THE COMBINATION OF GELATIN WITH HYDROCHLORIC ACID.

II. New Determinations of the Isoelectric Point and Combining Capacity of a Purified Gelatin.

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I.

Introduction.

About 6 years ago the writer[1] reported the results of hydrogen electrode titrations and conductivity titrations of gelatin with hydrochloric acid. These experiments led to the conclusion that 1 gm. of gelatin was capable of combining with 8.6 to 8.9 $\times 10^{-4}$ equivalents of HCl. The latter figure appeared to represent a maximum which was not exceeded even in the presence of a large excess of acid. In this earlier work the experimental data were corrected on the assumption that gelatin was isoelectric and combined with no measurable acid at pH 4.70. Hence the validity of the figure for the combining capacity is limited by this assumption, as well as by the fact that it was obtained only with gelatin obtained from one particular source (Peter Cooper's Glue Factory) and purified in one particular way (Loeb's method of washing).

The difference between purified gelatins of different origin was brought out by the work of Kraemer and Dexter[2] in their study of the effect of pH on the light-scattering capacity of gelatin sols and gels. They assumed the pH where this effect was at a maximum to be

[1] Hitchcock, D. I., (*a*) *J. Gen. Physiol.*, 1921–22, iv, 733; (*b*) *J. Gen. Physiol.*, 1923–24, vi, 95; (*c*) *J. Gen. Physiol.*, 1922–23, v, 383; (*d*) *J. Gen. Physiol.*, 1923–24, vi, 201.

[2] Kraemer, E. O., and Dexter, S. T., *J. Phys. Chem.*, 1927, xxxi, 764.

that of the isoelectric point, and found this pH to be close to 5 for hide gelatins, 5.5 for an ossein gelatin, and 8 for a pigskin gelatin. They concluded that previous estimates of the isoelectric point of hide gelatins were too low, and showed that many of the data of previous workers were not inconsistent with an isoelectric point at pH 5.

The combining capacity of "Difco" gelatin, purified by Loeb's method, was found by Chapman, Greenberg, and Schmidt[3] to be 10.4×10^{-4} equivalents of acid dye per gram of gelatin. They suggested that the writer's lower figures were due to the fact that the second basic groups of lysine, arginine and histidine were only 80 to 90 per cent dissociated (combined with H^+) in HCl even at pH 1. If this is true for these groups in gelatin, the curve of combined H^+ in HCl should not be horizontal between pH 2 and 1, but still ascending.

The present paper records an attempt to locate more exactly the isoelectric point of a gelatin from the same source as that used in the earlier experiments, to determine whether or not its combination curve with hydrogen ion is horizontal between pH 2 and 1, and to determine as exactly as possible the extent of its combining capacity for each of the ions of hydrochloric acid. The latter determinations have been made by electromotive force measurements of a cell without liquid junction, of the type used in exact thermodynamic studies in inorganic chemistry, but not previously used, so far as the writer is aware, in studies of protein solutions.

II.

Isoelectric Point.

The gelatin used in this work was part of a single 5 pound package of Cooper's gelatin, purchased from Peter Cooper's Glue Factory, Gowanda, N. Y. It was purified in 50 gm. lots by Loeb's method of washing, as improved by Northrop and Kunitz.[4] It was dried by alcohol and ether in the air. Solutions containing about 13 per cent gelatin by weight were prepared by dissolving 50 gm. of this air-dry preparation in 250 cc. of distilled water. The exact concentration of each stock solution was obtained by weighing the solution delivered at 40° by a 10 cc.

[3] Chapman, L. M., Greenberg, D. M., and Schmidt, C. L. A., *J. Biol. Chem.*, 1927, lxxii, 707.

[4] Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1927–28, xi, 477.

pipette, and evaporating to dryness another sample delivered by the same pipette under the same conditions. Dry weights were considered constant when they decreased less than 0.2 per cent per day, which was the case after 5 to 15 days at 110°C. This gelatin was analyzed for total nitrogen by the Kjeldahl method, and found to contain 18.0 per cent N on a dry weight basis. Its ash content was found to be 0.04 per cent, which may be compared with the value 0.1 per cent obtained[5] for gelatin purified by Loeb's original method. Its freedom from electrolytes may also be inferred from the conductivity data given in Table I.

The isoelectric point of the gelatin used in this work was determined by two methods, the pH of minimum osmotic pressure and the pH of

TABLE I.

Specific Conductivity at 30° of Purified Gelatin in Distilled Water.

Concentration of gelatin, gm. per 1000 gm. H_2O	Specific conductivity, $10^5 \times$ reciprocal ohms		
	Solution	Water	Difference
52.1	2.98*	0.12*	2.86
89.3	3.94*	0.12*	3.82
156.0	5.59†	0.58†	5.01

* Twice distilled water.

† Once distilled water.

maximum turbidity. Osmotic pressure measurements were made at 30°C., essentially as described by Loeb,[6] using 1 per cent and 2 per cent gelatin solutions whose pH was varied by very small additions of HCl or NaOH. The differences in level of the inside and outside solutions were measured to the nearest millimeter after 1 or 2 days. Each reading was corrected by subtracting the capillary rise of water in the tubes used, about 6 mm. The concentration of gelatin inside each membrane was determined by dry weight determinations, and the results were brought to a common basis by dividing the corrected height in millimeters of solution by the concentration of gelatin in grams per kilo of water. The pH values were obtained with bubbling hydrogen electrodes at 30°C., and are based on Scatchard's value[7]

[5] Loeb, J., *J. Am. Chem. Soc.*, 1922, xliv, 213.

[6] Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 1st edition, 1922; 2nd edition, 1924.

[7] Scatchard, G., *J. Am. Chem. Soc.*, 1925, xlvii, 696.

of 0.841 for the activity coefficient of H^+ in 0.1 M HCl (pH = 1.075). This standard is 0.04 pH higher than that previously used by the writer. Liquid contact was made by bridges of saturated KCl in agar-agar, and liquid junction potentials were assumed constant.

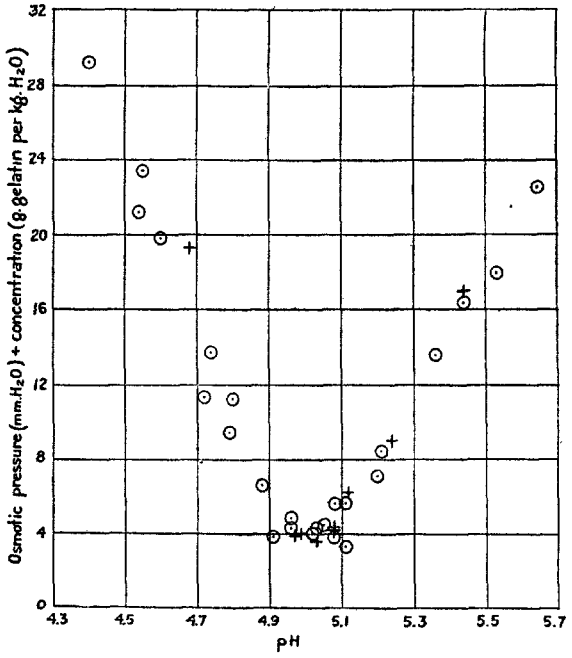

FIG. 1. Effect of pH on osmotic pressure of gelatin solutions near the isoelectric point. Ordinates are osmotic pressures in millimeters of water divided by concentrations in grams gelatin per kilo of water. Abscissæ are pH values. Circles represent experiments with about 10 gm. gelatin per 1000 gm. H_2O; crosses represent experiments at twice this concentration. The points indicate that the minimum osmotic pressure, and hence the isoelectric point of this gelatin, was located at pH 5.02 $\pm$ 0.05.

The pH values obtained with these agar bridges differed by less than 0.01 pH from those obtained with a liquid junction made around a ground glass stopper.

The results of six osmotic pressure experiments are plotted against pH in Fig. 1. The points show no minimum in the vicinity of pH

4.7, but indicate that this sample of gelatin was isoelectric at pH 5.02 ±0.05.

Confirmation of this location of the isoelectric point of the gelatin used was obtained from observations of the effect of pH on the opacity of gels. The pH was varied by small additions of HCl or NaOH, and measured, as described above, at 30°. No attempt was made to get quantitative photometric measurements of the scattered light, but the gel of maximum turbidity was located by inspection of 10 cc. samples in test-tubes after standing overnight in a refrigerator at about 8°C. The results are given in Table II. Evidently for this gelatin the pH of maximum opacity is 5.05 ±0.05. It appears to be independent of the concentration of the gelatin, and to differ very little from the pH

TABLE II.

Effect of pH on the Opacity of Gelatin Gels.

Concentration of gelatin, gm. per 1000 gm. H_2O	Composition of solvent	pH of maximum opacity	pH of next samples in series
2	0.00005 N HCl	5.05	4.95, 5.11*
10	0.0001 N HCl	5.03	4.93, 5.06*
20	H_2O	5.07*	4.99, 5.17

* pH of gelatin + water.

of the gelatin in water, 5.08 ±0.03, which also seems to vary in no regular way with the concentration. Hence the isoelectric point of this gelatin may be taken as pH 5.05 ±0.05, and any correction for acid or base contained in the purified gelatin is so small as to be safely neglected in the calculations of combining capacity reported below.

III.

Combining Capacity for Hydrogen Ion.

The curves previously obtained by the writer[1a,c] for the amount of hydrogen ion bound by gelatin as a function of pH are open to criticism because of the scattering of the points between pH 1 and 2. This scattering was due to the fact that, for 1 per cent solutions, the quantity plotted was a small difference between two larger concentrations of acid, each of which might be affected by the error inherent in a pH

determination. Hence the percentage error in the calculated amount of bound acid was often unreasonably large. It seems likely that such errors may account for some of the peculiar combination curves of protein with acid obtained by others.[8] In the present work it has proved possible to reduce this error by using higher concentrations of gelatin.

The solutions were made up by weight from hydrochloric acid solutions standardized by Na_2CO_3 and AgCl, and gelatin solutions standardized by dry weight determinations at 110°C. The pH determinations were made in wide test-tubes, using platinized wire electrodes and bubbling hydrogen. Contact was made with a saturated KCl calomel electrode through bridges of saturated KCl in 3 per cent agar-agar jelly. The bridge tubes were drawn out and bent up at the end to minimize diffusion of KCl into the solutions. The bridges were dipped into the solutions only while readings were being taken. The E. M. F. of the cells was measured to 0.1 millivolt with a Leeds and Northrup Type K potentiometer. In some cases 2 or 4 hydrogen electrodes were used with samples of the same solution in different tubes; in other cases a single electrode was used with a given solution, but the cell was refilled and the measurement repeated. Readings were considered constant when they checked within 0.5 millivolt (often less) and showed no drift over a period of 20 or 30 minutes. The E.M.F. readings were corrected to 1 atmosphere of hydrogen by the tables of Clark.[9] The cells were kept at 30° ±0.02 by an electrically controlled water bath.

The pH values were based on the standard mentioned above. Repeated measurements with a single calomel electrode (not made with especially purified materials) against hydrogen electrodes in 0.1 M HCl gave the E_0 value for this cell at 30° as 0.2405, and the pH values were obtained from the Nernst formula in the form

$$\text{pH} = \frac{E - 0.2405}{0.06015}.$$

[8] Lloyd, D. J., and Mayes, C., *Proc. Roy. Soc. London, Series B*, 1922, xciii, 69. Hoffman, W. A., and Gortner, R. A., in Holmes, H. N., Colloid symposium monograph, New York, 1925, ii, 209.

[9] Clark, W. M., The determination of hydrogen ions, Baltimore, 2nd edition, 1922.

TABLE III.

Determination of H^+ Combined with Gelatin from Hydrogen Electrode Measurements with KCl-agar Junction.

m	g	E	pH	a_H	γ_H	m_H	b_H	$\frac{b_H}{g} \times 10^4$
0	2	0.5470	5.11	7.8×10^{-6}	1.000	7.8×10^{-6}	0	0
0	10	0.5445	5.06	8.7×10^{-6}	1.000	8.7×10^{-6}	0	0
0	20	0.5455	5.07	8.5×10^{-6}	1.000	8.5×10^{-6}	0	0
0	40	0.5445	5.06	8.7×10^{-6}	1.000	8.7×10^{-6}	0	0
0.01029	31.3	0.4850	4.07	8.6×10^{-5}	0.921	9.3×10^{-5}	0.01020	3.26 ± 0.00
0.0550	90.0	0.4525	3.52	3.0×10^{-4}	0.860	3.5×10^{-4}	0.0546	6.07 ± 0.00
0.02058	31.4	0.4425	3.36	4.4×10^{-4}	0.895	4.9×10^{-4}	0.02009	6.40 ± 0.00
0.0543	70.4	0.4270	3.10	7.9×10^{-4}	0.861	9.2×10^{-4}	0.0534	7.58 ± 0.00
0.0844	90.2	0.3910	2.50	0.0032	0.846	0.0037	0.0807	8.95 ± 0.00
0.0531	51.8	0.3790	2.30	0.0050	0.861	0.0058	0.0473	9.13 ± 0.02
0.0830	70.1	0.3510	1.84	0.0146	0.847	0.0172	0.0658	9.39 ± 0.04
0.0521	34.2	0.3465	1.76	0.0174	0.852	0.0204	0.0317	9.27 ± 0.15
0.1131	89.5	0.3375	1.61	0.0245	0.839	0.0292	0.0839	9.37 ± 0.07
0.0816	51.6	0.3335	1.55	0.0282	0.847	0.0333	0.0483	9.36 ± 0.16
0.1052	71.7	0.3300	1.49	0.0324	0.841	0.0385	0.0667	9.30 ± 0.13
0.1110	70.3	0.3255	1.41	0.0389	0.840	0.0463	0.0647	9.20 ± 0.16
0.0795	34.7	0.3245	1.40	0.0398	0.848	0.0470	0.0325	9.37 ± 0.32
0.1046	56.1	0.3225	1.36	0.0437	0.841	0.0519	0.0527	9.39 ± 0.22
0.1087	51.6	0.3190	1.30	0.0501	0.840	0.0597	0.0490	9.51 ± 0.26
0.1035	41.2	0.3160	1.25	0.0562	0.841	0.0668	0.0367	8.91 ± 0.36
0.1062	34.7	0.3135	1.21	0.0617	0.840	0.0735	0.0327	9.43 ± 0.46
0.1019	27.3	0.3120	1.19	0.0646	0.841	0.0768	0.0251	9.20 ± 0.65
0.2310	121.2	0.3005	1.00	0.1000	0.836	0.1196	0.1114	9.19 ± 0.23
0.2192	73.0	0.2935	0.88	0.1318	0.836	0.1576	0.0616	8.44 ± 0.49

m = molality of HCl.

g = gm. gelatin per kilo H_2O.

E = observed E.M.F. in volts, corrected to 1 atm. H_2.

$$\text{pH} = \frac{E - 0.2405}{0.06015}.$$

a_H = activity of H^+ = antilog $(-\text{pH})$.

γ_H = activity coefficient of H^+, from Scatchard's data.

$m_H = \frac{a_H}{\gamma_H}$ = molality of free H^+.

b_H = molality of combined $H^+ = m - m_H$.

$\frac{b_H}{g} \times 10^4$ = mols of H^+ combined with 10,000 gm. gelatin.

The numbers following the ± sign represent the effect of an error of ± 0.5 millivolt in E.

The amounts of combined hydrogen ion were calculated by the method used by Cohn,[10] which differs slightly from that used by Loeb[6] and by the writer[1] in previous work. If b_H = molality of bound H^+, m = total molality of HCl, and a_H = activity of H^+ as given by pH measurements, then

$$b_H = m - \frac{a_H}{\gamma_H}.$$

Here γ_H was taken as the activity coefficient of H^+, according to Scatchard[7], in a pure HCl solution of molality m. This is equivalent to assuming the ionic strength principle of Lewis and Randall[11] and assigning to the gelatin-H^+ complex an effective valence of one. In the method of calculation previously used the terms m and b_H represented volume normal concentrations and γ_H was taken as the activity coefficient of H^+ in that concentration of pure acid having a measured hydrogen ion activity equal to that found for the gelatin-HCl solution. This was equivalent to assuming that the gelatin chloride present had no effect on the activity of H^+ in the excess HCl. The present assumption seems more reasonable than that formerly used because of the results with acid-salt mixtures cited by Lewis and Randall.[11]

The values of b_H were divided by the concentrations of gelatin to get the number of equivalents of H^+ combined with unit weight of gelatin. This quantity is given in the last column of Table III, and is plotted against pH in Fig. 2. The curve in Fig. 2 was drawn to represent the best smooth relation between the experimental points. It will be noted that the points do not show any evidence of a further upward trend with decrease of pH from 2 to 1, as would be expected if the explanation proposed by Schmidt[3] and his co-workers were correct. Except for two points which lie below the curve, the points lie fully as close to a horizontal line between pH 2 and 1 as could be expected from the effect of a possible error of 0.5 millivolt in the E.M.F. of the cell. Although in many cases the agreement of duplicates was

[10] Cohn, E. J., *Physiol. Rev.*, 1925, v, 349.

[11] Lewis, G. N., and Randall, M., *J. Am. Chem. Soc.*, 1921, xliii, 1112; Thermodynamics and the free energy of chemical substances, New York and London, 1923.

better than this, the measurements with HCl alone differed so much that the precision is probably not better than ±0.5 millivolt or ±0.01 pH for these agar bridge measurements.

The experiments thus confirm the writer's earlier conclusion that the amount of H^+ bound by 1 gm. of gelatin is constant between pH 2 and 1. For this gelatin this quantity is 9.35×10^{-4} equivalents of H^+ per gram of gelatin. This figure was confirmed by conductivity titrations of gelatin with HCl, carried out by the method previously

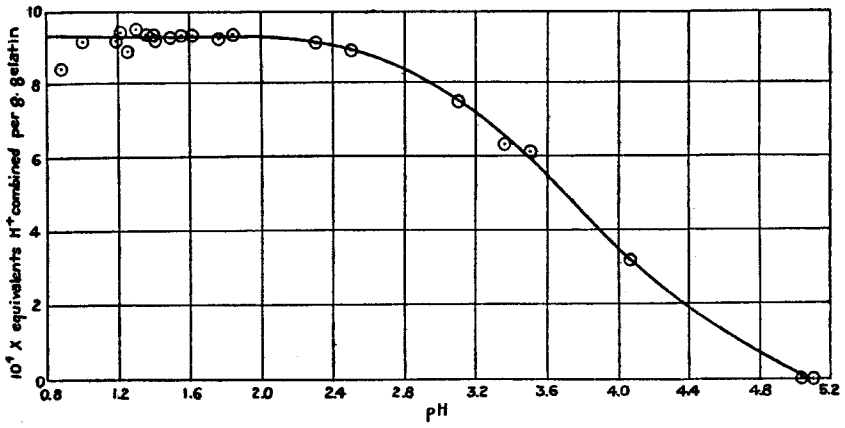

FIG. 2. The combination of gelatin with hydrogen ion from HCl. Ordinates are equivalents of H^+ combined with 1 gm. of gelatin, multiplied by 10^4, as calculated from hydrogen electrode measurements with an agar-KCl junction. Abscissæ are pH values of the gelatin-HCl solutions, obtained from the same measurements. The points indicate a maximum of 9.35×10^{-4} equivalents of H^+ combined with 1 gm. of gelatin between pH 2 and 1.

described.[1,d] The end-points of three titrations, using different concentrations of gelatin, gave the following results: 9.39, 9.37, 9.47 $\times 10^{-4}$; average, 9.41×10^{-4} equivalents per gram.

The difference between these values and those obtained earlier (8.9 by hydrogen electrode, 8.6 by conductivity) is more than accounted for by the different values assigned to the isoelectric point. Such differences might well be due to the very doubtful uniformity of the original commercial gelatin. It should be possible to avoid such differences and uncertainties by the use of gelatin prepared from definite materials in a definite way. A committee of the Leather and

Gelatin Division of the American Chemical Society has proposed tentative specifications for a standard gelatin for scientific purposes, and the writer hopes to be able to report later on the acid-combining capacity of gelatin prepared in accordance with these specifications.

IV.

Combination with Hydrogen and Chloride Ions.

A recalculation of old data[1,e] and a few new experiments with silver-silver chloride electrodes in gelatin-HCl solutions seemed to indicate that gelatin combined to some extent with chloride ion, with a maximum combining capacity of 1.5 to 2.5 $\times$ 10^{-4} equivalents of Cl^- per gram of gelatin. It seemed possible to determine more exactly this combination with chloride ion, as well as that with hydrogen ion, by measurements of the cell without liquid junction: Ag, AgCl, HCl + gelatin, H_2.

Four silver-silver chloride electrodes were prepared by electroplating, as in the previous work.[1,e] They were replated whenever any two differed by 0.2 millivolt in the same solution, 0.1 M HCl. The hydrogen electrodes were short coils of platinized platinum wire, and were completely immersed in the solutions, with hydrogen bubbling up around them. Commercial tank hydrogen was used. Its purity was tested by hydrogen electrode measurements in 0.1 M HCl with hydrogen passed through alkaline permanganate, alkaline pyrogallate, and distilled water. Identical readings were obtained with only the washing in water at 30°; accordingly the rest of the purifying train was omitted in the experiments. The vessels used were simple U-tubes about 2 cm. wide in the vertical arms and 1 cm. at the bend. A few measurements were made with the AgCl and H_2 electrodes in a single test-tube, but the E.M.F. so obtained decreased with time. The U-tubes, by protecting the AgCl from H_2, gave readings which were constant to 0.2 millivolt for several hours. In each experiment 4 cells were set up with the same solution, and readings taken to the nearest 0.05 millivolt for 3 to 5 hours. The E.M.F. values were plotted against time and an average value selected from those points which could best be represented by a horizontal line over a period of at least 1 hour. The values so obtained are probably reliable to 0.1

millivolt or better as a measure of the true E.M.F. in any one experiment. Many experiments had to be discarded because of lack of constancy and reproducibility in the E.M.F. It was found in some cases that the erratic behavior was due to toluene which had been used to protect the stock solution of gelatin. In the course of repeated meltings and settings of the gelatin, the toluene seemed to become permanently emulsified. Other solutions which had been in contact with thymol gave low and rapidly drifting E.M.F. values, an effect which was reproduced with HCl containing thymol. The data reported below were obtained with gelatin to which no preservative was added. The readings were obtained with AgCl electrodes which had not been freshly plated for 2 or 3 months, but had been kept in 0.1 M HCl. After each day's use in a gelatin solution they were washed with hot water, distilled water, and 0.1 M HCl. At the end of this series of experiments the E.M.F. obtained with these electrodes in the cell Ag, AgCl, HCl (0.1 M), H_2 was just as constant and reproducible as at the start of the series, but was 0.6 millivolt lower. This lowering was either a slow time effect or else an effect of the contact with gelatin. The calculations were based on this final HCl reading as a standard rather than on the initial reading, although the latter was again reproduced on replating the electrodes.

The data and calculations are given in Table IV. Each figure is the mean of two experiments, each done with four sets of electrodes. The E.M.F. of the cell Ag, AgCl, HCl, H_2 at 30°C. is given by the thermodynamic equation

$$E = E_0 - 0.06015 \log a_H \, a_{Cl} \tag{1}$$

in which a_H and a_{Cl} represent the activities of the ions and E_0 is the E.M.F. of a hypothetical cell in which the product $a_H \, a_{Cl}$ is equal to one. The determination of E_0 by extrapolation has been discussed elsewhere.[12] For the present purpose it is not necessary to know the absolute value of this quantity. Equation (1) may be written

$$E = E_0 - 0.1203 \log \gamma - 0.06015 \log m_H \, m_{Cl} \tag{2}$$

where γ is the geometrical mean activity coefficient of the two ions and m_H and m_{Cl} are their respective molalities. This form of equation

[12] Hitchcock, D. I., *J. Am. Chem. Soc.*, 1928, 1, 2076.

has been applied to the calculation of the activity coefficient of HCl in the presence of other chlorides. The results of Harned,[13] as quoted by Lewis and Randall,[11] show that in mixtures of HCl with LiCl, NaCl,

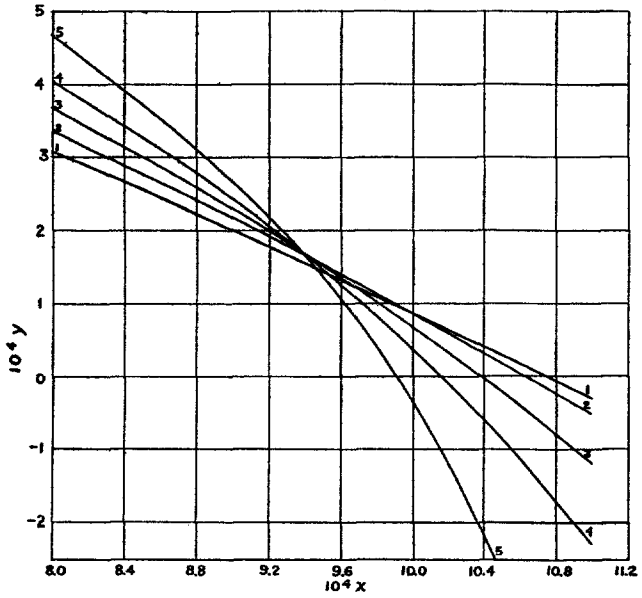

FIG. 3. Graphical determination of the combining capacity of gelatin with H^+ and Cl^- from E.M.F. measurenents of the cell Ag, AgCl, HCl + gelatin, H_2, without liquid junction. Each experiment leads to an equation containing two unknowns, x and y, the equivalents of H^+ and Cl^- combined with 1 gm. of gelatin. In the figure the abscissæ are values of $10^4\,x$ and the ordinates are $10^4\,y$. Each curve represents the relation between these variables for one experiment, and is marked with the number of the experiment. The true values of these quantities, if constant for all the experiments, should be given by a single intersection of all the curves. The figure shows that four of the five curves intersect at the point where $x = 9.4 \times 10^{-4}$ and $y = 1.7 \times 10^{-4}$.

or KCl, at a constant total molality of 0.1 or 0.2, the activity coefficient is the same as that in pure HCl of the same total molality. The writer has confirmed this by experiments with 0.1 M HCl partly neutralized by NaOH, KOH, or NH_4OH. Accordingly in the calcula-

[13] Harned, H. S., *J. Am. Chem. Soc.*, 1920, xlii, 1808.

tion of the gelatin experiments it will be assumed that the same principle applies; in other words, the partial neutralization of dilute HCl by gelatin is assumed not to affect the mean activity coefficient of its ions. On this basis the expression $E_0 - 0.1203 \log \gamma$ is assumed constant and equal to the value obtained without gelatin. An experiment done at the conclusion of the gelatin series with 0.1008 M HCl gave E =

TABLE IV.

Combining Capacities of Gelatin for H^+ and Cl^- from E.M.F. of Cells without Liquid Junction.

Experiment No.	m	g	E (observed)	$\frac{E - 0.2305}{0.06015}$	$m_H\, m_{Cl}$	E (calculated)
1	0.1005	13.5	0.3546	2.063	0.00865	0.3547
2	0.1014	27.3	0.3589	2.135	0.00733	0.3589
3	0.1025	44.5	0.3652	2.239	0.00577	0.3652
4	0.1036	59.8	0.3721	2.354	0.00443	0.3721
5	0.1048	77.4	0.3827	2.530	0.00295	0.3828

m = molality of HCl.

g = concentration of gelatin in gm. per kilo H_2O.

E (observed) = E.M.F. in volts of cell Ag, AgCl, HCl + gelatin, H_2 (1 atm.).

0.2305 = $E_0 - 0.1203 \log \gamma$, from E (observed) = 0.3504 for 0.1008 M HCl without gelatin.

$m_H\, m_{Cl}$ = antilog $\frac{0.2305 - E}{0.06015}$ = product of molalities of ions.

E (calculated) = $0.2305 - 0.06015 \log (m - gx)(m - gy)$ where m and g have values in columns 2 and 3, $x = 9.4 \times 10^{-4}$, and $y = 1.7 \times 10^{-4}$. The latter values were obtained graphically in Fig. 3 from the values of $m_H\, m_{Cl}$ in column 6 and the relation $m_H\, m_{Cl} = (m - gx)(m - gy)$.

0.3504. From this it follows that $E_0 - 0.1203 \log \gamma$, which is equal to $E + 0.1203 \log m$, is 0.2305. Now equation (2) may be rewritten

$$\frac{E - 0.2305}{0.06015} = -\log (m - g\,x)(m - g\,y) \qquad (3)$$

where x and y are numbers of equivalents of H^+ and Cl^- combined with 1 gm. of gelatin, and g is the concentration of the gelatin in grams per kilo H_2O. For each experiment E, m, and g were measured; hence the equation contains the two unknowns x and y. The concentrations in the experiments were such that x and y might be expected to be constant,

the gelatin being combined to the maximum possible extent with both H^+ and Cl^-. This constancy may be tested, and the equation solved for both x and y, by plotting y as a function of x for each experiment. This was done by using the observed values of E, m, and g, assuming values for x, and calculating y. If the values of x and y are really constant, all the curves should intersect in a single point whose coordinates are the true values of x and y. The curves in Fig. 3 were obtained from the data of Table IV in this way, and the validity of the assumed constancy of x and y is justified by the single intersection of four curves. The form of equation (3) is such that algebraically x and y are interchangeable. The experiments in the preceding part of this paper indicate that the higher value belongs to x, the equivalents of H^+ combined with 1 gm. of gelatin. The experiments therefore lead to the conclusion that 1 gm. of gelatin in 0.1 M HCl combines with a maximum of 9.4×10^{-4} equivalents of H^+ and 1.7×10^{-4} equivalents of Cl^-. The consistency of these figures with the experimental data is shown by the last column in Table IV, which gives the E.M.F. calculated by equation (3), using the observed values of m and g and the above values of x and y. The agreement of these with the observed values shows that the failure of Curve 1 in Fig. 3 to intersect the others at a single point is not due to an unreasonable experimental error in the E.M.F., but simply due to the magnification of error inherent in an experiment with a low protein concentration. These figures are probably more reliable than those obtained from the agar bridge data because of the absence of liquid junction errors in these cells. It is hoped that this method may be used later in a study of the standard gelatin mentioned above, as well as with other proteins.

V.

Summary.

1. Cooper's gelatin purified according to Northrop and Kunitz exhibited a minimum of osmotic pressure and a maximum of opacity at pH 5.05 $\pm$0.05. The pH of solutions of this gelatin in water was also close to this value. It is inferred that such gelatin is isoelectric at this pH and not at pH 4.70.

2. Hydrogen electrode measurements with KCl-agar junctions were

made with concentrated solutions of this gelatin in HCl up to 0.1 M. The combination curve calculated from these data is quite exactly horizontal between pH 2 and 1, indicating that 1 gm. of this gelatin can combine with a maximum of 9.35×10^{-4} equivalents of H^+.

3. Conductivity titrations of this gelatin with HCl gave an end-point at $9.41\ (\pm 0.05) \times 10^{-4}$ equivalents of HCl per gram gelatin.

4. E.M.F. measurements of the cell without liquid junction, Ag, AgCl, HCl + gelatin, H_2, lead to the conclusion that this gelatin in 0.1 M HCl combines with a maximum of 9.4×10^{-4} equivalents of H^+ and 1.7×10^{-4} equivalents of Cl^- per gram gelatin.

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