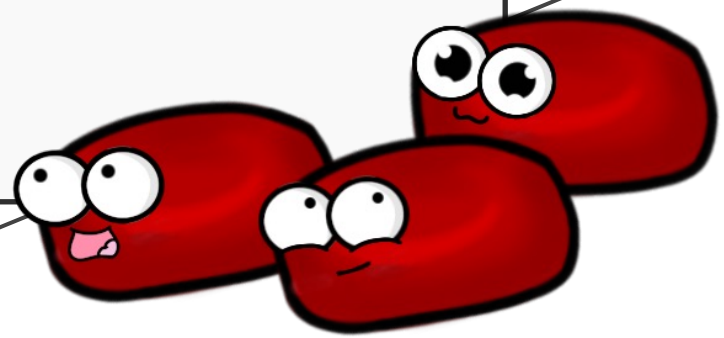
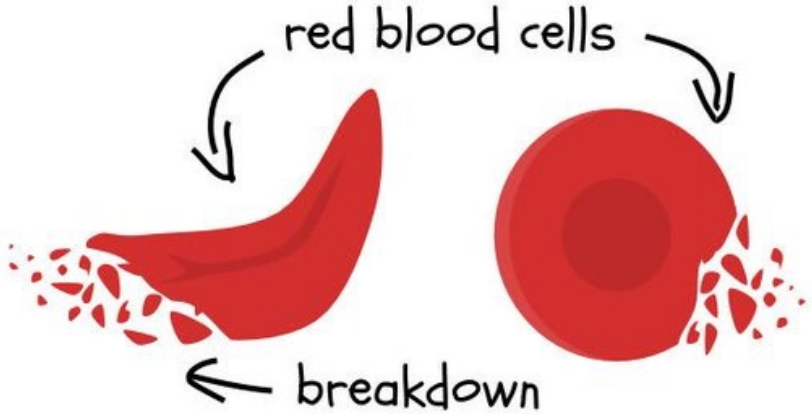
Blood Biochemistry BCH 220 [Practical]

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.*
- *In vivo*: in the living organism.
- *In vitro:* it happens outside of a living organism.



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

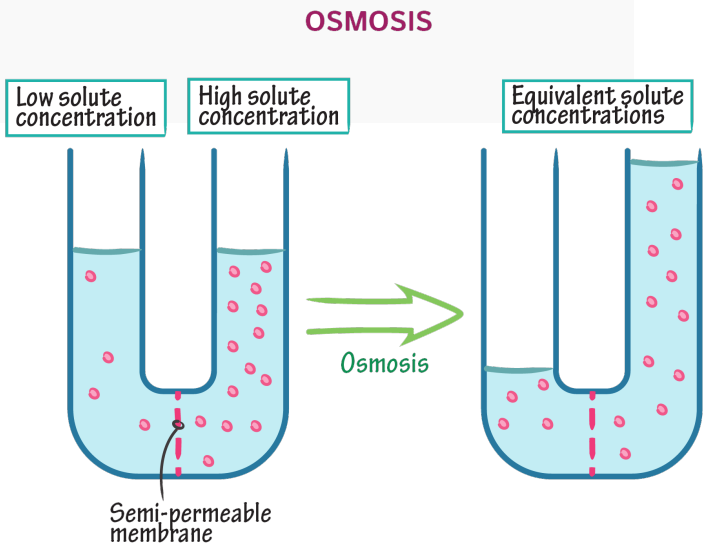
Osmosis and Osmotic Pressure

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.



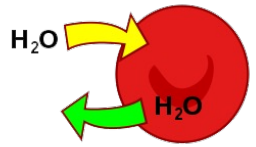
Tonicity

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%,** 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



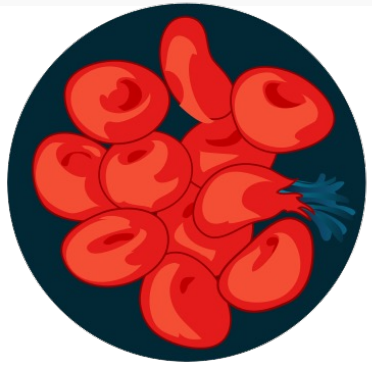
Tonicity

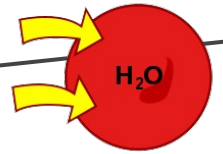
Types of solutions:

➢ **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





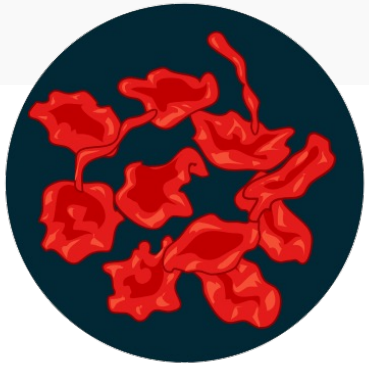
Tonicity

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.

Experiment (1): Hemolysis Test

Method

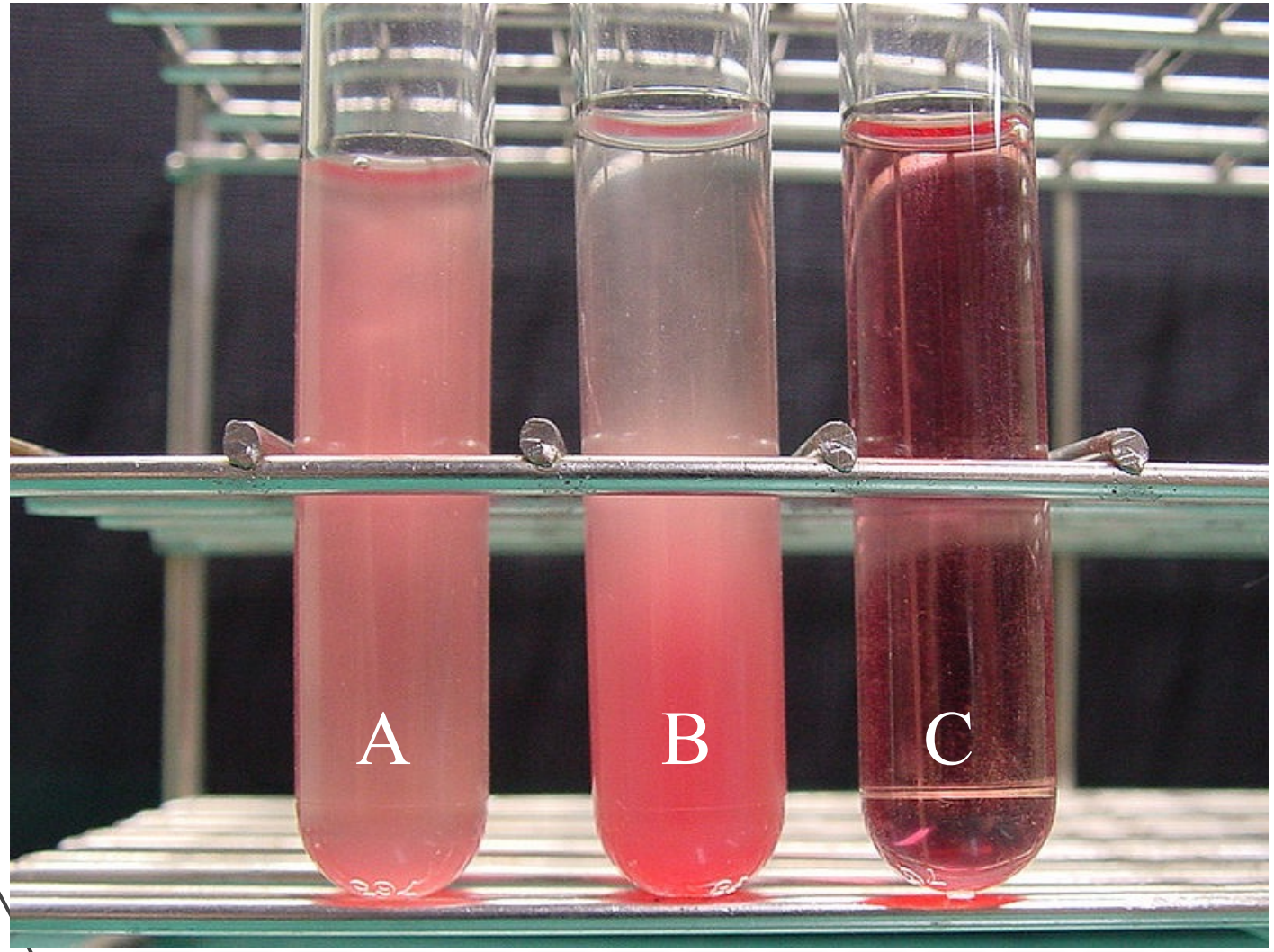
1. Label 6 tubes (A→ F). Then, add 1 ml of RBCs suspended in saline into each tube

	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. When is 0.45% saline used as treatment?

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