

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)
 - CH_3COOH (**Weak acid**)
 - CH_3COONa (**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)
 - NH_3 (**Weak base**)
 - NH_4Cl (**conjugated acid –its salt-**)

- Mechanism of action:

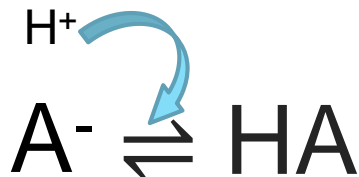
- How buffers can resist the change in PH?

-Example using [HA/A⁻] buffer:

- Where: HA is a Weak acid and A⁻ is conjugated base [its salt].



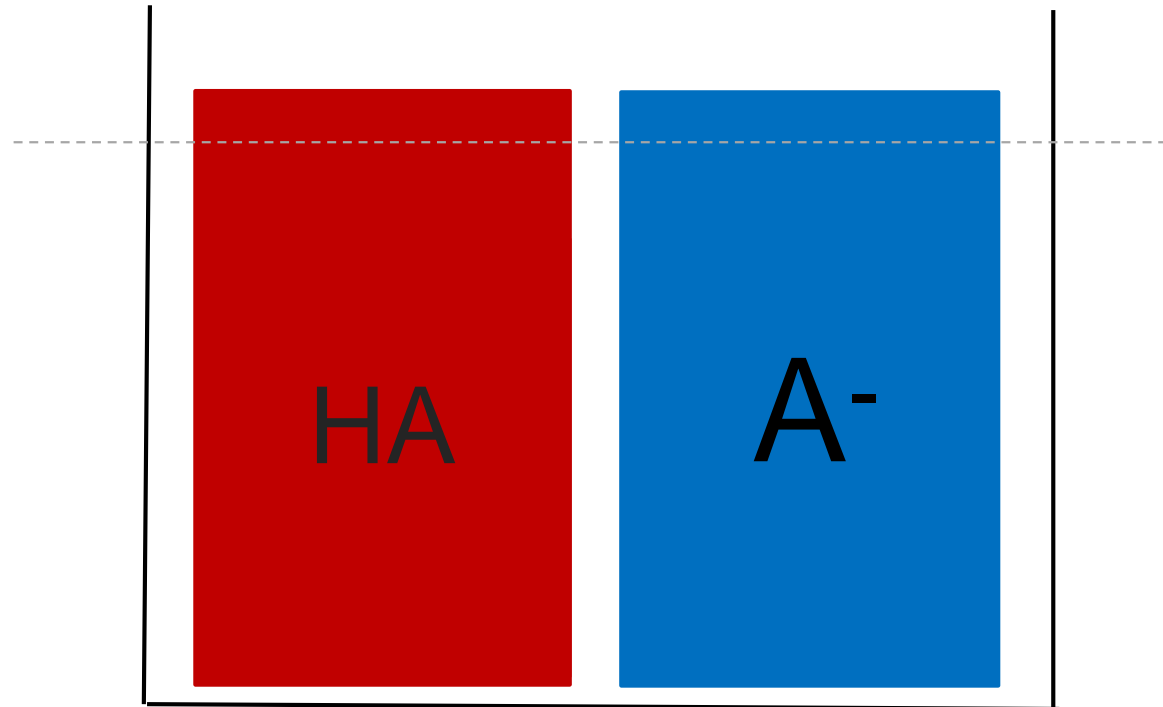
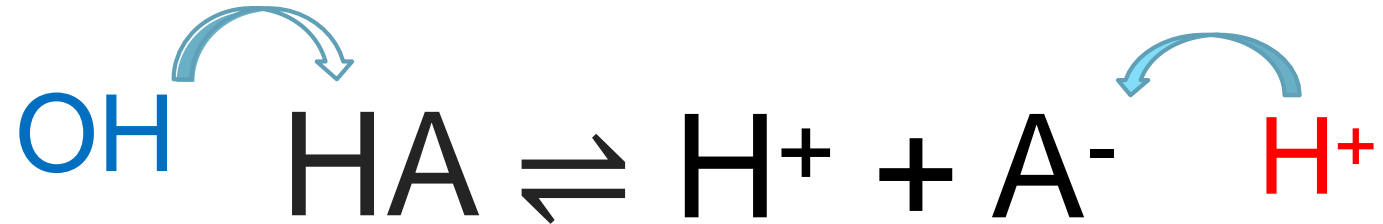
If **H⁺ (acid)** is added to this buffer system → **H⁺** will react with conjugated base → to give **conjugated acid**.



If **OH⁻ (base)** is added to this buffer system → **OH⁻** will react with **conjugated acid** → to give conjugate base and H₂O.



Mechanism of action



- Mechanism of action cont':

- Example:

Conjugated base
 CH_3COO^-

Weak acid
 CH_3COOH

- Buffer system: CH_3COOH / CH_3COO^-

- 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 a buffer can protect against pH changes from added H^+ or OH^- ion as long as there is sufficient basic and acidic forms respectively.



The Henderson-Hasselbalch equation

- It is often used to:

1. Calculate the pH of the Buffer.
2. 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.

- This equation is derived from acid dissociation constant:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

- 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].

$$pH = pka + \log 1$$

$$pH = pka + 0$$

$$pH = pka$$

- Buffer capacity:

- Quantitative measure of 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^+/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 expressed as β and can be derived from Henderson Hasselbalch equation:

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

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

Where: β = the buffer capacity , $[H^+]$ = the hydrogen ion concentration of the buffer ,

$[C]$ = concentration of the buffer and K_a = acid dissociation constant

Calculating the PH

For weak acid [not buffers]

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

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

For weak base [not buffers]

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

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

For buffers

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

$\text{pH} + \text{pOH} = \text{pKw}$

[Pkw : number of dissociation constant of H₂O].

Practical part



- Objectives:

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



A) Natural of buffer:

1. You are provided with: 0.2M solution of CH_3COOH , 0.2M solution of CH_3COONa .

→ Determine which is the weak acid and which is the conjugated base [or its salt].

2. Calculate the volume that you must take from CH_3COOH and CH_3COONa to prepare the following mixtures with final **volume of the solution =20 ml** :

1. 100% [HA]
2. 75% [HA] , 25% [A^-]
3. 50% [HA] , 50% [A^-]
4. 25% [HA] , 75% [A^-]

3. Calculate the pH for each solution [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 CH_3COOH and CH_3COONa 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 CH_3COOH

A- : as CH_3COONa

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

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

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

- Mix 15 ml HA and 5 ml A- and misure the pH, note the total volume is 20 ml.

$$- [15\text{ml} + 5\text{ml}] = 20 \text{ ml}$$

□ The same way for other mixtures ...

- Calculation cont':

2. To Calculate the pH for the previous mixtures with pKa of CH₃COOH = 4.76 :

(A) 100% [HA]:

$$\text{pH} = \frac{(\text{pKa} + \text{p[HA]})}{2} \rightarrow \text{p[HA]} = -\log 0.2 = 0.69 \rightarrow \text{pH} = \frac{(4.76 + 0.69)}{2} = \underline{2.72}$$

(B) 75% [HA], 25% [A⁻]:

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

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

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

- Calculation cont':

(C) 50%[HA], 50%[A⁻]:

$$\text{pH} = \text{pK}_a + \log [\text{A}^-] / [\text{HA}] \rightarrow \text{pH} = 4.76 + \log [\text{A}^-] / [\text{HA}] \rightarrow [\text{HA}] = C_1 \times V_1 = C_2 \times V_2 \\ = 0.2 \times 10 = C_2 \times 20 = \text{C}_2 = 0.1\text{M}$$

$$\rightarrow [\text{A}^-] = C_1 \times V_1 = C_2 \times V_2 \\ = 0.2 \times 10 = C_2 \times 20 = \text{C}_2 = 0.1\text{ M}$$

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

(D) 25% [HA], 75% [A⁻]:

$$\text{pH} = \text{pK}_a + \log [\text{A}^-] / [\text{HA}] \rightarrow \text{pH} = 4.76 + \log [\text{A}^-] / [\text{HA}] \rightarrow [\text{HA}] = C_1 \times V_1 = C_2 \times V_2 \\ = 0.2 \times 5 = C_2 \times 20 = \text{C}_2 = 0.05\text{M}$$

$$\rightarrow [\text{A}^-] = C_1 \times V_1 = C_2 \times V_2 \\ = 0.2 \times 15 = C_2 \times 20 = \text{C}_2 = 0.15\text{ M}$$

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

- Method:

Solutions	HA CH ₃ COOH (ml)	A ⁻ CH ₃ COONa (ml)	Final volume (ml)	Calculated pH	Measured pH	2M HCl (ml)	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		

- Preparation of buffers:

- 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=4.7).

- Calculation:

- Solid sodium acetate [as A⁻].
- 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⁻]
 $0.19 = [HA] + [A^-]$

Required

- Calculation 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 acid and conjugated base that make up the buffer with 0.19M:

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

So:

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

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

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

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

So,

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

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

- Calculation 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.

- Testing for buffering behavior:

- 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.

Solution	Measured pH	Add 2M HCl	Measured pH
0.19M acetate buffer		0.1 ml	
0.2M KCl		0.1 ml	