Buffers, pH & Gastric Acid: An Overview

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How are acids and bases defined in biochemical systems?

• **Bronsted** and **Lowry** definition of Acids and Bases are the most convenient for the biochemical systems:
  - Conjugate Acid / Conjugate Bases concept

• **An acid is a Proton Donor;**

• **A base is a Proton Acceptor;**

• **Acid is always accompanied by Conjugate Base;**

• **Base is always accompanied by conjugate acid**
• Examples:
• Consider a weak acid: \( \text{HA} \), it dissociates thus:
  \[
  \text{HA} \xleftrightarrow{\text{dissociation}} \text{H}^+ + \text{A}^-
  \]
  • HA is undissociated acid,
  • \( \text{A}^- \) is its conjugate base; it is a proton acceptor,

• Consider \( \text{H}_2\text{O} \): It can act as a base and as an acid
• Water is Amphoteric thus:
  \[
  \text{H}_2\text{O} + \text{H}_2\text{O} \xleftrightarrow{\text{dissociation}} \text{H}_3\text{O}^+ + \text{OH}^-
  \]

• Can you identify the conjugate base and conjugate acid is this expression?
Dissociation Constant; “Apparent Dissociation Constant”

• Dissociation of weak acid can be expressed thus:

\[ HA \rightleftharpoons H^+ + A^- \]

• \( Ka \) (moles/litre) is Dissociation constant for weak acid;

• “Apparent dissociation constant” \( (Ka') \) is used in Biochemical and Clinical applications;

\[
\begin{align*}
Ka &= \frac{[A^-][H^+]}{[HA]} = Keq \\
Ka' &= \frac{[A^-][H^+]}{[HA]}
\end{align*}
\]
What do you understand by “Concentration” of Acid or Base?

• Concentration of an acid or base depends on amount (in grams) of Acid or Base in 1000 ml of solution;

• 0.5 M HCl is less concentrated than 2.0 M HCl solution;

• 0.5 M Acetic acid is less concentrated than 2.0 M Acetic acid solution;

• 0.5 M HCl solution and 0.5 M Acetic acid solution have the same concentration, but the HCl adds more H⁺ ions to the solution than the Acetic acid because HCl is a strong acid;

• Concentration is represented as [ ],
  • Example: [HCl] = 0.5 M
TAKE NOTE

• 1.0 molar (1.0 M) solution contains the molecular weight in grams of the compound in 1000ml of solution;

• 0.5 M NaCl solution will contain $0.5 \times 58.4\text{g}$ of NaCl in 1000ml of solution {Note: 58.4 is the mol wt of NaCl};

• 11.0mM solution of Glucose will contain $11 \times 180\text{mg}$ of Glucose in 1000ml of solution {Mol wt of Glucose = 180}
How is $K_a$ relates to $pK_a$ of a weak acid?

• Relative strengths of weak acids and weak bases are expressed quantitatively as their Apparent Dissociation Constants, which express their tendency to dissociate;

• By definition: $pK_a = -\log_{10}K_a$

• For weak acid: $pK_a$ is the pH at which the concentrations of Protonated and Unprotonated species are equal in solution $\{HA = A^-\}$;

• Tendency of a weak acid to dissociate can be evaluated from the $K_a$ or $pK_a$ value;
• Ka` is directly proportional to strength of the acid;

\[ \text{HA} \leftrightharpoons \text{H}^+ + \text{A}^- \]

\[ \text{Ka`} = \frac{[\text{A}][\text{H}^+]}{[\text{HA}]} \]

• The **smaller** the Ka` value, the lower the tendency of the acid to dissociate and the **weaker the acid**;

• The **larger** the Ka` value the higher the tendency of the acid to dissociate and the **stronger the acid**
• The smaller the $K_a^+$, the larger the $pK_a$;
  \[ pK_a = -\log K_a^+ \]

• $pK_a$ is inversely related to strength of the acid;
• The larger the $pK_a$ the weaker the acid;
• The smaller the $pK_a$ the stronger the acid;
Some relevant weak acids and their conjugate bases

<table>
<thead>
<tr>
<th>Acid</th>
<th>Conjugate base</th>
<th>pKa`</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium ion (NH₄⁺)</td>
<td>Ammonia (NH₃)</td>
<td>9.25</td>
</tr>
<tr>
<td>Carbonic acid (H₂CO₃)</td>
<td>Bicarbonate ion (HCO₃⁻)</td>
<td>6.37</td>
</tr>
<tr>
<td>Dihydrogen phosphate ion (H₂PO₄⁻)</td>
<td>Monohydrogen phosphate ion (HPO₃²⁻)</td>
<td>6.86</td>
</tr>
<tr>
<td>Lactic acid (CH₃CHOHCOOH)</td>
<td>Lactate ion (CH₃CHOHCOO⁻)</td>
<td>3.86</td>
</tr>
</tbody>
</table>
Handerson-Hasselbalch equation is the relationship between pH of a solution containing a weak acid and the $K_a$ (or $pK_a$) of the acid;

Equation for a weak acid can be expressed as follows:
What are some uses of Henderson-Hasselbalch equation?

• Preparation of buffer solutions of known pH
• Calculating the pH of biochemical solutions;
• Representation of Acid – Base balance during metabolism;
• Helps to predict the effects of various alterations in Acid – Base balance;
What is a Buffer Solution?

• Buffer solution is a solution that resists change in pH when small amounts of acid or base are added.

• Two main types of buffer solutions:
  • **Acidic buffer** solution:
    • Made up of a weak acid and salt of the weak acid;
  • **Basic buffer** solution:
    • Made up of a weak base and salt the weak base;
What are the major buffers in the metabolic system?

• Major buffers with Conjugate Acid/Conjugate Base pairs:

• Bicarbonate buffer system: \( \text{H}_2\text{CO}_3 / \text{HCO}_3^- \);
  • NB: actual value of \([\text{H}_2\text{CO}_3] = \{[\text{H}_2\text{CO}_3] + [\text{CO}_2\text{ dissolved}]\}\)

• Haemoglobin buffer system: \( \text{HHb} / \text{Hb}^- \)

• Oxyhaemoglobin buffer system: \( \text{HHbO}_2 / \text{HbO}_2^- \)

• Phosphate buffer system: \( \text{H}_2\text{PO}_4^- / \text{HPO}_4^{2-} \)

• Protein buffer system: \( \text{RCOOH} (\text{NH}_3^+) / \text{RNH}_2 (\text{COO}^-) \)
• In RBC: main buffer systems are:
  • Haemoglobin buffer,
  • Oxyhaemoglobin buffer,
  • Bicarbonate buffer;

• Blood plasma: main buffer systems are:
  • Bicarbonate buffer,
  • Protein buffer,
  • Phosphate buffer;
How is the Bicarbonate Buffer system represented?

• An expression for Bicarbonate buffer system in blood is:

Carbonic Anhydrase

\[ \text{CO}_2(\text{gas}) \leftrightarrow \text{CO}_2(\text{diss}) + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \]

• This expression can be separated into two:

• Carbonic Anhydrase (Zn activator) catalyses reaction:

\[ \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \]

• Carbonic Acid rapidly dissociates thus:

\[ \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \]

• Combining the two expressions give:

\[ \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^- \]
• Equilibrium expression can be written as:

• CO$_2$ can be written as $P_{co_2}$ (Partial Pressure CO$_2$ in solution)

• Concentration of dissolved CO$_2$ (meqL$^{-1}$) is obtained by multiplying $P_{co_2}$ by factor $\alpha$;

• Thus, $\alpha P_{co_2} = $ meq L$^{-1}$

• $\alpha = 0.03$meq L$^{-1}$ mmHg$^{-1}$ at 37°C
• Substituting for $[\text{CO}_2]$ in the equilibrium expression gives

$$K_a' = \frac{[\text{H}^+][\text{HCO}_3^-]}{0.03 \text{ Pco}_2}$$

• Henderson – Hasselbalch equation for Bicarbonate buffer system becomes

$$\text{pH} = 6.1 + \log_{10} \frac{[\text{HCO}_3^-]}{0.03 \text{ Pco}_2}$$

• Note: in this equation $[\text{HCO}_3^-]$ is expressed in meq L$^{-1}$
GASTROINTESTINAL ACID PRODUCTION (GAP)

- **H⁺ ions** or **HCO₃⁻ ions** are secreted into the gut lumen;
- Example:
  - **H⁺ ions** are secreted in the Stomach,
  - **HCO₃⁻ ions** are secreted in the Colon,

- Production of **HCO₃⁻ ions** and **H⁺ ions** inside Gut mucosal cells occur via the same net reaction:

\[
\text{CO}_2 + \text{H}_2\text{O} \xleftrightarrow{=} \text{HCO}_3^- + \text{H}^+
\]

Let us consider two different cases: **Fig. 1 & Fig. 2**
When \( \text{H}^+ \) ions are secreted into the Gut Lumen, \( \text{HCO}_3^- \) ions are also produced (Fig. 1)
When $\text{HCO}_3^-$ ions are secreted into the Gut Lumen $\text{H}^+$ ions are also produced (Fig. 2)
• In Fig. 1, the $H^+$ ions produced do not stay in the Gut lumen because they are not needed;

• In Fig. 2, the $HCO_3^-$ ions produced also do not stay in the Gut lumen because they are not needed;

• WHAT THEN HAPPENS TO THESE IONS IN BOTH CASES?
• Both ions are moved in the direction opposite to the Gut lumen;

• That is, into the Interstitial Fluid and Blood as shown in the respective figures; (Fig. 1 and 2);
  • If an organ secretes $H^+$ ions into the Gut Lumen, it must secretes $HCO_3^-$ ions into the Blood;
  • If it secretes $HCO_3^-$ ions into the Gut Lumen, it must secretes $H^+$ ions into the Blood;

• Effects on Blood and Gut Lumen are equal and opposite because a One-to-one ratio of $H^+$ ion and $HCO_3^-$ ion are produced by each reaction;
What happens in different segments in the GIT?

STOMACH:

• Under fasting (basal) conditions: Parietal cells secrete $\text{H}^+$ ions into Gastric lumen at about 10mM per hour;

• Following meals (Postprandial): Rate of secretion can reach as much as 50mM per hour;
  • Secretion of $\text{H}^+$ ions lowers pH to 1.0 in Chyme;

• Stomach releases $\text{HCO}_3^-$ in Blood at rest and during meals

• Resulting in very slight Postprandial Rise in $[\text{HCO}_3^-]$ in plasma, which often cause, after a delay, Renal Spillage of $\text{HCO}_3^-$ ions;

• Rise in $[\text{HCO}_3^-]$ that occurs in urine after a meal is called the “Alkaline tide”;


DUODENUM AND ASSOCIATED ORGANS

- HCO$_3^-$ are secreted into Duodenal Chyme from 3 sources:
  - Pancreas,
  - Gall Bladder,
  - Duodenal Mucosa,

- [HCO$_3^-$] in Pancreatic Fluid is about 25mM (Basal) and 150mM (Postprandial);

- In 24 hours, [HCO$_3^-$] Pancreatic secretion is about 200mM

- [HCO$_3^-$] in Bile from Gall Bladder is about 40mM;

- Duodenal Mucosa also generates and secretes HCO$_3^-$ ions;

- In all cases equi-molar amount of [H$^+$] is release in blood
JEJUNUM, ILEUM, and COLON

• Jejunum secretes small quantities of $H^+$ ions into the Gut lumen, thereby Alkalinising the Blood;
• Ileum secretes $HCO_3^-$ ions, thus Acidifying the Blood;
• Colon secretes over 200mM $HCO_3^-$ ions per day into Lumen; thus has a major Acidifying effect on blood;
GENERAL CONCEPT

General view is as follows:

• Stomach secretes:
  • $H^+$ ions into Gut lumen, thus Acidifying its contents,
  • $HCO_3^-$ ions into the Blood, thus Alkalinising it;

• Almost all the segments below the Pylorus have the opposite effect: They secrete
  • $HCO_3^-$ ions into the Gut lumen, Alkalinising it;
  • $H^+$ ions into the blood, thus Acidifying it;
Simplified mechanism for Production of Gastric Acid

- Parietal (Oxyntic) cells are major source of Gastric HCl
- Source of H$^+$ is Carbonic Anhydrase formation of H$_2$CO$_3$ from H$_2$O and CO$_2$ (Fig. 4)
- Alkaline urine follows Ingestion of Meals (‘‘Alkaline tide’’), as a result of formation of HCO$_3^-$ ions in the process of HCl secretion;
- Secretion of H$^+$ into lumen is an active process driven by a membrane-located H$^+$- K$^+$ ATPase;
- Parietal cells contain numerous Mitochondria needed to generate ATP used for H$^+$ - K$^+$ ATPase to function;
- HCO$_3^-$ ions pass into Plasma in exchange for Cl$^-$, which is coupled to secretion of H$^+$ into lumen;
Fig. 4: Production of Gastric Acid (HCl) in Parietal cell using H\(^+\) - K\(^+\) ATPase (H\(^+\) - K\(^+\) – Pump)
What are some of the effects of Gastric acid?

• Gastric acid caused proteins to denature, making them more accessible to the action of Proteases;
• Low pH has the effect of destroying most microorganisms entering the GIT;
• Some clinical conditions may arise from defects in digestive processes, such as ulceration by Gastric HCl or diminished secretion of HCl causing **Achlorhydria**;
• Parietal cells may secrete HCl at concentration of 160 mM (equivalent to pH of 0.8);
• Despite the high acidity the epithelium of the stomach is intrinsically resistant to damage by Gastric acid;
• Excessive secretion of Gastric acid may lead to Gastritis, Gastric ulcers and Peptic acid disease;
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