Experiment No 13. Estimation of Succinate Dehydrogenase activity in the eggs/ tissue.

Aim: To estimate succinate dehydrogenase activity in silkworm eggs.

Principle: Succinate dehydrogenase is one of the mitochondrial enzymes, which catalyzes the conversion of succinate to fumerate. In this reaction FAD reduces to $FADH_2$. In *in vitro*, the lemon yellow colored INT accepts electrons and becomes red coloured farmazan which can be measured at 495 nm.

Reagents Required:

1. **Sodium phosphate buffer (0.1M, pH 7.4):** Mix 16 ml (0.2 M) of monobasic and 84 ml (0.2 M) of dibasic and makeup to 200 ml with distilled water.
2. **INT** [2(4-iodophenyl)-3(4-nitrophenyl)-5-Phenyltetrazolium chloride]: 1mg/ml in distilled water.
3. **Sodium succinate:** 15mM
4. **Glacial acetic acid, Toluene *etc*.,**
5. **Sample:** Prepare 0.2% egg (5 days old or more) homogenate in cold phosphate buffer/ distilled water using mortar and pestle. Centrifuge the homogenate at 3000 rpm for 10 min, collect the clear supernatant and use as sample.

Procedure for Standard Curve:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of INT in to the series of labeled test tubes.
2. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
3. Now add 1 ml of buffer, 1 ml of sodium succinate and 1 ml of sample (egg homogenate) to all the test tubes including the test tubes labeled 'blank' and 'test'.
4. Mix the contents of the tubes by vortexing / shaking the tubes and incubate at 37°C for 24 h or until complete reduction of INT.
5. Now add 6 ml of glacial acetic acid to stop the reaction.
6. Then add 6 ml of toluene, mix and keep them in a refrigerator for separation of toluene layer.
7. Now collect the upper red coloured toluene layer containing farmazan in to a tube, cool to room temperature and record the absorbance at 495 nm against blank.

8. Then plot the standard curve by taking concentration of farmazan (equivalent to INT) along X-axis and absorbance at 495 nm along Y-axis.

For drawing the standard graph please refer last page figure 1.

Procedure for Test:

1. **Blank:** Take 1 ml of phosphate buffer, 1 ml of sodium succinate, 1 ml of INT and 1 ml of inactivated enzyme sample (distilled water may be used) in a clean dry test tube, and incubate at 37°C for one hour.
2. **Test:** Take 1 ml of phosphate buffer, 1 ml of sodium succinate, 1 ml of INT and 1 ml of enzyme sample in a clean dry test tube, and incubate at 37°C for one hour.

After incubation add 6 ml of glacial acetic acid to both blank as well as test to stop the reaction. Then add 6 ml of toluene to each tube, mix and keep them in a refrigerator to separate the red farmazan. After separation, collect the upper red coloured toluene layer containing farmazan in to a cuvette and record the absorbance at 495 nm against blank.

Observations and Calculations:

Standard Curve:

INT (ml)	H_2O (ml)	Buffer (ml)	Succinate (ml)	Sample (ml)	Mix, incubate at 37ºC for 24 h.	Acetic acid (ml)	Toluene (ml)	Mix & keep in a fridge for 24 h. Then collect the upper layer	A_{495}
0.0	1.0	1	1	1		6	6		
0.2	0.8	1	1	1		6	6		
0.4	0.6	1	1	1		6	6		
0.6	0.4	1	1	1		6	6		
0.8	0.2	1	1	1		6	6		
1.0	0.0	1	1	1		6	6		

Test:

Ex.	INT (ml)	Buffer (ml)	Succinate (ml)	Sample (ml)	Mix & incubate at 37ºC for 1hr	Acetic acid (ml)	Toluene (ml)	Collect the farmazan as above	A_{495}
B	1	1	1	1		6	6		0.00
T	1	1	1	1		6	6		

B - Blank

T - Test

Optical density of the test: ---

SDH activity level = $\frac{\text{Standard Curve Value X 60}}{\text{Tissue taken (mg) X Incubation time (min)}}$

= ----- µg of farmazan formed per hour per mg at 37°C.

Report: The SDH activity in the given sample is ----- µg of farmazan formed per hour per mg tissue at 37°C.
