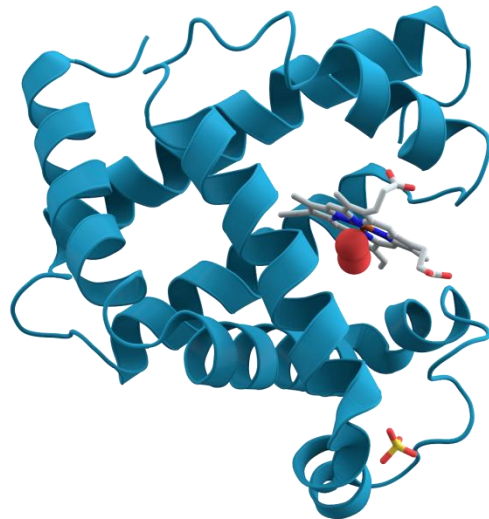


Qualitative tests of protein



Certain functional groups in proteins can react to produce characteristically colored products.

-The **color intensity** of the product formed by a particular group varies among proteins in **proportion** to the number of reacting functional or free groups present and their accessibility to the reagent.



Biuret test:

This test is specific for the **peptide bond**. Substances containing not less than two peptide linkages give this test.

Objective:

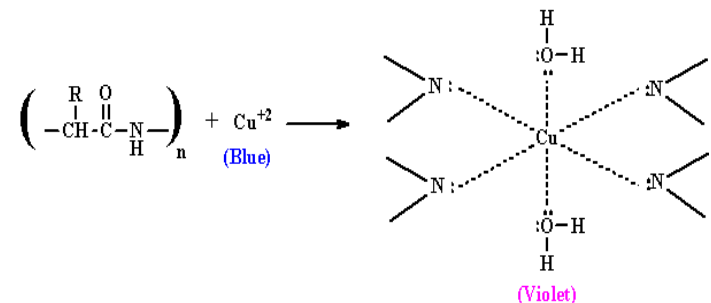
detect the presence of peptides or proteins in a sample.

Principle:

When proteins and peptides (i.e peptide bonds) treated with an alkaline solution of dilute copper sulfate a violet color is formed . A positive test is indicated by the formation of a **violet color**.

Note: the color density is **proportional** to amount of proteins present.

* **biuret reagent** is alkaline copper sulfate solution.



Method:

- 1- Add 2ml of protein Albumin
- 2- Add 1 ml of biuret reagent and mix well.

Result:

protein	Observation	Comment
Albumin		



Protein precipitation

- Is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants.
- The solubility of proteins is affected by pH, temperature, salts, heavy metal salts etc.



```
graph TD; A[1. Effect of salt concentration on the protein solubility] --> B[2. Precipitation of proteins by acids.]; B --> C[3. Precipitation of protein by salts of heavy metals.]; C --> D[4. Protein denaturation.];
```

1. Effect of salt concentration on the protein solubility

2. Precipitation of proteins by acids.

3. Precipitation of protein by salts of heavy metals.

4. Protein denaturation.

1. Effect of salt concentration on the protein solubility:

Objective: to investigate the effect of different salt concentration on protein solubility.

Principle:

The **low salt concentration** solutions make protein **solubility easier** using the attraction of salt ions to the functional groups of the protein (**salting in**).

On contrast, **high salt concentration** or solids dissolved in the reaction medium up till saturation solutions causes the protein to **precipitate** since salt ions, in this case, compete with the protein molecules in binding water molecules (**salting out**).

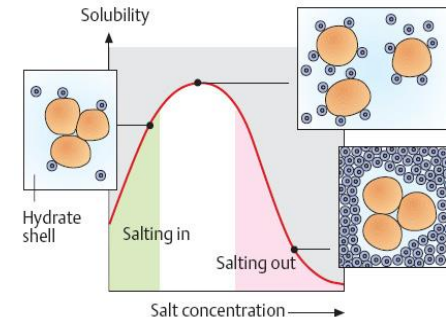
So the salt it just cause **protein dehydration**. e.g $(\text{NH}_4)\text{SO}_4$

Note: Each protein can be precipitated at specific salt concentration.

There is **inverse relationship** between the Mw of protein and the concentration of salt)

High Mw need low concentration salt (low percentage of saturation)

Low Mw need high conc. of salt (High percentage of saturation)

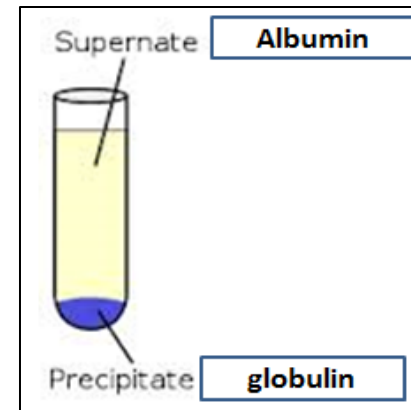


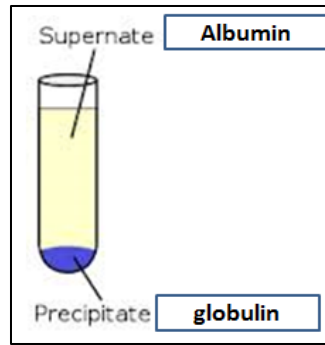
Egg Proteins:

Albumin and globulin

Separated by centrifugation at 3000 for 20 min.

The **albumin** is the **supernatant** because it has **low Mw.** and **globulin** is the precipitate which is **higher Mw** than albumin.





+ NaCl

(low salt concentration)

Globulin dissolves (salting in)

+ 50% saturated

$(\text{NH}_4)_2\text{SO}_4$

Globulin precipitate (salting out)

Globulin has high MW so it precipitate at lower salt concentration

+ 50% saturated

$(\text{NH}_4)_2\text{SO}_4$

No precipitation

Albumin has low MW so it need higher salt concentration to precipitate

+ 100% saturated

$(\text{NH}_4)_2\text{SO}_4$

Albumin precipitate (salting out)

Albumin has low MW so it precipitate at high salt concentration

Method:

T1	T2	T3
Take your globulin sample	Take 2 ml of your albumin sample	Take your T2 tube
Add 4 ml of NaCl solution to your globulin tube	Slightly add 1 ml of 50% saturated (NH ₄) ₂ SO ₄ solution	Add a few amount of 100% solid (NH ₄) ₂ SO ₄
Shake it well and write your observation	record your observation .	Shake it well and write your observation

Result:

Tube	Observation	Comment
Globulin + NaCl		
Albumin+50% saturated (NH ₄) ₂ SO ₄		
(Albumin+50% saturated (NH ₄) ₂ SO ₄) + 100% saturated (NH ₄) ₂ SO ₄		

2. Precipitation of proteins by acids:

Objective:

To investigate the effects of strong acids on the protein solubility.

Principle:

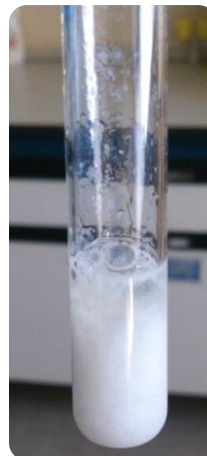
This test depend on affecting solubility of the protein as a function of changes in pH. In highly **acidic media**, the protein will be positively charged, which is attracted to the acid anions that cause them to precipitate.

Method

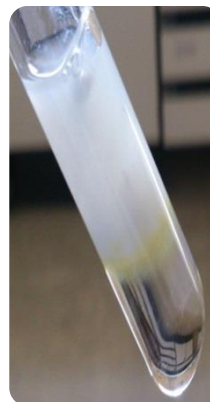
A	B
In a test tube, put 3ml of conc. nitric acid carefully	Put 3 ml of the albumin solution
Using a dropper add to (albumin) on the inner wall of the tube to form a layer up the acid	add 5-7 drops of T.C.A solution carefully
Record your observation	Record your observation

Result:

Tube	Observation	Comment
Conc. HNO ₃ + Albumin		
Albumin + TCA		



Precipitation of albumin using Trichloroacetic acid [TCA]



Precipitation of albumin using concentrated nitric acid.

3. Precipitation of protein by salts of heavy metals:

Heavy metal salts usually contain Hg^{+2} , Pb^{+2} , Ag^{+1} , Tl^{+1} , Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.

Objective:

to identify the effect of heavy metal salt on protein.

Principle:

-Heavy metal salt will **neutralize** the protein:

By the negative charge of protein will bind with positive charge of metal ion . Then the protein will precipitate as insoluble metal protein salt .

Method:

A	B
In a test tube, put 1 ml of Albumin sample	In a test tube, put 1 ml of Albumin sample
Using a dropper add to (albumin) few drops of AgNO_3	Using a dropper add to (albumin) few drops of HgCl_2
Record your observation	Record your observation

Result:

Tube	Observation	Comment
Albumin + AgNO_3		



4. Protein denaturation:

Objective:

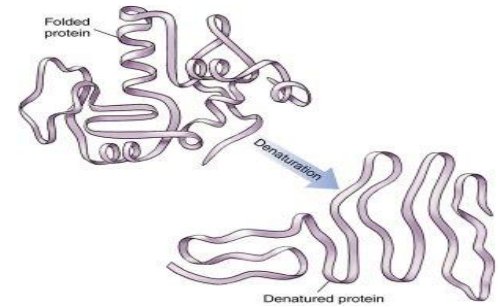
to investigate the effect of high temperature on protein structure.

Principle:

Non-covalent bond can be broken by heating, leading to protein denaturation and the precipitation.

Denaturation is a process in which the proteins losing its quaternary structure, tertiary structure and secondary structure, by application of some external factor or compound such as a strong acid or base, a conc. inorganic salt, an organic solvent (e.g., alcohol or chloroform), or heat.

* without alteration of the molecule's primary structure, i.e., without cleavage of any of the primary chemical bonds that link one amino acid to another.

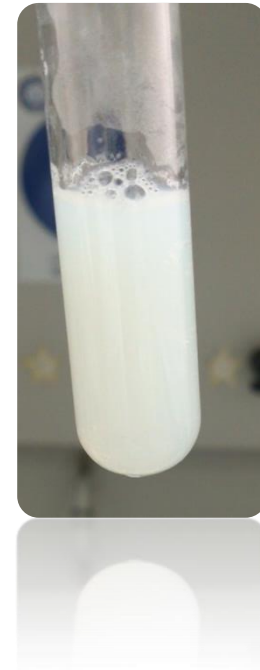


Method:

- 1- Take 1 ml of protein Albumin .
- 2- Place it in a boiling water bath for 5-10 minutes.
- 3-Remove aside to cool to room temperature.
- 4-Note the change.

Result:

protein	Observation	Comment
Albumin		



Questions :

Do you think free amino acids will give a positive result with biuret test ? why?

What is the least number of amino acids bonded together by peptide bonds that will respond positively to biuret test?

After heating albumin at high temperature, does it still biologically active? Why?

Can we use salting out method in fractionating mixture of proteins? Explain your result with example.