

Viscosity of galactomannans at alkaline and neutral pH: evidence of ‘hyperentanglement’ in solution

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Galactomannans occur as storage polysaccharides in the seed endosperm of certain leguminous plants (Dea & Morrison, 1975). The polymer backbone consists of (1→4)-linked β -D-mannose and is solubilised by α -D-galactose attached as irregularly spaced single-sugar sidechains at O(6) of a proportion of the backbone residues. The two galactomannans of greatest commercial significance are guar gum, from *Cyamopsis tetragonolobus*, and locust bean gum (LBG), from *Ceratonia siliqua*, which have mannose:galactose ratios of ~ 1.55 and ~ 3.55 , respectively.

In solution, these materials exist as disordered mobile coils. However, their rheological properties have been found to depart from those of typical ‘random coil’ polysaccharides (Morris et al., 1981). In particular, double-logarithmic plots of ‘zero shear’ specific viscosity against degree of space-occupancy show an abrupt change of slope at $c[\eta] \approx 2.5$, in comparison with $c[\eta] \approx 4$ for most other disordered coils, and the subsequent slope is unusually high (~ 4.5 in comparison with ~ 3.3). Similar departures from ‘normal’ concentration dependence have been observed for solutions of konjac glucomannan (Jacon et al., 1993) and tamarind seed galactoxyloglucan (Gidley et al., 1991) which, like the galactomannans, have linear backbones of (1→4)- diequatorially linked sugars.

This anomalous behaviour has been rationalised (Morris et al., 1981) in terms of intermolecular association (hyperentanglement) between unsubstituted regions of glycan chains, in addition to normal topological entanglement. However, although it has been demonstrated that consecutive runs of more than six unsubstituted mannose residues in galactomannans can form stable associations on freezing and thawing (Dea et al., 1986) there is not yet any direct evidence of spontaneous association in solution.

In the present investigation, the concept of ‘hyperentanglement’ has been probed by comparison of solution properties in strong alkali and at neutral pH at the same overall ionic strength. At high pH, hydroxyl groups become ionised, converting neutral polysaccharides to polyelectrolytes. This will have the effect of expanding molecular dimensions by intramolecular electrostatic repulsion, and would, therefore, be expected to increase the viscosity of solutions of independent disordered coils. It will also, however, destabilise intermolecular associations by introducing electrostatic repulsions between the constituent chains. In particular, the essentially unsubstituted mannan from ivory nuts (*Phytalephas macrocarpa*) is insoluble in water but dissolves in strong alkali (Dea & Morrison, 1975), and partial dissociation (swelling) of cellulose fibrils under alkaline conditions (8-10% NaOH) has extensive industrial applications in the preparation of cellulose derivatives (Kamide & Saito, 1987). Exposure to strong alkali might, therefore, be expected to abolish any ‘hyperentanglements’ between galactomannan chains in solution, with possible reduction in viscosity rather than the increase anticipated for isolated chains.

Viscosity measurements were made using concentric cylinder geometry, with inner and outer radii of 5.5 and 6.0 mm, respectively, on a Contraves Low Shear 30 viscometer. LBG (Meypro fleur M-175 from Meyhall) was allowed to hydrate overnight in distilled deionised water (at $\sim 2\%$ w/w) and was dissolved by autoclaving for 20 min at 120°C. Solutions of guar gum (from

Hercules) were prepared by mechanical stirring at ambient temperature. All reagents were AnalaR grade from BDH.

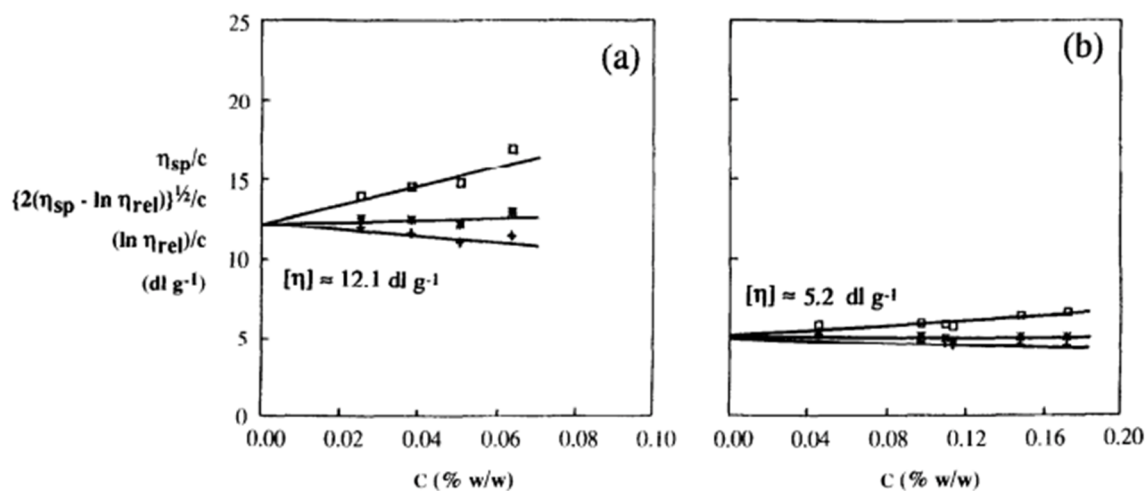


Fig. 1. Determination of intrinsic viscosity (20 C) for LBG in (a) salt (1 M NaCl) and (b) alkali (1 M NaOH) by combined Huggins (□), Kraemer (+) and single point (*) extrapolation of, respectively, η_{sp}/c , $(\ln \eta_{rel})/c$ and $\{2(\eta_{sp} - \ln \eta_{rel})\}^{1/2}/c$ to zero concentration.

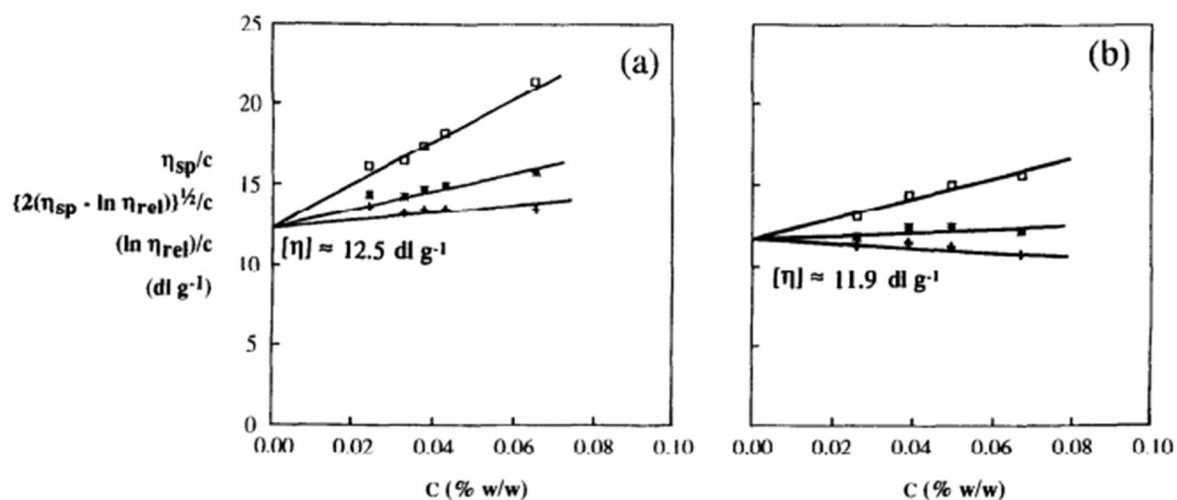


Fig. 2. Determination of intrinsic viscosity (20 C) for guar gum in (a) salt (1 M NaCl) and (b) alkali (1 M NaOH); symbols as in Fig. 1.

Figure 1 shows Huggins, Kraemer and 'single-point' extrapolations to intrinsic viscosity (Morris, 1984) for LBG in 1 M NaOH and in 1 M NaCl. The value of $[\eta]$ at neutral pH is more than twice that in alkali (12.1 dl g⁻¹ in comparison with 5.2) consistent with a substantial reduction in hydrodynamic volume by dissociation of intermolecular 'hyperentanglements'. Similar effects were observed (Fig. 2) for guar gum, but were much smaller in magnitude (reduction in $[\eta]$ from 12.5 dl g⁻¹ in salt to 11.9 dl g⁻¹ in alkali), consistent with a much lower content of unsubstituted sequences capable of forming intermolecular associations.

An obvious problem in interpretation of the above results, however, is that polysaccharides are susceptible to degradation under alkaline conditions, by a 'peeling' reaction initiated at the reducing end of the chain for (Aspinall, 1970). The observed reductions in intrinsic viscosity might therefore be due simply to reduction in molecular weight rather than to suppression of

intermolecular association. This possibility was tested preparing solutions of galactomannans in alkali (1 M NaOH) and comparing their viscosity before and after neutralisation with HCl. Experimentally, the volume changes during neutralisation were measured by accurate weighing, and the polysaccharide concentration in the alkali solution was adjusted to the same value by addition of the appropriate volume of 1 M NaOH. Comparison was also made with the same concentration of galactomannan dissolved directly in 1 M NaCl.

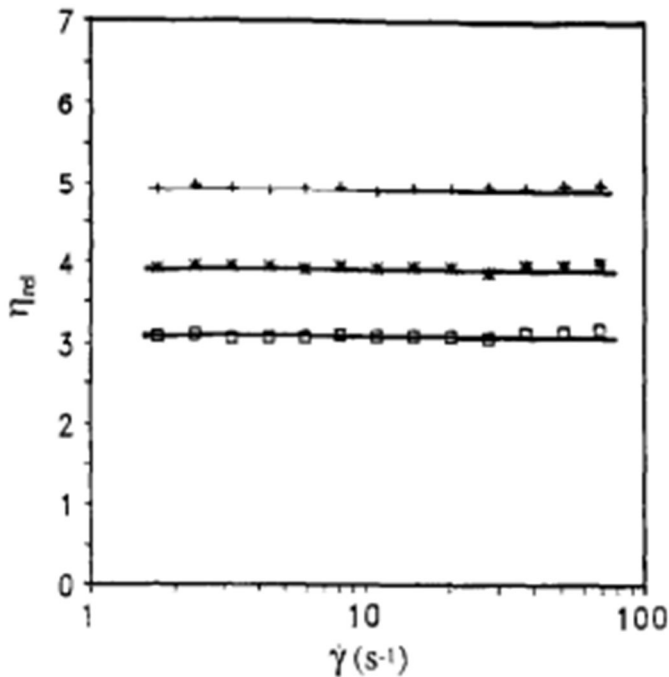


Fig. 3. Shear-rate dependence of relative viscosity (20°C) for solutions (0.37% w/w) of LBG prepared in 1 M NaOH (□); prepared in 1 M NaCl (+); and prepared in 1 M NaOH and neutralised to pH 7.0 (*)

Figure 3 shows the results obtained for LBG at a final concentration of 0.37% w/w. Neutralisation caused a substantial increase in viscosity (from $\eta_{rel} \approx 3.1$ to $\eta_{rel} \approx 4.0$) demonstrating directly that the reduction in intrinsic viscosity under alkaline conditions (Fig. 1) cannot be due solely to depolymerisation. The viscosity obtained after neutralisation from alkali, however, was appreciably lower than that of LBG dissolved at neutral pH (at the same overall ionic strength). The difference is likely to be due, in part, to depolymerisation under alkaline conditions prior to neutralisation. It is also possible, however, that a proportion of the intermolecular associations between LBG chains in the solid state may persist on dissolving at neutral pH but dissociate in alkali, with only partial re-association when the solution is neutralised.

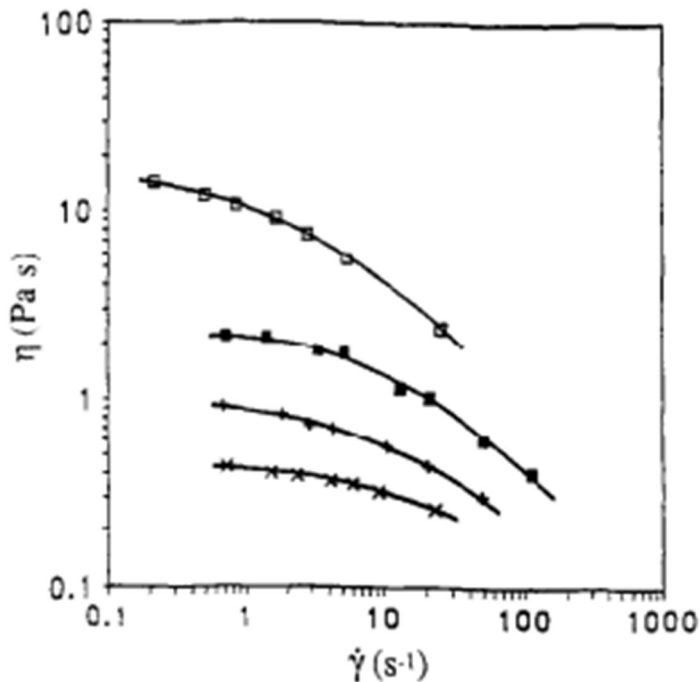


Fig. 4. Shear-rate dependence of viscosity (20°C) for guar gum prepared in 1 M NaOH and neutralised to pH 7.0; 0.37% w/w, before (x) and after (+) neutralisation; 1.3% w/w, before (■)

Large increases in viscosity on neutralisation from alkaline solutions were also observed for guar gum. As shown in Fig. 4, the degree of enhancement became greater as the polymer concentration was increased (from 0.37% to 1.3% w/w), as would be expected from simple mass-action considerations for formation of transient 'hyperentanglements' between chains. The smaller of the two concentrations used to obtain the values shown in Fig. 4 is still far above the concentration range required for measurement of intrinsic viscosity (~0.025-0.07% w/w; Fig. 2) which would explain why the magnitude of the pH-dependent changes is so much greater.

We therefore conclude that topological entanglement in solutions of galactomannans, and related plant polysaccharides with (1→4)-diequatorial linkage geometry, is augmented by alkali-labile non-covalent associations (hyperentanglements) and that, as proposed initially (Morris et al., 1981), it is these associations that give rise to departures from the general form of concentration dependence for other disordered polysaccharides.

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