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Xanthan–locust bean gum interactions and gelation

D.F. Zhan

Institute of Resource Development and Applied Science, Wuhan University, Wuhan 430072, People's Republic of China

M.J. Ridout, G.J. Brownsey & V.J. Morris*

AFRC Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich, Norfolk, NR4 7UA, UK

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The gelation of xanthan–locust bean gum (LBG) mixtures has been investigated. It has been observed that, over the ionic strength range chosen, the melting temperatures of the gels (T_g) remains constant, whereas the order–disorder transition temperature (T_m) for xanthan alone increases with increasing ionic strength. The preparation temperature (T_p) of the mixed gels is known to affect gelation. Studies have been made on the effect of T_p relative to T_g and T_m on gelation. It has been observed that gelation occurs at all T_p, even when $T_p < T_m$. At a given ionic strength, increasing T_p leads to an increase in the measured storage modulus (G') which closely follows the degree of disordering of the xanthan molecules. Similarly, for a fixed T_p, increasing the ionic strength stabilises the ordered xanthan conformation and is found to reduce the measured storage modulus (G'). These results are discussed in the light of the current models for mixed-biopolymer gelation.

INTRODUCTION

Synergistic interactions between polysaccharides are of commercial interest because they offer the prospect of generating novel functionality, or of producing given rheological or textural characteristics using reduced levels of polysaccharides, and possibly reduced cost. Xanthan–locust bean gum (LBG) mixtures are used industrially as thermoreversible gelling agents. The xanthan–LBG system is of particular interest because gelation results from the admixture of two 'non-gelling' components. Xanthan gum is an anionic heteropolysaccharide produced by the bacterium *Xanthomonas campestris* (Jeanes *et al.*, 1974). The primary structure of the polysaccharide consists of a cellulose backbone (β-(1 → 4)-D-glucose) substituted at C3 on alternate glucose residues with a charged trisaccharide side-chain (Jansson *et al.*, 1975; Melton *et al.*, 1976). Non-carbohydrate substituents include *O*-acetate, as 6-*O*-acetyl-D-mannose on the inner mannose residue of the side-chain, and pyruvic acid, as the sugar ketal, 4,6 (1-′-carboxyethylidene)-D-mannose, on the terminal mannose residue. The substitution is incomplete. The secondary structure of xanthan has been investigated by the use of X-ray diffraction studies of orientated fibres and by the use of molecular modelling methods, and has been shown to form a five-fold helical structure of pitch 4·7 nm. (Moorhouse *et al.*, 1977; Okuyama *et al.*, 1980). Recent studies (Millane & Narasaiah, 1990; Millane & Wang, 1992) on a family of xanthan-like polysaccharides, based on the xanthan structure but containing elongated or reduced side-chains, have revealed that the branch–backbone linkage is crucial in determining the helical conformation. Analysis of the X-ray patterns is limited by the poor crystallinity of the fibre samples, and it is not possible at present to distinguish between sterically acceptable single and double helical structures (Moorhouse *et al.*, 1977; Okuyama *et al.*, 1980; Millane & Narasaiah, 1990; Millane & Wang, 1992). In solution, xanthan undergoes an 'order–disorder' transition attributed to a 'helix–coil' transition in which the ordered (helical) structure is stabilised at low temperature and/or high ionic strength, and the transition mid-point temperature (T_m) increases with increasing ionic strength (Morris *et al.*, 1977; Norton *et al.*, 1984).

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*To whom correspondence should be addressed.

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LBG is a galactomannan obtained from *Ceratonia siliqua*. Galactomannans are polysaccharides consisting of a mannan backbone (β-(1 → 4)-D-mannose) incompletely and irregularly substituted at C6 with α-D-galactose (Dea & Morrison, 1975). The mannose:galactose (M/G) ratio is dependent on the source of the galactomannan and the method of extraction (Dea & Morrison, 1975). LBG has a value of M/G ≈ 3·55.

Xanthan forms transparent thermoreversible elastic gels when mixed with certain galactomannans such as LBG and tara gum (Dea & Morrison, 1975; Dea *et al.*, 1977; Morris *et al.*, 1977). All current models for gelation are based on an association between xanthan and the galactomannan but differ in the description of the intermolecular binding. There is considerable experimental evidence favouring intermolecular binding (McCleary & Neukom, 1982; Cheetham *et al.*, 1986; Cheetham & Mashimba, 1988, 1991; Cheetham & Punruckrong, 1989; Williams *et al.*, 1991).

The earliest models for intermolecular binding proposed a binding of unsubstituted regions of the galactomannan to the ordered xanthan helical structure (Dea & Morrison, 1975; Morris *et al.*, 1977). The tendency of galactomannans to show synergistic interactions with xanthan parallels their tendency for self-association, with both gel strength and gel melting point increasing with decreasing galactose content (Dea & Morrison, 1975). However, gelation depends on the detailed distribution of galactose residues as well as the M/G ratio, and this has led to the proposal that binding involves not just unsubstituted regions but also probably regions of galactomannan backbone substituted on one side (McCleary *et al.*, 1979).

Tako *et al.* (1984) have proposed an alternative model in which xanthan is in the ordered helical structure and intermolecular binding arises from an interaction of the xanthan side-chains with unsubstituted regions of the galactomannan backbone.

Mixing experiments (Cairns *et al.*, 1986, 1987) suggest that denaturation of the xanthan helical structure is an important factor promoting xanthan–galactomannan gelation. Indeed experimental evidence from enzymatic studies (McCleary & Neukom, 1982), GPC studies (Cheetham *et al.*, 1986), gel melting point measurements (Cheetham & Mashimba, 1988), and optical rotation studies using depolymerised galactomannans and/or depolymerised xanthan (Cheetham & Mashimba, 1988, 1991) support this theory. X-ray diffraction patterns for aligned fibres prepared from mixed gels show novel X-ray patterns (Cairns *et al.*, 1986, 1987). The best patterns were obtained for xanthan–LBG gels. These patterns show a xanthan pattern plus a new pattern which is related to that of pure carob. Qualitative analysis of this data has been taken to suggest that a small fraction of the disordered xanthan chain co-crystallises with the galactomannan (Morris, 1990, 1991). The sensitivity to galactose content and distribution has been attributed to the need to accommodate the long xanthan side-chain within this galactomannan crystalline lattice (Morris, 1990, 1991). Modelling studies (Millane & Wang, 1990) have shown that disordered xanthan can adopt a two-fold structure consistent with such a model. Cheetham and co-workers (Cheetham *et al.*, 1986; Cheetham & Mashimba, 1991) have proposed similar models involving intermolecular binding between denatured regions of the xanthan chain and the galactomannan.

Recently it has been observed that mixing xanthan and LBG at temperatures below T_m leads to gelation (Williams *et al.*, 1991; Mannion *et al.*, 1992). These authors proposed that gelation may occur by two mechanisms. Gels prepared by heating to temperatures above T_m, and then cooled, are considered to involve intermolecular binding to denatured segments of the xanthan chain, whereas gels prepared by heating at temperatures well below T_m, and then cooled, are believed to involve binding to the ordered xanthan helix.

In the present studies the melting point of the xanthan–LBG mixed gels (T_g) and the xanthan order–disorder transition temperature (T_m) have been measured. Addition of sodium chloride has been used to vary T_m. The mixing temperature (T_p) has been varied relative to T_m in order to assess the effect of disordering the xanthan helix on the rheology of xanthan–LBG gels.

EXPERIMENTAL

Xanthan gum (Keltrol) was purchased from Kelco-AIL. The xanthan was converted to the sodium salt by passing 0·1% aqueous solution (clarified by passing through a 3 μm filter) through a H^+ Dowex column. The acidic solution was then neutralised with dilute (0·1 M) sodium hydroxide and freeze-dried. Solutions were prepared by heating the xanthan in water for 0·5 h at 85°C then adding sodium chloride solution or water to give the required concentration. The sample was then heated at 85°C for a further 1 h in a sealed tube which was vigorously shaken. The solution was then passed through a 1·2 μm filter, cooled, left overnight and diluted as required.

LBG was purchased from Sigma and used without further purification. Solutions were prepared in the same manner as for the xanthan solutions.

Mixed gels were prepared by combining the appropriate xanthan and LBG solutions at 85°C, mixing, and then holding the mixtures at 85°C for 1 h in sealed tubes. The solutions were then poured into moulds and allowed to cool overnight.

Rheological studies were made using an Instron 3250 mechanical spectrometer fitted with a temperaturecontrolled chamber around the plates. The mixed gels were subjected to a sinusoidal oscillation at low strains (< 0·5), over a range of frequencies (0·011–10 Hz) between flat plates of diameter 40 mm. Gels were tested 24 h after preparation. All results shown are for gels tested at 20°C.

Optical rotation studies were undertaken with a JASCO DIP-360 polarimeter using a cell of path length 100 mm. A mercury light source was used with a selected wavelength of 436 nm.

Solutions were poured hot into the cell at 85°C, held at this temperature for 1 h, in order to allow any air bubbles to escape from the solution, cooled over a 3-h period to 20°C, and held at this temperature overnight. Most optical rotation–temperature studies were carried out using a heating cycle.

Gel melting points were routinely measured using the falling ball method. Once the gels had set in a glass tube, a steel ball was placed on the gel surface and the tube sealed. The gels were heated at a heating rate of ≈ 1°C/min, and the melting point was determined as that temperature at which the ball started to fall through the gel.

RESULTS AND DISCUSSION

Optical rotation (α) measurements on 0·5% xanthan solutions were used to monitor the variation of the mid-point transition temperature with added sodium chloride. Figure 1 shows optical rotation data for an aqueous solution of xanthan and also a solution containing 0·4 M NaCl. The transition temperature (T_m) was determined by curve-fitting the α-temperature (T) data in the vicinity of the mid-point transition, and defining T_m as the point of inflection $d^2\alpha/(dT)^2 = 0$.

Figure 2 shows a plot of $1/T_m$ versus $\log_{10}$ (salt concentration). The linear relationship obtained is characteristic of that expected for the screening of a charged helical polysaccharide.

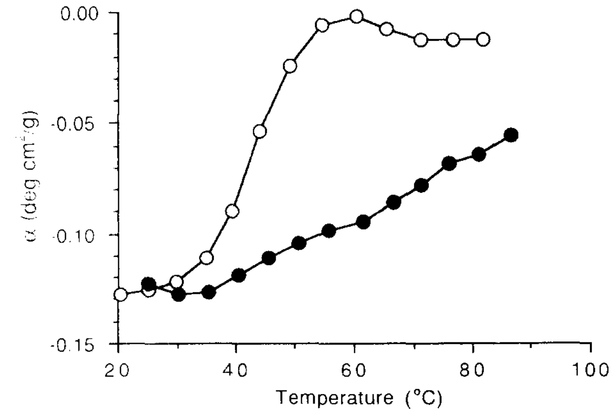

Fig. 1. Order–disorder transition for 0·5% xanthan solutions: ○, in water; and ●, in 0·4 M NaCl.

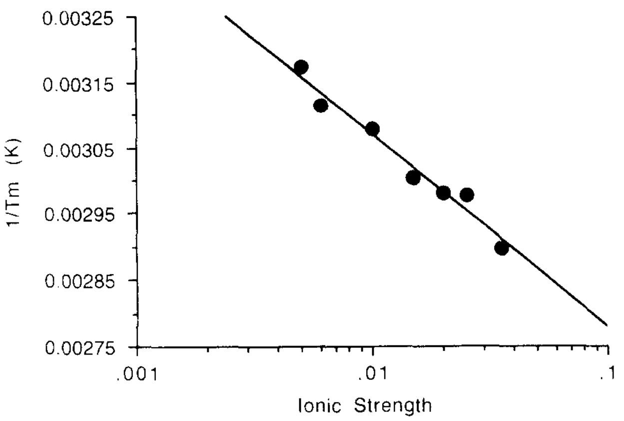

Fig. 2. Dependence of the order–disorder transition temperature (T_m) of xanthan solutions on the level of added NaCl.

The melting points (T_g) of mixed gels as shown in Fig. 3 suggest that, for a given ratio of xanthan:LBG, the melting temperature (T_g) appears to be independent of ionic strength. For 1:1 xanthan:LBG the melting point was found to lie in the range 46–50°C and showed no dependence on ionic strength. These data support the studies of Williams *et al.* (1991) who argued that gel formation occurred at ≈ 49°C, independent of the presence of added salt.

From Figs 2 and 3 we note that for the 1:1 aqueous xanthan:LBG mixture at 0·5% polymer concentration, T_g of the gels is equal to the T_m value measured for xanthan only. By addition of salt to the mixtures it should be possible to achieve conditions such that the T_m for xanthan can be made much higher than T_g for the mixed gels. By suitable choice of the preparation temperature (T_p), and the level of added salt, the following conditions should be possible for preparation of the gels: $T_p < T_g < T_m$, $T_g < T_p < T_m$, and $T_g < T_m < T_p$. This was achieved in practice by using added salt concentrations of 0, 0·02 and 0·4 M NaCl, for

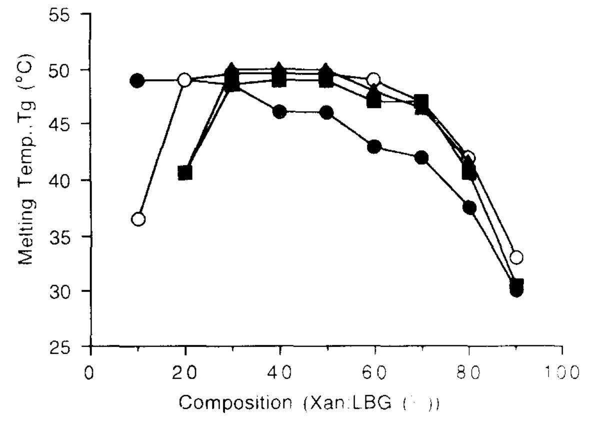

Fig. 3. Melting temperatures for a series of xanthan:LBG gels under differing salt conditions. ●, Water; ○, 0·01 M NaCl; ■, 0·02 M NaCl; and ▲, 0·04 M NaCl.

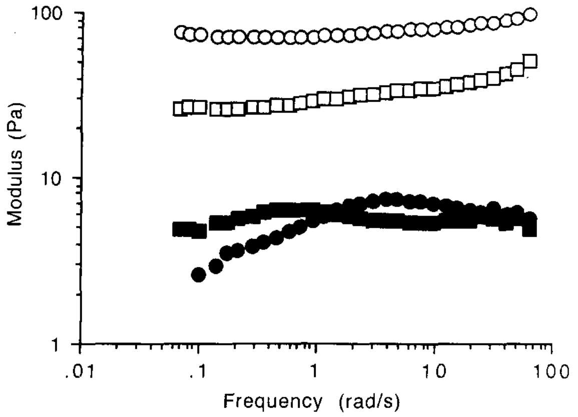

Fig. 4. Frequency dependence of the storage (G') and loss (G'') moduli of gels prepared in the regimes: ○, $T_m < T_g < T_p$; and □, $T_p < T_g < T_m$. ○, □, storage modulus; ●, ■, loss modulus.

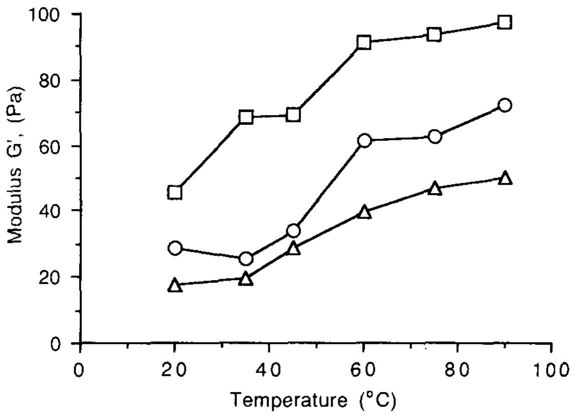

Fig. 5. Effect of added salt and preparation temperature (T_p) on (G') for 1:1 xanthan:LBG mixtures. □, Water; ○, 0·02 M NaCl; and △, 0·4 M NaCl.

which T_g is constant (46–50°C), and T_m = 45, 50 and 115°C (estimated from Fig. 2), respectively. Preparation temperatures used were 20, 35, 45,60, 75 and 90°C.

Mechanical spectroscopy was used to assess whether the mixtures had gelled. Figure 4 shows typical spectra for the conditions $T_p < T_g < T_m$ and $T_g < T_m < T_p$ (mixtures in 0·02 M NaCl). In both cases the storage modulus (G') is greater than the loss modulus (G'') and both are effectively independent of frequency, implying gel-like behaviour. For all systems prepared, G' was greater than G''. Hence, all systems were considered to have gelled. Phase angles were seen to drop with an increase in T_p for any given concentration of added salt and to increase with ionic strength for a chosen value of T_p. However, the changes were small, as for most systems the phase angle was less than 10°, implying highly elastic gels in all cases. As T_p was increased G' increased significantly. Figure 5 shows how G' increases with increasing T_p for each of the systems containing different levels of added salt. Since T_g is independent of salt content, and the gels become significantly stronger with increasing T_p, these data suggest that the primary determinant of gel properties is the value of T_p relative to T_m. This would suggest that the nature of the gels is determined by the level of disorder induced in the xanthan molecules prior to mixing. To test this assumption the dependence of G' on T_p for the mixed gels has been compared with the temperature dependence of the optical rotation of xanthan solutions measured under the same salt conditions (Fig. 6). The increase in G' with an increase in T_p clearly follows the variation of α with temperature for the xanthan solutions.

If G' can be taken as a measure of the density of cross-links per unit volume then the experimental data suggests that the level of interaction between xanthan and LBG increases with the level of disorder induced in the xanthan molecules. The xanthan order–disorder transition is an equilibrium process and the level of disordered xanthan will depend on the temperature of the xanthan sample relative to T_m. If LBG interacts with disordered segments of the xanthan chain then the addition of LBG would create a second equilibrium process involving such xanthan–LBG association. Thus, a favourable xanthan–LBG interaction would drive the xanthan order–disorder process to produce more disordered xanthan. The level of interaction achieved would depend on the experimental conditions and the relative equilibrium constants for the two processes. Thus, gelation would most likely be time dependent with higher levels of disordered xanthan favouring high G' and a closer approach to the expected equilibrium G' values.

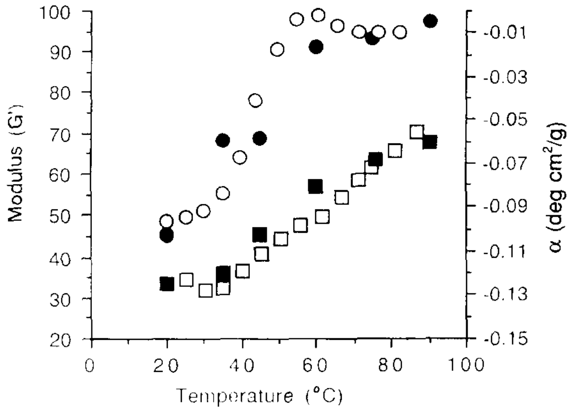

Fig. 6. Comparison of optical rotation–temperature data for 0·5% xanthan solutions with the variations of G' for 1:1 xanthan:LBG at various preparation temperatures. (T_p) ○, □, optical rotation data; ●, ■, modulus data; ○, ●, samples prepared in water; □, ■, samples prepared in 0·4 M NaCl.

In previous studies (Cairns *et al.*, 1986, 1987; Morris, 1990, 1991) it has been noted that the nature of xanthan:LBG mixtures is determined by the relative values of T_p and T_m. In these experiments three preparation regimes were employed. Xanthan–LBG mixtures were prepared at room temperature and compared with similar mixtures prepared at 95°C with $T_p > T_m$, and then cooled to room temperature. The third regime involved addition of calcium chloride to shift T_m above 100°C, mixing at 95°C ($T_p < T_m$) and cooling to room temperature. It was observed that, whereas hot mixing ($T_p > T_m$) prior to cooling resulted in free-standing elastic gels, the other two regimes yielded pourable mixtures. In the present study it has been demonstrated that all the mixtures prepared can be technically described as gels. This is not perhaps surprising since, for studies at appropriately low strain xanthan alone exhibits weak gel-like behaviour. What is important is the dependence of G' on the level of disordered xanthan present on mixing. Recently, Morris (1992) has argued that these differences in rheological measurements are due to differences in the process of mixing. It is argued that for cold mixed xanthan:LBG samples in the absence of salt, the mixing process results in the break down in the gel structure as it is formed. Thus, the differences in the mechanical properties between hot and cold mixtures are attributed to differences between an intact gel and a smashed gel or slurry, and not to differences resulting from the effect of the conformation of the xanthan molecule on intermolecular binding. Thus, it is maintained that the experimental data can be explained on the basis that the interaction occurs between LBG and the xanthan helix. Such a criticism is valid for a comparison of hot and cold mixed samples. However, it has been shown in the present studies, and elsewhere (Williams *et al.*, 1991), that T_g is independent of added salt content. Thus, a comparison of samples prepared containing different levels of added salt, but with $T_p > T_g$, should identify the effects of different degree of xanthan disordering on the rheology of the samples.

Thus, previous (Cairns *et al.*, 1986, 1987) and present experimental data are consistent with a model in which intermolecular binding occurs between LBG and disordered segments of the xanthan chain. By considering the fact that addition of LBG may perturb the normal xanthan order-disorder transition it is possible to explain gelation without invoking two separate gelation mechanisms, as has been suggested recently (Williams *et al.*, 1991; Mannion *et al.*, 1992). Such a model can also explain the dependence of G' on the M/G ratio of selectively prepared LBG fractions (Mannion *et al.*, 1992). These authors showed that G', for mixtures prepared at room temperature, showed little dependence on the M/G ratio of the LBG fraction. However, heating these samples even at temperatures where $T_p < T_m$, and then cooling to room temperature, led to increases in the values of G' for the gels, which were more noticeable for higher M/G LBG fractions. On the basis of the present model, the lower the value of T_p relative to T_m, the less xanthan would be available to bind to the allowable sites on the LBG molecules. As T_p is raised the amount of xanthan available for binding would increase. Fractions with higher M/G ratios can accommodate more xanthan segments and should result in progressively higher G' values.

CONCLUSIONS

It has been confirmed that xanthan-LBG mixtures will gel when prepared at temperatures $T_g < T_p < T_m$ or even $T_p < T_g < T_m$. However, it has been demonstrated that the storage modulus of the gels, and hence the level of xanthan-LBG interaction, is related to the degree of disordering of the xanthan molecule at the preparation temperature. Gelation can be explained by means of a single mechanism, involving binding of disordered segments of the xanthan chain to LBG chains, both for hot setting ($T_p > T_m$) and cold setting ($T_p < T_m$) gels.

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