ELSEVIER

Carbohydrate Research 305 (1998) 101–108

CARBOHYDRATE
RESEARCH

Effect of deacetylation on the synergistic interaction of acetan with locust bean gum or konjac mannan

Cordelia Ojinnaka [a], Geoffrey J. Brownsey [b], Edwin R. Morris [a,*], Victor J. Morris [b]

[a] *Cranfield University, Silsoe College, Silsoe, Bedford MK45 4DT, UK*
[b] *Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, UK*

Received 11 July 1996; accepted 15 July 1997

Abstract

It has been discovered that deacetylation of the bacterial polysaccharide acetan promotes synergistic interactions with either locust bean gum (LBG) or konjac mannan (KM). Acetan is similar in structure to xanthan, and adopts a similar 5-fold conformation in the solid state. Like xanthan, it shows a thermally reversible order (helix)–disorder (coil) transition in solution. Both polymers have a cellulosic backbone with charged (anionic) sidechains attached at O-3 of alternate glucosyl residues, but the sidechains in acetan are longer (pentasaccharide rather than trisaccharide) and do not contain pyruvic substituents. Acetan has two sites of acetylation, one at O-6 of the inner mannosyl residue of the carbohydrate sidechains (as in xanthan) and the other on the polymer backbone (believed to be at O-6 of the branched glucosyl residues). Solutions of acetan or deacetylated acetan were equilibrated against 10 mM potassium chloride (to stabilise the ordered conformation) and were mixed (at 25 °C) with solutions of LBG or KM, also equilibrated against 10 mM potassium chloride. Unlike xanthan, native acetan showed no evidence of synergistic interaction with either LBG or KM. After deacetylation, however, large enhancements were observed in dilute-solution viscosity, and thermoreversible gels were formed at higher concentrations. With KM as co-synergist, gel melting was accompanied by an intense endotherm in differential scanning calorimetry. The magnitude of this endotherm increased with storage time at 25 °C, reaching a final value of $\Delta H \approx 15.9$ J/g (in comparison with $\Delta H \approx 5.0$ J/g for the order–disorder transition of deacetylated acetan alone). It is suggested that interaction occurs by formation of heterotypic junctions between the acetan backbone and unsubstituted regions of the plant

---

[*] Corresponding author. Tel.: +44-1525-863302; fax: +44-1525-863363.

PII S0008-6215(97)00277-2

polysaccharide, and that the acetate groups on native acetan promote solubility and hence inhibit association.

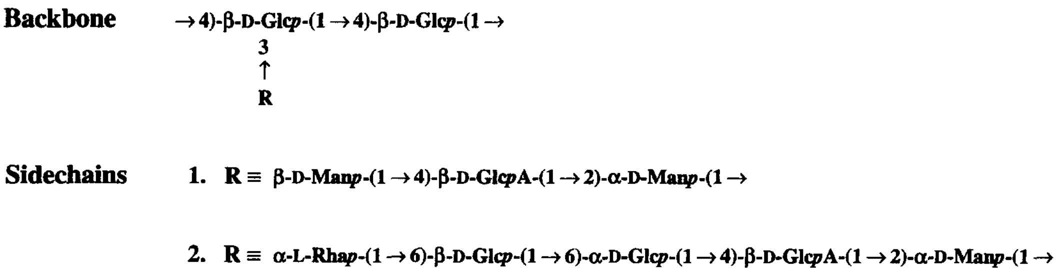
Keywords: Deacetylation; Synergistic interaction; Acetan; Differential scanning calorimetry; Rheology

1. Introduction

Certain binary mixtures of polysaccharides exhibit unusual and often unexpected synergistic interactions [1]. There may be an unexpected increase in viscosity, the gelling ability of one component may be enhanced, or the mixture may gel under conditions where the individual components are non-gelling. Striking examples of the latter behaviour occur when the bacterial polysaccharide xanthan gum is mixed with certain plant galactomannans or glucomannans [1,2]. There is convincing evidence that the synergistic gelation of xanthan occurs by direct binding to the co-synergist, rather than by thermodynamic incompatibility. In particular, studies by gel permeation chromatography have shown co-elution of complexes of xanthan and locust bean gum (LBG) at a retention time substantially different from that of the galactomannan alone [3]. With the glucomannan konjac mannan (KM) as co-synergist, the most direct evidence of binding is the appearance of intense DSC (differential scanning calorimetry) transitions coincident with the formation and melting of the mixed gel [4]. Xanthan also shows massive increases in viscosity and the development of gel-like properties when mixed with LBG or KM in very dilute solution, where exclusion effects are likely to be negligible [5–7]. The nature of the interaction, however, is still controversial.

At high temperature and low ionic strength xanthan exists in solution as a disordered coil, but on cooling and/or on addition of salt it converts into an extended, ordered conformation [8]. The ordered structure has been identified as a 5-fold helix by X-ray fibre diffraction in the solid state [9,10], although it has not yet been established whether the helix is single-stranded or double-stranded. The xanthan disorder–order transition persists in the presence of galactomannans, prompting the initial suggestion [11,12] that synergistic gelation occurs by binding of 'smooth' regions of the mannan chain (i.e., without galactose substituents) to the surface of the 5-fold helix. More recently, however, it has been found that X-ray diffraction patterns obtained from oriented fibres prepared from synergistic gels of xanthan with certain galactomannans or with KM are new patterns, and not simple sums of the diffraction patterns of the component polysaccharides [13–15]. This evidence has been interpreted in terms of the formation of heterotypic junction zones in which the xanthan helices are denatured, and the backbone of the galactomannan or the glucomannan binds directly to the backbone of the xanthan molecule, with both constituents in, or close to, the 2_1 (cellulosic) conformation of the (1 → 4)-diequatorially linked chains. An attractive feature of this proposal is the strong stereochemical similarity between the cellulosic backbone of xanthan, the mannan backbone of the galactomannans, and the mixed backbone of KM. Molecular modelling studies [16] have shown that denatured xanthan can adopt a stereochemical structure compatible with such proposed interactions.

Acetan is a new bacterial polysaccharide with a chemical structure [17,18] very similar (Fig. 1) to that of xanthan gum [19,20]. Both polysaccharides possess cellulosic backbones solubilised by substitution of charged sidechains on alternate glucosyl residues. Xanthan contains a trisaccharide sidechain and acetan



Backbone $$\rightarrow 4)\text{-}\beta\text{-D-}\underset{\begin{array}{c}3\\ \uparrow\\ \mathrm{R}\end{array}}{\mathrm{Glc}p}\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow$$

Sidechains 1. $\mathrm{R} \equiv \beta\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\mathrm{A}\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow$

2. $\mathrm{R} \equiv \alpha\text{-L-Rha}p\text{-}(1\rightarrow 6)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 6)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\mathrm{A}\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow$

Fig. 1. Primary structure of (1) xanthan and (2) acetan. Both polymers are acetylated at O-6 of the mannosyl residue adjacent to the cellulosic backbone. Xanthan has 4,6-linked pyruvic ketal groups on the terminal mannosyl groups. Acetan has an additional site of acetylation on the polymer backbone, probably at O-6 of the branched glucosyl residue. All substituents occur in variable, non-stoichiometric proportions.

a pentasaccharide sidechain. Both polysaccharides have been shown to form similar 5-fold helical structures in the solid state [21,22]. Acetan [21], like xanthan [8], exhibits thermally reversible order (helix)–disorder (coil) transitions and the ordered (helical) structures are stabilised at low temperature and/or high ionic strength. In view of its structural and conformational similarity to xanthan, acetan might also be expected to show analogous binding interactions with LBG or KM. The aim of the present investigation was to determine whether or not such interactions do, in fact, occur. A preliminary account of some aspects of the work has been published elsewhere [23].

2. Materials and methods

Acetan (synthesised by *Acetobacter xylinium* NRRL B42 strain M1) was prepared by the methods described by MacCormick et al. [24]. The charged acetan polysaccharide was purified by selective precipitation with cetyltrimethylammonium bromide (CTAB) using the procedure of Scott [25]. This results in a lyophilised sample in the sodium salt form. The deacetylated polymer was prepared using the following procedure. A 0.5% w/v solution of acetan in 125 mM NaOH was flushed for 10 min with dry nitrogen, sealed, held at 45 °C for 6 h, neutralised with 0.5 M HCl, dialysed extensively against distilled deionised water, and freeze-dried. Complete deacetylation was checked by infrared spectroscopy. Konjac mannan (isolated from *Amorphophallus konjac*) was obtained from Senn Chemicals, Dielsdorf, Switzerland. Locust bean gum was supplied by Marine Colloids Division of FMC, Rockland, ME, USA. The mannose: galactose ratio, as determined by optical rotation [1], was 4.2:1. Potassium chloride was AnalaR grade from BDH. Distilled deionised water was used throughout.

Solutions of acetan or deacetylated acetan were prepared by overnight stirring at ambient temperature, and were clarified by filtration through 0.65-μm Millipore filters. KM and LBG were dissolved by autoclaving for 20 min at 120 °C and clarified by centrifugation. Final concentrations were determined by freeze-drying a known volume of each solution. Stock solutions of all four polysaccharides were dialysed to equilibrium against 10 mM potassium chloride and the dialysate was then used for dilution to the required final concentrations, in order to ensure that any changes observed after mixing were not trivial changes arising from variations in ionic conditions. Mixtures were prepared by combining KM or LBG solutions with solutions of acetan or deacetylated acetan at 25 °C. Previous studies [21,23] have shown that, under the salt conditions used in the present work (10 mM potassium chloride), acetan and deacetylated acetan remain conformationally ordered at this temperature.

Viscosity measurements, also at 25 °C, were made on a Contraves Low Shear 30 rotational viscometer using cup and bob geometry (inner and outer radii, 5.5 and 6.0 mm). Low-amplitude oscillatory measurements of storage modulus (G′), loss modulus (G″) and complex dynamic viscosity (η^*) were made using cone and plate geometry (diameter, 5 cm; cone angle, 0.05 rad) on a sensitive prototype rheometer designed and constructed by Dr. R.K. Richardson (Silsoe College). In both cases temperature was controlled using a Haake circulating water bath and measured by a thermocouple in contact with the stationary element. DSC measurements over a temperature range of 5–95 °C were made on a Seteram microcalorimeter, using the solvent as reference. Sample and reference pans were balanced to within 0.5 mg.

3. Results

Interactions in dilute solution.—The following simple procedure [26] was used to screen for intermolecular binding of LBG or KM to acetan or deacetylated acetan at very low concentrations, where exclusion effects should be negligible. Solutions of the two polysaccharides under investigation were prepared at approximately the same specific viscosity (η_{sp} = 0.7–0.8). These solutions were then mixed (at 25 °C) in various proportions, and the viscosities of the mixtures were measured over a range of low shear-rates. The same technique has been applied previously to mixtures of LBG or KM with xanthan or deacetylated xanthan, and showed massive enhancement of viscosity [7].

As shown in Fig. 2, the viscosity of mixed solutions of native acetan (in its ordered conformation at 25 °C) with either LBG or KM remained the same, to within experimental error, as the common viscosity of the starting solutions, irrespective of the composition of the mixtures. Deacetylated acetan, under the same experimental conditions, however, showed clear evidence of interaction with LBG and KM. In both cases the viscosity varied systematically with mixing

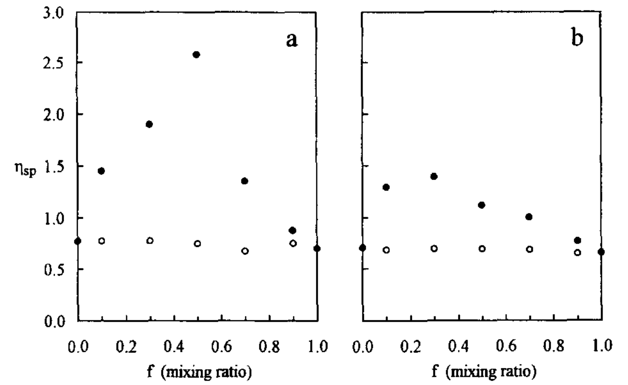


Fig. 2. Specific viscosity (25 °C) for mixtures of solutions of (a) LBG (~ 0.07% w/v) or (b) KM (~ 0.1% w/v) with solutions of native (○) or deacetylated (●) acetan (~ 0.03% w/v). The starting solutions were equilibrated against 10 mM potassium chloride and mixed at 25 °C; f denotes the proportion of acetan solution (native or deacetylated) in each mixture.

ratio, passing through a maximum. With KM as co-synergist (Fig. 2b), the maximum value of η_{sp} was about twice that of the individual starting solutions. With LBG (Fig. 2a), the increase in specific viscosity was more than 3-fold.

Mixed gel rheology.—Figs. 3a and b show mechanical spectra (at 25 °C) for, respectively, deacetylated acetan and LBG at 1% w/v in 10 mM potassium chloride. In both cases, viscous flow (loss modulus, G″) predominates over elastic response (storage modulus, G′), and the complex dynamic viscosity (η^*) shows little variation with frequency (ω), behaviour typical of semi-dilute polysaccharide solutions [27]. The spectrum obtained for a mixture of the two starting solutions (at a ratio of 3:7 deacetylated acetan:LBG), however, is typically gel-like (Fig. 3c); G′ shows little variation with frequency and is substantially higher than G″ throughout the accessible

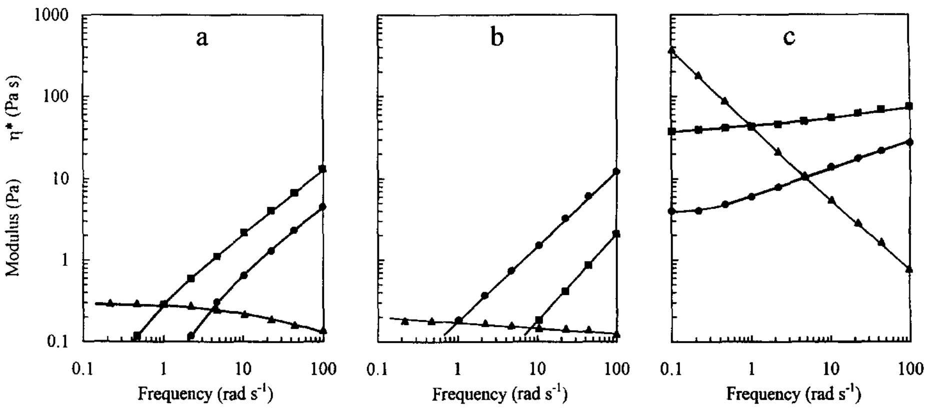


Fig. 3. Mechanical spectra (25 °C; 2% strain) showing the frequency-dependence of G′ (■), G″ (●), and η^* (▲) for (a) deacetylated acetan (1.0% w/v), (b) LBG (1.0% w/v), and (c) a mixture of the two at a ratio of 3:7 (acetan:LBG). The starting solutions were equilibrated against 10 mM potassium chloride and mixed at 25 °C.

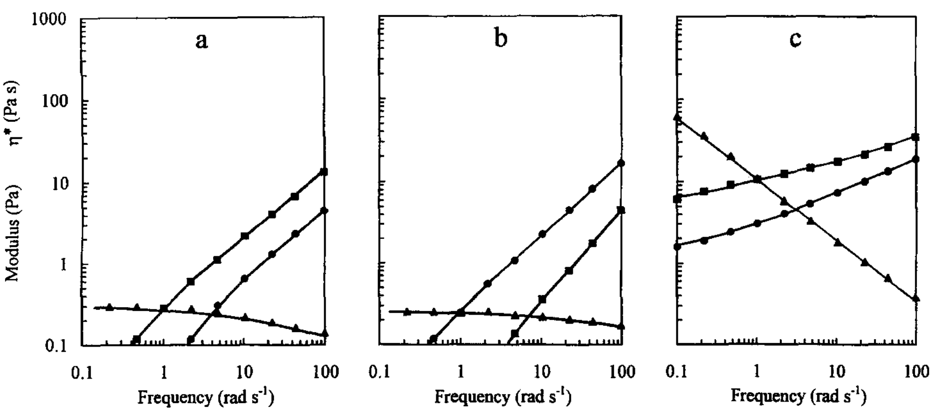


Fig. 4. Mechanical spectra (25 °C; 2% strain) showing the frequency-dependence of G′ (■), G″ (●), and η^* (▲) for (a) deacetylated acetan (1.0% w/v), (b) KM (1.0% w/v), and (c) a mixture of the two at a ratio of 3:7 (acetan:KM). The starting solutions were equilibrated against 10 mM potassium chloride and mixed at 25 °C.

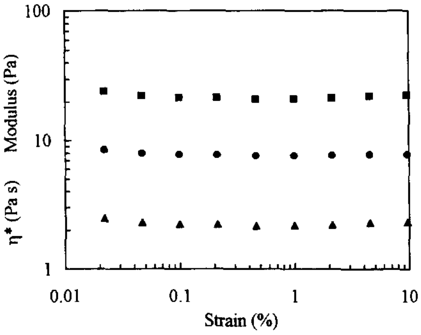


Fig. 5. Strain-dependence (25 °C; 10 rad s^{-1}) of G′ (■), G″ (●), and η* (▲) for the deacetylated acetan–KM mixture from Fig. 4c.

range, and log η* decreases linearly with increasing log ω. There is also a gross increase in the absolute values of the rheological parameters in comparison with those of the starting solutions (e.g., about a 1000-fold increase in η* at the lowest accessible frequency, 0.1 rad s^{-1}).

With KM as co-synergist (Fig. 4), the increase in η* at low frequency was smaller (~200-fold at 0.1 rad s^{-1}), consistent with the smaller enhancement in dilute-solution viscosity (compare Fig. 2a and b), and the separation between elastic and viscous response (G′ and G″) was also somewhat lower. The mechanical spectrum of the mixed system (Fig. 4c), however, is still gel-like (G′ > G″, with little frequency-dependence in either modulus; linear reduction in log η* with increasing log ω). As shown in Fig. 5, the moduli of the mixed gel remain independent of the amplitude of oscillation (i.e., linear viscoelastic response) up to at least 10% strain. Similar linear

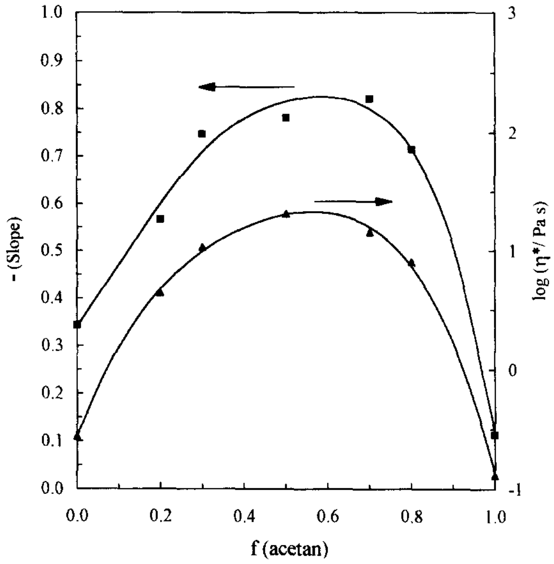


Fig. 7. Variation of η* (▲) at 1 rad s^{-1} and of the slope of log η* vs. log ω (■) with the proportion of deacetylated acetan in mixtures with KM at a total polymer concentration of 1.0% w/v; conditions as in Fig. 6.

viscoelasticity was observed for the mixed gel of deacetylated acetan with LBG whose mechanical spectrum is shown in Fig. 3c.

As illustrated in Fig. 6 for deacetylated acetan in combination with KM at a total polymer concentration of 1% w/v, varying the mixing ratio of the two components causes an initial increase and subsequent decrease in elastic response and in overall moduli. Fig. 7 shows the composition-dependence of η* at low frequency (1 rad s^{-1}), chosen as a representative index of network strength, and of the slope of log η*

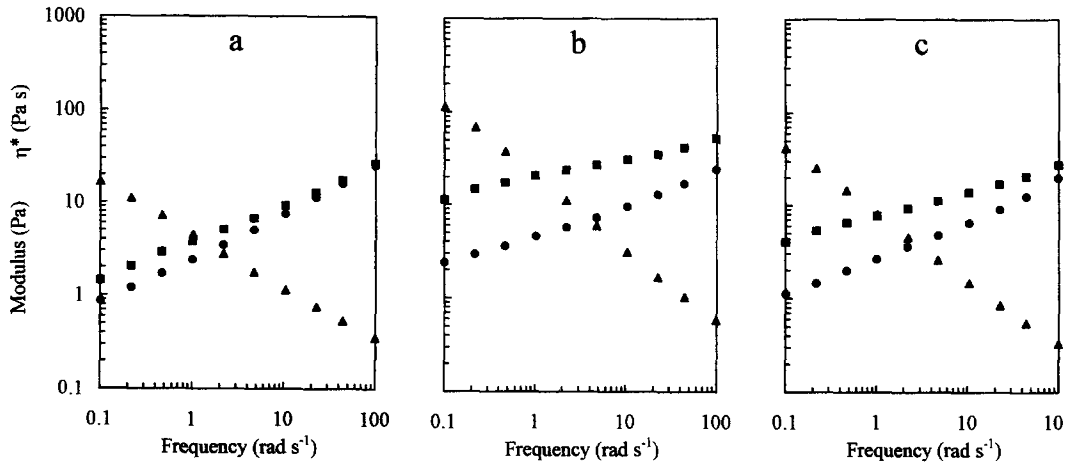


Fig. 6. Mechanical spectra (25 °C; 2% strain) showing the frequency-dependence of G′ (■), G″ (●), and η* (▲) for mixtures of deacetylated acetan (1.0% w/v) with KM (1.0% w/v) at acetan:KM ratios of (a) 2:8, (b) 5:5, and (c) 8:2. The starting solutions were equilibrated against 10 mM potassium chloride and mixed at 25 °C.

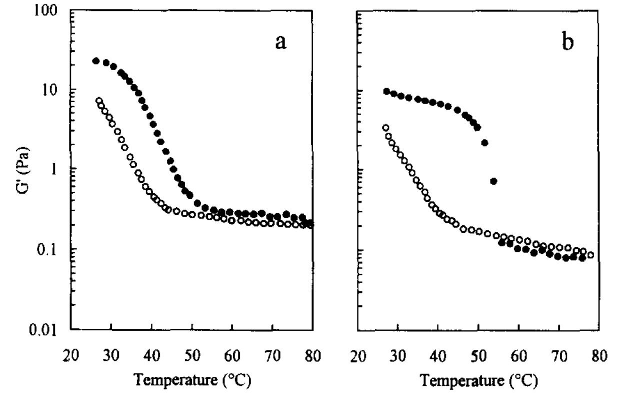


Fig. 8. Temperature-dependence of G' (10 rad s^{-1}; 2% strain) for mixtures of deacetylated acetan (0.3% w/v) with (a) LBG or (b) KM (0.7% w/v) in 10 mM potassium chloride on heating (●) and cooling (○) at 1 °C/min.

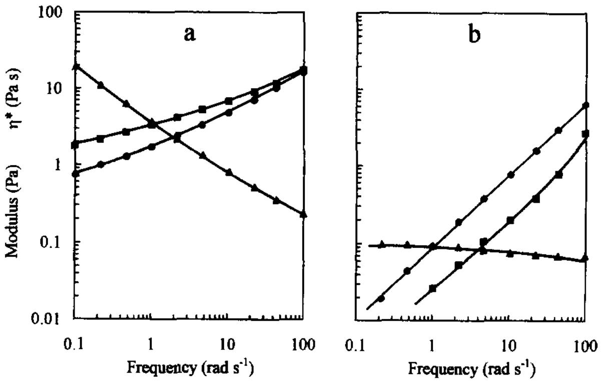


Fig. 9. Mechanical spectra (2% strain) showing the frequency-dependence of G' (■), G'' (●), and η^* (▲) for mixed gels of deacetylated acetan (0.3% w/v) with KM (0.7% w/v) in 10 mM potassium chloride after heating from 25 °C to (a) 40 °C and (b) 60 °C.

vs. log ω, as an index of gel-like character. Both pass through maximum values as the proportion of acetan present is increased, paralleling the behaviour observed (Fig. 2) for viscosity enhancement in dilute solution.

In contrast to the clear evidence of synergistic gelation for deacetylated acetan, mixtures of native acetan with either LBG or KM (prepared at 25 °C with a total polymer concentration of 1% w/v) gave mechanical spectra approaching to those of normal polysaccharide solutions ($G'' > G'$; strong frequency-dependence of both moduli, and only slight reduction in log η^* with increasing log ω), with no evidence of gel-like properties beyond those of acetan alone [23] This is consistent with the absence of any detectable enhancement in dilute-solution viscosity (Fig. 2), and further demonstrates that the presence of acetate substituents inhibits synergistic interaction of acetan with KM or LBG.

Melting and setting behaviour of synergistic gels. —On initial mixing of deacetylated acetan (0.3% w/v) with KM (0.7% w/v) in the presence of 10 mM potassium chloride at 25 °C (as in Fig. 4), the mixture remained fluid for about 5 min before forming a cohesive network. With LBG as co-synergist, under identical experimental conditions, gelation occurred within about 30 s, consistent with the rheological evidence of more extensive association in dilute solution (Fig. 2a and b) and in mixed gels (Fig. 3c and Fig. 4c). Fig. 8 shows the changes in G' (at 10 rad s^{-1} and 2% strain) observed when the resulting gels were heated and cooled (at 1 °C/min). In both cases, there is a well-defined melting process on heating, with recovery of network structure at lower temperature on cooling. Melting occurs more sharply and at higher temperature when the co-synergist is KM (Fig. 8b) rather than LBG (Fig. 8a). The restructuring process, however, is closely similar for both, giving greater hysteresis in the system incorporating KM. Mechanical spectra recorded at temperatures just below and just above the sharp melting transitions in Fig. 8 (at 40 °C and at 60 °C) illustrate the change from gel-like to solution-like response (Fig. 9).

The thermal changes accompanying the gelation and melting processes shown in Fig. 8 were investigated by DSC (at a scan rate of 0.5 °C/min). As

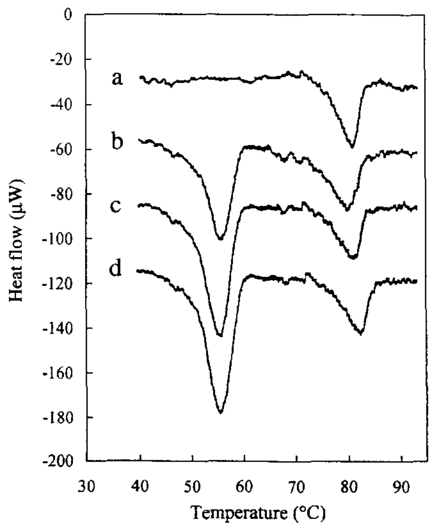


Fig. 10. DSC heating scans (0.5 °C/min) for deacetylated acetan (0.3% w/v) in 10 mM potassium chloride, alone (trace a) and in combination with KM (0.7% w/v) after mixing at 25 °C and holding for (b) 1 h, (c) 16 h, and (d) 24 h. The individual traces have been displaced vertically by arbitrary amounts to avoid overlap.

shown in Fig. 10, mixed gels of deacetylated acetan with KM gave two sharp endotherms on heating, centred at ~ 55 °C and ~ 80 °C. The first transition is coincident with the sharp melting process shown in Fig. 8b, and was found to increase in magnitude (traces b–d in Fig. 10) as the samples were held for progressively longer times at 25 °C (from $\Delta H \approx 9.5$ J/g when heating commenced 1 h after mixing to $\Delta H \approx 15.9$ J/g after storage for 24 h). In the cooling direction, this transition was much broader, and was displaced to lower temperature, consistent with the difference between the gelation and melting profiles shown in Fig. 8. The second endotherm corresponds to the order–disorder transition of deacetylated acetan alone (Fig. 10a); its magnitude was independent of storage time at 25 °C ($\Delta H \approx 5.0$ J/g) and a corresponding exotherm, with the same position and magnitude, was obtained on cooling. Similar behaviour was observed for mixtures of deacetylated acetan with LBG, but the lower-temperature transitions accompanying formation and melting of the mixed gel network (Fig. 8a) were smaller and broader, and could not be quantified with acceptable precision.

4. Discussion and conclusions

The main finding from this investigation is that deacetylated acetan, which at the concentrations used in the present work is non-gelling [23], forms synergistic gels with locust bean gum and konjac glucomannan, which are also non-gelling. There is strong evidence that gelation occurs by direct binding between the participating polysaccharides, rather than by an exclusion mechanism. The reasons for this belief are the following: (1) The interaction persists in very dilute solution (Fig. 2), where exclusion effects are likely to be negligible. (2) Gelation and melting occur as co-operative processes (Fig. 8) at a temperature well below the conformational transition of deacetylated acetan alone (Fig. 10a). (3) Melting of mixed gels incorporating KM as co-synergist is accompanied by an intense endothermic transition in DSC (traces b–d in Fig. 10). For native acetan, by contrast, no evidence of synergistic interaction was observed under the experimental conditions used (mixing at 25 °C in the presence of 10 mM potassium chloride), indicating that acetate substituents inhibit formation of heterotypic junctions.

Recent NMR studies [28] suggest that there are two sites of acetylation in the heptasaccharide repeating unit of acetan (Fig. 1). One is at O-6 of the inner mannosyl residues of the carbohydrate sidechains, as in xanthan. The other is at O-6 of the branched glucosyl residues in the polymer backbone. Deacetylation enhances the synergistic gelation of xanthan, but the unmodified polysaccharide also forms mixed gels with LBG or KM, even in mixtures prepared at temperatures below the helix coil transition [1]. An obvious interpretation would be that the presence of *O*-acetyl substituents on the polymer backbone of acetan, but not of xanthan, causes direct blocking of heterotypic association. However, recent studies in Norwich, which will be reported in detail elsewhere [29], have shown that if mixtures of native acetan with LBG or KM are heated to above the temperature range of the order–disorder transition of the acetan component and then re-cooled, a synergistic network gradually develops over time. It seems more likely, therefore, that the main effect of the *O*-acetyl groups is to provide an entropic drive to solubility, and that their removal shifts the entropy—enthalpy balance in favour of ordered association.

As shown in Fig. 8, there is substantial thermal hysteresis between formation and melting of the synergistic gels produced by deacetylated acetan in combination with either LBG or KM. Synergistic gels of xanthan or deacetylated xanthan with LBG or KM, by contrast, show no such hysteresis [7]. The temperature-course of gelation (as monitored by low-amplitude oscillatory measurements of G′) coincides closely with the melting profile and in all cases the onset of network formation on cooling and final loss of structure on heating occur close to 60 °C, irrespective of whether the xanthan is acetylated or non-acetylated and of whether the co-synergist is LBG or KM. The melting profiles obtained for synergistic gels of deacetylated acetan with LBG (Fig. 8a) or KM (Fig. 8b) comply with this generality. In both cases, final loss of structure (i.e., completion of the steep decrease in G′) occurs just below 60 °C. The setting profiles, however, are displaced to substantially lower temperature, and are closely similar with either KM or LBG as co-synergist. It would therefore appear that the heterotypic junctions formed initially by deacetylated acetan on cooling in the presence of LBG or KM are less stable than those in synergistic gels of xanthan, but that they undergo subsequent conversion into structures of comparable stability (giving similar melting behaviour).

The differences in behaviour observed for deacetylated acetan with KM or LBG mirror similar effects observed for deacetylated xanthan [7]. It is possible that these differences result from different mechanisms of association of the two bacterial polysaccharides with either KM or LBG, or that differences in the substitution patterns of KM or LBG determine the extent of initial interactions required prior to the formation of heterotypic junction zones.

Finally, displacement of acetan (or xanthan) from their normal helix structure to the conformation required for heterotypic association with mannan or glucomannan will occur only if the enthalpic stability of the synergistic junctions is greater than that of the 5-fold helix. The DSC traces shown in Fig. 10 demonstrate that the heat required to melt the junctions formed between deacetylated acetan and KM is about three times greater than that needed to disrupt the 5-fold helix of the acetan component. By contrast, it has been shown [30] that the genetically engineered polytetramer variant of xanthan (in which the terminal mannosyl groups of the carbohydrate sidechains are absent), gives a much more stable helix structure, and shows no evidence of synergistic interaction with either LBG or KM.

Acknowledgements

We thank Mr. M.J. Ridout, Ms. J.E. Harris and Mr. C.A. MacCormick for help and advice in the preparation of acetan, and Dr. R.K. Richardson for generous access to his protoype rheometer. We also thank the AFRC (now BBSRC) for support of the work at the Institute of Food Research and for the award of a co-operative studentship to one of us (C.O.).

References

[1] E.R. Morris, in P. Harris (Ed.), *Food Gels*, Elsevier, London, 1990, pp 291–359.

[2] I.C.M. Dea and A. Morrison, *Adv. Carbohydr. Chem. Biochem.*, 31 (1975) 241–312.

[3] N.W.H. Cheetham, B.V. McCleary, G. Teng, F. Lum and Maryanto, *Carbohydr. Polym.*, 6 (1986) 257–268.

[4] P.A. Williams, S.M. Clegg, D.H. Day, G.O. Phillips, and K. Nishinari, in E. Dickenson (Ed.), *Food Polymers, Gels and Colloids*, RSC Special Publication No. 82, Royal Society of Chemistry, Cambridge, 1991, pp 339–348.

[5] G. Cuvelier and B. Launay, *Carbohydr. Polym.*, 8 (1988) 271–284.

[6] R.O. Mannion, C.D. Melia, B. Launay, G. Cuvelier, S.E. Hill, S.E. Harding, and J.R. Mitchell, *Carbohydr. Polym.*, 19 (1992) 91–97.

[7] F.M. Goycoolea, T.J. Foster, R.K. Richardson, E.R. Morris, and M.J. Gidley, in G.O. Phillips, P.A. Williams, and D.J. Wedlock (Eds.), *Gums and Stabilisers for the Food Industry 7*, IRL Press, Oxford, 1994, pp 331–344.

[8] I.T. Norton, D.M. Goodall, S.A. Frangou, E.R. Morris, and D.A. Rees, *J. Mol. Biol.*, 175 (1984) 371–394.

[9] R. Moorhouse, M.D. Walkinshaw, and S. Arnott, *ACS Symp. Ser.*, 45 (1977) 90–100.

[10] K. Okuyama, S. Arnott, R. Moorhouse, M.D. Walkinshaw, E.D.T. Atkins, and C. Wolf-Ullish, *ACS Symp. Ser.*, 141 (1980) 411–427.

[11] E.R. Morris, D.A. Rees, G. Young, M.D. Walkinshaw, and A. Darke, *J. Mol. Biol.*, 110 (1977) 1–16.

[12] I.C.M. Dea and E.R. Morris, *ACS Symp. Ser.*, 45 (1977) 174–182.

[13] P. Cairns, M.J. Miles, V.J. Morris, and G.J.Brownsey, *Carbohydr. Res.*, 160 (1987) 411–423.

[14] P. Cairns, M.J. Miles, and V.J. Morris, *Nature (London)*, 322 (1986) 89–90.

[15] G.J. Brownsey, P. Cairns, M.J. Miles, and V.J. Morris, *Carbohydr. Res.*, 176 (1988) 329–334.

[16] R.P. Millane and B. Wang, *Carbohydr. Polym.*, 13 (1990) 57–68.

[17] R.O. Couso, L. Ielpi, and M.A. Dankert, *J. Gen. Microbiol.*, 133 (1988) 2123–2135.

[18] P.-E. Jansson, J. Lundberg, K.M.S. Wilmalasariri, and M.A. Dankert, *Carbohydr. Res.*, 245 (1993) 303–310.

[19] P.-E. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 45 (1975) 275–282.

[20] L.D. Melton, L. Mindt, D.A. Rees, and G. Sanderson, *Carbohydr. Res.*, 46 (1976) 245–257.

[21] V.J. Morris, G.J. Brownsey, P. Cairns, G.R. Chilvers, and M.J. Miles, *Int. J. Biol. Macromol.*, 11 (1989) 326–328.

[22] R.P. Millane, in R. Chandrasekaran (Ed.), *Frontiers in Carbohydrate Research 2*, Elsevier, New York, 1992, pp 168–190.

[23] C. Ojinnaka, E.R. Morris, V.J. Morris, and G.J. Brownsey, in G.O Phillips, P.A. Williams, and D.J. Wedlock (Eds.), *Gums and Stabilisers for the Food Industry 7*, IRL Press, Oxford, 1994, pp 15–26.

[24] C.A. MacCormick, J.E. Harris, A.P. Gunning, and V.J. Morris, *J. Appl. Bacteriol.*, 74 (1993) 196–199.

[25] J.E. Scott, *Methods Carbohydr. Chem.*, 5 (1965) 38–44.

[26] E.R. Morris, in G.O. Phillips, D.J. Wedlock, and P.A. Williams (Eds.), *Gums and Stabilisers for the Food Industry 2*, Pergamon Press, Oxford, 1984, pp 57–78.

[27] S.B. Ross-Murphy, in H.W.-S. Chan (Ed.), *Biological Methods in Food Research*, Blackwell, 1984, pp 138–199.

[28] I.J. Colquhoun, M. Defernez, and V.J. Morris, *Carbohydr. Res.*, 269 (1995) 319–331.

[29] M.J. Ridout, G.J. Brownsey, and V.J. Morris, *Macromolecules*, submitted.

[30] T.J. Foster and E.R. Morris, in G.O Phillips, P.A. Williams, and D.J. Wedlock (Eds.), *Gums and Stabilisers for the Food Industry 7*, IRL Press, Oxford, 1994, pp 281–289.