University of Ahel AL-Bait
College of Pharmacy
Practical of Biochemistry

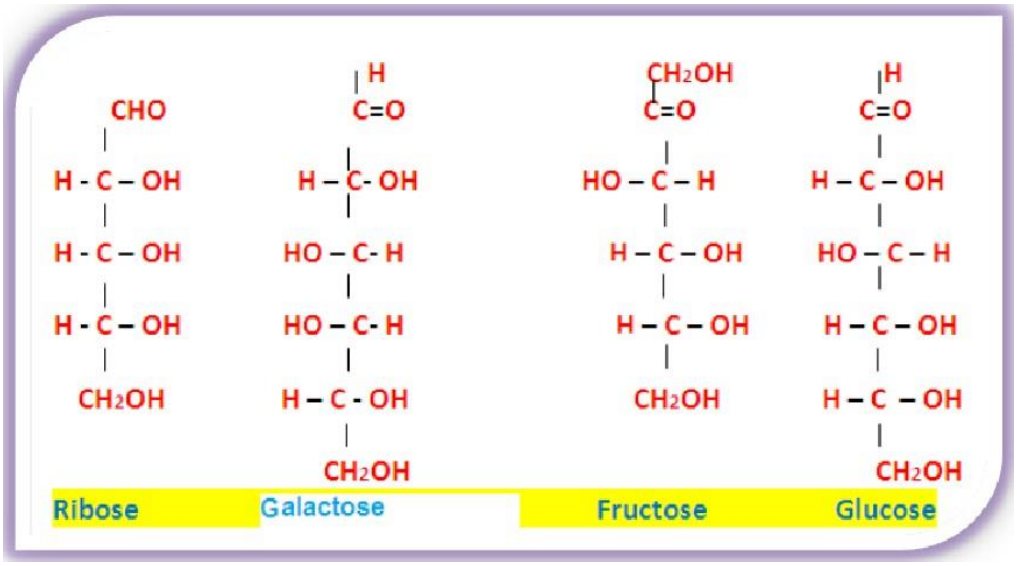
Prepared by
Dr. Radwan Asal
MSc. Zahraa Sabbar Omran

CARBOHYDRATE

carbohydrates contain carbon hydrogen and oxygen in same water ratio 1:2 according to that carbohydrate general formula shall be $C_nH_{2n}O_n$ carbohydrate are the main sources of energy in the body . Brain and cells and RBCs are almost wholly dependent on carbohydrates as the energy source. The carbohydrates could also defined as aldehyde or ketone group derivatives of polyhydric alcohols, giving rise to the reducing ability and are known as an aldoses or ketoses . the basic units of the carbohydrates are monosaccharaides which cannot be split further by hydrolysis these monomers are named according to the number of carbon atoms in the chain thus tetroses , pentoses and hexoses contain four ,five and six carbon atoms respectively .

Classes of carbohydrates:-

1-Monosaccharides (also called simple sugar) Sample for monosaccharide is Glucose Fructose Galactose Ribose



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Important monosaccharides are

1.Glucose known as grape sugar which is popular Aldohexoses saccharide enter in tissues ; biological fluids & blood in human and animal , the phosphorous form of it representing saccharides form in blood also its forming starch glycogen cellulose also enter in sucrose and lactose composition.

2.Fructose Known as fruit sugar which is popular Ketohexoses saccharide found in free form in many fruits and its more sweeting than sugar beet.

3.Galactose : Aldohexoses saccharide forming part of lactose and it cannot be found in free from of building tissues.

4.Mannose : Aldohexoses saccharide it is popular , found in free form in external epicarp of citrus & some fruits ,

What's concerning us in our scientific study is pentose's (5 carbon atoms) like Ribose ,Arabinose and hexoses like Glucose , Galactose (aldoses) and Fructose (ketoses).

2-Disaccharides (also called oligosaccharides) are composed of two monosaccharides bounded together with elimination of water molecule by GLYCOSIDIC- Linkage.

This compounds found in number of plants and animals such :

- Maltose.
- lactose,
- Sucroseo

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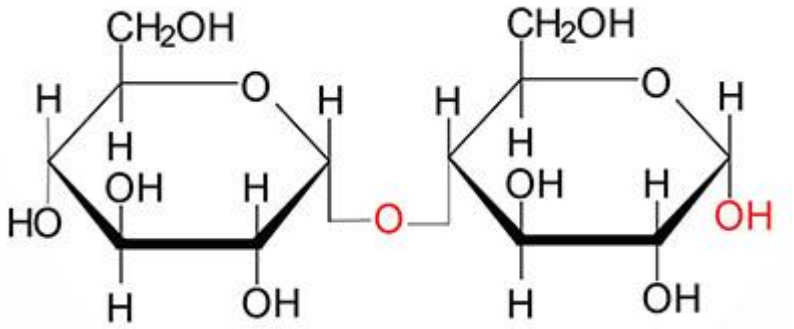
All these compounds have the same molecular formula but different in formula ; properties and sources.

1• Maltose

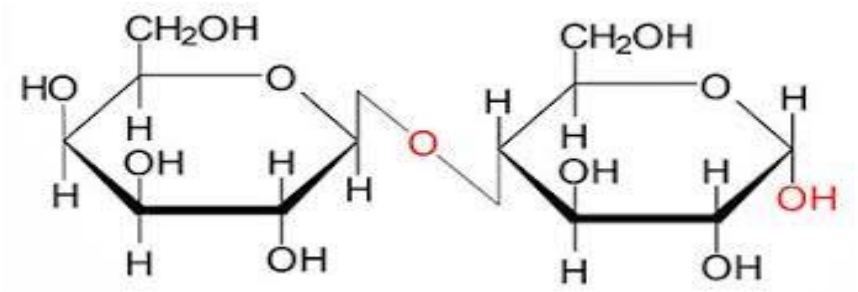
Called malt sugar because it found basely in malt , maltose formed through combining of two D-glucose molecules by glycoside linkage. The glycoside linkage in maltose is α-1,4 glycoside whereas this sugar fermented to ethyl alcohol and C02, maltose is reduced sugar due to the free non bonded aldehyde group



Maltose break up under acids or Maltase enzyme effect to two α- Glucose molecules

2-Lactose

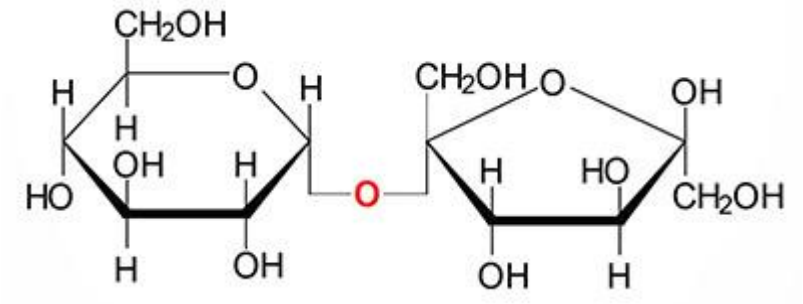
Called milk sugar because it found in milk , lactose composed form combining α-Glucose unit with β- Galactose with glycoside linkage Glycoside bond is β - 1,4 , this sugar is fermented sugar ; reduced due to free aldehyde group.



lactose break up under acids or lactase enzyme effect α- Glucose molecules and β –Galactose .

3-Sucrose

Regular table sugar most important source in sugar cane and beet sugar , Sucrose composed from combining of alpha Glucose with beta fructose by glycoside linkage.
the glycoside bond type is (α1- β2) this sugar is no fermented no reduced because it doesn't contain free aldehyde group.



. Sucrose break up to two molecules (alpha glucose and beta fructose) under acids or sucrose enzyme effect.

Polysaccharides

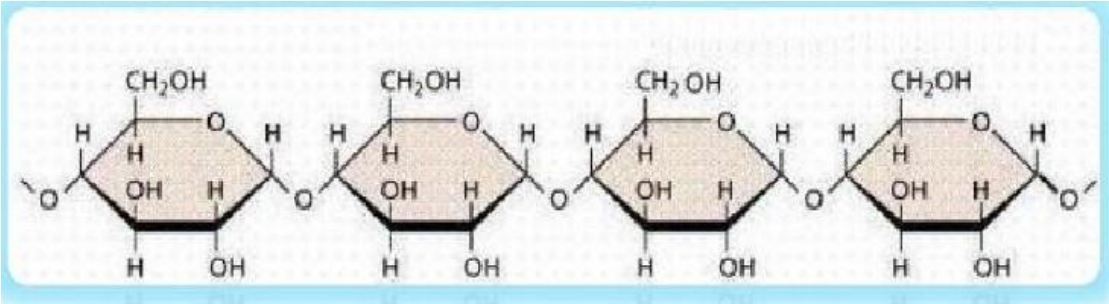
In the nature there are polysaccharides like long saccharides chain composed from uniting of big number of monosaccharaides units or their derivatives or mixture of it connect together with glycoside linkage in high molecular weights spread in plants and animals on tasteless & no smell compounds non crystalized non soluble ; straight or branched chains.
Poly saccharide classified according to formation to two types :
1- Homogenous polysaccharides : which produce the same sugar when decomposition .

2- Heterogeneous polysaccharides : which produce different types of sugar when decomposition and inorganic compounds.

The most important polysaccharides are :

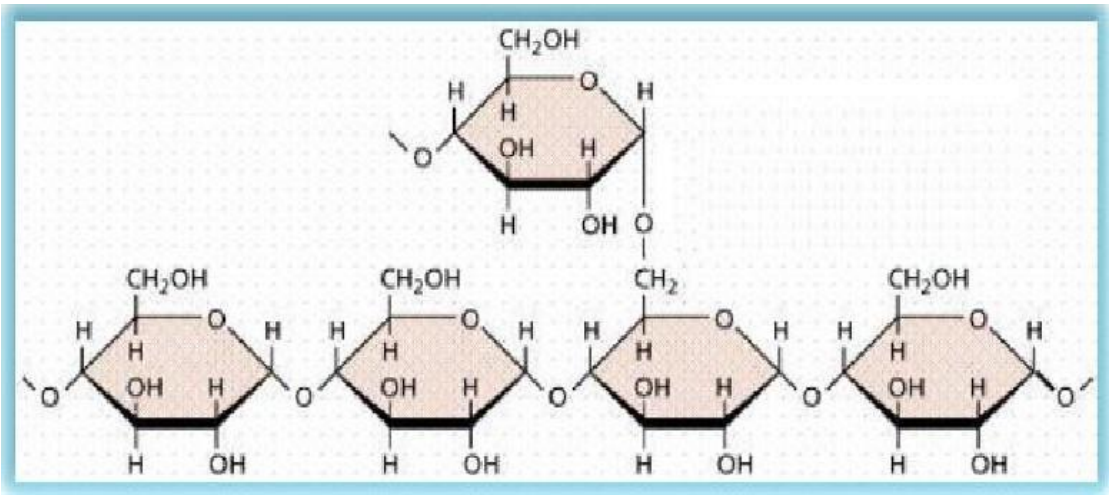
1- Starch

Plant storage sugar , found in plants like potatoes , rice , wheat , corn and barley.
Starch doesn't dissolve in cold water but form glycolic solution in hot water.
starch combined of two parts Amylose & Amylopectin.
Amylose : forming 10—30 % of starch , composed from conjunction of (200 — 1000) alpha — Glucose units by 1,4 — Alpha glycoside linkage on liner chains.



Amylose

Amylopectin : from 70 -90% of starch , composed from conjunction of more than 1300 alpha - glucose units in 1,4 & 1,6 alpha glycoside linkage thefore it form branched chains.



Amylopectin

Prepared by
Dr. Radwan Asal
MSc. Zahraa Sabbar Omran

Starch completely decompose to glucose when heat it with diluted acids , also starch decomposition by Amylase excess in saliva and gastric to maltose after passing in chain of intermediate molecules called Dextrin's.

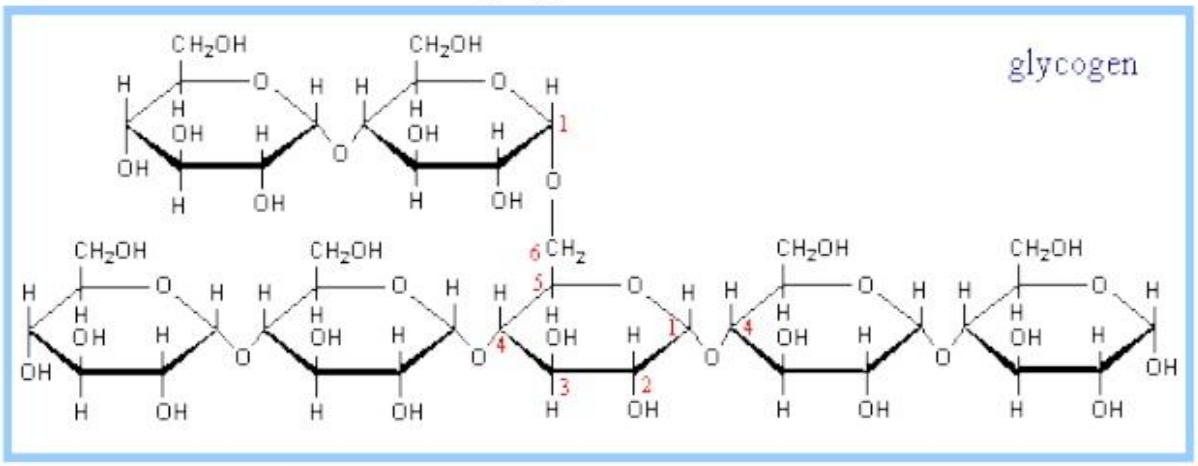
Starch → Amylose → Maltose → D-glucose

Starch → Amylopectin → dextrins → maltose → D-glucose

2. Glycogen

Animal storage sugar , store in liver and muscles . regardless the fact that glycogen total amount in in muscles more than it amount in liver but muscles glycogen more dynamic than liver glycogen whereas it dedicated to muscles consumption. Glycogen composition similar to amylopectin that alpha — glucose connect together in branched chains whereas 1-4, α glycoside linkage give liner chains and 1-6, α represent the point of branching , glycogen more branched than amylopectin and have higher molecular weight compering with amylopectin , it MWT approx. 4000000 , glycogen doesn't dissolve in water and give red purple color with iodine.

Structure of Glycogen molecule



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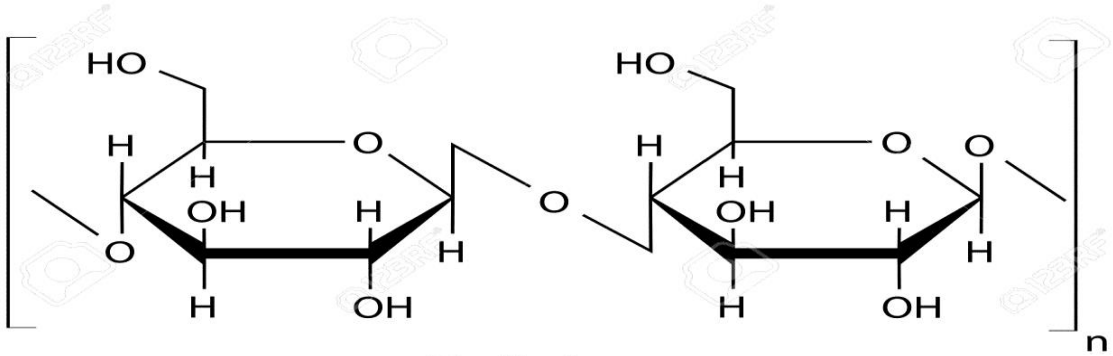
3- Cellulose

Building sugar in plants , enter in cellular walls in plants ... forming their fibers and giving it their firm shape.

Cellulose is non-soluble in water and doesn't digest in human digestive duct, cellulose decompose under sulfuric acid effect to give D-glucose while partial decomposition give Cellobiose (Di-Saccharide).

Cellulose consist of liner chains not branching resulted from beta - Glucose conjunction by 1-4 ,β glycoside linkages have molecular weight close to amylase

Cellulose chains have the ability to take parallel positions allowing hydrogen linkages to form between hydroxyl groups for the parallel chains tighten each other firmly proportion with it function in plant body & cell wall as a pillar plus their non-solubility in water.



Cellulose

Cellulose is very important for intestine because it promote intestine constriction without that cellulose not represent any nutrition value because is not digested.

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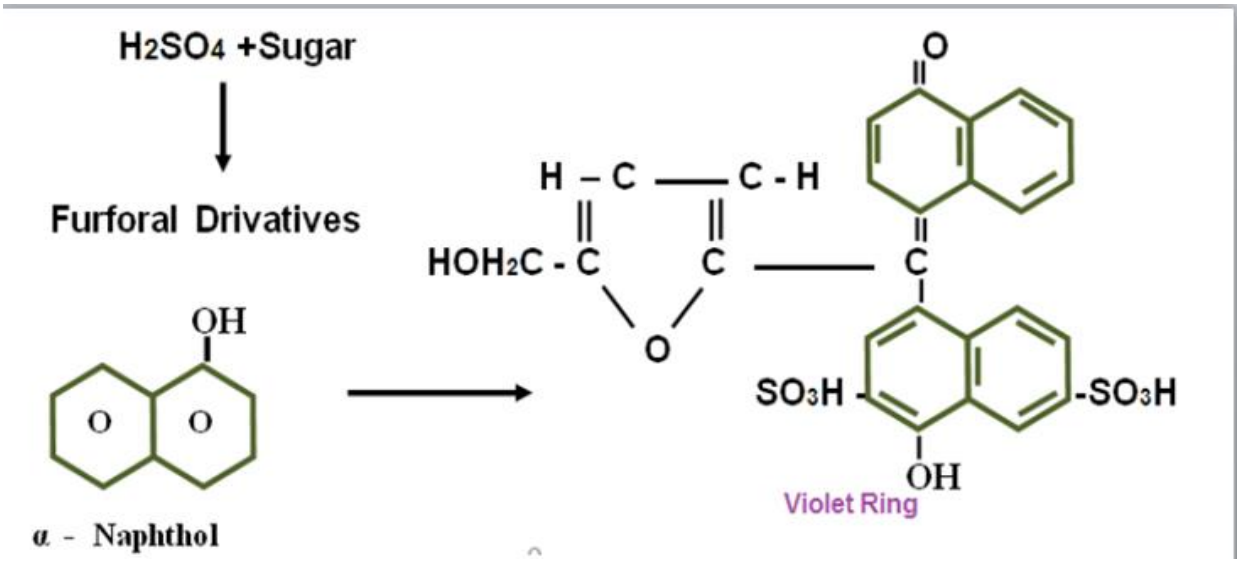
In our practical study for carbohydrates we will study Mono — Di — poly saccharides with demonstration to saccharides liquids examinations.

Examinations

1- Molisch's test

Test Principle :

Concentrated sulfuric acid (H_2SO_4 con.) break up glycoside linkages giving monosaccharide , these monosaccharide lose water giving furfural or it derivative whose combined with alcoholic alpha Naphthol….. violate complex appears as ring .



This is general test for carbohydrate and organic compounds that give furfural under concentrated sulfuric acid effect which is (Dehydrating agent)but not (oxidizing agent) ..thymol can use instead of alpha Naphthol because it will not color during storing and more stable compound .

Compounds & Reagents

1- conc. Sulfuric acid
2- alpha Naphthol liquid/ alcoholic : prepare by diluting 5gm of alpha Naphthol in 100 ml Ethanol .

Method :

Add 2 drops of Alpha Naphthol to 2ml of sugar liquid place in test tube, stir well then add 1 ml of conc. Sulfuric acid very slowly & carefully to the inner side of test tube and let the acid slide to the bottom of tube …2 layers will forms sugar in the top and acid in the bottom then in the borderline violate ring will appear .

Notes:

1- you may note green ring under the violate ring due to sulfuric acid reaction with the alpha-Naphthol (this green blush ring not important in our test).
2- The compounds that give this test is furfural , Glucouronic acid , glyceraldehyde, oxalic acid , formic acid, lactic acid ..etc therefore this test can consider as general test for all saccharides but in the same time it not specific for it. The negatively for this test for carbohydrate special importance because it give assurance that unknown samples is free of carbohydrates .
3- We can't use this test to examine glucose in urine, because glucouronic acid that exist naturally in urine give positive result for the test .

2- Fehling's test

Test Principle :

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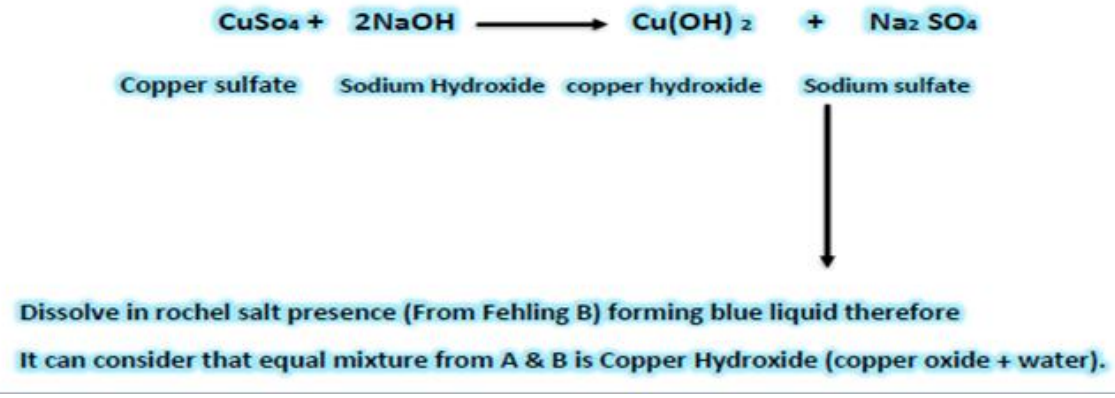
Dr. Radwan Asal
MSc. Zahraa Sabbar Omran

This test depend cupric ions reduction to cupreous ions by reduced saccharides. Fehling reagent consist of 2 liquids : (Fehlings A) which is copper sulfate & (Fehlings B) which is mixture of NaOH and Rochel salt (sodium potassium tarttrate) .

When use this reagent …equal amount from fehlings A&B , it is observed during the mixing of A&B gelatin substance with light blue color forming which is copper hydroxide then this compound dissolve in presence of rochel salt giving dark blue compound in this point rochel salt importance appears which help to make copper hydroxide turn to liquid form :



In case reduce sugar(s) react with fehling .. reaction can illustrate as below

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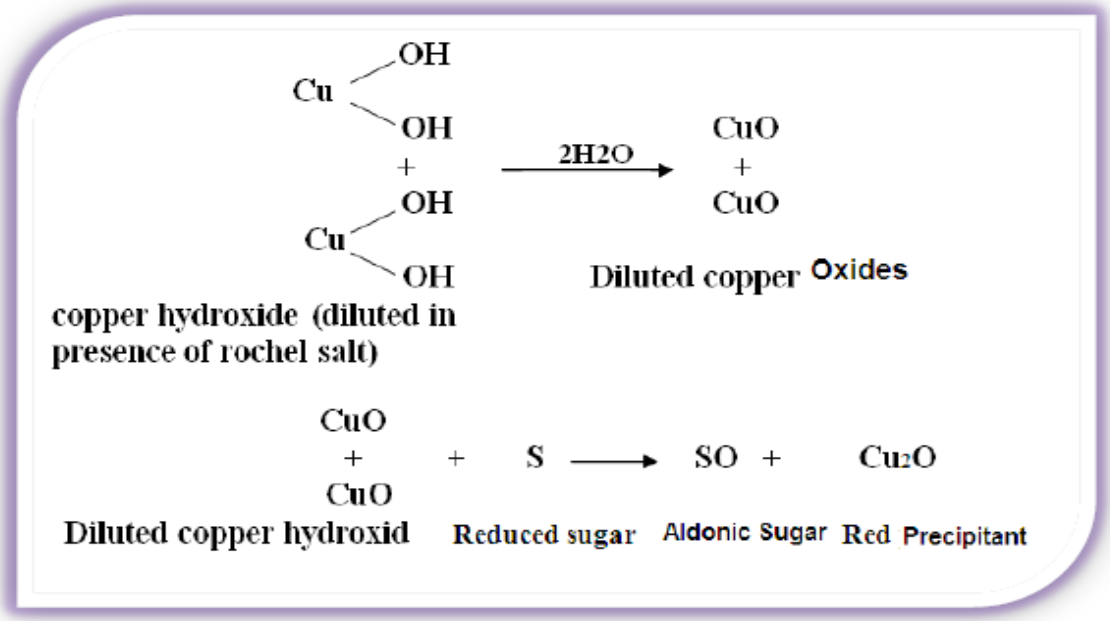
MSc. Zahraa Sabbar Omran



Compound and reagent

1- Copper sulfate (liq. 1%)
2- NaOH (10%) liq.
3- Rochel salt (30%) liq.
4- Sample of sugar liquid (Monosaccharide) such Arabinose – Fractose – Galactose (1%)
5- Fehling A : prepare by dissolving 69.70 gm copper sulfate in 1 L D.W.
6- Fehling B : prepare by dissolving 120 gm of sodium hydroxide and 246gm Rochel salt in 1L D.W.

Method

Mix 3 ml of Fehling A with the same amount Fehling B .. you will notice of blue gelatin precipitant quickly dissolve to dark blue liquid .

Boil this liquid to insure Reactivity the liquid consider good if color doesn't change by boiling .

If any yellow or brown precipitant yield during boiling the liquid , this mean reagent is not active .

After checking fehling activity , add 2ml of sugar solution to the 6 ml (A&B fehling's) then heat over direct flame till boiling .

If there any reduced sugar (all monosaccharide and some disaccharide) yellow or brown precipitant will result , this precipitant is Cu_2O (+ve test).

If there is no reduced sugar (sucrose and starch) the solution color remain blue without any change (- ve test).

Note :

1- To improve test sensitivity dilute fehling's (A&B) with D. water in ratio 1:5 by this the red precipitant can see regardless it small amounts , because sometimes the dark blue color make red precipitant recognizing difficult .

2- Cu_2O color as following : Yellow ⟶ orange ⟶ Red ⟶ Brown the difference in **Cu_2O** color due to molecules size whereas small molecules give yellow bigger molecules give red and brown

3- Fehling 's problems:

a) Sodium hydroxide within fehling's solution component have bad effect on saccharides.

b) It can't be test reduced sugar in acidic medium without neutralizing the acid to make the medium neutral or week alkalinity .

c) Fehling's solution need 2 separated bottles , it can't mix the 2 solution and leave them for long time to avoid auto reduction if this happen boiling fehling solution alone will give red precipitant for this reason it is important to demonstrate reactivity test on fehling's solution before use it .

d) Ammonia and ammonium salt presence in this test affect negatively on fehling test because it react with copper oxide (red) giving colorless solution , therefore it is difficult to test the biological liquid contain small amount of reduced saccharides saturated with ammonium sulfate … now if ammonium

sulfate founded within glucose solution , the solution must boiled first with alkali to let ammonium salt volatile as ammonia gas .

e) Fehling solution important because it help to determine glucose in urine (diabetes) however fehling's has been replaced (because the above problems) with solution have more sensitivity & accuracy which is Benedict's .

Fehling solution fault in finding glucose in urine due to natural compounds exist in urine (in amount higher than normal level)

And reduce fehling's solution as example ascorbic acid , uric acid ….etc , therefore it is better to not depend on fehling test to detect glucose in urine while benedict test represent the best replacement for it sensitivity & accuracy .

3- Benedict's test

Test Principle :

Benedict test depend on same scientific idea of fehling test , Benedict's differ from fehling's as follow : compose of one solution , blue color , consist of copper sulfate – sodium carbonate & potassium citrate , note the copper sulfate is common compound in both tests , sodium carbonate in benedict replaced sodium hydroxide in fehling and potassium citrate replaced rochel salt in fehling benedict's test consider as excellence modification for fehling test for more sensitivity and disposal of fehling's too many problem , therefore this test successfully replaced fehling to detect glucose in urine (diabetes) .

Compounds & reagent

1- Qualitative benedict test :prepare by adding 173 gm of potassium citrate + 100g anhydrous sodium carbonate in 800 ml D.W. then heating , after that leave the solution for cooling then do filtration . add add to filtrate solution copper sulfate solution (prepare by adding 17.3 gm copper sulfate in 100 ml D.W.) complete the volume after that with D.W. to become one liter .

2- Prepare the following sugar solution : glucose , Ribose , Galactose , Fructose (1%)

Method

1- Add 0.5 from each sugar solution to 1ml qualitative benedict reagent shake well
2- Place all test tube in boiling water bath 5 minutes.
3- You will note forming of red precipitate … compare result in test tubes.

Note

1- In positive tests precipitant color grade from yellow to brown (**Yellow** ⟶ **orange** ⟶**Red** ⟶ **Brown**) this precipitant colors is similar fehling's tests result . in negative results solution remain blue .

Fehling Sol.	Copper Sulfate (blue Color)	Sodium Hydroxide + rochel salt (colorless)
Benedict Sol.	**Copper sulfate + Sodium carbonate + potassium citrate (blue)**	

2- Benedict test used extremely in glucose detection in urine . Benedict test preferred than fehling test for the following points:
a) Qualitative benedict sol. Distinguish by it highly sensitivity to glucose in urine comparing with fehling because only small amount of urine required for the test (8 drops of urine of 5ml of benedict sol) while in fehling equal amount of urine and fehling sol. Needed . thus the small amounts of urine in benedict test will not contain amount of reduced compounds naturally found with urine like **Vitamin C , Uric acid** which reduced benedict solution if exist in high amounts .

b) In benedict sol. Sodium carbonate replaced sodium hydroxide in fehling sol. And the last compound have bad impact on glucose while sodium carbonate doesn't have such impact .

c) Benedict sol. Store in one in one bottle and more stable than fehling .

d) It can consider benedict Sol. As successful try to develop fehling solution by make it one solution instead of 2 sol. With more sensitivity and stability .

e) The below table show comparison between benedict Sol. & fehling Sol.

4- Barfoed 's test

Test Principle :

Barfoed reagent is acidic solution (due to acetic acid existence) of copper acetate barfoed test differ from fehling and benedict test because cupric ions reduction occur in acidic medium while the medium is alkaline in benedict test and fehling test .

As reduction is difficult in acidic medium comparing with alkaline medium therefore the reduction doesn't occur in this case except for Monosaccharide which characterized in their strong reduction ability comparing with Mon. ,Di. & polysaccharide , because in monosaccharide each molecule contain one reduction group while disaccharide there is 2 molecule but one reduction group therefore the importance of barfoed test relay on it ability of distinguishing between Monosaccharide and disaccharide with regard to difference in reduction abilities for each type . also the time play important role to decrease test positively that if heating time increased the disaccharides will give positive result due to it hydrolysis in the acidic medium (acetic acid) to monosaccharide beside heating therefore it is important to control time in this test & avoid any additional heating time .

Compounds & reagent

1- Barfoed reagent prepared by dissolving 13.3 gm of crystal copper acetate in 200 ml D.W. then the solution should filter then 1.9 ml glacial acetic acid should add to the filtered solution .
2- Sugar solutions (Monosaccharide) like glucose , fructose and ribose (1% con.)

Method

1- Add 1ml of monosaccharide (all given sugar Sol.) to 2ml of Barfoed , shake well .
2- Place all test tube in one time in water bath for 5 minute (do not add any additional time).
3- After time is over remove the test tubes out the water bath & place it in tubes rack for cooling down .

Note that if there any MonoS. (+ve test) red precipitate will appear (few amount) in tube bottom , while (-ve test) like reduce Disaccharide or unreduced saccharide ..the solution remain clear blue with no red precipitant

Note

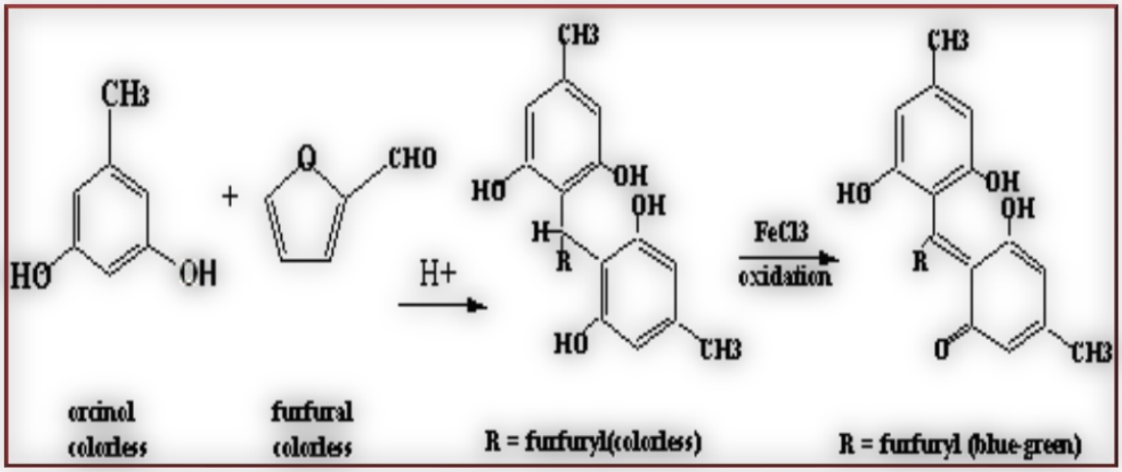
1- This test can used to distinguish between (Mono & Di) reduced sugar .
2- Disaccharide may give +ve test with this reagent if the solution remain in water bath more 5 minute due to the hydrolysis happen to disaccharide under glacial acid effect in this reagent …the disaccharide changed to 2 molecules of monosaccharide.
3- This test can't use to detect monosaccharide (glucose) in urine due to chlorides presence like NaCl in urine which conflict with this test producing green precipitant .

5-Bail's Test

Prepared by
Dr. Radwan Asal
MSc. Zahraa Sabbar Omran

Test Principle :

Bial's test is modification to Moliche's test , the concentrated sulfuric acid replaced with concentrated hydrochloride acid & Alpha Naphthol replaced with ORCINOL, In this test under specific conditions (heat & Acid effects) five carbons sugars change to Furfural which unite with Orcinol (in Ferric ions presence) giving green blush compound.



Compounds & Reagents :

1- **Five carbon sugar\ Solution (Ribose or Xylose 1% concentration).**
2- **Concentrated HCl.**
3- **Bail's reagent prepared by dissolving 1.5 gm of Orcinol in 500 ml of Conc. HCl then add 20 drops of $FeCl_3$ (10% Conc.)**

Work Method :

Add 0.5 ml of sugar solution to 1 ml of bail reagent … heat in boiled water bath for 2 to 3 minutes , solution will color to green blush with pentoses.

Notes :

Some compounds existence in this test could conflict with reagent therefore in case uncertainty regard test positivity … the solution must dilute by adding 10 ml of D.W then add 5 ml of Amyl Alcohol or butyl alcohol (2 layers will noticed) shake the tube well.

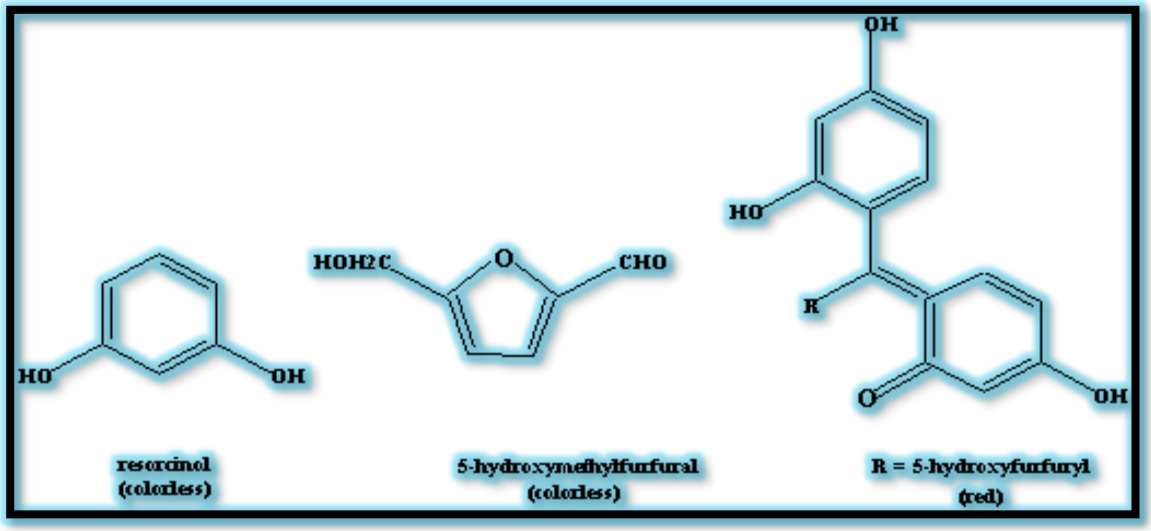
Prepared by
Dr. Radwan Asal
MSc. Zahraa Sabbar Omran

If solution contain pentose's alcohol layer will colored with Green.

6-Selliwanoff Test

Test Principle :

This test is particular for ketones saccharides (Fructose) , this test can consider as modification of moleche's test whereas sulfuric acid replaced by hydrochloric acid , alpha naphthol replaced by Resorcinol without that both tests are similar in scientific idea.



Compounds & Reagents :

1- Solution of fructose sugar 1%.

2- Seliwanoff reagent prepared by dissolving 0.5 gm or resorcinol in 1 liter of HCL in 3 Molar concentration.

Work method :

Put 1 ml of reagent in test tube then add 0.5 ml of sugar Sol. Heat for 10 minutes , solution will become red.

Notes :

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1- Sucrose give positive result with this test because it decompose under acid HCl effect to glucose and fructose , then fructose react with reagent giving red color.

Sucrose $\xrightarrow{HCl}$ Glucose + Fructose

Fructose + Seliwanoff reagent ⟶ Red Sol.

2- This reaction depend on principle that aldose doesn't give furfural when boil with conc. HCl therefore it gives negative result with this reagent.

3- This test give clear result in presence of traces of $FeCl_3$ (1%) or H_2O_2 .

4- Pent saccharides give blue color with this reagent.

This test depend on ketone group in fructose .

POLYSACCHARIDES TESTS

7-Iodine Test

Test Principle:

This test depend on iodine adsorption on starch & dextrin surface giving blue color with starch ; violate with dextrin & red color with glycogen.

This test consider as high sensitive for starch & dextrin, also it sensitive for heat & reaction medium whereas this test give positive result in neutral or acidic medium and give negative result in alkaline medium. the blue color resulted from starch reaction with iodine vanish when we add sodium thiosulfate (or potassium) from this, starch importance appears as internal indicator in quantitative analytical chemistry in iodine solution calibration by sodium thiosulfate.

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Dr. Radwan Asal
MSc. Zahraa Sabbar Omran

Poly saccharide

Compounds & Reagent :

1- **Starch solution 1%.**
2- **Dextrin Solution 1%.**
3- **Glycogen Sol. 1%.**
4- **hydrous iodine Sol. 1%.**
5- **Iodine reagent prepare by dissolving 30 gm of (KI) in one litter of D.W then dissolve 12.7 gm of iodine in previous solution.**

Work Method :

Acidize the medium by adding 1 drops of HCl 3N to 1ml of sample solution … put the mixture in boiling water bath for 2 minutes then add 1 drops of iodine solution , compare the resulted colors.

Notes :

1- **Dextrin solution give violate color with iodine.**
2- **Iodine test should perform in neutral or acidic solution of starch ,it is not right to do test in alkaline solutions because free iodine vanish in alkaline medium changing to iodide salt or iodate salt as below :**

Whereas free iodine is the main reactant in this test , therefore this test give negative result and no blue color appear but when we add mineral acid (HCl) free iodine released again through the reaction of iodate with iodide in acidic medium then blue color appear once again.

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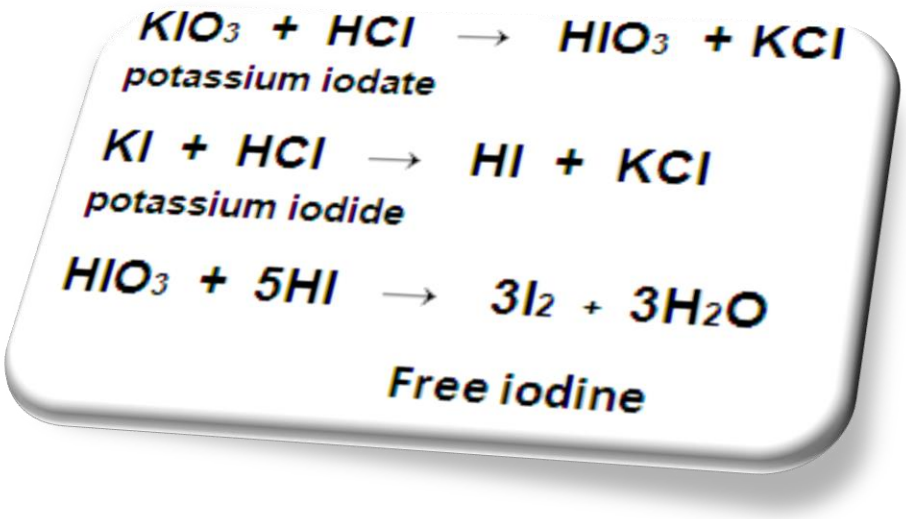

3- When execute iodine test starch or dextrin solution should be in room temperature because head not help iodine adsorption on starch or dextrin surface.

4- The blue color (result from add iodine to starch) vanishing due to sodium thiosulfate reaction with iodine :

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

It noticed that due to the above reaction the free iodine disappear from reaction medium which consider essential for blue color appearance.

Disaccharides decomposition (Sucrose decomposition)

The aqueous decomposition (hydrolysis) of sucrose give mixture of glucose & fructose which are reduced mono-Saccharides in benedict solution, decomposition occur under concentrated acids & heat condition.

Compounds & reagent :

1- Solution contain glucose & sucrose 1% for each one.

2- Benedict quantitative solution.

3- NaOH 40%.

4- **Conc. HCl.**

5- **Litmus paper.**

Work Method :

Put 3 ml of sucrose solution in test tube add 1 ml of HCl , boil the mix ,cool then add NaOH 40% till neutralization (use litmus paper).

Add 2 ml of benedict reagent to the solution then put the tube in water bath … notice the brown – yellow precipitant indicating the reduction .

Poly saccharides decomposition :

Hydrolysis of starch by diluted Acids or saliva

This test stand to proof that in starch hydrolysis the process pass through the following stages :

Starch → Amilodextrin → Arthrodextrin → Achrodextrin → Maltose → Glucose

Blue with iodine violate with iodine red iodine doesn't give color with iodine

this decomposition happen by diluted mineral acids or Amylase enzyme in saliva. The hydrolysis resulted from diluted acids effect differ from saliva products results , that glucose resulted from acids effect while maltose is the result from saliva effect.

To follow decomposition process step by step , we can benefit from the following tests :

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A- **Iodine test : give distinguished colors with starch –Blue , amylodextrin – violate, arthrodextrin – red , the other compounds give negative test.**

B- **Benedict test : this test can detect reduced saccharides produced during the reaction like lower dextrin's ; maltose & glucose.**

C- **Ozason test : through this test maltose and glucose existence can proof because they producing characterized crystals from equivalent ozazonse.**

Compounds & Reagents :

1- **Starch 1%.**
2- **Diluted HCl 20%.**
3- **Iodine solution 1%.**
4- **Benedict Reagent .**
5- **Sodium Hydroxide 5%.**

Work method :

Mix 10 ml of starch Sol. With 2 ml of HCl , put the test tube in boiled water bath, take 1 drop of mix in the beginning of heating and after each 3 minutes take few drops of mix and do iodine test to detect the hydrolysis of products. You will note changing the color from blue (starch) to violate then red then the color sop changing due to achrodextrin & the other next products . when this point reached iodine color will not change any more so leave the test tube inside the water bath for 10 minutes, then take little of the solution and neutralize acidity by adding NaOH 5% then execute benedict test then proof glucose existence in solution by osazone test , notice benedict solution reduction. Detect reduced sugar presence by osazone test you will notice forming Glucozazon compound indicating that the final product of starch decomposition by diluted acids is glucose , this is prove that starch compose of many units of glucose. Repeat the experiment using diluted saliva instead of diluted acid.

Notes :

1- In starch hydrolysis by diluted acids the final product will be glucose , while maltose is the final product for starch hydrolysis by saliva amylase , this ease the distinguishing between glucose & maltose by ozazone test.

2- This test support the scientific idea of starch decomposition by amylase enzyme in saliva also highlight saliva role in starchy digestion.

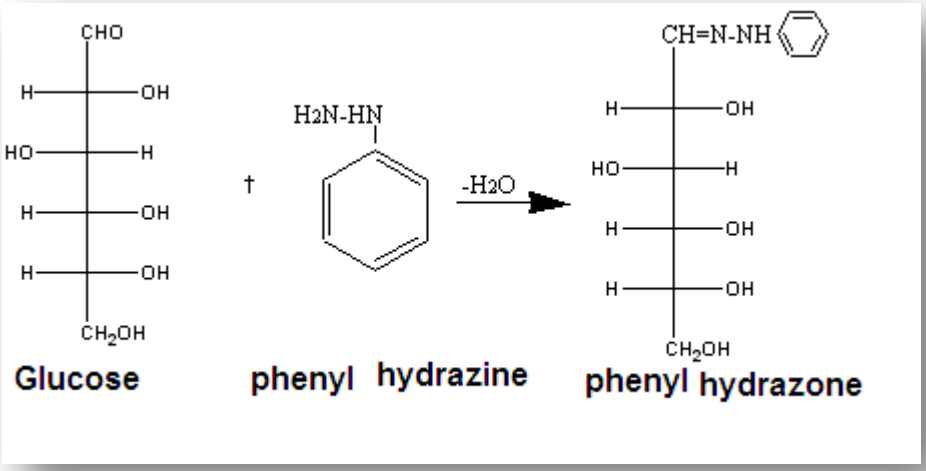
Osazone compounds

Phenyl hydrazine effect on monosaccharaides

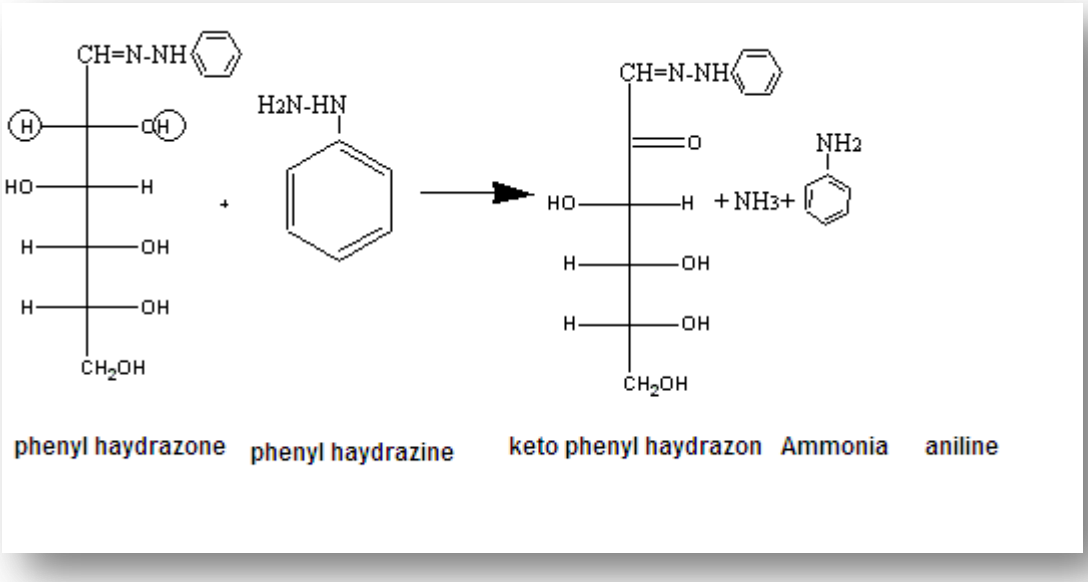
In first step glucose react by aldehyde group with phenyl hydrazine giving the main component of this test ;

All Monosaccharaides also di reduced saccharides characterized by its ability to unit with phenyl hydrazine producing compounds (yellow distinguished crystals) can see under microscope, this test persuade after making sure the sugar sample is reduced sugar (by benedict or Fehling tests). Now if we review reaction steps of this test , phenyl hydrazine with glucose (aldose monosaccharaide) to form osazone (glucozazone in this case) :

a- In first step glucose react phenyl haydrazine by it terminus aldehyde group giving phenyl hydrozone , this process consider as reduction for glucose.

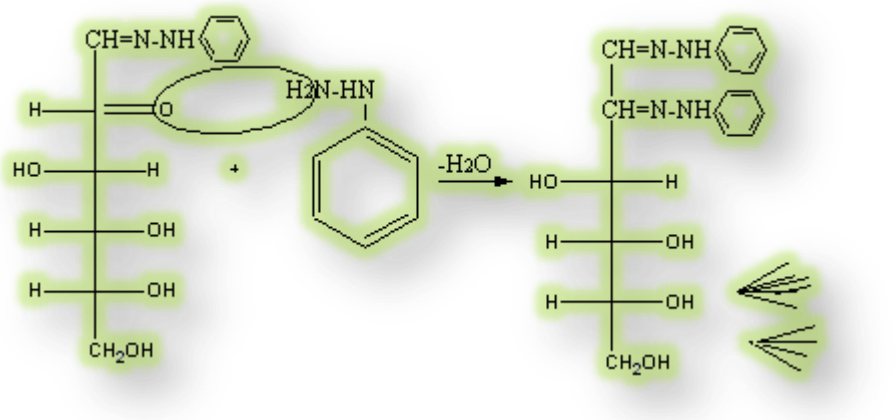


b- In second step oxidation happen to phenyl haydrazone , the compound loss the 2 hydrogen atoms attached to second carbon atom then transform to keto phenyl hydrazine and these 2 hydrogen atoms reduce phenyl hydrazone to aniline & ammonia :



c- In third step keto phenyl haydrzone combine with third molecule of phenyl hydrazine (through ketone group on second carbon atom in the compound)giving glucozazone which separated as yellow needle crystals, this happen while the test tube inside boiling water bath, this step consider as reduction of keto phenyl hydrazine.

Prepared by
Dr. Radwan Asal
MSc. Zahraa Sabbar Omran



Keto phenyl hydrazine **Glucozazone**

If ketone mono saccharide like fructose react with phenyl hydrazine the uniting start with second carbon atom(the once contain the ketone group) then the first carbon atom (reverse glucose) finally fructozazone resulted, which is similar to glucozazone in chemical & natural properties (glucozazone & fructozazone representing one compound chemically – check chemical equations).

The osazones resulted from reduced mono & di saccharides (like maltose , lactose) marked by their yellow colors , some of it precipitate in high temperature like glucozazone the others precipitate after cooling test tube slowly in room temperature like lactozazone , any way all of it distinguished by remarkable shapes under microscope and this is become essential to distinguishing between different kinds of reduced sugars.

Compounds & reagents :

1- Phenyl hydrazine reagent prepare by mixing equal amounts phenyl hydrazine hydrochloride with anhydrous sodium acetate.
2- Samples of monosaccharide's like Glucose ; Fructose & Glactose 1%.

Work Method

1- To 0.5 g of phenyl hydrazine hydrochloride add 0.1 g of sodium acetate and 10 drops of glacial acetic acid. To this mixture add 5ml of test solution and heat in boiling water bath for about half an hour, allow the tube to cool slowly and examine the crystals under microscope.

2- Note that some osazones forming while the test tube in boiling water bath (like glucozazone) in case the osazones doesn't yield while test tubes in the water bath … remove the tube from water bath and leave it to cool slowly put it in tube rack and don't attempt to cool it fast by cold water , note the precipitants crystals.

3- Glucose , fructose and mannose produce needle – shaped yellow osazone crystal, whereas lactosazone is mushroom –shaped. Different osazones show crystals of different shapes. Maltose produces flower shaped crystals.

Note :

1- Osazone test not limited only as mean to identify some saccharides but also it help to define the stereo and chemical composition of it, for example glucose & fructose give same osazone indicating that third – forth – fifth – sixth carbon atoms are completely similar ,the stereo distribution of H & OH atoms around CARBON ATOM are similar and difference between it lie in first & second carbon atoms which overcame by osazone reaction . glactose give different weights because it different from other saccharides in their steric configuration (H & OH) in Forth carbon atom.

2- Hydrochloride phenyl hydrazine is toxic material , the famous scientist Emil Fischer is the first researcher who used this compound to detect carbohydrate, and he was just about to lose his life because this compound toxin , therefore it is important to use it very carefully.

3- This reaction occur in three steps.

4- This test doesn't give positive result unless excess amount of phenyl hydrazine reagent added because each reduced saccharide molecule need three molecule of reagent , therefore if the amount of phenyl hydrazine not enough the reaction will not complete and no crystals yield.

5- Osazones formation depend on free aldehyde or ketone groups in saccharide molecule, therefore this test doesn't persuade without insuring the saccharide is reduced , normally the non-reduced saccharides like sucrose starch dextrin glycogen not give this test because it's not contain aldehyde or ketone groups.

6- Due to phenyl haydrazine instability hydrochloride phenyl hydrazine use instead of it also sodium acetate added to create suitable medium reaction.