CLXI. THE ABSORPTION OF WATER BY GELATIN.

V. THE INFLUENCE OF CALCIUM HYDROXIDE.

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THE absorption of water by gelatin may occur by at least three different mechanisms. When immersed in distilled water, electrically neutral (isoelectric) gelatin absorbs water by imbibition. This water, which is held by capillary forces, may be regarded as penetrating throughout the system. In solutions of salts at about p_H 5, gelatin absorbs more water than the water of imbibition. In these conditions, both positive and negative ions of the salt are apparently absorbed equally by the gelatin, causing hydration of the gelatin. This type of swelling is referred to as lyotropic or salt swelling. In either acid or alkaline solution, water absorption is induced by a different mechanism, the gelatin, acting as a weak base or a weak acid, combines with the electrolyte present to form ionisable salts with a non-diffusible ion. This leads to the production of an internal osmotic pressure within the gel which induces further water absorption. This type of swelling is referred to as osmotic swelling. Electrolytes may exert both an electrostatic and a lyotropic influence on the physical properties of gelatin. The electrostatic influence depends solely on the charge carried by the ions and, in the same conditions, is equal for the same concentration of different ions of similar charge. The lyotropic effect, however, is dependent on the specific nature of the ions. Generally, the electrostatic influence predominates in the more dilute solutions, and the lyotropic influence does not become patent until a greater concentration is reached.

The curves for the water absorption of gelatin in hydrochloric, nitric and sulphuric acids coincide from p_H 5.0–3.2, but at concentrations greater than 0.001 *N*, the specific nature of the acids becomes apparent [Pleass, 1929]. The most important observation is that the p_H value of the position of maximum swelling is determined by the anion of the acid. Hydrochloric and nitric acids induce maximum swelling of 6500 and 7000 % respectively, on the dry weight of the gelatin at p_H 2.6, while the maximum in sulphuric acid is only about 5100 % and occurs at p_H 3.0. Previously it had been stated [Loeb, 1922] that bivalent acids induce swelling which at the maximum is only about half as great as that produced by univalent acids. The author, however, found that

maximum swelling in sulphuric acid is about four-fifths of the maximum in hydrochloric or nitric acid. It is obvious, therefore, that even in solutions of acid, where swelling has been assumed to be entirely electrostatic, the specific nature of the ions plays a part. The author has extended the study to that of calcium hydroxide in order to draw a comparison between the bivalent ion calcium and the univalent ion sodium.

EXPERIMENTAL.

The water absorption has again been measured by the percentage change in weight, calculated on the air-dry weight at 100°, of pieces of purified leaf gelatin when immersed in the experimental solutions for a period of 3 days at a temperature of 18° or above; in experiments at 12°, the pieces were allowed to remain for 4 days and at 0° for 7 days. The solutions were prepared, and in every instance brought to the temperature of the experiment before the pieces of gelatin were added. The experimental material was prepared as previously described [Jordan-Lloyd and Pleass, 1927], but extreme care was taken to exclude carbon dioxide from the solutions. All bottles used were coated internally with paraffin wax and either sealed with wax or else allowed to stand in containers in the presence of soda-lime during the whole experiment. When determining the p_H value of dilute solutions of lime, a modification of a method described by Cameron [1929] was used. A titration curve has been obtained by means of the glass electrode for 10 cc. of B.D.H. "universal buffer solution" with $N/10$ hydrochloric acid and $N/10$ sodium hydroxide solution over a p_H range from 1.5–10. 0.05 cc. of a suitable indicator and 5 cc. of each of the experimental fluids are measured into comparator test-tubes, in this order and without shaking. The test-tubes are corked immediately and are then arranged in order of their p_H values. 0.1 cc. of the same indicator and 10 cc. of the "universal buffer solution" are measured into a small flask. This solution is titrated with $N/10$ hydrochloric acid or $N/10$ sodium hydroxide solution until the colour matches that of each of the experimental tubes in succession, exactly as described by Jordan-Lloyd [1930]. For solutions more strongly alkaline than p_H 9.5, either the glass electrode or the closed hydrogen electrode was used for the p_H determination.

Only solutions of calcium hydroxide without the addition of any mineral salt have been examined, over the p_H range 5 to 12.5 and at temperatures of 0, 12, 18, 22 and 25°. The curves correlating water absorption and final p_H value at the various temperatures are shown in Fig. 1.

Swelling at 18°. The curve for 18° is generally similar in form to that for the sodium hydroxide system at the same temperature [Jordan-Lloyd and Pleass, 1927], but it shows some marked differences. The two curves nearly coincide from p_H 5 to 8, suggesting that in very dilute solutions the physico-chemical properties of the two hydroxides are almost identical. In more concentrated solutions, the difference becomes more pronounced. In solutions of sodium hydroxide, the swelling of gelatin increases rapidly with increase in

the p_H value until it reaches its maximum at about p_H 10. The curve of the calcium hydroxide system, however, has a much longer portion of low gradient, extending from p_H 7 to 9.5, and reaches its maximum at p_H 11. Complete solution of the gelatin occurs in both systems in solutions more alkaline than p_H 12.1. The maximum swelling in solutions of sodium hydroxide is 4700 %, while for the calcium hydroxide system, the maximum is 4200 %. Loeb [1922] gives curves correlating the relative volume of 1 g. of solid gelatin with the internal p_H value of the melted gel in solutions of uni- and bi-valent bases.

Fig. 1. The water absorption of leaf gelatin over a p_H range 5 to 12.5 at temperatures of 0°, 12°, 18°, 22°, 25°, in presence of $\text{Ca}(\text{OH})_2$.

Although the maximum of the curve for the bivalent systems relates to solutions more alkaline than the point of maximum swelling in the univalent systems, the difference between his two curves is much greater than that between those of the author. In Loeb's curves, at all corresponding p_H values, the swelling in solutions of bivalent bases is about half that in solutions of the univalent bases. This is a very important difference from the results of the author who finds that in dilute solutions the curves are nearly identical, and that at the maxima the swelling in the bivalent system is about seven-eighths of that in the univalent system. The author's results for swelling of gelatin in

solutions of uni- and bi-valent acids are also at variance with those of Loeb. At 0° , however, the maximum increase in water absorption above that of the water of imbibition of the gelatin at the same temperature is approximately half as great as that in the sodium hydroxide system. It is possible that the "valency rule" postulated by Loeb should refer only to the increase in water absorption above that of the water of imbibition and in conditions in which hydrolysis and protein degradation are reduced to a minimum, *i.e.* at low temperatures.

The influence of temperature. The influence of temperature on the magnitude of the water absorption of gelatin from solutions of calcium hydroxide is shown

Fig. 2. The influence of temperature on the water absorption of leaf gelatin at the maximum, at p_H 10.0 and at p_H 8.1, in presence of $\text{Ca}(\text{OH})_2$.

in Fig. 1. Speaking generally, the swelling is less the lower the temperature (see Fig. 2) and at the lower temperatures the swelling varies less with change in p_H value. At 0° , the limits of water absorption lie within 1100 and 2000 % for the whole range of p_H from 5 to 12.5. At low temperatures, the peak of maximum swelling is flatter and less clearly defined than at higher temperatures. With increase in temperature, the curves not only run to a much sharper maximum, but also the maximum occurs at lower p_H values, 11.55 at 0° , 11.3 at 12° , 11.0 at 18° , 10.8 at 22° and 10.55 at 25° (see Fig. 3). From 0° to 18° , the portion of the curve which is of very low gradient becomes shorter with

rise of temperature, while at 22° it has almost disappeared and a second maximum has occurred at p_H 8.1, which is the precursor of solution at 25°. Jordan-Lloyd has found similar results with the sodium hydroxide system [1930], but sodium hydroxide has a greater swelling and solvent action than calcium hydroxide. At 25°, the gelatin dissolves in all solutions of sodium hydroxide of p_H value greater than 9.7, while, although in solutions of calcium hydroxide at this temperature the gelatin is very soft and swollen, it does not dissolve completely in solutions less alkaline than p_H 11.3 except in solutions of p_H value between 7.5 and 8.3.

Since osmotic swelling is due to osmotic forces acting in opposition to the cohesive forces of the gel, the increase in swelling with increase in temperature is probably largely due to the decrease in rigidity of the gel with increasing temperature. The author [1930] has investigated the setting of gelatin and finds that in the presence of sodium hydroxide at 0° and 12°, the minimum concentration of gelatin which will form a gel increases very slightly as the

Fig. 3. The influence of temperature on the p_H value of maximum water absorption in presence of $\text{Ca}(\text{OH})_2$.

alkalinity of the solution is increased from p_H 5.0 to 10.0. From p_H 10.0 to 11.0, the gradient of the curve becomes steeper until, in solutions of greater alkalinity, gelation necessitates the presence of an increasingly high concentration of gelatin. In all the experimental solutions of p_H value greater than 11.5, the formation of a gel is inhibited. At 18°, the gradient of the gelation curve is steeper and setting is inhibited in all solutions of greater alkalinity than p_H 10.2, while at 25° the gradient of the setting curve is increased to such an extent that gelation is inhibited in solutions more alkaline than p_H 5.6. This is most probably due to some chemical change in the gelatin, possibly hydrolysis, which proceeds much more rapidly as the temperature is increased.

Titration curves for a 1 % solution of gelatin with hydrochloric acid or sodium hydroxide were obtained by measuring the p_H value of the solution immediately after mixing the electrolyte with the gelatin solution and again after keeping the solution for a week at 18°. (Atmospheric carbon dioxide was

rigidly excluded.) These curves are reproduced in a paper by Jordan-Lloyd [1929]. The p_H values of the acid solutions of gelatin are practically unaltered by keeping the solutions, but the p_H values of the alkaline solutions of greater alkalinity than p_H 6.5 decreased appreciably during one week. This suggests that sodium hydroxide causes considerable chemical change in the gelatin at 18°, which is probably responsible for the absence of a maximum on the setting curves for the alkaline solutions and thus reduces the symmetry of the system about the isoelectric point. The maximum in these setting curves for the acid gels occurs at about p_H 2.4 at all temperatures between 0° and 18°. Jordan-Lloyd [1930] also found that the p_H of maximum swelling in solutions of hydrochloric acid occurs at p_H 2.5–2.6 both at 0° and at 18°. It is very interesting to note that at 0° the maximum swelling of gelatin occurs at p_H 11.5–11.6 in both sodium hydroxide and calcium hydroxide solutions and at this p_H value the hydroxyl ion concentration is practically identical with the hydrogen ion concentration of maximum swelling in hydrochloric and nitric acid solutions between 0° and 18°, *i.e.* in each case in conditions in which hydrolysis and protein degradation have been reduced to a minimum. It seems probable, therefore, that the shift of the p_H of maximum swelling of the gelatin in alkaline solutions with increase in temperature may be caused by some chemical change in the gelatin molecule at higher temperature which leads to reduction in the strength of the gel. The shift in the p_H value at which maximum swelling occurs with increase in temperature is considerably greater in the sodium hydroxide system than in the calcium hydroxide system.

These results support Jordan-Lloyd's opinion [1930] that in order to simplify the study of the physical properties of gelatin observations should be made at a temperature of 0°, at which temperature hydrolysis is reduced to a minimum. Much of the work on the physico-chemical properties of gelatin, especially that relating to combination with bases and equivalent weight estimations, is certainly open to criticism if the condition of the gelatin in the presence of alkali is altering both with time and increase of temperature. The zone of p_H value from 5 to 9.5 presents a difficult problem. The author is unable to offer any explanation of the portion of the curve of low gradient which occurs in the most dilute alkaline solutions or to explain the fact that at 25° the gelatin dissolves completely at p_H 8 and does not do so in solutions at p_H 9.5. The discussion in a paper by Jordan-Lloyd [1930] contains some relevant ideas.

SUMMARY.

1. The influence of calcium hydroxide solutions on the water absorption of gelatin over a range of p_H from 5 to 12.5 and at temperatures from 0° to 25° has been investigated.
2. It has been found that the gelatin swells more at higher temperatures than at low.
3. The p_H value at which maximum swelling occurs is dependent on the temperature. At 0° the maximum occurs at p_H 11.5 and at 25° at p_H 10.5.

4. The magnitude of the swelling in solutions of calcium hydroxide is always less than, but much greater than half, that of the swelling in solutions of sodium hydroxide at the same temperature and p_H value. At 0° , however, the maximum increase in water absorption from solutions of calcium hydroxide above that of the water of imbibition of the gelatin at the same temperature is about half as great as that in the sodium hydroxide system. Also, at 0° , maximum swelling occurs at p_H 11.5 in both the calcium hydroxide and sodium hydroxide systems and at this p_H value the hydroxyl ion concentration is approximately equal to the hydrogen ion concentration of maximum swelling in hydrochloric acid and nitric acid. The author stresses the importance of working at low temperatures, at which the gelatin less readily undergoes chemical change.

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