

Rockefeller University

Digital Commons @ RU

---

Collection of Reprints by Jacques Loeb

Special Collections

---

5-1919

**Amphoteretic Colloids. V. The Influence of the Valency of Anions  
Upon the Physical Properties of Gelatin**

Jacques Loeb

Follow this and additional works at: <https://digitalcommons.rockefeller.edu/collection-of-reprints-loeb>

---

## AMPHOTERIC COLLOIDS.

### V. THE INFLUENCE OF THE VALENCY OF ANIONS UPON THE PHYSICAL PROPERTIES OF GELATIN.

By JACQUES LOEB.

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

(Received for publication, February 21, 1919.)

It was shown in preceding papers that while all gelatin salts with univalent cation show a comparatively high osmotic pressure, a high degree of swelling, a high viscosity, and a high alcohol number, the gelatin salts with bivalent metal (and trivalent metal) show for the same pH and gelatin concentration a low osmotic pressure, low degree of swelling, low viscosity, and a low alcohol number. Since the conductivities of the two types of gelatin salts were found to be practically the same, the valency effect could not be attributed to differences in the degree of ionization and "hydratation" of the gelatin ions. The writer suggested as a tentative explanation the assumption of an aggregate formation of a stoichiometrical character. He had shown that two gelatin ions combine with one bivalent cation and it was suggested that in the process of electrolytic dissociation these two gelatin ions form one anion with two charges. This suggestion is able to explain quantitatively the results actually observed.<sup>1</sup>

In one of his previous publications the writer had already called attention to the fact that bivalent anions differ in their valency effect from bivalent cations.<sup>2</sup> While  $\text{SO}_4$  has a similar depressing effect upon the physical properties of gelatin as Ca or Ba, the same does not seem to be true for other bivalent and trivalent anions like oxalate, succinate, tartrate, citrate, or phosphate. The acids of these latter anions seemed to act as if they were monobasic acids, one molecule of these acids being capable of combining with one gelatin ion only.

<sup>1</sup> Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 483.

<sup>2</sup> Loeb, J., *J. Biol. Chem.*, 1918, xxxiv, 489.

It seemed very important to test this possibility, since a decision might serve as a crucial test for or against our purely chemical conception of the behavior of colloids, according to which the effect of an electrolyte upon the physical properties of the colloid is due to the formation of real chemical compounds between the colloid and one of the ions of the electrolyte. If it can be shown that  $\text{SO}_4$  has a similar depressing effect as a bivalent cation we must also be able to prove that  $\text{SO}_4$  combines with two molecules of gelatin; and if it can be shown that  $\text{PO}_4$ , citrate, oxalate, succinate, etc., have no such depressing effect upon the physical properties of gelatin but behave like Br, Cl,  $\text{NO}_3$ , and acetate, we must also be able to prove that one molecule of each of these acids combines with only one molecule of gelatin.

We are able to decide this question in favor of the purely chemical theory of colloids.

The decision was rendered possible through the proof that we can ascertain the amount of acid in combination with a given mass of gelatin by titration with NaOH.<sup>3</sup> If we treat gelatin with acid and titrate afterwards with NaOH, we obtain the mass of acid in combination with the gelatin with the aid of two corrections; namely, first by deducting from the titration value a constant value which is 1.8 cc. 0.01 N NaOH for 10 cc. of a 1 per cent gelatin solution, titrating to neutrality (pH = 7.0). This constant value 1.8 cc. 0.01 N NaOH is the quantity of alkali required to bring 0.1 gm. isoelectric gelatin to pH 7.0. The correctness of this method was demonstrated in the case of HBr and HCl in which it was possible to compare the amount of NaOH required for the titration of a 1 per cent gelatin solution with the results of volumetric analysis for Br according to Volhard's methods. It was found that the Br (or Cl) numbers agreed with the NaOH numbers when the constant value mentioned above was deducted. In that paper<sup>3</sup> we had titrated for pH = 9.0, while in the experiments reported in the present paper we titrate for pH = 7.0; as a consequence the value of the constant given in the first paper was higher than 1.8 cc. 0.01 N NaOH.

<sup>3</sup> Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 363.

In addition a second correction is needed as explained in a previous paper. The gelatin treated with acid contains some free acid, the titration value of which has also to be deducted from the titration number. This correction can be found by titration of the same acid at the same pH but free from gelatin.

With the aid of these two corrections we are also able to investigate the effect of acid on gelatin in the presence of the acid. It was observed that the maximal osmotic pressure of gelatin chloride or sulfate is reached at a pH of between 3.3 to 3.5. This maximum is never reached when we wash the gelatin four or six times with H<sub>2</sub>O after a treatment with an acid of moderate concentration, as was done in our previous experiments, since in this washing the acid formed by hydrolytic dissociation is removed, which causes new hydrolysis and in this way in successive washings a rapid decline of the pH results, as will be shown more definitely in a subsequent paper.

Since it was necessary for our purpose to be always sure of ascertaining the maximal osmotic pressure a 1 per cent solution of isoelectric gelatin can reach when treated with different acids, we were forced to adopt a different method from that described in the preceding papers. We melted 1 gm. of gelatin rendered isoelectric in the manner described in a previous paper and then added different quantities of 0.01 N or 0.1 N acid and made up the volume to 100 cc.<sup>4</sup> This solution was put into a collodion bag of a capacity of about 50 cc. and the bag was put into a beaker containing 400 cc. of the same percentage of acid in distilled water as that added to the gelatin solution. The collodion bag was closed tightly with a perforated rubber stopper containing a glass tube serving as a manometer to measure the osmotic pressure of the gelatin solution. After about 18 hours, when osmotic equilibrium was reached, the pH, the conductivities, and the titration numbers of the acid inside and outside the bag were determined, as were also the osmotic pressures.

<sup>4</sup> For the sake of briefness we shall designate such solutions as 1 per cent gelatin solutions, although this does not take into account the increase in weight of the gelatin due to the combination with another ion.

Table I gives the data of one experiment with a 1 per cent solution of isoelectric gelatin made up in 100 cc. of  $H_2O$  containing varying quantities of 0.01 N HBr, stated in the upper horizontal row. The second horizontal row of figures gives the pH of the gelatin solution at the end of the experiment, and the third row the figures for the cc. of 0.01 N Br found by Volhard's method, in 10 cc. of the gelatin solution. These latter figures are too high since they include the free HBr in the solution. We can find the value for the free HBr solution from the pH and this value must be deducted from the titration number for Br. This deduction gives us the corrected Br values of the horizontal Row 4. Row 5 gives the number of cc. of 0.01 N HBr found in 10 cc. of the gelatin solution by titrating with NaOH to pH = 7.0. This value demands, as stated, two corrections; first, a deduction of the amount of NaOH needed to bring 10 cc. of 1 per cent isoelectric gelatin solution to pH = 7.0. This value is 1.8 cc. of 0.01 N NaOH for 10 cc. of a 1 per cent solution of gelatin. The second correction is that for the free HBr present in the gelatin solution which can be measured by titrating free HBr of the same pH as that of the gelatin solution to pH = 7.0. Row 6 gives the titration values for NaOH after the two corrections have been made. These corrected figures should give us the amount of HBr in combination with 0.1 gm. of gelatin. The reader will notice that the corrected numbers for the direct titration for Br after Volhard (Row 4), and the corrected numbers for titration with NaOH (Row 6), are, within the limits of the accuracy of our method, identical, as they should be. This identity is of the greatest importance for the subject we intend to discuss in this paper, since in the case of other acids (with the exception of HCl and  $H_3PO_4$ ) we can ascertain the amount of acid in combination with 0.1 gm. of gelatin only indirectly by titrating with NaOH. It was, therefore, necessary for us to be sure that this titration gives us the correct amount of acid in combination with 0.1 gm. of gelatin if we make the two corrections mentioned, and the identity of the titration numbers by the direct Volhard method for Br and by the indirect method of titrating with NaOH gives us this certainty.

Row 7 gives the osmotic pressure in mm. of the height of a column of the 1 per cent gelatin solution. Row 8 gives us the conductivity



$\left(\frac{10,000}{\text{ohms}}\right)$  corrected for the pH (*i.e.*, after deduction of the conductivity of a free acid solution with the pH found in the gelatin solution).

Rows 9 to 12 give the measurements of the pure acid solution (free from gelatin) surrounding the collodion bag containing the gelatin solution. The pH in the outside solution is always lower than inside the bag and this difference may find its explanation on the basis of the theory of equilibrium developed by Donnan for such cases. The values for titration for Br by the Volhard method and for the titration for HBr by titration with NaOH are practically identical for this pure acid solution, thus showing that the degree of accuracy of the method of titration was adequate.

In Table II the measurements for 1 per cent isoelectric gelatin treated with  $\text{H}_2\text{SO}_4$  are given. Here we can ascertain the amount of  $\text{SO}_4$  in combination with 0.1 gm. of gelatin only indirectly by titrating with NaOH, applying the two corrections mentioned. All the other measurements are the same as in the experiment with HBr.

A comparison of the corrected NaOH values in Row 4, Table II, with those in Row 6, Table I, shows that they are practically identical for the same pH. Thus for pH = 3.35 the corrected NaOH value is 4.4 in the case of gelatin sulfate, and 4.35 for gelatin bromide. Since the experiments with HBr have shown that these corrected NaOH values are identical with the Br values we can state, that for a given pH 1 gm. of gelatin is in combination with twice as many atoms of Br as with radicles of  $\text{SO}_4$ ; or in other words, that  $\text{H}_2\text{SO}_4$  behaves toward gelatin as a dibasic acid. Fig. 1 illustrates this identity of the curves for NaOH values of the two acids. In spite of the identity of equivalents of these two ions in combination with 1 gm. of gelatin the osmotic pressures are very different for the two types of gelatin salts. This is illustrated by Fig. 2, where the ordinates represent the osmotic pressures and the pH are the abscissæ. It is obvious that the values of the osmotic pressure of gelatin bromide are considerably higher than the values for the osmotic pressure of gelatin sulfate. The reader will notice that the maximum osmotic pressure for gelatin bromide is about 325 mm., which is identical with the maximal osmotic pressure found for gelatin salts with univalent cation, *e.g.* sodium gelatinate; while the maximum value for gelatin sulfate is about 130, almost identical with the maximum for calcium

TABLE II.

1 per cent solution of isoelectric gelatin made up in 100 cc. H <sub>2</sub> O containing varying quantities of m/100 H <sub>2</sub> SO <sub>4</sub> .															
	45	40	35	30	25	22.5	20	17.5	15	12.5	10	7.5	5	2.5	0
1. Cc. m/100 H <sub>2</sub> SO <sub>4</sub> .....	2.4	2.45	2.5	2.65	2.75	2.85	2.9	2.95	3.0	3.1	3.2	3.35	3.55	4.1	4.8
2. pH of gelatin solution.....	15.2	14.4	13.8	12.5	11.6	10.9	10.0	10.4	9.1	8.3	7.8	6.6	5.5	3.8	1.8
3. Cc. 0.01 N NaOH required to bring 10 cc. of gelatin solution to pH 7.0.....	9.55	9.2	9.0	8.9	8.2	6.7			6.2	5.7	5.4	4.4	3.4	1.85	0
4. Corrected NaOH values.....															
5. Osmotic pressure in mm. of height of column of a 1 per cent gelatin solution.....	63	74	77	80	87	90	91	92	114	123	129	132		112	34
6. Conductivity $\left(\frac{10,000}{\text{ohms}}\right)$ , corrected for pH.....	31.0		30.1		26.0		18.1		15.25	10.85		9.8	7.3	3.95	0.52
Measurements of pure acid solution (free from gelatin) in the outside beaker.															
7. pH of acid solution.....	2.1	2.2	2.2	2.3	2.35	2.4	2.45	2.5	2.55	2.65	2.75	3.0	3.3	3.5	5.0
8. NaOH number.....	8.0	7.3	6.4	5.2	4.4	3.85	3.4	3.0	2.4	1.8	1.4	1.1	0.7	0.3	
9. Conductivity $\left(\frac{10,000}{\text{ohms}}\right)$ .....	81.3	74.0	65.8	55.3	47.6	47.9	35.5	33.2	27.4	21.4	16.1	11.75	7.46	3.22	0.22



gelatinate. The maxima for both gelatin bromide and gelatin sulfate are found at the same pH, namely between 3.3 and 3.5. van't Hoff's theory would ascribe the difference in the maximal osmotic pressures of the two acids to a corresponding difference in the number of particles in solution; while the colloid chemists would ascribe the difference in osmotic pressure to a difference in the "hydratation" due to a difference in the ionization of the two gelatin salts.

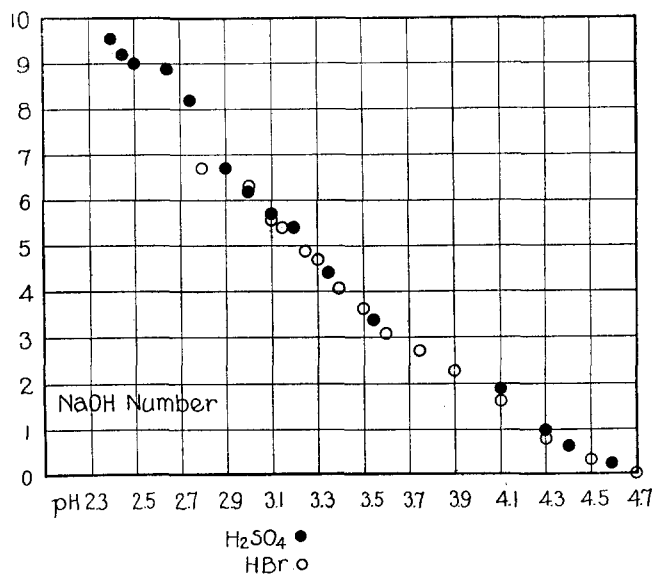


FIG. 1. Relative amount of HBr and  $H_2SO_4$  in combination with gelatin. Abscissæ represent pH; ordinates, amount of 0.01 N HBr and  $H_2SO_4$  bound by 0.1 gm. of gelatin. The identity of the curves for the two acids proves that  $H_2SO_4$  combines with gelatin as a dibasic acid.

The curves for conductivity of the two gelatin salts (plotted according to the corrected conductivity values of Table I and Table II) differ but slightly (Fig. 3). As a matter of fact, if other experiments with the two acids are taken into account (one of which will be given later) the curves for the conductivities of the two gelatin salts are practically identical if plotted over pH as abscissæ. This identity of the conductivity curves contradicts the hydratation hypothesis of the colloid chemists.

We therefore are confronted with the same situation as in the case of sodium gelinate and calcium gelinate, where we also found practically equal conductivity combined with a ratio of osmotic pressures of 3:1 for the two metal gelinates mentioned. In the latter case we offered the tentative assumption that the Ca ion combines

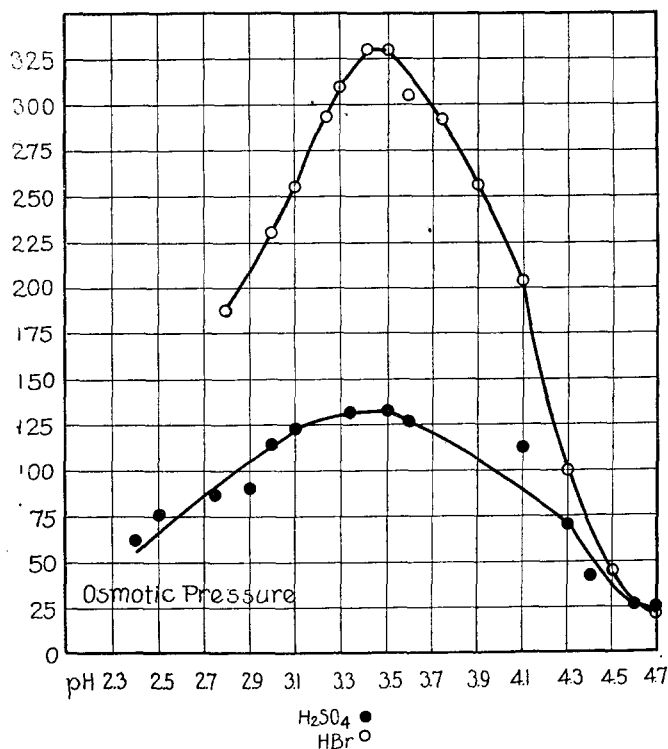


FIG. 2. Osmotic pressure curves for gelatin sulfate and gelatin bromide. Abscissæ represent pH; ordinates, osmotic pressure, showing that for the same pH the osmotic pressure is higher when HBr than when H<sub>2</sub>SO<sub>4</sub> is added to gelatin. The maximal osmotic pressure for both acids is found at the same pH (about 3.5).

with two gelatin anions, these two gelatin anions remaining together in a single aggregate when electrolytic dissociation occurs. If the compound Ca<sub>2</sub> gelatin<sub>4</sub> dissociates into two Ca ions and one aggregate of four gelatin anions carrying four charges, the same number of charges, namely eight, would be carried by the dissociation of four

sodium gelatinate molecules yielding eight charges and eight ions. This would produce equality of charges and a ratio of osmotic pressures of 3:8. The same assumption applied to gelatin bromide and gelatin sulfate would demand that gelatin<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> dissociate into three particles, two negative SO<sub>4</sub> ions and one aggregate (gelatin<sub>4</sub>) with four positive charges; while in the case of gelatin bromide the same number of charges would be carried by eight separate ions, four Br and four separate positive gelatin ions. This would again produce equality in the number of charges and a ratio of 3:8 in the number of particles.

When pH becomes less than 3.3 the values for osmotic pressure drop again. This drop which had been noticed by previous investigators, especially by Pauli, is ascribed by the latter to a diminution in the degree of electrolytic dissociation of the gelatin salt. This assumption meets, however, with a difficulty in the fact that the conductivity of the gelatin salts does not show this drop but continues to rise steeply for values of pH less than 3.3.

*Antagonism between HBr and H<sub>2</sub>SO<sub>4</sub>.*

The real crux between the colloidal and the chemical conception seems to lie in the question of the justification of the assumption of "hydratation" as the cause of osmotic pressure of protein solutions. The writer does not question the possible correctness of the idea that ions are in general surrounded by a jacket of water molecules; he only doubts the correctness of the idea that this possible hydration of the protein ion is the cause of the osmotic pressure, the swelling, and the other physical properties of proteins as Pauli, Michaelis, and others assume. The following experiment seems to be a further proof against this application of the hydratation hypothesis. We had seen that a 1 per cent solution of both gelatin bromide and gelatin sulfate reaches the maximal osmotic pressure at a pH of about 3.5 and that for this pH the same amount of gelatin is combined with equivalent amounts of Br and SO<sub>4</sub>, namely 0.1 gm. of gelatin binding about 3.6 cc. of 0.01 N Br or SO<sub>4</sub>. Both solutions have the same conductivity, namely about 9.7. In order to produce gelatin bromide or gelatin sulfate of a pH of about 3.4 it is necessary

that 1 gm. of isoelectric gelatin should be melted and added to 15 cc. of 0.01 N HBr or  $\text{H}_2\text{SO}_4$  and that the volume be made up to 100 cc. by adding distilled water. By mixing HBr and  $\text{H}_2\text{SO}_4$  in various

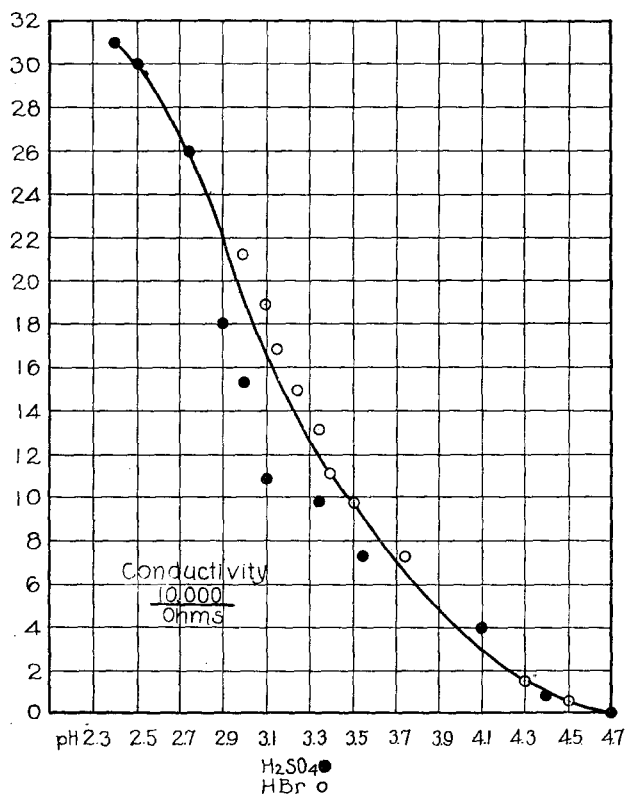


FIG. 3. Conductivity curves for gelatin sulfate and gelatin bromide. Abscissæ represent pH; ordinates, conductivity of gelatin sulfate and gelatin bromide, showing that aside from a few irregular values the curves are practically identical for the two acids. This disproves the assumption that the differences in osmotic pressure, as shown in Fig. 2, can be attributed to differences in ionization and "hydration" of the two gelatin salts.

proportions but always adding 15 cc. of 0.01 N acid to 1 gm. of isoelectric gelatin, melting the latter, and making up the volume to 100 cc. by the addition of water, the amount of gelatin salt formed and its degree of ionization, *i.e.* the conductivity, should remain the

same, while the osmotic pressure should vary according to the relative proportion of HBr and  $\text{H}_2\text{SO}_4$  used. HCl was used in this experiment instead of HBr since we had found that HCl and HBr act qualitatively and quantitatively alike on the physical properties of gelatin. The mixtures in which the 15 cc. of 0.01 N acid were prepared were made up as follows: 15 cc. 0.01 N HCl + 0 cc. 0.01 N  $\text{H}_2\text{SO}_4$ ; 14 cc. 0.01 N HCl + 1 cc. 0.01 N  $\text{H}_2\text{SO}_4$ ; 13 cc. 0.01 N HCl + 2 cc. 0.01 N  $\text{H}_2\text{SO}_4$ , etc., and finally 0 cc. 0.01 N HCl + 15 cc. 0.01 N  $\text{H}_2\text{SO}_4$ . Table III gives the numerical values for the pH, the corrected titration number, the conductivity, and the osmotic pressure of each gelatin solution. While the titration number (Row 2) and the values for conductivity (Row 3) are everywhere practically identical, the osmotic pressures vary from 300 to 128. Fig. 4 gives the graphical expression of the result.

It is obvious that the values for osmotic pressure are not the algebraic mean between the values for pure HCl and pure  $\text{H}_2\text{SO}_4$ . The curve is convex towards the axis of abscissæ, thus showing that the depressing effect of  $\text{SO}_4$  increases more rapidly than the quantity of  $\text{SO}_4$  added. This is characteristic for a true antagonistic salt effect.

The main result is unequivocal; namely, that the differences in the osmotic pressures are not due to differences in ionization and hydration of protein ions as the colloid chemists assume, since the conductivities remain constant while the osmotic pressures vary considerably.

What is true for the relative influence of the two acids on osmotic pressure is also true for their relative influence on swelling, and viscosity of gelatin as is shown in Figs. 5 and 6.

*Proof that Dibasic and Tribasic Acids (with the Exception of  $\text{H}_2\text{SO}_4$ ) Behave Practically like Monobasic Acids toward Gelatin.*

Of the other dibasic and tribasic acids which the writer has thus far investigated, namely oxalic, tartaric, succinic, citric, and phosphoric acid, none shows the valency influence. We are able to give the explanation for this peculiar behavior by proving that all these acids react with gelatin practically as if they were monobasic acids.

TABLE III.

1 per cent solution of isoelectric gelatin made up in 100 cc. H <sub>2</sub> O containing varying quantities of 0.01 N HCl and H <sub>2</sub> SO <sub>4</sub> .																
Cc. 0.01 N HCl.....	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
Cc. 0.01 N H <sub>2</sub> SO <sub>4</sub> .....	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. pH of gelatin solution.....	3.4	3.35	3.4	3.35	3.4	3.35	3.4	3.35	3.4	3.35	3.35	3.35	3.35	3.35	3.35	3.35
2. Corrected NaOH values.....	3.3	3.6	3.6	3.6	3.3	3.5	3.8	3.9	3.7	3.9	3.9	3.8	4.0	4.1	4.1	4.1
3. Conductivity $\left(\frac{10,000}{\text{ohms}}\right)$ , corrected for pH.....	9.2	10.4	9.1	9.5	8.4	8.1	9.2	9.2	9.5	9.1	9.5	9.3	9.6	9.3	9.4	9.2
4. Osmotic pressure in mm. of height of column of a 1 per cent gelatin solution.....	300	282	254	236	210	205	172	185	162	166	151	148	136	134	127	128

We repeated the experiments represented in Tables I and II with a number of other acids, the method of adding acid to gelatin being the same in all cases, though the quantity of acid needed to produce

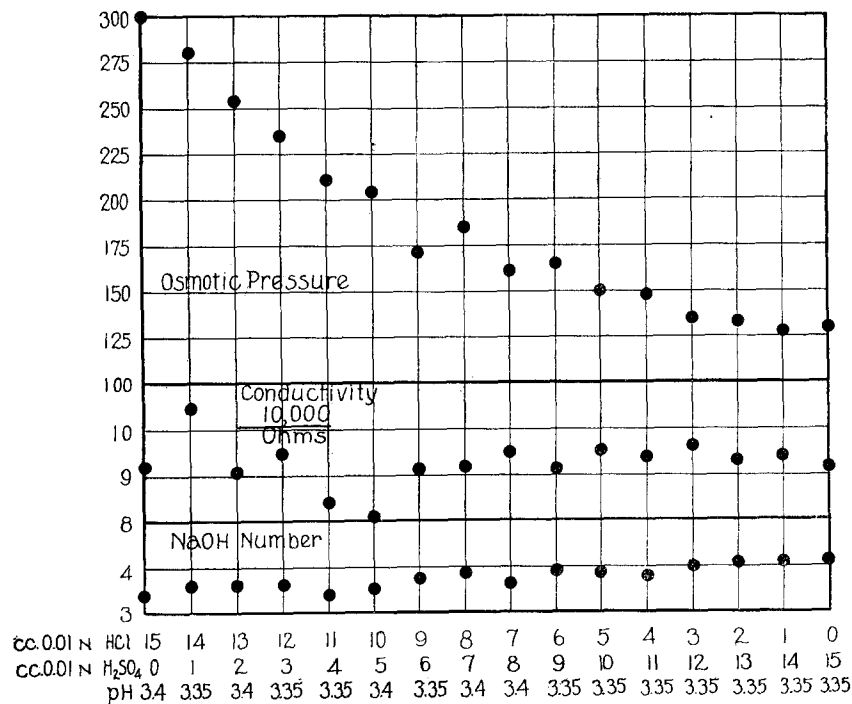


FIG. 4. Effect of mixtures of HCl and H<sub>2</sub>SO<sub>4</sub> upon conductivity and osmotic pressure of gelatin. Abscissæ represent mixtures of the two acids; ordinates of lower curve, amount of acid in combination with 0.1 gm. of gelatin. The curve is a straight line, proving that equivalent amounts of H<sub>2</sub>SO<sub>4</sub> and HCl are bound by 0.1 gm. of gelatin. Ordinates of middle curve, corrected conductivities of gelatin treated with different proportions of a mixture of the two acids. The curve is also practically a straight line. Upper curve, osmotic pressure curve, showing that the osmotic pressure is the greater the greater the proportion of HCl is in the mixture. The curve is convex to the axis of abscissæ, which is characteristic for antagonism. Notice identity of conductivities and the difference in osmotic pressure, contradicting the hydration theory of colloid chemists.

the same pH was different for different acids. In the case of phosphoric acid we determined directly (with the uranylacetate method) the PO<sub>4</sub> in combination with 10 cc. of a 1 per cent gelatin solution

treated with different quantities of  $H_3PO_4$ . In the case of  $HNO_3$  and oxalic acid we determined the amount of acid in combination with the same amount of gelatin by titration with NaOH for pH = 7.0,

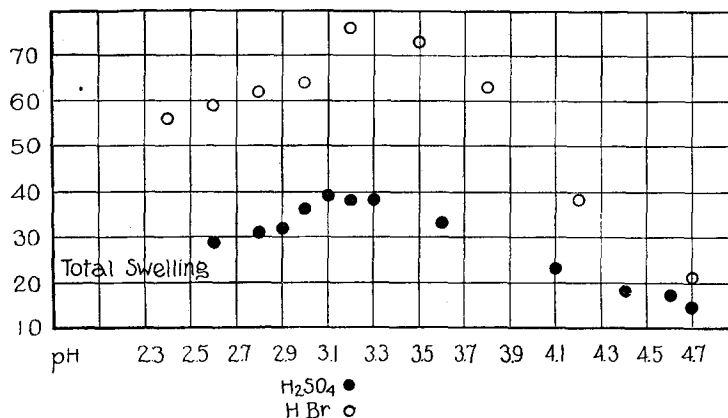


FIG. 5. Relative influence of HBr and  $H_2SO_4$  upon swelling.

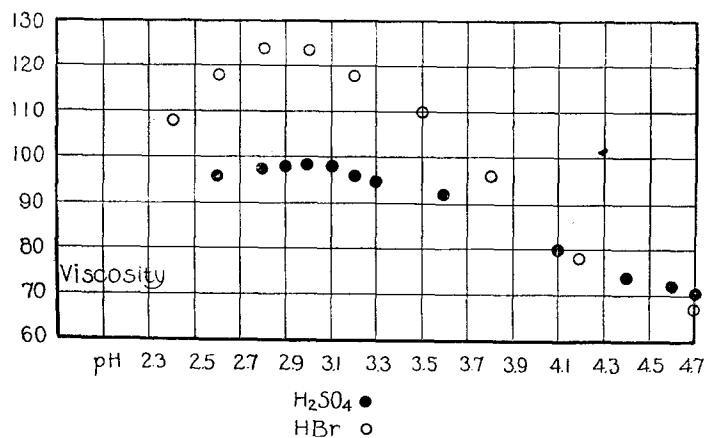


FIG. 6. Relative influence of HBr and  $H_2SO_4$  upon viscosity.

and making the two corrections discussed before. Table IV gives the equivalents of these three acids in combination with 0.1 gm. of gelatin for different pH in terms of cc. of 0.01 N NaOH used for titration.



The values found for  $\text{HNO}_3$  are slightly less than those found for  $\text{HBr}$  and  $\text{HCl}$ . This harmonizes with the fact that the effect of  $\text{HNO}_3$  on the physical properties of gelatin is also slightly less than the effect of  $\text{HCl}$  or  $\text{HBr}$  for the same pH. A comparison of the figures for  $\text{NaOH}$  values for  $\text{HNO}_3$  and for the  $\text{PO}_4$  values, found by titration for  $\text{PO}_4$  (Table IV, Rows 1 and 3) shows for the two values practically the ratio of 1:3 at the same pH; *i.e.*, three times as much  $\text{H}_3\text{PO}_4$  as  $\text{HNO}_3$  is in combination with the same mass of gelatin. The figures for  $\text{HNO}_3$  and oxalic acid (Rows 1 and 2, Table IV) give the ratio of approximately 1:2.

We therefore see in these figures the proof that actually one molecule each of phosphoric and oxalic acids is in combination with only one molecule of gelatin; and we see in this the explanation for the

TABLE IV.

Cc. of 0.01 N acid in combination with 10 cc. of a 1 per cent gelatin solution at different pH.										
pH	3.1	3.2	3.3	3.4	3.5	3.7	3.9	4.1	4.2	4.3
1. $\text{HNO}_3$ .....	4.35	4.1	3.6	3.2	2.85	2.45	1.9	1.45		0.75
2. Oxalic acid.....	9.6	8.75	7.6	6.7	6.00	4.3	3.0		1.65	
3. $\text{H}_3\text{PO}_4$ .....		12.4	10.4	9.8	9.00	7.4	5.8	4.5	2.6	2.1

fact that they do not depress the osmotic pressure as does  $\text{H}_2\text{SO}_4$ , which behaves like a dibasic acid towards gelatin.

What has been proved for oxalic and phosphoric acids holds also for tartaric, succinic, and citric acids. These latter two acids are very weak and thus relatively large quantities of acid have to be added to bring the solution to the desired pH. The same is true for acetic acid. The equilibrium conditions seem to be such that a very large amount of undissociated acid is found inside the collodion bag and it seems for the present impossible to determine by titration with  $\text{NaOH}$  the amount of these acids in actual combination with gelatin.

The fact that all the dibasic and tribasic acids mentioned (with the exception of  $\text{H}_2\text{SO}_4$ ) behave towards gelatin practically like monobasic acids is a further proof for the theory that the influence of acids on gelatin is determined by the relative amount of gelatin salt formed

through the addition of acid by gelatin. If  $\text{H}_3\text{PO}_4$  behaves towards gelatin like a monobasic acid the curves for osmotic pressure for gelatin phosphate should be identical with the curves for gelatin nitrate when plotted over pH as abscissæ. Fig. 7 shows that this is actually the case. The maximum for the two curves lies between pH 3.3 and 3.5 and is about 300 mm. Fig. 8 shows that the osmotic pressure curves for gelatin acetate and citrate are also identical and

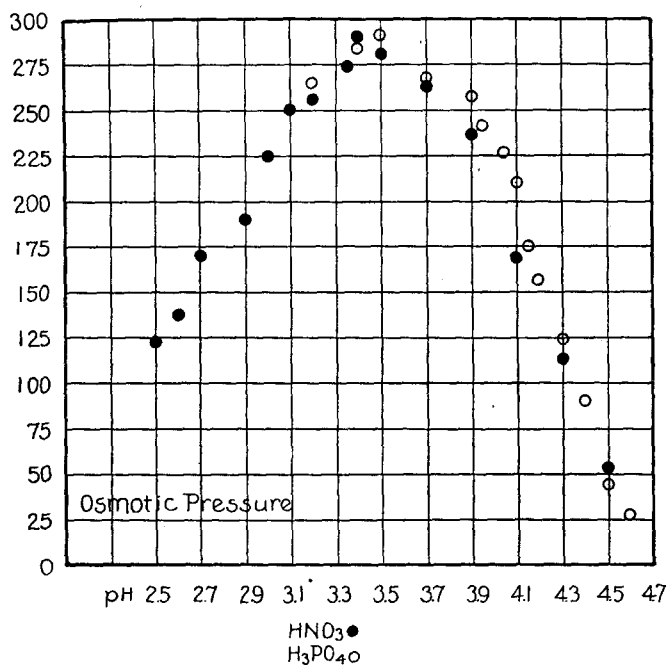


FIG. 7. Showing identity of influence of  $\text{HNO}_3$  and  $\text{H}_3\text{PO}_4$  upon osmotic pressure of gelatin solution for the same pH.

that they agree with the curves for gelatin nitrate and gelatin phosphate as well as with those for gelatin bromide (Fig. 1).

What is true for the osmotic pressure curves is also true for the curves for the other physical properties of gelatin. Thus Fig. 9 gives the curves for viscosity of 1 per cent solutions of gelatin bromide, oxalate, and tartrate, showing that they are practically identical when plotted with pH as abscissæ. Fig. 10 gives the curves

for swelling for gelatin oxalate, and chloride, which are also approximately identical.<sup>5</sup> The fact that the curves for viscosity and swelling are similar to those for osmotic pressure has been pointed out so often in previous papers that we need not dwell further on this point.

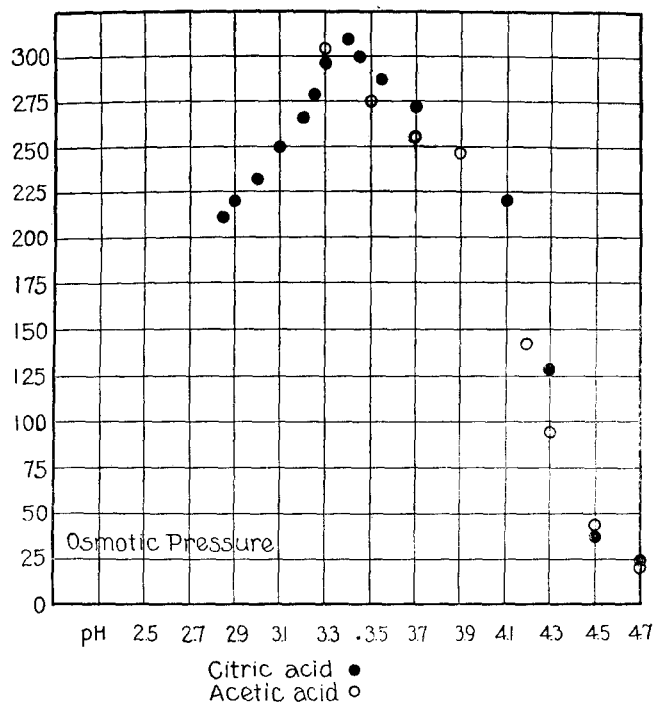


FIG. 8. Showing identity of influence of citric and acetic acids upon osmotic pressure of gelatin solution for the same pH. Curves in Fig. 8 are identical with those in Fig. 7.

<sup>5</sup> The close proximity of the effects of different acids does not harmonize with the contention of the colloid chemists who report a typical difference in the effects of the acids according to the nature of the anion. This is an error due to the fact that they failed to determine the pH of their solutions and to compare the behavior of gelatin solutions of the same pH, comparing instead the effects of equimolecular concentrations of different acids, without making any of the necessary corrections discussed in this paper.

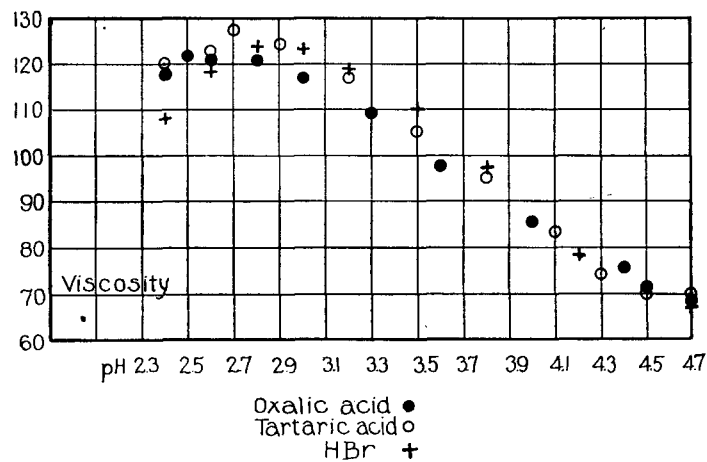


FIG. 9. Viscosity curves of gelatin treated with HBr, tartaric, and oxalic acids are identical when plotted over pH as abscissæ.

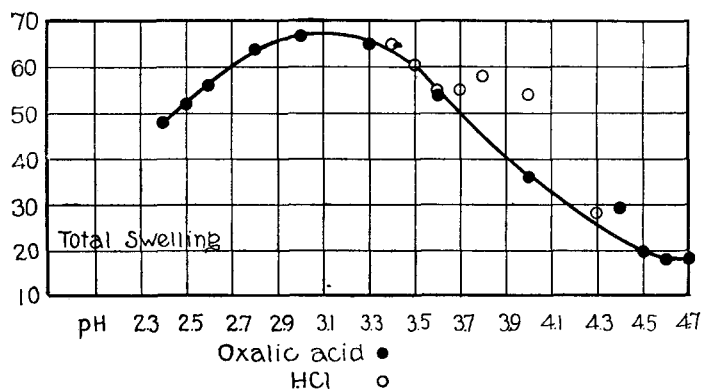


FIG. 10. Curves showing the influence of HCl and oxalic acid upon swelling of gelatin. The curves are identical when plotted over pH.

*Antagonism Experiments between HCl and Phosphoric and Oxalic Acids.*

It was to be expected that when  $H_3PO_4$  or oxalic acid is added to HCl or HBr it can produce no or only a slight antagonistic effect. The results of actual experiments support this expectation. The same experiment as that with HCl and  $H_2SO_4$  (represented in Fig. 4) was made by mixing different proportions of HCl and  $H_3PO_4$ .

15 cc. 0.01 N HCl + 0 cc. 0.01 N  $\text{H}_3\text{PO}_4$ ; 13.5 cc. 0.01 N HCl + 4.0 cc. 0.01 N  $\text{H}_3\text{PO}_4$ ; 12 cc. 0.01 N HCl + 8 cc. 0.01 N  $\text{H}_3\text{PO}_4$  to 3 cc. 0.01 N HCl + 32 cc. 0.01 N  $\text{H}_3\text{PO}_4$  and 0 cc. 0.01 N HCl + 40 cc. 0.01 N  $\text{H}_3\text{PO}_4$  were added each to 1 gm. of isoelectric gelatin and the solution was made up to 100 cc., the osmotic pressure of which was determined against 400 cc. of a pure acid solution of the same constitution and concentration as that added to gelatin. The pH was the same in all gelatin solutions, namely about 3.5, and the osmotic pressure was also the same, about 300 mm. (Fig. 11).

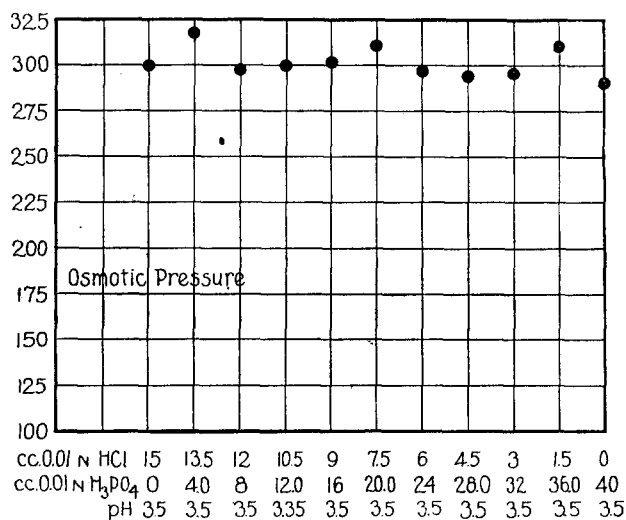


FIG. 11. Showing that  $\text{H}_3\text{PO}_4$  has no antagonistic effect on the influence of HCl upon the osmotic pressure of gelatin. The curve of osmotic pressure of different mixtures of the two acids for the same pH is a straight line.

There is a slight antagonistic effect when we mix HCl and oxalic acid (Fig. 12). This is due to the fact that the amount of oxalic acid in combination with a given mass of gelatin is less than twice the amount of HCl (or HBr) in combination with the same mass of gelatin. It is possible that a small fraction of the oxalic acid acts like a dibasic acid on gelatin while the greater part acts like a monobasic acid.

The fact that the dibasic and tribasic anions which have no or only a slight antagonistic effect act like monobasic acids towards gelatin harmonizes with the hypothesis of aggregation. Our idea of aggregation is a stoichiometrical one, making the number of gelatin ions forming one aggregate of gelatin ions a simple multiple of the valency number of the polyvalent ion with which they are in combination. Since phosphoric, citric, tartaric, succinic, and practically also oxalic acids behave like monobasic acids, *i.e.* since they can bind only one gelatin molecule, they cannot be expected to cause any aggregate formation and hence cannot produce any antagonistic salt action.

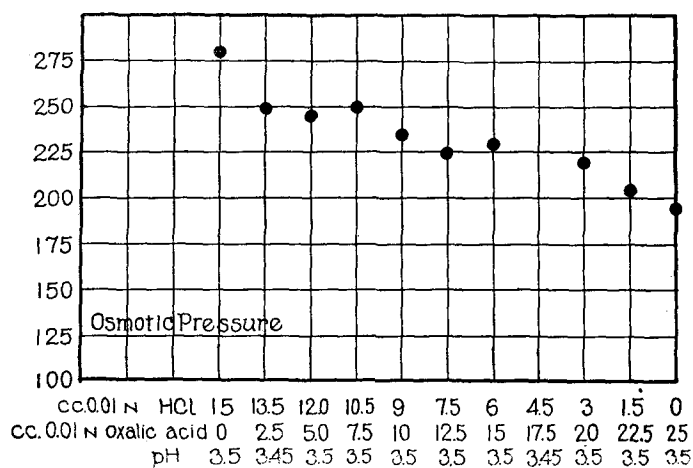


FIG. 12. Showing that oxalic acid has practically no antagonistic effect on the influence of HCl upon the osmotic pressure of gelatin.

#### SUMMARY.

1. When we plot the values of osmotic pressure, swelling, and viscosity of gelatin solutions as ordinates over the pH as abscissæ, practically identical curves are obtained for the effect of monobasic acids (HCl, HBr, HNO<sub>3</sub>, and acetic acid) on these properties.

2. The curves obtained for the effect of H<sub>2</sub>SO<sub>4</sub> on gelatin are much lower than those obtained for the effect of monobasic acids, the ratio of maximal osmotic pressures of a 1 per cent solution of gelatin sulfate

and gelatin bromide being about 3:8. The same ratio had been found for the ratio of maximal osmotic pressures of calcium and sodium gelatinate.

3. The curves representing the influence of other dibasic and tri-basic acids, *viz.* oxalic, tartaric, succinic, citric, and phosphoric, upon gelatin are almost identical with those representing the effect of monobasic acids.

4. The facts mentioned under (2) and (3) permit us to decide between a purely chemical and a colloidal explanation of the influence of acids on the physical properties of gelatin. In the former case we should be able to prove, first, that twice as many molecules of HBr as of  $H_2SO_4$  combine with a given mass of gelatin; and, second, that the same number of molecules of phosphoric, citric, oxalic, tartaric, and succinic acids as of  $HNO_3$  or HCl combine with the same mass of gelatin. It is shown in the present paper that this is actually the case.

5. It is shown that gelatin sulfate and gelatin bromide solutions of the same pH have practically the same conductivity. This disproves the assumption of colloid chemists that the difference in the effect of bromides and sulfates on the physical properties of gelatin is due to a different ionizing and hydrating effect of the two acids upon the protein molecule.