CCVIII. THE ABSORPTION OF WATER BY GELATIN.

PART VII. THE INFLUENCE OF TEMPERATURE ON SWELLING IN ACID SOLUTIONS AND THE COMBINA- TION OF GELATIN WITH HYDROCHLORIC, NITRIC OR SULPHURIC ACID.

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It has long been known that the influence of aqueous solutions of inorganic substances on the physical properties of proteins is modified by the nature of the ions present. Loeb [1922] has stated that at the same p_H dibasic acids and diacidic bases induce only half as much water absorption by gelatin as monoacidic bases and monobasic acids.

The simple mathematical relationship postulated by Loeb has been denied by various workers: Ostwald, Kuhn and Böhme [1925], investigating the swelling of gelatin, found that the volume at maximum swelling was not related to the valency of the anion of the acid; Küntzel [1926], working on the contraction of tendons from the tail of the rat in acid solutions, and also the author [1929], working on the increase in weight of purified leaf gelatin, found that at the same p_H the influence of nitric acid is slightly greater than that of hydrochloric acid, which in turn is considerably greater than that of sulphuric acid at $p_H < 3.2$. In very dilute solutions however no difference in the effects of the three acids can be distinguished. Isgarischev and Pomeranzeva [1926] have measured the swelling of powdered caseinogen in $N/20$ solutions of a large number of organic acids and suggest that the pre-dominating factor is the nature and structure of the anion of the acid.

The author [Pleass, 1929] discussed the question of the magnitude of the swelling and also reported that the p_H for maximum swelling was determined by the anion of the acid. Recently published work [Jordan-Lloyd, 1930; Pleass, 1930] has shown that, although this p_H in solutions of hydrochloric acid is independent of the temperature between 0° and 18° , in solutions of sodium hydroxide and calcium hydroxide the p_H for maximum swelling becomes greater as the temperature is decreased. It was decided, therefore, that the influence of temperature on solutions of sulphuric acid should also be investigated.

The experimental material and method were exactly similar to that previously described [Jordan-Lloyd and Pleass, 1927; Pleass, 1930]. Pieces of purified leaf gelatin were weighed and immersed in solutions of sulphuric acid of varying concentrations at constant temperatures.

After 7 days' immersion at 0°, 4 days at 12°, or 3 days at 18° or 25°, the pieces of gelatin were removed from the solutions, lightly blotted and weighed. The final weights are expressed as a percentage of the initial weight, corrected to its weight after drying at 100°. The figures for the percentage weights of the gelatin are plotted graphically against the final p_H of the solutions.

In Fig. 1 are plotted the curves for the water absorption of gelatin from solutions of sulphuric acid of final p_H 0.1–5.0 at temperatures of 0°, 12°, 18° and 25°.

Fig. 1. The influence of temperature and p_H on the absorption of water by gelatin in solutions of sulphuric acid.

The first inspection of the curves shows that the lower the temperature, the less the water absorption. At 25°, as the p_H moves from 5.0 to 3.3, the water absorption of the gelatin increases to so great a degree that in the more concentrated solutions of sulphuric acid the gelatin is too soft to weigh. At 12° and 0°, the degree of swelling is much less than at 18°, and also at the lower temperatures the peak of maximum swelling is less sharply defined. Over the whole range of p_H studied, the magnitude of the swelling lies between 1000 and 1800 at 0°, 1100 and 2800 at 12° and 1300 and 5100 at 18°. At 0° and 12°, the gelatin does not dissolve in solutions of sulphuric acid as acid as p_H 0.1. In an analogous manner, Jordan-Lloyd [1930] found that gelatin did not dissolve in *N* hydrochloric acid at 0°, but at 18° the gelatin dissolved in all solutions of $p_H < 0.9$.

A closer examination of the curves reveals that not only is the degree of swelling and the sharpness of the peak dependent on the temperature, but

that the maximum occurs at p_H 3.0 at 18°, at p_H 2.6 at 12° and at p_H 2.4 at 0°.

The fact that the p_H for maximum swelling of gelatin in solutions of sulphuric acid varies with temperature in an analogous manner to the alteration in solutions of sodium and calcium hydroxides, while the p_H for maximum swelling in solutions of hydrochloric acid is independent of temperature (from 0 to 18°) is very striking. Jordan-Lloyd [1930] and the author [Pleass, 1930] suggested that in the alkaline systems this phenomenon might be due to some structural change in the gelatin molecule, possibly hydrolysis, which proceeded to a greater extent at the higher temperature. This opinion was based largely on the fact that gels containing sodium hydroxide decrease in p_H on keeping for several days at 18°, even when atmospheric carbon dioxide is rigidly excluded. In order to investigate more fully the possibility of the

Fig. 2. Titration and acid fixation curves of a 1 % solution of gelatin with hydrochloric, nitric or sulphuric acid at 18–20°.

hydrolysis of gelatin occurring in the presence of mineral acids, the p_H of a 1 % solution of gelatin containing various known quantities of hydrochloric, nitric or sulphuric acid were determined by means of the glass electrode and plotted against the normality of the acids present (see Fig. 2) and after keeping for a week at 18–20° these gels were re-examined. It was found that within the limits of the experimental error no changes in the p_H were detected. Thus, no evidence was obtained of hydrolysis of the gelatin on keeping in any of the acid solutions.

The p_H of solutions of the acids alone were also measured and the results are plotted against the corresponding normality of the acids (Fig. 2). The amount of acid fixed by the gelatin has been derived by plotting the differences between the curve for the acids alone and the curve for acid in the presence of the gelatin at each p_H . The curves for the combination of acid are

remarkably similar for the three acids examined—in fact they are probably identical within the experimental error of the method. With all three acids, a flat portion of the curve occurs from about p_H 2.4 to 1.9 and at a normality of acid of 0.0085. This indicates that the combining weight of gelatin is the same for the three acids (approximately 1180) from p_H 2.4 to 1.9. Assuming that the gelatin behaves as a weak base and combines with strong acids in accordance with the following equation in solutions of $p_H > 1.9$

therefore

$$\frac{[\text{G}][\text{HX}]}{[\text{G.HX}]} = K.$$

In very dilute solutions, the reaction will proceed predominantly from left to right since there is an excess of gelatin. That is, most of the acid is in combination with the gelatin (*cf.* Fig. 2 where the curve for gelatin and acid is almost superimposed on the curve for gelatin combined with acid).

In moderately dilute acid solutions, say p_H 3.5–2.5, the reaction proceeds in both directions since there is neither an excess of acid nor of gelatin to force the reaction to proceed to completion. An indication of the degree of dissociation of the gelatin-acid compound is given by the shaded area in the graphs (Fig. 2).

In more concentrated acid solutions (p_H 2.5–1.9), the excess of acid causes the reaction to proceed from left to right. The flatness of the dotted curve over this range shows that the full quota of acid for the complete neutralisation of the most strongly basic groups (probably the amino-groups) has been taken up. It can be seen that half neutralisation of the gelatin occurs at about p_H 3.7 from which it may be deduced that the first basic dissociation constant is 5×10^{-11} . This value is identical within the limits of experimental error with that obtained by Atkin and Douglas [1924] working at 24° and using the hydrogen electrode for the determinations of the p_H . In strongly acid solutions (p_H 1.9) the gelatin takes up more acid, probably by means of the acid combining with the weaker basic groups (possibly the imino-groups). This is in agreement with the work of Jordan-Lloyd and Mayes [1922] and Atkin and Douglas [1924].

Thus the p_H for maximum swelling of gelatin in solutions of hydrochloric and nitric acids at temperatures between 0° and 18° and in solutions of sulphuric acid at 0° approximates very nearly to the highest p_H at which the first stage of fixation of these acids by the gelatin at 18–20° is complete, namely p_H 2.4. Since the combination of gelatin with all three acids occurs stoichiometrically at 18–20°, the differences in the magnitude of the swelling and the p_H for maximum swelling of the gelatin in solutions of the three acids at this temperature may possibly be explained by the specific characteristics of the anions and their influence on the physico-chemical properties of water, since these differences become less as the temperature is decreased until at 0°, the freezing point of water, they are negligible.

Jordan-Lloyd has recently [1931] established that the magnitude of the water absorption of both dried and undried gelatin gels is proportional to the square of the centigrade temperature from 0 to 18° for all the external conditions studied, *i.e.* water, solutions of acids, bases and salts. She develops the idea that the influence of temperature on the activity of the water, one of the common factors in all the systems, may be the direct cause of the influence of temperature on swelling. She emphasises the point that the swelling of the gelatin is proportional to the square of the degrees on a scale of which the zero is based on a property of water.

As previously indicated [Pleass, 1930], at 0° at the p_H of maximum swelling in hydrochloric and sulphuric acids, the hydrogen ion concentration is almost equal to that of the hydroxyl ion concentration at which maximum swelling occurs at 0° in the alkaline system (p_H 11.5–11.6). On superficial examination, therefore, it would appear that, assuming that the p_H of the solutions in which maximum swelling of the gelatin occurs gives an indication of the basicity of the gelatin in acid solutions and of the acidity of the gelatin in alkaline solutions, the isoelectric point of the gelatin should coincide with the p_H of neutrality. Owing, however, to the logarithmic nature of the p_H scale, small variations in the ionic concentration at the positions of maximum swelling are inappreciable, while similar variations in ionic concentration are of considerable magnitude near the neutral point when expressed on the p_H scale.

It should also be indicated that in all the experiments on the swelling of gelatin dealt with in this series of papers, the p_H values considered have been those of the external solutions, since the determination of the p_H of the internal gel involves the melting of the gel and may be attended by unknown errors. It is probable however that an accurate knowledge of the internal p_H of the gel would be very valuable when correlating the swelling of gelatin with the combination of gelatin with acids and alkalis.

SUMMARY.

The water absorption of purified leaf gelatin in solutions of sulphuric acid from p_H 0.1 to 5 at 0°, 12°, 18° and 25° has been studied. Increase in temperature causes an increase in water absorption until at 25° in all solutions more acid than p_H 3.2 the gelatin is too soft to weigh.

Maximum swelling occurs at p_H 3.0 at 18°, p_H 2.6 at 12° and p_H 2.4 at 0°.

Curves for the combination of gelatin with hydrochloric, nitric and sulphuric acids from p_H 5.0 to 1.7 are given. The three curves are practically identical, exhibiting a flat portion from p_H 2.4 to 1.9, and indicate a combining weight for gelatin of 1180 and a first basic dissociation constant of 5×10^{-11} .

No evidence of hydrolysis of gelatin in these acid solutions (*i.e.* $p_H > 1.5$ at 18–20°) has been obtained.

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