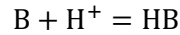


SUPPLEMENTARY NOTE

Deriving buffering capacity

In any biological system, a large pool of H^+ ions is held on buffers. This is represented chemically by the equilibrium involving the buffer's ionised (B) and protonated (HB) states:



The acid-dissociation constant of this equilibrium is defined as:

$$K_a = \frac{[H^+] \cdot [B]}{[HB]} \quad (\text{Eq 4})$$

The system's buffering capacity can be expressed as the ratio between the change in [B] (or [HB]) and the measured $[H^+]$ response. In a closed system, the sum of [B] and [HB] is constant (C_B). By applying this to Eq 4, it is possible to derive an expression for [B]:

$$[H^+] = K_a \cdot \frac{C_B - [B]}{[B]}$$

$$[B] = \frac{K_a \cdot C_B}{K_a + [H^+]}$$

Buffering capacity, quantified as $\Delta[B]/\Delta[H^+]$, becomes a derivative for infinitesimal $[H^+]$ changes:

$$\frac{d[B]}{d[H^+]} = -\frac{K_a \cdot C_B}{(K_a + [H^+])^2}$$

Since pH is the reporting standard for changes in $[H^+]$, a more appropriate quantification for buffering is $d[B]/dpH$, which can be expanded by a mathematical formula called the chain rule:

$$\begin{aligned} \frac{d[B]}{dpH} &= \frac{d[B]}{d[H^+]} \cdot \frac{d[H^+]}{dpH} = \left(-\frac{K_a \cdot C_B}{(K_a + [H^+])^2} \right) \cdot (-\ln(10) \cdot [H^+]) \\ &= \ln(10) \cdot C_B \cdot \frac{K_a \cdot [H^+]}{(K_a + [H^+])^2} = \ln(10) \cdot C_B \cdot \frac{10^{pK_a - pH}}{(1 + 10^{pK_a - pH})^2} \end{aligned} \quad (\text{Eq 5})$$

Note that $dpH/d[H^+]$ is the first derivative of $-\log_{10}([H^+])$. Eq 5 shows that peak buffering capacity is attained when medium pH aligns with the buffer's pK_a . Under these circumstances, the buffer's protonated and unprotonated forms are equal in concentration (Eq 1).

In contrast to closed buffer systems, CO_2/HCO_3^- is considered an open system because its components can be biologically regulated. For media inside a CO_2 incubator, the concentration of the acidic form of the buffer (rather than the sum of the acidic and basic forms) is constant. Adapting this to Eq 4,

$$K_a = \frac{[H^+] \cdot [HCO_3^-]}{[CO_2]}$$

$$[HCO_3^-] = \frac{K_a \cdot [CO_2]}{[H^+]}$$

$$\frac{d[HCO_3^-]}{d[H^+]} = -\frac{K_a \cdot [CO_2]}{([H^+])^2}$$

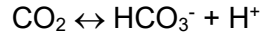
$$\frac{d[HCO_3^-]}{dpH} = \ln(10) \cdot \frac{K_a \cdot [CO_2]}{[H^+]} = \ln(10) \cdot [HCO_3^-]$$

Henderson-Hasselbalch correction

According to the Henderson-Hasselbalch equation, the concentration of HCO_3^- required to attain a given target pH ($\text{pH}_{\text{target}}$) at 5% CO_2 (1.2 mM) is:

$$[\text{HCO}_3^-] = 1.2 \times 10^{\text{pH}_{\text{target}} - 6.15}$$

When a medium containing a salt of HCO_3^- and intrinsic buffers (e.g. serum proteins) is placed in a 5% CO_2 incubator, the CO_2 hydration reaction proceeds until equilibrium is reached:



Intrinsic buffers will chelate some of the H^+ ions, allowing the reaction to proceed towards a combination of higher $[\text{HCO}_3^-]$ and lower $[\text{H}^+]$. Consequently, the standard Henderson-Hasselbalch equation will underestimate the pH of solutions prepared with low $[\text{HCO}_3^-]$. The necessary correction can be derived mathematically by solving a system of differential equations:

$$\frac{d\text{pH}}{dt} = - \frac{k_h \cdot [\text{CO}_2] - k_r \cdot [\text{HCO}_3^-] \cdot [\text{H}^+]}{\beta_{\text{intrinsic}}}$$

$$\frac{d[\text{HCO}_3^-]}{dt} = k_h \cdot [\text{CO}_2] - k_r \cdot [\text{HCO}_3^-] \cdot [\text{H}^+]$$

Here, k_h is the CO_2 hydration constant (0.19 s^{-1}), k_r is the rate constant of the reverse reaction (equal to K_a/k_h) and $[\text{CO}_2]$ takes a constant value of 1.2 mM in a 5% CO_2 incubator. Intrinsic buffering capacity is given by a constant $\beta_{\text{intrinsic}}$. The equation can be solved for a given starting $[\text{HCO}_3^-]$ to obtain a steady-state relationship (i.e. $d\text{pH}/dt = d[\text{HCO}_3^-]/dt = 0$) between added $[\text{HCO}_3^-]$ and pH at 5% CO_2 . Assuming serum has a pH of 7.4, it can be shown that the correction to starting $[\text{HCO}_3^-]$ is:

$$\Delta[\text{HCO}_3^-] = \beta_{\text{intrinsic}} \times (\text{pH}_{\text{target}} - 7.4)$$

Thus, $[\text{HCO}_3^-]$ required to produce a medium at $\text{pH}_{\text{target}}$ in 5% CO_2 is given by:

$$[\text{HCO}_3^-] = 1.2 \times 10^{\text{pH}_{\text{target}} - 6.15} + \beta_{\text{intrinsic}} \times (\text{pH}_{\text{target}} - 7.4) \quad (\text{Eq 6})$$

Sera used in this study produced a $\beta_{\text{intrinsic}}$ of 1.11 mM pH^{-1} (**Fig 1C**), determined by best-fit, but will likely vary depending on the type and concentration of serum used.