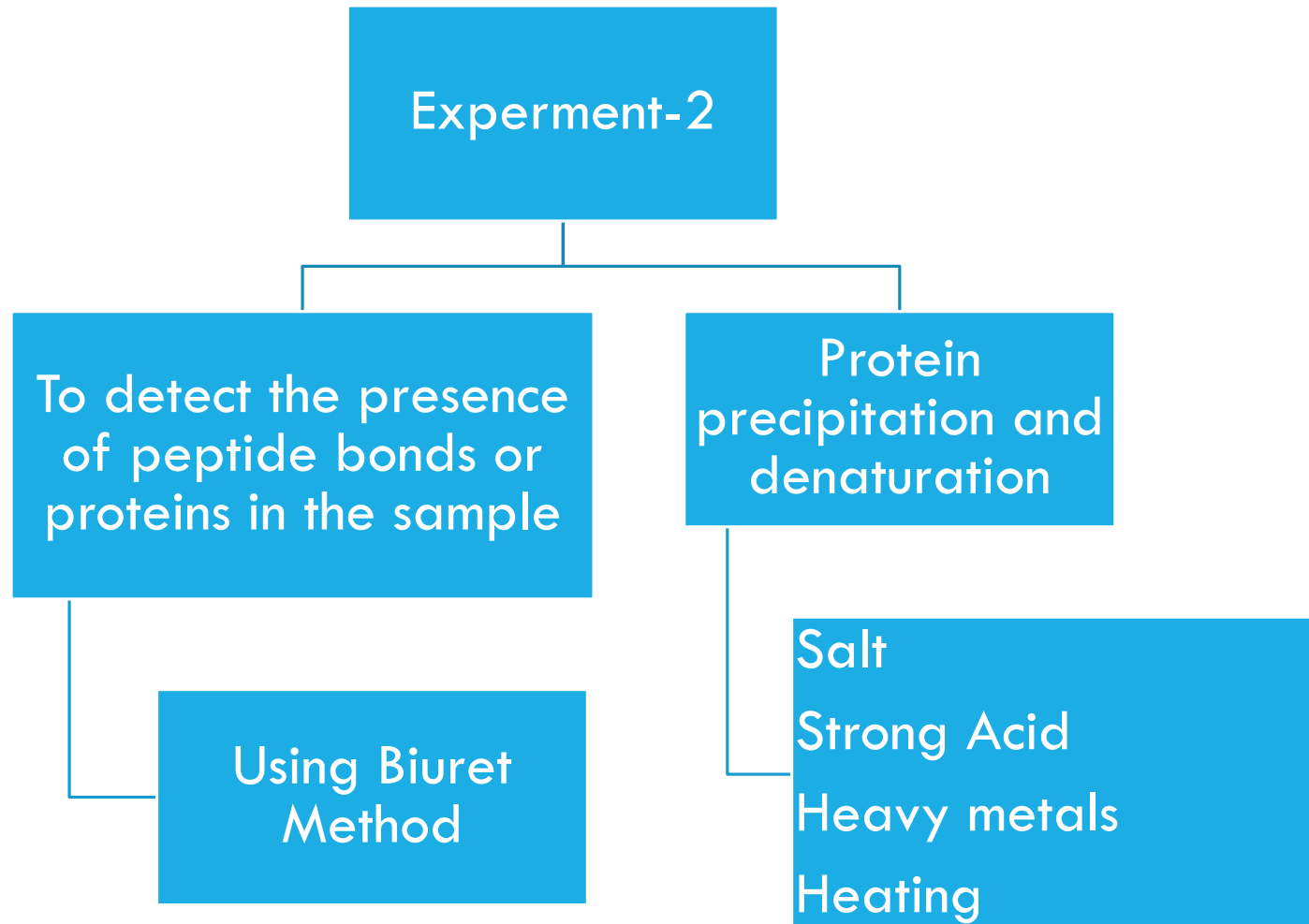


QUALITATIVE TEST OF PROTEIN |

OUTLINE



BIURET TEST

Objective:

- To detect the presence of a protein or peptides.
- Positive result (purple color) will be given if the substance has **two or more peptide bonds** (three or more amino acids)

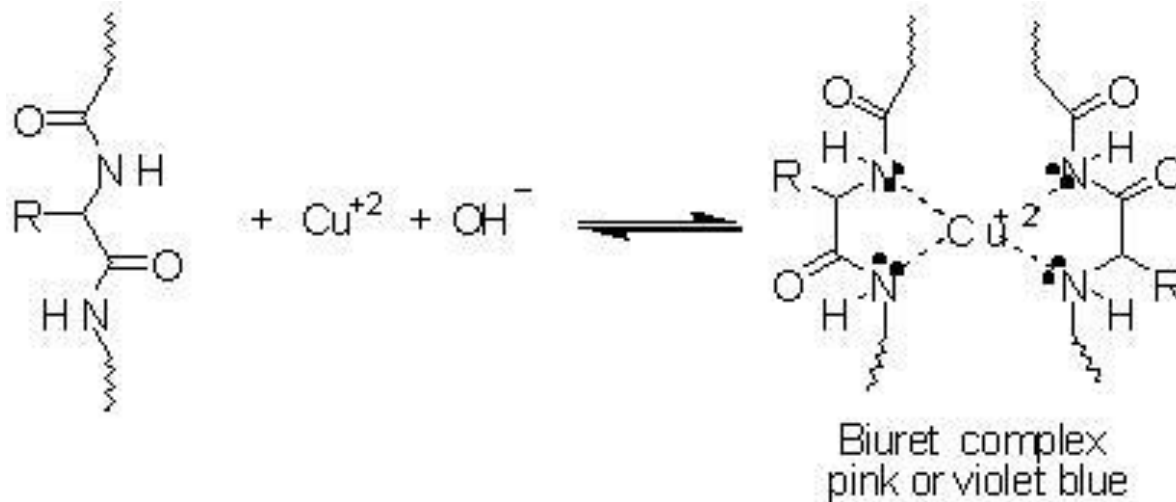
Note: Despite its name, the reagent does not in fact contain biuret ($(\text{H}_2\text{N}-\text{CO}-)_2\text{NH}$). The test is so named because it also gives a positive reaction to the peptide-like bonds in the biuret molecule.



PRINCIPLE:

- In this reaction, proteins form a pink-purple colored complex with CuSO_4 in a strongly alkaline solution.

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**.



METHOD:

- 1- Add 3ml of protein Albumin
- 2- Add 1 ml of 10M NaOH
- 3- Add 0.5 ml of CuSO₄ and mix well.

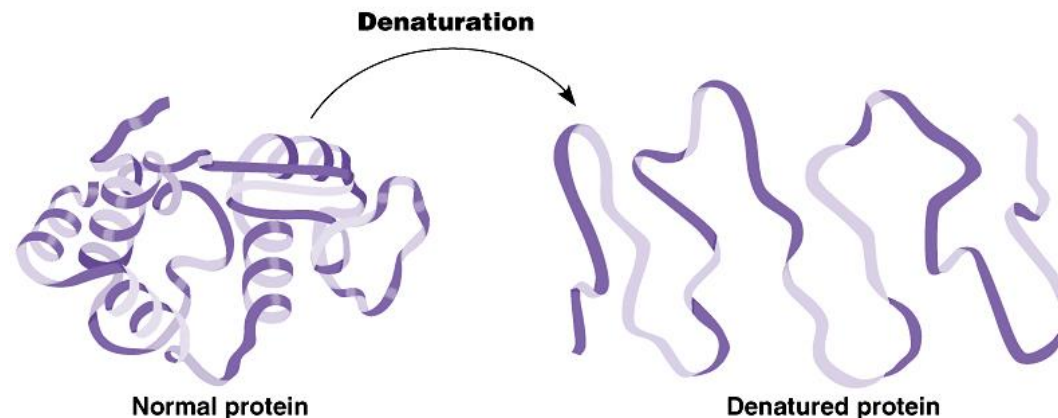
protein	Observation	Comment
Albumin		

2-PROTEIN PRECIPITATION

- The solubility of proteins is affected by pH, temperature, salts, heavy metal salts ..etc
- The change of one of these factors will lead to protein precipitation and/ or denaturation.
- Proteins will get denatured while using some factors that lead to precipitation.
- Is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants.

DENATURATION OF PROTEINS

- **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, an organic solvent (e.g., alcohol or chloroform), or heat.
- Protein will become more viscous, decreased solubility and aggregation, and protein become inactive



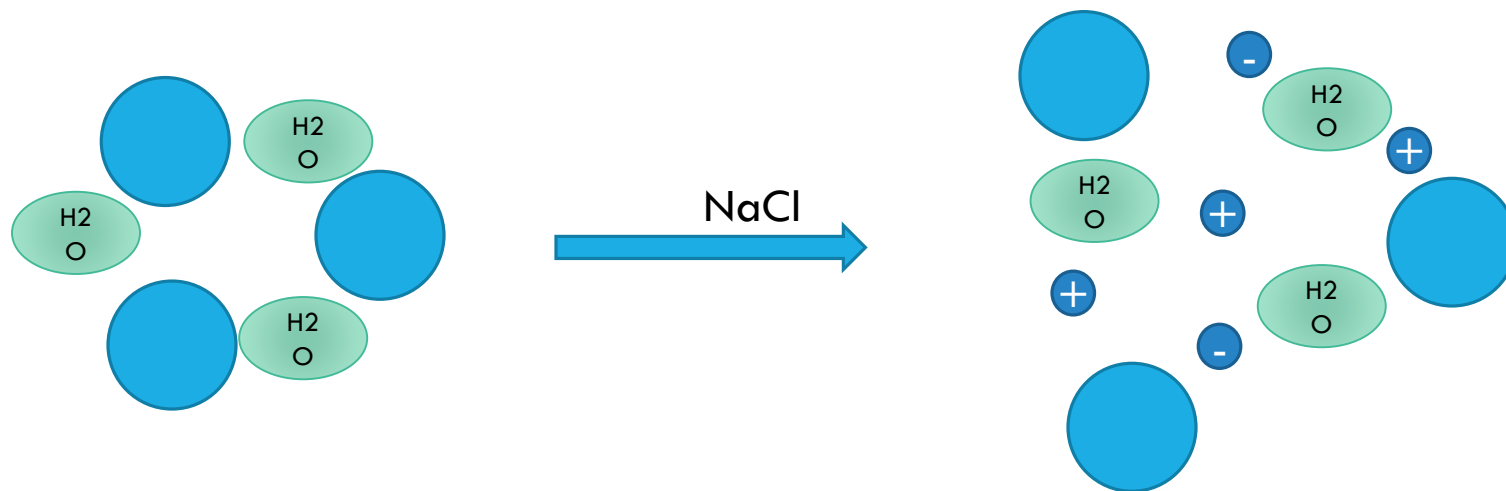
EXPERIMENT (2): EFFECT OF SALT CONCENTRATION ON THE PROTEIN SOLUBILITY :

Objective:

- To investigate the effect of different salt concentration on protein solubility.
- When low concentrations of salt is added to a protein solution the solubility increases (This is called salting in)
- At some point, solubility begins to decrease as salt increases-"salting out"
- Each protein can be precipitated at specific salt concentration.
- It is Reverse process, the protein can again become soluble when we add water
- It could used in the process of protein isolation

PRINCIPLE: SALTING IN

- Low concentrations of salt → the solubility increases. This could be explained by the following:
- Salt molecules stabilize protein molecules by :
- Decreasing the electrostatic energy between the protein molecules which increase the solubility of proteins.

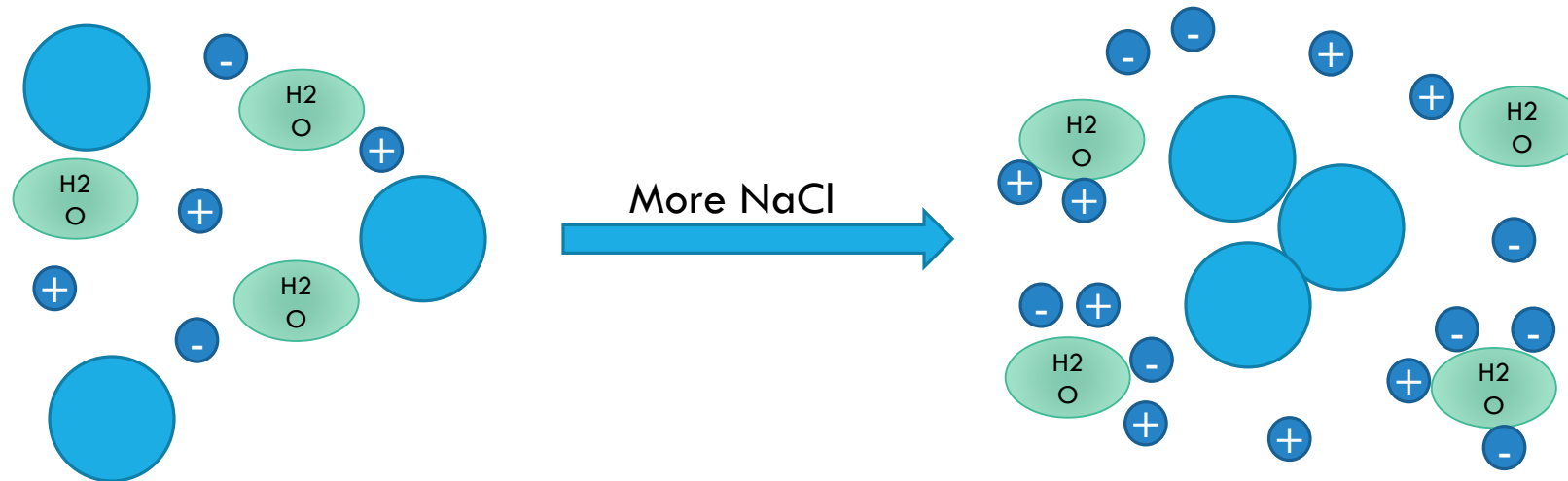


PRINCIPLE: SALTING OUT

High concentration of salts \longrightarrow the solubility decreases, and protein precipitates.

This could be explained by the following:

because the excess ions (not bound to the protein) compete with proteins for the solvent. The decrease in solvation allows the proteins to aggregate and precipitate.



METHOD:

A	B
Take 2 ml of your albumin sample	On the same tube
Add of 0.1M NaCl solution	Add a few amount of 100% solid (NH₄)₂SO₄
Concentrate your vision on the tube while adding	Shake it well and write your observation
record your observation .	Compare between the tube of A and B

RESULTS:

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

**Discusses each result and Compare between them
what and why you obtain it ...**

EXPERIMENT(3):ACID PRECIPITATION OF PROTEINS

Objective:

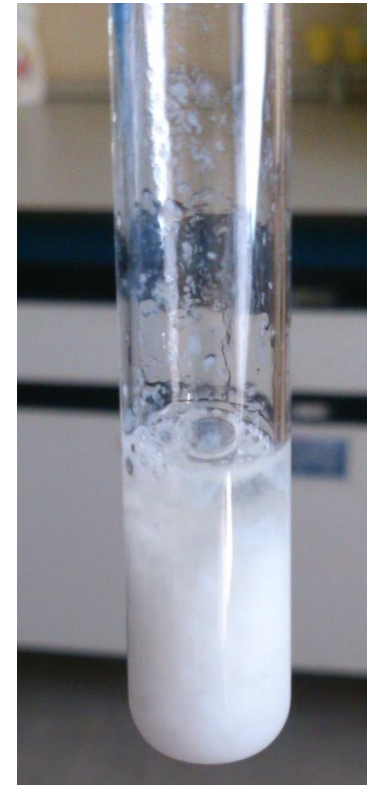
To investigate the *effects of strong acids* on the protein solubility.

Applications:

- Separation and purification
- Detection of small amount of protein in urea sample
- Stop the enzyme reaction

PRINCIPLE:

- This test depends on affecting the 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

RESULTS:

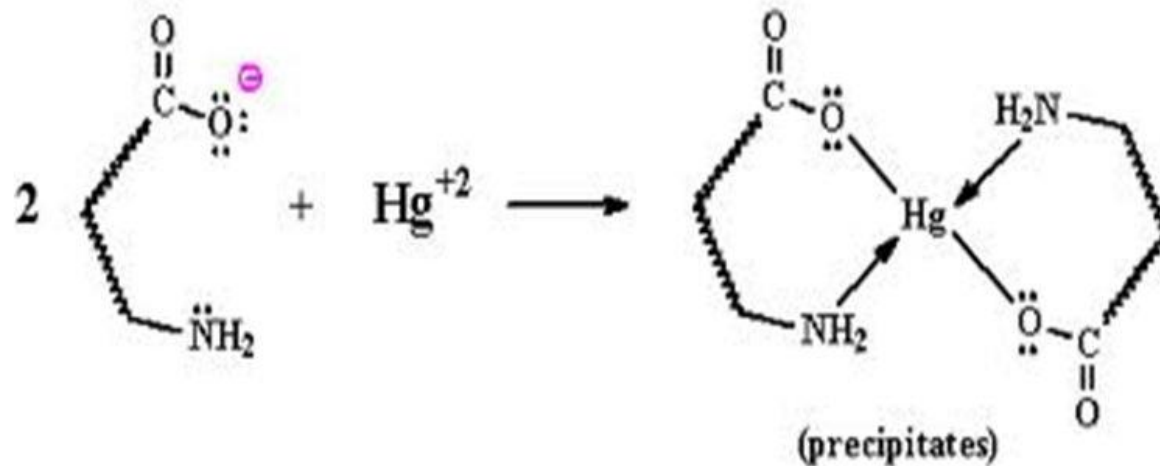
Tube	Observation	Comment
Conc. HNO ₃ + Albumin		
Albumin + TCA		

**Discusses each result
what and why you
obtain it ...**

EXPERIMENT(4):PRECIPITATION OF PROTEINS BY SALTS OF HEAVY METALS:

Objective:

to identify the effect of heavy metal salt on protein



PRINCIPLE

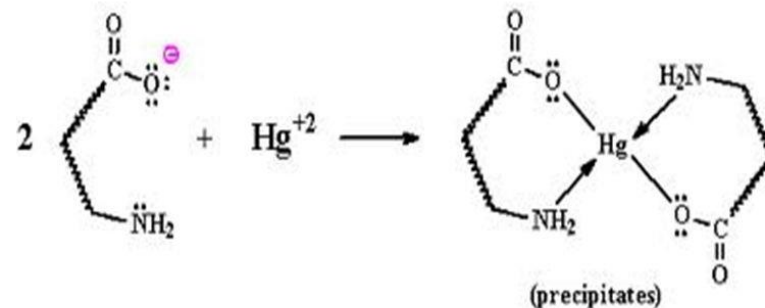
Heavy metal salts usually contain Hg^{+2} , Pb^{+2} , Ag^{+1} , Tl^{+1} , Cd^{+2} and other metals with high atomic weights.

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 .

Application::

To eliminate the poisoning by palladium Pb^{++} ,.....mercury salts Hg^{++}



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

RESULTS:

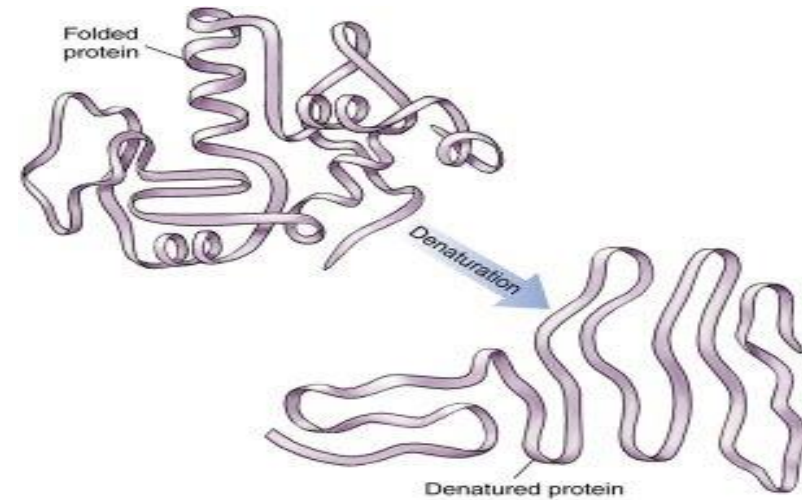
Tube	Observation	Comment
Albumin + AgNO ₃		

**Discusses each result
what and why you
obtain it ...**



EXPERIMENT(5):PROTEINS DENATURATION BY HEATING

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



METHOD:

- 1- Take 3 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+ heating		

