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Microscopy of xanthan/galactomannan mixtures

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Polarization microscopy has been used to investigate the structure of 50/50 xanthan/galactomannan (guar gum or locust bean gum) mixtures in aqueous solution, the total concentration ranging from 0.5 to 4%. By the use of polarized light microscopy birefringent areas resulting from the formation of cholesteric mesophases in xanthan gum was clearly seen as has previously been reported by several authors. In xanthan/galactomannan mixtures, we also observed birefringent areas. Moreover, these zones in the blend appeared more anisotropic than with xanthan gum alone. This suggests that xanthan molecules organize themselves as liquid crystalline mesophases in definite enriched xanthan areas resulting from a concentration of xanthan inside these birefringent zones. Upon heating, this anisotropy disappears at a temperature well below the helix–coil transition temperature of xanthan molecules. In fact, this loss of order of the mixed system occurs at the same temperature as the melting temperature of the gel, as assessed by the use of rheological measurements. Since the ordered helical structure of the xanthan molecules still exists beyond the melting temperature while anisotropy disappears, this suggests that the xanthan molecules are no longer concentrated in specific areas but more evenly distributed in the medium. Gel melting would, therefore, be the result of the disappearance of these xanthan enriched areas.

INTRODUCTION

Xanthan is a high molecular weight extracellular polysaccharide produced by fermentation with the bacterium *Xanthomonas campestris*. The backbone is a chain of (1–4)-ß-D-glucose residues. This polysaccharide exhibits a polyelectrolyte character due to every second glucose residue carrying a charged trisaccharide side chain. The conformation of this biopolymer will change through an order (helix)-to-disorder (coil) transition upon heating. The stability of this ordered conformation is extended to higher temperature at increasing ionic strength. Xanthan polymer–polymer association is promoted by increasing salt concentration, owing to a reduction in intermolecular repulsion (Smith *et al.*, 1981). Recently, scanning tunnelling microscopy has been performed on xanthan gum (Gunning *et al.*, 1993). The images revealed aligned arrays of rod-like, stiff macromolecules with 'diameters' consistent with those expected for individual helices, but were of insufficient quality to permit any analysis of helical structure. It is well known that rod-like macromolecules in solution undergo a transition from an isotropic state to a state of partial long-range orientational order (usually called 'lyotropic liquid crystalline') at a critical concentration. Although ultra-high molecular weight xanthan may thus be regarded as a flexible polymer, it is still capable of forming mesophases in aqueous solutions. Highly concentrated solutions of xanthan gum in aqueous systems can also form lyotropic liquid crystals. The anisotropic nature of xanthan gum is a result of the helical structure probably via the involvement of hydrogen bonds. Allain *et al.* (1988) and Oertel and Kulicke (1991) have studied the viscosity behaviour of highly concentrated xanthan solutions. They found that when the viscosity is plotted as a function of concentration and shear rate, xanthan gum exhibits a behaviour typical of lyotropic liquid crystals. Using polarization microscopy, they then described the liquid crystal phase transition and the corresponding rheological properties. The relationship between rheological behaviour and molecular association has also been studied by Lim *et al.* (1984) using dynamic oscillatory measurements, stepshear rate measurements and optical birefringence. According to these authors, for concentrations of 1% and greater, liquid crystalline regions are formed. In contrast, they reported that for concentrations around 0.5%, the

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structure is not liquid crystalline in nature, but is due to networks formed by self-association, possibly through hydrogen bonding. However, the presence of mesophases has been detected at a lower xanthan concentration. Birefringence has been reported for concentrations of xanthan as low as 0.25% in water solution (Milas & Rinaudo, 1979) or for a concentration of 0.8% in 0.02 M KCl (Carnali, 1991). This latter author showed the appearance of an anisotropic phase at xanthan concentrations in close proximity to the onset of the weak-gel properties. Thus, at the lower concentration, the texture consisted of birefringent patches that resembled those more clearly evidenced at a higher concentration, namely 3% but which became more widely spaced and thinner as the concentration decreased. These latter results suggest that the cause of the unusual rheology of xanthan is the presence of interacting domains of dispersed anisotropic phase, in contrast to the Ross-Murphy *et al.* (1983) assumption which suggests that the peculiar rheology of xanthan solution is due to association of ordered chain segments, giving rise to a weak three-dimensional network.

Galactomannans have a linear backbone of (1–4)-linked ß-D-mannose residues substituted to varying degrees with (1–6)-linked monosaccharide sidechains of α-D-galactose. The most familiar examples are locust bean gum (LBG) and guar gum, whose mannose/galactose ratios are approximately 3.5 and 1.8, respectively.

Xanthan/galactomannan (guar gum and LBG) mixtures are widely used in the food industry due to the synergistic properties they develop. Although the molecular mechanism underlying these synergistic phenomena is still a matter of debate, it is generally considered that these properties arise from specific interactions between xanthan and galactomannan chains. There is, therefore, a need for further investigation of such systems.

The aim of the present study was to investigate the liquid crystalline properties of xanthan in xanthan/galactomannan mixtures. These properties were considered as a function of polymer concentration, ionic strength and temperature and compared with the properties of the polymer alone. Polarized light microscopy and viscoelastic measurements were used for this purpose.

MATERIALS AND METHODS

Materials

A commercial food grade xanthan powder was a gift from Rhône-Poulenc (France). The galactomannans (guar gum and LBG) used were obtained from Meyhall Chemical (Switzerland). Intrinsic viscosity values of these samples were 1980 ml/g for guar gum and 1490 ml/g for LBG, respectively, and 6000 ml/g in 0.13 M KCl for xanthan gum. Galactomannan samples have been purified by precipitation in ethanol (Lopes da Silva & Gonçalves, 1990).

Preparation of the mixtures

Xanthan/galactomannan mixtures (50/50) at a total concentration of 0.5% or 2% (w/w) were obtained by dispersion of appropriate amounts of the powder in distilled water or KCl and by mixing the blend at 80°C for 30 min under magnetic stirring. The mixtures in water were also concentrated to 4% (w/w) by evaporation.

Methods

The mesophases were observed with an Olympus Vanox polarizing microscope. The hot polymer samples were placed between a slide and a coverslip and sealed to prevent fluid evaporation. The thin sample and slide allows rapid heat transfer. A heating stage enables the sample to be heated between 20°C and 80°C every 5°C. At each temperature, the extent of birefringence was observed after 5 min. Meanwhile, dynamic measurements were performed using a Rheometrics Fluid Spectrometer (RFSII) with a Couette system (R_i = 16 mm; R_e = 17 mm; H = 33 mm). The solutions were transferred at 80°C into the rheometer and rapidly cooled to 25°C. A layer of paraffin oil was added to protect the sample from dehydration. A frequency–temperature sweep was then performed using step-by-step heating every 5°C (30 min at each temperature). The frequency range was between 0.01 and 100 rad/s at a strain of 1%. The mechanical spectrum was thus obtained at intervals of 5°C at temperatures ranging from 25°C to 80°C. The linearity of the viscoelastic behaviour was checked both in the sol and the gel states.

RESULTS AND DISCUSSION

Microscopy of xanthan solutions and xanthan/galactomannan mixtures

Figures 1a and b show the structures of concentrated aqueous xanthan solutions in water (no salt added) at 2 and 4% (w/w), respectively. Observation under the polarizing microscope shows that immediately after the preparation the sample was entirely dark. Gradually, white areas corresponding to anisotropic phases appeared. Thus, in our case the observation was made after 12 h at 4°C. As a rule, an isotropic solution is indicated by a black field, whereas an anisotropic medium illuminates the field due to birefringence. In the present work, we found a low degree of birefringence for a concentration of approximately 0.8%. The surface

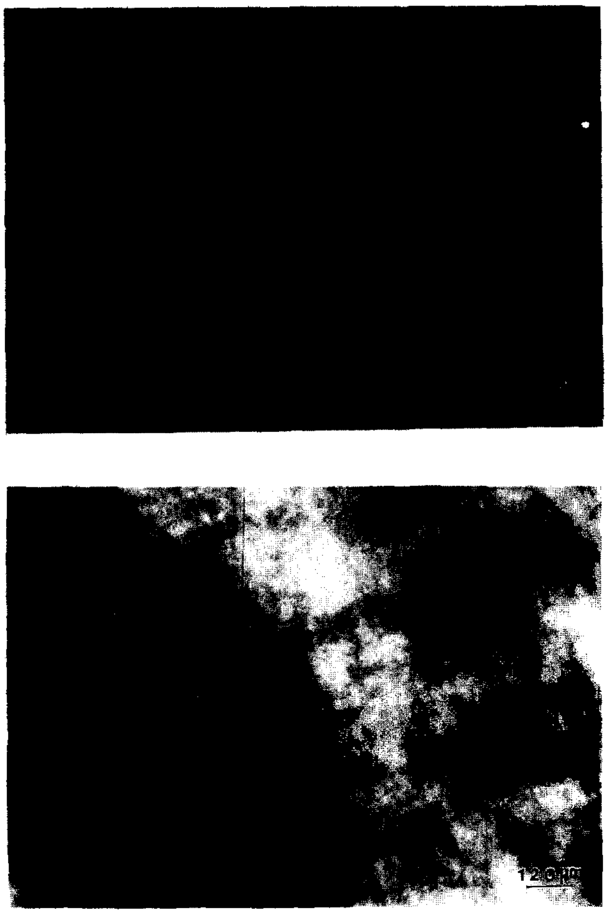

Fig. 1. Micrographs at 20°C of a xanthan gum solution (no salt added) at (a) 2% (w/w); and (b) 4% (w/w) after 12 h at 4°C.

of these white areas increased with concentration showing the formation of liquid crystal phases in solutions of high molecular weight semi-flexible molecules of xanthan. Although there was a strong birefringence at a concentration of 4%, no regular texture was visible, even under different conditions. The reason for this absence may lie in the flexibility of the polymer and the high viscosity of the solution. However, the cholesteric organization of these liquid crystalline phases has been described with partially hydrolysed polymers and at high concentrations (Maret *et al.*, 1981; Bouligand & Livoland, 1984; Livolant, 1986). The present results are in good agreement with those obtained by Lim *et al.* (1984). These authors found that for concentrations of 1% (w/w) and greater, liquid crystalline regions are formed by the shear field, and these liquid crystal regions persist after the cessation of shear. However, they suggested that for a concentration around 0.5% (w/w), the structure is not liquid crystalline in nature, but is due to a network formed by self-association as observed using dynamic light scattering. Allain *et al.* (1988) from viscosity measurements and optical properties of xanthan also reported anisotropic phase formation at low polymer concentrations (0.7% w/w) in the case of purified solutions. Carnali (1991) observed the appearance of an anisotropic phase at xanthan concentrations in close proximity to the onset of the weak gel properties, i.e. 0.8% (w/w) in 0.02 M KCl. The anisotropic phase was identified by a static, permanent birefringence. Microscopic evidence indicates that this anisotropic phase is present as a dispersion and that its volume fraction increases with polymer concentration in the usual way. In salt solutions, similar structures have been seen but the birefringence appeared at a slightly higher concentration as reported by Milas and Rinaudo (1979). Clearly, in concentrated xanthan solutions, in the absence or presence of external salts, the observed birefringence indicates an orientation of the molecules. These birefringence measurements demonstrate that structures in xanthan solutions above 0.8% (w/w) in water are liquid crystalline in nature. There is clearly no structural difference between low and highly concentrated xanthan gum solutions. The disappearance of birefringence as the concentration decreases is only the result of the decrease in the volume fraction of the anisotropic areas.

The effect of the presence of the galactomannan (guar gum or LBG) on the formation of mesophases has been investigated for xanthan solutions alone. Figure 2a, which shows a 50/50 xanthan/LBG mixture at 1% (w/w) (no salt being added), shows that birefringence appeared for a concentration much lower than the required concentration of xanthan. The same phenomenon was observed for a 50/50 xanthan/guar gum mixture at 1% (w/w) (Fig. 2b). Further experiments have been performed on xanthan/guar gum mixtures at higher concentrations. It has been shown that the higher the concentration, the stronger the birefringence, as reported for xanthan solutions alone. Similar structures have also been obtained with added salt. Clearly, we have provided evidence that xanthan gum, when blended with a galactomannan (guar gum or LBG) in an aqueous medium, forms liquid crystalline mesophases which are much more anisotropic than for a xanthan gum solution at an identical concentration. Moreover, the organization of the gels resembles the structure of the xanthan gum solutions. These results suggest that xanthan molecules organize themselves as liquid crystalline mesophases in some areas, due to the presence of guar gum or LBG. To make these areas highly birefringent requires a concentration of xanthan gum inside these zones.

Effect of temperature

The microstructure of these systems has been investigated with varying temperatures from 25 to 80°C. Experiments were first carried out on xanthan gum solutions alone at 1% (w/w) in water and in 0.13 M KCl. For the xanthan gum solution at 1% (w/w)

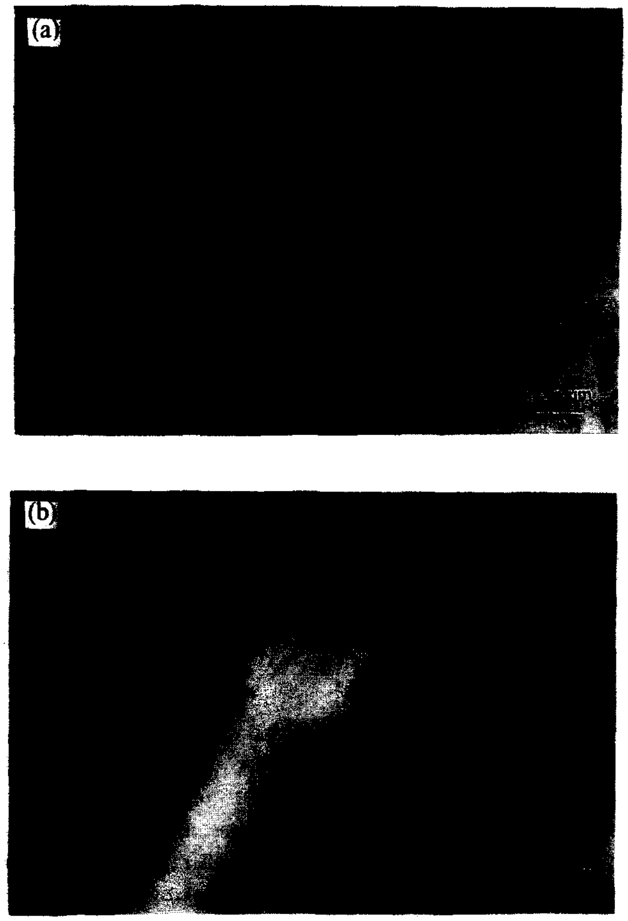

Fig. 2. Micrographs at 20°C of (a) xanthan/locust bean gum mixture; and (b) xanthan/guar gum mixture at a ratio of 50/50, at 1% (w/w), no salt being added after 12 h at 4°C.

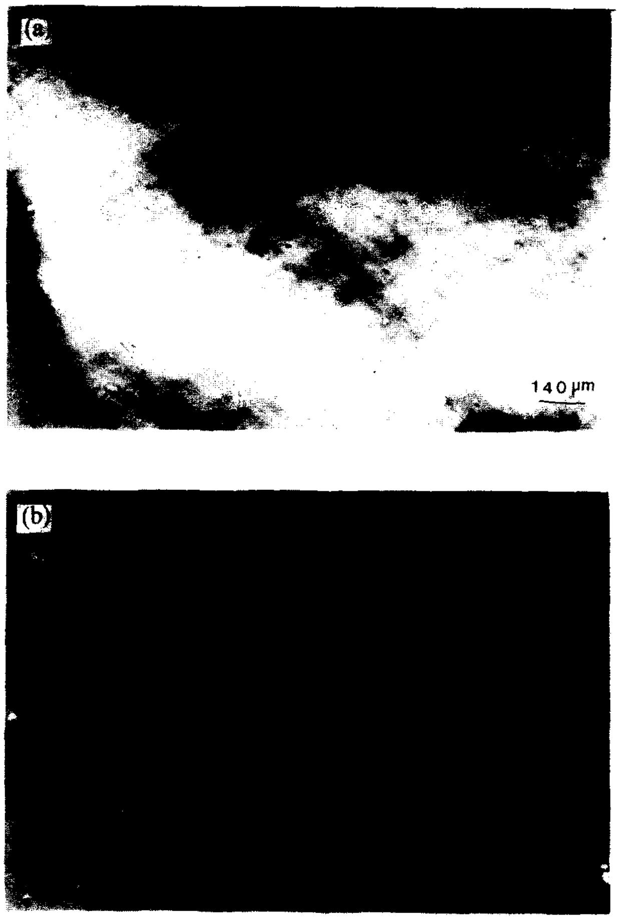

Fig. 3. Micrographs of a 50/50 xanthan/guar gum mixture at 2% (w/w) in 0.13 M KCl at (a) 25°C; and (b) 65°C.

in water (no salt added) we observed a decrease of birefringence as the temperature increased and particularly the disappearance of the anisotropy at approximately 55°C. As estimated from differential scanning calorimetry (DSC) studies, the xanthan midpoint of the helix–coil transition at this concentration in water occurred at 55°C. This result is in agreement with the data reported by Milas and Rinaudo (1979). These authors found that the birefringence of concentrated xanthan solutions disappears at a temperature corresponding exactly to the helix–coil transition temperature of the xanthan molecules. The same experiment has been performed on the xanthan gum solution at 1% (w/w) in 0.13 M KCl. For temperatures ranging from 25 to 80°C, the birefringence decreased slightly but persisted at 80°C. The conformational transition of xanthan molecules under these ionic conditions, as checked by DSC experiments, takes place at 110°C (Schorsch & Doublier, unpublished). Therefore, birefringence of xanthan would only completely disappear at 110°C.

Figures 3a and b show the microstructure of a 50/50 xanthan/guar gum mixture at 2% (w/w) in 0.13 M KCl at temperatures of 25 and 65°C, respectively. These experiments were carried out for the same xanthan concentration as used in the previous study. The loss of birefringence with increasing temperature is clearly seen. At 25°C, the system was strongly birefringent, whereas at 65°C, the mixture appeared isotropic without any anisotropic area. This contrasts with the results for xanthan solution alone. Concurrently, dynamic measurements have been performed on this mixture. A result is illustrated in Fig. 4 for the 50/50 xanthan/guar gum mixture at 2% (w/w) in 0.13 M KCl. Increasing the temperature from 25 to 80°C resulted in a progressive loss of structure, the system exhibiting the properties of a gel at 25°C and behaviour close to that of a solution at 75°C. The melting temperature of the mixture was in the vicinity of 70°C under these conditions. Clearly, these data show that the loss of the birefringence occurred at the same temperature as the melting temperature of the xanthan/guar gum gel. Furthermore, we checked that the helix–coil transition of xanthan was not altered in the presence of guar gum under ionic conditions (Schorsch & Doublier, unpublished). Therefore, the loss of birefringence in the blend did not coincide with the helix–coil transition of the xanthan molecules. It is thus clear that, beyond the melting temperature of the gel,

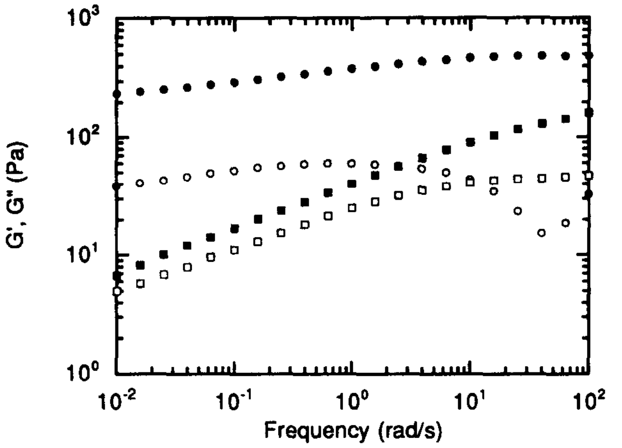

Fig. 4. Viscoelastic spectra, G' and G'' against frequency of a 50/50 xanthan/guar gum mixture at 2% (w/w) in 0.13 M KCl at 25°C (●, O: G', G'') and 75°C (■,□: G', G'').

xanthan molecules remain in an ordered conformation. As a result, the liquid crystalline mesophases should also remain as previously reported for xanthan solutions alone. This suggests that the xanthan molecules are no longer concentrated in definite areas but, in contrast, are distributed evenly in the overall available volume. It infers that the enriched xanthan mesophases disappear and the birefringence is no longer detectable.

CONCLUSION

The thermal behaviour of concentrated xanthan/galactomannan mixtures was investigated by means of polarization microscopy and rheology. It was seen that not only xanthan solutions but also xanthan/galactomannan mixtures exhibit birefringence which has been ascribed to lyotropic phases. Qualitatively, similar results have been obtained whatever the type of galactomannan, guar gum or LBG. A stronger anisotropy was seen in the presence of guar gum or LBG than with xanthan alone at equal concentration. This suggests a concentration effect of xanthan gum by the galactomannan. This liquid crystalline organization disappears at the temperature which corresponds closely to the melting temperature of the gel. This melting temperature lies well below the helix–coil transition temperature of xanthan. This means that beyond the melting temperature of the gel, xanthan molecules, which are concentrated in some definite areas due to the presence of the galactomannan, are able to propagate homogeneously in all the available volume. Although it has been largely neglected in favour of the junction zone model, the concept of mutual incompatibility between the two polysaccharide entities could be favoured for the formation of a mixed network. Such mixed networks have been reported for protein/polysaccharide systems (Tolstoguzov, 1990). Thermodynamic incompatibility of the two components of the mixture with exclusion of one from the domain of the other should produce phase domains rich in each component, thus increasing the effective concentration of the individual species.

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