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***College of Pharmacy
third level***



practical lab.(1) (Carbohydrates)

Carbohydrates

- Carbohydrates are an important components of the nutrition, because they are easy to digest compared to other nutrients such as fats and proteins.

Carbohydrates

- **Sugars have two sources:-**
- **A- A plant source** that is involved in the synthesis of the cell walls of a plant
- **B- The animal source** is found in (blood, urine, milk) concentrated in the form of a multiple sugar called glycogen, which is found mainly in the liver and muscles.

Carbohydrates

- It means carbon water



(Carbo)



(Hydrate)

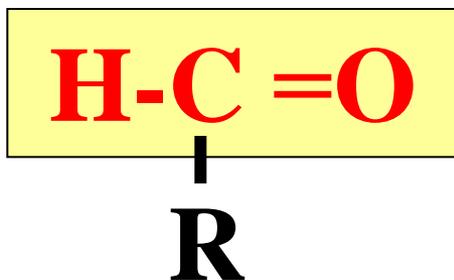
- Compounds that contain in their composition carbon, hydrogen and oxygen in a ratio of 1:2

Definition of carbohydrates

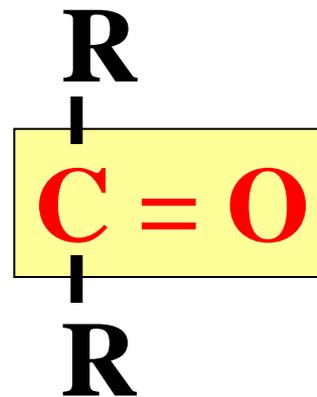


- They are organic compounds aldehydes or polyhydric ketones,
- Hydrolysis, give aldehydes or polyhydric ketones.

Aldehyde and ketone

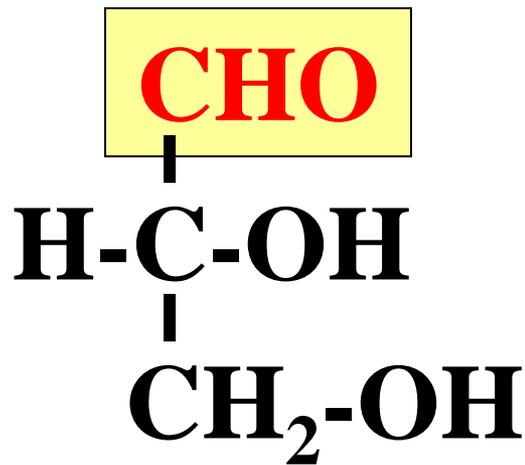


Aldehyde

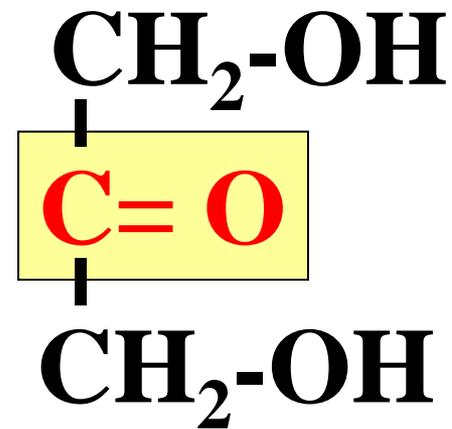


Ketone

Polysaccharides exist in the form of aldehyde or polyhydric ketone



جليسرالدهيد
(ألدوترايوز)
Aldotriose



ثنائي هيدروكسي أسيتون
(كيتوترايوز)
Ketotriose

Classification of carbohydrates

Disaccharides

2
Molecule of
monosaccharides

Polysaccharides

More than 10
Molecule of
monosaccharides

Monosaccharides

8-3
Corn carbon

Oligosaccharides

10-3
Molecule of
monosaccharides

The importance of carbohydrates

- Carbohydrates are one of the primary sources of energy, especially the brain and nervous system.
- Some types of sugars are involved in the structure of cells and tissues of plants and animals.
- A source for synthesis of a large number of important organic compounds such as nucleic acids.
- Heparin is a polysaccharide and anti-clotting agent.
- It is involved in many industries such as the textile industries based on cotton cellulose fibers.

Monosaccharides

- **General composition (CH₂O)_n**

They are simple sugars that cannot be broken down into simpler substances such as glucose, fructose, and galactose.

- **Divide based on the number of carbon into:**

Tri – Tetr – Pent – Hex – Hept – Oct -

- **Divide according to the presence of the effective group (aldehyde or ketone)**

The most important mono sugar

- **sugar (hexose)** glucose, fructose, galactose
- **sugar (pentose)** sugar Raibose -----
RNA
- **sugars (triose)** glyceraldehyde,
dihydroxyacetone ----- metabolism
intermediates



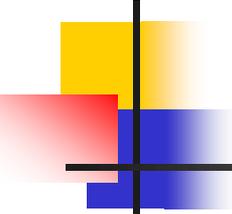
Monosaccharides

- **include:**
- **A. Glucose:**
- It is the simplest type of carbohydrate and is called blood sugar.
- it is in the form of natural sugar in food or the body can provide it through the digestion of complex carbohydrates such as the starches found in rice, pasta and potatoes.



Monosaccharides

- **B. Fructose:**
 - It is found in fruits and honey. It is the sweetest type of sugars and starches in terms of taste.
 - combines with **glucose** to form **sucrose**.
- **C. Galactose:**
 - combines with **glucose** to form **lactose**, "milk sugar"



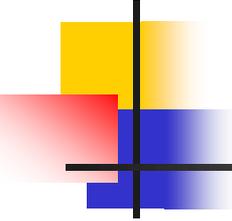
Experiments

1- Molisch Test :

It is a general test for CHO .

Principle:-

- The sulfuric acid (H_2SO_4) strips 3 water molecules to form the furfural compound and binds with the alpha-naphthol to a **violet ring**.

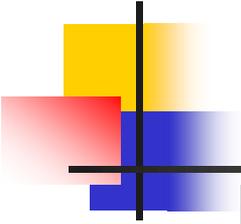


Experiments

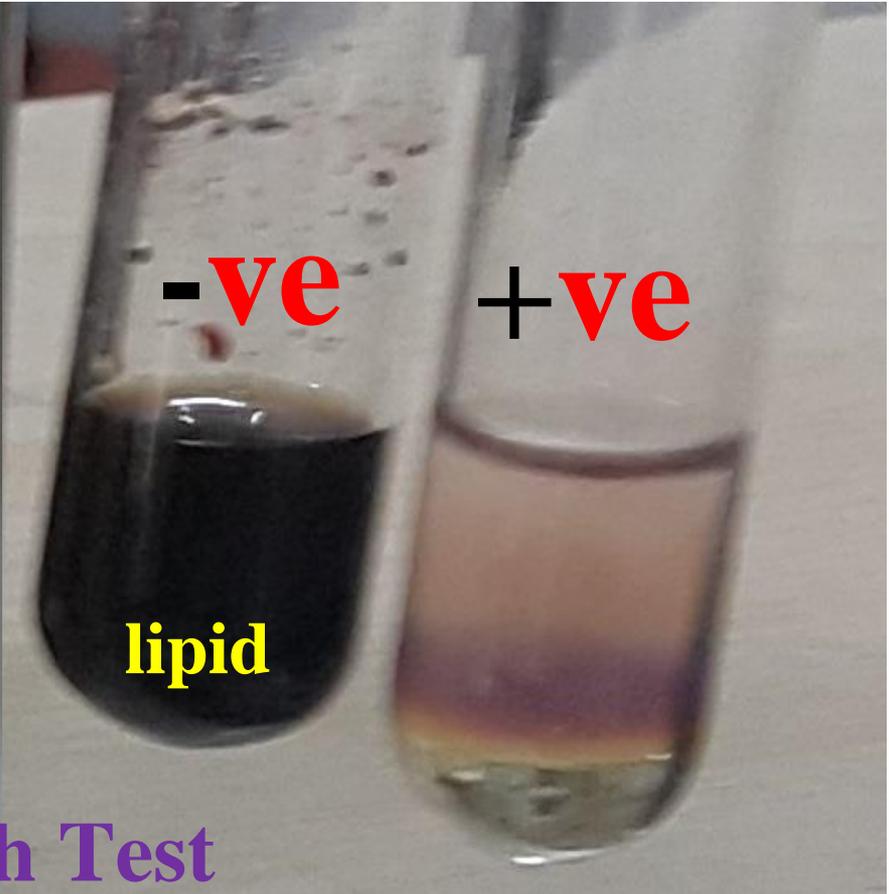
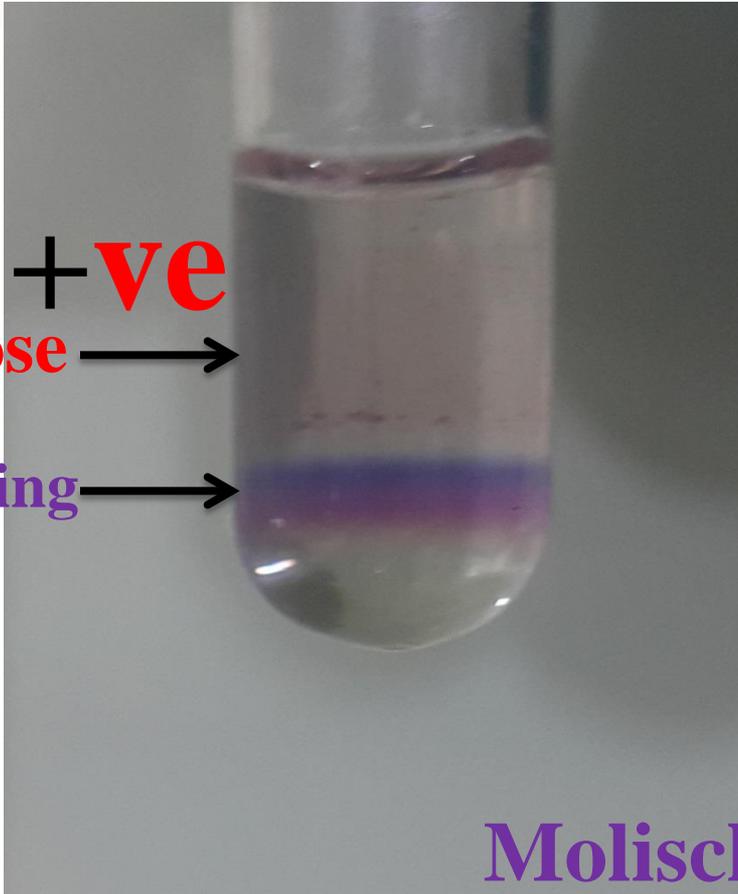
Molisch Test :

Method:-

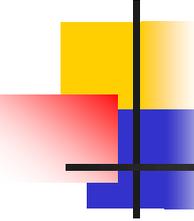
- 1ml test solution + 2 drops of α -naphthol
- mix well
- add 2-3 ml of conc. H_2SO_4
- **violet ring** appears at the junction of two layers.



+ve
Glucose →
violet ring →



Molisch Test

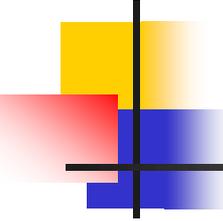


2- Benedict Test:

This test is to differentiate between reducing mono, disaccharides and non-reducing disaccharides.

Principle:-

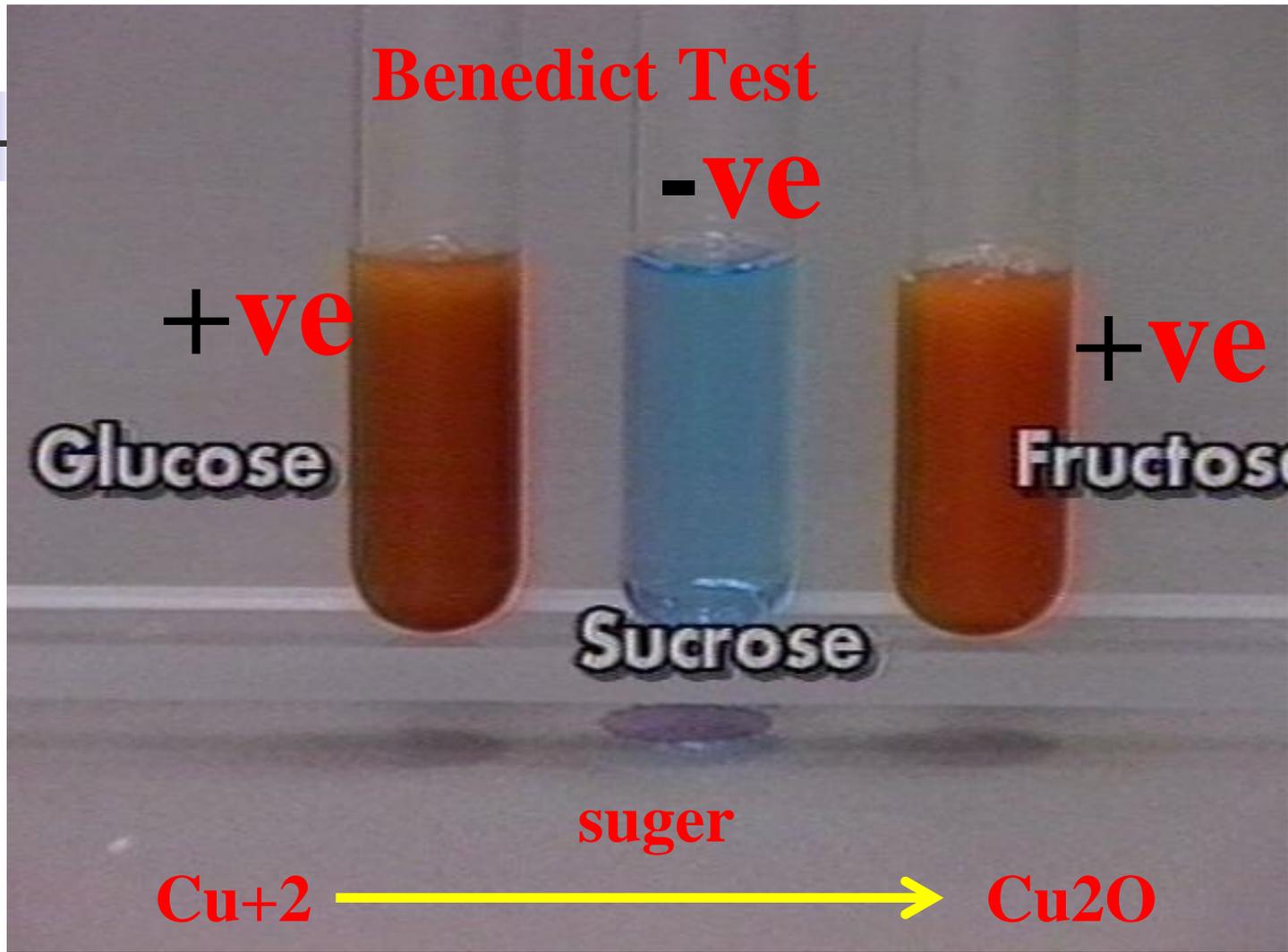
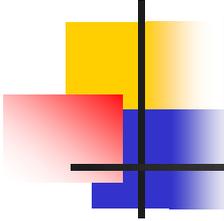
It is based on the **reduction** of copper ions to **copper oxide** in a hot **alkaline medium**.



Benedict Test:

Method:

- 1ml test solution + 1ml Benedict's reagent
- heat the mixture in Boiling Water Bath for (3min)
- **Reddish brown ppt.**



Benedict Test

-ve

+ve

Glucose

+ve

Fructose

Sucrose

suger

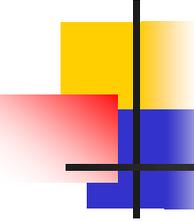
Cu⁺²



Cu₂O

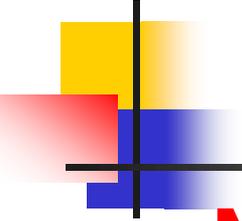
(in hot alkaline medium)

red ppt



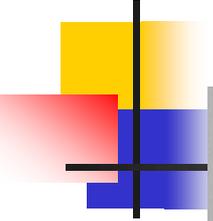
3- Barfoed's Test:

This test is used to distinguish between monosaccharides and disaccharides, since the monosaccharide reduces the Cu^{+2} ion faster than disaccharide in the hot **acidic medium**.



Method:

- 1ml of the solution to be tested +2ml of Barfoed's reagent.
- test tubes into a boiling water bath and heat for 2 minutes.
- Remove the tubes from the bath and allow to cool.
- Formation of a **red precipitate of Cu_2O** .



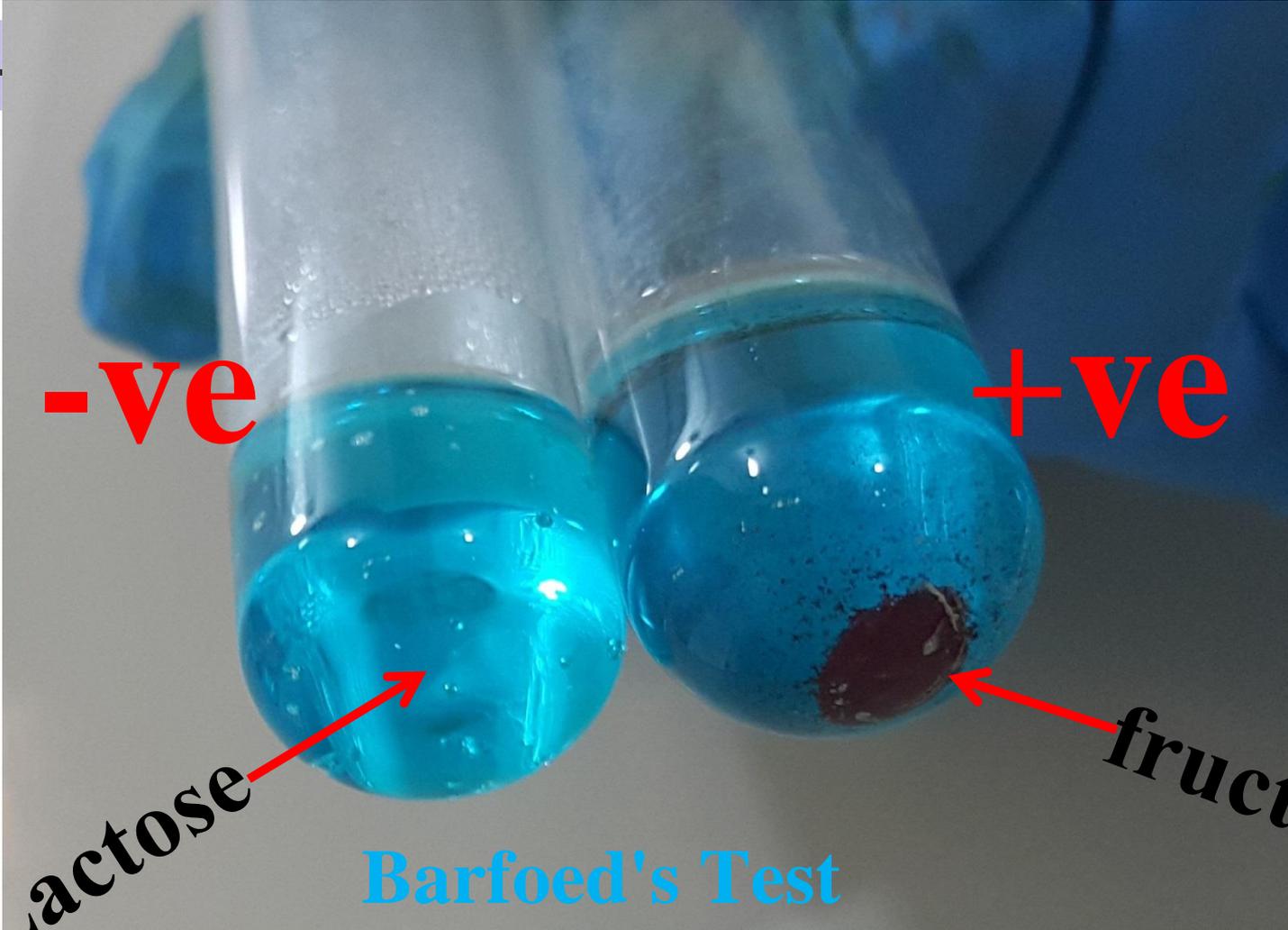
-ve

+ve

Lactose

fructose

Barfoed's Test



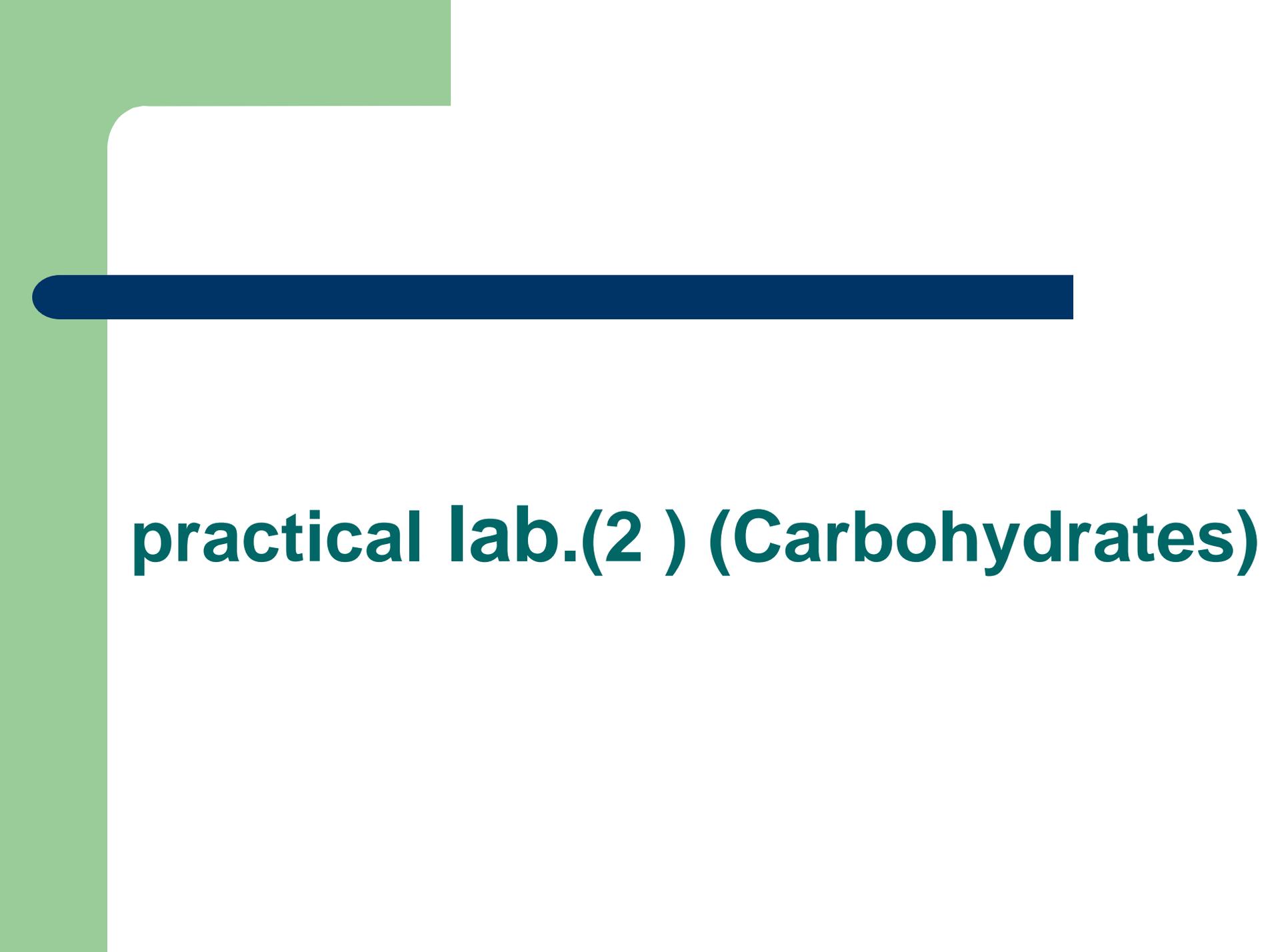
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practical lab.(2) (Carbohydrates)

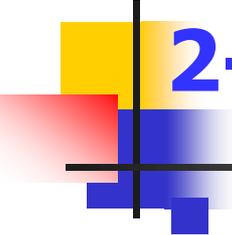
Disaccharides

1-Maltose: (malt sugar)

It is the simplest reducing disaccharide consisting of :-

glucose + glucose

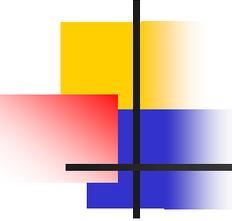
- found in barley or in saliva and pancreatic juice.



2-Lactose:(milk sugar)

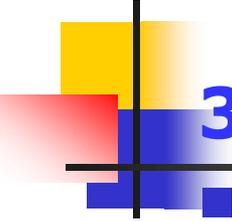
It is a disaccharide in nature and is known as milk sugar because it is present in milk only.

- It consists of two molecules:
glucose + galactose
- it is also a reducing sugar.



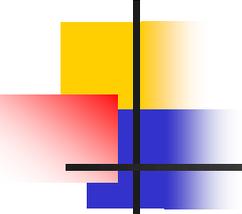
Lactose

- It is possible for lactose to be present in the urine of a woman during pregnancy, and that its lack of absorption in the intestine can cause diarrhea.



3-Sucrose : (cane sugar, table sugar)

- It is one of the most important disaccharides present in nature.
- It consists of two parts
glucose + fructose
- it is a non-reducing sugar because the Link between both groups is active (the aldehyde group in glucose and the ketone group in fructose called dextrose and is found in sugar cane and beet.



Poly saccharides

■ Starch:

- It is a multiple sugar found in the plant.
- It consists of two main:
 - A-** non-branching glucose chains called amylose.
 - B-** Branching chains called amylopectin

■ Glycogen:

- It is called animal starch. It is similar to vegetable starch but is more branched. It stores in the liver and muscles.

Experiments

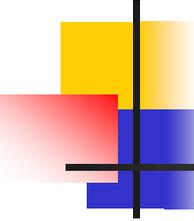
4- Seliwanoff's Test:

This test is used to distinguish between the aldoses and ketoses due to the presence of the **ketone group**

Principle:-

Ketone sugars differ from aldehyde sugars in that they lose water and form **furfural** more easily. When **fructose sugar** is heated with **HCl**, furfural is formed and condensed with **resorcinol** to form a **red complex**.

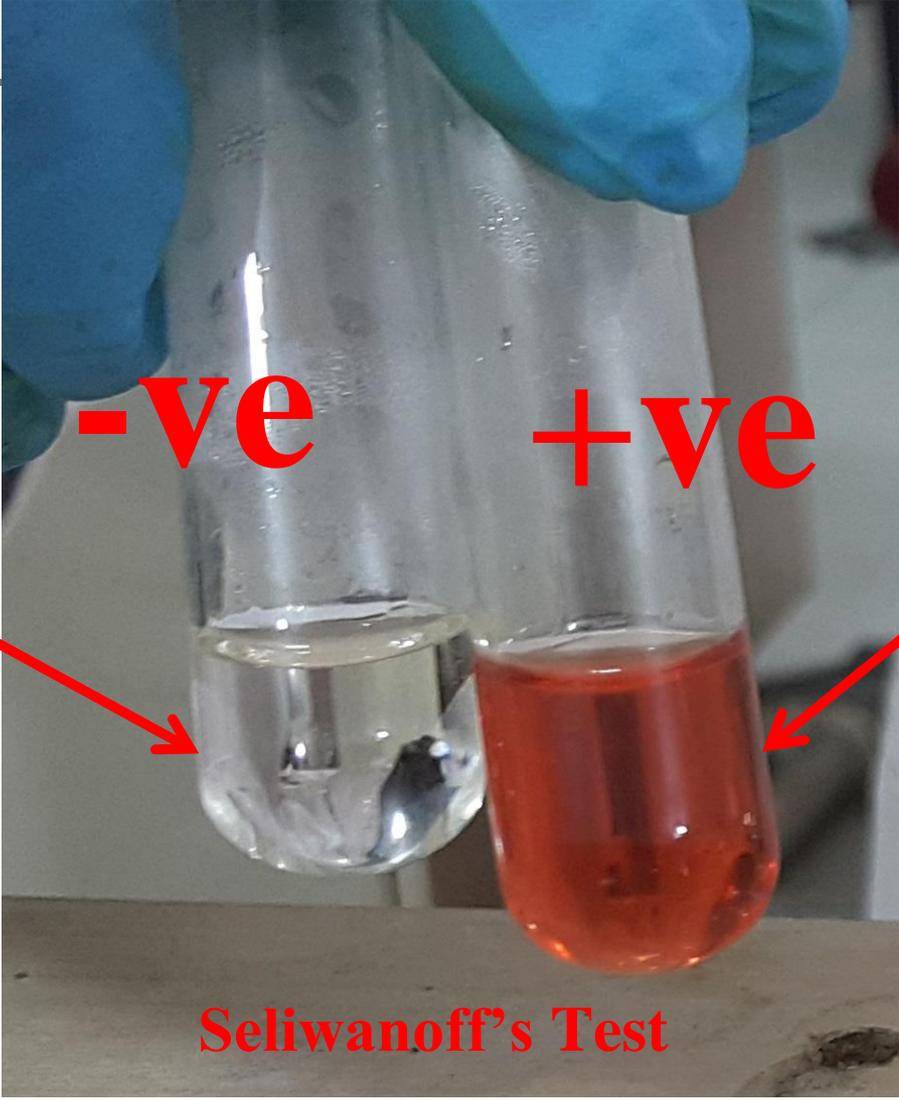
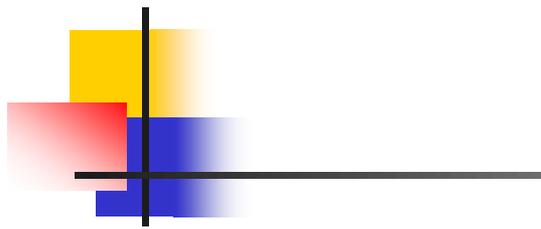
Experiments



Seliwanoff's Test:

Method:

- 1ml of a sugar solution+ 2ml of Seliwanoff's reagent
- heat the mixture in Boiling Water Bath for (2min)
- A positive test is indicated by the formulation of a **red product**



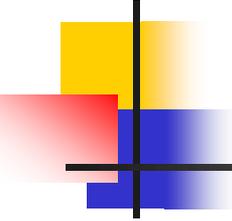
Glucose

-ve

+ve

Fructose

Seliwanoff's Test



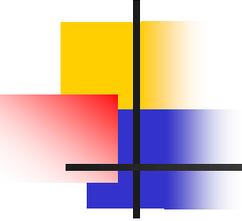
5- Bial's Test:

This test is used to distinguish pentose sugars.

Principle:

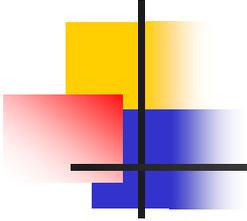
Arabinose(pentoses) + HCl _____ Furfural

Furfural+ orcinol ___ Fe⁺³ _____ Deep green complex



Method:

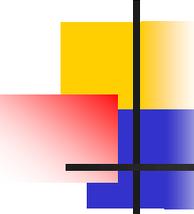
- 0.5 ml of suger solution+ 1ml of Bial's reagent
- heat the mixture in Boiling Water Bath for (2min)
- cooled the solution become for green, deep green than blue green.



Deep green complex



Bial's Test

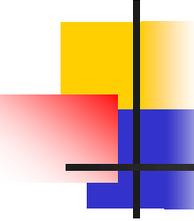


6-Iodine Test:

This test is used to distinguish for polysaccharides.

Principle:-

The principle of this test is based on the interaction of iodine ions with chains of the **starch molecule (amylose)** and the formation of **blue complexes**.



Iodine Test:

Method:

- 1ml of starch solution + 1 drop of the iodine solution

- A deep blue colour is produced.

Starch ----- deep blue colour

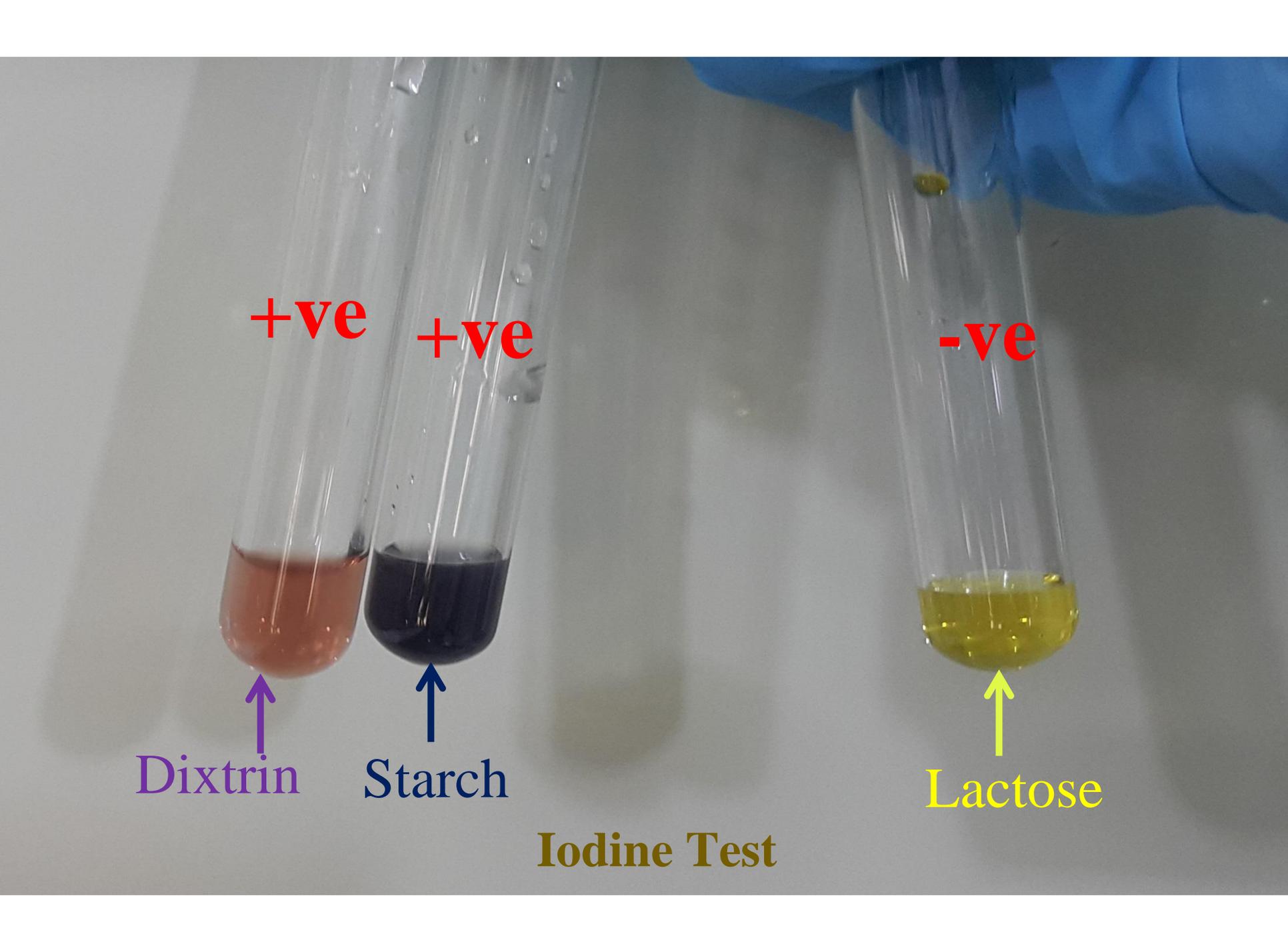
Dixtrin ----- purparal colour

Glycogen ----- Reddish brown colour.

+ve **+ve** **-ve**

Dixtrin Starch Lactose

Iodine Test



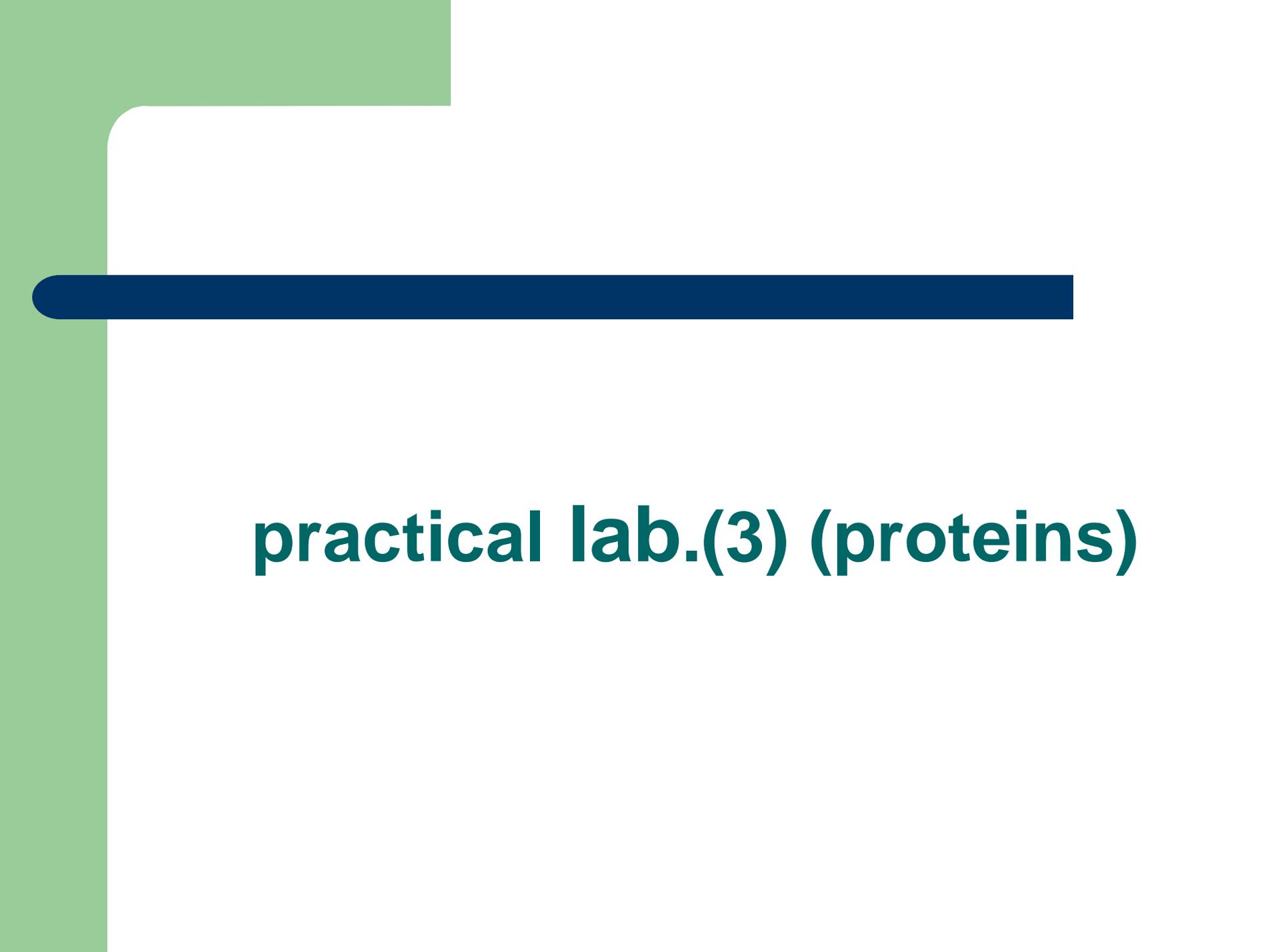
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practical lab.(3) (proteins)

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.

The biological and physiological functions of proteins

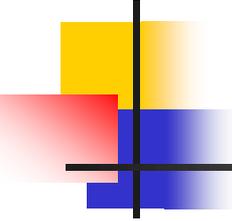
- The body's need to grow and build body tissues.
- A source of energy.
- Repair, replace and build body tissues.
- Maintains the balance of acidity and base in the body.
- It is involved in a number of biologically important compounds, such as **enzymes**, a number of **hormones** and **antibodies**.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

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

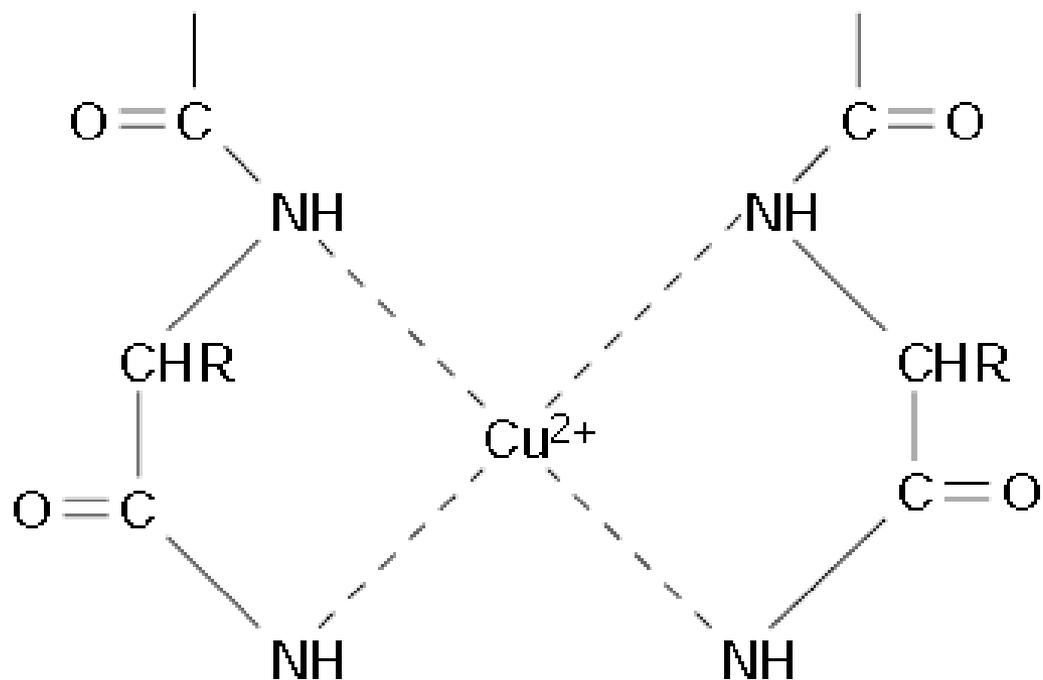
Biuret reaction :

Principle:

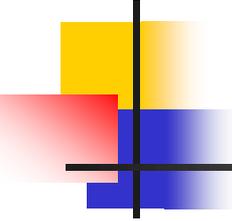
This reaction depends on the protein that gives a **violet color** with a solution of copper sulfate in **alkaline medium**. Finally, a **coordination complex** is formed between the copper ions and **amino groups** in the peptide bonds.

Experiments

Biuret reaction :



Copper coordination complex



Experiments

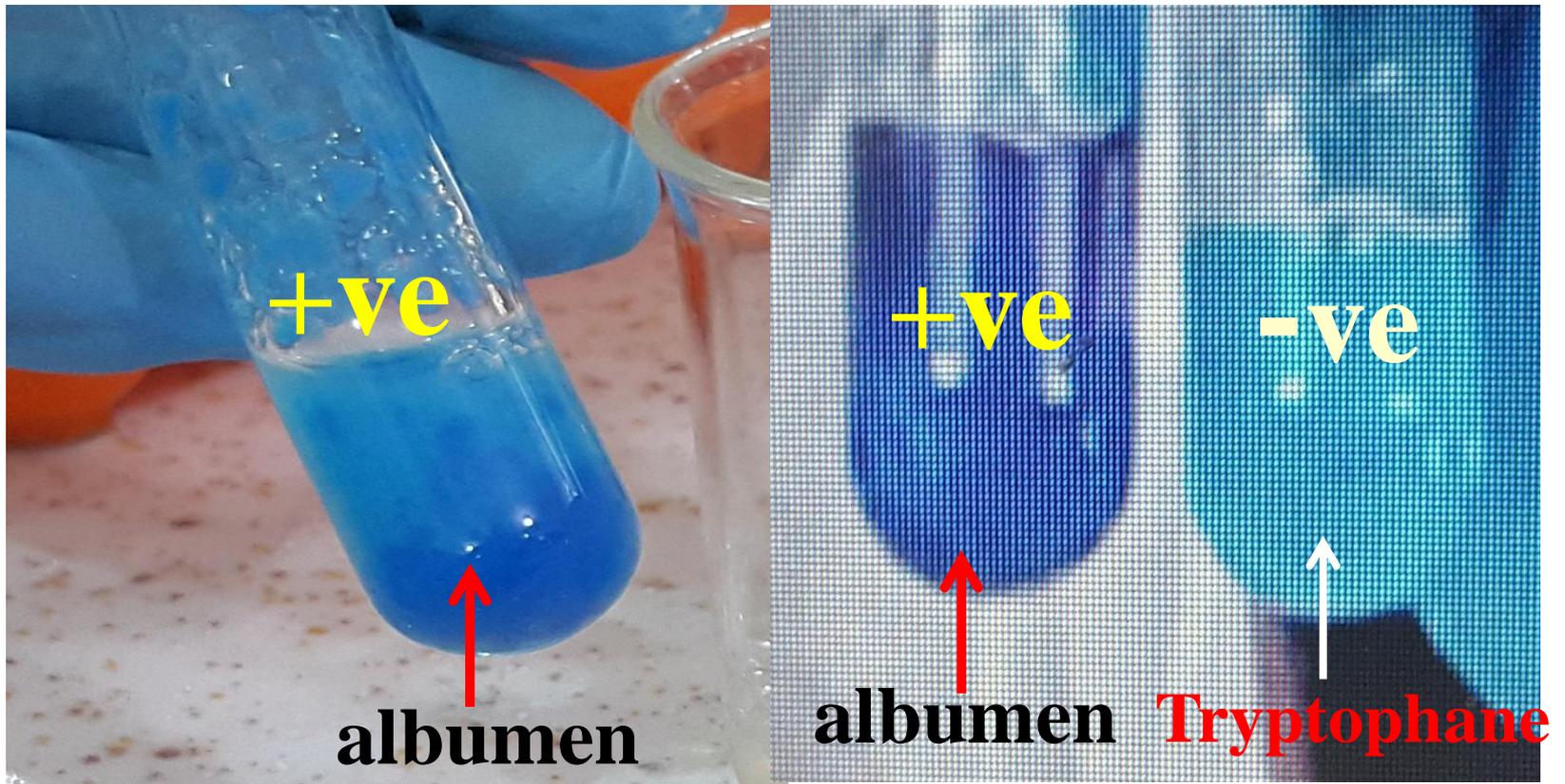
Biuret reaction :

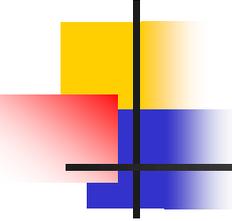
Method:

- 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
- **A violet colour** appears. It indicates the presence of **protein**

Experiments

Biuret reaction





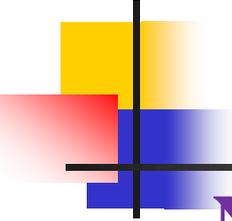
Experiments

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:

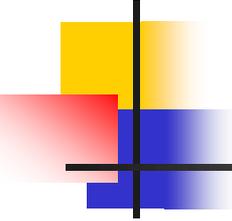
Method:

- 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





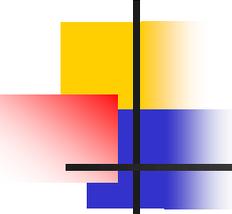
Experiments

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

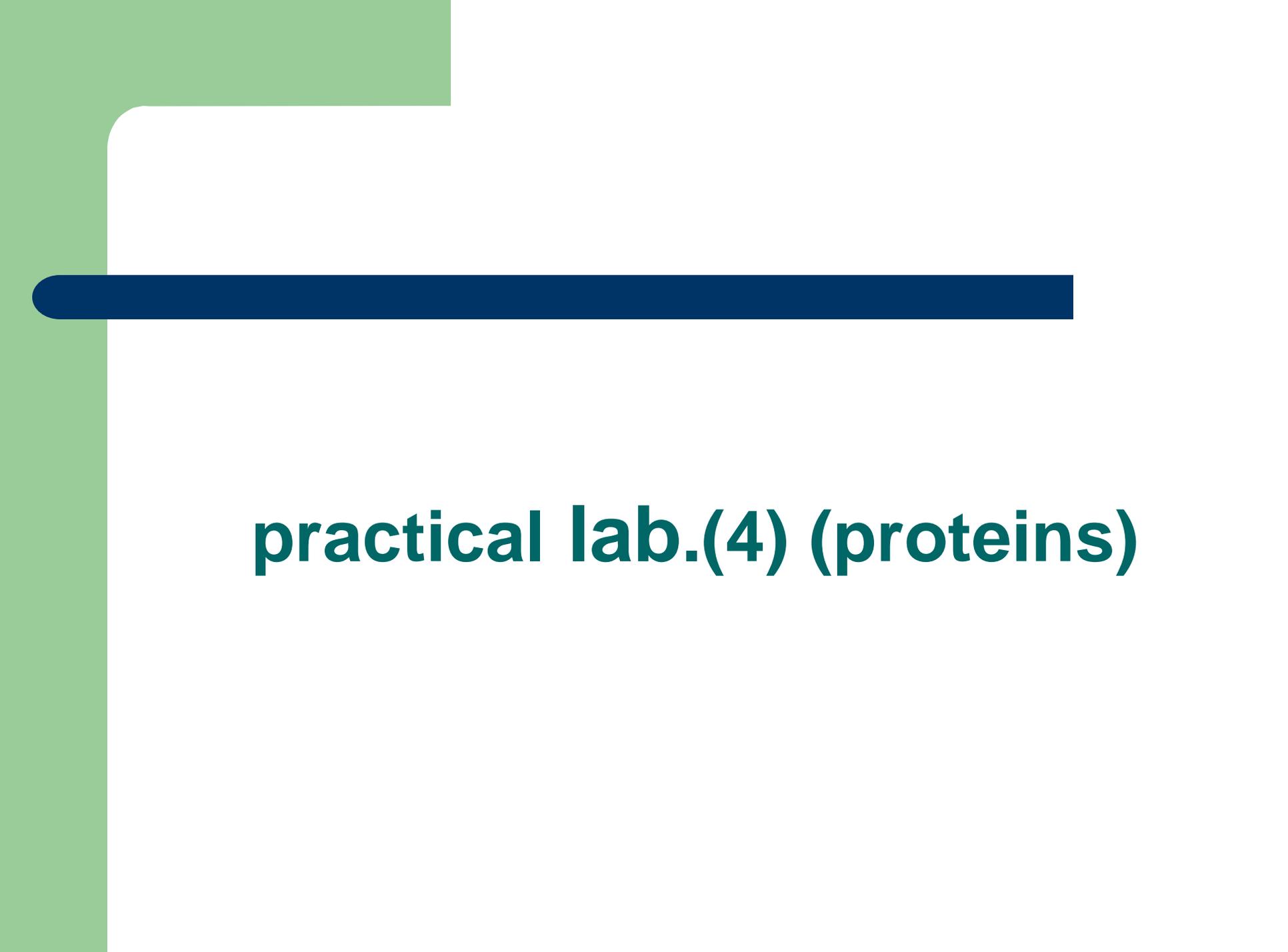


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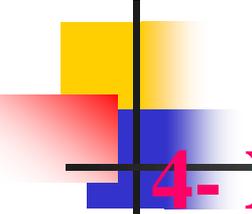


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practical lab.(4) (proteins)



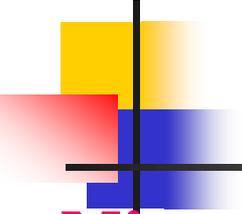
Experiments

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

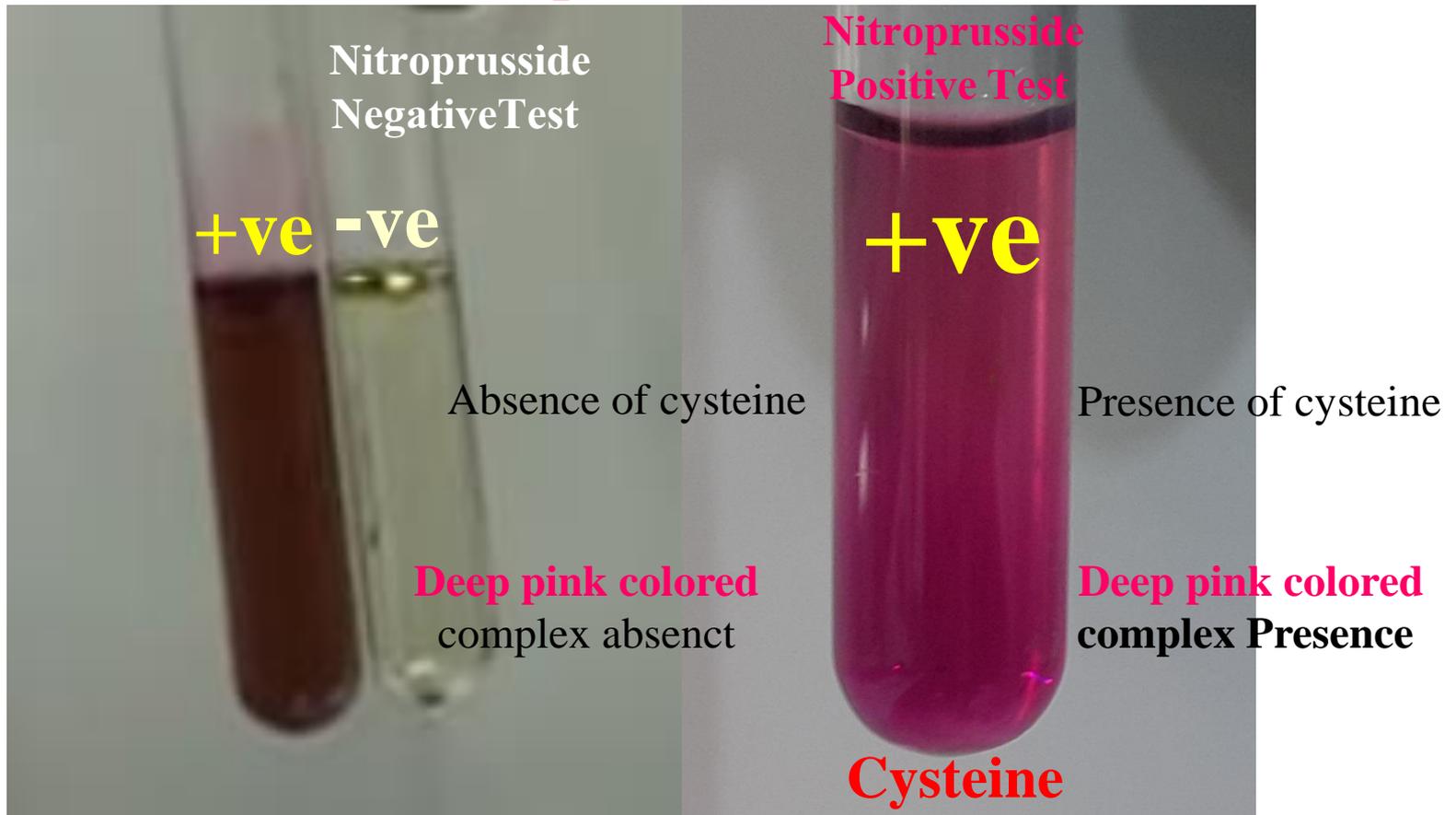
Nitroprusside reaction:

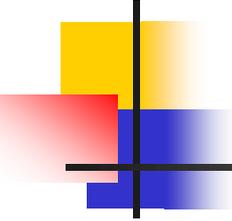
Procedure:-

- 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





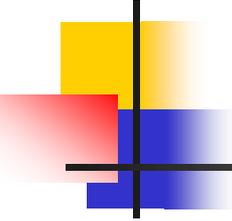
Experiments

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**.



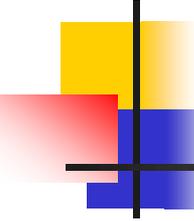
Experiments

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**



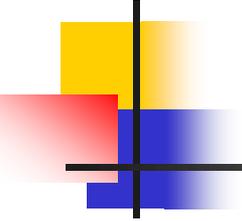
Rosenheim reaction:

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

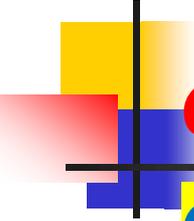
**No Purple ring
present**



**Positive
Acree-Rosenheim
Test**

Tryptophan Present

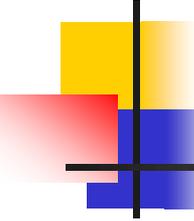
**Purple ring
present**



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**.

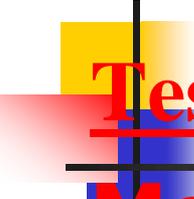


Test for cystein and cysteine:

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**.

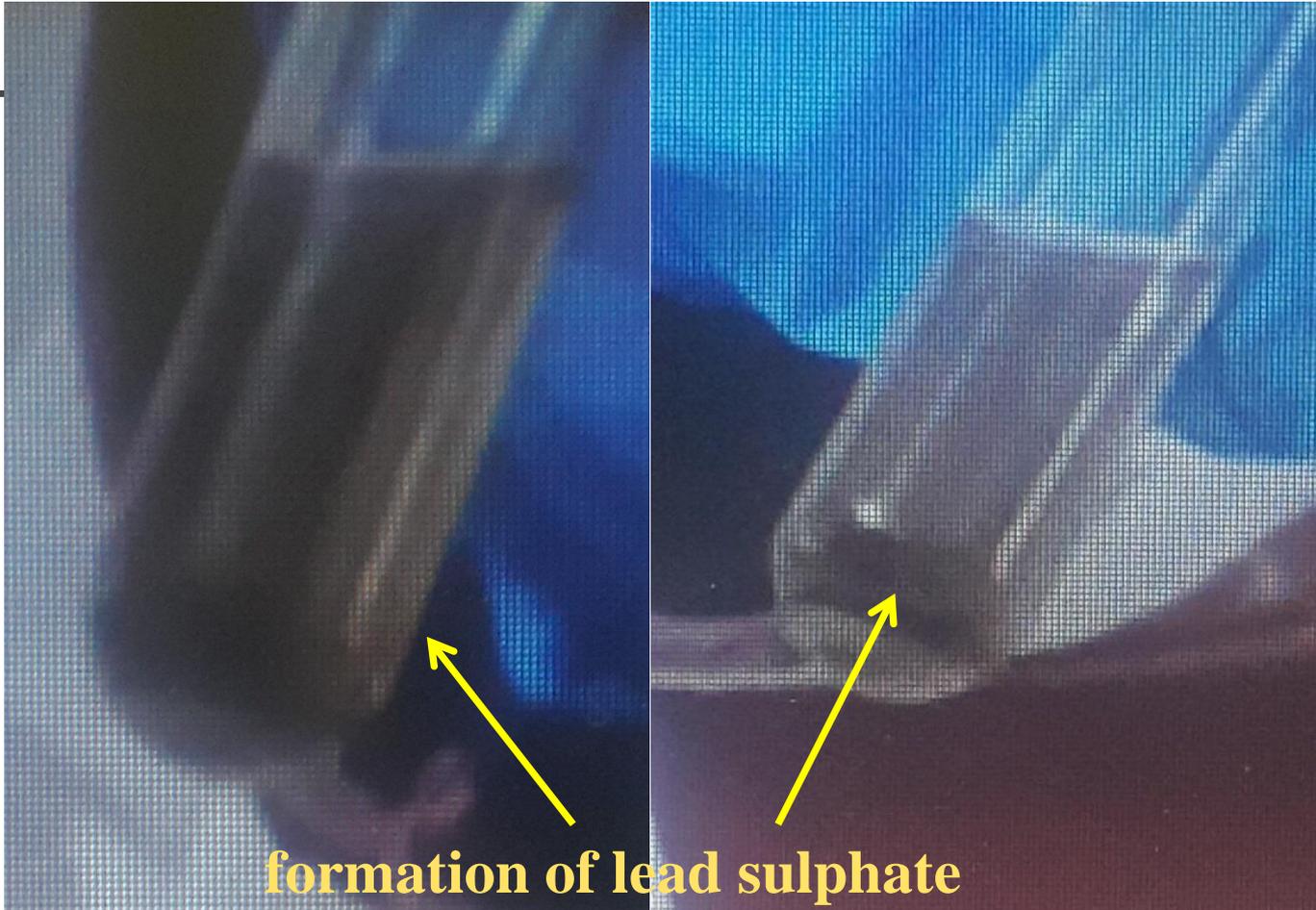


Test for cystein and cysteine:

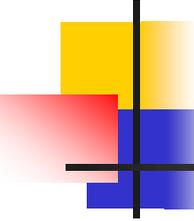
Method:-

- 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

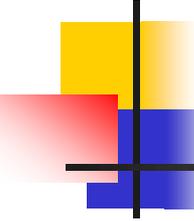


Cystein & cystine + 40%NaOH $\xrightarrow{\text{boil}}$ Na₂S $\xrightarrow{\text{Lead acetate}}$ PbS



7- Xanthoproteic Reaction :

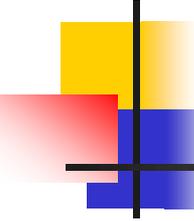
This test is general for proteins, because it is positive with aromatic amino acids (**aromatic groups**) that contain a benzene ring in their composition, as protein is not devoid of those acids.



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**.

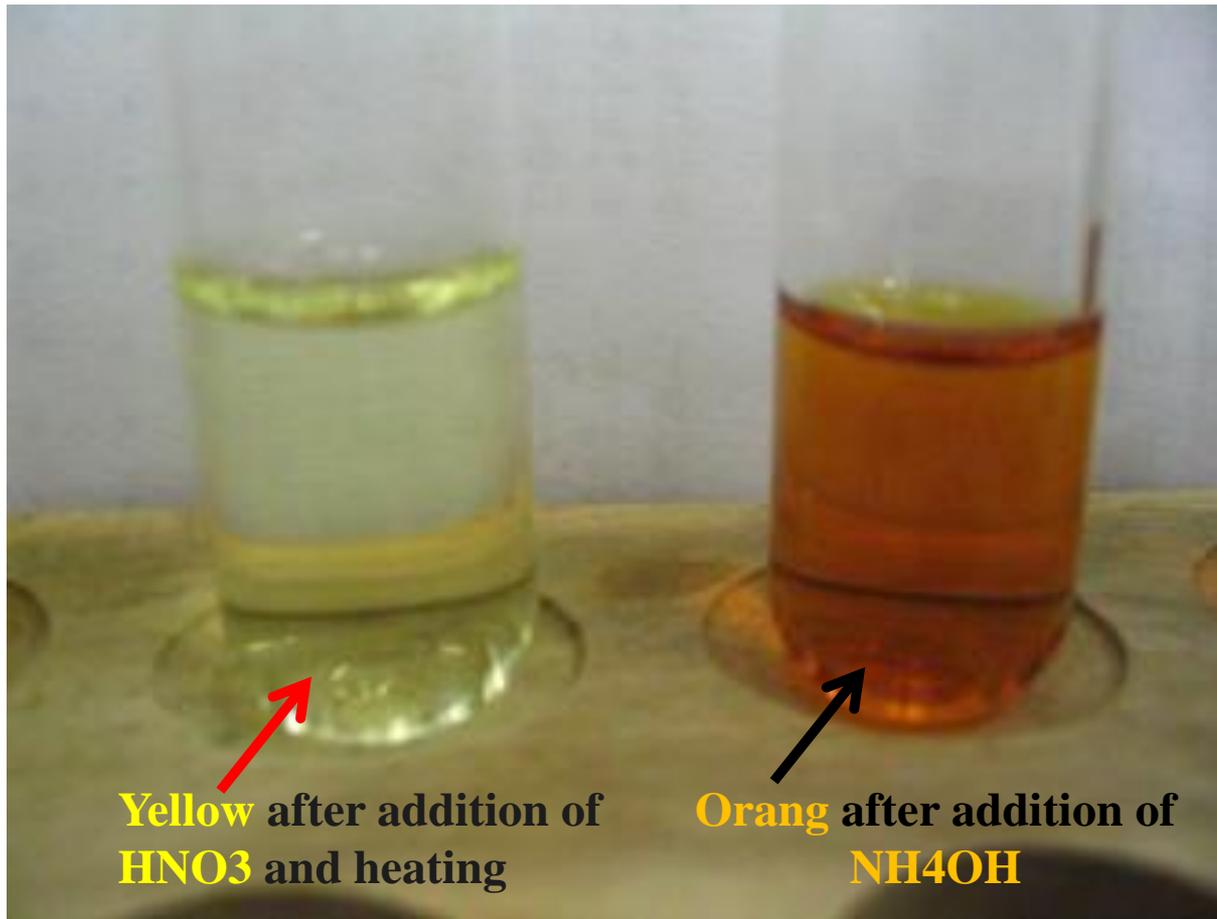


Xanthoproteic Reaction :

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





thank you for
listening



Assistant Lecturer. Zuhair A. ALrawi

***College of Pharmacy
third level***



practical lab.(5) (Lipids)

Lipids

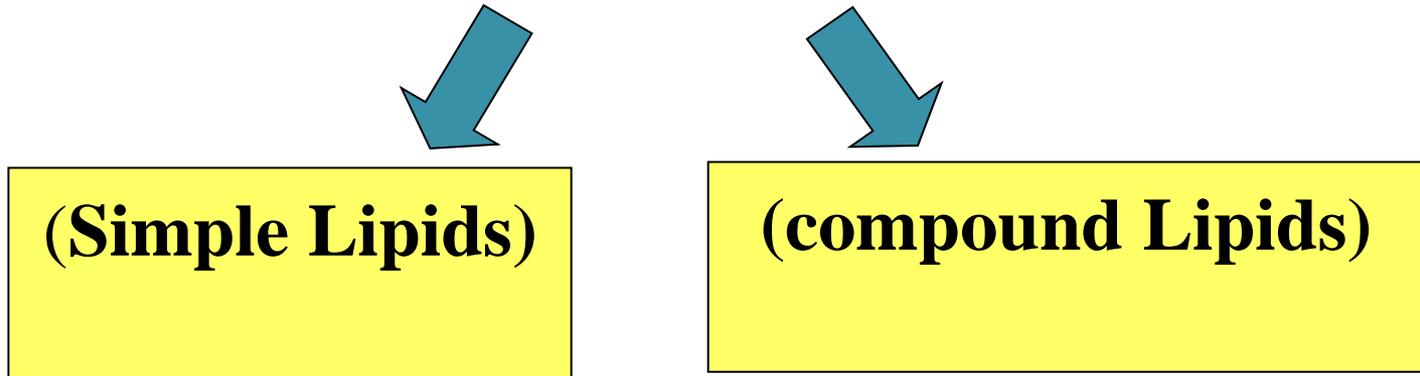
- It is a group of heterogeneous organic compounds that do **not dissolve** in **water** and **dissolve** with non-polar solvents such as **alcohol**, **ether**, **chloroform**, and Carbon tetrachloride **CCl₄**. It makes up **50%** of the composition of a living cell.
- Fats contain **C**, **H**, **O**, and some of them contain **P** and **N**.

The most important functions of lipids

- energy source.
- Provides the body with essential fatty acids necessary **for growth.**
- **Protecting** the internal organs in the body such as the **heart, kidneys, and spleen.** keeping them from shocks.
- **A source** of fat-soluble **vitamins** (**A, D, E, and K**).
- It enters into the **cell structure**, especially the cell membrane.

Classification of Lipids

- The fat is classified into:-

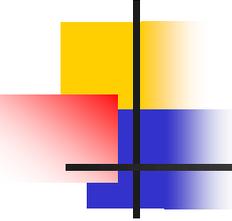


1- Simple Lipids : They are esters produced from the reaction of **fatty acids** with **alcohols**.

- **include:-**
- **Fat and Oil:** It is the most common fats in nature. These are **glycerol esters (triple alcohol)**. **Hydroxyl** (with **monohydroxy fatty acids**).
- **Waxes:** Such as **bees wax**, which are **esters** of long-chain **fatty acids** with **alcohols Monohydroxylate**.

2- compound Lipids :

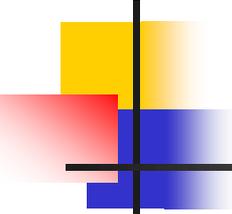
- They are esters produced from the reaction of fatty acids with alcohols, and include in their composition molecular groups that contain phosphorous or nitrogen.
- **Include:-**
- **Phospholipids.**
- **Glycolipids.**
- **Lipoproteins.**



Experiments

1- Copper Acetate Test

This test is used to distinguish between saturated and unsaturated fatty acids and Triglycerides.

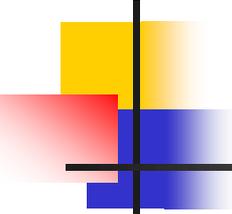


Experiments

1- Copper Acetate Test

- Principle:

This experiment is based on the fact that free saturated fatty acids combine with copper acetate to form **Copper salts**. In the lower water layer is **Bluish green** copper precipitate. While unsaturated fatty acids give **green** copper salts in the upper layer of petroleum ether.

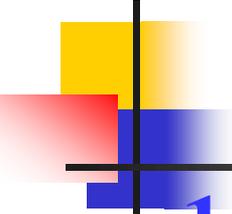


Experiments

1- Copper Acetate Test

Method:

- In a test tube, place a few drops of **lipid** and 5 mL **petroleum ether**, then add 3 mL **copper acetate**. $(\text{CH}_3\text{COO})_2\text{Cu}$
- Shake the mixture well, then leave the tube until the mixture separates into two layers and note the following: -



Experiments

1- Copper Acetate Test

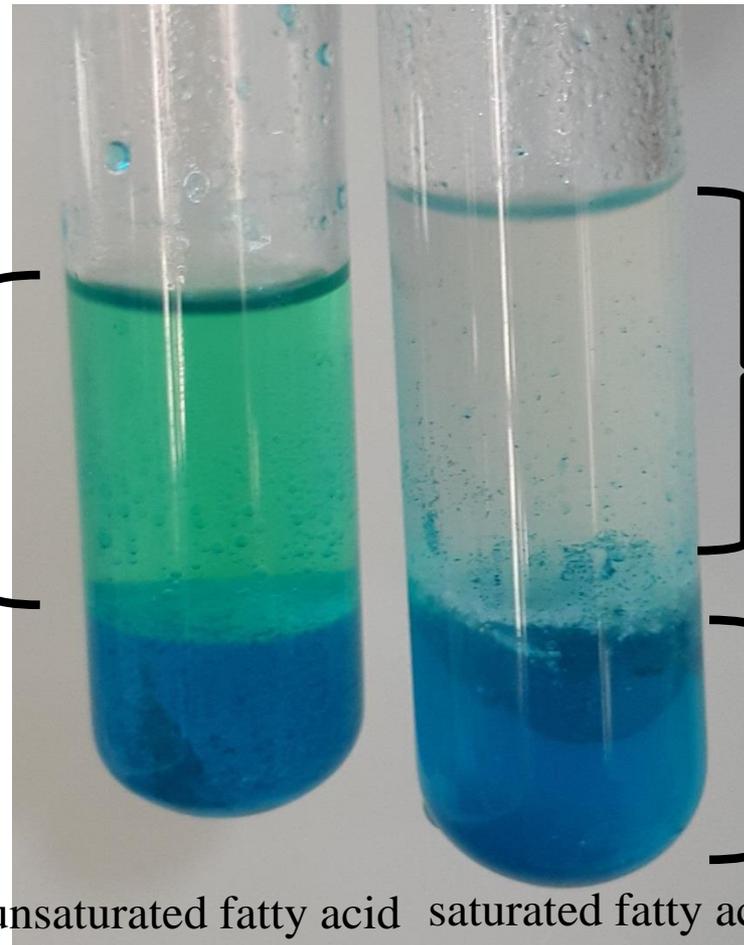
Method:

- If the ethereal **upper layer** appears **green** and the aqueous **lower layer** appears **blue**, the fat is **unsaturated** fatty acid.
- If the **upper layer** remained **clear** and without color with the appearance of a **greenish blue** precipitate in the **lower layer**, the fat was **saturated** fatty acid.

Experiments

Copper Acetate Test

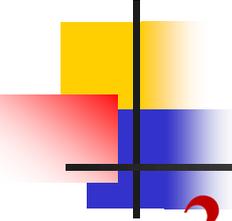
The ether layer is dissolved in the oil



the upper layer remained clear and without color

copper acetate
(CH_3COO) $_2\text{Cu}$

unsaturated fatty acid saturated fatty acid



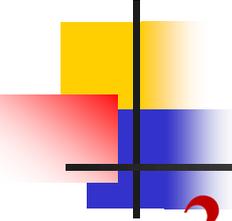
Experiments

2- Salkowski reaction:

This reaction is specific to cholesterol.

Principle:

The Salkowski's test for **cholesterol**, where it interacts with concentrated sulfuric acid (H_2SO_4) that absorbs water and forms two products, one dissolving in **sulfuric acid** and the other in **chloroform**.



Experiments

2- Salkowski's reaction:

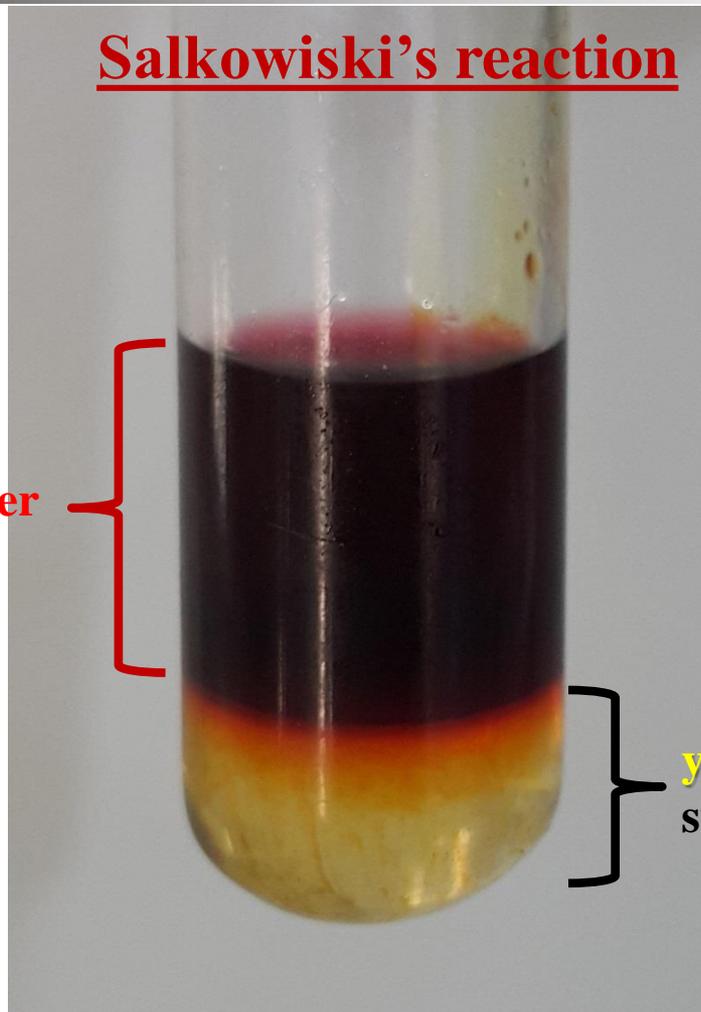
Method:

- put in test tube, 2 ml of a **chloroform cholesterol** solution + 2 ml of slowly **concentrated H₂SO₄** along the side of the test tube.
- Shake the tube well, then leave it until the mixture settles.
- We will notice the formation of two **colored layers**, the **upper layer** which is the **red chloroform layer**, and the **lower** the **acid layer** and it is **yellow**.
- Indicates the presence of **cholesterol**.

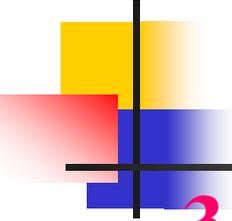
Experiments

Salkowski's reaction

red chloroform layer



yellow layer concentrated sulfuric acid (H_2SO_4)

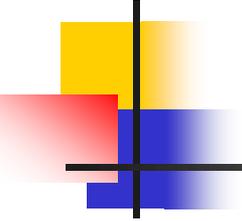


Experiments

3- Rancidity Test:

It is the detection of unwanted fats and oils left behind for a long time.

It is a chemical change that occurs to fats that are left over for a **long period of time**, to have a distinct taste and smell due to the fatty acids released from them.



Experiments

3- Rancidity Test:

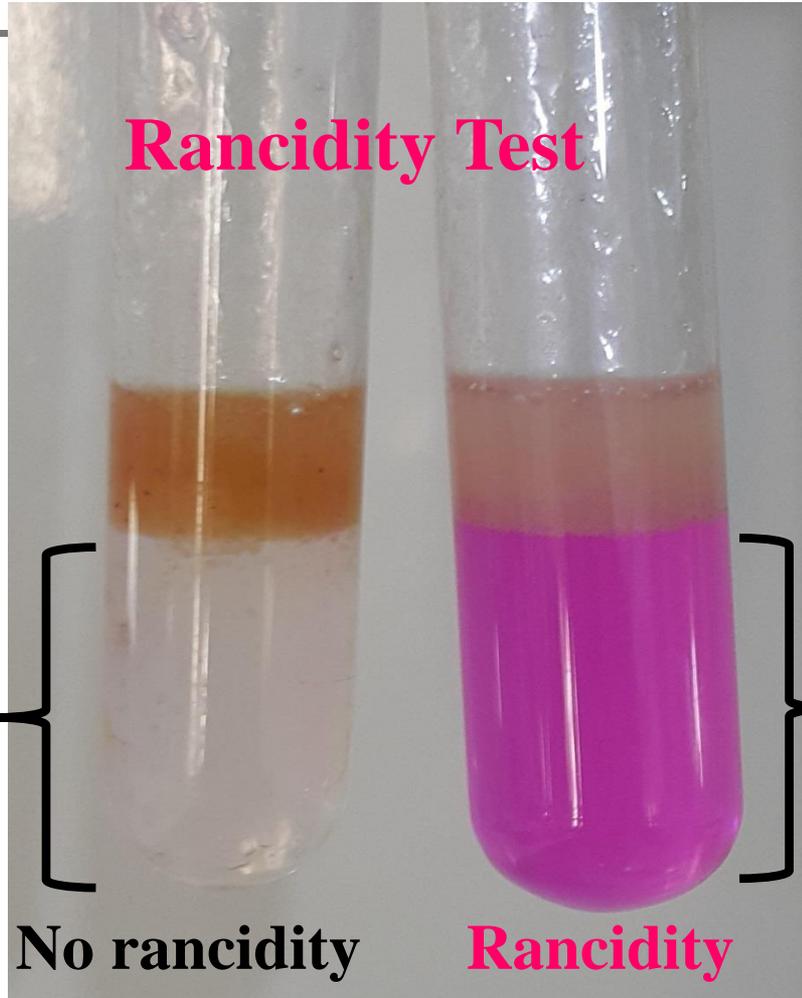
Method:-

- put in test tube, 2ml of NaOH + 3 drops of α -naphthol.
- Add 2 ml of fat or any oil, then shake the mixture well.
- If rancidity is present, it gives a **red** or **pinkish** color.
- If the color appears clear, watery, there is **no rancidity**.

Experiments

Rancidity Test

appears clear, watery



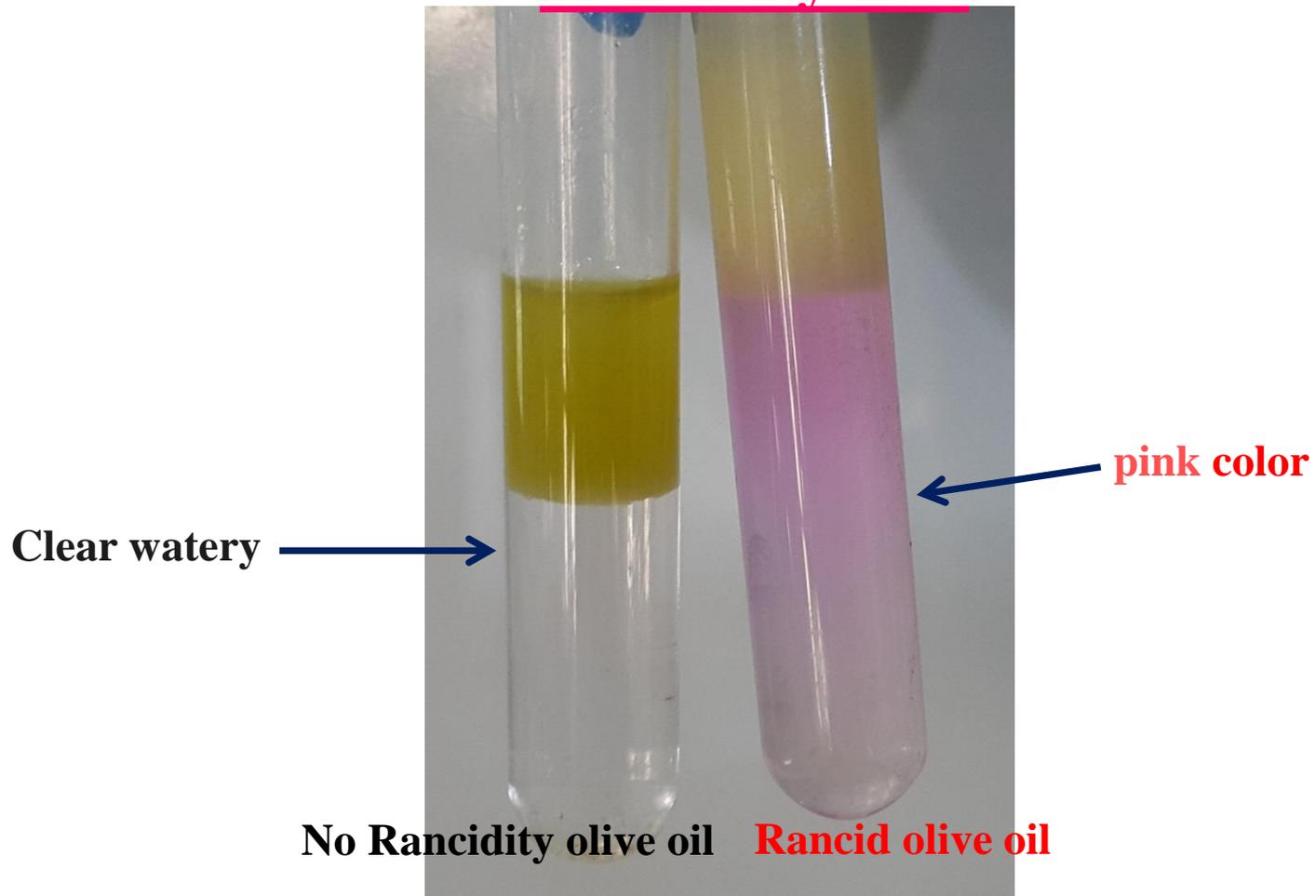
No rancidity

Rancidity

red or pinkish color

Experiments

Rancidity Test





**thank you for
listening**