國立中正大學

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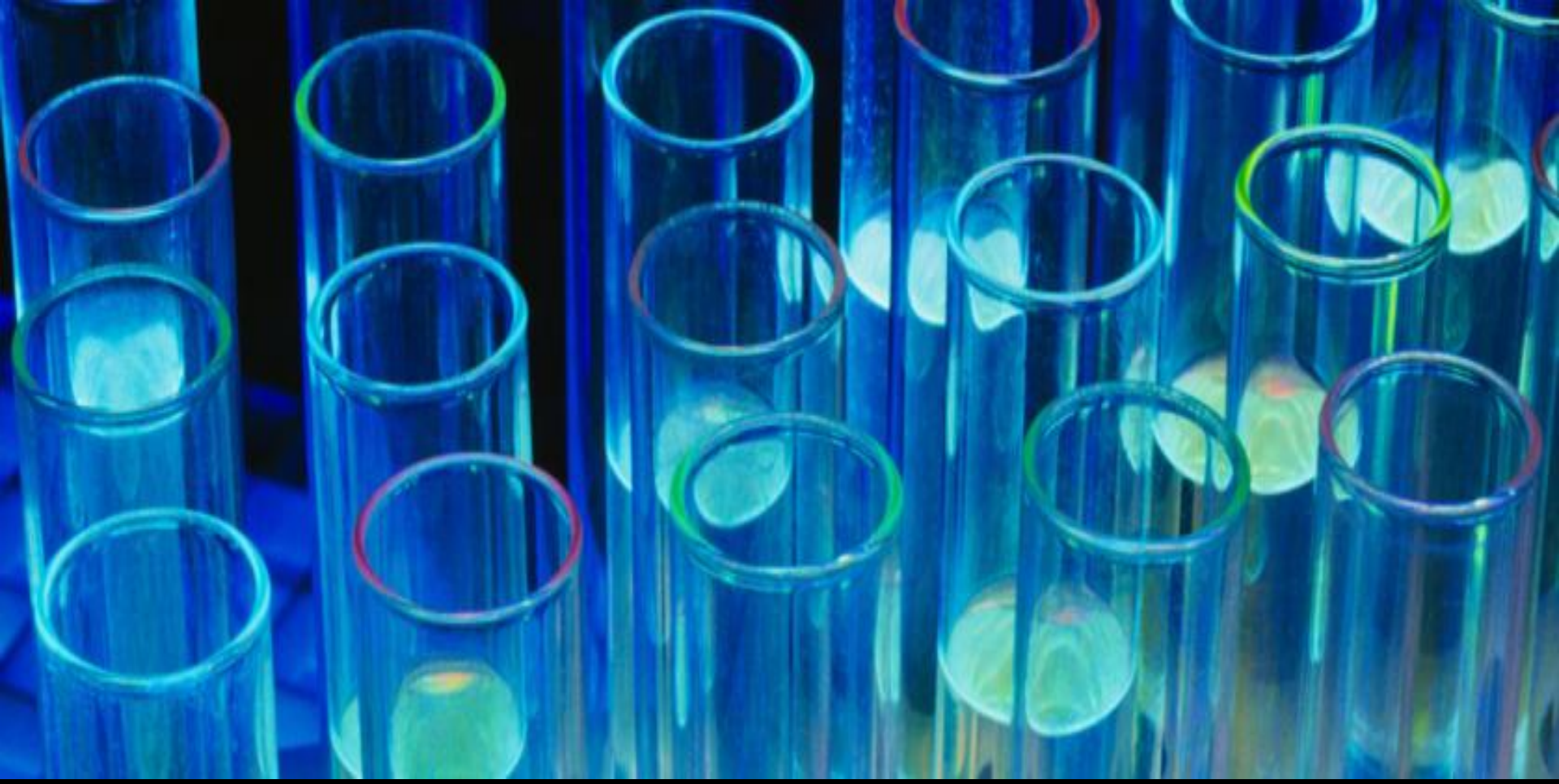


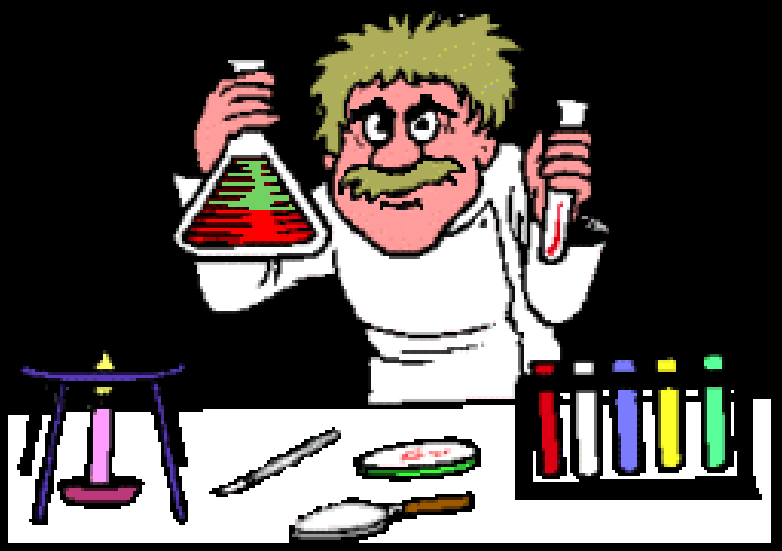
115-2

Supplementary Material 8 - Basic Analysis and Characterization Tests of Carbohydrates

115.03.09 ~ 115.03.15 (time required：2 hours)

1.Purpose

- This laboratory experiment utilizes chemical methods to perform fundamental analyses of carbohydrates, a class of substances essential to biological processes. By characterizing their unique properties, we establish a foundation for further exploration into the complex mechanisms of biochemistry.



2.Principle

2.1 Definition of Carbohydrates

- Compounds composed of carbon, hydrogen, and oxygen, with the general formula $C_x(H_2O)_y$; therefore, they are commonly referred to as **carbohydrates**

2.2 Types of Carbohydrates

(1) monosaccharides ($C_n(H_2O)_n$)
(2) disaccharides ($C_n(H_2O)_{n-1}$)
(3) oligosaccharides ($(C_6H_{10}O_5)_x$ ，x > 6)

4. **Monosaccharides** contain **three to nine carbon atoms** and **possess either an aldehyde group (RCHO) or a ketone group (R_2CO), enabling them to reduce metal ions such as Cu^{2+} and Ag^{+} in alkaline solution.**

* **Examples include glucose, fructose, galactose, mannose, and xylose.**

5. **Disaccharides are formed by the condensation of two monosaccharide molecules and , upon hydrolysis, still yield two monosaccharides. For example, one molecule of sucrose can be hydrolyzed to produce one glucose and one fructose. However, because sucrose itself lacks a free aldehyde or ketone group, it does not react in tests for aldehydes or ketones (e.g., the Benedict's test).**

Benedict Test

- Aldoses

$(R\text{-}COH)_{(aq)} + 2CuSO_{4\,(aq)} + 3H_2O_{(l)} \rightarrow \underline{2CuOH}_{(aq)} + R\text{-}COOH_{(aq)} +$

$H_2SO_{4\,(aq)} \xrightarrow{\triangle} Cu_2O_{(s)}\downarrow + 2H_2O_{(l)}$

- Ketoses

$(RC{=}OCH_2OH)_{(aq)} + 4CuSO_{4\,(aq)} + 6H_2O_{(l)} \rightarrow \underline{4CuOH}_{(aq)} +$

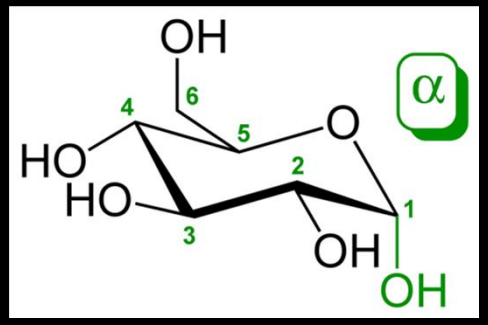
$R'C{=}OCH_2OH_{(aq)} + R''\text{-}COOH_{(aq)} \xrightarrow{\triangle} 2Cu_2O_{(s)}\downarrow + 4H_2O_{(l)}$

$\triangle$ indicates heating

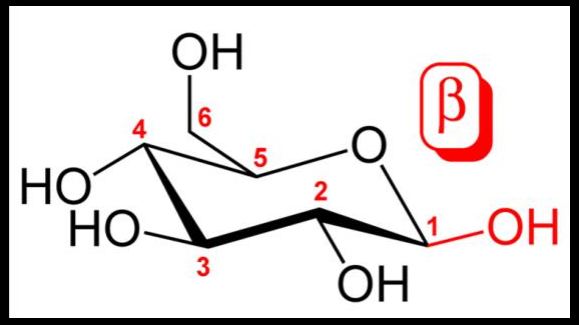
- Polysaccharides are formed by the polymerization of many linked monosaccharide units and have very large molecular weights(ranging from 160,000 to 14,000,000).Upon hydrolysis, they can also yield monosaccharides.

Examples：

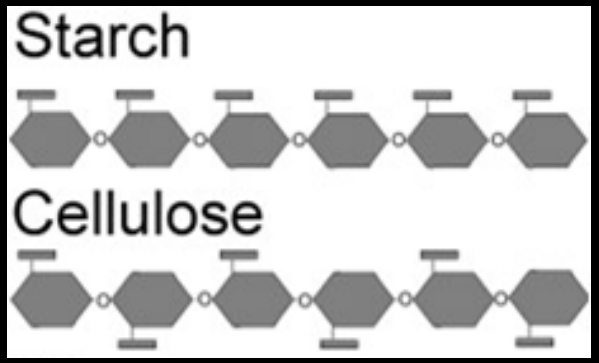
* Starch : glucose units are linked in the α form and can be digested by humans.
* Cellulose : glucose units are linked in the β form, and it cannot be digested by humans.



starch



cellulose



Iodine Test、Polysaccharide Test

- Polysaccharides such as glycogen and amylopectin form colored, light-absorbing complexes with iodine. In this reaction, iodine interacts with amylopectin to produce a reddish-brown complex. In contrast, the amylose component of starch forms a helical, light-absorbing colored complex with iodine, in which iodine is located within the center of the helix, yielding a deep-blue complex. If both components are present, the color appears purple.

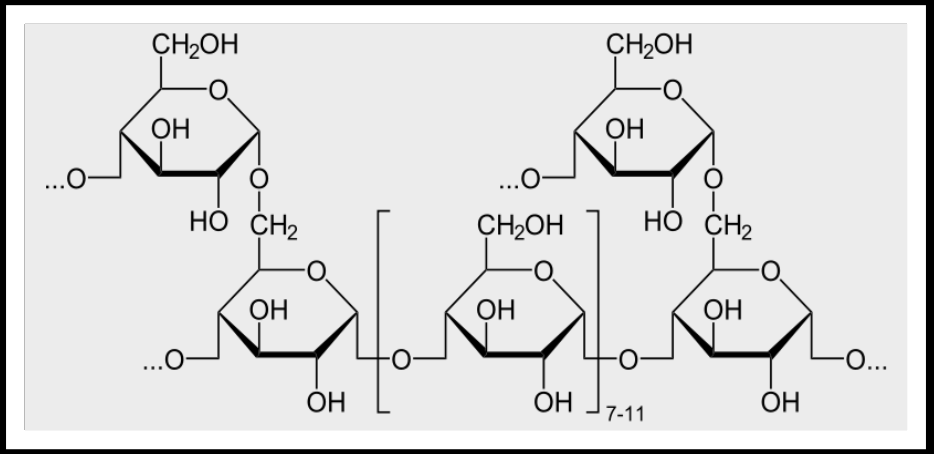


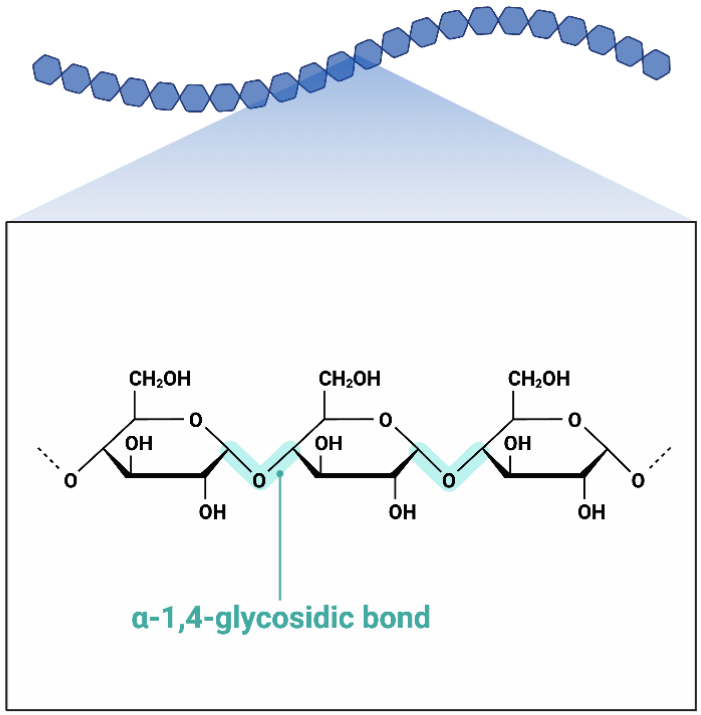
Figure 1. Glycogen

Figure 2. Amylose Complex

Types of Starch

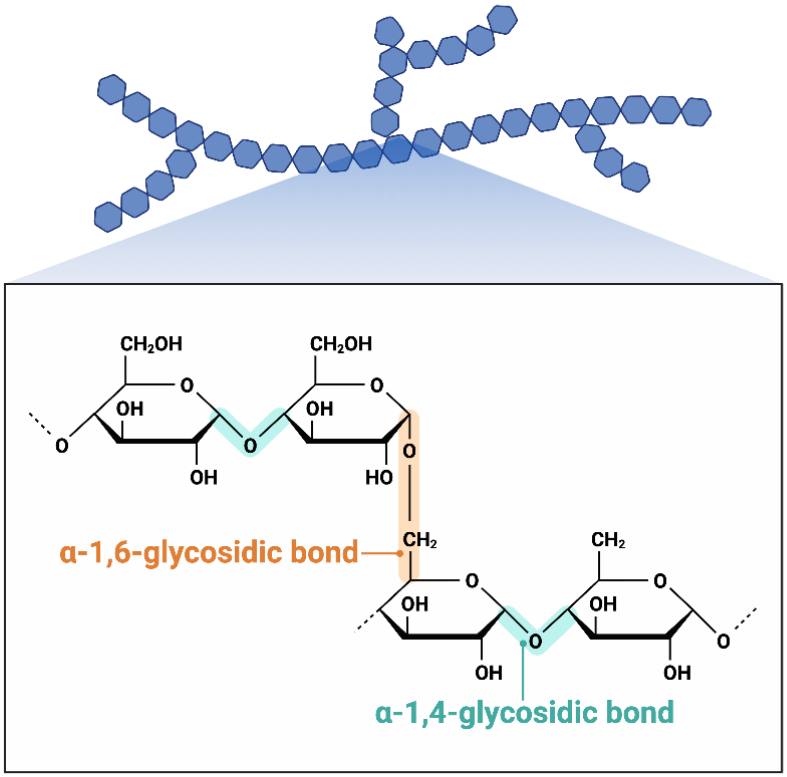
Structure of Amylose vs. Amylopectin

Amylose



Amylose is composed of glucose molecules connected by α-1,4-glycosidic bonds.

Amylopectin



Straight-chain portions of amylopectin are connected by α-1,4-glycosidic bonds, whereas the branches are connected by α-1,6-glycosidic bonds.

3. Equipment and Reagents

Medicine

A. Benedict test：

- Benedict reagent，including：

a. 10.0 wt% anhydrous Na_2CO_3 ***

b. 17.3 wt% Na-citrate *

c. 17.3 wt% anhydrous $CuSO_4$ ***

- 10.0 wt% sugar test solutions：glucose、fructose、sucrose
- 1.00 wt% sugar test solutions： starch

Figure 3. Benedict Reagent

* ：Toxic * ：Irritant * ：Corrosive

References : https://pubchem.ncbi.nlm.nih.gov/

Medicine

B. Iodine Color Reation (Polysaccharide Test)：

- Iodine test solution，containing：
  - (a) 1.27 wt% iodine (I2) ***
  - (b) 2.00 wt% potassium iodide (KI) **
- 3.0 M NaOH****
- 3.0 M HCl ****
- 1.00 wt% sugar test solutions：starch、cellulose

Figure 4. Iodine test solution.

* ：Toxic * ：Dehydrating * ：Flammable * ： Corrosive * ：Irritant

References:https://pubchem.ncbi.nlm.nih.gov/

Equipment

Group equipment cabinet	Provided by the TA
Test tubes (3)	Plastic dropper
10 mL volumetric flask (1)	
250 mL beakers (2)	
Hot plate stirrer	

4. Experimental Procedure

A. Benedict's test

1. Pipette 2.00 mL of each sugar test solution into a test tube.
2. Add 2.00 mL of Benedict's reagent and mix thoroughly.
3. Place all test tubes in an 80.0°C water bath simultaneously, and observe the results after 5 minutes.

   Add a stir bar to the boiling water bath to prevent bumping.

B. Iodine Color Test(Polysaccharide Test)

I. Starch

1. Pipette 2.00 mL of the starch solution into two separate test tubes
2. Add 3 drops of 3.0 M NaOH to one test tube, and 3 drops of water to the other.
3. Add 1 drop of iodine test solution to each tube and carefully observe the color.
4. Heat the tubes in an 80.0°C water bath for 15 minutes, then observe the differences.

Experiment note：The color change in this step occurs because the iodine solution cannot remain stable in an alkaline solution.

B. Iodine Color Test(Polysaccharide Test)

II. Cellulose

1. Pipette 2.00 mL of the cellulose solution into two separate test tubes.
2. Acidify one test tube by adding 5 drops of 3.0 M HCl, and add 5 drops of water to the other.
3. Add 2 drops of iodine test solution to each tube.
4. Heat the tubes in an 80.0°C water bath for 15 minutes, then observe the differences.

5. Precautions

1. Add a stir bar to the boiling water bath to prevent bumping.
2. The cellulose test solution is difficult to dissolve; when dispensing, take only the clear supernatant from the upper layer.
3. Wear a lab coat and personal protective equipment (PPE), including gloves, safety goggles, and a face mask, during the experiment.
4. All chemicals used in this experiment are toxic and pose certain hazards. Follow laboratory safety regulations and the teaching assistant's instructions at all times to ensure safety.
5. Do not remove any chemicals or laboratory equipment from the laboratory.

6. Experimental Results

A. Benedict's Test

	Fructose	Glucose	Sucrose	Starch
Initial solution	Clear	Clear	Clear	White turbidity
Benedict	**Blue**	**Blue**	**Blue**	**Blue**
Hot water (80.0°C) (5 min)	Orange-red	**Red**	**Blue**	**Blue**

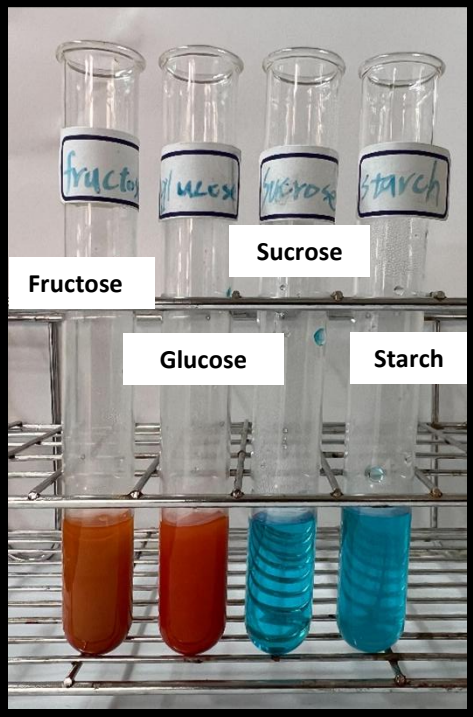


Figure 5. Color Change of the Test Solution

B. Iodine Color Test(Polysaccharide Test)

I. Starch

	Starch (NaOH)	Starch (H_2O)
Initial solution	White turbidity	White turbidity
After dropwise addition of the solution	Clear solution White precipitate	White turbidity
Iodine solution	Clear solution White precipitate	Blue-black solution
After heating	Clear solution White precipitate	Blue-black solution

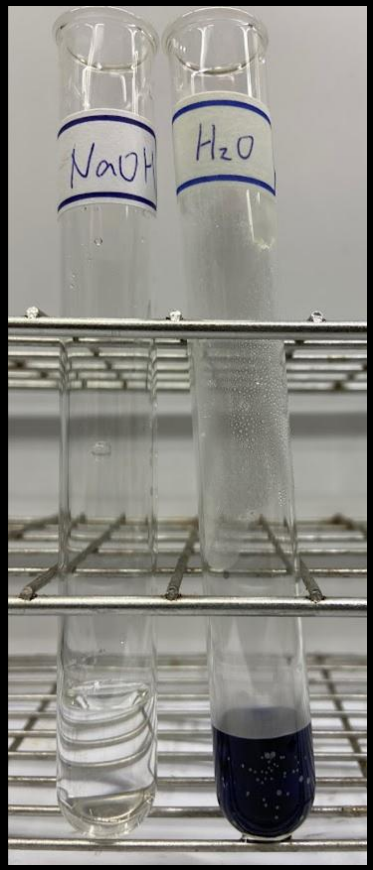


Figure6. Change of the Test Solution

B. Iodine Color Test(Polysaccharide Test)

II. Cellulose

	Cellulose (HCl)	Cellulose (Water)
Initial solution	Clear and colorless	Clear and colorless
After adding the solution dropwise	Clear and colorless	Clear and colorless
Iodine solution	Light brown	Light brown
After heating	Light brown	Light brown

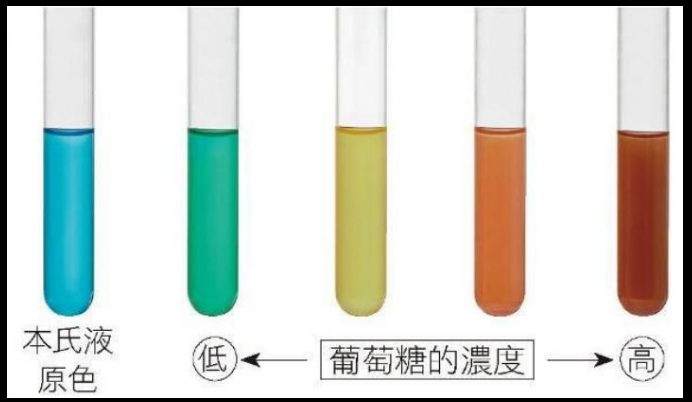
Figure.7 Change of the Test Solution

7. Questions and Discussion

1. **In the Benedict's test, if the sample contains reducing sugars, different color changes will be observed depending on the amount of reducing sugars present. If the observed colors are green, orange, red, and blue, please arrange these samples in order of reducing sugar content.**

Ans:

Red > orange > green > blue. Because **cuprous oxide (Cu_2O)** is **reddish-brown**, a **greater amount of Cu_2O** makes the solution appear **closer to red**. When the amount of Cu_2O is **lower**, mixing with the **blue color of Benedict's reagent** results in a color that appears closer to green.



2. In this experiment, the iodine color test (polysaccharide test) requires heating for 15 minutes. Why does the Benedict's test require heating for only 5 minutes ?

Ans:

Benedict's reagent reacts only with monosaccharides (e.g., glucose, fructose, and galactose). However, because disaccharides and polysaccharides can be broken down into monosaccharides during heating and then react with Benedict's reagent, the mixture should not be heated for too long

3. A. Please explain how Benedict's reagent is used to qualitatively analyze sugars, and describe the reaction process and the observed results.

   B. Besides Benedict's reagent, what other reagents can be used to detect the presence of carbohydrates ?

Ans:

A. To perform Benedict's test, mix the sample with Benedict's reagent and then heat the mixture. Reducing sugars reduce the copper(II) ions in Benedict's reagent, producing an orange to reddish-brown precipitate (Cu_2O). The formation of a precipitate indicates that the sample contains reducing sugars.

B. Other reagents used to detect carbohydrates include Fehling's reagent and Barfoed's reagent.

4. Why are some disaccharides, such as maltose, reducing sugars, whereas sucrose is non-reducing ?

Ans:

Maltose consists of two glucose units linked together; one glucose unit retains a free anomeric carbon (hemiacetal) that can open to form a free aldehyde group, so maltose is a reducing sugar. In contrast, sucrose is composed of glucose and fructose, and the glycosidic bond involves both anomeric carbons; therefore, neither a free aldehyde nor a free ketone group is available, and sucrose is non-reducing.

8. Grading Criteria

1. Color Changes
2. Accuracy of the Discussion Answers
3. Data Review and Analysis
4. Photo Attachment Required

9. Estimated Amount of Reagents

- **Benedict : 8 mL X 28 X 4 X 1.5 = 1344 mL**
- **Iodine test solution : 0.5 mL X 28 X 4 X 1.5 = 84 mL**
- **10 wt% glucose : 0.2 g X 28 X 4 X 1.5 = 33.6 g**
- **10 wt% fructose : 0.2 g X 28 X 4 X 1.5 = 33.6 g**
- **10 wt% sucrose : 0.2 g X 28 X 4 X 1.5 = 33.6 g**
- **1 wt% starch : 0.06 g X 28 X 4 X 1.5 = 10.08 g**
- **1 wt% cellulose : 0.04 g X 28 X 4 X 1.5 = 6.7 g**

10. Waste solution

- **Benedict : 1500 mL**
- **Iodine test solution : 100 mL**
- **10 wt% glucose : 500 mL**
- **10 wt% fructose : 500 mL**
- **10 wt% sucrose : 500 mL**
- **1 wt% starch : 1000 mL**
- **1 wt% cellulose : 600 mL**
- **Total: 4.5 L Waste solution**

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