# XXIV. DIAGRAMMATIC CO-ORDINATION OF PHENOMENA RELATING TO AGGREGATION OF SOLS.

By GEORGE STANLEY WALPOLE.

*From the Wellcome Physiological Research Laboratories, Herne Hill, S.E.*

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The published investigations of the following phenomena embrace a considerable portion of the facts so far accumulated on the causes inhibiting or actuating the aggregation of sols, especially when they are mixed with proteins and electrolytes:

1. *The action of electrolytes on sols*—causing the running together of sol particles when added to the sol in sufficient concentration.

2. "*Protection*"—the property of proteins and certain other substances in preventing the aggregation of sols by electrolytes.

3. *Change of sign of the electrostatic charge on the protected sol particle.*

4. *Non-coincidence of point of maximum flocculation and point where sign of observed particle changes.*

5. *Change of sign of charge on the protein at its isoelectric point.*

6. *Mutual precipitation of dissimilarly charged colloids* in solutions free from electrolytes, including the particular case where one of the colloids is amphoteric and has the properties of "protection," e.g. is of a protein nature; also the solubility of the precipitate formed in excess of either constituent.

7. "*Irregular series*" (Bechold) and "*Pre-zone phenomenon*" (Buxton).

8. "*Reversible and irreversible aggregation.*"

Up to the present these phenomena have been generally investigated one at a time. Each worker has naturally chosen experimental conditions particularly suitable for the study of one phenomenon only. Little or no co-ordinated work has been done, linking up on a quantitative basis the known facts, and demonstrating them as parts of a well-ordered scheme of things, easy to remember, and, on the surface, easy to understand.

The plotting of results, obtained in this class of work, in chart form, undertaken primarily for my own use, has proved so helpful to me in

following out these relationships that I feel in a position to recommend them for general use in expressing the results of experiments of this type.

In working with a very finely divided oil emulsion, the particles of which were so small that, without misapplying the term, it could be called an "oil sol," I found, in common with previous experience, that the addition of hydrochloric acid in sufficient strength caused the particles to run together and the oil to separate. The presence of a sufficient quantity of gelatin, however, prevented this from happening; the sol was "protected" in this case. When the gelatin was in certain concentrations, not too small and not too great, the oil particles aggregated immediately if just the right amount of acid was added, but not if the acid was too strong or too weak.

This seemed to point to some new and peculiar phenomenon where the gelatin acted as an "activator" to the acid, for in this case the acid was far too weak to aggregate the sol alone. It was not till later that it was seen that this fitted into the general scheme of things and was capable of expression in terms of things known, and that the invention of a new "phenomenon" and a corresponding vocabulary was unnecessary. In other cases where gelatin of a certain strength caused immediate aggregation of the oil in the presence of a small quantity of acid incapable of doing this alone, it was found that all strengths of acid greater than this brought about the same result.

To simplify the matter of making and studying these mixtures it was decided that each should contain 2 cc. of oil sol, 2 cc. of gelatin solution and 2 cc. of hydrochloric acid solution. Rows of these mixtures were put up in test tubes. In any one row the 2 cc. of acid placed in each tube was of the same strength throughout; the concentration of the 2 cc. of gelatin solution added varied from tube to tube. The strength of acid used was changed from row to row.

As very dilute solutions were sometimes used it was found necessary from the outset to express their concentrations as powers of ten, either of actual concentration or normality. For instance the strength of a gelatin solution was expressed in terms of its concentration. A 1 in 10 solution was expressed as "gelatin $10^{-1}$," for example, and a 1 in 15,000 solution as $10^{-4\cdot2}$. Similarly 0·001 N hydrochloric acid was written $10^{-3}$ N HCl.

After the mixtures had been made two hours they were examined carefully, and notes made of those which showed to the eye no change of state of the sol, those in which partial aggregation had occurred, and those in which the separation of the oil was complete.

Observations in the electric field using the microscope method showed

that the oil particles in some of these mixtures were positively and in others negatively charged. The sign of the charge did not depend upon the state of aggregation—either charge was observed in tubes containing mixtures which did not aggregate just as in those that did.

When the results of these two sets of observations were expressed in tabular form little that was intelligible could be made of them; in the form of a chart they are all summarised in Fig. 1.

### Aggregation Diagram No. 1.

It will be remembered that each mixture consisted of 2 cc. of oil sol, 2 cc. of gelatin solution and 2 cc. of hydrochloric acid. As ordinates are expressed negative exponents of normality of the hydrochloric acid put into the tube; as abscissae the negative exponent of the concentration of gelatin. The tubes were considered one at a time and a mark plotted on the chart at the point corresponding with the concentration of acid and gelatin in the tube indicating (1) that no change had taken place in the distribution of

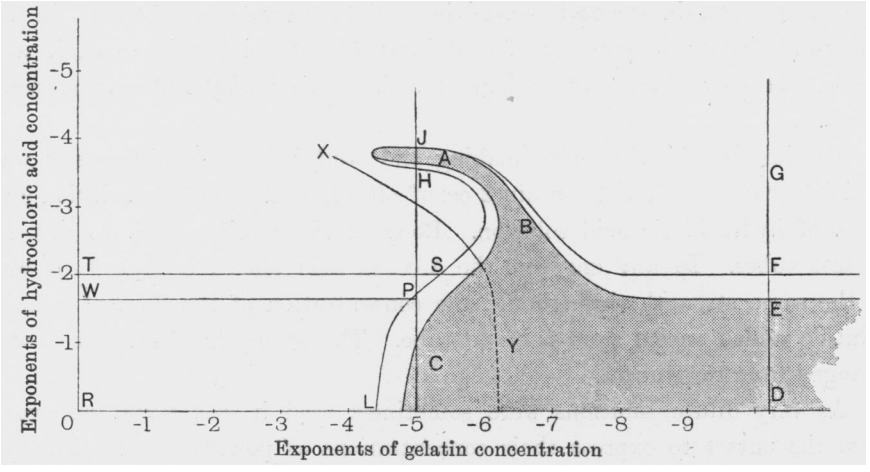


**Fig. 1. Aggregation diagram No. 1. Oil sol-gelatin-hydrochloric acid.**

the oil particles, (2) that complete aggregation and separation of the oil had occurred, or (3) that aggregation was only partial.

The sign of the oil particles, whether aggregated or not, was indicated by a small + or −. Lines were now drawn on the diagram by the help of these distinguishing marks. The first, *LHJF*, enclosed all points where aggregation, whether partial or complete, was observed. Another, *CABE*, enclosed all points where separation of the oil was complete.

The line *XY* was drawn through all points where the oil particles appeared to have no electric charge; that is, they did not wander in the electric field. In all mixtures represented by points to the right of this line, the particles were negatively charged. All points to the left of this line correspond to mixtures in which particles were positively charged.

The chart—called for convenience an "aggregation diagram"—represents the state of aggregation and sign of the dispersed particle, two hours after mixing, of every possible mixture of equal volumes of oil sol, gelatin solution and hydrochloric acid. Mixtures stronger in HCl than 0·33 normal were not plotted in the present instance simply to avoid unnecessary complication of the diagram.

At this stage it will be well to examine this chart with reference to the list of phenomena given at the beginning of this paper.

1. *The action of electrolytes on sols.* The action of electrolytes in the absence of protein is seen in the extreme right of the diagram. In the particular case shown in Fig. 1 the protein in a dilution of one in one hundred million or more has no effect. The lines bounding the aggregation area become parallel to the abscissae, and may be produced to infinity to the point where the protein is infinitely dilute. The ordinate *DEFG* represents, therefore, the behaviour of mixtures of 2 cc. sol, 2 cc. water and 2 cc. of every strength of electrolyte solution, in this case HCl. No aggregation occurs with extremely dilute acid, but when the strength of acid used is $10^{-2}$ N a slight separation of the sol occurs. As the strength of acid increases the amount of sol separation increases until at $10^{-1\cdot6}$ N and all concentrations above this it is described as "complete."

In the case of an electronegative sol and an electrolyte with a polyvalent cation the ordinate *DEF* would be much shorter—a result obtained equally if the sol were electropositive and the electrolyte contained a polyvalent anion.

At present the actual mechanism of electrolyte precipitation of sols is not very well understood. It is hoped that in the course of this work some phenomena may be observed which can be co-ordinated with the facts already known about the conditions determining the stability or otherwise of sols.

2. "*Protection.*" Any point in the area *WPLR* corresponds to a mixture in which the gelatin has prevented the acid from aggregating the oil sol. Enough acid is present to bring this about completely if the gelatin were not present. It may therefore be referred to as the "area of complete protection against aggregation." In the same way the area *WPST* is "the area of complete protection against partial aggregation," for in the mixtures

the behaviour of which is represented by points in this area the acid alone would only partially aggregate the sol in two hours.

Areas of "partial protection against partial aggregation" and "partial protection against complete aggregation" are to be found in the diagram and may some day become matters of study.

3. *The change of sign of the "protected" sol particle.* It is interesting to note that the "area of protection" lies entirely inside the area where the sign of the sol particles, or rather the sol-gelatin complexes, is positive. Also, and this is shown better in Fig. 2 where mastic sol was used, it is possible to have a sol protected by gelatin in a mixture containing as much as one-third of its volume of N HCl, while a mixture exactly similar except that the acid is 6000 times more dilute, flocculates immediately. A result of this kind was observed by Mines [1912] using a gold sol.

4. *Non-coincidence of point of maximum flocculation and point where the sign of the observed particle changes.* The type of flocculation which may be observed in extremely dilute acid concentration if the concentration of the protein be adjusted with great care, is remarkable in that no change of sign of the electronegative sol particle necessarily takes place.

In Fig. 1 the ordinate *CPHJ* passes through points representing a series of mixtures in which are placed say 2 cc. of oil sol, 2 cc. gelatin $10^{-5}$, and 2 cc. of hydrochloric acid decreasing in concentration from tube to tube. From normal acid to decinormal acid ($10^{-1}$) complete flocculation is observed, from there to $10^{-1\cdot7}$ the flocculation is incomplete, and as far as can be made out in solutions containing so much electrolyte the sign of the visible particles is +. With acid strengths from $10^{-1\cdot7}$ to $10^{-3\cdot5}$ there is no change whatever visible in the state of aggregation of the sol, but at HCl $10^{-3}$ N no charge can be detected on the particles, while at $10^{-3\cdot1}$ and all tubes following, which contain weaker acid, the sign of the particles is −. Following on the series; partial separation is observed with acid $10^{-3\cdot6}$; at $10^{-3\cdot7}$ and $10^{-3\cdot8}$ the separation is complete; at $10^{-3\cdot9}$, $10^{-4}$ and onwards no change in sol aggregation can be seen.

The change of sign of the sol particles with constant gelatin concentration and diminishing acid takes place outside either of the ranges where aggregation occurs; and this assumes a particular interest when it is remembered that globulin suspensions are negatively charged at the point of maximum flocculation, and on increasing the acid concentration they are well dispersed before the visible particles are electrically neutral. This observation recorded by Chick [1913] I have confirmed. The figures given in the paper referred to are $H^{\cdot}$ concentration $32 \cdot 10^{-7}$ for the point of maximum flocculation [cf.

Michaelis and Rona, 1910]. At H· concentration $748 \cdot 10^{-7}$ the sign is still –, changing to + at $1140 \cdot 10^{-7}$.

5. *Change of sign of the protein at its isoelectric point.* A line may be drawn through all points having H· ion concentration equal to the isoelectric point of the protein. This will divide the diagram into two parts representing mixtures containing + charged and – charged protein respectively.

6. *Mutual precipitation of dissimilarly charged colloids.* Biltz [1904] in his classical confirmation of earlier work by Picton and Linder [1897], and Lottermoser [1901] expressly state that *in the absence of electrolytes* oppositely charged colloidal solutions precipitate one another from solution when mixed in certain definite proportions, and that the precipitate is soluble in excess of either constituent. Whether this limiting condition is ever fulfilled in practice is questionable; but still the mixtures containing only hydrochloric acid $1/3 \cdot 10^{-3}$ N HCl may be considered in the class of mixtures of colloidal solutions to which Biltz referred. Following the abscissa representing a row of mixtures made by mixing say 2 cc. acid $10^{-3}$ N, 2 cc. oil sol and 2 cc. gelatin solution, decreasing in concentration from tube to tube, it is seen that mutual precipitation only occurs within a certain range of gelatin-hydrosol ratios. The large number of the variables from tube to tube, however, all of which affect aggregation, makes the analysis of this phenomenon in the present state of our knowledge very difficult, and postpones the complete understanding of the curious shape of the aggregation area.

7. "*Irregular Series,*" and "*Pre-zone phenomenon.*" The first of these terms was used by Bechold [1904] to describe the results obtained by aggregating various sols and bacterial suspensions with solutions of ferric and aluminium chlorides. Buxton and Rahe [1910] saw in these experiments the demonstration of the action of the acid solution when concentrated, and the precipitating action of a colloidal solution in greater dilutions. In order to avoid confusion they referred to their numerous flocculation experiments in which irregular series occurred from other causes as demonstrations of the "pre-zone phenomenon."

8. *Reversible and Irreversible Aggregation.* Sols such as gold, oil and mastic are referred to as irreversible hydrosols because when flocculated by electrolytes they do not disperse again when the electrolytes are removed by dialysis, and, when flocculated by traces of acid, the reversal of this effect is not obtained by addition of corresponding traces of alkali.

In the aggregation area of Fig. 1 there may be recognised two types of flocculation. To the extreme right, where separation of the sol has occurred

in the absence of protein, the aggregation is not reversible by adding alkali. At *JH* where the protein plays an all-important *rôle* in the flocculation, the addition of a trace of alkali results at once in the almost complete reversal of the phenomenon. In other parts of the aggregation area it would appear as if both the reversible and irreversible type of aggregation were found varying in relative amount with the position on the diagram. When the sol is of oil the former type shows a grape bunch appearance under the ultramicroscope, while the latter aggregates are single drops of oil formed by the coalescence of the minute particles. With gold sol-gelatin-HCl mixtures both types are also readily observed.

With mastic-gelatin-HCl mixtures these phenomena may be observed with even greater readiness. If care be taken to adjust the proportions so that aggregation occurs with a maximal quantity of gelatin and traces only of hydrochloric acid, the electronegative aggregates are, as would be expected from the diagram, dispersed by traces of alkali or acid. The reversibility of the flocculation diminishes on standing just as globulin suspensions after a time become insoluble. When in addition it is found that the flocculae are dispersed and not, as would be expected, aggregated further by the addition of a small quantity of sodium chloride, the analogy to euglobulin appears so striking that the possibility of its being a complex physically comparable with the mastic-gelatin or oil-gelatin complexes here dealt with cannot be overlooked[1].

## Aggregation Diagrams Nos. 2, 3 and 4 a.

After this preliminary description of the method of investigation pursued in coordinating various phenomena connected with the aggregation of sol-protein complexes, three particular experiments may be considered. These refer to the behaviour of mixtures of equal volumes of a particular *mastic* sol, gelatin and an electrolyte solution, and may be called for convenience experiments 2, 3 and 4. This particular sol I found more easy to work with than an oil sol, for the various stages of aggregation from a slight clouding of the blue opalescence in twenty-four hours to an immediate and complete flocculation of the whole of the material could be picked out more or less accurately and recorded.

The difference between the three experiments lay in the nature of the

[1] The flocculated complex made from pseudoglobulin (horse) and a sol of the lipoid from euglobulin (horse) resembles euglobulin still more closely. Even after several days' standing it is instantly and completely dispersed by traces of alkali or acid.

electrolyte constituents of the mixtures. In all three experiments the electrolyte varying in amount from row to row was hydrochloric acid. In experiment 3, however, every mixture was in addition 0·011 N with respect to sodium chloride: in experiment 4, every mixture was in addition 0·011 N with respect to sodium acetate. When the mixtures had been prepared and

*Mastic-gelatin-electrolyte Aggregation Diagrams.*

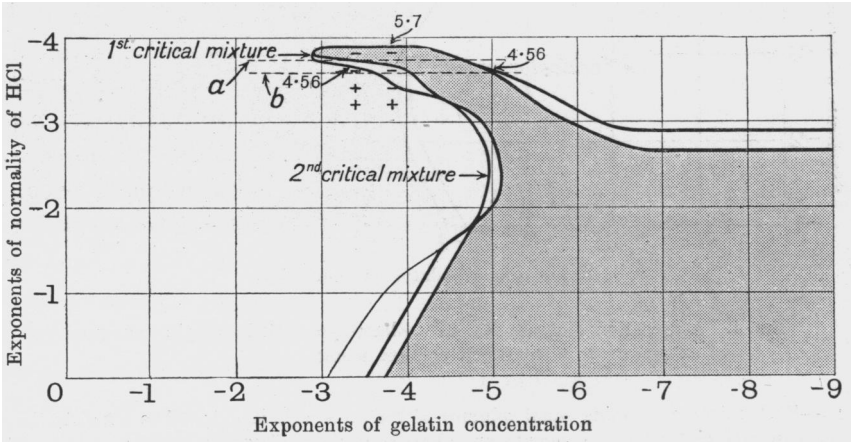


**Fig. 2. Aggregation diagram No. 2. Electrolyte HCl in varying amounts.**

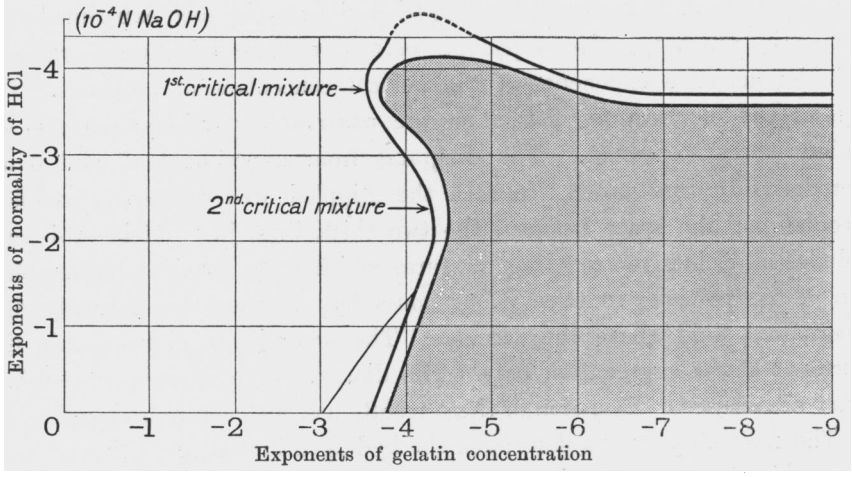


**Fig. 3. Aggregation diagram No. 3. Electrolyte HCl in varying amounts +0·033 N sodium chloride.**

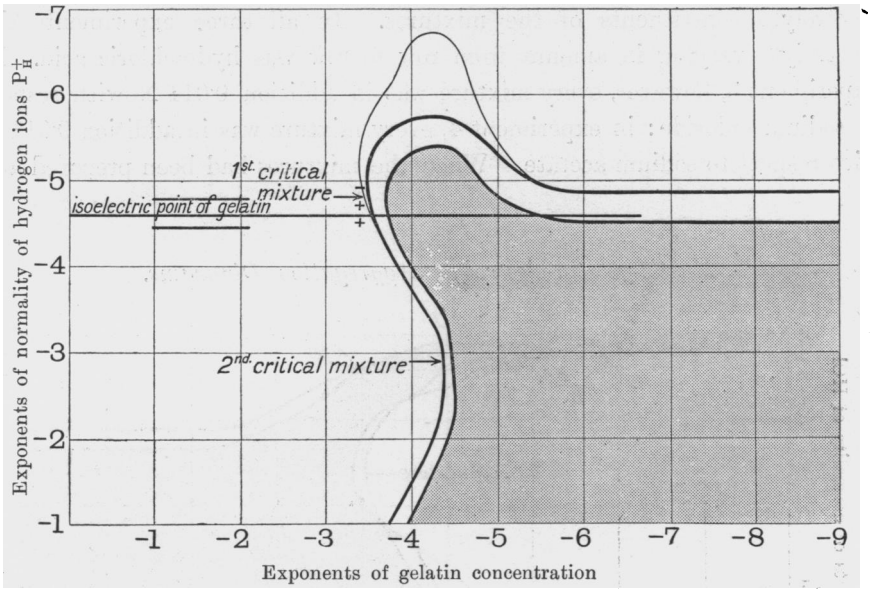


**Fig. 4. Aggregation diagram No. 4A. Electrolyte HCl in varying amounts +0·033 N sodium acetate.**

**NOTE.—Each mixture contains equal volumes of the three constituents. The concentrations of gelatin and HCl whose exponents are plotted in these diagrams are the concentrations used to make the mixtures. To find the actual concentration in a mixture add log 3 = ·477 to the negative exponent. In Fig. 4 the $H^{\cdot}$ ion concentrations, plotted as ordinates, refer to the $H^{\cdot}$ ion concentrations of the mixtures.**

allowed to stand 24 hours, one aggregation diagram for each experiment was plotted in which negative exponents of acid concentration were ordinates and the negative exponents of the gelatin concentration abscissae. The sign of the electrostatic charge on the observable particles was also recorded where expedient. The diagrams from experiments 2 and 3 are reproduced in this paper. In each the stippled area represents complete flocculation: the space between the two thick lines just outside this area includes all points representing mixtures which show considerable aggregation of the particles in twenty-four hours but do not leave perfectly clear supernatant fluid above the flocculae. The areas bounded by a thin line represent slight aggregation only. The diagram from experiment 4 which is not necessary for discussion of these results has not been reproduced.

The next step was to determine the hydrogen ion concentration of every mixture in the three experiments.

In experiments 2 and 3, the rows containing acid less than $10^{-3\cdot4}$ N

presented very considerable difficulties. The reaction inertia and the hydrogen ion concentration were low, and in those tubes to which no sodium chloride was added the conductivity was also low. By working with sufficient care accurate results were obtained in tubes where no flocculation had occurred but in the remaining tubes the results were of such a nature that they are best considered separately and recorded with certain reservations.

In experiment 4 the mixtures all lent themselves readily to accurate $P_H^+$ determinations and so a second aggregation diagram could be prepared in which the ordinates were the negative exponents of hydrogen ion concentration. This diagram 4A has been reproduced (Fig. 4). A comparative study of this diagram and the two preceding is instructive.

It is possible to regard diagram 3 as being derived from diagram 4A by submitting the ordinates to a process similar to that of opening out a telescope, the telescope having a large number of joints which do not slide out necessarily at the same rate. This suggests that if the influence of changing hydrogen ion concentration be ignored, sodium chloride and sodium acetate in strength 0·011 N behave very similarly in relation to the flocculation of mastic-gelatin mixtures. A similar relationship between diagrams 2 and 4A does not hold.

Hydrogen ion determinations for certain important mixtures in experiments 2 and 3 were made quite satisfactorily and a few are recorded on the diagram 2, but in view of the labour involved, corresponding diagrams, 2A and 3A, in which the ordinates would have been hydrogen ion concentrations, could not be prepared complete for the present paper (see p. 182).

In experiment 4 it was found that throughout each row of mixtures the H· ion concentration did not vary appreciably, that is to say, the mixtures along the row were *isoxyntic* (i.e. of equal H· ion concentration) in spite of a variation in gelatin content from $10^{-3\cdot48}$ to infinite dilution. At this stage certain relationships can be established by comparing diagrams 2, 3 and 4A.

(1) *Relationships between composition and possibility of flocculation.*

(*a*) In each of the three experiments there is one mixture which is just on the verge of flocculation and in which flocculation would not have occurred at all if the gelatin concentration had been a little greater. Neither would it have flocculated if a little more acid or a little less acid had entered into its composition. Its reaction is invariably in the region of the isoelectric point of gelatin. It may be conveniently called the "1st critical mixture" of the system mastic-gelatin-electrolyte. In experiment 2 the "1st critical mixture" contains five times as much gelatin as in experiments 3 and 4.

(*b*) It is seen that at the upper limits of HCl concentration flocculation is not appreciably influenced by the presence of 0·011 N NaCl. The effect of this concentration of sodium chloride is shown markedly, however, when the minimal concentrations of hydrochloric acid at which complete flocculation of mastic alone can be observed are examined.

In experiment 2 if the mixture made from HCl $10^{-2\cdot4}$ N, gelatin $10^{-5\cdot0}$ and mastic be considered it is seen that partial flocculation has occurred. If stronger acid with the same gelatin solution, or weaker acid with the same gelatin solution, or weaker gelatin with the same acid strength, were used to make the mixture flocculation would be complete.

Similarly in experiment 3 if the mixture made from HCl $10^{-2\cdot4}$ N, gelatin $10^{-4\cdot4}$ and mastic be considered the same fact is evident. More acid, or less acid, or less gelatin, other constituents of the mixture being left unaltered, will give mixtures flocculating to the full extent. And, again, in experiment 4 there is one mixture made from gelatin $10^{-4\cdot4}$ to which the same considerations apply.

The three mixtures, one in each experiment, which have this curious relationship to those contiguous to them may also be called "critical mixtures." To distinguish them from those of the type described in (*a*) they may be called "2nd critical mixtures."

Critical mixtures whether "1st" or "2nd" have this in common—a slight increase in gelatin concentration would inhibit flocculation completely. They differ in that a "1st critical mixture" is not flocculated by more acid or less acid, while a "2nd critical mixture" is.

The three "2nd critical mixtures" considered—one from each of the experiments 2, 3 and 4—were found experimentally to have the same hydrogen ion concentration $P_H^+ = 2\cdot9$.

They are made from gelatin $10^{-5\cdot0}$ when electrolyte is not added and $10^{-4\cdot4}$ when sodium chloride or acetate is present, i.e. those which are 0·011 N with respect to sodium salt contain four times as much gelatin as the other. It is suggested that the increased thickness of coating of gelatin (at $P_H^+ = 2\cdot9$ in all three cases) resulting from the inclusion of four times as much gelatin per unit volume in the mixture, balances the flocculation effect of 0·011 N sodium ions. On p. 186 an explanation of the existence of the "2nd critical mixture" has been attempted.

In Figures 2 and 3 it is seen that as the acid concentrations increase towards the foot of the diagram, the effect of the added sodium chloride in experiment 3 becomes less and less noticeable. The apparent discrepancy of diagram 4A in this respect disappears when the nature of the ordinates

is recognised. Diagrams 2A, 3A (not reproduced) and 4A are identical towards the base line—$P_H^+ = 10^{-0}$ (normal $H^{\cdot}$ ion concentration).

*Relationships between flocculation area and (a) the isoxyntic line corresponding to the isoelectric point of gelatin; (b) the line passing through all points where the observed particles are electrically neutral.*

## Experiment 4.

In experiment 4 the following determinations were made with a transport apparatus similar in design to that used by Michaelis [1909]. It was made of glass of one cm. bore all in one piece. Rubber was dispensed with entirely. Non-polarisable electrodes, Cu in $CuCl_2$, and Ag in N NaCl, were employed, and many test experiments were made which left no doubt as to the accuracy of the results.

Fluid in side tubes—32 cc. water + 30 cc. acetate mixture + 30 cc. 5 per cent. alcohol.
Fluid in middle tube—30 cc. gelatin $10^{-3.6}$ + 30 cc. acetate mixture + 30 cc. mastic sol.

| Composition of acetate mixture | $P_H^{\pm}$ of middle tube | Direction of migration | |
|---|---|---|---|
| 3·3 cc. N sod. acetate + 2·2 cc. N HCl + water to 100 cc. | 4·28 | + | Isoelectric |
| ,, ,, 2·0 ,, ,, ,, | 4·47 | + | point of |
| ,, ,, 2·0 ,, ,, ,, | 4·52 | + | gelatin |
| ,, ,, 1·5 ,, ,, ,, | 4·75 | + | 4·6 |
| ,, ,, 1·2 ,, ,, ,, | 4·92 | − | (Michaelis) |
| ,, ,, 1·2 ,, ,, ,, | 4·92 | − | |
| ,, ,, 1·0 ,, ,, ,, | 5·05 | − | |

The meniscus of the moving mastic suspension remained sharp and could be observed with great accuracy. After about an hour the direction could be ascertained definitely even in the solutions $P_H^+ = 4{\cdot}75$ and $P_H^+ = 4{\cdot}92$. Gelatin concentration $10^{-3.6}$ was used throughout these determinations because it was the weakest solution that could be used without involving flocculation.

On the diagram + and − signs represent the results of some of the determinations just described. It is remarkable to note that the observed particles in the 1st critical mixture are almost without any electric charge. The isoelectric point of gelatin is possibly slightly more acid than this, indicating that the uncharged particles result from a combination of neutral or possibly slightly negative gelatin and the electronegative nucleus.

### Experiment 2.

In the 1st critical mixture of experiment 2, in which electrolytes were excluded as far as possible, the effect of the electronegative nucleus on the charge of the mastic-gelatin complex will be seen to be much more marked.

As previously stated a considerable time was spent in determining under the best experimental conditions the hydrogen ion concentrations of certain mixtures in experiment 2 which had not flocculated. In every case a series of mixtures was made simultaneously in which the position of the flocculation area was verified and then the non-flocculated mixtures of this same series examined with respect to their hydrogen ion concentration and the sign of the electrostatic charge of the observable particles by the microscope method. The mixtures were prepared and the observations repeated many times with concordant results.

Flocculated mixtures were also examined in the same way. The particles were invariably electronegative but the hydrogen ion concentrations did not furnish results sufficiently trustworthy to be considered in this connection.

The results of the observations are given below. Some are recorded on the diagram.

*Mixtures of equal volumes of mastic sol, gelatin solution and HCl taken from Exp. 2.*

| Exponent of gelatin concentration used | Exponent of normality of HCl used | E.M.F. | $P_H^+$ | Sign of observed particle |
|---|---|---|---|---|
| −3·6 | −3·0 | 0·455 | 3·53 | + |
| ,, | −3·4 | 0·490 : 0·488 | 4·12 | ± |
| ,, | −3·6 | 0·515 : 0·515 | 4·56 | − |
| ,, | −3·9 | 0·580 | 5·70 | − |
| −4·0 | −3·0 | 0·455 | 3·54 | + |
| ,, | −3·4 | 0·491 : 0·485 | 4·10 | − |
| ,, | −3·6 | 0·502 : 0·507 | 4·40 | − |
| ,, | −4·0 | 0·534 : 0·544 | 5·00 | − |
| −4·4 | −3·0 | 0·455 | 3·54 | + |
| ,, | −3·4 | 0·492 | 4·18 | − |
| −5·0 | −3·5 | 0·513 | 4·54 | − |
| ,, | −3·6 | 0·515 | 4·56 | − |

From the evidence of these figures the following statements may be made:

(*a*) In the "horn" like part of the flocculation area the observed particles are negatively charged. In mixtures, just below this, which have not flocculated, the particles are also negatively charged. Comparison with aggregation diagram No. 1 where an oil sol was used shows that the

line $XY$ passing through all mixtures where the observed particles of the diagram are electrically neutral does not pass so close to the "horn" like part of the flocculation area as in diagram 2. The other striking difference between the two diagrams is the relative stability of the two sols to $H^{\cdot}$ ion concentration. It is suggested that these two phenomena have a common factor.

(*b*) *Passing upwards through the "horn"* along a series of mixtures made from gelatin solution of concentration $10^{-3\cdot6}$ *the $H^{\cdot}$ ion concentration is changing very rapidly,* viz. from $P_H^+ = 4\cdot56$ at acid $10^{-3\cdot6}$ N to $P_H^+ = 5\cdot70$ at acid $10^{-3\cdot9}$.

It is therefore established that the isoxyntic lines $P_H^+$ 4·45 and $P_H^+$ 4·80 representing the upper and lower limits of the isoelectric range of gelatin as measured by Michaelis, either both pass below the "horn" or that the upper $P_H^+$ line 4·8 passes through the lower part of the "horn."

The precise positions of these lines cannot be determined with the same accuracy as in experiment 4, so that they are represented by dotted lines in the diagram labelled *a* and *b*.

(*c*) Mixtures which, if the available data with regard to the isoelectric point of gelatin be correct, would show migration of gelatin to the cathode nevertheless contain observable particles carrying a definite negative charge.

These results from experiments 4 and 2 may be summarised as follows:

It is established, within the limits of experimental error, that, in the presence of 0·011 N sodium acetate, the 1st critical mixture contains electrically neutral particles, and has the hydrogen ion concentration at which no wandering of gelatin can be detected in transport experiments. The sign of the particle in this and contiguous mixtures is, therefore, that of its coating: the electronegative character of the mastic nucleus does not manifest itself at all.

In the absence of electrolytes except minimal traces of HCl, and gelatin of such concentration that when the HCl content is increased the line representing the series of mixtures passes downwards through the middle of the "horn" like projection of the aggregation area, the above coincidences do not occur. Instead the following phenomena occur in the following order:

(1) No aggregation; gelatin negative; all observable particles negative.

(2) Aggregation; gelatin negative; all observable particles negative.

(3) Aggregation; gelatin sign not discoverable by transport experiments; all observable particles negative.

(4) No aggregation; gelatin positive; all observable particles negative.

(5) No aggregation; gelatin positive; all observable particles positive.

The isoelectric range of gelatin was determined in the presence of sodium acetate and hence the above results, in so far as they depend upon this determination, are open to discussion. It is extremely probable, however, that, in the absence of added electrolyte, the electrically neutral particle is made up of an electropositive gelatin coating and an electronegative mastic nucleus. On the other hand it may be stated definitely that the particles in the 1st critical mixture and all other mixtures represented by points in the "horn" like part of the aggregation area are electronegative. All mixtures containing electrically neutral particles are relatively more acid, and show no sign of aggregation.

I can offer no explanation of the results. Every endeavour has been made to discover faults in the observations.

Liberation of alkali from the glass of the microscopic cell was at first considered a possible source of error, but a considerable number of experiments showed that this did not account for the effects observed.

## Theoretical discussion of the observed results.

The existence of the "horn" in Figs. 1 and 2, or rather its suppression in Figs. 3 and 4, due to the presence of 0·011 N sodium chloride or sodium acetate, is the manifestation of a phenomenon the explanation of which may for the present be referred to the conjectures concerning the salt dispersion of globulin [Hardy, 1905; Mellanby, 1905; Chick, 1913], denaturated serum protein [Chick and Martin, 1912] and methylimino compounds [Schryver, 1910].

Since, in experiments 3 and 4, 0·011 N sodium salt was present, while it was not present in experiment 2, the series containing gelatin $10^{-5\cdot0}$ in experiment 2 is taken as being comparable with those containing $10^{-4\cdot4}$ in experiments 3 and 4. The reasons for this are stated on p. 180. These series are now to be considered first of all independently, and then with regard to their mutual relationship.

*Experiment* 2. *Series of mixtures made from gelatin* $10^{-5}$ *and hydrochloric acid of increasing concentration.*

The facts observed are that with acid from infinite dilution to $10^{-3\cdot7}$ no flocculation occurs; with acid $10^{-3\cdot6}$ there is a partial flocculation; with all stronger acids flocculation occurs except with one strength of acid, namely,

$10^{-2.4}$ N, which is on the verge of flocculation, and is the "2nd critical mixture."

Also, the $P_H^+$ of the mixture which is on the verge of flocculation made from $10^{-3.6}$ acid is 4·56, and the observed particles of this mixture and all the neighbouring mixtures are negatively charged. It will be remembered that the isoelectric range of gelatin is $10^{-4.45}$ to $10^{-4.8}$.

Among the phenomena known to be concerned in this flocculation or non-flocculation are the surface tension effects at the surfaces of the particles. These are partly of an electrical nature with purely surface effects as concomitant factors. Practically the whole of the gelatin present is in this series on the surfaces of the mastic particles—a point probably capable of demonstration by differential filtration experiments. The series may be divided into two parts. The first consisting of mixtures containing acid from infinite dilution to say $10^{-2.9}$ N HCl and the latter from this strength to normal acid.

In the first part of the series it is known that hydrogen ions are not present in sufficient quantity to flocculate mastic at all even when not coated with gelatin.

This factor can therefore be put aside for the moment and purely surface effects—electrical and otherwise—considered. Gelatin is present in the solution in three forms—just as is any other electrolyte. In accordance with the mathematical investigations of Michaelis and others, the proportion of it which is un-ionised is a maximum at its isoelectric point, and the proportions which are negatively charged and positively charged respectively are equal in amount. If the solution be made more acid the concentration of positively charged gelatin increases. On the alkaline side of the isoelectric point electropositive gelatin ions also exist in concentrations, compared with the total amount of the gelatin present, diminishing with diminishing acidity.

Their existence is necessitated on theoretical grounds but they cannot be detected by the direct transport method. Is it not possible that the flocculation observed on the alkaline side of the isoelectric range established by transport observations is due to traces of electropositive gelatin—and, in fact, that the flocculation of mastic is a very sensitive indicator of gelatin in this condition? The particles are observed to be electronegative—the effect of the electronegative mastic nucleus is not abolished—but it is suggested that the tendency to minimise the surface energy of the system through flocculation becomes operative in spite of electrostatic repulsion when this repulsion is diminished through the acquisition by the particle

of a film of gelatin originally electropositive. The behaviour of the series of mixtures all made from gelatin $10^{-5}$ and HCl acid from infinite dilution to $10^{-2\cdot9}$ N is seen to be in accordance with the above hypothesis. Continuing the same series from acid $10^{-2\cdot9}$ N to normal acid the phenomenon of the "2nd critical mixture" is observed.

Throughout these mixtures purely surface effects may be taken as being overwhelmed by phenomena of an electrical nature. There are two factors involved. The *first*, which did not come into consideration over the first half of the series, is the flocculating effect of the hydrogen ions on the gelatin-coated mastic particles. The *second* is a factor having the opposite effect—the increasing electropositiveness of the coat, a quantity which may be referred directly to gelatin cation concentration. Adopting the nomenclature used by Sörensen [1912] and one of the equations deduced from fundamental principles quoted by him—

$$\rho = \frac{1}{1 + \frac{k_a}{H^{\cdot}} + \frac{k_b H^{\cdot}}{k_w}},$$

an expression may be obtained giving the rate of change of gelatin cation concentration at any $H^{\cdot}$ ion concentration,

$$A^{\cdot} = \frac{k_b x}{OH'} = \frac{k_b A \rho}{OH^{\cdot}} = \frac{k_b A \rho H^{\cdot}}{k_w}$$

$$= \frac{k_b A H^{\cdot}}{k_w \left(1 + \frac{k_a}{H^{\cdot}} + \frac{k_b H^{\cdot}}{k_w}\right)}.$$

Differentiating with respect to $H^{\cdot}$,

$$\frac{dA^{\cdot}}{dH^{\cdot}} = \frac{k_w k_b A H^{\cdot} (H^{\cdot} + 2k_a)}{(k_w k_a + k_w H^{\cdot} + k_b H^{\cdot 2})^2}.$$

Putting the dissociation constants and $A$, the total gelatin concentration, equal to unity and substituting for $H^{\cdot}$ 0·01, 0·10, 0·20, 0·25, 0·33, 0·50, 1·00, 1·50, 2, 10, the values for the expression are 0·02, 0·19, 0·28, 0·32, 0·37, 0·41, 0·33, 0·19, 0·16, 0·01.

From these figures it is seen that the rate of change of gelatin cation concentration increases and then diminishes again with increasing $H^{\cdot}$ ion concentration from its isoelectric point. On the other hand, the rate of change of the tendency to flocculate, that is the factor above, probably increases steadily when referred to the same variable.

The actual result is the algebraic sum of these two factors and it follows from the above relationship that there is a certain $H^{\cdot}$ ion concentration where this algebraic sum is a minimum. This I conceive to be the case

in the mixture which contains acid $10^{-2\cdot4}$ N, and which I have called the "2nd critical mixture."

*Experiments* 3 *and* 4. *Series of mixtures made from gelatin* $10^{-4\cdot4}$ *with addition of sodium chloride* + HCl, *and sodium acetate* + HCl *respectively. Hydrogen ion concentration increases from each mixture to the next.*

Throughout the series in experiment 4 the same general phenomena are observed with one important difference. The range of hydrogen ion concentration over which flocculation is observed extends to $P_H^+ = 6{\cdot}56$. In Fig. 2 flocculation ceases before the value of hydrogen ion concentration falls to $P_H^+ = 4{\cdot}56$ at the gelatin concentration $10^{-5}$ which is taken as comparable; and at $P_H^+ = 5{\cdot}7$ under the peculiar circumstances of the series with gelatin $10^{-3\cdot6}$. The addition of 0·011 N sodium acetate makes all this difference. In similar fashion in Fig. 3 complete flocculation was observed in tubes to which no acid at all was added, and almost complete flocculation in tubes containing $10^{-4}$ NaOH.

If this result of added electrolyte is taken as indicating a widening of the isoelectric range it is comparable with Michaelis and Davidsohn's observations on the isoelectric point of oxyhaemoglobin. In transport experiments they showed [1912] that though the position of the $P_H^+$ range over which no definite inference could be drawn as to the direction of wandering remained unchanged, its upper and lower limits widened with increasing electrolyte addition.

Proceeding along these two series "2nd critical mixtures" are again observed at $P_H^+ = 2{\cdot}9$. The explanation is probably the same in all three cases even if that put forward is not correct.

## Experimental details.

*Materials used.* The electronegative sols used were of oil, mastic and gold. The *oil sol* found most convenient was the result of many trials and was prepared in the following manner. To 94 cc. of Kahlbaum's acetone, 6 cc. of water are added and 20 cc. of olive oil "sublime." After thorough shaking and settling 40 cc. of the acetone layer are blown in from a pipette as quickly as possible into 1000 cc. of "conductivity" water. The rapidity of mixing of the acetone solution of oil and the water is a contributing factor to the success in preparing a finely divided sol. It should show to the naked eye only traces of oil on the surface, even after standing many months, and appear transparent with a blue fluorescence. A "white"

appearance indicates that the oil particles are much larger than when the solutions are transparent and fluorescent. The material extracted by wet acetone from the olive oil is probably richer in free fatty acid than the original oil, and to this I have ascribed the fine emulsions obtained by this method. Adding the same oil drop by drop to 100 cc. acetone + 6 cc. water until no more dissolves even on prolonged shaking, I obtained from the solution coarse "white" emulsions on blowing 4 cc. of it into 100 cc. of water [Donnan, 1910; Dubrisay, 1913].

A freshly prepared oil sol does not differ in appearance from one many months old. It is found, however, that when an endeavour is made to prepare a flocculation diagram with some protein and an electrolyte solution the sol does not seem to be so sensitive to minute differences of protein or electrolyte concentration after long standing. And the sharp well defined arcs on the diagram give way to regions similar in shape but of blurred outline.

The *mastic sol* used in all these experiments was prepared from commercial gum mastic in the following manner. Excess of the gum was boiled under a reflux condenser with 99 per cent. alcohol for several hours, the vessel cooled and then allowed to stand for several days. The clear solution resulting contained 1·7 per cent. of total solids. Five cc. blown forcibly from a pipette into 95 cc. of conductivity water furnished an excellent sol, pale straw colour by transmitted light, and with a marked blue fluorescence. If the alcoholic solution is run out from the pipette into the water in the normal way a milky fluid results, the particles of the disperse phase of which are comparatively large. The difference between the solution resulting is in the two cases very striking; much more so than with oil or any other suspension I have observed.

Unlike the particles of the oil sol the mastic particles increased in size hourly in a very marked manner, though even after months of standing no flocculation occurred. Fresh suspensions were therefore prepared for each experiment. As their magnitude increased the particles seemed to change in size at a progressively lower rate.

Contrary to expectation it was found quite possible to measure hydrogen ion concentrations with accuracy in solutions containing mastic or oil in the colloidal condition.

The mastic and oil sols used contained small quantities of acetone and alcohol respectively. It was thought that possibly 1·7 per cent. of alcohol or acetone might affect the charge on the protein particles in the mixtures made, but no evidence of this could be observed. Samples of sols were

distilled until quite free from alcohol or acetone under diminished pressure, and then half made up to the original volume with the correct proportion of alcohol, and half made up with water. No difference in the behaviour of these two sols on flocculating with traces of gelatin and acid could be observed either in the rapidity or the limits of flocculation. Scarcely any difference was observable between the original sol and the same sol after distillation and making up to the original volume with the correct proportion of alcohol. In transport experiments the addition of 1·7 per cent. of alcohol to both middle and outer limbs made no difference to the direction of migration of gelatin in the neighbourhood of the isoelectric point.

The *gold sols* ($Au_F$) were prepared by Zsigmondy's [1901] formalin method followed by dialysis. The removal of the alkali rendered these sols less stable, and after several days' dialysis an increase in size of the particles was invariably observed. The finer sols ($Au_P$) obtained by the ethereal phosphorus method were also prepared.

*Gelatin.* Coignet Fils et Cie's Gold Label Gelatin was soaked in frequent changes of conductivity water in a hard glass vessel for several days till twelve hours' contact at 0° did not change 3 gemmho water to more than 5 gemmhos. No signs of mould or bacterial growth were observed. A solution, approximately 1 per cent., was prepared by dissolving in boiling distilled water and the exact strength determined by refractive index at 17·5°. Suitable dilution gave a concentration $10^{-3}$ from which other solutions could be made. A typical $10^{-3}$ gelatin solution had conductivity 9·6 gemmhos.

The effect of the dialysis could be demonstrated by comparing different concentrations of dialysed gelatin with corresponding concentrations of undialysed gelatin in their effect on mastic sol and N/10 sodium acetate. Equal volumes of gelatin solution, mastic sol and N/10 sodium acetate solution are taken in each test tube. Flocculation is more rapid and complete in the latter case and extends over a wider range of gelatin concentration—a result attributable to the additional electrolytes present.

*Apparatus.* Determinations of $H^{\cdot}$ ion concentration were in all cases made electrometrically, using a gas electrode of convenient type in conjunction with Michaelis' saturated KCl calomel electrode. The connecting fluid was saturated KCl and no correction for contact potential of the fluid investigated and saturated KCl was made. For nearly all of the determinations an electrode vessel of borosilicate glass with the electrode mounted in the same material and a ground joint [Walpole, 1914] was used.

Conductivity determinations were made by the Kohlrausch method, at

18°, in stoppered vessels of the Henry-Zörkendörfer pattern, using well-blacked electrodes.

As far as possible all solutions were stored in special bottles of hard glass. The test tubes employed were remarkable for their insolubility in water. As the interactions observed were many of them almost instantaneous, the slow absorption of carbon dioxide from the atmosphere by the mixture could be ignored.

The *ultramicroscopic* apparatus was Leitz' 1913 pattern illuminated by 5 amp. arc light. Observations on direction of migration in the electric field could be made in solutions containing only small quantities of electrolytes by using a cell 0·3 mm. deep provided with platinum electrodes [Chick and Martin, 1912].

Migrations in the electric field were also observed in an apparatus similar to that employed by Michaelis. Every due precaution was taken to avoid the fallacies contingent to the method.

In connection with the experimental part of this work I wish to express my indebtedness to my assistants Messrs R. Defries and S. Scott.

## Summary.

A method is given by which the relationship of the phenomena of the inhibition or actuation of the aggregation of mixtures of sols and electrolytes, especially in the presence of proteins, may be coordinated diagrammatically on a quantitative basis.

This method has been applied to oil and mastic sols to which gelatin and hydrochloric acid have been added and the effect of the addition of sodium chloride and sodium acetate to the mastic-gelatin-hydrochloric acid mixtures has been studied in a similar manner.

The chief phenomena observed may be summarised here:

1. In the presence of not too much gelatin flocculation of mastic or oil sols may occur by the addition of a trace of acid quite unable to flocculate the sol alone. The same quantity of gelatin "protects" the sol against acid 6000 times more concentrated.

2. This aggregation may be observed equally well with gold sols and in all cases it is reversible.

3. In the absence of electrolyte except HCl, two critical mixtures are observed which are on the verge of flocculation. These have been worked out in the case of one particular mastic sol and called the "1st" and "2nd" critical mixtures.

*1st critical mixture.* The hydrogen ion concentration of this mixture is in the neighbourhood of the isoelectric point of gelatin but is probably on the alkaline side of this point. The particles are negatively charged in this mixture, and in contiguous mixtures whether flocculated or non-flocculated. Mixtures differing from this by having more gelatin, more acid or less acid do not flocculate.

*2nd critical mixture.* The hydrogen ion concentration of this mixture is $P_H^+ = 2{\cdot}9$. Mixtures having more acid or less acid flocculate, but here again more gelatin inhibits flocculation. The particles are positively charged in this mixture and in contiguous mixtures whether flocculated or non-flocculated.

4. In the presence of hundredth normal sodium chloride or sodium acetate there are also two critical mixtures showing precisely similar flocculation changes with changing acid or gelatin concentration. The following differences are noticeable:

(*a*) The two critical mixtures approximate much more closely than before in gelatin concentration.

(*b*) The *1st critical mixture* is either at or only just on the alkaline side of the isoelectric point of gelatin. The gelatin coated mastic particles are almost exactly electrically neutral. The *2nd critical mixture* in the presence of added electrolyte resembles the *2nd critical mixture* in the absence of added electrolyte in all respects including $H^{\cdot}$ ion concentration except that four times as much gelatin is present in the former case. Apparently the additional thickness of gelatin coat corresponding to the greater gelatin concentration counterbalances the flocculating effect of the added electrolyte.

5. Whether sodium chloride or acetate be present or no, the *1st critical mixture* contains more gelatin than the *2nd critical mixture.*

6. In any series of oil-gelatin or mastic-gelatin mixtures containing minimal traces of electrolyte, and differing only in $H^{\cdot}$ ion concentration, those which are flocculated are not those which contain electrically neutral particles. This lack of coincidence of the "point of maximum flocculation" and the point where the particles have no "electric charge" was pointed out in an earlier paper: an endeavour has been made here to analyse this phenomenon further.

7. Miss H. Chick's observation that "electrolyte-free" globulin at the reaction of maximum flocculation is not electrically neutral has been confirmed.

8. The wide difference of gelatin concentration (100 times) between the *1st critical mixtures* with and without salt addition is related to an area

of aggregation over which this phenomenon is more or less reversible experimentally by acid, alkali, or sodium chloride. The reversal by alkali is much more readily observed than that by acid.

9. A possible physical similarity between the particles of a mastic gelatin mixture and those of a euglobulin suspension is suggested. Preliminary attempts to make a suspension strictly comparable with euglobulin from pseudoglobulin and a sol from the lipoid of euglobulin have been made, and work on this subject is being continued.

These simple systems were originally examined in the hope that the results would assist in the elucidation of the phenomena met with in the investigation of the acid agglutination of bacteria. The system in the case of a bacterial suspension is, however, more complicated and this paper can only be regarded as a contribution to the general subject of flocculation.

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