Benedict's Test





KEY OBJECTIVES

To detect the presence of reducing sugar in the sample solution

To estimate the concentration of reducing sugar in the sample solution

To differentiate and identify the extracted carbohydrates

INTRODUCTION

Benedict's Test is a chemical analytical method used for the detection of reducing sugar in a solution. Benedict's Test is a qualitative test often used for the differentiation of carbohydrates (saccharides/sugars) into reducing and non-reducing types.

Principle of Benedict's Test: Sodium carbonate in the Benedict reagent increases the pH of the sample-reagent solution mixture. Under warm alkaline conditions reducing sugars are tautomerism to strong reducing agents, enediols. These enediols reduce the cupric ions (Cu2+) (present as Copper Sulfate (CuSO4)) of Benedict reagent into cuprous ions (Cu+). The cuprous particles are present in form of insoluble Copper (I) oxide or cuprous oxide (Cu2O) which is of red color. These red-colored copper oxides get precipitated.

The concentration of reducing sugar in the sample differs from the intensity and shade of the color of the reaction mixture. This shade of color can be used to estimate the concentration of reducing sugar in the sample. Color may vary from greenish to yellow to orange-red to brick-red. As the concentration of reducing sugar increases color gradually changes from greenish to yellowish to orange to brick-red.



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GUIDING QUESTIONS

How can you detect sugar in fluids?

?

MATERIALS & PREPARATION

- Sample solution of carbohydrate
- Test-tubes and test-tube holders
- Pipette
- Bunsen burner
- Benedict's Reagent

Preparation of Benedict's Reagent:

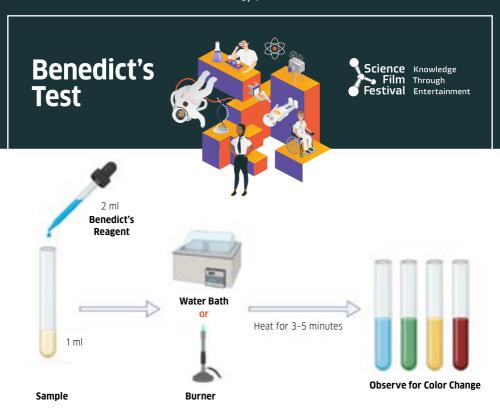
- Measure 17.3 grams of copper sulfate (CuSO4), 173 grams of sodium citrate (Na3C6H5O7), and 100 grams of anhydrous sodium carbonate (Na2CO3) (or 270 grams of sodium carbonate decahydrate (Na2CO3.10H2O).
- Put all the measured chemicals in a volumetric flask of 1000 ml.
- Pour distilled water up to 1000 ml marking.
- Dissolve all the components properly by shaking gently.

TASKS & PROCEDURE

- 1) In a clean test tube add 1 ml of sample solution (urine or carbohydrate solution).
- Add 2 ml of Benedict's reagents over the sample.
- Place the test tube over a boiling water bath and heat for 3–5 minutes or directly heat over a flame.
- 4 Observe for color change.

Important notes:

- Measurement must be accurate.
- Don't heat the mixture quickly. It is best to heat over a water bath slowly.
- During heating the solution, use a test-tube holder
- Don't face the test tube towards oneself or others during heating.
- Heating should be done at least thrice before reporting negative.



Result Interpretation / Observation of Benedict's

Any change in color from blue to green or yellow or orange or red within 3 minutes indicates a positive

Benedict test i.e. presence of reducing sugar in the sample. For semiquantitative evaluation, the concentration of reducing sugar can be estimated based on the shade of developed color as follows;

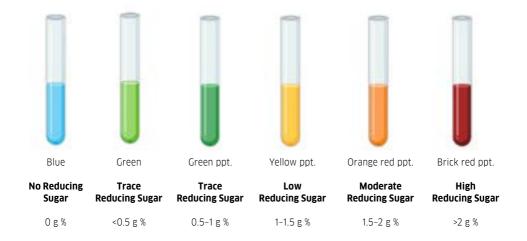
SHADE OF COLOR	APPROX. CONCENTRATION OF REDUCING SUGAR (IN g %)	INDICATION
Blue	0	No reducing sugar
Green Solution	<0.5	Trace reducing sugar
Green ppt.	0.5-1	Trace reducing sugar
Yellow ppt.	1-1.5	Low reducing sugar
Orange-red ppt.	1.5-2	Moderate reducing sugar
Brick-red ppt.	>2	High reducing sugar

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INTERPRETATION/OBSERVATION OF BENEDICT'S TEST



POSSIBLE EXTENSIONS

Applications of Benedict's Test:

In biochemistry for analysis and identification of unknown carbohydrate extracts.

In clinical diagnosis for rapid presumptive diagnosis of diabetes mellitus.

In quality control for detecting simple sugar and their quantification.

SOURCES

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