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Preparation of half-deacetylated chitosan by forced penetration and its properties

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Abstract

A novel rapid preparation of half-deacetylated chitosan with relatively large molecular weight was studied. Chitin from shrimp was first undergone forced penetration in vacuum and then deacetylated using concentrated NaOH with control of the reaction time. The degree of deacetylation (DD) was monitored using potentiometric titration and ^{1}H NMR. The half-deacetylated chitosan showed a distinct change in Scanning electronic microscopy (SEM) compared to chitin material, and behaved viscous and translucent in water. They were turned into microfibrils with a diameter of around 1 μm, which facilitate near homogeneous deacetylation. After degradation by pectinase, they were soluble in aqueous solution at all pH. The results of Intrinsic viscosity, FT-IR, and powder X-ray diffraction (XRD) show that the molecular weight and DD are collectively responsible for the solubility in the condition of random deacetylation of acetyl groups, which resulted from the intermolecular force.

Keywords: Chitin; Chitosan; Solubility; Penetration; Crystalline index; Molecular weight; Degree of deacetylation

1. Introduction

Chitin (poly-β-(1 → 4)-*N*-acetyl-D-glucosamine) is a natural polysaccharide found particularly in the shells of crustaceans such as crab and shrimp, the cuticles of insects, and the cell walls of fungi. It is the second most abundant polysaccharides receiving worldwide interest for its industrial, agricultural and medical applications. However, its application is quite restricted for its limited affinities with solvents due to the strong intermolecular hydrogen bonding and rigid crystalline structure. Chitosan, its deacetylated derivative, is soluble in aqueous medium in the presence of a small amount of acids such as AcOH, lactic acid, HCl and so on, but precipitates at neutral or high pH regions. Consequently, various studies were conducted to make water-soluble derivatives of chitin or chitosan by partial deacetylation, acetylation or other chemical modification (Kurita, 2001). The latter has the possibility of losing the original physicochemical and biochemical activities, and bring some toxicity by changing the fundamental skeleton of chitin and chitosan. It is known that chitosan with about 50% degree of N-deacetylation (DD), produced by homogeneous acetylation of chitosan, was soluble in water for its random distribution of *N*-acetyl groups on the amino groups (Aiba, 1991; Kurita, Kamiya, & Nishimura, 1991), the lower the molecular weight, the higher the solubility (Kubota, Tatsumoto, Sano, & Toya, 2000). These methods seem more tedious and expensive than that by deacetylation of chitin. However, heterogeneous deacetylation concentrated in the amorphous region of chitin to give block-type distribution of acetyl groups along the polymer chain (Kurita, Sannan, & Iwakura, 1977), which resulted from its partially swollen with sodium hydroxide solution and thus led to locally impotent deacetylation (Ottoy, Varum, & Smidsord, 1996). Sannan, Kurita, and Iwakura (1976) reported a homogeneous deacetylation of chitin, but it

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was time-consuming (over two days) and procedure-complicated. In present work, we report a new method to prepare half-deacetylated chitosan, which is rapid, simple, low-cost and efficient.

2. Experimental

2.1. Materials

Chitin powder (17%DD by FT-IR, particle size <80 mesh) from shrimp was supplied by Laizhou Haili Marine Biotechnology Co. Ltd., China, and pectinase (*Aspergillus niger*) was purchased from Sigma.

2.2. Preparation of deacetylated chitosan

Chitin from shrimp was mixed with 45% sodium hydroxide solution at a ratio of 1:10, removing air bubbles present in the chitin thoroughly by vacuum. The samples were then put in a thermostated water bath kept at 84 °C, stirred for 15, 20, 45 min, and 10 h, respectively, after which they were cooled immediately in cold water to room temperature and centrifuged for 15 min at 5000 rpm to remove the supernatant of concentrated sodium hydroxide solution. The precipitate was washed with aqueous 70% EtOH until it was neutral, and then washed with anhydrous EtOH to remove the residual water, air-dried at 60 °C overnight and finally stored in a desiccator with silica gel.

2.3. Enzymatic hydrolysis

Five milligram per milliliter deacetylated chitosan in pH 4.4, 0.2 mol/L CH_3COOH/0.1 mol/L CH_3COONa buffer solution was added with pectinase (25 μg protein/75 mg substrate), incubated for 3.5 h, heated to inactivate the enzymatic activity, dialyzed against deionized water, and then lyophilized.

2.4. Viscosity measurement

The molecular weight (M) of chitosan was measured by the viscometer based on the well-known Mark–Houwink equation $\eta = \kappa M^{\alpha}$. The intrinsic viscosity (η) of chitosans were measured with an Ubbelonde viscometer in 0.2 mol/L CH_3COOH/0.1 mol/L CH_3COONa aqueous solution at 30 ± 0.1 °C. The κ and α in the equation have been determined in the literature and were adjusted by the DD according to the following equation (Wang, Bo, Li, & Qin, 1991): $\kappa = 1.64 \times 10^{-30} \times \mathrm{DD}^{14}$, $\alpha = -1.02 \times 10^{-2} \times \mathrm{DD} + 1.82$, where DD is expressed as the percentage, suitable for the determination of chitosan with over 60%DD.

2.5. Degree of deacetylation measurement

The DD of chitosan samples were measured by alkaline titration method and ^{1}H NMR spectroscopy.

In the potentiometric titration method, we followed Jia and Li's method (2001) with a slight modification. About 0.100 g sample was dispersed in 10 ml of deionized water, mixed with 0.10 N HCl of standard solution, well dissolved and then titrated with 0.10 N NaOH of the standard solution using a pH meter (PHS-25, Shanghai) equipped with a glass electrode.

^{1}H NMR spectra were recorded on a JNM-ECP 600 MHz spectrometer (Japan Electronic Optic Laboratry). Each sample was air-dried (Zhang, Xue, Xue, Gao, & Zhang, 2005) and then immediately dissolved in D_2O containing 1% DCl, at a concentration of 10 mg/ml. The experiments were based on Lavertu's method (2003) and run at 70 °C. The chemical shifts are given on the δ scale relative to sodium-2.2-dimethy1-2-silapentane-5-sulfonate (DSS). DD was calculated using the following equation: DD (%) = 100 × H1D/(H1D + HAc/3), where H-1D and HAc are the integrals of the peak of the H-1 anometric proton of deacetylated monomer (H-1D) and of the peak of the three protons of *N*-acetyl group (H-Ac), respectively. This method is one of the most powerful absolute techniques, allowing a direct determination of DD, where the solvent (HOD) peak does not interfere with those of H-1D and HAc.

2.6. Estimation of solubility

Water solubility of the deacetylated chitosans were evaluated from the turbidity. The transmittance of the solution was recorded on a UV–vis spectrophotometer using a quartz cell with an optical path length of 1 cm at 600 nm (Kubota et al., 2000). The pH dependence of the water solubility of these chitosans were estimated from the transmittance of the solution, which were previously prepared with 0.2 mol/L acetate buffer by the stepwise addition of concentrated NaOH. The sample concentrations were 5 mg/ml.

2.7. Scanning electronic microscopy (SEM)

The morphology of half-deacetylated chitin was observed on a scanning electron microscope (SEM) (JSM-6700F, Japan Electron Optic Laboratory) after gold coating.

2.8. Powder X-ray diffraction (XRD)

X-ray diffraction patterns on powders were obtained using a Bruker AXS D8 Advance X-ray diffractometer 40 kV and 40 mA with Cu Kα_1 radiation at λ 1.54184 Å. The relative intensity was recorded in the scattering range (2θ) of 5–40° with steps of 0.1°, 0.1° per second, and the crystalline index (CrI; %) was determined by using the equation $\mathrm{Cr}I = (I_{11\mathrm{O}} - I_{\mathrm{am}}) \times 100/I_{110}$, where I_{110} is the maximum intensity at around about 20°, and I_{am} is the intensity of amorphous diffraction at 16°. (Focher, Beltranme, Naggi, & Torri, 1990; Kuma, Varadaraj, Lalithac, & Tharanathan, 2004).

2.9. FT-IR spectra

FT-IR spectra were obtained by Impact 360 FT-IR spectrometer under dry air at room temperature using KBr pellets. The samples were dehydrated as 2.5 described, and then cooled in a desiccator with CaO. DD of chitin material was calculated according to the following equation: $DA = 100 \times (1 - (A_{1655}/A_{3450})/1.33)$ which was derived for these absorbances (Brugnerotto et al., 2001; Domard & Rinaudo, 1983; Duarte, Ferreira, Marvao, & Rocha, 2002). Crystalline Index (CrI) was calculated from the ratio of absorbances at A_{1382}/A_{2920} cm^{-1} (Focher et al., 1990).

3. Results and discussion

3.1. Optimization of preparing half deacetylated chitin

3.1.1. Effect of vacuum penatration on the deacetylation of chitin

Chitin molecules from shrimp, mostly α-form, is aligned in an antiparallel fashion with strong intermolecular hydrogen boning, and formed as a lattice of highly crystalline microfibrils with an organized rigid network (Gow, Gooday, Russell, & Wilson, 1987). Chitin can be dissolved into aqueous NaOH as the alkoxide form (Kurita, 2001), which is the basis of homogeneous deacetylation. However, it takes a long time for chitin to completely swell into high viscous concentrated NaOH because of the pore blockage in chitin particles and the internal and external diffusional mass transfer effects. As a result, the molecules at the surface of the particles are more deacetylated than those at the centre of the particles, and thus induced heterogeneity of DD. Under vacuum, the air in chitin particles were pumped out; the mass transfer of NaOH was accelerated, and in turn, the swollen procedure was readily completed. Fig. 1 showed a distinct change of deacetylated chitin by vacuum penetration for about 1 h with respect to the powdered chitin material as a control. Interestingly, the chitin particles after deacetylation were turned into microfibrils with a diameter of around 1 μm, which facilitate homogeneous deacetylation.

3.2. Effect of reaction time on the deacetylation of chitin

The swollen chitin sample thus obtained was deacetylated in aqueous NaOH solution to enable the reaction to proceed under the same and homogeneous condition at 84 °C for different time, as shown in Table 1. The degree of deacetylation was increased with the increase of time, but crystalline index was decreased. Considering that the K and α in Mark–Huwink equation with DD were limited at DD > 60%, the molecular weights below 60% DD, in present work can only be estimated as the minimum values

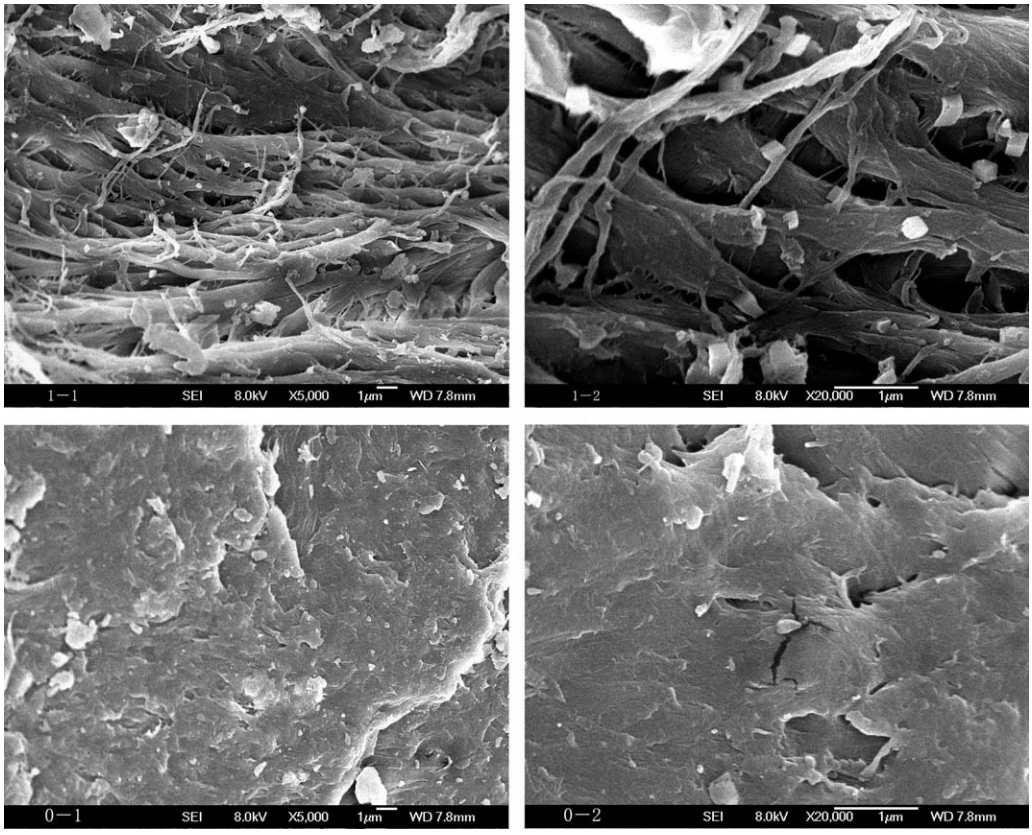

Fig. 1. SEM micrograph of half deacetylated chitin by vacuum penetration for about 1 h with respect to the powdered chitin matrix as a control. chitin matrix: 0–1, 5000× and 0–2, 20,000×; half-deacetylated chitosan: 1–1, 5000× and 1–2, 20,000×.

Table 1
Effect of reaction time on deacetylation of chitin

Sample	Time (min)	[η] ml/g	M (Da) (×10^{-5})	Degree of deacetylation (DD, %)		Crystalline index (%)	
				Titration	^{1}H NMR	FT-IR	XRD$_{110}$
No.0	Chitin	–	–	–		77	90.7
No.1	15	385(172)[b]	18.1[a]	49.51 ± 0.47[c]	49.8	41(33)[b]	63.5(27)[b]
No.2	15	473(198)[b]	18.1[a]	50.70 ± 0.28[c]	50.2	39(31)[b]	62.9(35)[b]
No.3	20	525(99.1)[b]	17.8[a]	56.84 ± 0.35[c]	57.4	42(34)[b]	62.8(38)[b]
No.4	45	737(192)[b]	17.4(5.35)[b]	68.26 ± 0.87[c]	66.8	36	58.7
No.5	600	851(283)[b]	9.32(2.89)[b]	84.74 ± 0.57[c]	86.4	29	59.9

[a] Represent the estimated molecular weight based on No.4 with 45 min of reaction time.
[b] Data in parenthesis beside No.1, No.2, No.3, No.4 and No.5 are related to corresponding enzymatic hydrolytes, No.6, No.7, No.8, No.9 and No.10, respectively.
[c] Mean ± s, $n = 3$.

based on that obtained at 45 min of reaction time, since the oxidative degradation could not be neglected within 45 min.

3.3. Solubility

Chitin and chitosan are macromolecules with different content of acetyl groups. The molecular weight and DD are collectively responsible for the solubility in the condition of random deacetylation of acetyl groups, as shown in Fig. 2. Since the apparent dissociation constant (pK_a) of chitosan increased with the increase of acetyl groups (Du, 2001), and that pK_a of chitosans with lower DD access to neutral, it is not impossible for half deacetylaed chitosan to dissolve in aqueous solution at around pH 7, resulting from the static electric repulsion among molecules. On the other hand, the higher molecular weight is, the more intensive intermolecular force, and in turn, the less solubility. Chitosan with less acetyl groups and/or chitin with more acetyl groups can readily form well ordered arrangement with van der waals force and hydrogen bonding, to some extent, exceeding the intramolecular chemical bonding (He, Chen, & Dong, 2001). It could be the reason why they are insoluble in water of alkali pH, and why chitin cannot be solubilized in acidic medium in relation with the insufficient protonated amino groups. As for half deacetylated chitosans, irregular molecular arrangements results in the least intermolecular force, as demonstrated by the least intrinsic viscosity among other chitosans with higher DD, as shown in Table 1. Thus they displayed viscous and translucent in water, indicating that some fractions with lower molecular weight were soluble in water; the others with high molecular weight were in swollen state, and then dissolved in concentrated alkali medium, which behaved the nature of chitin (Kurita, 2001). After enzymatic degradation by pectinase (Kittur, Kumar, & Tharanathan, 2003; Kittur, Kumar, Gowda, & Tharanathan, 2003) for 3.5 h, respectively, the hydrolyzed chitosan samples with around half-DD were soluble at all pH (Sample 6, 7 and 8), displayed in Fig. 2; in addition, the other two hydrolytes with higher DD (Sample 9 and Sample 10) were soluble at around pH 7 of aqueous solution but insoluble in alkali range, implying the high maintenance of intermolecular force.

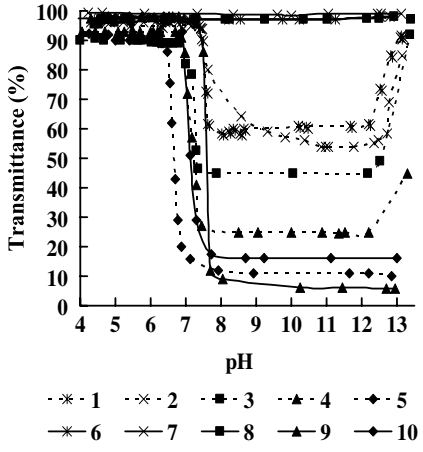

Fig. 2. Effect of pH on the solubility of chitosan with different degree of deacetylation and molecular weight. Sample numbers are described below Table 1.

3.4. Spectra comparison of X-ray diffraction

Fig. 3 shows the X-ray diffraction profiles of chitin with its deacetylated derivatives displayed in Table 1, and enzymatic degraded chitosans (No.6, No.7 and No.8). Five crystalline reflections were observed in the 2θ range of 5–40°. They were indexed as 020, 110, 120, 101 and 130 from the lower angle (Feng, Liu, & Hu, 2004; Li, Revol, & Marchessault, 1997; Wada & Saito, 2001). It was noted that the intensity of 020 reflection, decreased with the increase of DD, which correlated with the crystallinity. Focher et al. (1990) proposed a crystalline index (CrI_{110}) expressed as $\mathrm{Cr}I_{110} = (I_{110} - I_{am}) \times 100/I_{110}$ suitable for the estimation of crystallinity. By peak fitting of the diffraction profiles in Fig. 3, we calculated the accurate *d*-spacing, as shown in Fig. 4. It was found that the *d*-spacing change of the (020) plane is the most obvious at around half DD, indicating there were more expansions of the crystal lattices than that of lower or higher DD. In the case of enzymatic degraded chitosans, their *d*-spacing increased to over

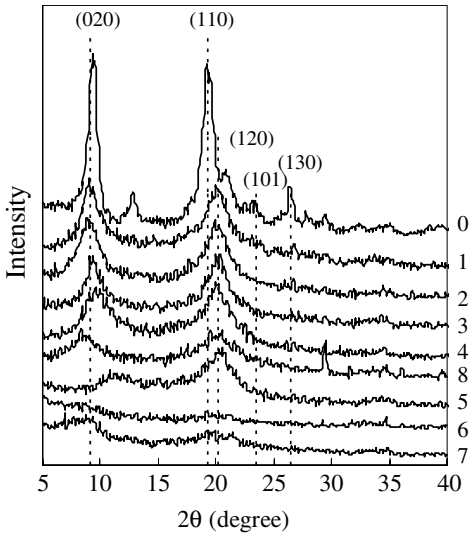

Fig. 3. XRD spectra of chitosans with different degree of deacetylation and molecular weight. The sample numbers are described below Table 1.

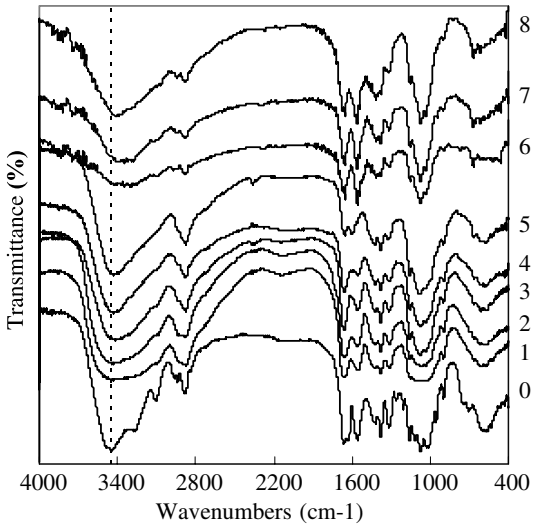

Fig. 5. FT-IR spectra of chitosans with different degree of deacetylation and molecular weight. The sample numbers are described below Table 1.

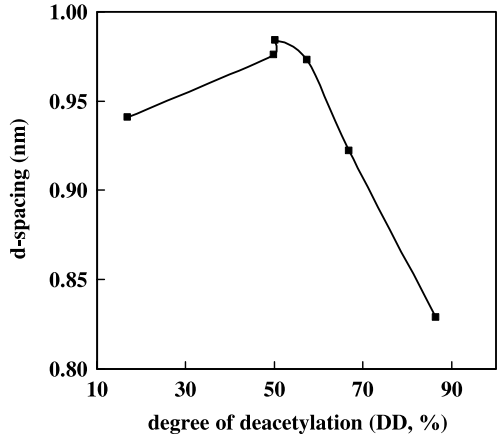

Fig. 4. Relationship of *d*-spacing at (020) plane with degree of deacetylation of chitin and chitosans.

1 nm, and CrI_{110} decreased distinctively with decreasing molecular weight, as shown in Table 1, and thus resulted in water-soluble derivatives with less intermolecular force.

3.5. Spectra comparison of FT-IR

The FT-IR spectra (Fig. 5) of chitin exhibited broad peaks at around 3450 cm^{-1} assigned to OH stretching, which became broader and moved to a lower frequency with increasing DD up to around 50% DD (Samples 1, 2 and 3), and with their decreasing molecular weight, indicating an increase in the disordered structure (Focher, Naggi, Torri, Cosanni, & Terbojevich, 1992b). Then the bands became narrow and moved back higher frequency (3450 cm^{-1}) with the increase of DD up to 86.4%DD (Sample 5), indicating a more ordered structure. This implies that there is less intermolecular force within molecules of half-deacetylated chitosan with the free hydroxyl group, amine group and the polymer chain end readily bonding with water (Gocho, Shimizu, Tanioka, Chou, & Nakajima, 2000). The crystalline Index (CrI), as shown in Table 1, agreed to that calculated from XRD.

4. Conclusion

The solubility of deacetylated chitosan are very much dependent on the degree of deacetylation and molecular weight of chitosan on the basis of the random distribution of acetyl groups. Forced penetration by vacuum is well utilized for homogeneous deacetylation and preparation of water-soluble chitosans with DD around 50%. This method is rapid, efficient, procedure convenient, and low-cost. Powder X-ray diffraction and FT-IR permitted the analyses of the polymorphs of chitin, deacetylated chitin and their degraded hydrolytes.

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