



SPECTROPHOTOMETRY

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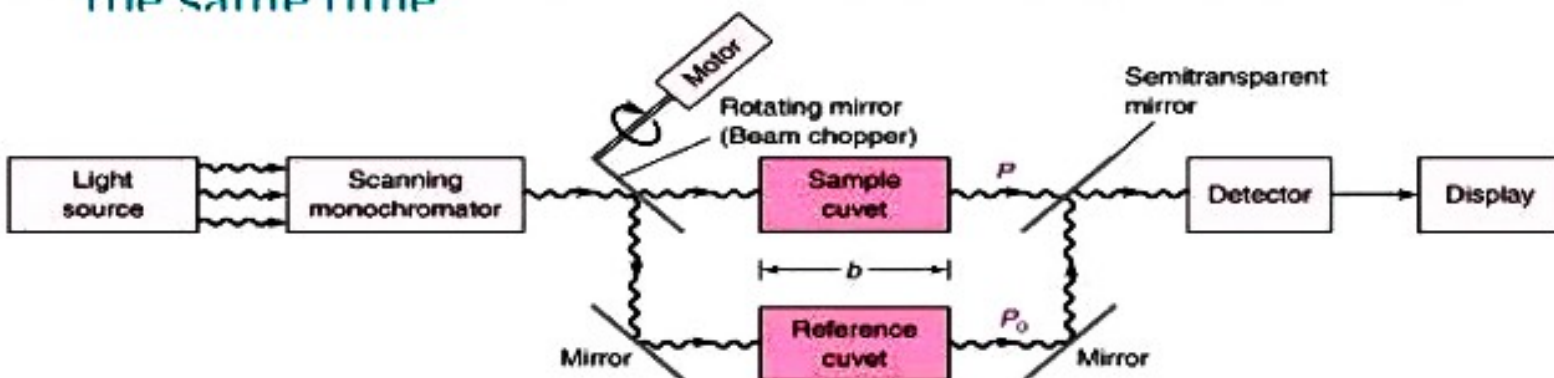
SPECTRPHOTOMETRY

- A method in which the absorption or transmission properties of a material is quantitatively measured as a function of wavelength.
- The basic principle behind this method is that :
“Each compound absorbs or transmits light over a certain range of wavelength”

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- A spectrophotometer is an instrument that measures the amount of photons absorbed by a sample after it is passed through its solution.
 - With the spectrophotometer , the amount of a known chemical substance can be determined by measuring the absorbance.

- **DOUBLE BEAM SPECTROMETER:**

In this type, before it reaches the sample, the light source is split into two separate beams. From these one passes through the sample and second one is used for reference. This gives an advantage because the reference reading and sample reading can take place at the same time





Based on the wavelength of light used it can be classified into :

- **VISIBLE SPECTROMETER**

Uses visible range (400 – 700nm) of electromagnetic radiation spectrum.

Visible spectrophotometers vary in accuracy. Plastic and glass cuvettes can be used for visible light spectroscopy.

- **UV SPECTROMETER**

Uses light over the UV range (180 - 400 nm).

UV spectroscopy is used for fluids, and even solids. Cuvettes, only made of quartz, are used for placing the samples.

- **IR SPECTROPHOTOMETER**

Uses light over infra red range (700 -15000) of electromagnetic radiation spectra.



DEVICE AND MECHANISM

- The spectrophotometer, in general, consists of two devices. They are the following :

1. SPECTROMETER :

A device that produce, typically disperse and measure the light.

2. PHOTOMETER :

Indicates the photoelectric dictator that measures the light.



- The spectrometer consists of the following parts :

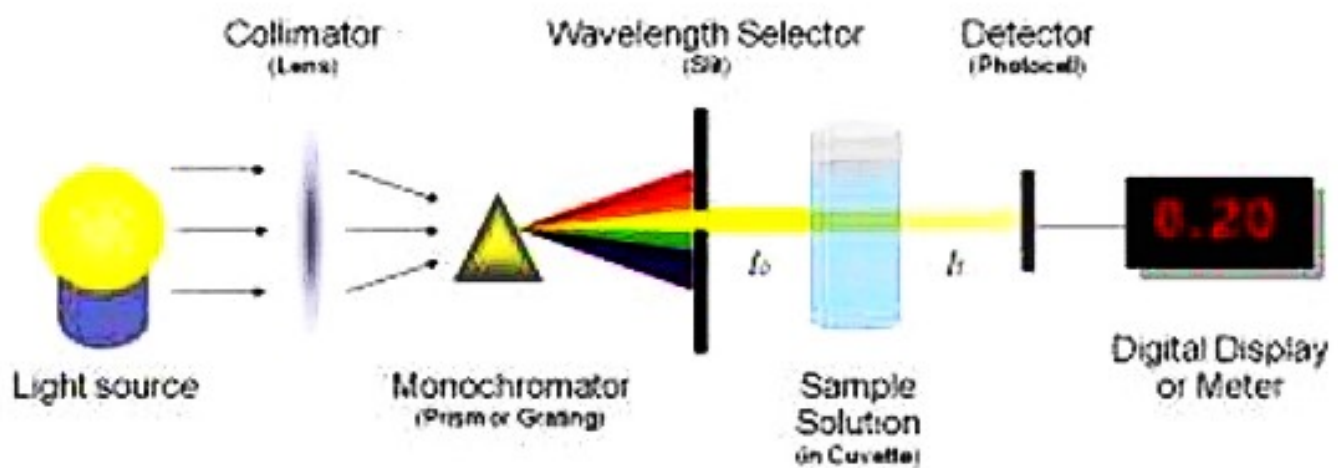
1. Light source : it produce a desired range of wavelength of light.

2. Collimator : transmits a straight beam of light.

3. Monochromator : split the light into its component wavelength.

4. Wavelength selector : transmits only the desired wavelength.

- The photometer detects the light absorbed by the sample as the light from the slit is passed through the solution. And then it sends signal to the galvanometer or digital display.





BEER – LAMBERT LAW

It states that the absorbance of light by a material in a solution is directly proportional to its concentration in that solution.

$$A = \epsilon lc$$

Where,

A - absorbance

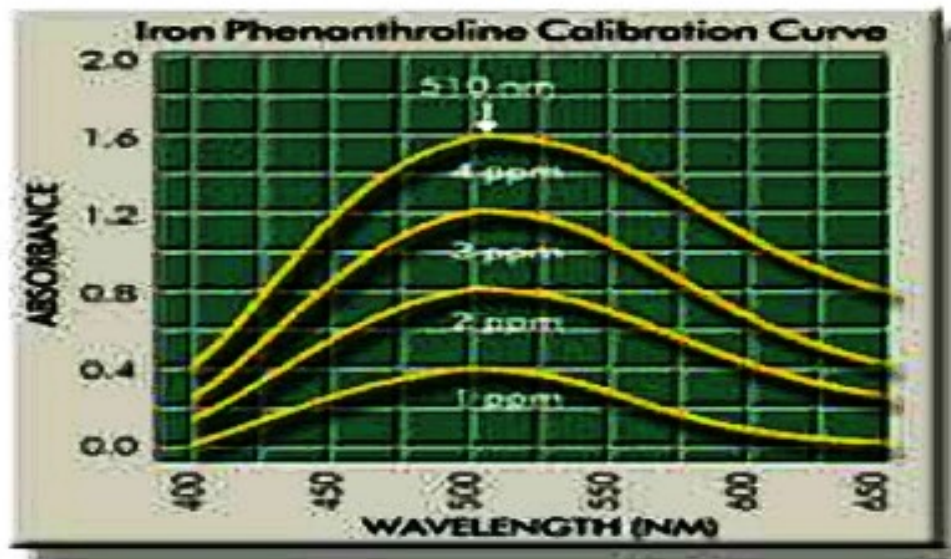
ϵ - molar absorptivity

l - length of solution

c - concentration

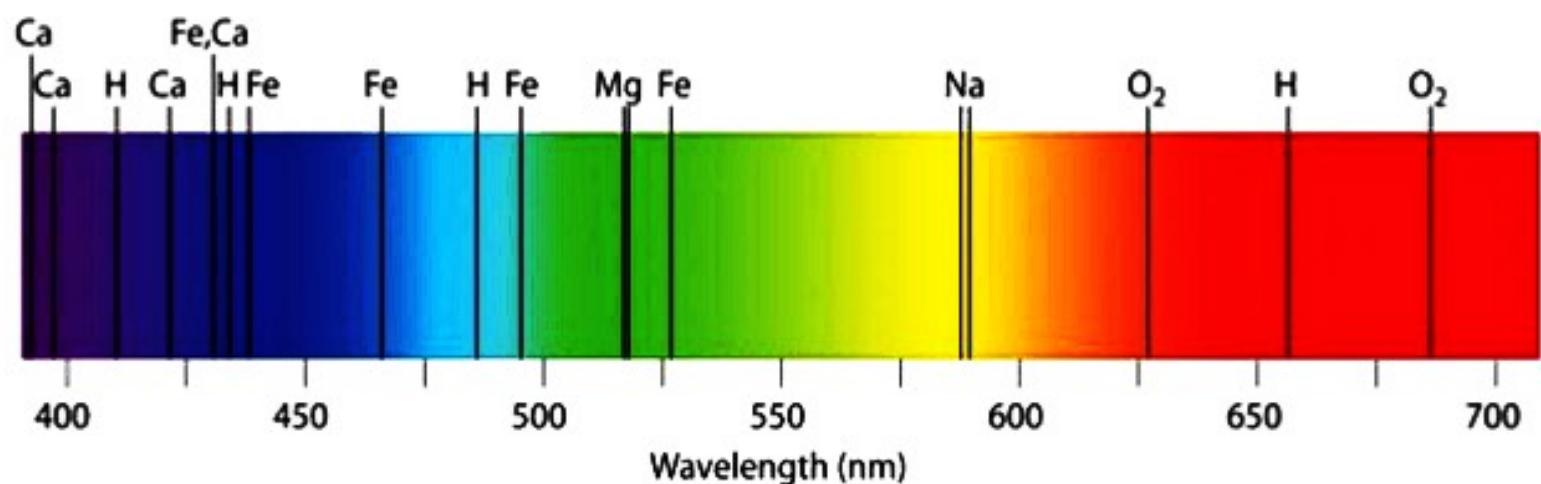
• STANDARDIZATION GRAPH

Standards (solutions of known concentration) of the compound of interest are made, treated, and their absorbances (ABS) and concentration values are used to create a **Standardization Graph**.

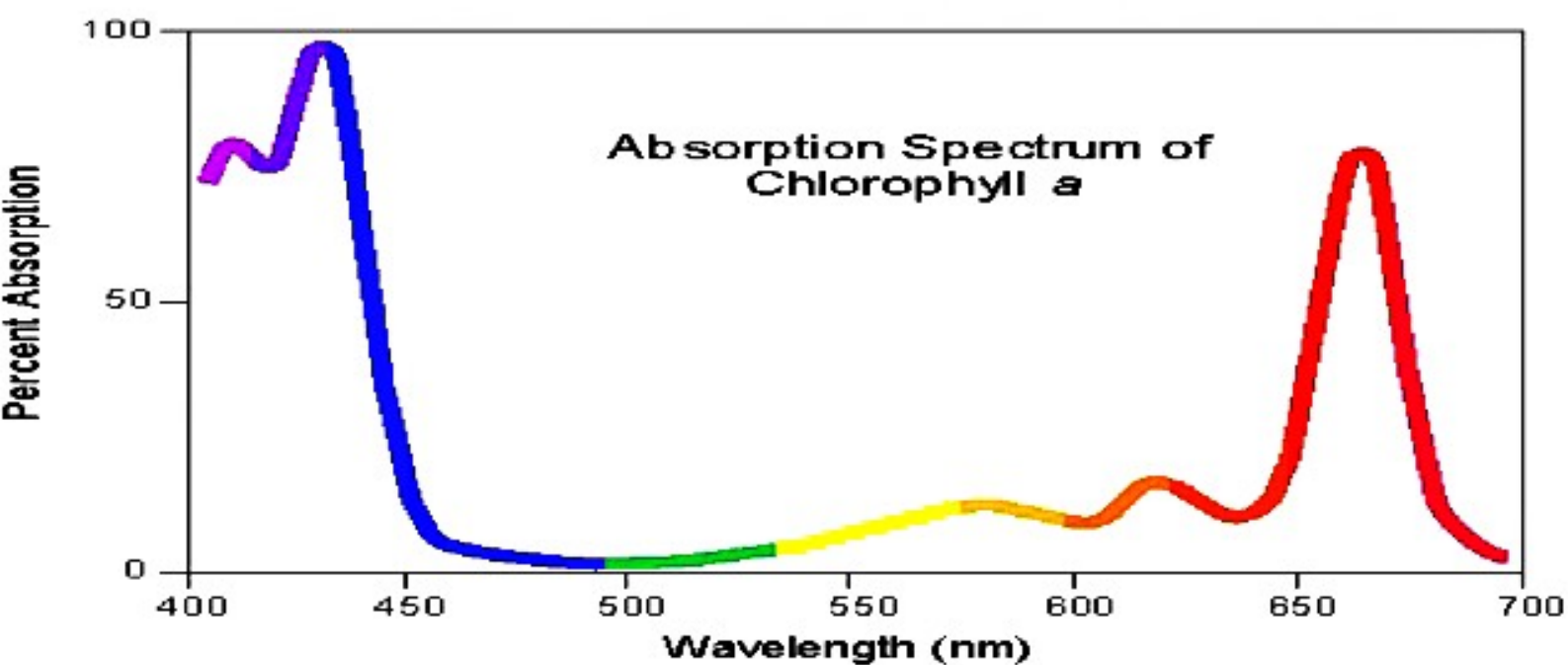
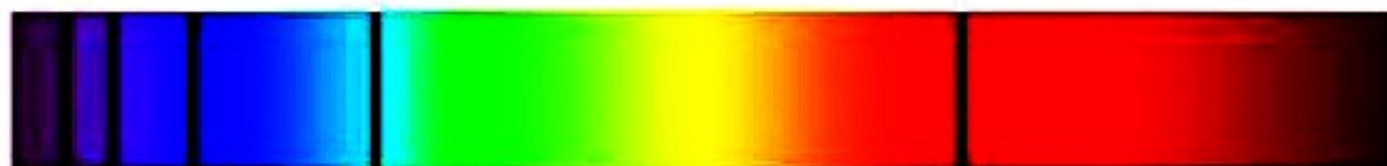


- Absorption spectra :

A spectrum of electromagnetic radiation transmitted through a substance, showing dark lines or bands due to absorption at specific wavelengths.



The Fraunhofer Absorption lines for the element Hydrogen





APPLICATIONS

1. Concentration measurement
2. Detection of impurities
3. Chemical kinetics
4. Detection of functional group
5. Molecular weight determination