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Influence of the Acetyl Substituent on the Interaction of Xanthan with Plant Polysaccharides — II. Xanthan–Guar Gum Systems

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ABSTRACT

A range of xanthans (Na^+ salt form) with varying levels of acetyl and pyruvic acid substitution were prepared by culturing different strains of Xanthomonas campestris *and by chemical deacetylation and depyruvylation. Oscillatory-shear measurements were used to characterize the behaviour of xanthan and guar gum alone, and of mixtures of the two in de-ionized water — the xanthan under these conditions was largely in the disordered form. The mechanical spectra of the blends resembled an 'entanglement network' system and showed some features characteristic of the individual components. However, evidence from both rheological and chiroptical measurements indicated a possible weak interaction between some low-acetyl xanthans and guar.*

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INTRODUCTION

As described in Part I of this study (Shatwell *et al.*, 1990*c*), xanthan, the exocellular polysaccharide slime from *Xanthomonas campestris* undergoes a 'synergistic interaction' with the plant galactomannans. We described how it interacts with locust bean gum (LBG) to form a thermoreversible-gel network and verified previous reports that the strength of the interaction depends markedly on the amount of acetyl carried by the bacterial polysaccharide. In Part II, we investigate the behaviour of guar gum, the polymer from the seeds of *Cyamopsis tetragonoloba*; in this case it is generally recognized that gelation does not occur, but there is evidence that the viscosity of the mixture is significantly enhanced.

According to Dea *et al.* (1977, 1986), xanthan interacts with the unsubstituted or poorly substituted regions of the mannose backbone or with regions substituted on one side only, and hence the strength of the interaction with the galactomannans is heavily dependent on the number and distribution of the galactose side-chains. LBG therefore, which carries typically 21–23% galactose and contains a significant number of unsubstituted blocks, gels quite strongly with xanthan, whereas guar gum, which contains 36–40% galactose and has the side-chains arranged mainly in pairs and triplets (McCleary *et al.*, 1985), does not. If however, galactose is removed from the guar gum, using a highly purified α-galactosidase, not only can gelation be induced, but the strength of the gel is observed to increase as the galactose content diminishes (McCleary *et al.*, 1981, 1984).

The nature of the supposed interaction between xanthan and guar gum leading to the enhanced viscosity is, so far, only poorly understood. Tako and Nakamura (1985) reported an enhanced interaction between deacetylated xanthan and guar gum. Dynamic viscoelasticity values measured for a series of xanthan–guar gum mixtures at a range of different polysaccharide ratios (total concentration, 0·2%) showed very little difference for mixtures of native xanthan, but with deacetylated xanthan the 'dynamic viscoelasticity increased', approaching an optimum at xanthan to guar-gum ratios of 2:1. On the basis of a whole series of rheological measurements, it was suggested that native xanthan and guar gum interact only weakly because the presence of the galactose side-chains on every 2–4 backbone residues prevents insertion of the xanthan side-chains into the galactomannan. To support their model they proposed that an increase in the flexibility of xanthan upon deacetylation facilitated greater association.

In the present paper, attempts are made to characterize the behaviour of xanthan–guar systems in de-ionized water using small-deformation, oscillatory-shear measurements. In particular, we have examined the interaction of guar gum with a range of xanthans with different levels of acetyl and pyruvic acid substitution. Using a total polymer concentration of 1·5% (1% guar: 0·5% xanthan), mechanical spectra have been measured for six different xanthan samples, and compared with the behaviour of the guar and xanthan samples on their own. Complementary optical-rotation measurements have been carried out on a limited number of samples to investigate the conformational behaviour of the mixed systems.

EXPERIMENTAL

Materials

Samples of xanthan were produced from the following bacterial strains grown in batch culture: *X. campestris* pv. *campestris* 646 (ATCC 13951), *X. campestris* pv. *phaseoli* 1128 and *X. campestris* pv. *phaseoli* 556 (both from the National Collection of Plant Pathogenic Bacteria, Harpenden, Herts, UK). The materials were recovered from the culture supernatant by precipitation in acetone, purified by ultracentrifugation and dialysis and converted to the sodium salt form by passage through ion-exchange resins. Deacetylation and depyruvylation were achieved by alkaline and acid hydrolysis, respectively. Details of the methods employed are given in Part I (Shatwell *et al.*, 1990*c*).

Guar gum was purified from a commercial flour (Hercules Ltd, London, UK) using the procedure described for LBG in Shatwell *et al.* (1990*c*).

Chemical and physical analyses

The neutral sugar content of the materials was measured by HPLC. The glucuronic acid content and the levels of acetyl and pyruvic acid substitution were determined by colorimetric assay.

Molecular weights were determined by static light scattering. Intrinsic viscosities $[\eta]$ were measured in 20 mM NaCl. Details of the methods employed are given in another publication (Shatwell *et al.*, 1990*a*).

Polarimetry

Optical rotation (OR) measurements were made on systems comprising 0·2% (w/w) xanthan and 0·4% (w/w) guar gum. Solutions of each polysaccharide were prepared at double the required concentration, by dispersing the polymer in de-ionized water (dissolution by stirring and heating at 90°C in a water bath) and filtering through a 0·45 μm Millipore filter. The solutions were then combined by weight in equal quantities, and stirred hot to ensure homogeneity.

Measurements were carried out at 365 nm (Hg emission line) using a Perkin-Elmer 241 polarimeter and 10-cm, quartz thermostatted cells. The cell temperature was controlled by a Haake F3 refrigerated water bath, and the temperature was measured with a chromel–alumel thermocouple. Cells were filled with the sample solution at 90°C, and after degassing the temperature was dropped to 15°C (bath cooling rate ≈ 1°C/min) and maintained at this overnight (16 h). The OR was determined over a temperature range of 15–91°C. The temperature was raised in 3°C increments, allowing 10 min at each step for the sample to equilibrate before taking the reading. For the cooling sweeps the time increments were the same, but measurements could be made immediately after degassing.

Mechanical spectroscopy

Steady and oscillatory-shear measurements were made using a Rheometrics Mechanical Spectrometer RMS-605 with automatic computation and plotting of results. Measurements on solutions of xanthan (2%, w/w) and guar gum (1%) were made using a 72-mm cone (cone angle 0·04 radian) and plate assembly in combination with the normal (TC-2000) transducer. Measurements on mixtures of xanthan (0·5%, w/w) and guar gum (1%, w/w) were made using 50-mm-diameter parallel plates (with a gap of 1 mm) and the TC-2000 transducer.

Samples were prepared by dissolving the freeze-dried polymers in de-ionized water in screw-top 1-oz bottles. Dissolution was achieved by high-speed shearing for 5 min, at 85°C, using a top-drive Atomix, autoclaving for 5 min at 110°C and then shearing again for 5 min at maximum speed. Air bubbles were removed by centrifugation. Single component systems were loaded onto the instrument at ambient temperature (22 ± 1°C). Mixed systems were reheated and loaded at 85°C and the periphery of the sample was sealed with a light silicon oil (Dow Corning 200/10 cs) to prevent water loss.

RESULTS

Chemical and physical analyses

A range of xanthans with very different levels of acetyl and pyruvic acid substitution (Table 1) was generated by culturing different strains of *X.*

TABLE 1

Oscillatory-Shear Measurements for Xanthan (0·5%)–Guar Gum (1·0%) Mixed Systems

Xanthan	*Acetyl substitution (%)*	*Pyruvic acid substitution (%)*	*Slope of η^* at low frequencies*	*Slope of η^* at high frequencies*	*Cross-over frequency (rad/s)*
ps.646	4·5	4·4	−0·53	−0·77	0·0143
ps.1128	7·7	1·7	−0·54	−0·69	0·082
DA ps.1128[a]	1·6	1·3	−0·73	−0·73	none
DA ps.1128[b]	1·5	1·3	−0·71	−0·77	none
ps.556	1·6	6·0	−0·75	−0·84	none
DP ps.556	1·1	1·0	−0·41	−0·70	0·3

[a] Batch 1.
[b] Batch 2.

campestris and by chemical deacetylation and depyruvylation. These materials were analysed chemically and their $[\eta]$ values were determined in 20 mM NaCl. (The molecular weights of three native materials were also determined by light scattering.) The data is summarized in full in the first paper of this series (Shatwell *et al.*, 1990*c*).

The guar-gum sample employed in the present work comprised 36% galactose and 64% mannose; $[\eta]$ was 15·6 dl/g. Using the Mark–Houwink parameters of Robinson *et al.* (1982), the weight-average molecular weight was $22{\cdot}5 \times 10^5$, and this may therefore be regarded as a typical high molecular weight sample.

Oscillatory-shear measurements on guar and xanthan solutions

Steady and oscillatory-shear measurements were used to characterize the behaviour of both guar and xanthan samples in de-ionized water. Figure 1 shows the so-called Cox–Merz plot for a solution of 1·0% guar gum. The behaviour is typical of an entanglement system (Robinson *et al.*, 1982; Richardson & Ross-Murphy, 1987). At low frequencies the response is predominantly liquid-like; G'' is greater than G', and the two are heavily frequency dependent. As the oscillatory frequency increases, however, G' rises faster than G'', and the two eventually cross at a frequency (ω) of about 1·5 rad/s. Above this, the behaviour resembles that of a solid; G' is greater than G'', and the two are far less frequency dependent; η^* shows the converse behaviour. At low frequencies it is essentially frequency independent, but the slope of η^* increases with the oscillatory frequency. The viscosity (η) of the guar-gum solution decreases with increasing shear rate, and η and η^* are closely superimposable, except at the highest frequencies. Such behaviour is typical of conformationally disordered polysaccharides.

A simple explanation for this type of behaviour is that, at high frequencies the interchain entanglements do not have sufficient time to come apart within the period of one oscillation, and so the solution behaves essentially like a gel. At lower frequencies, the principal response is rearrangement of the network to accommodate strain (flow), and so here G'' becomes predominant, as is the case for dilute solutions.

Guar gum appears to obey the Cox–Merz rule (Richardson & Ross-Murphy, 1987). This states that for many polymer solutions the frequency dependence on η^* and the shear rate dependence of η are closely superimposable when the same numerical values of ω and $\dot{\gamma}$ are compared (except for slight deviations which occur for dilute solutions and at high frequencies). This empirical correlation is followed by most solutions of disordered polysaccharides (Morris & Ross-Murphy, 1981).

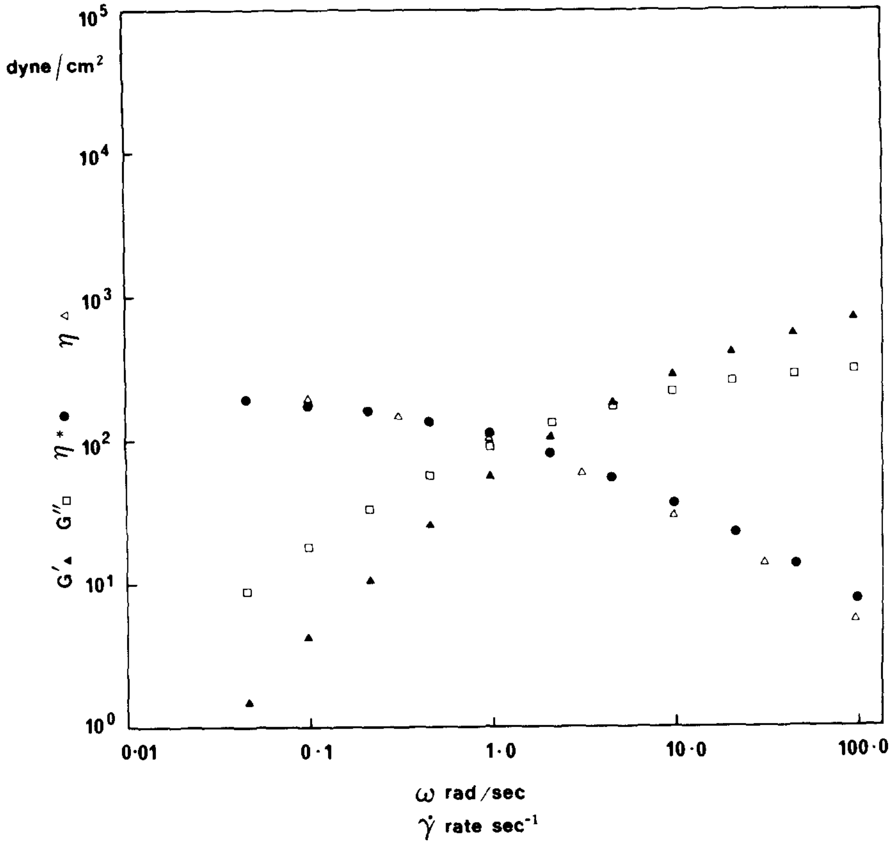

Fig. 1. Steady and oscillatory shear measurements for 1% guar gum in de-ionized water (Cox–Merz plot).

Figure 2 shows the Cox–Merz plot for a 2% solution of ps.646 in deionized water (attempts to measure a 0·5% solution were unsuccessful, as inadequate torque was generated). In salt solution, xanthan showed typical weak gel-network behaviour, but in the absence of added salt the response was quite different. The viscosity of the solution was significantly lower than that of the same sample in the presence of salt, and reliable data was therefore obtainable only at the higher frequencies. The frequency sweep actually looks more like the high-frequency region of an entanglement system than that of a weak gel. G′ is only slightly greater than G″, and the two approach one another at low frequencies. η and η^* are almost superimposable. Similar behaviour was observed for ps.1128, and deacetylated (DA) ps.1128, although the behaviour of the latter was slightly more ‘gel like’.

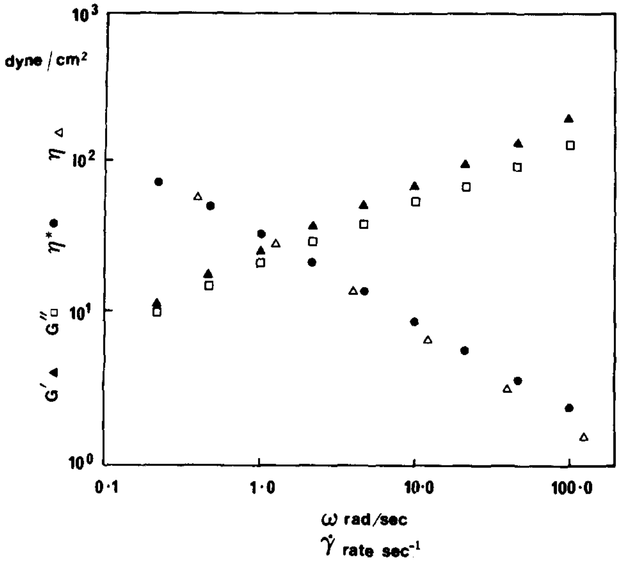

Fig. 2. Cox–Merz plot for 2% ps.646 in de-ionized water.

The very different behaviour of xanthan in de-ionized water may be explained by considering the nature of the polymer under the conditions used in the experiment. The sample was prepared as for the mixed-gel experiments and the solution was then allowed to cool to ambient temperature before loading onto the RMS. Optical-rotation studies on xanthan (Shatwell *et al.*, 1990*b*) revealed that in the absence of added salt the polymer took longer to re-adopt the ordered conformation on cooling, than it did to undergo the initial helix–coil transition. This suggests that under the conditions of the experiment, xanthan existed predominantly in the disordered state. It seems likely, therefore, that in the disordered state the intermolecular interactions between xanthan molecules were reduced and the viscoelastic behaviour of the polymer was governed by purely topological entanglements, as it is for other polymers, particularly random-coil polysaccharides. (Had the polymer solution been allowed longer to cool, the system may have become more gel-like.)

Oscillatory-shear measurements of xanthan–guar gum mixtures

Oscillatory-shear measurements were made upon mixed systems comprising 0·5% (w/w) xanthan and 1·0% (w/w) guar galactomannan in de-ionized water. The protocol for these experiments is given in Shatwell *et al.* (1990*c*).

Figure 3 shows a typical cooling sweep for a xanthan (ps.646)-guar gum mixed system (measurements at 10% strain and 10 rad/s). As the temperature drops from 75 to 25°C, G′ increases very slightly and tan δ decreases, but G″ remains essentially unchanged. The lack of any significant transition is consistent with the known inability of xanthan to gel with guar gum. The tan δ plots for the same system upon cooling and reheating are not given, but there was only a very slight decrease in tan δ on cooling, and no thermal hysteresis.

The strain sweep for the xanthan-guar gum system is also not shown. However, at a frequency of 10 rad/s, G′, G″, G* and tan δ showed no strain dependence (except at the very lowest strains where instrument sensitivity is limited) up to a value of 25%.

The frequency sweep for ps.646 and guar gum is shown in Fig. 4. The spectrum resembles that of an entanglement system. At high frequencies, G′ is significantly greater than G″, and the two are fairly frequency independent. At lower frequencies they approach one another, the frequency dependence increases and the two eventually cross at a ω of 0·0143 rad/s. η^* decreases with increasing frequency and becomes more frequency dependent. (Similar behaviour was reported by Clark (1988) for a 0·5% xanthan-guar gum blend (ratio 1:1) in 0·01 M NaCl).

Table 1 gives the slope of η^* values for each system at both high and low frequencies, and the cross-over frequency for G′ and G″ where present.

The behaviour of ps.1128 and depyruvylated (DP) ps.556 was similar to that described above and appeared to be a composite of that for the individual polymers in de-ionized water (Figs 1 and 2). That of ps.556 and DA ps.1128 was a little different and is typified by the frequency sweep shown in Fig. 5. Here, G′ and G″ show markedly less frequency dependence at the lower frequencies, and instead of crossing over, run almost parallel. This may be an indication of some weak intermolecular association within the system, and it is interesting to note that with the exception of depyruvylated ps.556 it was the polymers with little or no acetyl substitution that appeared to interact in this way.

Polarimetry

Polarimetry has been used to study the interaction behaviour of a number of mixed polysaccharide systems including, for example, the interactions of both agarose and κ-carrageenan with the galactomannans (Dea *et al.*, 1972, 1986). Dea *et al.* (1977) used optical rotation to study the interaction of xanthan with tara gum, LBG and guar galactomannan.

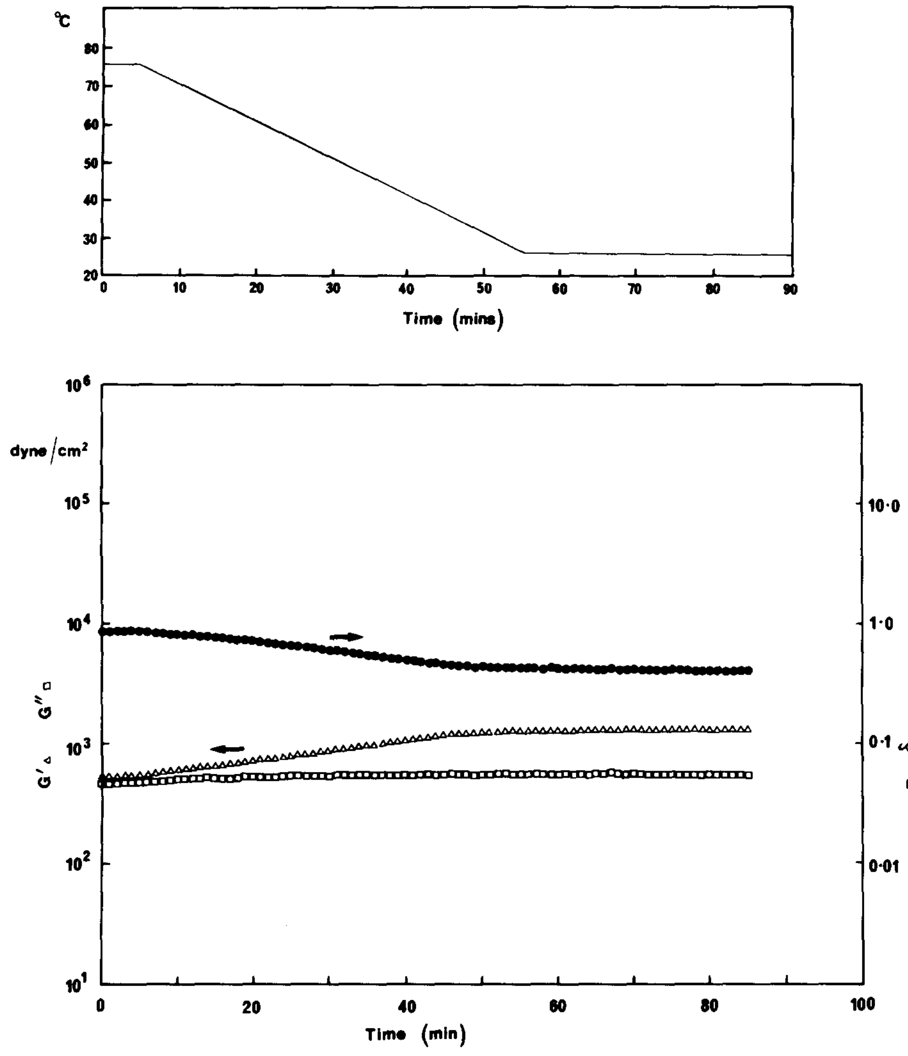

Fig. 3. Temperature sweep and cooling profile, for a mixture of 0·5% ps.646 and 1% guar gum.

It was observed that, in the presence of tara gum, the order–disorder transition for xanthan remained but was shifted to a higher temperature by approximately 10°C. This was interpreted as evidence for stabilization of the xanthan helix by interaction with the galactomannan. A significant flattening of the temperature profile below the gel point was also noted. This, they suggested, was due to inhibition of xanthan aggregation by network constraints. Xanthan–LBG gels appeared to show analogous behaviour, although measurements of optical activity below the gel point

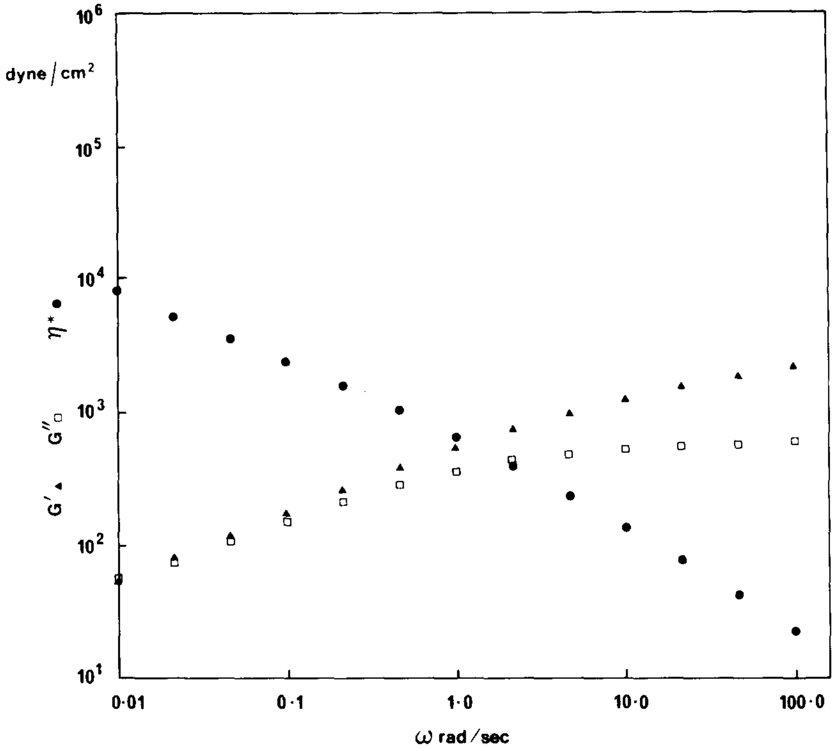

Fig. 4. Frequency sweep for a mixture of 0·5% ps.646 and 1% guar gum.

in this system were irreproducible, and this was attributed to stress birefringence. Guar gum, like the other galactomannans, was found to stabilize the xanthan helix to a temperature of approximately 10°C higher, but in this case the OR profile at low temperature was hardly affected, consistent with the absence of gel network formation.

In this investigation, polarimetry proved to be an unsatisfactory method for studying the interaction between xanthan and both LBG (Part I) and konjac mannan (Part III of this series), because of large irreproducible optical artifacts that occurred at about the onset of gelation. However, interesting data was obtained for combinations of ps.1128 and DA ps.1128 with guar gum. Figure 6 shows the temperature profiles obtained for ps.1128, DA ps.1128 (batch 1) and guar gum in isolation, and as mixtures of xanthan and galactomannan. The ps.1128 and DA ps.1128 samples showed typical helix-coil transition behaviour for xanthan, with transition midpoints close to those previously reported for the same samples (Shatwell *et al.*, 1990*b*). There was significant hysteresis between the heating and cooling curves. Guar gum, on the

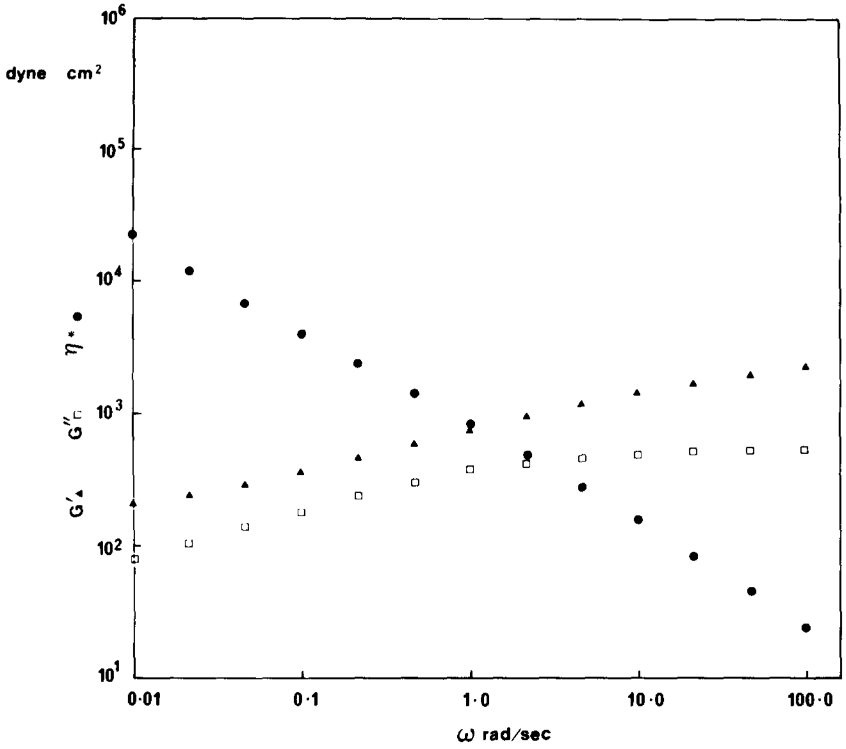

Fig. 5. Frequency sweep for a mixture of 0·5% ps.556 and 1% guar gum.

other hand, showed only a slight and gradual decrease in OR with increasing temperature, consistent with a slightly stiffened random coil, and there was no thermal hysteresis.

The heating and cooling curves for a mixture of ps.1128 and guar gum appeared to be a composite of those of the two individual components. The transition curve for xanthan was, in effect, shifted upwards, but the shape was essentially unaltered except in the high temperature plateau region of the curve, where there was a slight drop in OR, consistent with that observed for the galactomannan; the thermal hysteresis was retained. With deacetylated ps.1128, the behaviour was somewhat different. The OR curve for xanthan was once again shifted upwards and the slight drop in OR in the high temperature plateau region of the curve remained, but this time there was a significant flattening of the transition curve at below 37°C and, at still lower temperatures, optical artifacts analogous to those observed for the gelling systems were found to occur. Furthermore, the cooling curve, which showed initial hysteresis, fell to meet the heating curve at about 30°C. This suggests the possibility of a

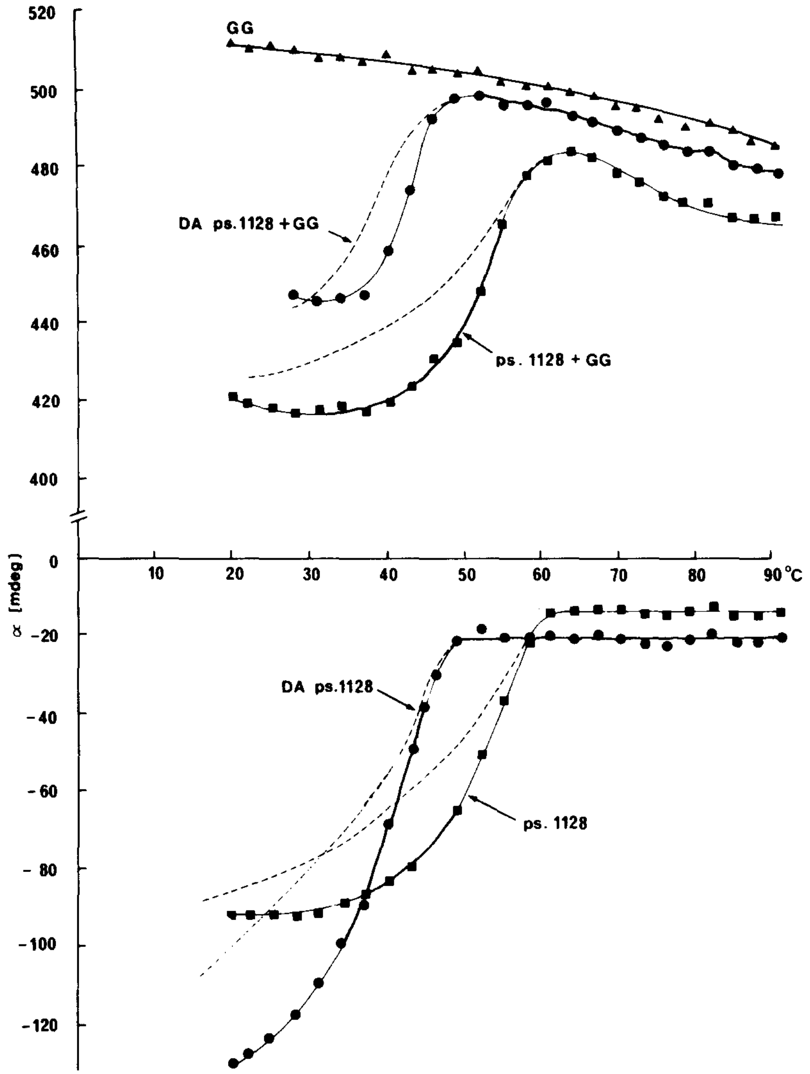

Fig. 6. Heating (——) and cooling (--) curves as monitored by optical rotation for xanthan (0·2%), guar gum (0·4%) and mixtures of the two.

weak interaction between the deacetylated derivative of ps.1128 and guar gum.

An alternative explanation involving the precipitation at low temperature of one or both of the polymers as a result of mutual incompatibility cannot, however, be completely ruled out, although no precipitate was visible in the cell. In contrast to the observations of Dea *et al.* (1977), there was no increase in the transition midpoint or in the temperature at which the upper plateau region was reached for either system. In other words, there was no evidence for stabilization of the xanthan helix by interaction with guar gum.

Statistical analysis

As in Part I of this series, an attempt was made to examine the behaviour of the different xanthan samples, and their degree of substitution by correlating these values against different rheological parameters using PROC RSQUARE (SAS, 1985). In the present case, only very limited success was obtained. From the outset, because the frequency dependence of G' was relatively pronounced, looking for correlations between G' measured at 10 rad/s and, for example, the percentage of acetyl substituents was a precarious procedure. At other (say lower) frequencies, the behaviour might be quite different. In fact, as mentioned above, G' and G'' actually showed a 'cross-over' for some of the samples.

As far as the log η^* versus log ω slopes were concerned, there was an obvious breakpoint in most of the traces, and for the statistical analyses the high and low-frequency slopes were treated differently. Overall, the apparent correlation coefficients calculated were rather poor, and few could be considered significant. Possible exceptions were the dependence of the high frequency slope of η^*, and the G' value at 10 rad/s on the percentage pyruvate substitution.

DISCUSSION

Evidence presented in this study indicates that for normal wild-type xanthan the enhanced viscosity apparent on mixing with guar gum is due solely to the presence of topological entanglements and not to a more specific intermolecular interaction, such as that which may occur with LBG (discussed in detail in Shatwell *et al.*, 1990*c*). Indeed, there is no intrinsic reason why the viscosity of a mixture of two polymers should be equal to the sum of the viscosities of the components. In fact, since the apparent volume of solvent available when one polymer is added to a solution of another is reduced, one might expect an increase in viscosity in the mixed system. For this reason, before concluding that there is a 'synergistic interaction' between two polymeric species, we need to establish a qualitative difference in the mechanical spectrum of the mixed system. Such a difference is seen, albeit to a limited extent, in Fig. 5. Thus, in agreement with the findings of Tako and Nakamura (1985), there is some evidence from both our OR studies and oscillatory-shear measurements to indicate a very weak interaction between low-acetyl xanthan and guar gum.

It should be noted, however, that whilst both ps.556 and deacetylated ps.1128 showed signs of weak association, depyruvylated ps.556, another low acetyl xanthan, did not. (This difference may be attributable to the somewhat lower molecular weight of the depyruvylated derivative.) A possibility not so far considered is that the apparent weak interaction occurred not between xanthan and guar gum but between the xanthan molecules alone. Both Jeanes *et al.* (1961) and Tako and Nakamura (1984) have produced evidence to suggest a stronger association between deacetylated xanthan molecules than between molecules of the native polymer (although presumably in both cases, unlike here, the xanthan was in the ordered state), and the frequency sweep that we obtained for a 2·0% solution of deacetylated ps.1128 in de-ionized water also showed slightly more gel-like behaviour than that of ps.646 and ps.1128, suggesting a possible alternative xanthan–xanthan interaction.

The apparent correlations between the high-frequency η^* slope and G' values and the pyruvate content mentioned above are presumably a reflection of the reduction in molecular weight on depyruvylation, since these parameters should depend upon molecular weight for an entanglement system (but, as discussed in Part I, not for a gel system above a critical molecular weight). The number of samples measured makes any further comment speculative.

The phase-separated model for the behaviour of two polymers in solution has already been mentioned briefly in Part I of this series. One expectation of such a model is that the turbidity of mixed samples (which would be related to the size of the phase domains) would be most pronounced for those samples at and very close to gelling conditions and for the 'strong' gels, and least pronounced for those systems where the lowest gel modulus was observed. In fact, this pattern of behaviour was observed over the course of this work, since optical-rotation measurements were only possible for the xanthan–guar system. This model is not inconsistent, for example, with the suggestion that on deacetylation, xanthan–xanthan interactions are favoured. This observation could be explained by a thermodynamic argument, since in a two polymer(A, B)–solvent(C) system, as well as polymer–solvent and solvent–solvent interactions, there can be A–A, A–B and B–B terms, and the respective proportion of these will reflect, for example, the free energy of mixing of the whole system.

Interestingly, no measurements seem to have been made of second virial coefficients (A_2) in xanthan–galactomannan systems, although Tolstoguzov and co-workers (Tolstoguzov, 1986) have demonstrated how powerful this approach can be when applied to other mixed biopolymer systems. Measurements of A_2 for xanthans with different substitution patterns would be particularly instructive.

Nevertheless, we believe our evidence favours a weak interaction between low acetyl xanthan and guar gum. The nature of this interaction, be it 'molecular' or 'thermodynamic' in origin, is still, and will certainly continue to be, in active dispute.

REFERENCES

Clark, R. C. (1988). In *Gums and Stabilisers for the Food Industry 4*, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams. IRL Press, Oxford, pp. 165–72.

Dea, I. C. M., McKinnon, A. A. & Rees, D. A. (1972). *J. Mol. Biol.*, **68**, 153–72.

Dea, I. C. M., Morris, E. R., Rees, D. A., Welsh, E. J., Barnes, H. A. & Price, J. (1977). *Carbohydr. Res.*, **57**, 249–72.

Dea, I. C. M., Clark, A. H. & McCleary, B. V. (1986). *Carbohydr. Res.*, **147**, 275–94.

Jeanes, A., Pittsley, J. E. & Senti, F. R. (1961). *J. Appl. Polym. Sci.*, **5**, 519–26.

McCleary, B. V., Amado, R., Waibel, R. & Neukom, H. (1981). *Carbohydr. Res.*, **92**, 269–85.

McCleary, B. V., Dea, I. C. M., Windust, J. & Cooke, D. (1984). *Carbohydr. Polym.*, **4**, 253–70.

McCleary, B. V., Clark, A. H., Dea, I. C. M. & Rees, D. A. (1985). *Carbohydr. Res.*, **139**, 237–60.

Morris, E. R. & Ross-Murphy, S. B. (1981). *Techniques in Carbohydr. Metab.*, **B310**, 1–46.

Richardson, R. K. & Ross-Murphy, S. B. (1987). *Int. J. Biol. Macromol.*, **9**, 250–6.

Robinson, G., Ross-Murphy, S. B. & Morris, E. R. (1982). *Carbohydr. Res.*, **107**, 17–32.

SAS (1985). *SAS Users Guide: Statistics.* Version 5, Edn. SAS Institute Inc., Cary, NC.

Shatwell, K. P., Sutherland, I. W. & Ross-Murphy, S. B. (1990*a*). *Int. J. Biol. Macromol.*, **12**, 71–8.

Shatwell, K. P., Sutherland, I. W., Dea, I. C. M. & Ross-Murphy, S. B. (1990*b*). *Carbohydr. Res.*, (in press).

Shatwell, K. P., Sutherland, I. W., Ross-Murphy, S. B. & Dea, I. C. M. (1990*c*). *Carbohydr. Polym.*, **14**, 29–51 (Part I of this Series).

Tako, M. & Nakamura, S. (1984). *Agric. Biol. Chem.*, **48**, 2987–93.

Tako, M. & Nakamura, S. (1985). *Carbohydr. Res.*, **138**, 207–13.

Tolstoguzov, V. B. (1986). In *Functional Properties of Food Macromolecules*, ed. J. R. Mitchell & D. A. Ledward. Elsevier Applied Science, Barking, UK, pp. 385–415.