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## AMPHOTERIC COLLOIDS.

### I. CHEMICAL INFLUENCE OF THE HYDROGEN ION CONCENTRATION.

BY JACQUES LOEB.

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

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#### I.

It is often stated (Pauli, Höber, and others) that both ions of a neutral salt affect the physical properties of an amphoteric electrolyte (ampholyte) simultaneously and in the opposite sense; so that the total result is the algebraic sum of the opposite action of the oppositely charged ions of the neutral salt.<sup>1</sup> The writer has been able to show by experiments on gelatin and on pig's bladder that this statement is not correct, but that apparently only one of the two ions of the neutral salt acts upon the ampholyte; namely, the one which has the opposite electric charge from that of the ampholyte.<sup>2</sup>

The hydrogen ion concentration of the gelatin solution used by the writer was  $10^{-7}$  (or in Sørensen's logarithmic symbol  $\text{pH} = 7.0$ ) and this gelatin reacted with neutral salts as if it were an anion capable of combining with the cation of a neutral salt. When, however, powdered gelatin is treated for some time with an acid, *e.g.* HCl, gelatin chloride (or hydrochloride) is formed, which is supposed to dissociate electrolytically into a positive gelatin ion and a negative chlorion. The writer found that when the supernatant

<sup>1</sup> This and other erroneous statements current in colloid chemistry are due to the fact that the previous investigators always studied the action of neutral salts upon proteins in the presence of the salt. The writer removed these salts after they had a chance to act on the gelatin. This procedure is necessary on account of the fact that the manifestation of the effect of the electrolyte upon the gelatin is repressed in the presence of the electrolyte.

<sup>2</sup> Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343; 1918, xxxiii, 531; xxxiv, 77, 395, 489; 1918, xxxv, 497.

acid is washed away from such powdered gelatin it is affected only by the anion of a neutral salt, while the cation of the salt used has apparently no effect.

The writer found that when powdered gelatin is treated for some time with a base, *e.g.* NaOH, the gelatin (after the supernatant NaOH is washed away) is only capable of combining with the cations of neutral salts, while the anions of neutral salts are without effect.

It seemed necessary to find out where the turning point for these two different modes of reaction lies, whether at the point of neutrality of the solution or at the isoelectric point of gelatin. The fact that gelatin solutions at the point of neutrality ( $\text{pH} = 7.0$ ) react with neutral salts as if they had previously received an alkaline treatment, suggests that the turning point lies at a more acid reaction of the gelatin and since the isoelectric point lies at a more acid reaction it was *a priori* likely that the isoelectric point for gelatin might be the turning point.

Amphoteric electrolytes are defined by the fact that they can dissociate electrolytically as acids as well as bases. When they dissociate as acids they are supposed to send more H ions than OH ions into solution and the ampholyte ion migrates in the electric field to the anode. When they dissociate as bases they are supposed to send more OH ions than H ions into solution, and the ampholyte migrates in the electric field as a cation. At the isoelectric point the ampholyte is supposed to send out an equal number of H and OH ions and it migrates in neither direction. Gelatin is naturally a stronger acid than base and hence migrates to the anode. By increasing the hydrogen ion concentration of the surrounding solution through the addition of some acid the dissociation of gelatin as acid can be repressed to the level of its dissociation as a base. This is the isoelectric point at which it migrates in neither direction and at which the total number of ions it sends into a solution is a minimum. With the addition of more acid it migrates to the cathode. Michaelis determined the isoelectric point of gelatin solutions by the migration method and found it in the neighborhood of  $C_{\text{H}} = 2.10^{-5}$  (or in Sørensen's logarithmic symbol  $\text{pH} = 4.7$ ).<sup>3</sup> Since the solution of gelatin used had a pH of 7.0 it was on the alkali side of the isoelectric point. We shall

<sup>3</sup> Michaelis, L., *Die Wasserstoffionenkonzentration*, Berlin, 1914.

show in this paper that gelatin on the alkali or less acid side of its isoelectric point can combine only with the cations of neutral salts, as we had actually found empirically in our previously published papers.

Moreover, it will follow from the experiments to be published in this paper that the gelatin ion can dissociate electrolytically only as one of the two, either as a cation or as an anion but not as both simultaneously (except to a negligible degree), and that the hydrogen ion concentration of the solution alone determines in which of the two ways it dissociates. We shall also show that the isoelectric point is not only the point where the gelatin sends out as many H as OH ions but that it is the point where it apparently sends out neither; *i.e.*, where it cannot exist in an ionized condition at all.

## II.

Our method of testing whether gelatin reacts with the cation or the anion of a neutral salt is based on the difference of the effects of univalent and bivalent ions upon gelatin. It was found in the writer's experiments on osmotic pressure, viscosity, alcohol precipitation, and swelling that a salt of the type  $\text{Na}_2\text{SO}_4$  (univalent cation, bivalent anion) of a certain molecular concentration, *e.g.*  $\text{m}/128$ , has quantitatively and qualitatively the same effect upon gelatin ( $\text{pH} = 7.0$ ) as a salt of the type  $\text{NaCl}$  at the molecular concentration  $\text{m}/64$ ; *i.e.*, twice the molecular concentration of the  $\text{Na}_2\text{SO}_4$ .<sup>4</sup> This proves that only the cation of the salt and not the anion acts upon the gelatin. It was found, moreover, that all neutral salts with monovalent cation (Li, Na, K,  $\text{NH}_4$ ) cause an increase in osmotic pressure, viscosity, alcohol number, and swelling of gelatin, while salts with a bivalent cation (Mg, Ca, Sr, Ba, Co, Mn) cause no such increase; and if a salt with bivalent cation is added in small quantity to a large quantity of a salt with univalent cation, the increase in osmotic pressure, viscosity, alcohol number, and swelling caused by the salt with univalent cation is inhibited (antagonistic salt action).<sup>5</sup> The same is

<sup>4</sup> Loeb, *J. Biol. Chem.*, 1918, xxxiv, 77, 489.

true for gelatin previously treated with NaOH. When such gelatin is subsequently treated with a salt with univalent cation its viscosity, osmotic pressure, swelling, etc., are increased, while when it is treated with a salt with bivalent cation this increase does not occur and its previous osmotic pressure, etc., are diminished. In all these cases the gelatin migrates as an anion, and the compounds formed are metal gelatinates where the metal is that of the alkali or salt used.

On the other hand, if gelatin has first been treated with an acid, *e.g.* HCl, gelatin chloride is formed which shows an increase in osmotic pressure, swelling, viscosity, and alcohol number. That such a gelatin (which is freed from the original acid by washing) reacts only with the anion of neutral salts is proved by the fact that a solution of a salt of the type  $\text{Ca}(\text{NO}_3)_2$  (univalent anion, bivalent cation) influences gelatin chloride quantitatively and qualitatively like a salt of the type  $\text{NaNO}_3$  (univalent anion, univalent cation) of half the molecular concentration; *e.g.*,  $m/128 \text{ Ca}(\text{NO}_3)_2$  acts like  $m/64 \text{ NaNO}_3$ .<sup>4</sup> This is only possible if the anions of the neutral salt react with the gelatin chloride, while the cations have no effect, since otherwise the depressing effect of Ca mentioned before should be noticeable, which is not the case. If gelatin chloride freed from the excess of acid by washing is treated with a sufficiently high concentration of a neutral salt with univalent anion the osmotic pressure, swelling, viscosity, etc., of the gelatin increase after the salt is washed away. If, however, the anion of the salt used is bivalent, *e.g.* sulfate, oxalate, succinate, it causes less increase or an actual decrease of the osmotic pressure, viscosity, alcohol number, and swelling of the gelatin.

Hence by making use of the depressing effects of bivalent ions and the opposite effect of the univalent ions we can determine at which pH the gelatin begins to combine with the cation and ceases to combine with the anion of a neutral salt. Our experiments show that this critical point lies for gelatin at a hydrogen ion concentration  $2.10 \cdot 10^{-5}$  (pH = 4.7), *i.e.* the isoelectric point of gelatin, and not at the point of neutrality.

<sup>5</sup> All these effects were measured after the excess of the electrolyte was washed away (see footnote 1).

Our method was the same as in our previous experiments. 1 gm. of finely powdered gelatin was put into each of a series of beakers containing 100 cc. of a solution of HCl of various concentrations (M/8 to M/8192). This series served as a control. We then experimented with HCl solutions of different concentrations (M/8 to M/8192), each containing a certain salt (*e.g.* Na<sub>2</sub>SO<sub>4</sub> or Ca(NO<sub>3</sub>)<sub>2</sub>) in the same concentration (M/16). The gelatin remained for 30 minutes in these solutions of a temperature of 20°C. The gelatin was then poured into cylindrical funnels of the same diameter, to allow the acid or acid-salt mixture to drain off, and the gelatin was then perfused three times with 25 cc. of distilled water of 10°C., and a fourth time with H<sub>2</sub>O of 20°C.<sup>6</sup> The gelatin was then melted and made into a 1 per cent solution. Dialysis experiments, titration experiments, and determinations of pH by Sørensen and Clark's indicator method<sup>7</sup> made it probable that the solutions contained no acid except that due to hydrolytic dissociation of the gelatin salt formed; it was also possible to show that the neutral salt added to the HCl solution had been washed away.

The following physical qualities of the washed gelatin were ascertained:

1. The conductivity  $\left(\frac{10,000}{\text{ohms}}\right)$ , at 24°C.
2. The osmotic pressure expressed in mm. height of the 1 per cent gelatin solution in the manometer tube.
3. The total swelling expressed in mm. of the height of the cylindrical mass of gelatin.
4. The alcohol number; *i.e.*, the cc. of 95 per cent alcohol required to precipitate 5 cc. of 1 per cent gelatin solution at 20°C.<sup>8</sup>

The results are given in the form of curves in which the logarithms of the concentration of the acid used are the abscissæ and the above mentioned values for conductivity, osmotic pressure, swelling, and alcohol number, the ordinates. The pH of each gelatin solution was

<sup>6</sup> The water was used at these temperatures to prevent excessive swelling and a retardation of the process of filtration.

<sup>7</sup> We are obliged to Dr. Dernby for demonstrating to us the use of these methods.

<sup>8</sup> The viscosity of the solutions was determined in a special set of experiments and the viscosity curves ran parallel to those for osmotic pressure. They may therefore be omitted in this paper.

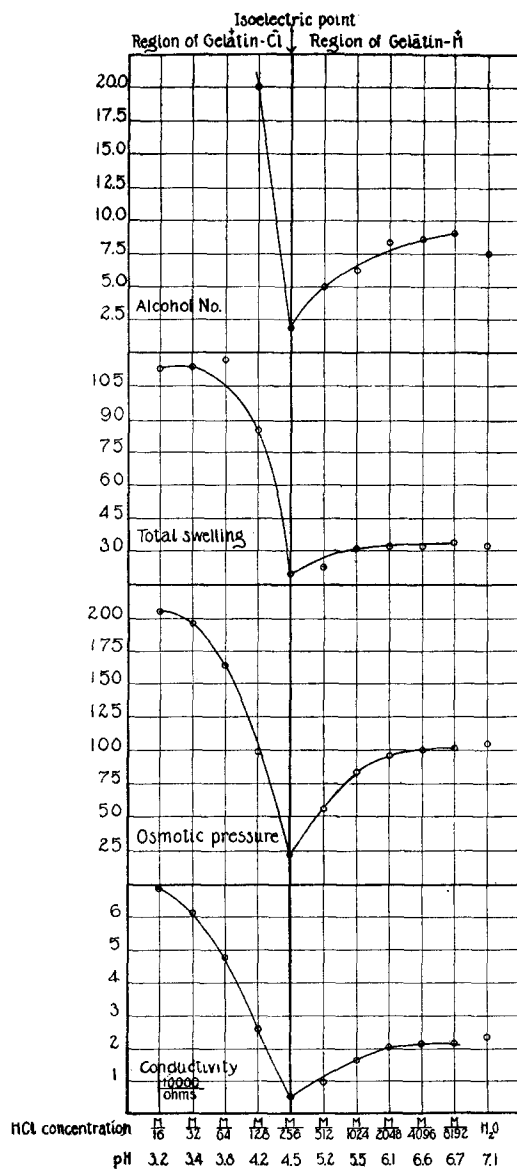


FIG. 1. HCl curves. Curves of the conductivity, osmotic pressure, swelling, and alcohol number of gelatin previously treated with various concentrations of HCl and then freed from excess of HCl by washing with water. On the axis of abscissæ are the logarithms of the HCl concentration used, and under each concentration is the pH of the 1 per cent gelatin solution in  $H_2O$  (made from the washed gelatin). The ordinates are the values for conductivity, osmotic pressure, swelling, and alcohol number for the gelatin. In the region of the isoelectric point ( $pH = 4.7$ ) all the curves have a minimum. On the left of this point gelatin exists in the form of gelatin chloride (with high ionization), the curves rising more rapidly than on the right of the isoelectric point where the gelatin exists in the form of common gelatin which dissociates as a very weak acid. All four curves are nearly parallel.

ascertained and is put under the figures for the logarithms of the concentration.

We now will in turn discuss the four main groups of curves.

Fig. 1 gives the curves for gelatin treated for 30 minutes with HCl alone and then freed from the supernatant and intercapillary acid (with the exception of that formed by hydrolysis of gelatin chloride). The striking fact is that the conductivity, osmotic pressure, swelling, and alcohol number (and viscosity, though this curve is omitted) have all a minimum near a point where the hydrogen ion concentration  $\text{pH} = 4.7$ ; *i.e.*, at about the isoelectric point as determined for gelatin solution by Michaelis on the basis of migration experiments. We may add that the transparency of the solution was also a minimum at the isoelectric point, where the solution was generally quite opaque after standing over night.

Though it was already known<sup>3</sup> that the viscosity and the swelling of gelatin are a minimum at  $\text{pH} = 4.7$ , our experiments for the first time furnish the proof that this is also the exact location of the minimum for conductivity, osmotic pressure, alcohol number, and transparency.

The second fact of significance is the sudden rise of the system of curves on either side of the isoelectric point. Since the conductivity curve is the direct expression of the degree of electrolytic dissociation existing in the gelatin solution (which had been freed from all ions except those formed by electrolytic and hydrolytic dissociation of the gelatin salt), these curves may be considered as a function of the degree of electrolytic dissociation of the gelatin.

Gelatin may exist in the form of non-ionized gelatin on both sides of the isoelectric point, but we shall endeavor to show that ionized gelatin exists in a different ionic state on the two sides of the isoelectric point. On the right, less acid side of the isoelectric point it can only exist as a negative ion (*e.g.*  $\overset{-}{\text{gelatin-H}}$  or  $\overset{-}{\text{gelatin-Na}}$ , etc.), while on the left, more acid side of the isoelectric point it can only exist as a cation (*e.g.*  $\overset{+}{\text{gelatin-Cl}}$  or  $\overset{+}{\text{gelatin-OH}}$ ). When we study the curves for gelatin treated previously with HCl and freed from the excess of HCl by washing as described, the two branches of the curves on the two sides of the isoelectric point are asymmetrical (Fig. 1).



The left branches rise higher than the right ones on account of the fact that on the left side of the isoelectric point we have gelatin chloride which as a salt has a high dissociation constant and which dissociates into gelatin<sup>+</sup> and Cl<sup>-</sup>, while on the right of the isoelectric point the curves are the expression of the electrolytic dissociation of common gelatin which dissociates like a very weak acid into gelatin<sup>+</sup> and H<sup>-</sup>. Hence the limited rise of conductivity on the right and the considerable rise on the left of the isoelectric point.

The parallelism between the curve for conductivity and that for the other physical properties of the gelatin solution is so complete that the idea is unavoidable that the degree of conductivity, *i.e.* of the ionization of the gelatin, determines all the other physical properties of the gelatin solution; the pH only entering to the extent as it influences the possibility and character of the ionization of the gelatin. We shall see presently, however, that this parallelism between the conductivity and the curve for the other physical properties of the gelatin solution does not hold throughout but that in addition to the degree of electrolytic (and hydrolytic) dissociation still another variable enters. The main fact for us is the typical asymmetry of the two branches of the curve to the left and to the right of the isoelectric point, the branch on the left (the more acid side) being steeper and much higher than the branch on the right.

A comparison of the curves of Figs. 2, 3, and 4 with those of Fig. 1 will prove that gelatin when it ionizes will ionize on the right, less acid side of the isoelectric point as an anion, on the left, more acid side of the isoelectric point as a cation.

Fig. 2 represents the effect of the same concentrations of HCl as Fig. 1, except that each acid solution contained so much Na<sub>2</sub>SO<sub>4</sub> as to make them all  $m/16$  for this salt. We will call this system of curves the Na<sub>2</sub>SO<sub>4</sub>-HCl curves to distinguish them from the pure HCl curves represented by Fig. 1. The powdered gelatin was put for 30 minutes at 20° into these acid-salt mixtures and then freed from all the supernatant free acid and salt by the process of washing described in the writer's previous papers. If it is true that the isoelectric point is also a chemical turning point and that gelatin can ionize only as an

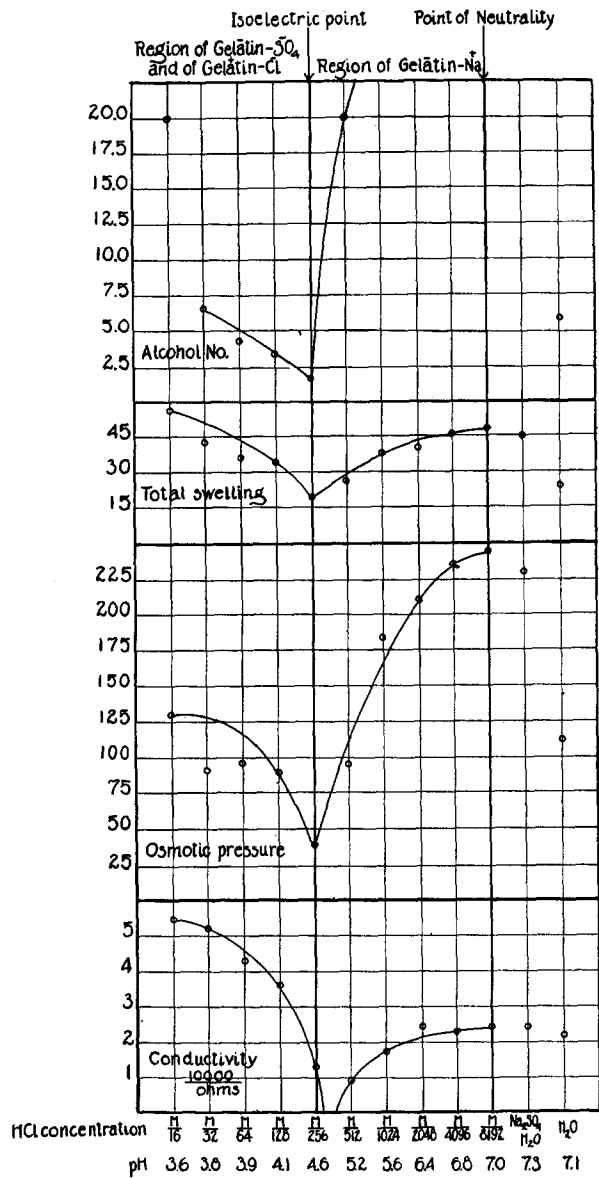


FIG. 2.  $Na_2SO_4$ -HCl curves. Isoelectric point and minimum again near  $pH = 4.7$ . Values for osmotic pressure, swelling, and alcohol number on right of isoelectric point, where gelatin exists as sodium gelatinate, higher than on left, where it exists as mixture of gelatin sulfate and gelatin chloride. The curves for osmotic pressure, swelling, and alcohol number are *not* parallel to the curve for conductivity.

anion on the right side of this point, and as a cation on the left side, we should be able to predict the difference between the two systems of curves, namely the HCl curves and the  $\text{Na}_2\text{SO}_4$ -HCl curves (Figs. 1 and 2), as far as osmotic pressure, swelling, and alcohol number are concerned. On the right, less acid side of the isoelectric point gelatin can dissociate only as a negative ion and hence, reacting with  $\text{Na}_2\text{SO}_4$ , it should be transformed into sodium gelatinate. On the left side, where gelatin can only exist as cation, in a mixture of HCl and  $\text{Na}_2\text{SO}_4$  gelatin chloride and gelatin sulfate should be formed. In the  $\text{Na}_2\text{SO}_4$ -HCl curves (Fig. 2) we should, therefore, expect to find on the right side of the isoelectric point ( $\text{pH} > 4.7$ ) sodium gelatinate, showing a high osmotic pressure, a high alcohol number, etc. On the left side of the isoelectric point where gelatin exists as gelatin chloride and gelatin sulfate, we should find lower values for osmotic pressure, alcohol number, etc., since we have shown in former experiments that the addition of  $\text{SO}_4$  to Cl represses the increase in osmotic pressure, viscosity, swelling, caused by Cl. Hence we should expect an asymmetry in this system of curves of exactly the reverse kind of that found in the pure HCl experiment. A glance at Fig. 2 shows that this is the case, for osmotic pressure, alcohol number, but not for conductivity and pH, which are the same for the same HCl concentrations as in the pure HCl experiment (Fig. 1). We shall return later to this difference which is of great theoretical importance.

Hence we reach the conclusion that the isoelectric point of the gelatin is also the turning point for the chemical reaction between gelatin and neutral salt, inasmuch as the gelatin on the less acid, right side of the isoelectric point can exist in an ionized state only as a negative gelatin ion capable of combining with cations but not with anions, while on the left (more acid) side of the isoelectric point it can exist in an ionized state only as a positive gelatin ion capable of combining with the anions of a neutral salt.

This conclusion is confirmed by the experiments of the  $\text{Ca}(\text{NO}_3)_2$ -HCl series. In this series, represented by the curves of Fig. 3, the same concentrations of HCl from  $\text{M}/8$  to  $\text{M}/4096$  were used and each HCl solution contained so much  $\text{Ca}(\text{NO}_3)_2$  as to make it  $\text{M}/16$  in regard to  $\text{Ca}(\text{NO}_3)_2$ . If our theory is correct, the asymmetry of the

curves for osmotic pressure, swelling, and alcohol number should be the reverse for the  $\text{Ca}(\text{NO}_3)_2$  series as that for  $\text{Na}_2\text{SO}_4$ . On the right side of the isoelectric point (for  $\text{pH} > 4.7$ ) gelatin should exist in the form of Ca gelatinate which according to our previous observations has a low osmotic pressure, a low alcohol number, low viscosity, etc. On the left side of the isoelectric point it should exist in the form of gelatin chloride and nitrate in which form gelatin has a high osmotic pressure, swells considerably, has a high viscosity, and a high alcohol number. The forms of the curves actually found are represented in Fig. 3, and show that the facts agree with our theory.

Our theory finally demands that the two branches of the curve should become almost symmetrical in regard to the isoelectric point, if we add to the HCl a salt with univalent cation and univalent anion, *e.g.* NaBr. NaBr was added to the HCl solutions so as to make the solution  $m/16$  in regard to this salt, and Fig. 4 gives the curves for gelatin treated with NaBr-HCl. It is obvious that the branches of the curves to both sides of the isoelectric point are much more symmetrical than was the case in the three other groups of curves. In this case we have on the right side of the isoelectric point Na-gelatin, on the left a mixture of gelatin bromide and chloride. On both sides of the isoelectric point we have gelatin in combination with univalent ions, which as we have shown in our previous papers yield a high osmotic pressure, a high swelling, etc.

The experiments were repeated with other salts,  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{Na}_2$  oxalate, etc., and our conclusions were confirmed. In the experiments described in this paper the reaction between the anions of the neutral salt with gelatin took place while the gelatin was in the acid solution; while the reaction between the cations of the neutral salt and gelatin could not take place while the gelatin was in the acid solution since all these concentrations were on the acid side of the isoelectric point of gelatin. This reaction must have taken place after the acid treatment and during the process of washing with  $\text{H}_2\text{O}$  when the gelatin previously treated with  $m/256$  HCl had reached a  $\text{pH} > 4.7$ .

This conclusion is confirmed by the experiments in which the gelatin was first treated with acid, then freed from the excess of acid by perfusions with distilled water, and was then treated with a salt and

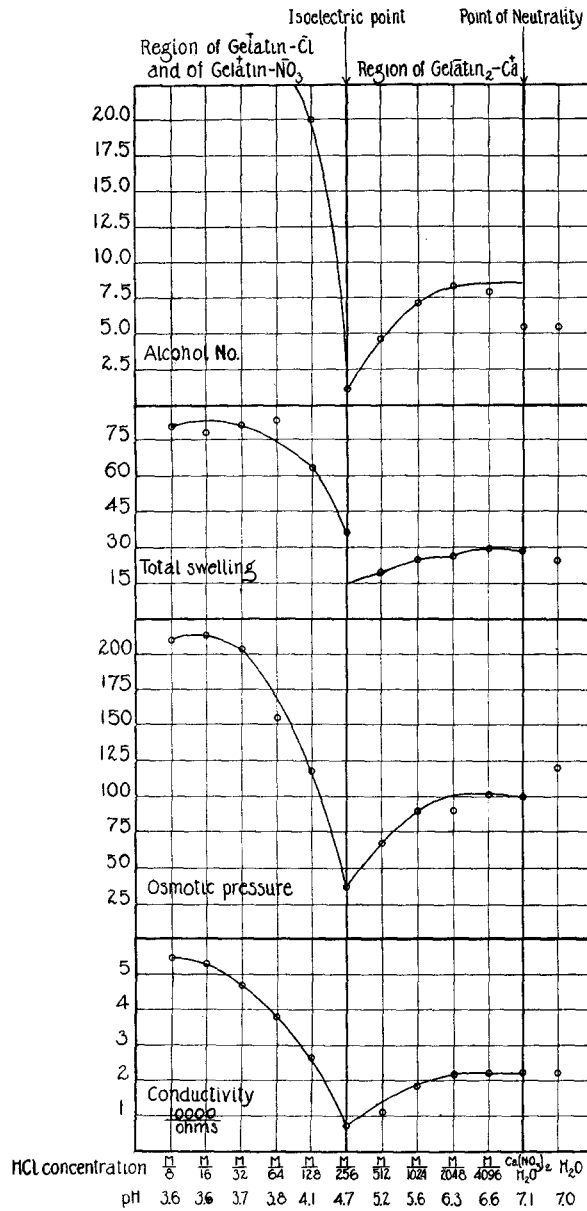


FIG. 3.  $\text{Ca}(\text{NO}_3)_2$ -HCl curves. Minima of all curves again at  $\text{pH} = 4.7$  (isoelectric point). The branches of the curves for osmotic pressure, swelling, and alcohol number on the left, where gelatin exists as mixture of gelatin Cl and gelatin nitrate, higher than the branches on right of the isoelectric point, where gelatin exists as calcium gelatinate.

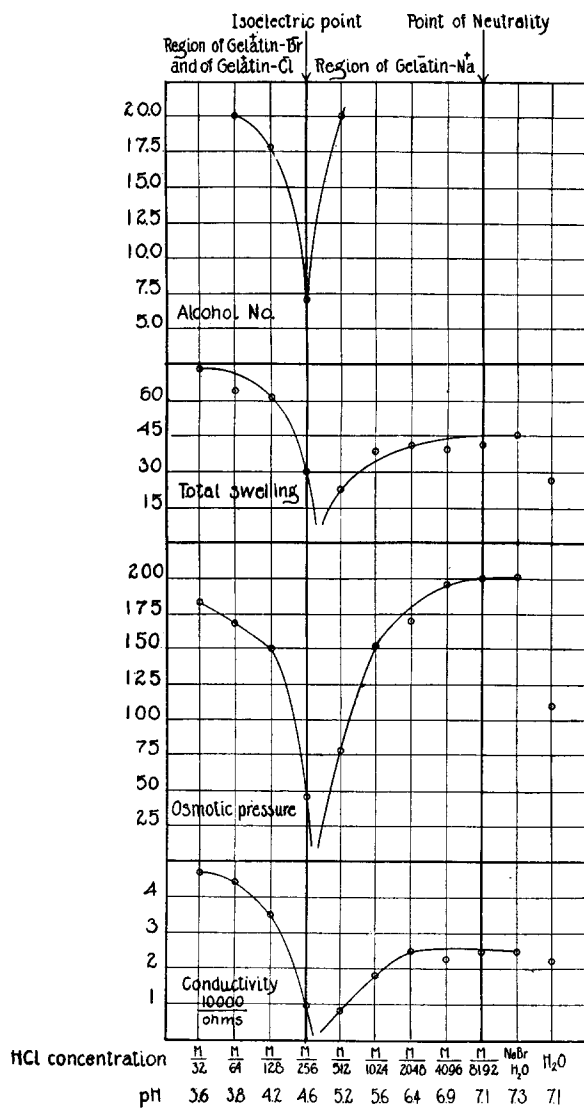


FIG. 4. NaBr-HCl curves. Minima again at  $\text{pH} = 4.7$  (isoelectric point). Curves for osmotic pressure, swelling, and alcohol number almost symmetrical in regard to isoelectric point. On left side gelatin chloride and bromide, on right side sodium gelatinate.

then freed again from the salt by perfusion with water.<sup>9</sup> In this case the same results were obtained as those stated in this paper; namely: *When pH of the gelatin solutions is >4.7, the gelatin can exist in an ionized condition only as an anion capable of combining with cations only to form metal gelatinates, while on the more acid side of the isoelectric point (pH <4.7) gelatin can exist in an ionized form only as a cation capable of combining only with the anion of the neutral salt.* This law holds probably for all amphoteric electrolytes. The question then arises: How does an amphoteric electrolyte react with neutral salts at its isoelectric point? Before we answer this question we will point out an important fact shown by our curves, though this has no direct bearing on the subject under discussion.

### III.

A comparison of the curves for the conductivities with those for the other properties of gelatin in Figs. 1 to 4 shows that the curves for conductivity are practically identical in all four figures as are also the values for pH; while the curves for osmotic pressure, etc., differ according to the valency of the ion combining with the gelatin. Since the conductivity curves are the direct expression of the degree of electrolytic dissociation, we can now say that the difference in the effect of univalent and bivalent ions demonstrated in the writer's previous publications is not merely a difference of the degree of ionization of the gelatin but is due to another effect upon the gelatin molecule. The important work by Langmuir and by Harkins on surface tension has suggested if not proved the idea of polarized molecules and of different degrees of solubility for different parts of the same organic molecule. It seems possible from their work that the solubility is confined to certain groups of the gelatin molecule or the protein molecule in general. It is quite possible that this will ultimately explain why the entrance of a univalent anion or cation into a gelatin molecule increases its solubility in water (or its attracting power for water molecules) and with this its osmotic pressure, alcohol number, viscosity, etc., while the entrance of a bivalent ion (com-

<sup>9</sup> In order not to increase the bulk of this paper we may be pardoned for omitting the details of these experiments.

binning possibly with two or more molecules of gelatin) will not cause or may even prevent such an increase, without altering the conductivity of the gelatin solution. The physical qualities of a gelatin solution depend, therefore, aside from the degree of its electrolytic dissociation and its hydrogen ion concentration upon a constitutional factor varying with the valency of the ion with which the gelatin is in combination. We intend to return to this question in a later publication.

#### IV.

The writer was curious to find out how gelatin reacts with neutral salts when brought to the isoelectric point. This was done by treating gelatin for 30 minutes with  $M/256$  acetic acid or with  $M/256$  or  $M/512$  HCl, etc., and then perfusing the gelatin repeatedly with 25 cc. of  $H_2O$  in the way described. That the gelatin had reached the isoelectric point was ascertained by measurements of pH, of conductivity, osmotic pressure, etc. When gelatin on either side of the isoelectric point is treated with  $M/4$  or  $M/8$  NaCl or any other salt with univalent anion or cation, it shows an increase in osmotic pressure, swelling, alcohol number, after the salt is washed away; when, however, gelatin rendered isoelectric in the way described is treated with the same solution of NaCl, all its physical properties, conductivity, osmotic pressure, swelling, alcohol number, transparency, remain unaltered after the salt is washed away. It is immaterial whether we treat such gelatin with NaCl,  $Na_2SO_4$ ,  $CaCl_2$ , or any other neutral salt; and it is also immaterial whether we use high or low concentrations of these salts; the physical qualities of the gelatin remain unaltered. Numerous experiments were made in which the nature of the acid, the concentrations, the number of washings, etc., used to reach the isoelectric point varied, but all yielded the same result, which means that *at the isoelectric point gelatin is chemically inert*, incapable of reacting with neutral salts. When, however, the pH of the gelatin deviates only slightly from that of the isoelectric point by becoming either  $>$  or  $< 4.7$ , the neutral salts react with gelatin in the way described in this and previous papers.

This is exactly what our theory demands, according to which gelatin can dissociate electrolytically only as an anion when pH is  $> 4.7$ , as



a cation when pH is  $<4.7$ . Hence it can dissociate as neither when  $\text{pH} = 4.7$ . Not being able to dissociate electrolytically, it cannot react chemically with the ions of a neutral salt, as was found to be the case.

This inability to dissociate electrolytically and hence to react with neutral salts at the isoelectric point should be a common property of all amphoteric electrolytes and this seems to be the case. While this note goes to press a paper has appeared by Van Slyke and Baker,<sup>10</sup> containing the following sentence. "At the isoelectric point casein combines with neither acid nor base;" *i.e.*, with neither cation nor anion.

### V.

The facts shown thus far support the idea that amphoteric electrolytes dissociate in a different way on the two sides of the isoelectric point; on the less acid side of that point they can ionize only as anions capable of combining with the cations of neutral salts, but not with their anions; on the acid side of the isoelectric point they can ionize only as cations capable of combining with the anion of a neutral salt, but not with its cation. The question then arises: What will happen when we bring a salt like sodium gelatinate in which gelatin is an anion on the acid side of the isoelectric point? Our theory demands that sodium gelatinate produced by treating gelatin with NaOH should not continue to exist when  $\text{pH} < 4.7$ , and that in this case gelatin should behave as if it had not been treated with NaOH. This is what actually happens. The writer made this experiment sometime before he had arrived at the theory published in this paper. He had undertaken the experiment in the expectation that gelatin first treated with alkali and then with acid should show a superposition of the effects of both treatments. 1 gm. of finely powdered gelatin was put for 30 minutes at  $20^{\circ}\text{C}$ . into each of a series of beakers all containing  $\text{m}/32$  NaOH. After this, each powder was put on a filter, the alkali was allowed to drain off, and each filter was perfused four times with 25 cc. of  $\text{H}_2\text{O}$  to remove the last traces of NaOH held in the capillary spaces (except the NaOH formed by the hydrolytic dissociation of the sodium gelatinate). Then each filter was perfused three

<sup>10</sup> Van Slyke, L. L., and Baker, J. C., *J. Biol. Chem.*, 1918, xxxv, 147.

times with 25 cc. of a HCl solution which varied for the different filters from  $M/8$  to  $M/8192$ , and after this each filter was perfused four times with  $H_2O$  to remove the HCl not bound by the gelatin.

The result is given in the curves of Fig. 5 for the conductivity, osmotic pressure, swelling, and alcohol number. The curves show a marked minimum at about  $pH = 4.8$ ; *i.e.*, the isoelectric point. The appearance of this low minimum of the values for conductivity, osmotic pressure, swelling, etc., characteristic of the isoelectric point, was at first a surprise and a puzzle to the writer. On the right side the curve of osmotic pressure rises to a value of 125 to 150 mm. which corresponds to the value for gelatin treated with  $M/32$  NaOH and washed ten times with  $H_2O$  (without any subsequent treatment with HCl). On the acid side of the isoelectric point the curve of osmotic pressure rises to that point which corresponds to the effect the same concentration of HCl has on gelatin not previously treated with alkali. Hence the result of the acid treatment is on the left, more acid side from the isoelectric point, exactly the same as when we use gelatin not previously treated with  $M/32$  NaOH and apply to it the same treatment with HCl as in this experiment; while on the right, less acid side of the isoelectric point, it is the same as if the gelatin had been treated with NaOH of increasing concentrations.

This permits of only one explanation, which in the light of the experiments published in the preceding parts of this paper is as follows. Sodium gelatinate, or perhaps more correctly a negative gelatin ion, cannot continue to exist except at hydrogen ion concentrations lower than that defining the isoelectric point. This means that in the acid solutions all or most of the sodium gelatinate must have given off its sodium ions and must have been retransformed into the weak gelatin acid; or some other constitutional (tautomeric?) change must have occurred in the gelatin molecule on the acid side of the isoelectric point. On the alkali side of the isoelectric point the sodium gelatinate could and did continue to exist.

This experiment was repeated with different concentrations of acids and alkalies, all confirming our conclusions. The experiment was also reversed, *i.e.* the gelatin was first treated with acid and then with alkali, and again our theory was confirmed.

It is of interest to see our view confirmed also by another set of facts. It has long been known that proteins and probably ampho-

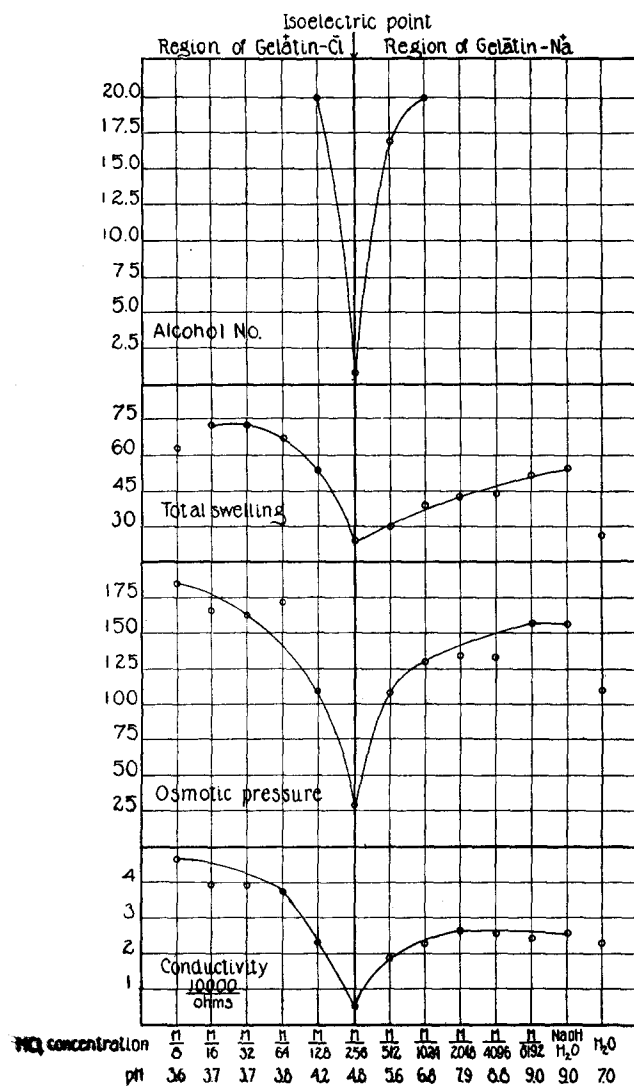


FIG. 5.  $m/32$  NaOH followed by HCl treatment. Minima at  $\text{pH} = 4.8$  (isoelectric point). Branches of curves on left side almost identical with those of Fig. 1 (where HCl alone was applied). On the left side of isoelectric point gelatin exists as gelatin chloride, on the right side as sodium gelatinate.

teric electrolytes in general stain with acid dyes in an acid but not in an alkali solution, and with basic dyes only in an alkaline solution. This agrees with our theory that an amphoteric electrolyte like gelatin can exist as an anion capable of combining with a basic dye (*e.g.* neutral red) only on the basic side of its isoelectric point, and that gelatin can exist as a cation capable of combining with acid dyes (*e.g.* acid fuchsin) only on the acid side of its isoelectric point. Our theory further demands that gelatin when stained with neutral red should give off its dye in solutions on the acid side of its isoelectric point, since on this side of the isoelectric point gelatin can no longer exist as an anion, while gelatin stained with acid fuchsin should give off its dye in solutions with a  $\text{pH} > 4.7$ , since here the gelatin can no longer exist as a cation. All this turns out as our theory demands. The writer intends to make further experiments on this problem, but the facts thus far observed support our theory that when dissociating electrolytically an amphoteric electrolyte is stable as an anion only on the basic side of its isoelectric point, and as cation on the acid side of its isoelectric point. Of course, in a non-ionized condition gelatin may exist at any pH.

Another apparent support of our theory lies in the old observation made by Hamburger<sup>11</sup> on the absorption of Cl and  $\text{SO}_4$  by the red blood corpuscles from serum when the  $\text{CO}_2$  of the blood rises. In this case it is probably simply a question of raising the pH of the blood above that of the isoelectric point for hemoglobin, which is near the point of neutrality. The whole problem of the retention and excretion of electrolytes by the body may become intelligible from the fact proved in this paper that proteins or amphoteric electrolytes in general can exist as cations only (and have a tendency to become cations) on the more acid side of the isoelectric point, while they can exist as anions only (and have a tendency to become anions) on the less acid side of their isoelectric point.

<sup>11</sup> Hamburger, H. J., and van Lier, G. A., *Arch. Physiol.*, 1902, 492. Hamburger, *Biochem. Z.*, 1918, lxxxvi, 309. See also, Hasselbalch, K. A., and Warburg, E. J., *ibid.*, 1918, lxxxvi, 410.

## SUMMARY.

1. It has been shown in this paper that while non-ionized gelatin may exist in gelatin solutions on both sides of the isoelectric point (which lies for gelatin at a hydrogen ion concentration of  $C_H = 2.10 \cdot 10^{-6}$  or  $\text{pH} = 4.7$ ), gelatin, when it ionizes, can only exist as an anion on the less acid side of its isoelectric point ( $\text{pH} > 4.7$ ), as a cation only on the more acid side of its isoelectric point ( $\text{pH} < 4.7$ ). At the isoelectric point gelatin can dissociate practically neither as anion nor as cation.

2. When gelatin has been transformed into sodium gelatinate by treating it for some time with  $M/32$  NaOH, and when it is subsequently treated with HCl, the gelatin shows on the more acid side of the isoelectric point effects of the acid treatment only; while the effects of the alkali treatment disappear completely, showing that the negative gelatin ions formed by the previous treatment with alkali can no longer exist in a solution with a  $\text{pH} < 4.7$ . When gelatin is first treated with acid and afterwards with alkali on the alkaline side of the isoelectric point only the effects of the alkali treatment are noticeable.

3. On the acid side of the isoelectric point amphoteric electrolytes can only combine with the anions of neutral salts, on the less acid side of their isoelectric point only with cations; and at the isoelectric point neither with the anion nor cation of a neutral salt. This harmonizes with the statement made in the first paragraph, and the experimental results on the effect of neutral salts on gelatin published in the writer's previous papers.

4. The reason for this influence of the hydrogen ion concentration on the stability of the two forms of ionization possible for an amphoteric electrolyte is at present unknown. We might think of the possibility of changes in the configuration or constitution of the gelatin molecule whereby ionized gelatin can exist only as an anion on the alkaline side and as a cation on the acid side of its isoelectric point.

5. The literature of colloid chemistry contains numerous statements which if true would mean that the anions of neutral salts act on gelatin on the alkaline side of the isoelectric point, *e.g.* the alleged effect of the Hofmeister series of anions on the swelling and osmotic pressure of common gelatin in neutral solutions, and the statement

that both ions of a neutral salt influence a protein simultaneously. The writer has shown in previous publications that these statements are contrary to fact and based on erroneous methods of work. Our present paper shows that these claims of colloid chemists are also theoretically impossible.

6. In addition to other physical properties the conductivity of gelatin previously treated with acids has been investigated and plotted, and it was found that this conductivity is a minimum in the region of the isoelectric point, thus confirming the conclusion that gelatin can apparently not exist in ionized condition at that point. The conductivity rises on either side of the isoelectric point, but not symmetrically for reasons given in the paper. It is shown that the curves for osmotic pressure, viscosity, swelling, and alcohol number run parallel to the curve of the conductivity of gelatin when the gelatin has been treated with acid, supporting the view that these physical properties are in this case mainly or exclusively a function of the degree of ionization of the gelatin or gelatin salt formed. It is pointed out, however, that certain constitutional factors, *e.g.* the valency of the ion in combination with the gelatin, may alter the physical properties of the gelatin (osmotic pressure, etc.) without apparently altering its conductivity. This point is still under investigation and will be further discussed in a following publication.

7. It is shown that the isoelectric point of an amphoteric electrolyte is not only a point where the physical properties of an ampholyte experience a sharp drop and become a minimum, but that it is also a turning point for the mode of chemical reactions of the ampholyte. It may turn out that this chemical influence of the isoelectric point upon life phenomena overshadows its physical influence.

8. These experiments suggest that the theory of amphoteric colloids is in its general features identical with the theory of inorganic hydroxides (*e.g.* aluminum hydroxide), whose behavior is adequately understood on the basis of the laws of general chemistry.

*Addendum.* Since this paper went to print the writer has been able to show by volumetric analysis that the view expressed in this paper is correct; namely, that on the alkaline side from the isoelectric point gelatin combines with the cation of a neutral salt,

while on the more acid side from the isoelectric point it combines with the anion of a neutral salt. The curve for the amount of cation or anion in combination with the gelatin runs parallel with the curves for osmotic pressure, swelling, etc. The new experiments will be discussed in the second number of this *Journal*.