LVII. EFFECT OF THE ADDITION OF SALTS ON THE p_H OF SOLUTIONS OF AMPHOLYTES.

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It has been found that the velocity of hydrolysis of starch in the system gelatin-starch-amylase is affected by the addition of calcium chloride. This effect may be due to change in the hydrogen ion concentration of the system.

Such a possibility was first pointed out by Spiro [1922], who found that the p_H of glycine solutions was lowered by addition of calcium or potassium chloride, and similar results were obtained by Mond [1923], Netter [1925], Simms [1929], and others. The majority of these authors interpreted the result of addition of electrolytes to colloidal sols as being essentially the same as in the case of crystalloid ampholytes, such as glycine.

Such a view is not, in our opinion, sufficiently justified by the experimental material available, as the effects of relatively high concentrations (0.15–0.25 M) of electrolyte only were examined, and, further, the dependence of the observed phenomena on the concentration of electrolyte was not investigated. The work of Freundlich [1930] on hydrophobe, and of Kruyt [1928, 1929] on hydrophile, colloids has shown that the influence of salts on the electrolytic properties of colloidal particles should rather be evidenced by low (0.02 M) than by high concentrations of salt.

In the present paper, experiments on the change in p_H of solutions of gelatin, ovalbumin, peptone, and glycine will be described, in relation to the concentration within the limits 0–0.025 M of salt added, to the valency of the anion and cation of the salt and to the original p_H of the solution.

EXPERIMENTAL.

Gelatin (Coignet et Cie, Paris) was twice washed with cold distilled water, and dissolved at 60°. 100 cc. portions of 0.5–2.0 % solutions were placed in flasks containing 10 cc. of water or of various salt solutions, and the p_H of the systems was determined potentiometrically at 20°, using a hydrogen-calomel electrode. Special attention was paid to the purity of the water and to the cleanliness of the vessels used, since even traces of impurity might affect the colloidal state of the gelatin. The salts used were sodium, potassium,

calcium, and magnesium chlorides and sodium and magnesium sulphates (Merck's "chemisch rein" chemicals). The p_H of the salt solutions was brought to that of the gelatin solution by the addition of 0.02–0.1 *N* sodium hydroxide, which was also used for bringing the gelatin solution to the desired p_H .

Similar experiments were performed using Witte's peptone (Poulenc Fr.); this peptone contained colloidal matter precipitable by the addition of 50 % alcohol. The ovalbumin used (Merck) contained globulin, which, however, separated on standing and was removed by filtration.

I. *Gelatin.*

These experiments (Table I) were performed on 1 % solutions of gelatin, the p_H of which was 5.8–5.9. It appears that whilst the salts used act quantitatively differently, yet in all cases the p_H of the system is lowered. This is best illustrated by Table I, which shows that the depression of p_H is, except at very low concentrations, proportional to the concentration of potassium chloride. Bivalent anions and cations act differently, being much more active per unit of concentration when present in small (1–10 *mM*) than in higher concentrations, and the curves obtained for such salts are of the type of adsorption isotherms.

Table I. *Variations in p_H of gelatin solutions.*

Molar concentration of salt	Lowering of p_H				
	CaCl ₂	MgCl ₂	MgSO ₄	Na ₂ SO ₄	KCl
0.0025	0.21	0.12	0.09	0.06	—
0.005	0.25	0.20	—	—	—
0.010	0.30	0.26	0.19	0.15	—
0.020	0.39	0.33	—	—	—
0.025	0.43	0.38	0.27	0.26	—
0.050	—	—	—	—	0.09
0.100	—	—	—	—	0.17

The results obtained in systems in which the concentration of calcium chloride is maintained constant whilst that of gelatin is varied (0.4–1.2 %) are given in Table II, in which we see that the depression in p_H caused by the salt is greater at lower than at higher concentrations of gelatin.

Table II. *Influence of concentration of gelatin.*

Gelatin %	Concentration of CaCl ₂ <i>M</i>	Lowering of p_H
0.4	0.010	0.38
	0.020	0.59
	0.010	0.48
0.6	0.020	0.50
	0.010	0.44
0.8	0.020	0.46
	0.010	0.44

The reaction of gelatin solutions was varied by adding 0.02 *N* sodium hydroxide solutions to the required p_H ; in this way three solutions were prepared, of p_H 5.90, 8.03, and 9.21, from the same preparation of gelatin, and of the same concentration. The greatest depression of p_H due to the addition of electrolytes was found at p_H 8.03 (Table III), 0.005 *M* calcium chloride lowering the p_H by 0.46, whilst at p_H 5.90 (Table I) the observed depression is only 0.25, and at p_H 9.21 0.03; the corresponding depressions observed in the presence of 0.01 *M* salt are respectively 0.54, 0.30 and 0.05. The solution remained clear at p_H 5.90 and 8.03, whilst at p_H 9.21 opalescence was observed on the addition of calcium chloride. Substantially similar results were obtained with magnesium chloride in place of calcium chloride.

Table III. *Influence of original p_H .*

Concentration of CaCl_2 <i>M</i>	p_H	Lowering of p_H
0.0	8.03	0.0
0.005	7.57	0.46
0.010	7.49	0.54
0.0	7.44	0.0
0.0025	7.23	0.21
0.0	9.21	0.0
0.005	9.18	0.03
0.010	9.16	0.05
0.025	9.01	0.25

II. Ovalbumin.

These experiments were performed on 2 % aqueous solutions of ovalbumin which were filtered overnight through filter-paper. The effect of adding calcium chloride or magnesium sulphate to such solutions is given in Table IV; the curve connecting variation in p_H with concentration of electrolyte is also of the adsorption isotherm type.

Table IV. *Ovalbumin solutions.*

Salt concentration <i>M</i>	p_H	Depression of p_H
CaCl_2 0.0	8.58	0.0
" 0.005	8.42	0.16
" 0.010	8.37	0.21
" 0.020	8.32	0.26
" 0.025	8.31	0.27
MgSO_4 0.010	8.47	0.11

III. Peptone.

The action of various concentrations of calcium and magnesium chlorides, magnesium and sodium sulphates, and sodium chloride on 3 % peptone solutions at p_H 4.8 and 5.7–6.0, was examined. At the isoelectric point for peptone (p_H 4.8) it was found that electrolytes possessing a bivalent anion (sodium sulphate) or cation (magnesium or calcium chloride), or both together (magnesium sulphate) produced only a negligible depression of p_H (Table V).

Table V. *Peptone*.

Concentration <i>M</i>	Depression of p_H			
	CaCl_2	MgCl_2	MgSO_4	Na_2SO_4
Original $p_H = 4.78-4.80$:				
0.005	0.05	0.06	0.05	0.05
0.010	0.07	0.09	0.07	0.07
0.025	0.08	0.12	0.09	—
Original $p_H = 5.8-6.0$:				
0.010	0.23	—	0.17	—
0.025	0.42	0.38	0.38	—

Solutions brought to p_H 5.7–6.0 by the addition of sodium hydroxide exhibit, on the other hand, a marked depression of p_H on the addition of bivalent ions. The magnitude of the change in p_H is a function of the initial p_H of the solution.

IV. *Glycine*.

The results obtained (Table VI) are identical with those obtained by earlier workers.

Table VI. *Glycine*.

Concentration of CaCl_2 <i>M</i>	p_H of solution	Lowering of p_H
0	6.78	—
0.025	6.71	0.05
0.050	6.66	0.12
0.100	6.64	0.14

DISCUSSION.

The results of the above experiments on the whole confirm those of earlier workers in this field, who found that the addition of salts depresses the p_H of solutions of amphoteric electrolytes, should this be above the isoelectric point. This phenomenon might be interpreted as being due to change in the activity factor, f_a , of the salts already present, according to the equation:

$$C_H = K [\text{acid}]/f_a [\text{salt of acid}].$$

This explanation does not, however, embrace the phenomena observed in their entirety. Thus, if we accept the existence of zwitterions, the dissociation not only of the carboxyl groups, but also that of part of the amino-groups has to be taken into consideration, when the action of added salts is the resultant of their action on both the acidic and the basic groups of the ampholyte. Next, apart from the activity factor, f_a , other factors, arising from the degree of dissociation of the salts added, may come into play, particularly in the case of salts giving rise to multivalent cations. Thirdly, at certain p_H values the salt may, as a result of hydrolytic dissociation, give rise to hydroxides possessing a smaller f_a than that of the base added to render the solution alkaline. Finally, the solubility of the salts formed with the ampholyte should

be taken into consideration; thus at certain p_H values the addition of calcium or magnesium chloride to ovalbumin solutions gives rise to turbidity.

We shall in a future communication examine more closely the factors capable of reducing the p_H of ampholyte solutions on addition of electrolytes; in this paper we desire to draw attention to the following facts.

The results obtained by adding different concentrations of electrolytes (0.0025–0.025 M) are very different from those observed in solutions of crystalloids. Michaelis and Krüger's [1921] experiments on the effect of salts on the p_H of solutions of phosphoric acid, glycine, *etc.*, have shown clearly that, within the limits of concentration of salt studied, these changes are quite inconsiderable and are proportional to the concentration of salt added. Our experiments show on the other hand that salts giving rise to bivalent cations or anions provoke, within certain p_H limits, considerable change in p_H , which is not proportional to the concentration of salt added. Thus, using 1 % gelatin solution at an initial p_H 5.9, the addition of calcium chloride to a concentration of 0.0025 M lowers the p_H by 0.21, the addition of a second portion of salt to 0.005 M lowers the p_H by only 0.04, whilst on addition of a third portion the difference is barely 0.025. Substantially the same results are obtained using magnesium chloride (first portion 0.12, second 0.08, third 0.03) or sulphate or sodium sulphate. This phenomenon is least marked with potassium chloride, in which case a concentration of 0.1 M , *i.e.* 40 times as great as for calcium chloride, has to be employed in order to achieve the same effect.

It will readily be seen from the above that the results obtained are quite different from those found for glycine, addition to which of calcium chloride to 0.0025 M reduces the p_H by only 0.02.

The reason for the different behaviour of univalent and bivalent ions may lie in the greater effect of the latter on the activity factor of the ampholyte, as follows from Eucken's [1930] formula:

$$\log f_a = -0.354 n_i^2 \sqrt{\Gamma},$$

where $\Gamma = \sum c_i n_i^2$, c_i is the ionic concentration, and n_i is the charge on the ions; the term n_i occurs twice.

A second reason for this difference may be differential aggregation of ions on the surface of the colloid, as a result of adsorption. The researches of Freundlich and of his collaborators led him to the conclusion that, even assuming equal adsorption of uni-, bi- and ter-valent ions, the effect will be the greater the higher the valency of the ions. It appears to us that the fact that the valency factor occurs twice in the formula expressing the activity factor can only partially explain the difference in the effect of varying the concentration of potassium chloride on the one hand and of calcium and magnesium chlorides and of sodium and magnesium sulphates on the other.

It is our opinion that the difference in the action of salts on the p_H of colloidal solutions of gelatin, ovalbumin, and peptone, in comparison with

their action in the case of crystalloids, is a consequence of the sorption of ions on the surfaces of the colloidal particles. This view is supported by the shape of the curves obtained, which resemble adsorption isotherms. The ions undergoing sorption are concentrated in the hydrosphere of the colloidal particles; since it is in this region that compounds responsible for the p_H of the solution, such as albuminates, are present, it would follow that the action of added salts should be greater in heterogeneous than in homogeneous systems. Should it be desired in such cases to determine $\log f_a$ and Γ , it would be necessary to substitute for C_i , the total ionic concentration per unit volume, not the concentration calculated for unit volume of the system as a whole, but for unit volume of the hydrosphere of the colloidal particles.

The differential actions of various electrolytes would be a consequence of the different valencies of their ions, and of differences in the extent to which they undergo sorption. Thus univalent ions are possibly only very feebly sorbed, and for this reason the curves obtained are of a similar type to those for solutions of crystalloids, whilst bivalent ions would be more powerfully sorbed. This conception is supported by the results obtained by Białyśzewicz [1928] and by his collaborator Zawadzki [1929], who have shown that the percentage of the ions of various electrolytes present in the dispersed phase of the ova of various animals varies greatly according to the valencies of the ions in question. In view however of the circumstance that univalent ions were in every case present in excess in the systems studied by these authors, the above hypothesis requires further experimental confirmation.

If we compare the actions of different salts giving rise to bivalent cations, it will be seen that the action of calcium chloride is very similar to that of magnesium chloride, as is evidenced both by the general similarity of the curves obtained and by the absolute values for reduction of p_H brought about by the presence of equimolar concentrations of both salts. The curves obtained from experiments in which bivalent cations are added to solutions of gelatin, ovalbumin, and peptone are not uniform for each colloid. Thus in the case of albumin a rapid decline in p_H , amounting to 0.21, is observed in concentrations of calcium chloride up to 0.01 M , whilst further addition of this salt up to 0.025 M lowers the p_H by barely 0.03. Gelatin solutions at first exhibit a rise in p_H of 0.30 in 0.005–0.01 M calcium chloride followed by a diminution in p_H amounting in 0.01–0.025 M solutions to about 0.13. The least characteristic curves, and the least resembling adsorption isotherms, are those obtained using peptone solutions, in which case the rapid decline in p_H persists up to 0.02–0.025 M calcium chloride. These differences on the whole support our view as to the fundamental difference in the action of ions on solutions of colloids and of crystalloids, although in all probability they are due partly to differences in the number of buffer groups and in those combining with ions on the surface of the colloidal particles present.

Those experiments performed on systems the p_H of which had been altered by the addition of alkali show that in the vicinity of the isoelectric point the

action of ions is least; as the p_H of the system increases the effect obtained is greater, until at a certain value a maximum effect is observed, after which it again declines. This phenomenon was observed with both gelatin and peptone solutions.

It is of interest that in systems the p_H of which is above the isoelectric point, and in which the colloidal particles should be exceptionally sensitive to differences in the valency of the ions added, salts giving rise to a univalent cation and a bivalent anion, *e.g.* sodium sulphate, have a considerably greater action than have univalent salts, such as sodium or potassium chloride.

The two following biologically important conclusions may be drawn from the above-described experiments. The first follows from the considerable reduction of p_H brought about by the presence in small concentrations of calcium and magnesium ions. The greatest effects are observed for concentrations within the limits 0.005–0.01 *M*. Since a 0.005 *M* solution of calcium chloride is equivalent to 55 mg./100 cc. CaCl_2 , or 19.5 mg./100 cc. Ca, and the calcium content of blood is normally about 10–12 mg./100 cc., it is apparent that small variations from the normal value may exert a certain influence on the p_H of the fluids of the organism. These variations in p_H would depend not on the total calcium content, but on the concentration of this ion in the hydrosphere of the colloidal particles of protein.

The action of calcium ions is, as appeared in the course of further experiments, to a large extent dependent on the presence of sodium and potassium ions, which greatly diminish the magnitude of the effect obtained. Thus 0.01 *M* calcium chloride reduces the p_H of a 1 % gelatin solution at p_H 5.9 by 0.31, whilst in the presence of 0.150 *M* potassium chloride the change is only 0.09.

Our second conclusion concerns enzymic reactions, the velocity of which is a function of the p_H of the medium. It follows from Myrbäck's [1926] work on ptyalin that the optimum reaction for this enzyme is at p_H 6.96, whilst at p_H 5.4 and 8.3 the velocity of reaction is only 50 % of that at p_H 6.96. If now the p_H is reduced from 8.3 to 7.8, a difference of 0.5, the observed hydrolysis will rise from 45 to 70 % of that at optimum p_H , *i.e.* the velocity of reaction will be increased by 56 %. The same reduction of p_H below 6.96 will lead to a corresponding inhibition of reaction. The possibility therefore arises that the velocity of enzymic reactions may to a certain extent be regulated by the presence of small concentrations of multivalent ions in systems containing protein.

Whether, under biological conditions, in the presence of high concentrations of univalent ions, this phenomenon plays any important part in the regulation of the velocity of enzymic reactions is questionable. Experiments are however in progress, with a view to the verification of this postulate, and to the determination of its practical importance in vital processes.

SUMMARY.

1. The addition of various mineral salts changes the p_H of solutions of gelatin, ovalbumin and peptone.
2. The depression of p_H is at a maximum on the alkaline side of the isoelectric point.
3. The action of salts is a function not only of their concentration but also of the valency of their ions.
4. The effect produced by unit concentration of salts is greater at very low than at higher concentrations.

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