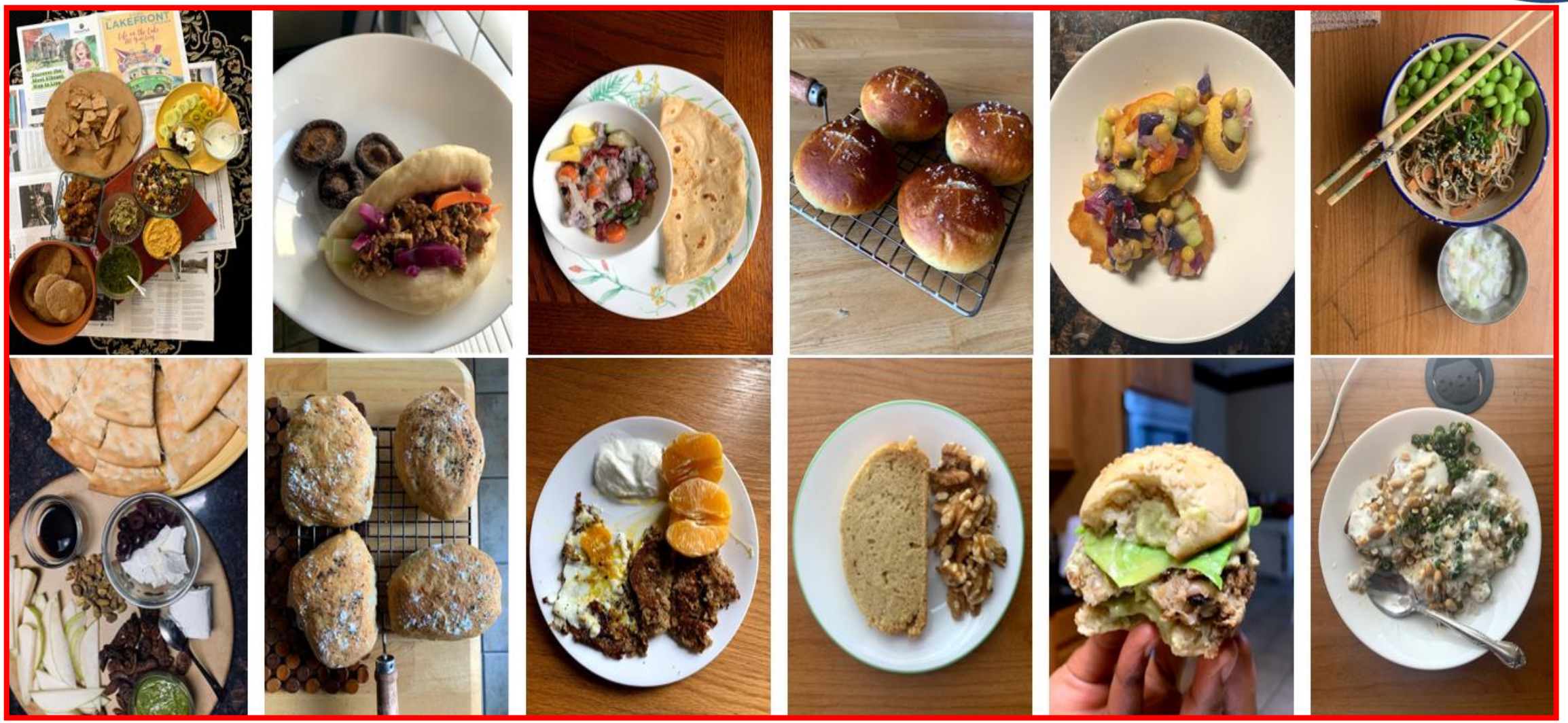
Lecture 3



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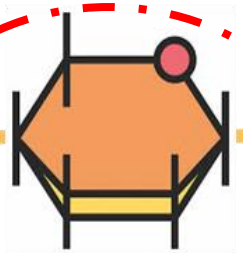

LAB 3: QUALITATIVE ANALYSIS OF CARBOHYDRATES

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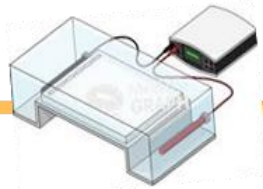

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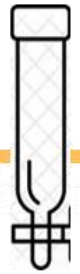
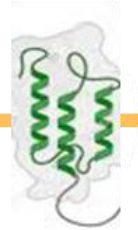


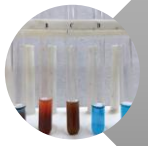
LAB 5: CHROMATOGRAPHIC SEPARATION OF PLANT



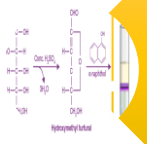
LAB 4: ANALYSIS OF PROTEINS - QUALITATIVE AND QUANTITATIVE METHODS

This week Lecture

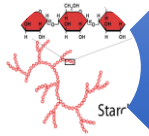
What are carbohydrates and why are they important?



An overview of the chemical tests we will use



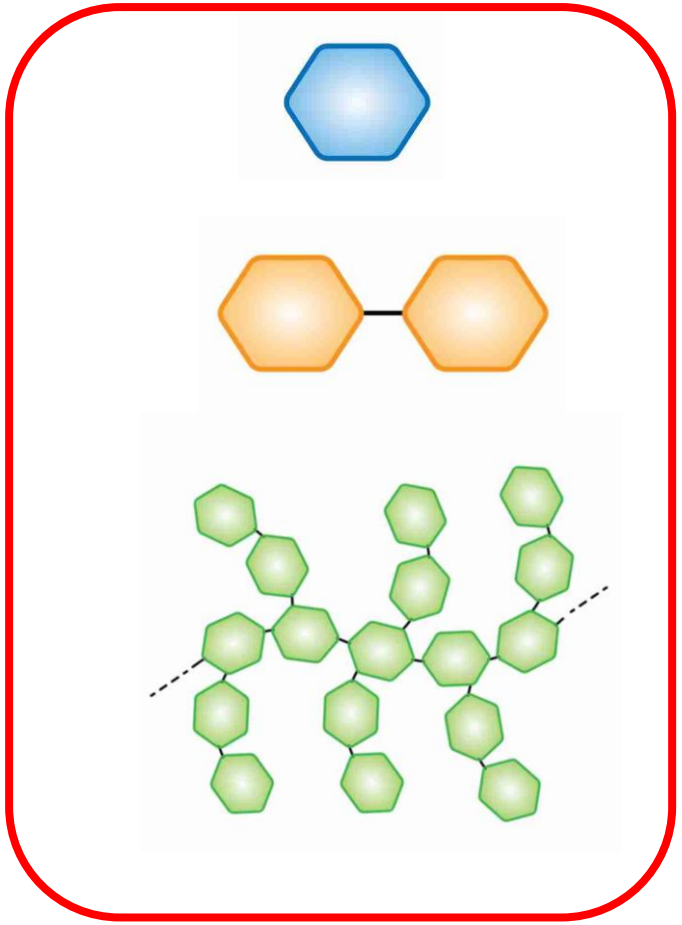
Understanding the principles behind how each test works



A step-by-step guide to the experimental procedure

How to interpret your results and identify your unknown

Qualitative Analysis of Carbohydrates

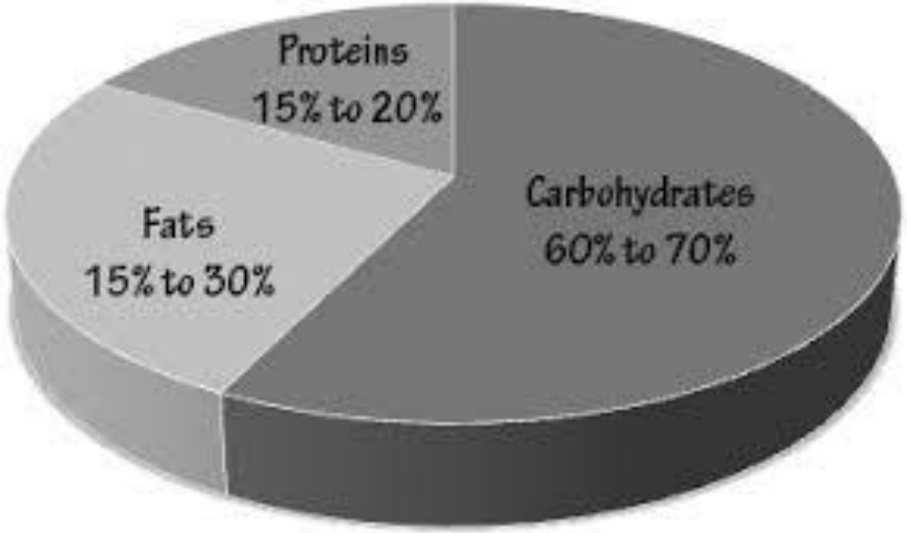


ATP

Understanding Carbohydrates

What are Carbohydrates?

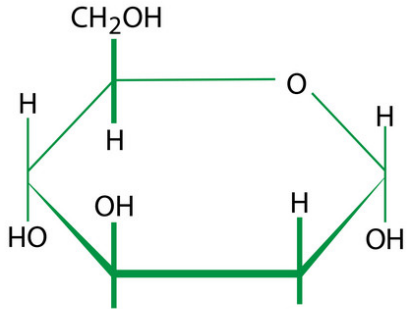
- The most **abundant class of organic compounds** in living organisms.
- Composed of **Carbon, Hydrogen, and Oxygen.**
- They are **the primary source of energy** for most living things, making up **60-70%** of a typical diet

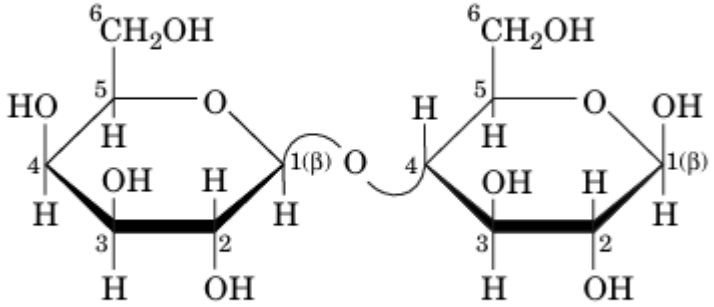


Classifying Carbohydrates

We classify them based on their size and complexity. For today's lab, we will work with:

- **Monosaccharides:** Simple sugars (e.g., Glucose, Fructose & Galactose).
- **Disaccharides:** Two sugars linked together (e.g., Sucrose, Maltose & Lactose).
- **Polysaccharides:** Long chains of sugars (e.g., Starch, cellulose, chitin)



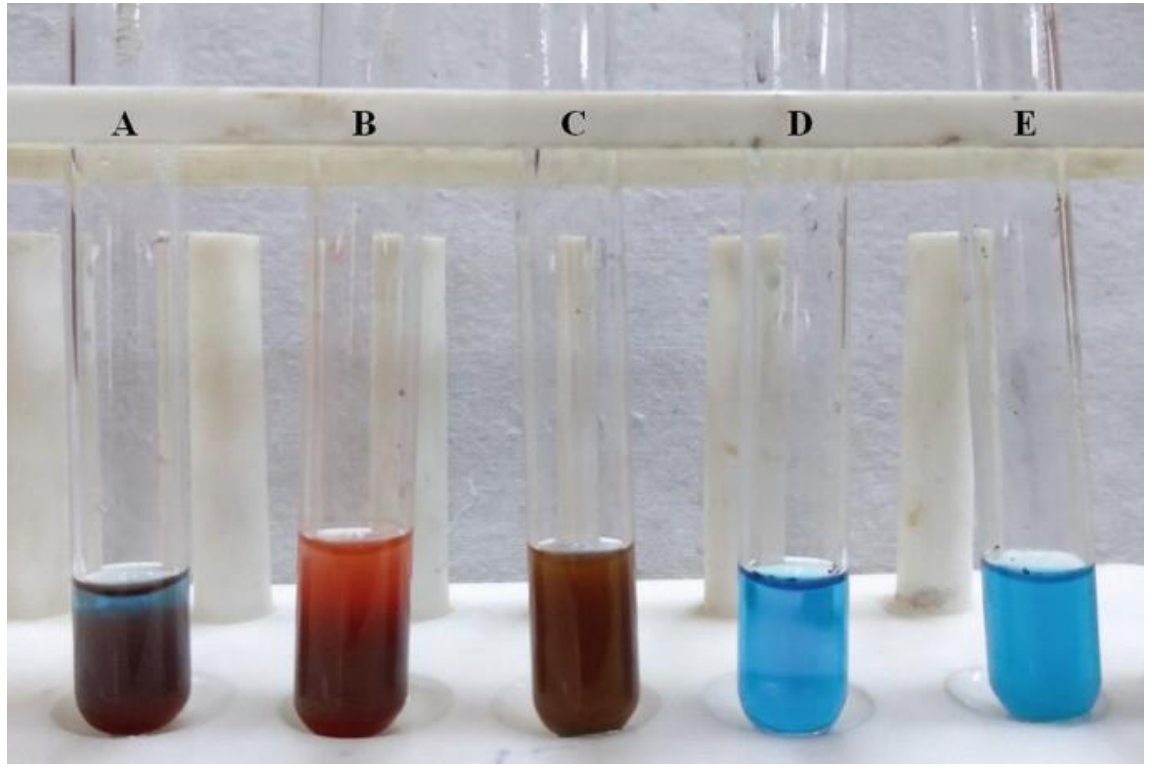


Galactose Glucose

The Central Question?

If given known & Unknown samples: How can we use chemical tests to determine the identity or class of your unknown carbohydrate.

- Glucose (monosaccharide)
- Fructose (monosaccharide)
- Sucrose (disaccharide)
- Starch (polysaccharide)
- Water (negative control)
- Unknown Sample.

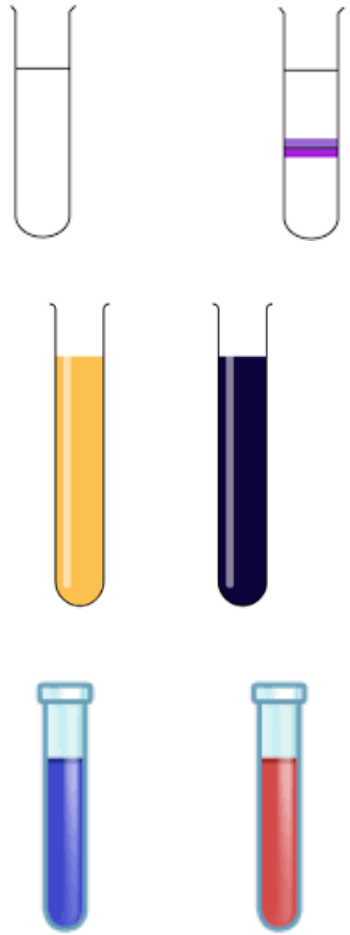


The Toolkit - Our Chemical Assays

Our Three-Step Investigative Process:

We will analyze our unknown using a logical sequence, from general to specific:

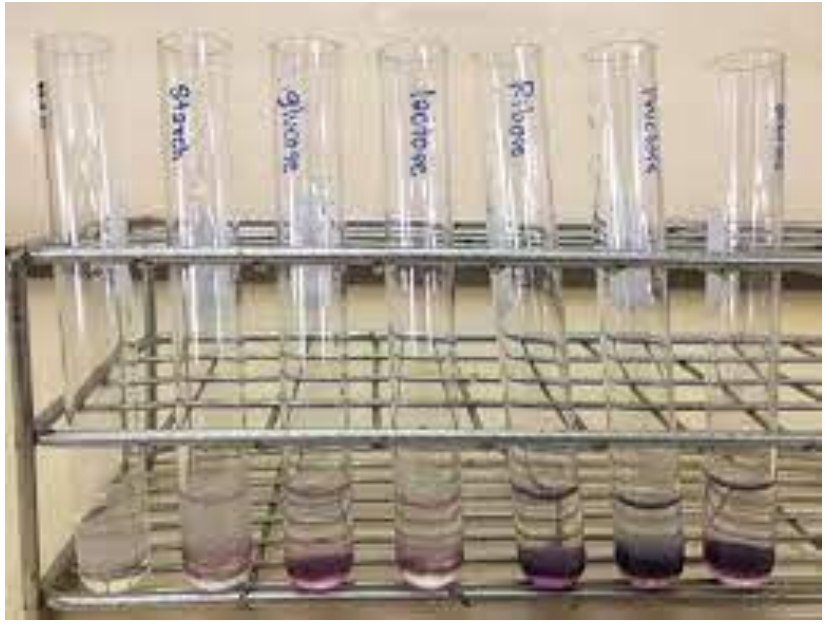
- **Molisch's Test:** Is it a carbohydrate at all?
- **Benedict's/Fehling Test:** The Reducing Sugar Test. Is my sample a monosaccharide or a reducing disaccharide?
- **Iodine Test:** The Polysaccharide Test. Is my sample Starch?

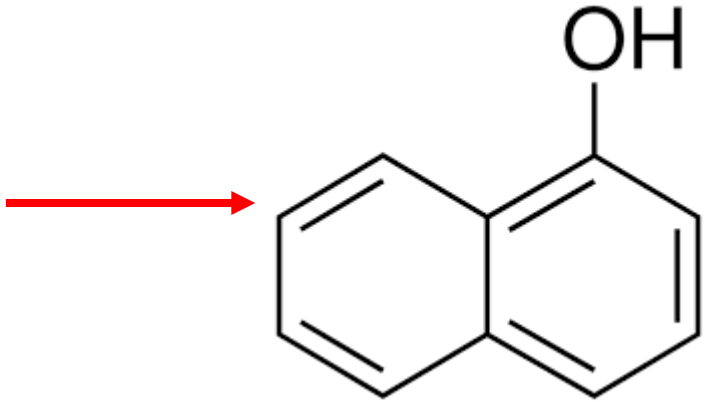


Test 1: Molisch's Test - The General Screen

Purpose: To detect the presence of any carbohydrate.

- It is the first test you should perform. If it's negative, you can be confident your sample is not a carbohydrate.
- **Reagent**: α-Naphthol in ethanol

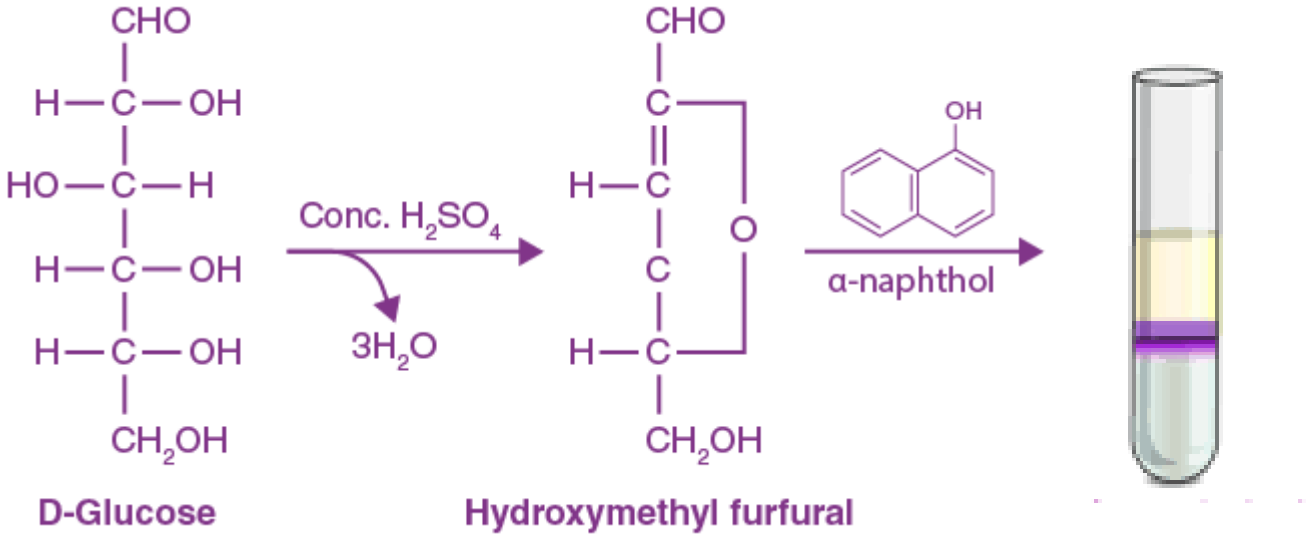




The Principle of Molisch's Test

A two-step, acid-catalyzed reaction:

- **Dehydration:** Concentrated Sulfuric Acid (H_2SO_4) acts as a dehydrating agent, removing water from the sugar to form an aldehyde (furfural or a derivative).
- **Condensation:** This aldehyde then reacts with two molecules of α-naphthol (from the Molisch's reagent) to form a deep purple-colored complex.



Procedure for Molisch's Test

- Pipette 1 mL of each sample into its respective labeled test tube.
- Add 2 drops of Molisch's reagent to every tube and mix gently.
- **SAFETY FIRST**: In the fume hood, tilt a tube at a 45° angle.
- Carefully and slowly, let ~2 mL of concentrated H_2SO_4 run down the inner wall to form a layer at the bottom. DO NOT MIX.
- Observe the interface for a purple ring. Record your observations.

Molisch's Test: A Positive Result

What to expect:

- **Positive Test:** A purple or reddish-violet ring forms at the interface between the dense acid layer and the aqueous sample layer.
- **CRITICAL:** DO NOT MIX the layers! The ring is the result.

Molisch's Test: SAFETY

EXTREME CAUTION

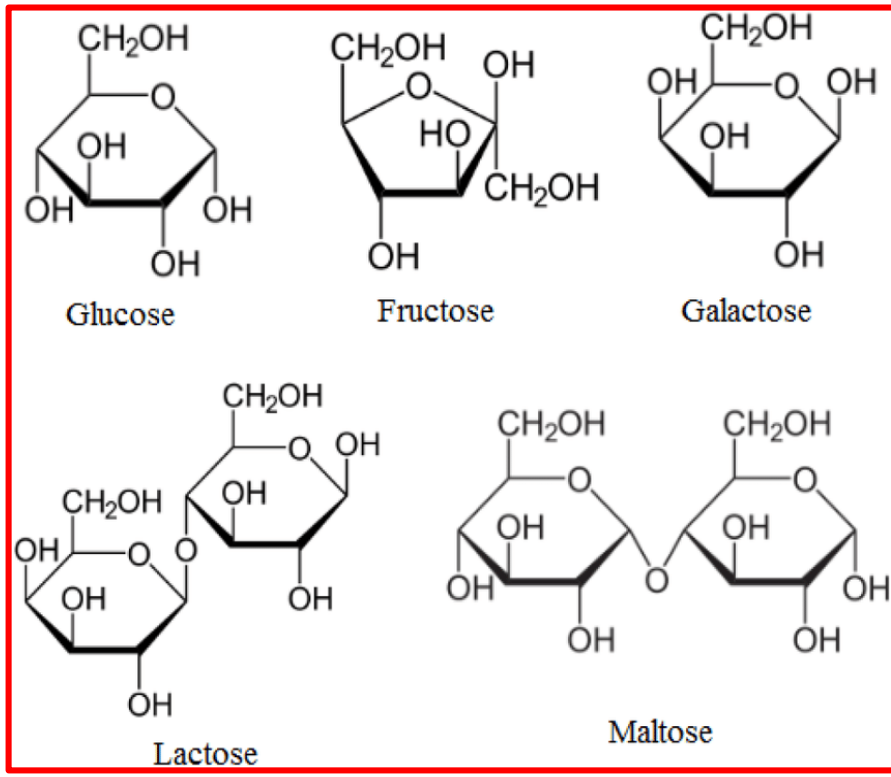
- Concentrated Sulfuric Acid (H_2SO_4) is highly corrosive and will cause severe burns.
- Always wear your safety goggles and gloves.
- Handle with extreme care. Report any spills immediately.
- **Remember the rule: Always Add Acid to Water.** For this test, you are carefully layering the acid under the aqueous sample.

The Reducing Sugar Tests: Benedict's & Fehling's

What is a reducing sugar?

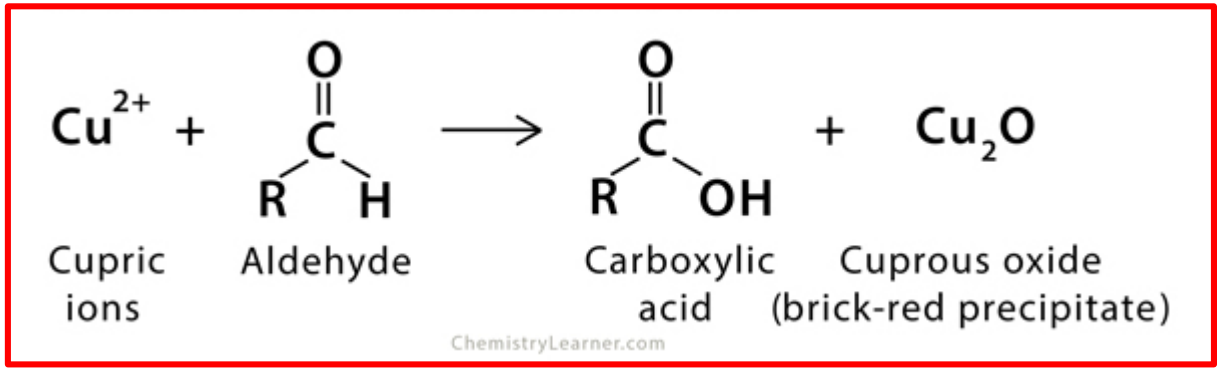
A sugar that has a free **aldehyde or ketone** group, which can be oxidized.

- To determine if **a carbohydrate** is a reducing sugar.
- **All monosaccharides** (glucose, fructose & galactose) are reducing sugars.
- **Most disaccharides** (e.g., lactose & maltose) are reducing sugars.
- Sucrose is a notable **exception.**



The Principle: A Shared Redox Reaction

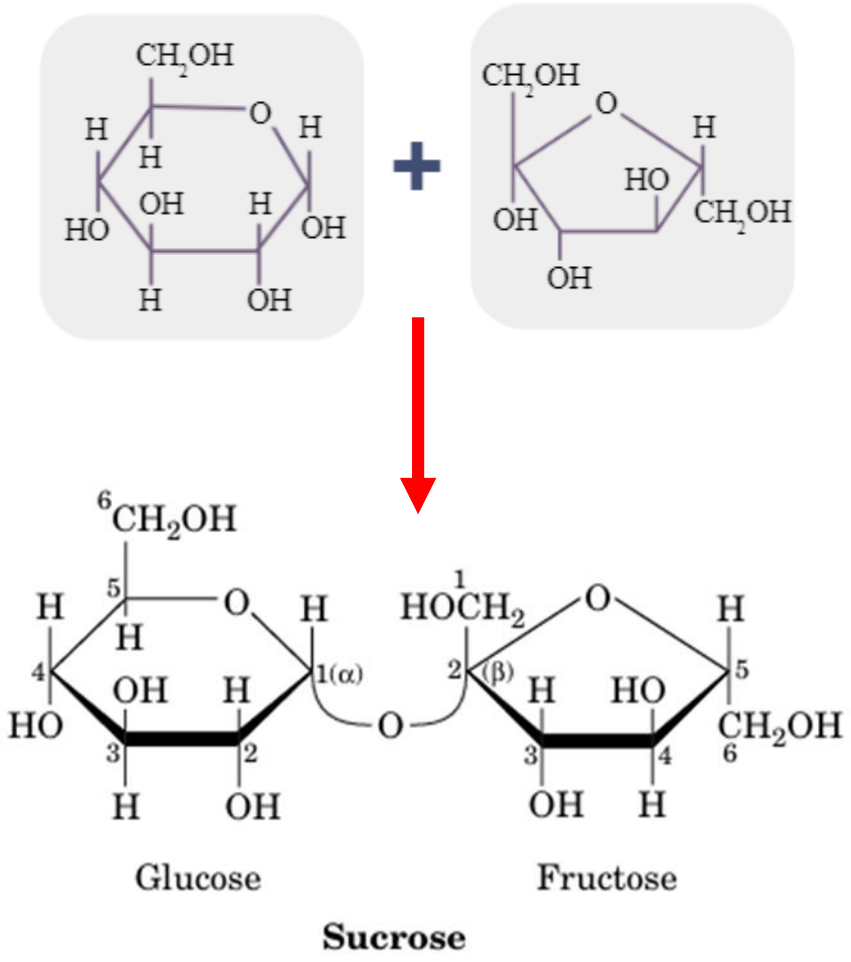
- Both **Benedict's** and **Fehling's tests** work on the same principle:
- The reducing sugar is **oxidized**.
- Blue, soluble **Copper(II) ions (Cu^{2+})** from the reagent are **Reduced**
- They become **red, insoluble Copper(I) Oxide (Cu_2O)**, which forms **a precipitate**.



The Key Exception

Why is Sucrose NON-Reducing?

- **In sucrose**, the reactive parts of both glucose and fructose (the anomeric carbons) are **locked together** in the **glycosidic bond**.
- Because there are **no free groups to react**, the ring cannot open to be oxidized.
- Sucrose will give a **negative result** in both tests.

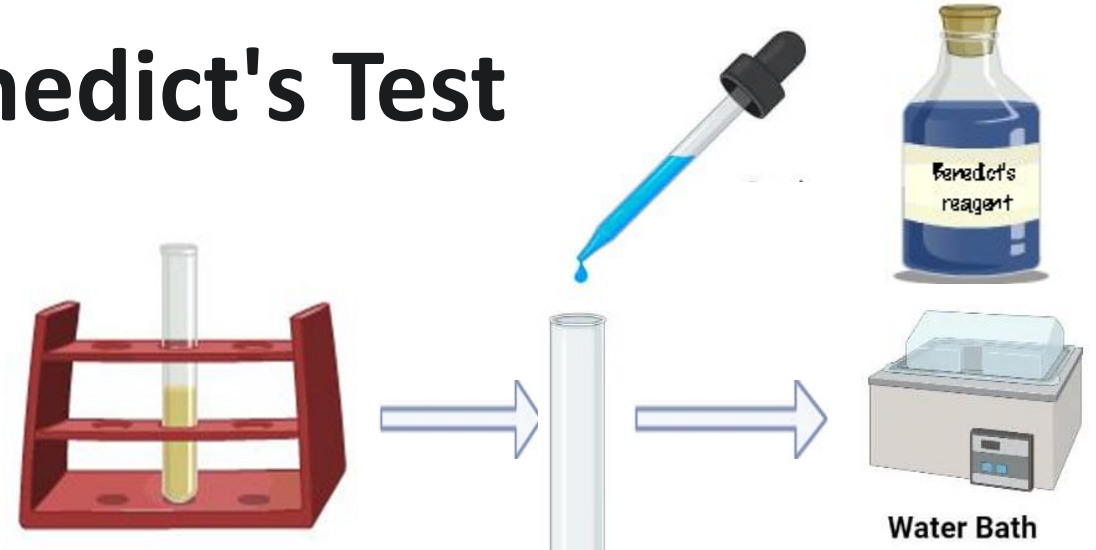


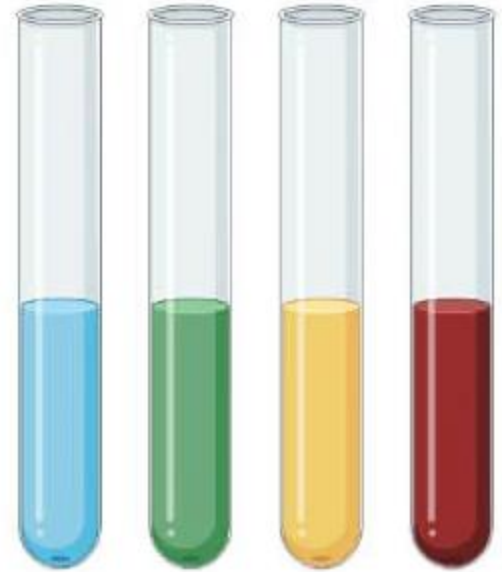
Benedict's Test

- **Reagent:** A single, stable solution using Sodium Citrate as the chelating agent.
- **Result**: A color change from blue to a final brick-red precipitate.

Procedure for Benedict's Test

- Set up a third clean set of 6 labeled test tubes.
- Pipette 1 mL of each sample into its respective tube.
- Add 1 mL of **Benedict's reagent** to each tube and mix well.
- Place all tubes in a **boiling water bath** for 3-5 minutes.
- Carefully remove the tubes and observe for a color change from the original blue. Record your observations.



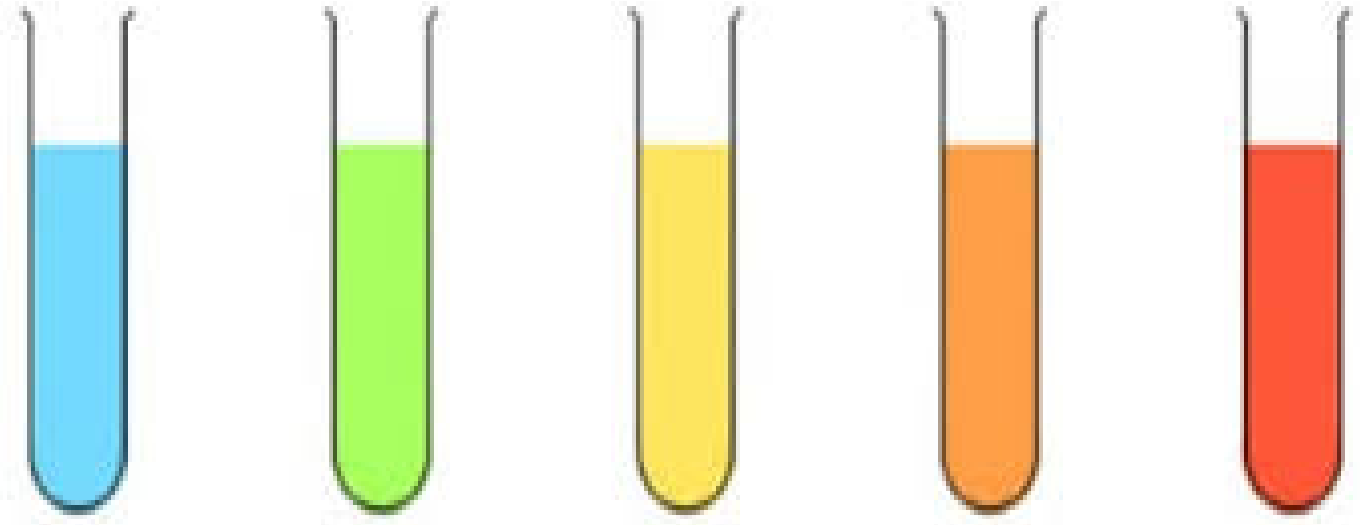


Benedict's Test: A "Semi-Quantitative" Result

The color of the final precipitate depends on how much Cu_2O was formed

Blue → Green → Yellow → Orange → Brick-Red

- **Blue:** No reducing sugar (0%)
- **Green:** Trace amounts
- **Yellow:** Low amounts
- **Orange:** Moderate amounts
- **Brick-Red:** High amounts



The Fehling's Test

- **Reagents:** Two separate, less stable solutions.
- **Fehling's A**: Copper(II) Sulfate solution.
- **Fehling's B**: Sodium Potassium Tartrate (chelating agent) and NaOH.


Banana Extract

Fehling's Solution A

Fehling's Solution B

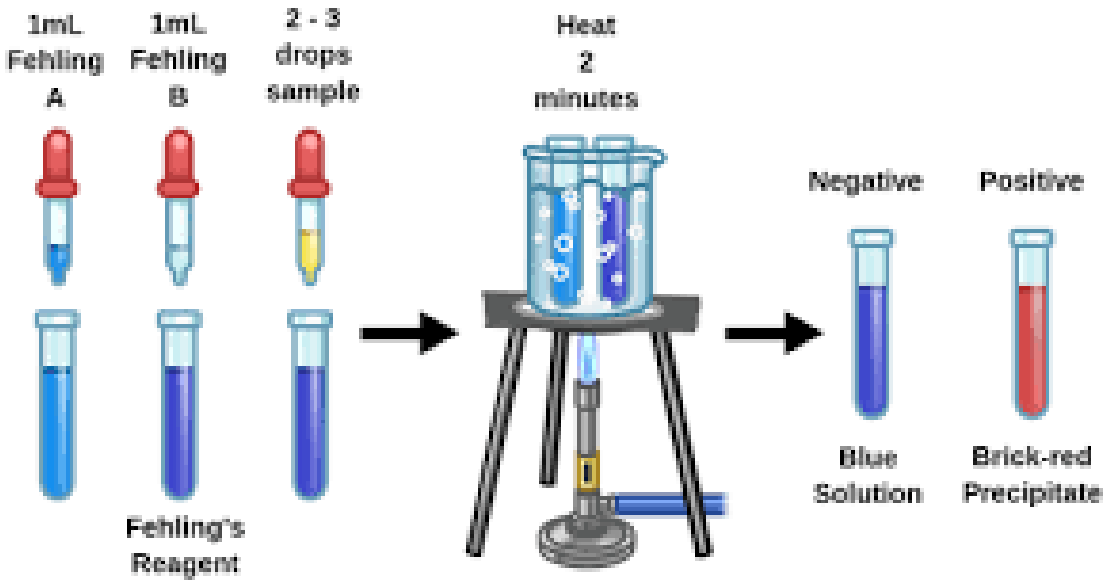

Burner


Dropper


Test Tube Holder

Procedure: Fehling's Test

- Set up a fourth set of 6 labeled tubes.
- Add 1 mL of each sample.
- Add 1 mL of Fehling's B, then 1 mL of Fehling's A and mix.
- Place all tubes in a boiling water bath for a few minutes.
- Observe for the formation of a colored precipitate.



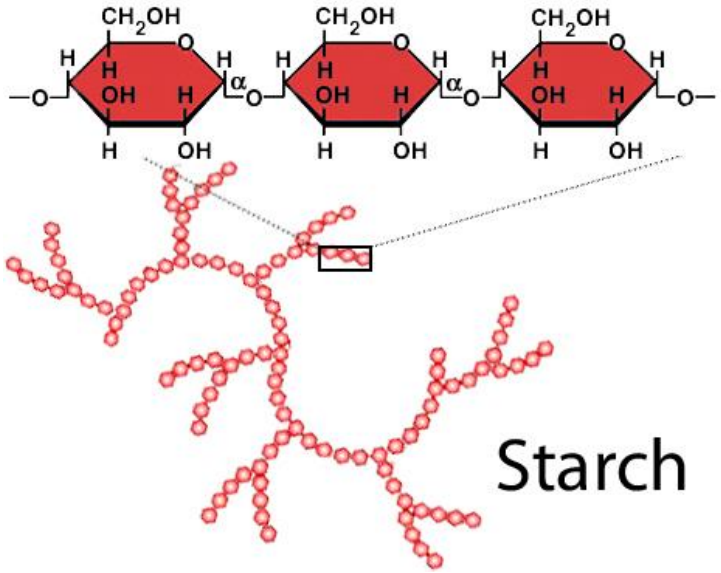
Benedict's vs. Fehling's: Key Differences

Benedict's Test	Fehling's Test
Stable, single solution	Unstable, two solutions (A+B)
Sodium citrate	Sodium potassium tartrate
Weak sodium carbonate	Strong sodium hydroxide)

The Iodine Test - The Starch Detective

Purpose: To specifically identify the polysaccharide Starch.

- The I_3^- ions in Lugol's solution fit perfectly inside the helical coil of starch, forming a blue-black colored complex.
- **Reagent:** Lugol's Iodine Solution (contains I_3^- ions).

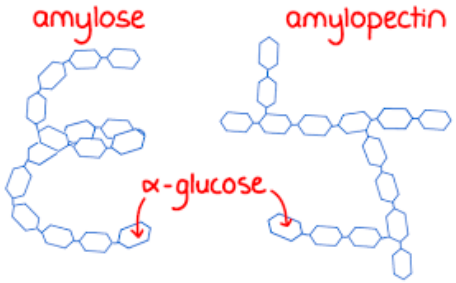


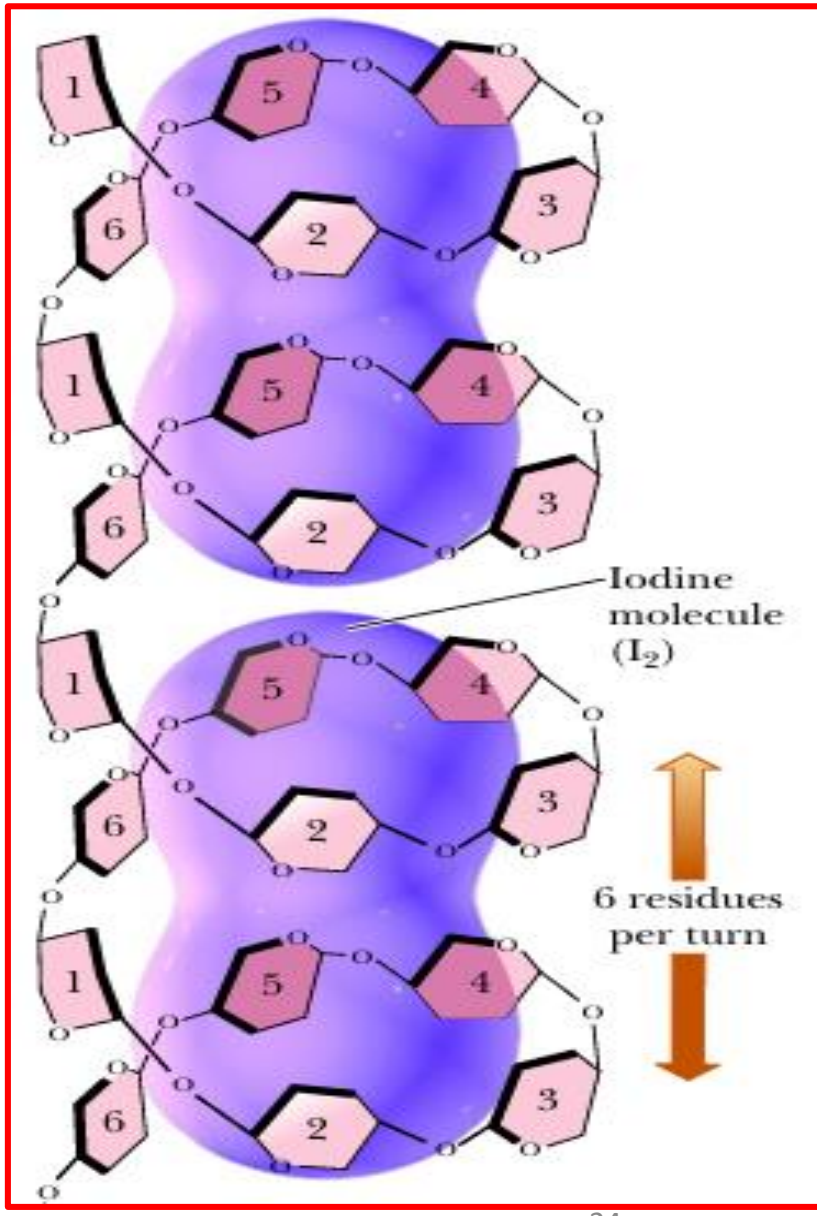
The Principle of the Iodine Test

- This is a **physical interaction**, not a chemical reaction.
- The linear **triiodide ions (I_3^-)** become trapped in the amylose helix.
- This interaction creates the **intense blue-black** color.

I_3^- + Starch → Dark blue complex

- The interaction is **heat-sensitive;** heating breaks the helix and the color disappears.

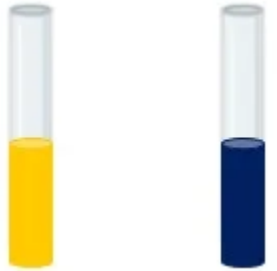




Procedure for Iodine Test

- Set up a new, clean set of 6 labeled test tubes.
- Pipette 1 mL of each sample into its respective tube.
- Add **2 drops of Lugol's Iodine Solution** to each tube.
- Mix and observe. A **deep blue-black color** is a positive result.
- Record your observations.



Iodine Test: A Positive Result

What to expect:

- **Positive Test:** The solution turns a deep blue-black color.
- **Negative Test**: The solution remains yellowish-brown (the color of the iodine reagent itself).

The Evidence & Interpretation

Recording Your Data

- Use the summary table in your lab manual.
- Record all observations neatly. Be descriptive. This is your primary evidence.

Sample Tested	**Molisch's Test** (Observation)	**Iodine Test** (Observation)	**Fehling's Test** (Observation)	**Benedict's Test** (Observation)
Glucose				
Fructose				
Sucrose				
Starch				
Unknown				
Water (Control)				

Summary of Our Chemical Tests

Test Name	What it Detects	Positive Result
Molisch's Test	Any Carbohydrate	Purple Ring
Benedict's Test	Reducing Sugars	Green/Yellow/Orange/Red Precipitate
Iodine Test	Starch	Blue-Black Color

What to Expect: The Knowns

Let's predict the results for our known samples. This helps you validate that your tests are working correctly.

Sample	Molisch's Test	Iodine Test	Benedict's Test
Glucose	+	-	+ (Red)
Fructose	+	-	+ (Red)
Sucrose	+	-	- (Blue)
Starch	+	+	- (Blue)
Water	-	-	- (Blue)

The Final Challenge: Identifying Your Unknown

Now, you use the results from your "Unknown" tube to solve the puzzle.

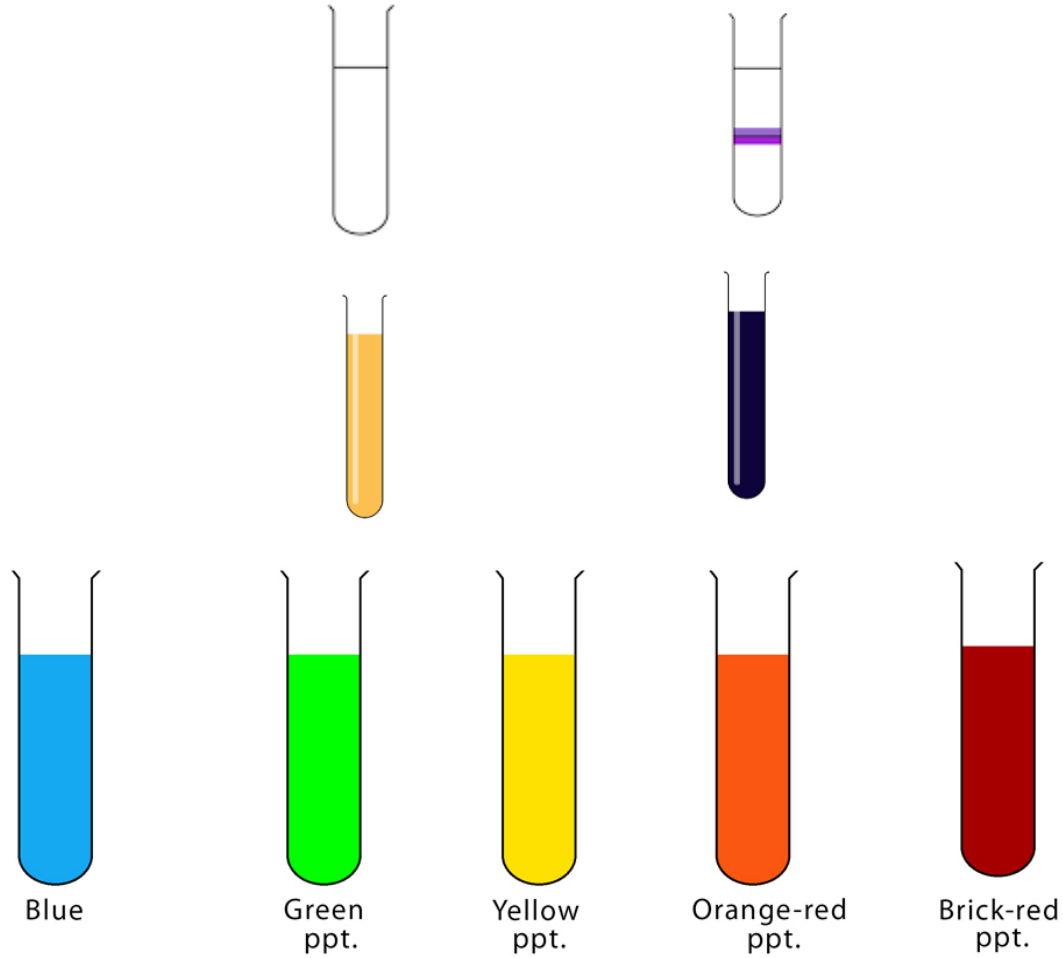
- **Step 1:** Look at the Molisch's Test. Is it positive? If not, you're done! (It's not a carbohydrate).
- **Step 2:** If Molisch's is positive, look at your other two tests.
- **Step 3:** Compare its pattern of results (+/-) to the pattern of the knowns.

Thinking Like a Scientist

Scenario A

Your unknown gives the following results:

- **Molisch's Test**: Positive (purple ring)
- **Iodine Test**: Negative (yellow-brown)
- **Benedict's Test**: Positive (orange-red precipitate)
- **Conclusion**: The unknown is a reducing sugar, most likely a monosaccharide like glucose or fructose.



Thinking Like a Scientist

Scenario B

Your unknown gives the following results:

- **Molisch's Test**: Positive (purple ring)
- **Iodine Test**: Positive (blue-black color)
- **Benedict's Test**: Negative (remains blue)
- **Conclusion**: The unknown is Starch.

Thinking Like a Scientist

Scenario C

Your unknown gives the following results:

- **Molisch's Test**: Positive (purple ring)
- **Iodine Test**: Negative (yellow-brown)
- **Benedict's Test**: Negative (remains blue)
- **Conclusion**: The unknown is a non-reducing sugar, most likely Sucrose.

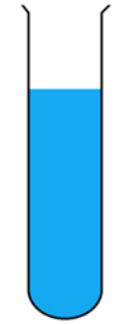
Application: The "Zero Sugar" Energy Drink

- **From your Study Questions**: A company claims its new drink has no glucose, fructose, or sucrose. It is sweetened with an artificial peptide and contains a novel polysaccharide ("EnerPlex") for texture.
- How would you test their claims using only our three tests?

Application: Expected Results

If the company's claims are TRUE:

- **Benedict's Test**: Should be Negative. (Confirms no glucose/fructose).
- **Iodine Test**: Result is Unknown. It depends on whether "EnerPlex" has a helical structure like starch. This would be an interesting finding!
- **Molisch's Test**: Should be Positive. Why? Because "EnerPlex" is a polysaccharide, which is a carbohydrate.
- This shows how our tests can be used to validate claims in a real-world scenario.



A Note on False Positives

- Could the peptide sweetener in the energy drink cause a false positive in the Molisch's test?
- Unlikely. The Molisch's test relies on the acid-catalyzed dehydration of a carbohydrate ring structure to form an aldehyde. Peptides do not have this structure and will not form furfural/HMF.
- Understanding the chemical principle of a test is key to identifying potential interferences.

Housekeeping & Waste Disposal

- **Acid Waste**: All solutions from the Molisch's test must go into the designated acidic waste container in the fume hood.
- **Benedict's Waste**: Can typically be disposed of in the designated aqueous waste container.
- **Iodine Waste**: Can also be disposed of in the aqueous waste container.
- Rinse all glassware thoroughly when you are finished.

Question

You are a food scientist working for a company that is developing a new line of sports drinks. You are given a sample of a new sweetener, "GlycoSweet," and asked to determine its carbohydrate properties. You perform the following tests with the results indicated:

- **Molisch's Test:** A purple ring is observed at the junction between the two liquids.
- **Iodine Test:** The solution turns a deep blue-black color.
- **Benedict's Test:** The solution remains blue, even after heating.

a) Based on these results, what type of carbohydrate is "GlycoSweet"? Explain your reasoning by interpreting the result of each test.

b) The marketing team wants to label the product as "contains no reducing sugars." Do your results support this claim? Explain why or why not, with specific reference to one of the tests performed.

c) If "GlycoSweet" had been lactose, what different results would you have expected for the Iodine and Benedict's tests?

d) Why is it critical that you DO NOT MIX the layers when observing the result for the Molisch's test?

Questions?