

Investigation 6.A: Testing for Macromolecules

Purpose: To perform qualitative tests to detect and identify the presence of carbohydrates, proteins, and lipids.

Question

How can you recognize and identify the presence of macromolecules in various samples?

Safety Precautions

Be careful when handling iodine, Benedict's solution, and Biuret reagent because they are toxic and can stain skin and surfaces. To prevent test tubes from breaking, do not allow the hot-water bath to boil vigorously. Clean up all spills immediately, with plenty of water, and inform your teacher immediately if a spill occurs.

Materials

- distilled water
- Biuret reagent
- albumin solution
- pepsin solution
- starch suspension
- iodine solution in dropper bottle
- Benedict's solution
- glucose solution
- onion juice
- large beaker (500 mL or larger) for hot-water bath
- potato juice
- vegetable oil
- butter or margarine
- 11 test tubes
- 3 test-tube racks
- 3 small squares of brown dropper bottle paper
- millimetre ruler
- wax pencil
- hot plate
- tongs

Procedure

Part 1: Test for Proteins

Biuret reagent has a blue colour that changes to violet in the presence of proteins or to pink in the presence of peptides.

1. Use a millimetre ruler and a wax pencil to mark and label four clean test tubes at the 2 cm and 4 cm levels. Fill each test tube as follows:
 - Test tube 1: Fill to the 2 cm mark with distilled water, and then add Biuret reagent to the 4 cm mark.
 - Test tube 2: Fill to the 2 cm mark with albumin solution, and then add Biuret reagent to the 4 cm mark. (Albumin is a protein.)
 - Test tube 3: Fill to the 2 cm mark with pepsin solution, and then add Biuret reagent to the 4 cm mark. (Pepsin is an enzyme.)
 - Test tube 4: Fill to the 2 cm mark with starch suspension, and then add Biuret reagent to the 4 cm mark.
2. Record the final colour of the contents of all four test tubes in the table on the following page.

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Biuret Test for Protein

Test Tube	Contents	Colour change	Conclusions
1	distilled water		
2	albumin		
3	pepsin		
4	starch		

3. Dispose of the contents of the test tubes as directed by your teacher. Clean and dry the test tubes.

Part 2: Test for Starch

Iodine solution turns from a brownish colour to blue-black in the presence of starch.

- Use a millimetre ruler and a wax pencil to mark and label two clean test tubes at the 1 cm level. Fill each test tube as follows:
 - Test tube 1: Fill to the 1 cm mark with starch suspension, and then add five drops of iodine solution. (Be sure to shake the starch suspension well before taking your sample.)
 - Test tube 2: Fill to the 1 cm mark with distilled water, and then add five drops of iodine solution.
- Note the final colour change. Record your results in the table below.

Iodine Test for Starch

Test Tube	Contents	Colour change	Conclusions
1	starch		
2	distilled water		

3. Dispose of the contents of the test tubes as directed by your teacher. Clean and dry the test tubes.

Part 3: Test for Sugars

Sugars react with Benedict's solution after being heated in a boiling-water bath. Increasing concentrations of sugar give a continuum of colours, as shown in the table below.

Typical Reactions for Benedict's Solution

Chemical	Chemical category	Benedict's solution (after heating)
distilled water	inorganic	blue (no change)
glucose	monosaccharide (carbohydrate)	varies with concentration: <ul style="list-style-type: none"> very low: green low: yellow moderate: yellow-orange high: orange very high: orange-red
maltose	disaccharide (carbohydrate)	varies with concentration (See results for glucose.)
starch	polysaccharide (carbohydrate)	blue (no change)

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- Use a millimetre ruler and a wax pencil to mark and label five clean test tubes at the 1 cm and 3 cm levels.
 - Test tube 1: Fill to the 1 cm mark with distilled water, and then add Benedict's solution to the 3 cm mark. Heat in a boiling-water bath for about 5 min.
 - Test tube 2: Fill to the 1 cm mark with glucose solution; add Benedict's solution to the 3 cm mark. Heat in a boiling-water bath for about 5 min.
 - Test tube 3: Put a few drops of onion juice in the test tube. Fill to the 1 cm mark with distilled water, and then add Benedict's solution to the 3 cm mark. Heat in a boiling-water bath for about 5 min.
 - Test tube 4: Put a few drops of potato juice in the test tube. Fill to the 1 cm mark with distilled water and then add Benedict's solution to the 3 cm mark. Heat in a boiling-water bath for about 5 min.
 - Test tube 5: Fill to the 1 cm mark with starch suspension; add Benedict's solution to the 3 cm mark. Heat in a boiling-water bath for about 5 min.
- Note the final colour change. Record your results in the table below.

Benedict's Test for Sugars

Test Tube	Contents	Colour (after heating)	Conclusions
1	distilled water		
2	glucose solution		
3	onion juice		
4	potato juice		
5	starch suspension		

- Dispose of the contents of the test tubes as directed by your teacher. Clean and dry the test tubes.

Part 4: Test for Fats

Fats leave a translucent, oily spot on paper. Liquid fats penetrate paper, while solid fats rest predominantly on top.

- Place a small drop of water on a square of brown paper. Describe the immediate effect.
- Place a small drop of vegetable oil on a square of brown paper. Describe the immediate effect.
- Place a small quantity of butter or margarine on a square of brown paper. Describe the immediate effect.
- Wait about 5 min. Examine each piece of paper to determine which test material penetrates the paper. Record your results in the table on the next page.

