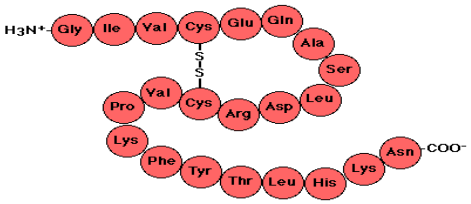
Effect of various factors on protein solubility and structure

BCH303 [Practical]

Proteins :

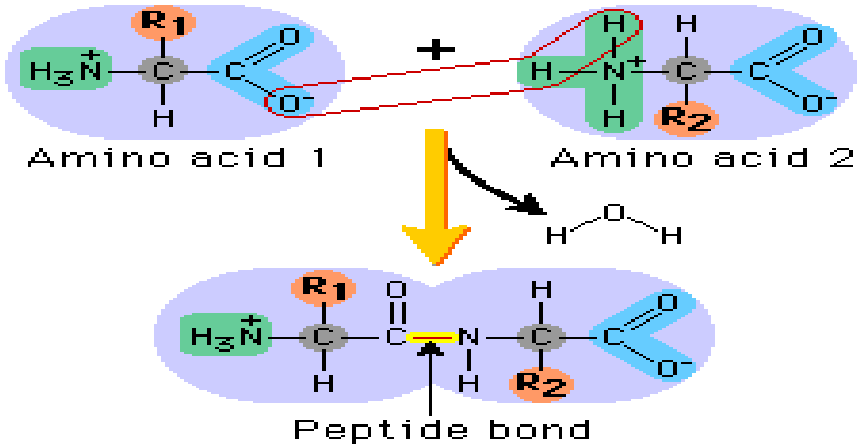
- Proteins are polymers of <u>amino acids.</u>



- **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.



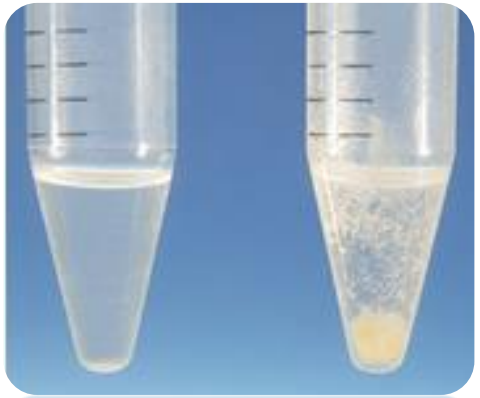
Proteins precipitation :

- What is ?

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

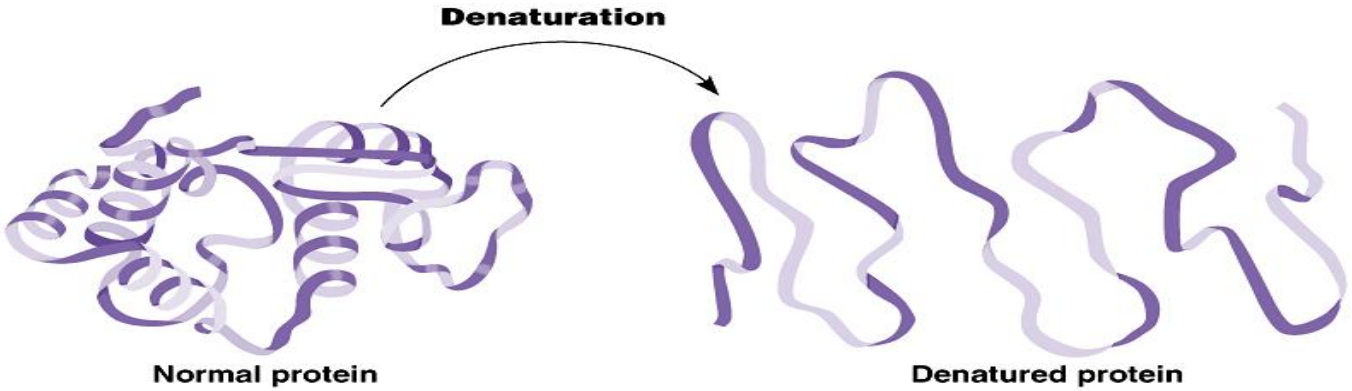
- Factors?

- The change of one of these factors will lead to protein precipitation and/ or denaturation.



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.
- Solubility ?
- **Activity?**

Practical part

Tests of proteins

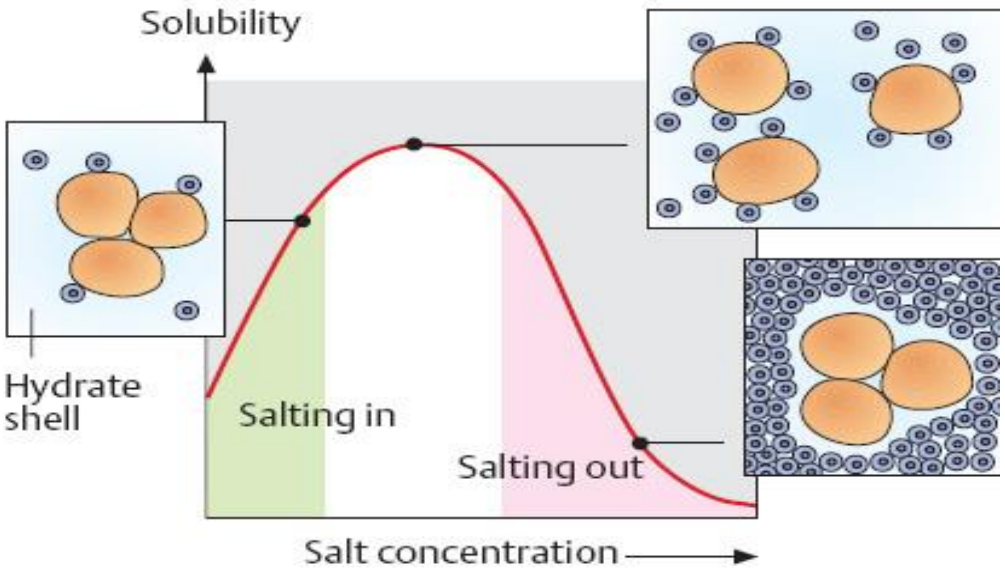
1. Effect of salt concentration on the protein solubility.
2. Effect of strong acids on protein solubility and structure.
3. Effect of salts of heavy metals on protein solubility and structure.
4. Effect of heat on protein solubility and structure.

Experiment 1 : Effect of salt concentration on the protein solubility

Objective:

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

Principle:



- Notes:

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

Experiment 1 : 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 $(NH_4)_2SO_4$, shake it well.
6. Record your results.
7. Compare between the two results.

Results:

Tube	Observation
Albumin + NaCl	
Albumin+100%saturate $(NH_4)_2SO_4$	

Experiment 2 : Effect of strong acids on protein solubility and structure

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 2 : Effect of strong acids on protein solubility and structure

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.

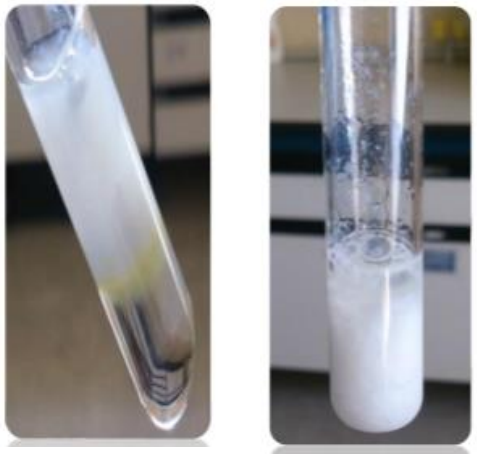
5. **In tube B:** Add 3 ml of the albumin solution.

6. Then add 5-7 drops of TCA solution CAREFULLY.

7. Record your results.

Results:

Tube	Observation
Albumin + HNO_3	
Albumin+TCA	



A **B**

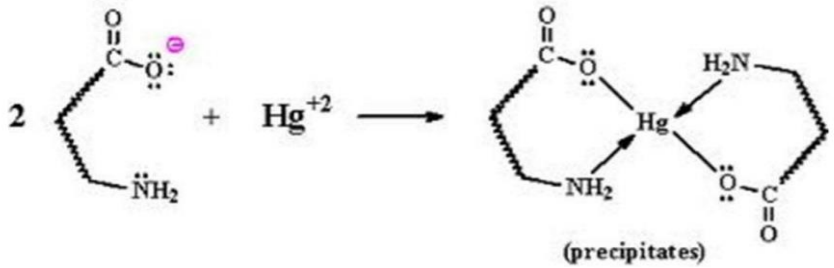
Experiment 3 : Effect of salts of heavy metals on protein solubility and structure

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**.
- The protein will <u>precipitate as insoluble metal protein salt.</u>



- <u>Applications:</u>

➢ To eliminate the poisoning by palladium Pb++ ,.....mercury salts Hg++

Experiment 3 : Effect of salts of heavy metals on protein solubility and structure

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 + $HgCl_2$	

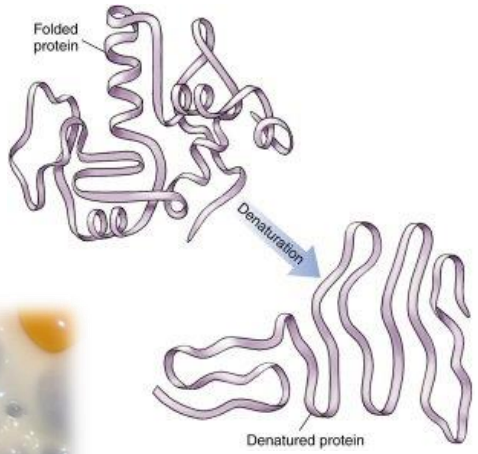
Experiment 4 : Effect of heat on protein solubility and structure

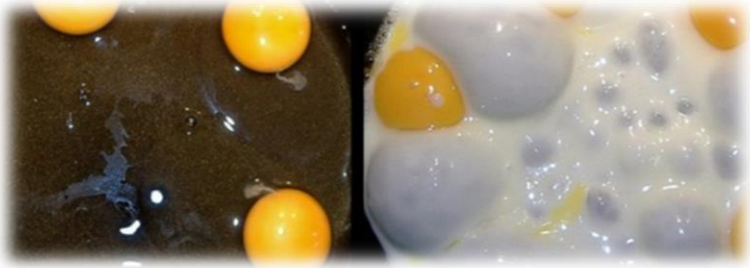
Objective:

- 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 4 : Effect of heat on protein solubility and structure

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	

Homework:

- **From today lab, which factors lead to protein denaturation and which lead to precipitation? Differentiate between them regarding the protein activity.**