Exp.9 Assay of carbohydrate (part I)

Introduction：

A. Molisch test (general test) for carbohydrates:

Molisch test is based on the fact that H_2SO_4 catalyzes the hydrolysis of complex carbohydrates to simple sugars and then dehydrates the sugars to form furfural (from pentoses) or hydroxymethyl furfural (from hexoses). These furfurals then condense with α-naphthol to form colored products.

B. Barfoed's test distinguishing mono-from disaccharides:

Barfoed's test is done under slightly acid rather than alkaline conditions. The acidity makes it a weaker oxidizing reagent, therefore, only monosaccharides may be hydrolyzed sufficiently by the acid, and the resulting monosaccharides will give a positive test. The test serves to distinguish between monosaccharides and disaccharides.

C. Benedict's test for reducing sugar:

Benedict's reagent is a solution containing copper sulfate, sodium carbonate, and sodium citrate. It is very stable and extremely reliable reagent for the examination of urine for pathological amounts of sugar.

Reagents:

1. 1% solutions of carbohydrates:
   xylose, glucose, fructose, maltose, sucrose, starch and glycogen
2. 1% glycine
3. conc. H_2SO_4
4. Molisch reagent: α-naphthol
5. Barfoed's reagent
6. Benedict's solution

Procedure：

A. Molisch test (general test) for carbohydrates:

Add 0.5 ml of the sample solutions into separate test tubes and add 1 drops of Molisch reagent into each tube. Mix well. **Slowly add** ~0.5mL of conc.H_2SO_4 down the side of each tube and **no shaking**, so that the acid forms a layer below the aqueous solution. A **purple-red** ring will appear at the interface between the acid and sample layers. Observe and record.

(NOTE: The test is extremely delicate so it is essential that the substance to be tested is free from traces of filter paper, the lassware is clean, and that the reagents are pure.)

B. Barfoed's test distinguishing mono-from disaccharides:

Add 1 ml of the sample solution into separate test tubes, then add 1 ml of Barfoed's reagent into each tube. Mix the solutions thoroughly. Heat these tubes in boiling water bath. Record the time required for the formation of a red precipitate in the bottom of each

tube.

C. Benedict's test for reducing sugar:

Add 0.5ml of the sample solution into separate test tubes, then add 0.5ml of Benedict's solution into each tube. Heat these tubes in boiling water bath for exactly 2 minutes. Record your results.

Results：

Record your results.

(有無反應請以+/-表示；以及顏色變化有無沉澱，如下 A-(1)範例)

Reagent / Sample		A Molisch Test	B Barfoed's Test	C Benedict's Test
(1)	Xylose	+ (紫環)		
(2)	Glucose			
(3)	Fructose			
(4)	Sucrose			
(5)	Maltose			
(6)	Starch		×	×
(7)	Glycine		×	×
(8)	Unknown			

Discussions：