

B. Sc. (Hons) Part-II Practical
Course: Zool. H. 211
Experiment 1
Detection of glucose/sugar in a test sample

Introduction

- Sugar is a mixture of fructose and glucose.
- Glucose in urine is called *glucosuria*.
- Glucose in urine or blood indicates *Diabetes mellitus* (DM) in patients;
- The presence of glucose in urine or a test sample is easily detected by Benedict's reagent (or Benedict's solution) which is clear blue in colour;
- Polysaccharides such as starches do not react with Benedict's reagent.

Stanley Rossiter Benedict (1884-1936):

- American chemist;
- Best known for discovering Benedict's reagent;
- a solution that detects reducing sugars like glucose, lactose & fructose;
- Benedict was born in Cincinnati, and went to the University of Cincinnati, USA;
- In 1909 he published a paper in *J. Biol. Chem.*

Composition of Benedict's reagent

One litre of Benedict's reagent is prepared as follows:

NaCO ₃ (anhydrous)	= 100g
Sodium citrate (Na ₃ C ₆ H ₅ O ₇)	= 173g
Copper sulphate (CuSO ₄ , 5H ₂ O)	= 17.3g
Distilled water (DH ₂ O)	= 1000mL

Note: The chemical test by Benedict's reagent is a *semi-quantitative test* because the colour of the precipitation only gives an idea about the quantity of sugar (e.g. trace, low, moderate and high) present in the sample.

Materials and Methods

The following apparatus and chemicals are required:

1. A test tube
2. A test tube holder
3. A dropper
4. A spirit lamp
5. Benedict's reagent
6. Test sample(s)

Procedures for Benedict's test

- Take 5 mL of Benedict's reagent in a test tube;
- Add 8-10 drops of the sample solution to it;
- Boil the mixture over a spirit lamp for 2-3 min;
- Allow to cool down the solution;
- Finally, observe the colour of the solution.



Fig. 1 Procedures for Benedict's test

2) BENEDICT'S TEST

Procedure

- Take 5 ml of Benedict's reagent.
- Add 8 drops of carbohydrate solution.
- Boil over a flame or in a boiling water bath for 2 minutes.
- Let the solution cool down.



(Negative Reaction)



(Positive Reaction)

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Fig. 2 Blue colour of the Benedict's solution is due to the presence of CuSO_4 ; Brick-red colour is due to the presence of CuO

Amount of glucose mg/dL	Test results	Colours of the test soln.
0	Negative (-)	Blue
30	Positive (trace)	Green (no ppt)
100	Positive (low +)	Yellow
300	Positive (moderate ++)	Orange-red
1000	Positive (high +++)	Brick-red
>1000	Positive (too high ++++)	Deep brick-red

Table 1 Colour of the test tube solution according to the amount of glucose in mg/dL

Results/Observations



Fig. 3 Colours of the test solution

Conclusion/Inference

1. The presence or absence of glucose/sugar in the supplied sample is indicated by the colour of the heated solution;
2. If the same blue colour persists after heating, no glucose/sugar is present in the sample;
3. If the colour of the solution changed to green, yellow, orange-red, brick-red or deep brick-red, the presence of glucose/sugar in the sample is confirmed.



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