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Determination of the degree of deacetylation of chitin and chitosan by X-ray powder diffraction

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Abstract—A new method to determine the degree of deacetylation (DD) of α-chitin and chitosan in the range of 17–94% DD using X-ray powder diffraction (XRD) is proposed. The results were calibrated using ^{1}H NMR spectroscopy for chitosan and FTIR for chitin, in comparison with the potentiometric titration method. The results showed a good linear correlation between the CrI_{020} from XRD and the calibrated DD value. This method is found to be simple, rapid and nondestructive to the sample.
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Chitin, a β-(1→4)-linked polymer of 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine), is mainly derived from the exoskeletons of insects, crustaceans and the cell walls of fungi and some algae.[1] There are three polymorphs in chitin, α-, β- and γ-chitin, which differ in regard to the arrangement of the chains within the crystalline regions.[2] The most abundant and stable form is α-chitin, which has been studied with X-ray diffraction and infrared spectroscopy.[3] It has a two-chain unit cell with $P2_12_12_1$ symmetry, indicating an antiparallel arrangement of the chitin chain with strong intermolecular hydrogen bonding.[4] Chitosan, its N-deacetylated derivative formed by the action of concentrated alkali and high temperature treatment, has received much attention because of its numerous and wide variety of applications ranging from biomedical uses to waste-water treatment and to uses in the fibre industry. The chemical behaviour of chitin and chitosan depends considerably on the degree of deacetylation (DD), a parameter defined as the mole fraction of deacetylated units in the polymorph chain. Therefore, the determination of DD has been one of the routine analyses performed for quality control on chitin and chitosan preparations. Processing adjustment based on the DD of chitin material is frequently required to facilitate the quick and feasible preparation of chitosan. Thus, the search for quick, user-friendly, low cost and accurate methods to determine the DD has been one of the major concerns over many decades. Determination by X-ray powder diffraction (XRD), if feasible, could provide these advantages. In the present work, we set out to find the relationship(s) between the crystalline state and DD by way of X-ray powder diffractograms.

Determination of DD by ^{1}H NMR and FTIR spectroscopy and by potentiometric titration. Chitin was mixed with highly concentrated NaOH in vacuum in order to pump out the air from chitin particles for accelerating the mass diffusion of NaOH to the inside of the chitin. This procedure provided a homogeneous deacetylation at high temperature and resulted in homogeneity of DD in a chitosan sample. Table 1 shows the results of these seven samples. Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful absolute techniques, allowing a direct determination of DD, where the solvent (HOD) peak does not interfere with H-1D and HAc, as shown in Figure 1. In this experiment, NMR spectroscopy was used to calibrate the

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Table 1. Degree of deacetylation (DD) of chitin and chitosan by potentiometric titration, FTIR and ^{1}HNMR related with corresponding crystalline index by FTIR and XRD

Sample	Potentiometric titration (%)	FTIR (%)		^{1}HNMR (%)	XRD (%)	
		DD	CrI		CrI_{110}	CrI_{020}
0	—	16.9	76.9	—	90.7	91.3
1	49.51 ± 0.47[a]	59.4	40.5	49.8	63.5	66.1
2	50.70 ± 0.28[a]	63.5	39.1	50.2	62.9	64.2
3	56.84 ± 0.35[a]	58.7	42.1	57.4	62.8	61.6
4	68.26 ± 0.87[a]	71.4	36.5	66.8	58.7	55.7
5	84.74 ± 0.57[a]	87.0	28.7	86.4	59.9	40.7
6	92.47 ± 0.11[b]	92.8	16.5	94.0	58.6	30.9

[a] Determination by Jia and Li's method (Ref. 6), mean ± SD, $n = 3$.
[b] Determined by Jiang, Chen and Zhong's method, (Ref. 5) mean ± SD, $n = 8$.

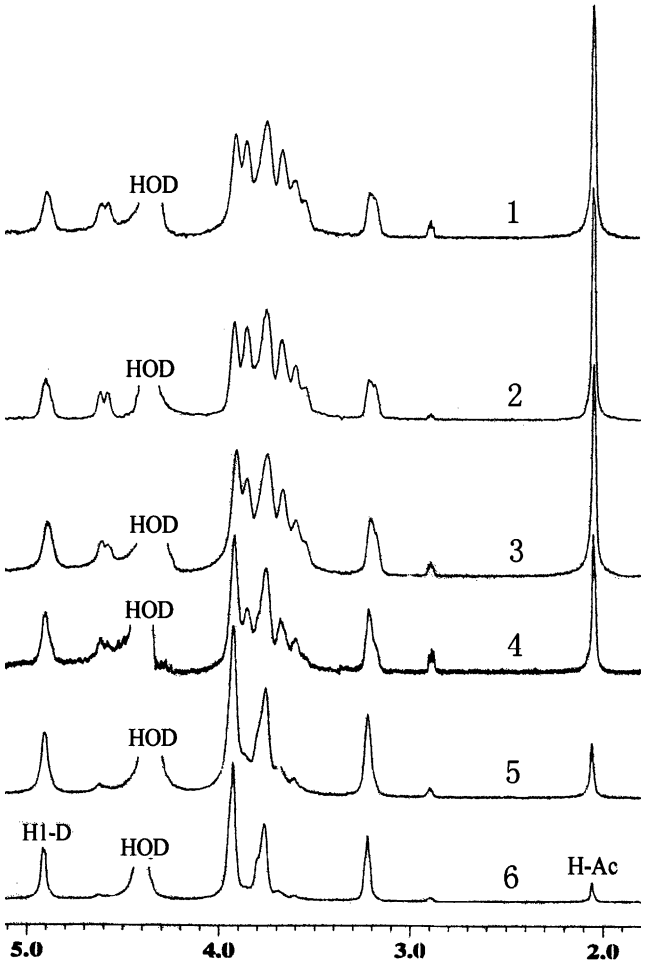

Figure 1. ^{1}H NMR spectra of chitosan with different degrees of deacetylation (DD), from top to bottom are Samples 1–6 described in Table 1. HOD, H-1D and H-Ac are the peaks of the HOD from H_2O exchange with D_2O, the anomeric proton of the deacetylated monomer and the *N*-acetyl proton, respectively.

other techniques including FTIR spectroscopy, potentiometric titration and XRD.

The results from the potentiometric titration agreed with that from the ^{1}H NMR determinations at lower DD values, but deviated at higher DD values, presumably due to the viscous hydrogel that adhered to the glass electrode. Since it is relatively difficult to remove trace impurities from industrialized chitosan, only the refined chitosan products were found to be appropriate for the linear potentiometric titration method,[5] which can avoid the adhesive hydrogel interference. Therefore, determination with two abrupt points was also utilized for approximate values,[6] although the adhesion problems still persisted.

The FTIR spectra (Fig. 2) of chitin exhibited broad peaks at ~3450 cm^{-1} assigned to OH stretching, which became broader and moved to a lower frequency with increasing DD up to ~50% (Samples 1, 2 and 3), indicating an increase in the disordered structure.[7] The bands then became narrow and moved back to higher frequency (~3450 cm^{-1}) with the increase of DD up to 94% (Sample 6), indicating a more ordered structure. This implies that there is less intermolecular force within molecules of half-deacetylated chitosan, with the free hydroxyl groups, amino groups and the polymer chain end readily bonding with water.[8] Therefore, under the same dehydration procedure, it was more difficult for the residual water to be thoroughly removed from the half-deacetylated chitosans than the other samples due to the high sorption of moisture, which may interfere with the determination. This result was inconsistent with that from Brugnerotto's method,[9] which used a chitosan sample with higher DD as an example for comparison.

X-ray diffraction analysis. Chitin and chitosan are polymorphic forms occurring as the α-form in shrimp and crab shell wastes. Figure 3 shows the XRD patterns of chitin and chitosans with different DDs. Five crystalline reflections were observed in the 2θ range of 5–40°. They were indexed as 020, 110, 120, 101 and 130 from

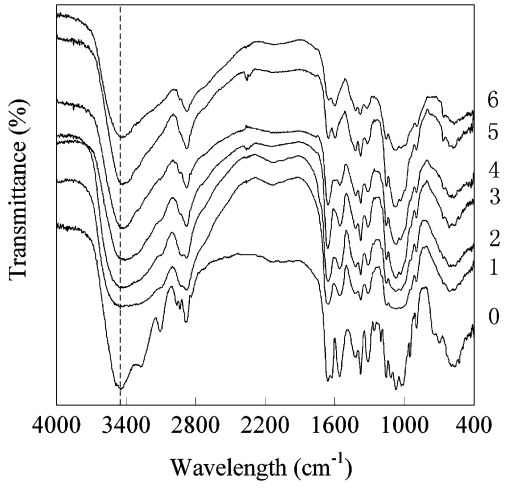

Figure 2. Comparison of FTIR spectra of chitin and chitosan with different degrees of deacetylation (DD). The samples from bottom to top are Samples 0–6, respectively, described in Table 1.

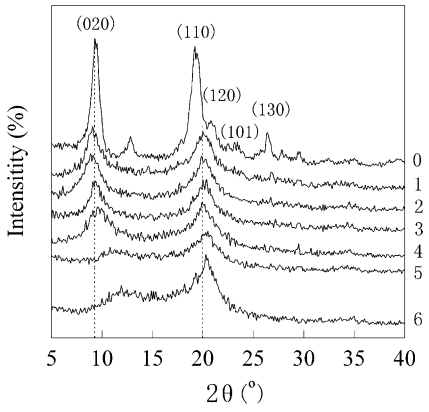

Figure 3. Comparison of X-ray powder diffractograms of chitin and chitosan with different degrees of deacetylation (DD). The samples from top to bottom are Samples 0–6, respectively, described in Table 1.

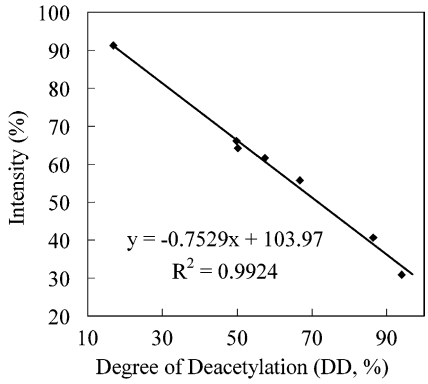

Figure 4. Crystalline index (CrI_{020}) as a function of the degree of deacetylation (DD).

the lower angle for chitin.[4,10,11] It is noted that the maximum peak of intensity at 020 reflection decreased with the increase of DD, and moved to a higher angle, and the second maximum peak of intensity at 110 reflection also decreased with the increase of DD, but the peak appeared at about the same reflection except that of chitin. Therefore, we proposed a crystalline index (CrI; %) expressed as $CrI_{020} = (I_{020} - I_{am}) \times 100/I_{020}$; another equation using I_{110} was expressed as $CrI_{110} = (I_{110} - I_{am}) \times 100/I_{110}$[12] as shown in Table 1. By peak fitting of the diffraction profiles in Figure 3, the *d*-spacing and relative intensity were calculated as shown in Table 2. It was found that the *d*-spacing change of the (020) plane was the most obvious at around 50% DD, with more expansions of the crystal lattices than that of lower or higher DD, and thus moved to a wider diffraction angle with the increase of DD. This result provided another piece of evidence for the half-deacetylated chitosan with less intermolecular forces in relation to moisture absorption, which may interfere with FTIR determinations.

Most importantly, it was found that CrI_{020} decreased linearly with the increase of DD (Fig. 4). This linear relationship between CrI_{020} and DD suggested a possibility for XRD to determine DD of macromolecular chitin and chitosan. Furthermore, we can estimate the DD according to the diffraction angle on the (020) plane for further correction. Generally, impurities cannot be detected, if the content does not exceed 3% total weight.[13] Therefore, trace impurities such as NaOH and NaCl present in the sample cannot interfere with the determinations. In this case three types of samples cannot be utilized with some methods required for sample weighing as UV spectrophometry,[14] first-derivative UV spectrophometry,[15] colloidal titration[16] and potentiometric titration.[6] It could be one of the reasons why an accurate value cannot be reached with these methods, which are suitable only for well-refined samples.

Samples for the determination of DD by the XRD method must meet some specifications. Firstly, the sample should be macromolecular. If the intermolecular force is disrupted, the amorphous pattern on the XRD profile would disappear. Secondly, the history of samples—temperature of preparation, storage and handling—should be identical. A variation in temperature can change their aggregation state, which may deform the profile.[17] Thirdly, the homogeneity of DD is necessary, considering that the (020) plane moved with the increase of DD and does not well overlap. In other words, the XRD method cannot determine the total DD of a mixture with different fractions of DD, for example, a mixture of a chitin sample (17% DD) and a chitosan sample (94%), which is a circumstance similar to that encountered in FTIR determinations.

Table 2. XRD parameters of chitin and its deacetylated chitosan with different DD

No.[a]	2θ (°)	*d*-Spacing (Å)	Relative intensity (%)
0	9.39, 19.22, 20.73, 23.41, 26.39	9.41, 4.61, 4.28, 3.79, 3.37	100, 94.2, 38.1, 21.9, 28.7
1	9.06, 20.00, 23.78, 26.85	9.76, 4.43, 3.74, 3.33	100, 92.8, 37.4, 35.1
2	8.98, 19.87, 26.67	9.84, 4.46, 3.34	100, 96.4, 31.3
3	9.08, 19.91, 26.52	9.73, 4.45, 3.36	96.9, 100, 29.8
4	9.58, 20.14	9.22, 4.40	95.6, 100
5	10.65, 20.20	8.29, 4.39	58.9, 100
6	11.91, 20.35	7.42, 4.36	59.9, 100

[a] The sample number is described in Table 1.

1. Experimental

1.1. Materials

Finely powdered (particle size <80 mesh) and purified chitin and its highly deacetylated chitosan, both from shrimp, were supplied by Laizhou Haili Marine Biotechnology Co. Ltd., China. The lower DD chitosan samples were prepared as follows: Chitin 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 thermostatted water bath kept at 84 °C, stirred for 15 min, 20 min, 45 min and 10 h, 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 anhyd EtOH to remove the residual water, air-dried at 60 °C overnight and finally stored in a desiccator with silica gel.

1.2. Determination of DD by ^{1}H NMR spectroscopy, potentiometric titration and FTIR spectroscopy

^{1}H NMR spectra were recorded on a JNM-ECP 600 MHz spectrometer (Japan Electronic Optic Laboratory). Each sample was air-dried at 80 °C for 10 h, followed by 105 °C for 1 h, sealed, cooled with an ice bag 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[18] 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 according to the following equation: DD (%) = 100 × H-1D/(H-1D + HAc/3), where H-1D and HAc are the integrals of the peak of the H-1 anomeric proton of deacetylated monomer (H-1D) and of the peak of the three protons of *N*-acetyl group (H-Ac), respectively, as shown in Figure 1.

As for potentiometric titration, we followed Jia and Li's method 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.

FTIR spectra were obtained with an Impact 360 FTIR spectrometer under dry air at room temperature using KBr pellets. The samples were dehydrated as described above, and then cooled in a desiccator with CaO. The DDs of chitin and chitosans were calculated according to the following equation:

$\mathrm{DD} = 100 \times (1 - (A_{1655}/A_{3450})/1.33)$ which was derived for these absorbances.[19,9,20]

1.3. XRD

X-ray diffractograms on powder samples were obtained using a Bruker AXS D8 Advance X-ray diffractometer under the following operating conditions: 40 kV and 40 mA with Cu $K\alpha_1$ radiation at λ 1.54184 Å and acceptance slot at 0.1 mm. About 20 mg of the sample was spread on a sample stage, and the relative intensity was recorded in the scattering range (2θ) of 5–40° in steps of 0.1°. The crystalline index (CrI; %) was determined in two ways: $\mathrm{CrI}_{020} = (I_{020} - I_{\mathrm{am}}) \times 100/I_{020}$, where I_{020} is the maximum intensity below 13° and I_{am}, the intensity of amorphous diffraction at 16°. For comparison, another crystallinity index was expressed as CrI_{110}, following the equation above and using I_{110}, where I_{110} is the maximum intensity at ~20°.[12,21]

References

1. Jiang, T. *Chitosan*; Chinese Chemical Industry Press: Beijing, 2001, pp 10–11.
2. Jiang, T. *Chitin*; Beijing: Chinese Chemical Industry Press: Beijing, 2003, pp 26–31.
3. Gow, N. A. R.; Gooday, G. W.; Russell, J. D.; Wilson, M. J. *Carbohydr. Res.* **1987**, *165*, 105–110.
4. Wada, M.; Saito, Y. *J. Polym. Sci., Part B: Polym. Phys.* **2001**, *39*, 168–174.
5. Jiang, X.; Chen, L.; Zhong, W. *Carbohydr. Polym.* **2003**, *54*, 457–463.
6. Jia, S.; Li, Q. *Chem. World* **2001**, *5*, 240–241.
7. Focher, B.; Naggi, A.; Torri, G.; Cosanni, A.; Terbojevich, M. *Carbohydr. Polym.* **1992**, *18*, 43–49.
8. Gocho, H.; Shimizu, H.; Tanioka, A.; Chou, T. J.; Nakajima, T. *Carbohydr. Polym.* **2001**, *41*, 87–90.
9. Brugnerotto, J.; Lizardi, J.; Goycoolea, F. M.; Argüelles-Monal, W.; Desbrières, J.; Rinaudo, M. *Polymer* **2001**, *42*, 3569–3580.
10. Feng, F.; Liu, Y.; Hu, K. *Carbohydr. Res.* **2004**, *339*, 2321–2324.
11. Li, J.; Revol, J.-F.; Marchessault, R. H. *J. Appl. Polym. Sci.* **1997**, *65*, 373–380.
12. Focher, B.; Beltranme, P. L.; Naggi, A.; Torri, G. *Carbohydr. Polym.* **1990**, *12*, 405–418.
13. Wang, X.; Wang, X. *Instrumental Analysis Technique*; Chinese Chemical Industry Press: Beijing, 2003, pp 151–155.
14. Aiba, S. *Int. J. Biol. Macromol.* **1986**, *8*, 173–176.
15. Tan, S.; Khor, E.; Tan, T.; Wong, S. *Talanta* **1998**, *45*, 713–719.
16. Terayama, H. *J. Polym. Sci.* **1952**, *8*, 243–253.
17. He, M.; Chen, W.; Dong, X. *Macromolecular Physics*; Fudan University Press: Shanghai, 2001, pp 34–37.
18. Lavertu, M.; Xia, Z.; Serreqi, A. N.; Berrada, M.; Berrada, M.; Rodrigues, A.; Wang, D.; Bushchmann, M. D.; Gupta, A. *J. Pharm. Biomed. Anal.* **2003**, *32*, 1149–1158.
19. Domard, A.; Rinaudo, M. *Int. J. Biol. Macromol.* **1983**, *5*, 49–52.
20. Duarte, M. L.; Ferreira, M. C.; Marvao, M. R.; Rocha, J. *Int. J. Biol. Macromol.* **2002**, *31*, 1–8.
21. Kuma, A. B. V.; Varadaraj, M. C.; Lalithac, R. G.; Tharanathan, R. N. *Biochim. Biophys. Acta* **2004**, *1670*, 137–146.