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Absorption behaviors and structure changes of chitin in alkali solution

Yongle Liu *, Zhaofeng Liu, Wanlian Pan, Qingji Wu

State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Donghua University, Shanghai 201620, China

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Abstract

Absorption behaviors of chitin in alkali solution under different treatment conditions of alkali concentration, soaking time, soaking temperature and added ethanol were studied. The solid state structural changes of the resulting chitin were investigated by X-ray diffraction and FT-IR spectroscopy. The results showed that the physical absorption of alkali solution occurred instantly when soaked in alkali solution, while the absorption equilibrium was established after soaking 4 h at room temperature. The absorbency increased as temperature decreased or alkali concentration increased. Maximum absorbency was achieved at freezing temperature with a 20 wt% NaOH solution. The addition of ethanol accelerated the alkali absorption of chitin. Hydrogen bonds in chitin were weakened by the alkali treatment and the crystallinity of chitin decreased significantly when soaked in higher concentration alkali solutions at room temperature. The molecular weight and degree of acetylation (DA) of chitin decreased significantly at treatment temperatures higher than 20 °C or treatment times longer than 4 h.

Keywords: Chitin; Alkali treatment; Absorption; Crystal structure; Hydrogen bond

1. Introduction

Chitin, poly-(1 → 4)-2-acetamido-2-deoxy-β-D-glucose, is one of the most abundant natural polymers with biodegradability, biocompatibility, non-toxicity, wound healing acceleration, the ability to chelate heavy metals and many other distinctive properties (Khor, 2001; Rinaudo, 2006). As a renewable natural material, chitin offers many potential applications in a number of fields, ranging from wastewater treatment, cosmetics, drug delivery, artificial skin, and many other novel applications (Kumar, 2000). However, chitin is not soluble in common solvents due to the existence of intra- and inter-molecular hydrogen bonds in chitin and its highly crystalline structure. This strongly restricts many applications of chitin.

The presence of amino and hydroxyl groups in the chitin repeat unit provides many opportunities for chemical modification (Kurita, 1998,2001). Etherification is one of the most important modifications used to prepare water soluble chitin derivatives, including carboxymethylchitin, hydroxyethylchitian, and hydroxypropylchitin (Hirano, 1988; Qin et al., 2006; Sini, Santhosh, & Mathew, 2005). Etherification of chitin is performed by treating alkali chitin with etherificants, and the first step is to prepare alkali chitin. Alkali treatment swells and decrystallizes the chitin structure, and improves the accessibility of chitin. The effects of failing to achieve uniform swelling and decrystallization are manifested in etherificant or etherificated product by poor solubility due to non-homogeneity. Martìn-Gil et al. (1992) reported that cooling between −13 and 31 °C reduced or broke crystalline linkages between the sheets of chains in chitin, and that the existence of alkali enhanced the extent of decryatallization. Similarly, Feng, Liu, and Hu (2004) reported that crystallinity of chitin decreased by half after alkali-freezing treatment in 50% NaOH solution for 3 days. Noishiki et al. (2003) studied the crystal conversion from β-chitin to α-chitin by alkali treatment,

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* Corresponding author. Address: College of Material Science and Engineering, Donghua University, 2999 North Renmin Road, Songjiang District, Shanghai 201620, People’s Republic of China. Tel./fax: +86 21 67792945.
E-mail addresses: lyl@mail.dhu.edu.cn, lyl_ly1979@163.com (Y. Liu).

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and reported the minimum NaOH concentration for chitin swelling was between 25 wt% and 30 wt%. Many articles (Morita & Jinno, 1988; Somorin, Nishi, Tokura, & Noguchi, 1979; Thor & Henderson, 1940; Wan, Creber, Peppley, & Bui, 2004) have reported the etherificant modifications of chitin or chitosan, and the alkali chitin or alkali chitosan were prepared at different conditions. However, unlike alkali treatment of cellulose (Heuser, 1944), the published work on alkali chitin is not systematic.

Chitin powder is capable of absorbing alkali from aqueous alkali solutions; the reactions involved may consist of simple physical absorption of alkali and chemical reaction between them. It is difficult to determine the exact relative importance of these two mechanisms, which are accompanied by the swelling of chitin powder. However, in order to understand the absorption behavior of chitin in alkali solution, it is important to determine the absorbency of chitin powder in aqueous alkali solution, the component of alkali chitin, and the changes in crystal structure and hydrogen bonds.

In this article, the absorption behaviors of chitin in alkali solution were studied. The solid-state structural changes of the resulting chitin powders were characterized by X-ray diffraction and FT-IR spectroscopy. Changes of molecular weight and degree of acetylation (DA) were also measured.

2. Experimental

2.1. Materials

Chitin (shrimp shell, flakes, Zhejiang Golden-shell Biochemical Co. Ltd., China) with 2.0 wt% ash was treated with 10 wt% diluted hydrochloric acid at 0–5 °C to further remove calcium carbonate. The product was washed with distilled water, dried and pulverized. The final ash content was below 0.8 wt%. The molecular weight and DA of the resulting sample was 5.1×10^5 Da and 89.4%, respectively. Other chemicals were of analytical grade and used without further treatment.

2.2. Measurement of absorbency

Chitin powder (0.1 g) was put into a 1.5 mL centrifugation tube which has pores at the bottom with viscose rayon to hold the solid samples, then NaOH solution was added and mixed. After soaked for a desired time under the appropriate conditions, the tube was centrifuged at 10,000 rpm for 10 min in a centrifuge (TGL-16G, Shanghai Medical and Analytical Apparatus Co. Ltd.) to remove the excess alkali solution. The total absorbency, including the absorbency of NaOH and water, was defined as the total mass absorbed by per 100 g dry chitin powder. NaOH absorbed in samples could be determined by titration, and its absorbency was described by grams per 100 g chitin. Then the water absorbency could be obtained from the total absorbency minus the NaOH absorbency.

2.3. Structure measurement

X-ray diffraction (D/max-2550PC diffractometer, 40 kV, 300 mA) patterns and FT-IR spectra (Nicolet, Nexus-670) were used to characterize the changes of crystal structure and hydrogen bonds in alkali chitin, respectively. The crystallinity were calculated by the Hermans–Weidinger equation (Zhang, Haga, Sekiguchi, & Hirano, 2000)

$$X_c(\%) = K \times I_c/(I_c + I_a) \times 100\% \tag{1}$$

where X_c, I_c, and I_a represent the crystallinity, diffraction intensity of crystalline region, and diffraction intensity of amorphous region, respectively. K is a coefficient, which is about 0.8–1.1.

The viscosity average molecular weight was calculated from the Mark–Houwink equation, and the constants a and K for chitin in N,N-dimethylacetamide/lithium chloride at 30 °C were 0.95 and 7.6×10^{-5} dl/g, respectively (Poirier & Charlet, 2002). DA was determined from infrared spectra as follows. The absorbencies of C–N stretching in secondary amide at 1320 cm^{-1} and C–H deformations at 1420 cm^{-1} were calculated from IR spectra, and described as A_{1320} and A_{1420}, respectively. DA(%) was given by $(31.92 \times A_{1320}/A_{1420} - 12.2)$ (Brugnerotto et al., 2001; Velde & Kiekens, 2004).

3. Results and discussion

3.1. Absorption behaviors

Chitin powders were soaked in 40 wt% aqueous NaOH solution for different time at room temperature. The effect of soaking time on the absorption behavior of chitin is shown in Fig. 1. The physical absorption of alkali solution occurred instantly. After soaking for 20 s, the total absorbency reached 136 g per 100 g chitin. As soaking time increased, absorbency increased steadily and the total absorbency reached 220 g per 100 g chitin after one hour soaking. Due to the highly crystal structure and

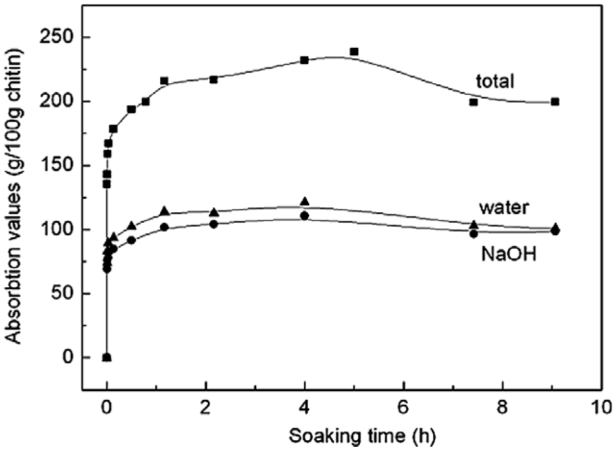

Fig. 1. Effect of soaking time on the absorption behaviors of chitin soaked at room temperature in 40 wt% alkali aqueous solution.

intra- and inter-hydrogen bonds in chitin, the penetration and diffusion of alkali solution in chitin is difficult and more soaking time was needed to obtain uniform alkali chitin. After soaked for 4 h, the absorption approached equilibrium and total absorbency reached 232 g per 100 g chitin. Further prolonging soaking time caused a slight decrease in absorbency. Therefore the alkali solution holding capability of chitin decreased after long time soaking. This is because of the degradation caused by alkali. The degradation was proved later by molecular weight measurements.

The effect of alkali concentration on the absorption behavior at room temperature is shown in Fig. 2. The absorbency increased slowly with increasing of alkali concentration. After NaOH concentration reached 25 wt%, a sharp increase in the absorbency was observed. The hydrating degree of NaOH in aqueous solution is related to the alkali concentration. In NaOH aqueous solution, the hydrated sodium ion and hydroxide ion were embraced by H_2O molecule, and the size of ions is too large to enter into the chitin structure. As the alkali concentration increased, the size of the hydrated ion decreased, and formed dipole hydrates which diffuse and penetrate more readily into the chitin powder matrix, therefore the NaOH absorbency increased. As the alkali concentration reached 35 wt%, the water absorbency began to decrease. However, the total absorbency was still increasing. This was probably due to the different proportion of NaOH and water combined with chitin molecules.

At freezing temperature (−18 °C to −20 °C), the effect of alkali concentration on absorption behaviors is shown in Fig. 3. When soaked in 20 wt% NaOH solution, the gelation of chitin occurred, and the total absorbency reached 800 g per 100 g chitin maximally. The NaOH and water combined by hydrogen bonds with chitin molecules could not be removed by centrifugation. As shown in Fig. 2, water absorbency made the major contribution to the swelling. When frozen, the volume of water expanded which further weakened the linkages between chitin macromolecule and decrystallized chitin structure, therefore enabled more alkali solution penetrate into chitin particles and form a gel. When NaOH concentration reached 30 wt%, the total absorbency began to decrease and stabilized at 200 g per 100 g chitin.

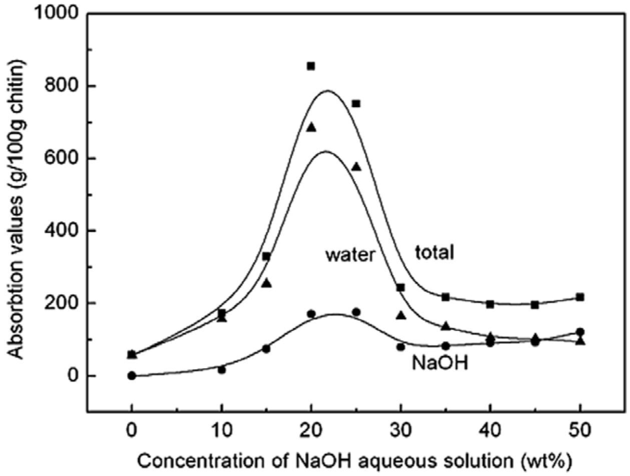

Fig. 3. Effect of NaOH concentration on the absorption behaviors of chitin soaked at freezing temperature for 4 h.

At different soaking temperatures, chitin showed different absorption behaviors. Fig. 4 shows the effect of soaking temperature on the absorption of chitin. All the absorbency values decreased as the temperature increased. The alkalization of chitin was an exothermic reaction; therefore the reverse reaction between hydroxyl groups and NaOH could be accelerated when temperature increased. To achieve the same absorbency, soaking temperature, and alkali concentration are interchangeable to a certain extent. Thus, the absorbency achieved with a 35–45 wt% alkali solution at 20 °C could also be achieved with a 30 wt% solution at 0 to −20 °C. As a result, maximum absorbency was achieved at lower temperatures with an alkali solution

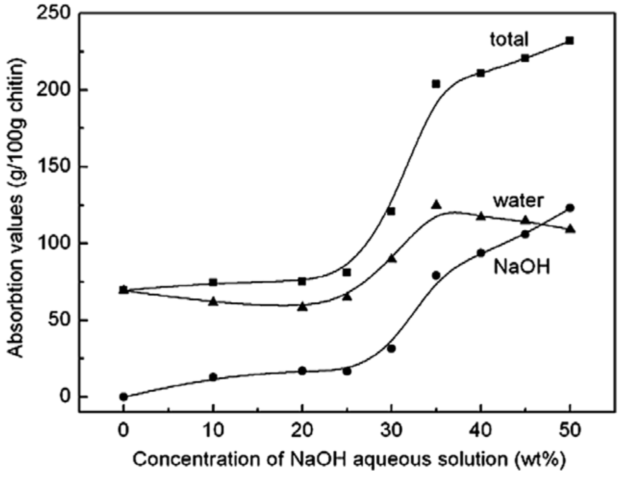

Fig. 2. Effect of NaOH concentration on the absorption behaviors of chitin soaked at room temperature for 4 h.

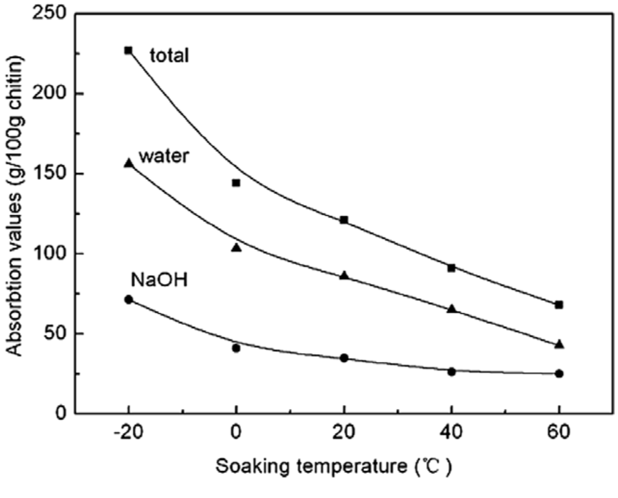

Fig. 4. Effect of soaking temperature on the absorption behaviors of chitin soaked in 30 wt% alkali aqueous solution for 4 h.

of lower concentration, as shown in Figs. 2 and 3. Also, lower temperature will be beneficial to reduce the side reactions of degradation and deacylation.

In the process for production of alkali cellulose, water-miscible inert organic diluents were used. Similarly, ethanol was added into the current system, and its influence on the absorption behavior of chitin is shown in Fig. 5. The result showed that the addition of ethanol enabled chitin to obtain higher absorbency. At the same soaking time and temperature, the absorbency in a 45 wt% alkali solution without ethanol could also be achieved with a 30 wt% alkali aqueous/ethanol solution (25/75, v/v), as shown in Figs. 2 and 5. Ethanol took effects in three ways. Firstly, ethanol could dissolve aqueous alkali in small amounts, and helped to transfer alkali from the portions of chitin which were rich in alkali to those portions which were lean in alkali. In this way, ethanol helped to distribute the alkali and water uniformly in the chitin matrix. Secondly, ethanol could reduce the size of hydrated NaOH ions, and enhance the penetration and diffusion of alkali solution in chitin matrix. Thirdly, as a mono-alcohol, ethanol could also reduce the cross links formed by hydrogen bonds between chitin molecules and water, therefore increasing the accessibility of chitin.

3.2. Changes of crystal structure and hydrogen bonds

Fig. 6 shows the X-ray diffraction patterns of chitin samples soaked in alkali solutions with various concentration at room temperature for 4 h. Five crystalline reflections were observed in the 2θ range of 5–30°. They were indexed as 020, 110, 120, 101, and 130, respectively (Wada & Saito, 2001; Zhang, Xue, Xue, Gao, & Zhang, 2005). It is noted that the peaks at 020, 110, and 120 reflections decreased dramatically when soaked in 40 wt% and 50 wt% NaOH solutions, and the crystallinity of treated chitin reduced from 51.9% for initial chitin to 29.3% and 30.8%, respectively. The X-ray diffraction pattern of chitin treated in 30 wt% NaOH solutions remained unchanged, and its crystallinity was 51.6%. Therefore, the minimum concentration required to induce decrystallization is more than 30 wt%.

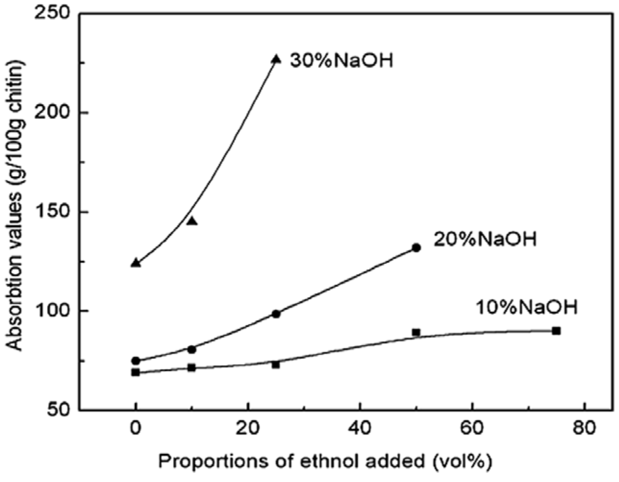

Fig. 5. Effect of ethanol on the absorption behaviors of chitin soaked at room temperature for 4 h.

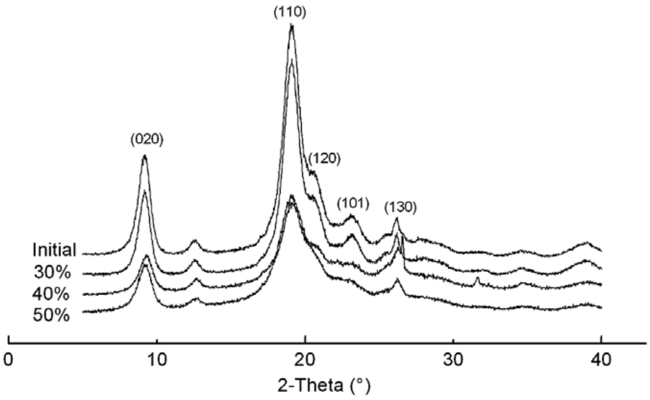

Fig. 6. X-ray diffraction patterns of initial chitin and chitin samples soaked in alkali solution with different concentrations at room temperature for 4 h.

To evaluate the changes of hydrogen bonds caused by alkali treatment, FT-IR spectra were measured and shown in Fig. 7. The bands at 3448 cm^{-1} and 3265 cm^{-1} were O–H and N–H stretching vibration, respectively. In dilute solution, free N–H stretching without the strong hydrogen-bond interaction fell in the regions of 3420–3500 cm^{-1} (Lin, Colthup, Fateley, & Grasselli, 1991). After alkali treatment, the band at 3265 cm^{-1} moved towards higher wavenumbers. The band of initial sample at 1659 cm^{-1} and 1625 cm^{-1} was attributed to the stretching vibration of C═O in amide , which was split by hydrogen bonds combining with neighboring N–H or O–H. However, in the FT-IR spectra of alkali treated samples, the double split peaks shifted to a single peak at 1660 cm^{-1}. The results indicated that the intra- and inter-hydrogen bonds were destroyed significantly by alkali treatment (Muzzarelli, 1973).

The alkali treatment may cause chemical degradation and deacylation of chitin. Therefore, the molecular weight and DA of chitin were measured after the alkali treatment, and the results are shown in Tables 1 and 2. When treated with alkali solution at room temperature within 4 h, no sig-

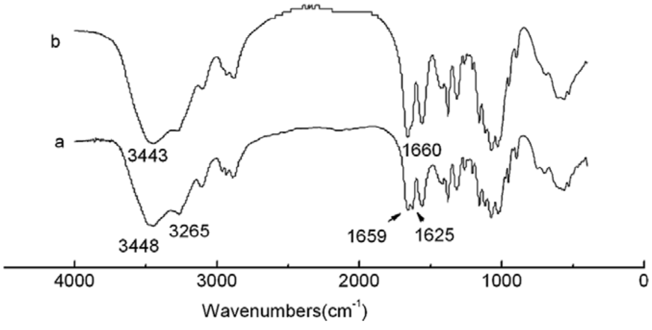

Fig. 7. FT-IR spectra of (a) initial chitin and (b) chitin treated with 40 wt% alkali solution at room temperature for 4 h.

Table 1
Molecular weight of alkali treated chitin samples

Treatment			$[\eta]$[a] (dL/g)	$\overline{M_v} \times 10^4$[b]
Alkali concentration (wt%)	Temperature (°C)	Time (h)		
Initial			20.1	51.0
30	20	4	19.3	50.0
40	20	4	19.2	48.7
40	20	19	17.0	42.8
40	40	4	14.6	36.3
50	20	4	18.9	47.5

[a] Measured in *N*,*N*-dimethylacetamide/lithium chloride at 30 °C.

[b] The constants a and K in Mark–Houwink equation were 0.95 and 7.6×10^{-5} dL/g, respectively.

Table 2
DA of alkali treated chitin samples (initial sample, DA = 89.4%)

Treating time[a] (h)	1	2	3	4	19
DA (%)	85.5	84.6	84.5	82.7	69.1
Treating temperature[b] (°C)	−20[c]	0	20	40	60
DA (%)	83.4	84.7	82.7	58.6	11.7
NaOH concentration[d] (wt%)	10	20	30	40	50
DA (%)	89.0	88.8	87.2	83.4	81.2

[a] Treated in 40 wt% alkali solution at room temperature.

[b] Treated in 40 wt% alkali solution for 4 h.

[c] Treated in 40 wt% alkali solution at −20 °C for overnight.

[d] Treated in 30 wt% alkali solution at −20 °C for overnight.

nificant decrease in molecular weight and DA of chitin was observed, which indicated that only slight depolymerization and deacetylation were induced in chitin. However, at higher treatment temperature or prolonged treating time, the chitin samples underwent extensive depolymerization and deacetylation. Therefore, in the procedure of preparing alkali chitin, higher temperature and prolonged treating time should be avoided.

4. Conclusion

The absorption behaviors of chitin in alkali aqueous solutions with a wide range of soaking time, alkali concentration, soaking temperature and the addition of ethanol were studied. The results showed that the mere absorption of alkali solution occurred instantly while the absorption equilibrium needed 4 h to be established. The absorbency increased as temperature decreased or alkali concentrations increased. The temperature and concentration of the alkali solution had a combined effect on the absorption behaviors of chitin in alkali aqueous solution and were interchangeable to a certain extent. Maximum absorbency was achieved at freezing temperature with a 20 wt% NaOH solution. The addition of ethanol increased the absorbency of chitin. X-ray diffraction patterns and infrared spectra measurements showed that chitin was decrystallized and hydrogen bonds in chitin were weakened by alkali treatment. The measurement on molecular weight and DA of chitin suggested that higher temperature and prolonged treatment time should be avoided during alkali treatment.

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