BCH 202 General Biochemistry [Practical]

Lab (1) Preparation of Buffer Solutions

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Hydrogen number (pH):

- The **acidity** of certain solutions can be described by using hydrogen number (pH).
- **pH defined as:** The negative logarithm of the hydrogen ion concentration.

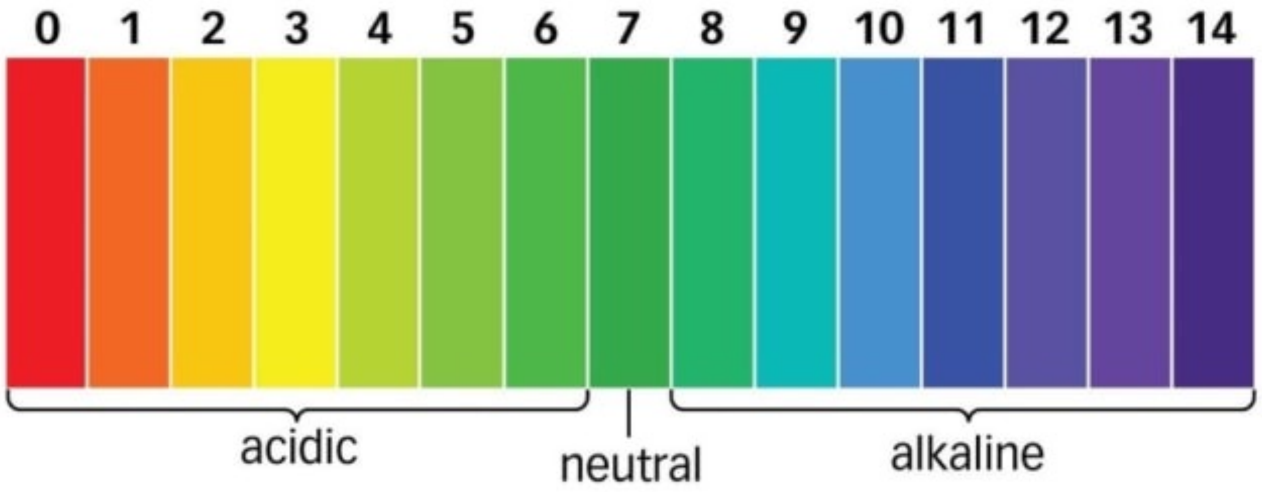
$$pH= - Log[H^+]$$

- When the pH increase the concentration of hydrogen ion decrease and vice versa.
- The pH range goes from 0 to 14.

Acidic → pH< 7

Neutral → pH=7

Basic → pH>7

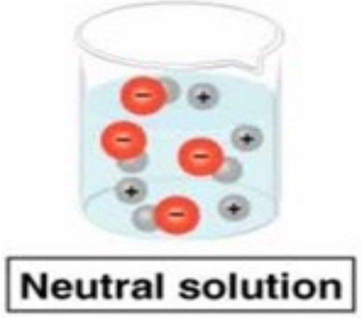


Acidic solution : has higher concentration of hydrogen ions [H^+] than hydroxyl ions [OH^-]

$H^+ > OH^-$

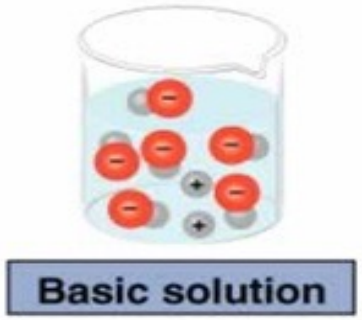
Neutral solution: has equal concentration of hydrogen ions [H^+] and hydroxyl ions [OH^-]

$H^+ = OH^-$



Acidic solution : has lower concentration of hydrogen ions [H^+] than hydroxyl ions [OH^-]

$H^+ < OH^-$



Measuring of hydrogen number:

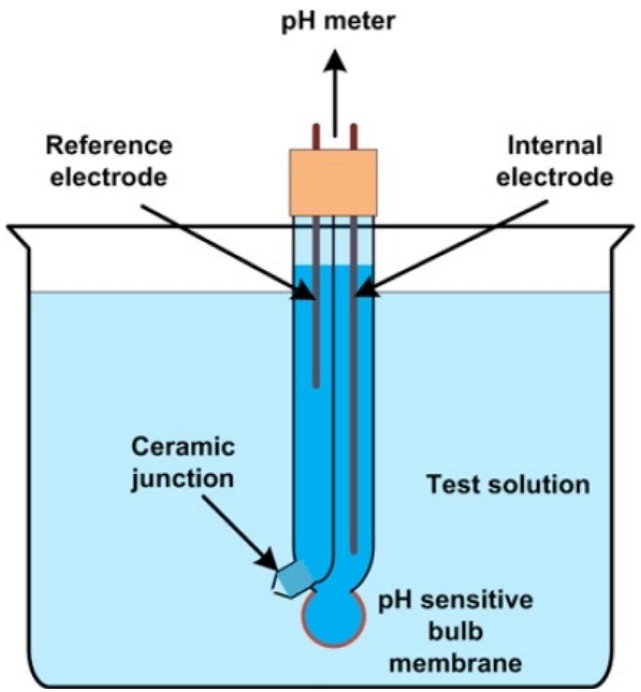
- To measure the hydrogen number in certain solution in very accurate way, we use a special instrument called **pH meter.** It's consist of two electrodes:

1. **Reference electrode:** contains silver-silver chloride wire immersed in saturated KCl solution.
2. **Glass electrode:** which contains a very thin bulb, that is sensitive to pH.

This device measures the difference between the electrodes, and converts it into a **pH from 0 to 14.**

- **Supporting materials:**

1. How pH meter works: shorturl.at/fkpL3
2. How to use the pH meter: shorturl.at/lKXZ4



Measuring of hydrogen number cont.:

Test strip (inaccurate):

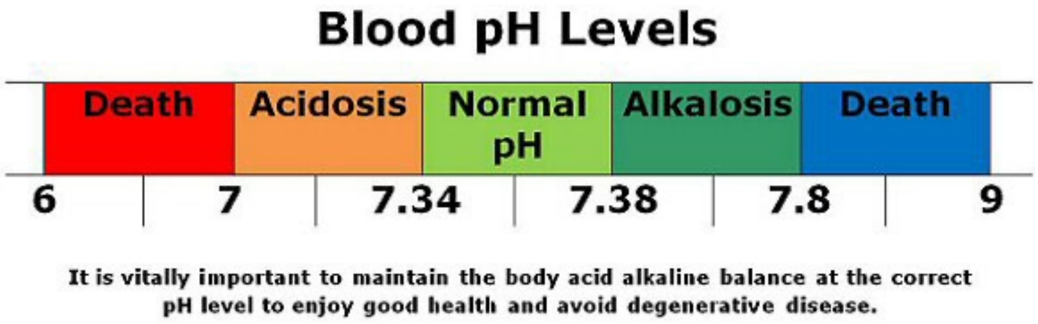
A pH test strip is a strip of litmus paper with which you can measure the pH value of a liquid which show a different color at different acidities.

pH and biological system:

- All biochemical reactions occur under strict conditions of the concentration of hydrogen ion.

- Biological life can not withstand large changes in hydrogen ion concentrations which we measure as the pH.

- So how to resist changes in pH ? **Buffers.**



Buffers:

- So, **buffers defines as:** the solutions that have the ability **to resist changes in pH** upon the addition of **limited** amounts of acid or base.

- <u>A buffer is made up of:</u>

a weak acid and its conjugate base.

Or

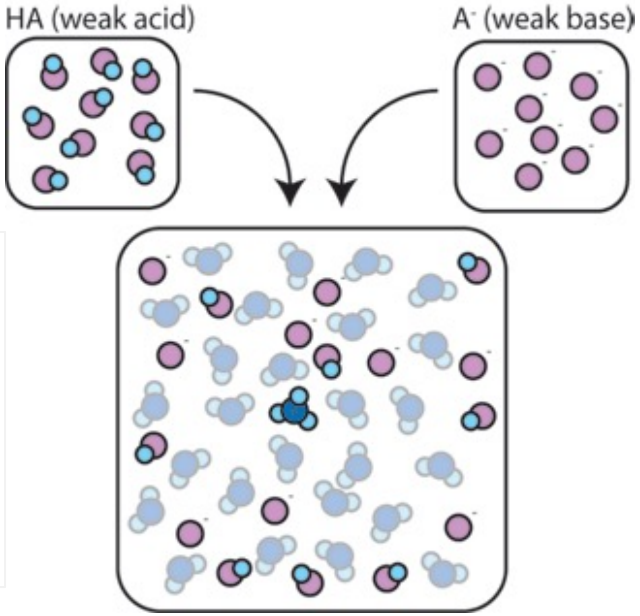
a weak base and its conjugate acid .

الحمض الضعيف + قاعدته المقترنة

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

القاعدة الضعيفة + حمضها المقترن

$$BOH \rightleftharpoons B^+ + OH^-$$



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.

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.

OH-

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

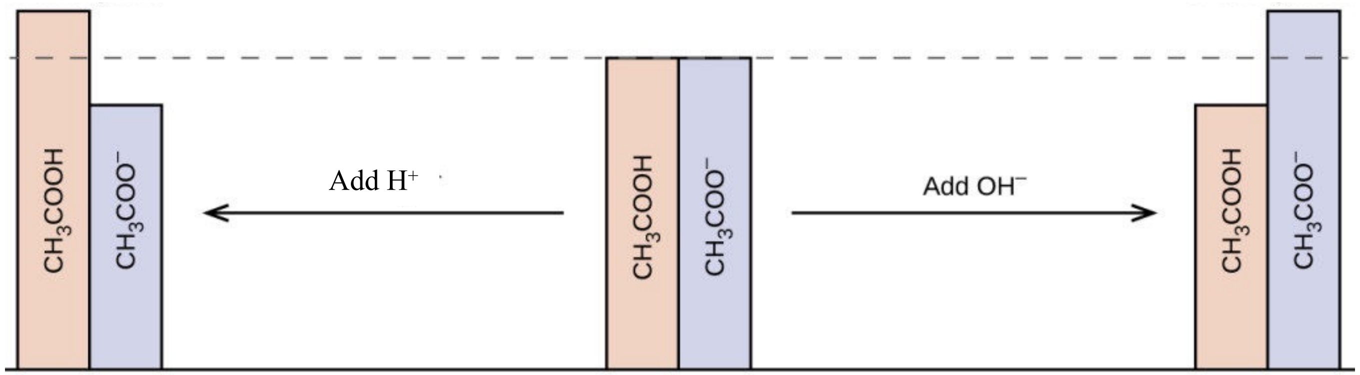
Mechanism of action cont.:

- **Example:**

Buffer system: CH_3COOH / CH_3COO^- , (CH3COOH :acid - CH3COO-: conjugated base)

-When acid [H+] added: ➔ CH3COO- + **H+** ➔ CH3COOH ↑

-When base [OH] added: ➔ CH3COOH + **OH -** ➔ CH3COO- ↑ + H2O



- **NOTE:** It resists pH changes when it's 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 ➔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:**

1. To prepare Buffer.
2. To calculate the pH of the Buffer.

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

- It relates the **Ka** [dissociation constant] of a weak acid, **[HA]** concentration of weak acid, **[A-]** concentration of conjugate base [salt of the weak acid] components and the **pH of the buffer**.

Choosing the proper buffer:

- 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].

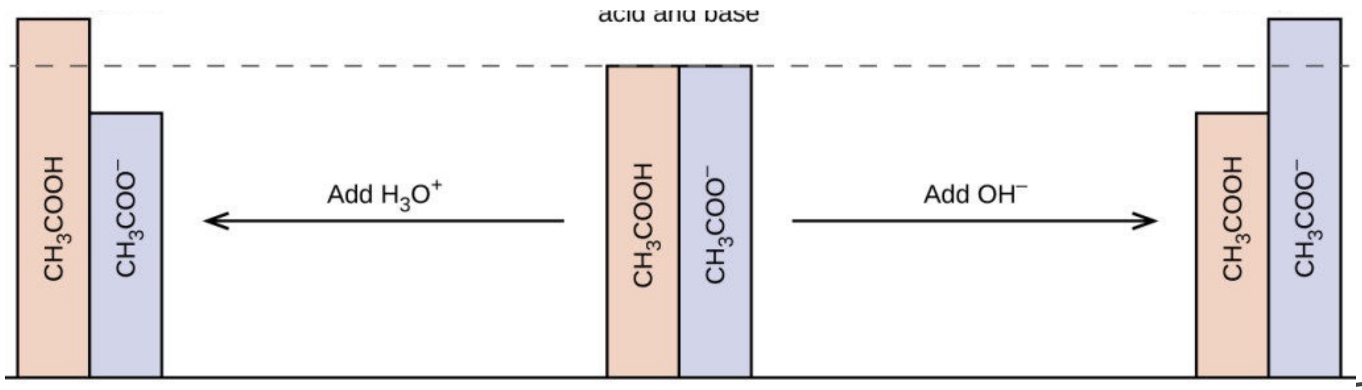
- **The buffer capacity is optimal when the ratio of the weak acid to its salt is 1:1; that is, when pH = pKa.**

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

$$pH = pka + \log 1$$

$$pH = pka + 0$$

$$\boxed{pH = pka}$$



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 litre of the buffer in order to decrease /increase the pH by one unit respectively.

- Buffer capacity is **directly proportional** to the buffer concentration.

A buffer has concentration of **0.5 M**

A buffer has concentration of **0.9 M** Which buffer has the highest capacity?

Practical Part

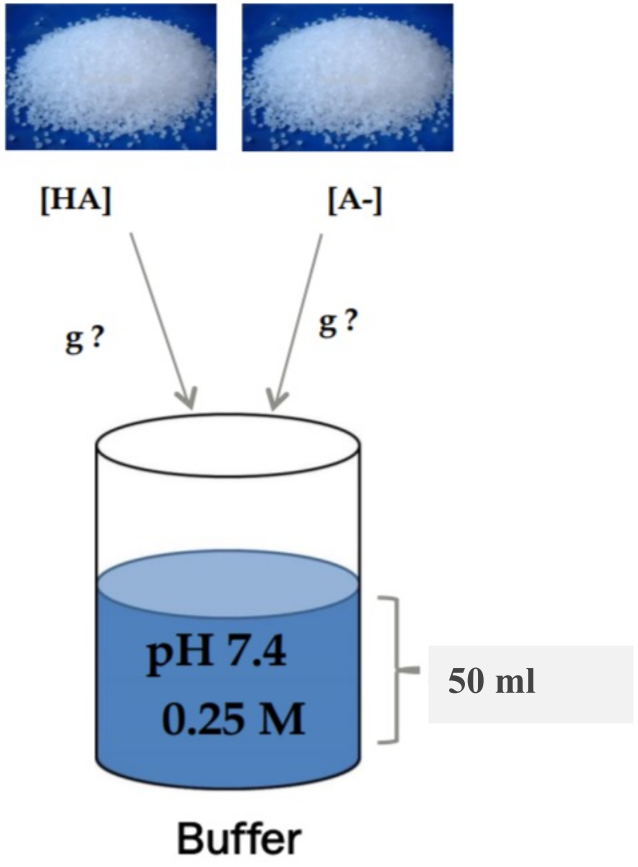
Objectives:

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

A. Preparation of phosphate buffer:

Prepare 50 ml from phosphate buffer with concentration 0.25M and pH=7.4, if you know that (pKa=7.2).

You are provided with buffer solution content: Monosodium dihydrogen phosphate $\underline{NaH_2PO_4}$ and Disodium hydrogen phosphate $\underline{Na_2HPO_4}$



Solution:

■ **Provided:**

pKa = 7.2 pH=7.4

Final volume of buffer =50 ml

Concentration of buffer = 0.25 M ➔ [HA] + [A^-]

■ **Required:**

Weight (g) of NaH2PO4 (as HA) and Na2HPO4 (as A).

Calculations:

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

$$pH = pKa + \log [A^-]\backslash[HA]$$

1. First calculate the concentration of the weak acid and its conjugated base that make up the buffer with 0.25 M:

➔ Assume [A-] = y and [HA] = 0.25 – y

So:

$7.4 = 7.2 + \log \frac{y}{0.25-y}$ ➔ $0.2 = \log \frac{y}{0.25-y}$

By taking the "Anti log for both sides" :

$1.6 = \frac{y}{0.25-y}$ ➔ y= 1.6 x (0.25 -y) ➔ y= 0.4 – 1.6 y ➔ y + 1.6 y = 0.4 ➔ 2.6 y = 0.4

y= 0.15 M [which is the concentration of $[A^-]$ in the buffer]
So, [HA] = 0.25 – 0.15 = **0.1 M** [which is the concentration of [HA] in the buffer]

} 0.15+ 0.1 = 0.25M

Calculations cont.:

Molecular weight
(A) Na_2HPO_4 = 142 g/mol
(HA) NaH_2PO_4 = 120 g/mol

2. Calculate the **weight** in (g) needed from [A-] to prepare the buffer, so number of mole of [A-] should be calculated first :

➔ **Calculate moles of A- in buffer: [1]**

No. of mole (of A-) = molarity (of A- calculated in the buffer) X volume L (volume of the buffer)
= 0.15 x 0.05 = 0.0075 mole

➔ **Calculate weight of A- needed: [2]**

Weight in (g) of [A-] = No. of moles x MW
= 0.0075 x 142 = **1.065 g**

3. Calculate the **weight** in (g) needed from [HA] to prepare the buffer, so number of mole of [HA] should be calculated first :

➔ **Calculate moles of HA in buffer: [1]**

No. of mole (of HA) = molarity (of HA calculated in the buffer) X volume L (volume of the buffer)
= 0.1 x 0.05 = 0.005 mole

➔ **Calculate weight of HA needed: [2]**

Weight in (g) of [HA] = No. of moles x MW
= 0.005 x 120 = **0.6 g**

[1] **Molarity** = $\frac{\text{No. of moles of solute}}{\text{Volume (L)}}$

[2] **No. of moles** = $\frac{\text{Wt(g)}}{\text{Mwt}}$

Homework:

- **You are provided with 0.15 M acetic acid and sodium acetate.**

Prepare 100 ml of a 0.2M acetate buffer, pH =5.2 if you know that pKa =4.76.

Hint: you will calculate ml of acetic acid and g of sodium acetate.