THE COMBINATION OF A STANDARD GELATIN PREPARATION WITH HYDROCHLORIC ACID AND WITH SODIUM HYDROXIDE

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I

INTRODUCTION

In a recent paper the writer (1930–31*b*) reported a study of the isoelectric point of gelatin which had been prepared and purified according to specifications of a committee of the Leather and Gelatin Division of the American Chemical Society (Davis, Sheppard, and Briefer, 1929; Hudson and Sheppard, 1929). As a further contribution towards the work of that committee, a study has been made of the maximum combining capacity of this gelatin for the ions of hydrochloric acid, by the method used previously (1928–29) with another gelatin preparation. This led to a search for an accurate and reproducible method of drying gelatin to constant weight, and to a determination, by the usual hydrogen electrode method, of the complete titration or dissociation curve of the gelatin in solutions containing either hydrochloric acid or sodium hydroxide. While it will probably not be advisable to include the results of all these measurements in the specifications for the standard gelatin, it seems worth while to report them as a step towards the better characterization of this widely studied protein material.

II

Dry Weight Determination

In unpublished reports of the standard gelatin committee, it had been suggested that moisture be determined by drying at 105°C., either to constant weight or for

[1] Most of the experimental work was done by the writer's assistants, Miss Ruth C. Belden and Mr. Angelo E. Benaglia.

a definite time, such as 24 hours. Careful determinations were made in this way by Dr. Rubert S. Anderson of this laboratory. The air-dry gelatin, in sheets about 0.2 mm. thick, was cut into pieces about 5 × 15 mm. and kept in a tightly stoppered bottle to insure uniformity in moisture content. The thick edges formed in the original drying of the gel were not used. Samples of 1 to 2 gm. were placed in aluminum dishes having tight covers, weighed, and kept in an oven at 105° ± 1°C. for about 3 weeks, being weighed daily during the first week. The weights approached a constant value only after 3 days in the oven, and then they were not

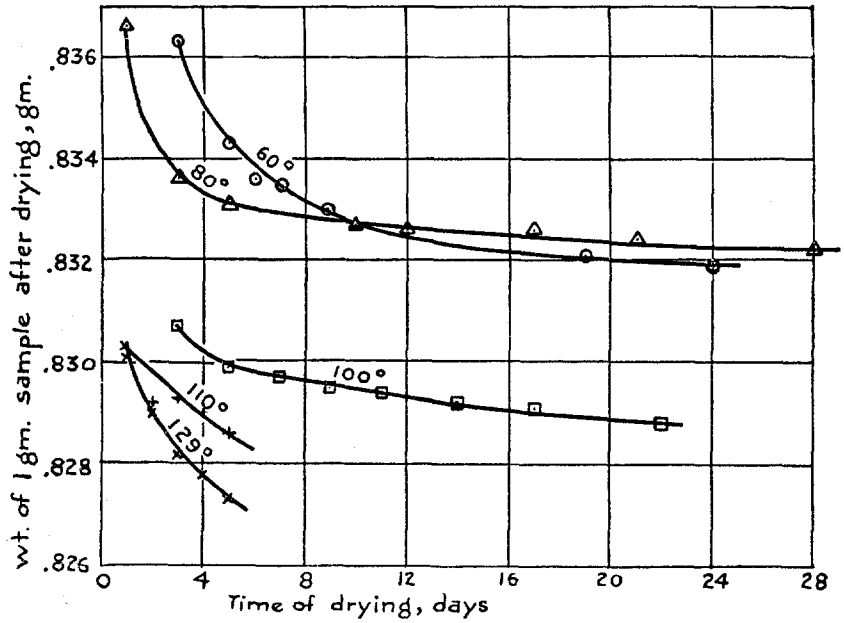

FIG. 1. Effect of temperature on the dry weights of 1 gm. samples of sheet gelatin in Abderhalden driers containing $MgClO_4 \cdot 3H_2O$. Each curve is marked with the temperature in °C. The abscissae represent times of drying in days; the ordinates are the weights in gm. of 1 gm. samples after drying.

truly constant, shifting up or down by 1 or 2 mg. according to the weather. This work was done during March, 1930, when the laboratory was heated artificially and the humidity was fairly low. Similar experiments carried out in July, during weather which was at times very hot and humid, demonstrated a marked effect of the water content of the atmosphere on the weight of gelatin after drying in a ventilated oven at 105°. In some of these experiments the weights of 1 gm. samples went up or down as much as 3 to 5 mg. in as many days. This work has led the writer to give up the ordinary drying oven as a means of determining the moisture content of gelatin.

The effect of drying the gelatin *in vacuo* at various temperatures was then studied, using Abderhalden driers with the vapors of different boiling liquids in the jackets. Since the tube of the drier had an inside diameter of only 11 mm., it was necessary to obtain special glass-stoppered weighing tubes to fit inside them. These were 10 cm. long and had an inside diameter of about 8 mm. A large quantity of gelatin was cut into small pieces at the beginning of the experiments and kept in a stoppered bottle which was opened as little as possible. The tubes were weighed by the use of a similar tube as counterpoise, to avoid the effect of possible condensation on the outer surface of the glass. The bulbs of the driers contained $MgClO_4 \cdot 3H_2O$ (sold under the trade name of "dehydrite").[2] The driers were evacuated within a few minutes by an oil pump, and were found to retain the vacuum without pumping during each drying period. The gelatin samples weighed from 0.9 to 1.1 gm. For convenience in comparison, the weights were recalculated as if each sample had weighed exactly 1 gm. at the start. Temperatures of 60, 80, 100, 110, and 129°C., as measured in the position of the drying tubes, were obtained by the use of boiling chloroform, benzene, water, toluene, and monochlorbenzene, respectively. The results of drying this gelatin (Eastman Standard Gelatin, Lot 48) are given in Fig. 1.

It will be observed that none of the weights became constant within the usual time for such determinations (48 hours or less), and that at 100° or above the samples showed no sign of ever attaining constant weight. The samples which were dried at 60° and 80°, however, lost weight so gradually after about 2 weeks in the driers that their weights may be taken as approaching a constant limiting value, in both cases 0.832 gm. for a 1 gm. sample of this particular lot of gelatin. It is this figure which has been used in calculating the gelatin and water contents of the solutions to be described in the remainder of this paper.

III

Analytical Data

The ash content of the standard gelatin was determined by burning 5 gm. samples in quartz crucibles, adding the gelatin in small amounts[3] and raising the temperature slowly to avoid loss due to swelling and spattering. After ignition to constant weight, the weights of ash found amounted to 0.041 and 0.046 per cent of the dry gelatin.

The nitrogen content of the material was determined by the Kjeldahl method, using essentially the Gunning-Arnold-Dyer modification as described by Sherman (1912) but with about one-tenth of the quantities of reagents there specified. The amount of gelatin taken for each analysis was 5 cc. of a 0.65 per cent solution. Its weight was known to about 1 part in 1000, as the solution was made up by

[2] In later experiments we have used anhydrous $MgClO_4$ (sold as "anhydrone").

[3] This bit of technique was suggested to the writer a few years ago by Miss Edith H. Lanman of the Department of Chemistry of Bryn Mawr College.

weight and the weight of solution delivered by the pipette was determined. The protein was digested over micro burners in 100 cc. Kjeldahl flasks with 2 cc. of concentrated H_2SO_4, 1 gm. of K_2SO_4, 0.075 gm. of HgO, and a piece of broken alundum to reduce bumping. Digestion was complete in 40 minutes, or about 20 minutes after the mixture became clear. After cooling, 35 cc. of water, 2.5 cc. of 4 per cent potassium sulfide solution, and 5 cc. of saturated NaOH were added, and the ammonia was distilled into 25 cc. of 0.02 N HCl through 2 Kjeldahl traps fused in series, as suggested by Northrop (1929–30). It was usually possible to distill over 25 cc. in 15 minutes before bumping became violent. The excess acid was titrated back with 0.02 N NaOH, using methyl red as indicator. Duplicate determinations generally agreed within 0.1 cc., or 1 part in 200, since the NH_3 was equivalent to about 20 cc. of 0.02 N HCl. Blank determinations ran from 0.04 to 0.08 cc., duplicates agreeing within 0.02 cc. By averaging four parallel determinations, rejecting any figure differing by more than 0.2 cc. from the mean of the others, the method is probably reproducible to within 2 parts in 1000. The figure obtained, from twelve determinations, for the nitrogen content of this gelatin was 18.15 per cent on the dry basis.

The specific conductivity of solutions of this gelatin at 30°C. was 3.8×10^{-5} reciprocal ohms for a 5.6 per cent solution and 5.0×10^{-5} for a 9.35 per cent solution. These figures have not been corrected for the conductivity of the water, which was about 0.2×10^{-5}.

The determination of the isoelectric point of this gelatin has been reported in a previous paper (1930–31*b*). The isoelectric point is probably at pH 4.85 ± 0.01, although the method of cataphoresis of collodion particles coated with gelatin gave the value 4.80 ± 0.01.

IV

Combination with Hydrogen and Chloride Ions from Hydrochloric Acid

This problem was studied by the method used previously with another gelatin preparation (1928–29) and with edestin (1930–31*a*). This consists in measuring the electromotive force, at 30°C., of cells without liquid junction, of the type Ag, AgCl, HCl + protein, H_2. The solutions were made up by weight to contain known proportions of gelatin, hydrochloric acid, and water. The hydrochloric acid concentration was kept equal to 0.1 M while the gelatin concentrations were varied. The technique was improved by referring the gelatin concentrations to the dry basis already discussed, and by using freshly plated silver-silver chloride electrodes for each new solution. The experiments were done several times by different workers before the need for these improvements was realized, and only the latest set of

measurements is given in Table I. While the data in this table may appear rather scanty, the results of the many earlier measurements agreed with them well enough so that little doubt is held as to their reliability. Each figure for the E.M.F. is the mean of values obtained with three separate cells, all agreeing to within 0.0002 volt, and each constant to 0.0001 volt for at least 1 hour. Similar reproducibility and constancy were attained on refilling the cells with fresh solution and taking readings for another hour after the electrodes had reached equilibrium. The experimental data are given in the first three columns of Table I.

TABLE I

Electromotive Force at 30°C. of the Cells Ag, AgCl, HCl + Gelatin, H_2

m	g	E (observed)	E'_0	E (calculated)	ΔE	pH (approx.)
0.1000	50.3	0.3708	0.2306	0.3709	+0.0001	1.3
0.1000	70.4	0.3841	0.2304	0.3840	−0.0001	1.5
0.1000	90.5	0.4084	0.2302	0.4084	0	1.9

m = mols HCl per 1000 gm. H_2O.
g = gm. dry gelatin per 1000 gm. H_2O.
E (observed) = E.M.F. in volts, corrected to 1 atmosphere dry H_2.
$E'_0 = E + 0.1203 \log m$, for cells containing HCl alone, of molality equal to $m - gy$ ($E'_0 = 0.2310$ for $m = 0.1002$).
E (calculated) $= E'_0 - 0.06015 \log (m - gx)(m - gy)$.
x = mols H^+ combined with 1 gm. gelatin $= 9.58 \times 10^{-4}$.
y = mols Cl^- combined with 1 gm. gelatin $= 2.0 \times 10^{-4}$.
$\Delta E = E$ (calculated) $- E$ (observed).
pH (approx.) $= -\log (m - gx)$.

The interpretation of these data depends on the following considerations. The E.M.F. of a cell such as those measured must be given by the thermodynamic equation

$$E = E_0 - 0.06015 \log m_H m_{Cl} \gamma^2 \qquad (1)$$

which holds for 30°C. Here E is the observed E.M.F. in volts, after correction to unit pressure of hydrogen, E_0 is a constant depending on the nature of the electrodes and the temperature, m_H and m_{Cl} are the molalities of free H^+ and Cl^- in the solution bathing the electrodes,

and γ is the geometric mean activity coefficient of the ions of HCl in the solution.

If some of the ions from the added HCl are combined with gelatin, it follows that $m_H = m - gx$ and $m_{Cl} = m - gy$, where m is the molality of the total HCl, free and combined, g is the gelatin concentration in gm. per 1000 gm. H_2O, and x and y are the numbers of mols of H^+ and Cl^-, respectively, combined with 1 gm. of gelatin. Equation (1) may be made more useful by these substitutions, and by letting $E'_0 = E_0 - 0.1203 \log \gamma$. It then becomes

$$E = E_0' - 0.06015 \log (m - gx)(m - gy). \qquad (2)$$

In order to solve equations of this form for x and y, it is necessary to have values for E'_0. This quantity includes E_0, which should not be changed by the presence of protein, and γ, which may well be changed by it. As a first approximation it was assumed that γ, and hence E'_0, was unchanged by the gelatin. Using for E'_0 the value of 0.2310, obtained from measurements with HCl free from protein, of molality 0.1002, the three equations resulting from the three experiments of Table I were represented graphically by assuming values for x, calculating y, and plotting y against x. The curves so obtained intersected at points corresponding to $x = 9.6$ to 9.7×10^{-4} and $y = 1.6$ to 1.8×10^{-4}. As a second approximation[4] it was assumed that E'_0 should be equal to the value obtained with pure HCl, not of molality m, but of molality $m - gy$. The reason for this is that gy represents a part of the HCl of which not only the H^+ but also the Cl^- is bound to gelatin; hence this part should have as little influence on the activity coefficient as a non-electrolyte. The difference $m - gy$ represents some free HCl and some HCl of which only the H^+ is bound to gelatin. If the ionic strength principle of Lewis and Randall (1921) applies to mixtures of HCl and an ionized protein hydrochloride, and if the positive protein-hydrogen ion is assumed to have an effective valence of one in its effect on the ionic strength, then the value of γ or E'_0 for

[4] This method of approximation, which is the same as that used in the previous study of edestin, was suggested by Dr. Rubert S. Anderson. The writer wishes to acknowledge his indebtedness to Dr. Anderson for much helpful discussion as well as for some careful experiments which were carried out in the preliminary stages of the work.

such a mixture should be the same as that for HCl of molality $m - gy$. For the second approximation y was assumed to be 1.7×10^{-4}, and values of E'_0 were read off from a plot of the values obtained for HCl in connection with the study of edestin (1930–31*a*), E'_0 being plotted against $\sqrt{m}$. These values were raised by 0.0001 volt because the value for 0.1 M HCl with the new electrodes was 0.2310 instead of 0.2309, as in the previous work. A graphical solution of the equations on this basis gave curves intersecting at points whose coordinates averaged 9.6 and 2.0×10^{-4}. A third approximation on the basis of $y = 2.0 \times 10^{-4}$ gave intersections for which the average value of y was again 2.0×10^{-4}, indicating that no further approximations were possible. The values of E'_0 corresponding to $m - gy$, using this value for y, are given in the fourth column of Table I. The fifth column gives values of E calculated from equation (2) by the use of these values for E'_0 and the values $x = 9.58 \times 10^{-4}$ and $y = 2.0 \times 10^{-4}$. The sixth column shows the extent to which the experimental data are fitted by these values. The last column gives the negative logarithm of the molality of hydrogen ion, which is approximately the pH value of the solutions. Table I shows that the data may be explained by assuming that each gm. of this gelatin in 0.1 M HCl was combined with 9.58×10^{-4} mols of H^+ and 2.0×10^{-4} mols of Cl^-, these amounts being independent of the gelatin concentration and hence of the pH, within the limits given.

The same data were treated in another way by solving equation (2) for three unknowns, E'_0, x, and y, using again the experimental figures in the first three columns of Table I. The resulting values were $E'_0 = 0.2307$, $x = 9.55 \times 10^{-4}$, and $y = 2.03 \times 10^{-4}$. From these values it is possible to get exact agreement between the observed and calculated values of E. This agreement, however, is a necessary consequence of the fact that only three equations were solved in getting the three unknowns. Theoretically E'_0 should not be constant, as already explained, and if it were constant it might be expected to have the value 0.2310 which was used in the first approximate calculation of x and y. Yet the agreement of the values for x and y found in this way with those given in Table I is an indication that the values found for x and y may be, to a certain extent, independent of the assumptions used in calculating them.

V

Combination Curve from pH Measurements

As a further means of characterizing this standard gelatin preparation, experiments were carried out to determine its complete dissociation curve, or curve of combination with H^+ and OH^-. This curve was obtained by making up a series of solutions of approximately constant gelatin concentration with varied concentrations of HCl or NaOH, rather than by the titration of a single gelatin solution. Solutions were made up by weight, as before, and concentrations were referred to 1000 gm. of water. The pH determinations were made at 30°C. with hydrogen electrodes in the vessels described by Simms (1923), liquid junction being made with saturated KCl in an open stop-cock. The standard of pH was 1.075 for 0.1000 molal HCl, possible variations in liquid junction potentials being neglected. Instead of plotting the total concentration of acid or alkali directly against pH, which would give an experimental titration curve, a calculation was made to get the number of equivalents of H^+ or OH^- combined with each gm. of gelatin. The curve of this quantity against pH is the dissociation or combination curve of the protein. The calculation was made by the method previously used (1928–29), which is essentially similar to that of Cohn (1925).

In the case of solutions containing HCl, the quantity plotted was $\frac{b_H}{g}$ where b_H is given by the relation

$$b_H = m - \frac{a_H}{\gamma_H}. \tag{3}$$

Here g is the gelatin concentration in gm. per 1000 gm. H_2O, b_H is the molality of combined H^+, m the total molality of HCl, and a_H the activity of H^+ as obtained from the pH measurements by the relation

$$pH = -\log a_H. \tag{4}$$

The quantity γ_H is the activity coefficient of the hydrogen ion. This was obtained from measurements of pH of HCl solutions without protein, the assumption being made that γ_H for a given value of m was not altered by the presence of protein. The values of γ_H so

obtained were practically identical with those given by Scatchard (1925) for 25°, as might be expected, since the standard of pH used in this work was obtained by arbitrarily assigning to 0.1 M HCl at 30° the value of γ_H which Scatchard found for 25°.

In the case of NaOH solutions, the quantity plotted was obtained by a similar equation,

$$b_{OH} = m - \frac{a_{OH}}{\gamma_{OH}}. \tag{5}$$

Here m is the total molality of NaOH, b_{OH} the molality of combined OH^-, a_{OH} the activity of free OH^-, and γ_{OH} its activity coefficient. The values of a_{OH} and γ_{OH} were obtained from pH measurements by the following method, which is believed to be new. Measurements were made of the pH of a series of NaOH solutions without protein, the values being referred to the HCl standard already mentioned. The pH values for NaOH solutions were not so reproducible as in the case of HCl, the divergence of duplicate solutions prepared and measured at different times being in some cases as much as 0.03 pH. By averaging four or five measurements at each concentration, fairly reliable values were obtained for 0.02, 0.04, 0.05, and 0.1 M. From these average pH values, values of the quantity pH $-$ log m were calculated and plotted against $\sqrt{m}$. The four points fell within 0.002 pH of a straight line whose equation was

$$\text{pH} - \log m = 13.724 - 0.4\sqrt{m}. \tag{6}$$

By extrapolating this line to zero concentration, it was possible to calculate values of the activity coefficient of OH^- for any solution of NaOH of molality up to 0.1. From the relations

$$a_H a_{OH} = K_w \tag{7}$$

and

$$a_{OH} = m\,\gamma_{OH} \tag{8}$$

it follows that, for pure NaOH solutions,

$$a_H = \frac{K_w}{m\gamma_{OH}} \tag{9}$$

or

$$\text{pH} - \log m = \text{p}K_w + \log \gamma_{OH}. \tag{10}$$

From the theoretical equation (10) and the empirical equation (6) it appears that pK_w corresponds to 13.724 and $\log \gamma_{OH}$ to $-0.4\sqrt{m}$. It may be noted that Michaelis (1922) gives 13.725 as the value of pK_w at 30°, while the limiting law of the Debye-Hückel theory states that $\log \gamma$ for a uni-univalent electrolyte should approach $-0.5\sqrt{m}$ in very dilute solutions.

Here use was made only of the empirical relation

$$pK_w + \log \gamma_{OH} = 13.724 - 0.4\sqrt{m} \tag{11}$$

as a means of getting values of $\frac{K_w}{\gamma_{OH}}$ for use in equation (12) with the pH measurements of the gelatin solutions containing NaOH. Equations (5) and (7) give

$$b_{OH} = m - \frac{K_w}{a_H \gamma_{OH}} \tag{12}$$

in which m is the total molality of the NaOH in the gelatin solution, a_H is obtained from the pH data by equation (4), and the ratio $\frac{K_w}{\gamma_{OH}}$ is obtained from equation (11). Finally the values of b_{OH} obtained by equation (12) were divided by g, the gelatin concentration, to get the number of mols of OH^- combined with 1 gm. of gelatin. The results of some of the measurements and calculations are given in Table II, and the complete dissociation curve of the gelatin is given in Fig. 2, which shows the amounts of combined H^+ or OH^- per gm. of gelatin as a function of pH.

It may be seen from Fig. 2 that on the acid side the amount of H^+ bound per gm. gelatin reached a limiting value of 9.6×10^{-4} equivalents, which is in good agreement with the value 9.58 found by the more precise method of Section IV. The curve crosses the line of zero combination at pH 4.85, which is the value found for the isoelectric point of this gelatin in the previous study (1930–31*b*). On the alkaline side the curve shows a flattening near pH 8, indicating that one set of groups is completely neutralized in this region. This is in agreement with the titration curves of Loeb (1922) and the writer (1923–24), as recalculated by Cohn (1925). The amount of bound OH^- at this

TABLE II

Combination of Gelatin with H^+ from HCl and OH^- from NaOH as Calculated from pH Measurements at 30°C.

A. Gelatin + HCl

m	g	pH	$-\log \gamma_H$	m_H	b_H	$\frac{10^4 b_H}{g}$
0.1022	20.4	1.157	0.075	0.0828	0.0194	9.51
0.0510	20.35	1.566	0.064	0.0315	0.0195	9.58
0.0306	20.3	2.015	0.056	0.0110	0.0196	9.65
0.01993	19.95	2.678	0.049	0.00235	0.01758	8.82
0.01529	20.3	3.230	0.046	0.00066	0.01463	7.21
0.00996	19.9	3.714	0.039	0.00021	0.00975	4.89
0.00498	19.9	4.188	0.031	0.00007	0.00491	2.46
0.00199	19.9	4.544	0.021	0.00003	0.00196	0.98
0	120.0	4.86	0	0.00001	0	0

B. Gelatin + NaOH

m	g	pH	$pK_w + \log \gamma_{OH}$	m_{OH}	b_{OH}	$\frac{10^4 b_{OH}}{g}$
0.00200	20.0	5.220	13.707	$10^{-8.5}$	0.00200	1.00
0.00500	20.0	6.000	13.696	$10^{-7.7}$	0.00500	2.50
0.00718	20.2	7.659	13.690	$10^{-6.0}$	0.00718	3.55
0.00821	20.2	8.763	13.689	0.00001	0.00820	3.73
0.01000	20.0	9.624	13.684	0.00009	0.00991	4.96
0.01235	20.1	10.155	13.680	0.00030	0.01205	6.00
0.01547	20.1	10.651	13.675	0.00095	0.01452	7.22
0.02000	20.0	11.219	13.668	0.00356	0.01644	8.23
0.0474	31.1	11.932	13.637	0.0197	0.0277	8.91
0.0974	41.4	12.361	13.599	0.0578	0.0396	9.57

m = molality of HCl or NaOH.

g = gm. gelatin per 1000 gm. H_2O.

$\text{pH} = 1.075 + \frac{E - E_{HCl}}{0.06015}$, where E = E.M.F. observed with calomel cell against H_2 electrode in gelatin solution, and E_{HCl} = E.M.F. observed with the same calomel cell against H_2 electrode in 0.1000 M HCl.

$-\log \gamma_H$ was obtained from a plot of $\text{pH} + \log m$ against $\sqrt{m}$, using data for HCl without protein.

m_H was obtained from the relation $-\log m^H = \text{pH} + \log \gamma$.

$b_H = m - m_H$.

$pK_w + \log \gamma_{OH}$ was obtained from a plot of $\text{pH} - \log m$ against $\sqrt{m}$, using data for NaOH without protein.

m_{OH} was obtained from the relation $pK_w + \log \gamma_{OH} - \text{pH} = -\log m_{OH}$.

$b_{OH} = m - m_{OH}$.

point was somewhat higher for the standard gelatin than for the preparations studied earlier, being about 3.6×10^{-4} equivalents per gm. This figure was roughly confirmed by a colorimetric titration with phenol red as indicator, which gave an end point of 3.3×10^{-4} equivalents NaOH per gm. gelatin at pH 7.9.

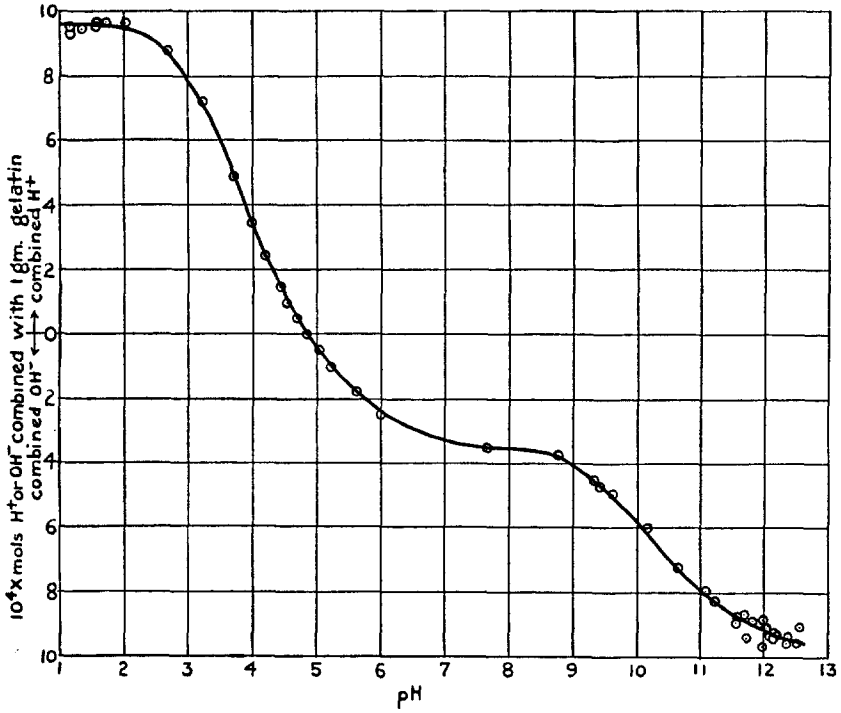

FIG. 2. Combination curve of the standard gelatin preparation with H^+ from HCl and OH^- from NaOH, as obtained from pH measurements at 30°C. The abscissae are pH values; the ordinates are $10^4 \times$ mols of H^+ or OH^- combined with 1 gm. of gelatin.

The remainder of the curve, in the more alkaline region, is quite different from Cohn's curve of collected data on various gelatins, in that it shows no flattening in the vicinity of 6×10^{-4} equivalents of combined OH^-. The scattering of the points near pH 12 is such that it cannot be said definitely that a maximum combining capacity for OH^- is indicated. At any rate it seems certain that this gelatin, or

its products of decomposition, combined with as much as 9 or 9.5 $\times$ 10^{-4} equivalents of OH^- between pH 12 and 12.5. This high combining capacity seems to have been characteristic of this particular lot of gelatin (Eastman Standard Gelatin, Lot 48). Another lot purchased later from the same source (Eastman Purified Gelatin, Lot 51) gave a combination curve practically identical with that here published, except that it flattened off at about 8.4 $\times$ 10^{-4} equivalents of combined OH^- per gm. between pH 11.5 and 12.5. Apparently it may be concluded either that the preparation of gelatin is in need of further standardization, or else that the maximum combining capacity for alkali is a property not well suited to the exact characterization of gelatin preparations.

VI

SUMMARY

It has been found possible to obtain constant dry weights of sheet gelatin only by drying *in vacuo* at temperatures below 100°C. for a period of several weeks. Values are given for the ash and nitrogen content, the specific conductivity, and the isoelectric point of a standard gelatin preparation. By the method of E.M.F. measurements of cells without liquid junction, of the type Ag, AgCl, HCl + gelatin, H_2, it has been found that this gelatin in 0.1 M HCl combines with a maximum of 9.58 $\times$ 10^{-4} equivalents of H^+ and 2.0 $\times$ 10^{-4} equivalents of Cl^-. By means of pH measurements with the hydrogen electrode and a KCl junction, the combination curve of this gelatin with H^+ from HCl and OH^- from NaOH has been determined between pH 1.1 and 12.5.

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