

UNIVERSITY OF PNG
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL Seminar
WATER, pH and BUFFER SOLUTIONS: Overview

Structure of Water (H₂O)

Brief describe the structure of water.

- o Molecule of water is written as H₂O
- o Oxygen atom is more electronegative than the Hydrogen atom
- o The bond angle in a molecule H₂O is about 104.5°
- o Distribution of electrons within the molecule of water is such that the portion of the molecule near the Oxygen atom is slightly negative, and the portion near the Hydrogen atom is slightly positive
- o Such a molecule is called a Dipole and is said to have a Dipole moment.
- o Water molecules interact with each other because the partial positively charged Hydrogen atom on one H₂O molecule is attracted to the negatively charged Oxygen atom on another water molecule
- o Forming a weak bond (called Hydrogen bond) between the two water molecules
- o The interaction results in the Tetrahedral structure of H₂O molecules
- o Five H₂O molecules form the tetrahedral structure of water (i.e., association of a central water molecule with four other water molecules by hydrogen bonding)
- o The tetrahedral structure is typical of Ice and to a lesser extent of Liquid water
- o Each hydrogen bond is relatively weak compared to a covalent bond, but the large number of hydrogen bonds between water molecules in liquid water is the reason for the stability of water

{Take note: Why is water liquid at room temperature, but Ammonia is gas at room temperature? What is Hydrogen bond?}

Water as a solvent:

Why is water a good solvent for most compounds?

- o The ability of water to serve as a solvent for Ions and some Organic and Bioorganic molecules is because of its dipolar nature and its ability to form Hydrogen bonds
- o Molecules that are capable of forming Hydrogen bonds can do so with H₂O
- o These molecules contain charges or dipoles
- o Their charges or Dipoles interact with positive or negative ions in H₂O
- o In aqueous solution these molecules are surrounded by several molecules of water, thus they become soluble (or dissolve) in water
- o Many Inorganic Ionic compounds dissolve in water and exist as separate ions surrounded by water; e.g. NaCl dissolves in water as Na⁺ and Cl⁻ ions
- o Biomolecules containing non-ionic but weakly Polar groups dissolve in H₂O because of the attraction of the polar groups to water molecules
- o Sugars and Alcohols are readily soluble in water for this reason

- o Non-polar (or Apolar) groups such as those present in Hydrocarbons do not form Hydrogen bonds therefore they are not soluble in water (i.e., they do not dissolve in water). They are said to be Hydrophobic!!!

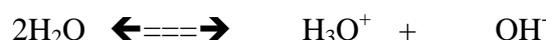
{Take note: Explain why non-polar (or Hydrophobic) molecules added to water usually form spherical droplets with minimum water-exposed surface (e.g., oil drops in water)?}

Dissociation of water:

- o Water molecules have a limited tendency to reversibly dissociate (i.e., ionize) into a **Hydrogen ion** or **Proton (H⁺)** and a **Hydroxyl ion (OH⁻)**.



- o An alternative description of this dissociation process is:



(Where H₃O⁺ is referred to as Hydronium ion).

- o The tendency of water to dissociate is given by the expression:

$$\mathbf{K} = \frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]} \quad \text{Expression 1.}$$

- o Where the terms in **square brackets []** represent **Molar concentrations** of Hydrogen ions, Hydroxyl ions and undissociated water molecules at equilibrium.
- o K represents the equilibrium constant or the dissociation constant of water. **(It is important at this point to make the assumption that at the Temperature of 25°C, the value of K is about 1.8 x 10⁻¹⁶ mol/L.)**

- o We can however calculate the Molar concentration (mol/L. or M) of Pure water.
- o Thus, One liter (1000g) of pure water (molecular weight = 18) contains 1000/18 = 55.56M.
- o This high Molar concentration (55.56M) of water is not significantly affected by dissociation, therefore it can be assumed to be constant.

Thus we can rewrite Expression 1 above as:

$$\mathbf{K} [\text{H}_2\text{O}] = [\text{H}^+][\text{OH}^-] \quad \text{Expression 2.}$$

As stated above K is a constant and [H₂O] is also a constant, therefore K [H₂O] represents a new constant, which is called the ionic product of water, K_w.

$$\text{Thus: } \mathbf{K_w} = \mathbf{K} [\text{H}_2\text{O}] = [\text{H}^+][\text{OH}^-] \quad \text{Expression 3}$$

At 25°C the value of K = 1.8 x 10⁻¹⁶ mol/L, and

**By calculation the value of [H₂O] = 55.56M. Since K_w = K [H₂O]
We can calculate the value of K_w as follows:**

$$\mathbf{K_w} = \mathbf{1.8 \times 10^{-16} \text{ mol/L} \times 55.56 \text{ mol/L} = 1.00 \times 10^{-14} \text{ (mol/L)}^2}$$

By calculation the value of $K_w = 1.00 \times 10^{-14} \text{ (mol/L)}^2$

This can be represented simply as $K_w = 10^{-14} \text{ M}^2$

We can now represent Expression 3 as follows:

$$K_w = [\text{H}^+][\text{OH}^-] = 10^{-14} \text{ M}^2$$

- o It must be realized that the Ion Product of Water, namely $[\text{H}^+][\text{OH}^-]$, is constant for all aqueous solutions, even those that contain dissolved acids or dissolved bases.
- o If a large amount of H^+ ions are added to pure water, the concentration of OH^- ions will decrease in order that the Ion Product $[\text{H}^+][\text{OH}^-]$ remain constant at 10^{-14} M^2 at 25°C .

What is the significance of the Ion Product of Water?

The expression $[\text{H}^+][\text{OH}^-] = 10^{-14}$ means that:

- o In pure water as well as in all aqueous solutions the product of $[\text{H}^+][\text{OH}^-]$ is constant and it is equal to 10^{-14} .
- o This simply means that none of these two concentrations can be changed alone.
- o If, for example an acid is added and $[\text{H}^+]$ increased, then $[\text{OH}^-]$ must decrease to maintain the Ion Product constant
- o It also means that in pure water or neutral solution, where $[\text{H}^+]$ and $[\text{OH}^-]$ are equal, each of them can be calculated as: $[\text{H}^+] = [\text{OH}^-] = \sqrt{10^{-14}} = 10^{-7} \text{ mol/L}$.
- o The calculation of $[\text{H}^+]$ can be calculated if $[\text{OH}^-]$ is known and vice versa.

How can the pH scale be related to the property of H_2O ?

The expression $[\text{H}^+][\text{OH}^-] = 10^{-14}$ can be converted to the p-scale of numbers, by first taking the logarithm of both sides. Thus:

$$\text{Log } \{[\text{H}^+][\text{OH}^-]\} = \text{Log } 10^{-14}$$

This expression can be written as:

$$\text{Log } [\text{H}^+] + \text{Log } [\text{OH}^-] = -14$$

By multiplying both sides by -1 the expression becomes:

$$-\text{Log } [\text{H}^+] - \text{Log } [\text{OH}^-] = 14 \quad \text{Expression 4.}$$

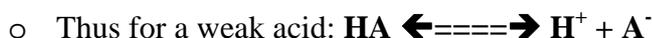
It must be noted at this point that by definition:

$$-\text{Log } [\text{H}^+] = \text{pH}; \quad -\text{Log } [\text{OH}^-] = \text{pOH}$$

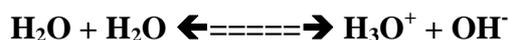
Thus expression 4 can be written as follows:

$$\text{pH} + \text{pOH} = 14$$

- o According to this concept a Conjugate Base always accompanies an Acid, and Conjugate Acid accompanies a Base.



- o Where **HA** is the undissociated acid and **A⁻** is its Conjugate base, since it is a proton acceptor. Water for example can act as a base and as an acid thus:



The dissociation of a weak acid can be described by the following expression:

$$\mathbf{K_a} = [\text{A}^-][\text{H}^+] / [\text{HA}] = \mathbf{K_{eq}}$$

(Where **K_a** is the dissociation constant for the weak acid, for acid ionization it is called Acidity constant.)

- o In **Biochemical and Medical applications**, it is usual to designate the concentration in terms of moles per liter in the expression for **K_a**.
- o In such a case, the constant **K_a** becomes the “**Apparent dissociation constant**” and is designated as **K_a'**
- o The Real or True (i.e., Thermodynamic) Dissociation Constant, **K_a** requires the use of ion activities in place of molar concentration for its calculation.
- o Thus, in Biochemical calculations we have to use the expression for the Apparent Dissociation Constant: The expression is: **K_a' = [A⁻][H⁺] / [HA]**
- o Note that the relative strengths of weak acids and of weak bases are expressed quantitatively as their Apparent Dissociation Constants, which express their tendency to ionize.
- o Thus the **pK_a'** value of an Acid group is that pH at which the Protonated Unprotonated species are present at equal concentrations.

Examples of some physiologically relevant weak acids and their conjugate bases:

Acid	Conjugate base	pK _a '
Ammonium ion (NH ₄ ⁺)	Ammonia (NH ₃)	9.25
Carbonic acid (H ₂ CO ₃)	Bicarbonate ion (HCO ₃ ⁻)	6.37
Dihydrogen phosphate ion (H ₂ PO ₄ ⁻)	Monohydrogen phosphate ion (HPO ₄ ²⁻)	6.86
Lactic acid (CH ₃ CHOHCOOH)	Lactate ion (CH ₃ CHOHCOO ⁻)	3.86

- o The tendency of a Conjugate Acid to dissociate could be evaluated from the **K_a'** or **pK_a'** value.
- o The smaller the **K_a'** the lower the tendency to dissociate and the weaker the acid.
- o The larger the **K_a'** values the higher the tendency to dissociate and the stronger is the acid.
- o Note also that the smaller the **K_a'**, the larger the **pK_a'**. Thus, the larger the **pK_a'** value the weaker the acid the smaller the **pK_a'** the stronger the acid.

What is the HENDERSON-HASSELBALCH EQUATION?

- o The relationship between the pH of a solution containing a weak acid and its acid dissociation constant can be stated using the Handerson-Hasselbalch equation.
- o This equation for a weak acid can be expressed as follows:

Briefly show how to derive the Handerson-Hasselbalch equation:

Consider a weak acid HA, that dissociates thus:



$$K_d = [\text{H}^+] [\text{A}^-] / [\text{HA}]$$

This can be arranged thus:

$$[\text{H}^+] = K_d ([\text{HA}] / [\text{A}^-])$$

Taking the Log of both sides gives:

$$\text{Log} [\text{H}^+] = \text{Log} K_d + \text{Log} ([\text{HA}] / [\text{A}^-])$$

Multiply both sides of the equation by -1 gives:

$$-\text{Log} [\text{H}^+] = -\text{Log} K_d - \text{Log} ([\text{HA}] / [\text{A}^-])$$

By definition: $-\text{Log} [\text{H}^+] = \text{pH}$, in addition $-\text{Log} K_d = \text{pKa}$

Thus substituting in the equation above gives the expression:

$$\text{Thus: } \text{pH} = \text{pKa} - \text{Log} ([\text{HA}] / [\text{A}^-])$$

This expression can be written as: $\text{pH} = \text{pKa} + \text{Log} ([\text{A}^-] / [\text{HA}])$

This expression is called the **Handerson-Hasselbalch** equation.

What are the major uses of the Handerson-Hasselbalch equation?

- o One major use of the Henderson-Hasselbalch equation is for the preparation of buffer solutions of known pH.
- o It is also important in calculating the pH of biochemical solutions.

What do you understand by the term buffer solution?

- o By definition a buffer solution is a solution that can resist change in pH when small amounts of acid or base are added to the solution.
- o **The Acidic buffer solution is made up of a weak acid and the salt of the weak acid.**
- o **The Basic buffer solution is made up of a weak base and the salt the weak base.**

List some of the factors that can affect the pH value of a buffer solution are:

The Handerson-Hasselbalch equation is used to prepare buffer solutions:

$$\text{pH} = \text{pKa} + \text{Log} ([\text{A}^-] / [\text{HA}])$$

- o The strength of the weak acid (K_a or pK_a) is the measure of acid strength and it is constant for a given weak acid or weak base.
- o The $[A^-]/[HA]$ ratio. Note that when the $[A^-] = [HA]$ then $pH = pK_a$
- o The greater the $[HA]$, the lower the pH of the buffer solution. Remember that lower pH means more acidity
- o An increase in temperature causes decrease in the value of pK_a and thus shifts the pH value lower.

What is the pH range of a buffer solution?

The **pH range of the buffer solution** can be estimated by using the limit over which the ratio of $[A^-]/[HA]$ can be varied.

That is when:

1. The ratio of $[A^-] / [HA] = 10:1$. At this ratio the $pH = pK_a + 1$
2. The ratio of $[A^-] / [HA] = 1:10$. At this ratio the $pH = pK_a - 1$.

Thus, the expression $pH = pK_a \pm 1$ is the range of the buffer solution.

What do you understand by the term Buffer Capacity?

The ability of a buffer solution to resist change in pH is called the buffer capacity.

By definition: **the Buffer capacity is the number of moles of protons or hydrogen ions required to change the pH of one liter of buffer solution by 1.0 pH unit.**

What are the factors that can influence the buffer capacity of a solution?

The buffer capacity can be influenced by the following:

- o **The final concentration of the buffer solution** – thus, the higher the concentration of the buffer solution the stronger the buffer capacity.
- o **The ratio $[A^-]/[HA]$.**
 - o When the **ratio is greater than one** the buffer capacity in the acid direction is greater than in the basic direction.
 - o When the **ratio is less than one** then the buffer capacity in the base direction is higher than in the acid direction.
 - o When the **ratio is equal to one**, then the buffer capacity in one direction is equal to the other. The buffer capacity is maximal at this point, i.e., when $pH = pK_a$.

Why does the pH of a buffer solution change when diluted?

- o Since the pH of a buffer solution depends upon the ratio $[A^-]/[HA]$, change is not expected in the pH of the buffer solution upon dilution of the buffer.
- o However, the pH does change.
- o This is because the ion activities of the conjugate base A^- and the conjugate acid HA change to different degrees upon dilution.

What are the BLOOD BUFFERS?

The important buffers in the metabolic system are as follows:

- o Bicarbonate buffer system H_2CO_3/HCO_3^- .

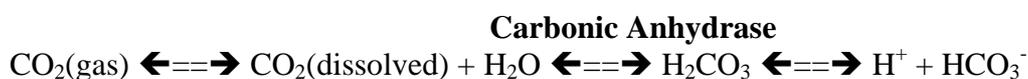
- o Note that the actual value of $[\text{H}_2\text{CO}_3] = \{[\text{H}_2\text{CO}_3] + [\text{CO}_2 \text{ dissolved}]\}$.
- o Hemoglobin buffer system HHb/Hb^-
- o Oxyhemoglobin buffer system $\text{HHbO}_2/\text{HbO}_2^-$
- o Phosphate buffer system $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$
- o Protein buffer system $\text{RCOOH}(\text{NH}_3^+)/\text{RNH}_2(\text{COO}^-)$

In the Red Blood Cells the main buffer systems are the Hemoglobin buffer, Oxyhemoglobin buffer and the Bicarbonate buffer.

In the Blood Plasma the main buffer systems are the Bicarbonate buffer, Protein buffer and Phosphate buffer.

What is the Bicarbonate Buffer system?

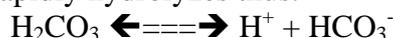
- An expression for Bicarbonate buffer system in the blood is



- This expression can be separated into two:
- Enzyme **Carbonic Anhydrase** catalyzes this reaction,
 - o (NB: zinc is activator for Carbonic Anhydrase)



- Carbonic Acid formed rapidly hydrolyzes thus:



- Combining this two expressions give:



- Equilibrium expression can be written as:



- o CO_2 can be written as P_{CO_2} (Partial Pressure of CO_2 in solution)
- Concentration of dissolved CO_2 (in meq L^{-1}) can be obtained by multiplying the P_{CO_2} by a conversion factor α

$$\text{Thus, } \alpha P_{\text{CO}_2} = \text{meq L}^{-1}$$

- Factor α has a value of $0.03 \text{ meq L}^{-1} \text{ mmHg}^{-1}$ (or $0.225 \text{ meq L}^{-1} \text{ kPa}^{-1}$) at 37°C
- Substituting for $[\text{CO}_2]$ in the equilibrium expression above, gives
- Henderson – Hasselbalch equation for the Bicarbonate buffer system becomes

$$\text{pH} = \text{pKa} + \log \left\{ \frac{[\text{HCO}_3^-]}{0.03 P_{\text{CO}_2}} \right\}$$

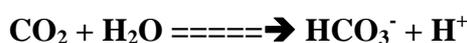
Bicarbonate buffer system is effective as a blood buffer because:

- o Equilibrium exists between gaseous CO_2 in the lungs and dissolved CO_2 in the plasma. This is an effective mechanism for the removal of CO_2 from the metabolic system and thus controls the H^+ ions in the blood.

- o The enzyme Carbonic Anhydrase catalyzes the interconversion of dissolved CO₂ to Carbonic Acid, thus regulating the concentration of dissolved CO₂ and consequently gaseous CO₂.
- o The equilibrium associated with the dissociation of Carbonic Acid is also under the control of Carbonic Anhydrase. The enzyme ensures that the Bicarbonate buffer functions as an Open System.

GASTROINTESTINAL ACID PRODUCTION (GAP)

- H⁺ ions or HCO₃⁻ ions are secreted into the gut lumen
- Example:
 - o H⁺ ions are secreted in the Stomach
 - o HCO₃⁻ ions are secreted in the Colon
- Production of HCO₃⁻ ions and all H⁺ ions inside Gut mucosal cells occur via the same net reaction:



Consider two different cases:

- When H⁺ ion is needed for secretion into gut lumen HCO₃⁻ ion is also produced (**see Fig. 1a**)
- When HCO₃⁻ ion is needed for secretion into gut lumen H⁺ ion is also produced (**see Fig. 1b**)
- In the first case the H⁺ that is produced and in the second case the HCO₃⁻ ion that is produced do not stay in the Gut lumen because they are not needed despite the fact that they are also produced

What then happens to the ions?

- Both ions are moved in the direction opposite to the gut lumen – that is, into the Interstitial Fluid and Blood (**see Figures 1a and 1b**)
- Therefore, if an organ secretes H⁺ ions into the Gut Lumen, it must secrete HCO₃⁻ into the Blood
- If it secretes HCO₃⁻ ions into the Gut Lumen, it must secrete H⁺ ions into the Blood
- **The effects on the Blood and Gut Lumen are equal and opposite because there is a One-to-one ratio of H⁺ ion and HCO₃⁻ ion produced by the reaction**

What happens in different segments in the GIT?

STOMACH:

- Under fasting (basal) conditions, Parietal cells secrete H⁺ ions into the Gastric lumen at about 10mM per hour

- Following meals (Postprandial), the rate of secretion can reach as much as 50mM per hour
- Secretion of H^+ ions lowers pH of Alimentary content (Chyme) to about 1.0
- Thus, stomach liberates HCO_3^- ions into the Blood, both at rest and, especially, during meals
- Release of HCO_3^- ions into the blood results in a very slight Postprandial Rise in Plasma concentration of HCO_3^- ions, which is often reflected, after a delay, in the Renal Spillage of HCO_3^- ions
- Rise in Urinary Concentration of HCO_3^- ions that results is sometimes called the “Alkaline tide”

DUODENUM AND ASSOCIATED ORGANS:

- HCO_3^- ions are secreted into Duodenal Chyme from **3** sources:
 - Pancreas, Gall Bladder, and Duodenal Mucosa
 - Pancreatic Fluid has HCO_3^- concentration of between 25mM (Basal) and 150mM (Postprandial)
 - In 24 hours, total Pancreatic HCO_3^- ions secretion is about 200mM
 - Bile from Gall Bladder has HCO_3^- ions concentration of about 40mM
 - Duodenal Mucosa also generates and secretes HCO_3^- ions
- As expected, these organs secrete equi-molar quantities of H^+ ions into the 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, thereby Acidifying the Blood
- Colon secretes more than 200mM HCO_3^- ions per day into the Lumen and thus has a major Acidifying effect on blood

Diagrammatic illustration of GIT secretion is presented in **Figure 2**.

- General view is as follows: Stomach adds:
 - H^+ ions to the Gut lumen, thus Acidifying its contents,

- HCO_3^- ions to the Blood, thus Alkalinising it
- Almost all the segments below the Pylorus have the opposite effect:
 - They add HCO_3^- ions to the Gut lumen and add H^+ ions to the blood

A simplified mechanism for the Production of Gastric Acid:

- Parietal (Oxyntic) cells are the source of Gastric HCl, which originates according to the reactions shown in **Fig. 3**
- Source of H^+ is Carbonic Anhydrase formation of H_2CO_3 from H_2O and CO_2
- Alkaline urine often follows Ingestion of a Meal (“Alkaline tide”), as a result of the formation of Bicarbonate ions in the process of HCl secretion
- Secretion of H^+ into the lumen is an active process driven by a membrane-located H^+ - K^+ ATPase
- Parietal cells contain numerous Mitochondria needed to generate the ATP used for H^+ - K^+ ATPase to function
- HCO_3^- ions pass into the Plasma in exchange for Cl^- , which is coupled to the secretion of H^+ into the lumen

Another model for explaining Gastric Acid secretion is as follows:

- H^+ ions are generated within the Parietal Cell from dissociation of water, OH^- ions formed in this process rapidly combine with CO_2 to form HCO_3^- ions catalysed by Carbonic Anhydrase
- HCO_3^- ions are transported out of the Parietal Cell in exchange for Cl^- ions
- Outflow of HCO_3^- ions into the Blood results in a slight elevation of blood pH known as the "Alkaline tide"
 - This process serves to maintain intracellular pH in the Parietal cell
- Chloride and Potassium ions are transported into the lumen of the Parietal cell via conductance channels, and such is necessary for secretion of acid
- H^+ ions are pumped out of the cell, into the lumen, in exchange for Potassium through the action of the H^+/K^+ ATPase or Proton pump; Potassium is thus effectively recycled
- Accumulation of Osmotically-active Hydrogen ion in the parietal cell generates an osmotic gradient across the membrane that results in outward diffusion of H_2O – the resulting Gastric Juice is 155mM HCl and 15mM KCl with a small amount of NaCl.

What are some of the effects of Gastric acid?

- As a result of contact with Gastric HCl, Proteins are denatured, making them more accessible to the action of Proteases.
- Low pH also has the effect of destroying most microorganisms entering the GIT
- Some clinical conditions arise from defects in the digestive processes, such as ulceration by Gastric HCl or diminished secretion of HCl causing **Achlorhydria**
- When stimulated, Parietal cells secrete HCl at a concentration of roughly 160 mM (equivalent to a pH of 0.8)
- Despite the high acidity the epithelium of the stomach is intrinsically resistant to the damaging effects of gastric acid
- Nonetheless, excessive secretion of Gastric acid is a major problem in human and can lead to Gastritis, Gastric ulcers and Peptic acid disease