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Short Communication

Role of conformation in synergistic interactions of xanthan

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The 'synertistic' gelation of xanthan with locust bean gum (LBG) and related plant polysaccharides has been studied extensively, but the mechanism of interaction remains controversial. An early proposal (Morris *et al.*, 1977) was that unsubstituted or sparingly substituted regions of the plant-polysaccharide backbone bind to xanthan in its five-fold ordered conformation (Moorhouse *et al.*, 1977; Okuyama *et al.*, 1980). Subsequently it was suggested, from X-ray diffraction studies and from enhanced gel strength after heating and cooling through the temperature range of the xanthan order–disorder transition, that attachment occurs to the cellulosic backbone of the xanthan molecule in its disordered form (Cairns *et al.*, 1986, 1987).

An alternative explanation (Morris, 1990) of increased cohesion after heating and cooling, however, is that when the two interacting polymers are mixed at a temperature below the gel melting point, they form an imperfect, disrupted network, with melting and resetting then giving a stronger, continuous gel. In an investigation reported in a recent issue of this journal, Zhan *et al.* (1993) used changes in salt concentration to vary the mid-point temperature (T_m) of the xanthan transition and studied the effect on the rheological properties obtained on mixing with LBG at a number of different temperatures, with the conclusion that disordering of xanthan, rather than gel melting, is the dominant factor. We now present evidence that their experimental results are more consistent with the converse interpretation.

Figure 1 shows the temperature course of gelation on cooling (1 deg/min) for mixed solutions of xanthan (0·3% (w/v)) and LBG (0·1% (w/v)) in water and in 30 mM NaCl. Measurements were made using low-amplitude oscillation (10 rad s^{-1}; 2% strain) on a sensitive prototype rheometer designed and constructed in this department by Dr R.K. Richardson. Development of solid-like structure is characterised by the reduction in dissipated energy (loss modulus, G'') relative to stored
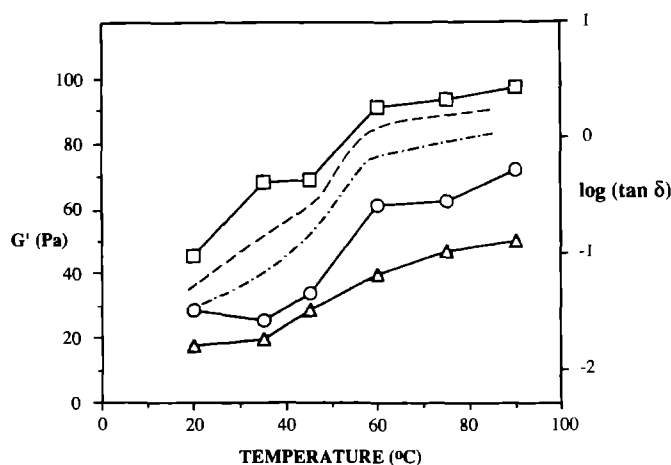

Fig. 1. Temperature course of gelation on cooling, and gel melting on heating, as monitored by changes in tan δ (10 rad/s; 2% strain; right-hand axis), for mixtures of xanthan (0·3% (w/v)) and LBG (0·1% (w/v)) in water (- - -) and in 30 mM NaCl (-·-·-). The other results included for direct comparison are reproduced, with permission, from Zhan *et al.* (1993), and show the effect of mixing temperature on gel modulus (G'; left-hand axis) measured at 20°C for mixtures of xanthan (0·25% (w/v)) and LBG (0·25% (w/v)) in water (□) and in 20 mM (○) and 400 mM (△) NaCl.

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energy (storage modulus, G'), expressed as the 'loss tangent' (tan $\delta = G''/G'$). The curves obtained on reheating the samples through the gel–sol transition were virtually identical (i.e. with no discernible thermal hysteresis between gelation and melting).

The onset of gelation occurs at essentially the same temperature (~60°C) in the presence or absence of salt. The xanthan T_m values, as determined by differential scanning calorimetry (Foster, 1992), however, are appreciably different. At the xanthan concentration used, the disorder–order transition in water is centred at ~45°C, well below the onset of gelation, so that association with LBG is clearly occurring with the xanthan component in its disordered form, as proposed by Cairns et al. (1986, 1987). The presence of 30 mM NaCl, however, raises T_m to ~70°C, so that the xanthan is then essentially fully ordered when network formation begins.

The remaining traces in Fig. 1 are reproduced directly from Zhan et al. (1993), and show G' at 20°C plotted against mixing temperature for xanthan (0·25% (w/v)) and LBG (0·25% (w/v)) in water, and in 20 mM and 400 mM NaCl. Although the polymer concentrations and mixing ratio are not identical to our own values, the difference is unlikely to be significant, since we have obtained gelation profiles closely similar to those shown in Fig. 1 for a wide range of systems, including combinations where the xanthan was deacetylated or konjac glucomannan was substituted for LBG (Foster, 1992; Goycoolea et al., 1994).

Taking the gel melting point as the temperature at which a steel ball placed on the surface of the sample began to sink, Zhan et al. obtained a constant value of 46–50°C, irrespective of the salt conditions used. As would be expected, this value is appreciably lower than the temperature of complete gel-melting that we have observed from small-deformation measurements (since a finite gel-strength will be required to support the weight of the ball), but it lies within the region of steepest ascent in tan δ (Fig. 1). Even lower values of gel melting-point from the falling-ball method have been reported previously (e.g. Dea et al., 1977), presumably reflecting the precise size of the ball and the way in which it was positioned in or on the sample. Although quantitatively different from the small-deformation criterion of melting, however, the observations of Zhan et al. agree with our own in showing no dependence upon salt concentration.

As shown in Fig. 1, the strength (G') of the mixed gels increased with mixing temperature at each of the salt conditions studied. This was attributed to the increasing proportion of disordered xanthan at the time of mixing. At each mixing temperature, there is also a systematic increase in G' with decreasing ionic strength, again attributed to the higher proportion of disorder present as the xanthan transition is displaced to a lower temperature.

For the ionic conditions used, the T_m values (from optical rotation) were 45°C in water, 50°C in 20 mM NaCl and 115°C in 400mM NaCl). In all three cases, however, the changes in G' with mixing temperature (Fig. 1) show a striking similarity to the temperature course of gelation and melting that we have observed by small-deformation oscillatory measurements. In particular, the greatest differences are between samples mixed at temperatures spanning the range of steepest change in tan δ, not between those prepared above and below T_m of the xanthan component. We therefore conclude that the strength of the gel network obtained after cooling from the mixing temperature to the temperature at which measurements were made (20°C) is determined predominantly by the extent of gelation during the mixing process (giving islands of 'smashed' gel, rather than a continuous network).

It must be emphasised that we do not disagree with the central tenet of the model proposed by Cairns et al. (1986, 1987). We fully accept that conformational re-arrangement of the xanthan helix to accommodate binding interactions with other polysaccharides is an entirely reasonable interpretation of current evidence. We also agree that formation of mixed gels can occur under conditions where the xanthan molecule is disordered; such behaviour is indeed clearly evident in our own results (Fig. 1) for xanthan/LBG in water. We do not, however, accept the postulate that the presence of disordered xanthan sequences is a necessary requirement for interaction to occur.

We propose instead that the crucial factor is the thermodynamic stability of the heterotypic mixed junctions formed between xanthan and its co-synergist, in comparison with that of the 5-fold helix structure of xanthan alone. If the xanthan molecule is ordered at the time of mixing, the conformation will be pulled over to the geometry required for efficient binding with the co-synergist; if it is disordered, the same heterotypic structure will be formed directly, in (enthalpically favourable) competition with adoption of the normal five-fold helix. Consistent with this proposal, we have recently found (Foster & Morris, 1994) that the polytetramer variant of xanthan, in which the terminal mannose residues of each side-chain have been eliminated by genetic manipulation (Betlach et al, 1987), shows no evidence of interaction with LBG or konjac mannan, and that its helical structure has an enthalpic stability more than twice that of xanthan ($\Delta H \approx 8\cdot5$ J/g in comparison with ~4·0 J/g).

Finally, the concept of competition between homotypic and heterotypic structures can be extended to explain the decrease in gel modulus with increasing salt concentration at equivalent mixing temperature (Fig. 1). It has been demonstrated (Ross-Murphy et al., 1983) that addition of salt induces aggregation of xanthan helices. A decrease in synergistic interaction would therefore be anticipated because, in simple terms, if the

xanthan is already stuck to itself it will have less capacity to stick to anything else, and if locked within an aggregate structure it will be less able to adjust its conformation to the geometry required for efficient packing within heterotypic junctions.

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