BT 210 Biochemistry Lab

Estimation of carbohydrate by the Anthrone method

Theory/Principle: Carbohydrates are dehydrated by conc.H_2SO_4 to form furfural. Active form of the reagent is anthranol, the enol tautomer of anthrone, which reacts by condensing with the carbohydrate furfural derivative to give a green colour in dilute and a blue colour in concentrated solutions, which is determined colorimetrically. The blue - green solution shows absorption maximum at 620 nm.

Reaction:

(i) **Hydrolysis** to monosaccharides

Disaccharide ⟶ Monosaccharide

(ii) **Dehydration**---product is a furfural

Monosaccharide ⟶ Furfural

(iii) **Reaction** of furfural with anthrone

Furfural + Anthrone reagent ⟶ Blue green complex

Methodology:

(a) Materials required:

(i) Equipments:

- UV Spectrophotometer
- Vortex mixer
- Mantle heater

(ii) Chemicals/Reagents:

- Anthrone Reagent
- Glucose
- Other carbohydrates if desired

(iii) Glass wares and others:

- Test tube, Test tube stand, Pipettes,Beaker,Ice
  Test tube caps or marbles, Tissue paper, Wash bottle.

(b) Reagents:

(i) **Anthrone reagent:** Dissolve 2g of Anthrone in 1 litre of concentrated H_2SO_4. Use freshly prepared reagent for the assay

(ii) **Glucose stock solution:** 200μg glucose per mL distilled water.

Note: Can include other carbohydrates of the same concentration if desired.

(c) Procedure:

1. Pipette out into a series of test tubes different volumes of glucose solution (follow up **Table 1**) from the supplied stock solution(200μg /ml) and make up the volume to 1 mL with distilled water.

2. Consider tube 1 as blank and tubes 2 through 9 for construction of a standard curve. Tubes 10-11 are for the unknown samples.
3. To each tube add 5 mL of the anthrone reagent (supplied) and mix well by vortexing.
4. Cool the tubes.
5. Cover the tubes with marbles on top and incubate at 90^o C for 17 minutes or boiling water bath for 10 minutes.
6. Cool to room temperature and measure the optical density at 620 nm against a blank.
7. Prepare a standard curve of absorbance vs. μg glucose.

Table 1

Sl. No.	Glucose		DH_2O (μL)	Anthrone reagent (mL)	A_{620}
	(μL)	(μg)			
1.	-	-	1000	5	
2.	50	10	950	5	
3.	100	20	900	5	
4.	200	40	800	5	
5.	300	60	700	5	
6.	400	80	600	5	
7.	500	100	500	5	
8.	750	150	250	5	
9.	1000	200	-	5	
10.	Unknown (A)	-	-	5	
11.	Unknown (B)	-	-	5	

(iv) **Calculation:** Determine the slope (y/x) from the standard curve, which will give the A_{620} per unit of glucose (μg). Hence, determine the amount of glucose in the unknown sample.

References:

1. E.E.Layne, (1975) *Methods in Enzymology*,**3:447**
2. David T. Plummer (1990) *An Introduction to Practical Biochemistry*,179 Third Edition