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Note

High-performance size-exclusion chromatography of guar gum*

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Guar gum (referred to as guar in this paper) is obtained from the endosperm of the seed of the guar plant, *Cyanaposis tetragonolobus*. Guar consists mainly of galactomannan with small amounts of protein, fiber and oils[1]. As shown in Fig. 1, the chemical structure of guar is composed of a linear chain of D-mannose units linked together by (1→4)-β-glycosidic linkages. On alternate D-mannose units there is a single D-galactose group attached to the mannose by an (1→6)-α-glycosidic linkage.

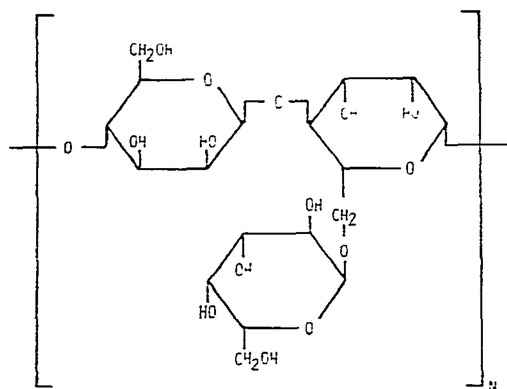

Fig. 1. Structure of guar gum.

The molecular-weight distribution (MWD) of guar is an important parameter in the characterization of this polysaccharide. However, a literature search has revealed no published reports on size-exclusion chromatography (SEC) of guar. The goal of this work is to apply a previously developed high-performance SEC procedure for polysaccharides[2–4] to determine the MWD of guar.

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EXPERIMENTAL

Apparatus

A Waters Assoc. 6000A liquid chromatograph and 401 differential refractometer were employed. The refractometer was thermostated to 23–24°C with a Haake FE water bath. Stagnant mobile phase was kept in the reference side of the refractometer. Samples were injected with a Rheodyne 70-10 injection valve.

Columns

The packing material consisted of a glycerylpropylsilyl layer covalently bonded to LiChrospher silica particles (10 μm). This was purchased prepacked in 25 cm × 4.1 mm I.D. stainless-steel columns from SynChrom (Linden, IN, U.S.A.). Nominal pore sizes used in this study were 100 and 4000 Å. Columns were arranged in series with the smaller pore-size support placed first.

Mobile phase

All mobile phases were prepared with distilled water and reagent-grade chemicals. They were filtered under vacuum by the use of a 0.22-μm membrane filter (Type GS; Millipore, Bedford, MA, U.S.A.). The previously used pH 3.7 mobile phase was prepared by first adding 60 ml of 4 *M* sodium acetate and 440 ml of 4 *M* acetic acid to a 1-l volumetric flask and filling to volume with water. This gives a pH 3.7 buffer of 0.24 *M* ionic strength. The ionic strength of this solution is then increased to 1.44 *M* by adding 0.4 moles of sodium sulfate to 1 l of the 0.24 *M* acetate solution. This solution is then diluted 1:1 with water and used as the mobile phase. The 1.44 *M* solution is used for sample preparation.

Standards

The following dextrans were obtained from Pharmacia (Uppsala, Sweden): T10, T20, T40, T70, T110, T150, T250, T500 and T2000. These have weight-average molecular weights of $10 \cdot 10^3$, $20 \cdot 10^3$, $40 \cdot 10^3$, $70 \cdot 10^3$, $110 \cdot 10^3$, $150 \cdot 10^3$, $500 \cdot 10^3$ and $2000 \cdot 10^3$, respectively. Glucose was used to determine the permeated column volume.

Viscosity determinations

Intrinsic viscosities were determined at 25°C with Ubbelohde capillary viscometers at six to eight concentrations and extrapolated to zero concentration. No shear rate corrections of the viscometers were made.

Recommended procedure

Sample preparation. Approximately 800 mg of guar was added to 400 ml of water and stirred for 1 h. The solution was then heated to 100°C, while stirring for 1 h. Stirring was continued overnight at room temperature. The resulting solution was sequentially filtered through Whatman No. 41 filter paper (20–25 μm particle retention), Whatman GF/F glass fiber filter (0.7 μm particle retention, 47 mm diameter) and finally through a 0.45-μm Millipore membrane filter (Type HA, 47 mm diameter). The solution was then diluted 1:1 with double strength mobile phase and further diluted with mobile phase to give a final concentration of <0.5 mg/ml. The concentra-

tion of guar was determined gravimetrically by heating an aliquot of the original solution at 70°C to dryness followed by 70°C in a vacuum oven for about 6 h.

Chromatographic conditions. With the 100–4000 Å column set and a refractometer attenuation set at ×1, 20 μl of sample solution were injected in triplicate at a flow-rate of 0.5 ml/min.

RESULTS AND DISCUSSION

Our previously established mobile phase, pH 3.7 acetate buffer of 0.7 ionic strength[2–4], was employed for the analysis of guar. Although this relatively high ionic strength buffer was developed specifically to chromatograph polyelectrolytes, it was applied to the analysis of guar, a non-polyelectrolyte. Thus, a lower ionic strength buffer might also have worked for these neutral polymers[5], but was not evaluated. According to the literature[6], guar is compatible with high concentrations of salt and stable over a wide pH range (1–10.5) without any significant viscosity change.

Because of the high molecular weight of guar, a 4000-Å pore-size packing was used. A 100-Å pore-size packing was also employed in order to separate salts and extraneous low molecular weight material from the polymer peak[2]. In order to minimize chromatographic viscosity effects, injected concentrations were kept below 0.05%[2,4].

Fig. 2 is a composite chromatogram of three guar samples which are described in Table I. For clarity the totally permeated peak has been omitted. Sample A is guar gum and samples B and C have been viscosity reduced with base. The order of elution, based on peak maxima, is in agreement with Brookfield viscosity data. As previously discussed[2], the 4000-Å column has poor resolution because of its large pore-size distribution. In order to increase resolution, additional 4000-Å columns could be employed.

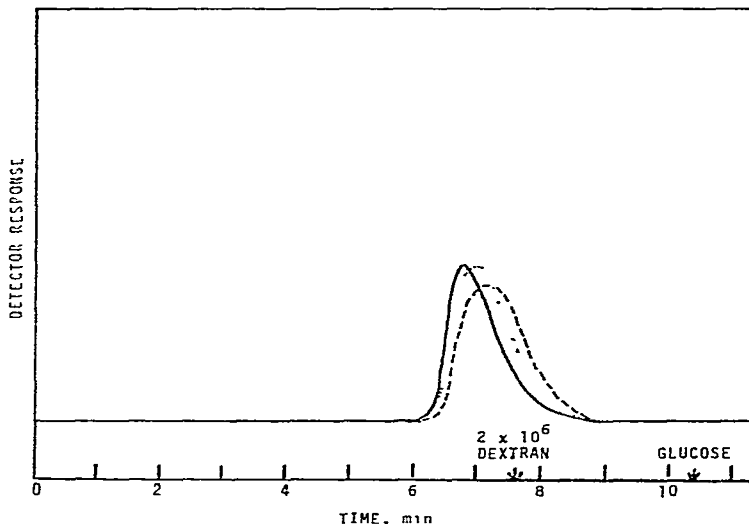

Fig. 2. High-performance SEC of guar gum. Mobile phase, 0.7 ionic strength acetate buffer, pH 3.7; column-set, 100–4000 Å SynChropak; injection volume, 20 μl; flow-rate, 0.5 ml/min (1000 p.s.i.); sample concentration, 0.35 mg/ml; refractive index detector attenuation, ×1; chart speed, 2 cm/min. Samples: ———, A; · · · · ·, B; ———, C.

TABLE I

CHARACTERISTICS OF GUAR SAMPLES

Parameter	*Sample*		
	A	*B**	*C**
Brookfield viscosity 1 %, cP**	5200	2900	830
Protein (%)	4.0	2.1	3.4
Solubles found in filtrate (%)***	71	72	73

* Base hydrolyzed.

** Viscosity measured on unfiltered solutions at 25°C using a number 4 spindle at 20 rpm.

*** Gravimetric analysis.

From the calibration curve shown in Fig. 3, the molecular weight of these guar samples was greater than the $2 \cdot 10^6$ molecular-weight dextran standard. Considering the highly branched structure of guar (Fig. 1), one would assume that, for a given molecular weight, it would be more compact than dextran, a more linear polysaccharide. Average molecular weights could not be calculated because of the lack of appropriate standards which would elute within the range of guar. According to the literature, weight-average molecular weights of guar have been reported to be $532 \cdot 10^3$ (ref. 7), $817 \cdot 10^3$ (ref. 7), $950 \cdot 10^3$ (ref. 8), $1.7 \cdot 10^6$ (ref. 9) and $1.9 \cdot 10^6$ (ref. 10). Furthermore, Koleske and Kurath[11] had prepared acetylated guar gum and fractionated this into a number of fractions. The two highest molecular weight fractions, which represented 46.3% of the original polymer, had weight average molecular weights of 4.8 and $5.3 \cdot 10^6$. One of these fractions had a molecular weight range of 0.5 to $11 \cdot 10^6$ as determined from sedimentation coefficients.

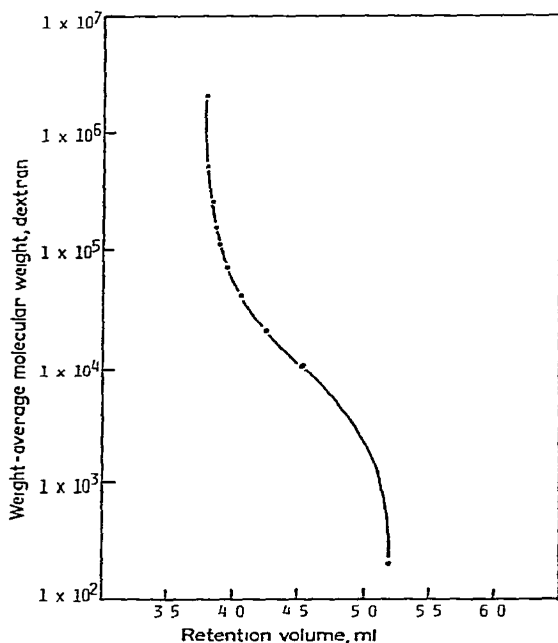

Fig. 3. Dextran calibration curve. See Fig. 2 for chromatographic conditions.

These large variations might be partially caused by the differences in the guar source and/or isolation methods of the galactomannan. Dea and Morrison[8] have suggested that the $1.7 \cdot 10^6$ value seems unreasonably high and may be due to molecular aggregation. A similar explanation was proposed by Hui and Neukom[10] who obtained a value of $1.9 \cdot 10^6$. They had observed a significant viscosity reduction of guar solutions when alkali was added and attributed this to the dissociation of galactomannan aggregates which might have been present in aqueous solution. Shcherbukhin *et al.*[12] have also reported the presence of aggregation of glucomannans in solution. Finally, Chudzikowski[13] speculated that the viscosity loss in alkali solutions may be due to the destruction of proteins which might form a complex with the polysaccharide.

To assure that molecular aggregation through hydrogen bonding was not occurring, samples were chromatographed in 0.7 ionic strength mobile phase containing 6 *M* urea. The results indicated that there was only a slight increase in elution volumes in the presence of urea.

In addition to adding urea to the mobile phase, the guar A sample was sonicated for 30 min at 80 W. The resulting chromatograms, shown in Fig. 4, clearly demonstrate a decrease in molecule size. However, it is not clear whether or not sonication lead to the disruption of aggregates or to cleavage of glycosidic bonds.

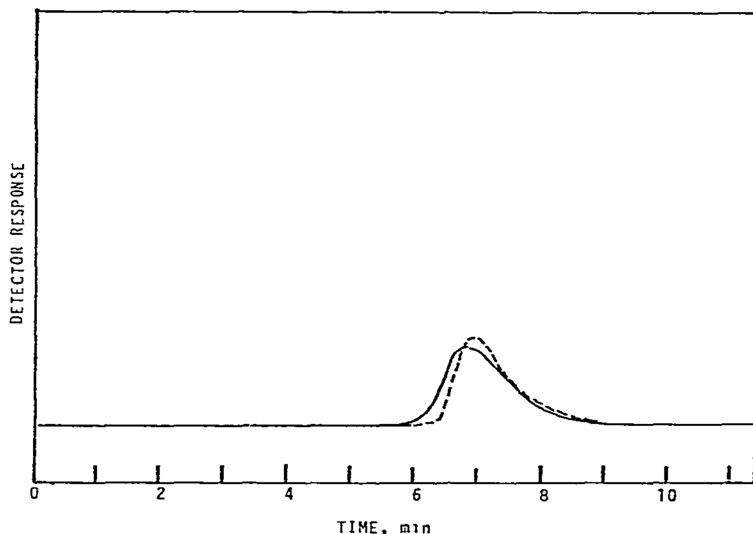

Fig. 4. High-performance SEC of guar gum, sample A, before (———) and after (- - - -) sonication for 30 min at 80 W. See Fig. 2 for conditions (refractive index detector attentuation, ×2).

The intrinsic viscosity of guar A was determined in water, 6 *M* urea in mobile phase, and 2 *M* guanidine–HCl. The data, presented in Table II, suggest that there was no significant viscosity change in 6 *M* urea, in agreement with SEC results. In 2 *M* guanidine–HCl, there was a small decrease suggesting that possibly a minimal amount of disaggregation had occurred.

The SEC method described in this paper has also been applied to carboxymethyl guar and hydroxypropyl guar.

TABLE II

INTRINSIC VISCOSITIES OF GUAR A

Solution	*[η]*
Water	17.4
6 *M* urea in mobile phase*	17.8
2 *M* guanidine-HCl	16.2

* 0.7 μ acetate buffer, pH 3.7.

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