

ON THE DISSOCIATION OF SOLUTIONS OF THE
"BASIC" CASEINATES OF SODIUM AND
AMMONIUM.

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In a previous paper¹ I have shown, from conductivity data, that the neutral caseinates of sodium and ammonium obey Ostwald's dilution-law for the salt of a monobasic acid. In this I propose to show that from the results of Sackur,² to which I inadvertently omitted to refer in my previous paper, it follows that the "basic caseinates" (alkaline to litmus but neutral to phenolphthalein)³ obey the same law.

The law, as formulated in my previous paper, is as follows:

$$m = \frac{\lambda}{U + v} + \frac{\lambda^2}{G(U + v)^2} \quad (1)$$

where m is the concentration of OH^- which has been neutralized by the casein, U is the specific ionic velocity of the cation of the base which is combined with the casein (in Cm-sec), v is that of the protein anion, $\lambda = 1.037 \times 10^{-2} x$, where x is the conductivity of the solution in reciprocal ohms, and G is the dissociation-constant of the salt; it is easily shown that this is identical with Ostwald's dilution-law for a binary electrolyte.⁴

Sackur's procedure was as follows: to a given amount of casein sufficient alkali was added to render the resulting

¹ T. Brailsford Robertson: Jour. Phys. Chem., 11, 542 (1907).

² O. Sackur: Zeit. phys. Chem., 41, 672 (1902). I am indebted to Mr. Wm. Sutherland for having in a private letter recalled Sackur's results to my attention.

³ Cf. van Slyke and Hart: Am. Chem. Jour., 33, 461 (1905). T. Brailsford Robertson: Jour. Biol. Chem., 2, 328, etc. (1907).

⁴ Page 545 of my previous paper.

solution exactly neutral to phenolphthalein, this solution was diluted and the conductivity measured at each dilution. Since the proportion of alkali to casein which is required to secure a solution neutral to phenolphthalein is the same, within wide limits, whatever the dilution of the alkali,¹ it follows that in all these solutions the OH^- concentration was the same, namely, about 10^{-5} .² The procedure was, therefore, identical with that adopted by me in the experiments described in my previous paper, save that I was working with solutions which were neutral to litmus, hence, my solutions contained less sodium (or ammonium) than Sackur's in the proportion of 5 to 8.³

Sackur's results with "basic" sodium caseinate were as follows:

TABLE I

Concentration of solution (in equivalent sodium)	κ = conductivity in reciprocal ohms.
250×10^{-4}	1160×10^{-6}
125×10^{-4}	641×10^{-6}
63×10^{-4}	351×10^{-6}
31×10^{-4}	197×10^{-6}
16×10^{-4}	109×10^{-6}

His results with "basic" ammonium caseinate were as follows:

TABLE II

Concentration of solution (in equivalent ammonium)	κ = conductivity in reciprocal ohms.
180×10^{-4}	1070×10^{-6}
90×10^{-4}	590×10^{-6}
45×10^{-4}	321×10^{-6}
23×10^{-4}	173×10^{-6}
11×10^{-4}	91×10^{-6}

¹ Van Slyke and Hart: l. c. T. Brailsford Robertson: Jour. Biol. Chem., 2, 328, etc. (1907).

² Cf. Salm: Zeit. phys. Chem., 57 (1906), Heft 4.

³ Cf. van Slyke and Hart: l. c. T. Brailsford Robertson: l. c.

Inserting the observations obtained with "basic" sodium caseinate in equation (1) and applying the method of least squares to the determination of the constants, we obtain¹

$$m = 1520\lambda + 0.463 \times 10^8 \lambda^2, \quad (2)^2$$

where $\lambda = 1.037 \times 10^{-2} x$. Inserting in this equation the experimentally ascertained values of λ and calculating therefrom the corresponding theoretical values of m we obtain:

TABLE III

m (Experimental)	m (Calculated)
250×10^{-4}	250×10^{-4}
125×10^{-4}	132×10^{-4}
63×10^{-4}	62×10^{-4}
31×10^{-4}	33×10^{-4}
16×10^{-4}	18×10^{-4}

Inserting the observations obtained with "basic" ammonium caseinate in equation (1) and, as before, applying the method of least squares to the determination of the constants we obtain

$$m = 1215\lambda + 0.370 \times 10^8 \lambda^2; \quad (3)$$

inserting in this equation the experimentally ascertained values of λ and calculating therefrom the corresponding theoretical values of m we obtain:

TABLE IV

m (Experimental)	m (Calculated)
180×10^{-4}	181×10^{-4}
90×10^{-4}	88×10^{-4}
45×10^{-4}	44×10^{-4}
23×10^{-4}	23×10^{-4}
11×10^{-4}	11×10^{-4}

¹ Since the concentration of free (unneutralized) hydroxyl ions in each solution is of the order of 10^{-5} while that of the neutralized base is, at its least, of the order of 10^{-3} we may, with considerably less than 1% error, consider m as being the total amount of base introduced into the solution.

² By a misprint in my former paper, the exponent of 10 in the second constant of equations similar to these is written —8.

The agreement in both cases is excellent and leaves very little room for doubt as to the validity of the law for these solutions. The precise interpretation which is to be placed upon these results is, however, not quite clear. Consider the equilibria in these solutions. Let the concentration of the protein anion XOH^- be represented by c while that of the sodium (or ammonium) ion is β , then, assuming that the casein under these conditions behaves essentially as a monobasic acid, we have

$$c\beta = GT, \quad (4)$$

where G is the dissociation constant of the casein salt and T is its concentration.

Let m be the concentration of OH^- which has been neutralized by the casein, then the amount of sodium (or ammonium) which is bound by protein ($= T$) is $m - c$, assuming that no sodium is bound in complex ions or in basic amphi-salts.

Hence,

$$c\beta = G(m - c). \quad (5)$$

Now equating the sum of the concentrations of the negative ions to the sum of the concentrations of the positive ions, assuming that the protein cation HX^+ is only present in negligible concentration, then, since the solutions are alkaline and the hydrion concentration is therefore negligible, we have

$$\beta = c + b. \quad (6)$$

Hence substituting in (5) we have

$$c(c + b) = G(m - c). \quad (7)$$

Now if x be the specific conductivity of the solution in reciprocal ohms and if $\lambda = 1.037 \times 10^{-2} x$, we have

$$\lambda = U\beta + Vb + vc, \quad (8)$$

but $\beta = c + b$ so that we have

$$c = \frac{\lambda}{U + v} - \frac{U + V}{U + v} b; \quad (9)$$

substituting in (7) we have

$$m = \frac{\lambda}{U + v} \left(1 - \frac{U + 2V - v}{(U + v)G} b \right) + \frac{\lambda^2}{G(U + v)^2} + \frac{(U + V)(V - v)}{(U + v)^2 G} b^2 \quad (10)$$

since in all these solutions b is of the order of 10^{-5} , b^2 is of the order of 10^{-10} and is negligible in comparison with m . Moreover, if we assume $U = 51 \times 10^{-5}$, $V = 196 \times 10^{-5}$ ¹ and the values of v and G experimentally ascertained for the neutral caseinates, namely, for sodium caseinate, 3×10^{-5} and 0.04^2 we find

$$\frac{U + 2V - v}{(U + v)^2 G} b = 0.002;$$

hence, this expression is negligible in comparison with unity and equation (8) becomes

$$m = \frac{\lambda}{U + v} + \frac{\lambda^2}{G(U + v)^2} \quad (9)$$

which is identical with equation (1). Since the constants have precisely the same meaning as in equation (1) this derivation indicates that the values of the constants should be the same for the neutral as for the "basic" caseinates, but this is far from being the case; in the accompanying table the values of v and of G (velocity of the protein ion and value of the dissociation-constant) for the "basic" caseinates, deduced from the above observations, are compared with those for the neutral caseinates:

TABLE V

	v		G	
	"Basic" caseinate	Neutral caseinate	"Basic" caseinate	Neutral caseinate
Sodium caseinate.....	15.2×10^{-5}	2.6×10^{-5}	0.0499	0.0395
Ammonium caseinate.	9.1×10^{-5}	— ^{ve}	0.0404	0.0428

¹ The specific ionic velocities of Na^+ and OH^- at 18° given by Kohlrausch and Holborn, "Leitvermögen der Elektrolyte," Leipzig, 1898, S. 200, multiplied by 1.14 to bring to 25° .

² Cf. my previous paper.

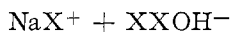
The difference between the two sets of results is surprising, but it is less so when we consider the behavior of solutions of the neutral caseinate towards the addition* of alkali. Five cc of N/10 alkali dissolves one gram of casein with the formation of a solution which is exactly neutral to litmus; on continuing to add alkali to this solution, the hydroxyl concentration changes very slowly until, when 3 cc of N/10 alkali have been added, it becomes neutral to phenolphthalein.¹ Thus if we consider in the first place, 100 cc of an N/100 solution of a neutral caseinate, by the addition of alkali, the concentration of Na in the solution has been raised by 0.006 N while that of the OH⁻ ions has only been raised by 0.00001 N. The solution of neutral caseinate cannot contain excess of free, undissociated casein because uncombined casein is insoluble. Hence, we should be inclined to suspect that since we are dealing with a polyvalent acid, in the solutions neutral to litmus only part of the carboxyl groups have been neutralized while, on the addition of alkali, more COOH groups are neutralized. But the facts that both the neutral and the "basic" caseinates behave towards dilution precisely as do the salts of monobasic acids and that the conductivity of the "basic" caseinates is greatly in excess of that of the neutral caseinates preclude this possibility. We are therefore driven to the conclusion that the neutral and the "basic" caseinates represent two totally different types of combination between the alkali and the casein or mixtures of constant composition of these two types. In this connection, two possibilities present themselves.

In my previous paper I pointed out that it makes no difference to the *form* of equation (1) whether we are really dealing with a sodium (or ammonium) salt of casein of the type $\text{Na}^+ + \text{XOH}^-$ or with the "ampho-salt" of the type $\text{NaX}^+ + \text{XOH}^-$. If this latter is the substance in solution, however, the sum of the velocities of the cations and anions

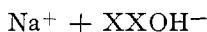
¹ van Slyke and Hart: l. c. T. Brailsford Robertson: Jour. Biol. Chem., 2, 317 (1907).

($U + v$) is no longer the sum of the velocities of the Na^+ and protein ions but is the sum of the velocities of the NaX^+ and XOH^- ions, and since the velocity of the NaX^+ ion would not probably be considerably less than that of the Na^+ ion, the *sum* of the velocities of the two ions in the solution might possibly be less than that of the non-protein ion in combination with the casein. From the fact that $U + v$ for neutral ammonium caseinate is less than U for NH_4^+ (leading to the apparently negative value for v) it was concluded that the neutral ammonium caseinate is really the amphi-salt of ammonium, and while for neutral sodium caseinate this is probably also the case, no positive proof could be adduced since $U + v$ is, in this case, slightly greater than U for Na^+ . It appears possible, therefore, that in solutions of the neutral caseinates we are dealing with the amphi-salts of the bases employed while in solutions of the "basic" caseinates we are dealing with the true salts of the bases employed; it is evident that in the formation of the amphi-salt, considerably more casein would be neutralized by a given amount of alkali than in the formation of the true salt.

The quantitative relations which are observed, however, are not satisfied by the supposition that the amphi-salt is $\text{NaX}^+ + \text{XOH}^-$ while the true salt is $\text{Na}^+ + \text{XOH}^-$, for in that case, nearly twice as much alkali would be required to form the true salt as to form the amphi-salt. If, however, we assume that the amphi-salt is

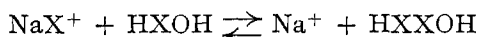


while the true salt is

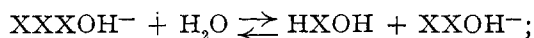


then from the amount of NaOH (5 cc $\text{N}/10$ to 1 gram) required to form the neutral salt, we find that the molecular weight of the substance HXXXOH is 2000, whence the molecular weight of the substance HXXOH would be 1340 and the amount of alkali required to neutralize 1 gram of casein to phenolphthalein would be 7.5 cc of $\text{N}/10$ which is very close to the experimental value.

There is, however, another possibility. It is obviously indifferent as regards the form of equation (1), whether in solutions of the neutral caseinates we are dealing with the salt $\text{Na}^+ + \text{XXXOH}^-$ or with the salt $\text{NaX}^+ + \text{XXOH}^-$ and either satisfies the quantitative relation observed between the amount of alkali required to form the neutral and that required to form the "basic" caseinates. Regarding the neutral caseinate as the amphi-salt of the base, in every solution of sodium caseinate there would be an equilibrium between the ions NaX^+ and Na^+ which, as the experimental data show, is shifted in the direction $\text{NaX}^+ \rightarrow \text{Na}^+$ by the addition of sodium hydrate to the system. Regarding the neutral caseinate as the salt of a molecule of casein which has undergone association, the equilibrium would be between the ions XXXOH^- and XXOH^- and would be shifted in the direction $\text{XXXOH}^- \rightarrow \text{XXOH}^-$ by alkali. Since, however, Ostwald's dilution-law for a binary electrolyte holds good, and moreover, the amount of alkali necessary to form either salt depends only upon the amount of casein present and not upon its dilution it follows that the equilibrium in question is not shifted by dilution but depends only upon the proportion between the masses of Na and of casein in the system. This is obviously a relation more to be expected if the equilibrium is of the type



than if it were of the type



this consideration, taken in conjunction with the fact that the sum of the velocities of the ions in a solution of neutral ammonium caseinate is less than the velocity of the ammonium ion is, I think, sufficient to enable us to state that the neutral caseinates probably represent "amphi-salts" of the bases combined with the casein while further addition of alkali converts the amphi-salts into true salts of the bases.

It may furthermore be pointed out that while casein is

very probably a polybasic acid, yet the fact that Ostwald's dilution-law for a binary electrolyte is obeyed both by the neutral and the basic caseinates indicates that, as is frequently the case with weak polybasic acids, only one COOH group is appreciably concerned in the neutralization of bases. This is in sharp contradiction to the conclusion reached by Sackur that casein acts towards bases as a 4-basic acid. He bases this conclusion upon the empirical law enunciated by Ostwald and Walden to the effect that $\frac{\Delta_{512} - \Delta_{32}}{\Delta_{512}} = 0.15$ for 2-basic, 0.22 for 3-basic and 0.29 for 4-basic acids, where Δ_{512} is the equivalent conductivity at dilution 512 and Δ_{32} is that at dilution 32. It is to be observed, however, that Sackur did not determine the equivalent conductivities at the exact dilutions employed by Ostwald and Walden, but for Na-caseinates employed the dilutions 640 and 40 and for NH_4 -caseinate employed the dilutions 890 and 55.6. The exact value of the ratio, obviously cannot be independent of the dilutions at which the equivalent conductivities are determined, and as a matter of fact, Ostwald finds that the quantity $\Delta_{1024} - \Delta_{32} = 10$ for Na salts of monobasic acids, 20 for Na salts of 2-basic acids, 30 for salts of 3-basic acids, etc.,¹ a relation which could not hold good were the relation $\frac{\Delta_1 - \Delta_2}{\Delta_1} = \text{const.}$ for the salt of an acid of given basicity true for all dilutions, even provided that Δ_1 approaches the molecular conductivity at infinite dilution. However, if by interpolation from Sackur's results for "basic" Na-caseinate, using formula (2), we calculate the values of Δ_{512} and of Δ_{32} we find that $\frac{\Delta_{512} - \Delta_{32}}{\Delta_{512}} = 0.31$, indicating a 4-basic acid. But if we similarly calculate Δ_{1024} and Δ_{32} and apply Ostwald's criterion quoted above, we find that $\Delta_{1024} - \Delta_{32} = 21.2$, indicating a dibasic acid. The two empirical equations, which we are not certain can be applied to the salts of proteins, therefore lead to mutually contradictory results while the

¹ W. Ostwald: *Zeit. phys. Chem.*, 1, 109 (1887) 529; 2, 901 (1888).

dilution-law which, as we have seen, does apply to solutions of the caseinates, indicates a monobasic acid. We must, I think, conclude that casein acts towards bases essentially as a monobasic acid and that empirical relations founded on measurements made upon quite different substances cannot with safety be directly applied to proteins.

Referring again to Table V, it may be pointed out that the values found for the velocity of the casein ion in solutions of the "basic" caseinates are of the same order as those found by Hardy¹ by direct observation by the boundary method, and as that estimated by myself from conductivity data,² for the velocity of the serum-globulin ion. It will also be observed from the values of G given in Table V, that the neutral caseinates and the "basic" caseinates are about equally dissociated and that to a fairly high degree.

Conclusions

1. In a previous paper it has been shown that solutions of the neutral caseinates (neutral to litmus) obey Ostwald's dilution-law for the salt of a monobasic acid; in this paper it is shown from Sackur's determinations, that solutions of the "basic" caseinates (neutral to phenolphthalein) obey the same law.

2. In explanation it is suggested that the neutral caseinates are "ampho-salts" of the combined base (type $\text{NaX}^+ + \text{XXOH}^-$) while the "basic" caseinates are true salts of the combined base (type $\text{Na}^+ + \text{XXOH}^-$); and that in every solution of a caseinate there is an equilibrium of the type $\text{NaX}^+ + \text{HXOH} \rightleftharpoons \text{Na}^+ + \text{HXXOH}$ which is independent of the dilution and depends upon the relative proportions of the base and of the casein.

3. It is concluded that only one COOH group of the casein molecule is appreciably concerned in the neutralization of bases.

¹ W. B. Hardy: *Jour. Physiology*, **33**, 251 (1905).

² T. Brailsford Robertson: *Jour. Phys. Chem.*, **11**, 437 (1907).

4. From Sackur's results it is estimated that the velocity of the casein ion in solutions of "basic" sodium caseinate is 15.2×10^{-5} centimeters per second under a potential gradient of a volt per centimeter, while in solutions of "basic" ammonium caseinate it is estimated to be 9.1×10^{-5} Cm-sec.

5. The dissociation constants of "basic" sodium and ammonium caseinates, estimated from Sackur's determinations, are respectively 0.0499 and 0.0404.