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Absolute Molecular Weight and Molecular Weight Distribution of Guar by Size Exclusion Chromatography and Low-angle Laser Light Scattering

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SUMMARY

A procedure to determine the absolute weight-average molecular weight (M_w) and molecular weight distribution (MWD) of guar by aqueous size exclusion chromatography coupled with low angle laser scattering is described. It is shown that for a rigorously purified sample of guar solution the values for M_w and MWD are $2{\cdot}2 \times 10^6$ and 1·9 respectively. The effect of sample preparation and purification on these molecular parameters are discussed. Limitations and challenges in the aqueous size exclusion chromatography of complex water soluble polymers such as guar are also explored.

INTRODUCTION

Guar gum is obtained from the endosperm of the seed of the guar plant *Cyamopsis tetragonolobus*. Guar consists mainly of galactomannan with small amounts of protein, hull fragments and oils (Whistler & Hymowitz, 1979). Due to its excellent thickening power in various aqueous environments and desirable rheological attributes, guar finds extensive use in many diverse areas of application (Goldstein *et al.*, 1973).

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The molecular weight and molecular weight distribution (*MWD*) of guar are fundamental parameters in influencing the properties and performance of this interesting polysaccharide. However, a comprehensive literature search reveals that information on the molecular weight of guar, though extensive, is not completely satisfactory. According to the literature, the weight-average molecular weight of guar has been reported to be $5{\cdot}32 \times 10^5$ (Sharman *et al.*, 1978), $8{\cdot}17 \times 10^5$ (Sharman *et al.*, 1978), $9{\cdot}5 \times 10^5$ (Dea & Morrison, 1975), $1{\cdot}7 \times 10^6$ (Deb & Mukherjee, 1963), $1{\cdot}9 \times 10^6$ (Hui & Neukom, 1964) and $1{\cdot}0 \times 10^6$ (Venkatiah & Mahadevan, 1982). Koleska & Kurath (1964), working with fractionated, acetylated guar, showed that the sample had several molecular weight fractions with molecular weights in the range $0{\cdot}5 \times 10^6$ to 11×10^6, as determined from sedimentation coefficients. More recently, Barth & Smith (1981) carried out preliminary size exclusion chromatography of guar to gain some information on the *MWD* of guar. However, they could not calculate any reliable molecular weight average and *MWD* of guar because of the lack of appropriate standards which eluted in the range of guar. Many of the problems and limitations with such literature data on guar can be related to the polydisperse nature of the sample, a poorly defined sample preparation technique, a lack of standards, and the use of empirical relationships that correlate viscosity/diffusion coefficient measurements to molecular weight.

More recently on-line low-angle laser light scattering (LALLS) detectors for size exclusion chromatography have become commercially available (McConnell, 1978). Also a considerable amount of progress has been made in the size exclusion chromatography (SEC) of high molecular weight water soluble polymers (Barth & Regnier, 1980; Pfannkoch *et al.*, 1980; Provder, 1980). Use of an on-line light scattering detector to analyze individual fractions of polymers separated by SEC eliminates the need for any external standard or universal calibration plot. The absolute molecular weight of each fractionated part of a polydisperse polymer sample is calculated from a few independently determined instrument and polymer constants. Knowing the molecular weight of each fraction (from on-line light scattering) and the corresponding mass (from the differential refractive index detector of the SEC unit), one can readily calculate, with the use of an on-line laboratory minicomputer, both the molecular weight averages and *MWD*. The main objective of this work was to develop a method for the SEC of guar coupled with LALLS which would be use-

ful in the determination of the absolute M_w and *MWD* of guar. A detailed account of the methods development procedure along with the effect of sample preparation and purity on the results are discussed.

In the literature (Beyer *et al.*, 1973; Bose *et al.*, 1982), the use of commercially available monodisperse polystyrene sulfonate polymers as a secondary calibration standard for aqueous SEC of high molecular weight water soluble polymers has been proposed. Such a procedure, if generally applicable, would be a great help in the characterization of water soluble polymers. Another objective of this work is to evaluate the validity of this suggestion, as judged from the SEC/LALLS results on guar. Finally, some of the general problems/challenges associated with sample preparation and purification, column packing and mobile phase selection for the SEC of complex water soluble polymers such as guar are also explored.

EXPERIMENTAL SECTION

Materials

Natural guar gum and chemically depolymerized guar gum were Celanese products. Sodium polystyrene sulfonate standards of the following molecular weight were obtained from Scientific Polymer Products, Inc., Ontario, New York.

M_w	*MWD*
$2{\cdot}4 \times 10^4$	1·10
$4{\cdot}0 \times 10^5$	1·10
$1{\cdot}2 \times 10^6$	1·10
$6{\cdot}0 \times 10^6$	1·20

Light scattering and aqueous size exclusion chromatography measurements

A Chromatix KMX-6 LALLS unit coupled with a Waters Associates model ALC/GPC 201 unit was used to obtain the absolute *MWD* of guar samples. A diagram of the set-up is shown in Fig. 1. All measurements were made at 25°C and a 6° scattering angle. To avoid excessive

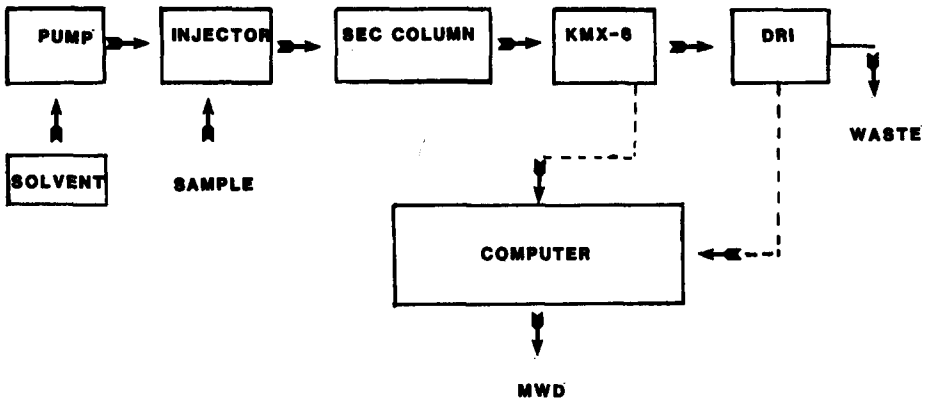

Fig. 1. Diagram of SEC/LALLS set up for on-line data processing.

back pressure on the differential refractive index (DRI) detector cell of the GPC, the light scattering cell was connected between the column and the GPC detector. The fractionated samples were examined; information on the excess Rayleigh factor ($\bar{R}_\theta$) was derived from the KMX-6 unit while signals arising from the corresponding mass (DRI of GPC) were collected on-line using a Chromatix KMX-6DP data processing system. The *MWD* was then computed from the $\bar{R}_\theta$, DRI responses and two independently determined polymer constants, namely the specific refractive index increment and the second virial coefficient (see next section). The theory and appropriate calculations for the determination of absolute *MWD* of polymers using LALLS/GPC are discussed in detail elsewhere (Ouano & Kaye, 1974; McConnell, 1978).

Refractive index increment (dn/dc) and second virial coefficient

A Chromatix KMX-16 differential refractometer operating with a He/Ne laser of 632·8 nm (the same wavelength as used in the KMX-6 LALLS unit) was used to determine (dn/dc). A rigorously clarified sample of guar (see 'Sample Preparation') was found to have a (dn/dc) of 0·153 ml g^{-1} in distilled water and in the mobile phase solvent (see 'Methods Development').

The second virial coefficient of guar in the mobile phase solvent was determined by determining $\bar{R}_\theta$ at four concentrations (c) of guar and plotting the data according to eqn (1), which relates $\bar{R}_\theta$ to M_w at the

small forward scattering angles employed in the KMX-6 (McConnell, 1978).

$$\frac{Kc}{\bar{R}_\theta} = \frac{1}{M_w} + 2A_2C \quad (1)$$

where C = the solute concentration in g ml^{-1}, A_2 = second virial coefficient in ml mol g^{-2}, K = polymer optical constant (McConnell, 1978).

A typical plot for guar is shown in Fig. 2. M_w and A_2 for a rigorously purified sample of guar in the mobile phase solvent were found to be $2{\cdot}1 \times 10^6$ and $5{\cdot}05 \times 10^{-4}$ respectively. The precision of the M_w and *MWD* measurements of a rigorously purified sample of guar was about ± 6%.

Intrinsic viscosity

Intrinsic viscosities at 25°C were calculated in various mobile phase media from flow times determined using a Cannon Ubbelhode dilution viscometer. No shear rate correction of the viscometer was made.

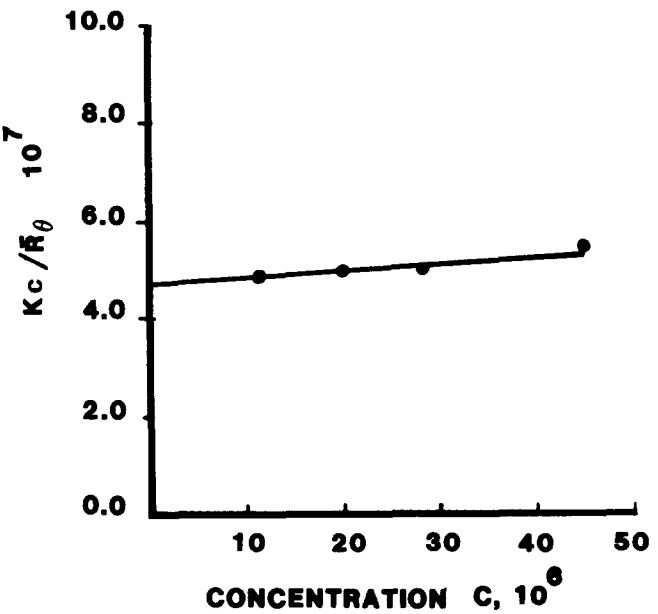

Fig. 2. Static LALLS data of guar. $M_w = 2{\cdot}1 \times 10^6$ and $A_2 = 5{\cdot}05 \times 10^{-4}$ ml mol g^{-2}.

Methods development: aqueous SEC of guar

Sample preparation

Due to the presence of a significant level of insoluble, non-gum material in the guar solution, extensive sample clean-up was required before SEC/LALLS analysis. The extreme sensitivity of the KMX-6 detector to small particulate contamination required that samples be free of particulates beyond the level normally required to protect column frits and the pumping hardware of the SEC. Also, since guar solutions are highly viscous even at low concentrations, they cannot be filtered as thoroughly as might be desired due to plugging of filter membranes.

After several trials, the following sample preparation procedure was adopted. A 0·1% solution of guar in an appropriate mobile phase (previously filtered through a 0·2 μm Gelman membrane filter) was prepared by slowly sprinkling the guar powder into the mobile phase solvent with stirring. The sample was allowed to stand overnight to permit complete hydration. Then the sample was centrifuged at 15 000 rpm (approximately 20 000 *g*) for 30 min in a Beckman Model L5-75B ultracentrifuge to remove cellular residues. This generally produced a clear solution which was further subjected to sequential filtration through 5·0 μm, 1·2 μm, 0·8 μm and 0·45 μm Gelman membrane filters. Extreme care was taken to keep dust and other contaminants out of the filtered sample.

Although the mobile phase was prefiltered through a 0·2 μm Gelman membrane before use and sample solutions were filtered further as described above, a 1·2 μm in-line Teflon filter prior to the KMX detector was necessary in order to obtain a reasonable signal/noise level.

It is worth mentioning here that extensive prefiltration of guar solutions as described above will strongly influence the distribution of various molecular fractions in the sample. Recent filtration data (Whitcomb *et al.*, 1980) shows clearly the extreme polydisperse nature of guar. However, we were forced to use such rigorous sample preparation techniques, recognizing full well that the molecular weight information thus obtained did not correspond to the total sample, but only to an extremely purified sample. A few guar samples with reduced levels of filtration were analyzed with limited success. These results will be discussed in a later section.

SEC column packing and mobile phase selection

Commercially available columns (Pfannkoch *et al.*, 1980) were found to be unsatisfactory in the molecular weight range of interest to guar solutions. A series of 120/230 mesh untreated fractosil silica (EM Laboratories Inc., Elmsford, New York) and CPG controlled pore glass (Electro-Nucleonics Inc., Fairfield, New Jersey) were selected for evaluation in the SEC/LALLS system based on literature recommendations (Barth & Regnier, 1980; Pfannkoch *et al.*, 1980; Omoroidon *et al.*, 1981). Evaluations of large pore sized Fractosil 2500 nm and 1000 nm columns indicated no resolution of samples as elution occurred at the total permeation volume for all sample injections. A smaller pore sized packing (CPG-350, mean pore diameter 33 nm) was found not to be useful at the opposite size extreme as the samples were totally excluded from the packing. A 2 ft column of 500 nm pore size Fractosil column was found to be satisfactory for the separation of guar sample. Increasing the column bank to 4 ft by the use of two such identical 2 ft columns in series helped in retention volume. However, peak broadening also increased proportionately with no net gain in resolution due to the use of a longer column length. The columns were dry packed.

Use of fairly large pore size untreated silica packing in aqueous SEC causes several problems which have been discussed recently (Pfannkoch *et al.*, 1980). One of them is that the surface of silica contains numerous active silanol sites on which adsorption and ion exclusion occur, thus leading to the operation of a non-size exclusion mechanism of separation (Barth & Regnier, 1980; Pfannkoch *et al.*, 1980). For instance, in distilled water about 50% of guar was adsorbed in a 2 ft column at a flow rate of 1 ml min^{-1}. Various additives were examined to minimize such adsorption using the procedure of Omoroidon *et al.* (1981). A mobile phase of the following composition (approximate ionic strength 0·1), 0·02% sodium azide, 0·035% Na_2SO_4, 1% methanol and 100 ppm Igepal CO-710 ethoxylated nonyl phenol non-ionic surfactant (GAF Corp.) was found to give a recovery of 90% of the guar injected into the column. Hence, in the mass calculation needed for the molecular weight computation, the sample SEC mass was assumed to be 90% of the injected mass.

Another problem in the use of large pore size, untreated columns is their low plate count (600 versus several thousand for commercial

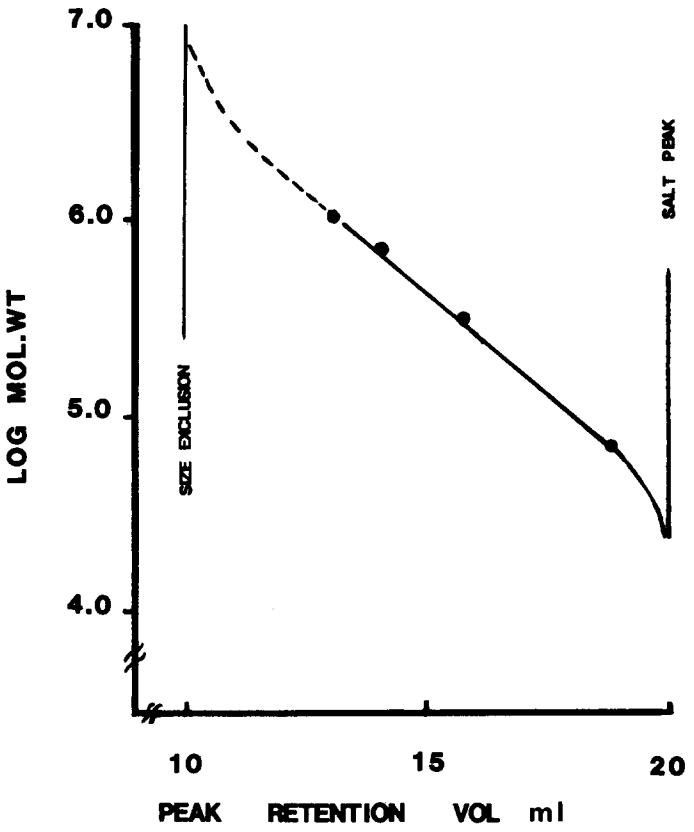

Fig. 3. SEC calibration plot of standard sodium polystyrene sulfonate. See text for details of SEC column conditions.

surface treated ones). This means that they have poor resolution and excessive band spreading, resulting in an underestimation of the width of the *MWD* (Hamielec, 1980).

The performance of a 2 ft long 500 nm pore size Fractosil column was checked out with the use of polystyrene sulfonate standards. The samples were made in the mobile phase described above and the calibration plot shown in Fig. 3 was obtained. It is seen that the calibration plot was linear over the molecular weight range $1{\cdot}2 \times 10^6$ to $6{\cdot}5 \times 10^4$.

Band spreading and resolution corrections to molecular weight averages were determined from Fig. 3 using the procedure of Hamielec (1980). The correction factor was found to be ±6%. It is generally believed that a band broadening correction factor of this magnitude may be ignored (Hamielec, 1980).

As mentioned earlier (Beyer *et al.*, 1973; Bose *et al.*, 1982), sodium polystyrene sulfonate of narrow molecular weight has been proposed as a secondary (molecular weight) standard in aqueous SEC. In order to check the general validity of this approach, the SEC data file of guar samples was recalculated to obtain the polystyrene sulfonate equivalent M_w and *MWD* of guar, using the calibration plot shown in Fig. 3. This assumed that the calibration plot was linear in the molecular weight range 2×10^4 to 3×10^6, even though a non-linear response was observed with a $6{\cdot}0 \times 10^6$ sample of polystyrene sulfonate.

After the bulk of the work with the evaluation of Fractosil columns was completed, the availability of a high efficiency μ Bondagel column (E-high μ Bondagel from Waters Associates) suitable for aqueous SEC of high molecular weight polymers came to our attention. The performance of this column was evaluated in our study. Sample recovery with E-high column was comparable to that of Fractosil. The theoretical plate count was better (1200 versus 600 for Fractosil) and the analysis time was reduced by half due to the smaller column size compared to the Fractosil column. However, bandspreading was seen to be higher. A calibration plot for polystyrene sulfonate samples was not totally linear. Attempts to extend the resolution to the lower molecular fraction by combining additional smaller porosity μ Bondagel columns were not successful. Overall, the performance of the Fractosil column was slightly better than the Bondagel column due to the lower band spreading and linear calibration curve.

Samples of guar solutions which had been prepared and rigorously cleaned up as described above were run under the following optimized SEC conditions:

Column: 500 nm pore size Fractosil, 2 ft long
Flow rate: 1·0 ml min^{-1}
In-line filter: 1·2 μm Teflon
Injected mass: 0·5 ml at 0·1% concentration or 5×10^{-4} g
SEC sample mass: 90% of above or $4{\cdot}5 \times 10^{-4}$ g

RESULTS AND DISCUSSION

A typical molecular weight distribution curve for a rigorously purified guar is shown in Fig. 4. M_w and *MWD* ($=M_w/M_n$, where M_n = number-average molecular weight) are calculated to be $2{\cdot}2\times10^6$ and 1·9 respectively. The M_w value of $2{\cdot}2\times10^6$ calculated from SEC/LALLS data agrees quite well with the static measurement value of $2{\cdot}1\times10^6$ reported earlier.

The sample has an intrinsic viscosity $[\eta]$ of 12·6 dl g^{-1} in the mobile phase solvent. Combining this value of the intrinsic viscosity with the diffusion coefficient (D_t) value of $2{\cdot}9\times10^{-8}$ cm^2 s^{-1} determined by the dynamic light scattering technique (Hardy, 1982), one can calculate a value of $2{\cdot}4\times10^6$ for $\bar{M}_{D,\eta}$ from the expression (McDonnell & Jamieson, 1977)

$$\bar{M}_{D,\eta}=\frac{3{\cdot}0\times10^{-29}}{[\eta]}\times\left(\frac{D_t^0.\eta_0{}^{-3}}{T}\right)$$

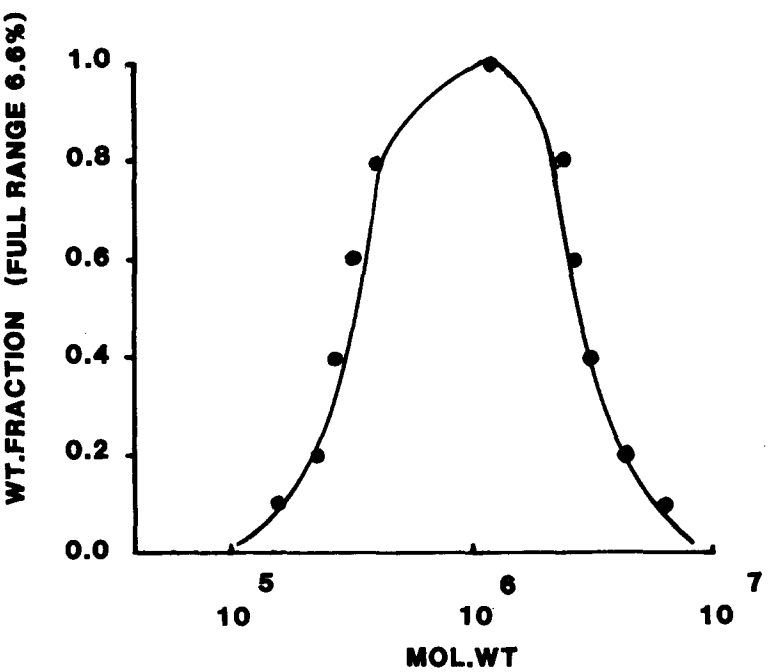

Fig. 4. SEC/LALLS *MWD* of guar.

The calculated $\bar{M}_{D,\eta}$ average for guar is slightly higher than the experimentally determined light scattering weight-average value of 2·1 to 2·2 × 10^{-6}.

It is interesting to apply the above equation to guar; however, it does raise some questions. For instance, the above equation from McDonnell & Jamieson (1977) was used for linear and flexible synthetic polymers and its application to a branched and fairly rigid polymer such as guar is perhaps not totally valid. Further, the molecular weight-average $\bar{M}_{D,\eta}$ calculated from the above equation corresponds to a complex average, intermediate in value between that of the weight-average (M_w, determined in this study) and the z-average (M_z), given by $M_{D,\eta} = M_w^{0.425} M_z^{0.575}$. In general, $M_z > M_w$ (Tanford, 1961). McDonnell & Jamieson (1977) have shown that this equation is applicable for a Schulz type distribution that has a unimodal *MWD* and the individual fractions approximate spherical symmetry. It is unlikely that these conditions are valid in a heterogeneous sample such as guar which is known to have individual fractions of broad molecular weight range (Koleska & Kurath, 1964; Barth & Smith, 1981). Further work with narrow fractionated samples of guar would be needed to satisfactorily settle these issues.

The *MWD* data of a guar sample filtered through a coarser (0·80 μm) filter instead of the normal 0·45 μm filter showed some interesting results. A 20–25% loss in mass was observed in filtration through a 0·45 μm filter. A 29% loss in mass was observed by Whitcomb *et al.* (1980) on a sample of guar under similar filtration conditions. The KMX signal of the sample filtered through a 0·80 μm filter showed the presence of 'large microgels', even though the DRI signal of GPC which is sensitive to mass did not show any abnormality. The KMX was very noisy and not totally reproducible on repeat injections. Hence the data from a 0·80 μm filtered sample could not be reliably analyzed using the SEC/LALLS computer program. Approximate calculations using this data showed that the M_w of a 0·8 μm filtered sample was in the range 8·0 to 10·0 million, a value considerably higher than the one found for the 0·45 μm filtered sample. Such a high value for the molecular weight of certain fractions of guar was observed by Koleska & Kurath (1964). Dynamic light scattering (Hardy, 1982) measurements on less rigorously filtered samples also showed the presence of very slowly diffusing large aggregates.

The SEC/LALLS results show that a rigorously purified sample of guar has an absolute M_w of 2·1 to 2·2 million and an *MWD* of 1·9. Such a value for the molecular weight of guar has been suggested by Dea & Morrison (1975) as unreasonably high and may be due to molecular aggregation. To be certain that molecular aggregation through hydrogen bonding was not occurring, samples were run in the above mobile phase containing 4 M urea. The SEC/LALLS results indicated very little change in the M_w and *MWD* values of guar in the presence of urea. The intrinsic viscosity of guar was also found to be unaffected in the presence of 4 M urea. The lack of any effect of urea on these parameters suggests that the observed molecular weight of guar is not due to molecular aggregation caused by hydrogen bonding. These observations on the effects of urea on the SEC and intrinsic viscosity of guar are in good agreement with those of Barth & Smith (1981).

Another objective of the study was to investigate the validity of using monodisperse sodium polystyrene sulfonate as an SEC standard for aqueous polymer characterization work. The SEC data file corresponding to Fig. 4 was recalculated using the calibration curve of polystyrene sulfonate shown in Fig. 3. This gave an M_w of $1{\cdot}3 \times 10^6$ and an *MWD* of 1·7, a result in poor agreement with that of SEC/LALLS data.

This shows that use of monodisperse polystyrene sulfonate polymers as a secondary calibration standard for the aqueous SEC of water soluble polymers such as guar is not satisfactory. The results to hand also show that use of polystyrene sulfonate calibration data to calculate the M_w and *MWD* of guar significantly underestimates these parameters. This suggests that a given retention volume, under the chromatographic conditions of this study, corresponds to a lower molecular weight of polystyrene sulfonate, as compared to that of guar.

Several factors could be responsible for the difference in the hydrodynamic size and shape of dissolved sodium polystyrene sulfonate and guar molecules in the mobile phase. One is due to electrostatic expansion of the sodium polystyrene sulfonate macroion at a moderate ionic strength (0·1 M) of the mobile phase (a feature common to polyelectrolytes but not important to non-ionic polymers such as guar). Another factor is the importance of ion exclusion of polyions by untreated packings, such as Fractosil, used in the study. It is possible to minimize these polyelectrolyte effects through the use of a higher ionic strength mobile phase. A minimum ionic strength of 0·5 M is

needed to fully swamp out the polyelectrolyte effect (Omoroidon *et al.*, 1981; Bose *et al.*, 1982). However, at these higher ionic strengths, the SEC resolution of polystyrene sulfonate was poor, a result in agreement with that of Omoroidon *et al.* (1981).

As discussed above, the size and shape of a dissolved polymer, especially a polyelectrolyte such as polystyrene sulfonate, and its chromatographic separation behavior are very sensitive to the environment of the mobile phase and other chromatographic conditions. Hence, it would be a very difficult task to optimize a set of conditions where polystyrene sulfonate could be run and used as a universal calibration standard for the aqueous size exclusion chromatography of all classes of water soluble polymers. However, although the use of sodium polystyrene sulfonate as a universal calibrant may not be applicable, it would seem to offer some value in obtaining useful comparative data under 'customized' optimal' conditions, as suggested by Omoroidon *et al.* (1981).

The above SEC/LALLS technique was also used to characterize a chemically depolymerized guar. The results are shown in Fig. 5. The

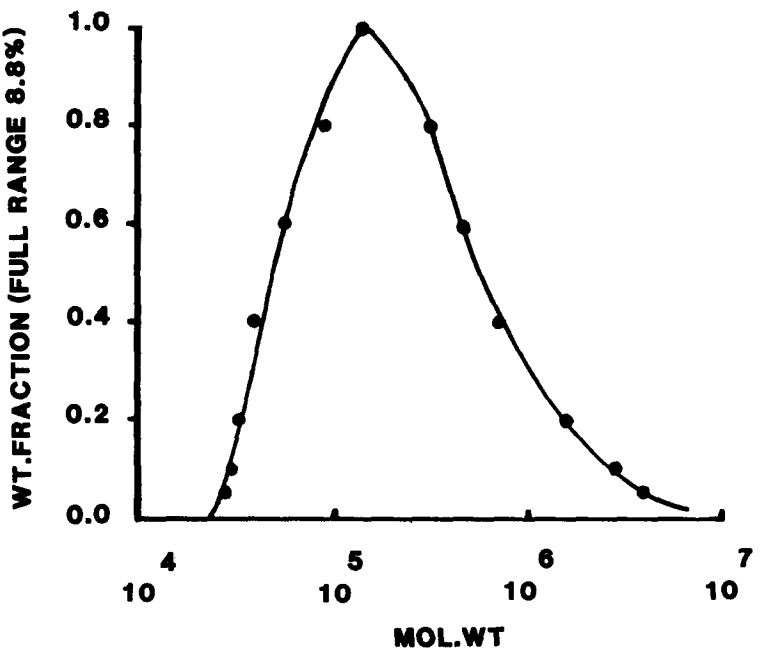

Fig. 5. SEC/LALLS *MWD* of chemically depolymerized guar.

M_w and *MWD* of the degraded sample were found to be $3{\cdot}1 \times 10^5$ and 3·0 respectively.

The SEC/LALLS described here has some inherent limitations which must be recognized not only when used on guar but also when used for the characterization of other water soluble polymers. In general, the technique underestimates the true molecular weight and *MWD* of the total sample. Due to the need for rigorous cleaning up of the sample, the data for the sample analyzed is biased and ignores the contribution of larger molecules removed during the sample preparation. We observed an adsorption of about 10% of the injected sample by the silica packing column. Usually the higher molecular weight fractions are adsorbed preferentially in polydisperse systems (Cohen Stuart *et al.*, 1980), thus tending to ignore the presence of the larger molecular weight fraction in the total sample. We also mentioned that the resolution of the untreated packing columns is poor. This means that a polymer fraction analyzed by the KMX detector is not monodisperse but polydisperse. This would give an M_w rather than a true M_n for the fractioned sample. This leads to an underestimation of the width of the *MWD*, but appropriate corrections can be made to this as discussed in some recent publications (Hamielec, 1980).

In summary, the SEC/LALLS technique outlined in this paper provides useful information on the absolute molecular weight and *MWD* of complex water soluble polymers. However, they are biased towards the lower molecular weight fractions of the sample. Availability of improved (reduced adsorption and polymer-packing interactions) and high efficiency (better resolution) columns for the aqueous SEC of high molecular weight water soluble polymers ($M_w > 10^6$) in the future would correct some of these limitations and would offer considerable progress in the better characterization of water soluble polymers.

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