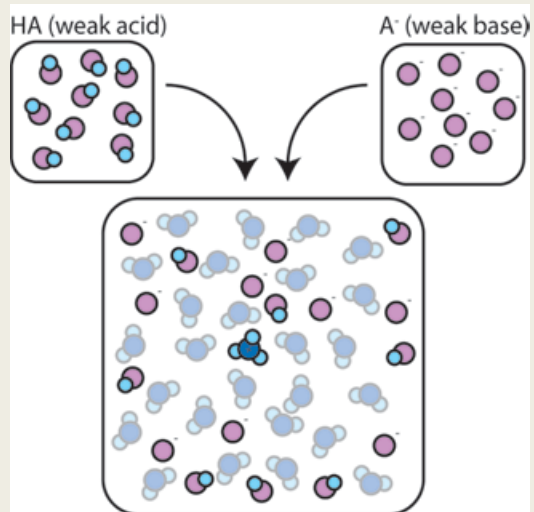


# Preparation Of Buffer Solution

BCH 202 [Practical]

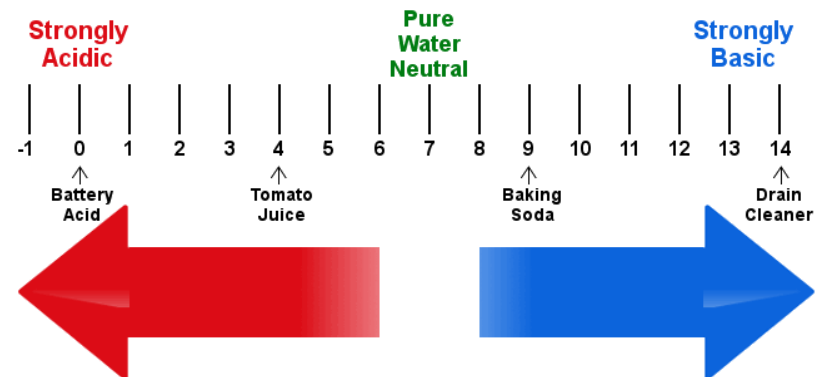


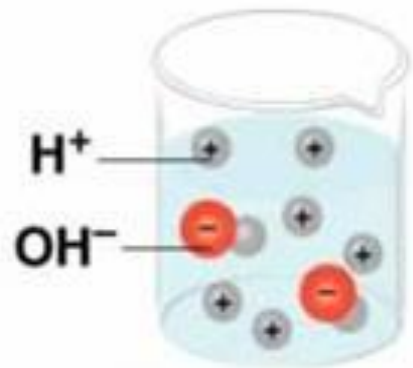
# Hydrogen number pH

- The formula that describe the acidity of certain solutions by using hydrogen number pH can be defined as: The negative logarithm of the hydrogen ion concentration.

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

- Notes: the pH increase when the concentration of hydrogen ion decrease and vice versa.

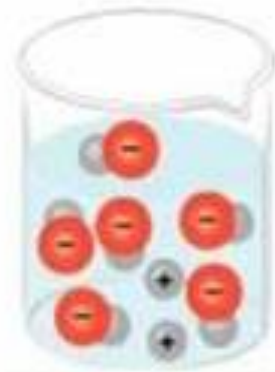




Acidic solution



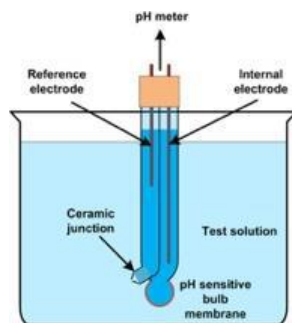
Neutral solution



Basic solution

# Measuring of hydrogen number

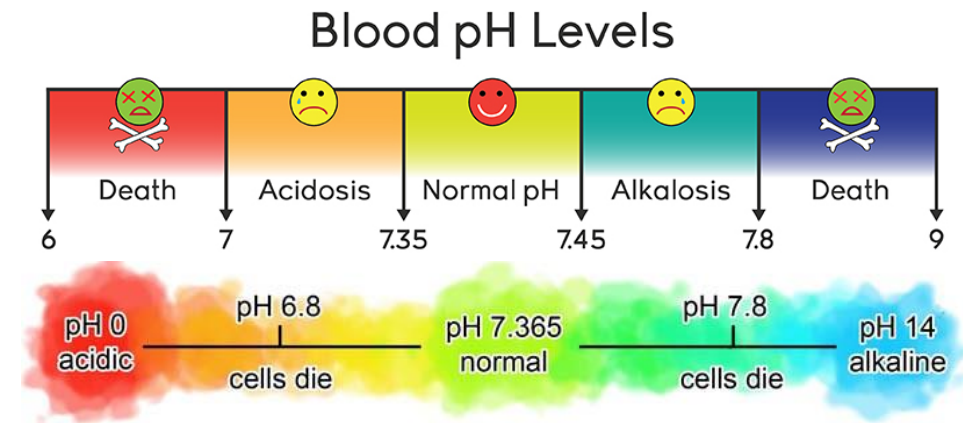
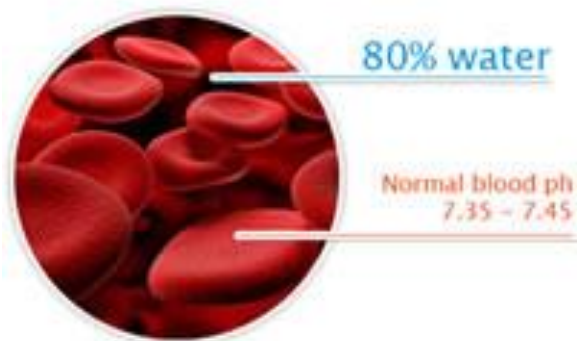
- ❑ To measure the hydrogen number in certain solution in very accurate way, we use a special instrument called pH meter.
- ❑ It's consist of glass electrode which contain a very thin bulb, blown onto a hard glass tube. which is sensitive to pH.
- ❑ The bulb contains a solution of hydrochloric acid and is connected to a platinum lead via silver - silver chloride electrode which is reversible with respect to hydrogen ions.



# Introduction:

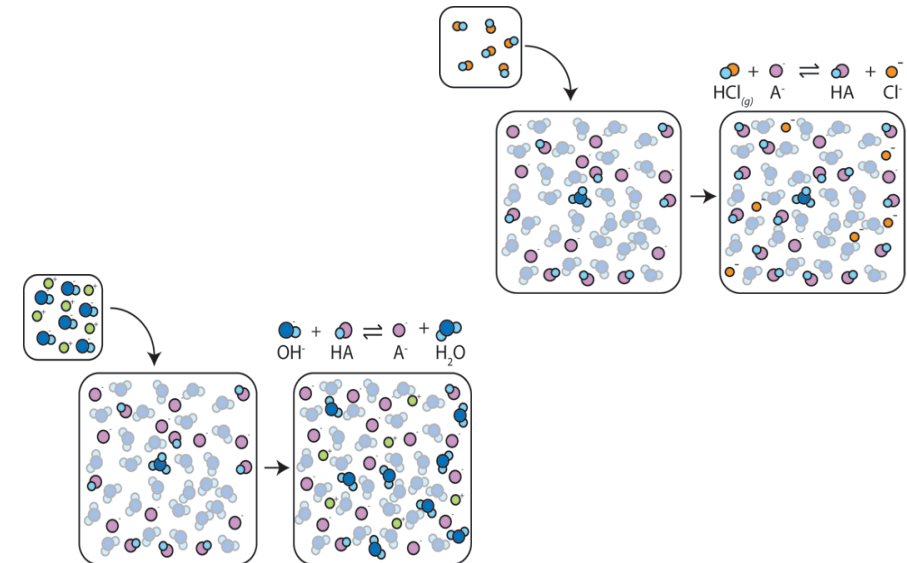
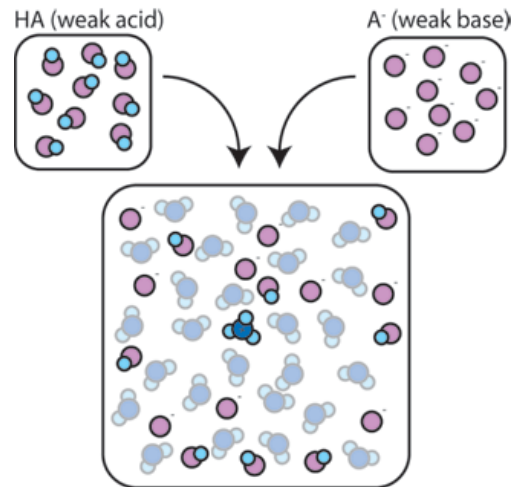
- All **biochemical reactions** occur under strict conditions of the **concentration of hydrogen ion**.
- **Biological life** cannot withstand large changes in hydrogen ion concentrations which we measure as the pH.

Those solutions that have the ability to resist changes in pH upon the addition of limited amounts of acid or base are called **Buffers**.



# Buffers

- are solutions that have the ability to **resist changes in pH**. upon the addition of limited amounts of acid or base.
- A buffer is made up of a weak acid and its conjugate base. Or a weak base and its conjugate acid.



# Mechanism of Action (Buffer):

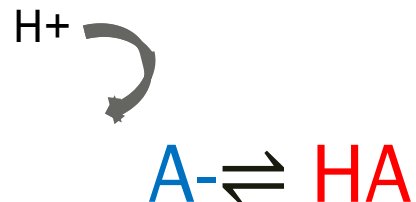
How buffers can resist the change in pH?

Example using [HA/A<sup>-</sup>] buffer

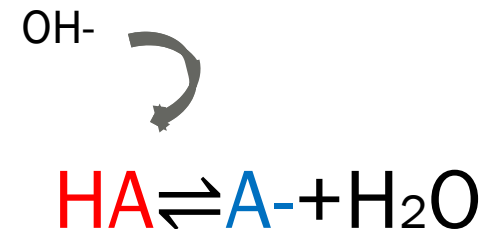
HA: Weak acid. A<sup>-</sup>: conjugated base [its salt].



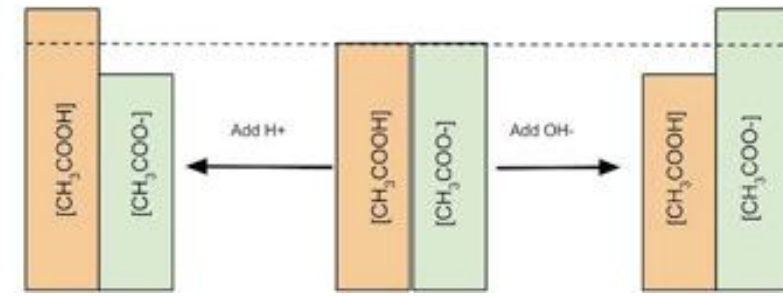
[a] If H<sup>+</sup> is added to this buffer system → H<sup>+</sup> will react with **conjugated base** → to give **conjugate acid**.



[b] If OH<sup>-</sup> is added to this buffer system [HA/A<sup>-</sup>] → OH<sup>-</sup> will react with **conjugated acid** to give **conjugate base** and H<sub>2</sub>O.



# Mechanism of Action (Buffer):



- **Example:**

- **Buffer system:**  $\text{CH}_3\text{COOH} / \text{CH}_3\text{COO}^-$ , ( $\text{CH}_3\text{COOH}$  :acid -  $\text{CH}_3\text{COO}^-$ : conjugated base )

- When acid  $[\text{H}^+]$  added:  $\longrightarrow \text{CH}_3\text{COO}^- + \text{H}^+ \longrightarrow \text{CH}_3\text{COOH}$  

- When base  $[\text{OH}^-]$  added:  $\longrightarrow \text{CH}_3\text{COOH} + \text{OH}^- \longrightarrow \text{CH}_3\text{COO}^-$   +  $\text{H}_2\text{O}$

- **NOTE:** It resists pH changes when it's two components are present in specific proportions.

- Thus a buffer can protect against pH changes from added  $\text{H}^+$  or  $\text{OH}^-$  ion as long as there is **sufficient** basic and acidic forms respectively. As soon as you run out of one of the forms you no longer have a buffer .



# Henderson-Hasselbalch equation

- The **Henderson-Hasselbalch equation** is an equation that is often used to:

1- To calculate the PH of the Buffer.

2-To prepare Buffer.

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

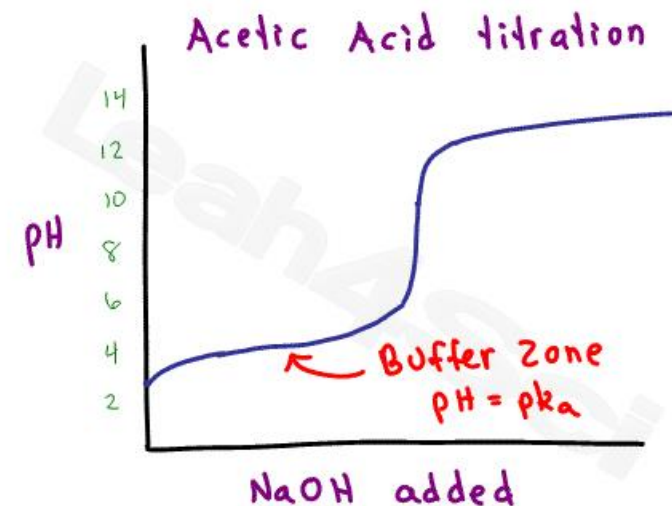
- It relates the **K<sub>a</sub>** [dissociation constant] of a weak acid, **[HA]** concentration Of weak acid, **[A<sup>-</sup>]** concentration Of conjugate base [salt of the weak acid] components and the **pH of the buffer**.

- A buffer is best used close to its pKa.[to act as a good buffer the pH of the solution must be within one pH unit of the pKa].
- The buffer capacity is optimal when the ratio of the weak acid to its salt is 1:1; that is, when  $\text{pH} = \text{pKa}$

$$\text{pH} = \text{pKa} + \log 1$$

$$\text{pH} = \text{pKa} + 0$$

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



# Buffer capacity

Quantitative measure of this resistance to pH changes is called **Buffer capacity**

- Buffer capacity can be defined in many ways, it can be defined as:
  - ▶ The number of moles of  $\text{H}^+$ / $\text{OH}^-$  ions that must be added to one liter of the buffer in order to decrease /increase the pH by one unit respectively.
  
- **Buffer capacity is directly proportional to the buffer concentration.**

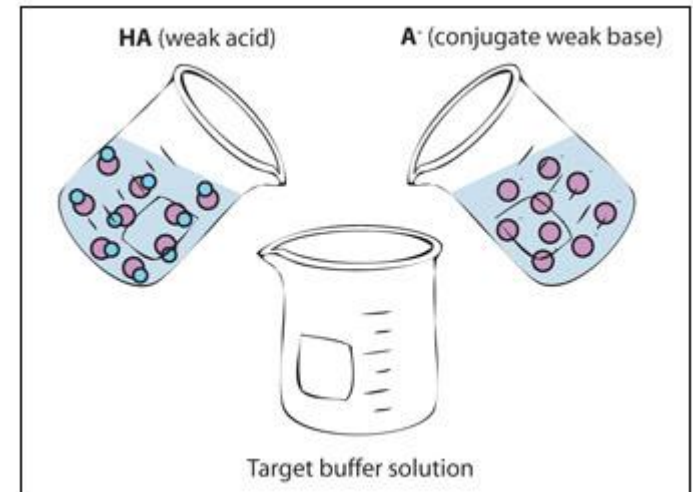
# Practical Part

Buffer solution preparation

Studying the properties of  
buffer solutions

# Objective:

- 1) To understand the nature of buffers solutions.
- 2) To learn how to prepare buffers.



# Preparation of phosphate buffer:

**Example: Prepare 500ml from phosphate buffer with concentration 0.25M and pH=7.4, if you know that (pKa=7.2)**

**You are provided** with buffer solution content; Monosodium dihydrogen phosphate  $\text{NaH}_2\text{PO}_4$  and Disodium hydrogen phosphate  $\text{Na}_2\text{HPO}_4$ .

## ■ Provided:

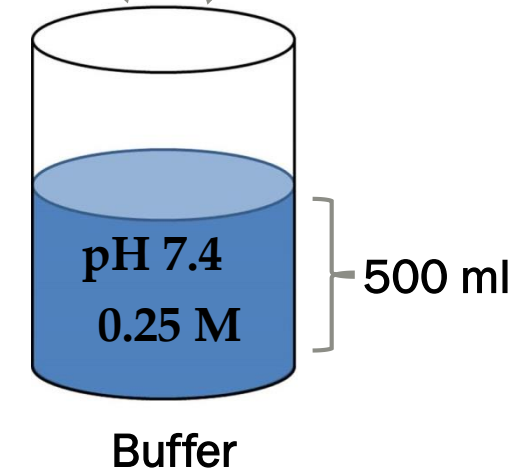
- $\text{pK}_a = 7.2$
- Final volume of buffer = 500ml
- $\text{pH} = 7.4$
- Conc = 0.25M

## ■ Required:

- Weight (g) of Monosodium dihydrogen phosphate  $\text{NaH}_2\text{PO}_4$
- Weight (g) of Disodium hydrogen phosphate  $\text{Na}_2\text{HPO}_4$



[HA]                      [A-]  
g?                              g?



# Calculations

- To prepare a buffer Henderson-Hasselbalch equation is used:

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

- 1) First calculate the concentrations of HA and A<sup>-</sup> in the buffer:

Assume  $[\text{A}^-] = y$  ,  $[\text{HA}] = 0.25 - y$

$$7.4(\text{of buffer}) = 7.2 + \log \frac{y}{0.25-y} \rightarrow 0.2 = \log \frac{y}{0.25-y}$$

by taking the “Anti log for both sides”:

$$1.6 = \frac{y}{0.25-y} \rightarrow y = 1.6 \times (0.25 - y) \rightarrow y = 0.4 - 1.6y \rightarrow y + 1.6y = 0.4 \rightarrow 2.6y = 0.4$$

- $y = 0.15 \text{ M}$  [which is the concentration of  $[\text{A}^-]$  in the buffer ]
- Buffer Concentration =  $[\text{HA}] + [\text{A}^-]$        $0.25 = [\text{HA}] + [\text{A}^-]$
- So,  $[\text{HA}] = 0.25 - 0.15 = 0.1 \text{ M}$  [which is the concentration of  $[\text{HA}]$  in the buffer ]

# Calculations Cont'

- 2) calculate the weight needed from [A-] to prepare the buffer, No. of mole of [A- ] should be calculated first :
- Calculate **moles** in buffer: No. of mole (of A-) = molarity (of A- calculated in buffer) X volume L (volume of buffer required)

$$= 0.15 \times 0.5 = \underline{0.075 \text{ mole}}$$

- Calculate the **wight** of stock A-: wt in (g) of [A-] = mole X Mw

$$= 0.075 \times 142 = \boxed{10.65 \text{ g}}$$

- 3) calculate the weight needed from [HA] to prepare the buffer, No. of mole of [HA ] should be calculated first :
- Calculate **moles** in buffer: No. of mole (of HA) = molarity (of HA calculated in buffer) X volume L (volume of buffer required)

$$= 0.1 \times 0.5 = \underline{0.05 \text{ mole}}$$

- Calculate the **wight** of stock A-: wt in (g) of [A-] = mole X Mw

$$= 0.05 \times 120 = \boxed{6 \text{ g}}$$



# Method

- Now take .....g from  $\text{NaH}_2\text{PO}_4$  and ..... g from  $\text{Na}_2\text{HPO}_4$  then complete the volume up to 500 ml by addition of water.
- Check the pH

## (2) Testing for buffering behavior:

### Method:

- In one beaker add 10ml of 0.25M Phosphate buffer that you have prepared, and in another beaker add 10ml of water.
- Measure the pH.
- Add 0.5ml from 0.1 M HCl to both solutions.
- Measure the pH after the addition.

<b>Solution (10 ml of each)</b>	<b>Measured pH</b>	<b>Add 2M HCl (0.1ml)</b>	<b>pH after HCl</b>
Phosphate buffer		0.1 ml	
water		0.1 ml	

## H.W

You are provided with **acetic acid** and **sodium acetate**.

Prepare **100 ml** of a **0.3M** acetate buffer pH =5.2 if you know that  $pK_a = 4.76$ .