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Influence of the Acetyl Substituent on the Interaction of Xanthan with Plant Polysaccharides — III. Xanthan–Konjac Mannan 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 interaction between these polymers and konjac mannan in de-ionized water and the data was analysed statistically. The majority of the polymers interacted to form a strong thermoreversible-gel network. Xanthan gelled with konjac mannan only at relatively high concentrations compared with xanthan–locust bean gum (LBG) systems but the gels formed had significantly higher melting and setting temperatures than xanthan–LBG gels of the same concentration. The transition from the liquid to the gel state was also much sharper. The strength of the gels was shown to be heavily dependent on the level of acetyl substitution.*

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INTRODUCTION

The previous papers in this series have shown that, in combination with locust bean gum (LBG), xanthan forms a strong thermoreversible gel network [Part I — Shatwell *et al.* (1990*c*)], but with guar gum weaker entanglement networks are usually formed [Part II — Shatwell *et al.* (1990*d*)]. In both cases, the number of acetyl substituents carried by the xanthan appears to influence the intensity of this interaction. In Part III, we examine the corresponding behaviour of xanthan with the glucomannan konjac mannan.

Konjac mannan is a β-(1→4)-linked copolymer of mannose and glucose derived from the tubers of *Amorphophallus konjac*. The mannose:glucose ratio is approximately 1·4:1·0 (Bewley & Reid, 1985), but the distribution of the residues is uncertain. Partial repeat-unit structures have been proposed by Kato *et al.* (1970) and Shimahara *et al.* (1975*b*), but the presence of only a few weak meridional reflections in the X-ray fibre-diffraction pattern indicates that there is in fact no regular repeating structure and no cellulosic or mannan blocks (Brownsey *et al.*, 1988). Some branching may occur through the C-3 of the glucose and mannose residues (Smith & Srivastava, 1959; Kato & Matsuda, 1973; Shimahara *et al.*, 1975*a*). The polymer carries about 4% acetyl (Dea *et al.*, 1977; Cheetham & Punruckvong, 1985), i.e. about one residue in seven is substituted, but the precise location of these groups is also unknown.

Native konjac mannan does not gel alone, but it will do so when warmed in the presence of alkali. Gelation is believed to be due to the elimination of the acetyl group under alkaline conditions (Maekaji, 1974). It has been suggested that the acetyl ester sterically inhibits molecular association (Dea, 1981), but the highly crystalline X-ray fibre-diffraction pattern obtained for the native polymer (Brownsey *et al.*, 1988) is not consistent with this view. Instead, as Maekaji has suggested, the acetyl group may suppress intermolecular hydrogen bonding, in which case its removal would decrease the solubility of the polymer by enhancing intermolecular association, leading eventually to gelation. In addition, the presence of the acetyl group could inhibit aggregation by contributing to the conformational entropy of the unassociated chains.

Konjac mannan will also gel when mixed with xanthan, although, to date, relatively little work has been carried out on this particular mixed system. Evidence from Dea *et al.* (1977) has indicated that xanthan–konjac mannan gels are stronger than those of the equivalent galactomannan systems, and that gels are formed at much lower total polysaccharide concentrations (as low as 0·02%). Brownsey *et al.* (1988)

studied the interaction using X-ray fibre diffraction and obtained evidence for intermolecular binding. However, such an interaction only appeared to occur when the xanthan was first denatured by heating. They were unable to suggest a model for the junction zones, since analysis of the diffraction patterns was complicated by the complex and possibly irregular structure of the glucomannan.

According to Dea *et al.* (1977), the acetyl group promotes gelation between xanthan and konjac. An approximate 20°C reduction in the gel melting and setting temperatures for mixtures of deacetylated xanthan and konjac mannan was reported. (Gel melting temperature may be regarded as one measure, albeit indirect, of gel strength, since for a similar class of materials we can assume that this is related to the difference between the setting temperature and room temperature). This paper sets out to test the assertion of Dea and coworkers, and to examine more rigorously the influence of the xanthan acyl substituents on the interaction with the glucomannan, by comparing the strength of gels formed between konjac mannan and a series of xanthans with different levels of acetyl and pyruvic acid substitution. Gel strength is tested directly using a combination of oscillatory-shear measurements and minimum gelling concentration experiments.

EXPERIMENTAL

Materials

As discussed in Parts I and II (Shatwell *et al.*, 1990*c*, *d*), samples of xanthan were produced from the following bacterial strains grown in batch culture: *X. campestris* pv. *campestris* 646, *X. campestris* pv. *phaseoli* 1128 and *X. campestris* pv. *phaseoli* 556. 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 treatment with ammonium hydroxide and trifluoroacetic acid, respectively.

Konjac mannan was prepared by purification of a commercial food grade konnjaku flour. The crude material (10 g) was washed at room temperature with 3 × 50-ml quantities of 50% aqueous ethanol to extract impurities. The residue was dissolved in 4 litres of distilled water, with heating (80°C) and stirring. The solution was then clarified by centrifugation, concentrated by dialysis against polyethylene glycol and lyophilized (Dea *et al.*, 1977).

Chemical and physical analyses

The ratio of the component sugars and the levels of acetyl and pyruvic acid substitution were determined for the polymers as described in Shatwell *et al.* (1990*c*).

Molecular weights (static light scattering) and intrinsic viscosities of the xanthans in 20 mM NaCl were measured as described by Shatwell *et al.* (1990*a*). The intrinsic viscosity of the konjac mannan was determined in exactly the same manner. Helix–coil transition midpoints were determined in de-ionized water using polarimetry (Shatwell *et al.*, 1990*b*). A limited number of studies were also performed on mixed xanthan–konjac mannan systems, as described for the guar systems in Shatwell *et al.* (1990*d*).

Estimation of minimum gelling concentrations

Starting at a concentration of 0·5% (w/w) xanthan and 1·0% (w/w) konjac mannan in distilled water, serial 1/2 dilutions were prepared at 80°C. The samples were homogenized using a vortex mixer and then cooled to room temperature. After 24 h ageing, they were examined visually for gel formation. As before (Part I: Shatwell *et al.*, 1990*c*) we have used the notation (+ +) strong gel; (+) soft gel; (+/−) structured solution; (−) free flowing solution (see Table 2).

Mechanical spectrometry

The response of the mixed gel systems to oscillatory shear was measured using a Rheometrics Mechanical Spectrometer RMS-605 with automatic computation and plotting of results. Conditions and loading procedures were as described in Part I (Shatwell *et al.*, 1990*c*).

Samples comprising 0·5% (w/w) xanthan and 1·0% (w/w) konjac mannan were prepared by dissolving the freeze-dried polymers in de-ionized water (total weight, 15·0 g) in screw-top universal bottles. Dissolution was achieved by high-speed shearing for 5 min, at 85°C, using a top-drive Atomix. The samples were pressure cooked for 5 min at 110°C and then sheared again for 5 min at maximum speed. Air bubbles were removed by centrifugation and the gel was remelted and loaded onto the instrument.

RESULTS

Chemical and physical analyses

Xanthans with different levels of acetyl and pyruvic acid substitution (Table 1) were analysed both chemically and physically. More extensive data are given in Table 1 of Shatwell *et al.* (1990*c*).

The sample of konjac mannan used in this study comprised 39% glucose and 61% mannose — a mannose:glucose ratio of 1·6:1·0. The acetyl content was 4·2% by weight. The intrinsic viscosity $[\eta]$ in 20 mM NaCl was 5·5 dl/g, somewhat lower than typical values reported in the literature and indicating a relatively low molecular weight.

Although several light-scattering molecular-weight studies have been carried out on konjac mannan and its chemically modified derivatives (Torigata *et al.*, 1952; Sugiyama *et al.*, 1972; Kishida *et al.*, 1978), as far as we are aware the Mark–Houwink parameters for konjac mannan itself have not yet been published. Kishida *et al.*, however, asserted that data for a partially methylated derivative could be employed for the unmodified polymer. They found that in the equation

$$[\eta] = K'M_w^{\alpha}$$

K' was $6{\cdot}37 \times 10^{-4}$, and α was 0·74. Using these values a M_w of $\approx 1{\cdot}9 \times 10^5$ is obtained for our sample. Both $[\eta]$ and the resultant M_w are very much lower than values found by this group for their own unhydrolysed konjac mannan samples; they reported typical values for M_w of $\approx 10^6$, and $[\eta]$ of $\approx$ 18–20 dl/g. The reason for the apparent very low M_w of our sample is not immediately obvious, but the Mark–Houwink parameters for guar obtained by Robinson *et al.* (1982) ($3{\cdot}8 \times 10^{-4}$ and 0·723, respectively) are rather different to those obtained by Kishida *et al.* (1978). In particular, the K' parameter, which is a measure of the local chain flexibility should be very similar for the two samples, since the mannose backbone in guar and the β-(1→4)-linked D-glucose and D-mannose backbone of konjac should have similar conformational flexibility. Although the parameters do not look very different, using the guar values with $[\eta] = 5{\cdot}2$ dl/g gives an apparent M_w of $5{\cdot}3 \times 10^5$, almost a 300% increase.

We feel that the chemical derivatization employed by Kishida *et al.* may have modified the polymers to a greater extent than they anticipated, and feel more confident about the recalculation based upon the guar data. Of course, the additional contribution to M_w made by the galactose side-chains of the galactomannan will affect the M_w estimate,

TABLE 1
Oscillatory-Shear Measurements for Xanthan (0·5%)-Konjac Mannan (1·0%) Mixed Systems

Xanthan	*Acetyl substitution (%)*	*Pyruvic acid substitution (%)*	*tan δ*	*G′*	*Slope of η**
ps.646	4·5	4·4	0·106	3000	−0·93
ps.1128	7·7	1·7	—	—	−0·74 (LF)
DA ps.1128[a]	1·6	1·3	0·16	1100	−0·93
DA ps.1128[b]	1·5	1·3	0·15	1140	−0·93
ps.556	1·6	6·0	0·075	2300	−0·94
DP ps.556	1·1	1·0	0·11	930	−0·92

LF — determined at low frequencies only.
[a] Batch 1.
[b] Batch 2.

but by much less than the approximately 300% difference in the M_w values estimated from the two sets of parameters discussed above.

One final comment can be made here: as mentioned in the Introduction, there are reports in the literature that konjac mannan has some long chain branching. However, the Mark–Houwink a value reported by Kishida *et al.* is not consistent with this, since their value (0·74), like that for guar, is close to the maximum expected for an 'excluded volume' linear chain in a good solvent. Long-chain branching would be expected to lower the exponent considerably. For example, a for high M_w dextrans is ≈ 0·2.

Oscillatory-shear measurements

As in Parts I and II (Shatwell *et al.*, 1990*c*, *d*), oscillatory-shear measurements were carried out on mixed systems comprising 0·5% (w/w) xanthan and 1·0% (w/w) konjac mannan in de-ionized water. The materials used were ps.646, ps.1128, deacetylated (DA) ps.1128 batches 1 and 2, ps.556, and depyruvylated (DP) ps.556. (Two different samples of deacetylated ps.1128 were included in the study to give an indication of the reproducibility of the data). Procedures are described in detail in Shatwell *et al.* (1990*c*).

Figure 1 shows a typical cooling sweep for a mixture of xanthan (in this case ps.646) and konjac mannan. The behaviour is characteristic of a thermoreversible gel system. At high temperatures (i.e. at the far left of

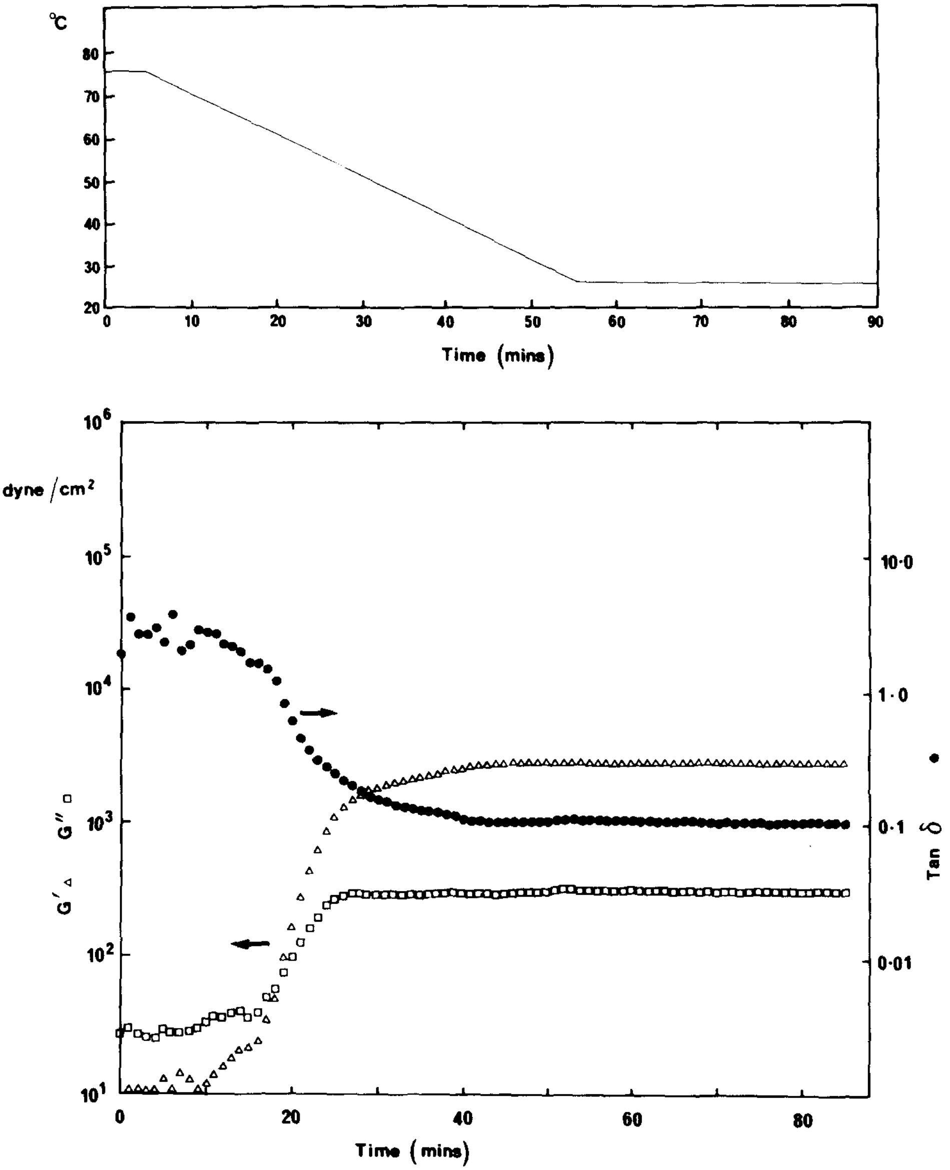

Fig. 1. Temperature sweep and cooling profile, for a mixture of 0·5% ps.646 and 1·0% konjac mannan.

the trace), the behaviour is essentially that of a liquid, G″ being greater than G′. As the temperature drops and the gel forms, G′ increases rapidly and tan δ falls. Broadly speaking, the trace resembles that obtained for the xanthan–LBG gels, but there are a number of significant differences. The konjac mannan gel in the melted state was far less viscous than the xanthan–LBG mixtures, and the torque generated was therefore lower. Consequently, the data on the far left of the spectrum is more erratic. The xanthan–konjac mannan gels set up and melted out at significantly higher temperatures, typically between 50 and 60°C, compared with 40–50°C for the LBG gels. This indicates that the xanthan–konjac mannan junction zones are more stable to heat. In addition, once the gel had formed, the G' and tan δ plots soon levelled off and there was little additional increase in gel strength with time. The plots of tan δ on cooling and reheating, therefore, showed no thermal hysteresis, unlike those of the LBG mixed systems. The strain sweep for this system was featureless, both G' and G'' were almost strain independent up to the maximum of 25%. The frequency sweep for the same system is given in Fig. 2. This quite clearly indicates a strong gel network; G' is much greater than G'', and both show very little frequency dependence; η^* decreases markedly with increasing frequency.

The majority of the xanthan/konjac mannan systems showed the same pattern of behaviour. However, ps.1128, which has a very high acetyl content, actually failed to form a recognizable gel. The mixture, at ambient temperature, flowed freely and was of too low a viscosity to generate sensible data on the RMS. Nevertheless, the frequency sweep for this system is shown in Fig. 3 and although the data is much more erratic than for other traces, due to the small torque values measured, there may be evidence for weak cross-linking at the lowest frequencies. Here G' and G'' do appear to be relatively frequency independent and η^* decreases with increasing frequency.

Comparative gel strengths

Three measures of gel strength were used in this study — the elastic modulus, i.e. G', the tan δ value at the end of the cooling sweep (i.e. at 25°C, 10% strain and 10 rad/s) and the slope of log η^* in the frequency sweep. Generally speaking, the higher the elastic modulus, the stronger the gel, and the lower the tan δ value, the more elastic the system. Likewise, the closer the slope of η^* to $-1{\cdot}0$, the more elastic the gel network is. (See the first paper in this series for a more detailed explanation of these parameters.)

Table 1 gives a summary of G', tan δ and slope η^* for the xanthan–konjac mannan systems. If one compares this data with that for the xanthan–LBG systems, one finds that in the konjac systems, G' was generally somewhat lower and tan δ a little higher. The slope of η^*, on the other hand, was very similar.

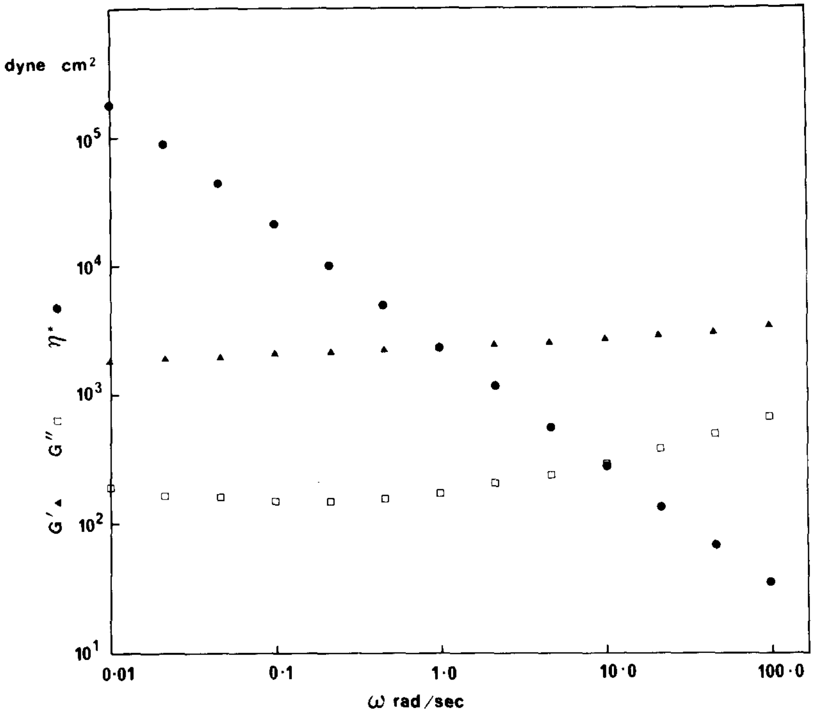

Fig. 2. Frequency sweep for a mixture of 0·5% ps.646 and 1·0% konjac mannan.

Most of the xanthans formed a relatively strong gel network with konjac mannan. Sample ps.646, the most representative wild-type xanthan, gave the highest modulus and almost the 'best' gels, although very similar results were obtained for the low acetyl sample ps.556. As already noted, ps.1128, with a 7·7% acetyl content, failed to gel at all at 25°C. As the modulus for this sample was too low to measure properly, no data is included for this polymer in Table 1 (see below) apart from an estimate of the slope of η^* at low frequencies (determined from Fig. 3). This value, $-0{\cdot}74$, is significantly lower than that for any of the other systems and approaches the expected result for an entanglement network. The deacetylated derivative of ps.1128, in contrast, formed quite a strong gel with konjac mannan, indicating that the acetyl group has an inhibitory effect upon gelation. Depyruvylation of ps.556 resulted in some decrease in the strength of the interaction with konjac mannan. G' fell significantly and tan δ rose. The slope of η^*, however, changed very little.

Table 2 shows the results of the minimum gelling concentration experiments. Serial 1/2 dilutions were performed on the xanthan-konjac

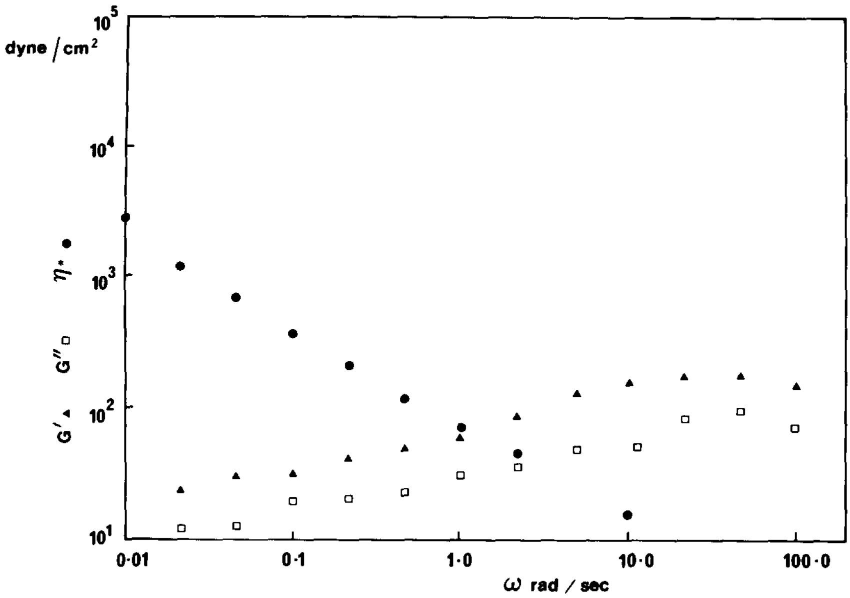

Fig. 3. Frequency sweep for a mixture of 0·5% ps.1128 and 1·0% konjac mannan.

TABLE 2
Estimated Minimum Gelling Concentrations for Xanthan–Konjac Mannan Mixed Systems

Xanthan	*Dilution*	*Gelation*				
		0	*1/2*	*1/4*	*1/8*	*1/16*
	~ % KM	*1·0*	*0·5*	*0·25*	*0·125*	*0·063*
	~ % Man	*0·5*	*0·25*	*0·125*	*0·063*	*0·031*
ps.646		+ +	+	+/−	−	−
DA ps.646		+ +	+ +	+	+/−	−
DP ps.646		+ +	+/−	−	−	−
ps.1128[a]		−	−	−	−	−
DA ps.1128		+ +	+ +	+	+/−	−
ps.556		+ +	+	+/−	−	−
DP ps.556		+ +	+	+/−	−	−

[a] Batch 1.

mannan systems in the melted state and, after cooling, the samples were assessed visually for gel formation. Compared to its behaviour with LBG, xanthan appeared to gel with konjac mannan only at relatively high concentrations. This is in marked contrast to the behaviour reported by Dea *et al.* (1977). They stated that recognizable gels were formed with konjac mannan down to a total polysaccharide concentration of 0·02%. However, in the present study, the lowest concentration at which a gel was formed was 0·375%. This was true for ps.646 and deacetylated ps.1128 (batch 1) and compares with a corresponding concentration of 0·094% for a mixture of deacetylated ps.1128 (batch 1) and LBG.

This evidence appears to conflict with the findings from mechanical spectrometry, i.e. that although the xanthan–konjac mannan gels were marginally weaker than the xanthan–LBG systems at 25°C, they were significantly more resistant to disruption by heat. The answer to the discrepancy may lie in the relative M_w of the galactomannan and glucomannan. As already discussed, whichever method was used, the M_w of the konjac mannan calculated from $[\eta]$ was rather low. While the effect of M_w upon gel strength has not been positively established for these systems, it is likely that although xanthan and konjac mannan interacted fairly strongly at high concentrations, because of its low M_w, the glucomannan was incapable of forming a well-defined, cross-linked gel network at concentrations as low as for the xanthan–LBG mixed systems.

Sample ps.1128 which failed to gel at all at room temperature, after deacetylation formed recognizable gels down to a total polysaccharide concentration of 0·375%. Deacetylation of ps.646 also led to a decrease in minimum gelling concentration, but the effect was not as marked as for ps.1128. The gels formed by all the deacetylated derivatives were observed to be firmer to the touch than those of the equivalent native polymers. This indicates once again that the acetyl substituent of xanthan has a strong inhibitory affect on the interaction with konjac mannan.

Depyruvylation of ps.646 produced a slight increase in the minimum gelling concentration but with ps.556 there was no change. The gels formed by both of the native polymers, however, were slightly firmer to the touch than those of their depyruvylated derivatives. This concurs with the evidence from mechanical spectrometry, indicating that depyruvylation caused a slight decrease in the strength of the gel formed with konjac mannan.

There appeared to be no consistent correlation between the M_w of the xanthan samples, as indicated by $[\eta]$, and the minimum gelling concentrations.

Statistical analysis

The apparent inhibitory effect of the xanthan acetyl group upon gelation, and the opposite effect indicated for the pyruvic acid residue, was tested statistically using a commercial computer package. The effect of M_w, as indicated by [η], was also considered. As in Part I (Shatwell *et al.*, 1990*c*), PROC RSQUARE from the SAS library (SAS, 1985) was used to carry out a multiple-regression analysis on the oscillatory-shear data. The model-dependent parameters, tan δ, G$'$ and slope of η^*, were regressed against the independent variables, acetyl, pyruvate and [η], and the full correlation matrix of dependent and independent variables was computed. This is shown in Table 3.

As no data were available for ps.1128, except for an estimate of the slope of η^* at low frequencies, values below the limit of resolution of the RMS-605, were included for this sample. Sensible values for tan δ (500) and for G$'$ (50 dyne/cm^2) were adopted. (This was considered necessary because the number of samples in the data set was already very small and to have omitted ps.1128 would not only have reduced the number still further but would have excluded what appeared to be one of the most important polymers in the study.) Again, for the purpose of this investigation, correlation coefficients $> 0{\cdot}85$ (or $< -0{\cdot}85$) were considered to be statistically significant.

A strong correlation was found between tan δ and the slope of η^*. This was to be expected since, as discussed in Part I (Shatwell *et al.*, 1990*c*), both are measures of 'gel strength'. A weak correlation was found between the percentage acetyl and tan δ, and the percentage acetyl and the slope of η^*. This appears to support our earlier observations, but from the statistical analyses the role of the acetyl group in the xanthan–konjac mannan mixed systems was rather less clear cut than for the xanthan–LBG systems. No significant correlations were found between gel strength and either the pyruvate content or [η].

Following the approach adopted previously, the regression method was applied to the rheological data for the gels and the helix-coil transition midpoint temperatures (T_m). G$'$, tan δ and the logarithmic slope of η^* values for the mixed systems were tested against the order–disorder T_m values. The resultant correlation matrix is shown in Table 4. At least some significant correlations appeared to be present, in particular between T_m and tan δ, and T_m and the slope of η^*, indicating that the higher the transition midpoint, the less elastic the gel.

The conclusions drawn from these computations, like those for the LBG–xanthan systems are, at best, provisional. They are included here because we feel they may help to guide other workers in any future studies. Further aspects are discussed in more detail below.

DISCUSSION

As for the xanthan–LBG systems, the acetyl substituent was shown to have an inhibitory effect upon the gelling interaction with konjac mannan.

TABLE 3

Correlation Matrix Relating Acetyl, Pyruvate and Intrinsic Viscosity to Gel Strength in Xanthan (0·5%)–Konjac Mannan (1·0%) Mixed Systems

	Acetyl substitution (%)	*Pyruvic acid substitution (%)*	*[η]*	*tan δ*	*G′*	*Slope of η^**
% Acetyl substitution	1·000	0·019	0·799	0·881	−0·254	0·874
% Pyruvic acid substitution		1·000	0·329	−0·216	0·788	−0·278
[η]			1·000	0·838	−0·235	−0·795
tan δ				1·000	−0·636	0·997
G′					1·000	−0·667
Slope of η^*						1·000

TABLE 4
Correlation Matrix Relating Gel Strength to the Helix-Coil Transition Midpoint

	tan δ	*G′*	*Slope of η^**	T_m
Tan δ	1·000	−0·681	0·997	0·939
G′		1·000	−0·713	−0·594
Slope of η^*			1·000	0·950
T_m				1·000

This was shown most distinctly by the failure of mixtures containing ps.1128 to gel at room temperature. Pyruvate appeared to promote gelation to some extent, but these experimental observations were not supported by the statistical analysis of the data.

To date, no plausible molecular model has been suggested for the xanthan–konjac mannan interaction, in marked contrast to the situation for the xanthan–galactomannan systems. This presumably reflects both the paucity of experimental studies, and the difficulties in interpretation.

As mentioned previously, the X-ray fibre-diffraction data obtained for this system (Brownsey *et al.*, 1988) is very difficult to explain convincingly. However, the stereochemical similarity between the xanthan (cellulose) and glucomannan backbones suggests a backbone–backbone interaction, somewhat akin to that described by Cairns *et al.* (1986, 1987) or Moorhouse *et al.* (1977) for the xanthan–LBG interaction. Of course, this model does not require the glucomannan chains to be linear; in fact, a degree of long-chain branching might actually promote gelation, providing that packing was not perturbed by the presence of branch points. Clearly, more detailed studies of the konjac mannan chain architecture are required.

Attempts were made to monitor the course of any chiroptical changes on gelation of the mixed xanthan–konjac mannan systems. These were conspicuously unsuccessful, as optical artifacts invariably occurred close to the onset of gelation. Possible explanations for such artifacts have already been mentioned, and are twofold. Stress birefringence has been reported in such mixed systems (Dea *et al.*, 1977), whilst incipient phase separation will also produce gross fluctuations in density and light scattering. The latter implies a thermodynamic mechanism for gelation in this system, but this does not exclude the involvement of 'molecular interactions', since these will also have thermodynamic consequences, as discussed in Part II (Shatwell *et al.*, 1990*d*).

In the absence of a satisfactory model, speculation as to the mechanism by which the acetyl group inhibits gelation is difficult. The ways in which the acetyl group could inhibit association include direct steric hindrance, suppression of the intermolecular attractive forces, (particularly hydrogen bonding) or via its influence on the ordered conformation. If, on the other hand, the pyruvate substituent promotes gelation, then, assuming that xanthan can only interact in the disordered state (Brownsey *et al.*, 1988), it might do so via its destabilizing effect upon the ordered conformation.

The role of the pyruvic acid substituent in gelation is by no means clear cut. The changes observed upon depyruvylation were small and any apparent trends were not reflected by the data from the statistical analysis. Furthermore, it is by no means certain that the changes observed were actually due to loss of the pyruvic acid group. There is evidence to suggest that autoclaving the polymer in de-ionized water can lead to a loss of pyruvic acid (Cheetham & Punruckvong, 1985). In the studies described in Part I (Shatwell *et al.*, 1990*c*), we were able to discount this effect for xanthan–LBG gels. For this reason, it was not considered necessary to perform such a control experiment for the konjac mannan mixed gels since the temperature regimes were identical. In addition, it is likely that any reduction in gel strength upon depyruvylation was due, not to the loss of the pyruvic acid group, but to the concomitant decrease in molecular weight that is believed to have occurred.

Evidence for depolymerization included both the reduction in the intrinsic viscosity, and the presence of low-molecular-weight degradation products in the reaction mixture. These were detected in the dialysate after depyruvylation. Nevertheless, no obvious correlation was found between the intrinsic viscosity of xanthan and the gel strength, and this was confirmed by the statistical analysis. However, there is evidence to suggest that the presence in some samples of high-molecular-weight aggregates formed upon freeze-drying and not subsequently broken down when the polymer was redissolved may have raised the apparent intrinsic viscosity. This was undoubtedly true in the case of ps.1128 (Shatwell *et al.*, 1990*a*).

CONCLUDING REMARKS

Throughout this and our previous studies it has been implied that for cold-setting gels there is a relationship between the gel melting temperature and 'gel strength' measured at some other lower temperature (i.e. those gels with the highest melting temperature are also the 'strongest' gels at room temperature). Whilst this remark satisfies intuition, it is less obvious than might at first be thought. The Eldrige–Ferry theory (and subsequent extensions — see, for example, Clark & Ross-Murphy, 1987) relates the gel melting temperature to concentration and molecular weight. These same two parameters also occur in expressions for the small deformation modulus, but in the Eldridge–Ferry theory, gel 'melting' is related to the enthalpy of crystallite formation. This enthalpy must itself be reasonably constant for a given series of similar samples before our intuition is satisfied. A complication occurs however if, as in the model of Cairns *et al.* (1986, 1987), xanthan can only gel from the disordered form, since the order–disorder temperature must lie somewhere below (but presumably close to) the gel melting temperature. Limited evidence from our own work has indicated a correlation between T_m and 'gel strength' but following the earlier argument, it is by no means clear to us whether or not there should be any such relationship. It appears that the physical chemistry in such a system has yet to be investigated.

Finally, and as a conclusion to all three papers in this series, it now seems unequivocal that the presence of acetyl substituents on xanthan does inhibit xanthan–galactomannan and xanthan–glucomannan interactions. The effect of pyruvate in these systems is far less clear-cut; it seems, at least as a first approximation, that there is very little, if any, effect on 'gel strength' for the range of samples investigated here.

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