[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN]

DIFFUSION-POTENTIAL MEASUREMENTS APPLIED TO HYDROCHLORIC ACID–GELATIN SYSTEMS. I. THE EQUIVALENT WEIGHT OF GELATIN[1]

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RECEIVED MAY 5, 1927 PUBLISHED AUGUST 5, 1927

In this article an apparatus is described which permits the approximate measurement of a static liquid-junction potential between hydrochloric acid of two concentrations and of more complex systems of hydrochloric acid–sodium chloride. The measurements are also extended to systems of hydrochloric acid–gelatin. The striking similarity between these systems and those of hydrochloric acid–sodium chloride favors the establishment of gelatin-acid solutions as true solutions. The stoichiometric character of the combination between gelatin and acid is demonstrated and the equivalent weight of gelatin determined.

If two different electrolytic solutions are placed in contact, there is developed at the junction a potential difference which is called a diffusion potential. This potential is caused by the unequal migration velocities of the ions in the two solutions, that is, in simple electrolytes or mixtures of electrolytes the potentials are determined by the ionic activities and the ionic mobilities.

In a boundary such as

$$0.10\ N\ \text{HCl} \mid 0.10\ N\ \text{HCl} \qquad (1)$$

the potential is zero since identical solutions are on each side of the contact layer. If, however, sodium hydroxide replaces part of the hydrochloric acid in one of the solutions in such a manner that the mixture always has a constant concentration of 0.10 N, a potential develops which becomes increasingly greater as the solution ultimately approaches 0.10 N sodium chloride, since the hydrogen ion is being replaced by the slower-moving sodium ion.

If in an identical arrangement as the above gelatin is substituted for the sodium hydroxide, the resulting potentials should give some information as to the physical state of the resulting mixtures, that is, whether as Loeb and others have maintained, gelatin reacts stoichiometrically with hydrochloric acid, forming highly ionized gelatin chloride,[2] or whether an adsorption complex of some sort is formed,[3] or whether the type of

[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] (a) Loeb, "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Co., New York, **1922**. (b) Hitchcock, *J. Gen. Physiol.*, **5**, 383 (1923). (c) Procter, *J. Chem. Soc.*, **105**, 313 (1914).

[3] (a) Shukov and Shchukarev, *J. Phys. Chem.*, **29**, 285 (1925). (b) Alexander, "Glue and Gelatin," Chemical Catalog Co., New York, **1923**. (c) de Izaguirre, *Kolloid-Z.*, **32**, 47 (1923). (d) Bracewell, THIS JOURNAL, **41**, 1511 (1919).

combination might be due partly to purely chemical forces and partly to forces of adsorption, as Hoffman and Gortner suggest.[4]

In order to carry out such measurements it was necessary to devise an apparatus that would permit the measurement of such potentials between solutions of high viscosity. Its reliability was first demonstrated with simpler electrolytic solutions.

Hydrochloric Acid Cells

The apparatus used in the investigation is illustrated by Fig. 1. A and B are two glass vessels filled with solutions M and M′. CGD is a three-way stopcock of 9 mm. bore. The two siphon arms are indicated.

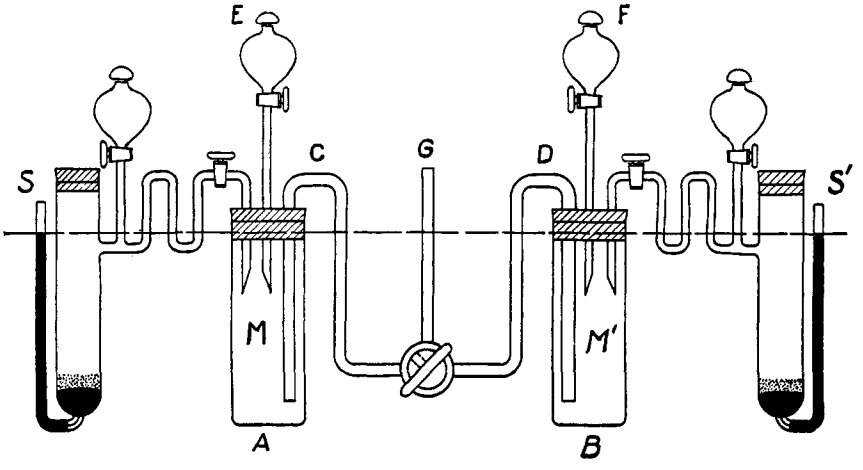

Fig. 1.—Apparatus with saturated potassium chloride electrodes for the measurement of diffusion potentials.

E and F are dropping funnels and S and S′ saturated potassium chloride electrodes. The liquid junction was made in the stopcock between the two siphon arms by proper manipulation of the stopcock and the application of suction through G.

Potential measurements were first made with a simple boundary which is illustrated by the following cell,

$$\underset{e_1}{\text{Hg, HgCl, satd. KCl}} \;||\; \underset{e_2}{\text{0.10 } N \text{ HCl}} \;|\; \underset{e_3}{\text{0.01 } N \text{ HCl}} \;||\; \underset{e_4}{\text{satd. KCl}}, \underset{e_5}{\text{HgCl, Hg}} \qquad (2)$$

Such a cell is made up of five potentials e_1, e_2, e_3, e_4 and e_5. Potentials e_1 and e_5 are equal and oppositely directed, and the same is considered

[4] Hoffman and Gortner, "Colloid Symposium Monograph II," Chemical Catalog Co., New York, **1925**, p. 209. This work has been criticized by Cohn, *Physiol. Rev.*, **5**, 349 (1925).

approximately true for e_2 and e_4.[5] Hence, the measured total potential of such a cell must be due almost entirely to the diffusion potential e_3.

The potential measurements were made by means of a Queen Gray Standard type potentiometer which gave readings to 0.01 mv. The standard cell was a Weston cadmium cell which was checked against another cell certified by the Bureau of Standards. All measurements were carried out in an oil thermostat at 25° regulated to ±0.015°. Observations were made by means of a telescope placed 12 feet from a Leeds and Northrup galvanometer No. 2500E with a sensitivity of 362 megs.

The calomel-electrode vessels were of the Lewis type.[6] The usual precautions were taken in making up the saturated potassium chloride electrodes and only the purest of chemicals used. The electrodes were checked with each other frequently and usually showed potentials below 0.10 mv. If at any time two reference electrodes showed potentials greater than 0.20 mv. they were discarded.

The hydrochloric acid solutions were made by dilution of a stock solution of 1 *N* hydrochloric acid which in turn was made from a standard, analyzed hydrochloric acid solution.

The concentration cells were made up and left in the bath for at least one hour before measurements were taken. When a cell had attained the temperature of the bath the stopcock was opened, the liquid junction established and a reading taken immediately. The stopcock was then closed. Another reading was taken shortly afterward. In all cases readings were taken with stopcock open; then the stopcock was immediately closed. Readings were taken in this manner at approximately hour intervals, until the potential of the cell started to drop rapidly. This usually resulted after a period of 40 hours.

The results obtained were very satisfactory. Three cells gave average values of 38.08, 38.08 and 38.11 mv. over a period of about 35 hours. These values represent the averages of about 20 readings taken over this period. The "time effects" were not large, amounting to less than 0.5 mv. It may be concluded, therefore, that the apparatus is capable of giving constant and reproducible potentials with this simple type of boundary.[7]

[5] Loomis and Acree [*Am. Chem. J.*, **46**, 585 (1911)], Fales and Vosburgh [THIS JOURNAL, **40**, 1291 (1918)] and others have shown that the diffusion potential between saturated potassium chloride and solutions of hydrochloric acid and sodium chloride below 0.1 *N* is zero or at any rate very small, that is, the order of a few tenths of a millivolt. More recently, Scatchard [*ibid.*, **47**, 696 (1925)] claims that the potential between saturated potassium chloride and concentrations of hydrochloric acid below 0.1 *N* is constant and has the value of 4.7 mv.

[6] Lewis, Brighton and Sebastian, *ibid.*, **39**, 2245 (1917).

[7] Calculated values of this boundary, by the Nernst equation, indicate that the potential closely approximates 38 mv.

Hydrochloric Acid-Sodium Chloride Cells

The cells next studied were of the general type,

$$\text{Hg, HgCl, KCl} \;\|\; \underset{\text{A}}{\text{satd. KCl}} \;\|\; \underset{\text{A}}{0.10\ N\ \text{HCl}} \;\Big|\; \underset{\text{B}}{(0.10-x)\ N\ \text{HCl} + x\ N\ \text{NaCl}} \;\Big|\; \underset{\text{C}}{(0.01-y)\ N\ \text{HCl} + y\ N\ \text{NaCl}} \;\Big|\; \underset{\text{D}}{0.01\ N\ \text{HCl}} \;\|\; \underset{\text{D}}{\text{satd. KCl}} \;\|\; \text{HgCl, Hg} \qquad (3)$$

E_1 E_2 E_3

The cells were made up of four solutions A, B, C and D. A represents 0.10 N hydrochloric acid, the same in all of the cells; B represents a mixture of hydrochloric acid and sodium chloride of total concentration 0.10 N, in which x varies in different cells from 0 to 0.10; C represents a mixture of hydrochloric acid and sodium chloride of total concentration 0.01 N, in which y is equal to x until the value 0.01 is reached and then remains constant while x in the other solution changes until it reaches its final value of 0.10; D represents a solution of 0.01 N hydrochloric acid, the same in all cells.

The sodium chloride solutions in the mixtures were formed by partial neutralization of the hydrochloric acid. The sodium hydroxide used for this purpose was made by dilution of the solution formed by the electrolysis of sodium amalgam. Its concentration was determined by titration with hydrochloric acid.

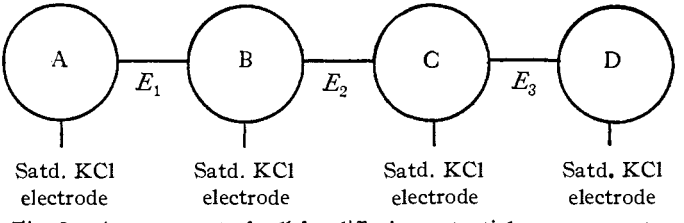

Fig. 2.—Arrangement of cell for diffusion potential measurements.

The total potential of the above cell is made up of three different diffusion potentials E_1, E_2 and E_3 at the solution contacts *A-B*, *B-C* and *C-D*. The cell shown in Fig. 1 permits the measurement of a single diffusion potential. By means of three such cells joined together, and with the use of four saturated potassium chloride electrodes, it is possible to measure each of the three boundary potentials E_1, E_2 and E_3 individually, or combinations of the three. The arrangement of the cell is illustrated by Fig. 2.[2]

The method of setting up the cells and the manner of taking the readings were exactly as described for the hydrochloric acid cells. After the cell had attained the temperature of the bath, readings were taken at hour intervals for a period of about 12 hours.

Table I serves as a typical example of the results obtained with Bound-

[8] It is apparent that each diffusion potential involves the measurement of perhaps three boundaries, but the two boundaries satd. KCl | solution and solution | satd. KCl, as noted before, probably have but little influence on the principal potential; hence, the diffusion potential is closely approximated in each case.

ary E_1. This is characteristic of the results with Boundaries E_2 and E_3. In the table the type cell is indicated at the top and the arrow from left to right indicates the direction of positive current through the cell. Duplicate cells were measured for each boundary and these are labeled Cell A and Cell B. The first entry under "Time" gives the hour at which the cell was first placed in the bath.

TABLE I

MEASUREMENTS WITH BOUNDARY E_1

Type Cell

$$\text{Hg, HgCl, satd. KCl} \;\|\; 0.10N\ \text{HCl} \;\big|\; \begin{matrix}0.08N\ \text{NaCl}\\ 0.02N\ \text{HCl}\end{matrix} \;\|\; \text{satd. KCl, HgCl, Hg} \qquad (E_1 \rightarrow)$$

Cell A			Cell B		
Time		Potential, mv.	Time		Potential, mv.
9:30	A. M.		9:45	A. M.	
11	A. M.	20.70	11	A. M.	21.11
12	M.	21.02	12	M.	21.37
1	P. M.	21.10	1	P. M.	21.34
2	P. M.	21.06	2	P. M.	21.17
3	P. M.	21.09	3	P. M.	21.03
4	P. M.	21.00	4	P. M.	21.07
5	P. M.	20.95	5	P. M.	20.88
6	P. M.	21.03	7	P. M.	20.97
7	P. M.	21.00	8	P. M.	20.90
8	P. M.	21.06			
10	P. M.	21.02			
	Av.	+21.00		Av.	+21.08

A summary of the measured values obtained with the various cells and the different boundaries is given in Table II. Col. 1 indicates the particular boundary measured; Col. 2, the value for the initial reading with Cell A; and Col. 3 the average of all readings for Cell A. Cols. 4 and 5 give similar readings with Cell B. Col. 6 gives the average of values in Cols. 3 and 5, and Col. 7, the averages of Cols. 2 and 4. The figures in parentheses after the potential values in Cols. 3 and 5 indicate the number of readings with the corresponding period in hours which the average value represents. For instance, (11–12) means 11 readings over a period of 12 hours.

An examination of the tables shows that the diffusion potentials of these more complex boundaries are as accurately reproducible as those of simpler systems, and in most instances appreciable "time effects" are not noticeable.

Hydrochloric Acid-Gelatin Cells

The hydrochloric acid-gelatin cells were of the following type,

$$\text{Hg, HgCl, satd. KCl} \;\|\; \underset{A}{0.10\ N\ \text{HCl}} \;\big|\; \underset{B}{0.10\ N\ \text{HCl}\ x\ \%\ \text{gel.}} \;\big|\; \underset{C}{0.01\ N\ \text{HCl}\ x\ \%\ \text{gel.}} \;\big|\; \underset{D}{0.01\ N\ \text{HCl}} \;\|\; \text{satd. KCl, HgCl, Hg} \qquad (4)$$

$$E_1(\text{G}) \qquad E_2(\text{G}) \qquad E_3(\text{G})$$

TABLE II

SUMMARY OF POTENTIALS FOR VARIOUS DIFFUSION BOUNDARIES

	Boundary		Initial E, Cell A, mv.	Av. total E, Cell A, mv.		Initial E, Cell B, mv.	Av. total E, Cell B, mv.		Av. total E, Cells A and B, mv.	Av. initial E, Cells A and B, mv.
$2E_1$	0.10N HCl	0.004 NaCl | 0.096N HCl	+ 0.79	+ 1.05	(11–12)	+ 1.27	+ 1.09	(13–13)	+ 1.07	+ 1.03
$3E_1$	0.10N HCl	0.01N NaCl | 0.09N HCl	2.12	2.18	(11–12)	1.96	1.91	(7–8)	2.04	2.04
$4E_1$	0.10N HCl	0.04N NaCl | 0.06N HCl	8.43	8.33	(12–12)	8.02	8.13	(10–12)	8.23	8.22
$5E_1$	0.10N HCl	0.08N NaCl | 0.02N HCl	20.70	21.00	(11–12)	21.11	21.08	(9–10)	21.04	20.90
$6E_1$	0.10N HCl	0.10N NaCl	30.30	29.94	(12–12)	30.37	29.99	(9–12)	29.96	30.33
E_2	0.10N HCl	0.01N HCl	38.52	38.08	(19–32)	38.57	38.08	(17–34)	38.08	38.54
$2E_2$	0.004N NaCl | 0.096N HCl	0.004N NaCl | 0.006N HCl	42.96	42.81	(11–12)	43.02	43.13	(11–12)	42.97	42.99
$3E_2$	0.01N NaCl | 0.09N HCl	0.01N NaCl	55.71	55.54	(11–12)	56.14	56.09	(7–8)	55.81	55.92
$4E_2$	0.04N NaCl | 0.06N HCl	0.01N NaCl	43.22	41.91	(12–12)	43.33	42.09	(11–12)	42.00	43.27
$5E_2$	0.08N NaCl | 0.02N HCl	0.01N NaCl	15.32	12.81	(11–12)	14.79	13.01	(9–10)	12.09	15.05
$6E_2$	0.10N NaCl	0.01N NaCl	—10.70	—10.59	(12–12)	—10.51	—10.61	(9–11)	—10.60	—10.60
$2E_3$	0.004N NaCl | 0.006N HCl	0.01N HCl	8.72	8.79	(11–12)	8.53	8.68	(11–12)	8.73	8.62
$3E_3$	0.01N NaCl	0.01N HCl	30.00	29.49	(12–11)	30.00	29.73	(11–11)	30.00	29.61

These cells were made up of four solutions, A, B, C and D. A represents a solution of 0.10 N hydrochloric acid, the same in all cells. B represents a solution of x% gelatin in 0.10 N hydrochloric acid, in which x is the number of grams of gelatin present in 100 cc. of solution, and varies from 0 to 17.4 in the different cells. C represents a solution of x% gelatin

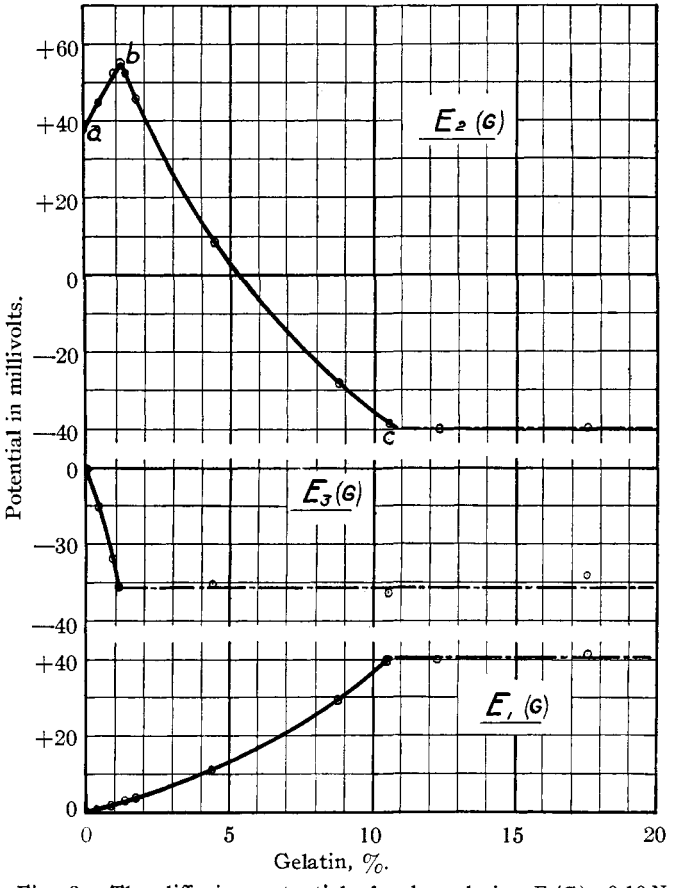

Fig. 3.—The diffusion potentials for boundaries E_2(G), 0.10N HCl + x% gelatin | 0.01N HCl + x% gelatin; E_3(G), 0.10N HCl + x% gelatin | 0.01N HCl; E_1(G), 0.10N HCl | 0.10N HCl + x% gelatin.

in 0.01 N hydrochloric acid, where x again represents the number of grams of gelatin in 100 cc. of solution and varies between the limits 0 and 17.4 in the different cells. D represents a solution of 0.01 N hydrochloric acid, the same in all cells.

The total potential of this cell is made up of three diffusion potentials formed by the contacts of Solutions A and B, B and C, and C and D.

These are E_1 (G), E_2 (G) and E_3 (G) as indicated. Four saturated potassium chloride electrodes, one dipping in each solution, permit the measurement of any boundary or combination of boundaries.[9]

The hydrochloric acid solutions were made by dilution of an analyzed solution of 1 N hydrochloric acid, as described with the previous cells.

The gelatin was a pure ash-free (0.04% ash) iso-electric gelatin obtained from the Research Laboratories of the Eastman Kodak Company. The moisture content of the gelatin was about 12.6%. This was determined by heating 2 to 3g. samples in the drying oven at 110° until constant weight was obtained. The moisture content was applied as a correction to all weighings of gelatin.

A uniform procedure was used in preparing the gelatin solutions. The manner in which a 2% solution (which corresponds to a 1.75% solution when the moisture correction is applied) of gelatin in 0.10 N hydrochloric acid was prepared serves as an illustration of the general way in which all solutions were obtained.

Ten g. of gelatin (8.74 g. of the dry gelatin) was weighed to the nearest milligram. This was dissolved in distilled water at a temperature of about 35°. The solution of gelatin was then poured into a 500cc. flask and water added so that the volume was between 300 and 400 cc. A volume of 50 cc. of 1 N hydrochloric acid was then run in by means of a calibrated pipet and water was added to bring the volume to 500 cc. The solution of 2% gelatin in 0.01 N hydrochloric acid was made up in exactly the same way, 50 cc. of the 0.10 N hydrochloric acid being used instead of 1 N hydrochloric acid.

There was no difficulty encountered in making up these solutions, although with higher concentrations of gelatin they were very viscous and set to gels within a short time. The gel was easily melted by placing the flask in warm water. After this procedure it would not set to a gel until some time afterward, so that there was no trouble in filling the cells and making the boundaries.

The cells were filled and the boundaries made in exactly the same manner as previously described with the hydrochloric acid-sodium chloride cells. Readings were extended over a much longer period than with the other sytems, usually over 24 hours. Since it seemed desirable to experiment with these cells under as rigorous conditions as possible, so that any consistent or reproducible results which might be obtained could not necessarily be ascribed to an empirical procedure, duplicate cells were measured under as varied conditions as possible, and in many instances entirely new gelatin solutions were made up. All measurements were carried out at 25°.

[9] With considerations as noted before (see Ref. 5).

Results

The information given in Table III is typical of the results obtained with other cells. This table gives measurements with cells containing 0.874% of gelatin.

TABLE III

MEASUREMENTS ON CELLS WITH 0.874% OF GELATIN

Type Cell

satd. Hg, HgCl, KCl || 0.10 *N* HCl | 0.10 *N* HCl 0.874% gel. | 0.01 *N* HCl 0.874% gel. | 0.01 *N* HCl || satd. KCl, HgCl, Hg

E_1(G) → E_2(G) → ← E_3(G)

Cell A

Time	E_1(G), mv.	E_2(G), mv.	E_3(G), mv.	E(G), mv.	E_6(G), mv.
		Boundaries			
5:30 P. M.					
7 P. M.	1.95	52.32	24.57	29.30	29.70
7:45 P. M.	1.87	52.32	23.57	30.70	30.62
8:30 P. M.	2.19	52.45	23.05	30.86	31.59
9:15 P. M.	1.86	52.31	23.03	31.03	31.14
10:15 P. M.	1.85	52.30	23.06	31.13	31.09
11:10 P. M.	1.87	52.34	23.06	31.16	31.15
9:15 A. M.	2.20	52.01	23.72	30.13	30.49
10:30 A. M.	1.95	52.10	23.72	30.41	30.33
11:45 A. M.	2.03	52.26	23.55	30.62	30.74
6:30 P. M	1.86	52.35	23.48	30.76	30.73
8 P. M.	1.88	52.30	23.55	30.53	30.63
10 P. M.	2.14	52.03	23.45	30.58	30.72
9:30 A. M.	1.86	52.04	23.24	30.59	30.74
Av.	+1.96	+52.24	−23.46	+30.59	+30.74

Cell B

10:30 A. M.					
12 M.	1.95	52.11	25.28	28.75	28.98
1:30 P. M.	2.07	52.02	24.83	29.26	29.26
2:30 P. M.	1.88	52.28	23.65	30.22	30.51
4 P. M.	1.93	52.25	23.84	30.41	30.34
5:15 P. M.	2.08	52.23	24.33	30.02	29.48
7 P. M.	1.90	52.45	23.97	30.32	30.41
8 P. M.	1.84	52.52	24.01	30.35	30.44
9 P. M.	1.82	52.28	24.05	30.28	30.05
10 P. M.	2.08	52.17	24.22	30.04	30.03
9 A. M.	1.77	52.05	23.82	29.82	30.00
12:30 P. M.	1.88	52.11	23.75	29.86	30.04
9:30 P. M.	1.85	51.97	23.72	29.93	30.10
Av.	+1.90	+52.20	−24.11	+29.95	+29.97

At the top of the table is indicated the type cell with the boundaries E_1(G), E_2(G) and E_3(G). Small arrows give the direction of flow of positive current in each boundary and a large arrow indicates the positive

or negative character of the entire cell; if from left to right the potential is considered to be positive and if from right to left negative. The first half of the table gives the measured values with Cell A and the second half those with Cell B. The first entry under "Time" gives the hour at which the cell was first placed in the thermostat bath. Successive entries give the time when measurements on the various boundaries were made. The readings in millivolts obtained with the three boundaries are given in the columns labeled $E_1(G)$, $E_2(G)$ and $E_3(G)$. In columns $E(G)$ and $E_s(G)$ are given the measured values for the total cell and also the values as determined from the algebraic sums of the boundaries.

It can be seen in both Cells A and B that in most instances the "time changes" in boundaries are very small, being of the order of a few tenths of a millivolt. The averages of the duplicate cells are very good.

Cells containing 0.437, 1.31, 1.75, 4.47, 8.74, 10.49, 12.23 and 17.48% of gelatin were treated in a manner similar to that illustrated in Table III.[10]

With higher concentrations of gelatin the "time changes" in boundaries $E_1(G)$ and $E_2(G)$ became more noticeable, although they consisted of fluctuations from hour to hour and did not show a general drift in any given direction. The 8.74% gelatin solutions became very viscous and overnight the gelatin in 0.01 N hydrochloric acid set to a gel. This had little effect on the boundary as comparison between readings taken at night and those taken the following morning showed only a change of 0.35 mv. and 0.20 mv. on two separate cells. With the 17.48% gelatin cells both gelatin solutions changed to gels within a few hours, but this change in state showed no noticeable effect on any of the potentials. In general, the agreement between duplicate cells and the deviations in readings in the single boundaries were not so good with the higher concentrations of gelatin, that is, above 10% of gelatin, but the potentials were perfectly definite. However, this irregularity at the higher concentrations introduced no difficulty since the significant conclusions are based upon results obtained at lower concentrations.

In practically every case values for duplicate cells agreed to less than 0.5 mv. and in most cases to within a few tenths of a millivolt. This shows that diffusion potentials with the supposedly complex gelatin systems are as accurately reproducible and as definite as with the simpler hydrochloric acid–sodium chloride systems.

In the cells containing more than 1.09% of gelatin, Boundary $E_3(G)$ showed a rather erratic behavior, the initial value for the boundary being much larger than any of the other values. This fall in potential amounted to as much as 10.7 mv. in ten hours. The reason for the apparent "time

[10] The original thesis may be consulted for detailed measurements on these cells.

effect" in this boundary and others was made the subject of a special investigation and has been satisfactorily explained.

The graphical representation of the changes in diffusion potential in the different cells with the amount of gelatin added is given in Fig. 3, Curves $E_1(G)$, $E_2(G)$ and $E_3(G)$. In all instances the percentage of gelatin added to the solutions is plotted against the potential in millivolts.

Curve $E_1(G)$ shows the change in potential for Boundary $E_1(G)$ with increasing amounts of gelatin where x, the amount of gelatin in 100 cc. of solution, varies from 0.437 to 17.4 g. This potential starts at zero, since there is 0.10 N hydrochloric acid on each side of the boundary, increases gradually as gelatin is added to one side and finally reaches a maximum of +40 mv., when the gelatin concentration is about 10.9%. Beyond this concentration, as more gelatin is added, the potential remains practically constant in the region of +40 mv.

Curve $E_3(G)$ shows the changes in potential for Boundary $E_3(G)$. The potential starts at zero, since 0.01 N hydrochloric acid is on both sides of the boundary. As gelatin is added to one of these solutions, that is, as x varies from 0.437 to 17.4, the potential becomes increasingly more negative very rapidly, and soon reaches a limiting value of —31.50 mv. at 1.09% gelatin concentration. With higher concentrations of gelatin the potentials become largely indeterminate, although they tend to remain in the region of —31 mv.

Curve $E_2(G)$ represents Boundary $E_2(G)$, where x again varies from 0.437 to 17.4. The potential for this boundary starts with that between 0.10 N and 0.01 N hydrochloric acid, increases gradually as equal amounts of gelatin are added to both solutions and finally reaches a maximum of +54.97 mv. at a concentration of 1.09%. As more gelatin is added to both sides, that is, as x continues to increase, the potential drops rapidly, becomes negative and finally reaches a minimum of about —39 mv. It then remains constant for higher gelatin concentrations. The minimum point on the curve corresponds to a gelatin concentration of about 10.9%.

In Fig. 4 are given graphically the results with the hydrochloric acid-sodium chloride cells which have been previously described. The boundaries are indicated under each curve. The number of cc. of sodium hydroxide solution added to produce the hydrochloric acid-sodium chloride mixtures are plotted against the resulting potentials in millivolts.

Equivalent Weight of Gelatin from Diffusion-Potential Curves

If the curves for the hydrochloric acid–sodium chloride systems are compared with the curves for the hydrochloric acid–gelatin systems, it will be noted that their general appearance is strikingly similar. This would seem to indicate that the mechanism which is the cause of the

potentials in the hydrochloric acid–gelatin systems is perhaps similar to that of the hydrochloric acid–sodium chloride systems and interpretations that might be applied to the latter could also be applied to the former.

In the hydrochloric acid-sodium chloride curves, the maximum and minimum points on each curve correspond to the condition where complete

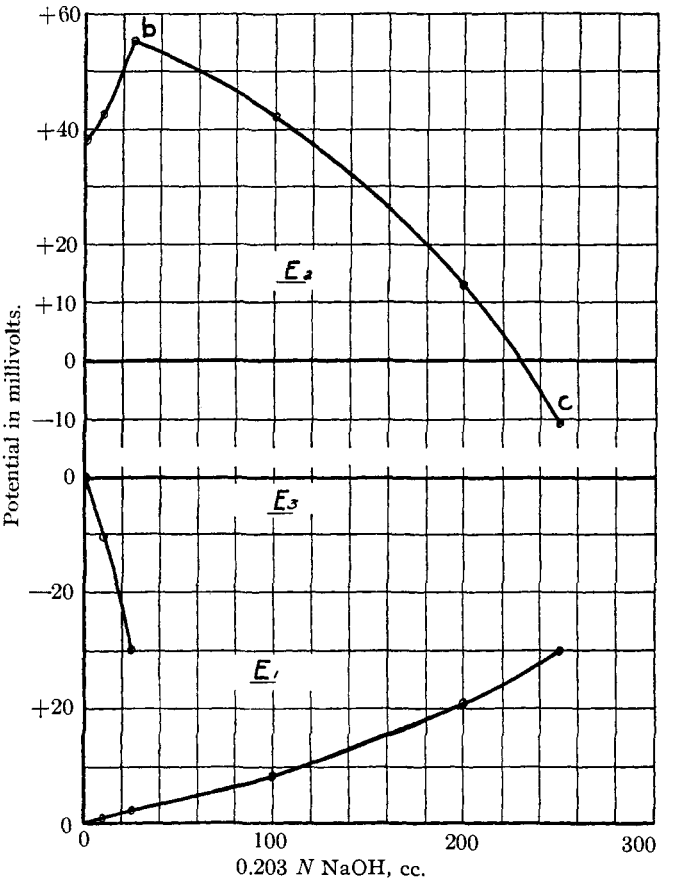

Fig. 4.—Diffusion potential changes with boundaries E_2 $(0.10-x)$ N HCl + x N NaCl | $(0.1-y)$ N HCl + y N NaCl; $E_3(0.01-y)$ N HCl + y N NaCl | 0.010 N HCl; E_1 0.10 N HCl | $(0.10-x)$ N HCl + x N NaCl.

combination between hydrochloric acid and sodium hydroxide has occurred. It seems clear from examination of the hydrochloric acid-gelatin curves that the four points of inflection on these three curves must indicate definite regions where complete combination between gelatin and hydrochloric acid has taken place. On both Curves E_2(G) and E_3(G) the first breaks occur at a gelatin concentration of 1.09%. This would correspond

to an equivalent weight of gelatin of 1090, since the solution at this point contains 1.09 g. of gelatin in 100 cc. of 0.10 N hydrochloric acid, and hence 1090 g. of gelatin would be necessary to combine with one equivalent weight of hydrochloric acid. On Curve E_1(G) the first break and on Curve E_2(G) the second break occur at a concentration of close to 10.9%, which corresponds to a condition where gelatin has combined with all the 0.10 N hydrochloric acid. This again gives the equivalent weight of gelatin as close to 1090.

These curves offer a striking proof that combination between gelatin and hydrochloric acid at concentrations ranging from 0.01 N to 0.10 N occurs in stoichiometric proportions, and the amount of gelatin which will combine with one equivalent weight of hydrochloric acid is 1090.

Other values which have been obtained for the equivalent weight of gelatin are given in a summarized form in Table IV. The results obtained in this work compare favorably with Hitchcock's values and also with those calculated by Greenberg and Schmidt.

TABLE IV

VALUES FOR THE EQUIVALENT WEIGHT OF GELATIN

Equiv. wt	Method	Equiv. wt.	Method.
665	Electrometric titration[a]	1319–2083	Titration[h]
839	Titration[b]	1180	Titration[i]
1428	Titration[c]	1087	Electrometric titration[j]
1176	Calcd.[c]	1120	Corr. of above and from diaminized gelatin[k]
1250	Estimated[d]	1160	Conductivity[l]
1063	Electrometric titration[e]	1135	Calcd.[m]
839	Catalytic action[f]	1180	Electrometric titration[n]
885	Electrometric titration[g]		

[a] Manabe and Matula, *Biochem. Z.*, **52**, 369 (1913).
[b] Procter, Ref. 2c.
[c] Bracewell, Ref. 3d.
[d] Lloyd, *Biochem. J.*, **14**, 147 (1920).
[e] Lloyd and Mayes, *Proc. Roy. Soc.*, **B93**, 69 (1922).
[f] Wintgren and Kruger, *Kolloid-Z.*, **28**, 81 (1921).
[g] Wintgren and Vogel, *ibid.*, **30**, 45 (1922).
[h] Oakes and Davis, *J. Ind. Eng. Chem.*, **14**, 706 (1922).
[i] Loeb, Ref. 2a.
[j] Hitchcock, *J. Gen. Physiol.*, **4**, 733 (1922).
[k] Hitchcock, *ibid.*, **6**, 95 (1923).
[l] Ref. *k*, p. 201.
[m] Greenberg and Schmidt, *Proc. Soc. Exptl. Biol. Med.*, **21**, 281 (1924).
[n] Atkin and Douglas, *J. Am. Leather Chem. Assocn.*, **19**, 528 (1924).

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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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN]

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

BY EGBERT K. BACON WITH ALFRED L. FERGUSON

RECEIVED MAY 5, 1927 PUBLISHED AUGUST 5, 1927

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

[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).