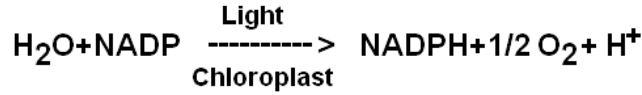


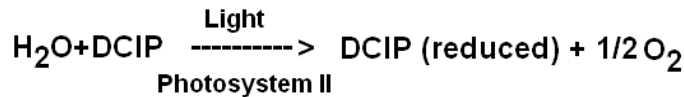
Ex. No. 8: Hill Reaction.

Aim: To study the Hill reaction.

Principle: One of the first steps of photosynthesis is the photolysis of water to donate two electrons to the reaction center, P680. This reaction is known as the Hill reaction. The electrons donated to P680 move through an electron transport chain to the reaction center P700, and eventually, to reduce NADP to NADPH. The complete reaction can be summarized as follows:



Other electron acceptors can be substituted for NADP, which allow the Hill reaction to be measured, and some of its components studied. The most commonly used electron acceptor is the dye 2,6 dichlorophenol indophenol (DCIP), which can accept electrons instead of P700. When DCIP is added to a chloroplast or thylakoid suspension, the following reaction occurs:



DCIP is a blue color in its oxidized form. When reduced, it is colorless. This property of DCIP allows the measurement of the rate of the Hill reaction.

Reagents Required:

1. **Potassium phosphate buffer:** 125 mM pH 7.5 with 0.3 M sucrose.
2. **DCIP:** 30 µg/ml DCIP in distilled water.

Procedure: Prepare 25% homogenate of fresh Spinach leaves in potassium phosphate buffer with 0.3M sucrose, filter and centrifuge the filtrate at 3000 rpm for 5 min. Re-suspend the chloroplast pellet in 10 ml of buffer and store on ice. In a test tube take 1.9 ml water, 3 ml buffer, 0.5 ml chloroplast suspension and 0.1 ml DCIP, mix and incubate in sunlight for 1 hour. A suitable control should also be prepared and incubate in dark. Prepare a standard curve using different concentrations of DCIP on X-axis against absorption at 550 nm on Y-axis.

For drawing Standard Graph please refer figure 1.

Then from this standard curve calculate the amount of DCIP reduced.

Standard Curve:

Sl. No.	Water	DCIP	Concentration of DCIP (µg)	A ₅₅₀
1	5.5	0.0	0	0.00
2	5.3	0.2	6	
3	5.1	0.4	12	
4	4.9	0.6	18	
5	4.7	0.8	24	
6	4.5	1.0	30	

Test:

Serial Number	Water	Buffer	Chloroplasts	DCIP	A ₅₅₀ against water
T-1 Incubated in dark	1.9	3	0.5	0.1	
T-2 Incubated in sunlight	1.9	3	0.5	0.1	

Calculation:

Amount of DCIP reduced = O D of T₁ – O D of T₂ =

Report: The given sample showed ----- µmole of DCIP reduced per hour.
