



Assistant Lecturer. Zuhair A. ALrawi

***College of Pharmacy
third level***



practical lab. (3) (proteins)

proteins

- They are complex organic materials with high molecular weights consisting of basic elements: (**H, N, O, C**) and also contain: (**Fe, S, P, Zn**).
- Proteins are the main component of both animal and plant tissues and cells in terms of structure and function. Where protein is the main component of the human body, as it represents about 20% of the body weight, as muscles, connective tissues, bones, blood, skin, nails, hormones and enzymes are all based on protein.

Definition of proteins:

- Compounds of high (large) molecular weights that contain **amino acids** as structural units linked together by peptide bonds.

Classification of proteins

- **Classified depending on composition :-**



(Simple Proteins)



(Conjugate proteins)

- **Classified depending on shape:-**

(Globular proteins)

(Fibrous proteins)

1-Simple Proteins

- Its types are classified on the basis of their **solubility**, include the following types:-
 - **Protamins**
 - **Albumins**
 - **Globulis**
 - **Prolamines**
 - **Scleroproteins**
 - **Keratin**
 - **Collagen**
 - **Elastin**

2- Conjugate Proteins

- They are proteins attached to non-protein substances such as sugars, fats and minerals.
- **Includes the following types :-**
- **Phosphoproteins**
- **Glycoproteins**
- **Chromoproteins such as Hemoglobin, chlorophyll**
- **Lipoproteins**
- **Nucleoproteins**

Classified depending on shape:-

- **Globular proteins:-**

- They are **spherical** or **oval-shaped** proteins that dissolve easily in water and are characterized by their abundance of wrapping, forming **spherical shapes** such as **albumin**, **globulin** and **protamine**.

- **Fibrous proteins:-**

- They are **long** or **needle-shaped** proteins and are insoluble in water and resist the action of enzymes that break down proteins. **They are of three types: keratin, collagen and elastin.**

The biological and physiological functions of proteins

- The body's need to grow and build body tissues.
- A source of energy.
- Repair, replace and build body tissues.
- Maintains the balance of acidity and base in the body.
- It is involved in a number of biologically important compounds, such as **enzymes**, a number of **hormones** and **antibodies**.
- Proteins form the structural basis of chromosomes.
- Maintaining the water balance in the body.

Amino acids

- It is the smallest building block in protein synthesis.
- They are organic acids that contain groups (**amine and carboxyl**).
- **Characteristics of amino acids:-**
 - Dissolve in **water**.
 - Does not dissolve with **ether**.
 - Little soluble with **alcohol**.
 - Solid compounds with high melting points.

Classification of amino acids

- **Aliphatic:** Glycine, Alanine, Valine, Leucine, Isoleucine, Serine, Threonine.
- **Aromatics:** Phenylalanine, Tyrosine.
- **Sulfur containing:** Cysteine, Cystine, Methionine .
- **Heterocyclic :** Proline, Histidine, Hydroxyproline.

Experiments

1- Biuret reaction :

This test is general for proteins.

It is **positive** for all proteins and peptides having **two** or **more** peptide bonds.



Experiments

Biuret reaction :

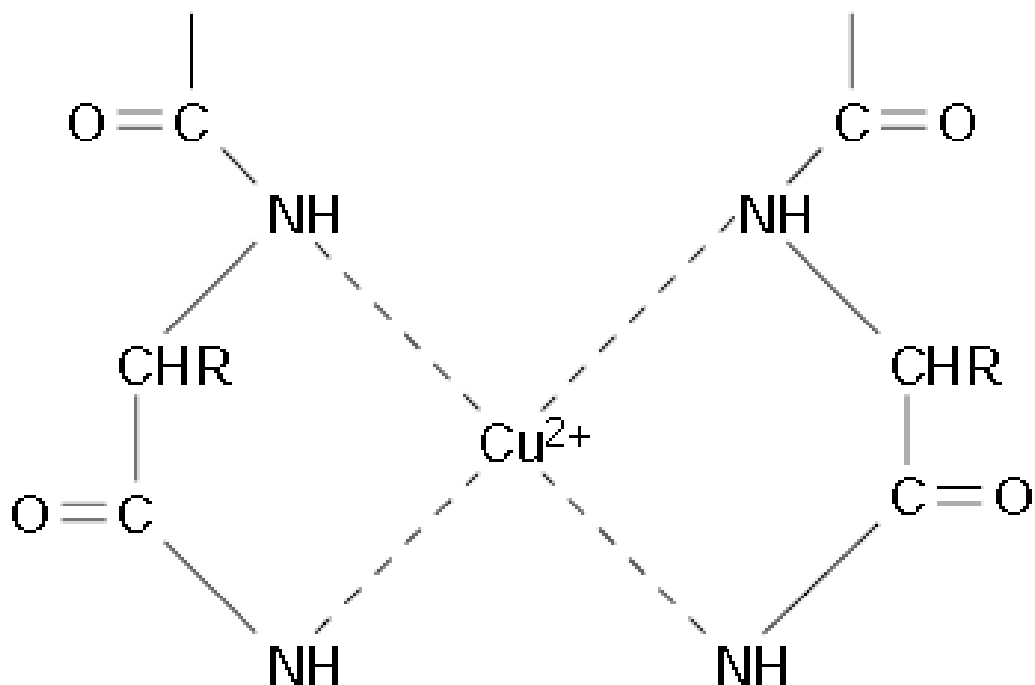
Principle:

This reaction depends on the protein that gives **blue** to **violet color** with a solution of copper sulfate in **alkaline medium**. Finally, a **coordination complex** is formed between the copper ions and **amino groups** in the peptide bonds.



Experiments

Biuret reaction :



Copper coordination complex



Experiments

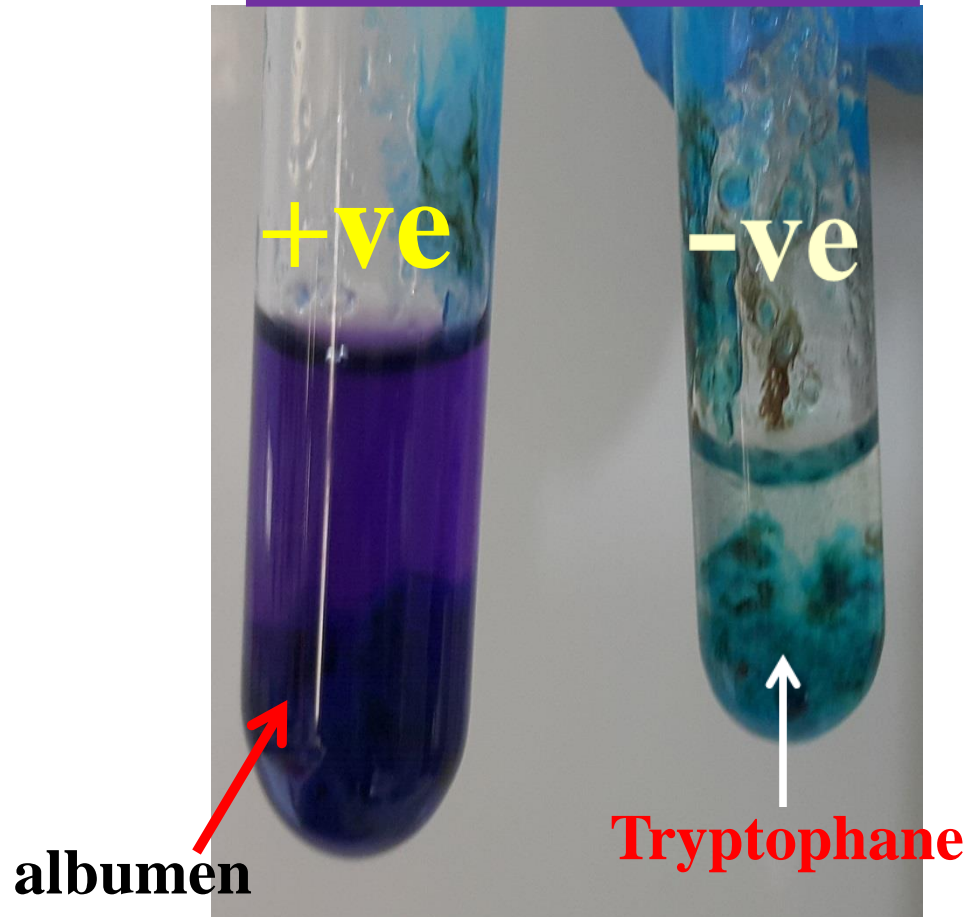
Biuret reaction :

Method:

- put 2ml protein solution (**Albumen**) into a test tube + 2ml 10% **NaOH** solution
- mix well
- add 2-8 drops of 10% **CuSO₄ solution** and mix
- **blue to violet colour** appears. It indicates the presence of **protein**

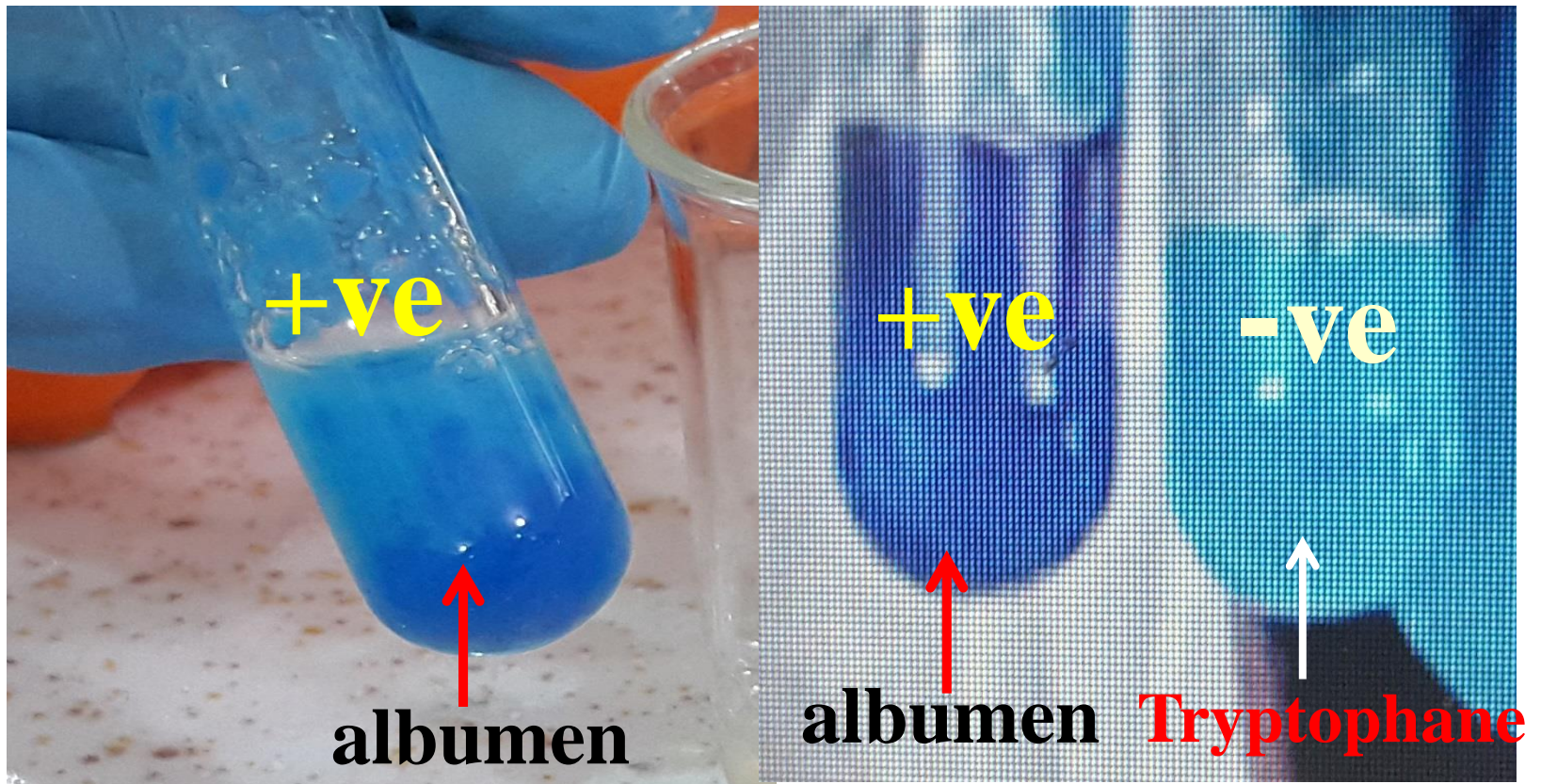
Experiments

Biuret reaction :



Experiments

Biuret reaction





Experiments

2- Ninhydrin reaction:

This reaction is used to detect the free amino group in peptide compounds and amino acids.

Principle:

- When a solution of ninhydrin is heated with the amino acid, which is considered a reducing agent, the amino acid is oxidized by removing the amino group from it by means of hydrindantine, and the ammonia is released and condensed with hydrindantine, forming a purple compound.



Experiments

Ninhydrin reaction:

Method:

- put 1 ml of **tryptophan amino acid** into a test tube+ 2 drops of a 0.2% **ninhydrin solution**.
- heat the mixture in a boiling Water Bath for (2-5min).
- **A violet color is produced.**

Experiments

Ninhydrin reaction





Experiments

3- Millon's reaction:

This test is for the amino acid **tyrosine**.

Principle:

The amino acid **tyrosine**, which is free or contained within the protein structure, reacts with **Millon's reagent**, forming the complex compound of mercury salts of di-nitro-tyrosine of **red-scarlet color**.



Experiments

Millon's reaction:

Method:

- 2ml **tyrosine** solution + 6drops of **Millon's reagent** .
- heat the mixture in a boiling Water Bath for (2-5min).
- **red-scarlet color** is appear.

Experiments

Tyrosine amino acid
(red-scarlet color)



Millon's reaction

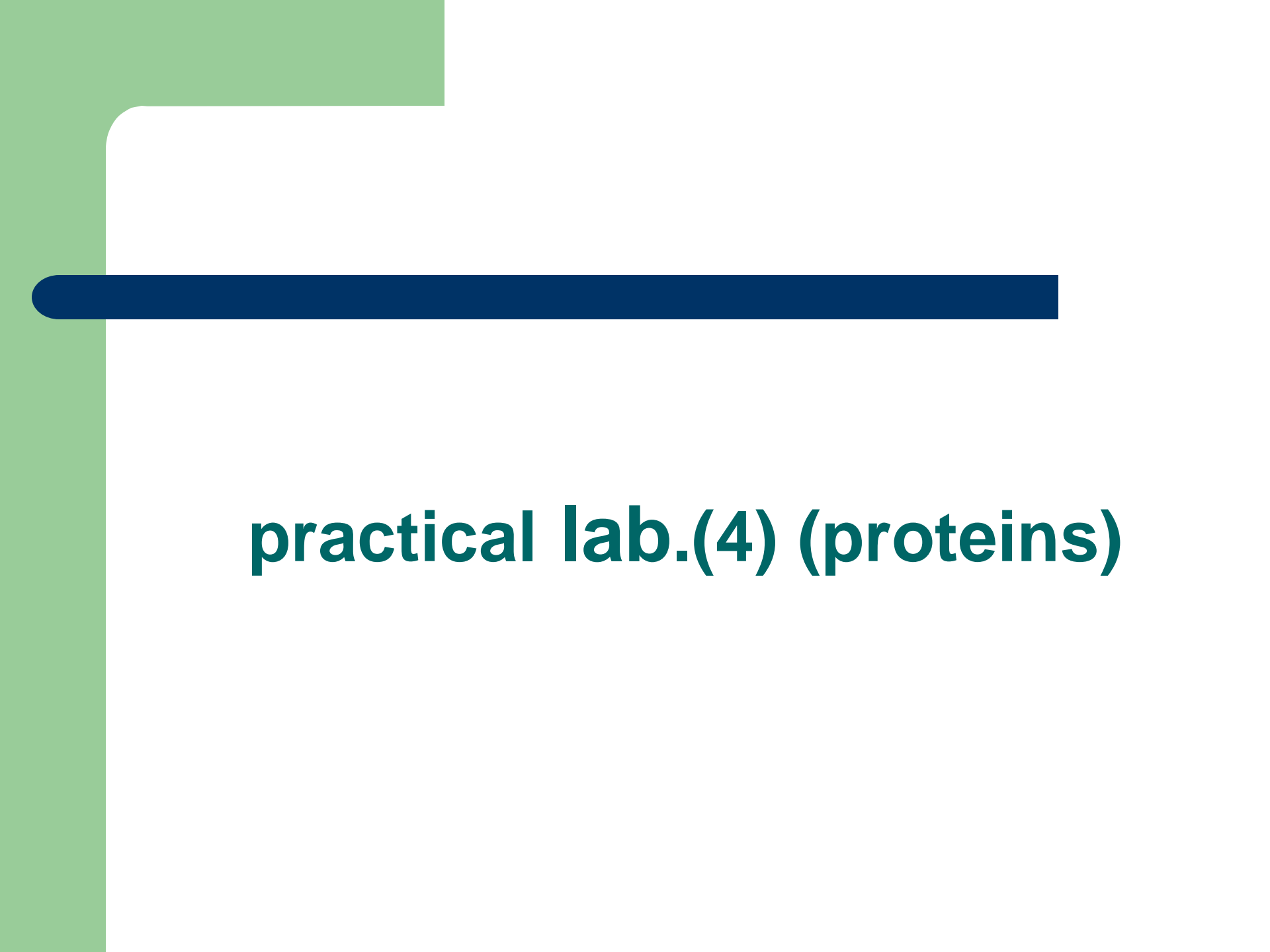


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practical lab.(4) (proteins)



Experiments

4- Nitroprusside reaction:

This test is for **amino acids** that contain a free **group of thiols (-SH)** as those found in the amino acid **cysteine**.

Principle:

The test is based on the detection of free **sulfur atoms** in an alkaline solution due to the decomposition of the SH-group. The sulfur then reacts with the nitroprusside ion to form a **Deep pink complex**.



Experiments

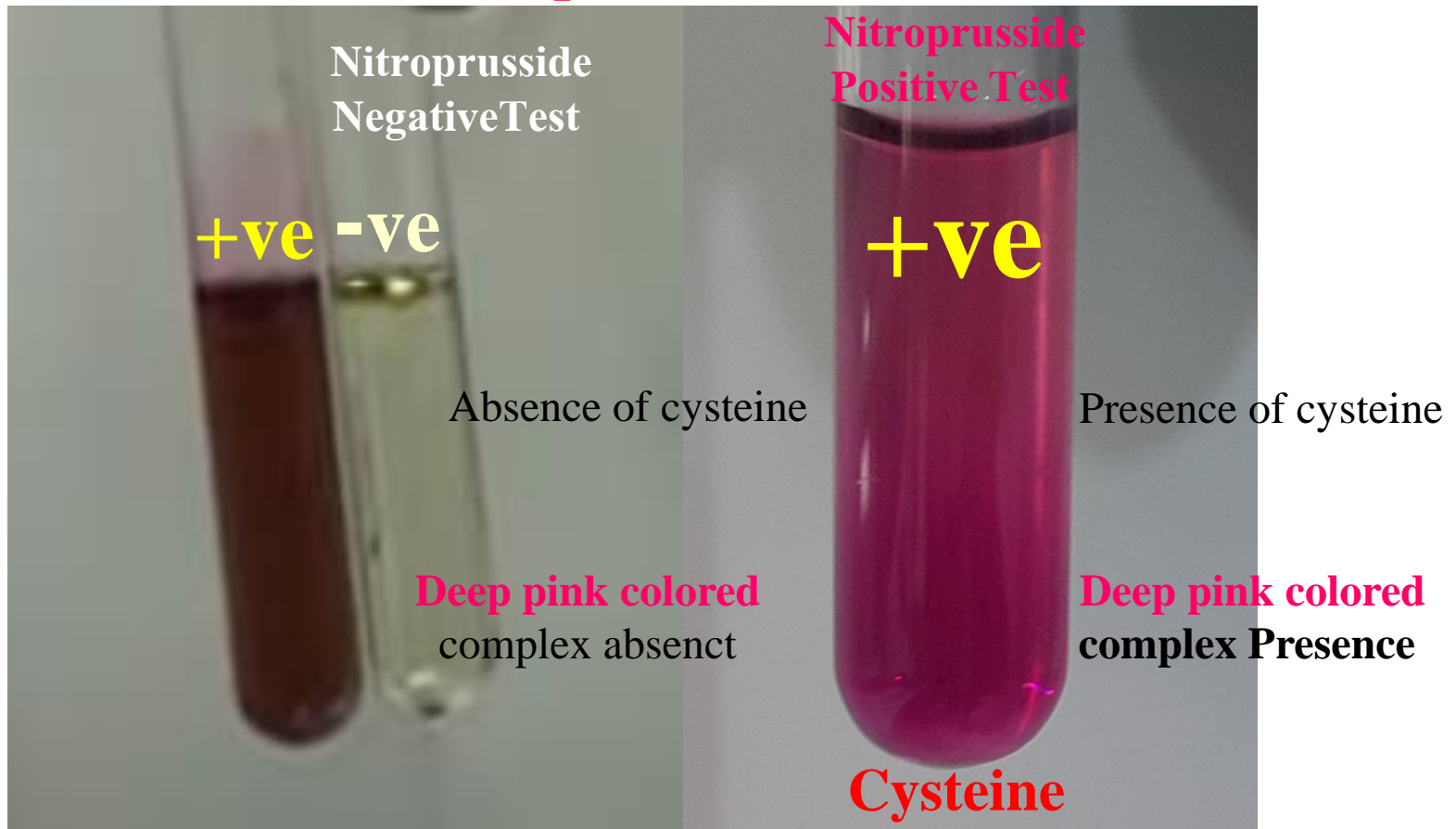
Nitroprusside reaction:

Procedure:-

- put 2 ml of a **Cysteine amino acid** into a test tube + 5 drops of **sodium nitroprusside solution** and mix well.
- Add 2 ml of ammonium hydroxide solution(**NH₄OH**).
- The appearance of a **Deep pink complex** indicates the presence of a thiol group.

Experiments

Nitroprusside reaction





Experiments

5- Rosenheim reaction:

this test is specific for **indole group** the only **amino acid** which containing **indole group** is **tryptophan**, so this test is specific for **tryptophan**.

Principle:

The indole rings of **tryptophan** react with formaldehyde in the presence of strong acid (rosenheim reagent) to form **Red-violet compounds** named **carboline**.



Experiments

Rosenheim reaction:

The rosenheim reagent only react with protein that contain **tryptophan**, the protein is hydrolysed by concentrated **sulphuric acid(H₂SO₄)** and the **tryptophan** to be free, so it react with formaldehyde to form **Red- violet compound**.

Protein + H₂SO₄_____ **tryptophan** + other A.A

Tryptophan + Formaldehyde H₂SO₄ **Red-Violet product**

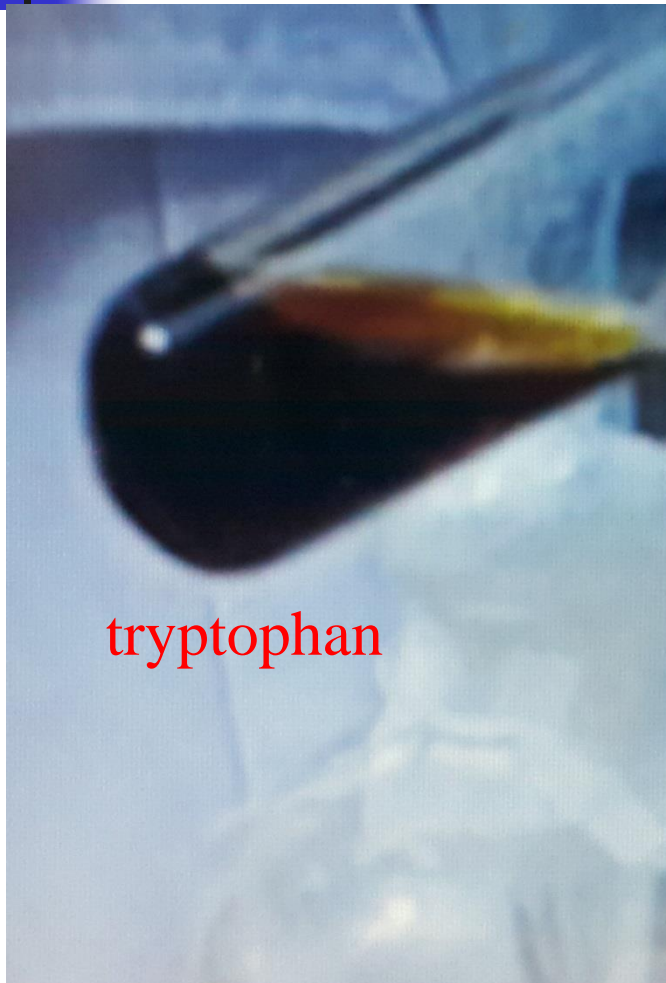


Rosenheim reaction:

Method:

- Add 2ml of the **test solution(tryptophan)** to a clean test tube.
- Add 10 drop of **FeCl₃** shaking more quickly and add 10 drops of **formaldehyde**.
- Mix well.
- Add 2ml of Concentrated **H₂SO₄** drop by drop to produce **Red to violet colour**.

Rosenheim reaction





Rosenheim Test



**Negative
Acree-Rosenheim
Test**

Tryptophan Absent

**No Purple ring
present**



**Positive
Acree-Rosenheim
Test**

Tryptophan Present

**Purple ring
present**



6- Test for cystein and cysteine:

Cysteine is a non-essential sulfur-containing amino acid in humans, related to cystine.

- Cysteine is important for protein synthesis and collagen production .
- detoxification, and diverse metabolic functions.
- Cysteine is a component of the **antioxidant glutathione**.



Test for cystein and cysteine:

Cystine is a sulfur-containing amino acid obtained by the oxidation of two cysteine molecules which are then linked via a **disulfide** bond.

Principle:

This will convert the organically combined sulphur of **cystein** and **cysteine** into **sodium sulphide**. When add a drop of **lead acetate** solution, **black** or **brown** colour appears due to the formation pf **lead sulphate**.

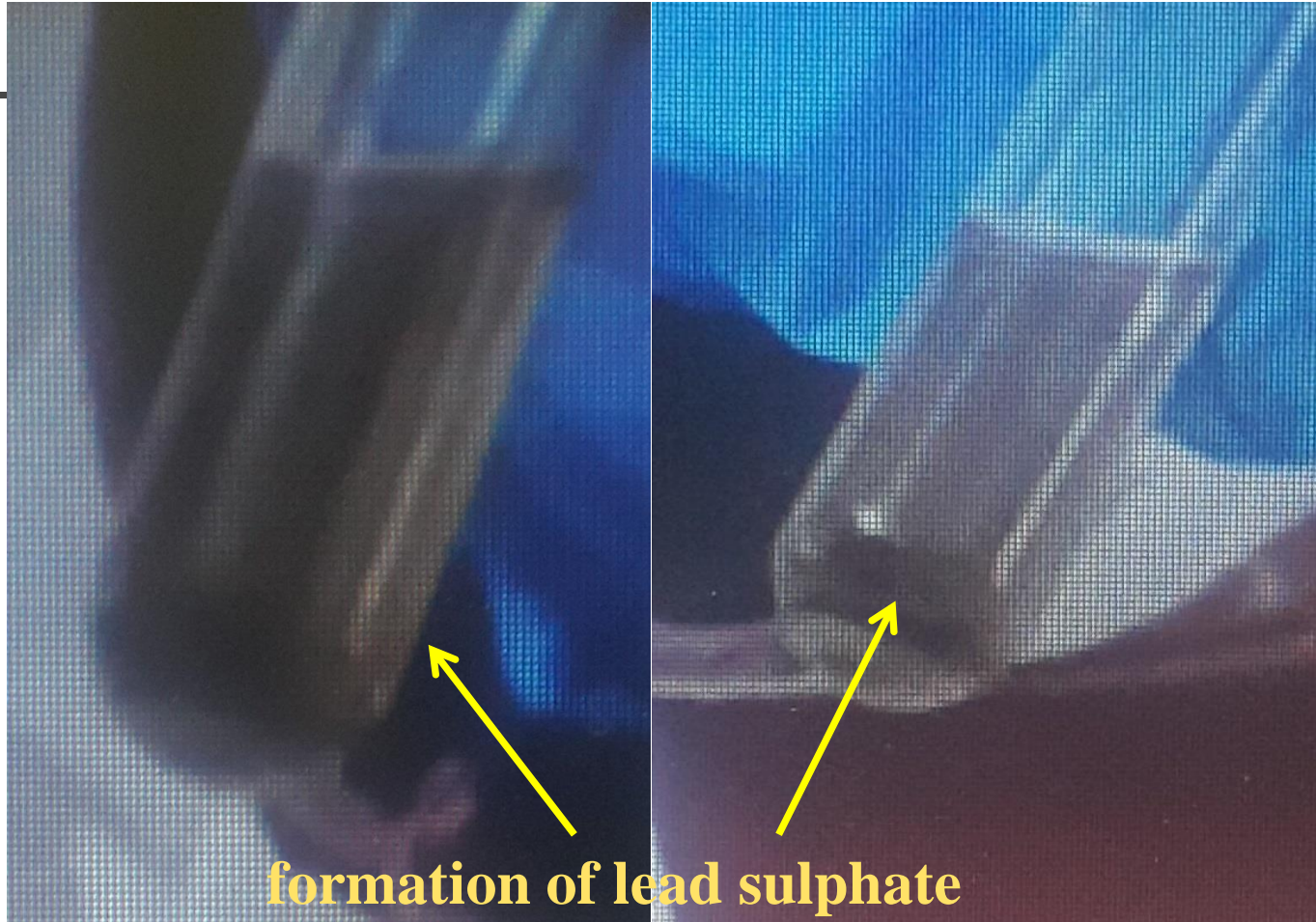


Test for cystein and cysteine:

Method:-

- 1ml protein solution + 1ml of 40% NaOH and boil for not less than one minute.
- add a drop of lead acetate solution.
- formation black or brown colour of lead sulphate.

Test for cystein and cysteine



Cystein & cystine + 40%NaOH $\xrightarrow{\text{boil}}$ Na₂S $\xrightarrow{\text{Lead acetate}}$ PbS



7- Xanthoproteic Reaction :

This test is general for proteins, because it is positive with aromatic amino acids (**aromatic groups**) that contain a benzene ring in their composition, as protein is not devoid of those acids.



Xanthoproteic Reaction :

Principle:

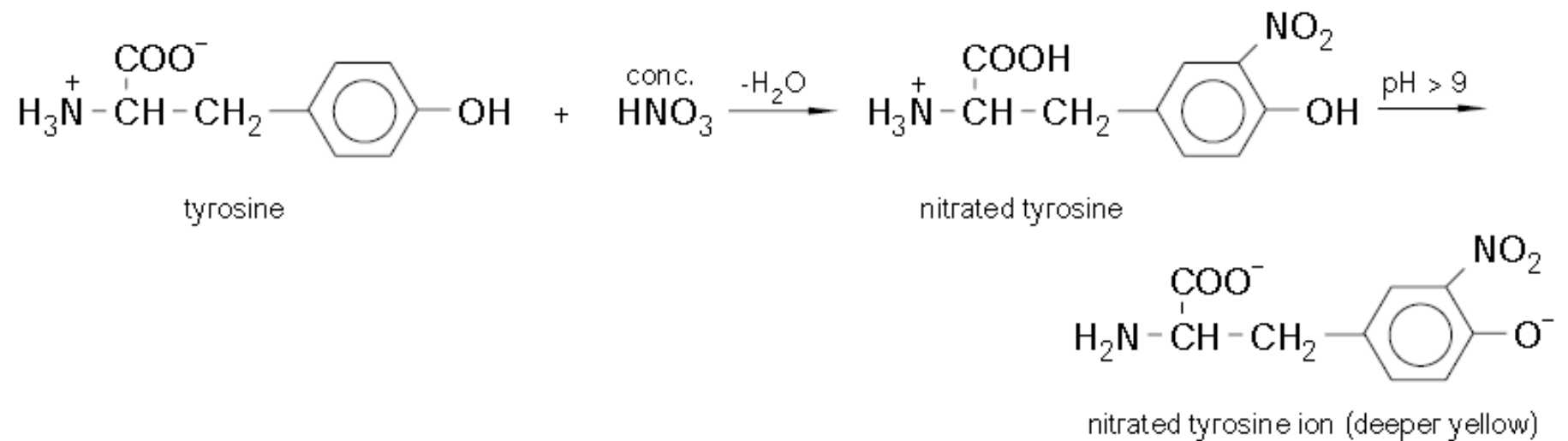
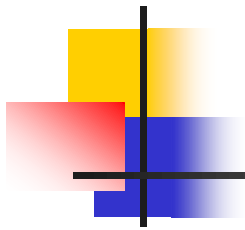
- This detector is based on the presence of benzene compounds, when **heated** with **conc. HNO₃**, the nitration process of the benzene ring in the amino acid occurs, to give a **yellow precipitate**, and because the resulting nitro compounds are yellow in color, so the detection is called xanthoproteic. (Yellow = xantho). When alkali is added to these nitro-derived salts, the color changes from **yellow** to **orange**.



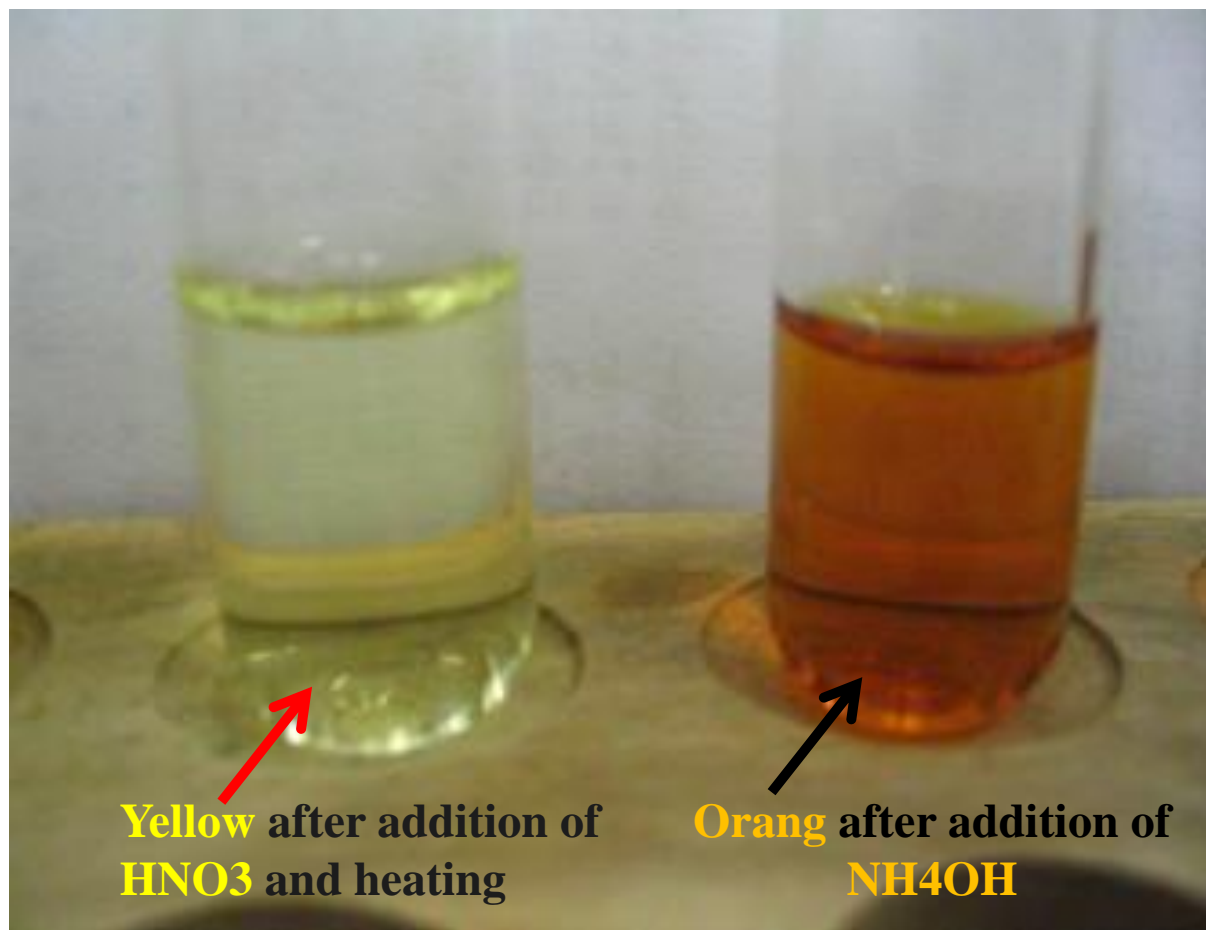
Xanthoproteic Reaction :

Procedures:-

- Take 2ml of **tryptophan amino acid** solution in dry test tube + 1ml of con. **Nitric acid (HNO₃)** and mix well.
- The mixture was heated in a boiling water bath for (1-2 minutes). A **yellow precipitate** is formed, then left to cool.
- add 10 drops of Con. **NH₄OH or NaOH** for test tube, become **orange**.



Xanthoproteic Reaction





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