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Promotion and inhibition of xanthan 'weak-gel' rheology by calcium ions

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Abstract

The effect of calcium ions on the rheology of xanthan has been studied by low-amplitude oscillatory measurements. Solutions of Na^+ xanthan were prepared at a fixed concentration of 5 g/l (~7.5 mN w.r.t. COO^-), with incorporation of 10 mN NaCl to ensure adoption of the ordered conformation, and with $CaCl_2$ concentrations varied between 0 and 100 mN. Measurements were made at 5 °C after cooling (at 1 °C/min) from the disordered state at 90 °C. G', G'' and the slope of $\log\eta^*$ *vs.* $\log\omega$ rose steeply as calcium ion concentration was increased to 7.5 mN (~100% stoichiometric equivalence), but then dropped sharply between 7.5 and 15 mN, before continuing to increase monotonically at higher concentrations of Ca^{2+}; converse behaviour was observed for $\tan\delta$. Thus the solutions pass through states of maximum and minimum gel-like character at ~100% and ~200% stoichiometric equivalence of Ca^{2+} (i.e. at Ca^{2+}:COO^- ratios of ~1:2 and ~1:1, respectively). Closely similar results were obtained for Me_4N^+ xanthan in the presence of 10 mN Me_4NCl and 4 M urea. The anomalous reduction in gel-like character at Ca^{2+} concentration between stoichiometric and twice stoichiometric is tentatively ascribed to partial replacement of intermolecular site-binding of calcium ions by binding to individual carboxyl groups, to maximise the degree of complexation.

Keywords: Rheology; Xanthan; Calcium ions; Site-binding

1. Introduction

Xanthan is an anionic bacterial exopolysaccharide whose primary structure (Jansson, Kenne, & Lindberg, 1975; Melton, Mindt, Rees, & Sanderson, 1976) is based on the pentasaccharide repeating unit shown in Fig. 1. It was first identified as a potentially useful material because of its unusual rheological properties (Jeanes, Pittsley, & Senti, 1961). Solutions of xanthan flow freely, like those of other polysaccharide thickeners, but are capable of holding solid particles in suspension as if trapped in a gel. These characteristic 'weak-gel' properties were central to the rapid commercial success of xanthan as an industrial hydrocolloid. The involvement of an ordered conformation was indicated by a preliminary report (Morris, 1973) of a sigmoidal change in optical rotation on heating and cooling, which was subsequently related (Morris, Rees, Young, Walkinshaw, & Darke, 1977) to sharp changes in the temperature-dependence of solution viscosity observed initially by Jeanes et al. (1961).

Since these early publications, the conformation and rheology of xanthan have been studied extensively. The transition from a disordered conformation at high temperature to an ordered structure at low temperature is fully reversible on heating, with no significant thermal hysteris, and, like the disorder–order transitions of other polyelectrolytes (Piculell & Nilsson, 1990), is displaced to progressively higher temperature with increasing ionic strength (e.g. Holzwarth, 1976; Morris et al., 1977; Milas & Rinaudo, 1979). The ordered structure, as characterised

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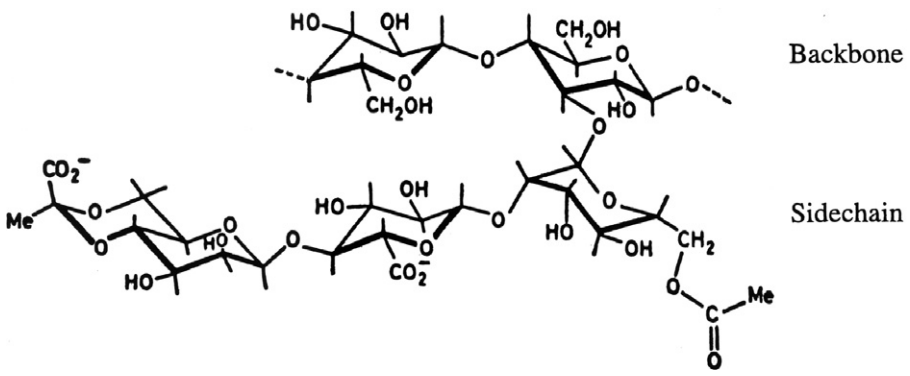

Fig. 1. Pentasaccharide repeating unit of xanthan. The polymer has a (1 → 4)-linked β-D-glucan (cellulosic) backbone with charged trisaccharide sidechains of: β-D-Man*p*-(1 → 4)-β-D-GlcA*p*-(1 → 2)-α-D-Man*p*-(1 →) attached at O(3) of alternate residues. In most of the repeat units, the inner mannose residue of each sidechain carries an *O*-acetyl substituent at C(6), as shown. In commercial xanthan (as produced by Kelco) the 4,6-linked pyruvate ketal substituent shown on the terminal mannose residue is present in ~39% of the sidechains.

by X-ray fibre diffraction in the solid state (Moorhouse, Walkinshaw, & Arnott, 1977; Okuyama et al., 1980), is a fivefold helix with a pitch of ~4.7 nm (i.e. with 10 backbone glucosyl residues per repeat); the persistence length in solution is about an order of magnitude greater than the length of the repeat unit (e.g. Muller, Anhourrache, Lecourtier, & Chauveteau, 1986; Ross-Murphy, Morris, & Morris, 1983; Tinland & Rinaudo, 1989), indicating a stiffness close to that of double-stranded DNA.

It is well established from light-scattering studies (e.g. Lecourtier, Chauveteau, & Muller, 1986; Liu, Sato, Norisuye, & Fujita, 1987; Norton, Goodall, Frangou, Morris, & Rees, 1984) that ordered xanthan can adopt a stable dimeric structure in solution, but whether the dimer is a co-axial double helix or is formed by lateral association of single helixes, individually stabilised by ordered packing of sidechains along the glucan backbone, is a subject of long-standing controversy (Mitchell, 1993); evidence cited in support of both proposals is reviewed elsewhere (Norton et al., 1984). There is, however, general agreement that the 'weak-gel' rheology (Morris, 1991) involves supramolecular associations that are inhibited by urea (Frangou, Morris, Rees, Richardson, & Ross-Murphy, 1982; Southwick, Lee, Jamieson, & Blackwell, 1980) and promoted by salt (Ross-Murphy et al., 1983; Smith, Symes, Lawson, & Morris, 1981). From comparison of the concentrations of urea required for inhibition of network properties, it has been concluded (Ross-Murphy et al., 1983) that the order of effectiveness of common counterions in promoting association of ordered xanthan increases through the series $Na^+ < K^+ \ll Ca^{2+}$. Studies of cation activity in the presence of xanthan (Lambert, Milas, & Rinaudo, 1985) indicate that the particular effectiveness of Ca^{2+} involves specific site-binding to the carboxyl groups of the polymer.

In the present work, we have explored the effect of calcium ions by the more direct procedure of observing the changes in small-deformation oscillatory rheology induced by addition of increasing amounts of $CaCl_2$ (0–100 mN) to solutions of xanthan (5 g/l) in the Na^+ salt form (stabilised in the ordered conformation by incorporation of 10 mM NaCl), or in the Me_4N^+ salt form in the presence of 10 mM Me_4NCl and 4 M urea (conditions chosen to minimise association in the absence of Ca^{2+}). In both systems we have found a large, sharp, decrease in gel-like character over a narrow range of Ca^{2+} concentration (between ~100 and ~200% stoichiometric equivalence to the carboxyl groups of the polymer), superimposed on the expected trend to greater intermolecular association with increasing concentration of Ca^{2+}.

2. Materials and methods

Distilled deionised water was used in all the preparative procedures described below; tetramethylammonium chloride (Me_4NCl) was from Aldrich; sodium chloride, calcium chloride dihydrate and urea were AnalaR grade from BDH. Commercial xanthan (Keltrol T) from Kelco (now CP Kelco), San Diego, USA, was converted to the Na^+ and Me_4N^+ salt forms by cation exchanged on Amberlite IR-120 resin, dialysed, and freeze dried. Solutions of the freeze-dried materials were prepared at a concentration of 10 g/l in water by mechanical stirring at 80 °C, and were then mixed 50/50 with appropriate solutions (80 °C) of the other constituents at twice the required final concentrations, to give 5 g/l xanthan in the presence of the corresponding chloride salt (Na^+ or Me_4N^+) at a fixed concentration of 10 mN, with Ca^{2+} concentrations in the range 0–100 mN (i.e. 0–50 mM) and, in the case of the Me_4N^+ salt form, with urea at a fixed concentration of 4 M. Some comparative studies were carried out using Na^+ xanthan (Rhodigel Clear) from Rhodia Food, Aubervilliers, France.

Small-deformation measurements of storage modulus (G'), loss modulus (G''), $\tan\delta$ (G''/G') and complex dynamic viscosity ($\eta^* = (G'^2 + G''^2)^{1/2}/\omega$, where ω is frequency in rad s^{-1}) were made using highly truncated cone-and-plate geometry (50 mm diameter; 0.05 rad cone angle; 0.5 mm gap) on a sensitive prototype rheometer designed and constructed by one of us (R.K.R.). Temperature was controlled by a Haake circulating water bath and measured

with a thermocouple attached to the stationary element. Samples were loaded onto the rheometer at 90 °C, and their periphery was coated with light silicone oil to minimise evaporation. The temperature was then lowered to 5 °C at 1 °C/min, with measurement of consequent rheological changes at 1 rad s^{-1} and 0.5% strain. After equilibration for ~15 min at 5 °C, the frequency-dependence of dynamic moduli was measured at a fixed strain of 0.5%, and the strain-dependence was then recorded at a fixed frequency of 1 rad s^{-1}.

Turbidity was characterised by measurements of optical density (1 cm pathlength; wavelength range 300–400 nm) on a Unicam SP 1800 spectrophotometer.

3. Results and discussion

On the basis of previous studies of the xanthan coil–helix transition by optical rotation (e.g. Holzwarth, 1976; Milas & Rinaudo, 1979; Norton et al., 1984), 10 mN monovalent salt was chosen as sufficient to ensure essentially complete conformational ordering on cooling to the measuring temperature of 5 °C. The associated changes in solution rheology are illustrated in Fig. 2, which shows the variation in G' and G'' (measured at 1 rad s^{-1} and 0.5% strain) for 5 g/l Na^+ xanthan in 10 mN NaCl (with no added Ca^{2+}) on cooling (at 1 °C/min) from the disordered state at 90 °C. Both moduli increase steeply on initial reduction in temperature, with a transition from predominantly liquid-like response ($G'' > G'$) to predominantly solid-like ($G' > G''$) at ~45 °C, and then level out to roughly constant values at lower temperature. The final mechanical spectrum at 5 °C (Fig. 3a) is in some respects similar to those seen (e.g. Morris, 1984; Ross-Murphy, 1984) for conventional polysaccharide gels ($G' > G''$; linear reduction in $\log\eta^*$ with increasing $\log\omega$), but the separation of G' and G'' is much smaller (i.e. higher $\tan\delta$) and the frequency-dependence of both moduli is much greater.

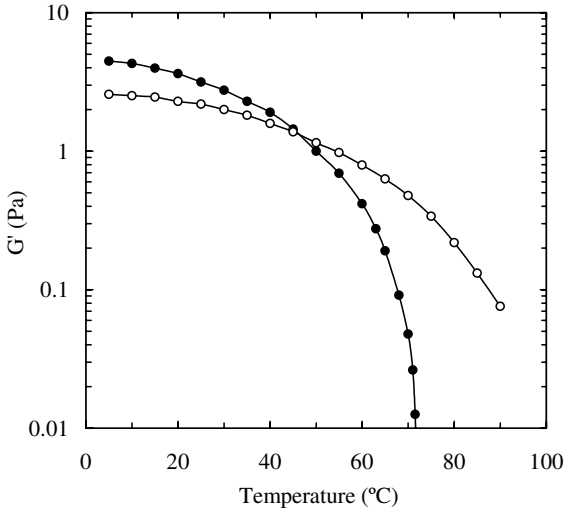

Fig. 2. Variation in G' (●) and G'' (○), measured at 1 rad s^{-1} and 0.5% strain, for Na^+ xanthan (5 g/l in 10 mN NaCl) on cooling from 90 to 5 °C at 1 °C/min.

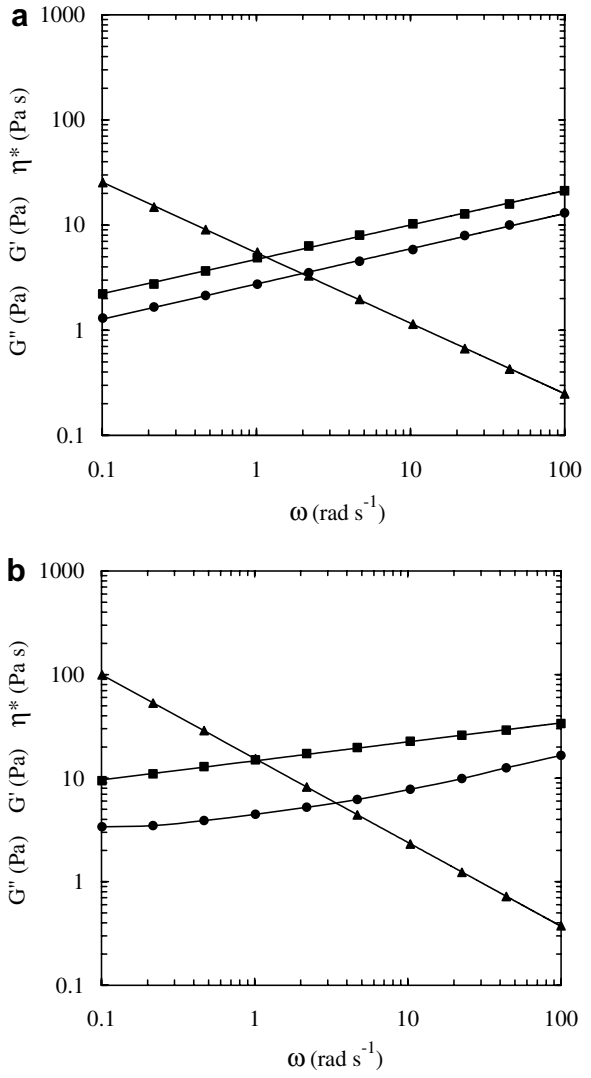

Fig. 3. Mechanical spectra (0.5% strain; 5 °C) showing the variation of G' (■), G'' (●) and η^* (▲) with frequency (ω) for Na^+ xanthan (5 g/l in 10 mN NaCl), (a) with no added Ca^{2+}, and (b) in the presence of 100 mN $CaCl_2$.

As anticipated, incorporation of $CaCl_2$ at the highest concentration studied (100 mN) gives a spectrum (Fig. 3b) that is more typically gel-like (lower $\tan\delta$; less variation of G' and G'' with frequency), and with substantially higher absolute values of both moduli. However, instead of the expected monotonic variation in 'weak gel' character between the two extremes of our experimental range (0 and 100 mN $CaCl_2$), the gel-like (elastic) response, as characterised by storage modulus (G'), passes through a sharp maximum (Fig. 4) at ~7.5 mN Ca^{2+} and then drops steeply to a minimum value at ~15 mN before increasing again at higher concentrations. Mechanical spectra (Fig. 5) show a transition from predominantly gel-like response at 7.5 mN Ca^{2+} (Fig. 5a) to behaviour approaching that of a normal polysaccharide solution at 15 mN (Fig. 5b). As shown in Fig. 6a, G'' also passes through

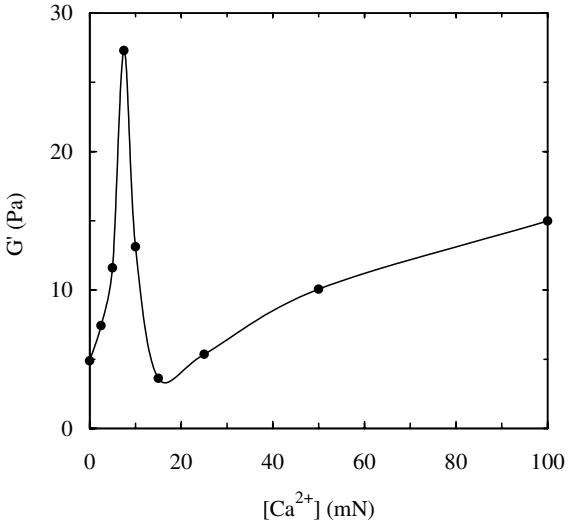

Fig. 4. Variation of G' (1 rad s^{-1}; 0.5% strain; 5 °C) with Ca^{2+} concentration for Na^+ xanthan (5 g/l) in 10 mN NaCl.

maximum and minimum values at the same Ca^{2+} concentrations as G', but the changes are smaller, and thus tan δ (G''/G') shows a converse pattern of behaviour (Fig. 6b), dropping to a minimum at ~7.5 mN Ca^{2+} and rising to a maximum at ~15 mN.

Fig. 7 shows the variation of $\log\eta^*$ with $\log\omega$ for some representative concentrations of $CaCl_2$ spanning the range studied. As found for G' and G'' (Fig. 6), the intercept (i.e. $\log\eta^*$ at $\log\omega = 0$; $\omega = 1$ rad s^{-1}) and slope of the log–log plots pass through maximum and minimum values at ~7.5 and ~15 mN Ca^{2+}, respectively (Fig. 8). Thus by all rheological criteria characterised (G', G'', η^*, tan δ, and the slope of $\log\eta^*$ *vs.* $\log\omega$), the weak-gel properties of Na^+ xanthan, stabilised in the ordered conformation by the presence of 10 mN NaCl, reach a maximum on incorporation of ~7.5 mN $CaCl_2$, and then drop to a minimum when the Ca^{2+} concentration is increased to ~15 mN.

As illustrated in Fig. 9, failure of the weak-gel network, as characterised by a reduction in elastic response (G'), occurs at ~3% strain. Thus the strain of 0.5% used in characterisation of the rheological parameters shown in Figs. 2–8 is well within the linear viscoelastic region. The magnitude of the reduction in G' beyond the point of failure increases systematically (Fig. 9) as the strength of the network decreases.

A similar pattern of Ca^{2+}-dependence was observed for the tetramethylammonium salt form in the presence of urea. Fig. 10 shows the mechanical spectrum obtained for 5 g/l Me_4N^+ xanthan in 10 mN Me_4NCl and 4 M urea at 5 °C. As anticipated from the known effectiveness of urea in inhibiting intermolecular association of xanthan (Ross-Murphy et al., 1983), and the inability of organic cations to form co-ordination complexes with polyanions, the spectrum is less gel-like than that obtained (Fig. 3a) for the Na^+ salt form in the absence of urea ($G'' > G'$; substantial frequency-dependence of both moduli; pronounced curvature in $\log\eta^*$ *vs.* $\log\omega$) and the absolute values of

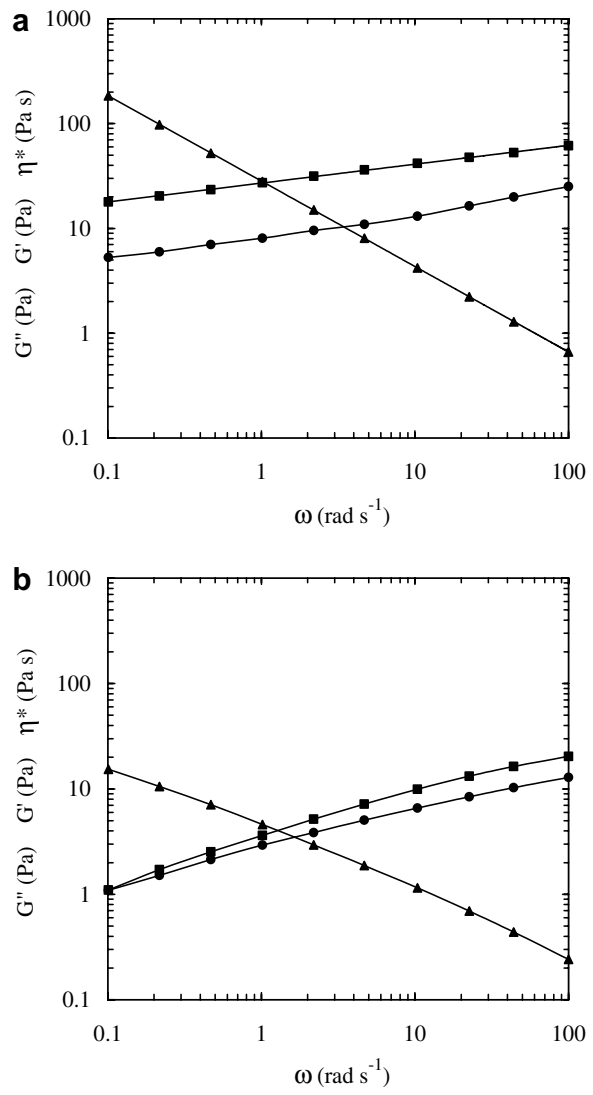

Fig. 5. Mechanical spectra (0.5% strain; 5 °C) showing the variation of G' (■), G'' (●) and η^* (▲) with frequency (ω) for Na^+ xanthan (5 g/l in 10 mN NaCl) in the presence of $CaCl_2$ at concentrations of (a) 7.5 mN and (b) 15 mN.

G' are much smaller (by about an order of magnitude at low frequency). The changes in G' (Fig. 11a), G'' (Fig. 11b) and tan δ (Fig. 12) observed on incorporation of increasing amounts of Ca^{2+}, however, are closely similar in form to those seen for Na^+ xanthan, although the maxima and minima are displaced to slightly higher calcium concentrations.

The formula weight per repeat unit (Fig. 1) for xanthan (Na^+ salt form) is 981 for units in which the sidechain carries a pyruvate group on the terminal mannose residue and 889 in the absence of pyruvate. The degree of pyruvate substitution for commercial xanthan as produced by Kelco is ~39% (Holzwarth, 1976; Smith et al., 1981), giving a molecular weight per charge of ~665. Thus a solution at the concentration used in the present work (5 g/l) is ~7.5 mN w.r.t. COO^- (5000/665). Residual moisture in the freeze-dried material will reduce this value slightly,

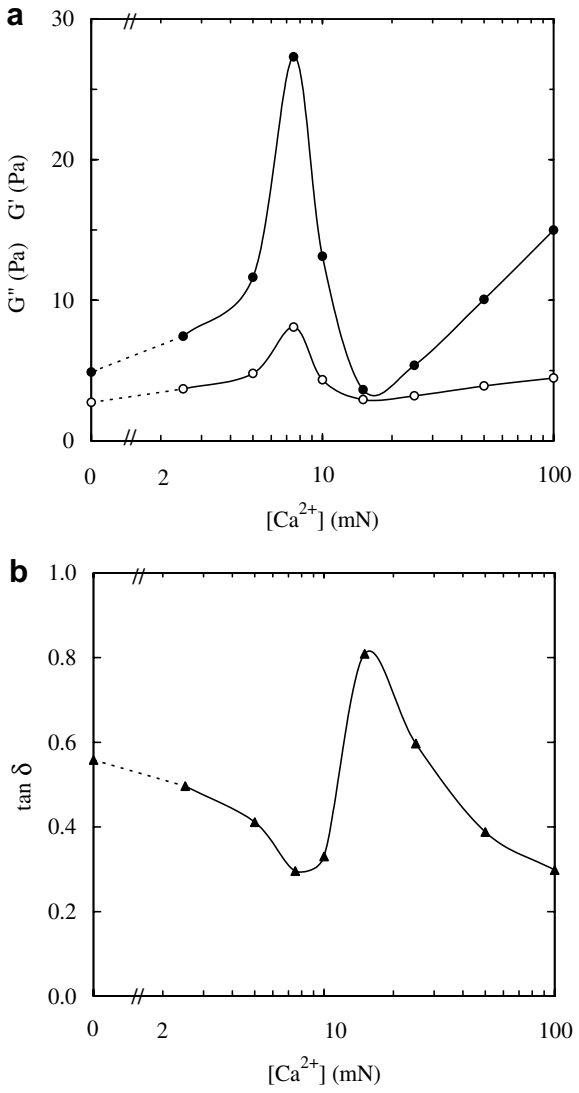

Fig. 6. Variation of (a) G' (●) and G'' (○) and (b) tan δ, measured at 1 rad s^{-1} and 0.5% strain, with increasing concentration of Ca^{2+} in solutions of Na^+ xanthan (5 g/l) in 10 mN NaCl.

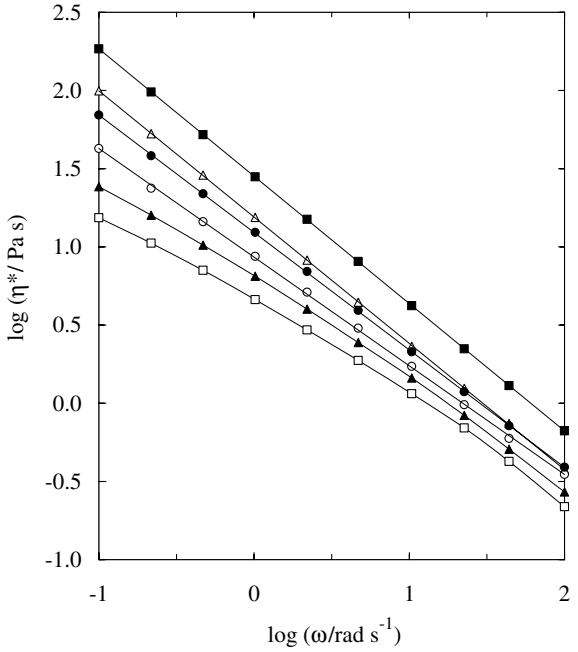

Fig. 7. Variation of $\log\eta^*$ (1 rad s^{-1}; 0.5% strain; 5 °C) with $\log\omega$ for solutions of Na^+ xanthan (5 g/l in 10 mN NaCl) in the presence of $CaCl_2$ at concentrations of 2.5 (○), 5.0 (●), 7.5 (■), 15 (□), 25 (▲) and 100 (△) mN.

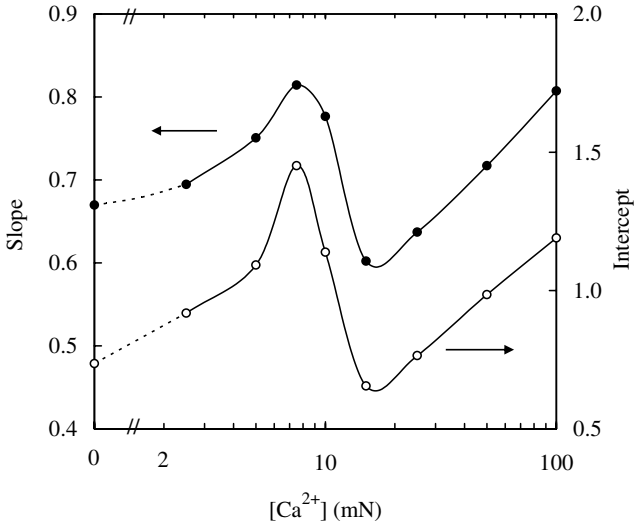

Fig. 8. Variation of the slope (●) and intercept (○) of $\log\eta^*$ *vs.* $\log\omega$ (Fig. 7) with increasing concentration of Ca^{2+} in solutions of Na^+ xanthan (5 g/l) in 10 mN NaCl.

but it seems reasonable to conclude that the maxima and minima in weak-gel properties (Figs. 6 and 8) correspond roughly to stoichiometric equivalence and twice stoichiometric equivalence between calcium ions and the carboxyl groups of the polymer (i.e. to Ca^{2+}:COO^- ratios of ~1:2 and ~1:1, respectively).

To explore the generality of the strange Ca^{2+}-dependence of rheological properties, and in particular to determine whether or not it is specific to the material produced by Kelco, some comparative experiments were carried out using xanthan from another supplier (Rhodigel Clear from Rhodia). The counterion to this polymer, as supplied, is predominantly Na^+, and therefore no ion-exchange procedure was used. The results obtained, although differing quantitatively from those reported above for Keltrol T (after ion-exchange to the Na^+ salt form), showed the same qualitative pattern of response: an initial increase in G' with increasing concentration of Ca^{2+}, followed by a reduction to a minimum at twice stoichiometric equivalence, and subsequent increase at higher concentrations.

The obvious interpretation of the initial increase in moduli (Figs. 4 and 11) at Ca^{2+} concentrations up to the stoichiometric value is that it arises from site-binding of calcium ions between pairs of carboxyl groups on separate helices, thus promoting intermolecular association and strengthening the 'weak gel' network. This interpretation is consistent with previous studies (Holzwarth, 1976; Lambert et al., 1985) in which binding of Ca^{2+} to ordered xanthan was

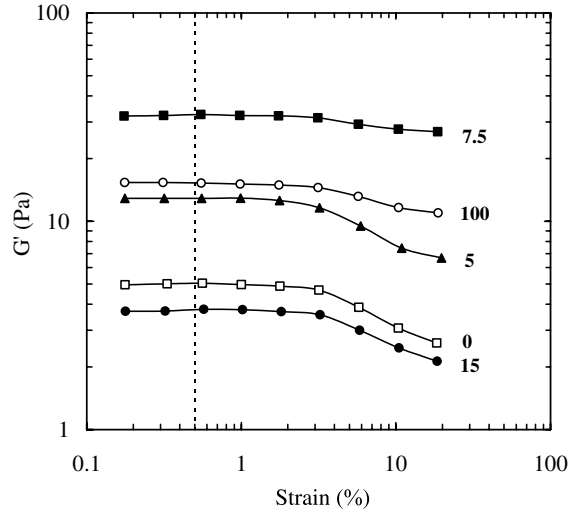

Fig. 9. Strain dependence of G′ (1 rad s^{-1}; 5 °C) for Na^+ xanthan (5 g/l in 10 mN NaCl) in the presence of $CaCl_2$ at concentrations of 0 (□), 5.0 (▲), 7.5 (■), 15 (●) and 100 (○) mN. The vertical dotted line shows the strain (0.5%) used in recording mechanical spectra.

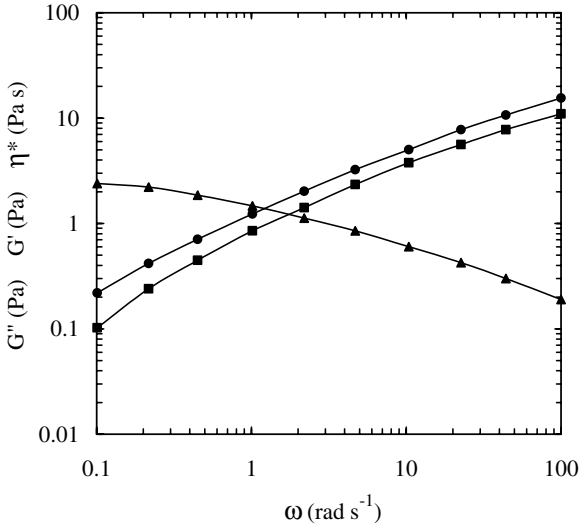

Fig. 10. Mechanical spectrum (0.5% strain; 5 °C) showing the variation of G' (■), G'' (●) and η^* (▲) with frequency (ω) for Me_4N^+ xanthan (5 g/l) in the presence of 10 mN Me_4NCl and 4 M urea.

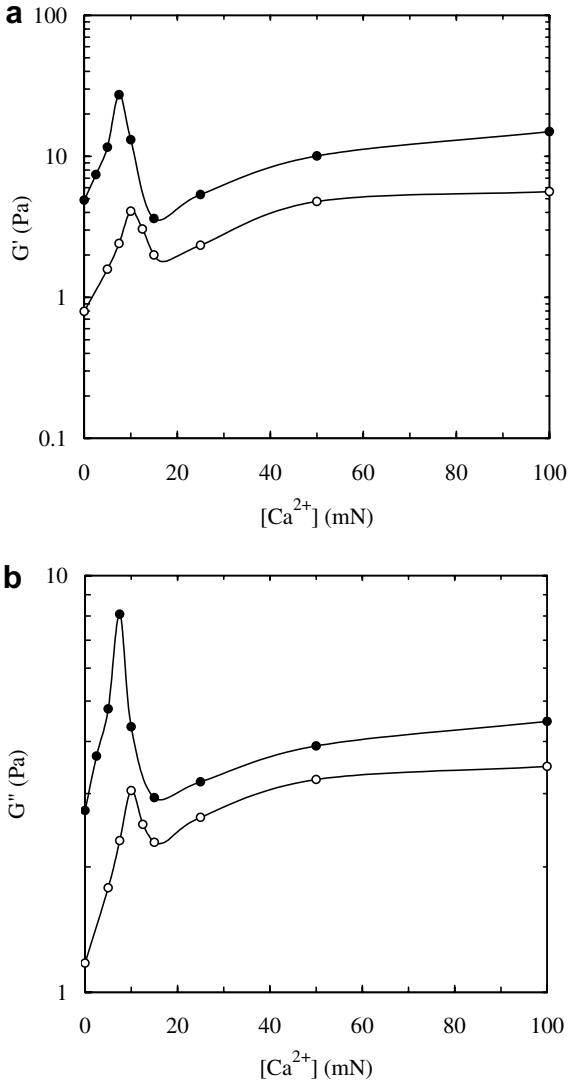

Fig. 11. Variation of (a) G' and (b) G'' (1 rad s^{-1}; 0.5% strain; 5 °C) with increasing concentration of Ca^{2+} in solutions of xanthan (5 g/l), in the Na^+ salt form with 10 mN NaCl (●) and in the Me_4N^+ salt form with 10 mN Me_4NCl and 4 M urea (○).

demonstrated directly by measurements of calcium ion activity. The subsequent sharp reduction in moduli at Ca^{2+} concentrations between stoichiometric and twice stoichiometric equivalence to the carboxyl groups of the polymer, however, is more difficult to explain.

One possibility we considered was that reduction in negative charge density along the polymer chain as Ca^{2+}-binding approaches stoichiometric might cause partial precipitation of xanthan. This possibility was tested by examining the optical density of solutions of Na^+ xanthan (from Keltrol T), prepared in the same way as in the rheological studies (i.e. at 5 g/l in 10 mN NaCl), with Ca^{2+} concentrations of 0, 7.5, 15 and 100 mN (as in Figs. 3 and 5). Measurements were made with the polymer in the disordered state at ~80 °C and in the ordered state at ~5 °C. As shown in Fig. 13, conformational ordering causes a large increase in turbidity, but at both temperatures the optical density values are essentially independent of calcium ion concentration, with no indication that the reduction in gel-like character between 7.5 and 15 mN Ca^{2+} is accompanied by the increase in turbidity that would be expected if it did indeed arise from partial insolubilisation of the polymer.

An alternative, highly speculative, interpretation is that as the Ca^{2+}:COO^- ratio is raised from 1:2 towards 1:1, intermolecular binding is progressively replaced by association of calcium ions with individual carboxyl groups (to maintain maximum complexation), with consequent reduction in the extent of crosslinking. If correct, this proposal would imply that the (initially negative) net charge on the xanthan helices should drop to zero at around stoichiometric

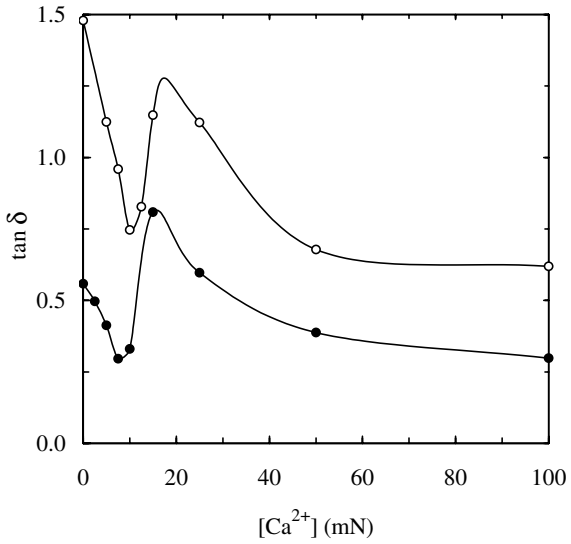

Fig. 12. Variation of tan δ (1 rad s^{-1}; 0.5% strain; 5 °C) with increasing concentration of Ca^{2+} in solutions of xanthan (5 g/l), in the Na^+ salt form with 10 mN NaCl (●) and in the Me_4N^+ salt form with 10 mN Me_4NCl and 4 M urea (○).

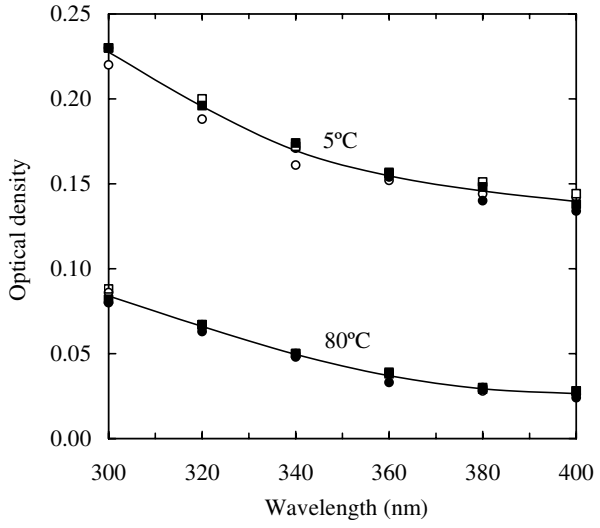

Fig. 13. Optical density values (1 cm pathlength), measured at ~80 °C (lower curve) and ~5 °C (upper curve), for Na^+ xanthan (5 g/l in 10 mN NaCl) in the presence of $CaCl_2$ at concentrations of 0 (○), 7.5 (●), 15 (□) and 100 (■) mN.

equivalence of Ca^{2+}, and then become positive at higher concentrations, due to binding of (divalent) Ca^{2+} ions to (monovalent) carboxyl groups (with the subsequent increase in moduli at higher concentrations of Ca^{2+} arising from non-specific screening of electrostatic repulsion between helices on further increase in ionic strength). One purpose of this publication is to encourage groups who specialise in electrochemistry of biopolymers to investigate whether or not there is indeed a change from polyanionic to polycationic properties as the concentration of Ca^{2+} in solutions of ordered xanthan is increased up to and beyond stoichiometric equivalence.

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