CXL. INVESTIGATIONS ON GELATIN. PART V. THE PROPERTIES OF A GELATIN PURIFIED BY FLOCCULATION IN AN ELECTRIC FIELD.

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IN the course of some of the experiments on the "recrystallisation" of gelatin, solutions were obtained occasionally which refused to separate on standing. At first it was thought that a stage of purification had been reached where a gelatin could form a stable suspension in water and that the presence of a trace of some impurity was necessary for precipitation.

But further investigation showed that the non-precipitation was actually due to the introduction of a small quantity of electrolyte as impurity, most probably ammonia due to a very slight hydrolysis of the gelatin. Traces of electrolyte present in the distilled water or dissolved from the glass vessels used may also play a part.

Such suspensions could, however, be completely and rapidly precipitated by placing them in an electric field. This method of precipitation has already been described [Knaggs and Schryver, 1924].

(The gel can be removed, rinsed with cold water and dried down. *This gelatin will again separate from a 1 % solution on cooling.*)

The upper liquid after electrical precipitation is perfectly clear and contains only traces of nitrogenous material. Thus analyses of several such preparations gave:

N content = 2.0–2.4 mg./100 cc. when upper pole +¹
 ,, = 1.4–1.6 mg./100 cc. ,, ,, ,, –.

In these experiments platinum was used for both the cathode and anode.

A sample of bone gelatin which, though ash free, yet contained break-down products as an impurity, gave the following results:

First precipitation 9.1 mg./100 cc.
Second ,, 3.5 mg./100 cc.
Third ,, 2.7 mg./100 cc.

These are the N content of the clear liquid at + pole.

(*Note.* This particular sample is being further investigated.)

¹ These numbers appear to vary slightly in different samples of gelatin and probably also with the current density used and other factors.

These low values of the N content of the clear solutions obtained after electrical precipitation reopened the question of the solubility of gelatin in water. The solutions obtained after ordinary flocculation of a solution of ash-free gelatin are usually very slightly opalescent. Occasionally they appear quite clear at first but invariably on standing develop this slight opalescence. As described in a previous report, these solutions contain always about 10 mg. N per 100 cc. It was decided to investigate the behaviour of these solutions in an electric field.

When placed in a U-tube and subjected to an E.M.F. the first action is to produce cataphoresis, the particles moving towards the anode. They are therefore negatively charged. The liquid round the cathode becomes perfectly clear and the moving boundary between clear liquid and the opalescent suspension is very marked. Approximately the particles move at the rate of $\frac{1}{2}$ cm. an hour under a potential gradient of 10 volts/cm. After a few hours, however, coagulation of the particles is noticeable, and gradually the gelatin is precipitated to the bottom of the U-tube, leaving the liquid in the upper part of the tube quite clear round both electrodes.

Further experiments were carried out in the larger apparatus described in the preceding paper (p. 1082) and the N content of the clear liquid determined. In every case it fell to between 1 and 2 mg./100 cc. after precipitation of the gelatin and was always a little higher round the + than round the - pole. It may here be recorded as supplementing previous observations [Knaggs, Manning and Schryver, 1923, Section IV], that if such a dilute solution is concentrated under reduced pressure at 40° to one-fifth of its bulk, the resulting solution flocculates on cooling with separation of the solid hydrated gelatin, *if the dilute solution is concentrated within 12 hours from being made up*. If it is concentrated after being allowed to stand three or more days this flocculation does not occur.

The gelatin remaining in solution after the flocculation of dilute solutions of ash-free gelatin is therefore present as a negatively charged suspension. In other words, the gelatin is peptised to the extent of 10 mg. N/100 cc. by adsorbed negative ions, probably OH ions, present in the water. The extreme sensitiveness of gelatin to OH ions, as shown by experiments described below, supports this conclusion.

The very minute amount of nitrogenous material remaining in solution after electrical precipitation of these suspensions is probably due to slight decomposition of gelatin at the two poles. There would probably be greater decomposition due to oxidation at the anode than to reduction at the cathode. The experimental results bear out this supposition. It may be noted here that the current used in the electro-dialysis or electro-precipitation of the purified gelatin does not exceed 10 milliamps.

EFFECT OF ADDITION OF ACID, ALKALI AND NEUTRAL SALTS TO
SOLUTIONS OF PURIFIED GELATIN.

A roughly quantitative comparison was made of the effect of acid, alkali and neutral salts in producing a clear gel or sol from dilute solutions of the purified gelatin.

The concentration of gelatin used was 0.5 %. On cooling this solution the usual flocculent precipitate and slightly opalescent supernatant liquid were obtained. Quantities of 100 cc. of this solution were transferred to 100 cc. flasks and the requisite quantity of acid, alkali or neutral salt added. The solutions were allowed to cool and observations were made after standing 3 days at room temperature (13° to 15°).

Ammonium hydroxide.

Concentration	Appearance of solution
0.00002 N	Flocculent precipitate, but the supernatant liquid more turbid than in water alone
0.00005 N	No separation of solid even on centrifuging. Solution completely peptised to a stable translucent gel
0.0001 N and higher concentrations up to 0.001 N	Solutions transmit more light with increasing concentration of alkali. All these solutions form weak gels.
0.001 N	Transparent gel

Hydrochloric acid.

Concentration	Appearance of solution
0.00001 N	Flocculent precipitate, but the supernatant liquid more turbid than in water alone
0.00002 N	Flocculent precipitate, but the supernatant liquid yet more turbid
0.00005 N	No separation of solid; a slightly translucent gel is formed
0.0001 N	No precipitation; opalescent gel
0.0005 N	Quite clear gel

Potassium chloride or barium chloride.

Concentration	Appearance of solution
0.0002 N	Flocculent precipitate; separation almost as complete as with water alone
0.001 N	Solid separated, but not so readily. Supernatant liquid slightly more turbid
0.005 N	Very weak opaque gel formed. On standing extensive syneresis occurred
0.025 N	Stable gel, but still rather opaque.
0.1 N	Nearly clear gel

It will be observed that only very small concentrations of acid or alkali are necessary to produce a clear and stable gel. On the other hand, a relatively large concentration of neutral salt is necessary to produce the same effect. In these respects, gelatin behaves like a typical globulin. If to a solution just sufficient acid is added to produce a clear stable gel, and if this gel is remelted at 35° and neutralised with an exactly equivalent quantity of alkali, on cooling, the gel again becomes white and opaque, and separates on standing into two phases. The salt formed by neutralisation of the acid is in concentration considerably below that necessary to produce a clear and stable gel. A similar phenomenon, though very much less marked, can be observed with some good commercial gelatins. By the careful addition of small quantities of acid a point

may be observed at which the gel exhibits a maximum turbidity (the iso-electric point). Generally however a commercial gelatin from which the inorganic impurities have not been removed gives a clear and stable gel even when brought to the iso-electric point by the addition of acid. That is to say, the "ash" in the original gelatin is much more effective as a clearing agent than the neutral salt added subsequently to the ash-free material.

THE ELECTRICAL CONDUCTIVITY OF GELATIN SOLUTIONS.

The electrical conductivity of these solutions proves a useful criterion of the degree of purity of the gelatin. Compare for example the following:

	Conductivity of a 1 % solution at 25° Reciprocal ohms
A high grade commercial gelatin. (Coignet's Gold Label)	259×10^{-6}
The same after electro-dialysis	20.5×10^{-6}
After further purification by "recrystallisation" and precipitation in an electric field	9.5×10^{-6}

These conductivities refer to 1 % solutions, calculated on the weight of air-dried material, made up at 35°, and measured at 25° after about an hour in a thermostat at the latter temperature. The solutions of the purified gelatin, when kept at 25°, alter slowly in conductivity over the first 24 hours and thereafter remain constant. Thus the solution quoted above, which had a specific conductivity of 9.5×10^{-6} after an hour at 25°, had increased in conductivity to a value 12.6×10^{-6} after 24 hours. This increase in conductivity appears to run parallel with the slow flocculation of the hydrated gelatin.

The conductivity of a solution kept at 35° does not change appreciably with the time.

The specific conductivity of solutions of the highly purified gelatin is not proportional to the concentration. It varies as indicated by the following results:

Concentration of solution	Specific conductivity at 25°
0.5 %	8.15×10^{-6}
1.0	9.5×10^{-6}
2.0	11.9×10^{-6}

Note. The specific conductivity of the water used for making up the solutions in all the conductivity determinations was between 1.0×10^{-6} and 1.5×10^{-6} .

Nelson Dale's Photographic Gelatin (a skin gelatin) gave similar results. Thus:

	Specific conductivity 1 % solution at 25°
Immediately after electro-dialysis	15.7×10^{-6}
After further purification	11.4×10^{-6}

The effect of heating the solution to 100° for a time, on the conductivity measured at 23°, is shown by the following results:

Initial specific conductivity of 1 % solution ...	8.1×10^{-6}
After 3 hours at 100°	18.2×10^{-6}
After 7 hours at 100°	24.2×10^{-6}

The increase in conductivity due to the degradation products on heating the solution at 100° is very marked.

France and Moran [1924] give the following results for the specific conductivity of solutions of a purified gelatin:

Concentration	Specific conductivity at 25°
1 %	40.09×10^{-6}
2 %	67.10×10^{-6}
(Water	1.0×10^{-6})

This gelatin had been purified by the method described by Ada M. Field [1921]. This is the method due originally to Loeb.

THE OPTICAL ROTATORY POWER OF GELATIN SOLS AND GELS.

The so-called "muta-rotation" of gelatin has been suggested by C. R. Smith [1919, 1920] as a criterion of the jelly strength, and therefore, presumably, of the freedom of the sample from breakdown products. The "muta-rotation" may be expressed as the ratio

$$\frac{\text{Equilibrium value of specific rotation at } 15^{\circ}}{\text{Equilibrium value of specific rotation at } 35^{\circ}}$$

For this ratio in a good sample of gelatin Smith gives the value 2.21. Poorer gelatins give lower values. Since, however, all samples of gelatin give very nearly the same rotation at 35°, the specific rotation at 15° may be used as the criterion of jelly strength of the sample.

Below are tabulated some values obtained for the purified Coignet Gold Label gelatin. All the measurements are made on a 5 % gel or sol. They are all equilibrium values, that is, the sol or gel was kept at the temperature mentioned until a constant value for the rotation was obtained.

Specific rotation at 35° in 0.1 N KCl	...	...	— 146°
" " 15° in 0.1 N KCl	...	...	— 313°
" " 15° in 1.0 N NaCl	...	...	— 313°
" " 15° in 1.0 N sodium salicylate			— 144°

The neutral salt was introduced because otherwise the gel is rather opaque and it is difficult to get accurate readings. With salts such as potassium chloride or sodium chloride there is no change in rotation over large changes in concentration of the salt.

It is interesting to observe that in the presence of a salt which liquefies gelatin, such as sodium salicylate, there is practically no change in rotation on cooling from 35° to 15°. If the salt is removed again by dialysis the solution forms a gel at 15°, and the rotation at that temperature reverts to its former value.

The effect of heating for some time at 100° is shown by the following results:

	Specific rotation at 15°	Specific rotation at 35°
Initially	— 345°	— 142°
After 4 hours at 100°	— 255°	— 144°
After 14 hours at 100°	— 209°	— 145°

Heating at 100°, therefore, has practically no effect on the rotation at 35°, but, on the contrary, a marked effect on the equilibrium value at 15°.

It seems to the present writer to be misleading to refer to the change of rotation on cooling a gelatin sol as *muta-rotation* and to attribute it to a chemical change [cf. Smith, 1919, 1920]. The change in rotation is associated rather with the physical change of sol to gel, and is indeed, as Smith has shown, a convenient way of following that change quantitatively.

VISCOSITY.

It is well known that, in determining the viscosity of a gelatin solution, the pre-treatment of the solution is of the first importance. For a comparison of viscosities of different gelatins exactly the same method must be followed in preparing the solutions of the different samples. Moreover, since subjection of the solution to a high temperature even for a short time produces an irreversible decrease in viscosity, it is desirable that in making up the solution as low a temperature should be used as is convenient. Another difficulty, which may be mentioned here, arises when attempts are made to measure viscosities of gelatin solutions at temperatures at which they possess appreciable rigidity. Measurements determined in a simple Ostwald, or similar type, viscometer are unreliable with solutions which do not obey Poiseuille's law [cf. Bogue, 1922]. This difficulty, however, does not arise if the determinations are carried out at 35°, or above, with solutions which have been given time for the transition gel $\rightarrow$ sol to be completed.

A study of the variation of the viscosities of sols of the purified gelatin with time was made at various temperatures from 35° to 40°. In all the experiments the results of which are quoted below, 5 g. of the air-dried material, which contained 22.0 % moisture, were made up to 100 cc. The gelatin was weighed out into a graduated flask, covered with water and allowed to soak overnight at room temperature. Water was then added up to the mark, and the flask was placed in a thermostat at the temperature at which the viscosity was to be measured. As soon as the gelatin had completely dissolved, the sol was transferred to the viscometer and observations of the time of flow were made at suitable intervals. A simple Ostwald viscometer was used. It will be noted that in this procedure the temperature is never raised above that at which the viscosities are measured.

The results, when plotted, gave always the same type of curve (Fig. 1). The viscosity at first falls off rapidly with the time, but after one or two hours the rate of fall becomes very much less. This fall in viscosity, however, is continuous. In no case was a constant viscosity reached, though some of the observations at 35° were carried over periods of more than 100 hours.

If the sol is removed from the thermostat, allowed to cool for some time, and then returned to the higher temperature, the variations in viscosity which occur are illustrated in Figs. 2 *a* and 2 *b*. If the cooling period is short, the viscosity-time curve continues at practically the same point that it had

Fig. 1. Variation of viscosity with time. 5 % purified Nelson Dale Photographic Gelatin at 35°. $p_H = 4.9$.

Fig. 2a. 5 % purified Coignet Gold Label Gelatin at 38°.

Fig. 2b. 5 % purified Coignet Gold Label Gelatin at 35°.

reached before cooling. With a longer period at the lower temperature a certain amount of recovery is observed. The recovery is more marked the lower the temperature at which the viscosities are measured (cf. Figs. 2 *a* and 2 *b*).

The initial fall in viscosity is due principally to the time taken by the transformation of gel to sol.

The results of these experiments show that even at 35° there is a slow *irreversible* decrease in viscosity of sols of highly purified gelatin.

The hydrogen-ion concentrations of the sols of the purified gelatin lay between p_H 4.90 and 4.95 at 35°. They did not vary appreciably with time.

It is evident that the initial viscosity as measured by the procedure outlined above is unsuitable as a basis for the comparison of the viscosities of different gelatins. Its rapid variation with time makes it dependent on the time taken for complete solution of the gelatin, which varies from sample to sample even of the same preparation. Moreover, as previously mentioned, reliable results cannot be attained by the use of an Ostwald viscometer until the transformation gel $\rightarrow$ sol is complete. The viscosities measured at a definite arbitrary length of time after immersion in the thermostat might be used. The time would have to be long enough for the rate of decrease of viscosity to have become very small; this would require at least 4 or 5 hours, which would be inconvenient.

If the sol is heated for a time to a higher temperature before being placed in the thermostat at 35° the initial rapid fall of viscosity is eliminated. Indeed, a 5 % sol which has been made up at 60°, and kept there for some time, exhibits a *rise* of viscosity with time on being brought to 35°. This rise, however, is only temporary. After a few hours the viscosity reaches a maximum and thereafter falls. By suitably adjusting the temperature at which the solution is made up and the time it is kept at that temperature, it is possible to keep the viscosity at 35° appreciably constant over a period of 3 or 4 hours. The period at the higher temperature has eliminated the initial steep portion of the curve mentioned above. When the solution is suddenly cooled to 35° there follows a slow rise in viscosity due to the time required by the sol to readjust itself to the new conditions, the "hysteresis" effect. This, superposed on the natural slow fall in viscosity at 35°, may result in an apparently constant viscosity. That the constancy is only apparent is shown by continuing the observations over a sufficiently long time; eventually the slow fall of viscosity reasserts itself.

The so-called "transition point," i.e. the temperature at which the viscosity does not change with time, observed by Davis and Oakes [1922] does not therefore possess the special significance they, and others [cf. Bogue, 1922], seem to attach to it. It is a function of the previous treatment of the sol. Davis and Oakes [1921] make up their solutions at 75°, which accounts for their finding a "transition point" about 38°. They give 38.03°.

If all that is required is a *comparison* of the viscosities of solutions of different

gelatins at 35°, the most convenient procedure is to make up the solutions at such a temperature and under such conditions that on cooling to 35° the viscosity remains appreciably constant for 2 or 3 hours. In obtaining the results quoted below, the solutions were made up at 50°. They were allowed to remain in the thermostat at 50° for one hour, and were then at once transferred to the thermostat at 35°. The subsequent variation of viscosity with time is illustrated by the typical curve shown in Fig. 3. The results are the average of readings taken repeatedly, especially during the first few hours.

Fig. 3. 5 % purified Nelson Dale Photographic Gelatin at 35°, after heating at 50° for one hour.

RESULTS WITH NELSON DALE PHOTOGRAPHIC GELATIN.

Viscosities of 5 % solutions at 35°, relative to that of water at the same temperature.

	Viscosity	p_H
Before purification	5.036	6.3
After „	4.822	4.9

The apparent decrease in viscosity on purification is more than accounted for by the change in H-ion concentration. Addition of acid or alkali to the purified gelatin at once raises its viscosity. The variation of viscosity with p_H when the purified gelatin is dissolved in dilute alkali solutions, is shown in the following table. The H-ion concentrations were measured electrometrically at the same temperature at which the viscosities were determined (35°).

Purified gelatin dissolved	Viscosity	p_H
(1) In water	4.822	4.9
(2) In 0.002 <i>N</i> ammonia	4.933	5.0
(3) In 0.006 <i>N</i> NaOH	5.399	5.7
(4) In 0.017 <i>N</i> NaOH	7.074	10.2

In the last-mentioned case, i.e. at p_H 10.2, the viscosity at 35° did not show the initial constant period, but decreased continuously with time. That given is the initial viscosity.

It is evident that, if the comparison is made at the same H-ion concentration, the method of purification used results in an increase of viscosity.

CONCLUSIONS.

(1) The amount of nitrogen remaining in solution after the electro dialysis of a gelatin forms a good criterion of its degree of separation from degradation products—this amount in the particular sample investigated lay between 1 and 2 mg. per 100 cc. when the lower pole in the dialysis apparatus was the anode and the proper movement of the gelatin in the electric field was in the same direction as gravity.

(2) The previous determination of the solubility (Knaggs, Manning and Schryver) of 0.056 part of gelatin in 100 cc. of water was too high, as complete equilibrium between the phases in the experiments described had not been attained, and the gelatin was probably not completely freed from the last traces of electrolytes.

(3) If a purified gelatin has any true solubility in water (i.e. if it can be "molecularly dispersed") this does not exceed 10 parts in 100,000.

(4) A gelatin is not a protein *sui generis*, but has nearly all the characteristic properties of a globulin. It is insoluble in water, but soluble (or dispersible) in very weak solutions of acids and alkalis. Very much stronger solutions of neutral salts such as potassium chloride are necessary to produce corresponding effects. It differs from most other globulins in its higher state of hydration, to which may be ascribed the great capacity for forming gels. The other globulins can also be made to form gels when they can be dispersed in a relatively small amount of dispersion medium. Thus, for example, edestin, a typical plant globulin, will yield a jelly when obtained in high concentration in a solution of sodium salicylate.

(5) Gelatin solutions show an irreversible diminution in viscosity when kept, even at 35°. The conditions to be observed for obtaining correct determinations with an Ostwald viscometer are discussed.

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