

CHAPTER III

THE DEVELOPMENT OF A MODIFIED HENDERSON-HASSELBALCH EQUATION FOR USE IN THE CASE OF HETEROGENEOUS SOLUTIONS.

As already hinted at several times the conditions in heterogeneous solutions are complicated by the fact that the concentration of the solute in the individual phases is not directly given by its mean concentration, as the solute cannot be assumed to be evenly distributed over the whole system. Further we cannot regard the activity coefficients in the single phases as already known, and lastly there is the possibility that the solute may be concentrated by adsorption in the interfaces between the different phases.

The influence of the first condition on the determination of reaction by Henderson's and Hasselbalch's equations has latterly been recognised by Fridericia [1920], Parsons [1917] and by Joffe and Poulton [1920], but they have not realised the full significance of it, only regarding serum-blood cells as a heterogeneous system, and failing to appreciate that serum (plasma) and the fluid of the blood cells are likewise heterogeneous systems.

Fridericia's and Parsons' contributions appeared while the experimental part of this research was in progress, but Joffe and Poulton's work only came to my notice after I had embarked upon the literary section. These investigations are of the greatest importance for the questions here considered and they will be fully dealt with in a later chapter of this work.

We will proceed with our calculations *under the assumption* that all the combined carbonic acid is present as bicarbonate (that no CO_2 is bound by complex combinations or adsorbed), and we will investigate how the experimental results accord with this assumption.

Let Vol. % combined carbonic acid = β ,
 β being the mean concentration in the heterogeneous solution. Let us first consider a system of two watery phases in mutual equilibrium, and in equilibrium with a gaseous phase containing CO_2 .

100 parts of the system consist of q parts of phase (2) and $100 - q$ parts of phase (1).

From this we get

$$100 \times \frac{\text{vol. of phase (2)}}{\text{vol. of phase (1) + vol. of phase (2)}} = q \dots\dots\dots(108)$$

Also
$$\frac{\text{Vol. \% combined CO}_2 \text{ in phase (2)}}{\text{Vol. \% combined CO}_2 \text{ in phase (1)}} = d \dots\dots\dots(109)$$

Seeking now the amount of combined CO_2 in the single phases expressed by β , d and q ,

$$\frac{V_{(1)}qd}{100} + \frac{V_{(1)}(100-q)}{100} = \beta, \dots\dots\dots(110)$$

$$V_{(1)} = \frac{100\beta}{100-q(1-d)} \text{ (vol. \% combined CO}_2 \text{ in phase (1)), } \dots(111)$$

and
$$V_{(2)} = \frac{100d\beta}{100-q(1-d)} \text{ (vol. \% combined CO}_2 \text{ in phase (2)). } \dots(112)$$

From (111) in conjunction with (95) we get

$$a_{\text{H}'(1)} = K'_{1(1)} \frac{100 - q(1-d)}{100} \times \frac{P_{\text{CO}_2} a}{7.60\beta}, \dots\dots\dots(113)$$

where $a_{\text{H}'(1)}$ is the apparent hydrogen ion activity in phase (1) and $K'_{1(1)}$ is K_1 divided by the apparent activity coefficient of HCO'_3 in phase (1).

Analogous to this we have

$$a_{\text{H}'(2)} = K'_{1(2)} \frac{100 - q(1-d)}{100d} \times \frac{P_{\text{CO}_2} a}{7.60\beta}. \dots\dots\dots(114)$$

$K'_{1(1)}$ and $K'_{1(2)}$ are not different from K'_1 in a homogeneous solution and the indices only signify that $F_a(\text{HCO}'_3)$ refers to a particular solution.

Comparing (95) with (113) and (114) it will be seen that the last two can be expressed in the same form as (95), viz. (113) in the form

$$a_{\text{H}'(1)} = \lambda_{(1)} \frac{P_{\text{CO}_2} a}{7.60\beta} \dots\dots\dots(115)$$

and (114) in the form
$$a_{\text{H}'(2)} = \lambda_{(2)} \frac{P_{\text{CO}_2} a}{7.60\beta}. \dots\dots\dots(116)$$

Since
$$\lambda_{(1)} = K'_{1(1)} \frac{100 - q(1-d)}{100}, \dots\dots\dots(117)$$

$$\lambda_{(2)} = K'_{1(2)} \frac{100 - q(1-d)}{100d}, \dots\dots\dots(118)$$

$\lambda_{(1)}$ and $\lambda_{(2)}$ occur in (115) and (116). These are only constants as long as the factors in them do not vary or the variations neutralise one another.

$K'_{1(1)}$ and $K'_{1(2)}$ will only vary with the reaction if the activity coefficient of the bicarbonate ion varies with it.

L. Michaelis [1920] has lately drawn attention to this condition, regarding it however from the classical standpoint of Arrhenius, and therefore assuming that K'_1 varies with the degree of dissociation of the bicarbonate. His views appear in the form of a criticism on the theory which H. Straub and Klothilde Meier [1918-1920] put forward for the carbonic acid combining power of the blood on the strength of a series of experiments carried out by them. Straub and Meier's experiments and theories are however subject to an elementary error, and as will later be shown they are quite fallacious, but Michaelis' comments naturally do not lose their interest on this account.

According to Bjerrum's¹ theory there is reason to expect that the activity coefficient of an ion will decrease in a solution containing ions with an opposite charge and this will be especially the case if these ions are polyvalent or have a large molecular volume.

If there are already rather many ions present in the solution the effect of the new-comers will be small, and possibly in special cases it will not be noticed at all, but it is not right, without due consideration, to regard $K'_{1(1)}$ and $K'_{1(2)}$ as constants, as it is a fact that the ionic concentrations in phases (1) and (2) vary with the reaction.

The factor $\frac{100 - q(1-d)}{100}$ in (117) and the corresponding factor in (118)

¹ I have repeatedly had the opportunity of discussing this condition with Prof. Bjerrum about which he has up to the present only published a rough sketch.

will vary provided that q and d vary. From S. P. L. Sørensen's [1915-1917] experiments on egg albumin there is every reason to expect they will vary with the reaction, but what influence the variations will have on the "total constants" experimental investigations alone can inform us at present.

As will be shown in one of the subsequent chapters $\lambda_{(1)}$ in serum and the liquid phase of the blood corpuscles is constant within the limits of the reactions investigated (experimental errors are not very small in experiments with the fluids of blood cells), and we will therefore continue our calculations under this assumption.

Let $a_{H'}$ in the liquid phase of serum = $a_{H'(s)}$,
 and $a_{H'}$ in the liquid phase of blood cells = $a_{H'(c)}$,
 $\lambda_{(1)}$ in serum = $\lambda_{(s)}$,
 $\lambda_{(1)}$ in blood cells = $\lambda_{(c)}$.

The volume of the blood cells expressed as a percentage of the total volume of the blood is Q . The partition of HCO'_3 between blood cells and serum is D , so that

$$\frac{C_{HCO'_3 \text{ in blood cells}}}{C_{HCO'_3 \text{ in serum}}} = D,$$

and lastly the mean concentration of combined carbonic acid in the blood expressed in volumes % = B , and

$$Q = \frac{\text{vol. of blood cells}}{\text{vol. of blood}} \times 100.$$

From (115) and (116) we then get

$$a_{H'(s)} = \lambda_{(s)} \frac{100 - Q(1 - D)}{100} \times \frac{PCO_2 \alpha}{7.60 B}, \dots\dots\dots(119)$$

$$a_{H'(c)} = \lambda_{(c)} \frac{100 - Q(1 - D)}{100D} \times \frac{PCO_2 \alpha}{7.60 B}. \dots\dots\dots(120)$$

(119) and (120) can be put in a form analogous with (95), viz.

$$a_{H'(s)} = \Lambda_{(s)} \frac{PCO_2 \alpha}{7.60 B}, \dots\dots\dots(121)$$

$$a_{H'(c)} = \Lambda_{(c)} \frac{PCO_2 \alpha}{7.60 B}, \dots\dots\dots(122)$$

$$\Lambda_{(s)} = \lambda_{(s)} \frac{100 - Q(1 - D)}{100}, \dots\dots\dots(123)$$

$$\Lambda_{(c)} = \lambda_{(c)} \frac{100 - Q(1 - D)}{100D}. \dots\dots\dots(124)$$

It follows from the above that in $\Lambda_{(s)}$ and $\Lambda_{(c)}$ four variables take part, of quite a different kind from those which determine K'_1 in (95), and it will thus be understood that we cannot immediately draw conclusions regarding the combination of carbonic acid in the blood from a numerical agreement between $\Lambda_{(s)}$ and K'_1 in any bicarbonate solution.

Fridericia [1920], Henderson [1908-1910], Hasselbalch [1916, 2], Parsons [1917-1920], Joffe and Poulton [1920], Michaelis [1920] and many others have not realised the full consequences of this condition (which in an almost

analogous way can be developed by the classical dissociation theory), and we are therefore compelled to investigate anew the questions mentioned in the introduction:

I. Can the hydrogen ion concentration of the blood be calculated by an equation similar to Henderson's and Hasselbalch's?

II. Is all the combined carbonic acid of the blood ionised?

I have attacked the first problem from a purely empirical standpoint. For practical work it is convenient to employ (121) in logarithmic form

$$p_{H'(s)} = p\Lambda_{(s)} + \log B - \log \frac{P_{CO_2} \alpha}{7.60}, \dots\dots\dots(125)$$

and transform it thus

$$p_{H'(s)} = p\Lambda'_{(s)} + \log B - \log f_0, \dots\dots\dots(126)$$

where f_0 is the mean concentration of dissolved CO_2 in the blood expressed in volumes %, and

$$p\Lambda'_{(s)} = p\Lambda_{(s)} - \log \Phi_a(CO_2), \dots\dots\dots(127)$$

while $\Phi_a(CO_2)$ is the reciprocal of the relative absorption coefficient in blood.

The relative absorption coefficient in serum is 0.975 according to Bohr [1905] and 0.81 in blood corpuscles, from which $\Phi_a(CO_2)$ can be calculated by the following equation:

$$\Phi_a(CO_2) = \frac{100}{100 - \frac{19Q}{100} - \frac{2.5(100-Q)}{100}}, \dots\dots\dots(128)$$

RÉSUMÉ.

I. The modified Henderson-Hasselbalch equation is adapted for use with heterogeneous solutions.

II. It appears from the resulting equation that nothing can be concluded from the size of the total constant about the way the carbonic acid is combined, without taking the heterogeneity into account.

CHAPTER IV

METHODS FOR THE ELECTROMETRIC DETERMINATION OF THE APPARENT HYDROGEN ION ACTIVITY IN SOLUTIONS CONTAINING CO_2 WITH THE HELP OF THE PLATINUM HYDROGEN ELECTRODE, AND THE TECHNIQUE EMPLOYED.

The general principles and methods of estimation by the potentiometrical method have been often described and are to be found in every handbook of physical chemistry. The method used here is that described in S. P. L. Sørensen's *Études enzymatiques*, II [1909-1910] and those interested are referred to this work for further information. Within the limits of reaction obtaining in the following experiments the reaction of a solution will be a function of the CO_2 tension with which it is in equilibrium. What effect a change of the CO_2 tension