

THE FLOCCULATION OF GELATIN AT THE ISO-ELECTRIC POINT

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(Accepted for publication, February 26, 1931)

It is well known that such properties of gelatin solutions as turbidity and Tyndall effect intensity, which depend upon the aggregation of the gelatin, show a maximum at the isoelectric point. This relation has been studied quantitatively by Kraemer and Dexter (1927), who have shown that above 40°C. the light-scattering capacity of gelatin is very slight and is nearly independent of temperature and hydrogen ion concentration, but that below this temperature, particularly with calfskin gelatin, the scattering increases very sharply within a few tenths of a pH unit in the region of the isoelectric point. It was pointed out by these investigators that this behavior cannot be explained on the basis of the solubility of a homogeneous substance. By its separation into a "soluble" and an "insoluble" fraction by Schryver and Thimann (1927) and by Kunitz and Northrop (1928) it has furthermore been shown that gelatin itself is not a truly homogeneous substance.

The writer had observed that when gelatin solutions, at the isoelectric point and sufficiently free from electrolytes and other impurities, are cooled to temperatures below 40°C., aggregation occurs to such an extent that gelatin separates from solution as a flocculant precipitate. It was considered worth while to study this flocculation of gelatin in some detail, as it appeared to offer a method for investigating the nature of gelatin solutions and their possible heterogeneity. It also seemed to offer a possible method for the fractionation of gelatin.

The particular problems selected for this study were (1) the nature of the equilibrium between the flocculated and the dissolved gelatin, and (2) the properties of these gelatin flocculates and of the gelatin remaining in solution at equilibrium at various temperatures.

I. EXPERIMENTAL MATERIAL AND METHODS

Solutions were prepared by soaking gelatin in distilled water for 1 hour at room temperature, heating to 60°C. for 10 minutes, and filtering. They were then dialyzed in an electro-ultrafiltration apparatus of the type described by Bechhold (1925). Using direct current at 110 volts, electro-dialysis was continued until the current passing through the solution had decreased to a constant low value, characteristic of the cell. Using a small dialyzer of 100 cc. capacity, it required less than 2 hours for the current to decrease to a constant value of 0.8 milliamperes. A larger dialyzer of 2 liters' capacity was less efficient, because of the relatively smaller electrode surface, and required 8 hours to reach a constant current of 8.0 milliamperes. Ultrafiltration membranes were prepared from a 10 per cent solution of collodion in glacial acetic acid; such membranes were found to give gelatin-free filtrates. The gelatin solutions were kept at 37°C. during dialysis to prevent the formation of a gel on the membranes and were stirred constantly by means of an electric stirrer.

The hydrogen ion concentration of those solutions which were not already at the isoelectric point changed rapidly during dialysis, finally reaching a value which remained constant during prolonged dialysis. On cooling such a dialyzed solution to temperatures below a range of 30–40°C., depending upon the concentration, gelatin separated out as a flocculant precipitate, which has been taken as the criterion that the solution is at its isoelectric point and free from electrolytes.

The pH value at which the solutions became isoelectric differed for various gelatins. Solutions of Coignet's Gold Label and of Coignet's Silver Label gelatin remained at a pH of 5.0 during dialysis, which is the isoelectric point for these gelatins. Solutions of Bacto-gelatin of the Digestive Ferments Company, originally at a pH of about 4.5 changed rapidly to a value within a range of pH 7.6 to 8.4, the isoelectric range for this gelatin, and then remained at this value during further dialysis. A pigskin gelatin (Ucopco gelatin of the United Chemical and Organic Products Company) became isoelectric at a pH of 6.8. A spray-dried powdered gelatin of commercial grade became isoelectric at a pH of 5.6.

It has been shown recently by Briefer (1929) that the isoelectric point of gelatin depends upon the previous chemical treatment. Kraemer and Dexter (1927) and Briefer and Cohen (1928) have obtained an isoelectric point at a pH of 5.0 for lime-treated calfskin gelatin and an isoelectric range around a pH of 8 for acid-treated pigskin gelatin. Sheppard and Houck (1930) have found a similar isoelectric point for calfskin gelatin and lime-treated pigskin gelatin, and isoelectric ranges of pH 7 to 8 and pH 6 to 7 for two samples of acid-treated pigskin gelatin. It would appear that the Ucopco gelatin and the Bacto-gelatin were of this latter character.

Before any comparison of the isoelectric flocculation of the various gelatins could be made, it was first necessary to study in detail the behavior of one particular gelatin in order to develop a suitable technique for the equilibrium study. Coig-

net's Gold Label gelatin was selected on account of its relative purity and has been used exclusively in the following experiments.

Solutions 2 and 3 were dialyzed in the 100 cc. dialyzer; Solutions 4 and 5 were dialyzed in the 2 liter dialyzer; a small amount of toluene was added before dialysis to prevent bacterial action during dialysis. Solutions 6, 7, and 10 were dialyzed in the 100 cc. dialyzer; no toluene was added to these solutions or to the following solutions. Solution 8 was dialyzed in the 2 liter dialyzer; portions of this solution were diluted to make Solutions 9 and 11. Solution 1 was dialyzed for a longer period of time (a total of 20 hours), and between periods of dialysis was heated four times to temperatures between 80°C. and 90°C. for periods of 10 minutes. All solutions were at a pH of 5.0 and had a specific conductivity of 6×10^{-6} to 10×10^{-6} reciprocal ohms.

When dialysis was completed each solution was pipetted into Pyrex test tubes, which were sealed in the blast lamp, and then heated to 80°C. for 10 minutes. Solutions treated in this manner showed no evidence of bacterial growth during the course of the experimental work and tubes now over 2 years old are still clear. This method of preventing bacterial action was found to be more efficient than the usual method in which antiseptics are employed and does not bring into question the effect upon flocculation of the addition of any foreign substance.

The chief objection which could be made to this method of preventing bacterial action is that irreversible changes are known to occur when gelatin solutions are heated. Sheppard and Houck (1930) have followed the rate of this change in concentrated gelatin solutions by measurement of viscosity. They found the change occurring at 40°C. at the isoelectric point to be extremely small and at 85°C. a 1 per cent change occurred in 10 minutes.

It was therefore necessary to conduct a preliminary investigation to determine a satisfactory time and temperature for the routine heating. It was soon found that heating solutions for 10 minutes at temperatures above 80°C. brought about an irreversible decrease in the amount of gelatin flocculating on subsequent cooling to 25°C., as well as a decrease in the rate at which this flocculation equilibrium is reached. It was also found that this effect increased rapidly with the temperature, apparently as a logarithmic function. At 80°C., no apparent effect on the flocculation was produced in 10 minutes while, as noted above, the bactericidal results seem entirely satisfactory; this time and temperature were accordingly adopted for all of the experimental work with the exception of Solution 1 which was used in Sections 1 and 2 following. The results of heating gelatin solutions at temperatures above 80°C. and for times longer than 10 minutes are of considerable interest and will be discussed subsequently in this paper.

After heating, the solutions were put into thermostats and, after flocculation began to occur, the gelatin, which remained in solution, was determined at regular intervals. A separate tube, not previously opened, was used for each determination, to avoid contamination with microorganisms.

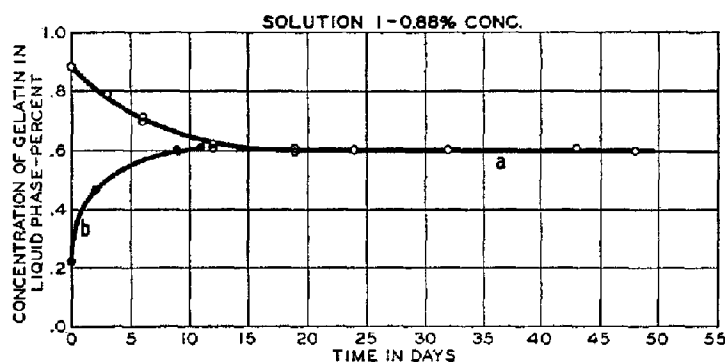
The concentration of gelatin in the solutions was determined by measurement

of the refractive index at 25°C. with a Zeiss immersion refractometer. A calibration, made by determining the refractive indices of solutions of known concentration, showed that the refractive index of a gelatin solution is a linear function of the concentration, as has been found also by Walpole (1913) and Sheppard and Houck (1930). Concentrations of gelatin could be determined by this means to within 0.01 per cent.

II. EXPERIMENTAL

1. *The Establishment of Equilibrium in Flocculating Gelatin Solutions*

The flocculation of gelatin from solutions is temperature reversible. Experiments were made to determine the extent of flocculation at a



GRAPH 1. Rate of flocculation at 25°C. and redispersion at 25°C. of gelatin previously flocculated at lower temperature.

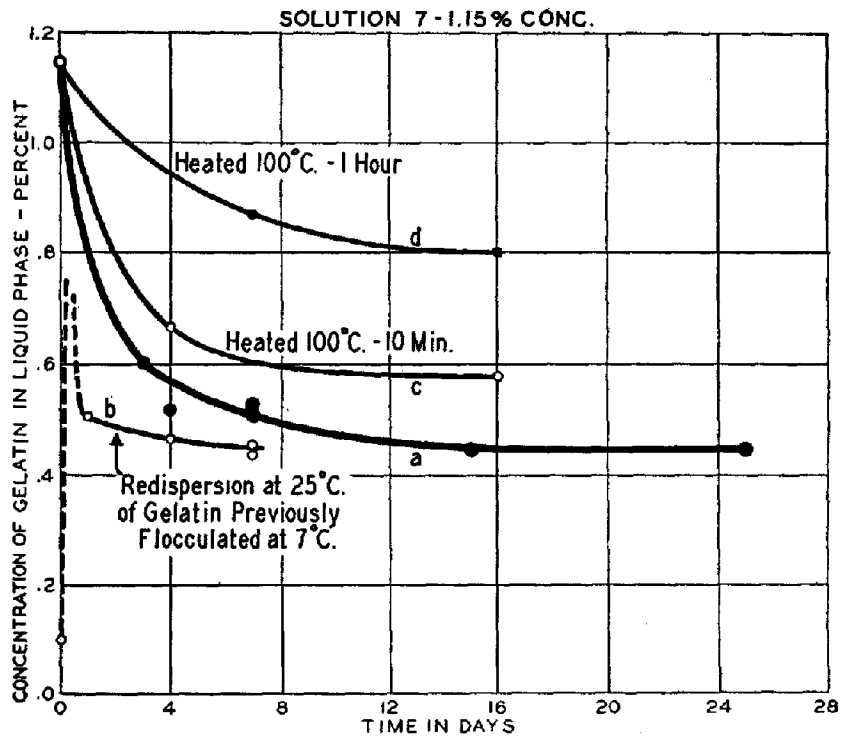
given temperature and whether the system would reach an equilibrium. Twelve tubes containing 0.88 per cent gelatin solution (Solution 1) were put in a thermostat at 25°C., immediately after their heating to 80°C., and were kept at this temperature for 45 days. The concentration of gelatin remaining in the liquid phase, determined at regular intervals, reached a constant value after 18 days, and at the end of 2 months this value had not changed (Graph 1, Curve *a*). Another set of tubes containing this same solution was brought to a temperature of 15°C. and flocculation allowed to occur. These tubes were then placed in the thermostat at 25°C. A portion of the gelatin flocculate redispersed (Graph 1, Curve *b*), and after 12 days at 25°C. the liquid

phase in these tubes contained the same concentration of gelatin as was obtained when flocculation occurred after direct cooling to 25°C.

Thus, at a given temperature, the same concentration of gelatin in the liquid phase is obtained whether the system is brought to that temperature from a lower temperature or from a higher temperature, and no further flocculation occurs after a certain length of time. This indicates that in such systems an apparent equilibrium exists between the gelatin flocculate and the gelatin remaining in solution at a given temperature.

2. Irreversible Change on Heating Gelatin Solutions

It has been mentioned that Solution 1 was heated several times to temperatures above 80°C. for periods of 10 minutes. This treatment



GRAPH 2. Rate of flocculation at 25°C. showing the effect of heating the solutions above 80°C.

produces irreversible changes; and, for this reason, the values at flocculation equilibrium for this solution cannot be compared with other solutions of the same concentration which were not heated above 80°C.

The magnitude of the effect of this irreversible change in decreasing the percentage of gelatin flocculation at any given temperature is shown in Graph 2. Curve *a* shows the rate at which flocculation occurred at 25°C. in Solution 7, of 1.15 per cent concentration, which had been heated only to 80°C. in the routine heating. Curve *c* shows the rate of flocculation at 25°C. for this same solution which was heated to 100°C. for 10 minutes, and Curve *d* for the same solution heated to 100°C. for 1 hour.

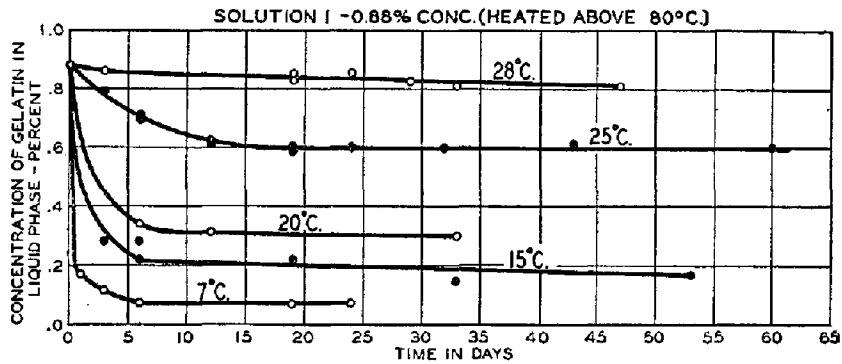
In solutions which have been heated at 100°C., even for several hours, there is, however, no change in the refractive index or hydrogen ion concentration. The fact that the effect upon flocculation of this heat treatment is not removed when the treated solutions are subsequently electro-dialyzed, indicates that this effect is not due to the presence of small amounts of ammonia, or other electrolytes, which might conceivably be produced by hydrolysis. In general, it may be said that there are no indications that the irreversible change on heating is due to any marked change in the chemical nature of the gelatin.

3. *The Relation between Temperature and Flocculation Equilibrium*

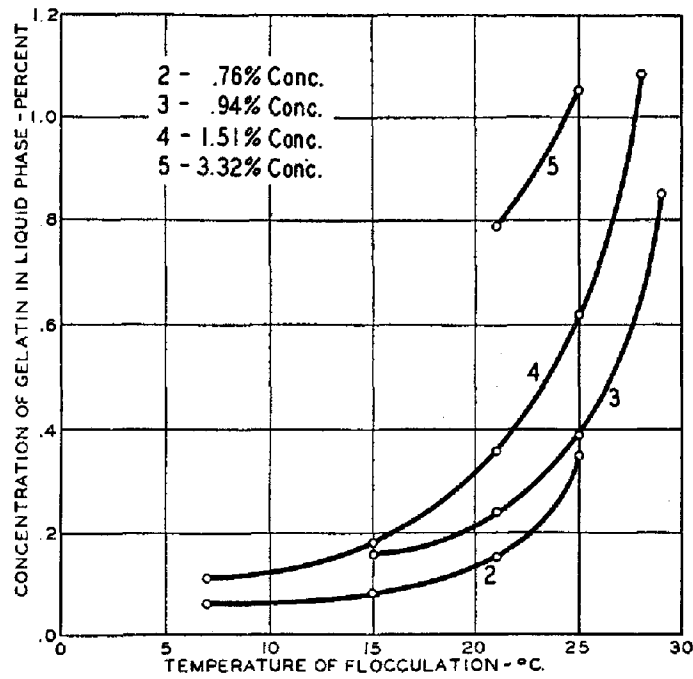
The flocculation equilibrium for Solution 1 was determined at a number of temperatures. It is seen from Graph 3 that flocculation was rapid at 7°C. and that after 6 days no further flocculation occurred. Flocculation equilibrium was approached more slowly at higher temperatures and at 28°C. had not been reached after 40 days.

The relation between the temperature of flocculation and the concentration of gelatin remaining in solution after flocculation has occurred is shown further for Solutions 2, 3, 4, and 5 in Graph 4. These curves resemble the temperature-gelation curve for gelatin systems which are not at the isoelectric point, or which contain sufficient electrolytes or other impurities to prevent flocculation. The aggregation process which results in flocculation of gelatin in the absence of electrolytes must be very similar to the aggregation process which results in gel formation when small amounts of electrolytes are present in the gelatin system. This aggregation is presumably the result of the loss

of thermal energy by the gelatin molecules. In the case of flocculation the coalescence of the molecules is more complete than in the formation of a gel.



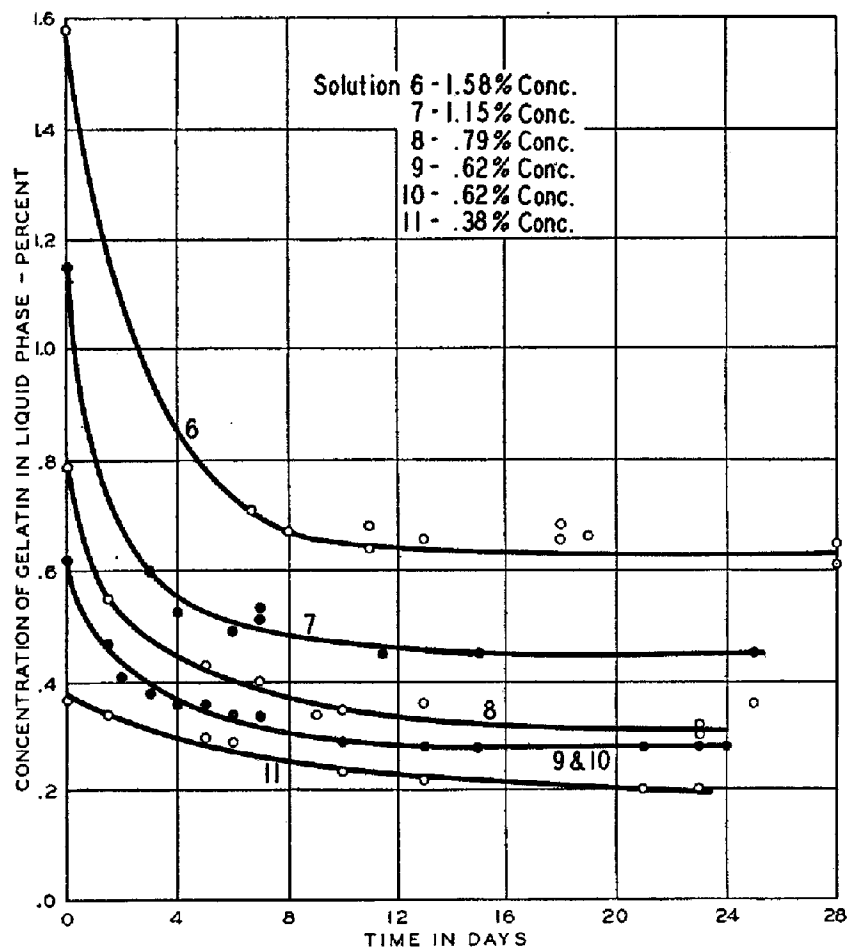
GRAPH 3. Rate of flocculation at various temperatures.



GRAPH 4. Temperature reversible flocculation of gelatin.

4. *Relation between Original Gelatin Concentration and Flocculation Equilibrium*

The flocculation equilibrium at 25°C. was determined for Solutions 6 to 11, inclusive, of concentrations ranging from 1.58 per cent to 0.38



GRAPH 5. Rate of flocculation at 25°C. for solutions of various concentrations.

per cent. The large differences in the concentration of gelatin remaining in the liquid phase after flocculation in these solutions is shown in Graph 5. The concentration of gelatin remaining in solution is evi-

dently dependent upon the concentration of the original solution, increasing nearly linearly with increasing amounts of gelatin in the original system. The slight departure from linearity may perhaps be accounted for by the fact that the rate at which equilibrium is established is lower for dilute gelatin solutions than for more concentrated solutions.

It has been shown above that a definite relation exists at any temperature between the flocculate and the gelatin remaining in solution. But this relation cannot be considered as an equilibrium condition of a single chemical substance of a definite solubility since the concentration of gelatin remaining in solution is dependent not only on the temperature but also on the amount of gelatin present in the system. A gelatin solution must, therefore, be considered either as a mixture of two or more chemical substances of different solubilities or as a mixture of particles similar in chemical nature but non-uniform in size, or some still more complex system must be assumed. In the following experiments an attempt is made to examine these several possibilities.

5. Flocculation from the Precipitate Fraction

After flocculation had occurred in Solution 2 (0.76 per cent) at 25°C., the precipitate containing 53.8 per cent of the gelatin present was separated from the liquid phase. After washing several times with distilled water and centrifuging, the flocculate was dispersed in distilled water by warming and gave a solution of 1.33 per cent gelatin content. This solution was sealed in Pyrex tubes, heated to 80°C. for 10 minutes and put in the thermostat at 25°C. 83 per cent of the gelatin flocculated from this solution within 1 day.

In a preliminary experiment, a solution of 1.16 per cent concentration was allowed to flocculate at room temperature (about 18°C.). After washing, redispersing, and reflocculating twice, with the removal of the liquid phase each time, the precipitate was dispersed to form a solution of 1.03 per cent concentration. 90 per cent of the gelatin flocculated from this solution at room temperature within 1 day.

Thus, it is seen that flocculation of gelatin occurs in solutions of these precipitate fractions to a much greater extent and at a greater rate than in the original solution of the same concentration and at the same temperature. It is probable that the gelatin which remains in

solution after reflocculation in these precipitate fractions is chiefly gelatin which would normally have remained in solution at the original flocculation temperature but which was carried down mechanically. It would appear possible that, if the precipitate could be washed, redispersed, and reflocculated a sufficient number of times, a solution would be obtainable from which the gelatin would entirely flocculate at the original temperature of flocculation. It is intended that a thorough investigation be carried out on this point.

6. The Redispersal of Low Temperature Flocculates

An interesting phenomenon was observed when tubes in which flocculation equilibrium was established at 7°C., were brought to a temperature of 25°C. As soon as thermal equilibrium was reached at 25°C., the flocculate entirely disappeared to give an opalescent solution from which reflocculation quickly occurred. After several days, equilibrium was again reached and the liquid phase contained the same gelatin concentration as was obtained when the flocculation equilibrium was reached by lowering the temperature from 80°C. directly to 25°C. This is shown in Graph 2. Curve *a* shows the rate at which equilibrium was reached when the tubes were placed directly in the thermostat at 25°C. Curve *b* shows the changes occurring at 25°C. in the tubes in which flocculation equilibrium had been reached previously at 7°C.

This behavior in dispersion is best explained as a result of the intimate mixture in the 7°C. flocculate of particles which would be in solution at 25°C. with those which would be flocculated at that temperature, so that on heating to 25°C. the more dispersible particles exercise a disruptive force on the entire flocculate.

7. Flocculation of Gelatin from the Liquid Fraction

The liquid phase of gelatin Solution 3 was removed after flocculation had occurred at 25°C., and concentrated in the electro-dialyzer from a gelatin content of 0.39 per cent to 1.17 per cent. This solution was then sealed in Pyrex tubes and heated to 80°C. as usual. At 25°C., this solution remained clear and fluid for over 30 days, no flocculation occurring. When the temperature of this solution was lowered to 21°C., 29 per cent of the gelatin had flocculated in 3 days and 48 per

cent in 30 days. At 15°C., 65 per cent flocculated in 2 days and 76 per cent in 30 days.

Similarly a solution was prepared by removing the liquid phase from Solution 2 after flocculation had taken place at 25°C. and concentrating from a gelatin content of 0.34 per cent to 1.22 per cent. No flocculation occurred as long as the temperature of this solution was kept at 25°C. At 15°C., 68 per cent of the gelatin precipitated in 3 days; and at 7°C., 83 per cent in the same time.

A solution containing 0.81 per cent gelatin was flocculated at 21°C., 75.3 per cent of the total amount of gelatin precipitating. The liquid phase of 0.20 per cent gelatin content, so obtained, was concentrated to 1.0 per cent. No flocculation occurred at 21°C. At 15°C., 40.8 per cent of the gelatin flocculated in 26 days leaving a liquid phase of 0.68 per cent gelatin concentration.

The flocculation of gelatin from liquid fractions resembles the flocculation from the original solution, but occurs only over a temperature range below the original flocculation temperature. This is further strikingly shown by the behavior of the liquid fraction obtained from a very concentrated gelatin solution (about 8 per cent) at 25°C. This liquid, containing 2.39 per cent gelatin, remained clear and fluid at 25°C. but at lower temperatures flocculation occurred.

The behavior of solutions of these liquid and precipitate fractions is difficult to explain by assuming that the non-uniformity of gelatin is due to the presence of only two constituents or of even a small number of constituents, whether they differ chemically or physically. There is evidence to indicate that this non-uniformity exists in the liquid fraction as well as in the original solution. It has been shown above that a liquid phase obtained at any temperature can be concentrated to one-tenth of its volume without flocculation occurring as long as the temperature is not lowered below that of the original flocculation. It must be assumed, then, that the constituents of the original precipitate are entirely insoluble in the liquid phase at that temperature. Otherwise, a further precipitation of these constituents would occur when the solution was concentrated. In the experiments with Solutions 2 and 3 above, the concentrated liquid fraction, obtained by flocculation at 25°C., gave no precipitate at 25°C. but, when cooled to 21°C., 15°C., and 7°C., an increasingly large partial flocculation occurred. It

is evident that these partial flocculations cannot be due to the separation of a residual portion of the constituent or constituents represented in the original flocculate, but must indicate a heterogeneity in the 25°C. liquid fraction. Confirming evidence of this heterogeneity of liquid fractions has also been obtained from the observation that, in them also, flocculation is a function of both temperature and concentration.

The same reasoning may be applied to any fraction. Since it appears that the number of fractions obtainable depends simply upon the number of temperatures at which flocculations and separations are made, it would seem that the number of species of gelatin constituents present must be assumed to be large. These gelatin constituents may represent molecules differing in chemical structure or they may be the result of different degrees of disruption of the collagen macromolecule, differing in size rather than in chemical constitution. As noted above, there is evidence favoring the latter assumption. In either case it would be expected that, at definite temperatures, particular constituents would lose sufficient thermal energy to form loose aggregates and flocculate from solution. On raising the temperature, these aggregates would be broken up to give a solution of the original constituents. The change occurring when gelatin solutions are heated to higher temperatures may be regarded as a further irreversible breaking up of the various gelatin constituents. According to this conception, when flocculation has occurred in gelatin solutions at 25°C., all gelatin constituents which tend to precipitate at this temperature have been removed from the liquid phase and no further flocculation should occur on concentration. On lowering the temperature, however, another group of constituents tends to precipitate.

8. The Salting-Out of the Flocculate Fraction and of the Liquid Fraction

Further evidence concerning the heterogeneity of gelatin is given by the different behavior of solutions of the liquid fraction and of the precipitate fraction when these solutions are salted out with ammonium sulfate.

Varying amounts of a saturated solution of ammonium sulfate were added to 10 cc. portions of a gelatin solution containing only the precipitate fraction, and to 10 cc. portions of a solution containing only the

liquid fraction, obtained by flocculation at 25°C. Both solutions were at 25°C. and of the same concentration, 0.90 per cent. In the case of the precipitate fraction, the solution became turbid when 5.6 per cent saturated with ammonium sulfate. The turbidity increased gradually on the addition of more ammonium sulfate. At 9 per cent of saturation, a small amount of precipitate had formed. The amount of this precipitate increased gradually with further addition of ammonium sulfate, until the solution was 16 per cent saturated, when the precipitate flocculated out completely leaving a clear solution. In the case of the liquid fraction, no turbidity appeared until the solution was 14 per cent saturated, when just a faint opalescence occurred. At 15 per cent of saturation, a heavy precipitation occurred.

The behavior of these fractions of gelatin with respect to precipitation by ammonium sulfate can also be explained by the non-uniformity of the gelatin constituents. Thus, solutions of the precipitate fraction, obtained at 25°C., contain those classes of constituents which are most easily precipitated. There are present in this fraction only a small number of constituents which are precipitated by a low concentration (5.7 per cent of saturation) of ammonium sulfate. But there also are present other classes of constituents which can be precipitated by higher concentrations of ammonium sulfate. Hence, on increasing the concentration of ammonium sulfate, the amount of precipitation increases gradually. In the case of the liquid fraction obtained at 25°C., no precipitation occurs for low concentrations of ammonium sulfate since all classes of constituents which can be precipitated by concentrations less than 15 per cent of saturation have already been removed from this fraction. From the large and sudden precipitation which occurs in this fraction at 15 per cent of saturation, it appears that gelatin contains a relatively large proportion of constituents of this group. The number of constituents which require more than 15 per cent of saturation to cause precipitation is relatively small.

At first sight it would seem that the data from the precipitate fraction are somewhat in conflict with those from the liquid fraction; that is, complete precipitation in the precipitate fraction occurred at 16 per cent of saturation while the heavy precipitation in the liquid fraction took place when only 15 per cent saturated with ammonium sulfate. This apparent overlap in the characteristics of the two solutions

can easily be explained. The degree of ammonium sulfate saturations, as stated, is only an indication of the amount of sulfate added and not of the actual concentration present. It is probable that a certain amount of ammonium sulfate is removed from the solution by the flocculate and that, in the case of the precipitate fraction, the nominal 16 per cent saturation at which the heavy precipitation took place was actually somewhat less. The seemingly complete salting out of the liquid fraction at 15 per cent of saturation indicates a carrying down of the less precipitable species during the heavy precipitation which occurs when the boundary between the original precipitate and liquid fraction is passed. This is apparently the same phenomenon that was observed in the case of rapid flocculations at low temperatures.

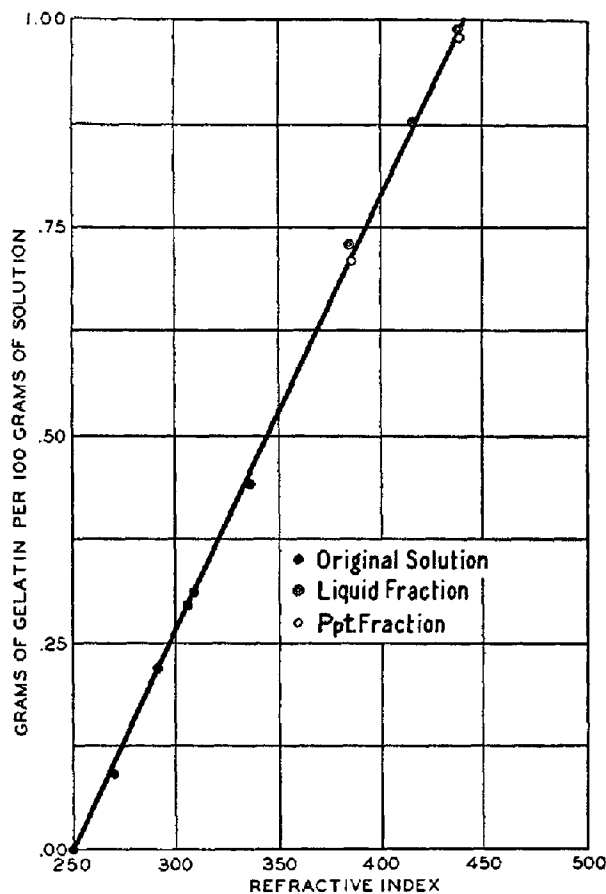
The same marked difference in the behavior of the precipitated fraction and of the liquid fraction toward salting out with ammonium sulfate was also observed at 60°C. This would seem to show that the characteristic differences between the gelatin constituents persist at temperatures above the isoelectric flocculation range. This is also indicated, of course, by the unchanging behavior of precipitate fractions when repeatedly redispersed and flocculated.

9. Refractive Indices of the Liquid and of the Precipitate Fractions

It is shown in Graph 6 that the refractive indices for solutions of the liquid fraction and of the precipitate fraction are, within the experimental error, the same linear function of the concentration as for the original solution. From both the liquid and precipitate fractions of Solution 1, flocculated at 25°C., solutions of two different concentrations were prepared. The refractive indices of these two sets of solutions were determined and those of the same concentration from either fraction were found to be identical within the experimental error. This indicates that there can be no very great chemical difference between the gelatin constituents of these fractions. Since refractive index is dependent upon the basic molecular structure and not upon the size of the constituents, the assumption that gelatin consists of a large number of constituents which differ only in the degree of association or orientation offers the best explanation for the observed facts.

If this explanation is correct, a marked difference should exist be-

tween the liquid and the precipitate fractions with respect to some physical characteristic, such as viscosity, which is dependent upon the degree of association or orientation.



GRAPH 6. Relation between refractive index and concentration. The figures on the abscissa are abbreviations for 1.33250 etc.

10. Viscosity of the Liquid and of the Precipitate Fractions

The previously discussed irreversible change, occurring on heating, causes a decrease in the viscosity of gelatin solutions and a decrease in the amount of gelatin which flocculates from solution at any given temperature. It is to be expected, therefore, that the liquid fraction

would have a lesser viscosity than the precipitate fraction. This was found to be the case. The viscosities of the original solution and of solutions containing the precipitate fraction flocculated at 25°C. and of the solutions containing the liquid fraction after flocculation at 25°C. were determined. Viscosities were measured in an Ostwald viscosimeter at 40°C., at which temperature it has been shown by Hatschek and Jane (1926) and others that the rate of flow through a capillary is a measure of true viscosity. It was found that the viscosity of the original solution is intermediate between the viscosity values of the precipitate fraction and of the liquid fraction and that flocculation can effect a separation into categories exhibiting a marked difference in a physical characteristic.

III. CONCLUSIONS

The results of this investigation show that a gelatin solution consists of a considerable number of constituents. At a particular temperature, certain gelatin constituents tend to aggregate and to flocculate from solution. When these particular gelatin constituents have completely flocculated, no further change occurs in the system and an apparent equilibrium exists. This is not a dynamic equilibrium between the gelatin flocculate as a whole and the gelatin remaining in the solution but a steady state determined for that system by the temperature.

It is also shown that gelatin can be separated into fractions in which the gelatin constituents are more nearly uniform and tend to flocculate over a much narrower temperature range. It should be possible to obtain a number of fractions in which all of the gelatin would flocculate at a definite temperature. The aggregation of the various gelatin constituents is presumably due to loss of thermal energy, and the temperature at which this occurs must be some function of the mass of the constituent. It is natural to assume, then, that the constituents which flocculate at a given temperature are larger than those which remain in solution at that temperature. Recently, Krishnamurti and Svedberg (1930) have obtained evidence with the ultracentrifuge that the constituents of a gelatin solution are heterogeneous as to mass, even at a pH value at which there is no tendency toward aggregation.

There is much reason to suppose that the gelatin constituents do not differ very greatly chemically since different fractions have the same refractive index and the same isoelectric point.

The data as a whole are best explained by considering the gelatin constituents to be different degrees of association of the same or very similar molecular structural units. This is in agreement with Shepard and Houck (1930), who consider that "the molecules of gelatin are fundamentally identical with those of collagen, the difference being only in the degree of association and orientation". Meyer and Mark (1928) have interpreted the x-ray data obtained from collagen as indicating that the micelles of the collagen fiber are built up of main valency chains of anhydro-amino acids. It may be supposed that during peptization of these fibers, the amino acid chains become separated, disorientated, and partially broken up, so producing the heterogeneous system which we know as gelatin. It is evident that the manner in which this breaking-up proceeds depends upon the chemical treatment previous to the peptization process and the gelatin produced from lime-treated collagen would be expected to differ from that from acid-treated collagen.

From the results herein reported it seems evident that the technique of isoelectric flocculation of electrolyte-free gelatin offers a profitable method for the study of gelatin and an extended investigation along these lines should yield much valuable information concerning the nature of gelatin. It is possible that this method may also be extended to other hydrophilic colloids.

In conclusion, the writer wishes to express her sincere appreciation of the sympathetic assistance of Dr. C. L. Alsberg, under whose guidance this work was carried out.

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