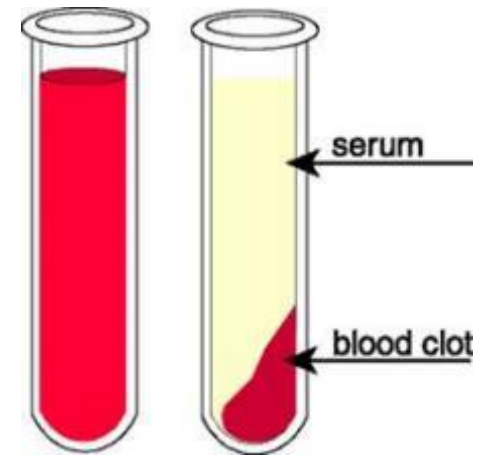
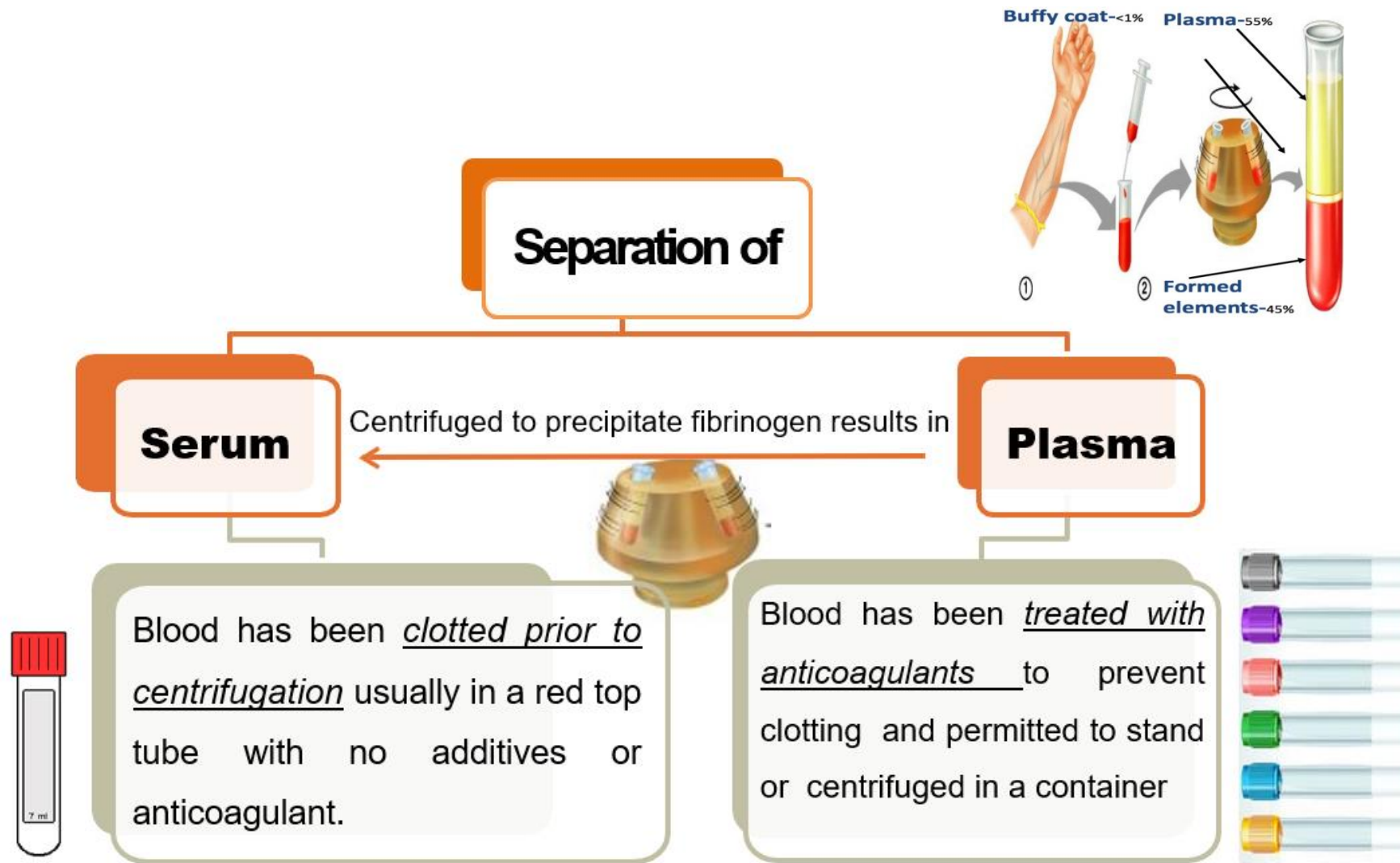


Separation of Plasma and Serum and Their Proteins from Whole Blood

BCH 471

[Practical]










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

Collection of Blood Specimens

If **whole blood** or **plasma** is desired, an anticoagulant must be added to the specimen.

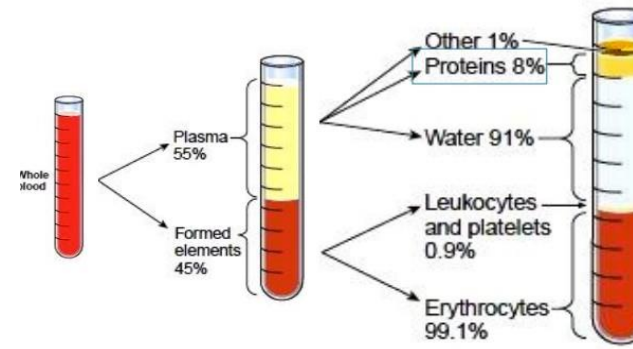
Tube Cap Color	Additive	Function of Additive	Common Lab Tests
 Green	Heparin	It inhibits the formation of thrombin from prothrombin and thus preventing the formation of fibrin.	-Routine Chemistry Tests -Cytogenetic
 Purple	EDTA	It is a chelating agent, it binds calcium , which is essential for the clotting mechanism.	-Hematology -Molecular Tests
 Light Blue	Sodium Citrate	It inhibits blood coagulation by converting calcium into a non-ionized form , and hence prevent clotting of blood.	Coagulation Test
 Dark Gray	Potassium Oxalate	It inhibits blood coagulation by forming insoluble complexes with calcium ions .	-Preserve glucose in whole blood
 Light Gray	Sodium Fluoride	It has been used chiefly as a preservative since it inhibits red cell metabolism and bacterial action .	-Some Chemistry Tests.

Changes in Blood on Keeping

- Loss of carbon dioxide.
- Conversion of glucose to lactic acid (glycolysis).
- Increase in plasma inorganic phosphate.
- Formation of ammonia from nitrogenous substances.
- Passage of substances through the red cell envelope.
- Conversion of pyruvate into lactate.



PLASMA PROTEINS



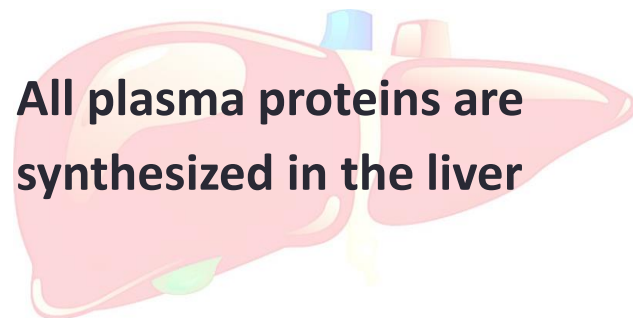
- **The main plasma proteins are:**

MW
↓

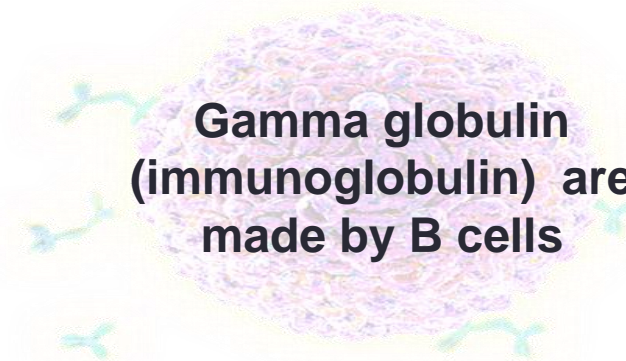
- ✓ Albumin (36-50 g/l), Mw 66.241kDa .
- ✓ Globulins (18-32 g/l), Mw of globulins Cover a wide range.
- ✓ Fibrinogen (2-4 g/l), Mw 340 kDa .

↑
Conc.

Site of synthesis



except



Note: All plasma proteins are water soluble

ALBUMIN

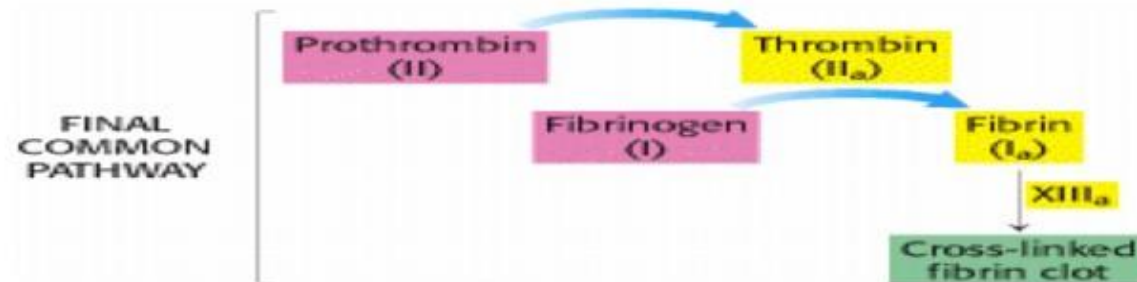
- It is the **most abundant** protein present in plasma.
- **It has many functions including:**
 - ☑ Maintenance of the blood osmotic pressure.
 - ☑ Adjusting blood pH.
 - ☑ Act as a transporter, transporting free fatty acids, bilirubin, drugs, steroid hormones, calcium and copper in the blood.

GLOBULINS

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

FIBRINOGEN

- It is a glycoprotein (proteins that contain oligosaccharide chains)
- It is converted by thrombin into fibrin during blood coagulation.

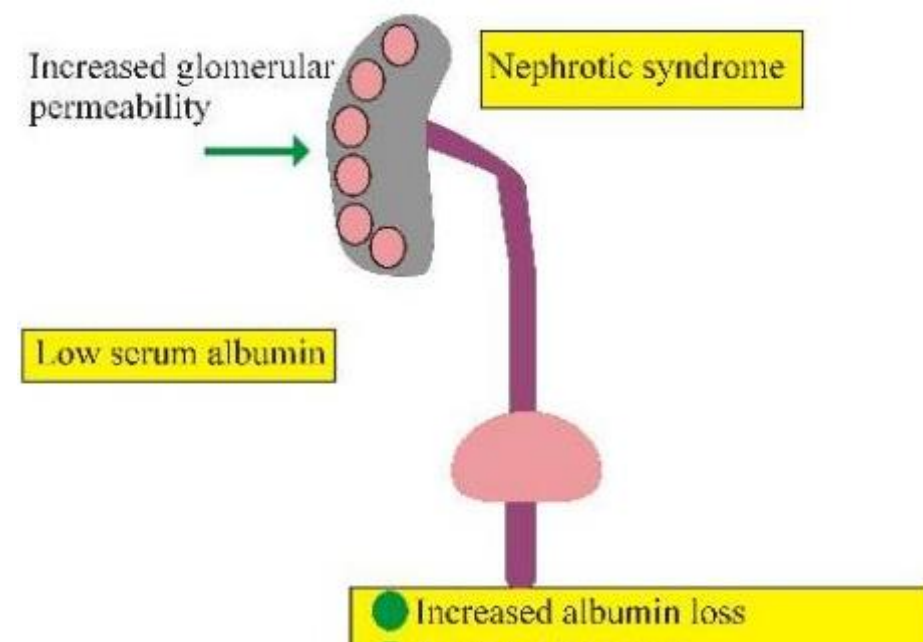


SERUM PROTEINS

- 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 a decrease in albumin fraction and the increase is due to an 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 may be due to:

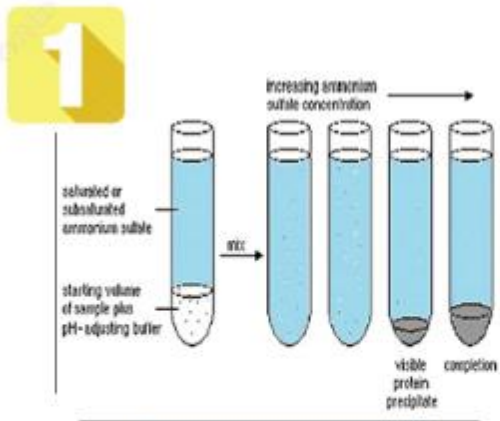
- A heavy loss of albumin in urine.
- Mal-absorption of protein from the digestive tract.
- Decreased formation by the liver due to defective liver.
- Increase catabolism of protein or due to insufficient intake of protein in diet.



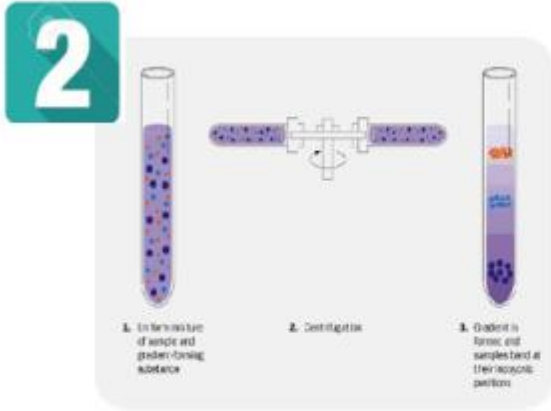
A high serum globulin occurs commonly in:

- Advanced liver disease.
- multiple myeloma.
- number of chronic infections.

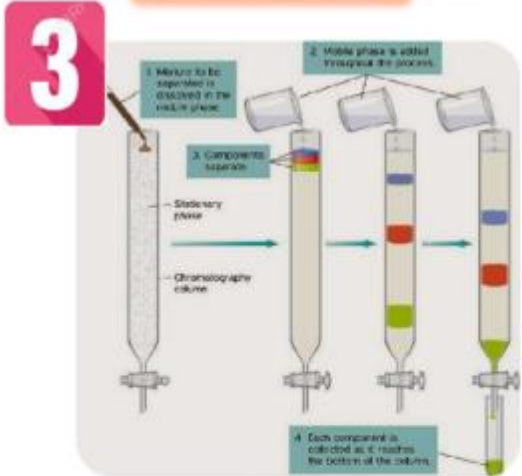
Serum and plasma proteins can be separated from each other by :



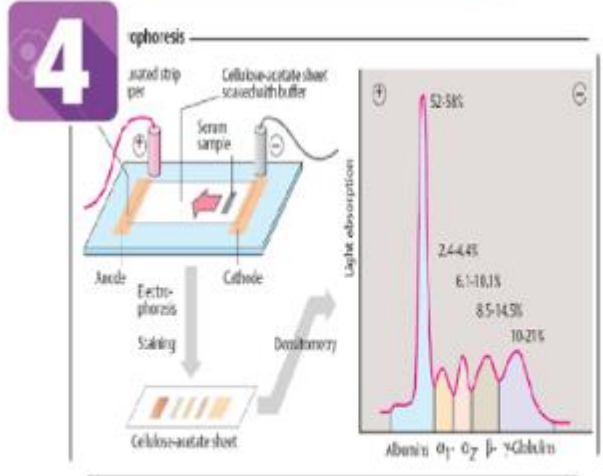
Salting out



Ultracentrifugation



Chromatography



Electrophoresis

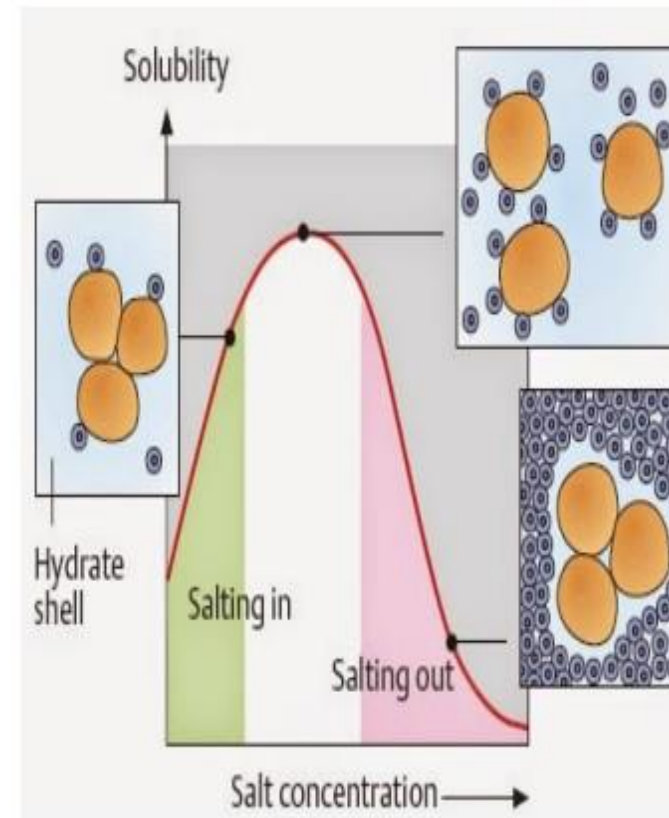
PRINCIPLE OF SALTING OUT

- 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.



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.



- 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 heating → protein precipitate (cloudiness)

PRACTICAL PART

Separation of Plasma and Serum from whole blood :



Centrifuge at
3000 rpm
for **10 minutes**



Plasma



Plasma



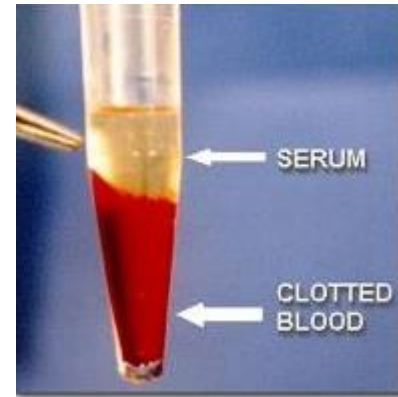
Red Blood
Cell



Centrifuge at
3000 rpm
for **10 minutes**



Serum



SERUM

CLOTTED
BLOOD

* Measure the volume of Plasma and Serum obtained

Separation of main proteins in Plasma and Serum :

Plasma



5 ml Plasma + 5 ml saturated NaCl solution

Centrifuge at 3500 RPM/10 min

Transfer the supernatant into other test tube

Take the precipitate (**Fibrinogen**) and dissolve it in 2 ml 0.9% saline

Biuret test

1 ml + 1ml Biuret

The Aim to.....

(Tube A)

Blue color

confirms the presence of protein
"fibrinogen"

Clotting Test

1 ml fibrinogen+1ml serum

The Aim to.....

(Tube B)

Clotting occurs

because serum contains active thrombin
which converts fibrinogen to insoluble
fibrin

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

The Aim to.....

Incubate at 37 °C / 10 min

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)

Separation of main proteins in Plasma and Serum :

Serum



4ml serum + 4 ml saturated ammonium sulphate solution
Aim to :.....

Centrifuge at 3500 rpm /10 min

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

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

Blue color
confirms the presence of protein

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

Cloudiness
confirms the presence of protein

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

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