RESEARCH PAPERS

THE MOLECULAR PROPERTIES OF GHATTI GUM: A NATURALLY OCCURRING POLYELECTROLYTE

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An authenticated sample of ghatti gum from Anogeissus latifolia has been fractionated by ethanolic precipitation (Fraction I), and chromatography on silica gel (Fractions 2 and 3). The equivalent weights found were 1,750, 1,800 and 2,040 respectively. The sodium salts of the fractions have been studied in various salt solutions by viscosity and light scattering methods. From the latter the molecular weights (= 2·1, 2·7, and 2·6 × 10⁶ respectively), radii of gyration, and some idea of molecular shape have been obtained. The molecule appears to be rod shaped. The expansion factors of the molecules calculated from the light scattering and the viscosity data agree with each other. Reasons for the difference in the values of the calculated and observed second virial coefficients are discussed.

GHATTI gum is obtained from *Anogeissus latifolia* (Combretaceae). During the latter part of the nineteenth century, until British control in 1898, troubles in the Sudan almost stopped the export of acacia. This shortage led to a search for substitutes, and to the introduction of ghatti gum.

Work on the chemical structure of the gum is not complete, but degradation studies have shown it to be a polysaccharide which consists of a backbone of galactose units to which other sugars are attached (Aspinall, Hirst and Wickstrøm, 1955; Aspinall, Auret and Hirst, 1958). The side chains can consist of arabinose residues and also of aldobiuronic acids.

Ghatti gum has a number of industrial applications but very little physico-chemical work has been done. The molecular weight of the neutral dialysed material was found to be 2,878 by freezing-point depression and 11,920 by osmotic pressure measurements (Shaw, 1939). Equivalent weights ranging from 1,340–1,735 were found. Electrophoresis studies on glass-fibre filter paper have been made by Lewis and Smith (1957), and the interaction of the gum with Prussian Blue solution has been observed (Carhart and Shaw, 1936). In this paper attempts to fractionate the gum and to determine its molecular nature in solution have been made. These are necessary preliminaries to studies of adsorption and electrophoresis of suspensions stabilised with this material.

EXPERIMENTAL

Fractionation of Ghatti Gum and Preparation of Sodium Ghattate

The material was first authenticated by comparison with a known sample of gum from *Anogeissus latifolia*. Whole tears of gum were slowly stirred in water at 20° overnight and the solution filtered to remove

any insoluble material. The free acid was prepared by careful acidification of the filtrate with hydrochloric acid and precipitation with ethanol. The precipitate was collected, washed well with ethanol, and redissolved in water. After dialysis for 24 hr. the solution was concentrated at not more than 40° using a rotary film evaporator. The free ghattic acid was then precipitated with ethanol. This precipitation was repeated twice. Finally, the material was dissolved in water, neutralised with sodium hydroxide solution, concentrated, and dried at 35° in a vacuum oven over P_2O_5 (Fraction 1).

A chromatographic fractionation was also attempted. The solution of crude gum was passed down a strong cation exchanger to convert it to the free acid. This solution was then dialysed and evaporated to dryness as before. 16.5 g. of the ghattic acid was dissolved in 525 ml. of a 1:2 water: methanol mixture. This solution was then passed down a column of 450 g. silica gel. Elution of the column with 1 litre of 33 per cent water in methanol gave 8 g. of material (Fraction 2). Slow increase of water content and finally elution with 1 litre of water gave, after evaporation, 3.5 g. material (Fraction 3). The remainder of the material on the column could not be eluted with water.



FIG. 1. Top view of light scattering cell. External diameter = 5 cm. A, incident beam. B-B viewing area.

Fractions 2 and 3 were evaporated to a small volume, neutralised as before with NaOH, using a titration curve to determine the end-point on a small part of the sample, and dried.

The equivalent weights of fractions 1, 2 and 3 were found to be 1,750, 1,800 and 2,040 respectively. The sodium hydroxide and sodium chloride used were Analar materials, and the water was twice redistilled from permanganate.

Light-Scattering Measurements

The apparatus described by Elworthy and McIntosh (1961) was used. The turbidity of Analar benzene measured immediately after an extensive calibration with Ludox solutions had been found to be $27\cdot2 \pm 0.4 \times 10^{-5}$ cm.⁻¹; eighteen months later four repeat measurements on benzene gave $27\cdot3 \pm 0.3 \times 10^{-5}$ cm⁻¹. This agreement indicated that there was no change in calibration constant, within experimental error, over this time period.

The cells originally described have been modified by the inclusion of semi-circular light traps cut into the back wall (Fig. 1), which eliminated stray light when measurements were made down to 30° to the incident

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beam. The cell was blackened inside by the Relanol process. Solutions were clarified by filtration through a No. 5 porosity gas filter tube. The concentrations of the solutions were checked on a Hilger-Rayleigh interferometer after filtration.

Specific Refractive Index Increments (dn/dc)

These were determined with a Hilger-Rayleigh interference refractometer using monochromatic light (Bauer, Fajans and Lewin, 1960). In sodium chloride solution the specific refractive index increments were measured at constant chemical potential as described by Vrij and Overbeek (1962).

Viscosity Measurements

A Couette viscometer, based on the design by Ogston and Stanier (1953) was constructed. A constant voltage transformer (Advance 250 w.) was used in addition to a variable speed motor control (Engelhard-Hanovia) to give a constant rotational speed for the outer cylinder. As this cylinder rotated a brass rod attached to it activated a Counting Instruments photo-transistor counter type 500. The speed of rotation could thus be measured at the same time as the deflections of the inner cylinder were observed. A suspended level dilution viscometer was also used.

Densities

The densities were measured by displacement of dry benzene.

All measurements were made at 20° ; $\pm 1^\circ$ for light scattering, and $\pm 0.05^\circ$ for all other experiments.

RESULTS AND DISCUSSIONS

Light scattering measurements were made on all fractions in 0.5N sodium chloride solutions and on fractions 1 and 2 in varying salt concentrations. Two representative Zimm plots are shown in Figs. 2 and 3 for fraction 2, where c in the concentration of sodium ghattate in g./ml. and S_{θ} is the scattering from the solute at an angle θ to the incident beam. The equations governing the two limiting lines of a Zimm plot are given below.

$$\left(\frac{\text{Hc}}{\text{T}}\right)_{\theta=0} = \frac{1}{M_{\text{w}}} + 2\text{Bc} \quad \dots \quad (1)$$

Zero concentration line:

in which $H = 32\pi^3 n_0^2 (dn^*/dc)^2/3\lambda^4 N$

T = turbidity, B = second virial coefficient, P () = particle scattering factor, Rg = radius of gyration, and M = molecular weight.

The asterisk on the (dn/dc) symbol indicates that the measurement was taken at constant chemical potential where salt was present.

The fraction prepared by ethanolic precipitation has the lowest molecular weight. There is considerable variation in the molecular weights determined for fraction 2 in various solvents. Several authors (Benoit, Holtzer and Doty, 1954; Robinson and Saunders, 1959; Elworthy and Macfarlane, 1962) have shown that errors in the determination of M can







FIG. 3. Zimm plot for fraction 2 in water. See text for symbols.

be as large as ± 10 per cent. Estimates of error have been based on a study of equation (1) using particles small enough for $P(\theta)$ to be taken as unity. The use of a Zimm plot introduces further error as extrapolated values of (Hc/T) $\theta = 0$ must again be used in an extrapolation procedure to obtain M.

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The radii of gyration are consistent with the molecular weights of the different fractions. As the salt concentration is decreased Rg increases, which is normal polyelectrolyte behaviour. It is possible that the radii of gyration may have lower limits or error than M as they are obtained from the ratio of the limiting slope of the zero concentration line to the intercept in the Zimm plots. As experimental errors would affect both parts of this ratio in the same sense, it may be that some cancellation is obtained.



FIG. 4. Comparison of calculated and experimental zero concentration lines of Zimm plots. Curve 1, coils with $M_w:M_n = 2:1$. Curve 2, monodisperse coils. Curve 3, monodisperse rods. Curve 4, rods with $M_w:M_n = 1\cdot1:1$ in graph A (fraction 1 in 0.5N NaCl). Rods with $M_w:M_n = 1\cdot1:1$ in graph B (fraction 2 in 0.5N NaCl). Rods with $M_w:M_n = 2:1$ in graph C (fraction 2 in water.)

For fraction 1 in 0.5N sodium chloride and fraction 2 in 0.5N sodium chloride and in water, the molecular model which fits the limiting line (c = o) of the Zimm plots was calculated. Using the observed Rg values calculations of particle scattering factors for monodisperse rods and coils, and for coils with a molecular weight distribution $M_z: M_w: M_n = 3:2:1$ were made. In all cases the rod model gave the closest approach to the experimental results (Fig. 4). The fit of calculated to experimental

results was not perfect even for the rod model, and hence the effect of polydispersity was explored using Reichmann's treatment (1959) where $x = (2\pi/\lambda_1) L \sin \frac{\theta}{2}$ in which L is the length of the rod (for experimental values see Table I) and z, w, and n are used to indicate the appropriate average.

Fraction	Solvent	dn*/dc	10-ªM	Rgz(Å)	10⁵B	L(Å)	ρ
1 1 2 2 * 2 3	0.5N NaCl 0.05N NaCl 0.5N NaCl 0.05N NaCl 0.005N NaCl Water 0.5N NaCl	0·137 0·139 0·136 0·138 0·143 0·143 0·148 0·130	2·1 2·1 2·2 3·3 2·7 2·7 2·6	727 821 768 873 1,005 1,061 740	4 10 4 10 41 260 2	2,520 2,840 2,660 3,030 3,460 3,680 2,560	0.017 0.016 0.027 0.027 0.020 0.048 0.033

 TABLE 1

 Data from light scattering measurements

* By dissymmetry method $\rho =$ depolarisation

The equation (Hc/T) $c = 0 = \frac{1}{M_w} \left(1 + \frac{x_z^2}{9}\right) \dots \dots \dots (3)$ holds up to $x_z = 1.5$.

For the upper part of the limiting line, approaching the asymptote where smaller molecules make a significant contribution to the scattered light:

(Hc/T)
$$_{c=0} = \frac{1}{M_{n}} \left(\frac{2}{\pi^{2}} + \frac{2x_{n}}{\pi} \right) \qquad \dots \qquad (4)$$

This equation holds down to x = 2. Its usefulness can be extended if higher terms of the series are included:

(Hc/T)
$$_{c = 0} = \frac{1}{M_{n}} \left(\frac{2x_{n}}{\pi} + \frac{2}{\pi^{2}} + \frac{2}{\pi^{3}x_{n}} + \frac{2}{\pi^{4}x_{n}^{2}} + \frac{2}{\pi^{5}x_{n}^{3}} \right) \dots$$
 (5)

Tests showed that this equation held satisfactorily down to $x_n = 1.5$. Equation (3) can simply be applied to the results as M_w and Rg_z (hence L_z and x_z) are directly determinable from Zimm plots. As light scattering measurements could not be made at high enough angles to give an idea of the asymptote to the limiting line of the Zimm plot, equation (5) was used by successive approximation. By assuming a molecular weight distribution $(M_z: M_w: M_n)$, as M_w was known, M_n could be calculated; the ratio of $L_z: L_w: L_n$ was taken in the same way. Starting with the monodisperse case, $M_z = M_w = M_n$, cases of increasing polydispersity were considered until the best fit to the experimental results were obtained (see Fig. 4).

The figure clearly shows that the rod model gives the best fit to the experimental results, although some deviations from a perfect rod shape could be present, indicating that this is the most probable shape for the molecule, and that it was maintained when the solvent was changed to water. It is interesting that an apparent change of molecular weight distribution occurs on altering the solvent. Both fractions 1 and 2 give a narrow

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distribution in 0.5N sodium chloride solution, but in water fraction 2 gives $M_{\rm w}$: $M_{\rm n} = 2:1$. This effect must be bound up with the expansion of the molecules (discussed later); on contraction, in the concentrated sodium chloride solution, differences between the various molecular lengths would be minimised, giving an apparently narrow distribution.

TABLE II

DISTRIBUTION GIVING	BEST	FIT	то	LIMITING	LINE	OF	ZIMM	PL	OT
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Fraction	Solvent	Lz:Lw:Ln
1 2 2	0.5N NaCl 0.5N NaCl Water	$\begin{array}{c} 1 \cdot 2 : 1 \cdot 1 : 1 \cdot 0 \\ 1 \cdot 2 : 1 \cdot 1 : 1 \cdot 0 \\ 3 \cdot 0 : 2 \cdot 0 : 1 \cdot 0 \end{array}$

Further information on molecular structure can be obtained from the viscosity measurements. From the viscosity results, following Schneider and Doty's procedure of plotting the viscosity intercepts $[\eta]$ against $1/N^{\frac{1}{2}}$ where N = normality of added salt, straight lines are obtained (Fig. 5) and these can be easily extrapolated to infinite ionic strength $(1/N^{\frac{1}{2}} = 0)$. In this state the molecule can be treated as being uncharged.



FIG. 5. Plots of $[\eta]$ against $1/N^{\frac{1}{2}}$ for: A, fraction 1; B, fraction 2.

$$[\eta] = \left(\frac{\eta^{\mathrm{sp}}}{c}\right)_{c=0} = v(\overline{v}_{2} + w_{1}v_{1}^{0}) \qquad \dots \qquad (6)$$

where v is Simha's shape factor (see Mehl, Oncley, and Simha, 1940), \overline{v}_2 = specific volume of solute, v_1° = specific volume of solvent and w_1 is the hydration of the macromolecule in g. water/g. material. The viscosity intercepts at infinite ionic strength are 112 and 94 c.c./g. for fractions 1 and 2 respectively. The size of those intercepts indicates that the molecule is either highly asymmetric or highly solvated, or both.

Water vapour adsorption studies have been used (Elworthy, 1961) to determine hydration; unpublished studies on sodium ghattate show $w_1 = 0.20 \text{ g./g.}$ for fraction 1 and 0.19 g./g. for fraction 2. The shape factor is calculated from equation (6) for two conditions, $w_1 = 0$, and for the values of w_1 given above. With this level of hydration it is apparent that the deviation of the viscosity intercept from the Einstein value of 2.5 is due to molecular asymmetry, and since light scattering indicates that rod like particles are present, the viscosity results have been interpreted on this basis. Mehl, Oncley, and Simha's table gives v in terms of the axial ratio a/b of a prolate ellipsoid, and for comparison with light scattering results, which give the length, L, of a rod shaped molecule, a/b is converted to L/d, where d is the diameter of the rod, by Tanford's 1961 (a) procedure:

From the light scattering molecular weights, densities, and values of hydration the volumes of the molecules can be calculated, and thus using the viscosity results, the molecular dimensions.

TABLE III MOLECULAR DIMENSIONS CALCULATED FROM VISCOSITY INTERCEPTS AT INFINITE IONIC STRENGTH

			w ₁ = 0		$w_1 = 0.20$ (fraction 1), $w_1 = 0.19$ (fraction 2).				
Fraction	p • (g./ml.)	a/b	L	d	a/b	L	d		
1 2	1·52 1·54	49·0 44·5	2,190 2,220	37 41	43·4 38·5	2,160 2,190	41 47		

* p = solute density, L and d in Å.

The lengths of the rods at infinite ionic strength (obtained by extrapolating the results from light scattering experiments as a plot of Rg^2 against $1/N^4$), were 2,500 Å and 2,580 Å for fractions 1 and 2 respectively. Considering the errors when two sets of experimental results are compared, the agreement between these lengths and those calculated from viscosity is good.

It is interesting to compare the structure of arabic acid as determined from light scattering experiments by Veis and Eggenberger (1954) with that found here for ghattic acid. Arabic acid appears to be a stiff coiled molecule with a root mean square distance between its ends varying from 555 Å when uncharged to 1.050 Å when fully charged. This expansion is proportionally greater than that exhibited by sodium ghattate, and may be due to the higher charge density of arabic acid (equivalent weight = 1,200). The molecular weight of arabic acid appears to be half that of the ghatti molecule. No further ideas on the shape of the arabic acid molecule are available as Zimm plots were not used. The viscous behaviour of both materials seems similar, in that the addition of electrolyte greatly reduces viscosity. In the presence of 0.005 N NaCl [n] for sodium arabate was 28 c.c./g. (Basu, Dasgupta, and Sircar 1951), compared with $[\eta] = 203$ c.c./g. for sodium ghattate. The higher viscosity of the ghatti solution is due to the larger size of the molecules.

Expansion of Molecule

The viscosity results obtained at different ionic strength are plotted in Fig. 6 for fraction 1, and results in water for the other fractions are given in Fig. 7. Fractions 1 and 2 were examined in water, 0.005 and 0.5N NaCl solution in the Couette viscometer, and showed Newtonian flow over the concentration range studies and up to a velocity gradient of 72 sec.⁻¹ (the maximum obtainable on the instrument). The capillary viscometer was used therefore for the remainder of the measurements (average velocity gradient 1,310 sec.⁻¹) as this is the more precise instrument. Using the same solution, the results obtained on both instruments agreed well (cf. Fig. 6).



FIG. 6. Plots of η_{sp}/c for fraction 1 in sodium chloride solutions with normalties of: A, 0.0017; B, 0.005; C, 0.05; D, 0.5; E, 2.0. X = points from couette viscometer.

In water the viscosity curves turn upwards as the concentration is reduced (Fig. 7). This behaviour has been observed for other polyelectrolytes in water and the intercepts can be determined (Fuoss and Strauss, 1948) by plotting c/η_{sp} against $c^{\frac{1}{2}}$. In this case the intercept at $c^{\frac{1}{2}} = 0$ is $1/[\eta]$. For fractions 2 and 3, $[\eta] = 2,600$ c.c./g. and 2,350 c.c./g. respectively.

The increase of both Rg and $[\eta]$ with decreasing salt concentration, can be taken as a measure of the expansion of the molecule. Pals and Hermans

(1952) determined the expansion factor α from $\frac{[\eta]}{[\eta]^{\circ}} = \alpha^2$, while Schneider and Doty used $\frac{Rg^2}{Rg_0^2} = \alpha^2$ where $[\eta]^{\circ}$ and Rg_0 are values obtained by extrapolation to infinite ionic strength.

TABLE IV EXPANSION FACTOR AT VARIOUS SALT CONCENTRATIONS

NaCl solution, normality Fraction 1. (visc) 1. (L/S) 2. (visc) 2. (L/S)	2∙0 1∙01	0·5 1·04 1·04 1·04 1·03	0.05 1.18 1.17 1.18 1.17	0.005 1.46 1.47 1.34	0·0017 1·69

There is excellent agreement between the values of α calculated by the two different methods. Although fraction 1 has the higher charge density, α is very similar for both fractions.



FIG. 7. Plots of η_{BD} /c against c for A, fraction 2; B, fraction 3; both in water.

Schneider and Doty did not obtain agreement between the two different methods of calculating α for the coil like molecule of sodium carboxymethyl cellulose (NaCMC). The square of the radius of gyration is proportional to h² for a coil and L² for a rod. The comparison of results at low ionic strength with those at infinite ionic strength (where $\alpha = 1$) should lead to a measure of α^2 . However $[\eta]$ is proportional to α^3 for a coiled up molecule, so that the ratio $\frac{[\eta]}{[\eta]^{\circ}}$ should yield α^{3} for this model.

A recalculation of Schneider and Doty's results shows that $\sqrt[3]{[\eta]}$ gives a

value of α in fair agreement with those obtained from light scattering data. The situation is different for rod like molecules. Simha's relationship between the shape factor v and the axial ratio a/b can be generalised, in the range of axial ratios of interest here, to v = constant (a/b)^{1.8} (Tanford 1961b).

As $[\eta]$ is proportional to v we would expect to obtain $\alpha^{1.8}$ from the $\frac{[\eta]}{[\eta]^{\circ}}$ ratio, provided the molecule is rod like. α^2 is obtained within the limits of experimental error. The discrepancy between these indices can be lessened as we must consider $\alpha^{1.8}$ to be the lower limit for the rod like molecules. It would be expected that as the length of the rod increases during the expansion process, its width would decrease, while it is assumed in the above discussion that as L increases d remains constant. The expansion should lead to a higher power than $\alpha^{1.8}$ and this is observed since α^2 is obtained and agrees with the light scattering data. It would be stretching the results too far to attempt a calculation of the degree of thinning of the rod on expansion, also charged groups present on the side chains may cause a "swinging out" of the side chains during ionisation.

There is a considerable expansion of the molecule length on passing from infinite ionic strength to pure water (from 2,580 Å to 3,680 Å for fraction 2), indicating that a certain amount of flexibility is present, It is possible that electrical forces help to maintain the rod like shape at large extensions, aided by the effect of unionised side chains giving a stiffening to the galactose framework.

Second Virial Coefficients

Firstly the molecule in its uncharged state at infinite ionic strength is considered. The value of B (see eqn. 1) under this condition was determined by extrapolating a plot of B against $1/N^{0.73}$ which gave a convenient straight line. In this state the counterions should be held close to the main part of the molecule, the charge Z does not contribute to intermolecular interactions, and B is expected to be a function of molecular shape and size only. Tanford (1961c) gives

$$\mathbf{B} = \mathbf{L} \overline{\mathbf{v}}_2 / \mathbf{d} M = \mathbf{L} \mathbf{V} / \mathbf{d} M^2 \qquad \dots \qquad \dots \qquad \dots \qquad \dots \qquad (8)$$

where V is the molecular volume. The length of the rod found from light scattering at infinite ionic strength is used in the calculation.

It can be seen that in uncharged state the value of B agrees reasonably with that calculated for a rod like molecule of dimensions determined by light scattering, particularly as there are large errors in the experimental determination of small values of B.

As the ionic strength is decreased the second virial coefficient increases. The shielding effect of added salt also decreases during this process which gives mutual repulsion between the macromolecules. Donnan-like

conditions should be approached with the counterions distributed between the ionic atmosphere and the solution. Tanford (1961d) gives:

$$B = \frac{1000. Z^2 v_1^{\circ}}{4M^2. m_3} \qquad \dots \qquad \dots \qquad \dots \qquad (9)$$

where m_3 is the molality of sodium chloride. The Donnan term has been calculated (Table V) and is considerably larger than the observed second virial coefficient. A similar state of affairs was observed for NaCMC where the Donnan term was 2 or 3 times greater than the observed second virial coefficient. For sodium ghattate the Donnan term is 4 to 40 times greater. This may be a consequence of the much lower charge distribution in the latter molecule than in the NaCMC molecule, and probably indicates that a fair amount of ion-binding exists, even in dilute salt solution. This bound layer of counterions will presumably tend to neutralise most of the macromolecular charge with respect to other large ions. However, the molecule expands as the ionic strength is lowered, so local shielding of one part of the molecule from another cannot be complete. NaCMC, being a coiled molecule, will occupy a much larger volume of space in solution than the sodium ghattate molecule, which is

TABLE V								
CALCULATED	AND	OBSERVED	SECOND	VIRIAL	COEFFICIENTS			

Normality		Fraction 1		Fraction 2			
	10 ⁵ B expt	10 ⁵ B eqn. 8	10 ⁸ B eqn. 9	10 ⁵ B eqn. 9	10 ⁵ B eqn. 8	10 ⁵ B eqn. 9	
0-5 0-05 0-005	3 4 10	2*	15 154 1,540	3 4 10 41	2*	16 163	

* Calculated for unhydrated molecule. No significant change in B is obtained if hydration is considered.

visualised as being a fairly compact rod, although the molecular weight of sodium ghattate is five times that of NaCMC. Much smaller interferences would be expected which is reflected by the value of B for sodium ghattate being 1/30 that of NaCMC in 0.5N sodium chloride solution. This provides an interesting contrast between the two types of polyelectrolyte.

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