



Effect of complexation conditions on xanthan–chitosan polyelectrolyte complex gels

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Abstract

Polyelectrolyte hydrogels formed by xanthan gum and chitosan can be used for encapsulation and controlled release of food ingredients, cells, enzymes, and therapeutic agents. In this study, xanthan–chitosan microcapsules were formed by complex coacervation. The effects of initial polymer concentration and chitosan solution pH on the crosslinking density of xanthan–chitosan network were investigated by swelling studies and modulated differential scanning calorimetry (MDSC) analysis. The crosslinking density was found to be less dependent on chitosan solution concentration than xanthan solution concentration and chitosan pH. The capsules were completely crosslinked at all conditions studied when initial xanthan solution concentration was 1.5% (w/v). The changes in the conformation of chitosan chains as chitosan pH approaches 6.2 were found to be important in achieving capsule network structures with different crosslinking densities. These findings indicate that the parameters studied cannot be viewed as independent parameters, as their effects on the degree of swelling are interdependent.

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1. Introduction

Polyelectrolyte complex (PEC) gels formed by the electrostatic attractions between two oppositely charged polyelectrolytes mixed in aqueous solution (Michaels & Miekka, 1961) are known to exhibit unique physical and chemical properties, as the electrostatic interactions within the PEC gels are considerably stronger than most secondary binding interactions (Lee, Lee, Song, & Park, 2003). In the last decade there has been an increasing interest in the use of PEC gels formed by chitosan and polyanions as carriers for drug delivery and in immobilized systems (Gupta & Kumar, 2001; Magnin, Lefebvre, Chornet, & Dumitriu, 2004; Patel & Amiji, 1996; Risbud & Bhone, 2000; Risbud, Hardikar, Bhat, & Bhone, 2000; Thacharodi & Rao, 1995). The unique properties of chitosan arise from its amino groups that carry positive charges at pH values below 6.5, which enables its binding

to negatively charged materials such as enzymes, cells, polysaccharides, nucleic acids, hair, and skin (Sandford, 1992). Chitosan, poly- β -(1 \rightarrow 4)-D-glucosamine, is the only natural polysaccharide with a cationic nature and is produced by alkaline deacetylation of chitin (Muzzarelli, 1977; Sandford, 1989). It shows excellent biological properties such as biocompatibility, biodegradability, lack of toxicity, and adsorption, as well as relatively high nitrogen content (Dutta, Dutta, & Tripathi, 2004; Felse & Panda, 1999; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Sashiwa & Aiba, 2004).

The idea of forming xanthan–chitosan PEC gels is not new (Chellat et al., 2000; Chu, Kumagai, & Nakamura, 1996; Dumitriu & Chornet, 1997; Dumitriu, Magny, Montane, Vidal, & Chornet, 1994; Magnin, Dumitriu, & Chornet, 2003). Xanthan gum, a microbial exopolysaccharide consisting of a cellulosic backbone with two mannose and one glucuronic acid side chains on every second glucose residue (Jansson, Kenne, & Lindberg, 1975; Melton, Mindt, Rees, & Sanderson, 1976), is considered an anionic polyelectrolyte (Richardson & Ross-Murphy,

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1987). The molecular weight of xanthan gum can reach up to 6 million Daltons, which makes it possible to create extremely viscous solutions at very low concentrations (Cottrell, Kang, & Kovacs, 1980; Lo, Yang, & Min, 1997). In addition to its enzymatic resistance, xanthan gum is stable over a wide range of temperatures and pH, which finds many applications in food, pharmaceutical, cosmetic, and oil-drilling industries (Garcia-Ochoa, Santos, Casas, & Gomez, 2000; Meyer, Fuller, Clark, & Kulicke, 1993; Nussinovitch, 1997; Sanderson, 1996). The hydrogel network formed through the ionic interactions between the amino groups of chitosan and carboxyl groups of xanthan shows pH-sensitive swelling characteristics, which enable the controlled release of entrapped materials such as therapeutic agents, enzymes and bacteria (Chellat et al., 2000; Chu et al., 1996; Chu, Sakiyama, & Yano, 1995). In particular, xanthan–chitosan hydrogels are recognized as promising candidates for targeted delivery and controlled release of encapsulated products for oral administration because only non-toxic metabolites are produced during degradation and the complex has relatively high enzymatic resistance (Chellat et al., 2000).

A variety of xanthan–chitosan PEC gels can be obtained by changing the molecular properties of the xanthan and chitosan polymers, such as molecular weight, degree of acetylation of chitosan, and pyruvic acid content of xanthan, as well as changing the complexation conditions, such as chitosan solution pH, polymer concentration, complexation time, and mixing ratio (Dumitriu, 2002; Dumitriu & Chornet, 1997; Dumitriu et al., 1994; Magnin et al., 2004). For many applications of xanthan–chitosan hydrogels, it might be more practical to vary the complexation conditions to obtain microcapsules with different crosslinking densities rather than changing the chemical structure of the polymers. Crosslinking density is an important factor determining the stability, pH-sensitive swelling behavior (thus the release properties), as well as the mechanical strength of hydrogel networks (Magnin et al., 2004; Mao, Kondu, Ji, & McShane, 2006; Peppas, Bures, Leobandung, & Ichikawa, 2000; Peppas, Wood, & Blanchette, 2004). By controlling the crosslinking density, xanthan–chitosan microcapsules with different properties can be prepared for a desired application. Nevertheless, to the authors best knowledge, there is no systematic study discussing the combined effects of complexation conditions on the network structure of the capsules formed by xanthan gum and chitosan.

This study addresses the relative importance of polymer concentration and chitosan solution pH in the complexation of xanthan gum and chitosan in the form of capsules. The swelling degree (SD) of microcapsules formed by different combinations of xanthan and chitosan was studied as an indication of the crosslinking density of the hydrogel membrane. An increase in the crosslinking density restricts the degree of swelling due to decreased chain mobility and reduces the pH-sensitivity by improving the stability of the network. The SD results were

statistically analyzed and both single and combined effects of these factors on the complex structure were evaluated. Differential scanning calorimetry (DSC) measurements were employed to further investigate the changes in the membrane structure as influenced by the hydrogel preparation conditions.

2. Materials and methods

2.1. Preparation of chitosan and xanthan solutions

Chitosan from crab shells with a minimum of 85% deacetylation and a molecular weight of 370 000 (reported by the supplier) was purchased from Sigma-Aldrich Chemicals (St. Louis, MO). A known amount of chitosan was dissolved in 1 N HCl by heating and agitating. The desired solution pH was adjusted by 1 M NaOH and DI water was added to bring it to the final volume. Xanthan gum with a molecular weight of 1.02 million (TICAXAN[®]) was kindly supplied by TIC Gums (Belcamp, MD). A predetermined amount of xanthan gum was dissolved in DI water under heating and agitation. Both solutions were autoclaved before use.

2.2. Capsule formation

In this study the extrusion (complex coacervation) method was used. Capsules were formed by dropwise addition of a solution of xanthan (50 ml) into a solution of chitosan (300 ml) using a manually operated syringe with a 0.7-mm cannula (Becton-Dickinson, Franklin Lakes, NJ). The chitosan solution was agitated continuously for 40 min to allow crosslinking and avoid coalescence of capsules. The capsules were filtered through a 160 µm Millipore nylon filter, washed twice with DI water, and then freeze-dried for 24 h.

2.3. Determination of the SD

The effect of three complexation parameters, namely initial xanthan solution concentration (0.5%, 0.7%, 1.0%, and 1.5% w/v), initial chitosan solution concentration (0.7% and 1.0% w/v) and chitosan solution pH (4.5, 5.5, and 6.2) on the degree of swelling of the resulting capsules were studied. Ten freeze-dried capsules were weighed and suspended in DI water overnight for each combination. The capsules were filtered, blotted to remove surface water, and weighed. The SD values were calculated using the following equation:

$$SD (\%) = \frac{W_s - W_d}{W_d} \times 100, \quad (1)$$

where W_s and W_d are the weight of swollen capsules and that of dry capsules, respectively. The averages of four replicates for each combination were reported.

2.4. DSC measurements

DSC measurements were carried out on a TA Instruments Q100 DSC (New Castle, DE). The cell resistance and capacitance calibrations were performed in two steps. The first step was heating an empty cell and the second step was heating the cell with equal weight sapphire disks on the sample and reference platforms. The cell constant and temperature calibrations were performed with an indium standard.

Standard DSC was used for the first heating and cooling runs. Approximately 5 mg of dry capsules were placed in sealed Al pans in small pieces. Each sample was heated up to 160 °C at a rate of 10 °C/min and cooled back to 40 °C at a rate of 5 °C/min to erase the thermal history of the polymers and eliminate the effect of moisture. Modulated differential scanning calorimetry (MDSC) curves were obtained from the second heating run at 2 °C/min. Samples were heated from 100 to 175 °C with a modulation period of 60 s and modulation amplitude of 0.318 °C. A nitrogen purge was applied for all experiments. The reversing signal was used to compare the glass transitions of the samples. In addition, chitosan in flakes and xanthan in powder form were subjected to the same experimental procedure to determine their glass transition temperatures.

2.5. Statistical analysis

Statistical analyses were conducted using SAS 9.1.2 Software (Cary, NC). Factorial analysis of variance was used to analyze the effect of xanthan concentration, chitosan concentration, and chitosan solution pH on the SD of the capsules. Differences in least square means were used for pairwise mean comparison. Analyses were performed using mixed procedure of SAS.

3. Results and discussion

3.1. Effect of polymer concentration and chitosan solution pH on the SD of xanthan–chitosan capsules

By extruding xanthan solution into moderate concentrations of chitosan (0.7% and 1.0% (w/v)), no stable capsules could be formed at xanthan concentrations below 0.5% (w/v). Xanthan concentrations exceeding 1.5% (w/v) resulted in formation of amorphous capsules. The degree of swelling decreased by approximately 50% when using 0.7% (w/v) xanthan solution instead of 0.5% (w/v). Therefore capsules formed from xanthan solutions at concentration of 0.5% (w/v) were not included in statistical analysis and DSC studies. The pH of chitosan solution was controlled, ranging from 4.5, where the ionization degree of chitosan is unity, to 6.2, since chitosan precipitates above its pKa value of approximately 6.3.

Xanthan concentration was found to have a very significant effect on the SD of xanthan–chitosan capsules at both chitosan concentrations ($P < 0.0001$) (Table 1).

Table 1
Swelling degrees of xanthan–chitosan capsules in DI water

Chitosan (% (w/v))	Xanthan (% (w/v))		
	0.7	1.0	1.5
0.7			
pH 4.5	2862 ⁱ	2116 ^j	1267 ^{ek}
pH 5.5	3959	3019	1601 ^{el}
pH 6.2	2049 ^m	1305 ^{an}	1520 ^{aeo}
1.0			
pH 4.5	2375 ^{hgi}	2164 ^{hij}	1573 ^{fk}
pH 5.5	2661 ^{eg}	2639 ^{ch}	1110 ^{fl}
pH 6.2	1738 ^{dm}	1187 ^{dn}	1467 ^{dfo}

Mean values with same letter are not significantly different at $P = 0.05$ level.

However, this effect was found to be dependent on chitosan concentration and pH. When 0.7% (w/v) chitosan was used, the higher the xanthan concentration the lower was the SD under all conditions studied except when xanthan concentration was increased from 1.0% to 1.5% (w/v) at pH 6.2. On the other hand, with 1.0% (w/v) chitosan, increasing xanthan concentration resulted in significantly lower SDs only when xanthan concentration was increased from 1.0% to 1.5% (w/v) at pH 4.5 and 5.5 ($P < 0.05$) and no significant difference was observed at other conditions. At both chitosan concentrations, the decrease in the SDs of the capsules with the increase in xanthan concentration was more pronounced when chitosan solution pH was 5.5 and least significant at pH 6.2.

The effect of chitosan solution pH on the degree of swelling was more significant when chitosan solution concentration was 0.7% (w/v) ($P < 0.0001$) than when it was 1.0% (w/v) ($P < 0.005$) (Table 1). With 1.5% (w/v) xanthan concentration, the increases in pH had no significant effect on the SD ($P > 0.05$), whereas at other xanthan concentrations, the degree of swelling decreased significantly when chitosan pH was increased from 4.5 to 6.2. Such significant decreases in SD with increasing chitosan pH could be attributed to the changes in the chain flexibility of chitosan polymer with the changes in the solution pH. The ionization degree of chitosan decreases from 1.0 to 0.5 as pH increases from 4.5 to 6.2 (Ikeda, Kumagai, Sakiyama, Chu, & Nakamura, 1995), which means that amino groups become less charged as pH increases. As a result, one may expect that fewer ionic linkages would occur between the two polymers, resulting in higher SDs as the pH approaches to its pKa value. However, since the charge density of the chitosan molecule is reduced by almost 50% as pH approaches 6.2 from a value of 4.5, the polymer chains become less extended with a smaller radius of gyration. This might result in a higher diffusion coefficient for chitosan chains at pH 6.2, consequently enhancing diffusion of chitosan into the xanthan–chitosan network and forming more linkages during the specified reaction time. This result suggests that

since the formation of xanthan–chitosan network is instantaneous upon contact, the diffusion of chitosan chains in the bulk solution through this network plays an important role to achieve a highly crosslinked structure with small SD.

Intriguingly, when 0.7% and 1.0% (w/v) xanthan solutions were extruded into 0.7% (w/v) chitosan solution, increases of chitosan pH from 4.5 to 5.5, first significantly increased the degree of swelling before it reached the lowest SD at pH 6.2. This significant increase in the SD when chitosan pH was raised from 4.5 to 5.5 might be associated with the slight decrease in the charge density of chitosan (ionization degree ca. 0.9) that lessens the ionic linkages between the two polymers, rendering higher SDs. Magnin et al. (2004) have demonstrated similar results where the SD of xanthan–chitosan matrix continued to increase with increasing chitosan solution pH from 3.5 to 5.8 in bulk form by using 0.65 wt% chitosan and 0.65 wt% xanthan solutions. Moreover, our results also showed that when 1.0% (w/v) chitosan concentration was used, pH change from 4.5 to 5.5 chitosan solution had no significant effect on the SD, suggesting that the SD was less affected by the decrease in the charge density of the chitosan chains as pH approaches to 5.5 when chitosan concentration is increased.

The effect of chitosan solution concentration on the SD of capsules was less pronounced than the effect of xanthan solution concentration and chitosan solution pH. Increasing chitosan concentration from 0.7% to 1.0% (w/v) significantly decreased the degree of swelling only when chitosan pH was 5.5 and at xanthan concentrations of 0.7% or 1.0% (w/v). No significant difference was observed at other conditions. These findings indicate that the parameters studied cannot be viewed as independent parameters, as the effect of one parameter on the degree of swelling depends on the other two parameters. While swelling studies were capable of identifying the combined effect of polymer concentration and chitosan solution pH on the crosslinking densities of xanthan–chitosan hydrogel capsules, further investigation is needed to understand the differences in the membrane structure as influenced by the xanthan–chitosan hydrogel preparation conditions. Therefore, DSC analysis was performed to compare the changes in thermal transitions in order to elucidate the changes in the crosslinking density of the capsule network.

3.2. DSC analysis of xanthan–chitosan capsules

Conventional DSC was used for the first heating and cooling runs. The first heating run of each sample gave single endothermic peak at about 100 °C, which attributes to the absorbed water. Samples were cooled back to 40 °C before the second heating. The MDSC technique was used for the second heating. MDSC applies two simultaneous heating rates to the sample. The linear heating rate provides total heat flow as conventional DSC and sinusoidal (modulated) heating rate provides the heat

capacity-related (reversing) component of the total heat flow. The reversing signal was used to quantify the glass transition since it separates the glass transition completely from other non-reversing processes (Gill, Sauerbrunn, & Reading, 1993; Reading, Elliott, & Hill, 1993). The transition enthalpies were calculated by integration of the peaks on the reversing heat capacity (Rev C_p) curves as is usually done for first-order phase transitions (Köhler, Möhwald, & Sukhorukov, 2006).

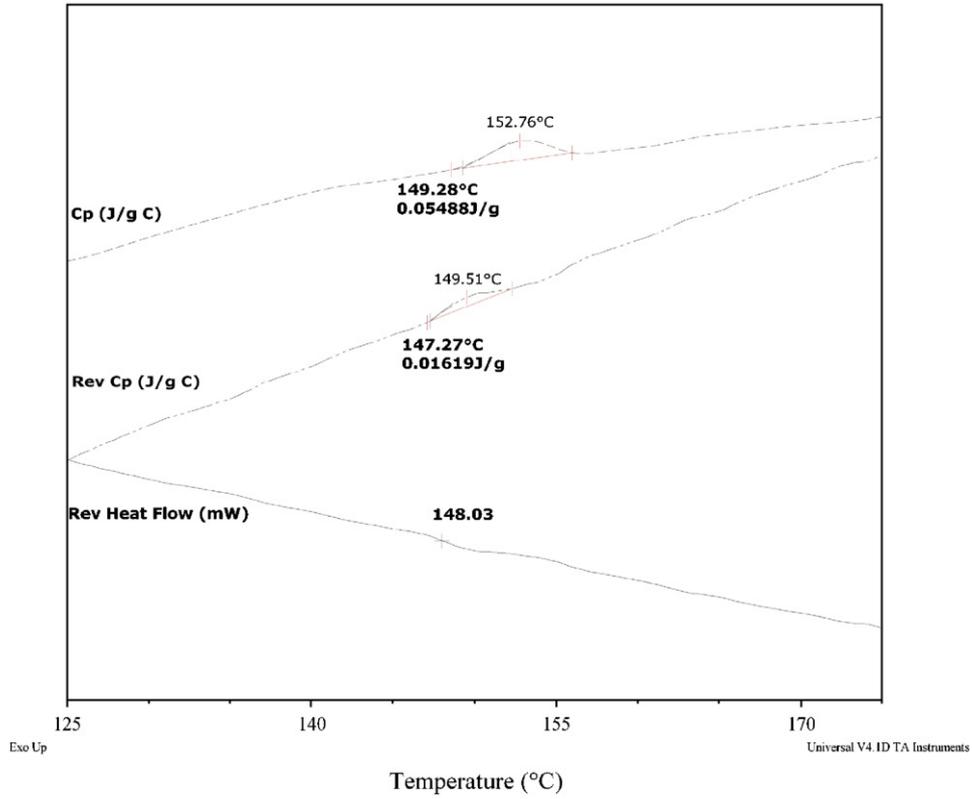
The small glass transitions observed for both chitosan and xanthan can be explained by the fact that both polymers are partially crystalline (Fig. 1). The inflection points of the peaks on the Rev C_p curves correspond to the glass transition temperature (T_g) of the samples. The T_g of chitosan was determined to be approximately 148 °C and the enthalpy of this transition (H) was calculated as 0.016 J/g. The T_g for xanthan gum was found to be at approximately 143 °C with a transition enthalpy of $H = 0.01$ J/g.

Representative reversing heat flow curves from the second heating runs of the freeze-dried hydrogel capsules are shown in Figs. 2 and 3. The transition enthalpies and the glass transition temperatures were determined as described above. It is evident from this data that the polymer complex shows weak transitions as expected in physically crosslinked networks. For this reason, the transition enthalpy of xanthan gum, 0.01 J/g, was selected as the threshold enthalpy to differentiate the noise from the actual transitions appearing on the MDSC curves of the hydrogel capsules.

Fig. 2 shows the effect of initial xanthan concentration on the capsule network structure. In Fig. 2a, the MDSC curves of capsules made from 0.7% (w/v) chitosan solution at pH 6.2 are shown. When xanthan solution was 0.7% (w/v), two glass transition temperatures at approximately 144 and 149 °C were observed. This indicates the presence of uncrosslinked xanthan gum and chitosan in the network. However, these transitions disappeared in the samples formed using 1.0% (w/v) xanthan, which might indicate complete crosslinking of both polymers. This increase in crosslinking density explains the significant decrease in the SD of capsules when the initial xanthan concentration was increased from 0.7% (SD = 2049) to 1.0% (SD = 1305). On the other hand, increasing xanthan concentration to 1.5% (w/v) did not result in a significant difference in the degree of swelling (SD = 1520) since the network was already completely crosslinked when 1.0% (w/v) xanthan was used.

Fig. 2b shows the MDSC curves of capsules prepared from 1% (w/v) chitosan solution at pH 4.5. The transitions appeared approximately at 147 and 148.5 °C in the 0.7% and 1.0% xanthan curves, respectively, can be attributed to the glass transition of chitosan and the transition appeared in 1.0% (w/v) xanthan curve at approximately 143 °C corresponds to the glass transition of xanthan gum. This suggests the presence of uncrosslinked polymer chains in both networks. When xanthan concentration was increased

a



b

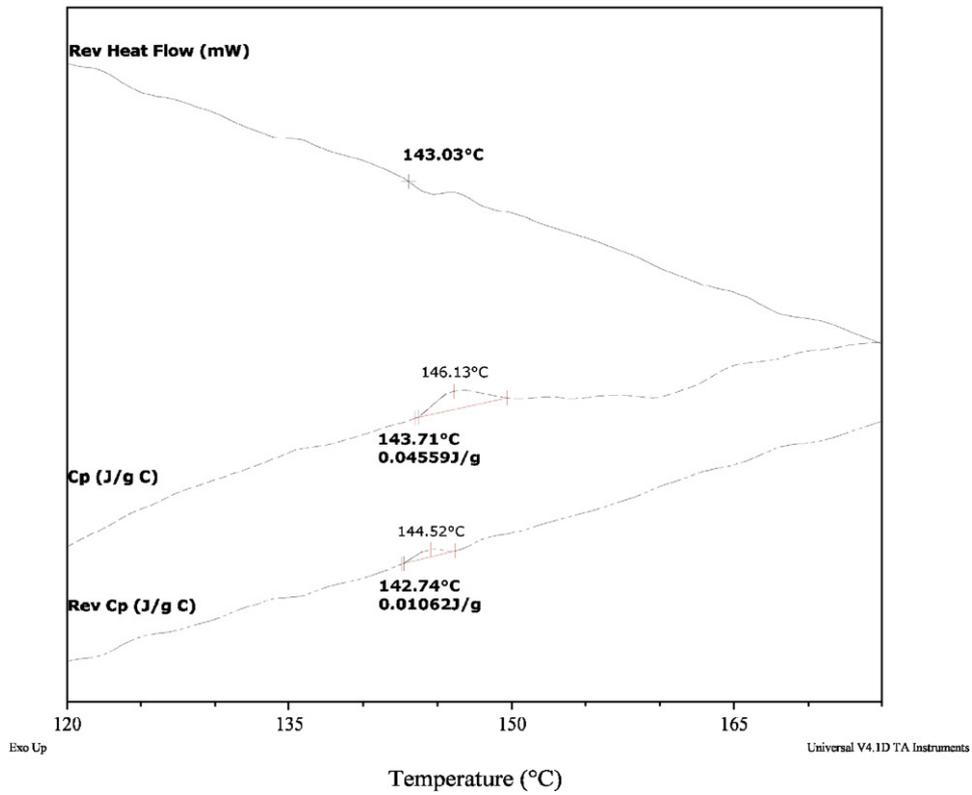


Fig. 1. MDSC curves of: (a) chitosan, (b) xanthan gum.

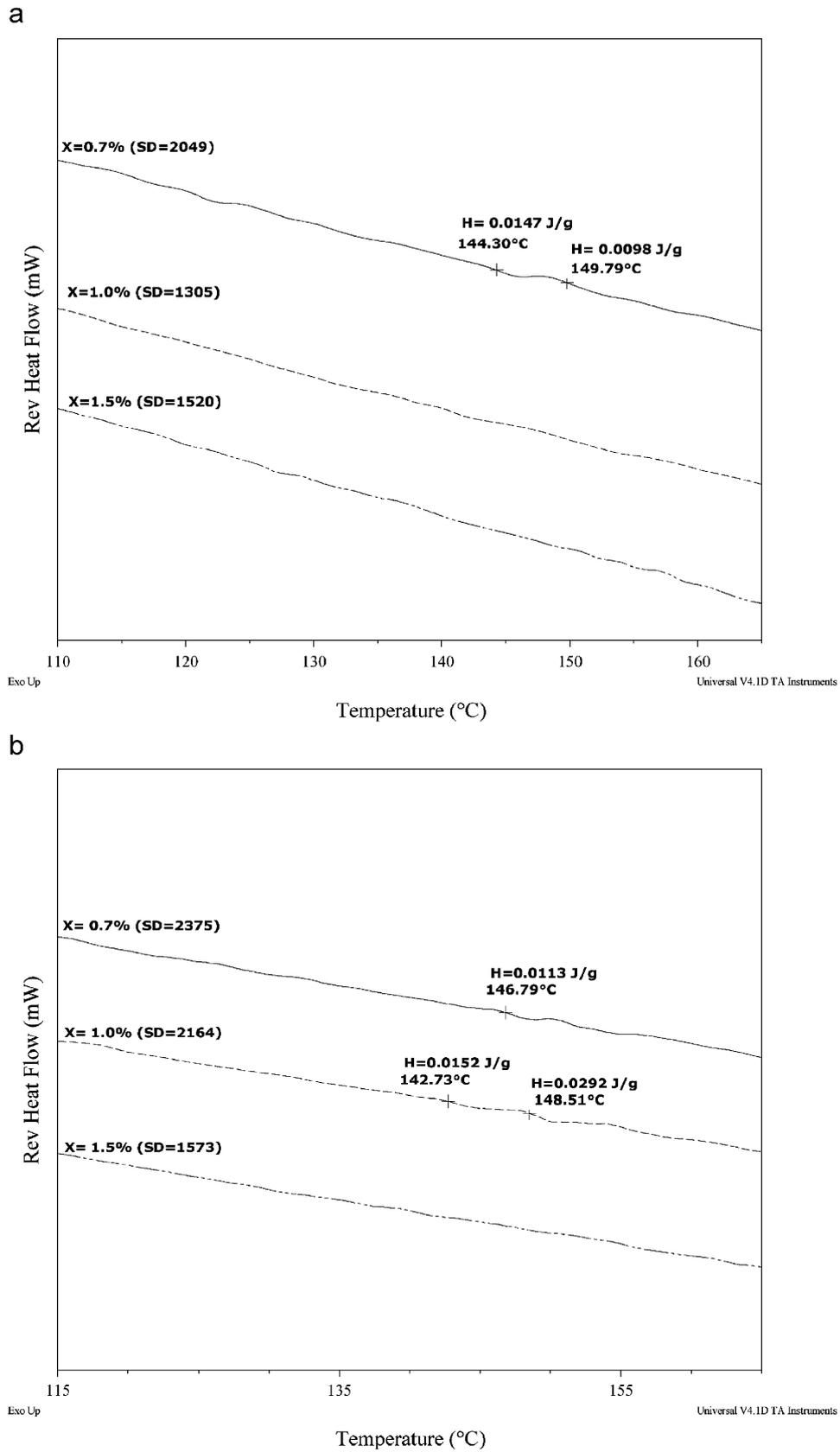


Fig. 2. MDSC curves of xanthan–chitosan capsules showing the effect of initial xanthan concentration on the resulting capsule network structure. (a) Chitosan: 0.7% (w/v) pH = 6.2; (b) Chitosan 1.0% (w/v) pH = 4.5.

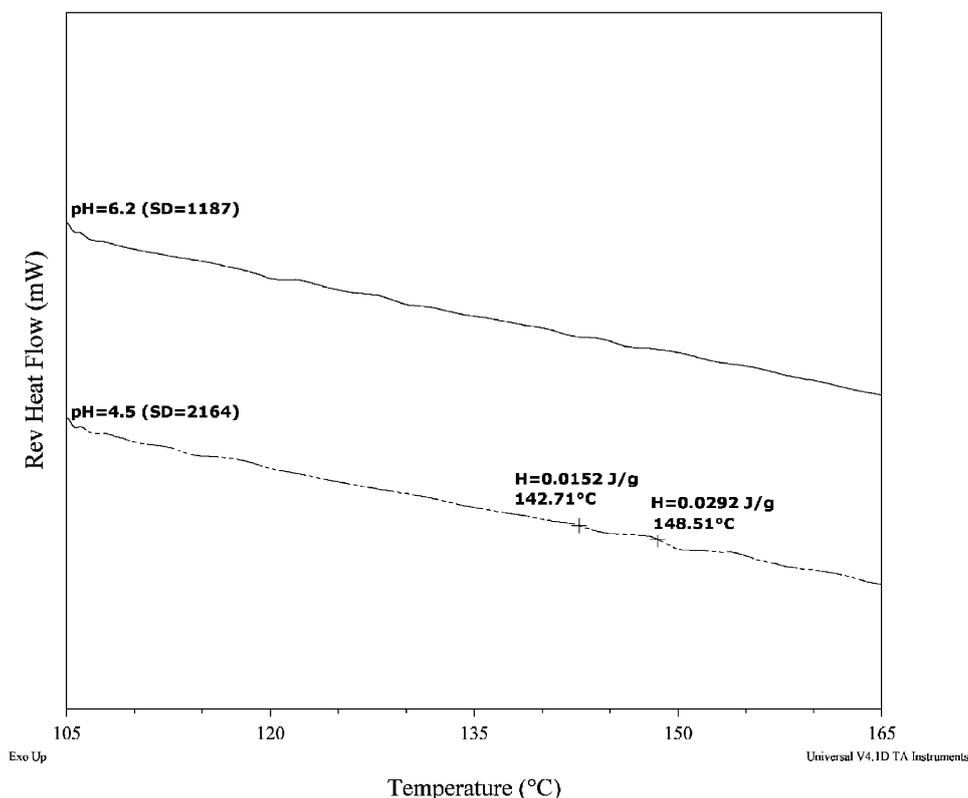


Fig. 3. MDSC curves of xanthan–chitosan capsules showing the effect of initial chitosan solution pH on the resulting capsule network structure. Xanthan: 1% (w/v), chitosan: 1% (w/v).

to 1.5% (w/v), no transitions were observed suggesting a completely crosslinked network. These results are in good agreement with the swelling study, since there was no significant difference in the SD when xanthan concentration was increased from 0.7% (w/v) ($SD = 2375$) to 1.0% (w/v) ($SD = 2164$) and with further increase in xanthan concentration to 1.5% (w/v), the degree of swelling decreased significantly ($SD = 1573$).

The effect of pH on the network structure of the capsules made from 1.0% (w/v) chitosan and 1.0% (w/v) xanthan solutions is shown in Fig. 3. Both transitions from xanthan gum and chitosan observed at pH 4.5 were eliminated when the pH was increased to 6.2. This result might suggest an incomplete crosslinking between the two polymers at pH 4.5 and a complete crosslinking at pH 6.2. The swelling data also show that there is a significant decrease in the degree of swelling when pH is increased from 4.5 ($SD = 2164$) to 6.2 ($SD = 1187$), which can be explained by the increase in the crosslinking density.

4. Conclusions

Characterization of factors contributing to the crosslinking density of xanthan–chitosan network is important in developing hydrogels with desired mechanical and controlled release properties. Results from the SD and DSC experiments showed that the crosslinking density of xanthan–chitosan network was dependent on the complexation conditions employed in the present study.

Xanthan concentration was found to be the most critical parameter in xanthan–chitosan network formation. The hydrogel capsules were completely crosslinked at all conditions studied when initial xanthan solution concentration was at 1.5% (w/v). The increases in xanthan concentration very significantly affected the degree of swelling of the hydrogel at both chitosan concentrations. On the other hand, the effect of chitosan solution pH on the degree of swelling was more pronounced at 0.7% (w/v) than at 1.0% (w/v) chitosan concentration. The SD was less dependent on chitosan concentration than xanthan concentration and chitosan solution pH. Conformational changes of chitosan polymer chains, which are dependent on the solution pH, were critical in determining the crosslinked network structure that affects the SDs of resulting gels. Results from this study showed that pH and concentration effects on the xanthan–chitosan network properties are dependent on each other. It can be concluded that, the xanthan–chitosan network properties can be easily modulated by changing operationally controllable parameters, especially xanthan concentration and chitosan solution pH.

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