THE SWELLING OF ISOELECTRIC GELATIN IN WATER.

I. EQUILIBRIUM CONDITIONS.

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If a block of isoelectric gelatin is placed in water it will imbibe water and increase in volume. The writer and Kunitz (1) were able to measure the pressure with which this water was drawn into the block and found that it increases with the concentration of the gelatin. According to the phase rule the concentration (osmotic pressure) of a solution in the presence of the solid is independent of the amount of solid. It was suggested therefore that gelatin consisted of two or more fractions some of which were insoluble at low temperature while others were soluble and so could exert osmotic pressure. The swelling of isoelectric gelatin then becomes a process of osmosis just as Wilson (2) showed was the case for the swelling of gelatin in acid, except that in the case of isoelectric gelatin the osmotic pressure is not due to the ions of an electrolyte but to the presence of a soluble constituent of the gelatin itself. Water therefore enters the gelatin until the elastic pressure is equal to the osmotic pressure. The present paper is an attempt to apply this mechanism quantitatively to the swelling of gelatin.

The general behavior of gelatin when placed in water has been described by a number of investigators and in detail by the very complete experiments of Arisz (3). The more striking peculiarities may be briefly described as follows. In general the swelling increases with the temperature and with the concentration of gelatin. A block of gelatin concentrated by allowing the water to evaporate swells much more than a similar block made by allowing a solution of the same concentration to solidify. Thin films of gelatin reach a value which increases only slowly while large blocks do not give any indication of a maximum value but continue to swell until dissolved. At higher
temperatures there is less indication of an equilibrium value. If a block of gelatin is allowed to remain in water until it has stopped swelling and then is raised to a higher temperature in air for a short time under such conditions that there is no change in volume, it will swell rapidly when replaced in water at the first temperature. These peculiarities are similar to those of any substance when under an elastic strain, and can be readily accounted for at least qualitatively by the mechanism of swelling stated above. When the block is placed in water, water enters owing to the osmotic pressure of the solution in the block. The fibres of solid material are thereby forced apart and the force with which they attempt to return to their original position opposes the entrance of the water. At the same time the osmotic pressure is decreasing owing to dilution. When the elastic force equals this osmotic pressure the process stops. If the fibres have been stretched beyond their elastic limit, however, or if the force is applied for too long a period of time the fibres become fatigued and the elastic force is lessened so that more water enters. As is the case with any elastic body therefore a true equilibrium value is never reached. The osmotic pressure increases with the temperature and at the same time the amount of solid material decreases and also probably becomes less elastic so that swelling increases very rapidly as the temperature rises. In a thin film the whole mass becomes filled with water before the fibres become fatigued whereas in a large block the outside layers, which swell first, become fatigued and take up more water before the water has diffused into the inner layers. This “secondary swelling” therefore overlaps the primary so that in the case of large blocks there is no indication of a maximum value. This mechanism will be discussed more in detail under the kinetics of the swelling process. When a block which has swollen at a low temperature is raised to a higher temperature the elasticity is destroyed and it therefore swells again when replaced in water. When the gelatin is concentrated by evaporation it decreases in size and is therefore under an elastic strain just as when it swells except that in the case of loss of water the elastic force is in the same direction as the osmotic pressure instead of opposite to it. The block therefore swells more than a similar one which is not under elastic strain at this concentration.
**Experimental Procedure.**

In order to avoid the difficulty of working with very thin pieces of gelatin, the gelatin was coated on glass rods about 0.2 by 15 cm. The rods were weighed, dipped in gelatin of the desired strength at a temperature of 40°C., weighed again in order to determine the amount of gelatin in the film and then placed in stoppered tubes containing a strip of wet filter paper in order to prevent loss of water by evaporation. The tubes were then put in the cold room at 5°C. Arisz found that gelatin swells more if placed in water at a low temperature immediately after solidifying than if kept at the same temperature in air for a time before placing in water. After the 1st day no further change occurred. These experiments were repeated and confirmed except that a change was noted for the first 3 days. All the gelatin used in these experiments was therefore kept at 5°C for 3 days before it was placed in water. If, as the writer assumes, solid gelatin contains a saturated solution of the material forming the network this is exactly the behavior expected since time is required to reach the equilibrium value. The experiment is analogous to that of Loeb (5) who found that the osmotic pressure of a gelatin solution cooled from 70°C to 37°C was higher at first than that of a solution raised from 10°C to 37°C, but later became the same. At the beginning of an experiment the rods were weighed again and then placed in water at 5°C and weighed at intervals after wiping dry with soft filter paper. Since warming even for a short time destroys the elasticity, it is necessary to weigh at the same temperature as that at which the swelling occurred. Some  

1 Schroeder found that gelatin which was in apparent equilibrium with water lost weight when suspended in saturated water vapor at the same temperature and this has been considered as contrary to the second law of thermodynamics. As Bogue (4) has pointed out, however, the force of gravity is acting on the gelatin in the air but not in the water and if the gelatin is supposed to consist of a network of capillaries, this is sufficient to account for the observation. Suppose a fine capillary is dipped into water and after the water has risen in the tube, the tube is removed and suspended vertically just above the surface of the liquid. A drop will be formed at the bottom of the tube and since this drop has a convex surface its vapor pressure will be higher than that of the body of water. Water will evaporate from the drop until the surface of the water at the bottom of the capillary is no longer convex.
experiments were also made with slides dipped in gelatin and with thin slices of gelatin cut from a cylinder. Spheres of gelatin made by dropping liquid gelatin into cold toluene were also used.

For convenience in the calculation and presentation of the results they have been expressed in terms of gm. of water per gm. of gelatin.

Measurement of the Swelling Pressure.—The pressure with which the water tends to diffuse into the gelatin was measured by the writer and Kunitz (1). The measurements were extended to higher concentrations using the same method as described. The results of these measurements of the swelling pressure of isoelectric gelatin at 5° are shown in Fig. 1.

These pressures were obtained with gelatin that had been made up to the concentration noted while liquid and it seemed quite possible that a different pressure would be obtained when the concentration was changed by a swelling of the solid gelatin. In order to test this point a Chamberland filter was coated with collodion and then with gelatin containing 40 gm. per 100 gm. of water. The thimble was then placed in water for 12 hours at the end of which time the concentration was 30 gm. of gelatin per 100 gm. of water. The filter and
gelatin were then removed from the water, the inside filled with water and a manometer tube attached as previously described (1). At first no pressure was obtained as the swelling pressure was balanced by the elasticity of the gelatin. As the elasticity decreased with time, however, the pressure gradually rose and after 4 days remained constant at 255 mm Hg pressure. This is the same, within the experimental error, as the value obtained with 30 per cent gelatin which had been prepared by adding water to the melted gelatin. The effect on

![Graph showing swelling of various concentrations of gelatin in water at 50°C.](image)

**Fig. 2.** Swelling of various concentrations of gelatin in water at 50°C.

the osmotic pressure caused by the addition of water is therefore the same whether the water is added to the solid or liquid gelatin.

The results of some typical swelling experiments are shown in Fig. 2. It is evident that at this temperature and with thin films of gelatin, a fairly constant maximum value is reached. The experiments also show that the final value for the swelling depends only on \( V_f \), the concentration of the gelatin at the time it solidified and, within the limits of variation used in these experiments, is independent of the concentration of the gelatin when it is put in water.
Calculation of the Equilibrium Conditions.

According to the mechanism of swelling outlined above equilibrium is reached when the osmotic pressure equals the elastic pressure. The elastic pressure in this case takes the place of the hydrostatic pressure in the case of osmosis experiments discussed in a preceding paper (6). At equilibrium then

\[ \text{OP} = \text{EP} \]  

(1)

In order to use this relation both pressures must be expressed in terms of the volume of water. In ideal dilute solutions the osmotic pressure may be written \( \text{OP} = \frac{P_o}{V} \) where \( P_o \) is a constant depending on the concentration and molecular weight of the solute, the molal volume of the solvent and the temperature, and \( V \) is the gm. solvent. It has been shown by Kunitz (7) that the osmotic pressure of gelatin solutions also obeys this law when the proper correction is made for the amount of solvent combined with the gelatin. The relation is rather complicated, however, and it is more convenient to use an empirical relation. It may be seen from Fig. 1, that at concentrations of between .1 and .4 gm. gelatin per gm. of water, the swelling pressure curve may be represented by the equation \( P = \frac{1330g - 140v}{v} \)

where \( g \) is the gm. gelatin and \( v \) the gm. water or \( P = \frac{1330 - 140V}{V} \)

where \( V \) is the gm. water per gm. gelatin.

The bulk modulus of an elastic body by definition is proportional to the elastic force divided by the difference between the volume when under no strain and the volume under the force applied. Or in terms of the original volume, it is the force required to increase the volume by an amount equal to the original volume.

\[ K_s = \frac{EP V_f}{V - V_f} \text{ and } \frac{EP}{V_f} = \frac{K_s (V - V_f)}{V_f} \]

where \( V_f \) is the volume when under no strain and \( K_s \) is the bulk modulus. Substituting these values of \( \text{OP} \) and \( \text{EP} \) in (1)

\[ \frac{1330 - 140V_s}{V_s} = \frac{K_s (V_s - V_f)}{V_f} \]
where \( V_e \) is the volume at equilibrium or

\[
K_e = \frac{1330(1 - 1.05V_e)}{(V_e - V_f) V}
\]

\[
V_f = \frac{K_e V^2_e}{1330 + V_e(K_e - 140)}
\]

\[
V_e = \frac{(K_e - 140)V_f}{2K_e} \pm \sqrt{\frac{1330V^2_f}{K_e} + \left[ \frac{(K_e - 140)V_f}{2K_e} \right]^2}
\]

Ordinarily the total volume would be used in the calculation of the bulk modulus but since in the case of the osmotic pressure it is better to use the volume of water rather than the total volume, the calculation is simplified by using the same value in the bulk modulus calculation. The use of the total volume instead of the volume of water would simply result in a different value for the bulk modulus. It may be noted that the volume of dry gelatin, however, cannot be used, as was done in an earlier paper since in that case the formula would predict that the swelling would depend only on the concentration of the block, which is not the case. The determining factor is the concentration at the time the solution solidified and not the concentration at the time it happened to be put in the water. In other words, as with any elastic body, it is necessary to define the change in volume as the change from the volume when under no elastic strain.

### TABLE I.

<table>
<thead>
<tr>
<th>( V_f )</th>
<th>( V_e )</th>
<th>( K_e )</th>
<th>( K_e \times .77 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H_2O ) per gm. gelatin when cast</td>
<td>( H_2O ) per gm. gelatin after swelling</td>
<td>( K_e \times .77 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Observed</td>
<td>Calculated ( K_e = 500 )</td>
<td></td>
</tr>
<tr>
<td>2.35</td>
<td>3.5 ± .1</td>
<td>3.48</td>
<td>490</td>
</tr>
<tr>
<td>3.35</td>
<td>4.3 ± .2</td>
<td>4.40</td>
<td>550</td>
</tr>
<tr>
<td>5.0</td>
<td>5.7 ± .2</td>
<td>5.86</td>
<td>670</td>
</tr>
<tr>
<td>5.8</td>
<td>6.8 ± .3</td>
<td>6.56</td>
<td>350</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>500</td>
</tr>
</tbody>
</table>

Ordinarily the total volume would be used in the calculation of the bulk modulus but since in the case of the osmotic pressure it is better to use the volume of water rather than the total volume, the calculation is simplified by using the same value in the bulk modulus calculation. The use of the total volume instead of the volume of water would simply result in a different value for the bulk modulus. It may be noted that the volume of dry gelatin, however, cannot be used, as was done in an earlier paper since in that case the formula would predict that the swelling would depend only on the concentration of the block, which is not the case. The determining factor is the concentration at the time the solution solidified and not the concentration at the time it happened to be put in the water. In other words, as with any elastic body, it is necessary to define the change in volume as the change from the volume when under no elastic strain.
As will be shown later this "elastic volume" is the actual volume when the solution solidified.

A number of experiments were made as previously described and the average value of \( K_e \) calculated. The results are given in Table I. \( K_e \) is expressed in mm. mercury pressure. The values of \( K_e \) vary somewhat but the equation is of such a form that the value of this constant is very sensitive to small differences in \( V_e \). The value is really constant for the range of gelatin used as may be seen by the fact that the values of \( V_e \) calculated by assuming a constant value for \( K_e \) are equal, within the probable error of the measurement, to the observed values. If the value of \( K_e \) be defined as the force required to increase the volume by an amount equal to the volume of the dry gelatin in the block rather than the volume of water, then the value given must be multiplied by the ratio of the volume of dry gelatin to that of the water. Since 1 gm. of gelatin occupies about .77 cc., the pressure required to increase the block by an amount equal to the volume of the dry gelatin contained in it will be \( K_e \times .77/V_e \). This value decreases in proportion to the concentration of gelatin, as would be expected. The same relation had been found by Sheppard (8) to hold approximately for the relation of modulus of elasticity to the concentration of the gelatin.

Since these experiments were made with gelatin on glass the gelatin is prevented from elongating by the glass rod. It might be expected therefore that gelatin alone would swell more. This is the case as shown by Table II which gives the equilibrium concentration for

### Table II

<table>
<thead>
<tr>
<th></th>
<th>Thin sheets cut from cylinder</th>
<th>Spheres</th>
<th>On slides</th>
<th>On glass rods</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_e )</td>
<td>7.4</td>
<td>7.3</td>
<td>370</td>
<td>6.7</td>
</tr>
<tr>
<td>( K_e \times .77/V_e )</td>
<td>140</td>
<td>130</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>( V_e )</td>
<td>5.8</td>
<td>6.7</td>
<td>350</td>
<td>6.8</td>
</tr>
</tbody>
</table>

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gelatin on slides, in the form of spheres or as thin pieces cut from a cylinder. It may also be noted that the value for the bulk modulus calculated on the basis of the volume of the dry gelatin agrees with that previously calculated indirectly from separate swelling and osmotic pressure experiments (9).

It has been assumed in these calculations that the gelatin when solidified is under no elastic strain and that this concentration is therefore the determining one for the swelling. As a corollary gelatin, the concentration of which has been changed after solidifying, is under elastic strain. It might be expected then that gelatin under these conditions would show the phenomenon of fatigue in common with other elastic bodies. That this is true is shown in Fig. 3. This experiment shows the result of keeping gelatin varying lengths of time at 20° after having been swollen or dried while solid. Curves 1 and 3 show that gelatin which has been allowed to solidify at 5° undergoes no change when kept in air at 20°. The swelling which occurs on replacing in water at 5° is the same whether or not the gelatin has been kept at 20°. Gelatin which has swollen at 5° before being placed at 20°, swells further on replacing at 5°, the amount of swelling depending upon the length of time the gelatin had been at 20°.
After the first 2 hours at 20° further exposure to this temperature causes no further change in the behavior of the gelatin. The swelling now is the same as that of freshly solidified gelatin of the same concentration. Gelatin which has been partially dried shows the same behavior in that it swells much less after exposure to 20° and the swelling soon reaches a constant value. This loss of elasticity occurs very rapidly at 20° but is much slower at 5°. This is shown by Fig. 4. In this experiment, three series of rods coated with 30 per cent gelatin were allowed to swell to the equilibrium value at 5°. All but two were then removed and placed in air at 5°. They were then returned to the water after varying periods of time. In the meantime the two rods which had been left in water continued the slow secondary swelling. On returning the other rods to water they swelled rapidly, the more so the longer the time since the beginning of the experiment, and very rapidly approached the value of those that had been in the water continuously. This shows that the secondary swelling is due to the loss of elasticity and that it is continually going on. As stated before a large block will therefore not show any maximum value since the

![Fig. 4. Effect of time at 5° in air, after swelling, on subsequent swelling when the gelatin is replaced in water.](image)
outside layers become fatigued and take in additional water before the internal layers have reached the equilibrium value. The same process will occur much more rapidly at a higher temperature so that even small blocks show no maximum. This secondary swelling will evidently continue until all the water has been taken up. If sufficient water is present a solution of gelatin will eventually result.

It may be noted that the secondary swelling in Fig. 4 is proportional to the square root of the time. The significance of this will be discussed under the kinetics of the process.

SUMMARY.

The swelling of isoelectric gelatin in water has been found to be in agreement with the following assumptions.

Gelatin consists of a network of insoluble material containing a solution of a more soluble substance. Water therefore enters owing to the osmotic pressure of the soluble material and thereby puts the network under elastic strain. The process continues until the elastic force is equal to the osmotic pressure. If the temperature is raised or the blocks of gelatin remain swollen over a period of time, the network loses its elasticity and more water enters. In large blocks this secondary swelling overlaps the initial process and so no maximum can be observed.

The swelling of small blocks or films of isoelectric gelatin containing from .14 to .4 gm. of dry gelatin per gm. of water is defined by the equation

\[ V_0 = \frac{(K_x - 140)}{2 K_x} V_f \pm \sqrt{\frac{1330 V_f}{K_x} + \left[ \frac{K_x - 140}{2 K_x} \right]^2} \]

in which \( K_x \) = the bulk modulus \( = \frac{P V_f}{(V_s - V_f)} = \frac{1330(1 - .105 K_x)}{(V_s - V_f)} \)

\( V_s \) = gm. water per gm. gelatin at equilibrium; \( V_f \) = gm. water per gm. gelatin when the gelatin solidified.

BIBLIOGRAPHY.