AMPHOTERIC COLLOIDS.

II. Volumetric Analysis of Ion-Protein Compounds; the Significance of the Isoelectric Point for the Purification of Amphoteric Colloids.

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INTRODUCTION.

In order to understand the influence of the hydrogen ion concentration upon the chemical and physical behavior of proteins we may well keep in mind the rôle of the hydrogen ion concentration upon the reactions of an amphoteric metal hydroxide, like aluminium or chromic hydroxide, whose chemical constitution is better known than that of the proteins.

"Aluminium hydroxide dissolves in acids. From its solution in hydrochloric acid, an aluminium salt, aluminium chloride $AlCl_3$, $6H_2O$, is obtained. It also combines with strong bases, dissolving for instance in a solution of sodium hydroxide and forming an aluminate, $NaAlO_2$.... According to the best knowledge we have on the subject, the molecule of aluminium hydroxide has the following structure or arrangement of its atoms $Al(-O-H)_3$. It is readily seen that the cleavage of the molecules may produce, either aluminium and hydroxide ions, characteristic ions of a base, or aluminate and hydrogen ions, characteristic ions of an acid:

$$Al^{+++} + 3 \overline{OH} \rightleftharpoons Al(-O-H)_3 \rightleftharpoons A\overline{lO_2} + H^+ + H_2O."[1]$$
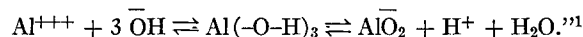
It is obvious that between the action of acids and of bases producing these two types of electrolytic dissociation there must be one hydrogen ion concentration in which aluminium hydroxide is practically neither able to form Al^{+++} nor $Al\overline{O_2}$ ions, and this would be the isoelectric point.

[1] Stieglitz, J., The elements of qualitative chemical analysis, New York, 1911, 171.

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We may apply the experience gained from aluminium hydroxide to proteins which are also amphoteric electrolytes and for some of which the exact location of the isoelectric point is known. For gelatin it lies at pH = 4.7. In a previous paper[2] it was shown that when pH > 4.7, gelatin is only able to dissociate as gelatin (capable of combining with metals to form metal gelatinates, e.g. sodium gelatinate); when pH < 4.7, gelatin is only capable of dissociating as gelatin (capable of combining with anions to form, e.g., gelatin chloride). At the isoelectric point it is practically not dissociated at all. There is this difference between amphoteric electrolytes of the type of proteins and that of metal hydroxides, namely that in the case of proteins the OH ions and the H ions are attached to different groups in the molecule.

We also have shown that a metal gelatinate must give off its metal ion when the hydrogen ion concentration is raised beyond that of the isoelectric point; and that a gelatin-anion compound must give off its anion when the hydrogen ion concentration is lowered below that of the isoelectric point. At the isoelectric point gelatin cannot be in combination with either anion or cation and such gelatin free from *ionogenic* impurities we designate as pure gelatin. It would thus appear that we can free proteins and amphoteric colloids in general from *ionogenic* impurities by bringing the ampholyte to its isoelectric point. Such impurities, however, as form part of the complex gelatin ion can, of course, not be removed by this method. In this and some following papers we intend to furnish additional proof for the correctness of these ideas. This can be done by making the gelatin combine with such ions as Ag or Br or CNS, etc., whose quantity can be easily determined volumetrically.

The Combination of Cation with Gelatin.

In all experiments in which the chemical or physical influence of an electrolyte upon an amphoteric colloid is to be investigated it is necessary to remove the excess of electrolyte after it has had time to act on the colloid; and the writer has shown that the neglect of this precaution has caused a good deal of the confusion which prevails in the

[2] Loeb, J., *J. Gen. Physiol.*, 1918, i, 39.

literature of colloid chemistry. By using gelatin in the condition of a fine powder we are able to wash away the excess of electrolyte after it has had time to act on the gelatin.

When we put 1 gm. of powdered gelatin for 30 minutes at 20 or 15° into 100 cc. of 3 $M/1024$ HCl or HNO_3, and then wash the gelatin on the filter to remove the excess of acid, we obtain a gelatin with pH of about 4.7; *i.e.*, of the isoelectric point. When we use a higher concentration than 3 $M/1024$ HCl, we get a pH< 4.7, and a gelatin which is on the acid side of the isoelectric point and which is able to act only as cation; when we use an acid less concentrated than 3 $M/1024$, we get after the excess of acid is washed away a gelatin whose pH > 4.7 and which being on the alkaline side of its isoelectric point can act only as an anion and can combine only with a cation.

In order to get clear results it is well to work with a series of different concentrations of an acid, *e.g.* $M/4$, $M/8$, $M/16$, etc., to $M/8192$ HCl or HNO_3, so that we obtain series of gelatin solutions with different pH on either side of the isoelectric point. After the excess of acid is washed away by several perfusions with H_2O, the gelatin is perfused three times with 25 cc. of the same solution of a salt, *e.g.* $M/8$ NaBr or $M/8$ NaCNS or $M/16$ $AgNO_3$, to bring about a reaction between salt and gelatin. It is necessary to stir up the gelatin on the filter while the salt solution is poured on to bring about as complete a contact between the powdered particles of gelatin and salt as possible. After the salt solution has drained off, the excess of salt is washed away by six perfusions with 25 cc. of H_2O each. The gelatin is then melted and made into a 1 per cent solution whose pH is determined and which is then analyzed for Br or silver, as the case may be. We shall describe some of the results obtained by this method.

1 gm. of finely powdered gelatin is put for 30 minutes at 15°C. into each of a series of beakers containing 100 cc. of HNO_3 varying from $M/8$ to $M/8192$; and into one beaker containing 100 cc. of distilled water serving as a control. The gelatin is then put on a filter, washed, and is perfused three times with 25 cc. of $M/16$ $AgNO_3$ in the way described, and subsequently six times with 25 cc. of H_2O to wash away the excess of $AgNO_3$. The swelling is measured while the gelatin is still in the cylindrical funnel, and the gelatin is melted and made into a 1 per cent solution. This part of the experiment is of course carried

out in a dark room.   Then the pH is determined, and 25 cc. of the solution are used to determine the quantity of silver in combination with the gelatin, according to Volhard's method.

Fig. 1 contains two curves the abscissæ of which are the logarithms of the concentrations of HNO_3 with which the gelatin was originally
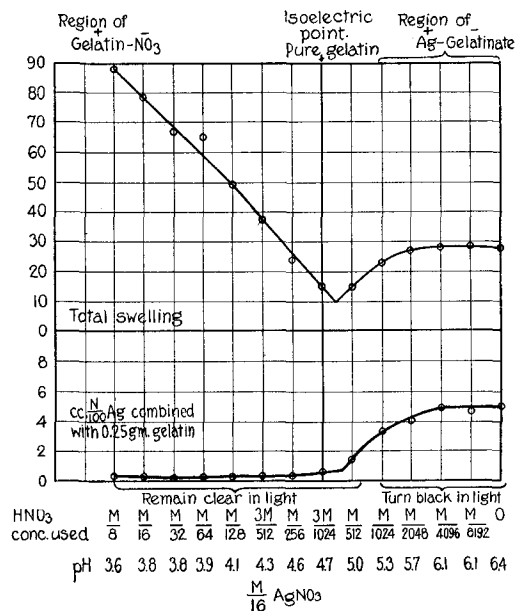

FIG. 1. Gelatin treated with different concentrations of HNO_3, from $M/8$ to $M/8192$, washed, and then treated with the same concentration of $AgNO_3$ ($M/16$), and then washed again.   Abscissæ show concentrations of acid used.   The final pH of the gelatin solution is found under the figure for the concentration of acid used.

The ordinates of the lower curve give the values for the silver found in combination with the gelatin.   The curve shows that at the isoelectric point (pH = 4.7) and on the acid side of the isoelectric point, the gelatin was practically free from silver.   On the more alkaline side the amount of silver found in combination with the gelatin increased with the pH.   This proves that gelatin can combine with a cation only on the alkaline side from the isoelectric point, and this is corroborated by the fact that on the alkaline side from the isoelectric point only was the gelatin darkened by light.   The ordinates of the upper curve are the values for the swelling of the same gelatin.   On the alkaline side from the isoelectric point, where the gelatin had combined with silver, the curve for swelling runs parallel to the curve for silver gelatinate formed.   It was, therefore, the relative mass of silver gelatinate formed which determined the physical properties of gelatin.

treated.   Under each concentration is put the pH found for the gelatin
solution at the end of the experiment.   The ordinates of the lower
curve are the numbers for the cc. of 0.01 N silver in combination with
0.25 gm. of gelatin.   The curve shows that at the isoelectric point
(pH = 4.7) the gelatin is free from ionic silver and that the same is
true for all the gelatin on the acid side from the isoelectric point (pH
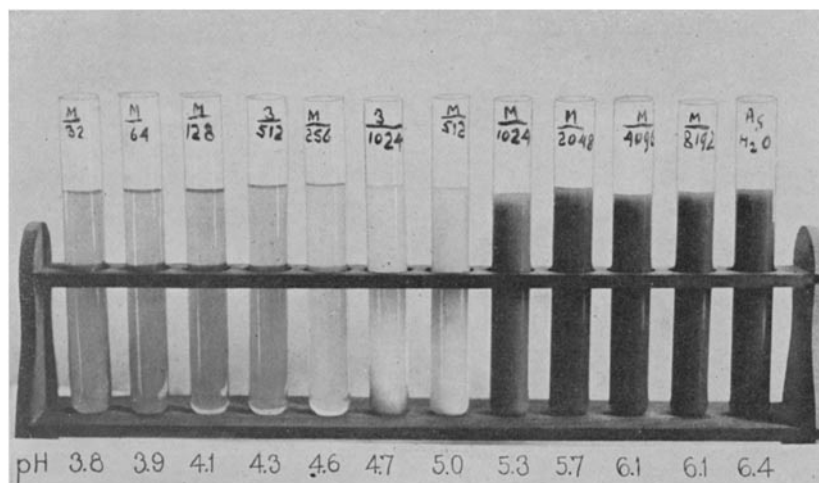3.6 to 4.7).   On the alkaline side from the isoelectric point the

FIG. 2. Photograph of the gelatin solutions whose curves are contained in Fig.
1, taken a week after the experiment was made.   The gelatin with a pH > 5.0
turned dark brown in a few hours when exposed to diffused daylight, while the
gelatin with a pH ≲ 4.7 was not affected by light after an exposure of over a week.
The photograph also shows that owing to the precipitate in suspension the tubes
containing the gelatin at the isoelectric point, pH = 4.6 and 4.7, were more
whitish than the rest, which were slightly yellow.

amount of ionic silver in combination with gelatin rises in a regular
curve increasing in height with the increase in pH.

20 cc. of each gelatin solution were put into a test-tube and exposed
to the light.   The five test-tubes that had been treated with M/1024
or less concentrated HCl turned rapidly dark or black in the light,
while none of the others did, even when made slightly alkaline and
though the exposure lasted for more than a week.   The gelatin treated

with 3 M/1024 acid (pH = 4.7) was quite opaque but remained perfectly white; while the test-tube treated with M/256 was only slightly opaque but remained also practically white. This shows that the gelatin treated with 3 M/1024 and whose pH was 4.7 was also entirely free from ionized silver. Fig. 2 is a photograph of these test-tubes. This experiment was repeated several times with the same result. It is a very striking demonstration experiment.

On the more acid side, where the gelatin was free from silver, the gelatin existed as gelatin nitrate as we shall prove presently. This then shows that at the isoelectric point and on the acid side from the isoelectric point a cation cannot combine with gelatin, while on the more alkaline side from the isoelectric point such a combination occurs.

Table I gives the cc. of 0.01 N Ag found in combination with 0.25 gm. of gelatin.

TABLE I.

Cc. 0.01 N Ag in combination with 0.25 gm. of gelatin at different pH.

pH	3.6	3.8	3.8	3.9	4.1	4.3	4.6	4.7	5.0	5.3	5.7	6.1	6.1	6.4
Cc. 0.01 N Ag combined with 0.25 gm. of gelatin	0.5	0.3	0.4	0.3	0.2	0.2	0.2	0.55	1.25	3.2	4.0	4.85	4.6	4.9

The small values of 0.2 cc. or even 0.5 cc. found in the gelatin on the acid side from the isoelectric point are due to incomplete removal by washing; an increase in the number of washings would probably have removed these traces also. Where 0.5 or less cc. of 0.01 N Ag was found in 0.25 gm. of gelatin, light no longer blackened the gelatin. As soon as pH became greater than 4.7 the value of Ag found rose suddenly.

Fig. 3 represents the physical properties of gelatin treated first with M/16 AgNO₃ and then brought to different hydrogen ion concentrations by treatment with different concentrations of HNO₃. 1 gm. of powdered gelatin was first put for 30 minutes into 100 cc. of M/16 AgNO₃ (pH about 6.9) and the excess of salt was then washed away (in the manner described). Then each gm. of gelatin was perfused three times with a definite concentration of HNO₃ and the excess of acid was washed away by four perfusions with distilled water. The
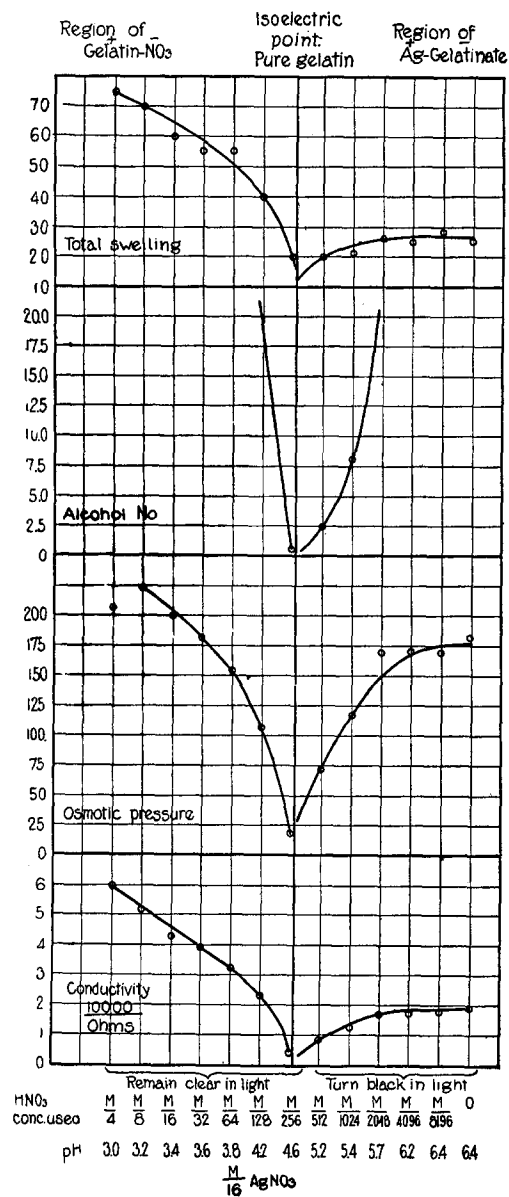
FIG. 3. Gelatin treated first with M/16 AgNO₃, washed, then treated with different concentrations of HNO₃, and washed again. Curves for conductivity. osmotic pressure, alcohol number, and swelling. On the right from the isoelectric point the gelatin exists as silver gelatinate, on the left as gelatin nitrate, and at the isoelectric point as pure, sparingly soluble gelatin.

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swelling of the gelatin was measured while it was in the cylindrical funnel and the height of the column of gelatin in mm. expresses the degree of swelling, all the funnels having the same diameter. The gelatin was then melted, made into a 1 per cent solution, and the conductivity, osmotic pressure (in mm. of the height of the gelatin solution), alcohol number (cc. of 95 per cent alcohol required to cause a definite degree of precipitation in 5 cc. of 1 per cent gelatin solution) were determined. There is an abrupt fall in all the curves near pH 4.6; i.e., at about the isoelectric point, and a rise on both sides. On the right from the isoelectric point (pH > 4.7) the gelatin exists as silver gelatinate, while on the left, more acid side from the isoelectric point (pH < 4.7) the gelatin exists as gelatin nitrate. At the isoelectric point it exists as non-ionized gelatin. This was proved by exposing the gelatin solutions to the light after completion of the experiment which was of course carried out in a dark room; all those gelatin solutions whose pH was from 5.3 to 6.4 turned black when exposed to light, while none of the others was affected by light even after an exposure of more than 2 weeks. Since the original treatment of the gelatin with $M/16$ $AgNO_3$ near the point of neutrality was the same for all the solutions, it is obvious that the silver gelatinate when its pH was 4.7 or less gave off part if not practically all its silver. This was actually proved to be the case by the analysis of the filtrate of such gelatin during and after the acid treatment.

When we treat the gelatin first with HNO_3, wash the acid away, and then treat the gelatin with $M/16$ $AgNO_3$ and wash away the excess of salt, we get an identical system of curves and an identical effect of exposure to light, proving that gelatin can only combine with Ag when its pH > 4.7, while it cannot combine with gelatin when its pH $\lessgtr$ 4.7.

The writer would like to call attention to the fact shown in Figs. 1 and 3 that for pH > 4.7 the curves for swelling and for conductivity are practically parallel to the curve for the amount of silver gelatinate formed.

The Combination of Anions with Gelatin.

In this chapter we intend to furnish the data necessary for proving that gelatin combines with anions only when pH < 4.7, while it can-

not combine or remain in combination with anions when pH $\gtrless$ 4.7. We chose for this purpose two anions, Br and CNS, for the volumetric determination of which we possess the convenient Volhard method. Powdered gelatin was first treated with different concentrations of HNO_3 and then washed free from the excess of acid. Then the gelatin in each funnel was perfused three times with 25 cc. of $M/8$ NaBr and this was followed by six perfusions with 25 cc. of H_2O. The swelling was plotted (upper curve in Fig. 4), the gelatin was melted and made into a 1 per cent solution, and analyzed for Br by the Volhard method. The lower curve gives the quantity of Br in combination with 25 cc. of gelatin. Table II gives the amount of Br found in combination with 0.25 gm. of gelatin. The table, as well as the curve, shows that the treatment of gelatin with $M/8$ NaBr did not lead to the formation of gelatin bromide when pH $\gtrless$ 4.7; while it led to the formation of gelatin Br when pH $<$ 4.7. The quantity of gelatin bromide formed increased with the hydrogen ion concentration.

TABLE II.

Cc. 0.01 N Br in combination with 0.25 gm. of gelatin at different pH.

pH	3.7	3.8	3.9	4.1	4.2	4.6	4.7	5.1	5.8	6.4	6.8	7.0	7.1
Cc. 0.01 N Br combined with 0.25 gm. of gelatin	7.5	7.4	7.1	5.0	2.95	1.4	0.1	0.15	0.1	0.15	0.2	0.1	0.1

In the experiment represented by Fig. 5 everything was the same except that the treatment of gelatin with HNO_3 was followed by three perfusions with $M/8$ NH_4CNS (instead of $M/8$ NaBr). The lower curve shows that when pH $\gtrless$ 4.7 the gelatin contains no CNS, but that gelatin can combine with CNS as soon as pH $<$ 4.7.

The results are given in Table III. The reader's attention is called to the sharp drop in the values for Br (Table II) as well as for CNS (Table III) at pH $=$ 4.7 and less.

TABLE III.

Cc. 0.01 N CNS in combination with 0.25 gm. of gelatin at different pH.

pH	3.5	3.5	3.9	4.1	4.3	4.4	4.7	4.9	5.5	6.2	6.3	6.4	6.5	7.0
Cc. 0.01 N CNS combined with 0.25 gm. of gelatin	9.4	9.2	6.7	4.1	3.4	1.95	0.15	0.2	0.1	0.1	0.1	0.1	0.1	0

When we reverse the order of treatment, *i.e.* when we treat gelatin first with M/8 NaBr at pH about 7.0, only sodium gelatinate should be formed according to our theory, and no Br should combine with the gelatin. This was found to be correct. When we treat gelatin first
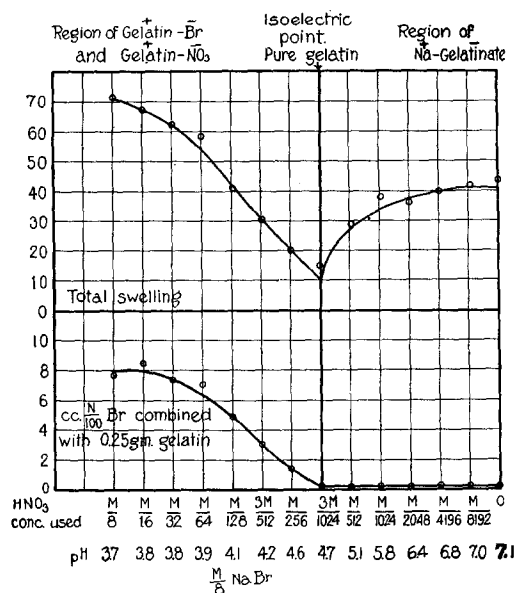

Fig. 4. Gelatin treated with different concentrations of HNO₃, washed, treated with M/8 NaBr, and washed again. The ordinates of the lower curve are the values of Br in combination with gelatin, showing that on the right (alkaline) side from the isoelectric point and at the isoelectric point gelatin contains no Br, while on the left, more acid side from the isoelectric point, the amount of the Br found increases with the pH. The ordinates of the upper curve represent the swelling of the same gelatin. The two curves on the left side are almost parallel, showing that the degree of swelling is determined by the relative mass of the gelatin bromide (or nitrate) formed.

with M/8 NaBr, then with varying concentrations of HNO₃, no Br should enter into ionogenic combination with the gelatin. This is confirmed by the volumetric analysis which shows that such gelatin is free from ionic Br. Only sodium gelatinate is formed under these conditions.

Figs. 4 and 5 confirm a fact already referred to in connection with Fig. 1. The curves for the swelling of gelatin for pH $<$ 4.7 are parallel to the curves representing the amount of gelatin Br and gelatin CNS formed. Fig. 6 gives the relation of the curve for swelling to the curves for the other physical properties of gelatin treated with acid and then with M/8 NaBr. These experiments prove definitely that on the acid side from the isoelectric point gelatin (and probably amphoteric colloids in general) can combine only with anions, on the
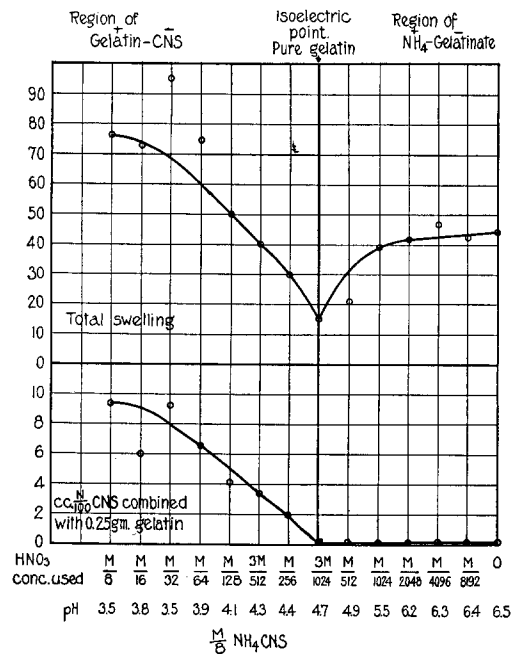

FIG. 5. Gelatin treated with different concentrations of HNO$_3$, washed, then treated with M/8 NH$_4$CNS, and washed again. Further explanation of figure corresponds to that for Fig. 4.

more alkaline side from the isoelectric point only with cations, and at the isoelectric point with neither.

Action of Heavy Metals and Polyvalent Ions.

When we treat finely powdered gelatin with a solution of M/10 copper acetate, the powder assumes a blue color due to the formation

Region of Gelatin-$\overset{+}{B}$r
and Gelatin-$\overset{+}{C}$l

Isoelectric
point
Pure gelatin

Region of
Na-Gelatinate

70
60
50
40
30

Total swelling

20

20.0
17.5
15.0
12.5
10.5
7.5
5.0
2.5
0

Alcohol No.

250
225
200
175
150
125
100
75
50
25

Osmotic pressure

4
3
2

Conductivity
10000
Ohms

HCl
conc. used $\dfrac{M}{4}$ $\dfrac{M}{8}$ $\dfrac{M}{16}$ $\dfrac{M}{32}$ $\dfrac{M}{64}$ $\dfrac{M}{128}$ $\dfrac{M}{256}$ $\dfrac{M}{512}$ $\dfrac{M}{1024}$ $\dfrac{M}{2048}$ $\dfrac{M}{4096}$ $\dfrac{M}{8192}$ 0

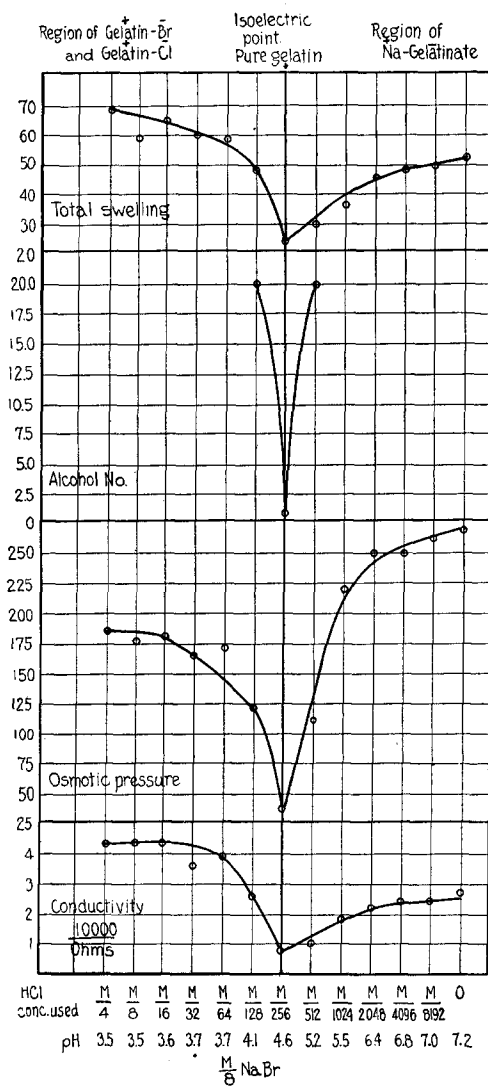pH   3.5 3.5 3.6 3.7 3.7 4.1 4.6 5.2 5.5 6.4 6.8 7.0 7.2

$\dfrac{M}{8}$ NaBr

FIG. 6. Gelatin treated first with varying concentrations of HCl, washed, then treated with M/8 NaBr, and washed again. Curves for conductivity, osmotic pressure, alcohol number, and swelling. On the right side from the isoelectric point gelatin exists as sodium gelatinate, on the left side as gelatin chloride or bromide, at the isoelectric point as pure, sparingly soluble gelatin.

of copper gelatinate (pH being about 6.3). When we wash off the excess of copper acetate solution, the blue color remains. When we treat such copper gelatinate with an acid to increase the hydrogen ion concentration, we notice that the gelatin loses its blue color as soon as its pH $\lessgtr$ 4.7. If after the acid treatment gelatin is perfused six times with distilled water, the gelatin keeps its blue color when pH > 4.7, and it is possible to demonstrate the presence of ionized copper in such gelatin with the ferrocyanide test. When the pH of the gelatin is $\gtrless$ 4.7, the ferrocyanide test shows that the gelatin is free from ionized copper. The water with which this gelatin was washed contains the copper, while the water with which the gelatin with a pH > 4.7 was washed is free from copper, except that which was originally contained in the capillary spaces between the granules of gelatin and which was carried away with the first perfusions.

Fig. 7 gives the curves of gelatin first treated for 30 minutes with $M/16$ copper acetate and subsequently with from $M/8$ to $M/8192$ HCl. For pH $\gtrless$ 4.6 all the values of the curves are as low as they are at the isoelectric point, and for the same reason; namely, copper gelatinate as well as the gelatin at the isoelectric point being sparingly soluble. (It is well to keep in mind that at the isoelectric point the gelatin contains no ionizable copper, while for pH > 4.7, the gelatin is in combination with ionic copper.) On the left, more acid side from the isoelectric point the gelatin exists in the form of gelatin chloride and gelatin acetate.

The experiment proves, therefore, that copper gelatinate cannot continue to exist when pH $\lessgtr$ 4.7, and that the gelatin gives off its copper at such pH.

The same experiment was made with $CeCl_3$ and Pb acetate$_4$, and almost identical curves were obtained as in the case of copper acetate, Ce as well as Pb forming sparingly soluble gelatinates. In the case of K_4FeCN_6 the sparingly soluble gelatin$_4$ FeCN$_6$ was formed only on the acid side of the isoelectric point as our theory demands. Fig. 8 gives the curves. The gelatin was first treated with different concentrations of HCl and then with $M/16$ K_4FeCN_6 in the manner described for the other cases.
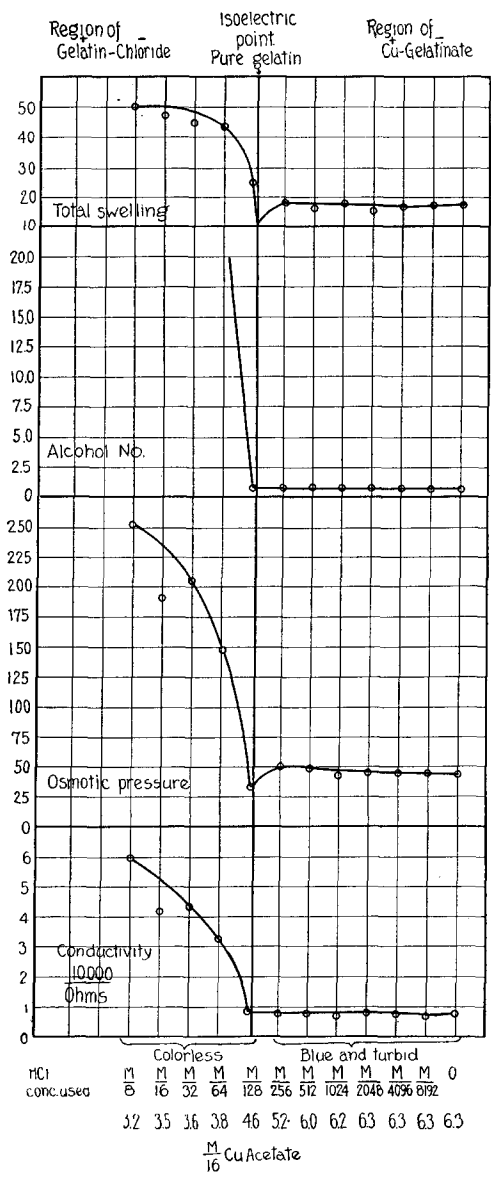
Fig. 7. Gelatin treated with m/16 copper acetate, washed, treated with different concentrations of HCl, and washed again. On the right side from the isoelectric point gelatin exists as copper gelatinate, stained blue, practically insoluble, and with low conductivity, osmotic pressure, alcohol number, and swelling. On the left side from the isoelectric point it exists as gelatin chloride (and acetate), highly soluble, and with high conductivity, osmotic pressure, alcohol number, and swelling; at the isoelectric point pure gelatin, practically insoluble.
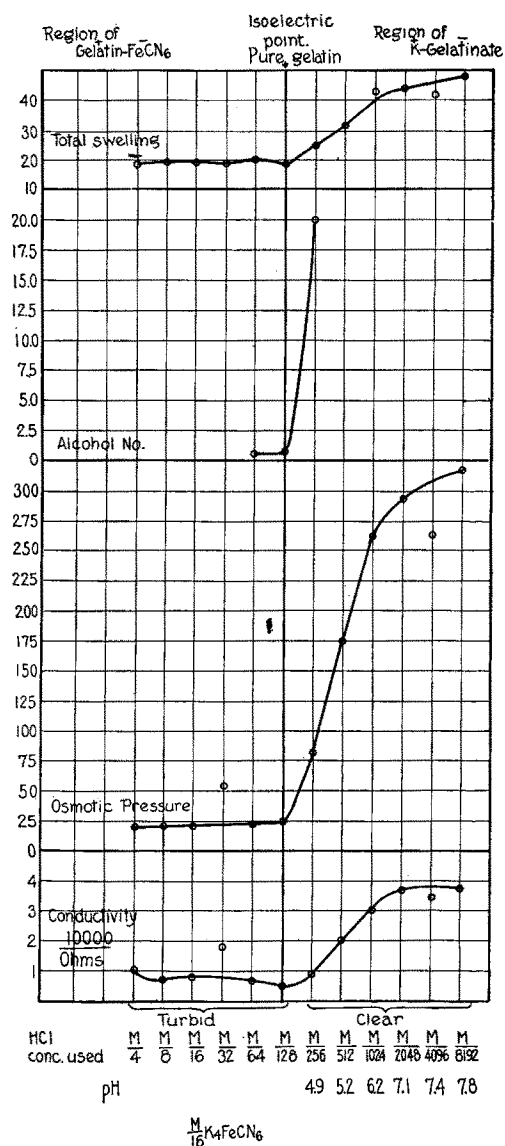
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FIG. 8. Gelatin treated with different concentrations of HCl, washed, then treated with M/16 K₄FeCN₆, and then washed again.   On the left, more acid side from the isoelectric point, gelatin exists as insoluble gelatin ferrocyanide, with low osmotic pressure, low conductivity, etc.; on the right, more alkaline side from the isoelectric point, gelatin exists as potassium gelatinate with high conductivity, osmotic pressure, etc.; at the isoelectric point, pure and sparingly soluble gelatin.

Experiments with Dyes.

Gelatin is readily stained with the basic dye neutral,red at the point of neutrality. Our theory demands that gelatin thus stained with neutral red should give off all its stain at the isoelectric point and on the acid side from the isoelectric point; while the neutral red should be retained when pH > 4.7. This turns out as the theory demands. 1 gm. of finely powdered gelatin was put for 20 minutes at about 15°C. into a series of beakers each containing 1 cc. of m/100 neutral red. The gelatin was then filtered and washed twice with 25 cc. of distilled water. All the gelatin was stained a beautiful deep red. Each funnel was then perfused three times with a definite concentration of HCl, the concentration for the various funnels varying from m/8 to m/8192, and this was followed by eight perfusions with 25 cc. of H_2O each. In the funnels treated with a concentration of HCl of m/512 or above, the gelatin became decolorized (the neutral red being washed away); while all the funnels treated with acid of a lower concentration retained their deep red color and did not give off their stain. The decolorized gelatin was then melted, made into a 1 per cent solution whose pH was determined. It was found that the gelatin previously treated with m/512 HCl and decolorized had a pH = 4.7, that treated with 3 m/1024 having a pH = 4.6, and so on. Hence the gelatin was not able to retain its neutral red when brought to the isoelectric point or to a pH ⪅ 4.7, as our theory demands.

Michaelis and Davidsohn[3] working with large blocks of gelatin, gained the impression that gelatin is slightly stainable with both basic and acid stains at the isoelectric point. We are inclined to believe that our method of staining gelatin in a finely divided condition and then ascertaining the pH where the dye is given off again by the gelatin, gives more reliable results than their method of working with large blocks of gelatin. We intend to return to the problem of staining in a future communication.

SUMMARY.

1. It is shown by volumetric analysis that on the alkaline side from its isoelectric point gelatin combines with cations only, but not

[3] Michaelis. L., and Davidsohn, H., *Biochem. Z.*, 1913, liv, 323.

with anions; that on the more acid side from its isoelectric point it combines only with anions but not with cations; and that at the isoelectric point, pH = 4.7, it combines with neither anion nor cation. This confirms our statement made in a previous paper that gelatin can exist only as an anion on the alkaline side from its isoelectric point and only as a cation on the more acid side of its isoelectric point, and practically as neither anion nor cation at the isoelectric point.

2. Since at the isoelectric point gelatin (and probably amphoteric colloids generally) must give off any ion with which it was combined, the simplest method of obtaining amphoteric colloids approximately free from ionogenic impurities would seem to consist in bringing them to the hydrogen ion concentration characteristic of their isoelectric point (i.e., at which they migrate neither to the cathode nor anode of an electric field).

3. It is shown by volumetric analysis that when gelatin is in combination with a monovalent ion (Ag, Br, CNS), the curve representing the amount of ion-gelatin formed is approximately parallel to the curve for swelling, osmotic pressure, and viscosity. This fact proves that the influence of ions upon these properties is determined by the chemical or stoichiometrical and not by the "colloidal" condition of gelatin.

4. The sharp drop of these curves at the isoelectric point finds its explanation in an equal drop of the water solubility of pure gelatin, which is proved by the formation of a precipitate. It is not yet possible to state whether this drop of the solubility is merely due to lack of ionization of the gelatin or also to the formation of an insoluble tautomeric or polymeric compound of gelatin at the isoelectric point.

5. On account of this sudden drop slight changes in the hydrogen ion concentration have a considerably greater chemical and physical effect in the region of the isoelectric point than at some distance from this point. This fact may be of biological significance since a number of amphoteric colloids in the body seem to have their isoelectric point inside the range of the normal variation of the hydrogen ion concentration of blood, lymph, or cell sap.

6. Our experiments show that while a slight change in the hydrogen

ion concentration increases the water solubility of gelatin near the isoelectric point, no increase in the solubility can be produced by treating gelatin at the isoelectric point with any other kind of monovalent or polyvalent ion; a fact apparently not in harmony with the adsorption theory of colloids, but in harmony with a chemical conception of proteins.

The writer wishes to express his thanks to Mr. M. Kunitz for his faithful assistance in these experiments.