CXXV. THE ABSORPTION OF WATER BY GELATIN. PART II.

THE NITRATE SYSTEM.

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IN a previous paper [Jordan-Lloyd and Pleass, 1927] the absorption of water by gelatin in a system containing hydrogen, hydroxyl, sodium and chlorine ions was considered. The present paper deals with a system which is similar, except that nitrate ions are present in place of the chlorine ions. The experimental material and method were precisely the same as described in the previous paper. The ash content of the gelatin used in the present experiments varied from 0.02–0.01 %.

Three variables influencing water absorption were examined: p_H , temperature (t) and concentration of sodium nitrate (M). The experimental results are summarised in the curves shown in the figures.

The influence of p_H (nitric acid or sodium hydroxide) on the swelling of gelatin at 18° is shown in Fig. 1. In the absence of inorganic salts the swelling curve shows a minimum at p_H 5.0, confirming the value previously found using hydrochloric acid. This value of p_H may therefore again be regarded as the isoelectric point. The curve shows a maximum in the acid range at p_H 2.6. The p_H value at the peak of the acid swelling curve coincides with the value found for the maximum swelling in the hydrochloric acid system, but the degree of swelling is slightly greater. In the absence of inorganic salts the system on the alkaline side of the isoelectric point is identical with that described in the previous paper, *i.e.* there is a well-defined maximum of alkaline swelling at p_H 10.0 and an additional broadly defined maximum between p_H 7.5 and 7.9. At p_H values less than 1 or greater than 11.6, the gelatin dissolves in the external fluid.

It will be convenient again to consider separately the behaviour of gelatin in the four zones of hydrogen ion concentration centring respectively on p_H 2.6 (acid zone); p_H 10.0 (alkaline zone); p_H 5.0 (isoelectric zone); and p_H 7.0 (neutral zone).

The acid swelling zone (p_H 1–4).

In the previous paper it was shown that in the absence of sodium chloride the swelling of gelatin in hydrochloric acid is in accordance with the predictions of the Proctor-Wilson equation [Procter, 1914; Procter and Wilson, 1916].

When sodium chloride is present, the repression of the swelling by the salt in concentrations less than 0.01 M occurs according to the predictions of Loeb's equation [Loeb, 1922]. In greater concentrations of sodium chloride, however, the repression of the swelling is considerably greater than the value calculated from this equation and coagulation of the gelatin may occur. In the nitrate system the form of the curves of swelling is similar to that of the corresponding curves for the chloride system, but certain differences are apparent. In the absence of mineral salts the degree of swelling in nitric acid is slightly greater than in hydrochloric acid at all values of p_H , the swollen weights at p_H 2.6, for instance, being 7000 and 6500 % respectively on the dry weight of the gelatin. The addition of sodium nitrate to the nitric acid system causes greater repression of swelling than does the addition of an equivalent concentration of sodium chloride to the hydrochloric acid system at all salt concentrations from 0.01 to 1 M . Coagulation, defined as a reduction of the swelling of the protein below the value found at the isoelectric point (1300 %), which appears at 0.5 M in the acid chloride system, is first observed in nitric acid solutions at a nitrate concentration of 0.7 M . Coagulation can occur in the chloride system at all reactions below p_H 4.0, in the nitrate system at p_H values less than 3.5. Coagulation, therefore, occurs less readily in the nitrate than in the chloride system, although in 2 M nitrate solutions, at p_H values less than 2.3, the degree of coagulation is greater than in the corresponding chloride systems, while at all p_H values greater than 2.3 the gelatin dissolves. The experimental curves showing the relationship between swelling and salt concentration in acid solutions (Fig. 7, p_H 2.0, 2.5 and 3.0) show marked differences from the corresponding curves for the chloride system and only agree approximately with Loeb's theoretical curve when the salt content is low. At p_H 2.0 the nitrate curve is similar in form to the chloride curve, suggesting that it represents a compound function, the sum of two (or possibly, in this case, three) simple functions of the salt concentration (M). When M is less than 0.2, the swelling curve, as in the chloride system, resembles in form the curve of Loeb's equation; when M is between 0.2 and 0.5 the curve is inflected and indicates that it may be turning to correspond to a negative linear function of $\log M$; later, however, at concentrations of nitrate greater than 0.5 M there is a further slight inflection of the curve which suggests that the more concentrated salt solutions have not only a coagulating, but also a solvent action on the gelatin. The curve, therefore, illustrates the influence of three effects of the nitrate ion. The solvent action of solutions more concentrated than 0.1 M is shown more emphatically at p_H 3.0 (Fig. 7). At this and higher values of p_H the solvent action completely masks any coagulating action.

Swelling in nitric acid is strongly influenced by temperature (Fig. 2). At temperatures below about 12° the temperature coefficient for the water absorption is not nearly as great as at higher temperatures. This may possibly be connected with the transformation of the gelatin from the gel form (gelatin A) to the sol form (gelatin B) [Smith, 1919], a change which begins at about

15°. In the absence of inorganic salts complete solution in the acid zone takes place at about 20° in nitric acid.

The alkaline swelling zone (p_H 8.0–11.5).

The maximum gelatin swelling in solutions of sodium hydroxide free from salts occurs at p_H 10.0 (Fig. 1). The effect of sodium chloride in the alkaline zone appears to be limited in salt concentrations up to 2 *M* to a repression of the swelling in accordance with the theoretical expectations of Loeb's equation. The effect of sodium nitrate in this zone shows some very marked differences

Fig. 2. t varying, M constant at zero, p_H constant at 2.0, 2.5, 4.2, 5.0, 6.0, 7.0 and 10.5.

Fig. 3. t varying, p_H constant at 5.0, M constant at 5 values varying from zero to 1 *M*.

from the effect of sodium chloride. At 18° in concentrations less than 0.1 *M* the nitrate represses the alkaline swelling in a manner similar to the chloride, although for equimolecular concentrations of the two salts the repression is greater with nitrates than with chlorides, showing that even in dilute salt solutions the specific nature of the ions must be considered. At concentrations of nitrate greater than 0.1 *M* the dominant effect of the salt is to increase water absorption (see Fig. 7, p_H 10.0). This undoubtedly arises from a solvent action. Complete solution at all p_H values in the alkaline zone occurs at 18° in all concentrations of nitrate greater than 1 *M* (Fig. 1).

The isoelectric zone (p_H 4–6).

The position of minimum swelling in a system free from all salts is at p_H 5.0. The presence of sodium nitrate in isoelectric or nearly isoelectric solutions causes increased absorption of water by gelatin, the amount absorbed at 18° being proportional to the logarithm of the salt concentration (M), where M is less than 0.1 (Fig. 7, p_H 5.0). At salt concentrations greater than 0.1 M the water absorption is directly proportional to the salt concentration (Fig. 7, p_H 5.0; and Fig. 8) until at concentrations of 1 M when the gelatin is on the verge of solution. The effect of temperature on the action of the salt is very great. At 0° and 12° the water absorption is proportional to the logarithm of the concentration of the sodium nitrate at all concentrations examined (Fig. 5). At 18° and 22° the swelling increases rapidly with increase of salt concentration and complete solution takes place at 1 M and 0.2 M respectively. Nitrates, therefore, have a much greater dispersing action on gelatin B, which is present in the system only at temperatures above 15°, than on gelatin A. The effect of temperature on the absorption of water at different concentrations of nitrates is shown in Fig. 3. Below 12° and in concentrations of salt less than 0.1 M the effect of the nitrates is similar to that of the chlorides and there is further a maximum of absorption of water at about 12° to 15°. A rise of temperature above 18° leads to a greater swelling and solvent action with chlorides than with nitrates.

The neutral zone (p_H 6–8).

In this zone, in solutions of sodium hydroxide free from salts, swelling increases with increase of alkalinity, reaching a broadly defined maximum extending from p_H 7.0 to 8.0 (Fig. 1). The effect of adding sodium nitrate in this zone is somewhat the same as the effect of adding sodium chloride, *i.e.* up to a concentration of nitrate of 0.1 M the salt depresses the alkaline swelling; in concentrations greater than this, however, gradual increase in the nitrate concentration decreases the repression of the swelling (Fig. 7) until at a nitrate concentration of 1 M at 18° swelling is very nearly the same as in the absence of all salts, but the gelatin is on the verge of solution. At 2 M solution occurs in this as in all other zones. The solvent action of nitrates, which is the factor responsible for the secondary swelling in the more concentrated salt solutions, is very nearly the same in neutral, isoelectric and alkaline solutions (Figs. 7 and 8), and in all three zones it first makes itself apparent at a nitrate concentration of 0.1 M . At 18° the solvent action in all three zones is greater for nitrates than for chlorides in equivalent concentrations.

The effect of temperatures at p_H 7.3 and at different concentrations of nitrates is shown in Fig. 4. The curves closely resemble the corresponding curves for the chloride system. In concentrations of nitrate less than 0.1 M they also resemble curves at p_H 5.0 (Fig. 3) in showing a temperature of maximum swelling, which at p_H 7.3 is at 18°. In solutions of 0.1 and 1 M swelling increases rapidly with temperature until solution is reached. The

curves at all temperatures show definite minima of swelling which are much more strongly marked than in the chloride system. The salt concentration at the minimum of swelling in nitrates varies inversely with the temperature, minima being obtained at 0.20, 0.13, 0.10 and 0.04 M at 0°, 12°, 18° and 22° respectively.

Fig. 4. t varying, p_H constant at 7.3, M constant at 5 values varying from zero to 1 M .

DISCUSSION.

The behaviour of gelatin in the presence of nitric acid or nitrates presents at many points a strong contrast to its behaviour in the presence of hydrochloric acid or chlorides, although when considered from a physical-chemical viewpoint these systems are very similar, both nitric and hydrochloric acids being strong monobasic acids which combine with sodium hydroxide to produce readily soluble, highly ionised salts. The obvious difference in the two systems lies in the relative positions of chloride and nitrate in the Hofmeister series. "Hofmeister" effects are generally associated with fairly strong solutions of salts, and the contrast between the two systems is certainly most marked when the concentration of the salt is greater than 0.1 M , although it is by no means limited to the higher concentrations of chlorides and nitrates. Nitric acid produces greater swelling than does hydrochloric acid and in the acid, neutral

and alkaline zones the depression of swelling caused by nitrates, even in dilute solution, is greater than that caused by chlorides at the same concentrations. Thus, even in dilute solutions, where the effect of the salts has been assumed to be entirely electrostatic, the divergence from a strictly quantitative relationship between the two systems suggests that the specific nature of the ions plays a part.

Fig. 5. M varying, p_H constant at 5.0, t constant at 0°, 12°, 18°, 22°.

It was shown in the earlier work on chlorides that the coagulation of gelatin by these salts depends on the p_H of the system. It will be seen on referring to Fig. 7 of the earlier paper [Jordan-Lloyd and Pleass, 1927] that at the position on the swelling-salt concentration curves where the swelling in acid solutions is equal to that in distilled water, there is a point of inflection which marks the beginning of coagulation. Coagulation may, therefore, be

given a numerical significance by defining it as the reduction of the water content of a gel below that amount which the isoelectric colloid would contain in equilibrium with distilled water. Chlorides coagulate gelatin at any p_H less than 4.0 if present in sufficient concentration. It can be seen that nitrates in acid solutions can also coagulate gelatin, the conditions of p_H and salt

Fig. 6. M varying, p_H constant at 7.3, t constant at 0°, 12°, 18°, 22°.

concentration which can bring this about being more strictly defined than in the chloride systems, coagulation only occurring at p_H values less than 3.5 (Figs. 7 and 9). Fig. 9 shows clearly that there is a change in system at the coagulation point, which depends on the p_H of the system. At p_H values less than 3.0, nitrate concentrations greater than 0.6 M (approx.) lead to coagulation.

At the corresponding point in the chloride system, 0.3 M salt was sufficient

to start coagulation. The coagulating action of the nitrates only exists over a limited range of p_H and salt concentrations. Beyond these narrow limits excess of nitrate leads to solution of the gelatin. At 1 M concentration nitrates dissolve gelatin over a p_H range from 4.0 to 5.0; at 2 M over all p_H values greater than 2.3 (Fig. 7). At p_H 4.1 the system is interesting—above 1 M concentration of sodium nitrate the gelatin dissolves, below 0.7 M the weight of the swollen gelatin is constant at a value of 1900 % on the dry weight, independent of the salt concentration. A similar point where water

Fig. 7. M varying, t constant at 18°, p_H constant at 2.0, 2.5, 3.0, 5.0, 7.0 and 10.0.

absorption is independent of salt concentration is found at p_H 4.4 in the chloride system, the swollen weight in this case being 1400. At p_H 3.5, increasing the nitrate concentration from 0.6 to 1 M leads to an increase of swelling followed by solution at higher concentrations. The effect of the nitrates at p_H 3.5 is, therefore, similar to its effect in the neutral and alkaline zones (see Fig. 8). In these zones nitrates at a concentration of less than 0.1 M depress swelling, at concentrations between 0.1 M and 1 M they lead to an increase in swelling proportional to the concentration of the salt. In the iso-electric zone sodium nitrate up to 0.1 M resembles sodium chloride in leading

to an absorption of water which is proportional to the logarithm of the concentration of the salt, and which is, therefore, probably due to a similar mechanism, possibly adsorption. At concentrations greater than $0.1\ M$ the effect of the addition of salt becomes identical with its effect in all other systems on the alkaline side of the isoelectric point (Fig. 7). The nitrates, therefore, have in solutions greater than $0.1\ M$ an additional effect on the system which is either absent from the chloride systems or does not show itself in concentrations as weak as $2\ M$ or less.

Fig. 8. M varying, t constant at 18° , p_H constant at 5.0, 7.0, 8.0, 9.0 and 10.0.

In the previous paper it was pointed out that the mechanism of water absorption was very different in acid or alkaline swelling on the one hand, and in swelling under the action of salts on the other. The contrast between these two types is emphasised by the amount of swelling which can take place before the gelatin loses its property of cohesion. In acid swelling gelatin can absorb 70 times its own weight of water and still retain its cohesive properties and in alkaline swelling the capacity for holding water in a jelly phase is of the same order. In salt swelling, however, the capacity for holding water is only about half this value and solution in salt solutions appears to set in very rapidly once a critical water content has been passed.

SUMMARY.

1. Sodium nitrate, up to a concentration of 0.6 *M*, in the presence of nitric acid suppresses the swelling of gelatin due to the acid. At greater concentrations its effect depends on the p_H . From p_H 4.0 to 3.5 increasing salt concentration causes swelling; at p_H 3.0 it causes coagulation of the gelatin; at 2 *M* coagulation occurs at all p_H values less than 2.3 but solution at all values greater than 2.3. At p_H 4.1 over a range of 0–0.7 *M* nitrates have no effect on swelling; beyond this range they dissolve the gelatin.

2. Up to a concentration of 0.1 *M* sodium nitrate in the presence of sodium hydroxide suppresses the swelling of gelatin due to the alkali. At concentrations greater than this, increasing concentration of nitrate is accompanied by

Fig. 9. *M* varying, *t* constant at 18°, p_H constant at 1.5, 2.0, 2.5, 3.0 and 3.5.

an increase in swelling, directly proportional to the concentration of the salt present. In solutions more concentrated than 0.1 *M* the gelatin is dissolved at 18°.

3. The swelling of gelatin due to nitric acid and sodium hydroxide increases as an exponential function of the temperature.

4. At the isoelectric point (p_H 5.0), sodium nitrate promotes water absorption, swelling being proportional to the logarithm of the concentration of the salt up to 0.1 *M*. At greater concentrations swelling is directly proportional to the concentration of the nitrate.

5. Over a p_H range from 5 to 10 and at concentrations of salt greater than 0.1 *M*, swelling in solutions of sodium nitrate is influenced only by the salt concentration and is independent of the p_H .

6. At p_H 5.0 a rise of temperature from 0° to 12° is accompanied by an increase in swelling which is the same in the absence of nitrate and at all concentrations of nitrate up to 1 *M*. In the absence of salt, swelling is at a maximum at 12° , in dilute solutions of nitrate at about 15° to 18° ; in stronger solutions there is no temperature of maximum swelling, the gelatin passing into solution as the temperature is increased. The temperature of solution is lower the greater the concentration of nitrate present.

7. At p_H 7.0, in the absence of salt or in dilute solutions of nitrates, the effect of temperature on swelling is similar to its effect at p_H 5. The temperature influences the position of minimum swelling at varying concentrations of nitrate, the salt concentration registered at this point diminishing with rising temperature.

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