



Philosophical Magazine Series 6

ISSN: 1941-5982 (Print) 1941-5990 (Online) Journal homepage: <http://www.tandfonline.com/loi/tphm17>

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To cite this article: William Sutherland (1908) XLV. Another method of measuring large molecular masses, *Philosophical Magazine Series 6*, 16:94, 497-512, DOI: [10.1080/14786441008636534](https://doi.org/10.1080/14786441008636534)

To link to this article: <http://dx.doi.org/10.1080/14786441008636534>



Published online: 21 Apr 2009.



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THE
LONDON, EDINBURGH, AND DUBLIN
PHILOSOPHICAL MAGAZINE
AND
JOURNAL OF SCIENCE.

[SIXTH SERIES.]

OCTOBER 1908.

XLV. *Another Method of Measuring large Molecular Masses.*
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IT has already been proposed to find the masses of molecules, which are too large for the ordinary methods, by determining their radii from their velocities of diffusion or their velocities in electrolytic conduction (Phil. Mag. [6] ix. p. 781 and xiv. p. 1). These methods have been applied to the large molecules of egg albumin and globulin. In the latter paper it was shown that they yield for the radius of the hydrogen molecule an absolute value agreeing well with that given by the kinetic theory of gases. The latter paper contains incidentally also another method of finding large molecular masses which will be worked out in the present communication and applied briefly to experimental data for certain peptones, casein, and globulin. The theoretical equation (13) of that paper gives the following connexion between the molecular conductivity λ and the concentration of a solution containing n gram-molecules per cu.³ of a solute whose molecule yields n_1 positive ions of valency ν_1 and n_2 negative ions of valency ν_2 , the conductivities of the ions at infinite dilution being Λ_{01} and Λ_{02} , and at concentration n being Λ_1 and Λ_2 :

$$\lambda_0 \eta_0 / \lambda \eta = 1 + 2\pi(\Lambda_1 + \Lambda_2) C \nu_1 \nu_2 \{n(n_1 + n_2) / h\}^{1/2} / 3KI \lambda_0, \quad (1)$$

in which C is a constant, η_0 is viscosity of solvent, η of solution, h is the mass of the atom of hydrogen, K is the

* Communicated by the Author.

dielectric capacity of the solvent, I the force in the solvent which ionizes the solute completely at all concentrations, and λ_0 is generally identical with $\Lambda_{01} + \Lambda_{02}$. In applying equation (1) to the refined measurements of Kohlrausch and his pupils on very dilute aqueous solutions I showed that $\nu_1 \nu_2$ did not suffice to express the whole dependence upon valencies, and that seemingly a factor $(\nu_1 + \nu_2)^2$ is introduced by $1/I$. Moreover, Kohlrausch has recently preferred to treat λ as linear in $n^{\frac{1}{2}}$ rather than in $n^{\frac{3}{2}}$, which was his discovery of years ago. I believe this discrepancy arises in the assumption made in calculating λ from the experimental measurements that the conductivity of the water is not altered by the presence of the solute. Now in the Molecular Constitution of Aqueous Solutions (Phil. Mag. [6] xii. p. 1) I showed that the H ions of acids and the OH ions of alkalis ionize H_2O powerfully. It is possible therefore and probable that other ions ionize H_2O to a small extent, variable with the concentration. Probably a very careful discussion of Kohlrausch's measurements would clear up the discrepancy between his experiments and (1) and would give the law of the ionization of H_2O by all ions. Equation (1) is well verified by measurements on solutions which are not too dilute, though even with them $\nu_1 \nu_2 (n_1 + n_2)^{\frac{3}{2}}$ does not suffice to give the whole of the dependence upon valency. But on the other hand equation (1), as it stands, expresses the valency rule discovered inductively by Ostwald from his experiments on dilute solutions of the Na salts of polybasic acids (*Ztschr. f. physik. Chem.* i. and ii.). The form in which Ostwald's valency rule is expressed by Bredig (*ibid.* xiii.) is equivalent to $d\lambda/dn = \nu_1 \nu_2 \phi(n)$, where $\phi(n)$ is a function of the concentration the same for all solutes. This is the result obtainable from (1) by differentiation. Thus (1) is in agreement with a large body of experimental evidence. But (1) for dilute solutions may be written approximately

$$\lambda_0/\lambda = 1 + 2\pi(\Lambda_{01} + \Lambda_{02})C\nu_1\nu_2^{\frac{1}{2}}n(n_1 + n_2)/h^{\frac{3}{2}}/3KI\lambda_0. \quad (2)$$

But a better value of the coefficient of $n^{\frac{3}{2}}$ can be obtained by improving the reasoning by which λ_0 comes into the right-hand side of (1). In (1) λ_0 measures the rate at which a positive ion and a neighbour negative one tend to relax the strain produced in them by electric force. But it will be better to regard each ion relaxing at its own rate, and so to replace $1/\lambda_0$ by $1/\Lambda_{01} + 1/\Lambda_{02}$. If in the usual way we are going to compare solutions of equivalent and not equi-

molecular concentrations, we have m the number of gram equivalents per cm.^3 equal to

$$n(n_1\nu_1 + n_2\nu_2). \quad \dots \quad (3)$$

So the relation between λ and m is

$$1/\lambda = \{1 + 2\pi C(\Lambda_{01} + \Lambda_{02})^2 \nu_1 \nu_2 (n_1 + n_2)^{\frac{1}{2}} m^{\frac{1}{2}} / 3h^{\frac{1}{2}} (n_1 \nu_1 + n_2 \nu_2)^{\frac{1}{2}} \} \text{KIA}_{01}\Lambda_{02} / (\Lambda_{01} + \Lambda_{02}). \quad (4)$$

For brevity we write this :—

$$1/\lambda = 1/(\Lambda_{01} + \Lambda_{02}) + bm^{\frac{1}{2}}, \quad \dots \quad (5)$$

where

$$b = 2\pi C \nu_1 \nu_2 (n_1 + n_2)^{\frac{1}{2}} (\Lambda_{01} + \Lambda_{02}) / 3h^{\frac{1}{2}} (n_1 \nu_1 + n_2 \nu_2)^{\frac{1}{2}} \text{KIA}_{01}\Lambda_{02} \quad (6)$$

and is the immediate subject of study.

In the important case of the Na salt of a ν_2 -basic acid we have $n_2 = \nu_1 = 1$, $n_1 = \nu_2$, and

$$b = 2\pi C \left(\frac{n_1 + n_2}{2n_1} \right)^{\frac{1}{2}} \frac{\nu_1}{\Lambda_{01}} \frac{\nu_2}{\Lambda_{02}} \frac{\Lambda_{01} + \Lambda_{02}}{3h^{\frac{1}{2}} \text{KI}}. \quad \dots \quad (7)$$

The experimental material from which Ostwald discovered his valency rule affords a suitable test for this theoretical deduction. It will be noticed that b , the coefficient of $m^{\frac{1}{2}}$, depends not only on $\nu_1 \nu_2$ but also upon both Λ_{01} and Λ_{02} , whereas in Ostwald's rule there is no mention of this latter dependence. The reason for Λ_{01} and Λ_{02} not entering into Ostwald's statement of his rule is that he worked only with Na salts, so that Λ_{01} was constant, and that although he worked with acids with basicity ranging from 1 to 6, there was a rough tendency for $\{(n_1 + n_2)/2n_1\}^{\frac{1}{2}} (\Lambda_{01} + \Lambda_{02})/\Lambda_{02}$ to be constant and so to disguise the dependence on Λ_{02} . In testing our equations we shall first apply (5) to Ostwald's data for the sodium salt of nicotinic or β -pyridine carboxylic acid $\text{C}_5\text{H}_4\text{NCOOH}$ converting them from the Siemens unit of resistance, which he used, to the ohm. I find $\Lambda_{01} + \Lambda_{02} = 89.8$, and $b = 0.0838$, which give the following comparison :—

$1/10^3 m$	32	64	128	256	512	1024
λ exp.	72.9	75.4	78.0	80.3	82.4	84.0
λ calc.	72.6	75.6	78.1	80.3	82.0	83.6

By availing himself of the carboxyl substitution compounds of pyridine up to pyridine pentacarboxylic acid, Ostwald obtained a series of similar ions with ν_2 ranging from 1 to 5, and by means of the sodium mellitate $\text{C}_6(\text{COONa})_6$ he carried his investigation up to $\nu_2 = 6$. The following table contains

the values of $\Lambda_{01} + \Lambda_{02}$ and b which I have calculated from Ostwald's data, as given by Bredig (*loc. cit.*), after I have converted them to the ohm as unit. The substances represented by their formulas are the sodium salts of pyridine-monocarboxylic acid, -tricarboxylic, -tetracarboxylic, and -pentacarboxylic acids, the place of the -dicarboxylic acid being taken by that of phenylpyridine-dicarboxylic acid, and the 6-basic salt being hexasodium mellitate :—

TABLE I.

$C_5NH_4CO_2Na.$	$C_5NH_2C_6H_3(CO_2Na)_2.$	$C_5NH_2(CO_2Na)_3.$	$C_5NH(CO_2Na)_4.$	
$\Lambda_{01} + \Lambda_{02} \dots$	89.8	106.1	144.8	166.5
$b \dots \dots$	0.0838	0.1257	0.1450	0.1796
$C_5N(CO_2Na)_5$	$C_6(CO_2Na)_6.$			
$\Lambda_{01} + \Lambda_{02} \dots$	192.1	204.2		
$b \dots \dots$	0.2197	0.2830		

From the data of Kohlrausch Λ_{01} for Na at 25° C. is 51.2, so that for the ions of these six acids we get for Λ_{02} the values 38.6, 54.9, 93.6, 115.3, 140.9, and 153.0. We have now all the data necessary for testing the implication of (7) that

$$b(\Lambda_{02}/\nu_2)\{2n_1/(n_1 + n_2)\}^{1/2}/(\Lambda_{01} + \Lambda_{02}) \text{ is to be constant. } (8)$$

Here are the products $\times 10^4$ for the six Na salts :—

361 358 358 363 382 423

We shall take the product to be 0.036 at 25°, becoming $0.036 \times 1.18 = 0.0425$ at 18°. The factor $\{2n_1/(n_1 + n_2)\}^{1/2}$ enters through the necessity for expressing as uniform a distribution as possible throughout the solution of the nm_1 positive ions, each charged with ν_1 electrons, and the nm_2 negative ions, each charged with ν_2 electrons. Mathematically it is difficult to specify such a distribution; it is still more difficult to make physical allowance for the effect of the different magnitudes of the charges. If we leave out $\{2n_1/(n_1 + n_2)\}^{1/2}$ the products $\times 10^4$ are

361 325 313 311 322 353

On the whole then the theoretical deduction (7) is verified by experiment, and Ostwald's valency rule in its amended form as given by (7) is established upon a theoretical physical basis. By its means the ratio Λ_{02}/ν_2 can be found for an ion which has Λ_{02} too small to be measured with sufficient accuracy in the ordinary way. From this ratio the volume of the large slow ion can be found by one of the

methods which I have proposed and applied for the measurement of large molecular masses. In the paper on Ionization in Solutions and Two New Types of Viscosity (*loc. cit.*), it was shown that for element ions this method is expressed for 18° C. by the equation

$$\frac{1}{B^{\frac{1}{3}}\Lambda_0} = \frac{0.0365}{B^{\frac{1}{3}}} + \frac{0.0022}{\nu(1 + 10.5/B^{\frac{1}{3}})}, \dots (9)$$

in which B is the volume of a gram-atom of valency ν . For the fatty acid ions from the acetic CH_3COO up to $\text{C}_5\text{H}_{11}\text{COO}$ the same formula holds except that 0.0022 is replaced by 0.0097, ν of course being 1. While for these five ions the formula expresses the experimental facts closely, yielding Λ_0 to 1 per cent., it fails for formic acid for which it makes Λ_0 too large by 18 per cent. Now for the pyridinecarboxylic acids I find that at 18° C., assuming that at 25° Λ_0 is 1.18 times its value at 18°,

$$\frac{1}{B^{\frac{1}{3}}\Lambda_0} = \frac{0.00547}{B^{\frac{1}{3}}} + \frac{0.0097}{\nu(1 + 10.5/B^{\frac{1}{3}})} \dots (10)$$

In the second term this is identical with the previous result for the fatty acid ions, the valency ν appearing specifically, as it does in the case of the element ions. But the coefficient of the first term on the right is only 0.15 times that for the fatty acid ions and the element ions. I take this striking result to be due to the fact that pyridine is a base giving a markedly alkaline reaction. The N atom in it carries a special electron pair $b\ddot{N}$, and the positive electrons of the acids derived from pyridine have their inductive effects mostly confined within the pyridinecarboxylic ions by this pair $b\ddot{N}$. Now the first term on the right originates in viscosity caused by the electron of the ion acting inductively on the molecules of the solvent. But if the induction is confined within the ion mostly, then the viscosity of electric inductive origin must become relatively small. In the paper just cited I gave reason for theoretically expecting this variable effect in large ions, and was surprised not to find it in the fatty acid ions. It appears now that the paraffin residue $\text{C}_n\text{H}_{2n+1}$ does not affect the inductive action in the solvent appreciably until in the formic ion it is reduced to H, whence the exceptional behaviour of the formic ion. It is very satisfactory in these circumstances to find the ordinary viscous resistance to the pyridinecarboxylic ions expressed by the same term as applies to the fatty acid ions with fulfilment of the additional theoretical condition that ν must appear as in (10)

with values ranging from 1 to 6. In the following table are compared the values of Λ_{02} previously derived in this paper from the experimental data with those derived from (10) and multiplied by 1.18 to convert from 18° to 25°. The values of B the limiting volume of a gram-ion are derived from the data of Table IV. of Further Studies on Molecular Force (Phil. Mag. [5] xxxix. p. 1). B for certain elements has the approximate values C 8, H 4, N 8, and O 6. The substances are taken in the same order as in Table I. of the present paper.

TABLE II.

B.....	98	172	121	138	155	176
Λ_{02} exp.	38.6	54.9	93.6	115.3	140.9	153.0
Λ_{02} calc.	37.8	55.8	95.9	117.3	136.3	151.9

It ought to be mentioned that the theoretical considerations here used have led to very different values for Λ_{02} from those found by Bredig from the application of Ostwald's original valency rule and other empirical considerations. For example, Bredig gives the ionic conductivity of the ion of pyridinepentacarboxylic acid as 89.2 in the Siemens unit, equivalent to 94 with the ohm, to be compared with the 140.9 of Table II. above, and there are similar differences with the other ions though they tend to vanish when $\nu=1$. The fact that the values Λ_{02} exp. in Table II. agree with those given by (10) is favourable to the conclusion that the theory has led to more correct limiting values Λ_{02} than Bredig could obtain at the time when he collected and calculated the wealth of data in his paper.

We can now see how the foregoing considerations lead to a new method of measuring large molecular masses, or more strictly large ionic masses from which the related molecular masses can be inferred. If the mass of an ion is large and ν is not large, then the ionic velocity, say Λ_{02} , is small compared with $\Lambda_{01} + \Lambda_{02}$, which is measured experimentally and is liable to error comparable with Λ_{02} , thus involving Λ_{02} in very considerable error. But if b is measured and used in (8) along with the measured $\Lambda_{01} + \Lambda_{02}$ it gives Λ_{02}/ν_2 which in (10) gives B. The conditions of the solution are best understood by re-writing (10) in the following form:—

$$\frac{\nu}{B^{\frac{1}{2}}\Lambda_0} = \frac{0.00547\nu}{B^{\frac{1}{2}}} + \frac{0.0097}{1 + 10.5/B} \dots \dots (11)$$

Here there is a second unknown ν in addition to B. But it occurs as factor of a term which is small when ν is small. So the values of B can be calculated for such small values of ν

as 1, 2, and 3. To each value of B corresponds a value of the gram-molecular mass of the ion. But the equivalent mass of the ion is generally known from a titration, and this compared with the values of M shows what must be the right value of ν , and so the right value of M is obtained. But (10) applies strictly only to the pyridinecarboxylic acids, where there is an amphoteric union of basic and acid properties. We may assume (10) and (11) to apply approximately to other amphoteric ions. The first term on the right is uncertain, being for the pyridinecarboxylic acids only 0.15 of the value for the fatty acids, but for the case of ν not large it is of minor importance. Indeed, in these equations if we neglect the first term on the right we get a form of relation corresponding to the relation $\nu/B^{\frac{1}{2}}\Lambda = K/280$, K being dielectric capacity of ion, which I used when first proposing to find the mass of an ion from its conductivity. For the large organic ions $K = 2$ nearly. When ν is large we shall have to proceed in a similar way with (11), but more warily. It will be best then to illustrate the method by applying it to some typical cases.

The most interesting group is that of the peptones shown by Siegfried and his pupils to be definite chemical substances (*Ber. d. Deutsch. Chem. Ges.* xxxiii.; *Zeitsch. f. physiolog. Chem.* xxxv., xxxviii., xlv.). The following are the names of these, their simplest formulas by analysis, and the corresponding provisional molecular weights along with equivalents discussed below.

		Equivalent		
		as acid.	as base.	
Trypsinfibrinpeptone	α ... $C_{10}H_{17}N_3O_5$	259	157	290
"	β ... $C_{11}H_{19}N_3O_5$	273	197	397
Pepsinfibrinpeptone	α ... $C_{21}H_{34}N_6O_9$	515	248	370
"	β ... $C_{21}H_{36}N_6O_{10}$	533		
Pepsinglutinpeptone $C_{22}H_{30}N_7O_{10}$	573	320	470
Trypsinglutinpeptone $C_{19}H_{30}N_6O_9$	486		

These are all amphoteric, but their acidic character is more pronounced than their basic. The equivalent weights for four of them both as acids and bases have been measured by Neumann according to the ingenious method of electric titration devised by Sjöqvist (Neumann, *Zeitsch. f. physiolog. Chem.* xlv. p. 216; Sjöqvist, *Skand. Archiv. f. Physiolog.* v. 1895). This method depends upon the exceptionally large electric conductivities of the H ion of acids and of the OH ion of alkalies. Suppose a normal solution of HCl has a normal solution of NaOH added to it in instalments, the specific electric conductivity of the mixture being measured after

each addition. At the point of exact neutralization the conductivity will be a minimum, namely, that of a normal solution of NaCl. If more NaOH solution is added, the conductivity increases till with an infinite amount it becomes that of normal solution of NaOH. The experimental method of using the method to find the equivalent weight of an acid is to start with a solution of NaOH 0.05 normal, as in Sjöqvist's investigation with albumin, and then to add the acid in instalments till it is present in great excess. Then if the results are graphed with amount of acid as abscissa and electric conductivity as ordinate, they give in the simplest cases two straight lines meeting at the point of minimum conductivity, whose abscissa gives the amount of acid required to make a known volume of 0.05 normal solution of the acid. In the more complex cases the graph does not give the two ideal straight lines near the point of minimum conductivity, but forms a curve with the two straight lines as fairly decided asymptotes. If these asymptotes are produced till they meet, their point of intersection gives the required datum. Neumann tested the method on glycocoll $\text{NH}_2\text{CH}_2\text{COOH}$ and asparagine $\text{C}_2\text{H}_3\text{NH}_2\text{CONH}_2\text{COOH}$ both as acids to NaOH and bases to HCl, and obtained by electric titration results agreeing with their known equivalents. Applying the method then to four of Siegfried's peptones, Neumann obtained the results given above under the headings "equivalent as acid, as base." These do not stand in any simple intelligible relation to the provisional molecular weights. The situation is a suitable one for applying the methods of the present paper. Neumann measured the equivalent conductivities of the four Na peptonates at strengths 1/32 and 1/1024 normal, as given in the next table along with the values of $\Lambda_{01} + \Lambda_{02}$ and b in (5) derived from them. His values of the conductivity of HCl correspond to the temperature $21^\circ.5$, according to Kohlrausch's latest data. At this temperature Λ_{01} for Na is 47, by which we obtain Λ_{02} for the peptone ions. Then by means of (8) with $(0.036 + 0.0425)/2 = 0.03925$ as the appropriate constant for $21^\circ.5$, and with tentative values of $n_1/(n_1 + n_2)$, we calculate Λ_{02}/ν_2 as given in the table, and so we obtain ν_2 .

TABLE III.

Peptone.	$\lambda(32)$.	$\lambda(1024)$.	$\Lambda_{01} + \Lambda_{02}$.	Λ_{02} .	b .	Λ_{02}/ν_2 .	ν_2 .
Trypsinfibrin α ...	72.98	90.76	102	55	.1246	29.2	1.88
„ β ...	82.2	104.7	120	73	.121	35.4	2.06
Pepsinfibrin α I. .	72.3	101.9	126	79	.186	23.2	3.4
„ II.	77.5	106.6	129	82	.163	27.1	3.02
Pepsinglutin	75.8	105.5	128.5	81.5	.172	25.6	3.18

It is to be noticed that four out of the five values of ν are nearly integers, while that for the first specimen of pepsin-fibrin peptone α is nearly 3.5. Such a fractional value might appear if a peptone was split up by NaOH into two ions, one of valency 3, the other 4. That there is a splitting of the peptone molecule we shall see immediately. But for the four cases where ν_2 is nearly a whole number we shall replace its value in the table by the nearest whole number, with which we shall then divide the tabulated value of Λ_{02} to get the following amended values of Λ_{02}/ν_2 for the four peptones in the above order:—

ν_2	2	2	3	3
$\Lambda_{02}/\nu_2 \dots$	27.5	36.5	27.3	27.2

These valencies are the same as Neumann found by the use of Ostwald's empirical valency rule. To use the values of Λ_{02}/ν_2 in (11) for finding B we must reduce them to 18° by dividing by 1.09 and then we get

	P e p t o n e.			
	Trypsinfibrin α .	Trypsinfibrin β .	Pepsinfibrin α .	Pepsinglutin.
B	150	83	144	144

The valencies of these ions must represent the number of COO groups they contain, so that $144/3=48$ represents the volume of a COO group in a trivalent peptone ion, together with the volume of the atoms associated with it. But B for COO is 20, so that the volume of an equivalent of a trivalent peptone ion is about $2\frac{1}{2}$ times the volume of COO, so its mass will be roughly $2\frac{1}{2}$ times that of COO, which is 44. Thus we find the order of magnitude of an equivalent to be about 110 at the most, and this is much smaller than 248 and 320 found by Neumann by electric titration for the trivalent peptones. It is plain then that we have to do with a splitting up of the peptone molecule by NaOH. Suppose M to be the molecular mass of peptone, which is split into n_2 ions of valency ν_2 and mass p , and a neutral residue of mass r , then $M=n_2p+r$, and the equivalent by titration is $M/n_2\nu_2=p/\nu_2+r/n_2\nu_2$. This $r/n_2\nu_2$ is the difference between our rough maximum of 110 found above for p/ν_2 and 248 or 320 for $p/\nu_2+r/n_2\nu_2$. It is important, then, to look into the values of B as closely as we can. In the trivalent ion with $B=144$ 3000 would contribute 60 to B. Knowing the amino-acid character of the peptones we may assume that possibly an NH_2 group is associated with one COO group or two, if two they contribute 32 to B. In B there remains only 52 to account for, which is most easily done by assuming

the presence of C and H groups, which for the trivalent ion in peptones give $\text{HCCH}_2\text{COO}(\text{CHNH}_2\text{COO})_2$ as a typical formula, having $B=144$. The actual formula must be decided by chemical analysis of the acid ions when separated from the residue. But as regards the two peptones yielding trivalent ions we can assert that the molecular mass of pepsinfibrinpeptone α must be a multiple of 515, the minimum possible by analysis, and also a multiple of three times 248, the equivalent found by electric titration. The smallest number which satisfies these conditions within the limits of experimental error in determining the equivalent is $515 \times 3 = 1545$, which would give an equivalent of 257, the experiments giving a possible range from 234 to 262. In the same way the molecular mass of pepsinglutin peptone must be a multiple of 573 and also of 3×320 . In this case 5×573 satisfies the conditions, since $2865/9$ gives an equivalent of 317, while the experimental range is from 317 to 328. Treating the divalent ions in the same way, we find for the ion of trypsinfibrinpeptone α such a formula as $(\text{CHNH}_2)_4(\text{COO})_2$, for which B is 152 instead of 150, and for pepsinfibrinpeptone β ions the formula $\text{C}(\text{NH}_2)_2(\text{COO})_2$, for which B is 80 instead of 83. For α the molecular mass is to be a multiple of 259, and also of 2×157 . The number 1295 is the lowest satisfying the conditions nearly, since $1295/8$ gives an equivalent 162, the experimental value ranging from 157 to 164. For the β form the molecular mass is a multiple of 273, and also of 2×197 , and 2730 meets the case. We have not obtained perfectly definite molecular masses for the peptones, because we have found that we really deal only with the acid ions split from the peptone molecule, and are without definite experimental information as to the mass of the neutral residue. The HCl compounds of peptone are not so definite in their behaviour as the NaOH compounds, so that Neumann's experiments with them do not enable us to add anything of importance to the foregoing. The molecular mass of the peptones seems to be of the order 1400 or 2800. If we use the estimated molecular masses for the four peptones along with the masses of the ions in $M = n_2\nu + r$ for calculating r , we get the values 1111, 2214, 479, and 1806. Apparently these residues are important substances still of considerable complexity.

In this connexion it will be useful to consider briefly the molecular conductivities of aqueous solutions of the peptones found by Neumann, these being calculated by him according to Siegfried's formulæ above, as if these gave correct molecular masses. I find that his results can be expressed by

the formula

$$\lambda = a + c/n^{\frac{1}{2}}, \quad (12)$$

where a and c are parameters characteristic of each peptone, and have the following values:—

	Peptone.			
	Trypsinfibrin α .	Trypsinfibrin β .	Pepsinfibrin α , commercial.	Pepsinfibrin α , Börkel.
a	10.2	10.56	12.7	8.78
c	0.0549	0.0275	0.0187	0.016

The following comparison is given to show how the formula expresses the experimental facts:—

1/1000 n .	Trypsinfibrinpeptone α .							
	8	16	32	64	128	256	512	1024
λ exp. ...	15.88	18.01	20.44	23.70	28.82	36.99	49.23	66.51
λ calc. ...	15.11	17.14	20.02	24.09	29.84	37.98	49.48	65.76

To explain the formula we may assume that the parameter a represents the molecular conductivity of a salt whose variation with concentration is not detectable at these dilutions in the presence of the rest of the peptone. The difference between 12.7 for the commercial sample of pepsinfibrinpeptone and 8.78 for Börkel's preparation may be ascribed to soluble impurity in the commercial sample. But in the other cases a may originate in some organic salt of an amine base which is split off from the peptone upon solution in water. Then the term $c/n^{\frac{1}{2}}$ arises from the conductivity of the acids separated from the peptone by water. Ostwald's dilution law for organic acids at high dilution makes their conductivity proportional to $1/n^{\frac{1}{2}}$. I have suggested that this law of Ostwald's originates in the organic acids consisting of double molecules which are partly dissociated by water according to the law, the dissociated molecules being completely ionized and the undissociated not at all. Thus, then, the formula (12) shows that either peptone is an organic acid or has organic acids split off from it, and that the single acid or mixture of acids forms so dilute a solution that its molecular conductivity can be accurately represented by $c/n^{\frac{1}{2}}$. With an amount of ionized dissociated di-acid proportional to $1/n^{\frac{1}{2}}$ the molecular conductivity ought to be proportional to $\Lambda_0/n^{\frac{1}{2}}(1 + bm^{\frac{1}{2}})$, where m is the actual concentration of the organic ions. The fact that this term $bm^{\frac{1}{2}}$ does not appear in (12) proves that the actual concentration of the organic ions is too small to make it appreciable. This is exactly what we should expect of such acids as we have found to form Na salts when peptone is neutralized

with NaOH. The phenomena of diffusion with peptones and their compounds must be considerably complicated by the ionizations which take place with them. The result of Kühne that pepsinpeptone diffuses only half as slowly as glucose (twice as fast) seems incompatible with the results of the present paper (see Cohnheim's *Chemie der Eiweisskörper*, p. 87). The diffusion of the peptones is worth thorough investigation for its bearings upon the whole physical chemistry of digestion.

By this method we shall now investigate the Na salts of globulin and casein. For Hardy's data for the conductivity of solutions of Na globulin I have found the following formula to hold with the units used in this paper (Proc. Roy. Soc. B. lxxix. p. 146):—

$$\lambda = 22 + 1/(0.005 + 3.1 m^{\frac{1}{2}}). \quad . . . \quad (13)$$

At infinite dilution this gives $\lambda = 222$, which is a little greater than the 218.4 of Kohlrausch for NaOH at 18° and infinite dilution. The remarkable point about this formula is the functioning of one half of the Na in quite a different capacity from the other, shown by the term $22 = 44/2$ which is independent of the concentration. Moreover, although at infinite dilution the conductivity is nearly that of NaOH, at ordinary finite dilutions it is much smaller than that of NaOH because the coefficient 3.1 is so large. This large coefficient is due to the globulin whose ions involve the half of the Na ions and all the OH ions in a greater viscosity of electric origin. The globulin ions act as an electric brake upon the electrically driven OH ions and half of the Na ions. Thus although the experiments with Na globulin are not yet refined enough in practice and theory to yield directly the conductivity of the globulin ion, they give us the viscosity of electric origin due to the globulin ion, and thus enable us to find its conductivity and volume by the methods of the present paper, if we accept Hardy's conclusion that ν_2 is probably 2. Let us write (13) in the general form

$$\lambda = \Lambda_{0b}/2 + 1/\{1/(\Lambda_{0b}/2 + \Lambda_{0a}) + bm^{\frac{1}{2}}\}, \quad . . \quad (14)$$

where Λ_{0b} represents the conductivity of the positive ion like Na, and Λ_{0a} represents that of OH. Probably Λ_{02} the conductivity of the globulin or similar ion ought to appear on the right side as an additional term, but it is best omitted till it emerges clearly from the experiments. For NH_4 globulin this formula holds just as for Na globulin. I shall now show that it applies to Na and NH_4 casein, thereby strengthening considerably the validity of its form

and interpretation. The data for the caseinates were obtained by Sackur (*Ztschr. f. physik. Chem.* lxi. 1902) at 25°. For Na casein they give $\Lambda_{ob}/2 = 28.15$, $\Lambda_{ob}/2 + \Lambda_{oa} = 219.3$, and $b = 1.678$. The sum of 28.15 and 219.3 is 247.4, while λ for NaOH at 25° and infinite dilution is 247.2. Kohlrausch's value for $\Lambda_{ob}/2$ at 25° is 25.6, so perhaps 28.15–25.6 represents a tendency for Λ_{o2} for casein to make its appearance. The following comparison shows the applicability of (14) to Na casein:—

1/1000 <i>m</i> ...	40	80	160	320	640
λ exp.	46.5	51.3	56.2	63.0	69.5
λ calc.	46.8	51.1	56.4	62.5	69.7

For NH₄ casein $\Lambda_{ob}/2 = 38.4$, $\Lambda_{ob}/2 + \Lambda_{oa} = 231.5$, and $b = 1.634$. For Hardy's NH₄ globulin at 18° the corresponding values are 2.9, 208.3, and 2.3, this last number differing unaccountably from the 3.1 for Na globulin.

It is necessary to form some conception of the way in which half of the positive ions in these solutions are exempt from the resistance of viscosity of electric origin. The case becomes clearer if we consider first the simpler one of the HCl compound of globulin for which (*loc. cit.*)

$$1/\lambda = 1/384.6 + 0.9 m^{\frac{1}{2}}. \quad . \quad . \quad . \quad (15)$$

Here at infinite dilution the conductivity is 384.6, which is almost the 384.0 for pure HCl, while 0.9 for b is so large that it must be due to large globulin ions. It seems, then, that HCl in water ionizes the globulin molecule into a positive and a negative globulin ion which we shall denote by the symbols G_b and G_a . The solution of globulin in HCl is a mixture of solutions of G_bCl and G_aNa , which are both completely ionized at all concentrations, so that at infinite dilution the conductivity is the sum of those for H, Cl, G_a , and G_b . But $\Lambda_{oa} + \Lambda_{ob}$ is too small compared with $\Lambda_{o1} + \Lambda_{o2}$ for HCl to be found from ordinary conductivity experiments, and therefore does not appear in (15). But to find the effect of the globulin ions on the value of b we may reason in the following manner. So far I have not considered the molecular conductivity of mixtures in general, though the subject ought to illustrate some of the details of viscosities of electric origin. But for the matter in hand we may formulate a simple approximate theory for our mixture of globulin compounds thus:—As the ions pass one another we shall have cases where G_b has G_a for its neighbour, and also others where Cl is its neighbour, and similarly with G_a . It is as if we had to do with a mixture of G_aG_b , G_bCl , G_aNa ,

and HCl. The slowly moving ions G_a and G_b cause most of the viscous resistance of electric origin; we shall assume that they cause it all. To find the appropriate $1/\lambda_0$ for introducing in the righthand side of (1) we note that with pure G_aG_b we should use $1/\Lambda_{0a} + 1/\Lambda_{0b}$, say $2/\Lambda_0$, and with pure G_aNa $1/\Lambda_{0a} + 1/\Lambda_{01}$, so that a mean value suitable for G_a would be $(3/\Lambda_0 + 1/\Lambda_{01})/2$, which is nearly $3/2\Lambda_0$. So for G_b we should get $3/2\Lambda_0$. With Λ_0 as a sort of mean value for both globulin ions we have $1/\lambda_0$ represented by $3/2\Lambda_0$.

Hardy has shown (Journ. of Physiology, xxxiii, p. 251) that the valency of globulin is probably even, so we shall try 2 as its simplest likely value, according to my globulin paper (*loc. cit.*). Now G_a when associated with G_b as its neighbour would give 2×2 for $\nu_1\nu_2$ in (2) or (6), and when associated with Na would give 2×1 . Hence for the mean value of ν for its neighbour we take $3/2$, and we can now adapt (8) to the case we are considering by introducing into its righthand side Λ_{01} or 44 for Na, which was omitted as a factor in comparing Na compounds amongst themselves. Hence for the mean globulin ion of assumed valency 2 we have

$$b(\Lambda_0/2)\{2n_1/(n_1+n_2)\}^{1/2}/(3/2)^2 = 0.0425 \times 44. \quad (16)$$

As each HCl produces two globulin ions, m should be replaced by $2m$ for G and H or Cl, and so the proper value of b to use in this equation is the mean of 0.9 and $0.9/2^{1/2}$. The appropriate value for $\{2n_1/(n_1+n_2)\}^{1/2}$ is the mean of those for $n_1=n_2=2$, and $n_1=2, n_2=1$, or 1.050. These give $\Lambda_0/2 = 4.965$, and so $\Lambda_0 = 9.93$, while Hardy's direct measurement by the method of Lodge shows that it is about 10. With 4.965 for Λ_0/ν in (11) we get $B = 9506$. Hence, with x for the number of C atoms in the globulin ion, by means of the following percentage composition of globulin and the values of B for the atoms

	C.	H.	N.	O.	S.
Per cent.	52.71	7.01	15.85	23.32	1.11
Atomic B	8	4	8	6	18

we get the equations $12x = 0.5271$ times the mass of the globulin ion ($H=1$), and

$$x \left\{ 8 + \frac{12}{52.71} \left(4 \cdot \frac{7.01}{1} + 8 \cdot \frac{15.85}{14} + 6 \cdot \frac{23.32}{16} + 18 \cdot \frac{1.11}{32} \right) \right\} = B. \quad (17)$$

These give $x=512$ and the molecular mass of the globulin ion as 11650, so that its equivalent is 5825. With HCl Hardy found the equivalent of globulin to be 5560, the sharper results with NaOH indicating 5000. Thus the value of b has given us values of Λ_0 and of the equivalent of globulin in excellent agreement with the direct determinations of experiment. There is good reason for believing, then, that the valency of the globulin ions produced by HCl in water is 2 and their molecular mass (weight) is 10000 (nearly). The formula for the globulin ion is $C_{512}H_{817}N_{132}O_{170}S_4$.

Returning to the cases of the globulin and the casein compound formed with NaOH we find the marked peculiarity that half of the Na ions move unaffected by viscosity of electric origin, while the other half of the Na ions and all the OH ions are affected by it to an unexpected extent. For NaOH globulin $b=3.1$, whereas for HCl globulin it is 0.9. For NH_4OH globulin it is 2.3, whereas we should expect the NaOH and NH_4OH compounds to have nearly the same value, as they do in the case of casein. If there were an excess of NaOH it would show almost the conductivity of pure NaOH, the ions of the excess appearing to escape the large viscosity due to the globulin. In reality, of course, all the Na and all the OH ions would move under a reduced average viscosity of electric origin, but the effect is the same as if those equivalent to the globulin suffered the full viscosity due to it, and those in excess moved as if free from that viscosity. But in the actual case half of the equivalent Na behaves as if free from the viscosity caused by the globulin. Here, then, we must have an arrangement of the Na, the OH, and the globulin ions, such as leaves half of the Na ions unconstrained by electric force from the rest of the ions. This arrangement, being different from the homogeneous distribution assumed in our theory, must be the cause of the increase in the value of b . But though in our ignorance of this arrangement we are not able to calculate from b the value of Λ_0 , and that of B for the globulin ion in presence of NaOH, we can use the values of b to compare the sizes of the ions of globulin and casein. We must first reduce the value of b for NaOH casein from 25° to 18° by the factor 1.18, thus $1.678 \times 1.18 = 1.98$, and then with 3.1 for b in NaOH globulin we have B for casein nearly $(1.98/3.1)^3$ times B for globulin. If we take 10000 for the mass of the globulin ion in presence of NaOH, we have that of the casein ion 10000 $(1.98/3.1)^3$ nearly or 2605. With the percentage composition of casein, C 53.07, H 7.13,

N 15.64, O 22.60, S 0.76, and P 0.80, and with 16 for the atomic B of P we can write down the corresponding formula for the casein ion, but it is better to derive this from the experimental equivalent of casein, as is done below.

If $\nu=2$, as in the case of globulin, the equivalent of casein is 1302. Now Laqueur and Sackur found 1135 for the equivalent of casein in its NaOH compound using phenolphthalein as indicator. The calculated result, as regards order of magnitude, is in satisfactory agreement with this. Hence the casein ion appears to be divalent, and its molecular mass by titration is 1135×2 or 2270. According to the percentage composition of casein and 2270 for the mass of its ion the formula of its ion is half of $C_{200}H_{322}N_{50}O_{64}SP$, and as we have seen by analogy with globulin the casein ions must be derived in pairs from the casein molecule, the formula just given is the simplest possible according to physical considerations. It is also the simplest according to purely chemical considerations, as the S atom or the P atom cannot be divided. When we speak of the casein ion being half of this formula we mean the average casein ion, the S may go with one of the ions and P with the other, or both S and P may be in one ion and neither of them in the other, the mean ion always containing $\frac{1}{2}S$ and $\frac{1}{2}P$. In the same way the formula of the globulin ion is half of $C_{1024}H_{1634}N_{264}O_{340}S_{88}$, which we take to be the formula for the globulin molecule. My previous estimate was dearly double this (*loc. cit.*). According to the theory of the colloidal state proposed by me in 'The Chemistry of Globulin' (*loc. cit.*) colloidal globulin and casein will have the molecules which are given by their formulas not present as free molecules, but as those three-dimensional patterns proposed to be called *semplars*, which are joined symmetrically to one another by some of the latent valencies of N and O being called into action. For a full account of many previous methods of estimating the mass of large protein molecules the reader is referred to *Die Grösse des Eiweissmoleküls* by F. N. Schulz (Jena, G. Fischer, 1903).

Melbourne, May 1903.