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.

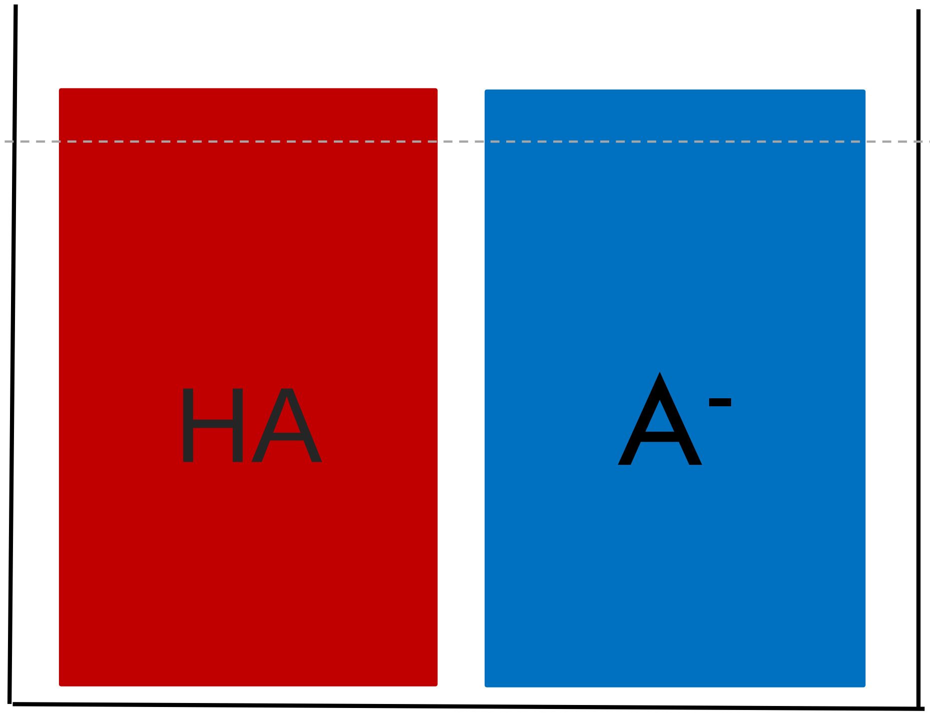
$$\overset{H^+}{A^-} \rightleftharpoons HA$$

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

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

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].

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

$$pH = pka + \log 1$$

$$pH = pka + 0$$

$$\boxed{pH = pka}$$

Calculating the pH:

For weak acid [not buffers]

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

* P[HA] = -log [HA]

For weak base [not buffers]

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

* 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) 100% [HA]:

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

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

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

$= 0.2 \text{ X } 15 = C_2 \text{ X } 20 =$ C2 = 0.15M

$\rightarrow$ **[A$^-$]** $= C_1 \text{ X } V_1 = C_2 \text{ X } V_2$

$= 0.2 \text{ X } 5 = C_2 \text{ X } 20 =$ 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

Method:

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	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		

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=4.7).

Calculations:

- 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

Calculations cont' (first method):

- **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:

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

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

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

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

So,

$[HA] = 0.19 - 0.11$

$= $ **0.08 M** [which is the concentration of $[HA]$ in the buffer]

$0.11 + 0.08 = 0.19M$

Calculations cont' (second method):

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

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

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

First: $4.86 = 4.76 + \log \frac{[A-]}{[HA]}$

$0.1 = \log \frac{[A-]}{[HA]}$ ➔ by taking the "Anti log for both sides" ➔ $\frac{[A-]}{[HA]} = 1.26 = \frac{1.26}{1}$

SO: $\frac{1.26}{2.26}$ of total = [A-] **and** $\frac{1}{2.26}$ of total = [HA]

[A-] = $\frac{1.26}{2.26} \times 0.19$ <u>**= 0.11 M**</u> [which is the concentration of $[A^-]$ in the buffer]

[HA-]= $\frac{1}{2.26} \times 0.19$ = <u>**0.08 M**</u> [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**

Problem 1-27 , p39
Problem 1-28 , p40

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	