

Exercise 29

Aim: To detect the presence of urea in the given sample of urine.

Principle: Urea is mainly excreted into urine via kidneys. The nitrogen of amino acids is removed as urea. Normally a healthy adult person excretes about 15g of nitrogen per day; 95% of this nitrogen is excreted as urinary urea. The amino groups of amino acids are ultimately removed as ammonia (NH_3). This NH_3 , is highly toxic, and is ultimately converted into urea. Normally urine is acidic. If the urine is kept exposed to atmosphere, it splits and ammonia gets released and thus the stored urine becomes alkaline.

At optimum pH and temperature urease enzyme decomposes urea into ammonia and carbon dioxide which form ammonium carbonate (an alkaline substance). which changes the slightly acidic solution to alkaline solution. When phenol red is used as indicator in this reaction mixture, the colour of solution changes from yellow to pink.

Requirement: Glasswares: test tubes, Chemicals: 2% Na_2CO_3 solution, 2% acetic acid, sodium hypobromite, sodium hydroxide, 1% acetic acid, urease tablet, phenol red, dilute NaOH solution, 1% CuSO_4 Solution Equipments: test tube holder, test tube stand, spirit lamp.

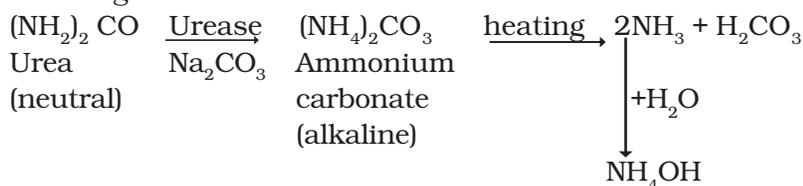
Procedure

(a) Urease test

- Take 2 mL of urine in one test tube and 2 mL of water in the other.
- Add a drop of phenol red indicator to each tube.
- Add 2% Na_2CO_3 solution drop by drop till the pink colour develops in both test tubes (just alkaline).
- Now add 2% acetic acid to each test tube drop by drop till the pink colour disappears (just acidic).
- Add a pinch of soybean powder (contains the enzyme urease) or a pinch of urease enzyme powder to each test tube and rotate the tubes between the palms or warm both the tubes to about 60°C . Overheating should be avoided to prevent denaturation of enzyme.
- The pink colour appears in the tube containing urine but not in the other tube containing water.

Discussion

The enzyme urease acting on urea releases ammonia as shown in the following reaction:



This test is a specific test for urea because the enzyme urease shows its specificity for the substrate urea. The optimum pH (just acidic) and temperature (60°C) must be maintained for the activity of the enzyme urease. Urea is formed in the liver from ammonia and carbon dioxide. Ammonia is the product of deamination of amino acids. Therefore, urea excretion in urine is dependent on the amount of protein ingested.

Note: In place of Soyabean powder or urease enzyme, the aqueous extract of *Cajanus cajan* (Arhar) can also be used as a source of urease.

(b) Biuret test

- Place a small amount of urea in a dry test tube and heat it on a low flame. Urea melts with the liberation of ammonia.
- On further heating it solidifies (in case of urine, the urine is heated till it is completely evaporated).
- Cool the tube. Add 3mL of water and shake.
- Add to it 1mL of dilute NaOH and 1 or 2 drops of 1% CuSO₄ solution. The pink colour develops indicating the presence of urea. Excess drops of CuSO₄ should not be added, otherwise CuSO₄ will form Cu(OH)₂ with NaOH forming a blue colour. This is sometimes mistaken for a positive Biuret test.

Discussion

Urea when heated decomposes with the liberation of ammonia and the formation of biuret. The biuret is dissolved in water and develops a pink/violet colour forming a complex with the alkaline copper sulphate solution.

(c) Sodium hypobromite test

- To the 2 mL of the given sample of urine in a test tube, add 2 drops of alkaline sodium hypobromite solution.
- Brisk effervescence of nitrogen appears in the test tube which indicates presence of urea in the sample.

