

## Dissolution and Stability of Chitosan in a Sodium Hydroxide/Urea Aqueous Solution

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**ABSTRACT:** Medium-molecular-weight chitosan (MMWC; 157.5 kDa) and low-molecular-weight chitosan (LMWC; 53.4 kDa) samples were dissolved in an NaOH/urea solution by freeze–thaw treatment. The factors affecting dissolution were optimized, and the stability of chitosan in the produced solution was investigated. NaOH and urea concentrations of 2 and 0.67 mol/L, respectively, and a treatment temperature of  $-18^{\circ}\text{C}$  were optimized as the dissolving conditions. MMWC and LMWC could be completely dissolved in the 2 mol/L NaOH/0.67 mol/L urea solution after six and five cycles of freeze–thaw treatments, respectively. Dissolution and storage in the 2 mol/L NaOH/0.67 mol/L urea solution slightly increased the deacetylation degree of chitosan and slightly decreased the molecular weight. The solution stability of LMWC was better than that of MMWC. MMWC tended to form a gel during storage. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2014**, *131*, 39819.

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### INTRODUCTION

Chitosan, is a cationic polysaccharide, obtained by the alkaline partial deacetylation of chitin, which originates from shells of crustaceans.<sup>1</sup> Because of its unique polycationic nature, chitosan has been proposed for applications in the food, pharmaceutical, and chemical industries.<sup>2–4</sup>

Chitosan is a semicrystalline material with numerous intermolecular and intramolecular hydrogen bonds; this makes it difficult to dissolve in neutral or alkali aqueous solution.<sup>5</sup> Acids are able to protonize the amino groups of chitosan and reduce the hydrogen bonding between chitosan molecules; this leads to the dissolution of chitosan. However, the dependence of dissolution on acid to some extent limits the widespread application and development of chitosan.

In recent years, novel alkali solvents containing urea or thiourea have been developed to dissolve macromolecules. Cai and Zhang<sup>6</sup> found that at low temperature, cellulose could be dissolved in LiOH/urea and NaOH/urea solutions, and the dissolving power of the former solvent was better. Qi et al.<sup>7</sup> found that the solubility of cellulose in an NaOH/urea system varied with the molecular weight and temperature. Chen et al.<sup>8</sup> extracted a water-insoluble polysaccharide from sclerotium of *Poria cocos*

and found that this polysaccharide existed as a flexible chain in a 0.5M NaOH/0.2M urea solution. Hu et al.,<sup>9</sup> Li et al.,<sup>10</sup> and Chang et al.<sup>11</sup> all demonstrated that NaOH/urea aqueous solutions could be used as solvents for chitin.

Until now, there have been only a few studies reporting the dissolution and properties of chitosan in urea- or thiourea-containing alkali solvents. Fan and coworkers<sup>12,13</sup> successfully dissolved chitosan in an LiOH/urea solution. Almeida et al.<sup>14</sup> indicated that chitosan could also be dissolved in an NaOH/thiourea aqueous solution, but the dissolution process would result in chain depolymerization. In this study, the NaOH/urea solution was tested to dissolve chitosan, and the stability of chitosan in the NaOH/urea solution was also studied.

### EXPERIMENTAL

#### Materials

Medium-molecular-weight chitosan (MMWC) and low-molecular-weight chitosan (LMWC) samples were prepared by the degradation method with hydrogen peroxide in our laboratory. The viscosity-average molecular weights ( $M_v$ ) of these two samples were 157.5 and 53.4 kDa, respectively, as determined by the method of Roberts and Domszy,<sup>15</sup> and their degrees of deacetylation (DDs) were 88.4 and 88.6%, as determined by the

method of Zhou et al.<sup>16</sup> Sodium hydroxide, sodium chloride, acetic acid, and urea (analytical purity) were supplied by Sino-pharm Chemical Reagent Co., Ltd. (Shanghai, China).

#### Dissolution of Chitosan in an NaOH/Urea Aqueous Solution

Chitosan powder (1.00 g) was dispersed in 100 mL of an NaOH/urea aqueous solution, and the resulting suspension was cooled to a designated temperature. After 12 h of treatment, the mixture was thawed at 25°C. After several cycles of freeze–thaw treatments, a chitosan solution was obtained and was stored at 25°C for further analysis.

The solubility of chitosan in the NaOH/urea aqueous solution after freeze–thaw treatment was determined by the procedure described by Hu et al.<sup>9</sup> with slight modification. When the ice in the thawed solution disappeared, the chitosan solution was immediately centrifuged at 3500 g for 20 min. To determine the amounts of dissolved chitosan, the insoluble chitosan was first washed with NaOH/urea aqueous solution and subsequently with water and then dried in a vacuum oven and weighed. The solubility ( $S$ ) was calculated with the following formula:

$$S = (1 - W_{is}) \times 100\% \quad (1)$$

where  $W_{is}$  is the weight of insoluble chitosan. All experiments were done in triplicate.

#### Microscopic Observation

The state of the chitosan particles in the NaOH/urea aqueous solution was observed with an Olympus BX51/BX52 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) at a magnification of 40 $\times$ .

#### Determination of the Molecular Weight and DD

The molecular weight of chitosan was determined with an Ubbelohde viscometer. The intrinsic viscosity ( $[\eta]$ ) of the chitosan samples was measured in a 0.2 mol/L NaCl/0.1 mol/L CH<sub>3</sub>COOH solution at 25°C.  $M_v$  was calculated with the Mark–Houwink equation:

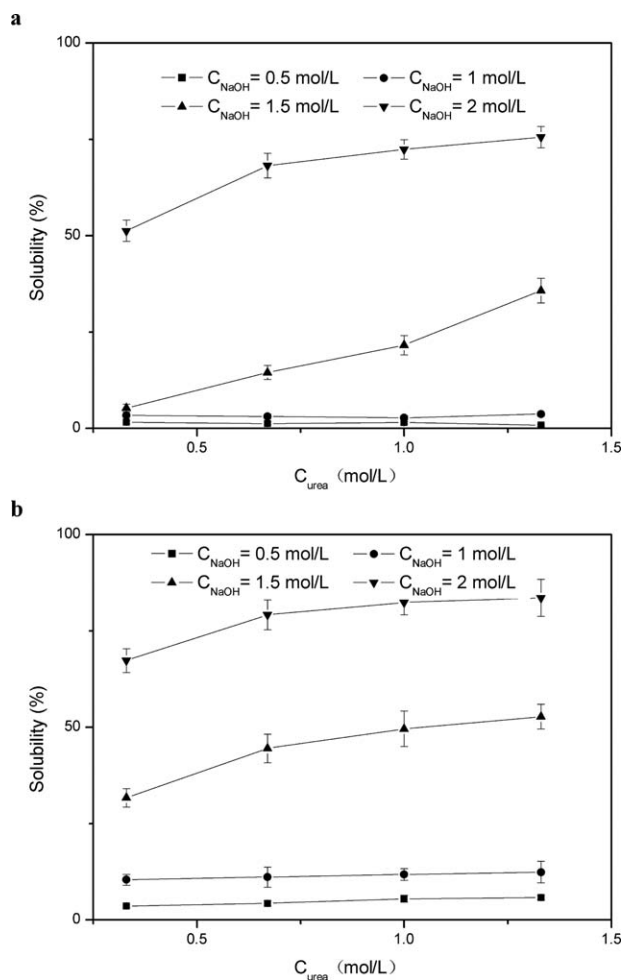
$$[\eta] = k(M_v)^\alpha \quad (2)$$

where the constants  $k$  and  $\alpha$  were  $1.81 \times 10^{-3}$  cm<sup>3</sup>/g and 0.93, respectively.<sup>15</sup> All of the experiments were done in triplicate.

The DD of chitosan was determined by the method of potentiometric titration, as described by Zhou et al.<sup>16</sup> The chitosan sample (0.2 g) was dissolved in 20 mL of 0.1 mol/L HCl, and excess HCl was back-titrated with a 0.1 mol/L NaOH solution with a DELTA-320-S pH meter (Mettler-Toledo, Halstead, United Kingdom). All of the experiments were done in triplicate. The differential and integral titration curves were drawn between the solution pH and the volume of alkali added; this produced an integral curve with two inflexions. The DD was calculated with the following equation:

$$DD\% = \frac{\Delta V \times C_{\text{NaOH}} \times 10^{-3} \times 16}{W \times 0.0994} \times 100\% \quad (3)$$

where  $\Delta V$  is the volume of NaOH between two inflexion points,  $C_{\text{NaOH}}$  is the concentration of the NaOH solution,  $W$  is the weight of the chitosan sample, 16 g/mol is the molecular weight



**Figure 1.** Effect of the solvent composition on the solubility of chitosan (freeze–thaw cycles = 4, freeze temperature =  $-18^{\circ}\text{C}$ ): (a) MMWC and (b) LMWC.

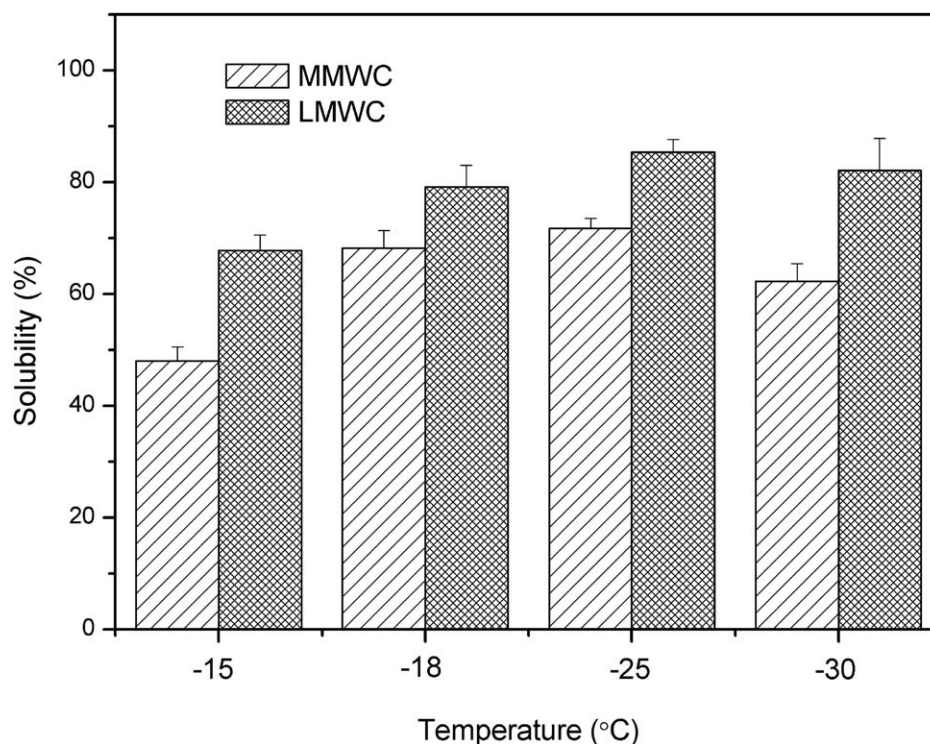
of the amino group, and 0.0994 is the theoretical weight fraction of the amino groups in chitosan.

#### Rheological Properties

Rheological measurements were performed on an AR1000 instrument (TA Instruments, New Castle, DE) equipped with a parallel-plate measuring system (40 mm in diameter).

**Steady-Shear Rate Test.** The apparent viscosities of these samples were determined rheometrically at 25°C at shear rates of 1–100 s<sup>-1</sup>.

**Temperature Sweep Measurement.** Samples were loaded into the space between parallel plates immediately after incubation, and the exposed rim was covered with mineral oil to prevent evaporation during the measurements. Oscillatory measurements were performed at 1 rad/s, and the temperature was increased at 1°C/min between 25 and 75°C. Oscillatory measurements were made at a fixed frequency of 1 rad/s and a strain amplitude of 10%, which was within the linear viscoelastic region as determined in preliminary tests. Changes in the storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were recorded throughout heating.



**Figure 2.** Effect of the treatment temperature on the solubility of chitosan in a 2 mol/L NaOH/0.67 mol/L urea aqueous solution (freeze–thaw cycles = 4).

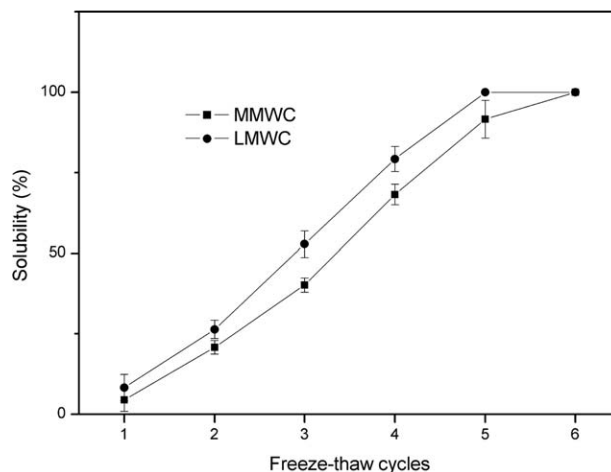
## RESULTS AND DISCUSSION

### Factors Affecting the Dissolution of Chitosan in an NaOH/Urea Aqueous Solution

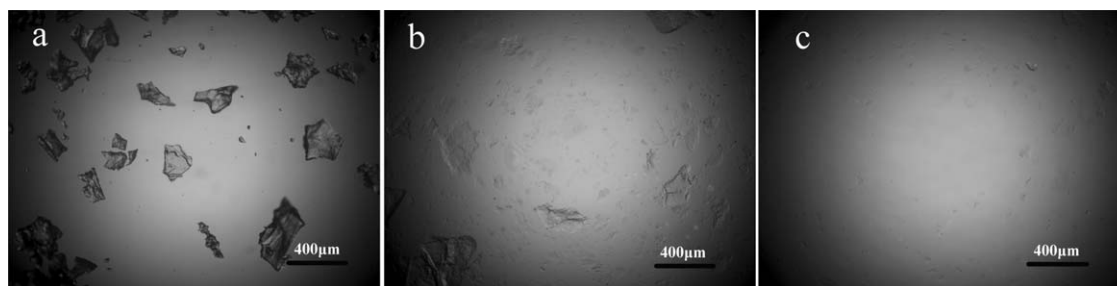
**Solvent Composition.** Sodium hydroxide/urea aqueous solutions have been used to dissolve biomacromolecules, with their composition and the procedure used affecting their solubility.<sup>9,17</sup> In this study, we chose a freeze–thaw treatment to destroy the particle structure and allow the solvent to penetrate to the inner part of the particles. The solubility of chitosan MMWC after four cycles of freeze–thaw treatments was determined to select the appropriate solvent composition for further experiments. As Figure 1 shows, at the four chosen urea concentrations ( $C_{\text{urea}}$ 's) from 0.33 to 1.33 mol/L, the solubilities of MMWC and LMWC were extremely low when  $C_{\text{NaOH}}$  was less than 1 mol/L but their solubilities increased dramatically when  $C_{\text{NaOH}}$  reached 2 mol/L. This result was consistent with those of Cai and Zhang<sup>6</sup> and Hu et al.,<sup>9</sup> whose research materials were cellulose and chitin, respectively. When  $C_{\text{NaOH}}$  was 2 mol/L, the solubility of chitosan was increased with increasing  $C_{\text{urea}}$  but showed no sign of a great increase after  $C_{\text{urea}}$  exceeded 0.67 mol/L. Thus,  $C_{\text{NaOH}}$  and  $C_{\text{urea}}$  were fixed at 2 and 0.67 mol/L, respectively.

**Treatment Temperature.** The solubilities of the two chitosan samples in 2 mol/L NaOH/0.67 mol/L urea solution at different treatment temperatures are shown in Figure 2. As the treatment temperature decreased from  $-15$  to  $-18$  or  $-25$ °C, the solubilities of MMWC and LMWC greatly increased. This phenomenon might have been related to ice crystal formation, as was suggested by Qi et al.<sup>7</sup> and Hu et al.<sup>9</sup> in their articles on chitin

and cellulose. The freezing point of the 2 mol/L NaOH/0.67 mol/L urea solution was around  $-18$ °C.<sup>9</sup> At high treatment temperatures, such as at  $-15$ °C, water molecules did not freeze and expand, and when the temperature decreased to the freezing point or below ( $-18$  and  $-25$ °C), the expansion of the formed ice was supposed to enhance the dissolution of chitosan. When the treatment temperature decreased from  $-25$  to  $-30$ °C, both chitosan samples showed a slight decrease in the solubility. As Hu et al.<sup>9</sup> indicated, when the treatment temperature was far below freezing point, the freezing and expansion



**Figure 3.** Effect of the freeze–thaw cycles on the solubility of chitosan in a 2 mol/L NaOH/0.67 mol/L urea aqueous solution (freeze temperature =  $-18$ °C).



**Figure 4.** Microscopic images of chitosan (MMWC) for different freeze–thaw cycles in a 2 mol/L NaOH/0.67 mol/L urea aqueous solution (freeze temperature =  $-18^{\circ}\text{C}$ ): (a) 0, (b) 2, and (c) 4 cycles.

**Table I.**  $M_v$  and DD Values of Chitosan after Dissolution and Storage in a 2 mol/L NaOH/0.67 mol/L Urea Aqueous Solution

Sample	Parameter	Original sample	Storage time after dissolution			
			Fresh solution	6 h	12 h	18 h
MMWC	DD (%)	$88.4 \pm 0.1$	$90.4 \pm 0.2$	$91.3 \pm 0.3$	$91.6 \pm 0.2$	$91.9 \pm 0.4$
	$M_v$ (kDa)	$157.5 \pm 6.9$	$150.3 \pm 5.7$	$148.6 \pm 4.2$	$143.1 \pm 6.8$	$140.3 \pm 5.8$
LMWC	DD (%)	$88.6 \pm 0.4$	$91.2 \pm 0.1$	$91.7 \pm 0.2$	$92.1 \pm 0.3$	$92.5 \pm 0.2$
	$M_v$ (kDa)	$53.4 \pm 1.3$	$52.8 \pm 1.5$	$52.3 \pm 1.8$	$51.5 \pm 2.1$	$50.8 \pm 1.6$

process was shorter, and the expanding effect became weaker compared with that at the freezing point or near temperature, which reduced the solubility of the material. The data in Figure 2 suggest that  $-18$  and  $-25^{\circ}\text{C}$  were good treatment temperatures for dissolving chitosan. From an economic standpoint,  $-18^{\circ}\text{C}$  was chosen as the treatment temperature for further study. Figure 2 also shows that the solubility of LMWC was higher than that of MMWC at the same treatment temperatures. This result suggested that the solubility of chitosan was influenced by its molecular weight. As estimated from the molecular weight, the degree of polymerization of MMWC was approximately 980, which was much higher than that of LMWC, which had a degree of polymerization value of about 330. Thus, the different solubilities of MMWC and LMWC in the NaOH/urea solution might have been due to the different entanglements of the chitosan chains.

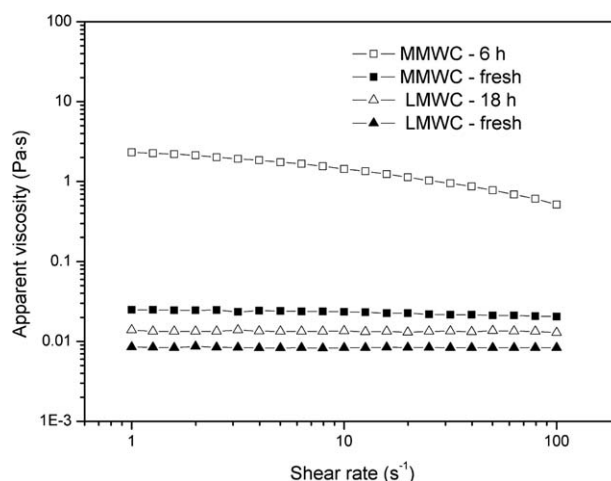
**Freeze–Thaw Cycles.** The number of freeze–thaw cycles was another important factor influencing the solubility of chitosan (Figure 3). As Figure 3 shows, the solubilities of MMWC and LMWC increased with increasing number of freeze–thaw cycles. For the same number of freeze–thaw treatment cycles, the solubility of LMWC was higher than that of MMWC. MMWC and LMWC were completely dissolved in the solvent after six and five freeze–thaw cycles, respectively. This result also indicated that chitosan with short chains was easier to dissolve in the NaOH/urea solution.

The dissolution states of chitosan for different numbers of freeze–thaw cycles are shown in Figure 4. As the number of freeze–thaw treatment cycles increased, large particles gradually dissociated into small pieces. The images were in accordance with the solubility data in Figure 3, with both demonstrating

that the solubility of chitosan increased with increasing freeze–thaw cycles.

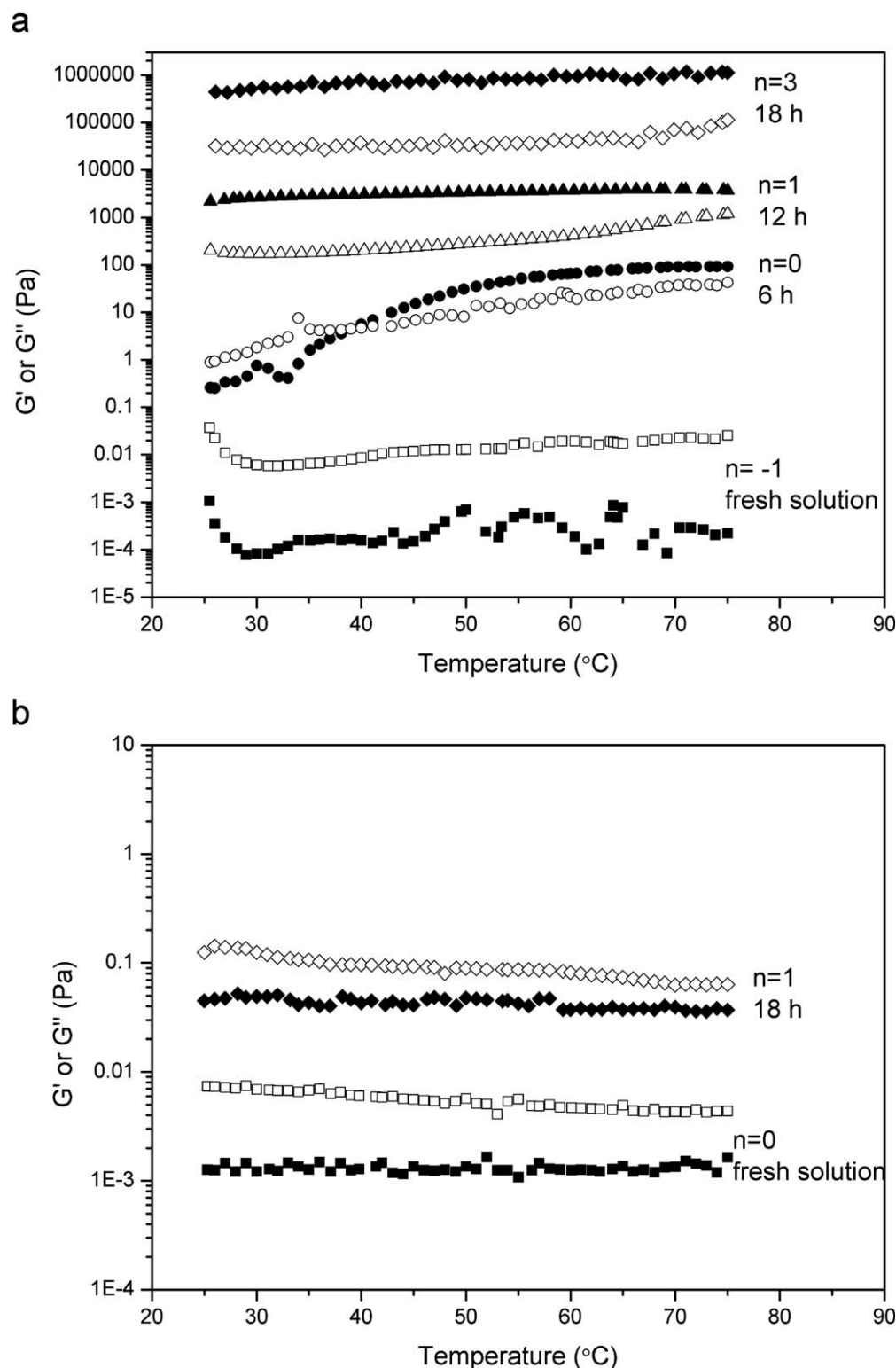
#### Molecular Stability of Chitosan in the NaOH/Urea Aqueous Solution

The DD and  $M_v$  of chitosan are shown in Table I. After complete dissolution in the 2 mol/L NaOH/0.67 mol/L urea aqueous solution, both MMWC and LMWC showed a slight increase in DD. This result was in agreement with the findings of Hu et al.,<sup>9</sup> who did similar research on chitin. Actually, NaOH is a common reagent for the alkaline deacetylation of chitin in the preparation of chitosan.<sup>18,19</sup> The  $M_v$  of the two chitosan samples did not show great change after dissolution and storage in the NaOH/urea aqueous solution; this indicated that the stability of chitosan molecular chains was excellent, with only slight chain degradation during short-term storage.



**Figure 5.** Dependence of the apparent viscosity on the shear rate for chitosan in a 2 mol/L NaOH/0.67 mol/L urea aqueous solution.





**Figure 6.** Temperature dependence of  $G'$  and  $G''$  of chitosan in a 2 mol/L NaOH/0.67 mol/L urea aqueous solution: (a) MMWC and (b) LMWC. The black and white symbols denote  $G'$  and  $G''$ , respectively. The data were shifted along vertical axes by  $10^n$  with a given value to avoid overlapping.

#### Solution Stability of a Chitosan/NaOH/Urea Aqueous Solution

**Steady-Shear Flow Behavior.** The apparent viscosities of chitosan in the 2 mol/L NaOH/0.67 mol/L urea aqueous solution at

different shear rates are shown in Figure 5. As Figure 5 shows, the apparent viscosities of the freshly prepared MMWC and LMWC solutions remained constant as the shear rate increased and showed the behavior of Newtonian fluids. After storage for

6 h, the apparent viscosity of MMWC was greatly increased, and the stored solutions showed shear-thinning characteristics, which indicated pseudoplastic fluids. As the storage time was extended to increase to 12 or 18 h, the MMWC solution lost its flow characteristics and formed a gel. In contrast to that of MMWC, the flow state of the LMWC solution was retained after storage. After 18 h of storage, the viscosities of LMWC were only slightly increased, and its Newtonian fluid characteristics did not change. The different changes in the LMWC and MMWC solutions in flow behavior after storage might have been due to their different chain lengths because the interactions between short chains, such as hydrogen bonding and chain entanglements, might have been weaker compared with those of long chains.

**Gelation Properties.** The study of the gelation properties of macromolecules in the NaOH/urea system (or similar solution systems) has been attracting more attention because it is of considerable academic and industrial importance. Hu et al.<sup>20</sup> found that a chitin/NaOH/urea solution was sensitive to temperature and would transform to a gel when temperature increased to physiological conditions. Cai and Zhang<sup>21</sup> found that the sol–gel transition temperature of cellulose/NaOH/urea solution decreased as molecular weight of cellulose increased. The temperature sweep measurements were performed to determine the gelation temperature of chitosan solution, which might reflect the gelation trend of chitosan solution. As Figure 6(a) shows, for the fresh MMWC solution,  $G'$  was higher than  $G''$  during the entire heating process. However, after 6 h of storage, the MMWC solution showed a sol–gel transition at a temperature around 38.5°C, where  $G''$  reached and exceeded  $G'$ . When the storage time was increased to 12 or 18 h, the MMWC solution was completely transformed to a gel. These results indicate that the molecular chains of MMWC in solution easily formed a network, which was incongruent with the results of the apparent viscosity. The freshly prepared LMWC solution and solutions stored for 18 h did show a sol–gel transition during the entire heating course [Figure 6(b)]; this indicated that the LMWC solution was more stable than MMWC under a storage environment. The superior stability of the LMWC solution over that of the MMWC solution might have been due to its short chain length, which might have made it harder for LMWC to form a network structure.

## CONCLUSIONS

The dissolution and stability of different MMWC (157.5 kDa) and LMWC (53.4 kDa) samples in an NaOH/urea aqueous solution were studied. The 2 mol/L NaOH/0.67 mol/L urea aqueous solution and a treatment temperature of  $-18^{\circ}\text{C}$  were considered the optimum dissolving conditions. MMWC and LMWC could be completely dissolved in the 2 mol/L NaOH/0.67 mol/L urea aqueous solution after six and five cycles of freeze–thaw treatments, respectively. During dissolution and storage in the 2 mol/L NaOH/0.67 mol/L urea solution, chitosan showed a slight increase in DD and a slight increase in  $M_v$ . The LMWC solution showed better solution stability compared with MMWC, and the MMWC solution gelled during storage.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Galed, G.; Miralles, B.; Panos, I.; Santiago, A.; Heras, A. *Carbohydr. Polym.* **2005**, *62*, 316.
2. Elsabee, M. Z.; Abdou, E. S. *Mater. Sci. Eng. C* **2013**, *33*, 1819.
3. Hu, L.; Sun, Y.; Wu, Y. *Nanoscale* **2013**, *5*, 3103.
4. Ngah, W. S. W.; Teong, L. C.; Hanafiah, M. A. K. M. *Carbohydr. Polym.* **2011**, *83*, 1446.
5. Pillai, C. K. S.; Paul, W.; Sharma, C. P. *Prog. Polym. Sci.* **2009**, *34*, 641.
6. Cai, J.; Zhang, L. *Macromol. Biosci.* **2005**, *5*, 539.
7. Qi, H. S.; Chang, C. Y.; Zhang, L. N. *Cellulose* **2008**, *15*, 779.
8. Chen, X. Y.; Xu, X. J.; Zhang, L.; Kennedy, J. F. *Carbohydr. Polym.* **2009**, *75*, 586.
9. Hu, X. W.; Du, Y. M.; Tang, Y. F.; Wang, Q.; Feng, T.; Yang, J. H.; Kennedy, J. F. *Carbohydr. Polym.* **2007**, *70*, 451.
10. Li, G.; Du, Y.; Tao, Y.; Liu, Y.; Li, S.; Hu, X.; Yang, J. *Carbohydr. Polym.* **2010**, *80*, 970.
11. Chang, C.; Chen, S.; Zhang, L. *J. Mater. Chem.* **2011**, *21*, 3865.
12. Fan, M.; Hu, Q. L. *Carbohydr. Res.* **2009**, *344*, 944.
13. Fan, M.; Hu, Q. L.; Shen, K. *Carbohydr. Polym.* **2009**, *78*, 66.
14. Almeida, E. V. R.; Frollini, E.; Castellan, A.; Coma, V. *Carbohydr. Polym.* **2010**, *80*, 655.
15. Roberts, G. A. F.; Domszy, J. G. *Int. J. Biol. Macromol.* **1982**, *4*, 374.
16. Zhou, H. Y.; Chen, X. G.; Kong, M.; Liu, C. S.; Cha, D. S.; Kennedy, J. F. *Carbohydr. Polym.* **2008**, *73*, 265.
17. Xu, X. J.; Zhang, L. N.; Zhang, Y. Y. *J. Appl. Polym. Sci.* **2005**, *97*, 1710.
18. Tolaimate, A.; Desbrières, J.; Rhazi, M.; Alagui, A.; Vincendon, M.; Vottero, P. *Polymer* **2000**, *41*, 2463.
19. Weska, R. F.; Moura, J. M.; Batista, L. M.; Rizzi, J.; Pinto, L. A. A. *J. Food Eng.* **2007**, *80*, 749.
20. Hu, X.; Tang, Y.; Wang, Q.; Li, Y.; Yang, J.; Du, Y.; Kennedy, J. F. *Carbohydr. Polym.* **2011**, *83*, 1128.
21. Cai, J.; Zhang, L. *Biomacromolecules* **2006**, *7*, 183.