THE VISCOSITY OF TOBACCO MOSAIC VIRUS PROTEIN SOLUTIONS

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It is well known that the viscosity of a liquid is a measure of its resistance to shear. Upon the introduction of small solid objects into a viscous medium, the internal motion of the liquid when caused to flow is made more complicated than it would be otherwise and the resistance to shear is thereby increased. Einstein (4) has derived the following formula relating the viscosity of a suspension of solid spheres ($\eta$) to the viscosity of the solvent ($\eta_0$) and the fraction of the total volume occupied by the spheres ($G$).

$$\frac{\eta}{\eta_0} - 1 = 2.5G$$  \hspace{1cm} (1)

If the spherical particles have electrical charges, the internal motion of the liquid will be complicated still further and the increase in viscosity will be greater than that given by the Einstein equation. Von Smoluchowski (19) has formulated the following relationship, taking into account this additional effect.

$$\frac{\eta}{\eta_0} - 1 = 2.5G \left(1 + \left(\frac{\xi D}{2\pi}\right)^2 \frac{R}{\eta_0 r^2}\right)$$  \hspace{1cm} (2)

where $\xi$ is the electrokinetic potential, $D$ is the dielectric constant of the medium, $R$ is the specific electrical resistance, and $r$ is the radius of the spheres.

The contribution to the internal friction of a liquid made by a rod-like particle oriented perpendicular to the direction of motion of the liquid is greater than that of a sphere of equal volume. However, the contribution of a rod-shaped particle oriented paral-
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The motion of a suspension of rod-like particles is less than when oriented perpendicularly. Kuhn (11) has derived the following equation for the viscosity of a suspension of randomly oriented rod-like particles.

\[ \frac{\eta}{\eta_0} - 1 = 2.5G + \frac{G}{16} \left( \frac{b}{a} \right)^2 \]  

where \( b/a \) is the ratio of length to diameter of the particles. The model which Kuhn used to approximate a rod consisted of a number of rigidly joined spheres. Guth (9) derived the same equation for a rod-like model consisting of an ellipsoid of revolution. An alternate equation has been derived by Eisenschitz (6) for a model consisting of an ellipsoid of revolution, which reduces to the following form for particles in which the length greatly exceeds the diameter.

\[ \frac{\eta}{\eta_0} - 1 = G \frac{(b/a)^2}{15(\ln 2(b/a) - (3/2))} \]  

The unsimplified form of this equation reduces to the Einstein equation just as does the Kuhn-Guth equation for particles in which \( b/a = 1 \). If one assumes that the particles are completely oriented by the streaming liquid, the contribution to the viscosity of the liquid will be much less and will be given, according to Eisenschitz (5), by the following equation.

\[ \frac{\eta}{\eta_0} - 1 = 1.15 \frac{G}{\pi} \left( \frac{b}{a} \right) \ln 2(b/a) \]  

It has been established that the molecules of the tobacco mosaic virus protein are rod-shaped (1-3, 13, 14, 22). Studies on the viscosity of solutions of the protein have been reported by Stanley (20), by Frampton and Neurath (8), and by Lauffer (12). The interpretation of such viscosity data is seen to be fraught with a considerable degree of uncertainty. If it is assumed that the rod-like particles are not appreciably charged (see Equation 2), that they are not appreciably oriented in the stream of the viscometer, and that they are not appreciably hydrated, it is permissible to apply the Kuhn-Guth or Eisenschitz equations (Equation 3 or 4). It is a known fact that the rod-like particles of the tobacco mosaic virus protein do orient somewhat in a flowing stream, for the
material shows stream double refraction under such circumstances. Furthermore, from the results of Mehl (15), one would expect a considerable degree of orientation of particles in a viscous system with a velocity gradient of the order encountered in a capillary viscometer. However, complete orientation should not be encountered in the rod-like particles of tobacco mosaic virus protein because of the finite thickness of the particles and because of the effect of Brownian movement. When either Equation 3 or Equation 4 is used, therefore, it must be remembered that the asymmetry calculated should be too small.

**Presentation and Discussion of Results**

*Viscosity As Function of Concentration*—The viscosities reported in this study were measured, with a high precision quartz viscometer, on very dilute solutions of several independently isolated samples of the protein prepared by ultracentrifugation repeated four or five times. The kinetic energy correction was applied in those few cases in which it was appreciable. In Table I are given the results of measurements of the specific viscosity of the tobacco mosaic virus protein dissolved in water at various concentrations. A linear relationship is seen to hold between specific viscosity and concentration in the lowest concentration ranges, showing that there is no interaction between particles at those concentrations. This linearity does not hold for solutions as concentrated as 1 per cent. Neurath and Saum (17) found anomalous diffusion for tobacco mosaic virus protein at a concentration of 1 per cent, which they explained as being due to interaction between the particles.

Interpreted by means of Equation 3, the results of Table I indicate a ratio of particle length to thickness of about 35:1, and

1 The author wishes to express his gratitude to Dr. D. A. MacInnes and Dr. L. G. Longsworth for the use of the quartz viscometer and the facilities of their laboratory.

2 Although the data found in this study indicate that the specific viscosity of ultracentrifugally isolated tobacco mosaic virus protein is a constant property from preparation to preparation, results obtained in another connection seem to indicate that the specific viscosity is somewhat dependent upon the treatment given the protein during its isolation. This observation may explain the difference between the results of Stanley (20) and those presented in this paper.
with use of Equation 4 this value turns out to be about 63. With
knowledge of the shape of the tobacco mosaic virus protein par-
ticles, the sedimentation studies reported by Eriksson-Quensel
and Svedberg (7) and by Wyckoff (24, 26) may be interpreted
somewhat more fully than was previously possible. An equation
which may take the following form has been derived, expressing
the dissymmetry factor used by Svedberg (21), \( f/f_0 \), as a function
of the ratio of the major to the minor axes of the colloidal particles
regarded as being rod-like ellipsoids of revolution (10, 18).

\[
\frac{f}{f_0} = \frac{(a/b)^4}{\sqrt{1 - (a/b)^2}} \log_e \left( \frac{1 + \sqrt{1 - (a/b)^2}}{a/b} \right)
\]  

Once this dissymmetry factor is known, the molecular weight of
the suspended particles may be calculated with the equation (21).

\[
\frac{f}{f_0} = \frac{M(1 - Vd)}{6\pi\eta_0 N_s a_0 (3MV/4\pi N)^{1/3}}
\]  

\( M \) is the molecular weight, \( V \) is the partial specific volume of the
protein taken to be 0.73 (1, 20), \( d \) is the density of the solvent,
\( N \) is Avogadro's constant, and \( s_{a0} \) is the sedimentation constant
taken to be \( 174 \times 10^{-13} \) (24). With the value for \( b/a \) obtained
from Equation 3, a molecular weight of \( 42.6 \times 10^6 \) is obtained
for the tobacco mosaic virus protein. This corresponds to par-
ticles 12.3 m\( \mu \) in diameter and 430 m\( \mu \) in length. With the value
of \( b/a \) obtained from Equation 4, a value for \( M \) of \( 63.2 \times 10^6 \),

TABLE I
Specific Viscosities of Aqueous Solutions of Tobacco Mosaic Virus Protein
at 20°

<table>
<thead>
<tr>
<th>( \eta - \eta_0 )</th>
<th>( C )</th>
<th>( (\eta/\eta_0) - 1 )</th>
<th>( C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm. protein per cc. solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0050</td>
<td>0.000099</td>
<td>50.05</td>
<td></td>
</tr>
<tr>
<td>0.0165</td>
<td>0.000296</td>
<td>55.74</td>
<td></td>
</tr>
<tr>
<td>0.0272</td>
<td>0.000458</td>
<td>59.36</td>
<td></td>
</tr>
<tr>
<td>0.0278</td>
<td>0.000494</td>
<td>56.28</td>
<td></td>
</tr>
<tr>
<td>0.0542</td>
<td>0.000920</td>
<td>58.91</td>
<td></td>
</tr>
<tr>
<td>0.0566</td>
<td>0.000988</td>
<td>57.20</td>
<td></td>
</tr>
<tr>
<td>0.6000</td>
<td>0.009200</td>
<td>65.25</td>
<td></td>
</tr>
</tbody>
</table>
corresponding to particles 11.5 m\(\mu\) in diameter and 725 m\(\mu\) in length, is obtained. Both of these sets of values are of the same order of magnitude as those determined by diffusion (8, 16, 17), stream double refraction (15), ultrafiltration (1, 23), and x-ray diffraction (2, 3) studies.

Even though no high degree of reliability can be ascribed to the results of any of the methods just considered, since the agreement obtained in the results of the several independent methods is fairly good, it seems reasonable to assume that the size and shape of the tobacco mosaic virus protein particles actually are of the order of those indicated by these studies. As a working model, the tobacco mosaic virus protein molecule may be regarded as being a cylindrical body, 12.3 m\(\mu\) in diameter and 430 m\(\mu\) in length, having a molecular weight of 42.6 \(\times\) 10\(^6\). With this model it is possible to interpret the rather puzzling observation that chemically isolated preparations of the protein and ultracentrifugally isolated preparations which have remained in contact with electrolytes show two sedimentation boundaries in the ultracentrifuge, one with a sedimentation constant of about 174 \(\times\) 10\(^{-13}\) and the other with a constant of 200 \(\times\) 10\(^{-13}\) (24). The most carefully prepared protein has a single constant of 174 \(\times\) 10\(^{-13}\). The simplest way to dispose of this second boundary is to regard it as being due to a second component formed by the association of the particles represented by the slower moving boundary. If 2 rod-shaped molecules having the dimensions of the working model from a supply having a sedimentation constant of 174 \(\times\) 10\(^{-13}\) associate end to end, they will contribute to a second supply having a molecular weight of 85 \(\times\) 10\(^6\) and a ratio of particle length to diameter of 70. With Equations 6 and 7, it can be shown that such a supply would have a sedimentation constant of 202 \(\times\) 10\(^{-13}\), a figure in very good agreement with the experimental value of 200 \(\times\) 10\(^{-13}\). Similar calculations made with a particle having the dimensions found from Equation 4 as a model would yield a value for the sedimentation constant of the second component somewhat under the observed one. Some model intermediate between the two would yield the exact value. This good agreement between calculated and observed sedimentation constants of the second component is evidence in favor of the essential correctness of the model chosen, as well as for the explanation of the origin of the second boundary.
By combining Equations 6 and 7 and by assuming, as was done above, that the faster moving boundary represents particles formed by the end to end association of two particles from the slower moving supply, it is possible to write two simultaneous equations involving only $M$ and $b/a$ of the original particles as variables. Hence $M$ and $b/a$ may be evaluated from the sedimentation constants of the two species present in double boundary preparations. Wyckoff (25) has given the sedimentation constants of double boundary preparations of the protein of aucuba mosaic virus, a strain of tobacco mosaic virus, as $185 \times 10^{-13}$ and $220 \times 10^{-13}$. According to the method here described, the molecular weight of the slower component of the aucuba mosaic virus protein is found to be about $32.5 \times 10^{6}$, corresponding to particles with a length of $267 \text{ m}_{\mu}$ and a diameter of $13.7 \text{ m}_{\mu}$. The molecular weight of the second component would be about $65 \times 10^{6}$, corresponding to particles $534 \text{ m}_{\mu}$ long and $13.7 \text{ m}_{\mu}$ in diameter. It should be emphasized that the nature of the assumptions made in this calculation cause the molecular weight derived from it to be a minimum value, for, if any type of association other than a perfect end to end type is assumed, the values obtained for the molecular weight and the dissymmetry of the original molecules will be greater than those obtained in this case.

In a manner similar to that used in the calculation of the sedimentation constant of the heavy component of tobacco mosaic virus protein, it can be shown that, if the tobacco mosaic virus protein represented by the sedimentation constant of $174 \times 10^{-13}$ is to be regarded as being in an associated state itself, as some workers believe, then the maximum sedimentation constant which could possibly be shown by the hypothetical original molecules (particles one-half as long but of the same diameter as the model) would be $145 \times 10^{-13}$. No evidence from the ultracentrifuge for any active component having such a sedimentation constant or a lower one has as yet been reported, even in studies on unpurified juice from diseased tobacco plants (26).

Viscosity As Function of pH—In Table II are presented the results of viscosity measurements on solutions of the protein in buffers of ionic strength of 0.02 at various values of pH. These measurements were carried out on solutions containing about 0.5 mg. of protein per cc. It is seen that in the alkaline range the
viscosity falls off gradually as pH increases. In the range between pH 5.5 and about 7, the specific viscosity is essentially constant, but it rises sharply between 5.5 and 4.2. Very near the isoelectric point there is a minimum with a value about equal to that obtained at pH 6. On the acid side of the isoelectric point there is a second maximum followed by a second minimum. Interpreted in terms of the Kuhn-Guth or Eisenschitz theories, these data indicate that in the alkaline range there is a dissociation of the molecule into simple, less asymmetrical units, and that, as one

### Table II

**Specific Viscosity of Tobacco Mosaic Virus Protein in Buffers at Various Hydrogen Ion Concentrations**

<table>
<thead>
<tr>
<th>Buffer system</th>
<th>pH</th>
<th>$\frac{(\eta/\eta_0) - 1}{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl-NaOH</td>
<td>10.61</td>
<td>9.4</td>
</tr>
<tr>
<td>Na$_2$B$_4$O$_7$-HCl</td>
<td>8.92</td>
<td>29.2</td>
</tr>
<tr>
<td>“</td>
<td>8.27</td>
<td>35.2</td>
</tr>
<tr>
<td>“</td>
<td>7.69</td>
<td>43.0</td>
</tr>
<tr>
<td>NaAc-HCl</td>
<td>6.51</td>
<td>47.6</td>
</tr>
<tr>
<td>“</td>
<td>6.03</td>
<td>47.6</td>
</tr>
<tr>
<td>“</td>
<td>5.53</td>
<td>49.0</td>
</tr>
<tr>
<td>“</td>
<td>4.97</td>
<td>139.4</td>
</tr>
<tr>
<td>“</td>
<td>4.24</td>
<td>233.0</td>
</tr>
<tr>
<td>“</td>
<td>3.74</td>
<td>50.2</td>
</tr>
<tr>
<td>NaCl-HCl</td>
<td>3.03</td>
<td>95.8</td>
</tr>
<tr>
<td>“</td>
<td>2.12</td>
<td>49.8</td>
</tr>
</tbody>
</table>

*The concentration of protein is expressed as gm. per cc., ionic strength, $S = 0.02$.

approaches the isoelectric point from either side, one encounters, first, an end to end association of molecules to form new particles of greater asymmetry, and, finally, a side to side association of the long particles to form less asymmetrical crystals of length and thickness within the range of microscopic visibility in which the rod-shaped molecules are arranged side by side and end to end.

Studies of the double refraction of flow of the tobacco mosaic virus protein in buffers of various pH values confirm in general the conclusion drawn from the viscosity data. As is seen in Fig. 1, the stream double refraction of tobacco mosaic virus protein,
measured by the method described by Lauffer and Stanley (14), is at a maximum in the region of the isoelectric point and decreases above pH 6 and below pH 3. The increased double refraction in the region of the isoelectric point may be regarded as being due to the end to end association of the rod-shaped molecules to form particles of greater length, which, therefore, orient to a greater extent. It should be noted that stream double refraction begins to increase at a pH value somewhat higher than that at which the viscosity begins to increase, and that no minimum is observed very near the isoelectric point. The difference in behavior be-

![Graph](http://www.jbc.org/)

**Fig. 1.** The effect of pH on the stream double refraction of tobacco mosaic virus protein. The measurements were made on solutions containing about 0.25 mg. of tobacco mosaic virus protein per cc. Double refraction is reported as galvanometer deflections in mm., to which it is approximately proportional.

between stream double refraction and viscosity very near the isoelectric point is probably due to the fact that the viscosity is a function of the length and thickness of the particles, whereas stream double refraction is concerned only with the length. If very thin long particles associate side to side at the isoelectric point, the viscosity should decrease, but the stream double refraction may well be unaffected. These stream double refraction data are entirely consistent with the observation of Mehl that the rotational diffusion constant of the protein, as estimated from stream double refraction data, is much less around pH 4.5 than at 6.8 (15). This rotational diffusion constant is regarded as being
inversely proportional to the cube of the particle length, and hence Mehl's results indicate particles of much greater length at pH 4.5 than at pH 6.8.

**Viscosity As Function of Ionic Strength**—In Table III are presented the results of a study of the effect of electrolytes on the specific viscosity of tobacco mosaic virus protein. It may be seen that the specific viscosity decreases with increasing ionic strength of the electrolyte. It seems reasonable to suppose that this change is due to the electroviscous effect (Equation 2) (19). The electrokinetic potential, $\zeta$, is regarded as being a function of the electrical charge on the colloidal particle and the thickness of the double layer; i.e., the distance between the charged surface and the electrical center of the atmosphere of oppositely charged ions in the liquid surrounding the particle. This distance turns out to be inversely proportional to the square root of the ionic strength. Hence, the electrokinetic potential, and therefore the electroviscous effect, would be expected to vanish in solutions of high ionic strength. In making a calculation of the asymmetry of the particle, it perhaps would seem to be well to use the minimum value for specific viscosity obtained in solvents of high ionic strength, because in such solvents both $R$ and $\zeta$ found in Equation 2 are low and, hence, the specific viscosity of the protein would be due largely to its shape. Such a procedure would result in a lower value of the asymmetry than that reported for the protein. However, since this low value would probably be too low because of the orientation of the particles due to streaming in the viscometer, it seems desirable to allow these two errors to compensate each other partially.

### Table III

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Ionic strength</th>
<th>pH</th>
<th>$(n/n_0 - 1)/C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>Very small</td>
<td>6.5</td>
<td>57.87</td>
</tr>
<tr>
<td>NaAc-HCl</td>
<td>0.020</td>
<td>6.5</td>
<td>47.6</td>
</tr>
<tr>
<td>K$_2$HPO$_4$-KH$_2$PO$_4$ (2.2:1)</td>
<td>0.053</td>
<td>7.3</td>
<td>46.0</td>
</tr>
<tr>
<td>&quot; (2.2:1)</td>
<td>0.119</td>
<td>7.1</td>
<td>39.6</td>
</tr>
<tr>
<td>&quot; (2.2:1)</td>
<td>0.238</td>
<td>7.2</td>
<td>42.7</td>
</tr>
</tbody>
</table>

* The concentration of protein is expressed as gm. per cc.
From measurements of the specific viscosity of tobacco mosaic virus protein solutions used in conjunction with sedimentation data, the size and shape of the tobacco mosaic virus protein molecule have been estimated. Two alternate sets of values are obtained, one corresponding to rod-like particles having a molecular weight of $42.6 \times 10^6$, a diameter of $12.3 \text{m}_{\mu}$, and a length of $430 \text{m}_{\mu}$, and the other corresponding to rod-like particles having a molecular weight of $63.2 \times 10^6$, a diameter of $11.5 \text{m}_{\mu}$, and a length of $725 \text{m}_{\mu}$. Both of these sets of values are of the same order of magnitude as the values obtained from stream double refraction, diffusion, ultrafiltration, and x-ray diffraction data. In terms of a model arbitrarily chosen to have the dimensions of the first set of values, it was shown that a second component, whose particles are formed by the end to end association of two rod-like molecules resembling the model, should have a sedimentation constant of $202 \times 10^{-13}$ as compared with $174 \times 10^{-13}$ for the original component. Preparations of tobacco mosaic virus protein showing double boundaries in the ultracentrifuge have components with sedimentation constants of $174 \times 10^{-13}$ and $200 \times 10^{-13}$.

The variations of viscosity and double refraction of flow of the protein with changes in hydrogen ion concentration are discussed. Both of these characteristics increase in the region of the isoelectric point, but only the viscosity falls sharply to a minimum very near the isoelectric point. This behavior is regarded as being due to the end to end association of rod-like molecules, followed by the side to side association of the long rods as one approaches the isoelectric point from either side. The viscosity was found to decrease upon the addition of electrolytes, an effect probably due to the electrokinetic potential of the particles.

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