HETEROGENEITY OF GUM ARABIC AND ITS SALTS

ROGER E. NELSON AND PAUL ANDER*
Department of Chemistry, Seton Hall University, South Orange, New Jersey 07079 (U. S. A.)
(Received April 17th, 1972; accepted for publication June 8th, 1972)

ABSTRACT

Moving-boundary electrophoresis experiments with the alkali-metal salts of gum arabic have shown the existence of a previously unreported, minor component. This component arises when gum arabic is kept in solution for several days, and it appears to be a hydrolysis product. From the frictional coefficients, calculated from the electrophoretic mobilities at infinite ionic strength, it was deduced that the hydrolysis proceeds with the removal of specific branches from the main backbone of the gum arabic molecule. Approximately 35 percent of the branches are more readily hydrolyzed than the others and, thus, are different from the rest of the branches. The labile branches appear to be made more susceptible to hydrolysis by a physical change, possibly dehydration, that occurs in crude gum arabic when it is stored in the dry state. The equivalent weight of the hydrolysis product is 1490, compared to 1190 for gum arabic. The electrophoretic data, combined with light-scattering data, indicate that its molecular weight is approximately 20 percent less than that of gum arabic, but that, in solution, its molecules are larger than those of gum arabic. The high molecular weights observed in light-scattering experiments have been shown to be due to the presence of the hydrolysis product, which appears to cause aggregation through hydrogen bonding. The aggregates are very loosely held together, however, and can break apart in an electric field and in a shear field. At high ionic strengths, both gum arabic and its hydrolysis product are free-draining, and both have very large frictional coefficients, indicative of the large size of the ionic segments in these molecules.

INTRODUCTION

Gum arabic, an ionic polysaccharide exuded by trees belonging to various species of the genus *Acacia*, has a chemical composition that has been reasonably well established[1,2]. Numerous degradation studies have shown that the fundamental monosaccharide moieties in gum arabic are residues of D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid. The accepted structure is that of a backbone

*To whom inquiries should be directed.

consisting exclusively of D-galactose residues joined by β-(1→3)-linkages[3]. The other monosaccharide residues exist in branches stemming from O-6 of the D-galactose residues in the backbone. The L-arabinose and L-rhamnose residues occupy terminal positions in the branches, and the D-glucuronic acid residues are at penultimate positions. On the basis of degradation studies, Hirst *et al.*[4] suggested a highly branched, globular structure, and Swenson *et al.*[3] reported evidence for the existence of a major backbone in gum arabic.

An equivalent weight of ~1200 for gum arabic has been reported by many investigators; a few spurious values[1] were probably attributable to the use of impure or degraded samples. Drying of gum arabic can apparently result in the formation of a partly insoluble polymer having a lower content of carboxylic acid as compared to that of the undried material[5,6]. The formation of ester crosslinks has been suggested to explain the experimental findings[7]. The molecular weight generally accepted for gum arabic and its salts[8,9] is in the range of 2.5 to 3.0×10^5. Veis and Eggenberger[10] and Mukherjee and Deb[11] respectively reported average molecular weights of 11 and 5.7×10^5 by light-scattering of arabic acid in aqueous solution. Sloniewsky and Ander[12] found that the average molecular weight by light-scattering for different preparations of potassium arabate lay between 5.7×10^5 and 28.8×10^5, depending on the way in which the sample was prepared. They attributed this variation to the formation, during the preparation of pure arabate salts from the crude gum, of stable aggregates, and characterized these aggregates in aqueous salt solutions by measurement of light-scattering and viscosity.

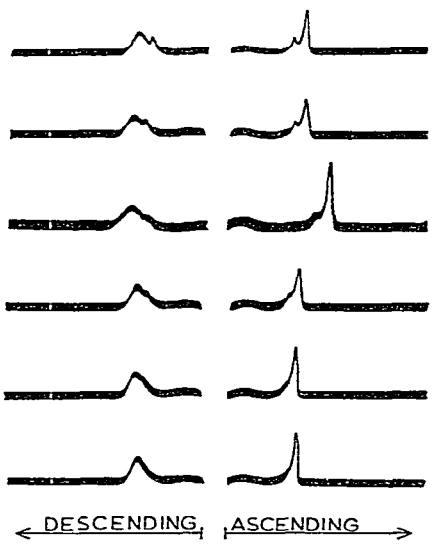

Fig. 1. Electrophoretic patterns obtained in 0.10M KCl for potassium arabate samples of different molecular weights. From top to bottom, molecular weights and concentrations of the samples are: M, 40.0×10^5 (0.400%); H, 28.8×10^5 (0.406%); F, 18.9×10^5 (0.581%); E, 15.0×10^5 (0.411%); C, 9.1×10^5 (0.410%); and A, 5.7×10^5 (0.414%).

Heidelberger *et al.*[13,14] showed that the portion of gum arabic precipitated by Type II antipneumococcus serum had a much lower content of L-rhamnose than had the original gum, and they suggested compositional differences between the precipitated and the non-precipitated fraction. Additional indications of the possible heterogeneity of gum arabic have been found by several other workers[15–18]; hence, when we quite inadvertently observed two electrophoretic peaks in experiments with certain preparations of a salt of gum arabic, it became of interest to investigate this matter further, in order to see how these results might be related to other published results indicating the possibility of heterogeneity.

RESULTS AND DISCUSSION

Samples of different preparations of the alkali-metal salts of gum arabic of various molecular weights exhibited different electrophoretic patterns. Fig. 1. shows the electrophoretic patterns in 0.10M potassium chloride of several samples of potassium arabate of different molecular weight. The molecular weights of the different samples shown in Fig. 1 decrease from the top to the bottom, and so does the proportion of the minor peak. Table I lists the weight-average molecular weight (M_w) of each

TABLE I

MOLECULAR WEIGHT, AND WEIGHT FRACTION OF MINOR PEAK FOR SEVERAL SAMPLES OF POTASSIUM ARABATE

Sample	$M_w \times 10^{-5}$	W_B
M	40.0	0.32
H	28.8	0.21
F	18.9	0.14
E	15.0	0.11
C	9.1	0.06
A[a]	5.7	0.00

[a]Probably contained a small proportion of fraction *B*, but this could not be determined experimentally.

sample[12], with the corresponding weight-fraction of the minor peak, W_B, properties found to be related by equation *1*.

$$M_w = (0.20 + 12\ W_B) \cdot 10^6 \qquad (1)$$

Equation *1* gives the value 2.0×10^5 as the molecular weight of the arabate molecule (*A*) without any of the minor fraction (*B*) present. It is interesting that this value is close to those reported for the arabate molecule[8,9], considered to be the major fraction *A*. The minor fraction *B* could not be an impurity, as all samples were prepared from the same batch of crude gum arabic with a conversion of >80 percent, and the proportion of fraction *B* present in some samples was as high as 32 percent of the total. Thus, the proportion of fraction *B* present depends on the method of preparation.

Sloniewsky[19] has shown that a sample of potassium arabate having a molecular weight of 10.8×10^5 in 0.02M potassium chloride had a molecular weight of 2.9×10^5 in 4M urea. As urea is known to dissociate aggregates of macromolecules, this observation was interpreted as meaning that the higher molecular weights found for certain samples of arabate differently prepared were stable aggregates of the arabate molecule having a molecular weight of 2.5×10^5 as determined in aqueous salt solutions. When sample *M*, containing 32 percent of the material giving the minor peak, in 0.10M potassium chloride plus 4M urea was examined electrophoretically, electrophoretic patterns the same as those observed in 0.10M potassium chloride were found. Because, in the absence of urea, two peaks indicate a high molecular weight, and because, in the presence of urea, the same two peaks were observed, these results indicate that fraction *B* does not have a high molecular weight, as it can be present under conditions where the molecular weight is low. Thus, it appears that the presence of fraction *B* causes an increase in the molecular weight, but fraction *B* does not necessarily have an extremely high molecular weight. Consequently, fraction *B* probably acts as a nucleus for aggregation during static, light-scattering measurements, but the aggregates may be very loosely held together and may break apart in the electric field during the electrophoretic measurements. Weakly bonded aggregates of macromolecules have been shown to break up[20] or to be deformed[21,22] in low-shear fields.

It was found that fraction *B* could not be removed from a given sample by dialysis, indicating that it is definitely not a material having a very low molecular weight whose presence causes aggregation. In fact, it was found that, once a two-peak sample had been prepared, the proportion of the minor peak present could not be altered by any normal means. For example, when dissolved in water and then isolated by precipitation or by freeze-drying, a sample exhibiting two peaks showed the same two peaks after both treatments. Likewise, a one-peak sample exhibited the same, single peak after these two treatments. Also, the electrophoretic patterns exhibited by a given sample were unaffected by filtration, centrifugation, dialysis, or treatment with an ion-exchange resin.

It was found that the method used in the preparation of these samples controlled whether one peak or two would be observed in the electrophoretic patterns. All samples that had been prepared by very quickly dissolving the crude gum in water exhibited only one peak, whereas samples prepared by dissolving the crude gum very slowly, over a long period of time, exhibited two peaks. In the product obtained by the latter procedure, the proportion of the minor peak was variable and was, in some instances, very small.

Having found that the method of initial dissolution of the crude gum played a major role in the properties of the sample ultimately isolated, two large batches of arabic acid were prepared in two different ways. In the first, the crude gum was ground up in a mortar, and the resulting powder was added in small portions to the vortex of rapidly stirred water. In the second, whole tears of the crude gum were immersed in water, and allowed to swell slowly and to dissolve during three days, with only very

gentle stirring. In both procedures, the final concentration of the solution of the crude gum was the same, and all subsequent steps in the preparations were identical. These two preparations yielded samples of arabic acid referred to as HAr(1) and HAr(2), respectively. The lithium, potassium and sodium, salts of both acids were then prepared; all three of the salts of HAr(1) were found to exhibit only one electrophoretic peak, whereas the three salts of HAr(2) all exhibited two peaks. Prior to the preparation of these samples, it had been found that grinding of the crude gum has no effect on the electrophoretic pattern. Thus, when dissolved very slowly, a sample of the ground, crude gum yielded a solution exhibiting two peaks.

The salts of HAr(1) and HAr(2) were prepared as representative one- and two-peak samples, respectively, to be subsequently used in comparative experiments. Most of the work was conducted with the potassium salts of the two samples of arabic acid, but the sodium or lithium salts could just as well have been used, because the electrophoretic properties of both the *A* and the *B* fractions were in all cases found to be independent of the counter-ion.

Figs. 2 and 3 show the electrophoretic patterns of KAr(1) and KAr(2), respectively, as a function of their concentration in 0.10M potassium chloride. The sharpness of the ascending boundaries, and the broadness of the descending boundaries, are due to concentration gradients and pH gradients across the boundaries, and are well known phenomena in electrophoretic experiments[23–25]. As may be seen, the ascending and the descending patterns become, in both cases, more enantiographic with decreasing concentration, as is normally found in electrophoretic experiments.

The patterns of KAr(2) as a function of concentration, depicted in Fig. 3, are of particular interest, because they show that the proportion of the minor peak is

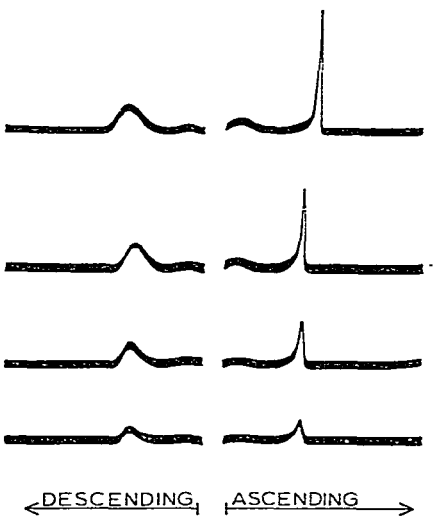

Fig. 2. Electrophoretic patterns of KAr(1) in 0.10M KCl as a function of the concentration. From top to bottom, the percent concentrations are 0.740, 0.583, 0.389, and 0.219.

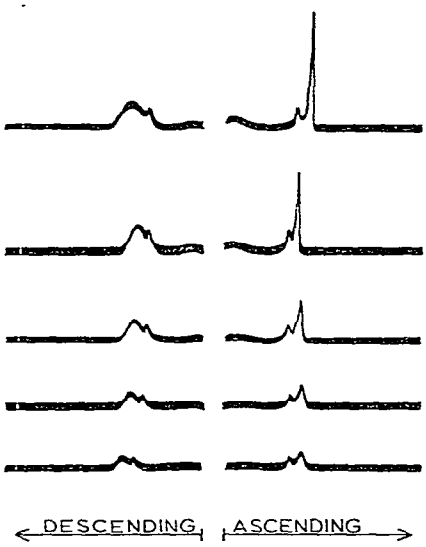

Fig. 3. Electrophoretic patterns of KAr(2) in 0.10M KCl as a function of the concentration. From top to bottom, the percent concentrations are 0.736, 0.586, 0.400, 0.244, and 0.176.

independent of the concentration. A number of proteins, such a β-lactoglobulin[24,26], have been shown to undergo reversible aggregation in moving-boundary experiments, and these phenomena have been successfully treated by the Gilbert theory[27]. This theory indicates that, for a rapidly equilibrating system, bimodal electrophoretic patterns are to be expected, but that the relative proportions of the two peaks should depend on the concentration. Because, in the arabate system, there are indications that fractions *A* and *B* aggregate under equilibrium conditions but dissociate under the electric field applied, re-equilibration must be slow, as, otherwise, the electrophoretic patterns would be dependent on the concentration.

The electrophoretic patterns of KAr(2) in several aqueous solutions of potassium chloride over the range of ionic strength of 0.02 to 0.20 showed identical patterns. Thus, the presence of fraction *B* in a given sample is independent of the ionic strength, as well as of the concentration.

It was found that the equivalent weight of a sample was dependent on the weight fraction of its minor peak, according to the linear relationship

$$EW = 1190 + 300\ W_B, \qquad (2)$$

where EW is the equivalent weight of arabic acid. From equation *2*, when $W_B = 0$, an equivalent weight of 1190 for fraction *A* is obtained. This result is in excellent agreement with the values previously reported for arabic acid[1,7], thus again indicating that fraction *A* is the gum arabic molecule studied by most of the other workers. When $W_B = 1$, an equivalent weight of 1490 is obtained for fraction *B*. The titration curves of HAr(1) and HAr(2) were identical[28], except for the positions of the inflection points, indicating that the carboxylic acid groups in both the *A* and the *B* fractions are the same.

It has been suggested by other workers that some of the carboxylic acid groups in gum arabic can exist in ester[7] or lactone[29] form. Occurrence of some of the carboxyl groups in fraction *B* in either or both of these forms would account for the higher equivalent weight of this fraction. However, infrared spectral analysis of HAr(1), HAr(2), and their respective potassium and sodium salts failed to reveal the existence of any such groups. Thus, all of the carboxyl groups in fraction *B* are in the acid form, and its higher equivalent weight must be due to a lower number of changes per unit weight.

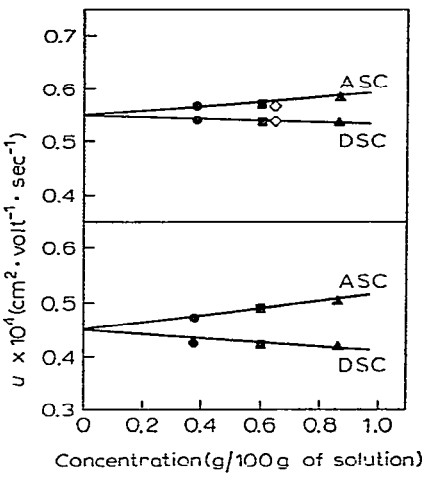

Fig. 4. Electrophoretic mobility of salts of gum arabic as a function of the polymer concentration in 0.20M simple electrolyte. Upper, fraction *A*; Lower, fraction *B*. ●, KAr in KCl; ▲, NaAr in NaCl; ■, LiAr in LiCl; and ◇, gum arabic, from Joubert[15].

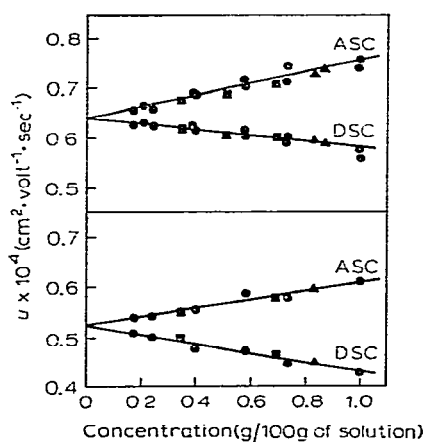

Fig. 5. Electrophoretic mobility of salts of gum arabic as a function of the polymer concentration in 0.10M simple electrolyte. Upper, fraction *A*; Lower, fraction *B*. ●, KAr in KCl; ▲, NaAr in NaCl; and ■, LiAr in LiCl.

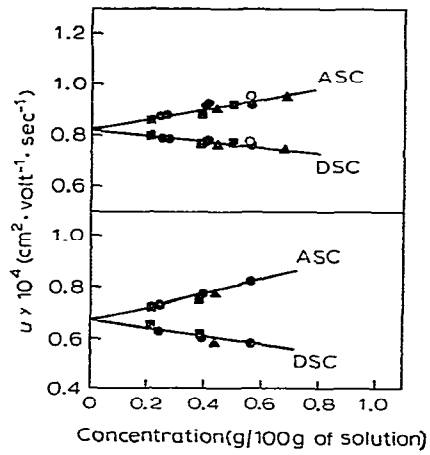

Fig. 6. Eletrophoretic mobility of salts of gum arabic as a function of the polymer concentration in 0.05M simple electrolyte. Upper, fraction *A*; Lower, fraction *B*. ●, KAr in KCl; ▲, NaAr in NaCl; ■, LiAr in LiCl; and ○, KAr in KBr.

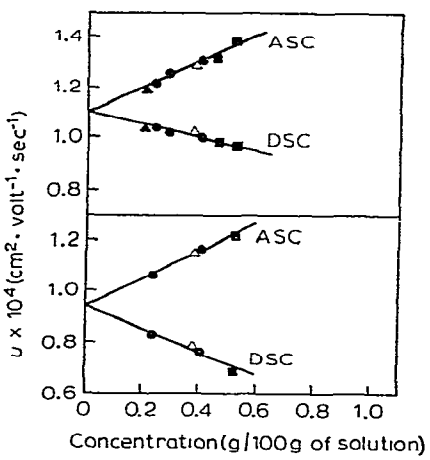

Fig. 7. Electrophoretic mobility of salts of gum arabic as a function of the polymer concentration in 0.02M simple electrolyte. Upper, fraction *A*; Lower, fraction *B*. ●, KAr in KCl; ▲, NaAr in NaCl; ■, LiAr in LiCl; and △, NaAr in KCl.

The electrophoretic mobilities of arabic salts containing one and two peaks are shown in Figs. 4–7 as a function of the concentration of the polymer in 0.20, 0.10, 0.05, and 0.02M simple electrolyte, respectively. The mobilities were in all cases negative; that is, the poly-ion was negatively charged, but the minus sign has been omitted from all mobilities reported. The mobilities of fraction *A*, determined from two-peak salts, were found to be identical, within experimental error, to those obtained for one-peak salts. These results were generally obtained in the presence of the alkali-metal chloride having the same cation as the arabic salt. However, in one

instance, NaAr was examined in the presence of potassium chloride (see Fig. 7), and KAr was studied in the presence of potassium bromide (see Fig. 6). Included in Fig. 4 are data points taken from the results of Joubert[15]. As may be seen, these points lie almost exactly on the curves for fraction *A*, indicating once again that this is the species that has been studied by most of the other workers.

It is obvious from these Figures that the mobility of the arabate anion, of either fraction *A* or *B*, is independent of the nature of the alkali-metal counter-ion, at all of the ionic strengths investigated. As may be seen, the linear plots from both the ascending and descending boundaries extrapolate to a common point, namely, the mobility of the arabate anion at infinite dilution (u°). The values of u° for both the *A* and the *B* fractions at several ionic strengths are listed in Table II. It may be

TABLE II

MOBILITIES AT INFINITE DILUTION OF FRACTIONS *A* AND *B* AT SEVERAL IONIC STRENGTHS

Ionic strength	$u^\circ \times 10^4$ ($cm.volt^{-1}.sec^{-1}$)	
	Fraction A	*Fraction* B
0.02	1.10	0.94
0.05	0.82	0.67
0.10	0.64	0.53
0.20	0.55	0.45
∞	0.31	0.24

noted that u° increases sharply with decreasing ionic strength (I), and it was found that u° is a linear function of $I^{-1/2}$; this is shown in Fig. 8, in which it may be seen that fractions *A* and *B* have very similar slopes (1.01×10^{-5} and 0.96×10^{-5}, respectively) but appreciably different intercepts at infinite ionic strength. The extrapolated values of u° at infinite ionic strength for fractions *A* and *B* are also given in Table II.

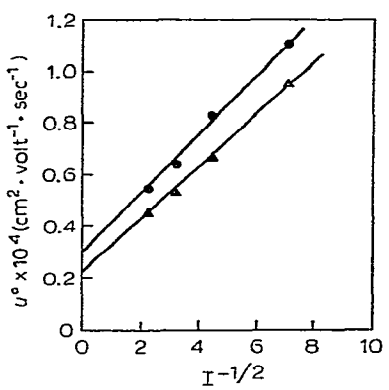

Fig. 8. Electrophoretic mobility at infinite dilution as a function of the reciprocal square root of the ionic strength. ●, fraction *A*; ▲, fraction *B*.

Hermans and Fujita[30] have shown that the mobility of a free-draining sphere having a low charge-density will have a finite value at infinite ionic strength, whereas, for an impermeable sphere having a high charge-density, the mobility tends toward zero with increasing ionic strength. Clearly, the former situation applies to the arabate system, as u° is finite at infinite ionic strength. Thus, fractions *A* and *B* are both free-draining. The equation derived by Hermans and Fujita[30], who used slightly different symbols, is

$$u^\circ = e/f + 2\rho/3\eta B^2 I, \qquad (3)$$

where f is the frictional coefficient of a polymer segment containing one electronic charge e, ρ is the charge density of the molecule, η is the viscosity of water, and B is a constant that relates the thickness of the ionic atmosphere to the ionic strength.

Equation *3* indicates that, at finite ionic strength, u° should depend on I^{-1}, not on $I^{-1/2}$, as found for the arabate system. However, the derivation of equation *3* neglected relaxation effects, which, according to Overbeek[31], would involve a correction term proportional to I^{-1}. The second term on the right-hand side of equation *3* also involves the charge density. Were the charge density to change with increasing ionic strength, the mobility would not be expected to be strictly proportional to I^{-1}. However, from combined measurements of viscosity and light-scattering, Swenson and co-workers[3] found negligible expansion of the gum arabic molecule with decreasing ionic strength, so that, in this system, the charge density is essentially constant, provided that, with decreasing *I*, there is no change in the degree of ionization of the poly-ion. Hermans and Fujita's treatment[30] also does not take into account the finite size of the counter-ions, whereas Gorin's treatment[32] of Henry's equation for the electrophoretic mobility of an impermeable sphere does consider it. Neither does Hermans and Fujita's study allow for the possibility of incomplete ionization of the poly-ion. Thus, the failure of equation *3* to conform to the experimental results probably stems from the authors' neglect of relaxation effects and of the finite size of the counter-ions in the theoretical derivation, and of the possibility of changes in ionization with decreasing ionic strength.

As the correction term in equation *3* becomes negligible at infinite ionic strength, the extrapolated mobilities in Fig. 8 can be used to calculate molecular parameters, and these parameters may then be compared with results obtained at finite ionic strengths, provided that e/f is constant with respect to ionic strength. As already noted, the work of Swenson and co-workers[3] indicates that the latter proviso is probably met in the arabate system. It is interesting that, for fractions *A* and *B*, when the u° values are plotted against $I^{-1/2}$, the resulting curves have only very slightly different slopes; this difference in slope, ~5 percent, is probably within the experimental error. Although these slopes cannot be interpreted in the light of any existing theories, it would seem that, as fractions *A* and *B* afford essentially the same slopes, the factors affecting the mobilities of both fractions are identical. The difference between the intercepts in Fig. 8, ~25 percent, is outside the experimental error and indicates that the frictional coefficient of fraction *B* is larger than that of fraction *A*;

this could be attributable to a higher molecular weight of a chain segment in fraction *B*; or, should both fractions have the same molecular weight per segment, to a greater resistance to movement of a chain segment in fraction *B*. Because the equivalent weight of fraction *B* is higher than that of fraction *A*, the form of its molecule would appear to be the main cause of the lower mobility of fraction *B*.

In the absence of any detailed knowledge of the gum arabic molecule, hydrodynamic arguments will here be employed to present a cursory model that can account for the experimental results obtained. For fraction *A*, on setting the mobility at infinite ionic strength equal to e/f, it was found that $f = 52$ ng.sec^{-1} for a chain segment carrying one electronic charge. By assuming the validity of Stokes' law, $f = 6\pi\eta r$, where η is the viscosity of water (namely[33], 17.87 mpoises at 0°) and r is the radius of a spherical chain segment, r was found to be 1.5 nm. Thus, the ionic groups in fraction *A* are ~3.0 nm apart.

Because, in the arabate molecule, on average, each segment contains approximately eight monosaccharide residues[34,35], the observed value of f for fraction *A* indicates that the value of f for each moiety is ~6.5 ng.sec^{-1}; this corresponds to a radius of ~0.19 nm for an average monosaccharide residue. This value agrees very well with radii reported for other monosaccharide residues in polysaccharides; for example, from electrophoretic studies, Napjus and Hermans[36] found that the radius of a D-glucose residue in sodium *O*-(carboxymethyl)cellulose is ~0.1 nm and, from X-ray studies, Sponsler and Dore[37] found the radius of the D-glucose residues in cellulose to be 0.256 nm.

As the molecular weight for fraction *A* is 2.5×10^5 and its equivalent weight is 1190, there is a total of 210 charges (and, therefore, segments) per molecule. If the radius of each segment is 1.5 nm and the overall molecule is spherical, as has been shown by other workers[3,12], the value of the molecular radius can be approximated by multiplying the volume of the segment by 210, and then calculating the radius of the sphere having this volume. This calculation yielded for the molecular radius of fraction *A* a value of 9.1 nm, which is very close to the values found by other workers by using different techniques. From light-scattering measurements, Swenson and co-workers[3] found that, in 0.35M sodium chloride, the radius of gyration is 10.0 nm, or less. From hydration studies, Oakley[38] found that the radius of sodium arabate is 11.1 nm, and estimated that the radius of the unhydrated molecule is 8.8 nm; from titration studies, Overbeek[39] obtained a value of 10.0 nm.

For fraction *B*, from the value of u° at infinite ionic strength, the frictional coefficient of a segment is 67 ng.sec^{-1}, corresponding to a spherical segment having a radius of 2.0 nm, in contrast to 1.5 nm for those in fraction *A*. If, as calculated for fraction *A*, the frictional coefficient of an average monosaccharide residue is 6.5 ng.sec^{-1}, it would appear that fraction *B* contains approximately ten monosaccharide residues per segment, that is, two more than in fraction *A*. The foregoing calculation makes the assumption that the frictional coefficients of the individual residues in a segment are additive. This assumption is apparently valid, as it leads to a very reasonable value for the radius of a single residue in fraction *A*.

The molecular weights of the neutral monosaccharides L-arabinose, L-rhamnose, and D-galactose are 150, 164, and 180, respectively. Thus, taking into account the loss of water, the addition of two molecules of L-arabinose to a segment of fraction *A* would increase its equivalent weight, which is actually the molecular weight of a segment, by 264; and the equivalent weight would increase by 292 by the addition of two molecules of L-rhamnose, and by 324 by the addition of two molecules of D-galactose. The observed equivalent weight of fraction *B* is 1490, which exceeds that of fraction *A* by 300, in very good agreement with these calculations, so that it would seem quite reasonable to assume that a segment in fraction *B* contains two more monosaccharide residues than does a segment in fraction *A*.

It is known that arabic acid can undergo acid hydrolysis and autohydrolysis, with the liberation of all four of the major monosaccharide residues[34,35,40]. The structure of gum arabic proposed by Glicksman and Schachat[1] has a backbone containing branches that consist of one of each of these major consituents. As all four can be hydrolyzed off rather readily, it would not be unreasonable to assume that hydrolysis can remove whole branches from the main backbone; this does not necessarily mean that the branches are removed intact, but only that the four residues in a branch can readily be removed. Thus, it may be that hydrolysis occurred during the slow dissolution of the crude gum, a 10 percent solution of which has pH 4.3.

In the situation just described, the molecular weight of the hydrolyzed material would be given by $M_A - 634y$, where M_A is the molecular weight of fraction *A*, y is the total number of branches removed during the hydrolysis, and 634 is the sum of the molecular weights of the four major monosaccharide residues less the water eliminated. The total number of segments (n) in the hydrolyzed material would be given by $210-y$, because the removal of one branch corresponds to the removal of one D-glucuronic acid residue, and the total number of these residues in the molecule is equal to the total number of segments. As the equivalent weight of fraction *B* is 1490, and is equal to the molecular weight divided by the total number of segments, the value of y can be determined from the relationship

$$(2.5 \times 10^5 - 634y)/(210-y) = 1490.$$

It was found that $y = 72$ and, thus, fraction *B* contains $(210-72)$, that is, 138 segments, a number ~ 34 percent less than for fraction *A*. The molecular weight of fraction *B* was then calculated to be 2.0×10^5, in contrast to 2.5×10^5 for fraction *A*. The segment radius for fraction *B* had previously been calculated from its electrophoretic mobility and found to be 2.0 nm. With this value and the value of n for fraction *B*, the molecular radius of fraction *B* was calculated as for fraction *A*, and was found to be 13.0 nm, in contrast to 9.1 nm for fraction *A*. All of the molecular parameters calculated for fractions *A* and *B* are summarized in Table III.

The lower molecular weight and larger size of the molecules in fraction *B* would at first glance seem to be contradictory. However, the situation can be explained in a number of ways. Firstly, the molecules of fraction *B* may not be spherical. Sphericity was assumed in all of these calculations because there is experimental evidence for

TABLE III

MOLECULAR PARAMETERS OF FRACTIONS *A* AND *B*

Parameter	*Fraction* A	*Fraction* B
$M_w \times 10^{-5}$	2.5	2.0
EW (g.equiv.$^{-1}$)	1190	1490
n (segments.mol^{-1})	210	138
$f \times 10^8$ (g.sec^{-1})	5.2	6.7
r (nm)	1.5	2.0
Molecular radius (nm)	9.1	10.3

sphericity for the molecules of fraction *A*, but the molecules in fraction *B* might not be spherical. Secondly, the rather small size of the molecules in fraction *A* may be due to intramolecular hydrogen-bonding between branches, which could possibly prevent the molecule from achieving a more expanded state. It is known that many macromolecules, such as gelatin[41], deoxyribonucleic acid[42], and several cellulose derivatives[43], are hydrogen-bonded in solution and that, when these hydrogen bonds are broken (for example, by urea), the molecules expand. Consequently, upon removal of some of the branches in the gum arabic molecule, less hydrogen-bonding between the remaining branches might occur, and this could account for the larger size of the molecules in fraction *B*.

The major conclusions to be drawn from the foregoing calculations are that (*a*) molecules of gum arabic can apparently undergo hydrolysis to yield a species, fraction *B*, having a lower molecular weight but a larger size than the original material, and (*b*) approximately 34 percent of the branches in the original material are more labile than the rest of the branches. Were all branches equally labile, only a single, broader electrophoretic peak would be expected, instead of the two distinct peaks found in the presence of the presumably hydrolyzed material.

There appear to be two possible explanations for the greater susceptibility to hydrolytic cleavage of certain branches in gum arabic. First of all, Heidelberger and co-workers[13,14] have shown that gum arabic apparently consists of two species, one of which has a higher content of L-rhamnose than the other. As this monosaccharide is known to exist only in the branches of gum arabic[1], it follows that the two species have different branches, and it may be postulated that the different branches are hydrolyzed differently. The causes of these different tendencies is, however, open to speculation. Were this model correct, the various parameters calculated for fractions *A* and *B*, given in Table III, would be somewhat erroneous, because, in making these calculations, the structure proposed for the whole gum was used, so that this structure represented an average structure for the two species and, as such, could not validly be used to describe fraction *A* only.

The second possible explanation involves an analogy with some of the results of Michie and co-workers[44], who found that certain of the (1→4)-D-glucosidic

linkages in regenerated cellulose are weaker than others, and that these undergo acid hydrolysis much faster than normal D-glucosidic linkages. They considered that the formation of these weaker linkages was not attributable to chemical factors, but was probably due to such physical factors as local strains in amorphous regions, or variations in the normal state of hydrogen bonding. Possibly, as the tears of crude gum arabic age, dehydration or other physical changes occur, thus causing some of the linkages to become weakened and more susceptible to hydrolysis.

It was noticed that the tears of gum arabic used in this study had an exterior shell that was physically different from the interior of the tear, and the exterior appeared to be drier than the interior. Consequently, about half of a single tear was allowed to dissolve slowly in water; the remaining half was then removed and allowed to dissolve in a fresh portion of water. Thereafter, both solutions were treated identically for preparation of the potassium salts. The salt prepared from the material from the exterior of the tear exhibited two electrophoretic peaks, whereas that from the material from the interior showed only one peak. It would therefore appear that, as tears of gum arabic age, certain of the linkages are weakened, possibly because of dehydration, and these undergo hydrolysis more readily than the other linkages. These weakened linkages would appear to lie between the main backbone and the branches.

The foregoing explanation indicates that gum arabic consists of either (*a*) a single molecular species having two different types of branches, or (*b*) two species having different branches. In (*a*), it may be postulated that one type of branch is preferentially weakened, and subsequently hydrolyzed off, during the slow dissolution process. In (*b*), a situation essentially the same as that suggested by Heidelberger and co-workers[13,14], it could be postulated that the branches in one species are more readily weakened than those in the other species. The present results do not permit a choice to be made between these two possibilities. However, it should be pointed out that the former hypothesis would be consistent with the results obtained by Heidelberger *et al.*[13,14], if it is assumed that the sample of gum arabic used by them was partially hydrolyzed before they conducted their measurements; two species, fractions *A* and *B*, having different branches, would then have been present, but they would not have been indicative of heterogeneity in the original gum. Thus, all of the suggestions of heterogeneity of gum arabic can be explained on the basis of a single, homogeneous molecule that has two different types of branches. However, the possibility of the presence of two distinct molecular species still cannot be completely ruled out.

The model of the gum arabic system described in the foregoing discussion is, admittedly, partly speculative. It is, however, gratifying that most of the conflicting reports in the literature concerning the nature of gum arabic are explicable on the basis of this model. For example, the various equivalent weights reported in the literature are in the same range as those found in the present studies. If the samples of arabic acid used by other workers had unwittingly been partially hydrolyzed in the way described here, the different equivalent weights found could be explained on

the basis of the various proportions of fractions *A* and *B*, which have different equivalent weights.

The variations in molecular weight and, in particular, the conflicting data of Sloniewsky and Ander[12] on molecular weight by light-scattering and viscosity can also be explained on the basis of this model by postulating that the hydrolyzed material, namely, fraction *B*, acts as a nucleus for aggregation. It is difficult to explain exactly why this might occur, but, possibly, because of the absence of some of the branches in this fraction, there is less intramolecular hydrogen-bonding and a greater possibility of intermolecular hydrogen-bonding, which, according to Sloniewsky and Ander[12] is responsible for the aggregation. They found[12] that, as the molecular weight increases, the intrinsic viscosity increases, but not as greatly as would be expected for the high molecular weights observed. In order to account for this observation, they postulated that, in a shear field, the aggregates are dissociated. However, in order to account for the higher (although not very much higher) intrinsic viscosities, this argument must also postulate that the aggregates are only partially, and reproducibly, dissociated in the shear field. A more satisfactory argument would result from a consideration of the results of the present work: if fraction *B* is responsible for the aggregation, complete dissociation in both a shear field and an electric field could be postulated, because, in this situation, the molecules in fraction *B* are larger than those in fraction *A* (despite its lower molecular weight) and the intrinsic viscosity would increase as the proportion of fraction *B* increased, but not because of aggregation.

EXPERIMENTAL

Preparation of arabate samples. — A single batch of crude gum arabic (Lot BXC578, a gift from S. B. Penick and Co.) was used to prepare all of the arabate samples used in this study. The source of this batch was *Acacia senegal*, a species of tree native to North Africa, which is one of the commonest sources of gum arabic.

For this investigation, the crude gum was converted into its lithium, potassium, and sodium salts. Two different methods of preparation were used, but, regardless of the subsequent steps, the first step in both methods was to dissolve the crude gum in water. The resulting solution was filtered through a Büchner funnel to remove insoluble impurities and a small amount of gelatinous material, and the filtrate (10–20% by weight) was then treated by one of the following methods.

Method 1. The solution was treated with an excess of hydrochloric acid, and the acid form of the gum was precipitated by the gradual addition of three volumes of 95% ethanol. This procedure was repeated three more times, and the final precipitate of arabic acid was washed until it was free from chloride ion. To obtain the various arabic salts, the arabic acid was dissolved in water, neutralized with the chosen alkali hydroxide, and the salt precipitated by the addition of 95% ethanol.

Method 2. This method of preparation of arabic salts does not involve isolation of the acid form of the gum. The filtered solution of the crude gum was treated with

Dowex 50 X-12 ion-exchange resin, in the appropriate cation form. The acid form of the resin was treated several times with 2M alkali, washed free of the excess of alkali with water, and then added to the solution of the gum, and the suspension was stirred for several hours. The resin was filtered off, and the process was repeated with fresh resin; the resin was filtered off, and the filtrate was freeze-dried in small batches to obtain the respective salt.

The samples of arabic acid and of its salts, prepared by either method, still contained appreciable proportions of moisture, even after being dried for several days in a vacuum desiccator. Moisture contents were determined, in triplicate, at least, by drying the samples to constant weight in a vacuum oven at 40°/15 torr.

All samples were tested for the presence of reducing sugars with Fehling solution. The crude gum gave a positive test, but all of the purified samples of the acid and its salts gave a negative test. The samples were checked for the presence of calcium and magnesium by using a Perkin–Elmer Atomic Absorption Spectrophotometer, Model 303. All were found to contain <0.1% of each of these ions, regardless of the method of preparation.

The equivalent weight of each sample of arabic acid was determined by titration with standard alkali solution, and the equivalent weights of the salts prepared from these acids were calculated by subtracting the atomic weight of hydrogen and adding the atomic weight of the cation to the equivalent weight of the parent acid. The equivalent weights of the salts that were not prepared from samples of isolated arabic acid were determined by ashing the salts in a platinum crucible and then calculating on the assumption that the residue was the appropriate alkali carbonate. All determinations of equivalent weight were performed in triplicate. The moisture content and equivalent weight for each sample are given in Table IV.

TABLE IV

CHARACTERISTICS OF SAMPLES[a] OF ARABATE

Sample	*Moisture (%)*	*Equivalent weight*
HAr(1)	3.80±0.03	1200±15
KAr(1)	4.75±0.04	1238±15
NaAr(1)	6.15±0.05	1222±15
LiAr(1)	9.40±0.03	1206±15
HAr(2)	4.40±0.05	1300±13
KAr(2)	4.35±0.05	1338±13
NaAr(2)	4.70±0.03	1322±13
LiAr(2)	5.35±0.03	1306±13
KAr(3)	9.65±0.05	1288±20
NaAr(3)	7.00±0.09	1268±23

[a]The first eight samples were prepared by method *1*, and the last two by method *2*.

Electrophoresis. — Electrophoretic measurements were made with a Perkin–Elmer electrophoresis apparatus, Model 238.

Conductance water having a specific conductance of $<0.8\ \mu ohm^{-1} \cdot cm^{-1}$ at 0°, was used to prepare all solutions used in the electrophoresis experiments. The solutions were made up, by weight, by dissolving the polymer in a salt solution of known ionic strength. The appropriate cell-compartments were then filled with this solution, and the rest of the compartments were filled with the salt solution. The pH of all solutions was in the range of 6.2 to 7.3.

A few experiments were performed in which the solutions were buffered and then dialyzed. The mobilities determined from these experiments were found to be identical to those obtained by using unbuffered, undialyzed solutions. Thereafter, no solutions were buffered or dialyzed. (Joubert[15] showed that the mobility of gum arabic is independent of pH in the range 6.34 to 7.79.)

Electrophoretic mobilities, u, were calculated in the usual way from the relationship

$$u = d/Xt,$$

where d is the distance (in cm) that the boundary had moved after time t (in sec) under an applied field-strength X (in volt. cm^{-1}). The field strength was kept in the range of 3.8 to 4.2 volt.cm^{-1} in all experiments, and was determined from the relationship

$$X = I/\kappa A,$$

where I is the current in amperes, measured by means of the milliameter on the electrophoretic instrument, A is the cross-sectional area of the cell (0.30 cm^2), and κ is the specific conductance of the polyelectrolyte solution. The specific conductance was determined before each experiment by using the procedure and the conductance cells previously described[45].

In order to measure the distances moved by the boundaries, and the relative peak areas, the photographs of the electrophoretic patterns were projected with a photographic enlarger onto a piece of grid paper and traced. From the magnification employed, the distances traveled could be obtained from the tracings to within $\sim \pm 0.8\%$. Because most of the boundaries were asymmetric, the mobilities were measured from the first moments of the peaks, instead of from the peak maxima. The current and the distances moved were the least accurate of the quantities used in calculating the mobilities, and led to a total accuracy of $\sim \pm 2\%$.

The relative peak-areas, which give the weight distribution of the two components[24], were determined by counting the grid squares of the tracings. The reproducibility in calculating the relative areas in this way was $\sim 5\%$.

ACKNOWLEDGMENT

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

REFERENCES

1 M. GLICKSMAN AND R. E. SCHACHAT, in R. L. WHISTLER (Ed.), *Industrial Gums*, Academic Press, New York, 1959, Chapter X.
2 F. SMITH AND R. MONTGOMERY, *The Chemistry of Plant Gums and Mucilages*, Reinhold, New York, 1959.
3 H. A. SWENSON, H. M. KAUSTINEN, O. A. KAUSTINEN, AND N. S. THOMPSON, *J. Polym. Sci., Part A-2*, 6 (1968) 1593.
4 D. M. W. ANDERSON, SIR E. HIRST, AND J. F. STODDART, *J. Chem. Soc. (C)*, (1966) 1959.
5 M. N. MOORJANI AND C. S. NARWANI, *Cur. Sci.* (India), 7 (1948) 123.
6 A. W. THOMAS AND H. A. MURRAY, *J. Phys. Chem.*, 32 (1928) 676.
7 L. K. H. VAN BEEK, *J. Polym. Sci.*, 33 (1958) 463.
8 H. B. OAKLEY, *Trans. Faraday Soc.*, 31 (1935) 136.
9 S. SAVERBORN, in *The Svedberg*, Almkvist and Wiksells, Uppsala, 1944.
10 A. VEIS AND D. N. EGGENBERGER, *J. Amer. Chem. Soc.*, 76 (1954) 1560.
11 S. N. MUKHERJEE AND S. K. DEB, *J. Indian Chem. Soc.*, 39 (1962) 823.
12 A. R. SLONIEWSKY AND P. ANDER, *Carbohyd. Res.*, 18 (1971) 103.
13 M. HEIDELBERGER AND J. ADAMS, *J. Exp. Med.*, 103 (1956) 189.
14 M. HEIDELBERGER, J. ADAMS, AND Z. DISCHE, *J. Amer. Chem. Soc.*, 78 (1956) 2853.
15 F. J. JOUBERT, *J. S. Afr. Chem. Inst.*, 7 (1954) 107.
16 M. A. JERMYN, *Aust. J. Biol. Sci.*, 15 (1962) 787.
17 B. A. LEWIS AND F. SMITH, *J. Amer. Chem. Soc.*, 79 (1957) 3929.
18 N. YOSHIDA AND C. THIES, *J. Colloid Interfac. Sci.*, 24 (1967) 29.
19 A. R. SLONIEWSKY, Ph. D. Dissertation, Seton Hall University, 1968.
20 N. ELIESZER AND A. SILVERBERG, *Biopolymers*, 5 (1967) 95, 105.
21 I. Y. Z. ZIA, R. G. COS, AND S. G. MASON, *Science*, 153 (1966) 1406.
22 H. L. GOLDSMITH, *Science*, 153 (1966) 1407.
23 H. A. ABRAMSON, L. S. MOYER, AND H. GORIN, *Electrophoresis of Proteins*, Hafner, New York, 1964.
24 M. BIER, *Electrophoresis*, Academic Press, New York, 1959.
25 L. G. LONGSWORTH AND D. A. MACINNES, *J. Amer. Chem. Soc.*, 62 (1940) 705.
26 R. TOWNEND AND S. N. TIMASHEFF, *J. Amer. Chem. Soc.*, 79 (1957) 3613; 80 (1958) 4433.
27 G. A. GILBERT, *Disc. Faraday Soc.*, 13 (1953) 159; 20 (1955) 68.
28 R. E. NELSON, Ph. D. Dissertation, Seton Hall University, 1969.
29 C. L. BUTLER AND L. H. CRETCHER, *J. Amer. Chem. Soc.*, 51 (1929) 1519.
30 J. J. HERMANS AND H. FUJITA, *Koninkl. Ned. Akad. Wetenschap. Proc., Ser. B*, 58 (1955) 182.
31 J. T. G. OVERBEEK, *Advan. Colloid Sci.*, 3 (1950) 97.
32 M. H. GORIN, *J. Chem. Phys.*, 7 (1939) 405.
33 R. A. ROBINSON AND R. H. STOKES, *Electrolyte Solutions*, 2nd ed., Butterworths, London, 1965.
34 E. L. HIRST AND A. S. PERLIN, *J. Chem. Soc.*, (1954) 2622.
35 C. L. BUTLER AND L. H. CRETCHER, *J. Amer. Chem. Soc.*, 52 (1930) 4509.
36 P. J. NAPJUS AND J. J. HERMANS, *J. Colloid Sci.*, 14 (1959) 252.
37 O. L. SPONSLER AND W. H. DORE, in H. B. WEISER (Ed.), *Colloid Symposium Monograph*, Chemical Catalog Co., New York, 1926.
38 H. B. OAKLEY, *Biochem. J.*, 31 (1937) 28.
39 J. T. G. OVERBEEK, *Bull. Soc. Chim. Belges*, 57 (1948) 252.
40 H. B. OAKLEY, *Trans. Faraday Soc.*, 33 (1937) 372.
41 H. BOEDTKER AND P. DOTY, *J. Amer. Chem. Soc.*, 58 (1965) 968.
42 P. ALEXANDER AND K. A. STACEY, *Biochem. J.*, 60 (1955) 194.
43 E. OTT, *Cellulose and Cellulose Derivatives*, Interscience, New York, 1943.
44 R. I. C. MICHIE, A. SHARPLES, AND A. A. WALTER, *J. Polym. Sci.*, 51 (1961) 85.
45 R. E. NELSON AND P. ANDER, *J. Phys. Chem.*, 75 (1971) 1691.