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. CH_3COOH / CH_3COONa (Pka)

➔ CH3COOH (Weak acid)

➔ CH3COONa (conjugated base –its salt-)

2. NaH_2PO_4 / Na_2HPO_4 (Pka)

Basic Buffer

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

Example:

1. NH_3 / NH_4Cl (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 Weak acid and A- is conjugated base [its salt].

$$HA \rightleftharpoons H^{+} + A^{-}$$

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

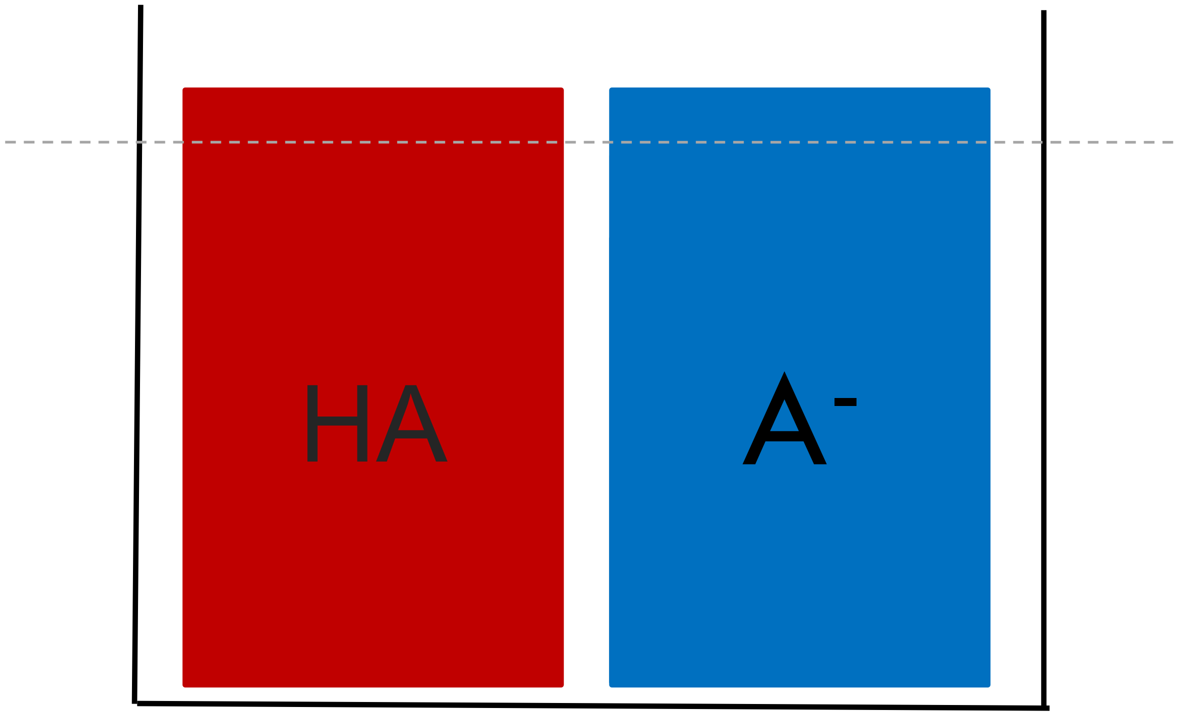
$$A^{-} \overset{H^{+}}{\rightleftharpoons} HA$$

If OH^- **(base)** is added to this buffer system → OH^- will react with conjugated acid ➔to give conjugate base and H_2O.

$$HA \overset{OH^{-}}{\rightleftharpoons} A^{-} + H_2O$$

Mechanism of Action

OH $\quad HA \rightleftharpoons H^+ + A^-$ $\quad H^+$



Mechanism of Action cont':

Example:

conjugated base	conjugated acid
CH_3COO^-	CH_3COOH

- **Buffer system:** CH_3COOH / CH_3COO^-
- When acid $[H^+]$ added:

$$CH_3COO^- + H^+ \longrightarrow CH_3COOH$$

- When base $[OH^-]$ added:

$$CH_3COOH + OH^- \longrightarrow CH_3COO^- + H_2O$$

- **NOTE:** It resists pH changes when it's 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 H^+ ions or OH^- ions added to the system respectively).

Henderson-Hasselbalch equation:

- **It is often used to perform:**
  1. To calculate the pH of the Buffer.
  2. To preparation of Buffer.

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

- It relates the Ka [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].

➔ <u>**The buffer capacity is optimal when the ratio of the weak acid to its salt is 1:1; that is, when pH = pKa**</u>

$$pH = pka + \log 1$$

$$pH = pka + 0$$

$$\boxed{pH = pka}$$

Buffer capacity:

- Quantitative measure of buffer 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.

- The 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 Ka= acid dissociation constant

Calculating the pH:

For weak acid [not buffers]

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

* P[HA] = -log [HA]

For weak base [not buffers]

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

* P[OH] = -log [OH]

For buffers

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

pH = pkw – pOH

[Pkw : number of dissociation constant of H_2O].

Practical Part

Objectives:

- 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 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 [pKa of CH_3COOH = 4.76].

4. Follow the table.

Calculations:

1. To Calculate the volume that you must take from CH3COOH and CH3COONa to prepare the previous mixtures with final volume of the solution =20 ml:

Note:
HA : as CH_3COOH.
A^- : as CH_3COONa.

(A) 100% [HA]:

The final volume is 20ml, So:

20 x 100% = (20 x 100)/100 = 20 ml

➔ Take 20ml of HA and measure the pH.

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

From HA= 20 x 75% = (75 x 20) /100 = 15 ml

From A^- = 20 x 25% = (25 x 20)/ 100 = 5 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 other mixtures …

Calculations cont':

2. To Calculate the pH for the previous mixtures with pKa of $CH_3COOH = 4.76$:

(A) **<u>100% [HA]:</u>**

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

(B) **<u>75% [HA] , 25% [A⁻] :</u>**

$pH = pka + \log [A^-] / [HA] \rightarrow pH = 4.76 + \log [A^-]/[HA] \rightarrow$ **[HA]** $= C_1 X V_1 = C_2 X V_2$

$= 0.2 X 15 = C_2 X 20 = C2 = 0.15M$

$\rightarrow$ **[A⁻]** $= C_1 X V_1 = C_2 X V_2$

$= 0.2 X 5 = C_2 X 20 = C2 = 0.05 M$

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

Calculations cont':

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

pH = Pka + log $[A^-]$ / [HA] → pH= 4.76 + log $[A^-]$/[HA] → [HA] = C_1 X V_1 = C_2 X V_2

=0.2 X 10 = C2 X 20 = C2 = 0.1M

→ [A-] = C_1 X V_1 = C_2 X V_2

= 0.2 X 10 = C_2 X 20 = C2 = 0.1 M

So, pH = 4.76+log 0.1/0.1 → pH = 4.76 +0 = 4.76 **[pH=pka]**

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

pH = pka + log [A-] / [HA] → pH= 4.76 + log [A-]/[HA] → **[HA]** = C_1 X V_1 = C_2 X V_2

=0.2 X 5 = C_2 X 20 = C2 = 0.05M

→ **$[A^-]$** = C_1 X V_1 = C_2 X V_2

= 0.2 X 15 = C_2 X 20 = C2 = 0.15 M

So, pH = 4.76+log 0.15/0.05 → pH = 5.24

Result:

Solutions	HA CH_3COOH (ml)	A^- CH_3COONa (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^-]			20 ml			0.1		
50%[HA],50%[A^-]			20 ml			0.1		
25%[HA],75%[A^-]			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:

$\left.\begin{array}{l}\text{o Solid sodium acetate [as A-].}\\ \text{o 0.2M Acetic acid [as HA].}\\ \text{o Pka = 4.76}\end{array}\right\}$ Provided

$\left.\begin{array}{l}\text{o Final volume of buffer =50ml}\\ \text{o pH=4.86}\\ \text{o Buffer concentration = 0.19 M}\\ \text{o Buffer Concentration = [HA] + [A}^-\text{]}\\ \qquad\qquad\quad 0.19 = [HA] + [A^-]\end{array}\right\}$ Required

Calculations cont':

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

$$pH = pka + \log [A-] / [HA]$$

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

➔ Assume $[A^-] = y$ and $[HA] = 0.19 - y$

So:

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

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

$y = 1.26 \times (0.19-y)$ ➔ $y = 0.24 - 1.26\,y$ ➔ $y + 1.26\,y = 0.24$ ➔ $2.26\,y = 0.24$

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

So,

$[HA] = 0.19 - 0.11$

$= \mathbf{0.08\ M}$ [which is the concentration of [HA] in the buffer]

$0.11 + 0.08 = 0.19M$

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 X 0.05 = 0.004mole

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

0.2 = 0.004 / V

➔ V = 0.02 L = **20 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 X 0.05 = 0.0055 mole

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

➔ =0.0055 x 82 = **0.451 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 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	
o.2M KCl		0.1 ml	

Homework:

- You are provided with 0.5M acetic acid and solid sodium acetate.
  Prepare 100ml of a 0.3M acetate buffer pH =4.78 if you know that (pKa=4.76).

➔''individually''