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Advances in Applied Science Research, 2012, 3 (3):1372-1382

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ISSN: 0976-8610
CODEN (USA): AASRFC

Characterization of the glass transition temperature of chitosan and its oligomers by temperature modulated differential scanning calorimetry

Prerna P. Dhawade* and Ramanand N. Jagtap

Department of Polymer and Surface Engineering, Institute of Chemical Technology, Matunga, Mumbai, India

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ABSTRACT

In differential scanning calorimetry (DSC), remnant moisture loss in samples often overlaps and distorts thermal events such as glass transitions. Temperature modulated DSC (TMDSC) is known for separating such overlapping processes. In this study, chitosan was depolymerized by oxidative degradation method. By this method, chito-oligomers of different molecular weights were produced. The glass transition temperature (Tg) of chitosan and its oligomers was analyzed by temperature modulated DSC. While performing this event hermetically sealed pans were used. Tg of chitosan and its oligomers was resolved by TMDSC-exhibiting glass transition temperature in the first heating curve. The structural formation of chito-oligomers was evaluated by FTIR and ^{1}H NMR. The water plasticizing effect on Tg of chitosan and its oligomers was discussed with the help of TMDSC measurements and the presence of water in them have been evaluated by FTIR and ^{1}H NMR. XRD had explained the crystallinity of chitosan and its oligomers and even the effect of water on crystallinity.

Key Words Temperature modulated DSC; Chitosan depolymerisation; Oxidative degradation; Moisture; Plasticizing effect.

INTRODUCTION

Differential scanning calorimetry is generally employed for finding glass transition temperature of biopolymers in presence of water [1]. Native structure of biopolymers is maintained in its steric form till the water is present in it. If the structure undergoes deformation then water is lost which destroys its steric structure affecting its properties, as biopolymers show biological activity in presence of water [1]. Even presence of water affects many properties of polymers, such as rheological, transport properties and glass transition temperature [2]. Presence of moisture in material gives a broad endothermic peak due to evaporation of water during DSC analysis. This affects the thermal measurements such as Tg. Here, water acts as plasticizer lowering the glass transition temperature. Dry samples of the same material will give different Tg than the wet material [3]. Therefore, materials which are hygroscopic in nature are subjected to advanced technique of thermal analysis ie. Temperature modulated differential scanning calorimetry (TMDSC).

Temperature Modulated differential scanning calorimetry (TMDSC) is an advanced technique of differential scanning calorimetry which involves the superimposition of a modulation on the conventional linear temperature programme, allowing separation of the total heat flow signal into its heat capacity and kinetic components, these being known as the reversing and non-reversing heat flows respectively. The glass transition temperature is obtained from reversing heat flow [4]. Whereas the crystallization occurs from the non-reversing heat flow as it is a non-reversing process which liberates heat. This technique offers a unique combination of high resolution and high

sensitivity [4]. It is mostly used to separate overlapping of thermal effects such as cold crystallization and glass transition of polymer blends [5]. This technique is widely used in pharmaceutical industries to determine Tg [6,7]. The effect of absorbed water on Tg of the amorphous system has been resolved in the first cycle of TMDSC [8]. This is how TMDSC is used in resolving the Tg when there is presence of water in the material.

Chitosan is a biodegradable polymer obtained by alkaline deacetylation of chitin. It is a linear copolymer of 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose joined by β (1,4) glycosidic bonds. Chitosan is nontoxic, biodegradable, biocompatible polymer having inherent film forming property which also exhibits biological activity [9]. Chemical modification of chitosan becomes feasible due to presence of amino and hydroxyl groups in it. It finds wide application such as biomedical [10], food [11], textile [12], waste water treatment [13], adsorption [14] and interior finishing coatings for formaldehyde adsorption [15]. The effect of water content on phase structure of water/ chitosan has been investigated by conventional differential scanning calorimetry [16]. Even a comparative study of water effect on starch and chitosan was carried out using conventional DSC [17]. Conventional differential scanning calorimetry of chitosan has provided different glass transition temperatures (Tg) [16, 18-20]. Chitosan is a high molecular weight polymer. Its chemical structure and molecular size [21] decides its applicability. Therefore to enhance its applicability it has been depolymerized. Chitosan has been degraded to different molecular weights by various methods of degradation. It consists of degradation of chitosan by sodium nitrite [22], hydrogen peroxide [23], enzymatic degradation [24], ozone treatment [25] potassium persulphate [26] etc.

In this study chitosan is depolymerised by oxidative degradation method and the effect of water on chitosan and its oligomers which are formed after deformation in the structure of a native chitosan has been studied. For this evaluation temperature modulated differential scanning calorimetry is used in order to obtain precise glass transition temperature and the effect of water on Tg.

MATERIALS AND METHODS

Chitosan obtained from shrimp shells was acquired from Sangam Laboratories (Mumbai, India). The degree of deacetylation (DDA) of chitosan was 85%. Chitosan was obtained in the solid form and its colour was whitish ivory. Acetic acid, sodium hydroxide, sodium nitrite, methanol used were purchased from S. D. Fine Chemicals Ltd. Acetate buffer for intrinsic viscosity measurement was procured from Himedia chemicals Ltd. Deuterated water (D_2O) and deuterated acetic acid (d_4-CD_3COOD) used for ^1H NMR was obtained from Sigma-Aldrich.

Depolymerization of Chitosan

The low molecular weight chitosan were prepared by oxidative degradation with $NaNO_2$ at room temperature [22]. 1g chitosan was dissolved in 1% (v/v) 100ml acetic acid solution. When chitosan was completely dissolved, $NaNO_2$ in different concentrations viz, 0.08, 0.1 and 0.2g was added slowly and stirred at room temperature for three hours. 1 N NaOH was used to neutralize the reaction mixture. Subsequently, chitosan was precipitated by adding above solution in excess of methanol. It was filtered, washed several times with methanol and dried at room temperature. After this various oligomers like C1 (Chitosan depolymerized by 0.08g of sodium nitrite), C2 (Chitosan depolymerized by 0.1g of sodium nitrite), C3 (Chitosan depolymerized by 0.2g of sodium nitrite) from pure chitosan (B) were formed.

Characterizations

Molecular weight of chitosan and its oligomers has been measured by intrinsic viscosity method. For measuring intrinsic viscosity of chitosan, pure chitosan and its oligomers were dissolved in acetate buffer. Ubbelohde capillary viscometer was used to determine intrinsic viscosity in a constant-temperature water bath at 25 ± 0.01 ◦C in triplicate. The capillary diameter used was 0.63 mm.Various concentrations were tested for each sample. The intrinsic viscosity was determined by the common intercept of both Huggins ($\eta sp/C \sim C$) and Kraemer ($\eta inh \sim C$) plots on the ordinate at $C = 0$.

While reduced viscosity of polymers is defined as:

$$\eta_{sp} / C = t\text{-}t_o / t_o C$$

and inherent viscosity is defined as:

$$\eta_{inh} = \ln (t/t_0)/C$$

where t_0 is the flow time for solvent and t is the flow time for tested solution.

Both $\eta sp/C$ and ηinh are plotted on the same graph. The common intercept of the plots on the ordinate at $C = 0$ gives:

$[\eta] = (\eta sp/C)_{C=0} = (\eta inh) C=0 = 3.85$ L/g

The viscosity-average molecular weights of chitosan were calculated using the classical Mark-Houwink equation:
$[\eta] = K (Mv)^a$

Where $[\eta]$ is the intrinsic viscosity of the depolymerized chitosan, K and a are constants for given solute–solvent system and temperature. For pure chitosan, degree of deacetylation (DD) value was 85%.

The constants reported in literature [21] are $K = 1.38 \times 10{-}5$ and $a = 0.85$

Fourier transformed infrared spectroscopy (FTIR) was conducted on FT-IR spectrometer (Shimadzu 8400 S) in the range between 3400 and 600 cm^{-1}. Spectra was evaluated on attenuated total reflectance using diamond crystal (angle of incidence = 45˚). 64 scans with a resolution of 2 cm^{-1} were given.

The structure of chitosan and its oligomers prepared above were analyzed by ^1H NMR spectroscopy. ^1H NMR was recorded on Bruker Avance 500 spectrometer. All samples were dissolved in 1% (v/v) d_4-CD_3COOD/ D_2O at a concentration of 2mg/ml. These samples were transferred in 5mm NMR tube. Internal standard used was d_4-CD_3COOD/ D_2O. ^1H NMR spectra of samples were acquired at 500 MHz; 25 °C; centre of peak: 4.28ppm; size of spectral window: 7002 Hz; time domain: 16384; acquisition time: 1.16 s; number of scans 256 and data size, 16 K.
The TMDSC experiments were conducted using a Modulated DSC TOPEM® (TOPEM® is modulated DSC optimized by Mettler Toledo) with a Refrigerated Cooling System (RCS) unit attached. The instrument was calibrated using the melting of indium standard. 'White spot' nitrogen was used as the purge gas which was flowing at a rate of 40 cc/min throughs DSC cell. Perkin Elmer aluminium hermetic pans were used throughout the study. The sample weight employed was constant at approximately 15mg. After loading the samples, the pans were sealed with a dry weld. The following parameters were selected: modulation amplitude of 0.159°C and a 30 s modulation period with a 2°C/ min underlying heating rate. The experimental method consisted of an initial 20-min isothermal period at 25°C to allow equilibration of the sample to the programmed temperature modulation, then heating to 130°C. Analysis of the results was carried out by MT Analysis software

Thermogravimetric analysis was carried out on a TGA 60H, Shimadzu. All analysis was performed with a 10mg sample in aluminium pans under a dynamic nitrogen atmosphere between 0-500 ˚C. The experiments were run at a scanning rate of 10K/min.

X-ray diffraction patterns of samples were measured by a Rigaku XRD-6000 diffractrometer and used a CuKa target at 40 kV and 30 mA at 21˚C.

RESULTS AND DISCUSSION

Depolymerization of chitosan

Depolymerisation of chitosan is a homogenous reaction and it is proportional to the amount of nitrous acid used. Sodium nitrite attacks the amino group of chitosan instead of N- acetyl group. Subsequently, the glycosidic linkage breaks and 2,5-anhydro-D-mannose forms. The concentration of sodium nitrite plays a significant role in the depolymerisation process of chitosan. Therefore by keeping chitosan concentration constant at 1g in 100ml of 1% acetic acid, the concentration of sodium nitrite has been changed. The molecular weights of the samples have been measured. It has been observed that the molecular weight decreases rapidly as the concentration of sodium nitrite increases. Depolymerisation of chitosan by sodium nitrite above 0.2g gives a rapid decrease in molecular weight. The relationship between concentration of $NaNO_2$ and the molecular weight (MW) of chitosan oligomers is shown in Figure 1. Furthermore, from the slope of the profiles, one can conclude that molecular weight of chitosan decreases progressively as the concentration of $NaNO_2$ increases. This may be explained by larger molecular dimensions of chitosan in solution, which increases the contact area with $NaNO_2$.

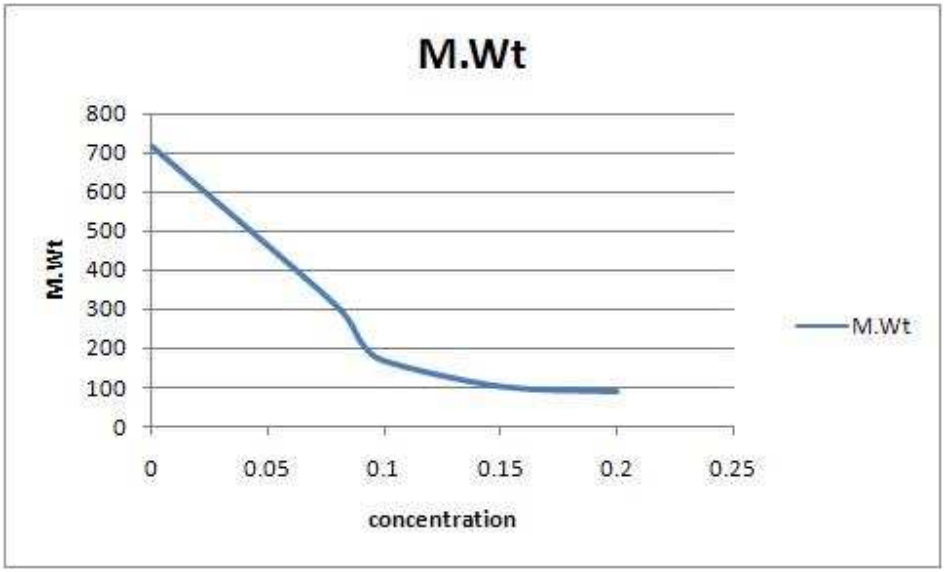


Fig-1 Relationship between concentration of $NaNO_2$ and the molecular weight (MW) in kDa of chitosan oligomers

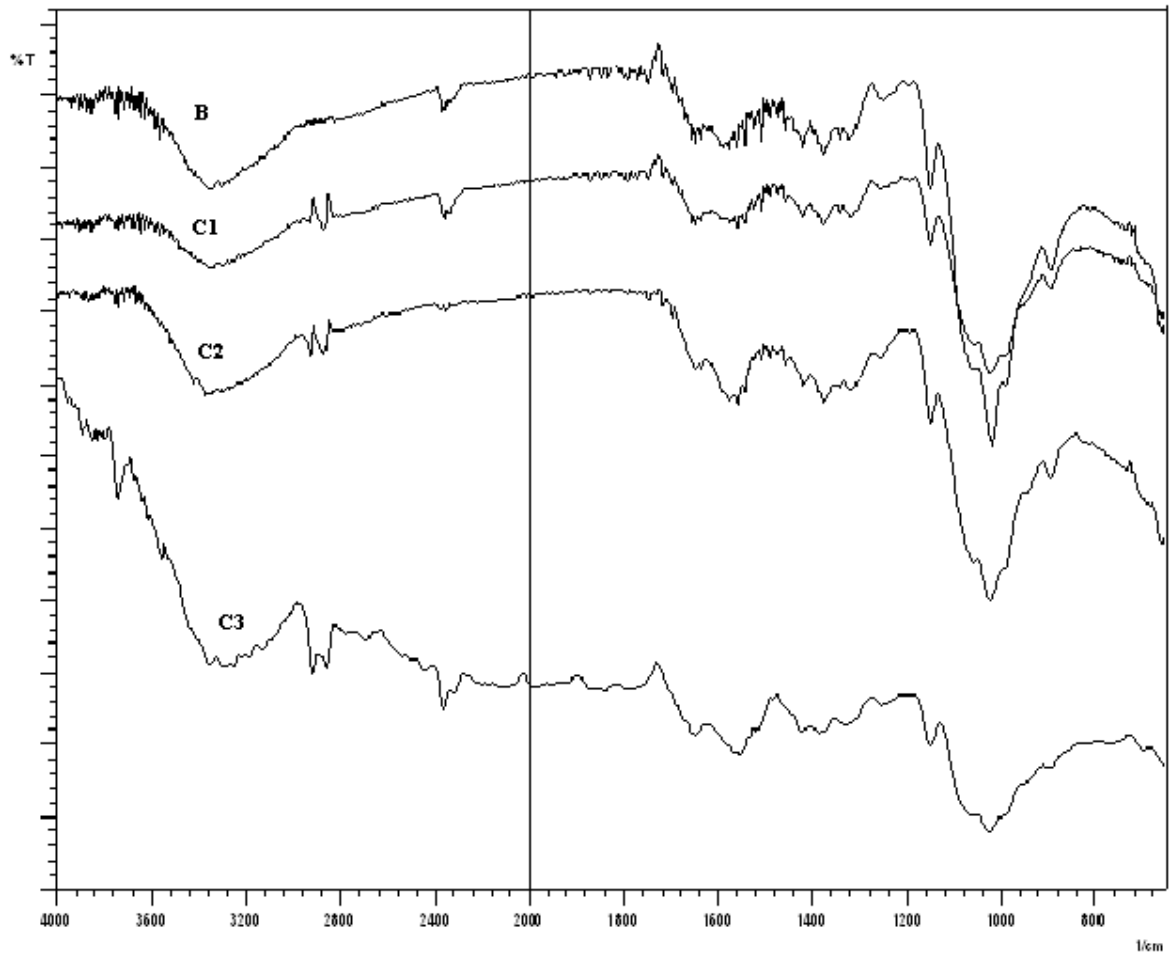
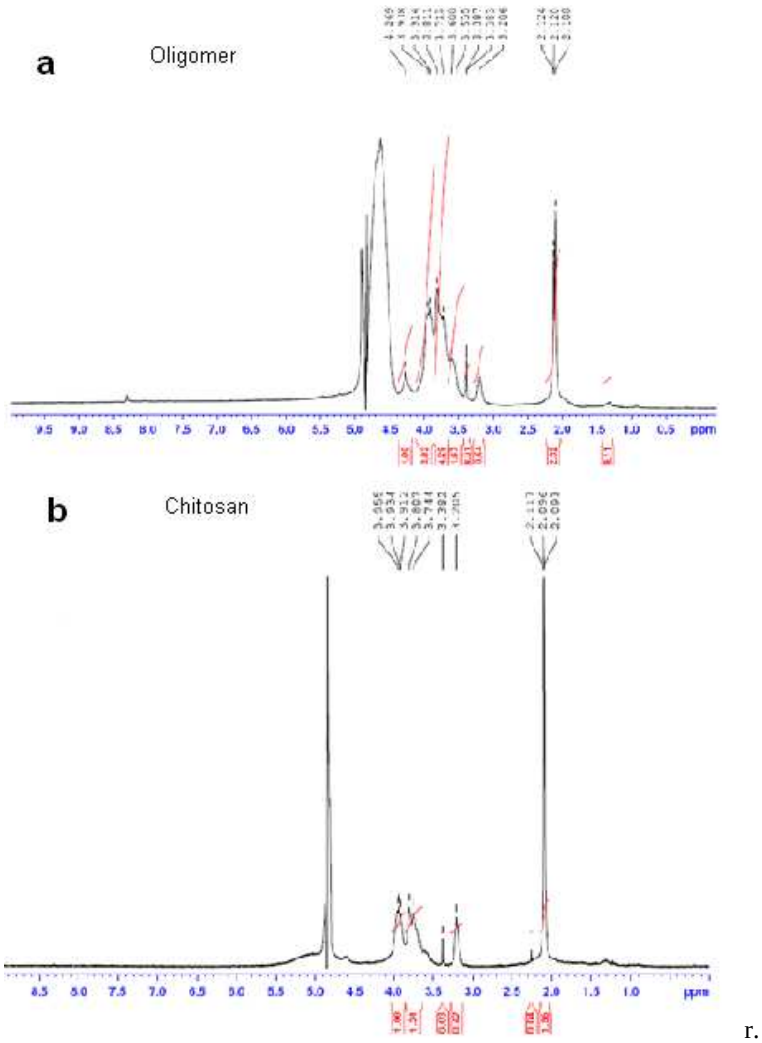


Fig-2 FTIR of pure chitosan (B) and its oligomers (C1, C2, C3)



r.

Fig-3 H^1 NMR spectra of oligomer (a) and pristine chitosan (b)

FTIR analysis of depolymerized chitosan samples

As shown in Figure 2, FTIR is used to depict the structural changes in the depolymerised chitosan. Chitosan showed characteristic peaks in IR spectrum which confirms its saccharide structure. These peaks are obtained at 1151, 1068, 1022, and 892 cm^{-1}. Characteristic strong amino peak was found at around 3425 cm^{-1}, 1651 cm^{-1} and 1317 cm^{-1} which are assigned to amide I and II bands respectively. The peak at 1418 cm^{-1} in C3 is the joint contribution of bend vibration of OH and CH. Its oligomers have shown the characteristic peaks of saccharide at 1151, 1072, 1018 and 892 cm^{-1}. The strong absorption at 1650 cm^{-1} are attributed to the absorption of δ_{N-H} (–NH2) and δ_{N-H} (amide II). With the decrease in molecular weight, the IR spectra of chitosan oligomers showed many fine absorption peaks compared to initial chitosan. As the aldehyde group in depolymerised chitosan is present in the hydrated form, the free aldehydic group absorption appearing at 1716 cm^{-1} does not appear. This is supported by H^1 NMR.

Presence of water in chitosan can be elucidated from IR spectra. In chitosan hydrogen bonded hydroxyl group gave a peak at 3353 cm−1. This peak in oligomers appears at lower frequency due to formation of five membered ring formed due to depolymerisation. The broadness of the peak is due to presence of water and subsequent hydrogen bonding. Along with chitosan, its oligomers also show such broadness in the peak appearing at around 3342 cm−1.

^{1}H NMR analysis of depolymerized chitosan

^{1}H NMR spectra of pure chitosan and its oligomers are represented in Figure 3a & 3b. ^{1}H NMR of pristine chitosan was obtained at 500 MHz. Internal standard used was d_4-CD_3COOD/ D_2O, its peak appears at 4.78 ppm. Two resonances H-1 and H-2 at 4.8ppm occur due to 2-amino-2-deoxy- D-glucopyranose. Peak at 4.8 ppm of 2-amino-2-deoxy-D-glucopyranose and peak of internal standard overlap each other and therefore peak of (-CH) of glucosamine is obscured. (-C<u>H</u>-NH_2) proton is represented by a peak at 3.20 ppm. Chemical shifts at 3.38 ppm and 3.744 ppm correspond to protons of –C<u>H_2</u>-OH. Chemical shifts from 3.91-3.95 ppm correspond to HOH_2C-C<u>H</u>-, CH-C<u>H_2</u>- and -C<u>H_2</u>-OH protons of glycoside ring. Peaks appeared between 3.2-3.9 ppm are broad due to presence of water in chitosan. ^{1}H NMR of depolymerised chitosan has exhibited same resonance as that of chitosan but they are more defined in oligomers. As the molecular weight of chitosan reduces, the water content in the oligomers decreases, but still the broadening of peaks in ^{1}H NMR spectra of oligomers is observed due to presence of water. The peak appearing at 5.1ppm in oligomers is merging with peak appearing at 4.8ppm which indicates presence of a hydrated aldehyde where a water molecule has been added to the aldehydic group which seems to be the only existing form of the reducing end in wate.

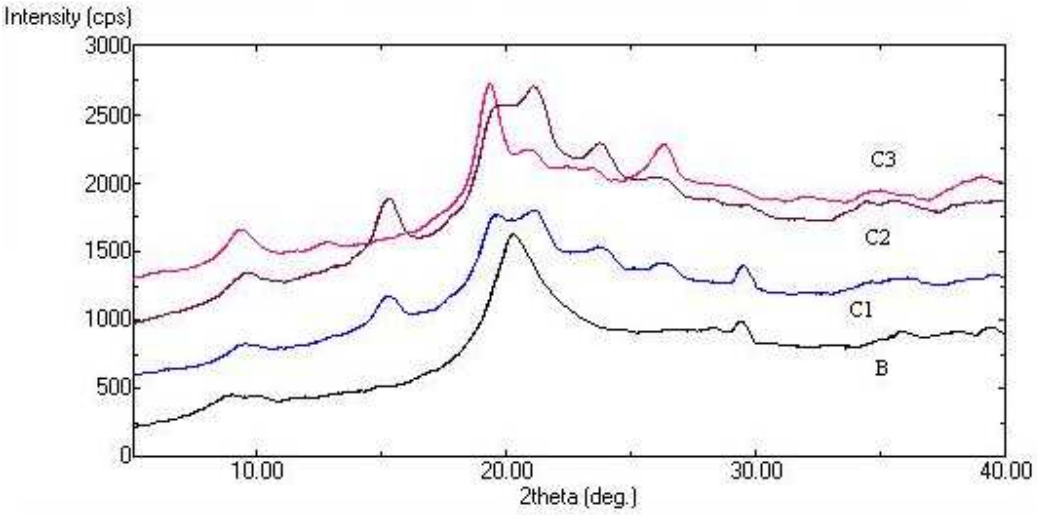


Fig-4 X-ray diffraction pattern of chitosan (B) and its oligomers (C1, C2, C3)

XRD of chitosan and its oligomers

In Figure 4, X-ray diffraction patterns of chitosan (B) and its oligomers (C1, C2, and C3) are shown. As reported previously initial chitosan shows characteristic peaks at 10.4º and 20.4º which coincides with the pattern of the 'L-2 polymorph' of chitosan. In oligomers a new peak is observed at 2θ = 21º and the other characteristic peaks at 10.4º and 20.4º have been increased. These have patterns characterized of chitosan polymorph which is referred to as the 'tendon hydrate polymorph' [27]. The other peaks are observed at 2θ = 15 º and 23 º which is referred to as the annealed polymorph' as described earlier by Ogawa [28]. The higher molecular weight chitosan did not show complete conversion to the annealed polymorph because of the low mobility of its polymer chain as presence of water forms hydrogen bond with hydroxyl and amine group. The crystallinity of chito-oligomers is increasing in the order B < C1 < C2 < C3 which is evident from (Fig. 4**)**. This confers that crystallinity increases with the increase in depolymerisation and decrease in water content due to which re-crystallization of short chain occurs.

Thermogravimetric analysis

TG curves of chitosan and its oligomers are shown in Figure 5. The first stage begins at 80ºC with weight loss of 6%. The second stage starts at 260 ºC and reaches maximum at 380 ºC with weight loss of 54%. The weight remains after 500 ºC is 40%. The first stage starts at 60 ºC with weight loss of 6% for chitosan due to the loss of water. Whereas the water loss in oligomers is more during thermal analysis which infers that their thermal stability is less as compared to chitosan. Even from the second stage of thermal analysis it can be observed that weight loss is more as the molecular weight decreases. As molecular weight decreases the amine group present in chitosan decreases due to which even the water content decreases, eventually the stability of oligomers reduces down. Therefore, in the first stage only the oligomers losses most of its weight and as the thermal analysis moves ahead it losses most of its weight. After 500 ºC the remaining weight of the oligomers goes down substantially. This analysis confirms

structural changes which occur during depolymerisation of chitosan. Thermal stability of chitosan is more than the oligomers as their molecular weight decreases.

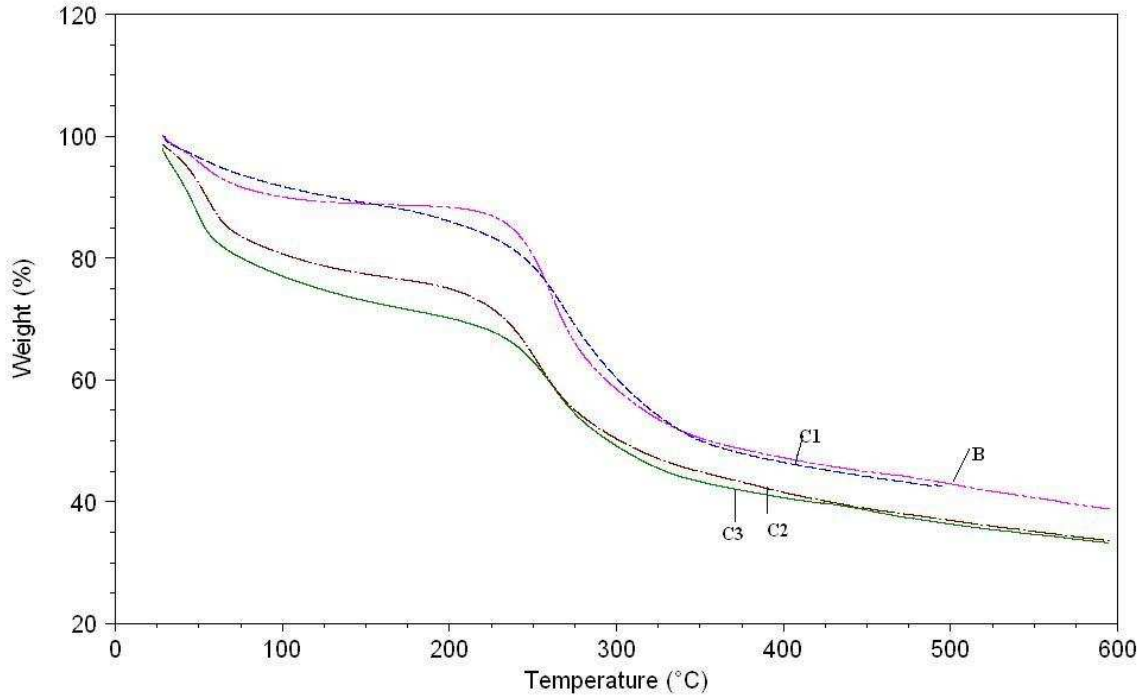


Fig-5 Thermogravimetric graphs of pure chitosan (B) and its oligomers (C1, C2 and C3)

Modulated Temperature Differential Scanning Calorimetry

Ratto et. al [16] have reported presence of water in chitosan and its effect on glass transition temperature. They have observed glass transition temperature of chitosan at 30˚ C for water content ranging from 8 to 30%. Lazaridou and Biliaderis [18] found Tg ranging from -23˚ to 67˚C as per the water content in them. This indicates plasticizing effect of water in both the above cases. Whereas Sakurai, Maegawa and Takahashin [19] reported Tg of chitosan at 203˚ C. While Kittur et. al [20] found no evidence for Tg suggesting that Tg for chitosan could lie at a higher temperature, where degradation prevents its determination.

Water is present in three forms in macromolecules: free freezing water loosely bound, freezing water with a melting point below that of pure water and water tightly bound to hydrophilic groups that does not freeze [2]. To determine exact Tg of chitosan and its oligomers, as water mask the Tg (TOPEM®) has been used because TMDSC is such a technique where reversing and non-reversing heat flow are separately detected. As the glass transition temperature is the change in sample heat capacity, it has been observed in reversing heat flow instead of non-reversing heat flow. This can be understood with the reference to the basic heat flow equation associated with TMDSC.

$$dQ/dt = Cp\ dT/\ dt + f\ (t, T) \qquad (1)$$

where dQ/dT is the total heat flow (J/s or W), Cp is the heat capacity (J/K) and dT/dt is the heating rate. The term Cp dT/dt is the reversing heat flow which is dependent on rate of change of temperature and heating flow. Whereas f (t, T) is the non-reversing heat flow component comes from kinetically controlled event which has heat flow contribution and it is dependent on both temperature and time. The Cp dT/dt component is calculated from the response to the oscillation via

$$A_{mhf} / A_{mhr} . K = Cp \qquad (2)$$

where A_{mhf} is the amplitude of the modulated heat flow signal, A_{mhr} is the amplitude of the heating rate signal and K is the heat capacity calibration constant. Eq.2 provides the non-reversing signal which is the difference between the total heat flow response and the reversing signal. As the limits of Tg measurement are less dependent on the baseline quality it is considered that the technique should give an enhancement in sensitivity, because ΔCp is calculated from the amplitude of the oscillation rather than an inflexion in the baseline. The assumption given by Reading et al. [29], Lacey et al.[30] that the response of the rate of the kinetic response to the temperature is linear over the modulation interval, hence the phase lag between the modulated heat flow and the derivative modulated temperature is negligible is used in this and subsequent deconvolutions.

TMDSC graphs of chitosan and its oligomers are illustrated in Figure 6. (a-d). Choice of pan during TMDSC measurement is important [8]. Therefore, the study of temperature modulated DSC of chitosan and its oligomers were carried out in hermetic sealed pans in order to avoid the loss of water present in them. Use of hermetic sealed pan helps in keeping the water content constant throughout the temperature programme. The presence of glass transition temperature appears on the reversing heat flow signal. The hermetic pan does not allow water to escape which leads to plasticization of the material which eventually lowers Tg. It has been confirmed by the mass of the hermetic pan remaining constant before and after the MTDSC run. TOPEM® graphs clearly demonstrate the ability of TMDSC to separate the associated endothermic relaxation from the glass transition of chitosan and its oligomers. The water loss peak appears in the non-reversing heat flow together with the endothermic relaxation peak as it is a kinetically controlled event.

From conventional DSC the Tg of glass transition temperature of chitosan has appeared at 118ºC in dry state. In presence of water and by temperature modulated DSC analysis, it came out to be at 61 ºC. This confirms that water does acts as plasticizer in chitosan. Water form an intermolecular hydrogen bonding with chitosan through amine and hydroxyl groups present in them. This helps in molecular rearrangement which eases the chain mobility in chitosan.

TOPEM® of chitosan Figure 6.a, shows same type of thermographs for total heat flow and non-reversing heat flow. Due to this Tg of the material cannot be elucidated, as the presence of water is masking the Tg. But when the total heat flow is further divided into reversing heat flow and non-reversing heat flow, the Tg becomes more prominent. In reversing heat flow Tg for chitosan is obtained at 61.37ºC. The water which is presenting in three different forms starts liberating at various stages. Freezing water with a melting point below that of melting point of water comes out at around -5 ºC, tightly bound water releases in the range of ±10 ºC- 100 ºC and free loosely bound water is liberated at about 150 ºC. From heat capacities obtained from non-reversing and reversing heat flow it can be concluded that the Tg values are correct to its order. TGA studies indicated that the endotherm appearing at a lower temperature corresponds to a weight loss process which it is reasonable to assume represented loss of water. As TMDSC analysis is performed in hermetically sealed pans the removal of water does not happen whereas it shows its plasticizing effect on glass transition temperature. Whereas in TGA, pan is not sealed, therefore; it shows removal of water peak and even on constant heating it leads to degradation.

Oligomers are prepared by oxidative degradation method in which amine group decreases. As amine groups also contribute in hydrogen bonding with water, their reduction eventually reduces water content in oligomers exponentially with the decrease in the amine group. But, as the water reduction is exponential with the decrease in molecular weight, it helps in providing the same plasticizing effect of water in oligomers which it has shown in chitosan. Therefore, TOPEM® of oligomers, Figure 6.b shows the same trend of chitosan. In sample C1 water liberates in the range of -5 ºC-150 ºC which masks the Tg occuring at around 40.76 ºC. But reversing heat flow eventually separates it from non-reversing and making it more prominent. The water content in this sample is 9.33% whereas it was 9.78% in pure chitosan. Due to depolymerisation the water content reduces down eventually decreasing the heat capacity required to provide Tg. But the water content does not reduces drastically because there is not much decrease in bound water which helps in providing Tg which is not very less in comparison to pristine one. Therefore, even though there is a drastic difference in molecular weights of chitosan and its oligomers, but there is a little decrease in Tg. Even the structural change is small which aids in restoring water content and provides the precise value of Tg for oligomers. The mobility of chain becomes less as the water content goes down and rearrangement of polymer chains becomes confined to glass transition of the amorphous regions. Same trend is observed in other two oligomers. The Tg of C2 is 32.22 ºC whereas the water content in it is 8.22%. Sample C3 attains the Tg at 12.17 ºC whose water content is 8.05%. Hence a clear manifestation of glass transition temperature of chitosan and its oligomers in presence of water has been obtained using TMDSC.

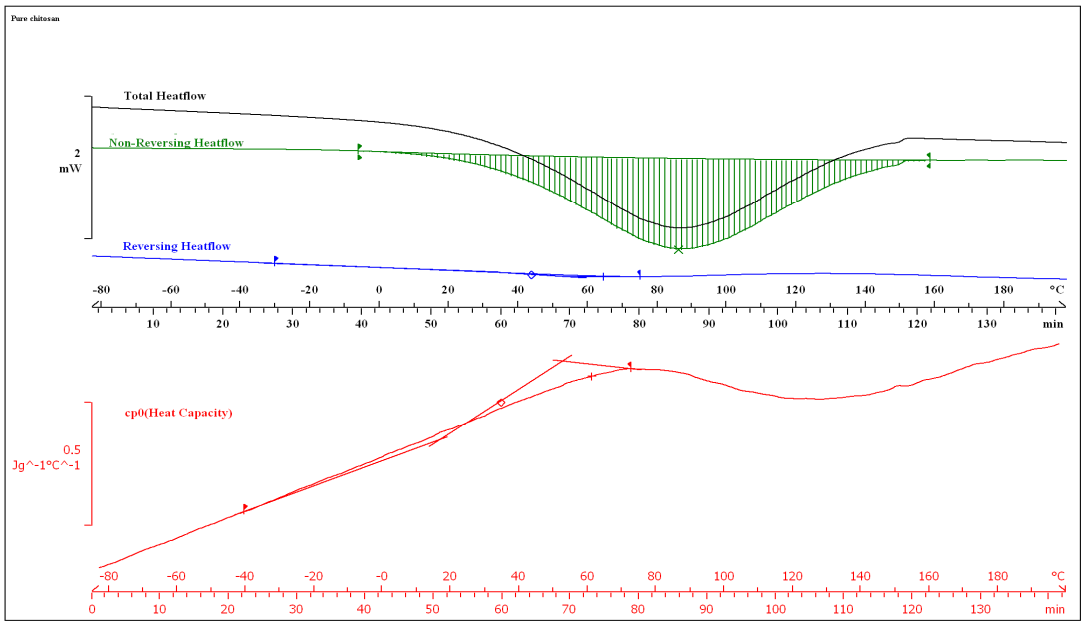


Fig-6.a Modulated DSC TOPEM® of pure chitosan (B)

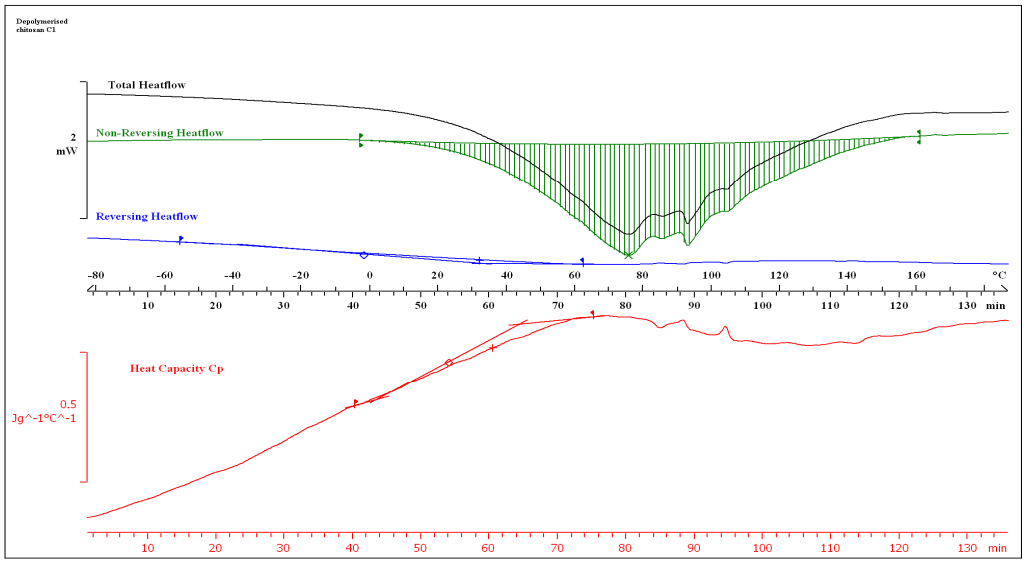


Fig-6.b Modulated DSC TOPEM® of chitosan oligomer C1

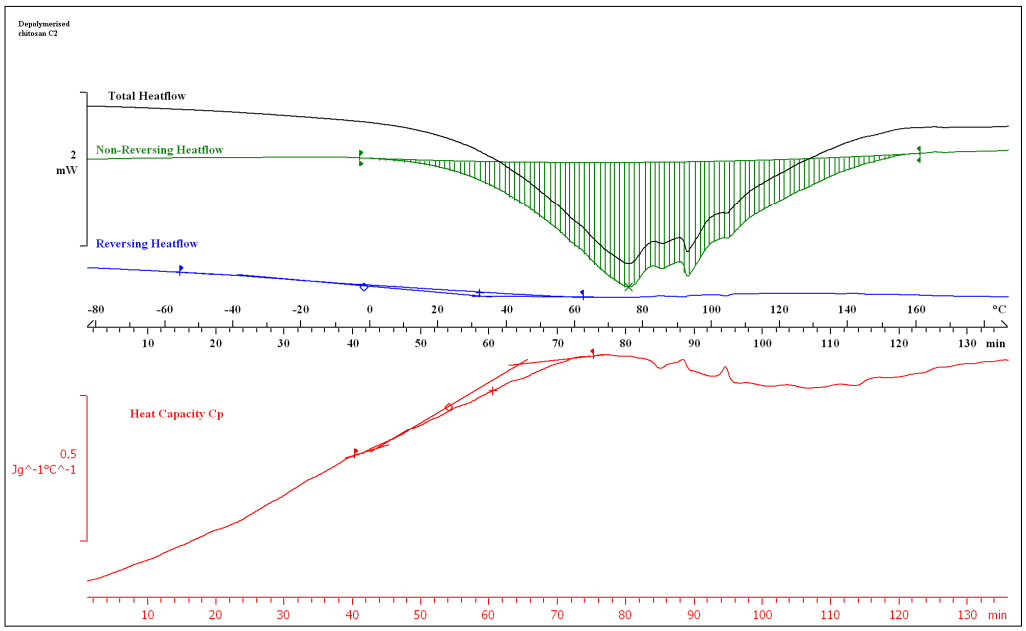


Fig-6.c Modulated DSC TOPEM® of chitosan oligomer C2

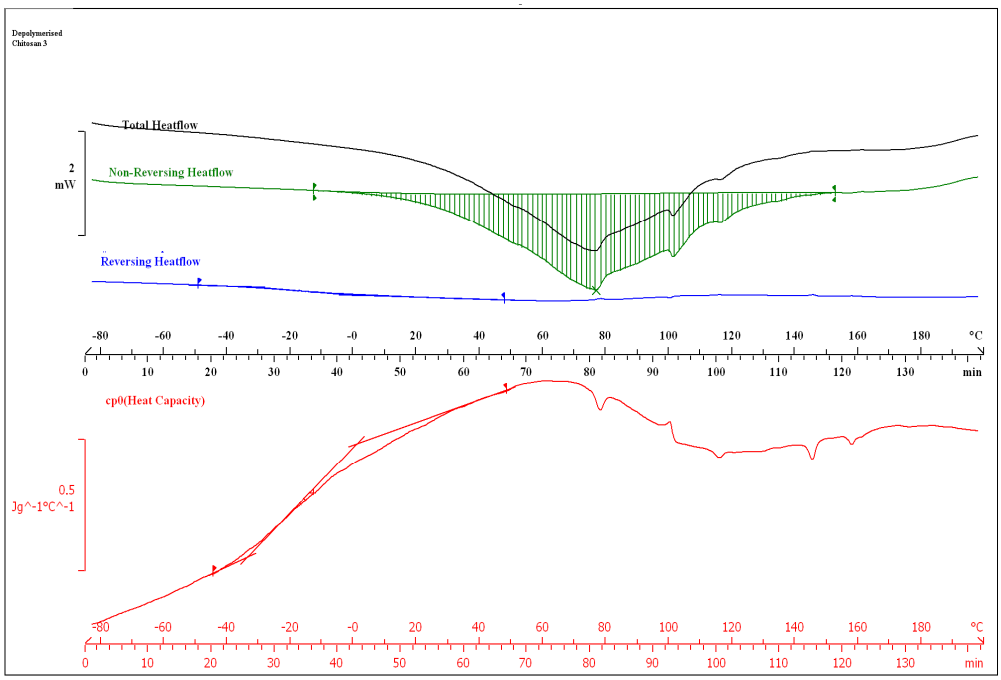


Fig-6.d Modulated DSC TOPEM® of chitosan oligomer C3

CONCLUSION

This study has explored the practicalities of measuring the *T*g of chitosan and its oligomers which are prepared by oxidative degradation method. The effect of water on Tg of chitosan and its oligomers have been evaluated using MTDSC ie. TOPEM®. An understanding of such factor underpins the use of glass transition measurements for further applications such as the study of the effects of water as a plasticiser on the mechanical properties of film

coats and the effects of chain substitution on the molecular mobility of these polymers. It has provided Tg of chitosan and its oligomers which falls in the range of required Tg which may be feasible for surface coating application.

Acknowledgements
The authors acknowledge UGC-SAP for financial support. The authors are thankful to Mr. Sarvesh Tiwari and Mr. Harshil Shah of Mettelor Tolledo for TMDSC analysis. We are also thankful to TIFR for their assistance in NMR.

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