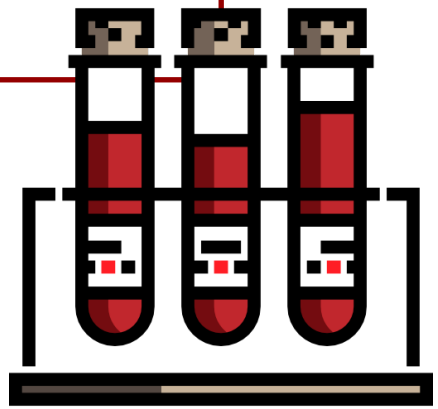
Blood Biochemistry BCH 471[Practical]

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.

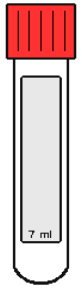
(Serum = Plasma – clotting factors)

Collection of Blood Specimens

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

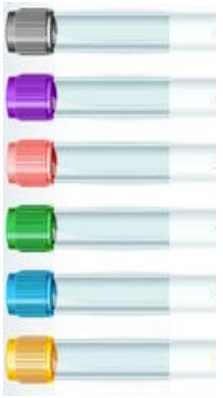
Separation of

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



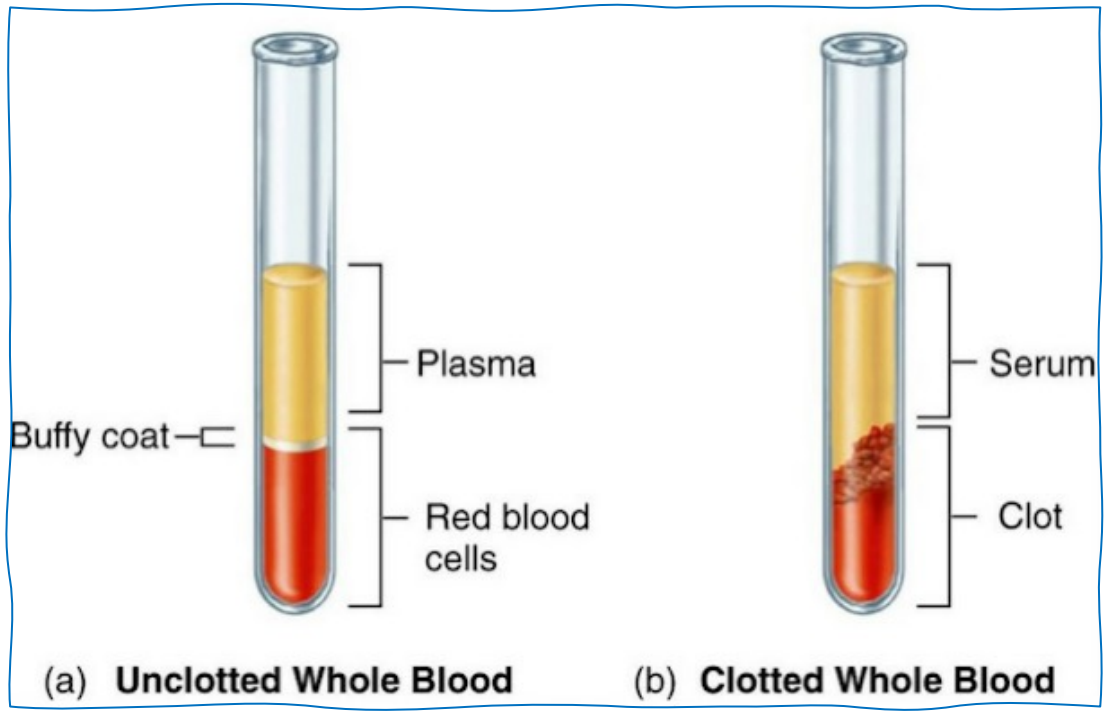
Blood has been *clotted prior to centrifugation* usually in a red top tube with **no additives or anticoagulant.**

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.

Collection of Blood Specimens



1. **Can you spot the differences between the two tubes?**
2. **Which tube will clot if it's allowed to set?**

Regarding the yellow layer.

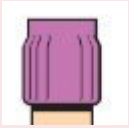
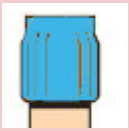
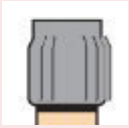
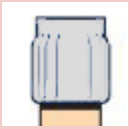
a. **Which tube contains clotting factors? Why?**

b. **Which tube contains anticoagulant? Why?**

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

Types of Anticoagulants

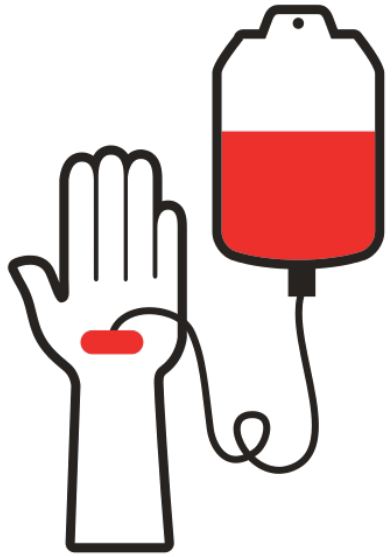
If **whole blood** or **plasma** is desired, an anticoagulant must be added to the specimen immediately after it is drawn or placed into the tube into which the blood is collected.

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.

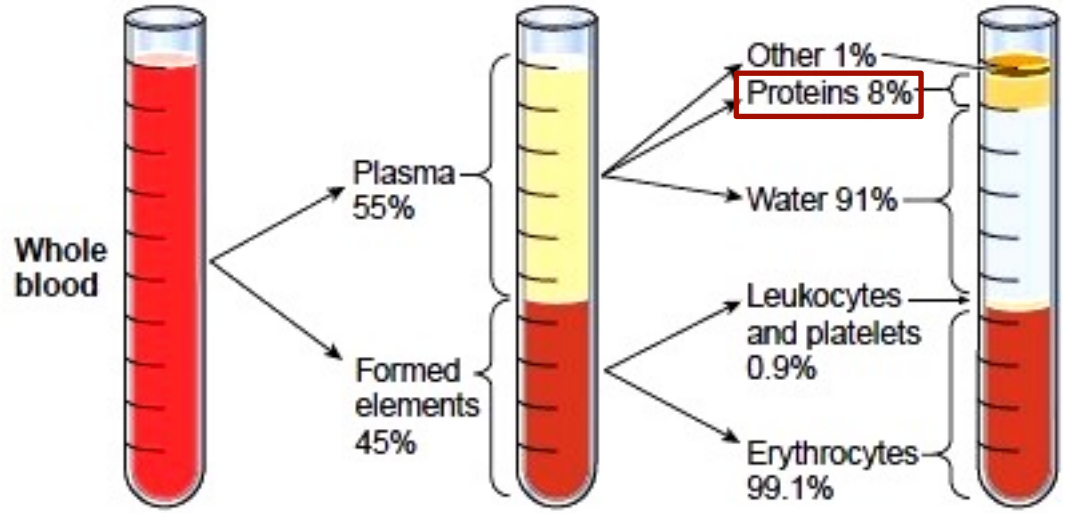
Biochemical Changes in Blood Upon Storage

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



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), Mw 66 kDa
- Globulins (18-32 g/l), Mw cover a wide range
- Fibrinogen (2-4 g/l). Mw 341 kDa

Conc. ↑

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 Plasma Proteins

➢ Albumin

It is the most abundant protein present in plasma, and synthesized solely in the liver.

Functions:

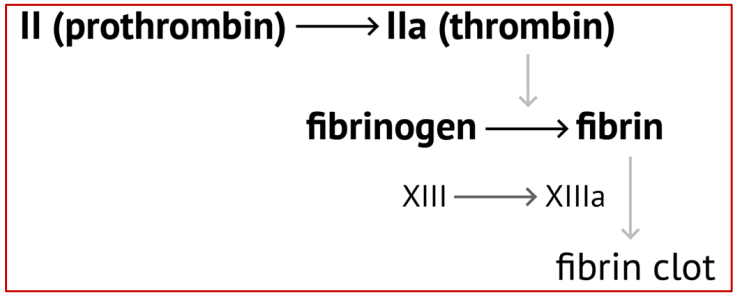
1. Maintenance of **colloidal osmotic pressure**, thus maintaining blood volume.
2. Serves in the **transport** of bilirubin, hormones, fatty acids, metals, vitamins, and drugs.
3. **Antioxidation activity,** via neutralization of free radicals.

➢ 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?**

Major 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

Major Serum Proteins

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. **Insufficient intake** of protein in the diet.
4. **Malabsorption** of protein from the digestive tract.
5. **Increase catabolism** of protein.

A high serum globulin (Hyperglobulinemia) occurs commonly in:

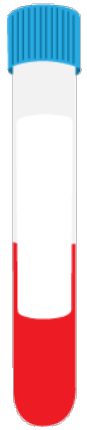
1. Advanced **liver disease**.
2. Certain types of **cancer**, such as multiple myeloma. (**γ-globulins**),
3. A number of chronic **infections**.

Practical Part 💉

Objectives

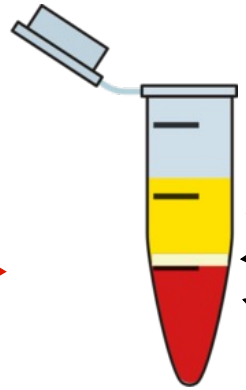
- To **separate plasma** and **serum** from whole blood.
- To separate **blood proteins** using **salting-out** method.
- Identification of blood proteins using **Biuret** and **heat coagulation** method.

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



Blood <u>with</u> Anticoagulants

Centrifuge at **3000 rpm** for **10 minutes**

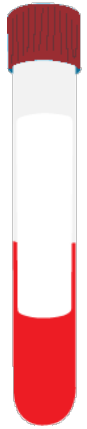


Plasma

Buffy coat (WBCs and platelets)

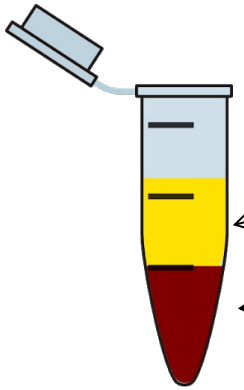
RBCs

Plasma



Blood <u>without</u> Anticoagulants

Centrifuge at **3000 rpm** for **10 minutes**



Serum

Clotted blood

Serum

Measure the volume of Plasma and Serum obtained

Experiment (2): Separation and identification of Proteins in Plasma/Serum

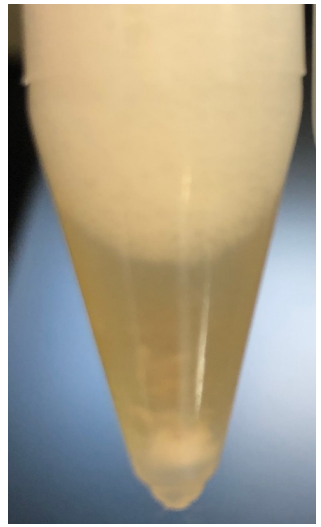
A) Plasma/Serum proteins can be separated by:

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

B) Plasma/Serum protein can be identify by:

1. Biuret test
2. Heat coagulation

A) Principle of Salting Out

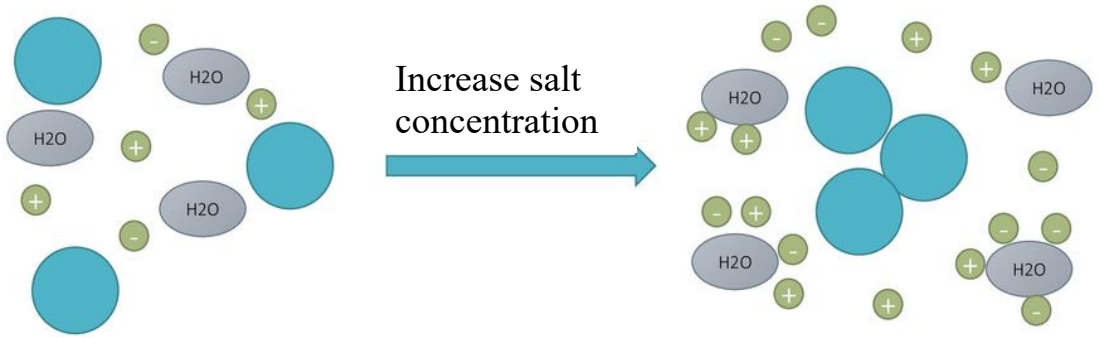


- When high concentrations of salt is added to the protein solution, the solubility decreases, and the protein precipitates.

This can be explained by the following:

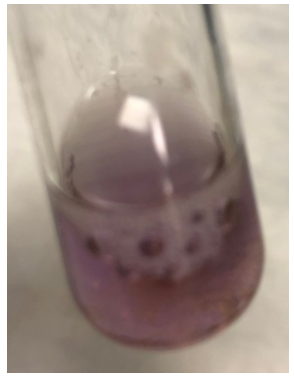
- The salt molecules compete with the protein molecules in binding with water, leading to **dehydration**.
- The salt concentration needed for the protein to precipitate out of the solution differs from protein to protein. How?

Salting Out



B) Principles of Identification Tests

Biuret test



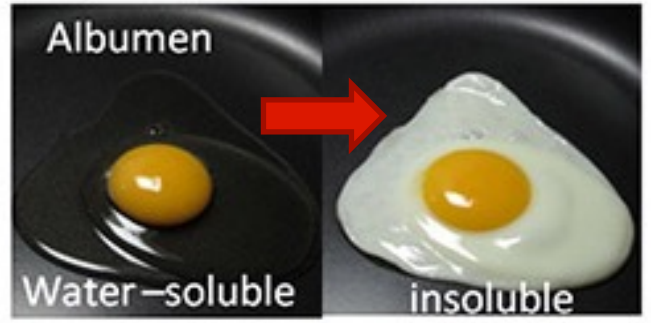
- In the presence of peptides that contain at least two peptide bonds (i.e. it is not given by **dipeptides and free amino acids**), a copper(II) ion forms violet/blue-colored complexes in an alkaline solution.

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**)



Homework

1. How albumin contribute to Kwashiorkor oedema?
2. How can fibrinogen be used as a drug?
3. How decreased temperature can contribute to frostbite?