DSC and rheological studies on aqueous dispersions of curdlan[1]

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Abstract

Differential scanning calorimetry (DSC) and dynamic viscoelastic measurements of aqueous dispersions of curdlan were performed to clarify the gelation mechanism. Second run DSC heating curves for aqueous dispersions of curdlan showed a single endothermic peak for dispersions heated to temperatures lower than 53°C, and splits into two peaks for dispersions heated to temperatures higher than 53°C, indicating that the structural disorders of at least two different energies are involved. Dispersions heated at temperatures higher than 120°C did not show any endothermic peak in the second run DSC heating curves indicating that structures formed at temperatures higher than 120°C are stable and thermo-irreversible. Mechanical spectra of the aqueous dispersions of curdlan showed solid-like behaviour both at 40 and 70°C although the mechanical loss tangent for the dispersion heated to a higher temperature was much smaller. All these results are consistent with previous findings that gels formed at higher temperatures are thermo-irreversible, and that gels formed at lower temperatures are thermo-reversible. © 1997 Elsevier Science B.V.

Keywords: Curdlan; Differential scanning calorimetry; Dynamic viscoelasticity; Gelation; Thermo-reversible

1. Introduction

Curdlan is a microbial polysaccharide, which consists of D-glucosyl residues connected by β-(1 → 3)-linkages, produced by *Alcaligenes faecalis var. myxogenes* 10C3 [1,2]. When the dispersion of curdlan is heated, it forms a gel. If it is heated at around 60°C and then cooled, it forms the so-called low-set gel which is thermo-reversible [3]. If it is heated above 80°C, it is said that it forms a thermo-irreversible gel [4]. Konno et al. [5] showed later that the gel formed by dispersions heated in the range 60–120°C was thermo-reversible, and that gels formed by heating above 145°C was thermo-irreversible.

In spite of these tremendous efforts, the gelation mechanism of curdlan aqueous dispersions still remains unclear. The dynamic viscoelastic measurements and differential scanning calorimetry (DSC) were carried out to throw some light on the mechanism in the present work.

2. Materials and methods

2.1. Preparation of dispersions

Powdered curdlan sample (Lot. DL-15A) was given by Takeda Chemical Industries. Powders were dispersed in distilled water by using a homogenizer at

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[1]Dedicated to the memory of late Prof. T. Harada.

1200 rpm and 0°C, then transferred into a vial which was soaked in ice-water, and sonicated for 10 min. The dispersion was stirred by using a magnetic stirrer at 40°C overnight.

2.2. Differential scanning calorimetry (DSC)

DSC measurements were carried out using a sensitive DSC apparatus, PTC-10D,8240A (Rigaku Denki, Tokyo). A powdered sample was sealed into a silver pan of 70 µl (Seiko Electronics, Tokyo) with distilled water. The sealed pan was left overnight before heating to attain equilibrium. Heating and cooling rates were 1°C min^{-1}. The details of the DSC measurements have been described previously [6].

2.3. Dynamic viscoelastic measurements

The storage shear modulus G' and the loss shear modulus G'' of dispersions of curdlan were observed as a function of frequency at a constant temperature, and as a function of temperature at a constant frequency in the linear viscoelastic regime (shear strain <4%) using a Fluids Spectrometer RFSII and a Dynamic Stress Rheometer from Rheometrics (NJ, USA). The details of the dynamic viscoelastic measurements were described previously [7,8].

3. Results and discussion

Fig. 1 shows heating DSC curves for 5, 7.5 and 10 wt% curdlan dispersions. An endothermic peak was observed at about 58°C, which shifted very slightly to higher temperatures with increasing concentration of curdlan. The endothermic enthalpy per 1 mg curdlan, ΔH, was 12.5 mJ, which did not depend largely on the concentration in the concentration range examined. These tendencies were in good agreement with a previous study [9]. The sharpness of the peak was estimated by the peak height (mJ s^{-1}) divided by the width $T_t - T_o$ (K), where T_t is the terminal temperature and T_o the onset temperature; the endothermic peak became sharper with increasing concentration of curdlan, the sharpness of the peak being 3.0×10^{-3}, 4.6×10^{-3} and 5.4×10^{-3} mJ s^{-1} K^{-1} for 5, 7.5 and 10% dispersions, respectively. The appearance of an endothermic peak on heating should be attributed to
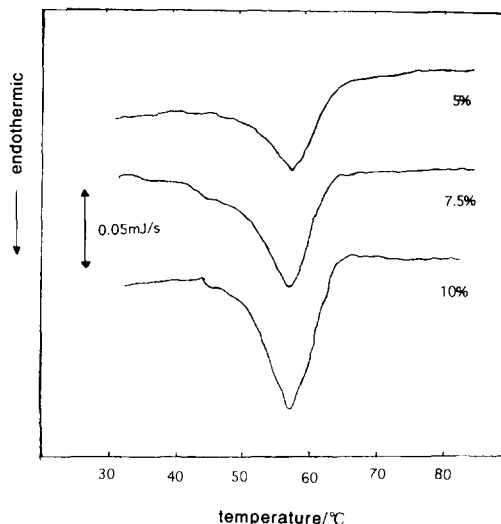

Fig. 1. DSC heating curves of 5, 7.5 and 15% aqueous dispersions of curdlan. Heating rate: 1°C/min.

the disordering of the structure, or swelling of curdlan particles as was observed for the gelatinisation of starch [10,11].

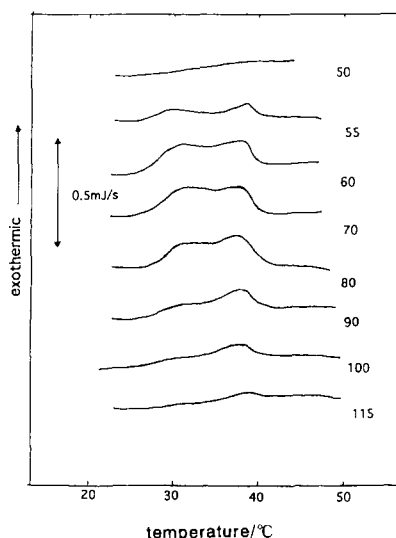Fig. 2 shows the cooling DSC curves for the 2% curdlan dispersions which had been heated at various

Fig. 2. DSC cooling curves of 2% aqueous dispersions of curdlan kept at various temperatures for 30 min. Cooling rate: 1°C/min. The numbers beside each curve represent the temperature in °C at which the dispersion was kept.
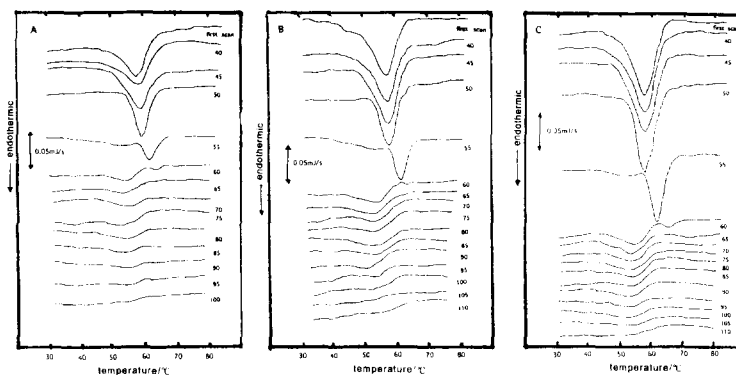
Fig. 3. Second run DSC heating curves of 5% (A), 7.5% (B) and 15% (C) aqueous dispersions of curdlan kept at various temperatures for 60 min. Heating rate: 1°C/min. The numbers beside each curve represent the temperature in °C at which the dispersion was kept.

temperatures for 30 min from 50 to 115°C. Cooling DSC curves for the solutions heated at temperatures lower than 50°C did not show any peak, while those heated at higher than 55°C showed double exothermic peaks at 38 and 31°C. These exothermic peaks were attributed to the structure ordering due to the formation of hydrogen bonds. The exothermic enthalpy was a maximum for the dispersion which was heated at 70°C. Since the curdlan suspension forms a gel on heating above the temperature from 70 to 80°C, the main molecular forces responsible for the formation of junction zones are considered to be hydrophobic interactions. The dispersions which were heated above 120°C showed no exothermic peak in the cooling DSC curves indicating that the gels formed on heating above 120°C became thermo-irreversible. The critical heating temperature, 120°C above, which the gels become thermo-irreversible is lower than that (145°C) observed by Konno et al. [5], and the reason for this is not clear at present. Junction zones formed on heating at temperatures higher than 120°C seem to be of a permanent character. Even if bonding energies of the formed hydrogen bonds are low, i.e. several k Cal mol^{-1}, the accumulated energies by the formation of enormous amount of hydrogen bonds may be high enough to behave as a thermally stable structure as observed in cellulosic materials and as proposed for the gel formation of konjac mannan in alkaline condition [12]. Heating at higher temperatures may be necessary to swell the curdlan molecules and rearrange so that they may form a more stable associated structure. Although the dispersions heated at tempera-

tures lower than 115°C showed exothermic peaks in cooling DSC curves which should be attributed to the formation of hydrogen bonds, dispersions heated at temperatures higher than 120°C may form junction zones of a permanent character. Further, gel formation by hydrogen bonding on cooling does not occur for these dispersions.

Fig. 3 shows the second run heating DSC curves for 5, 7.5 and 10% dispersions of curdlan which had been heated at various specified temperatures for 60 min, and then quenched to 10°C by liquid nitrogen. An endothermic peak appeared at about the same temperature as the first run heating DSC curve around 58°C. Dispersions heated at temperatures higher than 60°C for 60 min showed an endothermic peak much broader at lower temperatures around 55°C.

Second run heating DSC curves for the 10% dispersions heated at temperatures from 50 to 63°C are shown in Fig. 4. The main endothermic peak around 58°C was shifted to higher temperatures with increasing heating temperature up to 60°C. At lower temperature, endothermic peak began to appear in a second run heating DSC curve for a dispersion heated at 53°C, and it also shifted to higher temperatures with increasing heating temperature. A dispersion heated above 60°C scarcely showed a higher temperature peak. At least two kinds of structural disordering with different thermal energies may occur in a dispersion heated in the range 51–60°C.

Fig. 5 shows the endothermic enthalpies estimated from a lower and a higher temperature peak in the second run heating DSC curves, shown in Fig. 4, and
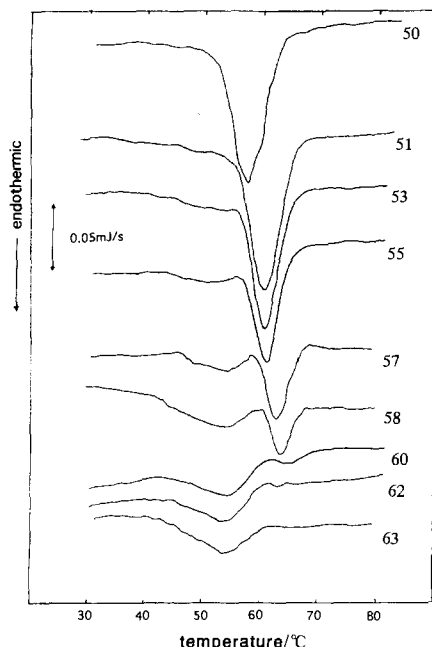
Fig. 4. Second run DSC heating curves of 10% aqueous dispersions of curdlan kept at various temperatures for 60 min. Heating rate: 1°C/min. The numbers beside each curve represent the temperature in °C at which the dispersion was kept.
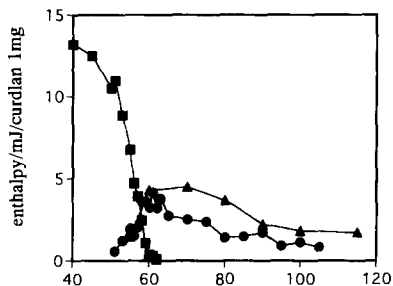

Fig. 5. Endothermic enthalpy for a higher and a lower temperature peak ΔH_h (■) and ΔH_l (●) in the second run heating DSC curves, exothermic enthalpy ΔH (▲) in cooling DSC curves.

the exothermic enthalpy in cooling DSC curves, shown in Fig. 2, as a function of heating temperature. The endothermic enthalpy ΔH_h for a main endothermic peak at a higher temperature in second run heating DSC curves decreased steeply with increasing heating temperature while the endothermic enthalpy ΔH_l for an endothermic peak at a lower temperature showed a maximum at a heating temperature around 60°C. The

exothermic enthalpy observed in cooling DSC curves also showed a maximum at around 60–70°C. An ordered structure formed by heating at temperatures higher than 120°C seemed to be a stable network as mentioned above, and it does not show any endothermic peak in the second run heating.

A structure formed by heating in the range 57–100°C is partially thermo-reversible because a dispersion heated at this temperature range showed exothermic peaks in cooling DSC curves and endothermic peaks on the second run heating DSC curves. This thermo-reversible network is formed by a weakly bound molecular chains, and can be strengthened by hydrogen bonds formed on cooling. The thermo-irreversible gels formed by heating at temperatures higher than 120°C seemed to have tightly packed structure, and did not show any exothermic peak on cooling because the tightly packed structure does not need the rearrangement of molecular chains by the formation of hydrogen bonds on cooling.

Fig. 6 shows the degree of thermo-irreversible gelation dG which is defined as $dG = 1 - \Delta H_{2T}/\Delta H_1$ for 10% aqueous dispersion of curdlan, where ΔH_{2T} is the endothermic enthalpy determined from the endothermic peak in the second run DSC heating curve for a curdlan aqueous dispersion kept at temperature T for 60 min, and ΔH_1 is the endothermic enthalpy in the first run heating. If gels formed by heating are completely thermo-irreversible, no endothermic peak
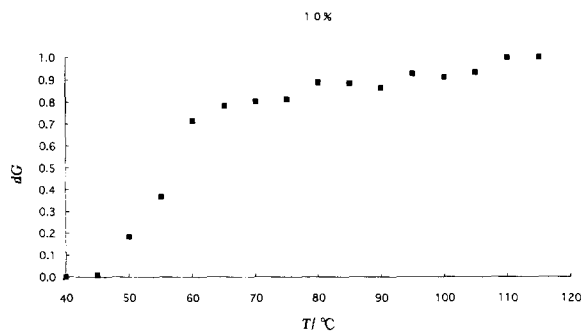

Fig. 6. The degree of thermo-irreversible gelation $dG = 1 - \Delta H_{2T}/\Delta H_1$ for a 10% aqueous dispersion of curdlan as a function of heating temperature T, where ΔH_{2T} is the endothermic enthalpy determined from the endothermic peak in the second run DSC heating curve for a 10% aqueous dispersion of curdlan kept at temperature T for 60 min, and ΔH_1 is the endothermic enthalpy in the first run heating.
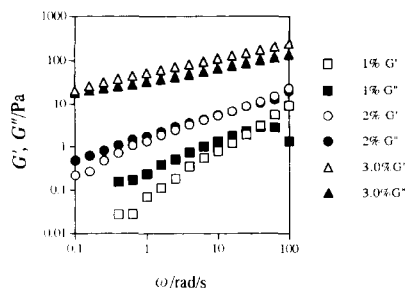
Fig. 7. Frequency dependence of storage shear modulus G' and loss shear modulus G'' for 1, 2 and 3% DMSO solutions of curdlan at 40°C. □ (G'), ■ (G''), 1%; ○ (G'), ● (G''), 2%; △ (G'), ▲ (G''), 3%.

Fig. 8. Frequency dependence of storage shear modulus G' and loss shear modulus G'' for 2, 3 and 4% aqueous dispersions of curdlan at 40°C. □ (G'), ■ (G''), 2%; ○ (G'), ● (G''), 3%; △ (G'), ▲ (G''), 4%.

should appear in the second run DSC heating curve. As it is clear from this figure, the degree of thermo-irreversible gelation increases steeply in the range 0–70°C, and then continues to increase gradually with increasing temperature. It seems that aqueous dispersions of curdlan heated in the range 50–70°C form a partially irreversible gel, and tend to become almost irreversible gels above 120°C. The temperature range at which dG increases steeply should shift to lower temperatures with increasing concentration of curdlan. How fast dG increases should depend not only on the concentration but also on the molecular weight of curdlan, and this should be explored in the future.

Fig. 7 shows the frequency dependence of storage and loss shear moduli, G' and G'', for DMSO solutions of curdlan with concentrations 1, 2 and 3% at 40°C. All these solutions behaved like a concentrated polymer solution: G'' predominates at lower frequencies but G' is larger than G'' at higher frequencies because molecular chains can disentangle during the long period of oscillation at lower frequencies while the entanglement points play a role of a temporary cross-link during short period of oscillation at higher frequencies. The cross-over frequency of G' and G'' shifted to lower frequencies as has been observed for many polymer solutions [13], including biopolymer solutions such as hyaluronic acid solutions [14] and gellan gum solutions [15]. The behaviour of curdlan in DMSO is quite different from that in water because it is not soluble in water while it dissolves in DMSO. The elastic modulus of gels formed by curdlan–DMSO–water was shown to be maximum at around 0.277 mol fraction DMSO in the range 15–85°C [9].
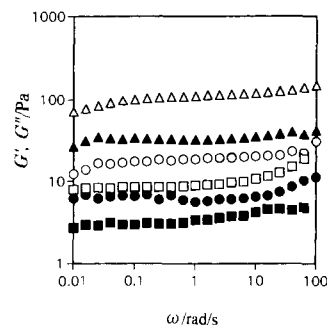
Frequency dependence of G' and G'' for aqueous dispersions of curdlan of 2–4% at 40°C is shown in Fig. 8. All these dispersions showed a solid-like behaviour; G' is larger than G'' at all frequencies and shows a plateau region even at lower frequencies. This is quite a similar behaviour seen in true elastic gels which are formed from polymer solutions [15,16]. Tada et al. [17] observed that mechanical spectra changed from a dilute solution behaviour to gel-like behaviour, where plateau region at lower frequencies is seen, for dispersions of curdlan–sodium hydroxide–water with decreasing content of sodium hydroxide. They also suggested that the association was promoted with decreasing content of sodium hydroxide by a dynamic light scattering. The similar rheological behaviour for dispersions of colloidal particles, which are not soluble in water, has been also observed for dispersions of monodisperse polystyrene lattices [18] and xanthan-konjacmannan [19]. The difference in the mechanical spectra of 'true' gels and the dispersions cannot be distinguished only by the measurement of the frequency dependence in the small deformation range [16]. These dispersions have been shown to have an yield stress, and to flow under the shear higher than this yield stress while the true gels break beyond a certain deformation.

Fig. 9 shows the frequency dependence of G' and G'' for 2–4% aqueous dispersions of curdlan at 70°C. Both moduli increased remarkably on heating, and tan $\delta = G''/G'$ is far smaller (about 0.1) for dispersions at 70°C than for dispersions at 40°C (about 0.3–0.4) indicating that dispersions changed into more solid-
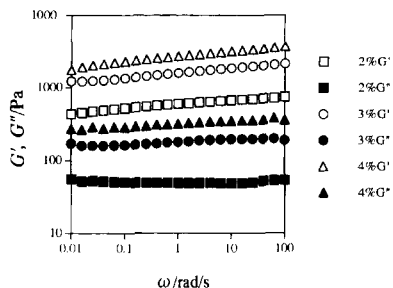
Fig. 9. Frequency dependence of storage shear modulus G' and loss shear modulus G'' for 2, 3 and 4% aqueous dispersions of curdlan at 70°C. □ (G'), ■ (G''), 2%; ○ (G'), ● (G''), 3%; △ (G'), ▲ (G''), 4%.
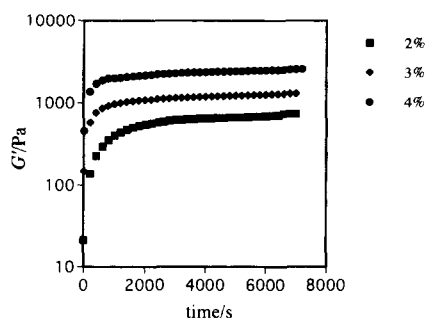

Fig. 10. The time course of storage shear modulus G' for 2, 3 and 4% aqueous dispersions of curdlan at 70°C and at $\omega = 1$ rad/s.

like state on heating; these gels formed at 70°C show fracture behaviour beyond a certain large deformation.

Storage modulus G' for 2–4% dispersions of curdlan as a function of time at 70°C is shown in Fig. 10. All these curves are approximated well by a first-order reaction equation: $G' = G'_s [1 - \exp(-k(t - t_0))]$, where G'_s represents the saturated value of the storage shear modulus after a sufficient long time, k the rate constant, and t_0 the latent time. In the present case, for curdlan dispersions kept at 70°C, t_0 is almost negligible, i.e. the gelation began to occur almost without delay. The rate constant k is 0.9×10^{-3}, 1.2×10^{-3} and 1.3×10^{-3} s^{-1} for 2, 3 and 4% dispersions, respectively, indicating that the gelation proceeds faster with increasing concentration of curdlan.

In conclusion, curdlan aqueous dispersions form thermo-reversible gels by heating it in the range 60–

100°C, and a further heating at higher temperatures leads to the formation of thermo-irreversible gels. The critical transition temperature should, however, depend on concentration, molecular weight of curdlan, and the difference between the thermo-reversibility which is not absolute but relative. Gels formed by heating at temperatures from 60 to 100°C are partially thermo-reversible. Further structural studies should be performed.

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