

Organic Compound Identification

Purpose

To recognize that all living things are made of cells and that cells contain carbohydrates, lipids, proteins, and nucleic acids

Materials

goggles, apron, science journal, Benedict's solution, materials for a water bath, 13 test tubes, test tube rack, graduated cylinder, pipets, glucose solution, distilled water, apple slice, stirring rods, starch suspension, solution of iodine and potassium iodide, ½ corn kernel, Sudan IV solution, knife or scalpel, peanut seed, cover slip, concave slide, water dropper, compound microscope, I₂KI solution in bottle with dropper, 1 mL vegetable oil, Biuret reagent

Monosaccharide (Simple Carbohydrate) Identification

Monosaccharides, or simple carbohydrates, can be identified when Benedict's solution is added and heat is applied. An orange or red precipitate will form when large amounts of glucose are present. If a small amount of glucose is present, then a yellow or green precipitate will form.

Read through the following procedure and write what you predict you will observe in each test tube:

1. Start a water bath by placing a 1 L beaker half-filled with water on a hot plate, and bringing water to a boil.
2. Label one test tube "glucose," one test tube "apple," and one test tube "water."
3. Add 5 mL of Benedict's solution to each of the three test tubes.
4. Using a clean pipet, add 10 drops of glucose solution to the "glucose" test tube.
5. Using a clean pipet, add 5 mL of distilled water to a test tube.
6. Place a piece of apple into the tube and crush it with a stirring rod.
7. Decant the water into the test tube labeled "apple."
8. Using a clean pipet, add 10 drops of distilled water to the "water" test tube.
9. Using a clean stirring rod each time, mix the solutions in each labeled test tube.
10. Place each of the labeled test tubes in the water bath for approximately two minutes.
11. Remove the test tubes from the water bath and place them in a test tube rack.
12. Record your observations in your science journal.

Questions

- A. What purpose did the "glucose" test tube serve?
- B. What purpose did the "water" test tube serve?
- C. Were your predictions correct? Why?
- D. Although all organisms contain simple carbohydrates, what other substances could you test and expect a positive identification?

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Organic Compound Identification (continued)

Polysaccharide (Starch) Identification

Starches are made up of chains of glucose molecules. Starches can be identified in substances by using a solution of iodine and potassium iodide (I_2KI). A color change from dark blue to black will occur.

1. Label one test tube “starch,” one test tube “potato,” and one test tube “water.”
2. Add 5 mL of distilled water to the test tube labeled “starch.”
3. Using a clean pipet, add 5 drops of starch suspension to the test tube.
4. Swirl the test tube and then add one drop of I_2KI .
5. Add 10 mL of distilled water to the test tube labeled “water.”
6. Add one drop of I_2KI .
7. Take half of a corn kernel and place a drop of I_2KI on its cut surface.
8. Record your observations in your science journal.

Questions

- A. What purpose did the “starch” test tube serve?
- B. What purpose did the “water” test tube serve?
- C. Did all of the kernel tissue react with the I_2KI ? Explain why you think this happened.
- D. What is the importance of starch in plant cells?

Lipid Identification

Polar compounds are compounds that are positively charged on one side and negatively charged on the other. Lipids are nonpolar substances. Lipids do not dissolve in polar substances such as water, but will dissolve in other nonpolar substances. This particular identification test targets triglycerides, or fats and oils. Triglycerides can be identified when they react with Sudan IV stain.

1. Label one test tube “oil,” and one test tube “water.”
2. Add 3 drops of Sudan IV solution to each test tube.
3. Add 1 mL vegetable oil to the test tube labeled “oil” and 1 mL distilled water to the test tube labeled “water.”
4. Using a clean stirring rod each time, mix the contents of each test tube.
5. Record your observations in your science journal.
6. Cut a thin slice of a peanut seed and make a wet mount using distilled water.
7. Using a compound microscope, observe the tissue under low and high power.
8. Remove the slide and place a drop of I_2KI on one edge of the cover slip. (Blot water from the other edge of the cover slip, if needed.)
9. Place the slide back under the microscope and record your observations in your science journal.
10. Add Sudan IV solution to your wet mount.
11. Observe the tissue under low and high power.
12. Record your observations in your science journal.

(continued)

Organic Compound Identification (continued)

Questions

- A. What purpose did the “oil” test tube serve? What did you observe when Sudan IV was added?
- B. What purpose did the “water” test tube serve? What did you observe when Sudan IV was added?
- C. What did the addition of I₂KI to the peanut seed show?
- D. Do you think that Sudan IV is a polar or nonpolar substance? Why?

Protein Identification

Proteins consist of sequences of amino acids. There are 20 different amino acids found in living organisms. The differences in combinations of amino acids and protein structures allow for thousands of different proteins to be found in organisms. Proteins consisting of particular amino acids can be identified when they react with a solution of sodium hydroxide and copper sulfate. A color change of purple indicates the presence of peptide bonds in the solution. This is called the Biuret test.

1. Choose four substances to test for protein identification, under the following conditions: One substance must be your known positive control and one substance must be your known negative control.
2. Label four test tubes with their corresponding substance identifications.
3. For each of the four test tubes, add 1 mL of the corresponding substance.
4. Add 1 mL of Biuret reagent to each of the four test tubes.
5. Using a clean stirring rod each time, mix the contents of each test tube.
6. Record your observations in your science journal.

Questions

- A. What substances did you choose as your positive and negative controls? Why?
- B. What other two substances did you choose to test for presence of proteins? Why?

Organic Compound Identification

Teacher Directions

- A. Monosaccharide (Simple Carbohydrate) Identification
- To prepare a 10% solution of glucose, add 10 g of glucose in powder form to a final volume of 100 mL distilled water.
 - You can purchase Benedict's solution from any biological supply company.
 - Cut apples into small pieces for students to crush in their test tubes.
 - Aid students as they prepare hot water baths. If more than one lab group is using a hot water bath, have students label their test tubes with their lab group identifications.
- B. Polysaccharide (Starch) Identification
- I₂KI preparation: Add 1.3 grams iodine to 2 grams of potassium iodide and add to 100 mL distilled water.
 - The day before the lab, soak the corn kernels in a bowl of water overnight to soften them. The day of the lab, cut the corn kernels longitudinally so each group of students can have one half of a corn kernel.
- C. Lipid Identification
- If needed, review with students how to prepare a wet mount.
 - You can purchase Sudan IV stain from most biological supply companies.
 - Since Sudan IV is nonpolar, it will move into the lipid droplets of the peanut seed tissue.
- D. Protein Identification
- You can purchase Biuret reagent from most biological supply companies.
 - The positive control can be albumin in powder form. To prepare a 10% albumin solution, add 10 g in a final volume of 100 mL distilled water.

Answer Key

- A. Monosaccharide (Simple Carbohydrate) Identification
- a. positive control
 - b. negative control
 - c. answers will vary
 - d. examples might be table sugar, honey, other fruits
- B. Polysaccharide (Starch) Identification
- a. positive control
 - b. negative control
 - c. No. Only parts of the kernel contain starch.
- C. Lipid Identification
- a. positive control; Sudan IV dissolved in the oil
 - b. negative control; Sudan IV separated from the water
 - c. presence of starch
 - d. nonpolar; it did not dissolve in water and did dissolve in oil (like dissolves like)
- D. Protein Identification
- a. Students should choose a known protein as the positive control and a known nonprotein (e.g., distilled water) as the negative control.
 - b. Answers will vary.