Graphic Representation of CO₂ Equilibria in Biological Systems

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As biology advances and multicomponent systems with wide pH, PCO₂, and temperature changes are encountered, it is necessary to extend the rather limited acid-base formulations of medicine and yet somehow retain comprehensibility. For example, in Rahn and Reeves’ analysis (12) of water-ion (OH⁻/H⁺) ratios in the animal kingdom and in its clinical applications (16), one must keep track of a bewildering array of reactants, products, and ionization constants, each varying differently with temperature. Even everyday cardiorespiratory problems arising from the difference between systems open and closed to CO₂ (4,10,11) need clarification.

We present the log C-pH diagram (1-3,9,14) as extended to open and closed biological systems (6) and derive a new water-ion balance method for determining equilibrium pH. The water-ion formulation allows hydroxyl ion and proton-transfer reactions to be treated with equal emphasis. The diagram makes immediately obvious which products of OH⁻ transfer and H⁺ transfer reactions are the “controlling products” in the sense that they determine pH. This approach may serve as an introduction to acid-base states in hypothermia and in organ systems in which the OH⁻/H⁺ ratio is considered to be important (12).

Water-Ion Balance Equation

Water, by itself, dissociates into equal numbers of protons and hydroxyl ions. When other species are added, the number of protons Hw and of hydroxyl ions OHw produced by such direct water dissociation is still equal, regardless of the equilibrium pH. This is the principle of water-ion balance.

An imbalance between total [H⁺] and [OH⁻] in an aqueous solution generally occurs when molecules other than water produce, directly or indirectly, water ions in excess of Hw and OHw. Species like NaOH and H₂SO₄ “donate” water ions directly. Species such as sodium acetate (NaAc) and CO₂ “free” OH⁻ and H⁺ by abstracting, respectively, protons and hydroxyls from water. Before dealing with CO₂ systems we will derive the water-ion balance equation using familiar nonvolatile species as examples.

Water ions in excess of Hw and OHw are measured by their product equivalents. For example, adding NaOH to water produces one Na⁺ for each OH⁻ so that at equilibrium

\[ [\text{OH}^-] = [\text{OH}_w] + [\text{Na}^+] \]

where [Na⁺] is the concentration of the product equivalent of the excess OH⁻ contributed by NaOH.

When H₂SO₄ is added to water, the equilibrium hydrogen ion concentration [H⁺] is

\[ [\text{H}^+] = [\text{H}_w] + 2[\text{SO}_4^{2-}] \]

since two excess protons are donated per sulfate ion.

The acetate ion adds hydroxyl indirectly by abstracting a proton when NaAc is added to water. Thus, for a solution of sodium acetate

\[ [\text{OH}^-] = [\text{OH}_w] + [\text{HAc}] \]

If all three species are added to water, water ions and their equivalents are produced in the following reactions

\[
\begin{align*}
\text{NaOH} &\rightarrow \text{OH}^- + \text{Na}^+ \\
\text{H}_2\text{SO}_4 &\rightarrow 2\text{H}^+ + \text{SO}_4^{2-} \\
\text{Ac}^- + \text{H}_2\text{O} &\rightarrow \text{OH}^- + \text{HAc} \\
\text{H}_2\text{O} &\rightarrow \text{H}_w + \text{OH}_w
\end{align*}
\]

The total water-ion concentrations are obtained by simply adding to [Hw] and [OHw] the concentrations of the product equivalents of the excess water ions

\[
\begin{align*}
[\text{H}^+] &= [\text{H}_w] + 2[\text{SO}_4^{2-}] \\
[\text{OH}^-] &= [\text{OH}_w] + [\text{Na}^+] + [\text{HAc}]
\end{align*}
\]

The principle of water-ion balance requires

\[ [\text{H}_w] = [\text{OH}_w] \]
Combining these three equations yields the water-ion balance equation

\[ [H^+] + [Na^+] + [HAc] = [OH^-] + 2[SOi^-] \]

Written generally, the equation is

\[ [H^+] + [OH^- \text{ equivalent(s)}] = [OH^-] + [H^+ \text{ equivalent(s)}] \] (I)

This equation determines the equilibrium pH expressed for simplicity in this paper as \(-\log [H^+]\). We will show how the log C-pH diagram is used to solve the equation.

Log C-pH Diagram

The diagram consists of dissociation curves, each representing the concentration of a reactant or product vs. pH. Their shapes are fixed by each chemical system. These shapes are not changed when other chemical reactants are added. The controlling products of reactions that involve water-ion exchange are established by inspection of the dissociation curves. The equilibrium pH is found at the intersection of the two controlling product curves.

The diagram can also be used to illustrate charge neutrality (6), proton balance (7), and classical analytical approaches (13), but these will not be considered in this paper.

To find an equilibrium pH, three steps are followed on the diagram. 1) Dissociation curves are derived from the equilibrium and mass balance equations for each open- or closed-system reactant and product. 2) The reaction expressions are used to identify the products resulting from water-ion exchange. 3) The water-ion balance equation is solved for the pH at the intersection of two curves established by substitution of the product equivalents in each side of the equation. The solutions can be achieved graphically by simple inspection of the diagram.

In explaining how these steps are carried out, each will be described separately in examples of biological interest, although in the actual use of the diagram, finding the equilibrium pH becomes (with practice) a single rapid visual process. The first example is the dissociation of pure water at 25 and 37°C.

Water

Dissociation Curves

The equilibrium equation is \([H^+] [OH^-] = K_w\). By definition \(K_w = -pK_w\). The dissociation equations, the first from the definition of pH, are

\[ \log[H^+] = -pH \]

and

\[ \log[OH^-] = pH - pK_w \]

Since both \(\log[H^+]\) and \(\log[OH^-]\) have the form \(\log C\), these equations are represented graphically as the two bold dissociation curves (here straight lines) in Figure 1. The line for \(\log[H^+]\) vs. pH has a slope of \(-1\), and the pH \(= 0\) when \([H^+] = 1\) M. The line for \(\log[OH^-]\) vs. pH has a slope of \(+1\), and pH \(= pK_w\) when \([OH^-] = 1\) M.

Reactants and Products

The reactant on the left and the products on the right of the reaction expression are written as

\[ H_2O \rightarrow Hw + OH_w \]

Equilibrium pH

The solution of the water-ion balance equation, obtained by substituting the products of water-ion transfer, and noting that there are no ions donated or freed by added species, is

\[ [H^+] = [OH^-] \] (Ia)

which establishes the equilibrium pH. At 25°C, where \(pK_w = 14\), the bold dissociation curves intersect at the point (solid circle) where pH \(= 7\) as indicated on the abscissa and where proton and hydroxyl concentrations both have the value C \(= 10^{-7}\) M as indicated on the ordinate.

At 37°C, where \(pK_w = 13.6\), the thin \([OH^-]\) dissociation curve intersects with the bold \([H^+]\) curve, which cannot move because it defines pH, at the open circle where pH \(= 6.8\) and where proton and hydroxyl concentrations both have increased to C \(= 1.58 \times 10^{-7}\). The pH at the neutral point has fallen to 6.8, reflecting the increased dissociation of water at the higher temperature.

The diagram lets the observer "see" quite precisely how much rising temperature raises \([H^+]\) and \([OH^-]\) by increasing water dissociation (changing position but not the shape of the dissociation curves) and by how much equilibrium pH falls (the horizontal distance that the open circle has moved to the left). The diagram continually reminds one of the definition of pH.

Open Unbuffered CO₂ System

The three steps needed to solve for equilibrium pH are now described for an open CO₂ system in which Pco₂ can be regulated by gas exchange.
Dissociation Curves

The system is described by the Henderson-Hasselbalch equations from which the dissociation equations are

\[ \log[\text{HCO}_3^-] = pH - pK'_1 + \log[\text{CO}_2^-] \]
\[ \log[\text{CO}_2^-] = 2pH - pK'_1 - pK'_2 + \log[\text{CO}_2] \]

The first is the logarithmic form of Eq. 5 below. The second results from combining the first with the logarithmic form of Eq. 6, \( \log[\text{CO}_2^-] = pH - pK'_1 + \log[\text{HCO}_3^-] \).

As with the water-ion dissociation equations, the terms on the left have the form \( \log C \). If \([\text{CO}_3^-] \) is held constant by gas exchange, as indicated by the horizontal line labeled \([\text{CO}_3^-] \), the equations are represented by the straight lines labeled \([\text{HCO}_3^-] \) and \([\text{CO}_2^-] \) in Figure 2. The \([\text{HCO}_3^-] \) line has a slope of +1 and intersects the \([\text{CO}_2^-] \) line at \( pH = pK'_1 \). The \([\text{CO}_3^-] \) line has a slope of +2 and intersects the \([\text{HCO}_3^-] \) line at \( pH = pK'_1 \) (see 3rd equation above). Carbonic acid concentration \([\text{H}_2\text{CO}_3] \) is a constant fraction \((1/400)\) of \([\text{CO}_2^-] \) as shown by the lower horizontal line located 2.6 scale divisions below the \([\text{CO}_2^-] \) line \((\log 1/400 = -2.6)\).

If \(PCO_2\) and hence \([\text{CO}_2^-] \) are raised at constant temperature, the entire system of four lines simply shifts upward, maintaining a fixed shape. (All crossings of carbonate lines with water-ion lines move to the left).

If temperature changes, different \(pK'_1, pK'_2\), and \([\text{CO}_2^-] \) solubility establish different crossings and a different \([\text{PCO}_2] \).

Reactants and Products

When \(\text{CO}_2\) and water react, the products appear on the right in the net reaction expressions as follows

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O} - 2\text{H}^+ + \text{CO}_2^- \]
\[ \text{H}_2\text{O} - \text{H}_2\text{O} + \text{OH}^- \]

The product equivalent of \(\text{H}^+\) in the first reaction is \([\text{HCO}_3^-]\), since one proton is freed per \(\text{HCO}_3^-\) product. But two protons are freed per \([\text{CO}_2^-]\), so the equivalent product of \(2\text{H}^+\) is \([2\text{CO}_2^-]\). Substituting the equivalents in Eq. 1 yields

\[ [\text{H}^+] = [\text{HCO}_3^-] + 2[\text{CO}_2^-] + [\text{OH}^-] \]

(1b)

Equation 1b applies to either an open or a closed system.

Equilibrium pH

The open-system dissociation curves are used to find the equilibrium \(pH\). The three terms on the right of Eq. 1b are expressed in each \(pH\) range of Figure 2 by the highest line representing a product of water-ion transfer. (The \([\text{CO}_3^-]\) and \([\text{H}_2\text{CO}_3]\) lines can be ignored as determinants of \(pH\) because they are not reaction products.) In this case the highest such line, up to \(pH = 10.3\), is the \([\text{HCO}_3^-]\) line. Thus Eq. 1b is, in practical form

\[ [\text{H}^+] = [\text{HCO}_3^-] + \ldots \]

which establishes equilibrium \(pH\). The \([\text{HCO}_3^-]\) lines intersect the \([\text{H}^+]\) line at \(pH = 4.51\) (solid circle), thus controlling equilibrium. Inspection of the diagram makes immediately obvious that \([\text{HCO}_3^-]\) is the "controlling product." That the other products of water-ion transfer are negligible can be seen from their millimolar concentrations read from the diagram where their dissociation curves intersect the vertical dotted line at \(pH = 4.51\).

It can also be seen from the fact that their dissociation curves are more than two scale divisions below \((1/100)\) of the controlling product curves.

The equilibrium \(pH\) can, of course, be found by solving simultaneously for six unknowns in Eq. 1b and the following five

\[ [\text{CO}_2] = S \cdot PCO_2 \]

where \([\text{CO}_2] = [\text{H}_2\text{CO}_3] + [\text{CO}_2]_{\text{diss}}\) (since carbonic acid and dissolved \(\text{CO}_2\) are indistinguishable in the sum) and \(S\) is the apparent solubility coefficient

\[ T_{\text{CO}_2} = [\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_2^-] \]
\[ K_w = [\text{H}^+] [\text{OH}^-] \]
\[ K'_1 = [\text{H}^+] [\text{HCO}_3^-]/[\text{CO}_3^-] \]
\[ K'_2 = [\text{H}^+] [\text{CO}_2^-]/[\text{HCO}_3^-] \]

where the activity coefficients are incorporated in the apparent equilibrium constants \(K'_1\) and \(K'_2\). The unknowns are \([\text{CO}_2]\), \(T_{\text{CO}_2}\), \([\text{HCO}_3^-]\), \([\text{H}^+]\), and \([\text{OH}^-]\). Standard texts show how to decide which of these six unknowns can be approximated in solving for \(pH\), but the graphic method makes these approximations obvious.
Once the pH is known for one equilibrium, an acid-base disturbance can often be quantitatively illustrated on the diagram. In this aqueous system, doubling PCO2 at the same temperature, and therefore at the same CO2 solubility, raises all carbonate curves 0.3 unit in Figure 2 (log 2 = 0.3). The pH falls 0.15 unit as can be seen from the leftward shift of the [H+]-[HCO3] intersection (insert in Figure 2). Similarly if a temperature fall should double the CO2 solubility, S, at constant PCO2, [CO2] again doubles because of Eq. 2. Thus the same leftward shift of the intersection is induced by cooling, if the slight temperature dependence of pK's is neglected (intersection at open circle).

Closed Unbuffered CO2 System

The three steps needed to solve for equilibrium pH are now described for a closed CO2 system in which TCO2 remains constant because gas exchange is prevented or because, as in many living systems, CO2 production equals CO2 elimination.

Dissociation Curves

For low pH, combining the mass balance Eq. 3

\[
\text{Tco}_2 = [\text{CO}_2] + [\text{HCO}_3] + \ldots \quad (\text{pH} < 8.2)
\]

with the equilibrium Eq. 5 the dissociation equations are

\[
[\text{CO}_2] = \frac{\text{Tco}_2 [\text{H}^+]}{[\text{H}^+] + K}\]

\[
[\text{HCO}_3] = \frac{\text{Tco}_2 K}{[\text{H}^+] + K}\]

Straight-line approximations to the logarithmic form of these equations are shown in the graph of Figure 3:

when \([\text{H}^+] >> K'(\text{pH} << K)\)

\[
\log[\text{CO}_2] = \log \text{Tco}_2
\]

\[
\log[\text{HCO}_3] = \text{pH} - K' + \log \text{Tco}_2
\]

when \([\text{H}^+] << K'(\text{pH} >> K)\)

\[
\log[\text{CO}_2] = - \text{pH} + K' + \log \text{Tco}_2
\]

\[
\log[\text{HCO}_3] = \log \text{Tco}_2
\]

For pH << K', \([\text{CO}_2] - \text{Tco}_2\) and \([\text{HCO}_3]\) vs. pH has a slope of +1. At pH = K', \([\text{HCO}_3] = [\text{CO}_2] = \frac{1}{2} \text{Tco}_2\). For pH >> K', \log \[\text{CO}_2]\) vs. pH has a slope of -1 and \([\text{HCO}_3] = \text{Tco}_2\). The straight line approximations around K' are similarly derived from \(\text{Tco}_2 = \ldots + [\text{HCO}_3] + [\text{CO}_2^+] + \ldots\) and Eq. 6.

In the vicinity of the K's, the graphs change. Each curve has the same shape or its mirror image about pK. \([\text{H}_2\text{CO}_3]\) is 2.6 orders of magnitude below \([\text{CO}_2]\) as was true in the open system. If \(\text{Tco}_2\) rises, all four curves shift upward but maintain the same shapes and relative positions. If temperature changes, the K's change as do the relative positions of the curves, but \(\text{Tco}_2\) does not.

Reactants and Products

When NaHCO3 is added to water, the following reactions lead to the products on the right

\[
\begin{align*}
\text{NaHCO}_3 & \rightarrow \text{H}^+ + \text{CO}_2^2 + \text{Na}^+ \\
\text{NaHCO}_3 & \rightarrow \text{OH}^- + \text{CO}_2 + \text{Na}^+ \\
\text{H}_2\text{O} & \rightarrow \text{H}_2\text{O} + \text{OH}_w
\end{align*}
\]

The equivalent of H+ is [CO2]. The equivalent of OH- is [CO2]. The Na+ is not a product of water-ion transfer, simply appearing by direct dissociation of the reactant. Equation 1 then becomes

\[
[\text{H}^+] + [\text{CO}_2] = [\text{CO}_2^+] + [\text{OH}^-] \quad (lc)
\]

This equation applies in either an open or closed system.

Equilibrium pH

When NaHCO3 is added to water to form a 25 mM solution, and CO2 exchange is prevented, the log C-pH diagram (Figure 3) immediately establishes equilibrium pH = 8.20 at the intersection of the dissociation curves of the controlling products [CO2] and [CO2+] (solid circle). The millimolar concentrations depicted in Figure 3 at this equilibrium pH are

\[
\begin{align*}
[\text{HCO}_3^-] &= 24.8 \\
[\text{CO}_2] &= [\text{CO}_2^+] = 0.2 \\
[\text{H}_2\text{CO}_3] &= 0.0005 \\
[\text{OH}^-] &= 0.004 \\
[\text{H}^+] &= 0.0000063
\end{align*}
\]

The diagram shows that at pH 8.2 the [OH-] line is about \(2 \times 10^{-1}\) units below the solid circle and represents a concentration \(1/50\) that of the controlling products. It is therefore not completely negligible in Eq. lc. Including [OH-] yields pH = 8.19.
The equilibrium pH could again be found by solving six equations (1e and 2-6) replacing $P_{CO_2}$ by $T_{CO_2}$ as an unknown. The algebraic solution is 

$$[H^+]^2 = K_1; K_1 + \ldots \text{ or } pH = 1/2(pK_1 + pK_2) + \ldots$$

The diagram clearly demonstrates the last expression by the fact that the symmetrical $[CO_2^-]$ and $[CO_3^{2-}]$ curves intersect at the equilibrium pH halfway between $pK_1$ and $pK_2$.

If more NaHCO$_3$ were added to this system, all carbonate curves would simply move upward but the equilibrium intersection would not move significantly to the right. This is a diagrammatic way of showing that sodium bicarbonate is not an alkalinizing agent when injected into a closed system, a chemical fact clinically emphasized by Odell and co-workers (10,11).

**Closed Buffered System, Temperature Effects**

The graphical concepts of this paper are next applied to the studies of Rahn and Reeves (12), who hypothesize that vertebrate blood acid-base systems regulate to maintain a state of constant protein ionization as the temperature varies. Blood is modeled as an aqueous solution of 130 mM imidazole (Im) in a closed CO$_2$ system with a 25 mM total CO$_2$ content.

**Dissociation Curves**

The CO$_2$ dissociation curves in this system (Figure 4) are the same as in Figure 3. The equilibrium and mass balance equations for Im are $K_{im} = [H^+] [Im]/[HIm^+]$ and $T_{im} = [HIm^+] + [Im]$. The $[HIm^+]$ dissociation curve looks like a $[CO_2^-]$ dissociation curve with $T_{CO_2}$ replaced by $T_{im}$ and $K_1$ by $K_{im}$. Only the dissociation curves of the controlling variables are shown in Figure 4 as bold lines at 37°C.

**Reactants and Products of the Reaction**

The reactions are

\[
\begin{align*}
\text{CO}_2 + H_2O &\rightarrow H^+ + HCO_3^- \\
\text{CO}_2 + H_2O &\rightarrow 2H^+ + CO_3^{2-} \\
\text{Im} + H_2O &\rightarrow OH^- + HIm^+ \\
H_2O &\rightarrow H_W + OH_W
\end{align*}
\]

The equivalents of $H^+$, $2H^+$, and $OH^-$ are the products $[HCO_3^-]$, $2[CO_3^{2-}]$, and $[HIm^+]$, respectively. Equation 1 then becomes

$$[H^+] \cdot [HIm^+] = [HCO_3^-] \cdot 2[CO_3^{2-}] \cdot [OH^-](Id)$$

At 37°C the equilibrium pH is 7.4.

**Equilibrium pH from Water-Ion Balance**

The equilibrium pH is found at the intersection of the curves that represent each side of Eq. 1d. Practically, Eq. 1d is determined by the "controlling products"

$$\ldots + [HIm^+] = [HCO_3^-] + \ldots$$

The same water-ion balance equation applies when the temperature decreases. The imidazole dissociation curves shift to the right by 0.017 pH unit/degree because the $pK_{im}$ increases. But the state of ionization of the HCO$_3^-$ and of the imidazole protein hardly changes simply because of the low $pK_1$ of the CO$_2$ system (6.1). (The HCO$_3^-$ line is almost horizontal and $[HIm^+]$ hardly increases at all with cooling.) This is true regardless of the nearly identical temperature tracking of the $pK_{im}$ and $pK_W/2$ as considered in the a-stat hypothesis (12). The pH increases very nearly in proportion to the $pK_{im}$ because nearly all of the total CO$_2$ content ($T_{CO_2}$) exists in the form of $[HCO_3^-]$. What is not apparent on the figure is that the solubility $S$ of CO$_2$ increases significantly with decreasing temperature; thus, at low temperatures a much lower $P_{CO_2}$ can sustain a constant $T_{CO_2}$.

**Open Buffered System, Temperature Effects**

Consider the same aqueous solution of imidazole in a system open to the exchange of CO$_2$, so that $P_{CO_2} = 40$ mmHg. The $[HIm^+]$ dissociation curves are the same as in the previous section. The $[HCO_3^-]$ dissociation curves are like those of Figure 2 for the open system. The reactants and products of the reaction are the same as in the previous section, and hence the water-ion balance equation is the same, as is its practical form. Equilibrium pH occurs at the intersection of the $[HIm^+]$ with the $[HCO_3^-]$ dissociation curves. The $[HCO_3^-]$ dissociation curve shifts upward with decreasing temperature because the in-
creased solubility of CO₂ more than offsets the increase in pK₁. Thus at constant Pco₂, the pH rises slightly while [HCO₃⁻] and TCO₂ rise significantly.

Keeping in vivo Pco₂ constant, a common strategy used during cardiopulmonary bypass, can be achieved by adding CO₂ to the respiratory gas mixture (8). Recent work suggests (12,16) that closely matching CO₂ elimination to CO₂ production to keep TCO₂ constant is physiologically advantageous. To achieve this strategy requires a level of ventilation sufficient to lower PCO₂ to 18 mmHg (20°C) or 6.5 mmHg (0°C) as shown by the open circles in Figure 5. This requires approximately constant or slightly reduced ventilation because metabolic CO₂ production falls with falling temperatures.

Summary

Most of the acid-base concepts we have considered were appreciated in the laboratory years ago. For example, Stadie et al. (15) anticipated many of Reeves and Rahn's equations. In 1908 Henderson (5) showed that the ability to keep Pco₂ constant in an open system greatly increases the buffering capacity of the body. But such biochemical truths often fail to be appreciated when expressed in the forbidding language of mathematics and are rediscovered years later at the bedside or in the operating room (4,10,11,16).

The log C-pH diagram is a means of displaying quantitatively the many variables, including temperature, that determine acid-base equilibria in biological systems. The relative importance of each species is indicated by the vertical separation of their dissociation curves at each pH. Negligible concentrations can be recognized by inspection. Concentration differences and ratios like [OH⁻]/[H⁺], on the other hand, are often not negligible, and the diagram shows how these can be important. The inconstancy of the Rosenthal factor is explained at low pH by the small difference between TCO₂ and [HCO₃⁻]. The fact that this difference is even smaller in the higher pH range of hypothermia clarifies the a-stat hypothesis. Both problems are illustrated by the relations between a straight line and a curve.

References