U. S. DEPARTMENT OF COMMERCE NATIONAL BUREAU OF STANDARDS

RESEARCH PAPER RP765

Part of Journal of Research of the National Bureau of Standards, Volume 14, February 1935

COMBINING WEIGHT OF COLLAGEN

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ABSTRACT

The adsorption of hydrochloric acid by collagen was determined in solutions varying in acid concentration from 0 to about 1.7 molal. The solutions were nearly or completely saturated with sodium chloride. The high concentration of salt was used to repress the swelling of the collagen, and to provide a reference substance for the measurement of the adsorption. The quantity of acid reacting with the collagen was found to vary continuously with the concentration of acid. The nature of the relation between the concentration and the quantity of acid combined indicates that at least 3 types of compounds are formed by the collagen and the hydrochloric acid. At low concentrations, the combining weight of the collagen is about 1,000. It is deduced that 3 nitrogen atoms in each unit of 38 are contained in strongly basic groups. The equivalent weight of collagen is indeterminate under the conditions of this experiment. So many types of nitrogen-containing groups are reacting with the acid that not even an approximate value of the true equivalent weight can be selected.

CONTENTS

I. INTRODUCTION

Determination in aqueous solution of the amount of acid which combines with collagen presents a serious difficulty. The collagen swells, taking up solution amounting to from six to eleven times its dry weight. It has been shown by Procter (1) [1] that concentration of acid in the gel phase is less than that in the free solution, the only condition being that a partially ionized salt be formed. No precise correction for the consequent error is possible in the present state of knowledge of the system.

This difficulty may be partly overcome by adding salt to the solution. This decreases swelling and tends to equalize the concentration of acid in the two phases. However, there is a question as to how closely the behavior of the collagen in the salt solution follows that in a solution free from salt.

[1] The figures, 1 to 10, given in parentheses here and elsewhere in the text correspond to the numbered references at the end of this paper.

V. Kubelka (2) measured the adsorption of hydrochloric acid on hide powder in 10 percent, 20 percent, and saturated (26 percent) solutions of sodium chloride. In the case of the saturated solutions, a remarkable relationship between concentration of acid and the amount of acid adsorbed was indicated. The curve showing the relation of adsorption to concentration exhibited a sharp break at about one-tenth normal and an adsorption of about 1.03 millimoles of acid per gram of dry hide powder. As there were only 12 determinations in the concentration range up to approximately normal acid, the form of the curve was not well defined. The purpose of the present work was to determine the nature of the relation between the adsorption and the concentration of acid in solutions nearly saturated with sodium chloride, with the object of determining the combining weight of collagen with respect to acid in dilute solution.

II. EXPERIMENTAL MATERIAL AND METHOD

The collagen was prepared from limed and unhaired steer hide. After deliming and bating, the hide was washed with distilled water, and dried by washing first with 95 percent ethyl alcohol and then with xylene. After drying, the grain and flesh surfaces were split off, and the remaining collagen was cut into narrow strips about 3 cm long. This material was prepared for sampling by conditioning it in an atmosphere in equilibrium with a solution saturated with sodium chloride.

Most of the determinations were made in solutions which were only 93 to 99 percent saturated with sodium chloride. Thirty determinations at low acid concentrations were made in solutions saturated with sodium chloride.

Approximately 5 g of collagen was used with about 50 ml of solution. One day was allowed for the system to approach equilibrium, a series of determinations having shown that equilibrium with respect to the adsorption was practically attained in this time. The final acid concentration was measured by pouring the solution into a beaker and titrating it, the weight of the solution poured off being measured by reweighing the flask and its contents. An excess of sodium hydroxide solution was added, which was then titrated with dilute hydrochloric acid. With the exception of the titration of this excess, all standard solutions were measured by weight. In case the solution was not saturated with sodium chloride, its concentration was determined by evaporating to dryness and heating the residue at 150° C. The weight of this residue was corrected for the collagen present, and for sodium chloride equivalent to the sodium hydroxide added in the titration. An additional correction of 0.27 percent was necessary because the sodium chloride retained that much water under these conditions. In each case the total nitrogen in the solution poured off was determined by the Kjeldahl method. This quantity was always small, exceeding the equivalent of 15 mg of collagen in only four determinations at the highest acid concentrations.

In 15 determinations the reversibility of the reaction was tested by adding about 45 ml of a nearly saturated solution of sodium chloride

to the collagen and solution remaining in the flask after the completion of a regular determination. The procedure of the regular determination was then repeated, using the known weight and concentration of the solution added to calculate the amount of sodium chloride in the system. These determinations will be referred to as "reversed" in the text and in figure 3.

In the determinations at the four highest acid concentrations a considerable hydrolysis of the collagen took place. The average collagen equivalent of the nitrogen in the solution poured off was 84 mg for these four while for the four determinations next lower in acid concentration the corresponding average was 8 mg. The presence of the dissolved products of hydrolysis interfered somewhat with

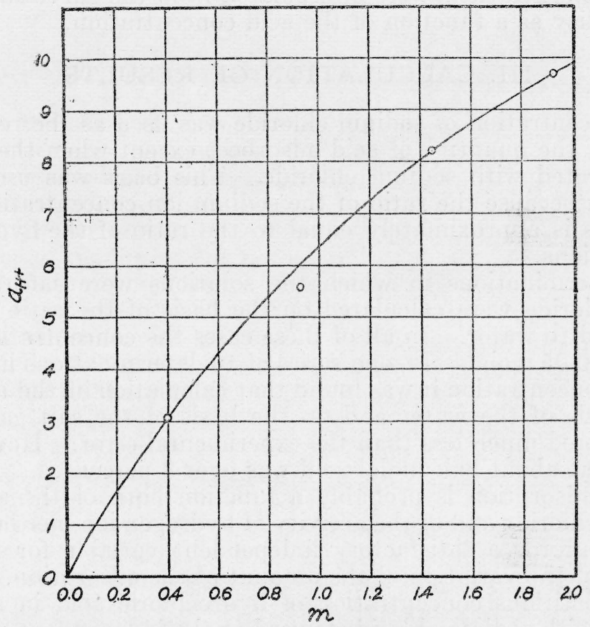

FIGURE 1.—*The activity of hydrogen ion in solutions of hydrochloric acid saturated with sodium chloride at 25° C.*

m is the molality of hydrochloric acid.

the titration. A more serious error arose from the failure of the system to reach equilibrium with respect to the distribution of the products of hydrolysis, apparently because the rate of hydrolysis was greater than the rate of diffusion of the products. For the determinations that were reversed from these four the average value of the collagen equivalent of the nitrogen in the solution poured off was 26 mg, while the average for the other determinations in the same range of concentration was 6 mg. The excess of hydrolytic products in the collagen phase makes the calculated value of the adsorption too high.

The hydrogen ion activities in a series of solutions of hydrochloric acid saturated with sodium chloride were measured with the hydrogen electrode. The results are shown in figure 1, where a_{H^+} repre-

sents the activity of hydrogen ion and m is the molality of hydrochloric acid.

The curve for interpolation was selected so as to be consistent with the results of Thomas and Baldwin (3) at low concentrations of acid. Activities of hydrogen ion in the experimental solutions were calculated from these data and those of H. C. Jones (4), assuming the linearity of the relationb etween pH and molarity of sodium chloride at constant molarity of acid.

Data presented by J. Erskine Hawkins (5) were used to calculate the activity of hydrochloric acid.

The solubility at 25° C of sodium chloride in hydrochloric acid solution was determined at four acid concentrations. Values at intermediate concentrations were interpolated from the curve showing the total molality as a function of the acid concentration.

III. CALCULATION OF RESULTS

The concentration of sodium chloride was used as the reference in calculating the quantity of acid adsorbed, except when the solutions were saturated with sodium chloride. This basis was used for the calculation because the ratio of the sodium ion concentrations in the two phases is approximately equal to the ratio of the hydrogen-ion concentrations.

The determinations in which the solutions were saturated with sodium chloride were calculated on the basis of the ratio of hydrochloric acid to water. In all of these cases the concentration of acid was below 0.05 molal. In one series of 10 determinations in the same range of concentration it was found that calculation of the adsorption on the basis of the water and on the basis of the salt gave results which differed much less than the experimental error. However, the difference at about half molal acid was over 3 percent.

As the adsorption is probably a function both of the activity of hydrogen chloride and of the activity of hydrogen ion, neither of these activities affords a satisfactory independent variable for the representation of the variation of the amount of the adsorption. For this reason a fictitious concentration of hydrochloric acid in a solution saturated with sodium chloride is used as the independent variable in presenting the data. This concentration is calculated as that giving the same activity of hydrogen chloride as that in the actual solution. This calculated concentration is designated as m. Concentrations of this type calculated from the activity of hydrogen chloride and from that of hydrogen ion are in close agreement at concentrations below 1 molal.

The quantity used to present the results is the fraction of the total nitrogen which reacts with the acid. This is calculated as the ratio of the number of millimoles of acid adsorbed to the number of milligram atoms of nitrogen in the sample of collagen. This fraction is designated by the letter f.

Figures 2, 3, and 4, show the experimental results. Some of the data at low acid concentrations are omitted from figures 2 and 3 because of the small scale of these figures.

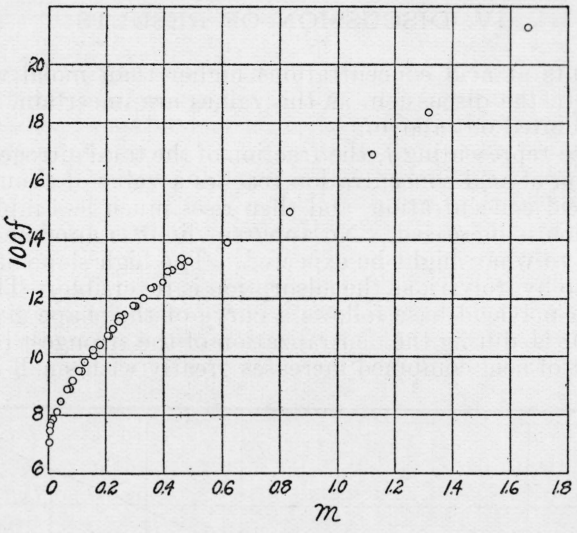

FIGURE 2.—*The adsorption of hydrochloric acid by collagen as a function of the molality of hydrochloric acid in solutions saturated with sodium chloride.*

f is the fraction of the total nitrogen which reacts with the acid.

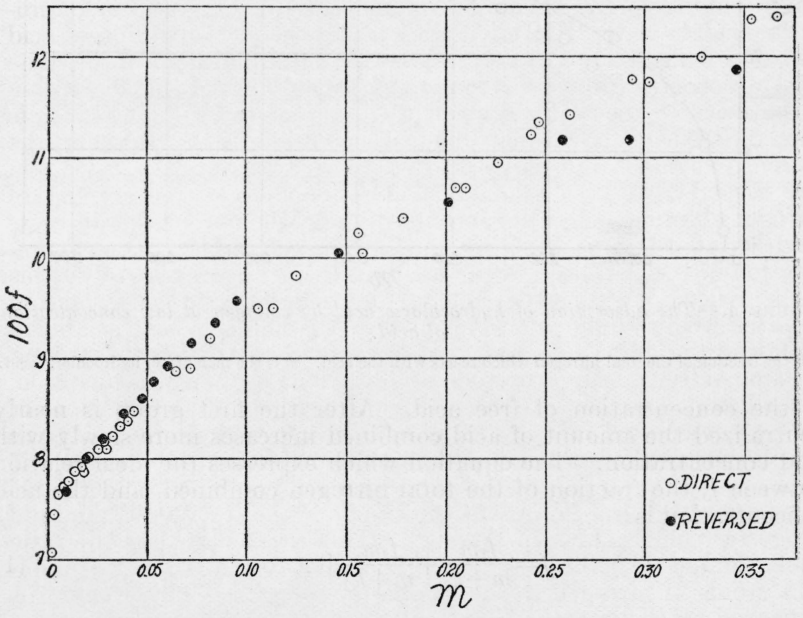

FIGURE 3.—*The results of the experiment testing the reversibility of the adsorption of hydrochloric acid by collagen in solutions saturated with sodium chloride.*

f is the fraction of the total nitrogen which reacts with the acid. *m* is the molality of hydrochloric acid.

IV. DISCUSSION OF RESULTS

The results at acid concentrations higher than molal will not be considered in the discussion, as the values are uncertain, because of the error pointed out above.

The curve representing f, the fraction of the total nitrogen reacting, as a function of acid concentration reaches a value of about 0.08 at a very low acid concentration, and then rises much less rapidly as the concentration is increased. No apparent limit is approached, which is contrary to what might be expected. The high slope of the curve is not due to hydrolysis, as the adsorption is reversible. The neutralization of a polyacid base follows a curve of the shape given by the data. That is, during the neutralization of the strongest basic group the amount of acid combined increases greatly with small increments

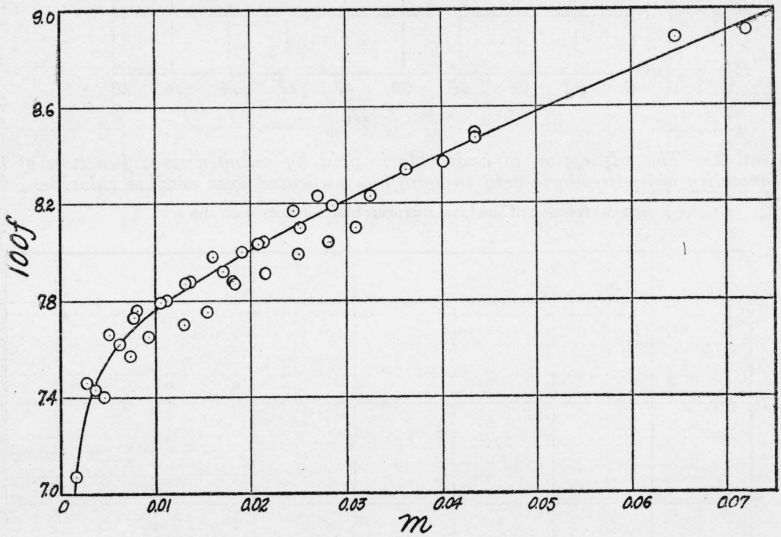

FIGURE 4.—*The adsorption of hydrochloric acid by collagen at low concentrations of acid.*

f is the fraction of the total nitrogen which reacts with the acid. m is the molality of hydrochloric acid.

in the concentration of free acid. After the first group is nearly neutralized the amount of acid combined increases more slowly with acid concentration. The equation which expresses the ideal relation between f, the fraction of the total nitrogen combined, and the acid concentration is

$$f=\frac{f_1 m}{m+k_1}+\frac{f_2 m}{m+k_2}+\cdots, \tag{1}$$

where f_1, f_2, etc., are the fractions of the total nitrogen contained in basic groups which form salts having the hydrolysis constants k_1, k_2, etc., respectively. Equation 1 is hardly better than a rough approximation, because the variations of the activity coefficient of the acid and the ionization of the collagen salts formed are not taken into account. However, the constants in the numerators have a precise significance, and it will be shown that one of them can be evaluated.

A plot of the results at low acid concentrations on a scale larger than that of figure 2 gives a better representation of the form of this part of the curve. Such a plot is shown in figure 4. It may be seen that on this scale there is very little curvature at concentrations higher than 0.01 molal. As the sum of all the terms beyond the first in eq 1 varies almost linearly with m it may be represented accurately by a term of the form $m/(a+bm)$, where a and b are em-

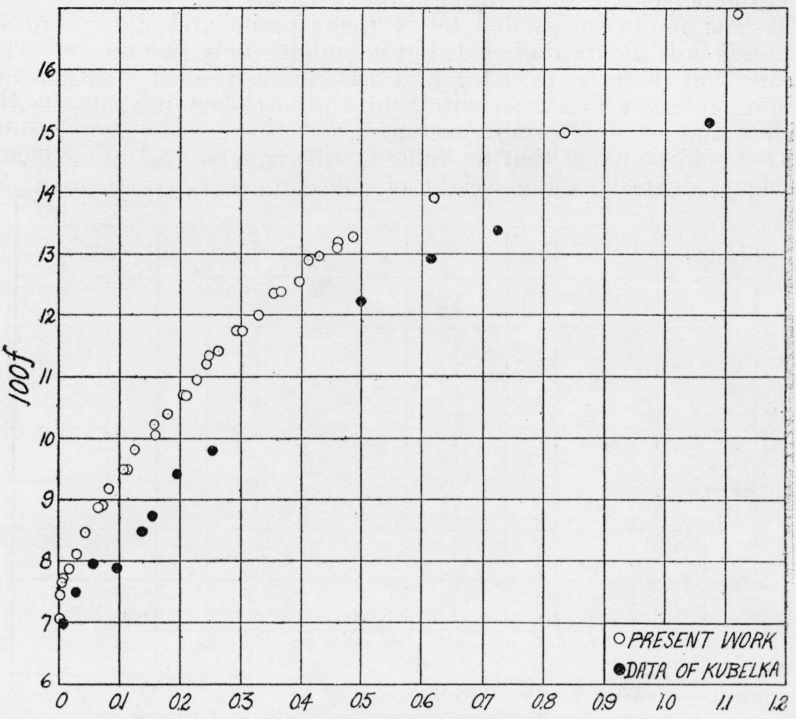

FIGURE 5.—*The adsorption of hydrochloric acid by collagen in solutions saturated with sodium chloride.*

The data of Kubelka (2) are compared with those of the present work. (f is the fraction of the total nitrogen which reacts with the acid. m is the molality of hydrochloric acid).

pirical constants having no physical significance. The smooth curve of figure 4 represents values calculated from the equation

$$f=\frac{0.0766\ m}{m+0.000136}+\frac{m}{11\ m+4.77}. \qquad (2)$$

The four constants were evaluated to make the calculated f fit four selected points. This expression fits the experimental data in this concentration range.

The constant 0.0766 in eq 2 represents the fraction of the total nitrogen which occurs in groups which are much more strongly basic than others in the collagen. It has been shown (6) that the number of nitrogen atoms in a structural unit of collagen is 38 or a multiple thereof. The constant 0.0766 is equal to 2.91/38. Thus it may be concluded that 3 nitrogen atoms of each group of 38 are involved in this first part of the neutralization.

One effect of the predominance of these 3 nitrogen atoms in the reaction with acid in dilute solution is to give the collagen an apparent equivalent weight of about 1,000 in dilute solution. This results from the fact that collagen contains about 17.9 percent of nitrogen, so that 78 g of collagen contains 1 g atom of nitrogen. Then the combining weight is (38/3)(78), or about 1,000.

More accurately, the combining weight is (177.4)(100/P), where P is the percentage of nitrogen in the collagen.

It was found impossible to fit the experimental data with an expression of the form of eq 1, but containing only two terms. This means that there are in collagen at least three types of nitrogen-containing groups which react with acid, the corresponding salts having widely different hydrolysis constants.

It should be noted that no value for the true equivalent weight of collagen, defined as the weight of collagen which reacts with one

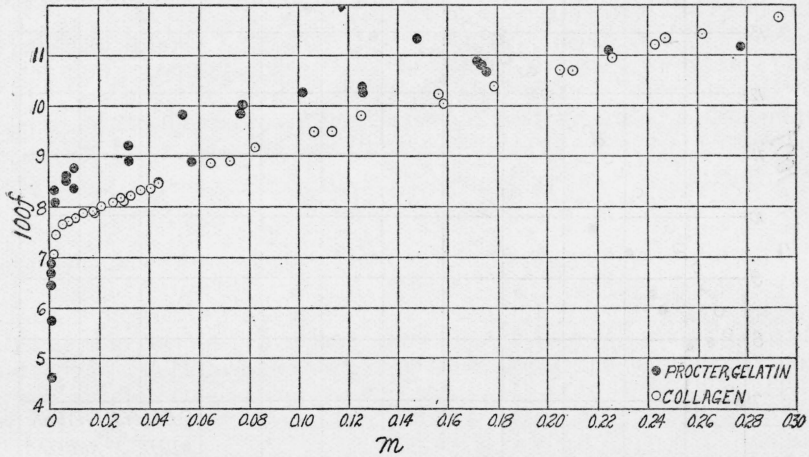

FIGURE 6.—*A comparison of the adsorption of hydrochloric acid, from solutions saturated with sodium chloride, by gelatin and collagen.*

The data for gelatin are taken from the work of Procter (1), and the data for collagen from the present work. f is the fraction of the total nitrogen which reacts with the acid. m is the molality of hydrochloric acid.

equivalent of acid at an indefinitely high concentration, can be derived from these data.

Figure 5 compares Kubelka's (2) results for hide powder with the present work. It does not seem probable that the apparent break exhibited in the graph of Kubelka's results is real. However, it is of interest to note that Kubelka offers a value of 977 for the combining weight of dry hide powder, deriving this figure from the amount of adsorption at the point where the break in his curve occurs.

Procter (1) measured the adsorption of hydrochloric acid by gelatin in solutions saturated with sodium chloride. Atkin and Douglas (7) criticized Procter's method of determining the total acid in the gelatin, pointing out that excess base was required to make the solution of gelatin alkaline to phenolphthalein. The result of this error was to give high values for the amount of acid combined with the gelatin. However, the correction suggested by Atkin and Douglas can hardly be applied quantitatively under the conditions of Procter's

experiment. Figure 6 shows Procter's results, calculated on the basis of 17.9 percent of nitrogen in the dry gelatin, compared with the present work on collagen.

Procter and Wilson (8) used Procter's data to derive an equivalent weight of 768 for gelatin. This value is open to doubt, because of the experimental error pointed out by Atkin and Douglas, and because the amount of acid combined was limited by the range of acid concentration covered in the experiments.

Kubelka and Wagner (9) measured the adsorption of hydrochloric acid by hide powder, and its swelling in acid solutions. From their data Beek (10) calculated the amount of acid combined with the collagen, and derived a value of 969 for the equivalent weight. This would naturally be lower than the value found in the present work, because no account was taken of the reaction of the acid with more than 1 type of nitrogen-containing group. The value corresponding to reaction of 3/38 of the total nitrogen is 993, when calculated on the basis of 17.86 percent of nitrogen.

Most of the published values for the combining weights of collagen and gelatin are between 1,050 and 1,150. No detailed examination of the methods used in obtaining these values is necessary. It is sufficient to point out that the error due to ionization of the protein salt formed was not taken into account, and that as a result the values are too high.

V. SUMMARY

The adsorption of hydrochloric acid by collagen was measured in solutions containing high concentrations of sodium chloride.

The graph showing the relation between the fraction of the total nitrogen in the collagen which reacts with hydrochloric acid, and the concentration of acid, is a smooth curve. The adsorption is reversible.

There is a series of at least three types of nitrogen-containing groups in the collagen which react with hydrochloric acid, forming salts having widely differing hydrolysis constants.

Three nitrogen atoms in each unit of 38 are in groups much more strongly basic than the remainder. Collagen has an apparent equivalent weight of about 1,000 in dilute acid solution.

VI. REFERENCES

1. J. Chem. Soc. **105,** 313 (1914).
2. Kolloid–Z. **23,** 57 (1918).
3. J. Am. Chem. Soc. **41,** 1981 (1919).
4. Carnegie Inst. Pub., Washington, no. 60, p. 93 (1907).
5. J. Am. Chem. Soc. **54,** 4480 (1932).
6. BS J. Research **8,** 549 (1932) RP434.
7. J. Am. Leather Chemists Assoc. **19,** 528 (1924).
8. The Chemistry of Leather Manufacture, 2d ed., **1,** 140. (The Chemical Catalog Co., Inc., New York, 1928).
9. Kolloid–Z. **46,** 107 (1928).
10. BS J. Research **5,** 1109 (1930) RP249; Ind. Eng. Chem. **22,** 1373 (1930).

WASHINGTON, December 19, 1934.

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