THE RECIPROCAL RELATION BETWEEN THE OSMOTIC PRESSURE AND THE VISCOSITY OF GELATIN SOLUTIONS.

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1. If we put a suspension of 1 gm. of finely powdered gelatin in 100 cc. of HCl, so that the gelatin has a pH of 3.4, into a collodion bag and dip the latter into a pure solution of HCl (without gelatin) of pH 3.0, the liquid will practically not rise in the manometer connected with the liquid in the collodion bag (except to that slight extent which may be caused by some of the solid gelatin going into solution); in other words, we get no osmotic pressure. The reason is as follows: in this experiment the Donnan equilibrium is established between each individual granule of solid gelatin and the solution of HCl inside the bag and since the latter diffuses freely through the membrane there is in this case no difference of pH inside and outside the collodion membrane, and hence no difference of osmotic pressure on the opposite sides of the membrane (unless some of the solid gelatin goes into solution).

If we make this experiment, however, with a 1 per cent solution of gelatin chloride of a pH of 3.4 inside the collodion bag, while on the other side of the membrane we have a HCl solution of pH 3.0 without gelatin, we notice a rapid rise of the column of water in the manometer tube inserted into the collodion bag and the osmotic pressure of the 1 per cent solution of gelatin will be about 450 mm. H₂O or a little more when equilibrium is established.¹ This proves that the osmotic pressure of a gelatin solution cannot be due to (submicroscopic) solid particles of a jelly, but must be due to particles causing a Donnan equilibrium across the collodion membrane. Such particles can only be the individual gelatin ions, since even the smallest

¹ Loeb, J., J. Gen. Physiol., 1920–21, iii, 691.
particle of gel must cause a Donnan equilibrium to be established between the solid particles and the solution inside the bag.

If we assume that certain protein solutions contain two different constituents, namely, first, isolated ions and molecules, and second, isolated submicroscopic particles capable of occluding water, these two constituents of such solutions must play a different rôle in osmotic pressure and in viscosity. The characteristic influence of electrolytes on the osmotic pressure of protein solutions must be due to the isolated protein ions since these alone can bring about the Donnan equilibrium across the membrane; while the similar influence of electrolytes on the viscosity of protein solutions must be due to the solid submicroscopic particles since these alone are capable of swelling and of giving rise to a Donnan equilibrium between each individual particle and solution. If this inference is correct, it should be possible to demonstrate the existence of a reciprocal relation between the viscosity and the osmotic pressure of those protein solutions which contain isolated protein ions as well as solid protein particles capable of occluding water; e.g., gelatin or casein solutions. It is the purpose of this paper to show that this reciprocal relation between the osmotic pressure and viscosity of such protein solutions actually exists.

Fig. 1 shows that the osmotic pressure of a 1 per cent solution of originally isolectric gelatin diminishes the more, the more we replace the dissolved gelatin by small granules of powdered gelatin. The ordinates of the upper curve represent the values of the osmotic pressure of a 1 per cent solution of originally isolectric gelatin at different pH, the pH serving as abscissa of the curves. The acid used was HCl, and the curve is the usual one given repeatedly in the writer's preceding publications. At the beginning of the experiment the gelatin solution was rapidly heated to a temperature of 45°C. and rapidly cooled to 20°C. and then kept at that temperature throughout the entire experiment. The pH is that of the gelatin solution at the end of the experiment.

The middle curve represents an experiment in which 0.5 gm. of the isolectric gelatin in solution was replaced by 0.5 gm. of isolectric powdered gelatin. The latter did not contribute to the osmotic pressure.

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pressure, the observed osmotic pressure being due to the isolated ions of the 0.5 per cent gelatin in solution which determine the Donnan effect, and in addition, to the gas pressure of the isolated gelatin ions and the isolated gelatin molecules. Theoretically, of course, the

![Graph showing osmotic pressure vs pH]

**Fig. 1.** A suspension of 1 gm. of a fine powder of gelatin in 100 cc. of water has practically no osmotic pressure (lowest curve), while a solution of 1 gm. of the same gelatin has a maximal osmotic pressure of over 500 mm. (uppermost curve). A mixture of 0.5 gm. of powdered and 0.5 gm. of liquid gelatin in 100 cc. water has practically the osmotic pressure of the 0.5 per cent liquid gelatin in 100 cc. of water (middle curve).
coarse particles of gelatin also participate in the osmotic pressure but this effect is negligible on account of the small number of such particles. (The gelatin particles used were of grain size slightly above one sixtieth of an inch diameter.) At the beginning of the experiment the 0.5 per cent solution of gelatin was rapidly heated to 45°C. and rapidly cooled to 20°C., and then the powdered gelatin was added. The pH is that of the 0.5 per cent gelatin solution at the end of the experiment.

The lowest curve represents the osmotic pressure of 1 gm. of powdered isoelectric particles in 100 cc. of HCl of different pH. The slight osmotic pressure observed is probably due to the fact that a little of the gelatin went gradually into solution. This apparently happened to a less extent in a repetition of this experiment and the osmotic pressures observed were still lower than in the lowest curve in Fig. 1. All these osmotic pressure experiments were made in a thermostat at 20°C.

The viscosity is affected in exactly the opposite way from the osmotic pressure if part of the dissolved gelatin is replaced by solid particles of gelatin. The more dissolved gelatin is replaced by solid particles of gelatin the higher the viscosity, a result to be expected from the experiment and conclusions published in the preceding paper.\(^2\)

Solutions of 0.5, 0.625, 0.750, 0.875, and 1.0 gm. of isoelectric gelatin were heated quickly to 45°C. and cooled quickly to 20°C. and so much powdered gelatin of pH 7.0 was added as to bring the total gelatin in 100 cc. to 1 gm.; i.e., to a 0.5 per cent solution of gelatin was added 0.5 gm. of powdered gelatin (between mesh sizes 100 and 120), and to a 0.875 per cent solution of liquid gelatin was added 0.125 gm. of powdered gelatin, while no powdered gelatin was added to the 1 per cent solution of liquid gelatin. The different mixtures were brought to different pH through the addition of different quantities of HCl and the solutions were allowed to stand for 1 hour before the viscosities were measured in order to give the powdered gelatin a chance to swell. The results of the measurements are represented in Fig. 2. The reader will see that within the range of the pH between 3.6 and 1.4 the viscosity is the greater, the more liquid gelatin is replaced by powdered gelatin. This supports the idea that the
influence of electrolytes on the viscosity of gelatin solutions is due to the influence of the electrolytes on the swelling of solid submicroscopic particles of gel in the solution.

![Graph showing the influence of replacing liquid by powdered gelatin on viscosity](image)

**Fig. 2.** The influence of replacing liquid by powdered gelatin on viscosity is exactly the reverse as on osmotic pressure. The more the powdered particles replace the liquid gelatin the higher the specific viscosity.

The nature of the curves in Fig. 2 between pH 4.6 and 3.8 requires an explanation. The curves are here the lower the more liquid gelatin is replaced by solid gelatin. This is due to the fact that it was neces-
sary to let the suspensions stand for at least 1 hour to allow the particles of powdered gelatin to swell before the viscosity measurements were made. During this time the liquid gelatin at, or near, the isoelectric point increases rapidly in viscosity while this increase in viscosity is suppressed where the hydrogen ion concentration is higher. This is proved by Fig. 3 which gives the viscosity of the supernatant solutions of gelatin (without the suspended particles) which had been standing for 1 hour. Inside the range of pH 4.4 and 4.6 the viscosity had risen more rapidly on standing than at the lower pH;

![Figure 3](image-url)

**Figure 3.** Increase of viscosity of gelatin solution at the isoelectric point on standing due to the formation of submicroscopic particles of gel.

which means that at, or near, the isoelectric point new submicroscopic particles of solid jelly are constantly formed from the molecules while this process is the slower the higher the hydrogen ion concentration. While thus the addition of acid to a solution of isoelectric gelatin retards the rate of formation of new submicroscopic particles of jelly, it increases the swelling of those already present when the acid is added. On the other hand, powdered particles of isoelectric gelatin in water of pH 4.7 do not increase their volume on standing.

The fact that the addition of acid to a solution of isoelectric gelatin inhibits or retards the formation of new solid particles on standing...
is strikingly illustrated by Fig. 4. Gelatin chloride solutions all containing 1 gm. of originally isoelectric gelatin were brought to various pH by adding HCl and were at the beginning of the experiment all heated rapidly to 45° and cooled at once to 20°C. Then the viscosity was measured immediately at 20°. The result is given in the lowest curve in Fig. 4. The solutions were kept at 20° for 1 hour and the viscosity measurements were repeated. The middle curve in Fig. 4 shows that at this time the viscosity of the isoelectric solution had increased considerably, that of the solutions of lower pH down to 3.0 had increased the less, the lower the pH. Below pH 3.0 no increase in the viscosity had occurred. 2 hours later another viscosity measurement was made; the results are represented in the upper curve of Fig. 4. At the isoelectric point the viscosity had increased enormously, but less and less at lower pH. During all the time and also
during the viscosity measurements the temperature of the solutions was 20°C.

A more exact demonstration of the inhibiting influence of acid on the increase of viscosity of gelatin solutions on standing is given in

![Graph](image.png)

**Fig. 5.** Increase of viscosity of gelatin sulfate solution of different pH on standing. The increase is most rapid at the isoelectric point, thus proving that the acid retards or prevents the formation of submicroscopic solid particles of jelly on standing.

**Fig. 5.** Gelatin sulfate solutions containing 2 gm. of originally isoelectric gelatin in 100 cc. were prepared and heated rapidly to 45°C, and cooled rapidly to 20°C, and then kept constantly at 20°C.
Viscosity measurements were made every 5 or 10 minutes. Fig. 5 gives the result. The lower the pH the less the viscosity increases on standing.

There exist, therefore two different and to some extent antagonistic effects of acid on the viscosity of gelatin solution. One effect is due to the swelling of the submicroscopic particles of solid gelatin already present in the solution at the time the acid is added. This effect is regulated by the Donnan equilibrium and increases when (not too much) acid is added to the isoelectric gelatin solution. The second effect consists in retarding the formation of solid particles of jelly on standing. This inhibitory effect of the acid seems to increase steadily with the hydrogen ion concentration of the solution without going through a maximum at a pH above 1.8.

If we now return to the discussion of the curves in Fig. 2 we may say that the results in that part of the curves which belongs to the abscissae of pH above 3.8 is the expression of the fact that that part of the viscosity which is due to the gelatin in solution had undergone an increase during the hour the solution had been standing at 20° after having been heated to 45°C.; and that the increase caused in the viscosity of the liquid gelatin was a maximum at the isoelectric point, being almost zero at a pH below 3.4; while the addition of acid had the opposite effect on the solid granules of gelatin, since their volume was increased according to the rules of the Donnan equilibrium.

It is necessary that we convince ourselves that a Donnan equilibrium exists when particles of solid gelatin are suspended in a solution of gelatin. That this is actually true was shown in the following way. 0.5 gm. of powdered gelatin was added to 100 cc. of a 0.5 per cent gelatin chloride solution of different pH. The different beakers containing these mixtures were kept for 3 1/2 hours at 20°C. The mass was then filtered through cotton wool and the pH of the filtrate (0.5 per cent gelatin solution) and of the solid gelatin granules were determined, that of the latter after they had been melted. It was found that the pH of the gelatin granules was higher than that of the solution and that the difference followed the Donnan equilibrium equation (Table I), though the result was slightly irregular owing to the fact that it is impossible to free the suspended particles of gelatin completely from the supernatant liquid. When we separate
TABLE I.

<table>
<thead>
<tr>
<th>pH of gelatin in suspension</th>
<th>5.60</th>
<th>4.69</th>
<th>4.18</th>
<th>3.73</th>
<th>3.43</th>
<th>2.93</th>
<th>2.68</th>
<th>2.34</th>
<th>2.09</th>
<th>1.86</th>
<th>1.53</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH of gelatin in solution</td>
<td>4.98</td>
<td>4.35</td>
<td>4.12</td>
<td>3.69</td>
<td>3.50</td>
<td>3.14</td>
<td>2.78</td>
<td>2.50</td>
<td>2.28</td>
<td>1.97</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Fig. 6. Influence of temperature on the variation of viscosity of gelatin solutions on standing. Below 35°C, the viscosity of a 2 per cent gelatin chloride solution of pH 2.7 no longer increases but diminishes on standing.
a gelatin solution from water by a collodion membrane we get two equilibria, one across the membrane caused by the protein ions on one side of the membrane; and a second one inside the protein solution caused by the solid particles of jelly.

2. These experiments in which liquid gelatin was replaced by solid particles of gelatin support the idea of a reciprocal relation between the osmotic pressure and the viscosity of gelatin solutions. It is, however, possible to give a more direct proof. It was noticed that the viscosity of solutions of gelatin chloride does not always increase on standing but that it diminishes when the temperature exceeds a certain limit. This is shown for a 2 per cent solution of gelatin chloride of pH 2.7 in Fig. 6. The viscosity of such a solution increases on standing very rapidly at 15°C., much less rapidly at 25°C., but diminishes when kept at a temperature above 35°C., and the more rapidly the higher the temperature. This we assume to be due to the fact that at a temperature above 35°C. the rate of melting of submicroscopic particles of solid jelly exceeds the rate of their formation from isolated ions or molecules.

Several liters of a 0.55 per cent solution of isoelectric gelatin were kept at about 10°C. for 48 hours and at 20°C. for the next 24 hours. Then the stock solution was divided into two parts. The one part was divided into parts of 90 cc. each, and each part was brought to different pH by adding 10 cc. containing different quantities of HCl. In this way the concentration of originally isoelectric gelatin was, therefore, in every case 0.5 per cent. The second portion was treated in the same way except that before adding the acid the gelatin was kept for 1 hour at 45°C. This was done to melt part of the submicroscopic pieces of jelly assumed to exist in the solution and thus to increase the concentration of the isolated ions and molecules and to diminish the relative quantity of solid submicroscopic particles capable of occluding water and thereby causing the high viscosity characteristic of gelatin solutions. After this second portion of the stock solution of isoelectric gelatin had been kept for 1 hour at 45°C. it was rapidly cooled to 20°C., the HCl was added in the way described for the first portion and the solutions were put into collodion bags to measure the osmotic pressure. Each collodion bag contained about 50 cc. of gelatin solution. The temperature now remained constant
at 20°C. for both sets of experiments. It was noticeable from the first that the osmotic pressure of the gelatin solution which had been kept for 1 hour at 45°C and which was therefore supposed to have melted into smaller particles was higher than that of the gelatin solution not previously heated. Fig. 7 shows the result after 22 hours. The maximum osmotic pressure was, for the gelatin solution that had been previously heated, 200 mm. H₂O, while it was only 170 mm. for the other gelatin solution not previously heated to 45°C.

Fig. 7. Showing that the osmotic pressure of a solution of gelatin chloride which has been previously heated to 45°C. for 1 hour and then rapidly cooled to 20°C. is higher than the osmotic pressure of the same solution of gelatin chloride not previously heated.

Then the viscosities were determined at 20°C and they gave the opposite result (Fig. 8), the viscosities being considerably higher in the solutions not previously heated to 45°C than in the solutions previously heated. This experiment then confirms our expectation that there exists a reciprocal relation between the viscosity of protein solutions and their osmotic pressure, inasmuch as a transformation of solid submicroscopic particles of jelly into isolated protein ions
and molecules diminishes the viscosity but increases the osmotic pressure of the gelatin solution.

As far as the quantitative relations are concerned, the difference in viscosity (Fig. 8) is more striking than the difference in osmotic pressure (Fig. 7). The osmotic phenomena are a more complicated function of the change caused by the transformation of larger aggregates into simple ions, inasmuch as there exists a Donnan equilibrium not only between the gelatin solution and the outside watery solution

![Viscosity Ratio vs pH](image)

**Fig. 8.** Showing that the influence of previous heating on the viscosity of 0.5 per cent solutions of gelatin chloride is the reverse of the influence of the previous heating on osmotic pressure (Fig. 7).

but also between each solid particle of gelatin and the true gelatin solution surrounding it.

It was expected that when we put a 1 per cent solution of gelatin of, e.g., pH 3.5, which had been kept for 1 hour at 45° and cooled to 20° into a 1 per cent solution of an identical gelatin chloride solution of pH 3.5, which had not been heated to 45° before being brought to 20°C, water would diffuse from the latter into the former solution. This experiment was carried out with a positive result.
These experiments support the idea expressed in the preceding paper that protein solutions are true solutions which may or may not contain solid particles of protein capable of swelling. In the case of gelatin solutions the formation of submicroscopic particles of solid jelly from isolated molecules or ions is a reversible process and this leads in this case to a reciprocal variation of osmotic pressure and viscosity of such solutions.

This probably explains a phenomenon which has puzzled the writer for a long time; namely, that the osmotic pressures of gelatin solutions of the same pH and concentration of originally isoelectric gelatin occasionally showed variations for which he could not account. It now becomes probable that this was due to a factor which was not taken into consideration, namely, that on standing at room temperature a gradual transformation of isolated molecules or ions into larger aggregates takes place, which must diminish the osmotic pressure but increase the viscosity. This source of variation was eliminated in the viscosity experiments in which the gelatin solution was always heated first to 45°C. and then as soon as this temperature was reached the solution was cooled to the temperature desired for the viscosity measurements. It is probable that the same uniformity of treatment is also required for the osmotic pressure experiments.

This reciprocal relation between osmotic pressure and viscosity exists probably also in the case of solutions of casein salts. Solutions of Na caseinate are less opaque than those of casein chloride (of the same concentration of originally isoelectric casein) which indicates that the Na caseinate solution contains more isolated casein ions and molecules and less submicroscopic solid particles than the solution of casein chloride.

The writer had already shown in a preceding paper that the maximal viscosity of a 1 per cent solution of casein chloride is higher than the viscosity of solutions of Na caseinate of equal concentration of originally isoelectric casein, while the osmotic pressures of solutions of the two salts show exactly the reverse relation, the maximal osmotic pressure of a 1 per cent solution of Na caseinate being almost 700 mm. H₂O while the maximal osmotic pressure of a 1 per cent solution of casein chloride is only about 200 mm.
The solutions of crystalline egg albumin seem to consist (at ordinary temperature and at not too high a concentration of albumin and of the hydrogen ions) exclusively or almost exclusively of isolated molecules or ions. Since the latter cannot diffuse through a collodion membrane they give rise to a Donnan equilibrium across the membrane and hence only the osmotic pressure of solutions of salts of crystalline egg albumin is influenced by electrolytes in the way demanded, while the viscosity shows such an influence only to a negligible degree.

3. Since in connection with viscosity we have assumed the existence of submicroscopic solid particles of jelly in gelatin solutions we must point out where our ideas agree and where they disagree with the speculations on the rôle of the degree of dispersion current in colloid chemistry. On the basis of these latter speculations it would be assumed that the osmotic pressure of a protein solution is determined directly by the concentration of the protein in solution according to van't Hoff's law. We assume the correctness of van't Hoff's law but the osmotic pressure is only in part determined by the gas pressure of the protein particles, the main source of the osmotic pressure being the unequal distribution of the crystalloidal ions on the opposite sides of the membrane due to the Donnan equilibrium. This has been overlooked by the believers in the dispersion theory. We agree with the believers in the dispersion theory that the melting down of larger aggregates of gelatin ions into isolated gelatin ions must raise the osmotic pressure but disagree in the explanation of this effect; since in our opinion the increase in the number of isolated protein ions must lead to an increase in the distribution of crystalloidal ions on the opposite sides of the membrane according to the Donnan equilibrium and this is the main reason why the osmotic pressure of a gelatin solution is increased when the solid submicroscopic particles of jelly are transformed into isolated ions.  

1 If we wish to measure the true osmotic pressure of a protein solution, free from the complication of the Donnan equilibrium, we must do so at the isoelectric point of the protein. This condition was approximately fulfilled in the experiments of Sørensen (Sørensen, S. P. L., Compt. rend. trav. Lab. Carlsberg, 1915-17, xii, 262) on solutions of crystalline egg albumin, and of Hüfner and Gansser (Hüfner, G., and Gansser, E., Arch. f. Physiol., 1907, 209) on solutions of hemoglobin.
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On the other hand, the high viscosity of gelatin solutions, e.g., that part of the viscosity which is due to the volume occupied by the solid particles of jelly, is entirely regulated by the Donnan equilibrium. The dispersion theory was not able to account for the influence of electrolytes on the viscosity and osmotic pressure of protein solutions while this influence is accounted for mathematically by the Donnan theory.

SUMMARY AND CONCLUSIONS.

1. These experiments confirm the conclusion that protein solutions are true solutions consisting of isolated ions and molecules, and that these solutions may or may not contain in addition solid submicroscopic particles capable of occluding water.

2. The typical influence of electrolytes on the osmotic pressure of protein solutions is due to the isolated protein ions since these alone are capable of causing a Donnan equilibrium across a membrane impermeable to the protein ions but permeable to most crystalloidal ions.

3. The similar influence of electrolytes on the viscosity of protein solutions is due to the submicroscopic solid protein particles capable of occluding water since the amount of water occluded by (or the amount of swelling of) these particles is regulated by the Donnan equilibrium.

4. These ideas are supported by the fact that the more the submicroscopic solid particles contained in a protein solution or suspension are transformed into isolated ions (e.g., by keeping gelatin solution for 1 hour or more at 45°C.) the more the viscosity of the solution is diminished while the osmotic pressure is increased, and vice versa.