

**Lab#1**  
**BCH 471**

# **Separation of Plasma and Serum and Their Proteins from Whole Blood**

## 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.

## Whole Blood:

Whole blood contains: (red blood cells, white blood cells, and platelets) suspended in fluid called plasma.

-The general functions of blood are in metabolism and its regulation (e.g., osmotic balance), transport, and defense or protection.

- **Plasma:** is the liquid portion of the blood in which red and white blood cells and platelets are suspended before the centrifugation. (contain albumin, globulins and **fibrinogen**).

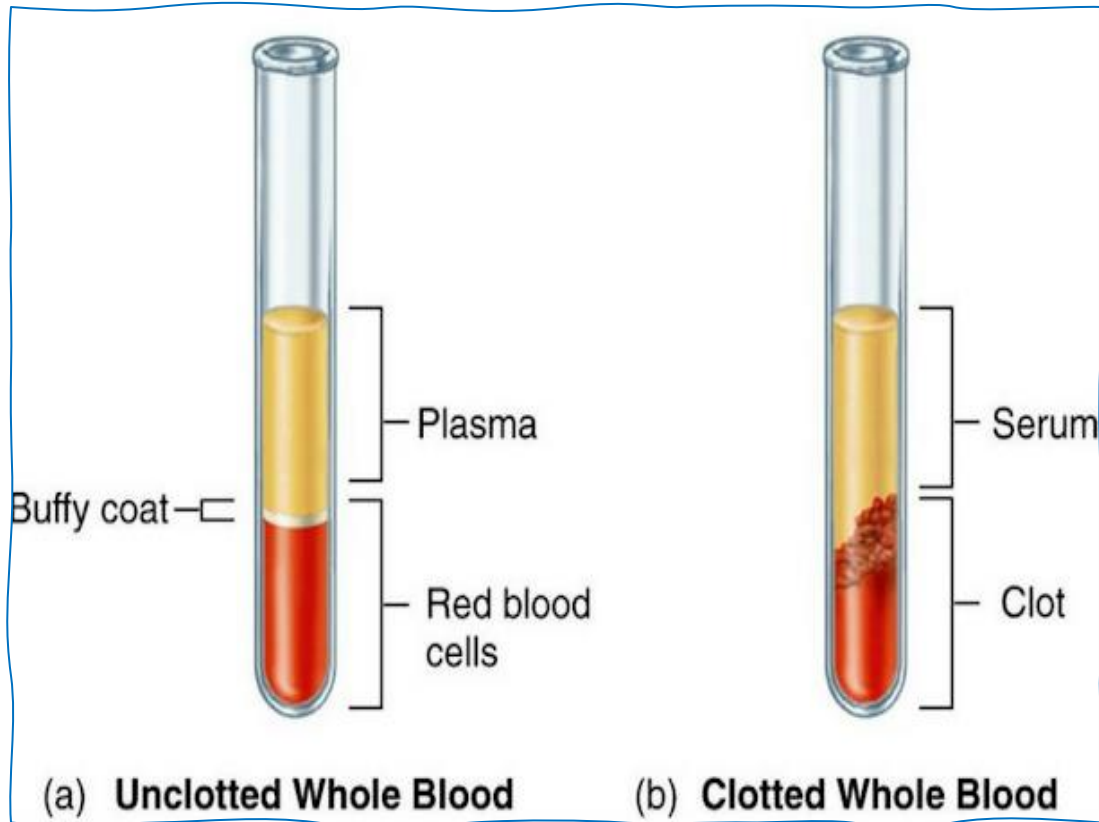
- **Serum:** resembles plasma in composition but lacks the coagulation factors ( such as fibrinogen). **But it will still contain active thrombin.**

-Serum, can be obtained by, letting a blood specimen clot prior to centrifugation or by centrifugation of plasma to precipitate Fibrinogen and the liquid phase will be the serum.

$$\text{Serum} = \text{Plasma} - \text{Fibrinogen}$$






In order for the blood to clot.....





-leukocytes.    -platelets(Thrombocytes)    -erythrocytes

# Collection of Blood Specimens

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

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



## Blood Collection Tube Red Cap: (No Additive Tube)

Used for serum determinations in chemistry, serology and immunohematology.

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

## Centrifugation of blood sample

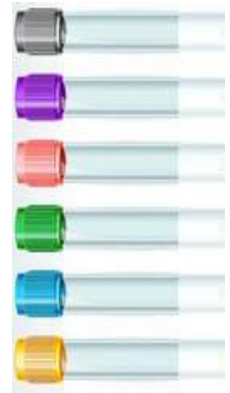
Serum

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



Plasma

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





# Biochemical Changes in Blood Upon Storage:

- 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 3 plasma proteins are:

## 1- Albumin, (36-50 g/l), MWt 66.241 kDa.

-It is the most abundant protein in the plasma.

-It has some functions such as:

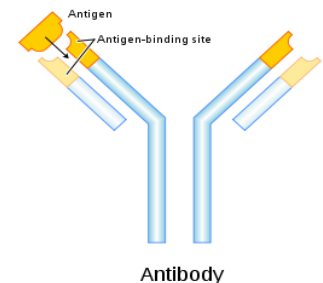
- Maintenance of the blood osmotic pressure.
- Adjusting blood pH.
- Act as transporter, transport free fatty acids, bilirubin, drugs, steroids hormones, calcium and copper in the blood.

## 2-Globulins, (18-32 g/l), MWt of globulins cover a wide rang.

Sub classified in to four types ( $\alpha$ - globulins,  $\alpha_2$ - globulins,  $\beta$  – globulins and  $\gamma$ -globulins ).

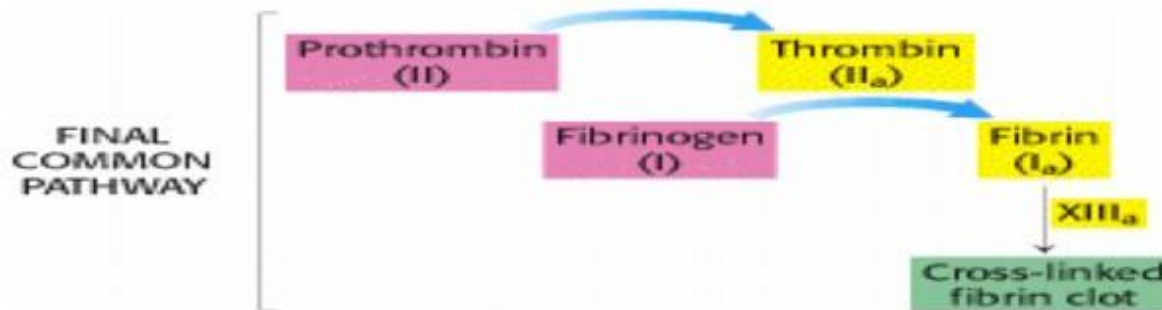
$\alpha_1$ ,  $\alpha_2$ , and  $\beta$ -globulins, which serve as carrier proteins.

and  $\gamma$ -globulins, which include the **immunoglobulins** responsible for immune responses.

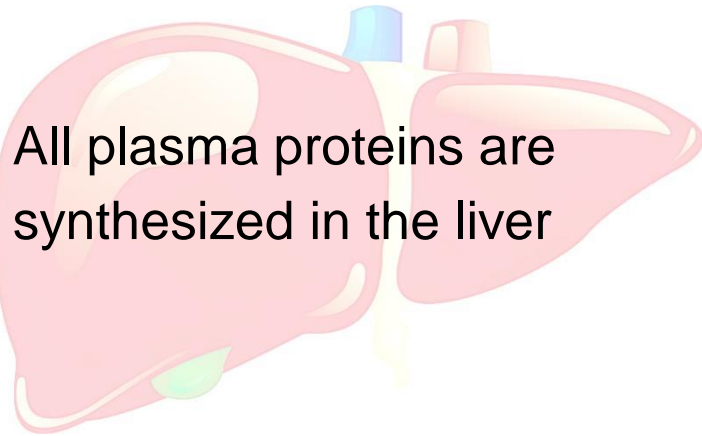


### 3-Fibrinogen, (2-4 g/l), MWt 340 kDa.

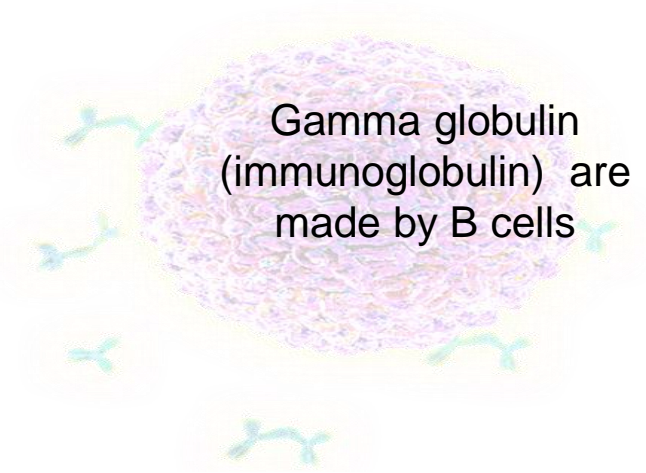
- Fibrinogen (or Factor I) is a soluble glycoprotein, with a central role in blood clotting.
- It is converted to fibrin by thrombin, during blood clotting.



## Site of synthesis of plasma proteins



**except**



**Note:** All plasma proteins are water soluble

## Major Serum Proteins:

- Total serum protein consists of two main fractions, **albumin (A)** and **globulin(G)**.
- 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.

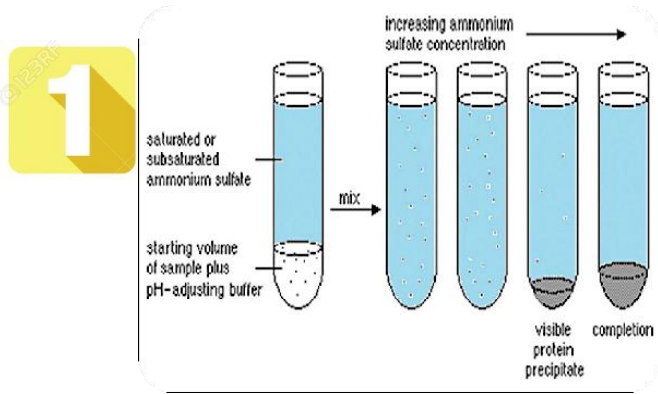
## **A low serum albumin may be due to:**

- A heavy loss of albumin in urine.
- Malabsorption of protein from the digestive tract.
- Decrease of synthesis by the liver. (defective liver).
- Increase catabolism of protein
- Insufficient intake of protein in diet.

## **A high serum globulin occurs commonly in:**

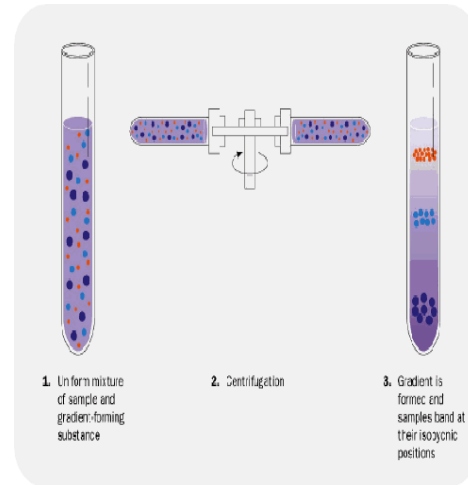
- Advanced liver disease.
- Certain types of cancer, such as multiple myeloma. ( $\gamma$ -globulins),
- number of chronic infections.

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



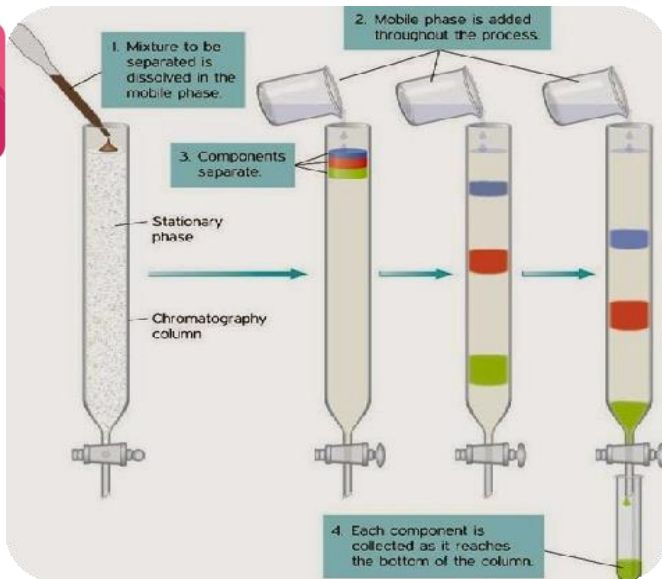
Salting out

**2**



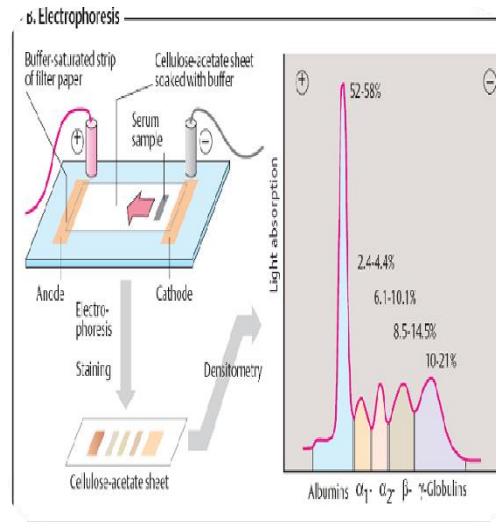
Ultracentrifugation

**3**



Chromatography

**4**



Electrophoresis

# Practical Part



**For the separation part in the lab:**

**Salting Out will be used**

**Principle:**

-When the proteins precipitate at high concentration of salts it called Salting out. The salt molecules compete with the protein molecules in binding with water.

In this case, the protein molecules tend to associate with each other because protein-protein interactions become energetically more favorable than protein-solvent interaction.

## For the identification part in the lab:

### 1. Biuret test

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.



### 2. Heat coagulation

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



