

OSMOTIC FRAGILITY TEST

- **By d Gehan el wakeel**

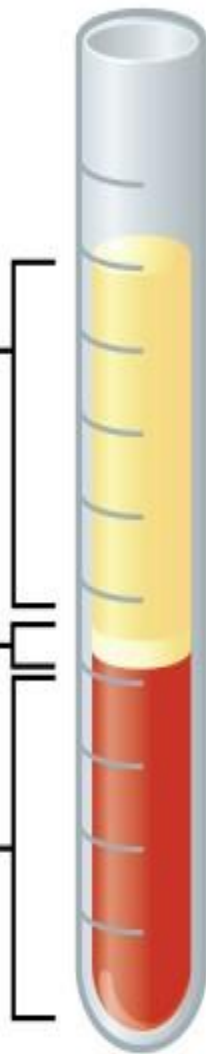
COMPOSITION OF BLOOD

- **1- Plasma: 55% of blood volume**
- **Composition:**
- **Water:90%**
- **Dissolved solutes:**
- **A) Organic:** Plasma proteins, lipids ,hormones ,enzymes ,nutrients and waste products
- **B)Inorganic constituents:**The various electrolytes :Na⁺,K⁺,Cl⁻,Ca²⁺andPO₄³⁻
- **2-Cells : 50% of blood volume**
- **Red blood cells , white blood cells and platelets**

Plasma:
- Water, proteins,
nutrients, hormones,
etc.

Buffy coat:
- White blood cells,
platelets

Hematocrit:
- Red blood cells



Normal Blood:

♀ 37%–47% hematocrit
♂ 42%–52% hematocrit



Anemia:

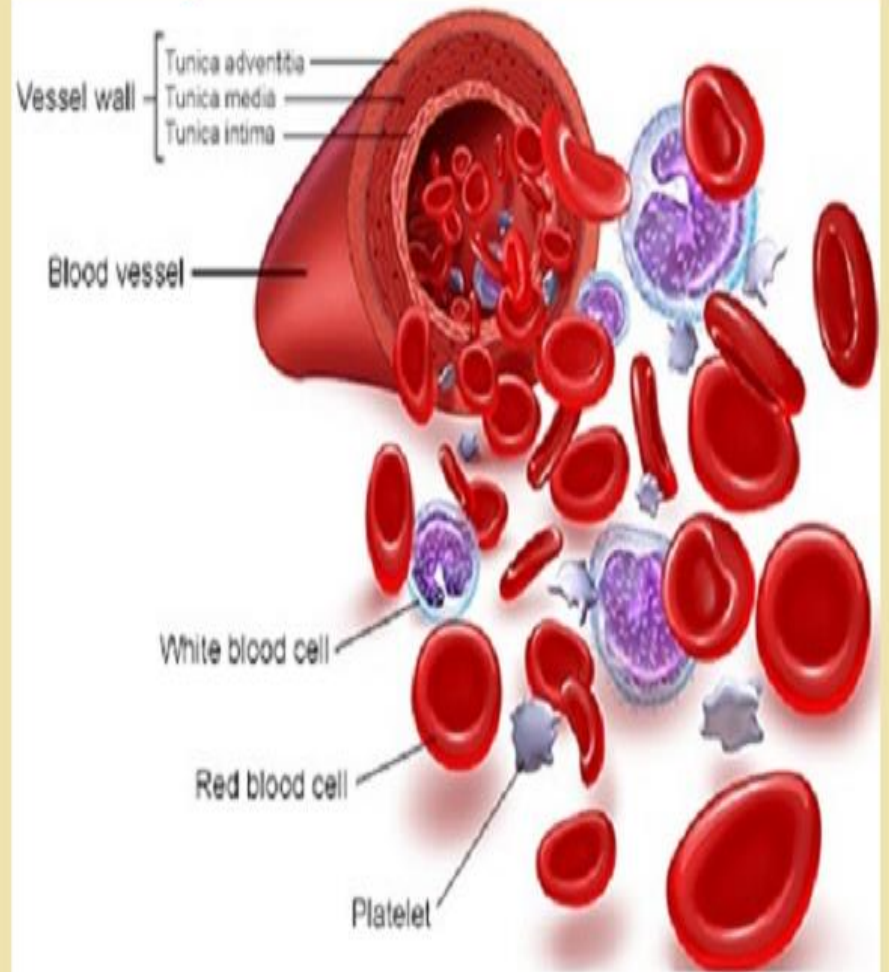
Depressed
hematocrit %



Polycythemia:

Elevated
hematocrit %

Composition and Functions of Blood

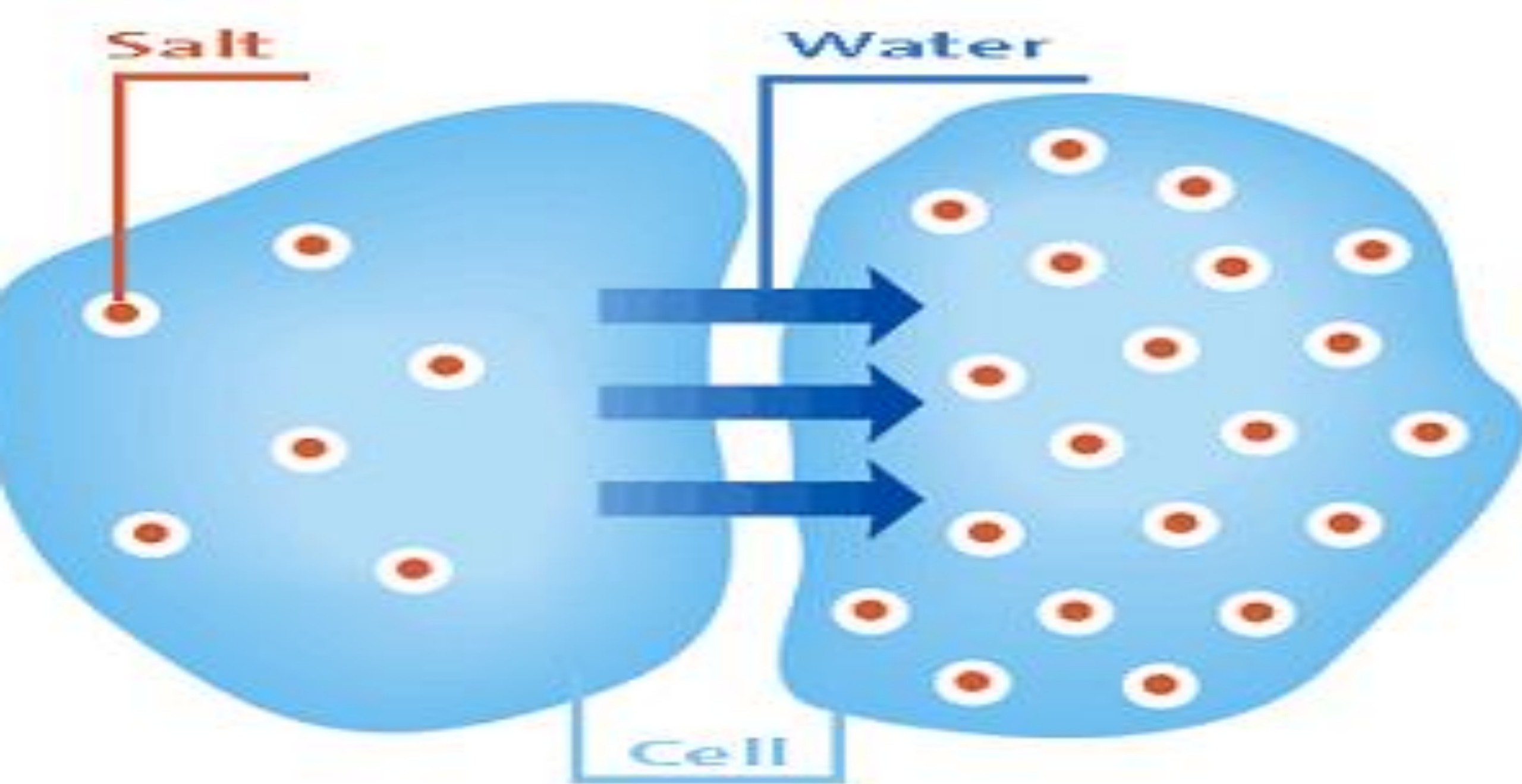


OSMOSIS

Definition

- It is the passive flow of water across a semi-permeable (selectively permeable) membrane down a concentration gradient of water

: means **from high concentration** of water **to low concentration** of water or **low concentration of solute** **to high concentration** of solute.



2) PHYSIOLOGICAL SIGNIFICANCE OF OSMOSIS:

At the cellular level:

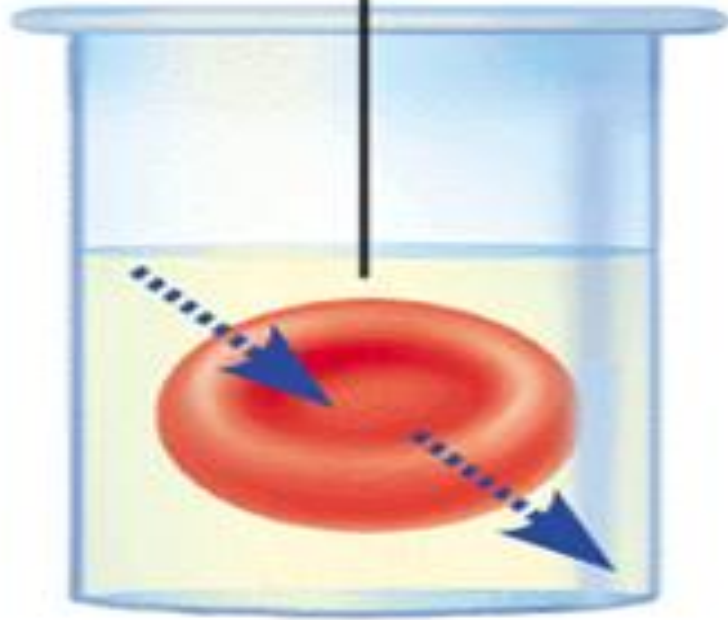
- The ECF and ICF are osmotically equal
- any change in plasma osmolarity causes cells to shrink or swell

Tonicity:

- It is the ability of a solution to affect fluid volume and pressure within a cell.
 - It depends on concentration and permeability of the cell to the solute
 - It is used to describe the osmolarity of a solution relative to plasma;
1. Isotonic solution has the same osmolarity as plasma and causes no change in cell volume e.g. NaCl solution 0.9 %.
 2. Hypotonic solution has osmolarity less than the plasma which causes drawing water into the cell resulting in cell swelling.
 3. Hypertonic solution has osmolarity higher than the plasma which causes drawing water out of the cell resulting in cell shrinkage.

Isotonic

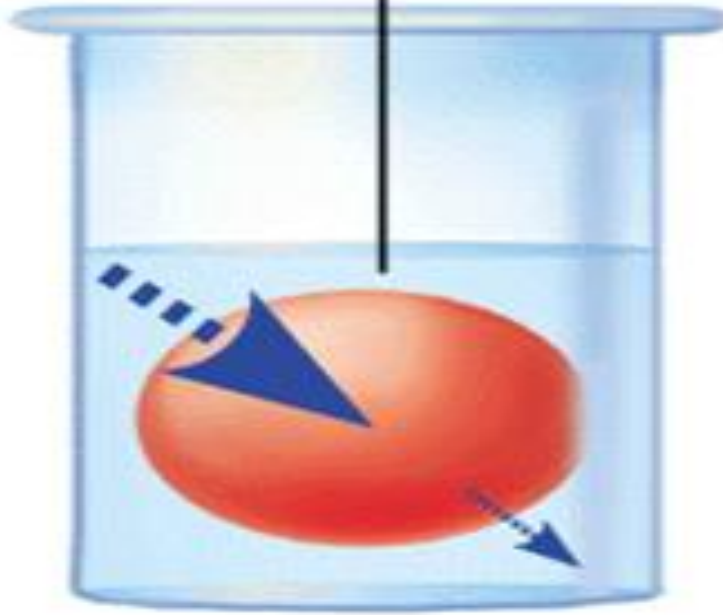
9 grams of salt in 1 liter of solution



no change in cell volume

Hypotonic

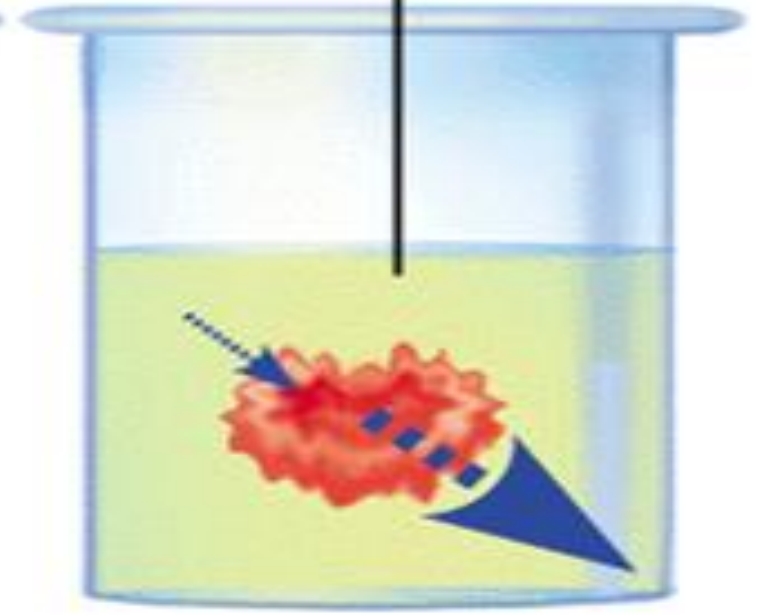
Pure water



cell swelling

Hypertonic

18 grams of salt in 1 liter of solution




cell shrinkage

RED BLOOD CELLS

- **Are non- nucleated circular biconcave discs**
- **Life span: 120 days**
- **Count:5 millions**
- **Structure:**

1- cytoplasm : formed of hemoglobin

2-cell membrane

- ❖ Its main function is to **keep haemoglobin** inside the cell
 - ❖ The red cell membrane is **permeable to various electrolytes**
 - ❖ It has **high degree of flexibility** which allow red cell to be deformed into any shape and pass through narrow blood channels then return to normal shape after passing
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OSMOTIC FRAGILITY OF RED BLOOD CELLS

- **Isotonic solutions: 0.9% NaCL (saline)** is isotonic with plasma
- When red blood cells are suspended in **hypertonic saline** solutions, water diffuses out of the cell and they shrink
- When suspended in **hypotonic saline** solution, water diffuses into the cell, it swells and may rupture
- The ability of the red cells to resist hemolysis in hypotonic solutions determines their osmotic fragility
- **The biconcave shape of red cells allows 45-65%** increase in their volume before they rupture

□ Haemolysis :

- ❖ It normally starts in about **0.5% NaCl solution**
- ❖ It completes in about **0.35% NaCl solution**

CONDITIONS IN WHICH THERE IS INCREASED RED CELL FRAGILITY

1-Inherited defects in red blood cells:

Hereditary spherocytosis: the cell is spherical so lack biconcave shape and is small and fragile so rupture easily

2-Old cells : decreased power of Na pump :Na accumulate inside the cell. With water osmosis

MATERIALS

- **1- One test tube rack containing ten test tubes.**

2 - Volumetric pipette (2ml)

3- A dropping pipette with a rubber teat.

4- Blood obtained by vein-puncture from a volunteer.

5- Nacl 1%.

6- 0.3 molar of Urea.

7- 0.3 molar of Glucose.

9- Soap | 10- 70% Alcohol

EXERCISE 1:

- 1. Label tubes from one to ten in sequence.**
- 2. Prepare from 1% NaCl solution different solutions of a progressively increasing concentration of NaCl (0.3 up to isotonic saline of 0.9 concentrations) as the table**
- 3. Dispense one drop of blood to each of the 10 test tubes. Mix well and let stand for 3 minutes.**
- 4. Centrifuge the test tubes at 3000 RPM for 2-3 minutes.**
- 5. Hold the rack of tubes up to the light and compare them:**
 - If the solution is red and transparent, hemolysis has occurred.**
 - If the solution transparent and there is a precipitate at the bottom, remix it**
- 6. Report the NaCl concentration at which: A) Complete hemolysis occurred b) Partial hemolysis occurred c) No hemolysis occurred.**
- 7. Explain the results.**

EXERCISE2

- To see the osmotic effect of substances that penetrate or damage the cell membrane:

1. **Prepare 10 ml of each of the following substances :**

NaCl 1%. 0.3 molar of Urea. 0.3 molar of Glucose. Soap 10- 70% Alcohol

2. Dispense a drop of blood to each tube, mix well then let stand for 3 minutes.

3. **Centrifuge at 3000 RPM for 1 minutes.**

4. Discuss the results of hemolysis and speed of hemolysis related to the type of tested substances and their osmolarities.

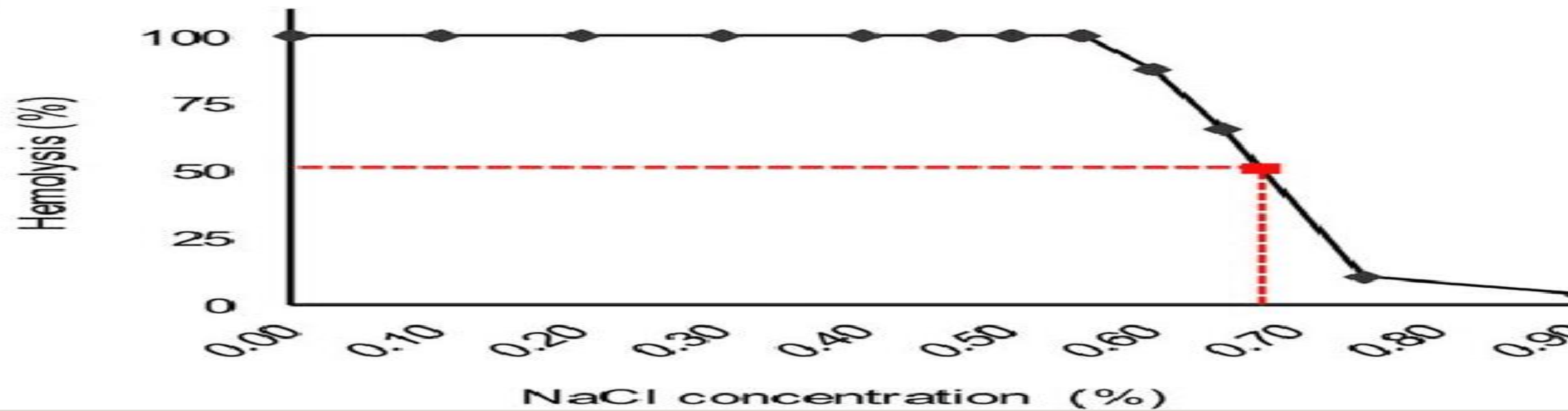
RESULTS OF EXERCICE 2

- **Ionic Nacl** : Non – permeable:**No hemolysis** (0.1 molarity-0.3osmolarity)
- **Polar Small size Urea** :Freely permeable:**Complete hemolysis** (0.3 molarity-0.3 osmolarity)
- **Polar Large size glucose** :Selectively permeable:**No hemolysis** (0.3 molarity-0.3 osmolarity)
- **Detergent Nacl + Soap** : Damages cell membrane :**Complete hemolysis**
- **Organic solvent Alcohol** : Dissolve the lipid of Cell membrane:**Complete hemolysis**



0.00 0.10 0.20 0.30 0.40 0.45 0.50 0.55 0.60 0.65 0.75 0.90

B



FIGURE

- The curve in the figure shows that:
- 1- At concentration **0.9%** and **0.8%** there is no hemolysis
- 2- At concentration **0.7%** hemolysis begins
- 3- Hemolysis increases from concentration **0.7%** to concentration **0.1%**
- 4- 100% hemolysis begins at **0.6%** till **0.1%**

I- WHICH IS A CONCENTRATION OF SOLUTION THAT DOES NOT CAUSE RED BLOOD HAEMOLYSIS?

- a) 0.1% Nacl solution
- b) 0.5% Nacl solution
- c) 0.9% Nacl solution
- d) 0.3% Nacl solution
- e) 0.6%Nacl solution

2-WHICH OF THESE SOLUTION CAUSES NO HEMOLYSIS WHEN MIXED WITH BLOOD?

- a) **Polar Small size Urea** : (0.3 molarity-0.3 osmolarity)
- b) **Polar Large size glucose : (0.3 molarity-0.3 osmolarity)**
- c) **Detergent Nacl + Soap**
- d) **Organic solvent Alcohol**
- e) **0.1% Nacl solution**

DEFINE OSMOTIC FRAGILITY AND MENTION 2 CAUSES OF INCREASED RED CELL FRAGILITY

Answer

Definition The ability of the red cells to resist hemolysis in hypotonic solutions determines their osmotic fragility

2 causes of increased fragility

1-Old cells : decreased power of Na pump :Na accumulate inside the cell. With water osmosis **1-Inherited defects in red blood cells:**

2-Hereditary spherocytosis:the cell is spherical so lack biconcave shape and is small and fragile so rupture easily

THANK

•you