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# Conformational Aspects of Xanthan–Galactomannan Gelation

Norman W. H. Cheetham & Ernest N. M. Mashimba

School of Chemistry, The University of New South Wales, PO Box 1, Kensington 2033, Australia

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*ABSTRACT*

*Gel melting temperatures have been used to probe some aspects of the mechanism of gel formation between xanthan gum and locust bean (carob) gum.*

*It is shown that homogeneous gels form in water between the disordered form of xanthan, and locust bean gum. In salt solutions, gel islands initially form, but can be converted to homogeneous gels by heating above the xanthan order–disorder transition temperature, $T_c$. Dialysis of gel islands also results in homogeneous gels. It is proposed that in water, junction zones are formed between the xanthan backbone in the flat ribbon (cellulosic) conformation and the locust bean gum backbone in a similar conformation. In the presence of salt, additional intermolecular associations occur which raise the gel melting point above that in water alone.*

## INTRODUCTION

Until recently, the model for gel formation between xanthan and galactomannans proposed by Dea, Morris and co-workers (Morris *et al.*, 1977; Dea *et al.*, 1977) has been widely accepted. It invokes interaction between the xanthan backbone in the ordered, rod-like conformation and galactose-free regions of the galactomannan backbone (Fig. 1). More recently, a slight but important modification was introduced to explain the strong interactions between xanthan and some highly-substituted galactomannans (McCleary, 1979). It was proposed that there were sections of the galactomannan having all the galactosyl residues located on the same side of the main chain, and that these sections could interact with the xanthan backbone to form junction zones in addition to any galactose-free sections (Fig. 2). In these models, there is usually the



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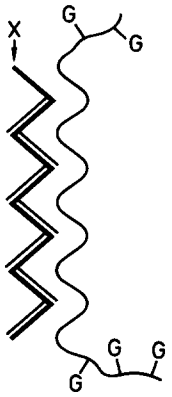


**Fig. 1.** Model for xanthan–galactomannan mixed junction zones (Morris *et al.*, 1977; Dea *et al.*, 1977).

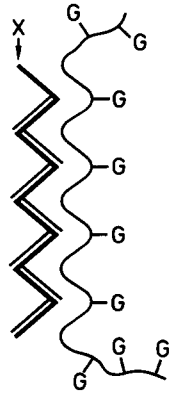


**Fig. 2.** Model for xanthan–galactomannan mixed junction zones (McCleary, 1979). X, xanthan in the ordered helical conformation; G, galactomannan with a galactose-free face interacting with xanthan.

implication that xanthan exists as single, rather than double chains, though debate over the double chains versus single chain remains active (Holzwarth & Prestridge, 1977; Holzwarth, 1978; Sato *et al.*, 1984*a*, *b*, *c*; Kitagawa *et al.*, 1985; Milas & Rinaudo, 1986; Cairns *et al.*, 1987). There seems little doubt that many disagreements on this question arise because xanthan solution properties depend on the history of the sample, on the method of dissolution, and probably on pyruvate content as well. Morris *et al.* (1983) showed that heating, especially in the presence of urea, produced true solutions of xanthan which were filterable, and that other dissolution methods produced dispersions containing aggregates or microgels. Efforts to use 'native' xanthan unaffected by isolation procedures still yield conflicting results. Lecourtier *et al.* (1986) used xanthan from unpasteurised fermentation broth which was thus a native product and had excellent filterability properties through a series of membranes. They concluded that native xanthan in sodium chloride solutions of 0·1 M or greater existed as a double helix ($\bar{M}_w \approx 4{\cdot}8 \times 10^6$ daltons). Reduction of the salt concentration to $10^{-6}$–$10^{-4}$ M, then readjustment to 0·1 M yielded a slightly lower $\bar{M}_w$ of $4{\cdot}1 \times 10^6$ daltons. The xanthan in $10^{-5}$ M NaCl had a $\bar{M}_w \approx 2{\cdot}1 \times 10^6$ daltons, and was thus single-stranded. The single-strand was stable in solution for a period of years (Muller *et al.*, 1986). Lecourtier's xanthan was highly pyruvylated (DS = 0·98 per side chain) and the dissociation into single strands occurred in $10^{-5}$ M NaCl without heating. Samples of lower pyruvate

content used by Milas & Rinaudo (1984) though heat-treated, apparently did not dissociate into single strands under the salt concentration and temperature range of the experiments, presumably because electrostatic repulsions were too weak.

Milas & Rinaudo (1986) also used unpasteurised culture both, but the $DS_{pyr}$ was only 0·4. Their conclusion that the native form of xanthan is single-stranded is at odds with the results of Lecourtier *et al.* (1986). Milas & Rinaudo (1986) propose the existence of three conformational states: I, the native, ordered form; II, the denatured, ordered form (heated to $T_m$, then cooled); and III, a disordered form (present at high temperature or low salt concentration, or both). All are single chains. Lecourtier *et al.* (1986) propose three ordered conformations: 1, the native, compact double helix; 2, the extended double helix; 3, the extended single helix, (which should be converted by heating to a disordered form analogous to the form III of Milas & Rinaudo).

It appears that most samples of xanthan used in gel-formation studies with galactomannans were single stranded, as considerable heating in water was used to dissolve them (Dea *et al.*, 1977; Morris *et al.*, 1977) and pyruvate levels were relatively low. It has been shown that considerable loss of pyruvate can occur as a result of heating xanthan solutions (Rinaudo *et al.*, 1983; Cheetham & Punruckvong, 1985). In such studies, interaction is strongest with galactomannans having high mannose/galactose (M/G) ratios. (Dea & Morrison, 1975; Morris *et al.*, 1977; McCleary, 1979; McCleary *et al.*, 1981, 1984; Cheetham *et al.*, 1986). Further, it has been clearly shown that the galactomannan fine structure affects gel strength. Thus the galactomannan from *Ceratonia siliqua* (carob or locust bean, 25% galactose) interacts more strongly than that from *Caesalpinia pulcherima* with 24% galactose (Dea *et al.*, 1986). Enzymic hydrolysis of the former galactomannan shows oligosaccharide patterns consistent with a non-random distribution of galactose side chains, and reveals a considerable proportion of unsubstituted blocks suitable for interaction with xanthan. *Caesalpinia pulcherima* galactomannan has a distribution approaching statistically random, and consequently has fewer blocks suitable for interaction. *Leucaena leucocephala* (40% galactose) galactomannan interacts strongly with xanthan, despite the high galactose content. It has frequent regions of the main chain in which every second D-mannosyl residue is substituted with D-galactose. This, in the ordered two-fold ribbon conformation, would have unsubstituted sides or faces suitable for interaction with xanthan (Fig. 2). A different model for gel formation has been proposed (Tako & Nakamura, 1984; Tako *et al.*, 1984; Tako & Nakamura, 1986). This involves interaction of the xanthan side chains with the mannan back-

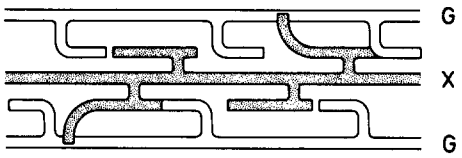


**Fig. 3.** Model for xanthan–galactomannan mixed junction zones (Tako & Nakamura, 1984; Tako *et al.*, 1984). X, xanthan; G, galactomannan.

bone (Fig. 3). Acetate-free xanthan interacted more strongly, as shown by dynamic viscoelasticity measurements with galactomannans, than the native material. The ordered conformation of the xanthan backbone has apparently been retained in this model.

The ordered (five-fold) helical backbone conformation of xanthan is stabilised by acetate (Smith *et al.*, 1981; Tako & Nakamura, 1984). The stronger interaction of acetate-free xanthan with galactomannans (Tako & Nakamura 1986) could be due to greater side chain mobility and/or greater backbone flexibility than is possible for native xanthan at room temperature. The precise conformation in which xanthan interacts with galactomannans is unknown. Care must be taken in interpretation of results. Only small portions of the galactomannan (McCleary *et al.*, 1984) and xanthan (Dea *et al.*, 1977) chains appear to be involved in junction zone formation. Xanthan only inhibited the action of $\beta$-mannanase on locust bean galactomannan if the mixtures were heated prior to addition of enzyme (McCleary *et al.*, 1981). A number of rheological studies have been carried out on aqueous dispersions of xanthan, rather than on true solutions (Morris *et al.*, 1983). It is possible that the gel formation in such samples involves the soluble portions of the xanthan and not the aggregates or microgels. If this is the case, larger portions of each soluble xanthan molecule could be involved in junction zone formation.

Recently Cairns *et al.* (1986; 1987) have proposed a different model for the gelation of xanthan and galactomannans, based on X-ray fibre diffraction studies on stretched gels. They found that mixing of xanthan solution with galactomannan solution (carob or tara gum) did not lead to gelation until they were heated above the helix–coil transition temperature ($T_c$) of xanthan and then cooled. The X-ray fibre diffraction patterns on the gels formed from heated samples were different to those from cold-mixed samples, in showing reflections which were not due to xanthan alone. Mixed junction zones were formed by interaction between the xanthan and galactomannan backbones, with the relative positions of xanthan side chains on either side of a sandwiched galactomannan molecule being staggered (Fig. 4(a), (b)). In order to achieve this,

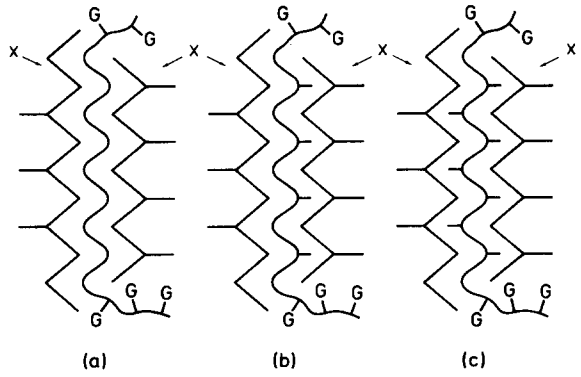


**Fig. 4.** 'Sandwich' models for xanthan–galactomannan mixed junction zones (Cairns *et al.*, 1986; 1987). (a) Two xanthan chains enclosing a galactose-free region of the galactomannan. Xanthan side chains are staggered and disordered to give an X-ray diffraction pattern with a repeat distance of 0·52 nm. (b) Partially-substituted galactomannan chains with one unsubstituted face must also be accommodated, to account for the effect of galactomannan fine structure on xanthan–galactomannan interactions. (c) Fully-substituted galactomannan chains apparently cannot be accommodated to a significant extent. X, xanthan; G, galactomannan.

the ordered conformation of the xanthan side chains needs to be disrupted. The X-ray results indicate a repeat distance of 0·52 nm. This is equivalent to half the axial advance per repeat unit of cellulose and mannan. Other binding schemes such as those previously proposed would be expected to give an axial advance per repeat unit of 1·04 nm. The junction zone stoichiometry is not obtainable from the above results, and several galactomannan molecules could be sandwiched between xanthan backbones. Fully-substituted galactomannan regions (Fig. 4(c)) cannot be accommodated, as shown by the effect of galactomannan fine structure on gel strength mentioned above. Two important conclusions arise from the work of Cairns *et al.*, 1987.

1. Specific intermolecular binding between xanthan and galactomannans can occur, due to structural compatibilities between the cellulose-like xanthan backbone and the mannan backbone. (Such specific binding does not occur between galactomannans and $\kappa$-carrageenan or furcellaran.)
2. The specific binding will only arise if the xanthan is in the non-helical form. This is in contrast to the proposals of the original model.

Gel melting points and optical rotation have been used to study the interaction between xanthan and locust bean gum in order to clarify some of the above points.

## 2 EXPERIMENTAL

The xanthan used was derived from 'crude' Keltrol (Kelco Division of E. Merk & Co. Inc., USA), 1·9% N. Cold-water dissolved xanthan (XCW) was prepared by stirring crude xanthan ( ≈ 0·3% w/v) in water at room temperature overnight. The resulting dispersion was centrifuged at 200 000 *g* for 5 h. The clear supernatant liquid was removed, dialysed against distilled water, passed through a column of cation-exchange resin (Dowex 50w ($Na^+$)), dialysed exhaustively and lyophylised. Yield: ≈ 45%; 4·3% pyruvate; 4·1% acetate; 0·65% N. This was redissolved in cold water to form a clear solution, which could be readily filtered (1·2$\mu$ Millipore) and was used for gel melting point experiments. Hot-water dissolved xanthan (XHW) was prepared from the crude material in a similar manner to that above, except that a temperature of 90°C for 30 min was used for dispersion. The yield of XHW was ≈ 79%. The gel-like centrifugate from each method was opaque, and of much higher viscosity than the corresponding supernatant solutions of the same total polysaccharide content. The centrifugate was redispersed in water, recentrifuged at 100 000 *g*, and also used in gel-forming experiments with locust bean gum (LBG).

Locust bean gum ($M_w$, $270 \times 10^3$, M/G = 5·1, LBG-c) was obtained by $\beta$-D-mannanase treatment of hot-water soluble locust bean gum as previously described (Cheetham *et al.*, 1986). Acetate and pyruvate levels in xanthan were determined by HPLC as previously described (Cheetham & Punruckvong, 1985). Acetate-free and pyruvate-free xanthan were prepared by alkali and acid treatment of xanthan solutions, as described by Bradshaw *et al.*, (1983). Residual acetate and pyruvate was 0 and 9·3% respectively. Gel melting points were determined by noting the temperature at which the gel failed to support a 2 mm steel ball bearing, as the temperature of the surrounding water bath was raised slowly. For gel melting points determined in water, 1% aqueous solutions of xanthan and LBG were diluted 1:1 with water then mixed. For melting points in salt, they were diluted 1:1 in 0·1 M KCl solution before mixing. Solid KCl or urea was added where indicated. Optical rotations were measured at 365 nm in a Perkin-Elmer 141 polarimeter, using a 1 ml thermostatted cell, path length 10 cm.

## 3 RESULTS AND DISCUSSION

The melting temperatures for xanthan–LBG gels should reflect some of the underlying molecular events, and should be capable of rationalisation in terms of those events. Though the absolute melting temperatures

reported here are somewhat arbitrary due to the method of determination, the relative values have yielded useful information.

The crude xanthan used as the starting material was not a native form, having undergone precipitation and drying during isolation from the fermentation broth, which probably means aggregation had occurred (Muller *et al.*, 1986). It was decided to prepare a 'purified' xanthan sample by simple cold-water dispersion, followed by high-speed centrifugation. This yielded XCW (45%). In this step no heat treatment was applied. A similar procedure was used for the isolation of xanthan from a hot-water dispersion, and yielded XHW (79%). The gel-forming properties of XCW and XHW were virtually identical. These purified xanthan samples formed solutions which were readily filterable and were considered to be true solutions. It is probable that preferential dissolution of some single-stranded, partially-disordered xanthan molecules occurred in the cold (XCW), and that more of these were formed during heating to yield XHW with the same properties as XCW. The nitrogen contents of XCW and XHW were reduced to about one third of the crude xanthan level in each case. Optical rotation versus temperature curves for XCW exhaustively (to constant conductivity; Fig. 5(a)) and

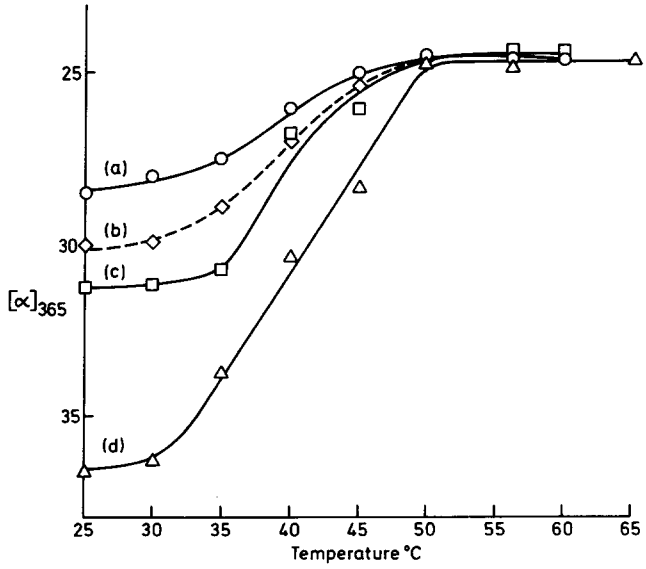


**Fig. 5.** Optical rotation versus temperature curves for various xanthan samples. (a) Cold-water soluble xanthan (XCW) in water, dialysed to constant conductivity in an ultrafiltration cell. (b) XCW from (a) after freeze-drying and redissolution in water. (c) Hot-water soluble xanthan (XHW) in water, dialysed to constant conductivity. (d) XCW in water, partially dialysed.

partially dialysed (Fig. 5(d)) show that the former has much less negative rotation than the latter. This indicates a lower degree of order for the fully-dialysed sample, which formed a gel at room temperature with LBG. The partially-dialysed XCW did not form a gel at room temperature. These results are consistent with considerable side chain disorder being necessary for strong interaction with LBG to form a gel. Figure 5(b) shows XCW after freeze-drying and redissolution in cold water. The slightly more negative rotation shows that a small increase in order has been brought about, possibly by concentration effects during freezing. Fully-dialysed XHW (Fig. 5(c)) retains slightly more order than XCW, but still gels with LBG without being heated.

The residue left after centrifugation to isolate XCW presumably consisted of cell debris, and microgels (aggregates) of xanthan molecules which were not appreciably soluble. The viscosity of these residual aggregates was much higher (by a factor of 3) than a solution of an equivalent concentration of XCW or XHW. Table 1 summarises and interprets the melting points of gels formed between XCW (0·5%) and LBG (0·5%) under a variety of conditions. The concentration of 0·05 M KCl was chosen for experiments as $T_c$ of the order–disorder transition is well below 90°C at this ionic strength, as determined by optical rotation. Lower ionic strengths did not give sufficient temperature enhancement of gel melting points relative to those in water. Overall conclusions are consistent with the xanthan in junction zones being in the disordered form to initiate interaction with LBG. Once the interaction is established, addition of KCl restores the ordered form in the non-junction zone parts of the xanthan chains and 'locks' the junction zones in place. KCl enhances xanthan–xanthan interactions via pyruvate (Smith *et al.*, 1981), side-by-side dimerisation (Morris *et al.*, 1983) or limited double-helix formation. Thus the final gel melting point in 0·05 M KCl (45°C) is slightly but consistently higher than in water (42°C). In 1 M KCl the gel melting point was 55°C. In Experiment 3 (Table 1) gel islands formed. These were quite small and could be centifuged to the bottom of the tube only when the temperature during centrifugation was reduced to 2°C. They remained as precipitate even when brought back to room temperature.

Xanthan dissolves readily in water when it consists mainly of partially-disordered single chains, e.g. XCW. However, the same xanthan only dissolves to the extent of ≈4% in 0·05 M KCl even at 95°C after 2 h. Small particles very similar to gel islands are formed. Self-association of ordered xanthan chains on the outside of each solid particle must prevent full penetration of solvent into the particle.

Gel island formation with LBG in the presence of KCl is interpreted as follows: the gel islands are tightly cross-linked mixed gels. The XCW

molecules in KCl occur not as single, largely-disordered chains (as they principally do in water; Fig. 6(a)) but partly as coaxial or side-by-side helices joined by some single-chain regions which have disordered side chains (Fig. 6(b)).

The xanthan backbone is in the flat ribbon conformation, making it structurally compatible with the galactomannan backbone. When LBG is added, junction zones form between the mannan and xanthan backbones to form tightly cross-linked structures which are the gel islands (Fig. 6(c)). When heated (Experiment 3, Table 1) above $T_c$ (the helix-to-coil transition temperature, which in 0·05 M KCl is below 95°C) the ordered conformation is largely destroyed, and on cooling in the presence of LBG, rearranged junction zones are formed (Fig. 6(e)). Ordered double-helix formation and association of xanthan is limited by network constraints imposed by the mixed junction zones, and the result is a more open, firm gel instead of gel islands. Experiment 5 (Table 1) shows that cooling separately followed by immediate mixing reforms gel islands. Experiment 7 (Table 1) shows that when mixed hot, the two polymer solutions gel on cooling as before. The LBG must be present when the disordered xanthan conformation is available for interaction, and before the xanthan chains can self-associate. Experiments in 0·1 M calcium chloride and in 0·2 M KCl led to immediate formation of fine gel islands, which could not be converted to gels by heating to 95°C. $T_c$ for xanthan is above 95°C under these conditions. This was confirmed by the lack of the sharp order–disorder transition when heating of xanthan solutions was followed by optical rotation measurements. The final optical rotation reading was well below that of disordered xanthan.

Extensive dialysis of gel islands in an ultrafiltration cell led to the formation of a firm homogeneous gel similar to that formed initially in water (Experiment 11, Table 1; Fig. 6(d)). Solid urea added to a mixed gel (Experiments 8 and 12) caused the formation of 'clumps' rather than gel islands. One hazy clump occupied most of the volume, together with a small water phase. Centrifugation even at 2°C did not collapse the clump to a precipitate. The clumps were more like gels, and could be converted to homogeneous gels by heating to 95°C and cooling (Experiment 9). Urea (4 M) stabilises the ordered xanthan conformation but does not raise $T_c$ above 95°C (Frangou *et al.*, 1982). Hence heating and cooling reformed homogeneous gels (Experiments 9 and 13) which were weaker than those before the addition of urea, showing that hydrogen bonding is at least partly involved in gel formation. The pyruvate effect was explored by experiments with pyruvate-free xanthan (PFXCW; Table 2). No gel forms in cold water at the same net concentration (0·25%) of XCW used above. Pyruvate removal stabilises the ordered form (Holzwarth, 1978) and no gel forms in water even on heating and

**TABLE 1**
Melting Points for XCW–LBG Gels (0·5% + 0·5% w/v)

| *Experiment* | *Melting point (°C)* | *Interpretation* |
|---|---|---|
| 1 XCW + LBG in cold water. Mix cold. | 40 | Enough single, non-helical regions are present to allow gelation. |
| 2 Heat[a] and cool gel from Experiment 1. | 42 | Heating causes rearrangement of xanthan and LBG to allow more favourable interactions. |
| 3 XCW + LBG in KCl.[b] Mix cold. | Gel islands | Very tight mixed gel forms. |
| 4 Heat and cool Experiment 3. | 45 | Non-helical xanthan interacts with LBG to form a firm gel on cooling. KCl enhances xanthan–xanthan interactions, and increases melting point. |
| 5 XCW + LBG in KCl. Heat and cool separately. Mix. | Gel islands | Though the helical form was largely destroyed on heating, it reformed on cooling and gel islands formed as in Experiment 3. |
| 6 Heat and cool Experiment 5. | 45 | Reasoning as in Experiment 4. |
| 7 XCW + LBG in KCl. Heat, mix hot, then cool. | 45 | Non-helical xanthan at 95°C cooled in the presence of LBG interacts strongly to yield a firm gel on cooling, as in Experiments 4 and 6. |

| | | | |
|---|---|---|---|
| 8 | Gels from either Experiment 4, 6 or 7. Add solid urea to 4 M. | Clumps[c] | Urea destroys the gel, indicating involvement of hydrogen-bonds. No gel islands. Solid urea has a shock effect. |
| 9 | Heat and cool Experiment 8. | 33 | Urea stabilises the helical form,[d] but destabilises the interaction with LBG, causing a lowered melting point. |
| 10 | (a) Add solid KCl to gel from Experiment 2. | Gel islands | Gel is destroyed on KCl addition, but is reformed after the heat–cool cycle. |
| | (b) Heat and cool. | 42 | Expected melting point is 45°C as in Experiments 4, 6 and 7. |
| 11 | Take solution from Experiment 3. Dialyse KCl away in stirred cell (without heating). | Firm gel ≈ 40 | Removal of KCl gives conditions similar to Experiment 1, and the gel is of similar strength. No exact melting point, as original volume was not exactly maintained during dialysis. |
| 12 | Add solid urea to gel from Experiment 2. | Clumps[c] | Reasoning as for Experiment 8. |
| 13 | Heat and cool Experiment 12. | 31 | Reasoning as for Experiment 9. Melting point is lower than in Experiment 9, as KCl in Experiment 9 enhances xanthan inter-chain interactions.[e] |

[a] 95°C for 30 min.
[b] 0·05 M.
[c] Clumps formed in the presence of urea differ from the gel islands formed in KCl, in being much larger. They do not centrifuge down at 2°C, whereas gel islands do so.
[d] Frangou *et al.* (1982).
[e] Smith *et al.* (1981).

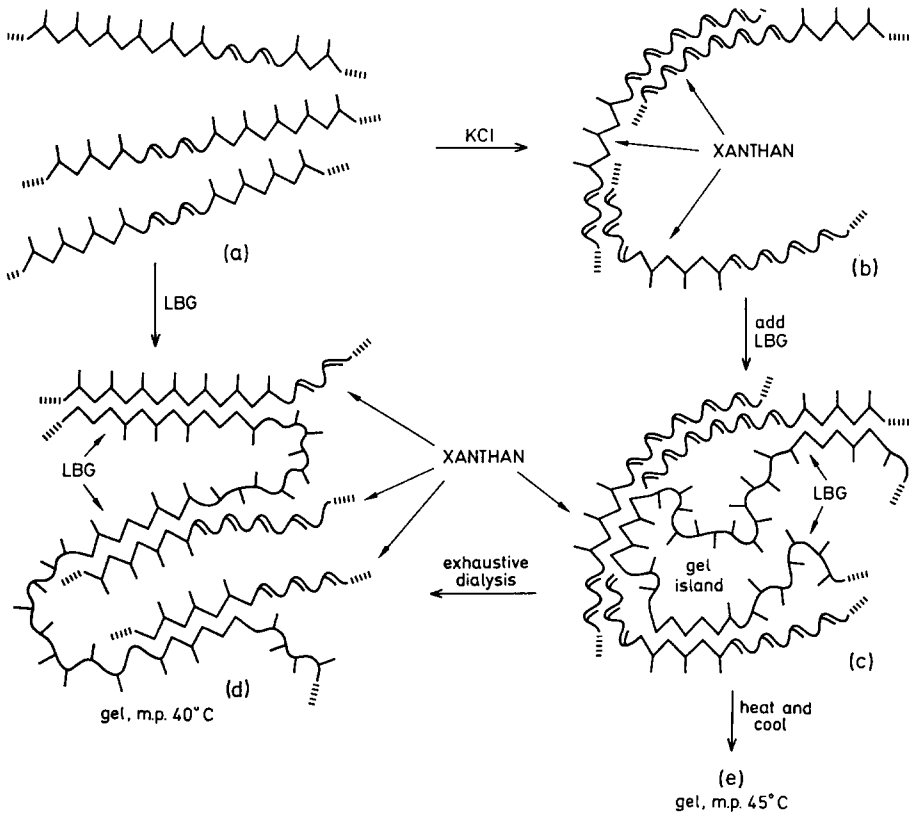


**Fig. 6.** Xanthan–galactomannan gel formation under various conditions. (a) Xanthan (XCW) after dialysis in water to constant conductivity. Zigzag regions represent xanthan in the disordered conformation with side chains extended. A small amount of ordered (helical) conformation is also present. (b) Xanthan (XCW) in 0·02 M KCl. Large regions are in the ordered conformation, which interact to form homogeneous junction zones, but a true gel is not formed. (c) Addition of locust bean gum (LBG) to (b) forms mixed junction zones with xanthan and gel islands result. (d) Exhaustive dialysis of (c) results in loss of most of the ordered regions, and hence loss of homogeneous junction zones. The result is a firm gel with m.p. ≈ 40°C instead of gel islands. (e) Heating of (c) above $T_c$ allows xanthan to rearrange the junction zones in the gel islands to form a homogeneous gel, m.p. 45°C.

cooling. This is an anomalous result, as the $T_c$ for the order–disorder transition should still be below 95°C. In KCl the PFXCW forms gel islands, and a gel is formed only after heating (gel m.p. 46°C). This is the highest melting point found in these experiments, and shows that when the destabilising pyruvate is removed, intermolecular xanthan–xanthan association is enhanced, and junction zones are more firmly locked in place.

**TABLE 2**
Melting Points for Acetate-free (AFXCW) and Pyruvate-free (PFXCW) Xanthan–LBG Gels

| | *Experiment* | *Melting point (°C)* |
|---|---|---|
| 1 | 0·5% AFXCW + 0·5% LBG in water. Mix cold. | No gel — viscous |
| 2 | Heat and cool Experiment 1. | 41 |
| 3 | 0·5% AFXCW + 0·5% LBG in KCl.[a] Mix cold. | Gel islands |
| 4 | Heat and cool Experiment 3. | 43 |
| 5 | 0·5% PFXCW + 1% LBG in water. Mix cold. | No gel |
| 6 | Heat and cool Experiment 5. | No gel |
| 7 | 0·5% PFXCW + 0·5% LBG in KCl. Mix cold. | Gel islands |
| 8 | Heat and cool Experiment 7. | 46 |

[a] 0·05 M KCl.

Acetate-free (AFXCW) xanthan at 0·25% net concentration in water does not gel with LBG until heated and cooled (Table 2). The gel melting point of 41°C at this concentration shows that there is similar interaction with LBG to the parent XCW (gel m.p. 42°C at 0·25% net concentration). It would appear that the presence of *some* ordered conformation enhances gel strength. The AFXCW in water interacts more strongly with LBG (m.p. 41°C) than does the pyruvate-free sample (no gel). Assuming that interaction requires the initial presence of some disordered xanthan side chains, the results with PFXCW and AFXCW are consistent with the observations that acetate stabilises the ordered form of xanthan (Tako & Nakamura, 1984; Smith *et al.*, 1981) and pyruvate destabilises it (Holzwarth, 1978). That *some* degree of ordered conformation enhances gel strength is again indicated by the increase in melting point from 41 to 43°C when AFXCW is changed from water to 0·05 M KCl, heated, and cooled (Table 2). Very similar results to those in Table 2 were obtained with XHW, i.e. material isolated from heated (90°C for 30 min) crude xanthan using the same centrifugation–filtration procedure as for XCW. Thus XHW and XCW appear to have similar molecular properties, but the XHW yield (79%) from the crude starting material is higher than that of XCW (45%), as might be expected.

Table 3 shows the gel melting points obtained with crude xanthan dissolved in cold (CXC) and in hot (CXH) water. In each case the gel melting point was lower than that for the corresponding purified xanthan sample (XCW and XHW respectively). This merely reflects the fact that the crude xanthan did not fully dissolve at room temperature or at 95°C (Morris *et al.*, 1983) and that mainly the soluble xanthan chains are involved in gel formation with LBG. To confirm the latter proposal, the

**TABLE 3**
Melting Points for Crude Xanthan Gels with LBG Dissolved Cold (CXC) and at 95°C (CXH)

| | *Experiment* | *Melting point (°C)* |
|---|---|---|
| 1 | 0·5% CXC + 0·5% LBG in water. Mix cold. | < 25 (very weak gel) |
| 2 | Heat and cool Experiment 1. | 34 |
| 3 | 0·5% CXC + 0·5% LBG in KCl.[a] Mix cold. | Gel islands |
| 4 | Heat and cool Experiment 3. | 39 |
| 5 | 0·5% CXH + 0·5% LBG in water. Mix cold. | Gel islands |
| 6 | Heat and cool Experiment 5. | 39 |
| 7 | 0·5% CXH + 0·5% LBG in KCl. | Gel islands |
| 8 | Heat and cool Experiment 7. | 40 |

[a] 0·05 M KCl.

following experiments were carried out. The centrifuged 'pellet' from the preparation of XCW was resuspended in cold water, and recentrifuged. Very little polysaccharide ( ≈ 5% of total) was recovered from the supernatant liquid, indicating that nearly all xanthan soluble under the conditions had been removed. The pellet was analysed for total carbohydrate, and the carbohydrate concentration was adjusted to 0·5%. Using this suspension–dispersion as the source of xanthan, mixing experiments with 0·5% LBG were performed as previously, in water and in 0·05 M KCl, by mixing cold, then heating. No gel or gel islands were formed except after heating in KCl (m.p. 41–42°C). Most of the truly soluble xanthan had been removed by the cold water extractions, but on heating sufficiently more dissolved to gel in the presence of KCl. The suspension–dispersion, though very viscous itself, did not interact sufficiently with LBG to form a gel. This is taken to confirm that fully soluble xanthan chains are the only ones involved in gel formation.

The above data is consistent with a sandwich model of junction zones similar to that proposed by Cairns *et al.* (1986; 1987) (Fig. 4). Any model would need to accommodate not only bare mannan backbones in the junction zones, but also at least partly-substituted ones. This is necessary to account for results such as those of Dea *et al.* (1986) where *Leucaena leucocephala* (40% galactose) galactomannan has frequent regions in which every second D-mannosyl residue is substituted by D-galactose, and which interacts strongly with xanthan, whereas the galactomannan from *Cyamopsis tetragonolobus* (guar, also 40% galactose), which has a non-regular distribution of D-galactosyl units, does not.

## 4 CONCLUSIONS

The model of Cairns *et al.* (1986; 1987) allows growth of a periodic sandwich structure in two dimensions, but not in the third. The present authors propose a modified structure for junction zones which requires substantial regions of disordered xanthan, rationalises to some extent the proven effect of galactomannan fine structure on xanthan–galactomannan interactions, explains the lack of growth in the third dimension (a matter of degree rather than an absolute requirement) and possibly the 0·52 nm repeat distance for the glucose units in xanthan observed by Cairns and co-workers. Figures 7, 8 and 9 show detailed representations of the mixed junction zones of Fig. 6(d). One galactomannan chain is sandwiched between two xanthan chains. The latter are in the disordered ribbon conformation and their side chains are arranged to form the repeat distance of 0·52 nm, as observed by Cairns *et al.* (1986; 1987). The xanthan chains are stacked above and below the LBG backbone, and chain directions are shown by the arrows. Side chains are represented by triangles. The xanthan trisaccharide side chain is perhaps misleadingly small, for the sake of simplification.

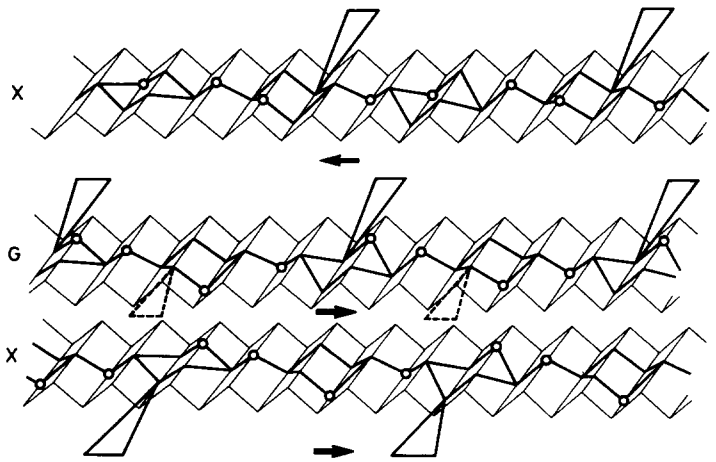


**Fig. 7.** A possible model for xanthan (X) and galactomannan (G) chains in a mixed junction zone. Chains are stacked vertically and are in the ribbon conformation. Chain directions are shown by arrows. The two xanthan molecules are arranged so that their side chains (triangles) are staggered. Galactomannan chains are substituted on one face only (solid triangles). Full substitution of the galactomannan chains (solid plus dotted triangles) is not accommodated because of steric interactions with xanthan side chains.

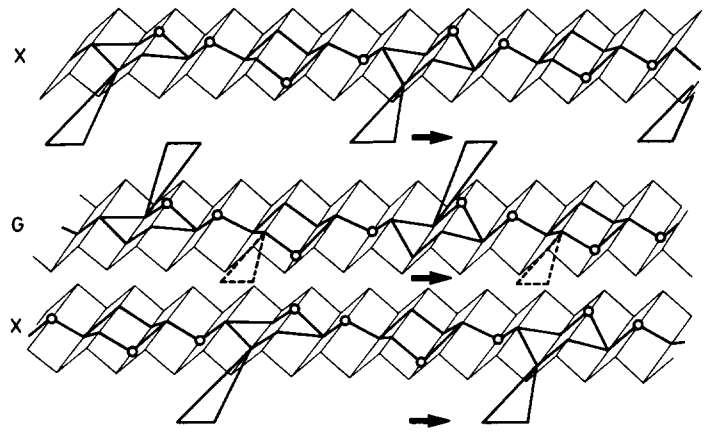


**Fig. 8.** An alternative model to that in Fig. 7. Xanthan side chains remain staggered, but are arranged on the same face, and could interact with side chains on the next xanthan chain below. Again, fully-substituted galactomannan side chains are not likely to be accommodated.

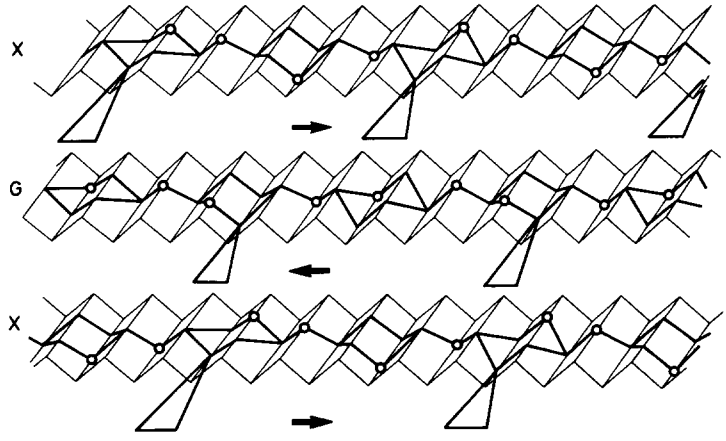


**Fig. 9.** One of a number of unlikely arrangements for xanthan–galactomannan junction zones. Steric interactions between side chains are obvious. Other possible combinations of chain directions, or rotation about either horizontal axis, suffer from similar problems.

There seems little to choose between Figs 7 and 8 on the grounds of steric interaction of side chains. The authors favour Fig. 7, as the large xanthan side chains are on opposite sides of the junction zone. Other LBG molecules could stack above and below the xanthan and extend the junction zones in two dimensions, but presumably not in the third.

Dotted side chains on LBG show that there would be substantial steric interactions for such fully-substituted chains. The models can thus accommodate both unsubstituted and single-face substituted LBG chains in junction zones. The arrangement in Fig. 9 is obviously ruled out, as are others which might be constructed. A specific directionality of chains being required for junction zone formation would help rationalise gel island behaviour. In salt, association of xanthan helices could also involve chain directionality. When LBG is added in the presence of salt, it would interact with the disordered parts of xanthan molecules which already have some directionality imposed on them. This directionality is destroyed on heating above $T_c$ or by exhaustive dialysis. On cooling in the presence of LBG, a rearrangement of junction zones occurs and a homogeneous gel is formed. Further work required to substantiate the above proposals is in progress.

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