Synthesis and Characterization of Low Molecular Weight Chitosan

Xue Zheng, Yihui Yin, Wei Jiang, Liyan Xing, and Junwen Pu *

Chitosan can be widely used in many areas owing to its unique properties, although its poor solubility in water is still a limiting factor. In the present study, low molecular weight chitosan (LMWC) was prepared by degradation with NaClO so that chitosan was able to dissolve in water. Chitosan to liquor ratio, NaClO content, temperature, and time were considered variables of NaClO degradation, and the Box-Behnken design was used to determine optimal conditions. There was good agreement between the experimental data and their predicted counterparts. The optimum conditions for chitosan degradation were estimated to be 1:67.91 of chitosan to liquor ratio, 22.03% of NaClO content, a temperature of 90.3 °C, and a time of 3.07 h. It was found that synthesis under these optimized conditions achieved the lowest molecular weight (10,937.4 Daltons). In addition, Fourier transform infrared spectroscopy, X-ray diffractograms, and thermogravimetric analysis showed that the structure of LMWC was similar to the original chitosan, while the crystallinity and thermal stability decreased after degradation.

Keywords: Chitosan; Low molecular weight; Sodium hypochlorite; Box-Behnken design

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INTRODUCTION

Chitin, a linear structure compound of β-(1,4)-linked-N-acetyl-D-glucosamine, is the second most abundant linear polysaccharide next to cellulose; approximately 100 billion tons of chitin are produced annually over the world (Qin *et al.* 2002; Rinaudo 2006). It is renewable and easily obtained, mostly from exoskeletons of crustaceans and arthropods, and from cell walls of fungi, insects, and yeasts (Tolaimate *et al.* 2000; Liu *et al* 2006). Chitosan, a polymer of β-(1,4)-linked-2-amino-D-glucosamine, is the N-deacetylated derivative of chitin. It is the only alkali-cationized polysaccharide in nature, and it has many unique properties such as antibacterial activity, biocompatibility, biodegradability, and nontoxicity. Due to these attributes, it has broad ranges of present and potential applications including biotechnology, drug delivery, cosmetics, agriculture, food science, textiles, *etc.* (Li *et al.* 1992; Remunan-Lopez and Bodmeier 1997; Okamoto *et al.* 2002; Cheng *et al.* 2003; Chung *et al.* 2003).

However, high molecular weight and the crystal structure of chitosan results in low solubility in water and organic solvents, which limits its applications. Therefore, it is important to improve the solubility of chitosan by decreasing the molecular weight. Several methods to prepare a low molecular weight, water soluble chitosan without altering its chemical structure have been tried. Among them, hydrogen peroxide (H_2O_2) is the most commonly used oxidation agent for the degradation of chitosan because it is

easy to handle and readily available (Qin *et al.* 2002; Du *et al.* 2009). The mechanism of H_2O_2 degradation is the formation of reactive hydroxyl radicals through the disassociation of hydrogen peroxide. Hydroxyl radicals can attack the glycosidic linkages of chitosan and subsequently break the original chains. As a result, chitosan can be broken into small molecules (Tian *et al.* 2004). Recently, there have been many studies about the degradation with H_2O_2 under the catalysis of phosphotungstic acid (Huang *et al.* 2007). Moreover, hydrochloric acid and acetic acid have also been employed to degrade chitosan (Rege and Block 1999; Liu *et al.* 2006). Nevertheless, in consideration of industrial production, H_2O_2 is expensive and acid is responsible for severe pollution to the environment; for this reason, looking for a cheap chemical with no pollution to the environment has attracted the authors' attention.

Sodium hypochlorite (NaClO) has been widely used in pulp bleaching on account of its nucleophilic addition reaction with lignin. At the same time, it is also responsible for the oxidative degradation of carbohydrate polymers. Oligosaccharides with different end groups, and even monosaccharides, can be obtained by the oxidization and fractionation of the main chains in cellulose, leading to the decrease in degree of polymerization (DP) and molecular weight. Due to the strong degradation of cellulose by NaClO, a protectant is always needed when bleaching. Given that chitosan has a similar structure to cellulose (Fig. 1), it was hypothesized that the degradation effect of NaClO on cellulose can also be applied to degrade chitosan to acquire low molecular weight chitosan (LMWC). Moreover, there has been no research about degrading chitosan with NaClO alone.

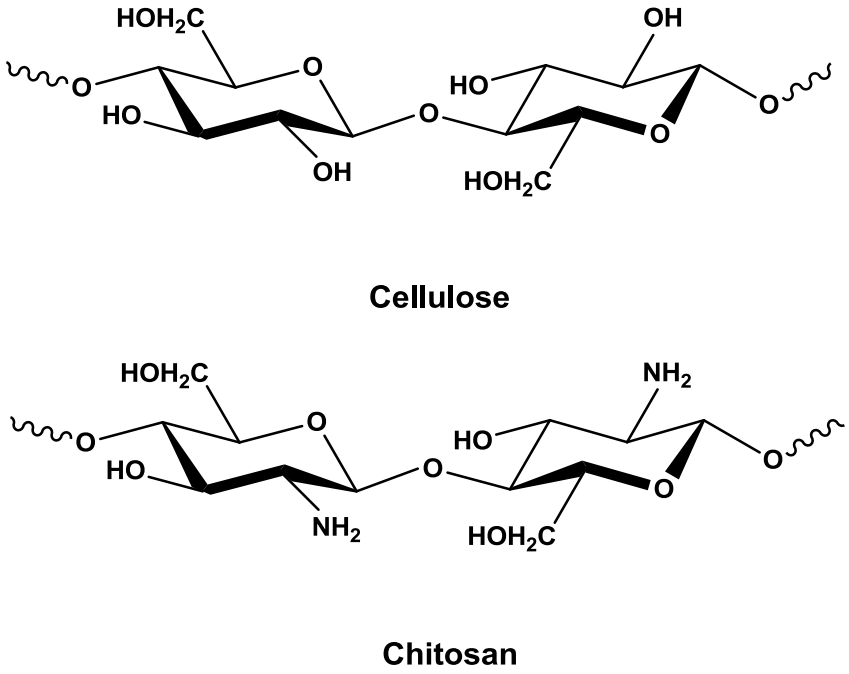

Fig. 1. The basic structure of cellulose and chitosan

Response surface methodology is a statistical method that is useful for mathematical modeling and simultaneously solving multivariate equations (Triveni *et al.* 2001). It can quantify the correlation between the independent input parameters and the dependent response (Aslan and Cebeci 2007). The combination of processing parameters

can be optimized through response surface methodology, and a higher efficiency can be obtained (Yang *et al*. 2008). Therefore, in the present study, NaClO was used for the depolymerization of chitosan. The degradation conditions, including chitosan to liquor ratio, NaClO content, temperature, and time, were optimized by response surface methodology, and the structure of the product was characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffractograms (XRD), and thermogravimetric analysis (TG).

EXPERIMENTAL

Materials

Original chitosan, whose degree of deacetylation is 91% and viscosity-average molecular weight is about 200,000, was obtained from Zhengzhou Ming Chemical Products Co., Ltd (Henan, China). Sodium hypochlorite (NaClO), acetic acid, sodium hydroxide (NaOH), absolute ethanol, sodium chloride (NaCl), and other reagents used were all analytical grade and without further purification.

Degradation of Chitosan

First, 3 g of chitosan was dissolved completely in a certain amount of 2% (v/v) acetic acid solution, and then a certain amount of NaClO was added to the solution. The amounts of 2% acetic acid and NaClO are discussed later. The reaction mixture was reacted at the desired temperature with continuous stirring for a period of time. After the reaction, the resultant mixture was neutralized using a NaOH solution and subsequently precipitated with two volumes of absolute ethanol. The suspension was centrifuged, and the solid was freeze-dried to collect the desired LMWC. During this experiment, chitosan to liquor ratio, NaClO content, reaction temperature, and time were evaluated.

Determination of Molecular Weight

Chitosan molecular weight (viscosity average) was calculated from the classical Mark-Houwink relationship: $[\eta]=k\cdot \mathrm{Mw}^{a}$, where $[\eta]$ is the intrinsic viscosity, M_w is the molecular weight, $k=1.81\times10^{-3}$ cm^3/g, and $a=0.93$ (Roberts 1992). Polymer solutions of known concentrations were prepared in a solvent system consisting of 0.1 M acetic acid and 0.2 M sodium chloride in deionized water. The viscosity measurements were made by recording the efflux times of the solutions in Ubbelohde viscometers (0.5 mm) maintained in a constant-temperature bath at 25 ±1 °C.

Box-Behnken Design

Response surface methodology was employed for experimental design, data analysis, and model building with Design Expert software (Trial Version 8.0.6, Stat-Ease, Inc., USA). A Box-Behnken design with four variables was used to establish the model. Four independent variables were chitosan to liquor ratio from 1:50 to 1:90 g:mL (A), NaClO content from 15 to 25% (B), temperatures from 85 to 95 °C (C), and time 2.5 to 3.5 h (D), with three levels for each variable, while the dependent variable was the molecular weight of chitosan. The symbols and levels are shown in Table 1. Three replicates at the central point of the designed model were used to estimate the pure error sum of squares. The degradation conditions were optimized to obtain the lowest molecular weight of chitosan by this software.

Statistical Analyses

Modeling of variables

For each of the response variables, a second-degree polynomial model was used to fit the data with the equation below (Eq. 1),

$$Y= a_0+a_1A+a_2B+a_3C+a_4D+a_{11}A^2+a_{22}B^2+a_{33}C^2+a_{44}D^2+a_{12}AB+a_{13}AC+a_{14}AD+a_{23}BC +a_{24}BD+a_{34}CD \quad (1)$$

where Y is the predicted response; A, B, C, and D are the variables; a_0 is the constant; a_1, a_2, a_3, and a_4 are the linear effects; a_{11}, a_{22}, a_{33}, and a_{44} are the quadratic coefficients; and a_{12}, a_{13}, a_{14}, a_{23}, a_{24}, and a_{34} are the interaction coefficients.

Statistical significance of the terms in the regression equation was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. In addition, P-value, lack of fit, coefficient of determination (R^2), and adj-R^2 were calculated to check the model adequacy. The above quadratic equation was used to build surfaces for the variables.

Optimization and verification procedures

The degradation conditions were optimized to obtain the lowest molecular weight of chitosan by this software. The LMWC prepared under the optimal conditions and the original chitosan were subjected to characterization.

Characterization of Original Chitosan and LMWC

Fourier Transform Infrared Analysis

FTIR spectra of original chitosan and LMWC were recorded on a Tensor 27 spectrometer (Bruker, Germany) with KBr pellets in the range of 4000 to 400 cm^{-1}.

X-Ray Diffractograms Analysis

Crystallinity of original chitosan and LMWC was measured by X-ray diffraction method using an XRD-6000X diffractometer with Cu Kα X-radiation between 2θ angles of 5° to 40°.

Thermal Analysis

TGA of original chitosan and LMWC were performed using a TGA Q5000 V3.15 Build 263 thermogravimetric analyzer (ShenZhen BoYuan Science and Technology Co., China). The samples weighing around 8 mg were heated from 50 to 500 °C at a heating rate of 10 °C/min under an inert atmosphere of N_2.

RESULTS AND DISCUSSION

Effects of Chitosan to Liquor Ratio, NaClO Content, Temperature, and Time on the Chitosan Molecular Weight

NaClO is a powerful oxidizing agent. It will attack β-(1,4) glucosidic bonds of chitosan to achieve the aim of depolymerization. The degradation mechanism is similar to that of H_2O_2 degradation (Tian *et al*. 2003). The reaction scheme is as follows (Fig. 2):

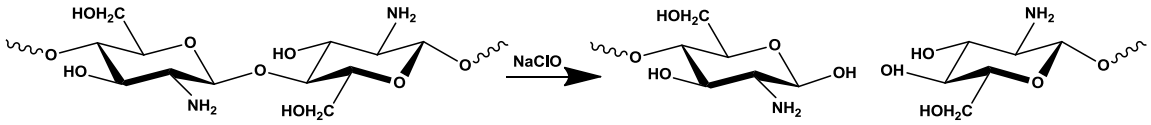

Fig. 2. Reaction scheme of chitosan degradation

The influence of chitosan to liquor ratio, NaClO content, temperature, and time on the molecular weight of chitosan as well as their interactions are shown in Fig. 3. The data were generated through keeping two variables at center values of the testing ranges and varying the other two within the experimental range. From the results obtained, it was obvious that each factor played an apparent role in the molecular weight of chitosan and that the effect direction of four factors was similar. When the variables were kept constant within the range under investigation, the molecular weight firstly decreased with the elevation of the variables, thereafter increasing at a turning point.

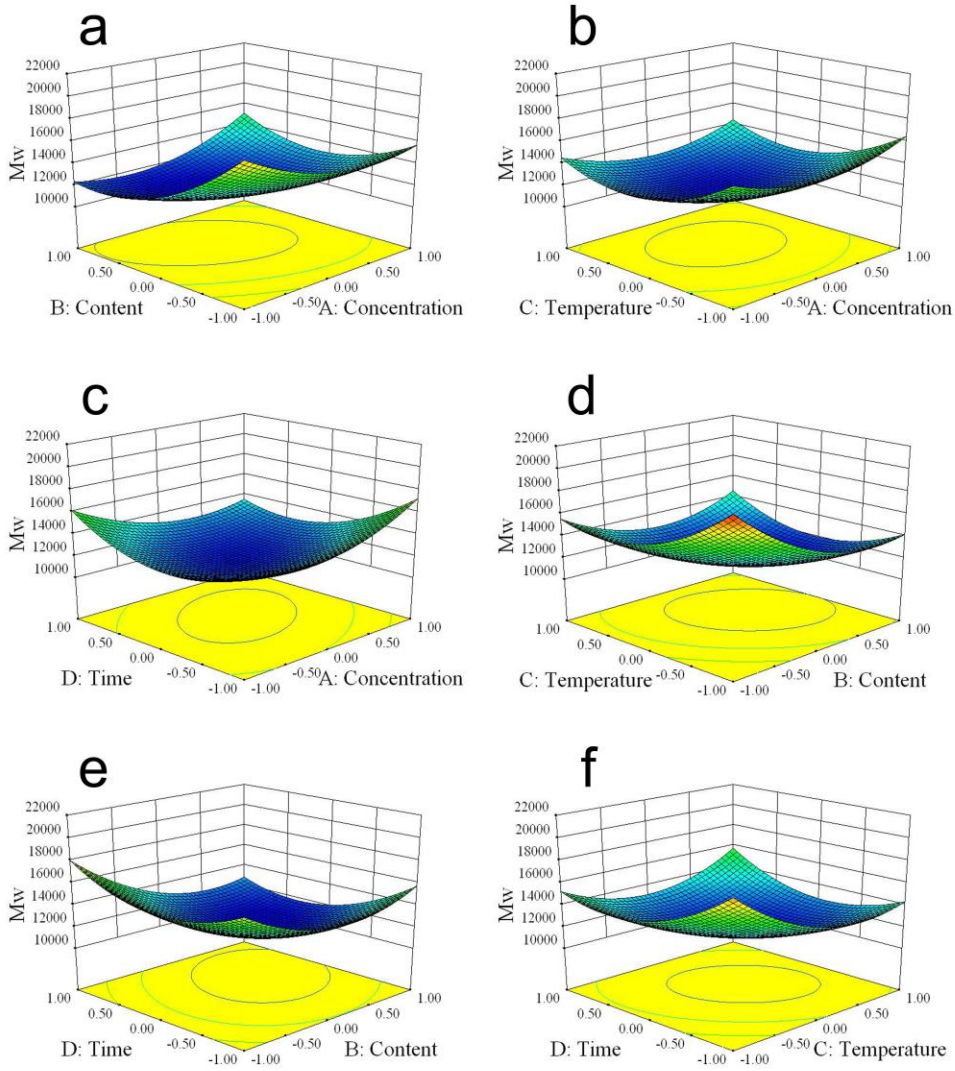

Fig. 3. Response surface plots for molecular weight; other variables are held at medium level

The chitosan to liquor ratio should be well controlled. The amount of acetic acid was not enough for the dissolution of chitosan. Instead, strong acidity resulted in too low of a pH, which inhibited chitosan degradation (Qin *et al.* 2002). Additionally, the protonation of $–NH_2$ restrained the degradation, suggesting that the reduction of molecular weight came from the oxidative cleavage of main chain.

As one can see, although a high NaClO content accelerated the cleavage of glycosidic bonds, adding too much NaClO was not advantageous for the preparation of LMWC because NaClO is a strong alkali, and a greater quantity of NaClO would result in high pH and chitosan precipitation, which was against for the degradation of chitosan.

The data also clearly demonstrated that moderate temperature could improve the chitosan degradation, which increased the deprotonation of $–NH_3^+$ and the concentration of oxidizing species. Higher temperature, on the contrary, could not promote the degradation of chitosan. This may be because when the reaction temperature was too high, the depolymerization of chitosan became rapid and severe. Some chitosan was depolymerized to the oligosaccharide with aldehyde side groups, and this oligosaccharide can react with unreacted chitosan or ethanol to produce a more complicated by-product. So the reaction temperature should not be too high during the depolymerization.

Appropriately extending the time of the reaction contributed to the synthesis of LMWC. However, if the time was too long, the solution would become a strongly acidic system when NaClO was completely consumed; for the same reason of protonation, the degradation of chitosan was restrained.

Model Fitting and Optimization

The mathematical model representing the molecular weight of chitosan as a function of the independent variables within the region under investigation was expressed by the following equation (Eq. 2),

$$Y=11345.15+12.91A-1663.01B-969.71C-512.04D+1734.15A^2+2305.90B^2+2377.11C^2 +2286.62D^2+1368.57AB-66.23AC-1349.08AD+1164.97BC-954.21BD+1218.60CD \quad (2)$$

where Y is the molecular weight of chitosan; A, B, C, and D are the coded variables of chitosan to liquor ratio, NaClO content, temperature, and time, respectively.

Table 1 shows the experimental conditions of the Box-Behnken design (BBD) along with the corresponding values observed for the four responses studied. Experimental data was fitted to the quadratic model by ANOVA. The ANOVA for the four responses is shown in Table 2.

Exploration and optimization of a fitted model may produce misleading results unless the model exhibits a good fitness. So checking the model adequacy is essential (Wang *et al.* 2008). The model P-value of the chitosan molecular weight was <0.0001, which indicated that the model fitness was significant, since a P-value less than 0.0500 indicated that the model term was perfect. In this case B, C, D, AB, AD, BC, BD, CD, A^2, B^2, C^2, and D^2 were significant model terms. The "lack of fit" test, which measured the fitness of the model, did not result in a significant F-value (0.0678), meaning that the model was sufficiently accurate for predicting the molecular weight of chitosan. The coefficient of determination (R^2) is another important index for measurement of the degree of fitness (Nath and Chattopadhyay 2007). The small value of R^2 indicated the poor relevance of the independent variables in the model.

Table 1. Box-Behnken Design and the Response for Chitosan Molecular Weight

Trial	Coded levels				Molecular weight
	A	B	C	D	
	Chitosan to liquor ratio (g : mL)	NaClO content (%)	Temperature (°C)	Time (h)	
1	1 (1:90)	0 (20)	1 (95)	0 (3.0)	14257.55
2	0 (1:70)	-1 (15)	0 (90)	1 (3.5)	16972.59
3	-1 (1:50)	0 (20)	0 (90)	-1 (2.5)	14306.11
4	0 (1:70)	1 (25)	-1 (85)	0 (3.0)	13502.11
5	-1 (1:50)	0 (20)	1 (95)	0 (3.0)	13873.12
6	0 (1:70)	1 (25)	0 (90)	-1 (2.5)	16162.22
7	1 (1:90)	0 (20)	0 (90)	-1 (2.5)	16522.43
8	0 (1:70)	-1 (15)	-1 (85)	0 (3.0)	20408.45
9	0 (1:70)	0 (20)	1 (95)	-1 (2.5)	14895.71
10	0 (1:70)	1 (25)	0 (90)	1 (3.5)	12576.95
11	-1 (1:50)	-1 (15)	0 (90)	0 (3.0)	18561.27
12	0 (1:70)	0 (20)	-1 (85)	-1 (2.5)	19069.68
13	0 (1:70)	-1 (15)	1 (95)	0 (3.0)	16130.25
14	0 (1:70)	0 (20)	-1 (85)	1 (3.5)	15427.85
15	-1 (1:50)	0 (20)	-1 (85)	0 (3.0)	15873.86
16	0 (1:70)	1 (25)	1 (95)	0 (3.0)	13883.77
17	1 (1:90)	1 (25)	0 (90)	0 (3.0)	15689.28
18	1 (1:90)	0 (20)	-1 (85)	0 (3.0)	16523.21
19	-1 (1:50)	1 (25)	0 (90)	0 (3.0)	12909.72
20	0 (1:70)	0 (20)	1 (95)	1 (3.5)	16128.28
21	0 (1:70)	-1 (15)	0 (90)	-1 (2.5)	16741.00
22	-1 (1:50)	0 (20)	0 (90)	1 (3.5)	16813.52
23	1 (1:90)	-1 (15)	0 (90)	0 (3.0)	15866.56
24	1 (1:90)	0 (20)	0 (90)	1 (3.5)	13633.53
25	0 (1:70)	0 (20)	0 (90)	0 (3.0)	11133.39
26	0 (1:70)	0 (20)	0 (90)	0 (3.0)	11352.57
27	0 (1:70)	0 (20)	0 (90)	0 (3.0)	11549.49

Table 2. Results of ANOVA for Molecular Weight

Source	Sum of squares	Df	Mean square	*F*-value	*P*-value	
Model	1.300E+08	14	9.289E+06	17.93	< 0.0001	significant
A	2000.90	1	2.001E+03	3.861E-03	0.9515	
B	3.319E+07	1	3.319E+07	64.04	< 0.0001	
C	1.128E+07	1	1.128E+07	21.78	0.0005	
D	3.146E+06	1	3.146E+06	6.07	0.0298	
AB	7.492E+06	1	7.492E+06	14.46	0.0025	
AC	17546.20	1	1.755E+04	0.03	0.8571	
AD	7.280E+06	1	7.280E+06	14.05	0.0028	
BC	5.429E+06	1	5.429E+06	10.48	0.0071	
BD	3.642E+06	1	3.642E+06	7.03	0.0211	
CD	5.940E+06	1	5.940E+06	11.46	0.0054	
A^2	1.604E+07	1	1.604E+07	30.95	0.0001	
B^2	2.836E+07	1	2.836E+07	54.73	< 0.0001	
C^2	3.014E+07	1	3.014E+07	58.16	< 0.0001	
D^2	2.789E+07	1	2.789E+07	53.81	< 0.0001	
Residual	6.218E+06	12	5.182E+05			
Lack of Fit	6.132E+06	10	6.132E+05	14.15	0.0678	not significant
Pure Error	86654.93	2	4.333E+04			
Cor Total	1.363E+08	26				

The model was able to fit well with the actual data when R^2 approached unity (Sin *et al.* 2006). By analysis of variance, the R^2 value of the model was determined to be 0.9544, which showed that the regression model defined the true behavior of the system well. The values of the adjusted determination coefficients (adj-R^2=0.9011) also confirmed that the model was highly significant.

The optimal conditions to obtain the lowest molecular weight of chitosan were predicted by a computer program to be 1:67.91 of chitosan to liquor ratio, 22.03% of NaClO content, a temperature of 90.3 °C, and 3.07 h of time. The predicted molecular weight was 10937.4.

These new prediction conditions were submitted to the same experimental procedures applied as those from the beginning of this study. The molecular weight of chitosan was determined to be 11,260.26. There was no significant difference between the estimated and observed values (P<0.05), suggesting a good fit between the models and the experimental data.

FTIR Analysis of Original Chitosan and LMWC

FTIR spectroscopy has been shown to be a powerful tool for the study of the physicochemical properties of polysaccharides. Curves (a) and (b) in Fig. 4 show the IR spectra of original chitosan and LMWC produced under the optimum reaction conditions.

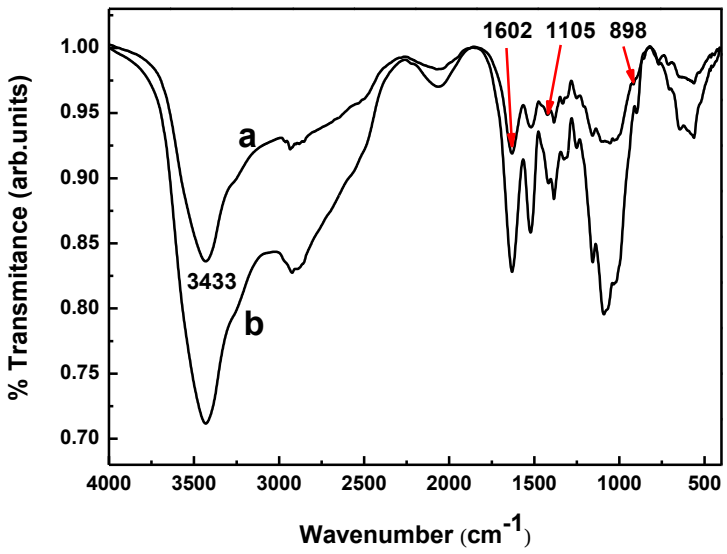

Fig. 4. FTIR spectra of (a) original chitosan and (b) LMWC

The main bands in the spectrum of original chitosan are as follows: both the N-H and O-H stretching vibrations could be characterized by a strong and broad band centered at 3433 cm^{-1}; the peak at 1602 cm^{-1} was assigned to the characteristic N-H absorption of NH_2; the peak at 1105 cm^{-1} was due to the C-O-C stretching vibration in glucose circle; and the band at 898 cm^{-1} corresponded to the β-(1,4) glucoside bond in chitosan.

In comparison with the FTIR spectrum of original chitosan, that of LMWC showed a similar spectrum. It can be seen that no band was observed between 1650 and 1900 cm^{-1}, which allowed us to conclude that oxidative groups such as carboxylic,

aldehyde, or carbonyl groups did not exist in LMWC. The results verified the rupture of β-(1,4) glucoside bonds in macromolecule to be the basic process during amino groups of chitosan protection by acids without resulting in the ring-opening oxidation of glucosamine repeating units (Shao *et al*. 2003).

XRD Analysis of Original Chitosan and LMWC

The X-ray diffractograms of original chitosan and LMWC are exhibited in Fig. 5. The strongest reflection of original chitosan appeared at 2θ=20.0°, which is assigned to (100) reflection (Tian *et al*. 2004). However, the diffraction angle's intensity of LMWC was weakened obviously. The crystallinity of original chitosan was 88.6%, and that of LMWC was reduced to 79.8%. As a result, the solubility of chitosan in water was improved. It was assumed that the degradation first took place preferentially in the amorphous region and then proceeded very moderately from the edge to the inside of the crystalline zones. That was to say, the chitosan in amorphous regions was the first to be degraded to water-soluble molecules, which were then promptly soluble in the water. With deeper degradation, the crystalline structure was destroyed and the crystallinity decreased.

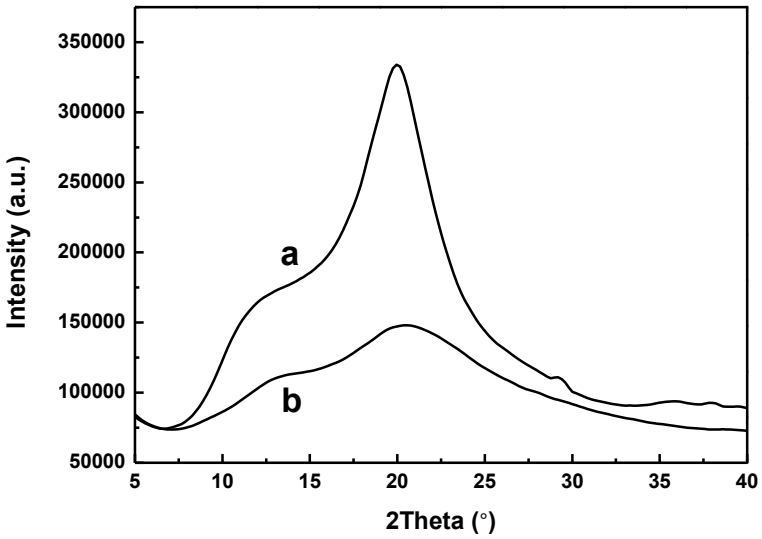

Fig. 5. XRD patterns of (a) original chitosan and (b) LMWC

TG Analysis of Original Chitosan and LMWC

As can be seen from Fig. 6, the TG curves of original chitosan and LMWC were similar, indicating they corresponded to the same thermal decomposition process. Both processes can be divided into three parts. For original chitosan, the first part was from 50 to 210 °C, in which water and crystal water were lost and the weight loss ratio was 13.71%. The second part was from 210 to 386 °C, in which the decomposition, oxidation, and combustion of chitosan occurred. The weight loss ratio was up to 42.26%. It can be found clearly from the figure that the greatest weight loss point in this part was at 230 °C. After that, the curve of chitosan was still on a slow decline, which meant that chitosan had not been decomposed completely.

Compared with original chitosan, the LWMC showed little difference in the thermal decomposition temperature. LMWC started to decompose from 202 °C, which

was lower than chitosan. Reasons for this could be the differences in their structures and molecular weight. Before degradation, chitosan needed to absorb numerous heats to break down the hydrogen bonds existing in the molecules. However, the degradation with NaClO led to the disintegration of intramolecular and intermolecular interaction and led to partial breaking of the molecular structure. As a result, the crystallinity and thermo stability decreased.

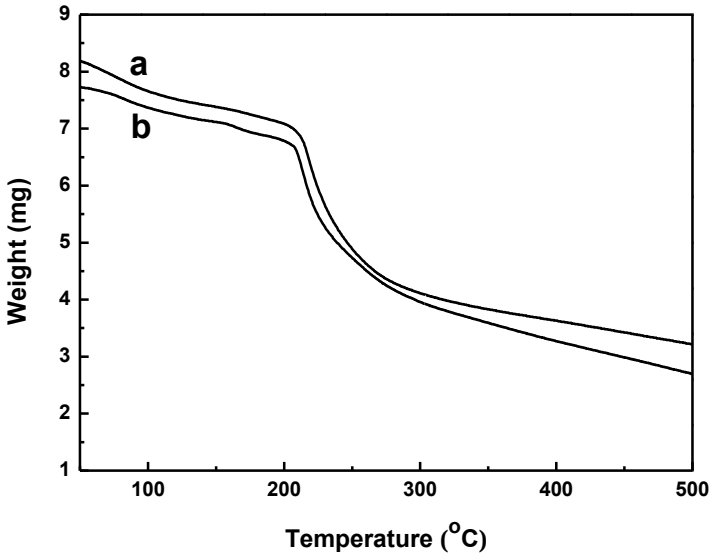

Fig. 6. TG curves of (a) LMWC and (b) original chitosan

CONCLUSIONS

1. From the results obtained, NaClO showed good potential for degrading the original chitosan into LMWC. A mathematical model between degradation conditions and molecular weight was built with good fitness through a Box-Behnken design.
2. Chitosan to liquor ratio, NaClO content, reaction temperature, and time were considered variables of NaClO degradation. Each factor showed a significant effect on the molecular weight. As a consequence, the optimal conditions to obtain the lowest molecular weight of chitosan were 1:67.91 for the chitosan to liquor ratio, 22.03% NaClO content, a temperature of 90.3 °C, and 3.07 h of time by means of response surface methodology. The predicted molecular weight was 10937.4.
3. The characterization of the original chitosan and LMWC under the optimum conditions showed that there was no change in chemical structure, but the crystallinity and thermal stability decreased after degradation.

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