Summary

1. A cell system is described which allows a close approximate measurement of diffusion potentials.

2. Reproducible diffusion potentials which show but small "time effects" have been measured with hydrochloric acid systems, hydrochloric acid–sodium chloride systems and hydrochloric acid–gelatin systems.

3. The reaction between hydrochloric acid and gelatin appears to be stoichiometric, purely chemical in nature.

4. A new and unusual method for determining the combining capacity of gelatin for hydrochloric acid is given. Four independent boundary measurements give the equivalent weight of gelatin as close to 1090.

Work of this nature is to be continued with di- and tri-basic acids and bases with gelatin, also for similar systems in which gelatin will be replaced by other proteins.

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DIFFUSION-POTENTIAL MEASUREMENTS APPLIED TO HYDROCHLORIC ACID-GELATIN SYSTEMS. II. THE COMPONENTS OF HYDROCHLORIC ACID-GELATIN SOLUTIONS[1]

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A previous article[2] has shown that the equivalent weight of gelatin can be obtained from diffusion-potential measurements on hydrochloric acid-gelatin systems and that the reaction between hydrochloric acid and gelatin appears to be a stoichiometric one. This article explains the irregularities that were shown in some of the previous measurements[3] and gives a more complete understanding of the conditions as existing in the various boundaries and solutions. All references to curves of Figs. 3 and 4 in this article refer to the same figures of the previous article.

The Effect of Uncombined Gelatin at the Diffusion Boundary

From comparison of curves in Fig. 3 with those in Fig. 4 it will be seen that the hydrochloric acid-gelatin solutions were not exactly similar to the hydrochloric acid-sodium chloride mixtures, as gelatin was added

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[1] The material presented in this article is constructed from a portion of the thesis submitted to the Graduate School of the University of Michigan by Egbert K. Bacon, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Ferguson and Bacon, THIS JOURNAL, **49**, 1921 (1927).

[3] Portions of Curve E_2(G) were concave upward, while similar portions of Curve E_2 were concave downward. Large "time effects" were observed in certain regions of curves E_1(G), E_2(G) and E_3(G).

to the hydrochloric acid without regard to the amount needed for complete combination. (In fact, this amount was not known until after the experiments were carried out and the equivalent weight was determined.) Thus it happened that in many cases there was uncombined gelatin present in the acid-gelatin mixtures.

With the hydrochloric acid-sodium chloride mixtures free sodium hydroxide was never present. If sodium hydroxide had been added to the 0.01 N hydrochloric acid after complete neutralization had occurred (which corresponds to the regions beyond the maximum point b on Curve E_2), the condition at the boundary would have been

$$(0.10 - x)\ N\ \mathrm{HCl},\ x\ N\ \mathrm{NaCl} \mid 0.01\ N\ \mathrm{NaCl},\ y\ N\ \mathrm{NaOH} \qquad (1)$$

At such a diffusion boundary there is a new source of potential, that between x N hydrochloric acid and y N sodium hydroxide which, according to Planck,[4] would be largely indeterminate because chemical action between sodium hydroxide and hydrochloric acid takes place at the boundary.

This appears to be similar to the condition which resulted in the b c region of Curve E_2(G). The point b on the curve corresponds to a condition of complete combination between gelatin and 0.01 N hydrochloric acid so that instead of writing the boundary as

$$0.10\ N\ \mathrm{HCl},\ x\%\ \text{gel.} \mid 0.01\ N\ \mathrm{HCl},\ x\%\ \text{gel.} \qquad (2)$$

a more exact condition is given as

$$(0.10 - x)\ N\ \mathrm{HCl},\ y\ \%\ \text{combined gel.} \mid 0.01\ N\ \text{combined gel.},\ z\ \%\ \text{uncombined gel.} \qquad (3)$$

Thus, there was free hydrochloric acid and uncombined gelatin in contact at the boundary, until complete combination between the 0.10 N hydrochloric acid and gelatin had occurred, that is, a concentration of 10.9% of gelatin.

"Corrected" Curve for Boundary E_2(G)

If the above is the explanation of the real conditions existing at the contact boundary in the region b c of Curve E_2(G), then a boundary such as

$$(0.10 - x)\ N\ \mathrm{HCl},\ y\%\ \text{combined gel.} \mid 0.01\ N\ \text{combined gel.} \qquad (4)$$

should correspond more nearly to the condition of the same region on curve E_2 of the hydrochloric acid-sodium chloride system,

$$(0.10 - x)\ N\ \mathrm{HCl},\ x\ N\ \mathrm{NaCl} \mid 0.01\ N\ \mathrm{NaCl} \qquad (5)$$

Accordingly, boundaries such as that given in (4) were measured. The results of these "corrected" measurements are given in Fig. 1 A, corresponding to 1.75, 4.37, 8.74 and 10.49% gelatin.

The path of the curve $b'c'$ between 1.09% of gelatin and 10.9% of gelatin is now concave downward, the same as in Curve E_2. It converges to the same point c', as does Curve E_2(G), when complete combination

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[4] Planck, *Wied. Ann.*, **40**, 561 (1890).

between 0.10 N hydrochloric acid and gelatin has taken place (10.9% of gelatin). This is further proof that the equivalent weight of gelatin is close to 1090, as shown in the previous article.

To show that uncombined gelatin present in the left-hand side of the boundary would have no appreciable effect on the potential after all of the free hydrochloric acid had combined with the gelatin, a boundary of the following type was measured.

$$0.10\ N\ \mathrm{HCl},\ 12.24\%\ \mathrm{gel.}\ |\ 0.01\ N\ \mathrm{combined\ gel.} \quad (6)$$

This should give a potential similar to a boundary such as

$$0.10\ N\ \mathrm{HCl},\ 12.24\%\ \mathrm{gel.}\ |\ 0.01\ N\ \mathrm{HCl},\ 12.24\%\ \mathrm{gel.} \quad (7)$$

since there is no free hydrochloric acid present in either system, but only combined and uncombined gelatin.

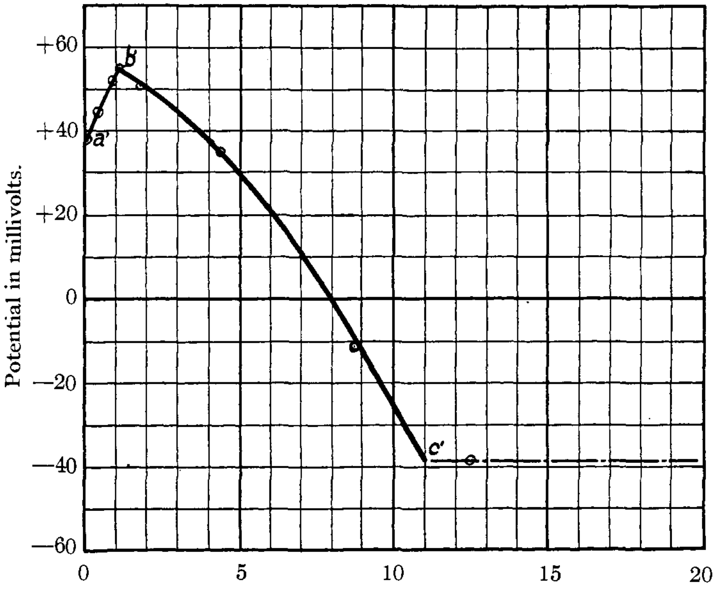

Fig. 1A.—$E_2(G)$, 0.01 N HCl + x% gelatin | 0.01N HCl + y% gelatin, where x varies from 0 to 10.9 and y varies from 0 to 1.09.

The potentials of these two boundaries were —37.69 mv. and —39.79 mv., respectively. This close agreement shows that an excess of gelatin beyond the amount required for complete combination with the acid present has no appreciable influence on the diffusion potential.

The Function of Free Hydrochloric Acid and Uncombined Gelatin at the Diffusion Boundary

From these considerations it seems clear that the difference in observed potentials, as shown by Curves E_2(G) and $2E_2$(G), between points b

and c and b' and c' is a function of the concentration of free hydrochloric acid on one side of the boundary and the concentration of the uncombined gelatin on the other. It is the presence of these components on opposite sides of the contact layer that is the cause of the differences in diffusion potentials.

This is illustrated more conclusively by the following considerations. Any point on the $b'c'$ region of Curve $2E_2$(G) represents the boundary condition as

$$(0.10 - x)\ N\ \text{HCl},\ y\ \%\ \text{combined gel.}\ |\ 0.01\ N\ \text{combined gel.} \qquad (8)$$

If z per cent. of iso-electric gelatin were added to the 0.01 N combined gelatin, any change resulting in the diffusion potential would be caused by the effect produced by the introduction of this excess of iso-electric gelatin. This is exactly the conditions that are represented in the region $b\ c$ of Curve E_2(G), only it gives the results of proportionally increasing amounts of uncombined gelatin in contact with decreasing amounts of hydrochloric acid. If one of these factors is kept constant, the effect produced by changing quantities of the other can be observed. A boundary of the type

$$0.10\ N\ \text{HCl},\ 1.75\%\ \text{gel.}\ |\ 0.01\ N\ \text{combined gel.},\ x\%\ \text{uncombined gel.} \qquad (9)$$

illustrates such a condition. Boundaries of this nature were measured.

The solution in the left-hand side contained 1.75% of gelatin in 0.10 N hydrochloric acid or 0.084 N hydrochloric acid plus combined gelatin. The composition of this solution was kept constant. Varying quantities of iso-electric gelatin (which remained in the uncombined state) were added to the combined gelatin solution on the other side of the boundary.

The results of these measurements are given graphically in Fig. 2A, Curve 2(G)A. The potential is plotted as a function of the value of x, and the values of x used are 0, 0.66, 1.53, 4.15, 5.03 and 11.25. The curve appears to reach a limiting value at a potential around +15 mv. Concentrations of gelatin beyond 5 or 6% produce little effect.

Curve 2(G)B represents a boundary of the type

$$0.10\ N\ \text{HCl},\ 5.24\%\ \text{gel.}\ |\ 0.01\ N\ \text{combined gel.},\ x\%\ \text{uncombined gel.} \qquad (10)$$

This is similar to Boundary 9 but represents a lower starting point on $b'c'$, that is, where the free hydrochloric acid on the left-hand side of the boundary has the lower concentration, 0.052. The values for x are 0, 4.15 and 5.9. Concentrations of gelatin beyond 4% show little effect on this boundary.

Curve 2(G)C shows the results when the free acid has the still lower concentration, 0.0084 N. Constancy in potential is reached between 1 and 2% of gelatin.

Curve 2(G)D shows a condition of constancy when there is no free hydrochloric acid left. Any concentration of gelatin now has no appreciable effect on the potential.

To show further the difference between the effect of combined and uncombined gelatin the following boundary was measured,

0.10 *N* HCl, 1.74% gel. | 4.37% solution *combined* gel. (11)

in which one solution represents 1.75% of gelatin in 0.10 *N* hydrochloric acid and the other a 4.37% solution of *combined* gelatin, based upon the combining weight 1090 for gelatin. The value —39.19 mv. was obtained for the potential of this boundary. A similar boundary which contained 4.37% of *iso-electric* gelatin in 0.01 *N* hydrochloric acid gave a value of —8.61 mv.

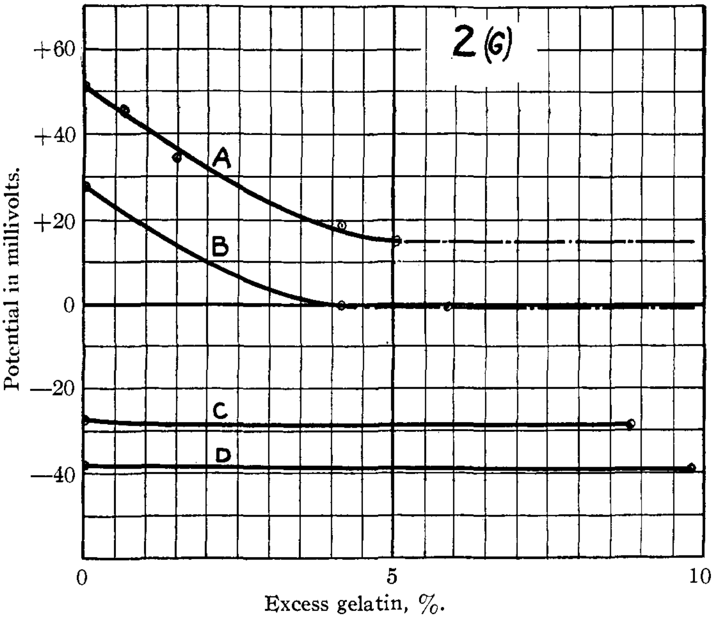

Fig. 2A.—The influence on diffusion potentials when uncombined gelatin is on one side of the boundary and free HCl on the other. Curve A, 1.7% gelatin, 0.084 *N* HCl; B, 5.2% gelatin, 0.052 *N* HCl; C, 10% gelatin, 0.008 *N* HCl; D, 10.9% gelatin, 0.000 *N* HCl.

Conclusions

The importance of defining hydrochloric acid-gelatin solutions in terms of free acid, combined and uncombined gelatin, is shown in these experiments. This can be done only if the stoichiometric character of the gelatin-acid combination is admitted and the equivalent weight of gelatin known. The understanding of the conditions existing in these hydrochloric acid-gelatin solutions and the cause of the observed potentials become clear when the following assumptions are made.[5]

(1) Iso-electric gelatin combines stoichiometrically with hydrochloric

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[5] These are essentially the same as those of Loeb and his co-workers.

acid, at least between concentrations of 0.10 *N* and 0.01 *N*. Other interpretations of the curves which have been discussed could hardly lead to other conclusions. Five independent boundary measurements establish the equivalent weight of gelatin as close to 1090.

(2) Iso-electric gelatin, although considered neutral, is able by its amphoteric character to combine with hydrochloric acid much in the same manner as hydrochloric acid combines with sodium hydroxide. A highly ionized salt, gelatin chloride, is formed which is dissociated into a gelatin cation and a chloride anion.

From a consideration of these assumptions the diffusion potentials of hydrochloric acid–gelatin systems become understandable. When the components of the solutions are gelatin chloride (combined gelatin) and free hydrochloric acid, the observed potentials are very much the same as if the components were hydrochloric acid and sodium chloride. The introduction of any uncombined gelatin at one of the diffusion boundaries where free hydrochloric acid is present results in interaction between hydrochloric acid and gelatin at the boundary layer, with the formation of gelatin chloride. Thus the diffusion layer would be largely indeterminate and the potential would have no specific value or precise meaning. Examination of the experimental data and curves prove this, as erratic results were obtained whenever free hydrochloric acid and uncombined gelatin were at the boundary. This is in accord with Planck's views that a diffusion potential which results at a boundary where there is chemical action is largely indeterminate.

Summary

Important conclusions resulting from this investigation may be summarized as follows:

1. The components of hydrochloric acid-gelatin solutions should be defined in terms of free acid, combined and uncombined gelatin.

2. Diffusion potentials of hydrochloric acid–gelatin systems are only understandable if the hydrogen chloride-gelatin combination results in the formation of highly-ionized gelatin chloride.

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