THE COMBINATION OF GELATIN WITH HYDROCHLORIC ACID.

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Titration curves for gelatin with different acids have been obtained by Loeb, and used to show that the protein reacts stoichiometrically with the different acids. The amount of a strong acid combined with the protein may be obtained from the titration curve (in which the abscissae are pH values and the ordinates concentrations of acid) by subtracting from the ordinates the amounts of acid necessary to bring the same volume of water, without protein, to the same pH values. In this way Loeb obtained a curve for the amount of HCl combined with 1 gm. of gelatin at different pH values. Similar curves were obtained by Tague for the combination of amino-acids with NaOH, and by the writer for the combination of edestin with acids. Each of these combination curves appeared to become horizontal beyond a certain pH; i.e., after enough acid (or alkali, in the case of Tague's experiments) had been added, the amount combined with the ampholyte became constant.

In a recent paper by Lloyd and Mayes a curve is given to represent the amount of HCl combined with 1 gm. of gelatin. This curve, however, does not become horizontal, but rises with increasing acidity in a rather discontinuous manner. These authors did not obtain the amount of combined HCl in the way just described, but calculated it by the formula

\[ n' = n - \frac{[H^+]_{corr.}}{\alpha} \]

where \([H^+]_{corr.} = \sqrt{[H^+][Cl^-]}\),

1 Loeb, J., J. Gen. Physiol., 1920-21, iii, 100.
N = normality of total HCl, a its degree of ionization, and N' = normality of combined HCl. The values which they used for the degree of ionization were conductivity ratios given by Lewis. Inasmuch as the results of Lloyd and Mayes differed from those obtained by Loeb, it seemed worth while to repeat and amplify his experiments.

Solutions were made up containing 1, 2.5, and 5 gm. of gelatin in 100 cc. of HCl of various concentrations. The gelatin was taken from a stock solution of isoelectric gelatin which had been rendered practically ash-free in the way described by Loeb, and was diluted to twice the concentration required for each set of experiments. 25 cc. samples of these solutions were measured out at 33°C. by a pipette, and each sample was diluted to 50 cc. by the addition of the proper amounts of 0.1 or 1.0 HCl and water from burettes. The concentration of the gelatin was checked by dry weight determinations of the amount of gelatin delivered by the 25 cc. pipette, and was accurate to about 1 part in 200. The concentration of the acid used was determined by titration against pure NaCO₃, and was accurate to 1 part in 500 or better. The pH of the solutions was determined at 33°C with the hydrogen electrode and potentiometer, using rocking cells of the Clark type connected by a salt bridge of saturated KCl to a saturated KCl calomel cell. The pH values are referred to 0.1000 m HCl as a standard, its pH being taken as 1.036 at 33°C. The E.M.F. readings obtained were reproducible to within 1 millivolt, which corresponds to about 0.02 pH.

The titration curves obtained in this way are given in Fig. 1, the abscissae being pH values, and the ordinates total concentration of HCl expressed in millimoles per liter, which is the same as cc. of 0.1 m acid per 100 cc. The curve for 1 per cent gelatin represents three experiments, one of which was performed by Mr. M. Kunitz.

In order to determine how much of the total HCl was not combined with the gelatin, a series of solutions containing only HCl and water was prepared, and the pH values were determined. The values are given in Table I.


### TABLE I.

**Titration of Water with Hydrochloric Acid at 33°C.**

<table>
<thead>
<tr>
<th>Concentration of HCl, per liter, millimoles</th>
<th>0.05</th>
<th>0.10</th>
<th>0.19</th>
<th>0.39</th>
<th>0.68</th>
<th>0.97</th>
<th>1.98</th>
<th>3.89</th>
<th>8.75</th>
<th>15.56</th>
<th>24.31</th>
<th>48.63</th>
<th>100.00</th>
<th>117.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.35</td>
<td>4.07</td>
<td>3.74</td>
<td>3.38</td>
<td>3.17</td>
<td>3.00</td>
<td>2.70</td>
<td>2.41</td>
<td>2.06</td>
<td>1.81</td>
<td>1.63</td>
<td>1.33</td>
<td>1.04</td>
<td>0.99</td>
</tr>
</tbody>
</table>

### TABLE II.

**Titration of Gelatin with Hydrochloric Acid.**

<table>
<thead>
<tr>
<th>pH of 1 per cent gelatin chloride*, millimoles</th>
<th>1.25</th>
<th>1.50</th>
<th>1.75</th>
<th>2.00</th>
<th>2.25</th>
<th>2.50</th>
<th>2.75</th>
<th>3.00</th>
<th>3.25</th>
<th>3.75</th>
<th>4.00</th>
<th>4.25</th>
<th>4.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total HCl per liter, millimoles</td>
<td>69.5</td>
<td>41.3</td>
<td>37.6</td>
<td>19.5</td>
<td>14.7</td>
<td>11.8</td>
<td>9.8</td>
<td>8.3</td>
<td>6.8</td>
<td>5.5</td>
<td>4.3</td>
<td>3.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Uncombined HCl, millimoles</td>
<td>59.0</td>
<td>33.1</td>
<td>18.0</td>
<td>10.0</td>
<td>6.6</td>
<td>3.1</td>
<td>1.7</td>
<td>1.0</td>
<td>0.5</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Combined HCl per 10 gm. gelatin, millimoles</td>
<td>10.5</td>
<td>8.2</td>
<td>9.5</td>
<td>9.5</td>
<td>8.1</td>
<td>8.1</td>
<td>8.1</td>
<td>7.3</td>
<td>6.3</td>
<td>5.2</td>
<td>4.1</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>pH of 2.5 per cent gelatin chloride, millimoles</td>
<td>1.00</td>
<td>1.18</td>
<td>1.38</td>
<td>1.63</td>
<td>1.78</td>
<td>1.91</td>
<td>2.14</td>
<td>2.32</td>
<td>2.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total HCl per liter, millimoles</td>
<td>131.3</td>
<td>93.8</td>
<td>65.7</td>
<td>46.9</td>
<td>40.8</td>
<td>35.0</td>
<td>30.2</td>
<td>27.2</td>
<td>24.3</td>
<td></td>
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<tr>
<td>Uncombined HCl, millimoles</td>
<td>114.0</td>
<td>70.5</td>
<td>43.5</td>
<td>24.3</td>
<td>16.7</td>
<td>12.3</td>
<td>7.3</td>
<td>4.8</td>
<td>2.6</td>
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<tr>
<td>Combined HCl per 25 gm. gelatin, millimoles</td>
<td>17.3</td>
<td>23.3</td>
<td>22.2</td>
<td>22.6</td>
<td>24.1</td>
<td>22.7</td>
<td>22.9</td>
<td>22.4</td>
<td>21.7</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Combined HCl per 10 gm. gelatin, millimoles</td>
<td>6.9</td>
<td>9.3</td>
<td>8.9</td>
<td>9.0</td>
<td>9.3</td>
<td>9.1</td>
<td>9.2</td>
<td>9.0</td>
<td>8.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH of 5 per cent gelatin chloride, millimoles</td>
<td>1.03</td>
<td>1.37</td>
<td>1.61</td>
<td>1.80</td>
<td>1.95</td>
<td>2.28</td>
<td>2.60</td>
<td>2.64</td>
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<tr>
<td>Total HCl per liter, millimoles</td>
<td>145.0</td>
<td>86.6</td>
<td>71.5</td>
<td>62.1</td>
<td>56.5</td>
<td>50.8</td>
<td>47.1</td>
<td>45.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncombined HCl, millimoles</td>
<td>103.0</td>
<td>44.4</td>
<td>25.5</td>
<td>15.9</td>
<td>11.2</td>
<td>5.3</td>
<td>2.5</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined HCl per 50 gm. gelatin, millimoles</td>
<td>42.0</td>
<td>42.2</td>
<td>46.0</td>
<td>46.2</td>
<td>45.3</td>
<td>45.5</td>
<td>44.6</td>
<td>42.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined HCl per 10 gm. gelatin, millimoles</td>
<td>8.4</td>
<td>8.4</td>
<td>9.2</td>
<td>9.2</td>
<td>9.1</td>
<td>8.9</td>
<td>8.6</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* The figures for 1 per cent gelatin chloride were obtained from the curve averaging three experiments.
These results were plotted on a large scale and a smooth curve was drawn through the points. From this curve were read off the concentrations of free HCl present at the pH actually measured in each gelatin chloride solution, and these values were subtracted from the total HCl concentrations of the respective gelatin chloride solutions. The differences accordingly represent the millimoles of HCl combined with the amount of gelatin present in 1 liter, or the cc. of 0.1 M HCl combined with the gelatin present in 100 cc. These values were divided by the number of grams of gelatin in 100 cc. to get the cc. of 0.1 M HCl combined with 1 gm. of gelatin, which is the same as the number of millimoles of HCl combined with 10 gm. of gelatin. Table II indicates the method of calculation, and the final results are plotted in Fig. 2.

It will be noticed that the points lie fairly close to a smooth curve, except in the most acid region, where a small error in the pH may lead to a large error in the difference between the ordinates of two steep curves. The curve is horizontal between pH 1 and 2, indicating that here the gelatin is all combined with the acid. There is no evidence of the discontinuous sections found in the curve of Lloyd and Mayes. This difference is due in part to differences in the experimental curves, but is also due largely to the method of calculation of the combined acid. The method used by Lloyd and Mayes involves the assumption that the uncombined HCl is ionized to the extent indicated by the conductivity ratio for a different concentration of HCl; namely, the concentration of the total HCl present. Moreover, these authors have neglected the difference between the conductivity ratios and the activity coefficients or hydrogen electrode values for HCl, which is clearly brought out by the table given by Lewis from which they obtained their ionization values. The method of calculation used by Loeb, Tague, and the present writer involves the assumption that the same concentration of uncombined HCl is necessary to furnish the same hydrogen ion concentration, as determined by the hydrogen electrode, whether or not gelatin is present. The latter assumption seems to lead to more reasonable results.

The maximum height of the curve in Fig. 2, 9.2 millimoles of HCl for 10 gm. of gelatin, indicates that a 1 per cent gelatin solution has a
normality of 0.0092 with respect to its combination with HCl, or that the combining weight of gelatin is \( \frac{16}{0.0092} \), or about 1,090. While the exact height of the maximum is still more or less uncertain, it is probable that this value of the combining weight is more nearly correct than the smaller values given by Procter, Wilson, Wintgen and Krüger, and Wintgen and Vogel, because the calculation involves simpler and more probable assumptions. Moreover, the earlier workers did not have ash-free or isoelectric gelatin at their disposal.

It is possible to calculate from these results an ionization constant for gelatin, assuming it to react as a mono-acid base. The simplest way of doing this is based on the resemblance between the combination curve and the dissociation curve of a base. The equation for the ionization of such a base is:

\[ \alpha = \frac{k_b}{k_b + [OH^-]} \]

where \( \alpha \) represents the fraction ionized and \( k_b \) the ionization constant. Since the ion product of water, \( k_w \), is equal to \([H^+] \times [OH^-]\), this becomes

\[ k_w = k_b \]

Michaelis has pointed out that if \( \alpha \) is plotted against \( \log [H^+] \), at the point where \( \alpha = \frac{1}{2} \), \( \log [H^+] = \log K \). Applying this to Fig. 2, \( \alpha = \frac{1}{2} \) where the ordinate = 4.6 or \( pH = pK = 3.625 \). Accordingly \( K = 2.4 \times 10^{-4} \). This is of the same order as the value obtained by Procter and Wilson and is intermediate between the values obtained by Wintgen and Krüger and Wintgen and Vogel, but differs by a whole power of 10 from that obtained by Lloyd and Mayes. Inasmuch as the use of this constant leads to only rough agreement with the combination curve in Fig. 2, it is not desired to lay any stress on this calculation. It is quite certain that gelatin is not a mono-acid base, though its combination curve may resemble the ionization curve of such a base.

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SUMMARY.

The amount of HCl combined with a given weight of gelatin has been determined by hydrogen electrode measurements in 1 per cent, 2.5 per cent, and 5 per cent solutions of gelatin in HCl of various concentrations, by correcting for the amount of HCl necessary to give the same pH to an equal volume of water without protein. The curve so obtained indicates that the amount of HCl combined with 1 gm. of gelatin is constant between pH 1 and 2, being about 0.00092 moles.

The writer wishes to express his gratitude for the advice of Dr. Jacques Loeb, under whose direction this work was done.