Experiment No. 10: Estimation of Haemolymph Glucose level.

Aim: To estimate haemolymph glucose level by DNS method.

Principle: Several reagents have been employed which assay sugars by using their reducing properties. One such compound is 3, 5- dinitrosalicylic acid (DNS) which in alkaline solution is reduced to 3-amino 5-nitrosalicylic acid.

Reagents Required:

1. **Sodium potassium tartarate:** Dissolve 300 gm of this salt in about 500 ml of water.
2. **3, 5-dinitrosalicylic acid:** dissolve 10 gm of this powder in 200 ml of 2 mol/lt sodium hydroxide.
3. **Dinitrosalicylic acid reagent:** Prepare this fresh by mixing solutions 1 and 2 and make up to 1 liter with water.
4. **Sodium hydroxide:** 2 mol/liter
5. **Stock glucose standard:** 1gm/liter solution in saturated benzoic acid.
6. **Working sugar standard:** Dilute stock standard solution in 1:1 ratio with distilled water to get 500 µg/ml glucose.

Preparation of protein free filtrate: To 1 ml haemolymph sample, add 8 ml distilled water, 0.5 ml of 2/3 N sulfuric acid and 0.5 ml of 10% sodium tungstate solution in a stoppered centrifuge tube and mix the contents. Then centrifuge at 3000 rpm for 10 min and collect the supernatant as sample.

Procedure:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
2. Pipette out 1 ml of the given haemolymph sample (protein free filtrate) in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
4. Now add 0.5 ml of DNS to all the test tubes including the test tubes labeled 'blank' and 'sample'.

5. Mix the contents of the tubes by vortexing / shaking the tubes and incubate for 10 min in a boiling water bath and cool to room temperature.
6. Then to the cooled test tubes add 2.5 ml of distilled water mix the contents and record the absorbance at 540 nm against blank.
7. Plot the standard curve by taking concentration of glucose along X-axis and absorbance at 540 nm along Y-axis. *For drawing the standard graph please refer last page figure 1.*

Now from this standard curve calculate the concentration of glucose in the given sample.

Observations and Calculations

Volume of standard glucose (500 µg/ml)	Volume of distilled water (ml)	Concentration of glucose (µg)	Volume of DNS reagent (ml)	Incubate for 10 min in a boiling water bath and cool	Volume of distilled water (ml)	A_{540}
0.0	1.0	000	0.5		2.5	0.00
0.2	0.8	100	0.5		2.5	
0.4	0.6	200	0.5		2.5	
0.6	0.4	300	0.5		2.5	
0.8	0.2	400	0.5		2.5	
1.0	0.0	500	0.5		2.5	
1.0 sample	0.0	To be estimated	0.5		2.5	

Result: The given haemolymph sample contains ----µg glucose/ml.
