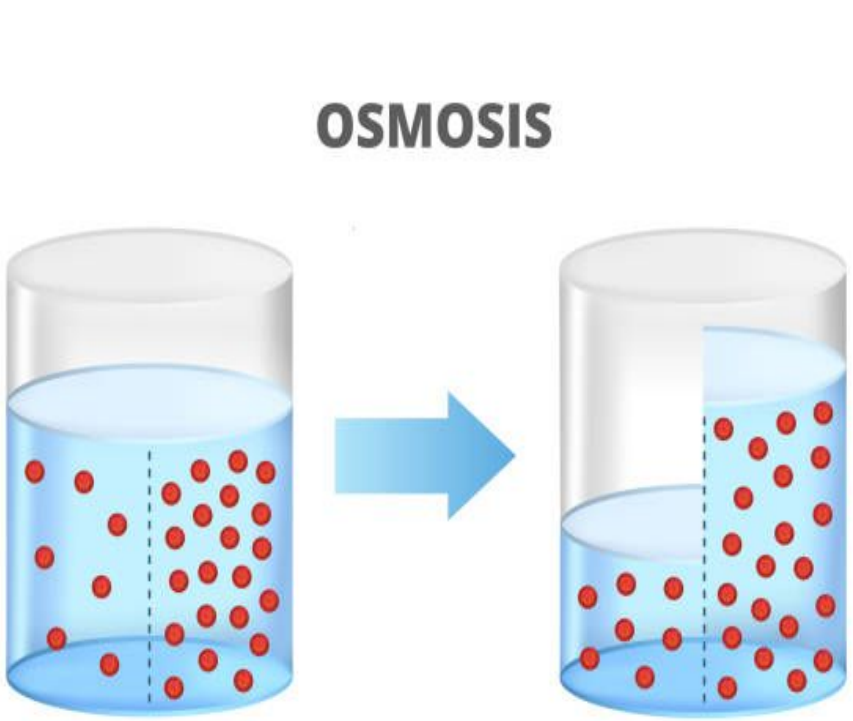
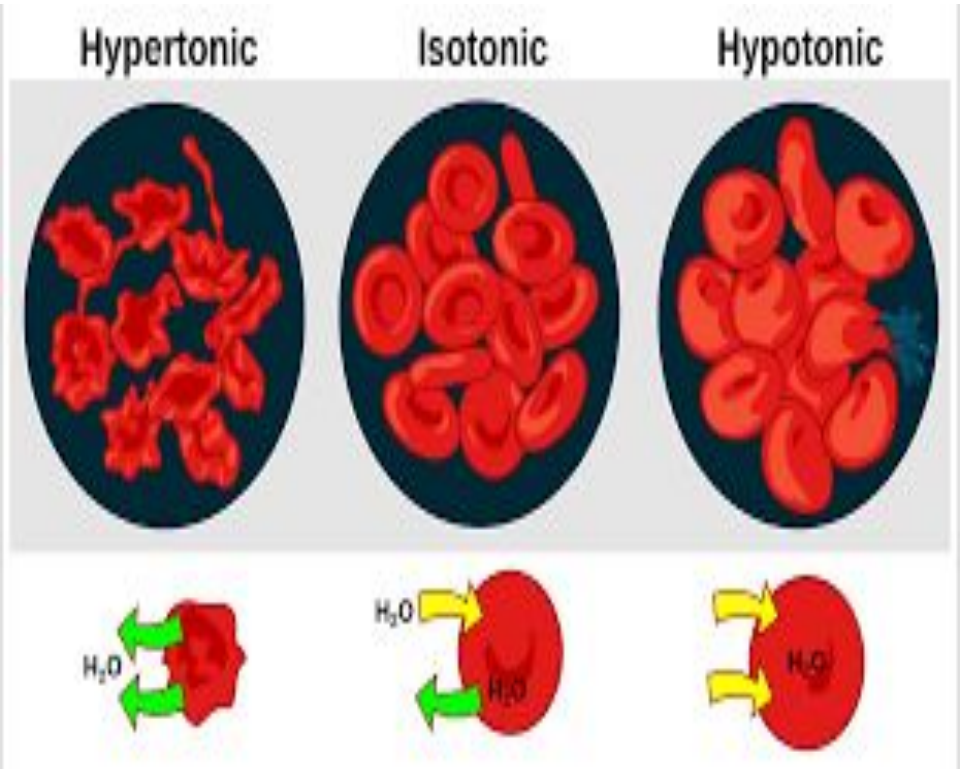
Osmotic Fragility Test





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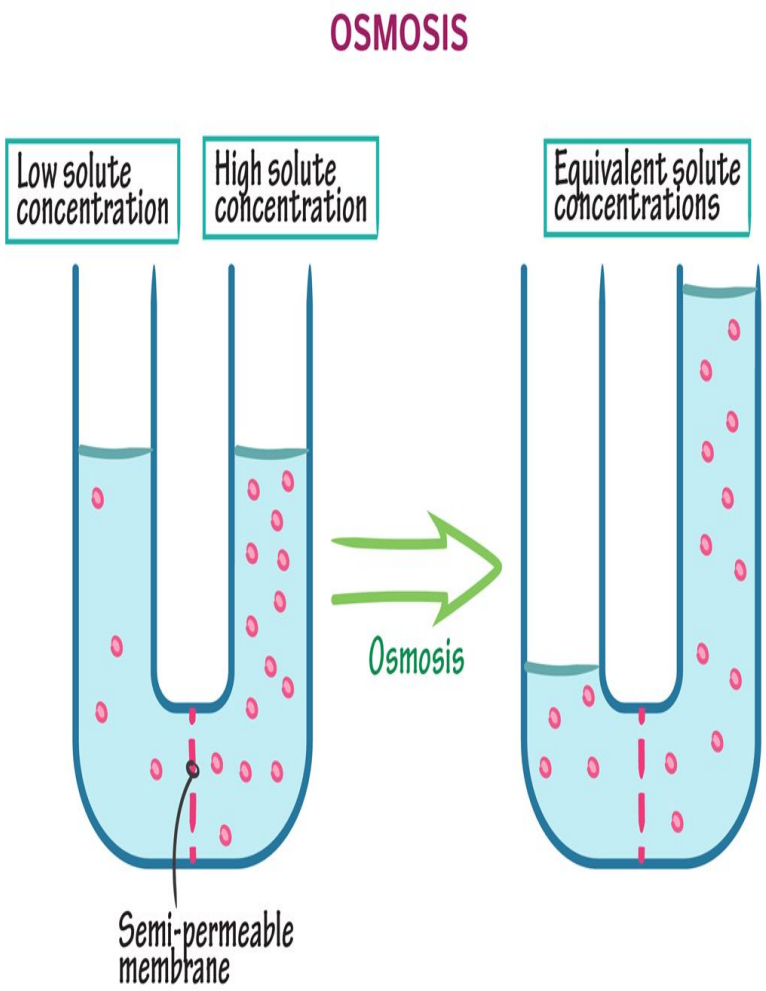
Osmosis

Osmosis is a process by which the molecules of a solvent pass from a solution of low concentration to a solution of high concentration through a semi-permeable membrane.

As the solvent moves, the concentrated solution is diluted and the concentration on both sides of the membrane is equalized.



Diffusion:-It is the movement of particles from an area of higher concentration to lower concentration to equalize concentration

Difference between Diffusion and Osmosis

Diffusion	Osmosis
Any type of substance moves from an area of highest energy or concentration to a region of lowest energy or concentration.	Only water or another solvent moves from a region of high energy or concentration to a region of lower energy or concentration.
Diffusion can occur in any medium, whether it is liquid, solid, or gas	Osmosis occurs only in a liquid medium.
Diffusion does not require a semipermeable membrane.	Osmosis requires a semipermeable membrane.
Diffusion does not depend on solute potential, pressure potential, or water potential.	Osmosis depends on solute potential.

Osmotic Solutions

0.9 g% NaCl corresponds to 9 g/L of NaCl

There are three different types of solutions:

Isotonic Solution

Hypertonic Solution

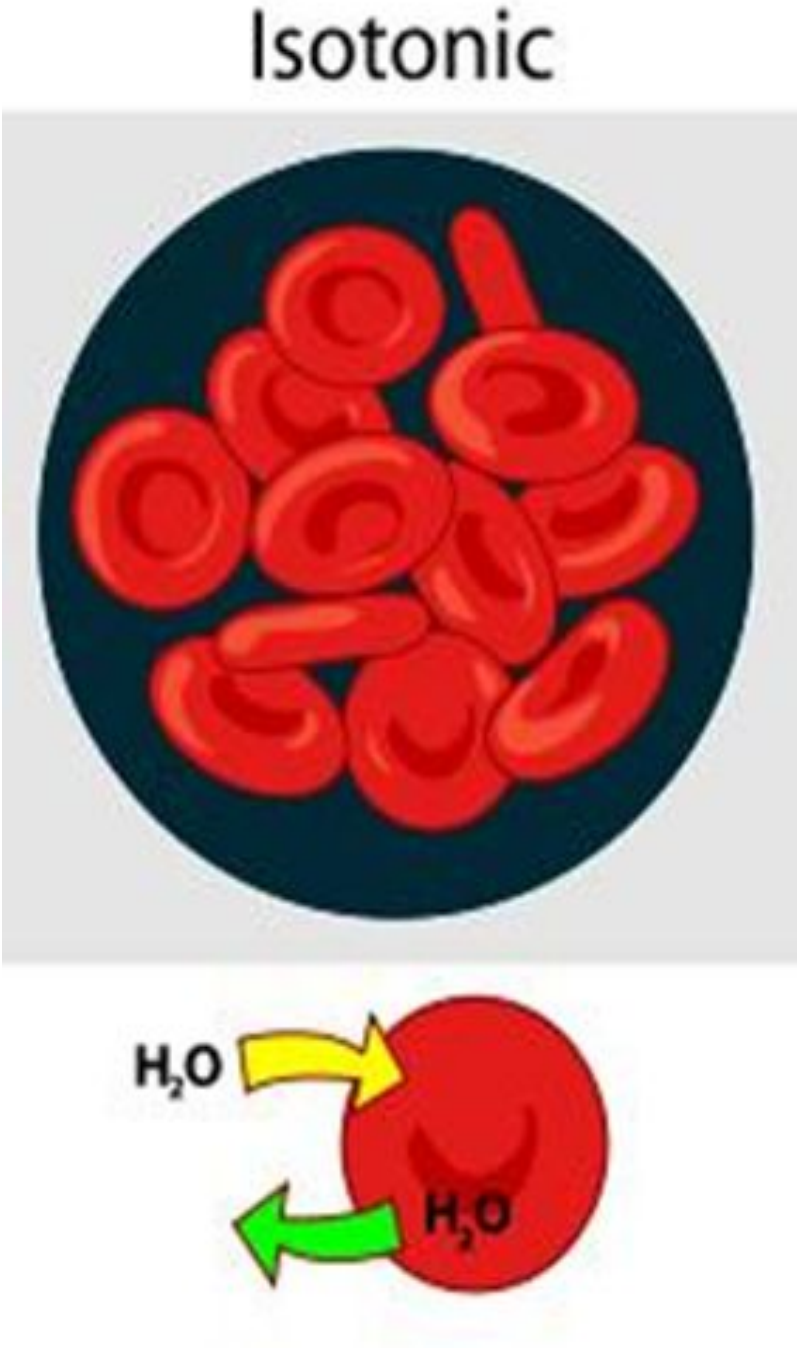
Hypotonic Solution

Isotonic solution

A solution that has the same salt concentration as cells and blood.

When cells are in isotonic solution, movement of water out of the cell is exactly balanced by movement of water into the cell.

A 0.9% solution of NaCl (saline) is isotonic .

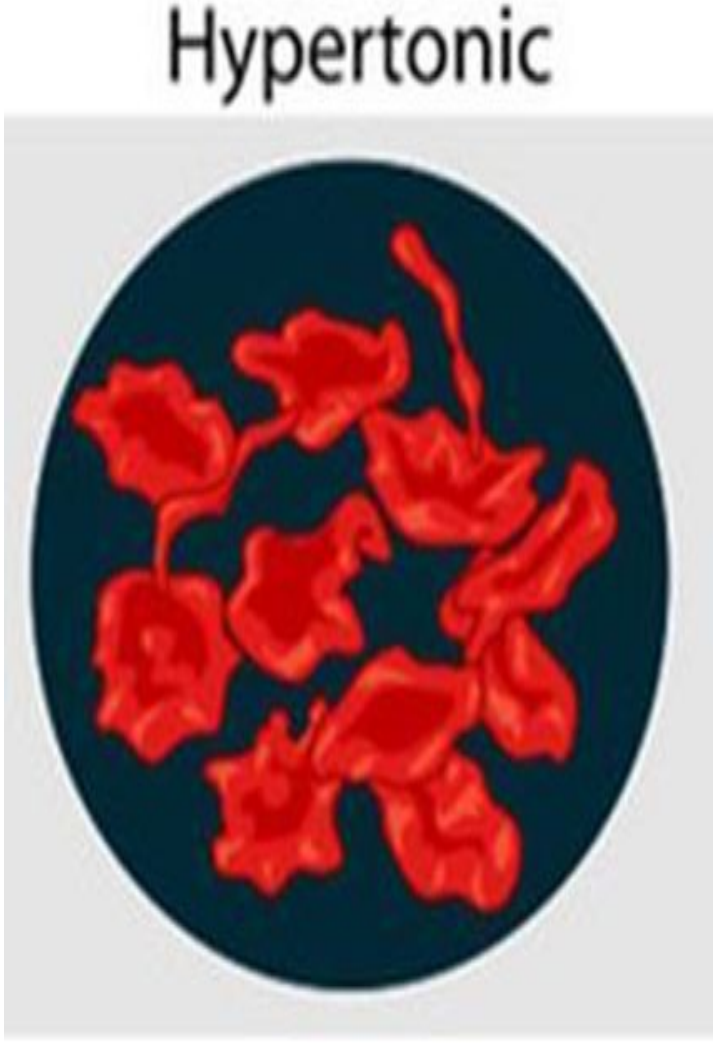


Hypertonic solution

In a hypertonic solution the total molar concentration of all dissolved solute particles is greater than the concentration in a cell.

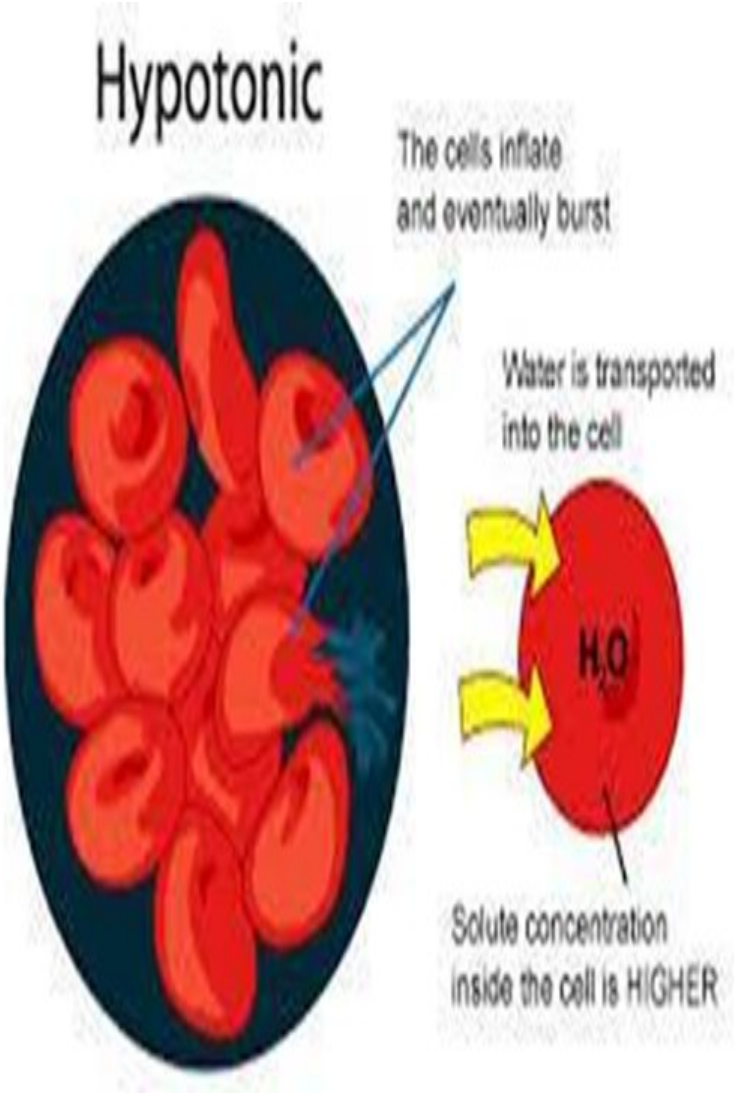
If concentrations of dissolved solutes are greater outside the cell, the concentration of water outside is correspondingly lower.

As a result, water inside the cell will flow outwards to attain equilibrium, causing the cell to shrink.



Hypotonic solution

It refers to a solution that contains a lower amount of solute as compared with the solute concentration in the other solution across a semipermeable membrane.



Osmotic fragility

Definition:

Osmotic fragility is a test to measures red blood cell (RBC) resistance to hemolysis when exposed to a series of increasingly dilute saline solutions.

The sooner hemolysis occurs, the greater the osmotic fragility of the cells.

The fragility of RBCs may be defined as inability of RBC membrane to resist the low concentrations of Nacl solutions (hypotonic solutions).

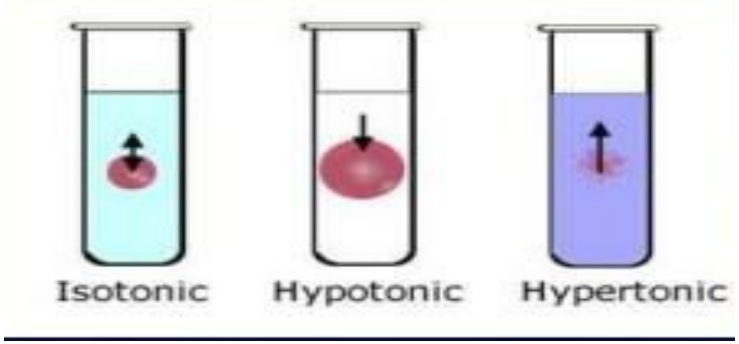
Factors affect the osmotic fragility

- Increased surface to volume ratio.
- Decreased surface to volume ratio.
- Functional state of the red blood cell membrane.

PRINCIPLE:

In the osmotic fragility test, whole blood is added to varying concentrations of buffered sodium chloride solution and allowed to incubate at room temperature.

The amount of hemolysis in each saline concentration is then determined by reading the supernatants on a spectrophotometer



Normal mature RBC are biconcave, round discs that are about 6-8μ in diameter.

The term used to indicate red blood cells of normal size and shape is normocytic.

The term used to indicate a normal color or central pallor (i.e.,normal hemoglobin content) is normochromic.

 A normal RBC count would be:

- **Men** – 4.7 to 6.1 million cells per microliter (cells/mcL)
- **women** – 4.2 to 5.4 million cells/mcL
- Normal life cycle of RBC is 120 days
- A high red blood cell count is a condition called Polycythemia Vera.
- A decreased red blood cell count is a condition called Anemia.

- In isotonic solution (0.9% of Nacl solution) the RBCs, can live without changing their shapes for several hours.
- In circulation RBCs live for 120 days and then become highly fragile and rupture i.e. Hemolysis occur on passing through minute capillaries.

Why the Test is performed?

This test is performed to detect

1-Thalassemia

2-Hereditary Spherocytosis.

- Thalassemia is an inherited condition that affects the portion of blood (hemoglobin) that carries oxygen.
- Some red blood cells are more fragile than normal, but a larger number are less fragile than normal.

Hereditary spherocytosis

- is a common disorder in which red blood cells are defective because of their round, ball-like (spherical) shape.

These cells are more fragile than normal.

- Spherical cells are said to have increased osmotic fragility because they are less likely to expand and break open in salter water than normal red blood cells (which are indented or curved inward on both sides).

- **Osmotic fragility decreased in:**
  - Thalassemia.
  - Iron deficiency anemia.
  - Sickle cell anemia.
  - Reticulocytes

- **Osmotic fragility of red cells increased in:**
  - Hereditary spherocytosis.
  - Acquired spherocytosis.
  - Poisoning
  - Severe Burn
  - Malaria
    Hemolytic Disease of the Newborn
    Acquired Hemolytic Anemia

Factors Affecting Osmotic Fragility test Interpretation

Interpretation of Osmotic Fragility of RBC and diagnosing associated medical conditions is not straightforward.

Various factors have to be taken into consideration as they can influence the test results.

1. **Temperature**: Higher temperatures increase osmotic fragility.
2. **pH**: Lower pH increases fragility, while higher pH decreases it.
3. **Age of blood sample**: Older samples tend to be more fragile.
4. **Incubation time**: Longer incubation can increase fragility.
5. **Ongoing medications** (e.g. aminoglycosides drugs) can alter red blood cell membrane stability.
6. **Lipid content of RBC membranes**: Higher lipid content can decrease fragility.
7. **Underlying health conditions**: Diseases affecting red blood cells can impact results.

Practical

Material:

- **Specimen:** whole blood
- **Collection Medium:** Na Heparin tube or Lithium Heparin tube.
- **Minimum:** 5 ml whole blood.
- **Rejection Criteria:** Hemolyzed specimen.
- **Methodology:** Spectrophotometer.

Osmotic Fragility Test Procedure

- Prepare a series of hypotonic saline solutions (0.1% to 0.9% NaCl).
- Collect a blood sample from the patient.
- Add a small, equal amount of blood to each saline solution.
- Incubate the mixtures at room temperature for 30 minutes and centrifuge the samples.
- Examine the supernatant for hemolysis.
- Determine the concentration at which hemolysis begins (minimum fragility).
- Identify the concentration at which hemolysis is complete (maximum fragility).
- Compare results to normal reference ranges for accurate osmotic fragility test interpretation.
- Correlate findings in context of possible red blood cell disorders.

PRECAUTIONS

1-The drop of blood should be added immediately to avoid clotting.

2-The tubes should be marked carefully.

3-The tube should be read over the printed page for complete hemolysis, partial hemolysis and no hemolysis.

4-The size of drops should be of appropriate as it will alter the concentration of solution.

Relevant Questions

What are important properties of the RBC membrane?

- The cell membrane of RBC has two properties
- 1-Elasticity
- 2-Selectivie Permeability

What is the effect of sodium chloride solution of different strength on RBC membrane

- A sodium chloride solution of different strengths will affect the red blood cell (RBC) membrane by causing it to shrink in hypertonic solutions due to water loss through osmosis,
- and swell and potentially burst (hemolysis) in hypotonic solutions (low salt concentration)
- as water moves into the cell; a solution with a salt concentration close to that of the RBC (isotonic) will cause no net change in cell volume.

what is the difference between osmolarity and osmolality

Osmolarity refers to the concentration of dissolved particles per liter of solution,

while osmolality refers to the concentration of dissolved particles per kilogram of solvent,

what is the osmolarity of body solutions

The osmolarity of body solutions, typically referring to blood plasma, is around 280-300 milliosmoles per liter (mOsm/L), with a normal range considered to be between 275 and 295 mOsm/kg.


Thank
You