Low Molecular Weight Water-Soluble Chitosans: Preparation with the Aid of Cellulase, Characterization, and Solubility

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ABSTRACT: Preparation of water-soluble chitosan (WSC) was made by treating partially N-deacetylated chitosan with acetic anhydride in aqueous acetic acid. The optimal conditions of preparing WSC were determined on the basis of orthogonal tests. Low molecular weight WSC with broad molecular weight (600–1.5 kDa) were obtained by the depolymerization of WSC using cellulase at optimum condition of pH 4.5 and 60°C. The solubility of WSC in water and aqueous organic solvents was investigated in detail. Weight–average molecular weight (M_w) and molecular weight distribution (M_w/M_n) of samples were measured by gel permeation chromatography. The structure of WSC and its degraded products were characterized by XRD, FTIR, and MALDI-TOF MS. The decrease of molecular weight led to transformation of crystal structure and the increase of solubility, but the chemical structures of residues were not modified compared to WSC, which was not hydrolyzed. The solubility of the WSC in water and aqueous organic solvents increased with the decrease of molecular weight. The solubility of the WSC with low molecular weight was rather high even in aqueous dimethylacetamide and dimethylsulfoxide.
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Key words: water-soluble polymers; enzymes; degradation; solubility

INTRODUCTION

Chitin and chitosan have been considered as biomaterials in fields such as biomedicine, pharmacology, and biotechnology due to their biocompatibility, biodegradability, and biological activities.[1–3] However, when it is used in biological fields, its applications are restricted because it is insoluble in water and can be dissolved in acidic aqueous solutions only where the amino groups are protonated. And the reason for the intractability of chitosan lies in the rigid crystalline structure and the acetamide or primary amino group residues, which are important in forming conformational features through intra or intermolecular hydrogen bonding.[4]

Because chitosan dissolves only in water-containing acetic acid, using acetic acid as a solvent is considered as another limitation for actual use from bioactive agents such as peptide or protein drugs, genetic material, and anticancer drugs, which may be affected by acetic acid.[5] Therefore, various studies were conducted to make water-soluble derivatives of chitin and chitosan by chemical modification techniques. However, when chemical modifications change the fundamental skeleton of chitin and chitosan, the modified chitin and chitosan lose the original physicochemical and biochemical activities.[6,7]

Chitosan become water soluble through controlling the degree of deacetylation, and the so-called "water-soluble chitosan (WSC)" with a deacetylation degree of about 50% can be obtained from chitin by hydrolysis with alkali[8] or from chitosan by N-acetylation with acetic anhydride.[9] This kind of WSC did not change the fundamental skeleton of chitosan and has been used as a starting material for the chemical modification of chitosan,[10] as a substrate for chitosanase and chitinase,[11] and as ecological and environmental friendly materials in agricultural, biomedical, cosmetic, and food additive fields.[12,13] Therefore, if WSC could be prepared in a simple manner, their biological and physiological applications would develop markedly.

And recently, evidence shows that WSC with weight–average molecular weight >5 kDa and with a degree of deacetylation of 58% reached the maximum inhibitory rate to sarcoma 180 tumor cells in the mice at 64.2% through intraperitoneal injection.[14] Low molecular weight chitosan can be prepared by chemical or enzymatic depolymerization of chitosan, of which the latter is preferred as the process can be easily

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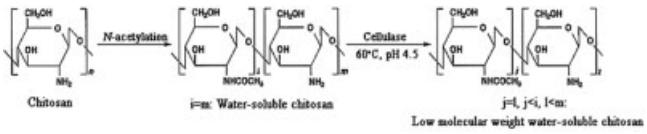

Scheme 1 The preparation of low molecular weight WSCs.

controlled, monitored, and the products can be obtained without any modification, which are normally seen with chemical hydrolysis.[15] Chitosanase and chitinase is the preferred enzyme for such depolymerization processes, but its cost, unavailability, and specificity limit their usage.[16] The structure of chitosan is very similar to cellulose, except for the amino group that replaces the hydroxyl group on the C-2 position. Therefore, cellulase, a kind of cheap, commercially available nonspecific enzyme, can hydrolyze chitosan efficiently.[17]

In the study, WSC was prepared in a simple manner. A series of WSCs with broad molecular weight (600–1.5 kDa) were prepared with the aid of cellulase (Scheme 1), and then characterized with Fourier transform infrared (FTIR), X-ray diffraction (XRD), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometers (MALDI-TOF MS). Potentiometric titration and elemental analysis have been used to measure the degree of deacetylation of chitosan investigated. The relation between molecular weight and water and aqueous organic solvents solubility is investigated in detail.

EXPERIMENTAL

Materials

Chitosan with a weight–average molecular weight (M_w) of 820 kDa and the degree of deacetylation (DD) of 82.4% was obtained from Yuhuan Biochemical Co. (Zhejiang, China). D-Glucosamine HCl were purchased from Seikagaku Corp. (Japan). All other chemicals were of reagent grade.

The cellulase was a product of Ningxia XiaSheng Industry Co. (China).

Preparation of WSC by N-acetylation

A portion (8 g) of chitosan was dissolved in a solution of acetic acid (200 mL), and the mixture of anhydrous ethanol and acetic anhydride was added into the chitosan solution with magnetic stirring. After stirring at room temperature for a predetermined time, the reaction mixture was adjusted to pH 9 by KOH, and dialyzed against distilled water for 3 days. The solution was concentrated with a rotary evaporator under reduced pressure and precipitated by adding anhydrous ethanol, then dried over phosphorus pentoxide in vacuum to get N-acetylated chitosans.

Batch experiments of enzymatic hydrolysis

The WSC powder (0.1 g) was introduced in a reactor containing 0.2*M* NaAc–HAc buffer. WSC to liquor ratio of 1:100 was used. After the mixture was stirred for 1 h, the reactor was kept in a thermostatic water bath and enzyme solution was added. The weight ratio of cellulase to substrate was 1:20. Details of the reaction conditions were given in the text. At various intervals, 0.5 mL of the reaction mixture was taken out. After heating in a water bath at 100°C, filtrate was analyzed by GPC.

Preparation of low molecular weight WSC by enzymatic hydrolysis

WSC (6 g) was dissolved in 150 mL distilled water. Then the solution was adjusted to pH 4.5 using acetic acid. The solution in the reaction vessel was placed in a thermostatic water bath at 60°C, and a solution (2 mL) in which the cellulase was dissolved in 0.2*M* acetate buffer (pH 4.5) was added to initiate reaction. After predetermined time, the mixture was taken out and boiled for 10 min to remove the enzyme. After filtering, the filtrate was concentrated with a rotary evaporator under reduced pressure followed by neutralization with 10% KOH to pH 9, and then precipitated by adding anhydrous ethanol. The precipitate was collected by filtration, and washed thoroughly with anhydrous ethanol. The low molecular weight WSCs were collected after drying over phosphorus pentoxide in vacuum.

Characterizations

The increasing amount of reducing sugars resulting from a cleavage of glycosidic linkage was monitored by spectrophotometric analysis on the basis of Schales' modified method[18] with D-glucosamine HCl as standard.

Weight–average molecular weight (M_w) and molecular weight distribution (M_w/M_n) of sample were measured by GPC. The GPC equipment consisted of connected columns (TSK G5000 PWXL and TSK G3000 PWXL (Tokyo, Japan)), TSP P100 pump (Thermoquest, San Jose, CA), and RI 150 refractive index detector (Thermoquest, San Jose, CA). 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 sample concentration was 0.4 mg/mL. The standards used to calibrate the column were TOSOH pullulan (Showa Denko, Tokyo, Japan). All data provided by the GPC system were collected and

analyzed using the Jiangshen Workstation software package (Dalian, China).

Intrinsic viscosity was determined by a literature viscometric method.[19] WSC samples were prepared in 0.2*M* CH_3COOH/0.1*M* CH_3COONa aqueous solutions. The relative viscosity, η, of WSC samples were measured using an Ubbelohde capillary viscometer at (30 ± 0.5)°C. Specific viscosity was determined using: $\eta_{sp} = (\eta_{solution} - \eta_{solvent})/\eta_{solvent}$.

Intrinsic viscosity, $[\eta]$, is defined as reduced viscosity, $\eta_{red,}$ extrapolated to a WSC concentration, C, of zero: $[\eta] = (\eta_{sp}/C)_{C\to 0} = (\eta_{red})_{C\to 0}$, where C is in g/mL.

FTIR spectra were recorded with KBr pellets on a Nicolet FT-IR 360 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 Kα 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]

MALDI-TOF MS analysis of chitooligomers was carried out using Voyager DE STR matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Applied Biosystems, USA) at an acceleration voltage of 20 kV, using 2,5-dihydroxybenzoic acid as the matrix.

Determination of the degree of deacetylation

The WSC (0.1 g) was dissolved in a known excess of 0.1*M* HCL (10 mL). From the titration of 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 the free amino groups in the solution.[21] The titration was performed with a DELTA-320-S pH meter.

The DD of chitosan that cannot dissolve in hydrochloric acid was determined by elemental analysis (EA).[22] Elemental analyses were performed at Elemental Analyzer-MOD 1106 (Carlo Erba Strumentazione) and the degree of deacetylation determined by EA was calculated using the following equation:

$$DD = 1 - [(W_C/W_N - 5.14)/1.72] \times 100\%$$

where W_C/W_N is the ratio (w/w) of carbon to nitrogen.

Estimation of water-solubility

The pH dependence of water solubility of WSC was evaluated from turbidity.[23] WSC was dissolved in 1% (v/v) acetic acid. The transmittance of the solution was recorded with the stepwise addition of concentrated NaOH solution on a Shimadzu UV-9100 spectrophotometer using a quartz cell with an optical path length of 1 cm at 600 nm. The solubility of the WSC in aqueous organic solvents was estimated from the transmittance of the solution, which was prepared in advance with deionized water, by adding organic solvents stepwise. The sample concentrations were 1.0% (w/v).

TABLE I
The Variables Investigated for Preparation Water-Soluble Chitosan and Their Levels

Variables investigated	Levels of each variable		
	1	2	3
A: concentration of acetic acid (%, v/v)	1	5	10
B: volume ratio of anhydrous ethanol to HAc solution	3:5	1:1	3:2
C: molar ratio of acetic anhydride to chitosan	2:5	3:5	4:5
D: reaction time (h)	1	3	5

RESULTS AND DISCUSSION

Preparation of WSC

The optimal condition for preparing WSC was studied by the orthogonal test. Four controllable variables, concentration of acetic acid (HAc) (% v/v), the volume ratio of anhydrous ethanol to HAc solution, the molar ratio of acetic anhydride to chitosan, and reaction time (h), were selected, each at three levels. The investigated variables and their test levels are listed in Table I. Reference to the experimental design theory, the orthogonal array $L_9(3^4)$ was selected to arrange the test program. The test results are listed in Table II. As results indicated, in our study range, the order of influence of each variable on the substitutes of N-acetylation is $C > B > A > D$. The data revealed that the N-acetylated chitosans were water soluble when the DD was 46.5–61.8%. However, dissolution of half N-acetylated chitosan was easier than that of the other samples. Thus the optimum reaction conditions were determined as follows: concentration of acetic acid, 5% (v/v); the volume ratio of anhydrous ethanol to HAc solution, 3:2; the molar ratio of acetic anhydride to chitosan, 2:5; and the reaction time, 3 h. The experiment showed that appropriate reaction condition was necessary to prepare WSC.

Influence of reaction condition on the depolymerization of WSC

Cellulase and WSC reacted in 0.2*M* NaAc–HAc buffer with different pH for 1 h under mild stirring at 60°C,

TABLE II
The Results of Orthogonal Test for Preparation Water-Soluble Chitosan

Sample code	*A*	*B*	*C*	*D*	DD(%)[a]	Solubility	
						water	1% (v/v) HAc
1	1	1	1	1	65.4	i	s
2	1	2	2	2	46.5	s	s
3	1	3	3	3	29.7[b]	i	i
4	2	1	2	3	61.8	s	s
5	2	2	3	1	35.4[b]	i	i
6	2	3	1	2	50.6	s	s
7	3	1	3	2	54.3	s	s
8	3	2	1	3	75.3	i	s
9	3	3	2	1	44.5[b]	i	i
K_1	141.6	181.5	191.3	145.3			
K_2	147.8	157.2	152.8	151.4			
K_3	174.1	124.8	119.4	166.8			
Variance	32.5	56.7	71.9	21.5			

i, insoluble; s, soluble.
[a] Degree of deacetylation measured by potentiometric titration.
[b] Degree of deacetylation determined by elemental analysis.

the weight ratio of cellulase to substrate was 1:20, and the concentration of WSC solution was 1% (w/v). The results are shown in Figure 1. It indicated that pH 4.5 was the optimum pH for depolymerizing WSC by cellulase.

In such condition, pH 4.5, 0.2*M* NaAc–HAc buffer solution, the weight ratio of cellulase to substrate was 1:20, the concentration of WSC solution was 1% (w/v), mild stirring, cellulase and WSC solution reacted at different temperatures for 4 h. The results are shown in Figure 2. In the investigated range, the optimum temperature was 60°C. The enzyme was relatively stable in the temperature range below 60°C, but was rapidly inactivated at higher temperatures.

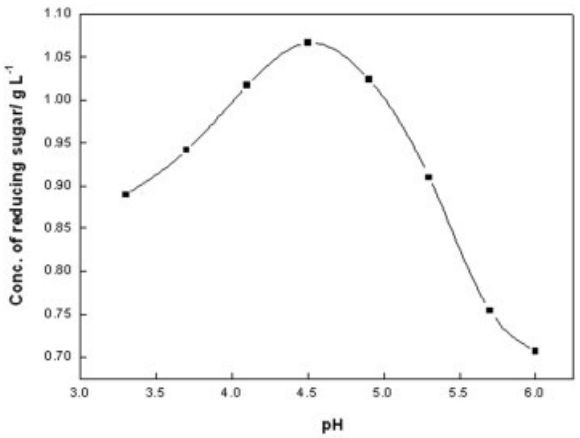

Figure 1 pH dependence of the enzymatic hydrolysis of WSC. The concentration of WSC was 1% (w/v) and the weight ratio of cellulase to substrate was 1:20. The concentrations of reducing sugar were analyzed after incubation at 60°C for 1 h.

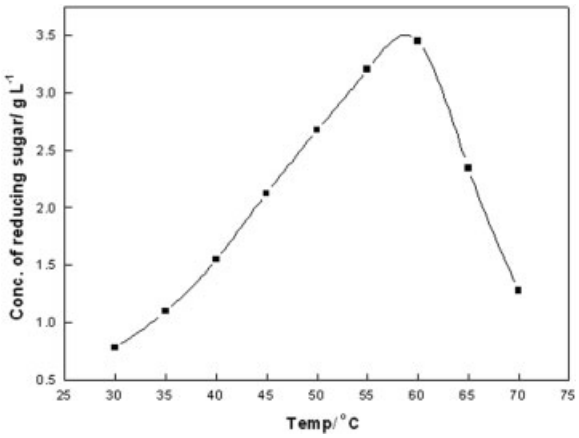

Figure 2 Effect of temperature on the enzymatic hydrolysis of WSC. The concentration of WSC was 1% (w/v) and the weight ratio of cellulase to substrate was 1:20. The concentrations of reducing sugar were analyzed after incubation in 0.2*M* NaAc–HAc buffer solution (pH 4.5) at different temperature for 4 h.

Degrading enzymes attacking polysaccharides are commonly classified according to whether they cleave a susceptible glycosidic bond situated at a terminal residue in a chain and successively release monomer units from the chain end (exo-action) or they depolymerize polysaccharides by an apparently random splitting of interior glycosidic bonds (endo-action). It is possible to differentiate between the two enzymatic functions in some cases by following the molecular weight of a polysaccharide as a function of the extent of reaction. In general, molecular weight drops rapidly in endo-type degradation process, but remains essentially constant for quite some time in exo-type reactions. Figure 3 illustrates GPC curves of WSC and its degraded products. Higher elution volumes correspond to a decrease in the molecular weight of chitosan. Obviously, the extent of degradation was increased by prolonging the duration. A rapid decrease in the molecular weight of WSC was seen in the early reaction stage. GPC measurements showed the information on degradation process and indicated that the enzymatic hydrolysis was endo-action.

The molecular parameters of degraded WSC

Table III lists the degradation conditions along with M_w, M_w/M_n of the low molecular weight WSC samples prepared with the aid of cellulase. A series of low molecular weight WSC with M_w from 600 to 1.5 kDa was obtained. As shown in Table III, the molecular weight varied according to the weight ratio of cellu-

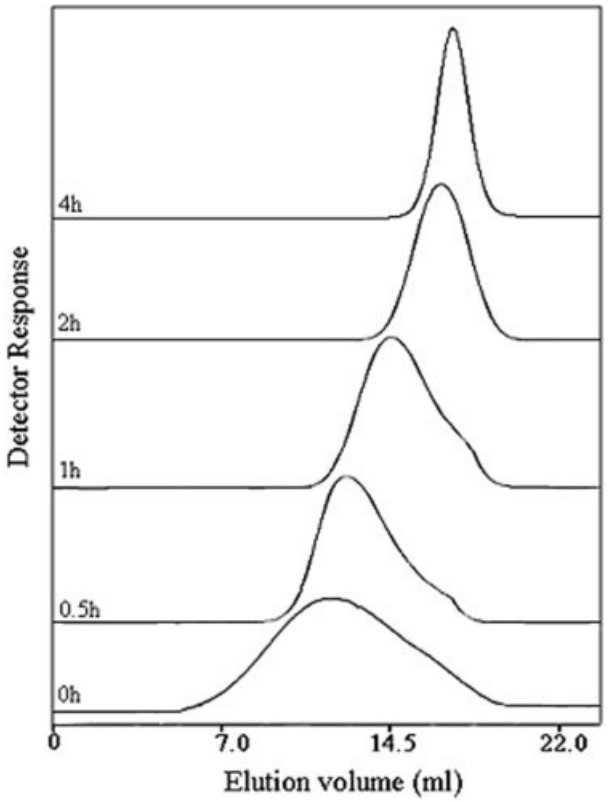

Figure 3 GPC profiles of enzymatic products of WSC at different reaction periods. The concentration of WSC was 1% (w/v), the weight ratio of cellulase to substrate was 1:20, and reaction condition was 60°C and pH 4.5.

lase to substrate (E/S) and the reaction time. Increasing the weight ratio of cellulase to substrate and prolonging the duration increased the extent of degradation. With the decreasing of molecular weight, the molecular weight distribution (M_w/M_n) decreased.

The DD of degraded WSCs were listed in Table III. The DD of the low molecular weight WSCs did not change with the decrease of molecular weight. It may

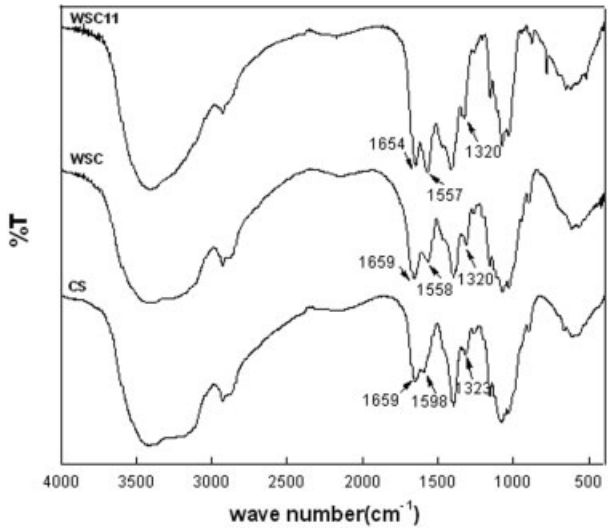

Figure 4 FTIR spectra of initial chitosan (a), WSC (b), and degraded water-soluble chitosan WSC11(c).

be due to the random distribution of almost equal amounts of glucosamine (GlcN) and *N*-acetylglucosamine (GlcNAc) units along the chain after the chitosan N-acetylated in homogeneous condition[23,24] and the cellulase cleaved glycosidic bonds of WSC in a random manner.

FTIR spectra

Figure 4 shows the IR spectra of initial chitosan, WSC, and WSC11. The absorption bands at 1659, 1598, and 1323 cm^{-1} in initial chitosan are, respectively, attributed to the amide I, N—H bending mode of —NH_2 and amide III band. The absorption band at 1558 cm^{-1} in WSC is considered as the contribution of amide II band, indicating the DD decreases.[25] And the IR spectra of WSC11 is similar to that of WSC, but the amide I shifts to low wave number, suggesting that carbonyl groups had more opportunity to form stronger hydro-

TABLE III
Degradation Conditions and the Parameters of WSC and Its Hydrolyzates

Sample code	Degradation condition		M_w (10^{-4})	M_w/M_n	[η] (mL/g)	DD (%)	Yield (%)
	E/S (% w/w)	Time (h)					
WSC	Untreated	—	79	7.42	621	51.3	—
WSC1	0.5	0.2	60	7.21	452	52.8	98.6
WSC2	1	0.2	34	7.04	255	50.3	97.2
WSC3	1	0.5	23	6.08	178	51.6	97.3
WSC4	2	0.5	17	5.37	127	51.4	95.3
WSC5	2	1	12	4.61	91	50.9	93.7
WSC6	3	1	9.8	4.25	74	51.4	91.8
WSC7	4	1	4.0	3.33	47	50.1	87.2
WSC8	5	2	1.1	2.04	18	51.9	85.0
WSC9	5	3	0.71	1.73	21	51.7	83.1
WSC10	5	4	0.33	1.36	13	50.1	83.3
WSC11	5	6	0.15	1.24	8	50.1	81.3

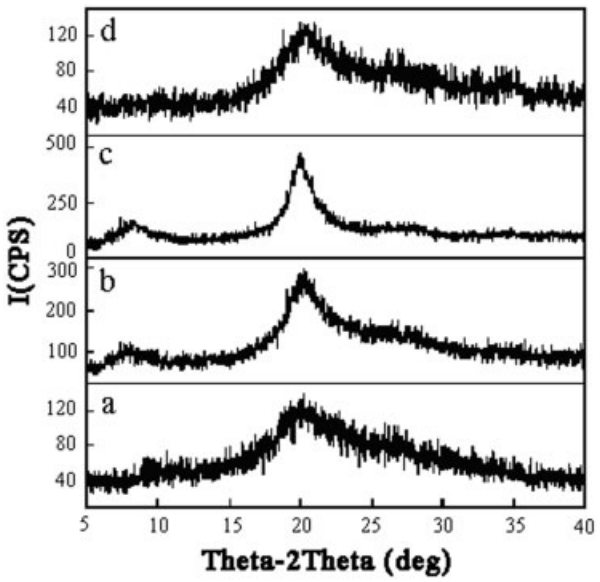

Figure 5 X-ray diffraction patterns of initial chitosan (a), WSC (b), and degraded water-soluble chitosan WSC4(c) and WSC11(d).

gen bonds in that the scission of polymer chains led to the increasing mobility of molecules. The IR spectra suggested that there was no significant difference between the residues of WSC before and after the cellulase hydrolysis.

X-ray diffraction analysis

Figure 5 shows the X-ray diffraction patterns of the main fraction of initial chitosan, WSC, and its hydrolyzates. X-ray diffraction pattern of initial chitosan shows its characteristic peaks at $2\theta = 10.4°$ and 19.8°, which coincided with the pattern of the "L-2 polymorph" of chitosan, reported previously.[26] The WSC showed a shift of this reflection to 8.0 ° because of larger *d*-spacing as a result of increase in the unit cell dimension. The intensity of the characteristic peak at $2\theta = 8.0°$ and 19.8 ° increased, indicating that the GlcNAc residues in WSC are distributed so as to form a highly ordered structure through hydrogen bonding with GlcNAc residues of neighboring chain, facilitating the incorporation of water molecules into such a network and forming a hydrated crystal. WSC4 displayed the same characteristic peak as WSC. And crystallinity measurements showed higher crystallinity for depolymerized WSC4 compared with WSC, which was attributed to the higher mobility of low molecular weight WSC chains as a consequence of decrease chain length and the hydrogen bond formed again. WSC11 had only one major peak and became amorphous.[9] That was to say, the chitosan in amorphous region was first degraded to water-soluble molecules and dissolved in water.[17] The crystalline structure was destroyed with deeper degradation.

And it is well known that annealed chitosan polymorphs (anhydrous form) are always formed because the molecular weight decreased and usually associated with the decreased solubility and thus loss of functionality.[27] Low molecular weight WSC prepared by cellulase hydrolysis did not appear annealed form. Therefore, they probably did not change the functionality and are more suitable for biomedical and food applications.

MALDI-TOF mass spectrum analysis

MALDI-TOF mass spectrum is an outstanding tool for the investigation of high-molecular-weight substances and for the potential possible analysis of end-group and repeat units of polymer monomer up to 30 kDa. In addition, MALDI-TOF mass spectra allow the relative quantities of constituents of a mixture to be determined.[28] 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.[29] Figure 6 shows the MALDI-TOF mass spectrum of WSC11, which revealed that the WSC11 was composed mainly of WSC oligomers with degree of polymerization (DP) 3–13. In the MALDI-TOF mass spectrum, WSC oligomers contained intensive quasi-molecular ions $[M + Na]^+$ and a $[M + K]^+$ ion. Because the protonation degree of saccharide is weaker than that of protein, saccharide can produce addition compound with Na^+ and K^+ in the matrix.[30] For example, in Figure 6, the peak appeared at 651.42 m/z ($[M + Na]^+$) is attributed to sodium form of $(GlcNAc)_3$ and the peak appeared at 666.46 m/z ($[M + K]^+$) is attributed to potassium form of $(GlcNAc)_3$.

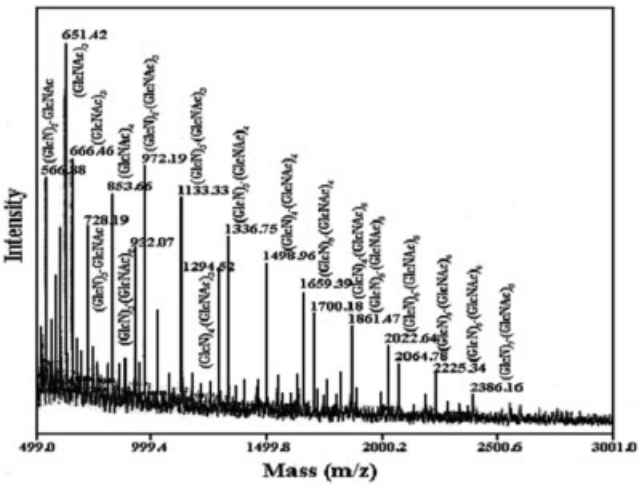

Figure 6 MALDI-TOF mass spectrum of WSC11.

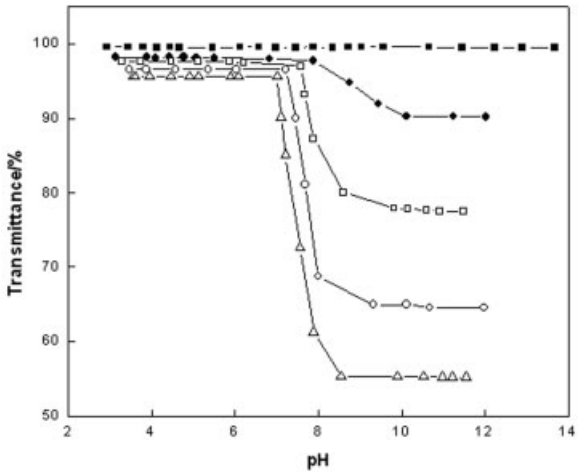

Figure 7 pH dependence of water solubility of different concentration of WSC. (■) 0.1%, (●) 0.3%, (□) 0.5%, (○) 0.7%, (△) 0.9% (w/v).

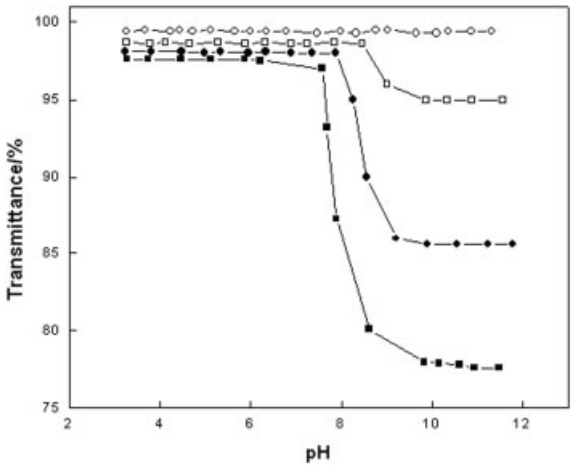

Figure 8 pH dependence of water solubility of different molecular weight WSC. (■) WSC, (●) WSC1, (□) WSC2, (○) WSC3. The sample concentrations were 0.5% (w/v) and sample codes correspond to those in Table III.

Figure 6 represents that the difference of the two adjacent peaks was 161. This means that it corresponds to the M_w of the GlcN unit. The MALDI-TOF mass spectrum has suggested that chemical structures of residues were not modified after being hydrolyzed by cellulase. These coincided well with the conclusion from FTIR analysis of WSC11.

Estimation of solubility

Figure 7 shows the solubility of WSC with different concentration in different pH value solutions. The results show that in the low concentration (0.1% (w/v)), the WSC with M_w = 790 kDa can dissolve in all pH range. With the increase of concentration, the solubility decreased within the alkaline region. It suggested that high concentration of WSC increased the intermolecular interactions, such as van der Waals forces, and then decreased the water solubility.

Figure 8 shows the solubility of different molecular weight WSC in different pH value solutions. The WSC3 with M_w = 230 kDa can dissolve in all pH range but chitosan can dissolve in all pH range only when the M_w below 1.5 kDa.[31] Thus the solubility of the WSC with about 50% of degree of deacetylation is probably accounted by the combination of many factors such as degree of deacetylation and the reduction of molecular weight. The latter might be important in some cases, but the degree of deacetylation seemed to be the most important reason why WSC can dissolve in all pH range solution. Made a comparison among all these WSC samples, the low molecular weight WSCs retained the water solubility over a wide pH range. The low molecular weight WSC with molecular weight below 230 kDa gave very high solubility, but the solubility in water of the rest decreases with increase in molecular weight in the alkaline region. The higher water solubility of low molecular weight WSCs is attributed to the decrease of intermolecular interactions, such as van der Waals forces; the lower the molecular weight, the lower the intermolecular attraction forces.[23] Therefore, the decreasing in water solubility of the WSC with high molecular weight is probably due to the high molecular weight itself and not the blockwise distribution of N-acetyl groups.

Figures 9 and 10 show the solubility of different molecular weight WSC in DMA and DMSO, respectively. DMA is known to be a good solvent for chitin when it is used with LiCl, and DMSO is a kind of very important solvent in chemical modification. Because chitosan shows poor affinity for organic solvents, which has made the controlled reactions difficult, it is

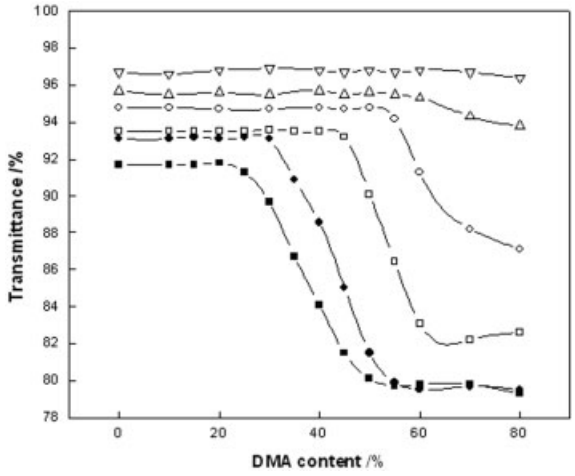

Figure 9 Dependence of solubility of WSC on DMA content: (■) WSC, (●) WSC1, (□) WSC2, (○) WSC3, (△) WSC8, (▽) WSC10. Sample codes correspond to those in Table III.

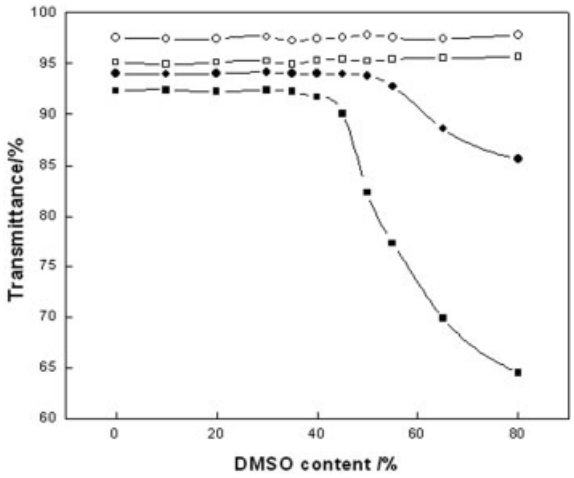

Figure 10 Dependence of solubility of WSC on DMSO content: (■) WSC, ● WSC1, (□) WSC5, (○) WSC8. Sample codes correspond to those in Table III.

very necessary to discuss the solubility of WSC in organic solvent. After the WSCs were previously dissolved in deionized water, DMA or DMSO was gradually added. As shown in Figures 9 and 10, the lower the molecular weight, the higher the solubility in aqueous DMA and DMSO. The solubility of the WSC in DMSO is slightly higher than that in DMA. And it is obviously that reducing the molecular weight prior to degree of deacetylation is one of the most effective methods of rendering WSC soluble in aqueous organic solvents.

CONCLUSIONS

WSC was prepared by controlling the N-acetylated degree with acetic anhydride under the homogeneous condition, and then was hydrolyzed with cellulase. The optimum reaction conditions of preparing WSC were obtained by an orthogonal test. Cellulase showed optimum depolymerization at pH 4.5 and 60°C, and the enzymatic hydrolysis was endo-action. The different reaction time and the weight ratio of enzyme and substrate gave WSC with different molecular weights. The decrease of molecular weight led to transformation of crystal structure and the increase of solubility in both water and aqueous organic solvents, but the chemical structures of residues were not modified after hydrolyzed by cellulase. And the degree of polymerization of WSC oligomers was mainly from 3 to 13. It is a very simple method to prepare WSC.

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