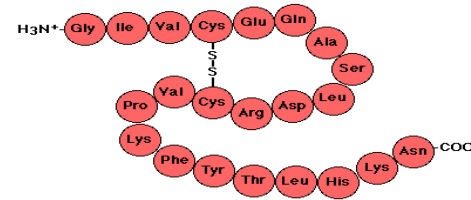


Qualitative tests of Proteins

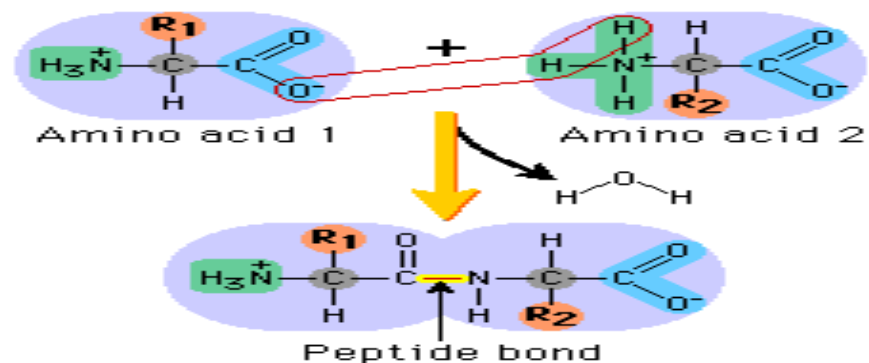
BCH302 [Practical]

Proteins :

- Proteins are polymers of amino acids.

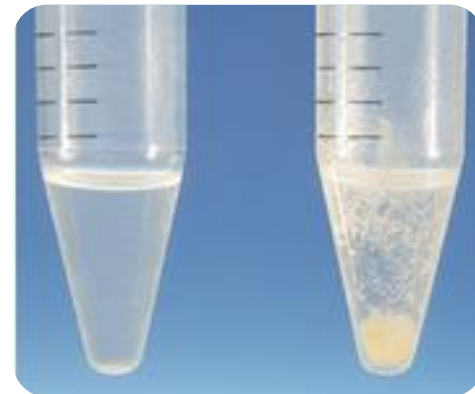


- Amino acid molecules in proteins are **covalently joined** together through a linkage, termed a **peptide bond**.
- How peptide bond formed?**
➔ By removal of the elements of water (dehydration) from the α -carboxyl group of one amino acid and the α -amino group of another.



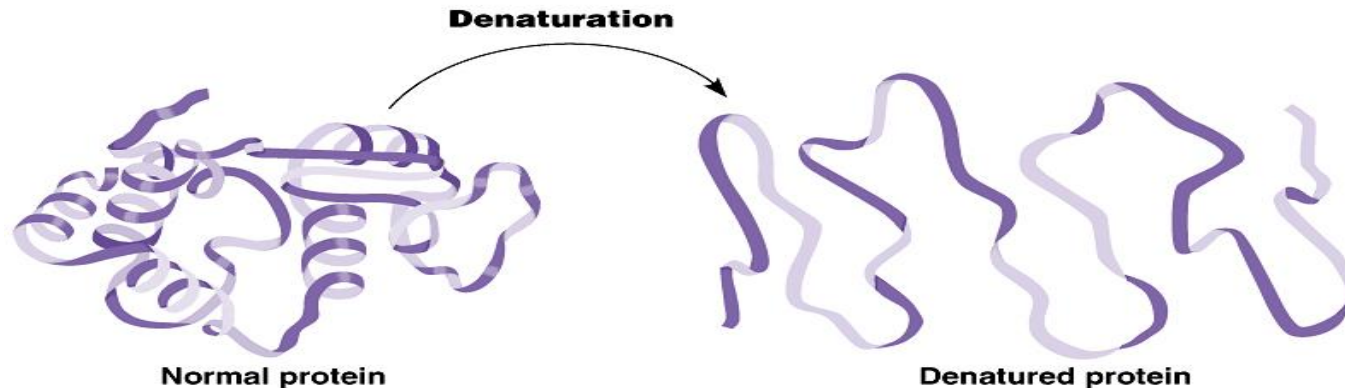
Proteins precipitation :

- 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 precipitation is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants.



Proteins denaturation:

- **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**.



- No alteration on the molecule's primary structure, i.e., without cleavage of any of the primary chemical bonds that link one amino acid to another.
- Protein will become more viscous, decreased solubility and aggregation, and protein become inactive.

Practical part

Qualitative tests of proteins

- 1 Biuret test: detect the presence of peptides or proteins.
- 2 Effect of salt concentration on the protein solubility.
- 3 Precipitation of proteins by acids.
- 4 Precipitation of protein by salts of heavy metals.
- 5 Protein denaturation.

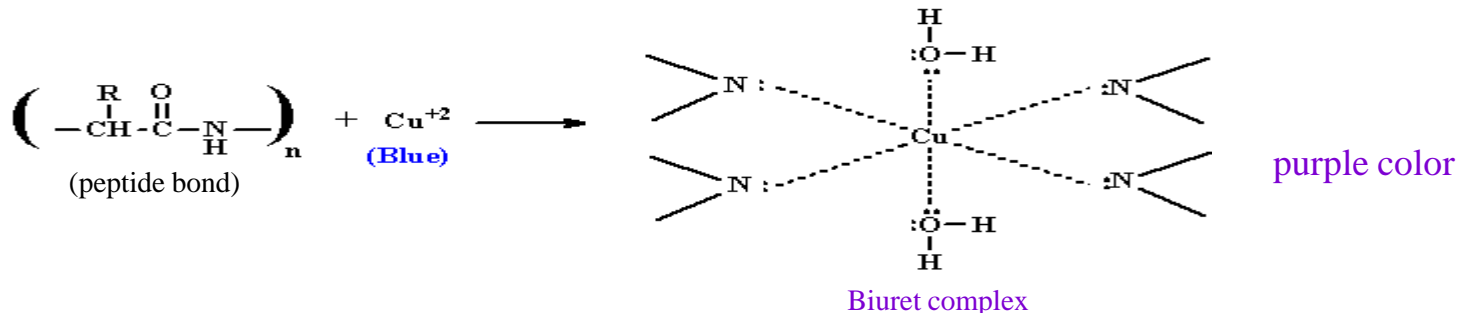
Experiment 1 : Biuret test

Objective:

- To detect the presence of a protein or peptides.

Principle:

- In this reaction, proteins form a **purple colored complex** with CuSO_4 (copper sulfate) in a strongly **alkaline solution**.
- When **peptide bonds** in proteins and peptides treated with an **alkaline solution** of dilute copper sulfate (Biuret reagent) a **violet** color is formed → A positive test is indicated by the formation of a **violet** color.
- The color density is **proportional** to amount of proteins present.
- This test is **specific for the peptide bond**, positive result (purple color) will given if the substance have **two or more peptide bonds** (three or more amino acids).



Note: Despite its name, the reagent does not in fact contain biuret, the test is so named because it also gives a positive reaction to the peptide-like bonds in the biuret molecule. 7

Experiment 1 : Biuret test

Method:

1. Add 2ml of protein Albumin in one tube.
2. In another tube add 2ml of water.
2. Add 1 ml of biuret reagent to all tubes and mix well.

Results:

Tube	Observation
Albumin (protein)	
water	



Blue color is the biuret reagent color

Experiment 2 : Effect of salt concentration on the protein solubility

Objective:

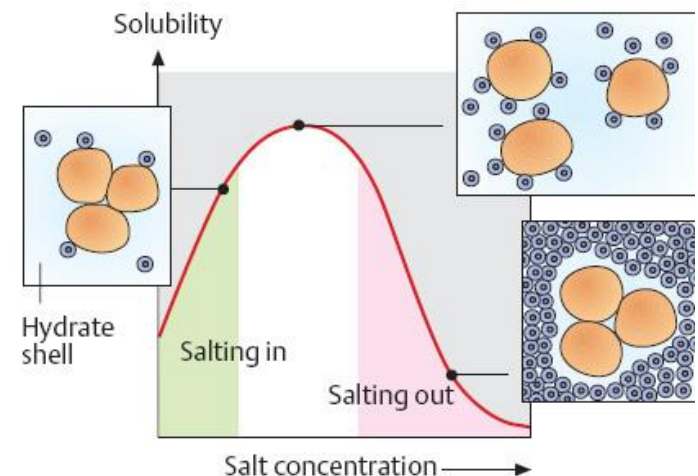
- To investigate the effect of different salt concentration on protein solubility.

Principle:

- The **low salt concentration** solutions make protein **solubility increased** using the attraction of salt ions to the functional groups of the protein (this called **salting in**).
- High salt concentration** causes the **protein to precipitate (decrease the solubility)** since salt ions, in this case, compete with the protein molecules in binding water molecules (this called **salting out**).

- Notes:**

- Each protein can be precipitated at specific salt concentration.
- It is reverse process, the protein can again become soluble when we add water .



Experiment 2 : Effect of salt concentration on the protein solubility

Method:

1. Label one tube as **A**.
2. Add 2ml of albumin.
3. Add drops of **0.1M NaCl** solution, Concentrate your vision on the tube while adding.
4. Record your results.
5. In the same tube add few amounts of 100% solid $(\text{NH}_4)_2\text{SO}_4$, shake it well.
6. Record your results.
7. Compare between the two results.

Results:

Tube	Observation
Albumin + NaCl	
Albumin+100% saturate $(\text{NH}_4)_2\text{SO}_4$	

Experiment 3 : Acid precipitation of proteins

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**.
- Applications:
 - Detection of small amount of protein in urea sample.
 - Stop the enzyme reaction.

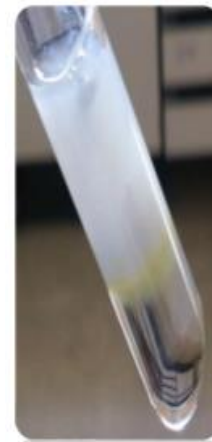
Experiment 3 : Acid precipitation of proteins

Method:

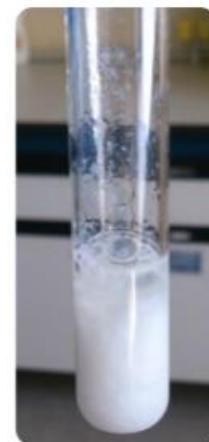
1. Label two tubes A and B.
2. **In tube A:** add 3 ml of conc. nitric acid (HNO_3) CAREFULLY.
3. Then, Using a dropper add drops of albumin on the inner wall of tube A to form a layer up the acid.
4. Record your results.
5. **In tube B:** Add 3 ml of the albumin solution.
6. Then add 5-7 drops of TCA solution CAREFULLY.
7. Record your results.

Results:

Tube	Observation
Albumin + HNO_3	
Albumin+TCA	



A



B

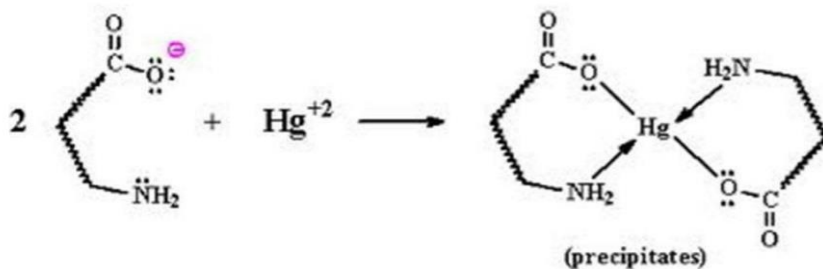
Experiment 4 : Precipitation of proteins by salts of heavy metals

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 \rightarrow then the protein will precipitate as insoluble metal protein salt.



• Applications:

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

Experiment 4 : Precipitation of proteins by salts of heavy metals

Method:

1. Label two tubes A and B.
2. In tube A and B add 1 ml of Albumin sample.
3. **In tube A:** using a dropper add few drops of AgNO_3 .
4. Record your results.
5. **In tube B:** using a dropper add few drops of HgCl_2 .
6. Record your results.

Results:

Tube	Observation
Albumin + AgNO_3	
Albumin + HgCl_2	



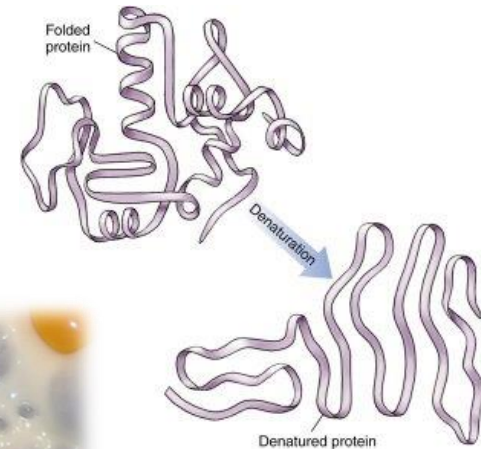
Experiment 5 : Protein denaturation by heating

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.



Experiment 5 : Protein denaturation by heating

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.

Results:

Tube	Observation
Albumin + heating	

