SHREE H. N. SHUKLACOLLEGE OF SCIENCE
(AFFILIATED TO SAURASHTRA UNIVERSITY)
Shree H.N. Shukla College Campus Nr. Lalpari lake, Behind old Marketing Yard,
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F.Y.B.SC.(SEM-1)(CBCS)

NEW PROPOSED SYLLABUS -JUNE 2019

BIOCHEMISTRY(101)

PHYSICAL AND CHEMICAL ASPECTS OF BIOCHEMISTRY

UNIT -3 PH, BUFFER AND PHYSIOLOGICAL BUFFERS

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- **Henderson-Hasselbalch equations ,simple numerical problems involving applications of this equation.**
- **Physiological buffers: types and importance**

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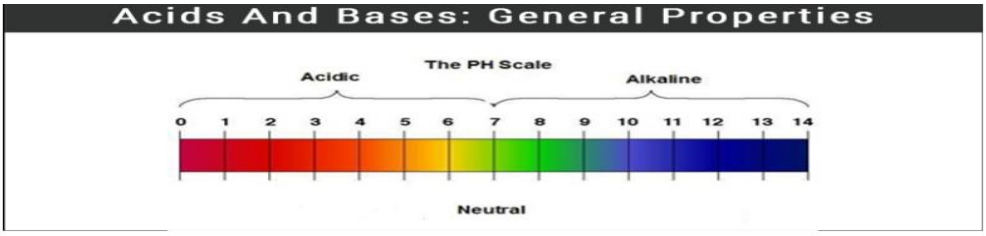
Properties of Acid and Base

What are Acids and Bases?

Acids are chemical substances which are characterized by a sour taste in an aqueous medium. They have the tendency to turn blue litmus red. On the other hand, **bases are chemical substances which are characterized by a bitter taste and are slippery to touch.** Some bases are soluble in water, while others are not.

Water soluble bases are known as alkalis. They have the tendency to turn red litmus blue. Acids and bases react with a wide range of chemical compounds to form salts.



Physical Properties of Acids and Bases

Acids	Bases	Salts
An acid is a substance which dissolved in water, ionizes and releases hydrogen ions [H^+ (aq.)] in solution.	An acid is a substance which dissolved in water, ionizes and releases hydroxide or hydroxyl ions [OH^-(aq.)] in solution.	A salt is a compound formed by the partial or complete replacement of the ionisable hydrogen atoms of an acid by a metallic ion or an electropositive ion.
They turn blue litmus paper to red.	They turn red litmus paper to blue.	No effect on litmus paper.
They have pH value less than 7.	They have pH value greater than 7.	They have pH value 7.

Chemical Properties of Acid and Bases

- **Reactions of Acids and Bases with Metals**

  When a metal reacts with an acid, it generally displaces hydrogen from the acids. This leads to the evolution of hydrogen gas. The metals combine with the remaining part of acids to form a salt. For example, the reaction of sulphuric acid with zinc.

$$H_2SO4 + Zn \longrightarrow Znso_4 + H_2$$

Alkalis (bases that are soluble in water) react with metals to produce salt and hydrogen gas. For example, reaction of zinc with sodium hydroxide.

$$NaoH + Zn \longrightarrow Na_2Zno_2 + H_2$$

2. The Reaction of Metal Carbonates/Metal Bicarbonates with Acids

Metal carbonates/metal bicarbonates react with acids to produce salt, carbon dioxide and water. For example the reaction of sodium carbonate/sodium bicarbonate with hydrochloric acid.

$$Na_2co_2 + HCL_{(aq)} \longrightarrow 2Nacl_{(aq)} + H_2o_{(l)} + co_2$$

3. The Reaction of Metal Oxide with Acids

Metal oxides react with acids to produce salt and water. For example reaction of copper oxide and dilute hydrochloric acid.

$$Cuo + 2Hcl \longrightarrow Cucl_2 + H_2$$

Shape of titration curves of strong and weak acids and bases

The general shape of the titration curve is the same, but the pH at the equivalence point is different. In a weak acid-strong base titration, the pH is greater than 7 at the equivalence point. In a strong acid-weak base titration, the pH is less than 7 at the equivalence point.

A titration is a controlled chemical reaction between two different solutions.

Introduction

The titration of a weak acid with a strong base involves the direct transfer of protons from the weak acid to the hydroxide ion. The reaction of the weak acid, acetic acid, with a strong base, NaOH, can be seen below. In the reaction the acid and base react in a one to one ratio.

$$C2H4O2(aq)+OH-(aq)\rightarrow C2H3O-2(aq)+H2O(l)$$

In this reaction a burets is used to administer one solution to another. The solution administered from the burets is called the titrant. The solution that the titrant is added to is called the analyte. In a titration of a Weak Acid with a Strong Base the titrant is a strong base and the analyte is a weak acid. In order to fully understand this type of titration the reaction, titration curve, and type of titration problems will be introduced.

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The Titration Curve

The titration curve is a graph of the volume of titrant, or in our case the volume of strong base, plotted against the pH. There are several characteristics that are seen in all titration curves of a weak acid with a strong base. These characteristics are stated below.

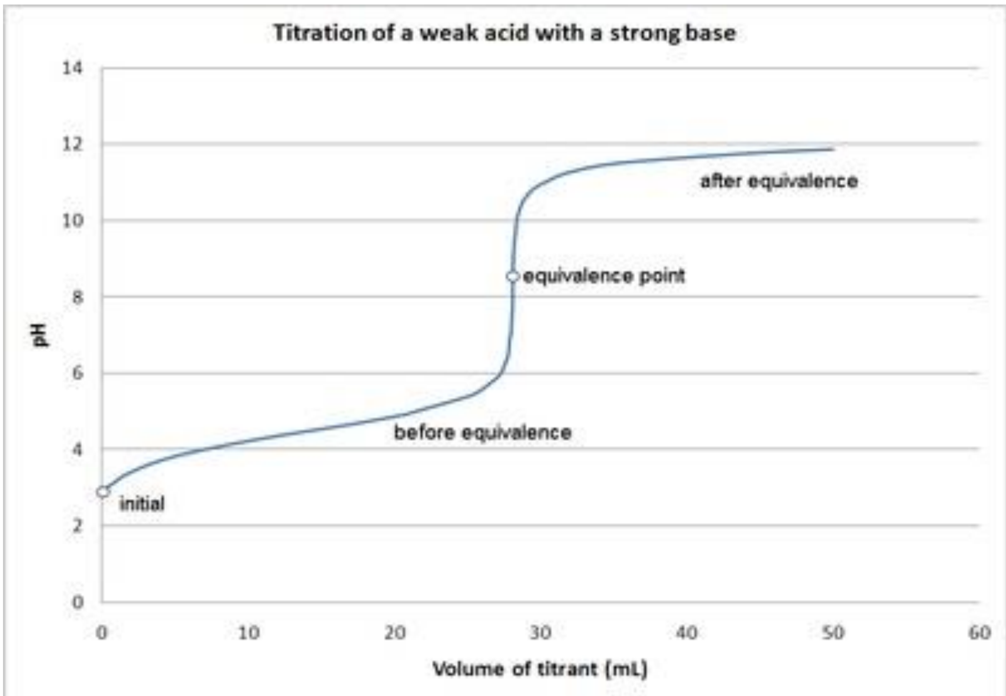
1. The initial pH (before the addition of any strong base) is higher or less acidic than the titration of a strong acid

2. There is a sharp increase in pH at the beginning of the titration. This is because the anion of the weak acid becomes a common ion that reduces the ionization of the acid.
3. After the sharp increase at the beginning of the titration the curve only changes gradually. This is because the solution is acting as a buffer. This will continue until the base overcomes the buffers capacity.
4. In the middle of this gradually curve the half-neutralization occurs. At this point the concentration of weak acid is equal to the concentration of its conjugate base. Therefore the pH=pK_a. This point is called the half-neutralization because half of the acid has been neutralized.
5. At the equivalence point the pH is greater then 7 because all of the acid (HA) has been converted to its conjugate base (A-) by the addition of NaOH and now the equilibrium moves backwards towards HA and produces hydroxide, that is:

$$A^- + H2O \rightleftharpoons AH + OH$$

6. The steep portion of the curve prior to the equivalence point is short. It usually only occurs until a pH of around 10.

The image of a titration curve of a weak acid with a strong base is seen below. All of the characteristics described above can be seen within it.



Meaning of k_a and pk_a values

What is pKa and Ka?

Ka is the acid dissociation constant. pKa is simply the -log of this constant. Similarly, Kb is the base dissociation constant, while pKb is the -log of the constant. The acid and base dissociation constants are usually expressed in terms of moles per liter (mol/L).
pKa is the negative log base ten of the Ka value (acid dissociation constant). It measures the strength of an acid. The lower the value of pKa, the stronger the acid and the greater its ability to donate its protons.

Acid Dissociation Constant From pH

The pH scale, or "power of hydrogen," is a numerical measure of a solution's acidity or basicity. In an aqueous solution, it can be used to compute the concentration of hydrogen ions $[H^+]$ or hydronium ions $[H_3O^{+}]$. Low pH solutions are the most acidic, whereas high pH solutions are the most basic.

The concentration of free hydrogen (or hydronium) ions in an aqueous acid solution is measured by its pH: pH equals -log $[H^+]$ or -log $[H_3O^+]$. The last equation can be rewritten as follows:

$[H_3O^+] = 10^{-pH}$

The above comparison allows you to determine the relative concentration of acid to conjugate base and estimate the dissociation constant Ka if you know the molar concentration of an acid solution and can detect its pH.

Ka Example

Question:

If the hydroxide ion concentration is 6.6×10^{-6}M, what is the concentration of hydronium ions in a solution?

Solution:

The product of the hydroxide ion concentration and the hydronium ion concentration in any acidic or basic solution equals 1×10^{-14}, the dissociation constant for water. To put it another way:

$2H_2O \rightleftharpoons H_3O^+ + OH^-$

Ksp = $[H_3O^+][OH^-] = 1*10^{-14}$

We can use this equation to calculate the hydronium ion concentration if we know the hydroxide ion concentration.

$[H_3O^+][OH^-] = 1*10^{-14}$

pH meter-types of electrodes, principle and working of pH meter.

What is pH?

pH indicates the concentration of Hydrogen ions in a solution. pH signify the power of hydrogen. To comprehend pH theory, it is necessary to comprehend water dissociation. The pH scale was formed from water's spontaneous dissociation. Water dissociates spontaneously into its H+ and OH- components. In pure water, the concentration of H+ ion is 1 x 10-7. This H+ ion concentration is neutral, meaning it is neither acidic nor alkaline.

What is pH Meter?

A pH meter is a valuable tool used in various fields to determine the acidity or alkalinity of water-based solutions. It provides precise measurements by assessing the movement of hydrogen ions within the suspension, which is then expressed as pH. The term "pH" is derived from the "p," which stands for the negative logarithm, and "H," the chemical symbol for Hydrogen.

- The fundamental principle behind a pH meter lies in potentiometric measurement. It involves detecting the variation in electrical potential between two electrodes: a pH electrode and a reference electrode. The pH electrode is specifically designed to respond to changes in hydrogen-ion concentration, while the reference electrode provides a stable electrical potential. By comparing these two potentials, the pH meter can determine the acidity or alkalinity of the solution.
- The pH scale ranges from 0 to 14, where pH 7 is considered neutral. A pH value below 7 indicates acidity, while a value above 7 indicates alkalinity. The mathematical relationship between the pH scale and hydrogen-ion concentration is expressed as pH = -log[H+]. This equation allows for the translation of electrical potential differences into meaningful pH values.

Definition of pH Meter

A pH meter is a scientific instrument that measures the acidity or alkalinity of water-based solutions by detecting the electrical potential difference between a pH electrode and a reference electrode. It provides precise pH measurements and is widely used in laboratories, quality control, and various scientific and industrial applications.

pH Measurement

The pH rate of a material is directly linked to the degree of the hydrogen ion [H+] and the hydroxyl ion [OH-] concentrations.

The quantitative data rendered via the pH meter shows the ratio of the movement of an acid or base in terms of hydrogen ion activity.

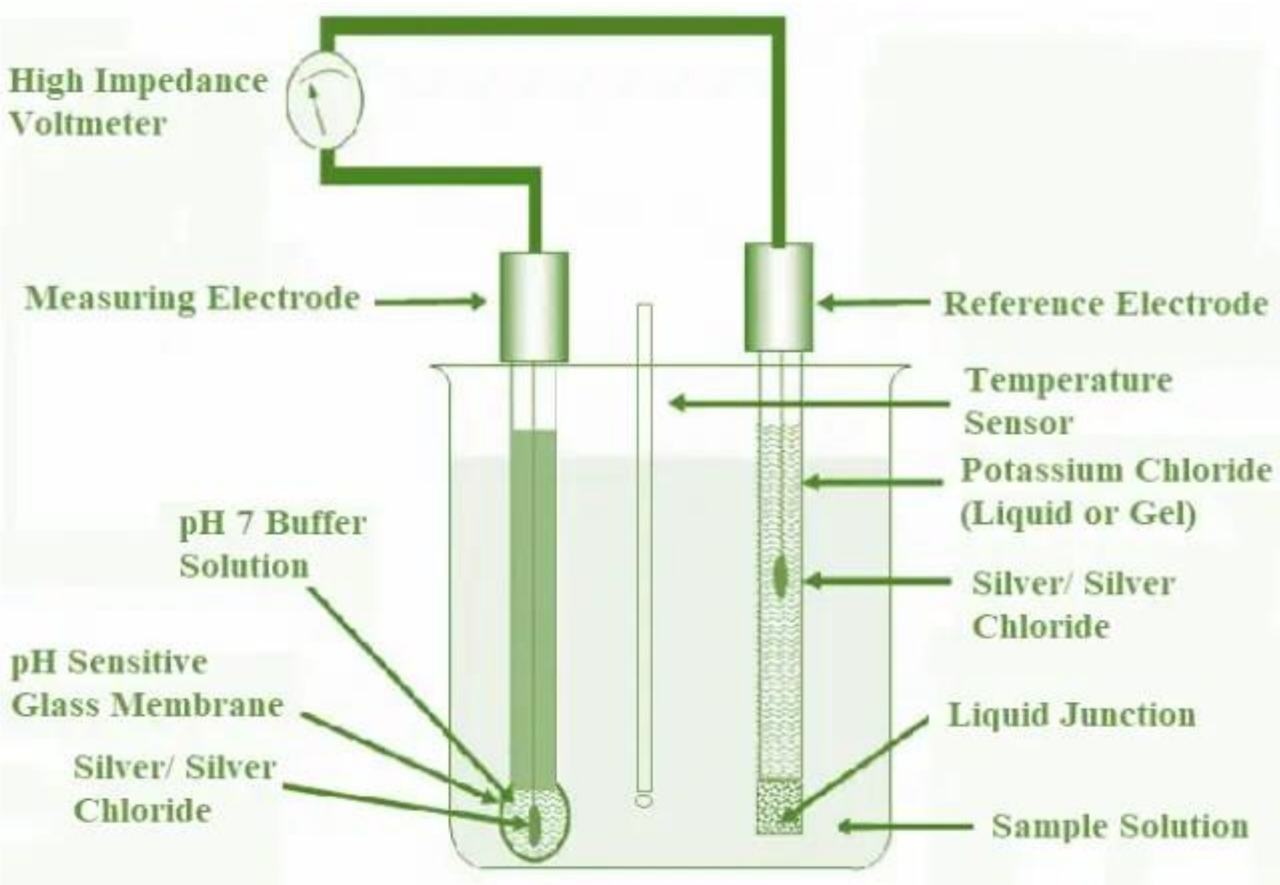
- If the H+ density is higher than OH-, the substance is acidic; i.e., the pH amount is less than 7.
- If the OH- intensity is higher than H+, the substance is basic, including a pH value higher than 7.
- If identical quantities of H+ and OH- ions are present, the substance is neutral, with a pH of 7

pH Meter Working Principle

The principle of operation for a pH meter is based on the exchange of ions between the sample solution and the inner solution of the glass electrode. This exchange occurs through the glass membrane, allowing the pH meter to measure the acidity or alkalinity of the solution.

- A pH meter consists of a pH probe that conducts electrical signals to the pH meter itself, which then displays the pH value of the solution.
- The pH probe comprises two electrodes: a sensor electrode and a reference electrode. The sensor electrode is filled with a pH 7 buffer, while the reference electrode is filled with saturated potassium chloride solution.
- The sensor electrode bulb is coated with metal salts and silica, forming a porous glass membrane.
- Then the pH probe is immersed in a sample solution to measure the pH, hydrogen ions accumulate around the bulb and replace the metal ions within the electrode.

- At the same time, some metal ions from the glass electrode transfer to the sample solution. The reference electrode, which has low sensitivity or complete insensitivity to pH changes, provides a constant voltage.
- This voltage generates an electrical current that is captured by a silver wire, creating a potential difference related to the activity of hydrogen ions.
- The pH meter compares this voltage to the reference electrode and converts it into a pH value.



A pH meter consists of several essential parts that work together to accurately measure the pH of a solution:

1) **Electrode**: The electrode is responsible for directly measuring the pH of the solution. It consists of a glass bulb filled with a pH-sensitive solution and a metal wire that extends into the solution.
2) **Reference electrode:** The reference electrode provides a stable reference point for pH measurement. It typically consists of a silver wire coated in silver chloride and is immersed in a solution of potassium chloride.
3) **Meter**: The meter is the display unit of the pH meter. It can be a digital display or an analog meter with a needle that indicates the pH reading.
4) **Temperature probe:** Some pH meters are equipped with a temperature probe. This probe measures the temperature of the solution being tested. Temperature can affect pH readings, so the temperature probe allows for temperature compensation.

5)**Calibration solution**: pH meters require calibration to ensure accurate readings.

6)**Amplifier**: The amplifier is a crucial component that amplifies the voltage signals generated by the electrodes. It improves the accuracy of the pH readings by enhancing the measurement sensitivity.

7)**High input impedance meter:** This component is responsible for processing the tiny electrode voltages and displaying the pH measurements in pH units. It consists of a microprocessor that reads the pH of the solution, calculates the temperature, and translates the amplifier voltage value.

8) **Combined electrode:** The combined electrode contains both the reference electrode and the pH glass electrode. It is where the actual pH measurement takes place. The reference electrode provides a stable voltage reference, while the pH glass electrode is sensitive to hydrogen ions and produces a millivolt output that corresponds to the pH of the solution.
9) **Thermometer probe:** Some pH meters feature a built-in thermometer probe. It measures the temperature of the solution being tested and incorporates temperature compensation into the pH reading. This feature is known as Automatic Temperature Compensation (ATC).

How to calibrate ph meter?

Calibration of the pH meter is a crucial duty that must be performed daily before to doing any tests with the pH meter.

Utilize the pH meter and electrode system in accordance with the manufacturer's instructions or the pertinent SOPs. All measurements should be conducted between 20 and 25 degrees Celsius. The device is calibrated with the potassium hydrogen phthalate buffer solution (primary standard) (buffer pH 4.0) and another buffer solution with a different pH, preferably buffer pH 9.2. The pH measurement of a third buffer with a pH of 7.0 must not vary by more than 0.05 units.

Buffer

What is Buffer solution ?

- A solution whose pH is not altered to any great extent by the addition of small quantities of either an acid or base is called buffer solution.
- Buffer is also defined as the solution of reserve acidity or alkalinity which resists change of pH upon the addition of a small amount of acid or alkali.
- Many chemical reactions are carried out at a constant pH. In nature, there are many systems that use buffering for pH regulation. For example, the bicarbonate buffering system is used to regulate the pH of blood, and bicarbonate also acts as a buffer in the ocean.
- Characteristics of buffer solution

(i) It has a definite pH.

(ii) Its pH does not change on standing for long periods of time.

(iii) Its pH does not change on dilution.

(iv) Its pH is slightly changed by the addition of small quantity of an acid or base.

Types of buffer solutions

(a) Acidic Buffer:

It is formed by the mixture of weak acid and its salt with a strong base.

Examples: (i) $CH_3COOH + CH_3COONa$, (ii) $HCN + NaCN$, (iii) Boric acid + Borax etc.

(b) Basic Buffer:

It is formed by the mixture of a weak base and its salt with strong acid.

Examples: (i) $NH_4OH + NH_4Cl$, (ii) $NH_4OH + NH_4NO3$, (iii) Glycine + Glycine hydrochloride

(c) Simple Buffer:

It is formed by a mixture of acid salt and normal salt of a polybasic acid,

example $Na_2HPO_4 + Na_3PO_4$

Or a salt of weak acid and a weak base. Example: CH_3COONH_4

Henderson's Equation (pH of buffer)

(a) Acidic Buffer:

It is a mixture of CH_3COOH and CH_3COONa

$$CH_3COOH \leftrightharpoons CH_3COO^- + H^+$$

$$CH_3COONa \rightarrow CH_3COO^- + Na^+$$

By the law of chemical equilibrium, $K_a = \{[CH_3COO^-]\,[H^+]\} / [CH_3COOH]$

$\therefore [H^+] = \{K_a\,[CH_3COOH]\} / [CH_3COO^-]$

Taking negative log both sides, we obtain that

$– \log[H^+] = – \log K_a – \log \{[CH_3COOH]/[CH_3COO^–]\}$

$pH = pK_a + \log \{[CH_3COO^–]/[CH_3COOH]\}$

$$\mathbf{pH = pK_a + \log \{[salt] / [acid]\}}$$

This equation is known as **Henderson's Equation**

Where, K_a = dissociation constant

$[CH_3COO^–]$ = initial concentration of salt

- $[CH_3COOH]$ = initial concentration of acid

(b) Basic Buffer:

It is a mixture of NH_4OH and NH_4Cl

$$NH_4OH \leftrightharpoons NH_4^+ + OH^–$$

$$NH_4Cl \rightarrow NH_4^+ + Cl^–$$

By the law of chemical equilibrium, $K_b = \{[NH_4^+] [OH^–]\} / [NH_4OH]$

$\therefore [OH^–] = \{K_b [NH_4OH]\} / [NH_4^+]$

Taking negative log both sides, we obtain that

$– \log [OH^–] = – \log K_b – \log \{[NH_4OH] / [NH_4^+]\}$

$pOH = pK_b + \log \{ [NH_4^+] / [NH_4OH]\}$

$\mathbf{pOH = pK_b + \log \{[salt] / [base]\}}$

This equation is known as **Henderson's Equation**

Where, K_b = dissociation constant

$[NH_4^+]$ = initial concentration of salt

$[NH_4OH]$ = initial concentration of base

pH + pOH = 14

- **Buffer capacity**
- Buffer capacity is defined as the number of moles of acid or base added in one litre of solution as to change the pH by unity.
- Buffer capacity (Φ) = No. of moles of acid or base added to 1 litre solution/change in pH

  $\Phi = \partial b / \partial(pH)$

- Where ∂b – No. of moles of acid or base added to 1 litre
- $\partial(pH)$ – change in pH

Applications of Buffer in biochemistry

(i) Buffers are used in industrial processes such as manufacture of paper, dyes, inks, paints, drugs, etc.

(ii) Buffers are also employed in agriculture, dairy products and preservation of various types of foods and fruits.

(iii) It is used to determine the pH with the help of indicators.

(iv) Blood is the natural buffer, it maintenance of pH is essential to sustain life because enzyme catalysis is pH sensitive process. The normal pH of blood plasma is 7.4.

(v) For the removal of phosphate ion in the qualitative inorganic analysis after the second group using $CH_3COOH + CH_3COONa$ buffer.

What is the Henderson-Hasselbalch Equation?

The Henderson-Hasselbalch equation provides a relationship between the pH of acids (in aqueous solutions) and their pK_a (acid dissociation constant). The pH of a buffer solution can be estimated with the help of this equation when the concentration of the acid and its conjugate base, or the base and the corresponding conjugate acid, are known.

Equation of Henderson-Hasselbalch

The Henderson-Hasselbalch equation can be written as:

$pH = pK_a + \log_{10} ([A^-]/[HA])$

Where $[A^-]$ denotes the molar concentration of the conjugate base (of the acid) and [HA] denotes the molar concentration of the weak acid. Therefore, the Henderson-Hasselbalch equation can also be written as:

$$pH = pKa + \log \frac{[\text{ conjugate base }]}{[\text{ acid}]}$$

An equation that could calculate the pH value of a given buffer solution was first derived by the American chemist Lawrence Joseph Henderson. This equation was then re-expressed in logarithmic terms by the Danish chemist Karl Albert Hasselbalch. The resulting equation was named the Henderson-Hasselbalch Equation.

Derivation of the Henderson-Hasselbalch Equation

he ionization constants of strong acids and strong bases can be easily calculated with the help of direct methods. However, the same methods cannot be used with weak acids and bases since the extent of ionization of these acids and bases is very low (weak acids and bases hardly ionize). Therefore, in order to approximate the pH of these types of solutions, the Henderson-Hasselbalch Equation is used.

Limitations of the Henderson-Hasselbalch Equation

The Henderson-Hasselbalch equation fails to predict accurate values for the strong acids and strong bases because it assumes that the concentration of the acid and its conjugate base at chemical equilibrium will remain the same as the formal concentration (the binding of protons to the base is neglected).

Since the Henderson-Hasselbalch equation does not consider the self-dissociation undergone by water, it fails to offer accurate pH values for extremely dilute buffer solutions.

Solved Example

1)A buffer solution is made from 0.4M CH_3COOH and 0.6M CH_3COO^-. If the acid dissociation constant of CH_3COOH is $1.8*10^{-5}$, what is the pH of the buffer solution?

As per the Henderson-Hasselbalch equation, $pH = pK_a + \log([CH_3COO^-]/[CH_3COOH])$

Here, $K_a = 1.8*10^{-5} \Rightarrow pK_a = -\log(1.8*10^{-5}) = 4.7$ (approx.).

Substituting the values, we get:

$pH = 4.7 + \log(0.6M /0.4M) = 4.7 + \log(1.5) = 4.7 + 0.17 = 4.87$

Therefore, the pH of the solution is 4.87.

Henderson Hasselbalch Equation

$$pH= pK_a + \log \frac{[conjugate\ base]}{[weak\ acid]} \text{ (for weak acid)}$$

$$pOH= pK_b + \log \frac{[conjugate\ acid]}{[weak\ base]} \text{ (for weak base)}$$

- Physiological buffers are chemicals used by the body to prevent large changes in the pH of a bodily fluid. Physiological buffer system usually consists of a weak acid and its conjugate base.

the protection against stressful experiences that is afforded by an individual's social support.

Solution

Buffer system:

1. A buffer is a solution that can withstand changes in pH.
2. It is caused by the addition of acidic or basic components.
3. Buffer solutions are broadly classified into two types:
4. acidic buffer solutions and alkaline buffer solutions.

Carbonic acid bicarbonate buffer system:

1. The carbonic acid bicarbonate buffer system is made up of carbonic acid.
2. They are weak acids, and their conjugate base is bicarbonate anion.

Phosphate buffer system:

1. Although it is not necessary as an extracellular fluid buffer.
2. It is essential in buffering renal tubular fluid and intracellular fluids.
3. When a strong base, such as NaOH, is added to the buffer system

Protein buffer system:

All proteins have the ability to act as buffers.

Proteins are made up of amino acid

It contains both positively and negatively charged amino groups and carboxyl groups.

These molecules' charged regions can bind hydrogen and hydroxyl ion.