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Diploma in Pharmacy 2nd Year

Biochemistry & Clinical Pathology

Experiment

To perform qualitative analysis of carbohydrates.

Aim:

To perform qualitative analysis of carbohydrates.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 1 – 4

Materials Required

Fehling's solution A and B, Molish reagent, phenylhydrazine hydrochloride, beaker, glass rod, measuring cylinder, funnel and test tube.

Theory :

1) **Fehling's Test** : This test serves as the carbohydrate reduction test. The blue alkaline cupric hydroxide solution in Fehling's solution reduces to yellow or red cuprous oxide and precipitates when heated with reducing sugars. OH

As a result, the test solution's ability to precipitate a yellow or brownish-red coloured substance aids in the identification of reducing sugars.

2) **Molisch's Test** : This test is a common test for all carbohydrates greater than tetroses. The test is based on the principle that pentoses and hexoses are dehydrated by concentrated sulphuric acid to yield

furfural and hydroxymethylfurfural, respectively. These products condense into a purple condensation product when combined with α -naphthol.

3) Osazone Test : In this test, Phenylhydrazine combines with the ketoses and aldoses to form a phenylhydrazone, which then reacts with two more molecules of phenylhydrazine to produce osazone.

Lactosazone produces mushroom shape crystals, whereas glucose, fructose, and mannose produce needle-shaped yellow osazone crystals. Different osazones will exhibit crystals in a various shapes. Maltose produces flower- shaped crystals.

Procedure

Common tests which are carried out for identification of carbohydrates are given below:

1) Fehling's Solution Test : In this test, 1-2ml each of the Fehling's solution A and B should be added to few drops of the test solution and boiled for a few minutes. A yellowish-red colour appears that confirms the presence of a reducing sugar.

i) Fehling's Solution A : This reagent should be prepared by dissolving 34.65gm copper sulphate in distilled water and making the volume up to 500ml.

ii) Fehling's Solution B: This reagent should be prepared by dissolving 125gm potassium hydroxide and 173gm Rochelle salt (potassium sodium tartrate) in distilled water and making the volume up to 500ml.

2) Molisch Test : All members of carbohydrates give a positive result for this test. In this test, 1-2 drops of Molisch's reagent (5% of 1-naphthol in alcohol) should be mixed with 1-2ml of test solution. About 1ml of concentrated sulphuric acid should be added along the side of the tube.

A colour develops at the junction of the two liquids due to the reaction between a-naphthol and furfural and/or due to the derivatives formed by dehydration of sugars with concentrated sulphuric acid.

3) Osazone Formation : A sugar should be heated with phenylhydrazine hydrochloride, sodium acetate, and acetic acid to form yellow crystals of osazone.

Result :

Qualitative analysis of Carbohydrates was Performed.

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