DONNAN EQUILIBRIUM AND THE PHYSICAL PROPERTIES OF PROTEINS.

IV. VISCOSITY—Continued.

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1. In order to complete the demonstration that the viscosity of suspensions of powdered gelatin in water is influenced by electrolytes in a similar way as the viscosity of solutions of gelatin, it is necessary to prove that the characteristic valency effect exists also in the case of the viscosity of suspended particles of solid gelatin in water.

This proof is furnished in Fig. 1. 0.5 gm. of finely powdered gelatin (going through a sieve of mesh size 100 but not through a sieve of mesh size 120) of pH 7.0 was put into a series of beakers containing each 100 cc. of HCl of different pH and kept in the solution over night at a temperature of 20°C. Simultaneously a similar series of beakers containing each 100 cc. of H₃P₀₄ and H₂S₀₄ of different pH (instead of HCl) were prepared, each receiving also 0.5 gm. of powdered gelatin. After 19 hours the viscosities of all these series of suspensions were determined at 20°C. Fig. 1 gives the result, the ordinates being the values for the viscosity ratios, gelatin suspension: water, and the abscissae are the pH of the solid gelatin particles at equilibrium. The curves show that the viscosity of suspensions of gelatin sulfate is a little less than half that of suspensions of gelatin chloride and phosphate of the same pH. The curves for the suspensions of gelatin chloride and gelatin phosphate are alike, with the exception of part of the descending branch.

Experiments on the influence of these three acids on swelling published in a previous paper¹ show that the curves for the relative volume of powdered gelatin in solutions of these three acids are similar to the

viscosity curves in Fig. 1 of this paper since the relative volume of gelatin sulfate was found to be not far from one-half of that of gelatin chloride or gelatin phosphate of the same pH. This demonstration completes the proof that the viscosity of suspensions of powdered gelatin in water of different pH is influenced in the same way by electrolytes as is the viscosity of solutions of the same gelatin salts, and that this influence is due, in the case of suspensions, to the influence of the Donnan equilibrium upon the swelling of the particles.

The volume V of gelatin occupied in 100 cc. of the suspension was determined by filtering and deducting the volume of the filtrate...
from the total volume of the suspension. Knowing the viscosity we can calculate Einstein's constant $c$ according to the formula

$$\left(\frac{\eta_s - 1}{\eta_s}\right) \frac{100}{V} = c$$

where $c$ should be 2.5 if $V$ is sufficiently small.

The values in Table I show that Einstein's formula gives the correct values for viscosity when the volume, $V$, of the gelatin is small, since in that case $c$ is equal, or nearly equal, to 2.5, as his formula demands.

When, however, the volume is larger, the value for $c$ exceeds 2.5. The fact that the value for $c$ exceeds 2.5 when the relative volume occupied by the particles in the solution is large, was found also by Hatschek, Smoluchowski, and Arrhenius. Hatschek replaced the value 2.5 in Einstein's formula by a larger one, namely 4.5. This, however, meets in our case with the difficulty that the value $c$ shows a drift reaching a maximum when the volume of the gelatin particles is a maximum. This difficulty is largely avoided in Arrhenius's formula and we have to change from Einstein's formula to that of Arrhenius whenever the relative volume of the particles in solution or suspension exceeds the limits of the applicability of Einstein's formula, as we shall see in the next chapter.

<table>
<thead>
<tr>
<th>$V$ (cc)</th>
<th>$\frac{\eta_s}{\eta_p}$</th>
<th>$c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1.292</td>
<td>2.5</td>
</tr>
<tr>
<td>17</td>
<td>1.480</td>
<td>2.8</td>
</tr>
<tr>
<td>18</td>
<td>1.792</td>
<td>4.4</td>
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<tr>
<td>20.5</td>
<td>2.064</td>
<td>5.1</td>
</tr>
<tr>
<td>20.5</td>
<td>2.020</td>
<td>4.9</td>
</tr>
<tr>
<td>20</td>
<td>1.855</td>
<td>4.2</td>
</tr>
<tr>
<td>18</td>
<td>1.625</td>
<td>3.5</td>
</tr>
<tr>
<td>16.5</td>
<td>1.542</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Hatschek, E., *Kolloid Z.*, 1913, xii, 238; 1920, xxvii, 163.


The experiments on the viscosity of suspensions of powdered gelatin in water have, therefore, led to the result, first, that the influence of pH, of the valency of ions, and of the concentration of neutral salts on the viscosity of suspensions of finely powdered gelatin in water is similar to the influence of these three agencies on the viscosity of gelatin solutions; second, that the influence of electrolytes on the viscosity of the suspensions is due to the variation of the swelling (or relative volume) of the suspended particles; and third, that this latter fact explains why the Donnan equilibrium determines also the variation of viscosity of these suspensions. If it could be shown that a solution of gelatin contains also some (submicroscopic) particles of solid jelly (capable of swelling), we should understand at once why electrolytes influence the viscosity of gelatin solutions in a similar way as they influence the swelling, osmotic pressure, or the p.d. of these solutions. We have only indirect means of testing this occlusion theory of viscosity in the case of gelatin.

2. If these ideas are correct it would follow that if we melt a suspension of 0.5 gm. of powdered gelatin, in 100 cc. of water the viscosity of the 0.5 per cent solution of gelatin should be considerably lower than the viscosity of the suspension if measured at the same temperature, since in melting, part of the solid particles of the suspension should be transformed into individual molecules or ions or into particles too small to occlude water. In other words, as a consequence of the melting there should be a diminution of the relative volume occupied by the solid gelatin held originally in suspension.

This experiment was tried in the following way: 0.5 gm. of powdered gelatin was put into 100 cc. of water containing 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12.5, 15, and 20 cc. of 0.1 N HCl to bring the gelatin to different pH. The suspension was allowed to stand 1 hour at 20° to bring about the swelling of the particles and the viscosity of the suspension was measured in a straight viscometer at 20°C. The time of outflow of water through the viscometer at 20° was 48.5 seconds. The upper curve in Fig. 2 gives the ratio of viscosity of suspensions to that of water at 20°C. (When the viscosity is high, the values obtained are a little too great owing to a gravity effect which causes the solid particles to collect above the upper opening of the capillary tube during a part of the time of the experiment, thus increasing
temporarily the density of the suspension.) After the viscosity of a suspension was measured, the suspension was transformed into a solution by heating the suspension to 45° for 10 minutes; after that the solution was rapidly cooled to 20° and the viscosity of the gelatin solution was now measured with the same viscometer. The lower curve in Fig. 2 shows that the viscosity was now considerably diminished. The abscissae are the pH of the gelatin solutions.

![Graph showing viscosity changes with pH](image)

**Fig. 2.** Difference in the viscosity of a suspension of 0.5 gm. of powdered gelatin in 100 cc. and of the solution of the suspension in the same liquid; both viscosities were measured at 20° C.

Another test of the occlusion theory of viscosity is as follows. When we heat a 1 per cent solution of isoelectric gelatin rapidly to 45° and cool it rapidly to a lower temperature, e.g., 20° C., the solution will ultimately set to a continuous gel but will steadily increase its viscosity before this stage is reached. It is natural to assume that the formation of a continuous jelly is preceded by the formation of smaller masses of jelly, which at first are submicroscopic and later
touch each other, forming a continuous jelly. Hence the longer a solution of isoelectric gelatin stands at 20°C, the greater the number of submicroscopic solid pieces of jelly formed in the solution. The submicroscopic pieces of jelly surrounded by a true solution of isolated molecules of gelatin in water are compelled to regulate the amount of water they occlude by the Donnan equilibrium. Hence when we add some HCl to a 1 per cent solution of isoelectric gelatin after

![Graph](https://example.com/graph.png)

**Fig. 3.** Increase in viscosity when acid is added to solutions of isoelectric gelatin after they had been standing for 3 and 17 hours respectively.

the solution had been standing for some hours at 20°C, we should expect to find a higher viscosity than when we add the same amount of acid to the gelatin solution immediately after it had been rapidly heated to 45°C, and rapidly cooled to 20°C.

This experiment turns out as expected, as is shown in Fig. 3. When we bring newly prepared 0.5 per cent solutions of isoelectric gelatin to different pH by the addition of HCl we obtain a viscosity curve like the lowest in Fig. 3. When, however, we let the 0.5 per cent iso-
electric gelatin solution stand for 3 hours at 20°C. before adding the
acid, we get a parallel curve but higher than the first one (middle
curve, Fig. 3), for the reason that during the 3 hours an additional
number of solid jelly particles capable of swelling has been formed.
If we let the solution of isoelectric gelatin stand for 17 hours at 20°C.,
the curve (upper curve, Fig. 3), is still higher though practically
parallel with the first curve except at the summit. It is probable
that on standing the size of individual particles also increases and
the greater the size the greater the viscosity, since the viscosity is
chiefly but not exclusively a function of the relative volume of the
particles.

These and similar experiments agree with the occlusion theory
but not with the hydration theory; since there is no reason why the
degree of hydration of gelatin should increase the longer isoelectric
gelatin is kept at 20°C.

3. The fact that the viscosity of certain protein solutions is compar-
atively high, especially in the case of gelatin, has led to the suggestion
that proteins might possess a different type of viscosity not present in
solutions of crystalloids and that this second type of viscosity might
be connected with a certain structure in the protein solution.

"Bearing in mind the possibility that protein solutions may contain
a preformed molecular structure analogous to that of the jellies or
coagula which they can form, we are strongly impelled towards the
belief that the type of viscosity which solutions of proteins exhibit
may in some manner owe its existence to this structure, and not to
the type of internal friction which hinders molecular and ionic motion.
Thus a netlike structure, such as a tennis net, will offer no hindrance
to the passage through it of a quickly moving body which is smaller
than its meshes, other than that which is due to the fact that the
material which composes the net occupies a small fraction of the
area which the body must traverse, but to any force which involves
deformation of the structure, for instance, a force which seeks to
drag it through a small tube, it will offer a very considerable
resistance."5

5 Robertson, T. B., The physical chemistry of proteins, New York, London,
Bombay, Calcutta, and Madras, 1918, 324-325.
This assumption of a second type of viscosity seems unnecessary since it is possible to account for the viscosities of protein solutions on the basis of Einstein's law when the relative volume occupied by the protein in solution is small, and on the basis of Arrhenius's formula when the volume exceeds the limits within which Einstein's formula holds. According to our view the former is true when the protein exists in the solution exclusively or almost exclusively in the form of isolated molecules or ions or particles too small to occlude water and this seems to be the case for solutions of crystalline egg albumin, at ordinary temperature, at a pH above 1.0 and when the concentration is not excessive.

The viscosity of such protein solutions is not only of a low order of magnitude but is not influenced by electrolytes in the way as is, e.g., their osmotic pressure. Where we have viscosities of a higher order of magnitude, as in the case of gelatin solutions, we notice that the viscosity is influenced by electrolytes in the same way as is the osmotic pressure of these solutions. In the case of such proteins we assume that both the high order of viscosity as well as the characteristic influence of electrolytes on the viscosity are due to the same cause; namely, the existence in the gelatin solution of a certain number of submicroscopic particles of solid jelly occluding large masses of water, the exact amount of which is regulated by the Donnan equilibrium. The occlusion of large masses of water increases the relative volume occupied by the gelatin in solution so that Einstein's formula is no longer applicable. We are dealing, however, in both cases with the same type of viscosity which is primarily a function of the relative volume occupied by the protein particles in the solution.

Measurements of the influence of concentration on the viscosity of solutions of crystalline egg albumin of pH 5.1, i.e., quite near the isoelectric point, were carried out at 15°C. and showed that the viscosity is under these conditions practically a linear function of the concentration (Fig. 4). If Einstein's formula \( \frac{\eta}{\eta_s} = 1 + 2.5 \varphi \) can account for the measurements, it should be possible to calculate the volume \( \varphi \) of the albumin in 100 cc. of solution from

\[
\varphi = \left( \frac{\eta}{\eta_s} - 1 \right) \frac{100}{2.5}
\]
By dividing the weight of albumin in solution by its volume, we should obtain the density of albumin which should be according to the direct determination not far from 1.36 (Arrhenius). Table II shows that if we calculate the density of solutions of albumin chloride on the basis of Einstein's formula we obtain values which only vary inside the limits of accuracy of the experiments from the density determined directly. The time of outflow of water through the viscometer was 227 seconds at 15°C.

Entirely different results should be expected in the case of solutions of gelatin if the high viscosity is due to swollen particles of solid jelly contained in the solution and not due to the existence of a network, as some authors assume. When we plot the curve for the influence of concentration on the viscosity of solutions of isoelectric gelatin we get.

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**TABLE II.**

<table>
<thead>
<tr>
<th>Concentration of crystalline egg albumin.</th>
<th>( \frac{n}{\eta_s} - 1 )</th>
<th>Calculated volume of albumin.</th>
<th>Calculated density of albumin.</th>
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<tr>
<td>per cent</td>
<td></td>
<td>cc.</td>
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<tr>
<td>14</td>
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<td>11.6</td>
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<tr>
<td>12</td>
<td>0.240</td>
<td>9.6</td>
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<tr>
<td>8</td>
<td>0.132</td>
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</tr>
<tr>
<td>6</td>
<td>0.100</td>
<td>4.0</td>
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</tr>
<tr>
<td>4</td>
<td>0.074</td>
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</tr>
<tr>
<td>2</td>
<td>0.042</td>
<td>1.7</td>
<td>1.17</td>
</tr>
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</table>
curves like those of Fig. 5, which are no longer straight lines as in the case of crystalline egg albumin but curved lines. The curvature is the smaller the higher the temperature.

![Viscosity curves of isoelectric gelatin](image)

**Fig. 5.** Influence of concentration on the viscosity of solutions of isoelectric gelatin.

In these experiments the gelatin solution was first heated rapidly to 45° and then brought rapidly to the temperature indicated in the figure. The formula of Einstein can no longer be applied but the formula of Arrhenius gives fair agreement

\[
\log \frac{\eta}{\eta_n} = \delta \phi
\]
where $\varphi$ is the relative volume and $\theta$ is a constant. In other words, if Arrhenius's formula can account for the viscosity of these solutions the logarithm of the viscosity ratio when plotted over the concentration (Fig. 6), should give a straight line. The agreement of the value for 45° and 35° with this theory is satisfactory (considering the limits of accuracy of the measurements), (Fig. 6) the logarithms of the viscosity increasing practically in direct proportion with the concentration (i.e., the relative volume) of the gelatin in the solution (Table III). At 60° the agreement is not quite so good but still recognizable. At 25°, however, it is satisfactory only at the lowest concentrations but at the higher concentrations the viscosity grows more rapidly than the concentration. The reason for this, however, is obvious since at this temperature the gelatin solution solidifies.

**Fig. 6.** Showing that the logarithms of the viscosity of solutions of isoelectric gelatin of different concentrations when plotted over the concentration as abscissae are straight lines.
so rapidly that the viscosity measurements were no longer possible for a concentration of 3.5 per cent gelatin solution, and for this reason the value of the viscosity of a 2 or 3 per cent solution is already too high on account of the mechanical hindrances of the flow of the solution through the viscometer owing to partial solidification.

From these experiments we may therefore draw the conclusion that since the viscosity measurements of gelatin solutions agree fairly well with Arrhenius's viscosity formula the variation of the viscosity of gelatin solutions must be due to variations in the relative volume occupied by the gelatin in solution; and since the gelatin used in these experiments was isoelectric, these variations cannot possibly be ascribed to a hydration of gelatin ions. The only other way by which the isoelectric gelatin particles can increase their volume is through the occlusion of water by submicroscopic particles of solid jelly. Since the amount of water occluded by solid jelly is regulated by the Donnan equilibrium this will explain why the influence of electrolytes on the viscosity of a gelatin solution is similar to that of electrolytes on the viscosity of suspensions of powdered gelatin in water.

The connection between the tendency of gelatin solutions to set to a jelly and their high viscosity lies therefore in this, that the setting to a jelly is preceded by the formation of submicroscopic solid particles of gelatin capable of swelling and of occluding comparatively large

### TABLE III.

<table>
<thead>
<tr>
<th>Concentration of solution of isoelectric gelatin</th>
<th>( \eta )</th>
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<tbody>
<tr>
<td></td>
<td>( 60^\circ \text{C.} )</td>
</tr>
<tr>
<td>per cent</td>
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<td>0.4321</td>
</tr>
<tr>
<td>4.0</td>
<td>0.5214</td>
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quantities of water whereby the relative volume occupied by the gelatin in the solution is enormously increased. That this interpretation is correct is proved by experiments on the viscosity of solutions of casein chloride which have no tendency to set to a jelly but which contain, side by side with isolated casein ions and molecules, small particles in suspension, occluding water and capable of swelling.

4. It has already been shown in a preceding paper that the pH influences the viscosity of casein solutions in a similar way as it influences the viscosity of gelatin solutions, and we have convinced ourselves that the addition of neutral salts has a similar depressing effect on the viscosity of casein chloride solutions as it has on the viscosity of solutions of gelatin chloride. Yet casein solutions have no tendency to set to a jelly (as far as the writer has been able to observe) and we cannot therefore attribute the influence of electrolytes on the viscosity of casein chloride solutions to a second type of viscosity. But in the case of casein chloride solutions we can prove directly that the influence of electrolytes on the viscosity has its source in the swelling of casein particles.

The material used in our experiments was a fine dry powder of nearly isoelectric casein prepared after VanSlyke and Baker.\(^6\) Particles of equal size of grain (between mesh 100 and 120) were sifted out and 1 gm. of such powder was put into 100 cc. each of solutions of HCl of different concentration to bring the casein to varying pH.\(^7\) A microscopic examination of the granules showed that they underwent a swelling which was a minimum at the isoelectric point, which increased with increasing hydrogen ion concentration until it reached a maximum and which then diminished again with a further increase in the hydrogen ion concentration. Hence the volume of the casein particles suspended in the HCl varied in a similar way with the pH as the volume of suspended particles of gelatin.

This swelling could also be observed when the suspension was put into 100 cc. graduates and the suspended particles were allowed to settle. The volume of the sediment was a minimum at the isoelectric

\(^7\) Loeb, J., \textit{J. Gen. Physiol.}, 1920-21, iii, 547.
point increasing with increasing hydrogen ion concentration of the solution and finally decreasing again. But the curves for swelling and for volume of sediment were only parallel at the beginning, since the swelling (which occurred at once) was followed by some of the casein going into solution, or into suspension in the supernatant milky liquid. The longer the experiment lasted the smaller the volume of the sediment became and the larger the mass which went into the supernatant solution. This is expressed in Fig. 7. The upper curve represents the volume of the sediment after 1 hour. The suspension of 1 gm. of casein in 100 cc. HCl of different concentration had been kept for 1 hour at 20°C, had been shaken repeatedly but not frequently and the suspension was then passed into 100 cc. graduates and allowed to settle at 20°C. After 2 hours the volume of the sediment was measured and the volumes are the ordinates of the curve marked “after 1 hour” in Fig. 7. A similar experiment was made in which the suspension of casein was kept for 22 hours at 20°C. and was allowed to settle during 6 hours also at 20°C. The volumes are the ordinates of the second curve in Fig. 7 marked “after 22 hours.” The abscissæ are the pH of the total solution and suspension.
The curve "after 1 hour" is clear, since it is chiefly the expression of the variation of the degree of swelling of the casein particles since not as much has gone into solution as after 22 hours. We notice that the volume occupied by the solid particles in the 1 hour curve is a minimum at the isoelectric point, that it rises steeply after pH 3.1, and it drops at 2.2, and that a second drop commences at pH 1.8. The two drops have a different cause. The drop at pH 1.8 is due to a diminution of the degree of swelling of the sediment, while the drop at 2.2 in the 1 hour curve is due to the fact that at 2.2 the solubility of casein chloride is a maximum. This conclusion is sup-

Fig. 8. Dry weight of sediment of casein chloride solutions after 1 and after 22 hours.

ported by the fact that the drop at 2.2 increases in time and is very considerable after 22 hours (Fig. 7), while otherwise the 1 hour and the 22 hour curves show only minor differences.

The proof that this interpretation in the volume curves of Fig. 7 is correct is furnished by Fig. 8 where the ordinates are the dry weights of the sediments, the volumes of which are given in Fig. 7. 1 gm. of powdered casein had when dried for 24 hours at between 90 and 100° a dry weight of 0.87 gm.

That part of the casein chloride which goes into the supernatant liquid (i.e., which is not contained in the sediment) consists of two
constituents, namely, first, solid submicroscopic (and microscopic) particles in suspension which in due time would have settled, and second, isolated casein ions and molecules. The solid particles in the supernatant liquid (unless they are below the limit required to occlude water) undergo the same swelling under the influence of the Donnan equilibrium as the particles of the sediment. In addition, however, we have individual casein ions in solution (the molecules being probably insoluble since isoelectric casein is practically insoluble) but these ions cannot undergo any swelling and hence do not add materially to the volume and the viscosity. As a consequence the more solid particles of casein chloride are dissolved into isolated casein ions or particles too small to occlude water the more the relative volume occupied by the casein in the solution should be diminished and this should be accompanied by a diminution in viscosity. If our theory of the origin of the viscosity of the gelatin solutions is correct, it should be possible to prove that where the solubility of the casein chloride solution is a maximum the viscosity curve should show a drop.

The correctness of this inference is supported by the viscosity curves in Fig. 9 which represent the viscosity after 1 hour and after 22 hours. The experiments are the same as those referred to in Figs. 7 and 8. The viscosity of the total suspension and solution
was measured in a straight viscometer with a time of outflow for water of 48.4 seconds at 20°C. The curve for the viscosities after 1 hour is the expression chiefly of the swelling, since casein chloride goes only slowly into solution at 20°C. The curve is almost continuous and has its maximum in the region between pH 2.1 and 2.4, where also the swelling is a maximum. There is, however, a slight depression at pH 2.2 where the solubility of the casein is a maximum.

The curve for the viscosities after 22 hours shows, however, a distinct saddle at pH 2.2 where the solubility of casein chloride is a maximum. This agrees with the assumption that the high viscosity is due to swollen particles of casein, a certain quantity of which had been dissolved at or near pH of 2.2. This solution of the particles capable of swelling beneath that size where they no longer can occlude water must diminish the relative volume of the casein and cause a diminution of the viscosity. Below a pH of 1.8 where the solubility of the casein is considerably diminished, the 1 hour and the 22 hours viscosity curves (Fig. 9) no longer differ materially. As a consequence of the saddle the maximum of the viscosity curve after 22 hours now lies at pH 2.6.

Since the point at issue, namely the diminution of the viscosity when solid submicroscopic particles, capable of swelling, are dissolved into particles so small that they no longer can occlude water is so fundamental for the theories of viscosity and of colloidal behavior in general that it seemed necessary to look for a more striking proof than that given in the experiment quoted. For this purpose measurements were made on 1 per cent casein chloride solutions prepared from very finely powdered casein particles sifted through a 200 mesh. In order to get a more rapid dispersion of the particles the experiment was carried out at 40°C. The time of outflow of water through the viscometer at 40°C was 35.5 seconds. Fig. 10 gives the results. The viscosity measurements were made at four different times; namely, first, immediately after the powdered casein was put into the HCl; then after 1, 3, and 6 hours. During this time the casein chloride solutions were kept at 40°C. The viscosity curve taken immediately after the suspensions were prepared is continuous and is the expression of the swelling which occurred in the few minutes which elapsed in the preparation of the suspensions and during
which the casein was at 40°C. The maximum swelling occurred at about pH 2.3. At this time the amount of casein dissolved into separate casein ions was negligible. The curve resembles the 1 hour curve in Fig. 9 except that the depression due to the solution of some casein chloride in Fig. 9 is lacking in Fig. 10. After 1½ hours the second measurements of viscosity were taken, and the reader will notice from Fig. 10 that the viscosity had dropped considerably in the neighborhood of pH 2.2, where the solubility of casein chloride is the greatest, and the maximum depression is at pH 2.1 where also the solubility is a maximum. With a further lowering of the pH the viscosity rises again. The maximal viscosity in the 1½ hours

![Graph](https://example.com/graph.png)

**Fig. 10.** Diminution of viscosity through solution of solid particles of casein chloride.

series is now at pH of about 2.7 or 2.8 where it was also in 22 hours series in Fig. 9. The later viscosity measurements, after 3 and 6 hours (Fig. 10), confirm these conclusions.

5. These experiments leave little doubt that the high viscosity of protein solutions is due to the existence of particles occluding large quantities of water the amount of which is regulated by the Donnan equilibrium while the isolated ions of proteins in solution or the particles too small to occlude water have no share in the causation of high viscosities.

The quantities of water which can be occluded in a solid jelly of gelatin are enormous. If we assume the molecular weight of gelatin
to be of the order of magnitude of about 12,000 a solid gel of 1 per cent originally isoelectric gelatin contains over 60,000 molecules of water to 1 molecule of gelatin. It is out of the question that such masses of water could be held by the secondary valency forces of the gelatin and water molecules. Swelling casein particles occlude much less water and for this reason the viscosity of casein chloride solutions never becomes as high as that of gelatin solutions, containing equal masses of protein per 100 cc. of solution.

It is well known (and we have made use of the fact earlier in this paper) that the viscosity of a gelatin solution that had been heated to 45°C. and is allowed to stand at 20°C increases steadily even if the concentration is not high enough to permit the setting of the solution to a jelly. This increase in viscosity on standing seems to be caused by the gradual increase in the number of solid particles of jelly due to the collision of gelatin molecules or ions, these solid particles occluding water and thus increasing the apparent volume occupied by the gelatin in the solution. The gradual formation of solid particles of jelly from isolated molecules is most rapid at the isoelectric point and is retarded by the addition of acid; while the addition of acid increases the swelling of those particles of jelly which already exist in the solution. It is well to keep in mind that the addition of acid to a solution of isoelectric gelatin has thus two opposite effects on the viscosity of gelatin solutions.

According to Zsigmondy, Smoluchowski has explained (in a paper which has not been accessible to the writer) the increase in the viscosity through coagulation of a solution of aluminium oxide by the assumption of an occlusion of liquid between the particles. Smoluchowski calculates from the increase of viscosity during coagulation of aluminium oxide that the coagulating particles occupy a volume 400 to 500 times as great as that occupied by the dry material itself. This apparent increase of volume he explained through the aggregation of needleshaped particles. It would be of interest to test whether or not the volume of these particles and the viscosity of the suspension of aluminium oxide is also controlled by the Donnan equilibrium.

We are therefore led to a conception of the nature of protein solutions which is somewhat different from that current in the literature of

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colloid chemistry, which assumes that proteins form no true solutions but suspensions or emulsions. According to our view protein salts form, as a rule, true solutions consisting of isolated protein ions and molecules, which, however, may contain in addition solid fragments occluding comparatively large quantities of water. These latter particles of solid material are responsible for both the comparatively high viscosity of solutions of certain proteins, and for the influence of electrolytes on viscosity.

6. It is of interest to see whether or not Arrhenius's formula can account for the influence of electrolytes on the viscosity of casein suspensions. If this were the case, the curves representing \( \log \frac{\eta}{\eta_0} \) should run parallel to curves representing the relative volume occupied by the casein in the solution. We get the values of \( \log \frac{\eta}{\eta_0} \) from our observations of the relative viscosity which give us \( \frac{\eta}{\eta_0} \), and we can calculate the volume from the measured volume of the sediment plus the calculated volume of the casein in the supernatant liquid. The latter value we obtain by deducting the dry weight of the sediment from the (known) dry weight of the whole mass of casein put into the water (1 gm. powdered casein, dry weight = 0.87 gm.), and assuming that the casein in the supernatant liquid consists exclusively of suspended particles. This is approximately correct for a 1 hour experiment at 20°. The ordinates in Fig. 11 represent the values for volume thus corrected and the values for \( \log \frac{\eta}{\eta_0} \) while the abscissae are the pH of the suspensions. The two curves are almost parallel.

It should be stated that these corrected volumes of casein include a certain amount of water between the granules. We are, however, in this case not concerned with the absolute but the relative volume occupied by the casein.

When we add NaCl in different concentrations to a casein chloride solution we notice that the viscosity is diminished as it is in the case of solutions of gelatin chloride. R. F. Loeb found by microscopic observation that this diminution of viscosity was accompanied by a diminution in the degree of swelling of the individual particles of casein which ran parallel to the depression of the viscosity.
Fig. 11. Similarity of curve for $\log \frac{\eta}{\eta_0}$ and of relative volume of casein chloride solutions.

Fig. 12. Similarity of curve for $\log \frac{\eta}{\eta_0}$ and of relative volume of casein chloride solutions.
1 gm. of powdered casein was put into 100 cc. of H₂O containing 12.5 cc. of 0.1 HCl and NaCl in concentrations varying from 0 to \( M_4 \). The mixture was shaken occasionally and kept for 16 hours at 20°. Then the viscosity, volume of sediment (after settling for 24 hours), dry weight of sediment (after deduction of the free NaCl contained in the sediment) were determined. When the corrected volume and the values for \( \log \frac{\eta}{\eta_0} \) are plotted as ordinates over the concentration of abscissae it is found that the two curves agree fairly well (Fig. 12) except where no or little salt was added and where therefore some casein particles had been completely dissolved. In this solution the calculated volume was too high and our curves express the fact. From these experiments we may conclude that the influence of electrolytes on the viscosity of casein solutions or suspensions is due to the swelling of particles of casein suspended in the solution of casein and that the volume of these particles is regulated by the Donnan equilibrium.

**SUMMARY.**

1. The proof is completed that the influence of electrolytes on the viscosity of suspensions of powdered particles of gelatin in water is similar to the influence of electrolytes on the viscosity of solutions of gelatin in water.

2. It has been suggested that the high viscosity of proteins is due to the existence of a different type of viscosity from that existing in crystalloids. It is shown that such an assumption is unnecessary and that the high viscosity of solutions of isoelectric gelatin can be accounted for quantitatively on the assumption that the relative volume of the gelatin in solution is comparatively high.

3. Since isoelectric gelatin is not ionized, the large volume cannot be due to a hydration of gelatin ions. It is suggested that this high volume of gelatin solutions is caused by the existence in the gelatin solution of submicroscopic pieces of solid gelatin occluding water, the relative quantity of which is regulated by the Donnan equilibrium. This would also explain why the influence of electrolytes on the viscosity of gelatin solutions is similar to the influence of electrolytes on the viscosity of suspensions of particles of gelatin.
4. This idea is supported by experiments on solutions and suspensions of casein chloride in which it is shown that their viscosity is chiefly due to the swelling of solid particles of casein, occluding quantities of water regulated by the Donnan equilibrium; and that the breaking up of these solid particles into smaller particles, no longer capable of swelling, diminishes the viscosity.

5. This leads to the idea that proteins form true solutions in water which in certain cases, however, contain, side by side with isolated ions and molecules, submicroscopic solid particles capable of occluding water whereby the relative volume and the viscosity of the solution is considerably increased. This accounts not only for the high order of magnitude of the viscosity of such protein solutions but also for the fact that the viscosity is influenced by electrolytes in a similar way as is the swelling of protein particles.

6. We therefore reach the conclusion that there are two sources for the viscosity of protein solutions; one due to the isolated protein ions and molecules, and the other to the submicroscopic solid particles contained in the solution. The viscosity due to the isolated molecules and ions of proteins we will call the general viscosity since it is of a similar low order of magnitude as that of crystalloids in solution; while the high viscosity due to the submicroscopic solid protein particles capable of occluding water and of swelling we will call the special viscosity of protein solutions. Under ordinary conditions of hydrogen ion concentration and temperature (and in not too high a concentration of the protein in solution) the general viscosity due to isolated ions and molecules prevails in solutions of crystalline egg albumin and in solutions of metal caseinates (where the metal is monovalent) while under the same conditions the second type of viscosity prevails in solutions of gelatin and in solutions of acid-salts of casein; and also in solutions of crystalline egg albumin at a pH below 1.0 and at higher temperatures. The special viscosity is higher in solutions of gelatin than of casein salts for the probable reason that the amount of water occluded by the submicroscopic solid gel particles in a gelatin solution is, as a rule, considerably higher than that occluded by the corresponding particles of casein.