

Super Absorption Behavior of Chitosan by Freeze-Blasting in Different Alkaline Solvents

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ABSTRACT The absorption behavior of chitosan in alkaline solution by freeze-blasting was studied. The influence of alkaline type, concentration, and small molecules was investigated, as well as the different roles of LiOH and NaOH in the absorption. Chitosan reached its maximum absorption rate when LiOH concentration was 4.8 wt% and NaOH 4.0 wt%, respectively. Chitosan showed better absorption capacity in LiOH solution. Urea could improve the absorption when its concentration was more than or equal to 4.0 wt%, and the improvement was greater in NaOH solution. Thiourea showed no obvious effect in LiOH solution, but showed an effect when NaOH concentration was 4.0 wt% and 6.0 wt%. During the absorption, NaOH only reacted with -OH of chitosan, while LiOH not only reacted with -OH of chitosan, but also reacted with the acetyl group of chitosan. LiOH was more powerful in destroying hydrogen bonding of chitosan. LiOH/urea solution may be a uniquely powerful solvent for chitosan.

KEYWORDS Chitosan, NaOH, LiOH, urea, thiourea

1 INTRODUCTION

Chitosan, a natural polysaccharide, is a functional biopolymer obtained from deacetylation of chitin [1–4]. It is nontoxic, regenerable, antibacterial, biocompatible and biodegradable and thus is considered as an environmentally friendly material [5–7]. Due to these advantages, it is considered for use in many fields, including food packaging, absorbents, drug delivery, tissue engineering and other fields [8–11]. However, its poor solubility has seriously restricted its application. Chitosan structure consists of randomly distributed β -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine, which provides a rigid and unbranched structure to chitosan. Massive inter- and intrahydrogen bonds make it hard to dissolve in most solvents except for dilute acid solutions. However, chitosan is, like other polysaccharides, unstable in acid. Its hydrolysis is accompanied with the cleavage of glycosidic-bonds, which results in decreased and higher dispersivity in molecular weights. Moreover, a polyelectrolyte solution was formed after the dissolution of chitosan in acid. This characteristic has brought many

problems to the processing of chitosan, such as high viscosity [12–15].

There has been a long-term effort to improve the solubility of chitosan and seek appropriate solvents for chitosan ever since its discovery. With respect to the solubility, various studies have been carried out, including modification of the degree of deacetylation, degradation and chemical modification [16–21].

Chitin, as the source of chitosan, can be dissolved when an alkaline suspension of the polymer is mixed with ice [22, 23], and this procedure has allowed the authors to cast transparent chitin film with good mechanical properties [18]. Plenty of work concerned with the system of chitin and alkali solutions have been carried out [24–27]. In addition, this alkali chitin solution has been applied to prepare chitin product, including hydrogels [28], fibers [14], membranes [29], and other products.

Chitosan is structurally similar to chitin; therefore, it is possible for it to dissolve or swell in alkali aqueous solution. However, few works have been carried out in this field. In our previous work [30, 31], we have proved that chitosan (Deacetylation Degree (DD) = 80%, $M_n = 2.7 \times 10^5$) can dissolve directly in LiOH/urea aqueous solution by freeze-thawing treatment, and the solution is relatively stable.

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In our current work, chitosan with high molecular weight ($M_n \geq 4.8 \times 10^5$) and different DD was used to study the absorption and swelling behavior of chitosan in alkali aqueous solutions. How the alkali type and the small polar molecules influenced the absorption behavior of chitosan, as well as the crucial role of LiOH and NaOH freeze-blasting were investigated. Finally, this work is focused on acquiring a basic understanding of the absorption behavior of chitosan in alkali aqueous solution and providing guidance for the further development of this new type of solvent for chitosan.

2 MATERIALS AND METHODS

2.1 Materials

α -Chitin and α -Chitosan (DD = 89.4%, $M_n = 5.6 \times 10^5$ Da) (shrimp shell, powders) supplied by Zhejiang Golden-Shell Biochemical Co. Ltd. (China) were used without further treatment.

Chitosans with different degrees of deacetylation (DD) were prepared in laboratory [32], which were coded as CS65, CS70, CS75 and CS80 according to their DD, as shown in Table 1.

The HCl, LiOH·H₂O, NaOH, urea and thiourea were obtained from Sinopharm Chemical Reagent Co. Ltd. (China). All the reagents were of analytical grade, and used without further purification.

Table 1 Characteristics of N-deacetylated chitosan.

Sample	DD / %	M_n / Da	CrI / %
CS65	64.2	5.6×10^5	44.4
CS70	71.9	5.3×10^5	59.4
CS75	76.7	4.8×10^5	30.2
CS80	81.4	4.4×10^5	57.9

Table 2 Composition of the alkaline solutions.

		LiOH / wt%					NaOH / wt%				
Urea wt%	2.0	1.2	2.4	3.6	4.8	6.0	2.0	4.0	6.0	8.0	10.0
	4.0	1.2	2.4	3.6	4.8	6.0	2.0	4.0	6.0	8.0	10.0
	6.0	1.2	2.4	3.6	4.8	6.0	2.0	4.0	6.0	8.0	10.0
	8.0	1.2	2.4	3.6	4.8	6.0	2.0	4.0	6.0	8.0	10.0
Thiourea wt%	2.5	1.2	2.4	3.6	4.8	6.0	2.0	4.0	6.0	8.0	10.0
	5.0	1.2	2.4	3.6	4.8	6.0	2.0	4.0	6.0	8.0	10.0
	7.5	1.2	2.4	3.6	4.8	6.0	2.0	4.0	6.0	8.0	10.0
	10.0	1.2	2.4	3.6	4.8	6.0	2.0	4.0	6.0	8.0	10.0

2.2 Methods

2.2.1 Absorption Experiment under Room Temperature

Alkaline solutions with different compositions were prepared (water, LiOH solution, NaOH solution, LiOH/urea solution or NaOH/urea solution) according to the design shown in Table 2.

Chitosan powders (0.500 g) were soaked in 100 g alkali aqueous solution. After stirring and standing for 24 h and absorbing fully, the sample was filtered with nylon net and its surface water was removed by filter paper. The mass of resultant sample was measured precisely and recorded as m_1 .

2.2.2 Absorption Experiment with the Treatment of Freeze-Blasting

Chitosan powders (0.500 g) were soaked in 100 g alkali aqueous solution, as shown in Table 1. After stirring and standing for 3 h and freezing for 5 h at -60°C , the sample was taken out and thawed at a low temperature of approximately 0 to 5°C . The obtained sample was filtered by nylon net and the mass was recorded as m_2 after precise measurement.

The absorption of sample was calculated from Equation 1:

$$A = \frac{m - 0.500\text{g}}{0.500\text{g}} \times 100\% \quad (1)$$

where m is the mass of the swollen samples, g

2.2.3 Measurement

The DD was determined by conductometric titrations using a conductivity meter DDS-307 equipped with a Pt electrode [33].

The M_n of prepared samples was calculated from the classical Mark-Houwink relationship,

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

where $[\eta]$ is the intrinsic viscosity, $\alpha = -1.02 \times 10^{-2} \times DD + 1.82$, $k = 1.64 \times 10^{-30} \times DD^{14} \text{ cm}^3 \cdot \text{g}^{-1}$ [34].

The FTIR spectra of chitosans were measured on a Vector 22 spectrometer (Bruker) in KBr pellets at ambient temperature. All spectra were recorded with an accumulation of 32 scans and a resolution of 4 cm^{-1} in the range from 4000 cm^{-1} to 500 cm^{-1} .

The XRD patterns of powdered samples were obtained on a Bruker AXS D8 Advance X-ray diffractometer (Cu $K\alpha$, 40 kV, 34 mA). The scattering range (2θ) was $5\text{--}60^\circ$ with steps of $0.1^\circ/\text{s}$. The crystalline index (CrI) was determined by the Hermans-Weidinger equation [35].

For each sample, three parallel experiments were carried out and the average values were taken. Data were analyzed by an analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range tests. Statistical analysis was performed using the software STATISTICA 6.0.

3 RESULTS AND DISCUSSION

3.1 Effect of Alkaline Solution Type and Concentration

3.1.1 Effect of LiOH Concentration

Effect of LiOH concentration on the absorption by chitosan after freeze-blasting is illustrated in Figure 1. Chitosan of four different deacetylation degrees (DD) could only absorb a small amount of solvent in water (LiOH concentration was 0 wt%), and showed little difference in absorption capacity. The absorption rates were all around 600%; CS65, CS70, CS75 and CS80 appeared as dispersed swelling particles. At room temperature, with the addition of LiOH, all four

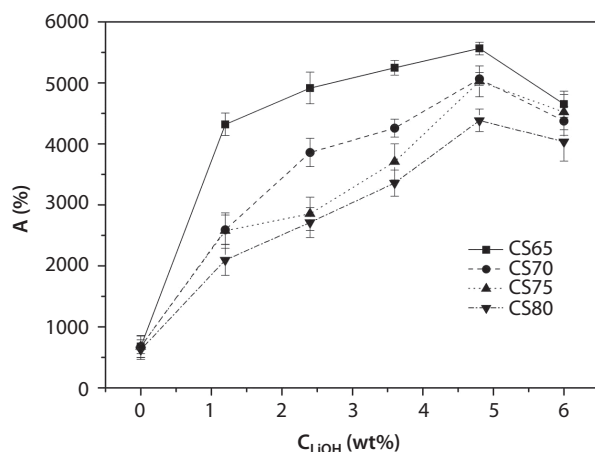


Figure 1 Influence of LiOH concentration on the absorption of chitosan.

chitosans also showed a particle state in different concentrations of LiOH solution, with the absorption rate ranging from 730% to 960%. However, after freeze-blasting, the absorption capacity of chitosan increased obviously and formed gel from dispersed particles. The absorption rate increased and leveled off with the increasing of LiOH concentration, and declined at a higher LiOH concentration. For CS65, for example, as the LiOH concentration increased from 0 to 1.2 wt%, the absorption rate of CS65 increased from 670% to 4300%, by 6 times; as LiOH concentration increased to 4.8 wt%, the absorption of CS65 reached its maximum 5560%, beyond which the value declined with the increasing of LiOH concentration. CS70, CS75 and CS80 displayed similar absorption behavior as CS65.

There are vast quantities of O, H and N atoms in chitosan molecule, forming strong hydrogen bonding among them [36]. Although chitosan is hydrophilic, it is difficult to dissolve in most solvents. Chitosan can absorb solvent and swell into gel in LiOH aqueous solution by freeze-blasting treatment. The reason may be that LiOH destroys the intramolecular and intermolecular hydrogen bonds in chitosan, resulting in the dominance of hydrophilicity of chitosan.

3.1.2 Effect of NaOH Concentration

Figure 2 shows the effect of NaOH concentration on the absorption behavior of chitosan after freeze-blasting. On the whole, CS65, CS70, CS75 and CS80 also exhibited significant absorption behavior in NaOH solution. With the increase of NaOH concentration, the absorption rate of all chitosan samples increased first and then declined, and reached its maximum when NaOH concentration was 4.0 wt%. The absorption capacity of chitosan decreases with the increase of deacetylation degree in NaOH solution, similar to that in LiOH solution.

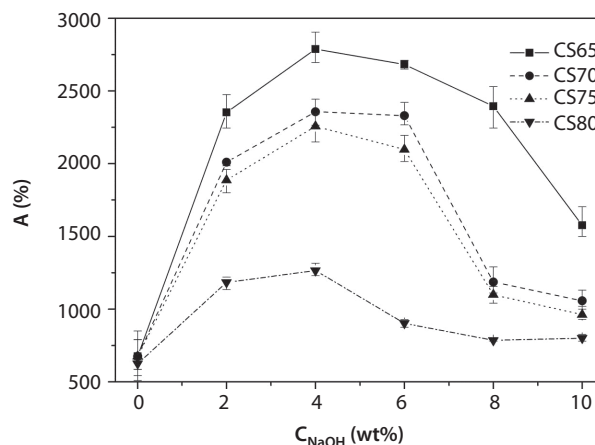


Figure 2 Influence of NaOH concentration on the absorption of chitosan.

Comparing Figure 1 and Figure 2, it is easy to find that chitosan had a better absorption to LiOH. For CS65, for example, it had the highest absorption capacity. The maximum absorption rate of CS65 in LiOH solution was 5560%, while in NaOH solution it was only 2700%. This significant difference was even enlarged in the case of CS80. Moreover, the concentrations at which chitosan reached its maximum absorption rate in these two alkaline solutions were different, that is, 4.8 wt% for LiOH and 4.0 wt% for NaOH.

The reasons for such differences between LiOH and NaOH solutions may be as follows: (1) the properties of LiOH and NaOH are different, perhaps due to the different alkalinity; (2) LiOH and NaOH play different roles in the absorption, which we will discuss in the following section.

Compared to the other three chitosans of different deacetylation degrees, CS65 showed better absorption performance. So, in the following, we studied CS65 sample to explore the effect of small molecules on chitosan absorption behavior.

3.2 Effect of Small Molecules

3.2.1 Effect of Urea

Figure 3 shows the effect of urea concentration on the absorption behavior of CS65 by freeze-blasting in alkali solutions; curve (a) is the absorption behavior of CS65 in LiOH/urea solution, and curve (b) is in the NaOH/urea solution. As shown in the figures, when LiOH concentration ranged from 1.2 wt% to 6.0 wt%, the addition of urea had a similar influence on the absorption behavior of CS65. When urea concentration was lower, urea weakened the absorption rate of CS65, the absorption rate reached its minimum when urea concentration was 2.0 wt%, then with the increasing of urea concentration, CS65's absorption rate gradually increased; when the urea concentration in the solvent system was greater than or equal to 4 wt%, adding urea could improve the dissolvability of CS65/LiOH system. Comparing the improvements of absorption of CS65 by urea, we can also find that when LiOH concentration was lower, the promoting effect of urea was more obvious; as LiOH concentration increased, the absorption capacity of CS65 increased, and the promoting effect was weakened. For NaOH solution, the effect of urea concentration on CS65's absorption behavior was similar to that on CS65/LiOH system. Comparing CS65/LiOH/urea system and CS65/NaOH/urea system, the maximum improvement of adding urea to CS65/LiOH system was 131%; while 155% to CS65/NaOH system. Urea had a better improving effect on CS65/NaOH system.

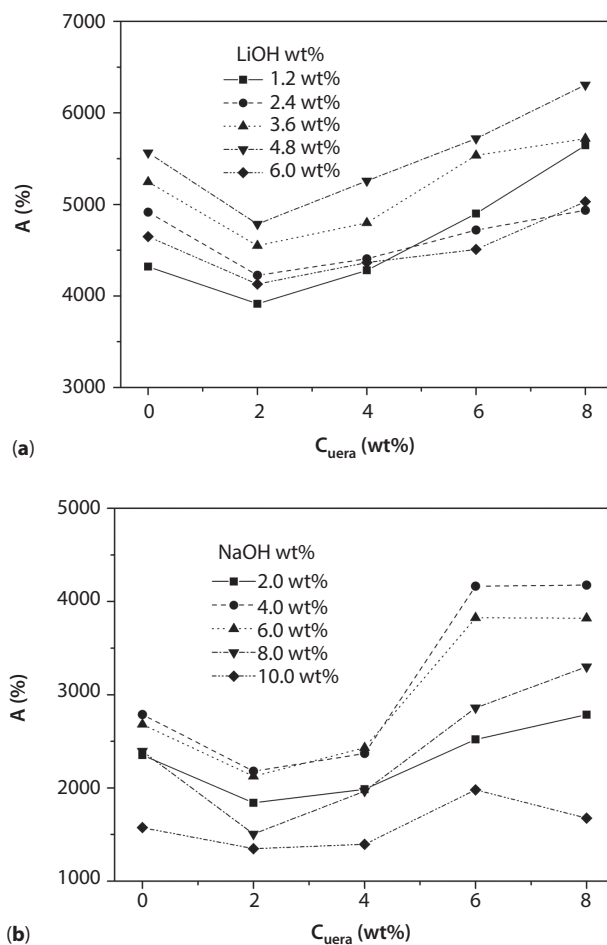


Figure 3 Influence of urea concentration on the absorption of CS65 in aqueous LiOH/urea solution (a) and NaOH/urea solution (b).

3.2.2 Effect of Thiourea

Figure 4 shows the effect of thiourea on the absorption behavior of CS65 in LiOH/thiourea solution and NaOH/thiourea aqueous solution. Curve (a) is the absorption behavior of CS65 in LiOH/thiourea solution, and curve (b) is that in NaOH/thiourea aqueous solution. As shown in the figure, the effect of thiourea on absorption behavior in different solvent systems was different. For LiOH solution, when LiOH concentration was 1.2 wt%, adding thiourea hindered the CS65 absorption of solvent; when LiOH concentration ranged from 2.4 wt% to 6.0 wt%, thiourea had no obvious effect on CS65/LiOH system.

Compared with CS65/LiOH, thiourea showed a totally different influence on CS65/NaOH system. When NaOH concentration was 4.0 wt% and 6.0 wt%, thiourea improved the absorption of CS65 in NaOH solution, and the improving effect was more prominent as thiourea concentration increased; for the other three NaOH concentrations, adding thiourea weakened the absorption.

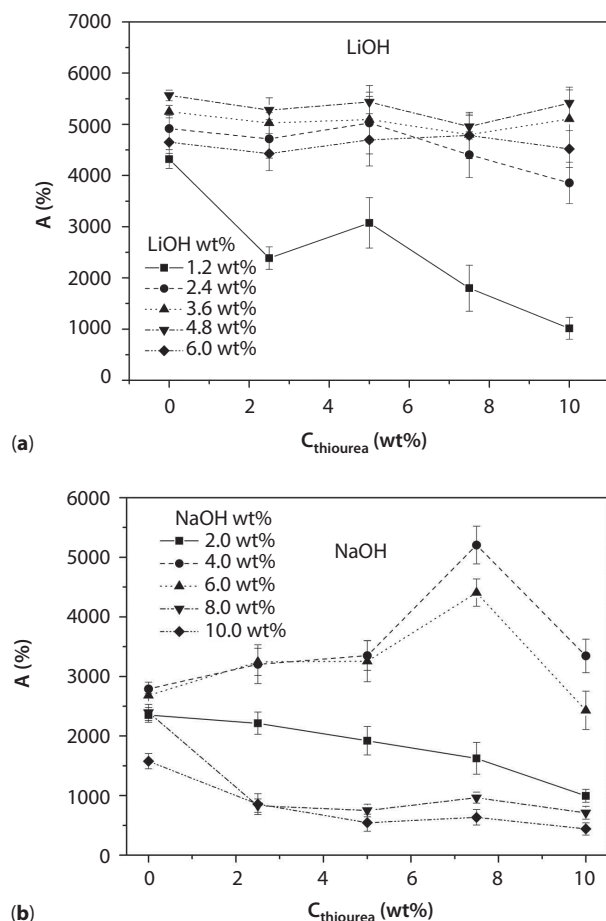


Figure 4 Influence of thiourea concentration on the absorption of CS65 in aqueous LiOH/thiourea solution (a) and NaOH/thiourea (b) solution.

Comparing urea with thiourea, urea showed better improvement on CS65's absorption in alkaline solution system than thiourea, and was greater for NaOH solution.

3.3 The Different Roles of LiOH and NaOH in Absorption of CS65

Figure 5 shows the infrared spectra and their partial enlargement of CS65 powder and freeze-dried powder of CS65 swelling hydrogels formed from the absorption in 4.8 wt% LiOH/4.0 wt% urea solution and 8.0 wt% NaOH/4.0 wt% urea solution by freeze-blasting treatment. Curve (a) is the infrared spectrum of CS65, curve (b) is freeze-dried powder of CS65/LiOH/Urea gel, and curve (c) is the freeze-dried powder of CS65/NaOH/Urea gel. It is easy to find that curve (c) is similar to curve (a), except the vibration peak at 670 cm⁻¹ marked with a #3 box, this peak may be caused by the replacement of -OH in CS65 by -O-Na⁺.

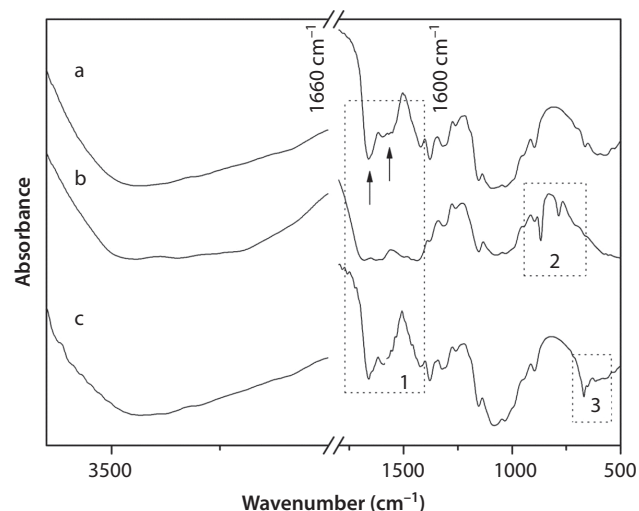


Figure 5 FTIR spectra of CS65 (a), freeze-dried powder of CS65 swelling hydrogels in 4.8 wt% LiOH/4.0 wt% urea solution (b) and CS65 in 8.0 wt% NaOH/4.0 wt% urea solution (c) by freeze-blasting treatment.

Comparing curve 5(b) with 5(a) and 5(c), the infrared spectrum is significantly different, and marked with boxes. There is no obvious difference in amide I peak at 1660 cm⁻¹ and amide II peak at 1600 cm⁻¹ in curve (a) and (c), but amide I peak in curve (b) is greatly weakened. The attenuation of amide I peak suggests that LiOH had an effect on the acetyl amino group of CS65. In our previous work [30], we have found that the molar ratio of absorbed LiOH and water decreases linearly with the increase of DD, which may also due to the reaction. However, further work is needed to clarify the reaction. The vibration peak at 3450 cm⁻¹ in curve (b) is significantly wider than that in (a), and tends to split into several smaller peaks. The peak at 3450 cm⁻¹ corresponds to the vibration of -NH and -OH of chitosan. The changing of this peak suggests a reaction of these two groups. According to the literature, -OH of chitosan can react with concentrated alkaline solution, so we speculate that the hydroxy group of chitosan might also have reacted with LiOH. There are two new vibration peaks at 2500 cm⁻¹ and 785 cm⁻¹ in curve (b), which might have been due to the hydrogen substitution on hydroxy group of CS65 with Li⁺ [37]. From the above analysis we speculate that during the absorption of NaOH solution by CS65, the NaOH reacted with -OH of CS65 to destroy hydrogen bonding of chitosan, while LiOH not only reacted with -OH of CS65, but also reacted with acetyl group of CS65, which led to attenuation of amide I peak, corresponding to the acetyl group on the infrared spectrum. Therefore, compared to NaOH, LiOH was more powerful in destroying hydrogen bonds of chitosan.

This may be why chitosan showed better absorption capacity in LiOH solution.

4. CONCLUSIONS

Four chitosan samples with different degree of deacetylation were used to study the absorption behavior of chitosan in alkaline solution. The effect of alkaline concentration, type of alkaline and the addition of small molecules on chitosan's absorption behavior was investigated. We also attempted to provide an explanation of the different roles of LiOH and NaOH in the absorption.

Chitosan can absorb a vast quantity of solvent and form gel after treated by freeze-blasting in LiOH or NaOH solution. It reached its maximum absorption rate when LiOH concentration was 4.8 wt% or NaOH concentration was 4.0 wt%. Chitosan possessed better absorption capacity in LiOH. Upon adding urea into the solvent system, when urea concentration was greater or equal to 4.0 wt%, urea would improve the absorption in these two systems, and such an effect was more significant in NaOH solution; upon adding thiourea into the system, thiourea had no improving effect on the absorption by CS65 in LiOH solution. In NaOH solution, the effect of thiourea divided into two situations. When NaOH concentration was 4.0 wt% and 6.0 wt%, thiourea improved the absorption of NaOH solution, and the improving effect was greater as thiourea concentration increases; when NaOH concentration was too high or too low, adding thiourea impeded the absorption. NaOH only reacted with -OH of CS65, while LiOH not only reacted with -OH of CS65, but also reacted with the acetyl group of CS65. LiOH was more powerful in destroying hydrogen bonding of chitosan. LiOH/urea might be a uniquely powerful solvent for chitosan.

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