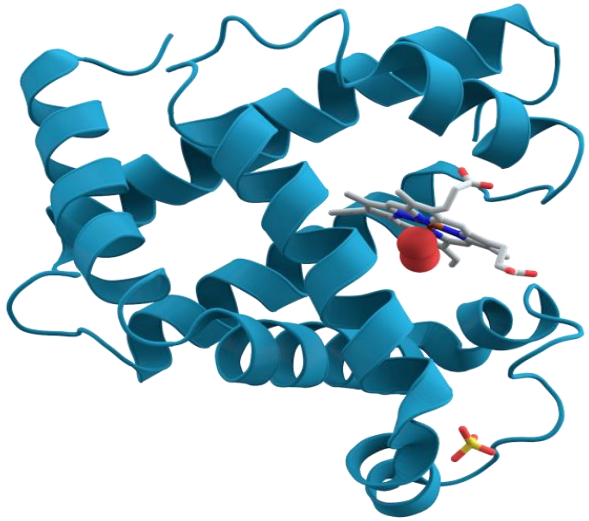
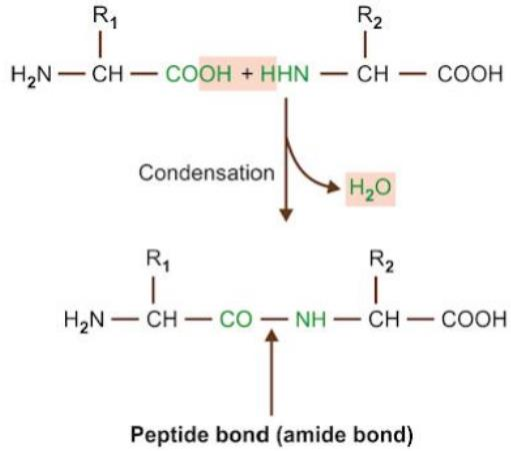
Effect of various factors on protein solubility and structure



Proteins:

- proteins made up of smaller unites called **amino acids**.
- Amino acid molecules in proteins are covalently joined together through a amide linkage, termed a **peptide bond**.
- formed by the removal of water (dehydration) from the α carboxyl group of one amino acid and the α-amino group of another

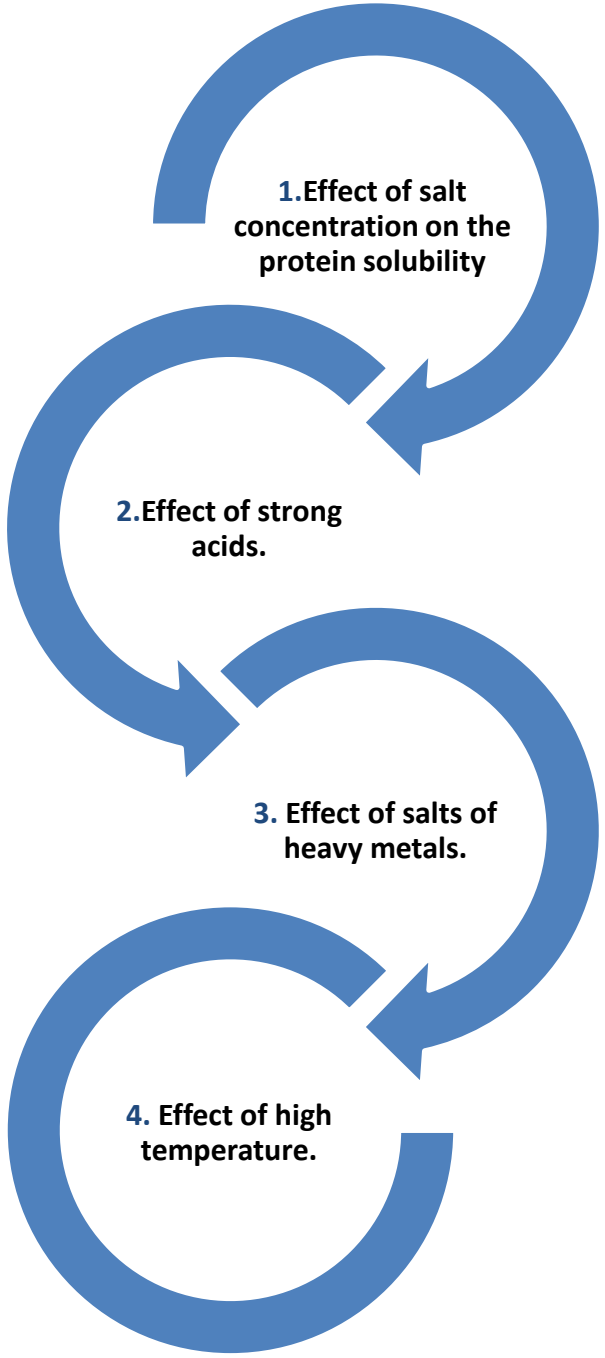


Protein precipitation

- Protein precipitation is the process of separating a protein from a solution as a solid by altering the protein solubility with addition of a reagent.

- 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.


1.Effect of salt concentration on the protein solubility
2.Effect of strong acids.
3. Effect of salts of heavy metals.
4. Effect of high temperature.

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**).

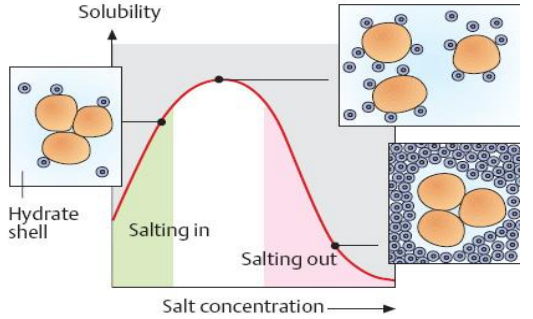
On contrast, **high salt concentration** or solids dissolved in the reaction medium up till saturation solutions causes the protein to **precipitate s**ince salt ions, in this case, compete with the protein molecules in binding water molecules (**salting out**).

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)

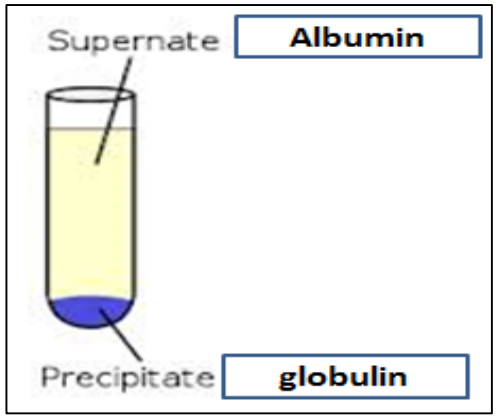


Egg Proteins:

Albumin and **globulin**

Separated by centrifugation at 3000 for 20 min.

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



Method:

1. In a tube add 2 ml of albumin.
2. Add drops of 0.1M NaCl solution, concentrate your vision on the tube while adding.
3. Record your results.
4. In the same tube add few amounts of 100% solid (NH4)2SO4, shake it well.
5. Record your results.
6. Compare between the two results

Result:

Tube	Observation
Albumin+ 0.1M NaCl	
Albumin+ 100% solid (NH2)SO4	

2.Effect of strong acid:

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. By changing the pH of the solution, the charge state of the solute changed. In highly **acidic media**, the protein will be positively charged, which is attracted to the acid anions that cause them to precipitate.

There are many **applications** of this test in laboratories, i.e. in the detection of small amounts of protein in urea sample, also to stop the enzymatic action of an enzyme.

Method

1. Label two tubes A and B.

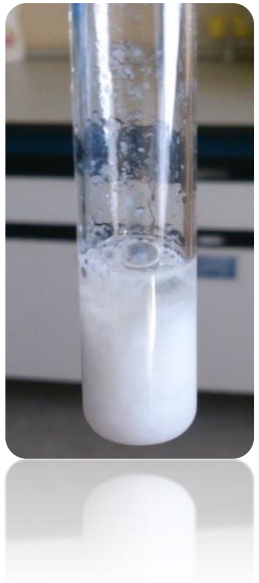
2. **In tube A**: add 3 ml of conc. nitric acid (HNO3) CAREFULLY.

3. Then, using a dropper add drops of albumin on the inner wall of tube A to form a layer up the acid.

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

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

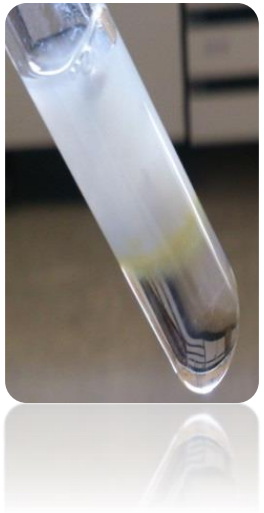
6. Record your results



Precipitation of albumin using Trichloroacetic acid [TCA]

Result:

Tube	Observation
Conc. HNO3 + Albumin	
Albumin + TCA	



Precipitation of albumin using concentrated nitric acid.

3. Effect of 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.

Objective:

to identify the effect of heavy metal salt on protein.

Principle:

Since salts are ionic they disrupt salt bridges in proteins (heavy metal salt will **neutralize** the protein). The negative charge of protein will bind with positive charge of metal ion which cause the protein to precipitate as insoluble metal protein salt.

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. In tube B: add few drops of $HgCl_2$.

5. Record your results.

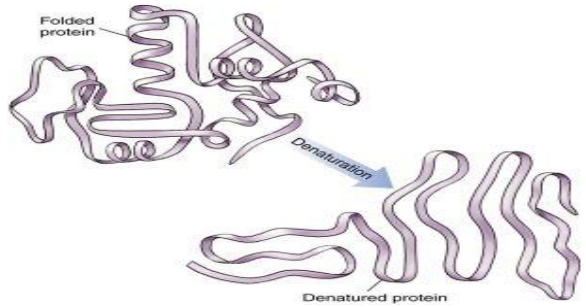
Result:

Tube	Observation
Albumin + $AgNO_3$	
Albumin + $HgCl_2$	

4. Effect of high temperature:

Denaturation is a process in which the proteins losing its quaternary structure, tertiary structure and secondary structure, by application of some external factor such as a strong acid or base, a conc. inorganic salt, an organic solvent (e.g., alcohol or chloroform), or heat. Denatured proteins lose their 3D structure and therefore cannot function.

*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.



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.

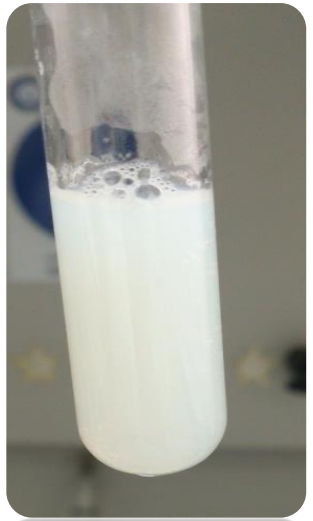
Method:

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:

Tube	Observation
Albumin + heating	

The proteins in eggs denature and coagulate during cooking. Other foods are cooked to denature the proteins to make it easier for enzymes to digest them. Medical supplies and instruments are sterilized by heating to denature proteins in bacteria and thus destroy the bacteria.

Questions :

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.