University of Baghdad
College of Science for Women
Department of Chemistry

BIOCHEMISTRY LAB

Third class/ First course

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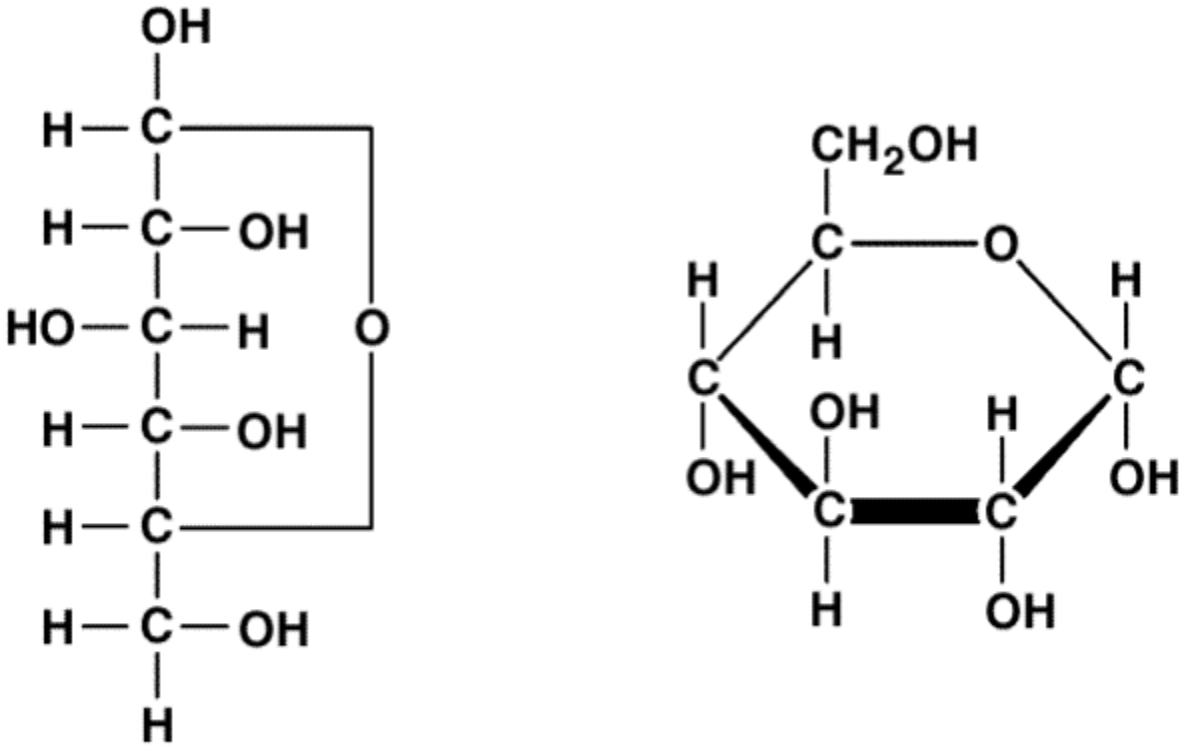
2025-2026

Carbohydrates

Carbohydrates are aldehyde or ketone derivatives of polyhydric alcohols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Glucose

- **They are widely distributed in plants and animals . Plants synthesize glucose by photosynthesis and it is converted mainly to storage form, the starch and structural frame work form, the cellulose.**

- **Animals largely depend on plant source to obtain carbohydrates though they can synthesize carbohydrates from non carbohydrates sources like glycerol and amino acids in their body (gluconeogenesis).**
- (استحداث السكر)

- **The Glucose is the major form of carbohydrate absorbed from the gut in humans.**
- **According to the metabolic status it has different fates:**
- **catabolized to release energy**
- **polymerized to form the storage fuel(the glycogen)**
- **sometimes converted to other sugars like fructose and galactose.**

- **Different carbohydrates are present in intracellular and extracellular fluids and are excreted in urine when the concentration of them rises in the blood as in certain diseases)**

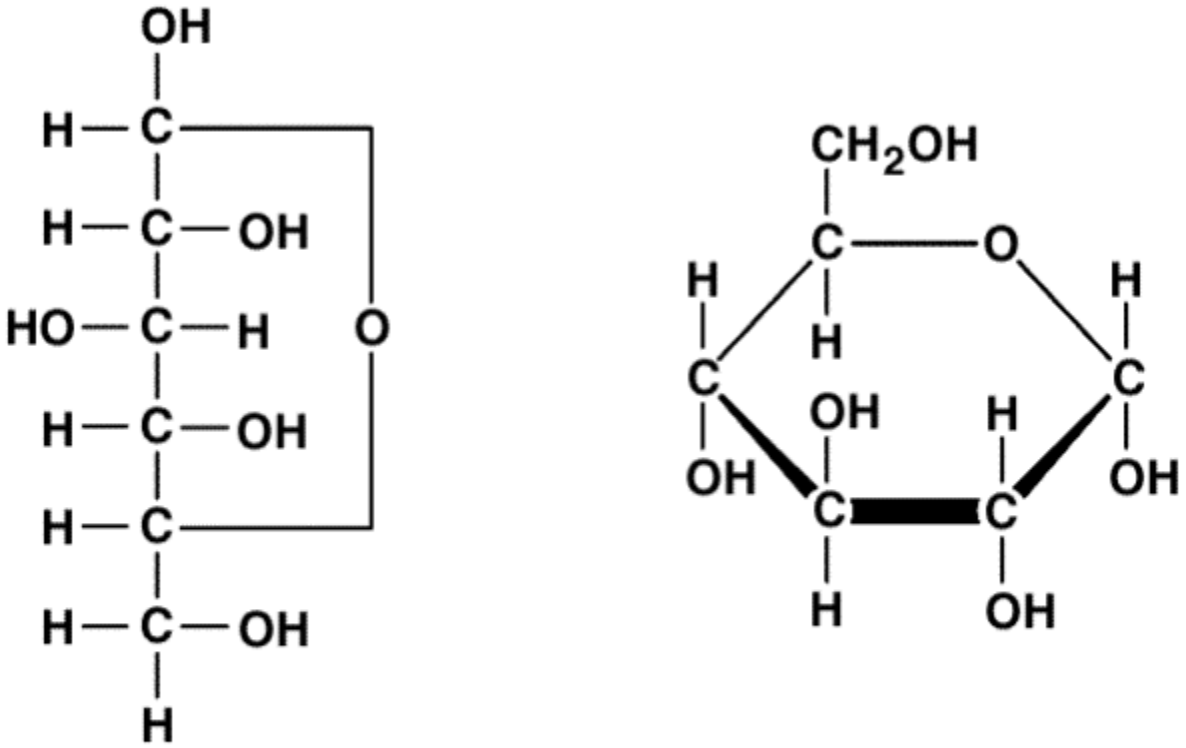
- (Glucose in urine in diabetes mellitus, fructose in urine in fructosuria , Galactose in urine in galactosemia). Hence, it is essential to understand the tests for their detection.

The classification of **carbohydrates** will be useful for the detection of various types of carbohydrates by different chemical tests.

The classification of **carbohydrates**

- **Monosaccharides:**
- Cannot be hydrolyzed into simpler carbohydrates. They are classified into trioses ,tetroses, pentoses, hexoses,heptoses based on the number of carbon atoms present in them. They are again divided into aldoses and ketoses based on the functional group present in them

Classification of Monosaccharides		
Monosaccharides	Ketoses	Aldoses
Trioses	Dihydroxyacetone	Glycerose
Tetroses	Erythrulose	Erythrose
Pentoses	Ribulose	Ribose
Hexoses	Fructose	Glucose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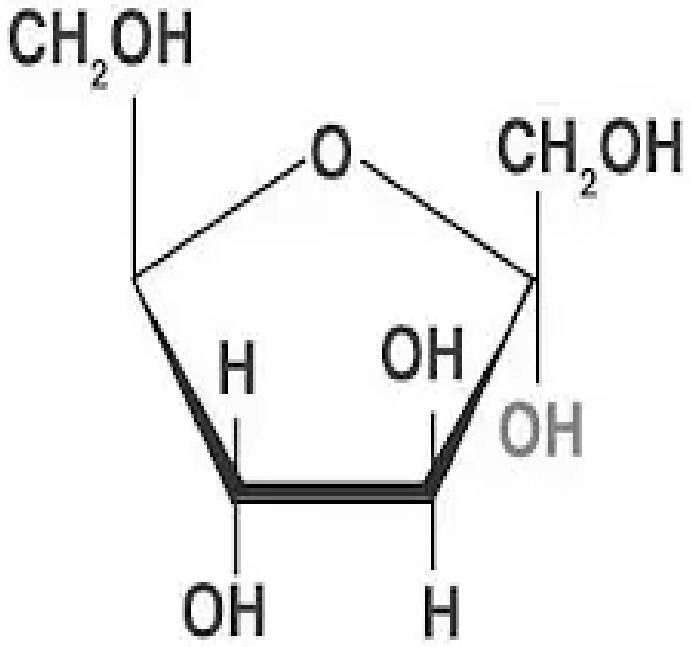
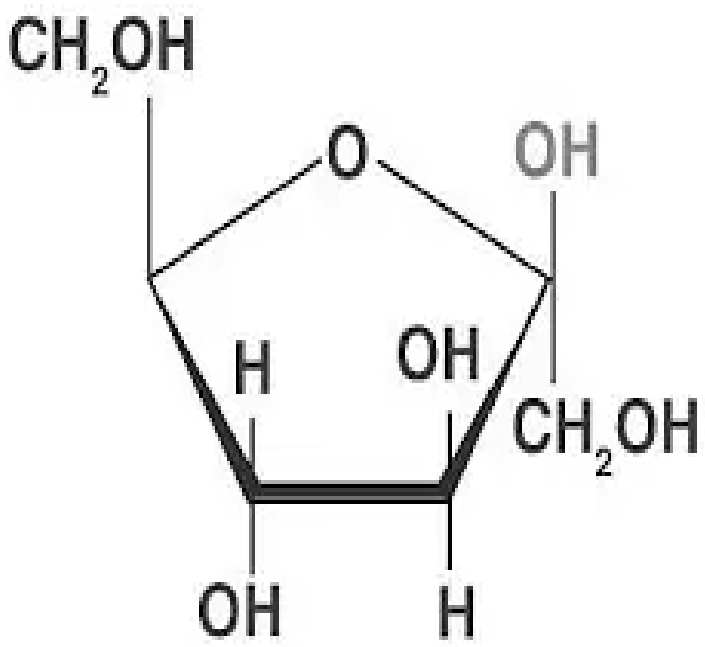
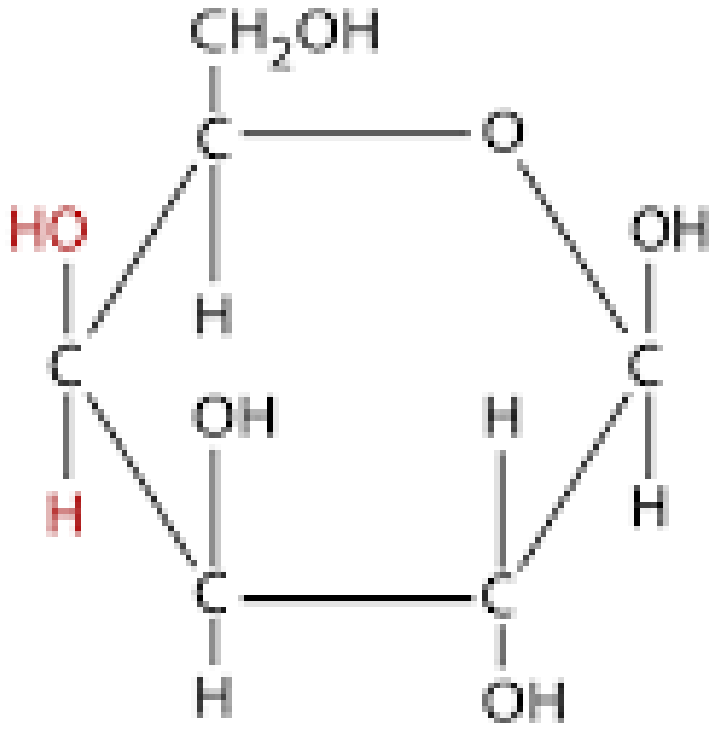
Glucose



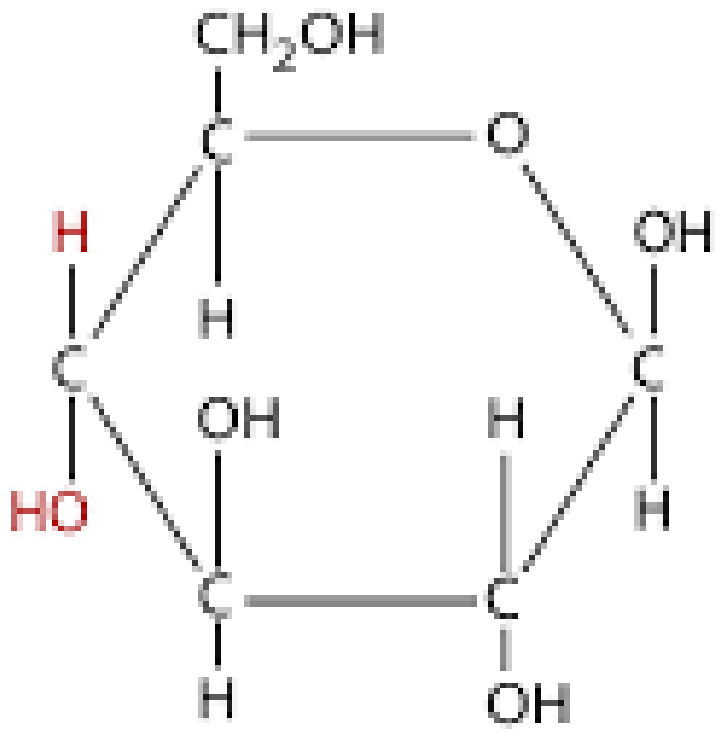
α-D- Fructose



β-D- Fructose



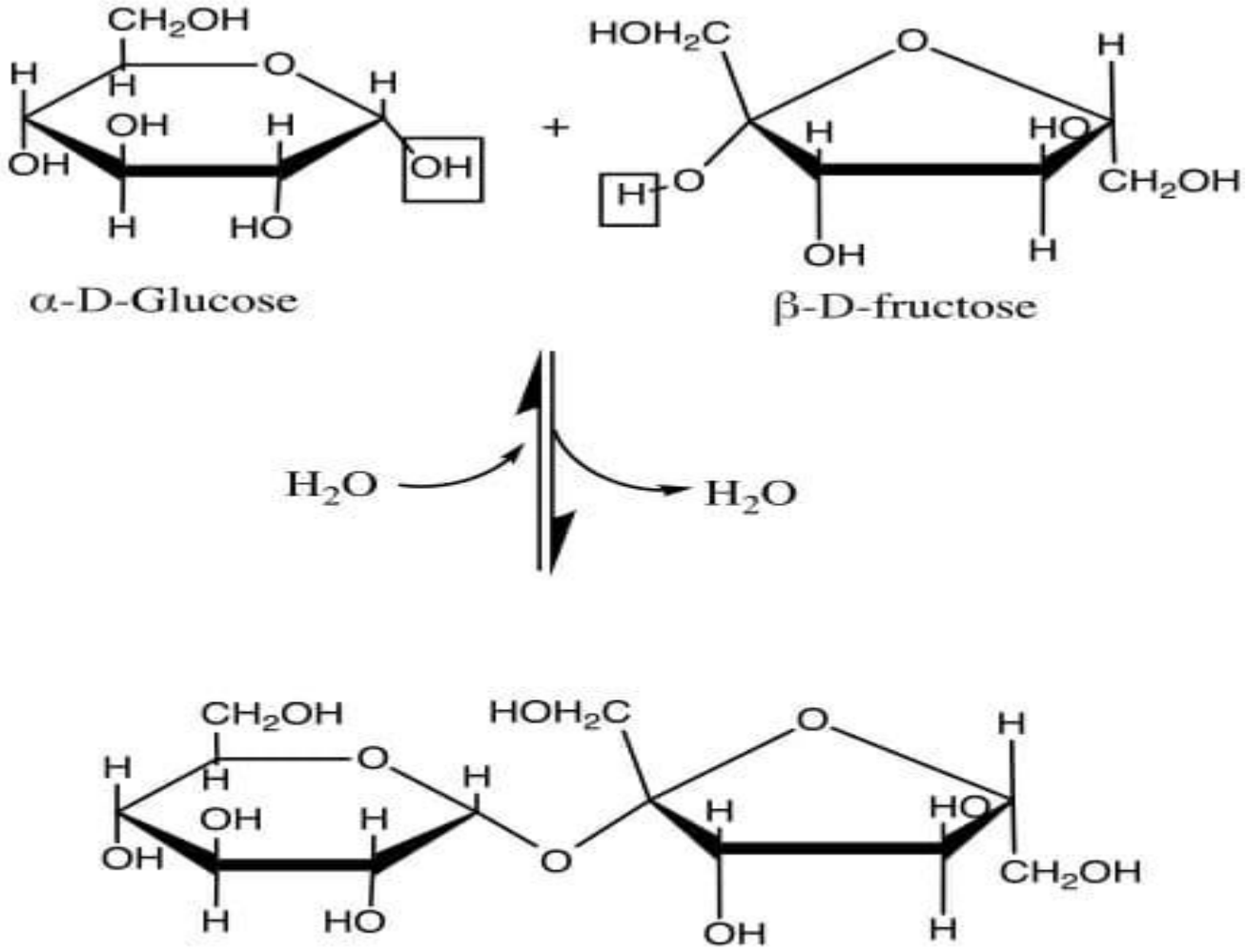
Galactose



Glucose

Disaccharides

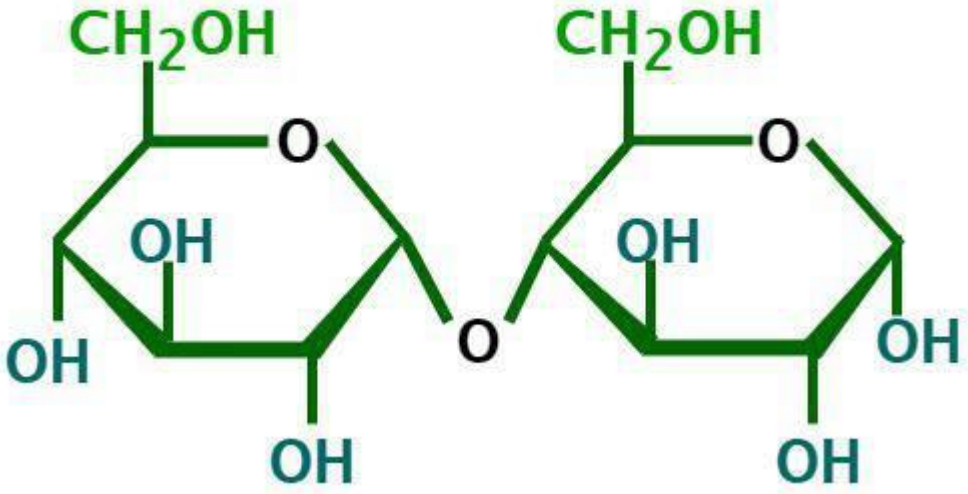
- : **Disaccharides** Give rise to two monosaccharide units upon hydrolysis
- E.g.: Sucrose (glucose + fructose)
  Lactose (glucose + galactose)
  Maltose (glucose + glucose)
-



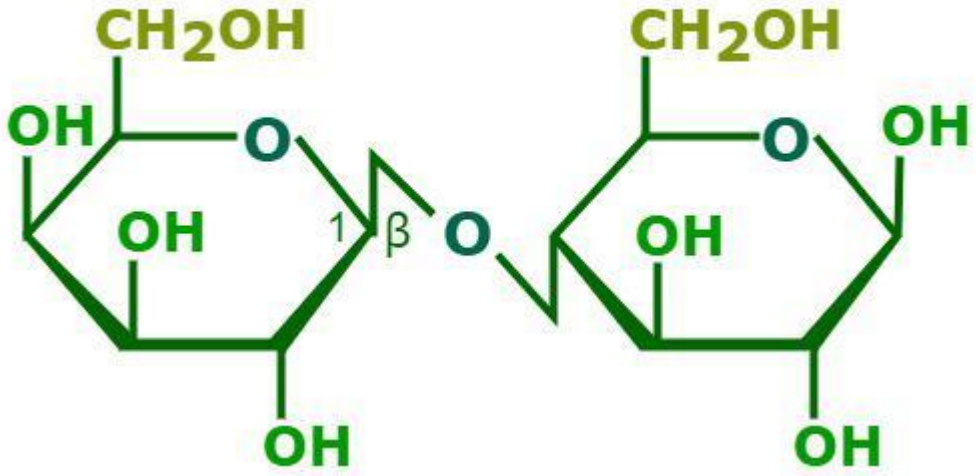
α-D-Glucose + β-D-fructose

α-D-glucopyranosyl-(1→2)-β-D fructofuranose
(Sucrose)

Maltose



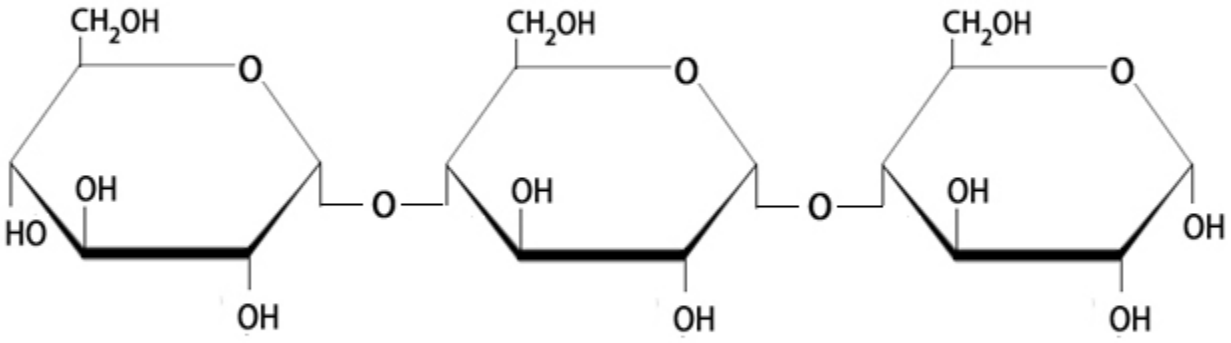
Lactose



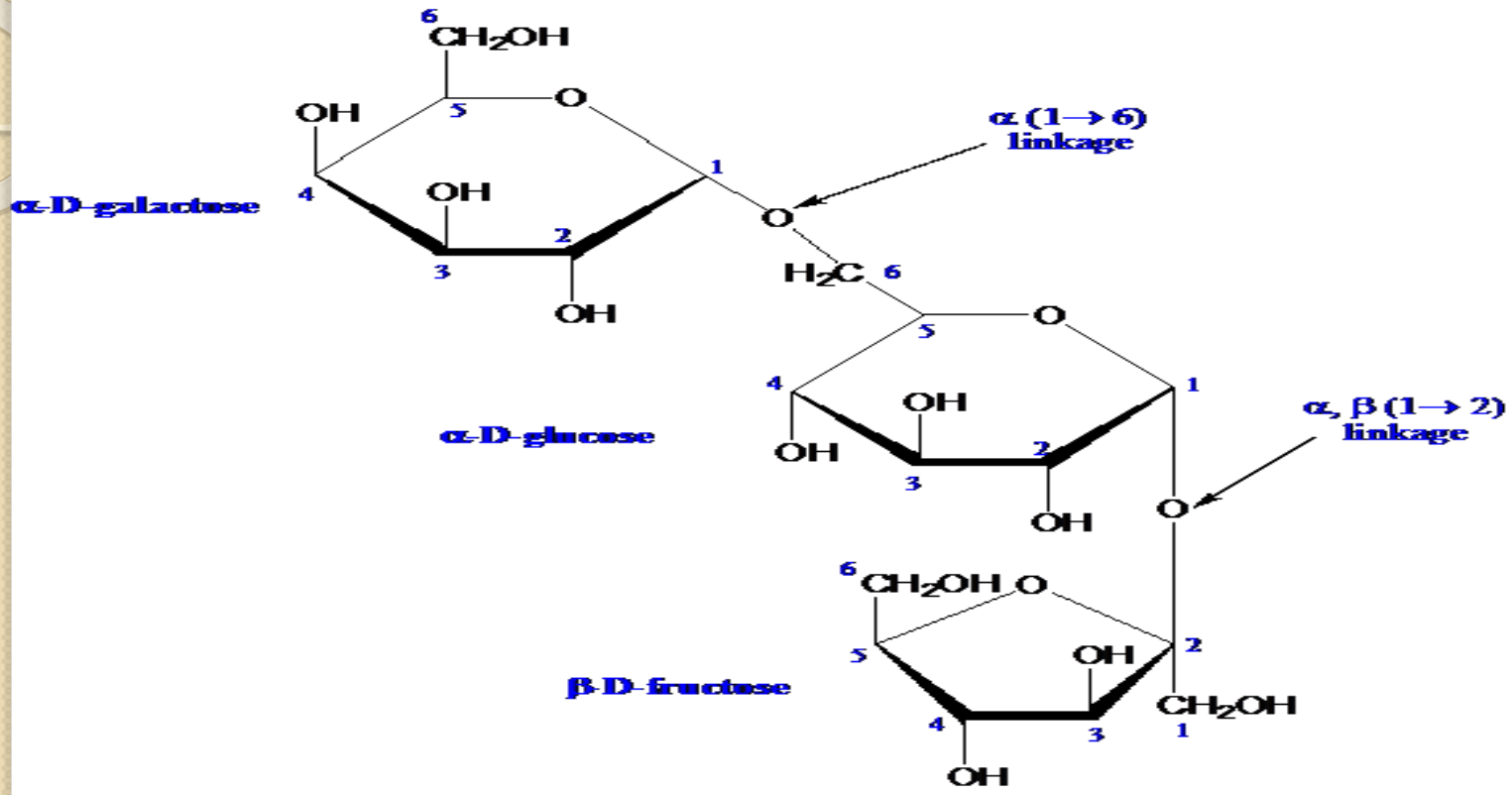
Oligosaccharides:

- **Oligosaccharides:** Yields less than ten monosaccharides.
- E.g.: Maltotriose (3 glucose units),
- Raffinose (glucose + fructose + galactose)

Maltotriose

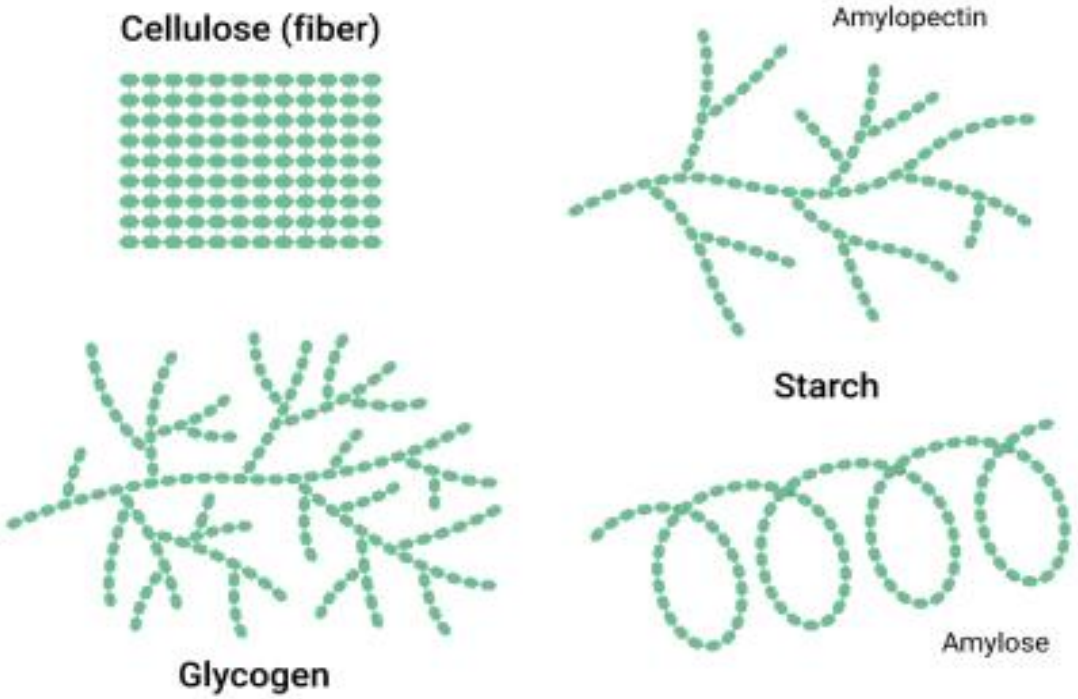


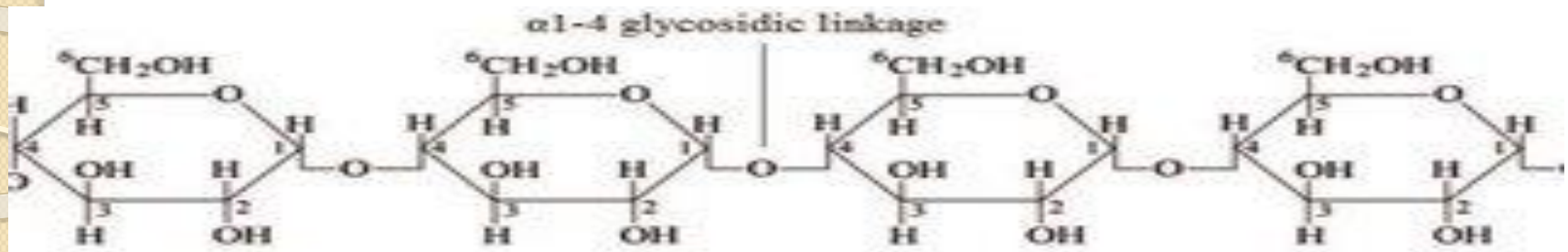
Glucose Glucose Glucose


α-D-galactose
α-D-glucose
β-D-fructose
α (1→6) linkage
α, β (1→2) linkage
RAFFINOSE

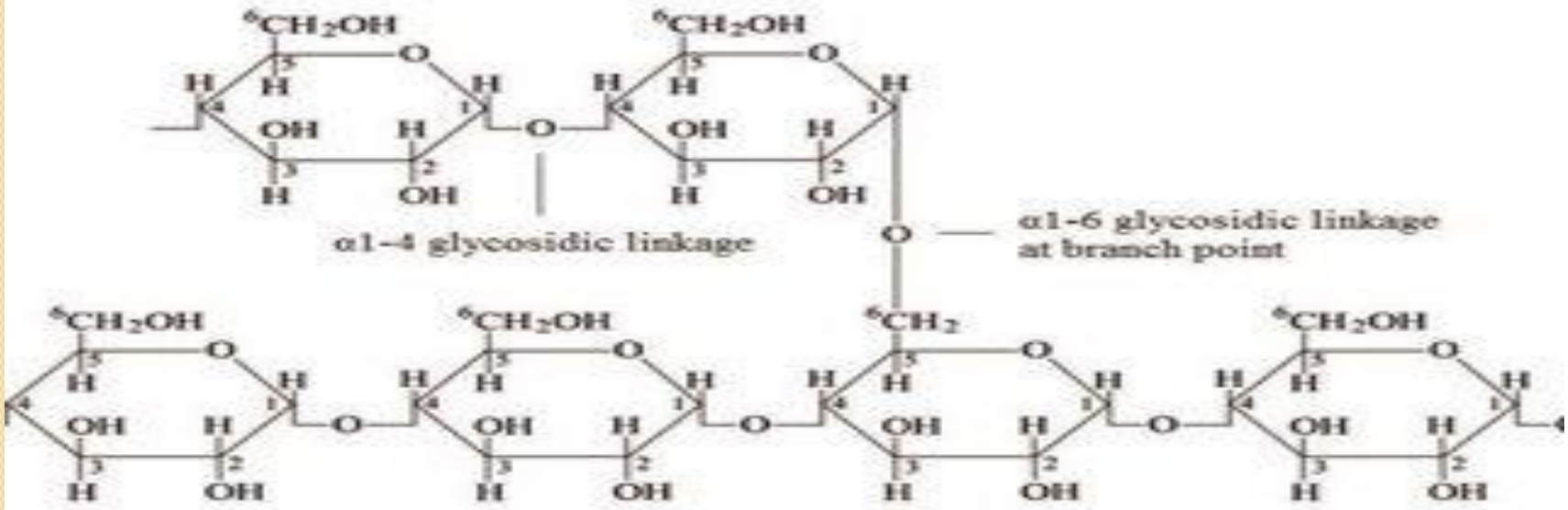
Polysaccharides:

- **Polysaccharides: Contain more than ten monosaccharide units**
  - **Homopolysaccharides (consisting of same type of monomeric units)**
- **Polymer of glucose: Starch, glycogen, cellulose Polymer of fructose: Inulin**
  - **Heteropolysaccharides (consisting of different types of monomeric units)**
- **Proteoglycans, e.g. Heparin (D-glucosamine sulfate + D-sulfated iduronic acid)**
- **Hyaluronic acid (D-b glucuronic acid + N-acetylglucosamine).**


Cellulose (fiber)
Amylopectin
Starch
Glycogen
Amylose



Amylose



Amylopectin

REACTIONS OF MONOSACCHARIDES

- Monosaccharides possess one or more hydroxyl groups and an aldehyde or keto group. Therefore many reactions of monosaccharides are the known reactions of alcohols , aldehydes or ketones. Many of the reactions shown by monosaccharides are exhibited by higher carbohydrates also. Differences in the structures of sugars often affect the rate of a reaction and sometimes the ability to react.

- **The reactions due to hydroxyl group:**
- Dehydration (e.g. Molisch test, Rapid furfural test , Seliwanoff's test)
- **The reactions due to carbonyl group:**
- Reduction (e.g. Benedict's test, Barfoed's test)
- Condensation (e.g. Osazone test)

- **Glucose, Fructose, Lactose, Maltose, Sucrose, Starch Solutions:** 1% solutions -Weigh 1 gm of respective sugars and dissolve in 100 ml of water

Molisch Test

- **Procedure:**
- To 5 ml of sugar solution in a test

tube add two drops of Molisch reagent. mix

- Add 3 ml of concentrated sulphuric acid along the sides of the test tube by slightly inclining the tube, thus forming a layer of acid (acid being heavier goes down beneath the sugar solution) in the lower part.

Molisch's Reagent:

Dissolve 5 g of a-naphthol in 100 ml of 95% of alcohol

- **Application of the test**

- Used as a general test to detect carbohydrates.

Principle:

- Concentrated acid dehydrates the sugar to form furfural (in the case of pentoses) or furfural derivatives (hexoses and heptoses) which then condense with a-naphthol to give a **reddish violet** colored complex

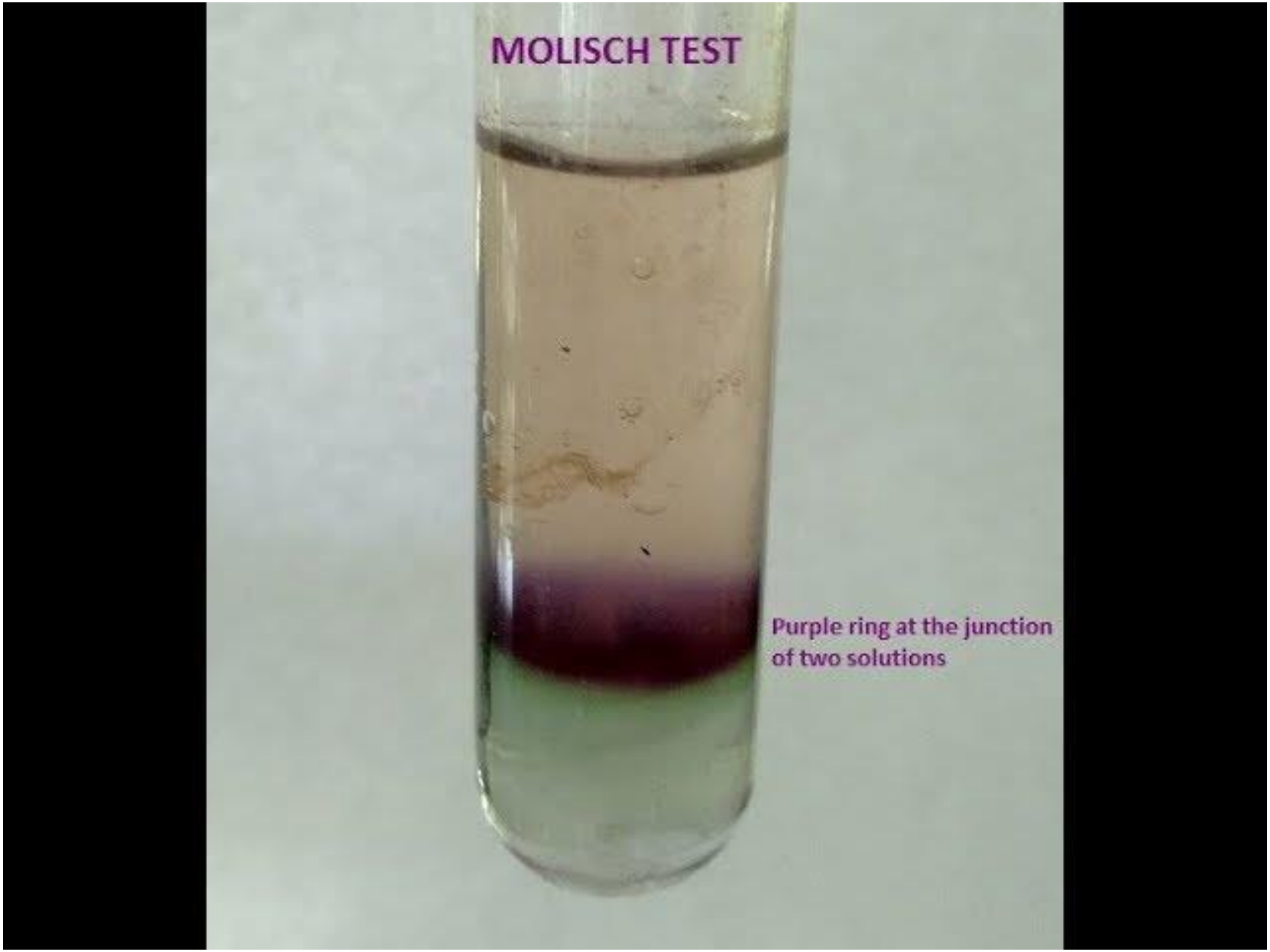
Observation:

- A **reddish violet ring** appears at the junction of two liquids.
- **Inference:** Indicates presence of a carbohydrate and hence the presence of monosaccharide.

https://youtu.be/I1qcTVs3F4Q?si=K3hMq_UJOMhrmiRh

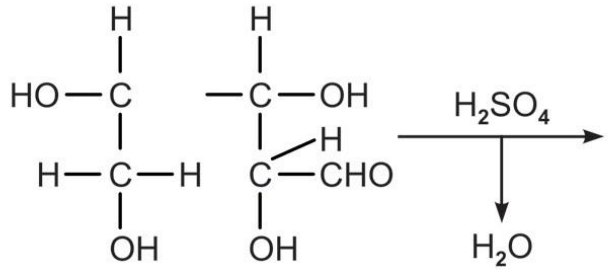
Aberrant Observations

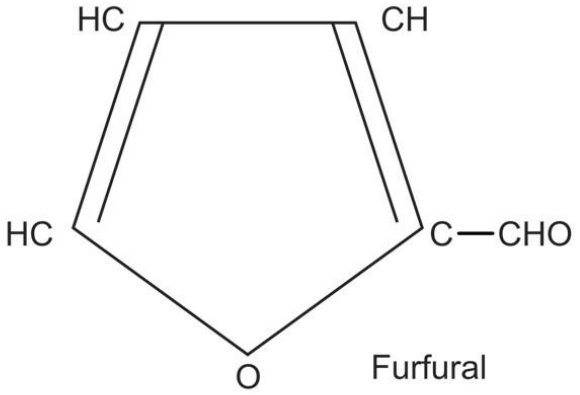
- Instead of a violet ring in the Molisch test, appearance of **dark brown color** indicates **charring of sugar** due to the **heat generated during the addition of acid** (acid water interaction generates heat). It will become obvious when the concentration of the sugar solution is high. To avoid charring, dilute the sugar sample solution with water as depicted in figure 1A-2 and repeat the Molisch test.
- Appearance of a **green color** while doing the test, which persist even after completion of the test suggest excess use of Molisch reagent than required or due to the presence impurities in the reagent.


MOLISCH TEST
Purple ring at the junction
of two solutions

Molisch test

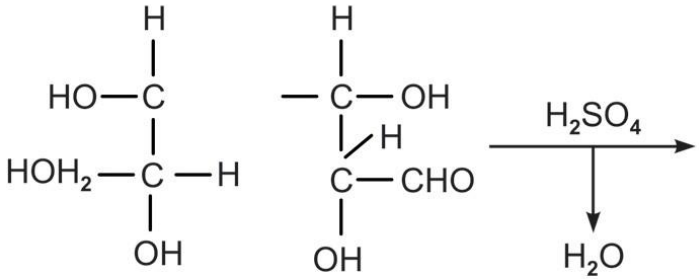
Pentose

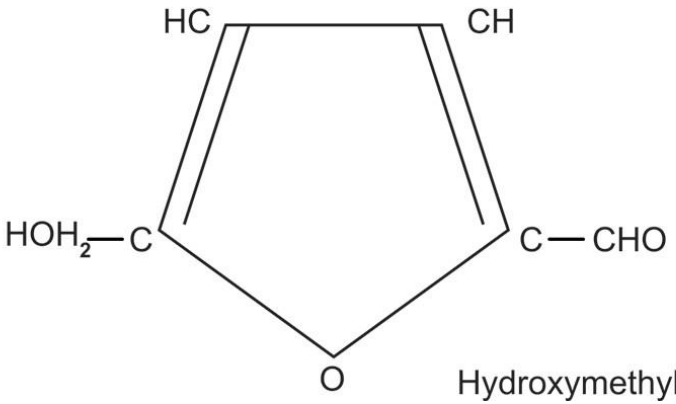




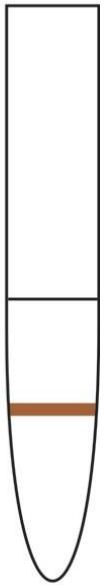
Furfural

Hexose





Hydroxymethylfurfural



Benedict's Test

- To 5 ml of Benedict's reagent in a test tube add exactly 8 drops of the sugar solution. Mix well.
- Boil the solution vigorously for two minutes or place in a boiling water bath for three minutes. Allow the contents to cool by keeping in a test tube rack. Do not hasten cooling by immersion in cold water

Application of the test:

- To detect reducing sugars. It is widely used in detecting glucose in urine even though not specific for glucose.

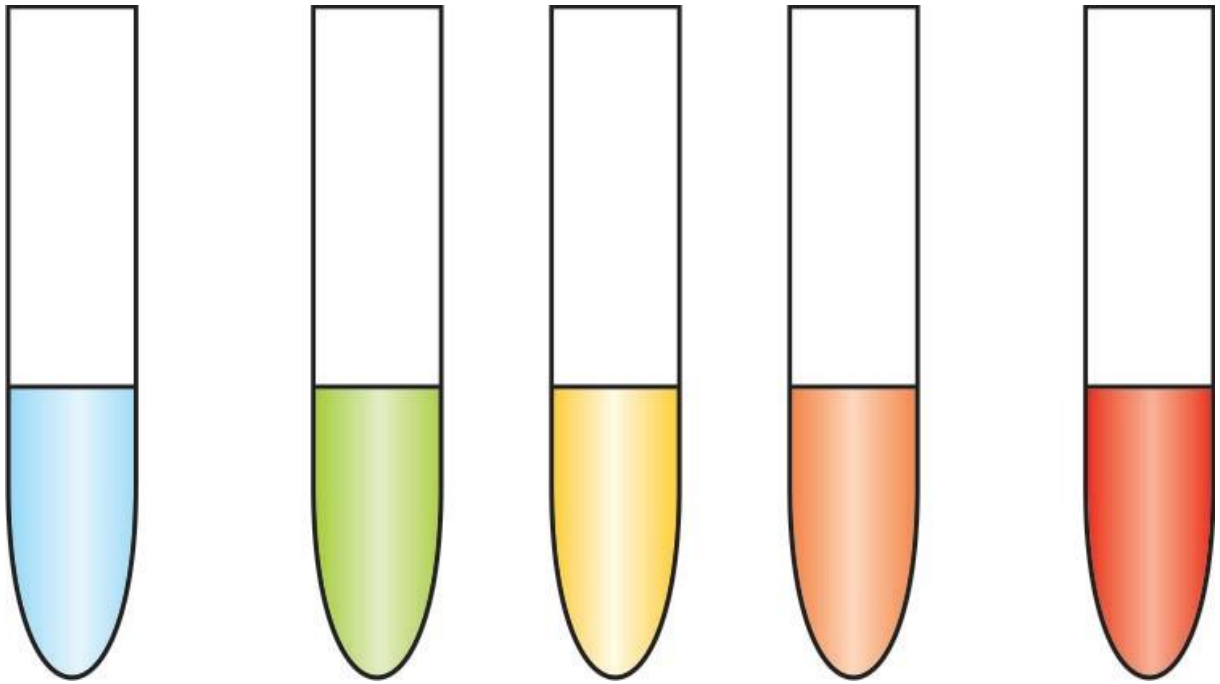
Observation:

- The entire body of the solution will be filled with a precipitate, the color of which varies with the concentration of the sugar solution : green, yellow, orange or red.
- In the absence of reducing substance, blue color of the Benedict's reagent remains as such.
- https://youtu.be/nlPHeqHOYpU?si=H-zwDpuPYtbH5OTy

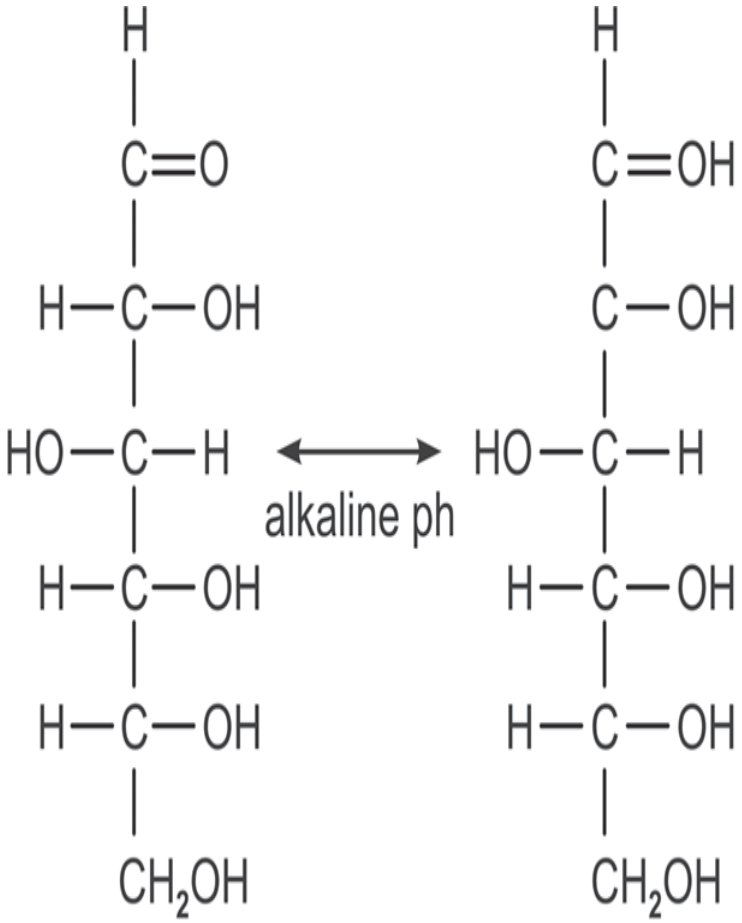
Inference:

- Reducing monosaccharides, glucose, fructose, galactose and mannose give a positive reaction with Benedict's reagent.
- The color of the precipitate give an idea about the concentration of the sugar solution as shown below.
- Blue : absence of reducing sugar
- Green :up to 0.5 gm%
- Yellow : > 0.5 to 1.0 gm%
- Orange : > 1.0 to 2.0 gm%

❖Thus, Benedict's test is described as a semi- quantitative test.

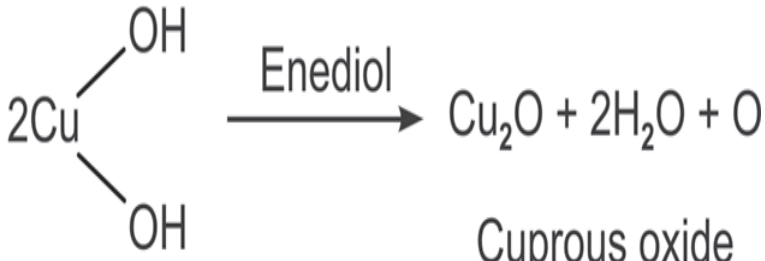


Benedict's test at different sugar concentrations



Glucose

Enediol



Cuprous oxide
(yellow to red)

Cupric hydroxide
(blue)

Principle:

- Carbohydrates with a free aldehyde or keto group have the ability to reduce various metallic ions. In this test cupric ions are reduced to cuprous ions by the enediols formed from sugars in the alkaline medium of Benedict's reagent.
-

Benedict's reagent

- **Benedict's reagent** contains copper sulphate, sodium citrate and sodium carbonate.
- ***Copper sulphate*** dissociate to give sufficient cupric ions (in the form of cupric hydroxide) for the reduction reactions to occur.
- ***Sodium citrate*** keeps the cupric hydroxide in solution without getting precipitated.
- ***Sodium carbonate*** (Na_2CO_3) make the pH of the medium alkaline.

The medium alkaline.

- In the alkaline medium sugars form enediols which are powerful reducing agents. They reduce blue cupric hydroxide to insoluble yellow to red cuprous oxide

Benedict's Qualitative Reagent

- Heat to dissolve 173 g sodium citrate and 100 g sodium carbonate in about 800 ml of water in a conical flask. Transfer to a graduated cylinder through a folded filter paper placed in a funnel or beaker of 1L capacity. Dissolve 17.3 g copper sulfate in about 100 ml of water. Add the copper sulfate solution slowly with constant stirring to the carbonatecitrate solution and make up to 1L

Barfoed's Test

- **Procedure:**
- To 5 ml of Barfoed's reagent in a test tube add 0.5 ml of sugar solution. Mix well.
- Keep in a boiling water bath for **2 minutes**. Keep the tube in a test tube rack and examine for precipitate after 10-15 minutes

Barfoed's Reagent

- Dissolve 13.3 g neutral copper acetate crystals in 200 ml water. Pass through a filter paper placed in a funnel to remove the particles if present to another graduated beaker. Then add 1.8 ml glacial acetic acid.

- **Application of the test:** Useful to distinguish between monosaccharides and disaccharides.

- **Chemistry of the test:** Reduction reaction as shown under Benedict's test.

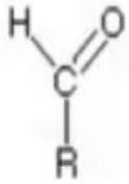
- **Observation:** A **red** precipitate clinging to the bottom most part of the test tube forms, in the presence of a monosaccharide**.**
- **Inference:** The test is answered by monosaccharides only, e.g. glucose, fructose, galactose, mannose.
- **Principle:** It is a reduction test. Reducing property owes to the carbonyl group (aldehyde or keto group). Barfoed's reagent is copper acetate in acetic acid.

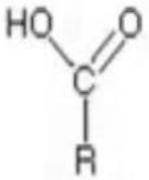
Points to Ponder

- It is important to keep the time limit (2 minutes) prescribed for Barfoed's test otherwise disaccharides will also respond to the test positively.
- Disaccharides when present in high concentrations (> 5 gm%) also will give positive response

- Unlike the Benedict's test, Barfoed's test is unsuitable for testing sugars in urine or any fluids containing chloride.
- The red precipitate is formed at the bottom of the tube. To see the precipitate, lift the tube to the eye level, otherwise the precipitate formed adhering to the bottom most part of the tube may escape notice.

Barfoed's Test





$$RCHO + 2Cu^{2+} + 2H_2O \rightarrow RCOOH + Cu_2O\downarrow + 4H^+$$

Monosaccharide (Eg: Aldose)

Cupric ion (from Cupric acetate of Barfoed's Reagent)

Carboxylic Acid

Cuprous Oxide (Brick Red Ppt.)

Seliwanoff's Test

- **Procedure:** To 5 ml of Seliwanoff's reagent in a test tube add 5 drops of fructose solution and heat the contents to just boiling.
- **Observation:** Positive reaction gives a **red color** within half a minute (Fig. 1A-8).
- **Inference:** This test is given by ketoses.
- e.g. fructose.
- https://youtu.be/BHRtMRM_o-o?si=ucbdNx8ZSjLKxdu8

Seliwanoff's Reagent:

- Dissolve 0.05 g resorcinol in 100 ml dilute HCl .

Principle:

- A dehydration reaction due to the hydroxyl groups of the sugar. Selivanoff's reagent is resorcinol in dilute hydrochloric acid. Ketoses (e.g. fructose) are more readily dehydrated by HCl than the aldoses to form hydroxymethyl furfural which then condenses with resorcinol of Seliwanoff's reagent to form a **red colored** complex.

Points to Ponder

- The test is sensitive up to 0.1 gm% of fructose in the absence of glucose.
- In the presence of glucose, the test becomes less sensitive to fructose.
- Large amounts of glucose gives the same color.
- If the boiling is prolonged a positive reaction may occur with glucose because of Lobry de Bruyn-van Ekenstein transformation of glucose into fructose in the presence of acid.

The precautions to be followed to get a positive test for fructose are given below:

- Concentration of HCl used must be less than 12%.
- The reaction must be observed ***within 20 to 30 seconds of performing the test.***
- Those reactions occurring after 20 -30 seconds, must not be taken into account.
- Glucose must not be present in amounts more than 2% or else it will interfere with the test.

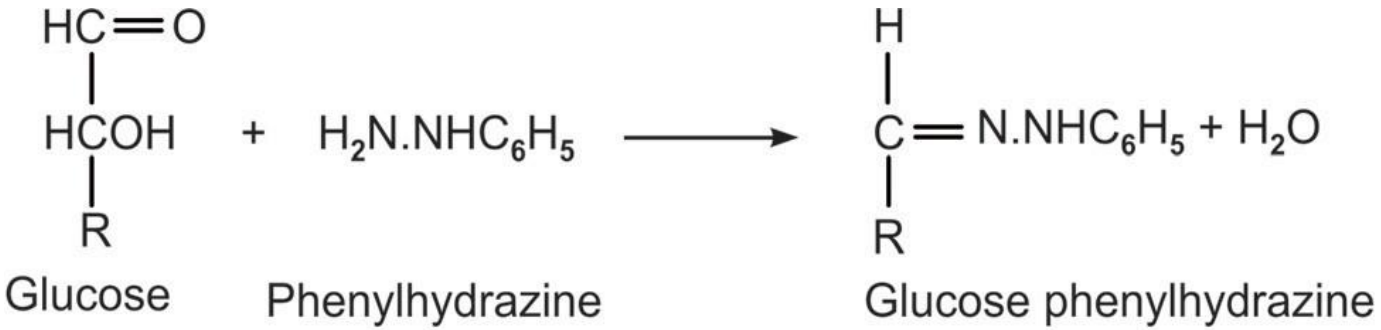
Osazone Test

- **Principle:** The reaction involves the carbonyl carbon (either aldehyde or ketone as the case may be) and the adjacent carbon. One molecule of sugar reacts with one molecule of phenyl-hydrazine to form phenylhydrazone which then reacts with two additional phenyl hydrazine molecules to form the osazones

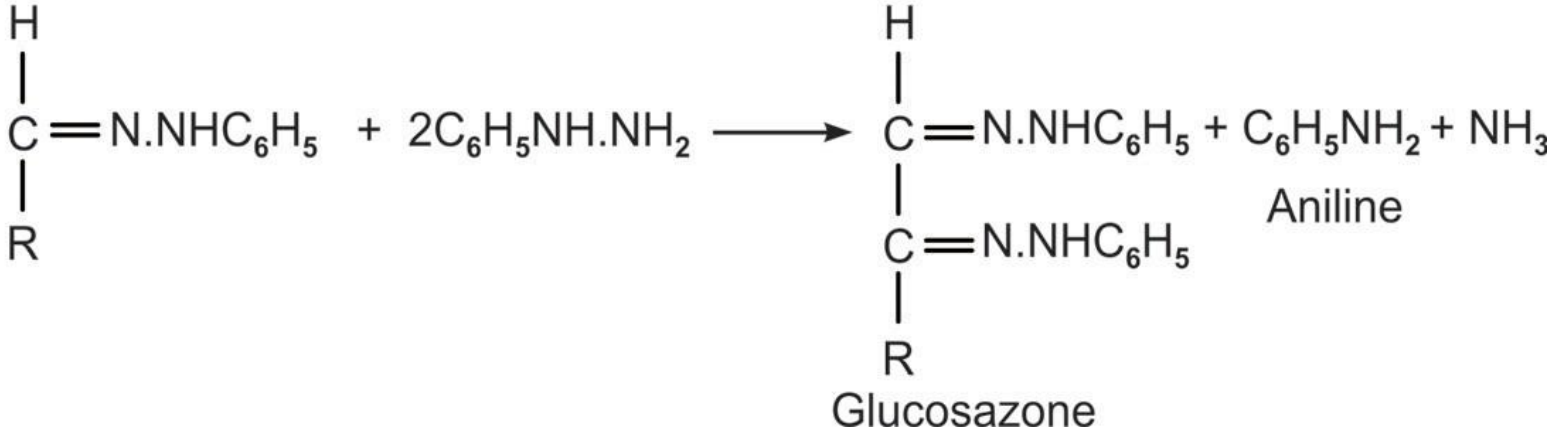
Phenylhydrazine Mixture

: Mix 2 parts phenyl- hydrazine hydrochloride and 3 parts sodium acetate by weight thoroughly in a mortar (Mixture with longer shelf life may be prepared by using equal weights of phenylhydrazine hydrochloride and anhydrous sodium acetate).

Chemistry of Osazone test



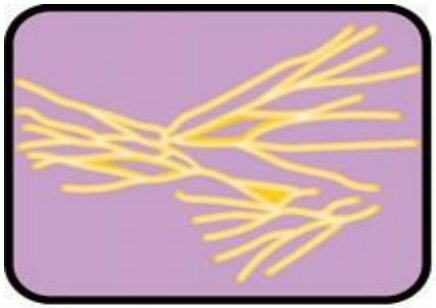
Glucose Phenylhydrazine Glucose phenylhydrazine



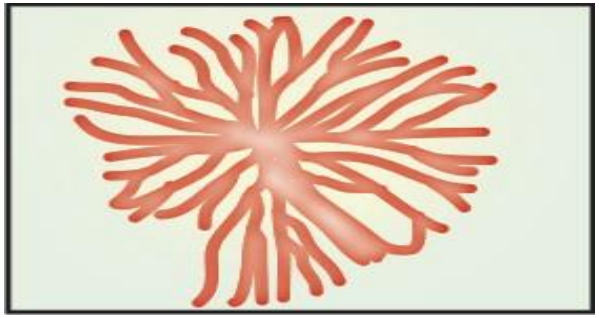
Aniline

Glucosazone

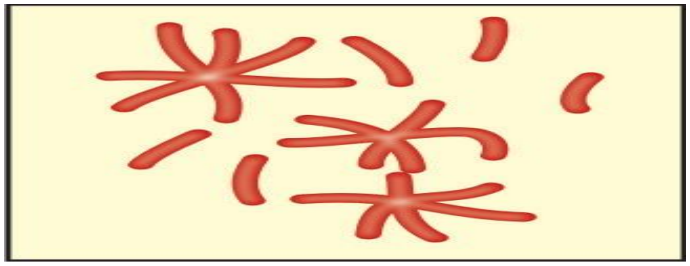
- Glucose, fructose, mannose yield the same shaped phenyl osazone crystals because of the elimination of differences in configuration about the carbon atoms 1 and 2 during osazone formation.



Glucose, fructose, mannose-single crystal has the shape of a needle or and when grouped look like a broom or a bundle of hay



Lactosazone (Puff shaped)



Maltosazone (Petal shaped)

DISACCHARIDES

- Disaccharides are glycosides in which both components are monosaccharides. The general formula of common disaccharides is $C_{12}H_{22}O_{11}$.
- **Maltose**
- **Lactose**
- **Sucrose**

Sucrose Hydrolysis Test

- This test is used to convert sucrose (non-reducing disaccharide) to glucose + fructose (reducing monosaccharides).

Principle:-

- Sucrose is the only non-reducing disaccharide so it does not reduce the Cu++ solution (Bendict's and Fehling's test) because there is no free aldehydic or ketonic group to give positive reducing properties. This bond can be hydrolysed by strong acid ; concentrated HCl and the individual components of sucrose (glucose + fructose) are then able to give positive reducing test.

Method:

- 1- Set up tow tubes add to each one 4ml of a sucrose solution ,Lable the tube : (Sucrose with HCL, Sucrose without HCl) 2- To only one tube add four drops of concentrated hydrochloric acid (HCl)
- 3-Heat both in boiling water bath for 15 minutes.
- 4- After 15 minutes of heating add 4 drops of concentrated NaOH to each tube???

- 5-From the tube containing HCl take 2ml in tow tube to do Benedict's test and Seliwanoff's test .
- Add 2 ml of Benedict's reagent and 2.5 ml of Seliwanoff's reagent WHAT do expect?
- 6-From the tube which contain only sucrose take 2 ml to do Benedict's test only (add 2 ml of Benedict's reagent) WHAT do expect?

POLYSACCHARIDES

- The polysaccharides are complex carbohydrates of high molecular weight, which on hydrolysis yields monosaccharides or products related to monosaccharides. The various polysaccharides differ from one another with respect to their constituent monosaccharide composition, molecular weight and other structural features.

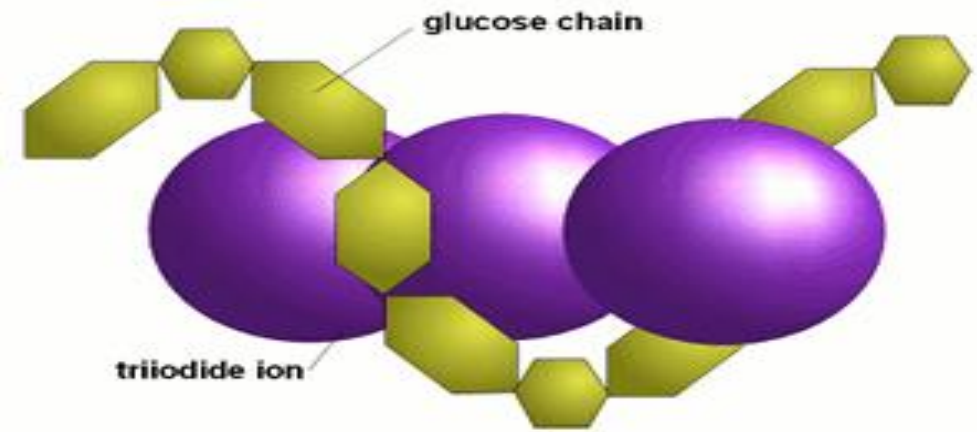
- In all types the linkage between the monosaccharide units is the glycosidic bond.
- This may be α or β which join the respective units through 1 , 2 or 1 , 3 or 1 , 4 linkages in the linear sequence or at branch points in the polymer.
- Polysaccharides are **classified** based on the type of monosaccharide units present in them.

- **1. Homopolysaccharide**
- Contains only one type of onosaccharide,
- e.g. Starch, Glycogen.
- **2. Heteropolysaccharide**
- Contains more than one type of monosaccharide units, e.g. Glycosaminoglycans (heparin, hyaluronic acid).

Iodine Test

I- Effect of temperature on adsorption

- **Procedure:** To 2-3 ml of starch solution add 2 drops of dilute (0.05 N) iodine solution.
- Observe the changes on heating and on subsequent cooling.
-



0.1 N iodine Solution

- Dissolve 1.27 g iodine and 3 g pure KI (potassium iodide) crystals in 100 ml distilled water. Dilute 1:10 in distilled water before use.

Inference:

- Starch forms an adsorption complex with iodine to give a blue color. The blue color disappears on heating due to the breaking of the Iodine starch adsorption complex and appears on cooling due to reformation of the adsorption complex

Iodine Test

1- Effect of pH change on adsorption

- Adsorption is not affected by acidic media.
- But it is affected by the basic environment because free iodine turns into iodide salts, Iodate and Iodide

$3I_2 + 6\,NaOH \longrightarrow 5NaI + NaIO_3 + 3H_2O$

$NaIO_3 + HCL \longrightarrow HIO_3 + NaCL$

$NaI + HCL \longrightarrow HI + NaCL$

$HIO_3 + 5HI \longrightarrow 3I_2 + 3H_2O$

Starch Hydrolysis Test

- **Procedure:**
- Take 25 ml of starch solution in a beaker. Add 10 drops of concentrated HCl and boil gently. At the end of each minute , transfer a drop (using glass tube) of the solution on to a plate for doing the iodine test and 3 drops to 5 ml of Benedicts solution (Set tubes containing 5 ml of Benedict's reagent in series) . Continue until the iodine test becomes negative. Then place the tubes for the Benedict's test in the boiling water bath for 3 minutes.

Response of Starch Hydrolysis Test				
Time in minutes	Color with I_2	Benedict's test	Hydrolysis	Product
1	Blue	Blue	No reduction	Starch
5	Violet	Green	Reduction starts	Amylodextrins
8	Reddish violet	Red	Initiation of reduction	Amylo and erythrodextrins
12	No color	Red	Partial reduction	Achrodextrins
20	No color	Red	Complete reduction	Glucose

- Starch → Soluble starch → Amylodextrins → Erythrodextrins Achrodextrins → Maltose → Glucose.

- When the hydrolytic stage reaches to the level of formation of maltose and glucose iodine test becomes negative and Benedict's test becomes positive

Starch precipitation

1-Starch precipitation by alcohol;

- Place 3 ml of starch solution in a test tube and add 3 ml of 95% Ethanol to it.
- Filter the solution and add a drop of iodine to the filtrate and precipitate. What do you notice??

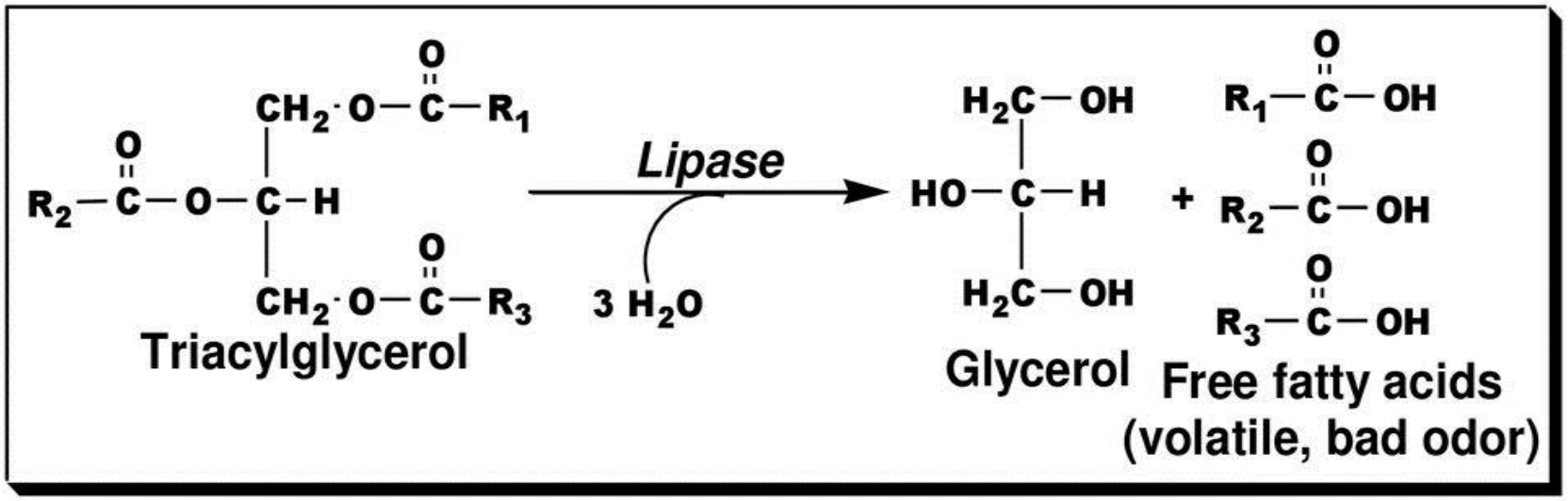
1-Starch precipitation by Ammonium sulfate.

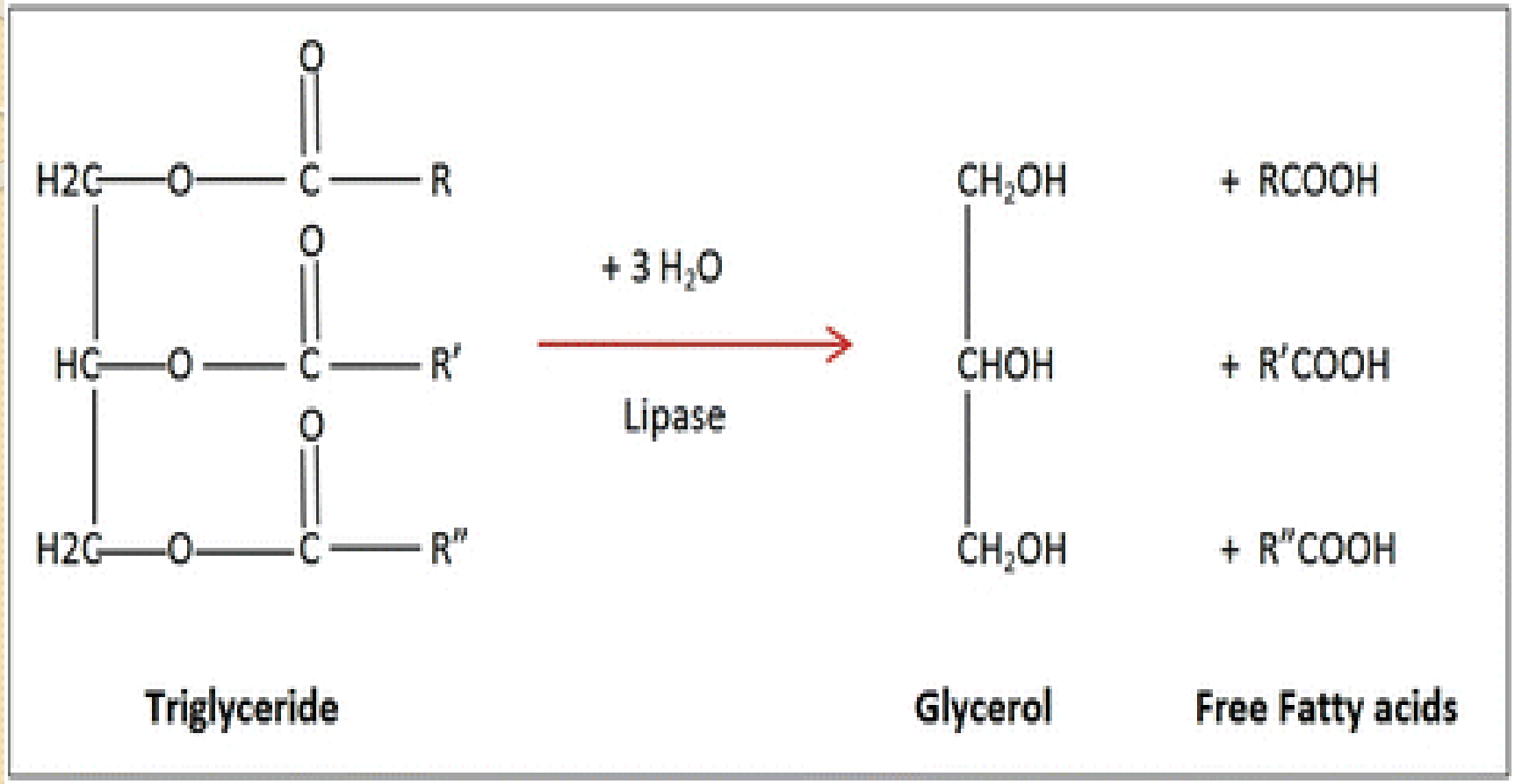
- Place 3 ml of starch solution in a test tube and add 3 ml of saturated Ammonium sulfate solution (or 0.25 gm solid Ammonium sulfate) to it. Mix
- Filter the solution and add a drop of iodine to the filtrate and precipitate. What do you notice??

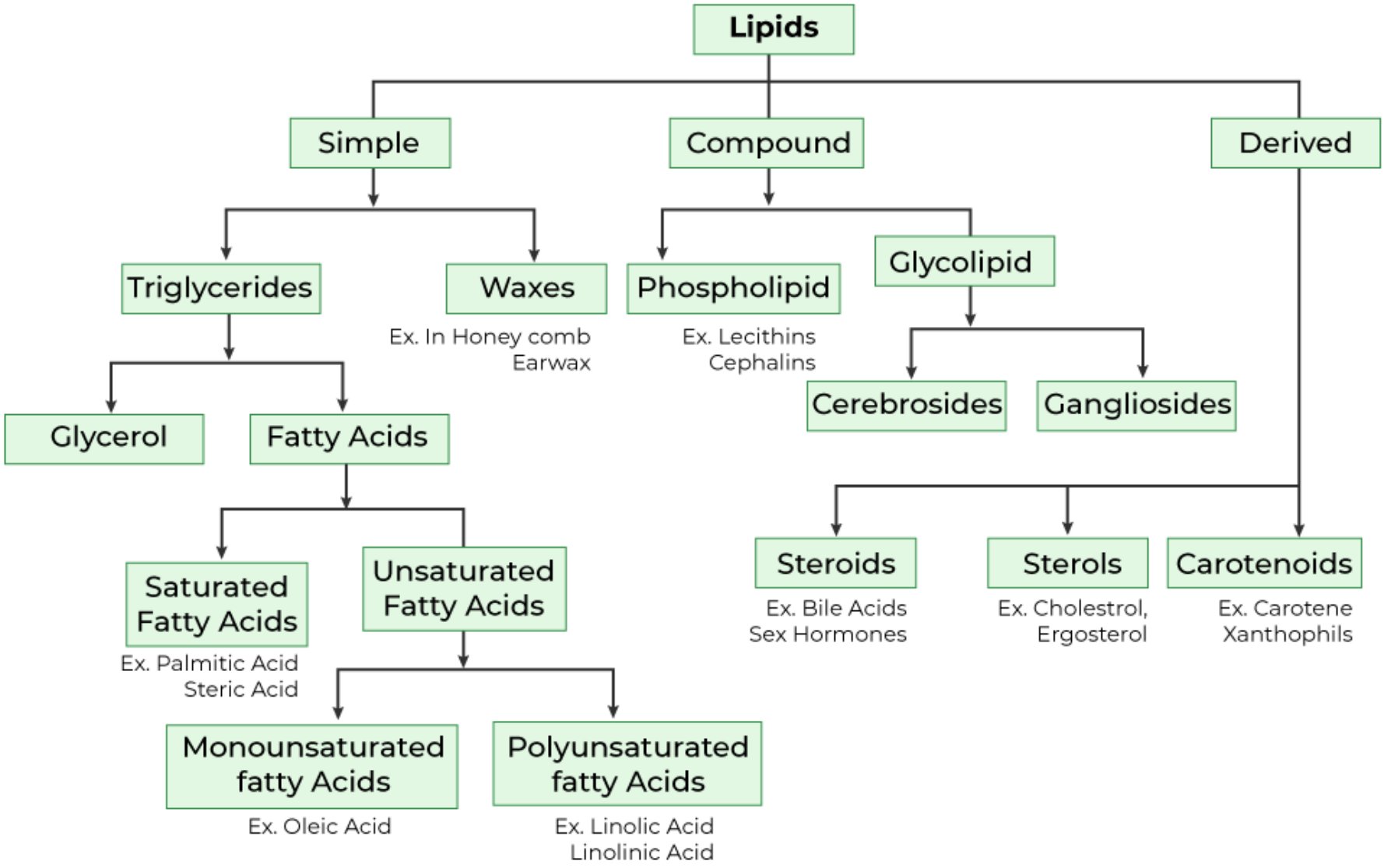
Lipids

- Lipids are naturally occurring heterogeneous group of substances found in all vegetables and animal matter. They are insoluble in water and soluble in solvents like ether, chloroform, boiling alcohol and benzene

- **Lipids are esters of fatty acids or substances capable of forming such esters**. To understand much about the different types of lipids, classification of lipids is useful.


Lipase
3 H2O
Triacylglycerol
Glycerol
Free fatty acids
(volatile, bad odor)


H2C—O—C—R
HC—O—C—R′
H2C—O—C—R″
O
+ 3 H2O
Lipase
CH2OH
CHOH
CH2OH
+ RCOOH
+ R′COOH
+ R″COOH
Triglyceride
Glycerol
Free Fatty acids


Lipids
Simple
Compound
Derived
Triglycerides
Waxes
Ex. In Honey comb
Earwax
Phospholipid
Ex. Lecithins
Cephalins
Glycolipid
Cerebrosides
Gangliosides
Glycerol
Fatty Acids
Saturated Fatty Acids
Ex. Palmitic Acid
Steric Acid
Unsaturated Fatty Acids
Monounsaturated fatty Acids
Ex. Oleic Acid
Polyunsaturated fatty Acids
Ex. Linolic Acid
Linolinic Acid
Steroids
Ex. Bile Acids
Sex Hormones
Sterols
Ex. Cholestrol,
Ergosterol
Carotenoids
Ex. Carotene
Xanthophils

- Fats can be classified according to their ability to be hydrolyzed by the base to form soap.
- **1- Saponifiable fats.**
- **Definition**: These are lipids that can be hydrolyzed (broken down) into their constituent fatty acids and glycerol in the presence of a strong base.
- **Examples**: Triglycerides (fats and oils), phospholipids, and waxes.
- **Chemical Structure**: They typically contain ester bonds between glycerol and fatty acid chains.
- **Saponification**: When treated with a strong base, saponifiable lipids undergo saponification, producing soap (salts of fatty acids) and glycerol.

- **2-Non-Saponifiable Lipids**
- **Definition**: These lipids **cannot** be hydrolyzed into fatty acids and glycerol and do not form soap upon treatment with a strong base.
- **Examples**: Steroids (like cholesterol), terpenes, and fat-soluble vitamins (like vitamins A, D, E, and K).
- **Chemical Structure**: They generally do not contain ester bonds and are often composed of complex ring structures or long hydrocarbon chains.
- **Behavior in Saponification**: Non-saponifiable lipids remain intact and do not produce soap when treated with strong bases.

Fatty acids

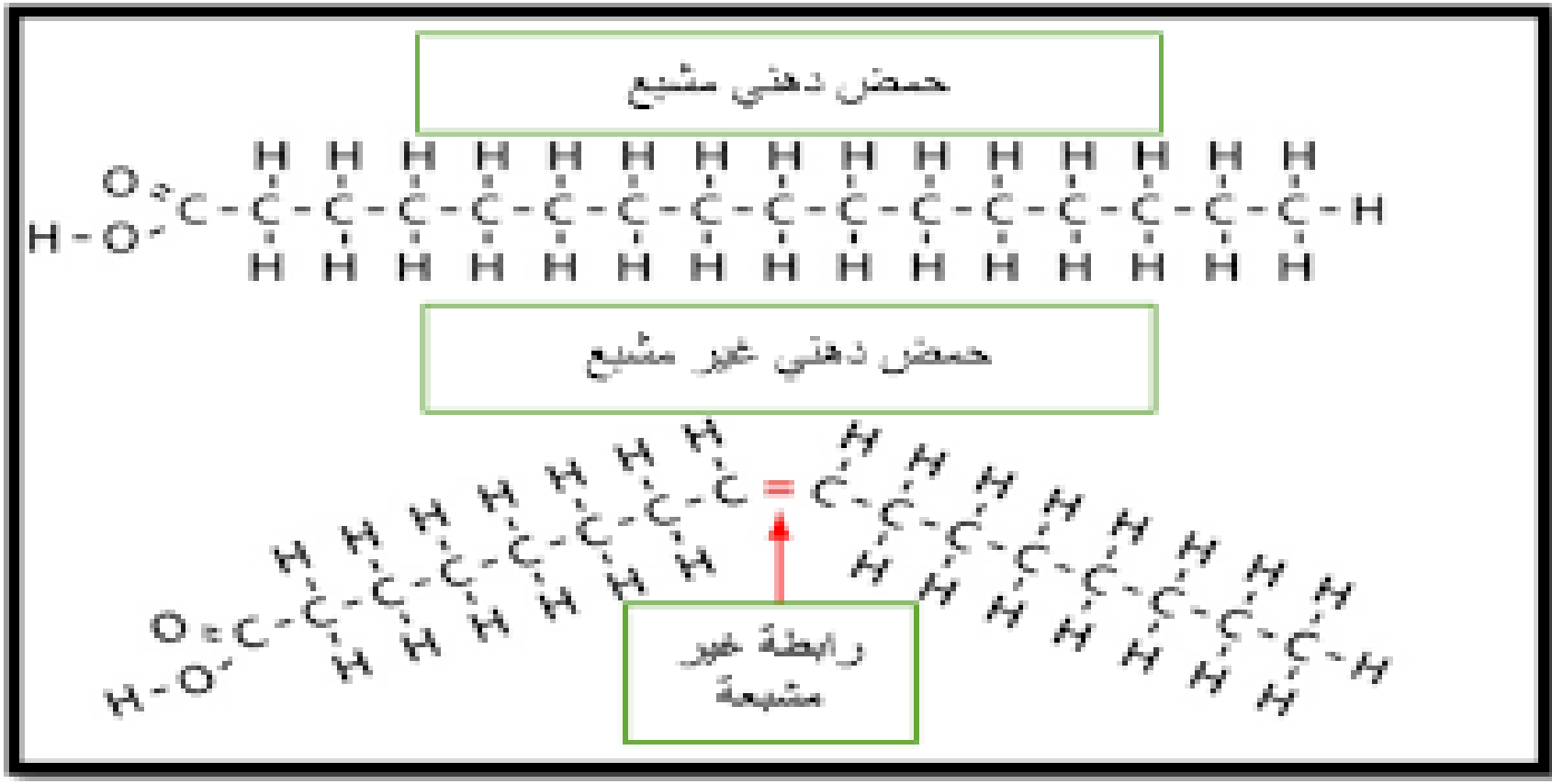
- **fatty acid** is a carboxylic acid with an aliphatic chain, which is either saturated or unsaturated

- . Saturated fatty acids

- Saturated fatty acids have no C=C double bonds. They have the formula $CH_3(CH_2)_nCOOH$, for different *n*. An important saturated fatty acid is stearic acid (*n* = 16), which when neutralized with sodium hydroxide is the most common form of soap.

- Unsaturated fatty acids
- Unsaturated fatty acids have one or more C=C double bonds.

- Most fatty acids are even-chained, e.g. stearic (C18) and oleic (C18), meaning they are composed of an even number of carbon atoms. Some fatty acids have odd numbers of carbon atoms; they are referred to as odd-chained fatty acids


حمض دهني مشبع
حمض دهني غير مشبع
رابطة غير مشبعة

9. Formulas of some fatty acids

Name	Formula	Semi-structural formula
lauric	$C_{11}H_{23}COOH$	$CH_3(CH_2)_{10}COOH$
myristic	$C_{13}H_{27}COOH$	$CH_3(CH_2)_{12}COOH$
palmitic	$C_{15}H_{31}COOH$	$CH_3(CH_2)_{14}COOH$
palmitoleic	$C_{15}H_{29}COOH$	$CH_3(CH_2)_4CH_2CH{=}CHCH_2\,(CH_2)_5CH_2COOH$
stearic	$C_{17}H_{35}COOH$	$CH_3(CH_2)_{16}COOH$
oleic	$C_{17}H_{33}COOH$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
linoleic	$C_{17}H_{31}COOH$	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$
linolenic	$C_{17}H_{29}COOH$	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COOH$
arachidic	$C_{19}H_{39}COOH$	$CH_3(CH_2)_{17}CH_2COOH$
arachidonic	$C_{19}H_{31}COOH$	$CH_3(CH_2)_4(CH{=}CHCH_2)_3CH{=}CH(CH_2)_3COOH$

REACTIONS OF FATS AND FATTY ACIDS•

Cupric acetate test:

- Application :
- Distinguish between fats and saturated and unsaturated fatty acids.
- Free fatty acids combine with cupric acetate to form fatty acid salts.
- The solubility of these salts in ether varies depending on the type of fatty acid

- Unsaturated fatty acids form soluble salts in ether.

- While saturated acids form insoluble salts (precipitate)

Procedure:

- 1- Take three test tubes and put a saturated fatty acid in the first, an unsaturated fatty acid in the second, and a neutral fat in the third.
- 2- Add 3 milliliters of petroleum ether to the three tubes and shake well to dissolve it
- 3- Add equal amounts of copper acetate to the three tubes. Mix well. Leave the tubes for a while and then record notes.

Notice

- 1- The tube containing the neutral fat shows the two layers clearly, and the upper layer is colorless
- 2- In the tube containing saturated fatty acid, a blue precipitate will form in the bottom layer and the upper layer will remain colorless.
- 3- In the tube containing the unsaturated fatty acid, the upper layer (the ether layer) appears colored green.

Cupric acetate test:

$CH_3(CH_2)_XCOOH + (CH_3COO)_2Cu$

saturated f.a

$\{CH_3(CH_2)_XCOO\}_2Cu + CH_3COOH$ (blue pt)

$CH_3(CH_2)_XCH=CHCOOH + (CH_3COO)_2Cu$

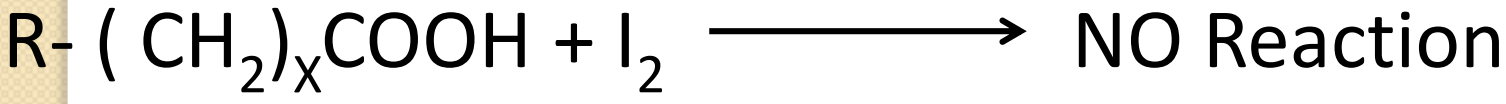
unsaturated f.a

$\{CH_3(CH_2)_XCH=CHCOO\}_2Cu + CH_3COOH$ (blue pt)

Halogenation Test

- **Procedure:**
- Take two test tubes and mark A and B respectively.
- Add 5 ml of petroleum ether to both tubes.
- Add 6-8 drops of oleic acid in tube A and a scoopful of stearic acid in tube B and shake well.
- Add a few drops of iodine solution and shake well.

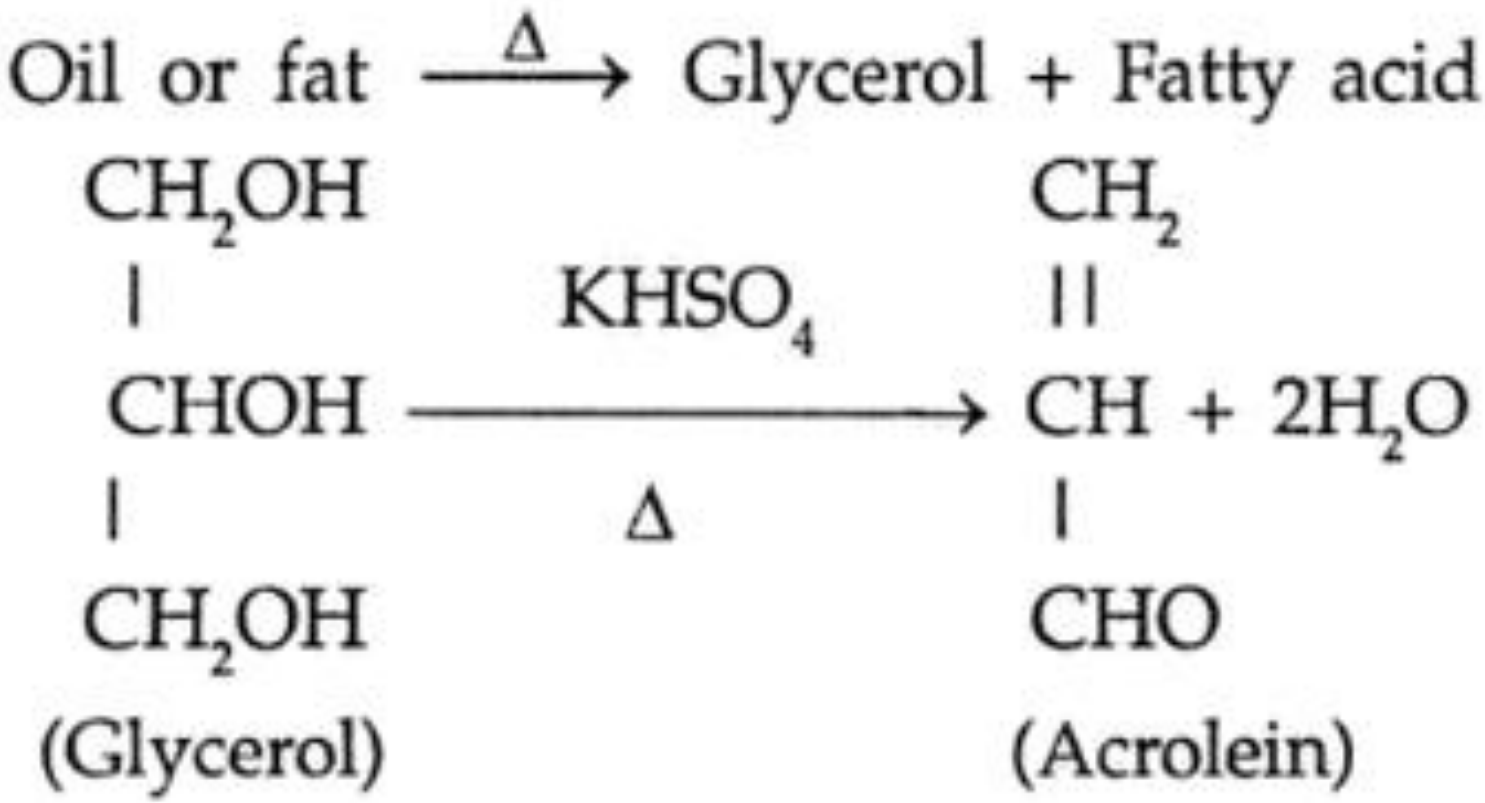

R- CH=CH-COOH + I2
R- CH-CH-COOH
I I


R- (CH2)XCOOH + I2
NO Reaction

Acrolein test

- It is used to distinguish between natural fats and fatty acids

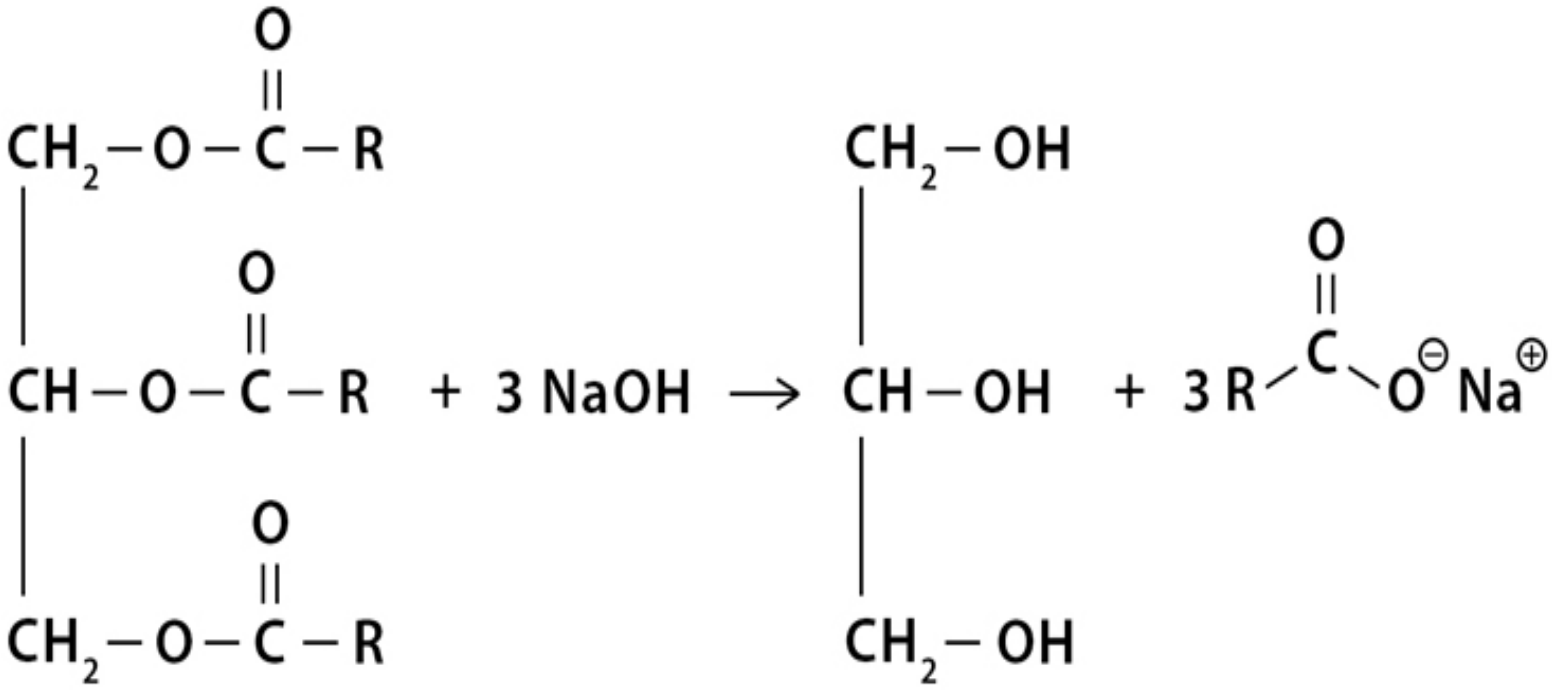
- Natural fats hydrolyze into their components of fatty acids and glycerol when heated with water under high pressure and in the presence of auxiliary factors such as acids.


Oil or fat
Δ
Glycerol + Fatty acid
CH2OH
CHOH
CH2OH
(Glycerol)
KHSO4
Δ
CH2
CH + 2H2O
CHO
(Acrolein)

(Saponification) :Decomposition of fats and oils by strong bases

- Fats or glycerides are broken down when treated with strong bases such as sodium or potassium hydroxide into glycerol and salts of the fatty acids that make up the fat.
- This process is called Saponification, as soap is salts of fatty acids

Saponification



Triglyceride | Sodium hydroxide | Glycerol | Soap

ChemistryLearner.com

Procedure

- 1- Put a little fat or oil in a test tube.
- 2- Add 2 ml of alcoholic potassium hydroxide solution, shake the solution well and heat for two minutes.
- 3- Add 10 ml of water and heat for 3 minutes.
- 4- Cool the solution and add litmus paper to it, then add drops of concentrated hydrochloric acid until the color of the litmus paper changes.

- 5-Transfer the layer of fatty acids collected on the surface to a second test tube, add some water and heat for several minutes
- 6- Add the base drop by drop with continuous shaking until the soap is formed, which is in the form of a clear solution.

Separation and precipitation of fatty acids

- The principle of the experiment depends on the precipitation of fatty acid from the soap solution (fatty acid salt) using hydrochloric acid.

- $RCOONa + HCL \longrightarrow RCOOH + NaCL$

- The fatty acid is released by adding acid and precipitates because it is insoluble in water

Procedure:

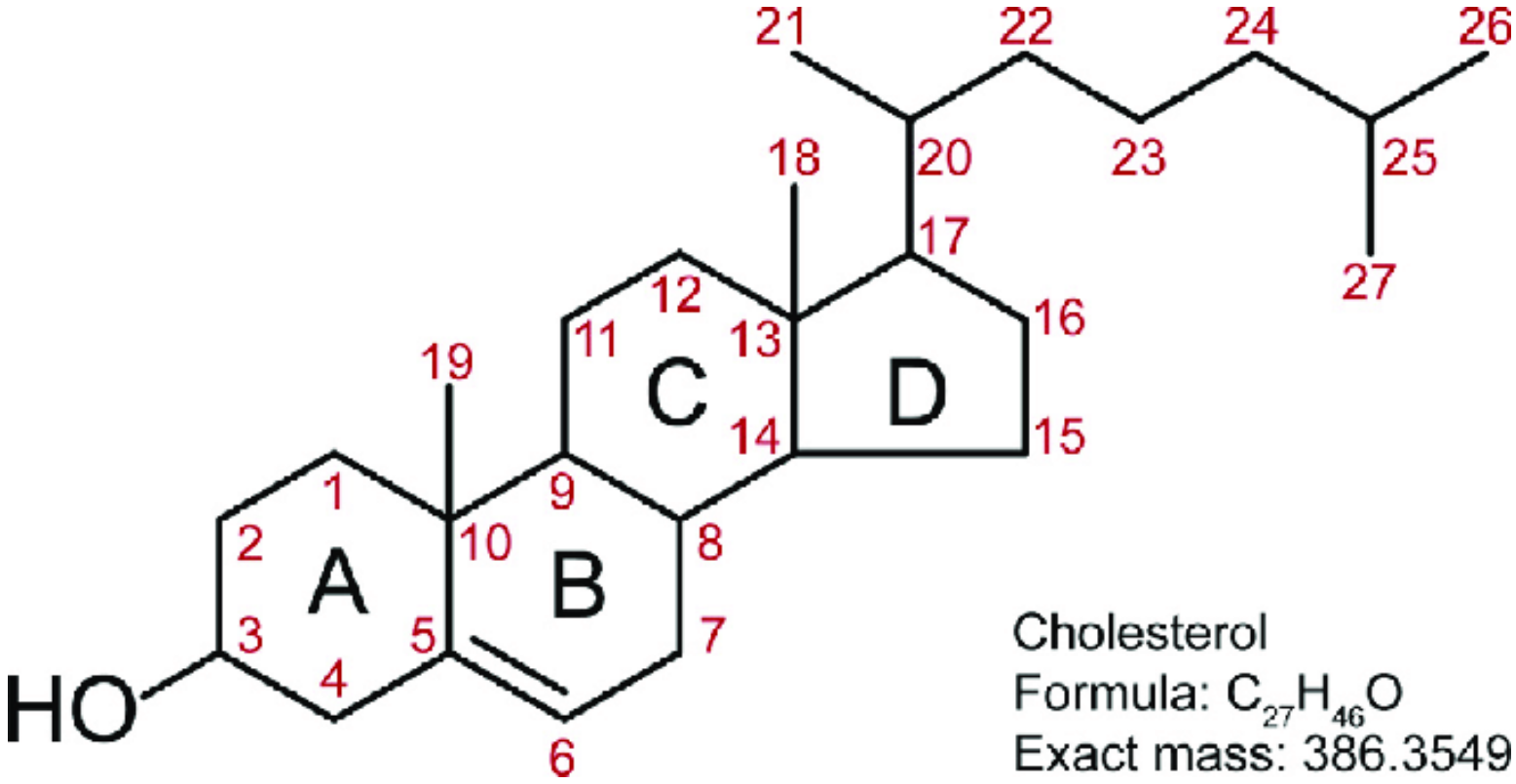
- 1- Take a small amount of soap solution in a test tube, put a litmus paper in it and add a few drops of hydrochloric acid to it.
- 2- Notice the formation of a white precipitate of fatty acids
- 3- Heat it for a short time and leave it for half a minute to cool. Note the separation of an oil layer that hardens after cooling

Separating the soap by salting

- Soap dissolves easily in water, but when table salt is added to the soap solution, it will precipitate. This is because table salt is more soluble in water than soap, so the soap separates in the form of a white precipitate.

Cholesterol

- It is an alcoholic compound containing 27 carbon atoms, one carboxyl group, and a double bond between carbon atoms 5 and 6.



Salkowski's Reaction

- **The Salkowski reaction is based on the interaction of cholesterol with concentrated sulfuric acid in a chloroform solvent.**

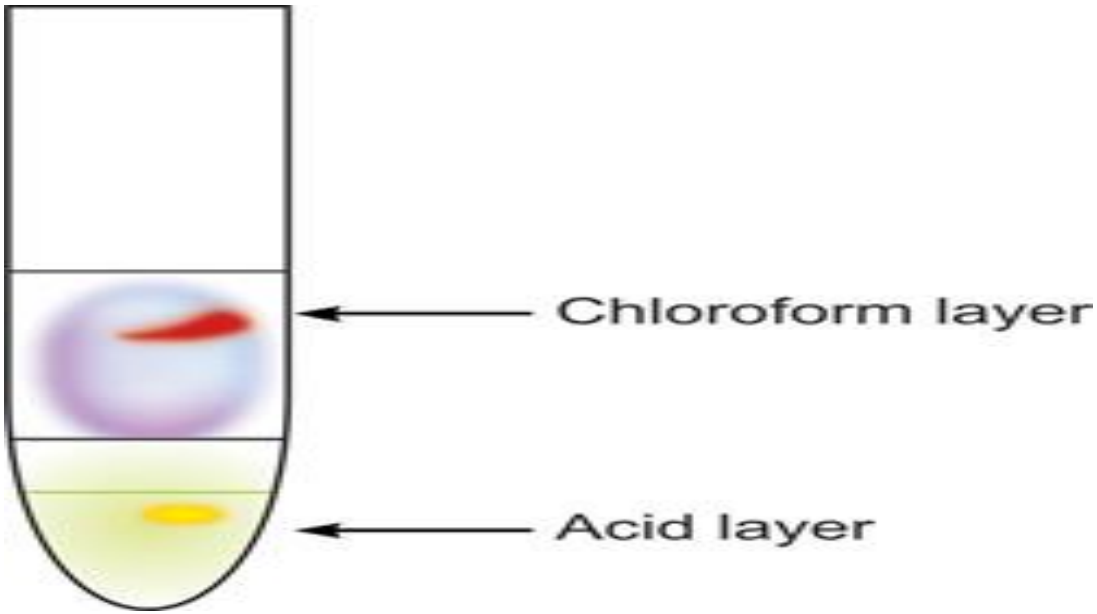
Procedure

1- Dissolve a few crystals of cholesterol in 2 ml of chloroform in a dry test tube.

2- add an equal volume of concentrated H_2SO_4 gently along the sides of the tube. The acid being heavier goes down.

Observation:

- The acid layer develops a yellow color with a green florescence. A play of colors from bluish red to cherry red to purple develops in chloroform layer.



Inference:

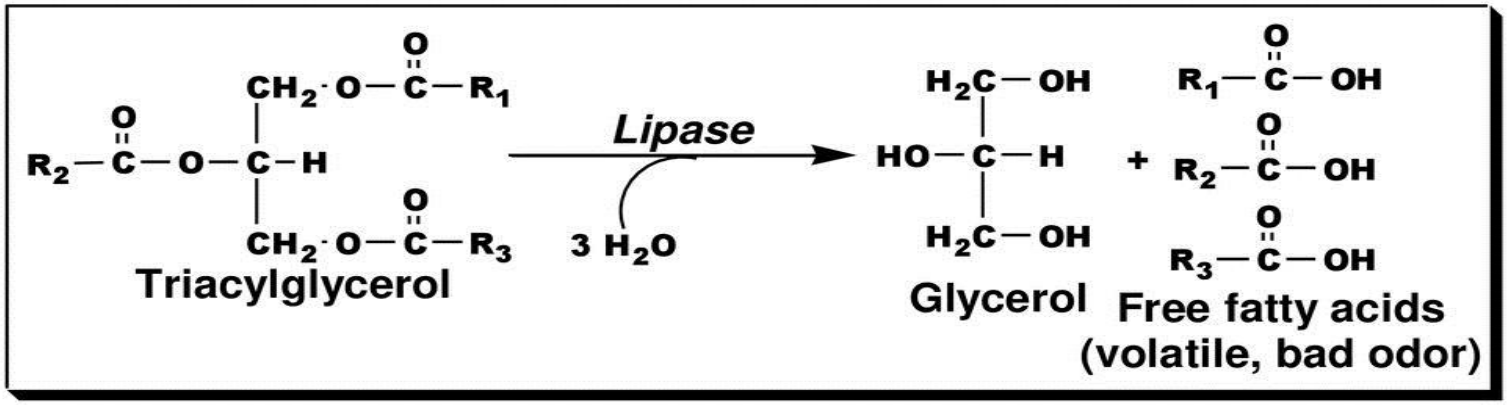
- Cholesterol is dehydrated by concentrated sulfuric acid to form 3,5 cholestadine or 2,4 cholestadine. They polymerize and react with sulfuric acid to form their sulfuric acid derivatives giving rise to a play of colors.

RANCIDITY

- It is complete or incomplete oxidation or slow, flameless combustion at normal temperatures.
- Or it is the process of hydrolysis or spontaneous oxidation of fats and their conversion into short-chain aldehydes or ketones that have unacceptable taste and smell.

Types of rancidity

- **1- Hydrolytic Rancidity**
- Hydrolysis in the presence of the lipase enzyme into fatty acids + glycerol due to increased humidity and high storage temperature. This process may occur due to some microscopic organisms that grow on the fat, and in the presence of sufficient moisture, it decomposes. It occurs mainly in the cell where the smelly butyric acid is separated.
- This enzyme can be eliminated by heat treatment (boiling or pasteurization). .


Lipase
3 H2O
Triacylglycerol
Glycerol
Free fatty acids
(volatile, bad odor)

2- Oxidative Rancidity

- Since fat contains fatty acids with unsaturated bonds, it is easy for it to be exposed to oxidative rancidity, especially since fatty tissue contains the lipoxygenase enzyme, which acts as a catalyst in adding oxygen to fatty acids, thus increasing viscosity and density.

- The factors that help this reaction are light energy, especially infrared radiation

- To prevent oxidation, antioxidants are added to fats during manufacturing

Procedure:

- 1- Take two test tubes and put two drops of good oil in the first and two drops of rancid fat in the second.
- 2- Add 1 ml of petroleum ether to each tube to dissolve the fat and shake well.
- 3- Add drops of phenolphthalein indicator dissolved in NaOH (why) and what will you notice??
- . **This test is used to distinguish between good and rancid fats**