Quantitative analysis of Carbohydrates

A- Introduction of carbohydrates:

This experiment is designed to introduce you to carbohydrates, one of the three major classes of macronutrients found in food. You will also learn a number of ways to classify carbohydrates and several tests used in carbohydrate analysis.

The experiment is composed of four parts (background, prelab, experiment, postlab) that should be completed in the order listed below.

If you are already familiar with carbohydrates, you may proceed directly to the section on carbohydrate tests.

The term carbohydrates is generally applied to the group of polyhydroxy aldehydes or ketones commonly known as sugars. 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.

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

Carbohydrates can be classified as simple or complex:

1- Simple carbohydrates, often called monosaccharides or simple sugars, contain one saccharide unit and cannot be broken down into smaller carbohydrates.

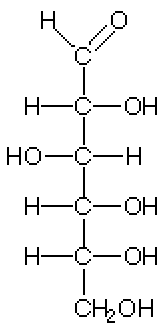
2- Complex carbohydrates are those containing more than one saccharide group.

- Disaccharides contain two monosaccharide units.
- Oligosaccharides contain 3-6 monosaccharide units.

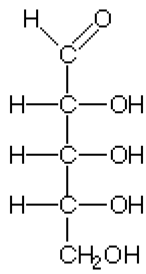
- polysaccharides can contain over 7 or more monosaccharide units. Complex carbohydrates can be broken down into smaller carbohydrate units through a process known as hydrolysis.

Monosaccharides can be classified in a number of ways. They can be classified by the number of carbon atoms they contain. While there are others, pentoses (5 carbons) and hexoses (6 carbons) are the most common.

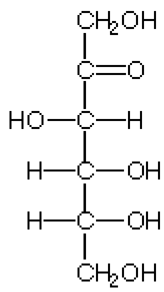
Monosaccharides can also be classified as ketoses or aldoses. A ketose contains a carbonyl group, in addition to having one or more hydroxyl groups. An aldose contains an aldehyde group in addition to the hydroxyl groups. The two descriptors are commonly combined into a single term like "aldohexose" for an aldose or "ketohexose" for ketose that also is a hexose. See below some examples of monosaccharides.



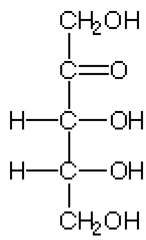
glucose
(an aldohexose)



ribose
(an aldopentose)

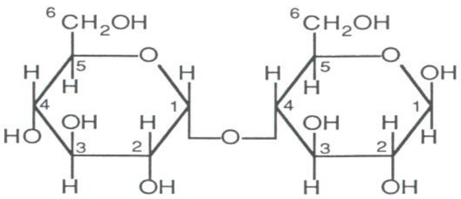


fructose
(a ketohexose)



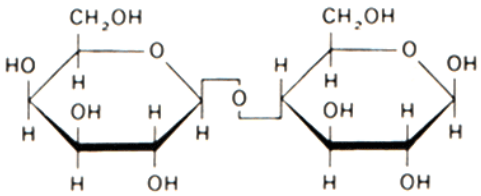
ribulose
(a ketopentose)

Disaccharides can be broken into two monosaccharide units by hydrolysis and lost one molecule of water, See below some examples of disaccharides.



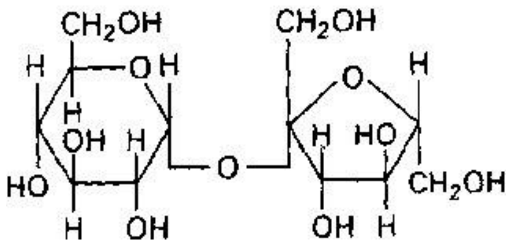
glucose **glucose**

Maltose



galactose **glucose**

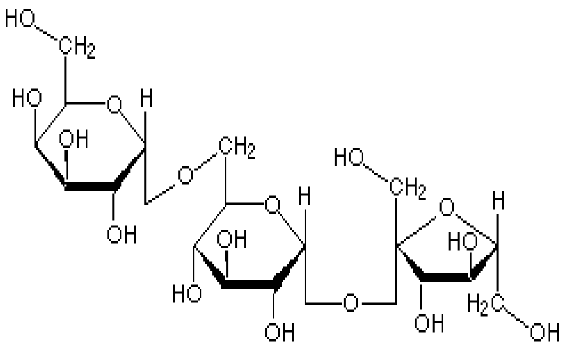
lactose



glucose **fructose**

Sucrose

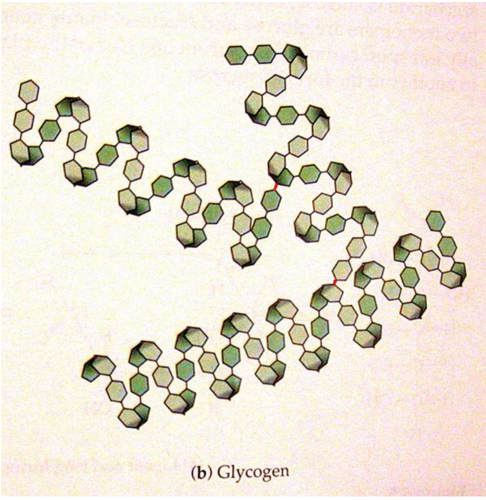
Oligosaccharides can be broken into 3-6 monosaccharide units by hydrolysis and lost one or more molecules of water, See below example of oligosaccharides.

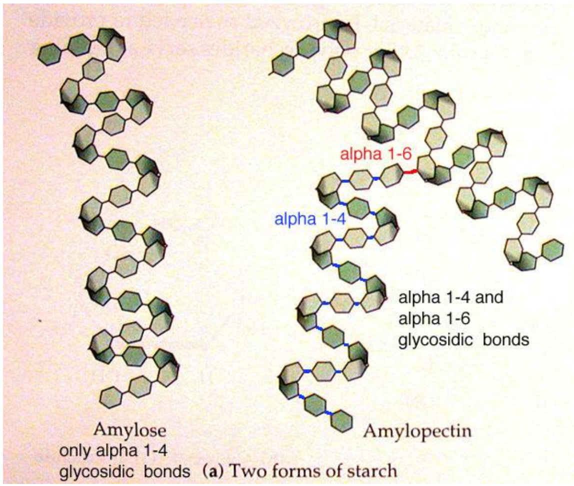


glucose **glucose** **fructose**

Raffinose

Polysaccharides can be broken into7 or morew monosaccharide units by hydrolysis and lost one or more molecules of water, See below example of polysaccharides.





B- Lab experiments:

- **Physical properties:**

  Solubility- Limos paper- Color- Shape.

- **Chemical properties:**

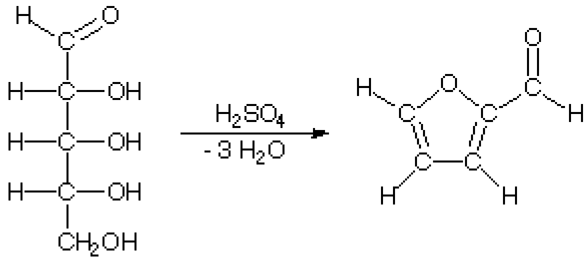
1- The Molisch Test:

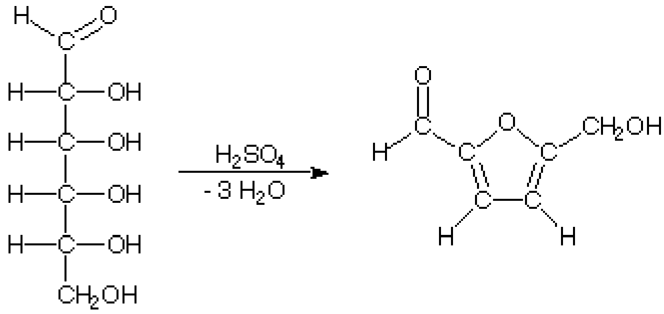
Shows positive test for:

All carbohydrates. Monosaccharides give a rapid positive test. Disaccharides and polysaccharides react slower.

Reactions:

The test reagent dehydrates pentoses to form furfural (top reaction) and dehydrates hexoses to form 5-hydroxymethyl furfural (bottom reaction). The furfurals further react with α-naphthol present in the test reagent to produce a purple product (reaction not shown).





How to perform the test:

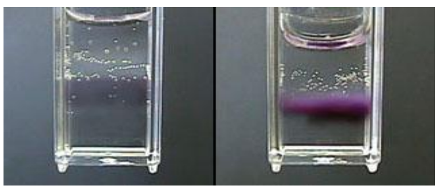
1- Two ml of a sample solution is placed in a test tube.

2- Two drops of the Molisch reagent (a solution of α-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.

A positive test is indicated by:

The formation of a purple product at the interface of the two layers.



a negative test (left) and a positive test (right)

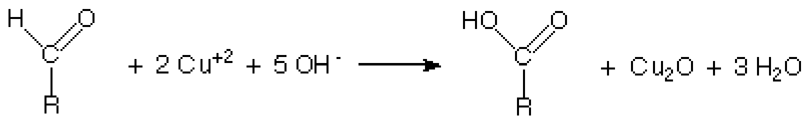
2- The Benedict's Test:

Shows positive test for:

Reducing sugars

Reactions:

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

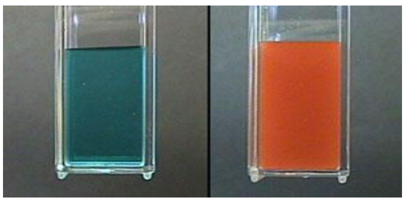


How to perform the test:

1- One ml of a sample solution is placed in a test tube.
2- Two ml of 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 three minutes.

A positive test is indicated by:

The formation of a reddish precipitate within three minutes.



a negative test (left) and a positive test (right)

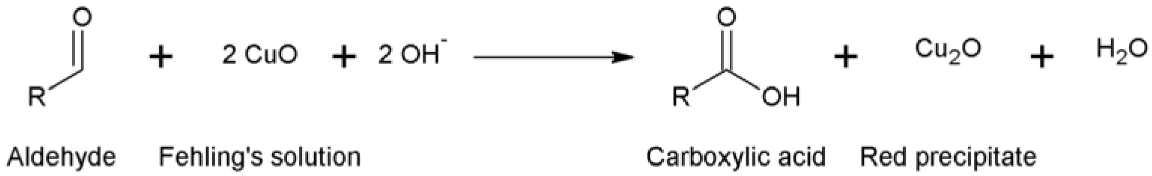
3- Fehling's Test:

Shows positive test for:

Reducing sugars

Reactions:

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



How to perform the test:

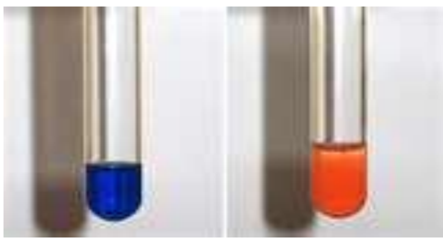
1- One ml of a sample solution is placed in a test tube.

2- One ml of fehling's reagent (fehling's A, uses 7 gm $CuSO_4.5H_2O$ 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 three minutes.

A positive test is indicated by:

The formation of a reddish brown precipitate within five minutes.



a negative test (left) and a positive test (right)

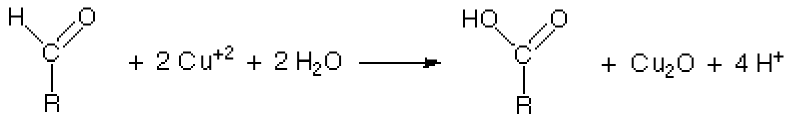
4- The Barfoed's Test:

Shows positive test for:

Reducing monosaccharides

Reactions:

Reducing monosaccharides are oxidized by the copper ion in 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.



How to perform the test:

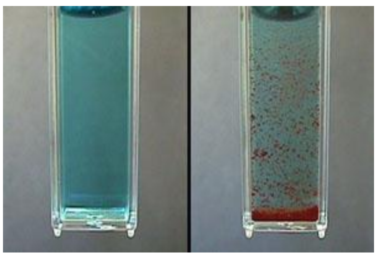
1- One ml of a sample solution is placed in a test tube.

2- Three ml of 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 three minutes.

A positive test is indicated by:

The formation of a reddish precipitate within three minutes.



a negative test (left) and a positive test (right)

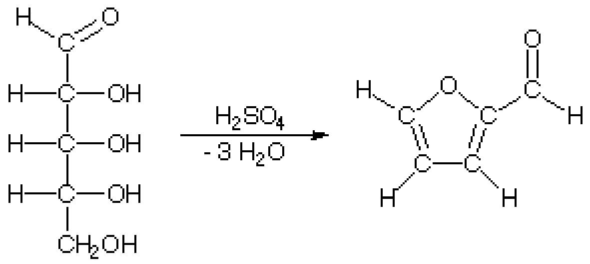
5- The Bial's Test:

Shows positive test for:

Pentoses

Reactions:

The test reagent dehydrates pentoses to form furfural. Furfural further reacts with orcinol and the iron ion present in the test reagent to produce a bluish product (reaction not shown).



How to perform the test:

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

2- Two ml of Bial's reagent (a solution of orcinol, HCl and ferric chloride) is added.

3- The solution is then heated gently in a Bunsen Burner or hot water bath.

4- If the color is not obvious, more water can be added to the tube.

A positive test is indicated by:

The formation of a bluish product. All other colors indicate a negative result for pentoses. Note that hexoses generally react to form green, red, or brown products.

two negative tests (left, middle) and a positive test (right)

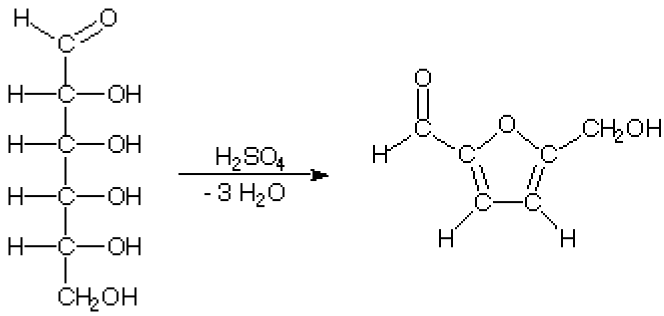
5- The Seliwanoff's Test:

Shows positive test for:

Ketoses

Reactions:

The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural further reacts with resorcinol present in the test reagent to produce a red product within two minutes (reaction not shown). Aldohexoses react to form the same product, but do so more slowly.

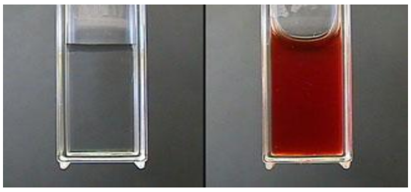


How to perform the test:

1- One half ml of a sample solution is placed in a test tube.
2- Two ml of Seliwanoff's reagent (a solution of resorcinol and HCl) is added.
3- The solution is then heated in a boiling water bath for two minutes.

A positive test is indicated by:

The formation of a red product.



a negative test (left) and a positive test (right)

7- The Hydrolysis Test:

Shows positive test for:

Sucrose

Reactions:

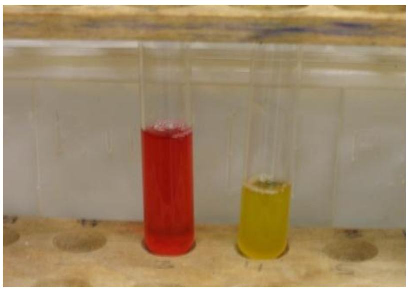
Sucrose is the only non-reducing sugar so it does not reduce the Cu solution (Bendict's and fehling's test). But can be hydrolysed to its component and then test for.

How to perform the test:

1- 6ml of a sample solution is placed in a test tube.
2- Two drops of concentrated hydrochloric acid (HCl).
3- Heat the tube in boiling water bath for 15 minutes.

A positive test is indicated by:

The formation of orange coloure.



a negative test (right) and a positive test (leftt)

8- The Iodine/Potassium Iodide Test:

Shows positive test for:

Starch

Reactions:

Iodine complexes with starch to form a blue-black product. Note that other polysaccharides may give other colors including blue or red.

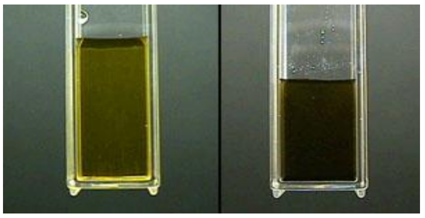
How to perform the test:

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

5- Two drops of iodine/potassium iodide solution and one ml of water are added.

A positive test is indicated by:

The formation of a blue-black complex.



a negative test (left) and a positive test (right)

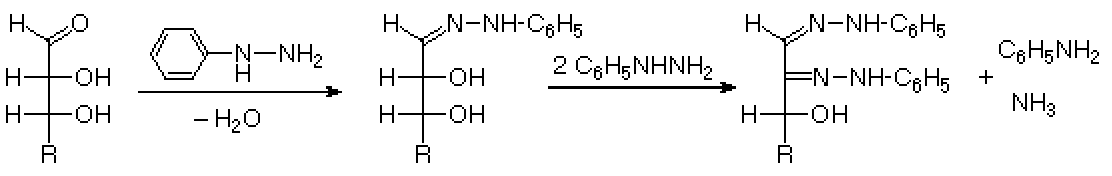
9- The Osazone Test:

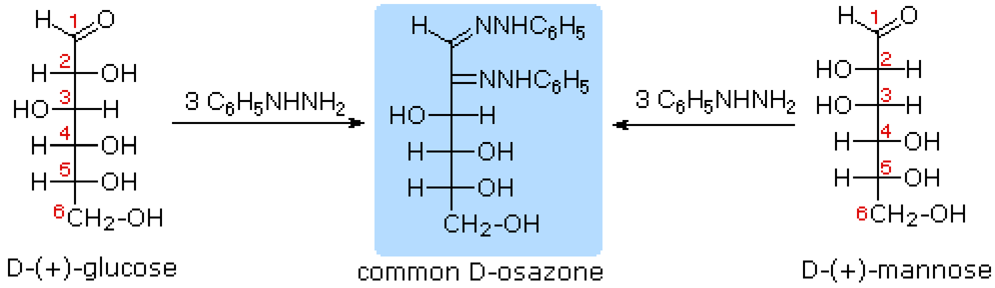
Shows positive test for:

All carbohydrates except sucrose and starch.

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-phenylhydrazone, 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.





How to perform the test:

1- Two gm of the sample in a test tube.
2- Add one and half gm of phenylhydrazine hydrochloride, 0.6 gm of crystallized sodium acetate, and 4 mL of distilled water.
3- Place the test tube in a beaker of boiling water.
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.

A positive test is indicated by:

The formation of precipitation around 20 min, the result is monosaccharides (glucose or fructose), formation of precipitation after cool, the result is disaccharides (maltose or lactose).

OSAZONE

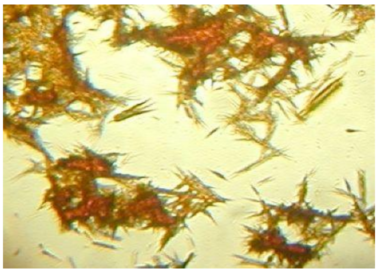
>30 minute, Fructose will be action

some picture which taken form http://www.didier-pol.net/3osazon.htm

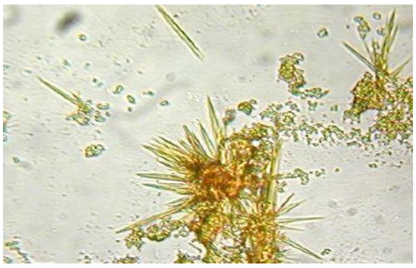
Here is Glucosazone x250

Here is Maltosazone x250

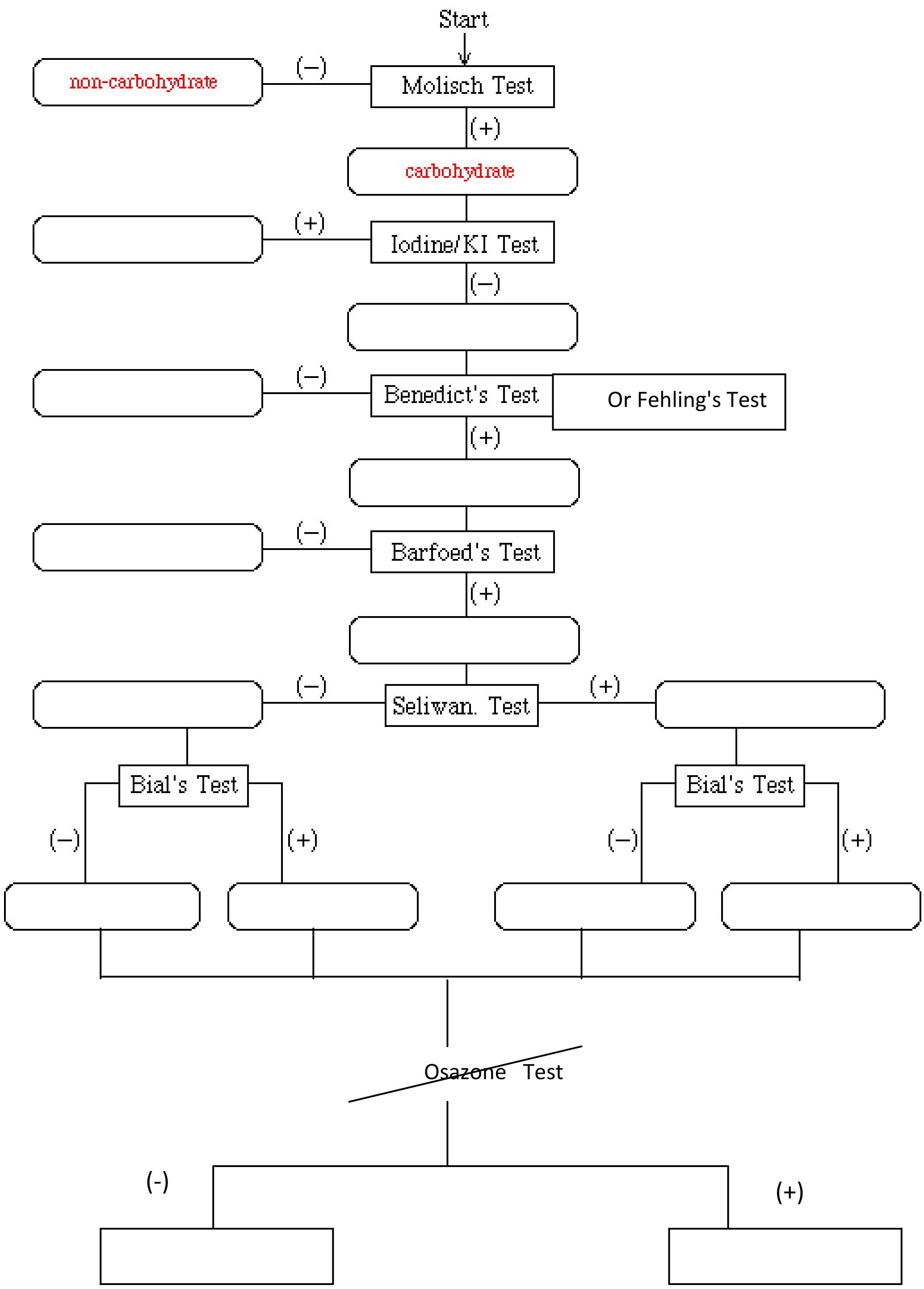
Here is Galactosazone x250



Here is lactosazone x250



This partially completed scheme was devised to make efficient use of the tests described in the background. Complete the scheme by filling in the empty boxes with words from the list below.



When you are finished collecting data, determine the identity of each unknown. Record physical and chemical properties of your test, observation and results in the tables below.

Unknown (1)

Test	Observation	Results
-Physical properties: Chemical properties:		

Unknown (2)

Test	Observation	Results
-Physical properties: Chemical properties:		

References:

http://www.harpercollege.edu/tm-ps/chm/100/dgodambe/thedisk/carbo/yback9.htm

http://www.wikipedia.org