

THE IONIC ACTIVITY OF GELATIN.

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I.

INTRODUCTION.

In two other papers¹ it was shown that the monoions of weak acids in dilute solution obey the limiting Debye-Hückel equation:

$$-\log f = a \nu^2 \sqrt{\mu} = a \nu^2 \sqrt{\frac{1}{2} i \nu^2} \quad (1)$$

while the polyvalent ions, in the presence of NaCl, obey a modified equation:

$$-\log f = a \nu^2 \sqrt{\frac{1}{2} i \nu^2} \quad (2a)$$

where

$$\nu^2 = \nu^2 - (\nu^2 - \nu) \frac{r_z}{18} \quad (2b)$$

where r_z is the distance in Ångstrom units between like charges in a polyvalent ion. However, these polyvalent ions show a large deviation in one direction with Mg^{++} ions and a small deviation in the opposite direction with SO_4^{-} ions. Furthermore, the ions of ampholytes obey the above equations in the presence of NaCl but are anomalous with $MgCl_2$. On the other hand, the cations of amines or ampholytes do not obey the Debye-Hückel law either with NaCl or $MgCl_2$.

¹ Simms, H. S., *J. Phys. Chem.*, 1928, pending publication.

The fact that anions of ampholytes obey the modified Debye-Hückel equation (*2a* and *b*) gives us a means for studying a protein. Gelatin was chosen for the purpose.

In the study of simple substances we plotted the square root of the ionic strength ($\sqrt{\mu}$) against values of the uncorrected dissociation indices (pK' values) for each index of the substance. The slopes of these curves equal Sa , where

$$\pm S = \nu^2 - (\nu - 1)^2 = (2\nu - 1) - (\nu - 1) \frac{r_2}{9} \quad (3)$$

where ν is the valence of the ion produced by a given step in ionization, and $\pm S$ has the sign of the valence. In other words, for a given dissociation index:

$$\text{pK}' - \text{pK} = Sa \sqrt{\mu} \quad (4)$$

where S has the value given by equation (3).

II.

*Ionic Activity of Proteins.*²

The above method cannot be applied to proteins since we cannot obtain pK' values with any accuracy. However, we may use the following method: Let us consider two solutions of a weak electrolyte in equal concentrations and having the same equivalents of base (*b'*) but differing in their ionic strength (due to the addition of salt to one of them). The difference (ΔpH) between the hydrogen indices of these two solutions will be the same as the difference ($\Delta\text{pK}'$) between the uncorrected dissociation indices and will equal $\Delta(-\log f)$, hence:

$$\Delta\text{pH} = \Delta\text{pK} = \Delta(-\log f) = S' \cdot a \cdot \Delta\sqrt{\mu} \quad (5)$$

or

$$S' = \frac{2 \Delta\text{pH}}{\Delta\sqrt{\mu}} \quad (6)$$

² Attempts to determine or correct for the activity of proteins have been made by others, for example, Cohn, *Physiol. Rev.*, 1925, v, 349. Sørensen, Lang, and Lund, *J. Gen. Physiol.*, 1925-28, viii, 543. Stadie and Hawes, *J. Biol. Chem.*, 1927, lxxiv, p. xxxi.

This S' is the correct value of S only if b' is the same in both solutions. If it is different (as will be the case at high or low pH) we must write:

$$S = \frac{\Delta\text{pH} - \Delta b'/\beta'}{\alpha \cdot \Delta\sqrt{\mu}} = S' - \frac{2 \Delta b'}{\beta' \cdot \Delta\sqrt{\mu}} \quad (7)$$

where $\beta' = \frac{\Delta b'}{\Delta\text{pH}}$ is a modification of Van Slyke's "buffer value"³ and represents the equivalents of base required to produce unit

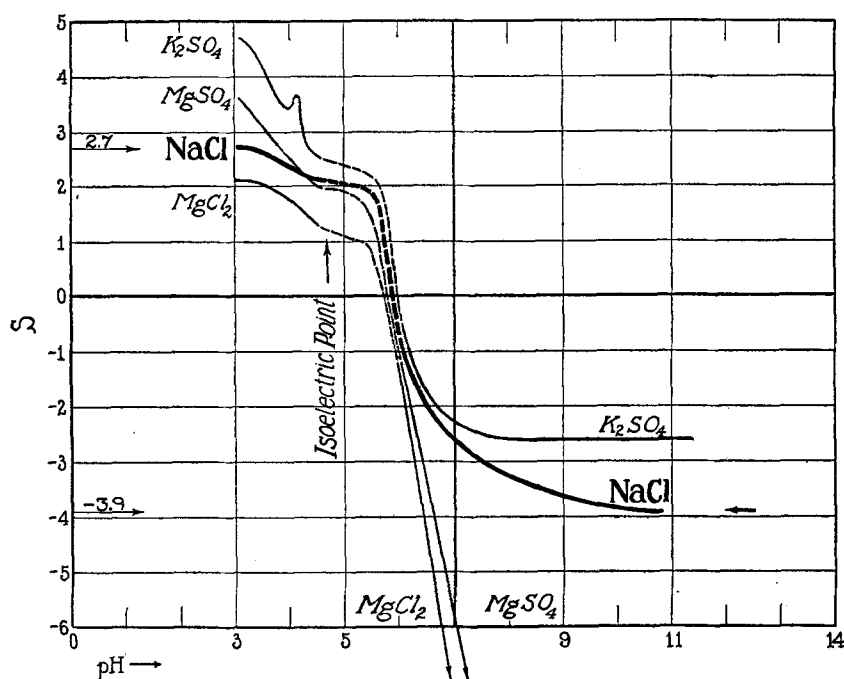


FIG. 1. Values of $S = \nu_x^s - (\nu_x - 1)^s$ for gelatin in the presence of 0.0375μ of salts. Slight experimental errors produce large differences in values of S . Each curve represents the mean of a large number of points.

changes in pH; $\Delta\text{pH} = \text{pH}_s - \text{pH}_o$; $\Delta b' = b'_s - b'_o$; and $\Delta\sqrt{\mu} = \sqrt{\mu_s} - \sqrt{\mu_o}$. The subscript s refers to solutions with salt and o without salt. S is less than S' when $\Delta b'$ is positive (algebraically).

³ Van Slyke, *J. Biol. Chem.*, 1922, lii, 525, and Simms, *J. Am. Chem. Soc.*, 1926, xlviii, 1249. The β' values used in Table IV are those of the gelatin titration curve without salt.

III.

Results of Study of Gelatin.

We have obtained data on gelatin from pH 3 to 11. At numerous values of $\frac{b-a}{c}$ (equivalents of base) we obtained the pH of solutions without salt and at two different concentrations each of NaCl, MgCl₂, K₂SO₄, and MgSO₄. The values of ΔpH and of⁴ $\Delta\sqrt{\mu}$ were used in equation (6) to calculate S' . The values of S were then calculated from equation (7) and are presented in Table IV and plotted in Fig. 1 for the dilute solutions (0.0375 μ) of salts. The curves for concentrated solutions (0.075 μ) are essentially the same but show slight deviations due to the higher ionic strength.

Previous data showed that in the presence of NaCl, anions of weak electrolytes behave normally (and cations show small deviations) while the presence of Mg⁺⁺ ions or SO₄⁻ ions causes large deviations.

⁴ The values of $\sqrt{\mu}$ were obtained as follows: The *total* ionic strength is:

$$\mu = \mu_s + \mu_o \quad (8)$$

where μ_s is the ionic strength due to added salt (0.0375 and 0.0750, respectively in the two concentrations) and μ_o is the ionic strength of the solution having no added salt. The latter is calculated as follows: If the "apparent valence" of protein is unity, we may write:

$$\mu_o = \pm b'c + h + oh \quad (9)$$

(where $\pm b'c$ is always a positive number), but with an "apparent valence" of ν_A we get

$$\mu_o = \pm \frac{\nu_A^2 + 1}{2} b'c + h + oh \quad (10)$$

Our preliminary estimation of S_M was 2.0 in acid solution and 3.0 in alkaline solution. Equation (15) gives values of ν_A equal to 1.5 and 2.0. These were used in equations (8) and (10) to obtain the μ values used in Table IV. The final values of S_M are 2.7 and 3.9 (corresponding to ν_A equals 1.85 and 2.45). Although we would expect the latter values would be more correct, they appear to be too high. As an approximation we have used the same value (1.5) of ν_A at all points in the acid titration and the same value (2.5) at all points in alkali titration and we have neglected h and oh in equation (10).

TABLE I.
Deviations (ΔS) Produced in Weak Electrolytes by 0.0375μ of Salts.

Substance	Distance r_2	Valence ν	Apparent valence ν_A	ΔS in acid titration MgCl ₂	ΔS in alkaline titration		
					MgCl ₂	MgSO ₄	K ₂ SO ₄
	Å						
Citric acid.....	7.4	2	1.7		-9		
Citric acid.....	7.4	3	2.2		-29		
Oxalic acid.....	4.5	2	1.8		-17		
Malonic acid.....	5.7	2	1.7		-8.3	-5.0	+0.7
Succinic acid.....	7.0	2	1.6		-1.3		
Azelaic acid.....	12.1	2	1.3		-0.8		
Aspartic acid.....	7.0	2	1.6		-9.5		
Aspartic acid.....		1	1	-0.2			
(Glycine).....		1	1	-0.2	-6		
(Aminoethanol).....		1	1	-0.8			

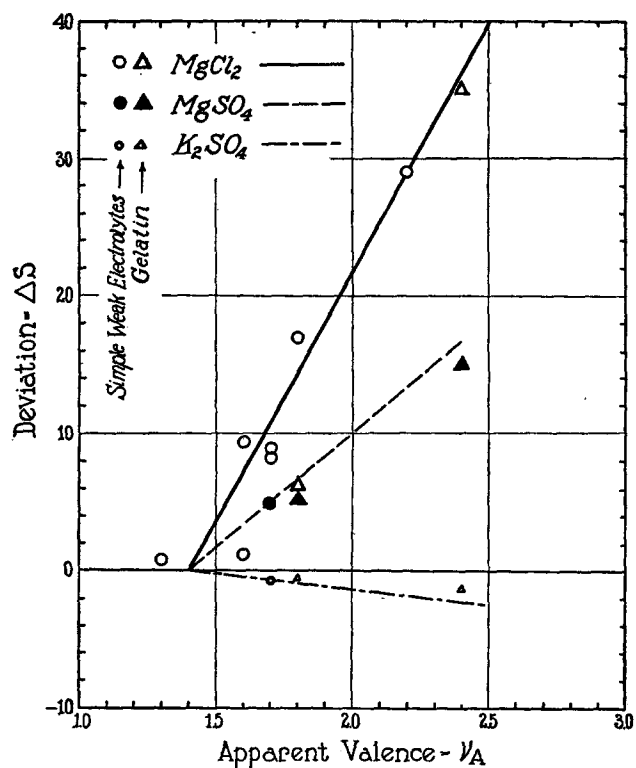


FIG. 2. Relation between "apparent valence" and the deviation produced by 0.0375μ of various salts on simple weak electrolytes and on gelatin. The lines correspond to equations (12a), (12b), and (12c).

Hence we will take the NaCl data as "normal" and note the deviations caused by other ions. This deviation will be:

$$\Delta S = S_o - S_x \quad (11)$$

where S_o is the value of S with NaCl, and S_x is the value of S with any other salt.

The values of ΔS for some ions of simple weak electrolytes are given in Table I and are plotted in Fig. 2. It will be seen that the data with MgCl_2 fall roughly on the empirical curve with the equation

$$r_A = 1.4 - \frac{\Delta S}{25} \quad (12a)$$

If we assume the same origin for the curves of MgSO_4 and K_2SO_4 , and use the data with malonic acid to determine the slope, we get, for MgSO_4

$$r_A = 1.4 - \frac{\Delta S}{17} \quad (12b)$$

For K_2SO_4 :

$$r_A = 1.4 + \frac{\Delta S}{2.3} \quad (12c)$$

These equations apply only to the effect of 0.0375μ of these salts on polyanions.

The effect of these salts on gelatin is given in Table II where the values found in alkaline titration (Columns 7 to 9) are seen to agree with those calculated by the above formulas (Columns 10 to 12). These points for gelatin are plotted in Fig. 2 (triangles) and are seen to agree with the curves found for the simple weak electrolytes.

In acid solution gelatin behaves like aspartic acid or glycine with MgCl_2 . Unfortunately we have no data on polycations with which to make quantitative comparison in acid solution. The qualitative behavior agrees with our expectations.

On the basis of these observations there is every reason to believe that gelatin in dilute solution (2.5 per cent or less) behaves like a polyvalent ampholyte with distant ionizable groups⁵ and that the

⁵ The nature and sources of these groups will be discussed in the following paper.

ionization is purely that of a weak electrolyte showing the same effects in the presence of Mg^{++} or SO_4^{-} ions that are shown by the simple weak electrolytes.

These deviations are similar to the combination of proteins with inorganic ions as observed by other methods⁶ and the combination of simpler substances with inorganic ions.⁷

TABLE II.
Deviations (ΔS) Produced in Gelatin by 0.075μ of Salts (Distance $r_x > 18 \text{ \AA}$.)

pH	Valence ν	Appar-ent valence ν_A	ΔS values									
			Found in acid titration			Found in alkaline titration			Calculated for alkaline titration			
			MgCl ₂	MgSO ₄	K ₂ SO ₄	MgCl ₂	MgSO ₄	K ₂ SO ₄	MgCl ₂	MgSO ₄	K ₂ SO ₄	
3.4	57	1.8	-0.6	+0.2	+1.1							
7.1	16	1.8				-6.3	-5.3	+0.5	-15	-6.5	+0.9	
11.0	43	2.4				-30 to -40*	-15*	+1.3	-36	-17	+2.3	

* The values for MgCl₂ (-30 to -40) and MgSO₄ (-15) at pH 11 were estimated by extrapolation.

IV.

Relation between Activity, Valence, and Distance.

Assuming a given molecular weight we may calculate the corresponding number of acid or basic groups from the base or acid "combining capacity." This calculated valence (ν_x) will be a large number

⁶ Northrop and Kunitz, *J. Gen. Physiol.*, 1925-26, ix, 351 and unpublished data. Hastings and Sendroy, *J. Biol. Chem.*, 1927, lxxi, 723. Loeb, R. F., and Nichols, *J. Biol. Chem.*, 1927, lxxiv, 645. Adair, *J. Biol. Chem.*, 1925, lxiii, 517, 529. Austin, Sunderman, and Camack, *J. Biol. Chem.*, 1926, lxx, 427.

⁷ Pfeiffer and collaborators, *Z. physik. Chem.*, 1924, cxxxiii-cxliii.

In this connection we will mention that a quantitative measurement of the interaction of glycine and phosphoric diion gives the mass action equation:

$$k = \frac{[\text{glycine}] \times [\text{HPO}_4^{-}]}{[\text{Combined}]} = 0.040$$

as shown by the effect of glycine on pK_2 of H_3PO_4 . The data will be published later. Similar, but more complex, relations have been found to apply to the effects of Mg^{++} and of SO_4^{-} on ions of oxalic acid.

which if used in the Debye-Hückel equation (1) will give impossible values for the effect of protein on ionic strength. This is because equation (1) assumes the charges to be located at a single point.

A polyvalent ion with charges very far apart would behave like a number of univalent ions. Equation (2) is derived to allow for finite distances between like charges. The mean distance r_x may be calculated from the equation (derived from equation (3)):

$$r_x = \frac{9(2\nu - S - 1)}{\nu - 1} \quad (13)$$

From the base-combining capacity (1.75 equivalents per 2500 gm.) and the acid-combining capacity (2.30), we may calculate the maxi-

TABLE III.

Assumed molecular weight	ν_x No. of acid groups	ν_x No. of basic groups	Mean distance r_x (in Å.u.)	
			Between acid groups ($-S_M = -3.9$)	Between basic groups ($S_M = 2.7$)
96,000	67	88	17.6	17.8
61,500*	43	57	17.4	17.7
30,000	21	27.5	16.7	17.4
20,000	14	18.4	16.0	17.1

*See Foot-note 8.

imum valence ν_M of acid and basic groups corresponding to various assumed molecular weights.

The NaCl curve in Fig. 1 reached a maximum (S_M) of + 2.7 and a minimum ($-S_M$) of -3.9 (in acid and alkaline solutions, respectively). With these values of ν_M and S_M we may calculate the mean distance r_x from equation (13). These are given in Table III for various assumed molecular weights.⁸

⁸ The value 96,000 was obtained by Smith, *J. Am. Chem. Soc.*, 1921, xliii, 1350. The more accurate value of 61,500 (at 25°C.) has since been obtained by Kunitz, *J. Gen. Physiol.*, 1926-27, x, 811. The arbitrary values 30,000 and 20,000 are included in Table III to show that the molecular weight has little effect when the valence is above 10.

Since the formula probably gives low values for long distances, we conclude that the distances are 18Å. u. or over .

The *probable* distance may be estimated if we consider that the *minimum* distance between acid groups in a protein molecule is about that in aspartyl-aspartic acid anhydride, which is 10Å.u. Only 1 amino acid molecule in 14 in gelatin is a dicarboxylic acid. Hence the probable mean distance is 30 to 60Å.u. ; but since some will be closer than others the *effective* mean distance will be less, probably between 18 and 25Å.u.

Another check on this distance is found in the "titration index dispersion."⁹ That calculated for 18Å.u. or over, is 0.7 or less. This agrees with the titration curve which has a dispersion of not more than 0.7, and apparently much less.

v.

The Gelatin Molecule.

The data indicate that the gelatin molecule is large; that the dielectric constant of the medium between these groups is not greatly different from that of water;¹⁰ and that the free ionizable groups are all functioning and are accessible to the inorganic ions in solution. We conclude that the protein molecule is spongy or arborescent in shape with molecules of solvent and of other solutes invading the interstices. It is reasonable to suppose, furthermore, that the shape and size of the molecule changes with pH since the like charges will repel each other¹⁰ and the increase in ionic strength may also influence the

⁹ The term "titration index dispersion" is used to refer to the difference between two "titration indices" having the same intrinsic indices. Thus since oxalic acid is symmetrical both groups have the same intrinsic indices (2.55); but its "titration index dispersion" ($pG_2 - pG_1$) is 2.93. In the case of sebacic acid $pG_2 - pG_1 = 0.88$. These values depend upon the distances r_d (4.5 and 14.3, respectively) and the distances r_m (1.2 and 4, respectively) between the charges in the diion and the monoion.

A divalent acid with $r_d = 18$ and $r_m = 5$ would have a dissociation index dispersion of $\Delta pK = 0.85$ or a *titration index dispersion of 0.7*, while a greater distance would give a smaller dispersion.

¹⁰ Simms, *J. Am. Chem. Soc.*, 1926, *xlvi*, 1251.

shape.¹¹ Such a change in shape and size would be roughly analogous to the opening and closing of a flower.

VI.

The Apparent Valence of Gelatin.

If we assume a point charge (an impossible condition) r_z in equation (3) will equal zero and we get:

$$\pm S = 2 \nu_A - 1 \quad (14)$$

or

$$\nu_A = \frac{\pm S + 1}{2} \quad (15)$$

where ν_A is the "apparent valence" under the assumption of a point charge ($\pm S$ is always a positive number). If we use the maximum ($S_M = 2.7$) and minimum ($-S_M = -3.9$) values of S obtained from Fig. 1 we find that the "maximum apparent valence" is $\nu_A = 2.4$ for acid groups (in alkaline solution) and $\nu_A = 1.8$ for basic groups (in acid solution). These apparent valences have no physical significance but we may substitute ν_A^2 in the unmodified equation (1) to obtain the *maximum* effect of gelatin on the ionic strength.⁴ The *true* effect appears to be even lower than the above values would indicate.

VII.

EXPERIMENTAL.

The gelatin used was ash-free isoelectric gelatin kindly furnished by Dr. John H. Northrop.

Most of the data were obtained on solutions which were 0.005 M per 2500 gm. (an arbitrary molecular weight), namely 1.25 per cent gelatin. The solutions at high and low pH were twice as strong

¹¹ The last two conclusions are contrary to Svedberg and Nichols (Svedberg and Nichols, *J. Am. Chem. Soc.*, 1927, xlix, 2920) whose primary assumptions were that the hemoglobin molecule is spherical and its size is independent of pH. See also Ghosh, *J. Chem. Soc.*, 1928, cxxxiii, 117.

TABLE IV.

Titration Data of Gelatin without Salt and in the Presence of 0.0375μ (D.—) and 0.0750μ (C.—) of Salts.

In order to condense this table we give only about half the experimental data used in Fig. 1.

Salt	pH	$\frac{b-a}{c}$	Mean β'	S'	S
None.....	1.808	-4.000*			
None.....	2.133	-3.000*			
None.....	2.448	-2.500*			
None.....	2.894	-2.000*			
None.....	3.010	-2.000	0.85		
D.—MgCl ₂	3.080	"		1.4	2.0
C.—MgCl ₂	3.113	"		1.2	1.7
D.—NaCl.....	3.109	"		1.8	2.6
C.—NaCl.....	3.133	"		1.4	1.9
D.—MgSO ₄	3.140	"		2.4	3.5
C.—MgSO ₄	3.196	"		2.1	3.0
D.—K ₂ SO ₄	3.189	"		3.3	4.7
C.—K ₂ SO ₄	3.256	"		2.8	3.9
None.....	3.074	-1.900	1.06		
D.—MgCl ₂	3.167	"		1.7	2.2
C.—MgCl ₂	3.199	"		1.4	1.8
D.—NaCl.....	3.194	"		2.2	2.9
C.—NaCl.....	3.216	"		1.6	2.0
D.—MgSO ₄	3.223	"		2.8	3.6
C.—MgSO ₄	3.275	"		2.3	2.9
D.—K ₂ SO ₄	3.270	"		3.6	4.7
C.—K ₂ SO ₄	3.329	"		2.9	3.7
None.....	3.245	-1.700	1.17		
D.—MgCl ₂	3.328	"		1.5	1.8
C.—MgCl ₂	3.370	"		1.4	1.6
D.—NaCl.....	3.362	"		2.1	2.5
C.—NaCl.....	3.390	"		1.6	1.9
D.—MgSO ₄	3.394	"		2.7	3.2
C.—MgSO ₄	3.431	"		2.0	2.4
D.—K ₂ SO ₄	3.438	"		3.5	4.2
C.—K ₂ SO ₄	3.497	"		2.8	3.3
None.....	3.391	-1.500	0.92		
D.—MgCl ₂	3.488	"		1.7	2.0
C.—MgCl ₂	3.534	"		1.5	1.7
D.—NaCl.....	3.522	"		2.3	2.7
C.—NaCl.....	3.553	"		1.7	2.0
D.—MgSO ₄	3.542	"		2.6	3.0
C.—MgSO ₄	3.586	"		2.1	2.4
D.—K ₂ SO ₄	3.588	"		3.5	4.0
C.—K ₂ SO ₄	3.649	"		2.8	3.2

* The asterisk on values of $(b - a)/c$ indicate that those solutions were 0.01 M per 2500 gm. (*i.e.*, 2.50 per cent). All other solutions were 0.005 M per 2500 gm. (*i.e.*, 1.25 per cent).

TABLE IV—Continued.

Salt	pH	$\frac{b-a}{c}$	Mean β'	S'	S
None.....	3.573	-1.300	1.12		
D.—MgCl ₂	3.671	"		1.7	1.8
C.—MgCl ₂	3.705	"		1.4	1.5
D.—NaCl.....	3.706	"		2.3	2.5
C.—NaCl.....	3.742	"		1.8	1.9
D.—MgSO ₄	3.720	"		2.5	2.7
C.—MgSO ₄	3.756	"		1.9	2.1
D.—K ₂ SO ₄	3.764	"		3.3	3.6
C.—K ₂ SO ₄	3.813	"		2.5	2.7
None.....	3.742	-1.100	1.4		
D.—MgCl ₂	3.842	"		1.6	1.8
C.—MgCl ₂	3.871	"		1.3	1.4
D.—NaCl.....	3.876	"		2.2	2.4
C.—NaCl.....	3.911	"		1.7	1.8
D.—MgSO ₄	3.882	"		2.3	2.5
C.—MgSO ₄	3.916	"		1.8	2.0
D.—K ₂ SO ₄	3.926	"		3.0	3.3
C.—K ₂ SO ₄	3.971	"		2.1	2.4
None.....	3.791	-1.000	1.4		
D.—MgCl ₂	3.898	"		1.7	1.8
C.—MgCl ₂	3.930	"		1.4	1.5
D.—NaCl.....	3.930	"		2.2	2.3
C.—NaCl.....	3.969	"		1.8	1.9
D.—MgSO ₄	3.947	"		2.5	2.6
C.—MgSO ₄	3.976	"		1.9	2.0
D.—K ₂ SO ₄	3.987	"		3.2	3.3
C.—K ₂ SO ₄	4.028	"		2.4	2.5
None.....	3.973	-0.800	1.5		
D.—MgCl ₂	4.077	"		1.6	1.6
C.—MgCl ₂	4.099	"		1.2	1.2
D.—NaCl.....	4.113	"		2.2	2.3
C.—NaCl.....	4.138	"		1.6	1.6
D.—MgSO ₄	4.116	"		2.2	2.3
C.—MgSO ₄	4.141	"		1.6	1.6
D.—K ₂ SO ₄	4.160	"		4.4	4.5
C.—K ₂ SO ₄	4.190	"		3.1	3.2
None.....	4.14	-0.600	1.0		
D.—MgCl ₂	4.256	"		1.6	1.6
C.—MgCl ₂	4.267	"		1.7	1.7

TABLE IV—Continued.

Salt	pH	$\frac{b-a}{c}$	Mean β'	S'	S
D.—NaCl.....	4.295	-0.600		2.2	2.3
C.—NaCl.....	4.304	"		1.5	1.5
D.—MgSO ₄	4.29	"		2.2	2.4
C.—MgSO ₄	4.30	"		1.5	1.5
D.—K ₂ SO ₄	4.328	"		2.6	2.7
C.—K ₂ SO ₄	4.349	"		1.9	1.9
None.....	4.41	-0.300	1.1		
D.—MgCl ₂	4.512	"		1.3	1.3
C.—MgCl ₂	4.503	"		0.8	0.8
D.—NaCl.....	4.554	"		1.9	1.9
C.—NaCl.....	4.566	"		1.4	1.4
D.—MgSO ₄	4.556	"		2.0	2.0
C.—MgSO ₄	4.542	"		1.1	1.1
D.—K ₂ SO ₄	4.596	"		2.5	2.5
C.—K ₂ SO ₄	4.607	"		1.8	1.8
None.....	4.7	0	1.0		
D.—MgCl ₂	4.847	"		>1	>1
C.—MgCl ₂	4.827	"		<1	<1
D.—NaCl.....	4.891	"		>2	>2
C.—NaCl.....	4.881	"		<2	<2
D.—MgSO ₄	4.855	"		>2	>2
C.—MgSO ₄	4.859	"		<2	<2
D.—K ₂ SO ₄	4.921	"		>2	>2
C.—K ₂ SO ₄	4.898	"		<2	<2
None.....	5.1	0.300	0.4		
D.—MgCl ₂	5.28	"		(2.7)	(2.7)
C.—MgCl ₂	5.23	"		(1.7)	(1.7)
D.—NaCl.....	5.38	"		(4.0)	(4.0)
C.—NaCl.....	5.31	"		(1.7)	(1.7)
D.—MgSO ₄	5.30	"		(2.7)	(2.7)
C.—MgSO ₄	5.26	"		(1.7)	(1.7)
D.—K ₂ SO ₄	5.38	"		(4.0)	(4.0)
C.—K ₂ SO ₄	5.36	"		(2.5)	(2.5)
None.....	6.10	0.500	0.17		
D.—MgCl ₂	6.019	"		-1.2	-1.2
C.—MgCl ₂	5.960	"		-1.4	-1.4
D.—NaCl.....	6.055	"		-0.8	-0.8
C.—NaCl.....	5.977	"		-1.2	-1.2
D.—MgSO ₄	6.033	"		-1.1	-1.1
C.—MgSO ₄	6.004	"		-1.0	-1.0
D.—K ₂ SO ₄	6.07	"		-0.5	-0.5
C.—K ₂ SO ₄	6.02	"		-0.8	-0.8

TABLE IV—Continued.

Salt	pH	$\frac{b-a}{c}$	Mean β'	S'	S
None.....	6.82	0.600	0.12		
D.—MgCl ₂	6.61	"		-3.3	-3.3
C.—MgCl ₂	6.51	"		-3.1	-3.1
D.—NaCl.....	6.68	"		-2.2	-2.2
C.—NaCl.....	6.63	"		-1.9	-1.9
D.—MgSO ₄	6.48	"		-5.4	-5.4
C.—MgSO ₄	6.65	"		-1.7	-1.7
D.—K ₂ SO ₄	6.65	"		-2.7	-2.7
C.—K ₂ SO ₄	6.45	"		-3.7	-3.7
None.....	7.72	0.700	0.11		
D.—MgCl ₂	7.17	"		-9.1	-9.1
C.—MgCl ₂	7.49	"		-2.3	-2.3
D.—NaCl.....	7.54	"		-3.0	-3.0
C.—NaCl.....	7.56	"		-1.6	-1.6
D.—MgSO ₄	7.53	"		-3.1	-3.1
C.—MgSO ₄	7.65	"		-0.6	-0.6
D.—K ₂ SO ₄	7.58	"		-2.3	-2.3
C.—K ₂ SO ₄	7.61	"		-1.1	-1.1
None.....	8.66	0.800	0.12		
D.—NaCl.....	8.46	"		-3.4	-3.4
C.—NaCl.....	8.48	"		-1.9	-1.9
D.—K ₂ SO ₄	8.49	"		-2.9	-2.9
C.—K ₂ SO ₄	8.56	"		-1.0	-1.0
None.....	9.414	0.900	0.19		
D.—NaCl.....	9.209	"		-3.6	-3.8
C.—NaCl.....	9.214	"		-2.1	-2.2
D.—K ₂ SO ₄	9.297	"		-2.0	-2.1
C.—K ₂ SO ₄	9.235	"		-1.9	-2.0
None.....	9.792	1.000	0.26		
D.—NaCl.....	9.592	"		-3.6	-3.9
C.—NaCl.....	9.580	"		-2.3	-2.5
D.—K ₂ SO ₄	9.646	"		-2.6	-2.9
C.—K ₂ SO ₄	9.629	"		-1.8	-2.0
None.....	10.408	1.300	0.57		
D.—NaCl.....	10.254	"		-2.9	-3.4
C.—NaCl.....	10.237	"		-2.0	-2.4
D.—K ₂ SO ₄	10.303	"		-2.0	-2.4
C.—K ₂ SO ₄	10.266	"		-1.6	-1.9

TABLE IV—*Concluded.*

Salt	pH	$\frac{b-a}{c}$	Mean β'	S'	S
None.....	10.573	1.400	0.40		
D.—NaCl.....	10.434	"		-2.6	-4.3
C.—NaCl.....	10.410	"		-1.9	-3.0
D.—K ₂ SO ₄	10.476	"		-1.8	-3.1
C.—K ₂ SO ₄	10.456	"		-1.3	-2.1
None.....	11.442	2.000*	0.18		
D.—NaCl.....	11.390	"		-1.3	(-6)
C.—NaCl.....	11.307	"		-1.1	(-4)
D.—K ₂ SO ₄	11.410	"		-0.8	-2.6
C.—K ₂ SO ₄	11.384	"		-0.8	-2.8

(0.01 M or 2.5 per cent). At each equivalent of base $(b - a)/c$ which was studied, two solutions were made up without salt, also one "dilute" (0.0375 μ) and one "concentrated" (0.0750 μ) of each of the salts: NaCl, MgCl₂, K₂SO₄, and MgSO₄. Each solution contained 5 cc. of a mother solution of gelatin (of twice the concentration) and was made up to 10 cc. All readings at a given value of $\frac{b-a}{c}$ were taken within

a few minutes of each other to reduce experimental error in the relative values to a minimum. One solution without salt was measured first and the other last, those with salt coming in between. The same two bubbling water-jacketed hydrogen electrode cells¹² were used in all measurements. Saturated KCl junction was assumed constant. The pH standard was 0.100 M HCl equals pH 1.075 at 25°C. The data are given in Table IV. The two values without salt are averaged in each case where there was any difference.

The values of b' used in equation (7) to calculate values of S' and S were calculated by the formula as described previously.¹³

$$b' = \frac{b-a}{c} + h - \frac{oh}{c}$$

¹² Simms, *J. Am. Chem. Soc.*, 1923, xlv, 2503.

¹³ Simms, *J. Am. Chem. Soc.*, 1926, xlviii, 1239.

VIII.

SUMMARY.

2.5 and 1.25 per cent gelatin have been titrated potentiometrically in the absence of salts and in the presence of two concentrations (0.0750 and 0.0375 μ) of NaCl, MgCl₂, K₂SO₄, and MgSO₄. The data have been used to calculate values of $\pm S = \nu^s - (\nu - 1)^s$, where $\nu^s = \nu^2 - (\nu^2 - \nu) r_x/18$.

The maximum and minimum values of S with NaCl were used to calculate the mean distance (r_x) between like charges in gelatin. This is found to be 18 Å.u. or over (between acid or basic groups) which agrees with the probable value and the titration index dispersion. Thus the data with NaCl are shown to be normal and to obey the equation found to hold for simple weak electrolytes; namely, $\text{pK}' - \text{pK} = Sa \sqrt{\mu}$ where S is related to the valence and distance by the above equations.

Using the NaCl data as a standard the deviations (ΔS) produced by the other salts are calculated and are found to agree quantitatively with the deviations calculated from equations derived for the simple weak electrolytes. This shows that in gelatin, as in the simple electrolytes, the deviations are related to the "apparent valences" (values which are a function of the true valence and the distance between the groups).

The maximum "apparent valences" of gelatin are 2.4 for acid groups (in alkaline solution) and 1.8 for basic groups (in acid solution). These values correspond to the hypothetical condition of zero distance between the groups. They have no physical significance but have a practical utility first as mentioned above, and second in that they may be used in the unmodified Debye-Hückel equation to give the maximum effect of gelatin on the ionic strength. The true effect is probably even lower than these values would indicate.

The data indicate that gelatin is a weak polyvalent ampholyte having distant groups and that the molecule has an arborescent structure with interstices permeated by molecules of the solvent and other solutes. The size and shape probably vary with the pH.