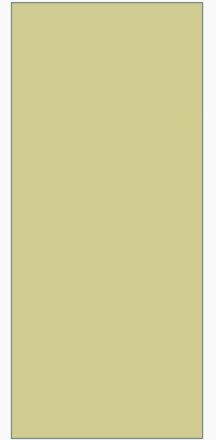


PREPARATION OF DIFFERENT BUFFER SOLUTION



OBJECTIVES

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

BUFFERS

- Biological life cannot withstand changes in hydrogen ion concentrations which we measure as the pH.
- All biochemical reactions occur under strict conditions of the concentration of hydrogen ion.
- **Buffers** are Those solutions that have the ability to resist changes in pH.

- A buffer is a solution that resists changes in pH upon the addition of **limited amounts** of acid or base.

There are two types of buffers:

Acidic buffer

are made from a weak acid and its salts

Example:

CH₃COOH-CH₃COONa

- CH₃COOH - weak acid
- CH₃COO⁻-Na⁺ -
SALT(CONJUGATED BASE)

Basic buffer

are made from a weak base and its salts

Example:

NH₃-NH₄Cl

- NH₃-weak base
- NH₄Cl –
SALT(CONJUGATED acid)

HOW BUFFERS CAN RESIST THE CHANGE IN PH?

- **Example:** acidic buffer (CH_3COOH , CH_3COO^-)
- When **H^+ ions** are added to the system they will react with the conjugate base in the buffer as follows,
- $\text{CH}_3\text{COO}^- + \text{H}^+ \longrightarrow \text{CH}_3\text{COOH}$
- When **OH^- ions** are added they will react with the conjugate acid in the buffer as follows,
- $\text{CH}_3\text{COOH} + \text{OH}^- \longrightarrow \text{CH}_3\text{COO}^- + \text{H}_2\text{O}$
- **NOTE:** It resists pH changes when it's two components are present in specific proportions
- 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 perform the calculations required in preparation of buffers for use in the laboratory.
- $pH = pK_a + \log \frac{[A^-]}{[HA]}$
- This equation is derived from acid dissociation constant:
 - $K_a = \frac{[H^+][A^-]}{[HA]}$
- A buffer is best used close to its pK_a
- To act as a good buffer the pH of the solution must be within one pH unit of the pK_a .

Exp (1) : Nature of buffers

- You are provided with : **0.2M** solution of CH_3COOH , **0.2M** solution of CH_3COONa .
- Determine for your acid-base pair which is the **acid component** and which is the **base component**.
- Calculate the volume that you must take from CH_3COOH and CH_3COONa (**the final volume of the solution = 20 ml**)
- pKa of CH_3COOH = 4.76**

SOLUTION	ml HA	ml A-	Final volume	CALCULATED pH	MEASURED pH
100%HA	20 ml	0	20 ml	2.72	
75%HA, 25%A-	15 ml	5 ml	20 ml	4.28	
50%HA, 50%A-	10 ml	10 ml	20 ml	4.76	
25%HA, 75%A-	5 ml	15 ml	20 ml	5.24	

PH CALCULATIONS

- **Calculated pH:**

** 100% HA :

$$\text{pH} = (\text{pK}_a + \text{p}[\text{HA}])/2$$

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

$$\text{pH} = (4.76 + 0.69)/2 = 2.72$$

** 75%HA , 25% A-

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

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

$$\begin{aligned} \text{no.of moles of A}^- &= M \times V (\text{ in L}) \\ &= 0.2 \times 0.005 = 0.001 \text{ moles} \end{aligned}$$

$$\begin{aligned} \text{No.of moles of HA} &= 0.2 \times 0.015 \\ &= 0.003 \text{ moles} \end{aligned}$$

$$\begin{aligned} \text{pH} &= 4.76 + \log (0.001/0.003) \\ &= 4.2 \end{aligned}$$

PH CALCULATIONS

** 50%HA , 50% A-

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

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

$$\begin{aligned} \text{no.of moles of } A^- &= M \times V \text{ (in L)} \\ &= 0.2 \times 0.01 = 0.002 \text{ moles} \end{aligned}$$

$$\begin{aligned} \text{No.of moles of HA} &= 0.2 \times 0.01 \\ &= 0.002 \text{ moles} \end{aligned}$$

$$\begin{aligned} pH &= 4.76 + \log (0.002/0.002) \\ &= 4.76 \end{aligned}$$

** 25%HA , 75% A-

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

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

$$\begin{aligned} \text{no.of moles of } A^- &= M \times V \text{ (in L)} \\ &= 0.2 \times 0.015 = 0.003 \text{ moles} \end{aligned}$$

$$\begin{aligned} \text{No.of moles of HA} &= 0.2 \times 0.005 \\ &= 0.001 \text{ moles} \end{aligned}$$

$$\begin{aligned} pH &= 4.76 + \log (0.003/0.001) \\ &= 5.24 \end{aligned}$$

EXP (2): PREPARATION OF BUFFER

- You are provided with 0.2M solution of acetic acid and solid sodium acetate , $pK_a = 4.76$). Prepare 45ml of a 0.2M acetate buffer $pH = 4.86$.

CALCULATIONS

- 0.2 M acetic acid
- Solid sodium acetate
- $\text{Pka} = 4.76$
- Final volume of buffer = 45ml
- Buffer concentration = 0.2 M
- Buffer Conc. = $[\text{HA}] + [\text{A}^-] = 0.2 \text{ M}$

$$PH = Pka + \log [HA]/[A]$$

$$\text{Assume } [A] = y$$

$$[HA] = 0.2 - y$$

$$4.86 = 4.76 + \log \frac{y}{0.2 - y}$$

$$0.1 = \log \frac{y}{0.2 - y}$$

$$1.258 = \frac{y}{0.2 - y}$$

$$y = 0.25 - 1.258 y$$

$$y + 1.258 y = 0.25$$

$$y = 0.11 \text{ M}$$

$$[HA] = 0.2 - 0.11$$

$$= 0.09 \text{ M}$$

- [HA] :

No. of mole = $0.09 \times 0.045 = 4.05 \times 10^{-3}$ mole

*M of buffer = no. of mole / V

$0.2 = 4.05 \times 10^{-3} / V$

$V = 0.0202 \text{ L} = 20 \text{ ml}$

-[A] :

No. of mole = 0.11×0.045

$= 4.95 \times 10^{-3}$ mole

* wt (g) of A- = $4.95 \times 10^{-3} \times 82$

$= 0.41 \text{ g}$

** take 20 ml from acetic acid and 0.41 g from Solid sodium acetate and complete volume to 45 ml H₂O.(0.2 M acetate buffer)

EXP(3): TESTING FOR BUFFERING BEHAVIOR

- **Table 3.** For the 0.2M acetate buffer prepare:

Solution(10 of each)	Measured pH	Add 2M HCl(0.1ml)	pH after HCl
0.2M acetate buffer.			
0.2M KCl.			