Pressure Cell Assisted Solution Characterization of Polysaccharides. 1. Guar Gum

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To reduce time-dependent aggregation phenomena and achieve true "molecular" solution, the "pressure cell" solubilization method of Vorwerg and co-workers was applied to solutions of guar galactomannans (three samples of different molecular weights), using various heating, time, and pressure profiles. Physicochemical characterization of the guar samples before and after pressure cell treatment included measurements of intrinsic viscosity [η] by capillary viscometry and M_w and radius of gyration from size exclusion chromatography coupled to multiangle laser light scattering (SEC/MALLS). Heating the guar solutions (100−160 °C) without pressurization produced chain degradation with [η] and M_w values being reduced significantly, whereas this effect was reduced substantially for samples subject to initial pressurization (∼5−10 bar). The constants in the Mark−Houwink−Sakurada equation, relating [η] and M_w were established and the characteristic ratio C_∞ and chain persistence length L_p were calculated using both the Burchard−Stockmayer−Fixman (BSF) method for flexible and semiflexible chains and the Hearst method more appropriate for stiffened chains. Definitive conclusions can now be drawn on the flexibility of the guar chain backbone, with $L_p \approx 4$ nm from the BSF plot, in good agreement with previously published work using such geometric methods. This contrasts with the higher values obtained from extrapolation of data for polyelectrolytes with a similar backbone geometry, such as sodium carboxymethyl cellulose, to "infinite" ionic strength.

Introduction

Galactomannans, those polysaccharides with a polymannose backbone and galactose side-chain substituents, are important from the viewpoint of applications because evidence shows their chains are semiflexible, and so they have a high intrinsic viscosity [η] for a given molecular weight (relative molecular mass). This, in turn, means that they typically form fairly viscous aqueous solutions at concentrations around 1% w/w. The most familiar of the galactomannans are guar, which usually has ∼40% galactose side-chains, and locust bean gum (LBG), containing ∼20−23% galactose. Both are employed in the pharmaceutical, food, and chemical industries, as water-soluble "thickeners", predominantly for the reason given above.

Macromolecular characterization of polymers by light-scattering methods, normally a most powerful and flexible tool, is limited somewhat for both starch polymers and nonstarch polysaccharides (NSPs) by their tendency not to form true solutions, i.e., not to completely hydrate. Consequently in the case of guar many "solutions" are, in fact, mixtures (dispersions) of essentially undissolved (at the molecular level) material in a solution of partly and fully dissolved galactomannan macromolecules. More explicitly, unless precautions are taken, all such solutions contain, as well as the individual molecules, small numbers of supramolecular particles that form clusters, whose size increases with time. As discussed in detail by Kratochvil[1] and others[2] these contribute very little to, say [η] because the very large aggregates are usually compact (i.e. pseudo-spherical). Further, for these MW distributions, [η] is often closer to a number average. Such aggregates, if present in the measured solution, will strongly affect light-scattering moments (weight and *z*-average); they also affect semidilute solution rheological properties, including normal stresses, which depend on higher moments of the MW distribution.

In particular, a nonhydrated "solution" can cause distortions in the angular dependence of scattered light and thus lead to errors in the determination in the intercept on the scattering intensity (Kc/R_θ) axis, leading to an overestimate of molecular weight.[1] The work of Vorwerg and co-workers on starches,[3−5] employing a pressure/heat ("pressure cell") treatment of the starch solution, suggested an approach that helps to eliminate such problems. They subjected dilute solutions of starch to high temperatures under an applied pressure of nitrogen gas. True "molecular" solution was achieved, and time-dependent aggregation phenomena were substantially reduced. The approach was subsequently applied successfully to solutions of a novel xyloglucan, detarium gum.[6]

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This paper is the first of a series which will investigate a number of NSPs using the pressure cell technique and the techniques of dilute and semidilute solution rheology, SEC/MALLS, and conventional static and dynamic light scattering. In the present paper, we consider the application of some of these approaches to guar galactomannan solutions.

Guar solutions have been quite well studied, and following work in the 1980s, galactomannans are generally accepted to be random coil semiflexible polymers, and, *under appropriate controlled conditions*, to form nonassociative entanglement solutions. We add the italicizing deliberately, because it is the case that both the chain flexibility and the nature of the semidilute solution rheology are still not fully appreciated. Early work,[7,8] including that by Robinson and co-workers,[2] established the so-called Mark−Houwink parameters. The latter paper also obtained an estimate of the chain characteristic ratio (and so the persistence length) which was consistent with a cellulose-like backbone. In the Robinson paper, the problem of preparing macromolecular solutions was discussed in some depth. These data have been widely cited and appear to be generally accepted. In the same paper, they also showed entanglement-like semidilute solution viscoelasticity. Subsequent work extended the characterization to nonlinear viscoelastic measurements.[9] In the intervening years, there have been quite a few other papers,[10−12] but the majority appear to have accepted the tenets of the Robinson publication.

As far as LBG solutions are concerned, it has been known since the classic review of Dea and Morrison,[13] that the lower galactose content makes these even harder to solubilize (polymannose itself is essentially water insoluble) and the reduction in galactose content apparently makes water a progressively poorer solvent. Indeed work by Richardson and co-workers[14,15] has studied kinetic aspects of the association of semidilute LBG solutions and shown that there is a substantial increase in the low-frequency extent of the G' plateau, with time. They regard this as a gelation effect although, in view of the comments above, it seems unlikely to be more than the expected increase in the extent of associative polymer behavior with time, rather than gelation per se. In this case, creep measurements may prove interesting in helping establish whether long-time flow, at effective times longer than, e.g., 100 s, occurs, and if it can be separated from the time-scale of (re-) association.

The recent paper by Wientjes and co-workers[12] has reinvestigated the linear viscoelastic behavior of aqueous (including buffered) guar solutions. They claim that exponents in the log(viscosity) vs log(concentration) plots are above expected values (typically 4 ± 0.5), up to $\sim$5.3. They also describe other deviations from "simple" entanglement behavior and derive a phenomenological "sticker" type model to rationalize their observations.

Similar high values of the viscosity exponent were given in the early paper by Morris and co-workers,[16] and attributed to "hyperentanglements". However, the slightly lower exponents reported by the same group the following year[2] are consistent with a more careful consideration of the nature of the guar "solutions". Indeed the various (albeit small) deviations reported by Wientjes et al.[12] are quite understandable in terms of the contribution of a small volume fraction of aggregated "particles" to the solution (dispersion).[17,18] Certainly the hydration regime employed by these workers, stirring for extended periods at 4 °C, is known to be inadequate.[19] This conclusion also explains the over large Mark−Houwink exponent ($\sim$1.05) they describe, presumably due to the distortion of light scattering by these particles. This value is much above the known value for a high molecular weight polymer chain, 0.5−0.8, and quite incompatible with the known chain conformation for mannan backbone polymers. Previously published and well-accepted estimates for this exponent for guar galactomannan are typically $\sim$0.7, a result reiterated in the present paper.

Here we describe our study of the effect of pressure cell treatment on guar solutions, employing different pressure and temperature conditions to maximize the solubilization, without degrading the polymer chain length.[20] Finally, we have adopted a self-consistent approach, which together with past published data allows definitive conclusions on the persistence length (flexibility) of the guar chain backbone to be reached.

Materials and Methods

Purified samples from commercial food grade guar gum flours of different average molecular weight M30, M90, and M150 (Meyprogat range, Meyhall Chemicals, A.G., Kreuglingen, Switzerland−Rhodia Group) were used in all experiments. The samples were purified from the flour using an isolation procedure devised by Girhammar and Nair[21] and modified by Rayment et al.[17] to allow complete hydration of the guar gum sample. The moisture content of the extracted polymer was determined by incubation overnight in an oven at 103 °C to a constant weight. Freeze-drying was carried out using an Ehrist ALPHA I-5 Freeze-dryer (DAMON/IEC (U.K.) Ltd.), and samples stored in a desiccator until used.

Sugar Analysis. the galactose/mannose ratio and other saccharide components in the purified guar samples were determined using a gas chromatographic method with alditol-acetate derivatized samples after the method of Englyst and Cummings.[22]

Solutions were prepared at 0.05 wt % by adding known weights of the freeze-dried samples to deionized water at 50 °C, containing 0.02% sodium azide. The temperature was raised to 80 °C as dry powder was added with stirring. The heating was stopped at 80 °C, and the solutions were left covered overnight, with stirring, at room temperature to allow hydration to occur.[19] The solution at this stage was used as reference material ("untreated sample"). Then 30 mL of this solution was added to the reaction chamber of a pressure/heating cell (HEL Ltd, Barnet, Herts., U.K.). These solutions were then subjected, under stirring, to a range of temperature and pressure conditions between 70 and 160 °C and 0−12 bar added pressure. Added pressure was applied using nitrogen gas while the reaction chamber was at a temperature of 50 °C. These conditions were applied for a range of times from 10 min to 2 h.

The treated solutions were then analyzed in various ways.

Table 1. Results from Alditol−Acetate Derivative Analysis of Guar Samples, Where Results Are Expressed as Sugars (mg/g) in the Hydrolysate and Are Uncorrected for Hydrolysis

sample	arabinose	mannose	galactose	gal/man	% galactose
M 30	12 ± 0.4	671 ± 20	430 ± 13	0.64 ± 0.02	39 ± 1
M 90	9 ± 0.3	663 ± 20	435 ± 13	0.66 ± 0.02	40 ± 1
M 150	10 ± 0.3	683 ± 20	449 ± 13	0.66 ± 0.02	40 ± 1

1. Capillary viscometry: The intrinsic viscosity [η] was determined using the Viscosity Measuring Unit AVS 350 (Schott-Geräte, Hoffheim, Germany), connected to the ViscoDoser AVS 20 piston buret (for automatic dilutions), to make automated measurements of the flow-through times in a capillary viscometer (Ubbelholde viscometer for dilution sequences). The viscometer was immersed in a precision water bath (transparent thermostat CT 1650, Schott-Geräte, Hoffheim, Germany) to maintain the temperature at 25 ± 0.1 °C. Results were analyzed using separate Huggins and Kramer extrapolations (linear regression, 99% confidence intervals) and the final result quoted in dL/g (1 dL/g = 100 mL/g = 0.1 m^3/kg).

2. Size exclusion chromatography coupled to multiangle laser light scattering (SEC/MALLS): The size exclusion chromatography system used consisted of a Jasco HPLC pump, a guard column, and TSK G5000 and G4000 columns. An on-line degasser was used to remove gas from the eluent. A flow rate of 0.8 mL/min for the mobile phase was used at room temperature. A DAWN-DSP multiangle laser light-scattering detector and an Optilab 903 refractometer (Wyatt Technologies, Santa Barbara, CA) were used for light-scattering intensity and concentration detection, respectively. The mobile phase was 0.02 wt % sodium azide in distilled de-ionized water. 100μL samples of the guar solutions were injected into the size exclusion system after filtering through 0.45μm filters (Whatman). Repeat injections were made for each sample. Data were captured and analyzed using the software package ASTRA (v. 4.20). Data returned are number, weight, and *z*-averages for molecular weight and root-mean-square radius of gyration.

Results

Sugar Analysis. The results for the three guar samples are shown in Table 1. For the three samples the average ratio of galactose to mannose is 0.65 ± 0.02, thus raising the effective molecular weight per backbone residue from 162 for unsubstituted mannan to 267 (162 × 1.65). There is basically no difference in the percentage of galactose present in the three samples (39−40%) and the only other saccharide detectable is a very small percentage of arabinose.

Pressure Cell Treatment and [η]. From intrinsic viscosity, it is possible to follow the effects of different factors such as temperature of heat treatment, effect of added pressure and the time duration of heat treatment. The results are presented in Figures 1−3. Figure 1 shows for guar M150 that an increase in the treatment temperature from 70 to 160 °C, under no added pressure and during a 10 min period of time reduces the intrinsic viscosity [η] significantly. The

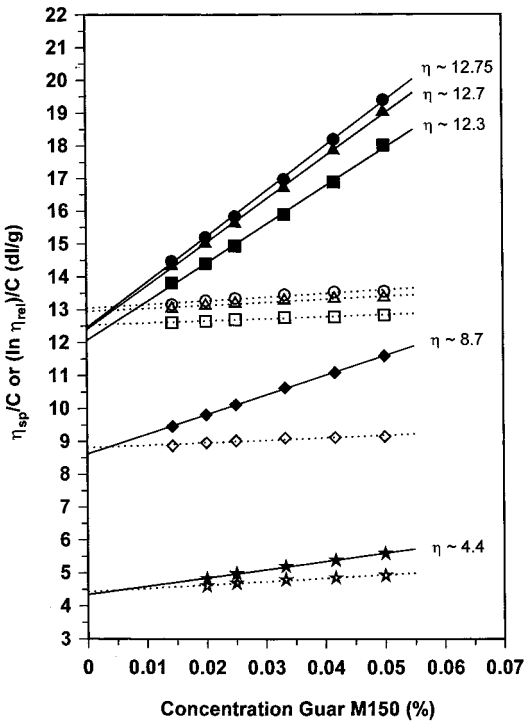

Figure 1. Intrinsic viscosity of guar M150 measured at 25 °C, estimated from plots η_{sp}/C vs C (%) (solid symbols) and (ln η_{rel})/C vs C (%) (open symbols). The samples are pressure cell treated during 10 min at (●) 70 °C, (■) 100 °C, (◆) 130 °C, and (★) 160 °C. The data for the untreated sample are represented by (▲).

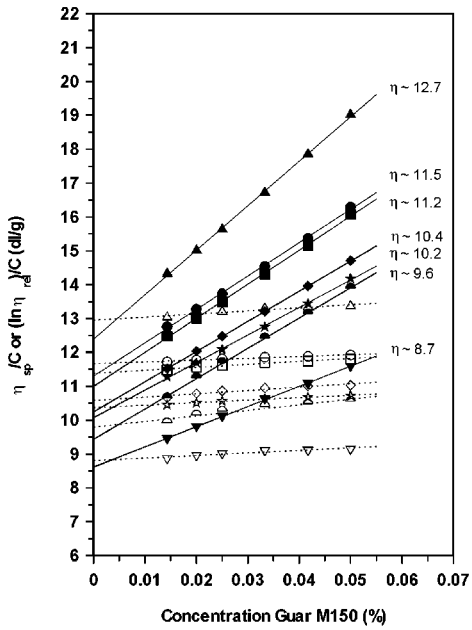

Figure 2. Intrinsic viscosity of guar M150 measured at 25 °C, estimated from plots η_{sp}/C vs C (%) (solid symbols) and (ln η_{rel})/C vs C (%) (open symbols). The samples are pressure cell treated at 130 °C during 10 min under (▼) 0, (half circle) 2, (★) 3, (◆) 5, (■) 8, and (●) 12 bar. The data for the untreated sample are represented by (▲).

values of [η] for the untreated sample and for the samples treated at a temperature below 100 °C are not significantly different (lying in the [η] error range, here ± 0.6 dL/g). Figure 2 illustrates for guar M150 the effect of pressure treatment (from 0 to 12 bar nitrogen, added pressure) on values of [η] at a fixed temperature (130 °C is chosen

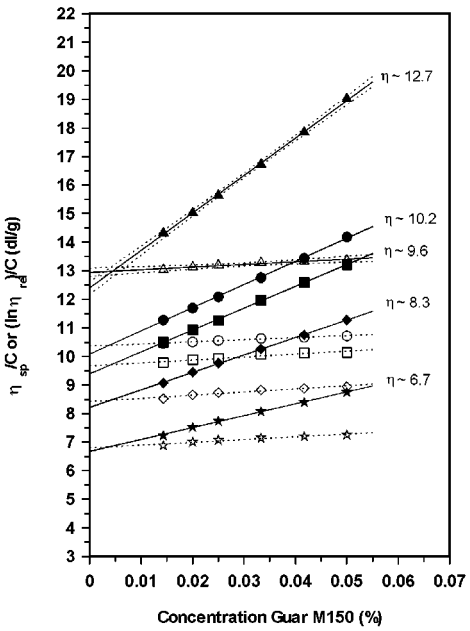

Figure 3. Intrinsic viscosity of guar M150 measured at 25 °C, estimated from plots η_{sp}/C vs C (%) (solid symbols) and (ln η_{rel})/C vs C (%) (open symbols). The samples are pressure cell treated at 130 °C under 3 bar, during (●) 10, (■) 30, (◆) 60, and (★) 120 min. The data for the untreated sample are represented by (▲).

because a significant reduction in $[\eta]$ is seen in the absence of pressure) and fixed time (10 min). From this, it clearly appears that pressure influences the intrinsic viscosity. Heating for a longer period of time (0 to 120 min) at a fixed significant temperature (130 °C, where chain degradation seems to occur), under 3 bar nitrogen is illustrated in Figure 3 and shows that the $[\eta]$ of guar M150 sharply decreases with time. Similar observations on $[\eta]$ have been reported for guar M90 and guar M30 (figures not presented here) when subjected to temperature and time variations. These data suggest that, within those we have applied, stable results, which we attribute to the best dissolution conditions, correspond to ∼130 °C, ∼4−12 bar pressure, and heating times of 10−40 min.

Parts a and b of Figure 4 summarize the behavior of $[\eta]$ for the three samples of guar studied in terms of temperature and time treatment. As can be seen from these figures, an increase in temperature, and in time, produces a decrease in $[\eta]$ for all non-pressure-modified samples.

Light Scattering. From light scattering, M_w (weight-average molecular weight) and R_g (z-average root-mean-square radius of gyration) were obtained. Parts a and b of Figure 5 show typical examples of the SEC−MALLS chromatograms obtained, in terms of cumulative weight fraction vs measured M_w. The M_w/M_n polydispersity obtained from the M_w distributions for all of the samples and treatments lie mainly in the range 1.3−2.8 (a Flory, or most probable distribution would give a value of 2.0). The value for untreated samples was, in fact, typically narrower than for those that had undergone pressure cell treatment as shown in Figure 5. However, this is an artificial result, because the guard column removes much of the highly aggregated, but small in terms of number fraction, material seen in "classical" light scattering of guar solutions.[2] The overall percentage recovery, deduced from the RI concentration detector system,

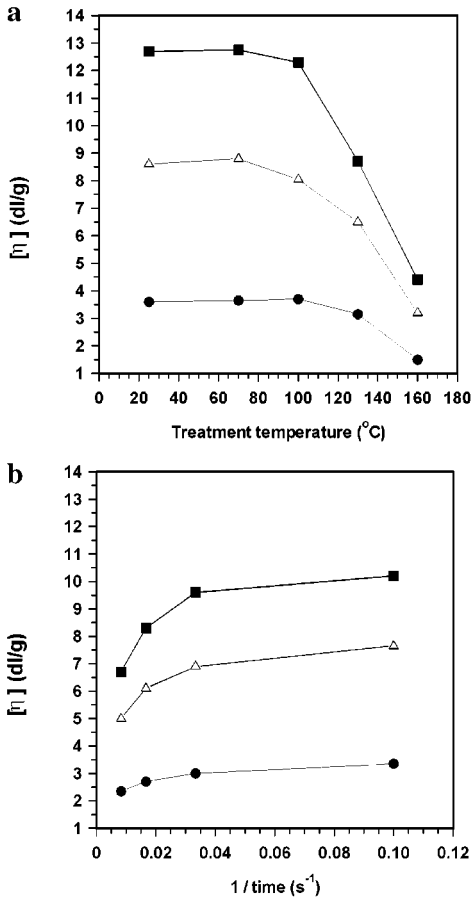

Figure 4. (a) Behavior of the intrinsic viscosity $[\eta]$ (measured at 25 °C) with respect to treatment temperature in the absence of pressure for (■) guar M150, (△) guar M90, and (●) guar M30. (b) Behavior of the intrinsic viscosity $[\eta]$ (measured at 25 °C) with respect to the effect of time (plotted reciprocally) at 130 °C under 3 bar of nitrogen for (■) guar M150, (△) guar M90, and (●) guar M30.

was typically ∼80−90%. We expected a rather higher percentage recovery for the pressure and/or heat-treated systems, and in some, but by no means all, cases this is seen. Overall, however, there was no real pattern with regard to this parameter; we discuss the measured M_w (and concomitant R_g distributions) in more detail below. Figure 6 shows the Mark−Houwink−Sakurada plot[23] (log $[\eta]$ vs log M_w) for our experimental data on the three samples of guar subjected to various heat/pressure/time treatments. The values of R_g are also plotted as a function of the molecular weight in Figure 7. Figure 8 illustrates the Burchard−Stockmayer−Fixman plot $[\eta]/M_w^{1/2}$ vs $M_w^{1/2}$ for all the data. The intercept enables calculation of the chain characteristic ratio and consequently the chain persistence length for guar galactomannan. Figure 9 shows the corresponding Hearst plot. This assumes $[\eta] \propto M_w$, which is only true for very stiff chains.

Discussion

Significance of $[\eta]$ Values. The purpose of the pressure heating process is, of course, to disrupt the nonspecific aggregation of polymer chains, which commonly occurs when they are dispersed in aqueous solvents. We make the comment "nonspecific" here, because there is some evidence, for both LBG and guar, that treatment with alkali can

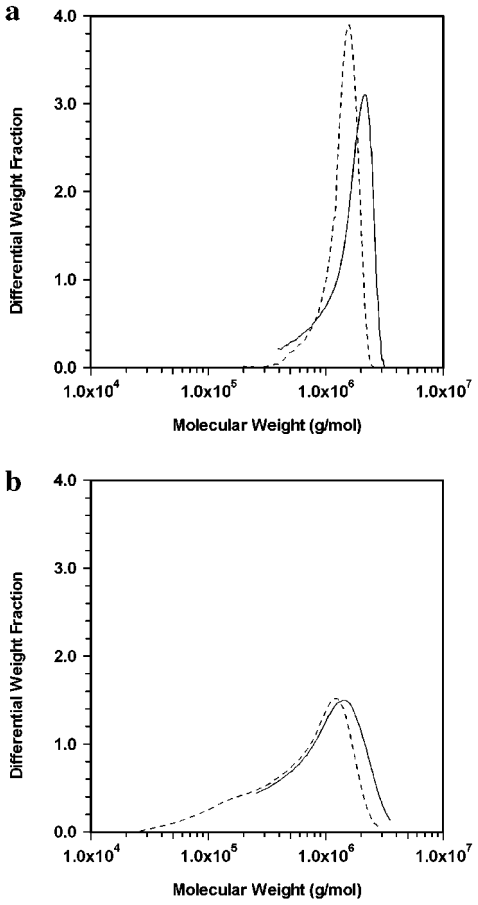

Figure 5. (a) Chromatograms representing the differential weight fraction vs M_w for guar M150 untreated (solid line) and guar M150 treated at 70 °C for 10 min (dotted line). (b) Chromatograms representing the differential weight fraction vs M_w for guar M150 treated at 130 °C for 10 min (solid line) and guar M150 treated at 130 °C for 10 min under 4 bar (dotted line).

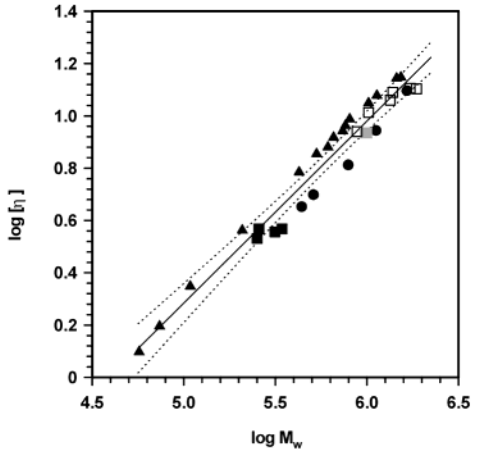

Figure 6. Mark−Houwink−Sakurada plot (log [η] vs log M_w) for various guar samples treated under different conditions; (●) Robinson et al.[2] data, (▲) Beer et al.[11] data and square symbols for this work ((□) M150, (gray box) M90, and (■) M30). The α parameter is 0.70 ± 0.03. Dotted lines indicate 99% confidence intervals.

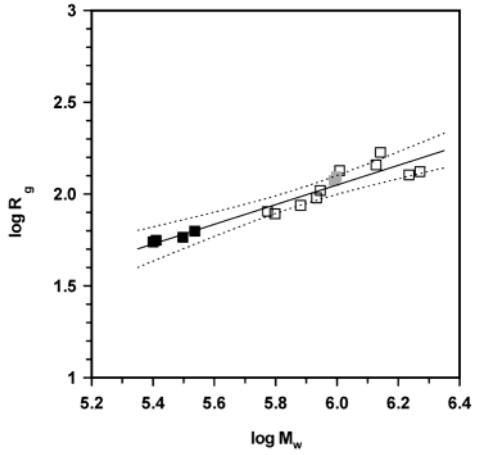

Figure 7. Values of R_g plotted as a function of the molecular weight for the data of the present work ((□) M150, (gray box) M90, (■) M30). The Flory exponent is 0.54 ± 0.05. Dotted lines indicate 99% confidence intervals.

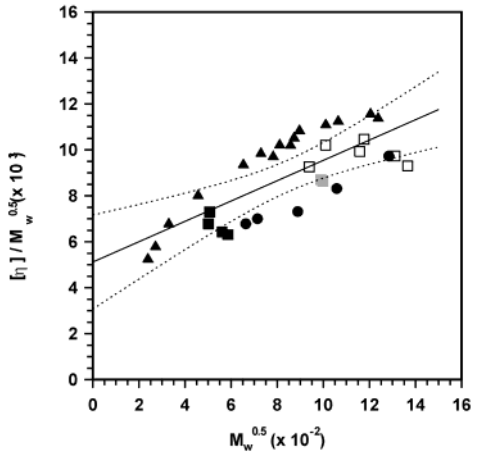

Figure 8. Burchard−Stockmayer−Fixman plot; [η]/$M_w^{1/2}$ vs $M_w^{1/2}$, all data as in Figure 6 with the same symbols. Dotted lines indicate 99% confidence intervals.

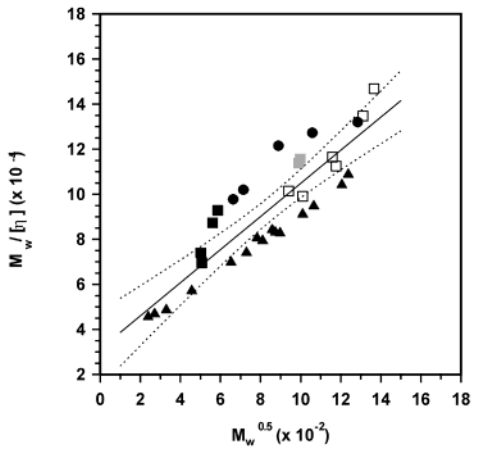

Figure 9. Hearst plot; M_w/[η] vs $M_w^{1/2}$, data as in Figure 6. The slope of this plot is $1/K_\theta$, so that K_θ is 1.36×10^{-3} m³·kg^{-1}. This gives $C_\infty \sim 28.2 \pm 1.8$ and $L_p \sim 7.6 \pm 1.2$ nm.

significantly lower the value of [η]. Work by Goycoolea and co-workers[24] at pH10 reduced the intrinsic viscosity of guar by ~5% (from 12.5 to 11.9 dL/g) and of LBG from 12.1 to 5.2 dL/g (~60%), values which were recovered on neutralization. Unfortunately their interpretation simply in terms of disruption of specific chain/chain associations may place too much emphasis on measurements of [η] alone. Our own view is that only by using a combination of an absolute determination of M_w, such as static light scattering, and [η]—as used in all classical polymer characterization work—can unequivocal conclusions be obtained.

Therein lies one limitation of the present approach—albeit one that we hope we have given due consideration. There is good evidence in the literature, particularly from Mitchell and co-workers,[25] that high temperatures can cleave the galactomannan backbone, producing a drastic reduction in M_w. This effect has to be controlled, or at least distinguished, from the process we wish to carry out, i.e., the disruption of the nonmolecularly dispersed aggregates to produce a "molecular" solution of chains, without concomitant degradation of these chains. The ideal result, therefore, is that we produce a significant decrease in the measured M_w, without a large decrease in $[\eta]$. This is because, as has been known for many years, and as repeated above, the number of aggregates is usually quite small, but their weight average contribution is very large. Consequently they contribute very significantly to the (low angle) light-scattering intensity, thus giving a falsely high apparent M_w, but alter $[\eta]$ far less, so its value typically reflects something between a number and weight average moment of the MW distribution.

The purpose then of the applied overpressure (that is the initial pressure applied, which is over and above the vapor pressure exerted by the solution in the sealed cell at temperatures above 100 °C) is apparently to protect the chain from some of the degradation effects.

The discussion is complicated by the fact that there is still no absolute agreement as to the value of various significant exponents in the dilute solution characterization of galactomannans and indeed some workers[12] still seem quite unaware of their significance. Of course the same applies to other water-soluble polysaccharides, and sadly too many totally improbable values of the mean square radius of gyration vs M_w exponent have appeared in the literature. We remind the reader that for a flexible random coil linear polymer, this exponent *must* lie between 0.5 (the Gaussian chain value) and 0.6, the corresponding exponent for an excluded volume chain. Indeed as we noted above, lower than physical exponents (~0.4) were obtained in some of the present measurements of individual fractions using SEC−MALLS; because of this, we have less faith in the elements making up the M_w distribution, than in its overall moments. Our rationale for this, is 2-fold, viz. that the latter, by contrast, give physically realistic and meaningful results, which also agree well with other literature data, including that measured[2] under different conditions, and a different light-scattering technique.

In the early literature (and indeed still in excellent work today),[26] the approach is adopted of chemically derivatizing the polysaccharide so that measurements can be carried out in nonaqueous solvents, where aggregation effects will be far less problematic. Such work by Kajiwara and co-workers (personal communication, unpublished) has recently confirmed the branched structure of the detarium gum polysaccharide (a xyloglucan similar to tamarind) which we first reported using static light scattering following pressure cell heating.[6] The difficulty of this approach is first that it is very time-consuming and requires special expertise and second that there is always a problem that the conditions involved in the derivatization step may themselves degrade the chain length. In this respect, the pressure cell route is both easier and cleaner.

Pressure Heating Process. Figures 1 and 2 illustrate the competition between an increase in temperature and an increase in pressure on values of $[\eta]$ following pressure cell treatment. Figure 1, for the highest M_w sample guar M150, clearly shows that measurements at 160 and 130 °C (following the time profile described in Methods) have produced a significant reduction in $[\eta]$, which strongly suggest the chain has been degraded. In contrast, Figure 2 shows by prepressuring the cell to different amounts, the reduction, which in the absence of overpressure is around 30%, from 12.7 to 8.7 dL/g, is substantially overcome, so that at 12 bar, it is around 10% (11.5 dL/g). What mechanism is involved we can only speculate—whether the pressure reduces agitation by raising the system pressure relative to the solution vapor pressure or whether other processes are involved. Certainly any of the anticipated oxygen/free radical induced degradation processes[27] would be enhanced by an increase in pressure. Unpublished preliminary experiments using either free radical scavengers or pressurizing under air and/or nitrogen showed little effect. Kinetic factors also, as we might expect, apply. Figure 3 shows the effect of heating for longer time at the same temperature and pressure. Clearly if the pressure cell process is to be employed in other systems, such preliminary experiments will be crucial. Interestingly the *expected* (simple kinetic) effect of increasing pressure would be to degrade the polymer faster, quite the opposite of what is seen.

The corresponding results (temperature and time variations) for the medium and low M_w samples, M90 and M30 follow the same general observations reported above for M150, as summarized in Figure 4, parts a and b. These show the behavior of $[\eta]$ with respect to (a) treatment temperature in the absence of pressure and (b) the effect of time (plotted reciprocally) under 3 bar of nitrogen at a fixed temperature.

Excellent though these data do appear to be, they do not, as pointed out above, help to decide if the reduction in $[\eta]$ is simply due to chain degradation, disaggregation, which is unlikely in view of the changes shown, or a combination of the two. This illustrates the limitation of measuring $[\eta]$ alone. However, by combining these data with the appropriate averages from SEC/MALLS, a clearer picture appears.

$[\eta]$ and Light Scattering Data. Table 2 shows the results of the latter experiments. It appears there is some reduction in M_w under almost all conditions but, more significantly, the initial change in M_w is generally rather larger than the corresponding reduction in $[\eta]$. We return to this point later. Before that we must consider how best to illustrate all of the data: since we have results for $[\eta]$ and M_w, we can plot these in the form of a (Staudinger−) Mark−Houwink−Sakurada plot of log $[\eta]$ vs log M_w, and they are shown in Figure 6, as the square symbols. The data show two main features. First the results for each sample are bunched reasonably closely, but show behavior as noted above, in which there is an initial approximately horizontal region extending over a factor of ~3 in M_w, where no obvious reduction in $[\eta]$ is seen. Second this is followed by a more obvious decrease in both M_w and $[\eta]$. We identify the first

Table 2. Summary of Intrinsic Viscosity [η] and Static Light Scattering Results

sample	treatment	[η] (dL/g)	M_w ($\times 10^{-6}$) (g/mol)	R_g[a] (nm)
Guar M 150	none	12.7	1.87	133
	70 °C, 10 min	12.75	1.72	127
	100 °C, 10 min	12.3	1.39	169
	130 °C, 10 min	8.7	0.88	105
	130 °C, 4 bar, 10 min	10.3	1.02	134
	130 °C, 12 bar, 10 min	11.5	1.34	144
	130 °C, 5 bar, 60 min		0.63	78
	160 °C, 3 bar, 10 min		0.59	80
	160 °C, 7 bar, 10 min		0.76	87
	160 °C, 8 bar, 10 min		0.86	95
Guar M 90	none	8.6	1.00	125
	100 °C, 4 bar, 10 min	8.6	0.98	118
Guar M 30	none	3.6	0.31	58
	70 °C, 10 min	3.7	0.34	63
	100 °C, 4 bar, 10 min	3.7	0.26	56
	130 °C, 5 bar, 10 min	3.4	0.25	55

[a] R_g = *z*-average root-mean-square radius of gyration.

behavior as due to disaggregation, followed by the second, chain degradation. In this respect, the double log plot reflects the behavior seen in Figures 4.

More significantly, all our data lie close to the old (arguably classical) results of Robinson et al.[2] and more recent results due to Beer and co-workers.[11] The Robinson results were collected by measurement of M_w only by extrapolating from wide-angle (>~60°) light scattering, whereas the Beer data show fractions cuts, measured using only a single (90°) light-scattering detector. Both sets of data might therefore also be criticized. Nevertheless, the agreement between all three is really quite good. It also adds to our understanding, because in making this plot, we have made the a priori assumption that different treatments can be regarded as different samples. This is true strictly only when the original sample had a random (Flory) MW distribution, and was degraded randomly, so that it retains a Flory distribution. This assumption appears to hold up quite well. Under these circumstances, we are justified to measure the slope (Mark−Houwink) exponent of all the data. This gives a value of 0.70 ± 0.03, slightly below, but within the error of the widely cited value (0.72) given by Robinson and co-workers,[2] and very close to the value from Beer.[11] We feel that there can now be little doubt that this value for guar is appropriate and can be regarded as reliable. It supports much previous work in concluding that guar behaves as a semiflexible linear polymer chain, with some excluded volume, since the exponent is closer to the excluded volume limit, 0.8, than the Flory-theta chain, 0.5, value. This conclusion is supported by Figure 7, which shows for our data the corresponding plot of log R_g (root-mean-square radius of gyration) vs log M_w; the slope here, the Flory exponent, is 0.54 ± 0.05, which lies almost exactly between the two limits 0.5 and 0.6.

These exponents appear to be in good agreement with our expectations, but the theoretical exponents are, of course, for monodisperse systems. What then is the effect of polydispersity? In principle, we could use the data from the SEC−MALLS to furnish the equivalent exponents for monodisperse fractions, but as we mentioned above, we already know that there are problems in this approach. The same applies to the alternative approach of using the ratio of M_w/M_n and the Schultz−Flory correction formulas. It is for this reason that we adopted the (otherwise less than rigorous but) pragmatic routine of comparing our data with that of Beer et al. who have such narrow fraction data. The fact that there is no *significant* difference in the separate *slopes* of Figure 6 from ourselves, Robinson,[2] and Beer,[11] albeit that the latter seem slightly greater in absolute terms, serves as a posteriori justification for our approach.

It might be also argued that it is inappropriate to include the nontreated samples in the overall data. Instead it would be better to include only those data for each sample that we consider correspond to "true" molecular solution—those treated for, e.g., 10−30 min at 130 °C and pressurized to 4−12 bar. We have performed this calculation, but it does not alter the results (statistically) significantly, *within our 99% confidence interval*, but gives slightly higher exponents, with an [η] exponent of 0.75 ± 0.04, while that for R_g is 0.58 ± 0.06 but still within the expected range.

Determination of C_∞ and Chain Persistence Length for Guar Galactomannan. With consistent and reasonable values of both the MKS and Flory exponents, we can employ one of the standard methods for determining the intrinsic chain flexibility for a polymer. The most widely employed of these is still the so-called Stockmayer−Fixman plot originally due to Burchard (sometimes referred to as a BSF plot).[23] Figure 8 shows this plot is ($[\eta]/M_w^{1/2}$ vs $M_w^{1/2}$) for all the data originally given in Figure 6. The intercept of this plot K_θ, which corresponds to the chain in the θ-state, i.e., where there is no excluded volume. Like other subsequent models, the BSF by extrapolating to zero on the $M_w^{1/2}$ axis, assumes that a very low M_w chain will have no excluded volume. K_θ may be related directly to the parameter C_∞, the chain characteristic ratio. This can, in turn, be used to calculate the Kuhn length, l_k, (sometimes referred to as *b*) the segment length of the equivalent random flight chain and thus, the chain persistence length, L_p (sometimes q).

From the Mark−Houwink−Sakurada equation

$$[\eta] = K' M_w^{\alpha} \tag{1}$$

and the Flory−Fox equation

$$[\eta] = \phi L^3 / M_w \tag{2}$$

when L is given by the real chain θ-state model, this gives

$$[\eta] = \phi l^3 (C_\infty/m)^{3/2} M_w^{1/2} = K_\theta M_w^{1/2} \tag{3}$$

Thus the Mark−Houwink−Sakurada K' and α parameters are here $\phi(C_\infty l^2/m)^{3/2} = K_\theta$ and 0.5, respectively.

Substituting the intercept value 5.1 ± 0.6 × 10^{-4} $m^3 \cdot kg^{-1}$ for K_θ, using l = 0.54 nm and m = 267 and ϕ, the viscosity constant, ~ 2.6 × 10^{26} in SI units, in eq 3 gives C_∞ = 14.7 ± 1.2.

In the wormlike chain approximation the characteristic ratio is simply equal to $2L_p/l$. We can then obtain for the persistence length a value L_p of 4 (± 0.3) nm. These values

agree quite well with the earlier value of C_∞ from Robinson and co-workers[2] and tabulated values of L_p for a number of cellulose derivatives,[28] whose β-1,4-linked *O*-glycosidic polyglucan backbone is comparable to the polymannan backbone of guar gum.

Here typical values of L_p for cellulose and derivatives lie mainly in the range 3−6 nm, depending upon the solvent and, for derivatives, the degree of substitution. Such values, including the classic work by Henley[29] and others[30] and that of Kamide and co-workers,[31] are reported in standard works.[32,33]

However, these values do disagree significantly from the recent computations and measurements of Petkowicz and co-workers,[34] who obtained L_p of 9.3 nm for a galactomannan ratio (M/G $\sim$ 1) polysaccharide molecule, and a still higher (computed) value (14.5 nm) for the unsubstituted polymannan. Other higher values do exist in the literature, including recent data by Frollini et al.,[35] for guar and by Hoogendam et al.[36] for cellulose derivatives, which are around 2$\times$ as great as our values. However, in neither paper is there any discussion of the reasonableness of the result obtained, although the Hoogendam paper does mention the effect that counterion condensation can make on the final result.

Both of the experimental papers described above use the Skolnick−Fixman−Odijk model[37,38] (or variants of it) to extrapolate values for polyelectrolytes[39] to the intrinsic (infinite ionic strength) persistence length. This is not at all the same as the method applied here for neutral polysaccharides, and it is well-known that the two do not always (or even often) agree. Indeed in the Hoogendam paper, they quote measurements for the same system by Rinaudo, one of the authors of the Frollini paper, giving a value of $\sim$5 nm, whereas Hoogendam et al. themselves actually obtain a value of $\sim$16 nm. The discrepancy is not untypical for this (different) approach, and would suggest future work could profitably compare all the different methods for estimating (what are actually rather different definitions of) chain persistence length.

In relation to the computations of Petkowicz et al.[34] and others[40] on the characteristic ratio of β-(1,4)-linked polysaccharide chains, it is worth noting that theoretical estimates of persistence length and/or characteristic ratio are usually higher than measured values, even going back to the classical calculations of Whittington[41] in the early 1970s.

As far as our experiments are concerned, it is worth noting, however, that even here L_p will depend on the extrapolation method employed. If we use the so-called Hearst plot[42] (Figure 9) we find a value of L_p of 7.6 ($\pm$ 1.2) nm, a factor of $\sim$2 of that from the BSF plot, although still lower than found by Petkowicz, a discrepancy that has been reported previously.[28] Although the Hearst method is sometimes employed for semiflexible polymers, it is actually more appropriate for stiff polymers. Further, all these methods are, of course, sensitive to MW distribution, but not, as we suggested above, to this extent.

Conclusion

The technique of pressure cell heating has been successfully applied to solutions of three purified guar samples of different molecular weights. A range of temperatures, pressures, and times has been used in order to achieve true molecular solution by reducing time-dependent aggregation phenomena. Both capillary viscometry and light-scattering techniques have enabled us to draw, together with past-published work, definitive conclusions on the flexibility of the guar chain backbone. Future work will involve the use of the pressure cell technique as an aid to characterize solutions of various nonstarch polysaccharides (NSPs).

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