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Ion Series and the Physical Properties of Proteins. III

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ION SERIES AND THE PHYSICAL PROPERTIES OF PROTEINS.

III. THE ACTION OF SALTS IN LOW CONCENTRATION.

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I. The Difference in the Effect of Acids, Alkalies, and Salts on Proteins.

The data concerning electrolytic dissociation and the behavior of electrolytes in general suggest that it is well to discriminate between solutions of electrolytes in low and in high concentrations. While no sharp line of demarcation can be drawn it will suffice for the problem we are discussing to designate a concentration of electrolytes below $M/16$ as low and those near the solubility limit of one of the components as high. In this paper we intend to deal with solutions of low concentrations; *i.e.*, $M/16$ or less.

It has been noticed by a number of authors that the influence of neutral salts on the physical properties of proteins differs from that of acids and bases; and various attempts have been made to find an expression for this difference. Pauli¹ states that while acids and alkalies form salts with proteins, neutral salts form "adsorption compounds" with "electrically neutral," *i.e.* non-ionized, protein molecules, both ions of the salt being simultaneously adsorbed by the protein molecule. This idea is no longer tenable for salt solutions of low concentration since the writer has shown through his experiments with powdered gelatin that only one (or practically only one) of the two ions of a neutral salt can combine at one time with a protein. At the isoelectric point, *i.e.* at pH 4.7, gelatin can combine with neither ion of a neutral salt; at a pH > 4.7 only the metal ion of the neutral salt can combine with the gelatin, forming metal gelatinate;

¹ Pauli, W., *Fortschr. naturwiss. Forschung*, 1912, iv, 223.

at a pH < 4.7 only the anion of the neutral salt is capable of combining with the protein, forming gelatin-acid salts.²

Lillie has made the statement that while acids and alkalies increase, salts depress the osmotic pressure of gelatin.³ This statement, while it was the expression of facts actually observed by Lillie, is no longer tenable owing to the fact that the influence of the hydrogen ion concentration of the gelatin solution was not taken into consideration. If we add acid to a gelatin-acid solution of a pH of 3.0 or below, the effect is practically the same as when we add a neutral salt, namely a diminution of the osmotic pressure of the solution; and when we add alkali, *e.g.* KOH, to a solution of a metal gelatinate of pH 11.0 or above, the effect is also a similar depression of the osmotic pressure as that caused by the addition of KCl. We also get a depression when we add some acid to a solution of metal gelatinate or when we add some alkali to gelatin-acid salts; since in both cases the gelatin is brought nearer to the isoelectric point.

It is also incorrect to speak of an antagonism between the effects of acids and salts, since the facts mentioned show that there is also an antagonism between little and much acid; thus if the pH of a gelatin-acid salt is 3.0 a further addition of acid depresses the osmotic pressure or viscosity. The question then arises, what is the correct expression of the facts in the case?

An analogy with another field of phenomena may be of service. The writer has recently published a series of articles on the influence of electrolytes on the rate of diffusion of water through collodion membranes, which have shown that water diffuses through such a membrane as if the particles of water were positively charged. When pure water is separated from a solution of an electrolyte of not too high a concentration, the positively charged particles of water diffuse through the collodion membrane into the solution as if they were attracted by the anion and repelled by the cation with a force increasing with the valency of the ion. In this case the oppositely charged ions of an electrolyte influence the rate of diffusion of water through the membrane in an opposite sense.

² Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 237; *Science*, 1920, lii, 449.

³ Lillie, R. S., *Am. J. Physiol.*, 1907-08, xx, 127.

The second fact which was brought out was that the relative strength of the apparent attractive and repelling action of the oppositely charged ions of an electrolyte upon the electrically charged particles of water varies with the concentration of the electrolyte in the solution. In the lower concentrations of the electrolyte the attractive action of the anion upon the positively charged particles of water increases more rapidly with increasing concentration of the salt than the repelling action of the cation; while when a certain concentration is reached the repelling action of the cation upon the positively charged particles of water increases more rapidly with a further increase in the concentration of the salt than the action of the anion.⁴

These facts offer an analogy which is helpful in understanding the difference in the action of acids and alkalies on the one hand, and of neutral salts on the other upon the physical properties of proteins.

When acids or alkalies are added to isoelectric gelatin both ions of the acid or alkali influence the physical properties of proteins but in an opposite direction. When we add acid to isoelectric protein the hydrogen ions increase, the anions depress the osmotic pressure and viscosity of the protein solution (and this depressing action increases with the valency of the anion of the acid). As long as little acid is added to isoelectric protein the augmenting action of the hydrogen ion on these properties increases more rapidly with increasing concentration of the acid than the depressing action of the anion; while when the pH of the solution falls below 3.3 or 3.0 the reverse is the case. This causes the drop in the curves for osmotic pressure, viscosity, and swelling below a pH of 3.0.

When we add alkali to isoelectric protein the OH ions (or the diminution of the concentration of hydrogen ions) increase the osmotic pressure, viscosity, etc., of the solution of metal proteinate while the cation of the alkali depresses these properties with a force increasing with the valency of the cation. In the lowest concentrations of the alkali added the augmenting action of the OH ion on the physical properties of the metal proteinate increases more rapidly with the concentration than the depressing effects of the cation of the alkali; while in higher concentrations, *i.e.* as soon as the pH becomes about 10.0 or 11.0, the reverse is the case.⁵

⁴ Loeb, J., *J. Gen. Physiol.*, 1919-20, ii, 173.

⁵ Loeb, J., *J. Gen. Physiol.*, 1920-21, iii, 85, 247.

When, however, neutral salts are added to protein solutions we no longer notice an opposite effect of the oppositely charged ions. When neutral salts are added to isoelectric gelatin no effect is noticed as long as the concentration of salt does not reach the value required for precipitation. When neutral salt is added to a protein solution on either side of its isoelectric point only a depressing action of that ion which has the opposite sign of charge as the protein ion is observed. No augmenting action of the ion with the same sign of charge as the protein is noticeable. Thus if we add CaCl_2 or Na_2SO_4 to a solution of gelatin chloride or gelatin nitrate we observe only a depressing effect of the Cl or SO_4 ion but no augmenting effect of the Ca or Na ion; while when we add these salts to a solution of a metal gelatin we observe only a depressing effect of the Ca or Na ion but no augmenting effect of the anion. We shall first show that this is the correct expression for the difference in the action of acids and bases on the viscosity of proteins on the one hand and of neutral salts on the other.

A 2 per cent solution of isoelectric gelatin is prepared and brought to a pH of 4.0. The solution is made 1 per cent in regard to the originally isoelectric gelatin by adding to 50 cc. of the 2 per cent solution either 50 cc. of H_2O or of a salt solution, *e.g.* NaCl , of different molecular concentration, from $M/8,192$ to 1 M , taking care that the hydrogen ion concentration remains the same. We determine the viscosity (*i.e.* the time of outflow through a viscometer) in the way described in a preceding publication and plot the reciprocal of time of outflow (counting that of water as 1) as ordinates over the pH as abscissæ (lower curve, Fig. 1). For the sake of brevity we beg leave to designate this value as specific viscosity.⁵ The addition of the NaCl causes only a drop, and no rise in the curve.

If, however, we mix the 2 per cent gelatin solution of pH 4.0 with various concentrations of HCl (upper curve, Fig. 1) instead of with NaCl we do not notice a drop but at first a rise followed by a drop when the concentration of the Cl ion is a little above $N/1,000$. In Fig. 1 the drop appears at a concentration of about $N/256$ HCl , but the reader must remember that on account of the fact that part of the acid combined with the gelatin the pH of the solution was about 3.0. In other words, while the addition of H ions increases the vis-

cosity of a solution of gelatin chloride of pH 4.0, the addition of Na ion does not have such an effect, but the Cl ion depresses the viscosity in both cases, no matter whether NaCl or HCl is added to the gelatin

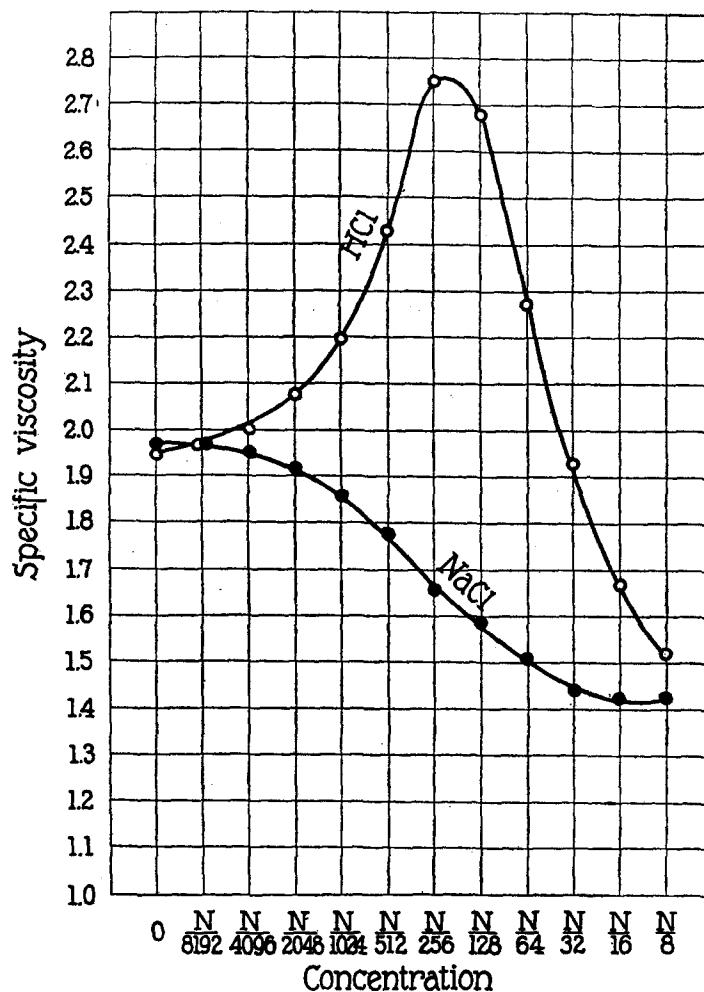


FIG. 1. Difference in the effect of different concentrations of NaCl and of HCl on the specific viscosity of a 1 per cent solution of gelatin chloride of pH 4.0. In the case of NaCl we observe only the depressing effect of the Cl ion; in the case of HCl we notice an augmenting effect of the H ion and a depressing effect of the Cl ion, the latter prevailing as soon as the concentration of acid added is $> N/256$.

solution; and the depressing action of the Cl ion increases with its concentration. Moreover, the increase of the viscosity by the H ions stops as soon as the pH of the solution reaches about 3.0.

When we repeat the same experiment with a gelatin solution of pH 3.0, the addition of NaCl immediately causes a drop also (Fig. 2)

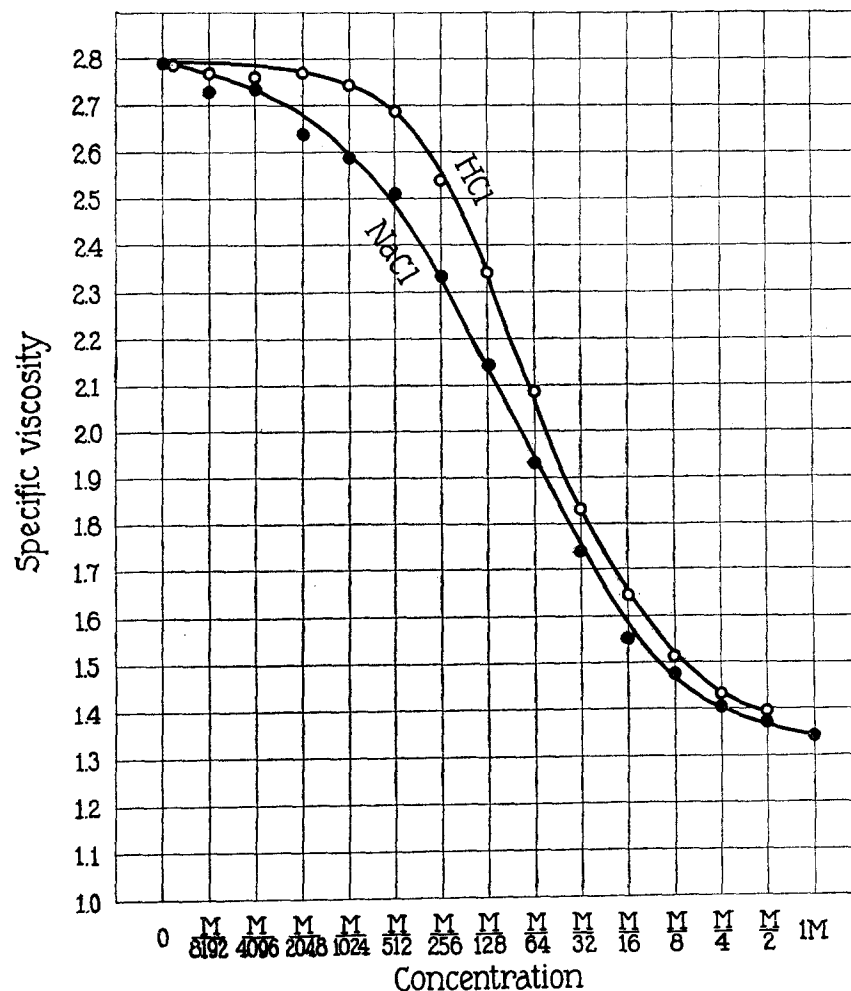


FIG. 2. The specific viscosity of 1 per cent solution of gelatin chloride of pH 3.0 is depressed almost equally by the Cl ion of HCl as of NaCl. The augmenting effect of the H ion in the case of HCl is no longer noticeable.

while the addition of HCl no longer causes a rise but the drop commences a little later than in the case of NaCl.

When, however, we make the same experiment with a gelatin solution of pH 2.5 (Fig. 3), we notice an immediate drop upon the

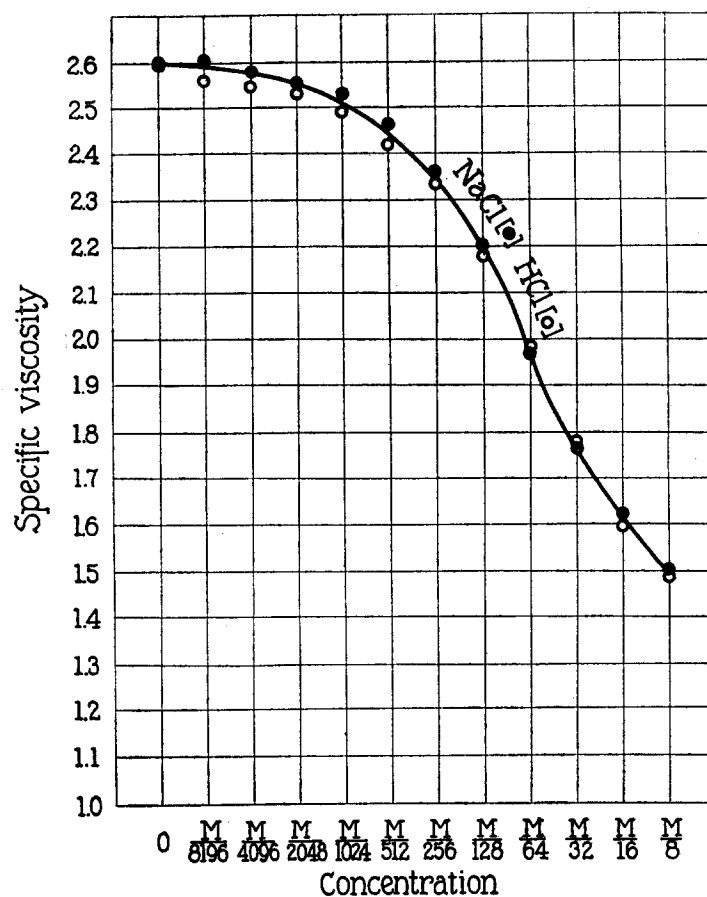


FIG. 3. When the gelatin solution has a pH of 2.5, HCl and NaCl depress the specific viscosity of the gelatin solution to the same degree.

addition of HCl as well as in the case of the addition of NaCl, and the curve for HCl coincides practically with that for NaCl, as our theory demands.

That the depression of the viscosity of gelatin chloride due to the presence of a salt is exclusively determined by the anion of the salt

and that the cation has no augmenting effect is shown in Fig. 4, where the influence of NaCl, CaCl₂, and LaCl₃ upon the viscosity of gelatin of pH 3.0 is represented. 50 cc. of a 2 per cent solution of gelatin chloride of pH 3.0 were added to 50 cc. of a solution of different concentrations of each salt as described, the pH being kept at 3.0. It is

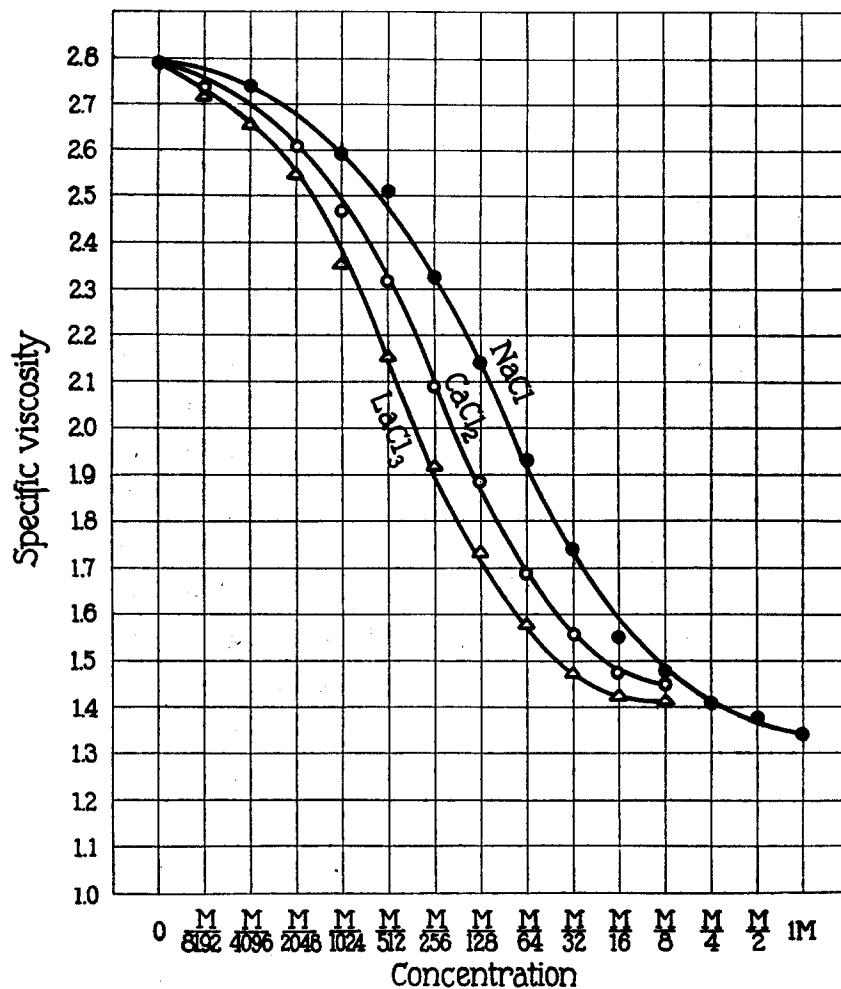


FIG. 4. The depressing effect of equal molecular concentrations of NaCl, CaCl₂, and LaCl₃ on the specific viscosity of 1 per cent gelatin chloride solution of pH 3.0 is in exact proportion to the concentration of the Cl ions in the solution; *i.e.*, as 1:2:3.

obvious from Fig. 4 that the molecular concentrations of NaCl , CaCl_2 , and LaCl_3 , which depress the viscosity to the same level are approximately in the ratio of 3:2:1. Thus when the effect of NaCl and CaCl_2 is plotted over the same concentration of the Cl ions the curves for the salts become identical (Fig. 5), and the same would be practically

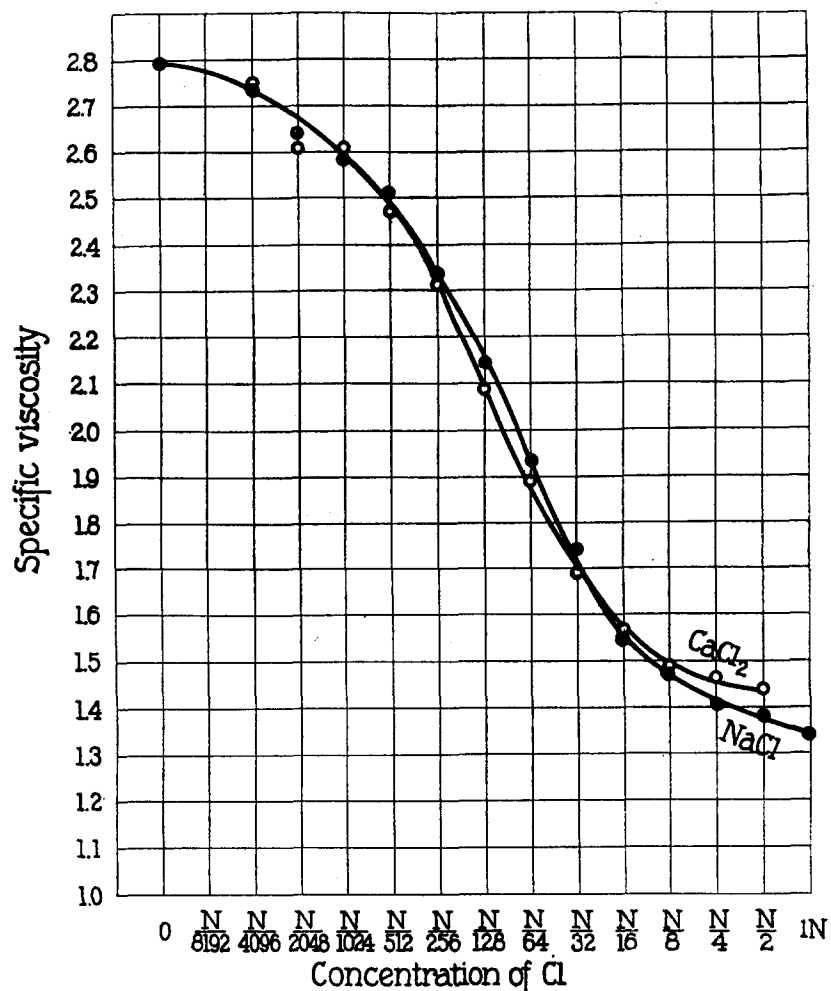


FIG. 5. Showing that NaCl and CaCl_2 have the same depressing effect on the viscosity of gelatin chloride of pH = 3.0 when the concentration of Cl ions is the same.

true for the LaCl_3 curve. From this it follows that the depressing effect of these three salts on gelatin chloride is practically exclusively a function of the concentration of the Cl ion, while no effect of the cation seems to be noticeable. In all these cases the pH of the gelatin solution was not altered by the addition of the salt.

When we prepare 1 per cent solutions of gelatin chloride of pH 3.0 in solutions of Na salts with the anion of a weaker acid, *e.g.* Na_2 oxalate, $\text{Na}_4\text{Fe}(\text{CN})_6$, the pH is increased and we are in danger of attributing erroneously a depressing effect to the anion which in reality is caused by the increase in pH. In Fig. 6 the effects of the addition of equal concentrations of NaCl, Na_2SO_4 , and $\text{Na}_4\text{Fe}(\text{CN})_6$ on gelatin chloride of pH = 3.0 are plotted. In the case of $\text{Na}_4\text{Fe}(\text{CN})_6$ only the lowest concentrations, from $\text{M}/8,192$ to $\text{M}/1,024$, could be used, since in these only did the pH of the protein solution remain = 3.0. Fig. 6 shows that the depressing effect of these salts increases rapidly with the valency of the anion. When the concentration of the salt was only $\text{M}/1,024$ a drop in the viscosity was already noticeable. This drop was small in the case of NaCl (from 2.8 to 2.6), was greater in the case of Na_2SO_4 (from 2.8 to 2.35), and considerably greater in the case of $\text{Na}_4\text{Fe}(\text{CN})_6$ (from 2.8 to 1.5). The objection might be raised that since Na_2SO_4 has twice as many cations as NaCl of the same concentration and $\text{Na}_4\text{Fe}(\text{CN})_6$ has four times as many cations, it was the difference in the concentration of the cations which caused the difference in the drop. This is refuted by the fact that Na_2SO_4 causes a drop to 1.8 at a concentration of $\text{M}/256$ while NaCl causes the same drop at a concentration of above $\text{M}/64$ which is about four times as high. If the concentration of the cation were responsible for the drop the two concentrations should be as 1:2. $\text{Na}_4\text{Fe}(\text{CN})_6$ causes the same drop of the viscosity to 1.8 at a concentration less than $\text{M}/1,024$. Hence the concentration of $\text{Na}_4\text{Fe}(\text{CN})_6$ required to cause the same diminution of the specific viscosity as that caused by $\text{M}/64$ NaCl is less than $\frac{1}{16}$ of the latter, while it should be at the least only $\frac{1}{4}$ if the cation were responsible for the drop. The depressing effect of the anion seems to increase almost in proportion to the square of its valency, as the Hardy-Whetham rule demands.

We have selected viscosity experiments, but experiments on osmotic pressure and on swelling lead to the same formulation of the difference in the effect of acids and salts.

What has been shown for the effect of acids on the physical properties of proteins can also be shown for the influence of alkalis. Thus the addition of KOH to Na gelatinate of pH 12.0 depressed the

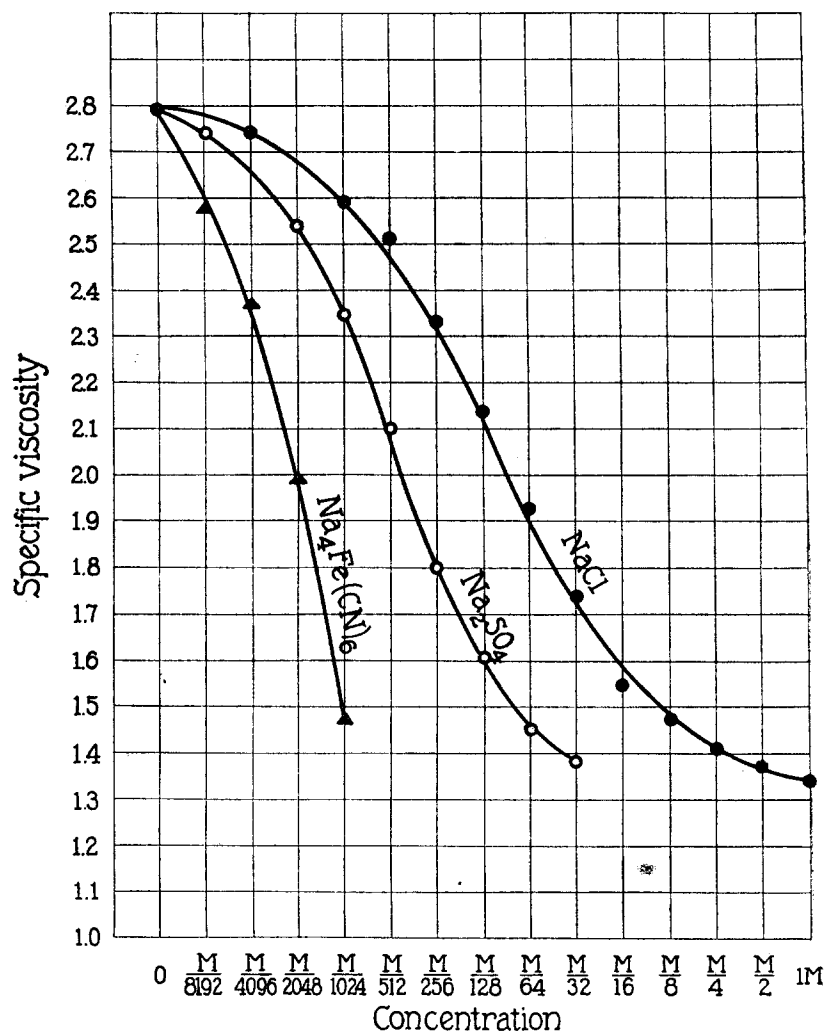


FIG. 6. The relative depressing effect of equal molecular concentrations of NaCl, Na₂SO₄, and Na₄Fe(CN)₆ on the specific viscosity of a gelatin chloride solution of pH 3.0 is approximately as 1:4:16.

viscosity in the same way as the addition of KCl (Fig. 7); while the addition of KOH to Na gelatinate of pH 4.8 to 8.0 increases the viscosity and the addition of KCl to Na gelatinate always depresses the viscosity. The depressing effect of salts on metal gelatinate is due to the cation of the electrolyte added, that of bivalent cations being greater than that of monovalent cations, while the valency of the anion has no effect.

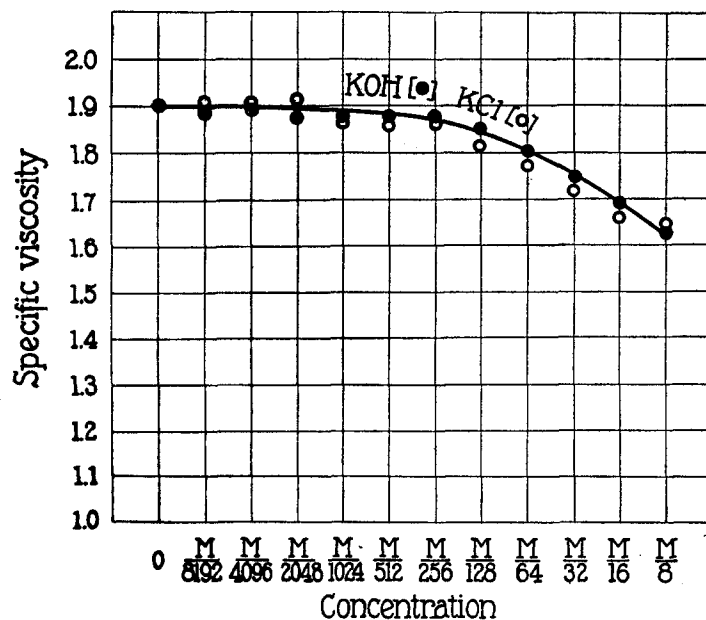


FIG. 7. The depressing effect of KOH and KCl on Na gelatinate of pH 12.0 is practically the same.

The addition of neutral salt to isoelectric gelatin leaves the viscosity and osmotic pressure of the solution practically unchanged.

The cause of this difference in the action of acids, alkalies, and salts is easy to understand on the basis of the purely chemical theory of the behavior of proteins. When we add acid to isoelectric gelatin we transform part of the latter into gelatin-acid salt and the proportion of gelatin-acid salt formed increases with the amount of the acid added. Near the isoelectric point the amount of gelatin-acid salt formed increases very rapidly with the addition of acid, but when

the pH approaches 3.0 the addition of the same amount of acid which near the isoelectric point caused a considerable change now causes only a slight change, while when the pH falls below 3.0 the depressing influence of the anion continues to increase with increasing concentration of the electrolyte.

II. Ion Series and the Action of Salts on Proteins.

We have shown in preceding papers that, as long as the concentration of the electrolyte is not too high, only the sign of the charge and the valency of an ion influence such physical properties as swelling, viscosity, solubility in alcohol, and osmotic pressure of proteins; while all the different ions of the same sign of charge and valency have either the same effect or if there is a difference in effect it is too small to be noticed with our present methods of work.⁵ This proof was furnished for the action of acids and alkalies on the physical properties of proteins just mentioned. We now wish to make the proof complete by considering also the action of salts. To do this we are compelled to compare the relative depressing action of low but equal concentrations of different salts upon the physical properties of a gelatin salt, for example gelatin chloride of a definite pH; *e.g.*, 3.0. As can be easily surmised the addition of a salt will in many cases alter the pH of the solution and this alteration will be larger in the case of certain salts, *e.g.* Na acetate, than in the case of others, *e.g.* NaCl. Unless we take into consideration these variations in the pH caused by the addition of salts we shall be in danger of erroneously ascribing the influence of a variation in the hydrogen ion concentration to an influence of the nature of the anion. The Hofmeister ion series are due to this error.

The method of our experiments was as follows. 50 cc. of a 2 per cent solution of originally isoelectric gelatin were brought to a pH of 3.0 by the addition of HCl. To this were added 50 cc. of H₂O or of a salt solution of different molecular concentration, and the viscosity of this mixture was measured using those precautions which were described in a preceding paper.

Fig. 8 gives the curves representing the depression of the specific viscosity of a gelatin chloride solution of pH 3.0 by different concentrations of salts with monovalent anion; namely, NaCl, NaH₂PO₄,

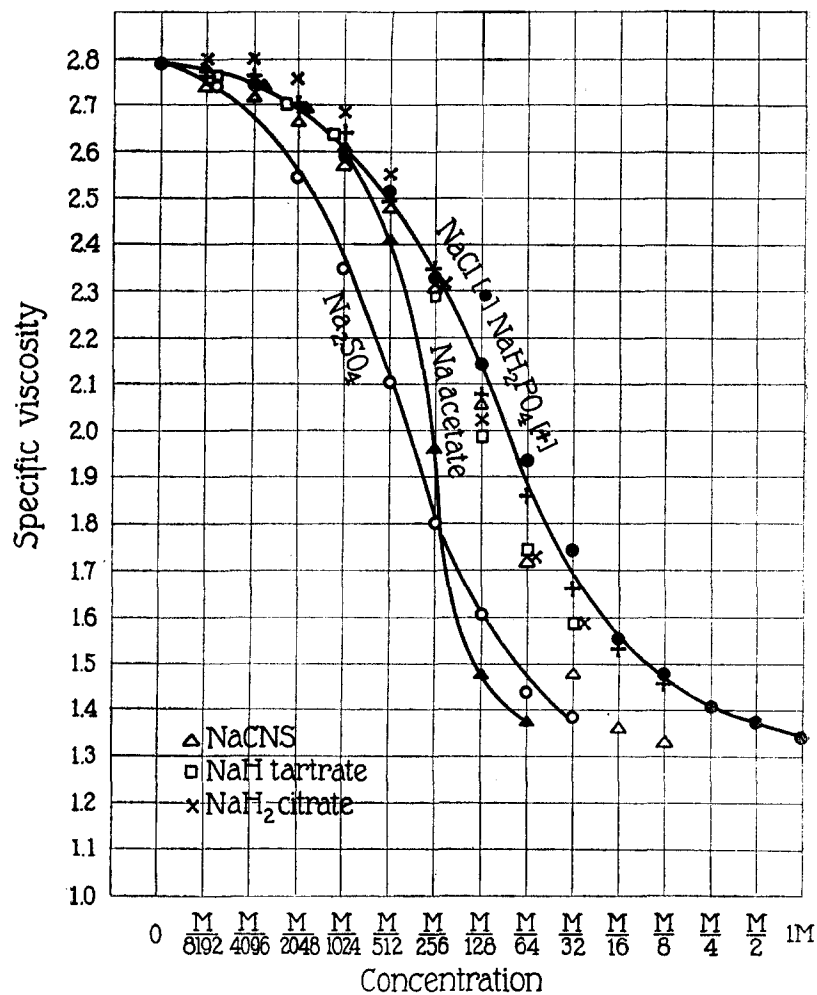


FIG. 8. The depressing effect of different salts with monovalent anion (NaCl, NaH₂PO₄, NaCNS, NaH tartrate, and NaH₂ citrate) on the specific viscosity of 1 per cent solution of gelatin chloride of pH 3.0. The effects of NaCl and NaH₂PO₄ are identical since the pH is not altered by the addition of these salts. The depression in the values for the specific viscosity is greater in the case of Na acetate than in the case of NaCl for the reason that the Na acetate raises the pH of the gelatin solution.

NaCNS, NaH tartrate, NaH₂ citrate, and Na acetate. The curve for Na₂SO₄ is added for comparison. The monosodium salts of weak dibasic and tribasic acids dissociate electrolytically into a Na ion and a monovalent anion, H₂PO₄, H tartrate, H₂ citrate, etc. All the salts mentioned in Fig. 8 are therefore salts with monovalent anion with the exception of Na₂SO₄. Our valency rule demands that the relative depressing effect of these salts (with the exception of Na₂SO₄) should be the same and that deviations from this rule should find their explanation in corresponding deviations of the pH due to the influence of certain of the salts. We will first consider this latter influence as given in Table I, which shows the results of the measurements of pH

TABLE I.

Changes in pH of 1 Per Cent Gelatin Chloride of pH = 3.0 upon Addition of Various Concentrations of Salts.

	Molecular concentrations of salts used.												
	0	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4
NaCl.....	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Na ₂ SO ₄	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.05	3.1	3.2	3.3	3.35
NaH ₂ PO ₄	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.1	3.2	3.3	3.4	3.45	3.5
NaCNS.....	3.0	3.0	3.0	3.0	3.0	3.0	3.1	3.2	3.3	3.6	3.9	4.2	4.4
NaH tartrate.....	3.0	3.0	3.0	3.0	3.0	3.0	3.1	3.3	3.45	3.5	3.55		
NaH ₂ citrate.....	3.0	3.0	3.0	3.0	3.0	3.1	3.2	3.4	3.6	3.7	3.75		
Na acetate.....	3.0	3.0	3.0	3.05	3.1	3.3	3.7	4.3	4.6				

in these different gelatin solutions after the addition of salts. The original gelatin chloride solution had a pH of about 3.0 and this was not altered by the addition of NaCl and only slightly by the addition of NaH₂PO₄ in concentrations below M/16. According to the valency rule the curves for the depressing effect of NaCl and NaH₂PO₄ should be almost identical and Fig. 8 shows that this is the case.

Table I shows that NaCNS, monosodium tartrate, and monosodium citrate raise the pH of the solution as soon as the concentration reaches M/128 or more. If we consider this effect, we must expect to find that the drop in the curves for NaCNS, monosodium citrate, and monosodium tartrate is a little steeper in concentrations of M/128 and

above than the curve for the depressing effect of NaCl. Fig. 8 shows that the curves for the depressing effect of these three salts are slightly lower than the curve for NaCl or NaH_2PO_4 . The greatest apparent deviation from the valency rule occurs in the curve for Na acetate whose depressing effect is of the order of that of Na_2SO_4 .

In the colloidal literature it is always stated that Na acetate acts like Na_2SO_4 and this is interpreted to mean that the acetate anion acts like the bivalent SO_4 anion and not like the monovalent Cl or NO_3 anion. Table I shows that Na acetate also depresses the hydrogen ion concentration more than NaCl or NaH_2PO_4 ; $\text{m}/64$ Na acetate brings the gelatin solution practically to the isoelectric point, and at the isoelectric point the viscosity of gelatin solution is a minimum. This lowering of the hydrogen ion concentration (and not the alleged influence of the acetate anion) explains the excessive depressing effect of Na acetate. That this interpretation is correct can be proved in the following way. We prepare 1 per cent solutions of gelatin acetate of pH 3.3 and gelatin chloride also of pH 3.3. The specific viscosity of these two solutions was practically the same (both were 1 per cent solutions in regard to originally isoelectric gelatin). The solution of gelatin acetate of pH 3.3 was made up in various concentrations of Na acetate of pH 3.3. The Na acetate solution of pH 3.3 was obtained by dissolving $\text{m}/16$ Na acetate in $1\frac{1}{2}$ M acetic acid and the various degrees of dilution of this $\text{m}/16$ Na acetate solution of pH 3.3 were brought about by dilution with pure acetic acid of pH 3.3. The non-dissociated molecules of acetic acid have no more depressing influence on the physical properties of proteins than have the molecules of any non-electrolyte. Fig. 9 gives the curve representing the depressing effect of Na acetate on gelatin acetate of pH 3.3 when the pH is kept constant.

The gelatin chloride solution of pH 3.3 was made up in different concentrations of NaCl and the depressing effect of NaCl on the viscosity of gelatin chloride is also plotted in Fig. 9. It is obvious from Fig. 9 that the depressing effect of Na acetate and NaCl are identical when the pH is kept constant and identical in both cases.

The same fact was confirmed in a somewhat different way. A 2 per cent solution of gelatin chloride of pH 3.0 was made up in various concentrations of Na acetate also of pH 3.0. In order to prepare Na

acetate solutions of pH 3.0 $M/4$ Na acetate was dissolved in $M/4$ HCl and the various dilutions required for the experiment were obtained by diluting the mixture of equal parts of $M/4$ HCl and $M/4$ Na acetate with $M/1,000$ HCl.

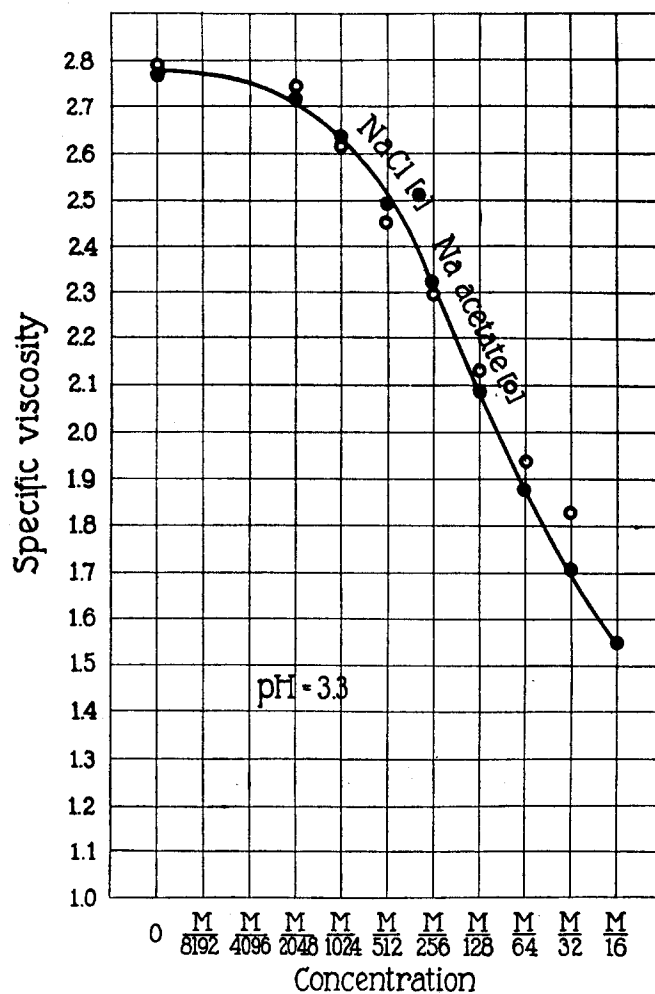


FIG. 9. When the pH is kept equal the depressing effect of equal concentrations of NaCl and Na acetate on the specific viscosity of a 1 per cent gelatin chloride or gelatin acetate solution of pH 3.3 is the same.

The 2 per cent gelatin chloride solution of pH 3.0 was diluted with 50 cc. of this mixture so that the resulting 1 per cent gelatin chloride solution of pH 3.0 contained various concentrations of Na acetate (or

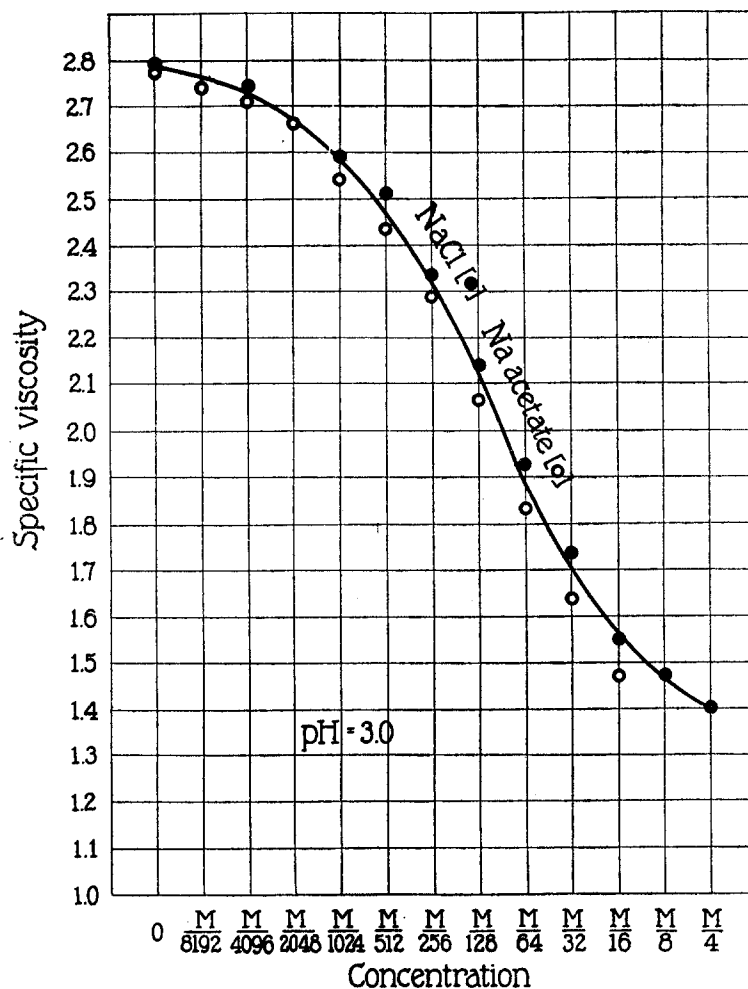


FIG. 10. See legend of Fig. 9, except that the pH of gelatin solution is 3.0.

more correctly of NaCl and Na acetate). The curve representing the depressing effect of this salt is given in Fig. 10, and is shown to be identical with the curve representing the depressing effect of the addition of NaCl to gelatin chloride of pH 3.0.

We can, therefore, state that sodium acetate has the same effect on the viscosity of gelatin chloride as the addition of any other salt with monovalent anion, and that the anomalous effect ascribed to the acetate anion in the colloidal literature is in reality due to the depression of the hydrogen ion concentration of the gelatin solution by the Na

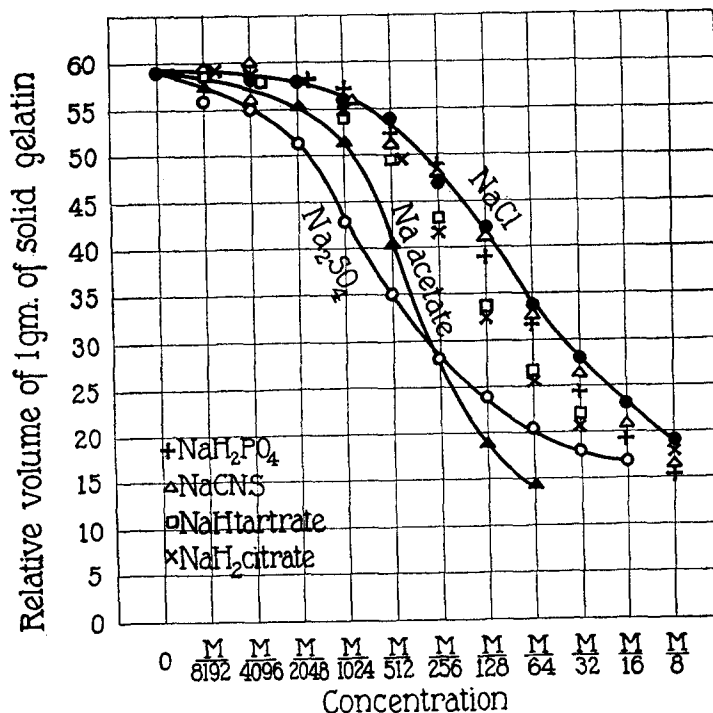


FIG. 11. Showing that the depressing effect of salts with monovalent anion on the swelling of gelatin chloride of pH 3.3 is similar to that on the specific viscosity. All salts with monovalent anion depress the swelling of gelatin chloride to the same extent, the seeming deviation from this rule being due to variation in the pH of the gelatin solution caused by buffer salts.

acetate which is a buffer salt. The failure to recognize the buffer character of salts, like the acetates, citrates, and tartrates, has led to the error of the Hofmeister ion series. In reality we find our valency rule confirmed whereby all salts with an anion of the same valency have the same relative depressing effect on the viscosity of a gelatin chloride solution if the pH of the solution is kept constant.

What has been demonstrated for the effect of these salts on the viscosity of gelatin solutions holds also for their effect on the swelling of gelatin. The same volumetric method for measuring the swelling effect was used which was described in the preceding paper. Fig. 11 gives the relative depressing effect of NaCl, NaH_2PO_4 , NaCNS, monosodium tartrate, monosodium citrate, and Na acetate on the swelling of gelatin chloride of pH 3.3 (the curve for Na_2SO_4 is added for comparison), and Table II gives the variation of the pH of the gelatin caused by the addition of these salts. Our theory demands that all these salts (except Na_2SO_4) should depress the swelling of gelatin chloride of pH 3.3 to the same amount, and that deviations from this

TABLE II.

Changes in pH of 1 Per Cent Gelatin Chloride of pH = 3.3 upon Addition of Various Concentrations of Salts.

	Molecular concentrations of salts used.											
	0	m/8,192	m/4,096	m/2,048	m/1,024	m/512	m/256	m/128	m/64	m/32	m/16	m/8
NaCl.....	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Na_2SO_4	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.35	3.4	3.5	3.6
NaH_2PO_4	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.4	3.5	3.6	3.7
NaCNS.....	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.35	3.4
NaH tartrate.....	3.3	3.3	3.3	3.3	3.3	3.4	3.5	3.5	3.6	3.7	3.7	3.7
NaH_2 citrate.....	3.3	3.3	3.3	3.3	3.3	3.4	3.5	3.6	3.8	3.85	3.9	3.9
Na acetate.....	3.3	3.3	3.3	3.4	3.45	3.5	3.8	4.3	4.8	5.2	5.4	5.5

rule must find their explanation in variations of pH caused by the addition of salt. Table II shows that the variations in pH are small for NaCl, NaCNS, and NaH_2PO_4 and hence the curves for the depressing effect of these three salts upon the swelling of gelatin are almost identical, as the valency rule demands. Monosodium citrate and tartrate have a greater depressing effect on the hydrogen ion concentration and Na acetate has a still greater depressing effect than these two salts. This explains the apparent deviation of the curves for these three salts from the valency rule.

Salts like disodium tartrate, disodium oxalate, and trisodium citrate offer an opportunity for an interesting test for our theory on account

of the difference between the electrolytic dissociation of weak dibasic or tribasic acids and the salts of the same acids. A weak dibasic

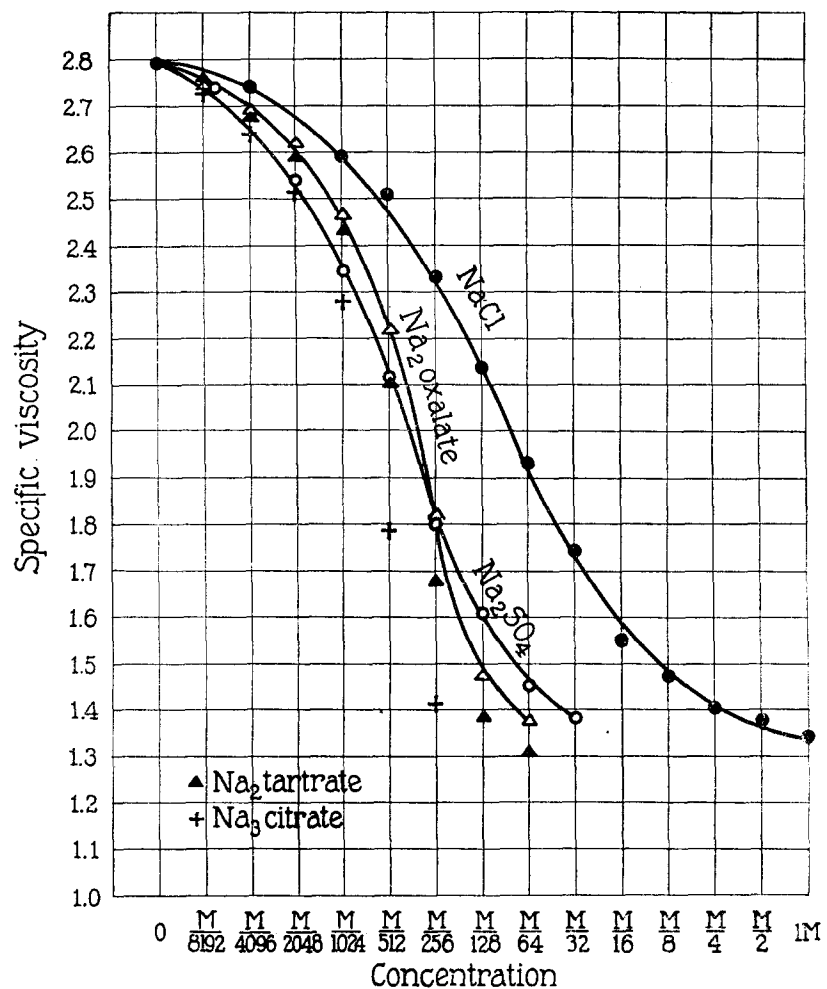


FIG. 12. Bivalent anions have an equally depressing effect as SO_4 on the specific viscosity of a 1 per cent gelatin chloride solution of pH 3.0.

acid like tartaric gives off one hydrogen ion easily but not both; sodium tartrate gives off both Na ions more readily. This is comprehensible on the idea that the oppositely charged ions in a molecule

are held together by electrostatic forces and that these forces are stronger in the case of a hydrogen ion which is free from electrons than in the case of a Na ion where the positive nucleus is separated by two shells of electrons from the valency electron of the oppositely charged ions by which it is held. For this reason the tartaric acid, or the oxalic acid, anion combines with proteins in the form of a monovalent acid tartrate ion while we should expect the anion of disodium tartrate or disodium oxalate to be a bivalent anion. Hence the tartrate and oxalate anions should act like the sulfate anion when disodium tartrate or disodium oxalate are added to a protein solution. This is confirmed, as Fig. 12 shows. The curves for the depressing effect of Na_2 oxalate and Na_2 tartrate practically coincide with the curve for

TABLE III.

Changes in pH of 1 Per Cent Gelatin Chloride of pH = 3.0 upon Addition of Various Concentrations of Salts.

	Molecular concentrations of salts used.												
	0	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4
NaCl.....	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Na_2SO_4	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.05	3.1	3.2	3.3	3.35
Na_2 oxalate.....	3.0	3.0	3.0	3.05	3.1	3.3	3.5	3.9	4.2	4.4			
Na_2 tartrate.....	3.0	3.0	3.0	3.1	3.2	3.3	3.7	4.0	4.35	4.7	4.8	5.0	
Na_3 citrate.....	3.0	3.0	3.05	3.2	3.3	3.8	4.4	5.2	5.7	6.1			

the depressing effect of Na_2SO_4 on the viscosity of gelatin solution, except in concentrations greater than M/256 where disodium tartrate and disodium oxalate cause also a depression of the hydrogen ion concentration (Table III) and where therefore the curves for these two salts drop more rapidly than the curve for Na_2SO_4 . Hence disodium oxalate and disodium tartrate act like Na_2SO_4 , while, as Fig. 8 shows, monosodium tartrate, monosodium citrate, and monosodium phosphate act like NaCl (if the necessary corrections for pH are made).

The osmotic pressure, viscosity, and swelling of Na gelatinate should be depressed by the cation of a salt and the more so the higher the valency of the cation. Fig. 13 shows that this is true for the swelling of Na gelatinate of pH about 9.3. The molecular concentration in

which the swelling is depressed by the same amount is about half as great for Na_2SO_4 as for NaCl (for molecular concentrations from $M/256$ to $M/32$), proving that the Na ion is responsible for the depression, while it is about eight times as high for NaCl as for CaCl_2 . The pH of the gelatin was practically the same in all solutions.

All these data confirm our valency rule, whereby ions of the same valency and the same sign of charge have, in the same concentration, the same depressing effect on osmotic pressure, swelling, solubility in

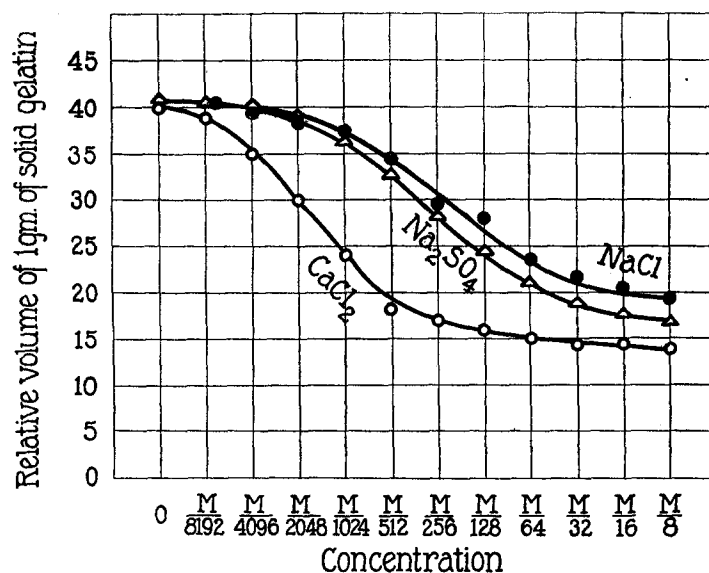


FIG. 13. The depressing effect of neutral salts on the swelling of Na gelatinate of pH about 9.3 is due to the cation of the salt, the depressing effect of NaCl being half as great as that of Na_2SO_4 of equal molecular concentration of Na_2SO_4 while that of CaCl_2 is considerably greater owing to the fact that Ca is bivalent.

alcohol, and viscosity of proteins; while the depressing effect increases rapidly with the valency. The Hofmeister ion series are due to the neglect of measuring the influence of the salts on the hydrogen ion concentration of the gelatin solutions. This neglect has given rise to the statement that salts, like sodium acetate, have the same depressing effect on the physical properties of proteins as the sulfates. A number of attempts to apply colloid chemistry to biology, pathology, and medicine are based on such errors.

SUMMARY.

1. Ions with the opposite sign of charge as that of a protein ion diminish the swelling, osmotic pressure, and viscosity of the protein. Ions with the same sign of charge as the protein ion (with the exception of H and OH ions) seem to have no effect on these properties as long as the concentrations of electrolytes used are not too high.

2. The relative depressing effect of different ions on the physical properties of proteins is a function only of the valency and sign of charge of the ion, ions of the same sign of charge and the same valency having practically the same depressing effect on gelatin solutions of the same pH while the depressing effect increases rapidly with an increase in the valency of the ion.

3. The Hofmeister series of ions are the result of an error due to the failure to notice the influence of the addition of a salt upon the hydrogen ion concentration of the protein solution. As a consequence of this failure, effects caused by a variation in the hydrogen ion concentration of the solution were erroneously attributed to differences in the nature of the ions of the salts used.

4. It is not safe to draw conclusions concerning specific effects of ions on the swelling, osmotic pressure, or viscosity of gelatin when the concentration of electrolytes in the solution exceeds $M/16$, since at that concentration the values of these properties are near the minimum characteristic of the isoelectric point.

Note.—The solutions of 1 per cent isoelectric gelatin were prepared by bringing 1 gm. of dry gelatin to the isoelectric point according to the method described in previous papers. It was found that in this process about 20 per cent of the gelatin was lost, so that the originally 1 per cent gelatin solution contained in reality only about 0.8 per cent isoelectric gelatin. This does not affect the contents of the paper or the conclusions since the gelatin concentration used was always the same.