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Viscosity Studies on the Association of Tobacco Mosaic Virus[1]

BY H. K. SCHACHMAN[1a]

Introduction

Several physico-chemical methods have been used to demonstrate the tendency of the rod-like particles of tobacco mosaic virus to aggregate. Double boundaries in the ultracentrifuge, high values of intrinsic viscosity, and finally direct measurements of electron micrographs furnish conclusive proof that many preparations of purified tobacco mosaic virus contain particles having lengths greater than that ascribed to the fundamental unit usually found in fresh, carefully prepared samples of purified virus. Viscosity measurements, because of their extreme dependence on the asymmetry of molecules, are ideally suited for the investigation of this phenomenon which invariably takes the form of end to end aggregation and thus larger and larger axial ratios. Lauffer,[2] in his excellent review, has shown how successful have been the theoretical approaches relating molecular size and shape to viscosity. In view of the recent strides in the theoretical treatment by Simha,[3] Onsager[3a] and others,[2] and because of the sensitive relationship between viscosity and particle shape, it seemed advisable to conduct viscosity studies designed to increase our knowledge of this association phenomenon.

Material and Methods

Rib grass virus used in these studies is a strain of tobacco mosaic virus discovered by Holmes[4] and subsequently studied from a chemical point of view by Knight and Stanley.[5] Of the many strains analyzed by these workers, this particular one seemed most unusual in amino acid composition, possessing higher tyrosine and lower phenylalanine contents than the other strains in that family of viruses. Observations on the rib-grass virus in the ultracentrifuge generally showed sharper boundaries than did similarly isolated samples of tobacco mosaic virus. Only rarely were double boundaries observed and, therefore, this particular strain was chosen for study since the qualitative evidence showed that it was more resistant to aggregation than the other strains of this family of rod-like viruses.

The preparations used in the study were isolated according to the well established procedure developed at the Rockefeller Institute for Medical Research at Princeton, New Jersey.[6] All of the virus preparations were obtained from young diseased Turkish tobacco plants. The juice expressed from the frozen and ground plants was clarified in the Sharples centrifuge by passage of the material through the rotating bowl at a rate of about one liter per minute. This preliminary clarification was followed by a run through the bowl at a rate of 10–15 cc. per min. The centrifuge was rotating at 50,000 r. p. m. for this run, and the temperature of the plant juice was maintained at about 20° by passage of cooling water through a coil surrounding the rotating bowl. This initial run served to effect about a thirty-fold concentration of virus. After a low speed clarification of the resuspended virus in a small laboratory centrifuge, three alternate high and low speed centrifugation cycles yielded preparations of virus which were reasonably pure and possessed the

(1) Paper presented before the Chemistry Section of the American Association for the Advancement of Science, 113th meeting, Cambridge, Mass., Dec. 26, 1946.

(1a) Junior Research Fellow of the National Institute of Health.

(2) M. A. Lauffer, *Chem. Rev.*, **31**, 561 (1942).

(3) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(3a) L. Onsager, *Phys. Rev.*, **40**, 1028 (1932).

(4) F. O. Holmes, *Phytopathology*, **31**, 1089 (1941).

(5) C. A. Knight and W. M. Stanley, *J. Biol. Chem.*, **141**, 39 (1941).

(6) W. M. Stanley, THIS JOURNAL, **64**, 1804 (1942).

characteristic bluish-white color of concentrated virus solutions. Slightly more than 80% of the virus protein was recovered and purified by this procedure.

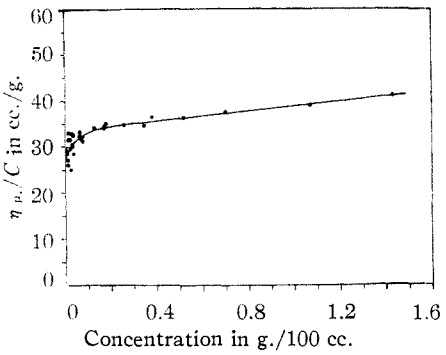

Fig. 1.—Specific viscosity divided by concentration plotted as a function of concentration for Preparation D.

Unfortunately, the histories of these virus preparations vary; two had aged for as long as four years because of the interruption caused by the war. Preparations A and B had been stored as concentrated solutions in distilled water at 4° for about four years. Preparations C and D were both freshly prepared and studied within a few months after final purification.

Viscosity measurements were performed in an Ostwald type viscometer having a capacity of 1 cc. of solution. The viscometer, constructed by Mr. Arthur D. Mack of the Naval Medical Research Institute, Bethesda, Maryland, possessed a relatively low velocity gradient of the order of 300 sec.$^{-1}$, and was so constructed that surface tension and kinetic energy corrections were kept to a minimum. The length of the capillary was about 90 cm. and the radius about 0.031 cm. In view of Lauffer's calculations,[7] it is expected that the degree of orientation of the particles in this capillary viscometer would be small. It would then follow that the viscosity results obtained in this instrument would not differ seriously from the random orientation viscosity, and the employment of the Simha equation should be valid. Bull[8] indicated that increased accuracy of measurement results from the use of narrow slits above and below the drainage bulb in preference to the device of calibration marks on the glass. However, as contrasted to the use of rubber tubing, best results were obtained by coating areas on each side of the bulb with plicene, and then, by means of a fine wire, cutting through the plicene to the glass surface to form very fine slits. Measurements were conducted in a water-bath maintained at 24.8 ± 0.005°. The use of a cathetometer served to increase the accuracy of the timing. A one-second electric stop watch enabling estimation to milliseconds was employed throughout, and readings were taken to the nearest one-hundredth of a second. Statistical treatment of the data showed reliability of measurements of the order of several hundredths of a second.

(7) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).
(8) H. B. Bull, *J. Biol. Chem.*, **133**, 39 (1940).

Results and Discussion

Figure 1 shows the results of 31 independent measurements of the viscosity of Preparation D dissolved in 0.1 *M* phosphate buffer at *p*H 7.3. The use of η_{sp}/C *vs.* C indicates that the concentration range of greatest interest is that close to zero. The conventional plot of η_{sp} *vs.* C unfortunately does not demonstrate the profound change as C approaches zero, and thus this newer method of presenting results is employed throughout. The importance of this method of approach can be realized by examination of the Einstein equation[9] and its modification by Guth,[10] and Guth and Gold[11] which can be represented by

$$\eta/\eta_0 = \eta_{rel} = 1 + 2.5G + 14.1G^2 + \ldots$$
$$\eta_{rel} - 1 = \eta_{sp} = 2.5G + 14.1G^2 + \ldots$$
$$\eta_{sp}/G = 2.5 + 14.1G + \ldots$$

In these equations, η_0 is the viscosity of the solvent, and η the viscosity of the solution whose concentration is expressed by G, the volume fraction of solute. Usually the Einstein equation is presented with only the first power of the concentration and to a large degree it has been justified for spherical particles at concentrations of solute which are very low. Obviously, then, a plot of η_{sp}/G *vs.* G would be expected to yield a curve which is practically horizontal and which approaches the value, 2.5 at $G = 0$. Carrying the analogy further to the situation embraced by the Simha treatment for asymmetric molecules, one would find coefficients in the power series which differ from those predicted by Einstein and Guth and Gold. The coefficient of the term containing the first power of the volume fraction is the factor called intrinsic viscosity and it can be employed in the Simha equation to evaluate axial ratios for asymmetric molecules.

$$\eta/\eta_0 = 1 + AG + A'G^2 + \ldots$$
$$\eta_{sp}/G = A + A'G + \ldots$$
$$[\eta_{sp}/G]_{G\to 0} = [\eta] = A$$

The curve of η_{sp}/G *vs.* G would approach A asymptotically as G approached zero and would be convex to the G axis. In the results presented in this paper the weight concentration, C, is used because of recent evidence[12] indicating considerable hydration of the virus particles. Correction for this factor would not alter the shape but only the position of the curve inasmuch as it is unlikely that the degree of hydration varies with virus concentration in the region under consideration. The rather sharp drop in the curve of Fig. 1 as C approaches zero demands attention. Many of the points in that region were obtained by having a second person record all readings so that the ex-

(9) A. Einstein, *Ann. Physik*, **19**, 289 (1906); **34**, 591 (1911).
(10) E. Guth, *Kolloid-Z.*, **74**, 147 (1936).
(11) E. Guth and O. Gold, *Phys. Rev.*, **53**, 322 (1938).
(12) H. K. Schachman and M. A. Lauffer, data to be published.

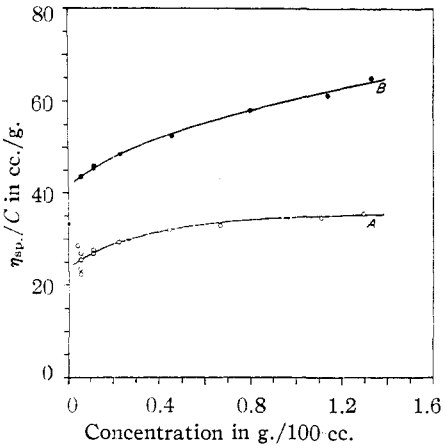

Fig. 2.—Specific viscosity divided by concentration plotted as a function of concentration for two different preparations: Curve A, O. Preparation A; Curve B, ●. Preparation B.

perimenter would not be exposed to subjective influences. In addition, a second person measured some of the points without knowledge of the concentrations involved and the recorded times. In this manner it is hoped that the human element has been virtually eliminated in that critical region where differences in outflow time between the virus solutions and the buffer are of the order of several tenths of a second. The similarity of the curves portrayed in Figs. 1–3 to molecular weight *vs.* concentration curves for substances like acetic acid in benzene suggests a dissociation of any polymerized virus into the fundamental unit. That the change is greatest in the low concentration region is in accord with the concepts of dissociation based on an equilibrium between monomer and dimer. Figure 2 shows the results for two other virus samples in 0.01 *M* phosphate buffer at *p*H 7.2. Preparation A gave a unimodal distribution curve for particle length and Preparation B gave a bimodal curve as shown by electron micrographs. Figure 3 is a similar plot for the partially aggregated virus, Preparation B, in two different buffers. It is readily seen that sodium chloride causes aggregation of the virus, but the influence of the salt has a vanishingly small effect at low concentrations of virus and thus the two curves seem to approach the same intercept at infinite dilution. That the salt has really caused polymerization of the virus is shown by examination of the boundaries obtained in the ultracentrifuge. A second, faster-moving component is observed in many runs in the presence of sodium chloride. In addition, there is a pronounced increase in flow birefringence of the virus solution in the presence of sodium chloride. At intermediate and high virus concentrations in sodium chloride buffer solution, Preparation B yielded continually increasing viscosity values. At low virus concentrations, however, this aggregation reaction proceeds slowly enough so as to yield essentially constant viscosity values.

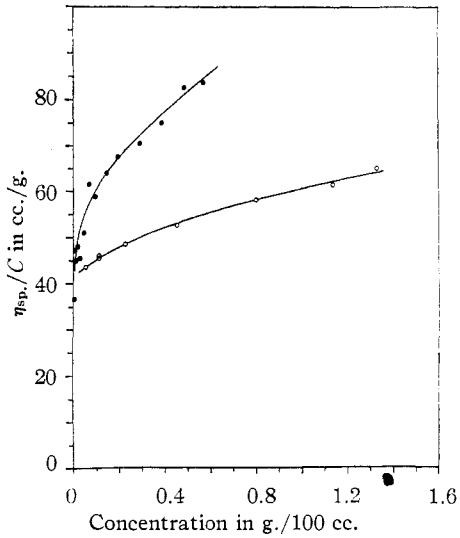

Fig. 3.—Specific viscosity divided by concentration plotted as a function of concentration for Preparation B: O, 0.01 *M* phosphate buffer at *p*H 7.2; ●, 0.2 *M* NaCl and 0.01 *M* phosphate buffer at *p*H 7.0.

In performing the experiments the diluted samples were spun for about five to ten minutes in a low speed centrifuge to remove dust particles. Viscosity measurements were then begun and continued for about twenty-five minutes. Occasionally solutions were allowed to stand in the viscometer for several hours to see whether the viscosity changed. A difference in outflow time of 0.1 second between successive measurements was readily detectable, and if a consistent drift was observed the time factor was carefully studied. With the exception of a few points for Preparation B in sodium chloride buffer solution, no time dependence was observed, and it is thus felt that the viscosity values represent equilibrium conditions.

It has been known for a long time that the rod-like virus particles aggregate in end to end fashion. The critical dependence of the state of aggregation on method of preparation has been amply demonstrated by the various values of particle lengths on different preparations.[7,13–17] It has been shown that aging of the purified solutions resulted in an aggregation which appears to be irreversible.[16,18] That the association does not stop at the dimer stage is abundantly clear from the viscosity studies of Lauffer[7] and from examination of electron micrographs. However, it had not been shown that there existed the opposite reaction in which the aggregated particles break up into smaller units, fully active in a biological sense and corresponding to what is considered to be the fundamental virus particle. From his studies of the stream double refraction of tobacco mosaic virus, Mehl[19] found that the aggregation caused by lowering the *p*H of the solution to 4.5

(13) M. A. Lauffer, *J. Biol. Chem.*, **126**, 443 (1938).
(14) V. L. Frampton and H. Neurath, *Science*, **87**, 468 (1938).
(15) J. R. Robinson, *Proc. Roy. Soc. (London)*, **A170**, 519 (1939).
(16) N. W. Pirie, *Advances in Enzymology*, **5**, 1 (1945).
(17) V. L. Frampton, *Arch. Biochem.*, **1**, 83 (1942).
(18) R. W. G. Wyckoff, *J. Biol. Chem.*, **121**, 219 (1937).
(19) J. W. Mehl, *Cold Spring Harbor, Symp. Quant. Biol.*, **6**, 218 (1938).

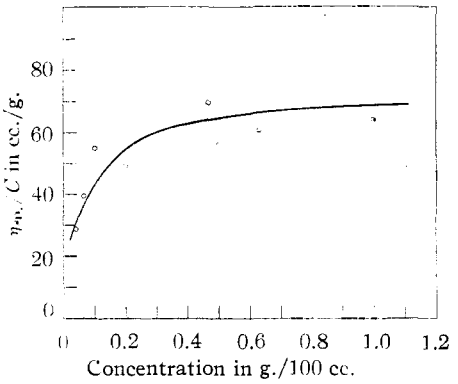

Fig. 4.—Specific viscosity divided by concentration plotted as a function of concentration for chemically isolated tobacco mosaic virus in 0.1 *M* phosphate buffer (data of Frampton and Neurath[12]).

was partially reversed by bringing the *p*H back to 6.8. Preparation C, kindly supplied by Dr. H. Gaw of the Rockefeller Institute for Medical Research, had a very high viscosity and exhibited considerable birefringence of flow. Continually decreasing viscosity values were obtained when this sample was dissolved in 0.1 *M* phosphate buffer. After about five hours, the viscosity reached a constant value corresponding to that expected for the monomer. The interpretation of this drop in viscosity as a function of time as being a dissociation reaction was verified by a simple experiment making use of the electron microscope. Two aliquots of the virus stock solution were diluted to a concentration of 3.5 mg./cc. One was diluted in distilled water and the second in 0.1 *M* phosphate buffer. After standing for five hours at room temperature, both solutions were diluted 1 to 50 in distilled water, and mounts were made and shadowed with gold. Pictures were then taken in the electron microscope. The average length of 100 particles of the water dilution was about 430 mμ while the average of an equal number of particles in the buffer solution was about 250 mμ. Rate studies now in progress may yield information regarding the energy of activation for the dissociation reaction and thence some clues as to the nature of the depolymerization reaction. Conversely, rate studies are being made of the aggregation reaction of essentially pure monomer.[20] The high viscosity of this aggregated virus solution is believed to be the result of the removal of most of the electrolyte. Dr. Gaw had dialyzed the virus solution against distilled water because his chemical studies required essentially electrolyte free material. This is in accord with the observations of Lauffer,[13] who studied the effect of ionic strength on the specific viscosity of tobacco mosaic virus. Lauffer found that the specific viscosity decreased with increasing ionic strength and suggested that the change was due to the electroviscous effect which vanishes in solutions of high ionic strength. In view of more recent knowledge obtained through electron microscopy, it seems more reasonable to consider this decrease in viscosity in the presence of electrolyte as due to a dissociation of the virus aggregates. This suggests that the interaction forces which cause aggregation of the virus can be damped out by increasing the ionic strength.

Early data of Frampton and Neurath[14] obtained on chemically isolated material show results similar to those in Figs. 1–3. The curve shown in Fig. 4 is obtained from their data for the viscosity of tobacco mosaic virus in 0.1 *M* phosphate buffer. More recent viscosity results by Frampton[17] indicate tremendously aggregated particles; but this is not surprising in view of the fact that his method of purification involved dialysis and electrodialysis of the virus solution. This treatment removes electrolytes, some of which appear to be essential for the stabilization of the monomer.

The role of electrolyte is somewhat complicated because of the specific effects of certain ions. In the case of the aggregated virus sample, Preparation C, the reaction can be made to go in either of two directions by varying the ions in the solvent but maintaining almost a constant *p*H and ionic strength. In a buffer containing 0.2 *M* sodium chloride and 0.01 *M* phosphate at *p*H 7 the viscosity increases. In 0.1 *M* phosphate buffer at *p*H 7, however, the viscosity decreases and finally levels off at a value approximately that of the monomer. In 0.01 *M* phosphate buffer, there is only a very slight decrease. In this manner, it is seen that certain ions favor the aggregation reaction and other ions favor the dissociation reaction.

In view of the demonstration of the two reactions

(1) monomer ⟶ dimer ⟶ trimer ⟶ . . . polymer
(2) polymer ⟶ . . . trimer ⟶ dimer ⟶ monomer

it seems quite reasonable that the two competitive reactions could ultimately result in equilibrium. An attempt to explain the viscosity curves on that basis was therefore begun. For the purposes of simplification of the mathematical treatment, it was assumed that equilibrium occurred between monomer and dimer. That this condition does not hold rigidly is evident from examination of electron micrographs.[21] Some of the particles present are definitely in a higher state of aggregation than the dimer stage. However, many electron micrographs do yield a bimodal distribution curve; thus the error in assuming a monomer–dimer equilibrium is not great.

It is of interest that Youden, Beale and Guthrie,[22] and Bald[23] developed a formula which quantitatively accounted for the biological activity of some preparations of purified tobacco mosaic

(20) H. K. Schachman, unpublished results.

(21) T. Sigurgeirsson and W. M. Stanley, *Phytopathology*, **37**, 26 (1947).

(22) W. J. Youden, H. P. Beale and J. C. Guthrie, *Contrib. Boyce Thompson Inst.*, **7**, 37 (1935).

(23) J. G. Bald, *Ann. Applied Biol.*, **24**, 33 (1937).

virus. The activity of other preparations, however, could not be accounted for by their equation. Bald[24] later suggested that the virus particles could aggregate with a consequent loss in activity on a weight basis. He assumed that the aggregation was reversible and that the aggregates dissociated into active particles on dilution. In this manner, he was able to account for the infectivity of those solutions which did not conform to the equation derived from the Poisson series.

On the basis of the results obtained from diffusion studies on tobacco mosaic virus, Frampton[17] suggested that the virus particles could aggregate reversibly. The diffusion curves for tobacco mosaic virus are skewed toward the solvent side of the boundary.[25] Apparently the virus particles on the solvent side of the boundary, where there is a high dilution, are diffusing at an accelerated rate. Frampton suggested that the skewness of the curves can be partially accounted for by the assumption that in the dilute region of the boundary the virus aggregates dissociate into smaller units which have higher diffusion constants.

In the following treatment, assume that the total concentration of virus in g./cc. is C, the molecular weight of the monomer is M and that of the dimer, $2M$. Let α equal the weight fraction present as dimer.

The molar concentrations of dimer and monomer are, respectively, $\frac{\alpha C}{2M}$ and $\frac{(1-\alpha)C}{M}$. According to the equilibrium, 2 monomer $\rightleftarrows$ dimer

$$K' = \frac{[\text{dimer}]}{[\text{monomer}]^2} = \frac{M\alpha}{2(1-\alpha)^2 C} \qquad (1)$$

The volume fraction of monomer, G_1, is $(1-\alpha)CV_1$, and the volume fraction of dimer, G_2, is αCV_2, where V_1 and V_2 are the partial specific volumes of the monomer and dimer, respectively.

The Einstein equation so modified as to consider two solutes in the same solvent can be written

$$\eta = \eta_0(1 + mG_1 + dG_2) \qquad (2)$$

where m and d are constants depending on the asymmetry of the monomer and dimer. It was shown by Treffers[26] that the fluidity of protein solutions is a linear function of the protein concentration. Moreover, Treffers,[26] and Bingham and Roepke[27] showed that the fluidity of solutions containing several protein components could be expressed approximately by the relation

$$\phi/\phi_0 = \eta_0/\eta = 1 - k_1C_1 - k_2C_2 \qquad (3)$$

where ϕ is the fluidity of the solution, ϕ_0 is the fluidity of the solvent and k_1C_1 and k_2C_2 are the fluidity lowering constants and the concentrations of the two proteins. At low concentrations, where square terms are considered negligible, equations 2 and 3 are identical. At higher concentrations the terms in the viscosity equation involving G_1^2, G_2^2 and G_1G_2 become appreciable and must be included. The slopes of the curves in Figs. 1–4 are small, at high concentrations, with the exception of that for Preparation B in sodium chloride buffer solution. This suggests that the interaction or square terms have a relatively small effect and therefore they are not included in this treatment. The coefficients m and d can be evaluated by the Simha equation[2] on the basis of a rod 280 by 15 mμ for the monomer. Substitution for G_1 and G_2 in equation 2 yields

$$\eta/\eta_0 - 1 = \eta_{sp} = m(1-\alpha)CV_1 + d\alpha CV_2 \qquad (4)$$

Inasmuch as the aggregation is end to end, it seems reasonable to expect the hydrations of monomer and dimer to be the same, and thus $V_1 = V_2$. The constants m and d now become M and D and equation 4 becomes

$$\eta_{sp}/C = M(1-\alpha) + D\alpha \qquad (5)$$

Let $K = \alpha/(1-\alpha)^2C = 2K'/M$ and solve for α.

$$\alpha = \frac{2KC + 1 - \sqrt{1+4KC}}{2KC}$$

Upon substitution of this value of α in (5), equation 6 results.

$$\eta_{sp}/C = M\left[\frac{-1+\sqrt{1+4KC}}{2KC}\right] + D\left[\frac{2KC+1-\sqrt{1+4KC}}{2KC}\right] \qquad (6)$$

For the purpose of comparison of the theoretical curve with the experimental data in the region of low concentrations, equation 6 was simplified by binomial expansion of $\sqrt{1+4KC}$. Equation 7 is the result of such treatment and the rearrangement of terms.

$$\eta_{sp}/C = M + K(D-M)C - 2K^2(D-M)C^2 \qquad (7)$$

The experimental curve can be considered as satisfied at low values of C if the following three conditions obtain: (1) the intercept is the coefficient M, (2) $d(\eta_{sp}/C)/dC > 0$, and (3) $d^2(\eta_{sp}/C)/dC^2 < 0$. That these requirements are satisfied can be seen by examination of equation 7 and the first and second derivatives. Condition (2) holds if $1 > 4KC$ which is true at low values of C.[28]

That equation 6 does not completely satisfy the experimental data is evident, for the theory requires η_{sp}/C to approach D at high values of C. This does not happen, as is demonstrated both by viscosity measurements and the observation in the ultracentrifuge of a major component corresponding to monomer. The results seem to indi-

(24) J. G. Bald, *Australian J. Exp. Biol. Med. Sci.*, **15**, 211 (1937).

(25) H. Neurath and A. M. Saum, *J. Biol. Chem.*, **126**, 435 (1938).

(26) H. P. Treffers, THIS JOURNAL, **62**, 1405 (1940).

(27) E. C. Bingham and R. R. Roepke, *ibid.*, **64**, 1204 (1942).

(28) It can be shown that an essentially similar equation results if the square terms are included in the viscosity equation. In that case the coefficients of the square terms and the cross product term are unknown. If it is assumed that the coefficient of the G_1G_2 term is the geometric mean of the coefficients of G_1 and G_2 then the resultant equation satisfies the above three criteria.

cate that in the purification process a certain fraction of the virus, depending upon the treatment to which it is subjected, is aggregated. This fraction upon dilution seems capable of dissociation and it appears to follow the well established principles of equilibrium reactions. In Fig. 1 the intercept is about 27 cc./g. On the basis of the Simha equation for a rod 280 by 15 mμ a value of about 25 cc./g. is expected. If complete equilibrium between monomer and dimer obtains η_{sp}/C should approach 79 cc./g. at high concentrations. In the case of Preparation D a value between 35 and 40 cc./g. is reached. This corresponds to about 89% monomer. Until greater knowledge is obtained as to the control of the aggregation process, it seems advisable to propose this treatment as only a first step in attempts to explain the data.

In view of the high dilutions of the solutions studied and the large surface area of the capillary the problem of adsorption of the virus on the glass surface must be considered. An attempt to determine the extent of adsorption of virus on the capillary, with the consequent decrease in the concentration of solution, was made by chemical analysis. Analyses of very dilute solutions indicated that the concentration of solutions which had been studied in the viscometer and stored there for hours did not differ detectibly from aliquots of the same dilution, which had been standing in a glass tube with a much smaller surface. In addition, there was excellent reproducibility of outflow times for the buffer; despite the fact that sometimes the viscometer was rinsed with only water after the virus solutions were studied and other times it was cleaned with chromic acid. In view of these results it seems that adsorption of the virus on the surface of the capillary is a less likely explanation of the results obtained than is the suggestion of a dissociation of the virus aggregates.

Viscosity measurements of solutions containing asymmetric particles such as tobacco mosaic virus indicate the existence of non-Newtonian flow. It is possible that even at the low shear gradients obtained in this viscometer there is a slight orientation of the particles. As the solutions become more dilute the outflow time decreases and thus the average shear gradient and degree of orientation increases. An increase in the amount of orientation of the rod-like particles could be the cause of the lower viscosity at high dilutions. However, most of the curvature in Fig. 1 occurs at about 0.1 g./100 cc. The change in shear gradient from 0.1 g./100 cc. to infinite dilution is only about 4% and it seems unlikely that this slight change in shear gradient could affect the orientation of the virus particles sufficiently to cause a drop in η_{sp}/C at the lower concentrations. A complete answer to this problem of the anomalous viscosity of tobacco mosaic virus requires studies in a Couette type viscometer. The anomalous viscosity may be due, in part, to a structural effect, in which case it is expected that η_{sp}/C would approach a definite value asymptotically as C approached zero.

Summary

Viscosity measurements made on preparations of the rib grass strain of tobacco mosaic virus indicate that it possesses a high resistance to aggregation as compared to the more common strain. One preparation after storage in distilled water for four years still had very few aggregated particles. The existence of a reversible aggregation process has been demonstrated by viscosity measurements and by the electron microscope. The role of electrolyte has been shown to be important and there is evidence for specificity of anions with the chloride ion favoring aggregation and the phosphate ion favoring dissociation. Measurements of viscosity at low concentrations indicate a falling off in values of η_{sp}/C at high dilutions. An equilibrium between monomer and dimer has been suggested to explain the data qualitatively and a theoretical equation has been derived for the variation of η_{sp}/C as a function of C. The general shape of the theoretical curve is similar to the experimental curves but there is disagreement at higher concentrations for which there is as yet no explanation. Other interpretations for the unusual viscosity results have been considered and evaluated.

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