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Phase equilibria and gelation in gelatin/maltodextrin systems — Part III: phase separation in mixed gels

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Co-gels of potato maltodextrin Paselli SA-6 with gelatin were prepared by rapid quenching of mixed solutions from 90°C. At fixed setting temperature and fixed concentration of gelatin, the time required to form a self-supporting network showed an initial steady decrease with increasing concentration of SA-6 (as expected from polymer exclusion), but then increased dramatically before again decreasing. The interpretation of this behaviour as phase inversion from a gelatin-continuous network with SA-6 inclusions to a (more slowly-forming) SA-6 network with gelatin inclusions was confirmed by differential scanning calorimetry (showing both components melting separately, with no evidence of specific interaction), mechanical spectroscopy (showing that the mixed gel network was destroyed completely by melting of the gelatin component at low concentrations of SA-6, but only weakened at SA-6 concentrations above the inversion point) and by light microscopy (showing the expected changes in distribution of the two polymers).

In similar studies using the faster-gelling potato maltodextrin Paselli SA-2, microscopy and gel-melting profiles again showed phase-inversion from a gelatin-continuous network at low concentrations of SA-2 to a maltodextrin-continuous network at higher concentrations. Inversion, however, occurred at a lower concentration of maltodextrin than in the gelatin/SA-6 systems, and the accompanying change in gelation rate was confined to a sharp decrease in the dependence of gel-time on SA-2 concentration.

1 INTRODUCTION

The preceding paper (Kasapis *et al.*, 1993*b*) described the behaviour of potato maltodextrins Paselli SA-6 and SA-2 in the presence of gelatin at a temperature (45°C) where the individual polymers are stable as disordered coils in solution over long periods of time. The present work explores their behaviour at lower temperatures, where the gelatin and maltodextrin components can both form gel networks within the timescale of the experiments.

Mixed gel networks may be classified (Morris, 1986) into three types: interpenetrating, coupled and phase-separated. Interpenetrating networks represent the simplest situation, where the two components gel separately, forming independent network structures. Both networks span the entire system, but any interaction between them is solely topological. Coupled networks, by contrast, involve direct association between the two polymers. The most common mechanism of coupling is by polyanion–polycation association (Stainsby, 1980), but covalent bonding (via ester or amide linkages) has been demonstrated in a few mixed biopolymer systems (McDowell, 1970; McKay *et al.*, 1985; Oates *et al.*, 1987*a*, *b*), and in a very limited number of others (notably the mixed gels formed between alginate and pectin at acid pH and the xanthan/galactomannan or glucomannan systems) there seems clear evidence of

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formation of heterotypic co-operative junctions, analogous to the junction zones in normal single-component polysaccharide gels (Morris, 1990). Finally, phase-separated networks may be regarded as composites where one of the components forms a continuous network across the entire system and the other serves as a gel filler (Tolstoguzov, 1986).

The results obtained in the present investigation demonstrate that, as would be anticipated from their primary structures, there is no direct coupling between gelatin and maltodextrin in mixed gels. It also appears that the gels are of the phase-separated type, with no indication of bicontinuous, interpenetrating networks in any of the systems studied, and that phase inversion from a gelatin-continuous network with maltodextrin inclusions to a maltodextrin-continuous network with gelatin as the dispersed phase occurs over a very narrow range of composition.

2 EXPERIMENTAL

Gelatin and maltodextrin samples were identical to those used in the first two investigations of this series (Kasapis *et al.*, 1993*a*, *b*): second extract limed-ossein gelatin (LO-2) and Paselli maltodextrins SA-6 and SA-2. Binary systems were prepared by first dissolving the individual components at elevated temperatures (Kasapis *et al.*, 1993*a*) and then mixing appropriate amounts of the stock solutions at 60°C.

The rate of formation of gelatin/maltodextrin mixed gels was monitored by visual estimation of the time (t_g) required for the development of a self-supporting network, after rapid quenching of a mixed solution from high temperature to the required observation temperature (Oakenfull & Scott, 1986, 1988). Differential scanning calorimetry (DSC) measurements were made on a Setaram microcalorimeter at a scan rate of 0·1°/min. Objective measurements of storage modulus (G') during gelation and melting were made on a Bohlin VOR rheometer, using parallel-plate geometry (15 mm radius; 1 mm separation) at 1% strain and a frequency of 1 Hz. Networks were left to develop for 25 000 s (about 7 h) at 5°C prior to melting. Finally, the distribution of gelatin and maltodextrin in mixed gels was visualised by interference contrast microscopy on a Leitz Ortholux II.

3 EVIDENCE OF PHASE-INVERSION FROM GEL-TIME MEASUREMENTS

The procedure used to estimate gel time by visual inspection was identical to that applied to maltodextrin alone (Kasapis *et al.*, 1993*a*). Because of the time-consuming nature of these experiments, the investigation was confined to a few representative concentrations of gelatin (LO-2) at temperatures where gel formation occurs on a reasonable timescale, with systematic variation of maltodextrin concentration in each case. The results obtained using SA-6 as the maltodextrin component are listed in Table 1.

The most spectacular behaviour was observed for a gelatin concentration of 5% (w/w) at 25°C. As shown in Fig. 1, the gel time showed a steady decrease with increasing maltodextrin concentration, from 40 min for gelatin alone to about 16 min in the presence of 15% (w/w) SA-6. This is an expected consequence of thermodynamic incompatibility between the two polymers driving the gelatin component to the more compact ordered form. On slight further increase in maltodextrin concentration, however, there was a dramatic decrease

Table 1. Time (t_g; min) required for formation of a self-supporting network from mixed solutions of LO-2 at the concentrations (% w/w) and temperatures shown, in the presence of increasing concentrations (c; % w/v) of SA-6

2%, 10°C		5%, 25°C		7%, 10°C		7%, 20°C		9%, 20°C	
c	t_g	c	t_g	c	t_g	c	t_g	c	t_g
0·0	14·7	0·0	40	0·0	6·3	0·0	10·5	0·0	8·9
4·0	12·6	4·0	30	5·0	5·9	5·0	9·4	6·0	8·3
6·0	11·8	8·0	21	10·0	5·3	10·0	8·7	12·0	7·8
8·0	10·6	12·0	18	15·0	4·9	15·0	8·2	17·0	6·7
10·0	10·1	15·0	16	16·0	4·8	16·0	7·8	18·0	6·3
12·0	9·8	16·5	800	17·0	4·7	17·0	7·6	19·0	14·7
13·0	9·6	17·5	750	18·0	9·0	18·0	16·2	20·0	9·1
14·0	9·4	20·0	400	19·0	5·7	19·0	11·6	22·0	8·0
14·5	9·1	22·5	235	20·0	5·0	20·0	10·4	24·0	6·3
15·0	41·7	25·0	165	22·0	4·1	22·0	9·0		
16·0	28·9	27·5	115						
17·0	21·6	30·0	95						
18·0	17·3								
19·0	14·1								
20·0	12·9								

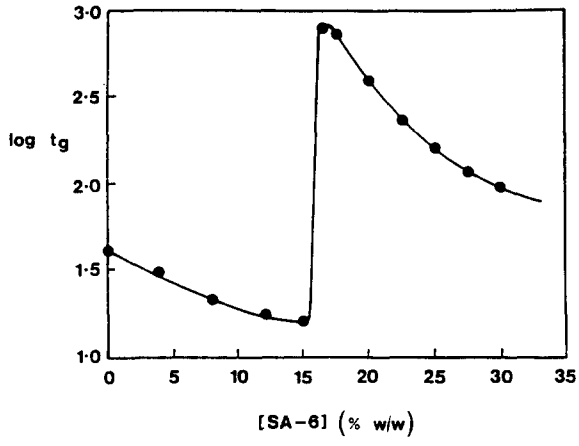


Fig. 1. Time (t_g, in min) at 25°C required for the formation of a self-supporting network from mixed solutions of 5% (w/w) LO-2 with varying concentrations of SA-6.

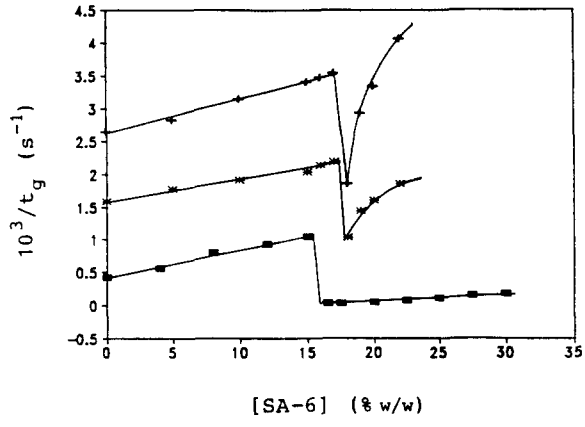


Fig. 2. Variation in gelation rate (reciprocal of gel time, t_g) with increasing concentration of SA-6 in mixed solutions with: 7% (w/w) LO-2 at 10°C (+); 7% (w/w) LO-2 at 20°C (*); and 5% (w/w) LO-2 at 25°C (■).

in the rate of gelation, with a gel time of over 13 h at 16·5% (w/w) SA-6. Similar, though less extreme, destructive interference with gelatin gelation has been observed previously with high concentrations of dextran (Tolstoguzov *et al.*, 1974) or high DE (10-13) maltodextrin (Marrs, 1982). The novel feature of the results reported here is that with a continued increase in SA-6 concentration the gel time again decreased steeply. A similar sharp increase in gel time at a critical concentration of SA-6, flanked by a steady decrease over lower and higher ranges of concentration, was observed (Table 1) at all other combinations of temperature and gelatin concentration studied.

Figure 2 shows some representative plots of the same data expressed as rate of gelation (i.e. as the reciprocal of t_g). The simple effect of polymer incompatibility in initial acceleration of gelatin gelation is evident in the linear increase in rate with increasing concentration of maltodextrin, and the sharp discontinuity at a critical concentration is again clear. An obvious interpretation which, as described later, is strongly supported by evidence from other techniques, is that the discontinuity corresponds to phase inversion from a gelatin-continuous network with SA-6 inclusions to a network in which the more slowly gelling SA-6 component forms the supporting, continuous phase, with gelatin dispersed as discrete particles of 'microgel'. This interpretation is fully consistent with the evidence presented in Fig. 3, where the dependence of gel time on concentration at fixed temperature (10°C) is shown for SA-6 alone, and in the presence of 2% and 7% (w/w) LO-2. The concentration-dependence in the mixed systems above the point of discontinuity has obvious parentage in the behaviour of SA-6 in isolation, with the presence of increasing amounts of gelatin causing progressive acceleration of SA-6 gelation in an analogous way to the acceleration of gelatin gelation by SA-6 at concentrations below the point of phase inversion.

Similar studies of gel time in mixtures of LO-2 with SA-2 rather than SA-6 also showed a discontinuity in the dependence of t_g on maltodextrin concentration (Fig. 4), but only as a slight decrease in slope rather than the sharp increase in t_g observed for SA-6. This can be readily explained by the much faster gelation of SA-2 (Kasapis *et al.*, 1993*a*) giving gel times comparable to those of gelatin, rather than the much longer times required for network formation by SA-6.

To test the validity of the interpretation of gel-time data in terms of phase inversion, the nature of the mixed gel structure was investigated by three different experimental approaches. First, the thermal changes accompanying gel melting were characterised by DSC. Secondly, the temperature-course of gel melting was monitored by mechanical spectroscopy. Finally, the ultrastructure of the intact gels was visualised by light microscopy.

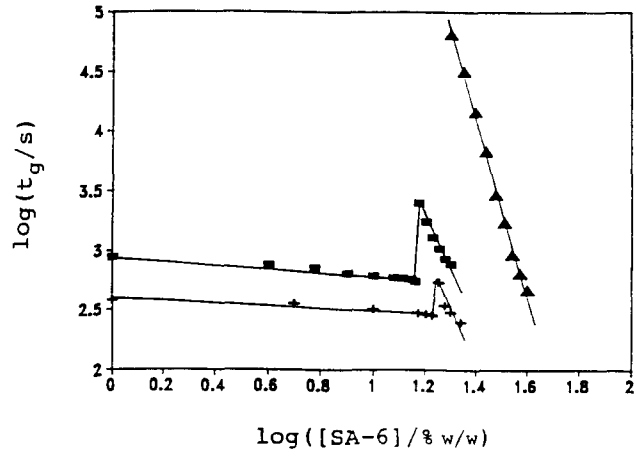


Fig. 3. Concentration-dependence of gel time (t_g; 10°C) for SA-6 alone (▲), and in the presence of LO-2 at concentrations of 2% (w/w) (■) and 7% (w/w) (+).

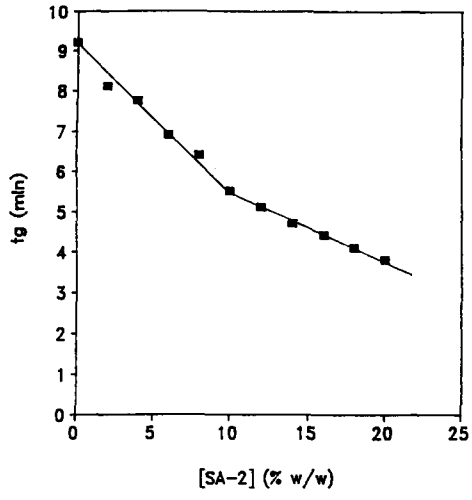


Fig. 4. Variation in gel time (t_g; 20°C) for 4% (w/w) LO-2 in the presence of increasing concentrations of SA-2.

4 CHARACTERISATION BY DSC

The DSC traces reproduced as parts (a) and (b) of Fig. 5 show the endotherms observed on melting of single-component gels of SA-6 (20% (w/w)) and LO-2 (2% (w/w)), with T_m values of about 59°C and 23°C, respectively. The corresponding melting profiles (obtained under identical experimental conditions) for mixed gels of 2% (w/w) LO-2 and 10% and 20% (w/w) SA-6 are reproduced as parts (c) and (d) of Fig. 5. These combinations correspond to SA-6 concentrations well below and well above the critical value of about 14·5% (w/w) at which the sharp increase in gel time is observed (Table 1). In both cases the thermograms show two transitions that correspond closely in position and general band-form to those of the individual components in isolation. The obvious conclusions from this evidence are:

(i) that both components form gel networks, and
(ii) that there are no specific interactions between them (i.e. no formation of heterotypic junctions).

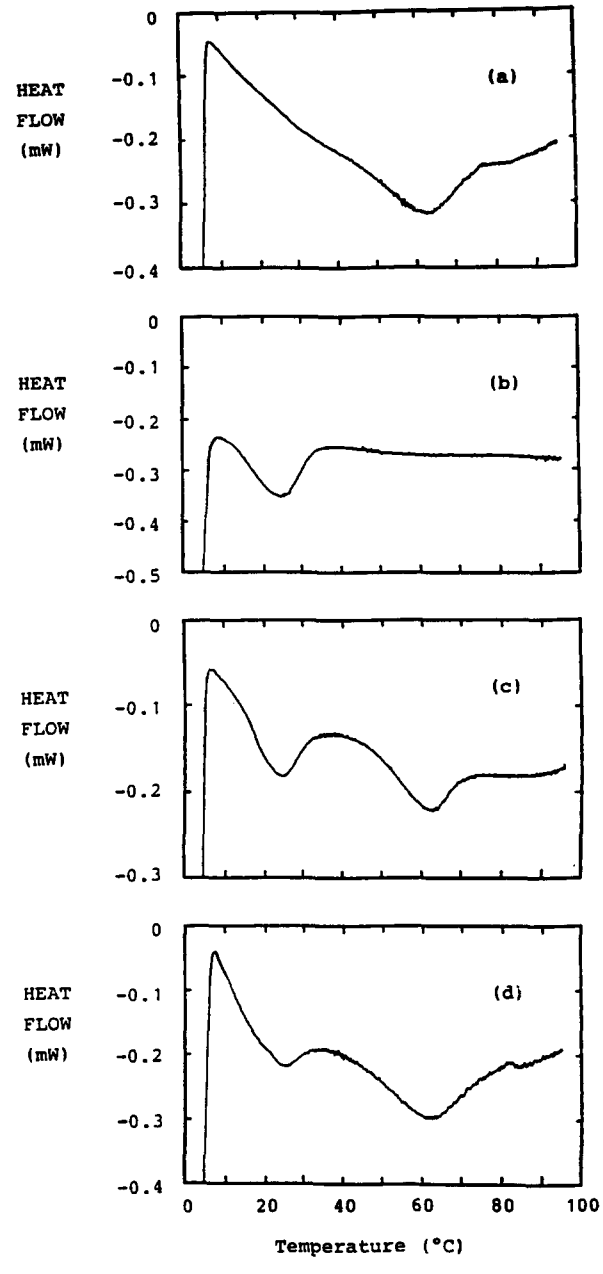


Fig. 5. Melting endotherms from DSC heating scans (5–95°C; 0·1°C/min) for: (a) 20% (w/w) SA-6; (b) 2% (w/w) LO-2; (c) 2% (w/w) LO-2 + 10% (w/w) SA-6; (d) 2% (w/w) LO-2 + 20% (w/w) SA-6. The apparent sharp peak at the low-temperature end of each trace comes from the initial thermal imbalance of the calorimeter.

5 TEMPERATURE-COURSE OF GEL MELTING

Figures 6 and 7 show the melting profiles of the same gels (2% (w/w) LO-2 with 10% and 20% SA-6) as monitored by small-deformation oscillatory measurements of G' and G''. At the lower concentration of SA-6, below the sharp increase in t_g, the network melts out completely (Fig. 6) over the temperature range of the DSC melting transition for the gelatin component (Fig. 5(b)). At the higher concentration of SA-6, above the inversion point, melting of the gelatin component is accompanied by a reduction in moduli (Fig. 7), but the gel remains intact until the higher temperature range associated with melting of the SA-6 network.

This behaviour is, of course, entirely consistent with the postulate that at lower concentrations of SA-6 the gelatin network forms the continuous, supporting phase (with consequent complete loss of cohesion when it melts), whereas at higher concentrations of SA-6, above the inversion point, the gelatin is dispersed as a discontinuous 'filler' in the maltodextrin-continuous matrix, so that its melting weakens the gel but does not destroy

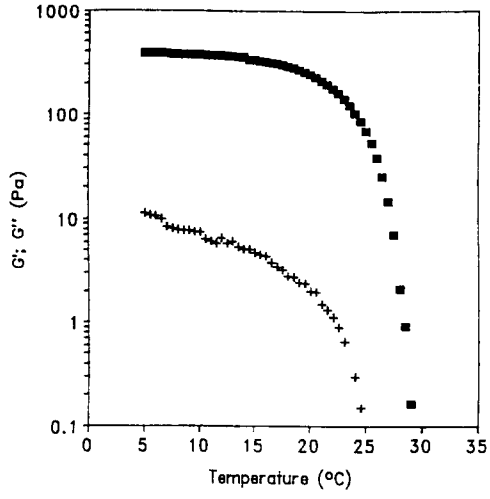


Fig. 6. Temperature-dependence of G' (■) and G'' (+) on thermal melting of a mixed gel of 2% (w/w) LO-2 + 10% (w/w) SA-6 set for 25 ks (about 7 h) at 5°C.

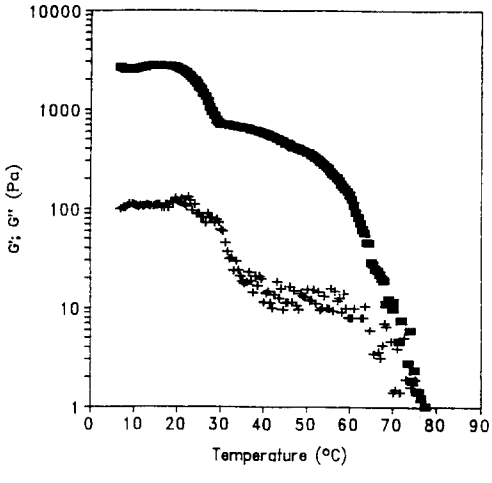


Fig. 7. Temperature-dependence of G' (■) and G'' (+) on thermal melting of a mixed gel of 2% (w/w) LO-2 + 20% (w/w) SA-6 (after 25 ks at 5°C).

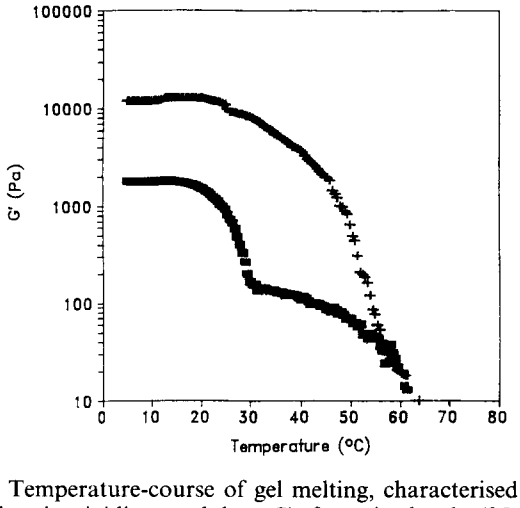


Fig. 8. Temperature-course of gel melting, characterised by a reduction in rigidity modulus, G', for mixed gels (25 ks at 5°C) of 2% (w/w) LO-2 with SA-6 at concentrations of 15% (w/w) (■) and 27·5% (w/w) (+).

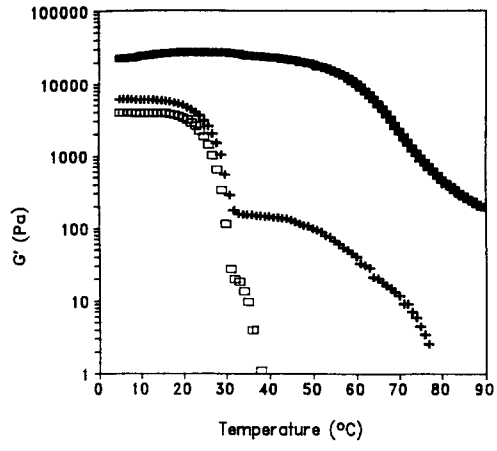


Fig. 9. Temperature-course of gel melting, characterised by a reduction in G', for mixed gels (25 ks at 5°C) of 4% (w/w) LO-2 with SA-2 at concentrations (% w/w) of 8 (□), 10 (+) and 22 (■).

it. As expected from this interpretation, the weakening is very evident (Fig. 8) at an SA-6 concentration of 15% (w/w) (just above the critical concentration of 14·5% for phase inversion) and barely detectable at much higher concentration (27·5% (w/w) SA-6) where the maltodextrin network dominates the overall mechanical properties.

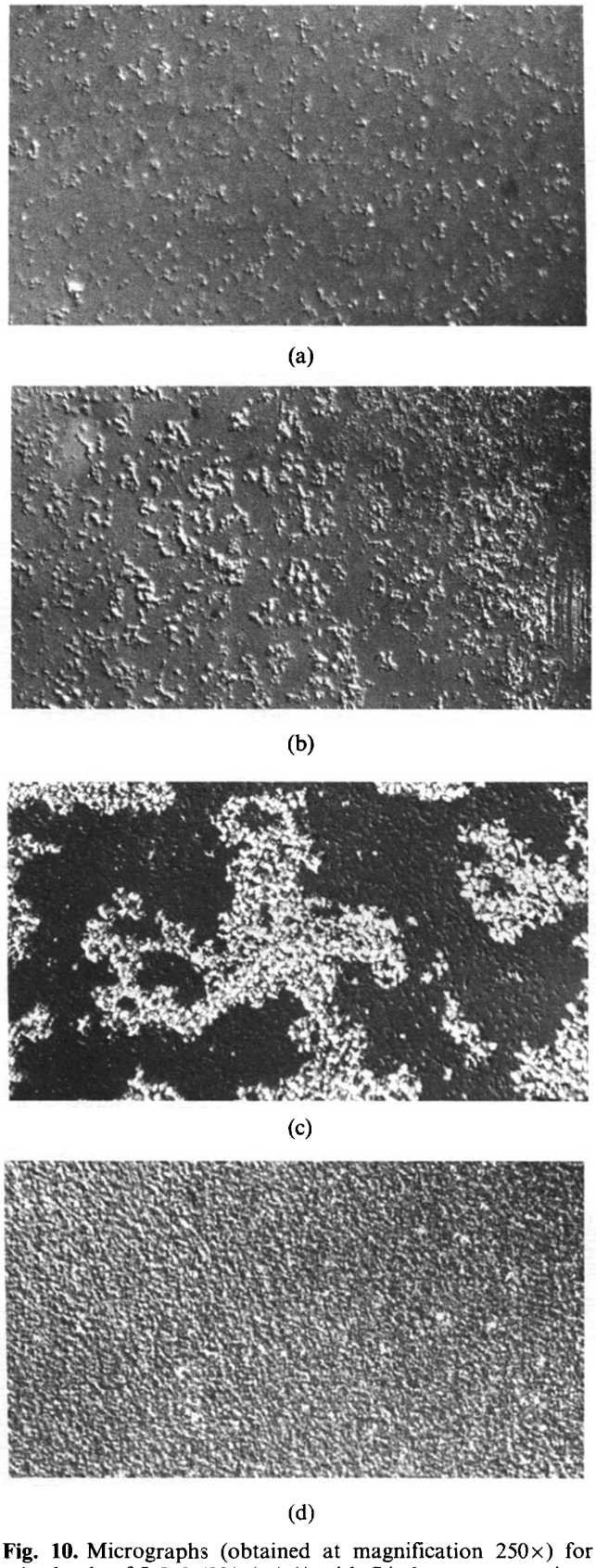
Figure 9 shows corresponding melting profiles for 4% (w/w) LO-2 in combination with SA-2, at maltodextrin concentrations (w/w) below (8%), at (10%) and well above (22%) the discontinuity in the concentration-dependence of gel time shown in Fig. 4. There is a clear progression of behaviour, with melting of the gelatin component causing complete loss of gel structure at 8% SA-2, very substantial weakening at 10%, and barely detectable change at 22%.

Comparison of Figs 7, 8 and 9 also highlights a significant difference between the two maltodextrin samples. The systems shown in Figs 7 and 8, where SA-6 forms the continuous phase, melt out completely below 80°C, at SA-6 concentrations as high as 27·5% (w/w) (Fig. 8), whereas the continuous network of SA-2 at 22% (w/w) (Fig. 9) retains appreciable moduli at the highest temperature accessible on the rheometer (90°C). The greater thermal stability of the SA-2 network is, of course, consistent with its higher chainlength giving rise to more stable ordered structures.

6 LIGHT MICROSCOPY

As final confirmation of phase inversion between low and high concentrations of maltodextrin at fixed gelatin concentration, the distribution of the two components within the mixed gel network was visualised directly by light microscopy, using interference-contrast optics.

Figure 10 shows the micrographs obtained for 2%



(a)

(b)

(c)

(d)

Fig. 10. Micrographs (obtained at magnification 250×) for mixed gels of LO-2 (2% (w/w)) with SA-6 at concentrations (% w/w) of (a) 5, (b) 10, (c) 15 and (d) 20. The abrupt increase in t_g for this system occurs at about 14·5% (w/w) SA-6. Black and white areas correspond, respectively, to gelatin and maltodextrin.

(w/w) LO-2 in combination with 5, 10, 15 and 20% (w/w) SA-6. The gelatin and maltodextrin components appear as black and white areas, respectively, because of their different phase-shifts. The progression from a gelatin-continuous network with (micron-sized) particulate inclusions of maltodextrin to a maltodextrin network with (similarly-sized) inclusions of gelatin is obvious. At 15% SA-6, which is very close to the discontinuity in concentration-dependence to t_g (about 14·5%), resolution of the two polymers into separate phases can be seen very clearly, with large agglomerates of maltodextrin separated by correspondingly large areas that seem essentially devoid of maltodextrin. The size of the agglomerates is of the order of 0·1 mm, which corresponds closely to the size of the maltodextrin particles precipitated from solution at higher temperature (45°C; Kasapis *et al.*, 1993*b*).

Similar characterisation (Fig. 11) of SA-2 in combination with 4% (w/w) LO-2 at maltodextrin concentration below (6%), just above (12%) and well above (18%) the discontinuity in concentration-dependence of t_g (10% (w/w); Fig. 4) again shows a clear progression from a gelatin-continuous to a maltodextrin-continuous network.

7 DISCUSSION AND CONCLUSIONS

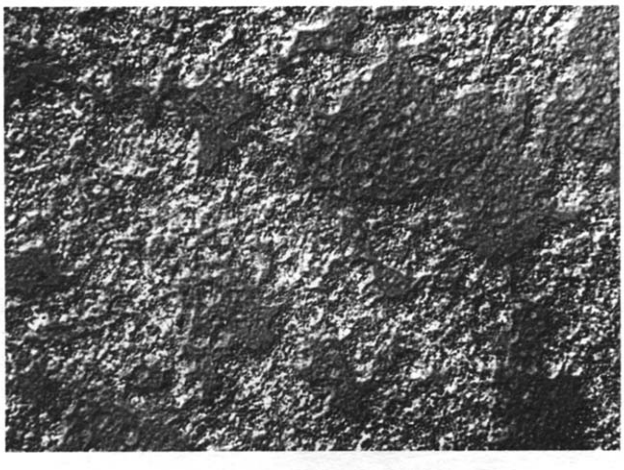
Comparison of DSC heating scans (Fig. 5) for gelatin and maltodextrin in single-component gels and in binary co-gels argues strongly against any direct coupling of the two polymers. The obvious interpretation of the observed behaviour is that each component forms its own network within the mixed gel.

In the preceding sections, we have interpreted our results in terms of resolution into a continuous and a discontinuous phase, but have not explicitly addressed the possibility of bicontinuous systems with the individual networks interpenetrating one another. It is obvious, however, that at low concentrations of maltodextrin, below the discontinuities shown in Figs 1–4, the maltodextrin network cannot span the entire system, since melting of the gelatin component is sufficient to eliminate long-range cohesion (Figs 6 and 9).

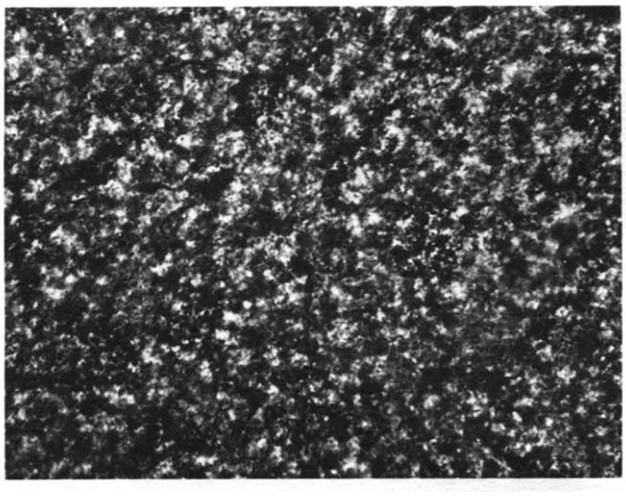
The evidence that the gelatin network becomes discontinuous at higher concentrations of maltodextrin is less direct. It does, however, seem reasonable to expect that if the gelatin network did remain continuous, with the maltodextrin network building in alongside it, the rate of initial gel formation should continue to follow the trends observed at lower concentrations of maltodextrin, rather than showing the abrupt changes observed experimentally (Figs 1–4).

The main conclusion from this investigation is therefore that gelatin/maltodextrin co-gels are of the phase-separated type, with phase-inversion from gelatin-

(a)



(b)



(c)

Fig. 11. Micrographs (conditions as in Fig. 10) for mixed gels of LO-2 (4% (w/w)) with SA-2 at concentrations (% w/w) of (a) 6, (b) 12, and (c) 18. The discontinuity in t_g for this system occurs at about 10% (w/w) SA-2.

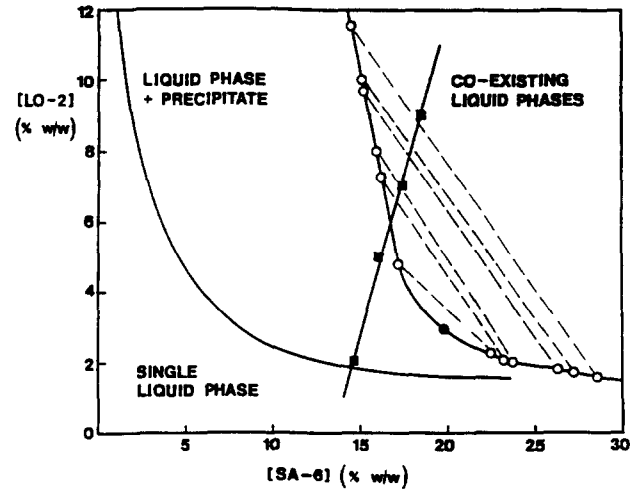


Fig. 12. Composition of LO-2/SA-6 mixed gels at the point of phase inversion (■), in comparison with the phase-diagram obtained (Kasapis *et al.*, 1993*b*) for the same system at 45°C. The binodal is defined by the composition (○) of co-existing liquid layers, joined by tie-lines (---), and converging to a single critical point (●).

continuous to maltodextrin-continuous occurring over a very narrow range of composition. Comparison of the gel-time data shown in Fig. 2 for SA-6 in combination with a fixed concentration (7% (w/w)) of LO-2 at 10°C and 20°C indicates that the critical concentration of SA-6 at which phase inversion occurs is not influenced by temperature. It does, however, increase linearly with increasing concentration of gelatin, and, as shown in Fig. 12, the line defining the composition of the mixed systems at the point of phase inversion passes smoothly through the binodal obtained from studies of mixed solutions in the disordered state at 45°C (Kasapis *et al.*, 1993*b*).

Quantitative analysis of the moduli of the final gels (Kasapis *et al.*, 1993*c*; part IV of this series) indicates that, at least for LO-2 concentrations up to 5% (w/w), formation of gelatin-continuous networks occurs prior to phase-separation, with subsequent gelation of the maltodextrin component then creating a separate discontinuous phase within the pores of the original continuous network. As discussed above, however, the gel-time measurements reported here argue strongly for maltodextrin-continuous networks forming after phase-separation has already occurred.

In the preceding paper (Kasapis *et al.*, 1993*b*) it was proposed that, at concentrations below the binodal, the presence of gelatin in the same solution phase promotes rapid ordering and aggregation of maltodextrin, with the consequent enhanced thermodynamic incompatibility between gelatin and the resulting maltodextrin assemblies then triggering phase separation. The indication of phase-separation prior to gel formation on quench-cooling initially monophasic solutions (Fig. 12) may be interpreted in an analogous way.

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