

Characterization of chitosan in acetic acid: Rheological and thermal studies

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Received 26.01.2009

This study examined the influence of temperature, concentration, shearing time, and storage time on the rheological properties, i.e. the dynamic viscosity and shear stress, as a function of shear rate of chitosan solubilized in weakly acid solutions. The results showed that shear thinning behavior (pseudoplastic non-Newtonian behavior) was pronounced at temperatures from 20 to 50 °C, but was more remarkable at lower temperature. In addition, the activation energy value derived from $\ln \eta$ vs. $1/T$ data was found to be 20.86 kJ mol⁻¹. When the effect of concentration was studied, the shear thinning behavior was pronounced at all concentrations. The effect of shearing time on the dynamic viscosity and shear stress of chitosan solutions did not show any significant differences at all shearing times applied in this study. Furthermore, an increase in viscosity was obtained with extending the period of storage to 3 months, after which a drop in viscosity was recorded. Thermal properties of chitosan films were also investigated by employing thermogravimetric analyses (TGA) and differential scanning calorimetry (DSC) in this work. Results of TGA revealed that the decomposition of the chitosan film proceeds in 2 distinct stages, which was confirmed with DSC curves.

Introduction

Chitosan, 1 → 4 linked 2 - amino, 2 - deoxy, β- D - glucan (Figure 1), is the only amino polysaccharide distributed in large amounts in nature.¹ It is the deacetylated derivative of chitin,²⁻⁵ the most abundant natural polymer on earth after cellulose,⁶⁻⁸ obtained from crustaceans,⁹⁻¹² such as shrimps, squids, and crabs. Chitosan has been used in many industries including wastewater treatment, medicine, food, and cosmetics.¹³⁻¹⁷

The rheological behavior of chitosan solutions has been reported in the literature.¹⁸⁻²⁶ For instance, Kienzle-Sterze et al.²⁰ demonstrated that the viscosity of concentrated chitosan solutions increased with increasing chitosan concentration and that shear thinning behavior is observed at polymer concentrations above

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0.50 g dL⁻¹. They also reported the independence and dependence of the zero shear viscosity on the ionic strength and pH of the media respectively. Wang and Xu²¹ reported that the non-Newtonian behavior of chitosan solutions increased with increasing degree of deacetylation (DD) attributed to the chains expanded structure and the increase of entanglements. Mucha²² also reported an increase in the shear stress and viscosity of chitosan solutions with increasing chitosan concentration due to the increase in entanglements between the macromolecular chains, which confirmed the results obtained by Kienzle-Sterze et al.²⁰ Hwang and Shin²³ revealed that the shear rate dependence of viscosity was more remarkable at higher chitosan concentrations. Martinez et al.²⁴ studied the influence of some parameters, such as temperature, acid type, and addition of salt, on the steady-shear rheology of concentrated chitosan solutions. Viscosity and normal stress decreased with increasing temperature and decreasing chitosan concentration. In addition, chitosan in hydrochloric acid solutions exhibited lower steady-shear viscosity and normal stress than in other acids solutions. However, salt addition was found to be the most effective parameter on the rheology of chitosan solutions. Mironov et al.²⁵ reported a decrease in the dynamic viscosity of chitosan solutions in acetic acid during storage due to polymer degradation.

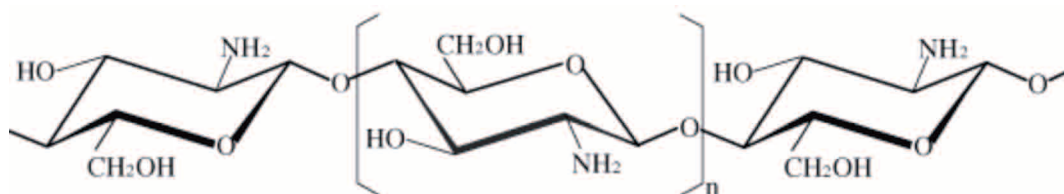


Figure 1. Chemical structure of chitosan.

Thermal analysis has been widely employed for the characterization of polymeric materials.^{27,28} Among the thermal techniques that can be used are thermogravimetry (TGA), differential scanning calorimetry (DSC), and dynamical mechanical thermal analysis (DMTA). In the case of chitosan, several studies have been reported in the literature regarding its thermal decomposition and stability.^{29–32}

The present work focused on studying the effects of shear rate, concentration, temperature, shearing time, and storage time on the dynamic viscosities and shear stress of aqueous solutions of chitosan solubilized in acetic acid. This work also aimed at studying the thermal properties of chitosan film with the view to further exploring the thin film properties and its applications.

Experimental

Materials

Shrimp source chitosan was purchased from a local company with a deacetylation percentage (DD) of 88.1% defined by UV method.³³ This chitosan was acid soluble, white, and flaky. Acetic acid (glacial 100%, pro analysis) was purchased from Merck (Darmstadt, Germany). Ultrapure water (Maxima Ultra Pure Water, Elga-Prima Corp, UK) with a resistivity greater than 18 MΩ/cm was used to prepare all solutions. All chemicals were used without further purification and freshly prepared solutions were used in all experiments.

Preparation of the solutions

Chitosan was dried in the oven until a constant weight was observed. Then 5 g of chitosan was dissolved in 500 mL of acetic acid (0.1 M) followed by mild stirring and heating at about 55 °C overnight to form 10 g L⁻¹ chitosan solution (pH 3.99). The solution was then filtered to remove dust and other traces of impurities and was used as a stock solution. The stock solution was then diluted with the acid to a range of concentrations between 2 and 10 g L⁻¹. Air bubbles were eliminated by keeping the solutions at room temperature for 2 h.

Film casting and drying

The prepared clear solution was cast onto polystyrene petri dishes for film formation, and then dried in the oven at 60 °C for 48 h. The obtained films were peeled off gently, and were further dried by keeping in a desiccator (with silica gel desiccant under vacuum) until use. All films obtained were transparent and free from air bubbles. The film thickness was about 0.012 mm measured with a digital micrometer (Mitutoyo, Japan).

Methods

The molecular weight of chitosan was about 5.5×10^5 g mol⁻¹ determined by gel permeation chromatography (GPC) with a Waters 1515 HPLC Pump and a Waters 2414 Refractive Index Detector. The column used was PL aquagel-OH 30 (8 μm, 300 × 7.5 mm) and the solvent used was 1% acetic acid.

The pH measurements of the chitosan solutions were carried out using a microprocessor pH meter (pH 211, Hanna Instruments). Experiments were carried out in triplicate, and the average and standard deviation values were reported.

Viscosity of chitosan solutions was determined at room temperature by using a Brookfield digital viscometer, model DV-II + Pro, with an attached UL adapter. The shearing time was 15 s. Solutions were kept at room temperature in glass bottles in a dark place until analysis. Viscosity and shear stress values were reported in centipoises (cP) and dyne cm⁻² (D cm⁻²), respectively. For the storage time measurements, solutions were kept at room temperature in glass bottles in a dark place until analysis. Each measurement was recorded as an average value of 5 readings when a constant shear rate (3.67 s⁻¹) was applied.

TGA was used to evaluate the thermal stability and to determine the decomposition temperature of chitosan. Thermogravimetric measurements were made using a Mettler-Toledo thermogravimetric analyzer model TGA/SDTA851e. The temperature range studied was 30-500 °C at a heating rate of 10 °C/min under a nitrogen atmosphere. The mass of the sample pan was continuously recorded as a function of temperature.

DSC studies were performed using a DSC Mettler Toledo (model DSC822e). The samples were scanned under a nitrogen atmosphere at a constant rate of 10 °C/min. The experiment consisted of 3 runs. The first run was from 40 to 500 °C, the second one was from 40 to 500 °C, after heating to 150 °C, holding for 1 min, and then cooling to 40 °C, and the third run was from 40 to 250 °C, after heating up to 250 °C and cooling to 40 °C.

Results and discussion

The effects of temperature and concentration on the shear viscosity of chitosan solutions

In this study, the shear rate-dependent viscosity of chitosan solutions as a function of shear rate at a range of temperature from 20 to 50 °C is presented in Figure 2. Non-Newtonian behavior is observed at all temperatures. However, increasing temperature leads to the appearance of Newtonian behavior. In other words, decreases in the viscosities were more pronounced at higher temperature i.e. lower shear thinning and higher viscosities are observed at lower temperature. The results obtained show good agreement with previous studies.²⁶ In addition, Figure 2 indicates that the temperature has more effect on the solutions at lower shear rate values, i.e. the viscosity increase associated with temperature is less important at high shear rates.³⁴

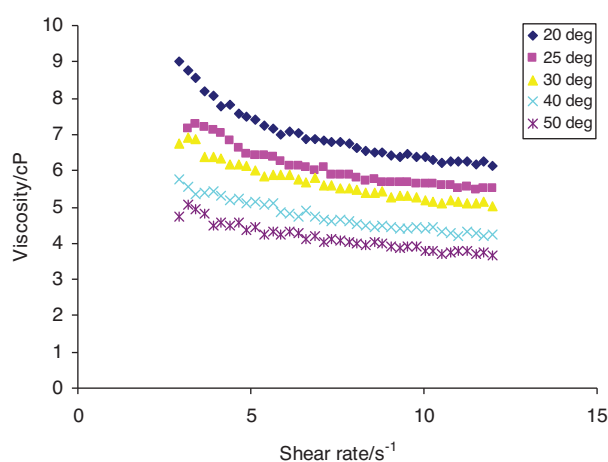


Figure 2. Influence of the shear rate and temperature on the rheological curves of chitosan solutions.

The relationship between shear viscosity (determined at a constant shear rate) and temperature for a chitosan concentration of 10 g L⁻¹ is demonstrated in Figure 3A. This graph shows an almost linear relationship ($R^2 = 0.947$) whereby viscosity of chitosan in acetic acid solutions decreases significantly with increasing temperature, i.e. it is a strong function of temperature. The viscosity varies from 9.3 cP for 20 °C to 4.2 cP for 50 °C. This linear decrease is attributed to the increase in thermal motion of the polymer with temperature.

The viscosity values obtained at a constant shear rate can be correlated with temperature according to the Arrhenius equation:

$$\eta = A \cdot e^{-E_a/RT}$$

where A is a constant related to molecular motion, E_a is the activation energy for viscous flow at constant shear rate, R is the gas constant, and T is the absolute temperature in K. Figure 3B presents an Arrhenius plot for the chitosan solutions with a concentration of 10 g L⁻¹. This graph also shows a linear relationship ($R^2 = 0.9904$) with an apparent activation energy of 20.86 kJ mol⁻¹. This value of the activation energy is in accordance with the reported values.^{21,24}

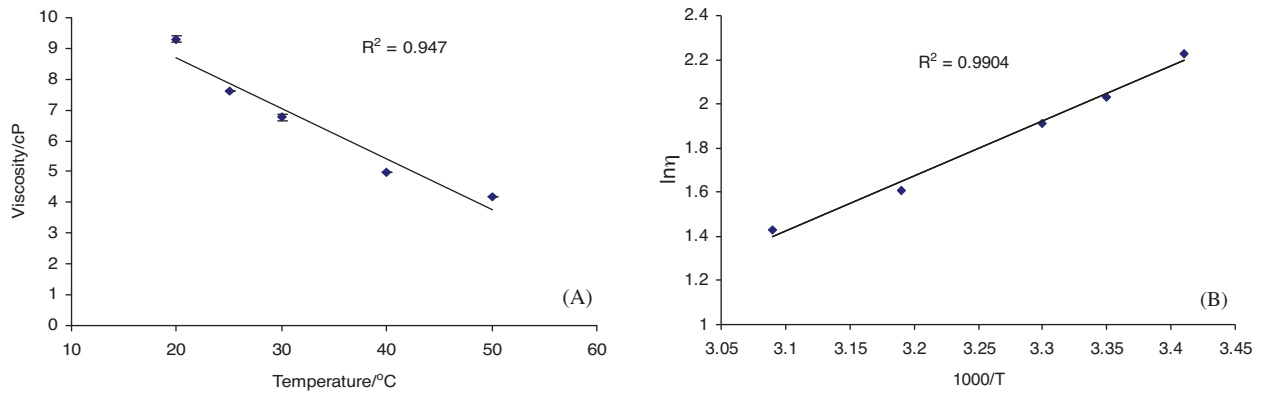


Figure 3. (A) Influence of temperature on viscosity of chitosan solutions at a constant shear rate and (B) the Arrhenius plot of $\ln \eta$ versus $1/T$ for chitosan in acetic acid solutions.

Figure 4A shows the effect of temperature on the shear stress of chitosan solutions as a function of shear rate. Shear stress increased with increasing shear rate. At the same shear rate, shear stresses were higher at lower temperature. In addition, increases in shear stress with increasing shear rate were more remarkable at lower temperatures. From the graph it is clear that the solutions exhibit pseudoplastic non-Newtonian behavior.

It is apparent in Figure 4B that this type of fluid displays increases in the shear rate with decreasing viscosity, suggesting pseudoplastic non-Newtonian behavior. Generally, shear thinning with higher viscosity is observed with increasing chitosan concentration. Much,²² Delben et al.,³⁴ and Kjønnsken et al.³⁵ reported stronger shear thinning at higher concentrations for different systems. This is probably attributed to increasing intermolecular entanglement as chitosan concentration increases, which in turn leads to more restriction in the freedom of movement of the individual chains.³⁶

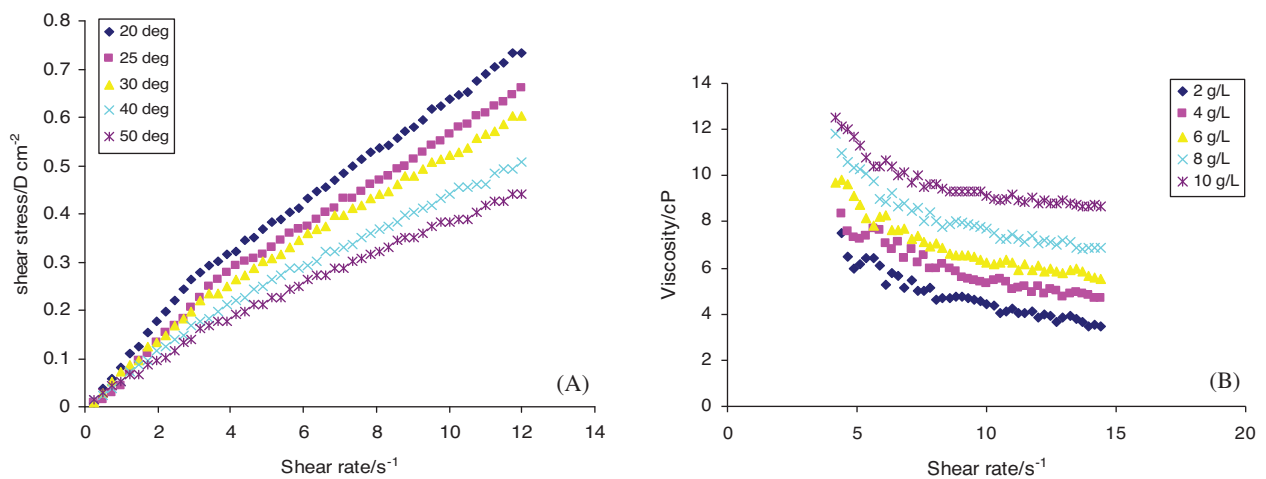


Figure 4. (A) Shear stress of chitosan in acetic acid solutions at different range of temperature versus shear rate and (B) Influence of the shear rate and concentration on the rheological curves of chitosan solutions at 25 °C.

The effects of shearing and storage time on the apparent viscosity of chitosan solutions

Viscosity measurements were obtained as a function of shear rate for 15, 30, 45, 60, and 75 s at 25 °C to study the shearing time effect (Figure 5). At all shearing time values, chitosan solutions exhibit similar behavior; the viscosity of chitosan solutions was shear rate dependent at low shear rates, but remained nearly constant at higher shear rates. Moreover, the shear stress as a function of shear rate curves shows a similar response for all shearing time values applied in this study (Figure 6). In addition, more shear thinning behavior and higher values of viscosity of chitosan solutions were observed when the period of storage was extended to 3 months, after which a drop in the viscosity values was recorded (Figure 7 and the Table). As for pH values, no significant changes were recorded as a function of time (Table).

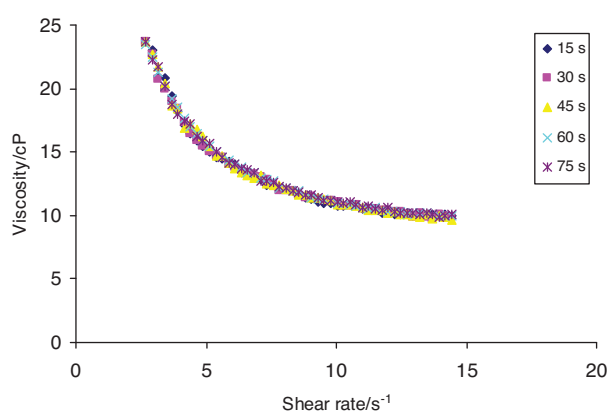


Figure 5. Influence of the shear rate and shearing time on the rheological curves of chitosan solutions at 25 °C.

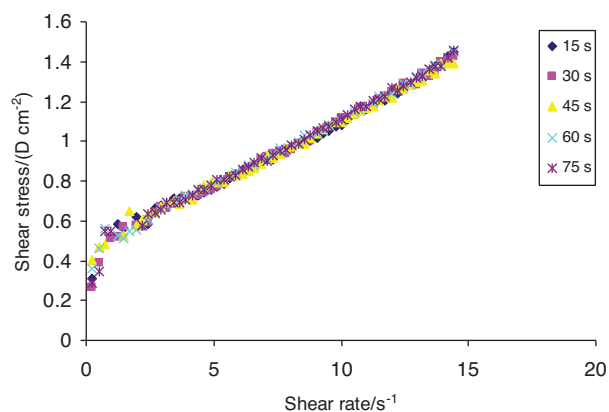


Figure 6. Influence of the shear rate and shearing time on the shear stress curves of chitosan solutions at 25 °C.

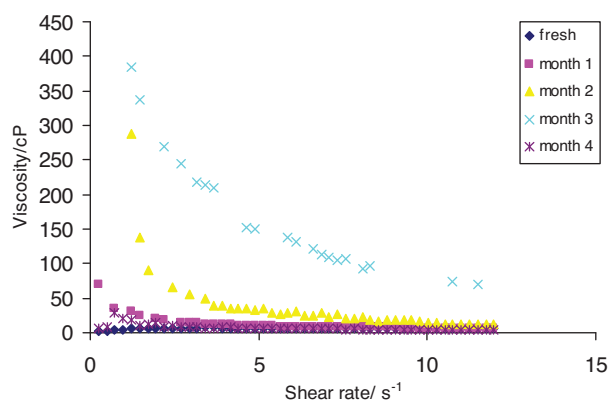


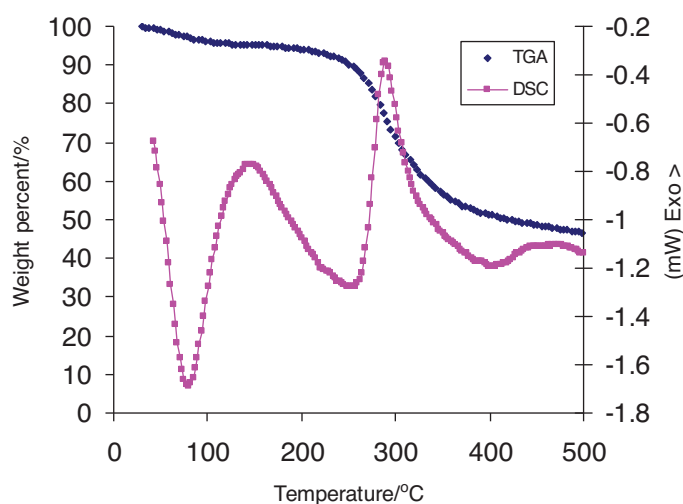
Figure 7. Influence of the shear rate and storage time on the rheological curves of chitosan solutions at 25 °C.

Table. Effect of storage time on dynamic viscosity, shear stress (determined at a constant shear rate) and pH of chitosan solutions (mean \pm SD).

Time (month)	Viscosity (cP)	Shear stress (D cm ⁻²)	pH
0	7.60 \pm 0.00	0.28 \pm 0.00	3.99 \pm 0.02
1	11.20 \pm 0.14	0.41 \pm 0.01	4.12 \pm 0.00
2	41.11 \pm 1.42	1.51 \pm 0.05	4.33 \pm 0.00
3	140.45 \pm 9.23	5.51 \pm 0.34	4.38 \pm 0.00
4	7.68 \pm 0.36	0.28 \pm 0.01	4.32 \pm 0.00

Thermal properties

Figure 8 shows the results of thermogravimetric analysis of chitosan film. Two weight losses are observed in the TGA curve. The first stage ranges between 30 and 145 °C and shows about 5% loss in weight. This is thought to be due to moisture vaporization. The second weight loss begins at about 150 °C and the corresponding weight loss of about 47% is attributed to the decomposition of chitosan. The total weight loss of the sample at about 500 °C is 52%.

**Figure 8.** TG and DSC first run curves of chitosan film.

The DSC first run curve of chitosan film is shown in Figure 8. After drying, the sample was kept in a desiccator before analysis. The film membrane exhibited a broad endothermic peak centered at about 79 °C. This peak is attributed to the loss of water associated with the hydrophilic groups of the polymer.^{37,38} This suggests that the sample was not completely anhydrous and that some bound water was still not removed when dried in the desiccator, which was confirmed by the results obtained from TG analysis. The exothermic peak, which appears in the temperature range between about 250 and 350 °C, corresponds to the decomposition of the polymer. Sakurai et al.³⁹ and Zeng et al.⁴⁰ also reported that the thermal degradation of chitosan begins at about 250 °C.

Since chitosan is apt to absorb moisture, a second heating run of the DSC, after first heating to 150 °C and holding it at that temperature for 1 min and then cooling to 40 °C, was done to eliminate the effect of moisture (Figure 9A). The first point to note is the absence of the endothermic peak, which confirmed that this peak is attributed to the water content in the sample. The exothermic peak centered at around 290 °C is due to the degradation of the chitosan chains as has been mentioned before.

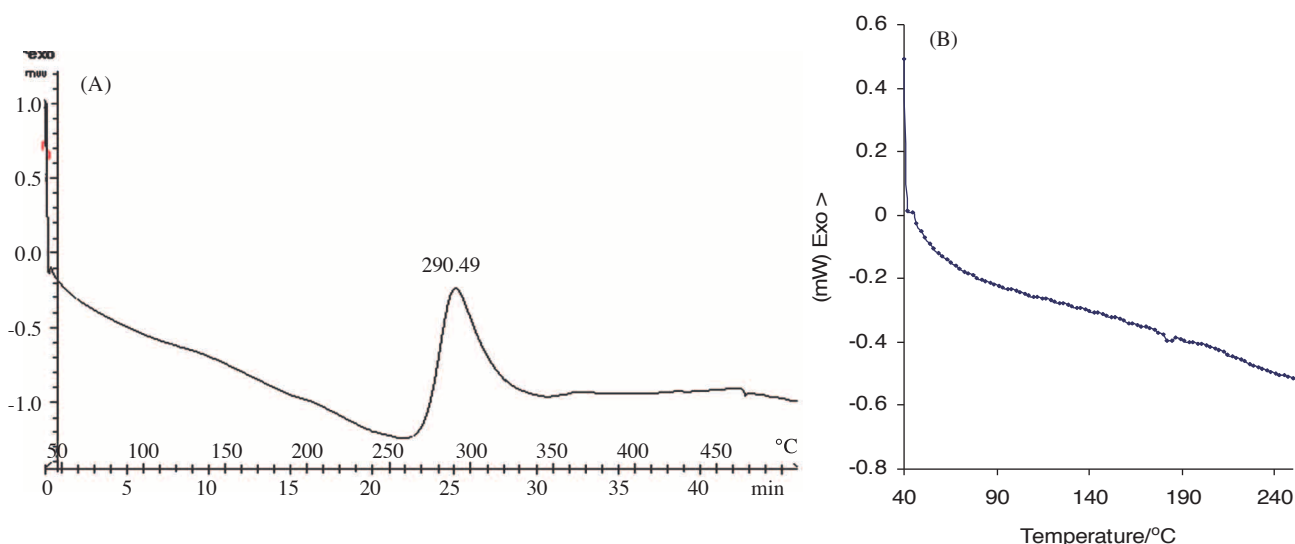


Figure 9. (A) DSC second run curve of chitosan film and (B) DSC third run curve of chitosan film.

A third run, after heating up to 250 °C and cooling to 40 °C, was also employed in this study (Figure 9B). The main feature in the curve is that there is a small endothermic peak at 179.4 °C. Zeng et al.⁴⁰ and Chuang et al.⁴¹ also reported an endothermic peak of chitosan at around 180 °C, which is very close to our value (179.4 °C). This peak may be due to the dissociation process of interchain hydrogen bonding of chitosan,⁴¹ which is strongly formed among the $-NH_2$ and $-OH$ functional groups.

Conclusion

This study showed that the dynamic rheological measurements of chitosan solutions in acetic acid suggested pseudoplastic non-Newtonian behavior. The shear thinning behavior was remarkable at temperatures between 20 and 50 °C. Chitosan solutions in acetic acid were found to obey the Arrhenius equation. In addition, chitosan in acetic acid solutions exhibited less shear thinning and an increase in viscosity with increasing concentration. This study has also shown that curves of the dynamic viscosity of chitosan solutions show similar behavior at all shearing times of 15-75 s and that less shear thinning behavior and higher values of viscosity of chitosan solutions were observed when the storage period was extended to 3 months. A general increase in viscosity with time was also observed at a constant shear rate, suggesting rheopexy behavior. However, a drop in viscosity was recorded in the fourth month. On the other hand, DSC results of chitosan film showed agreement with those obtained by TG measurements concerning the thermostability and degradation.

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