HYDRATION OF GELATIN IN SOLUTION.

By M. KUNITZ.

(From the Laboratories of The Rockefeller Institute for Medical Research, Princeton, N. J.)

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The high viscosity of gelatin solutions indicates that even at temperatures as high as 40°–50°C, gelatin is hydrated, i.e., that the ultimate gelatin units (particles or molecules) in the solution are in some way combined with a certain amount of water. This is true not only in the case of ionized gelatin, where the hydration is regulated by the Donnan equilibrium, but also in the case of isoelectric gelatin, the viscosity of which, even at high temperatures, is quite high as compared with that of crystallized egg albumin of the same concentrations, as shown in Fig. 1. The hydration of gelatin is indicated also by measurements of osmotic pressure of various concentrations of gelatin solutions. In this respect gelatin differs from egg albumin. The curve, Fig. 2, for osmotic pressure vs. concentration for isoelectric albumin (sp. cond. about 8 × 10⁻⁵ in 15 per cent sol.) at 20°C. is a straight line, while in the case of gelatin the osmotic pressure increases much faster than the concentration, and the curve is convex toward the concentration axis. This difference in the behavior of gelatin and egg albumin with respect to osmotic pressure is explainable by the difference in their degree of hydration. As was shown elsewhere, the osmotic pressure of dilute molal solutions of hydrated substances may be expressed as

\[ P = K \frac{C}{100 - \varphi} \]  

where \( K = \frac{RT}{M} \) (\( M = \) mol weight of solute).

\( C = \) gm. of solute per 100 cc. of solution.

\( \varphi = \) volume of the hydrated solute.


which means that the osmotic pressure would be proportional to the concentration if the concentration were corrected for the volume occupied by the hydrated solute.

![Graph showing viscosity-concentration curves of isoelectric gelatin at 35°C. and of isoelectric egg albumin at 20°C.]

In the case of egg albumin the correction is small, and if $C$ is expressed in gm. per 100 cc. of $H_2O$, there is then practically no correction, and the plotted curve is a straight line. In the case of gelatin, on the other hand, the volume occupied by the hydrated solute is quite large, so that the active concentration of gelatin as expressed in gm.
per 100 cc. of H₂O is much greater than the one taken from dry weight measurements. Hence the osmotic pressure increases much faster than the dry weight concentration. The curves, as said before, should become straight lines if the concentrations of the gelatin solutions were corrected for the volume of the hydrated solute. A means of finding this correction is afforded by viscosity measurements.

**Fig. 2. Osmotic pressure-concentration curves of isoelectric gelatin at 35°C. and of isoelectric egg albumin at 20°C.**

**A Method for Measuring Hydration of Gelatin.**

In a recent publication, the writer showed that the viscosity of a number of colloidal solutions, as well as of various sugar solutions, as measured by means of an Ostwald viscosimeter, may be well represented by the equation

\[
\frac{\eta}{\eta_0} = \frac{1 + 0.5 \phi}{(1 - \phi)^4}
\]  

(2)

where \( \eta \) is the absolute viscosity of the solution, \( \eta_0 \) is the absolute viscosity of the solvent, and \( \phi \) is the volume occupied by the solute expressed as a fraction of the total volume of the solution. In the case of sugars or other substances that are hydrated to a very small extent, the value of \( \phi \) when expressed as cc. per 100 cc. of solution actually equals the volume of the dissolved dry substance, while in the case of highly hydrated or solvated substances, such as casein in water or rubber in benzene, \( \phi \) represents the volume of the dry substance plus the volume of the solvent associated with it. This was checked for rubber by substituting the values for \( \phi \) as obtained from viscosity measurements into the equation for osmotic pressure, (equation (1)). The values of \( K \) for various concentrations of rubber were constant. The data of Caspari\(^4\) were used in both cases.

This method of testing the validity of the viscosity formula is applied in this paper to isoelectric gelatin. A series of curves for the osmotic pressure of various concentrations of isoelectric gelatin at various temperatures were plotted. All the curves showed the characteristic convexity toward the concentration axis. The concentrations were expressed as gm. of dry gelatin per 100 cc. H₂O, as determined by drying for 24 hours at 100°C., definite weights of gelatin solution taken from the osmometers after equilibrium was reached. At the same time viscosity measurements were carried out by means of an Ostwald viscosimeter on samples of the same gelatin solutions at the same temperatures at which the osmotic pressure measurements were done. The viscosities of freshly prepared gelatin solutions were also measured and gave practically identical results. The various values of \( \phi \) were then read off from the theoretical viscosity curve shown elsewhere,\(^5\) and the concentrations in the osmotic pressure curves were then corrected. Fig. 3 shows that the corrected points for the osmotic pressure values lie on straight lines in the range of the dry weight concentration from 1 to 10 gm. per 100 cc. of H₂O for the various temperatures used, thus proving that the values of \( \phi \) as obtained from the viscosity represent the true volumes of the hydrated gelatin particles, and hence affording a method of determining the degree of hydration of gelatin.

**Molecular Weight of Gelatin.**

One of the difficulties usually experienced in calculating the mol weight of gelatin from osmotic pressure through the application of van't Hoff's formula

\[
P = \frac{RT}{M} C \quad (C = \text{gm./cc.}),
\]

is the fact that \( P/C \), as usually plotted, is not constant. But if, on the other hand, the concentration is corrected for the "water of hydration" by means of viscosity, then \( RT/M \) becomes a constant value, expressed as the slope of the corrected curve, or it can be calculated more exactly from the relation

\[
\frac{RT}{M} = \frac{P \times (100 - \phi)}{C}
\]  \( (3) \)

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At 35°C, the value of \( RT/M \) is 313, as shown in Table I.

Hence

\[
M = \frac{RT}{313}
\]

\( R \) being the gas constant equals

\[
\frac{P_0 V_0}{T_0} = \frac{22.4 \times 760 \times 1000}{273} = 62,400 \text{ cc:mm.Hg/degree}
\]
or

\[
M = \frac{62,400 \times 308}{313} = 61,500.
\]

**TABLE I.**

<table>
<thead>
<tr>
<th>C (gm./100 cc. of solution)</th>
<th>( \eta )</th>
<th>( \eta/\eta_0 )</th>
<th>( \varphi )</th>
<th>( C/100-\varphi )</th>
<th>( P )</th>
<th>( RT )-uncorrected</th>
<th>( RT )-corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.43</td>
<td>7.75</td>
<td>1.08</td>
<td>3.5</td>
<td>350</td>
<td>324</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.06</td>
<td>15.05</td>
<td>2.35</td>
<td>7.5</td>
<td>375</td>
<td>319</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.96</td>
<td>21.80</td>
<td>3.84</td>
<td>12.0</td>
<td>400</td>
<td>312</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.24</td>
<td>27.90</td>
<td>5.55</td>
<td>17.0</td>
<td>425</td>
<td>306</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.00</td>
<td>33.40</td>
<td>7.50</td>
<td>23.0</td>
<td>460</td>
<td>307</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.20</td>
<td>38.10</td>
<td>9.70</td>
<td>29.5</td>
<td>492</td>
<td>304</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.85</td>
<td>42.18</td>
<td>12.1</td>
<td>37.5</td>
<td>537</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>13.9</td>
<td>45.52</td>
<td>14.7</td>
<td>47.0</td>
<td>588</td>
<td>320</td>
<td></td>
</tr>
</tbody>
</table>

Average .......................................................... 313

This value represents the weight of dry gelatin which, if dissolved in 1000 cc. of \( H_2O \), will produce an osmotic pressure of \( 22.4 \times \frac{308}{273} \) atmospheres at 35°C.

*The Mechanism of Hydration of Gelatin.*

The experiments of Loeb\(^6\) on viscosity of gelatin at various p\( H \) led him to the conclusion that gelatin solutions contain a number of

submicroscopic particles of solid jelly which are able to take up water and swell when there is a greater osmotic pressure inside the particles than outside. On the addition of acid or alkali to a solution of iso-electric gelatin a Donnan equilibrium is established between the diffusible ions inside and outside of the particles with the result that, owing to the larger concentration of gelatin inside, there are more ions inside than outside, and the particles then increase in volume on account of the difference in osmotic pressure. According to this view, then, these submicroscopic particles of solid jelly behave with respect to the Donnan equilibrium in agreement with the Procter-Wilson\(^7\) theory of swelling of blocks of gelatin. According to this theory a block of gelatin under the influence of a higher ion activity inside than in the surrounding medium takes up water until the difference in the osmotic pressure between the inside and the outside solution is balanced by the stress in the elastic structure of the block, which appears to obey Hooke's law, \(i.e.,\)

\[ e = E V \]

where \(e\) is the osmotic pressure due to the difference in the activity of ions, \(E\) is the bulk modulus, and \(V\) is the increase in volume.

This theory appears to hold also for the swelling of blocks of iso-electric gelatin, as was shown by Northrop and the writer.\(^8\) In the case of isoelectric gelatin, where ions are practically absent, an osmotic pressure exists in a block of solid gelatin due to the presence of a water-soluble constituent of gelatin held in a network of insoluble fibers. This was confirmed by actually isolating from gelatin, by fractional precipitation with alcohol, two fractions, one of which is soluble in cold water, does not set to a jelly, and has a low viscosity and a high osmotic pressure, and a second one which is insoluble at ordinary temperatures, sets to a jelly in very low concentration, and swells much less than ordinary gelatin. When a block of ordinary solid isoelectric gelatin is immersed in water, the water enters the gelatin which swells until the osmotic pressure of the soluble fraction of the


gelatin inside of the block is equal to the elasticity pressure of the block, \( P = E \frac{V_s - V_0}{V_0} \)

where \( P \) is the osmotic pressure in the block which can be measured directly as described, \( E \) is the bulk modulus of elasticity, \( V_s \) is the final volume of the block at equilibrium, and \( V_0 \) is the volume of the dry gelatin.

It will now be shown from the viscosity measurements of various concentrations of isoelectric gelatin at 35°C. that the apparent hydration of gelatin at this temperature is due to the swelling of the ultramicroscopic particles of solid jelly brought about by the same mechanism as the swelling of large blocks of gelatin,—namely, by the osmotic pressure of the soluble gelatin which is included in the insoluble particles of solid jelly, or micellae as they were named by Naegeli. The particles swell until the difference between the osmotic pressure inside and outside of the particles is balanced by the elastic pressure of the particle.

Let \( q \) be amount of water in cc. held by 1 gm. of gelatin, as calculated from viscosity, \( i.e. \)

\[
q = \frac{\varphi}{C} - .75, \text{ the last value being the volume of 1 gm. of dry gelatin.}
\]

Let also \( n \) be the number of micellae per gm. of gelatin.

\[
\frac{q}{n} = \text{cc. H}_2\text{O per micella, under the assumption that all the water of hydration is associated with the micellae only.}
\]

\[
s = \text{gm. of soluble gelatin per micella.}
\]

\[
K = \text{osmotic pressure constant for soluble gelatin, } i.e.,
\]

\[
K = \frac{RT}{M}
\]

Then the osmotic pressure inside at equilibrium equals \( \frac{Ks}{q/n} = \frac{Ksn}{q} \).

The osmotic pressure outside is the osmotic pressure \( P \) as determined directly, while the elastic pressure is

\[
E \frac{q/n}{V_0} = E \frac{q}{V_0^n}
\]

\(^8\) Northrop and Kunitz, \(^8\) p. 162.
where $E$ is the bulk modulus of the micella at the given temperature, and $v_0$ is the volume of the micella before swelling. The equilibrium condition is then

$$\frac{Km}{q} - P - E \frac{q}{v_0} = 0;$$

or if instead of $sn$ the symbol $\alpha$ is used, where $\alpha$ designates the fraction of 1 gm. of gelatin which is found in a soluble state inside of the micella, then the equation becomes

$$\frac{K\alpha}{q} - P - E_1 q = 0,$$

(4)

where

$$E_1 = \frac{E}{v_0}.$$

As the concentration of the gelatin approaches zero, $P$ approaches it likewise, and we have then

$$\frac{K\alpha}{q} - E_1 q = 0$$

[$P \cong 0$]

or

$$\frac{K\alpha}{E_1} = q^2$$

[$P \cong 0$]

If a curve is plotted for the values of $q$ as obtained from viscosity measurements against the concentrations of gelatin used and if the curve is continued until it crosses the $q$ axis, the value of $q$ at the interception may then be introduced into the last equation. The actual value of $q^2$ as read off from the curve is close to 52. Thus we have

$$\frac{K\alpha}{E_1} = 52.$$
When $P$ is not equal to zero the equilibrium equation becomes

$$\frac{52}{q} - \frac{P}{E_1} - q = 0 \quad (5)$$

or

$$\left(\frac{52}{q} - q\right) + P = \frac{1}{E_1} \quad (5a)$$

The following table shows the value of $1/E_1$ for various concentrations of isoelectric gelatin at 35°C.

<table>
<thead>
<tr>
<th>TABLE II.</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm./100 cc. of solution</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
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<tr>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td><strong>Average</strong></td>
</tr>
</tbody>
</table>

Substituting into equation (5) for $1/E_1$ its value of .123, and for $P$ the relation

$$P = \frac{K_1 C}{100 - \varphi} \quad \text{(equation (3))}, \ \text{where}$$

$$K_1 = 313^* \ \text{and} \ \varphi = (q + .75)C$$

we get

$$\frac{52}{q} - \frac{38C}{100 - (q + .75)C} - q = 0 \quad (6)$$

* Table I.
Fig. 4 shows the plotted theoretical curve for equation (6), obtained by assuming various values for $q$ and solving for $C$. On the same figure are also shown the experimental values for $q$ at various values of $C$, which are very close to the theoretical line. This agreement between the theoretical values of the water of hydration of gelatin with the values obtained from viscosity measurements confirms the theory that a solution of isoelectric gelatin even at 35°C. contains a definite number of small blocks of gelatin filled with a definite weight of soluble gelatin the osmotic pressure of which is higher than the osmotic pressure of the gelatin outside of the blocks, i.e. higher than the total osmotic pressure of the gelatin solution as a whole. Owing to this difference in pressure, each little block swells until its osmotic pressure is balanced by the total osmotic pressure of the solution and the elastic resistance of the block to stretch.

As the concentration of the gelatin is increased the total osmotic pressure

\[
\frac{52}{q} - \frac{38C}{100 - (q + .75)C} = 0.
\]
pressure of the solution is increased. The number of little blocks is also increased, but the amount of soluble gelatin per block is unchanged, with the result that the difference in osmotic pressure between the inside and the outside of the block continuously decreases with the increase in the total concentration of the gelatin solution. The swelling of the little blocks is thus decreasing gradually as shown on the curve. This is also clear from equation (5a) where \( \frac{52}{q} - q = .123 P \). An increase in \( P \) must be followed also by an increase in the value of \( \frac{52}{q} - q \), which is possible only when \( q \) is diminishing in its value.

The Quantitative Interpretation of pH Viscosity Curves.

If the equation \( \frac{K\alpha}{q} - P - E_1 q = 0 \) is true for isoelectric gelatin then at any other pH outside of the isoelectric point the equation should be \( \frac{K\alpha}{q} - P + D.P. - E_1 q = 0 \) where \( P \) equals

\[
\frac{313C}{100 - (q + .75)C}
\]

and \( D.P. \) is the difference between the osmotic pressure inside and outside of the micelle due to the difference in the ion activity brought about by the Donnan equilibrium established between the micelle and the gelatin solution outside of the micelle. This will be true only if the addition of acid or alkali does not modify the values of \( \alpha \) and \( E_1 \). That this is the case for HCl, at least, was demonstrated by the fact of the reversibility of the swelling of gelatin in an HCl solution, since on washing away the HCl the swollen gelatin returns to the original volume of isoelectric gelatin.\(^{10} \) This is also true with respect to osmotic pressure. Hence, if values could be substituted for \( K, \alpha, \) and \( E_1 \), then it would be possible to calculate the internal Donnan pressure of gelatin at various pH from the viscosity measurements. But, as found above,

\[
\frac{K\alpha}{E_1} = 52
\]

and

\[ \frac{1}{E_t} = .123 \quad \text{or} \quad E_t = 8.1 \]

Hence

\[ K \alpha = 420 \]

Substituting these values, the equation for the internal Donnan pressure of a solution of gelatin chloride at various pH is then

\[ D.P. = P + 8.1 q - \frac{420}{q} \tag{7} \]

There is also another way of obtaining the approximate values of the internal Donnan pressure of the micelles; namely, through an analysis of the ion distribution between the micelles and the outside gelatin solution.

Let

- \( H_t \) = hydrogen ion activity in the micelles.
- \([\text{HCl}_t]\) = total HCl concentration in the micelles.
- \( H_0 \) = hydrogen ion activity outside.
- \([\text{HCl}_0]\) = total HCl concentration outside.

If it is to be assumed that the gelatin has only a slight effect on the activity of the Cl ion,\(^{11}\) and that the concentration of HCl outside of the micelles is not much different than the average concentration of HCl in the gelatin solution, then according to the Donnan equilibrium relation

\[ H_t \gamma_{\text{Cl}_t} [\text{HCl}_t] = H_0 \gamma_{\text{Cl}_0} [\text{HCl}_0] \]

where \( \gamma_{\text{Cl}_t} \) and \( \gamma_{\text{Cl}_0} \) are the activity coefficients for Cl ion,

or

\[ H_t [\text{HCl}_t] = H_0 [\text{HCl}_0] \frac{\gamma_{\text{Cl}_0}}{\gamma_{\text{Cl}_t}} \tag{8} \]

The calculations of Loeb\(^{12}\) on osmotic pressure of gelatin have shown that the Donnan osmotic pressure of a solution of gelatin in a collodion membrane is equal approximately to the sum of the activities of the


\(^{12}\) Loeb,\(^6\) p. 217.
ions in the gelatin solution, as measured electrometrically, multiplied by $RT/1000$. This same pressure is also acting against the Donnan pressure of the micelle, which in its turn equals the sum of the activities of the ions in the micelle. Hence the difference in the Donnan pressure is

$$D.P. = \frac{RT}{1000} (H_i \times Cl_i - H_s - Cl_o)$$

where

$$Cl_i = \gamma_{Cl_i} [HCl_i] \text{ and } Cl_o = \gamma_{Cl_o} [HCl_o].$$

Equation (8) may also be put in the form of

$$H_i \left( \frac{H_i}{\gamma_{H_i}} + [HCl_i] C \right) = \frac{\gamma_{Cl_i} \alpha}{\gamma_{Cl_i}}$$

where $[HCl_i] = \text{equivalent concentration of HCl combined with 1 gm. of gelatin when dissolved in 100 cc. of solvent, } C = \text{concentration of gelatin in gm. per 100 cc. H}_2O, \text{ and } \alpha = H_4[HCl_o]$.

or

$$H_i^2 + H_i [HCl_i] \gamma_{H_i} C = \gamma_{H_i} \frac{\gamma_{Cl_i} \alpha}{\gamma_{Cl_i}}$$

If it is to be assumed, as a first approximation, that

$$\gamma_{Cl_i} = 1$$

and that

$$\gamma_{H_i} = \gamma_{H_o}$$

then

$$H_i^2 + H_i [HCl_i] \gamma_{H_o} C = \gamma_{H_o} \alpha$$

or

$$H_i^2 + H_i [HCl_i] C_i = \alpha_1$$

where

$$C_1 = \gamma_{H_o} C \text{ and } \alpha_1 = \gamma_{H_o} \alpha$$

It will be shown later that of every gm. of gelatin dissolved at 35°C., 0.48 gm. is found in the micelle. Hence if $q$ is the amount of $H_2O$ of hydration per gm. of gelatin, as obtained from viscosity measurement, the concentration of the gelatin in the micelle, is

$$C = \frac{0.48}{q}$$

since the water of hydration is associated, according to theory developed here, with the micelle only.
At any pH the amount of HCl combined per gm. of gelatin in the micelles can be obtained from the titration curve of gelatin, since it was shown by experiment that there is practically no difference in the titration curves of the soluble and the insoluble fractions of gelatin. Fig. 5 shows the titration curve for 1 per cent solution of gelatin with HCl. The curve was corrected, from the data in Table IV, for the free HCl, i.e. for the HCl required to bring H₂O to the corresponding

![Graph showing titration curve](image)

**Fig. 5. Corrected titration curve of 1 per cent isoelectric gelatin with HCl.**

pH. At any value of H, the value of HCl, is thus determinable by reading it off the curve. The correction for the free HCl was based on the relation 

\[
[HCl] = \frac{H}{\gamma_H} + [HCl]C
\]

where

- \([HCl]\) = total concentration of HCl in the gelatin solution.
- \(H\) = activity of the H ion as obtained from pH measurement.
- \(\gamma_H\) = activity coefficient of H at the ionic strength equal to \([HCl].\)
- \([HCl]\) and \(C\) are the same as defined before.

* Gelatin appears to affect neither the ionic strength of a solution nor the activity coefficients of the various ions. See Northrop and Kunitz, p. 29.
Values for $\gamma_H$ (as well as for $\gamma_{Cl\cdot}, \gamma_{H\cdot},$ and $\gamma_{Cl\cdot}$ used in the later calculations) at the various values of [HCl] were calculated, by the method of Lewis and Randall,\textsuperscript{13} from the recent data of Scatchard\textsuperscript{14} on the mean activity coefficients of KCl and HCl at 25°C. The curves for $\gamma_H$ and $\gamma_{Cl\cdot}$ at various concentrations of HCl are shown on Fig. 6. All pH measurements were done electrometrically at 35°C., and the values for pH were based on 0.100 M HCl as a standard, its pH being taken as 1.085 at 35°C. It is thus possible, by means of equation (10b), to calculate approximately the activities of the ions in the micelle from the known data on the whole solution and from the additional information on the concentration of the gelatin in the micelle from the viscosity measurements, especially since [HCl].

\textsuperscript{13} Lewis, G. N., and Randall, M., Thermodynamics and the free energy of chemical substances, New York and London, 1923, 381.
can be easily expressed as a function of $H_i$ by means of the equilibrium equation representing the titration curve. The actual calculations were done graphically by assuming various values of $H$ and solving for $a_i$ at a given value of $C_1$. This was repeated for the identical values of $H$ and another value of $C_1$. A family of curves were then plotted for $C_1$ of 2.0, 2.5, 3.0, 3.5, and 4.0 of pH as abscissae and values of $a_i$ as ordinates. These curves were then used for finding the pH of the micelle for various values of $\gamma_{H_a}$ and $\gamma_{H_i}C$, thus giving values of $H_i$. On substituting the found value of $H_i$ in equation (8), and assuming that $\gamma_{Cl} = 1$, the value of $[HCl_i]$ was obtained, namely $[HCl_i] = a_i \\ H_i$

Using the obtained approximate values of $[HCl_i]$ as the ionic strength of the solution in the micelle, values for $\gamma_{H_i}$ and $\gamma_{Cl}$ were
read off the curves. New values of $H_i$ and $[HCl]$ were then obtained in the same manner as before except instead of $a_i = \gamma_{H_0} a$ and $C_i = \gamma_{H_0} C$, values of $a'' = \gamma_{H_0} \frac{\gamma_{Cl}}{\gamma_{Cl_i}} a$ and $C'' = \gamma_{H_0} C$ were employed; also in equation (8) the actual values of $\frac{\gamma_{Cl}}{\gamma_{Cl_i}}$ were used. The new values of $H_i$ and $[HCl]$ happened to be almost identical with those obtained on the first approximation and hence they were taken as the correct ones. The sum of the activities of the ions inside of the micelle is then $H_i + \gamma_{Cl_i} [HCl]$ and outside $H_0 + \gamma_{Cl_0} [HCl_0]$, and the Donnan pressure is

$$19.2 \times 10^3 \times (H_i + \gamma_{Cl_i} [HCl] - H_0 - \gamma_{Cl_0} [HCl_0])$$

where $19.2 \times 10^3$ is the theoretical osmotic pressure of a molar solution in mm. of Hg at 35°C. The following is an example of the calculations.

A solution of 0.97 per cent gelatin containing 8.13 cc. N/10 HCl per 100 cc. gave a pH reading of 3.01. Its relative viscosity at 35°C. was found to be 2.50 with a value for $q$ of 18.65.

$[HCl] = 8.13 \times 10^{-4}$

\[
\begin{align*}
\gamma_{Cl_0} &= .910 \\
\gamma_{H_0} &= .928
\end{align*}
\]

At ionic strength of $8.13 \times 10^{-4}$

$H_0 = 9.77 \times 10^{-4}$ from pH

\[
\begin{align*}
a &= H_4 [HCl] = 7.94 \times 10^{-4} \quad &\gamma_{H_4} a = 7.56 \times 10^{-4} = a_1 \\
C &= \frac{.48}{18.65} = 2.57 \text{ gm./100 cc. H}_2\text{O} \quad &\gamma_{H_2} C = 2.39 = C_1
\end{align*}
\]

pH inside (from curve for $C_1 = 2.5$ and extrapolated for 2.39) = 3.31

$H_i$ from pH$_i$ = $4.90 \times 10^{-4}$

$[HCl] = \frac{a}{H_4} = \frac{7.94 \times 10^{-4}}{4.79 \times 10^{-4}} = 16.2 \times 10^{-3}$

$\gamma_{H_4} = .906$ and $\gamma_{Cl_i} = .878$ for the ionic strength of $16.2 \times 10^{-3}$
\[ a = \gamma_{H_2O}a = 0.906 \times \frac{0.911}{0.878} \times 7.94 \times 10^{-4} = 7.46 \times 10^{-4} \]

\[ C_\ell = \gamma_{H_2O}C = 0.906 \times 2.57 = 2.33 \text{ gm./100 cc. } H_2O \]

Corrected pH \( H_\ell = 3.30 \) (from curve for \( C_\ell = 2.5 \) and extrapolated for 2.33)

Corrected \( H_\ell = 5.01 \times 10^{-4} \)

Corrected \( [HCl] = \frac{\gamma_{H_2O}a}{\gamma_{Cl_\ell}}H_\ell = 0.911 \times \frac{7.94 \times 10^{-4}}{5.01 \times 10^{-4}} = 16.4 \times 10^{-4} \)

Corrected \( Cl_\ell = \gamma_{Cl_\ell}[HCl] = 14.4 \times 10^{-4} \)

\[ Cl_\ell = \gamma_{Cl_\ell}[HCl] = 7.4 \times 10^{-4} \]

Total activity of ions inside = \( H_\ell + Cl_\ell = 5.01 \times 10^{-4} + 14.4 \times 10^{-4} = 14.9 \times 10^{-4} \)

Total activity of ions outside = \( H_\ell + Cl_\ell = 9.8 \times 10^{-4} + 7.4 \times 10^{-4} = 8.4 \times 10^{-4} \)

Donnan pressure = \( 19.2 \times 10^{4} \times (14.9 - 8.4) \times 10^{-4} = 125 \text{ mm. Hg} \)

This Donnan pressure when calculated by means of the pressure-elasticity equation is

\[ D.P. = P + 8.1 \frac{q}{q'} = 3.5 + 151 - 22.5 = 132 \text{ mm. Hg} \]

which is identical, within the limits of error, with the value of 125 as calculated above.

Table III shows the values of the difference in Donnan pressure in mm. of Hg between inside and outside of the micelle in 0.97 per cent gelatin solution of various pH as calculated by both methods. The same is shown on Fig. 8. These results confirm quantitatively the theory of viscosity of gelatin of various pH, as developed by Loeb, namely, that the viscosity is regulated by osmotic forces due to the Donnan equilibrium. A difference in the activities of the ions inside and outside of the micelle is established because the concentration of the gelatin in the micelle is greater than the outside concentration of the gelatin in the solution.
| Equivalent concentration of HCl | 0 | $2.03 \times 10^{-2}$ | $4.06 \times 10^{-3}$ | $6.10 \times 10^{-1}$ |
|---------------------------------|--------------------------|--------------------------|--------------------------|
| pH                             | 4.8 | 4.20 | 3.76 | 3.38 |
| Relative viscosity              | 1.40 | 1.62 | 1.99 | 2.32 |
| q                              | 6.35 | 9.85 | 14.05 | 17.25 |
| Concentration of gelatin in the micelles | 7.22 | 4.87 | 3.41 | 2.78 |
| $\Delta P$                     | $-9.2 \times 10^{-3}$ | $4.17 \times 10^{-3}$ | $5.6 \times 10^{-3}$ |
| $\Delta P$                     | $6.10 \times 10^{-1}$ | $41.0$ | $88.0$ | $120$ |
\[ \text{II.} \]

\[ 97 \text{ Per Cent Gelatin-HCl Solution at } 35^\circ\text{C.} \]

<table>
<thead>
<tr>
<th></th>
<th>8.13 \times 10^{-3}</th>
<th>9.14 \times 10^{-4}</th>
<th>11.2 \times 10^{-4}</th>
<th>14.2 \times 10^{-4}</th>
<th>20.3 \times 10^{-4}</th>
<th>30.5 \times 10^{-4}</th>
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</thead>
<tbody>
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<td>3.01</td>
<td>2.83</td>
<td>2.58</td>
<td>2.29</td>
<td>2.01</td>
<td>1.73</td>
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<tr>
<td>2.50</td>
<td>2.54</td>
<td>2.52</td>
<td>2.40</td>
<td>2.19</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td>18.65</td>
<td>18.95</td>
<td>18.85</td>
<td>18.75</td>
<td>16.05</td>
<td>14.10</td>
<td></td>
</tr>
<tr>
<td>2.57</td>
<td>2.53</td>
<td>2.54</td>
<td>2.56</td>
<td>2.99</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>9.77 \times 10^{-4}</td>
<td>1.48 \times 10^{-4}</td>
<td>2.63 \times 10^{-4}</td>
<td>5.13 \times 10^{-4}</td>
<td>9.8 \times 10^{-4}</td>
<td>18.6 \times 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>7.4 \times 10^{-4}</td>
<td>8.3 \times 10^{-4}</td>
<td>10.0 \times 10^{-4}</td>
<td>12.6 \times 10^{-4}</td>
<td>17.6 \times 10^{-4}</td>
<td>25.6 \times 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>5.01 \times 10^{-4}</td>
<td>7.59 \times 10^{-4}</td>
<td>1.48 \times 10^{-4}</td>
<td>2.95 \times 10^{-4}</td>
<td>5.98 \times 10^{-4}</td>
<td>12.9 \times 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>14.4 \times 10^{-4}</td>
<td>16.1 \times 10^{-4}</td>
<td>17.8 \times 10^{-4}</td>
<td>21.9 \times 10^{-4}</td>
<td>28.8 \times 10^{-4}</td>
<td>36.9 \times 10^{-4}</td>
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</tr>
<tr>
<td>125</td>
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<td>128</td>
<td>138</td>
<td>138</td>
<td>107</td>
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<tr>
<td>132</td>
<td>136</td>
<td>135</td>
<td>133</td>
<td>108</td>
<td>88</td>
<td></td>
</tr>
</tbody>
</table>
Effect of Concentration of Gelatin on the pH—Viscosity Curves.

With the increase in the total concentration of the gelatin solution the difference in the gelatin concentration between the micelle and the outside is gradually diminished. Hence the increase in viscosity at pH 3.0 over that of isoelectric gelatin, which as shown above is brought about by the difference in the gelatin concentration of the micelle, should become less conspicuous with increase in the total concentration of the gelatin solution. That this is exactly what happens is shown in Table IV.

The effect of the concentration of the gelatin solution on the
viscosity-pH curves is shown still more strikingly if instead of the relative viscosity values the values of \( q \), i.e., of the volume of \( H_2O \) taken up by a gm. of gelatin as calculated from equation (2), are used in plotting the curves. This is shown on Fig. 9. The enormous effect of the concentration of the gelatin on the viscosity that is caused by the Donnan equilibrium between the micelles and the outside solution is apparent.

The curves show that at a concentration of 10 gm. of dry gelatin per 100 cc. of \( H_2O \) the Donnan effect on the viscosity of the solution disappears entirely. This indicates that at this concentration of gelatin there is no difference between the concentration of the gelatin inside and outside of the micelles. Let \( \alpha_1 \) be the fraction of each gm.

### TABLE IV.

**Viscosity Measurement of Various Concentrations of Gelatin, pH 4.7 and pH 3.0, at 37°C.**

<table>
<thead>
<tr>
<th>Concentration in gm. per 100 cc. solution</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>8.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative viscosity of gelatin, pH 4.7.....</td>
<td>1.10</td>
<td>1.43</td>
<td>1.95</td>
<td>2.75</td>
<td>3.83</td>
<td>5.28</td>
<td>6.70</td>
<td>12.4</td>
<td>21.3</td>
</tr>
<tr>
<td>Additional viscosity = relative viscosity - 1</td>
<td>0.16</td>
<td>0.43</td>
<td>0.95</td>
<td>1.75</td>
<td>2.83</td>
<td>4.28</td>
<td>5.70</td>
<td>11.4</td>
<td>20.3</td>
</tr>
<tr>
<td>Relative viscosity of gelatin, pH 3.0.....</td>
<td>1.84</td>
<td>2.39</td>
<td>3.44</td>
<td>4.54</td>
<td>5.78</td>
<td>7.12</td>
<td>9.06</td>
<td>14.2</td>
<td>22.0</td>
</tr>
<tr>
<td>Additional viscosity</td>
<td>0.84</td>
<td>1.39</td>
<td>2.44</td>
<td>3.54</td>
<td>4.78</td>
<td>6.12</td>
<td>8.06</td>
<td>13.2</td>
<td>21.0</td>
</tr>
<tr>
<td>Ratio of additional viscosity, pH 3.0/pH 4.7</td>
<td>5.24</td>
<td>2.32</td>
<td>2.57</td>
<td>2.02</td>
<td>1.69</td>
<td>1.43</td>
<td>1.42</td>
<td>1.21</td>
<td>1.03</td>
</tr>
</tbody>
</table>

of dry gelatin found in the micelles, then the concentration of the gelatin inside of the micelles is \( \alpha_1/q \) since \( q \) is the volume of \( H_2O \) containing \( \alpha_1 \) gm. of gelatin. Hence in a solution of gelatin containing 10 gm. of dry gelatin per 100 cc. of \( H_2O \)

\[
\frac{\alpha_1}{q} = \frac{10}{100}
\]

Substituting the value of \( q \), we get

\[
\frac{\alpha_1}{4.8} = \frac{10}{100}
\]

or

\[
\alpha_1 = 0.48
\]
This value of $\alpha_1$ has been used in calculating the concentration of the gelatin in the micelles of a 1 per cent solution of gelatin at various pH on the assumption that the relation between the fractions of the soluble and insoluble components of gelatin is not affected, within
certain limits, by the total concentration of the gelatin or by the pH of the solution. The agreement between the values for the Donnan pressure in the micellæ as calculated by two independent methods shows that the assumption is correct.

SUMMARY.

1. It was shown that the high viscosity of gelatin solutions as well as the character of the osmotic pressure-concentration curves indicates that gelatin is hydrated even at temperatures as high as 50°C.

2. The degree of hydration of gelatin was determined by means of viscosity measurements through the application of the formula

\[
\eta = \eta_0 \left(1 + 0.5 \varphi \right)^2
\]

3. When the concentration of gelatin was corrected for the volume of water of hydration as obtained from the viscosity measurements, the relation between the osmotic pressure of various concentrations of gelatin and the corrected concentrations became linear, thus making it possible to determine the apparent molecular weight of gelatin through the application of van't Hoff's law. The molecular weight of gelatin at 35°C proved to be 61,500.

4. A study was made of the mechanism of hydration of gelatin and it was shown that the experimental data agree with the theory that the hydration of gelatin is a pure osmotic pressure phenomenon brought about by the presence in gelatin of a number of insoluble micellæ containing a definite amount of a soluble ingredient of gelatin. As long as there is a difference in the osmotic pressure between the inside of the micellæ and the outside gelatin solution the micellæ swell until an equilibrium is established at which the osmotic pressure inside of the micellæ is balanced by the total osmotic pressure of the gelatin solution and by the elasticity pressure of the micelle.

5. On addition of HCl to isoelectric gelatin the total activity of ions inside of the micellæ is greater than in the outside solution due to a greater concentration of protein in the micellæ. This brings about a further swelling of the micellæ until a Donnan equilibrium is established in the ion distribution accompanied by an equilibrium in the osmotic pressure. Through the application of the theory developed here it was possible actually to calculate the osmotic pressure difference.
between the inside of the micellae and the outside solution which was brought about by the difference in the ion distribution.

6. According to the same theory the effect of pH on viscosity of gelatin should diminish with increase in concentration of gelatin, since the difference in the concentration of the protein inside and outside of the micellae also decreases. This was confirmed experimentally. At concentrations above 8 gm. per 100 gm. of H₂O there is very little difference in the viscosity of gelatin of various pH as compared with that of isoelectric gelatin.

The writer wishes to acknowledge his indebtedness to Dr. J. H. Northrop for valuable advice and suggestions.