STUDIES ON THE OXYGEN-, ACID-, AND BASE-COMBINING PROPERTIES OF BLOOD.

V. EXTENSION OF THE DEBYE-HÜCKEL THEORY OF IONIC INTERACTION TO HEMOGLOBIN, BICARBONATE-SODIUM CHLORIDE SYSTEMS.

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INTRODUCTION.

The Debye-Hückel theory of solutions was formulated in 1923. It has stimulated the reconsideration of a large amount of pre-existing data and the projection of further experiments to test its

1 A table of the symbols used will be found at the end of this paper. 303
range of validity. Practically all of this work deals with simple aqueous solutions of one or at most two electrolytes, although there is a limited amount of data in such solutions as water-alcohol-salt and water-sugar-salt. The extension of the theory to biological systems in which there are water, a colloidal electrolyte (protein) in high weight concentration, many salts, and non-electrolytes, is beset with theoretical and practical difficulties. Nevertheless, the general validity of the theory has been so well established that this extension will undoubtedly prove profitable. For this reason we have considered the Hb, H⁺, NaCl, HCO₃⁻, CO₂ equilibria reported in the previous paper in the light of the Debye-Hückel theory and have attempted to determine the extent of conformity and non-conformity of the theory to our data.

We have discussed the subject always with reference to the particular system water, Hb, H⁺, HCO₃⁻, NaCl, CO₂. No pretense is made that the treatment is complete; much more experiment, specifically designed in the light of the theory, is needed before any extensive analysis of that behavior of water-protein-salt solutions can be given. In general, however, we may say that our data are satisfactorily in accord with the theory, but lack of direct or other indirect experimental data on the effect of hemoglobin on the dielectric constant of water makes it impossible at present to offer the agreement as proof. In other words, the Debye-Hückel theory of solutions offers us the only plausible explanation with which we are acquainted of the effect of hemoglobin on the activity of the bicarbonate ion.

Résumé of Experimental Data on γHCO₃ in Hemoglobin Solutions.—Let us for the moment disregard any theory as to the behavior of CO₂ in solutions containing NaHCO₃, NaCl, and hemoglobin or its derivatives, HbCO, MtHb, HbCN, HbNO. We have defined certain quantities, viz. pK₁, pK₁', pK₂, β₀, β, γHCO₃, γ⁺, γ₀, and Γ, which may or may not have their usual theoretical significance. These values as defined by us are calculable from the experimentally determined CO₂ tension, total base, total CO₂, pao₂, chloride, and water content in a series of solutions whose hemoglobin content varied from 0 to 20 mM per liter. Our data clearly establish the following facts.
1. In water, as found by Hastings and Sendroy (1925), the relation $pK_1$, $pK'_1$, $\beta_0$, and $\sqrt{\bar{m}}$ is:

$$pK'_1 = pK_1 - \beta_0 \sqrt{\bar{m}}$$  \hspace{1cm} (1)

$pK_1 = 6.33$ from which

$$\log_{\text{HCO}_3^-} = - \beta_0 \sqrt{\bar{m}}$$  \hspace{1cm} (2)

$\beta_0$ was found to be 0.54, practically the theoretical value.

2. In hemoglobin solutions we have found a similar relation for $pK'_1$, $pK_\ast$, $\beta$, and $\sqrt{\bar{m}}$:

$$pK_1 = pK_\ast - \beta \sqrt{\bar{m}}$$  \hspace{1cm} (3)

It was also found that

$$pK_\ast = pK_1 - \rho[Hb]$$  \hspace{1cm} (4)

and

$$\beta = \beta_0 - \sigma[Hb]$$  \hspace{1cm} (5)

$\rho$ and $\sigma$ are constants characteristic of the derivative of hemoglobin.

The activity coefficient $\gamma_{\text{HCO}_3^-}$ when divided into two coefficients, i.e.,

$$\log \gamma_{\text{HCO}_3^-} = \log \gamma_\ast + \log \gamma_0$$  \hspace{1cm} (6)

could be calculated from the equations

$$\log \gamma_\ast = - \rho[Hb]$$  \hspace{1cm} (7)

$$\log \gamma_0 = - [\beta_0 - \sigma[Hb]] \sqrt{\bar{m}}$$  \hspace{1cm} (8)

We will show that the Debye-Hückel theory of the behavior of electrolytes in solutions may be extended to concentrated hemoglobin solutions and that it offers a satisfactory explanation of these facts.

**Debye-Hückel Theory in Aqueous Solutions.**—The properties, *e.g.* vapor pressure, freezing point, boiling point, osmotic pressure, membrane equilibria, electromotive force phenomena, *etc.*, of very
Dilute aqueous solutions of non-electrolytes and electrolytes can be quite satisfactorily explained by the application of the laws for ideal solution and the assumption that the (strong) electrolytes are completely ionized. In more concentrated solutions, however, the departure from the ideal state is great. Debye and Hückel (1923) explain in a satisfactory manner the behavior of strong electrolytes not only in water but in any solvent even in concentrated (3 to 5 M) solutions. Their theory evaluates the electrical effect of the charges of all the ions in the solution upon a given ion, A, in terms of the valence and concentration of all the ions, the valence, concentration, the effective ionic diameter of the given ion, and the dielectric constant of the solution.

The theory assumes nothing about ionization; it merely measures the effect on the properties of any given ion species of all ions present. Experiments on strong electrolytes show that as a rule they must be assumed to be completely ionized to account for this effect.

The salient features of the theory with emphasis on those aspects which relate to our problems will be discussed here. Since our discussion is limited to univalent ions (except in the case of the hypothetical hemoglobin ion discussed below), the Debye-Hückel equation may be written here

$$\log \gamma_i = -\frac{\beta_0 \sqrt{T}}{1 + B \alpha \sqrt{T}} + G\gamma$$  \hspace{1cm} (9)

where

- $\beta_0 = \frac{1.8 \times 10^6}{D_0 \gamma}$ $\gamma$  \hspace{1cm} $1.8 \times 10^6$ is calculated from universal constants.

- $D_0$ = dielectric constant of water.
- $T$ = absolute temperature.
- $\beta_0$ = 0.505 at 25°.
- $\beta_0$ = 0.532 at 38°.
- $B = 50.4a \times 10^8$ $50.4 \times 10^8$ is calculated from universal constants.
- $B_{23}$ = 0.328α $\times 10^8$.
- $B_{34}$ = 0.334α $\times 10^8$.

$G$ is a function which gives the variation of the dielectric constant of the solvent with $\gamma$. $\gamma = \frac{1}{2} \sum z[c]$; i.e. one-half the summation of the product of the concentration $[c]$ (in mols per liter) of each ion by its valence $z$ squared, is the ionic concentration.

$\gamma_i$ is the activity coefficient of the ion.
\( a = \text{mean ionic collision sphere or distance in cm. of closest approach between the center of the ion } A \text{ and the center of any surrounding positive or negative ion.} \)

Relation of \( \Gamma \) and \( \mu \).—The equation

\[
\sum \frac{\Gamma_i}{[c_i]} = \sum \frac{\mu_i}{(c_i)} \tag{10}
\]

gives the relation between \( \Gamma \) the ionic strength per liter and \( \mu \) the ionic strength per kilo of solvent. It is important to note that Equations 9 and 10 required that the ionic strength be expressed as mols per liter = \( \Gamma \). Since

\[
r = \frac{3}{2} z^2 \quad \text{[c]} = \frac{3}{2} [I] \quad z^2 \quad \text{[c]} = \frac{[w]}{\Gamma}
\]

Equation 10 becomes

\[
\log \gamma_i = -0.53 \sqrt{[\Gamma]} \mu \tag{11}
\]

The use of Equation 11 without the \([\Gamma]\) factor leads to erroneous results in concentrated protein solutions; e.g., in the red blood cell \([\Gamma] = 0.65 \) and \( \mu = 0.170 \) mols per kilo of H\(_2\)O. If we assume hemoglobin to be without effect, \( -\log \gamma_{\text{HCO}_3} = 0.176 \). By use of Equation 14 without the \([\Gamma]\) term, \( -\log \gamma_{\text{HCO}_3} = 0.219 \). The corresponding pK\(_t\) values are 6.154 and 6.111. In serum \( \mu = 0.160 \) and \([\Gamma] = 0.9 \) approximately. The above error is then only 0.010.

Valence of Hemoglobinate Ion in Calculation of \( \Gamma \) and \( \mu \).—In alkaline hemoglobin solutions a considerable part of the available base may exist as B\(_2\)b; i.e., we may have a fairly high concentration (10 to 40 \( \mu \)M per liter) of the colloidal ion H\(_b\)-\( \text{ze} \). There is enough evidence (Stadie and Martin, 1924) to show that 1 molecule of hemoglobin can bind 10 to 20 univalent base ions; i.e., its valence is 10 to 20. Since \( \Gamma = \frac{1}{2} \sum z^2 [c_i] \), the contribution of H\(_b\)-\( \text{ze} \) ion to \( \Gamma \) may be 100 to 400 times its concentration. The above value of \( z \) is approximated for a molecular weight of hemoglobin of 16,700, but \( z \) becomes 40 to 80 on the more probable value of 4 \( \times \) 16,700 which would make the maximum \( \Gamma \) factor \( \frac{6400}{4} = 1600 \). Should we not multiply \([\text{Hb-ze}]\) by some such factor
in calculating $\Gamma$? In view of the following consideration we believe this question should be answered negatively.

The introduction of $z^2$ into the ionic strength arises as follows: Call $h_B$ the effect which charged ions of the $A$ sort have upon given $B$ ions. Using the Boltzman principle, Debye and Hückel derived an expression for the $h_B$ which for simplicity we write

$$h_B = \text{funct.} \left( n_A z_A^2 e P_A \right)$$

where

- $n_A$ = number of $A$ ions in unit volume.
- $z_A$ = valence of $A$.
- $e$ = elementary electronic charge.
- $P_A$ = potential about $B$ due to charges on $A$ ions.

Figure 1. Log $\gamma_{\text{HCO}_3}$ in 10 mM carbon monoxide. Average $B$Hb = 16 mM per liter.

To evaluate $P$ they used Poisson's equation for the variation of the potential about a point having a charge $\pm ze$. The expression may be briefly written

$$P = \text{funct.} \left( z_A e \right)$$

from which they obtained

$$h_B = \text{funct.} \left( n_A z_A^2 e^2 \right) = \text{funct.} \left( z_A^2 e_A \right)$$

The assumption of Poisson's equation, that the charges on the ions $A$ act as if at a point, is a sufficiently close one for ions of the
Fig. 2. Log $\gamma_{\text{HCO}_3}$ in 10 mm carbon monoxide. Average BHb = 28.8 mm per liter.
### TABLE I (Fig. 1)

*Log γ\(_{\text{HCO}_3^-}\) at Varying Γ and Varying BHb.*

\(\text{HbCO} = 10 \text{ mM per liter.}\)

<table>
<thead>
<tr>
<th>I</th>
<th>BHb (mM per l.)</th>
<th>-Log γ(_{\text{HCO}_3^-})</th>
<th>(\sqrt{\Gamma})*</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.2</td>
<td>0.26</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>15.6</td>
<td>0.24</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>16.1</td>
<td>0.18</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>16.4</td>
<td>0.21</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>17.4</td>
<td>0.27</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>17.5</td>
<td>0.31</td>
<td>0.52</td>
<td></td>
</tr>
</tbody>
</table>

**Average**

16.4

<table>
<thead>
<tr>
<th>II</th>
<th>BHb (mM per l.)</th>
<th>-Log γ(_{\text{HCO}_3^-})</th>
<th>(\sqrt{\Gamma})*</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.1</td>
<td>0.31</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>22.9</td>
<td>0.22</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>23.2</td>
<td>0.31</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>24.0</td>
<td>0.20</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>24.5</td>
<td>0.21</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>24.9</td>
<td>0.23</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>25.5</td>
<td>0.23</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>26.7</td>
<td>0.29</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>26.8</td>
<td>0.25</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>27.4</td>
<td>0.46</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>27.7</td>
<td>0.41</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>28.1</td>
<td>0.33</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>28.2</td>
<td>0.36</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>31.1</td>
<td>0.17</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

**Average**

26.6

* Calculated assuming Hb\(\text{--}^{\text{eq}}\) univalent.

**TABLE II**

*Log γ\(_{\text{HCO}_3^-}\) at Constant Γ and Varying BHb and HbCO\(_4\).*

\(\Gamma = 133 \text{ mM per liter.}\)*

\(\text{Hb} = 10.6 \text{ " " "}\)

<table>
<thead>
<tr>
<th>BHb (mM per l.)</th>
<th>HbCO(_4) (mM per l.)</th>
<th>-Log γ(_{\text{HCO}_3^-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.3</td>
<td>0.0</td>
<td>0.35</td>
</tr>
<tr>
<td>6.7</td>
<td>0.0</td>
<td>0.41</td>
</tr>
<tr>
<td>0</td>
<td>8.2</td>
<td>0.33</td>
</tr>
<tr>
<td>0</td>
<td>9.6</td>
<td>0.33</td>
</tr>
<tr>
<td>0</td>
<td>13.5</td>
<td>0.37</td>
</tr>
</tbody>
</table>

**Average**

0.36 ± 0.026

* Calculated assuming Hb\(\text{--}^{\text{eq}}\) monovalent.
order of $10^{-8}$ cm. The colloidal hemoglobin ion has dimensions of an entirely different order of magnitude and we might readily suppose that compared to the ordinary ions the charged points are at infinite distances from each other. Obviously each point would act independently in its influence on B, as if it possessed a

![Figure 3](http://www.jbc.org/)

Fig. 3. Theoretical relation of $\log \gamma$ to $\sqrt{\Gamma}$ showing the effects of the three terms of the expanded Debye-Hückel equation.

charge $\pm z$ where $z = 1$. Whereas a hemoglobin ion may have a stoichiometrical valence of $z$, its ionic strength valence may be 1 or at least small.

That the charges on the hemoglobin ion act as if at an infinite
distance from each other, or, in other words, that the ionic strength valence of Hb\textsuperscript{−ze} is 1, is indicated by the following experiments. Two series of pK\textsubscript{i}' were determined in hemoglobin solution of constant concentration of Hb but varying concentrations of BHb; i.e., Hb\textsuperscript{−ze}. The pK\textsubscript{i}' values are plotted against $\sqrt{\Gamma}$ (calculated with $z_{\text{Hb}} = 1$) in Figs. 1 and 2 from which we have calculated the log $\gamma_{\text{HCO}_3}$ values given in Tables I and II.

Log $\gamma_{\text{HCO}_3}$ of the first six points (Table I) with average BHb of 16.4 mM per liter when plotted against $\sqrt{\Gamma}$ may be represented with small deviation by $- \log \gamma_{\text{HCO}_3} = 0.10 + 0.46 \sqrt{\Gamma}$. The remaining fifteen points with an average BHb = 26.6 mM with somewhat more dispersion give $- \log \gamma_{\text{HCO}_3} = 0.11 + 0.42 \sqrt{\Gamma}$. A 60 per cent increase in Hb\textsuperscript{−ze} produced no appreciable change in log $\gamma_{\text{HCO}_3}$. Similarly (Table II) even larger changes in the concentration of Hb\textsuperscript{−ze} (positive or negative) produced no change in log $\gamma_{\text{HCO}_3}$ outside the experimental error.

It is clear than an ionic strength valence of Hb\textsuperscript{−ze} much greater than 1 should have changed $\Gamma$ sufficiently to alter log $\gamma_{\text{HCO}_3}$. The absence of this effect is in accord with the assumption that it may be taken as 1.

**Ionic Collision Sphere of the Bicarbonate Ion.**—Debye and Hückel first considered all the ions as charged points. This simplification of Equation 9 gives

$$\log \gamma_i = - \beta_i \sqrt{\Gamma}$$

the limiting law for very dilute solutions (Fig. 3, heavy line). They next considered the case in which a given ion, $B$, was assumed to be a sphere of finite radius. They state (Debye and Hückel, 1923) that the magnitude $a$ clearly then does not measure the ion radius but represents a length which pictures a mean value for the distance to which the surrounding ions, positive as well as negative, can approach to the given ion. Scatchard (1927) has called $a$ the ionic collision sphere. The value of $a$, however, should approximate molecular dimensions as determined by totally independent methods and the striking agreement of the theory in this respect is one of its important experimental confirmations. For the common univalent ions $a$ ranges between 2.5 to $5 \times 10^{-8}$ cm.
This assumption of an ionic collision sphere of finite diameter makes Equation 9

\[ \log \gamma_i = -\frac{\beta_0 \sqrt{\Gamma}}{1 + B a \sqrt{\Gamma}} \]  

(13)

The effect upon the activity coefficient is shown (dotted line) in Fig. 3 in which \(-\log \gamma_i\) is plotted against \(\sqrt{\Gamma}\). For practically all salts studied (since they have a finite values) the \(\log \gamma_i - \sqrt{\Gamma}\) relations are curves of this character. The quite exact adherence (in water) of \(\log \gamma_{HCO_3^-}\) against \(\sqrt{\Gamma}\) to a straight line with theoretical slope from a concentration \(\Gamma = 0.010\) to 0.5 M is unusual. The alternative conclusions to be drawn from this are that \(a\) is small or zero or our calculation of \(\gamma_{HCO_3^-}\) is erroneous. It should be emphasized that this discrepancy of \(\gamma_{HCO_3^-}\) from theory and experience with mean ion activity coefficients determined by more rigid thermodynamic methods (e.g. in cells without liquid junction) is a serious one and hence the values should be accepted as tentative, especially in the higher concentrations. Without discussing the matter in detail we may point out that the bicarbonate ion is not completely unique in giving \(a = 0\) by Equation 9. For example the mean ion activity coefficients, i.e. \(\gamma_* = \sqrt{\gamma_+ \gamma_-}\) of NaNO₃ and NaIO₃ (determined from the freezing points) and \(\gamma_*\) of some cobalt amine salts in NaCl (determined by solubilities), follow Equation 10 up to \(\Gamma = 0.3\) to 0.5; i.e., the values of \(a\) by Equation 9 are zero. The measurements are of course independent of liquid junction potential. Furthermore, Gronwall (1927) has shown that Equation 9 is a special case of a more generalized equation. Briefly Gronwall's conclusion is that the observed 0 or even negative values of \(a\) obtained by Equation 9 become finite and positive when calculated by his more general equation. Specifically a value of \(a = 0\) by Equation 9 becomes \(2.12 \times 10^{-8}\) cm. We may say then that the adherence of \(\log \gamma_{HCO_3^-}\) to Equation 10 is not necessarily contrary to the theory or experiment with other ions.

**Dielectric Constant of Aqueous NaHCO₃-NaCl Solutions.**—Hückel next determined the effect of ions upon the dielectric constant of the solution. He introduced the \(\Delta \Gamma\) term into Equation 9, which gives as a complicated function of \(\Gamma\), the change of
the dielectric constant of the solution with increasing ionic strength. The effect of this dielectric change is to cause log $\gamma_i$ to pass through a minimum and increase (dash line, Fig. 3). This effect is usually appreciable only at high (1 M) salt concentration. It is absent in the NaHCO$_3$-NaCl solutions studied (up to 0.5 M) as we have seen; hence we shall disregard it completely in the subsequent discussion.

*Hydration in Hemoglobin Solutions.*—In any aqueous solution of ions or molecules it is necessary to consider the possible occurrence of hydration. We will not discuss the hydration of the ions here except to emphasize Hückel’s statement that activity measurements can give no categorical answer as to their existence. On the other hand, a solute like hemoglobin might be considerably hydrated and in a solution of 30 per cent by weight (20 mm per liter) such hydration might have an appreciable effect on the properties of the solution. As in the case of the ions, activity measurements yield no direct answer to the question which must be sought by other methods (optical, chemical).

We can assume either that there is hydration or no hydration; the agreement of the experimental facts with one or the other hypothesis will govern our choice. No hydration of hemoglobin is assumed here since, as we shall subsequently show, the experimental data may be satisfactorily explained on this basis.

Existence of $X_{HbCO}$.—In part we have already discussed the possible occurrence of $X_{HbCO}$ in solutions of Na$^+$, HCO$_3^-$, CO$_2$, Hb. We define it no more exactly than to say it is not HCO$_3^-$ ion and have, for simplicity, assumed it $= 0$. In the main, our data are apparently in accord with this assumption. However, since there is no conclusive evidence on this point, if subsequently $X_{HbCO}$ can be evaluated our conclusions will have to be modified in part or in whole. As in the case of hydrates, activity measurements cannot determine $X_{HbCO}$ or any similar protein ion compound. To call $X_{HbCO}$ undissociated hemoglobin bicarbonate is to assume that NaHCO$_3$ is completely dissociated while HbHCO$_3$ is partially dissociated. This is a possible hypothesis, but in view of our conclusion that HbHCO$_3$ behaves as NaHCO$_3$ in this respect we believe it unlikely.

*Debye-Hückel Theory in Hemoglobin Solutions.*—In our hemoglobin solutions, *i.e.* a mixture of electrolytes and non-electrolytes,
a combination of the Debye-Hückel theory for electrolytes and the principles discussed by Debye and McAulay (1925) and Scatchard (1927) for non-electrolytes gives a satisfactory theoretical approach to our problem. It is necessary to derive a function for the free energy of the bicarbonate ion under all of our experimental conditions in terms of $\gamma_{\text{HCO}_3^-}$.

Effect of Hemoglobin on the Free Energy of the Bicarbonate Ion.—Transfer 1 gm.-ion of $\text{HCO}_3^-$ at constant concentration and infinite dilution, first, from pure water to a solution of ionic strength $= 0$, hemoglobin $= [\text{Hb}]$, the free energy change $= \Delta F_*$; second, at constant hemoglobin $= [\text{Hb}]$ to a solution of ionic strength $= \Gamma$. The free energy change $= \Delta F_0$. The total free energy change in both steps is

$$\Delta F_{\text{HCO}_3^-} = \Delta F_* + \Delta F_0 \tag{14}$$

We may express these free energy changes as activity coefficients; i.e.,

$$\log \gamma_{\text{HCO}_3^-} = \log \gamma_* + \log \gamma_0 \tag{15}$$

$\Delta F_0$ is the free energy change at constant dielectric constant and is due to the interionic electrical effects at varying $\Gamma$. $\log \gamma_0$ is the corresponding activity coefficient, and, as we have already seen, Hückel equated this to

$$\log \gamma_0 = -\beta_0 \sqrt{\Gamma} \tag{16}$$

$\Delta F_*$ is the free energy change of transfer from a solution of dielectric constant $D_0$ to one of dielectric constant $D_*$ when $\Gamma = 0$. $\log \gamma_*$ is the corresponding activity coefficient.

We will evaluate these free energy terms for hemoglobin solutions and then we can calculate $\log \gamma_{\text{HCO}_3^-}$ and the corresponding $pK_1$ at any [Hb] or $\Gamma$. We will consider $\Delta F_0 = R T \ln \gamma_0$ first.

Dielectric Constant of Hemoglobin Solutions.—In order to calculate $\log \gamma_0$ we must properly evaluate the $\beta$ coefficient in hemoglobin, and to do this it is necessary to be aware of the meaning of $D_0$, the dielectric constant factor contained therein. With rare exceptions solutions of non-electrolytes in water decrease the dielectric constant. The effect of salts except in high con-
Debye-Hückel Theory in Hb Solutions

centration is small; the enormous electrical forces about an ion, however, (in water a univalent ion at a distance of $3 \times 10^{-8}$ cm. from its center develops a potential difference of $2 \times 10^6$ volts per cm.) must produce a change (increase) in the dielectric constant in the immediate neighborhood of the ion. We have to distinguish, then, three dielectric constants: $D_0$, that of the pure solvent; $D_s$, that of the solution as a whole; and $D_i$, that in the immediate neighborhood of the given ion whose activity is under consideration. The theoretical value of $\beta$ will vary with our choice of one or the other. On the theoretical grounds Debye and Pauling (1925) excluded $D_i$ from consideration, and in dilute solutions of salts, where $D_s$ is practically constant and equal to $D_0$, they conclude that the dielectric constant of the pure solvent should be used. The experiments of Brönsted and La Mer (1924) in very dilute solutions, and those reported by Hastings and Sendroy (1925) and ourselves on $\gamma$ for aqueous solutions of $\text{HCO}_3^-$, are in agreement with this hypothesis.

Non-electrolytes, however, may have as a rule a marked effect on the dielectric constant of water. It is obvious that in Equation 17 $\beta_0 = \text{constant} \cdot \frac{D_0}{D_0^3}$ must be replaced by $\beta = \text{constant} \cdot \frac{D_s}{D_s^3}$ where $D_s$ is the dielectric constant of the hemoglobin solution. We will assume that the dielectric constant $D_s$ of a solution of hemoglobin is increased proportionately to the hemoglobin concentration and that the increase is different for each derivative of hemoglobin.

On the basis of this assumption alone we will show that the Debye-Hückel theory will account for the behavior of the $\text{HCO}_3^-$ ion in our systems and that the two major effects of hemoglobin, viz. (1) the decrease of $\gamma$ at $\Gamma = 0$ and increasing hemoglobin and (2) the decrease of $\beta$ with increase of hemoglobin, are in harmony with the assumption.

Calculation of $\beta$ at Varying Hemoglobin Concentrations.—Represent the dielectric constant of a solution of any given hemoglobin derivative by an interpolation formula

$$D_s = D_0 (1 + \delta[Hb] + \delta'[Hb]^2 + \ldots)$$

$\delta$ and $\delta'$ are constants characteristic of each hemoglobin derivative and give the per cent increase of the dielectric constant per mm of Hb. Experience with other non-electrolytes indicates that we
may neglect higher powers of [Hb] over a short range of concentration; i.e.,

\[ D_\infty = D_0 (1 + \delta \text{[Hb]}) \]  

(17)

Introducing \( D_\infty \) into \( \beta \) we get

\[ \beta = \frac{1.86 \times 10^4}{D_\infty T^3} = \frac{1.86 \times 10^4}{D_\infty T^3} (1 + \delta \text{[Hb]})^{-\frac{2}{3}} \]

Expanding \((1 + \delta \text{[Hb]})^{-\frac{2}{3}}\), and neglecting higher powers of \( \delta \text{[Hb]} \), which is subsequently shown to be always less than 0.4, we get as a sufficient approximation, \( \beta = \beta_0 (1 - \frac{3}{2} \delta \text{[Hb]}) \).

Since

\[ \beta_0 = 0.54 \]

\[ \beta = \beta_0 - 0.81 \delta \text{[Hb]} \]  

(18)

The qualitative significance of Equation 18 is clear: In hemoglobin solutions \( \beta \) (i.e. the slopes of the log \( \gamma \) vs. \( \sqrt{T} \) lines) should decrease from \( \beta_0 = 0.54 \), the theoretical value in water. Further, the plot \( \beta \) against [Hb] should give a straight line and if \( \delta \) varies in different derivatives the \( \beta \)-Hb lines should be different. This agrees exactly with our experimental results given in Fig. 13 of the previous paper and Equation 5; viz., \( \beta = \beta_0 - \sigma \text{[Hb]} \).

From Equations 5 and 18

\[ \delta = 1.23 \sigma \]  

(19)

The characteristic experimental constant \( \sigma \) appears on the basis of the theory to be the characteristic constant 0.81 \( \delta \) giving the
specific effect of each hemoglobin derivative on the dielectric constant of water.

From the observed values of $\sigma$ in the previous paper, we have calculated $\delta$ for the various pigments (Table III). For $[\text{Hb}] = 15$ mM per liter, the calculated dielectric constants at $38^\circ$ are:

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Calculated $D_\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbCO</td>
<td>88</td>
</tr>
<tr>
<td>RHb</td>
<td>101</td>
</tr>
<tr>
<td>MtHb</td>
<td>107</td>
</tr>
<tr>
<td>HbCN</td>
<td>94</td>
</tr>
<tr>
<td>HbNO</td>
<td>94</td>
</tr>
</tbody>
</table>

To test these calculations we need data on the dielectric constants of hemoglobin solutions directly determined. Complete data of this character are not at hand. Fürth (1923) determined directly by the method of Drude (1897) the dielectric constant of some half dozen biological protein-containing fluids and found that only whole blood, dried red blood cells, and serum gave higher dielectric constants than water. In view of the difficulty of the direct determination of dielectric constants of conducting solutions, we can only regard this evidence as suggestive. It is well to emphasize that if hemoglobin decreases the dielectric constant, as do most non-electrolytes, $\beta$ must increase. Experimentally we have found the contrary to be the case. This, together with the fact that $\beta$ decreases proportionately to the hemoglobin concentration (Equation 5), gives support to our assumption and we tentatively conclude that the decrease of $\beta$ in hemoglobin solutions is due to the increase of the dielectric constant. The hemoglobin derivatives are divisible roughly into two groups according as $\delta$ is $>\ or< 0.017$. The dielectric constants of solutions of the same concentrations should be different. We have no independent evidence on this point.

Calculation of Log $\gamma_*$.—Let

$F_I =$ free energy of $\text{HCO}_3^-$ ion in the ideal state; i.e. in water $[\text{Hb}] = 0$, $\sigma_{\text{HCO}_3^-} = 0, \Gamma = 0$.

$F =$ free energy at hemoglobin $= [\text{Hb}], \sigma_{\text{HCO}_3^-} = 0, \Gamma = 0$.

The change of free energy in the transfer is given by Debye and McAulay (1925) as

$$F - F_I = \frac{N e^* n_{\text{HCO}_3^-}}{2D \, b}$$
Differentiating with respect to \( n_{\text{HCO}_3^-} \) and \( D \) and integrating between the limits \( n_{\text{HCO}_3^-} = 0 \) to 1 and \( D = D_0 \) to \( D_8 \), we get as the change in free energy in the transfer of 1 gm.-ion

\[
\Delta F^*_e = RT \ln \gamma^*_e = \frac{N e^2}{2b} \frac{D_0 - D_s}{D_0 D_s}
\]

\( N = \) Avogadro's number = \( 6.07 \times 10^{23} \).

\( \epsilon = \) elementary electronic charge = \( 4.77 \times 10^{-19} \) e.s.u.

\( D_s = \) dielectric constant of the solution.

\( b = \) ionic radius of \( \text{HCO}_3^- \).

\( R = \) gas constant.

\( T = \) absolute temperature.

\( n_{\text{HCO}_3^-} = \) No. of \( \text{HCO}_3^- \) ions per unit volume.

From Equations 21 and 18 we get

\[
F^*_e = RT \ln \gamma^*_e = -\frac{N e^2 \delta[\text{Hb}]}{2bD_0 (1 + \delta[\text{Hb}])} = -\frac{N e^2 \delta[\text{Hb}]}{2bD_0}
\]

by expansion of \((1 + \delta[\text{Hb}])^{-1}\) neglecting higher powers of \( \delta[\text{Hb}] \).

Substituting numerical constants (in calories), noting that \( D_{38^*} = 72.5 \), we obtain, in calories per equivalent

\[
\Delta F^*_e = RT \ln \gamma^*_e = -\frac{2.29 \times 10^{-8}}{b} \delta[\text{Hb}]
\]

from which

\[
\log \gamma^*_e = -\frac{1.06 \times 10^{-8}}{b} \delta[\text{Hb}]
\]

The qualitative significance of Equation 23 is clear. Log \( \gamma^*_e \) (or \( pK^*_e \)) plotted against [Hb] should give a straight line having a value of log \( \gamma^*_e = 0 \) (or \( pK^*_e = pK_1 \)) at [Hb] = 0 with decreasing values of log \( \gamma^*_e \) (or \( pK^*_e \)) as [Hb] increases. The slopes of these lines \( = (1.6 \times 10^{-8} \frac{\delta}{b}) \) should vary with the value of \( \delta \); i.e., with the nature of the hemoglobin derivative. These are precisely the
Debye-Hückel Theory in Hb Solutions

results observed as shown in Fig. 12 of the previous paper and Equation 7 of this paper; i.e.,

$$\log \gamma^* = -\rho[Hb]$$  \hspace{1cm} (7)

From Equations 23 and 7

$$\rho = \frac{N e^2 \delta}{2 \times 2.3 D_0 RT b} = \frac{1.6 \times 10^{-8} \delta}{b}$$  \hspace{1cm} (24)

The characteristic constant $\rho$ appears to be equal to the ratio of two constants: $\delta$, characteristic of the hemoglobin, and $b$, characteristic of the bicarbonate ion, multiplied by a factor which includes universal constants and $D_0$, characteristic of the solvent.

**TABLE IV.**

<table>
<thead>
<tr>
<th>Ionic Diameter of $HCO_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>HbCO</td>
</tr>
<tr>
<td>RHb</td>
</tr>
<tr>
<td>MtHb</td>
</tr>
<tr>
<td>HbCN</td>
</tr>
<tr>
<td>HbNO</td>
</tr>
</tbody>
</table>

Calculation of the Ionic Radius of the Bicarbonate Ion.—Debye and McAulay assumed in deriving Equation 20, that the electric charge of an ion is distributed over the surface of a sphere of radius $b$, which for simplicity they considered equal to $a$, the radius of the collision sphere. Similar to $a$ it should have molecular dimensions. From Equation 24

$$b = \frac{1.6 \times 10^{-8} \delta}{\rho}$$  \hspace{1cm} (25)

Using the $\delta$ values calculated from the slopes of the pK$_1$' lines and the $\rho$ values from the previous paper calculated from $\log \gamma^*$, we get the value for $b$ given in Table IV. Considering the number of simplifying assumptions made, the agreement of values with each other and with the magnitude of ionic diameters in general as measured by totally independent methods is good.

Restatement of Log $\gamma_{HCO_3^-}$ in General Terms.—Equations 1 to 8 give in terms of $\beta$, $\rho$, $\sigma$, $\Gamma$, and $[Hb]$ the variations of pK$_1$ and $\log \gamma_{HCO_3^-}$ from the experimental data of the preceding paper. It
is now possible to restate these relations in terms of \( \delta \), [Hb], \( b \), \( \Gamma \), and universal constants.

\[
\log \gamma_{\text{HCO}_3^-} = \log \gamma_0 + \log \gamma_* \tag{26}
\]
\[
\log \gamma_0 = -\beta_0 \left( 1 - \frac{2}{3} \delta[\text{Hb}] \right) \sqrt{\Gamma} \tag{27}
\]
\[
\log \gamma_* = -\frac{N e^2 \delta[\text{Hb}]}{2.3 \times 2 b D_0 RT} = -\frac{1.6 \times 10^{-8} \delta[\text{Hb}]}{b} \tag{28}
\]
\[
pK_1 = 6.33 + \log \gamma_{\text{HCO}_3^-} \tag{29}
\]

\( b = 2 \times 10^{-8} \text{ cm} \). The values of \( \delta \) are given in Table III.

**DISCUSSION.**

The demonstration of marked and specific effects of different hemoglobin pigments upon the pK1' of carbonic acid rests upon purely experimental grounds and reveals an interesting and unsuspected buffer mechanism in the acid-base equilibrium of the blood. The theoretical explanation of this effect by the Debye-Hückel theory, while simple and plausible, rests upon an almost wholly unsupported assumption which can only be tested experimentally by determinations of the dielectric constants of highly conducting solutions. Obviously, while the explanation is satisfactory, it must be accepted at present as a possible but not exclusive one.

**SUMMARY AND CONCLUSIONS.**

1. The Debye-Hückel theory is discussed with special reference to its application to concentrated solutions of hemoglobin.

2. Theoretical and experimental reasons are given to support the assumption that the colloidal hemoglobin ion has an ionic strength valence of 1.

3. The relations of the ionic collision sphere of HCO\(_3^-\), the dielectric constant of NaHCO\(_3\)-NaCl solutions, the hydration of hemoglobin, and the possible existence of X\(_{\text{HCO}_3^-}\) to the activity coefficient of the HCO\(_3^-\) ion are outlined.

4. It is shown theoretically that \( \gamma_{\text{HCO}_3^-}\), the activity coefficient of the bicarbonate ion in hemoglobin solutions, may be divided into two activity coefficients, \( \gamma_* \) and \( \gamma_0 \).

5. \( \gamma_* \) measures the effect of hemoglobin on the HCO\(_3^-\) ion. It is theoretically a function of the dielectric constant of the
hemoglobin solution, whereas $\gamma_0$ measures the effect of salt on the HCO$_3^-$ ion. Equations were derived giving $\gamma_*$ and $\gamma_0$ as functions of two constants; viz., $b$, characteristic of the HCO$_3^-$ ion, is its ionic diameter, and $\delta$, characteristic of hemoglobin, gives the effect of the hemoglobin on the dielectric constant of water.

6. The values of $b$ and $\delta$ calculated from the observed $pK_1'$ of carbonic acid are consistent with measurements by independent methods. Hemoglobin, contrary to most non-electrolytes, appears to increase the dielectric constant of water.

7. On the basis of the theory it is possible to calculate in terms of universal constants and the characteristic constants $b$ and $\delta$, the activity coefficients of HCO$_3^-$ ion (and hence $pK_1'$) in solutions with Hb varying from 0 to 20 mm per liter and salt from 0 to 0.5 M per liter. The activity coefficients so calculated agree within the limits of experimental error with the observed. The equations for the calculations are

$$\log \gamma_0 = \log \gamma_* + \log \gamma_0$$

$$\log \gamma_* = \frac{-Ne^2\delta[Hb]}{2.3 \times 2bD_0RT} - \frac{1.6 \times 10^{-8}\delta[Hb]}{b}$$

$$\log \gamma_0 = -\beta_0(1-\frac{1}{2}\delta[Hb])\sqrt{T}$$

From these equations $pK_1 = 6.33 + \log \gamma_0$. $b = 2 \times 10^{-8}$ cm. The $\delta$ values are given in Table III. On the basis of $\delta$ the hemoglobin derivatives are divided into two groups. (1) reduced hemoglobin, methemoglobin, and cyanhemoglobin; (2) carbon monoxide hemoglobin and nitric oxide hemoglobin. The first group should increase the dielectric constant of water more than the second.

We wish to take this opportunity to thank the H. K. Mulford Company of Philadelphia, who generously supplied us with large amounts of horse blood for the preparation of the crystalline hemoglobin used in these experiments.

**BIBLIOGRAPHY.**


*Explanation of Symbols.*

\( a, a_i \) = activities of molecules or ions; distance of nearest approach.

\( \gamma_{\text{HCO}_3}, \gamma_* \), \( \gamma_0 \) = activity coefficients of the bicarbonate ion; Equations 4 and 5, Paper IV.

\([ \ ]\) = concentrations mm per liter.

\(( \ )\) = concentrations mm per kilo of \( \text{H}_2\text{O}\).

\([W]\) = kilos of \( \text{H}_2\text{O} \) per liter.

\( P_{\text{H}_2}, P_{\text{CO}_2}, \text{etc.} \) = partial pressures of \( \text{H}_2, \text{CO}_2, \text{etc.} \).

\( \alpha^{\text{CO}_2}, \alpha^{\text{CO}_2} \) = solubility coefficients of \( \text{CO}_2 \) in water and in a given solution.

\( E, \text{ e.m.f.} \) = electromotive force.

\( L \) = liquid junction potential.

\( F \) = 1 faraday.

\( F \) = free energy.

\( \epsilon_0 \) = e.m.f. of reference cell; Equation 3, Paper III.

\( \Gamma, \mu \) = ionic strength; Equation 10, Paper V.

\( z_i \) = valence.

\( K_1, K_1', K_* \) = dissociation constants of \( \text{H}_2\text{CO}_3 \); Equations 3 and 4, Paper IV.

\( \rho, \sigma \) = constants; Equations 37 and 38, Paper IV.

\( \delta, a, b \) = constants; Equations 9, 18, and 21, Paper V.

\( \beta_{\text{H}_2}, \beta \) = constants; Equation 14a, Paper IV, and Equation 9, Paper V.

\( D_0, D_* \) = dielectric constants of water and a given solution.

\( \epsilon \) = elementary electronic charge.

\( N \) = Avogadro's number or gm. equivalents.

\( \text{Hb} \) = hemoglobin in general.

\( \text{RHB} \) = reduced hemoglobin.

\( \text{HbCO} \) = carbon monoxide hemoglobin.

\( \text{MtHb} \) = methemoglobin.

\( \text{HbCN} \) = cyanhemoglobin.

\( \text{HbNO} \) = nitric oxide hemoglobin.

\( \ln \) = natural logarithm.

\( \log \) = 10 base logarithm.

\( R \) = gas constant.

\( T \) = absolute temperature.
STUDIES ON THE OXYGEN-, ACID-, AND BASE-COMBINING PROPERTIES OF BLOOD: V. EXTENSION OF THE DEBYE-HÜCKEL THEORY OF IONIC INTERACTION TO HEMOGLOBIN, BICARBONATE-SODIUM CHLORIDE SYSTEMS
William C. Stadie


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