334 MBIO
Biochemical Instrumentation Techniques

- Lab 4 -

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Spectrophotometer

What is a Spectrophotometer?

- **A spectrophotometer,** one of the most useful lab equipment, is a combination of two devices:

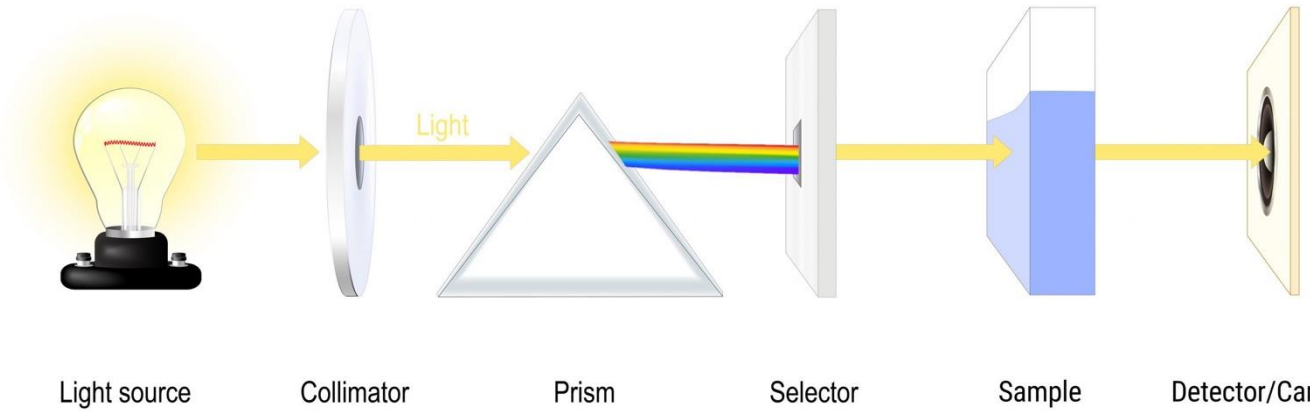
A Spectrometer ⟶ used for producing light of any selected wavelength or colour.

A Photometer ⟶ used for measuring the intensity of light.

Definition

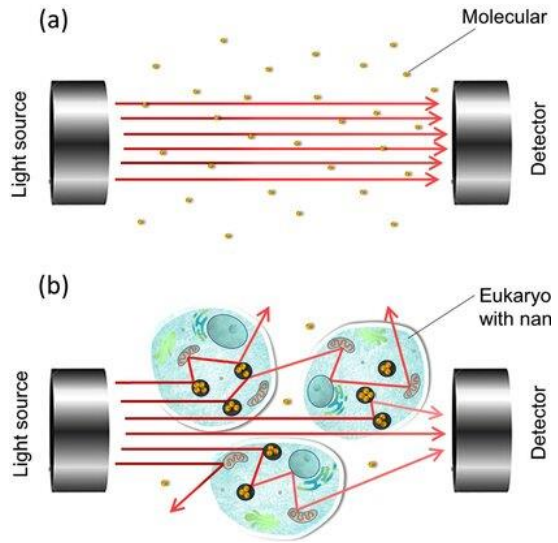
- A spectrophotometer is a spectroscopic instrument that resolves polychromatic radiations into different wavelengths.

- It measures the light intensity at several wavelengths.



Principle of a Spectrophotometer

- Spectrophotometer techniques are used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer.



- It is designed for the measurement of:

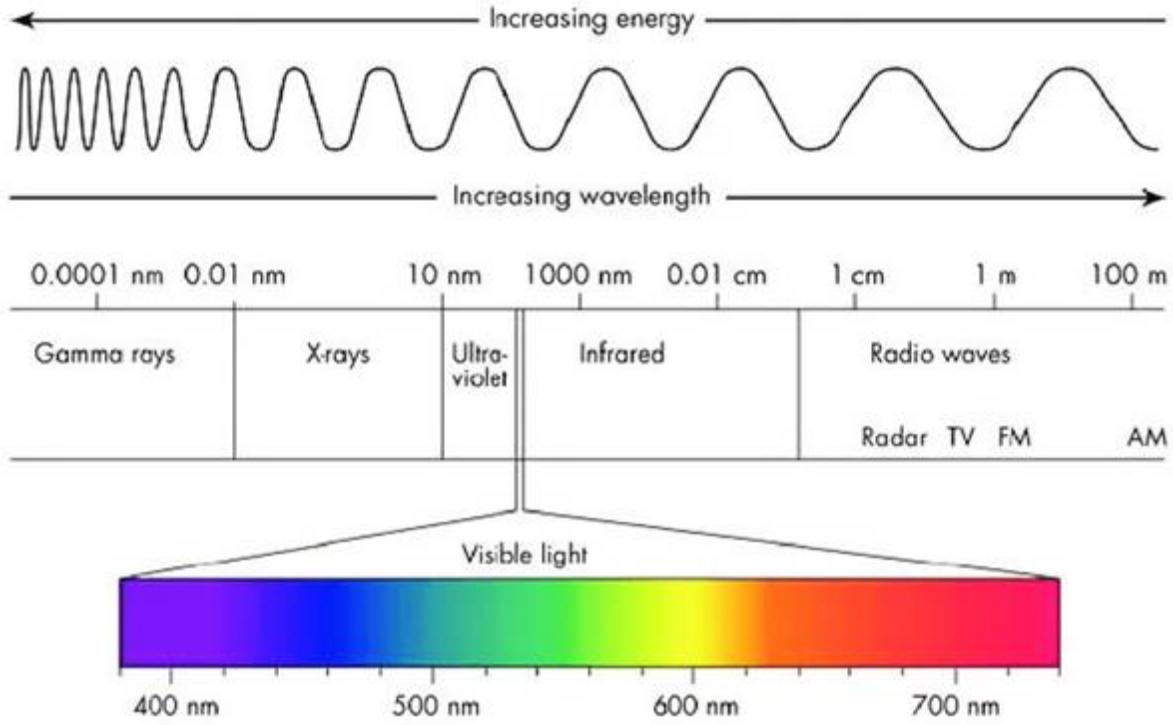
ultraviolet, visible, and infrared rays.

Principle of a Spectrophotometer

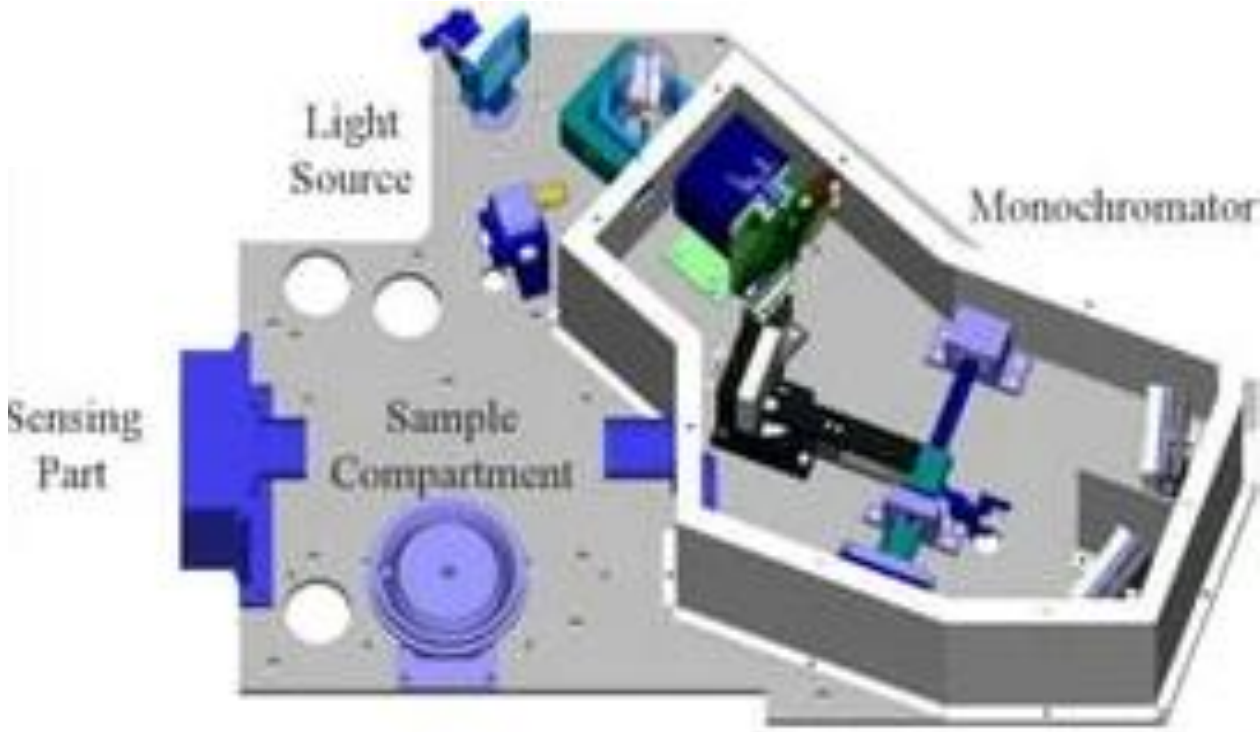
- The interaction of light (electromagnetic radiation EMR) with matter depends on the energy of the light.
- When a light source illuminates a sample, it excites its components—nucleus, electrons, and bond pairs—based on the provided energy.

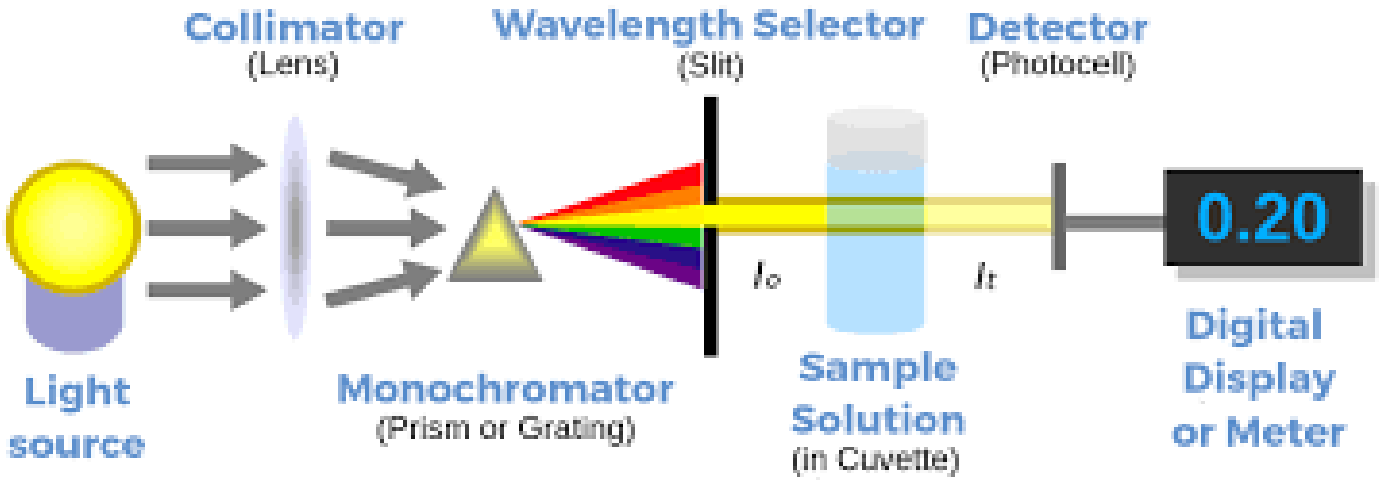
Principle of a Spectrophotometer

- Different wavelengths produce different effects:
  - Radio waves (low energy) resonate with nuclear energy.
  - UV-visible light excites valence electrons.



Components of Spectrophotometer

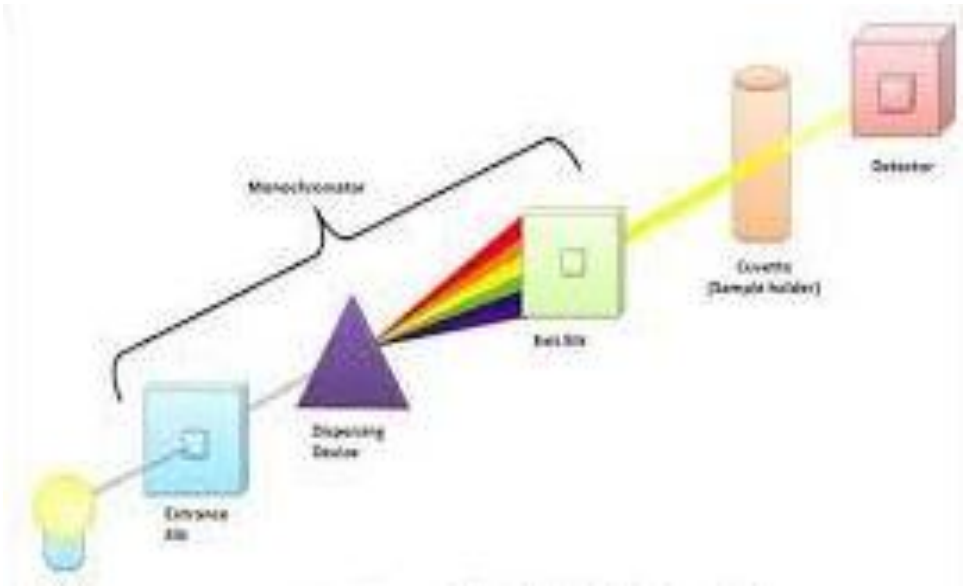




1. **Light Source:** to provide a sufficient of light which is suitable for marking a measurement.

There are three type of lamp:

- Tungsten Lamp
- Hydrogen Lamp
- Xenon Lamp



- Tungsten Lamp is the most common light source used in spectrophotometer wavelength , range of about 330 to 900 nm

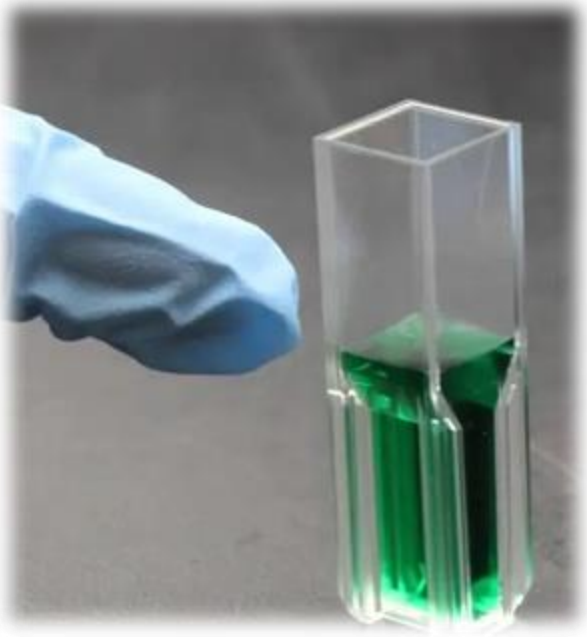
2. **Dispersion devices:** Monochromator accepts polychromatic input light from a lamp and outputs monochromatic light.

- Dispersion devices causes a different wavelength of light to be dispersion at different angles. **There are two types of Dispersion:**

Prism ⟶ used to isolate different wavelength.

Filters ⟶ separate different parts of the electromagnetic spectrum by absorbing or reflecting certain wavelengths and transmitting other wavelengths.

3. **Absorption cells (Cuvettes):** A cuvette is a kind of cell (usually a small square tube) sealed at one end, made of plastic, glass or optical grade quartz.



- It designed to hold samples for spectroscopic experiments.

4. **Detectors:** Any photosensitive device can be used as a detector of radiant energy.

- The photocell and phototube are the simplest photo detectors, producing current proportional to the intensity of the light striking them.

5. **Display devices:** The data from a detector are displayed by a readout device, such as an analogue meter, a light beam reflected on a scale, or a digital display , or liquid crystal display (LCD) .

- The output can also be transmitted to a computer.

The differences between the Spectrometer and Spectrophotometer

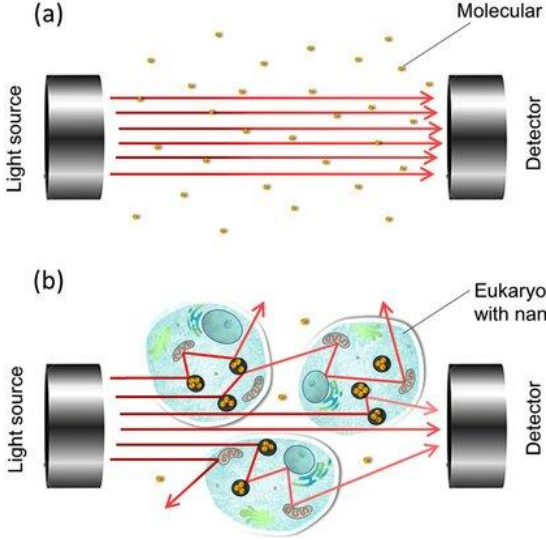
Spectrometer

A component of a spectrophotometer, that detect the range of wavelengths

Spectrophotometer

A device to analyse the sample analyte and qualifies the basis of absorption and emission of characteristic wavelengths.

How it Works



1. A sample solution is placed inside the spectrophotometer.

2. A light source shines light toward the sample.

3. A monochromator splits the light into each colour, or rather, individual wavelengths.

4. The wavelength of light hits the sample, which is held in the cuvette.

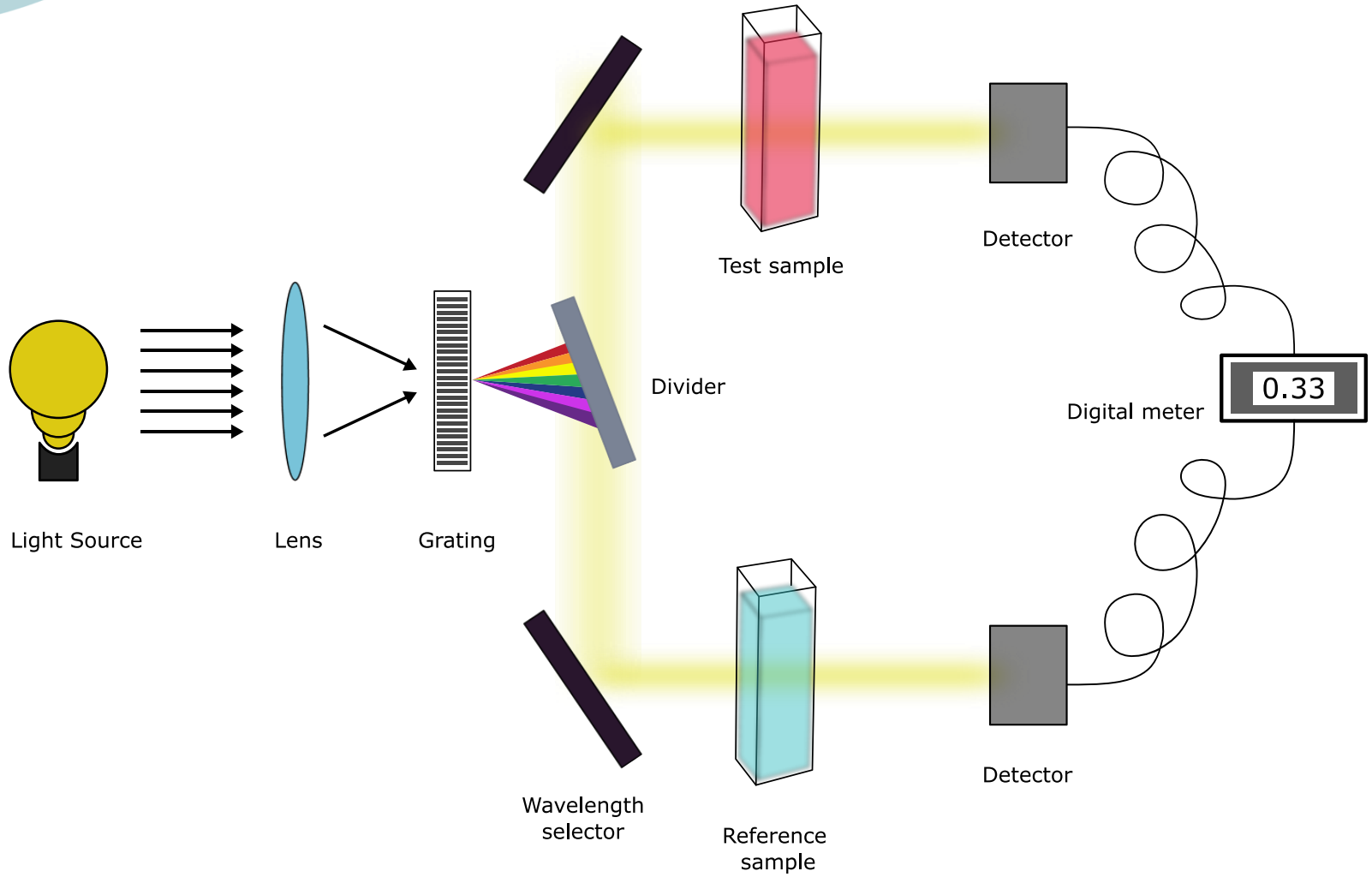
5. Whatever light passes through the sample is read and displayed on the output screen

Types of Spectrophotometer

- Spectrophotometry is classified based on the use of light sources and the type of interaction studied.

1. Single and double UV-Visible spectrophotometer
2. Nanodrop spectrophotometer
3. IR spectrophotometer
4. Atomic Absorption Spectrophotometer (AAS)
5. Fluorescence spectrophotometer
6. NMR spectrophotometer

1. Double Beam UV-Visible Spectrophotometer

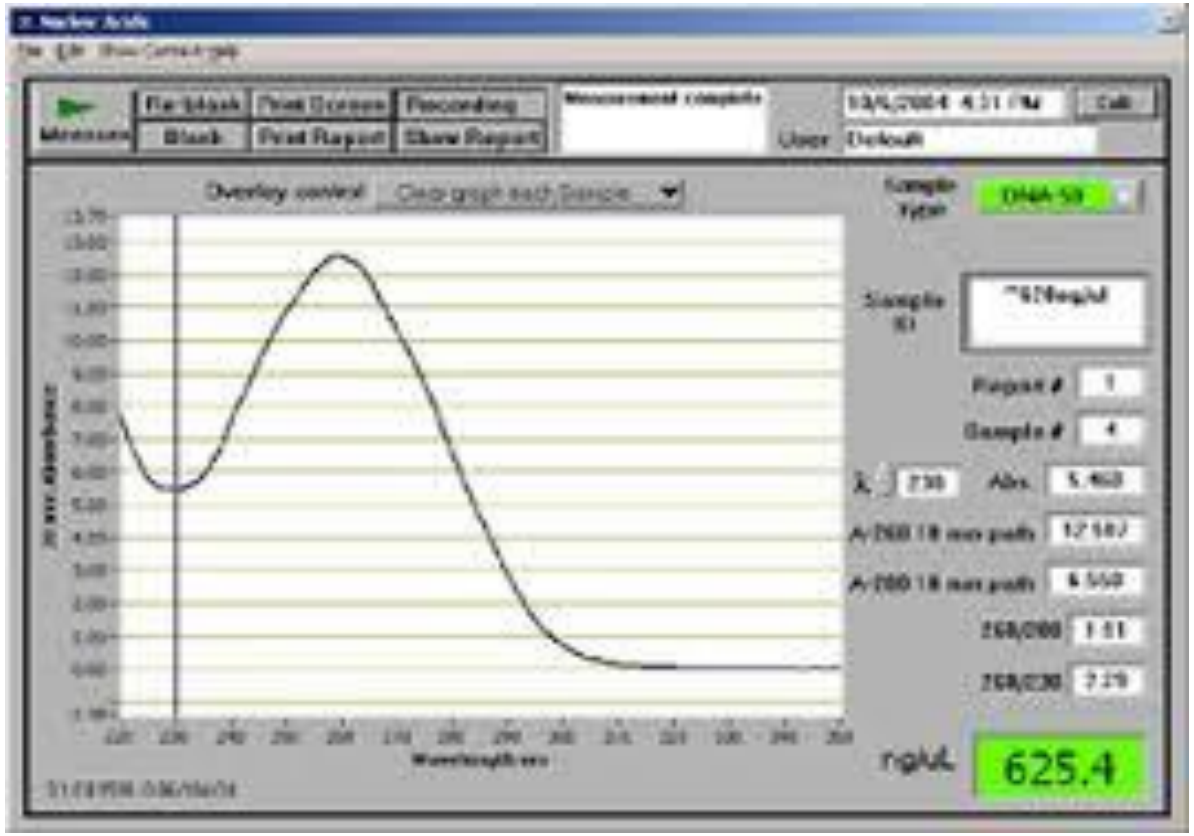


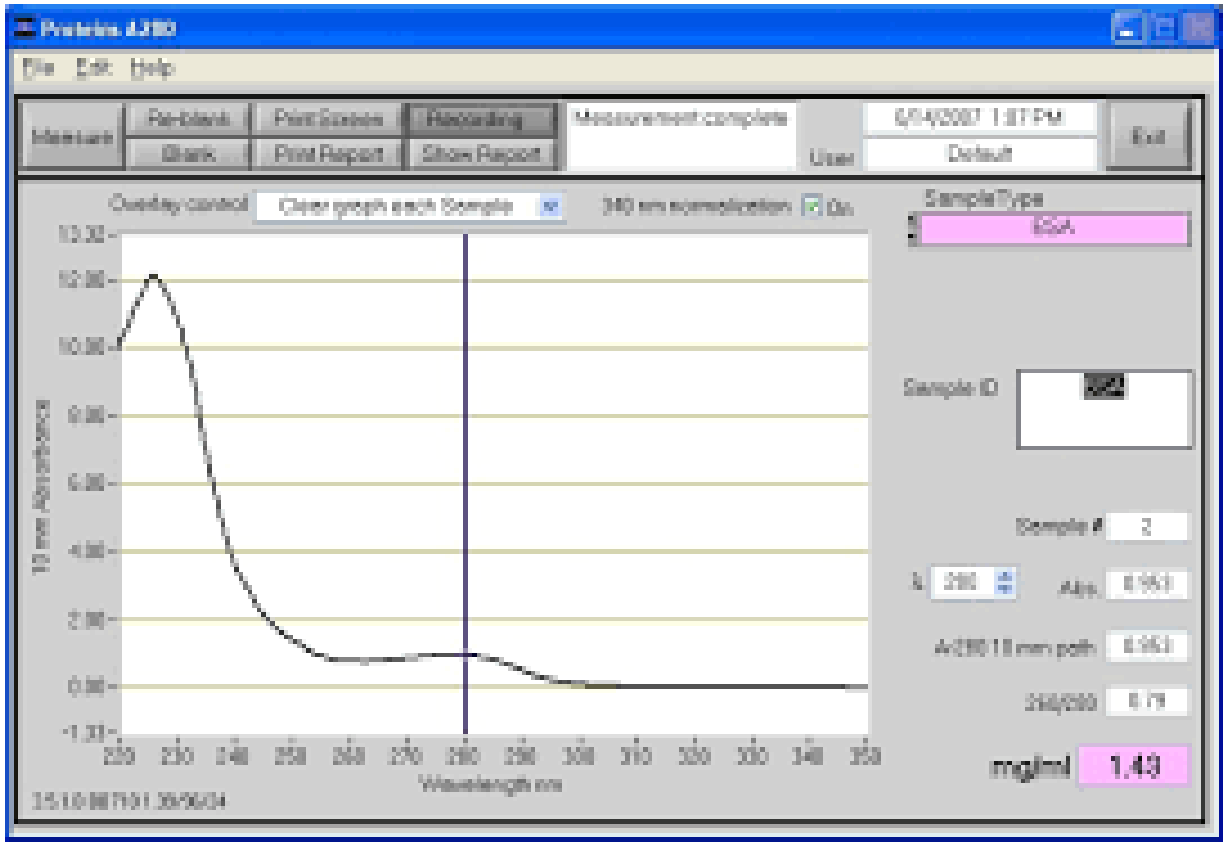
2. Nanodrop spectrophotometer

- Is a specialised instrument used to measure the concentration and purity of nucleic acids (DNA, RNA), proteins, and other biomolecules.

- It requires very small sample volumes (as low as 1–2 µL).
- It works by using UV-Vis spectrophotometry, analysing absorbance at specific wavelengths (e.g., **260 nm for nucleic acids**, **280 nm for proteins**)

2. Nanodrop spectrophotometer

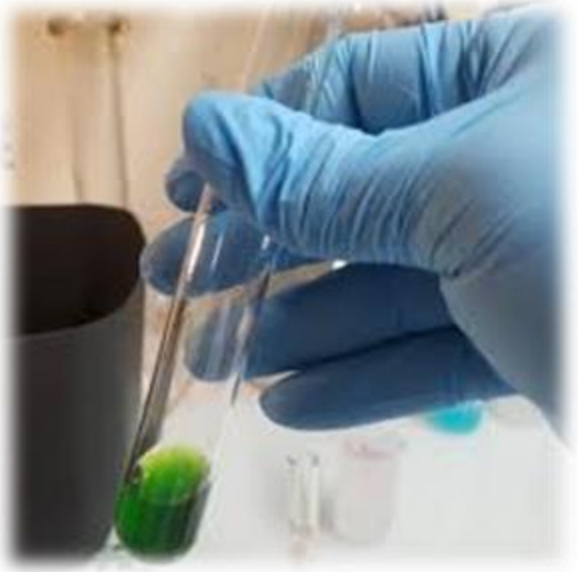




Experiment 1:
Assessment of Chlorophyll Concentration in Plants

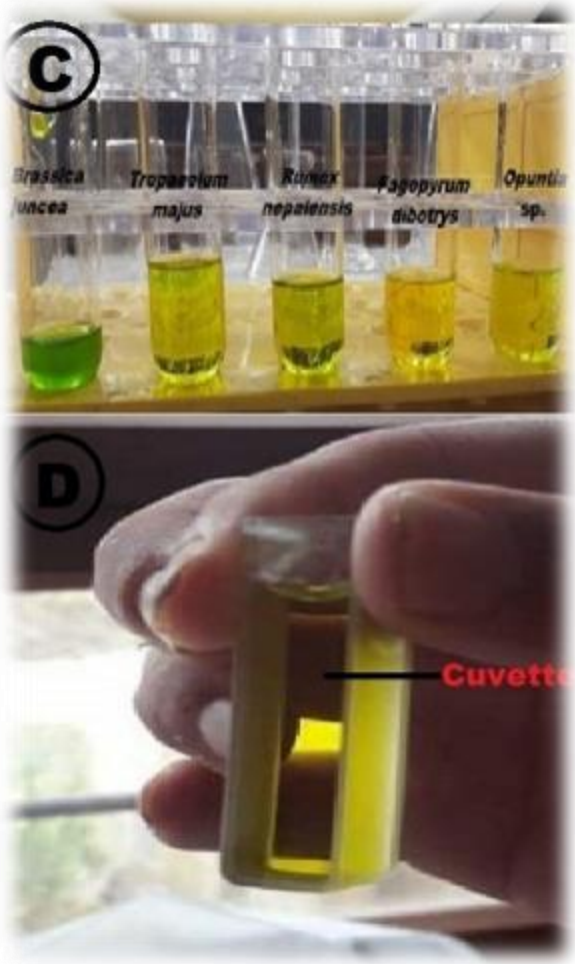
Method

- **Sample Preparation:** Weigh 3g of fresh leaf tissue and immerse in 30 mL of 80% acetone in a sealed container.
- **Incubation:** Let the sample sit for 15 minutes, shaking every 5 minutes.
- **Filtration & Centrifugation:** Transfer the supernatant to a fresh tube and centrifuge and transfer the supernatant to a new tube.



Measuring the Optical Density (O.D)

- Use a spectrophotometer to measure absorbance at:
  - 663 nm ⟶ Chlorophyll A.
  - 645 nm ⟶ Chlorophyll B.



Calculation Activity

- Assess the Chlorophyll concentration knowing that:

- **Chlorophyll A (mg/g)** = $(13.95 \times O.D_{663}) - (6.88 \times O.D_{645}) \times V / W \times 1000$
- **Chlorophyll B (mg/g)** = $(24.96 \times O.D_{645}) - (7.32 \times O.D_{663}) \times V / W \times 1000$

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