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Thermal gelation of aqueous curdlan suspension: preparation of curdlan jelly

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Abstract

Curdlan jelly was prepared by heating an aqueous curdlan suspension at 70 °C for 5 min. Theophylline, as a model drug, was entrapped in the jelly network. Curdlan jelly had a hardness comparable with that of commercially available jelly for confectionary. Syneresis was observed for 8 days after the preparation and was not detected during the experimental term from the gel prepared from 10% w/v curdlan suspension. Release of theophylline from the jellies was sustained, and was increasingly delayed with an increase in the curdlan concentration. An aqueous curdlan suspension was studied by means of differential scanning calorimetry (DSC) up to 80 °C and down to −25 °C, and subsequent re-heating to 30 °C. Enthalpy increased with the concentration of curdlan, while the temperature at the onset of the endothermic peak decreased with the concentration of curdlan. The enthalpy due to thermal gelation of 1 mg curdlan was 12.2 mJ. An increase in curdlan concentration decreased the enthalpy and lowered the onset temperature of the endothermic peak during the DSC re-heating scan. The results are due to an increase in the amount of non-freezing water and freezing bound water and a decrease in free water. The number of water molecules entrapped in the curdlan jelly as non-freezing water was 8.1 per glucopyranose residue.

Introduction

Curdlan is an extracellular bacterial polysaccharide, β-1,3-D-glucan, produced by *Alcaligenes faecalis* var. *myxogenes* 10C3 (Harada 1965; Zhang et al 2002). It is soluble in alkaline solution but insoluble in water or acidic solution. It forms gel when its alkaline solution is neutralized or its aqueous suspension is heated (Harada et al 1968). It is tasteless, has no evident toxicity (Spicer et al 1999) and produces retortable and freezable food gels. Consequently, it has been approved and used as a food additive (The Ministry of Health and Welfare of Japan 1986; FDA 1996), which is added to minced pork gel (Funami et al 1998), meatballs (Hsu & Chung 2000), doughnuts (Funami et al 1999), sausages, tofu (i.e., soybean curd), noodles (Nakao et al 1991), etc. Despite its various application to food, curdlan has not yet been used in practical pharmaceutical dosage forms.

Jelly-like preparations are desirable for oral administration to elderly patients because of their easy handling, chewing and swallowing (Hanawa et al 1995). In this study, we prepared curdlan jelly by heating an aqueous suspension of curdlan, and theophylline was incorporated in the jelly as a model drug. Release of theophylline was delayed by incorporation into the jelly. Its hardness was similar to that of commercially available confectionary jelly. Therefore, curdlan has potential as a drug carrier for sustained release by oral administration to elderly patients.

Materials and Methods

Materials

Curdlan was purchased from Wako Co. (Osaka, Japan) and used without further purification. Its average molecular weight was approximately 8.0×10^4. Commercially

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available jellies for confectionaries, of which gel hardness was measured, were Fruits Jelly (Kuze Co, Gifu, Japan) and K22 Mix-jelly (Kaneshiro Co., Toyohashi, Japan).

Preparation of curdlan jelly and assessment of its physical properties

Aqueous curdlan suspension (1 mL; 3 or 6% w/v) containing 10 mM theophylline as a model drug was placed in a glass test tube (ϕ = ca. 14 mm). The curdlan suspension was incubated at 120 strokes per min at 70 °C for 5 min in a water bath, TAITEC Personal-11 (Tokyo, Japan), resulting in a curdlan jelly.

Dissolution media used were distilled water and solutions no. 1 (pH 1.2) and 2 (pH 6.8) specified in Japanese Pharmacopoeia XIV. The dissolution medium (10 mL) was placed on the curdlan jelly and the tube containing the jelly was incubated at 120 strokes per min at 37 °C. Samples (1 mL) of the media were withdrawn at appropriate time intervals and the same volume of the media was added to the tubes. The percentage of theophylline released from the jelly was obtained after determination of the concentration in the medium by means of spectrophotometry at 271 nm with a UV-1600PC (Shimadzu Co., Kyoto, Japan).

The hardness of curdlan jelly was measured by a curd tension meter, Curdmeter MAX ME-500 (I'techno Engineering Co., Tokyo, Japan). Curdlan jellies prepared from 7 mL aqueous suspensions (curdlan concentration: 4, 5, 7 or 10% w/v) were removed from the glass tubes used for the preparation. Water was not liberated from the jelly by this procedure. Each jelly was divided into 4 columns with almost equal volumes. The fraction was placed on a mobile plate of the curd tension meter and the plate was raised at 1.21 mm s^{-1}. The force when the sensor axis penetrated into the jelly, F(g) was measured and hardness of the jelly, P(kPa) was calculated using equation 1.

$$P = 4gF/\pi d^2 \quad (1)$$

where d is diameter of the sensor axis (= 1 mm) and g the gravitational acceleration (= 9.80 m s^{-2}). The gel hardness of commercially available jellies for confectionaries was measured for comparison.

Jellies composed of 3, 5 and 10% w/v curdlan (10 mL) containing no theophylline were prepared in a similar manner and allowed to stand. Syneresis of the jelly was observed at appropriate time intervals. The degree of syneresis was calculated by dividing the volume of supernatant aqueous phase separated from the jelly layer by total volume of the sample (= 10 mL). The volume of the supernatant phase was calculated from the height of the phase measured with a scale ruler.

Differential scanning calorimetry (DSC) analysis

The DSC scans were carried out using an EXSTAR 6000 calorimeter (Seiko Instruments Co., Tokyo, Japan). Curdlan suspensions (ca. 50 mg) were placed in silver pans and silver lids were crimped in position. Each sample was initially held at 30 °C for 1 min before analysis. These samples were heated to 80 °C at 1 °C min^{-1}, maintained at this temperature for 0.5 min, cooled to −25 °C at 5 °C min^{-1}, held isothermally at these temperatures for 0.5 min and subsequently re-heated to 30 °C at 1 °C min^{-1}. Onset temperatures of endothermic peaks in DSC are defined as the temperatures where the baselines intersected the extrapolated tangents at the midpoints of the peaks (Hatakeyama & Quinn 1999). Although this analysis slightly overestimates or underestimates the onset temperature, it diminishes the personal bias of judgement of the onset (Hino et al 2001). Water liberation from the gel was not observed while homogeneity of the gel in the pan was confirmed when the lid was opened after the DSC scan.

Statistical analysis

The number of runs employed in the same measurement is shown by n. Statistically significant difference was assessed by 1- or 2-dimensional analysis of variance using F-test. The level of risk was described as P. Fitness of the plots of experimental data for regression lines was analysed by regression analysis with F-test. The level of risk was described as P.

Results and Discussion

Preparation of curdlan jelly by thermal gelation of aqueous suspension

White turbid curdlan jelly with elasticity was prepared by heating aqueous suspension. It did not show fluidity even when the glass tube was laid on a table or turned upside down. Container-free jelly was obtained as a whole mass without fragmentation by inclining the tube after cooling to room temperature.

It is reported that low-set gel prepared by heating curdlan aqueous suspension at around 60 °C is soft and contains much water, while high-set gel prepared by heating the suspension at above 120 °C has a tightly packed structure (Hirashima et al 1997). We prepared the curdlan jelly by heating at 70 °C because of the large encapsulation efficiency of the aqueous phase in low-set gel and avoidance of thermal decomposition of the drug to be entrapped during preparation.

Gelation of curdlan suspension occurred during the heating process rather than cooling as for heated gelatin or agar solution. It is considered that drug molecules are homogeneously distributed in the free water retained in the network structure of curdlan jelly. Undesirable recrystallization of drug might be caused if the gelation occurs during the cooling process like with gelatin or agar. However, such a problem will not be caused because gelation of curdlan occurs during the heating process.

Mechanical properties of curdlan jelly

The hardnesses of the curdlan jellies prepared from 4, 5, 7 and 10% w/v curdlan suspensions were 90.8 ± 4.8, 166.0 ±

7.5, 240.3 ± 3.5, and 283.1 ± 6.7 kPa, respectively (n = 12). The hardnesses of Fruits Jelly and K22 Mix-jelly were 8.7 ± 2.3 and 175.6 ± 6.4 kPa, respectively (n = 12). Curdlan jelly had a gel hardness comparable with that of commercially available jellies for confectionaries in Japan. Gel hardness at 4 different fractions, from the top to the bottom, of the jelly was measured. However, difference in the hardness due to the position in the jelly could not be observed. Consequently, curdlan jelly prepared by us had homogeneous and moderate hardness, which was easily chewed and swallowed by elderly patients. The hardness increased with increasing curdlan concentration ($P < 0.001$).

The time course of the degree of syneresis of water from curdlan jellies is shown in Figure 1. Significant difference between the three preparations was observed ($P < 0.001$) and syneresis was suppressed by increasing the concentration of curdlan. Jelly prepared from 10% w/v curdlan suspension was so stable that syneresis could not be detected within 8 days.

Release of theophylline from curdlan jelly is shown in Figure 2A. A sustained-release pattern was exhibited and the release was significantly delayed by increasing the concentration of curdlan ($P < 0.001$). Theophylline release from the jelly into JP XIV no. 1 or 2 solution was closely in accord with that into water (data not shown). Dependence of drug release on the pH of the dissolution medium could not be observed. Untreated curdlan powder is practically insoluble in water or acidic solution but soluble in strong alkaline solution. The dissolution media used in this study had too low a pH to dissolve curdlan. Curdlan jelly was prepared by heating aqueous suspension not via solution state in this study. Curdlan gel prepared by neutralizing its alkaline solution showed a pH-sensitive drug release pattern in a preliminary study (data not shown) while the jelly in this study was prepared by a different gelation mechanism from the gel prepared by neutralization. Consequently, drug release did not depend on the pH of the dissolution media.

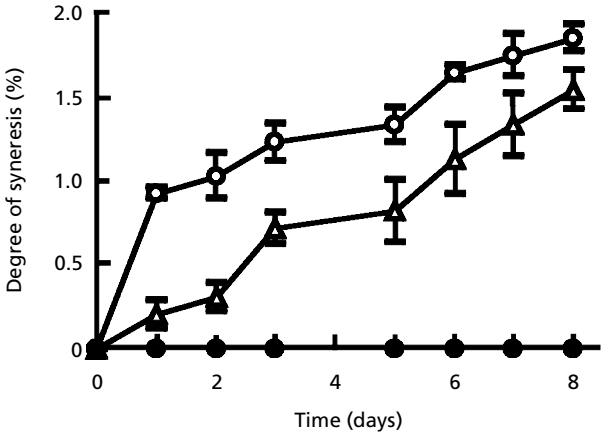

Figure 1 Time course of degree of syneresis from curdlan jellies (○, 3% w/v; △, 5% w/v; ●, 10% w/v). Horizontal bars represent mean ± s.d. (n = 4).

Higuchi (1963) expressed that the amount of drug released from a matrix dosage form (Q) is proportional to the square root of time (t). A similar relationship between Q and $t^{\frac{1}{2}}$ was theoretically derived, by Narashimhan &

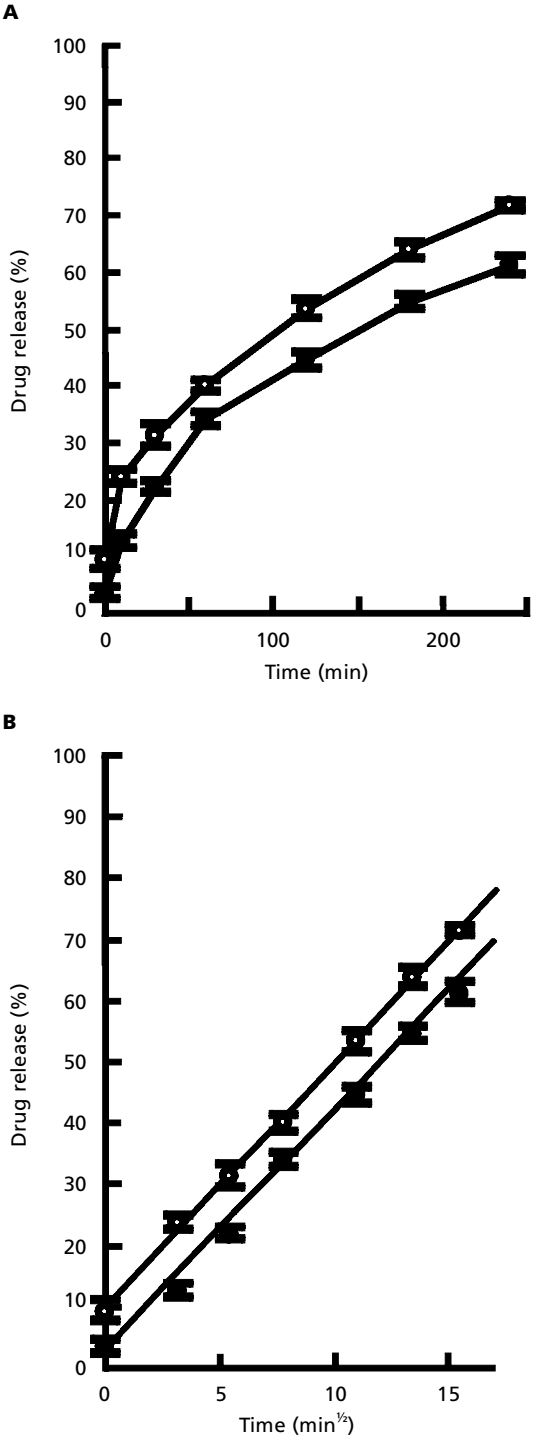

Figure 2 Release of theophylline from curdlan jellies (○, 3% w/v; ●, 6% w/v). A. Release profile. B. Replotted data based on Higuchi plots. Dissolution medium was water. Horizontal bars represent mean ± s.d. (n = 4).

Peppas (1997), for diffusion-controlled drug release from a polymeric carrier. Figure 2B shows replotted data of Figure 2A, which denotes the relationship between $t^{\frac{1}{2}}$ and Q. Good linear relationships were obtained and regression of the data to each line was good ($n = 7$; $P < 0.001$).

For theophylline release from jelly prepared from 3% w/v curdlan suspension,

$$Q = 3.99t^{1/2} + 9.74 \quad (2)$$

For theophylline release from jelly prepared from 6% w/v curdlan suspension,

$$Q = 3.85t^{1/2} + 2.20 \quad (3)$$

where units of Q and t are % and min, respectively. The correlation coefficient, r, of the data for jellies prepared from 3 and 6% w/v curdlan suspensions was 0.9994 and 0.998, respectively.

Release rate constants were almost the same in the two jelly preparations and the difference in drug release (Figure 2A) is considered to be due to initial burst release because the two lines are almost parallel in Figure 2B. The initial release was caused by disturbance of the jelly surface by exposure to dissolution media. The disturbance of the surface was suppressed by increasing the curdlan concentration, due to the increase in gel hardness. Further investigation of drug release from jellies taking account of chewing might be needed.

DSC study

DSC heating scan

DSC heating scans of aqueous curdlan suspensions are shown in Figure 3. Each scan exhibited one endothermic peak. The peak became broader, and the peak area increased, with increase in the concentration of curdlan. These peaks were due to gelation of suspensions.

Figure 4 shows the onset and offset temperatures and enthalpies of the endothermic peaks shown in Figure 3. Onset temperature significantly lowered and the offset slightly increased with increase in the concentration of curdlan. These data clarify the broadening of the endothermic peak caused by increasing the concentration of curdlan. Peak temperatures were almost constant (60.2–60.8 °C, data not shown) and any relationship between peak temperature and concentration of curdlan could not be observed. Onset temperature reflects the temperature when the threshold of the energy for a thermal event is supplied to, or absorbed from, the sample, while peak and offset temperatures are influenced by the dynamic equilibrium between supplied or absorbed heat and the content of the remaining portion which is gradually converted to another state in the sample (Hino et al 2001). Peak and offset temperatures are also affected by sample weight and the filling state of the sample. Consequently, the onset temperature reflects the initiation of a thermal event more precisely than the peak or offset temperatures. Onset temperatures are used for characterization of peaks hereinafter.

The enthalpy of the endothermic peak in Figure 3 per unit weight of the sample, ΔH ($mJ\,mg^{-1}$) is shown in Figure 4B. ΔH was directly proportional to the concentration of curdlan, x (% w/v), as in equation 4 ($r = 0.981$, $n = 5$; $P < 0.01$).

$$\Delta H = 0.122x \quad (4)$$

According to this equation, it is considered that 12.2 mJ of heat is absorbed for gelation of 1 mg curdlan during the heating procedure. Hirashima et al (1997) reported that the endothermic enthalpy per 1 mg curdlan was 12.5 mJ, which did not depend largely on the concentration in the concentration range examined (5–10% w/v). Similarity of the enthalpies and good regression of our data to equation 4 indicate good agreement of our result with their work.

DSC re-heating scan

DSC has been employed to study the state of water within some polymer gel systems. The state of water within polymer gels is normally classified to following three types: free water (i.e., unbound water), of which transition phase temperatures, enthalpies and peak shapes in DSC curves are equal to those of bulk water; non-freezing water (i.e., bound water), which does not undergo a detectable phase

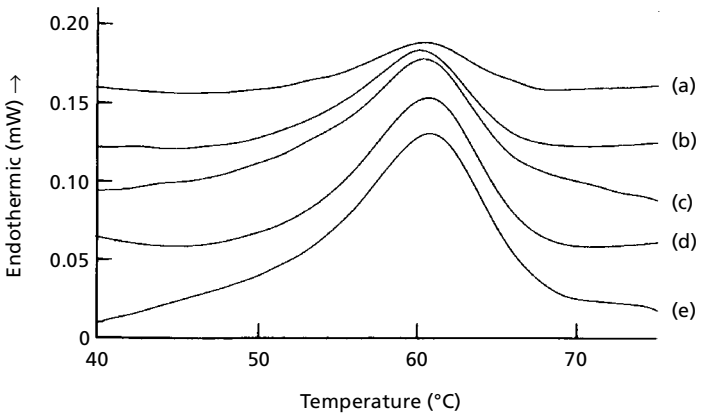

Figure 3 DSC heating scans of curdlan suspensions (3% w/v (a), 5% w/v (b), 7% w/v (c), 10% w/v (d), 12% w/v (e)).

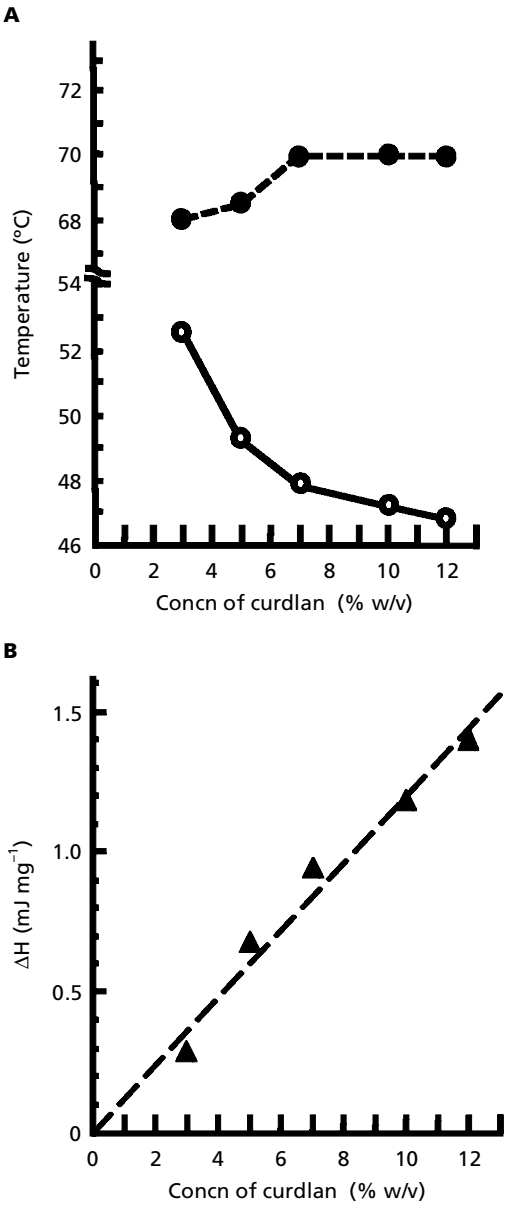

Figure 4 Onset and offset temperatures and enthalpies of endothermic peaks of DSC heating scans of curdlan suspensions. A. Onset temperature (○) and offset temperature (●). B. Enthalpy per unit weight of the sample.

transition by DSC; freezing bound water, which has a phase transition temperature lower than that of bulk water due to a weak interaction with the polymer chain (McCrystal et al 1999).

The amount of free water is calculated from the enthalpy of the exothermic peak in the cooling scan or that of the endothermic peak in the re-heating scan after cooling by DSC (Ford 1999). The latter calculation method provides a more precise and more reproducible value than the former method (McCrystal et al 1999) because crystallization occurs via nucleation, which is an uncontrolled phenomena (Bottom 1999).

DSC re-heating scans of curdlan suspensions after heating and subsequent cooling are shown in Figure 5. Each re-heating scan exhibited one endothermic peak. The onset peak temperature and the peak area decreased with increasing concentration of curdlan.

The onset temperature and enthalpy of the endothermic peak in the DSC re-heating scan (Figure 5) of curdlan suspensions are shown in Figure 6. It was confirmed that the onset temperature of the endothermic peak and the enthalpy decreased with an increase in the concentration of curdlan. The relationship between the enthalpy of the endothermic peak per unit weight of the sample, ΔH (mJ mg^{-1}), and the concentration of curdlan, x (% w/v), is expressed as equation 5 ($r = 0.993$, $n = 4$; $P < 0.01$).

$$\Delta H = -6.943x + 364.5 \quad (5)$$

The number of molecules of bound water per polymer repeating unit (p) is calculated from the enthalpy of the endothermic peak per unit weight of the sample, ΔH (mJ mg^{-1}) and the concentration of curdlan, x (% w/v), as shown in equation 6.

$$\Delta H = -0.01\{1 + (M_W\ p/M_c)\}\ H_0 x + H_0 \quad (6)$$

where, M_W and M_c are the molecular weight of water (=18) and the formula weight of polymer repeating unit (glucopyranose residue, $-C_6H_{10}O_5-$; = 162), respectively. H_0 is the enthalpy for melting water (mJ mg^{-1}).

Comparison of the parameters of equations 5 and 6 results in $H_0 = 364.5$ mJ mg^{-1} and $p = 8.14$ (water molecules/glucopyranose residue). The difference in the enthalpy of melting water between its theoretical (=334 mJ mg^{-1}) and experimental (364.5 mJ mg^{-1}) value might be caused by the formation of various polymorphic forms of ice (Ford 1999) and slight deviation of the experimental data from the regression line of equation 5, which is based on the assumption that p-value is constant independently of curdlan concentration.

A hexagonal unit cell of curdlan hydrate crystal ($a = b = 15.56$ Å, $c = 18.78$ Å) contains 18 anhydroglucopyranose residues and 36 molecules of water (Chuah et al 1983). The number ratio of water molecules to glucopyranose residue in the curdlan hydrate crystal is 2. The number of water molecules bound to one glucopyranose residue in curdlan jelly was 8.14. Curdlan molecules bound with much more water molecules in jelly than those in hydrated crystal. Curdlan jelly had the ability to accommodate more water molecules as bound water than hydroxypropylmethylcellulose gel ($p = 2.4$–7.1 (water molecules/polymer repeating unit)) (McCrystal et al 1999).

The lowered onset temperature of the endothermic peak of the DSC re-heating scan with increasing curdlan concentration (Figure 6) indicates an increase in the amount of freezing bound water.

Mechanism of thermal gelation of curdlan suspension

Curdlan is insoluble in water. Curdlan anhydride is composed of triple-stranded helix structure in its crystal, which has hexagonal unit cells ($a = b = 14.41$ Å, $c = 5.87$ Å).

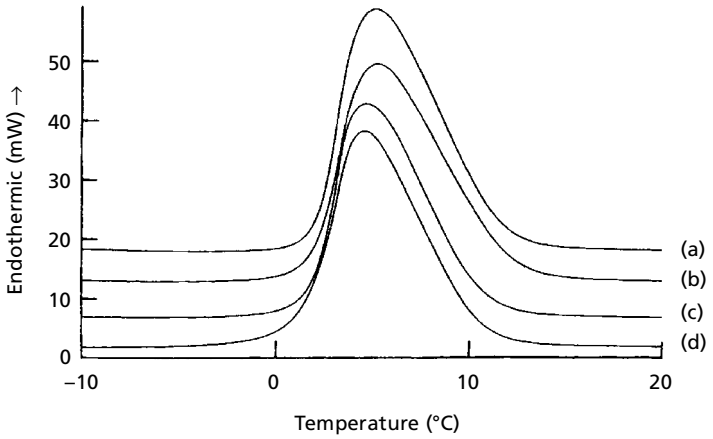

Figure 5 DSC re-heating scans of curdlan suspensions (3% w/v (a), 5% w/v (b), 7% w/v (c), 10% w/v (d)) after heating and subsequent cooling.

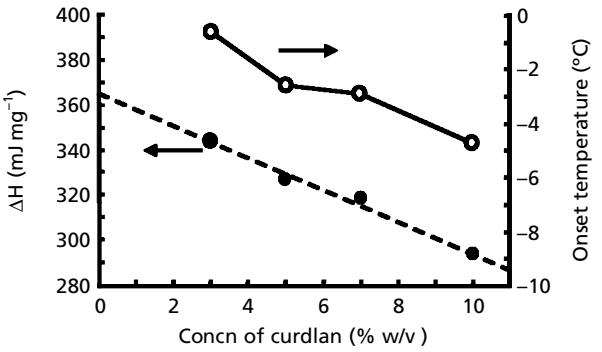

Figure 6 Onset temperatures (○) and enthalpies (●, per unit weight of the sample) of endothermic peaks of DSC re-heating scans of curdlan suspensions after heating and subsequent cooling.

The three strands of the triplex are linked together through triads of hydrogen bonds between O2 hydroxyls, and the helices are linked together through hydrogen bonds involving O4 and O6 hydroxyls. All hydroxyl oxygens participate in at least one hydrogen bond (Deslandes et al 1980).

Curdlan particles swell after exposure to water. Hydration of curdlan disrupts interhelical and intrahelical hydrogen bonds of curdlan resulting in disorder of the helical structure.

The thermal gelation process is caused by hydrophobic interactions between polymer chains (Sarkar & Walker 1995; Ford 1999). At lower temperatures, curdlan molecules are hydrated and there is little polymer–polymer interaction apart from simple entanglement. As the temperature rises, molecules absorb translational energy and gradually lose their hydrated water. Eventually, a polymer–polymer association takes place due to hydrophobic interactions, resulting in gelation.

The onset temperature of the endothermic peak during DSC heating scan was lowered by increasing the curdlan concentration (Figures 3 and 4A). The onset temperature indicates initiation of thermal gelation. Lowering of the temperature is caused by increasing the frequency of polymer–polymer interaction due to increase in the concentration.

Each heating scan in Figure 3 exhibited one endothermic peak. Some of the peaks showed slight shoulders at the lower temperature side of the peaks. These shoulders may be caused by complicated phenomena involving hydration–dehydration processes of curdlan particles. Further investigation including detailed discussion about thermal gelation involving hydration–dehydration process is in progress.

Conclusion

Curdlan jelly was prepared by heating aqueous suspension at 70 °C for 5 min. Theophylline as a model drug was entrapped in the jelly. The jelly had moderate hardness. Syneresis was not detected within 8 days after the preparation of the jelly from 10% w/v curdlan suspension. Release of theophylline from the jellies was sustained, and was delayed with increase in the concentration of curdlan. Curdlan was expected to be a candidate for oral administration to elderly patients.

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