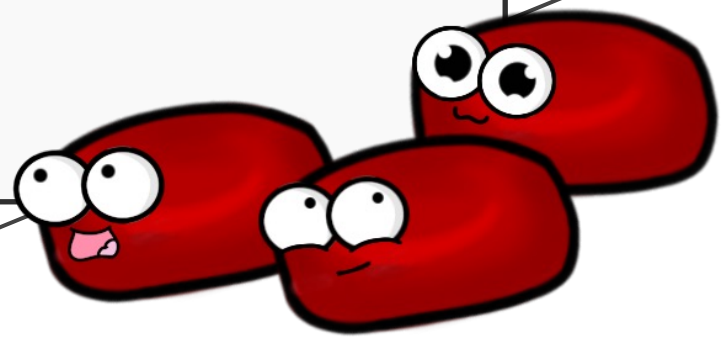
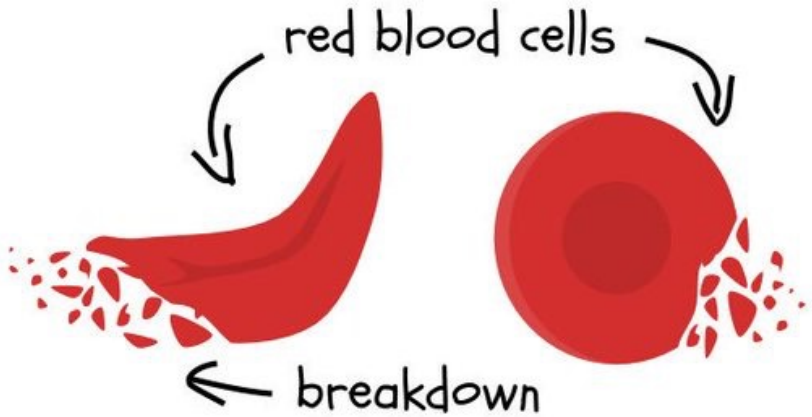
Blood Biochemistry BCH 471 [Practical]

Lab (4) Hemolyzing Agents & Detection of Blood



Blood Hemolysis

- **Hemolysis** (from the Greek **Hemo**: meaning blood, **lysis**, meaning to break open).
- It is the **breaking open** of red blood cells and the release of hemoglobin and the red cell contents into the surrounding fluid (plasma).
- Hemolysis may occur *in vivo* or *in vitro.*



Hemolysis *in-vivo*

- **Conditions that can cause hemolysis include:**

1. Immune reactions
2. Infections
3. Medications
4. Toxins and poisons

Hemolysis *in-vitro*

1. Improper technique during collection (e.g. incorrect needle size, excessive suction)
2. pH imbalance (addition acid or base)
3. Placing RBCs in a hypotonic solution

Note: In this lab blood hemolysis will be done by using hypotonic solutions and pH imbalance.

When Blood Hemolysis Should Be Done?

- Breaking down RBCs to release their content
- Estimation of hemoglobin
- To obtain erythrocyte free preparation of leukocyte and platelet

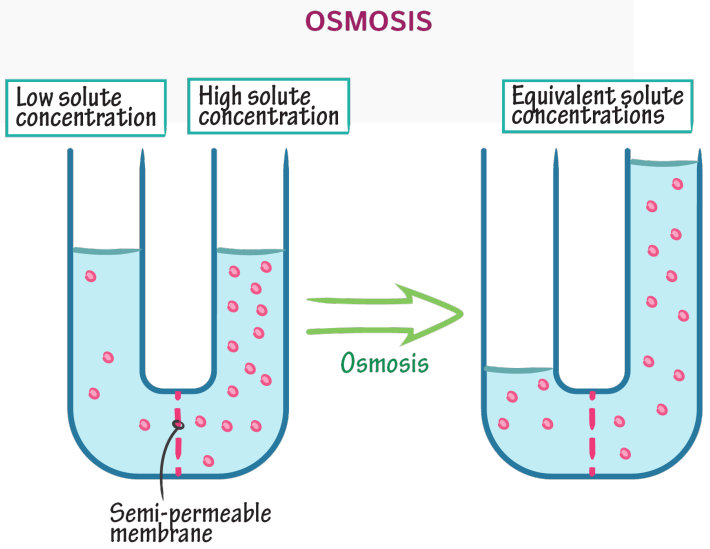
Osmosis and Osmotic Pressure

Osmosis:

- It is the diffusion of solvent molecules across a semi-preamble membrane into a region of higher solute concentration.
- Once an *equilibrium* is reached the flow of water stops.

Osmotic pressure: the pressure exerted by a solvent passing through a semi-permeable membrane in osmosis.

Tonicity: the concentration of a solution as compared to another solution.



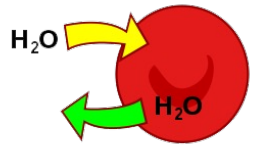
Tonicity

Types of solutions:

➢ **Isotonic**

- A solution that has the same solutes concentration as the normal cells of the body and the blood, having equal osmotic pressure.
- Example of Isotonic solution is **sodium chloride 0.9% (normal saline),** have the same osmotic pressure as serum and they do not affect the membranes of the RBCs.
- In hospitals, intravenous fluids are isotonic.

Solute inside the cell = Solute outside the cell



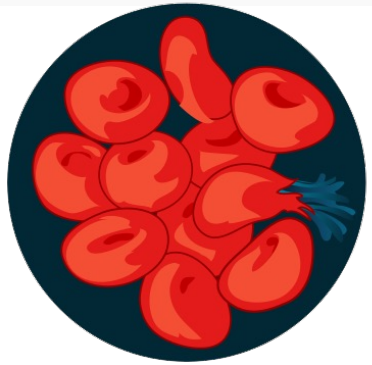
Tonicity

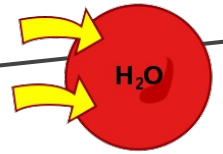
Types of solutions:

➢ **Hypotonic**

- In a hypotonic solution, there is a lower concentration of solute outside a cell, creating an environment with lower osmotic pressure than what is contained within the cell.
- The RBCs will burst or hemolyzed.
- Any concentration of NaCl that is **lower than 0.9%,** will be considered hypotonic for cells.

Solute outside the cell < Solute inside the cell





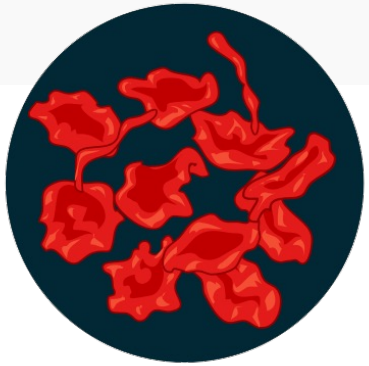
Tonicity

Types of solutions:

- **Hypertonic**

- In a hypertonic solution, there is a higher concentration of solute outside a cell, creating an environment with higher osmotic pressure than what is contained within the cell.
- The RBCs will be shrink.
- Any concentration of NaCl that is **higher than 0.9%,** will be considered hypertonic for cells.

Solute outside the cell > Solute inside the cell



Practical Part 💉

Objectives

1. To detect the presence of hemolysis in blood sample.
2. To detect the presence of blood in a biological sample.

Calculations

How many grams of NaCl are needed to prepare 100 ml of isotonic solution, knowing that the osmolarity of RBC = 0.308 Osmolar ?

First: Calculate the molarity from osmolarity equation: [1]

Osmolarity = 0.308 Osmolar

No. of dissociation particles = 2, since NaCl → $Na^+ + Cl^-$

$$\rightarrow \mathbf{M} = \frac{\textbf{Osmolarity}}{n} = \frac{0.308}{2} = 0.154\ \text{M}$$

[1] Osmolarity = M x n

Where:

M = molarity

n= No. of dissociation particles

Pause and think Why do you think it is important to prepare isotonic solutions?

Calculations

Second: Calculate the No. of moles expressed in (w/v %): [2]

To calculate in w/v % → **M= No. of moles / V (in L)**

→ **No. of moles = M x V (in L)** =

→ 0.154 (from step 1) x 0.1 (100 ml, because you want it as %)= 0.0154 moles

Third: Calculate weight in grams knowing that Mwt of NaCl = 58.5 g/mol: [3]

→ Wt (g) = No. of moles x Mwt =

→ 0.0154 (from step 2) x 58.5= 0.9 g in 100 ml **then 0.9% w/v**

= 0.9 % → the concentration of NaCl that will make an isotonic solution

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

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

Experiment (1): Hemolysis Test

Method

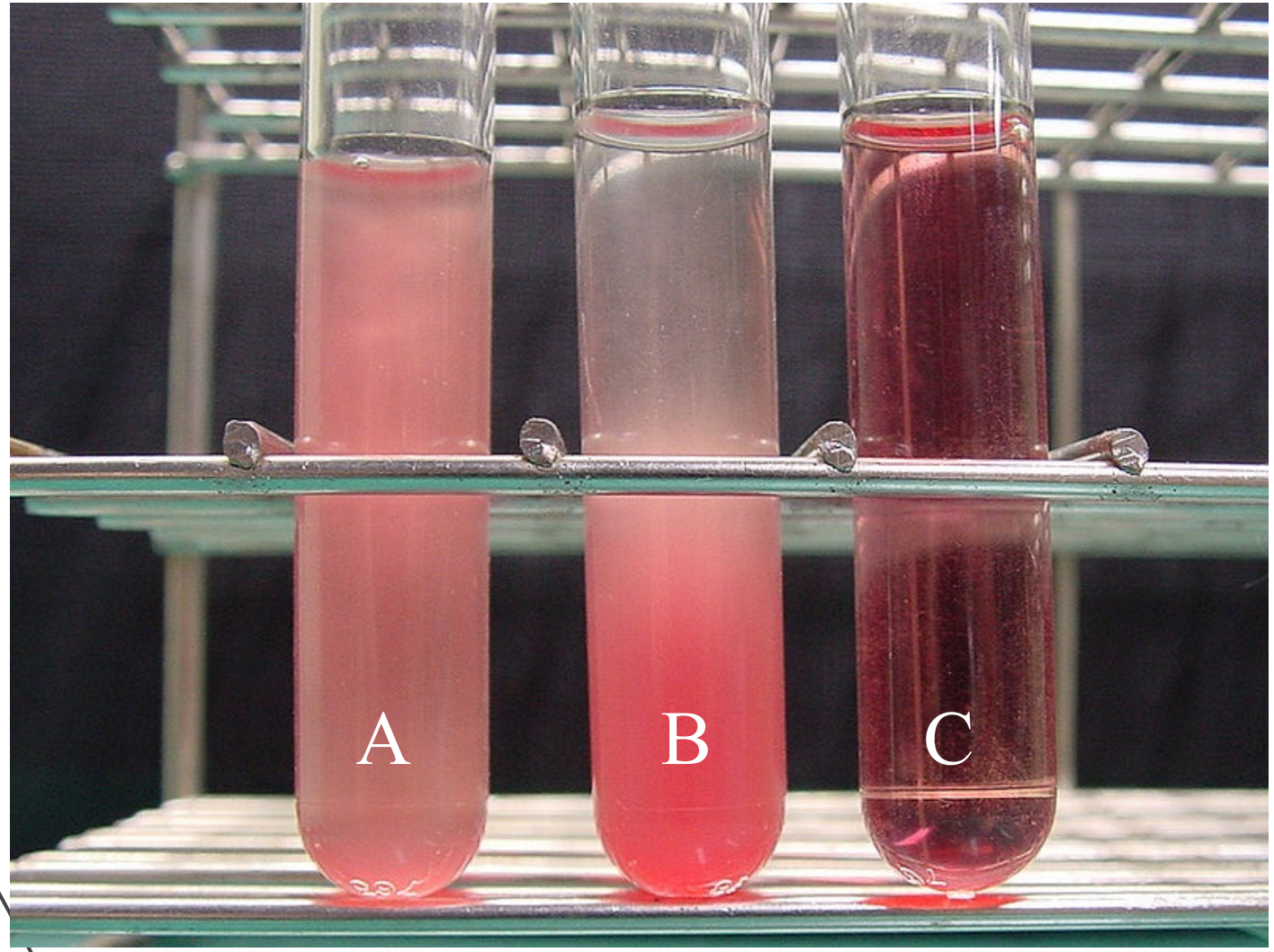
1. Label 6 tubes (A→ F). Then, add 1 ml of RBCs suspended in saline into each tube

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F
NaCl 0.45%	5 ml					
NaCl 1.2%		5 ml				
Sucrose 6%			5 ml			
NaOH 2 M				3 drops		
HCl 0.1 M					3 drops	
Dis. Water						5 ml
NaCl 0.9%				5 ml	5 ml	

2. Wait 30 min
3. Observe wither hemolysis has taken place

💡 Pause and Think **What type of solution is distilled water considered?**

Results



A Normal, non-hemolyzed sample

B Sedimented after one hour

C Hemolyzed sample

Note: the hemolyzed sample is transparent, because there are no cells to scatter the light.

Experiment (2): Detection of Blood by Benzidine Test

- It is often necessary to detect the presence of small quantities of blood in urine, stomach contents etc.

Principle

- This method depend on the fact that the heme group of hemoglobin possesses a peroxidase-like activity which catalyzes the breakdown of hydrogen peroxide (H_2O_2).
- The oxidizing species formed in this reaction can then react with benzidine giving blue greenish color.

Heme (hemoglobin) + H_2O_2 → **H_2O + [O]**

[O] + benzidine → blue greenish complex

Note: the test is **not specific** for blood as peroxidases present in milk, potatoes and pus, as well as the ions of Fe^{+3}, Cu^{+2} and K^{+1} will give false positive results

Experiment (2): Detection of Blood by Benzidine Test

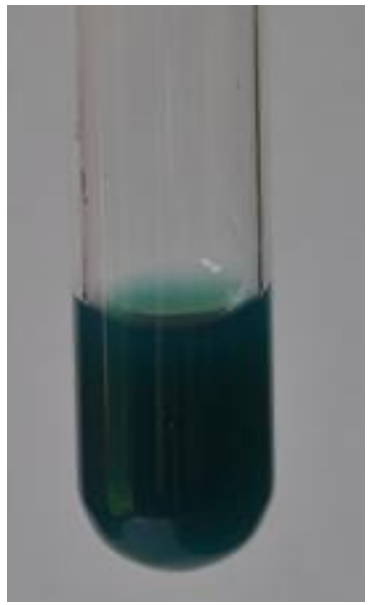
Method

- Place 3 ml of sample in a boiling water for 3 min
- Cool it under tap water
- Add 2 ml Benzidine + 1 ml H_2O_2

Results

- If the test is **negative** → blood is absent from sample.
- If the test is **positive** → blood is probably **not definitely** present in sample.

➢ For this reason these tests are often described as **"presumptive tests"** .



Positive results

Homework:

a. Why 0.9 saline solution is used in vaccine ?

b. Why does salt water help to reduce swollen gums?

c. How many grams of sucrose are needed to prepare 100 ml of isotonic solution expressed in (w/v%), knowing that the osmolarity of RBC = 0.308 Osmolar ?