

Experiment 28

Carbohydrates

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Name:

Date:

Key Objectives

1. Structure of Carbohydrates
2. Physical properties of Carbohydrates
3. Diagnostic tests for Carbohydrates

Discussion

General

Carbohydrates are one of the three principal classes of foods, and is a major source of energy in our diets. Carbohydrates are the major constituents of plants, and are also found in some animal tissues. Carbohydrates are compounds composed of carbon, hydrogen and oxygen containing an aldehyde or ketone functional group and many alcohol groups, and can also be called polyhydroxy aldehydes or polyhydroxy ketones. Molecules that when hydrolyzed yield polyhydroxy aldehydes or ketones are also considered carbohydrates.



Figure 28.1: Carbohydrates are found in many foods. credit: <https://pxhere.com/en/photo/954347>

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Carbohydrates can be characterized in a number of different ways based on the number of carbons (pentose, hexose), the functional group (aldose, ketose), formation of rings (furanose, pyranose), and isomers (D or L) and the number of monosaccharide units in the molecule (mono, di, and polysaccharides). A more detailed discussion of these features can be found in Hein 27.2 pages 764-766.

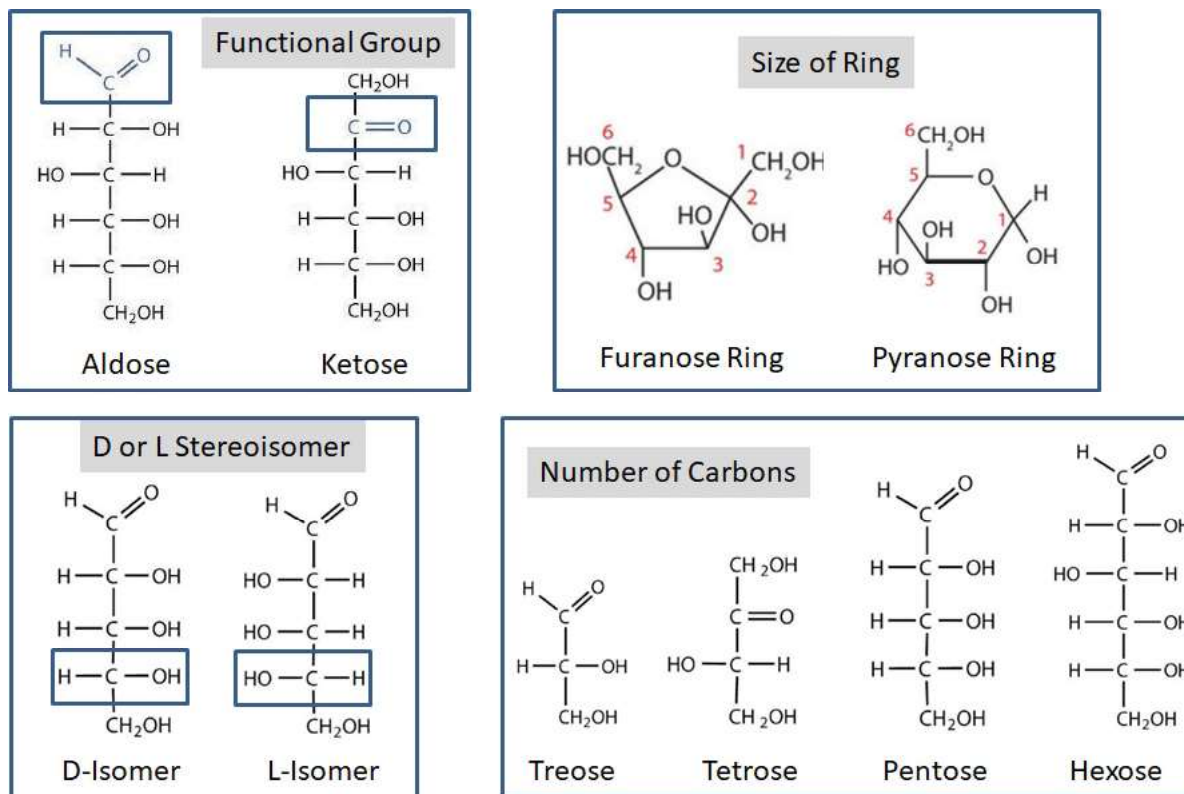


Figure 28.2: Carbohydrates can be classified according to Functional Group, Ring Size, D or L Isomer, and Number of Carbons. credit: author modified from "The Basics of GOB Chemistry (Ball et al.)" by LibreTexts is licensed under CC BY-NC-SA.

Chemical Tests for Carbohydrates

Numerous tests have been devised for the determination of the properties and for the differentiation of carbohydrates. A brief description of the most common tests follows.

Molisch Test

This is a general test for carbohydrates designed to distinguish between carbohydrates, lipids and proteins. The test proceeds in two steps, the first is based on the dehydration reaction of a carbohydrate with concentrated sulfuric acid to form a furfural or hydroxyfurfural which in the second step reacts with the Molisch reagent (α -naphthol) to yield a colored product, generally a purple ring at the interface of the two layers. Lipids and proteins can not be dehydrated therefore give a negative result.

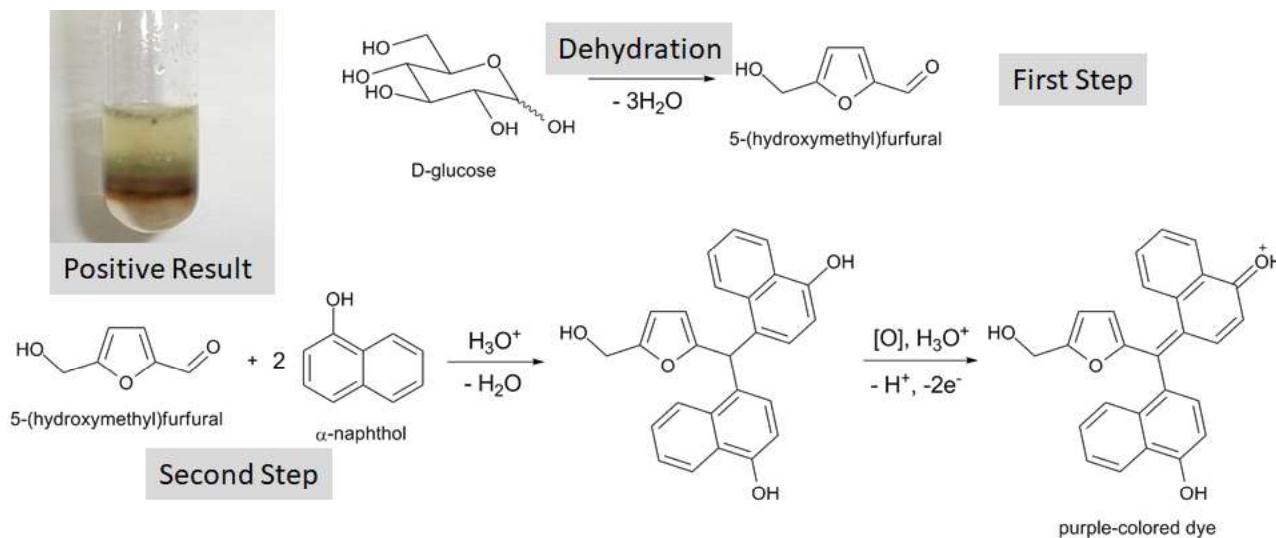


Figure 28.3: (a) Molisch test distinguishes between carbohydrates, lipids and proteins. credit: https://en.wikipedia.org/wiki/Molisch%27s_test

Seliwanoff Test

This test distinguishes a ketohexose from an aldohexose and disaccharides. The test is based on the fact that, when heated, ketoses are more rapidly dehydrated than aldoses. The reaction between a ketohexose and the Seliwanoff reagent (resorcinol and dilute HCl) occurs rapidly (within 1 minute) in boiling water and results in the formation of a reddish colored solution. The color intensifies with further heating. Other carbohydrates and aldohexoses will give a faint reddish color if the heating is prolonged due to the conversion of glucose to fructose by the HCl or hydrolysis of a disaccharide or polysaccharide to yield fructose.

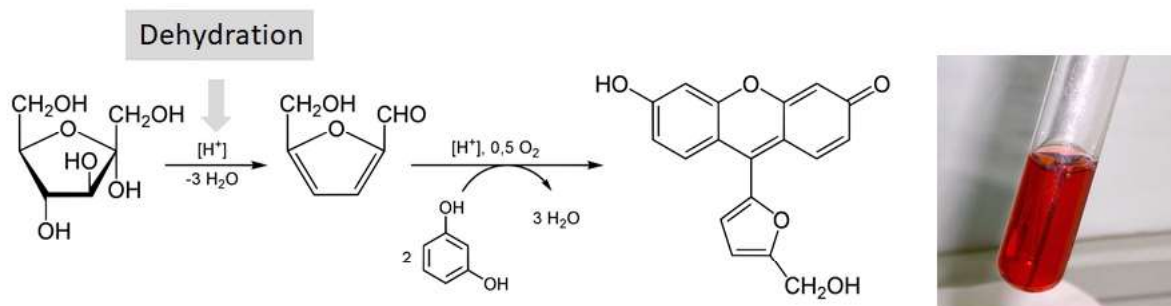


Figure 28.4: (a) Seliwanoff test distinguish aldohexoses from ketohesoses. credit: https://en.wikipedia.org/wiki/Seliwanoff%27s_test

Benedicts Test

This tests for reducing sugars. Free aldehyde groups, α -hydroxy ketones and hemiacetals are capable of reducing metal ions in solution. All common monosaccharides are reducing sugars. Some easily hydrolyzed disaccharides and polysaccharides will give a positive test, while harder to hydrolyze di- and poly sacchrides may initially be nonreducing, but react after extended time or upon heating in acidic solution by being hydrolyzed to monosaccharides.

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In the Benedict's test Cu^{2+} ions (blue in solution) are reduced to Cu^{+1} ions that form Cu_2O which is a brick-red precipitate. Depending on the amount of precipitate produced, a positive Benedict's test can appear green, yellow, orange, or red. Specifically for the Benedict's reagent solution is prepared by mixing copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), and sodium carbonate (Na_2CO_3) in distilled water. The generalized reaction is shown.

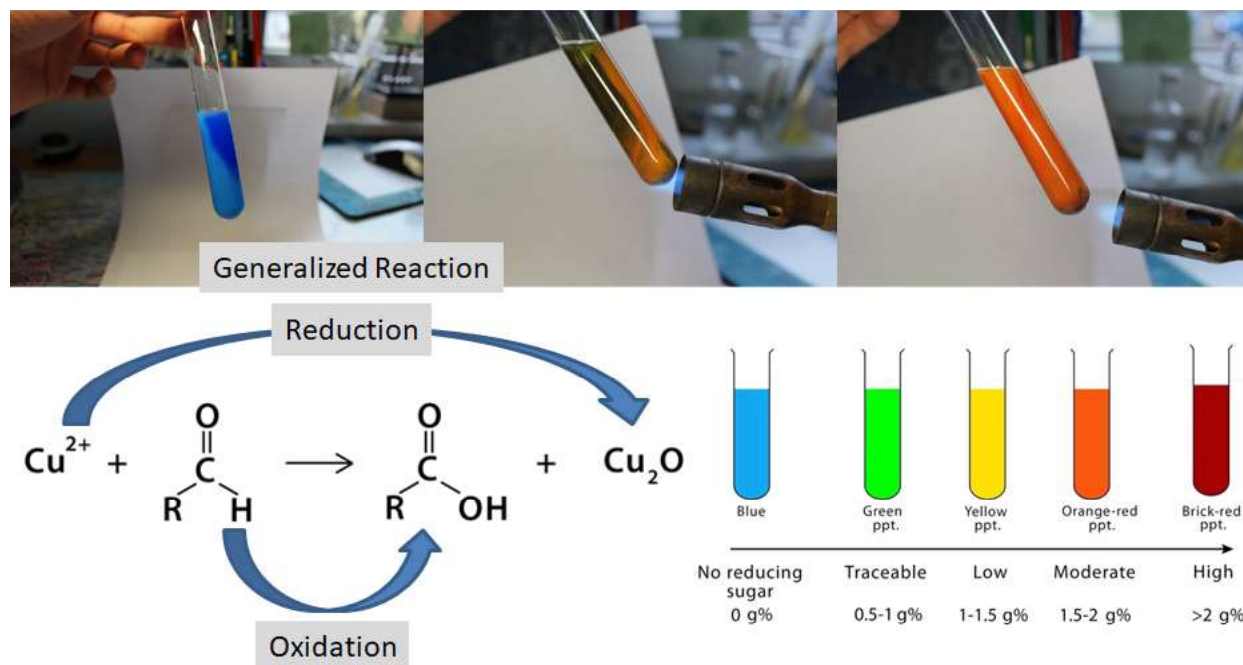


Figure 28.5: Benedict's test for reducing sugars. credit: By Kubawlo - Own work, CC BY-SA 4.0, <https://commons.wikimedia.org/w/index.php?curid=19455438>

Barfoeds Test

The Barfoeds test is similar to the Benedict's test, but differs in the specific reagents used (copper (II) acetate and acetic acid) which is less reactive than the Benedict's reagent. It is used to differentiate between monosaccharides and disaccharides. When boiled in water, monosaccharides will react in under five minutes, while disaccharides will take longer. If heated long enough (10-15 minutes) disaccharides will give a positive result due to hydrolysis to form monosaccharides. As in the Benedict's test above, the primary reaction is the reduction of Cu^{2+} ions to Cu_2O which forms a brick red precipitate.

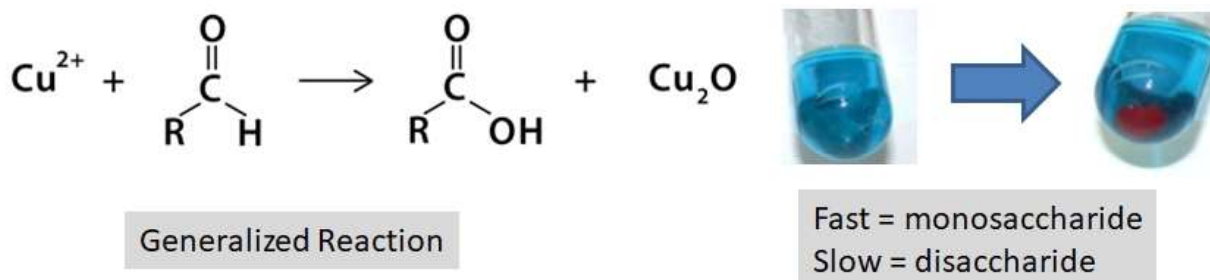


Figure 28.6: Barfoeds test for monosaccharides. credit: author

Bial Test

This test is used to distinguish between pentoses and hexoses. The Bial reagent contains orcinol (5-methyl resorcinol) dissolved in concentrated HCl with a small amount of iron (III) chloride present. When mixed with a monosaccharide, pentoses are converted to a furfural to yield a blue-green product. Hexoses, with prolonged heating, yield a muddy red precipitate.

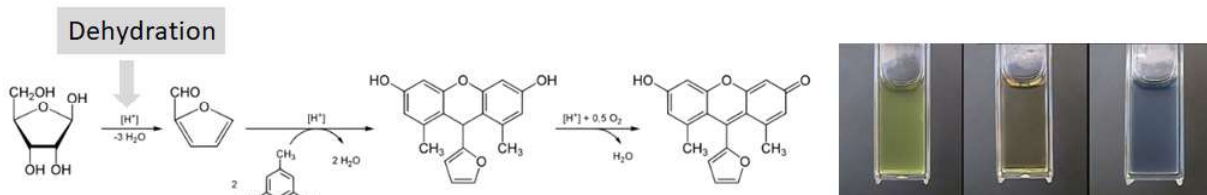


Figure 28.7: Bials test for pentose sugars. credit: https://en.wikipedia.org/wiki/Bial%27s_test and <http://dept.harpercollege.edu/chemistry/chm/100/dgodambe/thedisk/carbo/bial/bials.htm>

Iodine Test

Iodine reacts with polysaccharides to give colored complexes. The helix structure of a polysaccharide wraps around the iodine ion (I^-), resulting in a color change from clear (I_2) to colored (I^-) solution. Specifically amylose gives a deep blue-black color, amylopectin, cellulose, form red to brown colors, and glycogen produces a reddish colored precipitate. Monosaccharides and Disaccharides are too small to trap the iodine molecules and do not form dark colored complexes. The iodine test can be used to distinguish between mono/disaccharides and polysaccharides. It may also be used to follow the course of a hydrolysis reaction.

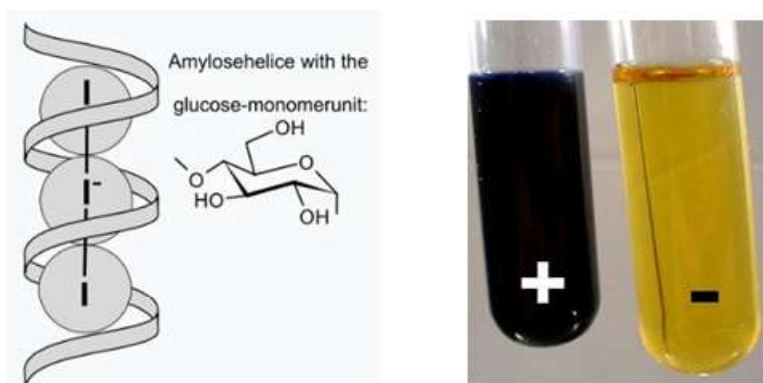


Figure 28.8: Iodine test for polysaccharides. credit: https://en.wikipedia.org/wiki/Iodine%E2%80%93starch_test

Procedure

A. Molisch Test

1. Place 5 mL of each solution to be tested in a test tube.
2. Add 3 drops of the Molisch reagent. Swirl to mix.
3. Tilt the test tube at a 45 degree angle and carefully add 40 drops of concentrated sulfuric acid by slowly dripping it down the side of the test tube. Do **NOT** mix the solutions. The sulfuric acid should form a layer beneath the carbohydrate layer.
4. Set the test tubes carefully in the rack and observe for evidence of a reaction over the next 15-20 minutes. Record your results.
5. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

B. Seliwanoff Test

1. Place 1 mL of each solution to be tested in a test tube.
2. Add 4 mL of the Seliwanoff reagent. Swirl to mix.
3. Place the test tubes in a boiling water bath for 2-3 minutes. Do not overheat.
4. Record your results.
5. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

C. Benedict Test

1. Place 1 mL of each solution to be tested in a test tube.
2. Add 4 mL of Benidicts Solution A and 1 mL of Benidicts Solution B. Swirl to mix.
3. Place the test tubes in a boiling water bath for 5 minutes. Do not overheat.
4. Record your results.
5. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

D. Barfoed Test

1. Place 1 mL of each solution to be tested in a test tube.
2. Add 5 mL of the Barfoed reagent. Swirl to mix.
3. Place the test tubes in a boiling water bath for 5 minutes. Do not overheat.
4. Record your results.

5. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

E. Bial Test

1. Place 2 mL of each solution to be tested in a test tube.
2. Carefully add 3 mL of the Bial's reagent. (Bial's reagent is dissolved in concentrated HCl. Swirl to mix.
3. Carefully heat (with agitation) each tube over a Bunsen burner until the mixture just begins to boil.
4. Record your results.
5. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

F. Iodine Test

1. Place 2 mL of each solution to be tested in a test tube.
2. Add 2-3 drops of the Iodine reagent. Swirl to mix.
3. Record your results.
4. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

G. Hydrolysis of Disaccharides

1. Before doing this part you must have completed parts B and C
2. Copy your results for Sucrose and Maltose (before hydrolysis from parts B and C) to the data table.
3. Place 10 mL of disaccharide in a test tube.
4. Add 5 drops of concentrated HCl to the test tube.
5. Place the test tubes in boiling water for 10 minutes.
6. Cool the solutions.
7. Neutralize the acid with 10% NaOH solution (15-20 drops), using red litmus paper as an indicator.
8. Using 2 mL portions of the resulting solutions run the Benedict and Seliwanoff tests.
9. Record your results.
10. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

H. Hydrolysis of a Polysaccharide

1. Perform the Iodine and Benedict test prior to doing this section.

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2. In a 250 mL beaker place 2.5 mL of a 1% starch solution, 200 mL of water, and 10 drops of concentrated hydrochloric acid.
3. Remove a 2-3 mL portion of the sample. Perform the Iodine test on the removed sample. Record your results.
4. Cover the beaker with a watch glass, and gently boil the starch solution for 15-30 minutes. Consider time zero to be when the solution first boils.
5. After the solution starts to boil, remove 2-3 mL of the solution and perform the Iodine test. Record your results.
6. Perform the iodine test every 5 minutes until a clear results is achieved. Record your results.
7. If your solution is running dry, add 50 mL of water to the beaker.
8. After the heating is completed, take a 2-3 mL portion and neutralize it with 10% NaOH solution, using red litmus paper as an indicator.
9. Perform the Benedict test on the sample.
10. Record your results.
11. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

I. Fruit Juices

1. Perform the Benedict and Seliwanoff test prior to doing this section.
2. Using the fruit juice samples provided run the Benedict and Seliwanoff tests using 1 mL samples of each juice.
3. Dispose of the contents of the test tubes in the waste bottle labeled "E26 Carbohydrate Waste".

Name: _____

Date: _____

Score: ____/80

Results

A-C. Characteristic Tests for Carbohydrates

	Molisch		Seliwanoff		Benedict	
	Observations	+/-	Observations	+/-	Observations	+/-
Water						
Arabinose						
Glucose						
Fructose						
Maltose						
Sucrose						
Starch						
Xylose						

D-F. Characteristic Tests for Carbohydrates

	Barfoed		Bial		Iodine	
	Observations	+/-	Observations	+/-	Observations	+/-
Water						
Arabinose						
Glucose						
Fructose						
Maltose						
Sucrose						
Starch						
Xylose						

G. Hydrolysis of a Disaccharide

	Sucrose		Maltose	
	Observation	+/-	Observation	+/-
Seliwanoff	Before Hydrolysis:		Before Hydrolysis:	
	After Hydrolysis:		After Hydrolysis:	
Benedicts	Before Hydrolysis:		Before Hydrolysis:	
	After Hydrolysis:		After Hydrolysis:	
Products of Hydrolysis (Names or Structures)				

H. Hydrolysis of Starch

	Control (H₂O)	0 min.	5 min.	10 min.
Observation				
	15 min.	20 min.	25 min.	30 min.
Observation				
	35 min.	40 min.	45 min.	50 min.
Observation				
	55 min.	60 min.	Benedicts	Draw a smiley face!
Observation				

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I. Fruit Juices

Fruit Juice ID	Benedicts		Seliwanoff	
	Observation	+/-	Observation	+/-

Questions

A. Molisch Test

1. Describe the evidence for a positive test.
2. What does a positive test indicate?
3. What is the chemical basis for a positive test?
4. Circle the names of the substances that gave a positive test.
Water Arabinose Glucose Fructose Maltose Sucrose Starch Xylose
5. List any substances that gave a false positive result and/or a false negative result. Explain.

B. Seliwanoff Test

6. Describe the evidence for a positive test.
7. What does a positive test indicate?
8. What is the chemical basis for a positive test?
9. Circle the names of the substances that gave a positive test.
Water Arabinose Glucose Fructose Maltose Sucrose Starch Xylose
10. List any substances gave a false positive result and/or a false negative result. Explain.
11. Upon prolonged heating, would you expect any of the substances that gave a negative result to yield a positive result? Explain.

C. Benedict

12. Describe the evidence for a positive test.

13. What does a positive test indicate?

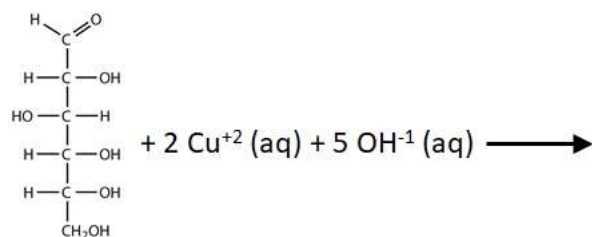
14. What is the chemical basis for a positive test?

15. Circle the names of the substances that gave a positive test.

Water Arabinose Glucose Fructose Maltose Sucrose Starch Xylose

16. List any substances gave a false positive result and/or a false negative result. Explain.

17. Complete the following reaction. Use a line drawing for the carbohydrate.



18. If the Cu^{+2} ions in the Benedicts Reagent are reduced what must have happened to the sugar? Explain.

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D. Barfoed

19. Describe the evidence for a positive test.

20. What does a positive test indicate?

21. What is the chemical basis for a positive test?

22. Circle the names of the substances that gave a positive test.

Water Arabinose Glucose Fructose Maltose Sucrose Starch Xylose

23. List any substances gave a false positive result and/or a false negative result. Explain.

E. Bials

24. Describe the evidence for a positive test.

25. What does a positive test indicate?

26. What is the chemical basis for a positive test?

27. Circle the names of the substances that gave a positive test.

Water Arabinose Glucose Fructose Maltose Sucrose Starch Xylose

28. List any substances gave a false positive result and/or a false negative result. Explain.

29. Write the structural formula **AND** name of two carbohydrates not tested that would give a positive Bial test.

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G. Hydrolysis of Disaccharides

30. What do the results of these tests indicate about the composition of sucrose and maltose? Explain.
31. Write a chemical reaction showing the hydrolysis of sucrose.

H. Hydrolysis of Starch

32. Describe the evidence for a positive test.
33. What does a positive test indicate?
34. Why does the disappearance of the initial blue/black color observed indicate the starch was hydrolyzed while being heated? Explain.
35. What additional evidence did you observe to indicate that starch was hydrolyzed? Explain.

I. Fruit Juices

36. Which carbohydrate(s) is proven to be present in each fruit juice? Explain.

37. Which fruit juice sample is really not a fruit juice? Explain.

J. Additional Questions

38. Explain what results would be observed when lactose reacts with each of the following reagents:
(i.e. Would a positive or negative result occur, and what visible evidence would be seen)

38(a) Benedict

38(b) Barfoed

38(c) Seliwanoff

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Identify the carbohydrate(s) that would give results consistent with the sample described on their expected behavior to each test. (i.e. if you observed unexpected behavior in lab, it does not apply here).

39. A carbohydrate solution gave a positive Molisch test and a negative Benedict, Barfoed, Bial, and Seliwanoff tests. When treated with HCl and boiled for several minutes, the solution showed a positive result for Benedict, Barfoed, and Seliwanoff tests, and a negative for Bials. What is the identity of the carbohydrate? The possible carbohydrates are limited to: arabinose, glucose, fructose, maltose, sucrose, and starch. Explain.

40. A student performed two tests on an unknown carbohydrate and saw the following results. For each indicate what possible carbohydrate could be in the unknown.

40(a) Test 1 - when tested with the Benedict reagent a Cu_2O precipitate was observed. What possible carbohydrates are present in the solution? The possible carbohydrates are limited to: arabinose, glucose, fructose, maltose, sucrose, and starch. Explain. (More than one answer)

40(b) Test 2 Using the solution above, the sample failed to give a Cu_2O precipitate with the Barfoed reagent. Of the possible carbohydrates from the previous test, which single carbohydrate must have been present? Explain.

Name: _____

Date: _____

Score: ____/20

Prelab Questions

1. Draw the structure of the following molecules and complete the table. If a structure contains more than one unit (ie maltose and sucrose) describe each unit. For example a disaccharide with two units might be a - A/K for Aldose/Ketose.

	Structure	(A)ldose or (K)etose	(P)entose or (H)exose	(M)ono, (D)i or (P)oly	(F)uranose or (P)yranose
Arabinose					
Glucose					
Fructose					
Maltose					
Sucrose					
Xylose					

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2. Complete the table below for each test. Include what functional group/feature of the molecule it test positive (+) and negative (-) for and the visual change which indicates each.

Test Name	Positive for:	Visual Change	Negative for:	Visual Change
Molisch Test				
Seliwanoff Test				
Benedict Test				
Barfoed Test				
Bial Test				
Iodine Test				