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Preparation and structure of chitosan soluble in wide pH range

Min Fan, Qiaoling Hu *, Kai Shen

Department of Polymer Science and Engineering, Zhejiang University, Key Laboratory of Macromolecule Synthesis and Functionalization, Ministry of Education, Hangzhou 310027, People's Republic of China

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ABSTRACT

Two kinds of chitosans, namely *N*-acetylated and *N*-deacetylated chitosan were prepared by the modified processes. They can dissolve in both acid and alkali solution. ^{13}C NMR was used to study the basic solution of chitosan, and XRD, FT-IR and SEM were used to study the structure of *N*-acetylated and *N*-deacetylated chitosan. The result from X-ray diffraction showed that a transformation of crystal structure occurred during the *N*-acetylation or *N*-deacetylation process with the decrease of crystallinity and expansion of crystal lattices. FT-IR spectra revealed that the intermolecular and intramolecular hydrogen bonds were destroyed by both treatments and a looser structure was observed by the SEM. The lower crystallinity, the decreased intermolecular interactions, the more disordered and looser structure were easy for the permeation of LiOH/urea aqueous solution and coordinated with the breakage of intermolecular and intramolecular hydrogen bond by LiOH at low temperature, the prepared chitosans dissolved in LiOH/urea/H_2O mixture.

1. Introduction

Chitosan, the main derivative of chitin, is an attractive linear aminopolysaccharide, composed primarily of repeating units of β-(1→4)2-amino-2-deoxy-D-glucose (D-glucosamine). As a unique natural alkali polysaccharide, chitosan has many potential applications including drug delivery, artificial skin, reinforced bone nail (Hu, Li, Wang, & Shen, 2004), absorbable suture and wound dressing (Kumar, 2000; Muzzarelli, 1994; Rinaudo, 2006) due to its distinctive properties, such as biodegradability (Xu, McCarthy, & Gross, 1996), biocompatibility (Muzzarelli,1994; Richardson, Kolbe, & Duncan, 1999; Risbud & Bhonda, 2000), non-toxicity, antibacterial activity, wound healing acceleration ability (Khor, 2001; Rinaudo, 2006). However, compared with collagen and hyaluronic acid, its natural materials counterparts, the widespread use of chitosan has been restricted. The limited utility of chitosan, principally, is the result of its insolubility at neutral or high pH region.

There are numerous intermolecular and intramolecular hydrogen bonds in chitosan molecules, which strongly stabilize the packing structure of chitosan in the three unit cell directions (Lamarque, Viton, & Domard, 2004), and make chitosan have no melting point and only dissolve in the acid pH range (Koide, 1998). Additionally, it only dissolves in some specific organic acids including formic, acetic, propionic, lactic, citric and succinic acid, as well as in a very few inorganic solvents, such as hydrochloric, phosphoric, and nitric acid (Wang, Turhan, & Gunasekaran, 2004).

The solubility of chitosan is mainly determined by three parameters: degree of deacetylation (DD), distribution of acetyl groups and degree of polymerization. By controlling the three parameters, the solubility of chitosan can be improved. A lot of research has been carried out in this field. The DD can be changed by either deacetylation with hot concentrated alkali (Sannan, Kurita, & Iwakura, 1976; Zhang, Xue, Li, Zhang, & Fu, 2006) or acetylation with acetic anhydride (Kurita, Kamiya, & Nishimura, 1991; Cho, Jang, Park, & Ko, 2000) under homogenous or heterogeneous conditions (Cho et al., 2000). Water-soluble chitosan with the DD of around 50% has already been prepared successfully by *N*-deacetylation or *N*-acetylation process. The distribution of acetyl groups was proved to be random (Kurita, Koyoma, Nishimura, & Kamiya, 1989; Kurita et al., 1991; Aiba, 1991). Chitosan with high solubility can also be produced by reducing the molecular weight of it. There are physical (Cho et al., 2000), acid-hydrolysis (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002), enzyme-hydrolysis (Nordtveit, Vårum, & Smidstrød, 1996) and irradiation degradation (Tahtat, Uzun, Mahlous, & Güven, 2007) methods to prepare chitosan with good solubility. In addition, many chemical modified chitosans with high solubility have also been prepared, such as carboxymethyl-chitosan, *N*-phthaloylchitosan, and chitosan-*g*-poly(ethylene glycol) (PEG) (Rinaudo, 2006).

However, to the authors' knowledge, the current deacetylation or acetylation process can only make chitosan soluble below pH 7.5. The chemical modification can cause chitosan soluble in wide pH range, while it has the possibility of losing the original physicochemical and biochemical activities, and bringing some toxicity by changing the fundamental skeleton of chitosan (Zhang et al., 2006).

* Corresponding author.
E-mail address: huql@zju.edu.cn (Q. Hu).

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Herein, partially *N*-acetylated, and *N*-deacetylated chitosan with relatively high molecular weight and DD were prepared in our lab by a modified process. The solutions of both chitosans in lithium hydroxide (LiOH)/urea/H_2O were obtained successfully through a freezing–thawing treatment. X-ray diffraction (XRD), ^{13}C nuclear magnetic resonance (NMR), Fourier infrared (FT-IR), scanning electron microscope (SEM) were used to study the structural change of chitosans and the dissolution behavior. The purpose of this work is to provide a new way to improve the solubility of chitosan and develop the production of chitosan materials, and to enable a broader application in wide pH range.

2. Materials and methods

2.1. Materials

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

Acetic anhydride was purchased from Shanghai Ling-Feng Chemical Reagent Co. Ltd. (China). Acetic acid, hydrochloric acid, LiOH·H_2O, NaOH, KOH and urea were obtained from Sinopharm Chemical Reagent Co. Ltd. (China). All the reagents were of analytical grade, and used without further purification.

2.2. Preparation of partially N-acetylated and N-deacetylated chitosan

N-acetylated chitosan was prepared by a modified *N*-acetylation process according to Hu, Fan, and Shen (2008). Chitosan (2 g) was dissolved in 2% (v/v) aqueous acetic acid (60 ml), and then the solution was diluted with 80 ml of methanol. After stirring for 10 min, acetic anhydride (acetic anhydride/amino group (n/n) = 1:4) was added to the diluted solution and stirred at room temperature for 15 min. Standing for 2 h, the mixture was precipitated by 0.5 M KOH/aqueous ethanol solution, filtered off, washed to neutral with deionized water and then dried at 60 °C under vacuum.

N-deacetylated chitosan was prepared under heterogeneous condition. About 10 g of dried α-chitin powder was stirred with 300 ml NaOH (55 wt%) at 90 °C in a flask for 4 h. Then the mixture was cooled by the addition of 1000 ml deionized water. After standing for one night, the solid was washed to neutral with deionized water, and then dried at 60 °C in oven.

The prepared *N*-acetylated and *N*-deacetylated chitosan were coded as CSa and CSd, respectively.

2.3. Preparation of chitosan–LiOH–urea solutions

LiOH·H_2O, urea, and deionized water with a mass ratio of 4.8:8:87.2 were mixed firstly to form a transparent solution, and then a certain amount of dried *N*-acetylated and *N*-deacetylated chitosan powder (2 wt%) were added and mixed. After freezing-thawing between −40 and 20 °C, the transparent solutions were obtained.

2.4. Measurement

The DD 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 (DD) of the chitosan (Raymond, Morin, & Marchessault, 1993).

The M_η of prepared samples was calculated from the classical Mark–Houwink relationship, $[\eta] = kM^\alpha$ (1) where $[\eta]$ is the intrinsic viscosity, $\alpha = -1.02 \times 10^{-2} \times DD + 1.82$, $k = 1.64 \cdot 10^{-30} \times DD^{14}\ cm^3 \cdot g^{-1}$. The intrinsic viscosity was measured with an Ubbelonde viscometer at 30 °C using 0.2 M CH3COOH–0.1 M CH3COONa as solvent (Roberts, 1992). The solutions were filtered through a P30 glass filter before determining $[\eta]$.

XRD and FT-IR were used to characterize the changes of crystal structure and hydrogen bonds in chitosans during modification and dissolution, respectively.

XRD patterns of powdered samples were obtained using a Bruker AXS D8 Advance X-ray diffract meter, 40 kV and 34 mA with Cu Kα 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 equation (Zhang, Haga, Sekiguchi, & Hirano, 2000) CrI (%) = $k \times I_c/(I_c+I_a) \times 100\%$ (2), where I_c, and I_a represent the diffraction intensity of crystalline region, and diffraction intensity of amorphous region, respectively. k is a coefficient.

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 to 500 cm^{-1}.

SEM (S-4800, Hitachi) was used to observe the morphology change of prepared chitosan, and the sample was treated by spray-gold before observation.

^{13}C NMR spectra of 4.8 wt% LiOH/8.0 wt% urea/D_2O and CS solution in LiOH/urea/D_2O and LiOH/D_2O were carried out on a NMK-300 MHz NMR spectrometer (Varian, Inc., USA) at ambient temperature. The chemical shifts were referenced to internal sodium 3-(trimethylsilyl)-1-propanesulfate (DSS).

The solubility of CSa and CSd at different pH was estimated from the transmittance of their solutions. A UV–vis spectrophotometer using a quartz cell with an optical path length of 1 cm at 600 nm was used to record the transmittance of the solutions (Kubota, Tatsumoto, Sano, & Toya, 2000). The sample concentration was 5 mg/ml. The pH value of the solution was adjusted by the stepwise addition of concentrated HCl.

3. Result and discussion

3.1. Dissolution of chitosan in LiOH/urea aqueous solvent

Fig. 1 shows the images of 2 wt% chitosan in aqueous 4.8 wt% LiOH/8.0 wt% urea, treated by freeze–thaw process. The result indicates that both the CSa and CSd can dissolve in LiOH–urea aqueous solution through a frozen process, while the commercial chitin and chitosan can only swell slightly. Obviously, the structural change of chitosan during the *N*-acetylation or *N*-deacetylation process plays a crucial role. Subsequently, more details will be given.

3.2. Effect of LiOH in the dissolution

^{13}C NMR spectra of solutions of CSd and CSa in 4.8 wt% LiOH/8wt% urea/D_2O and in 4.8 wt% LiOH/D_2O are shown in Fig. 2. Basically, CSd has similar ^{13}C NMR spectra to CSa except for the weakness of several peaks, which perhaps due to their different structure and DD.

The chemical shifts of C1 (105.3p.p.m), C4 (81.7p.p.m), C6 (78.7p.p.m), C3 (77p.p.m), C6 (63.6p.p.m), C2 (60.1p.p.m) and –CH_3 of acetyl groups (19.9p.p.m) for CSd and CSa in 4.8 wt% LiOH/8.0 wt% urea/D_2O are similar to those of chitosan in hydrochloride (HCl) (Vårum, Anthosen, Grasdalen, & Smidsrød, 1991), trifluoroacetic (CF_3COOH) (Hasegawa, Isogai, Onabe, & Usuda, 1992), which are good solvents for chitosan. There is no new peak

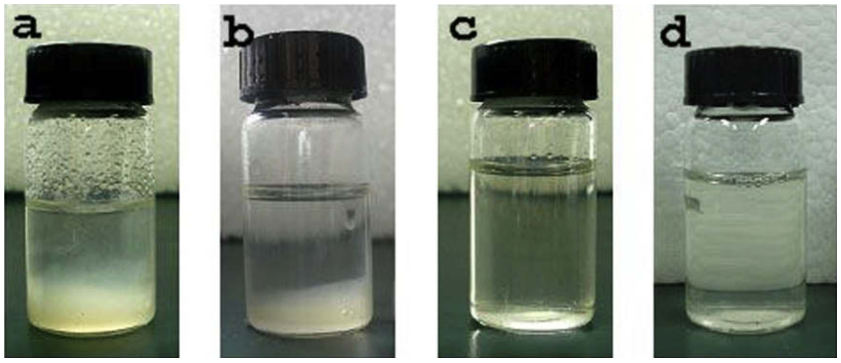

Fig. 1. Solutions of 2 wt% chitosan in 4.8 wt% LiOH/ 8 wt% urea/H_2O. (a) Commercial chitosan (b) commercial chitin (c) CSd (d) CSa.

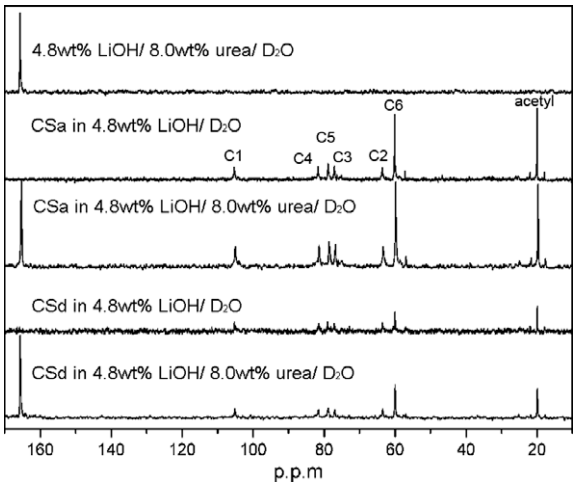

Fig. 2. ^{13}C NMR spectra of CSd in 4.8 wt% LiOH/D_2O and in 4.8 wt% LiOH/8 wt% urea/D_2O at ambient temperature.

in the obtained ^{13}C NMR spectra. The results show that chitosan can be dissolved in 4.8 wt% LiOH/8.0 wt% urea aqueous solution with no derivatization. It is notable that the chemical shifts of the CSd and CSa solutions in LiOH/urea/D_2O are similar to those in LiOH/D_2O, suggesting that LiOH mainly contributes to the breakage of intramolecular and intermolecular hydrogen bonds in chitosan, and causes it soluble in LiOH/urea/H_2O mixture.

3.3. Structure changes during the N-acetylation or N-deacetylation process

It is interesting that commercial chitin and chitosan can swell only slightly in aqueous LiOH–urea solution, while the *N*-acetylated and *N*-deacetylated chitosan can completely dissolve through freezing–thawing process. What's the difference between them leading to the dissolution? To evaluate the changes of crystal structure and hydrogen bonds caused by *N*-acetylation and *N*-deacetylation treatment, SEM, X-ray diffraction, and FT-IR are used and the results are shown in Figs. 3–5, respectively. The changes of DD, M_η, CrI and *d*-spacing are summarized in Table 1.

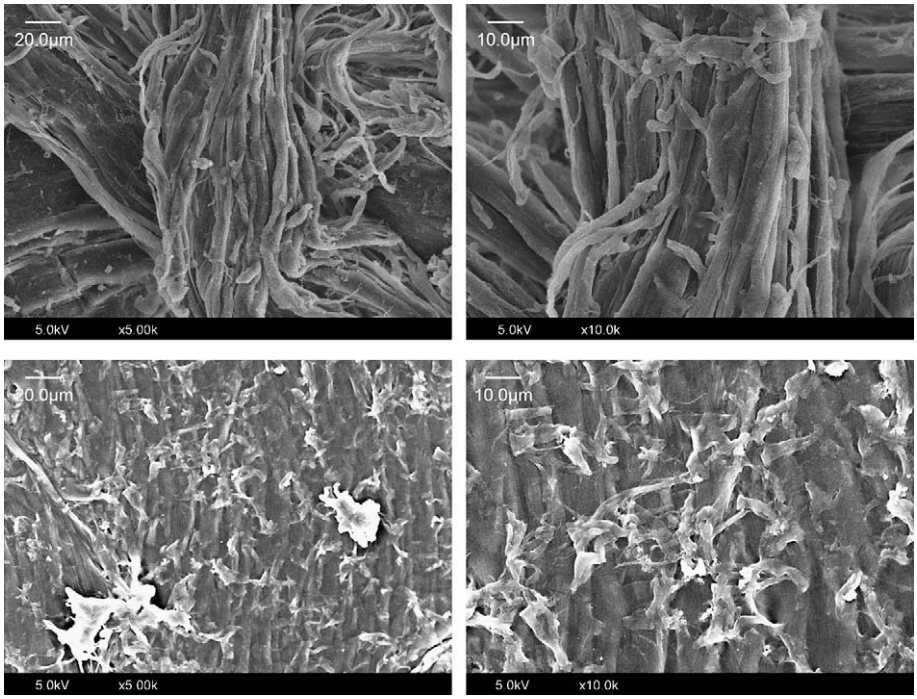

Fig. 3. SEM micrographs of powder chitin and CSd. Chitin: a-5000×, b-20,000×; N-deacetylated chitosan: c-5000×, d-20,000×.

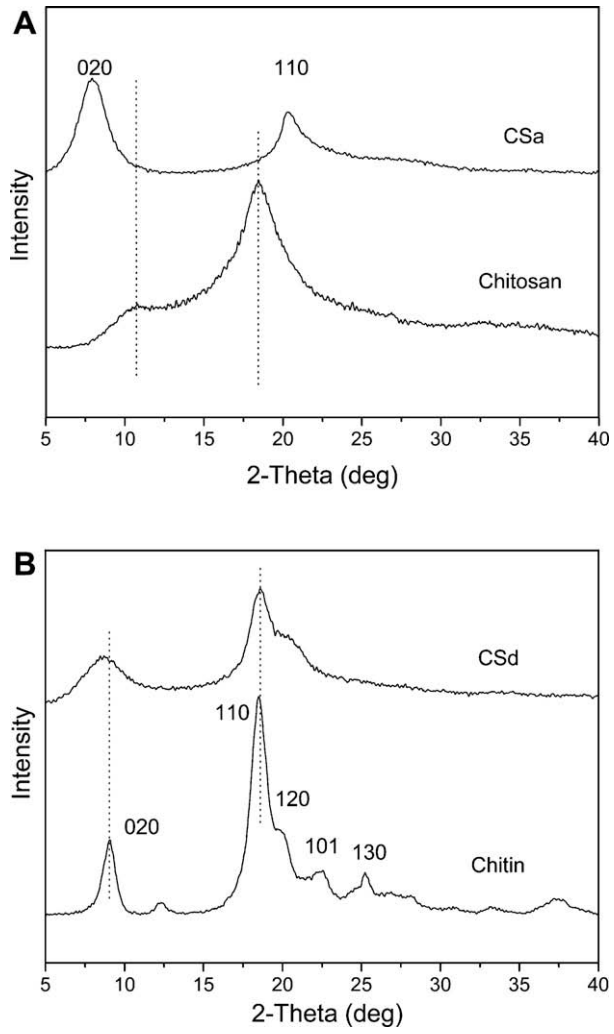

Fig. 4. XRD spectra of (A) original chitosan and CSa, (B) original chitin and CSd.

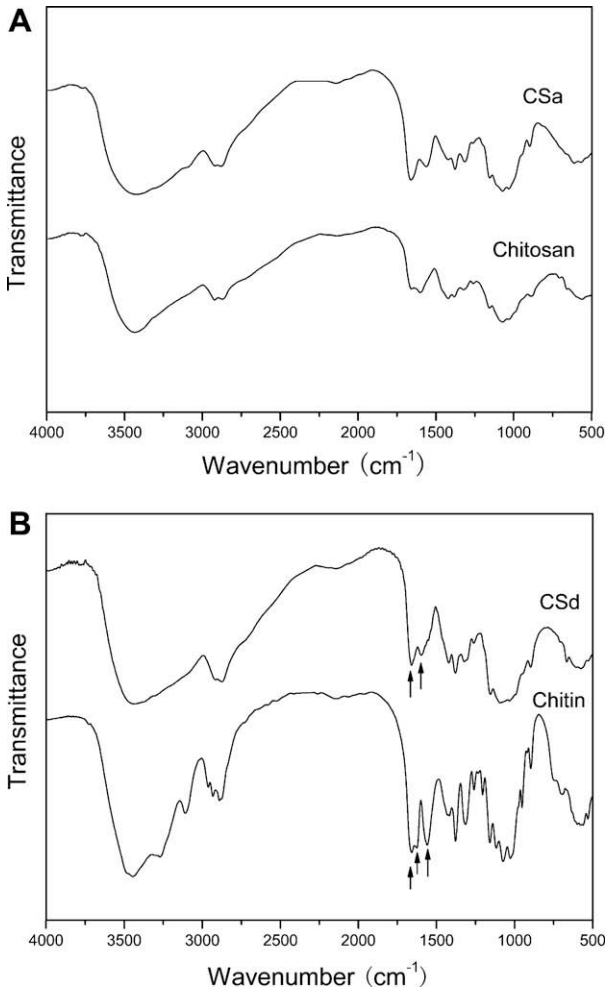

Fig. 5. FTIR spectra of (A) commercial chitosan and CSa, (B) commercial chitin and CSd.

3.3.1. Observation of the structure change by SEM

Fig. 3 gives the SEM micrographs of chitin powder and its *N*-deacetylated derivative, CSd. As shown in the graphs, there is a significant change in the structure of CSd in comparison to the original chitin. The crystal of original α-chitin is formed by large microfibril bundles with an organized rigid network. However, after the modified *N*-deacetylation process we proposed, the microfibril bundles with the diameter around 6 μm of the initial chitin particles were broken, and turned into much finer microfibrils with a diameter around 2 μm. Moreover, numerous micro voids formed during the *N*-deacetylation treatment. The looser structure of CSd is more beneficial to the permeation of LiOH and urea hydrates.

3.3.2. Crystal transformation by XRD

As can be seen from Table 1, the DD value of CSa decreases, while for the CSd, its DD increases, suggesting an introduction or a losing of bulk acetyl groups through the *N*-acetylation or *N*-deacetylation process, respectively. Viscosity-average molecular weight (M_η) of CSa decreases slightly, indicating that chitosan chains underwent a slight degradation during the *N*-acetylated treatment. Transformation of the crystal of chitin and chitosan occurred with the CrI decreasing dramatically and *d*-spacing increasing.

Fig. 4 shows the XRD patterns of chitosan with its *N*-acetylated derivative CSa and chitin with its *N*-deacetylated derivative CSd.

Table 1
Characteristics of original chitin, chitosan and prepared chitosan.

Sample	DD (%)	M_η/Da	CrI (%)	*d* (Å)	
				0 2 0	1 1 0
Chitosan	89.4	5.6×10^5	66.84	8.50	4.31
CSa	65.6	4.3×10^5	47.56	11.06	4.37
Chitin	–	–	79.29	9.70	4.79
CSd	80.2	2.7×10^5	45.73	10.23	4.78

For the chitosan and CSa, two crystalline reflections are observed in the 2θ range of 5–40°. With the decrease of DD, the intensity of reflection at around $2\theta = 10.7°$ increases remarkably with a shift to a lower angle, from 10.7° to 7.9°. As for reflection at about $2\theta = 18.5°$, it shifts to a higher angle, with the intensity decreasing significantly. The original chitosan shows the strongest reflection at $2\theta = 18.5°$, coinciding with the pattern of the "Form II" crystal, while CSa shows the strongest reflection at $2\theta = 7.9°$, much like the structure of "Form I" crystal, although the *d*-spacing ($d = 11.06$ Å) value is bigger than "Form I" ($d = 7.76$ Å) (Gao, Wan, & Zhang, 2007). The results indicate that a crystal structure transition occurs during the *N*-acetylation. Specifically speaking, the "Form II" crystal with constrained chain conformation of chitosan has converted to "Form I" crystal with a more extended chain structure, much of the structural compactness has been lost.

As for the chitin and CSd, five crystalline reflections are observed in the 2θ range of 5–40°. They were indexed as 0 2 0, 1 1 0, 1 2 0, 1 0 1, and 1 3 0, respectively (Wada & Saito, 2001; Zhang, Xue, Xue, Gao, & Zhang, 2005). It is notable that the maximum peak of intensity at 1 1 0 reflection (2θ = 18.5°) decreases with the increase of DD, and the second maximum peak of intensity at 0 2 0 reflection (2θ = 9.1°) also decreased with the increase of DD, with a shift to a lower angle 8.3°. The crystal structure of CSd is rather like that of chitosan than chitin, although the crystallinity of CSd is lower than that of chitosan. Both the shift to a lower angle of crystallization peaks in CSa and CSd is attributed to the expansion of the crystal structure.

By peak fitting the diffraction profiles, we calculated the accurate *d*-spacing and CrI of each sample, as shown in Table 1. It is found that the *d*-spacing of CSa and CSd increases compared with chitosan and chitin, respectively. Obviously, the CrI decreases. The results indicate that there are expansions of the crystal lattices in CSa and CSd due to the change of contents of the bulky acetyl groups in both chitosan and chitin. The ordered structures of initial materials are destroyed by the *N*-acetylation or *N*-deacetylation process, resulting in the lower crystallinity (Li, Revol, & Marchessault, 1997).

3.3.3. Change of hydrogen bonds

Infrared spectroscopy is useful in studying hydrogen bonding and other interactions, to evaluate the changes of hydrogen bonds caused by *N*-acetylation and *N*-deacetylation treatment. FT-IR spectra were measured and shown in Fig. 5. The wavenumbers of the IR absorption bands in the region from 4000 to 500 cm^{-1} were consistent with the assignments for α-chitosan (Blackwell, 1988) and α-chitin (Brugnerotto et al., 2001).

The bands of initial chitosan at 1658 and 1600 cm^{-1} are attributed to the stretching vibration of C=O and deformation of $-NH_2$. For chitosan and CSa, *N*-acetylation is associated with a progressive strengthening of the band occurring at 1660 cm^{-1}, and a shifting of the band at 1600–1562 cm^{-1}. The results indicate that *N*-acetylation has introduced acetyl groups, causing an increase of disordered structure (Focher, Naggi, Torri, Cosanni, & Terbojevich, 1992). For chitin and CSd, the amide I band, which is split into two peaks, probably by the hydrogen bonds of C=O with the hydroxymethyl group (Rinaudo, 2006), significantly weakens, and turns into one peak, indicating a losing of both acetyl groups and hydrogen bonds. The peaks at 3270 cm^{-1} and 3108 cm^{-1} assigned to the intermolecular and intramolecular hydrogen bonds in the original α-chitin disappears in the CSd, suggesting the decrease of both intermolecular and intramolecular hydrogen bonds in chitin molecules.

The broad band at 3440 cm^{-1} in both CSa and CSd, which is assigned to stretching vibrations of –OH and –NH, becomes broader and moves to lower wavenumber slightly because of the change in intermolecular and intramolecular hydrogen bonds (Maréchal & Chanzy, 2000; Ngono, Maréchal, & Mermilliod, 1999).

The results show that the association of molecules through hydrogen bonds and the crystal structures of chitin and chitosan are destroyed by *N*-acetylation or *N*-deacetylation treatment, leading to much more free hydroxyl groups, amino groups and the polymer chain end (Gocho, Shimizu, Tanioka, Chou, & Nakajima, 2001). The result of FT-IR is consistent with experimental evidence obtained from the X-ray diffraction, previously discussed.

As mentioned before, the solubility of chitosan is mainly determined by the DD, the distribution of acetyl groups on the molecule backbone and the molecular weight. It was found that chitosan with less acetyl groups and chitin with more acetyl groups can readily form well ordered arrangements (Ng, Hein, Ogawa, Chandrkrachang, & Stevens, 2007).

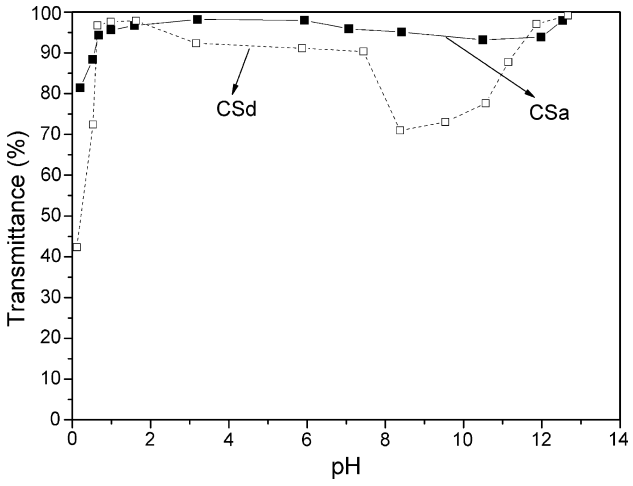

Fig. 6. Effect of pH on the solubility of CSa and CSd.

The penetration and diffusion of the alkali solution occurs from more amorphous regions to less amorphous regions (Ng et al., 2007). Due to the highly crystal structure and intra- and inter-hydrogen bonds, the penetration and diffusion of the alkali solution is difficult. The expanded crystal planes and increased amorphous region, the destroyed intermolecular and intramolecular hydrogen bonds, as well as the disordered and looser structures of CSa and CSd are of great help to small molecules such as water, LiOH hydrates and urea hydrates to impregnate and permeate thoroughly. Then when being frozen, the volume of water expands, which further weakens the linkages between chitosan macromolecules and decrystallized the structure. It therefore enables more alkali solution to penetrate into chitosan particles and finally form a transparent solution. Moreover, the solution of chitosan in LiOH/urea aqueous solvent is more stable than that of chitosan in 0.2 M HCl. The glycosidic (1→4) – linkages in cellulose are found to be alkali stable when the temperature is lower than 170 °C (Knill & Kennedy, 2003), chitosan has a very similar backbone to cellulose, and therefore, chitosan is also alkali stable at ambient temperature.

3.4. Solubility of CSd and CSa at different pH values

Fig. 6 shows the transmittance of the solution of CSa and CSd at different pH values. The solution of CSa is transparent when the pH >0.7, and the solution becomes cloudy when the pH <0.7, suggesting that CSa is soluble under the acid, neutral and basic conditions. Compared to CSa, the transmittance of the solution of CSd shows a different change. The solution of CSd is transparent in the pH range from 0.6 to 7.4 and 11.8 to 12.7, and becomes opaque when the pH <0.6 or between 7.4 and 11.2, suggesting that CSd is soluble under acid and strong basic conditions, but insoluble under alkalescent conditions. The different solubility of CSa and CSd perhaps results from their structural difference. CSd has less acetyl groups and less random distribution of acetyl groups than CSa. Therefore, CSd can form better ordered arrangement with van der waals force and hydrogen bonds than CSa (Zhang et al., 2006), which results in weaker solubility of CSd. It could be the reason why CSd is insoluble under weak basic condition while CSa is soluble.

4. Conclusion

N-acetylated chitoan from chitosan and *N*-deacetylated chitosan from chitin are prepared in the lab by the modified processes. It is found that both the *N*-acetylated and *N*-deacetylated chitosan

can dissolve in 4.8 wt% LiOH/8.0 wt% urea aqueous solution without derivatization, and the change in structure plays an important role in their dissolution behavior. XRD, FT-IR and SEM are used to study the transformation of the microstructure. It seems that the solubility of CSa and CSd in alkali solvent is attributed to the decrease of intermolecular interactions, the lower crystallinity, and the more disordered and looser structure, which are easier for the permeation of water, LiOH hydrates and urea hydrates. At low temperature, LiOH further breaks the intramolecular and intermolecular hydrogen bonds in CSa and CSd, and leads to the dissolution of CSa and CSd in LiOH/urea/H_2O mixture and the forming of a stable solution. The result may contribute to a new method of development for the production of chitosan materials and enable a broader application in wide pH range.

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