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Solution characteristics of the xyloglucan extracted from *Detarium senegalense* Gmelin

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The macromolecular solution properties of detarium xyloglucan, a seed extract of the plant *Detarium senegalense* Gmelin, were investigated by steady and dynamic shear rheometry, and static light scattering. The main polysaccharide of detarium gum is a xyloglucan, consisting of a cellulosic backbone with single-unit α-D-xylopyranosyl substituents attached to carbon-6 of the glucosyl residues, and with some of the xylose residues further substituted at carbon-2 by β-D-galactopyranosyl residues. In this paper, all the semi-dilute solution characterisation work seems to be very largely consistent with much of the published data for the rheology of other polysaccharide solutions and suggests that detarium gum is a well behaved linear polymer entanglement network system. It has been established that when $C < \sim C^*$, $\eta_{sp} \propto C^{1.3}$, while at $C > \sim 2C^*$, $\eta_{sp} \propto C^{4.0}$. The static light scattering technique was successfully applied to examine the molecular weight and architecture of the detarium xyloglucan macromolecule by employing pressure heating treatment of the samples. The scattering profile for detarium xyloglucan is not consistent with that of a linear macromolecule, but instead strongly suggests a small degree of long chain branching. The implications of this finding are discussed. © 1997 Elsevier Science Ltd

INTRODUCTION

Detarium senegalense Gmelin is an underexploited and previously largely uncharacterised leguminous seed crop belonging to the family Caesalpiniaceae. We have recently established that detarium seeds contain a high concentration of water-soluble non-starch polysaccharide (s-NSP) (~59.8 g per 100 g dry matter) located in highly thickened cell walls (Wang *et al.*, 1996). We have also confirmed that the main polysaccharide is a xyloglucan, consisting of a cellulosic backbone with single-unit α-D-xylopyranosyl substituents attached to carbon-6 of the glucosyl residues, and with some of the xylose residues further substituted at carbon-2 by β-D-galactopyranosyl residues (Wang *et al.*, 1996). Such xyloglucans occur as massive deposits in the cotyledonary cell walls of a variety of dicotyledenous seeds, such as those of nasturtium and tamarind (Reid, 1985). The present material, here denoted 'detarium gum', is structurally similar to that extracted from tamarind gum, although the proportion of galactose, relative to xylose and glucose, is somewhat lower than that found in tamarind gum, as seen in Table 1. In the present paper, we extend our previous characterisation of detarium, by including measurements of viscosity at finite concentrations, both above and below C^*, the coil overlap concentration, together with detailed studies of the molecular weight and chain properties obtained from intensity light scattering measurements.

In common with many polysaccharide gums extracted from plant materials, detarium xyloglucan has potentially important commercial applications, particularly in the pharmaceutical and food industries for controlling drug release and modifying texture, respectively (Lapasin and Pricl, 1995). Moreover, it has been known for many years that such gums have important nutritional and therapeutic benefits, notably

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Abbreviations—In this manuscript an unambiguous shorthand nomenclature for xyloglucan oligosaccharides is used. Each (1→4)-β-linked D-glucosyl residue in the backbone is given a one-letter code according to its subsituents. Thus G = unsubstituted glucose residue; X = xylose-substituted glucose residue; L = galactosylxylose-substituted glucose residue; sequences always read towards the reducing end of the molecule.

Table 1. Results of Dionex HPAE chromatography showing quantitatively oligosaccharide fractions converted from detarium xyloglucan and tamarind xyloglucan when treated with *endo*-1,4-β-glucanase. From Wang *et al.* (1996)

	Oligosaccharides ratio				Deduced monosaccharides		
	XXXG[a]	XLXG	XXLG	XLLG	Xylose	Galactose	Glucose
Tamarind	1.00	0.42	2.07	6.20	1.00	0.51	1.34
Detarium	1.00	0.30	5.60	6.20	1.00	0.46	1.33

[a] See abbreviations on p. 115.

in the treatment of metabolic disorders such as diabetes mellitus and hyperlipidaemia (Ellis, 1994). In recent clinical trials, significant reductions in the postprandial rise in blood glucose and insulin concentrations were seen in healthy (Onyechi *et al.*, 1993) and diabetic (Onyechi, 1995) human subjects given foods supplemented with detarium seed flour. More recent animal studies have shown that the physiological effects of guar gum and similar hydrocolloids are strongly dependent on their capacity to generate high levels of viscosity in the upper gastrointestinal tract (Ellis *et al.*, 1995).

Therefore, one aspect of this study was to characterise further the solution properties of purified xyloglucan, so that this can be related to therapeutic activity and other functional properties of the polymer. More generally, few detailed investigations of the solution properties of xyloglucans have appeared in the literature, and the only extensive study is that on tamarind gum (Gidley *et al.*, 1991; Lang and Burchard, 1993). However, the light scattering measurements in these studies were complicated by time dependence and strong aggregation effects. In the present work we have employed the pressure heating method of Vorwerg and Radosta (1993). Recent work by these authors (Aberle *et al.*, 1994) has shown that reliable scattering results can be obtained for starch polymers by employing this technique. The present work is the first application of this method to s-NSP.

MATERIALS AND METHODS

Extraction and purification of detarium xyloglucan

The detarium xyloglucan was extracted and purified using the method described in our previous paper (Wang *et al.*, 1996). This includes a maceration step after extraction of lipid with ethanol, enzyme digestion to remove starch and protein and the ethanol precipitation of the polymer followed by freeze drying. Subsequent solutions were prepared by dispersing the known weights of a freeze-dried sample of purified detarium gum in deionized water for 1 h at 80°C and then mixing overnight using a magnetic stirrer at room temperature. The xyloglucan content of the purified detarium was determined by using a modified procedure (Wang *et al.*, 1996) of the Englyst method (Englyst *et al.*, 1992).

Intrinsic viscosity measurement

The intrinsic viscosity, $[\eta]$, was determined by using a dilution capillary viscometer (Cannon Ubbelohde Dilution B glass viscometer. Glass Artefact Viscometers, Braintree, UK) immersed in a water bath to maintain the temperature at 25±0.1°C. The solution was filtered through a 0.45 μm syringe filter before measurements were taken. The concentrations of detarium gum used were based on the xyloglucan content of the purified samples. Further details of the intrinsic viscosity method are given in our earlier paper (Wang *et al.*, 1996).

Steady shear flow measurements

Steady shear experiments on the detarium gum solutions prepared at different concentrations (0.1–3.0% w/w) were performed on the Rheometrics Fluids Spectrometer (RFSII, Rheometric Scientific Ltd, Epsom, UK) with a cone and plate configuration (diameter 50 mm, cone angle 0.02 rad). The transducer system has a dual torque range of 0.002–10 g cm and 0.02–100 g cm, which corresponds to a stress range of 0.006–300 Pa with this geometry. Most of the steady shear measurements were carried out at shear rates of 0.05–1000 s^{-1} with a reduction in rate at the higher concentrations. All the measurements were conducted at 25°C.

Dynamic frequency sweep experiments

All the oscillatory experiments were conducted on the RFSII using the same geometry as for the steady shear measurements described above. In this apparatus, a sinusoidal strain wave with frequency ω was applied to the lower plate and the response of the sample exerted on the upper plate was detected by the transducer system (Ross-Murphy, 1994). Strain sweep experiments were carried out in order to determine the linear viscoelastic range of studied polysaccharide solutions. The complex shear modulus G^* and dynamic viscosity (η^*) were measured at a strain range of 0.1–100% in 5% increments at a frequency of 1 rad s^{-1} or 10 rad s^{-1}.

In frequency sweep measurements the strain was selected at 35%, which was within the linear viscoelastic limit established from the above

experiments. The frequency of the applied strain wave was varied from 0.05–100 rad s^{-1} with 5 points per decade. The viscoelastic parameters, namely the dynamic viscosity (η^*), storage modulus (G') and loss modulus (G'') were extracted.

Light scattering measurements

Static light scattering measurements were performed at 25°C with a fully computerised and electronically modified SOFICA photogoniometer (Baur, Instrumentenbau, Hausen, Germany) in the angular range from 30° to 150° in steps of 5° (25 angles). An argon ion laser (Uniphase; $\lambda_0 = 488$ nm) was the light source, and the scattering of toluene was used as the primary standard. The instrument automatically repeats measurements until data less than a predefined noise level (2%) are obtained. The refractive index increment, dn/dc, was chosen as 0.152 ml g^{-1}.

Solutions for light scattering were prepared by heating *c.* 20 ml of solution close to C^* (~0.1% w/w) to high temperatures (130 to 160°C), sealed under a pressure of 15 bar N_2 for varying times. The autoclaved solution and serial dilutions (1.0, 0.8, 0.6, 0.4, 0.2, 0.1) were then filtered (×3) directly into the cylindrical light scattering cuvettes (total volume *c.* 2 ml) using 0.22 μm Millipore syringe filters. All solution preparation stages were carried out in a laminar air flow cabinet to minimise contamination with dust.

Following the pressure heating process, a small amount of undissolved material was seen. In order to see if this made up a significant fraction of the sample, 50 ml of the *c.* 100 ml of the solution was freeze dried after filtering, and the recovery of dissolved material was found to be ~95.5% of the original sample. In subsequent determinations the presence of this undissolved fraction was ignored. Filtration through 0.45 μm Millipore filters was easy, because no real pressure had to be applied. This intimated that the solutions were largely aggregate free.

In two subsequent experiments, $[\eta]$ was also measured following the pressure heating and filtering processes, to examine whether or not this caused a noticeable reduction in molecular weight.

RESULTS

Intrinsic viscosity $[\eta]$

The $[\eta]$ was determined with the glass capillary viscometer at polysaccharide concentrations ranging from 0.01 to 0.1% (w/v). In this case, the viscosity relative to that of the solvent (water) lies in the range $1.2 < \eta_r < 2.0$. The experiments carried out at higher concentrations and at a range of different shear rates suggest that under these conditions (i.e. $1.2 < \eta_r < 2.0$) the solution viscosity is essentially Newtonian. The $[\eta]$ of detarium gum was found to be ~8.9±0.2 dl/g (Fig. 1), which is significantly higher than the value reported for tamarind-seed xyloglucan ($[\eta] = 6.0 \pm 0.5$ dl/g) (Gidley *et al.*, 1991). Since the $[\eta]$ of detarium gum is rather high, this gives a very low C^* (i.e. $C^* = 1/[\eta] = 1/8.9$ g/dl = 0.11% w/w), which means of course that solutions of detarium polysaccharide have significant viscosity effects even at relatively low concentrations.

Steady shear flow measurements

The response of semi-dilute detarium xyloglucan solutions to steady shear rate experiments were studied over a wide range of concentration (0.1–3.0%, w/w). Figure 2 shows the shear viscosity versus shear rate range 10^{-2}–10^3 s^{-1} on a double logarithmic scale. No shear rate viscosity dependence was shown in the range of $\dot{\gamma}$ used for concentrations lower than 0.3%. However,

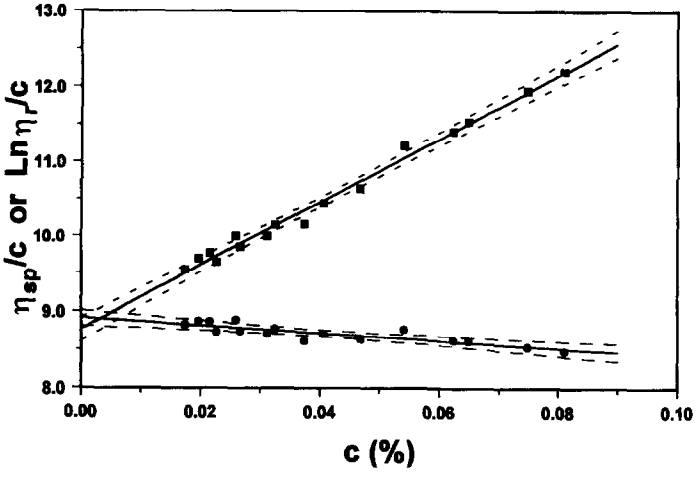

Fig. 1. Estimating intrinsic viscosity of detarium polysaccharide from plots η_{sp}/C vs. C (%) (■) and $\ln(\eta_r)/C$ vs. C (%) (●). Dotted lines indicate 95% confidence intervals.

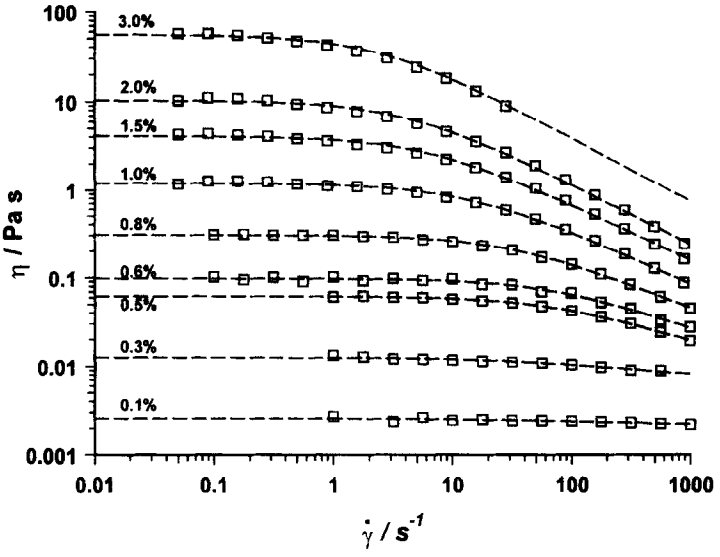

Fig. 2. Shear rate ($\dot{\gamma}$) dependence of viscosity (η) for different concentrations of detarium xyloglucan solutions. Dotted lines represent the least squares fitted Cross equation.

for solutions of concentration above this level, obvious shear-thinning behaviour was found, and as expected, this shear thinning became more pronounced as the concentration increased.

Most polysaccharide solutions of sufficiently high molecular weight and concentration, detarium polysaccharide being no exception, form an entangled network system. Many attempts have been made to explain and describe the shear-thinning behaviour of these systems (Launay *et al.*, 1986). According to Launay *et al.* (1986) and our own observations, amongst all these models the Cross equation (Cross, 1965) is the best *empirical* model for describing the flow behaviour of semi-dilute polysaccharide solutions. The Cross equation is written as:

$$\eta = \eta_\infty + (\eta_0 - \eta_\infty)/[1 + (\tau\dot{\gamma})^{m'}].$$

η_0 and η_∞ are limiting viscosities at zero and infinite shear rate, respectively. τ is a relaxation time, $\dot{\gamma}$ is the the shear rate, and m' is a power law exponent. The parameter m' is found to lie in the range $0 < m' < 0.73$, and corresponds to the usual limit exponent in the power law representation of η versus $\dot{\gamma}$.

The Cross equation has been used to calculate the zero-shear viscosity η_0 of the steady shear measurements by employing a nonlinear least squares fit. From these data, the concentration dependence of the zero-shear specific viscosity (η_{sp}) can be presented conveniently as a double logarithmic plot of η_{sp} against the coil overlap parameter, $C[\eta] = C/C^*$ (Fig. 3). In Fig. 3, the experimental points between polymer concentrations 0.02 and 0.08% (w/w) were obtained using the glass capillary viscometer, while the rest of the data were obtained with the RFSII. Two distinct linear regimes of slope can be identified from this plot, which is consistent with data seen for many other

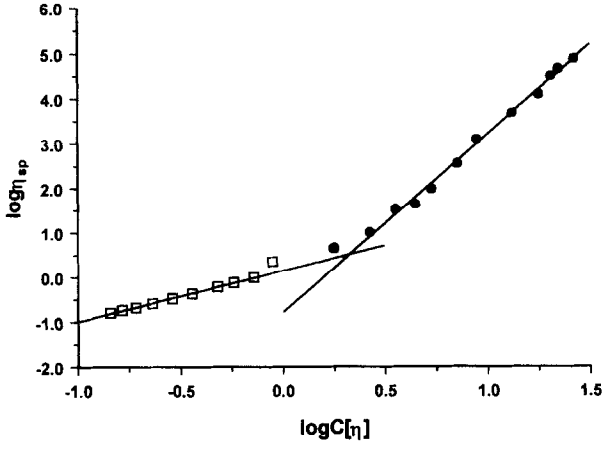

Fig. 3. Variation in the zero-shear specific viscosity of detarium xyloglucan solutions. Experimental points (□) between concentrations 0.02–0.08% (w/w) were obtained by using the glass capillary viscometer. Other experimental points (•) were obtained with the RFSII.

polysaccharide solutions. The first regime with a slope of 1.3 corresponds to the dilute solution, and the second one with a slope of 4.0 represents semi-dilute solutions. This means that when $C < C^*$, $\eta_{sp} \propto C^{1.3}$, while at $C > C^*$, $\eta_{sp} \propto C^{4.0}$, which is very similar to data published for guar gum (e.g. Robinson *et al.*, 1982). The highest slope value (i.e. 4.0) is almost the same as that obtained for most linear polymers interacting by purely topological entanglements. From the intersection between the extrapolations of the two straight lines the consequent concentration C_{cr} was calculated to be 0.21%. It is also worth mentioning that between the two linear regions there is an intermediate region in which our data shows a continuous curvature. Although some workers have

apparently introduced a third linear region to describe this behaviour, we are certainly not of the view that this has any real significance, particularly for polydisperse materials.

Dynamic measurements

As far as dynamic viscoelastic measurements are concerned, the coupling of strain γ and strain rate is critical, since only in the small-strain limit will G^* and η^* be independent of strain. This upper limiting strain depends strongly on the nature of the system and must be determined by careful experiments. The dependence of G^* on strain γ for 1%, 2% and 3% detarium gum solutions is illustrated in Fig. 4. G^* is reasonably independent of strain at all three concentrations up to $\gamma \sim 0.35$. There is no clear evidence that this limit was affected by different concentrations in the concentration range studied. Therefore, all the following frequency sweep experiments were conducted at $\gamma \sim 0.35$.

The storage and loss moduli G' and G'' for 1, 2 and 3% solutions of detarium xyloglucan are plotted against frequency ω in Fig. 5. G'' was always greater than G' for all three concentrations when in the low frequency range ($< 10\,\text{rad}\,\text{s}^{-1}$), indicating, therefore, that the solutions were predominantly liquid-like. Since G' increased faster than G'' with increasing frequencies, there was a crossover point between G' and G'' at 100, 35, 15 $\text{rad}\,\text{s}^{-1}$ for solutions of 1, 2 and 3%, respectively. This denotes a change of solution response from predominantly liquid-like to solid-like behaviour, which occurs at low frequencies for solutions of high polymer concentration.

The dynamic viscosity $\eta^*(\omega)$, which is defined as $(G'^2+G''^2)^{1/2}/\omega$, exhibits a similar profile when frequencies change as $\eta(\dot{\gamma})$ vs. $\dot{\gamma}$. At low frequencies a Newtonian plateau is seen while at high frequencies shear-thinning behaviour is exhibited. The η_0^* values were also calculated using the Cross equation in order to compare corresponding $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ values.

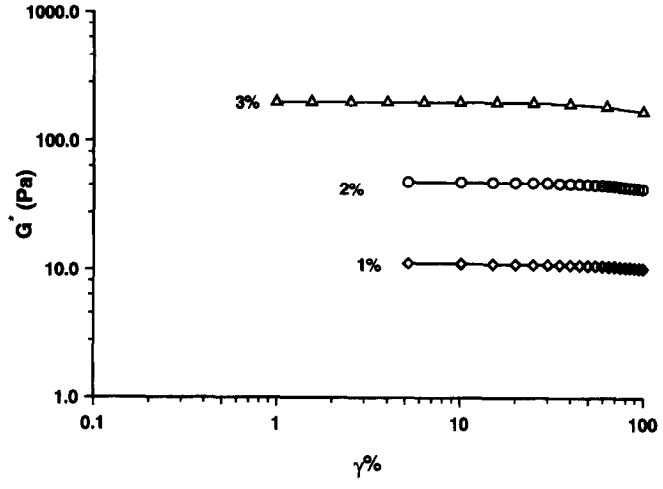

Fig. 4. Dependence of G^* on strain γ for 1, 2 and 3% (w/w) detarium xyloglucan solutions.

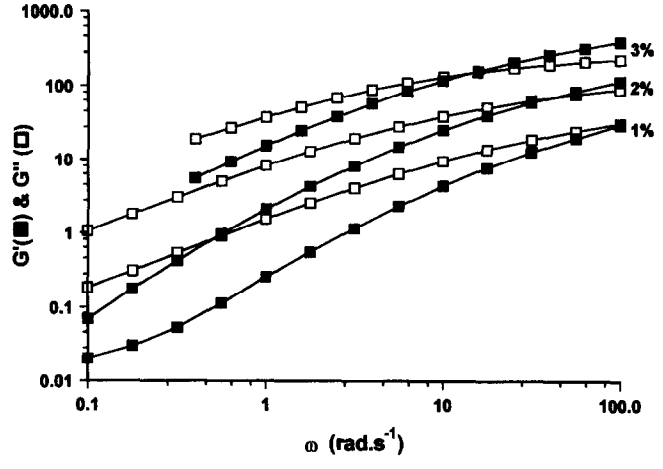

Fig. 5. G′ and G″ for different concentrations of detarium xyloglucan solutions.

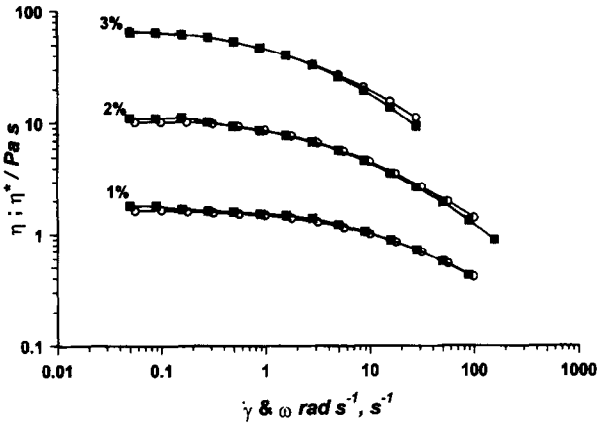

Fig. 6. Steady (■) and dynamic (○) viscosity data for 1 (i.e. $C/C^* \sim 9$), 2 and 3.0% (w/w) detarium xyloglucan solutions.

Good superposition of $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ was found for solutions of concentration from 1.0 to 3.0%. Within experimental error, and as always seen, within the lower shear rate ranges, the Cox–Merz rule (Ross-Murphy, 1994; Lapasin and Pricl, 1995) is obeyed perfectly, as shown in Fig. 6.

Light scattering measurements

Preliminary light scattering measurements carried out in Freiburg (Dr H. Urakawa, Kyoto Institute of Technology, personal communication) suggested that a wide range of molecular weights could be obtained for detarium gum, some of them $>10^7$, depending on pre-measurement heating temperature (at atmospheric pressures) and solvent. This suggested that a more rigorous solubilisation technique was required if reliable measurements were to be obtained using deionised water, which, in turn, suggested the employment of the pressure heating cell.

In all experiments the scatter of data obtained for deionised water at scattering angles $<40°$ was very large, a consequence of the use of water as the scattering medium, and the small cell size and scattering volume. Other measurements were very reproducible and good data could be obtained for all higher angles and concentrations $<C^*$. Because of the obvious curvature seen in all Zimm plots, the data were replotted in the Berry form, that is plotting the square root of the abscissa (Kc/R_θ) of a conventional Zimm plot (Burchard, 1983). This curvature reflects the structure-dependent angular behaviour of the scattering data, which is analysed below.

Results from the light scattering measurements are given in Table 2, and a typical Berry plot is illustrated in Fig. 7. The weight average molecular weights, MW_w, from independent zero angle and zero concentration extrapolations matched well in almost all cases, and good data were obtained for the (root of z-average mean square) radius of gyration, R_g (Table 2). As is usual for water-soluble neutral polysaccharides of high molecular weight, estimates for the second virial coefficient, A_2, were far less reliable, and, although all values were positive, they were quite small.

Most importantly, the MW_w and R_g showed no significant change either with length or maximum temperature of heating, or even when remeasured after 24 h (Table 2). The latter, in particular, is a clear sign that the very pronounced time-dependent supramolecular aggregation, almost always seen in light scattering of polysaccharides, and most other water-soluble polymers (Huglin, 1972), appears to have been eliminated by the high temperature and pressure treatment. The former effect severely reduces the reliability of much of the literature data on polysaccharide molecular weights by light scattering. Furthermore, the observation that the MW_w obtained are effectively independent of heating temperature and time strongly suggests that we are measuring a true molecular property, rather than a time and temperature dependent molecular weight degradation effect. This justifies *a posteriori* our strategy of treating the data for different treatments statistically as if they were strict replicates.

Table 2. Summary of static light scattering results

Sample	Treatment	MW_{W1}[a] ($\times 10^{-6}$)	MW_{W2}[a] ($\times 10^{-6}$)	R_g[b] (nm)	A_2[c] ($\times 10^4$)
XG1	130°C for 20 min	2.75	2.75	119	1.5
XG2	Same sample after 24 h		Scattering essentially unchanged		
G3	Repeat of XG1	2.58	2.53	112	0.4
XG4	130°C for 120 min	2.75	2.75	123	3.1
XG5	160°C for 20 min	2.72	2.72	114	1.9
Mean			2.69	117	
±Standard deviation			0.08	4.3	

[a] MW_{W1} and MW_{W2} correspond to the zero concentration and zero angle extrapolations of the Berry plot.
[b] R_g = z-average root mean square radius of gyration.
[c] A_2 = second virial coefficient in units of reciprocal concentration, viz. mol ml/g.
XG = xyloglucan.

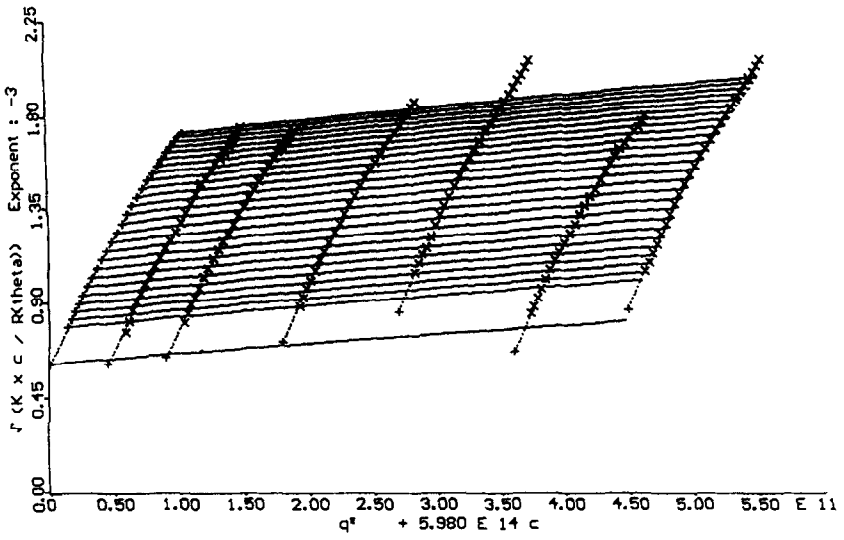

Fig. 7. Berry plot for sample XG5. The vertical shifts of some of the experimental data reflect slight concentration errors.

Intrinsic viscosity of heated samples

In order to confirm our supposition that no macromolecular changes had been induced by the pressure heating process, two subsequent experiments were carried out. Firstly, one of the freeze dried samples from Freiburg was remeasured in London. It was noticeable how readily this sample dissolved compared to the untreated polymer. A further heat and pressure experiment was also carried out in London, although this had to be carried out using a laboratory autoclave (121°C, 15 bar total pressure) rather than the specialist pressure cell. The $[\eta]$ values obtained for the sample after 20 min heating under 121°C autoclaved and for the rehydrated sample originally heated to 130°C in the pressure cell they were 9.3 dl/g and 8.4 dl/g, respectively. Although the latter value was a little lower than that seen for the original untreated sample, 8.9 dl/g, this in no way suggested a significant decrease due to depolymerisation. Instead, the data are very consistent with the influence and removal of a small number fraction (but large weight and *z*-average fraction) of supramolecular aggregates on the $[\eta]$ of the non-heat-treated samples.

DISCUSSION

The present discussion would be extremely short if it were not for the light scattering data. All the semi-dilute solution characterisation work (Fig. 2) seems to be largely consistent with much of the other data in the literature for the rheology of polysaccharide solutions. Nevertheless, the quality of the data are, in our view, very impressive. Figure 3 illustrates the expected variation of viscosity with dimensionless overlap, with values of the two slopes quite consistent with most other published data. The shear rate dependence of viscosity at different concentrations (Fig. 2), and the dynamic mechanical data are all completely normal (Fig. 5), the linear viscoelastic strain region is quite pronounced (Fig. 4), and there is very strong evidence for Cox–Merz superposition of η and η^* at corresponding shear rates and oscillatory frequencies, even at relatively high concentrations (Fig. 6). All of this suggests that detarium gum is a well behaved linear polymer entanglement network system (Morris *et al.*, 1981; Robinson *et al.*, 1982; Richardson and Ross-Murphy, 1987a, b; Ross-Murphy, 1984, 1994; Lapasin *et al.*, 1995). However, the molecular weight and angular dependence of scattering data suggest that this conclusion is partly illusory.

In our earlier paper (Wang *et al.*, 1996), we suggested that on the basis of a comparison of the intrinsic viscosity of the present sample with tamarind gum, the measured molecular weight for tamarind (Gidley *et al.*, 1991) and the bounds of the Mark Houwink equation for linear flexible macromolecules (0.5–0.8), the MW_w for detarium gum should lie in the (albeit generously bounded) range $1.3–1.9\times10^6$. However, the measured MW_w is very significantly larger than this (about two times greater). There are several potential explanations for this discrepancy, of which one at least is the questionable reliability of the tamarind gum results. These data (Lang and Burchard, 1993), for which one of the present authors was a coauthor, were themselves subject to significant sample-to-sample and day-to-day fluctuations, due mainly to very pronounced time-dependent aggregation effects. Another explanation is that the detarium gum is not an essentially linear macromolecule, but is instead slightly branched. This would lower the Mark Houwink exponent and so increase the estimated value of MW_w.

Because of the high quality of the zero concentration light scattering data it should be possible to analyse this and examine the shape of the macromolecule. This

approach, although widely employed for synthetic macromolecules using small angle X-ray scattering (or, for high molecular weight samples, light scattering) can be applied very rarely for light scattering of polysaccharides, because the overall data are not generally reliable, nor are the molecular weights high enough. Indeed, successful application of this approach (Burchard, 1994) is limited, for example, to amylopectin and glycogen, which are both of extremely high MW_w ($>2\times10^7$), or to very stiff macromolecules such as xanthan. As far as we are aware, this procedure has never previously been applied successfully to any of the galactomannan, glucomannan or xyloglucan series of polysaccharides.

Figure 8 shows just such a procedure, using a Kratky plot of $u^2P(u)$ vs. u. Here $u=qR_g$, $P(u)\equiv R_\theta/R_{\theta=0}$ is the so-called particle scattering factor, which reflects the angular dependence of the scattered light, and q is the scattering vector ($=4\pi\lambda/\sin(\theta/2)$). The parameter u, which is dimensionless, measures the intramolecular probe distance relative to the incident light wavelength, and $P(u)$ can be calculated for different chain architectures. The data from different experiments are in very good agreement (this is a particularly testing strategy) and show that at wide angles (high u) they reach an apparent plateau, where $u^2P(u)$ is *c.* 1.5.

The overall grouping of data lies much below the expected profile for a Gaussian (flexible) chain, which itself lies below that for semiflexible chains or rods (not illustrated in Fig. 8). The data lie above the curve for a very high degree of random homogeneous branching, and qualitatively, at least, resemble the traces calculated for low degree (three or four arm) star-branched macromolecules (also not shown in Fig. 8). Such a model cannot be taken too literally without including the effects of polydispersity, but what can be stated unequivocally is that the scattering profile for detarium gum is not consistent with that of a linear macromolecule, but instead strongly suggests a small element of long chain branching. The level of such branching need not be very great, say perhaps 2–10 branch points per chain. This is much below the level detectable biochemically (J.S.G. Reid, personal communication), but is quite sufficient to affect the overall chain profile. For example, at the same MW, a single tetrafunctional branch point could halve the radius of gyration (Zimm and Stockmayer, 1949).

By analogy with the entanglement behaviour of branched polymer melts (Evans and Edwards, 1981), the implications of this conclusion for the rheological data are that we might expect to see, at very high concentrations, a more pronounced slope in the η_{sp} vs. $c[\eta]$ trace. Examination of Fig. 3 does not reveal this, however, although the breakpoint value of $c[\eta]$ is rather low at ~2 (the corresponding value for most other polysaccharides is ~4; Morris *et al.*, 1981). Such a high slope value has been observed in an apparently very highly branched polysaccharide from bacterial levan (Kasapis *et al.*, 1994). However, the exponential increase in η_0 suggested from theory, and sometimes observed (Ball and McLeish, 1989) for star-branched polymer melts does not seem to be as obvious when considering solution behaviour.

One additional remark needs to be made. How can we be sure that the branching observed is not a consequence of either entanglements or some noncovalent interaction? For example, recent work by Goycoolea *et al.* (1995) has examined the effect of alkali treatment of polysaccharides, with a consequent reduction in $[\eta]$. Although alkali was used, in other respects the temperature regime was far less rigorous than employed in the present work. We feel that almost all non-covalent interactions would have been disrupted by the process used in the present work. Moreover, the time and heat treatment independence also argues against the presence of non-covalent bonds.

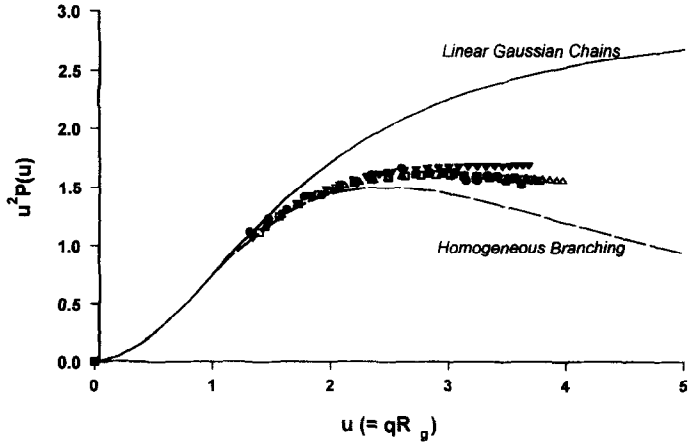

Fig. 8. Kratky plot for detarium gum samples. Theoretical curves calculated for models as in Burchard (1994). The upper line is for homodisperse linear chains, since both polydispersity and chain stiffness tend to increase the slope of this curve. Experimental points for samples XG1 (□), XG3 (•), XG4 (△), XG5 (▲).

Finally, we need to consider the longer term implications for the branched chain model. The assumption is made for a number of polysaccharides that these are linear macromolecules, although the positive evidence for this is nearly always absent. Biochemically it may even be simpler for plants to synthesise slightly branched macromolecules rather than linear chains, and the scattering part of this work, at least, suggests that there is a considerable future in re-examining the solution behaviour of a wide range of polysaccharides, employing the pressure–temperature method to create time-stable molecular solutions. The use of the method to produce more readily rehydratable materials is also worth examining.

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