

BIOLOGICAL BUFFER SYSTEMS

**Gunanidhi Sahoo
Department of Zoology
Utkal University, Bhubaneswar, Odisha, PIN – 751 004**

- ❖ Almost every biological process is pH-dependent; a small change in pH produces a large change in the rate of the process.
- ❖ True both for reactions occurring **with or without H⁺ ions**.
- ❖ The enzymes and many of their substrates, contain **ionizable groups** with characteristic **pK_a** values.
- ❖ The **protonated amino and carboxyl groups of amino acids** and the **phosphate groups of nucleotides**, for example, function as weak acids; their ionic state is determined by the pH of the surrounding medium.
- ❖ Cells/ organisms maintain a specific and constant cytosolic pH, usually near **pH 7**, keeping biomolecules in their optimal ionic state.
- ❖ In multicellular organisms, the pH of **extracellular fluids** is also tightly regulated.
- ❖ Constancy of pH is achieved primarily by **biological buffers**.

Ionization/Dissociation of **Water**, **Weak Acids**, and **Weak Bases**

- Pure water ionizes slightly, forming equal numbers of hydrogen ions (H_3O^+) and OH^- ion.
- The extent of ionization is described by an **equilibrium constant**,
The **ion product of water** (K_w) is derived from K_{eq} .

$$K_{\text{eq}} = \frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]}$$

- **Weak acids** partially ionize to release a H^+ , and lowers the pH of the aqueous solution. **Weak bases** accept a H^+ and increases the pH.
- The **extent of these processes is characteristic of each particular weak acid or base** and is expressed as an acid dissociation constant:

$$K_{\text{eq}} = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = K_{\text{a}}$$

- The **pKa** expresses, on a logarithmic scale, the relative strength of a weak acid or base:

$$\text{p}K_{\text{a}} = \log \frac{1}{K_{\text{a}}} = -\log K_{\text{a}}$$

- The stronger the acid, the smaller its pKa; the stronger the base, the larger its pKa. The pKa can be determined experimentally; **it is the pH at the midpoint of the titration curve for the acid or base.**

The Henderson-Hasselbalch Equation

- Relates to the dissociation of weak acids in equilibrium.



$$\text{Dissociation constant } (K_a) = \frac{[\text{H}^+] + [\text{A}^-]}{[\text{HA}]}$$

$$[\text{H}^+] = \frac{K_a \times [\text{HA}]}{[\text{A}^-]}$$

Taking log on both the sides, $\log [\text{H}^+] = \log K_a + \log [\text{HA}]/[\text{A}^-]$

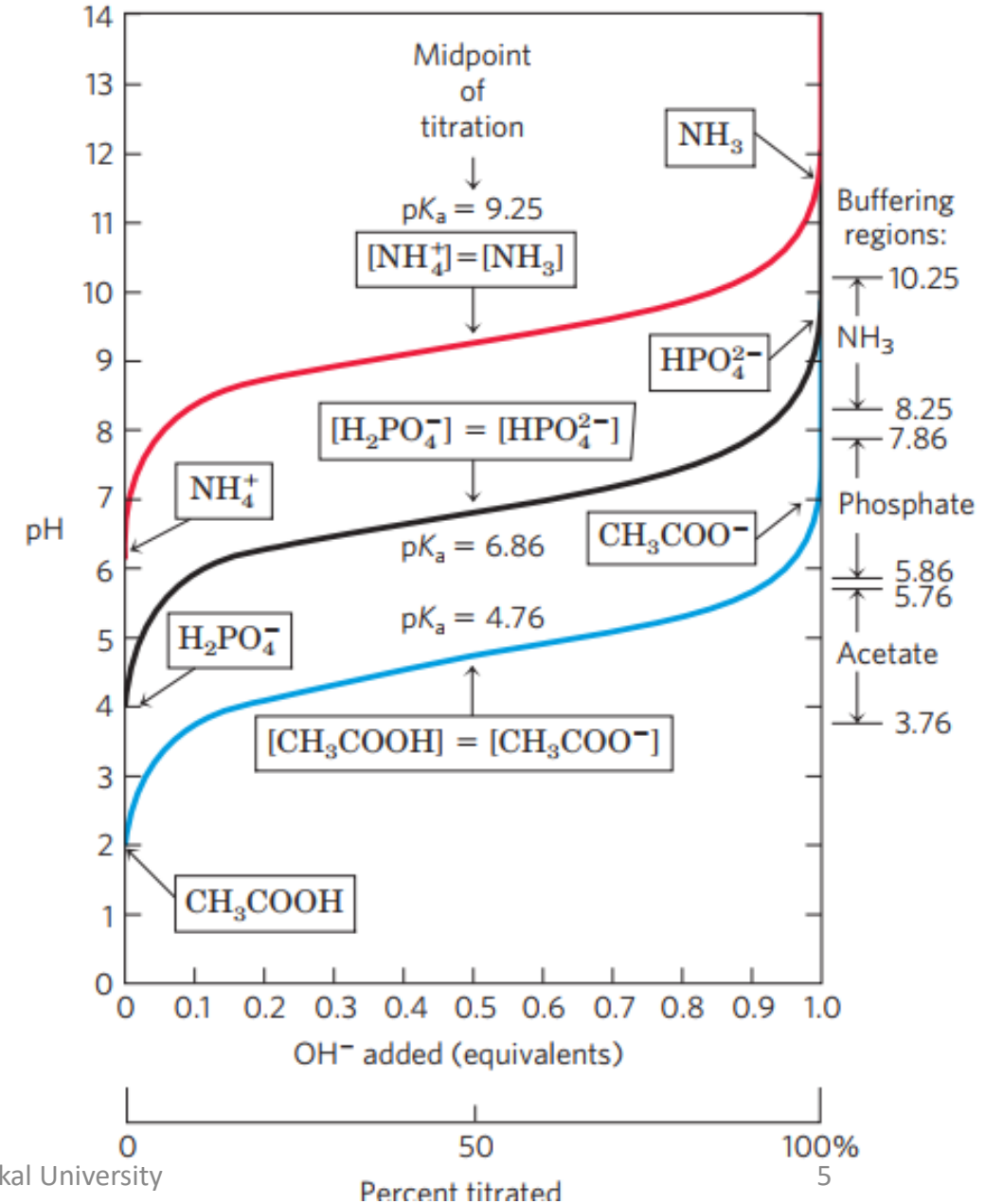
Changing signs on both the sides, $-\log [\text{H}^+] = -\log K_a - \log [\text{HA}]/[\text{A}^-]$

$$\text{pH} = \text{pk}_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad \text{or, } \text{pH} = \text{pk}_a + \log \frac{[\text{Proton acceptor}]}{[\text{Proton donor}]} \quad (\text{HH Equation})$$

This equation fits to the titration curve of all weak acids.

Titration curves reveal the Pka of weak acids

- Titration determines the amount of an acid in a given solution. A measured volume of the acid is titrated with a solution of a strong base, usually NaOH, of known concentration.
- A **titration curve** is the plot of the pH of the analyte solution vs. the volume of the titrant added as the titration progresses.
- A titration curve can be used to determine:
 1. The equivalence point of an acid-base reaction (the point at which the amounts of acid and of base are just sufficient to cause complete neutralization).
 2. The pH of the solution at equivalence point is dependent on the strength of the acid and strength of the base used in the titration.
- For strong acid-strong base titration, pH = 7 at equivalence point
- For weak acid-strong base titration, pH > 7 at equivalence point
- For strong acid-weak base titration, pH < 7 at equivalence point



- Comparison of the titration curves of three weak acids: Acetic acid, dihydrogen phosphate and ammonium ion.

Buffer

- Mixtures of weak acids and their salts of strong bases **OR** Mixtures of strong acids and their salts of weak bases.

Example: mixture of **acetic acid** and **sodium acetate**. $\text{CH}_3\text{COOH} + \text{CH}_3\text{COONa} \rightarrow \text{Na}^+ + \text{H}^+ + 2\text{CH}_3\text{COO}^-$

Resists change in pH on the addition of acid (H^+) or base (OH^-). **Weak acid: H^+ donor, Weak base: H^+ acceptor.**

Addition of alkali (NaOH) or acid (HCl): Salt is formed, but no free H^+ or OH^- will be available.



❖ **Two factors determine effectiveness/capacity of buffers:**

1. Molar conc. of buffer components: Directly proportional.
2. Relative conc. of the conjugate base and the weak acid.

Ideal buffer: Equal concentrations of acidic and basic components.

Biological Buffer systems

- Buffer systems in the human body are **extremely efficient**.
- **Different systems work at different rates.**
- Takes only **seconds** for the chemical buffers in the blood to make adjustments to pH. The respiratory tract can adjust the blood pH upward in minutes by exhaling CO₂ from the body. The renal system can also adjust blood pH through the excretion of H⁺ and the conservation of bicarbonate, but this process takes hours to days to have an effect.
- Principal buffers of **ECF**: Bicarbonate buffer, Protein buffer
ICF: Phosphate buffer, Protein buffer
RBC: Hemoglobin buffer

1. The Bicarbonate buffer system

- Principal buffer in blood plasma.
- Consists of H_2CO_3 (proton donor) and HCO_3^- (proton acceptor).
- Neutralizes stronger dietary acids and metabolic acids (HA) by converting them to weak bases (A^-) and increase in H_2CO_3 .

Strong base (B) \longrightarrow Weak acids (BH+) with rise in HCO_3^- .



$[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ of 20:1 is required to maintain pH of plasma at 7.4.

- Neutralization of any acid or base and subsequent change in buffer ratio or blood pH is neutralized by respiratory elimination of H_2CO_3 as CO_2 or urinary elimination of HCO_3^- .
- **As cells contain much lower amounts of HCO_3^- , imp of this buffer in cells is negligible.**

Acidosis

Accumulation of acids or loss of alkali \longrightarrow Fall of $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ below 20. **Ratio in favor of H_2CO_3 .**

Two types:

1. **Metabolic acidosis:** Conc. of plasma HCO_3^- decreased due to excessive loss of bases in renal failure, diabetic ketosis and severe diarrhea.
2. **Respiratory acidosis:** Retention of CO_2 due to hypoventilation \longrightarrow Rise in H_2CO_3 lowers the ratio. Occurs due to chronic respiratory obstructive airway disease (asthma), prolonged anesthesia, unconsciousness, etc.

Alkalosis

Accumulation of alkali or loss of acids \longrightarrow Increase in ratio \longrightarrow Rise in pH.

1. **Metabolic alkalosis:** High intake of alkaline substances, severe vomiting, indiscriminate use of antacids, etc.
2. **Respiratory alkalosis:** Excess removal of CO_2 from blood due to hyperventilation \longrightarrow Decrease in H_2CO_3 .

2. The Phosphate buffer system

- Consists of H_2PO_4^- (H^+ donor) and HPO_4^{2-} (H^+ acceptor).



- Acts in **cytoplasm of all cells. More effective in pH range of 5.8 to 7.8.**
- **pH of ICF = 6.0 – 6.9**, close to its pKa (6.86).
- Plasma $\text{HPO}_4^{2-} : \text{H}_2\text{PO}_4^-$ is 4.0. Hence, $\text{pH} = \text{pKa} + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$, **pH = 6.86 + log4 = 7.4**

If $\text{HPO}_4^{2-} : \text{H}_2\text{PO}_4^-$ tends to be changed by **more H_2PO_4^-** , **renal elimination of H_2PO_4^-** occurs and pH remains unchanged.

- Works exactly like acetate buffer system **except pH range.**
- Conc. of phosphate buffer very low in **plasma**, hence less effective.

3. The Protein buffer system

- Very imp in plasma and ICF, but conc. low in CSF, lymph and intestinal fluids.
- Proteins exist as anions serving as conjugate bases (Pr^-) at blood pH 7.4 and form conjugate acids (HPr) accepting H^+ .
- Have the capacity to buffer some H_2CO_3 in blood.



4. The Hemoglobin buffer system

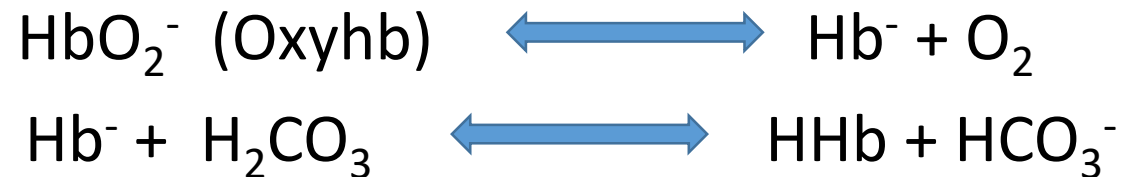
- Buffers CO₂ inside RBC, capacity depends on oxygenation/deoxygenation of Hb.



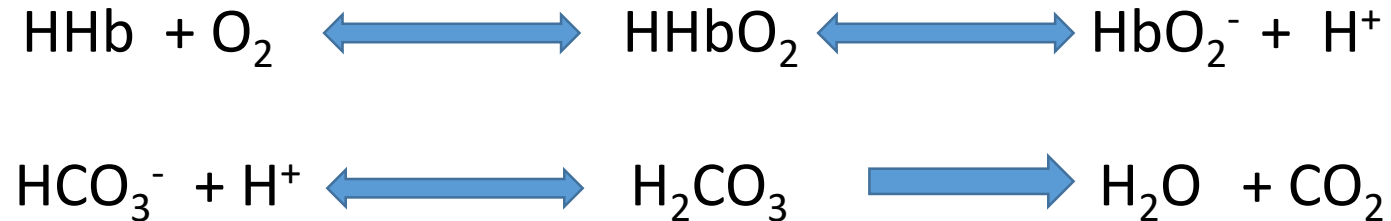
- At blood pH (7.4), $\text{H}_2\text{CO}_3 \longrightarrow \text{H}^+ + \text{HCO}_3^-$, needs instant buffering.

Oxyhemoglobin (HbO₂⁻) loses O₂ to form deoxyhb (Hb⁻) which remains undissociated (HHb) by accepting H⁺ from the ionization of H₂CO₃.

Thus, Hb⁻ buffers H₂CO₃ in RBC.

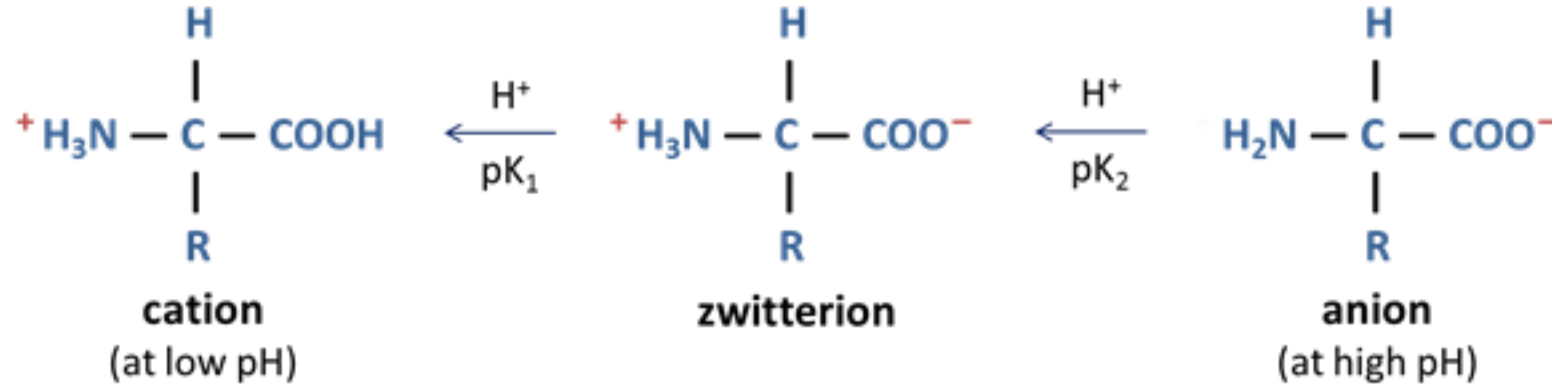


- ❖ Some HCO_3^- diffuse out into plasma and maintain the balance between ICF and plasma HCO_3^- . This causes influx of some Cl^- into RBC (**Chloride shift**).
- ❖ HHbO_2 , produced in lungs by oxygenation of HHb , instantly ionizes to H^+ and HbO_2^- .
- ❖ H^+ buffered by HCO_3^- in RBC forming H_2CO_3 which is dissociated into H_2O and CO_2 . CO_2 diffuses out of RBC and escapes into alveolar air.
- ❖ Some HCO_3^- return from plasma to RBC in exchange of Cl^- and changed to CO_2 .



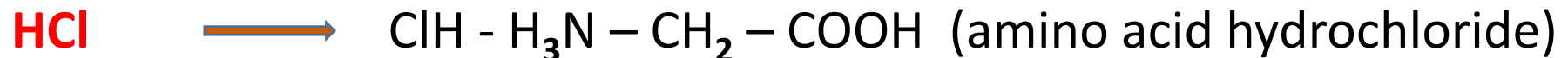
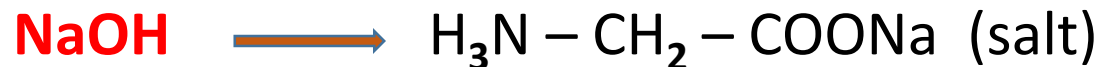
5. The Amino acid buffer system

- Contains both acidic (**COOH**) and basic (**-NH₂**) groups, H⁺ can pass between this two groups.
- **Zwitterion form:** Addition or removal of H⁺ to or from zwitterion produces either cation or anion.



- **Addition of: OH⁻** to a solution of aas, they take up H⁺ to form H₂O + anion.

H⁺, they are taken up by zwitterions to produce cationic form.



Thus, an aa solution is not neutral, but acidic or basic depending on the form in which it presents in greater concn.

Different aas used to buffer different pH values, a mixture has wider range.