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Carbohydrate Polymers 50 (2002) 27–33

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Solid state structure of chitosan prepared under different N-deacetylating conditions

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Received 4 October 2001; accepted 20 November 2001

Abstract

Changes in the crystallinity and polymorphic nature of chitosan, as a function of N-deacetylation of chitin under different conditions were studied. Viscosity average molecular weight suggests a higher degree of polymerization (DP) for chitosan prepared in the presence of thiophenol, as an oxygen scavenger. FT-IR spectra exhibited a progressive weakening of the bands at 1655, 3265 and 3100 cm^{-1} during N-deacetylation and A_{1382}/A_{2920} cm^{-1} ratios of 0.65, 0.56 and 0.46 indicated a higher order structure for chitosan prepared with thiophenol than those prepared under N_2 atmosphere or otherwise. The differences in crystallinity indices of chitosans were further substantiated by X-ray diffraction data. Splitting of C_1 and C_4 signals in CP-MAS ^{13}C NMR spectra suggested the possible occurrence of newer conformational polymorphs. DSC thermograms showed higher thermal stability for chitosan with higher DP. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Chitin; Chitosan; Deacetylation; Viscosity; Molecular weight; Polymorphism

1. Introduction

Chitin, a β-1,4-linked polymer of *N*-acetyl-D-glucosamine, is derived from the cell walls of lower plants and in the skeletal tissues of lower animals including arthropods and molluscs (Jeuniaux, Voss-Foucart, Poulicek, & Bussers, 1989). Chitosan is an exceptionally useful polysaccharide, which can be easily derived from chitin by N-deacetylation with hot alkali. In the recent decade, chitosan has received much attention because of its extraordinary properties and for its inexpensive abundant resources.

Chitin can be converted to chitosan by either homogeneous or heterogeneous alkaline N-deacetylation (Chang, Tsai, Lee, & Fu, 1997) or by enzymatic means (Araki & Ito, 1975; Martinou, Kafetzopoulas, & Bouriotis, 1995). Despite numerous attempts, the N-acetyl groups cannot be completely removed, without inducing degradation of the polysaccharide backbone (Lindberg, Lonngren, & Sevensson, 1975). In the presence of alkali, the polysaccharide chains were found to undergo some degradation because of high concentration of reagents used and prolonged reaction time required, obtaining complete deacetylation (Whistler & BeMiller, 1958). Nevertheless, the deacetylation reaction, when performed under heterogeneous conditions leads to sparsely as well as non-uniformly accessible block copolymers of *N*-acetyl-D-glucosamine and D-glucosamine residues, whose physicochemical properties appeared different from those of chitosan randomly deacetylated under homogeneous conditions (Aiba, 1991). In the present work, N-deacetylation of chitin was performed by alkali treatment under controlled conditions (under N_2 atmosphere and in the presence of thiophenol as an oxygen scavenger) and compared with traditionally N-deacetylated chitosans. The main objective of this study was to look for the influence of varying deacetylation conditions on the solid state structures, i.e. crystallinity and polymorphic nature of chitosan, which definitely have a bearing on understanding the multifaceted functionality attributes of chitosan. It is known that chitosan polymorph is very much dependent on the preparative steps it is subjected to (Ogawa, Yui, & Miya, 1992).

2. Experimental studies

2.1. Chitosan preparation

Chitosan A was prepared from shrimp chitin by N-deacetylation with 40% sodium hydroxide solution at 100 °C for

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PII: S0144-8617(01)00371-X

Table 1
Characteristics of chitosans prepared under different conditions

Sample	Conditions	Intrinsic viscosity [η] (dl g^{-1})	Viscosity average MW $\bar{M}_V$ (Da)	Degree of N-acetylation (%)	
				IR	^{13}C NMR
Chitosan A	Uncontrolled	2.15	96,000	12.0	11.2
Chitosan B	Under N_2 atmosphere	2.5	117,000	15.0	15.6
Chitosan C	With thiophenol	2.6	123,000	13.0	13.4

1 h. Chitosan B was prepared by carrying out N-deacetylation in the presence of nitrogen atmosphere, whereas chitosan C was prepared in the presence of 1% (v/v) thiophenol which was added before refluxing with 40% sodium hydroxide. After the reaction, chitosan samples were washed thoroughly with distilled water and freeze dried.

2.2. Viscosity measurements

The viscosity average molecular weight (MW) of chitosans was calculated by Mark–Houwink equation $[\eta] = K_m M^a$ where $K_m = 3.5 \times 10^{-4}$ and $a = 0.76$ (Rinaudo, Milas, & Dung, 1993). The viscosity measurements in 0.5 M acetic acid–0.2 M sodium acetate buffer were done using Ostwald viscometer and recording the efflux time of the solution in a constant temperature bath at 25 ± 0.1 °C. The mean of four replicates was taken for the calculation.

2.3. Infrared spectroscopy

IR spectra were obtained in Impact 410 Nicolet FT-IR spectrometer under dry air at room temperature using KBr pellets. The latter were prepared by thoroughly mixing KBr (200 mg) and chitosan (6 mg) and made into pellets. Crystallinity index (CrI) was determined from the ratio of absorbances at A_{1382}/A_{2920} cm^{-1} (Focher, Beltrame, Naggi, & Torri, 1990).

Degree of acetylation (DA) of chitosan was determined by the method of Miya, Iwamoto, Yashikawa, and Mima (1980). Considering the –OH band at 3450 cm^{-1} as a reference, the acetyl content (%) was determined from the ratio of absorbances: (A_{1655} cm^{-1}/A_{3450} cm^{-1})100/1.33, which was calculated by baseline drawing for these absorbances (Domard & Rinaudo, 1983). These values were correlated with the calculated DA deduced from the relative intensities of the resonances of the ring carbon (I_{C_1}, I_{C_2}, I_{C_3}, I_{C_4}, I_{C_5}, I_{C_6}) and methyl carbon (I_{CH_3}) obtained from solid state ^{13}C NMR spectra (Ottoy, Varum, & Smidsord, 1996), by adopting the following equation:

$$\mathrm{DA} = \frac{(I_{CH_3})}{I_{C_1} + I_{C_2} + I_{C_3} + I_{C_4} + I_{C_5} + I_{C_6}/6}$$

2.4. X-ray diffractometry

Powder X-ray diffraction patterns of lyophilized chitosan samples were obtained by using a EG-7G solid state germanium liquid nitrogen cooled detector Sintag XDS-2000 instrument equipped with a θ–θ goniometer, under the following operating conditions 30 kV and 25 mA with Cu Kα_1-radiation at λ 1.54184 Å. The relative intensity was recorded in the scattering range (2θ) of 4–60°. The CrI was determined as per the method of Focher et al. (1990) by using the equation $\mathrm{CrI} = I_{110} - I_{am}/I_{110}$ where I_{110} is the maximum intensity ($2\theta = 20°$) of the (110) lattice diffraction and I_{am} is the intensity of amorphous diffraction at $2\theta = 16°$.

2.5. CP-MAS ^{13}C NMR spectroscopy

The solid state spectra were recorded at 75.4 MHz in a BRUKER (Karlsruhe) DSX 300 spectrometer. The cross-polarization sequence was utilized for all samples, which were spun at the magic angle at 6 kHz. A contact time of 1 ms and a pulse (repetition) time of 5 ms were used and more than 1000 scans were accumulated for each spectrum. The chemical shifts were measured with respect to intensity, and approximately 300 mg of dry sample was inserted into a ceramic rotor.

2.6. Differential scanning calorimetry

This was carried out using a Rheometric Scientific (UK) equipment supported by thermal software S42 on a Compaq computer which is calibrated as per standard procedures. The equipment was provided with the autocool system. Accurately weighed (5–8 mg) dry material was placed in an aluminium cup and hermatically sealed. Empty cup was used as reference and runs were performed in duplicates. Samples were analyzed under continuous flow of dry nitrogen gas (10 ml min^{-1}) at a heating rate of 20 °C min^{-1} from 5 to 400 °C.

3. Results and discussion

3.1. Viscosity measurement

One of the simplest and most rapid methods for determining the MW of polymers is viscometry, which requires the determination of constants through correlation of intrinsic viscosity $[\eta]$ with MW (Roberts & Domszy, 1982). The most commonly used equation relating $[\eta]$ values with MW is the Mark–Houwink equation $[\eta] = K_m M^a$ where a

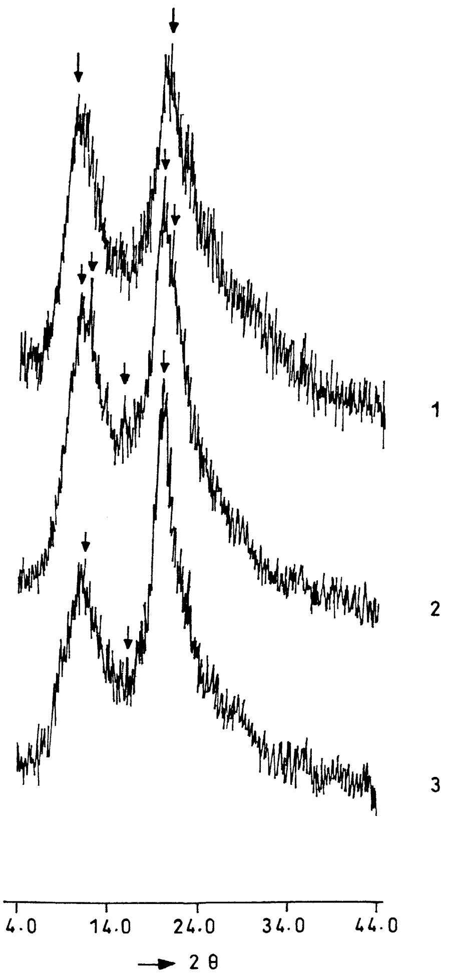

Fig. 1. X-ray diffraction patterns of: (1) Chitosan A, (2) Chitosan B, (3) Chitosan C.

and K_m are constants that are independent of MW over a wide range of values. The MW of chitin could not be determined due to its practical insolubility in the solvent system used. From the results presented in Table 1, it can be seen that the MW of chitosan C (123,000 Da) was more when compared to those of chiosans B (117,000 Da) and A (96,000 Da). This could be attributed to depolymerization of chitosan chains due to peeling reactions as well as the possible oxidative cleavage of the glycosidic bonds, as also reported before (Hasegawa, Isogai, & Onabe, 1994). Thiophenol (used in chitosan C) acts as a good oxygen scavenger and possibly arrests such depolymerization reactions.

3.2. X-ray diffraction

In Fig. 1, is given the powder X-ray diffraction patterns of chitosans A–C in dry state. Being hydrated polymorphs (refers to the bound water, ~10% present in chitosan samples, even after the material was freeze dried), all chitosan samples showed a strong reflection at 2θ around 9–10° (Table 2). In the hydrated crystals, the incorporation of bound water molecules into the crystal lattice gives rise to the more abundant polymorph, 'Tendon' form (Ogawa et al., 1992) of chitosan A, having this reflection at 9.9° (d = 8.93 Å) whereas chitosan B, exhibiting a 'L-2' type polymorph showed this reflection at 10.6° (d = 8.30 Å) and in addition a small peak at around 15.1° (d = 5.86 Å) which is 120 reflection, due to anhydrous crystal lattice (Ogawa & Yui, 1993; Yamamoto, Kawada, Yui, & Ogawa, 1997). The latter is characteristic of annealed polymorph, which is shown to exhibit minimal chemical and biological reactivities. Nevertheless, splitting of the peaks indicated multiple polymorphs in the crystal lattice. In chitosan C, appearance of a broad peak at around 10° is indicative of probably a better hydrated polymorph than chitosans A and B. Although chitosans A–C were prepared by heating (to ~100 °C) chitin in the presence of strong alkali, the diffraction peak at 15° was seen conspicuously only in chitosan B, very faintly in chitosan C, but not at all in chitosan A. This indicates their differential content of hydrated and anhydrous crystals. Chitosan B had slightly a higher degree of N-acetylation (15%). A heating regimen of >160 °C was reported (Ogawa, 1991) necessary to obtain the diffraction peak at 15°, which is attributed to anhydrous crystal lattice in 16% N-acetylated chitosan. On the contrary, such a diffraction pattern was observed in both chitosans B and C, with degree of N-acetylation of 15 and 13%, respectively, although the heating regimen during their preparation was at around 100 °C. Chitosan C showed relatively a higher CrI of 58% compared to other two samples.

3.3. CP-MAS ^{13}C NMR spectroscopy

Solid state CP-MAS ^{13}C NMR is known to be very sensitive to changes in the local order structure. The line widths in the spectra of chitosans (Fig. 2) were relatively small, indicating the samples to be more crystalline (Focher, Naggi, Torri, Cosanni, & Terbojevich, 1992b). The chemical shift values of carbons 1–6 and CH_3 are shown in Table 3. The degree of acetylation, calculated from the intensity of

Table 2
CrI and d-spacing values of chitosans prepared under different conditions

Sample	CrI (%)		2θ (°)	d-Spacing (Å)
	X-ray	IR		
Chitosan A	42.67	46.00	9.9, 20.0	8.92, 4.41
Chitosan B	48.57	56.00	10.6, 11.6, 15.1, 19.8, 20.7	8.30, 7.64, 5.86, 4.48, 4.28
Chitosan C	58.28	65.00	10.7, 19.9	8.24, 4.45

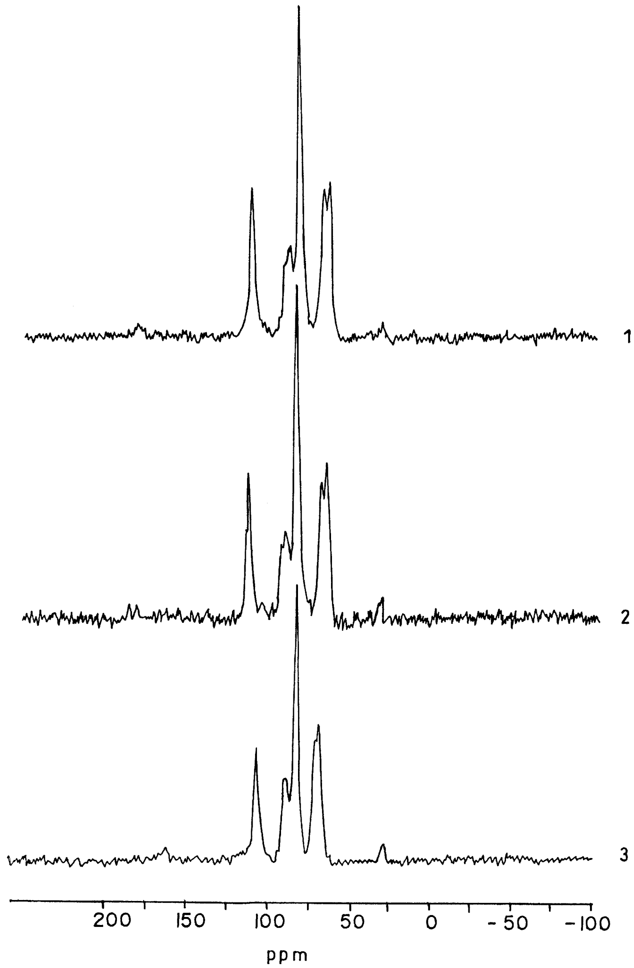

Fig. 2. CP-MAS ^{13}C-NMR spectra of: (1) Chitosan A, (2) Chitosan B, (3) Chitosan C.

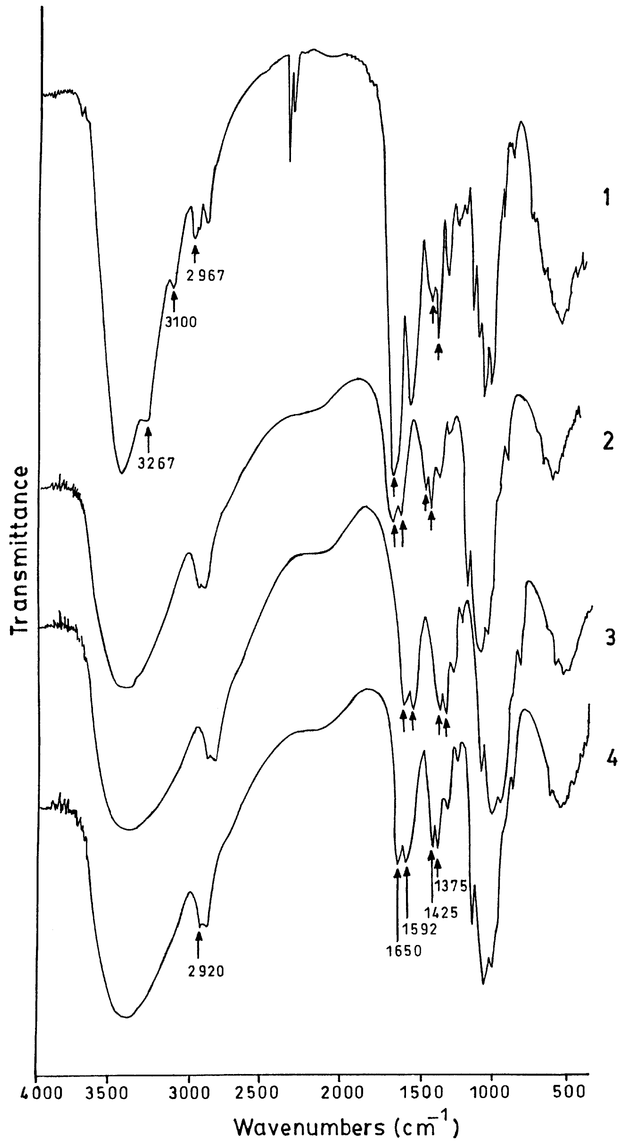

Fig. 3. FT-IR spectra of: (1) Chitin, (2) Chitosan A, (3) Chitosan B, (4) Chitosan C.

methyl and ring carbon was in good agreement with that deduced from FT-IR (Table 1). Multiplicity of C-2 and C-6 as well as of CH_3 and carbonyl signals (acetamide group) in chitosans A and B was attributed to polymorphic molecular heterogeneity, which may induce different crystallographic sites due to independent chains in the unit cell. The chemical shifts of C-1 and C-4 carbon in 1,4-linked carbohydrates are believed to be highly sensitive to any conformational change at the glycosidic linkage (Tanner, Chanzy, Vincendon, Roux, & Gaill, 1990). Chitosan A showed a weak splitting of C-4 signal, which is associated with external influences like temperature, concentration of alkali, etc. and was not seen in other two chitosans, deacetylated under controlled conditions. Chitosan B showed a splitting of C-1 signal indicating the possible existence of different polymorphic conformation, as also indicated by its X-ray diffraction data.

Table 3
Chemical shifts values (ppm) for chitosans prepared under different conditions

Sample	C_1	C_4	C_3/C_5	C_6	C_2	CH_3
Chitosan A	109.97	86.58	80.75	65.64	62.43	29.15
Chitosan B	111.47, 110.16	90.03, 87.83	80.68	65.28	62.57	28.83
Chitosan C	108.05	86.05	78.19	60.34	60.34	25.88

3.4. FT-IR spectroscopy

The FT-IR spectra (Fig. 3) of chitosans A, B and C though similar to each other as a whole, showed subtle differences in the absorption intensities. As expected, N-deacetylation is associated with a progressive weakening of the band occurring at 1665 cm^{-1} (amide I). Furthermore the vibrational mode of amide II at 1550 cm^{-1} appeared at 1604, 1598 and 1592 cm^{-1} for chitosans A, B and C, respectively. The absence of sharp absorptions around 3500 cm^{-1} in all the three samples indicated that there are no free –OH

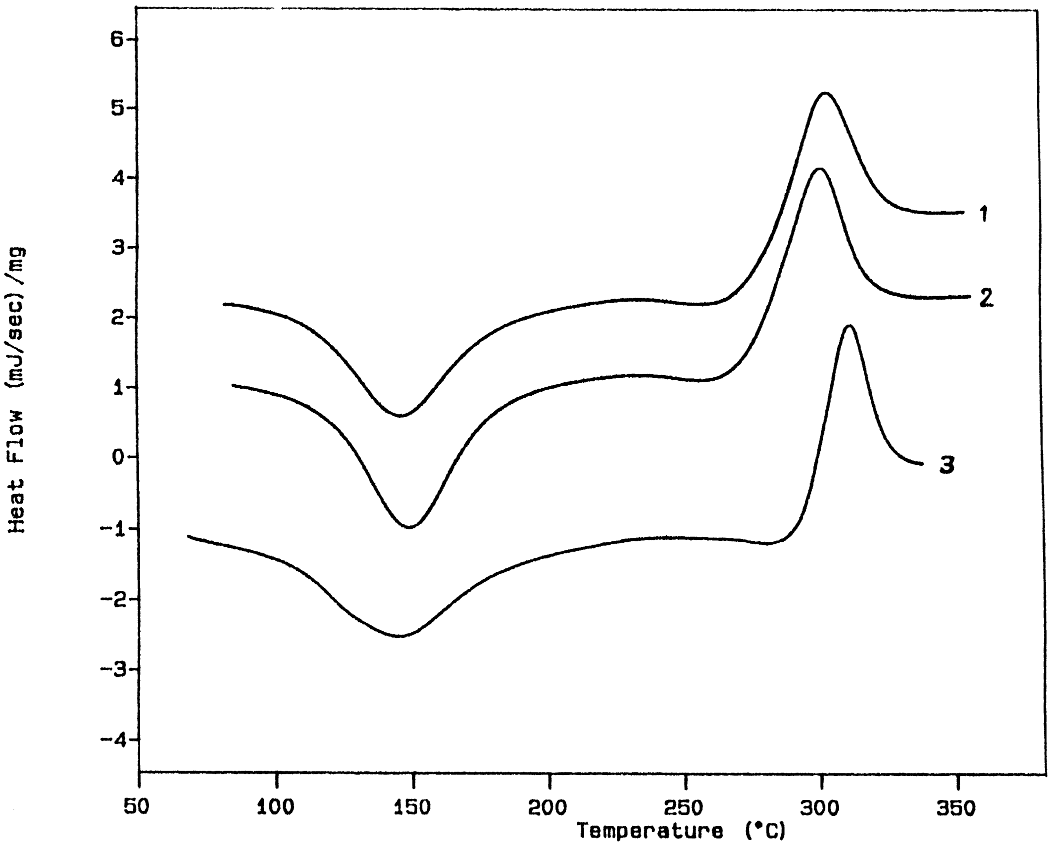

Fig. 4. DSC thermograms of: (1) Chitosan A, (2) Chitosan B, (3) Chitosan C.

groups. Both OH···3 and CH_2OH···6 are involved in intra- and intermolecular hydrogen bonds. The region above 3000 cm^{-1} was centered at 3395 cm^{-1} for chitosan A, 3407 cm^{-1} for chitosan B and 3419 cm^{-1} for chitosan C. Such a band shift to a higher frequency indicated an increase in the ordered structure (Focher et al., 1992b). The region 1500–1200 cm^{-1} is related to the local symmetry. The band around 1425 cm^{-1} for chitosans A, B and C was assigned to CH_2 bending, due to the rearrangements of hydrogen bonds on the most favorable orientation of the primary –OH groups, at least in the amorphous regions of the polysaccharides (Focher, Naggi, Torri, Cosanni, & Terbojevich, 1992a; Focher et al., 1992b). The vibration mode of chitosans A and B at 1381 cm^{-1} has been assigned to CH bending and C–CH_3 deformation mode. For chitosan C it appeared at 1375 cm^{-1}.

A marked modification of the shape and the intensity of CH_2 stretching bands of chitosan suggested a different arrangement of primary hydroxyl groups, as shown by the analysis of the bending mode of –CH_2 groups in the 1425 cm^{-1} region. Compared to chitosans A and C, chitosan B showed a large intensity at this point. The latter is considered conformational sensitive for polysaccharides (Focher et al., 1992a). Both shifts and intensity changes of the bands have been related to modifications in the environment of CH_2OH group, although the high degree of bond coupling in this region makes it difficult to assess the kind of chain packing or hydrogen bond network.

3.5. Differential scanning calorimetry

Polysaccharides usually have a strong affinity for water and in the solid state these macromolecules may have disordered structures which can be easily hydrated. As is known, the hydration properties of these polysaccharides depend on the primary and supra-molecular structures and the presence of bound water also has a strong influence on the overall polymorphic nature of the macromolecule. Therefore, the endotherm related to the evaporation of bound water is expected to reflect chemical and molecular changes during N-deacetylation.

Close examination of thermograms in Fig. 4 reveals that there are differences in the endotherm peak area and position, indicating that these macromolecules differ in their water holding capacity and strength of water–polymer interaction. The first thermal event registered in all the three samples was a wide endothermic peak centered between 140 and 150 °C with an onset at around 100 °C. In the case of chitin and its N-deacetylated forms, the endothermic peak area and ΔH increased with increase in N-deacetylation indicating a possible correlation between the water

Table 4
Thermal transitions of chitin and chitosans prepared under different conditions (T_o—onset temperature, T_p—peak temperature, T_c—completion temperature, ΔH—enthalpy. The values presented are average of two experiments with standard deviation)

Polymer	Endotherm (°C)				Exotherm (°C)			
	T_o	T_p	T_c	ΔH (mJ mg^{-1})	T_o	T_p	T_c	ΔH (mJ mg^{-1})
Chitin	96.1 ± 0.1	139.0 ± 0.1	204.8 ± 0.1	158.3 ± 0.1	350.7 ± 0.6	397.0 ± 1.1	414.2 ± 0.2	−25.7 ± 0.2
Chitosan A	100.2 ± 0.1	146.2 ± 0.1	207.1 ± 0.1	216.1 ± 0.0	256.1 ± 0.2	300.0 ± 0.0	334.6 ± 0.1	−193.5 ± 0.1
Chitosan B	111.4 ± 0.1	148.5 ± 0.1	209.2 ± 0.1	193.1 ± 0.1	276.5 ± 0.1	300.2 ± 0.1	329.2 ± 0.1	−184.1 ± 0.1
Chitosan C	94.3 ± 0.0	143.8 ± 0.1	204.5 ± 0.1	183.5 ± 0.1	284.4 ± 0.1	311.4 ± 0.1	337.6 ± 0.1	−146.6 ± 0.2

holding capacity and chemical and supra-molecular structure of these polymers (Table 4).

The second thermal event registered was a wide exothermic peak, whose area was used to express the overall exothermic effect connected with decomposition, ΔH. Due to differences in structural characteristics, remarkable differences in the exothermic transition for chitosan A and chitosan C were observed. The exothermic peak in chitosans A and B occurring at 300 °C was shifted to a higher temperature (311 °C) for chitosan C with a concomitant decrease in peak area. Such a shift in the latter to a higher temperature is attributed to reduce oxidative glycosidic bond cleavage because of oxygen scavenging by thiophenol. Nevertheless, the crystallinity of chitosan C was relatively more. A linear correlation between degree of polymerization (DP) and the decomposition peak temperature has been observed (Kittur et al., Unpublished data). From Table 1, it is clear that the intrinsic viscosity and average MW of chitosan C is more because of the annealed polymorph present in the polymer, which is also evident from other spectroscopic data.

4. Conclusions

Based on these results, it is obvious that chitosan functionality depends upon the degree of deacetylation and DP of the macromolecule, which in turn depends on the preparation procedure. Deacetylation under N_2 atmosphere results in introducing anhydrous crystal lattice. Use of thiophenol during deacetylation resulted in a product (chitosan C) with a higher DP and viscosity average MW, and a higher order structure. It also showed relatively a higher CrI compared to other two samples. Shift to a higher temperature of the exothermic peak suggested thermal stability due to reduced oxidative bond cleavage.

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