CLXXI. THE ABSORPTION OF WATER BY GELATIN.

VI. THE INFLUENCE OF THE THICKNESS AND ORIGINAL CONCENTRATION OF THE GEL.

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(Received August 13th, 1931.)

INTRODUCTION.

In the previous papers in this series on the behaviour of dry leaf gelatin when immersed in watery media, the effects of variations in the composition of the external medium have been studied [Jordan-Lloyd, 1920, 1930; Jordan-Lloyd and Pleass, 1927, 1928; Pleass, 1929, 1930]. It is, however, obvious that if (as frequently postulated in the course of the work) gelatin gels are systems with a definite internal architecture, variations in the methods used in preparing the dry gelatin might also be expected to influence the behaviour of the system. It has indeed been recognised from the earliest days of investigations into the physical chemistry of gelatin that the characteristics of the particular sample of gelatin used must be taken into consideration. Hofmeister [1890] and Pauli a few years later [1897] both introduced a factor allowing for the thickness of the dry gelatin in equations expressing the rate of swelling of gelatin in water. The idea that both external and internal dimensions of the dried gelatin play a part in its subsequent behaviour in water has indeed been latent in much of the literature, the ratio of surface to mass, for instance, having been considered by other workers besides Hofmeister and Pauli. The problem of the influence of the internal structure of the gelatin has, however, only been investigated in comparatively recent times.

Procter [1911] showed that the swelling of dried gelatin gels in water was partly controlled by the percentage concentration at which the original gelatin jelly had been allowed to set. Using gelatin dried from 5, 10 and 20 % jellies, he showed that swelling in water was inversely proportional to the original concentration. Gortner and Hoffmann [1922] confirmed this observation; using gelatin gels dried from jellies of 2·5, 5, 10, 15 and 20 % concentration, they showed that both the rate and amount of swelling in water were inversely proportional to the concentration at setting. Their experimental figures also give an indication that in contact with water the 10 % gel has a stability of concentration greater than that of gels which are more concen-

trated or more dilute, for, while gels dried down from more concentrated jellies regain more water on soaking than they lost during evaporation, and gels dried down from less concentrated jellies do not regain as much water as they lost, the gel dried from a 10 % jelly returns approximately to its former state on soaking in water. The special stability of the 10 % gelatin jelly has been demonstrated in a very attractive manner by Kunitz [1928], who used jellies of different gelatin concentrations from 3 to 20 % and immersed them without previous drying in $M/1000$ acetate buffer solutions at p_H 4.7. All the jellies which contained more than 10 % of gelatin absorbed water and all those which contained less gave up water to the surrounding fluid. Northrop [1927] and Northrop and Kunitz [1927] had previously shown that the equilibrium weight of gelatin jellies in water could be expressed by an equation involving the concentration at setting as a variable, and Kunitz, in his later paper, showed that the equation applied equally whether that equilibrium were attained by either gaining or losing water.

The work reported in the present paper deals with the influence of both thickness and original concentration of the jelly on the swelling of gelatin gels in water and in salt solutions, and at acid and alkaline reactions. The experimental evidence shows that both the thickness of the gelatin pieces and the original concentration of the gels at setting influence the amount of water absorbed at equilibrium by gels under different external conditions. This is equally true whether the gels are placed in the experimental conditions in the undried state or whether they have been taken to dryness as a first stage. The experiments, however, also show that the process of drying the gels at temperatures below their melting points very greatly reduces the capacity to absorb water under all the experimental conditions studied (the influence of the drying being inversely proportional to the original concentration) and that, therefore, changes occur in the gelatin gels during drying, which are not fully reversed on restoring to an aqueous medium.

Method and materials.

Most of the experiments were carried out with Coignet's Gold Label Gelatin, purified by the method previously described [Jordan-Lloyd and Pleass, 1927], but as large quantities of material were required, the commercial gelatin was sometimes used without purification. The latter possesses the practical advantage over the purified gelatin of being sufficiently soluble to make a 50 % gel. The gelatin used is, in every case, indicated in the experimental records. The gelatin was dissolved by soaking in cold water and then warming to 50° and adjusting the volume to the required concentration. Gels were cast by pouring the warm sol into moulds of hard paraffin wax and, when required for use in the dry condition, were dried as rapidly as possible at room temperature under an electric fan.

The experiments were carried out by placing the weighed gelatin pieces in stoppered glass jars with the experimental solution and a small amount

of toluene to prevent bacterial growth. Water absorption was followed in every experiment by weighing at suitable intervals of time until equilibrium (or substantial equilibrium) had been attained. Time curves are not given in this paper, but equilibrium may be considered to be reached at 23° in 3 days, at 18° in 4 days, at 7° in 7 days, at 0° in 10 days. Gels of pure gelatin come to a true equilibrium at 0° and 7°, but continue to absorb water slowly for an indefinite period at higher temperatures, due possibly to the solubility of the gelatin at these temperatures or else to the development of "fatigue" [see Northrop, 1927]. Gels of commercial gelatin do not come into a true equilibrium at any temperature, though at low temperatures they come to a state in which further changes are slow. In most cases, the absorption of water from (1) distilled water, (2) 0.2 *M* sodium chloride solution, (3) *N*/1000 hydrochloric acid, (4) *N*/1000 sodium hydroxide was followed at various temperatures. In some cases, experiments on absorption of water from salt solutions were extended to include a range of concentration from 0.1 to 2.0 *M* and also to include sodium nitrate and sulphate as well as chloride.

Experiments on the thickness of the gelatin.

Experiments on the influence of the original thickness of the experimental pieces were carried out, both on undried and on dried gels. For comparative purposes, the thickness of the undried gels was not measured in the undried condition but was measured from the unused portion of the experimental castings after drying. Gels of 5, 10 and 15 % original concentration were used in the experiments on thickness and a range of from 0.06 to 0.65 mm. of dry thickness was covered. Thin sheets of gelatin cannot be cast from the 15 % gels since the surface forces prevent the warm sol from spreading over the wax moulds unless a minimum thickness can be attained. Experimental results at 0° both for dried and undried gels in water, salt, acid and alkaline solutions are shown in Fig. 1 (*a*) and (*b*). It can be seen that with water and sodium chloride solution the effect of the thickness is comparatively slight, although in all cases the thin pieces contain a higher percentage of water than the thick ones. With the acid and alkaline solutions, however, the thickness of the pieces has a very considerable influence on the amount of water absorbed. In Fig. 1 (*c*) these swelling curves for undried 5 % gels have been plotted against the logarithm of the thickness and it can be seen that the points lie on two straight lines. The same relation holds for the 10 and 15 % gels. Hence the relation of swelling to the thickness of the pieces can be expressed by the equation:

$$S = -k_1 \log d + k_2,$$

where *S* is the swollen weight at equilibrium, *d* is the dry thickness and *k*₁ and *k*₂ are constants. The values of *k*₁ and *k*₂ depend partly on the original concentration of the gel and partly on such factors as *p*_H and temperature. For any particular value of the initial concentration, it seems highly probable

that $k_2 = f(k_1)$, under conditions including both acid and alkaline swelling. There are not, however, sufficient experimental data to establish this definitely.

Further, experiments on the influence of thickness on water absorption of gels after drying bring out two points clearly: firstly, that under similar experimental conditions very much less water is absorbed than by undried

Fig. 1.

gels; secondly, that the influence of thickness of the gels has become less in the case of the acid and alkaline systems. The equation connecting swelling and thickness of the gel in water and salt solutions over the range of thickness covered by these experiments approximates to the form

$$S = -k_3 d + k_4.$$

The curve for swelling in acid and alkaline solutions also approximates to the linear form, though obviously the value for k_3 is considerably greater than in the case of water and salt solutions.

The effect of drying the gel is a little difficult to explain, but it is clear that the lower the original concentration of the gel, the greater the effect of the drying. It, therefore, seems possible that during drying there is a re-organisation of the molecules of gelatin and of water within the gel of such a kind that the dried gel corresponds in internal structure to a more concentrated system than the jelly from which it has been dried. A re-arrangement of this type would lead to a reduction in the amount of water that would be absorbed on re-soaking. Details of the relation between swelling and the concentration of gelatin in the jelly are dealt with in the next section.

The effect of the initial concentration of the gel.

Experiments were made both with the original freshly set jellies and with gels which had been brought to the dry condition. The water absorption of both types was studied under various conditions. Experiments with pure gelatin were made at 5, 10, 15 and 20 % original concentration; experiments with commercial gelatin were made at 5, 10, 20 and 50 % original concentration. Observations were made at 0°, 7°, 18° and 23°. The factor for thickness was controlled, 0.25–0.30 mm. being usually chosen.

The freshly set jellies in water at 0° reach a steady equilibrium in the course of a few days, which remains steady up to 3 weeks, beyond which their behaviour was not followed. The 5 % gel lost weight; the 10 % changed very little, generally, however, showing a slight gain; the 15 and 20 % gels both gained in weight (see Figs. 1 (a) and 2 (a)). At all other temperatures all the gels gained in weight. In 0.2 *M* sodium chloride solution at 0° the gels also reach a steady equilibrium. Under these conditions the 5 % gel shows very little change of weight; the other gels all gain. At higher temperatures all gain. In dilute hydrochloric acid and sodium hydroxide solutions all gels absorb water freely at 0° and 7° and pass into solution at 18° and 23° after a few days.

The weight gained under various conditions, plotted against the original concentration, is shown in Fig. 2 (a). Under all circumstances, the total amount of water in the gel when it is in substantial equilibrium with its surroundings is an inverse logarithmic function of the original concentration. In Fig. 2 (b) the same experimental data shown in Fig. 2 (a) have been plotted against $1/\log G$ as abscissa, where G is the original concentration of the gelatin in the jelly. It can be seen from both figures that all the curves of swelling against concentration form a single family of curves in spite of variations of temperature and of the nature and concentration of the electrolyte in the system. The influence of initial concentration on swelling can, therefore, be expressed by the general equation:

$$S = k_5 \left(\frac{1}{\log G} \right) + k_6,$$

where S is the swelling, G the original concentration and $k_6 = f(k_5)$.

Swelling, therefore, is uniformly modified under all conditions by the

properties of the gel. These properties remain fundamentally the same under different physical and chemical conditions and are, therefore, to be attributed to the mechanical properties of the gel. Without any great straining of probabilities, the influence of concentration on swelling may be attributed to the mechanical resistance of the tangled micellae in the gel to being moved, either further apart or nearer together. If the effect of concentration on swelling under any particular conditions be considered, it follows from relations of the curves to each other that the resistance to swelling diminishes not only with a fall in the initial concentration, but also under all conditions which

Fig. 2.

lead to an increase in the amount of water present in the gel, or in other words, the ease with which the micellae are pushed apart increases with the distance between them.

The influence of the original concentration of the gelatin in dried gels is shown in Fig. 3 (a) and (b). Curves for swelling plotted against $1/\log G$ are given both for pure gelatin (Fig. 3 (a)) and for commercial gelatin (Fig. 3 (b)). As in the experiments on thickness, the most striking effect of drying the gels before putting them under the different experimental conditions is that under all circumstances the amount of water absorbed into the system is very considerably reduced, especially in the weaker gels. As long as the original concentration of the gelatin is not greater than 20 %, the general effect of concentration on swelling can still be expressed by the general equation:

$$S = k_5 \left(\frac{1}{\log G} \right) + f(k_5),$$

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

but the values of the constants k_5 and $f(k_5)$ have become different. The points obtained for the 50 % (commercial) gels do not, however, lie on the curve calculated from the general equation. It seems highly likely that both in undried jellies and dried gels this equation does not hold if the original concentration of the gelatin rises above about 20 %.

The curves for the swelling of the commercial gelatin cover a wide range of external conditions. The swelling of the gelatin has been followed not only for sodium chloride, but also for sodium nitrate and sodium sulphate. Further experimental data for these three salts over a range of concentrations up to $2M$ are given in Fig. 5. It can be seen that the influence of concentration on the water absorption remains similar with all three salts over the range of concentration studied. The values of the constants in the general equation will, however, be affected by the nature and concentration of the salt. Considering each salt individually, it is again found that the curves of swelling for different concentrations of salt form a family of curves which, if extended, come to a point at a value of $S = 100$, $G = 100$; *i.e.* under conditions where the gel contains no water, the presence of salt would fail to cause swelling. The same relation holds for the series of curves of swelling in hydrochloric acid at different values of p_H (Fig. 6).

The effect of temperature.

The effect of temperature on water absorption by gels of different thicknesses and original concentration have been studied both for the undried jelly and the dried gelatin. Experimental data are given in Fig. 7. For all systems studied, *i.e.* for water, salt, acid and alkaline solutions, the curves of weight plotted against temperature are true parabolas over a temperature range of 0° – 18° , *i.e.* $S \propto t^2$, where t is the temperature in degrees centigrade. The curves for the pure gelatin gels (undried and dried) are not shown with S plotted against t^2 , but Fig. 4 shows the data for commercial gelatin under a number of experimental conditions plotted on this system. The resulting curves are linear, hence under all conditions

$$S = k_7 t^2 + k_8,$$

where t is the temperature (centigrade) within the limits 0° to 18° . Where the experimental curves depart from the general parabolic form as the temperature rises, the gelatin has begun to dissolve in the surrounding medium. The relative positions of the curves (Fig. 7) suggest that the constant k_8 is influenced by both the thickness and the original concentration of the gel, but these two factors do not influence the value of the partial differential dS/dt , except possibly when the gels were set either at a low percentage of gelatin or in a very thin sheet. (In this connection, it will be noticed, on comparing the data given here with those published in the previous papers of the series, that a parabolic curve was not previously obtained for variations of swelling with temperature in water (p_H 5.0) and very dilute alkalis (p_H 7.0).

The sheet gelatin used in the previous work was obtained by dialysing commercial gelatin and was very thin, the average thickness of the pieces being 0.06 mm. compared with the 0.20–0.30 mm. used in the present work. The relatively greater surface exposed to solvent action is probably sufficient to account for the difference in experimental data.)

The value of the constant k_7 is evidently influenced by the nature of the electrolytes in the system, being very much greater in acid or alkaline than in water or salt solutions (Figs. 4 and 7). The value of the constant k_7 does not appear to be influenced by drying the gel, while the value of k_8 is considerably affected. The suggestion already put forward in this paper that a re-organisation occurs in the gel during drying in such a way as to bring

Fig. 7.

about an approximation in structure to one which set at a higher concentration of gelatin, receives support from the influence of drying on the value of k_8 , which, it has already been shown, is influenced by the original concentration.

The influence of temperature on swelling is, however, so similar in nature under all external conditions that it seems clear that the predominating effect of the temperature is on some factor common to all the systems; this factor might be the gelatin itself or it might be the water. It has already been shown by Smith [1919] that gelatin in solution in water undergoes a reversible mutarotation with change of temperature. Below 15°, the "gel" form, gelatin B with $[\alpha]_D = 141^\circ$, is the only stable form; at temperatures above 35°, the "sol" form, gelatin A with $[\alpha]_D = -313^\circ$, is the only stable form. This transformation of the gelatin with change of temperature might be considered

responsible to some extent for lowering of the cohesive forces of the gelatin which occurs with rise of temperature, but from Smith's experimental figures only 3 % of gelatin B will be present at 18°, while from the results recorded in this paper, as soon as the concentration of gelatin B rises above this figure, solution of the gelatin sets in and equation $S = k_7 t^2 + k_8$ no longer holds. The mutarotation of gelatin with temperature, therefore, is not a factor in this equation. The influence of the temperature on the activity of the water is, therefore, probably to be regarded as the direct cause of the influence of temperature on swelling. The fact that $S \propto f(t^2)$ on the centigrade scale, where the zero is based on the property of water, is noteworthy in this connection.

In acid and alkaline solutions temperature has such a profound influence over the whole range that there seems little doubt that it influences not only the activity of the water but also the osmotic and other properties of the gelatin salts formed under these conditions.

The special stability of the 10 % gel.

It has already been mentioned in the introduction to this paper that under certain conditions 10 % gels are more stable than those of any other concentration. This observation, originally recorded in the literature by Kunitz for experiments made at 5°, is fully confirmed by the experiments recorded here. The stability of the gelatin jellies in a system free from electrolytes is not independent of either the temperature or the purity of the gelatin. It has been observed constantly throughout this work that commercial gelatin never comes into true equilibrium with its surroundings, but after nearly attaining equilibrium continues to absorb water and gain in weight for an indefinite period. The lower the temperature, the more nearly is a true equilibrium reached, and an approximate equilibrium may be taken as a measure of the change in weight due to imbibition. With pure gelatin, a true equilibrium is reached at low temperatures (0° and 7°) but not at higher ones (18° and 23°), a situation possibly to be attributed partly to the appearance of Smith's soluble B form of gelatin at the higher temperatures. The rate at which the end of the true swelling of the gelatin is reached varies with the temperature, but the following times were found by experiment to represent the end of the first rapid water absorption: 0° in 10 days, 7° in 7 days, 18° in 4 days, 23° in 3 days.

Table I shows the weights attained in equilibrium in water by gels which had been set over a range of concentration from 5 to 50 %. Figures are given both for undried jellies and gels after drying.

It will be seen here again that, at the low temperatures, all gels tend to move towards the final concentration of 10 % or 1000 parts by weight of swollen gelatin to every 100 parts of gelatin in the system. At the higher temperatures, where imbibition is undoubtedly followed by slow solution, all gels tend to draw water into the system.

Table I.

Concentration of jelly at setting ...	5 %	10 %	15 %	20 %	50 %
Original weight of jelly for 100 g. gelatin (g.)	2000	1000	670	500	200
Weight of gel after soaking in water for 100 g. gelatin (g.)					
<i>Pure gelatin jellies (undried)</i> (dry thickness 0.24–0.28 mm.)					
10 days at 0°	1700	1050	760	550	—
7 „ 7°	1800	1070	800	570	—
4 „ 18°	2300	1350	830	810	—
3 „ 23°	∞	∞	1070	1000	—
<i>Pure gelatin gels (dried before soaking)</i> (thickness 0.27–0.32 mm.)					
10 days at 0°	1100	750	700	—	—
7 „ 7°	1150	800	770	—	—
4 „ 18°	1100	970	950	—	—
3 „ 23°	1200	1050	1000	—	—
<i>Commercial gelatin gels (dried before soaking)</i> (thickness 0.22–0.25 mm.)					
10 days at 0°	920	850	—	750	450
7 „ 7°	1020	950	—	800	470
4 „ 18°	1750	1500	—	1250	750
3 „ 23°	2000	1700	—	1600	900

The special stability of the 10 % gel is shown most clearly in systems which have not been dried. After drying, the re-organisation which has occurred has partly masked the effect of the original concentration. The special stability of the 10 % gel is not apparent in systems where electrolytes are present. The presence of acid, alkali or salt alters the equilibrium between the gelatin and the water. There is some evidence, however, that the tendency for gels of originally higher or lower concentration is to move in their final equilibrium towards the final equilibrium of the 10 % gel.

DISCUSSION.

The fact that gelatin jellies absorb water and swell in aqueous solutions until they have reached an equilibrium weight has always been recognised as evidence of cohesive forces due to the gelatin itself [Procter, 1914]. These cohesive forces have also always been regarded as of a mechanical nature. It has been shown by the experiments recorded in the present paper that the total cohesive force of any gel is influenced both by its ratio of surface to mass (thickness) and by the original concentration of the jelly at setting. With dried gelatin the cohesion has been further raised by the fact of drying.

A fact which emerges clearly from a review of the experimental data given in this paper is that under most circumstances the resistance of any gel to swelling is directly proportional to the amount of gelatin present in unit volume. (An apparent exception to this generalisation is found in freshly set jellies containing less than 10 % of gelatin, which eliminate water, *i.e.* undergo actual shrinkage. Equilibrium in water differs, however, from equilibrium in solutions of electrolytes, since neither osmotic forces nor hydration due to

salt action are factors in the final equilibrium.) It is obvious, therefore, that there is some definite structure in the gel which is imposing a restraint on swelling, and that this force of inhibition becomes increasingly greater, the greater the concentration of gelatin at the moment of setting. It is not unreasonable to suppose that this force acting against the swelling forces is due to a tangle of long threadlike micellae formed in the gel, and the purely mechanical nature of the cohesive forces is confirmed by the fact that thin sheets of gelatin offer less resistance to swelling than thick ones.

The absorption of water by gelatin is, therefore, affected, not only by the properties of the solution, but also by those of the gelatin gels. Three independent factors are to be attributed to the gels themselves: (1) a tendency for all gels in the presence of water to come to an equilibrium at 10 % concentration of gelatin; (2) an elastic resistance to swelling, which is purely mechanical and can be attributed to the forces required to move the entangled, threadlike micellae apart, and which falls off as the interstitial spaces between the micellae increase in volume; (3) in dry gelatin only, an additional effect which is to be attributed to some re-organisation occurring during drying.

SUMMARY.

1. The absorption of water under varying conditions by discs of gelatin is influenced by the thickness of the disc. The swelling of both dried and undried gelatin gels in water or salt solutions is not greatly influenced by the thickness of the gel; in acid or alkaline solutions swelling is a negative function of the logarithm of the thickness.

2. The absorption of water by gelatin is, under all conditions, an inverse logarithmic function of the original concentration at which the jelly was set. This is most clearly shown in the undried jellies.

3. The amount of water absorbed by gelatin from water, solutions of salts, acids or alkalis is a function of the square of the temperature (degrees centigrade), as long as solution of the gel itself is inappreciable.

4. The influence of the original concentration of the gel depends on the amount of water in the system, irrespective of whether the water has penetrated into the jelly through the direct action of temperature, p_H value of the solution or the nature of the salts present. The effect of the original concentration is, therefore, purely mechanical in nature.

5. At low temperatures swollen gels in equilibrium with water tend to approximate to a concentration of 10 %, regardless of the original concentration of the gel.

6. In similar conditions gels which have been dried contain, at equilibrium, less water than those which have not been dried.

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