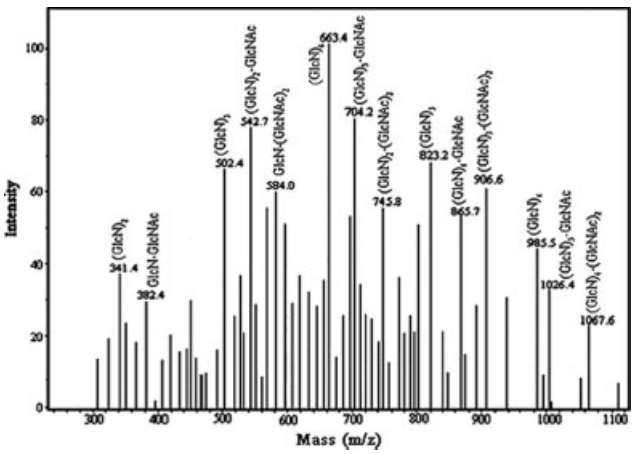

Figure 8 ESI-MS spectrum of DCs4

angle of the two kind of low molecular weight chitosan prepared by sonolysis disappeared, which corresponding to large spacing indicated that degradation of chitosan lead to decrease of the crystalline perfection. To compare the X-ray diffraction patterns of DCs1 with DCs3, the peak at $2\theta = 19.8^\circ$ of DCs3 slightly narrows which means that the number and size of the crystalline regions have decreased further and that the number of defects increased (Fig. 7). The intensity of the characteristic peak at $2\theta = 19.8^\circ$ of DCs3 decreased compare with DCs1, which indicated that the DCs3 had lower crystallinity than DCs1.[20] The percent relative crystallinities are 19.1%, 35.9%, and 23.4%, corresponding to CS4, DCs1, and DCs3, respectively. DCs4 had only one major peak and became amorphous.[32] That was to say, the chitosan in amorphous region was first degraded by ultrasound and our results confirm the splitting of ultrasound may be much more random and produced more than two fragments from one original molecule. With deeper degradation, the crystalline structure was destroyed.

ESI-MS analysis of DCs4

Chitosan with low degree of polymerization have been shown to exhibit similar signal strengths, irrespective of structure, when examined by mass spectrometry.[33] Although the relative amount of each oligomer product could not be determined from relative intensity in the spectrum since a linear correlation between the relative ion-intensity and the quantification of the product has not yet been established, it has been accepted that the relative ion-intensity can reflect the quantification of the products. Electrospray ionization (ESI) mass spectrometer has been used for structure characterization of saccharide.[34,35] Figure 8 shows the ESI-MS spectrum of DCs4, which was directly analyzed by ESI-MS after ultrasound treatment without precipitated by alkaline solution and dialysis. As can be seen, ultrasound treated chitosan not only could produce hemooligomers, such as $(GlcN)_2$, $(GlcN)_3$, $(GlcN)_4$, $(GlcN)_5$, and $(GlcN)_6$, but also produced mixture of heterooligomers, each of which carries one or two GlcNAc residue. It is also consistent with the conclusion ultrasound-treated chitosan not only at the mid-point of chain but also can produce chitosan with low degree of polymerization when the ultrasound treated time is enough.[26]

CONCLUSIONS

Ultrasound treatment has the potential to place chemical or enzymatic methods that are currently used to modify the molecular weight chitosan. Our experiments have shown that the degree of deacetylation is almost unchanged but the application of ultrasound reduces the molecular weight, which is often desirable for its use in pharmaceutical and food applications. The reduction in molecular weight of the resulting chitosan led to the transformation of the crystal structure. IR spectra confirmed that the chemical structures of residues were not modified. The molecular weight characterized by GPC and the structure of chitosan with low degree of polymerization analyzed by ESI-MS confirm that the splitting of ultrasound to chitosan chain is much more random and produced many fragments from one original molecule. The utilization of ultrasound offers advantages over chemical and enzymatic methods for the preparation of low molecular weight chitosan and more suitable and safer for biomedical and food applications.

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