

CHAPTER II

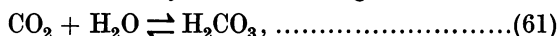
THE HYDRATION OF CARBON DIOXIDE AND THE DISSOCIATION OF CARBONIC ACID.

The Derivation of Henderson's and Hasselbalch's Equations and the Modification of these in accordance with Bjerrum's Theory. The Limits within which the Derived Equations are Valid.

In 1 cc. of water in equilibrium with a gas mixture in which the tension of CO₂ is P_{CO₂} mm. Hg, there will be dissolved according to Henry's law (equation (1)),

$$a = \frac{P_{CO_2}}{760} \alpha \text{ cc. CO}_2 \text{ (at } 0^\circ \text{ and } 760 \text{ mm. Hg).}$$

Some of the dissolved carbon dioxide is hydrated, forming carbonic acid,



and the mass action law gives for this reaction

$$\frac{A_{CO_2} a_{H_2O}}{a_{H_2CO_3}} = k_0. \dots\dots\dots(62)$$

Since a_{H₂O} can be regarded as constant under the experimental conditions,

$$\frac{a_{H_2CO_3}}{A_{CO_2}} = K_0, \dots\dots\dots(63)$$

and therefore from (7) and (11),

$$a_{H_2CO_3} = P_{CO_2} \alpha K_0 0.00005910. \dots\dots\dots(64)$$

This equation in accordance with what was said on p. 157 applies to watery solutions also, with the proviso that K₀ is the same for pure water and for watery solutions. How far this is the case cannot be estimated at present, but as from (62) and (63)

$$K_0 = \frac{a_{H_2O}}{k_0} \dots\dots\dots(65)$$

and we regard a_{H₂O} as constant, K₀ will only vary from one solution to another if k₀ varies.

Such a variation under the conditions investigated is improbable, but if it should happen it would be without importance for the following argument and would be included in the total constants. Only in the investigation of the extent to which the activity of the bicarbonate ion conforms to Bjerrum's [1916, 1918, 1919] equation for the calculation of the activity coefficient, would such a condition be of real importance and invalidate the proof. Assuming however that Bjerrum's theory is correct the above-mentioned investigation can be used as a proof that K₀ does not vary appreciably with the ionic concentration within the limits of concentration investigated.

In equation (63)

$$\frac{a_{H_2CO_3}}{A_{CO_2}} = K_0,$$

a_{H₂CO₃} is the apparent activity of carbonic acid, and in so far as it is not

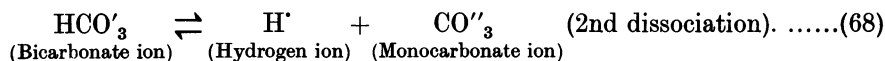
hydrated (*i.e.* it is not H_2CO_3 , H_2O for example), we have $a_{\text{H}_2\text{CO}_3} = A_{\text{H}_2\text{CO}_3}$. The error involved by putting

$$\frac{a_{\text{H}_2\text{CO}_3}}{A_{\text{CO}_2}} = \frac{C_{\text{H}_2\text{CO}_3}}{C_{\text{CO}_2}} \dots\dots\dots(66)$$

will in all cases be small, as the activity coefficient of non-electrolytes is an expression of the salting out effect which ought to be about the same for all non-electrolytes. K_0 indicates therefore how much of the dissolved CO_2 is hydrated. Experiment has shown that K_0 is small. Thus Thiel and Strohecker [1914] have calculated from the rapidity with which CO_2 neutralises bases that only $\frac{1}{2}$ % of the dissolved CO_2 is hydrated and therefore our K_0 is roughly 0.005.

Walker and Cormack [1900] make some remarks in their experiments on conductivity in CO_2 solutions about the value of K_0 . They show that such solutions of carbonic acid fulfil the requirements of the mass action law when the total amount of dissolved CO_2 is substituted for the carbonic acid in the equation. If only a small part of the dissolved CO_2 was hydrated the mass action law would not be satisfied in the form employed and they think therefore they are right in concluding about half the dissolved CO_2 is hydrated (which means that our K_0 should be 0.5). Walker and Cormack's determinations however certainly do not justify them in drawing these conclusions.

Carbonic acid dissociates in the following manner:



From the mass action law

$$\frac{a_{\text{H}^+} a_{\text{HCO}'_3}}{a_{\text{H}_2\text{CO}_3}} = k_1 \quad \text{(1st dissociation of carbonic acid),} \dots\dots(69)$$

$$\frac{a_{\text{H}^+} a_{\text{CO}''_3}}{a_{\text{HCO}'_3}} = K_2 \quad \text{(2nd dissociation of carbonic acid).} \dots\dots(70)$$

Multiplying equations (63) and (69) we get

$$\frac{a_{\text{H}^+} a_{\text{HCO}'_3}}{A_{\text{CO}_2}} = k_1 K_0, \dots\dots\dots(71)$$

and therefore

$$\frac{a_{\text{H}^+} a_{\text{HCO}'_3}}{A_{\text{CO}_2}} = K_1. \dots\dots\dots(72)$$

Since, almost without exception before the appearance of Bjerrum's theory, we put

$$a_{\text{H}^+} = C_{\text{H}^+}, \dots\dots\dots(73)$$

$$a_{\text{CO}_2} = C_{\text{CO}_2}, \dots\dots\dots(74)$$

$$a_{\text{HCO}'_3} = C_{\text{HCO}'_3} \gamma = (C_{\text{HCO}'_3} f_\mu), \dots\dots\dots(75)$$

where γ was the "conductivity dissociation" of the bicarbonate in the concentration under investigation, (72) became

$$\frac{C_{\text{H}^+} C_{\text{HCO}'_3} \gamma}{C_{\text{CO}_2}} = K_1, \dots\dots\dots(76)$$

which was transformed into

$$C_{\text{H}^+} = \frac{K_1}{\gamma} \frac{C_{\text{CO}_2}}{C_{\text{HCO}'_3}}. \dots\dots\dots(77)$$

This is Henderson's equation.

Equations like (76) and (77) have repeatedly been used in physical chemistry, for instance in W. Nernst's experiments on the dissociation constants of weak monovalent acids (cited from Datta and Dhar [1915]), in Datta and Dhar's [1915] determinations of the second dissociation constant of weak divalent acids, and in investigations on the second dissociation constant of carbonic acid by McCoy and Smith [1911] and McCoy and Test [1911], Auerbach and Pick [1912], and Seyler and Lloyd [1917, 1, 2], and lastly in work upon the solubility of the carbonates of the alkaline earths, for example, by Johnston [1916].

An equation analogous to (72) is one derived by Bjerrum and Gjaldbaek [1919, p. 82, equation (24)].

In logarithmic form (77) becomes

$$p_{\text{H}^+} = pK_1 + \log C_{\text{HCO}_3'} - \log C_{\text{CO}_2} + \log \gamma. \quad \dots\dots\dots(78)$$

As will be shown later in considering the limits between which an equation similar to (77) is valid, $C_{\text{HCO}_3'}$ in the cases treated by Hasselbalch and Henderson (as always in these investigations) can be assumed to have originated from the combined carbonic acid, as the amount of HCO_3' derived from the dissociation of the dissolved carbonic acid is negligible in comparison with it. If we know the total quantity of CO_2 in a solution and its pressure in a gaseous phase in equilibrium with the solution, the temperature and the relative absorption coefficient of the solution, it is easy to calculate the quantity of combined carbonic acid by subtracting the amount dissolved from the total amount. *If now it is assumed all the combined carbonic acid is in the form of bicarbonate*, and the CO_2 is expressed in volumes per cent., (77) compared with (5) becomes

$$C_{\text{H}^+} = \frac{K_1 \text{ vol. } \% \text{ dissolved } \text{CO}_2 \cdot 0.0004492}{\gamma \text{ vol. } \% \text{ combined } \text{CO}_2 \cdot 0.0004492}, \quad \dots\dots\dots(79)$$

$$C_{\text{H}^+} = \frac{K_1 \text{ vol. } \% \text{ dissolved } \text{CO}_2}{\gamma \text{ vol. } \% \text{ combined } \text{CO}_2}. \quad \dots\dots\dots(80)$$

This is Henderson's equation in a simplified form, expressed in the same way as that of Hasselbalch.

Hasselbalch [1916, 2] regarded carbonic acid as a divalent acid and put the concentrations in (77) in terms of normality, assuming a solution which contained a gram-molecule of CO_2 in a litre to be twice normal as regards carbonic acid, while he regarded a solution which contained a gram-molecule of CO_2 bound as bicarbonate in a litre, as normal with respect to bicarbonate¹.

Equation (77) under these circumstances becomes

$$C_{\text{H}^+} = \frac{K_1 \text{ vol. } \% \text{ dissolved } \text{CO}_2}{\gamma \text{ vol. } \% \text{ combined } \text{CO}_2} \cdot 2. \quad \dots\dots\dots(81)$$

And since Hasselbalch substitutes K_{I} for $\frac{K_1}{\gamma}$ his equation becomes

$$C_{\text{H}^+} = K_{\text{I}} \frac{\text{vol. } \% \text{ dissolved } \text{CO}_2}{\text{vol. } \% \text{ combined } \text{CO}_2} \cdot 2. \quad \dots\dots\dots(82)$$

¹ Parsons [1919] and L. Michaelis [1920] have recently pointed out that Hasselbalch uses this unusual convention and Michaelis has protested against it.

If we now compare (80) with (82) it will be seen that

$$\frac{K_1 \text{ (Henderson)}}{\gamma} = 2K_I \text{ (Hasselbalch)}. \quad \dots\dots\dots(83)$$

Hasselbalch's equation in logarithmic form becomes

$$p_{H^+} = p_{K_I} + \log \text{ vol. } \% \text{ combined CO}_2 - \log \text{ vol. } \% \text{ dissolved CO}_2 - 0.3010,$$

and when (83) is put in logarithmic form we get

$$pK_I \text{ (Henderson)} + \log \gamma = pK_I \text{ (Hasselbalch)} - 0.3010 \dots\dots(84)$$

Although the above appears to be correct it should be noted that Hasselbalch *determined his total constant* by the potentiometrical method. If this is recognised (83) becomes

$$\frac{K_1 \text{ (Henderson)}}{F_a \text{ (HCO}'_3)} = 2K_I \text{ (Hasselbalch)} \quad \dots\dots\dots(85)$$

and (84) is transformed accordingly, but at the same time a correction ought to be introduced into all Hasselbalch's calculations so that p_{K_I} always becomes 0.048 times larger (given by the difference between Sørensen's and Bjerrum's E_0).

If we abide by what is the usual custom in the literature of physical chemistry there is no doubt we ought to use molar concentration and not normality in such equations, and as a similar convention also has priority in physiological literature, Hasselbalch's mode of expression should be given up.

As the assumptions underlying (73), (74) and (75) are no longer tenable the equation should be modified in accordance with Bjerrum's requirements. When the mols in the equation with the exception of a_{H^+} are expressed by their concentrations, (72) becomes

$$\frac{a_{H^+} C_{HCO'_3} F_a \text{ (HCO}'_3)}{C_{CO_2} F_a \text{ (CO}_2)} = K_1. \quad \dots\dots\dots(86)$$

It will be seen from (54) that

$$1.117 a_{H^+} = C_{H^+} \text{ (Sørensen)}, \quad \dots\dots\dots(87)$$

and we thus obtain

$$\frac{C_{H^+} \text{ (Sørensen)} C_{HCO'_3}}{C_{CO_2}} = K_1 \frac{F_a \text{ (CO}_2)}{F_a \text{ (HCO}'_3)} 1.117 \quad \dots\dots\dots(88)$$

Substituting

$$K'_1 = \frac{K_1}{F_a \text{ (HCO}'_3)} \quad \dots\dots\dots(89)$$

we get from (88) and (85)

$$K'_1 = \frac{1.793 K_I \text{ (Hasselbalch)}}{F_a \text{ (CO}_2)}, \quad \dots\dots\dots(90)$$

and from (83) and (90)

$$K'_1 = \frac{0.896 K_1 \text{ (Henderson)}}{\gamma F_a \text{ (CO}_2)}. \quad \dots\dots\dots(91)$$

If the relative absorption coefficient for blood is taken as 0.92 (Bohr [1905]), $F_a \text{ (CO}_2)$ becomes 1.087 and we get for blood

$$\text{Blood } \begin{cases} K'_1 = 1.65 K_I \text{ (Hasselbalch)}, & \dots\dots\dots(92) \\ pK'_1 = pK_I \text{ (Hasselbalch)} - 0.218. & \dots\dots\dots(93) \end{cases}$$

Now that we have developed the relations between the various constants

we will revert to (72) and express it in the same way as Hasselbalch's equation by using (89)

$$a_{H^+} = K'_1 F_a (CO_2) \frac{\text{vol. \% dissolved } CO_2}{\text{vol. \% combined } CO_2} \dots\dots\dots(94)$$

or

$$a_{H^+} = K'_1 \frac{P_{CO_2 a}}{7.60 \text{ vol. \% combined } CO_2} \dots\dots\dots(95)$$

Putting (94) in logarithmic form we get

$$p_{H^+} \text{ (Bjerrum)} = pK'_1 + \log \text{ vol. \% combined } CO_2 - \log \text{ vol. \% dissolved } CO_2 - \log F_a (CO_2) \dots\dots\dots(96)$$

We have now worked out the relations between Henderson's, Hasselbalch's and our own equations as expressed in (90) and (91), but a considerable qualification of their significance must be made.

Henderson's and Hasselbalch's equations were evolved, as will be remembered, on the assumption that the degree of dissociation measured by conductivity gave the degree of activity of the bicarbonate, but since this is incorrect as Bjerrum later has shown, (73), (74), (75), (76), (77), (78), (79), (80), (81), (82), (83), (84), and (91) are only approximations to the true equations and have *only mathematical significance*, while (88) and (90) are correct actually as well as mathematically.

It is perhaps also worth pointing out that (90) only holds good if K_I (Hasselbalch) and K'_1 are calculated from the same experiments or from experiments which give the same results with the same method of calculation, and that Sørensen's E_0 (50) should be used for the calculation of K_I (Hasselbalch), while E_0 (Bjerrum) (51) should be used for the calculation of K'_1 . If (90) is not satisfied by a correct calculation from two series of experiments it is proof that the experimental results do not agree.

In the equations evolved up to the present we have assumed without hesitation that all the combined carbonic acid is present as bicarbonate. *We will now inquire how far the equations are valid if the second dissociation of carbonic acid (68) takes place to any considerable extent.* We will first find out, however, the reaction at which this happens.

Equation (70)
$$\frac{a_{H^+} a_{CO'_3}}{a_{HCO'_3}} = K_2$$

can be transformed into
$$\frac{C_{CO'_3}}{C_{HCO'_3}} = \frac{K_2 F_a(HCO'_3)}{a_{H^+} F_a(CO'_3)} \dots\dots\dots(97)$$

The value of the right side of the equation is still not accurately known in spite of a great deal of work on the subject, since McCoy's [1911], Auerbach and Pick's [1912], Shield's [1893], and Seyler and Lloyd's [1917, 1] results must be recalculated according to the new ideas. Bjerrum and Gjaldbaek [1919] give $K_2 = 10^{-10.22}$ from which we may expect to get a good approximation by putting

$$K_2 \frac{F_a(HCO'_3)}{F_a(CO'_3)} = 10^{-10} = K'_2 \dots\dots\dots(98)$$

It should however be noted that the value may vary somewhat with the ionic concentration.

If with this value $\frac{C_{CO'_3}}{C_{HCO'_3}}$ is calculated from (97) the table below is obtained.

Table IV.

$a_{H'}$	$p_{H'}$	$\frac{C_{CO'_3}}{C_{HCO'_3}}$ room temp.	$a_{H'}$	$p_{H'}$	$\frac{C_{CO'_3}}{C_{HCO'_3}}$ room temp.
1×10^{-7}	7.00	0.001	0.25×10^{-8}	8.60	0.040
0.5	7.30	0.002	0.2	8.70	0.050
0.3	7.52	0.003	0.17	8.77	0.059
0.2	7.70	0.005	0.15	8.82	0.067
0.15	7.82	0.007	0.13	8.89	0.077
1×10^{-8}	8.00	0.010	0.11	8.96	0.091
0.5	8.30	0.020	1×10^{-9}	9.00	0.100
0.3	8.52	0.033			

It will be seen from the table that at $p_{H'} 7.00$ the ratio is only $\frac{1}{1000}$, at $p_{H'} 8.00$, $\frac{1}{100}$ and at 9.00 it is $\frac{1}{10}$. It may be concluded from this that in reactions of physiological importance all the carbonate may be considered to be in the form of bicarbonate—a result which numerous workers have previously arrived at in a similar way (*e.g.* L. Meyer [1857], Heidenhain and L. Meyer [1863], Zuntz [1868], Chr. Bohr [1909], van Slyke and Cullen [1917], Parsons [1919], Bayliss [1915] and others). We shall revert briefly to this subject in discussing the variations of volume of the blood corpuscles.

It will now be shown that with a little alteration the equations evolved are valid even if the reaction is so alkaline that a large amount of monocarbonate ions are present in the solution. Instead of the concentration, the corresponding amounts of CO_2 are substituted expressed in volumes per cent., and the amount

corresponding to C_{CO_2} is called S_0 ,

„ $C_{HCO'_3}$ „ S_1 ,

„ $C_{CO'_3}$ „ S_2 .

$$(94) \text{ then becomes } a_{H'} = K'_1 F_a (CO_2) \frac{S_0}{S_1} \dots\dots\dots (99)$$

And (97) combined with (98)

$$a_{H'} = K'_2 \frac{S_1}{S_2} \dots\dots\dots (100)$$

Calling the total amount of combined CO_2 , B , we get

$$S_1 + S_2 = B, \dots\dots\dots (101)$$

and from (100) and (101)

$$S_1 = \frac{a_{H'} B}{K'_2 + a_{H'}} \dots\dots\dots (102)$$

When this is substituted in (99) we get a quadratic equation

$$a_{H'} = \frac{S_0 K'_1 F_a (CO_2)}{2B} \pm \sqrt{\frac{(S_0 K'_1 F_a (CO_2))^2 + 4S_0 K'_1 F_a (CO_2)^2 K'_2 B}{4B^2}}$$

The numerator under the square root sign is equal to

$$(S_0 K'_1 F_a (CO_2) + 2K'_2 B)^2 - 4K'_2^2 B^2.$$

$4K'_2^2 B^2$ can be neglected as K'_2^2 is very small, and as the negative sign is without physical significance, we get

$$a_{H'} = \frac{K'_1 F_a (CO_2) S_0}{B} + K'_2 \dots\dots\dots (103)$$

The equation (103) thus developed is valid generally (independent of hydrolysis of the ions), so long as the concentration of the combined carbonic acid is large compared with the concentration of the dissolved CO₂, and so long as all the combined carbonic acid is present as monocarbonate and bicarbonate.

It will be noticed that it is identical with the earlier equation (94) (the modified Henderson-Hasselbalch equation) when K'₂ is small compared with

$$K'_1 F_a(\text{CO}_2) \frac{\text{vol. \% dissolved CO}_2}{\text{vol. \% combined CO}_2}.$$

In the following short table the first column contains values of

$$K'_1 F_a(\text{CO}_2) \frac{\text{vol. \% dissolved CO}_2}{\text{vol. \% combined CO}_2},$$

the second column contains the corresponding values of

$$K'_1 F_a(\text{CO}_2) \frac{\text{vol. \% dissolved CO}_2}{\text{vol. \% combined CO}_2} + K'_2,$$

K'₂ being again 10⁻¹⁰; in the last column the corresponding p_H values are given.

Table V.

$K'_1 F_a(\text{CO}_2) \frac{S_0}{S_1}$	$K'_1 F_a(\text{CO}_2) \frac{S_0}{S_1} + K'_2$	p _H
10 ⁻⁸	1.010 × 10 ⁻⁸	7.996
10 ⁻⁹	1.100 × 10 ⁻⁹	8.959
10 ⁻¹⁰	2.000 × 10 ⁻¹⁰	9.699

It will be seen from the table that at reactions more acid than 10⁻⁸ the correction is negligible and the equation becomes identical with the modified Henderson-Hasselbalch equation.

We have still to investigate how small the quantity of combined carbonic acid can be without being small in comparison with the amount of bicarbonate arising from the dissolved CO₂.

The hydrogen ions in a solution containing dissolved CO₂ may either

- (1) exclusively be derived from the dissociated carbonic acid (except for the minimal amount due to the dissociation of water), or
- (2) partly come from the carbonic acid and partly from another acid, or
- (3) the reaction may be so acid that the carbonic acid is undissociated, in which case there can be no carbonate present in the solution and the equation does not apply.

We will only consider the case when carbonic acid is the sole source of hydrogen ions, because the field is more restricted than in the other cases.

Let us call the concentration of bicarbonate ions which is equivalent to the amount of available base, C_m, the bicarbonate ions which are derived from the dissociation of carbonic acid being, as already mentioned, equal to C_H. Equation (86) taken in conjunction with (89) and (85) then gives

$$\frac{C_H \cdot F_a(\text{H}^+) (C_m + C_H)}{C_{\text{CO}_2}} = K'_1 F_a(\text{CO}_2), \dots\dots\dots(104)$$

from which

$$C_H = -\frac{C_m}{2} \pm \sqrt{\frac{C_m^2}{4} + \frac{K'_1 F_a(\text{CO}_2) C_{\text{CO}_2}}{F_a(\text{H}^+)}} \dots\dots\dots(105)$$

in which the negative sign is without actual meaning. If C_m is equal to 0, (105) reduces to

$$a_{H^+} = \sqrt{K'_1 F_a(\text{CO}_2) F_a(H^+) C_{\text{CO}_2}} \dots\dots\dots(106)$$

(106) applies to reactions in solutions of CO_2 where there is no combined carbonic acid.

A hard and fast rule for using (105) instead of the modified Henderson-Hasselbalch equation is difficult to give, but a good approximate rule may be arrived at in the following way.

(104) is compared with (86) and (89) in the form

$$\frac{C_{H^+} F_a(H^+) C_m}{C_{\text{CO}_2}} = K'_1 F_a(\text{CO}_2) \dots\dots\dots(107)$$

The difference between (104) and (107) is due to the difference between the factors C_m and $(C_{H^+} + C_m)$. If C_{H^+} is small in comparison with C_m , the equations become the same. Making the approximate assumption that C_{H^+} is equal to a_{H^+} we will evaluate $\frac{C_{H^+}}{C_m}$. a_{H^+} is calculated from (94) which only differs from (105) in form, and an estimation of the value of $\frac{a_{H^+}}{C_m}$ is made. If this does not exceed 0.01 the use of equation (94) is admissible.

Table VI.

a_{H^+}	C_m	Vol. % combined CO_2	mm. Hg, CO_2
10^{-6}	10^{-4}	0.22	0.563
10^{-5}	10^{-3}	2.24	53.2
10^{-4}	10^{-2}	22.39	4710

In the table the values which C_m must have at different reactions so that the above requirements for the applicability of (94) may be fulfilled, are given. In the third column C_m is expressed in volumes per cent. of CO_2 and in the fourth column the corresponding tension of CO_2 calculated with the help of the constants determined for sodium bicarbonate in Chapter VIII.

RÉSUMÉ.

From the above results it may be said that in homogeneous solutions:

I. Henderson's equation is in agreement with the equation evolved on the basis of Bjerrum's new dissociation theory when $K_1 \frac{F_a(\text{CO}_2)}{F_a(\text{HCO}_3')}$ is substituted for $\frac{K_1}{\gamma}$ in the former.

II. Hasselbalch's $K_I = K_1 \frac{F_a(\text{CO}_2) \cdot 0.557}{F_a(\text{HCO}_3')}$.

III. The value found then becomes a_{H^+} (Bjerrum), not C_{H^+} (Sørensen), and instead of p_{H^+} (Sørensen) we have p_{H^+} (Bjerrum).

IV. The limits for the modified Henderson-Hasselbalch equation are given.

V. The complete equations for the relation between the hydrogen ion activity, the amount of dissolved CO_2 and the combined carbonic acid are evolved.