



Buffers I

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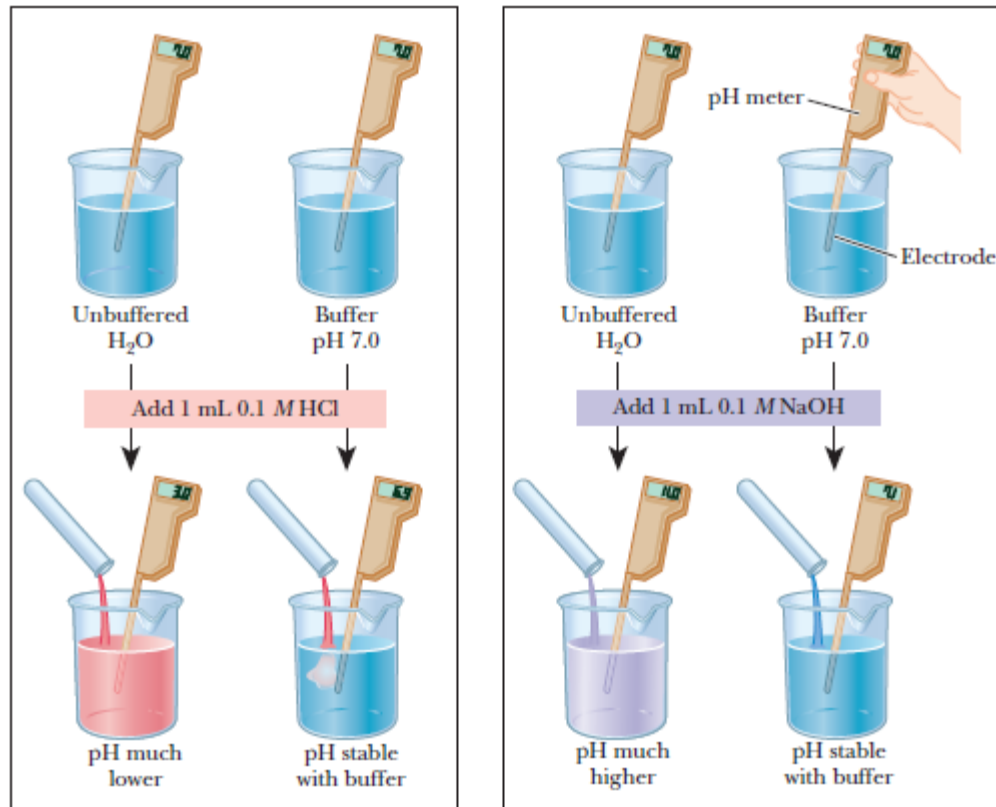
Lecture 4

MD summer 2014

Buffers

Significant changes in pH are harmful, ex. disruption of DNA double helix, disruption of protein structure, etc...

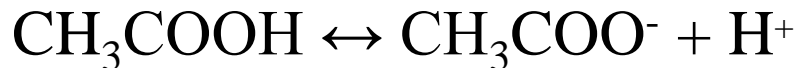
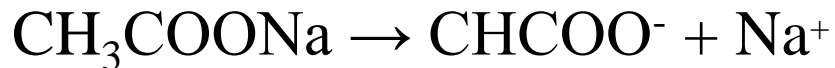
Buffers are solutions that resist abrupt and sudden changes in pH.



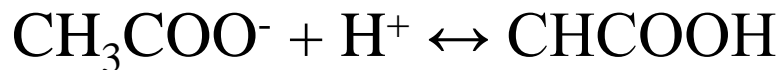
Buffer Action

Resists changes in PH when small amount of Acids or Alkali are added.

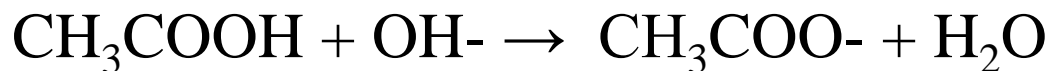
Acidic Buffer = Weak acid + salt of the acid (Acid + strong Base)



If an acid is added, the extra H^+ combine with acetate \rightarrow un-dissociated HA, so $[\text{H}^+]$ remains constant.



If an alkali is added, the OH^- ions are removed by reactions with un-dissociated and to form water so $[\text{H}^+]$ remains constant



Buffering action

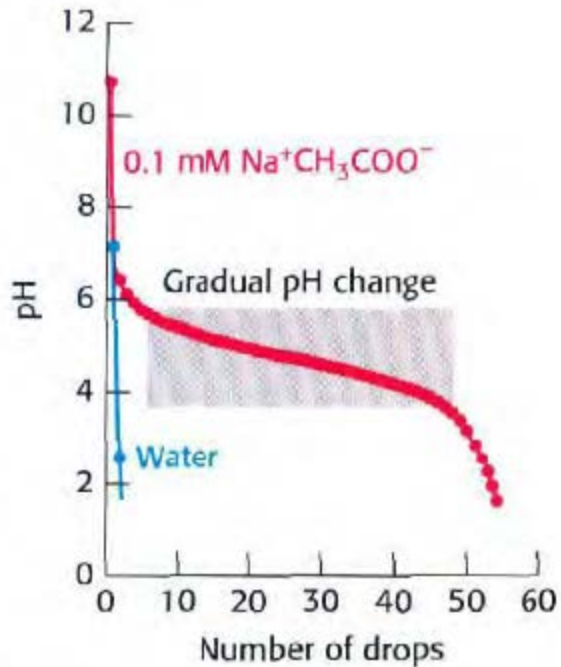


Figure 1.17 Buffering action. The addition of a strong acid, 1 M HCl, to pure water results in an immediate drop in pH to near 2. In contrast, the addition of the acid to a 0.1 M sodium acetate ($\text{Na}^+ \text{CH}_3\text{COO}^-$) solution results in a much more gradual change in pH until the pH drops below 3.5.

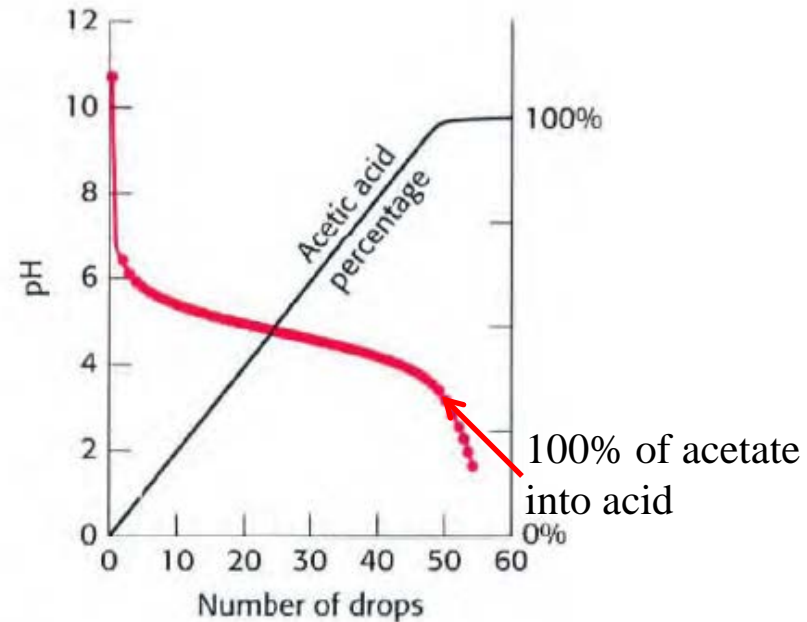


Figure 1.18 Buffer protonation. When acid is added to sodium acetate, the added hydrogen ions are used to convert acetate ion into acetic acid. Because the proton concentration does not increase significantly, the pH remains relatively constant until all of the acetate has been converted into acetic acid.

Preparation of buffers

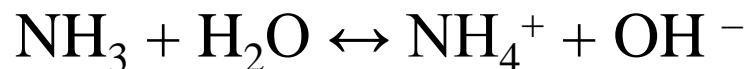
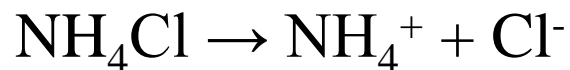


Buffer solution

Alkali Buffer:

PH > 7.0

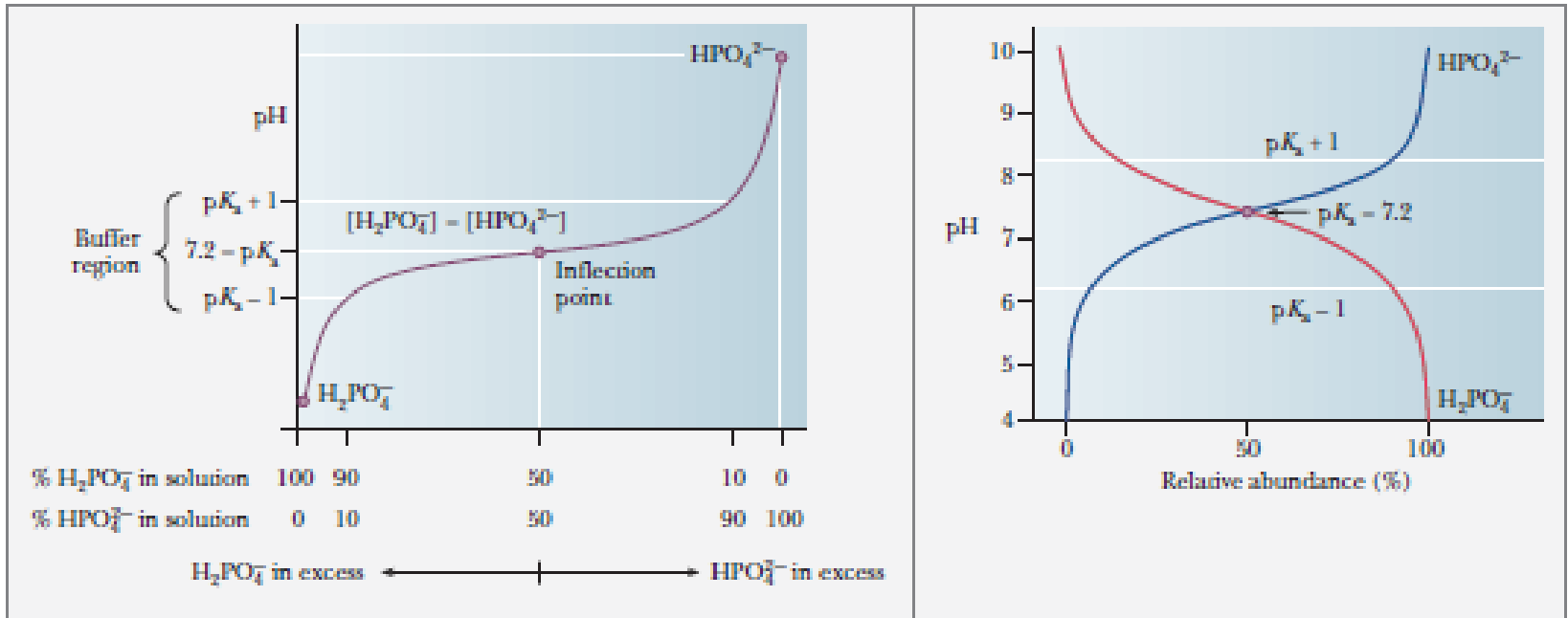
Weak base + Salt of the base



Excess H^+ combines with OH^-

Excess OH^- combines with $\text{NH}_4^+ \rightarrow \text{NH}_3$

Titration curve and buffer action



1 The titration curve of H_2PO_4^- , showing the buffer region for the $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ pair.

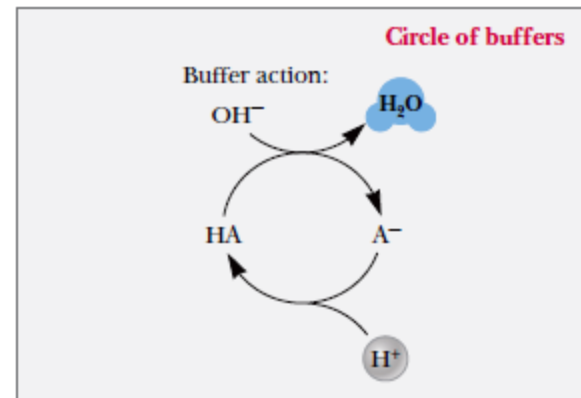
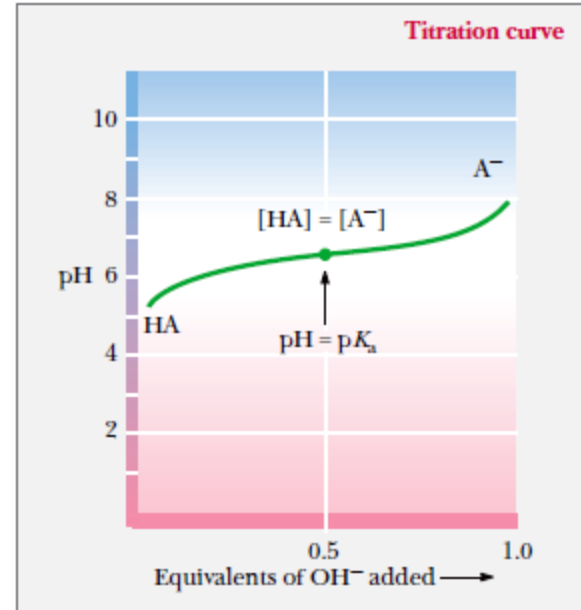
2 Relative abundance of H_2PO_4^- and HPO_4^{2-} .

■ **FIGURE 2.16** The relationship between the titration curve and buffering action in H_2PO_4^- .

Buffering action

Buffers work because the concentration of the weak acid and base is kept in the narrow window of the acid titration curve.

The buffer capacity is the amount of acid or base that can be added to a buffer solution before a significant change in pH occurs



■ **ACTIVE FIGURE 2.16** Two ways of looking at buffers. In the titration curve, we see that the pH varies only slightly near the region in which $[HA] = [A^-]$. In the circle of buffers, we see that adding OH^- to the buffer converts HA to A^- . Adding H^+ converts A^- to HA.

Factors affecting buffering capacity

Buffering Capacity depends on:

1. Buffer concentration
2. pKa of the buffer
3. The desired pH



How do we choose buffers?

Buffer selection criteria:

1. Suitable pKa for the buffer.

pKa ± 1 pH unit from the pH of the reaction
pKa $\pm \frac{1}{2}$ pH unit is even better

2. No interference with the reaction.

3. Suitable buffering capacity.

4. No precipitation of reactants or products due to presence of the buffer.

5. Nonbiological nature of the buffer.

Biochemical buffers

Table 2.8

Acid and Base Form of Some Useful Biochemical Buffers

Acid Form		Base Form	pK _a
TRIS—H^+ (protonated form) $(\text{HOCH}_2)_3\text{CNH}_3^+$	N— <i>tris</i> [hydroxymethyl]aminomethane (TRIS)	TRIS (free amine) $(\text{HOCH}_2)_3\text{CNH}_2$	8.3
TES—H^+ (zwitterionic form) $(\text{HOCH}_2)_3\text{CNH}_3^+\text{CH}_2\text{CH}_2\text{SO}_3^-$	N— <i>tris</i> [hydroxymethyl]methyl-2-aminoethane sulfonate (TES)	TES^- (anionic form) $(\text{HOCH}_2)_3\text{CNHCH}_2\text{CH}_2\text{SO}_3^-$	7.55
HEPES—H^+ (zwitterionic form) $\text{HOCH}_2\text{CH}_2\text{N}^+\text{CH}_2\text{CH}_2\text{SO}_3^-$	N—2—hydroxyethylpiperazine-N'-2-ethane sulfonate (HEPES)	HEPES^- (anionic form) $\text{HOCH}_2\text{CH}_2\text{NCH}_2\text{CH}_2\text{SO}_3^-$	7.55
MOPS—H^+ (zwitterionic form) $\text{O}^+\text{NCH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$	3—[N—morpholino]propane-sulfonic acid (MOPS)	MOPS^- (anionic form) $\text{O}^-\text{NCH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$	7.2
PIPES—H^+ (protonated dianion) $\text{O}_3\text{SCH}_2\text{CH}_2\text{N}^+\text{CH}_2\text{CH}_2\text{SO}_3^-$	Piperazine—N,N'- <i>bis</i> [2-ethanesulfonic acid] (PIPES)	PIPES^{2-} (dianion) $\text{O}_3\text{SCH}_2\text{CH}_2\text{NCH}_2\text{CH}_2\text{SO}_3^-$	6.8



pH in the body

- Blood pH is between 7.36 and 7.44
- Intracellular pH is between 6.9 and 7.4
- The widest range of extracellular pH over which the metabolic functions can be maintained is 6.8 to 7.8
- When $\text{pH} < 7.36$, acidosis results. $\text{pH} < 7.25$ life threatening
- Acidosis causes CNS depression and coma
- $\text{pH} < 7$ death occurs.

Buffer systems in the body:

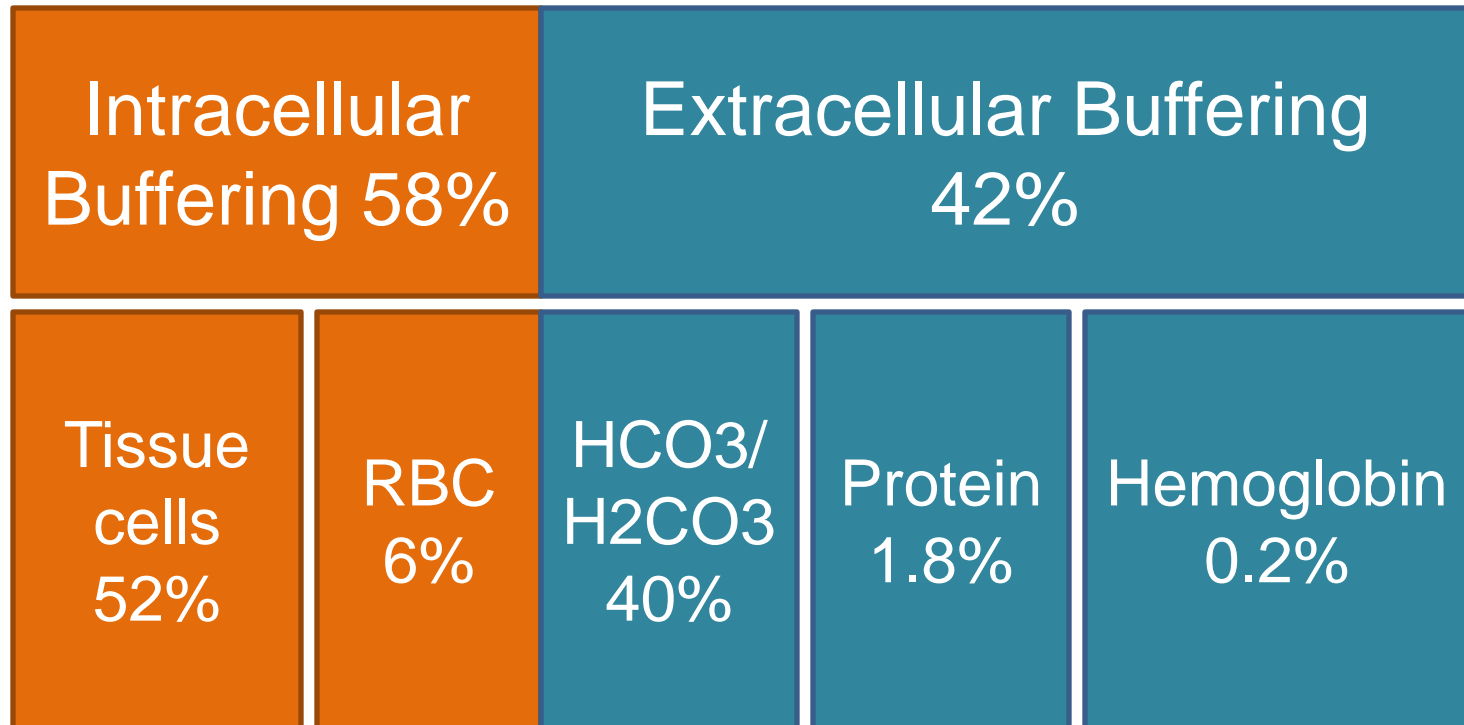
- 1.The bicarbonate–carbonic acid buffer system (ECF)
- 2.The hemoglobin buffer system in RBCs
- 3.The phosphate buffer system in all types of cells
- 4.The protein buffer system of cells and plasma.

-Buffers act quickly but not permanently

-Respiratory and renal mechanisms essentially act for final elimination.

-Buffers do not remove acids or replenish alkali in the body

Relative capacity of the buffer systems in the body



Regulatory Mechanism against changes in [H⁺] of blood

- Buffer Mechanism- first line of defense
- Respiratory Mechanism -second line of defense
- Renal Mechanism- third line of defense

The first two lines of defense keep the [H⁺] from changing too much until the more slowly responding third line of defense, the kidney's can eliminate the excess acid or base from body

The principal buffers of the blood

Buffer System	Plasma Extra cellular buffer	Erythrocyte Intra cellular buffer
Bicarbonate	$\text{NaHCO}_3 / \text{H}_2\text{CO}_3$	$\text{KHCO}_3 / \text{H}_2\text{CO}_3$
Phosphate	$\text{Na}_2\text{HPO}_4 / \text{NaH}_2\text{PO}_4$	$\text{K}_2\text{HPO}_4 / \text{KH}_2\text{PO}_4$
Protein	Na. protein / H. protein	KHb/ H.Hb KHbO ₂ /H.HbO

Types of acids produced in the body

Two types of metabolic acids produced:

1. Fixed Acids, non-gaseous

- Phosphoric and Sulphuric acids

Produced from Sulphur and Phosphorus of proteins and lipoprotein

- Organic Acids as pyruvic, lactic, ketoacids (e.g acetoacetic, B-hydroxy butyric acid and uric acid). Buffered and then H^+ is excreted by the kidneys

2. Volatile Acids

The physiological importance = Carbonic acid

Amount produced daily equivalent to 36 liters of 1.0 M acids

Excreted as CO_2 by the lungs

20,000 mEq/day

Acidosis

-Metabolic acidosis:

Starvation, untreated diabetes,
high-protein diet, low-fat diet

-Respiratory acidosis

- When $\text{pH} < 7.36$, acidosis results. $\text{pH} < 7.25$ life threatening

-Acidosis causes CNS depression and coma

- $\text{pH} < 7$ death occurs.

Alkalosis

-pH > 7.44

-pH > 7.55 is dangerous and > 7.60 results in death.

-Metabolic alkalosis:

Excess clinical administration of salts and metabolic acids as Na lactate and NaHCO_3
Deamination of amino acids, citrate
Severe vomiting

-Respiratory alkalosis :

Hyperventilation (heavy breathing)
Hysteria, anxiety or altitude sickness

Example:

Calculate the ratio of the concentration of acetate ion to the concentration of acetic acid in a titration process of sodium acetate. The pKa of acetic acid is 4.75.

At:

- A. pH 4.75
- B. pH 9
- C. pH 3

$$\text{pH} = \text{pK}_a + \log\left(\frac{[\text{Acetate ion}]}{[\text{Acetic acid}]}\right)$$

$$\frac{[\text{Acetate ion}]}{[\text{Acetic acid}]} = 10^{(\text{pH} - \text{pK}_a)}$$

A. $\frac{[\text{Acetate ion}]}{[\text{Acetic acid}]} = 1$

B. $\frac{[\text{Acetate ion}]}{[\text{Acetic acid}]} = 10^{4.25} = 17,782.79 \approx 18,000$

C. $\frac{[\text{Acetate ion}]}{[\text{Acetic acid}]} = 10^{-1.75} = 0.0178 \approx 0.02$

Buffer Calculations

Example 1:

A buffer consist of 0.2 mole CH_3COO^- in 500 ml of 0.1 M CH_3COOH . ($K_a = 1.8 \times 10^{-5}$ M). What is the PH?

$$\text{PH} = \text{p}K_a + \log \text{Base/ Acid}$$

$$\text{PH} = - \log 1.8 \times 10^{-5} + \text{Log } 0.4/0.1$$

OR

$$K_a = [\text{H}^+] \times 0.4 / 0.1 = 1.8 \times 10^{-5}$$

$$[\text{H}^+] = 4.5 \times 10^{-6} \text{ M}$$

$$\begin{aligned} \text{PH} &= - \log 4.5 \times 10^{-6} \\ &= 5.4 \end{aligned}$$

Buffer Calculations

Calculate the mass of sod. propionate to be dissolved in 1L of 1M propionic acid (M.W = 96.08 gr/mole) to give a buffer of PH=4.5 (pKa = 4.87)

$$\text{pH} = 4.5 \quad [\text{H}^+] = 10^{-4.5}$$

$$\text{pKa} = 4.87 \quad \text{Ka} = 10^{-4.87}$$

$$\text{Ka} = [\text{H}^+] [\text{propionate}] / [\text{propionic acid}]$$

$$\begin{aligned} [\text{propionate}] &= 1 \times 10^{-4.87} / 10^{-4.5} \\ &= 0.427 \text{ M} \end{aligned}$$

$$\text{Mass} = 0.427 \times 96.08 = 41.0 \text{ gr}$$

Buffer Calculations

Calculate pH of a buffer when 18 ml of 0.1 M HCL is added to 32 ml of 0.1 M NH₃ (pK_b = 4.75)

Calculate excess NH₃ and salt forms:

$$\text{HCL moles} = 18/1000 \times 0.1 = 1.8 \times 10^{-3}$$

$$\text{NH}_3 \text{ moles} = 32/1000 \times 0.1 = 3.2 \times 10^{-3}$$

$$\text{Ammonium salt} = 1.8 \times 10^{-3}$$

$$\text{Excess Ammonia} = 3.2 \times 10^{-3} - 1.8 \times 10^{-3} = 1.4 \times 10^{-3}$$

$$\begin{aligned} \text{Molar conc. of salt} &= 1.8 \times 10^{-3} / (50/1000) \\ &= 3.6 \times 10^{-2} \end{aligned}$$

$$\begin{aligned} \text{Molar conc. of NH}_3 &= 1.4 \times 10^{-3} / (50/1000) \\ &= 3.2 \times 10^{-2} \end{aligned}$$

$$K_b = [\text{NH}_4^+] [\text{OH}^-] / \text{NH}_3$$

$$10^{-4.75} = (3.6 \times 10^{-2} \times [\text{OH}^-]) / 3.2 \times 10^{-2}$$

$$\begin{aligned}[\text{OH}^-] &= 0.889 \times 10^{-4.75} \\ &= 1.58 \times 10^{-5} \text{ M}\end{aligned}$$

$$\begin{aligned}\text{p OH} &= -\log 1.58 \times 10^{-5} \\ &= 4.8\end{aligned}$$

$$\begin{aligned}\text{p H} &= 14 - 4.8 \\ &= 9.2\end{aligned}$$

OR

$$\begin{aligned}\text{p OH} &= \text{p Kb} + \log [\text{Salt}] / [\text{weak Base}] \\ \text{p OH} &= 4.75 + \log [3.6 \times 10^{-2}] / [3.2 \times 10^{-2}] \\ &= 4.75 + 0.05 \\ &= 4.8\end{aligned}$$

$$\begin{aligned}\text{PH} &= 14 - 4.8 \\ &= 9\end{aligned}$$

Indicators

- pH indicator is a dye that appears in different colors in its protonated and deprotonated forms.
- Often organic dye
- Different indicators change color over different pH ranges
- Effective indicators change color rapidly at the equivalence point to an end-point color.

Property	Phenolphthalin	Methyl orange
pKa	9.6	3.7
pH	8.3-10.0	3.1-4.4
Color in acids	Colorless	Red
Color in alkali	Pink	Yellow
Uses	Titrations of strong bases	Titrations of strong acids

Questions