

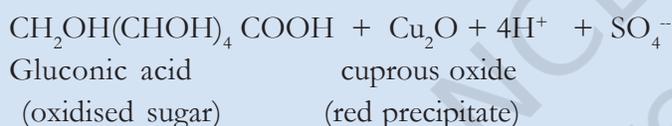
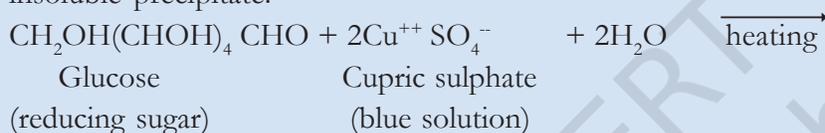
Exercise 30

Aim: To test the presence of sugar in the given sample of urine.

Principle: In normal urine, practically there is no glucose. Presence of glucose in urine is called glucosuria. To detect reducing sugars, such as glucose, fructose etc. in urine Benedict's or Fehling's tests are done.

CuSO_4 present in Benedict's solution or Fehling's solution is reduced on boiling by the reducing substances (glucose, fructose etc.) to form the coloured precipitate of cuprous oxide. The light green, green, yellow and brick red precipitates of cuprous oxides depend on the concentration of reducing substances present in urine.

Glucose reduces the blue cupric sulphate of Benedict's reagent or Fehling's reagent to a coloured insoluble precipitate.



Requirement: Glasswares: Test tubes, beakers, spirit lamp, pipette; Chemicals: Benedict's solution, Fehling's solution A and B, Seliwanoff's reagent, Miscellaneous: Test tube holder, test tube stand, urine sample.

Preparation of Reagents

- (i) Benedict's reagent Mix: 173 g of sodium citrate and 100 g of anhydrous sodium carbonate in 600 mL of water in a beaker and warm gently (solution A). Dissolve 17.3 g of hydrated CuSO_4 in 100 mL of distilled water (solution B). Add solution B to solution A with constant stirring. Cool and transfer to a one litre flask and make up to the mark with water.
- (ii) Fehling's reagent A: Dissolve 6.93 g of copper sulphate in 100 mL of distilled water.
- (iii) Fehling's reagent B: 20 g of KOH and 34.6 g of sodium-potassium tartarate (Rochelle's salt) dissolved in 100 mL of distilled water.

- (iv) Seliwanoff's reagent: Dissolve 50 mg of resorcinol in 33 mL of concentrated hydrochloric acid and dilute it to 100 mL with distilled water.
- (v) In absence of appropriate samples containing abnormal components of urine, these components (glucose, albumin) can be added in the normal urine sample.

Procedure

(a) Benedict's test

- Take 5 mL of Benedict's reagent in a test tube. Add 0.5 mL (8 drops) of freshly passed urine to it.
- Boil for 2 minutes holding the test tube firmly with a test tube holder (during boiling, the contents of the test tube get a tendency to spurt out. Hence, it is wise to keep shaking the test tube after holding it in the inclined position near the flame to avoid overboiling).
- A light green, green, yellow and brick red precipitate indicates the presence of reducing substances in urine.
- The various coloured precipitates depend on the concentration of reducing sugars in urine which gives a rough estimate of the concentration given below:

| Colour of precipitate | % of reducing suger present |
|-----------------------|-----------------------------|
| Light green ---- | 0.1 to 0.5 |
| Green ----- | 0.5 to 1.0 |
| Yellow ----- | 1.0 to 2.0 |
| Brick red ----- | above 2 |

(b) Fehling's test

- Take equal volumes (2 mL) of Fehling's solution A and B in a test tube. Mix them well.
- Add the above solution drop wise to 1 mL of urine sample taken in a test tube. Heat the test tube after each drop is added.
- A yellow or orange or brick red precipitate is formed which indicates the presence of reducing sugar in urine.

Exercise 30

Note: Benedict's and Fehling's test are not necessarily indicative of only glucose in urine but it may also indicate the presence of other reducing sugars, such as lactose (in case of pregnant woman and lactating mothers), fructose (in fructosuria), galactose (in galactosuria), homogentisic acid (in alkaptonuria), glucuronates and mucin.

(c) Seliwanoff's test

The qualitative Benedict's test is not very specific test for glucose, since other reducing sugars also give positive tests, such as fructose, galactose, lactose, maltose, pentose. Other urinary constituents, drugs and contaminants in the urine may give false positive results. For detecting whether glucose or fructose is present in the urine, Seliwanoff's test should be performed.

This test is to be performed when urine sample gives positive test for Benedict's or Fehling's test.

- Take 3 mL of Seliwanoff's reagent and add 1 mL of urine sample.
- Boil for two minutes. Appearance of red to orange colour indicates the presence of fructose.
- If no colour appears in 2 minutes, continue boiling for 5 minutes.
- If faint orange or no colour appears, then it indicates the presence of glucose.

Discussion

Test is sensitive between 50-80 mg glucose/100 mL urine. Less than this amount in the urine will not be detected by this test.