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STRUCTURAL CHANGE OF WATER BY GELATION OF CURDLAN SUSPENSION

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Phase transition of water restrained by curdlan suspension annealed at a temperature from 20 to 110°C was investigated by differential scanning calorimetry (DSC). The melting temperature of water restrained by annealed curdlan discontinuously decreased at around 60°C, while the amount of bound water calculated from enthalpy of melting increased at 60°C, regardless of water content. Using a highly sensitive DSC, curdlan suspension with various concentrations was studied. It was found that an endothermic transition was observed at ca. 58°C in a wide range of concentrations. The transition observed at 60°C is thermo-reversible and both temperature and transition enthalpy are constant even after gel formation. Well equilibrated suspension at a temperature lower than 60°C formed no gel.

Keywords: *annealing, bound water, curdlan, DSC, gels, transition enthalpy*

Introduction

Curdlan is a bacterial polysaccharide composed entirely of (β 1–3) linked *D*-glucose as shown in Fig. 1 [1]. Curdlan is a unique water insoluble polysaccharide which can form gels by heating [2]. It has been reported that thermo-irreversible hydrogels are formed when curdlan suspension is annealed at a temperature higher than ca. 60°C [3, 4]. The above type of gels is designated as high set gels (gel II). When the annealing temperature is lower than 60°C, gels are thermoreversible and designated as low set gels (gel I). It is considered that the above categorization is not always acceptable from DSC results [4] for the following reasons; (1) transition at around 60°C attributed to gel formation is an endothermic peak, (2) the suspension was heated to 80°C, and a similar size of exothermic peak was reversibly detected in the cooling curve, in spite of the 'thermo-irreversible' gel formation.

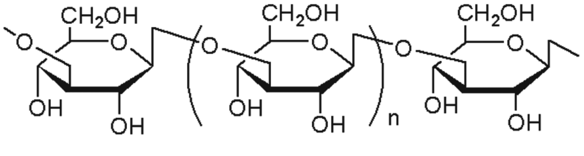

Fig. 1 Chemical structure of curdlan

Methylcellulose (MC) is a water-soluble polysaccharide and also is known to form soft gels when aqueous solution is heated. MC hydrogels are thermoreversible and transform to the sol state by cooling [5]. Sol-gel and gel-sol transition of MC are clearly seen by visual observation. In contrast, gelation of curdlan aqueous suspension seems to occur in a more complex manner, although the higher–order structure of curdlan gels prepared at various conditions, such as the presence of cations has been reported by many researchers [3, 4, 6–9]. By X-ray diffractometry, it is shown that curdlan forms mainly single stranded helical structures and the residual part forms triple helices in wet conditions [10]. The above results are supported by ^{13}C solid state nuclear magnetic spectroscopy (NMR) [11]. It is reported that the triple helix is entirely formed when curdlan is lyophilized from the displacements of the ^{13}C chemical shifts [12].

By thermoanalytical measurements, it is reported that an endothermic peak was observed at around 50–60°C [4, 13]. It is notable that thermal properties of curdlan sample are strongly affected by the amount of water in the suspension since the amount of water swollen in curdlan molecular assemblies affects markedly the hydrogelation. When curdlan gels are formed, it is thought that water molecules restrained by curdlan molecules play an important role. In this study, the thermal properties of curdlan suspension are investigated using a highly sensitive differential scanning calorimeter (DSC) in order to examine the proposed gelation mechanism [4, 13, 14], at the same

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time, structural change of water restrained in different types of gel is observed through melting of ice by standard DSC.

Experimental

Sample preparation

Curdlan granules (Wako Pure Chemicals) were ground, filtrated and 9 fractions with diameter from 15 to 100 μm were obtained. For the examination of gel forming ability of curdlan by visual and weighing method, the samples were prepared as followings; (1) water (10 mL) was added to weighed dried curdlan granules, (2) glass bottle was tightly sealed, (3) maintained at 20°C for 15 min and (4) heated at 105°C for 20 min. Various concentrations of curdlan aqueous suspensions (0.2 to 3.0 wt %) were prepared. Another series of samples were maintained at a temperature lower than 60°C for various times, and then annealing was carried out. The suspension portion was removed using a syringe and gel ratio was calculated as follow.

$$\text{Gel ratio} = m_{\text{gel}}/(m_{\text{gel}}+m_{\text{sol}}) \qquad (1)$$

where m_{gel} and m_{sol} were mass of gel and sol, respectively. After weighing the gel portion, the gel was heated at 100°C for 24 h in an air oven and curdlan content included in gels was calculated. Water content in the gel was also calculated.

For the thermal measurement, curdlan granules (38 μm) were placed in an aluminium sample pan and water was added using a micro syringe. The sample pan with curdlan and water was placed on a microbalance and water content was adjusted by vaporization. The pan was hermetically sealed and weighed. After maintaining the sealed sample pan for 24 h at 25°C, no mass loss was confirmed by weighing. The samples thus obtained were used for the measurement using a standard DSC. For highly sensitive DSC, weighed curdlan granules were placed in a silver pan and 50 μL water was added using a micro syringe. The silver pan was maintained at 25°C for 24 h before measurements.

When the amount of water in the sample was small, water content were calculated as follows,

$$\text{Water content} = W_c = m_{\text{water}}/m_{\text{dry curdlan}} \qquad (2)$$

where m_{water} and $m_{\text{dry curdlan}}$ were mass of water and dry curdlan, respectively. When W_c was more than 20 g g^{-1}, curdlan-water systems were defined by concentration (mass%).

Measurements

Two types of differential scanning calorimeters, a standard DSC (Seiko Instruments 220) and a highly sensitive DSC (Seiko Instruments 120) were used. Aluminium sealed type pans (sample mass=ca. 5 mg) were used for highly concentrated curdlan suspensions. Temperature was in a range from –120 to 120°C and heating rate was 10°C min^{-1}. Silver sealed type pans (sample mass=50 μL) were used for suspensions of 1 to 5 mass%. Temperature was in a range from 0 to 100°C and heating rate was 1°C min^{-1}.

Results and discussion

When curdlan suspension with a concentration ranged from 0.2 to 5 % was annealed, fragile gels were obtained by annealing at a temperature ranged from 60 to ca.80°C and rigid gels formed from 90 to 110°C by visual observation. However, turbid suspension was maintained and no gels were observed until 60°C. The samples obtained at a temperature higher than 60°C, gel portion and suspension could be separated. Figure 2 shows the relationship between concentration of gel and that of suspension. Gel forming conditions are shown in the caption. The fact that concentration of gels is higher than that of suspension, suggests that syneresis occurs by gelation.

Curdlan content in the gel annealed at various temperatures is shown in Fig. 3 (left hand figure) together with water content in the gel (right hand figure). Both curdlan content and water content in curdlan gel show a maximum point at around 80°C.

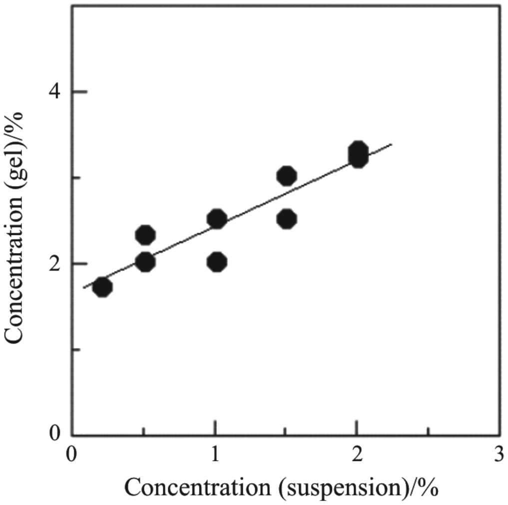

Fig. 2 Relationship between concentration of suspension and that of gel holding temperature= 25°C, holding time=10 min, annealing time = 20 min in a sealed vessel

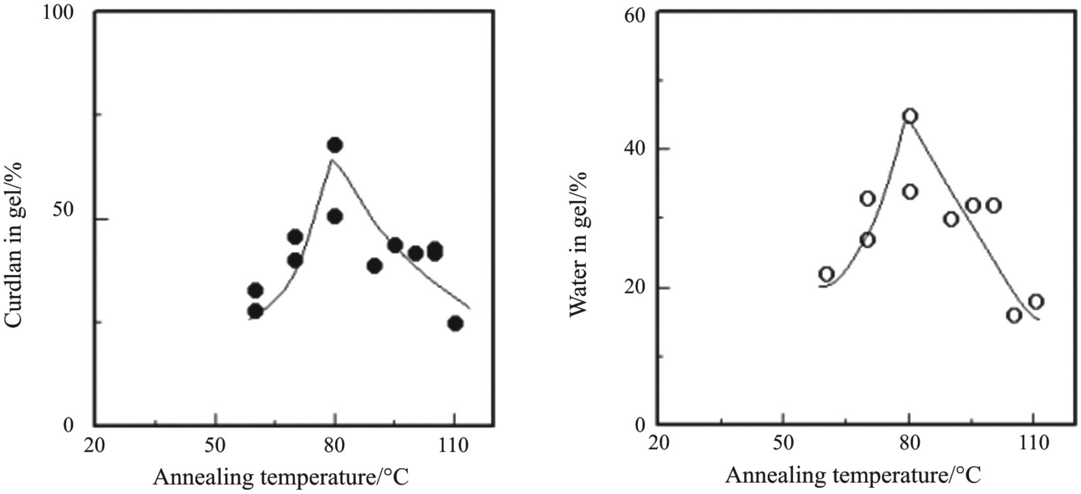

Fig. 3 Curdlan content in gel and water content in gel as a function of annealing temperature. Concentration of curdlan suspensions (diameter of granule 38 μm) =1.5 mass%, holding temperature= 25°C, holding time=10 min annealing time = 20 min in a sealed vessel

This strongly indicates that structural change occurs in the gel at around 80°C.

It is known that gel forming ability of polysaccharides is markedly affected by conditioning at the sol state, since the molecular relaxation of polysaccharide molecules in the presence of water is crucial for cross-linking formation [15–17]. It was observed that not only gelling conditions but also prehistory of curdlan samples affected the gel ratio, suggesting the cross linking formation is controlled by equilibration of curdlan molecules in aqueous suspension. Gel ratio of curdlan hydrogels prepared by the procedure as described in the experimental section increased with increasing diameter of granules, i.e. gel ratio was 0.32 for the sample with diameter 20 μm and 0.48 for diameter 80 μm. Water content of obtained gels decreased with increasing granule size.

This suggests that water diffusion inside the molecular assemblies is affected by the macroscopic shape of curdlan. When suspension with various concentrations was maintained at a temperature from 20 to 60°C, gel formation ability markedly decreased at a critical holding time, i.e. 10^2 min at 50°C and 20 min at 60°C as shown in Fig. 4. The critical holding time increased with increasing granule size. The above facts suggest that curdlan gels are formed only when curdlan molecules are not equilibrated in aqueous media at a temperature lower than 60°C. Hence, it is important to investigate the role of water during both equilibration and gelation.

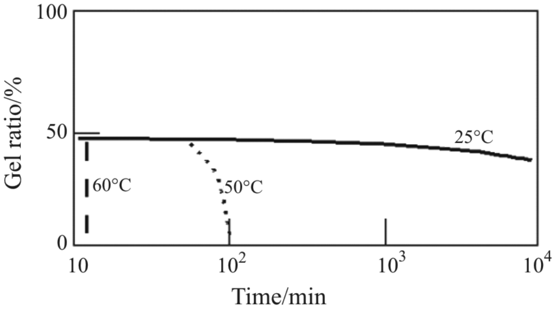

Fig. 4 Relationships between gel ratio and holding time; holding temperatures are shown in the figure

In order to analyze the phase transition behaviour of water restrained by curdlan molecules, differential scanning calorimetry (DSC) was extensively carried out. Curdlan suspension with various water contents were annealed at a temperature from 20 to 110°C for 10 min, cooled to –70° C at cooling rate of 10°C min^{-1}, maintained at –70°C for 1 min and then heated to a predetermined temperature at heating rate of 10°C min^{-1}. Figure 5 shows representative DSC melting curves of water restrained by curdlan gels. The left hand figure shows the system with water content 0.77 g g^{-1} and the right hand figure shows the system with water content 2.52 g g^{-1}. As clearly seen, the shoulder peak can be observed when water content of the systems was 0.77 g g^{-1}. Similar results were obtained for samples with a water content smaller than ca. 1.1 g g^{-1}. When water content exceeded 1.1 g g^{-1}, monotonous endothermic peak was observed. Cooling curves were also obtained for samples with various water contents annealed in a temperature range from 20 to 120°C. From DSC curves, starting temperature of melting (T_{mi}'), intercept temperature between tangential line of endothermic peak and sample baseline (T_{mi}), melting peak temperature (T_{mp}) and crystallization peak temperature (T_{cp}), were

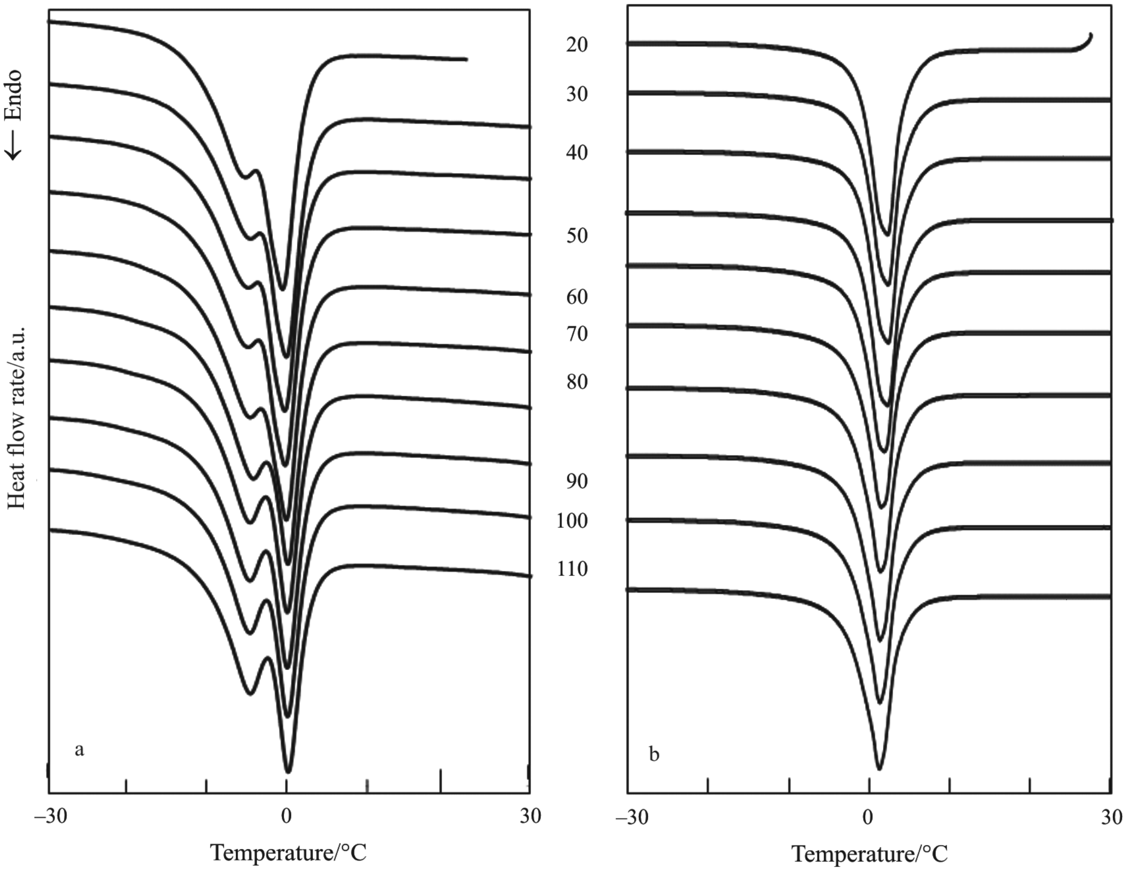

Fig. 5 DSC melting of water restrained by curdlan-water systems annealed at various temperatures, Numerals in the figure show annealing temperature, Heating rate= 10°C min^{-1}. a – water content=0.77 g g^{-1}, b – water content 2.52 g g^{-1}

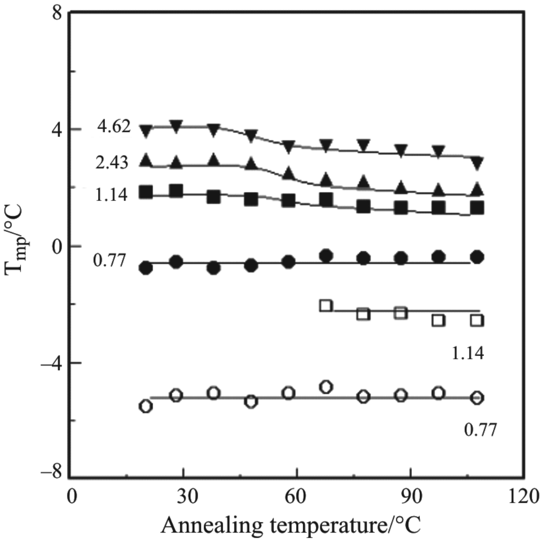

Fig. 6 Relationships between peak temperature of melting (T_{mp}) and annealing temperature of curdlan-water systems. Water content is shown as numerals in the figure. Open circles and rectangles are T_{mp} of the low temperature side peak

determined and values were correlated with annealing temperature or water content.

Figure 6 shows relationships between annealing temperature and T_m of curdlan-water systems. It is clearly seen that a discrete gap is observed at around 60°C except for the sample with water content 0.77 g g^{-1}. It has been reported that curdlan suspension transformed into gel by annealing at a temperature higher than ca 60°C [3, 4, 11, 13, 14]. The results shown in Fig. 3 suggest that a distinct structural change of water occurs at around 60°C. T_{mi}', T_{mi} and T_c shifted to the low temperature side when annealing temperature exceeded 70°C, although the figures are not shown. It was found that T_{mi}', T_{mi} and T_c increased with increasing water content. When water content was less than 1.14 g g^{-1}, melting peaks separated into two peaks (Fig. 5). T_{mp} of the low temperature side peak was markedly affected by water content.

Figure 7 shows the relationship between T_{mp} and water content. T_{mp} values of gel are always observed at a temperature lower than those of suspension, indicating that water molecules are strongly restrained by gel structure due to the introduction of defects in ice. The fact that melting peak is observed at the low tem-

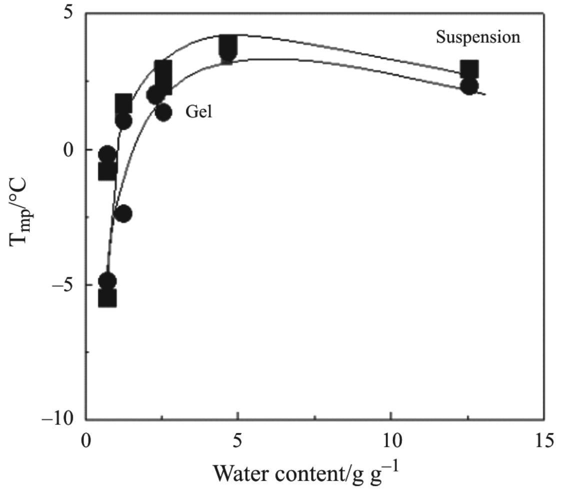

Fig. 7 Relationships between peak temperature of melting and water content of curdlan gel and suspension

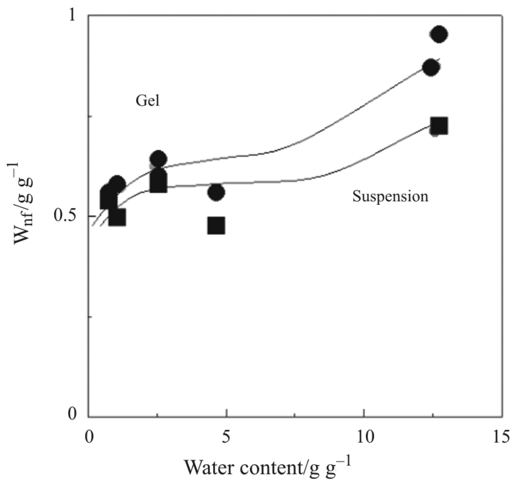

Fig. 9 Relationships between the amount of bound water and water content of curdlan in gel and suspension

perature side suggests that the irregular ice formed in the vicinity of curdlan molecules, especially in the junction zone. The above fact suggests that structural change of curdlan molecules accompanies with rearrangement of bound water molecules occurs. Bound water content calculated from enthalpy of melting [18, 19], increased discontinuously at 60°C. Figure 8 shows relationships between the amount of non-freezing water (W_{nf}) and annealing temperature. The bound water content changed discontinuously at around 70°C regardless of water content. This suggests that the structure of molecular assemblies drastically changed at around this temperature.

Water content dependency of the amount of bound water is clearly seen, as shown in Fig. 9. The above results showed no accordance with those of water soluble polysaccharides obtained in the similar experimental procedure [18, 19]. Generally, the amount of bound water depends on the number of hydrophilic groups or ionic groups in the molecular chain, if the water-polysaccharide systems are homogenously equilibrated. In the case of water insoluble polysaccharide, such as cellulose, the amount of bound water is affected by the higher order structure, mainly the number of molecular chains in the amorphous region. Accordingly, the variation of number of bound water molecules is moderate in a wide range of water content, even if the higher order structure changes slightly in water [20]. As shown in Fig. 7, W_{nf} of curdlan drastically increases. The results shown above strongly indicate that the higher order structure of curdlan in both states depends on water content.

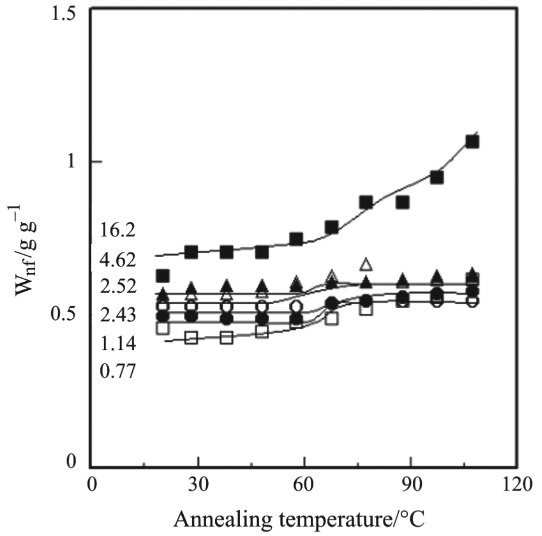

Fig. 8 Relationship between the amount of bound water (W_{nf}) and annealing temperature. Numerals in the figure show water content

Suspensions with low curdlan concentrations were studied using a highly sensitive DSC. Figure 10 shows DSC heating curves of curdlan suspensions with various concentrations. An endothermic peak

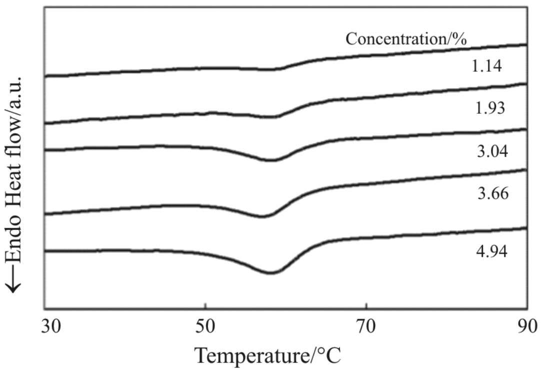

Fig. 10 DSC heating curves of curdlan suspension with various concentrations; heating rate = 1°C min^{-1}. Numerals in the figure show concentration (mass%)

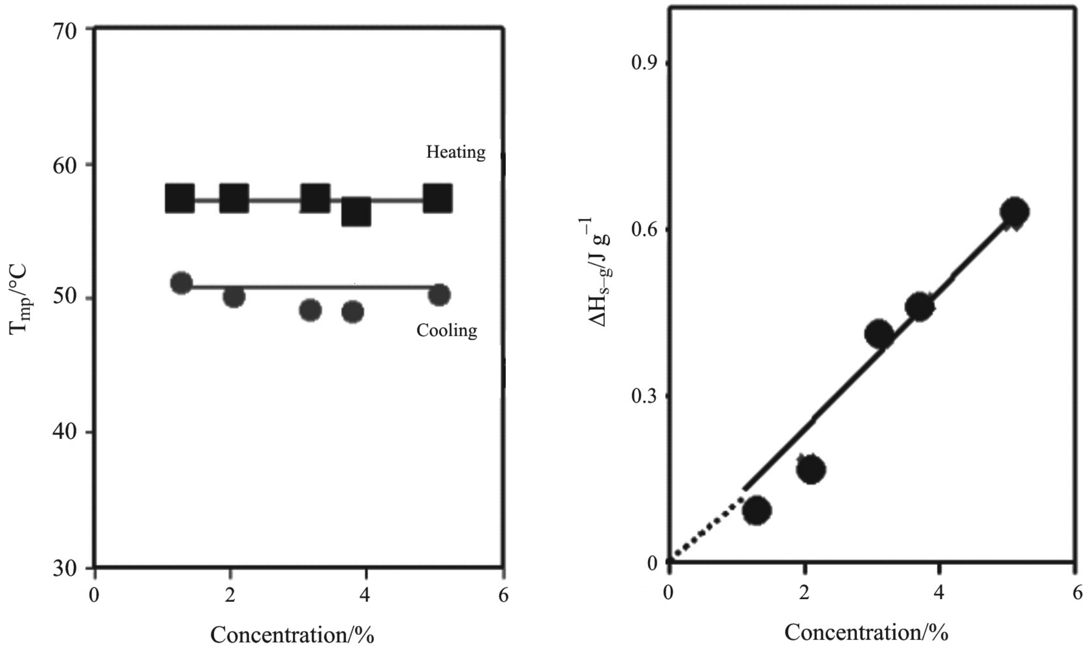

Fig. 11 Relationship between peak temperature of transition and enthalpy of transition ($\Delta H_{s–g}$) and concentration

was clearly observed. Starting temperature (T_i) of the transition was 50.0°C and peak temperature (T_p) was maintained at 57.5°C regardless of concentration. It is notable that transition was endothermic, although gel was formed at a temperature higher than the transition. This strongly indicates that the transition is attributable to decomposition of curdlan molecular assemblies in the presence of excess amount of water.

Figure 11 shows the peak temperatures obtained from heating and cooling curves (left hand figure) and enthalpy of transition ($\Delta H_{s–g}$) (right hand figure) as a function of concentration. Peak temperatures obtained from both heating and cooling curves maintain a constant value. Transition enthalpies increase linearly with increasing concentration. The values of transition enthalpy were 0.1 to 0.6 J g^{-1}, which are far smaller than conformational transition enthalpies of other polysaccharides, such as shizophyllan [21]. The above facts strongly suggest that the amount of water in the suspension affect the transition phenomena. In order to investigate the effect of amount of water in extreme condition, a highly concentrated suspension was investigated using a standard DSC.

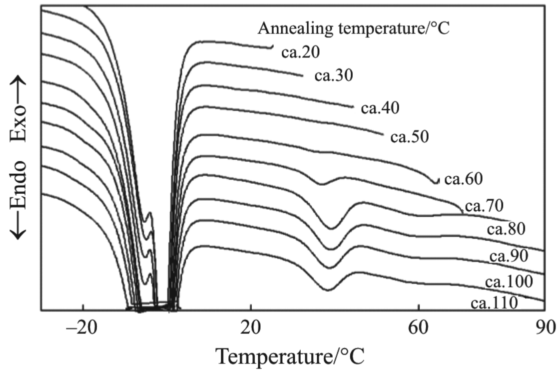

Fig. 12 DSC heating curves of curdlan suspension with water content 0.77 g g^{-1} annealed repeatedly. Annealing temperature is shown in the figure. Heating rate = 10°C min^{-1}

Figure 12 shows DSC heating curves of highly concentrated curdlan suspension repeatedly annelaed at various temperatures. Two endothermic peaks are observed at a temperature higher than melting of ice, when annealed temperature exceeded 60°C. By cooling, two exotherms were clearly observed, although DSC curves are not shown here. Peak temperatures were designated as T_{mp1} and T_{mp2} from the low to high temperature and results are shown in Fig. 13 (left hand figure) together with results (T_{cp1} and T_{cp2}) obtained by cooling curves. Enthalpy of transition of the two peaks obtained from both heating and cooling curves is shown in Fig. 13 (right hand figure). As shown in this figure, enthalpy values of the low temperature side peak (ΔH_{m1} and ΔH_{c1}) increase abruptly from 60°C and the maximum is found at 80°C. In contrast, enthalpy values of the high temperature side peak (ΔH_{m2} and ΔH_{c2}) maintain a constant at around 2.5 J g^{-1}. The values were far larger than those obtained for the systems with a large amount of water. As shown in Fig. 11, enthalpy of transition of samples with concentrations from 1 to 5 mass% was in a range from 0.1 to 0.7 J g^{-1}.

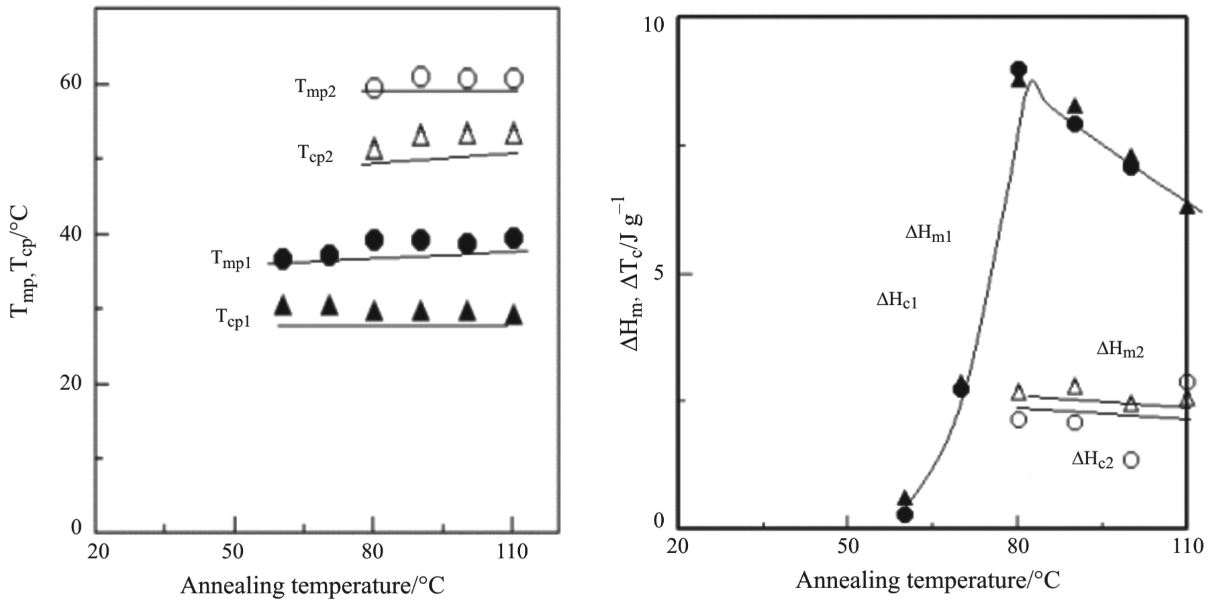

Fig. 13 Peak temperatures and transition enthalpies of curdlan-water system (W_c=0.77g g^{-1}) as a function of annealing temperature

Conclusions

From the above results, the following facts were clarified: (1) hygrogels can be formed at a temperature higher than ca. 60°C by visual observation, (2) an endothermic peak at around 60°C is observed in a wide range of water content, even when the water content of curdlan is extremely small to form suspension (3) transition at 60°C is reversibly observed by DSC, even when the maximum temperature of measurements was 100°C, (4) transition enthalpy increases with decreasing water content (5) melting temperature of water restrained by curdlan molecules and the amount of bound water varied and discontinuously decreased at around 60°C, (6) the amount of curdlan aggregated in gel structure and water content in the gel show the maximum at around 80°C. The repeatability of the above results is confirmed when curdlan suspension equilibrated in the same conditions.

From the above facts, it is reasonable to conclude that two different types of gel exist, however the temperature forming the two types of gel obtained in this study is different from that of the reported temperature, i.e. one type of gel is formed from 60 to 80°C and the other type of gel~~s~~ is formed at a temperature higher than 80°C. Distinct differences can be seen by visual observation in this study. However, the border of two types is ambiguous depending on experimental conditions. Structural change of water between two types of gel was undetectable. The thermal transition observed at around 60°C in curdlan-water system is thought to be a precursor transition before gelation and is attributable to decomposition of curdlan molecular assemblies. When the amount of water is not sufficient to form suspension, molecular assemblies decompose in a short range which can be observed as a characteristic peak at a temperature lower than the transition at 60°C, however, further investigation is necessary concerning this new peak. The facts that preparation conditions of suspension markedly affect the gelation indicate that curdlan gels can be formed when suspension is in a non-equilibrium state.

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