

EXPERIMENT NO: 10

DETERMINATION OF CHLOROPHYLL CONTENT OF GIVEN LEAF SAMPLE AND ESTIMATION OF ABSORPTION SPECTRUM OF CHLOROPHYLL

Principle:

Chlorophyll is a green pigment present in plant leaves that plays a critical role in photosynthesis. Chlorophyll extraction involves grinding the plant material to release the chlorophyll from the cell walls, and then using an organic solvent such as acetone to extract the pigments. The pigments can be separated and quantified by measuring their absorbance at specific wavelengths using a spectrophotometer.

Absorption spectrum of chlorophyll is an important technique used to understand the light absorption properties of chlorophyll.

Materials required:

Fresh plant tissue (leaves or stems)

Acetone (80% v/v)

Mortar and pestle

Volumetric flask(25 ml)

Whatmann's No-1 filter paper

Spectrophotometer

Quartz cuvettes

Extraction Procedure:

Collect fresh plant tissue and rinse with distilled water to remove any debris or contaminants. Grind the plant tissue using a mortar and pestle to obtain a homogenous sample. Add approximately 10 ml of 80% acetone to the sample, and grind the mixture until the tissue is completely macerated. Filter the content into a 25 ml volumetric flask using Whatmann's No-1 filter paper. Rinse the mortar and

pestle with additional 80% acetone, and transfer the rinse to the same volumetric flask and make up the volume to 25 ml.

Procedure for determination of chlorophyll content

Use a blank solution of 80% acetone to calibrate the spectrophotometer and zero the readings. Measure the absorbance of the extract at two wavelengths - 660 nm and 645 nm to calculate chlorophyll a and b content.

Calculation

Calculate the chlorophyll content using the following formulas

$$\text{Chl. a (mg/g)} = \frac{(12.7 * A_{660} - 2.69 * A_{645}) * V}{1000 * W}$$

$$\text{Chl. b (mg/g)} = \frac{(22.9 * A_{645} - 4.68 * A_{660}) * V}{1000 * W}$$

$$\text{Total Chlorophyll (mg/g)} = \text{Chl. a} + \text{Chl. b}$$

Here

A₆₆₀- Absorbance at 660 nm

A₆₄₅- Absorbance at 645 nm

V- Final volume of extract in ml

W- Weight of sample taken in gm

Procedure for estimation of Absorption Spectrum of Chlorophyll

Use a blank solution of the solvent to calibrate the spectrophotometer and zero the readings. Measure the absorbance of the extract at different wavelengths in the visible spectrum (400-700 nm) in 20 nm interval. Plot the absorbance readings against the corresponding wavelengths to obtain the absorption spectrum of chlorophyll.

Conclusion