SIZE AND SHAPE OF POTASSIUM AND SODIUM ARABATE AGGREGATES IN AQUEOUS SALT SOLUTIONS

ALEXANDER R. SLONIEWSKY AND PAUL ANDER*
Department of Chemistry, Seton Hall University, South Orange, New Jersey 07079 (U. S. A.)
(Received December 7th, 1970)

ABSTRACT

The average molecular weights obtained by light-scattering for several different preparations of sodium and potassium arabate covered a wide range of values; this variation was attributed to aggregation. The aggregates were found to be stable in solutions of simple salts. The extent of aggregation is apparently determined during the initial stages of preparation of the pure arabate salts from the crude gum. Analysis of the light-scattering data suggests a rod-like shape for the aggregated arabate particles. In 20 mM potassium chloride, the relationship between the molecular weight of the arabates and their intrinsic viscosities is described by the relationship $[\eta] = 0.103\ M^{0.42}$ ml.g^{-1}. The exponent of this equation is consistent with a highly branched, macromolecular model.

INTRODUCTION

Undegraded gum arabic is a naturally occurring, ionic polysaccharide that is a complex copolymer of D-galactose, L-rhamnose, L-arabinose, and D-glucuronic acid residues. The molecular weight of gum arabic and its salts generally accepted is in the range of 25×10^4 to 30×10^4. Such values were first reported by Oakley[1], who obtained them from osmotic-pressure experiments, and later by Saverborn[2], who employed sedimentation–ultracentrifugation techniques, as well as equilibrium ultracentrifugation. Both Oakley[1] and Saverborn[2] performed measurements on samples of gum arabic acid and its sodium and calcium salts. The diffusion patterns obtained by Saverborn[2] for sodium arabate indicated that this material was not highly polydisperse; however, he could not ascertain whether the close agreement between his values and those of Oakley showed that the arabate samples used by the two workers were nearly identical and monodisperse, or whether the agreement was merely coincidental. By use of the method of light-scattering, Veis and Eggenberger[3] studied the molecular weight, size, and shape of arabic acid in a series of aqueous solutions differing in pH; the molecular weight was reported to be 1.1×10^6. Later, the light-scattering results of Mukherjee and Deb[4] gave a molecular weight of 57×10^4 for

*To whom enquiries should be directed.

arabic acid. Swenson *et al.*[5] have since found the osmotic-pressure molecular weight of gum arabic to be 25×10^4 and the light-scattering molecular weight to be 36.5×10^4. As compared to the values reported by Oakley[4], the higher molecular weights obtained by light-scattering were, in both instances, attributed to presumed polydispersity of the arabate systems. Veis and Eggenberger[3] also suggested that the gum could have undergone some hydrolysis during the lengthy osmotic-pressure experiments, and that that could have accounted for the lower molecular weights reported by Oakley[1].

The viscous behavior of arabic acid and its salts has been investigated in several laboratories[6–10]. It appears, in general, that a change in the ionic strength or pH of the solvent affects the viscosity of arabates in a manner typical of polyelectrolytes. However, it has long been recognized that the viscosities of aqueous arabate solutions are unusually low[11]. This fact, together with the reported lack of shear dependence of viscosity, led to the assumption that, in solution, the arabate molecules exist as spheres having a radius of about 10 nm. This model has sometimes been used[12] for evaluating other molecular parameters. However, other results pointed to a somewhat different molecular shape. From the analysis of their light-scattering data, Veis and Eggenberger[3] concluded that the arabate molecules exist as stiff coils which become extended and somewhat rod-like when they are appreciably charged. The reasons for these discrepancies became apparent to the present authors when, in the course of some preliminary experiments, several samples of potassium arabate prepared by seemingly identical techniques exhibited different molecular parameters. This was true even when the starting material, *i.e.* the crude gum, was known to be a homogeneous sample. It therefore seemed desirable to prepare several samples of arabate salts and to evaluate their molecular weights, sizes, and shapes in solution by employing light-scattering and viscometric methods. The systems studied were sodium and potassium arabate; arabic acid was not used, as occurrence of strong interactions in this system has been suggested[2].

The molecular properties of the various samples were varied by introducing small changes in the preparative techniques; for the most part, this was done by trial and error. Finally, the experimental conditions responsible for the change were recognized, and this knowledge was used in making further preparations.

RESULTS AND DISCUSSION

The light-scattering data were evaluated by the method of Zimm. Representative plots are shown in Figs. 1 and 2. From these plots, the molecular weights, radii of gyration, and second virial coefficients were determined by employing the equations

$$\left(\frac{Kc_2}{R(\theta)}\right)_{\theta=0} = \frac{1}{(1-D)^2}\left(\frac{1}{M_w} + 2A_2c_2\right) \tag{1}$$

and

$$\left(\frac{Kc_2}{R(\theta)}\right)_{c_2=0} = 1 + \frac{16\pi^2}{3\lambda^2} \cdot R_g^2 \sin^2\frac{\theta}{2}, \tag{2}$$

where $K = 2\pi^2 n_0^2 (\Delta n/c_2)^2 / N\lambda^4$, n_0 is the refractive index of the solvent, N is the Avogadro number, λ is the wavelength of light *in vacuo* (436 nm), $\Delta n/c_2$ is the increment in refractive index, $R(\theta)$ is the Rayleight ratio, c_2 is the concentration of polymer in g/ml, M_w is the weight-average molecular weight, R_g is the root-mean-square (r.m.s.) radius of gyration of the polymer, A_2 is the second virial coefficient, and D is a thermodynamic correction-factor for a three-component system.

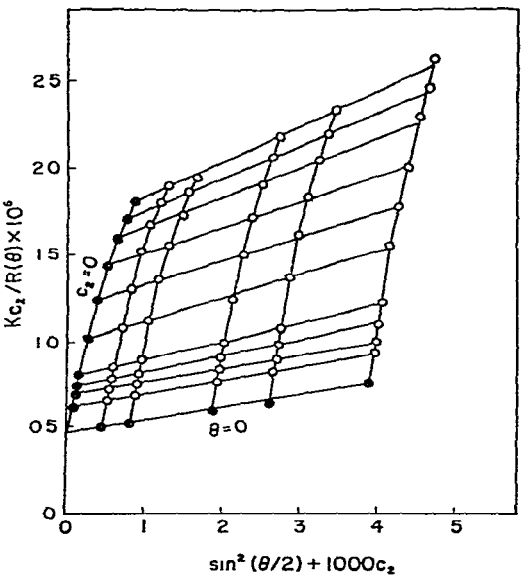

Fig 1. Zimm plot for potassium arabate, sample *G* in 20mM KCl.

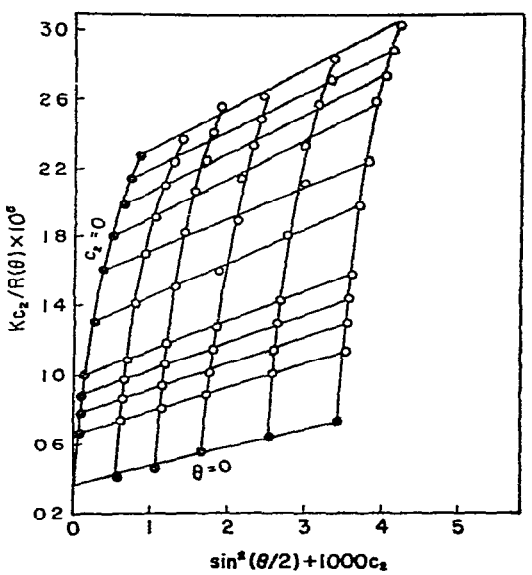

Fig. 2. Zimm plot for potassium arabate, sample *H* in 20mM KCl

The experimentally determined values of the refractive-index increment $\Delta n/c_2$ was 150 μl.g^{-1} for 20mM sodium chloride and potassium chloride, giving a value for the optical constant K of 3.70×10^{-7}.

The values of M_w and R_g calculated from Eqns. 1 and 2 are listed in Table I. Values for A_2 ranged from 6 to 15×10^{-5} ml.mol.g^{-2}. Compensation was made for the presence of a third component in the systems by use of the method suggested by Strauss and Wineman[13]. To use the nomenclature of these authors, the correction factor $(1-D)^{-2}$ was 1.02 for both of the 20mM salt solutions.

TABLE I

LIGHT-SCATTERING RESULTS OBTAINED WITH ARABATE SALTS IN AQUEOUS KCL AND NaCl SOLUTIONS

Sample	*Solvent*	$M_w \times 10^{-6}$	R_g (*nm*)	$R_g/M_w \times 10^3$
A	20mM KCl	0.57	25 0	4.4
B		0.86	47 2	5.5
C		0.91	51.3	5.6
D		1.38	71.4	5.2
E		1.50	89.7	5 8
F		1.89	95.9	5.1
G		2 25	103.9	4.6
H		2 88	154 0	5 4
H	5mM KCl	2 78	172.2	6 2
H		2.80	145.0	5.2
J	20mM NaCl	1.08	65 0	6.0
K		1.14	64.8	5.7
L		2.49	105.7	4 2

As may be seen from Table I, the various samples exhibited a wide range of molecular weights. This molecular-weight variation is attributed to the formation of stable aggregates of the fundamental arabate molecule, whose molecular weight has been reported[1,2,5] to lie in the range from 25 to 36×10^4. From the data presented, it is evident that the aggregation of gum arabic is in some manner effected during the preparation of pure samples from the crude gum. Once formed, the aggregates appeared to be stable; the results shown in Table I were readily reproduced in solutions having a wide range of concentrations of the simple salt. Also, no changes were observed after the solid samples had been kept under refrigeration for three months.

An attempt was therefore made to identify the experimental conditions during which this aggregation occurred. Eventually, it was recognized that the molecular weights of the products depended on the length of time that was initially needed in order to dissolve the crude gum in water. This behavior was made evident with samples *A* and *I*. For the first, where the dissolution time was purposely short, the polymer had a relatively low molecular weight. In contrast, in the preparation of sample *I*, the crude gum was dissolved over a long period of time; the molecular weight of that sample was the highest that was obtained in the present study, as

evidenced from the viscosity results. This observation finds some support in the literature; it has been reported[14] that such solution properties of crude gum arabic as viscosity may depend on the method of preparation of the solutions.

As regards the shapes of the dissolved arabate particles, it is of interest to accord some further consideration to the data obtained by light-scattering. The radii of gyration listed in Table I are appreciably higher than those that could be expected from more-or-less coiled molecules. Examination of Table I reveals a *linear* dependence of R_g on M_w, which indicates a rod-like shape for the arabate molecules in solution. If this is so, the particle scattering functions, $P^{-1}(\theta)$, constructed for uniform rods of appropriate length may be superposable on those obtained experimentally. The theoretical values of $P(\theta)$ were calculated by use of the equation[15]

$$P(\theta) = \frac{1}{x}\int_0^{2x} \frac{\sin w}{w}\,dw - \left(\frac{\sin x}{x}\right)^2, \tag{3}$$

where $x = 2\pi L \sin(\theta/2)/\lambda$, w is the integration variable, and L is the length of the (presumably uniform) rod-like molecules.

The values obtained were plotted as $P^{-1}(\theta)$ against $(R_g)^2h^2$, where

$$h = \frac{4\pi \sin(\theta/2)}{\lambda}. \tag{4}$$

The results are presented as solid lines in Figs. 3 and 4. The points in Fig. 3 are the experimental values of $P^{-1}(\theta)$ for sample H, calculated from Fig. 2 by means of the equation

$$\left(\frac{Kc_2}{R(\theta)}\right)_{c_2=0} = \frac{1}{M_w} P^{-1}(\theta). \tag{5}$$

The value of R_g used in that case was 154.0 nm. The points in Fig. 4 show a similar result for sample G. The radius of gyration R_g was 104.0 nm. From Figs. 3 and 4, it may be seen that very good agreement exists between the experimental and the theoretical values of $P^{-1}(\theta)$. These results indicated that samples H and G were largely uniform. It may be shown that, with the other samples, the experimental points fall below the theoretical curves, as expected of polydisperse, scattering systems. It is not possible to state definitely the extent to which the superposition of the $P^{-1}(\theta)$ curves constitutes an unequivocal proof of the rod-like nature of the scattering particles. For polydispersed samples, there exists a multitude of possibilities in which the different sizes and shapes of particles may be arranged. Hence, it is conceivable that a particular distribution may be found such that the light-scattering patterns produced are misleading in terms of shapes. On the other hand, such a condition would seemingly be coincidental, and would scarcely be expected to occur in a manner as consistent as that indicated in the foregoing discussion.

The reduced viscosities were obtained by extrapolation to zero rate of shear. Whereas the viscosity was independent of the shear rate for the samples having low molecular weight, some shear-dependence was noted for samples of higher molecular

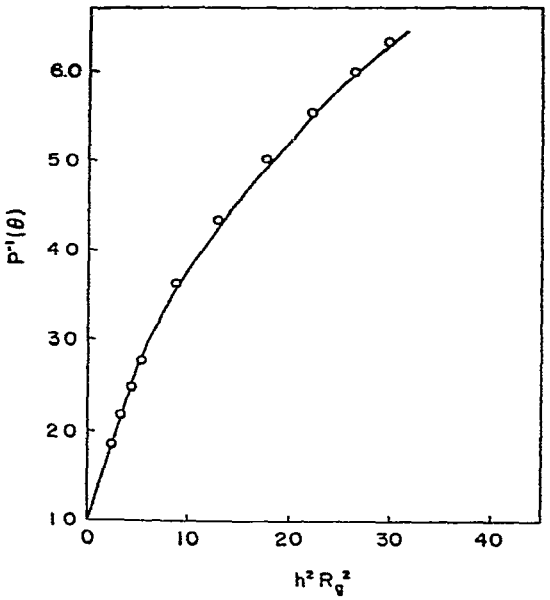

Fig. 3. A comparison of the experimental and theoretical values of $P^{-1}(\theta)$ for rods for sample *H*. The points are experimental values and the line is theoretical

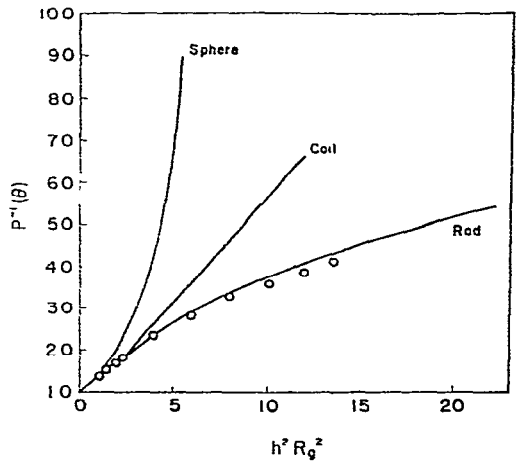

Fig. 4. A comparison of the experimental and theoretical values of $P^{-1}(\theta)$ for rods for sample *G*. The points are experimental values and the lines are theoretical

weight. The intrinsic viscosities $[\eta]$ and the values of the Huggins constant k' were determined in the usual way. The viscosity results are summarized in Table II.

A logarithmic plot of the intrinsic viscosities and their corresponding molecular weights gave a straight line, as shown in Fig. 5. It is well known that a small proportion of relatively large, random aggregates (or impurities) can produce a significant change in the values obtained by light-scattering. The viscosity is, however, little, if at

TABLE II

VALUES OF INTRINSIC VISCOSITY AND HUGGINS CONSTANT OBTAINED FOR POTASSIUM ARABATE IN 20mM KCl AND NaCl

Sample	*Solvent*	$[\eta]$ (*ml/g*)	k'
A	20mM KCl	25.8	1.01
C		32.5	0.95
E		39.3	0.47
G		46.0	0.51
H		52.4	0.39
I		81.0	0.37
J	20mM NaCl	36.0	0.62

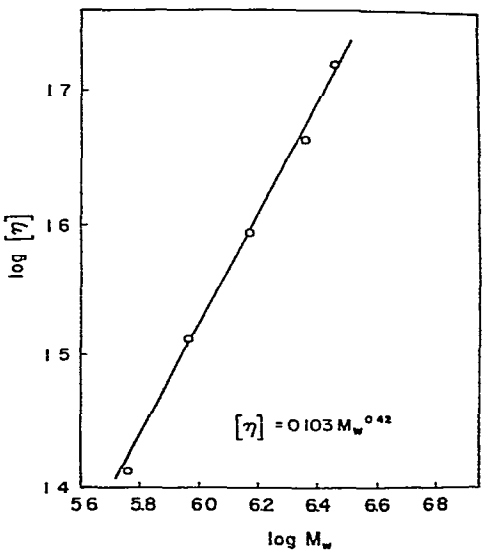

Fig. 5 Variations of intrinsic viscosity with the molecular weight, for several samples of potassium arabate in 20mM KCl

all, affected by this type of aggregation[15]. Because, in Fig. 5, the viscosity of the arabates is seen to vary with the molecular weight, and as this variation is well defined and quite significant, it is indicated that the high values obtained by light-scattering cannot be attributed to experimental artifacts. Rather, it seems obvious that the observed differences in molecular properties are real and that the mechanism by which the various arabate samples are made to differ leads to products having characteristic properties.

The relationship shown in Fig. 5 may be expressed in the form of the Mark–Houwink equation

$$[\eta] = KM^a \qquad (6)$$

where, for the present system, $K = 0.103$ and $a = 0.42$, when $[\eta]$ is expressed in $ml.g^{-1}$. On this basis, the intrinsic-viscosity value of 81.0 $ml.g^{-1}$ obtained for

sample *I* corresponds to a molecular weight of 7.9×10^6. The determined value of K is similar to that found for several other polysaccharides. For example, in 0.33M aqueous potassium chloride at 25°, amylose[17] showed $K = 0.115$, and cellulose tricaproate in *p*-dioxane[18] at 35° had $K = 0.125$ when $[\eta]$ was expressed as $ml.g^{-1}$. Values of that order were also reported[19] for some other derivatives of cellulose and for lower molecular-weight fractions of dextran.

If it is assumed that the weight-average and the viscosity-average molecular weights in the various samples are not very different from one another, the determined value of a in Eqn. 6 may be taken as an indication that the arabate particles in solution resemble highly branched macromolecules. As was shown from theoretical considerations[20] and also borne out by experiment[21], in such cases the values of a may be less than 0.5 and may become as low as 0.25. Taking into account the high degree of branching shown[22] to be present in the molecules of gum arabic, the foregoing description seems quite reasonable. In addition, the observed magnitudes of the shear dependence of viscosity seem also to be nearer to what has been observed for spherical models[23] than for rods.

The data obtained from light-scattering consistently follow relationships for long, rod-like structures. On the other hand, the low values of the viscosity, the observed dependence of intrinsic viscosity on the molecular weight, and the shear effects of viscosity provide evidence for a spheroid shape. It is interesting that the light-scattering results of Veis and Eggenberger[3] were also taken as being evidence for elongated structures, whereas the hydrodynamic data were, in general, more consistent with spheroid shapes.

The results of the present study indicate that salts of gum arabic can form stable, rod-like aggregates in aqueous salt solutions. Also, it is suggested that these aggregates are held together by such weak forces that they could be deformed or break up in a low-shear field, thereby giving rise to relatively low intrinsic viscosities and to low shear dependence of the viscosity. These interpretations are in accord with several recent, interesting studies. Eliezer and Silberberg[24] have demonstrated that branched poly(α-amino acids) form aggregates, as evidenced from light-scattering measurements. However, they have shown[25] that these aggregates break down in extremely mild viscosity-gradients. Also, it has been reported[26,27] that large, macromolecular aggregates can be deformed in low velocity-gradients to produce low values of viscosity.

EXPERIMENTAL

Preparation of arabate samples. — All samples used in the present study were made from one lot of "hand-picked, select" Acacia Senegal, kindly provided by S. B. Penick & Co. The gum tears were picked at random from this lot. To ensure homogeneity in the starting material, preparations designated *A*, *C*, *E*, and *F* were made from a single sample of powdered, thoroughly mixed gum.

The sodium and potassium arabates were prepared by two techniques. (1) Samples *A*, *B*, *C*, *E*, and *F* were prepared by a conventional method[6]. A 10 to 20%

solution of gum arabic in water was adjusted to pH ~1.5 with hydrochloric acid, and arabic acid was recovered from the solution by precipitation with alcohol. The acid was redissolved in water, and neutralized with the appropriate base (sodium hydroxide or potassium hydroxide) to pH 8.0, and the salt precipitated by the addition of alcohol, and dried *in vacuo*.

(2) Samples *D*, *F*, *G*, *H*, *I*, *J*, and *K* were prepared as follows. A 10 to 20% solution of the gum in water was filtered to remove solid impurities, and the filtrate was mixed with Dowex 50W X12 ion-exchange resin in the sodium or potassium form. The amount of resin used was such that, for each equivalent of gum, assuming its equivalent weight[28] to be 1200, 5 equivalents of resin were present; the suspension was stirred for 3 h, and the solution was decanted. A new portion of fresh resin was added to it, and the process was repeated After a total of 4 such batch exchanges, the solution was clarified by centrifugation in a Sorvall preparative centrifuge at 17,000 r.p.m. for 10 h at 3°, and then freeze-dried.

Within the two general procedures just outlined, certain experimental conditions were varied slightly in order to determine whether any of the following factors could affect the properties of the final arabate: (*a*) partial hydrolysis of the gum when in contact with 0.1M hydrochloric acid for up to 6 h, (*b*) degradation of the crude gum by mechanical grinding, and (*c*) presence in the final product of a substance, reportedly present[29] in the crude gum, which, although initially insoluble, dissolves on being peptized by the already soluble gum. None of those factors seemed to be responsible for the observed variations in the molecular weight and viscosity.

It is of interest to consider the way in which the gum was initially dissolved in water. Ordinarily, this was done by introducing the crude gum, in the form of the original tears or as a powder, into water, with subsequent stirring for several hours. On several occasions, however, the gum was dissolved in a different way. A plastic dish was filled to the top with water, and was then covered with a sheet of filter paper of fine porosity, so supported by a fine screen of stainless-steel wire that the water barely wetted the paper. The filter paper was covered with a layer of finely ground gum, which then slowly diffused into the water. This technique, designed for other purposes[1], effectively provided relatively long times for dissolution. Thus, in the preparation of sample *I*, the dissolution time (~12 h) was longer than that for any other sample. On the other hand, to prepare sample *A*, the finely powdered, crude gum was dissolved in water, with vigorous stirring, within 30 min.

Samples *K*, *L*, and *J* were prepared as sodium salts, and the rest as potassium salts.

The amount of water present in the samples was found by determining the losses in weight that occurred when portions of these samples were heated at 60° under vacuum; drying for 1 to 2 days was needed, to give constant weight. The losses in weight were in the range of 4 to 6%.

The equivalent weight was determined by using sample *I*. The value of 1270 obtained was in agreement with the results in the literature[6].

Light-scattering. — The solutions were clarified with sintered-glass filters of

"ultrafine" porosity. A solution of the polymer in water was filtered into a dust-free cell, and then an approximately equal volume of the salt solution was added, also by filtration. The final concentrations were determined by weight, the initial concentrations of polymer and salt, and the necessary densities, being taken into account. The constancy of the concentration of the polymer before and after filtration was checked by measurements of the differences in refractive index. Lower concentrations of polymer were obtained by successive dilution in the cell with 20.0 mM salt solution. The filtration procedure just described was adopted after it had been found that repeated filtrations of polymer in aqueous salt solutions (but not in pure water) often resulted in diminution of the scattering and of the dissymmetry values. Light-scattering measurements could not be performed with sample *I*, because, in the process of filtration, this polymer appeared to undergo fractionation on the sintered glass.

The light-scattering measurements were performed with a Brice–Phoenix photometer and light of 436 nm. The measurements were made at scattering angles (θ) of 30, 35, 40, and 45°, and then at 15-degree intervals up to 135°. The calibration of the instrument was checked by measuring the Rayleigh ratio, $R(90)$, of spectroscopic-grade benzene[30] and by determining the molecular weight of a sample of standard polystyrene obtained from the National Bureau of Standards. By both procedures, the results compared favorably with the values reported.

The cylindrical, light-scattering cells used in all determinations were calibrated against the standard, semioctagonal cell with a 0.25% solution of Ludox. Optical uniformity and symmetry of the cylindrical cells, and their alignment with respect to the incident beam, were checked by use of a dilute solution of fluorescein. The intensities measured were within $\pm 0.7\%$ of the $\sin \theta$ relationship expected.

The contributions of fluorescence and depolarization to the scattering ratio were found to be too small to necessitate compensation; hence, they were ignored.

The measurements of increment in refractive index were performed in a Brice–Phoenix differential refractometer calibrated with aqueous solutions of potassium chloride.

Viscosity. — The measurements of viscosity were performed at 25.00° in a Cannon–Ubbelohde viscometer, and the values were obtained at three different rates of shear. Owing to the design of the instrument, the kinetic-energy corrections were negligible. The concentrations of polymer were varied by dilution in the viscometer. The densities of the polymer solutions and of the solvent, which were needed for the calculations of viscosity, were determined at the same temperature with a 10-ml pyknometer.

ACKNOWLEDGMENT

This investigation was supported, in part, by U. S. Public Health Service Grant GM 11158.

REFERENCES

1 H. B. OAKLEY, *Trans. Faraday Soc.*, 32 (1936) 1360; 33 (1937) 372
2 S. SAVERBORN, *The Svedberg* (Mem. Vol.), (1944) 508.
3 A. VEIS AND D. N. EGGENBERGER, *J. Amer. Chem. Soc.*, 76 (1954) 1560.
4 S. N. MUKHERJEE AND S. K DEB, *J. Indian Chem. Soc.*, 39, No. 12 (1962) 823.
5 H. A. SWENSON, H. M. KAUSTINEN, O. A. KAUSTINEN, AND N. S THOMPSON, *J. Polym. Sci*, *A 2*, 6 (1968) 1593.
6 A. W. THOMAS AND H. A. MURRAY, *J. Phys. Chem*, 32 (1928) 676.
7 R H. SCHLEIF, T. HIGUCHI, AND L. W. BUSSE, *J. Amer. Pharm. Assoc., Sci. Ed*, 40 (1951) 98
8 R. TAFT AND L. E. MALM, *J. Phys. Chem*, 35 (1931) 874.
9 R. H. SCHLEIF, T. HIGUCHI, AND L. W. BUSSE, *J. Amer. Pharm. Assoc., Sci. Ed*, 40 (1951) 221
10 S. BASU, P. C. DASGUPTA, AND A K. SIRCAR, *J. Colloid Sci.*, 6 (1951) 539.
11 J. COUMON, *Chem. Weekblad*, 32 (1935) 426.
12 J. T. G. OVERBEEK, *Bull Soc. Chim. Belg.*, 57 (1948) 252.
13 U. P. STRAUSS AND P. L. WINEMAN, *J. Amer. Chem. Soc*, 80 (1958) 2366.
14 C. L. MANTELL, *The Water-Soluble Gums*, Reinhold, New York, 1947.
15 E. P. GEIDUSCHECK AND A. HOLTZER, *Advan. Biol. Med. Physics*, 6 (1958) 431.
16 G. SITARAMAIAN AND D. A I. GORING, *J. Polym. Sci.*, 58 (1962) 1107.
17 W. W. EVERETT AND J. F. FOSTER, *J. Amer. Chem. Soc.*, 81 (1959) 3484.
18 W. R. KRIGBAUM AND L. H. SPERLING, *J. Phys Chem.*, 64 (1960) 99.
19 J. BRANDRUP AND E. H. IMMERGUT (Eds.), *Polymer Handbook*, Interscience, New York, 1966.
20 B. W. ZIMM AND R. W. KILB, *J. Polym. Sci.*, 37 (1959) 19.
21 F. R. PRICE, J. H. GIBBS, AND B. H. ZIMM, *J. Phys. Chem*, 62 (1958) 972
22 D. M. W. ANDERSON, E. L. HIRST, AND J. F. STODDARD, *J. Chem Soc. (C)*, (1966) 1959
23 T. G FOX, P. C. FOX, AND P. J. FLORY, *J. Amer. Chem. Soc*, 73 (1951) 1901.
24 N. ELIEZER AND A. SILBERBERG, *Biopolymers*, 5 (1967) 95.
25 N. ELIEZER AND A. SILBERBERG, *Biopolymers*, 5 (1967) 105.
26 H L GOLDSMITH, *Science*, 153 (1966) 1407.
27 I. Y. Z. ZIA, R G. COX, AND S. G. MASON, *Science*, 153 (1966) 1406.
28 M. GLICKSMAN AND R. E. SCHACHAT, *Industrial Gums*, Academic Press, New York, 1959.
29 L. AMY, *Ann. Chim*, 2 (1934) 295.
30 J. P. KRATOHVIL, G. DERELIC, M. KERKER, AND E. MATIJEVIC, *J. Polym. Sci.*, 57 (1962) 59.