Benedict's Quantitative Solution

A Quantitative Test for Reducing Sugars

Introduction

Benedict's Quantitative Solution is a test reagent used for detecting and quantitatively determining the amount of reducing sugars present in a substance. All monosaccharides and some disaccharides are reducing sugars—that is, they contain a free aldehyde or α-hydroxyketone group that is capable of reducing copper(II) or iron(III) ions. Sucrose, a disaccharide, is a notable exception in that it is not a reducing sugar.

Concepts

- Reducing sugars
- Functional groups
- Oxidation–reduction reactions

Materials

Benedict's Quantitative Solution, 100 mL
Sodium carbonate, Na_2CO_3, 20 g
Sugar solution
Water, distilled or deionized, 1000 mL
Balance
Beaker, 100-mL
Beaker, 250-mL
Beaker, 2-L
Boiling stones, 2–3
Buret
Erlenmeyer flask, 125-mL
Funnel with filter paper
Hot plate stirrer with stirring bar
Pipet, 10-mL

Safety Precautions

This activity requires the use of hazardous components and/or has the potential for hazardous reactions. Copper(II) sulfate is a skin and respiratory irritant and is moderately toxic by ingestion and inhalation. Sodium carbonate is mildly toxic by ingestion. Potassium thiocyanate is moderately toxic by ingestion and emits toxic fumes of cyanide if heated to decomposition or if in contact with concentrated acids. Potassium ferrocyanide is slightly toxic by ingestion and emits toxic fumes of cyanide if heated to decomposition or if in contact with concentrated acids. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wear insulated gloves or use tongs when handling the hot Erlenmeyer flask. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

Preliminary Titration

Note: The reduction of copper(II) ions by reducing sugars is an example of a nonstoichiometric reaction—one which does not follow a defined pathway and cannot be described by an equation either qualitatively or quantitatively. Therefore, this procedure is only accurate over a small range of sugar concentrations. To obtain accurate results, the volume of sugar added must lie within 6–12 mL per 10 mL of Benedict's Quantitative Solution. Thus, the preliminary titration is performed to ensure that the sugar solution is within this concentration range.

1. Pipet 10 mL of Benedict's Quantitative Solution into a 125-mL Erlenmeyer flask. Add 2 g of anhydrous sodium carbonate and several boiling stones to the flask. Shake the mixture well to suspend the sodium carbonate.
2. Place the flask on a hot plate. Heat the contents in the flask to boiling. Keep the contents of the flask boiling steadily throughout the experiment. If the volume of the liquid in the flask is reduced, add distilled or deionized water to keep the volume constant.
3. Add 3 mL of the sugar solution to the flask. If testing monosaccharides, allow the sugar solution to boil for 30 seconds. If testing disaccharides, allow the sugar solution to boil for 1 minute. (Disaccharides are oxidized more slowly than monosaccharides). If the blue color is not removed after boiling for the prescribed amount of time, add another 3 mL of the sugar solution and boil again for the prescribed amount of time. Continue this process of adding 3-mL aliquots

and boiling until the blue color disappears from the solution and the conditions outlined in step 4 are met.

4. If the blue color is completely removed after adding 6 mL of the sugar solution, dilute a fresh sample of sugar solution in half and repeat steps 1–3. If the blue color still remains after adding 12 mL of the sugar solution, concentrate a fresh sample of sugar solution (by heating it and allowing some water to evaporate) to half its original volume and repeat steps 1–3. When the preliminary titration requires between 6 and 12 mL to remove the blue color, the sugar solution is within the proper concentration range to proceed.

Quantitative Determination of Reducing Sugars

5. Fill a buret with about 15 mL of the correctly diluted (or concentrated) sugar solution prepared in steps 1–4 above. Clamp the buret so that it is positioned above the hot plate.

6. Pipet 10 mL of Benedict's Quantitative Solution into a 125-mL Erlenmeyer flask. Add 2 g of anhydrous sodium carbonate and two boiling stones to the flask. Shake the mixture well to suspend the sodium carbonate.

7. Place the flask on a hot plate situated underneath the buret. Heat the contents in the flask to boiling. Titrate the Benedict's Quantitative Solution in the flask with the sugar solution in the buret. The endpoint of this titration occurs when the blue color disappears from the solution in the flask. Keep the contents of the flask boiling steadily throughout the experiment. If the volume of the flask is reduced, add distilled or deionized water to keep the volume constant.

8. Calculate the concentration of the unknown sugar solution using the following procedure and conversion factors.

   a. Find your sugar in the table below. Note the mass in grams of sugar required to completely reduce 10 mL of Benedict's Quantitative Solution. This is the number of grams of sugar that was titrated into the 10-mL sample of Benedict's Quantitative Solution before reaching the endpoint.

Sugar	Number of grams required to reduce 10 mL of Benedict's Quantitative Solution
Glucose	0.0200 g
Fructose	0.0202 g
Lactose	0.0271 g
Maltose	0.0282 g
Sucrose*	0.0196 g

*Non-reducing sugars, such as sucrose, are estimated by first hydrolyzing the sugar with dilute hydrochloric acid solution into its component monosaccharides, then titrating the hydrolyzed mixture.

   b. Record the number of mL of sugar solution required to reach the endpoint.

   c. Take the preliminary titration into account. For example, if the unknown solution had to be diluted in half, divide the volume required to reach the endpoint by 2.

   d. Divide the mass of sugar (step 1) by the adjusted volume (step 3) to determine the concentration of the sugar solution. Your answer will be in g/mL. Convert to mg/mL.

Example Calculation

Suppose you have a lactose solution of unknown concentration. The preliminary titration required that the original solution be diluted in half. The diluted sugar solution was then titrated and 8.20 mL were required to reach the endpoint.

a. According to the table above, 0.0271 g of lactose is required to completely reduce 10 mL of Benedict's Quantitative Solution. Therefore, 0.0271 g of lactose was titrated into the 10-mL sample of Benedict's Quantitative Solution before reaching the endpoint.

b. The volume required to reach the endpoint as read off the buret was 8.20 mL.

c. Taking the preliminary titration into account, the volume required to reach the endpoint (8.20 mL) must be divided in half. Therefore, 4.10 mL of the original lactose solution was actually required to reach the endpoint.

d. By dividing the mass of lactose (step 1) by the adjusted volume (step 3), the concentration in g/mL of the unknown lactose solution can be determined: 0.0271 g/ 4.10 mL = 0.00661 g/mL. Converting to mg/mL gives 6.61 mg/mL.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. All solutions may be flushed down the drain with excess water according to Flinn Suggested Disposal Method #26b.

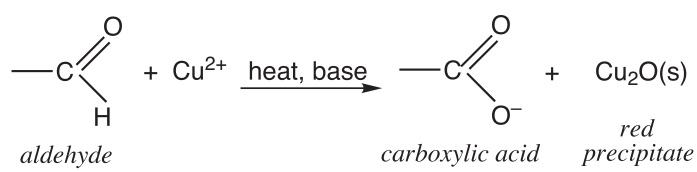
Tips

- Near the endpoint, the solution will become cloudy and begin to lose its blue color. Begin titrating more slowly at this point, allowing between 30 seconds to 1 minute (as in step 3 of the preliminary titration) between additions of sugar solu tion to allow the Benedict's Quantitative Solution and the sugar solution to react completely. The endpoint is when the blue color completely disappears from the solution and only a white cloudy solution remains.
- Stir the sugar solution frequently, especially after each addition of sugar solution to the flask. A stir bar may be used for constant stirring.
- The calculations shown here apply only to sugar solutions containing a single sugar, such as lactose or glucose. The cal culations for sugar solutions containing multiple sugars would need to take into account the conversion factors for the various sugars and ratios at which they are present.

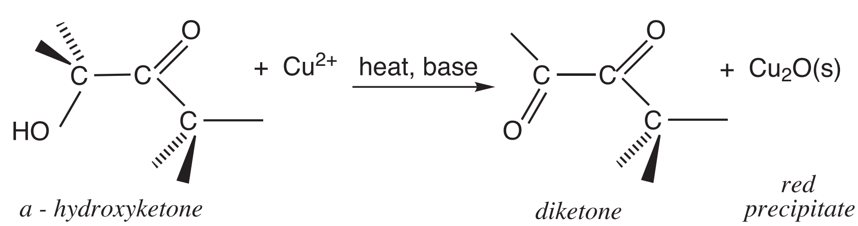
Discussion

Benedict's Quantitative Solution allows for the quantitative determination of reducing sugars. It is based on the redox reaction between copper(II) ions and reducing sugars. The copper(II) ions in Benedict's solution impart a characteristic blue color to the solution. When added to another solution containing a reducing sugar, the blue copper(II) ions are reduced by the reducing sugar, generally resulting in the formation of a precipitate and a color change.

A reducing sugar is a sugar that contains a free aldehyde or α-hydroxyketone group that is capable of reducing iron(III) or copper(II) ions. In a reaction with iron(III) or copper(II) ions, an aldehyde is oxidized to a carboxylic acid (see Reaction 1), while an α-hydroxyketone is oxidized to a diketone (see Reaction 2). All monosaccharides and some disaccharides are reducing sugars. Examples of reducing sugars include glucose, fructose, maltose, galactose, and lactose. Notably, sucrose is not a reducing sugar.



Reaction 1



Reaction 2

Benedict's Quantitative Solution contains copper(II) sulfate, sodium citrate, sodium carbonate, potassium thiocyanate, and potassium ferrocyanide. Each plays a role in the determination of reducing sugars:

- Copper(II) sulfate provides the copper(II) ions that are reduced by the reducing sugar.
- Sodium carbonate provides a basic environment, which is necessary for the redox reaction to occur.
- If the carbonate ions from the sodium carbonate were to combine with the copper(II) ions from the copper(II) sulfate,

insoluble copper(II) carbonate would form. To prevent the formation of a copper(II) carbonate precipitate, sodium citrate is added. The citrate ions chelate with the copper(II) ions before the copper(II) ions can precipitate with the carbonate ions.

- The reducing sugar reduces the copper(II) ions to copper(I) ions, which combine with the thiocyanate ions from the potassium thiocyanate to form white copper(I) thiocyanate. This makes the endpoint of the titration, the transition from blue to white, readily visible.
- The small amount of potassium ferrocyanide prevents any reoxidation of the copper(I) ions.

Reference

Frais, F. *Practical Biochemistry: An Introductory Course;* Butterworths: London, 1972; pp 30–32, 150–151.

Materials for *Benedict's Quantitative Solution* are available from Flinn Scientific, Inc.

Catalog No.	Description
B0062	Benedict's Quantitative Solution
G0024	Glucose Solution
GP1080	Buret, Straight Bore, Glass Stopcock, 50-mL

Consult the Flinn Scientific website for current prices.