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## Hydrophilic and Hydrophobic Colloids and the Influence of Electrolytes on Membrane Potentials and Cataphoretic Potentials

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# HYDROPHILIC AND HYDROPHOBIC COLLOIDS AND THE INFLUENCE OF ELECTROLYTES ON MEMBRANE POTENTIALS AND CATAPHORETIC POTENTIALS.

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## I.

### *Hydrophilic and Hydrophobic Colloids.*

It has been recognized for some time that it is necessary to subdivide colloids into two groups, the hydrophilic and the hydrophobic colloids, according to the influence of electrolytes on their precipitation. To the hydrophilic colloids belong among others proteins and soaps, and to the hydrophobic colloids, collodion, mastic, graphite, metal oxides, etc. The hydrophobic colloids (such as mastic) are precipitated from aqueous suspensions by comparatively low concentrations of salts and the precipitating effect of a salt increases rapidly with increasing valency of that ion of the salt which has the opposite sign of charge to that of the particle. Hydrophilic colloids, such as genuine crystalline egg albumin or gelatin, are only precipitated in very high concentrations of electrolytes and the sign of charge of the precipitating ion has no relation to the sign of charge of the protein. All attempts to account for this difference in the action of electrolytes on the two kinds of colloids by a common theory have failed for the reason that the forces which prevent the particles from coalescing in an aqueous solution are entirely different in the two cases. Hydrophilic colloids, such as genuine proteins, form true solutions in water, *i.e.* the forces by which the protein molecules or protein ions are kept in solution are the strong forces of chemical affinity between molecules of water and protein;<sup>1</sup> while the forces

<sup>1</sup> Loeb, J., *Proteins and the theory of colloidal behavior*, New York and London, 1922, 243. Loeb, J., and Loeb, R. F., *J. Gen. Physiol.*, 1921-22, iv, 187. Loeb, J., *Arch. néerl. physiol.*, 1922, vii, 510. Cohn, E. J., and Hendry, J. L., *J. Gen. Physiol.*, 1922-23, v, 521.

which prevent particles of hydrophobic colloids from coalescing are the weak forces of electrostatic repulsion due to the existence of an electrical double layer surrounding each particle. This explains why the influence of electrolytes on the precipitation of the two types of particles is so different that it cannot be covered by the same theory.

It has been known for some time that certain hydrophilic colloids prevent the precipitation of suspensions of hydrophobic colloids by low concentrations of salts and this effect has been called the protective action of colloids. Zsigmondy has especially investigated the protective action of gelatin on suspensions of gold in water, and he and others reached the conclusion that this is due to the formation of a film of gelatin around the gold particle.<sup>2</sup> The writer has shown that this protective action of a film of gelatin on the surface of hydrophobic colloids is due to a change in the nature of the forces which prevent the particles from coalescing when they collide. While particles of gold or collodion are prevented from coalescing only when the P.D. of the electrical double layer surrounding the particles exceeds the critical value of about 12 to 15 millivolts, the same particles when coated with gelatin will not coalesce even when their cataphoretic P.D. is zero, for the reason that the particles coated with gelatin are kept from coalescing not by the electrostatic repulsion due to their electrical double layer but by the forces of attraction between the polar (or "aqueous") groups of the molecules of gelatin and water. The writer was able to prove this by a comparison of the effect of electrolytes on the precipitation of solutions of gelatin and of suspensions of particles of collodion coated with gelatin.<sup>3</sup> It was found that the effect of electrolytes is qualitatively and quantitatively the same in both cases. Since gelatin forms true solutions in water (like the amino-acids from which it is built up), it follows that the protective action of gelatin is due to the attraction between gelatin and water. It follows further that it would be wrong to assume that all suspensions of solid particles in water are hydrophobic colloids; solid particles of gelatin in water or gelatin-coated particles of collodion, gold, etc., are hydrophilic suspensions, since the forces which keep

<sup>2</sup> Zsigmondy, R., *Kolloidchemie*, Leipsic, 2nd edition, 1918.

<sup>3</sup> Loeb, J., *J. Gen. Physiol.*, 1922-23, v, 479.

the particles from coalescing are the strong forces of attraction between water and gelatin which are destroyed only by high concentrations of salt. The sign of charge of the precipitating ion bears in this case no relation to the sign of charge of the particle.<sup>4</sup>

Crystalline egg albumin forms also true solutions in water and hence is a hydrophilic colloid, but loses its high affinity for water and becomes practically insoluble when boiled. The particles of such boiled or "denatured" egg albumin are prevented from coalescing only by the electrostatic forces of repulsion between the particles due to their double layers, and they are therefore hydrophobic colloids. The writer has shown that when collodion particles are coated with genuine crystalline egg albumin, the albumin becomes denatured in the film and the particles are kept from coalescing only by the weak forces of electrostatic repulsion due to the electrical double layers. Crystalline egg albumin has little or no protective action on hydrophobic colloids. The fact that crystalline egg albumin becomes insoluble when it forms a film on the surface of collodion particles may be due to a change in the configuration or orientation of the albumin molecules in the film.<sup>3</sup>

While it is true that the influence of salts on the precipitation of the two types of colloids cannot be explained by the same theory, it does not follow that there exists no analogy in the effects of salts on any of the colloidal properties of the two groups. Such an analogy actually exists if instead of the action of electrolytes on precipitation the action of electrolytes on certain other properties of the two types of colloids is compared.

It has been shown that genuine proteins are true crystalloids in regard to chemical reactions, solubility, and probably kindred proper-

<sup>4</sup> Some authors use the term emulsoids for hydrophilic colloids, and suspensoids for hydrophobic colloids. The terms emulsoids and suspensoids should be abandoned in this case, since proteins form true solutions in water which have nothing in common with emulsions. Furthermore, micelles of gelatin or particles of collodion coated with gelatin are kept in solution by the same forces which are responsible for true solution and it would be wrong to apply to them the term suspensoids. The terms hydrophilic and hydrophobic colloids are logical and lead to no contradiction.

ties such as surface tension. Genuine proteins show colloidal behavior only when their large ions are prevented from diffusing through membranes or gels which are readily permeable to the small ions of ordinary electrolytes. When, under such conditions, osmotic equilibrium is established between a protein solution or a protein gel and the outside solution, the total molar concentration of the diffusible ions is greater inside the protein solution or the protein gel than in the surrounding aqueous solution free from protein, and this excess of the total molar concentration of diffusible ions inside the protein solution or gel over that outside varies with the concentration and nature of an electrolyte. This and nothing else is the cause of the influence of electrolytes on the colloidal behavior of proteins and this was proved by the fact that when this difference in the molar concentration of the diffusible ions inside and outside the protein solution or gel is known, the colloidal properties can be predicted quantitatively. The colloidal properties of proteins thus affected are membrane potentials, osmotic pressure, swelling, and that type of viscosity which depends on the swelling of protein micelles.<sup>5</sup>

It is found that these four properties of proteins are influenced in a similar way by electrolytes as is the stability of hydrophobic colloids. Low concentrations of electrolytes suffice to bring the values of the membrane potentials, the osmotic pressure, the colloidal type of viscosity of protein solutions, and the acid or alkali swelling of protein gels to zero, and the active ion of an electrolyte has always a sign of charge opposite to that of the protein ion.

It is natural then to raise the question whether this analogy in the effects of electrolytes on the colloidal behavior of hydrophilic and hydrophobic colloids is merely accidental or whether it can be explained by a common theory.

In order to simplify our problems we will compare the influence of electrolytes on the membrane potentials of hydrophilic colloids (proteins) and on the cataphoretic potentials of hydrophobic colloids (particles of collodion and mastic).

In order to avoid confusion, it will be necessary to distinguish between the charging effect and the depressing effects of an electrolyte

<sup>5</sup> Loeb, J., *Proteins and the theory of colloidal behavior*, New York and London, 1922.

on the two types of potentials. The charging effect of electrolytes is different for the two kinds of potentials while the depressing effect is similar.

The charging effect of electrolytes on the membrane potentials of proteins is confined to acids and alkalies. This is due to the fact that the charging effect depends in this case on the formation of protein ions, which are responsible for the Donnan equilibrium on which the colloidal behavior of proteins rests. The ionization of a protein is a minimum at the isoelectric point and the addition of acids and alkalies to isoelectric protein causes a formation of protein salt dissociating electrolytically. The addition of a neutral salt of the pH of the isoelectric point of protein to an isoelectric solution of protein, however, causes no formation of protein salt and protein ions. For this reason neutral salts have no charging effect on the membrane potentials of protein solutions.<sup>5</sup>

The addition of little acid or little alkali to a protein solution causes at first an increase in the ionization and membrane potentials of the protein solution until a maximum potential is reached. When this maximum is once reached, a further addition of acid or alkali will cause a depression of the membrane potentials of protein solutions.

The charging effect of electrolytes on the *cataphoretic* P.D. is not confined to alkalies and acids, but is produced also by salts at neutral reaction.<sup>6</sup> The reason for this is that the cataphoretic P.D. of particles of non-electrolytes like collodion or mastic is not caused by a salt formation and ionization of this material but by the formation of an electrical double layer between particle and water, one film of which adheres to and moves with the particles. This film has as a rule a negative charge in the case of particles of collodion and mastic owing to the fact that as a rule negative ions are driven in excess into the film directly enveloping the particle; while an equal excess of cations exists in the adjacent film. The forces which cause this unequal distribution of oppositely charged ions between the two films of the double layer may be either forces inherent entirely in the water or forces of attraction between particles and one of the two oppositely charged ions; or a combination of both forces. For this reason salts

<sup>6</sup> Loeb, J., *J. Gen. Physiol.*, 1922-23, v, 109, 479, 505; 1923-24, vi, 105, 215.

as well as alkalies or acids can act as charging electrolytes for the cataphoretic P.D. of particles of dielectrics in water.<sup>6</sup>

The cataphoretic P.D. seems never to exceed a maximal value of about 70 millivolts, but the maximal P.D. may be considerably less than 70 millivolts, according to the nature of the particle and the nature of the charging electrolyte. If the maximal P.D. characteristic for the particle and the charging electrolyte has been reached, the addition of any electrolyte, salt, acid, or alkali will have a depressing effect.<sup>7</sup>

There can therefore be no common theory for the charging effects of electrolytes on membrane potentials of protein solutions and on the cataphoretic P.D. of particles of dielectrics. The only question which can be raised is whether the depressing effect of electrolytes on the two potentials can be derived from a common theory. In order to answer this question it will be necessary to compare the depressing effects of salts on the two types of potentials.

## II.

### *The Valency Rule for the Depressing Effect of Salts on Membrane Potentials.*

In these experiments the charging electrolyte was NaOH. 2 gm. of originally isoelectric gelatin were dissolved in 100 cc. of water containing 16 cc. of 0.1 N NaOH which brought the pH of the solution to exactly 11.0. These were diluted with equal parts of solutions of five different salts, LiCl, NaCl, KCl, CaCl<sub>2</sub>, and BaCl<sub>2</sub>, also of pH 11.0 but of different molar concentration. Collodion bags were filled with 50 cc. of this mixture and submerged in an aqueous solution free from protein but containing the same salt in the same molar concentration as that in the protein solution; and, moreover, this outside solution was also brought to pH 11.0 through the addition of NaOH. To prevent the effect of the CO<sub>2</sub> of the air on the pH, the outside solution was kept in closed flasks in which the air communicated with the outside air through tubes filled with soda lime. After about 5 hours, osmotic equilibrium was established and the membrane potentials between the inside gelatin solution and the outside aqueous solution were measured with the Compton electrometer as described.

<sup>7</sup> Loeb, J., *J. Gen. Physiol.*, 1923-24, vi, 215.

Fig. 1 gives the effect of the five salts on the membrane potentials. The abscissæ are the concentrations of the salts, the ordinates, the P.D. in millivolts. It is obvious that the valency rule holds as had already been shown in previous publications on the subject. The values for the depressing effect of LiCl, NaCl, and KCl on the membrane potentials of Na gelatinate solutions lie all on one curve, and so

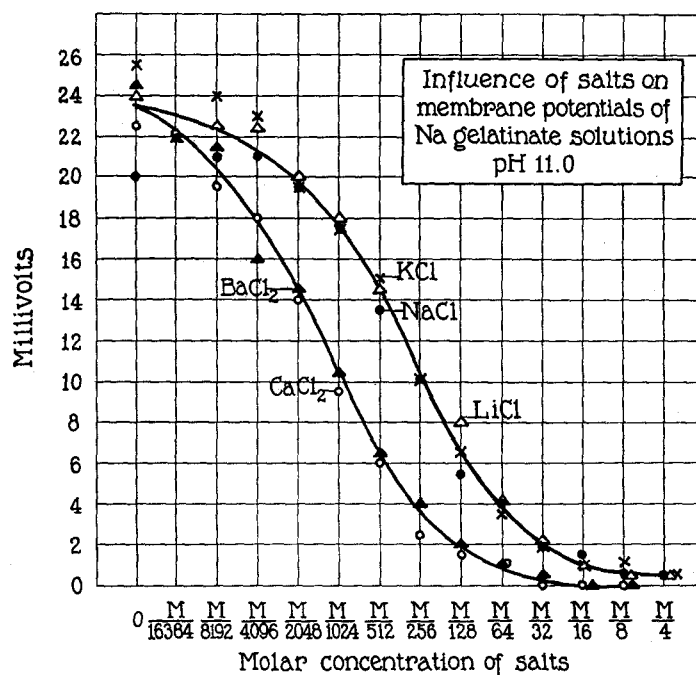


FIG. 1. Depressing effect of salts on the membrane potentials of a 1 per cent solution of Na gelatinate at pH 11.0. The abscissæ are the molar concentrations of salts, ordinates, the membrane potentials in millivolts. Only the valency but not the chemical nature of the cation determines the depressing effect.

do the values for  $\text{CaCl}_2$  and  $\text{BaCl}_2$ .<sup>8</sup> In this case the protein solution is negatively charged and hence the depressing effect increases with the increasing valency of the cation of the depressing salt. Only the valency of the cation of a salt but not the chemical nature of the cation influences the membrane potentials between solutions of metal gela-

<sup>8</sup> This figure is taken from Mr. Kunitz's doctor's thesis.



tinates and an outside aqueous solution. Such a valency rule seems to be one of the proofs that the membrane potentials are due to a membrane equilibrium, since (as far as the writer is aware) only those properties which depend upon membrane equilibria show this exclusive influence of valency and complete absence of influence of the chemical nature of the ion. This has been discussed sufficiently in previous papers.

## III.

*Proof of the Valency Rule for the Depressing Effect of Salts on the Cataphoretic Potentials of Non-Electrolytes.*

J. A. Wilson has suggested that the cataphoretic p.d. between particles and an aqueous solution was due to a Donnan equilibrium. He uses as an illustration a suspension of gold particles stabilized by KCl and assumes that the Cl ions combine with the gold imparting their negative charge to the particle.

"But the potassium ions are still left in solution, although their field of motion is restricted to the thin film of solution wetting the particles because they must continue to balance the negative charges on the particles. The volume of the film of aqueous solution enveloping a particle will be measured by the surface area of the particle and the average distance that the potassium ions are able to travel from the surface.

"Let us now consider the case where an amount of potassium chloride is present in the sol. too small to cause precipitation. The enveloping film will contain potassium ions balancing the charges on the particles as well as ionized potassium chloride. The surrounding solution will have potassium and chloride ions only in equal numbers. In the surrounding solution let

$$x = [K^+] = [Cl']$$

in the enveloping film let

$$y = [Cl']$$

and

$$z = [K^+] \text{ balanced by charges on the particles.}$$

whence

$$y + z \text{ represents the total concentration of potassium ion.}$$

As was shown in the discussion of Donnan's theory, the product  $[K^+] \times [Cl']$  must have the same value both in the enveloping film and in the surrounding solution at equilibrium. Hence

$$x^2 = y(y + z).$$

The surface layer of solution will have a greater concentration of ions than the surrounding solution by the amount  $2y + z - 2x$ . This unequal distribution of ions will give rise to a difference of potential between the enveloping film and the surrounding solution whose measure is

$$E = \frac{RT}{F} \log \frac{x}{y} = \frac{RT}{F} \log \frac{2x}{-z + \sqrt{4x^2 + z^2}}$$

But now, if we increase  $x$  without limit while  $z$  remains constant,  $E$  must decrease, approaching zero as a limit, since

$$\lim_{x \rightarrow \infty} E = \frac{RT}{F} \log \frac{2x}{\sqrt{4x^2}} = 0.$$

It is thus evident that the difference of potential between the enveloping film and the surrounding solution will be a maximum when there is no free potassium chloride present and will decrease, approaching zero, as the concentration of potassium chloride is increased without limit."<sup>9</sup>

Wilson's assumption that the charging effect of the KCl on gold is due to a chemical combination of the gold with the Cl ions may or may not be correct, but it is doubtful whether collodion or mastic particles combine with Cl ions. It is, however, not necessary to decide the question by which forces the Cl (or other anion) is held on the surface of the particle as long as some force other than the osmotic pressure is responsible for the excess of anions on the immediate surface of the particle. Given a maximal charge of this nature, the question arises whether the equation for the Donnan equilibrium can account for the depressing effects of electrolytes on the cataphoretic P.D. of collodion or mastic particles as Wilson suggests. In order to test this suggestion quantitatively, it would be necessary to measure three quantities, first, the cataphoretic P.D.; second, the value of  $z$ , *i.e.* the concentration of K ions bound by the Cl ions which are "adsorbed" by the solid particles; and third, the value of  $x$ , *i.e.* the molar concentration of K and Cl ions in the bulk of the solution. Only the first and the third of these three quantities can be measured directly, while it is impossible to measure  $z$  directly.

There is, however, an indirect method of testing Wilson's suggestion. If it is true that the depressing effect of electrolytes on the cataphoretic

<sup>9</sup> Wilson, J. A., *The chemistry of leather manufacture*, New York, 1923, 128-129.

p.d. finds its explanation in the Donnan equilibrium, we must expect that only the valency but not the chemical nature of the active ion of an electrolyte should influence the depressing effect; or, in other words, that the same valency rule should hold for the depressing effect of electrolytes on the cataphoretic p.d. as on the membrane potentials.

To test this possibility, the depressing effect of salts with monovalent and bivalent cations on the cataphoretic p.d. of particles of collodion, mastic, and graphite was compared. It was found that the valency rule holds. NaCl was used as a charging electrolyte for the particles of collodion, mastic, and graphite and it was used in that concentration in which its charging effect was about a maximum; namely,  $M/256$ . The solutions in these experiments had always a pH of 5.8; *i.e.*, they were as near the point of neutrality of water as was conveniently possible. At this pH the cataphoretic p.d. of collodion particles varied in  $M/256$  NaCl between 52 and 47 millivolts and that of the mastic particles between 60 and 55 millivolts. In order to test the depressing effect of other chlorides, LiCl, KCl, RbCl,  $MgCl_2$ ,  $CaCl_2$ , etc., 50 cc. of  $M/128$  NaCl were mixed with 50 cc. of some other salt, *e.g.*  $M/8$  LiCl. In this case the mixture consisted of  $M/256$  NaCl and  $M/16$  LiCl, where  $M/256$  NaCl was considered the charging electrolyte and LiCl the depressing electrolyte. In all these experiments, NaCl was always present in the concentration of  $M/256$  while the concentration of the depressing salts varied from  $M/16$  to  $M/2,048$ . The cataphoretic p.d. was calculated with the aid of the Helmholtz-Perrin formula from measurements of the rate of migration of particles in an electrical field under the microscope in the way described in preceding papers.

Fig. 2 gives the depressing effect of ten salts, LiCl, NaCl, KCl, RbCl,  $MgCl_2$ ,  $CaCl_2$ ,  $SrCl_2$ ,  $BaCl_2$ ,  $MnCl_2$ , and  $CoCl_2$ , on the particles of collodion charged to the maximum by  $M/256$  NaCl. The abscissæ are the molar concentrations of these depressing salts and the ordinates are the cataphoretic p.d. in millivolts. The pH of all solutions was 5.8. It is obvious that the values for the depressing effect of the four salts with univalent cation, LiCl, NaCl, KCl, and RbCl, lie (within the limits of the accuracy of the experiments) on one curve; and that the values for the depressing effect of the chlorides with divalent cation lie also all on one curve, which is, however, consid-

erably lower than that for the monovalent cations. The results are so clear that they leave no doubt that the valency rule holds for the depressing effect of these salts on the cataphoretic P.D. of the negatively charged collodion particles.

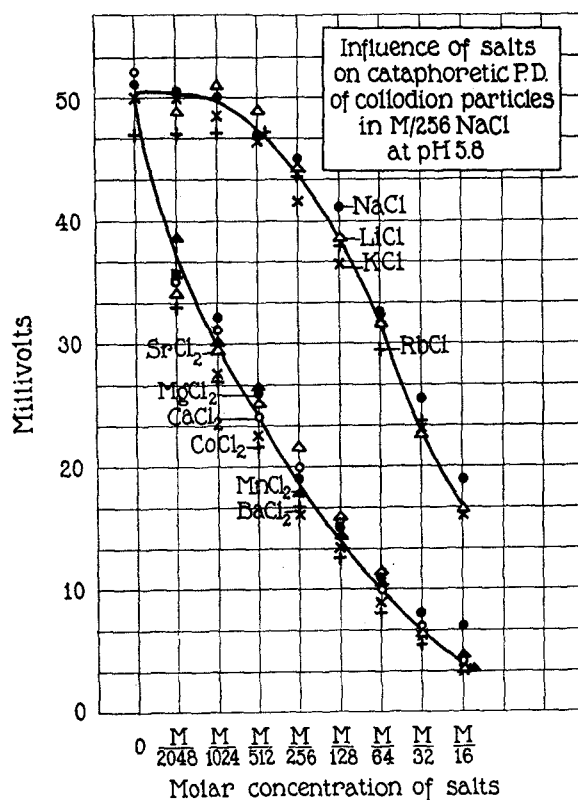


FIG. 2. Depressing effect of salts on cataphoretic P.D. of collodion particles in M/256 NaCl at pH 5.8. Only the valency but not the nature of the cation determines the depressing effect.

The maximal charging effect of CaCl<sub>2</sub> alone is reached at a concentration of about M/2,048, where the collodion particles have a P.D. of about 27 millivolts. When we add various concentrations of NaCl, the P.D. is never increased (Fig. 3) for the reason that the charging ion is the Cl ion and that the maximal charge possible by Cl ions in

the presence of Ca is already reached at a concentration of  $M/2,048$   $\text{CaCl}_2$ . This explains why the difference in the depressing effect between salts of the type of  $\text{NaCl}$  and  $\text{CaCl}_2$  is greater in the case of cataphoretic potentials than in the case of membrane potentials, as shown in a comparison between Figs. 1 and 2.

Fig. 4 shows that at the same pH of 5.8 the depressing effect of  $\text{NaCNS}$  and  $\text{NaNO}_3$  is practically identical with that for  $\text{NaCl}$ .

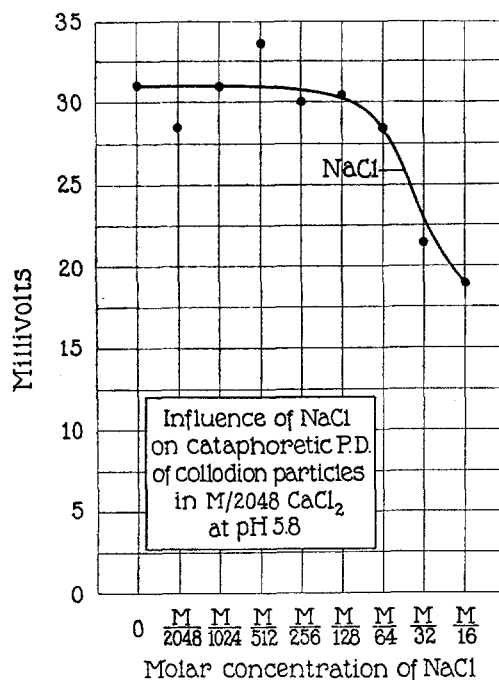


FIG. 3. Proof that the addition of  $\text{NaCl}$  to  $M/2,048$   $\text{CaCl}_2$  has no charging effect on collodion particles.

It has been shown in preceding papers that there exists a critical P.D. below which suspensions are no longer stable and this P.D. is in the neighborhood of about 14 millivolts. This is true only for suspensions of hydrophobic colloids the stability of which is due exclusively to electrical double layers. The fact that the critical P.D. is almost the same, regardless of the nature of the particle or of the salt, leads to the assumption that the critical P.D. is that P.D. which no

longer suffices to prevent electrostatically the collision of particles moving with average velocity in the liquid. We should therefore expect that the precipitating concentration of all salts with univalent cations and univalent anions for collodion particles should be the same and that the same should be true for salts with divalent cations and uni-

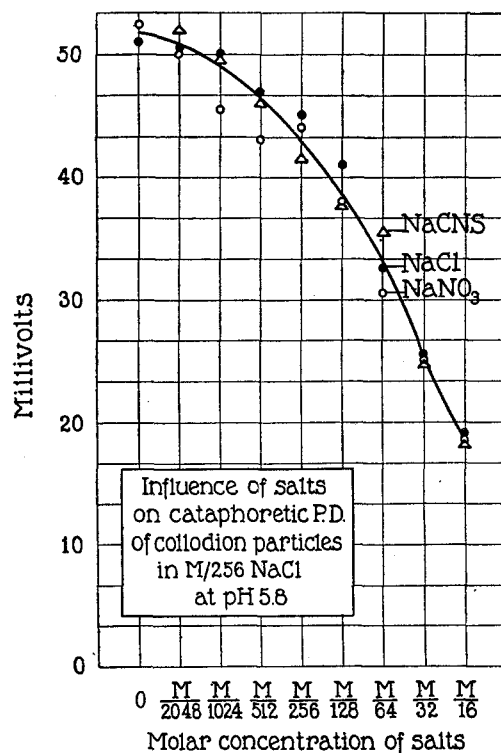


FIG. 4. Proof that the depressing effect of NaCl, NaNO<sub>3</sub>, and NaCNS on the cataphoretic P.D. of collodion particles in M/256 NaCl is identical (pH 5.8).

valent anions, except that it should be lower for the latter than the former. In a preceding paper<sup>7</sup> it was shown that this postulate of the theory is fulfilled (for the same pH) for a number of hydrophobic colloids, *e.g.* collodion, mastic, gold, graphite, etc. There is only one exception. It has always been observed that the salts of heavy metals have a greater precipitating effect than salts of other cations of the

same valency; thus  $\text{AgNO}_3$  has a greater precipitating effect than  $\text{NaNO}_3$ . Fig. 5 shows that  $\text{AgNO}_3$  has a greater depressing effect on the cataphoretic P.D. of collodion particles than  $\text{NaNO}_3$  at the same concentration and pH. This effect may or may not be due to the formation of complex salts by the ions of the heavy metals. In these

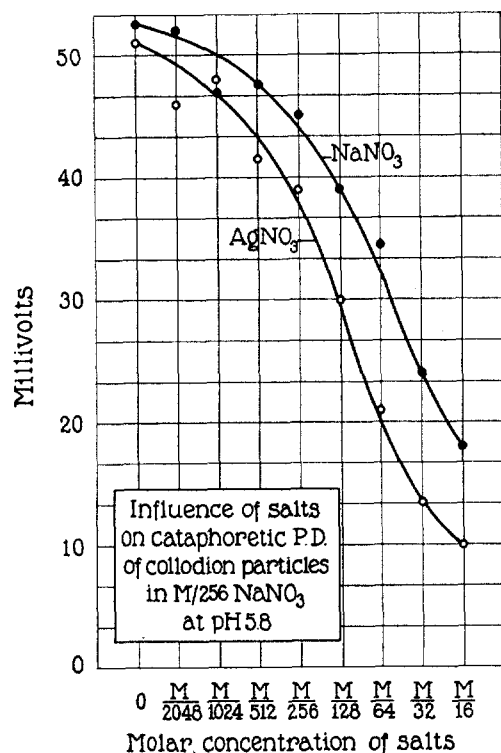


FIG. 5. Proof that the depressing effect of  $\text{AgNO}_3$  on cataphoretic P.D. of collodion particles in M/256  $\text{NaNO}_3$  is greater than that of  $\text{NaNO}_3$ .

experiments M/256  $\text{NaNO}_3$  was used as charging salt instead of  $\text{NaCl}$ . Acids may also cause complications by reacting chemically with the particles.

Fig. 6 shows that the valency rule holds also for the depressing effect of salts on mastic particles as it does in the case of collodion particles. It may be added that similar results confirming the valency rule for

the depressing effect of salts on the cataphoretic P.D. were also obtained in the case of particles of Acheson's graphite.

These results, then, leave no doubt that the valency rule holds for the depressing effect of salts on the cataphoretic P.D. of non-electro-

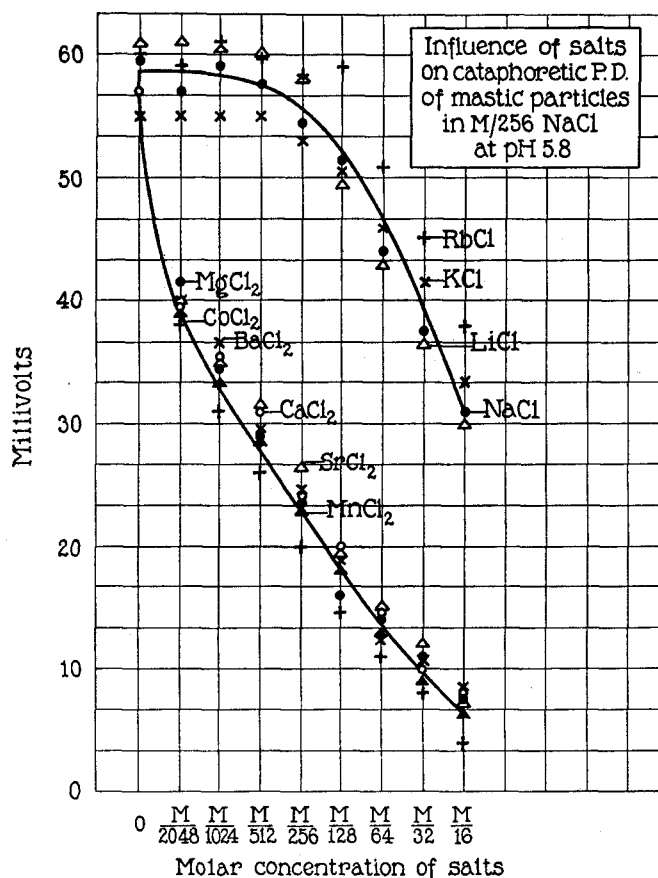


FIG. 6. Depressing effect of salts on the cataphoretic P.D. of mastic particles in M/256 NaCl at pH 5.8.

lytes; except in such cases where chemical complications may be expected. Unless it can be shown that the valency rule holds under other conditions than those of the Donnan equilibrium, these experiments confirm Wilson's hypothesis.



## IV.

*Proof of the Valency Rule for the Cataphoretic P.D. of Solid Protein Particles.*

The cataphoretic P.D. of solid protein particles has a double origin. In the first place isoelectric protein forms salts with acids and alkalies, which dissociate electrolytically. This ionization of the protein is one source of the cataphoretic charge of solid protein particles. When salts are present there exists a second source of cataphoretic P.D. This charging effect of salts is superposed on that of acids or alkalies. In the case of salts with tetravalent ions this second charging effect may reverse the sign of charge due to ionization. Thus solid casein chloride particles of pH 4.0 have a positive cataphoretic charge, due to the ionization of casein chloride, but if  $\text{Na}_4\text{Fe}(\text{CN})_6$  is added in low concentration (care being taken that the pH remains the same), the casein chloride particles will assume a negative cataphoretic charge. It will therefore be advisable to discuss the influence of acids and of salts on the cataphoretic P.D. of solid protein particles separately.

For the investigation of the influence of acid on the cataphoretic P.D. of protein, collodion particles coated with gelatin were used. Collodion particles were kept overnight in a 0.1 per cent solution of isoelectric gelatin (at pH 4.7). Next morning the particles were separated from the solution by centrifuging, washed in water of pH 4.7, and centrifuged again, and then put into water of pH 4.7 to serve as stock suspension. 2 drops of this stock suspension were put into 50 cc. of acid solution of different pH and their velocity of migration in the galvanic field was measured. Fig. 7 gives the result for five acids, HCl,  $\text{HNO}_3$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{SO}_4$ , and sulfosalicylic acid. It may be stated that in the range of pH used in these experiments  $\text{H}_3\text{PO}_4$  dissociates as a monobasic acid while sulfosalicylic acid dissociates like a strong dibasic acid. The abscissæ are the pH of the acid solutions in which the cataphoretic P.D. was measured, while the ordinates are the values of the cataphoretic P.D. in millivolts.

It is obvious that the five acids are separated into two distinct groups, one group including the three monobasic acids, HCl,  $\text{HNO}_3$ , and  $\text{H}_3\text{PO}_4$ , and the second group including the two strong dibasic acids, sulfuric and sulfosalicylic acid.

What is of importance is that the values for the three monobasic acids lie—within the limits of experimental accuracy—on one curve; and that the same is true for the two dibasic acids. This proves that only the valency but not the chemical nature of the anion of the acid determines the cataphoretic P.D.

The degree of accuracy of measurements is too small to permit us to lay stress on quantitative relations; and it may therefore be rather accidental that the approximate value of 0.66 for the ratio of the cata-

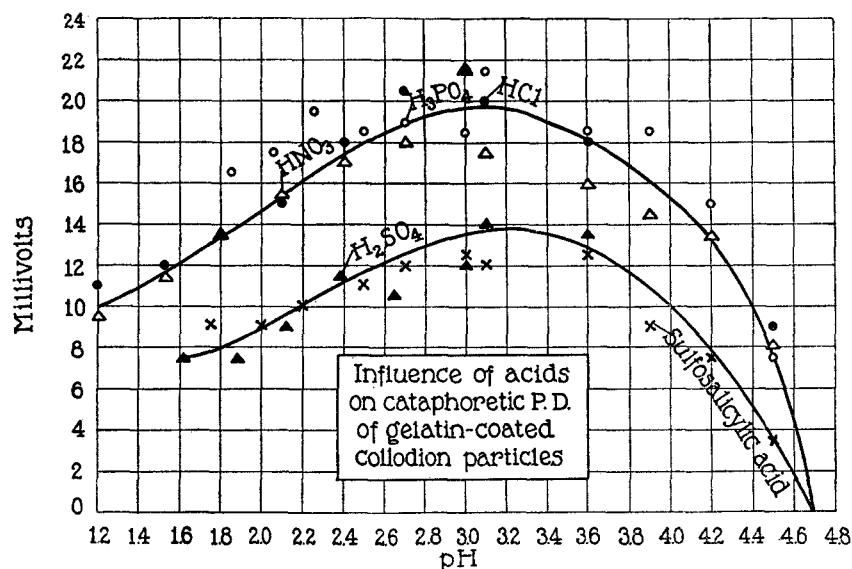


FIG. 7. Proof of valency rule for the influence of acids on the cataphoretic P.D. of gelatin-coated collodion particles. Abscissæ are the pH of acid solutions, ordinates, the cataphoretic P.D. in millivolts.

phoretic P.D. of dibasic and monobasic acids prevails at the same pH. This is the value demanded by the Donnan theory.<sup>5,10</sup> Omitting the values of P.D. below 10 millivolts, we find the following ratios (Table I).

Whatever may be the theoretical explanation of these measurements, it is certain that the forces due to the ionization of the protein

<sup>10</sup> Loeb, J., and Kunitz, M., *J. Gen. Physiol.*, 1922-23, v, 665.

itself outweigh in this case any influences which forces inherent in the water itself might have on the cataphoretic P.D. under the influence of acids.

The effect of salts on the cataphoretic P.D. of protein particles was tried on negatively charged particles of denatured egg albumin, to make the results comparable to the experiments on negatively charged particles of collodion and mastic. A 1 per cent solution of isoelectric crystalline egg albumin ( $\text{pH} = 4.8$ ), prepared after Sørensen's method, was heated to  $90^\circ\text{C}$ . The gross particles of the coagulated mass were removed by filtering through a coarse filter. The filtrate which was opalescent was used as the stock suspension for the experiments. 3 drops of this stock suspension were added to 50 cc. of a solution and

TABLE I.

pH	Cataphoretic P.D. for acids.		Ratio: $\frac{\text{Dibasic}}{\text{Monobasic}}$
	Dibasic.	Monobasic.	
	<i>millivolts</i>	<i>millivolts</i>	
4.0	10	15	0.66
3.8	11.6	16.6	0.70
3.6	13	18	0.72
3.4	13.6	19	0.72
3.2	14	19.5	0.72
2.8	13	19	0.68
2.4	11	17	0.65
2.2	10	16	0.63

after 20 minutes the speed of migration of the particles in an electric field was measured.

We used as a charging electrolyte  $\text{M}/5,000$  NaOH which transformed the isoelectric albumin into sodium albuminate (with negative albumin ions). The cataphoretic P.D. of the particles in this solution varied between 32 and 38 millivolts. In such solutions of  $\text{M}/5,000$  NaOH were contained various salts, LiCl, NaCl, KCl, RbCl,  $\text{CaCl}_2$ , and  $\text{BaCl}_2$ , as depressing electrolytes in concentrations varying from  $\text{M}/2,048$  to  $\text{M}/16$ . Fig. 8 gives the results, showing again a clear valency effect.

The salts with univalent cation, LiCl, NaCl, KCl, and RbCl, had an equal depressing effect on the cataphoretic P.D. of the particles

of denatured egg albumin, all the values lying on one curve. The values for the depressing effect of  $\text{CaCl}_2$  and  $\text{BaCl}_2$  also lie on one curve, which is, however, considerably lower than that for salts of the type  $\text{NaCl}$ . The curves in Fig. 8 resemble the corresponding curves in Fig. 2 for collodion particles and Fig. 6 for mastic particles.

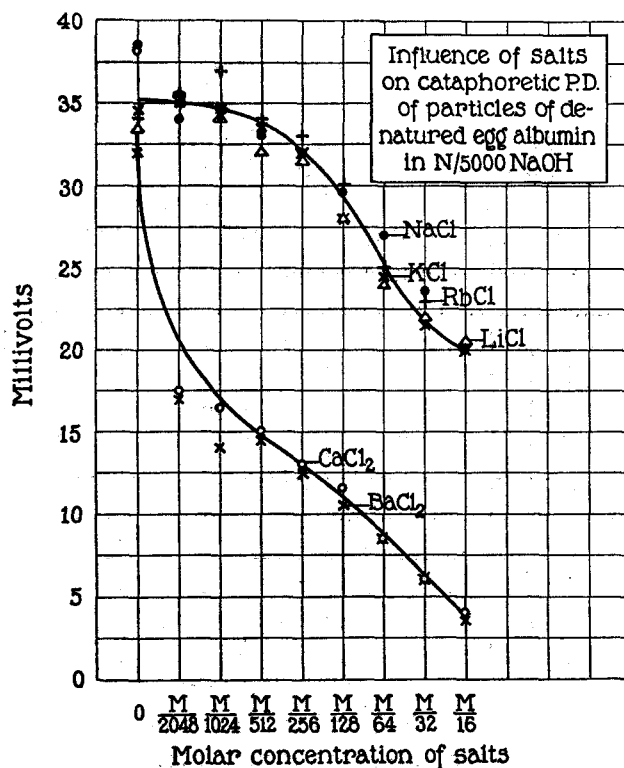


FIG. 8. Depressing effect of salts on the cataphoretic P.D. of denatured egg albumin.

We may therefore draw the conclusion that regardless of the chemical nature of the particles the depressing influence of electrolytes on the cataphoretic P.D. is determined by the valency but not by the nature of that ion of an electrolyte which has a sign of charge opposite to that of the particles. The only exceptions are where the electrolyte changes the chemical constitution of the particle (as possibly in the

case of heavy metals). In the case of the influence of acids on the cataphoretic P.D. of collodion particles coated with gelatin the cataphoretic P.D. seems to be determined by the ionization of the protein in a way as if the Donnan equilibrium might be the determining factor.

## V.

*Theoretical Remarks.*

If it is actually true that the depressing effect of electrolytes on the cataphoretic P.D. finds its explanation in the existence of a Donnan equilibrium between the film which adheres to and moves with the particles in cataphoresis and the oppositely charged film of the double layer, we are forced to form a somewhat different conception of this adhering film than that usually accepted. When gold particles are negatively charged in a NaCl solution, it is usually assumed that the Cl ions are directly responsible for the negative charge of the gold particles while the excess of Na ions is assumed to be responsible for the positive charge. If Wilson's suggestion is correct, the film which moves with the particle of gold is thicker, containing not only the Cl ions but also the Na ions held by the "adsorbed" Cl ions and in addition free NaCl. The film moving with the particle has therefore an excess of cations, while the other film of the double layer has a corresponding excess of anions. The situation is the same as when a solution of Na gelatinate is separated by a membrane from an aqueous solution with which it is in equilibrium. In this case the gelatin solution has an excess of cations and is negatively charged. The only difference is that in the case of the double layer the inequality in the concentrations of cations in the enveloping film and the bulk of solution is not due to a membrane but to the forces which press the Cl ions in excess on the solid particle. This leads to an excess of cations in the film which adheres to and moves with the particle.

In his recent papers the writer has made use of the older conception of the double layer, but the proof of the validity of the valency rule for the depressing effect of ions on the cataphoretic P.D. forces us to consider Wilson's conception of the constitution of the electrical double layer.

The measurements of the rate of migration of the particles were carried out by the writer's assistant, Mr. M. Kunitz.

## VI.

### SUMMARY AND CONCLUSIONS.

1. In order to be able to compare the effects of electrolytes on membrane potentials and cataphoretic potentials it seems necessary to distinguish between the charging and depressing effect of electrolytes on these potentials. Only low concentrations of acids and alkalies have a charging effect on the *membrane* potentials of proteins, while low concentrations of neutral salts have only a depressing effect; in the case of the *cataphoretic* potentials, low concentrations of salts have a charging effect as have also low concentrations of alkalies and in some cases low concentrations of acids. This difference finds its explanation in the difference of the origin of the two potentials and there can therefore be no common theory for the charging effect of electrolytes in the two cases.

2. There exists, however, an analogy in the depressing action of electrolytes on the two types of potentials inasmuch when the maximal P.D. is reached, all three kinds of electrolytes, acids, alkalies, and neutral salts, have a depressing effect on both types of potentials (taking into due consideration the effect of changes in the hydrogen ion concentration).

3. This depressing effect is adequately explained for the membrane potentials of protein solutions and protein gels on the basis of the Donnan equilibrium, and the question arises whether the same explanation may also hold for the cataphoretic potentials.

4. The active ion in the depressing action of electrolytes on membrane potentials as well as on cataphoretic potentials has the opposite sign of charge from that of the colloidal particle. It had been shown before<sup>5,10</sup> that only the valency but not the chemical nature of the active ion determines the depressing effect in the case of membrane potentials and it is shown in this paper that the same is true for the cataphoretic potentials of particles of collodion, mastic, Acheson's graphite, and denatured egg albumin.

5. It is shown that the same valency rule holds also for the effect of acids on the cataphoretic potentials of collodion particles coated with gelatin, and that the ratio of the effect of dibasic to that of monobasic acids is approximately 0.66, as Donnan's theory of membrane potentials would demand.

6. If we have a right to conclude from the validity of the valency rule for cataphoretic potentials that the depressing effect of electrolytes on the cataphoretic P.D. is determined by the Donnan equilibrium, we can understand the analogy between the depressing action of electrolytes on membrane potentials of hydrophilic colloids and on the cataphoretic potentials of hydrophobic colloids. We can also understand the analogy between the influence of electrolytes on the precipitation of hydrophobic colloids and on the depression of the values of all those properties of hydrophilic colloids which depend on the Donnan equilibrium, since the precipitation of hydrophobic colloids occurs when the cataphoretic P.D. is depressed below a critical value.