



Preparation of Alkali Swelling Chitosan and its Freezing-Swelling Behaviour

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Chitosans with different structure were prepared by deacetylation of chitin by means of orthogonal design experiment and research on the swelling behaviours of the chitosan after LiOH-freezing treatment was conducted. It was found that low temperature and the presence of LiOH played a significant role in promoting the swelling of chitosan. The degree of swelling first increased and then decreased with the increase of LiOH concentration and reached the maximum swelling ratio when the concentration of LiOH was 2.5 mol L⁻¹. According to the experimental results, alkali concentration, reaction temperature and time in deacetylation reaction had a more obvious impact on alkali swelling degree of chitosan. FT-IR spectra and X-ray diffraction showed that deacetylation made the structure of chitosan looser, which facilitated the diffusion and penetration of little molecules. By changing the alkali concentration and regulating the temperature and deacetylation time, chitosan with higher molecular weight and capable of swelling in alkali solution can be produced.

Key Words: Chitosan, LiOH, Freezing, Swelling.

INTRODUCTION

Chitin is the second most abundant natural polymer after cellulose in the natural world. As one of the most important derivatives of chitin, chitosan is prepared by deacylation of chitin. This technique has the advantages of abundant resources, non-toxicity, good biocompatibility and biodegradability, with broad prospect of application. With the progress of research on chitosan, its application has extended to the fields of chemical engineering, food, environmental protection, medical care and pharmaceutical preparations¹⁻³.

However, the poor solubility of chitosan restricts its applicability in many fields. Similar to chitin, due to strong intramolecular and intermolecular hydrogen bonding, it is difficult for ordinary solvents to enter chitosan molecules⁴. Strong acids can bind the amidogen of chitosan molecules to form salt, inducing the swelling of chitosan and even dissolution⁵. However, chitosan is likely to be degraded in acids and its spatial structure will be damaged, which affects its performance⁶.

The research conducted by Saito *et al.*⁷ showed that the dissolution of chitin was accompanied by severe degradation in 8 mol/L hydrochloric acid solution and the spatial structure of the molecule was transformed from pattern α to pattern β . As chitosan and chitin share many similarities in structure and

performance, the impact of acids on molecular structure of chitosan is easy to predict.

Liu *et al.*⁸ studied the swelling behaviour and structural changes of chitin in NaOH solution. It was proved that alkali solution has much less destructive effect on chitin molecules.

Chitosan is chemically similar to chitin, so it is possible for it to dissolve or swell in alkali aqueous solution with high stability and to be processed into products with good mechanical properties. However, few works have been carried out in this field^{9,10}.

In this study, a series of chitosan with variable structures was prepared by changing the process conditions using orthogonal experimental design. Taking LiOH solution as the swelling agent, the swelling behaviour of chitosan under freezing-blasting conditions and the effect of preparation conditions on the swelling properties of chitosan were studied.

EXPERIMENTAL

α -Chitin (shrimp shell, powders) supplied by Zhejiang Golden-shell Biochemical Co. Ltd. (China) was used without further treatment.

LiOH·H₂O, NaOH, NaCl, HCl and CH₃COOH were obtained from Sinopharm Chemical Reagent Co. Ltd. (China). All reagents were of analytical grade and were used without further purification.

Method

Preparation of chitosan specimens: Chitosan specimens were prepared according to Fan *et al.*⁹ and the orthogonal design experiment conditions were listed in Table-1. The specimens prepared were numbered according to Table-2.

TABLE-1
VARIABLES INVESTIGATED AND THEIR LEVELS

Variables investigated	Levels of each variable			
	1	2	3	4
A: NaOH concentration	45	50	55	60
B: reaction temperature (°C)	80	85	90	95
C: reaction time (h)	1	2	3	4

TABLE-2
EXPERIMENTAL ARRANGEMENT AND TEST RESULTS

Sample	Reaction condition			M _n (10 ⁴)	DD (%)
	C _{NaOH} (Wt %)	T (°C)	T (h)		
1	45	95	1	199.15	56.5*
2	45	80	2	280.98	49.2*
3	45	85	3	247.74	50.4*
4	45	90	4	258.98	53.7*
5	50	95	2	264.64	70.7
6	50	80	1	244.72	64.7
7	50	85	4	257.17	77.4
8	50	90	3	259.51	72.2
9	55	95	3	255.18	77.6
10	55	80	4	281.78	71.3
11	55	85	1	262.24	67.6
12	55	90	2	278.07	62.0
13	60	95	4	223.38	81.5
14	60	80	3	256.54	73.8
15	60	85	2	255.76	65.3
16	60	90	1	250.97	68.8

*DD of specimens No. 1-4 was determined by infrared spectrum because they could not completely dissolved in HCl solution.
DD = Degree of deacetylation.

Determination of M_n¹¹: The M_n of prepared samples was calculated from the classical Mark-Houwink relationship,

$$[\eta] = kM^\alpha \quad (1)$$

where $[\eta]$ is the intrinsic viscosity, $\alpha = -1.02 \times 10^{-2} \times DD + 1.82$, $k = 1.64 \times 10^{-30} \times DD^{14} \text{ cm}^3 \text{ g}^{-1}$. The intrinsic viscosity was measured with an Ubbelohde viscometer at 30 °C using 0.2 M CH₃COOH-0.1 M CH₃COONa as solvent. The solutions were filtered through a P30 glass filter before determining $[\eta]$.

Determination of degree of deacetylation (DD)¹²: The degree of deacetylation was determined by conductometric titrations using a conductivity meter DDS-307 equipped with a Pt electrode. A curve of the conductivity against the volume of NaOH with two inflectional points was obtained. The difference of the volumes of these two points corresponds to acid consumed for the protonation of amino groups and allows the determination of the degree of deacetylation of the chitosan.

Determination of the saturated water absorption rate (W_s) of specimens: The chitosan specimens were dried to constant weight in the furnace at 80 °C. Then 0.010 g of chitosan specimen was accurately weighed and loaded into a centrifuge tube. Distilled water was added into the centrifuge tube to enable saturated water absorption of the specimen for

more than 4 h. Later, wet weight m₀ was weighed. The saturated water absorption was calculated by

$$W_s (\%) = \left(\frac{m_0}{0.010} \right) \times 100 \% \quad (2)$$

Each specimen was measured for three times and the average value was taken.

Determination of alkali swelling property of specimens^{13,14}: Using different concentrations of LiOH solution as solvent, the swelling properties of the specimen were determined. Accurately weighed 0.100 g of chitosan specimen was loaded into a centrifuge tube and then 20 mL of LiOH solution was added. The specimens were left to stand at room temperature for more than 4 h before being put into the freezer for 12 h. The specimens were taken out after complete freezing and thawed at room temperature. The mass of the swelled specimen m_s was weighed. The swelling ratio (SR) was calculated by the following formula:

$$SR (\%) = \left(\frac{m_s}{0.100} \right) \times 100 \% \quad (3)$$

The procedure was repeated for three times and the average value was taken.

FT-IR spectra of specimens: FT-IR 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 4000-500 cm⁻¹.

XRD pattern of specimens: XRD patterns of powdered samples were obtained using a Bruker AXS D8 Advance X-ray diffract meter, 40 kV and 34 mA with CuK_α radiation at k 1.5406 Å. The relative intensity was recorded in the scattering range (2θ) of 5-60° with steps of 0.1° per second. The crystalline index (CrI) was determined by the Hermans-Weidinger equation¹⁵,

$$CrI (\%) = \frac{k \times I_c}{(I_c + I_a)} \times 100 \% \quad (4)$$

where I_c and I_a represent the diffraction intensity of crystalline region and diffraction intensity of amorphous region, respectively. k is a coefficient.

RESULTS AND DISCUSSION

Characteristics of prepared chitosans: Table-2 shows the experimental arrangement, the molecular weight and degree of deacetylation of prepared chitosans.

According to the results, the concentration of alkali had significant impact on the degree of deacetylation of the product under the experimental conditions and the degree of deacetylation increased significantly with the increase of alkali concentration. Temperature and reaction time had a more significant impact on degree of deacetylation than molecular weight of the product. The higher the temperature and the longer the reaction time, the greater the degree of deacetylation was. However, alkali concentration did not significantly affect molecular weight and the possible reason might be that under the experimental conditions, alkali concentration differences between groups were not big enough. Therefore, its impact on the molecular weight was not fully shown.

Saturated water absorption of prepared chitosan:

Table-3 shows the measured saturated water absorption of prepared chitosans, which was an indicator of the hydrophilicity of chitosan specimens. Though chitosan molecule contains a large number of hydrophilic groups such as hydroxyl and amidogen, the strong intramolecular and intermolecular hydrogen bonding of chitosan specimens cause a decrease in capacity to bind water molecules². The swelling of chitosan occurred as a result of freezing or adding LiOH polar small molecules, which broke hydrogen bond of chitosan molecules. The swelling behaviour and impact factors of chitosan in such process are mainly discussed.

TABLE-3
SATURATED WATER ABSORPTION
OF PREPARED CHITOSAN (W_s %)

Sample	W _s (%)	Sample	W _s (%)
1	247.4	9	214.8
2	263.9	10	211.2
3	255.1	11	236.1
4	254.2	12	238.0
5	239.4	13	219.4
6	251.6	14	241.6
7	224.5	15	227.5
8	233.1	16	240.8

Impact of degree of deacetylation on alkali swelling properties of prepared chitosans: As specimens No. 9, 14, 15 and 16 had similar molecular weights but significantly different degrees of deacetylation, we selected these specimens to study the impact of degree of deacetylation on swelling properties of chitosan. Table-4 shows the measured swelling properties of these specimens. Compared with the results of water absorption experiments at room temperature (Table-3), the swelling ability of chitosan was substantially increased. And the SR-LiOH concentration curves of four chitosan specimens were obtained as shown in Fig. 1. With the increase of LiOH concentration, the curves of the four specimens showed a similar variation trend. The degrees of swelling of specimens No. 14, 15 and 16 in 2.5 mol L⁻¹ LiOH reached the maximum, which indicated that freezing and the presence of LiOH promoted the swelling of chitosan molecules. This swelling phenomenon might be due to multiple factors. Firstly, in the polymer chain, there was a hydrophilic-hydrophobic balance, which moved to the hydrophilic direction at low temperatures. This was favorable for the forming of hydrogen bonds between hydrophilic groups of chitosan molecules and water molecules, which promoted swelling. In addition, low temperature activated Li⁺ so that the site at which the hydrogen bonds were formed in chitosan molecules was susceptible to attack¹⁶. Secondly, the free water volume increased by 10 % after freezing,

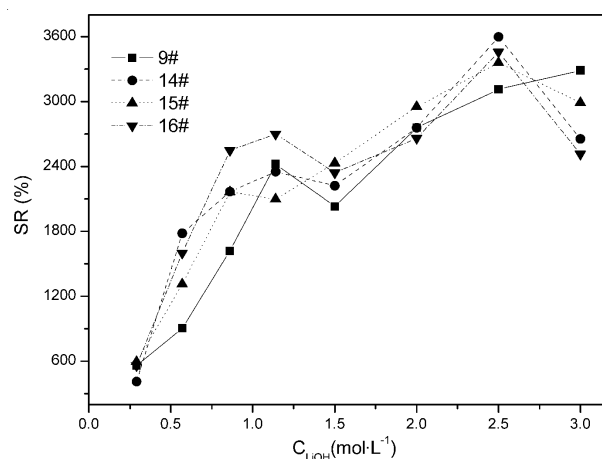


Fig. 1. Effect of concentration of LiOH on the swelling ratio of different chitosan

which increased the distance between chitosan molecules; the hydrogen bonds of chitosan were broken so that small molecules could easily enter. Moreover, LiOH molecules with strong polarity attached to chitosan molecules to form sol (double electric layer-structure); the electrical properties of sol resulted in mutual repulsion among chitosan molecules. This might also hinder the formation of hydrogen bonds among the chitosan molecules, thereby promoting swelling. Finally, Li⁺ existed in the form of hydrated ion in water and with the increase of LiOH concentration, the number of water molecules binding each Li⁺ decreased. Consequently, the volume of [Li(H₂O)_n]⁺ decreased, which facilitated its entry into chitosan molecules¹⁶.

We also found in the experiment that when the concentration of LiOH reached 3 mol L⁻¹, the swelling degrees of specimens No. 14, 15 and 16 were significantly reduced. This might be due to the fact that excessive amount of Li⁺ prevented the entry of water molecules and led to re-aggregation of the sol. Another possible reason was the enhanced shielding effect of OH⁻ on -NH₂. The local contraction of chitosan molecular chains in the gel would cause the overall contraction of polymer network. As a result, the degree of swelling of gel network decreased.

Specimen No. 9 had a special swelling behaviour under the experimental conditions. Firstly, when LiOH concentration was less than 3 mol L⁻¹, the degree of swelling of the specimen was generally smaller than that of other specimens; when LiOH concentration reached 3 mol L⁻¹, the degree of swelling continued to rise and finally exceeded that of other specimens. This might be related to its higher degree of deacetylation and molecular regularity. However, further investigation remains to be conducted for specific reasons.

TABLE-4
SWELLING PROPERTIES OF PREPARED CHITOSANS

Sample	Swelling ratio (%) in different concentration of LiOH solutions							
	C _{LiOH}							
	0.29	0.57	0.86	1.14	1.50	2.00	2.50	3.00
9	557	906	1618	2420	2031	2759	3112	3288
14	411	1782	2168	2352	2221	2759	3596	2656
15	594	1311	2165	2096	2431	2951	3357	2989
16	560	1601	2547	2700	2343	2662	3459	2516

According to the experimental results, alkali concentration, reaction temperature and time in deacetylation process had a significant impact on alkali swelling degree of chitosan. Chitosan with high molecular weight and degree of swelling in alkali solution can be prepared by changing alkali concentration and controlling the temperature and time of deacetylation.

Chitin and chitosan specimens No. 9, 14, 15 and 16 showed diverse swelling behaviours, which was closely related to their structure. Deacetylation of chitin not only changed acetyl content of chitosan, but also the distribution of acetyl groups and molecular weight of chitosan. Moreover, deacetylation also broke hydrogen bonds of chitin.

Fig. 2 shows the infrared spectra of chitin and specimens No. 9, 14, 15 and 16. The amide peak of chitin located at 1660 cm^{-1} was split into two smaller peaks, which might be caused by the formation of hydrogen bonds between carbonyl group in acetyl and the adjacent hydroxymethyl group. The amide peak of chitin was significantly weakened after deacetylation and the two smaller peaks merged into a single peak. It was indicated that the number of acetyl groups was reduced after deacetylation of chitin and the hydrogen bond between carbonyl group in acetyl and the adjacent hydroxymethyl group was also destroyed. The peaks of chitin at 3260 and 3100 cm^{-1} represented intermolecular and intramolecular hydrogen bonding of chitin. As deacetylation reaction proceeded, the two peaks disappeared, which indicated that the intermolecular and intramolecular hydrogen bonds of chitin were destroyed⁸.

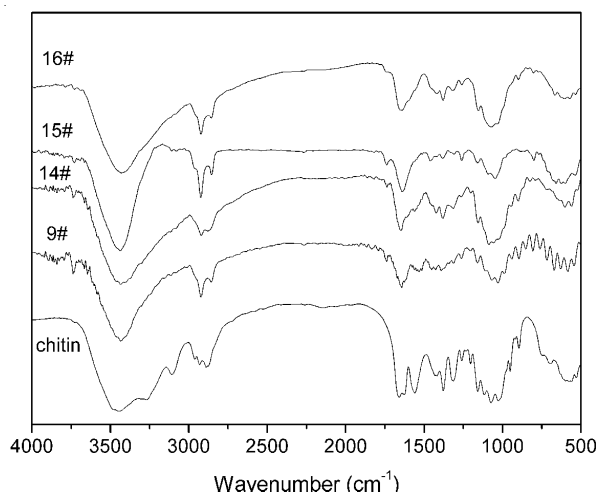


Fig. 2. Infrared spectra of chitin and prepared chitosans

Fig. 3 shows the XRD patterns of chitin and chitosan, which reflect the changes in crystal structure before and after deacetylation of chitin. In the range of $2\theta = 10\text{--}40^\circ$, there are 5 diffraction peaks for chitin, respectively at 010 , 110 , 120 , 101 and 130 ⁸. However, there are only two main crystalline peaks for specimens No. 9, 14, 15 and 16, which indicates that the crystal structure underwent significant changes after deacetylation of chitin.

Through the fitting analysis of their XRD patterns, the crystallinity index of chitin and specimens No. 9, 14, 15 and 16 are listed in Table-5. We can find from the table that after the deacetylation of chitin, the acetyl groups on the main chain

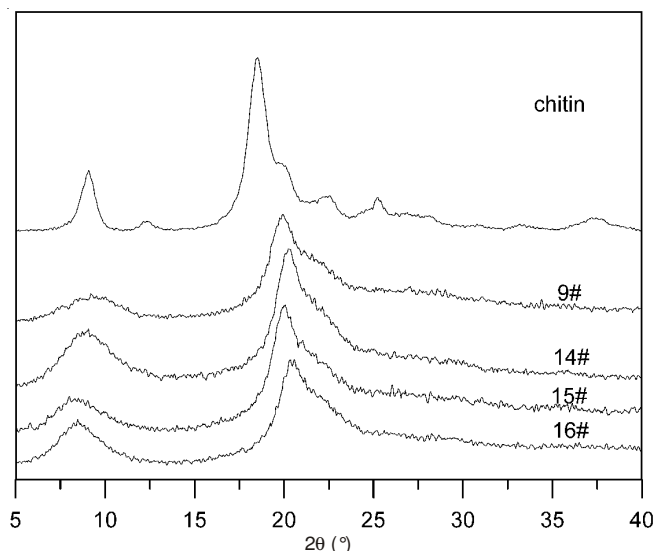


Fig. 3. XRD patterns of chitin and prepared chitosans

Sample	CrI (%)	Sample	CrI (%)
Chitin	79.29	15	68.86
9	56.19	16	47.97
14	69.01	—	—

were removed and the ordered structure of chitin was damaged, leading to decreased crystallinity index.

Conclusion

In this experiment, 16 chitosan specimens with different structures were prepared through the deacetylation of chitin by using orthogonal experiment design and further research on the swelling behaviours of the chitosan in LiOH solution by freezing treatment was conducted.

The swelling behaviour of chitosan in LiOH solution after freezing was also studied. It was found that low temperature and the presence of LiOH played a significant role in promoting the swelling of chitosan. The degree of swelling first increased and then decreased with the increase of LiOH concentration. Specimen No. 9 showed a distinctive swelling behaviour, which might be related to the high degree of deacetylation and molecular regularity. FT-IR spectra and X-ray diffraction showed that deacetylation made the structure of chitosan looser, which facilitated the diffusion and penetration of little molecules.

According to the experimental results, alkali concentration, reaction temperature and time in deacetylation reaction had a more obvious impact on alkali swelling degree of chitosan. By changing alkali concentration and regulating the temperature and deacetylation time, chitosan with higher molecular weight and capable of swelling in alkali solution can be produced.

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