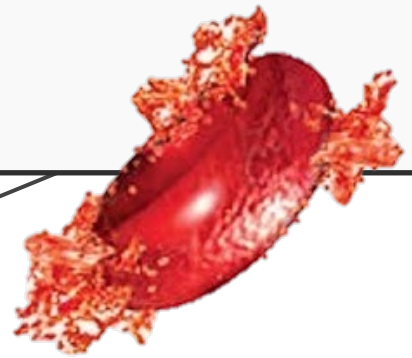
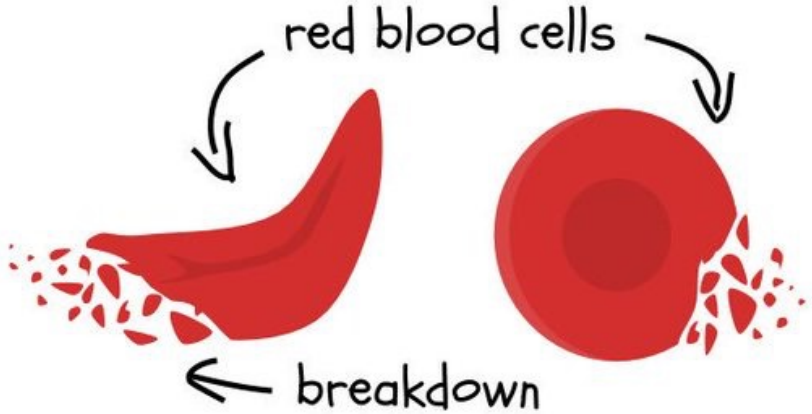
Blood Biochemistry BCH 471 [Practical]

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:**

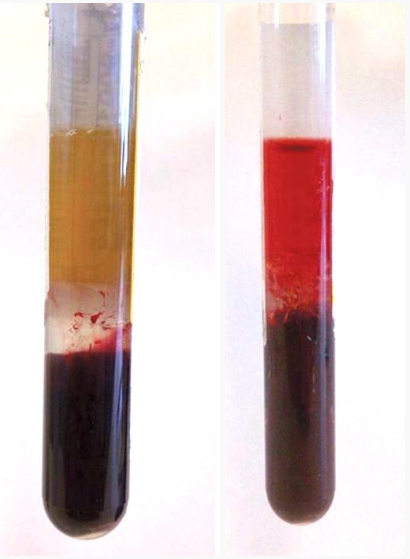
1. Immune reactions (e.g. during blood transfusion)
2. Infections (e.g. malaria infection)
3. Medications (e.g. cisplatin, a chemotherapy drug)
4. Toxins and poisons

- Since the potassium concentration inside red blood cells is much higher than in the plasma, elevated potassium in plasma is usually found in biochemistry **tests of hemolyzed blood**.

Hemolysis *in-vitro*

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

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



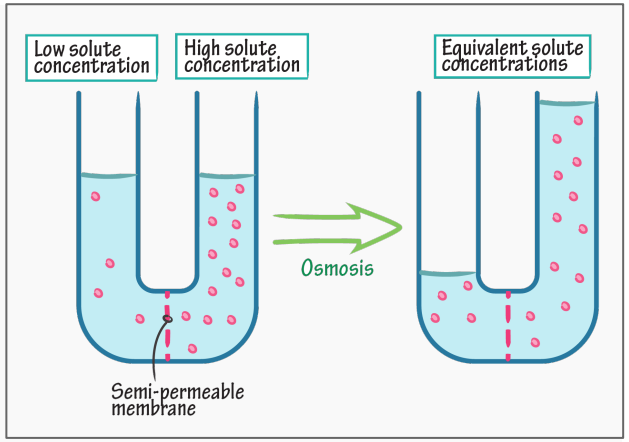
(Normal) (Hemolyzed)

- **When blood hemolysis should be done?**

1. Breaking down RBCs to release their content
2. Estimation of haemoglobin
3. To obtain erythrocyte-free preparation of leukocyte and platelet

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

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 passes through a semi-permeable membrane in osmosis.

Tonicity:

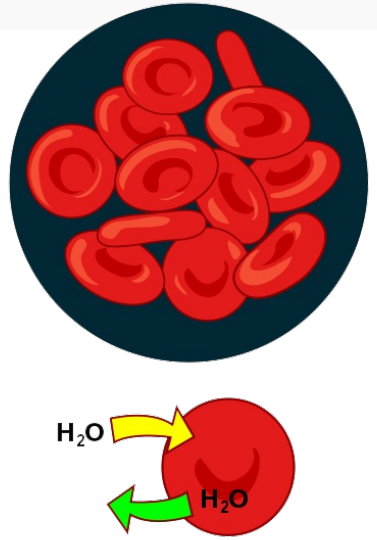
Is the ability of an extracellular solution to make water move into or out of a cell by osmosis.

- Accordingly, there are three types of solutions: **Isotonic**, **Hypotonic**, and **Hypertonic**.

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.

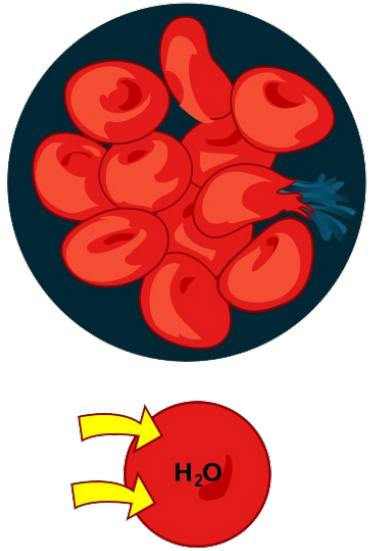
Solute inside the cell = Solute outside the cell



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.

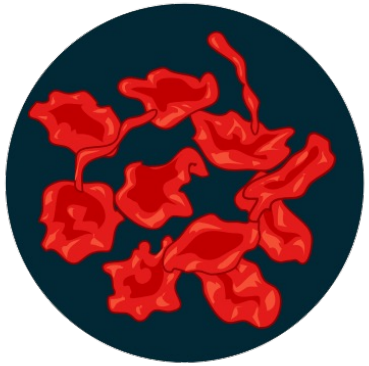
Solute outside the cell < Solute inside the cell



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 evaluate the effects of different solutions in blood hemolysis.
2. To detect the presence of blood in a biological sample.

Calculate the concentration of an isotonic solution of a specific substance

For example, what is the concentration of NaCl (w/v%) that will make an isotonic solution for RBC?

knowing that the osmolarity of RBC = 0.308 Osmolar.

First: Calculate the molarity from the 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

To Calculate in w/v% expression:

Second: Calculate the No. of moles: [2]

→ **No. of moles = M x V (in L) =**

→ 0.154 M x 0.1 L = 0.0154 moles

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

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

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

→ Wt (g) = No. of moles x Mwt

→ 0.0154 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

Experiment (1): Hemolysis Test

Method

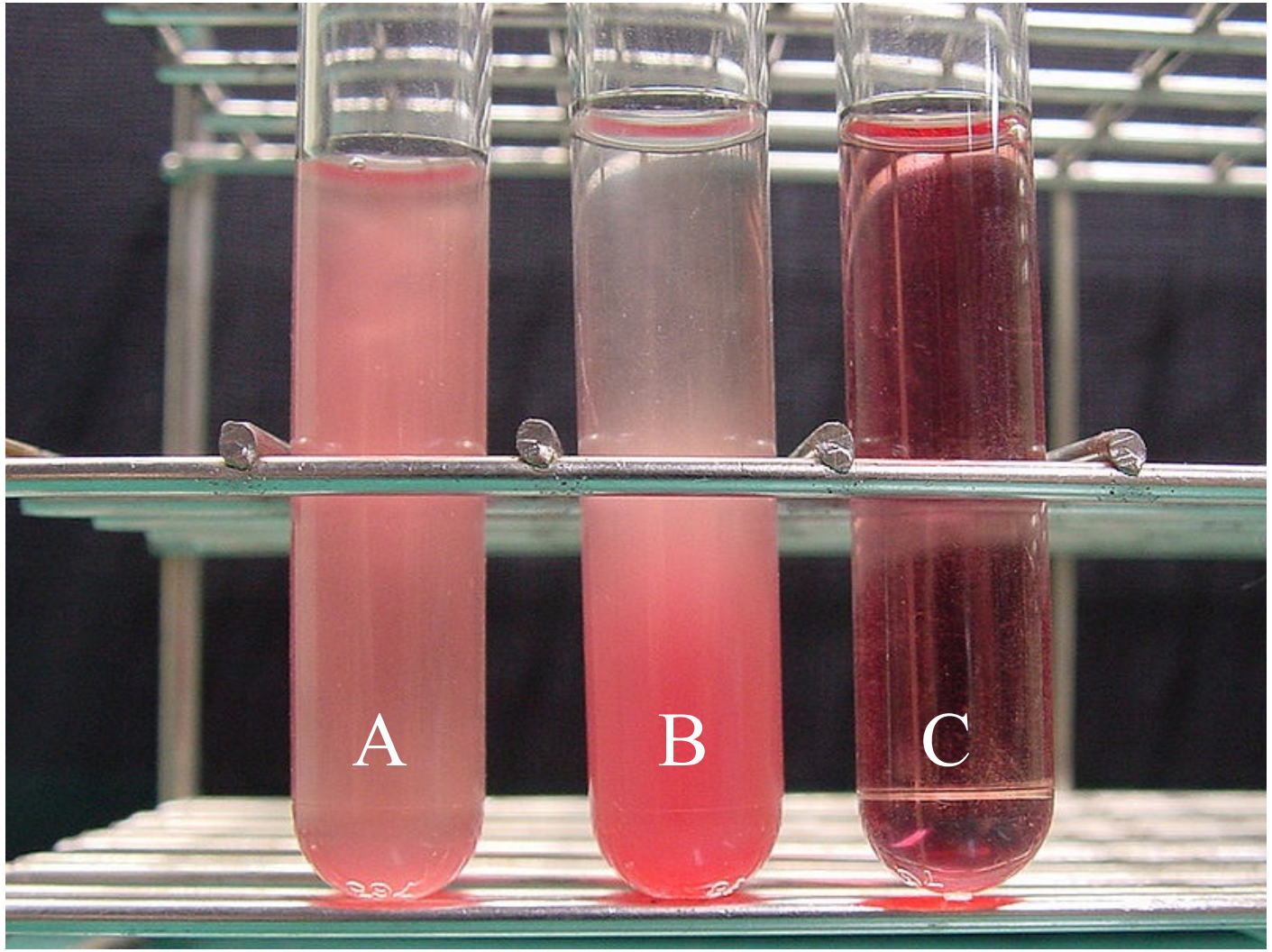
1. Label 6 tubes (A→ F). Then add as following:

	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 0.1 M				3 drops		
HCl 0.1 M					3 drops	
Dis. Water						5 ml
NaCl 0.9%				5 ml	5 ml	

2. Add 1ml of Sample A into each tube
3. Wait 10 - 30 min
4. Observe whether 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

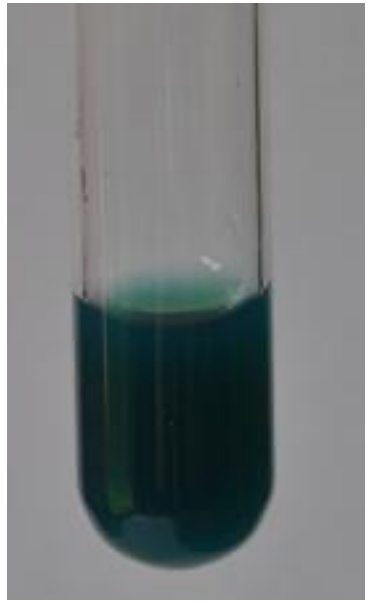
In a test tube add:

- Pinch of benzidine powder.
- 2ml glacial acetic acid.
- 3ml hydrogen peroxide (H_2O_2).
- Add 1 – 2 ml of the sample B.

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