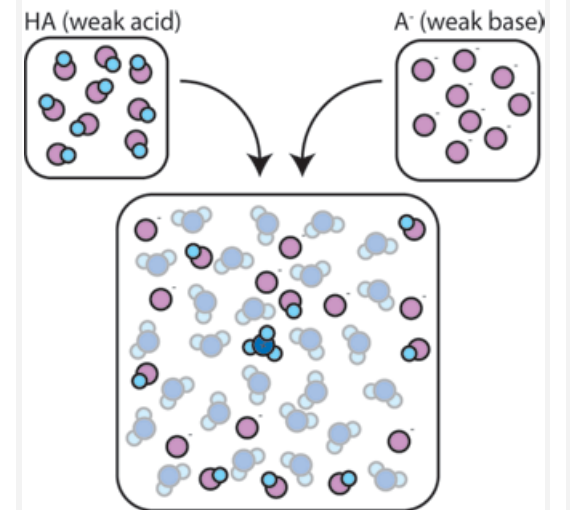


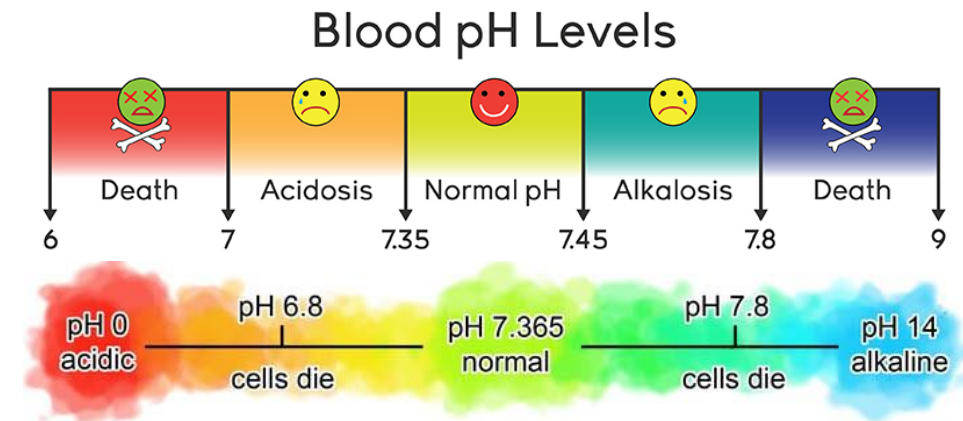
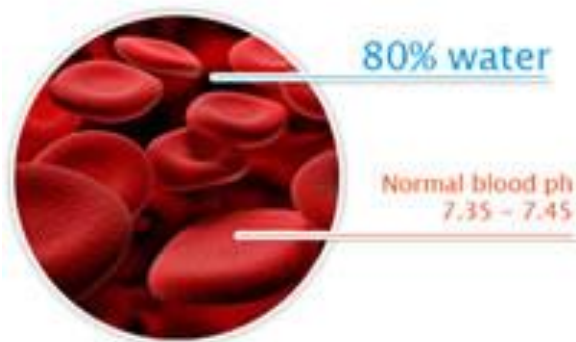
# Preparation Of Different Buffer Solutions



# Introduction:

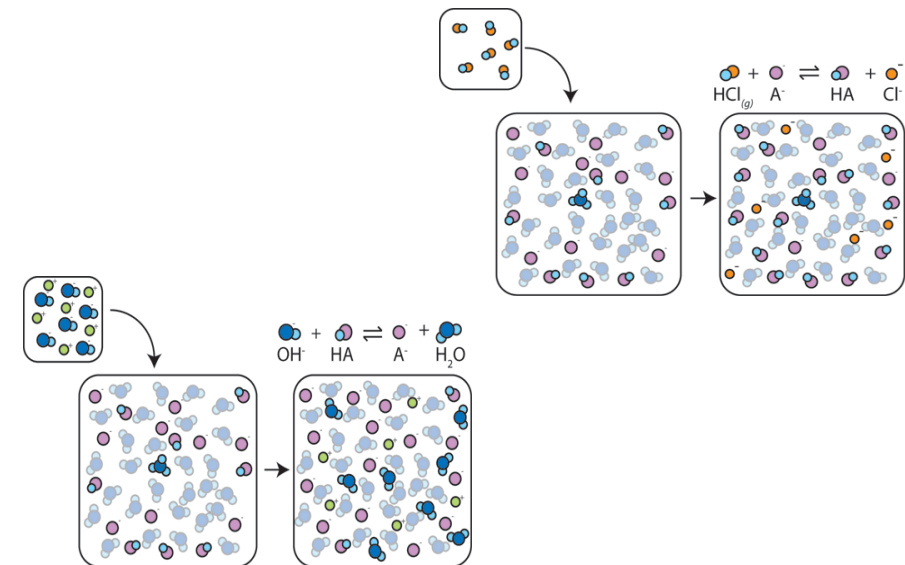
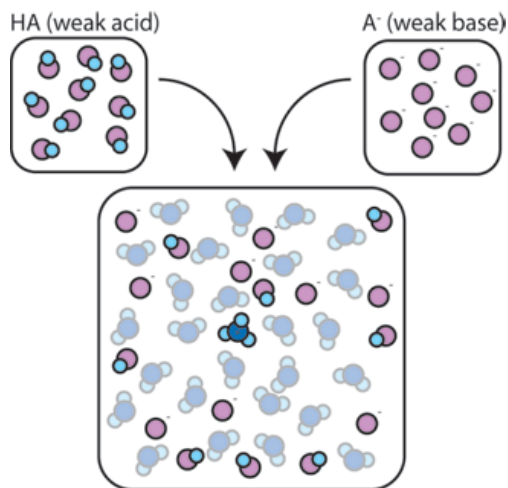
- All **biochemical reactions** occur under strict conditions of the **concentration of hydrogen ion**.
- **Biological life** cannot withstand large changes in hydrogen ion concentrations which we measure as the **pH**.

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.



# Types of Buffer:

## [acidic buffer]

- Is made of a Weak acid and its conjugated base [ its salt]

Example:

1.  $\text{CH}_3\text{COOH} / \text{CH}_3\text{COONa}$  (Pka)

→  $\text{CH}_3\text{COOH}$  (Weak acid)

→  $\text{CH}_3\text{COONa}$  (conjugated base -its salt)

2.  $\text{H}_2\text{CO}_3 / \text{HCO}_3\text{Na}$  (Pka)

3.  $\text{NaH}_2\text{PO}_4 / \text{Na}_2\text{HPO}_4$  (Pka)

## [basic buffer]

- is made of Weak base and its conjugated acid [its salt]

Example:

$\text{NH}_3 / \text{NH}_4\text{CL}$  (Pkb)

→  $\text{NH}_3$  (Weak base)

→  $\text{NH}_4\text{CL}$  (conjugated acid -its salt)

# Mechanism of Action (Buffer):

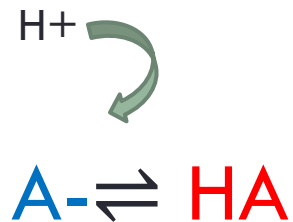
How buffers can resist the change in pH?

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

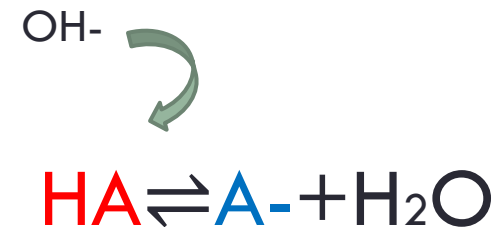
HA: Weak acid. A<sup>-</sup>: conjugated base [its salt].

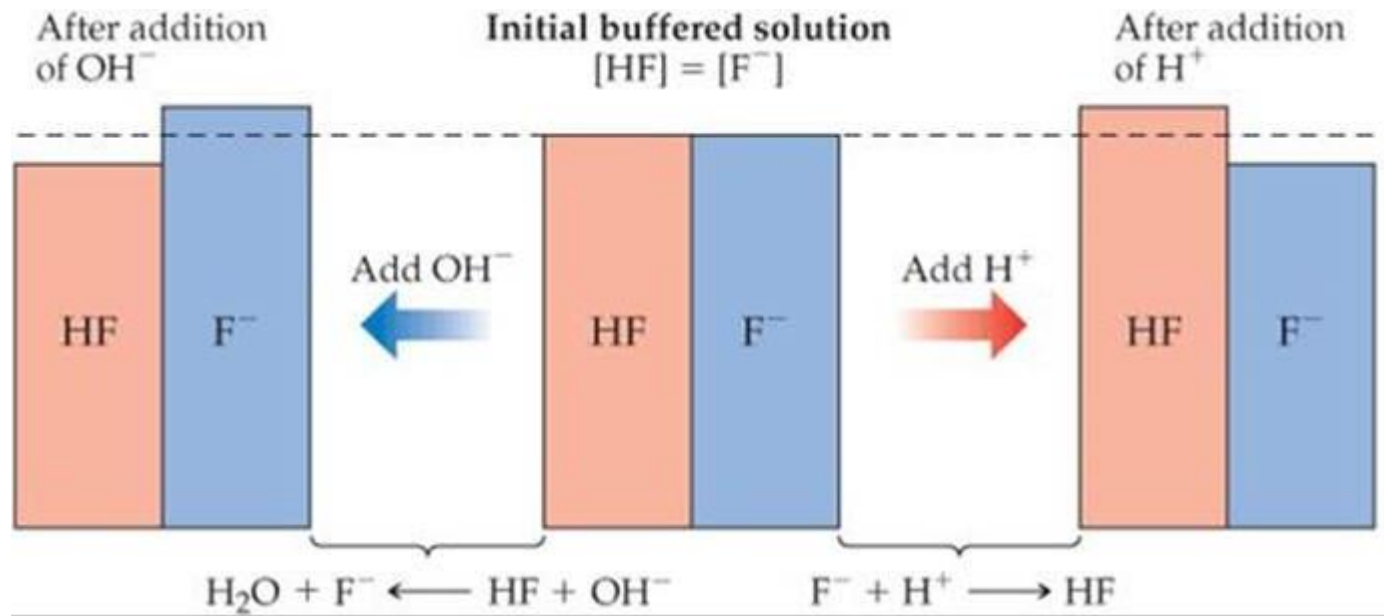
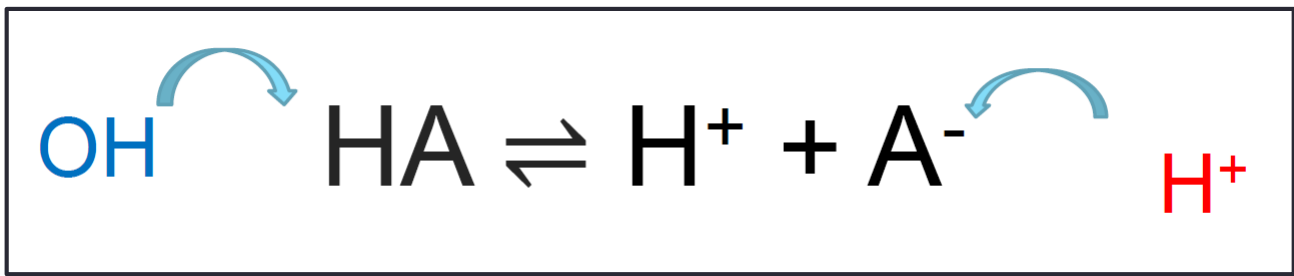


[a] If H<sup>+</sup> is added to this buffer system → H<sup>+</sup> will react with conjugated base → to give conjugate acid.

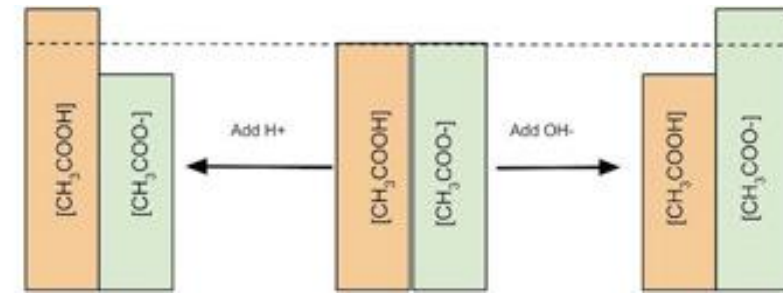


[b] If OH<sup>-</sup> is added to this buffer system [HA/A<sup>-</sup>] → OH will react with conjugated acid to give conjugate base and H<sub>2</sub>O.





# 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:  $\text{CH}_3\text{COO}^- + \text{H}^+ \longrightarrow \text{CH}_3\text{COOH}$  ↑

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

- **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**.

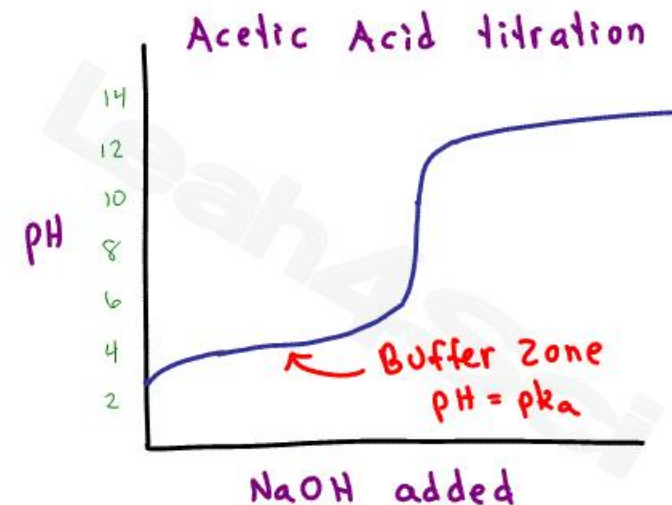


- A buffer is best used close to its pKa. [to act as a good buffer the pH of the solution must be within one pH unit of the pKa].
- The **buffer capacity** is optimal when the **ratio of the weak acid to its salt is 1:1**; that is, when **pH = pKa**

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

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

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



# 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 H<sup>+</sup>/OH<sup>-</sup> ions that must be added to one liter of the buffer in order to decrease /increase the pH by one unit respectively.

$$\beta = \frac{2.3 K_a [H^+][C]}{(K_a + [H^+])^2}$$

From the equation the buffer capacity is **directly proportional** to the buffer concentration.

□ The buffer capacity is **expressed** as  $\beta$  and can be derived from Henderson Hasselbalch equation:

□ Where :  $\beta$  = the buffer capacity , [H<sup>+</sup>] = the hydrogen ion concentration of the buffer , [C] = concentration of the buffer and **K<sub>a</sub>**= acid dissociation constant

# pH Calculation

1- for weak acid [not buffers]:

$$\text{pH} = \frac{\text{pka} + \text{p[HA]}}{2}$$

\*  $\text{P[HA]} = -\log [\text{HA}]$

2- for weak base [not buffers]:

$$\text{pOH} = \frac{\text{pkb} + \text{p[OH]}}{2}$$

\*\*  $\text{P[OH]} = -\log [\text{OH}]$

3- for buffers:

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

\*  $\text{PH} = \text{Pkw} - \text{POH}$ .

$\text{pH} = \text{pkw} - \text{pOH}$

□ [Pkw : number of dissociation constant of H2O].

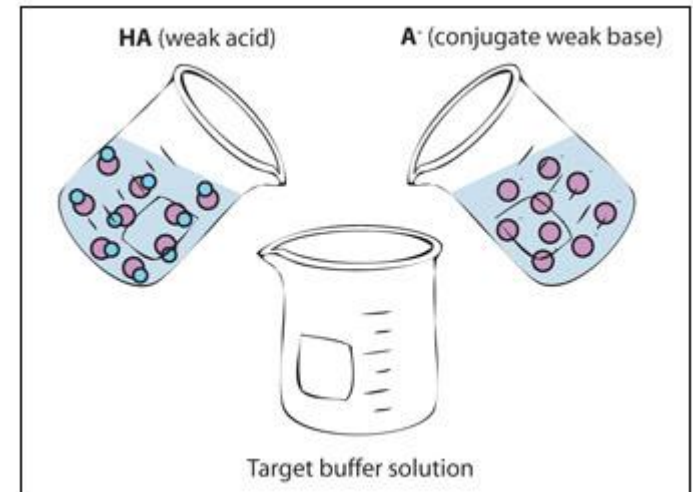
The concentration of HA and A<sup>-</sup>  
in the buffer



# Practical Part

# Objective:

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



# A. Nature of Buffers:

## Method:

You are provided with: 0.2M solution of  $\text{CH}_3\text{COOH}$  , 0.2M solution of  $\text{CH}_3\text{COONa}$ .

1. Determine which is the weak acid and which is the conjugated base [or its salt].
2. Calculate the volume that you must take from  $\text{CH}_3\text{COOH}$  and  $\text{CH}_3\text{COONa}$  to prepare the following mixtures with **final volume of the solution =20 ml** :
  - a. 100% [HA]
  - b. 75% [HA] , 25% [A-]
  - c. 50% [HA] , 50% [A-]
  - d. 25% [HA] , 75% [A-]
3. Calculate the pH for each solution [ $\text{pK}_a$  of  $\text{CH}_3\text{COOH} = 4.76$ ].
4. Follow the table.

# Calculations



## 1- Volume calculation

To Calculate the volume that you must take from CH<sub>3</sub>COOH and CH<sub>3</sub>COONa to prepare previous mixtures in final volume of 20 ml :

□ **(A) 100% [HA]:**

The final volume is 20ml, So:

$$20 \times 100\% = (20 \times 100) / 100 = 20 \text{ ml}$$

→ take 20ml HA and measure the PH.

□ **(B) 75% [HA], 25% [A-]:**

$$\text{From HA} = 20 \times 75\% = (20 \times 75) / 100 = 15 \text{ ml}$$

$$\text{From A-} = 20 \times 25\% = (20 \times 25) / 100 = 5 \text{ ml}$$

→ Mix 15ml HA and 5 ml A- and measure the PH (measured PH) note that the total volume is 20 ml [15ml +5ml =20ml]

□ The same way for the other mixtures.

# Calculations Cont'

## 2- pH calculation

To calculate the pH for the previous mixtures with pKa of CH<sub>3</sub>COOH = 4.76 :

- (A) [100%] HA :

$$\text{pH} = \frac{\text{pka} + \text{p[HA]}}{2}$$

$$\text{p[HA]} = -\log [\text{HA}] = -\log 0.2 = 0.69$$

$$\text{pH} = \frac{4.76 + 0.698}{2} = 2.72$$

- (B) 75%HA , 25% A- :

$$\text{PH} = \text{Pka} + \log [\text{A}^-]/[\text{HA}]$$

$$\text{PH} = 4.76 + \log [\text{A}^-]/[\text{HA}]$$

$$[\text{HA}] = C_1 \times V_1 = C_2 \times V_2 = 0.2 \times 15 = C_2 \times 20 = 0.15\text{M}$$

$$[\text{A}^-] = C_1 \times V_1 = C_2 \times V_2 = 0.2 \times 5 = C_2 \times 20 = 0.05 \text{ M}$$

- So, PH = 4.76+log 0.05/0.15 , pH = 4.282

- (C) 50%[HA] , 50%[A] :

$$\text{pH} = \text{Pka} + \log [\text{A}^-]/[\text{HA}], \text{pH} = 4.76 + \log [\text{A}^-]/[\text{HA}]$$

$$[\text{HA}] = C_1 \times V_1 = C_2 \times V_2 = 0.2 \times 10 = C_2 \times 20 = C_2 = 0.1\text{M}$$

$$[\text{A}^-] = C_1 \times V_1 = C_2 \times V_2 = 0.2 \times 10 = C_2 \times 20 = C_2 = 0.1 \text{ M}$$

$$\text{So, pH} = 4.76 + \log 0.1/0.1, \text{pH} = 4.76 \quad \boxed{\text{[pH=pka]}} \quad \text{Best}$$

- (D) 25% [HA], 75% [A-] :

$$\text{pH} = \text{pka} + \log [\text{A}^-]/[\text{HA}], \text{pH} = 4.76 + \log [\text{A}^-]/[\text{HA}]$$

$$[\text{HA}] = C_1 \times V_1 = C_2 \times V_2 = 0.2 \times 5 = C_2 \times 20 = C_2 = 0.05\text{M}$$

$$[\text{A}^-] = C_1 \times V_1 = C_2 \times V_2 = 0.2 \times 15 = C_2 \times 20 = C_2 = 0.15 \text{ M}$$

$$\text{So, pH} = 4.76 + \log 0.15/0.05, \text{pH} = 5.24$$



- You are provided with: **0.2M** solution of  $\text{CH}_3\text{COOH}$ , **0.2M** solution of  $\text{CH}_3\text{COONa}$ .
- Calculate the volume that you must take from  $\text{CH}_3\text{COOH}$  and  $\text{CH}_3\text{COONa}$
- With (the final volume of the solution = 20 ml) and  $\text{pK}_a$  of  $\text{CH}_3\text{COOH} = 4.76$

Solution	ml HA [ $\text{CH}_3\text{COOH}$ ]	ml A- [ $\text{CH}_3\text{COONa}$ ]	Final vol. [ml]	Calculated pH	Measured pH	Add 0.1 ml 2M Hcl	Measured pH	The difference
100%HA	20 ml	0	20 ml	2.729		0.1		
75%HA, 25%A-	15 ml	5 ml	20 ml	4.28		0.1		
50%HA, 50%A-	10 ml	10 ml	20 ml	4.76		0.1		
25%HA, 75%A-	5 ml	15 ml	20 ml	5.24		0.1		

## (2) Preparation of buffer:

You are provided with **0.2M acetic acid** and **solid sodium acetate**.

Prepare **50ml** of a **0.19M** acetate buffer  $\text{pH} = 4.86$  if you know that  $\text{pK}_a = 4.76$ .

### □ Calculations:

### □ Provided:

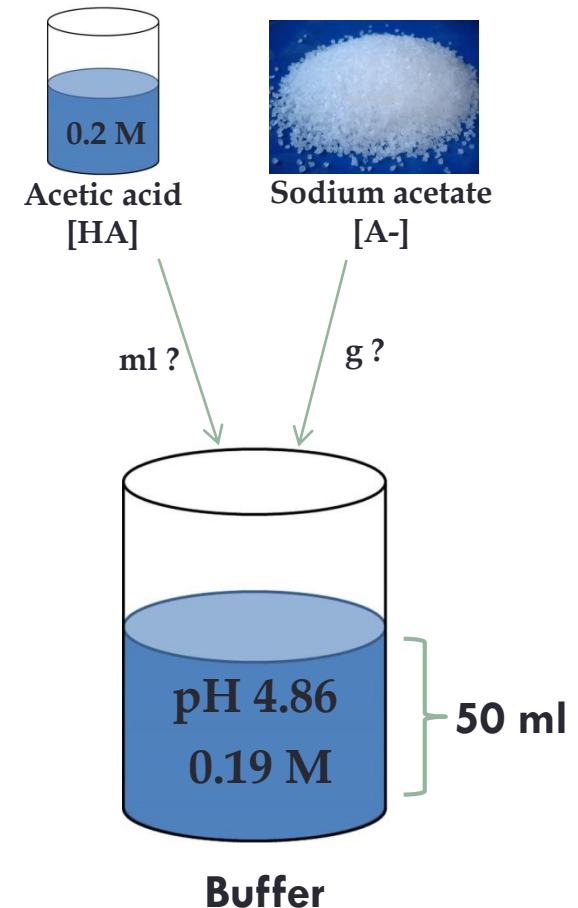
- Solid sodium acetate [as  $\text{A}^-$ ]
- 0.2M Acetic acid. [as HA]
- $\text{pK}_a = 4.76$

### □ Required:

- Final volume of buffer = 50ml
- $\text{pH} = 4.86$
- Buffer concentration = 0.19 M

$$\text{Buffer Concentration} = [\text{HA}] + [\text{A}^-]$$

$$0.19 = [\text{HA}] + [\text{A}^-]$$



# Calculations

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

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

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

Assume  $[\text{A}^-] = y$  ,  $[\text{HA}] = 0.19 - y$

$$4.86 \text{ (of buffer)} = 4.76 + \log \frac{y}{0.19 - y} \rightarrow 0.1 = \log \frac{y}{0.19 - y}$$

by taking the "Anti log for both sides":

$$1.26 = \frac{y}{0.19 - y} \rightarrow y = 1.26 \times (0.19 - y) \rightarrow y = 0.24 - 1.26y \rightarrow y + 1.26y = 0.24 \rightarrow 2.26y = 0.24$$

- $y = 0.11 \text{ M}$  [which is the concentration of **[A<sup>-</sup>]** in the buffer ]
- Buffer Concentration =  $[\text{HA}] + [\text{A}^-]$        $0.19 = [\text{HA}] + [\text{A}^-]$
- So, **[HA]** =  $0.19 - 0.11 = 0.08 \text{ M}$  [which is the concentration of **[HA]** in the buffer ]

# Calculations Cont'

2- To calculate the volume 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)  
$$= 0.08 \times 0.05 = 0.004 \text{ mole}$$

□ Calculate the **volume of stock HA**:  $M \text{ (of stock HA in Q)} = \text{no. of mole (of HA calculated)} / \text{Volume in Liter (volume of HA ?)}$   
$$0.2 = 0.004 / V$$
  
$$V = 0.02 \text{ L} = \boxed{20 \text{ ml}}$$

□ 3- To 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)  
$$= 0.11 \times 0.05 = 0.0055 \text{ mole}$$

□ Calculate the **wight of stock A-**: wt in (g) of [A-] = mole X Mw  
$$= 0.0055 \times 82 = \boxed{0.45 \text{ g}}$$

# Method

- Now take 20 ml from 0.2M acetic acid and 0.45 g from solid sodium acetate then complete the volume up to 50 ml by addition of water.
- Check the pH

### (3) Testing for buffering behavior:

#### Method:

- In one beaker add 10ml of 0.19M acetate buffer that you have prepared, and in another beaker add 10ml of 0.2M KCl.
- Measure the pH.
- Add 0.1ml from 2M HCl to for 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>
0.19M acetate buffer.		0.1 ml	
0.2M KCl.		0.1 ml	

## H.W

You are provided with **0.5M acetic acid** and **0.5M sodium acetate**.  
Prepare **100 ml** of a **0.3M** acetate buffer pH =5.2 if you know that  $pK_a = 4.76$ .