

# Preparation of Different Buffer Solutions

# Buffers:

- 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**.

# Two types of buffers

A buffer is made up of a **weak acid** and its conjugate base.

Or

A **weak base** and its conjugate acid.

## Acidic Buffer

Are made from 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.  $\text{NaH}_2\text{PO}_4 / \text{Na}_2\text{HPO}_4$  (pka)

## Basic Buffer

Are made from weak base and its conjugated acid [its salt].

### Example:

1.  $\text{NH}_3 / \text{NH}_4\text{Cl}$  (pkb)

→  $\text{NH}_3$  (Weak base)

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

# Mechanism of action:

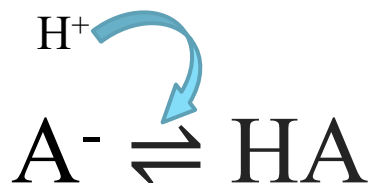
- How buffers can resist the change in pH?

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

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



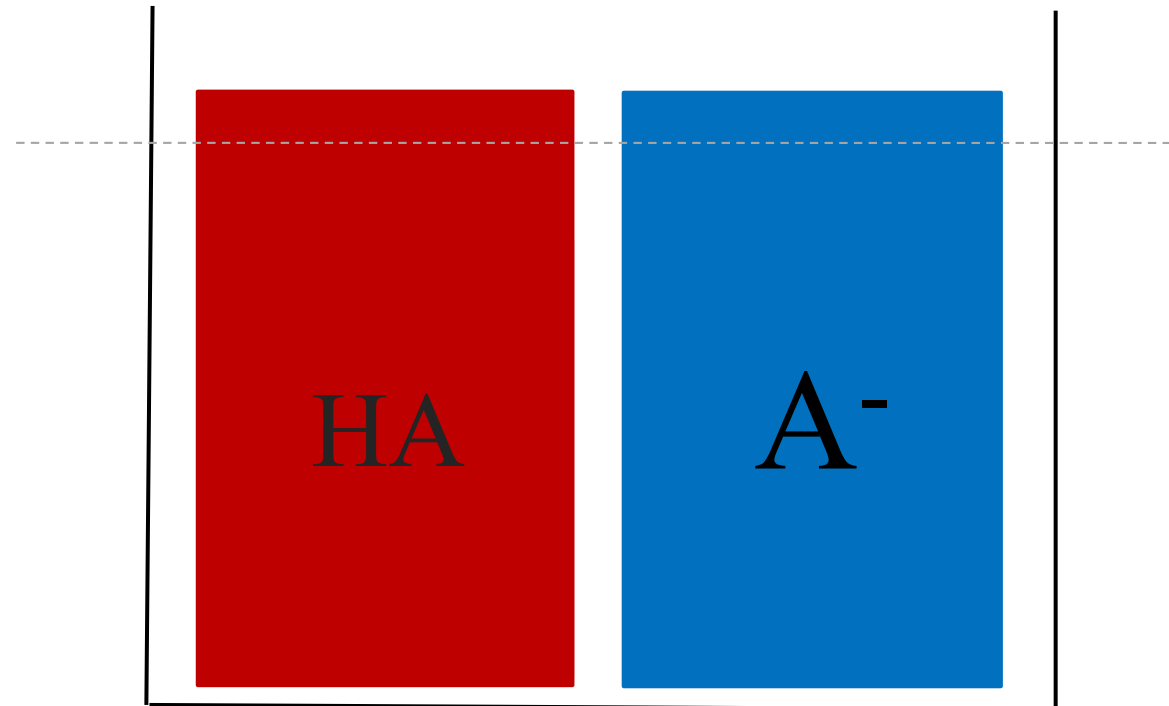
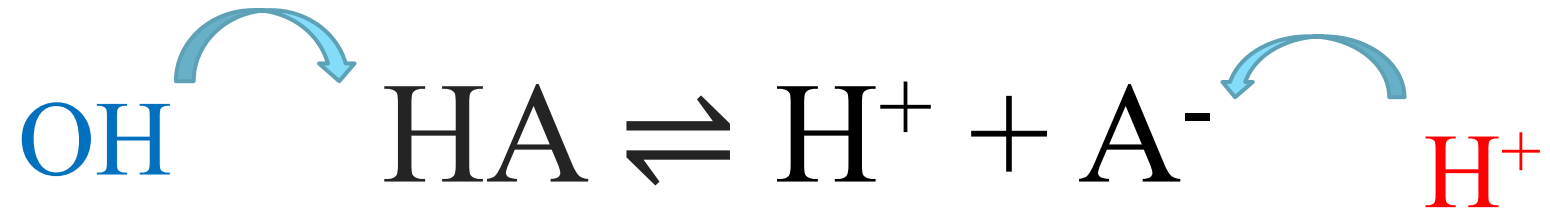
If H<sup>+</sup> (acid) is added to this buffer system → H<sup>+</sup> will react with conjugated base → to give conjugate acid.



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



# Mechanism of action



# Mechanism of action cont':

## □ Example:

Buffer system:  $\text{CH}_3\text{COOH}$  /  $\text{CH}_3\text{COO}^-$

conjugated base

$\text{CH}_3\text{COO}^-$

conjugated acid

$\text{CH}_3\text{COOH}$

When acid  $[\text{H}^+]$  added:



When base  $[\text{OH}^-]$  added:



□ **NOTE:** It resists pH changes when its two components are present in **specific proportions**.

→ Thus the buffer is effective as long **as it does not run out** of one of its components (There are enough conjugated base and conjugated acid to absorb the  $\text{H}^+$  ions or  $\text{OH}^-$  ions added to the system respectively).

# Henderson-Hasselbalch equation:

- It is often used to perform:
  - a. To calculate the pH of the Buffer.
  - b. To preparation of Buffer.

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

- It relates the  $K_a$  [dissociation constant] of a weak acid,  $[HA]$  concentration of weak acid component,  $[A^-]$  concentration of conjugate base [salt of the weak acid] component and the pH of the buffer.
- The equation is derived from the acid dissociation constant.

# Henderson-Hasselbalch equation cont':

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

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

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

$$\text{pH} = \text{pka}$$



# Calculating the pH:

For buffers

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

For weak acid [not buffers]

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

$$p[HA] = -\log [HA]$$
$$pK_a = -\log K_a$$

For weak base [not buffers]

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

$$p[OH] = -\log [OH]$$
$$pK_b = -\log K_b$$

# Practical Part

# Objectives:

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- To understand the behaviour and nature of buffers solutions.
- To learn how to prepare buffers.

# A) Nature of buffers:

## Method:

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

→ 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** :

100% [HA]

75% [HA] , 25% [ $\text{A}^-$ ]

50% [HA] , 50% [ $\text{A}^-$ ]

25% [HA] , 75% [ $\text{A}^-$ ]

3. Calculate the pH for each solution [ $\text{pK}_a$  of  $\text{CH}_3\text{COOH} = 4.76$ ].

4. Follow the table.

# Calculations:

1. To Calculate the volume that you must take from  $\text{CH}_3\text{COOH}$  and  $\text{CH}_3\text{COONa}$  to prepare the previous mixtures with final volume of the solution =20 ml:

(A) 100% [HA]:

The final volume is 20ml, So:

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

→ Take 20ml of HA and measure the pH.

Note:

HA : as  $\text{CH}_3\text{COOH}$ .

$\text{A}^-$  : as  $\text{CH}_3\text{COONa}$ .

(B) 75% [HA], 25% [ $\text{A}^-$  ]:

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

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

→ Mix 15ml HA and 5 ml  $\text{A}^-$  and measure the pH (measured PH) note that the total volume is 20 ml [15ml +5ml =20ml]

□ The same way for other mixtures ...

# Calculations cont':

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

(A) 100% [HA]:

$$\text{pH} = \frac{(\text{pKa} + \text{p}[\text{HA}])}{2} \quad \rightarrow \quad \text{p}[\text{HA}] = -\log 0.2 = 0.7 \quad \rightarrow \quad \text{pH} = \frac{(4.76 + 0.7)}{2} = \underline{2.73}$$

(B) 75% [HA], 25% [A<sup>-</sup>]:

$$\text{pH} = \text{pKa} + \log [\text{A}^-] / [\text{HA}] \quad \rightarrow \quad \text{pH} = 4.76 + \log [\text{A}^-] / [\text{HA}] \quad \rightarrow \quad [\text{HA}] = C_1 \times V_1 = C_2 \times V_2$$
$$= 0.2 \times 15 = C_2 \times 20 = \underline{C_2 = 0.15\text{M}}$$

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

So,  $\text{pH} = 4.76 + \log 0.05/0.15 \rightarrow \text{pH} = \underline{4.282}$

# Calculations cont':

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

$$\begin{aligned} \text{pH} = \text{Pka} + \log [A^-] / [HA] &\rightarrow \text{pH} = 4.76 + \log [A^-]/[HA] \rightarrow [HA] = C_1 \times V_1 = C_2 \times V_2 \\ &= 0.2 \times 10 = C_2 \times 20 = \mathbf{C_2 = 0.1M} \end{aligned}$$

$$\begin{aligned} \rightarrow [A^-] &= C_1 \times V_1 = C_2 \times V_2 \\ &= 0.2 \times 10 = C_2 \times 20 = \mathbf{C_2 = 0.1 M} \end{aligned}$$

$$\text{So, } \text{pH} = 4.76 + \log 0.1/0.1 \rightarrow \text{pH} = 4.76 + 0 = \mathbf{4.76 \text{ [pH=pka]}}$$

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

$$\begin{aligned} \text{pH} = \text{pka} + \log [A^-] / [HA] &\rightarrow \text{pH} = 4.76 + \log [A^-]/[HA] \rightarrow [HA] = C_1 \times V_1 = C_2 \times V_2 \\ &= 0.2 \times 5 = C_2 \times 20 = \mathbf{C_2 = 0.05M} \end{aligned}$$

$$\begin{aligned} \rightarrow [A^-] &= C_1 \times V_1 = C_2 \times V_2 \\ &= 0.2 \times 15 = C_2 \times 20 = \mathbf{C_2 = 0.15 M} \end{aligned}$$

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

# Result:

Solutions	HA CH <sub>3</sub> COOH (ml)	A <sup>-</sup> CH <sub>3</sub> COONa (ml)	Final volume (ml)	Calculated pH	Measured pH	2M HCl (ml)	Measured pH	The difference
100% [HA]			20 ml			0.1		
75% [HA], 25% [A <sup>-</sup> ]			20 ml			0.1		
50% [HA], 50% [A <sup>-</sup> ]			20 ml			0.1		
25% [HA], 75% [A <sup>-</sup> ]			20 ml			0.1		



# B) Preparation of buffer:

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

Prepare 50ml of a **0.19M acetate buffer** pH =4.86 if you know that (pKa=4.76).

## Calculations:

- Solid sodium acetate [as A<sup>-</sup>].
  - 0.2M Acetic acid [as HA].
  - pKa = 4.76
- } Provided

- Final volume of buffer =50ml
  - pH=4.86
  - Buffer concentration = 0.19 M
  - Buffer Concentration = [HA] + [A<sup>-</sup>]  
0.19 = [HA] + [A<sup>-</sup>]
- } Required

# Calculations cont':

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

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

First calculate the concentration of the weak acid and its conjugated base that make up the buffer with 0.19M:

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

So:

$$4.86 = 4.76 + \log \frac{y}{0.19 - y}$$

$$0.1 = \log \frac{y}{0.19 - y} \quad \rightarrow \text{by taking the "Anti log for both sides"} \quad \rightarrow \quad 1.26 = \frac{y}{0.19 - y}$$

$$y = 1.26 \times (0.19 - y) \quad \rightarrow \quad y = 0.24 - 1.26y \quad \rightarrow \quad y + 1.26y = 0.24 \quad \rightarrow \quad 2.26y = 0.24$$

$y = 0.11 \text{ M}$  [which is the concentration of  $[\text{A}^-]$  in the buffer ]

So,

$$\begin{aligned} [\text{HA}] &= 0.19 - 0.11 \\ &= \underline{0.08 \text{ M}} \quad \text{[which is the concentration of } [\text{HA}] \text{ in the buffer ]} \end{aligned}$$

$$0.11 + 0.08 = 0.19 \text{ M}$$

# Calculations cont':

To calculate the volume needed from [HA] to prepare the buffer, No. of mole of [HA] should be calculated first:

**No. of mole = Molarity x Volume of solution in L**

$$= 0.08 \times 0.05 = 0.004 \text{ mole}$$

So, **M of stock = no. of mole / Volume in Liter**

$$0.2 = 0.004 / V$$

$$\rightarrow V = 0.02 \text{ L} = \underline{20 \text{ ml}}$$

To calculate the weight needed from [A-] to prepare the buffer, No. of mole of [A- ] should be calculated first:

**No. of mole = Molarity x volume of solution in L**

$$= 0.11 \times 0.05 = 0.0055 \text{ mole}$$

**weight in (g) of [A-] = No. of moles x MW**

$$\rightarrow = 0.0055 \times 82 = \underline{0.451 \text{ g}}$$

# Method:

- Now take 20 ml from 0.2M acetic acid and 0.451 g from solid sodium acetate and then complete the volume up to 50 ml by addition of water.

# C) Testing for buffering behaviour:

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

<b>Solution</b>	<b>Measured pH</b>	<b>Add 2M HCl</b>	<b>Measured pH</b>
0.19 M acetate buffer		0.1 ml	
0.2 M KCl		0.1 ml	