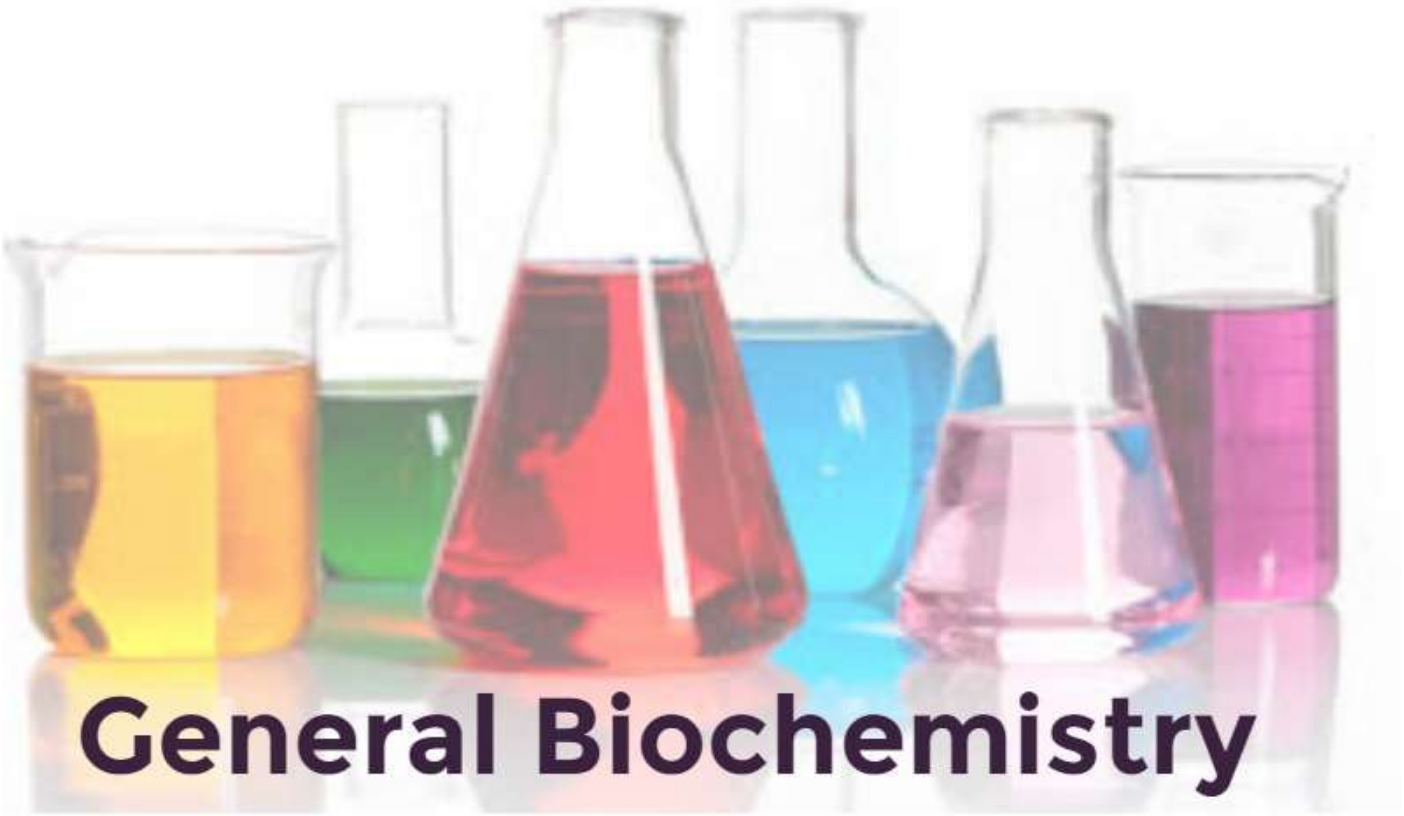
Kingdom of Saudi Arabia
Ministry of Education
King Abdulaziz University
Faculty of Sciences - AL Faisaliah Campus



General Biochemistry

Organized by

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Lecturer\Dalal Al-Saedi

Name:

Computer no.

Section

In the laboratory

Wear

Personal Protective Equipment (PPE)

Avoid

Eating and Drinking

Messing

Change dropper from bottle to another

Keep

Lab notbook

Calm

Lab safety and follow instruction

State of Materials

Solid

Liquid

Terms

Concentrated

Diluted

lab Equipments

Test Tubes 

Test Tube Holder 

Test Tube Rack 

Test Tube Brush 

Dropper 

Bottels 

Microscope Slides

Chemicals

Acids

Bases

Reagents

Hazards

lab Instruments

Water Bath

Light Microscope

Biosafety cabinet

Vortex

Table of content

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Qualitative analysis of carbohydrates

Introduction of carbohydrates

Carbohydrates are organic molecules which contain carbon, hydrogen and oxygen and are the most abundant class of organic compounds found in living organisms.

Carbohydrates are produced from carbon dioxide and water by plants through the process of **photosynthesis**. They are easily digested by animals where they are converted back into carbon dioxide and water, with a concurrent release of energy. The formulas of many carbohydrates can be written as carbon hydrates, $C_n(H2O)_n$, hence their name.

$$n\,CO_2 + n\,H_2O + \text{energy} \longrightarrow C_nH_{2n}O_n + n\,O_2$$

The function of carbohydrates

It is the main energy source for animals as they breakdown carbohydrates during the process of metabolism to release energy. Carbohydrates also serve as a structural material (cellulose), a component of the energy transport compound ATP, recognition sites on cell surfaces, and one of three essential components of DNA and RNA.

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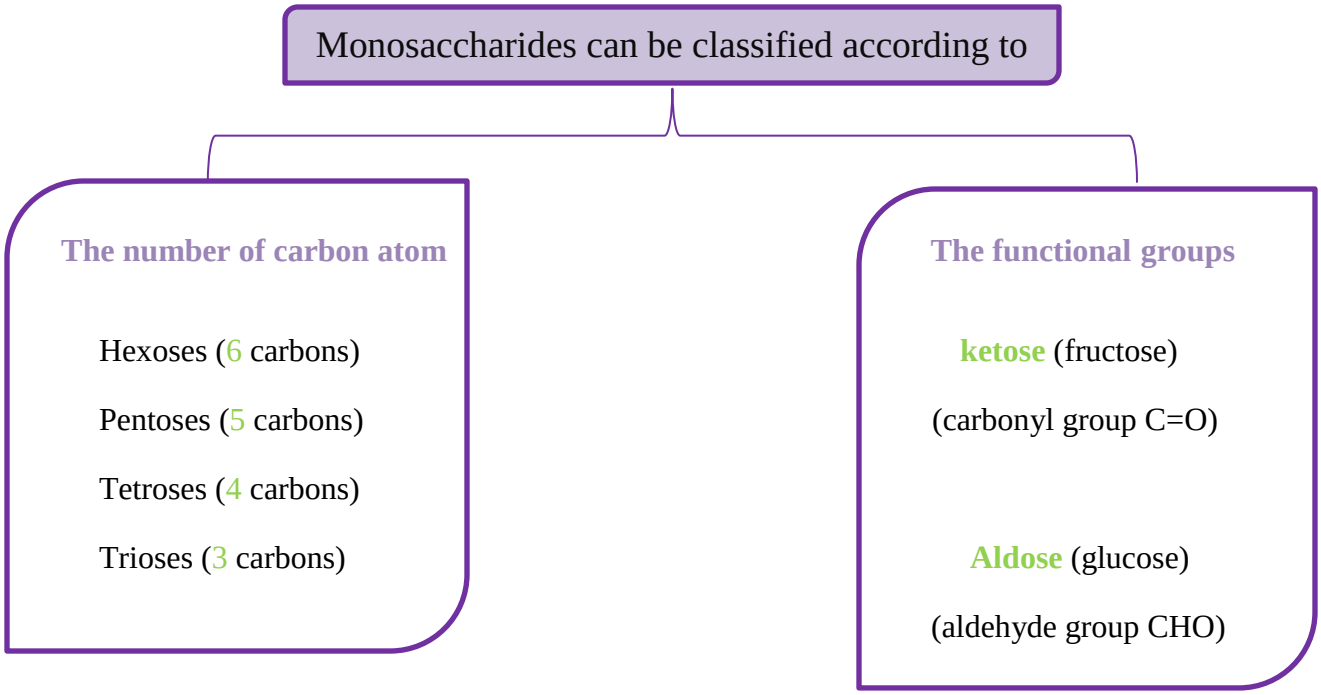
The classification of carbohydrates

1- Simple carbohydrates, often called **monosaccharides**, **contain one saccharide unit** and cannot be broken down into smaller carbohydrates**.** These are the simplest form of sugar and are usually colorless, water soluble and crystalline solids, e.g. **glucose**, **fructose**, **galactose** etc.



The two descriptors are commonly combined into a single term like "aldohexose" for an aldose or "ketohexose" for ketose that also is a hexose. See below some examples of monosaccharides.

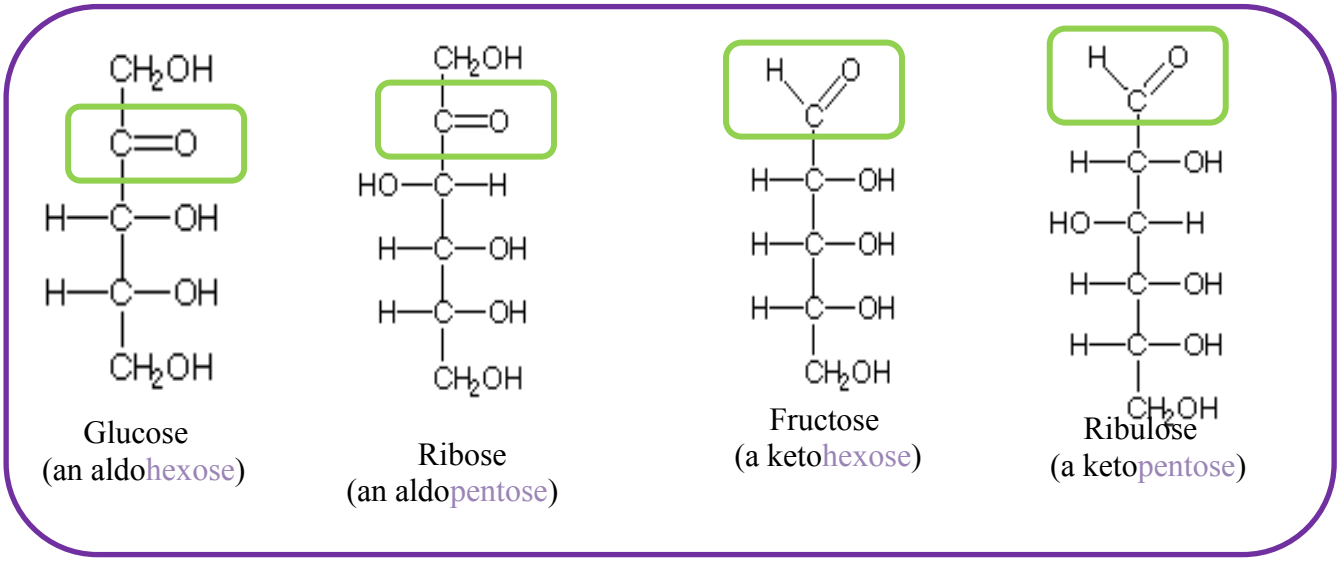


Figure 1: The classified of monosaccharides according to the number of carbon atoms and functional groups.

2- **Disaccharides** **contain** **two** **monosaccharide** unit and can be broken into two monosaccharide units by hydrolysis and lost one molecule of water. See below some examples of disaccharides, e.g. **maltose**, **lactose** and **sucrose**.

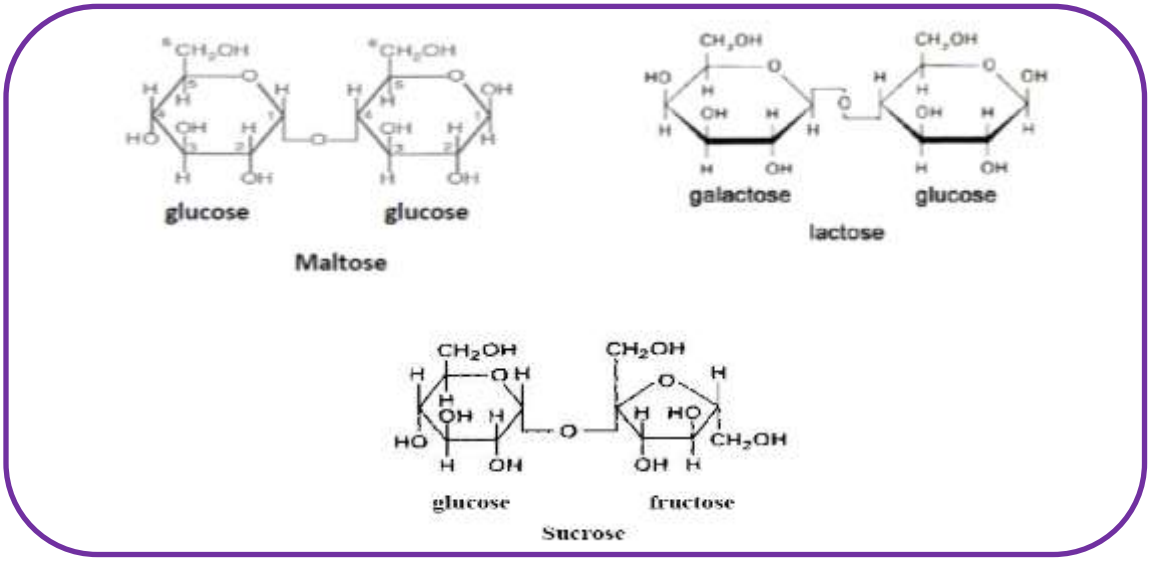


Figure 2: The example of disaccharides

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3- **Oligosaccharides contain 3-10 monosaccharide** unit and **can be** broken into 3-6 monosaccharide units by hydrolysis and lost one or more molecule of water. See below some examples of oligosaccharides, e.g. **Raffinose**.

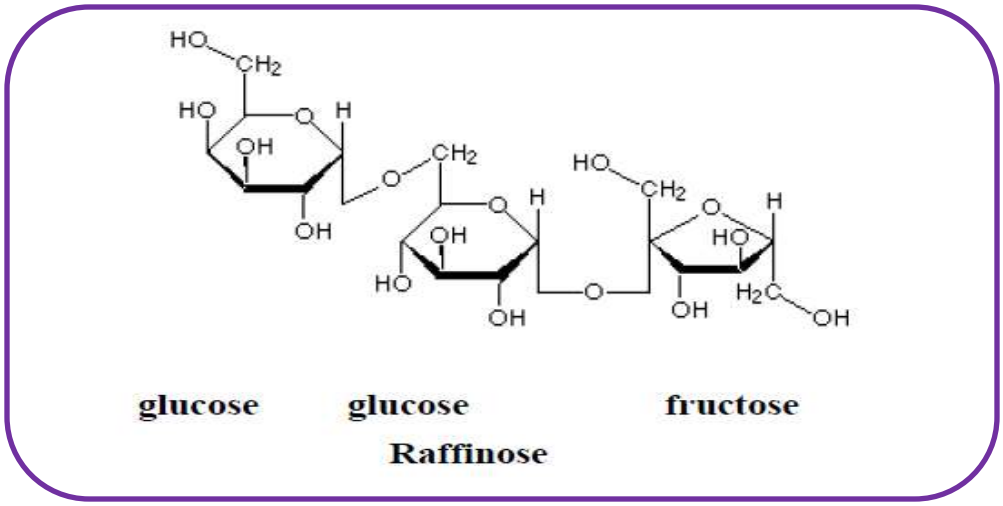

Figure 3: The example of oligosaccharides

4- **Polysaccharides can contain over 10 or more monosaccharide** unit and can be broken into 7 or more monosaccharide units by hydrolysis and lost one or more molecule of water. See below some examples of polysaccharides, e.g. **starch.**

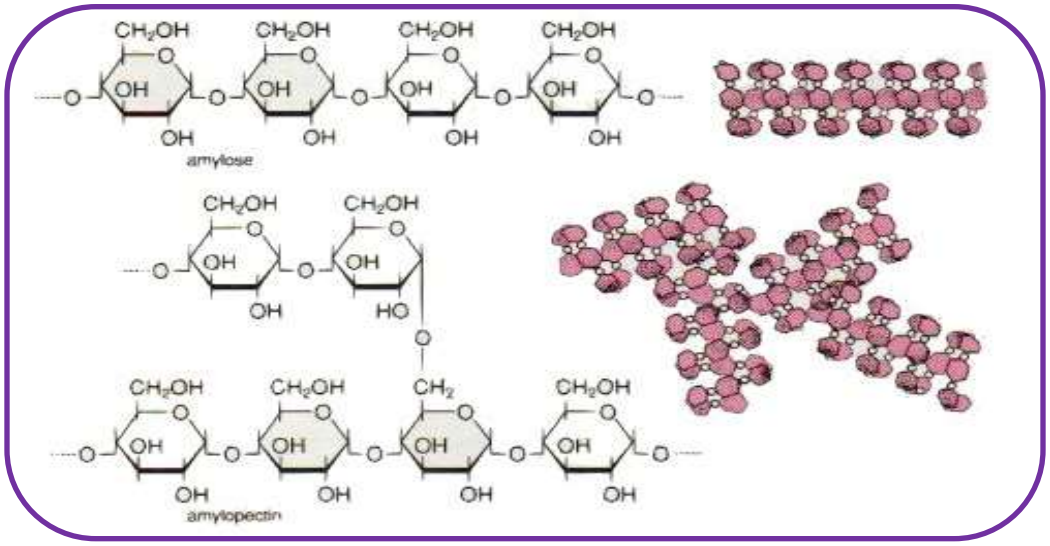

Figure 4: The example of polysaccharides

Lab experiments

A- Physical properties

Solubility- color- shape.

B- Chemical properties

Principle

Presence of carbohydrates can be confirmed **qualitatively** by several tests. Due to the presence of different numbers of sugar units specific carbohydrates exhibit typical color reactions that form the basis for their identification. In the following tests will be performed for the qualitative analysis of carbohydrates:

1- The Molisch test

General test for **all carbohydrates**, Monosaccharides give a rapid positive test. Disaccharides and polysaccharides react slower.

Reactions:

The test reagent dehydrates **pentoses** to form **furfural** (first reaction) and **dehydrates hexoses** to form **5-hydroxymethyl furfural** (second reaction). The furfurals further react with **ά-naphthol** present in the test reagent to produce **a purple product**

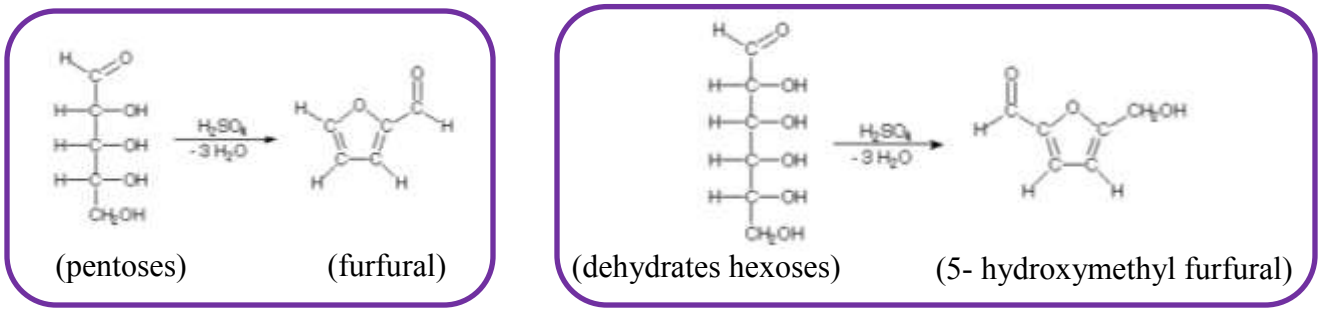

Figure 5: The reaction of molisch test

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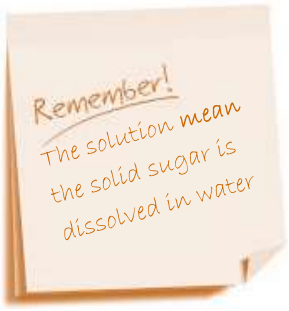
How to perform the test

1- Two ml of a sample solution is **placed** in a test tube.

2- Two drops of the molisch reagent (a solution of ά-napthol in 95% ethanol) is **added**.

3- The solution is then **poured** slowly into a tube containing two ml of **concentrated** sulfuric acid so that two layers form.



Result

Positive test is indicated by the formation of a violet ring at the interface of two layers.

Negative test

Positive test

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2- The Benedict test

It is used to **differentiate** between the reducing and non-reducing sugar.

Reactions

Benedict's test is **based** upon the participation of the **aldehyde** and **ketone groups** in a chemical reaction.

Reducing sugars are **oxidized** by the copper ion in **alkaline medium** to form a carboxylic acid and a reddish precipitate of copper (I) oxide.

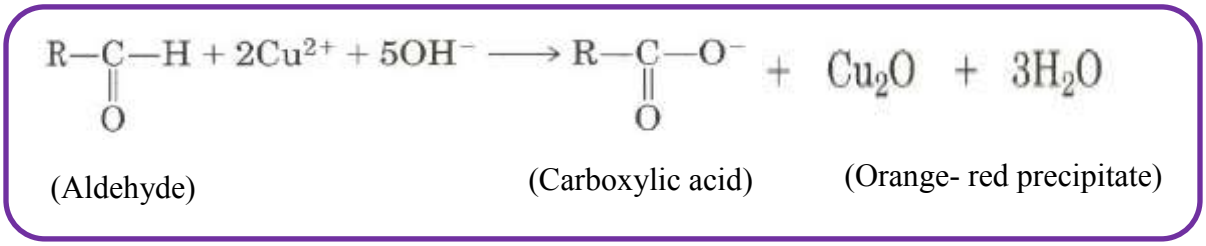

Figure 5: The reaction of benedict's test

How to perform the test

1- One ml of a sample solution is **placed** in a test tube.

2- Two drops of the Benedict's reagent (a solution of sodium citrate and sodium carbonate mixed with a solution of copper sulfate) is **added**.

3- The solution is then **heated** in a boiling water bath for two minutes.

Result

Show positive test for **reducing sugars,** is indicated by the formation of an orange-brown precipitate within two minutes.

Negative test **Positive test**

3- The Fehling's test

It is also used to **differentiate** between the reducing and non-reducing sugar.

Reactions:

Reducing sugars are **oxidized** by the copper ion in **alkaline medium** to form a carboxylic acid and a reddish precipitate of copper (I) oxide. Fehling's reagent is commonly used for reducing sugars but is known to be not specific for aldehydes.

(Aldehyde) (Fehling's solution) (Carboxylic acid) (Red precipitate)

Figure 6: The reaction of Fehling test

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How to perform the test:

1- One ml of a sample solution is **placed** in a test tube.

2- One drops of the fehling's reagent (fehling's A, uses 7 gm CuSO4.5H2O dissolved in distilled water containing 2 drops of dilute sulfuric acid. Fehling'a B, uses 35 gm of potassium tartarate and 12 gm of NaOH in 100 ml of distilled water) is **added**.

3- The solution is then **heated** in a boiling water bath for two minutes.

Result:

Show positive test for **reducing sugars,** is indicated by the formation of a red- brown precipitate within two minutes.

Negative test

Positive test

Notes

Sucrose is **non-reducing sugar** (does not react with Fehling's or Benedict's reagent**)** because the anomeric carbon of glucose is involved in the glucose-fructose bond and hence is not free to form the aldehyde in solution. Although the glucose and fructose alone are **reducing sugar**.

4- The Barfoed's test

The Barfoed's test is used to **distinguish** between reducing monosaccharides and disaccharides.

Reactions

Reducing monosaccharides are **oxidized** by the **copper ion** in acidic solution to form a carboxylic acid and a **reddish precipitate** of copper (I), oxide **within three minutes**. Reducing disaccharides undergo the same reaction, but do so at **a slower rate.**

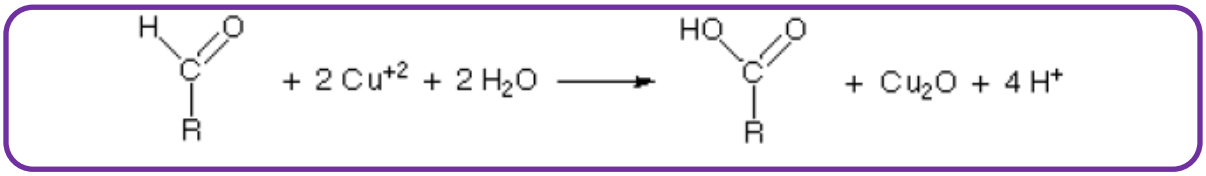

Figure 7: The reaction of Barfoed's test

How to perform the test

1- One ml of a sample solution is **placed** in a test tube.

2- Two ml of the Barfoed's reagent (a solution of cupric acetate and acetic acid) is **added**.

3- The solution is then **heated** in a boiling water bath for 1-3 minutes.

Result

Show positive test for **reducing monosaccharides,** is indicated by the formation of a reddish precipitate in the bottom of tube within 1-3 minutes.

Negative test

Positive test

Notes

In the Barfoed's test:

The water bath must be **boiling.**

The sugar solution must be **concentrated.**

The time taken to formation of reddish precipitate in the bottom of tube must be **calculate carefully**.

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5- The Seliwanoff's test

The Seliwanoff's test is used to **distinguish** between ketoses and aldoses. If a sugar contains a ketone group it is called ketose and if it contains an aldehyde group then it is called aldose.

Reactions

The test reagent **dehydrates** ketohexoses to form 5-hydroxymethyl furfural. 5-Hydroxymethyl furfural further **reacts** with resorcinol present in the test reagent to produce a **red product** within five minutes. Aldohexoses react to form the same product, but do so more slowly.

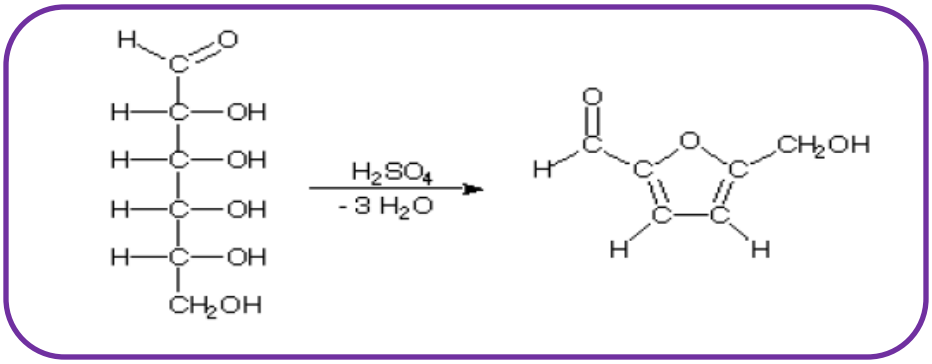

Figure 8: The reaction of Seliwanoff's test.

How to perform the test

1- One half ml of a sample solution is **placed** in a test tube.

2- Two ml of the Seliwanoff's reagent (a solution of resorcinol and HCl) is **added**.

3- The solution is then **heated** in a boiling water bath for five minutes.

The **sugar solution** in Seliwanoff's must be **concentrated**

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Result

Show positive test for **ketoses,** is indicated by the formation of a red color.

Negative test

Positive test

6- The Hydrolysis test for sucrose

Sucrose is the only **non-reducing sugar** so it does not reduce the alkaline Cu solutions (Fehling and Benedict). The sucrose first must be hydrolyzed to its component and then test it.

How to perform the test

1- Two ml of a sample solution is **placed** in a test tube.

2- Two drops of concentrated hydrochloric acid HCl is **added**.

3- The solution is then **heated** in a boiling water bath for 15 minutes.

Result

Show positive test for **Sucrose,** is indicated by the formation of a light orange color.

Negative test

Positive test

7- The Iodine/Potassium Iodine test:

Iodine forms colored complexes with polysaccharides. The color of the complex depends upon the three dimensional structure of the polysaccharide. **Starch** is a coiled structure which turns **blue** when bound to Iodine.

How to perform the test:

1- Two ml of a sample solution is **placed** in a test tube.

2- Two drops of Iodine/Potassium Iodine solution and one ml of water are **added**.

Result

Show positive test for **Starch,** is indicated by the formation of a blue- black color.

Negative test

Positive test

8- The hydrolysis test for starch

The experiment illustrates the **conversion** of starch to a reducing sugar (maltose) by the action of sulfuric acid at **boiling point**. The longer the starch is exposed to the acid the **further** hydrolysis proceeds.

How to perform the test

1- One ml of starch solution + One ml of sulfuric acid are **placed** in a test tube.

2- The solution is **heated** in a boiling water bath for 15 minutes.

3- **Add** drop from (Iodine/ Potassium Iodine) ⟶ Solution with **yellow color.**

4- One ml of yellow solution + One ml of Bendict or fehling reagent, then **heat** in boiling water bath for 1 minutes.

Result

Show positive test for **Starch,** is indicated by the formation of a yellow color, and then **converted** to orange precipitate.

9- The Osazone test

The Phenylhydrazine ($C_6H_5NHNH_2$) **reacts** with carbons no. 1 and no. 2 of reducing sugars to form derivatives called **osazones**. The formation of these distinctive crystalline derivatives is useful for comparing the structures of monosaccharides and disaccharides.

Reactions

The osazone reaction was developed and used by Emil Fischer to **identify** aldose sugars differing in configuration only at the **alpha-carbon.** The upper equation shows the general form of the osazone reaction, which effects an alpha-carbon **oxidation** with formation of a bis-phenyl hydrazone, known as an osazone. Application of the osazone reaction to D-glucose and D-mannose demonstrates that these compounds differ in configuration only at C-2.

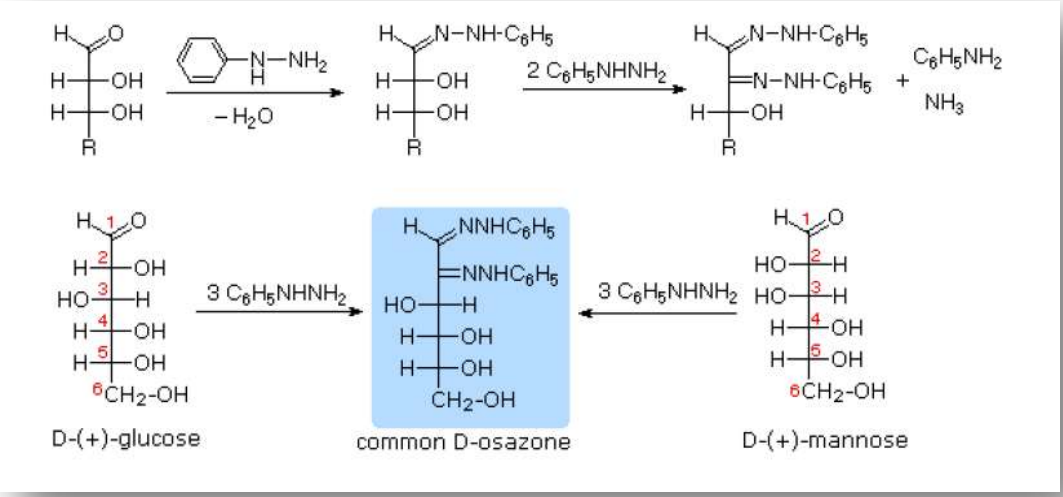


Figure 9: The reaction of Osazone test

How to perform the test

1- One gm of the sugar sample is **placed** in a test tube.

2- Two gm of phenylhydrazine hydrochloride + 3 gm of crystallized sodium acetate, and 4 mL of distilled water are **added**.

3- **Place** the test tube in a beaker of boiling water bath.

4- Note the time that the test tube was immersed and the time of the precipitation.

5- After 20 -45 min, **remove** the test tube from the hot water bath and set it aside to cool.

6- A small amount of the liquid and solid is **poured** on a watch glass.

7- Tip the watch glass from side to side to spread out the crystals, and **absorb** some of the mother liquid with a piece of filter paper**, taking care not to crush or break up the clumps of crystals.**

8- **Examine** the crystals under a low-power microscope (about 80-100×), and **compare** with photomicrographs.

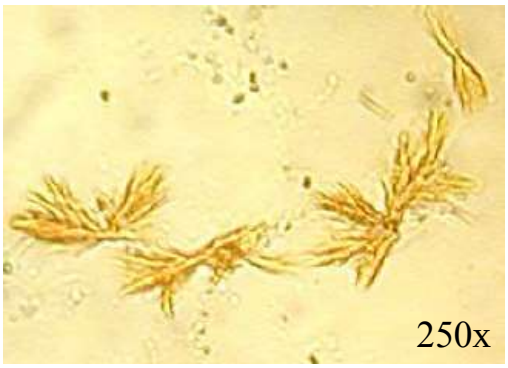
Result

Show positive test for **all carbohydrates** except sucrose and starch.

The formation of precipitate for **monosaccharides** (glucose or fructose) **before cooling**, while the formation of precipitate **after cooling** for **disaccharides** (maltose or lactose).

Glucosazone + Fructosazone

(Needle shaped)



Maltosazone

(Sunflower shaped)

Lactosazone

(Cotton shaped)

RESULTS & LAB REPORT

Unknown (glucose)

Physical properties

Chemical properties

Test	Observation	Result

RESULTS & LAB REPORT

Unknown (fructose)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (sucrose)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (lactose)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (maltose)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (starch)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT FOR SCHEME

Unknown (1)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT FOR SCHEME

Unknown (2)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT FOR SCHEME

Unknown (3)

Physical properties

Chemical properties

Test	Observation	Result

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Qualitative Determination of Lipids

Introduction

The term "**lipids**" applies to a class of compounds that are **soluble** in nonpolar organic solvents (e.g. alcohol, benzene, ether and chloroform) and **insoluble** in polar water. Lipids contain carbon, hydrogen and oxygen but have far less oxygen proportionally than carbohydrates.

Lipids are an important part of living cells. Together with carbohydrates and proteins, lipids are the main constituents of plant and animal cells.

Fatty acids: They are the building blocks of lipid. Fatty acid have a **long hydrocarbon chain** containing a carboxyl group at the end. They are divided into: saturated fatty acids and unsaturated fatty acids (unsaturated contain double bonds). The general formula for fatty acids {$CH_3 (CH_2)_n COOH$}

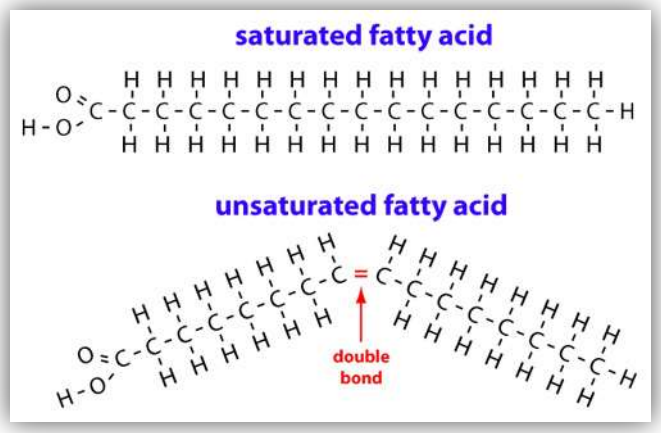

Figure 1: Types of fatty acid

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Lipid can be divided according to their chemical composition to

I. **Simple lipids:** They are esters of fatty acids with various alcohols. They include oils and fats which are esters of fatty acids with glycerol (e.g. **triglycerides**). The triacyglycerol is the simplest and most common fat. It is the form in which lipids are stored in the cell.

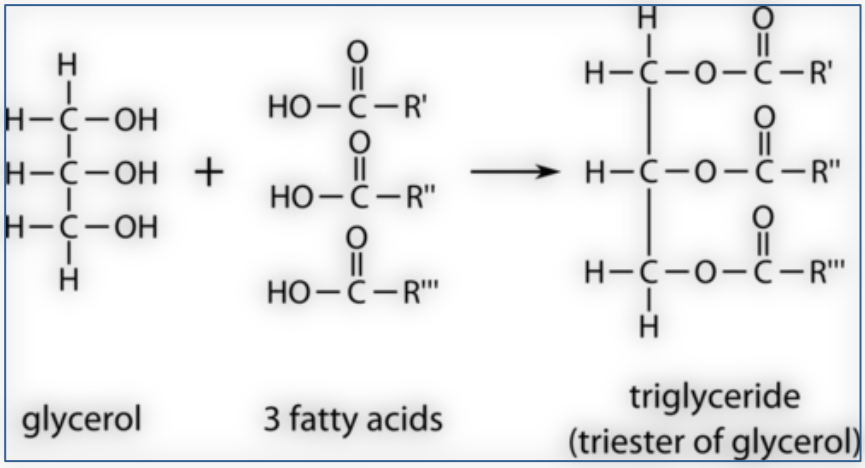

Figure 2: A triglyceride is a lipid that is formed by the esterification of glycerol with three fatty acid molecules

II. **Complex lipids:** Lipids are linking with other compounds, such as phosphate (e.g. **phospholipids**), carbohydrates (e.g. **glycolipids**) and proteins (e.g. **lipoproteins**).

II. **Precursor and derived lipids:** They include fatty acids, cholesterol, steroid hormones, fat-soluble vitamins (vitamin A, D, E & K) and eicosanoids, which include **prostaglandins**, **leukotrienes** and **thromboxanes**.

Biological functions of lipids

1. Lipid provide energy storage and metabolic fuels. They give more energy than carbohydrate and proteins.

2. Cholesterol is a precursor of steroid hormones, bile acids (which help in digestion of dietary fats) and vitamin D.

3. Lipids are found naturally in all living organisms. They have functional and structural components of the cell membrane.

4. Lipoproteins (e.g. LDL & HDL) are a mean for transporting lipids in blood.

5. Imbalance in lipid metabolism can lead to major clinical problems, such as obesity and atherosclerosis.

I. Chemicals reactions of lipids

A- Solubility Test

Objective: to test the solubility of oils in polar & non-polar solvent.

How to perform the test?

1-**Add** about 2ml of the provided oil sample to 5ml of water in a test tube and **try to mix** oil with water. By shaking, oil and water mix initially; however, they gradually separate out to form 2 layers.

2- **Repeat** the experiment using chloroform instead of water.

Result

Oil **dissolves** in the **organic solvent** "chloroform" but **doesn't dissolves** in **water**, because oils contain long hydrocarbon tails which are hydrophobic in nature.

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II- Chemical reaction of fatty acids

Fatty acids: **soluble** in (organic solvents**)** chloroform and ether, **insoluble** in water

A - Cupper Acetate Test

Objective

To **distinguish** between saturated and unsaturated fatty acid.

How to perform the test?

1- **Add** 1ml of each fatty acids (stearic and oleic acids) in 2 test tubes.

2- Each of test tube contain **add** 3ml of petroleum ether, shake tubes until fatty acid dissolve.

3- **Add** 1ml of cupper acetate ($Cu_2(CH_3COO)_4$), **shake** well for minute and wait .

Result

Stearic acid

Oleic acid

The **Blue/green precipitate** is appearing in **the bottom** of tube if fatty acid is **saturated** (palmitic, stearic). But if it is **unsaturated** fatty acids (oleic) the dark **green color** in the **upper solution** will appear.

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B – Un saturated Test

Objective

To **indicates** the presence of double bonds in the lipid sample.

Principle

Halogens **react** across double bonds .The decolorization of solution of iodine by lipid indicates the presence of double bonds in the lipid sample.

The halogens are non-metallic elements such as fluorine, chlorine, bromine, iodine, astatine

How to perform the test:

1- **Add** 1ml of each fatty acids (stearic and oleic acids) in 2 test tubes.

2- **Add** 1ml of chloroform, **shake** tubes until fatty acid dissolve.

3- **Add** drops iodide alcohol (drop by drop) , **shake** well.

Result

Unsaturated acids (Oleic acid): Brown color of the iodine **disappears**

Saturated acids (Stearic acid): Brown color of the iodine **remains**

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III- Chemical reaction of glycerol

Glycerol (or glycerin, glycerine) is a simple **polyol** compound. It is a colourless, odourless, viscous liquid that is widely used as a solvent and in pharmaceutical formulations. Glycerol has three hydrophilic hydroxyl groups (**trihydroxyalcohol**) that are responsible for its solubility in water and its hygroscopic nature. The glycerol **backbone** is central to all lipids known as triglycerides. Glycerol is sweet-tasting and of low toxicity.

Polyol (Sugar alcohols): are organic compounds, typically derived from sugars

A- Denestan experiment:

How to perform the test:

1- **Put** 1ml of borax solution in clean test tube.

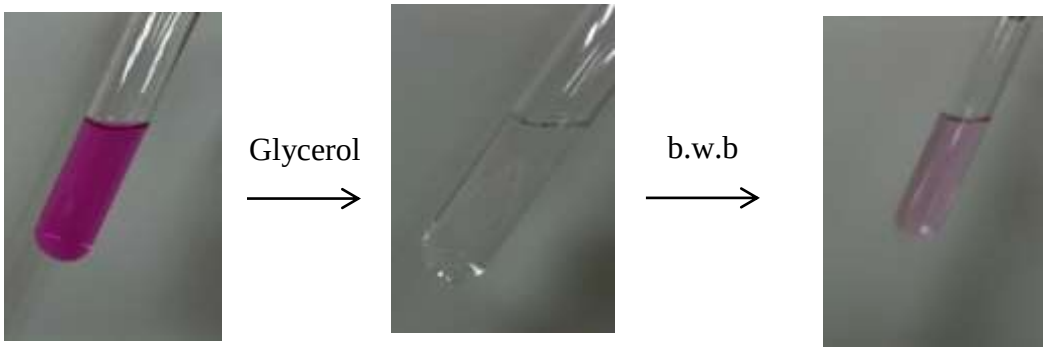
2- **Add** one drop of phenolphthalein (ph.ph), the **pink color** is formation.

3- **Add** dilute glycerol until the red color disappears.

4- **Heat** in the boiling water bath until → **pink color produced again.**

Remember! b.w.b = boiling in water bath

Result



Pink color | **Disappears Pink color** | **Pink color produced again**

Pink color **disappears** and produced **again** after heating

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B- Oxidation test

Objective

To **identify** redox reactions and exothermic reactions of glycerol

Principle

The potassium permanganate **oxidizes** the glycerol to carbon dioxide and water (hence the steam) and is itself reduced.

Potassium permanganate is a strong oxidizing agent and **glycerol** is an easily oxidized substance

How to perform the test

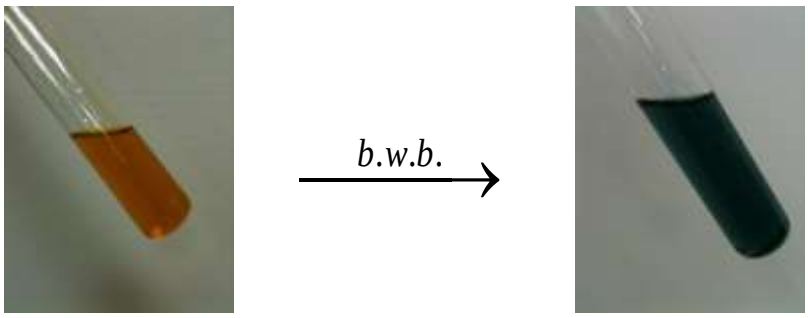
1- **Put** 1ml of glycerol solution in test tube.

2- **Add** one ml of potassium dichromate($K_2Cr_2O_7$).

3- **Add** one drop of concentrated sulfuric acid (H_2SO_4).

4- **Heat** in the boiling water bath few minuets

Result



The solution turns to **green color**

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VI- Chemical reaction of cholesterol

Cholesterol is a waxy substance, which is made in the body by the liver but is also found in some foods also can be taken in from food. It soluble in chloroform and insoluble in water

A- Salkowski test

Objective

To **detect** the presence of cholesterol

Principle

The cholesterol is react as a typical alcohol with a strong concentrated acids and the product are **colored substances**.

How to perform the test

1- **Dissolve** small amount of cholesterol in 2 ml of chloroform (in a dry test tube)

2-**Add** 2 ml of conc. sulfuric acid (H_2SO_4)

Result

The solution becomes **red glow yellow**

B-Liebermann-Burchard test:

Liebermann-Burchard

more specific than **Salkowski**

to detect the cholesterol

Objective

To **detect** the presence of cholesterol

Principle

The cholesterol is react as a typical alcohol with a strong concentrated acids and the product are **colored substances**. Acetic anhydride are used as solvent and dehydrating agents, and the sulfuric acid is used as dehydrating and oxidizing agent .A positive result is observed when the solution becomes red , then blue, and finally bluish –green color.

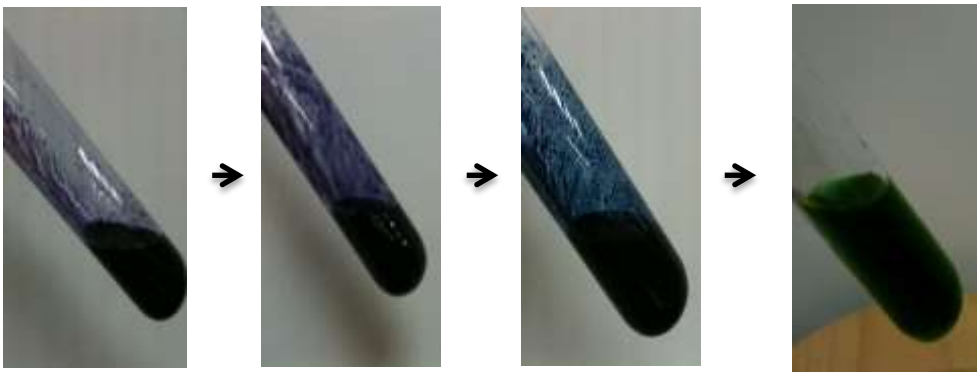
How to perform the test

1- **Dissolve** small amount of cholesterol in 2 ml of chloroform (in a dry test tube)

2- **Add** 10 drops of acetic anhydride ($C_4H_6O_3$), mix.

3-**Add** 2 drops of conc. sulfuric acid (H_2SO_4)

Result



The solution becomes red , then blue, and finally **bluish–green color**

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RESULTS & LAB REPORT

Unknown (fatty acid –stearic acid)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (fatty acid –oleic acid)

Physical properties

Chemical properties

Test	Observation	Result

RESULTS & LAB REPORT

Unknown (glycerol)

Physical properties

Chemical properties

Test	Observation	Result

RESULTS & LAB REPORT

Unknown (cholesterol)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (1)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (2)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (3)

Physical properties

Chemical properties

Test	Observation	Result

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Qualitative analysis of proteins and amino acids

Introduction of protein

Protein is a complex, high-molecular-weight organic compound that consists of amino acids joined by peptide bonds, its contain C, H, O, and Nitrogen.

Common structure: Central C, with an H, amino group (NH_2), and an acid group (COOH), and a side group (R), proteins made up of about 20 different amino acids. **Unique Side Groups,** differ in size, shape, electrical charge.

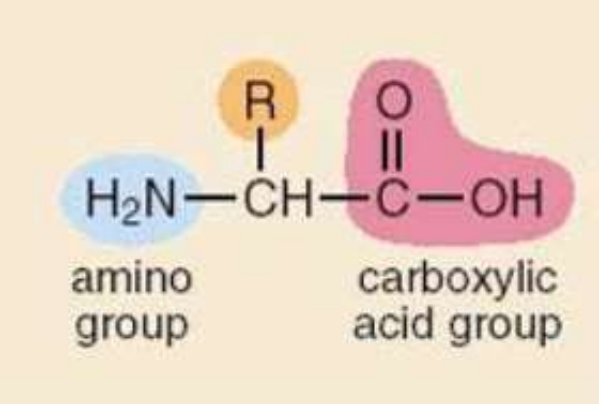


Figure 1: The structure of amino acid

The function of protein

Proteins are probably **the most important class** of biochemical molecules, although of course lipids and carbohydrates are also essential for life. They are the **basis** for **the major structural components** of animal and human tissue.

Proteins are **essential** to **the structure** and **function** of all **living cells** and **viruses**. Many proteins are **enzymes** or **subunits of enzymes**, which is catalyzing chemical reactions. Other proteins play structural or mechanical roles, such as those that form the struts and joints of the cytoskeleton, serving as biological scaffolds for the mechanical integrity and tissue signaling functions. It can be **hydrolyzed** by acids, bases or specific enzymes.

The classification of Proteins based on solubility and composition

According to this classification, proteins are divided into three main groups as **simple**, **conjugated** and **derived** proteins.

1- Simple proteins

This group includes proteins **containing only amino acids**, as structural components. These proteins are further classified based on their solubility in different solvents as well as their heat coagulability (The ability to coagulate, of being coagulable). Such as **Egg albumin** and **Globulins**.

2- Conjugated or compound proteins

These are simple proteins **combined** with **some non-protein substances** known as **prosthetic groups**. The nature of the non-protein or prosthetic groups is the basis for the sub classification of conjugated proteins. Such as **nucleoproteins** (combination of simple basic proteins) and **phosphoproteins** (combination with phosphoric acid, e.g. **Casein** of milk).

3- Derived proteins

These are **proteins derived by partial to complete hydrolysis** from the simple or conjugated proteins by the action of heat, enzymes or chemical reagents. This group also includes the artificially-produced polypeptides. Such as **peptone** and **gelatin**.

Lab experiments.

A- Physical properties

Solubility- color- shape.

Solubility of proteins

Identify the solubility of different types of proteins in different solvents.

Principle

Simple proteins dissolve easily in water while **soluble protein complex** by low solubility in distilled water. The proteins are derived insoluble in water but soluble in alkaline solutions.

How to perform the test:

1- Test the solubility of each **(albumin, casein, gelatin, peptone)** in cold water and then in hot water and then in sodium hydroxide solution.

2- Record solubility of all proteins in cold water and hot water and sodium hydroxide solution in the following table of results.

Protein	Albumin	Casein	Gelatin	Peptone
Distilled water				
Hot water				

Hydroxide sodium (NaOH)				

B. Chemical properties (color test)

1- Biuret Test

Principle

It is the **general test** for all proteins. Biuret reagent is dilute **Copper (II) sulfate** ($CuSO_4$) in strong alkaline medium. Alkaline CuSO4 **reacts** with all compounds containing 2 or more peptide bonds to give **a blue-violet color**.

How to perform the test:

1- **Put** 2ml from protein solution (albumin, casein, gelatin, and peptone) in 4 test tubes.

2- **Add** 1 ml of biuret reagent (2 ml of NaOH 10% + 1 drop of $CuSO_4$ 1%).

3- **Mix** well.

Result

Show positive test for **all proteins,** is indicated by the formation of a violet color.

Positive test

Notes

The **reaction with peptone** may be gave pink color when the solution is not concentrated.

2- Ninhydrin Test

Principle

Amino acids **contain** a free amino group and a free carboxylic acid group that **react** together with ninhydrin to produce a colored product. When an amino group is attached to the first, or alpha, carbon on the amino acids carbon chain, the amino group's nitrogen atom is part of **a blue-purple product.** Proteins also contain free amino groups on the alphacarbon and can react with ninhydrin to produce a blue-purple product.

How to perform the test:

1- **Put** 1 ml from protein solution (albumin, casein, gelatin, and peptone) in 4 test tubes.

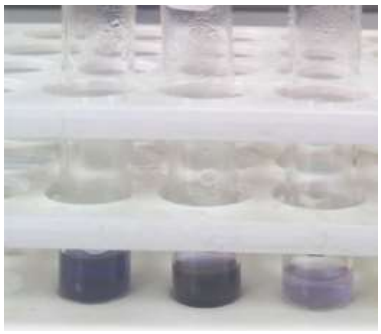
2- **Add** 1 ml of 0.5% ninhydrin – ethanol reagent solution.

3- **Mix** well

4- **Place** the test tubes into the boiling-water bath for 5 minute.

Result

Show positive test for **all proteins,** is indicated by the **formation of a blue-purple**



Positive test

Ninhydrin is a strong oxidizing agent, it should be handled with care, and applied apart from contact with skin or eyes. The gloves and mask is a must be used.

3- Coagulation (clotting) Test

Principle

In this test we see the **denaturating proteins at high temperature** due to change shape, chemical and physical properties of proteins.

How to perform the test

1- **Put** 1ml from protein solution (albumin, casein, gelatin, and peptone) in 4 test tubes.

2- **Heat** the flame directly on the tube and slightly inclined.

Result

Show positive test for **albumin only,** is indicated by the formation of coagulation of the solution

Positive test

4- Freezing Test

Principle

In this test we see the **change shape at low temperature** due to change shape, chemical and physical properties of proteins.

How to perform the test

1- **Put** 3ml from protein solution (albumin, casein, gelatin, and peptone) in 4 test tubes.

2- **Put** the tube in the Cup by the snow and leave for five minutes.

Result

Show positive test for **gelatin only,** is indicated by the formation of freezing solution

Positive test

5- Phosphorus Test

Principle

The basic idea of this test is to **detect** the presence of amino acids that contain the element of **phosphorus**

How to perform the test

1- **Put** 1ml from protein solution (albumin, casein, gelatin, and peptone) in 4 test tubes.

2 - **Add** 1ml of alkaline solution of 20% NaOH.

3 - **Heat** in a boiling- water bath for 10 minutes.

4-**Add** in the order as follows: (1ml of Molbidic acid and + 1ml sodium sulphate + 1ml of 0.5% hydroquinone) respectively.

Result

Show positive test for **casein only,** is indicated by the formation of blue color.

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6- Sulfur Test

Principle

The basic idea of this test is to **detect** the presence of amino acids that contain sulfur such as **cysteine**.

How to perform the test

1- **Put** 1ml from protein solution (albumin, casein, gelatin, and peptone) in 4 test tubes.

2 - **Add** 1ml of alkaline solution of 40% NaOH.

3 - **Heat** in a boiling- water bath for 10 minutes.

4-**Cool** solution and add 1ml 1% lead acetate.

Result

Show positive test for **albumin,** is indicated by the formation of **black-brown precipitatio**

Show positive test for **peptone,** is indicated by the formation of **yellow precipitation.**

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7- Sakaguchi Test

Principle

The basic idea of this test is to **detect** the presence of amino acids that contain guanidine group such as **arginine**.

How to perform the test:

1- **Put** 2ml from protein solution (albumin, casein, gelatin, and peptone) in 4 test tubes.

2- **Add** 1ml of alkaline solution of 10% NaOH.

3 - **Add** 1ml of 10% ά-naphthol.

4 - **Mix** well and cool in ice.

5- **Add** 1ml alkaline hypochlorite solution.

Result

Show positive test for **albumin, casein, gelatin and peptone** are indicated by the formation of color.

8- Xanthoproteic Test

Principle

The basic idea of this test is to **detect** the presence of amino acids that contain **benzene ring** such **phenylalanine**, **tryptophan** and **tyrosine**.

How to perform the test:

1- **Put** 2ml from protein solution (albumin, casein, gelatin, and peptone) in 4 test tubes.

2- **Add** 1ml of concentrated nitric acid (HNO_3).

3 - **Heat** the mixture in water bath for 30 minutes.

4-**Cool** and add drop-wise 40% NaOH to render the solution alkaline.

Result

Show positive test for **albumin, casein and peptone** are indicated by the formation of **orange color or orange precipitation.**

Notes

The gelatin ⟶ remains yellow color.

The peptone ⟶ convert to orange color.

The albumin and casein ⟶ convert to orange precipitation**.**

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RESULTS & LAB REPORT

Unknown (albumin)

Physical properties:

Chemical properties:

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (casein)

Physical properties

Chemical properties

Test	Observation	Result

Organized by: Duaa Zahim & Dalal Al-Saedi

RESULTS & LAB REPORT

Unknown (gelatin)

Physical properties

Chemical propertie

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (peptone)

Physical properties

Chemical properties

Test	Observation	Result

RESULTS & LAB REPORT

Unknown (1)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (2)

Physical properties:

Chemical properties:

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (3)

Physical properties:

Chemical properties:

Test	Observation	Result

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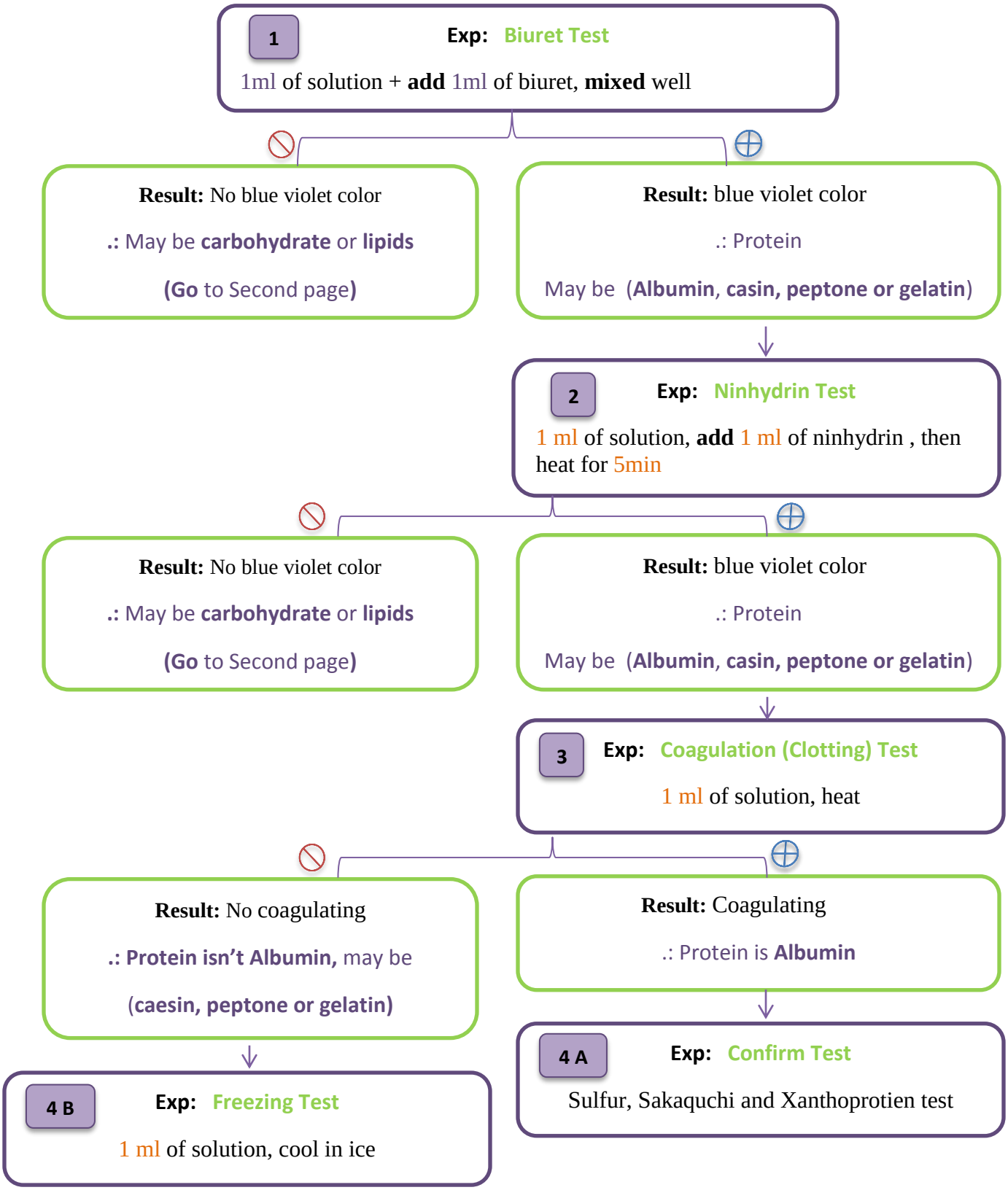
Scheme for Unknown Proteins



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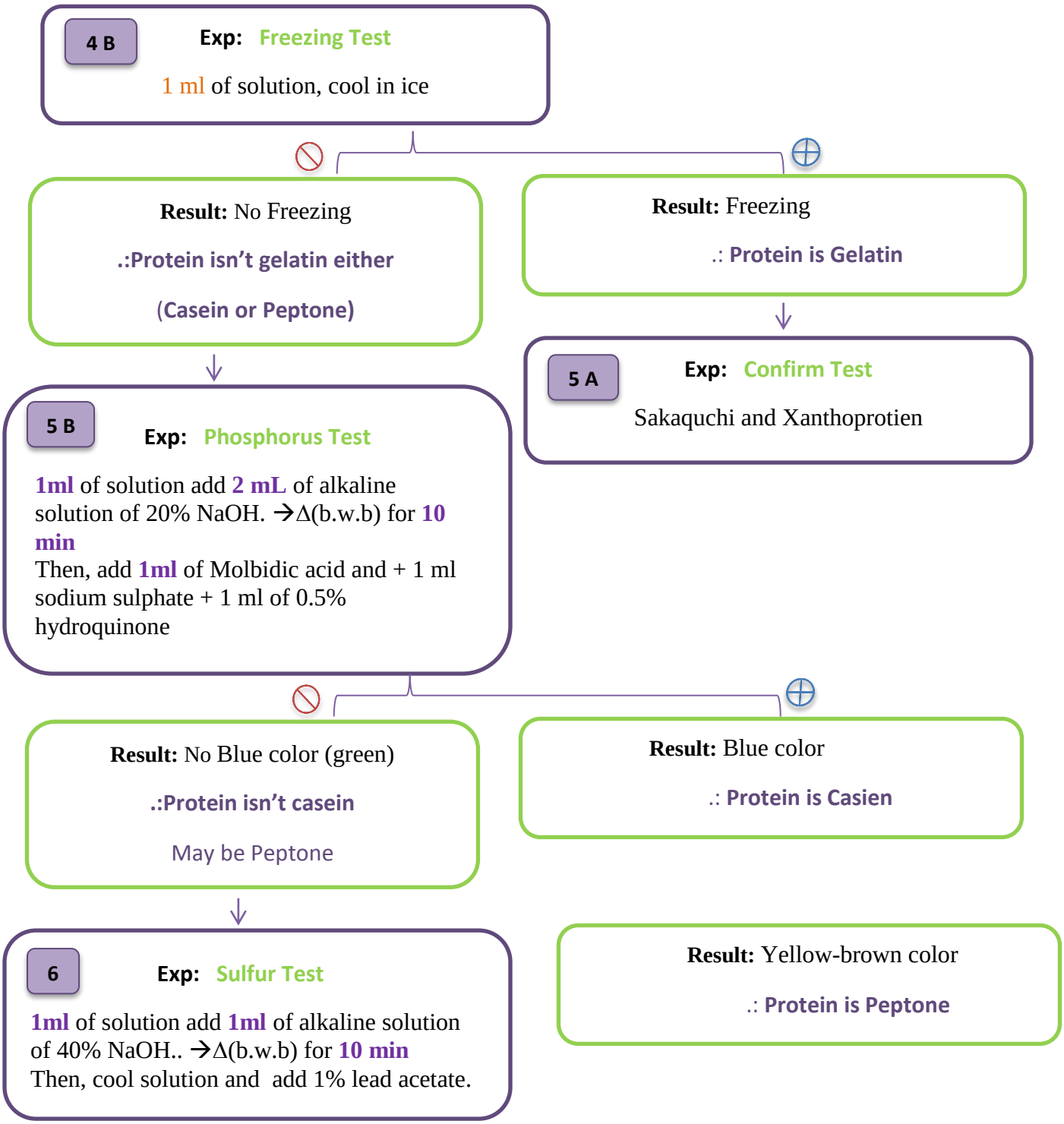

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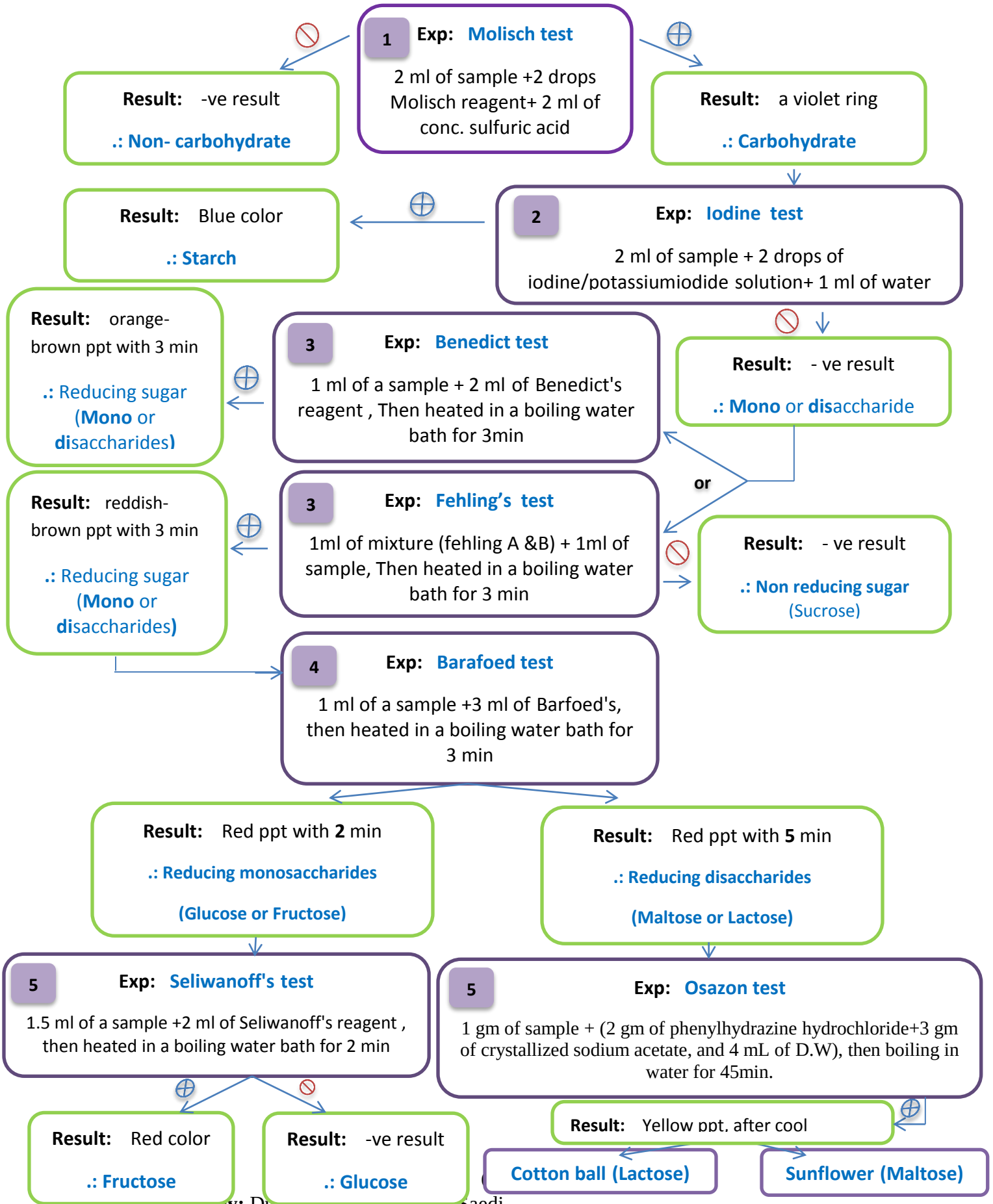
Scheme for Unknown Carbohydrates



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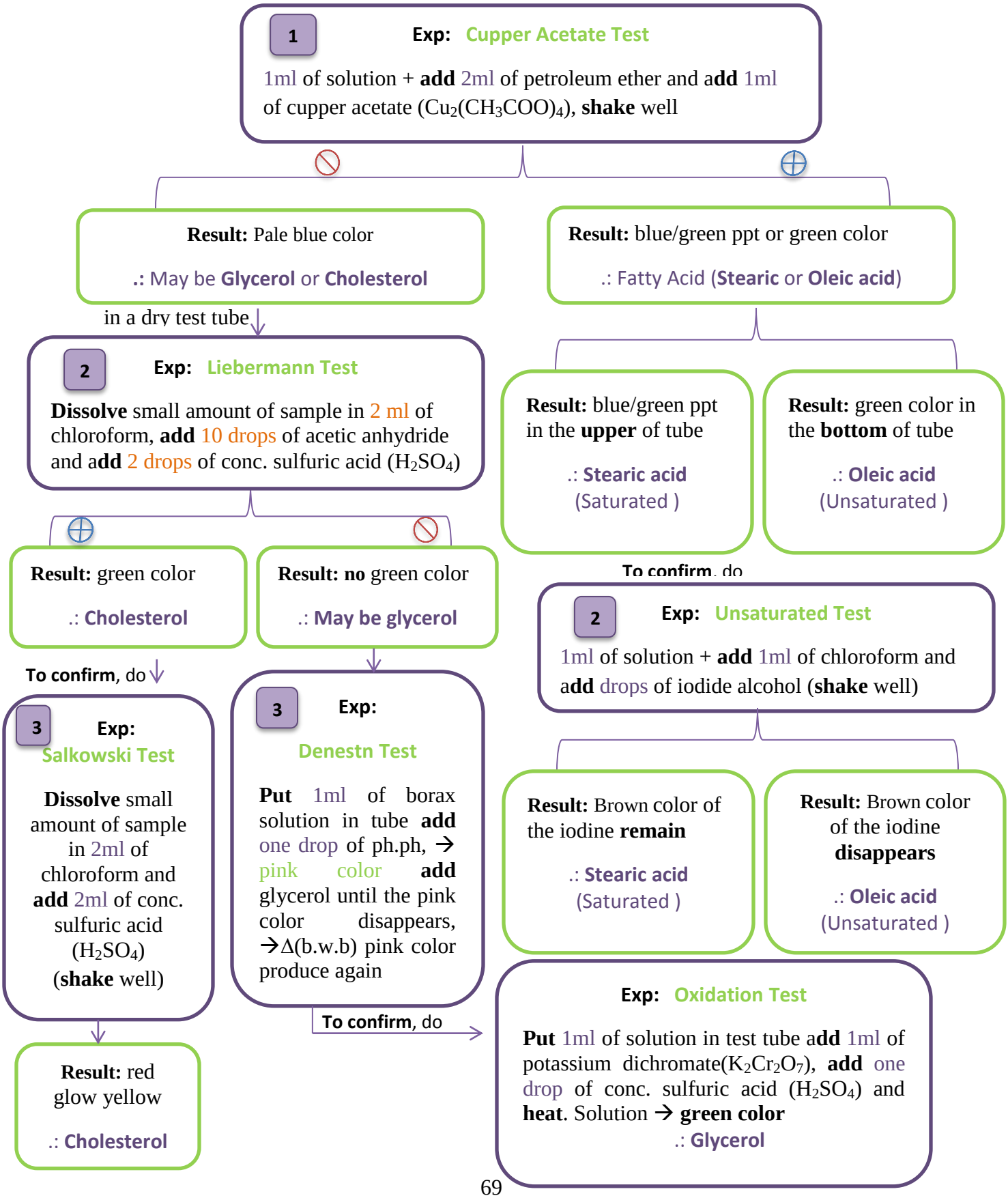
Scheme for Unknown Lipids



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RESULTS & LAB REPORT

Unknown (1)

Physical properties

Chemical properties

Test	Observation	Result

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RESULTS & LAB REPORT

Unknown (2)

Physical properties

Chemical properties

Test	Observation	Result

Organized by: Duaa Zahim & Dalal Al-Saedi

RESULTS & LAB REPORT

Unknown (3)

Physical properties

Chemical properties

Test	Observation	Result

Organized by: Duaa Zahim & Dalal Al-Saedi