LXXXV. THE DISSOLUTION OF GELATIN.

By FRED FAIRBROTHER.

(*From the Chemical Department, The Victoria University of Manchester.*)

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IN a previous communication under this title by Fairbrother and Swan [1922] evidence was put forward to show that when gelatin is put into water or dilute acid or alkali, some of the gelatin goes into solution in the liquid surrounding the gel. This dissolved gelatin was estimated by precipitation with tannic acid. No experimental evidence was there put forward to show the particular molecular complexity of the substance in solution, although it was suggested that the phenomenon of dissolution was not principally due to hydrolytic decomposition of the gelatin.

Several workers have criticised the conclusions arrived at in the above paper. Liesegang [1923] states that the solution (*i.e.* cold water which has stood in contact with gelatin) can be strongly concentrated by careful evaporation without forming a jelly, and hence that the solution consists only of hydrolysis products of gelatin.

Knaggs, Manning and Schryver [1923] come to the conclusion that the dissolution of gelatin in water is chiefly, if not entirely, due to thermal degradation and goes on continuously over many months, and hence that the 10 days allowed by Fairbrother and Swan [1922] did not represent an equilibrium time. Liesegang, and Knaggs, Manning and Schryver consider only the dissolution in water.

Alexander [1923] after quoting the results of Fairbrother and Swan states: "They should be repeated with ash-free gelatin properly freed from products of hydrolysis, for it is probable that only the hydrolysis products dissolve in cold water."

In the present paper experimental evidence is given to show that whatever be its ultimate fate as regards hydrolysis, gelatin dissolves spontaneously to some extent, in water, dilute acid or dilute alkali in a form which can be recovered and made into a jelly again.

EXPERIMENTAL.

A sample of Coignet's Gold Label Gelatin was used which was the same as "B" in the work carried out by Fairbrother and Swan.

One gram quantities were put in sterile flasks with 100 cc. water and a little thymol, and the flasks tightly stoppered. The flasks were kept at about 20° in an electrically heated incubator, and occasionally gently shaken.

Flasks which had stood thus for 7, 10, 32 and 42 days respectively were removed, the contents filtered and 3 to 4 times the volume of redistilled acetone added to each. In each case a milky white precipitate was produced which settled slowly. After standing for 2 to 3 days this was filtered off, dried, and 1 cc. cold water added. The precipitate which was in the form of a greyish powder swelled quickly to a loose, wrinkled gelatinous mass on the filter paper. This was liquefied by warming and poured into a small tube. On cooling the sol set to a turbid gel which was quite firm at room temperature in the case of 7, 10 and 32 days experiments and slightly less firm in that of 42 days.

A similar experiment was performed with 1 g. Nelson's Photographic Gelatin No. 1 which was allowed to stand for 7 days in 100 cc. of water at 20°. This also gave a precipitate with acetone, which when made into a 5 % sol with water, formed a firm gel on cooling.

Hence, the gelatin which goes into solution in water at 20° can, to some extent at least, be recovered as gelatin.

These experiments show that the dissolution in water is not primarily a thermal degradation or breakdown of the gelatin molecule.

With a view to a re-examination of the time factor as a consequence of the findings of Knaggs, Manning and Schryver [1923] determinations were made of the dissolution of this same Gold Label Gelatin, and of another sample of the same brand which had been purified by the method of Miss Field [1921] followed by precipitation with alcohol. The dissolution experiments were carried out at 20°. The experimental details of the estimations were as given by Fairbrother and Swan.

The values given below are the mean of two determinations.

Time in days	Weight of precipitate in g. given by tannic acid with 25 cc. of solution	
	Unpurified gelatin	Purified gelatin
4	0.027	0.027
7	0.029	0.034
14	0.040	0.042
21	0.047	0.049
42	0.053	0.050

These figures show an increase with time, of the gelatin dissolved, although, if the weight of precipitate be plotted against the time, the curve appears to be approaching a limiting value.

It is to be expected that in any case some slow continuous increase with time of the dissolution will take place, just as a jelly will continue to swell in water for many months although the major part of the swelling has taken place within the first few days. The "maximum" of dissolution is probably of a similar nature to the "maximum" swelling of a gel.

It should be pointed out that the above experiments were carried out at 20° as were those of Knaggs, Manning and Schryver.

The experiments of Fairbrother and Swan were performed at a lower temperature, at which one would expect any thermal degradation to be less marked.

It is probable that a small rise of temperature causes a more rapid physical, and reversible disintegration of the micellae rather than a chemical molecular decomposition. This breaking up of the micellae will increase very rapidly as the solution temperature is approached.

The above figures, however, do not show any marked difference in behaviour as between the commercial and the purified gelatin. Knaggs, Manning and Schryver state that gelatin which has been treated with dilute hydrochloric acid, and washed, does not yield as much "diffusible nitrogen" as untreated gelatin.

Further evidence that the dissolution during the first few days is not merely solution of degradation products already present in the gelatin, is given by experiments at a lower temperature. The amount of precipitate obtained by the addition of tannic acid as before to 25 cc. of water, 100 cc. of which had stood in contact with 1 g. of gelatin in a refrigerator (0° – 2°) was as follows:

Days:	4	7	32
Weight of precipitate g.	0.005	0.005	0.005

In connexion with the dissolution in dilute acid and alkali the following experiments were carried out.

One g. of Gold Label Gelatin was placed, with a little thymol in 100 cc. *N*/10 hydrochloric acid and kept at 20° . After 10 days the gelatin, which had dissolved completely, was neutralised with sodium hydroxide, leaving the reaction a little on the acid side of the isoelectric point. This solution was allowed to stand in the laboratory for three days, when it was found to have set to a very weak jelly. The concentration of dry gelatin was about 0.8 %. The jelly was melted, the gelatin precipitated by acetone, dried and made up into a 5 % sol. This set to a stiff jelly on cooling, indicating that, in accord with experiments of Miss D. J. Lloyd [1920], gelatin does not dissolve in dilute hydrochloric acid as a consequence of a molecular breakdown.

It has, however, also been found possible to recover gelatin from solution in sodium hydroxide, although the mechanism of dissolution is clearly different from the former case. Two g. of Gold Label Gelatin allowed to dissolve in 100 cc. *N*/10 sodium hydroxide for three days at 20° when neutralised with *N* hydrochloric acid did not form a gel on standing. The gelatin was then precipitated by alcohol. The precipitate was of quite a different character from that yielded by a solution in hydrochloric acid precipitated by alcohol; it was more gelatinous in nature and could only with great difficulty be filtered. It was centrifuged, washed with alcohol, dried and made into a 2.5 % sol. This formed a gel on cooling. Acetone was found to be much better than alcohol as a precipitant; the precipitate was not gelatinous and was more easily separated.

In another experiment 1 g. of gelatin dissolved in 100 cc. *N*/10 NaOH at 20° in 48 hours. The solution was filtered and neutralised with hydrochloric acid using methyl red as indicator, one drop of a solution of sodium hydroxide was then added. The final reaction was well on the alkaline side of the isoelectric point, *i.e.* the indicator was quite yellow. The gelatin was

precipitated by acetone and the precipitate made into a sol of about 5 %, which set to a firm gel on cooling. Hence the recovery of gelatin from solution in alkali is not entirely a question of the degree of neutralisation as suggested by Wilson [1923], but probably depends rather on the time the gelatin is left in contact with the alkali, since solutions of gelatin in alkali which have stood for longer than a few days, give on neutralisation and precipitation, products which yield progressively weaker gels.

It appears, therefore, that when a gel is put into dilute acid or alkali, gelatin can exist as such, in the outer liquid in equilibrium with the gel. If a Donnan membrane equilibrium is set up in such a case, as the work of Loeb, Proctor and others indicates, then it follows that the concentration of ionised gelatin within the gel must be greater than without. The present work makes it appear unlikely that this is caused by outer layers of gelatin acting as a semi-permeable membrane preventing the diffusion of the gelatin ions within the gel. A Donnan equilibrium would be set up if the gelatin cations were held from diffusing in some other way than by such a semi-permeable membrane. This makes it appear probable therefore that the gelatin cations are held as integral parts of the framework of the gel, which consists therefore, not of isoelectric gelatin alone, as postulated by Miss Lloyd, but also of aggregates of gelatin ions, which latter also exist in solution in the interstitial spaces within the gel and in the liquid without the gel.

An approximate calculation of the effect upon the Donnan equilibrium, which would be caused by the dissolved gelatin, shows that the effect would be very small.

SUMMARY.

1. Gelatin dissolves on standing in contact with water, dilute acid, or dilute alkali at ordinary temperatures primarily as gelatin. Some subsequent hydrolysis probably occurs.
2. The framework of a gelatin gel must consist not only of isoelectric gelatin but also of gelatin cations.

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