

THE EFFECT OF VALENCY OF CATIONS AND ANIONS  
ON NEGATIVELY AND POSITIVELY CHARGED  
RED BLOOD CELLS.

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It is a well established fact that the ion of an electrolyte which acts on the charge of a suspended particle is that one which bears a charge of the opposite sign to that of the particle. Thus the negatively charged particles of a collodion suspension have their charges reduced and may be flocculated by the cation of an added salt, while with the positively charged particles of a copper sol the anion is the active ion.

The efficiency of the active ion in producing its effect on the charge of a particle, or on the stability of the suspension, varies with its valency. Exact quantitative relations have not, however, been established, as widely varying results have been obtained by different observers in their attempts at an experimental determination of the correlation. A summary of this work may be found in Burton's monograph (1).

In the earlier investigation we have studied the effect of electrolytes on red blood cells suspended in isotonic sucrose (2). Under these conditions the cells are negatively charged and the changes in the P.D. of the cells which occur as the electrolyte is added depend in part on the valency of the cation. The effect of valency may, however, be masked by the action of H ions in the suspending fluid, and in the case of hydrolyzing salts this becomes a factor of considerable importance.

In the present study we wish to contrast the effect of the valency of the cation on negatively charged cells with the effect of valency of the anion on positively charged cells. It is possible to obtain cells positively charged by increasing the H ion concentration of the

suspending fluid. Above the isoelectric point of the cells, using this term in the sense of Michaelis, their original negative charge is reversed. Now under such conditions Loeb (3) has shown that proteins combine not with the cation of the electrolyte present, but with the anion, and Coulter (4) has demonstrated that the same principle holds for red blood cells. There seems, therefore, strong *a priori* evidence to support the hypothesis that the electrokinetic effects produced by the anion on positively charged red cells will be analogous to those produced by the cation on the negatively charged cells.

#### *Methods.*

Rabbit cells washed as free as possible of electrolyte with isotonic sucrose and suspended in the same fluid were used in all the experiments. The electric charge of the cells was determined with a Michaelis cataphoresis chamber and the results expressed in millivolts by means of the usual formula. The suspension of positively charged cells was produced by adding HCl to give a concentration of  $m/250$  in the total volume of suspension.

#### *The Effect of the Cation.*

In studying the action of the cation on the two types of cells three salts, NaCl, NiCl<sub>2</sub>, and AlCl<sub>3</sub>, were used with cations which are respectively monovalent, bivalent, and trivalent, the anion being the same in all cases. Two sets of series of tubes were prepared, one containing negatively charged cells in isotonic sucrose and the other positively charged cells in the same fluid containing  $m/250$  HCl. To both sets of tubes were added increasing concentrations of the three salts and the p.d. of the cells determined. As has been shown (2), the p.d. of the negatively charged cells in a weak concentration of electrolyte is not a permanent one. The curves that are illustrated are those of the "immediate charge" as these alone give a full demonstration of the effect of the added electrolyte. With the positive cells the charge is permanent, as there is sufficient electrolyte in the form of HCl to stabilize the charge.

Fig. 1 illustrates the cation effect of the different salts on the two types of cells. Above the isoelectric line the charge is negative,

below, it is positive. Valency effect of the cation is evident in the action of the salts on the negative cells. NaCl produces a slow depression of the charge which reaches an isoelectric point at a

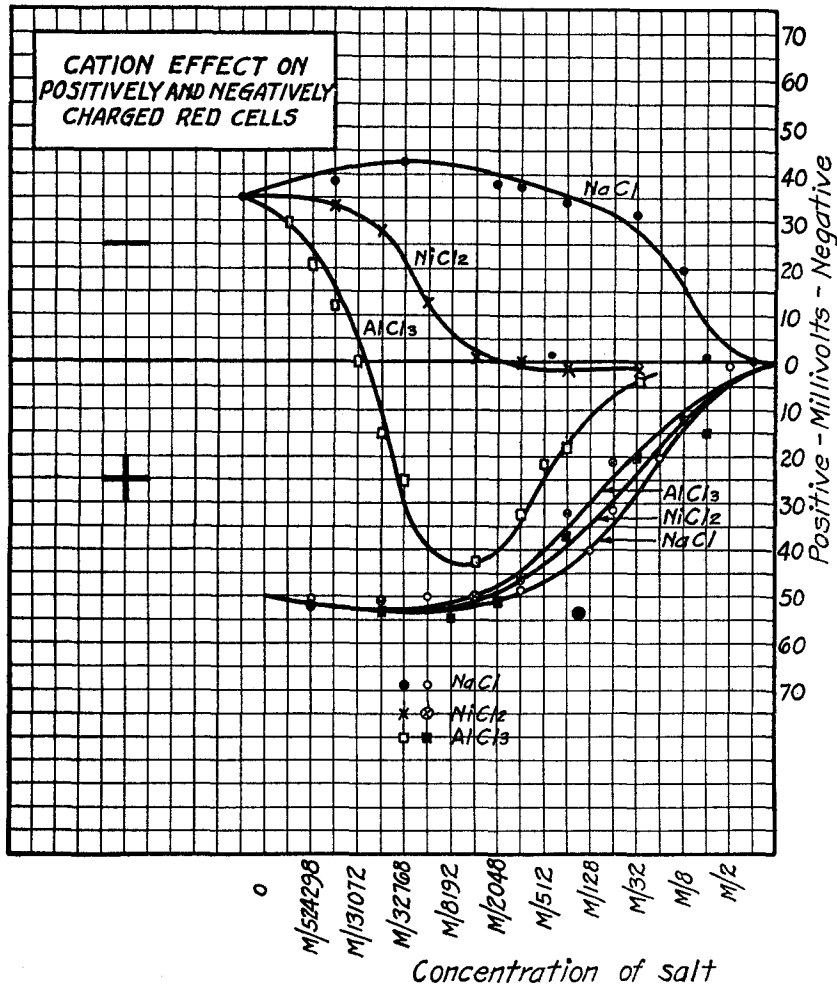


FIG. 1.

concentration of M/4. NiCl<sub>2</sub> with a bivalent cation, produces a much more sudden drop in the P.D. of the cells so that the isoelectric point is reached at a concentration of M/4,000. AlCl<sub>3</sub> with a triva-

lent cation causes a sudden fall in P.D., the isoelectric point being reached at  $m/131,072$ ; the charge is then reversed and the typical trivalent ion effect then follows.

With the positive cells there is no difference between the actions of the three salts. The monovalent anion produces a gradual depression of the P.D. until the isoelectric point is reached at a concentration of  $m/4$ . The presence of a trivalent cation such as Al produces no more effect than does that of a monovalent one such as Na. The curve of the monovalent anion, Cl, is the complete analogue of that of the monovalent cation, Na, in the case of the negative cells.

#### *The Effect of the Anion.*

In this experiment salts with the same cation, Na, were selected and different anions, Cl,  $SO_4$ , and  $PO_4$ . The procedure with the negatively and positively charged cells was the same as previously described.

Fig. 2 shows the results. With the negatively charged cells the action of the salts is identical within the limits of error of the method. The monovalent cation acts in the same manner whether it is in the presence of the monovalent anion, Cl, or the trivalent anion,  $PO_4$ . All gradually depress the P.D. of the cells and produce an isoelectric condition at a concentration of  $m/4$ .

With the positively charged cells the three salts produce strikingly different effects, for now the valency effect of the active anion comes into play. With NaCl the monovalent Cl causes a gradual depression of the charge and the curve of P.D. resembles that produced by the monovalent cation, Na, on negatively charged cells. With  $Na_2SO_4$  the bivalent anion produces a much more sudden drop in potential, the isoelectric point lying at a concentration of about  $m/256$ . This curve resembles that produced by the bivalent cation, Ni, on the negatively charged cells (Fig. 1).  $Na_3PO_4$  produces, by means of its trivalent anion, a more sudden drop in charge and a reversal of charge at  $m/512$ . It resembles, therefore, the curve of P.D. produced by the trivalent Al cation on the negatively charged cells (Fig. 1).

*Hydrogen Ion Concentration and the Observation of the Effect of Valency of Ions of Hydrolyzing Salts.*

Extensive investigations have been made in attempts to determine the exact relation between the effect of an ion and its valency. In

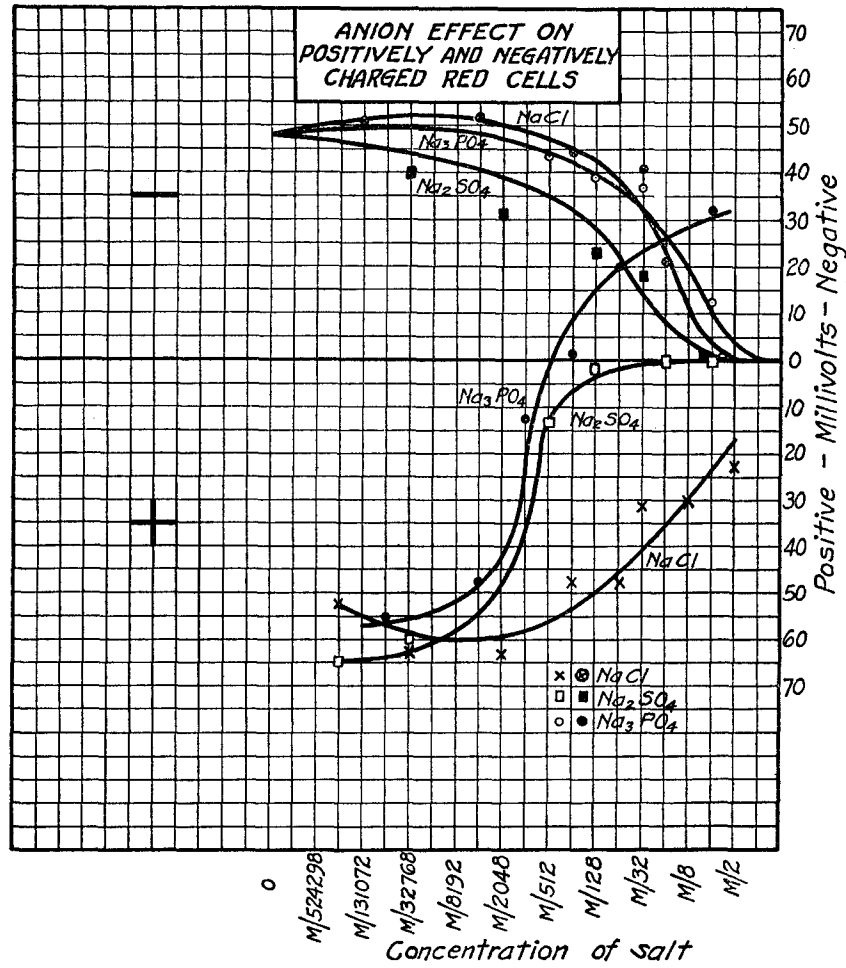


FIG. 2.

most cases the effect studied was the coagulating value of the salt, which, with suspensoid colloids, at least, is largely determined by

the electrokinetic action of the ion. The results of these investigations have been very unsatisfactory so far as determining any quantitative relationship. One of the earliest of such attempts is the Schulze-Linder-Picton law of coagulation, which states that the coagulative value of monovalent, bivalent, and trivalent ions is as  $1:x:x^2$ ,  $x$  being a constant of the value of 30 or 40. Burton (1) has shown in his critical summary how unsatisfactory is this ratio when it is applied to the experimental results of different workers. Moreover, as pointed out by Burton, the same experimenter does not obtain the same series order for metal cations when he uses different salts, *e.g.* chlorides, nitrates, and sulfates.

Freundlich (5) has suggested that differences in the degree of absorption of the active ions may explain these variations. With an amphoteric colloid such as is involved in a suspension of red blood cells, another factor which will obviously obscure the effect of valency is the variation in H ion concentration depending on the degree of hydrolysis of the salt.

Fig. 3 which represents the effect of the cation, Ca, on the p.D. of the negatively charged cells, illustrates this point. The cation is derived from four different salts, the acetate, the chloride, the nitrate, and the chromate. It is seen that there is a wide variation in the apparent activity of the cation, Ca, on the negative cells, the concentration required to bring the p.D. of the cells to an isoelectric point being  $M/64$ ,  $M/256$ ,  $M/2,048$ , and  $M/2,048$ , respectively. The last two salts even produce a slight reversal of the charge. This would indicate the presence of H ions, and a determination of their concentration shows indeed that the pH of the fluid in which reversal occurs is below the isoelectric point. As shown in Fig. 3, the pH of the chromate and nitrate is 4.4, whereas that of the chloride and acetate, which show the "normal" bivalent effect, is 5.5 and 5.3.

For the same reason a salt with bivalent cation, which hydrolyzes, may produce more effect, both coagulative and electrokinetic, than a salt with a trivalent cation which is not greatly hydrolyzed. Fig. 4 shows the effect on the p.D. of the cells and on the stability of the suspension of strongly hydrolyzed  $\text{CuCl}_2$  with bivalent cation as contrasted with the weakly hydrolyzed  $\text{CeCl}_3$  with trivalent cation.

The salt of the bivalent ion is more active than that of the trivalent ion in all respects. Both depress the charge of the cells to an iso-electric point at a concentration of about  $M/32,000$ ; but the  $CuCl_2$

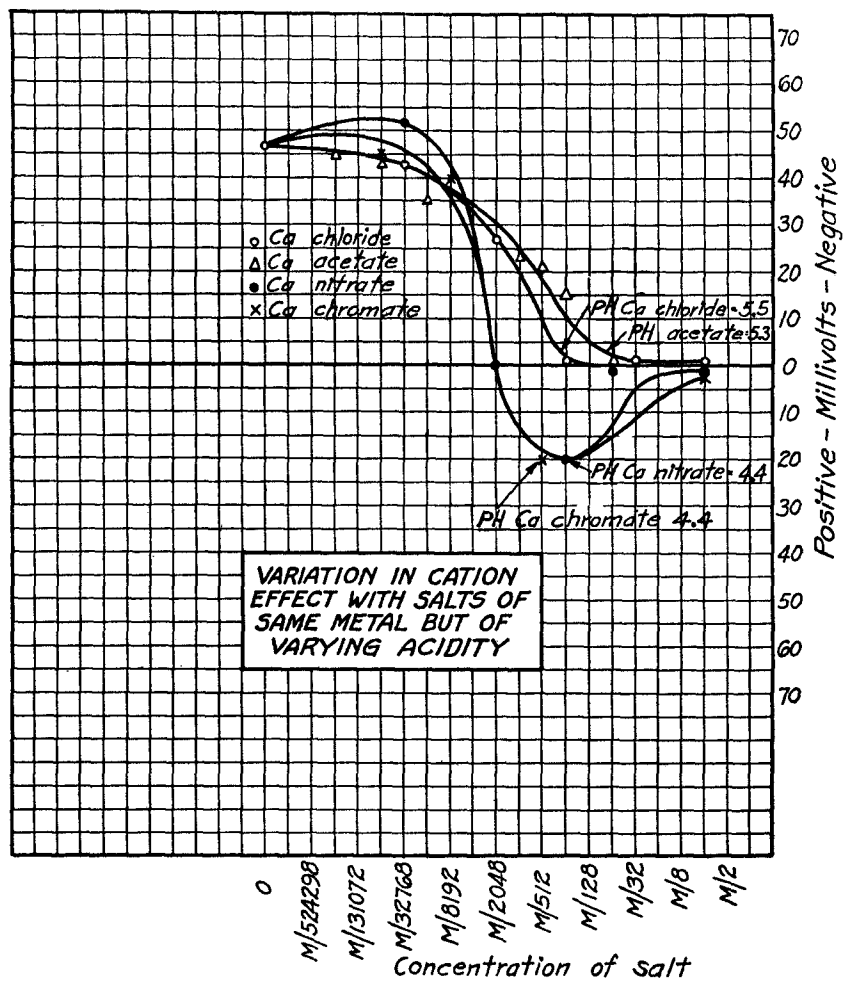


FIG. 3.

produces a strong positive charge which is sufficient to cause a zone of stability, whereas the  $CeCl_3$  does not produce a sufficient charge to stabilize the unstable suspension. The difference in the pH of

the same molar concentrations of the two salts is striking. At  $m/1,000$   $\text{CuCl}_2$  has a pH of 2.7, while that of the  $\text{CeCl}_3$  is 4.5.

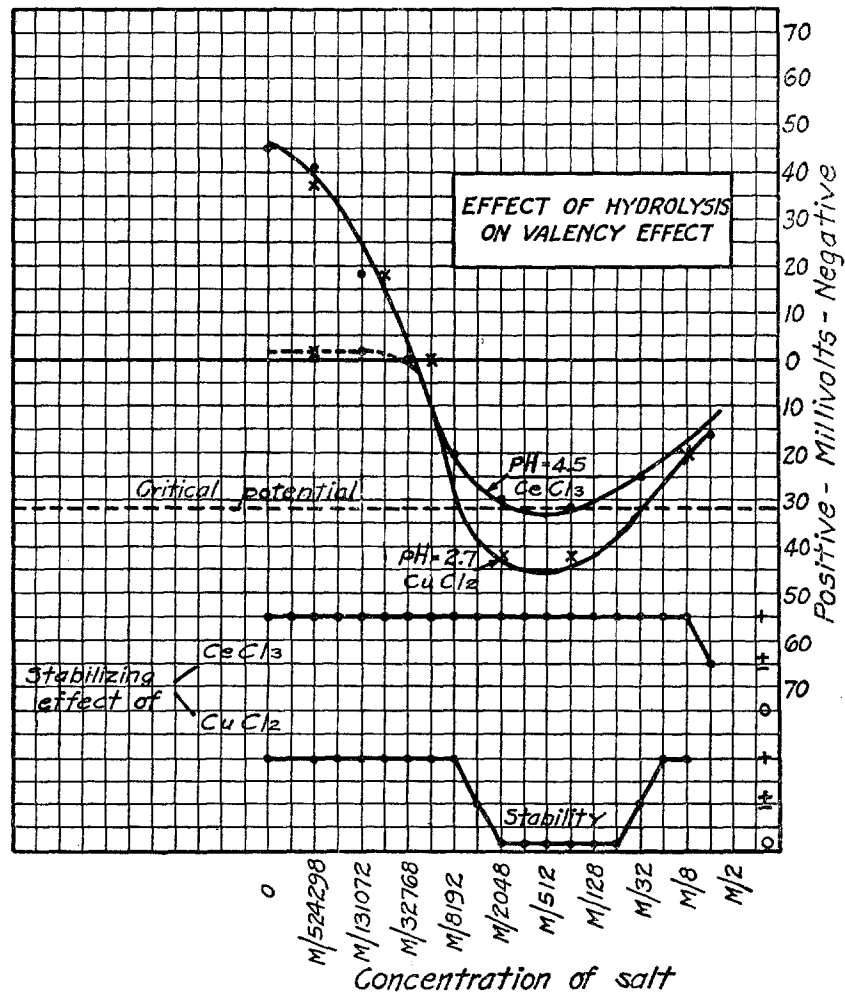


FIG. 4.

## DISCUSSION.

When the H ion concentration varies but little as was the case in certain of our experiments (Figs. 1 and 2), the effect of valency may



be satisfactorily demonstrated both with cations and negatively and with anions and positively charged red cells. If the H ion concentration varies too much (Figs. 3 and 4), the effect of valency is entirely obscured.

These results show how impossible it is to establish any quantitative relation between the activity of different salts on the basis of the valency of their ions alone. Two factors at least are concerned in the production of electrokinetic and coagulative phenomena, the general ion activity and the specific action of the H ion. With ampholytes the latter is of great importance. In fact Michaelis' theory of the isoelectric point (6) is based on a consideration of the H ion concentration alone, and the term isoelectric point has come to mean for many biologists, who are concerned largely with amphoteric proteins, a certain degree of acidity. As Freundlich<sup>1</sup> and Svedberg (7) point out, and as Michaelis himself has shown experimentally (8), the activity of all the ions must be considered.

#### CONCLUSIONS.

1. Under comparable conditions, valency effect may be demonstrated with a suspension of red blood cells and the cations and anions of salts.
2. The valency of the cation determines the degree of the effect on negatively charged cells, the valency of the anion, the effect on positively charged cells.
3. Anomalies in valency effects with different salts and red cell suspensions are in part due to variations in H ion concentration, depending on the degree of hydrolysis of the salt.

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<sup>1</sup>Freundlich (5), p. 784.