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Macromolecular Distribution of *Acacia senegal* Gum (Gum Arabic) by Size-Exclusion Chromatography*

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SUMMARY

Fractionation of Acacia senegal *gum has been carried out on Sephacryl gels S-400 and S-500. The shape and relative height of the chromatograms are sample dependent.*

Physicochemical data including circular dichroism and the weight-average molecular weights distribution show that about 70% of the material is composed of homogeneous polysaccharide chains with a very low nitrogen content. The remaining material is a combination of polysaccharide and nitrogen moieties (probably an arabinogalactan-protein complex).

Our data are consistent with the new and simplified structural models proposed recently for this polysaccharide.

INTRODUCTION

The gum exuded from *Acacia senegal* has important uses. Although many interesting results have been published previously, the mechanism of biosynthesis and detailed structure still remain unknown. These issues are reviewed in the literature (Anderson & Dea, 1971; Glicksman & Sand, 1973; Clarke *et al.*, 1979; Churms *et al.*, 1983, Street & Anderson, 1983; Ullmann, 1983).

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Direct evidence of heterogeneity has been obtained using both electrophoresis (Lewis & Smith, 1957) and ion-exchange chromatography (Jermyn, 1962). Jermyn concluded that gradient elution (one single peak) and stepwise elution demonstrate the heterogeneity 'but it cannot be said whether this heterogeneity is physical or chemical'.

Chemical and physicochemical analysis of materials prepared by fractional precipitation with sodium sulphate (Anderson & Stoddart, 1966; Anderson *et al.*, 1967) have led the authors to propose the term heteropolymolecularity for *A. senegal* gum, i.e. 'a polymer system having either a variation in monomer composition and/or a variation in the mode of linking and branching of the monomer units, in addition to a distribution in molecular weight'.

In the ensuing study, size-exclusion chromatography on Bio-gel P 300 has been used to estimate number-average molecular weights of the fractions. However, the gel is not efficient enough as a fractionation medium for the whole gum, which possesses both a very high molecular weight and size.

Recently, Fujiwara & Arai (1982) have reported the elution pattern of gum arabic on Sepharose 6B in comparison with the water-soluble polysaccharide of *Phellodendron amurense Ruprecht* harvested in Japan (P-WSPS). The latter exhibits a simple sharp peak, contrary to gum arabic whose elution pattern is discontinuous and broad. The authors concluded that P-WSPS is highly homogeneous without further investigation into gum arabic.

Introduction of new chromatographic materials can considerably improve the resolution. Also, accurate molecular weight data without the clarification problems previously reported (Veis & Eggenberger, 1954; Anderson & Rahman, 1967; Anderson *et al.*, 1967) can now be obtained by low angle laser light-scattering.

In addition, further information about the heteropolymolecularity can be reached by spectroscopic methods. For example, although the optical rotation values at 589 nm are quite constant whatever the samples, this wavelength is far from the electronic transitions of chromophores and a detailed study of optical rotatory dispersion and circular dichroism in correlation with absorption spectra can provide new information when comparing the properties of different fractions. Therefore, the new results arising from a number of samples fractionated on Sephacryl S-400 and S-500 which are given in this paper supplement and complete the earlier studies.

EXPERIMENTAL

Materials

The samples were kindly supplied by Iranex S.A. and were collected in the Province of Kordofan (Republic of the Sudan). Admixture with species other than *A. senegal* can be safely disregarded in view of some of the characteristics that we have determined (e.g. equivalent weight and specific rotation), complementary analytical data by ^{13}C-n.m.r. (Defaye & Wong, 1984) and g.l.c. studies of derivatives of some of these same materials (Ullmann, 1983).

Samples 1–6, 23 and 24 are individual nodules (in general from 5 g–8 g), light in colour and clean, which were studied one by one. Sample 13 is a mixture of industrial nodules of which the viscosity lies between 13·3 and 20·0 ml g^{-1}. Samples 20, 21 and 25 were prepared in the laboratory by admixing in each case about 100 g of three different batches of nodules.

Purification

The gum was usually dissolved in water, filtered and electrodialysed or precipitated by the addition of ethyl alcohol (Thomas & Murray, 1928). Electrodialysis leads to arabic acid with the risk of excessive heating. Ethyl alcohol can induce fractionation of low molecular weight species. In order to ensure that the purification procedure did not disturb the samples, the following procedure was adopted.

Crude nodules and commercial atomized or ground gum were dissolved by magnetic stirring in deionized water at room temperature and at a concentration of from 5% to 10%. After standing overnight, solutions were filtered by successive passage through 8-, 1·2-, 0·65- and 0·45-μm Millipore filters and then subjected to ultrafiltration using a Millipore low retention volume system equipped with Pellicon membranes of a nominal molecular weight limit of 10^4 daltons. Ultrafiltration was considered complete when the resistance in the filtrate channel was larger than 6×10^5 ohms. There are considerable theoretical as well as the practical time saving (less than 1 h) advantages using ultrafiltration when compared with bag dialysis. The purified aqueous solutions were either stored at 4°C, or freeze-dried to constant weight.

Determination of concentrations of polysaccharides

The concentrations of dissolved polysaccharides were determined by oven drying overnight at 104°C. Redissolved freeze-dried samples led to the same results within a variation of 1%.

Size-exclusion chromatography

Aliquots (5 ml) of the solution of polysaccharide (about 1% in molar sodium chloride) were injected into a column (2·6 × 60 cm) of Sephacryl S-400 or Sephacryl S-500 (Pharmacia Fine Chemicals, Uppsala, Sweden) previously equilibrated in the same media. Elution was also performed by molar sodium chloride at a flow-rate of 135 ml h^{-1} (25·5 cm h^{-1}) and controlled by ultraviolet absorption at 214 nm using a single path monitor UV-1/214 (Pharmacia Fine Chemicals) detector equipped with a flow cell of optical path length 3 mm. The void volumes of these columns were determined by chromatograms of Blue Dextran T-2000.

Semi-preparative size-exclusion chromatography

A preliminary experiment first checked that injection of samples up to 10 ml (3% of the column volume) and at concentrations of up to 3% did not affect significantly the chromatograms at the same flow-rate (~ 135 ml h^{-1}). Three arbitrary fractions were recovered, as shown in Fig. 1, after several repetitive injections of 10 ml of 2% samples (sample 20, 4 injections; sample 21, 7 injections; sample 25, 8 injections and 14 injections at 3·17%). Identical eluates were mixed and desalted by ultrafiltration using a Millipore low retention volume cassette system, as described previously. Solutions were then finally concentrated up to about 20 ml using an ultrafiltration cylindrical cell equipped with the same membranes (PTGC Millipore). They were then freeze-dried.

Some loss of material appeared in the successive operations and the total yield when compared with the quantity injected initially was 69% for sample 21, 85% for sample 22 and 72% for sample 25.

For samples 13 and 25, fractions of 19·5 ml have been collected in which the concentration of *A. senegal* gum was measured by polarimetry at 589 nm. It has been established that viscosities and molecular

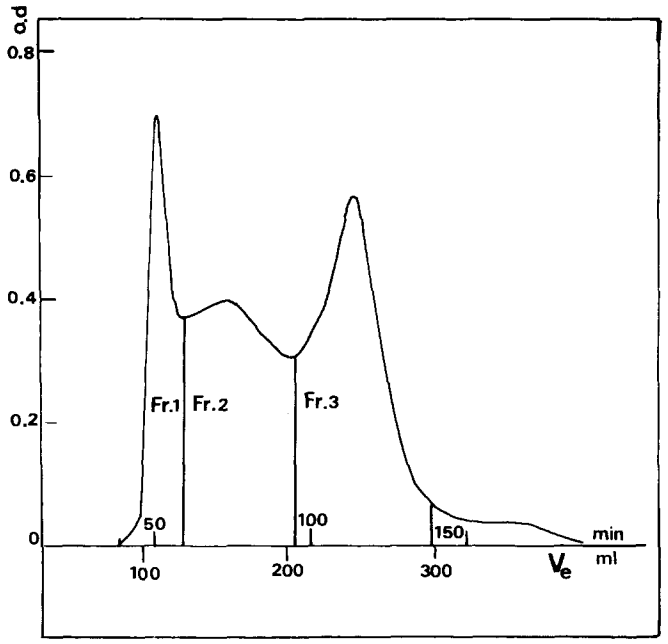

Fig. 1. Size-exclusion chromatography of *A. senegal* gum (sample 25) on Sephacryl S-500 gel in molar sodium chloride. Injection of 10 ml of sample (C = 2·04%). Column 2·5 x 64 cm, flow-rate 132 ml h^{-1}. Intrinsic viscosity 22·8 ml g^{-1}. U.v. detection at 214 nm, optical pathlength 0·3 cm.

weights determined on the fractions are near to intrinsic values, as measurements were performed under approximately θ conditions.

Light scattering

Light-scattering measurements were carried out at room temperature by using a low angle laser KMX-6 Chromatix light-scattering photometer. Solutions were filtered through 0·22 μm Millipore membranes to eliminate dust; no polymer adsorption was observed.

The techniques offer many advantages: measurements at low angles (2–7°) do not require double extrapolation (zero angle and concentration) which has been stated to be tedious for gum arabic solutions (Veis & Eggenberger, 1954; Anderson *et al.*, 1967), it is sufficient to

prepare some ml of the more highly concentrated solution at about 0·1%. The weight-average molecular weight $\bar{M}_w$ is calculated from

$$\frac{KC}{\Delta R_\theta} = \frac{1}{\bar{M}_w} + 2A_2C$$

where K is a constant depending on geometrical factors of the apparatus, refractive index of the solvent and refractive index increment of the solution; C is the concentration; A_2 is the second virial coefficient; and ΔR_θ is the measured excess of Rayleigh factor between solution and solvent. The refractive index increment was determined by a Brice-Phoenix differential refractometer at 546 nm and 25·0°C calibrated with KCl. A value of 0·150 ml g^{-1} was found for various samples in agreement with previous literature data (Veis & Eggenberger, 1954; Swenson *et al.*, 1968; Anderson & Dea, 1971). Taking into account this value, the constant K is $1{\cdot}623 \times 10^{-7}$.

Viscometry

Viscometry was conducted in an Ubbelohde Fica Viscomatic automatic system at 25·0°C. The flow time for the solvent (molar sodium chloride) was 69·5 s.

Equivalent weight

Arabic acid was prepared by passing *A. senegal* gum through a column containing Dowex 50 W X-8 (H^+ form, 20–50 mesh) ion-exchange resin. The solution was titrated with sodium hydroxide added with a Gilmont S 3200A microburette.

Absorption spectroscopy

Absorption data were recorded at room temperature with a Cary 15 spectrophotometer. At wavelengths less than 210 nm the apparatus was purged by nitrogen. Extinction coefficients were expressed in 10^3 cm^2 $mole^{-1}$ using 1250 as equivalent weight.

Extinction coefficients of isolated fractions at 214 nm

These were measured with the same detector as during separation. To simplify, ϵ is expressed in Tables 2–4 using % weight concentrations, i.e. in 10^2 cm^2 g^{-1}.

Optical rotation

The o.r.d. curves were recorded with a Fica Spectropol I spectropolarimeter at 25°C. Some optical rotation measurements were determined at 589 nm using a Perkin–Elmer 241 polarimeter. The specific rotation $[\alpha]$ is defined, as usual, by $[\alpha] = 100\alpha/lC$ where α is degree of rotation, l the pathlength in dm and C = the concentration in g (100 ml)$^{-1}$.

Circular dichroism

Circular dichroism spectra were recorded with a Jasco J-40B dichrometer at room temperature.

All spectroscopic measurements were recorded with Hellma QS quartz cells.

Nitrogen determination

Nitrogen was determined by the Service Central d'Analyse of the Centre National de la Recherche Scientifique.

RESULTS AND DISCUSSION

To overcome polyelectrolyte effects in size-exclusion chromagraphy (Rochas *et al.*, 1980), all experiments have been conducted in molar sodium chloride. Such a concentration was chosen because earlier physicochemical parameters in the literature had been measured in this medium (Anderson & Rahman, 1967; Anderson *et al.*, 1967). Physicochemical data on some of the unfractionated samples investigated are given in Table 1. (These results are typical of what has been observed in a larger batch of samples.)

TABLE 1
Physicochemical Data for some *A. senegal* Gums

Sample	$[\eta]^a$ *(ml g^{-1})*	$\bar{M}_w{}^b$ *(daltons)*	$M_{eq}{}^c$ *(g)*	$[\alpha]_{589}^{25}{}^d$ *(degrees)*
1	40·0	$2{\cdot}2 \times 10^6$	1102	–
2	16·2	$4{\cdot}4 \times 10^5$	1184	−33·0
3	24·0	$8{\cdot}3 \times 10^5$	1237	−34·4
4	31·0	$1{\cdot}4 \times 10^6$	1113	−32·3
5	15·5	–	1224	−29·6
6	21·8	$7{\cdot}1 \times 10^5$	1264	–
23	30·0	$1{\cdot}3 \times 10^6$	1285	–
24	16.0	$4{\cdot}0 \times 10^5$	1160	–

[a] Intrinsic viscosity in molar sodium chloride.
[b] Weight average molecular weight determined by laser light-scattering photometry.
[c] Equivalent weight.
[d] Specific rotation at 589 nm in molar sodium chloride.

The chromatograms on gel S-400 are shown in Fig. 2. *A. senegal* gum was always eluted in a rather complicated system of several peaks, the shape and relative height of bands being viscosity dependent.

Three or four main peaks, identified as A, B, C and D, invariably appeared. Peak A corresponded to the void volume V_0 of the column, also determined by chromatography of Blue Dextran T-2000 ($V_0 \sim$ 105 ml). The geometrical inner volume of the column, V_t was of 313 ml and it can be seen that peak D was located just prior to V_t. It was also established that low molecular weight molecules such as galactose were eluted in this same region.

When peak A was very large, peak B did not appear. Peak C was always present.

Peak B appeared as a shoulder of peak A when intrinsic viscosities were around 20 ml g^{-1}. It was thus interesting to investigate the behaviour of *A. senegal* gum on Sephacryl S-500 which is more porous.

The results for two samples whose intrinsic viscosities were 30 ml g^{-1} (23) and 16 ml g^{-1} (24) respectively are shown in Fig. 3. When viscosity was high, it can be seen that Sephacryl S-500 was more efficient and could partially resolve peak A observed on S-400. When viscosity was

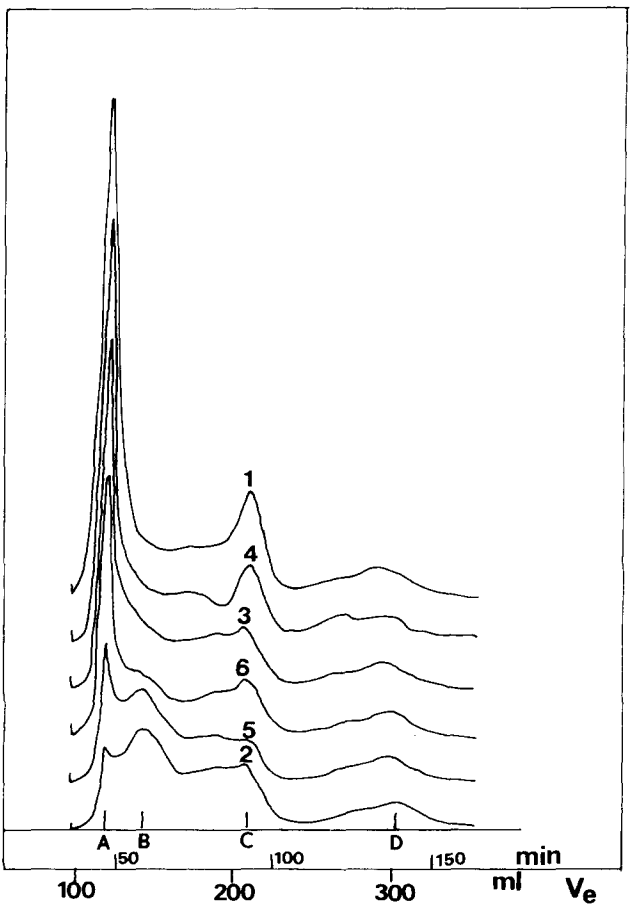

Fig. 2. Size-exclusion chromatography of *A. senegal* gum on Sephacryl S-400 gel in molar sodium chloride. Injection of 5 ml of sample (C = 1%). Column 2·5 x 59 cm, flow-rate 134 ml h^{-1}. Intrinsic viscosities: (1), 40·0 ml g^{-1}; (4), 31·0 ml g^{-1}; (3), 24·0 ml g^{-1}; (6), 21·8 ml g^{-1}; (5), 15·5 ml g^{-1}; (2), 16·2 ml g^{-1}.

low, no more material was eluted in the void volume of Sephacryl S-500.

At this stage, we have been able to demonstrate polydispersity because of the availability of new materials for size-exclusion chromatography.

Besides qualitative data, size-exclusion chromatography can give valuable quantitative information on molecular weight distribution.

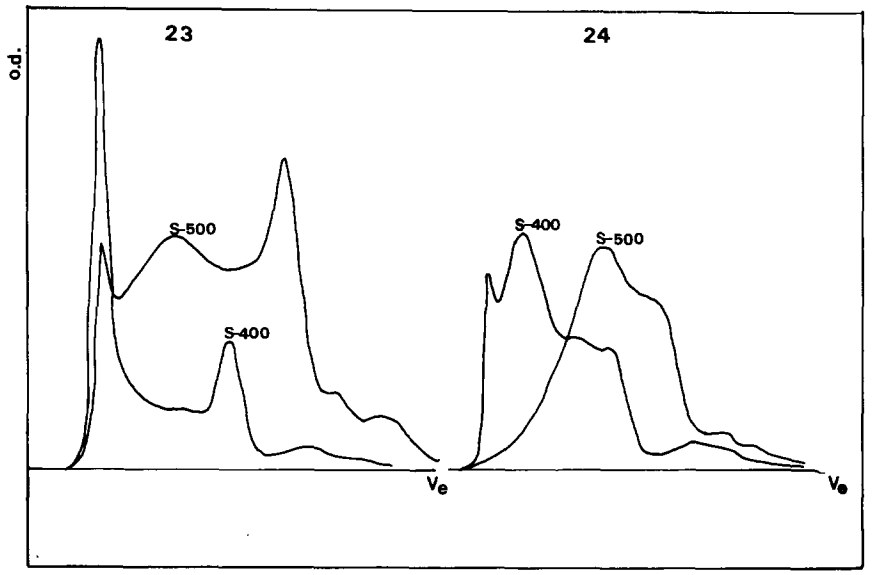

Fig. 3. Size-exclusion chromatography of *A. senegal* gum on Sephacryl S-400 and S-500 gels. Same conditions as in Fig. 2. Intrinsic viscosities: sample 23, 30 ml g^{-1}; sample 24, 16 ml g^{-1}.

A calibration plot of elution volume using known dextran fractions can be obtained, but it is well known that steric-exclusion chromatography is mainly governed by the size of macromolecules, i.e. both viscosity and molecular weight; such an attempt seems unjustified for *A. senegal* gum due to its highly branched nature when compared with dextran. It is also interesting to evaluate the relative area under the various peaks. This assumes that extinction coefficients are not fraction dependent whatever the elution volume. We have observed that the whole area under the chromatograms is decreasing for samples 1–6 in the same order as the viscosities; the ratio between 1 and 2 is about 1·5 for the same injected mass. Thus, integration by use of optical density data is not a safe method and has been rejected. An explanation of this observation is given below.

A reliable plot for $\bar{M}_w$ versus V_e for demonstrating polydispersity needs semi-preparative work to obtain fractions. Moreover, valuable information about heterogeneity can be reached if enough material of particular fractions is prepared. Accumulation and purification of three

TABLE 2
Physicochemical Data for Fractionated *A. senegal* Gums

Sample	$[\eta]^a$ *(ml g^{-1})*	$[\alpha]_{589}^{25\,b}$ *(degrees)*	$\epsilon_{214}{}^c$	x^d *(%)*	$M_{eq}{}^e$ *(g)*	*N%*^{f}
20	20·6 (20·1)^j	−29	6·8 (6·7)^k	–	1125	0·29
20-1^g	40·0	−27	15·7	4·5	–	–
20-2^h	40·0	−31	13·3	26·0	–	–
20-3^i	12·5	−26	4·0	63·6	1240	–
21	21·0 (22·6)^j	−27	7·3 (7·2)^k	–	1173 (1217)^l	0·30
21-1^g	84·0	−34	21·0	4·6	970	–
21-2^h	41·0	−29	14·7	26·0	1295	–
21-3^i	12·0	−25	3·6	67·5	1238	<0·1
25	22·8 (18·8)^j	−32	6·0 (5·8)^k	–	1183 (1264)^l	0·30 (0·26)^m
25-1^g	74·0	−30	19·9	6·0	1026	0·86
25-2^h	34·0	−26	11·0	21·3	1254	0·63
25-3^i	9·3	−28	2·9	76·6	1222	<0·1

[a] Intrinsic viscosity in molar sodium chloride.
[b] Specific rotation at 589 nm in molar sodium chloride.
[c] Extinction coefficients at 214 nm in 10^2 cm^2 g^{-1} using concentration in %.
[d] $x = 100\ (\epsilon/\epsilon_F)\ (s_F/s)$ where ϵ and ϵ_F are the extinction coefficients of whole gum and its fractions and s and s_F are, respectively, the full and fractional areas under the chromatograms.
[e] Equivalent weight.
[f] Percent of the dry and purified material.
[g,h,i] Fractions 1, 2 and 3, respectively (see Fig. 1).
[j] Reconstituted viscosity from x.
[k] Reconstituted extinction coefficient from x.
[l] Reconstituted equivalent weight from x.
[m] Reconstituted *N*% from x.

arbitrary fractions as shown in Fig. 1 for sample 25 have been performed for three samples (20, 21, 25) whose intrinsic viscosities are around 20 ml g^{-1}. Physicochemical data are given in Table 2. Extinction coefficients of the fractions were found to be very different from one another. Also, the respective recovery in each fraction was not connected to the relative area under the chromatograms. Therefore, ultraviolet detection is only useful as a qualitative tool though more convenient than colori-

metric methods such as the phenol–sulphuric acid reaction (Dubois *et al.*, 1956). The experimental method used to recover and purify the fractions leads to some loss of material. In order to calculate the relative ratio x of each fraction F, we have used the following relation between extinction coefficients and areas under the chromatograms: $x = 100\ (\epsilon/\epsilon_F)\ (s_F/s)$, where s and s_F are, respectively, the total and fraction areas under the chromatograms and ϵ, ϵ_F the extinction coefficients of the whole gum and its fraction F. This method leads to fair results within experimental errors: the total balance (sum of relative ratios) is near 100%, the reconstituted values for whole gum using x_F and viscosities, extinction coefficients, equivalent weight and N% of fractions are close to the experimental ones.

It can be seen in Table 2 that around 70% of the material is composed of the low viscosity species (fraction 3). Our results are in general agreement with Anderson's data (Anderson & Stoddart, 1966; Anderson & Rahman, 1967). The yield of high viscosity fractions by sodium sulphate precipitation (33·5 ml g^{-1} and 25·4 ml g^{-1} in two different

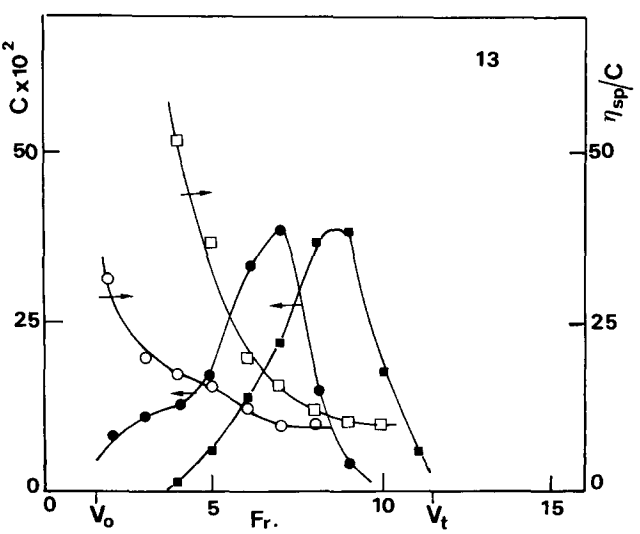

Fig. 4. Size-exclusion chromatography of sample 13 on Sephacryl S-400 and S-500 gels in molar sodium chloride. See conditions in Table 3. Concentrations of fractions (g dl^{-1}): ●, S-400; ■, S-500. Viscosities of fractions (ml g^{-1}): ○, S-400; □, S-500.

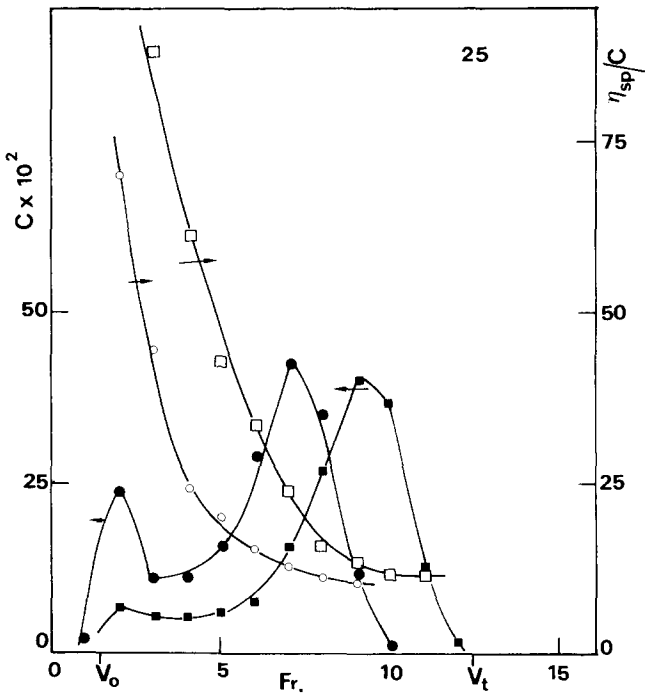

Fig. 5. Size-exclusion chromatography of sample 25 on Sephacryl S-400 and S-500 gels in molar sodium chloride. See conditions in Table 4. Concentrations of fractions (g dl^{-1}): ●, S-400; ■, S-500. Viscosities of fractions (ml g^{-1}): ○, S-400; □, S-500.

experiments) is of about the same order of magnitude as our fractions 1 and 2, but we have been able, by size-exclusion chromatography, to obtain very high viscosities of up to 84 ml g^{-1}. In our opinion, as fraction 1 is mainly eluted in the void volume, it cannot be arbitrarily dissociated from fraction 2 as discussed below.

The quasi constancy in $[\alpha]_D$ (Table 2) allows us to monitor the relationship between concentration and V_e. Two samples, 13 and 25, the properties of which are typical of non-viscous and viscous *A. senegal*, have been subjected to gel-filtration chromatography on both Sephacryl S-400 and S-500. Elution patterns are reported in Figs 4 and 5. Physico-chemical data, reduced viscosities, weight-average molecular weights and absorption have been measured on each fraction. Results are reported in Tables 3 and 4.

TABLE 3
Physiochemical Parameters of Fractions of *A. senegal* Gum (sample 13 [η] 15·5 ml g^{-1} $\bar{M}_w$ 4×10^5)

Fraction number	$C\% \times 10^2$ [a]	η_{sp}/C [b]	$\bar{M}_w$ [c]	ϵ [d]
A [e]				
2	8·7	31·9	$1·7 \times 10^6$	10·4
3	11·6	19·8	$9·4 \times 10^5$	12·5
4	12·6	18·5	–	11·1
5	17·6	15·1	$4·7 \times 10^5$	4·8
6	34·0	12·2	$2·8 \times 10^5$	–
7	39·3	9·8	$1·8 \times 10^5$	1·7
8	15·0	10·2	–	–
B [f]				
4	2·0	57·2	$3·4 \times 10^6$	17·8
5	5·7	37·4	$1·5 \times 10^6$	17·4
6	14·3	20·4	–	7·3
7	22·3	16·7	$4·2 \times 10^5$	6·0
8	37·0	12·7	$2·7 \times 10^5$	3·0
9	39·3	10·4	–	2·6
10	18·0	10·7	$2·1 \times 10^5$	–
11	6·7	–	–	–

[a] g (100 ml)$^{-1}$ obtained by measurements of rotation.
[b] ml g^{-1}.
[c] Direct determination.
[d] At 214 nm, using concentration in % (ϵ in 10^2 cm^2 g^{-1}).
[e] Gel Sephacryl S-400, column 2·6 x 58·1 cm, media M NaCl, flow rate 131 ml h^{-1} injected volume 10 ml, concentration 3·0%, V of each fraction 19·5 ml.
[f] Gel Sephacryl S-500, column 2·6 x 57·4 cm, media M NaCl, flow rate 133 ml h^{-1} injected volume 10 ml, concentration 3·0%, V of each fraction 19·5 ml.

The macromolecular distribution of 13 and 25 is clear from Fig. 6. It is possible to ensure that the figure for the material balance is similar for both gels and also that the opportunity of using either Sephacryl S-400 or S-500 is obvious for high viscosity fractions. For both samples, around 70% of the material exhibits viscosities lower than 15 ml g^{-1}. The difference between the two samples can be mainly attributed to higher viscosity fractions in the remaining 30% of material in 25 when

TABLE 4
Physicochemical Parameters of Fractions of *A. senegal* Gum (sample 25 $[\eta]$ 22·8 ml g^{-1} $\bar{M}_w$ $9{\cdot}1 \times 10^5$)

Fraction number	$C\% \times 10^2$ [a]	η_{sp}/C [b]	$\bar{M}_w$ [c]	ϵ [d]
A [e]				
2	24·4	70·1	$4{\cdot}0 \times 10^6$	13·6
3	10·9	45·1	$2{\cdot}0 \times 10^6$	12·2
4	10·9	24·7	$7{\cdot}7 \times 10^5$	6·9
5	15·6	20·2	–	3·7
6	29·1	15·3	$3{\cdot}2 \times 10^5$	2·3
7	43·2	12·9	–	2·0
8	35·7	11·5	$1{\cdot}8 \times 10^5$	2·9
9	11·3	12·4	–	–
B [f]				
2	7·2	121·1	$4{\cdot}1 \times 10^6$	20·2
3	5·0	89·6	$4{\cdot}0 \times 10^6$	18·2
4	5·4	61·3	$2{\cdot}4 \times 10^6$	15·6
5	6·3	43·9	$2{\cdot}1 \times 10^6$	13·1
6	6·9	34·5	$1{\cdot}1 \times 10^6$	10·5
7	15·1	19·3	–	4·8
8	27·0	16·6	–	2·9
9	40·9	14·3	$2{\cdot}5 \times 10^5$	2·4
10	37·4	12·2	–	2·6
11	13·5	12·7	–	3·6
12	2·2	–	–	–

[a] g (100 ml)$^{-1}$ obtained by measurements of rotation.
[b] ml g^{-1}.
[c] Direct determination.
[d] At 214 nm, using concentration in % (ϵ in 10^2 cm^2 g^{-1}).
[e] Gel Sephacryl S-400, column 2·6 × 58·1 cm, media M NaCl, flow rate 120 ml h^{-1} injected volume 10 ml, concentration 3·17%, V of each fraction 19·5 ml.
[f] Gel Sephacryl S-500, column 2·6 × 57·4 cm, media M NaCl, flow rate 125 ml h^{-1} injected volume 10 ml, concentration 3·17%, V of each fraction 19·5 ml.

compared with 13. For $10^5 < \bar{M}_w < 10^6$, the Mark-Houwink equation is of the type $[\eta] = 1{\cdot}6 \times 10^{-2}\, \bar{M}_w^{0{\cdot}53}$ for the fractions investigated, in fair agreement with the previous results of Anderson & Rahman (1967): $[\eta] = 1{\cdot}3 \times 10^{-2}\, \bar{M}_w^{0{\cdot}54}$.

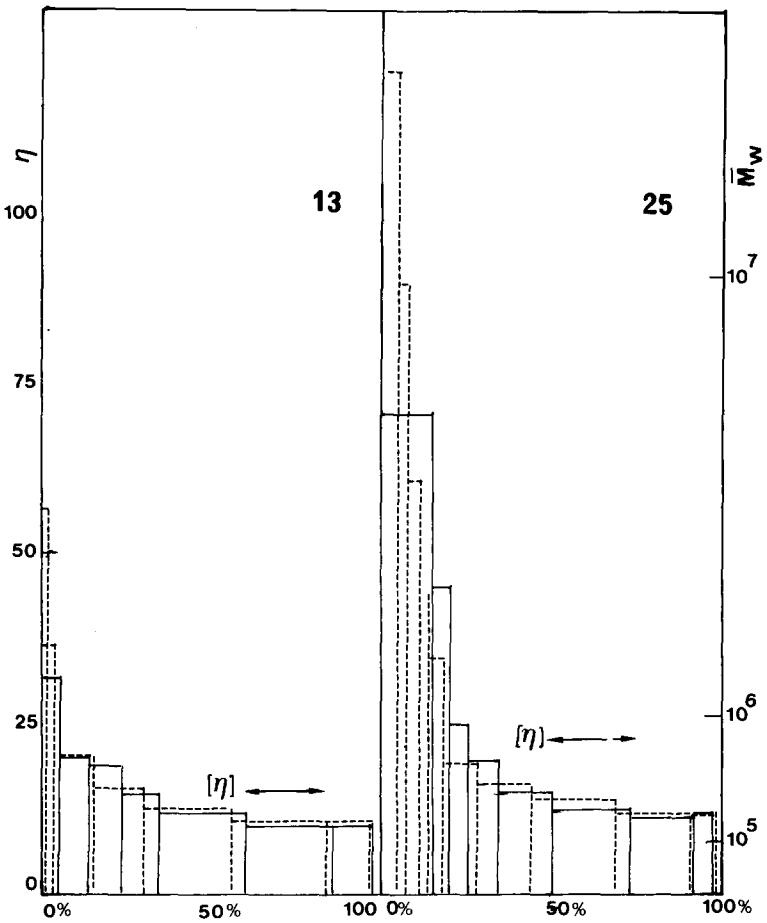

Fig. 6. Viscosity (left scale, ml g^{-1}) distribution of sample 13 and 25. Dotted lines Sephacryl S-500 gel, full lines Sephacryl S-400 gel. Calculated molecular weight (right scale) are given using the Mark-Houwink relation.

The efficiency of gel-filtration can be quantitatively expressed using K_{av}, the partition coefficient between the liquid and gel phases (where $K_{av} = (V_e - V_0)/(V_t - V_0)$) and also by a plot of $[\eta].\bar{M}_w$ versus the elution volume ($[\eta].\bar{M}_w$ is related to the hydrodynamic volume of the macromolecule and is often used for universal calibration (Grubisic *et al.*, 1967)). V_e values have been taken at the top of peaks of rechromatographed fractions. Figure 7 shows an example of the efficiency of the

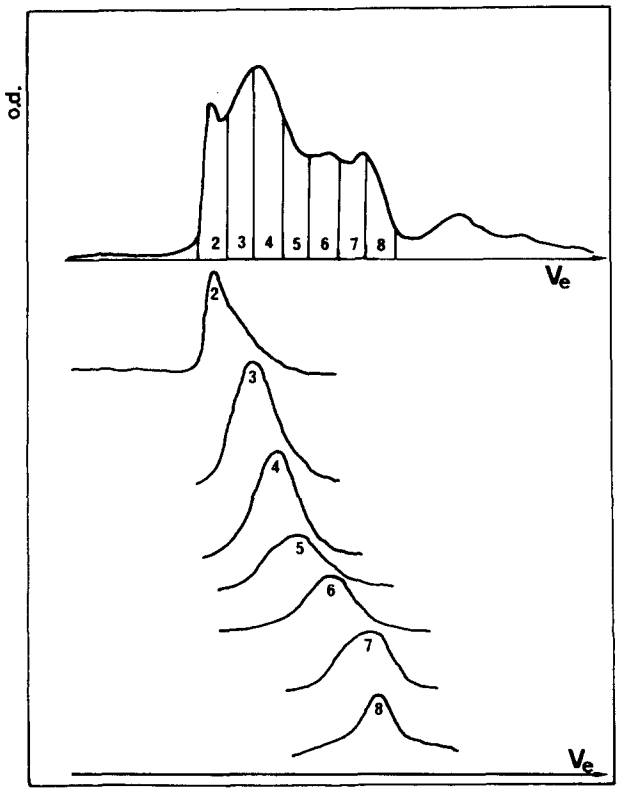

Fig. 7. Rechromatography of material from fractions 2-8 of sample 13 after separation on Sephacryl S-400 gel in molar sodium chloride.

process. A single linear plot of K_{av} versus $\bar{M}_w$ is observed for the two samples investigated, for both gels (Fig. 8). This representation clearly demonstrates the exclusion limits for each support. Figure 9 shows a moderately linear plot between $\log [\eta] . \bar{M}_w$ and V_e/V_0.

The use of these calibration methods is not directly applicable to the analysis of an unknown *A. senegal* gum sample. In fact, the chromatograms are always broad and the well defined peaks one might expect for a homogeneous compound are not observed. The macromolecular distribution is, however, easily deduced from size-exclusion chromatography.

Physical and chemical data (Tables 1-4) can be roughly classified into two types which may or may not be sample or fraction dependent.

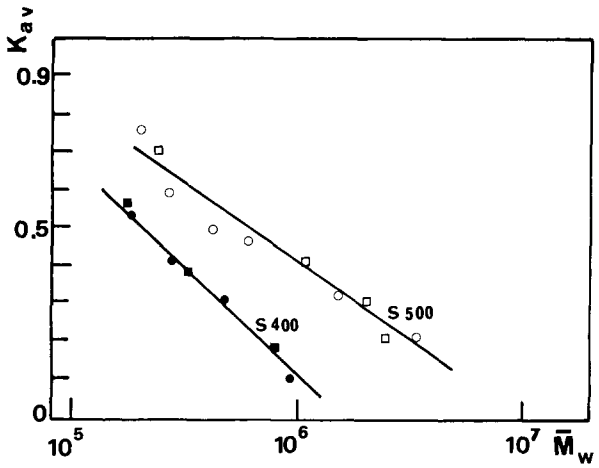

Fig. 8. Partition coefficients on Sephacryl S-400 (●, 13; ■, 25) and Sephacryl S-500 (○, 13; □, 25) gels.

The gum apparently contains two systems, both polydisperse and heteropolymers as defined by Anderson.

Many hypotheses have been put forward about the biosynthesis process of gums, which imply arabinogalactan-protein complexes (Clarke *et al.*, 1979). A recent paper (Akiyama *et al.*, 1984) has produced evidence that gum arabic consists of neutral sugars, uronic acid, protein and hexosamine, the main amino-acid being hydroxyproline. Neutral sugars determinations on samples and some of their fractions show (Table 5) that there is no correlation between each sugar content and other variable parameters such as viscosity or % *N*. U.v. and o.r.d. can be helpful to demonstrate the presence of the hetero-compounds (Figs 10 and 11). The chromophores of *A. senegal* gum absorb only in the far u.v., the electronic transitions being located in the range 200–210 nm for CO_2H and CO_2^- and below 180 nm for hydroxyls (Davidson, 1967). The carboxyl transition is masked by the base of the hydroxyl bands for which the extinction coefficients and also molar ratios in the gum are higher. Spectra of other samples exhibit the same general features, though extinction coefficients can vary. Evaluation of the o.r.d. behaviour which is featureless over the spectral range (230–600 nm) confirms the conclusions from the u.v. spectra. Application of Drude's equation gives some indication of λ_c, the wavelength of the optically

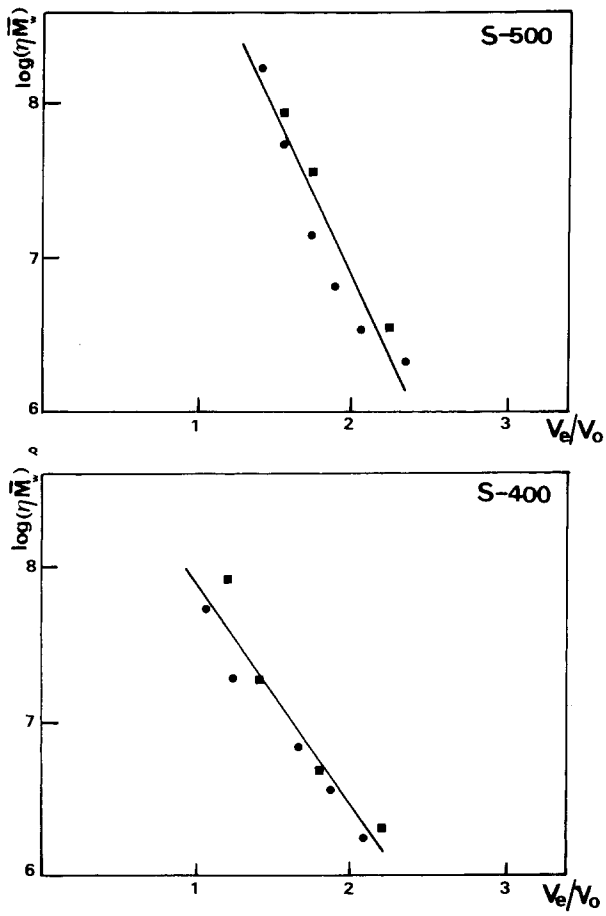

Fig. 9. Universal calibration plot for ●, 13 and ■, 25 on Sephacryl S-400 and S-500 gels.

active electronic transition; this is in the range 160–170 nm where hydroxyl functional groups are known to have an absorption band (Davidson, 1967).

The c.d. spectra of whole gum and derived fractions, as shown in Fig. 12, are of greater interest than those of o.r.d. The spectra of fraction 3 are similar to that of sodium glucuronate (Buffington *et al.*, 1977) whereas spectra of fractions 1 and 2 show the same general features as those of whole gum.

TABLE 5
Neutral Sugars Analysis of Unfractionated and Fractionated Samples of *A. senegal* Gums[a]

Neutral sugar \ *Sample*	*Content (%)*									
	1	*5*	*20*	*20-3*	*21*	*21-2*	*21-3*	*25*	*25-2*	*25-3*
Rhamnose[b]	17·4	15·2	17·1	17·4	14·6	15·1	14·2	17·1	18·2	16·6
Arabinose[b]	38·0	37·5	37·8	35·5	35·6	39·5	36·8	37·8	36·2	34·9
Galactose[b]	41·3	45·1	43·1	44·4	47·4	43·5	45·6	43·1	43·4	45·4
Glucose[b]	3·3	2·2	2·0	2·7	2·4	1·9	3·4	2·0	2·2	3·1

[a] From Ullmann, 1983.
[b] Values obtained by total acidic hydrolysis (H_2SO_4 72%, 1 h; H_2SO_4 2N, 3 h, 100°C).

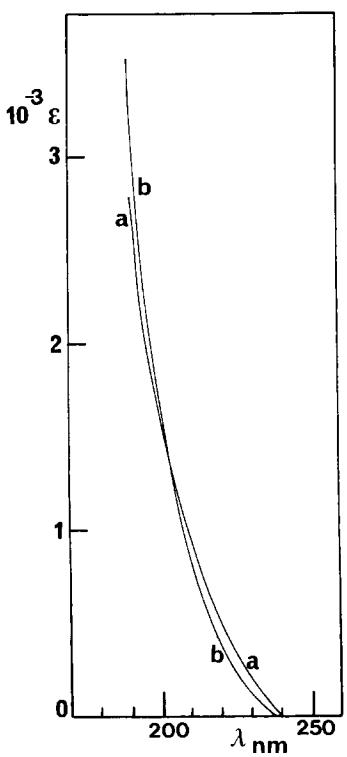

Fig. 10. Ultraviolet spectra of salt-free aqueous solutions of 13: (a) arabic acid; (b) sodium arabate, $C_a = 2{\cdot}12 \times 10^{-3}$ eq litre^{-1}; $l = 0{\cdot}1$ cm.

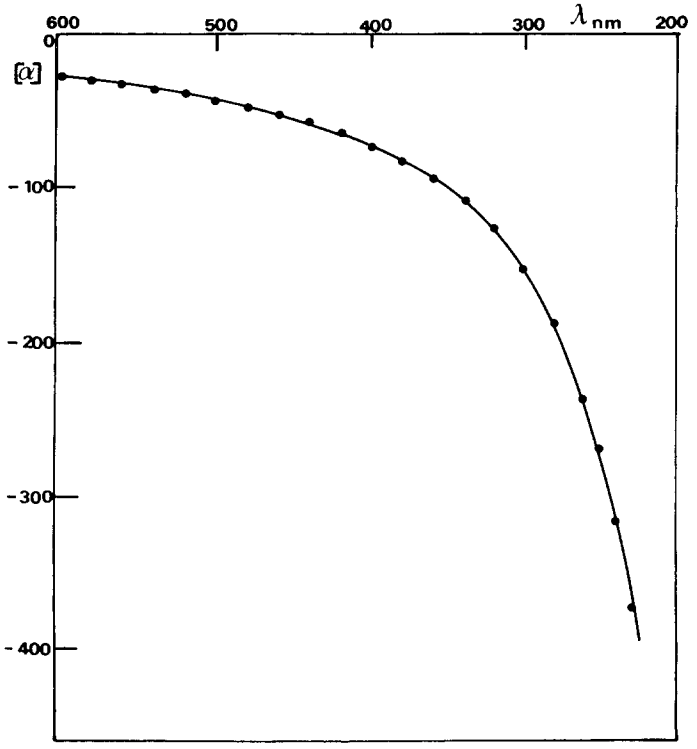

Fig. 11. O.r.d. spectra of sodium arabate (sample 13, C = 1·01 g dl^{-1}, l = 0·1 dm) in salt-free aqueous solution. Full line experimental; •, points calculated by the equation $[\alpha] = (-9{\cdot}44 \times 10^6)/(\lambda^2 - 2{\cdot}77 \times 10^4)$.

These anomalies in the c.d. results for fractions 1 and 2 are surely of the same origin as for the absorption spectra. A whole gum sample contains two components: one of high viscosity and in which almost all nitrogen is present; in this sense, fractions 1 and 2, though possessing different physicochemical data, are of the same nature and are only arbitrarily defined by the performance of the size-exclusion chromatography column. Fraction 3, which represents the main material, is made up of only the polysaccharide. The constancy in some physicochemical data can be thus interpreted by the following explanation of heteropolymolecularity: the chemical heterogeneity is due to the protein. We have been able to separate easily, by size-exclusion chromatography, the nitrogen-containing part of samples which seems to represent at the most about one fourth of material.

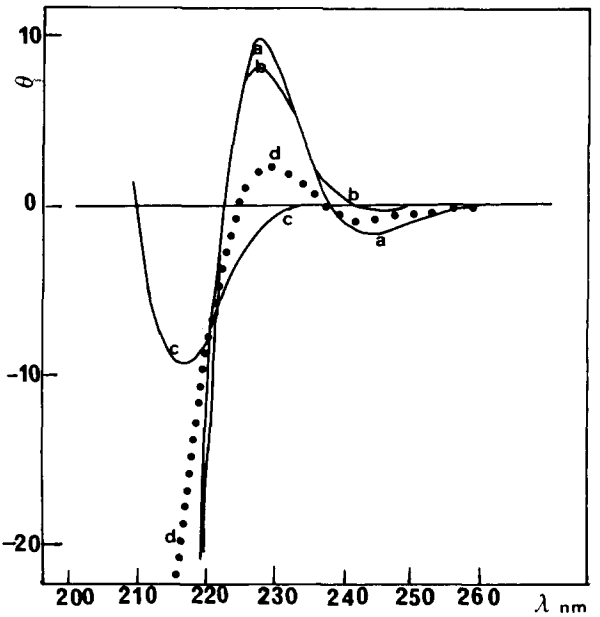

Fig. 12. C.d. spectra in water of sample 25: (a) fraction 1, C = 0·173 g dl^{-1}; (b) fraction 2, C = 0·159 g dl^{-1}; (c) fraction 3, C = 0·168 g dl^{-1}; (d) unfractionated, C = 1·0 g dl^{-1}. θ in m degrees. For a, b and c pathlength 0·1 dm; for d, 0·02 dm.

A consistent explanation of the reported data is that the biosynthesis process involves a nitrogenous carrier; for reasons which are still unknown, polysaccharide chains without nitrogenous components and which present well defined physicochemical and macromolecular characteristics are easily released (viscosity around 12 ml g^{-1}, i.e. $\bar{M}_w$ 2·4 × 10^5) and are primary constituents of *A. senegal* gum. Thus, the origin of heteropolymolecularity has to be looked for in the nitrogen containing part of the samples. Refinements of the structures previously proposed are in favour of the occurrence of regular sub-units in the molecular structure. (Churms *et al.*, 1983) or at least are consistent with more ordered rather than random structures (Street & Anderson, 1983). Our results are not contradictory with this new approach.

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