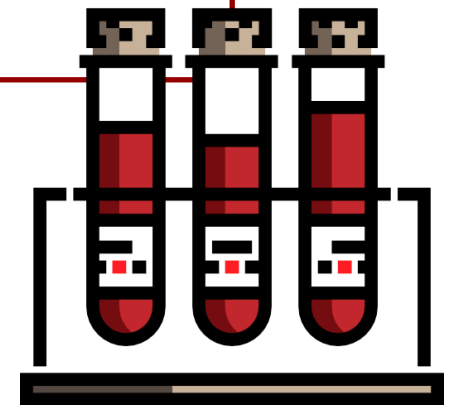


Blood Biochemistry BCH 220 [Practical]

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



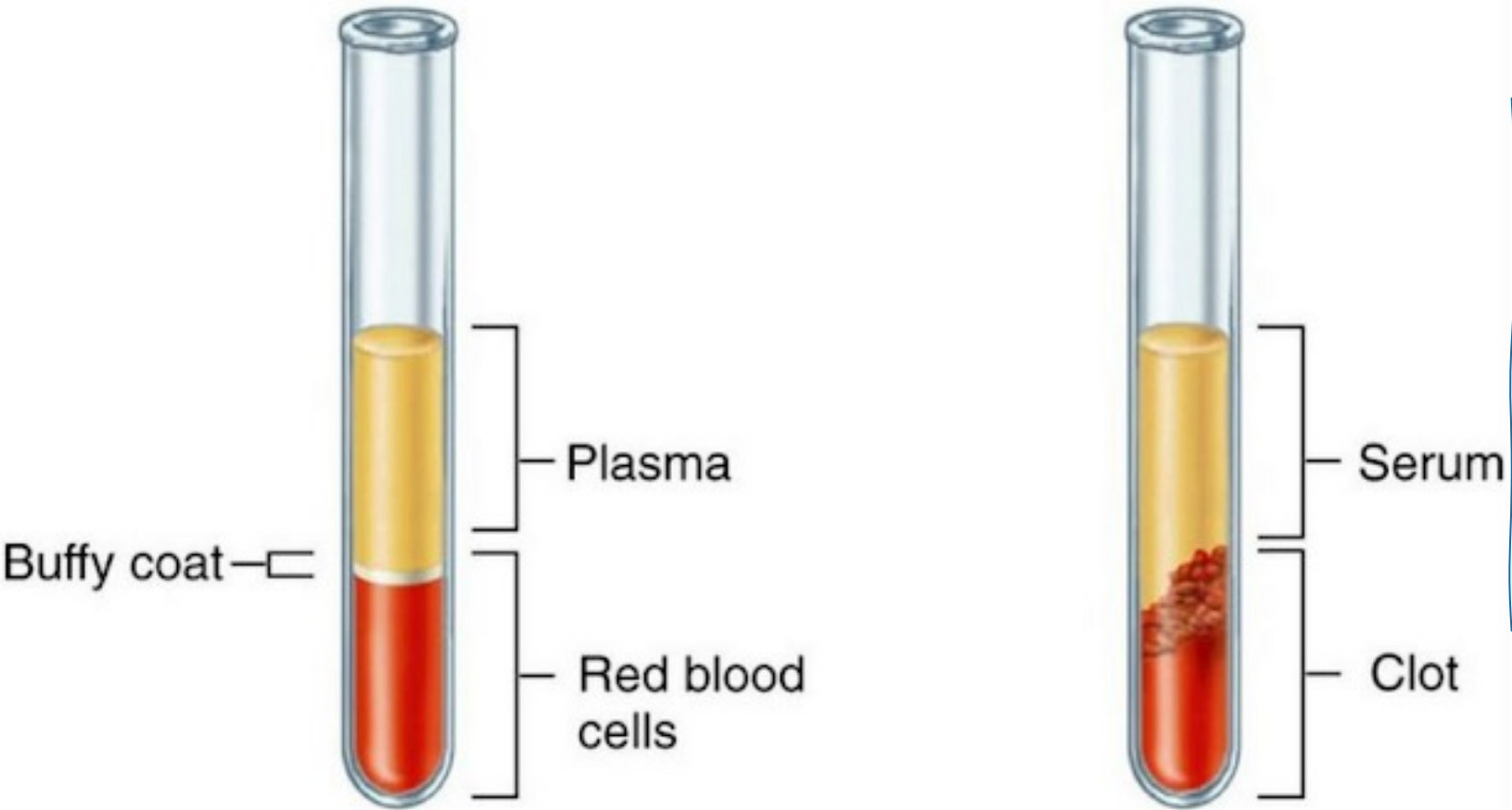
Blood Terminology

- **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)

Serums	Plasma
Fluid obtained when coagulated blood has been separated	Anti-coagulated blood has been separated
No anticoagulant	Anti-coagulants are needed for separation
Clotting factors are absent	Clotting factors are present in plasma

Blood Plasma vs Blood Serum



(a) **Unclotted Whole Blood**

(b) **Clotted Whole Blood**

1. Can you spot the differences between the two tubes?

Regarding the yellow layer.

- Which tube contain clotting factors? Why?
- Which tube contain anticoagulant? Why?
- Can plasma be converted to serum?

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 Proteins

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

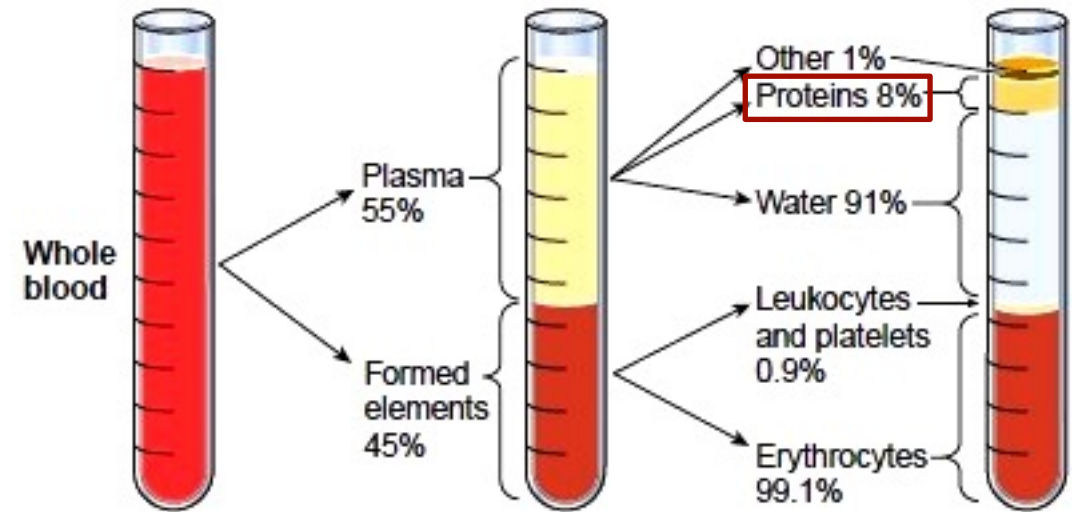
The main plasma proteins are:

Mwt ↓	▪ Albumin (36-50 g/l)	↑ Conc.
	▪ Globulins (18-32 g/l)	
	▪ Fibrinogen (2-4 g/l)	

Site of synthesis

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

Note: All plasma proteins are water soluble, Why?



Major Blood Proteins

➤ Albumin

It is the most abundant protein present in plasma.

Functions:

1. Maintenance of **blood volume**.
2. Serves in the **transport** of bilirubin, hormones, metals, vitamins, and drugs.

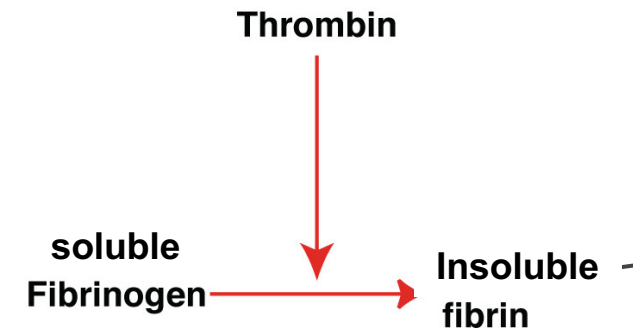
➤ Globulins

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 with a central role in **blood clotting**.
2. It is converted by **thrombin** to fibrin during blood coagulation.

💡 **Pause and Think** Which of these proteins is absent from serum? Why?



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.

A low serum albumin:

1. A **heavy loss** of albumin in urine.
2. **Insufficient intake** of protein in diet.


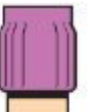



A high serum globulin:

1. Advanced **liver disease**.
2. Number of chronic **infections**.

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

Types of Anticoagulants

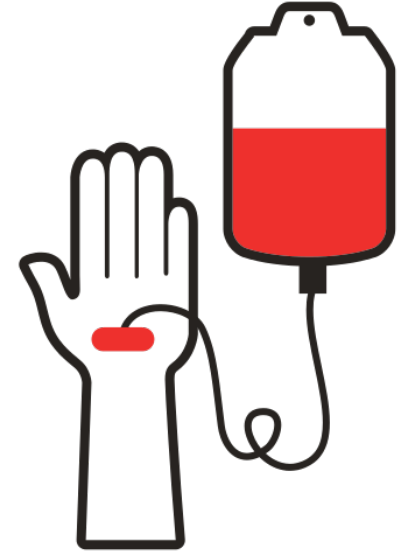
If **whole blood** or **plasma** is desired, an anticoagulant must be added to the specimen immediately after it is drawn or placed into the tube into which the blood is collected.

Tube Cap Color	Anticoagulants
	Green Heparin
	Purple EDTA
	Light Blue Sodium Citrate
	Dark Gray Potassium Oxalate
	Light Gray Sodium Fluoride



Biochemical Changes in Blood Upon Storage

1. Loss of carbon dioxide.
2. Increased lactic acid.
3. Increase in plasma inorganic phosphate.
4. Increased ammonia.
5. Passage of intracellular materials of RBC into plasma.



Practical Part

Objectives

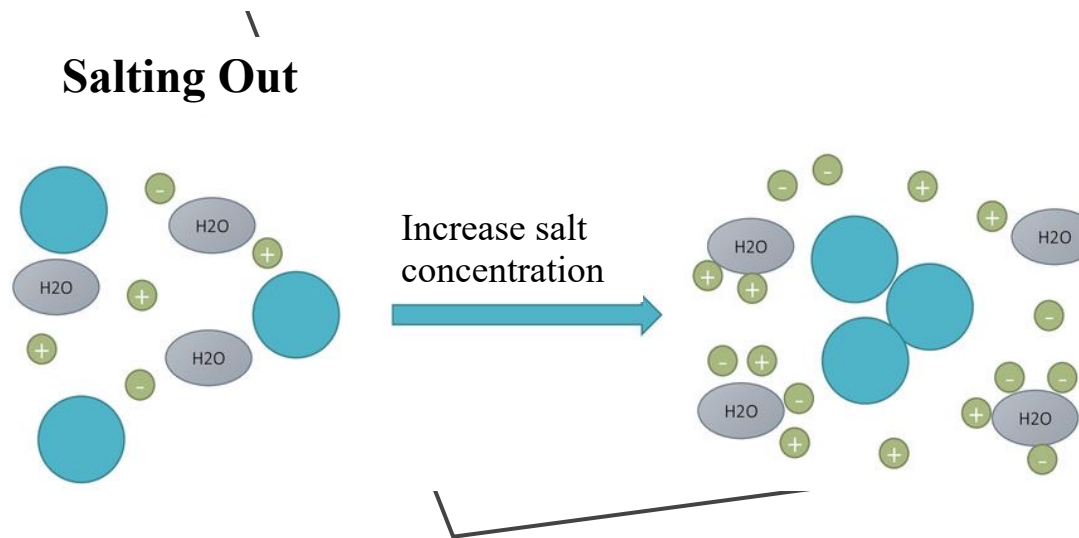
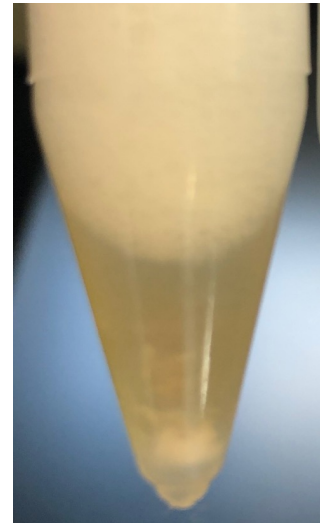
- To **separate plasma** and **serum** from whole blood.
- To separate **blood proteins** using **salting-out** method.
- Identification of blood proteins using **Biuret** and **heat coagulation** method.

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. How?



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.

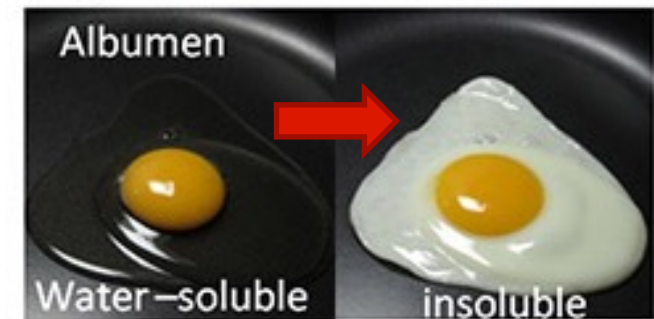
Protein + Biuret reagent → violet/blue color

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

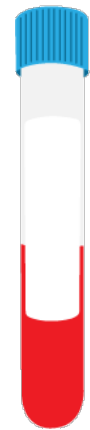


Heat coagulation

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

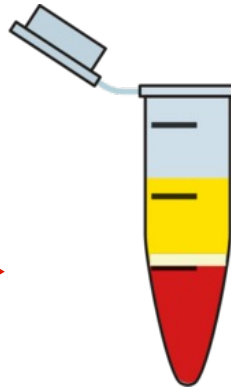


Experiment (1): Separation of Plasma and Serum from Whole Blood



**Blood with
Anticoagulants**

Centrifuge at
3000 rpm for
10 minutes

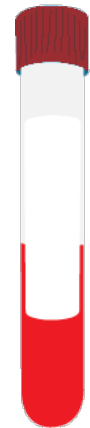


Plasma

Buffy coat (WBCs and platelets)

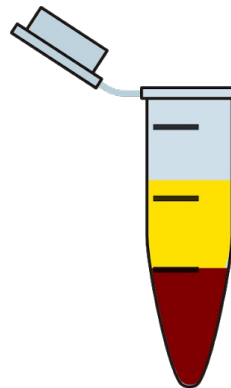
RBCs

Plasma



**Blood without
Anticoagulants**

Centrifuge at
3000 rpm for
10 minutes



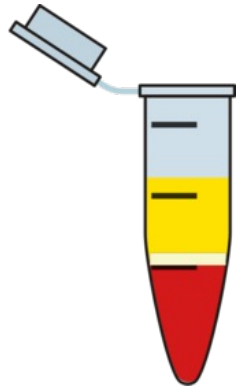
Serum

Clotted blood

Serum

Measure the volume
of Plasma and Serum
obtained

Experiment (2): Separation of Main Proteins in Plasma



.....ml Plasma + ml saturated NaCl solution

Add an **equal volume** of saturated NaCl to the plasma

Centrifuge at 5000 rpm/10 min

Transfer the supernatant into other test tube

Filtrate (**Serum**) + Few drops of 5 % CaCl₂

Dissolve the precipitate (**Fibrinogen**) in 2 ml 0.9% saline

incubate at 37 °C / 10 min

Biuret test
1 ml + 1ml Biuret (Tube A)

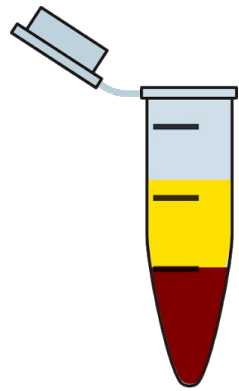
Purple color
confirms the presence of protein "fibrinogen"

Clotting Test
1 ml fibrinogen+1ml serum (Tube B)

Clotting occurs
because serum contains active thrombin which converts fibrinogen to insoluble fibrin

No clotting occurs
although calcium ions are required in the clotting process, no clotting occurs because of the absence of the fibrinogen in the solution)

Experiment (2): Separation of Main Proteins in Serum



.....ml serum + ... ml saturated ammonium sulphate solution

Add an **equal volume** of saturated ammonium sulphate to the plasma

Centrifuge at 3500 rpm /10 min

Globulin Precipitate , Dissolve the precipitate in 2ml 0.9% saline

Transfer the supernatant (**Albumin**) in to other test tube

Biuret test
1 ml + 1ml Biuret (Tube A)

Purple color
confirms the presence of protein

Heat Coagulation Test
(Tube B) 1 ml + drops of 2M acetic acid

Cloudiness
confirms the presence of protein

Divide the filtrate into **2 tubes**

Salting out
(Tube A) Add solid ammonium sulphate

albumin is precipitated
confirms the presence of protein

Heat Coagulation Test
(Tube B) Add drops of 2M acetic acid

Cloudiness
confirms the presence of protein



Homework:

- a. How albumin contributes to kwashiorkor oedema?
- b. How fibrinogen can be used as a drug?

