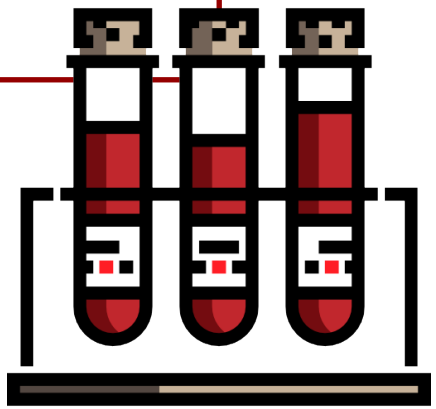
Blood Biochemistry BCH 471[Practical]

Lab (1) Separation of Plasma and Serum and Their Proteins from Whole Blood



Blood Terminology

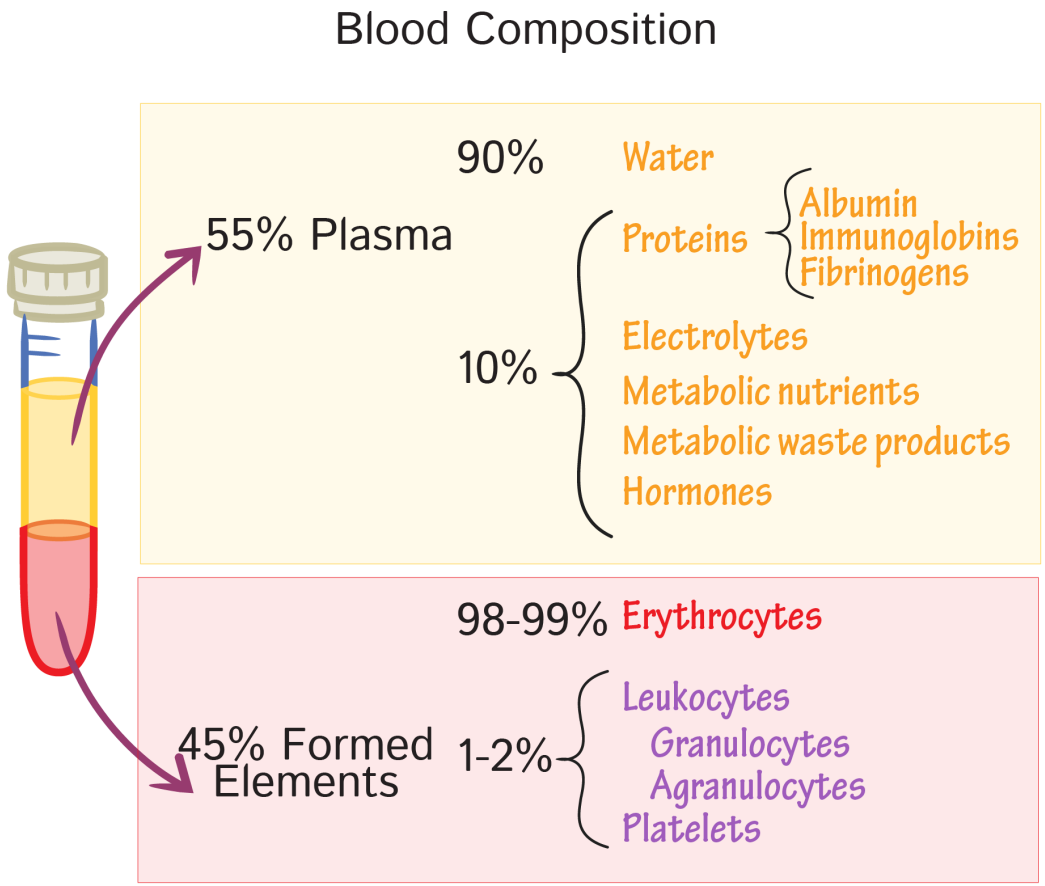
- **Blood**, fluid that transports oxygen and nutrients to the cells and carries away carbon dioxide and other waste products.
- **Plasma** is the liquid portion of blood, it constitutes about 55 % of blood volume and 90% of plasma is water.
- **Serum** resembles plasma in composition but lacks the coagulation factors.

(Serum = Plasma – clotting factors)

Blood Functions

- Supplying **oxygen** to cells and tissues.
- Providing essential **nutrients** to cells, such as glucose.
- Removing **waste** materials, such as carbon dioxide.
- **Protecting** the body from infection and foreign bodies through the white blood cells.
- **Transporting** hormones from one part of the body to another.
- **Regulating** acidity (pH) levels and body temperature.

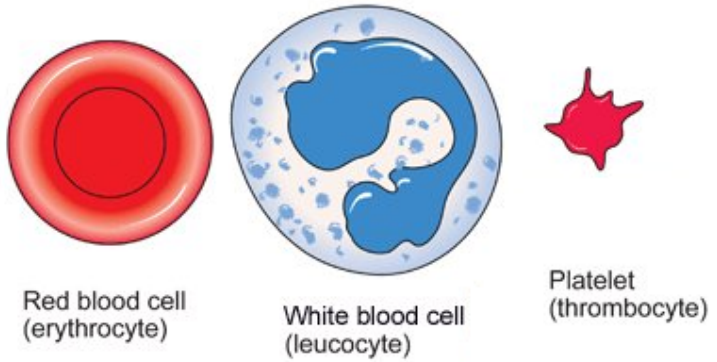
Blood Compositions



Formed Elements (BLOOD CELLS):

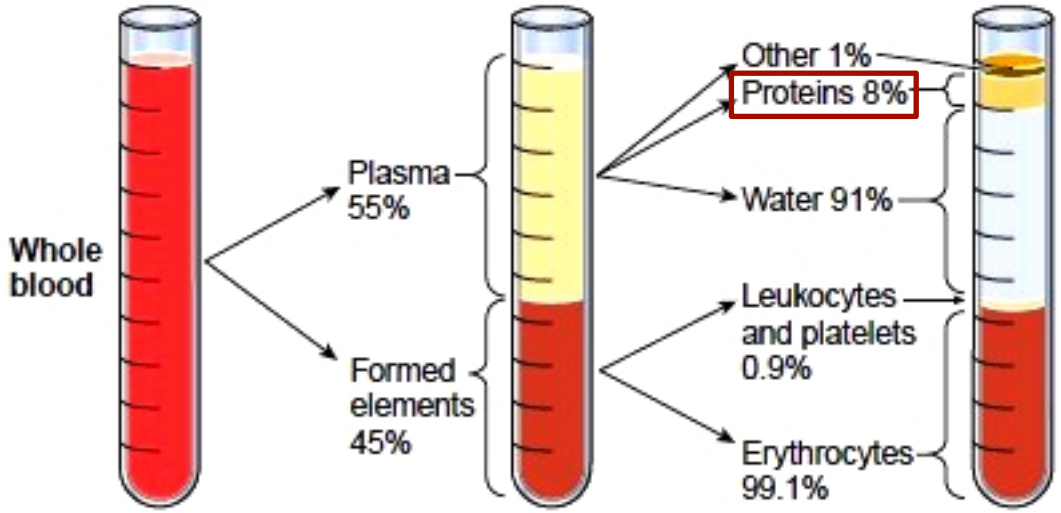
- Red blood cells (erythrocytes)
- White blood cells (leukocytes)
- Platelets (thrombocytes)

The cells are produced primarily by **bone marrow** and account for blood "solids".



Blood Proteins

- Blood contains 8% proteins.
- Plasma Proteins (PPS) Plasma contains >300 different proteins.
- Many pathological conditions affect level of PPS.



The main plasma proteins are:

Mwt ↓

- Albumin (36-50 g/l)
- Globulins (18-32 g/l)
- Fibrinogen (2-4 g/l)

Conc. ↑

Site of synthesis

- All plasma proteins are synthesized in the liver **except** gamma globulins (immunoglobulins) are synthesized by B cells.

Note: All plasma proteins are water soluble

Major Blood Proteins

➢ Albumin

It is the most abundant protein present in plasma.

Functions:

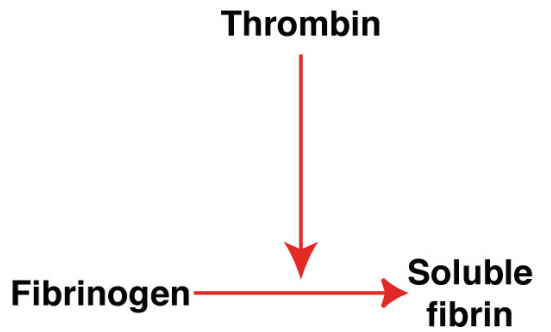
1. Maintenance of **colloidal osmotic pressure**, thus maintaining blood volume.
2. Serves in the **transport** of bilirubin, hormones, metals, vitamins, and drugs.
3. Adjusting blood pH.

➢ Globulins

Alpha (**α**) and beta (**β**) globulins are **transport proteins**, but gamma (**γ**) globulins are part of the **immune system**.

➢ Fibrinogen

1. Is a soluble glycoprotein (GP) with a central role in **blood clotting.**
2. It is converted by thrombin to fibrin during blood coagulation.



Serum Proteins

- Total serum protein consists of two main fractions, **albumin** and **globulin**.
- In normal people the A/G ratio is from **1.2** to **1.5**.
- Generally, the decrease in total protein is due to decrease in albumin fraction and increase is due to increase in globulin components.
- **Dehydration** is one condition in which the increase in total protein is due to increase in both albumin and globulin fractions because of haemoconcentration ➜ In this case the A / G ratio remains unaltered.

Note: ↓ albumin ↓ A/G ratio
↑ globulin ↑ A/G ratio
In dehydration: ↑ globulin ↑ albumin = A/G ratio

Serum Albumin and Globulin

A low serum albumin (Hypoalbuminemia) may be due to:

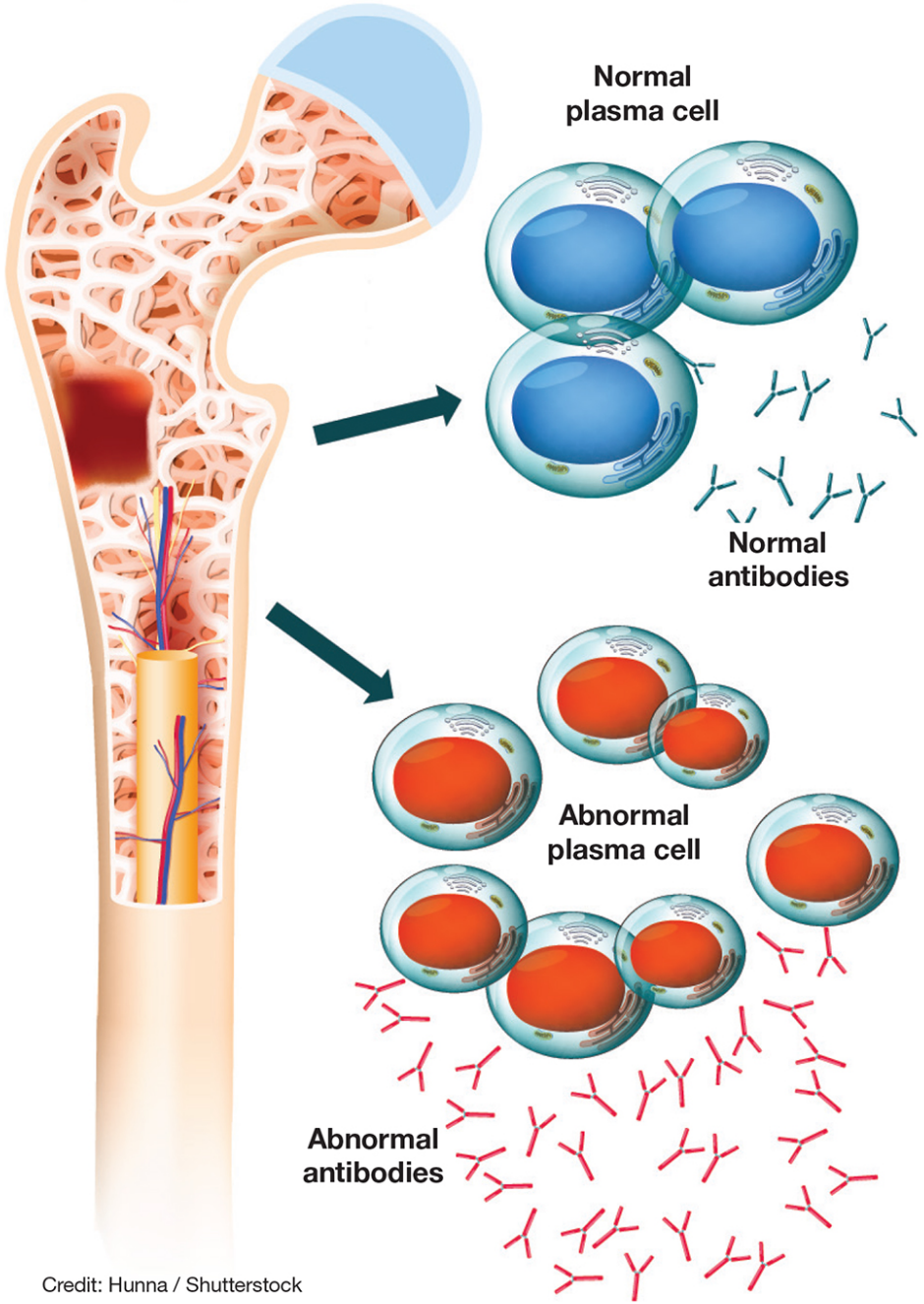
1. A heavy loss of albumin in urine.
2. Decreased synthesis by the liver due to defective liver.
3. Mal-absorption of protein from the digestive tract.
4. Increase catabolism of protein.
5. Insufficient intake of protein in diet.

A high serum globulin (Hyperglobulinemia) occurs commonly in:

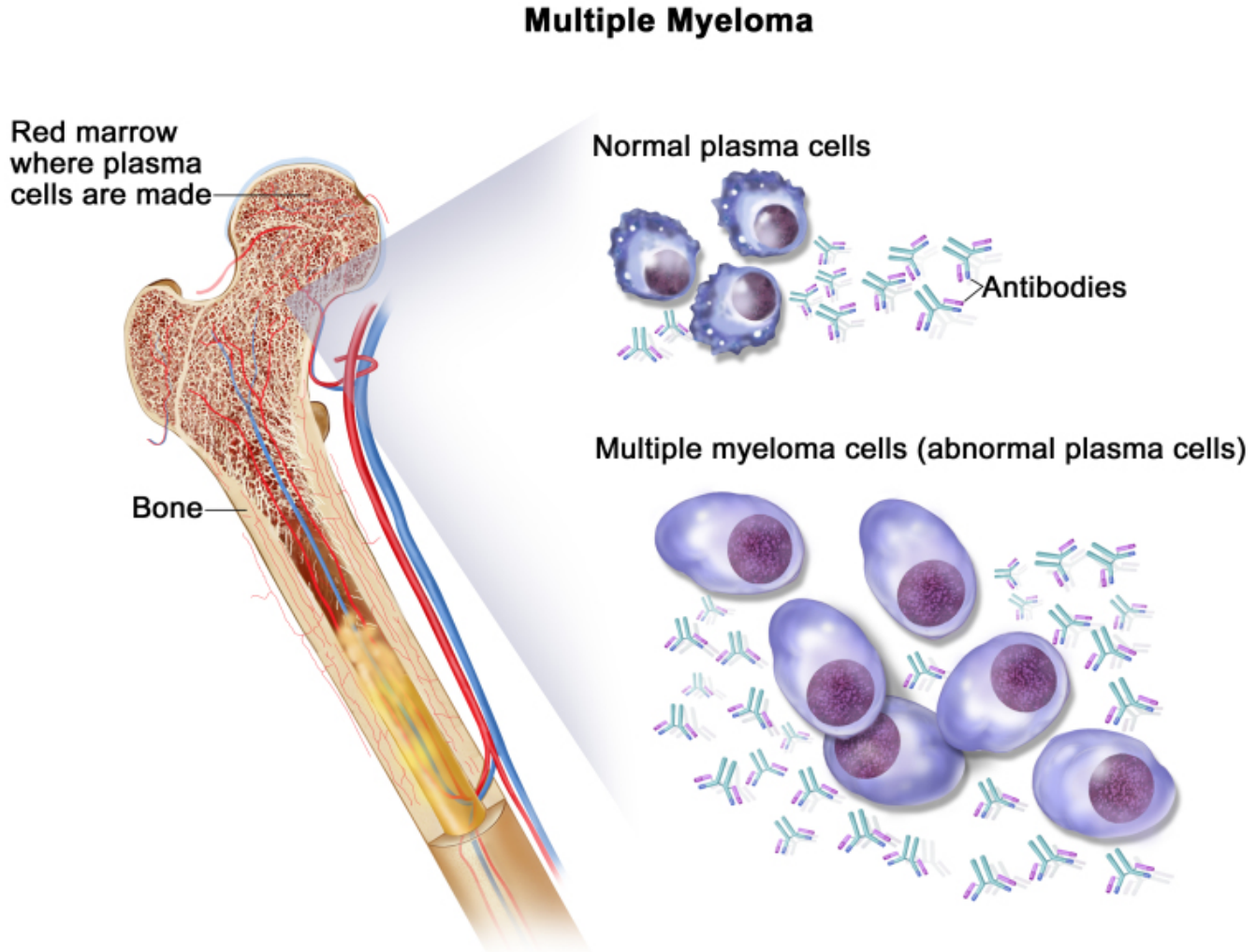
1. Advanced liver disease.
2. Certain types of cancer, such as multiple myeloma.
3. Number of chronic infections.

FIGURE 1

Multiple Myeloma in Bone Marrow



Credit: Hunna / Shutterstock

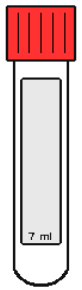


Collection of Blood Specimens

Blood tests could be performed on serum, plasma, or whole blood depending on the type of the test.

Separation of

Serum — Centrifuged to precipitate fibrinogen results in — **Plasma**

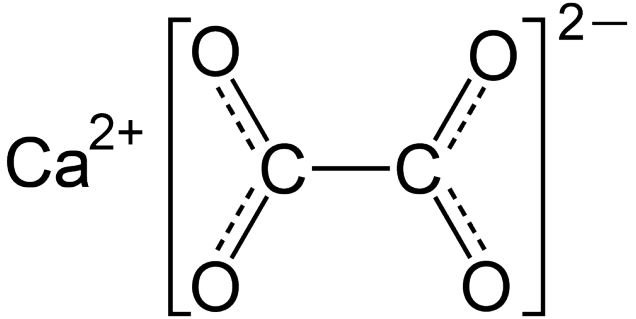


Blood has been *clotted prior to centrifugation* usually in a red top tube with **no additives or anticoagulant.**

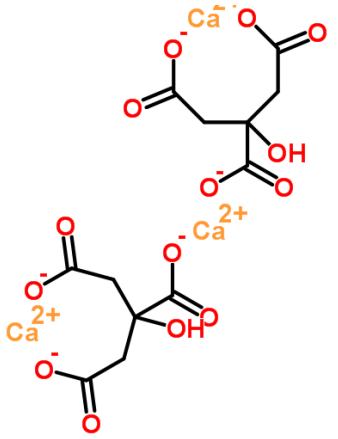
Blood has been *treated with anticoagulants* **to prevent clotting** and permitted to stand or centrifuged in a container.

Note: Serum is preferred for many tests (e.g. determination of lactate dehydrogenase) as the anticoagulants in plasma can sometimes interfere with the results.

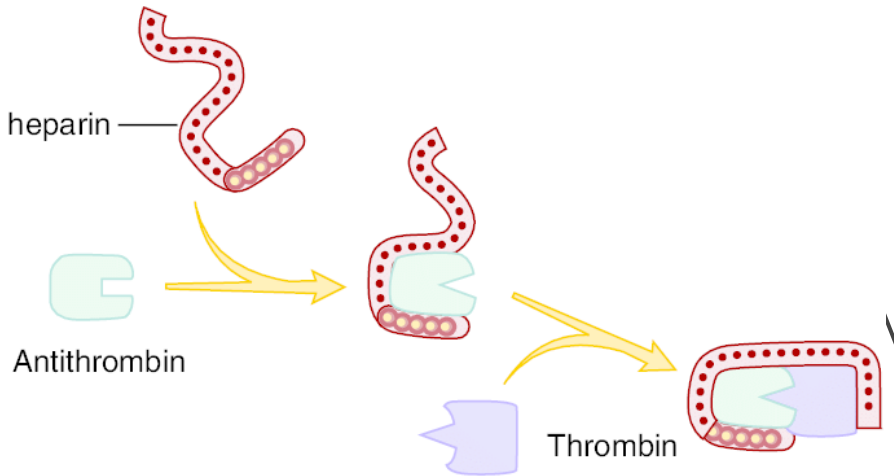
Anticoagulants Mechanism of Action

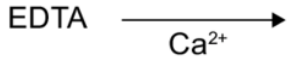


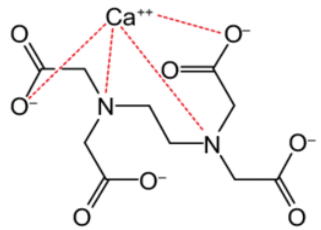
calcium oxalate



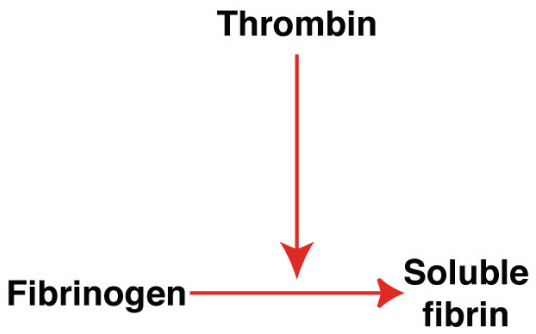
calcium citrate





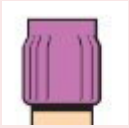
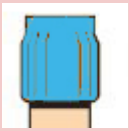
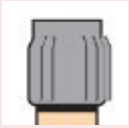


Calcium-EDTA



Types of Anticoagulants

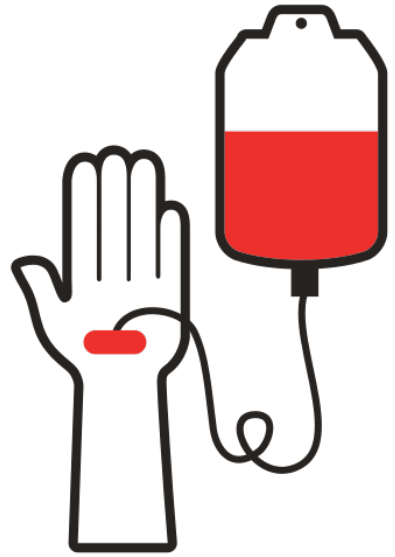
If whole blood or plasma is desired, an anticoagulant must be added to the specimen immediately after it is drawn or placed into the tube into which the blood is collected.

Tube Cap Color	Anticoagulants	Function
 Green	Heparin	It **inhibits the formation of thrombin** from prothrombin and thus preventing the formation of fibrin from fibrinogen.
 Purple	EDTA	It is a chelating agent, **it binds calcium**, which is essential for the clotting mechanism.
 Light Blue	Sodium Citrate	It inhibits blood coagulation by **converting calcium into a non-ionized form**, and hence prevent clotting of blood.
 Dark Gray	Potassium Oxalate	It inhibits blood coagulation by **forming insoluble complexes with calcium ions,** which is necessary for coagulation.
 Light Gray	Sodium Fluoride	It has been used chiefly as a preservative since it **inhibits red cell metabolism and bacterial action.**

Note: Free ionized calcium is required for most events in the blood coagulation process.

Biochemical Changes in Blood Upon Storage

1. Loss of carbon dioxide.
2. Conversion of glucose to lactic acid (glycolysis).
3. Conversion of pyruvate into lactate.
4. Increase in plasma inorganic phosphate.
5. Formation of ammonia from nitrogenous substances.
6. Passage of intracellular materials of RBC into plasma.



Separation of Plasma Proteins

Plasma protein can be separated from each other by:

1. **Salting-Out**
2. Ultracentrifuge
3. Electrophoresis
4. Chromatography

Practical Part 💉

Objectives

- To separate plasma and serum from whole blood.
- To separate blood proteins using **salting-out** method.
- Identification of blood proteins using **Biuret** and **heat coagulation** method.

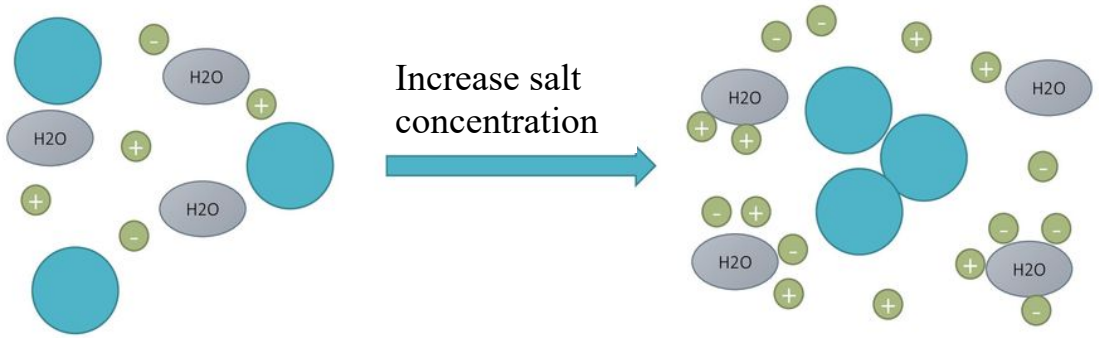
Principle of Salting Out

- When high concentrations of salt is added to the protein solution, the solubility decreases, and the protein precipitates.

This can be explained by the following:

- The salt molecules compete with the protein molecules in binding with water, leading to **dehydration**.
- The salt concentration needed for the protein to precipitate out of the solution differs from protein to protein.

Salting Out



Principles of Identification Tests

Biuret test

- In the presence of peptides that contain at least two peptide bonds (i.e. it is not given by **dipeptides and free amino acids**), a copper(II) ion forms violet/blue-colored complexes in an alkaline solution.

Protein + Biuret reagent → violet/blue color

- The intensity of the color is proportional to the number of peptide bonds and thus is a measure of the concentrations of proteins.

Heat coagulation

Protein + weak acid $\xrightarrow{\text{Heating}}$ protein precipitate (cloudiness)