



PROTEINS

- 1- Proteins contain the following elements : **Carbon - Hydrogen - Oxygen - Nitrogen - Sulfide - Phosphorous** ; nitrogen compose **16%** from protein weight.
- 2- Proteins formed by unitizing big number of amino acid combined with each other by peptide bonds.
- 3- Protein burning gives smell like **burned feathers**.
- 4- All proteins give biuret test. Amino acids or dipeptides doesn't give this test alone while this test positivity conditioned by two peptide bonds presences at least in molecule composition, proteins also give Ninhydrine test.
- 5- Because proteins consist of different amino acids, therefore proteins differ from each other's by their response (positivity) to colored test which depend on the presence of particular kinds of A.A's.
 - Zanthoprotein test : special for all **aromatic** A.A (also aromatic compounds).
 - Millon test : special for **tyrosine** (also phenolic compounds).
 - Rosenheim test : special for **tryptophan** (indole group).
 - Sakachugi test : special for **arginine** (Guanidoino group).
 - Pauly & Irish test : special for **histidine**(imidazole group).
 - Lead acetate : special for **cysteine & cysteine**(sulfur in two form S & SH).
- 6- Proteins differ in their **solubility** in different solvents like :
 - Albumin dissolve in D.W.
 - Globulin dissolve in salt 1%.
 - Casein dissolve in alkyl solution (Sodium hydroxide).
 - Gelatin dissolve in hot water.



Proteins precipitate by :

- A. Organic & complex inorganic acids like picric acid & tungstic acid.
 - B. Heavy mineral salts like magnesium chloride & sulfate and ammonium sulfate (half saturation & full saturation).
 - C. Alcohol.
- 7- Heat coagulation.
 - 8- Proteins may precipitate in their native state like protein precipitation by mineral salts (ammonium sulfate) in this case no change occur in protein molecule, the precipitated protein in this case dissolve again in their solvent. In some conditions precipitation happen with protein denaturation like protein precipitation by alcohol or by heat coagulation ... this case called irreversible changing.
 - 9- Amino acid divided to essential & non-essential amino acid , essential amino acid is A.A which human body cannot make it inside the body thus it is necessary to get it by food or the body will suffer from sickness changing.
 - 10- Albumin can detect in urine by heat coagulation.
 - 11- Protein can standardly determine by using chromatic techniques by Biuret method.
 - 12- Biuret test not special & selective for proteins, other compounds can give this test , the most important yield for this reaction is the product result from dry heating of urea.
 - 13- Amino acids is amphoteric compounds react with acids and bases due to acidic **carboxylic** group and **alkyl amine** group.
 - 14- Each A.A have special **isoelectric point**, A.A can participate at this point.
 - 15- Cysteine A.A can prepare by hair or wool.
 - 16- Proteins can classified into :
 - A. Simple protein : protein result from amino acid unitizing by peptide bonds like albumin



B. Complex protein : proteins contain amino acids and other groups not related to protein like phosphorous protein (Casein).

C. Derivatives proteins : result from simple proteins decomposition like gelatin.

17- Gelatin doesn't contain essential amino acids, therefore it gives negative result with the majority of color chromatic tests (mentioned previously).

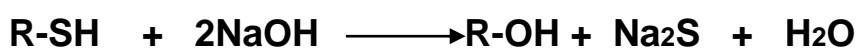
18- Albumin & globulin distinguished between other A.A's by their coagulation by heat.

Proteins General Examinations

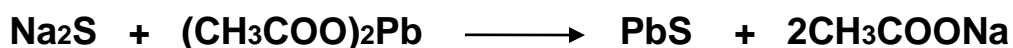
Sulfur Test

Test principle :

This test depend on boiling protein solution with sodium hydroxide, the organic sulfur founded in cysteine & cystine react with sodium hydroxide giving sodium sulfide which react with lead acetate giving lead sulfide (black color) according to following equations :

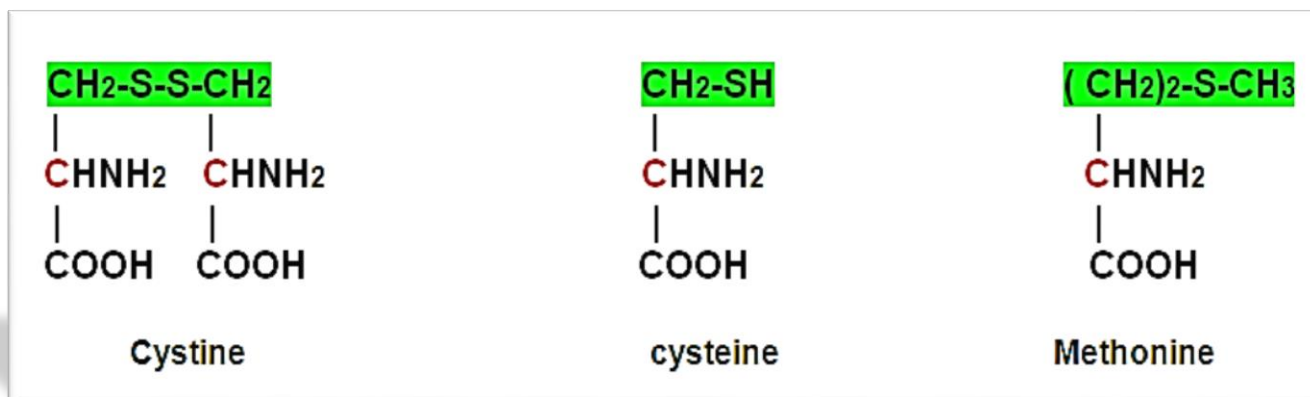


Sodium sulfide



Black lead sulfide

There are three A.A in proteins contain sulfur in their formation :



sulfur atom linkage in Cystine & Cysteine is weak and it is easy to dissociate by reacting with NaOH whereas sodium sulfide formed Na_2S then react with lead acetate giving black precipitate PbS while sulfur atom in methionine is stable & doesn't give this test in alkaline medium.

Reagents & compounds :

- 1- Samples of the following proteins : Albumin , Casein , Gelatin 1%.
- 2- Sodium hydroxide solⁿ 40%.
- 3- Lead acetate solⁿ 10%.

Work Method:

Add 2 ml of protein solution to 2 ml of sodium hydroxide 40%. Boil well for 3 minutes , cool the solution then add 5 drops of lead acetate 2%. Note the change in solution color (dark color between yellow – Black) most likely grey color.

Notes :

milk casein give negative test regardless it contain methionine amino acid which contain sulfur.

- 1- What is the A.A that contain sulfur? Write the structural formula.
- 2- Explain how you can identify amino acids contain sulfur? Explain why this test fail to detect sulfur in methionine ?



3- Why casein give negative test with lead acetate regardless the existence of A.A contain big amounts of sulfur?

Proteins Chromatic Tests

Demonstrate this tests on following proteins : Albumin – Casein – Gelatin
this testes depend on peptide linkages or special chemical groups in amino acids composition which forming protein chain , this tests are :

Biuret Test

Test principle :

This is general test for all proteins give positive result with all compounds contain at least two peptides linkage , thus this test being positive with all proteins also their hydrolysis products till Tripeptide stage while dipeptide & Amino acids doesn't give this test because first compound contain peptide linkage and the second compound doesn't have this linkage.

According to Haurowitz who explain biuret test by forming what called (COPPER COORDINATION COMPLEX) , there is some compounds called Oxamide not related by any mean to proteins but give positive biuret test also Biuret compound which prepare by heating Urea crystals in dry tube. This test named by this final compound.

Reagent & compounds:

- 1- Samples of Albumin – Gelatin – Casein . 1%
- 2- Sodium hydroxide Solⁿ (10%).
- 3- Acetic acid 20%.
- 4- Copper sulfate Solⁿ (1%).

Work Method :

Add 2ml of protein solution to 2ml of diluted NaOH then add 1 drop of 1%(CuSO₄) note the violate or pink color. Compare the color with reference sample. Acidulate the solution with acetic acid 20% then note :

A- In peptones no precipitant yield.



B- In other proteins white color appear.

Notes :

- 1- Peptones & protiozate give pink color , other proteins give violate color.
- 2- In diluted proteins solutions test demonstrate as below :
Add to diluted protein solution sodium hydroxide 10% then dilute copper sulfate 1%, add all this to solution note the violate color in separation surface.
- 3- The importance of this test due to prove peptide groups in protein molecule chain, also the fact that this test not special for proteins but respond to other compound doesn't decrease it importance.
- 4- Write down your results in table.

Proteins Precipitation

Proteins precipitation by heavy minerals

Reagents & compounds :

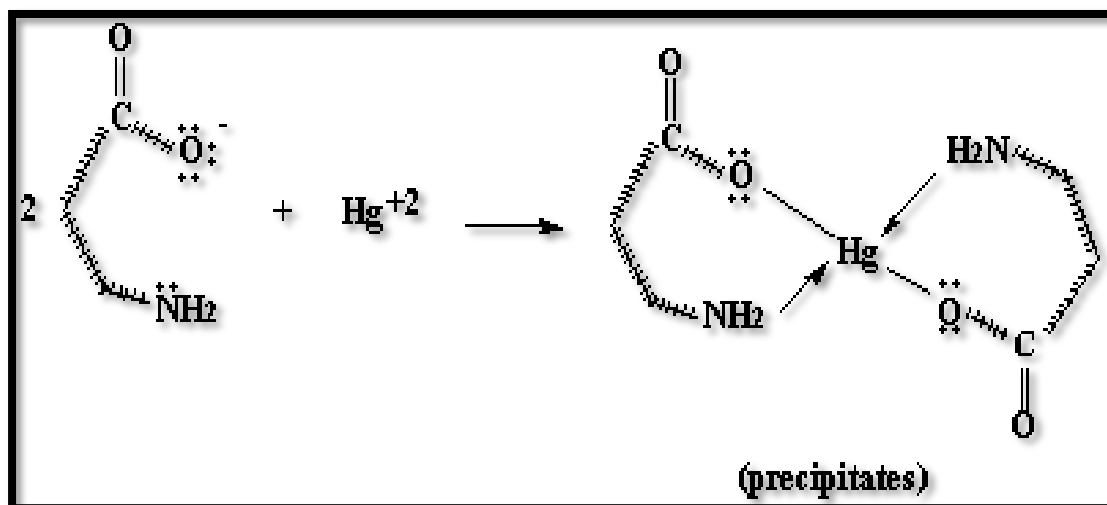
- 1- Albumen (white of egg).
- 2- Silver nitrate 1% Solⁿ
- 3- Lead acetate 1% Solⁿ.

Prepare 3 test tubes and add 3ml of proteins solution to each one :

- 1- First tube : add 2-3 drops of mercuric chloride solution 1%.
- 2- Second tube : add 2-3 drops of silver nitrate 1%.
- 3- Third tube : add 2-3 drops of lead acetate 1%. Note what happen !

Notes:

Proteins precipitation by heavy minerals carry special importance that it use in metal poisoning cases whereas egg white can use as antidote and in this case the heavy metal precipitate as heavy metal albumenate which leave the body with stool without absorption by that the metal loss it toxin.



Question :

- 1- What is the effect of adding heavy metal salt solution to proteins solution?
- 2- If you know that enzymes is proteins compounds, illustrate the effect of heavy metal salt solution on their enzymatic activity ?

Proteins precipitation by alcohol

Reagents & compounds :

1. Albumen – egg white.
2. Ethylic alcohol 90%.

Work Method:

Add 4ml of alcohol to 2ml of protein solution ... note what happen, try to dissolve the precipitated protein in the solvent which been dissolved already inside it.

Notes:

Alcohol doesn't precipitate protein completely .

Some proteins like Glialins or Prolamins not precipitate with alcohol,.

Questions:

- 1- Mention the effect of adding ethylic alcohol to proteins solution?
- 2- What happen to the physical properties of protein precipitated by alcohol?

Protein precipitation by concentrated mineral acids

Reagents & Compounds :



- 1- Egg albumen.
- 2- Conc. HCl.
- 3- Conc. H₂SO₄.
- 4- Conc. HNO₃.

Work Method :

Add to 5ml of clear albumen solution few drops of HCl (Conc.), mix well ... note if precipitate form or not? Continue to add HCl drop by drop note in the formed precipitant dissolve by Acid excess or not? Repeat the experiment with concentrated HNO₃ & H₂SO₄ , note in HNO₃ case the precipitant doesn't dissolve easily in excess of this acid.

Hailer Test : add 3ml of Conc. HNO₃ to test tube, and while the tube in inclined position add similar amount of albumen solution 0.5% by pipet , note white precipitate ring formed between the two solution layers. Dilute egg albumen solution and estimate the less dilution measure that give positive test. Repeat the experiment using urine of healthy person and urine of patient contain albumen.

Question:

1. why concentrated inorganic acids precipitate the proteins? And why the precipitated proteins dissolve in excess amount of same acid?
2. You have sample of egg albumen , mention the effect of nitric acid addition in room temperature & when boiling ? why?