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Solution Properties of Gum Exudates from *Sterculia urens* (Karaya Gum)

D. Le Cerf,[a] F. Irinei[b] & G. Muller[a]

[a]URA 500 du CNRS 'Polymères, biopolymères, membranes, Faculté des Sciences de l'Université de Rouen, 76134 Mont Saint Aignan Cédex, France
[b]Société Alland et Robert, 125, grande rue Port-Mort, 27940 Aubevoye, France

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ABSTRACT

Dilute solution properties of Karaya gum from Sterculia urens *were studied using size-exclusion chromatography, static and dynamic light scattering and viscosity experiments in 0·1* M *aq. NaCl.*

Solubility in water was found to be strongly dependent on the degree of acetylation. The native acetylated Karaya gum assumes a rather compact and branched conformation in aqueous solution, as evidenced by the low values of power-law exponents. In contrast, the fully deacetylated Karaya gum assumes a more expanded conformation and behaves as a random coil.

INTRODUCTION

Karaya gum, a dried exudate from trees of the genus *Sterculia*, has emulsifying, stabilizing and thickening properties, therefore making it of commercial importance to the food and pharmaceutical industry. The majority of commercial material comes from the *S. urens* tree growing in North and Central India, although other species, e.g. *S. setigera* (from Africa) or *S. villosa* (India), have been reported to have similar chemical composition and physicochemical properties (Hirst *et al.*, 1949; Anderson *et al.*, 1982; Khristova *et al.*, 1982). Native Karaya gum is a complex, partly acetylated anionic polysaccharide. The primary structure has been shown to be composed of D-glucuronic acid, D-galacturonic acid, D-galactose and L-rhamnose, in proportions differing according to the quality, type of gum and origin (Aspinall & Fraser,

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1965; Aspinall & Nasir-Ud-Din, 1965; Aspinall *et al.*, 1965; Aspinall & Sanderson, 1970; Aspinall *et al.*, 1987). The polysaccharide contains 40% of uronic acid residues. The native gum nodules swell in water but do not dissolve in it, probably as a consequence of the presence of acetyl groups. Heating such a dispersion improves the solubility only, whereas chemical deacetylation of the native gum with dilute ammonia results in a water-soluble polysaccharide (Lecompte, 1937).

Only limited data are available in the literature concerning the solution properties of Karaya gum (Kubal, 1948; Kubal & Gralen, 1948; Money, 1951).

This lack of precise information on both the conformation and macromolecular behaviour of Karaya gum, probably due to the fact that studies were performed on ill-defined Karaya solutions, has led us to begin a detailed study of Karaya gum in dilute solutions.

The present paper reports viscosity, static and quasi-elastic light-scattering (QELS) data from which some useful information concerning the conformation of Karaya gum, particularly with regard to the extent of acetylation, can be deduced.

EXPERIMENTAL

Materials and solutions

Several nodules of Karaya gum from *S. urens* were ground to obtain a fine powder which was screened on 200 mesh. An aqueous dispersion (2 g/litre) was first prepared by pouring the powdered material into cold water. The suspension was allowed to disperse at room temperature while being gently stirred for 5 h. Cold-water-soluble (GK_{CWS}), hot-water-soluble (GK_{HWS}) and deacetylated-soluble (GK_D) fractions of Karaya gum were thereafter prepared according to the solubilization procedure shown in Fig. 1.

Approximately 10 and 30% of the native gum was soluble in cold and hot water, respectively. Following the mild alkali treatment, about 90% of the native gum passed into solution. The solutions were purified by filtration through Millipore filters (pore size $8 - 0{\cdot}45$ μm), then by ultra-filtration against distilled water using a stirred ultra-filtration cell (Amicon model) equipped with Amicon XM100 membranes (MW cut-off 100 000) and, thereafter, the samples were freeze-dried. Solutions were prepared with weighed samples in volumetric flasks. For light-scattering measurements, the optical clarification of the solutions was carried out by passing the solutions through Millipore filters (0·45 μm).

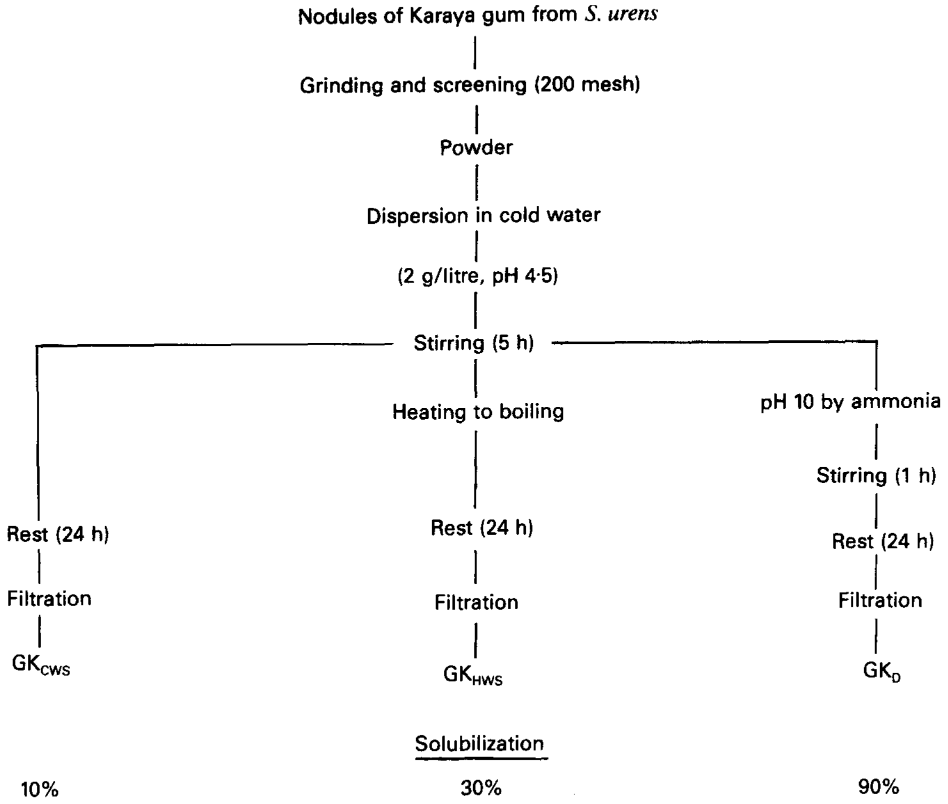

Fig. 1. Experimental procedure for the preparation of Karaya gum solutions.

Measuring methods

IR spectra of films of Karaya samples cast from aqueous solutions on calcium fluoride (CaF_2) windows were recorded using a FTIR spectrometer (Perkin-Elmer 1760).

The equivalent weight of native and acetyl-free acidic samples was determined from potentiometric and conductimetric measurements performed on GK solutions, prepared by dissolving the freeze-dried samples in water and then percolating them through an ion-exchange resin into the acid form (Dowex 50WX8 20–50 mesh).

Rheological measurements were performed in 0·1 M aq. NaCl at low shear rate using a Contraves LS30 viscometer and at high shear rate with an Ubbelohde capillary viscometer Viscomatic (FICA).

The weight-average molecular weight ($\bar{M}w$) and the second virial coefficient (A_2) were computed from scattered light intensities using a plot of the excess Rayleigh factor (R_θ) as a function of polymer concentration (C), measured with the help of a low-angle laser light-scattering (LALLS) photometer Chromatix KMX-6. At very low scattering angle, R_θ is related to C by

$$KC/R_\theta = 1/\bar{M}w + 2A_2C$$

where the optical constant (K) contains the refractive index increment dn/dC ($dn/dC = 0{\cdot}148$ ml/g in 0·1 M aq. NaCl) (Kubal & Gralen, 1948).

A variable-angle (20–150°) photometer Macrotron (AMTEC) was used for measuring the radius of gyration, $R_{g(z)}$, from the angular dependence of the scattered light.

QELS measurements were performed in the dilute concentration range (10^{-4}–5.10^{-4} g/ml) and at low angle ($\theta \simeq 5°$), by using a 64-channel real-time autocorrelator (K7025 Malvern Instruments) connected to the LALLS Chromatix KMX-6 for determining the translational diffusion coefficient (D_t) and, therefore, the hydrodynamic size (hydrodynamic radius, R_h) of the scattering species. In our experimental conditions, no extrapolation to zero scattering angle and zero concentration were necessary to obtain the true diffusion coefficient.

For size-exclusion chromatography (SEC) experiments, a mobile phase containing 0·1 M aq. NaCl was pumped at 0·5 ml/min through a TSK G6000PW column (Toyo Soda). After elution from the column, the injected polymer was detected using a refractive index (RI) detector (Model 6A, Shimadzu).

Samples of lower $\bar{M}w$ were obtained by sonication with a Vibracell sonifier for various times according to the degree of chain scission desired. The molecular weight change on sonication was monitored by SEC.

RESULTS AND DISCUSSION

Potentiometric titration with sodium hydroxide of uronic acid groups of native and deacetylated Karaya solutions prepared from freeze-dried, purified samples indicate that equivalent weights are 460 and 520 for GK_{CWS} and GK_D, respectively. The observed difference (near 12%) is in good agreement with the reported acetyl extent of the native gum (Aspinall *et al.*, 1965; Jefferies *et al.*, 1977).

This means that deacetylation with dilute ammonia was complete, and this is fully confirmed by IR data. FTIR spectra of GK_{CWS} and GK_D are shown in Fig. 2. Typical absorption bands were observed at around 3300, 1675–1610 and 1730–1240 cm^{-1} and were assigned to hydroxyl uronic acid and acetyl groups, respectively (solid line). Clearly the bands assigned to the acetyl groups have completely disappeared for GK_D (broken line), agreeing closely with the potentiometric data.

Figure 3 shows the elution profiles for the three Karaya preparations previously described. Comparing them, it is obvious that both CWS and HWS fractions are eluted very close to each other, whereas the acetyl-free sample appears less retarded. This is in favour of a higher hydrodynamic volume for the latter. Therefore, it appears that Karaya gum could be fractionated on the basis of its solubility in water, the fully deacetylated soluble fraction showing the largest molecular size and probably the highest molecular weight.

Care needs to be taken when comparing the molecular weight of CWS fraction and the fully deacetylated one. It is clear that only a small quantity of the native gum (10%) is solubilized in cold water and that the solubilized molecules are those of lower $\bar{M}w$ (but at the same acetyl extent). Treatment with dilute ammonia leads to the recovery of material of higher molecular weight than is solubilized by cold water. A similar effect was reported for other poorly water-soluble plant-gum exudates; the solubility of which being greatly improved by treatment with aqueous

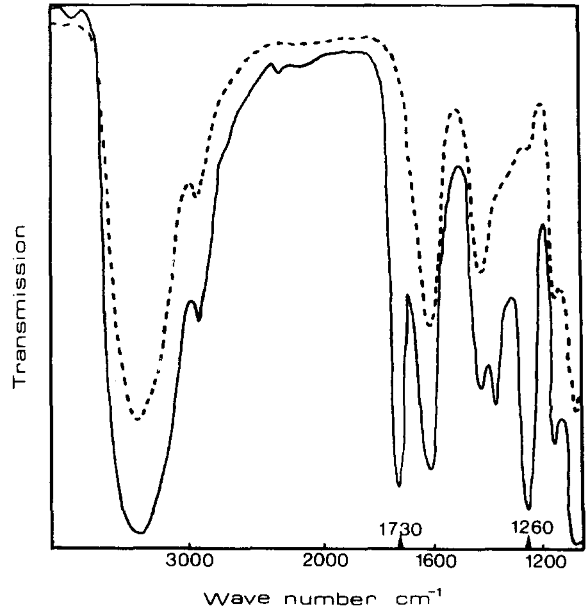

Fig. 2. FTIR spectra of GK_{CWS} (solid line) and GK_D (broken line) films.

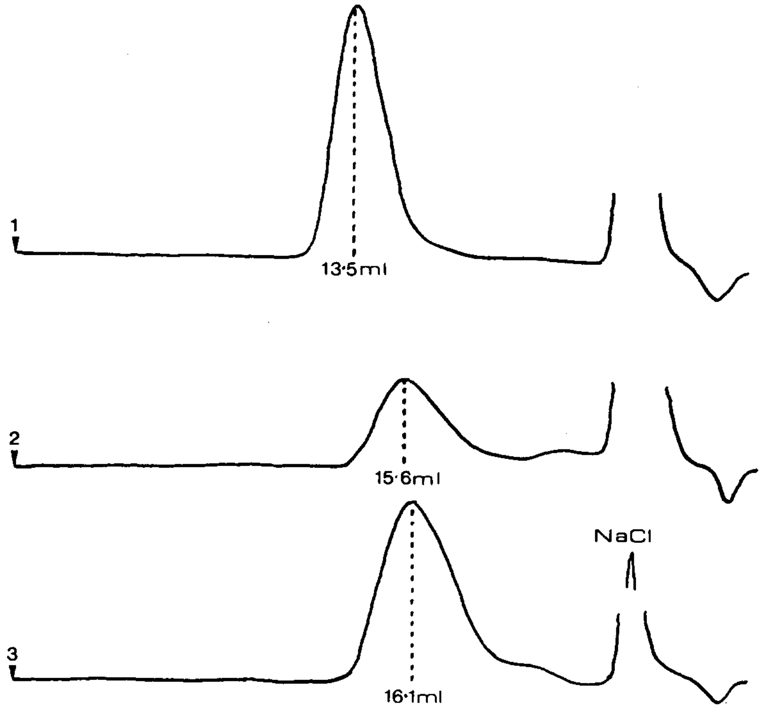

Fig. 3. Elution profiles for the three Karaya solutions to 0·1 M aq. NaCl. 1:GK_D 2:GK_{HWS} 3:GK_{CWS} (V_0 = 12·5 ml, V_t = 25 ml).

sodium borohydride concomitantly with an increase in the Mw of the solubilized material (Anderson *et al.*, 1972). We observed that the treatment of Karaya gum with sodium borohydride has the same effect that treatment with dilute ammonia has, i.e. leading to the solubilization of higher-$\bar{M}$w species with deacetylation.

The SEC data are corroborated by viscosity and light-scattering data. The shear rate ($\dot{\gamma}$) dependence of the viscosity of CWS and acetyl-free samples in 0·1 M aq. NaCl reported in Fig. 4 indicates a higher viscosity and a larger pseudoplasticity for acetyl-free Karaya compared to CWS fraction.

Plots of KC/R_θ versus concentration for both samples in 0·1 M aq. NaCl are given in Fig. 5. In each case the data can be fitted by a linear relationship. The similarity in the slopes reflects similarity in A_2, but the large difference in the intercepts indicates a significant difference in $\bar{M}$w between the two samples.

As measurements are not made as a function of angle, no information regarding the static molecular size (R_g) is available with the use of

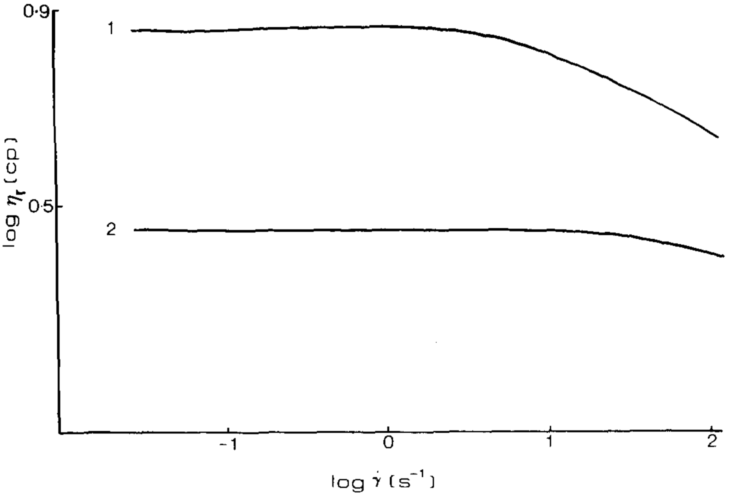

Fig. 4. Shear-rate dependence of relative viscosity for solutions of GK$_D$ (1) and GK$_{CWS}$ (2) in 0·1 M aq. NaCl (polymer concentration = 1 g/litre).

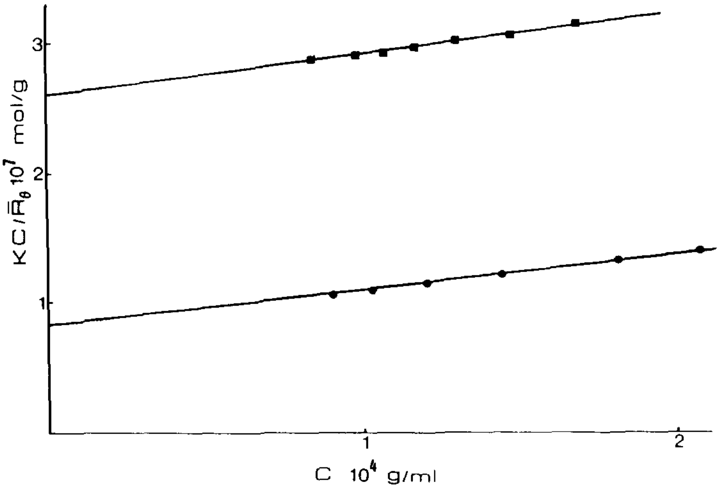

Fig. 5. Concentration dependence of scattered light (at $\theta \simeq 5°$) from solutions of GK$_D$ (●: $\bar{M}w = 12·6 \times 10^6$ daltons, $A_2 = 1·4 \times 10^{-4}$ cm^3/mol g^2) and GK$_{CWS}$ (■: $\bar{M}w = 3·8 \times 10^6$ daltons, $A_2 = 1·6 \times 10^{-4}$ cm^3/mol g^2) in 0·1 M aq. NaCl.

LALLS. A double extrapolation (Zimm's method) of scattered-light data to zero angle and zero concentration is therefore necessary for allowing the determination of R_g. Figure 6 shows a Zimm plot corresponding to solutions of CWS fraction in 0·1 M aq. NaCl. As shown, a curvature in the angular dependence of reciprocal excess scattering is observed, making

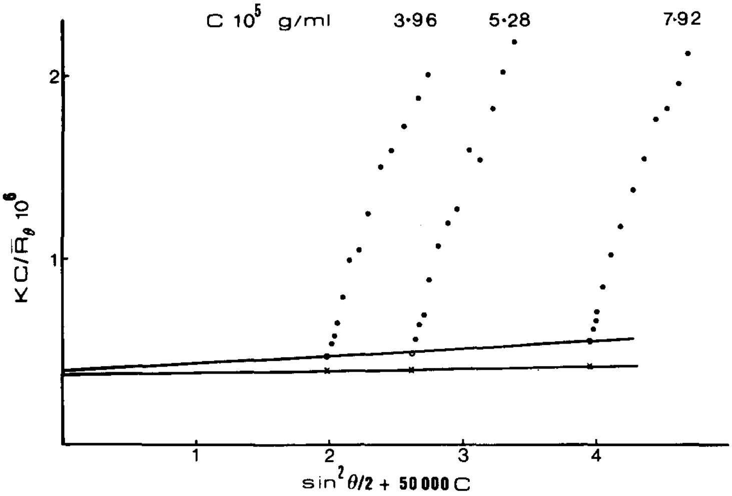

Fig. 6. Zimm plot of the static light-scattering measurements from GK_{CWS} in 0·1 M aq. NaCl. ● — measured data (20–120°C); ○ — extrapolated points to $\theta = 0°$ of the wide angle measurements. The cross shaped points are the experimental data from the low-angle measurements ($\theta \simeq 5°$).

difficult the unambiguous determination of R_g as it can be observed for other types of polysaccharides (Doublier & Launay, 1981; Robinson *et al.*, 1982; Muller *et al.*, 1988). Such a curvature appears stronger if the experimentally measured values for the light scattered at very low angle ($\theta \simeq 5°$) are taken into consideration and can be the consequence of a small quantity of very high Mw but probably compact particles. If they are taken into account, an overestimated value for R_g could result. On the other hand, it is well known that measurements need to be performed at sufficiently small scattering angles to permit extrapolation to zero angle, particularly in the case of high-$\bar{M}$w polymer.

For this reason, a compromise needs to be reached. Therefore, two methods of extrapolation of experimental data have been used for determining R_g. The first uses the measured values of the scattered light from both the wide-angle (20–150°) and the low-angle (5°) instruments, and gives R_g. In the second method, the radius of gyration (R'_g) is determined from extrapolation of $\theta = 0$ of the wide-angle measurements only.

As shown in Fig. 6, the two methods of extrapolation only slightly affect the intercept, i.e. Mw. This is not the case for R_g as indicated by data listed in Table 1. The difference between R_g and R'_g can be an indication of the 'quality' of solutions, and the larger differences observed for the CWS can be related to the presence of a greater extent of compact 'superstructures' compared to the acetyl-free sample. The

TABLE 1
Molecular Parameters of Karaya Gum Solutions in 0·1 M aq. NaCl (Solutions Prepared from Different Nodules)

Sample	*$\bar{M}w$*	*$[\eta]_{\gamma \Rightarrow 0\,s^{-1}}$ (ml/g)*	*$[\eta]_{\gamma = 800\,s^{-1}}$ (ml/g)*	*R_g (nm)*	*R'_g (nm)*	*R_h (nm)*
GK_{CWS}	$2–5 \times 10^6$	700–1100	240–360	210–320	160–220	78–130
GK_D	$12–16 \times 10^6$	2100–2600	900–1100	360–420	320–380	175–230

macromolecular parameters reported in Table 1 refer to different CWS and acetyl-free Karaya preparations made from various nodules differing in size and colour. The observed variations in the solution properties, e.g. viscosity and Mw, are probably in relation with the quality of the dried exudates, including various factors such as location, period of crop, storing conditions, etc. Two facts should be noted; first, deacetylation makes possible water solubilization of high-Mw polymeric chains; second, the small measured values of the intrinsic viscosity $[\eta]$, despite high $\bar{M}w$, suggest a rather compact conformation in 0·1 M aq. NaCl which agrees with the low values of A_2, therefore indicating that the aqueous salt solution is not a thermodynamically good solvent for Karaya gum. The intrinsic viscosity values found for the native gum (CWS fraction) are in agreement with those reported by Anderson *et al.* (1982) and definitively higher than those quoted by Kubal and Gralen (1948) for a HWS sample of $\bar{M}w = 9{\cdot}5 \times 10^6$ as determined from sedimentation experiments.

A better characterization of the overall conformation of Karaya gum in dilute 0·1 M aq. NaCl solution can be obtained from examination of the $\bar{M}w$-dependence of the size and shape parameters ($[\eta]$, R_g, R_h). As the solution properties of Karaya preparations coming from different nodules have been found to show variations (Table 1), lower-Mw Karaya fractions resulting from sonication of CWS ($\bar{M}w = 4{\cdot}9 \times 10^6$) and deacetylated ($\bar{M}w = 16 \times 10^6$) preparations originating from the same nodule were used.

Figure 7 shows the change of elution profiles of acetyl-free sample after sonication for various times.

The Mw dependences of $[\eta]$, $(R'_g)_z$ and R_h (computed from measured D_t with the Stokes–Einstein equation) are shown in Fig. 8. The $\bar{M}w$-dependence of these parameters is well described by power laws. The calculated values for the various exponents are listed in Table 2.

The reported data show that GK_{CWS} is characterized by low values of power-law exponents. Particularly, the value for the Mark–Houwink

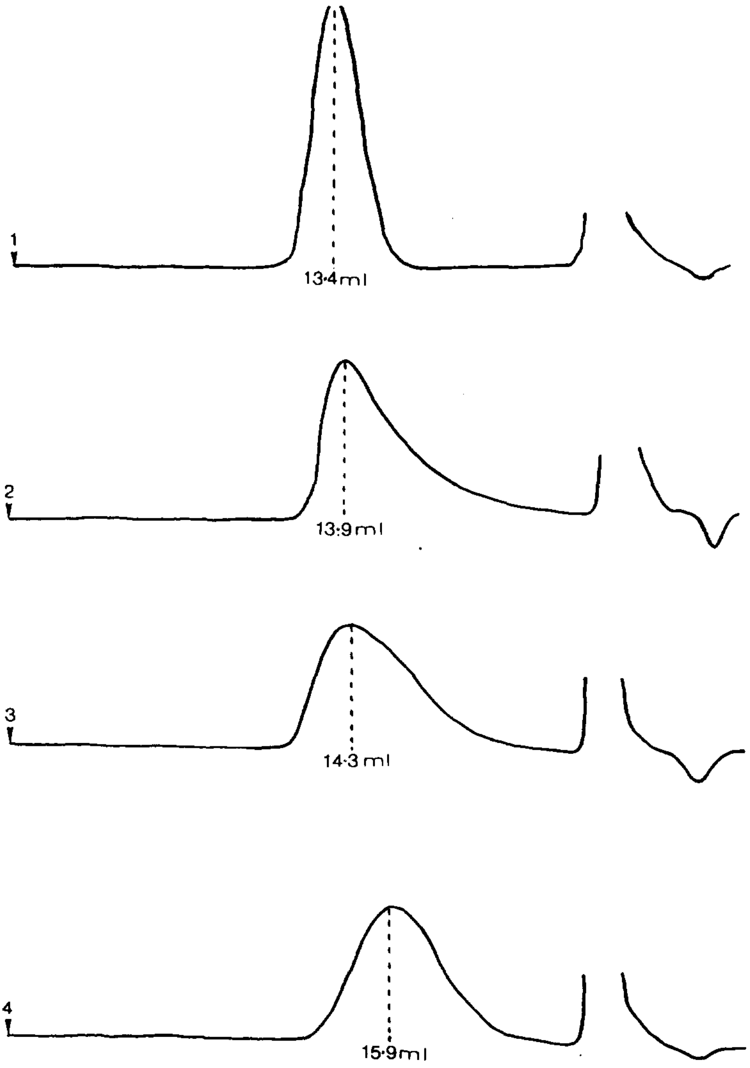

Fig. 7. Effect of sonication on the elution profile of acetyl-free Karaya sample (GK_D) in 0·1 M aq. NaCl. Before sonication ($\bar{M}w = 16 \times 10^6$) (1), and after sonication during 1 min ($\bar{M}w = 7·7 \times 10^6$) (2), 2 min ($\bar{M}w = 4·7 \times 10^6$) (3) and 5 min ($\bar{M}w = 1·4 \times 10^6$) (4).

exponent $\alpha \simeq 0·4$ is much lower than that expected for linear random coils and suggests that the particles are probably built up in another way. The existence of a branched structure seems probably responsible for the observed behaviour. GK_D shows a more expanded conformation and behaves as a random coil. For the fully deacetylated sample, the exponent of the Mark–Houwink expression $\alpha \simeq 0·7$, which is related to

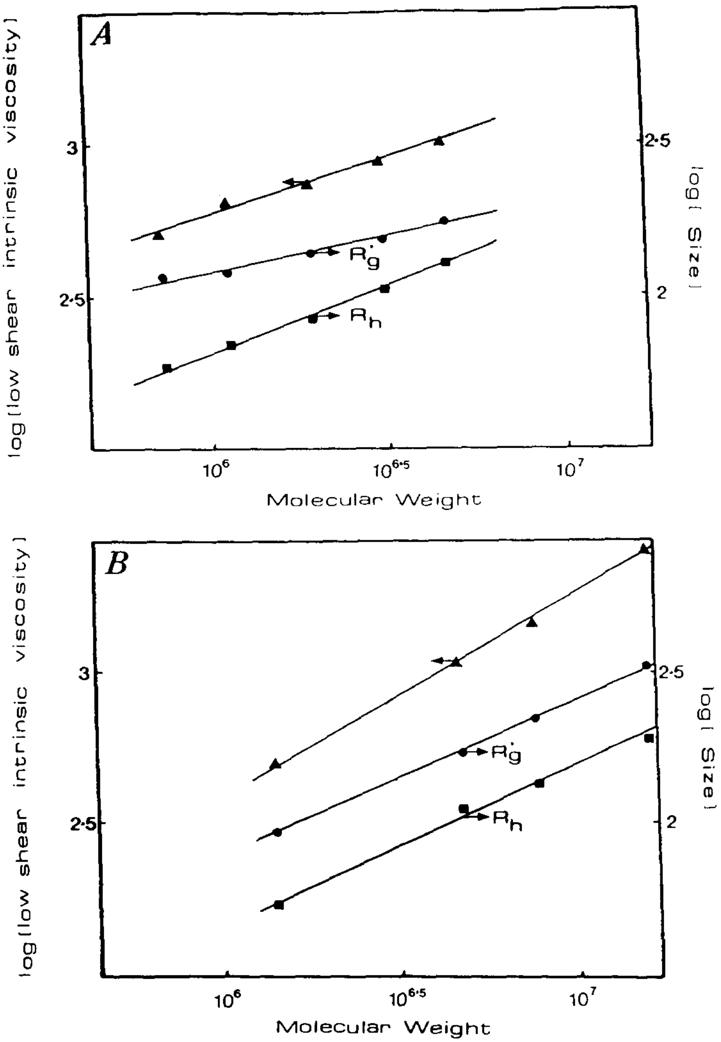

Fig. 8. Log–log Mw-dependence of the intrinsic viscosity $[\eta]$ and the size (R'_g, R_h) for GK_{CWS} (A) and GK_D (B) in 0·1 M aq. NaCl. ($[\eta]$ in ml/g and the size in nm).

TABLE 2

Power-Law Exponents of Karaya Gum Preparation in 0·1 M aq. NaCl

Sample	α	α'	β	β'	γ
GK_{CWS}	0·37	0·40	0·41	0·23	0·52
GK_D	0·66	0·75	0·51	0·49	0·60

$[\eta]_{\gamma \Rightarrow 0} = K_v \bar{M}w^{\alpha}$
$R_g = K_r \bar{M}w^{\beta}$
$R_h = K_h \bar{M}w^{\gamma}$

$[\eta]_{\gamma = 800\,s^{-1}} = K'_v \bar{M}w^{\alpha'}$
$R'_g = K'_r \bar{M}w^{\beta'}$

the diffusion $\bar{M}w$ power-law exponent by $\gamma = (\alpha + 1)/3$, predicts $\gamma = 0{\cdot}56$ in fair agreement with the found values (Table 2).

This study was mainly performed on both native and fully deacetylated Karaya samples of identical origin, i.e. *Sterculia urens*. Differences in solution behaviour are observed, depending on nodules from which solutions were made. Therefore for a better understanding of the overall properties of these high-$\bar{M}w$ polysaccharides, further work is needed concerning the effect of various factors which govern the overall comparison with *Sterculia* gums from other sources, e.g. Africa. This can help for finding new applications for exudates of *Sterculia*, e.g. in the food industry.

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