

Biological Chemistry: Testing for Reducing Sugars with Benedict's Reagent

Benedict's reagent is used to detect reducing sugars, not all sugars. Reducing sugars or simple sugars include the monosaccharides and some disaccharides. When simple reducing sugars are combined with Benedict's reagent, the sugar reduces or donates electrons to the copper compound in the reagent changing its color. Benedict's reagent is blue. In the presence of reducing sugars the reagent turns red. Small amounts of reducing sugar produce a yellow-green color, with more reducing sugar the reagent turns orange and with even more the reagent turns a brick red color. The color is produced by a precipitate (solid) that will with time settle to the bottom of the tube.

Benedict's test is interesting because it is not only important in clinical and food sciences but historically it was the only test diabetics could use in their homes to monitor their glucose. A diabetic patient would have to test their urine daily using Benedict's reagent to determine if they were excreting glucose and needed to alter their insulin injections.

Materials

Test tubes - 14

Test tube brush

Glass rod, scoopula

Large beaker (500 mL)

Test tube rack

Hotplate or Bunsen burner (if using a Bunsen burner you will also need a ring stand, wire gauze and ring clamp)

Test tube clamp or hot glove

Distilled water

Pipettes

Various foods (cereal, bread, glucose solution, apple, orange juice, potato, sucrose solution, whole milk, ground beef, artificial sweetner)

Note on food preparation: If you are performing tests for lipids, proteins and carbohydrates you only need to prepare the solid foods once. There are slight differences in the food stuffs used between these activities. The protein/biuret lab uses the most extensive food list. There should be enough extract to perform all tests from one food preparation. If you have already prepared the solid foods extracts, start on step 7.

Develop a hypothesis for this activity. Write your hypothesis here. _____

Procedure

1. Use a sharpie or permanent marker to label one set of test tubes: Control, cereal, bread, glucose, apple, orange juice, potato, sucrose, whole milk, artificial sweetner. Mark these tubes 3 cm from the bottom of the tube.
2. Use a sharpie or permanent marker to label a second set of test tubes: artificial sweetner, cereal, bread, apple, and potato. Mark this set of tubes at 1 and 6 cms from the bottom of the tube. These tubes will be used to grind and suspend the solid foods.
3. To this second set of tubes add the appropriate solid materials to the 1 cm mark. Finely chop the apple and potato pieces before placing these materials in the tubes.
4. Add water to the 6 cm mark on the test tubes containing cereal, bread, apple and potato.
5. Use the glass rod or scoopula to crush the solid foods.
6. Allow the solid foods to settle for 5 minutes. Continue to step 7.
7. Fill the beaker between $\frac{1}{3}$ and $\frac{1}{2}$ full with tap water. Add boiling beads to the beaker if available. Place the beaker on the hot plate and turn the hot plate on. If you are using a Bunsen burner and ring stand your instructor will provide directions.
8. Retrieve the first set of empty labeled tubes. Add Benedict's reagent to each tube to the 3 cm line.
9. Use a pipette to add 1 mL or 20 drops of water to the control tube.
10. Use a pipette to add 1 mL or 20 drops of orange juice to the tube marked 'orange juice'.
11. Use a different pipette to add 1 mL or 20 drops of milk to the tube marked 'milk'.
12. Use a new pipette to add 1 mL or 20 drops of the artificial sweetner solution to the tube marked artificial sweetner.
13. Use a new pipette to add 1 mL or 20 drops of the glucose solution to the tube marked 'glucose'.
14. Use a new pipette to add 1 mL or 20 drops of the sucrose solution to the tube marked 'sucrose'.
15. If it has been at least 5 minutes and the ground food has settled, you can now pipette the supernatant (liquid above the solids). Remove only the liquid, do not removes solids from the tubes.
16. Use a new pipette to add 1 mL or 20 drops of cereal extract to the tube marked 'cereal'. Use only the fluid above the food, do not pipette food chunks.
17. Use a new pipette to add 1 mL or 20 drops of bread extract to the tube marked 'bread'.
18. Use a new pipette to add 1 mL or 20 drops of apple extract to the tube marked 'apple'.
19. Use a new pipette to add 1 mL or 20 drops of potato extract to the tube marked 'potato'.
20. Place the tubes in the beaker of water. The water should be boiling. Boil the tubes for 5 minutes.
21. Use the test tube clamp or hot glove to remove the test tubes from the boiling water.
22. Record your results on the next page.
23. Clean your test tubes and return materials to the appropriate location.

Record your results in the table below. Based on the color produced in the Benedict's reaction, rank the level of reducing sugar on a scale of 1-10, with 1 indicating the lowest level (bluest color) of reducing sugars.

Food	Color	Are reducing sugars present?	Ranking
Water	Blue	No	1
Cereal			
Bread			
Glucose			
Apple			
Orange juice			
Potato			
Sucrose			
Whole milk			
Nutrasweet			

Glucose is the sugar found in your blood. Sucrose is table sugar. Review your results above. Are both glucose and sucrose reducing sugars?

Was your hypothesis supported? Why or why not?

What was the control? What was the dependent variable? What were the independent variables?