

Concept of pH

The biochemical reactions that are necessary for life are profoundly influenced by the pH of cellular solutions. This lecture discusses the chemical nature of pH, its measurement and its biological importance. The acidic or basic nature of the medium. The various biochemical reactions in the living system are also controlled by the state of pH in the biological medium.

An understanding of acid-base chemistry and dissociation of water is essential to understanding pH and buffer.

General Characteristics of Acids and Bases:

Acids are generally a class of substances that **taste sour** (but do not use this method to identify a compound), **such as vinegar**, which is a dilute solution of acetic acid. **Bases**, or alkaline substances, are characterized by their **bitter taste** and **slippery feel**. The first precise definition of an acid and base was given by Svante Arrhenius and is referred to as the Arrhenius Theory.

Arrhenius Theory:

In the 1830s, it was known that all acids contain hydrogen, but not all hydrogen containing compounds are acids. In 1889, Swedish chemist Svante Arrhenius, connected acidic properties with the presence of hydrogen (H^+) ions and basic properties with presence of hydroxyl (OH^-) ions. Arrhenius defined an acid as a substance that ionizes in water to give hydrogen ions, and a base as a substance that ionizes in water to give hydroxide ions.

If a solution contains more H^+ than OH^- ions, then it is acidic.

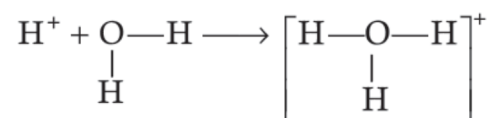
If a solution contains more OH^- than H^+ ions, then it is basic.

If H^+ and OH^- ions are equal, then it is a neutral solution.

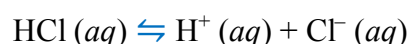
Hydrochloric acid (HCl) is a strong acid and is very soluble in water. It dissociates into its component ions in the following manner:



The hydrogen ion interacts strongly with a lone pair of electrons on the oxygen of a water molecule. The resulting ion, H_3O^+ , is called the **hydronium ion**.

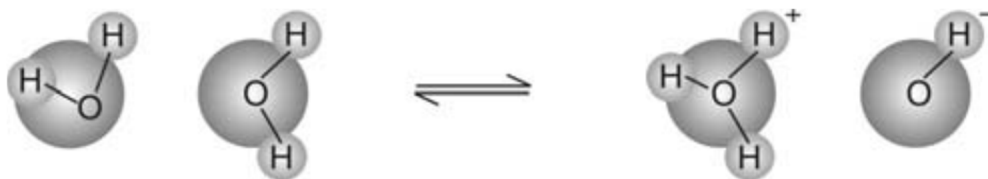


Acidic solutions are formed when an acid transfers a proton to water. The reaction of HCl with water can be written in either of the following ways:

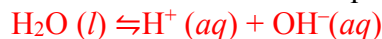


The Dissociation of Water:

Pure water is a poor conductor of electricity because it does not ionize to a great extent. So essentially it is a non-electrolyte. It consists almost entirely of H₂O molecule. A very small percentage of H₂O molecules undergo ionization and it can act as both a proton donor and acceptor for itself. A proton can be transferred from one water molecule to another, resulting in the formation of one hydroxide ion (OH⁻) and one hydronium ion (H₃O⁺).



This is called the **auto-ionization** or **dissociation** of water. This equilibrium can also be expressed as:



In the above equilibrium, water acts as both an acid and a base. **The ability of a species to act as either an acid or a base is known as amphoterism.** The concentrations of H₃O⁺ and OH⁻ produced by the dissociation of water is equal. The corresponding equilibrium expression for the auto-ionization reaction is:

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

In pure water the concentration of H₂O at 25°C is 55.6 M

$$[\text{H}_2\text{O}] = 55.6 \text{ M}$$

This value is relatively constant in relation to the very low concentration of H⁺ and OH⁻.

Molarity is one method of expressing concentration. It is the number of moles in one liter of the solution.

$$\text{Moles} = \frac{\text{Weight in grams}}{\text{Molecular weight}}$$

$$\text{Therefore, molarity of water} = \frac{1000}{18} = 55.6 \text{ M}$$

Therefore,

$$K_{\text{eq}} = \frac{[\text{H}^+][\text{OH}^-]}{55.6 \text{ M}}$$

Rearranging gives:

$$K_{\text{eq}} (55.6 \text{ M}) = [\text{H}^+][\text{OH}^-]$$

K_{eq} calculated from electrical conductivity measurement, $K_{\text{eq}} = 1.8 \times 10^{-16} \text{ M}$

$$1.8 \times 10^{-16} \text{ M} \times (55.6 \text{ M}) = [\text{H}^+][\text{OH}^-]$$

$$[\text{H}^+][\text{OH}^-] = 1 \times 10^{-14}$$

Therefore,

$$[\text{H}^+] = \frac{1 \times 10^{-14}}{[\text{OH}^-]}$$

$$[\text{OH}^-] = \frac{1 \times 10^{-14}}{[\text{H}^+]}$$

The ion-product constant *always* remains constant at equilibrium (as the name implies). Consequently, if the concentration of either H^+ or OH^- rises, then the other must fall to compensate. In acidic solutions, $[\text{H}^+] > [\text{OH}^-]$, and in basic solutions $[\text{H}^+] < [\text{OH}^-]$. A solution for which $[\text{H}^+] = [\text{OH}^-]$ is said to be neutral.

In pure water: $\text{H}^+ = \sqrt{K_w} = \sqrt{1.0 \times 10^{-14}}$ or $[\text{H}^+] = [\text{OH}^-] = 10^{-7} \text{M}$

pH Scale:-

The pH scale is used as a measure of acidity or the concentration of H^+ ions in aqueous medium. The symbol pH is an abbreviation for “*pondus hydrogeni*”, of French (translated as **potential hydrogen**), meaning “**hydrogen power.**” It is defined as the negative logarithms (10^{-n}) of the hydronium ions (H_3O^+) or hydrogen ions (H^+) concentration **expressed in moles per liter of water**. Dr. Sorensen has been credited as the founder of the modern pH concept.

$$\text{pH} = -\log([\text{H}_3\text{O}^+]) \text{ or } ([\text{H}^+])$$

- To obtain a mole of any substance, simply measure out its molecular weight in grams.
- One gram of hydrogen atoms is a mole.
44 grams of CO_2 is a mole (the atomic weight of C = 12, that of O = 16, and $12 + 2(16) = 44$).
- A mole of any substance contains the same number of particles, Avogadro's number, 6.02×10^{23} .
- The liter is a particular volume of water.
Thus, a pH of 0 means a hydrogen ion concentration of 10^0 molar or moles per liter, which is one mole.

TABLE 2.1 pH and its corresponding H^+ ion concentration.

pH	Concentration of H^+ ions in moles per liter		
0	10^0	=	1.0 molar
1	10^{-1}	=	0.1 molar
5	10^{-5}	=	0.00001 molar
7	10^{-7}	=	0.0000001 molar
10	10^{-10}	=	0.0000000001 molar

Since normal dissociation of water molecules into H^+ and OH^- yields 10^{-7} moles of H^+ , pure water has a pH of 7, the neutral point in the scale. Since the pH scale is logarithmic, **a difference in 1 unit is a 10-fold increase or decrease in concentration of hydrogen ions.**

$$pH = -\log [H^+]$$

$$pH = -\log 10^{-7} = 7$$

From this it is obvious that at 25°C:

pH < 7-solution is acidic

pH = 7-solution is neutral

pH > 7-solution is basic

Notice that the pH of a solution measures the concentration of dissociated protons and not the total concentration of acid in a solution.

The negative log scale is useful for measuring other minute quantities, for example, to measure $[OH^-]$:

$$pOH = -\log [OH^-]$$

Knowing this, we obtain the following useful expression:

$$pH + pOH = -\log K_W = 14.00$$

TABLE 2.2 the relationship between pH and pOH.

pH	pOH	$[H^+]$ mol/L	$[OH^-]$ mol/L
0	14	1.0	10^{-14}
2	12	0.01	10^{-12}
4	10	0.0001	10^{-10}
6	8	10^{-6}	10^{-8}
8	6	10^{-8}	10^{-6}
10	4	10^{-10}	0.0001
12	2	10^{-12}	0.01
14	0	10^{-14}	1.0

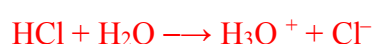
Buffers:

In any biological system, one of the most important parameters of an aqueous solution is the concentration of protons ($[H^+]$) or the pH. Although the $[H^+]$ is quite low, typically 10^{-6} to 10^{-8} M, it must be maintained within this range for life to exist. **If an aqueous solution is able to maintain its pH near constant, it can be called a buffer. A buffer is a solution of a weak acid or a weak base and its corresponding salt that keeps pH constant under diverse conditions. Weak acids and bases are those that dissociate very little in solution (as opposed to strong acids and bases like HCl and NaOH which dissociate completely).** The weak acid

or base is in equilibrium with its dissociated salts. **By the law of mass action, an increase in the concentration of either reactant or product forces the reaction in the opposite direction, thereby maintaining the original equilibrium. An acidic buffer (pH below 7) contains a weak acid and a salt of the acid (conjugate base).** A basic buffer (pH above 7) contains a weak base and its conjugate acid (salt of the base).

Strong Acids and Bases:

A strong acid undergoes complete dissociation into ions in aqueous solution. For example, when added to water HCl undergoes ionization as follows:



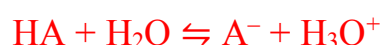
NaOH is a strong base because it completely dissociates into Na^+ and OH^- ions in water. When there is complete ionization like this that solution cannot act as a buffer.

Weak Acids and Bases:

A buffer is a solution of a weak acid or a weak base and its corresponding salt that keeps the pH constant under diverse conditions. Weak acids and bases are those that dissociate very little in solution (as opposed to strong acids and bases like HCl and NaOH that dissociate completely). **In water, a weak acid and base dissociate partially.** The reaction can be represented as follows:



The actual reaction in water is:



Since the concentration of water is constant, it can be represented more precisely by the first equation.

The weak acid or base is in equilibrium with its dissociated salts. By the law of mass action, an increase in the concentration of either reactant or product forces the reaction in the opposite direction, thereby maintaining the original equilibrium.

Weak acids and bases can therefore serve as reservoirs that can react with any extra hydrogen or hydroxide ions that may enter the medium. For example, if excess acid is added to a buffer solution of a weak acid and its salt (left), the introduced hydrogen ions initially increase the pH (middle). But as the weak acid's anions (negatively charged ions) take up the extra H^+ the original pH becomes reinstated (right). If a base is added, the free protons combine to form additional salt. In both cases, the ratio of (buffer acid) to (buffer salt) remains constant and so does the pH.

The law of mass action can explain the above reversible reaction and the dissociation constant for this reaction is given by

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

According to the **Henderson-Hasselbalch equation**

$$\text{pH} = \text{pKa} + \log\left[\frac{[\text{A}^-]}{[\text{HA}]}\right]$$

So if the pKa of a weak acid or base is known, it is easy to make a buffer of the desired pH by taking appropriate proportion of acid and base. **At equilibrium, when the concentration of the acid ([HA]) is equal to its conjugated base ([A⁻]).**

$$\text{pH} = \text{pKa}$$

All concentrations must be in molar units (*M*).

[Base] is the concentration of the form of the buffer without the proton.

[Acid] is the concentration of the form of the buffer with the proton.

[Base] > [acid] means pH > pKa

[Base] = [acid] means pH = pKa

[Base] < [acid] means pH < pKa

Example

Take the case of the weak acid. **Acetic acid** is a weak acid that dissociates incompletely into its ions as follows:



According to the law of Mass action

$$\text{Equilibrium constant } K = \frac{[\text{CH}_3\text{COO}^-][\text{H}_3\text{O}^+]}{[\text{CH}_3\text{COOH}][\text{H}_2\text{O}]}$$

In the case of a dilute solution, the concentration of H₂O is a constant and that of solute is always below 1M.

$$K \cdot [\text{H}_2\text{O}] = \frac{[\text{CH}_3\text{COO}^-][\text{H}_3\text{O}^+]}{[\text{CH}_3\text{COOH}]}$$

K · [H₂O] = K_a, the dissociation constant.

$$K_a = \frac{[\text{CH}_3\text{COO}^-][\text{H}_3\text{O}^+]}{[\text{CH}_3\text{COOH}]}$$

Therefore, when applying the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pKa} + \log\left[\frac{[\text{A}^-]}{[\text{HA}]}\right]$$

$$\text{pH} = \text{pKa} + \log\left[\frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}\right]$$

If we know the pKa of a weak acid or base, it is very easy to make the buffer of any desired pH by weighing out appropriate concentrations of salt and acid. For making the acetate buffer we need sodium acetate and acetic acid in appropriate quantities.

Titration Curves reveal the pKa of Weak Acids:-

Titration is used to determine 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 sodium hydroxide (NaOH), of known concentration. The NaOH is added in small increments until the acid is consumed (neutralized), as determined with an indicator dye or a pH meter. **The concentration of the acid in the original solution can be calculated from the volume and concentration of NaOH added.**



A typical plot of change in pH against the amount of NaOH added for acetic acid is given in Figure. In the beginning, acetic acid is present majorly as CH_3COOH but with the addition of NaOH, OH^- will neutralize H^+ to form H_2O and promotes further dissociation of acetic acid to give H^+ (Figure). At the midpoint of where 0.5 equivalent NaOH has been added, solution has an equal concentration of CH_3COOH (proton donor) and CH_3COO^- (proton acceptor). At the end of titration, all un-dissociated CH_3COOH will be converted into the CH_3COO^- .

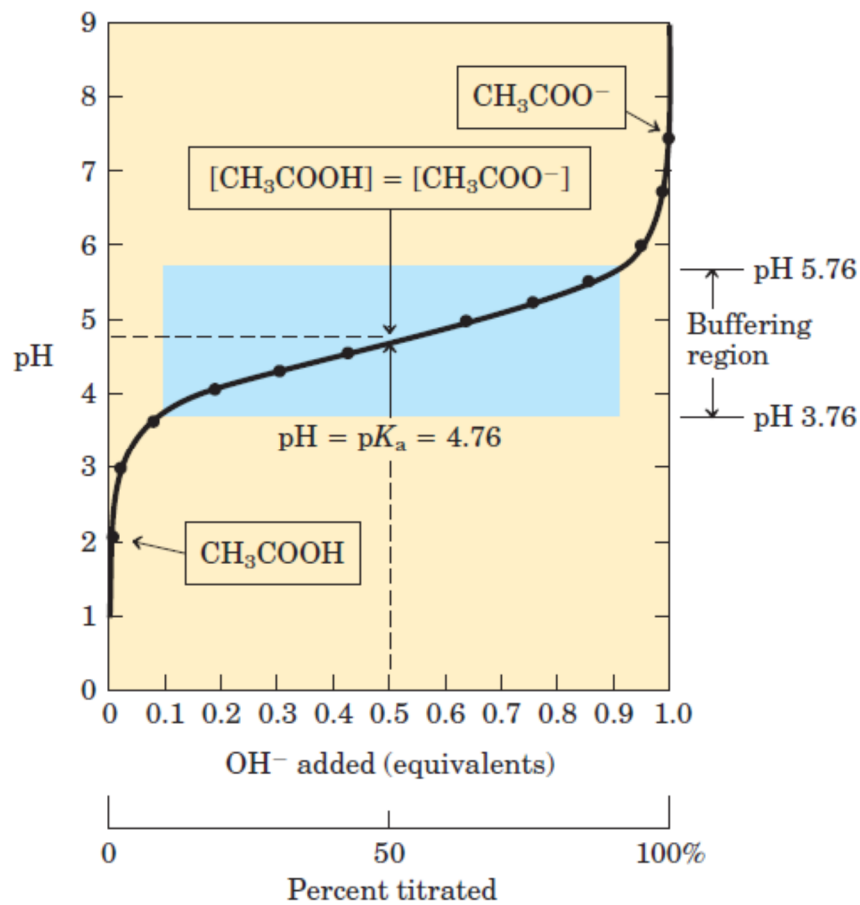


Figure Titration curve of acetic acid

Role of buffer in biological system:-

Each and individual organism keeps a constant pH to maintain homeostasis and growth. A number of biological buffers (including proteins) are known to maintain a constant pH within different tissues of human body. Few buffers are specific to the organ or tissue whereas many buffers are universally present. Biological reactions as well as macromolecules are sensitive to the change in pH and therefore buffer plays vital role for their optimal activity.