# DETECTION OF PROTEINS

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# REACTIONS FOR PROTEINS

## 1. GENERAL REACTIONS:

### i. BIURET REACTION:

This reaction is given by all substances containing *two or more peptide linkages* i.e. proteins and their hydrolytic products (proteoses and peptones). Dipeptides and amino acids do not give this reaction. The name "Biuret" was given to a compound which was produced after urea was heated at 180°C. This compound on reaction with dilute solution of copper sulphate gave a violet colour. Both biuret and peptides contain –CONH– (peptide linkages) and give positive biuret reaction, though biuret is not a protein in nature.

**PRINCIPLE:**

The peptide nitrogen atoms form a coordination complex with the cupric ions and a violet colour is produced.

**PROCEDURE:**

To 2ml of original solution (protein solution) in a test tube add 2 drops of 2% copper sulphate solution and 1ml of 5% sodium hydroxide solution. Mix thoroughly. A violet colour is produced with proteins, a bluish violet colour with gelatin, whereas peptones will give a pink colour.


Biuret
Harnstoff
Eiweiß

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ii. HEAT COAGULATION OF PROTEINS

Albumins, globulins and other proteins on heating undergo coagulation whereas gelatin, peptones and peptides do not coagulate on heating.

PROCEDURE:

Fill 2/3$^{rd}$ of the test tube with original solution (protein solution). Heat to boil. A white coagulum is formed.

Add few drops of 2% acetic acid to the boiled solution and note that the coagulum does not re-dissolve, but it persists.

**SEPARATION OF THE ALBUMIN AND GLOBULINS**


COAGULASE
TEST
POSITIVE
NEGATIVE

the coagulum does not re-dissolve, but it

## 2. SEPARATION OF THE ALBUMIN AND GLOBULINS

### i. AMMONIUM SULPHATE SATURATION TEST:

Half-saturation (50% saturation) with ammonium sulphate will result in precipitation of globulins, whereas albumins are precipitated after full-saturation (100% saturation) of the test sample with ammonium sulphate.

### a. 50% Ammonium Sulphate Saturation Test:

To 2ml of original solution (protein solution) add 2ml of saturated ammonium sulphate solution. Mix thoroughly. The solution is now half-saturated. A bulky precipitate of globulin is formed. Allow the precipitate to settle down and then filter through a filter paper into a clean and dry beaker. Filtrate if cloudy, should be re-filtered through the same filter paper, to get a clear filtrate. No precipitation will indicate the presence of peptones in the test sample.

b. 100% Ammonium Sulphate Saturation Test:

To 2ml filtrate from 50% ammonium sulphate saturation test, add ammonium sulphate crystals. Shake vigorously in order to dissolve crystals. Continue adding ammonium sulphate crystals to the filtrate until some crystals remain un-dissolved at the base of the test tube. The solution is now fully saturated and white precipitate of albumin is formed. Filter out the precipitated albumin. Absence of precipitation indicates the presence of peptones.

## 3. SEPARATION OF GELATIN

### MAGNESIUM SULPHATE SATURATION TEST:

To the precipitate from the 50% ammonium sulphate saturation test, add 2ml of distilled water and heat, the precipitate dissolves. Cool under tap water. Now saturate the solution in the test tube with magnesium sulphate crystals. A white precipitate is formed indicating the presence of gelatin.

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## B. TESTS FOR AMINO ACIDS

### 1. GENERAL REACTIONS OF AMINO ACIDS *(Free or Combined)*:

NINHYDRIN REACTION *(Triketo Hydrindene Hydrate)*:

Ninhydrin reaction is given by both free and combined α-amino acids.

PRINCIPLE:

Amino acids, on heating with ninhydrin, are oxidatively decarboxylated, producing carbon dioxide, ammonia ($NH_3$), and an aldehyde. Reduced ninhydrin then reacts with the liberated ammonia and a blue or purple coloured complex is produced.

PROCEDURE:

To 1ml of original solution (protein solution) in a test tube add 2 - 3 drops of freshly prepared 0.5% ninhydrin solution and heat to boil. A blue or purple colour is produced if proteins, peptides or amino acids are present.

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Characteristics colour of ninhydrin test

## 2. XANTHOPROTEIC REACTION *(Test For Benzoid Radicals)*:

### PRINCIPLE:

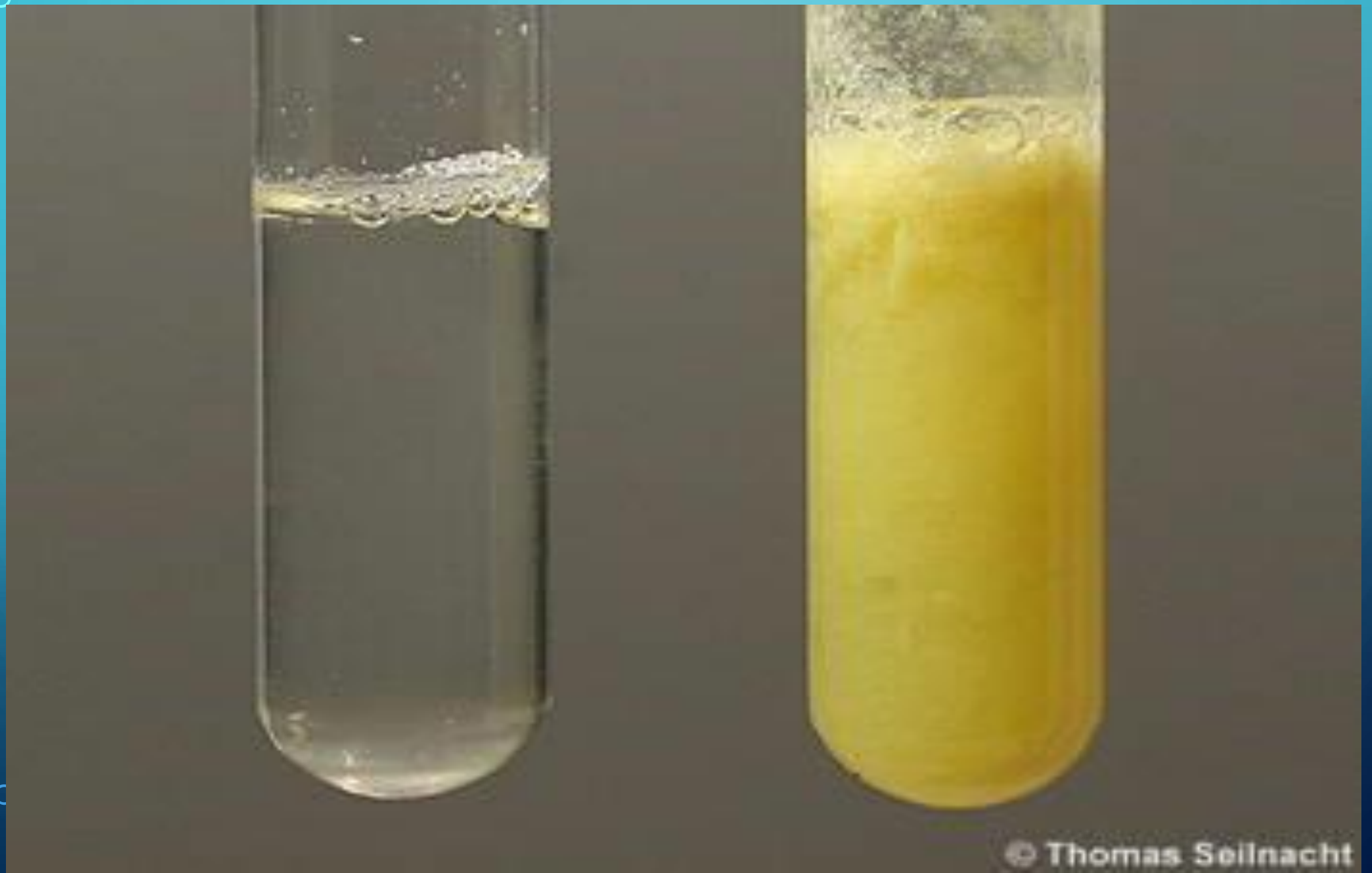
Nitration of benzoid radicals present in the amino acid side chain occurs due to reaction with nitric acid, giving the solution a yellow colouration.

### PROCEDURE:

To about 1ml of original solution (protein solution) in a test tube add 5 drops of concentrated nitric acid. A white precipitate is formed due to denaturation of proteins by nitric acid. Heat to boil for half minute. The colour of the precipitate turns yellow and then partially gets dissolved to give a yellow coloured solution. Cool under the tap water and add about 10 drops of strong aqueous ammonia, or sodium hydroxide. The yellow colour is intensified and changes to orange.

Yellow colour solution indicates the presence of aromatic amino acids (Tyrosine, Tryptophan and Phenylalanine) in the protein.

Phenylalanine like other amino acids contain benzene ring, but nitration of its benzene ring with nitric acid occurs with a great difficulty, and the process of nitration can not be performed ordinarily by this method.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## 3. MILLON'S REACTION *(Test For Hydroxybenzene Radicals)*:

Amino acid Tyrosine (Hydroxyphenylalanine) and other phenolic compounds give this reaction.

### MILLON'S REAGENT:

Mixture of mercurous and mercuric nitrates.

### PRINCIPLE:

The mercurous and mercuric nitrate reacts with the hydroxybenzene radicals (Phenols) forming a red coloured compound.

### PROCEDURE:

To 2ml of original solution (protein solution) in a test tube add a few drops of the Millon's reagent. Boil gently for half a minute. The solution will turn red or a red precipitate will be formed.

The proteins, on the addition of Millon's reagent, form a white precipitate first due to denaturation of proteins by mercury salts, which upon heating turn red. Tyrosine is the only amino acid which gives this reaction.

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Characteristics colour of Millon's test

## 4. CYSTEINE TEST *(Test For Sulphur)*:

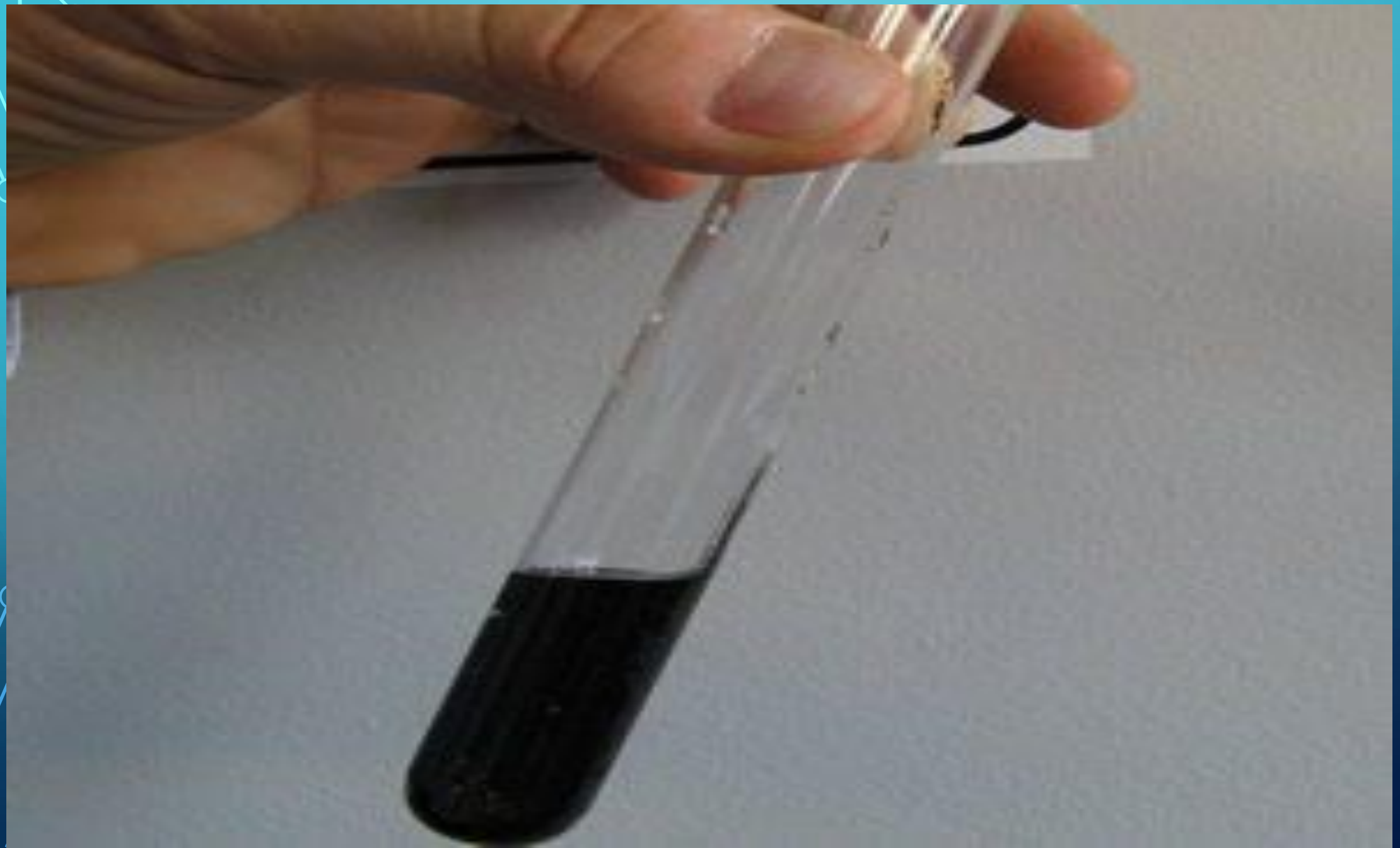
### PRINCIPLE:

Protein or amino acids when heated with sodium hydroxide, sulphur splits out as sodium sulphide, which on reaction with lead acetate, forms greyish brown to black precipitate of lead sulphide (PbS).

### PROCEDURE:

To 1ml of original solution (protein solution) in a test tube add 1ml of 20% sodium hydroxide and 0.5ml of 2% lead acetate. A white precipitate is obtained due to denaturation of proteins. Now boil the mixture. The white precipitate turns grayish brown or black indicating the presence of sulphur.

A positive reaction is due to the presence of a sulphur containing amino acid in the protein. This can be cysteine or methionine.



## 5. HOPKINS-COLE REACTION *(Test For Tryptophan)*:

### HOPKINS-COLE REAGENT:

Glyoxalic acid.

### PROCEDURE:

To 1ml of original solution (protein solution) in a test tube add 1ml of Hopkins-Cole reagent. Mix thoroughly and add 1ml of concentrated sulphuric acid, pouring it down along the side of the test tube. A deep violet or purple ring forms at the junction of the two liquids. This indicates the presence of tryptophan.

Gelatin and other proteins which do not contain tryptophan do not give this reaction.

## 7. TEST FOR PHOSPHATE

### PROCEDURE:

To 1ml of original solution (protein solution) in a test tube add 1ml of ammonium molybdate reagent. Heat to boil. A yellow precipitate is formed. This confirms the presence of phosphate.

Casein and other phosphoproteins give this test positive.

# Thank You