Qualitative test of protein-LAB2

1- Qualitative chemical reactions of amino acid protein functional groups:

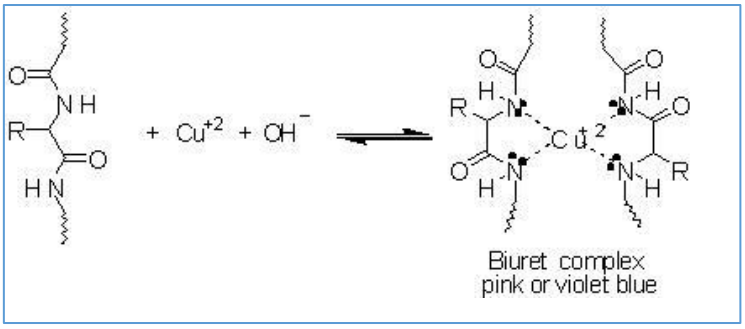
Certain functional groups in proteins can react to produce characteristically colored products. The color intensity of the product formed by a particular group varies among proteins in proportion to the number of reacting functional or free groups present and their accessibility to the reagent. In this part of the experiment we will use various color producing reagents (dyes) to qualitatively detect the presence of certain functional groups in proteins.

1-Biuret test:

This test is specific for the peptide bond. Substances containing not less than two peptide linkages give this test. In this reaction, proteins form a pink-purple colored complex with CuSO 4 in a strongly alkaline solution. Objective: to detect the presence of peptides or proteins in a sample.

2-Principle:

This test is used to detect the presence of proteins and peptides (i.e peptide bonds) by treating them with an alkaline solution of dilute copper sulfate . A positive test is indicated by the formation of a pink-violet color. The name of the test is derived from a specific compound, biuret which give a positive test with this reagent.



3-Materials:

- Proteins solutions [2% gelatin , 2%BSA, or 2% raw egg albumin dissolved in 0.1 NaCl and 1% casein] (casein is to be dissolved in diluted NaOH) .
- NaOH (3M).
- Copper sulfate (1% CuSO4 .5H2O, Fehling's solution A diluted 1/10 with water).
- Test tube .
- Method: ▯ in 3 different test tubes put 2ml of each protein solution. ▯ to each tube add 1ml 3M NaOH. ▯ Add 0.5 ml of CuSO4 .5H2O, and mix well. ▯ Observe the colors produced and write your observation in the table.

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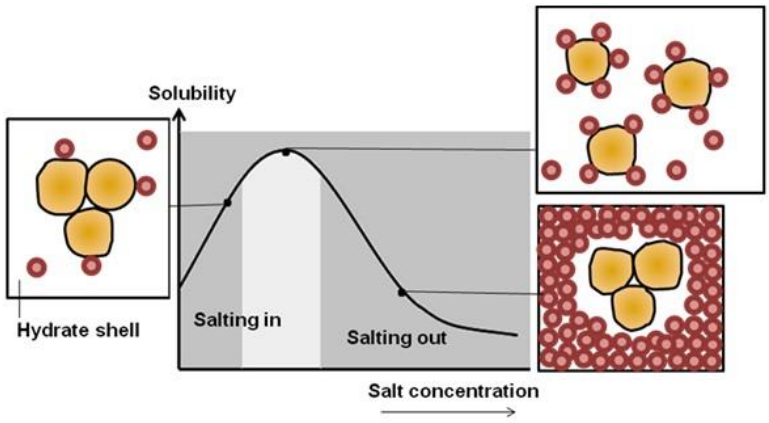
2-Effect of salt concentration on the protein solubility:

This experiment is used to separate different proteins using salting-out theory. Each protein can be precipitated at specific salt concentration.

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

2-Principle:

The low salt concentration solutions make protein solubility easier using the attraction of salt ions to the functional groups of the protein. 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.



3-Materials:

- Albumin
- Sodium chloride NaCl -0.1M.
- Ammonium sulfate (NH4)2SO4-solid.

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**

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3-Precipitation of proteins by acids:

This experiment is used to precipitate different proteins using strong acid solution. Each protein can be precipitated at specific acid concentration.

Objective: **to investigate the effect of concentrated acids on protein solubility.**

Principle:

Strong acids cause proteins to precipitate by affecting different bonds of the molecule, there are many applications of this test in laboratories, i.e in the detection of small amounts of protein in urea sample, also in the separation and purification of proteins or to stop the enzymatic action of an enzyme. - 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 changed, which is attracted to the acid anions that cause them to precipitate.

Materials:

 Concentrated nitric acid. Trichloroacetic acid (TCA). BSA (bovine serum albumin) 0.5%, or 2% egg albumin dissolve in 0.1 NaCl solution

Method:

 In a test tube, put 3ml of conc. nitric acid. Using a dropper add to the tube the protein solutions you have (albumin, casein, gelatin) drop-wise on the inner wall of the tube to form a layer up the acid. Note the white precipitate at the inner face of the protein in contact with the conc. acid. To another tube add 3 ml of the protein solution add T.C.A drop-wise till a precipitate forms.

4-Protein denaturation:

Denaturation is destruction of the usual nature of a substance, as by the addition of methanol or acetone. Most globular proteins exhibit complicated three-dimensional folding described as secondary, tertiary, and quarternary structures. These conformations of the protein molecule are rather fragile, and any factor that alters the precise geometry is said to cause denaturation. Extensive unfolding sometimes causes precipitation of the protein from solution. Denaturation is defined as a major change from the original native state 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.**

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Principle: - Treatment of proteins with strong acids or bases, high concentrations of inorganic salts or organic solvents (e.g., alcohol or chloroform), heat, or irradiation all produce denaturation to a variable degree. Loss of three-dimensional structure usually produces a loss of biological activity. - This test illustrates the importance of week bonds in globular protein's tertiary structure (the functional structure). Acid and heat disrupt ionic bonds and hydrogen bonds, respectively causing loss of the quaternary structure. This leads to denaturation and loss of biological function.

Materials:

 Albumin (2% raw egg albumin in 0.1 NaCl), 1% gelatin ,1% casein,1% globulin in 0.1 NaCl. Diluted acetic acid.

Method: Add 10 ml of protein solutions in different test tubes Add 3 drops of acetic acid to each tube. no need Place them in a boiling water bath for 5-10 minutes; Remove aside to cool to room temperature. Note the change in each tube.

5-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 investigate the effect of heavy metals on protein structure.**

Principle: - At pH7 the protein is normally negatively charged. Addition of the heavy metal cation will neutralize those negative charges and will cause the protein to precipitate, but any elevation of pH of the medium to higher than 7 (to basic) will precipitate protein as hydroxides, whereas more of metal cations will dissolve this precipitate. - The family's application of this technique is to eliminate poisoning by palladium (Pb++) and mercury salts (Hg++).

Materials:

- Albumin
- (2g $AgNO_3$) dissolved in 100ml distilled water

Method:

- In different test tubes take 1ml of protein solutions.

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- Add to each tube 0.5ml of $AgNO_3$ (be careful).
- Repeat the process using $HgCl_2$ instead of $AgNO_3$, compare the results.

- **How can this technique help eliminating poisoning by Pb^{++} from water pipes or accidental poisoning of Hg?**

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- **Write the chemical formula of this reaction and the resulting copper complex.**

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- **Do you think free amino acids will give a positive result with this reaction? why?**

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

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