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Effects of pH and DMSO content on the thermal and rheological properties of high methoxyl pectin–water gels

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It was shown that high methoxyl pectin forms a gel with a high elastic modulus in the presence of dimethyl sulfoxide (DMSO). Differential scanning calorimetry (DSC) and dynamic viscoelastic measurements were carried out for high methoxyl pectin–DMSO gels prepared at pH 2·4 and 6·86. Elastic moduli of pectin gels as a function of DMSO content showed a maximum around 0·2 mole fraction (mf) DMSO; however, acidic gels (pH = 2·4) needed less DMSO to form a gel and the elastic moduli for the acidic gels were larger than those for neutral gels (pH = 6·86). Endothermic peaks accompanying the gel-to-sol transition observed in heating DSC curves were sharpest for the acidic pectin gels with 0·168 mf DMSO and for neutral pectin gels with 0·277 mf DMSO. A small amount of DMSO, less than 0·3 mf, promotes gel formation, whilst excessive amounts of DMSO lower the gelling ability. The mean end-to-end distance r_m of chains which connect junction zones decreases, and the bonding energy ε increases with increasing DMSO content up to 0·277 mf; then r_m increases and ε decreases beyond this DMSO content. Judging from the heat absorbed on forming 1 mole of junction zones, junction zones in acidic gels are more heat-resistant than those in neutral gels. The electrostatic repulsion between carboxylate ions is reduced at lower pHs because of the suppression of the dissociation of carboxylic groups, hence the elastic modulus, the endothermic enthalpy accompanying gel-to-sol transition, and gel-to-sol transition temperature increase with decreasing pH.

INTRODUCTION

Pectin is a block copolymer consisting of D-galacturonic acid and its methyl ester. Some L-rhamnosyl groups are 1,2-linked and these substituents make the backbone irregular. Pectin whose methoxyl content exceeds 50% is called high methoxyl pectin and forms a gel in the presence of sugars at acidic pH lower than 3. The ester groups are the hydrophobic parts, while the other areas of galacturonic acid are the hydrophilic parts of the high methoxyl pectin. The mechanism of gel formation in high methoxyl pectin molecules is believed to be governed by both hydrogen bonds and hydrophobic interaction (Walkinshaw & Arnott, 1981). It is reported that electrostatic repulsion of pectin molecules is shielded by reducing negative charges at lower pH, which promotes aggregation and gelation (Morris *et al.*, 1980; Oakenfull & Scott, 1984; Clark & Ross-Murphy, 1987).

It is well known that dimethyl sulfoxide (DMSO) interacts strongly with water and changes the structure of water (Cowie & Toporowski, 1961; Rasmussen & MacKenzie, 1968), hence it affects the gel properties of agarose (Watase & Nishinari, 1988) and poly(vinyl

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alcohol) (Watase & Nishinari, 1989). Study of the effects of pH and DMSO on thermal and rheological properties of pectin-water systems will be useful in understanding the gelation mechanism and the structure of junction zones.

EXPERIMENTAL

Materials

Genu pectin of citrus origin (Batch No. 138050) from the Copenhagen Pectin Factory (Little Skensved, Denmark) was dissolved in water, and precipitated by ethanol. The precipitate was washed several times by 80–90% ethanol, and kept in ethanol overnight and then freeze-dried. The methoxyl content was 64%. The purified material was dissolved in distilled water, and then moulded into a cylindrical shape of 20 mm diameter and 30 mm in length for rheological measurements. A lower pH (2·4) gel and a neutral pH (6·86) gel were prepared with and without citric acid, respectively. The pH of the gels was measured at 15–18°C using a pH meter HM-7A from Toa Electronics Ltd, Tokyo, Japan.

Measurements

Cylindrical gels were subjected to longitudinal vibrations at a frequency of 2 Hz and an amplitude of 100 μm using a Rheolograph Gel (Toyo Seiki Seisakusho Ltd, Tokyo, Japan). The storage Young's modulus E' and mechanical loss tangent tanδ were determined at each temperature, after 20 min equilibration at that temperature.

A sample (15–50 mg) of the pectin gel was weighed into a 70 μl silver pan for differential scanning calorimetry (DSC) and hermetically sealed. The temperature was then raised at 2°C/min.

The details of the experimental procedures have been described previously (Watase & Nishinari, 1988; Watase & Nishinari, 1989).

RESULTS AND DISCUSSION

Figures 1(a)–(e) shows the temperature dependence of the storage Young's modulus E' and tanδ for pectin gels prepared at pH 2·4 with various pectin concentrations and DMSO contents. E' decreased gradually, and beyond a certain temperature it began to decrease rapidly with increasing temperature. The temperature T_0 at which E' begins to decrease rapidly and tanδ begins to increase rapidly shifted to higher temperatures up to 0·277 mole fraction (mf) DMSO and then shifted to lower temperatures with increasing DMSO content.

The temperature dependence of the elastic modulus of this type of thermoreversible gel has been explained on the basis of a reel–chain model (Nishinari *et al.*, 1985). The flexible chains were assumed to be Langevin chains, i.e. the ratio of the end-to-end distance of the chain to the extended chain length is given by the Langevin function, so that the case of strong stretching of chains can be taken into account. Both ends of each chain are bound to the junction zones by weak secondary interactions such as hydrogen bonds. The number of captured segments depends upon temperature, i.e. the segments are released from the junction with increasing temperature. According to this model, the temperature dependence as shown in Fig. 1 may be explained in terms of bonding energy ε, the mean end-to-end distance r_m of chains which connect junction zones, and the ceiling number ν, i.e. the upper limit of the number of segments which can be liberated from junction zones just before the transition from gel to sol occurs. According to this treatment, the elastic modulus E increases monotonically for large values of bonding energy ε, mean end-to-end distance r_m, or the ceiling number ν, while E decreases monotonically for small values of these three parameters. Rubber is a typical example of the first family which shows monotonic increasing behaviour; pectin shown in Fig. 1 as well as carrageenan, gelatin, and many other thermoreversible gels belong to the second family, which shows the monotone decreasing behavior. The mean end-to-end distance r_m may become shorter at higher concentrations in concentrated pectin gels by newly created junction zones through hydrogen bonds and hydrophobic interactions.

Initially, the storage modulus E' increased with increasing DMSO content at a constant temperature as shown in Fig. 2, and therefore r_m should decrease with increasing DMSO content up to 0·277 mf. Thus, the shift in the temperature T_0 at which E' began to decrease rapidly to higher temperatures with increasing DMSO content should be attributed to an increase in the bonding energy ε or the ceiling number ν. In contrast, E' decreased with increasing DMSO content at a constant temperature beyond 0·277 mf, indicating that r_m increased with increasing DMSO content. The shift of T_0 to lower temperatures with increasing DMSO content beyond 0·277 mf suggests that ε or ν should decrease with increasing DMSO content beyond 0·277 mf because the increase in r_m shifts T_0 to higher temperatures.

The gradients of the straight lines in the double logarithmic plot of the concentration and E' of pectin gels with various DMSO contents prepared at pH 2·4 and 25°C are about 2·2, irrespective of DMSO content. There have been many reports that the elastic modulus of gels is proportional to the concentration squared (Clark & Ross-Murphy, 1987). Although this is very close to the value of 2·25 predicted by a scaling treatment (de Gennes, 1979), this does not conclusively

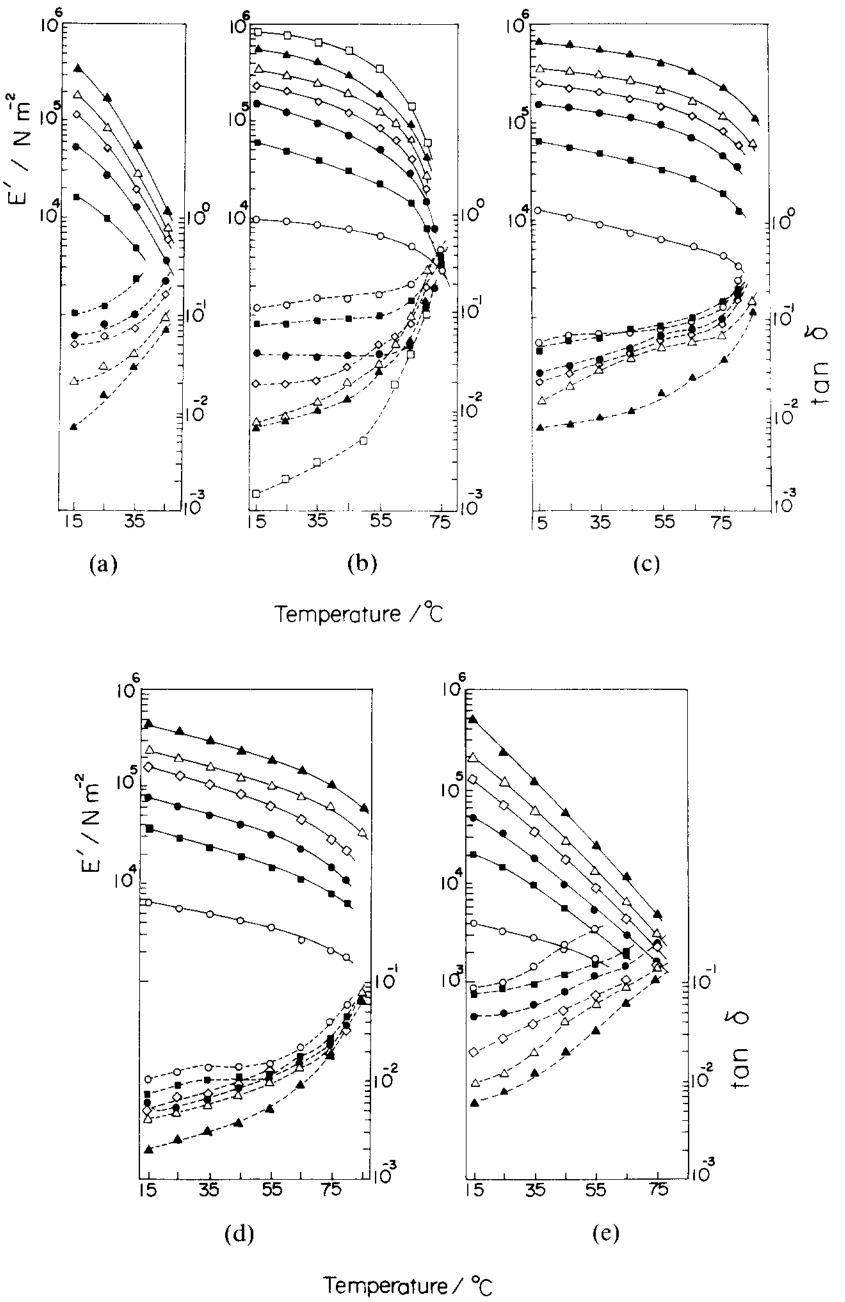


Fig. 1. Temperature dependence of storage Young's modulus E' (solid line) and mechanical loss tangent tanδ (broken line) for pectin gels with various DMSO contents: (a) 0·095 mf; (b) 0·168 mf; (c) 0·277 mf; (d) 0·352 mf; (e) 0·450 mf. Pectin concentrations: ○, 1%; ■, 2%; ●, 3%; ◇, 4%; △, 5%; ▲, 7%; □, 9%; pH = 2·4.

support the scaling treatment. The concentration dependence of pectin gels should be explored in more detail over a wider concentration range. Strictly speaking, the double logarithmic plot of the pectin concentration and E' of pectin gels with various DMSO contents prepared at pH 2·4 and 25°C showed a slightly convex curve: the gradient of the plot is steeper in the lower concentration range. This was explained by modified rubber elasticity theories (Oakenfull, 1984; Clark & Ross-Murphy, 1985). The gradient of the double logarithmic plot of E' against the polymer concentration was significantly larger, around 2·5–4·0 for pectin gels at pH 6·86. This indicates that the critical concentration for gel formation is far lower for lower-pH gels than for higher-pH gels.

Figure 2 shows the DMSO content dependence of E' of pectin gels prepared at pH 2·4 and 6·86 and at 25°C for various concentrations. E' as a function of DMSO content shows a maximum at around 0·25 mole fraction (mf) DMSO at pH 2·4 and 0·30 mf DMSO at pH 6·86 respectively, irrespective of pectin concentration. It seems that DMSO enhances the gelling ability of pectin up to 0·22 mf; however, the excessive DMSO inhibits the gelation. Since the interaction between

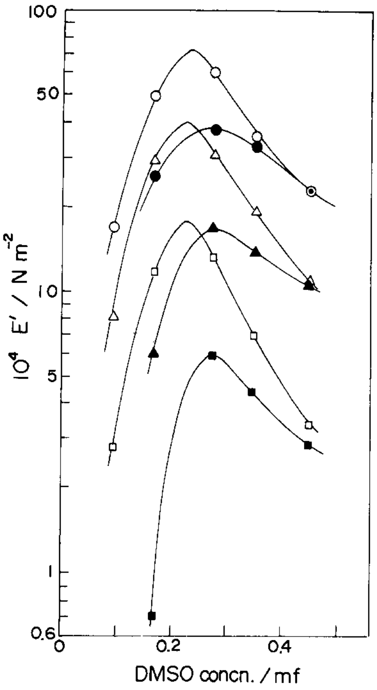
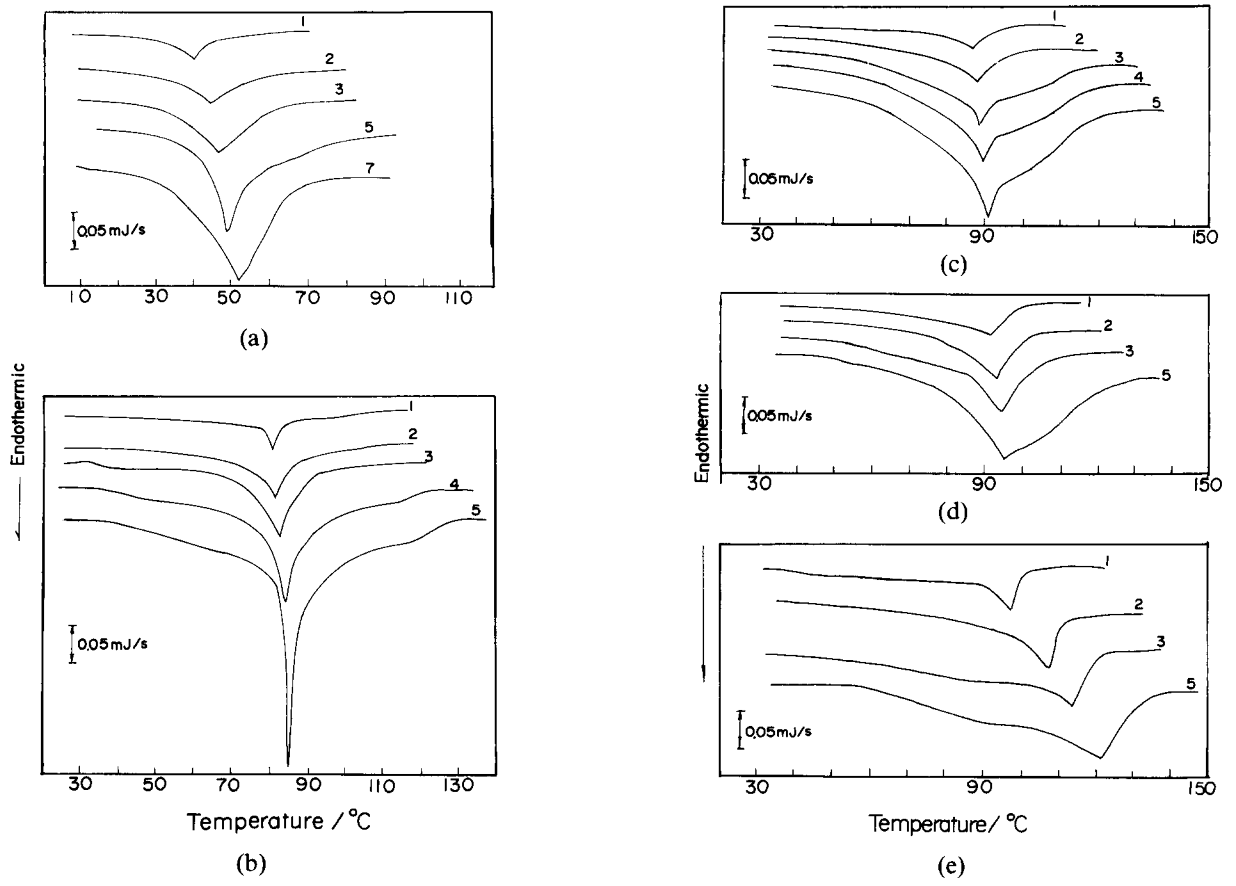


Fig. 2. Storage Young's modulus E' at 25°C as a function of DMSO content for various pectin concentrations at pH 2·4 (open symbols) and 6·86 (closed symbols). Pectin concentrations: □, ■, 3 wt %; △, ▲, 5 wt %; ○, ●, 7 wt %.

water and DMSO is very strong, the mixture of water and DMSO shows peculiar characteristics. The structure of a hydrated compound of DMSO has been shown to be $(CH_3)_2SO.2H_2O$ by thermodynamic and NMR investigations (Cowie & Toporowski, 1961). However, other research has shown it to be $(CH_3)_2SO.3H_2O$ (Rasmussen & MacKenzie, 1968). Note that 0·277 mf is just between these two. On adding DMSO to pectin-water systems, which do not normally form a gel, gelation occurs when the DMSO content exceeds a certain value. The solubility of polysaccharides will increase beyond the DMSO mole fraction 0·277 with increasing DMSO content because the quantity of non-hydrated DMSO increases.

Around 0·22 mf DMSO content, the decrease in E' with increasing temperature was suppressed, as shown in Fig. 1(b), (c) and (d), but at lower DMSO content (0·095 mf, Fig. 1 (a)) or higher DMSO content (0·45 mf. Fig. 1(e)), E' showed a rapid decrease with increasing temperature.

Figure 3(a)–(e) shows the heating DSC curves of pectin gels prepared at pH 2·4 with various pectin concentrations and with various DMSO contents. Endothermic peaks accompanying gel-to-sol transition shifted to higher temperatures with increasing



(a)

(b)

(c)

(d)

(e)

Fig. 3. Heating DSC curves for pectin gels of various concentrations at pH = 2·4. DMSO content: (a) 0·095 mf; (b) 0·168 mf; (c) 0·277 mf; (d) 0·352 mf; (e) 0·450 mf. Figures beside each curve represent pectin concentrations in wt %.

pectin concentration, but this shift is more pronounced in a gel with lower DMSO content (0·095 mf, Fig. 3(a)) or in a gel with higher DMSO content (0·45 mf, Fig. 3(e)). Endothermic peak temperature will be denoted as the melting temperature T_m hereafter.

The Eldridge–Ferry plot (Eldridge & Ferry, 1954) of the logarithm of pectin concentration against the inverse of the melting temperature T_m in K for pectin gels with different DMSO contents results in straight lines. According to Eldridge and Ferry, the gradient of these straight lines is proportional to the heat absorbed on forming 1 mol of junction zones, ΔH_m; ΔH_m as a function of DMSO content showed a maximum around 0·22 mf DMSO, as shown in Fig. 4. Since the bonding energy ε in the reel-chain model is proportional to ΔH_m, the maximum in T_0 at 0·277 mf DMSO can be explained in terms of the bonding energy. It is not necessary to invoke changes in ν.

The maxima of the melting temperatures T_m and of the endothermic enthalpies ΔH for pectin–water–DMSO gel around 0·22 mf DMSO as a function of DMSO content may be interpreted as follows. The content of free water, which does not interact so strongly with DMSO, decreases with increasing DMSO content. Therefore, DMSO plays the part of a thickener so that the concentration of pectin may increase, and as a result, T_m and ΔH increase with increasing DMSO content. It is well known that many polysaccharide gels consist of junction zones that are somewhat crystalline and of long flexible chains surrounded by a solvent which is amorphous, as mentioned above. Thus, the gel is formed by a balance between crystallinity and solubility. This balance shifts to the solubility side at a content of DMSO higher than a certain value around 0·2 mf because of the high dissolving power of DMSO.

Figure 5(a)–(d) shows the temperature dependence of E' and $\tan\delta$ of pectin gels prepared at pH 6·86 with various pectin concentrations and with various DMSO contents. The general features are similar to those seen in pectin gels prepared at pH 2·4 (Fig. 1(a)–(e)). At each pectin concentration and DMSO content, E' of pectin gels prepared at pH 6·86 is far smaller than E' of pectin gels prepared at pH 2·4. The decrease in E' with

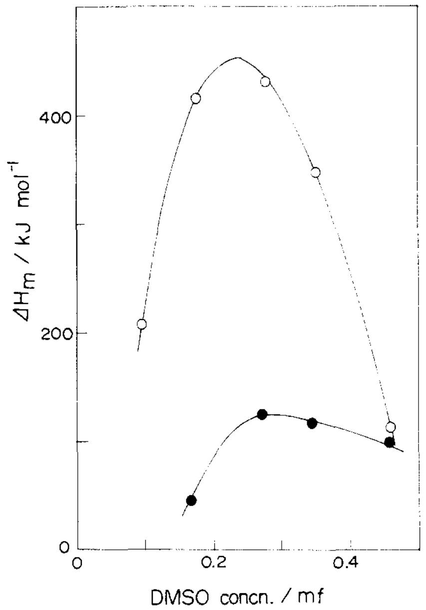


Fig. 4. The heat absorbed on forming 1 mole of junction zones ΔH_m for pectin gels at pH 2·4 (○) and 6·86 (●) as a function of DMSO content.

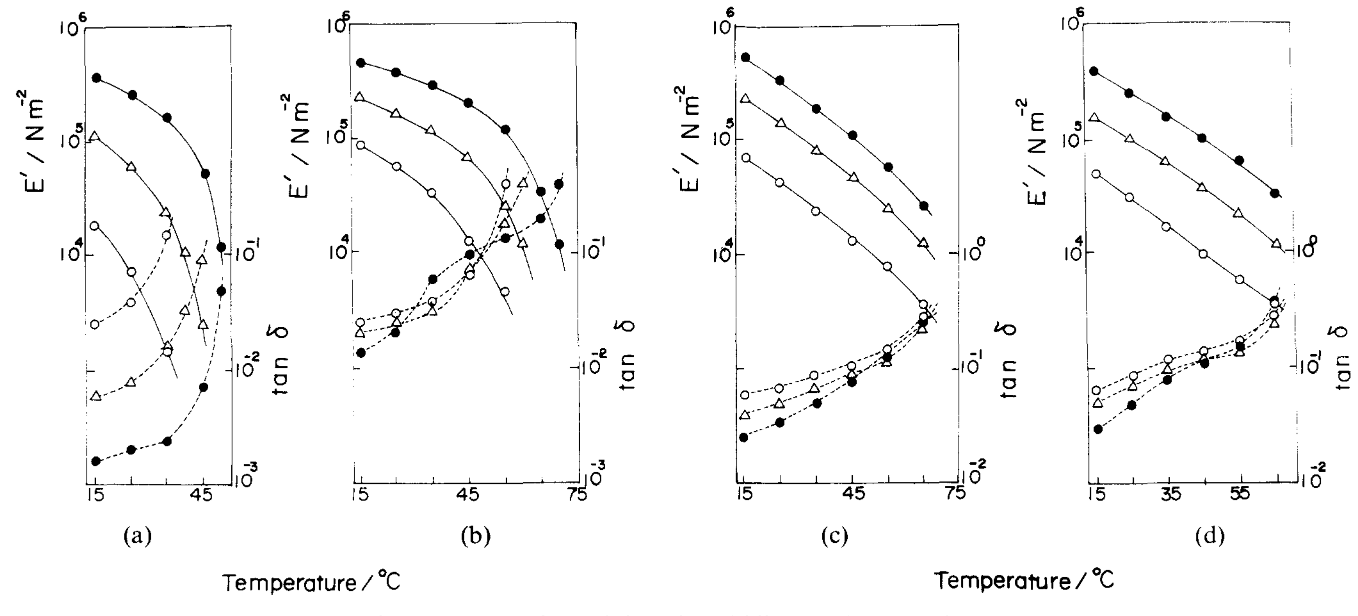


Fig. 5. Temperature dependence of storage Young's modulus E' (solid line) and mechanical loss tangent $\tan\delta$ (broken line) for pectin gels with various DMSO content: (a) 0·168 mf; (b) 0·277 mf; (c) 0·352 mf; (d) 0·450 mf. Pectin concentrations: ○, 3%; △, 5%; ●, 7%; pH = 6·86.

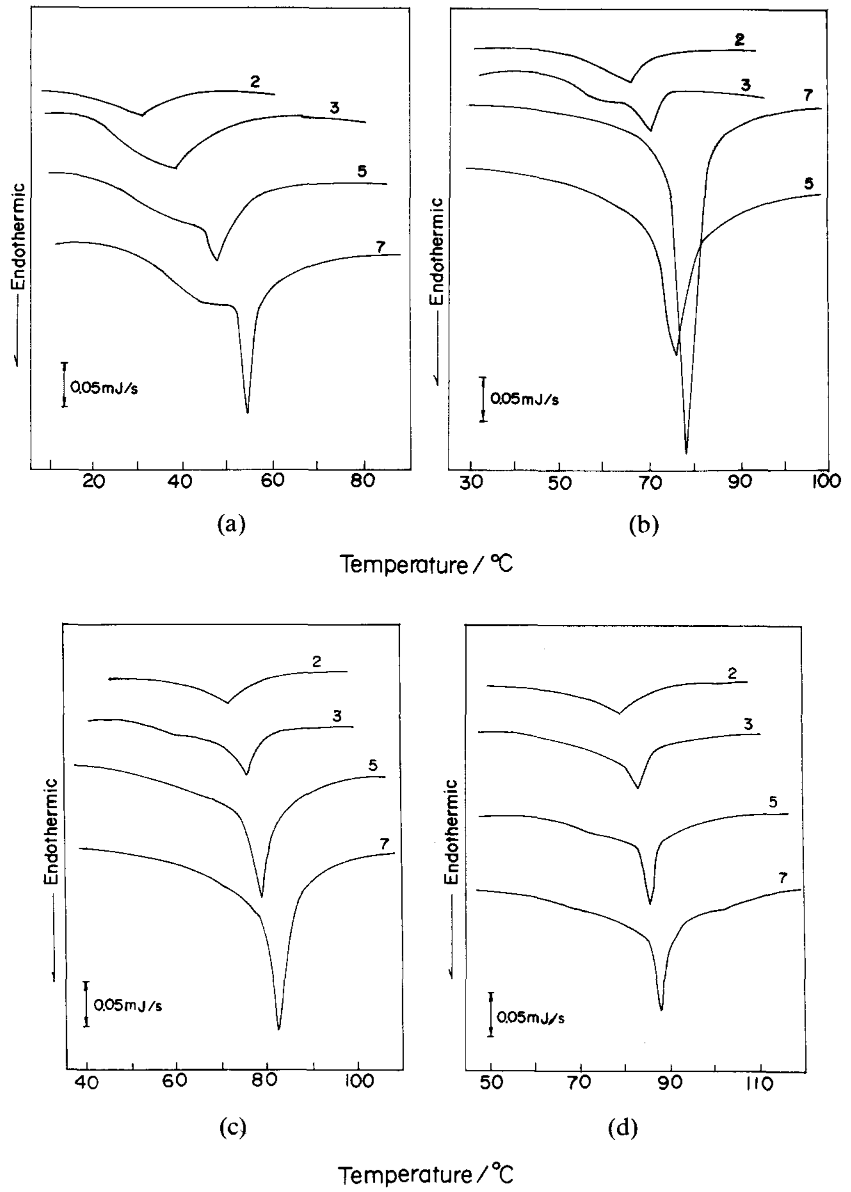


Fig. 6. Heating DSC curves for pectin gels of various concentrations at pH = 6·86. DMSO content: (a) 0·168 mf; (b) 0·277 mf; (c) 0·352 mf; (d) 0·450 mf; pH = 6·86. Single figures beside each curve represent the concentration of pectin in wt %.

increasing temperature is more conspicuous in higher pH gels than in lower pH gels. A pectin solution with a lower DMSO content (0·095 mf) at pH 6·86 does not form a self-supporting gel but only a sloppy gel, which is quite different from pectin gels prepared at pH 2·4 (Fig. 1(a)).

Figures 6(a)–(d), show the heating DSC curves of pectin gels prepared at pH 6·86 with various pectin concentrations and with various DMSO contents. The general features are again similar to those observed in pectin gels prepared at pH 2·4 (Fig. 3(a)–(e)). However, the melting temperature T_m was far lower than that for pectin gels prepared at pH 2·4. These endothermic peaks were sharpest in 0·277 mf DMSO pectin gels prepared at pH 6·86 while those prepared at pH 2·4 were sharpest in gels with 0·168 mf DMSO. The heat absorbed on forming 1 mole of junction zones, ΔH_m, determined from the Eldridge–Ferry plot for pectin gels prepared at pH 6·86 as a function of DMSO content, are shown in Fig. 4. As observed in pectin gels prepared at pH 2·4, ΔH_m showed a maximum around 0·22 mf DMSO; however, the absolute value of ΔH_m is far smaller than those for gels prepared at pH 2·4.

These findings suggest that junction zones in pectin gels prepared at pH 2·4 are more heat-resistant than those in pectin gels prepared at pH 6·86, and that pectin solutions at higher pH need more DMSO than those at lower pH to form a gel, although excessive amount of DMSO lowers the gelling ability. Pectin molecules will be extended by the electrostatic repulsion between carboxylate ions at higher pH. This will be reduced at lower pH because the dissociation of carboxylic groups is suppressed in the presence of abundant hydrogen ions. Therefore, the aggregation of pectin molecules is promoted at lower pH, leading to the increase in the number of junction zones, and thus also the elastic modulus, the endothermic enthalpy accompanying the gel-to-sol transition and the gel-to-sol transition temperature.

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