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AMPHOTERIC COLLOIDS.

IV. THE INFLUENCE OF THE VALENCY OF CATIONS UPON THE PHYSICAL PROPERTIES OF GELATIN.

BY JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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I. INTRODUCTION.

In 1901 and 1902 the writer¹ published a series of investigations in which he showed that low concentrations of bivalent cations, practically without regard to their chemical nature, *e.g.* Mg, Ca, Sr, Ba, Zn, Co, Pb, etc., inhibited the toxic action of high concentrations of salts with univalent cations, upon the eggs of *Fundulus*. Trivalent cations like AlCl₃ and CrCl₃ seemed to act still more effectively than the bivalent ions though it was obvious that secondary influences (*e.g.* the high hydrogen ion concentration) restricted the limit of their antagonistic influence. This influence of valency the writer attributed to the effect of the electric charges of the ions upon the physical state of the colloidal material. He also found that polyvalent anions, *e.g.* SO₄, oxalate, citrate, etc., had under the same conditions no antagonistic effect. Since that time cases of an antagonistic action of polyvalent anions have come to light, but this phenomenon is not only less common but also less striking than the antagonistic effect of polyvalent cations.

It had been shown in the writer's preceding publications on gelatin² that the salts of gelatin with univalent cations possess a comparatively high osmotic pressure, a high viscosity, a high degree of swell-

¹ Loeb, J., *Arch. ges. Physiol.*, 1901-02, lxxxviii, 68; *Am. J. Physiol.*, 1901-02, vi, 411.

² Loeb, J., *J. Biol. Chem.*, 1918, xxxiii, 531; xxxiv, 77, 395, 489; xxxv, 497; *J. Gen. Physiol.*, 1918-19, i, 39, 237, 363.

ing, and a high alcohol number, while the gelatin salts with bivalent cations have a much lower osmotic pressure, lower viscosity, etc. It was also found that the addition of a certain amount of a salt with a bivalent or polyvalent cation depresses the effect of salts with monovalent cation on osmotic pressure, viscosity, swelling, etc., of the gelatin.

Pauli³ and Michaelis⁴ seem to ascribe the variations in the swelling and the viscosity of protein solutions to variations in the degree of ionization and to a "hydratation" they assume to be connected with the ionization of the protein. According to this view we should have to assume that sodium gelatinate has a higher osmotic pressure than calcium gelatinate of the same concentration, because the former is more strongly ionized. In an earlier paper the writer tentatively accepted Pauli's hypothesis, but a closer scrutiny of the literature showed that neither Pauli nor Michaelis measured the effect of electrolytes upon the conductivity of their protein solutions, probably on account of the fact that they did not remove the excess of electrolyte after it had acted on the protein. The writer's method of removing the excess of electrolytes after they have had time to react with the gelatin made measurements of conductivity possible, and these measurements in connection with measurements of osmotic pressure and of the quantity of metal in combination with the gelatin led to a very definite explanation of the influence of the valency of ions on the properties of gelatin. *With the same equivalent of metal in combination with a given mass of gelatin the maximal osmotic pressure of a 1 per cent solution of gelatin salts with univalent cation, e.g. Na gelatinate, is almost exactly three times as great as that of gelatin salts with a bivalent metal, e.g. Ca gelatinate, while the conductivities of the solutions of the two types of gelatin differ little or not at all.* This indicates that the gelatin salts with univalent metal have at the point of maximal osmotic pressure about three times as many particles in solution as the same mass of gelatin salts with bivalent metal, while the number of electrical charges is about the same in both cases. The identity of the conductivities of gelatin salts of the type of sodium gelatinate and calcium

³ Pauli, W., *Fortschr. naturwiss. Forschung*, 1912, iv, 245.

⁴ Michaelis, L., *Die Wasserstoffionenkonzentration*, Berlin, 1914.

gelatinate proves that the difference in the influence of the valency of the cation upon the physical properties of gelatin cannot be ascribed to a difference in the degree of ionization of the two types of salts, but is due to some other as yet unknown factor. It seems possible to explain all the phenomena on the basis of the tentative assumption that gelatin salts with a bivalent cation dissociate into cations and aggregates of four, or six, or eight gelatin ions, each individual gelatin ion of the aggregate retaining its original negative charge, while the anions of salts of sodium gelatinate consist of only one gelatin ion each. The quantitative data suggest that the number of gelatin anions contained in each aggregate is of a stoichiometrical order, being a simple multiple of the number representing the valency of the polyvalent metal ion.

II. Amount of Alkali Combining with Gelatin.

We used powdered Cooper's non-bleached gelatin which is impure, having a pH of about 7.0 and consisting to a large extent of calcium gelatinate. It is necessary to purify this gelatin before using it by bringing it to the isoelectric point $\text{pH} = 4.7$.⁵ This is done by putting 1 gm. of gelatin for $\frac{1}{2}$ hour into 100 cc. of 3 M/1024 HCl or M/128 acetic acid, then putting the gelatin on a filter, allowing the excess of solution to drain off, and washing the gelatin two or three times with 25 cc. of distilled water at 5°C. If we wish to transform the pure isoelectric gelatin into a metal gelatinate we treat it subsequently with the hydroxide of the metal—*e.g.* NaOH, $\text{Ca}(\text{OH})_2$, etc.—with which we wish the gelatin to combine.

We always used finely powdered gelatin rendered isoelectric in the manner described. When we intended to prepare sodium gelatinate we treated different doses of isoelectric gelatin of 1 gm. each with 75 or 100 cc. of a NaOH solution varying from M/4 to M/8192 NaOH, after-

⁵ Isoelectric gelatin does not react with neutral salts like NaCl but will react with NaOH, since in this latter case the pH is raised beyond that of the isoelectric point. Common Cooper's gelatin, with a $\text{pH} = 7.0$, consisting of gelatin salts, especially Ca gelatinate, will be influenced in the same way by a treatment with NaOH as with NaCl, since in both cases a replacement of Ca by Na will occur. This is supported by the writer's previously published papers.

wards washing away the excess of alkali. In each case sodium gelatin-ate was formed but the amount formed varied with the concentration of the alkali solution used; and the pH varied correspondingly. It was of importance to measure the amount of Na or Ca in combination with the gelatin, to make sure that we were dealing with phenomena of a stoichiometrical character; it was especially necessary to make sure whether or not Na and Ca combine with gelatin in equivalent proportions. The metal gelatin-ate used for this purpose was not only washed as indicated, but was also dialyzed over night through collodion bags against 400 cc. of distilled water.

Our former experiments⁶ allow us to measure the amount of metal contained in a given mass of gelatin when the pH is known. Metal gelatin-ates can only exist on the alkaline side from the isoelectric point of gelatin, this point being defined by a pH = 4.7. The Na or K or Ca in combination with the gelatin at each pH can be calculated in the following way. We determine the cc. of 0.01 N NaOH required to bring 25 cc. of 1 per cent isoelectric gelatin of different pH (lying between 4.7 and 7.0) to the point of neutrality (pH = 7.0). By deducting this value from the quantity required to bring 25 cc. of 1 per cent gelatin solution from the isoelectric point to pH = 7.0—which was always found to be about 4.5 cc. of 0.01 N NaOH⁷—we obtain the amount of 0.01 N Na or of $\frac{1}{2}$ Ba or $\frac{1}{2}$ Ca in combination with the 25 cc. of 1 per cent gelatin at any pH between 4.7 and 7.0. When pH is > 7.0 we ascertain the amount of 0.01 N HCl required to bring the 25 cc. of gelatin to pH = 7.0, and add this to the value 4.5. In Fig. 1 the abscissæ are the pH, the ordinates the cc. of 0.01 N Na, K, or $\frac{1}{2}$ Ba found in combination with 25 cc. of gelatin for each pH. The results of three different experiments with the three different alkalies named are plotted (Fig. 1), showing the degree of agreement of the results. The curves are exactly the same whether gelatin has been treated with KOH, NaOH, LiOH, Ba(OH)₂, or Ca(OH)₂. It is also obvious from the curves that we are dealing with a simple salt formation of

⁶ Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 363.

⁷ In our preceding paper we determined the cc. of 0.01 N NaOH required to bring 25 cc. of 1 per cent gelatin from the isoelectric point to the turning point of phenolphthalein (pH = 9.0) and found 5.5, which agrees with our present result where we neutralize to pH = 7.0 instead of to 9.0.

a stoichiometrical nature and that one Ba or Ca replaces two Na (or two Li, K, or NH_4). The maximum amount of salt formed is practically reached not far beyond $\text{pH} = 8.0$, which is so near the point of neutrality that practically no corrections for the values for titration and only slight corrections for the values for conductivity are required. Our conductivity curves are the corrected curves, *i.e.* from the measured values the conductivities of the pure alkali solutions of the same pH are deducted.

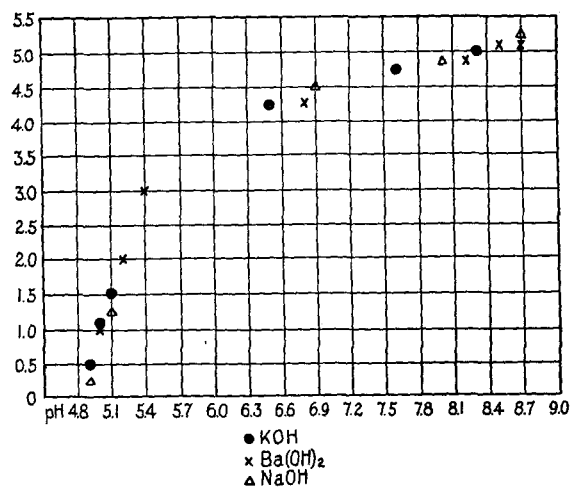
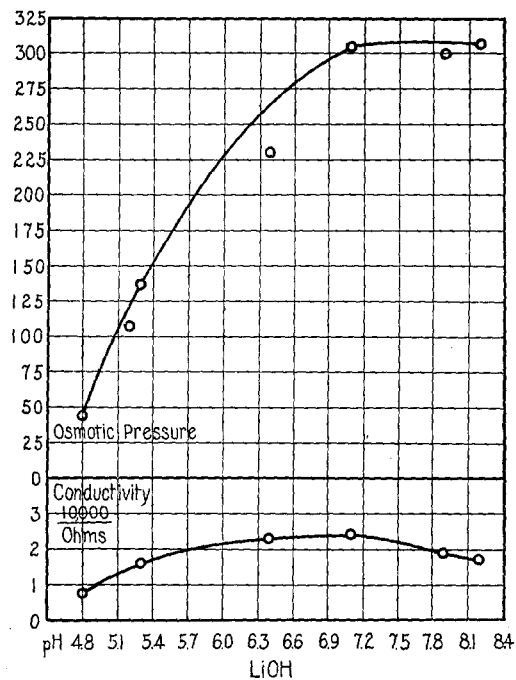


FIG. 1. Ordinates represent amount of Na, K, and $\frac{1}{2}$ Ba (expressed in cc. of 0.01 N Na) in combination with 25 cc. of 1 per cent gelatin solution, previously treated with NaOH, KOH, or $\text{Ba}(\text{OH})_2$ respectively and freed from the excess of salt by washing. Abscissæ represent pH of the solution. Curves in all three cases are identical, showing that one Ba replaces two Na or K.

III. Action of Different Alkalies on the Conductivity and Osmotic Pressure of Gelatin.

In order to obtain constant results we brought the powdered gelatin, as stated, to the isoelectric point by treating it for 30 minutes with $\text{m}/128$ acetic acid at 20°C . The gelatin was then put on a filter, the acid allowed to drain off, and then it was washed twice with 25 cc. of distilled water at about 5°C . Then such gelatin, while on the filter, was perfused three times with 25 cc. of a solution of NaOH or KOH,

etc., of a definite concentration, varying from $M/4$ to $M/8192$ at 15°C . During this perfusion the powdered gelatin was sufficiently stirred to allow intimate contact between all the gelatin particles and the alkali, and then after all the excess of alkali had been allowed to drain off the mass on the filter was washed once with 25 cc. of H_2O at 5° and



FIGS. 2 TO 7. Curves for osmotic pressure (in terms of mm. of a 1 per cent gelatin solution) and conductivity $\left(\frac{10,000}{\text{ohms}}\right)$ of a 1 per cent gelatin solution first rendered isoelectric and then treated with concentrations of an alkali, *e.g.* NaOH , varying from $M/8$ to $M/8192$ to cause a varying proportion of the gelatin to form a metal gelatin. Abscissæ represent pH of the solution after dialysis; ordinates of upper curve, osmotic pressure, and of lower curve, conductivity of the solution found at different pH. Curves for the osmotic pressures for gelatin salts with univalent ion, Li, Na, K, and NH_4 (Figs. 2 to 5) are alike and high, reaching a maximum of about 325 mm. Curves for the osmotic pressures of salts with bivalent metals, Ca and Ba, are very much lower than those for metal gelatinates with univalent metal, reaching a maximum of only 125 mm. Curves for conductivity are almost identical for both types of gelatin salts.

once with 25 cc. of H_2O at $20^\circ C$. Then the mass was made into a 1 per cent solution and put into collodion bags to determine the osmotic pressure. Each collodion bag was surrounded by a beaker containing 400 cc. of distilled water and the temperature was kept constant at $24^\circ C$. This allowed any excess of alkali left to diffuse out of the bag. The next day the osmotic pressure was measured, the pH of the gela-

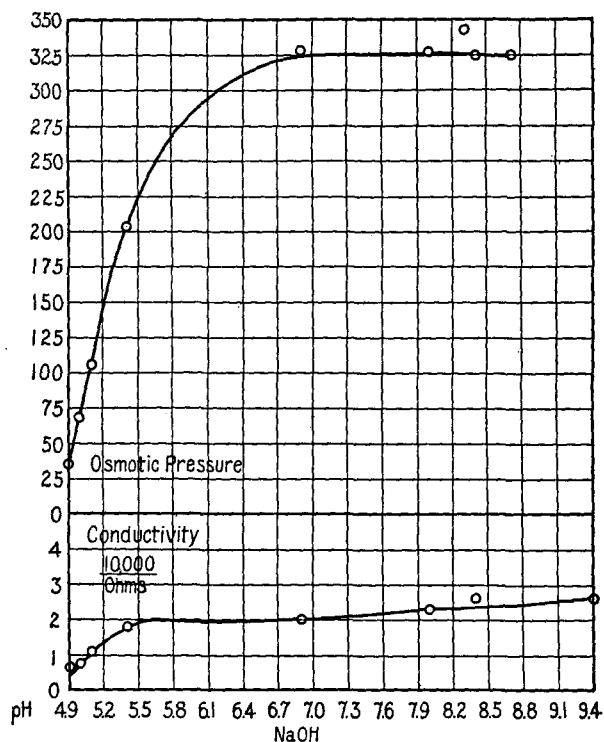


FIG. 3. See explanation under Fig. 2.

tin solution was determined, the solution titrated to determine the quantity of Na or K, etc., in combination with the gelatin, and the conductivity of the solution was measured.

The results of these measurements are contained in Figs. 2 to 7. The abscissæ are the pH, the ordinates the values for conductivity and osmotic pressure for these pH. The reader will notice that the curves representing the influence of Li, Na, K, and NH_4 on the osmotic

pressure (and the other physical properties) of gelatin are identical, if we use the pH as abscissæ. This result contradicts the statements current in colloid chemistry according to which these four cations have a different effect. The colloid chemists who make such statements have failed to measure the hydrogen ion concentration of their solutions. Our experiments show that the effects of the same cation on

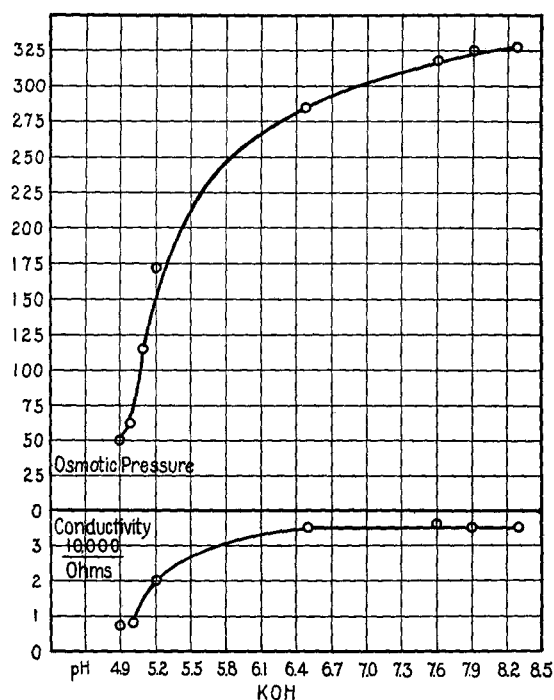


FIG. 4. See explanation under Fig. 2.

gelatin differ for different pH and hence we cannot be sure that apparent differences in the effect of two cations on a protein are the expression of differences in the structure of the two cations, unless we are certain that the pH is the same in both cases. The colloid chemists have, moreover, compared the effects of neutral salts of Li, Na, K, and NH_4 , in the presence of an excess of these salts, which introduces a second error.

It is obvious that the osmotic pressure in the curves for LiOH, NaOH, KOH, and NH₄OH (Figs. 2 to 5) reaches the same maximum (at pH between 7.0 and 8.0), namely that of a column of about 325 mm. of a 1 per cent gelatin solution; and that the curves for osmotic pressure of the Ca gelatinate and Ba gelatinate (Figs. 6 and 7) reach also an equally definite maximum of about 125 mm. osmotic pressure at about the same pH. In both cases we have probably to deduct

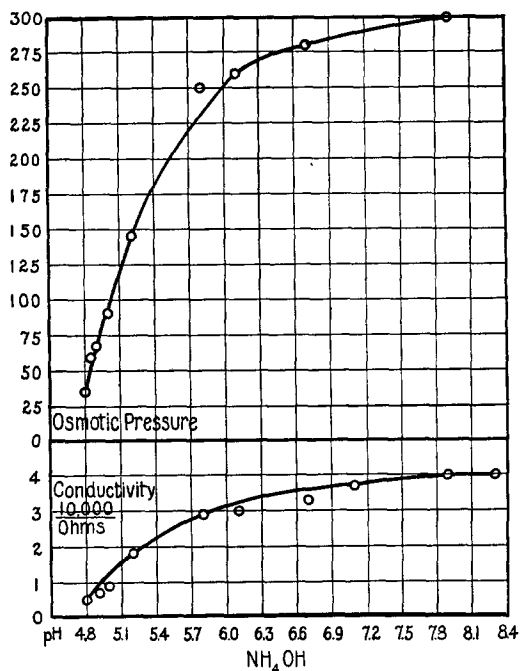


FIG. 5. See explanation under Fig. 2.

from these values about 25 mm.; namely, the osmotic pressure of a 1 per cent gelatin solution at the isoelectric point which includes the necessary capillary correction. This then leaves the following characteristic and constant values for the maximum osmotic pressure of the two types of metal gelatinates:

- Li, Na, K, NH₄ gelatinate.....300 mm. (uncorrected value 325 mm.)
- Ca and Ba gelatinate.....100 " (" " 125 ")

The constant character of this ratio of the two pressures of 1:3 (or 3:8 for the uncorrected value) for the two valencies, but regardless of the other qualities of the ions, betrays a stoichiometrical basis for the influence of valency. The experiments were repeated to guard against error, the results remaining the same.

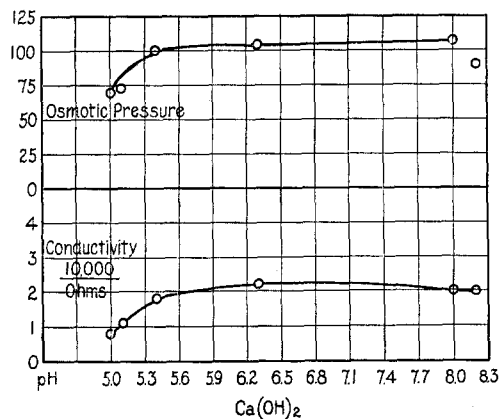


FIG. 6. See explanation under Fig. 2.

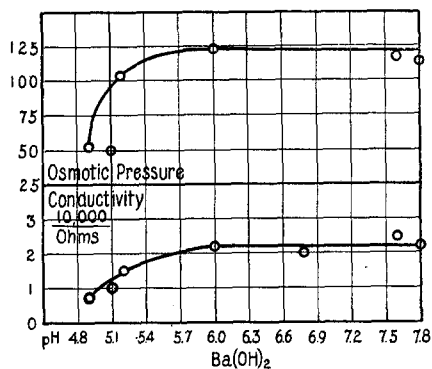


FIG. 7. See explanation under Fig. 2.

This means that in a 1 per cent solution of a metal gelatinate we have approximately three times as many particles in solution or suspension when the metal is univalent as when it is bivalent. Before we can draw any further conclusions we have to consider the relative conductivities of the same gelatin solutions.

A glance at the curves for conductivity shows that those for Na, Li, Ba, and Ca are almost identical, while the curves for K and NH₄ are a little higher than the others. These experiments were repeated and the same values were obtained. Fig. 8 shows that while the curves for conductivity of gelatin salts with univalent and bivalent metals (Na and Ba) are almost identical, the curves for the osmotic pressure of the two types of salts are very different.

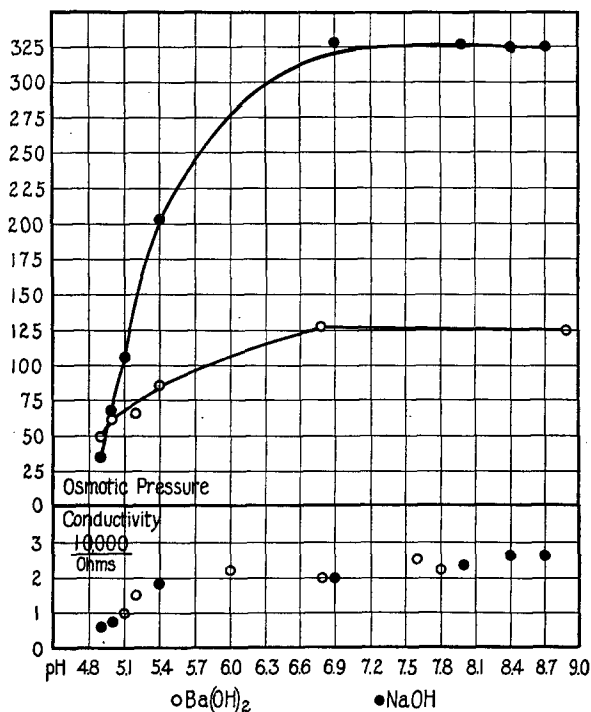


FIG. 8. Showing that while the curves for conductivity of sodium and barium gelatinate are practically identical, the curves for the osmotic pressures are very different.

There are two possible explanations for the fact that the ratio of conductivities of the two types of salts (Ca gelatinate and Na gelatinate) is 1:1 while the ratio of osmotic pressures is 1:3. The one explanation is that the degree of electrolytic dissociation of Ca gelatinate is so much smaller than that of Na gelatinate as to produce a

ratio of 1:3 particles in solution. This would account for the difference in the ratio of osmotic pressures but would leave unexplained the identity of conductivity of the two solutions. It would therefore be necessary to make a second assumption; namely, that the lower viscosity of a 1 per cent Ca gelatinate solution would raise the conductivity of a Ca gelatinate solution enough to compensate for the smaller degree of electrolytic dissociation.

In order to account for the ratio of 1:3 in osmotic pressure of the solutions of the two types of salts we have to assume that only about 20 to 25 per cent of the Ca gelatinate molecules are dissociated, while the dissociation of the sodium gelatinate is complete (five molecules of Ca gelatinate, one of which dissociates, would yield seven particles while the same amount of gelatin would form ten molecules of sodium gelatinate, yielding with complete dissociation twenty particles; this would result in a ratio of 7:20 for the relative number of particles in solution). The four electric charges of the one dissociated Ca gelatinate molecule would have to give the same conductivity as the twenty electric charges of the sodium gelatinate.

Our present knowledge speaks against such an influence of the viscosity of gelatin solutions upon conductivity. We prepared 1 per cent solutions of sodium, potassium, magnesium, and calcium gelatinate, of pH = 7.0, by putting 1 gm. of finely pulverized commercial Cooper's gelatin (probably mostly calcium gelatinate) for 1 hour at 20°C. into 100 cc. of $m/4$ NaCl or KCl, or $MgCl_2$ or $CaCl_2$, and allowed the excess of salt solution to drain off by putting the gelatin on a filter. We then washed the gelatin on each filter six times in succession with 25 cc. of H_2O , melted the gelatin by heating to about 50°C., and added enough water to make a 1 per cent gelatin solution. The solution was cooled to 24°C. and the time of outflow through a viscometer, as well as the conductivity of each solution, was measured immediately, at 24°C. (Table I). We found the usual typical difference in viscosity between Ca and Mg gelatinate on the one hand, and Na and K gelatinate on the other. It is well known that the viscosity of a gelatin solution prepared by melting will increase on standing, especially at a low temperature. The gelatin solutions were kept at about 2°C. for 2 hours and were then heated to 24°C., and their viscosity and conductivity were again measured. All the

viscosities had increased considerably and the viscosity of magnesium and calcium gelatinates was now as great as was originally that of the sodium gelatinates. Yet the conductivities were practically unaltered. The experiment was continued as indicated in Table I and enormous viscosities resulted, practically without any increase in the conductivities.

The reader will notice incidentally from the continuation of the experiment that upon heating to 50°C. and cooling to 24° the viscosity went practically back to its original level for the four different gela-

TABLE I.

Variation of Viscosity and Conductivity of Gelatin Solutions upon Standing.

Viscosity in Seconds of Outflow, Conductivity, $\frac{10,000}{\text{Ohms}}$.

All Measurements at 24°C.

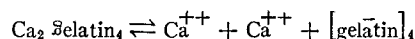
Treatment.	Na gelatinates.		K gelatinates.		Mg gelatinates.		Ca gelatinates.	
	Viscos- ity.	Conduc- tivity.	Viscos- ity.	Conduc- tivity.	Viscos- ity.	Conduc- tivity.	Viscos- ity.	Conduc- tivity.
	<i>sec.</i>	$\frac{10,000}{\text{ohms}}$	<i>sec.</i>	$\frac{10,000}{\text{ohms}}$	<i>sec.</i>	$\frac{10,000}{\text{ohms}}$	<i>sec.</i>	$\frac{10,000}{\text{ohms}}$
Immediately after melting...	99	2.31	102	3.07	88	2.10	86	2.12
After 1 hr. in refrigerator and heating to 24°C.....	135	2.22	143	2.94	138	2.06	130	2.12
After 18 hrs. in refrigerator and heating to 24°C.....	180	2.28	250	3.03	240	2.07	200	2.09
After being kept at 24° for 2 hrs.....	170	2.35	210	3.09	194	2.10	173	2.13
After heating to 50° and cooling to 24°.....	94.5	2.31	95.5	3.11	86	2.12	83.5	2.14

tin salts. In this case the influence of the "history" upon the colloidal solution is entirely reversible.

These experiments seem to exclude the assumption that the degree of electrolytic dissociation of calcium gelatinates is so much smaller than that of sodium gelatinates that it produces a ratio of 1:3 in the osmotic pressure of the two solutions; and that the difference in the degree of dissociation is compensated by the influence of viscosity upon conductivity in such a way as to make the conductivities of the solution of the two types of salts equal.

The second possible explanation is based on the assumption that the equality of conductivity is due to the fact that both types of solutions in equal concentration and for the same pH possess an approximately equal number of charges.

The identity of equivalents combining with gelatin demands that twice as many gelatin molecules must combine with one atom of Ca as with one atom of Na. For the sake of simplification we assume that one Na atom combines with one gelatin atom. This would mean that calcium gelatinate exists in the form of Ca gelatin_2 or $\text{Ca}_2 \text{ gelatin}_4$ or $\text{Ca}_3 \text{ gelatin}_6$, and sodium gelatinate in the form of Na gelatin. In this case all phenomena will find their explanation if we assume that in the dissociation of $\text{Ca}_2 \text{ gelatin}_4$ the four gelatin ions remain aggregated in one group with four negative charges



Such a dissociation would therefore yield three ions, one of which contains an aggregate of four negative gelatin ions. In order to obtain the same number of charges, four molecules of Na gelatin would be required, dissociating into four positive Na ions and four separate negative gelatin ions, making eight ions in all. This would demand a ratio of osmotic pressures for the two gelatin solutions of 3:8, which is slightly less than the ratio observed. The electrical charges would be the same for the two solutions and the conductivities would only show the difference due to differences in the ionic mobilities.

If the dissociating complex in the case of calcium gelatinate is $\text{Ca}_3 \text{ gelatin}_6$ resulting in the formation of three Ca ions and one aggregate gelatin_6 anion carrying six charges, the same number of charges would be carried by six molecules of sodium gelatinate dissociating into twelve ions. This would yield exactly the ratio of 1:3 for the osmotic pressure of solutions of calcium gelatinate and sodium gelatinate of the same concentration and conductivity.

If the aggregates consist of eight gelatin anions with four Ca ions the ratio of osmotic pressures would be 5:16 which is also approximately 1:3.

It is in reality only necessary to assume the existence of compounds of the form Ca gelatin_2 , the two anions of which form one aggregate of two gelatin anions, and to assume further that two, three,

or more such aggregates of two gelatin anions join to form larger aggregates of four, six, or eight gelatin anions, every one of which keeps its original charge. This would account for all the phenomena observed.

We are only able to estimate the relative difference in the mobilities of the cations. They are higher for K and NH_4 than for Na and Li, and we also find that the observed conductivities of K and NH_4 gelatinate in our experiments are higher than those of Na and Li gelatinate, possibly to the amount the difference in mobility of the ions named demands.

Bayliss in comparing the osmotic pressure and the electrolytic dissociation of solutions of Congo red found that it is ionized 80 per cent in a dilution of 500 liters, yet,

“The osmotic pressure [of such solutions] found experimentally, both by direct measurement and by vapour pressure, is, throughout a wide range of concentration, uniformly between 95 and 100 per cent. of what it would be if no dissociation existed. Since it should be from one and a half to three times this value, according to the concentration, it is plain that there are some abnormal conditions present.”

Bayliss suggests an explanation similar to the one given above; namely, “the possibility of aggregated simple ions carrying the sum of the charges of their components.”⁸

IV. Influence of Valency upon Swelling and Viscosity.

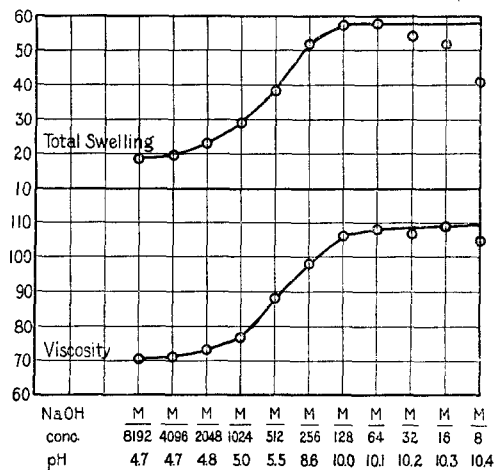
It has been demonstrated in previous papers that the curves for the values of viscosity and swelling are similar to the curves for osmotic pressure and it has been stated that these properties must also be a function of the relative number of metal gelatinate molecules and ions formed.

In Figs. 9 and 10 we give the curves for viscosity and swelling of sodium and potassium gelatinate and in Figs. 11 and 12 the same curves for barium gelatinate and calcium gelatinate. The values for viscosity are given in times of outflow and the values for swelling in terms of the height of cylinders of gelatin of the same diameter.⁹ On

⁸ Bayliss, W. M., *Proc. Roy. Soc. London, Series B*, 1911, lxxxiv, 253, 254.

⁹ Loeb, J., *J. Biol. Chem.*, 1918, xxxiv, 77, 395.

the axis of abscissæ are the logarithms of the concentrations of the alkali solution used. The pH of the gelatin is given under each concentration. The values for Na and K gelatinate are practically iden-



FIGS. 9 to 12. Curves for viscosity and swelling of sodium gelatinate (Fig. 9), potassium gelatinate (Fig. 10), calcium gelatinate (Fig. 11), and barium gelatinate (Fig. 12). The curves for sodium gelatinate differ from the curves for Ca and Ba gelatinate in the same sense and almost the same degree as the curves for osmotic pressure.

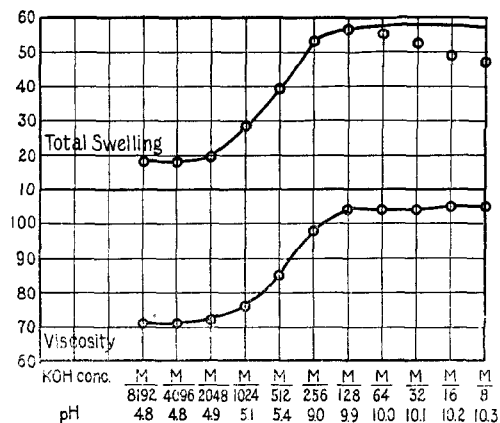


FIG. 10. See explanation under Fig. 9.

tical, and so are the values for Ca and Ba gelatinate. But the values for Na gelatinate and Ca gelatinate differ. The reader will notice that the values for pH are higher than in the curves for osmotic pressure. This is due to the fact that in the latter case the pH was determined after the excess of alkali had been removed by washing and dialysis, while the nature of the experiment made it necessary for us

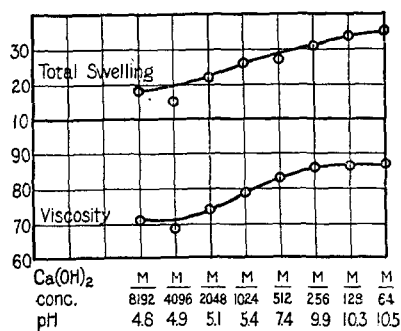


FIG. 11. See explanation under Fig. 9.

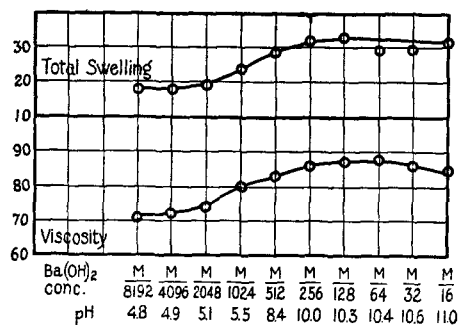


FIG. 12. See explanation under Fig. 9.

to determine the pH in the experiments on swelling and viscosity only after washing away the greater part of the excess of alkali, without using dialysis.

Neither the values for swelling nor for viscosity are as accurate as those for the osmotic pressure and hence the former cannot well serve for the purpose of drawing conclusions in regard to the molecular

basis of these properties. The viscosity rises for some time after the melting of gelatin and only approximately constant values can be obtained by measuring the time of outflow immediately; *i.e.*, within 5 minutes after the melting. The swelling is influenced by the resistance of the gelatin to the expanding forces leaving aside other sources of error. The osmotic pressure in connection with measurements on conductivity seems by far the most valuable property for the analysis of the molecular character of the influence of electrolytes upon the physical behavior of colloids.

The approximate parallelism between the curves for viscosity, swelling, and osmotic pressure excludes the idea that swelling and viscosity are determined by a hydration due to the degree of ionization of proteins as Pauli and many other colloid chemists assume. These hypotheses were developed without measurements of conductivity.

V. *Action of Trivalent Cations.*

The facts of the preceding chapters furnish a suggestion for the understanding of the action of trivalent metals. If the same tentative assumption may be made, namely that the gelatin ions held by the polyvalent metal ion dissociate in one aggregate, thus causing a diminution in the number of particles present in the solution without a diminution in the number of charges, it follows that this anionic aggregate must be the greater the greater the valency of the metal ion—leaving aside the atomic volume. Hence trivalent metals like Ce or Al should, if in combination with gelatin, lead to anionic aggregates consisting of a simple multiple of three. We cannot test this inference but we can show that gelatin compounds with a trivalent metal like cerium and aluminium are practically insoluble, and this insolubility might be the consequence of the increase in the number of gelatin anions forming an aggregate with the valency of cations.

1 gm. of finely powdered Cooper's gelatin (which is essentially calcium gelatin of pH = 7.0) was put for 1 hour into each of a series of beakers containing 100 cc. of a solution of Ce_2Cl_6 varying in concentration from $M/4$ to $M/16,384$, then drained on a filter and washed four times with distilled water to remove the excess of salt solution. The conductivity osmotic pressure. alcohol number, and total swell-

ing were ascertained. The results are plotted in Fig. 13. Gelatin treated with Ce_2Cl_6 solution from $M/4$ to $M/2048$ becomes insoluble and the cerium gelatinate forms a precipitate. The values for conductivity, etc., were as low as those obtained for gelatin at the isoelectric point. The gelatin treated with $M/4096$ or less concentrated Ce_2Cl_6 was clear and gave values for conductivity, etc., rising

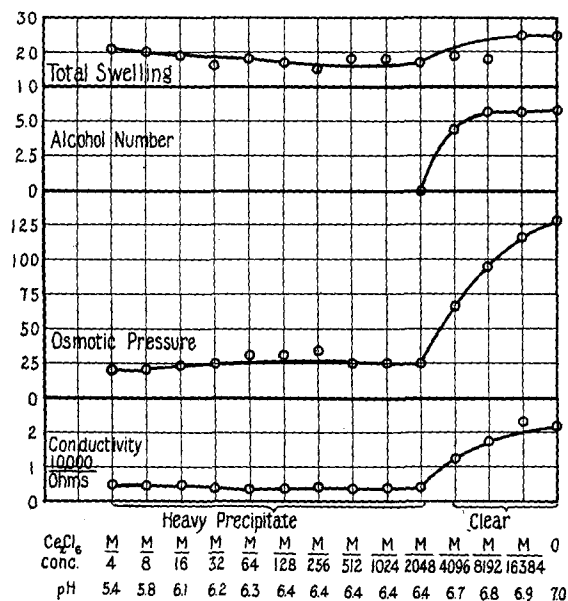


FIG. 13. Impure gelatin (chiefly calcium gelatinate) of pH 7.0 treated with concentrations of Ce_2Cl_6 varying from $M/4$ to $M/16,384$, then freed from excess of salts. pH of the gelatin solution is given under each solution. Cerium gelatinate is insoluble and hence the values for swelling, alcohol number, osmotic pressure, and conductivity are almost zero except when the concentration of the Ce_2Cl_6 used was low ($M/4096$ and less), when only a fraction of the original calcium gelatinate was transformed into cerium gelatinate.

with increasing solutions of Ce_2Cl_6 . In these cases not all the Ca gelatinate was transformed into Ce gelatinate. The less Ce gelatinate was formed the higher the value for conductivity, osmotic pressure, alcohol number, and swelling. The pH was between 5.4 and 7.0; *i.e.*, on the alkaline side of the isoelectric point of gelatin. Lead and copper acetates gave a similar result, as was to be expected.

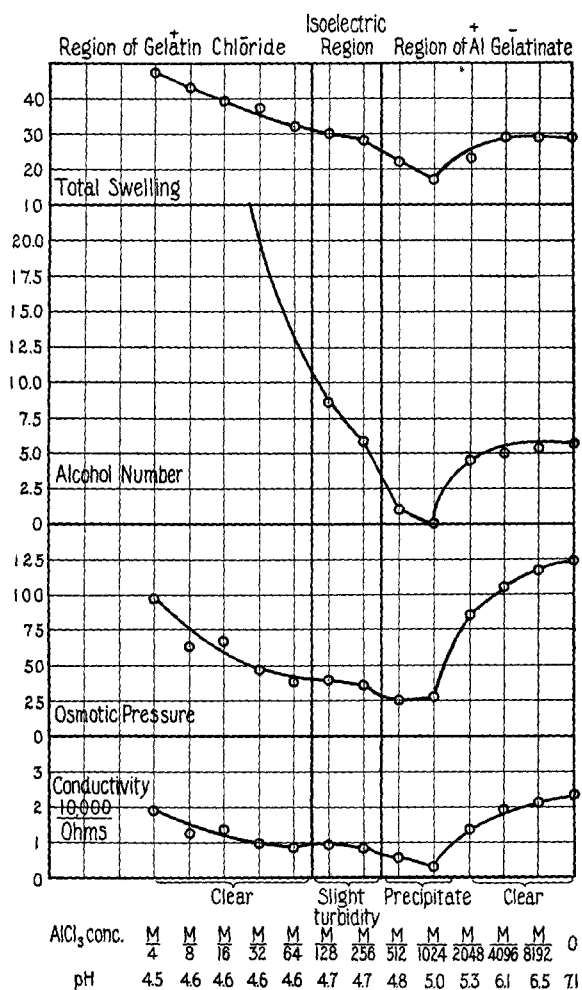


FIG. 14. Impure gelatin (chiefly calcium gelatin) treated with different concentrations of an acid salt with trivalent cation, namely, AlCl_3 . As long as $\text{pH} < 4.7$ no aluminium gelatin is formed and the gelatin exists in the form of gelatin chloride which gives a clear solution. At the isoelectric region $\text{pH} = 4.7$ gelatin is insoluble and all the curves show the low level characteristic of gelatin at the isoelectric point. When $\text{pH} > 4.7$ gelatin exists as Al gelatin. As long as the concentration of AlCl_3 used was high enough to transform all the original calcium gelatin into aluminium gelatin, $\text{m}/512$ to $\text{m}/1024$, all the gelatin was transformed into the insoluble aluminium gelatin which formed a precipitate as in the case of cerium gelatin. When the concentration of the AlCl_3 solution used was less than $\text{m}/1024$ not all the calcium gelatin was transformed into aluminium gelatin and the solution became gradually clear again.

AlCl_3 forms acid solutions and it seemed of interest to study the effect of a treatment of gelatin with AlCl_3 . We know now that for $\text{pH} < 4.7$ gelatin treated with AlCl_3 must form gelatin chloride and cannot be affected by the aluminium. The same is true also for $\text{pH} = 4.7$, where gelatin exists as sparingly soluble pure gelatin. For $\text{pH} > 4.7$ aluminium gelatinate can be formed which should be insoluble. If, however, the concentration of AlCl_3 , becomes too low, less and finally practically no aluminium gelatinate should be formed. These predictions were all fulfilled as the complicated curves in Fig. 14 show. 1 gm. of Cooper's powdered gelatin (*i.e.* chiefly Ca gelatin) was put for 1 hour at 20° into a series of beakers, each containing 100 cc. of a solution of AlCl_3 varying in concentration from $M/4$ to $M/8192$. The gelatin was then freed from the excess of salt solution by washing as described before. In Fig. 14 the abscissæ are the logarithms of the concentration of the AlCl_3 solution used and the final pH of the washed gelatin is found under each concentration. For $\text{pH} < 4.7$ no effect of aluminium is noticeable; the curves are typical gelatin chloride curves. For $\text{pH} = 4.7$ we find a region where gelatin behaves like isoelectric gelatin. For $\text{pH} = 4.8$ and 5.0 we have a precipitate formation due to the formation of aluminium gelatinate. When the concentration of AlCl_3 solution used is less than $M/1024$ the solution becomes clear again and the curves rise with increasing dilution of the AlCl_3 solution used. In other words, for $\text{pH} > 4.7$ the curves for aluminium gelatinate and cerium gelatinate run parallel as our theory demands.¹⁰

This curve suggests incidentally why in the writer's former experiments on *Fundulus* eggs the antagonistic influence of AlCl_3 to the toxic action of a sodium chloride solution was so restricted. On the acid side of the isoelectric point of amphoteric colloids AlCl_3 cannot act on the colloid, and on the basic side the concentration of Al is too low. It is the writer's intention to repeat these experiments with Ce_2Cl_6 or LaCl_3 , the solutions of which are less acid.

¹⁰ One wonders what interpretation such a curve as that for AlCl_3 would have found on the part of the colloid chemists who have persistently ignored not only the hydrogen ion concentration of their protein solutions but who have also overlooked the chemical significance of the isoelectric point.

SUMMARY.

1. A method is given by which the amount of equivalents of metal in combination with 1 gm. of a 1 per cent gelatin solution previously treated with an alkali can be ascertained when the excess of alkali is washed away and the pH is determined. The curves of metal equivalent in combination with 1 gm. of gelatin previously treated with different concentrations of LiOH, NaOH, KOH, NH_4OH , $\text{Ca}(\text{OH})_2$, and $\text{Ba}(\text{OH})_2$ were ascertained and plotted as ordinates, with the pH of the solution as abscissæ, and were found to be identical. This proves that twice as many univalent as bivalent cations combine with the same mass of gelatin, as was to be expected.

2. The osmotic pressure of 1 per cent solutions of metal gelatinates with univalent and bivalent cation was measured. The curves for the osmotic pressure of 1 per cent solution of gelatin salts of Li, Na, K, and NH_4 were found to be identical when plotted for pH as abscissæ, tending towards the same maximum of a pressure of about 325 mm. of the gelatin solution (for pH about 7.9). The corresponding curves for Ca and Ba gelatinates were also found to be identical but different from the preceding ones, tending towards a maximum pressure of about 125 mm. for pH about 7.0 or above. The ratio of maximal osmotic pressure for the two groups of gelatin salts is therefore about as 1:3 after the necessary corrections have been made.

3. When the conductivities of these solutions are plotted as ordinates against the pH as abscissæ, the curves for the conductivities of Li, Na, Ca, and Ba gelatinates are almost identical (for the same pH), while the curves for the conductivities of K and NH_4 gelatinates are only little higher.

4. The curves for the viscosity and swelling of Ba (or Ca) and Na gelatinates are approximately parallel to those for osmotic pressure.

5. The practical identity or close proximity of the conductivities of metal gelatinates with univalent and bivalent metal excludes the possibility that the differences observed in the osmotic pressure, viscosity, and swelling between metal gelatinates with univalent and bivalent metal are determined by differences in the degree of ionization (and a possible hydration of the protein ions).

6. Another, as yet tentative, explanation is suggested.