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Effect of sugars, galactose content and chainlength on freeze–thaw gelation of galactomannans

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Abstract

Cryogels of locust bean gum (LBG) were prepared by freezing and thawing 1.0 wt% solutions incorporating sucrose, glucose, fructose or sorbitol at concentrations of 40, 45, 50, 55 and 60 wt%, and were characterised by compression testing. Gel strength showed an initial increase and subsequent decrease with increasing concentration of sugar. Maximum strength was attained at 45 wt% fructose, 50 wt% sucrose or sorbitol, and 55 wt% glucose, but increased in the same order: fructose < sucrose ≈ sorbitol < glucose. The initial increase in gel strength is attributed to the reduction in water content with increasing concentration of sugar; the subsequent decrease is tentatively ascribed to inhibition of polymer–polymer association by binding of sugar molecules to the polymer chains, with differences in gel strength arising from differences in strength of binding.

Galactomannan samples with mannose:galactose (M/G) ratios spanning that of LBG were prepared by treatment of guar gum with α-galactosidase, and cryogels (1.0 wt%) were prepared at a fixed concentration (50 wt%) of sucrose. A sharp increase in gel strength was observed at M/G ratios around, and above, that of LBG, and is attributed to increasing content of unsubstituted sequences of mannan backbone long enough to form stable associations as junctions in the cryogel network. The samples of partially debranched guar gum had substantially lower molecular weights than LBG (attributed to some slight β-mannanase activity in the α-galactosidase used in their production), but the mechanical properties of the LBG cryogels fitted in reasonably well with the M/G-dependence observed for the other samples indicating that within the range studied (~10 to ~2000 kD.), chainlength has little effect.

Keywords: Freeze–thaw; Cryogelation; Rheology; Sugars; Guar gum; Locust bean gum

1. Introduction

Galactomannans occur widely in the seed endosperm of plants in the *Leguminoseae* family. They have a linear backbone of (1→4)-diequatorially linked β-D-mannose residues, some of which carry single-sugar sidechains of α-L-galactose attached by (1→6) linkages. The galactose content can vary from essentially zero to one galactose on every mannose residue, depending on the botanical source (Dea & Morrison, 1975), and is normally characterised by the molar ratio of mannose to galactose (M/G).

Like cellulose, which is also (1→4)-diequatorially linked, unsubstituted mannan is insoluble. Solubility of galactomannans increases with increasing content of galactose (i.e. with decreasing M/G ratio), presumably because the galactose substituents inhibit solid-state packing of mannan chains and contribute to conformational entropy in the solution state by freedom of rotation about the (1→6) linkages.

The two galactomanans of greatest practical significance as thickeners and stabilisers in industrial applications are guar gum from the annual plant *Cyamopsis tetragonolobus* and locust bean gum (LBG) from the seed pods of the carob tree (*Ceratonia siliqua*). Commercial guar gums normally have an M/G ratio of ~1.6, and can be dissolved in cold water. LBG is less highly substituted (M/G ≈ 3.5–4.0) and dissolves fully only at comparatively high temperature. Solubility at lower temperatures is confined to chains whose galactose content is higher than the average value for the whole gum, and indeed fractions of different M/G ratio can be obtained by dissolving at progressively higher temperatures.

Solutions of LBG, and particularly of fractions with high M/G ratio that dissolve only in hot water, are unstable. Over

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time, the polymer gradually precipitates from dilute solutions, and more concentrated solutions form gels; both processes are accelerated by freezing and thawing (Dea, 1987; Dea et al., 1977; Lozinsky, Damashkaln, Brown, & Norton, 2000; Richardson, Clark, Russell, Aymard, & Norton, 1999; Tanaka, Hatakeyama, & Hatakeyama, 1998a, 1998b), and can be attributed to intermolecular association of unsubstituted regions of the mannan backbone.

An investigation in which computer modelling was used to relate the extent of precipitation on freezing and thawing to the distribution of galactose sidechains along the polymer backbone, as determined by degradation with enzymes of known specificity, led to the conclusion that association occurs only above a critical threshold of about six consecutive mannose residues devoid of galactose substituents (Dea, Clark, & McCleary, 1986). A study of the effect of chaotropic and anti-chaotropic substances (urea and sodium sulfate, respectively) on the freeze–thaw gelation of LBG indicated that hydrogen bonding makes a major contribution to the stability of the mannan–mannan junctions (Lozinsky et al., 2000).

Gelation of LBG from unfrozen aqueous solutions occurs very slowly (over a period of several months), even when using a fraction that can be dissolved only in hot water (Richardson et al., 1999). The gels formed by the more rapid process of freezing and thawing (cryogels) are unstable, with a strong tendency to network contraction and syneresis (Dea et al., 1977). More stable gels can, however, be formed by incorporation of high concentrations (50–60 wt%) of sucrose (Dea et al., 1977; Richardson & Norton, 1998) or other co-solutes such as ethylene glycol or glycerol (Dea et al., 1977) in unfrozen solutions of LBG.

In the present work, we have explored the effect of combining freeze–thaw gelation with incorporation of co-solutes, and obtained strong, stable cryogels. Four co-solutes were used: sucrose, glucose, fructose and sorbitol, at concentrations of 40–60 wt%, in combination with LBG at a fixed concentration of 1.0 wt%. We have also used digestion of guar gum with α-galactosidase to prepare four galactomannan samples with M/G ratios spanning that of LBG, and have used a fixed concentration (50 wt%) of one co-solute (sucrose) to explore the effect of galactose content on cryogelation. Since the samples of partially debranched guar gum were substantially lower in molecular weight than LBG, comparison of rheological measurements also gave an indication of the effect of chainlength on the mechanical properties of the networks formed on freezing and thawing.

2. Materials and methods

The sucrose used was normal food grade, purchased locally; glucose, fructose and sorbitol were 'chemically pure' grade from Riedel-de Haën AG, Seelze, Germany. Although sorbitol (the C(1) alcohol of glucose) is not, strictly, a sugar, these four co-solutes will be described collectively as 'sugars'. LBG was a normal food grade sample supplied by Quest International. The other four galactomannan samples studied were prepared by treatment of guar gum with α-galactosidase for progressively longer times. We will refer to these as samples A, B, C and D, in order of decreasing galactose content.

For preparation of cryogels, the galactomannans were dispersed in distilled deionised water by mechanical stirring at room temperature, and were dissolved by continued stirring at 80 °C. The required quantity of co-solute was then added, and stirring was continued until it was fully dissolved. Where necessary, losses due to evaporation were corrected by addition of hot water, to bring the final concentration of galactomannan to 1.0 wt%. The solutions were then filled in 100 g aliquots into cylindrical plastic beakers of internal diameter 7 cm (giving a depth of ~2.5 cm) and placed immediately in a freezer at −20 °C. After 24 h at −20 °C, the beakers were transferred to a refrigerator at 5 °C, and allowed to thaw over a further period of 24 h. The resulting cryogels (at 5 °C) were characterised by compression testing on a TA-XT2 Texture Analyser from Stable Microsystems, using a cylindrical probe of diameter 3.5 cm and compression rate of 0.1 mm/s, with the samples contained in the cylindrical beakers in which they were formed. All samples were measured in triplicate.

The M/G ratios of the five galactomannans were determined from measurements of optical rotation at 20 °C on a Perkin–Elmer 241 polarimeter, using a cell of pathlength 1 cm. Their intrinsic viscosities, and hence their molecular weights, were obtained from measurements of dilute-solution viscosity, again at 20 °C, using a Contraves Low-Shear 30 rotational viscometer.

3. Results

3.1. Characterisation of galactomannan samples

The composition of the galactomannan samples, as characterised by the molar ratio of mannose to galactose (M/G), was estimated from optical rotation measurements (at 20 °C) using the relationship (Eq. (1)) proposed by Morris (1990).

$$\mathrm{M/G} = (235 - [\alpha]_D)/(50 + [\alpha]_D) \quad (1)$$

where $[\alpha]_D$ is the specific rotation measured at the sodium D-line (589 nm), defined as

$$[\alpha]_D = \alpha/(c \times l) \quad (2)$$

where α is the observed optical rotation (in degrees), c is concentration (in g/ml) and l is pathlength (in dm). Eq. (1) was derived initially from studies of a range of galactomannans by vacuum ultraviolet circular dichroism (Buffington, Stevens, Morris, & Rees, 1980), but was found to agree well (Morris, 1990) with published data from conventional measurements of D-line optical rotation.

To improve the precision of the $[\alpha]_D$ values, optical rotation measurements (1 cm pathlength; polymer concentration 1.0%, w/v) were made using the emission lines from a mercury lamp at wavelengths (λ) of 365, 436, 546 and 578 nm and the values of optical rotation at 589 nm were then derived from linear Drude plots of $1/\alpha$ versus λ^2. The Drude plots obtained for the four samples of partially debranched guar gum are shown in

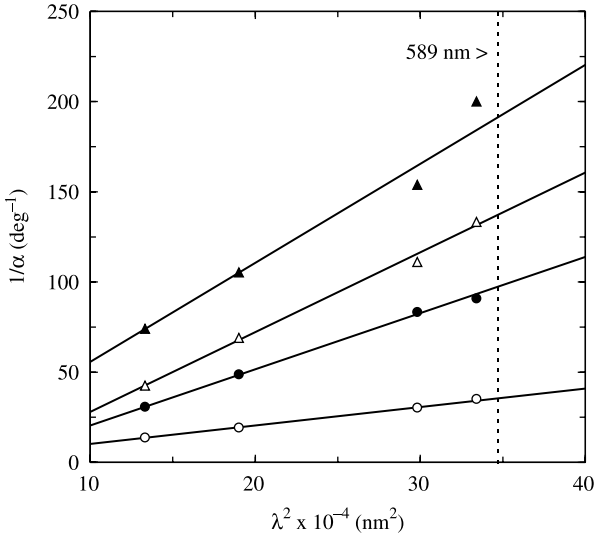

Fig. 1. Drude plots used to determine optical rotation (α) at 589 nm (sodium D-line) from measurements (at 20 °C) using mercury emission lines at lower wavelengths (λ), for galactomannan samples A (○), B (●), C (△) and D (▲).

Fig. 1. The dispersion curves (wavelength-dependence of optical rotation) corresponding to these Drude plots are shown in Fig. 2, where they are compared directly with the experimental readings, to show the standard of agreement between observed and fitted values. The curves obtained for LBG were very close to those for sample C, and are omitted from the figures to avoid a confusing clutter of symbols. The values of $[\alpha]_D$ derived from the Drude plots for all five galactomannan samples, and the corresponding values of M/G (from Eq. (1)), are given in Table 1.

Molecular weight was characterised by measurement of intrinsic viscosity, [η], using the following standard relationships (Bohdanecký & Kovár, 1982)

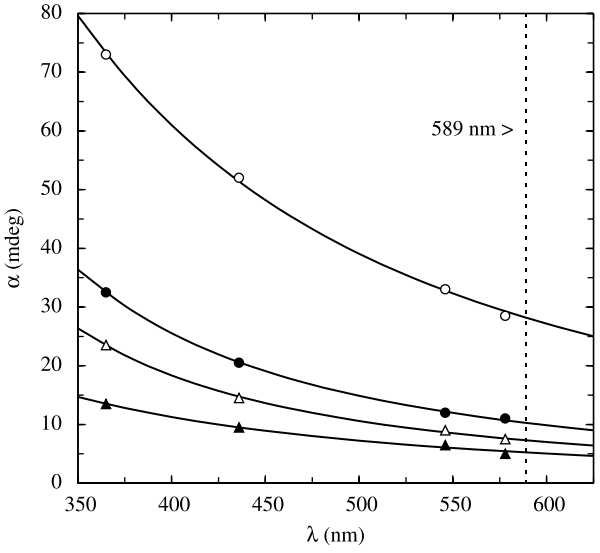

Fig. 2. Comparison of dispersion curves (solid lines) derived from the linear Drude plots in Fig. 1 with experimental values of optical rotation (20 °C; polymer concentration 1.0 wt%) for galactomannan samples A (○), B (●), C (△) and D (▲).

Table 1
Molecular weight and composition of galactomannan samples

Sample	[η] (dl/g)	M_r (kD)	$[\alpha]_D$	M/G
A	1.48	72.6	28.2	2.65
B	0.86	33.4	10.3	3.73
C	1.30	60.0	7.29	3.97
D	0.36	9.8	5.23	4.16
LBG	16.23	2148	7.69	3.94

Molecular weight (relative molecular mass, M_r) was derived from intrinsic viscosity, [η], using the Mark-Houwink equation proposed by Picout and Ross-Murphy (2002); the ratio of mannose to galactose (M/G) was calculated from specific rotation at 589 nm ($[\alpha]_D$) by the relationship proposed by Morris (1990).

$$\eta_{rel} = \eta/\eta_s \tag{3}$$

$$\eta_{sp} = (\eta - \eta_s)/\eta_s = \eta_{rel} - 1 \tag{4}$$

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \tag{5}$$

$$(\ln \eta_{rel})/c = [\eta] + k''[\eta]^2 c \tag{6}$$

where η and η_s are the viscosities of the solution and solvent, respectively, η_{rel} and η_{sp} are the (dimensionless) parameters of relative and specific viscosity, c is the concentration and k' and k'' are constants. Eqs. (5) and (6), which are known, respectively, as the Huggins and Kraemer equations, are valid only for solution viscosities up to about twice that of water (i.e. $\eta_{rel} \approx 2$), beyond which higher-order terms (c^2, c^3, etc.) are no longer negligible. Experimental measurements of solution viscosity (at 20 °C) were therefore confined to the approximate range $\eta_{rel} = 1.2$–2.0, the lower limit being imposed by the increasing error in values of η_{sp} (η_{rel}–1) as η_{rel} approaches 1. Within this range, plots of η_{sp}/c against c (Huggins plot) and $(\ln \eta_{rel})/c$ against c (Kraemer plot) should both be linear, extrapolating to a common intercept of [η] as $c \to 0$. The combined extrapolation is illustrated in Fig. 3 for locust bean gum.

The molecular weight (relative molecular mass, M_r) of each galactomannan sample was calculated from the measured value of intrinsic viscosity using the Mark-Houwink relationship (Eq. (7)) derived by Picout and Ross-Murphy (2002)

$$\log[\eta] = 0.706 \log M_r - 3.26 \tag{7}$$

The intrinsic viscosities and calculated molecular weights for the five galactomannan samples are reported in Table 1.

3.2. Analysis of compression curves

As an illustrative example, the compression curves obtained for sample C (1.0 wt% in combination with 50 wt% sucrose) are shown in Fig. 4a. The experimental scatter between the three replicates is typical of that seen for the other cryogels. There is a progressive increase in resistance (force) with increasing depth of penetration (distance) up to the point at which the gels break, when the resistance decreases. The erratic values at higher distance of penetration come from further rupture of the broken network.

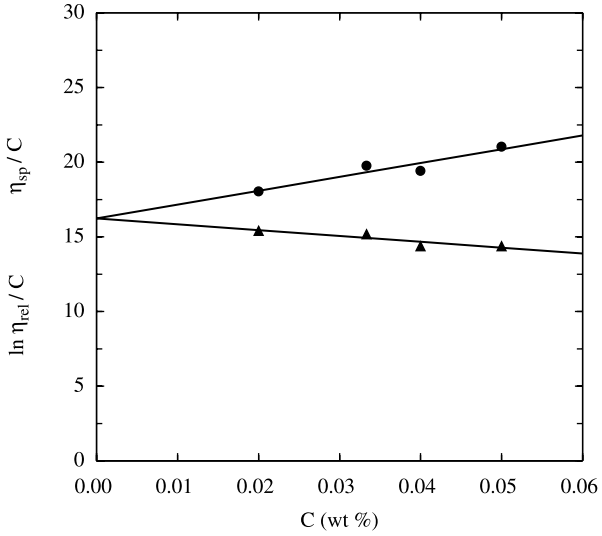

Fig. 3. Determination of intrinsic viscosity (20 °C) of LBG from Huggins and Kraemer plots of, respectively, η_{sp}/c (●) and (ln η_{rel})/c (▲) against concentration (c).

For quantitative comparisons between the different cryogels, the replicates for each sample were averaged, and the force–distance curves were converted to stress–strain curves. The test geometry used, in which a cylindrical probe was driven into gels contained in plastic beakers (Section 2), was chosen to allow the weakest samples, some of which were not self-supporting, to be included in the analysis. The deformations that occur in penetration tests of this type are complex (Gregson, Hill, Mitchell, & Smewing, 1999), but since the purpose of the measurements was to make comparisons between the cryogels, rather than to obtain rigorously valid rheological parameters, the data were treated in the same way as those from conventional compression testing, to yield 'apparent' values of stress, strain and moduli. The applied force (strictly, mass) in grams, as recorded by the Texture Analyser, was converted to Newtons ($F = m \times a$), and divided by the surface area of the probe (diameter 3.5 cm) to give stress (σ). The conversion factor between force (g) and stress (Pa) is 10.2 (i.e. an applied force of 1000 g corresponds to a stress of 10.2 kPa).

Extent of compression (distance) was converted to 'true' (Hencky) strain (ε), which is given (Ross-Murphy, 1984) by

$$\varepsilon = \ln(H_0/H) = \ln(H_0/(H_0 - d)) \quad (8)$$

where H_0 is the initial height of the sample (25 mm), H is the height to which it has been compressed, and d is the distance moved by the probe.

As illustrated in Fig. 4b, which shows the force–distance curves from Fig. 4a over the first 5 mm of compression, the initial region of the compression curves, up to ~10% 'true' strain ($\varepsilon \approx 0.1$), is essentially linear. The slope in this initial region was used to determine Young's modulus (E):

$$E = \sigma/\varepsilon \quad (9)$$

The other parameters derived from the averaged compression curves were the values of stress and strain at which the networks broke (break stress, σ_b and break strain, ε_b) and the modulus at break (E_b):

$$E_b = \sigma_b/\varepsilon_b \quad (10)$$

The standard deviation of these four parameters over three replicate compression curves for each cryogel was approximately 10%.

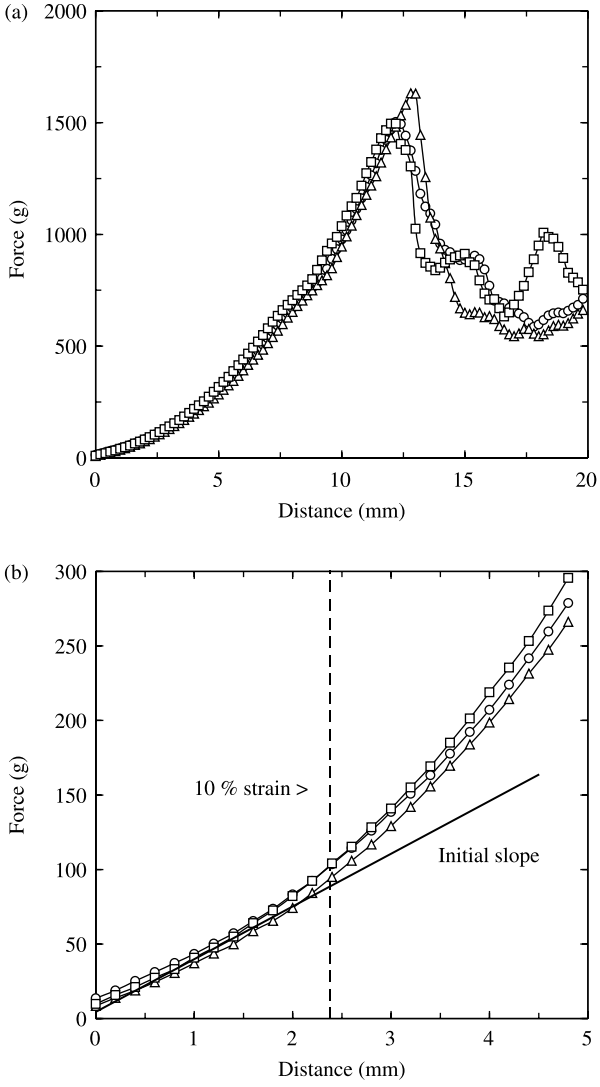

Fig. 4. (a) Force–distance curves (0.1 mm/s; 5 °C) for three replicate cryogel preparations of galactomannan sample C (1.0 wt%) in combination with 50 wt% sucrose. (b) Curves from (a) over the first 5 mm of compression.

3.3. Effect of sugars on cryogelation of LBG

Each of four sugars used was studied at five concentrations: 40, 45, 55, 50 and 60 wt%, in combination with 1.0 wt% LBG. The (averaged) compression curves obtained are shown in Figs. 5, 6, 7 and 8 for sucrose, glucose, fructose and sorbitol, respectively. Fracture of the gel network (reduction in stress with increasing strain) was observed for all preparation apart from two: 1.0 wt% LBG in combination with 40 wt% sucrose (Fig. 5a) or 60 wt% sorbitol (Fig. 8b). Values of break stress,

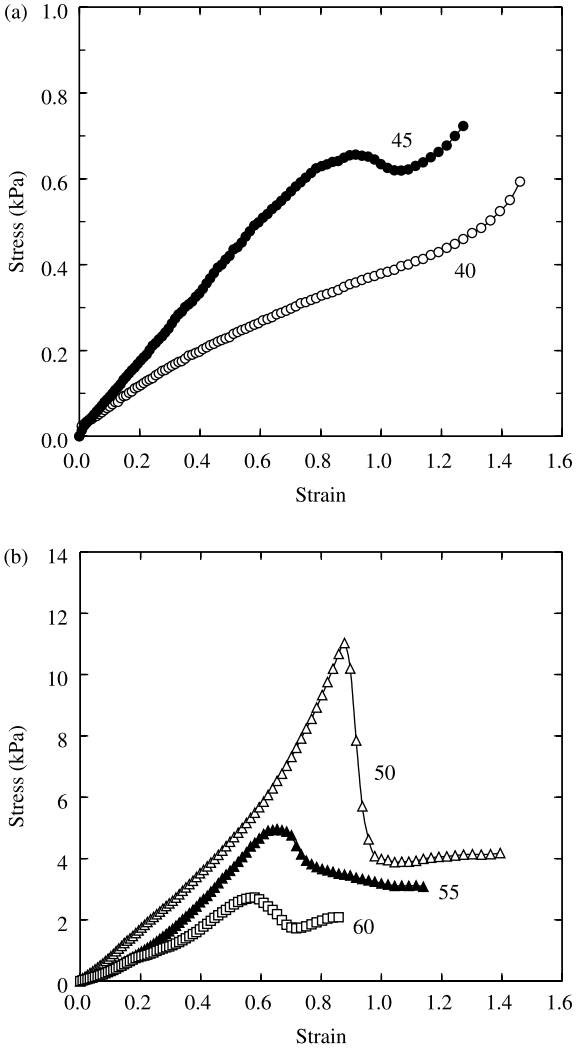

Fig. 5. Averaged compression curves (0.1 mm/s; 5 °C) for cryogels of 1.0 wt% LBG in combination with sucrose at concentrations of (a) 40 (○) and 45 (●) wt% and (b) 50 (△), 55 (▲) and 60 (□) wt%.

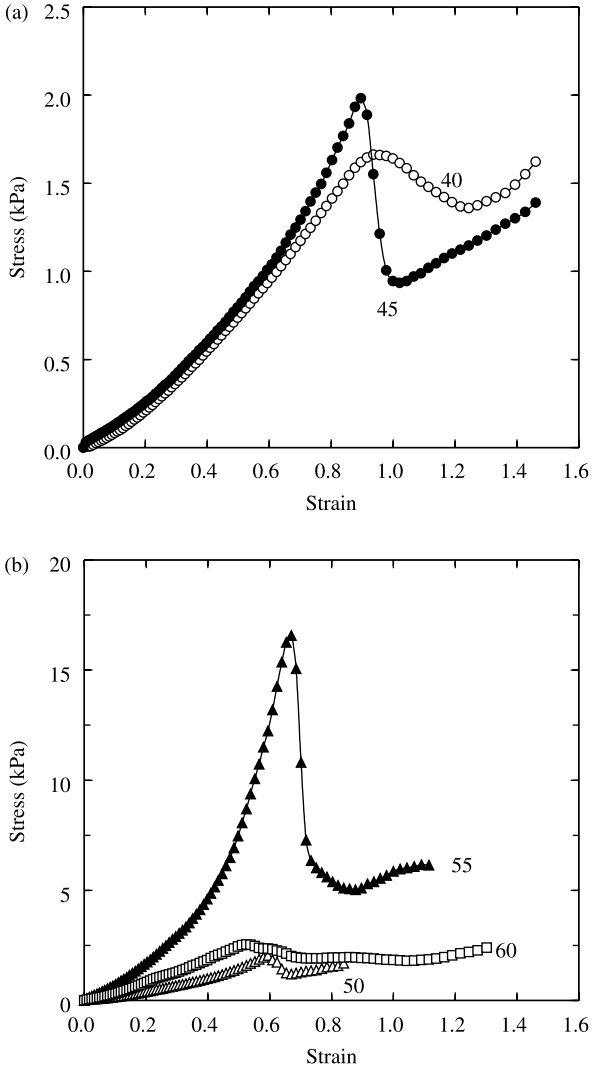

Fig. 6. Averaged compression curves (0.1 mm/s; 5 °C) for cryogels of 1.0 wt% LBG in combination with glucose at concentrations of (a) 40 (○) and 45 (●) wt% and (b) 50 (△), 55 (▲) and 60 (□) wt%.

break strain and modulus at break cannot, therefore, be reported for these samples.

As shown in Fig. 9, Young's modulus (obtained from the slope of the initial linear region of the compression curves) passes through a maximum value with increasing concentration of each of the sugars studied. For sucrose and sorbitol (Fig. 9a), the maximum occurs at ~50 wt%; for glucose, the concentration giving maximum cryogel modulus (Fig. 9b) is higher (~55 wt%) and for fructose it is lower (~45 wt%). However, the maximum modulus obtained with fructose as co-solute is substantially lower than with the other three sugars.

The variation of break stress with varying concentration of each of the four co-solutes is broadly similar (Fig. 10) to the variation in Young's modulus (Fig. 9). As shown in Fig. 11, there is an essentially linear decrease in break strain with increasing concentration of each of the four sugars, irrespective of the strength (break stress) of the same samples. The magnitude of this decrease is greater for sorbitol than for the other three co-solutes.

Fig. 12 shows the variation in modulus at break with increasing concentration of each of the four co-solutes studied. There is a systematic progression (Fig. 12a) in maximum modulus and sugar concentration at which the maximum is reached through the series: fructose < sucrose < glucose. The maximum value of modulus at break with sorbitol as co-solute (Fig. 12b) is similar to that obtained with sucrose (Fig. 12a), and occurs at about the same sugar concentration (~50 wt%).

3.4. Effect of galactomannan composition and chainlength

The (averaged) compression curves for the cryogels formed by partially debranched guar gum samples A–C, in combination with 50 wt% sucrose, are shown in Fig. 13a; the corresponding curves for sample D and LBG, again with 50 wt% sucrose, are shown in Fig. 13b. It is immediately evident that sample D, which has the highest M/G ratio (Table 1), gives the strongest cryogels.

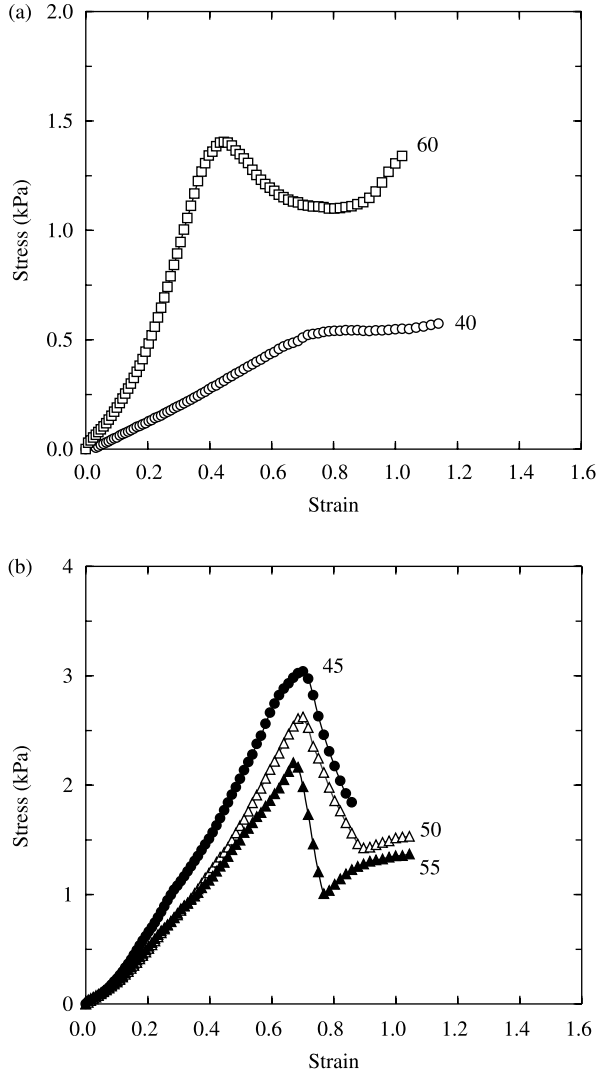

Fig. 7. Averaged compression curves (0.1 mm/s; 5 °C) for cryogels of 1.0 wt% LBG in combination with fructose at concentrations of (a) 40 (○) and 60 (□) wt% and (b) 45 (●), 50 (△) and 55 (▲) wt%.

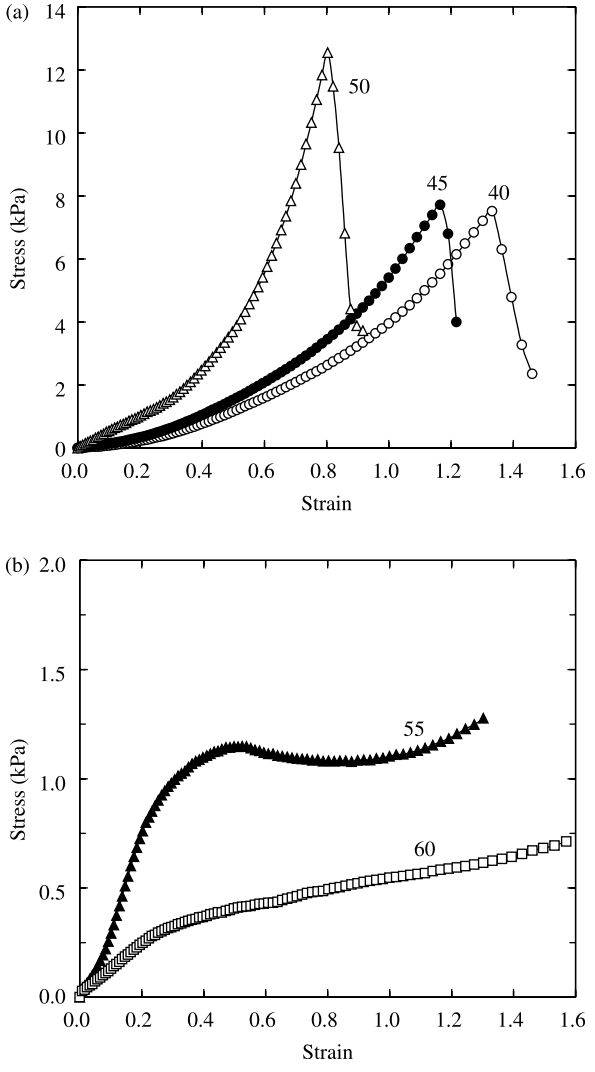

Fig. 8. Averaged compression curves (0.1 mm/s; 5 °C) for cryogels of 1.0 wt% LBG in combination with sorbitol at concentrations of (a) 40 (○), 45 (●) and 50 (△) wt% and (b) 55 (▲) and 60 (□) wt%.

As shown in Fig. 14, there is a progressive increase in Young's modulus (obtained from the initial slope of the compression curves) with increasing M/G ratio (i.e. with increasing content of unsubstituted mannose residues in the polymer backbone). The increase is steepest at M/G ratios above ~4.0. The cryogel modulus for LBG fits in smoothly with those for the samples of partially debranched guar gum, despite its much higher molecular weight (Table 1).

The variation of break stress with varying M/G ratio (Fig. 15) is broadly similar to the variation in Young's modulus (Fig. 14), with the value obtained for LBG again fitting in smoothly with those for the cryogels of partially debranched guar gum.

As shown in Fig. 16, the break strain of the cryogels formed by the samples of partially debranched guar gum also increases with increasing M/G ratio, the increase again being steepest at M/G ratios above ~4.0. However, in contrast to the results shown for Young's modulus (Fig. 14) and break stress (Fig. 15), the break strain for the cryogel formed by LBG is higher (by ~30%) than would be anticipated from the corresponding values for the cryogels of partially debranched guar gum and, in consequence, the modulus at break (Fig. 17) is correspondingly lower.

4. Discussion and conclusions

4.1. Galactose content and molecular weight of galactomannans

As shown in Table 1, the samples of partially debranched guar gum have much lower intrinsic viscosities than LBG, and there is a general trend to lower molecular weights with decreasing content of galactose (from 72.6 kDa for sample A to 9.8 kDa for sample D). Both effects can be explained by some slight β-mannanase activity in the α-galactosidase used for removal of galactose.

The values of Young's modulus (Fig. 14), break stress (Fig. 15), break strain (Fig. 16) and modulus at break (Fig. 17)

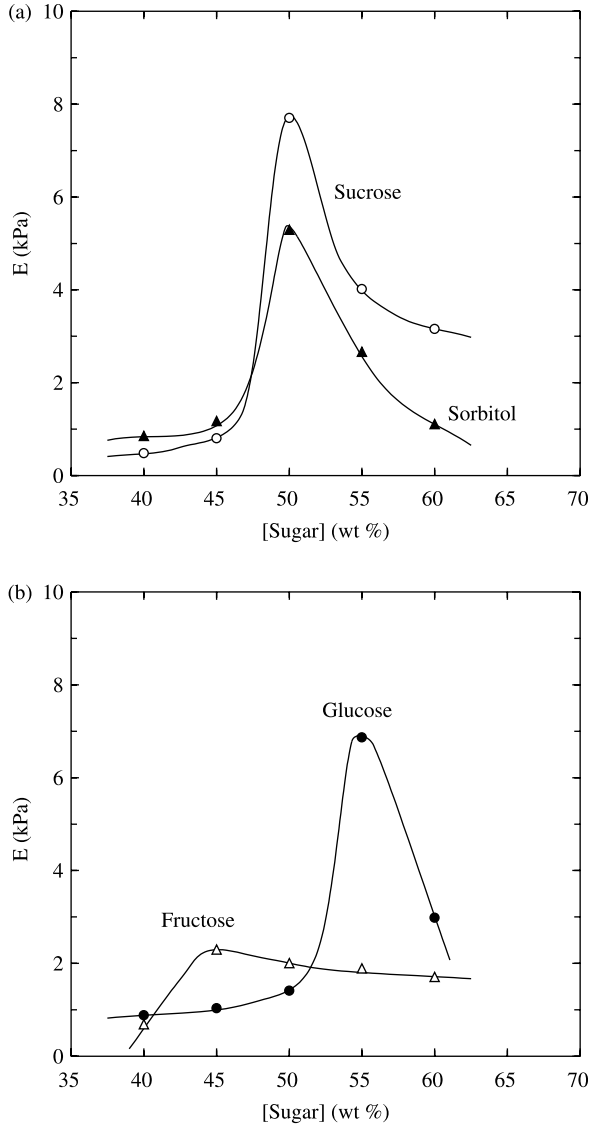

Fig. 9. Variation of Young's modulus with sugar concentration for cryogels of 1.0 wt% LBG in combination with: (a) sucrose (○) and sorbitiol (▲); and (b) glucose (●) and fructose (△).

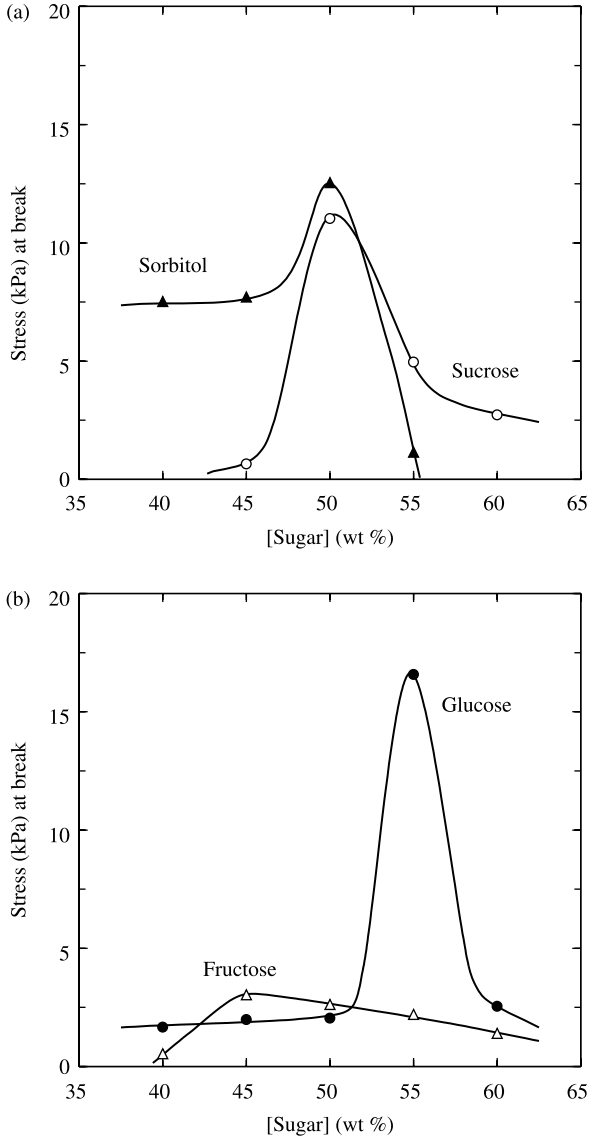

Fig. 10. Variation of break stress with sugar concentration for cryogels of 1.0 wt% LBG in combination with: (a) sucrose (○) and sorbitiol (▲); and (b) glucose (●) and fructose (△).

for the cryogels formed by the samples of partially debranched guar gum (1.0 wt% in combination with 50 wt% sucrose) vary smoothly with galactose content and, in particular, all four parameters increase steeply at M/G ratios above ~4.0. This sharp enhancement in mechanical properties can be explained by the decrease in galactose content increasing the probability of occurrence of unsubstituted mannan sequences longer than the threshold value of ~6 consecutive residues required (Dea et al., 1986) for intermolecular association.

The cryogelation properties of LBG fit in reasonably well (Figs. 14–17) with those of the other galactomannan samples, despite its much higher molecular weight (over 2000 kDa; Table 1). The greatest divergence is in break strain (Fig. 16), where the value for LBG lies substantially above the curve obtained for the samples of partially-debranched guar gum. This may be because the LBG molecules, being longer, form a larger number of intermolecular junctions, and therefore require greater deformation of the cryogel network before they separate from one another.

In contrast, the values of Young's modulus (Fig. 14) and modulus at break (Fig. 17) for LBG lie somewhat below the curves obtained for the samples of partially debranched guar gum. A possible explanation is that enzymic debranching gives a more block-like structure than in LBG, and consequently a higher proportion of unsubstituted sequences capable of forming intermolecular junctions at equivalent overall M/G ratio.

These differences between LBG and the samples of partially debranched guar gum, however, are relatively minor. The overall conclusions from this part of the investigation are that there is a large enhancement in the mechanical properties of galactomannan cryogels (1.0 wt% in combination with 50 wt% sucrose) at M/G ratios around and above that of LBG (because of the increasing proportion of long stretches of unsubstituted mannan backbone capable of forming stable intermolecular

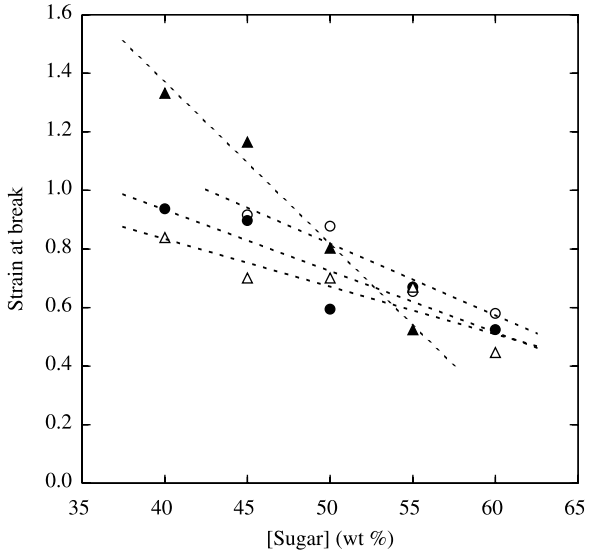

Fig. 11. Variation of break strain with sugar concentration for cryogels of 1.0 wt% LBG in combination with sucrose (○), glucose (●), fructose (△) or sorbitiol (▲).

junctions), and that molecular weight, within the range studied (from ~10 to ~2000 kDa; Table 1), has comparatively little effect.

4.2. Role of sugars in cryogelation

One important factor in the stability of aqueous solutions of galactomannans and other polysaccharides is the conformational entropy of disordered coils in solution, which is lost if the chains pack together in the solid state or in the intermolecular junctions of hydrated networks. Entropy becomes increasingly significant as temperature is raised ($\Delta G = \Delta H - T\,\Delta S$), which explains the progressive increase in solubility of LBG and other galactomannans with increasing temperature. The other determinant of solubility/insolubility is the enthalpic balance between polymer—solvent interactions (such as hydrogen bonding) which promote solubility, and polymer–polymer interactions favouring intermolecular association. In cryogelation, formation of ice crystals reduces the amount of liquid water available to interact with the polymer chains, thus shifting the balance towards intermolecular association.

Incorporation of high concentrations of sugars or other co-solutes in place of water also reduces the number of polymer–solvent interactions that can occur in competition with binding interactions between the polymer molecules. The solvent-quality of the remaining water is further reduced by competition of co-solute–solvent interactions with the polymer–solvent interactions which promote stability of the solution state. Neither of these effects, however, can explain why the initial increase in the strength of LBG cryogels with increasing concentration of added sugar (Figs. 9, 10 and 12) is followed by a decrease at higher concentrations.

A tempting interpretation is that the decrease comes from progressive depression of freezing point as the sugar

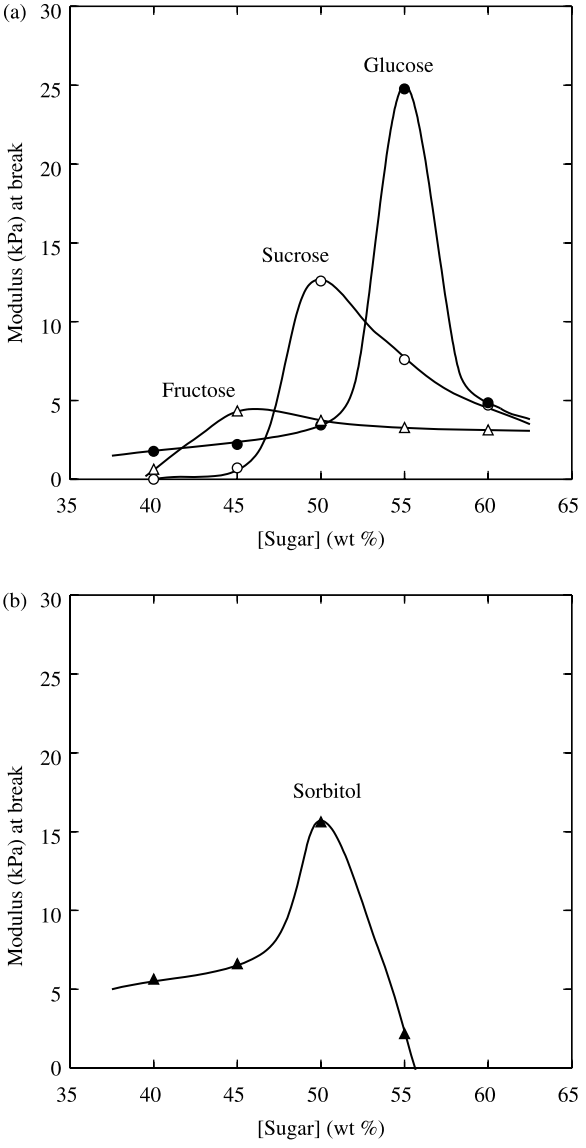

Fig. 12. Variation of modulus at break with sugar concentration for cryogels of 1.0 wt% LBG in combination with: (a) sucrose (○), glucose (●) and fructose (△); and (b) sorbitiol (▲).

concentration is increased, with therefore progressively less conversion of liquid water to ice. However, in an investigation of the effect of sucrose on the gelation of LBG from unfrozen solutions (Richardson & Norton, 1998), a maximum in gel strength was also observed, at the same sucrose concentration (50 wt%) as found (Figs. 9, 10 and 12) for the cryogels studied in the present work. It would appear, therefore, that the explanation must lie in some more general effect of co-solutes on the polymer–water system.

High concentrations (above ~70%) of sugars can inhibit or abolish conformational ordering of biopolymers by trapping the system in a vitrified state (Al-Amri, Al-Adawi, Al-Marhoobi, & Kasapis, 2005; Deszczynski, Kasapis, MacNaughton, & Mitchell, 2002, 2003; Evageliou, Kasapis, & Hember, 1998; Kasapis, 2006a, 2006b). This phenomenon, however, is unlikely to be a relevant factor at the lower concentrations of co-solute used in the present investigation (40–60 wt%, chosen to span the

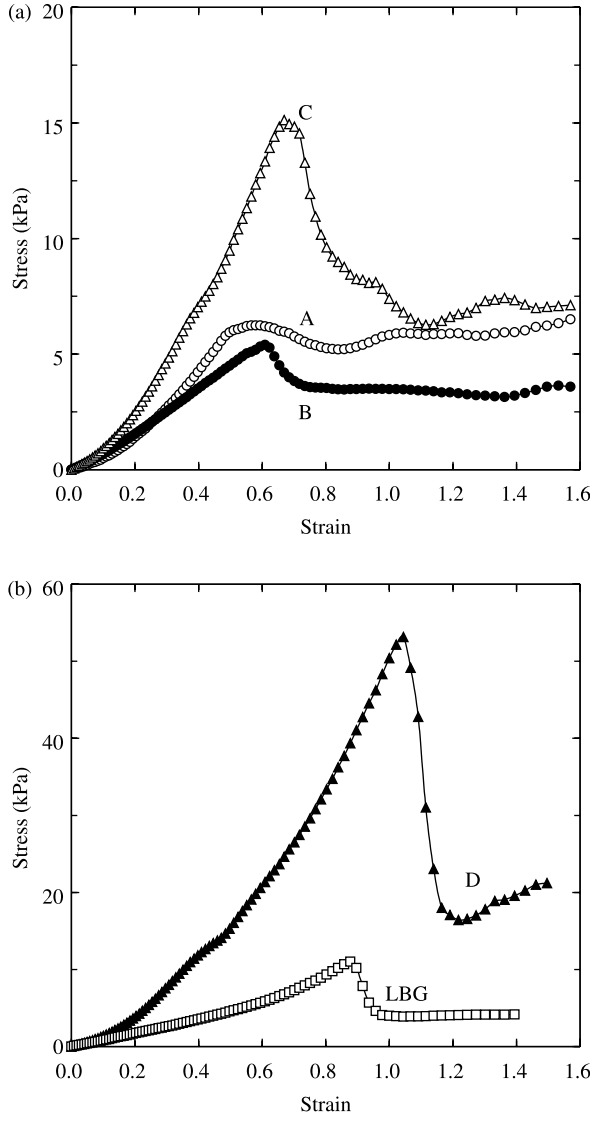

Fig. 13. Averaged compression curves (0.1 mm/s; 5 °C) for cryogels incorporating 50 wt% sucrose in combination with 1.0 wt% of: (a) galactomannan samples A (○), B (●) and C (△); and (b) galactomannan sample D (▲) and LBG (□).

range over which the strength of the galactomannan cryogels passes through its maximum value). At concentrations within this range, co-solutes normally promote or stabilise ordered association of biopolymers.

As discussed above, one obvious effect of introducing large concentrations of a co-solute into aqueous biopolymer systems is to reduce the concentration of water, with consequent decrease in the effectiveness of polymer–solvent interactions in competing with polymer–polymer interactions, thus promoting self-association of the polymer chains. Different co-solutes, however, differ substantially in their effectiveness in promoting association of biopolymers into intermolecular junctions (Back, Oakenful, & Smith, 1979; Evageliou, Richardson, & Morris, 2000b; Kohyama & Nishinari, 1991; Nishinari & Watase, 1992; Nishinari, Watase, Williams, & Phillips, 1990; Oakenful & Scott, 1986; Watase, Kohyama, & Nishinari, 1992; Watase, Nishinari, Williams, & Phillips, 1990). These

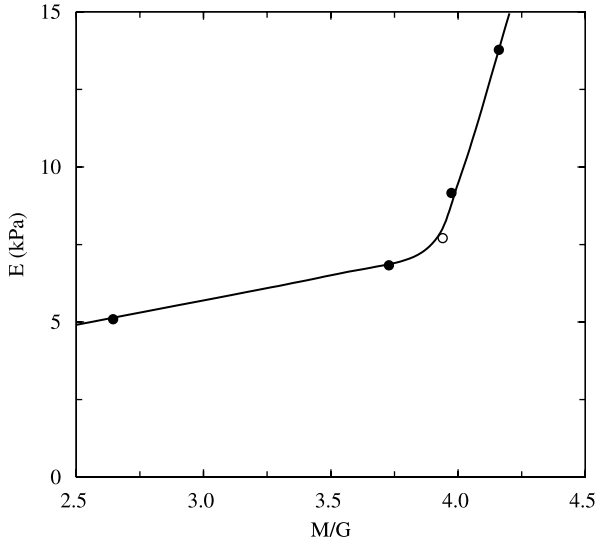

Fig. 14. Variation of Young's modulus with M/G ratio of the galactomannan component for cryogels incorporating 50 wt% sucrose in combination with 1.0 wt% of LBG (○) or partially debranched guar gum samples (●).

differences are normally attributed to modification of water structure by the co-solute, with consequent modification of the competition between polymer–water and polymer–polymer interactions.

For sugars, the order of effectiveness can often be correlated with the content of equatorial hydroxyl groups (Nishinari & Watase, 1992; Nishinari, Watase, Miyoshi, Takaya, & Oakenfull, 1995; Watase et al., 1992), whose spacing is particularly compatible with the 'lattice' structure of water (Tait, Suggett, Franks, Ablett, & Quickenden, 1972). However, from an analysis in which all possible pairwise interactions between the three constituents were considered explicity, Nilsson, Piculell, and Malmsten (1990) concluded that although water–co-solute interactions may dictate the overall

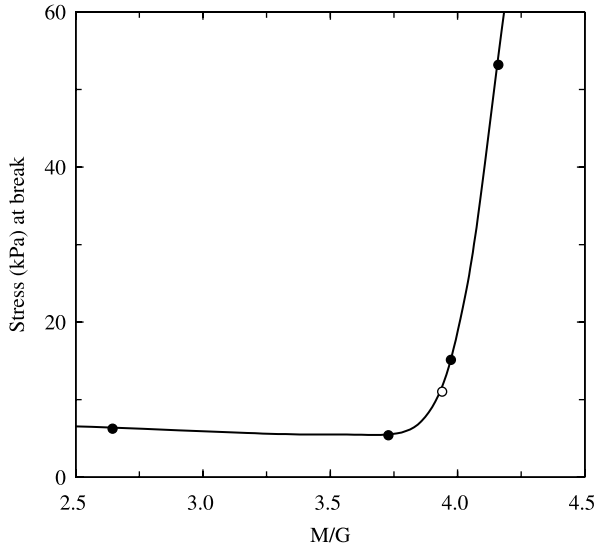

Fig. 15. Variation of break stress with M/G ratio of the galactomannan component for cryogels incorporating 50 wt% sucrose in combination with 1.0 wt% of LBG (○) or partially debranched guar gum samples (●).

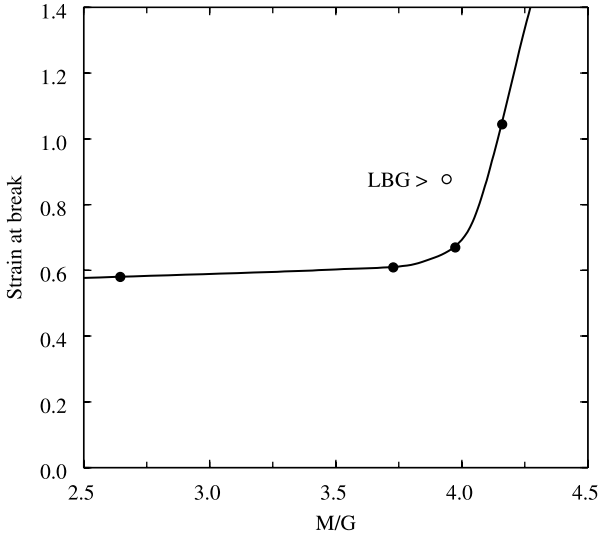

Fig. 16. Variation of break strain with M/G ratio of the galactomannan component for cryogels incorporating 50 wt% sucrose in combination with 1.0 wt% of LBG (○) or partially debranched guar gum samples (●).

properties of the system (thus explaining why the same order of effectiveness is often found for the same co-solutes in combination with different biopolymers), they act indirectly, by competition with polymer–co-solute interactions, which are the direct determinant of changes in conformational stability.

In an investigation of the gelation of high-methoxy pectin in the presence of high concentrations of sugar at low pH (as in jam-making), Evageliou, Richardson, and Morris (2000a) found that equivalent concentrations of sucrose, glucose or fructose gave large differences in gel strength, in the order: fructose < sucrose < glucose. The proposed interpretation was that sugars can inhibit intermolecular association of polysaccharide chains by binding to them through hydrogen bonds; that primary hydroxyl groups, being more acidic (polar) than secondary hydroxyls, form stronger hydrogen bonds (Plaschina et al., 1986); and that fructose, having two primary hydroxyl groups per monosaccharide (at C(1) and C(6)) has a greater inhibitory effect than glucose (one primary hydroxyl per monosaccharide, at C(6)), with sucrose (three primary hydroxyls per dissaccharide, i.e. 1.5 per monosaccharide) giving intermediate behaviour. As shown in Fig. 12a, the strength (modulus at break) of the LBG cryogels studied in the present work follows the same sequence (fructose < sucrose < glucose), and can perhaps be explained in the same way.

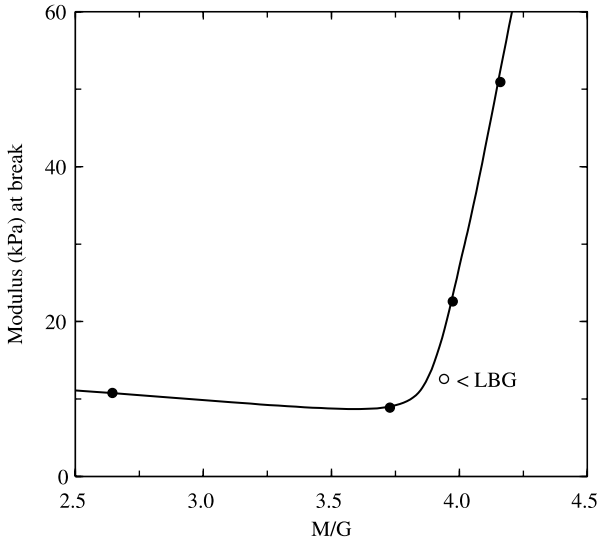

Fig. 17. Variation of modulus at break with M/G ratio of the galactomannan component for cryogels incorporating 50 wt% sucrose in combination with 1.0 wt% of LBG (○) or partially-debranched guar gum samples (●).

In terms of this interpretation, the initial increase in modulus with increasing concentration of sugar arises from the accompanying reduction in the amount of water available to maintain the polymer chains in solution. As the sugar concentration is increased further, the drive to increase in polymer–polymer association from reduction in water content continues, but is eventually outweighed by the inhibitory effect of sugar molecules binding to the polymer chains and blocking the chain–chain associations required to form the junctions of the cryogel network. The transition from enhancement to depletion of gel strength comes at progressively lower concentrations of sugar as the strength of the polymer–sugar interactions increases, with fructose binding more strongly than sucrose, which in turn binds more strongly than glucose.

The concept of direct binding of sugar molecules to polysaccharide chains is, of course, speculative, and indeed a recent investigation by NMR (Kumagai, MacNaughton, Farhat, & Mitchell, 2002) showed that the presence of polysaccharide (κ-carrageenan) at a concentration (0.9 wt%) similar to that used in the present work had no effect on the mobility of sugar molecules, arguing against any binding interaction. However, the water content of the samples studied was very low (16–3.5 wt%), in the range where vitrification effects are dominant. At higher water content, there is perhaps some experimental evidence of binding. In a study of high-methoxy pectin gels by differential scanning calorimetry (DSC), Tsoga, Richardson, and Morris (2004b) found that gels formed with fructose (60 wt%) as co-solute showed a very intense endotherm on heating over the temperature range at which network structure was formed on cooling, followed by an almost equally intense exotherm. The proposed interpretation was that (i) the two primary hydroxyl groups of fructose form strong hydrogen bonds to the ordered structure of pectin; (ii) the endotherm on heating is dominated by the heat required to break these hydrogen bonds; (iii) the intense exothermic process comes from re-attachment of fructose to the disordered chains liberated by dissociation of the initial complex. Although this interpretation is again speculative, it is consistent with the observation (Tsoga, Richardson, & Morris, 2004a) that the gels incorporating fructose were anomalously weak in comparison with those obtained using other co-solutes.

Finally, the maximum strength of the LBG cryogels formed with sorbitol as co-solute is similar to that obtained using sucrose (Figs. 9, 10 and 12) and occurs at the same sugar concentration (50 wt%). Unlike sucrose, however, sorbitol does not induce dental caries (Moynihan, 1998). Galactomannan cryogels incorporating sorbitol as co-solute might

therefore have useful practical application in 'tooth friendly' products.

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