

## **Experiment No 14. Estimation of Glycogen in Silkworm Fat body/Tissue.**

**Aim:** To estimate the amount of glycogen.

**Principle:** Glycogen is released from the tissue by heating with strong alkali and precipitated by the addition of ethanol. Sodium sulphate is added as co-precipitant to give a quantitative yield of glycogen.

Simple sugars, oligosaccharides, polysaccharides, and their derivatives, give an orange yellow color when treated with phenol and concentrated sulfuric acid, which can be read at 490 nm.

### **Requirements:**

1. **30% KOH.**
2. **Ethyl alcohol:** 95% ethanol
3. **2 N H<sub>2</sub>SO<sub>4</sub>**
4. **Glycogen Standard:** 100µg/ml in double distilled water.
5. **Phenol:** 5% solution.
6. **H<sub>2</sub>SO<sub>4</sub>:** 96-98%.

### **Procedure:**

#### **Isolation of Glycogen:**

1. Place 1 g of freshly collected silkworm tissue into a calibrated stoppered centrifuge tube containing 2 ml of 30% KOH and heat in a boiling water bath for 20 min with occasional shaking.
2. Cool the tubes in ice, add 0.2 ml of saturated Na<sub>2</sub>SO<sub>4</sub>, and mix thoroughly.
3. Precipitate the glycogen by adding 5 ml of 95% ethanol, stand on ice for 5 min, and centrifuge.
4. Discard the supernatant and dissolve the precipitated glycogen in about 5 ml of water with gentle warming, then dilute with distilled water to the 10 ml calibration mark and mix thoroughly. One ml of this sample is equivalent to 100 mg of tissue.

#### **Estimation of glycogen:**

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of glycogen standard in to the series of labeled test tubes.
2. Pipette out 1 ml of isolated glycogen 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 1 ml of phenol. Then rapidly pipette out 5 ml of H<sub>2</sub>SO<sub>4</sub> to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
5. Mix the contents and incubate on ice bath for 30 min.
6. Then record the absorbance at 490 nm against blank.
7. Then plot the standard curve by taking concentration of glycogen standard along X-axis and absorbance at 490 nm along Y-axis.
8. Then from this standard curve calculate the concentration of glycogen in the given test sample.

**Observations and Calculations:**

Volume of standard glycogen (ml)	Volume of distilled water (ml)	Concentration of glycogen (ml)	Volume of phenol (ml)	Volume of H <sub>2</sub> SO <sub>4</sub>	Incubate on Ice bath for 30 min	A <sub>490</sub>
0.0	1.0	00	1	5		0.00
0.2	0.8	20	1	5		
0.4	0.6	40	1	5		
0.6	0.4	60	1	5		
0.8	0.2	80	1	5		
1.0	0.0	100	1	5		
1.0 ml sample	0.0	To be estimated	1	5		

$$\begin{aligned} \text{Amount of glycogen silkworm litter} &= \frac{\text{Standard Curve Value}}{\text{Silkworm Tissue taken (mg)}} \\ &= \text{-----}\mu\text{g of glycogen/mg tissue} \end{aligned}$$

**Result:** The given unknown sample contains ---- $\mu\text{g}$  glycogen /mg tissue.

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