

THE EFFECT OF TEMPERATURE ON THE TITRATION CURVE OF CASEIN

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(Accepted for publication, July 11, 1932)

I

INTRODUCTION

Hoffman and Gortner (1) recently investigated the acid and base-binding properties of various proteins, among them casein. In respect to the influence of temperature, their conclusion was "... that at 35°C. much less acid or alkali is bound by the protein than at 25°C., while considerably more is bound at 15°C. than at 25°C."

Contemporary development in the theory of ionization of ampholytes points to the peculiar significance of the effect of temperature. We therefore undertook a recalculation of some of Hoffman and Gortner's data to bring their findings into conformity with recent advances in the theory of ampholytes. We believe that our investigation makes apparent certain phenomena which are not visible in the conclusions of Hoffman and Gortner. Furthermore it seems to us that neither the experimental error, nor certain suppositions made in the recalculations in any way vitiate the qualitative side of the conclusions. The quantitative side, on an absolute scale, is much less certain. We must remember the disheartening fact that there exist many casein preparations and probably two or more caseins, and also that the measurements of Hoffman and Gortner were made in a cell involving a liquid junction.

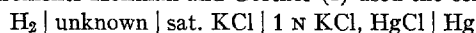
The recalculation itself is somewhat involved and may be subdivided into the following steps: first, the calculation of the E.M.F. of the half cell used by Hoffman and Gortner (1) at several temperatures; secondly, the calculation of the pH of casein solutions with acid or base at various temperatures, using these values, and finally the cal-

ulation of the acid or base bound by casein at various temperatures. These calculations are described below in detail.

II

The Calculation of the E.M.F. of the 1.0 N KCl Calomel Half Cell at Various Temperatures

In their measurements Hoffman and Gortner (1) used the cell:



They calculated the pH with the help of Schmidt and Hoagland's tables (2) and the amount of acid or base bound by the proteins, by a method described in detail in their communication. They believed that "... no temperature correction was necessary in this method of calculation as the hydrogen ion concentration was used only to determine the normality of the equilibrium solution" (3).

The hydrogen ion activity of hydrochloric acid is related to the E.M.F.'s observed and to the reference electrode by the familiar expression:

$$\text{pH} = \text{p}\gamma_{\text{H}^+} + \text{pHCl} = \frac{\text{E.M.F.}_{\text{observed}} - \text{E.M.F.}_{\text{calomel}}}{0.0001984 T} \quad (1)$$

in which $\text{p}\gamma_{\text{H}^+}$ is the negative logarithm of the hydrogen ion activity coefficient and pHCl is the negative logarithm of the normality of hydrochloric acid.

In their investigation Hoffman and Gortner (4: Table XX) carried numerous measurements with such cells at 15°, 25°, and 35°C., in which both the hydrochloric acid solution and the reference electrode were kept at the same temperature.¹

It is apparent from Relation I that if the E.M.F. of the calomel half cell is unknown, as it is safe to assume in our case, it could be calculated, provided the values of $\text{p}\gamma_{\text{H}^+}$ were known, since all other values are experimentally given.

There are several determinations of γ_{H^+} of hydrochloric acid available. In this calculation we accepted the γ_{H^+} as given by Lewis and Randall (5). A smooth curve was drawn through these values. The γ for 0.1 N HCl determined by Scatchard as given by Clark (6) agrees very well with the values given by Lewis and Randall. From this

¹ Private communication by Professor Gortner to one of the writers.

curve the values recorded in Table I, Column (2) have been interpolated.

Whether the activity coefficient of individual ions can be actually determined, is a somewhat debatable question. But, as we shall see presently, in the comparison of the properties of casein at various temperatures γ_{H^+} is involved as a constant. Therefore its exact value is immaterial. This conclusion follows from the following

TABLE I
The E. M. F. of 1.0 N KCl Calomel Half Cell at Various Temperatures

HCl normality	γ_{H^+}	E.M.F. of the half cell at:		
		15°	25°	35°
(1)	(2)	(3)	(4)	(5)
0.003	0.965	0.2860	(0.2755)	(0.2705)
0.006	0.945	0.2875	0.2810	0.2760
0.009	0.935	0.2875	0.2815	0.2765
0.012	0.925	0.2870	0.2815	0.2770
0.018	0.910	0.2855	0.2815	0.2775
0.024	0.900	0.2850	0.2805	0.2770
0.030	0.890	0.2850	0.2810	0.2780
0.045	0.875	0.2855	0.2810	0.2770
0.060	0.860	0.2845	0.2805	0.2775
0.075	0.855	0.2835	0.2790	0.2750
0.090	0.845	0.2840	0.2790	0.2755
0.105	0.835	0.2845	0.2790	0.2760
0.120	0.835	0.2850	0.2780	0.2755
0.150	0.825	0.2840	0.2780	0.2745
Average accepted.....		0.2855	0.2800	0.2765

considerations. In dilute solutions (to about 0.15 N) the activity coefficient of hydrochloric acid is practically independent of temperature (7, 8). It is therefore possible to use the same value of γ_{H^+} at different temperatures provided sufficiently dilute hydrochloric acid solutions are used.

We are now in a position to calculate the E.M.F. of the half cell Sat. KCl | 1 N KCl, HgCl | Hg from the data of Hoffman and Gortner (4: Table XX) with the help of Equation I. This has been done in Table I where the E.M.F.'s of this reference electrode are calculated

at three different temperatures and at normalities of hydrochloric acid ranging from 0.003 to 0.150.

The values obtained are far from perfect. They display variations greater than such measurements should warrant. But this fluctuation cannot be remedied by a selection of another set of γ and is probably inherent in the measurements themselves. They are however sufficiently accurate for our purpose.

The casein solutions in which we are interested were measured by Hoffman and Gortner at $22 \pm 1^\circ$ and 35°C . The value of the reference electrode at 22° was interpolated from a smooth curve down through values obtained in Table I.

III

The Amount of Acid or Base Bound by Casein at Various Temperatures

As a next step we recalculated the hydrogen ion activities of casein solutions in equilibrium with sodium hydroxide and hydrochloric acid. This was done with the help of Equation I, the E.M.F.'s of the calomel half cell arrived at in the preceding section, and the E.M.F.'s observed by Hoffman and Gortner (1: Tables LXXX, LXXXIV, LXXXVIII, and XCII). The data cover the titration of casein with moderate amounts of HCl and NaOH at 22° and 35°C .

The pH values of the system casein-sodium hydroxide were converted into pOH terms by subtracting the pH values from 14.100 at 22° and from 13.670 at 35° .

The results of these calculations are found in Figs. 1 and 2, except for larger concentrations of HCl and NaOH. The data in this case were corrected for the free NaOH or HCl in the following manner: Cohn (9) suggests that the amount of NaOH bound by the protein might be obtained from the following considerations:

$$\text{pH} + \text{pOH} = \text{p}K_w \quad (\text{II})$$

$$\text{pOH} + \text{p}\gamma_{\text{OH}} = \text{pNaOH} \quad (\text{III})$$

$$\text{NaOH added} - \text{NaOH free} = \text{NaOH bound} \quad (\text{IV})$$

Experimentally we know the pH; using the $\text{p}K_w$ given above we can find the pOH. Taking the values given by Cohn (9) for the activity coefficients of OH, we can obtain pNaOH and NaOH free. The

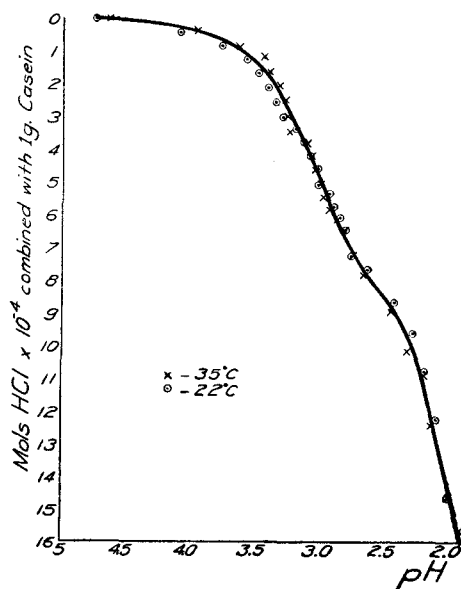


FIG. 1. The titration curve of casein with hydrochloric acid at 22° and 35°C.

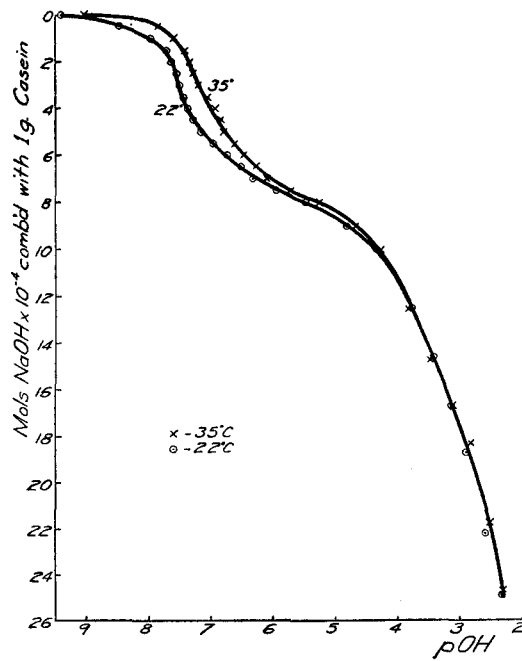


FIG. 2. The titration curves of casein with sodium hydroxide at 22° and 35°C.

latter is the antilog of pNaOH. Subtracting the free NaOH from the total NaOH added, we obtain the value for the NaOH bound by the protein.

A similar calculation was carried out for hydrochloric acid using the activity coefficients given in Table I.

The correction for the free NaOH is necessary only through a small region of the titration curve with NaOH. In the case of HCl it is significant almost throughout the titration curve.

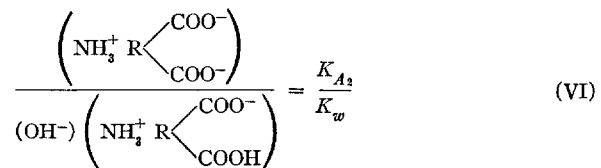
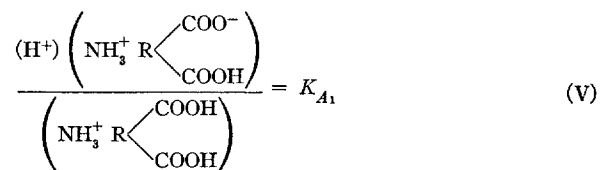
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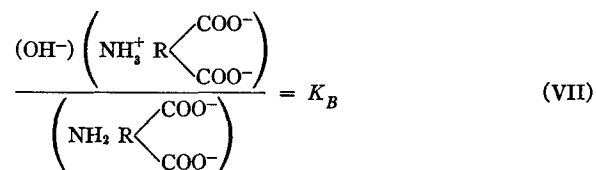
DISCUSSION

In Figs. 1 and 2 are reproduced the results of these calculations. A superficial examination of these data convinces us that we are dealing with two distinct phenomena in the titration of casein with sodium hydroxide. In terms of the negative logarithm of the hydroxyl ion activity (pOH) the effect of the temperature is considerable beginning from the isoelectric point to about pOH 6 (pH 8), but in a more alkaline region the two curves practically coincide.

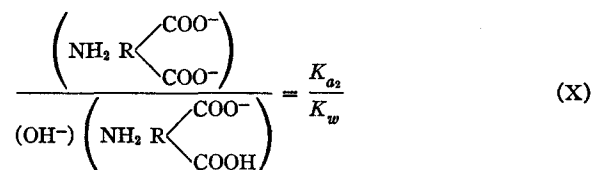
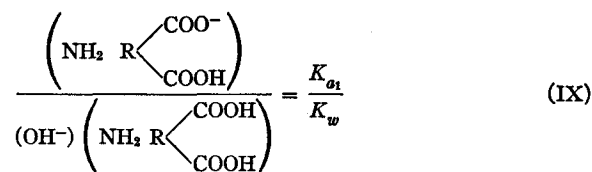
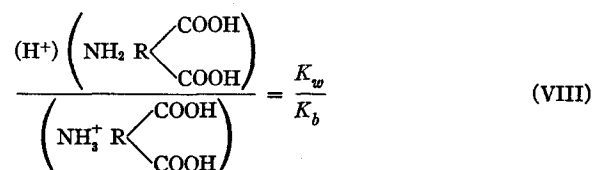
Similarly, the acid side of the titration curve in terms of pH (Fig. 1) is practically identical at 22° and 35°C. There is a slight discrepancy between the two curves between pH 4 and 3.2, but casein in combination with acid is slow in coming to equilibrium and we are rather inclined to attribute this discrepancy to an experimental error.

In 1923 Bjerrum (10, 11) suggested that a dicarboxylic amino acid should dissociate in the following way:





The corresponding constants according to the classical dissociation theory are (in the same order):



At first the whole problem seems to be one of notation, but on more careful examination we may note the following important differences:

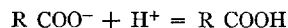
1. According to the classical theory the dissociation of an amino acid in acid solution, in terms of H^+ , involves K_w (VIII), in the case of the new theory (V) it does not.

2. In the case of the second classical constant, K_{a2} (X), in terms of OH^- , the situation is the same as in 1.

3. In the case of the first classical dissociation constant, K_{a1} (IX), as far as K_w is concerned, there is no difference between the old and new theory.

Casein in many ways resembles a multiple monoamino-dicarboxylic acid (12, 11). The important difference however is that these reacting groups are numerous since the molecular weight of casein is in the neighborhood of 200,000.

In regard to the effect of temperature on the types of ionization described in Equations V to X we know that the K_w is considerably influenced by the temperature (ΔH equals about 14,000 calories), while the ionization described by other constants is, or may be, little affected. For instance the reaction:



involves only from $-1,300$ to $+1,800$ calories (11). To be more specific one must expect that equilibrium (Equations V to X) involving the K_w must be very much influenced by temperature, while those which do not include the K_w are likely to be little affected.

In applying these criteria to the experimental findings (Figs. 1 and 2) we at once see that the type of ionization suggested by Bjerrum fits the experimental facts in a far better way than the classical one:

1. On the acid side of the titration curve of casein (Fig. 1) the classical theory predicts (VIII) a large temperature coefficient, Bjerrum's (V) a small one. The experimental findings indicate a small temperature coefficient.

2. On the alkaline side of the titration curve of casein (more alkaline than about pOH 6, Fig. 2) the classical theory (X) suggests a large temperature coefficient, while the theory of Bjerrum (VIII) a small one. The experimental facts again favor the Bjerrum theory.

3. In the range from the isoelectric point to about pOH 6 both theories predict a large temperature coefficient (Equations VI and IX). The experimental facts are in perfect agreement on this point.

On the whole, we must therefore conclude that the type of ionization suggested by Bjerrum adequately describes the effect of temperature on the titration curve of casein with acid or base. The study of the effect of temperature on the titration curves of proteins may thus become a useful tool in identifying the various types of ionization involved.

V

SUMMARY

The influence of temperature on the titration curve of casein may be accounted for by the Bjerrum theory of ionization of ampholytes.

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