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Role of conformation and acetylation of xanthan on xanthan–guar interaction

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The synergistic effect obtained by mixing xanthan and guar solutions were examined by low shear viscosity measurements in relation to the temperature. Native and deacetylated xanthan samples were used in mixtures in which the total polymer concentrations were 1 g/litre and 0·5 g/litre. Gelation was observed for temperatures lower than 15 °C for the native xanthan–guar system (weight ratio 1/1) in 10^{-2} M NaCl and at 22–24 °C for the same system in water; in this last case, it is known that the xanthan is in the disordered conformation. For a mixture of deacetylated xanthan–guar, gelation was observed at a temperature below 26 °C in water. The results confirm that there is a stronger interaction between deacetylated xanthan and guar than native xanthan and guar because of enhanced xanthan–guar gum backbone association in the former case.

INTRODUCTION

Xanthan gum is an anionic polysaccharide produced by the microorganism *Xanthomonas campestris*, whose primary chemical structure consists of (1 ⟶ 4)-β-D-glucose repeating units, as the main chain, with a trisaccharide substituent on alternate glucose residues. Two mannose units and a glucuronic acid unit make up the side chain. The terminal β-D-mannose unit of the side chain may have a pyruvic acid residue linked to its 4— and 6— positions. Also, the non-terminal D-mannose unit on the side chain may have an acetyl group at the 6— position (Janson *et al.*, 1975; Melton *et al.*, 1976). The acetyl and the pyruvate contents vary due to the bacterial strain, culture and postfermentation conditions (Slonecker & Jeanes, 1962; Cadmus *et al.*, 1976; Tait *et al.*, 1986).

Galactomannan polysaccharides occur as reserve materials in a wide range of legume seeds. Their structure consists of a (1 ⟶ 4)-β-D-mannan backbone with single (1 ⟶ 6)-α-D-galactopyranosyl unit attached at the 0—6— position of certain D-mannopyranosyl residues. Guar galactomannan has a high and irregular distribution of D-galactosyl residues (Mc Cleary *et al.*, 1984). Both xanthan and guar gums are used as thickeners.

Much effort has been made to elucidate the behaviour of xanthan–galactomannan mixtures (Dea & Morrison 1975; Dea *et al.*, 1977; Morris *et al.*, 1977). It was shown that the observed synergistic interaction based on cooperative interaction depends on the mannose/galactose (M/G) ratio and also on the fine structure of the galactomannan. Higher M/G ratios as well as unsubstituted D-mannose blocks seem to favour the interaction (Dea *et al.*, 1972; Morris *et al.*, 1977; McCleary, 1979; Dea *et al.*, 1986). This is the case when carob gum is one of the components of the system. The mixture xanthan–carob gums also gelifies aqueous systems. These results have been explained by a model in which the helical xanthan interacts with the galactomannan backbone (Dea *et al.*, 1977). Another model was suggested to explain the participation of both unsubstituted and D-galactosyl substituted blocks of the galactomannan in junction zones. In this model, the D-galactosyl residues would be located on the same side of the main chain, allowing interaction between the two backbones (McCleary, 1979).

Dynamic viscoelasticity measurements have been

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carried out with mixtures of xanthan and guar gums. Gelation has been observed at low temperatures. The values obtained for the transition temperatures showed a stronger interaction between deacetylated xanthan and guar than with native xanthan. It was suggested that the greater flexibility of the deacetylated xanthan side chain would be responsible for the effect (Tako & Nakamura, 1984, 1985).

Evidence of specific intermolecular binding was found from the results of X-ray fibre diffraction of xanthan–carob and xanthan–tara gels. Gelation was observed only after submitting the water mixtures to heat treatment followed by cooling. The similarity between the D-glucosyl xanthan backbone and the D-mannosyl galactomannan backbone favours the interaction when xanthan is in the disordered conformation (Cairns *et al.*, 1986, 1987). Gel melting points and optical rotation measurements have been carried out with xanthan–carob mixtures. Homogeneous gels were observed in water between the disordered conformations of xanthan and carob gums. Deacetylated xanthan would participate in the junction zones in a similar way. (Cheetham & Mashimba, 1988).

Recently, the 2_1 conformation was shown to be sterically accessible to the xanthan backbone (Millane & Wang, 1990) and compared with that of cellulose I. This conformation will allow a cooperative interaction with the mannan backbone of guar.

In the present work, the relative viscosities at low shear ratio of native xanthan and guar mixtures of varying compositions were determined in 10^{-2} M NaCl and water in the temperature range 10–30 °C. Mixtures of deacetylated xanthan and guar in water were also investigated.

EXPERIMENTAL

Materials and Methods

A culture broth of xanthan gum, supplied by Rhône Poulenc, was diluted with 10^{-2} M NaCl in order to preserve the native structure (Milas & Rinaudo, 1986). This solution was filtered through 3-μm and 0·8-μm membranes. The sodium salt of xanthan was precipitated by the addition of ethyl alcohol, then submitted to successive washings in water ethyl alcohol mixtures from 30/70 to 0/100 and finally dried under vacuum. The $\bar{M}_W = 3{\cdot}7 \times 10^6$ of xanthan gum was determined by light scattering, using a Chromatix KMX-6 photometer. The yield in pyruvate and acetate substituents were 0·59 and 0·88 respectively.

Deacetylation (Callet *et al.*, 1987) was carried out by adding 20 ml of 10^{-1} M NaOH to a solution of 300 mg xanthan in 300 ml water and stirred for 24 h at 5 °C. The solution was then neutralised with 10^{-1} M HCl and filtered through 0·8 μm membranes. The product was precipitated as the Na salt by addition of 30 g NaCl and 400 ml ethyl alcohol, submitted to the same washing procedure and dried under vacuum. This procedure destroys the native structure.

Partial ^{1}H-NMR spectra of the native and deacetylated xanthan samples were obtained in 5·7 g/litre D_2O solutions at 85 °C (Bruker WP 100 MHz spectrometer), with suppression of the hydroxyl proton signals by irradiation. Sodium acetate was used as the internal standard (Fig. 1). Figure 1 shows the partial ^{1}H-NMR spectra of the native and the deacetylated xanthan samples. The peak at 2·1 ppm, attributed to the acetyl protons, are only observed in the spectrum of the native xanthan sample.

Guar gum (Hercules Inc.) was dissolved in water, submitted to the same filtration procedure, precipitated in ethyl alcohol, washed and dried under vacuum. The $\bar{M}_V = 2{\cdot}5 \times 10^6$ was calculated from the Mark-Houwink equation, using values (Robinson *et al.*, 1982) of $K = 3{\cdot}8 \times 10^{-4}$ and $a = 0{\cdot}723$. The intrinsic viscosity $[\eta] = 1600$ ml/g was determined in water and in 10^{-2} M NaCl in a Fica Viscomatic MS (Ubbelohde capilar)

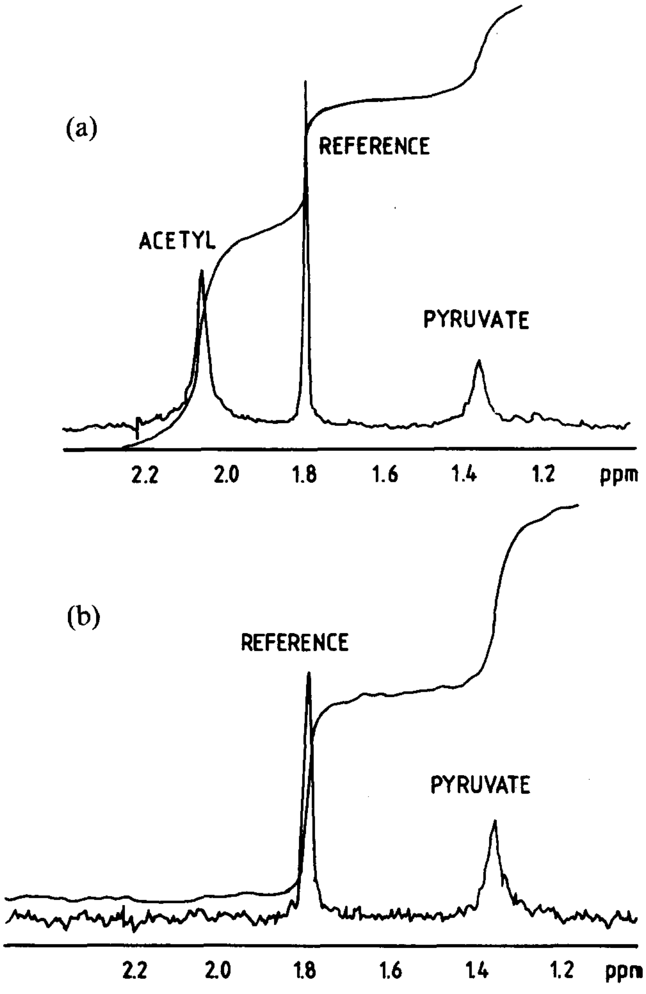

Fig. 1. Partial ^{1}H-NMR spectra of (a) native and (b) deacetylated xanthan samples in D_2O.

viscometer at 25 °C. The galactose/mannose ratio of guar gum, G/M = 0·56, was obtained after total hydrolysis in 1 M H_2SO_4, at 100 °C, and neutralization of the product in barium carbonate, by HPLC (Waters Associates Inc.), using a Bio-Rad HPX-87P ion exchange column and water as the eluant, at 85 °C (Bouffar-Roupe & Heyraud, 1987).

Viscosity measurements were carried out in a Contraves Low Shear-30 rheometer equipped with coaxial cylinders and a Rheoscan 30 programmer, at the lowest measurable shear rates. The solutions were obtained by mixing solutions of the pure gum solutions previously prepared in 10^{-2} M NaCl or in water at the appropriate temperatures. The water contents of both products were evaluated thermogravimetrically in a Setaram G-70 thermobalance and were taken into consideration in all solution preparations.

RESULTS AND DISCUSSION

Figures 2 and 3 show the viscosity behaviour of xanthan, guar gum and their mixtures at 25 °C, at a total polymer concentration of 0·5 g/litre respectively in 10^{-2} M NaCl and water allowing the influence of conformation on the intermolecular interaction to be determined; xanthan is in the disordered conformation in water but in the ordered conformation in 10^{-2} M NaCl (Milas & Rinaudo, 1986).

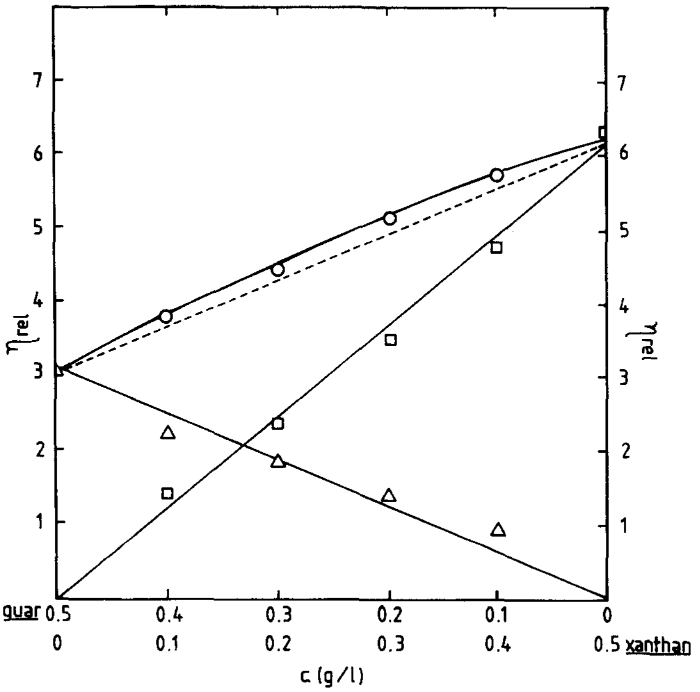

Fig. 2. Relative viscosity of pure polysaccharide solutions and their mixtures, at a total concentration of 0·5 g/litre in 10^{-2} M NaCl, at 25 °C: (□) xanthan, (△) guar, (○) xanthan–guar mixtures, (- - -) values calculated for mixture assuming no interaction.

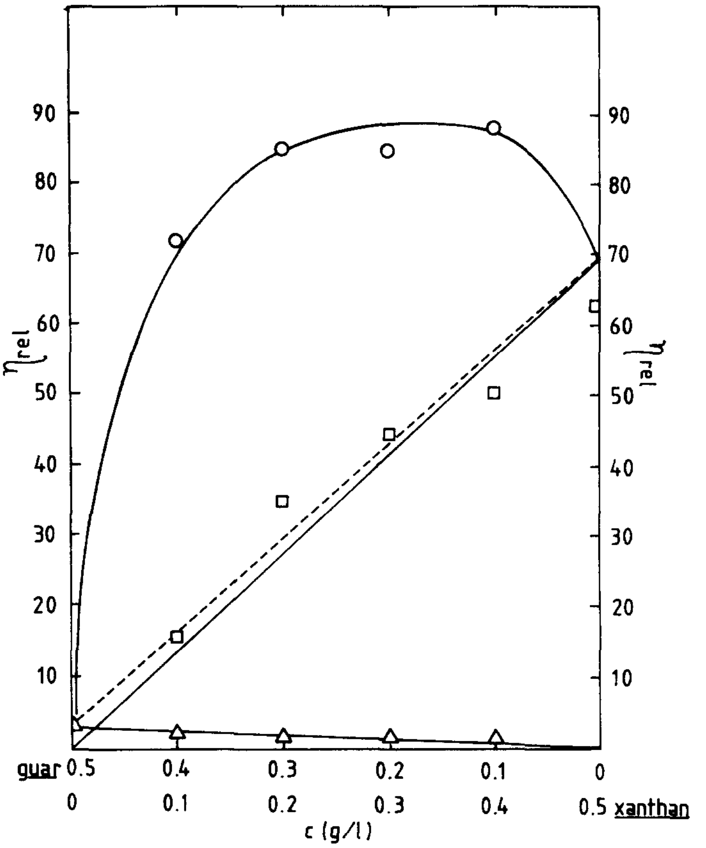

Fig. 3. Relative viscosity of pure polysaccharide solutions and their mixtures, at a total concentration of 0·5 g/litre in water, at 25 °C: (□) xanthan, (△) guar, (○) xanthan–guar mixtures, (- - -) values calculated for mixture assuming no interaction.

In both figures, the viscosities were taken in the Newtonian plateau for the pure polysaccharides. The same range of shear rates ($\dot{\gamma} = 2 \times 10^{-2}\ s^{-1}$) were also used for the mixtures although in this case, even at these low shear rates the Newtonian plateau was not reached. In order to eliminate previous shear effects, the readings were taken after 5–10 min of rotation.

In 10^{-2} M NaCl, a small synergistic effect is observed in Fig. 2 when the two polysaccharides are mixed. At the same total concentration, in the absence of salts, a much stronger interaction is observed (Fig. 3). It can be seen that the maximum viscosity corresponds to a xanthan–guar ratio of around 0·6. The cooperative interactions were studied as a function of temperature for a xanthan–guar ratio 1 : 1 which is not far from the optimum ratio for interaction.

To obtain information about the role of temperature on the interaction, viscosity measurements were carried out with each gum in the temperature range 10–30 °C. It was shown that there is a linear decrease in the relative viscosity with temperature for xanthan and guar gums in 2 g/litre 10^{-2} M NaCl and water solutions (Fig. 4). The observed behaviour indicates that under these conditions there is no change in the conformation nor in the intermolecular interaction for the pure polymers.

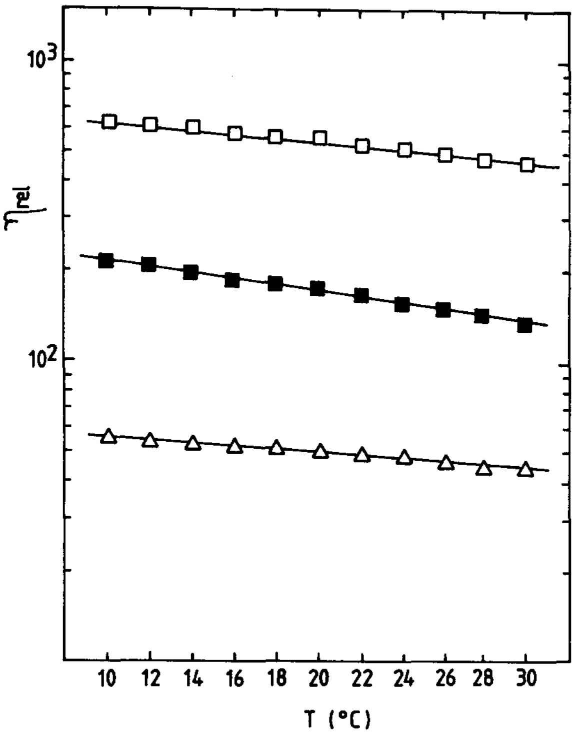

Fig. 4. Relative viscosity of pure polysaccharide solutions as a function of temperature, at the concentration of 2 g/litre: (□) xanthan gum in water, (■) xanthan gum in 10^{-2} M NaCl, (△) guar gum in 10^{-2} M NaCl and in water.

This data when compared with Fig. 3, clearly demonstrated that cooperative interactions are formed when disordered xanthan is mixed with guar at 25°C.

In addition, the larger relative viscosity of xanthan in water confirms the polyelectrolyte effect screened in the presence of 10^{-2} M NaCl. When native and guar solutions are mixed at a total concentration of 1 g/litre and 0·5 g/litre, a non-linear increase in the relative viscosity is observed with a decrease in temperature from 30 to 10°C, as shown in Figs 5 and 6. This may be attributed to interchain interaction, which at higher concentrations would result in gelation. The subsequent increase in temperature shows a similar but not coincident behaviour. This may be attributed to the formation, followed by dissociation, of specific intermolecular interactions between the two polymeric components of the system as the temperature changes. Much larger increases in viscosity with decreasing temperature are obtained when xanthan is in the disordered conformation (Fig. 6).

A critical temperature, T_C, resembling a gel point can be obtained for the native xanthan–guar from the intersections of the logarithmic relation between η_{rel} of the mixtures and the temperature (Fig. 7). Gel formation (or at least aggregation) was observed at total concentration as low as 0·2 g/litre and 0·1 g/litre for the two polysaccharides in water (Fig. 7). For 1 : 1 mixtures of xanthan and guar, the total concentration weakly affects T_C, thus it is found that T_C varies by only 5°C as

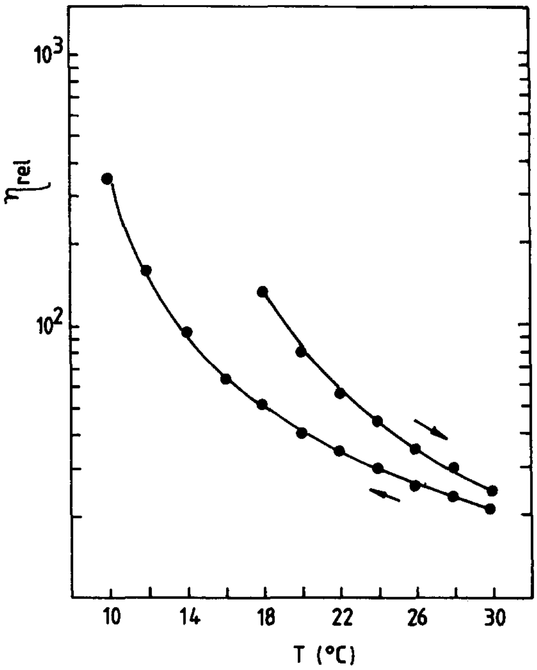

Fig. 5. Relative viscosity of xanthan–guar gums 1 : 1 mixture as function of temperature, at a total concentration of 1 g/litre, in 10^{-2} M NaCl.

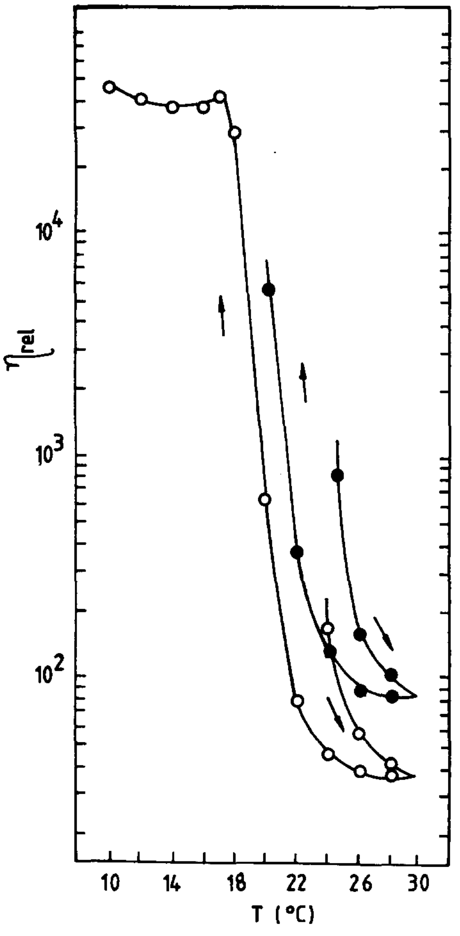

Fig. 6. Relative viscosity as function of the temperature for xanthan–guar gum mixtures at the total concentration of 1 g/litre (●) and 0·5 g/litre (○) in water.

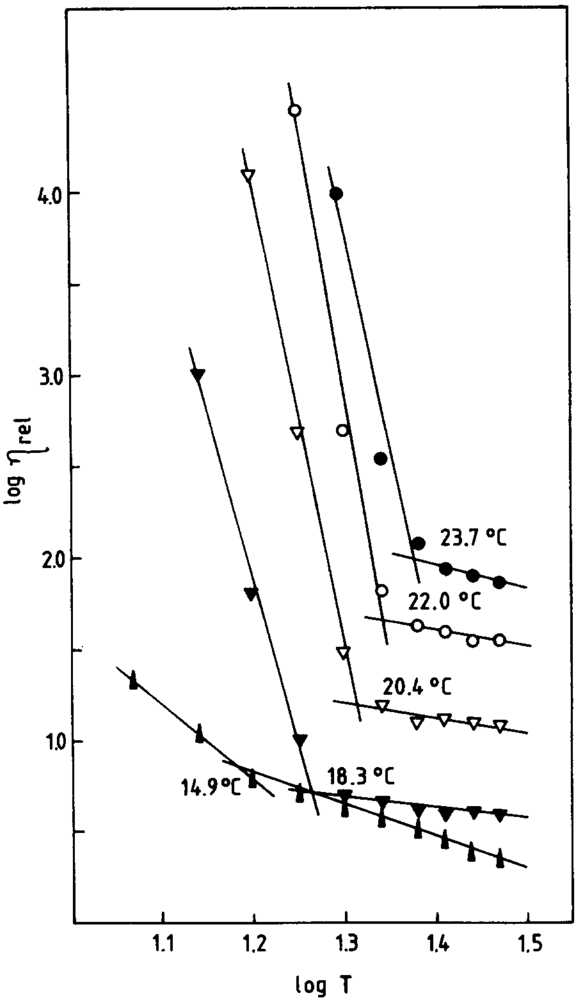

Fig. 7. Determination of the critical temperature of gelation for xanthan–guar gum mixtures at 1 g/litre in 10^{-2} M NaCl (▲) and in water (●); and at 0·5 g/litre (○), 0·2 g/litre (▽), 0·1 g/litre (▼) in water.

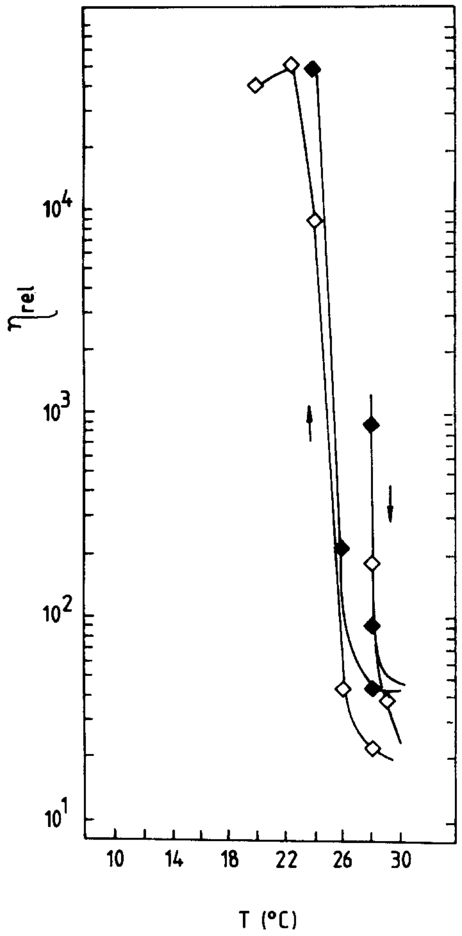

Fig. 8. Relative viscosity as function of the temperature for deacetylated xanthan–guar gum mixtures, at 1 g/litre (♦) and 0·5 g/litre (◇) in water.

the concentration is changed from 1 g/litre to 0·1 g/litre. The lowest T_C is the one obtained in 10^{-2} M NaCl solution; this is important evidence to show that the interaction between xanthan and guar is looser when the xanthan assumes the ordered conformation.

The same series of viscosity experiments were carried out with mixtures of deacetylated xanthan and guar in water solution at the total concentrations of 1 g/litre and 0·5 g/litre. Figure 8 shows a much more pronounced non-linear increase in viscosity as the temperature is lowered. This result can be attributed to the stronger intermolecular specific interactions in this system, as a consequence of removing the hydrophobic acetyl group from the xanthan side chain.

Figure 9 shows the graphical determination of T_C for the system deacetylated xanthan–guar gums in water at 1 g/litre and 0·5 g/litre. Critical temperatures near 26°C are observed. The higher T_C values are thought to be due to the increase in the stability of the mixed junction zones (Rochas & Rinaudo, 1984; Landry, 1987).

CONCLUSION

The experimental results obtained in this paper demonstrate specific and cooperative interactions between xanthan and guar gum at a ratio by weight of around 0·6. The interaction gives a large increase in viscosity indicating the formation of a gel-like structure with a hysteresis between increasing and decreasing temperature. An estimation of the critical temperature T_C for aggregation is suggested which varies slightly with the polymer concentration but demonstrates clearly the role of the xanthan conformation and that of its degree of acetylation. The stability of guar–xanthan interactions increases when the degree of acetylation decreases and when the conformation of the xanthan is disordered. These results imply that the junction zones are formed between mannan blocks and the cellulose-like conformation of xanthan segments as suggested recently by Millane and Wang (1990).

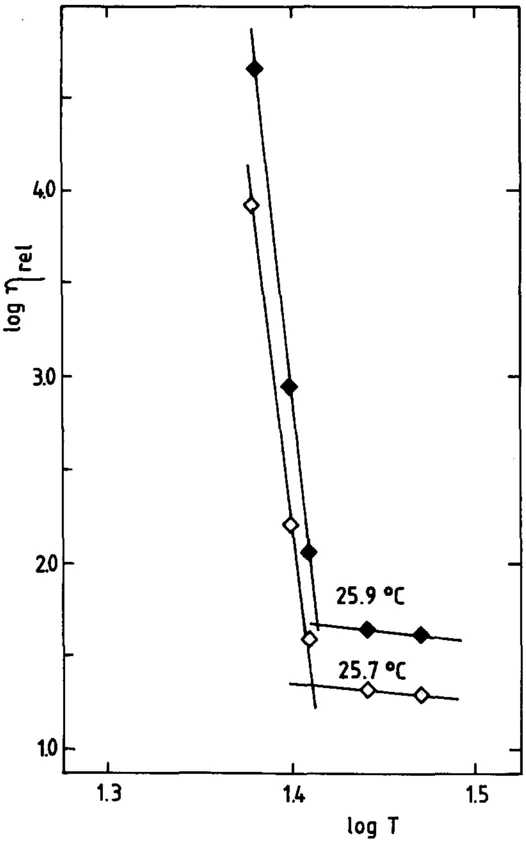

Fig. 9. Determination of the critical temperature of gelation for deacetylated xanthan-guar gum mixtures, at 1 g/litre (◆) and 0·5 g/litre (◇) in water.

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