Biologic buffers and pH control

A buffer solution counteracts pH variations due to adding moderate amounts of acids or bases

A buffer is made of:

A weak acid and its conjugate base
(e.g. acetic acid and sodium acetate)

A weak base and its conjugate acid
(e.g. ammonia and ammonium chloride)

$$pH = pK_A + \log \frac{[C_S]}{[C_A]}$$

- pH control

Buffer systems

- CO_2 (P_{CO2}) control

Respiration

- HCO_3^- control

Kidney filtering

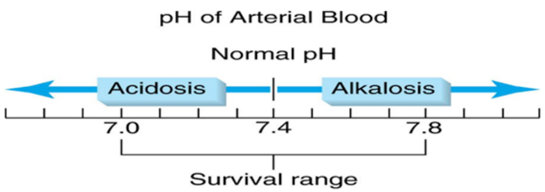
Intracellular and extracellular pH

Arteria blood pH = 7.40

Venous blood pH = 7.35 (per la CO_2 rilasciata dai tessuti)

pH > 7.40 o < 7.35 pathological condition acidosis or alkalosis

Intracellular pH ~ 7.2 (cell metabolism leads to production of acids)



Concentration of H^+

About 50-100 m moles of H^+ are released daily due to metabolism

[H^+] is maintained between 35 and 45 nano mol\L. (40nmol/L=pH 7.4)

Daily CO_2 production

About 0.7 Kg di CO_2, (16 moles), 400 liters at 37 °C

Mechanisms of pH control

1) Buffer solution: instantaneous absorption of H^+

2) Control of respiration: regulation of P_{CO2} and therefore of carbonic acid concentration (H_2CO_3)

Fast response

3) Kidney activity: urinary elimination of bicarbonate (HCO_3^-)

Slow response

Buffers present in plasma: rapid H+ elimination

A) Bicarbonate buffer → highest concentration

B) Phosphate buffer → optimal pK_a

C) Protein buffering power → intra and extracellular

Bicarbonate buffer

Bicarbonate (HCO_3^-) is the conjugate base of carbonic acid (H_2CO_3).

$$CO_2\,(aq) + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+$$

Arterial blood:

$H_2CO_3 \rightarrow CO_2 + H_2O$

Venous blood:

$CO_2 + H_2O \rightarrow H_2CO_3$

Carbonic anhydrase greatly accelerates these reactions

Carbonic anhydrase plays a key role in pH adjustment and also in the balance of fluids in different parts of our body. In recent years, carbonic anhydrase inhibitors are used to treat glaucoma, to prevent the pressure build up that can damage the optic nerve.

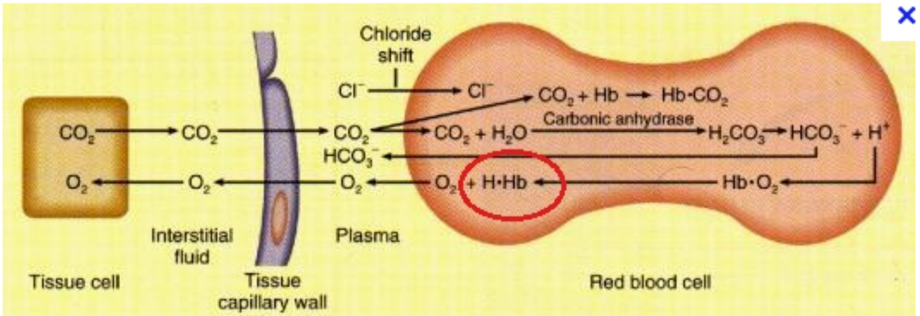
The carbonic acid bicrabonate is the main extracellular buffer system.

$$CO_2\,(gas) \overset{k}{\leftrightarrows} CO_2\,(aq) + H_2O \overset{K_{eq}}{\leftrightarrows} H_2CO_3 \overset{K_1}{\leftrightarrows} HCO_3^- + H^+$$

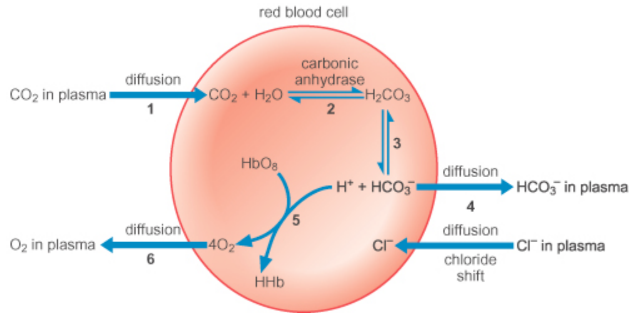
The carbonate ion (CO_3^{--}) is scarcely populated at physiological pH (pK_{a2} = 10.8)

H_2CO_3 is in equilibrium with CO_2 (P_{CO_2}), its pressure can be evaluated.

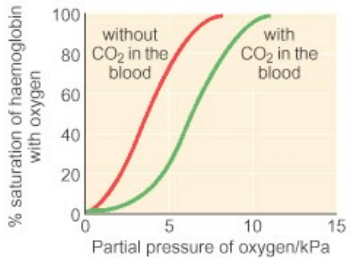
The carbonic acid bicrabonate is the main extracellular buffer system.



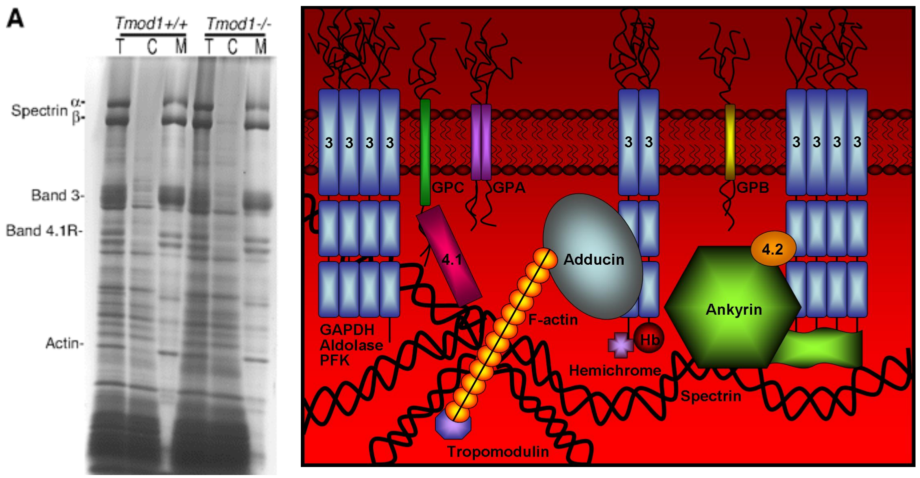
Chloride shift (exchange bicarbonate/chloride) maintains elecroneutrality across the celle membrane
(Band 3: anion exchanger)



Carbonic acid stimulates O_2 dissociation by hemoglobin due to the release of H^+



Band 3 is a membrane protein found in the RBC



$$\begin{array}{c} \qquad\qquad\qquad K_{eq} \qquad\quad K_{a1} \\ CO_2\ (gas) \leftrightarrows CO_2\ (aq) + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+ \end{array}$$

The equilibrium constant K_{eq} for the reaction $CO_2\ (aq) + H_2O \leftrightarrows H_2CO_3$

$$K_{eq} = \frac{[H_2CO_3]}{[CO_2]_{AQ}} = 5 \times 10^{-3}$$

The equilibrium constant K_{a1} for the reaction $H_2CO_3 \leftrightarrows HCO_3^- + H^+$

$$K_{a1} = \frac{[H^+] \times [HCO_3^-]}{[H_2CO_3]} = 1.58 \times 10^{-4}$$

CO_2 (gas) ⇆ CO_2 (aq) + H_2O ⇆ H_2CO_3 ⇆ HCO_3^- + H^+

The resulting constant (K'_{a1} between CO_2 in solution ($[CO_2]_{AQ}$), $[H^+]$ and $[HCO_3^-]$ is given by:

$$K'_{a1} = K_{eq} \times K_{a1} = \frac{[H_2CO_3]}{[CO_2]_{AQ}} \times \frac{[H^+] \times [HCO_3^-]}{[H_2CO_3]} = 7.9 \times 10^{-7}$$

$$pK'_{a1} = 6.1$$

We can consider that practically HCO_3^- e CO_2 in solution are the components of the buffer system, writing the equation in such terms

$$K'_{a1} = \frac{[H^+] \times [HCO_3^-]}{k \times P_{CO_2}}$$

$k \times P_{CO2} = [CO_2]_{AQ}$

If we solve for $[H^+]$ and consider the definition of pH, we obtain the Henderson-Hasselbach equation

$$pH = pK'_{a1} + \log \frac{[HCO_3^-]}{[CO_2]_{AQ}}$$

The pH of blood at 37 °C is equal to 7.4.
Form Henderson-Hasselbach

$$7.4 = 6.1 + \log \frac{[HCO_3^-]}{[CO_2]_{AQ}}$$

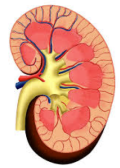
We can then determine the ratio base/acid in physiological conditions

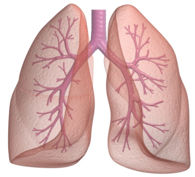
$$\log \frac{[HCO_3^-]}{[CO_2]_{AQ}} = 7.4 - 6.1 = 1.3$$

$$\Rightarrow \frac{[HCO_3^-]}{[CO_2]_{AQ}} = 20$$

The pH of blood does not depend on the concentrations of HCO_3^- e CO_2 but on their ratio

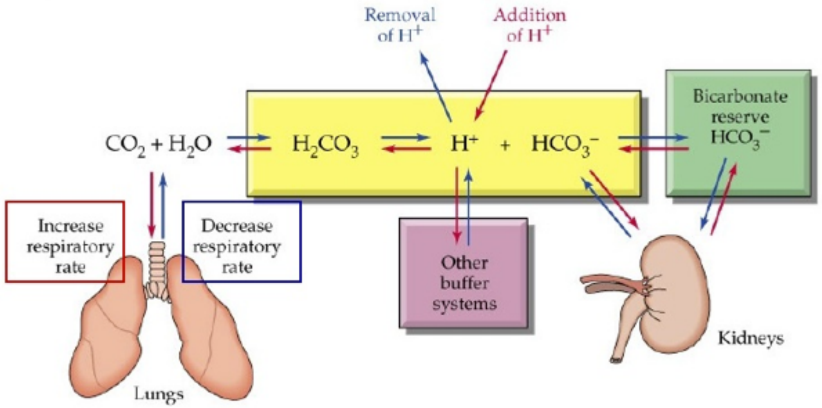
$$pH = pK'_{a1} + \log\frac{[HCO_3^-]}{[CO_2]_{AQ}}$$





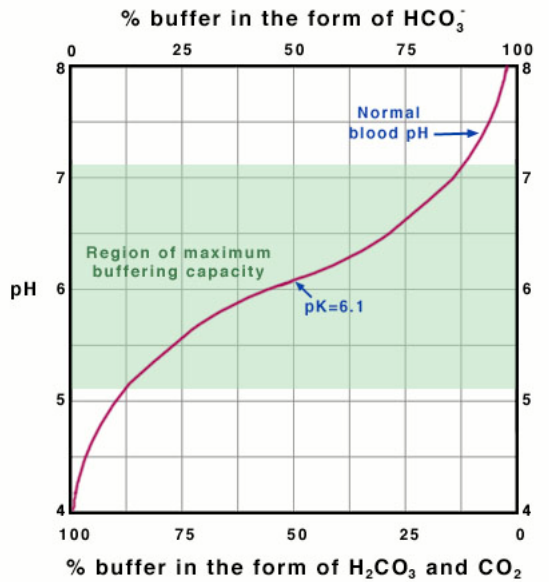
The concentration of HCO_3^- is controlled by kidney, and the CO_2 concentration depends on P_{CO2}, which is controlled by respiration.

The bicarbonate buffer is under the control of two systems that can indirectly control pH.


Removal of H+
Addition of H+
$CO_2 + H_2O$
H_2CO_3
H^+ + HCO_3^-
Bicarbonate reserve HCO_3^-
Increase respiratory rate
Decrease respiratory rate
Other buffer systems
Kidneys
Lungs

TITRATION CURVE OF BICARBONATE BUFFER

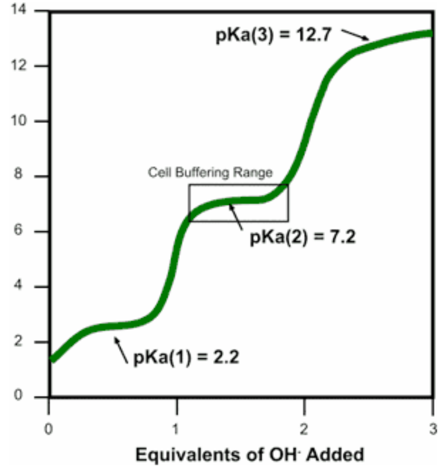
$$CO_2\ (gas) \leftrightarrows CO_2\ (aq) + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+$$



A pH = 7.4 other mechanisms are also acting to control the concentrations of HCO_3^- e CO_2 necessary to keep pH constant

Phosphate buffer (second dissociation) controls pH in the intracellular environment.

$$H_3PO_4 \overset{K_{a1}}{\leftrightarrows} H_2PO_4^{-} + H^+ \overset{K_{a2}}{\leftrightarrows} HPO_4^{--} + H^+ \overset{K_{a3}}{\leftrightarrows} PO_4^{---} + H^+$$



Titration curve of phosphoric acid.

The couple dihydrogen phosphate – hydrogen phosphate is relevant for intracellular pH control

$$\underset{acid}{} \; H_2PO_4^- \overset{K_{a2}}{\leftrightarrows} H^+ + \underset{base}{HPO_4^{--}} \qquad pK_{a2} = 7.2$$

$$7.4 = 7.2 + \log\frac{[HPO_4^{--}]}{[H_2PO^-{}_4]}$$

The ratio acid/base at physiological condition is therefore:

$$\log\frac{[HPO_4^{--}]}{[H_2PO^-{}_4]} = 7.4 - 7.2 = 0.2$$

$$\Rightarrow \quad \frac{[HPO_4^{--}]}{[H_2PO^-{}_4]} = 1.6$$

ALTERATIONS OF THE ACID BASE BALANCE

RESPIRATORY ACIDOSIS: the pH of the blood decreases due to a reduced respiration which leads to an increase of the dissolved CO_2 and then carbonic acid. (Asthma, emphysema, smoking)

METABOLIC ACIDOSIS: the blood pH decreases due to the increase of acidic substances in the blood (intense exercise, diabetes, nutrition).

RESPIRATORY ALKALOSIS: the pH increases due to hyperventilation that leads to excessive CO_2 elimination from the blood with the consequent decrease of H_2CO_3. (Fainting in case of hyperventilation slows breathing)

METABOLIC ALKALOSIS: the pH increases due to the release of alkaline substances in the blood. (Bicarbonate overuse, prolonged vomiting)

CO_2 (P_{CO2}) control through respiratory centers and lungs

The partial pressure of CO2 in plasma is normally 5.3 kPa (40 mmHg) and depends on the balance between the production through metabolism and elimination through the lungs.

CO_2 CONTROL

- The rate of breathing, and thus the CO2 elimination rate, is controlled by chemoreceptors of the respiratory center in the brain. The receptors respond to changes in [CO2] and [H +] of the plasma or cerebrospinal fluid.   IF

1 – P_{CO_2} exceeds 5.3 kpa.

2 - **pH decreases**.

Asthma, emphysema, pneumonia, smoking

→ The respiration rate increases.

CO_2 (gas) ⇆ CO_2 (aq) + H_2O ⇆ H_2CO_3 ⇆ HCO_3^- + H^+

CO_2 CONTROL

An increase of basic substances in the blood determines the increase of $[HCO_3^-]$, and therefore the ratio $[HCO_3^-] / [H_2CO_3]$ with a consequent **increase in pH** (ALKALOSIS).

➔ La velocità di respirazione diminuisce e aumenta la $[CO_2]$**.**

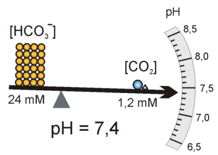
$$CO_2\ (gas) \leftrightarrows CO_2\ (aq) + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+$$

The buffer system CO2 / bicarbonate is efficient only in a open system because it is far from the maximum buffering power

In normal conditions

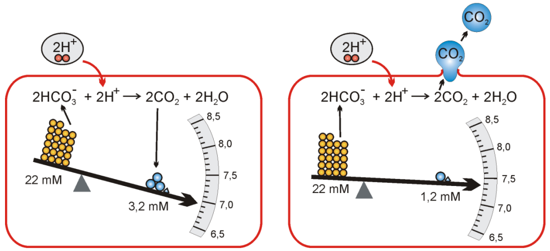
$$\frac{HCO_3^-}{CO_2} = \frac{20}{1}$$

$$\frac{[HCO_3^-]}{[CO_2]} = \frac{20}{1}$$



Increased acidity

$2H^+$

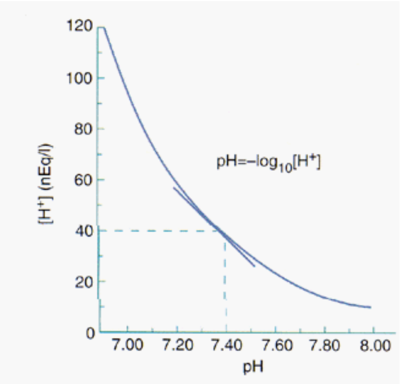


sistema “chiuso” pH = 6,93 sistema “aperto” pH = 7,36

An increase in the speed of the CO_2 exchange between capillary and alveolus is sufficient in an open system to partially compensate for the increase of H^+ (acidosis) or OH^- (alkalosis)

How do you measure the acid-base balance?

ABG (arterial blood gas): measurement of pH and of the concentrations of carbon dioxide and bicarbonate dissolved in arterial blood samples

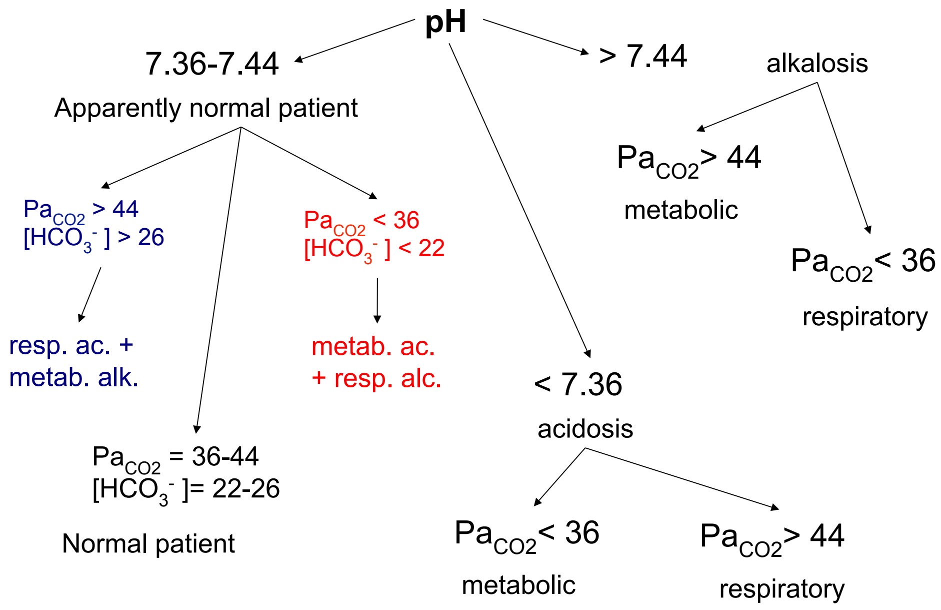


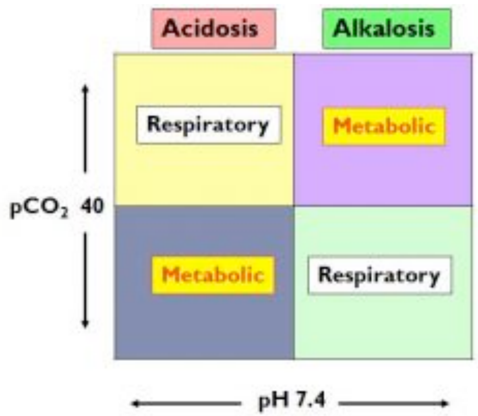
Physiological values

$[H^+]$ = 40 nmol/L (36-44 nM)
→ pH=7.4 (7.36-7.44)

P_{CO2} = 40 mmHg (36-44 mmHg)
$[HCO_3^-]$ = 24 mmol/L (22-26 mM)

ABG evaluation




Acidosis
Alkalosis
Respiratory
Metabolic
pCO2 40
Metabolic
Respiratory
pH 7.4

R.O.M.E.

Respiratory = Opposite
Metabolic = Equal

Respiratory = Opposite

pH is high, PCO2 is low ↑↓
(Alkalosis, Respiratory)

ph is low, PCO2 is high ↓↑
(Acidosis, Respiratory)

Metabolic = Equal

pH is high, HCO3 is high ↑↑
(Alkalosis, Metabolic)

ph is low, HCO3 is low ↓↓
(Acidosis Metabolic)