9-1918

The Influence of Electrolytes Upon the Osmotic Pressure of Gelatin Solutions

Jacques Loeb

THE INFLUENCE OF ELECTROLYTES UPON THE OSMOTIC PRESSURE OF GELATIN SOLUTIONS.

By JACQUES LOEB.

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

(Received for publication, July 12, 1918.)

I.

It is generally stated in the literature of colloid chemistry that gelatin is influenced by both ions of a neutral salt, and that the influence of the anion of the salt is greater than that of the cation. The different anions are said to differ in their relative efficiency in a definite order, the now famous Hofmeister anion series. The writer has shown in a series of articles that all these statements are contrary to fact, and that the real facts are as follows: Common gelatin (*i.e.* gelatin near the point of neutrality) is affected only by the cations of a neutral salt, and not by the anions, univalent cations causing an increase in swelling, alcohol number, and viscosity, while bivalent cations cause no such change. The anion of the salt has no effect whatever.[1–5] The error into which the colloid chemists have fallen is due to the fact that they always investigated the effect of a neutral salt on a protein in the presence of the salt, while the writer took the precaution to wash the excess of salt away after it had time to act on the gelatin. In order to be able to do this he used gelatin in the form of a fine powder. A 1 per cent solution of the gelatin used for his experiments had a hydrogen ion concentration of 10^{-7} (pH = 7) (determined by Dr. Dernby). Since according to Michaelis[6] the

---

[1] Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343.

[2] Loeb, *J. Biol. Chem.*, 1918, xxxiii, 531.

[3] Loeb, *J. Biol. Chem.*, 1918, xxxiv, 77.

[4] Loeb, *J. Biol. Chem.*, 1918, xxxiv, 395.

[5] Loeb, *J. Biol. Chem.*, 1918, xxxiv, 489.

[6] Michaelis, L., Die Wasserstoffionenkonzentration, Berlin, 1914.

isoelectric point of gelatin is at $C_H = 2.10^{-5}$ (pH = 4.7) the hydrogen ion concentration of the solution was less than that of the isoelectric point. In a paper which will appear in the first number of *The Journal of General Physiology* the writer will show that on the alkaline side from the isoelectric point gelatin (and amphoteric electrolytes in general) can exist in an ionized condition only as anions, and hence is capable of combining only with the cation of a neutral salt, as we had actually found. When the hydrogen ion concentration of the solution is greater than that corresponding to the isoelectric point, the gelatin (and amphoteric electrolytes in general) can exist in an ionized condition only as cations and hence can combine only with the anion of a neutral salt. Hence the statement that always both ions of a neutral salt act on the gelatin, and that the effect of the anion prevails in the case of gelatin is contrary to fact as well as theory.

In a paper generally accepted by colloid chemists Lillie[7] has claimed that while salts depress the osmotic pressure of gelatin solutions, acids and bases have the opposite effect; and he also expresses the general belief that both ions of a salt are effective and that the relative depressing effect of the anions of neutral salts follows the order of the Hofmeister anion series.

Lillie's conclusions are as follows:

> "Acid and alkali increase the osmotic pressure of gelatin solutions; in general these substances affect the osmotic pressure of gelatin solutions in the same manner as they do the rate of swelling of solid gelatin plates immersed in water.
>
> "Addition of salts depresses the osmotic pressure of both colloids [gelatin and albumin]; the degree of depression is a function of the nature of both the anion and the cation of the salt. It increases in the order: alkali metals < alkali earths < heavy metals (for cations); and CNS < I < Br < NO_3 < Cl < F < plurivalent anions—SO_4, tartrate, citrate, phosphate (for anions)."[8]

The statement that acid and alkali increase the osmotic pressure while salts depress it is as we shall show merely due to the fact that Lillie worked with low concentrations of acids and bases in which the repressing effect of the presence of the electrolyte is less noticeable; while he worked with high concentrations of

---

[7] Lillie, R. S., *Am. J. Physiol.*, 1907–08, xx, 127.

[8] Lillie, *Am. J. Physiol.*, 1907–08, xx, 169.

the neutral salts in which the repressing effect of the presence of the electrolyte was noticeable. Had he used stronger concentrations of alkalies and acids he would have noticed that the depressing effect which he observed in the case of salts is equally pronounced in the case of acids and bases. We shall show in this paper that the influence of electrolytes upon the osmotic pressure of gelatin runs parallel to the influence of the electrolytes upon the swelling, viscosity, and alcohol number of gelatin, demonstrated by our previous papers, inasmuch as only the cation of a neutral salt influences the osmotic pressure, while the anion is without influence upon common gelatin. We shall show, moreover, that while the salts with monovalent cation increase the osmotic pressure, the salts with bivalent cation have no such effect. The same difference exists between the influence of bases with univalent and bivalent cation and between monobasic and dibasic acids. In order to show this we have to wash away the excess of the electrolyte after it has had time to act on the gelatin.

The osmotic pressure was determined by the simple method devised by Lillie.[7] Small bags in the shape of Erlenmeyer flasks were made of collodion and the neck of each bag was fitted into a perforated rubber stopper and made tight with the aid of rubber bands. A vertical glass tube of about 2 mm. diameter went through the hole of the stopper into the gelatin solution which at the beginning filled the bag and the lower end of the glass tube to about 20 mm. All air bubbles were carefully removed from the bag. The latter was then put into a large beaker containing about 250 cc. of distilled water. The beakers were in a water bath kept at a temperature near 20°C. The final equilibrium of the column of liquid in the glass tube was reached over night and remained constant for the next 24 hours. Our final measurements were usually made after about 16 hours.

It was found that our collodion bags were easily permeable for salts like NaCl, but were practically impermeable for gelatin. When a bag was filled with a M/2 NaCl solution and put into distilled water, the liquid in the bag or the glass tube serving as manometer rose during the first half hour to a height of about 70 mm. and then fell rapidly. In a few hours the osmotic pressure of the solution was zero. When the same experiment was made with a 1 per cent gelatin solution put into a beaker with distilled water, the pressure rose slowly to a height of about 80 mm. and remained there for several days—in fact as long as we continued the experiments. A test made of the distilled water surrounding the bag failed to give the biuret test after 16 hours.

Lillie's method may not be sufficiently accurate to satisfy the demands of the physicist,[9] but it is adequate to allow us to show that our former statements concerning the influence of neutral salts upon the physical properties of gelatin (swelling, viscosity, and resistance to precipitability by alcohol) hold also for the influence of neutral salts upon the osmotic pressure of gelatin solutions.

II.

On the basis of our previously published experiments we should expect that a treatment of powdered gelatin with a neutral salt with univalent cation should cause an increase in the osmotic pressure of the gelatin, after the excess of the salt solution is washed away, since the treatment of the gelatin with neutral salts should lead to the formation of metal gelatinates. The higher the concentration of the salt solution used, the greater the mass of metal gelatinate formed and the greater the increase in osmotic pressure to be expected. On the other hand, treatment of gelatin with neutral salts with bivalent metal should on the basis of our previously published experiments not cause any increase in osmotic pressure after the excess of salt has been washed away. Table I shows that these theoretical expectations are all fulfilled.

The method was the same as that used in the experiments on alcohol precipitability. 1 gm. of powdered gelatin going through 50 mesh sieve but not through one of 60 was put for 1 hour into 100 cc. of the solution of a neutral salt to bring about a chemical reaction between gelatin and salt. The powder was then put on a filter, the salt solution allowed to drain off, and the gelatin was perfused six times with H_2O to remove the last traces of salt

---

[9] Two factors in our present method seem to cause a variation in the results; first, possible differences in the permeability of the membranes, and second, differences in the degree of washing away the electrolytes in solution and, if the washing is excessive, in combination with the gelatin,

solution held in the capillary spaces or adhering to the surface of the granules. The gelatin was then heated for about 10 minutes in a water bath of 50°C. so as to liquefy the gelatin, and distilled water was added to make the solution 1 per cent. With this gelatin solution free from the salt with which the gelatin had been treated the influence of salts upon the osmotic pressure was determined.

TABLE I.

	Osmotic pressure in mm. of 1 per cent gelatin solution in distilled water, made from powdered gelatin treated for 1 hour with one of the following solutions and then perfused six times with 25 cc. H_2O to wash away the excess of salt solution.												
	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	M/4096	M/8192	H_2O
NaCl	134	122	123	107	99	93	81	80	77	77	71	74	77
NaBr	136	133	123	118	107	88	78	79	82	78	76	78	79
NaCNS		132		118	107	94	94	82	88	78	75	73	74
Na acetate	170	151	139	127	108	92	89	80	79	78	77	75	81
KCl	116	120	116	100	95	80	72	75	68				70
$LiNO_3$	121	120	104	102	91	84	81	73	73				73
NH_4Cl	145	138	137	127	105	94	86	80	83	81	77	77	77
Na_2SO_4	159	166	156	151	136	107	102	83	80				73
Na_2 tartrate	166	159	142	129	116	110	98	77	60	73	78		72
Na_2 succinate		170	163	144		132	112	91	83	86	81		84
Na_2 oxalate		100	91	88	90	94	90	88	81	78	82	81	
Na_2 malate		166	152	142	135	121	102	89	76	79	77	73	
$MgCl_2$		79	83	82	82	77	78	81	81	75	78	78	80
$CaCl_2$	77	76	82	76	84	84	83	80	81				80
$SrCl_2$	75	81	78	80	83	81	82	82	76				78
$BaCl_2$		69	70	73	76	76	75	80	82	81	81	80	82
$MgSO_4$	84	80	80	82	83	82	79	81	80	73	82	82	81

In looking at the results in Table I it is strikingly clear, first, that a treatment of the gelatin with a neutral salt with a bivalent metal, $MgCl_2$, $CaCl_2$, etc., leads to no increase in osmotic pressure, even if we go up to M/4 solutions. (We cannot go beyond this limit on account of the liquefying effect of the higher concentrations upon the gelatin and the difficulty of freeing the solution from the excess of salt.)

Second, it is obvious that $MgSO_4$ acts exactly as $MgCl_2$, thus indicating that the anion has no influence.

Third, it is obvious that the salts with univalent cation increase the osmotic pressure of the gelatin.

Fourth, it is obvious that the molecular concentration required to cause a perceptible rise (to 90 mm. or above) in the osmotic pressure is for salts of the type NaCl (univalent cation, univalent anion), higher than for salts of the type Na_2SO_4 (univalent cation and bivalent anion). For the former it is between M/128 and M/256, and for the latter between M/256 and M/512; indicating that the concentration of the cation alone (or practically exclusively) determines the effect of the salt regardless of the nature of the anion, though this latter result does not come out as strikingly as in the measurements of the precipitability of gelatin with alcohol or the limiting concentration for additional swelling.[2, 3] Within the limits of the imperfections of our present osmotic pressure determinations the results on osmotic pressure confirm our theory of the action of neutral salts upon proteins expressed in our former papers. The only exception noticed is the oxalate solution, which apparently caused a precipitate in the gelatin.

III.

Our theory furthermore demands that bases should influence the osmotic pressure of gelatin in a sense parallel to that of the corresponding neutral salts; namely, a previous treatment of powdered gelatin with NaOH and KOH should cause a considerable increase in osmotic pressure (after the free base is washed away) while $Ba(OH)_2$ and $Ca(OH)_2$ should cause no or at the best only a slight increase in osmotic pressure under the same conditions of experimentation. According to our theory bases act upon proteins of the type of gelatin (provided that $pH > 4.7$) like neutral salts, both causing the formation of metal proteinates, while the anion of the salt has apparently no influence upon the protein. A glance at Table II shows this expectation to be correct. 1 gm. of powdered gelatin was put for half an hour into the solutions of the different bases and then treated in a similar way as in the case with salts. The excess of alkalies was allowed to drain off and the gelatin was washed four times with

25 cc. of H_2O. A treatment of the gelatin with $Ca(OH)_2$ leads to no increase in the osmotic pressure of the solution, while a treatment with $Ba(OH)_2$ may lead to a slight increase after the excess of base has been washed away. On the other hand, treatment of the gelatin with NaOH and KOH leads to a considerable increase in the osmotic pressure after the excess of alkali has been washed away.

TABLE II.

	Osmotic pressure in mm. of 1 per cent gelatin solution in distilled water, made from powdered gelatin treated for 30 min. with 100 cc. of one of the following solutions and then perfused four times with 25 cc. H_2O to wash away the excess of salt solution.									
	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	M/4096	M/8192	H_2O
NaOH.....	172		165	129	115	102	98	90	88	90	76
KOH......	154	155	147	120	98	96	95	88	87	79	77
$Ba(OH)_2$..		77	95	95	97	99	95	90	89	88	
$Ca(OH)_2$..			85	89	92	87					78

IV.

The fact that salts or bases with bivalent cation do not lead to an increase in the osmotic pressure (or the swelling, the viscosity, and resistance to alcohol precipitation) of proteins might be interpreted to mean, that such salts or bases do not combine with proteins. Such a view is, however, repudiated by the facts of antagonistic salt action, inasmuch as it can be shown that the addition of a comparatively small amount of a salt with bivalent cation inhibits the increase in osmotic pressure (and also the increase in swelling, viscosity, and resistance to alcohol precipitation)[1–4] caused by a treatment of the proteins with a salt or base with univalent cation after the excess of salt solution is washed away.

The method of our experiment was as follows: 1 gm. of powdered gelatin was put for 1 hour into 100 cc. M/8 NaCl containing 0, 1, 2, 4, 6, 8, 12, 16, and 32 cc. M/8 $CaCl_2$ or $BaCl_2$. After 1 hour the gelatin was put into a funnel, the salt solution was allowed to drain off, and the powder was then perfused six times with 25 cc. of H_2O to remove the last traces of the salt solution. After this, 1 per cent solutions of these different gelatins in distilled water were made, and the osmotic pressures were determined. It was found that the addition of Ca or Ba diminishes the rise in osmotic pressure which is caused by the pure solution of NaCl, and this diminution is the greater the more Ca or Ba had been added. The addition of 1 or 2 cc. of $CaCl_2$ or $BaCl_2$ had already a noticeable effect (Table III).

This proves that the salts with bivalent cation prevent the increase of osmotic pressure caused by the salts with univalent cation. The same antagonism has already been demonstrated in the writer's previous papers for additional swelling, increase in viscosity, and resistance to alcohol precipitation. The reader will remember that in all these experiments the excess of salt solution was washed away after the salt had had time to act on the gelatin. These experiments prove that the salts with bivalent cations react with gelatin.

TABLE III.

	Osmotic pressure in mm. of 1 per cent gelatin solution in distilled water, made from powdered gelatin treated for 1 hour at 20° with 100 cc. of an M/8 NaCl solution containing various amounts of M/8 $CaCl_2$ or $BaCl_2$, and then perfused six times with 25 cc. H_2O to wash away excess of salt.									
100 cc. M/8 NaCl + cc. $CaCl_2$ or $BaCl_2$	0	1	2	4	6	8	12	16	24	32
NaCl + $CaCl_2$	131	129	120	107	106	99	94	90	85	84
NaCl + $BaCl_2$	134	125	111	98	110	29	97	83	86	74

According to our theory the same antagonism as between NaCl and $BaCl_2$ should exist between NaOH and $Ba(OH)_2$. This was found to be true.

Powdered gelatin was put into 100 cc. of M/32 NaOH (free from carbonate) containing 0, 1, 2, 4, 6, 12, 16, 24, and 32 cc. M/32 $Ba(OH)_2$. The mixture was contained in tightly stoppered bottles, and the powder remained in the mixture 30 minutes. After this the gelatin was put into funnels, the alkali solution allowed to drain off, and the gelatin was perfused four times with 25 cc. of H_2O to remove the last traces of the original solution from the capillary spaces between the granules. Table IV shows that the expected antagonism between NaOH and $Ba(OH)_2$ was found, and in addition that the antagonistic action exists also for swelling and precipitation with alcohol.

These experiments are only intelligible on the assumption that the salts and bases with bivalent cation influence the protein in the same way and that, moreover, the bivalent cations inhibit the effect of the salts and bases with univalent cation.

TABLE IV.

	1 gm. powdered gelatin put for 30 min. into one of the following solutions and then perfused four times with 25 cc. H_2O.								
100 cc. M/32 NaOH + cc. M/32 $Ba(OH)_2$	0	1	2	4	8	12	16	24	32
Osmotic pressure in mm. of 1 per cent gelatin solution.....	185	147	148	147	133	126	115	109	105
Total swelling in mm. of height of cylindrical mass of gelatin......................	70	71	70	61	50.5	51	46	45	31
Cc. 95 per cent alcohol required to precipitate 5 cc. of 1 per cent gelatin solution......................	∞	∞	∞	∞	∞	14.9	11	10.4	7.1

V.

We will now show that if we do not remove the excess of solution of electrolyte with which the gelatin is treated, the effects of the electrolyte will be repressed and that this repression varies with the concentration and nature of the electrolyte. The measurements for the effect of electrolyte upon the osmotic pressure of gelatin will thus be the resultant of opposite influences of the electrolyte, the relative quantity of which is unknown in each case; and what appears to be the effect of the electrolyte upon the gelatin is in reality the difference between the unknown effect of the electrolyte upon the gelatin and the equally unknown repressing effect of the presence of the excess of electrolyte. Instead of arriving at the clear results which our method gives, we arrive at indefinite results which misled Hofmeister into the erroneous statement concerning the effect of anions upon the swelling;[10] and the authority of Hofmeister obviously induced Lillie to look for a confirmation of Hofmeister's statement.

The experiments represented in Table V were made in a similar way as those of Lillie. A 1 per cent solution of gelatin was made up in the solution of the electrolyte whose effect was to be studied, and the same solution of the electrolyte was put into the beaker surrounding the collodion bag containing the 1 per cent gelatin solution. Since the membrane is entirely permeable to crystalloids, the osmotic pressure of the gelatin solution can be measured in this way.

TABLE V.

	Osmotic pressure in mm. of 1 per cent gelatin solution in presence of electrolyte.													
	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	M/4096	M/8192	M/16384	H_2O
I.														
HNO_3	47	48	58	46	104	169	286	352	330	205	81	26		
NaOH			71	70	85	124	122	213	206	185	134	112		90
NaCl		37	33	38	35	41	42	63	74	83	90	84		88
II.														
H_2SO_4				39	48	62	90	121	93	140	103	34	46	81
$Ba(OH)_2$					60	49	50	71	102	107	115	110		
$BaCl_2$		35	32	30	31	33	33	32	38	42	60	73		
III.														
$NaNO_3$		32	34	28	38	39	50	61	62	81	78	91		87
LiBr		35	35	42	42	39	58	62	71	70	89	87		
Na acetate		43	43	45	Lost.	52	66	70	80	101	118	110		91
NaCNS			43		55	56	60		85	95	100	100		102
Na_2 tartrate			40	37	38	41	62	56	60		100	105	103	
Na_2SO_4		27	25	41	46	35	41	47	50	62	92	80		90

Group I, Table V, gives the osmotic pressure of a 1 per cent gelatin solution in the presence of a monobasic acid, HNO_3, a base with univalent cation, NaOH, and a neutral salt, NaCl. It is obvious that the presence of the base NaOH depresses the osmotic pressure below that of a non-treated gelatin solution as soon as the concentration of NaOH is M/64 or above; and the

---

[10] Hofmeister, F., *Arch. exp. Path. u. Pharmakol.*, 1891, xxviii, 210.

same is true as soon as the concentration of the acid is M/32 or above. According to Table I NaCl only begins to cause a marked increase in osmotic pressure in concentrations above M/128 or M/64, but the presence of the salt in these or higher concentrations prevents the manifestation of this increase.

Hence the behavior of gelatin in the presence of electrolytes conforms with the results and conclusions from our former experiments.

Furthermore, a glance at Group II, Table V, brings out a further confirmation of our theory; namely, that neutral salts with bivalent cation, $BaCl_2$, behave like bases with bivalent cation, $Ba(OH)_2$, and acids with bivalent anion, H_2SO_4, inasmuch as in all three cases the osmotic pressures run lower than the corresponding pressures in Group I, which was to be expected according to our previous publications.

Group III, containing different salts of Na, shows that there is no basis for assuming the validity of the Hofmeister series upon the osmotic pressure. The variations between the effects of sodium salts with different anions are within the limits of error of the method or due to differences in the repressing effect of the different salts present. Neither do Lillie's own observations support the validity of the Hofmeister series. He states:[11] "Individual variability is so strongly marked in colloidal solutions that little reliance can be placed on the order of action observed in a single series of determinations. . . . ." When we free the solution from an excess of salt, the results become clear and leave no doubt that the alleged anion effect of neutral salts upon gelatin does not exist.

SUMMARY.

1. It is generally stated in colloid chemistry that only acids and bases cause an increase in the swelling, the viscosity, and the osmotic pressure of hydrophilic colloids, while neutral salts of the type of NaCl have little or, according to Lillie, a directly opposite effect. The writer has found that neutral salts act exactly like acids and bases inasmuch as neutral salts and bases with univalent cation and acids with univalent anion

---

[11] Lillie, *Am. J. Physiol.*, 1907–08, xx, 152.

increase the swelling, the viscosity, and resistance to precipitation by alcohol of gelatin, while salts and bases with bivalent cation and acids with bivalent anion (with the exception of some organic acids) have no or only a slight effect. In this paper it is shown that the same is true for the influence of electrolytes upon the osmotic pressure of gelatin.

2. It was found by the writer that only the cation of neutral salts acts upon gelatin in neutral solutions or in solutions with $pH > 4.7$, while the anion of neutral salts does not influence the osmotic pressure and the other physical qualities of gelatin (swelling, viscosity, and alcohol precipitability).

3. These experiments contradict the statements generally made in colloid chemistry that the influence of neutral salts upon a protein is the algebraic sum of the opposite effects of the oppositely charged ions of the neutral salt. They also contradict the statement that common gelatin (at the point of neutrality) is influenced more strongly by the anion than by the cation of a neutral salt.

4. The difference between our results and those of the older experimenters is due to the fact that in the older experiments the effects of neutral salts upon gelatin were always measured in the presence of the salts. We were able to show that the presence of the salt represses the manifestation of its effects upon the gelatin and that it is therefore necessary to remove the excess of the salt after it has had time to act on the protein.

5. Our results are in agreement with a theory of the behavior of amphoteric electrolytes, which will appear in *The Journal of General Physiology;* namely, that on the more acid side of their isoelectric point amphoteric electrolytes can exist, when ionized, only in the form of cations, while on the less acid side of their isoelectric point they can exist, when ionized, only as anions. In the former case the ampholyte can combine only with the anion, in the latter only with the cation of a neutral salt. Since gelatin has its isoelectric point at $pH = 4.7$, while the gelatin used by us had a $pH = 7$, such gelatin could react only with the cation of neutral salts.