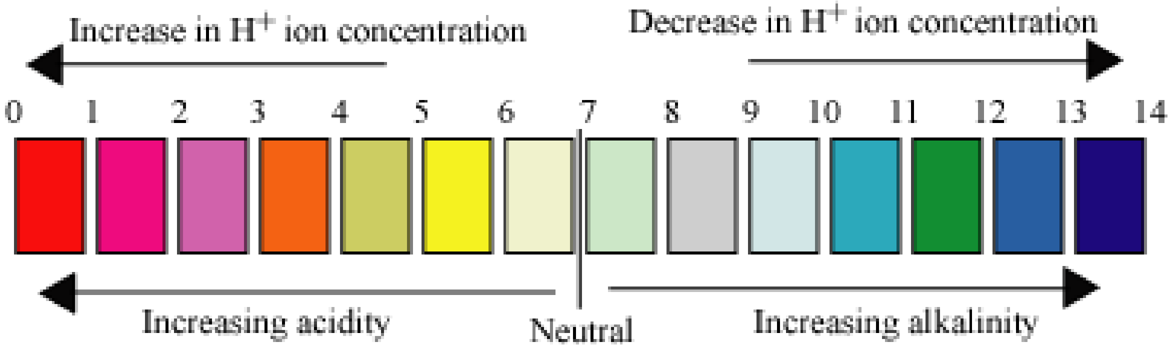
Preparation of Buffer Solutions

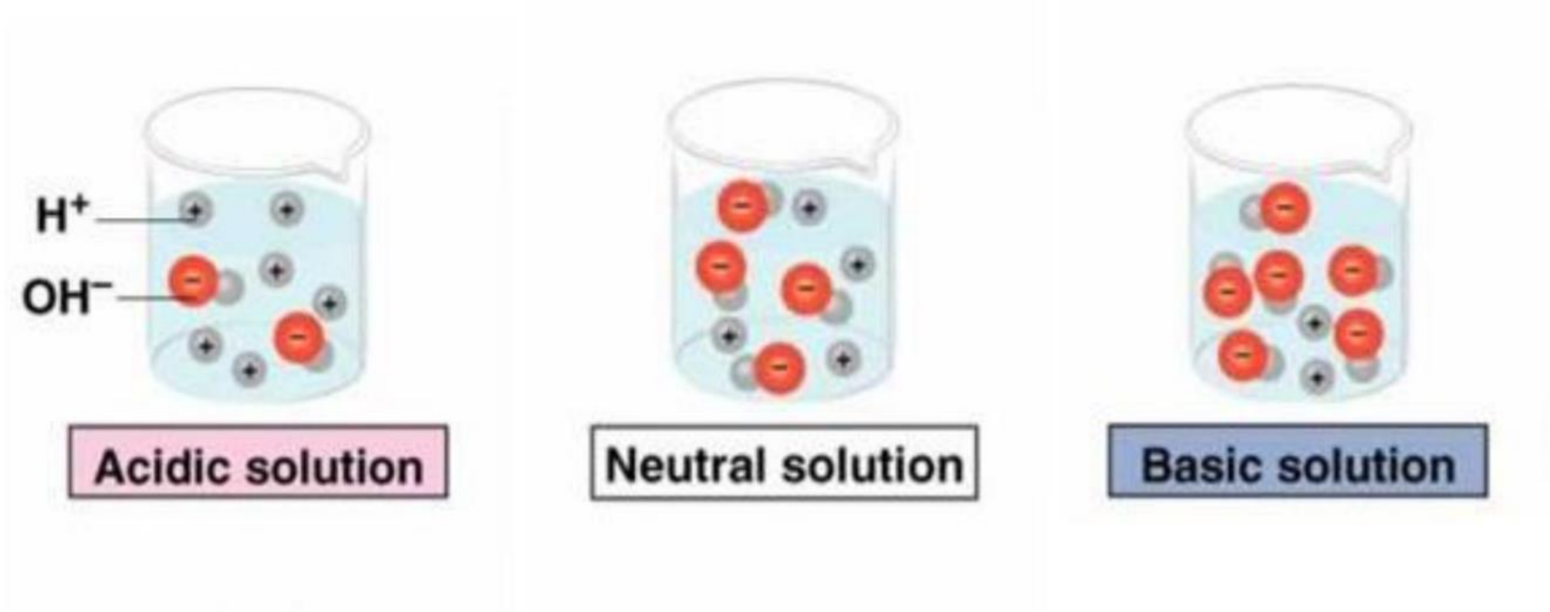
Hydrogen number (pH) :

- 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.




H+
OH−
Acidic solution
Neutral solution
Basic solution

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 glass electrode which contain a very thin bulb, blown onto a hard glass tube which is sensitive to pH.

- The bulb contains a solution of hydrochloric acid and is connected to a platinum lead via silver -silver chloride electrode which is reversible with respect to hydrogen ions.

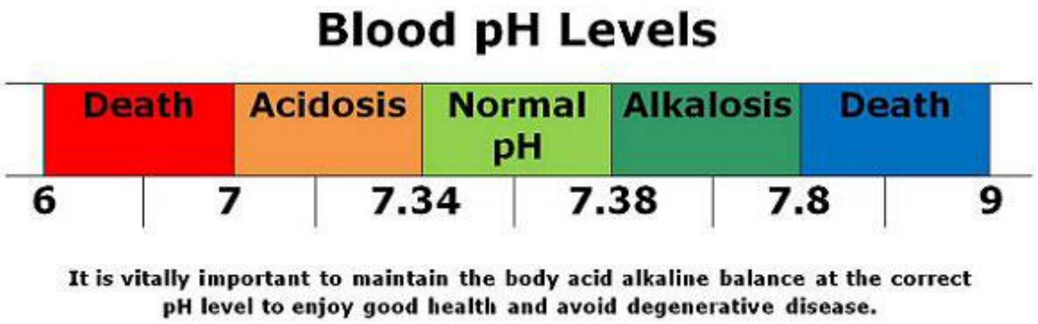
- Other methods?

- **Supporting materials:**

1. How pH meter works: https://youtu.be/P1wRXTl2L3I
2. How to use the pH meter: https://youtu.be/vwY-xWMam7o

pH and biological system:

- All biochemical reactions occur under strict conditions of the concentration of hydrogen ion.
- Biological life <u>can not withstand large changes in hydrogen ion concentrations</u> which we measure as the pH.
- So how to resist changes in pH ? **Buffers.**



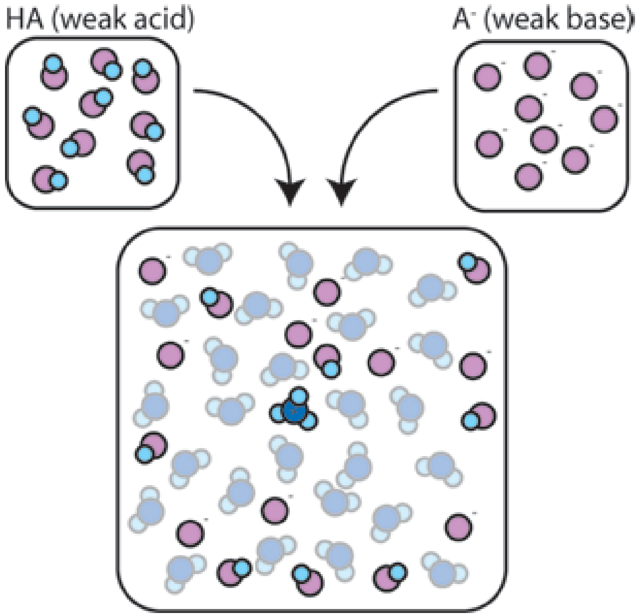
Buffers:

- 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.

- A buffer is made up of:

a weak acid and its conjugate base.
Or
a weak base and its conjugate acid .

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



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-

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

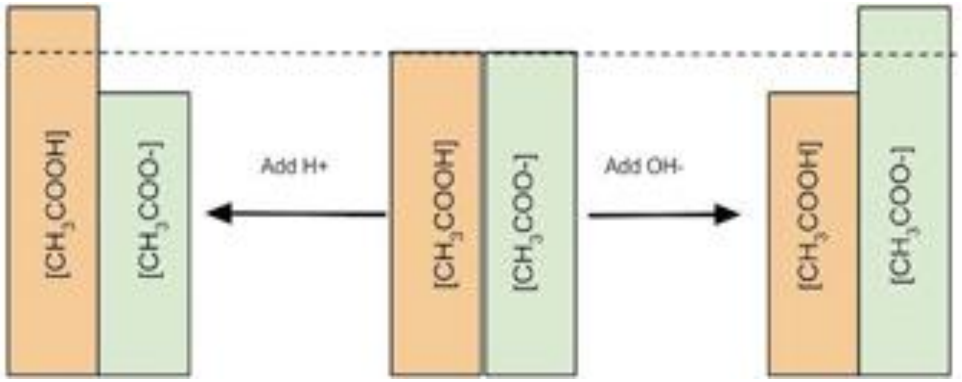
Mechanism of Action cont':

- **Example:**

Buffer system: CH3COOH / CH3COO- , (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 <u>specific proportions</u>.
- 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 calculate the pH of the Buffer.

  2-To prepare Buffer.

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

- 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.**

$$pH = pka + \log 1$$

$$pH = pka + 0$$

$$\boxed{pH = pka}$$

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.

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

Practical Part

Objectives:

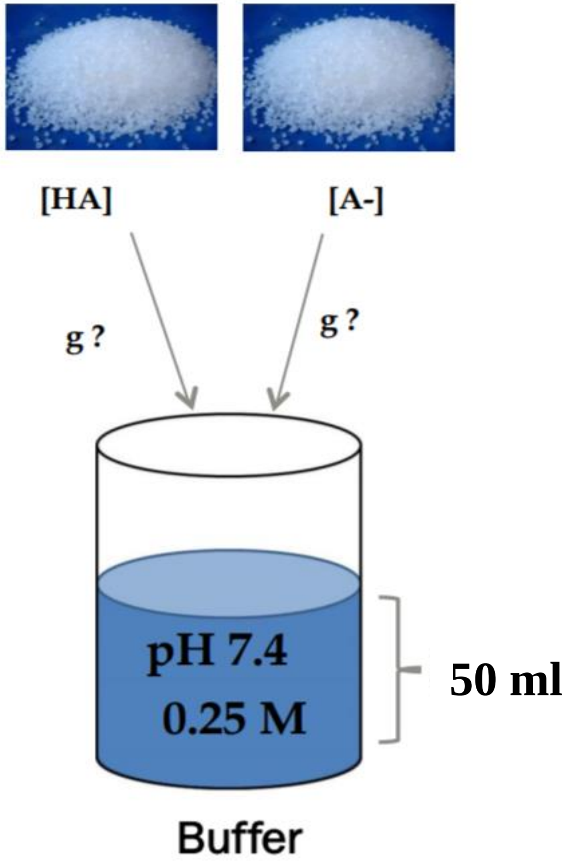
- To learn how to prepare buffers.
- To understand the behaviour and nature of buffers solutions.

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

<u>NaH_2PO_4</u> and Disodium hydrogen phosphate <u>Na_2HPO_4.</u>



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 NaH_2PO_4 (as HA) and Na_2HPO_4 (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:

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

By taking the "Anti log for both sides" :

$$1.6 = \frac{y}{0.25-y} \Rightarrow y = 1.6 \times (0.25 - y) \Rightarrow y = 0.4 - 1.6\,y \Rightarrow y + 1.6\,y = 0.4 \Rightarrow 2.6\,y = 0.4$$

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':

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:**

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:**

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:**

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:**

Weight in (g) of [HA] = No. of moles x MW

= 0.005 x 120 = **0.6 g**

Method:

- Now take 0.6 g from NaH2PO4 and 1.065 g from Na2HPO4 dissolve them in a volume of a distal water (less than 50 ml).

- Check the pH, then complete the volume up to 50 ml by addition of distal water using a volumetric flask.

B.Testing for buffering behavior:

Method:

1. In one beaker add 10 ml of 0.25M phosphate buffer that you have prepared, and in another beaker add 10 ml of KCl.
2. Measure the pH.
3. Add 0.1 ml from 2 M HCl to both solutions.
4. Measure the pH after the addition.

Solution	Measured pH	Add 2M HCl	Measured pH
0.25M Phosphate buffer		0.1 ml	
o.2M KCl		0.1 ml	