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Characterization of chitin, chitosan and their carboxymethyl derivatives by differential scanning calorimetry

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Abstract

Chitin, chitosan and their *O*,*N*-carboxymethyl derivatives were characterized by differential scanning calorimetry (DSC) mainly focusing on changes in physical and chemical structures at different levels of acetyl and carboxymethyl contents. The thermograms were characterized by endo- and exotherms corresponding to water evaporation and decomposition of the polymer, respectively. However, each endo- or exothermic peak temperature and area changed as a function of primary and higher order structures of the macromolecule. It was found that the enthalpy value for endotherm increased with increase in amino and carboxymethyl contents. Further, in the case of carboxymethyl derivatives, no glass transition was observed despite the presence of substantial amount of amorphous content. During decomposition, the decomposition peak temperature and area changed as a function of molecular weight (MW), acetyl and carboxymethyl contents. A theoretical basis was adopted to correlate the heat of the reaction, ΔH, to the degree of deacetylation (%DD) and carboxymethylation (DS). A good correlation was obtained when the corresponding peak area and peak height were plotted against %DD and DS.

Keywords: Chitin; Chitosan; Carboxymethyl derivatives; Differential scanning calorimetry; Enthalpy; Glass transition; Degree of deacetylation; Degree of substitution

1. Introduction

Chitin 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 one of the most abundant biopolymers next to cellulose. Chitin is substantially composed of 2-acetamido-2-deoxy-D-glucopyranose (*N*-acetyl-D-glucosamine, GlcNAc) units linked by β-(1,4-) linkage. Chitosan obtained from chitin, mainly by *N*-deacetylation with an alkaline treatment is chiefly composed of 2-amino-2-deoxy-D-glucopyranose (D-glucosamine, GlcN) units. Chitin/chitosan and their carboxymethyl derivatives have received much attention in the recent decades for their special properties (Nishimura, Nishi & Tokura, 1986; Sandford, 1989) and inexpensive, abundant resources. It is well known that some of the structural characteristics such as degree of acetylation (DA), degree of substitution (DS) and molecular weight (MW) of chitin/chitosan and their derivatives greatly influence various properties such as solubility, physiological activities (Hirano & Nagano, 1989; Kendra, Christian & Hadwiger, 1989; Suzuki, Watanabe, Mikami & Suzuki, 1990), chemical reactivity and biodegradability.

Invariably chemical (Muzzarelli, Tanfani, Emanuelli & Mariotti, 1982; Pangburn, Trescony & Heller, 1984), chromatographic (Niola, Basora, Chornet & Vidal, 1993; Sato, Mizutani, Tsuge, Ohtani, Aoi, Tak-asu et al., 1998) and spectroscopic (Aiba, 1986; Moore & Roberts, 1980; Varum, Anthonsen, Grasdalen & Smidsord, 1991) methods are used to study structures of these polymers. Thermal methods, such as thermogravimetry (TGA), differential scanning calorimetry (DSC) and differential thermal analysis (DTA) have emerged as powerful thermoanalytical techniques to monitor characteristic physical and chemical changes in both natural and synthetic polymers. These methods yield curves that are unique for a particular composition of matter and even slight changes in chemical structure and composition or molecular architecture will bring about discrete and reproducible variations in the thermograms. Further, some of these changes involve the loss of material in volatilization which can be quantitatively measured by TGA.

In fact DSC is ideally suited for rapid screening and finger

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printing purpose of materials, in solid state, as a function of temperature. Miyashita, Kobayashi, Kimura, Suzuki and Nishio (1997), studied the transition behavior and phase structure of chitin/poly (2-hydroxyethyl methacrylate) composites by DSC. The effect of *N,O*-carboxymethyl chitosan on phase behavior of chitosan/viscose rayon blends was studied by Yunlin, Liu, Fu, Li and Ya (1998). A survey of recent literature shows that thermal methods have been rarely used for monitoring the chemical and physical changes during various modifications of chitin and chitosan. In the present work the feasibility of using DSC for such studies is further explored.

2. Materials and methods

2.1. Preparation of chitosan samples

Chitosan samples (chitosan A–E) were prepared through heterogeneous alkaline deacetylation of shrimp chitin to different degrees. Chitosan D and E were further purified by dissolving in 1% aqueous acetic acid followed by filtration to remove insoluble material. The soluble chitosan was precipitated with 1 N sodium hydroxide, washed thoroughly with double distilled water and lyophilized. Chitosans with different MW but of same acetyl content were prepared by enzymatic hydrolysis using *A.niger* pectinase. The viscosity average MW of the chitosan was calculated by the Mark-Houwink equation; $[\eta] = K_m M^a$, where $K_m = 3.5 \times 10^{-4}$, $a = 0.76$ (Rinaudo, Milas & Ledung, 1993).

2.2. Preparation of carboxymethyl derivatives of chitin and chitosan

Carboxymethyl chitin was prepared by the method of Tokura, Nishi, Tsutsumi and Somarin (1983), *O*-carboxymethyl chitosan was prepared by *N*-deacetylating carboxymethyl chitin in aqueous 10% sodium hydroxide containing sodium borohydride (0.1%) for 10 h at 80°C (Hirano, Hayashi & Hirochi, 1992).

2.3. Preparation of N,O-carboxymethyl chitosan

To a chitosan (5 g) suspension in 50 ml isopropanol, 13 ml of 10 M sodium hydroxide was added in six equal portions over a period of 20 min under agitation. The alkaline slurry was stirred for an additional 45 min. Different amounts of monochloroacetic acid were added to achieve different degrees of substitution. The reaction mixture was heated at 60°C for 2 h, cold distilled water (5 ml) was added and its pH was adjusted to 7.0 with glacial acetic acid. The reaction mixture was filtered and the solid *N,O*-carboxymethyl derivative was washed with 70% methanol, and dried in an oven at 60°C (Hayes, 1986). The derivatives were purified by dissolving in water to remove insolubles (scarcely substituted product), filtered, dialyzed against double distilled water and lyophilized.

2.4. Infrared spectroscopy

IR spectra were recorded in KBr discs on a Impact 410 Nicolet FTIR spectrometer under dry air at room temperature. Degree of acetylation (%DA) of chitosans was determined following the method of Miya, Iwamoto, Yoshikawa and Mima (1980) and degree of carboxymethylation (DS) was determined from the ratio of the absorbencies at 1418 and 1312 cm^{-1} (Nahalka, Nahalkova, Gemeiner & Blanarik, 1998). Degree of deacetylation (%DD) was computed from %DA.

2.5. High performance size exclusion chromatography (HPSEC)

This was performed on Shimadzu HIC-6A system controller and CR-YA Chromatopack integrator units, fitted with columns E-linear and E-1000 μ-Bondapack (30 cm × 3.9 mm, i.d.) and an RI detector connected in series with a guard column. The columns were eluted with distilled water at a flow rate of 1.0 ml min^{-1}. The operating temperature was 27°C and the injection volume was 10.0 μl. The MW of carboxymethyl derivatives of chitosan was determined from a calibration curve (plot of log MW versus retention time) prepared for standard dextrans (T-10 to T-2000) of known MW.

2.6. X-ray Diffractometry

Powder X-ray diffraction patterns were obtained by using a EG-7G solid state Germanium liquid nitrogen cooled detector Scintag XDS-2000 instrument equipped with a $\theta-\theta$ goniometer, with the following operating conditions: 30 kV and 25 mA with a CuKα radiation at 1.54184 nm. The relative intensity was recorded in scattering range (2θ) of 4–60°. The crystallinity index (CrI) was determined as per the method of Struszezyk (1987).

2.7. Differential scanning calorimetry

This was carried out using a Rheometric Scientific (UK) equipment using thermal software version 5.42 supported on Compaq computer and calibrated as per the standard procedures. The equipment was provided with an autocool accessory for the programmed cooling. Accurately weighed (5 mg) material was placed into aluminium cup and sealed. An empty cup was used as reference and runs were performed at least in triplicate and the average mean values are given. The experiment consisted of two separate series, one heating the samples from 50–550°C and the other in the temperature range 3–220°C. In the latter case, the samples were heated upto 220°C, cooled to 3°C followed by immediate rescanning in the same temperature range. For each series, two separate pans of the same sample were prepared and analyzed under continous flow of dry nitrogen gas (10 ml min^{-1}) at a heating rate of 20°C min^{-1}.

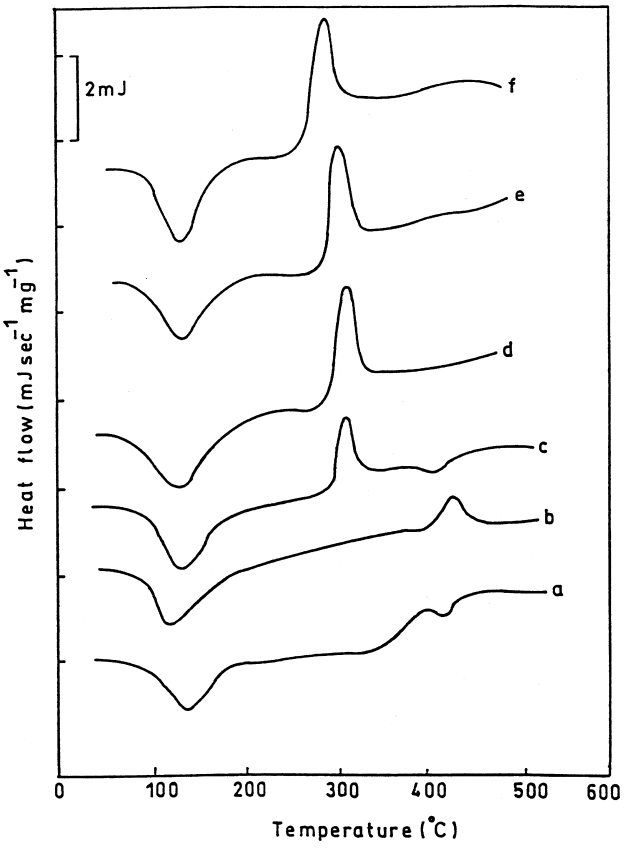

Fig. 1. DSC thermograms of (a) chitin, (b) chitosan-A (%DD = 25), (c) chitosan-B (%DD = 48), (d) chitosan-C (%DD = 79), (e) chitosan-D (%DD = 84) and (f) chitosan-E (%DD = 89).

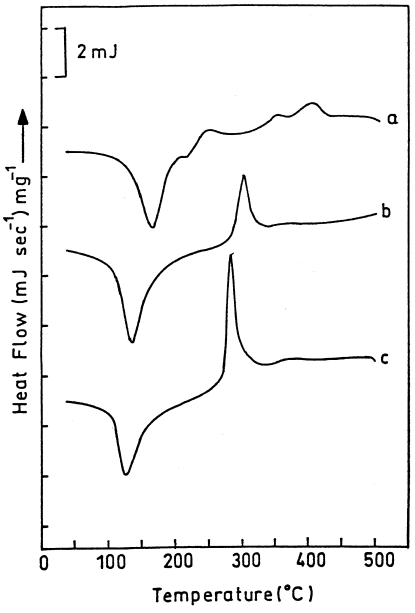

Fig. 2. DSC thermograms of (a) *O*-carboxymethyl chitin, (b) *O*-carboxymethyl chitosan, (c) *N*,*O*-carboxymethyl chitosan.

3. Results and discussion

Figs. 1 and 2 show the transitions detected in the thermograms of chitin, chitosan and their carboxymethyl derivatives. The first thermal event (series 1) registered in all samples was a wide endothermic peak centred between 125–150°C with an onset at 90–108°C. The minimum amount of the polysaccharide required to detect this endothermic peak varied considerably. In the case of carboxymethyl derivatives, a minimum of 2 mg of material was needed to detect this transition, which was related to the evaporation of water present in the sample. Values for the transition temperatures and their associated enthalpies are given in Table 1. Heating of the samples up to 220°C followed by a immediate second run in the same temperature range (series 2) yielded a thermogram with a reduced peak area (Fig. 3), supporting the view that water evaporation occurred during the first DSC scan.

The loss in weight (~15%) was confirmed by weighing the cups before and after the DSC runs. Moisture release during heating of the polymer material equilibrated over P_2O_5 suggests that the samples were not truely 'anhydrous' and that some bound water was not completely removed during drying in the desiccator. It is suggested that desiccators be evacuated to accomplish faster and complete removal of water. The onset of endothermic peak should be related to pressure build up because of water evaporation inside the cups. The pressure at which the seams of the cup started to leak can be estimated ~2.0 bar, which corresponds to approximate vapour pressure of water.

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 supramolecular structures (Kacurakova, Belton, Hirsch & Ebringerova, 1998; Phillips, Takigami & Takigami, 1996). Therefore, the endotherm related to the evaporation of water is expected to reflect physical and molecular changes during *N*-deacetylation and carboxymethylation. Close examination of thermograms in Figs. 1 and 2 (and Table 1) reveals that there are differences in the peak area and position of peak temperature of endotherm, indicating that these macromolecules differ in their water holding capacity and strength of water-polymer interaction. In the case of chitin and its *N*-deacetylated analogs (Fig. 1), the endothermic peak area increased with increase in *N*-deacetylation. A similar behavior was also observed in the case of *N*,*O*-carboxymethyl chitosan when the DS was increased from 0–1.27 (results not shown). *O*- and *N*-carboxymethyl derivatives of chitin and chitosan showed higher ΔH values than chitosans (Table 1). The fact that the ΔH increased with increase in *N*-deacetylation and carboxymethylation indicates that a definite correlation exists between the water holding capacity and chemical and supramolecular structures of these polymers. In chitin, the initial water molecules are associated with hydrophilic hydroxyl groups. As the deacetylation and carboxymethylation proceeds, the newly created hydrophilic centres, i.e.

Table 1
Thermal transitions of chitin, chitosan and their carboxymethyl derivatives (T_o, onset temperature, T_p peak temperature T_c completion temperature, ΔH enthalpy,-not detected)

Polymer	Endotherm (°C)				Exotherm I (°C)				Exotherm II (°C)			
	T_o[a]	T_p[a]	T_c[a]	ΔH[a] (mJ mg^{-1})	T_o[a]	T_p[a]	T_c[a]	ΔH[a] (mJ mg^{-1})	T_o[a]	T_p[a]	T_c[a]	ΔH[a] (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	98.8 ± 0.1	125.1 ± 0.1	164.1 ± 0.1	160.8 ± 0.1	406.3 ± 0.1	425.5 ± 0.0	453.5 ± 0.1	−51.9 ± 0.1				
Chitosan-B	95.7 ± 0.1	140.8 ± 0.1	199.0 ± 0.0	187.7 ± 0.0	291.8 ± 0.0	313.7 ± 0.0	337.5 ± 0.0	−75.9 ± 0.1	–	–	–	–
Chitosan-C	108.6 ± 0.0	144.6 ± 0.1	200.4 ± 0.1	200.0 ± 0.0	294.5 ± 0.1	314.1 ± 0.1	338.4 ± 0.1	−135.1 ± 0.1				
Chitosan-D	94.5 ± 0.1	143.7 ± 0.0	204.5 ± 0.1	218.9 ± 0.1	284.4 ± 0.1	311.1 ± 0.1	338.1 ± 0.1	−150.0 ± 0.3	–	–	–	–
Chitosan-E	100.2 ± 0.0	146.2 ± 0.0	193.1 ± 0.1	232.6 ± 0.0	256.1 ± 0.0	302.5 ± 0.0	333.1 ± 0.1	−170.2 ± .03	–	–	–	–
O-carboxymethyl chitin	102.8 ± 0.1	164.7 ± 0.1	207.5 ± 0.1	335.1 ± 0.1	217.5 ± 0.1	251.7 ± 0.1	281.6 ± 0.0	−66.0 ± 0.9	313.3 ± 0.1	407.3 ± 0.0	436.1 ± 0.1	–172.8 ± 0.3
O-carboxymethyl chitosan	81.6 ± 0.0	134.8 ± 0.1	201.4 ± 0.0	405.3 ± 0.1	266.7 ± 0.0	302.7 ± 0.1	333.0 ± 0.0	−157.5 ± 0.1	–	–	–	–
N,*O*-carboxymethyl chitosan	94.0 ± 0.1	126.3 ± 0.1	200.1 ± 0.1	280.1 ± 0.1	259.3 ± 0.1	283.3 ± 0.1	372.0 ± 0.1	−235.0 ± 0.0	–	–	–	–

[a] The values presented are average of three experiments with standard deviation.

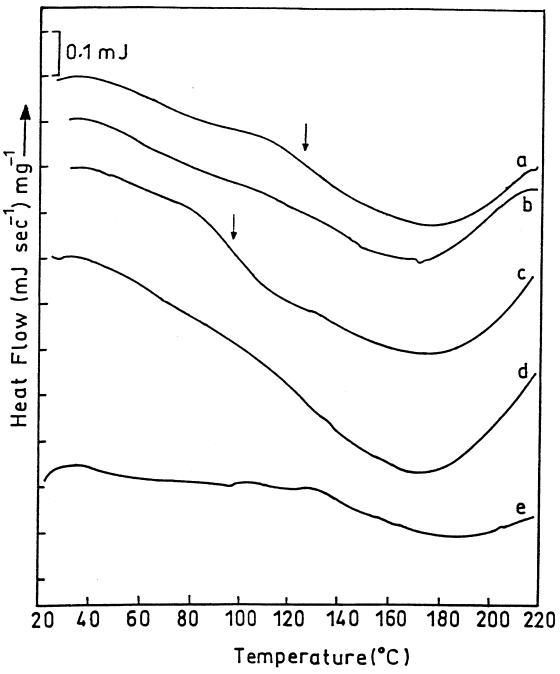

Fig. 3. Second run DSC curves of (a) chitin, (b) chitosan, (c) *O*-carboxymethyl chitin, (d) *O*-carboxymethyl chitosan, (e) *N*,*O*-carboxymethyl chitosan.

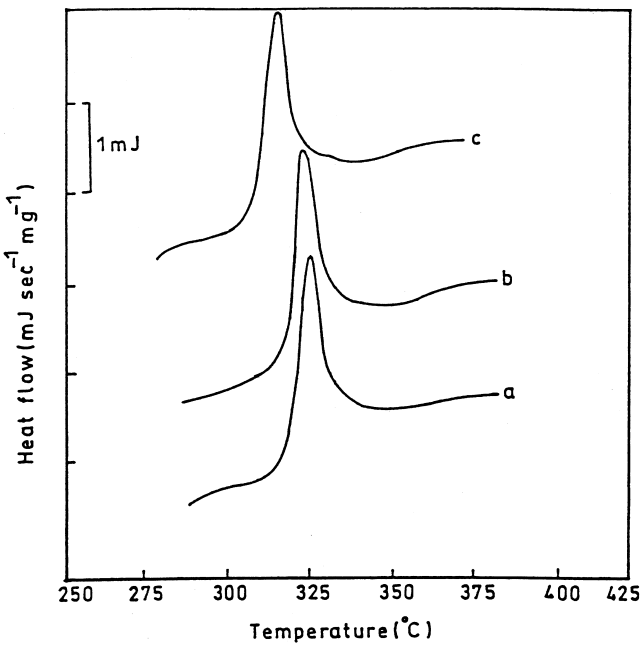

Fig. 4. DSC thermograms of chitosans with same acetyl content but with different molecular weights. Curve a (native chitosan, MW, 95,000), curve b (depolymerized chitosan, MW, 30,000), curve c (deploymerized chitosan, MW, 8000).

amine and carboxymethyl groups in the polysaccharide chain bind more number of water molecules and hence increase the content of bound water. Furthermore, the decrease in ordered structure due to chemical modification may contribute significantly towards increase in the content of sorbed water.

Penetration of alkali into the chitin crystallites with the cleavage of acetyl groups conceivably modifies the initial order and chemical structure of chitin macromolecule. Ottoy, Varum and Smidsrod (1996) observed this on chitosans prepared under heterogeneous conditions. They related the broadening of CP MAS-^{13}C-NMR signal to the presence of amorphous regions. Carboxymethylation is also expected to reduce the crystallinity of chitin and chitosans as it involves drastic reaction conditions. We confirmed the above hypothesis by X-ray diffractometry and IR spectroscopy. Chitosan A (Fig. 1, curve b) showed a %DD of 25% and CrI of 70. On the other hand chitosans B, C, D and E (Fig. 1, curves c,d, e and f) indicated %DD of 48, 79, 84, and 89 and CrI of 65, 60,54 and 45, respectively. In the case of carboxymethyl derivatives, X-ray diffractometry indicated a drastic decrease in crystallinity upon carboxymethylation. CrI values for *O*-carboxymethyl chitin, *O*-carboxymethyl chitosan and *N*,*O*-carboxymethyl chitosan were 33, 19 and 22, whereas the DS values were 0.7, 0.7 and 1.27, respectively (Fig. 2). On the basis of these results it can be deduced that, the increase in the content of polar groups together with increase in amorphous region, causes an increase in the water holding capacity upon *N*-deacetylation and carboxymethylation.

Fig. 3 shows the second heating scans after quenching the initially heated samples with liquid N_2. The temperature range of 3–220°C was selected for two reasons; 1) to avoid endothermic signal related to the melting of frozen water around 0°C and 2) to limit the possible sample degradation due to decomposition. As can be seen from the thermograms, a broad endothermic peak with T_p occurring around 175°C reappeared. This transition can be considered as due to re-evaporation into free state of bound water associated with the hydrophilic groups in the macromolecular chain. As is known, higher temperature forces both sorbed and firmly bound water into the free state away from the influence of polymer network. On cooling, a part of it reverts back to bound state. This state of water, i.e. non-freezing bound water, has been shown to remain relatively a constant event after successive cycles of heating and cooling (Kacurakova et al., 1998).

Appelqvist, Cooke and Gidley (1993) and Gidley and Bociek (1988) reported an endothermic peak in the range 50–70°C for a range of polysaccharides at a moisture level between 5 and 25%, which they attributed to enthalpic association between water and carbohydrate. However, the current consensus for the structural origin of this endotherm has been shown to favor an enthalpy relaxation (Livings, Breach, Donald & Smith, 1997), a common feature of physical ageing of many synthetic polymers and their blends (Berens & Hodge, 1982; Hodge & Berens, 1982). In the present study, the origin of an endotherm due to enthalpy relaxation seems very unlikely since, P_2O_5 drying of the samples impedes relaxation process and weight loss

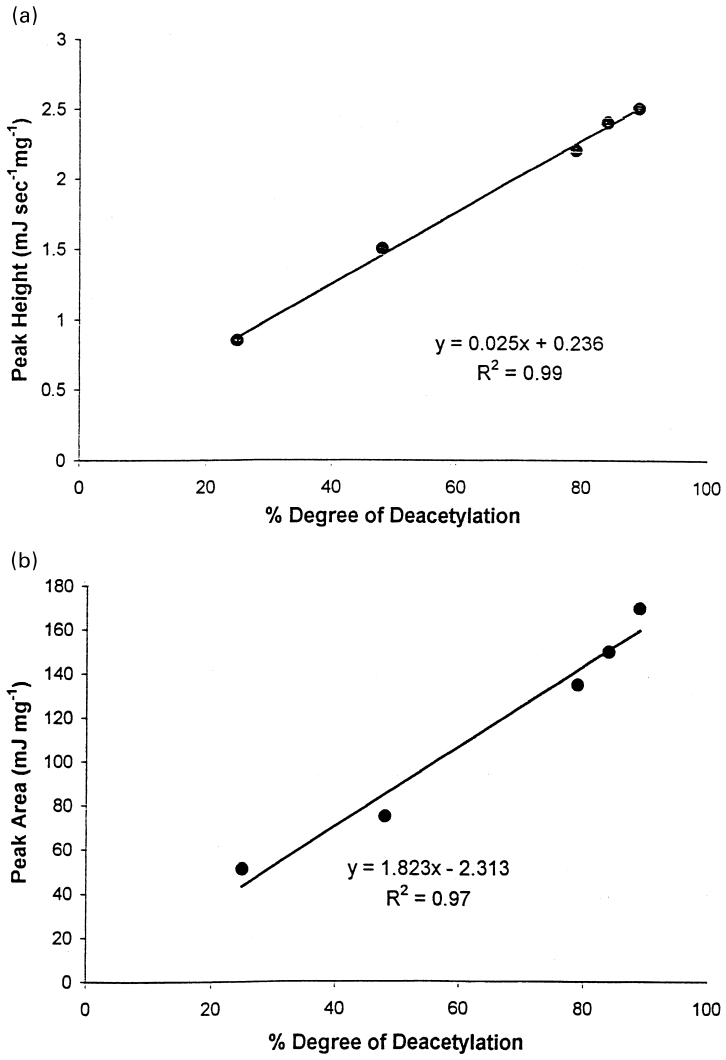

Fig. 5. (a) Correlation of DSC peak height with %DD of chitosan. (b) Correlation of DSC peak area with %DD of chitosan.

observed during the first scan reflects a mechanism other than enthalpy relaxation.

No glass transition (T_g) was observed in the second heating scans of chitin, chitosan and their carboxymethyl derivatives. Concerning the T_g of chitin and chitosan, it has been predicted previously to be latent in the decomposition temperature >230°C (Jiang, Su, Caracci, Bunning, Cooper & Adams, 1999), as in the case of cellulose. However, in the thermogram of chitin (curve a) shown in Fig. 3, there appears a gradual change in the slope of the curve in the neighbourhood of 125°C, just before the endotherm. This transition, however, appeared below 120°C for carboxymethyl chitin (Fig. 3, curve c). The origin of this transition was interpreted due to local relaxation of the backbone chain of chitin (Pizzoli, Ceccorulli & Scandola, 1991) and not due to T_g. The size of the step change in specific heat (C_p) in the thermogram depends on the fraction of amorphous phase present. In the case of carboxymethyl derivatives as discussed previously, a substantial amount of amorphous phase is already present. This, therefore, in theory, should exhibit a clear T_g. However, the present studies showed no evidence in favor of T_g; the DSC measurements showed no stepwise increase in specific heat, suggesting that the T_g value of carboxymethyl derivatives probably lies at rather higher temperature, where degradation prevents its determination.

The second main thermal event registered for these polysaccharides (Figs. 1 and 2) was a wide exothermic peak when heated between 50 and 550°C, whose area was used to express the overall exothermic effect connected with decomposition, ΔH. Due to differences in chemical and structural characteristics, remarkable differences in the exothermic transitions in chitin and chitosan were observed (Fig. 1). The exothermic peak in chitin and chitosan A (Fig. 1, curve a and b) occurring in the range 390–430°C was shifted to lower temperature concomitantly with an increase in peak area with increase in *N*-deacetylation. Such a shift to lower temperature was attributed to a decrease in thermal stability as a consequence of decreased acetyl content and degree of polymerization (dp). De-*N*-acetylation of chitin

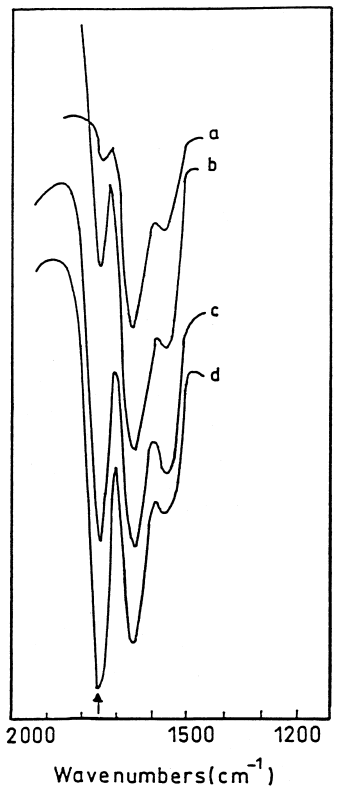

Fig. 6. Infrared spectra of *N,O*-carboxymethyl chitosan (a) DS,0.22 (b) DS,0.55 (c) DS,1.10 (d) DS,1.27.

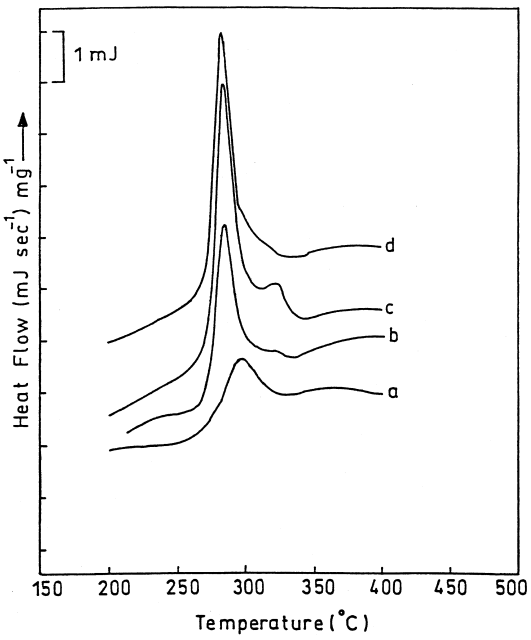

Fig. 7. DSC thermograms of N,O-carboxymethyl chitosans (a) DS,0.22 (b) DS,0.55 (c) DS,1.10 (d) DS,1.27.

under alkaline conditions causes substantial depolymerization due to glycosidic bond cleavage as a result of 'peeling' reaction. Therefore, it is reasonable to assume that a decrease in dp could also lead to decrease in thermal stability. This was further confirmed by analysis of chitosans with the same acetyl content but with different MW values. Chitinases, chitosanases and their related enzymes catalyze the cleavage of only the glycosidic bond keeping the acetyl groups intact. Therefore, the products of enzymatic hydrolysis essentially differ with respect to their MW. Fig. 4 shows the DSC thermograms for native and enzyme depolymerized chitosans. As can be seen, DSC curves are progressively shifted to lower temperature with decrease in MW. Curve a (native chitosan, $T_p = 322$°C) was shifted to 320°C with decrease in MW from 95,000 to 30,000 Da (curve b). The shift was, however, more significant in the case of chitosan with a MW 8000 Da (curve c, $T_p = 312$°C). In brief it is concluded that the decomposition peak temperature (T_p) decreases with decrease in acetyl content and dp.

The observed lower ΔH value for decomposition of chitin (highly crystalline) suggests that the decomposition efficiency might be different from its *N*-deacetylated analogues, because of differences in the mechanism of cleavage of *N*-acetyl groups. The increase in ΔH upon deacetylation, therefore, should reflect a different mechanism for the chitosan due to the presence of free unsubstituted amine groups. With this in mind, it was decided to investigate if there is any relationship between ΔH and DA. A theoretical basis proposed by Chatterjee and Schwenker (1972), that relates the heat of reaction, ΔH, of an endothermic or exothermic peak generated by the thermal response of the substituent group to DS was adopted. The values of peak height and area when plotted against %DD, good correlation coefficients R^2 of 0.99 and 0.97, respectively (Fig. 5a and b) were observed. From the Fig. 5a and b, the relationship between ΔH and %DD given by Chatterjee and Schwenker (1972) is proven to be true and helped to deduce %DD from the DSC data.

Fig. 2 shows the thermal transitions for *O*- and *N*- carboxymethyl derivatives of chitin and chitosan. In each case a well defined new peak appeared, consistent with backbone cleavage, which in some way may also involve simple cleavage of substituent groups. Curve a (carboxymethyl chitin) exhibited exotherms at 250°C and 407°C, in addition to a small exotherm observed in chitin (curve a, Fig. 1). The exotherm at lower temperature can be attributed to decomposition of highly substituted regions in the polysaccharide. Conversion of carboxymethyl chitin to carboxymethyl chitosan (curve b) resulted in the disappearance of both high and low temperature exotherms, and a new peak appeared in the temperature range of 270–300°C. Since the carboxymethyl groups are stable under the conditions of alkaline deacetylation, the shift of decomposition peak to lower temperature can be attributed to decrease in acetyl content and dp during deacetylation, as discussed previously. This was further confirmed by HPSEC, which indicated a relative MW of 12×10^5 and 7.9×10^5 Da for carboxymethyl derivatives of chitin and chitosan, respectively. The thermograms of

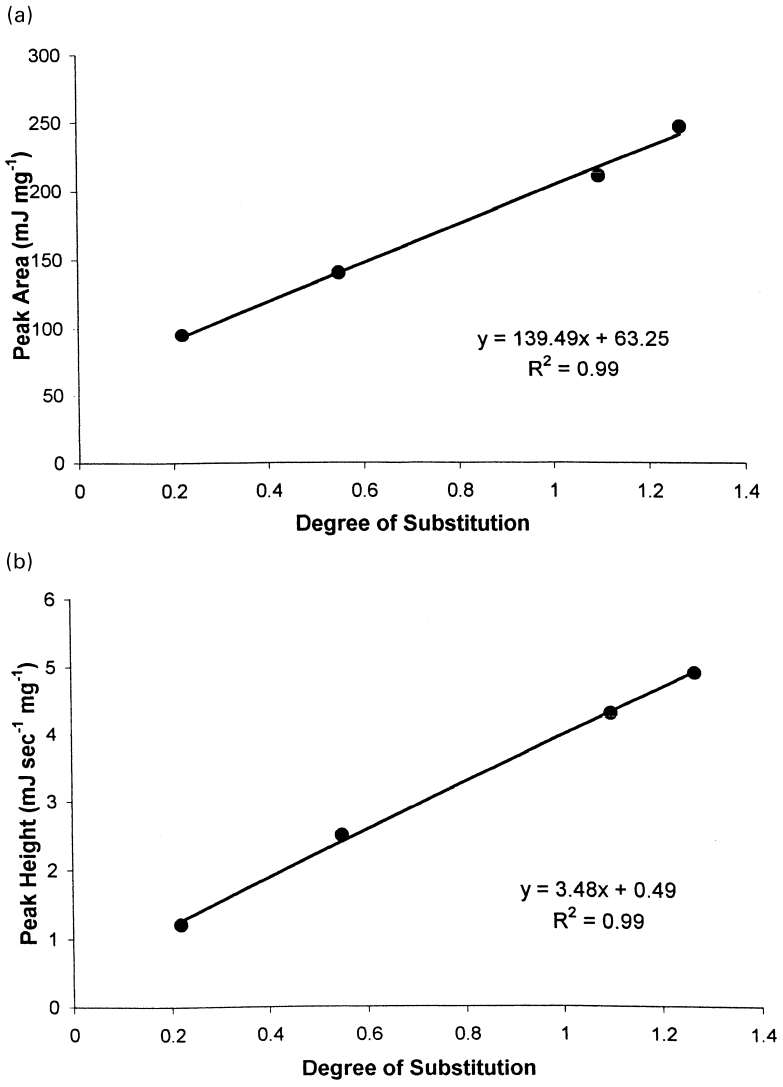

Fig. 8. (a) Correlation of DSC peak height with DS of *N,O*-carboxymethyl chitosan. (b) Correlation of DSC peak area with DS of *N,O*-carboxymethyl chitosan.

O-carboxymethyl and *N,O*-carboxymethyl chitosans were similar, except for the decomposition which appeared at lower temperature. Nonetheless, each curve was distinctly and measurably different from the base curve (Fig. 1, curves a and b) so that their unequivocal identification was assured.

It was decided to study the effect of carboxymethylation on chitosan and to determine the relationship between peak area and peak height wiith DS. *N,O*-Carboxymethyl chitosan was selected because of ease of preparation with high DS value. Fig. 6 shows the FTIR spectra for the variously carboxymethylated chitosans. As can be seen, the intensity of the band at 1735 cm^{-1} (–COOH) increased with increase in carboxymethylation. Fig. 7 shows the representative DSC curves for N,O-carboxymethyl chitosans with DS = 0.22, 0.55, 1.10 and 1.27. A new exothermic peak, T_p in the range 280–300°C was observed which was generated during earlier stages of chitosan decomposition, overlapping with the actual chitosan decomposition peak (T_p = 316°C). In order to determine the peak area, it was necessary to extrapolate the high temperature side back to the baseline and to measure the area thus enclosed. At higher DS value, the area became fully resolved. Also an increase in carboxymethylation led to an increase in ΔH. These differences indicate different mechanisms of chitosan decomposition taking place in the presence of carboxymethyl groups. Additionally, a plasticizing action is also envisaged for carboxymethyl group (Biliaderis, 1982).

Fig. 8a and b show the calibration curves obtained by plotting the peak area and peak height versus DS of *N,O*-carboxymethyl chitosan. A linear regression coefficient R^2 of 0.99, a slope of 139.5 and a *Y*-intercept of 63.2 was obtained based on peak area. On the other hand, when the peak height was used, the corresponding linear regression coefficient, slope and *Y*-intercept were 0.99, 3.48 and 0.49, respectively. Thus, both the height and area appear to be parameters to measure and correlate the DS. These results indicate that the DS of any sample can be determined from the DSC data using the calibration curves shown in Fig. 8a and b.

4. Conclusions

DSC was demonstrated to be an effective technique to evaluate the process of *N*-deacetylation and carboxymethylation. The thermal transitions for chitin, chitosan and their carboxymethyl derivatives took place in two stages reflecting changes in both chemical and physical structures. During decomposition stage, a decomposition peak characteristic of a modified chitin and chitosan appeared as a function of MW, acetyl and carboxymethyl contents. Furthermore, in the case of chitin and *N*,*O*-carboxymethyl chitosan, ΔH value increased with increase in amine and carboxymethyl contents. The relationship between ΔH and DS was proved to be true. Fig. 5a and b and Fig. 8a and b showed how well the peak parameters correlate with DS. It is possible that the %DD/DS of chitosan and *N*,*O*-carboxymethyl chitosan of any acetyl/carboxymethyl content can be obtained by this relationship, showing ΔH to be a good measure to determine the former.

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