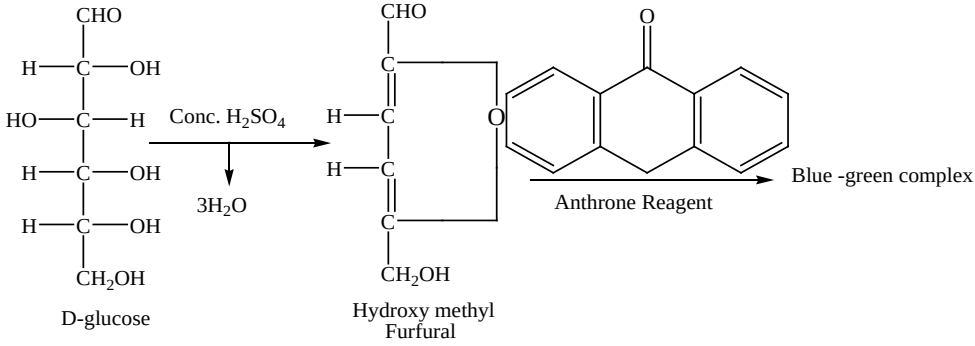
Aim 3

To Estimate the Amount of Glucose in given Sample

Introduction

In concentrated H_2SO_4 carbohydrates undergo dehydration to form furfural or hydroxyl – methyl furfural. Furfural on reaction with anthrone reagent gives a blue green coloured complex which shows maximum absorbance at 620 nm.

Reaction



Reagents

1. Anthrone reagent (0.2 % in Conc. H_2SO_4)
2. Glucose (0.1 g/litre)

Procedure

1 % stock solution of glucose is prepared.

1 gm/100 ml

Or

1000 mg/100 ml

Or

10 mg/1 ml

While 0.1 g/L of glucose concentration is required.

That means 0.1 g/litre

Or

100 mg/1000 ml

Or

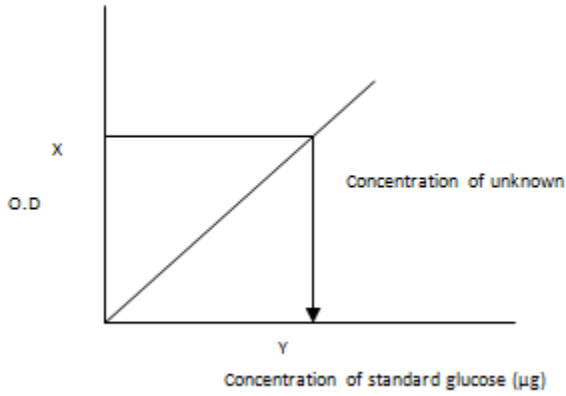
10 mg/100 ml

2. Different volumes of standard glucose solution are pipette out and mix test sample into series of test tubes.
3. 0.5 ml and 1.0 ml of test sample is pipeted out in test tubes.
4. One test tube is taken as blank.
5. Add distilled water in each test tube as given in observation table to make the final volume to 1 ml.
6. 4 ml of anthrone reagent is added in each test tube and mix well.
7. Put the tubes in boiling water bath for 10 minutes.
8. Cool to room temperature.
9. Take a blank tube containing 1 ml distilled water and 4 ml of anthrone reagent and note the O.D. at 620 nm.
10. Draw the standard curve between absorbance (O.D.) and concentration of glucose.
11. Calculate the concentration of the unknown sample from the standard curve.

Observation table

S. No.	Glucose volume (ml)	Glucose Conc. (mg)	Distilled water volume (ml)	Anthrone reagent (ml)	O.D.
Blank	-	0	1.0	4	
1	0.1	10	0.9		
2	0.2	20	0.8		
3	0.3	30	0.7		
4	0.4	40	0.6		
5	0.5	50	0.5		
6	0.6	60	0.4		
7	0.7	70	0.3		
8	0.8	80	0.3		
9	0.9	90	0.1		
10	1.0	100	-		
Unknown	0.5		0.5		
	1.0		-		

Standard curve:



Calculation:

O.D. of taken sample - X

From standard curve

Suppose X, O.D. corresponds to y mg of Glucose

Test sample taken – 0.5 ml

So 0.5 ml of the unknown sample contains y mg of Glucose.

Glucose (mg %) = Y/1.0*100

Alternatively, the concentration can be calculated by the formula.

O.D. test -……

O.D. standard -…….

Glucose (mg %) = (O.D. test/ O.D. standard X concentration of standard / volume of sample in ml)* 100.

Precautions

1. Distilled water should be used.
2. Solutions should be pipette out accurately.
3. Anthrone reagent should be stirred well.
4. The sample should be weighed properly and accurately.
5. Test tubes should be covered with aluminum foil before keeping them in water bath.