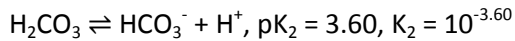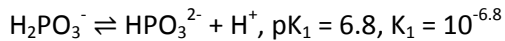# pH Buffering and the Bicarbonate System

The major buffers in plasma are the bicarbonate system and inorganic phosphate. The important dissociation reactions in the regime of physiological pH = 7.4 are:

$H_2PO_3^- \rightleftharpoons HPO_3^{2-} + H^+$, $pK_1 = 6.8$, $K_1 = 10^{-6.8}$

$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$, $pK_2 = 3.60$, $K_2 = 10^{-3.60}$

Thus, at pH = 7.4, the majority of the phosphate will be in the HPO_3^{2-} form, and nearly 100% of the bicarbonate will be in the unprotonated HCO_3^- form.

Bicarbonate is formed via the carbonic anhydrase reaction

$CO_2 + H_2O \rightleftharpoons H_2CO_3$, $K_{eq} = 2.7 \times 10^{-3}$.
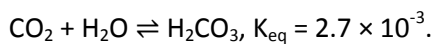
Although the equilibrium constant for this reaction is very low, the relatively small amount of H_2CO_3 formed will be associated with a much higher amount of HCO_3^-, given the dissociation reaction detailed above. To see how these systems work in the body, consider the following problems.

(a.) At a normal pH of plasma is 7.4, and a total carbon dioxide concentration $[\Sigma CO_2] = [CO_2] + [H_2CO_3] + [HCO_3^-]$ = 30 mM, and assuming that the carbonic anhydrase reaction is in equilibrium, what is the free concentration of carbon dioxide in the plasma?

To solve this problem, express the above sum for total carbon dioxide concentration $[\Sigma CO_2]$ in terms of the unknown $[CO_2]$ and the known pH:

$$[\Sigma CO_2] = [CO_2] + K_{eq}[CO_2] + \frac{K_2[H_2CO_3]}{[H^+]},$$

or

$$[\Sigma CO_2] = [CO_2] + K_{eq}[CO_2] + \frac{K_2 K_{eq}[CO_2]}{[H^+]} = [CO_2]\left(1 + K_{eq} + \frac{K_2 K_{eq}}{[H^+]}\right).$$

We can solve this problem in MATLAB using the following commands:

```
% Input constants
K1 = 10^(-6.8);
K2 = 10^(-3.60);
Keq = 2.7e-3;
pH = 7.4;
H = 10^(-pH); % H+ activity
SCO2 = 30e-3; % total CO2 (M)
% Computed aqueous CO2
CO2 = SCO2 / (1 + Keq + K2*Keq./H )
```

These calculations yield $[CO_2]$ = 1.66 mM, meaning about 94% of the ΣCO_2 is in the form of bicarbonate.

(b.) In Chapter 2 of the Silverthorn textbook [1], Question 22 asks, "What effect does hard work by your muscles have on the pH of the blood?" Here, we examine that question quantitatively in terms of the bicarbonate

system. Assume the concentrations from the above problem as an initial condition, with the addition of 1.5 mM total inorganic phosphate in the plasma. How does pH change as additional CO_2 is injected into the system?

Your solution will require simultaneously solving three conservation relationships. The first is for total phosphate

$$P_0 = [H_2PO_3^-] + [HPO_3^{2-}].\tag{1}$$

The second is for total carbon dioxide

$$[\Sigma CO_2] = [\Sigma CO_2]_0 + \Delta CO_2 = [CO_2] + [H_2CO_3] + [HCO_3^-],\tag{2}$$

where ΔCO_2 is the additional CO_2 injected into the system.

The third conservation relationship is for total exchangeable hydrogen ion, which is the sum of free plus bound hydrogen ions:

$$H = [H^+] + [H_2PO_3^-] + [H_2CO_3],\tag{3}$$

Where the value of H must be obtained based on the amount of exchangeable hydrogen ion created stoichiometrically by the carbonic anhydrase reaction.

The first step is to express these three conservation expressions in terms of three unknowns $[H^+]$, $[CO_2]$, and $[HPO_3^{2-}]$.

The value of H in the third equation is obtained based on the initial concentrations plus the turnover of the carbonic anhydrase reaction:

$$H = [H^+]_0 + [H_2PO_3^-]_0 + [H_2CO_3]_0 + \Delta H_{CA} = [H^+] + [H_2PO_3^-] + [H_2CO_3],$$

where ΔH_{CA} is obtained from the initial and final bicarbonate concentrations:

$$\Delta H_{CA} = [HCO_3^-] + [H_2CO_3] - ([HCO_3^-]_0 + [H_2CO_3]_0).$$

(In other words, what the above equation does is count how many times the CA reaction has turned over. There is a hydrogen ion generated every time that reaction turns over.) Substitution yields

$$[H^+]_0 + [H_2PO_3^-]_0 - [HCO_3^-]_0 = [H^+] + [H_2PO_3^-] - [HCO_3^-]$$

for the final conservation relation. Expressing the conservation relationships in terms of unknowns $[H^+]$, $[CO_2]$, and $[HPO_3^{2-}]$, we get

$$P_0 = [HPO_3^{2-}]\left(1 + \frac{[H^+]}{K_1}\right),$$

$$[\Sigma CO_2]_0 + \Delta CO_2 = [CO_2]\left(1 + K_{eq} + \frac{K_2 K_{eq}}{[H^+]}\right), \text{ and}$$

$$[H^+]_0 + [H_2PO_3^-]_0 - [HCO_3^-]_0 = [H^+] + \frac{P_0}{\left(1 + \frac{[H^+]}{K_1}\right)} - [CO_2]\frac{K_2 K_{eq}}{[H^+]}.\tag{4}$$

Here we have three equations and three unknowns. In MATLAB we can solve systems like this using the function fsolve. The first step is to write a function that computes the three equations as a function of the three variables:

```matlab
function f = conserve3(x,delta_CO2,Po,SCO2o,Ho,H2PO3o,HCO3o)
% Chemical constants.
K1 = 10^(-6.8);
K2 = 10^(-3.60);
Keq = 2.7e-3;
% The three unknowns
HPO3 = x(1);
CO2 = x(2);
H = x(3);
% Conservation equations (should equal 0)
f(1,:) = Po - HPO3*(1 + H/K1);
f(2,:) = SCO2o + delta_CO2 - CO2*(1 + Keq + K2*Keq/H);
f(3,:) = Ho + H2PO3o - HCO3o - H - Po*(H/K1)/(1+H/K1) + CO2*K2*Keq/H;
```

Notice that the output of the function, f, is a vector that is equal to 0 when Equation (4) is satisfied. In addition to the vector of unknowns, x, the function accepts as inputs the injected CO_2, the total phosphate, initial total CO_2, initial hydrogen ion activity, initial protonated phosphate, and initial protonated bicarb: `delta_CO2, Po, SCO2o, Ho, H2PO3o, HCO3o`.

To call the fsolve function and compute the equilibrium concentration, we use the following syntax

```matlab
delta_CO2 = +10e-3;
x = fsolve(@conserve3,x,[],delta_CO2,Po,SCO2o,Ho,H2PO3o,HCO3o);
pH = -log10(x(3))
```

This calculation yields a pH of approximately 6.6, illustrating that injection of CO_2 causes a drop in pH. (What happens to the predicted drop in pH if the total phosphate concentration is increased? You should find that increasing the phosphate concentration results in smaller changes in pH for a given amount of CO_2 injected.)

To make a plot of pH versus CO_2 injected we can use a simple loop:

```matlab
for i = 1:25
  delta_CO2(i) = (i-1)*1e-3;
  x = fsolve(@conserve3,x,[],delta_CO2(i),Po,SCO2o,Ho,H2PO3o,HCO3o);
  pH(i) = -log10(x(3));
end
plot(delta_CO2*1e3,pH); xlabel('\Delta CO_2 (mM)'); ylabel('pH');
```