DISTRIBUTION OF HYDROCHLORIC ACID IN GELATINE GELS

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In a recent series of publications (1), E. J. Bigwood has observed the presence of a permanent concentration gradient of hydroxyl ion when, under suitable conditions, dilute aqueous sodium hydroxide diffuses into a gelatine gel. We sought to reproduce similar concentration gradients and to measure them quantitatively in order to find conditions under which we could test the theoretical expressions derived by F. G. Donnan (2) for such a system. This theory presupposes a gradient of protein micelle concentration, naturally or artificially formed. We thought that the phenomenon described by Bigwood ought to satisfy these conditions but our attempts produced no system that could not, in our opinion, be satisfactorily explained by the laws of simple diffusion. There was nothing to indicate the presence of a protein concentration gradient.

In our experiments Coignet's Gold Label gelatine was used, purified by electrolysis (3) (ash content 0.01-0.02 per cent), and containing traces of thymol. 9 cc. columns of 3 per cent, 5 per cent, and 8 per cent gels containing equal amounts of brom-phenol blue were cast in test-tubes and covered with 5 cc. of 0.015 N HCl and 1 cc. of toluene. The tubes were then sealed and kept in the refrigerator. A control tube containing water instead of the acid showed no color change in the indicator throughout the experiment. The distance diffused, \( d \), was measured as cc. from the top of the gel to the boundary of the indicator change. After about the fifteenth day readings become less sharply defined because of the diffuseness of the zone of color change. From Fig. 1 it will be seen that \( \frac{d}{\sqrt{t}} \), which would be a constant in ordinary diffusion, is here a decreasing quantity. This might suggest an approach to a stable concentration gradient within the gel at equilibrium.

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However, in 3 weeks the indicator in the 3 per cent gel was yellow throughout, in 2½ months the color of the 5 per cent gel was uniform throughout but corresponded to a pH of about 4, i.e., within the range of the indicator. The 8 per cent gel still had a yellow, very diffuse zone at the top.

We repeated these experiments using 0.01 N HCl whose concentration was kept constant. For this purpose a gently flowing reservoir and constant level siphon system was used. It will now be observed that \( \frac{d}{\sqrt{t}} \) remains constant within experimental error (Fig. 2). Sobotka and Sabin (4) have also found that the constant gives lower values at the beginning of the experiment. If an equilibrium were being approached, one would expect \( \frac{d}{\sqrt{t}} \) to decrease continuously. The
decreasing values for the first experiments can, however, be explained as in simple diffusion by the exhaustion of the diffusing substance. Much more precise forms of the equation \( \frac{d}{\sqrt{t}} = k \) have been formulated by Adair, Stiles, and others (5), but it was not thought necessary to use them here.

It will thus be seen that all the conditions imposed in Bigwood's experiments would lead one to expect an extreme case of slow diffusion which might be easily mistaken, with relatively crude measuring methods, for a stationary system. His rather concentrated gels (8 per cent) were in contact with a limited amount of very dilute alkali (usually less than 0.004 N) whose pH, as he shows, was continuously decreasing. No mention was made of a preliminary purification of the gelatine which ordinarily contains 2–3 per cent ash. The active concentration of the alkali is further decreased by combination with the gelatine.* Sobotka and Sabin (4) have demonstrated the influence of this factor on speed of diffusion. Using the average value for the diffusion constant found in these experiments for an 8 per cent gel

* I cannot agree with Bigwood's conclusion that the gelatine in an 8 per cent gel is a negligible source of neutralisation for diffusing OH⁻ ions when the pH of the outside solution is equal to or greater than 10 (Reference 1c, p. 711).
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in contact with continuously renewed 0.01 N HCl, calculations show that it would take 3½ months for the indicator to change throughout the 9 cm. column and that toward the end the boundary, if clear, would take 2 weeks to move 0.2 cm. It is not remarkable, then, that this should appear to be a system in stationary equilibrium.

The fact that dilute acid was used in these experiments instead of alkali should not affect the results as long as the gels were not, at the end, at the isoelectric point but within the zone of color change of the chosen indicator.

In reference to his more recent experiments Bigwood points to the shifting of ion concentration, due perhaps to the liberation of acid valency in proportion to the evolution of the gel structure. In our opinion, this reversal of the various pH niveaux after a time can be explained by an examination of the ordinary form of diffusion curves for different concentrations (Fig. 3). If the concentration of diffusing substance were originally A and gradually dropped to B, the concentration at any one point in the gel would return to the value for the curve for B at that time. At a certain time this difference in concentration would mean a return to the original color of the indicator.

In any case, a reversal of the zone of color change after replacing the supernatant solution with water does not prove that a slow diffusion had not been taking place toward the interior of the gel. It may be a question of simply varying the relative intensity of diffusion in each direction.

![Diagram](image-url)
The theory of the existence of a permanent concentration gradient of hydrogen ion is based on the assumption of a permanent gradient for protein micelles. Light-scattering experiments, carried on by and with the kind assistance of Dr. K. Krishnamurti in these laboratories, do not indicate such a gradient under the given conditions. When he allowed uniform gelatin gels to imbibe small amounts of distilled water and measured the light-scattering power at various heights in the column, he found that it became identical throughout if sufficient time were allowed. Similarly, he measured 5 cm. columns of 3 per cent isoelectric gelatine in contact with 8.5 cc. 0.3 N HCl. The change in turbidity was the same throughout the gel in 2 weeks. The greater concentration of acid used in this investigation allowed equilibrium to be reached in a shorter time but the method is sensitive enough to detect any appreciable gradient (6).

Since we were unconvinced that permanent concentration gradients could be obtained in this way, we prepared one by layering equal amounts of gelatin sols of varying concentrations upon each other in a test-tube, being careful to avoid air-gaps or sharp interfaces within the gel. There was a uniform concentration of brom-phenol blue in the column which was then covered with 10 cc. 0.03 N HCl. This was renewed in 4 days and removed in 11 days. After 4 1/2 months a color gradient still persisted within the gel, the hydrogen ion concentration not having distributed itself evenly as had been the case with uniform gels under similar conditions. An attempt was made to test the conclusion that, with a protein concentration gradient and a diffusing electrolyte, the concentration of each ion would also be a gradient but in opposite directions. This is expressed by Donnan (2) in the following differential equations:

\[
\frac{dA}{dx} = \frac{c(A^-)}{2(1 + bh) + cp + \frac{cp}{1 + bh}}, \frac{dp}{dx}
\]

and

\[
\frac{dA^+}{dx} = \frac{c(A^+)}{2(1 + bh) + cp + \frac{cp}{1 + bh}}, \frac{dp}{dx}
\]
where \( h \) and \( A^- \) are the concentrations of the free cations and anions respectively of the added electrolyte at place \( x \) in the gel, \( c \) and \( b \) are constants in the simple Langmuir equation for the reversible adsorption of cations, and \( p \) is the micelle concentration. Therefore, if \( \frac{dp}{dx} > 0 \), then \( \frac{dh}{dx} < 0 \) and \( \frac{d[A^-]}{dx} > 0 \).

After 4½ months the gel was removed by cracking the tube, divided into sections, and analyzed. The indicator showed a pH increasing gradually from Sections 1 to 9. Table I gives the results of analysis.

Of course, a gradient of protein concentration does not necessarily mean a gradient of micelle concentration but it is unnecessary to repeat here the simplifying assumptions that were used as the basis and limitations of Donnan's theory.

It will be seen that the conclusions drawn by Donnan are verified

<table>
<thead>
<tr>
<th>No.</th>
<th>Weight of gel (gm.)</th>
<th>Concentration gelatine (gm. per cent)</th>
<th>Cl⁻ (total) (mg.)</th>
<th>Cl⁻ per gm. gel (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.698</td>
<td>1.89</td>
<td>1.115</td>
<td>0.408</td>
</tr>
<tr>
<td>2</td>
<td>3.925</td>
<td>1.87</td>
<td>1.503</td>
<td>0.399</td>
</tr>
<tr>
<td>3</td>
<td>3.865</td>
<td>2.07</td>
<td>1.752</td>
<td>0.445</td>
</tr>
<tr>
<td>4</td>
<td>3.497</td>
<td>2.23</td>
<td>1.752</td>
<td>0.493</td>
</tr>
<tr>
<td>5</td>
<td>2.756</td>
<td>2.54</td>
<td>1.381</td>
<td>0.494</td>
</tr>
<tr>
<td>6</td>
<td>4.629</td>
<td>....</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>7</td>
<td>2.967</td>
<td>4.28</td>
<td>2.036</td>
<td>0.673</td>
</tr>
<tr>
<td>8</td>
<td>4.527</td>
<td>....</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>9</td>
<td>1.943</td>
<td>6.49</td>
<td>1.540</td>
<td>0.780</td>
</tr>
</tbody>
</table>

The last column is corrected for the small blank on the gelatin. The chlorides were estimated by a method similar to that given by Van Slyke, D. D., *J. Biol. Chem.*, 1923, 58, 52.
in a general way, since the anion (Cl\(^-\)) concentration varies in the same direction as the protein concentration, while the cation (H\(^+\)) varies in the opposite direction.

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BIBLIOGRAPHY


