

(4)

## Ninhydrin test

=> Protein soln + 1% ninhydrin

+  
Boil

then cool.

Result → [ Blue colour ]

( presence of amino acid )

Expt.

(vii)

(viii)

③ ~~see~~ Millon's test

⇒ given solution (protein) in T.T.

+  
mercuric sulphate  
(by pipette)

+  
Boil

~~Result~~ [Yellow ppt] → then cool

+ add 1%  $\text{NaNO}_2$   
(sodium nitrite)

+ Heat

Result → red

(showing presence of

tyrosine)

Imp. (2) Ring Biuret test :->

protein soln. in T.T +  
40% NaOH + 1% Copper sulphate  
over the surface of liquid  
(2 fluids do not mix)  
then rotate

Result -> Pink/violet ring formed  
at the junction of the  
two fluids.

cooling and reappears on cooling.

### C. COLOUR REACTIONS OF PROTEINS

Proteins show colour reactions which are due to the presence of constituent radicals in the complex protein molecule. Since different proteins contain different groups, all proteins do not give positive reaction with all colour experiments. Sometimes, non-proteins or prosthetic groups also respond to certain colour reactions and hence several tests must be done before drawing any conclusion. For colour reaction experiments prepare 5% egg white solution.

#### **Experiment (8) : Biuret reaction.**

**Procedure :** Take 3 cc of 5% protein solution in a test tube, add 1 cc of 40% NaOH solution to make it strongly alkaline, and then add 2 drops of 1% copper sulphate solution.

**Result :** Violet or pink colour appears. This reaction is due to the peptide linkage and so it is positive with all proteins.

#### **Experiment (9) : Ring biuret test.**

**Procedure :** Take 3 cc. of 5% or even more dilute egg white solution in a test tube, add 1 cc of 40% NaOH, then add by means of a pipette 1 cc of 1% copper sulphate over the surface of the liquid very gently so that the 2 fluids do not mix. Rotate gently.

**Result :** A pink or violet ring is formed at the junction of the two fluids. Proteoses and peptonoses give rose colour. Gelatin gives bluish pink or violet colour.

#### **Experiment (10) : Xanthoproteic reaction for tyrosine, phenylalanine and tryptophane.**

**Procedure :** Take 3 cc of 5% egg white solution in a test tube, add 1 cc of conc.  $\text{HNO}_3$  and boil. First white precipitate is formed which changes to yellow. The liquid also becomes yellow. Cool the test tube and add excess of 40% NaOH or ammonia to make alkaline.

**Result :** The yellow colour changes to orange. The proteoses and peptonoses do not form precipitate with  $\text{HNO}_3$  but their solution turns yellow to orange in  $\text{HNO}_3$  (nitric acid). The yellow colour is due to formation of metaproteins insoluble in  $\text{HNO}_3$ .



## Test on Protein

Colour reactions of Protein

① Biuret reaction :

⇒ Protein sol. in T. T. +  
40% NaOH (~~alkaline~~)  
(alkaline)

then 2 drops of 1% Copper sulphate  
soln.

Result ⇒ Violet / pink colour

[due to peptide linkage]

nitro-compounds from the protein molecule containing benzene ring. When made alkaline, the nitro-compounds ionize freely and produce deep yellow or orange colour.

**Experiment (11) : Millon's test for tyrosine.**

**Procedure :** In a test tube take 3 cc of 1% egg solution, and 2 cc of mercuric sulphate reagent by pipette and heat cautiously for a minute. A yellowish precipitate generally formed. Cool the tube and add 2 drops of 1%  $\text{NaNO}_2$  (sodium nitrite). Heat again.

**Result :** The solution and the precipitate become red showing the presence of tyrosine.

**Experiment (12) : Aldehyde test for tryptophane.**

**Procedure :** In a test tube take 3 cc of protein solution (5% egg white), then add one drop of 0.2% of 40% formalin, then add 0.5 cc of mercuric sulphate reagent. Shake well and then add 2.0 cc of conc.  $\text{H}_2\text{SO}_4$ . Shake.

**Result :** Violet or purple colour develops. Sometimes, a little heat is required for the colour to appear.

**Experiment (13) : Glyoxalic acid test for tryptophane.**

**Procedure :** In a test tube take 3 cc of 5% egg white solution for protein and add 3 cc of glyoxalic reagent. Now add this solution very carefully to another test tube containing 5 cc of conc.  $\text{H}_2\text{SO}_4$  in such a manner that the two fluids do not mix. Rotate the tube gently.

**Result :** Purplish violet colour develops at the junction of the fluids. The purple or violet colour is due to the presence of tryptophane, which forms condensation product with the aldehyde.

**Experiment (14) : Arginine test for arginine.**

**Procedure :** In a test tube take 3 cc of 5% egg white protein solution, then add 2 drops of 1%  $\alpha$ -naphthol solution, then add 1 cc of 40%  $\text{NaOH}$  solution, and then add 2 drops of sodium hypobromide ( $\text{NaOBr}$ ).

**Result :** Bright colour is obtained. This reaction is specifically meant for arginine which is present in all proteins.

**Experiment (15) : Sulphur test for cystine and cysteine.**

**Procedure :** In a test tube take 3 cc of protein solution (5% egg white), then add 5 drops of lead acetate which causes precipitation. Now add 40%  $\text{NaOH}$  drop by drop till the precipitate dissolves. Boil.

**Result :** Black or brown precipitate is formed, which shows the presence of cystine or cysteine group.

**Experiment (16) : Molisch's test for carbohydrate group attached to protein molecule.**

**Procedure :** In a test tube take 3 cc of 5% egg white protein solution, then add 2 drops of 5% alcoholic thymol, now incline the tube and gently add 3 cc of conc.  $\text{H}_2\text{SO}_4$  (the acid should go by the side of the tube wall) in such a way that the fluids do not mix. Rotate the tube gently.

**Result :** Purple-violet ring, at the junction of the fluids, is formed which shows the presence of carbohydrate group attached to the protein molecule.

**Experiment (17) : Ninhydrin test.**

**Procedure :** Take 1 cc of 5% egg white protein solution and add 4 drops of 0.1% ninhydrin solution and boil for one minute. Cool the test tube.

**Result :** Blue colour develops. The test gives positive results by all amino acids and their derivatives except proline and hydroxyproline.

Experiment  
3. Test  
From and  
color  
organic  
acids and  
and the

then add twice the amount of sodium hydroxide solution to the solids. Filter the solution and keep the filtrate in boiling water bath to examine.

## 2. Test on Proteins

Proteins, found in animals and plants, are important building blocks formed by amino acids, condensed together by peptide linkage. All proteins contain carbon, hydrogen, oxygen, nitrogen and with a few exceptions sulphur also. The alimentary composition of proteins consists of approximately C = 45-55%, H = 6-8%, O = 19-25% and N = 14-20%. Proteins have high molecular weight. They contain free amino and carboxyl groups and so they can combine with bases and acids depending upon the pH of the medium. On hydrolysis, proteins break into peptones, proteoses, peptides and amino acids. Chemical behaviour of the proteins is due to the amino acids in the protein molecules.

For experiments fresh 5% solution of egg white is prepared. The egg white is filtered through cotton. Dissolve 5 cc of egg white into 95 cc of distilled water for 5% egg white solution. Proteins show both precipitation and colour reactions.

### A. PRECIPITATION OF PROTEINS WITH HEAVY METALS

**Experiment (1) : Mercuric chloride test.**

**Procedure :** In a test tube take 3 cc of 5% egg white solution, then add mercuric chloride drop by drop.

**Result :** White turbidity is produced first which becomes thick and granular. The heavy metal salts precipitate protein solutions. This precipitate is generally soluble in excess of the salt solutions.

**Experiment (2) : Ferric chloride test**

**Procedure :** Take 3 cc of 5% egg white solution and add 0.5% ferric chloride solution drop by drop.

**Result :** On addition of first drop, turbidity appears and it increases on addition of subsequent drops. If  $\text{FeCl}_3$  is added in excess, the turbidity disappears.

### B. PRECIPITATION OF PROTEINS BY ALKALOID REAGENTS

**Experiment (3) : Sulphosalicylic acid test**

**Procedure :** In a test tube take 3 cc of 5% egg white solution and add 20% sulphosalicylic acid

**Result :** White precipitate is obtained.

