Supplementary Handout 8 : Basic Analysis and Property Tests of Carbohydrates

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I. Objective

To use chemical methods to analyze carbohydrates and observe key properties, as an introduction to basic biochemistry.

II. Technique

Benedict's test detects reducing sugars via boiling; use anti-bumping chips. Iodine complexation analyzes polysaccharide structures through heating-cooling cycles. Acid-base treatments and salivary amylase evaluate carbohydrate stability and enzymatic specificity.

III. Introduction

Carbohydrates (saccharides) contain C, H, and O and are often written as $Cx(H_2O)y$. They include monosaccharides ($Cn(H_2O)n$), disaccharides ($Cn(H_2O)n_{-1}$), and oligosaccharides ($(C_6H_{10}O_5)x$, $x > 6$).

Sugars usually refer to sweet, simple mono- or disaccharides. Monosaccharides (3–9 carbons) have an aldehyde (–CHO) or ketone (–C(=O)–) group and can reduce Cu^{2+} or Ag^{+} in alkaline solution. Common examples are glucose, fructose, galactose, mannose, and xylose; they are water-soluble.

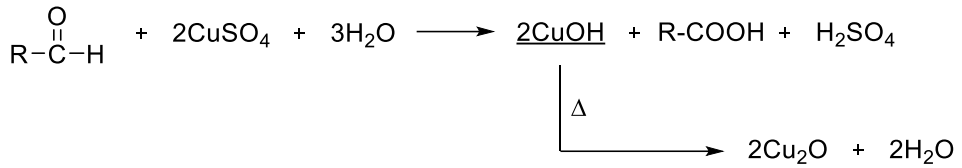
Disaccharides are formed from two monosaccharides and yield two monosaccharides after hydrolysis. Sucrose hydrolyzes to glucose + fructose, but sucrose itself does not respond to aldehyde/ketone tests (e.g., Benedict's test).

Polysaccharides are polymers with large molecular weights (16,000 to 14,000,000). Starch and cellulose can yield monosaccharides after hydrolysis. Starch has α-linkages (digestible), while cellulose has β-linkages (not digestible).

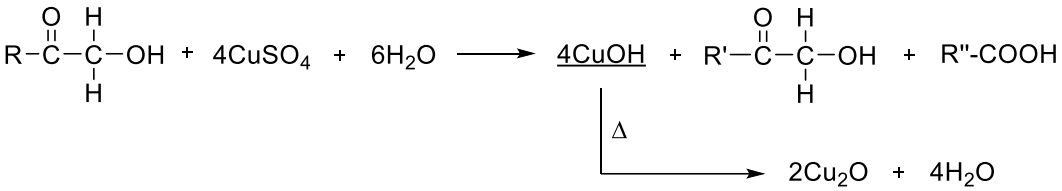
Benedict Test

Benedict's test is based on Fehling's test. Benedict's reagent is light blue, convenient, and stable. Sugars with a free (or readily formed) aldehyde/ketone group reduce alkaline copper(II) to colored cuprous oxide (Cu_2O), from yellow to brick red; these are reducing sugars. Aldoses form aldonic acids, and ketoses form lower-molecular-weight products. This reaction can also be used for sugar quantification.

Aldose:



Ketose:



Iodine Color Test / Polysaccharide Test (Iodine Test)

Polysaccharides such as glycogen and starch form colored, light-absorbing complexes with iodine. Iodine resides within the polysaccharide helix. Amylopectin appears reddish-brown, amylose deep blue, and mixtures often purple.

III. Chemicals

- **Benedict Test**

Preparation: Add Solution B to Solution A, then dilute to 1 L.
Solution A: 10 wt% anhydrous Na_2CO_3 (100 g) + 20 wt% Na-citrate (173 g) in 800 mL water.
Solution B: 1.8 wt% $CuSO_4$ (17.3 g) in 100 mL water.
10 wt% sugar solutions: glucose, fructose, sucrose.
1 wt% sugar solution: starch.

- **Iodine Color Test / Polysaccharide Test**

Iodine solution: 1.27 wt% iodine (I_2; 0.127 g) + 2 wt% KI (0.2 g), dilute to 100 mL.
3 M NaOH; 3 M HCl.
1 wt% sugar solutions: starch; cellulose.

IV. Procedure

- **Benedict Test**

1. Place 2 mL of a sugar solution in a test tube.
2. Add 2 mL Benedict's reagent and mix well.
3. Heat all tubes in a boiling water bath for 5 min, then record observations. Add a stir bar to prevent bumping.

- **Iodine Color Test / Polysaccharide Test (and effects of pH)**

I. Starch

1. Dispense 2 mL starch solution into three test tubes.
2. Add 3 drops of 3 M NaOH (tube 1), 3 drops of water (tube 2), and saliva (tube 3).
3. Add 1 drop iodine solution to each tube and note the color.
4. Heat the colored tube(s), cool, and note any color change.
5. Compare with a blank (water).

II. Cellulose

1. Place 2 mL cellulose solution into two test tubes.
2. Acidify one with 5 drops of 3 M HCl. Add saliva to the other.
3. Add 2 drops iodine solution to each tube.
4. Heat in a boiling water bath for 15 min and compare results.

V. Experimental Data and Results

Record all observations clearly (solution color and appearance).

Benedict Test Results

	glucose	**fructose**	**sucrose**	**starch**
Initial solution (2 mL)				
2 mL Benedict's reagent				
Boiling water bath (5 min)				

Iodine Color Test Results

I. Starch

	starch (NaOH)	**starch (H_2O)**	**starch (saliva)**
Initial solution (2 mL)			
NaOH			
H_2O			
Saliva			
Iodine solution			
After heating			

II. Cellulose

	cellulose (HCl)	**cellulose (saliva)**
Initial solution (2 mL)		
HCl		
Saliva		
Iodine solution		
After heating		

VI. Questions and Discussion

1. What is the purpose of adding Na-citrate to Benedict's reagent?
2. What causes the color change in the iodine test?
3. How does saliva affect starch and cellulose?
4. If you need 10 mL of Benedict's reagent and 10 mL of iodine solution, what are the component ratios?
   Benedict's reagent: Na_2CO_3, Na-citrate, $CuSO_4$
   Iodine solution: I_2, KI
5. Why does fructose (a ketose) still produce a Cu_2O precipitate in Benedict's test?