CCXCVIII.—*The Effect of Proteins on the Coagulation of Bentonite Suspensions by Electrolytes.*

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It is well known that when a hydrophilic colloid such as gelatin is added in sufficiently high concentration to a hydrophobic one, it protects it against coagulation by electrolytes. At low concentrations, however, it often produces the reverse effect, either coagulating the hydrophobic sol or making it sensitive to the action of electrolytes. The sensitising action of albumin on ferric hydroxide sol, discovered by Pauli and Flecker (*Biochem. Z.*, 1912, **41**, 470), was investigated by Brossa and Freundlich (*Z. physikal. Chem.*, 1915, **89**, 306), who found that addition of pure electrodialysed albumin diminishes the cataphoretic velocity of the ferric hydroxide sol and greatly facilitates its coagulation by electrolytes. Similar results have been obtained with other pairs of hydrophilic and hydrophobic colloids (compare Freundlich, " Colloid and Capillary Chemistry," pp. 582—589). Low concentrations of gelatin coagulate Carey Lea's silver sol, but high concentrations protect it.

In many cases, the hydrogen-ion concentration of the sol (or, more correctly, that of the final mixture) has been found to be an important factor. Thus, gelatin coagulates a negative gold sol if it is feebly acidic, but protects it if slightly alkaline. Freundlich (*op. cit.*) at first attributed the sensitising action of the hydrophilic sols to their capacity to form colloid ions bearing a charge opposite to that of the hydrophobic colloid. Later investigations have, however, shown that this simple explanation does not cover all the known facts. Kruyt and co-workers (*Z. physikal. Chem.*, 1922, **100**, 250; *Kolloid-Z.*, 1922, **31**, 338) have observed that tannin transforms many hydrophilic into hydrophobic colloids, *i.e.*, it sensitises them, and Brossa (*Kolloid-Z.*, 1923, **32**, 107) has shown that it makes both positive and negative dye sols sensitive to the action of electrolytes. Tannin is only slightly dissociated in solution and is not amphoteric like the proteins; hence its sensitising action cannot be due to the formation of colloid ions. Kruyt (*loc. cit.*) therefore suggests that tannin molecules can be considered as polar, and when they come into contact with agar or gelatin particles they orient themselves in such a way that their hydrophobic ends project into the solution. It may be mentioned that non-colloidal non-electrolytes, such as amyl alcohol, phenol, thymol, etc. (compare Kruyt and van Duin, *Koll.-Chem. Beih.*, 1914, **5**, 269; Freundlich and Rona, *Biochem. Z.*, 1917, **81**, 1871), also sensitise the hydrophobic colloids. The phenomenon is therefore very complicated.

This has been clearly recognised by Freundlich, who says (Bogue, " Colloidal Behaviour," Vol. I, p. 313) : " In any case, we have the following choice in dealing with this problem. If we desire a general solution, the influence of colloidal ions is certainly not sufficient and it is necessary to test the hypothesis employed in the case of tannin to determine if it is generally valid. Or we may abandon the hope of a general solution and assume that the increased sensitivity is due to different factors in different cases." The present work was undertaken with a view to determine the effect of protein sols in the coagulation of bentonite suspensions by electrolytes at different p_H.

The proteins used were gelatin, casein, and egg albumin. The first two were purified by repeated washing with dilute acetic acid solution and then with distilled water, as suggested by Loeb (*J. Gen. Physiol.*, 1918, **1**, 45). The ash content of gelatin was 0·2%, and that of casein 1·2%. The egg albumin sol was prepared from commercial dried material without purification. The suspension was prepared by shaking finely powdered bentonite with distilled water in a Pyrex-glass bottle for about 4 hours, and diluting it till it contained 1 g. of bentonite per 100 c.c. of sol. Every time before use the bottle containing the suspension was vigorously shaken. On the alkaline side of the isoelectric points of the proteins the order in which the constituents were added was : bentonite suspension, protein sol, water, and finally electrolyte. The volume of water added was so adjusted that in every case the final volume of the mixture was 50 c.c. The coagulation experiments were carried out in tall measuring cylinders. When the fine particles of bentonite aggregate and begin to settle, a sharp boundary is generally formed at the top of the suspension, and the rate of fall of the coagulated particles can therefore be determined by measuring the rate at which this boundary settles. The results are given in Table I. The p_H of the bentonite suspension was 8·2, that of the 2% gelatin sol 8—8·2, and the final p_H 8—8·2.

It will be seen from the above data that even at p_H 8—8·2 the rate of coagulation of the bentonite suspension by sodium or barium chloride is accelerated by the addition of small quantities of gelatin, but further quantities retard the coagulation and finally the suspension is protected. The data in Table I further show that for the same amount of sodium chloride the rate of coagulation of the suspension is greater the lower its concentration : for 15 c.c. of 12% sodium chloride the 0·5% suspension settles only 2 mm. in $3\frac{1}{2}$ hours, whereas the 0·1% suspension settles 4·0 mm. per hour. Further, it appears that, other factors remaining constant, the protective action exerted by a given amount of gelatin is the greater

TABLE I.

(*a*) Electrolyte, NaCl.

Volume of 1% bentonite suspension used = 25 c.c.

Vol. of 12% NaCl added, c.c. ...	0	0	15	15	15	15	15
Vol. of 2% gelatin added, c.c. ...	0	5	0	0·1	0·5	2·5	5·0
Boundary fall, mm./3½ hours ...	0	0	2	4·5	30	24	15

(In the last solution, the liquid at the top was turbid, showing that protection had begun.)

Volume of 1% bentonite suspension used = 5 c.c.

Vol. of 12% NaCl added, c.c.	0	0	15	15	15	15
Vol. of 2% gelatin added, c.c.	0	1·0	0	1·0	5·0	20·0
Fall of boundary, mm. per 7 mins.	0	0	0	2·0	0	0
Fall of boundary, mm. per 1 hour	0	0	4·0	—	2	0

(*b*) Electrolyte, $BaCl_2$.

Volume of 1% bentonite suspension used = 10 c.c.

Vol. of 0·1*N*-$BaCl_2$ added, c.c.	0	0	2·0	2·0	2·0	2·0
Vol. of 2% gelatin added, c.c.	0	1·0	0·0	0·2	1·0	10·0
Fall of boundary, mm. per 4 mins.	0	0	0	18	17	0
Fall of boundary, mm. per 15 mins.	0	0	2	—	—	—

the less concentrated the suspension, for in presence of 5 c.c. of 2% gelatin and 15 c.c. of 12% sodium chloride the rate of clearance of a 0·5% suspension is 15 mm. in 3½ hours or 4·3 mm. per hour, whereas under the same conditions in the case of the 0·1% sol it is only 2 mm. per hour.

Some experiments were carried out in which 1 c.c. of 0·1*N*-sodium hydroxide was added to 10 c.c. of 1% bentonite suspension, and the effect of gelatin on its rate of coagulation by sodium chloride was determined. The results are given below, the final volume being 50 c.c. and the final p_H 10·8—11·2.

Vol. of 12% NaCl added, c.c.	0	0	10·0	10·0	10·0	10·0
Vol. of 2% gelatin added, c.c.	0	5·0	0	0·2	1·0	2·0
Fall of boundary, mm. per 15 mins.	0	0	0	2	6	6
Fall of boundary, mm. per 37 mins.	0	0	3	6	17	16

The above data show clearly that even at p_H 11 gelatin sensitises the bentonite suspension. It should also be noticed that within p_H 8—11 gelatin alone cannot precipitate the suspension.

Experiments similar to those described above were also tried with casein and egg albumin sols. The sols were prepared by dissolving 2 g. of the solid in 20 c.c. of 0·1*N*-sodium hydroxide and diluting the volume to 100 c.c. The results are given in Table II, 10 c.c. of 1% bentonite suspension being used in a final volume of 50 c.c. in each case.

TABLE II.

Casein and sodium chloride.

Vol. of 12% NaCl added, c.c.	0	0	10·0	10·0	10·0	10·0
Vol. of 2% gelatin added, c.c.	0	2·0	0	0·2	1·0	5·0
Fall of boundary, mm. per 10 mins.	0	0	0	9	8	0
Fall of boundary, mm. per 70 mins.	0	0	6	—	—	0

Casein and barium chloride.

Vol. of 0·1N-$BaCl_2$ added, c.c.	0	0	2·0	2·0	2·0	2·0	2·0
Vol. of 2% casein added, c.c.	0	2·0	0	0·2	1·0	10·0	20·0
Fall of boundary, mm. per 4 mins. ...	0	0	0	2	1·5	1·5	0
Fall of boundary, mm. per 15 mins. ...	0	0	2	10	7	5	0

Egg albumin and sodium chloride.

Vol. of 12% NaCl added, c.c.	0	0	10	10	10	10
*Vol. of albumin added, c.c.	0	1·0	0	0·2	1·0	20·0
Fall of boundary, mm. per 10 mins.	0	0	0	2	20	3
Fall of boundary, mm. per 75 mins.	0	0	6	30	—	—

(In the last case, the upper liquid was slightly turbid, protection having started.)

* The concentration of the albumin sol was originally 2%, but, as part of the albumin separated on standing, it was somewhat less at the time of the experiments.

In the above experiments the p_H of the mixture in which the bentonite separated quickly, leaving a clear liquid at the top, was found to be 8—8·4. The data show clearly that, like gelatin, at low concentrations casein and egg albumin sensitise the bentonite suspension, but at high concentrations they begin to exert a protective action; in this respect egg albumin appears to be inferior to the other two.

Some results are recorded in Table III in which the hydrogen-ion concentration of the mixtures was on the acid side of the isoelectric points of the proteins. As the bentonite suspension was slightly alkaline, a small quantity of hydrochloric acid was added to it to diminish the p_H. In each case 10 c.c. of 1% bentonite suspension were used together with 1 c.c. of 0·1N-hydrochloric acid.

It will be noticed that on the acid side of their isoelectric points the proteins can coagulate the bentonite suspension without the addition of any salt. In these cases the hydrogen-ion concentration is such that the proteins can form a sufficient number of colloid kations and thus neutralise the negative charge of the suspension. In this region, therefore, the Freundlich theory of colloid-ion formation can account for the observed facts. The real difficulty, however, is apparent when one tries to explain the sensitisation observed between p_H 8 and 11. The isoelectric points of the three proteins used in these experiments all lie below p_H 5; it is therefore doubtful whether there can exist any protein kations at p_H 8, and still more

TABLE III.

Gelatin.

Final p_H of mixture, 3·4—3·6.

Vol. of 1% gelatin sol added, c.c.	Distance fallen by the boundary in 15 mins.
0	0
0·2	15 mm. Upper liquid slightly turbid.
1·0	20 mm. Upper liquid quite clear.
5·0	The suspension settles partly; upper liquid very turbid.

Casein.

Final p_H of mixture, 3·2—3·6.

Vol. of 2% casein sol added, c.c.	Distance fallen by the boundary in 5 mins.
0	0
0·2	2 mm. Upper liquid clear.
1·0	12 mm. Upper liquid clear.
5·0	11 mm. Upper liquid slightly turbid.

Egg albumin.

*Vol. of egg albumin sol added, c.c.	Distance fallen by the boundary in 15 mins.
0	0
0·2	10 mm. Upper liquid clear.
1·0	10 mm. Upper liquid clear.
38·0	2 mm. Upper liquid turbid.

* The concentration of the sol was somewhat less than 2% (see footnote, Table II).

so at p_H 11. The proteins (*e.g.*, gelatin), however, can sensitise the suspension at both these p_H's, so it is highly improbable that the sensitisation is due to the partial neutralisation of the negative charge by the protein kations at p_H 8 or p_H 11. It has already been stated that non-electrolytes such as thymol, urethane, etc., can sensitise a hydrophobic colloid; in their case it is generally assumed that they diminish the dielectric constant of the medium and thereby the electrokinetic potential of the colloid particles. It is possible that the proteins are strongly adsorbed on the surface of the bentonite particles and hence considerably diminish the dielectric constant in their immediate neighbourhood, thus causing a decrease in the electrokinetic potential and therefore in the stability. It is also possible that the proteins on being adsorbed on the surface of the bentonite particles undergo an irreversible change similar to those occurring in the process of denaturisation. Freundlich (Bogue, *op. cit.*, Vol. I, p. 307) cites evidence of such a change in the case of albumin–ferric hydroxide sol. A change of this type is also likely to affect the force of attraction between the bentonite particles as well as their electrokinetic potential.

Summary.

The effect of addition of gelatin, casein, and egg albumin on the coagulation of bentonite suspensions by sodium and barium chlorides at different p_H's has been studied. It has been found that on the alkaline side of their isoelectric points the proteins cannot coagulate the suspension, but make it markedly sensitive to the action of electrolytes; on the acid side, however, they effect coagulation without the addition of any salt.

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