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Effect of gamma irradiation on the macromolecular integrity of guar gum

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Abstract

Irradiation is becoming an increasingly important method of processing foodstuffs. Our understanding of its effects on the molecular integrity of food polysaccharides such as guar gum is however still very limited. In this study a range of techniques was used to determine absolute weight-average molecular weights and conformational parameters for a homologous series of irradiated guar gum samples. It was found that molar masses and viscosities of the irradiated samples decreased with increasing irradiation dose. However, this decrease was not linear but appeared to occur in two stages. Mark–Houwink parameters confirmed that there was no significant change in the gross conformation due to irradiation. Evaluation of the radiation yield through so-called $G_{(\text{scission})}$ values gave an average value of 3.8 which is lower than expected for polysaccharides indicating some resistance to radiolytic degradation. The amount of guar gum recovered after sample work-up increased with increasing radiation dose. This suggests concentrated solution properties of guar gum are significantly influenced by non-hydrated/undissolved material.

Keywords: Gamma irradiation; Food industry; Guar gum viscosity; Polysaccharide; β-D-Mannose

1. Introduction

The functional polysaccharide of guar gum, guaran, consists of a β-D-mannose backbone which is substituted by single α(1 → 6) linked galactose residues. The ratio of mannose to galactose residues is approximately 2:1. The solution properties of guar gum depend primarily on the molecular weight of the guaran. Although galactomannans are

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neutral polysaccharides and, therefore, not expected to undergo intermolecular associations, there is some evidence for the presence of supramolecular particles [1] and/or hyperentanglements [2].

The main uses of guar gum in the food industry are based on its stabilising and water binding (at low concentration) properties. These properties depend on the size of the guar molecule and knowledge of molar mass and solution conformation is therefore useful.

Molar mass, hydrodynamic parameters and fine structure of guar gum have been previously investigated by a number of workers [1,3–6]. The work described in the current paper was performed on a series of irradiated guar gum samples, all originating from the same source, therefore allowing measurements to be taken from a series of homologous materials. Radiation treatment is known to affect the molecular size of polysaccharides, whether it is applied to a solution or on the solid state, although the decrease in molecular size is larger when the polysaccharide is in solution [7,8]. The need for irradiating polysaccharides such as guar gum arises mainly because of their journey from the country of origin (in the case of guar gum mainly India and Pakistan) to the end user and/or the long storage times. Previously, disinfestation was carried out using ethylene dibromide or ethylene oxide, however, this is becoming less acceptable as small residual amounts of these chemicals still present in the gum are suspected to be harmful to the consumer, whereas irradiation methods are clean and are not thought to produce any harmful side-products.

This paper describes the application of an absolute method of molar mass determination, size-exclusion chromatography/multi-angle laser light scattering (SEC/MALLS), with sedimentation velocity, intrinsic viscosity and shear viscosity measurements as gross conformation probes (via Mark–Houwink–Kuhn–Sakurada a and b coefficients and the Wales–van Holde ratio).

2. Materials and methods

Irradiated guar gum samples and controls were a gift from Dr. K. King of Queen's University Belfast, and irradiation techniques and doses are as described elsewhere [9]. All other reagents were AnalR grade.

Preparation of guar gum solutions.—Except for concentrated solution viscosity measurements samples were routinely dissolved by adding an accurately weighed portion of the gum into the vortex of magnetically stirred phosphate/chloride buffer ionic strength 0.1, pH 6.8 (ref. [10]), to give a final concentration of approx. 3 mg/mL and stirred for one hour. The samples were then left to hydrate overnight in a refrigerator and stirred for a further one hour period the following morning. The insoluble fraction was removed by centrifugation (4000 rpm, 2500 g, 20 min) using an MSE Multex (Measuring and Scientific Equipment Ltd, Crawley, UK) bench top centrifuge. Supernatants were carefully decanted into sample vials, with further pre-analysis treatment depending on the analytical method used.

Recovery of guar gum.—The response of a RI detector was used to give the amount of guar gum under the main peak using a dn/dc value of 0.143 mL/mg [12].

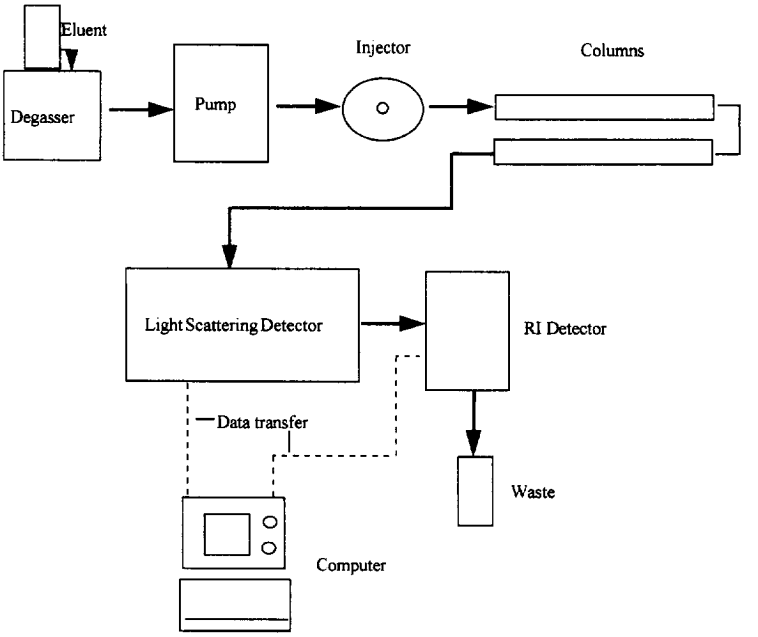

Fig. 1. Schematic diagram of SEC/MALLS instrumentation.

Percentage recovery was then calculated using original amount of guar dissolved/dispersed and amount of guar detected from column system.

Concentrated solution viscosities were obtained from 10 mg/mL solutions of guar gum which had been prepared by stirring the powder in double-distilled water with a high speed paddle stirrer for two hours.

Absolute molar mass determination by SEC / MALLS.—A schematic description of the SEC/MALLS system is shown in Fig. 1 (ref. [11]). It consisted of a degasser (Degassys DG-1200, HPLC Technology, Macclesfield, UK), a programmable solvent delivery system (Model 590, Waters, Millipore, Watford, UK), a Rheodyne Model 7125 injection valve (Rheodyne Inc., Cotati, CA, USA) fitted with a 100 μL loop, a guard column, and TSK G6000PW, G5000PW and G4000PW analytical columns (Anachem, Luton, UK) connected in series providing a separation range for polyethylene oxide from $> 2 \times 10^6$ to $\sim$ 1000 g/mol. The exclusion volume of the column system was found to be 18.7 mL and the total permeation volume 32.8 mL. The eluent was pumped at a flow rate of 0.8 mL/min at ambient temperature and the injection volume was 100 μL. Column effluent was monitored using a Dawn F laser light scattering photometer (Wyatt Technology, Santa Barbara, CA, USA) fitted with a 5 mW He–Ne laser ($\lambda = 632.28$ nm) and an Optilab 901 interferometric refractometer (Wyatt Technology, Santa Barbara, CA, USA).

Molecules are separated according to size by the size-exclusion columns and the light scattering signal from the eluting molecules is collected at up to 15 angles simultaneously. The corresponding concentration trace is obtained from the refractive index

detector and molar mass values $M_{w,i}$, at each fraction, i, are calculated according to the following equation:

$$\frac{Kc_i}{R_{\theta,i}} = \frac{1}{M_{w,i}P(\theta)}(1 + 2A_2c + \ldots)$$

where K is the polymer constant $2\pi^2 n_0(dn/dc)^2/\lambda^4 N_A$ [n_0 is the refractive index, dn/dc (mL/g) is the differential refractive index increment, λ is the wavelength in vacuo (cm) and N_A (mol^{-1}) is Avogadro's number], c_i is the polymer concentration at point i [calculated by using a dn/dc value of 0.143 mL/g (ref. [12]) for guar gum], $R_{\theta,i}$ is the excess Rayleigh ratio at point i and A_2 is the second virial coefficient. If c is sufficiently small, (i.e., ≤ 0.2 mg/mL) $A_2c \approx 0$. Scattering intensities at angles ranging from 36° to 158° were used for data analysis.

A plot of R_θ/Kc versus $\sin^2\theta/2$ (so-called Debye plot) will then give M_w at the intercept.

From the distribution of $M_{w,i}$ the whole distribution average molar mass M_w can be evaluated together with other averages such as the number (M_n) and z average (M_z) [13].

Sedimentation velocity.—Sedimentation velocity experiments were carried out using the MSE Centriscan 75 ultracentrifuge equipped with scanning Schlieren optics and using a monochromatic filter at a wavelength of 546 nm. Sample and reference cells (single sector of 20 mm optical path length) were filled with 800 μL and 900 μL of sample and solvent respectively, solute concentrations ranging from 1.2 to 3 mg/mL. The samples were run at a temperature of 20 °C and a speed of 47,000 rpm. A series of five to eight scans were taken at fixed time intervals. The distance between scans was measured using a digitising tablet connected to an Apple personal computer and data were analysed using software written by Dr. A.J. Rowe (Leicester University) giving the following information:

(a) the sedimentation coefficient $s_{T,b}$ for the sample under experimental conditions, (b) the standard error for a given set of scans, (c) the average radial dilution.

$s_{T,b}$ was converted into $s_{20,w}$ according to [14]:

$$s_{20,w} = \frac{(1 - \bar{v}\rho)_{20,w}}{(1 - \bar{v}\rho)_{T,b}} \times \frac{(\eta_{T,b})}{(\eta_{20,w})} \times s_{T,b}$$

Concentrations as calculated for the dissolved fractions were corrected by the radial dilution factor and $s^0_{20,w}$ and k_s (the concentration dependence regression factor) were obtained from a plot of $1/s_{20,w}$ versus concentration for each sample and extrapolating to zero concentration:

$$\frac{1}{s_{20,w}} = \frac{1}{s^0_{20,w}}(1 + k_s c)$$

Viscosity measurements.—Intrinsic viscosity. Flow times for the guar gum solutions were measured using an Automated Measuring Unit AVS 310 (Schott Geraete, Hofheim, Germany) at (25.00 ± 0.01 °C). Solution concentrations ranged from 0.5 to 1.5 mg/mL.

Intrinsic viscosities [η] were determined for each irradiated guar gum sample by calculating relative viscosities, i.e., η_r = flow time of solution/flow time of solvent converting to specific viscosity ($\eta_{sp} = \eta_r - 1$) and extrapolating to zero concentration using the Huggins relationship [15], i.e.,

$$\frac{\eta_{sp}}{c} = [\eta] + K_H[\eta]^2 c$$

where K_H is the Huggins constant. Correction of (kinematic) η_r values for solution densities was deemed unnecessary because of the low concentrations involved. Intrinsic viscosities are given by the intercept of extrapolation to zero concentration.

Shear viscosity. Shear viscosities of 1% guar gum solutions were measured using a Bohlin C.S. Rheometer (Bohlin Reologi AB, Lund, Sweden) consisting of a rotating upper cone (40 mm diameter, 4° angle) and a fixed lower plate with the sample contained between them. The temperature was at 24.8 °C within limits of ±0.05 °C. Data was collected for each irradiated sample and analysed using the dedicated Bohlin software which produced plots of viscosity versus shear rate. Zero shear viscosities were obtained using the Cross equation [16]

$$\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{(1 + (K\dot{\gamma})^m}$$

where η_0 and η_∞ refer to the asymptotic values of viscosity at very low and very high shear rates, respectively. K is a constant with dimension of time and m is a dimensionless constant, $\dot{\gamma}$ refers to the shear rate.

$G_{(scission)}$ *values.*—Degradation indices known as $G_{(scission)}$ values were calculated by the method described by McLaren [17] using the weight-average molecular weights obtained from SEC/MALLS measurements.

The average number of scissions per gram of guaran is given by:

$$S = \left(\frac{dp_1}{dp_2}\right) \times \left(\frac{N}{dp_1 \times 512}\right)$$

where dp_1 = degree of polymerisation for non-irradiated guaran, dp_2 = degree of polymerisation of irradiated guaran, N = Avogadro's number, 512 g/mol = molar mass of guaran repeating unit.

The amount (in g) of guaran per 1000 glycosidic bonds is given by:

$$\text{g guaran } (1000)^{-1} = \frac{1000 \times dp_1 \times 512}{(dp_1 - 1)} \times N$$

The number of scissions per 1000 glycosidic bonds (S_{1000}) in guaran is given by:

$$S_{1000} \cong 1000\left[\frac{1}{dp_2} - \frac{1}{dp_1}\right]$$

and

$$G_{(\text{scission})} = \frac{S_{1000} \times 100}{\text{dose}\,(\text{eVg}^{-1}) \times \text{g}(1000\,\text{bonds})^{-1}}$$

$1\ \text{Gy} = 6.24 \times 10^{15}\ \text{eVg}^{-1}$.

3. Results

The results from all measurements are summarised in Table 1.

Absolute molar masses and molar mass distributions.—There are a number of reports on the effect of γ-irradiation on physical properties such as viscosity of polysaccarides, but only little attention has been paid to the effect of γ-irradiation on molar mass, in particular absolute molar mass. Kusama et al. [18] reported the molar masses and molar mass distributions of irradiated cellulose fibres, however, their results were obtained by calibrated (using polystyrene and cellulose trinitrate standards) gel permeation chromatography, a technique which is based on the difference in hydrodynamic volume rather than molar mass between different molecules.

The variation of weight-average molecular weight with radiation dose is shown in Fig. 2 for SEC/MALLS (see also Table 1). This indicates that there is a significant decrease in molar mass with irradiation dose. At the low irradiation doses (i.e., 0.1 kGy to 0.8 kGy) this decrease is relatively steep, and then bottoms out at the higher doses.

According to Charlesby [19] "any molecular weight distribution which is initially of a random character will retain this character, although with different parameters, if it is subject to further random fracture". An overlay of the molar mass distribution of the three samples shown in Fig. 3 confirms that the breakdown in the irradiated guar gum samples was of a random nature.

Table 1
Weight-average molecular weights and hydrodynamic parameters for control and irradiated guar gum samples

Sample	$10^{-6} \times M_w$ light scattering	$[\eta]$ (mL/g)	η_0 (Pas)	$10^{13} \times s^0_{20,w}$	k_s (mL/g)	$k_s/[\eta]$
Control	2.70	1576	21.4	4.59	242	0.153
0.113	2.03	1467	19.87	5.01	222	0.151
0.204	2.32	1360	12.91	4.65	211	0.155
0.373	1.78	1111	8.27	5.14	241	0.216
0.498	1.87	957	5.18	4.39	190	0.198
0.649	1.66	1092	4.64	4.60	223	0.204
0.860	1.49	894	5.17	5.36	288	0.322
1.700	1.24	964	1.71	5.06	233	0.242
5.072	0.866	736	0.46	3.99	157	0.213
9.071	0.565	471	0.12	3.94	191	0.406

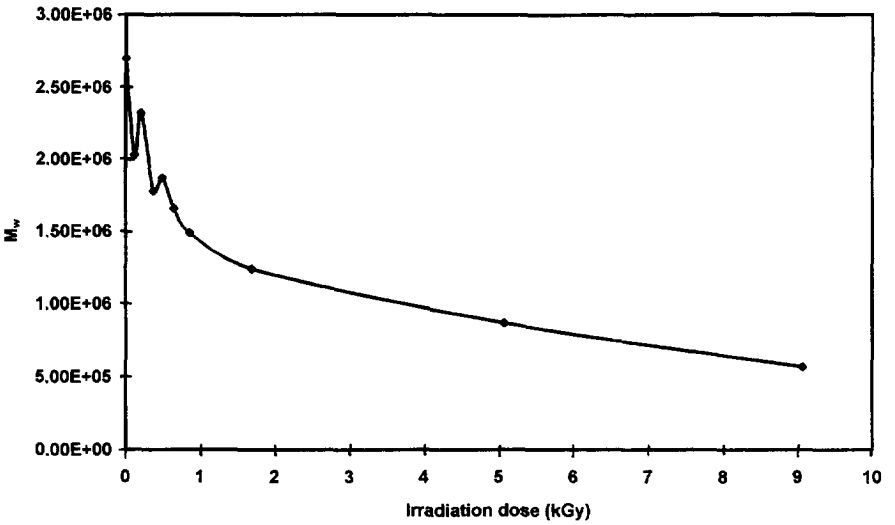

Fig. 2. Variation in absolute weight-average molecular weight values (from SEC/MALLS) with irradiation dose.

Intrinsic viscosity.—The intrinsic viscosity $[\eta]$ describes the property of an isolated macromolecule in solution. The value of $[\eta]$ depends on the shape and specific volume of the molecule and is related to the molar mass via its radius of gyration (R_g) [13].

The variation of intrinsic viscosity with irradiation dose given in Fig. 4 not only confirms the basic trend towards lower molecular size with increasing irradiation dose but also the rapid decrease at low irradiation doses followed by a much slower decrease at the higher doses. A Mark–Houwink Kuhn–Sakurada (MHKS) plot of intrinsic

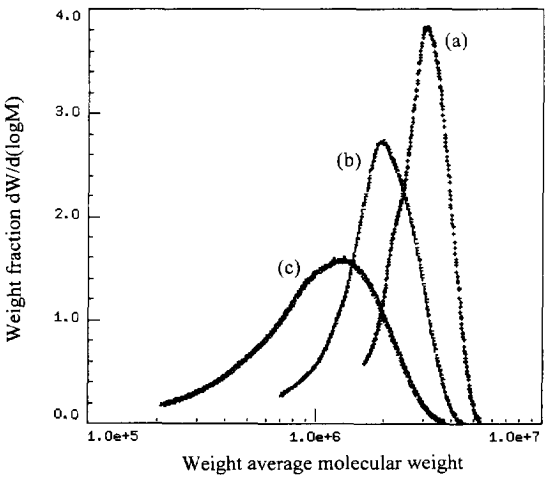

Fig. 3. Molar mass distributions of (a) non-irradiated, (b) 0.204 kGy, and (c) 1.700 kGy samples.

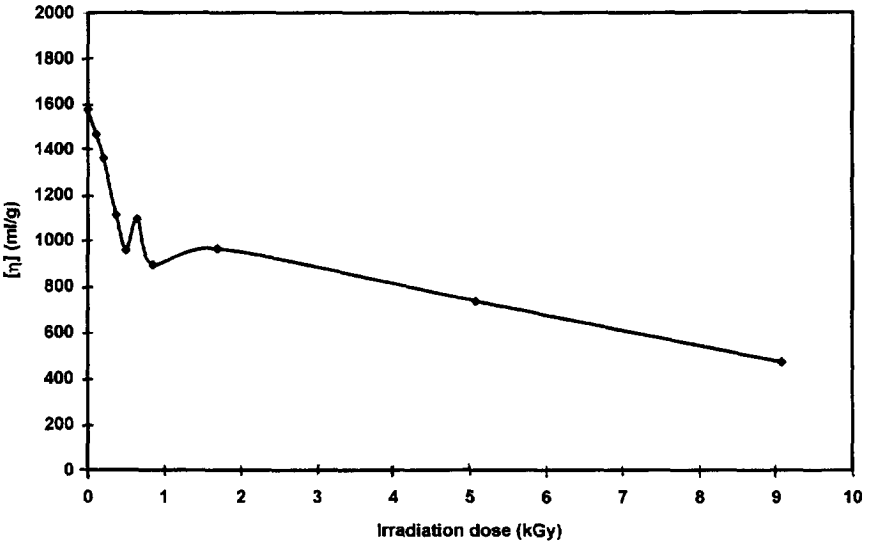

Fig. 4. Variation in intrinsic viscosity with radiation dose.

viscosity versus molecular weight for the range of samples investigated (see Fig. 5) gave a MHKS "a" coefficient of 0.725 which not only fits in well with the established model of a random coil solution conformation [20] but also agrees with the values found by other workers for guar gum [1,3].

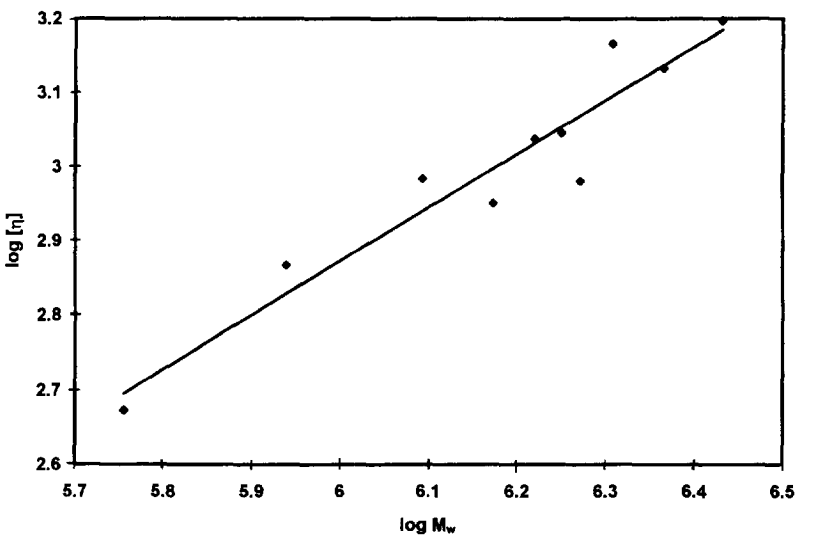

Fig. 5. Double-log plot of intrinsic viscosity versus molar mass.

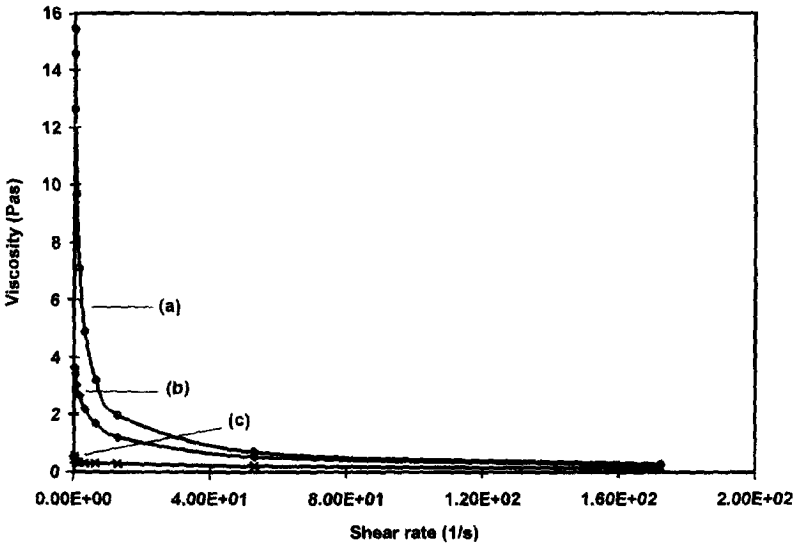

Fig. 6. Flow curves for (a) non-irradiated, (b) 0.86 kGy, and (c) 5.072 kGy irradiated guar gum samples.

Shear viscosity.—Examples of three flow curves are shown in Fig. 6. They demonstrate a significant change in rheological behaviour with increasing irradiation dose. Whilst the control sample and the low irradiated samples indicate a large shear rate dependence of viscosity, the samples irradiated at 5 kGy and above approach Newtonian behaviour. In order to compare the concentrated solution viscosities of all the samples the zero shear viscosity, η_0, was established via the Cross equation [16] and a graphical description of η_0 versus irradiation dose is given in Fig. 7. This shows a significant decrease in η_0 at low irradiation doses (0.1–0.6 kGy), a stabilisation up to a radiation dose of 1.0 kGy and then a second decrease at approx. 2 kGy. This is in good agreement with the trends found from molecular weight and intrinsic viscosity data. These results differ slightly from those reported at a shear rate of 54 s^{-1} by King and Gray [9] who used the identical guar gum samples. They found a much smaller decrease in shear viscosity with irradiation dose, only samples dissolved at 80 °C showed a similarly marked decrease in viscosity at the low irradiation doses.

Sedimentation velocity.—The rate of movement of a molecule in a centrifugal field is primarily dependent on the mass of the molecule and the viscous drag opposing this movement as the molecule moves through the solution. The sedimentation coefficient may therefore provide information on molecular conformation and in conjunction with the diffusion coefficient on the molar mass via the Svedberg equation [14].

Schlieren boundary traces obtained from a sedimentation velocity run using one of the irradiated guar gum samples are represented in Fig. 8. These traces are very sharp (hyperfine peaks) and would ordinarily indicate that the system is monodisperse. However, polysaccharides are rarely monodisperse even when fractionated and in our case these hyperfine peaks are an indication of (a) a highly non-ideal system and/or (b) a highly polydisperse system [21].

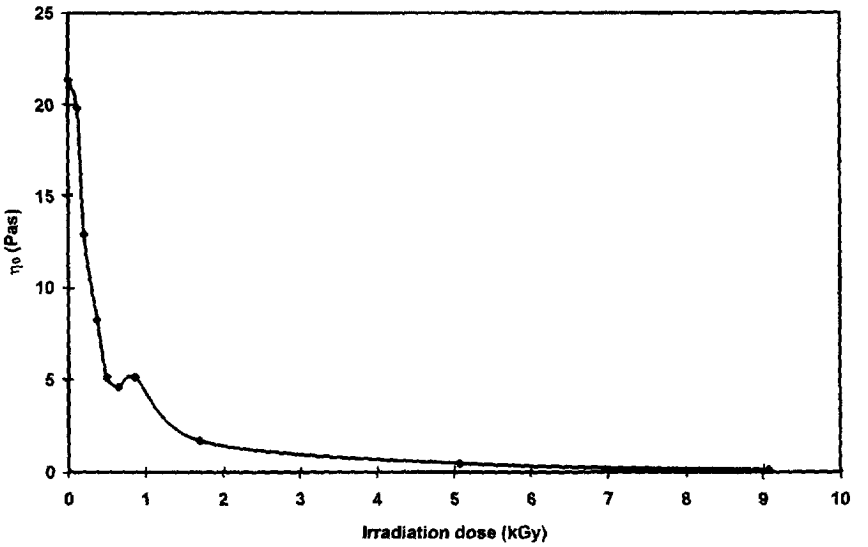

Fig. 7. Variation in zero shear viscosity with radiation dose.

Sedimentation coefficients corrected to standard conditions (i.e., zero concentration, water at 20 °C, $s^0_{20,w}$) were found to have values between 3.7 and 5.2 Svedbergs, S (1 S = 10^{-13} s), which for molecules of very large molar mass are indicative, qualitatively, of large expansion through solvation and/or large asymmetry.

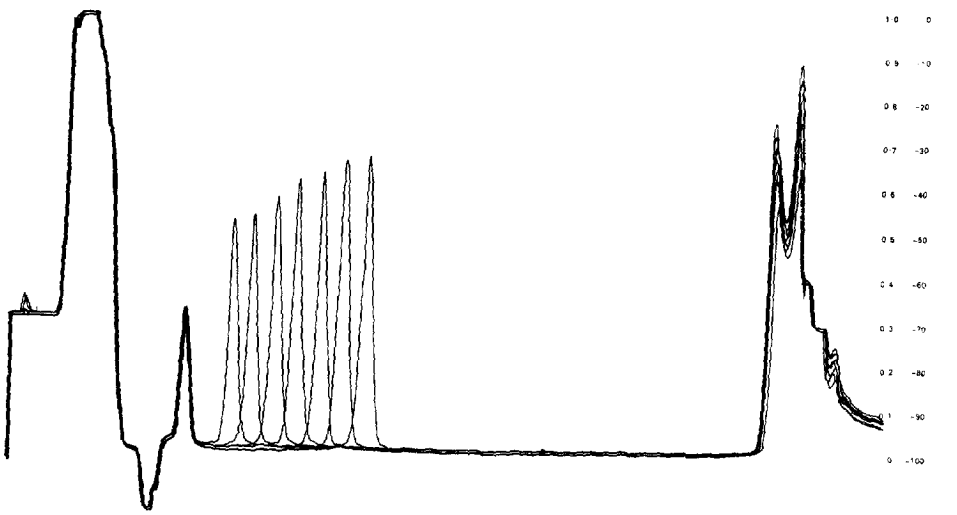

Fig. 8. Sedimentation velocity profiles from 0.649 kGy sample. Sample concentration = 1.8 mg/mL, rotor speed = 47,000 rpm, temperature = 20 °C.

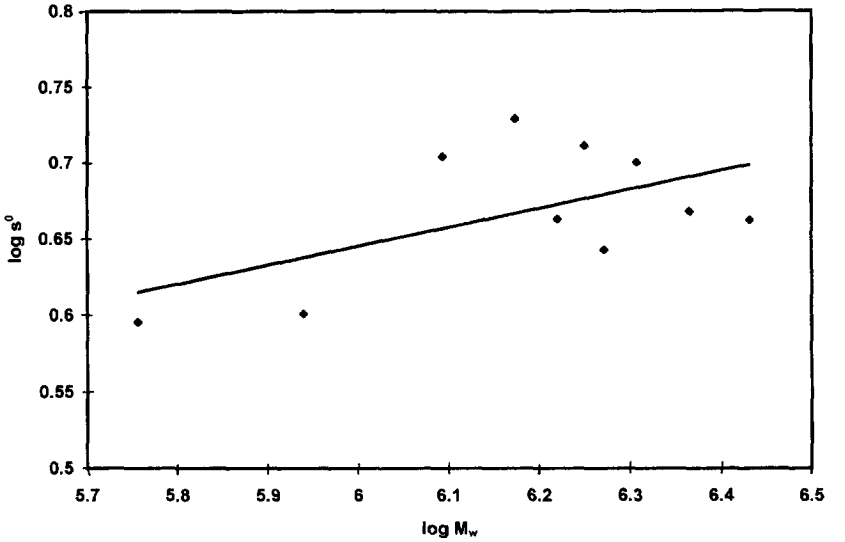

Fig. 9. Double-log plot of $s^0_{20,w}$ versus molar mass.

Further conformational information may be obtained through the Wales–van Holde ratio, $k_s/[\eta]$ (ref. [22]), and the MHKS relationship of sedimentation coefficient versus molecular weight. The Wales–van Holde ratios for all the guar samples are given in Table 1 and they are consistent with a deviation from spheroidal conformation. The MHKS plot (see Fig. 9) gave a MHKS "*b*" coefficient of 0.124 which is more consistent with a rigid rod conformation [20]. However, there is considerable scatter in these results and the value of 0.124 should not be taken in isolation but compared with the MHKS coefficient from viscosity data which is too high for a pure random coil conformation and points to at least an extended coil behaviour.

$G_{(scission)}$ *values.*—The average number of chain breaks per molecule over the doses investigated was found to be 3.78. This value is lower than would usually be expected for a polysaccharide [23] and suggests some degree of resistance to radiation by the guar gum investigated. It may be possible that the reason for this resistance to irradiation is due to impurities still present in the guar gum investigated. The material had not been purified prior to irradiation and the impurities may have acted as electron traps, for example, aromatic compounds would be able to delocalize and, therefore, stabilize the radical. McLaren [17] found that the cellulose in wood was far more resistant to radiation than purified celluloses.

A closer look at the $G_{(\text{scission})}$ value for the individual doses (see Table 2) reveals that except for the 0.204 kGy sample the G values at the lower irradiation doses are higher than at the higher irradiation doses, i.e., more molecules are affected by the low irradiation doses.

Guar gum recovery.—Guar gum does not dissolve completely in aqueous solutions in the cold but for most of the methods applied in this study, a molecularly dissolved

Table 2
$G_{(scission)}$ values for irradiated guar gum samples calculated using M_w from SEC/MALLS measurements

Radiation dose (kGy)	$G_{(scission)}$ value
0.113	10.34
0.204	2.96
0.373	5.00
0.498	3.14
0.649	3.37
0.860	3.40
1.700	2.48
5.072	1.49
9.071	1.48

sample was required. For this reason the sample work-up described in the methods section was used. However, centrifugation and filtration meant that a large amount of the sample was removed prior to analysis and a knowledge of the amount of actual dissolved material was necessary. The easiest way to determine this was to use the amount of material detected by the interferometric refractometer in the SEC/MALLS system and compare this to the amount of material originally used. In doing so it had to be assumed that all the material injected onto the column system was eluted. A graphic description of the change in guar gum recovery with irradiation dose is given in Fig. 10 which shows that there is an increase in guar gum solubility with increasing irradiation dose.

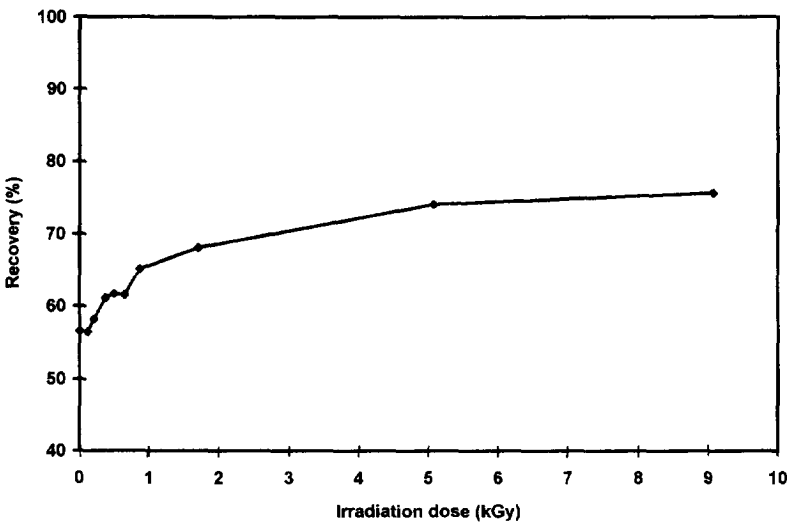

Fig. 10. Variation in guar gum solubility with irradiation dose.

4. Discussion

The results from the above techniques are all in fairly good agreement with each other, indicating that guar gum is most likely to exist in an extended coil conformation in solution. Also, as expected, an increase in irradiation dose is leading to a reduction in molecular size. Our findings are also in good agreement with those reported by King and Gray [9] who investigated the formation of radicals and the effect on concentrated solution viscosity after irradiation. They suggested that the decrease in viscosity at the low irradiation doses was due to the disruption of supramolecular particles and that at higher irradiation doses hydrolysis of the mannose backbone was responsible for the decrease in viscosity. However, in the light of our data we would propose a slightly different explanation for the behaviour of guar gum at the various irradiation doses.

It is not clear whether dissolved guar gum can be regarded as a true homogeneous solution. Except for the concentrated solution viscosities, all of the techniques discussed in this paper required molecularly dispersed solutions which was achieved by using the supernatant of the centrifuged solution and — for SEC/MALLS — subsequent filtering. For this reason, guar gum recoveries were measured and it is evident that recovery increases with increasing radiation dose and it is this recovery from the columns which gives a measure of the true concentration of the guar gum solutions. We consider as a consequence of this that the shear viscosities are a measure of the whole system (containing aggregates, non-hydrated guar gum and small amounts of extraneous materials), whereas intrinsic viscosity, molar mass and sedimentation velocity are measures of the molecularly dispersed polymeric material. This would explain the discrepancy between the measured and estimated (from concentrated solution viscosities and Robinson master plot) intrinsic viscosities at the lower irradiation doses (see Table 3), whereas the difference between the two sets of values decreases with increasing radiation dose. This could give a rationale for the anomalously high slope for guar gum in the η_0 versus $c[\eta]$ plot (ref. [1]), since at high molar mass and/or concentration more

Table 3
Comparison of measured and estimated intrinsic viscosity values

Intrinsic viscosity (mL/g)	
Measured	Estimated from master plot (ref. [1])
1576	1780
1467	1780
1360	1420
1100	1200
960	1180
1100	1040
890	1180
960	840
740	680
470	500

of the non-molecularly dispersed fraction will be present. The nature of the reduction will thus change with increasing $c[\eta]$ values.

5. Conclusions

The investigation into the effects of irradiating guar gum in the solid state has shown that there is a decrease in molecular weight, intrinsic viscosity and zero shear viscosity with irradiation dose. This is in good agreement with the results of other workers.

The actual solution conformations as established by MHKS plots and the Wales–van Holde ratio remain very similar to those found for non-irradiated guar gum. Such behaviour would be expected if there is no change in molecular structure except for a shortening of the chain. Similarly, the molecular weight distribution was moved to lower molar masses but essentially remained identical to the non-irradiated material.

Comparison of measured intrinsic viscosities and those estimated from zero shear viscosities via the Robinson master plot revealed higher estimated values at the low irradiation doses but convergence of estimated and measured intrinsic viscosities with increasing irradiation doses. This indicates that the difference between guar gum and the other polymers in the master curve may be due mainly to undissolved and extraneous materials rather than an intrinsic property of guar gum itself.

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