



Xanthan/locust bean gum interactions at room temperature

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Rheological and ultracentrifugation studies have been conducted on heated and unheated mixtures of xanthan with whole locust bean gum, and temperature fractions of the latter possessing different mannose:galactose ratios. The results suggest that xanthan and galactomannans may interact by two distinct mechanisms. One takes place at room temperature, gives weak elastic gels, and has little dependence upon the galactose content of the galactomannan, whilst the second requires significant heating of the polysaccharide mixture, gives stronger gels, and is highly dependent upon galactomannan composition. The results are discussed with reference to existing models proposed for the xanthan/galactomannan interaction.

INTRODUCTION

The unusual solution properties and the tendency to undergo rheological modification in the presence of other polysaccharides have led to extensive commercial utilisation of xanthan gum. Xanthan solutions have a high viscosity which is maintained over a wide range of salt concentrations, pH, and temperature, and is also insensitive to the electrolyte species present (Southwick *et al.*, 1983). However, they are very pseudoplastic, with viscosities falling rapidly on the application of an increased rate of shear, but recovering almost instantaneously when the shearing force is removed (Morris *et al.*, 1977).

Xanthan gum is the extracellular polysaccharide of *Xanthomonas campestris*, the primary structure being a pentasaccharide repeating unit (Jansson *et al.*, 1975) with a side-chain consisting of a glucuronic acid residue between two mannose units. The terminal mannose may possess an O-4 and O-6 linked pyruvate moiety; the mannose nearest the main chain carries a single acetyl group at C-6. The rheological behaviour of xanthan solutions has been attributed to the ordered molecular structure adopted under the majority of

environmental conditions (Morris *et al.*, 1977). At high temperature and low ionic strength, xanthan molecules are thought to be present in a disordered-coil conformation but, on cooling, a disorder/order transition takes place and a rigid, helical conformation is adopted. In distilled water, the mid-point of this transition (T_m) has been found to be 55°C, but increasing the ionic strength elevates T_m (Morris *et al.*, 1977; Southwick *et al.*, 1983; Norton *et al.*, 1984). The debate as to whether the ordered form of xanthan is a single or double helix remains active, with convincing arguments being proposed to support both structures (Morris *et al.*, 1977; Norton *et al.*, 1984; Milas & Rinaudo, 1986; Cairns *et al.*, 1987). The high viscosities of xanthan solutions at low-shear have been attributed to weak associations between the helices; a network easily destroyed but rapidly restored on removal of the shearing force (Morris *et al.*, 1977; Dea *et al.*, 1977).

Galactomannans are a series of natural polysaccharides from various sources sharing a similar chemical structure of a β -1-4-D-mannose backbone substituted to varying degrees at the 6 position with single α -linked D-galactose residues, the extent of galactose substitution being a function of the source species. Guar gum from *Cyamopsis tetragonolobus* has a mannose:galactose (M:G) ratio of approximately 2:1,

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whereas locust bean gum from *Ceratonia siliqua* has a M:G ratio of about 3.5:1.

Xanthan and galactomannans interact in solution to give significant synergistic increases in viscosity or gel strength (Dea *et al.*, 1977) and various models have been proposed for the mechanism of interaction. The original model proposed an association between the backbone of the xanthan helix and unsubstituted regions of the galactomannan (Dea *et al.*, 1977; Morris *et al.*, 1977). This model is supported experimentally by a decrease in interaction magnitude with increasing galactose substitution (Dea & Morrison, 1975) and by the apparent ability of galactomannans to stabilise xanthan helices and hence increase the value of T_m . The model was subsequently modified (McCleary *et al.*, 1979) in order to account for the strong interactions between xanthan and certain high-galactose galactomannans. It was found that galactomannan from *Leucaena leucocephala* interacts more strongly with xanthan than would be expected for a galactomannan of such high galactose content (M:G ratio 31:16). However, as approximately one-quarter of the backbone is composed of regions of alternate galactomannan substitution, it was suggested that these regions, where the substituents are all located on one side of the mannan backbone, were also capable of participating in interactions with xanthan.

The model proposed by Tako *et al.* (1984) suggested an association between the side-chains of xanthan and the backbone of the galactomannan, and the structure proposed by these workers assumes that xanthan is in a single-stranded helical conformation in the post-interaction product.

An alternative model has been proposed in which the xanthan helix appears not to be retained in the product of the interaction. Results of X-ray diffraction studies indicated that the original xanthan helix may not be present in the post-interaction structure and the difference in reactivity between sparsely and highly substituted galactomannans was attributed to the inability of highly substituted chains to fit into the space between adjacent xanthan molecules (Cairns *et al.*, 1986). The same authors also noted that addition of salt to the mixtures in order to maintain the xanthan ordered structure whilst undergoing heating, did not result in gel formation and they concluded that the interaction can only occur if the xanthan molecules are in a non-helical form.

Association between xanthan and locust bean gum of very high M:G ratio (5:1), at temperatures below T_m has been noted (Cheetham & Mashimba, 1988) and these authors suggested that the interaction occurs between the mannan backbone of the locust bean gum and disordered sections of the otherwise highly ordered xanthan molecules. It was proposed that heating above the order/disorder transition temperature of xanthan is necessary to rearrange the junction zones and enable

the formation of an homogenous gel network and these observations therefore support the model proposed by Cairns *et al.* (1986).

This study is concerned with the association of xanthan and galactomannans below the order/disorder transition temperature of xanthan. The effect of polysaccharide ratio, galactomannan galactose content and total polysaccharide concentration were investigated using rheological and ultracentrifugation techniques and the results are discussed with reference to the previously described models for the xanthan/galactomannan interaction.

EXPERIMENTAL

Materials

Locust bean gum was obtained from the Sigma Chemical Company, Poole, UK. Xanthan gum (Keltrol F) was a gift of the Kelco division of Merck & Co. Inc., Westminster, UK.

Fractionation of locust bean gum

The method used to separate whole locust bean gum (LBG) was based upon that described by Gaisford *et al.* (1986) and provides fractions according to the minimum temperature at which water solubility can be attained.

(LBG) (20 g) was heated to 90°C for 15 min in 200 ml of 80% v/v ethanol, cooled in an ice bath and excess ethanol decanted. The residue was washed with two 100-ml aliquots of ethanol, suspended in 2 litres of cold distilled water using a Silverson mixer and allowed to hydrate for 1 h at 35°C in a water bath. Insoluble matter was removed by centrifugation for 30 min at 5000 rpm and the solubilised galactomannan precipitated by pouring into twice the volume of ethanol. The precipitate was collected by filtration under vacuum and dried in a vacuum oven overnight at 50°C. Identical extractions were performed on the remaining insoluble portion after hydration at 50°C, 65°C and 80°C. On completion, the LBG had been separated into four fractions having solubilities of less than 35°C (LBG35), 35–50°C (LBG50), 50–65°C (LBG65), and 65–80°C (LBG80). Material insoluble at 80°C was discarded. After drying, the fractions were ground to a fine powder using a hammer mill (Glen Creston, Stanmore, UK).

Mannose:galactose (M:G) ratios of the locust bean gum fractions were determined by GLC analysis of their alditol acetate derivatives.

Preparation of solutions

The xanthan and galactomannan solutions used for rheological experiments were prepared in 0.1 M sodium

chloride solution with 0.005 g litre⁻¹ sodium azide added to prevent bacterial degradation. An amount corresponding to the desired dry weight of polysaccharide was dispersed in the solvent, the vessel sealed, and the temperature raised to 80°C for 30 min with continuous stirring at high shear rate. All solutions were heated to this temperature to give all samples equal exposure to heat, as it would have been necessary to attain this temperature to achieve solubilisation of the LBG80 fraction. Solutions were allowed to cool to room temperature overnight prior to mixing.

Mixing of xanthan and galactomannan solutions

Portions of xanthan and galactomannan solutions were mixed for 30 s at a high shear rate using an Ultra Turax mixer. All mixed and single polysaccharide solutions were allowed to stand for a further 24 h before rheological measurements were commenced.

Rheological measurements

Oscillatory rheological measurements were performed at 25°C using a Rheometrics Fluids Rheometer Model 7800, fitted with Couette geometry ($R_1/R_2 = 0.92$) over a frequency range of 10⁻¹ to 10 radians s⁻¹. Prior to a frequency sweep, a strain sweep was executed to ensure that studies were being performed in the linear viscoelastic region. Unless otherwise stated, all solutions had a total polysaccharide concentration of 0.1 g litre⁻¹ and a strain amplitude of 0.5% was used. The solutions containing equal proportions of xanthan and LBG temperature fractions were also tested after heating to 60°C and cooling overnight prior to repeating the frequency sweep.

Dilute solution continuous shear viscometry

Stock solutions containing approximately 0.01 g litre⁻¹ were prepared, and Newtonian viscosities were obtained for a series of dilutions using a Contraves Low Shear 30 viscometer fitted with Couette geometry ($R_1/R_2 = 0.92$). Intrinsic viscosities were obtained by extrapolation of the reduced viscosities to zero concentration using the Huggins equation (1):

$$\eta^{\circ}sp/C = [\eta] + K[\eta]^2C \quad (1)$$

where $\eta^{\circ}sp$ is the zero shear specific viscosity at concentration C , and K is the Huggins coefficient.

For mixtures, solutions of xanthan and LBG35 were mixed in equal proportions by gentle shaking, a series of dilutions prepared and the viscosity of each determined as above at a range of shear rates commencing at the maximum possible with this machine and geometry (118.2 s⁻¹).

Ultracentrifugation

Sedimentation coefficients (sedimentation velocity/field strength) for xanthan, LBG35, and a 50:50 mixture were determined at 25°C in 0.1 M aqueous sodium chloride over a range of concentrations using a MSE Centriscan analytical ultracentrifuge fitted with Schlieren optics and an appropriate multiplexing system. Loading concentrations were less than 2 mg ml⁻¹ for the single polysaccharides and 1 mg ml⁻¹ for the mixture. A rotor speed of 5000 rpm was used initially, but increased to 10 000 rpm and 47 000 rpm as sedimentation of single polysaccharides was followed using Schlieren optics whereas scanning absorption optics were used to follow sedimentation of the complex in the mixed solutions. Sedimentation coefficients were extrapolated to zero concentration and the values obtained adjusted to standard conditions (Van Holde, 1971) where possible.

RESULTS AND DISCUSSION

Dynamic rheology of unheated xanthan and whole locust bean gum (LBG) mixtures

The mechanical spectra of 0.1 g litre⁻¹ solutions of xanthan, LBG, and an unheated mixture containing an equal proportion of the two are shown in Fig. 1 and illustrate the different mechanical properties of the three solutions. The LBG spectrum is typical of a viscous solution where the loss modulus is greater than the storage modulus at all frequencies studied, and both parameters are dependent on oscillation frequency. The spectrum of xanthan gum clearly illustrates that, when subjected to the small displacements applied during the measurements, it behaves as a gel rather

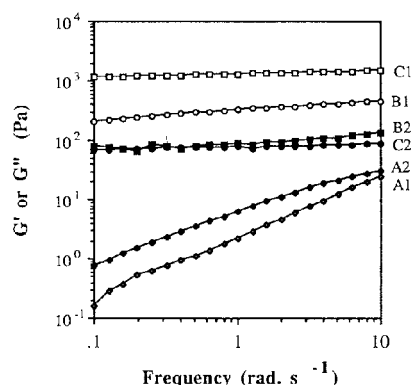


Fig. 1. Rheological spectra for (A) xanthan gum, (B) whole locust bean gum, and (C) a 50:50 cold-mixed solution. Total concentration 0.1 g litre⁻¹ in 0.1 M NaCl. Curves A1, B1, C1 are storage moduli (G') and A2, B2, C2 are loss moduli (G'').

than a viscous solution. The storage modulus is greater than the loss modulus at all the frequencies studied and both parameters are less frequency dependent than they are for LBG. The mechanical spectrum of the mixed polysaccharide system shows that it also behaves as a gel under the experimental conditions employed, but has a much greater storage modulus than xanthan alone.

Figure 2 shows how the dynamic rheological parameters of xanthan/LBG mixtures vary with respect to polysaccharide ratio. It shows that an interaction is occurring when xanthan and LBG are mixed at room temperature, resulting in significantly enhanced storage moduli. The loss modulus alters little, suggesting that the synergy is primarily an increase in the quantity or duration of cross-linking between the two polysaccharides. Maximum synergy occurs with mixtures containing equal proportions of the two polysaccharides where the storage modulus at 1 radian s^{-1} , was more than 4 times that of xanthan alone.

The solutions were mixed at room temperature and the rheological experiments were performed at 25°C in 0.1 M NaCl and it is generally accepted that at this temperature and ionic strength the xanthan molecules will be in the helical conformation (Morris *et al.*, 1977; Southwick *et al.*, 1983; Norton *et al.*, 1984). At first sight, therefore, this result cannot be accommodated by the model proposed by Cairns *et al.* (1986) which would require xanthan to be in the disordered conformation in the presence of the galactomannan. The result could be interpreted as per Cheetham & Mashimba (1988) as indicating that portions of the xanthan molecule are in the disordered conformation under these conditions, however, the observation that a significant interaction occurs under these conditions, where the majority of evidence suggests the xanthan will be entirely in the helical conformation (Southwick *et al.*, 1983; Norton

et al., 1984), supports the original model described by Dea *et al.* (1977) and Morris *et al.* (1977).

Effect of galactomannan temperature fraction

The storage moduli of cold-mixed solutions containing xanthan and different locust bean gum temperature fractions are shown in Fig. 3 and the M:G ratios of the fractions in Table 1. In general, the magnitude of the rheological synergy in these mixtures showed little dependence on galactomannan temperature fraction and the slight variability observed may simply be a reflection of the different self-association tendencies of the various fractions. Table 1 shows that the storage moduli of the galactomannan solutions increased dramatically with increasing M:G ratio and, in physical terms, the LBG35 fraction was a viscous solution, whilst the LBG80 fraction was a soft, flexible gel. Galactomannan self-association requires the presence of sections of mannan backbone with a low degree of galactose substitution (Dea *et al.*, 1977) and obviously these will become more plentiful with increasing M:G ratio. There are several possible explanations why the interaction between cold-mixed xanthan and galactomannan solutions appears independent of galactomannan composition within the range studied. For example, the stacking of xanthan helices may be disrupted relatively little on mixing, resulting in few binding sites on the xanthan molecules being available. Hence, even the LBG35 galactomannan fraction, which has the highest galactose content, may possess sufficient galactose-free regions to make maximum use of these binding sites. However, the relative positions of the curves shown in Fig. 3 do not support this to any great extent as, if availability of binding sites on the

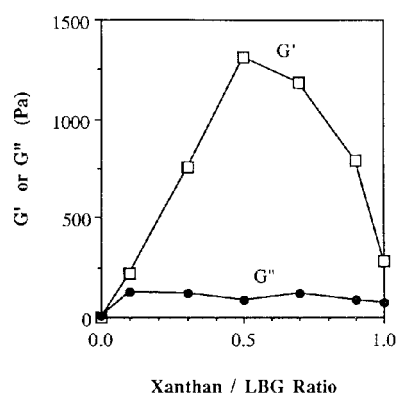


Fig. 2. Storage (G') and loss (G'') moduli of cold-mixed xanthan/whole locust bean gum (LBG) mixtures at 1 radian s^{-1} .

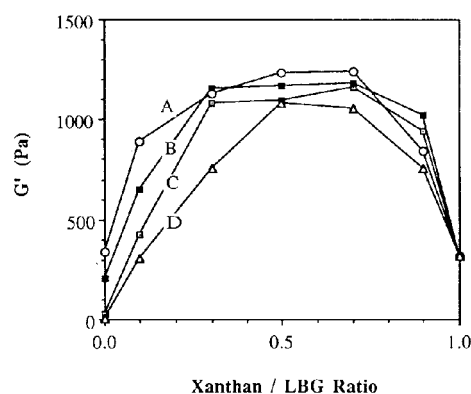


Fig. 3. Storage moduli of xanthan cold-mixed with different locust bean gum temperature fractions. (A) LBG80, (B) LBG65, (C) LBG50, (D) LBG35.

Table 1. Properties of locust bean gum fractions

Fraction	Intrinsic viscosity (d litre g ⁻¹)	Huggins constant	Storage modulus (Pa)	Loss modulus (Pa)	M:G ratio
LBG35	10.3	1.11	5.5	27.0	2.85
LBG50	12.1	1.00	29.5	66.0	3.55
LBG65	13.0	1.10	203.2	107.9	4.56
LBG80	9.6	2.71	337.7	124.4	5.25

Dynamic rheological values were determined at a frequency of 1 radian s⁻¹.

xanthan helices was the limiting factor, the curves of the different temperature fractions would be expected to diverge as the proportion of xanthan in the mixture is increased. An alternative explanation is that the greater self-association of the higher temperature galactomannan fractions may lead to a more heterogeneous mixture, reducing the possibility of xanthan and galactomannan binding sites being adjacent, and balancing out any enhanced synergy as a result of increased numbers of galactose-free regions on the galactomannan backbone. In addition, galactomannan self-association may reduce the number of galactose-free sites available for binding with xanthan if the same binding sites are involved.

Although the results do not permit a decision as to which, if any, of these explanations are correct, they do show that the factors affecting the extent of synergy of cold- mixed solutions are significantly different from those of hot- mixed solutions.

In Fig. 3, there is a broad region where the ratio of the two polysaccharides has little effect upon the storage modulus of the mixture and for all LBG fractions studied, the maximum storage modulus was between 3 and 4 times that of a xanthan solution of the same total concentration. The LBG35 fraction did give a slightly lower peak than the others, which may suggest a threshold M:G ratio below which the interaction

cannot occur but, alternatively, this observation may be due to differences in the storage moduli of the galactomannans being expressed in the mixtures.

Rheology of mixed solutions after melting and cooling

Table 2 shows the values of the storage moduli of 50:50 mixtures after melting at 60°C and allowing to reset at room temperature. The temperature to which the mixtures were heated is significantly below the T_m for xanthan at an ionic strength of 0.1 M, and other workers have found no gelation under these conditions (Cairns *et al.*, 1986), however, whereas the storage moduli of the xanthan/LBG35 mixture remained relatively unchanged, those of the higher temperature fractions were significantly increased.

There are several reasons why storage moduli may be increased by melting at temperatures below T_m and resetting. Firstly, heating the mixtures above the melting temperature may allow more complete molecular mixing. If the mixtures of xanthan with the higher temperature galactomannan fractions are progressively more heterogeneous as a result of galactomannan self-association, then this would account for the increasing differences in storage modulus seen between heated and unheated mixtures. Secondly, the input of energy may facilitate inter-

Table 2. Effect of galactomannan extraction temperature on the storage modulus of heated and cooled mixtures of xanthan and locust bean gum fractions

Fraction	Storage modulus (Pa)
LBG35	1162
LBG50	1615
LBG65	1942
LBG80	3429

Dynamic rheological values are those obtained at 1 radian s⁻¹ for solutions containing equal amounts of xanthan and galactomannan.

actions which are not possible at room temperature and, if so, the total synergy observed after heating will be the sum of a two-interaction process, one which can take place at room temperature and depends little upon galactomannan composition, and the other requiring a significant energy input which is sensitive to M:G ratio. It is also possible that one type of interaction takes place with xanthan in the ordered form, and the second occurs with disordered areas of xanthan, generated at temperatures significantly less than the published values for T_m . Detailed studies of the xanthan coil/helix transition (Norton *et al.*, 1984) suggest that the concept of a sharp conformational change occurring at T_m may be oversimplistic. Results obtained by these workers indicate that within a given temperature/ionic strength window, xanthan may consist of alternate ordered and disordered sections and that the minimum temperature at which this conformational heterogeneity occurs is 70°K below T_m . Using eqn (2) to estimate T_m using values for the constants m and P calculated from data obtained by Norton *et al.* (1984), gives an estimated T_m of 114°C at an ionic strength of 0.1 M.

$$1/T_m(^{\circ}\text{Kelvin}) = m \log I + P \quad (2)$$

Previous studies (Dea *et al.*, 1977) have shown that the presence of galactomannan increases the order/disorder transition temperature of xanthan by approximately 10°C and hence the T_m of xanthan in the mixtures examined in this study will be approximately 124°C. Conformational heterogeneity would therefore be expected at temperatures above 55°C and the disordered sections of the xanthan molecules will be free to interact with LBG according to the Cairns model. As was found in this work, the storage moduli of gels produced at 60°C would depend upon galactomannan galactose content. According to this model, at 25°C xanthan will be present only in the helical form and, at this temperature, the magnitude of the interaction was found to be almost independent of the galactomannan fraction. This, and the stabilising effect which galactomannans exert on xanthan helices (Dea *et al.*, 1977), suggests a second, alternative interaction process occurring at low temperature in which the xanthan helix is retained. The results here do not permit this association to be modelled other than speculatively.

Dilute solution studies

Viscosity/shear rate relationships for 50:50 mixtures of xanthan and LBG35 are shown in Fig. 4. A high shear viscosity-plateau was observed for each concentration studied. The extreme shear-thinning at relatively low shear rates, and the existence of an easily attained high-shear Newtonian region is indicative of the breaking down of a weak intermolecular network to give isolated

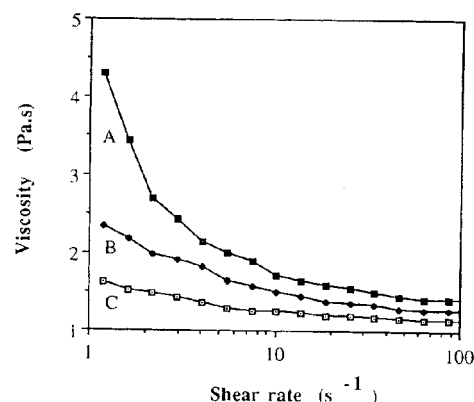


Fig. 4. Dilute solution viscosity/shear rate relationships for 50:50 mixtures of xanthan with fraction LBG35. Total concentration (A) 9×10^{-4} g litre $^{-1}$, (B) 1.1×10^{-3} g litre $^{-1}$, (C) 1.2×10^{-3} g litre $^{-1}$.

aggregates. Hence the results support the hypothesis of Cuvelier & Launay (1988) that xanthan and galactomannans in combination form a super-aggregate network which may be reversibly disrupted by shearing to give aggregates.

Concentration dependence of synergy in cold-mixed solutions

Figure 5 shows how the storage moduli of xanthan, LBG and 50:50 mixtures change with concentration. The ratio of the storage moduli of mixed solutions to those of xanthan at the same total polysaccharide concentration stays approximately constant (Fig. 6) enabling the following relationship to be derived:

$$G'(\text{mix}) = Q G'(\text{xanthan})$$

Where Q is a constant, the magnitude of which indicates the extent of synergy and which, over the

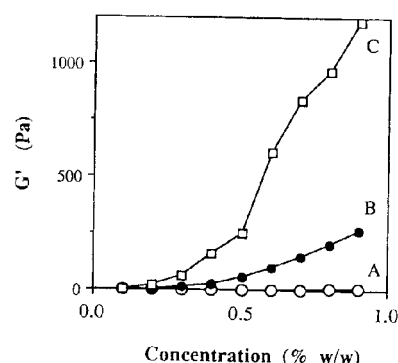


Fig. 5. Storage moduli/concentration relationships for (A) xanthan, (B) whole locust bean gum and (C) a 50:50 mixture.

Xanthan/locust bean gum interactions

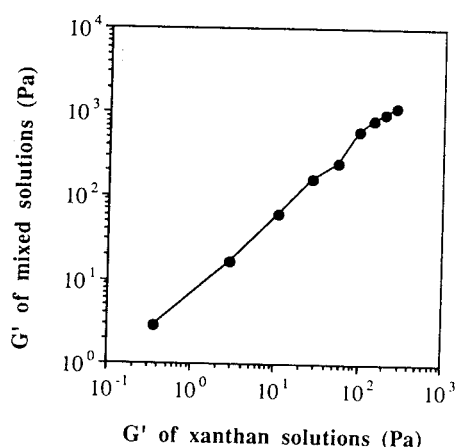


Fig. 6. Storage moduli of 50:50 xanthan/whole locust bean gum combinations against those for xanthan at the same total polysaccharide concentration.

Table 3. Sedimentation coefficients of xanthan, LBG35, and the product formed after cold-mixing

Polysaccharide	$S_{20, w}$
Xanthan	7.52 Svedbergs
LBG35	3.26 Svedbergs
Xanthan/LBG35 mix	60 Svedbergs (approximate ^a)

^aNot corrected to standard conditions.

concentration range studied, was found to be approximately 6.

Ultracentrifugation

Table 3 shows the sedimentation coefficients of xanthan, LBG35, and a 50:50 mixture. The much higher sedimentation coefficient of the polysaccharide combination was obtained without adjustment for the viscosity and density of the solution and such an adjustment would have further increased the sedimentation coefficient of the mixed system. The results suggest the presence of large aggregates in the mixed solution.

CONCLUSION

The results suggest that xanthan and galactomannans may interact by two distinct mechanisms. The first

requires heating of the mixture to within 70°C of the T_g of xanthan, giving storage moduli that depend on the galactose content of the galactomannan. The storage moduli of the gels produced tend to be significantly higher than those of unheated gels of the same composition. The second mechanism takes place at room temperature and gives weaker, more flexible gels whose rheological properties do not depend on galactose content, and in which the xanthan helix is retained in the post-interaction product.

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REFERENCES

- Cairns, P., Miles, M.J. & Morris, V.J. (1986). *Nature*, **322**, 89.
- Cairns, P., Miles, M.J., Morris, V.J. & Brownsey, G.J. (1987). *Carbohydr. Res.*, **160**, 410.
- Cheetham, N.W.H. & Mashimba, E.N.H. (1988). *Carbohydr. Polym.*, **9**, 1.
- Cuvelier, G. & Launay, B. (1988). *Carbohydr. Polym.*, **8**, 271.
- Dea, I.C.M. & Morrison, A. (1975). *Adv. Carbohydr. Chem. Biochem.*, **31**, 241.
- Dea, I.C.M., Morris, E.R., Rees, D.A., Welsh, E.J., Barnes, H.A. & Price, J. (1977). *Carbohydr. Res.*, **57**, 249.
- Gaisford, S.E., Harding, S.E., Mitchell, J.R. & Bradley, T.D. (1986). *Carbohydr. Polym.*, **6**, 423.
- Jansson, P.E., Kenne, L. & Lindberg, B. (1975). *Carbohydr. Res.*, **45**, 275.
- McCleary, B.V., Clark, A.H., Dea, I.C.M. & Rees, D.A. (1979). *Carbohydr. Res.*, **71**, 205.
- Milas, M. & Rinaudo, M. (1986). *Carbohydr. Res.*, **158**, 191.
- Morris, E.R., Rees, D.A., Young, G., Walkinshaw, M.D. & Darke, A. (1977). *J. Mol. Biol.*, **110**, 1.
- Norton, I.T., Goodall, D.M., Frangou, S.A., Morris, E.R. & Rees, D.A. (1984). *J. Mol. Biol.*, **175**, 371.
- Southwick, J.G., Jamieson, A.M., Blackwell, J. (1983). *Carbohydr. Res.*, **99**, 117-27.
- Tako, M., Asato, A. & Nakamura, S. (1984). *Agric. Biol. Chem.*, **48**, 2995.
- Van Holde, K.E. (1971). *Physical Biochemistry*, 2nd edn. Prentice-Hall, Englewood Cliffs, N.J., p. 105.