



# Spectrophotometry

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# Introduction

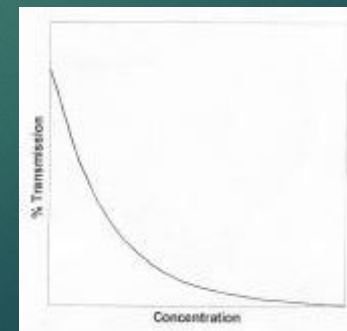
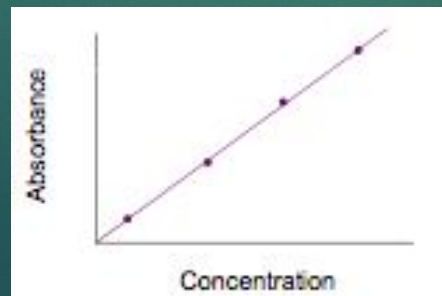
- ∪ Body fluids such as blood, csf and urine contain organic and inorganic substances.
- ∪ Organic substances – glucose, urea, uric acid etc.
- ∪ Inorganic substances – potassium, calcium, phosphorus, chloride , etc.
- ∪ These substances are in an equilibrium as` the rate of production and loss are equal.

# Introduction

- ∪ Normal concentration of these substances vary within very narrow limits.
- ∪ Because of small concentration of these substances and difficulty in isolating them photometric methods are frequently used.

# Introduction

- ⋮ compounds absorb light radiation of a specific wavelength.
- ⋮ the amount of light radiation absorbed by a sample is measured.
- ⋮ The light absorption is directly related to the concentration of the compound in the sample.



# Optical techniques

- Based on **estimation of light absorbing nature** of the substances
1. Calorimetry - can analyze only coloured compounds
  - 2 .Spectrophotometry – both coloured compounds and colourless as well.



# Optical techniques

- ↳ Based on estimation of light emitting nature of the substances
  - 1.Flame photometry
  - 2.Fluorimetry

# Spectrum

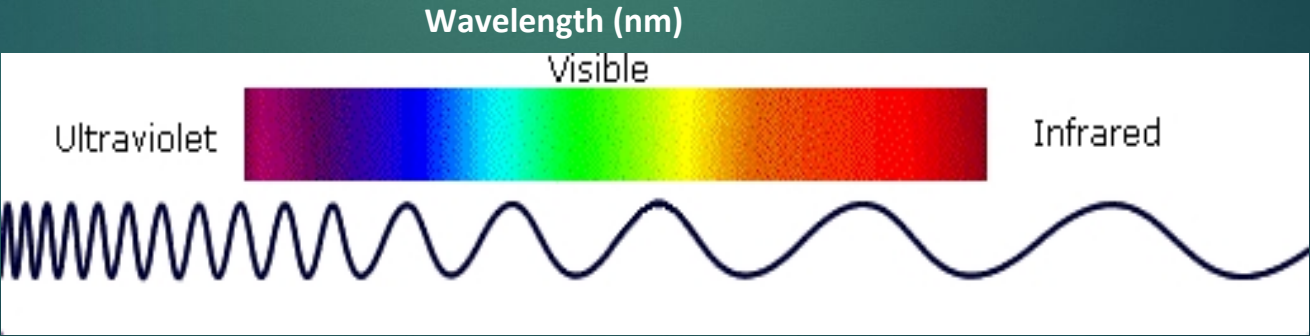
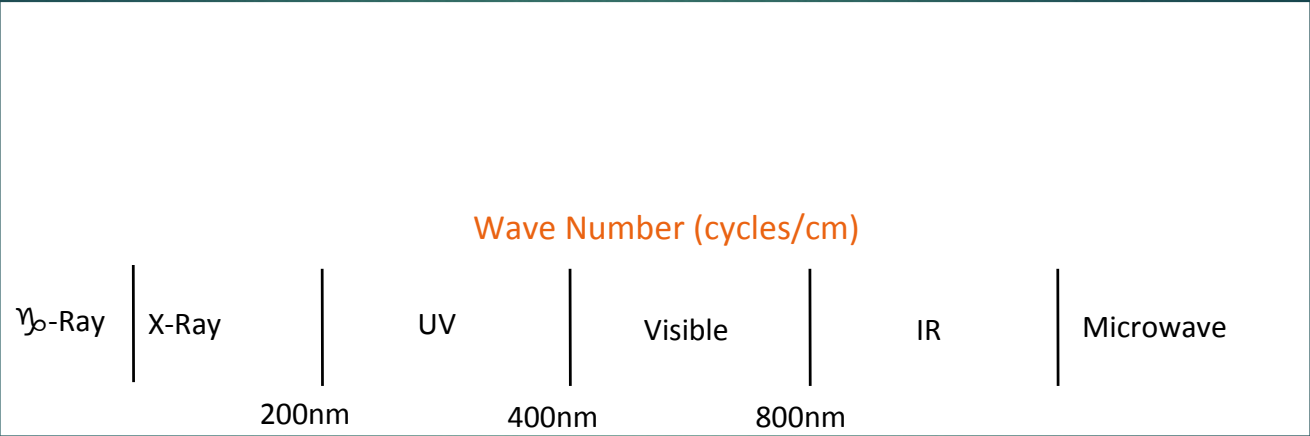
- ∪ It is not 2G spectrum scam
  - ∪ by which parliament was adjourned unendingly.
  - ∪ The word most heard till recently
- ∪ How does our spectrum differs from 2G ?



# Spectrum

- In Latin spectrum means "image" or "apparition"
- In the 17th century the word spectrum was introduced into optics, referring to
  - the range of colors observed when white light was dispersed through a prism
- It has since been applied by analogy to many fields other than optics.
- Thus, one might talk about the spectrum of political opinion, or the spectrum of activity of a drug

# Distribution of radiant energy



# Spectrophotometry

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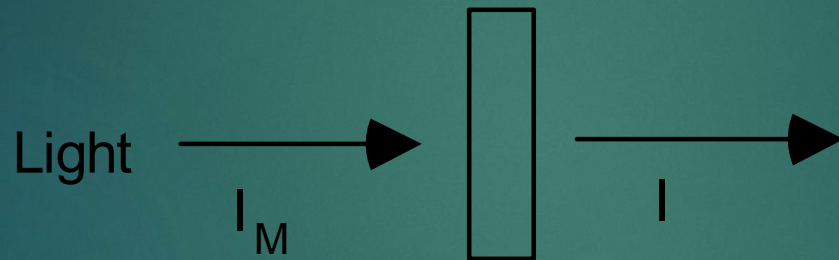
- **Photometry** – is defined as measurement of light
- Modern instruments isolate a narrow wavelength range of spectrum for measurements.
- Those that use
  - filters – Filterphotometer
  - prism or gratings - **Spectrophotometer**

# Spectrophotometer

- An instrument which can measure the absorbance of a sample at a particular wavelength.

# General principles

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Glass cell filled with  
concentration of solution (C)

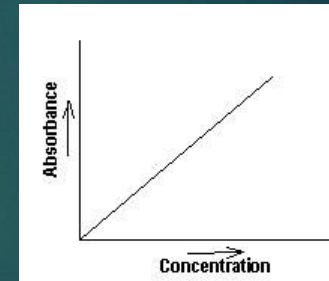
# Beer's law

- Absorbance of a solution is directly proportional to the concentration of a solution {  $A \propto C$  }

or

- Transmittance of a solution decreases exponentially with the increase in concentration of the solution

$$T = e^{-kc}$$



A = absorbance

T = transmittance

C = conc. of solution



## Lambert 's law

- Absorbance of a solution is directly proportional to the thickness of the optical path {  $A \propto t$  }

or

- Transmittance of a solution decreases exponentially with the increase in thickness of the optical path

$$T = e^{-kt}$$

A = absorbance

T = transmittance

C = conc. of solution

t = thickness of the optical path

## Beer – Lambert Law

- If both these are combined,

$$A \propto Ct \text{ or } T = e^{-kct}$$

A = absorbance

T = transmittance

C = conc. of solution

t = thickness of the optical path

K = constant for the substance

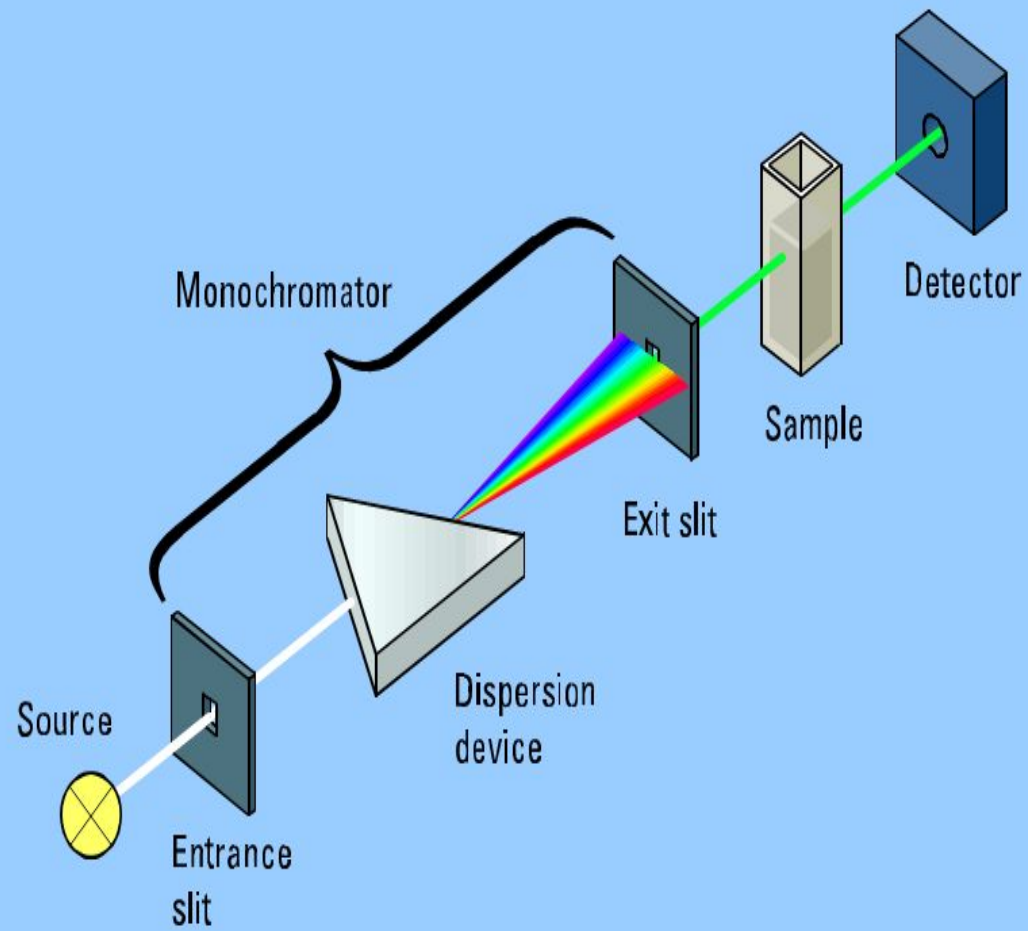
# Spectrophotometric analysis

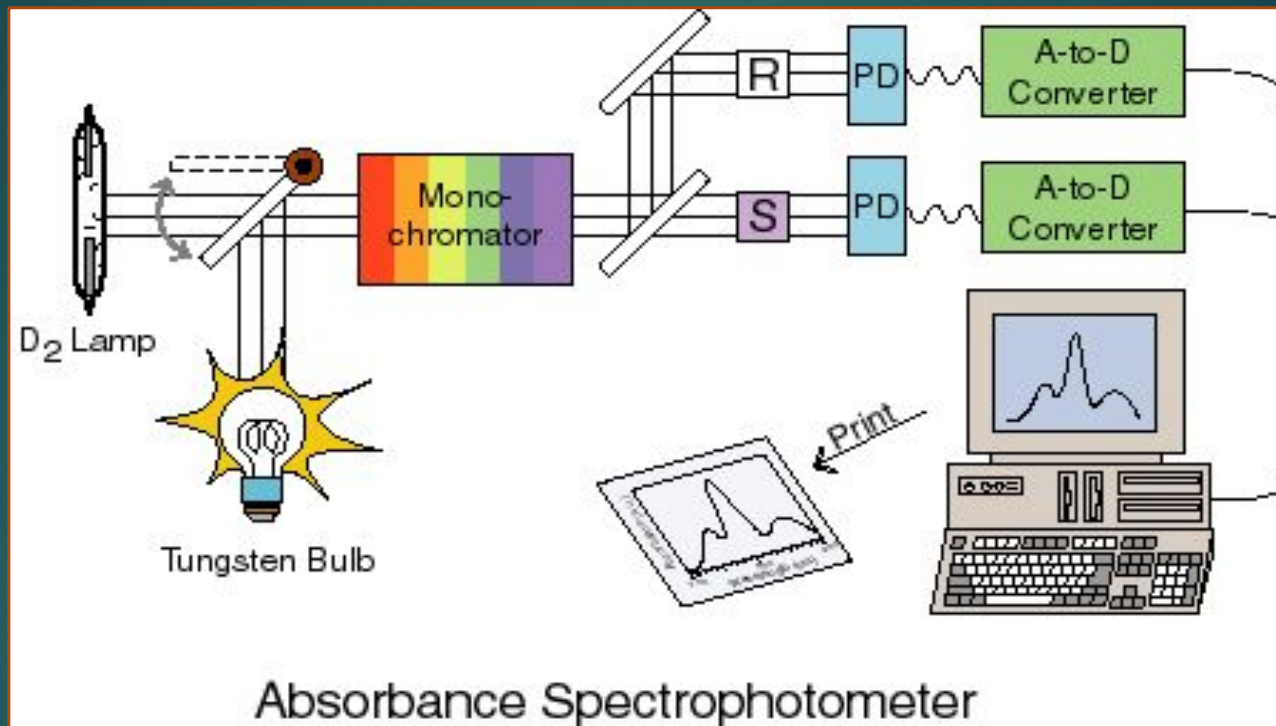
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- Widely used method of quantitative and qualitative analysis
- Accurate and sensitive

# Spectrophotometers

- ∪ Two types
  - ∪ Single beam
  - ∪ Double beam







# Instruments

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## Light source

- provide a sufficient of light which is suitable for making a measurement.
- The light source typically yields a high output of polychromatic light over a wide range of the spectrum.

# Light sources

1. Incandescent lamps
2. Laser sources

# Incandescent lamps

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- ∪ UV light

- ∪ 1. Deuterium lamp
- ∪ 2. Hydrogen Gas Lamp
- ∪ 3. Mercury Lamp

- ∪ Visible light

- ∪ 1. Tungsten Lamp (wavelength ranges from 360 – 700nm)

# Light Sources

## Laser sources

- ∪ Laser - Light amplification by stimulated emission of radiation
- ∪ They are used as
  - ∪ they provide intense light of narrow wavelength
  - ∪ Allow beam diameters in the range of several microns
  - ∪ Produce monochromatic light
- ∪ Wavelength output ranges from 550 – 1810 nm

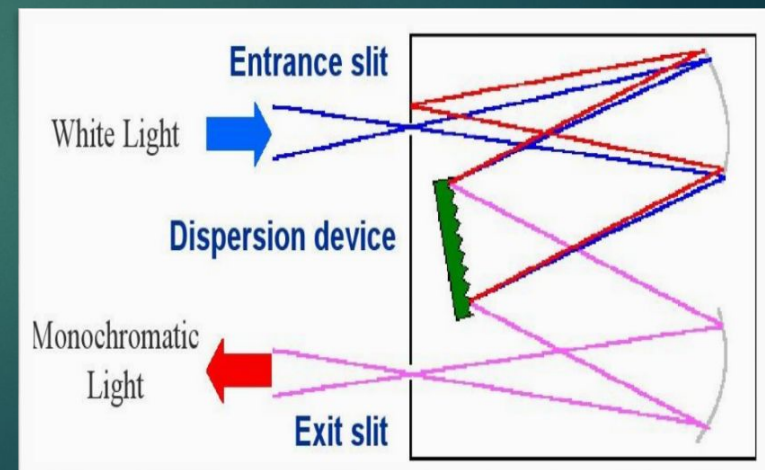
# Monochromator

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- A system for isolating radiant energy of a desired wavelength and excluding that of other wavelengths is called monochromator.
- Modern instruments isolate a narrow wavelength of the spectrum for measurements - **Isolation of spectrum**

# Monochromator

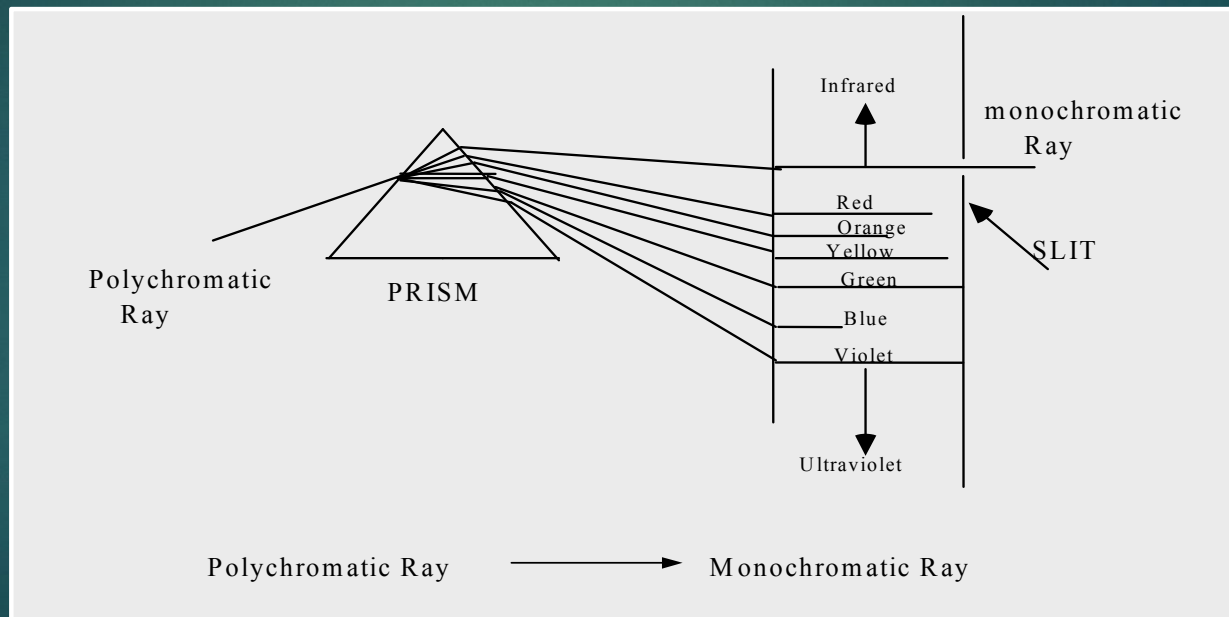
- Glass filters (coloured glass) – calorimeter
- Prisms or gratings – Spectrophotometers
- A glass filter is not true monochromatic light as it transmits light over a wide range of wavelengths.





## Dispersion of Polychromatic Light with a Prism

Prism - Spray out the spectrum and choose the certain wavelength ( $\lambda$ ) that you want by slit.



# Slit

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## Slit

- this is to allow a narrow beam of selected monochromatic light to pass through the sample solution
- Improve its chromatic purity

# Cuvette

- A small vessel used to hold a liquid sample to be analyzed.
- Shape – round, square, rectangular
- Constructed from glass, silica quartz
- Square or rectangular cuvettes have plane-parallel optical light surfaces and a constant light path
- Most have a 1cm light path



# Cuvette

- It is to be filled  $\frac{3}{4}$  th of its height
- Quartz cuvette is used in UV analysis



## Cuvette - care

- ⋮ Must be clean and optically clear ,as etching or deposits on the surface affect absorbance values
- ⋮ Glass cuvette – cleaned by copious rinsing with tap water and distilled water
- ⋮ Cuvets may be cleaned by detergent or soaked in a mixture of conc HCL: water : ethanol (1:3:4)

## Photodetector

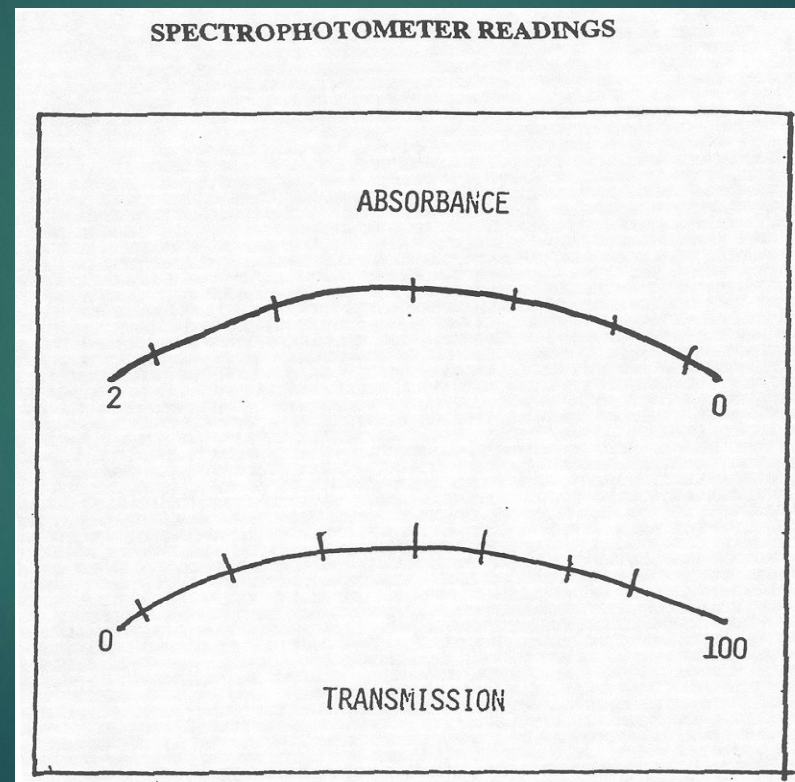
- Devices that convert light into electric signal that is proportional to the number of photons striking its photosensitive surface.
- **Photomultiplier tubes** (PMT) are commonly used as detectors
- Photodiodes are used in modern instruments



## Readout device

- The electrical signal from the PMT is amplified and then recorded by the galvanometer.
- Usually the recorders are calibrated in such a way that they directly give the absorbance or converted values of concentration.
- Now we have digital readout devices
- These operate on the principle of selective illumination of a portions of a bank of light-emitting diodes (LEDs)

# Spectrophotometer Reading



## Applications

In clinical diagnostic and research laboratories

□ Quantitative estimation of different compounds in various biological fluids.

E.g. Blood glucose, urea, cholesterol, creatinine, bilirubin and CSF protein etc.

∪ The absorption spectrum of a compound is characteristic for it

∪ Useful in identification and determination of purity and structure of a compound

# UV Spectrophotometer Application

Protein

Amino Acids (aromatic)

Pantothenic Acid

Glucose Determination

Enzyme Activity (Hexokinase)

## Visible Spectrophotometer Application

Niacin

Pyridoxine

Vitamin B12

Metal Determination (Fe)

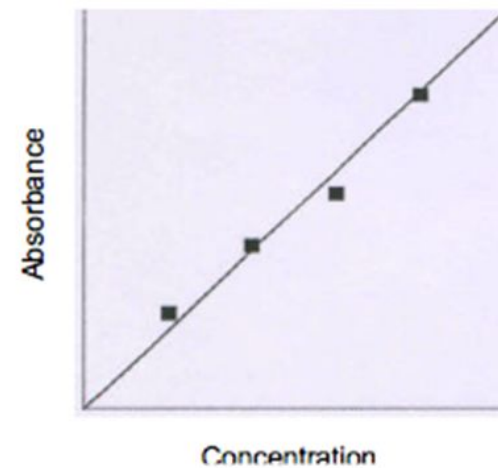
Enzyme Activity (glucose oxidase)

# Applications

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## 1. Concentration measurement

- Prepare samples
- Make series of standard solutions of known concentrations

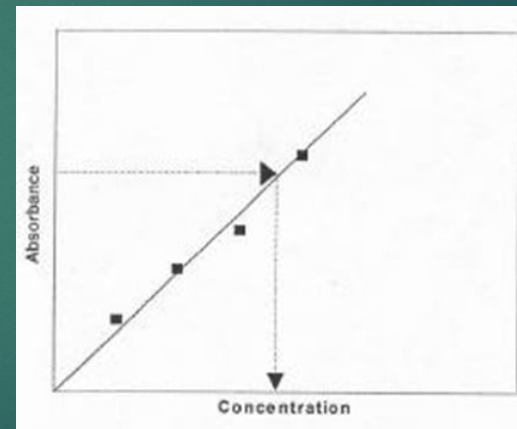
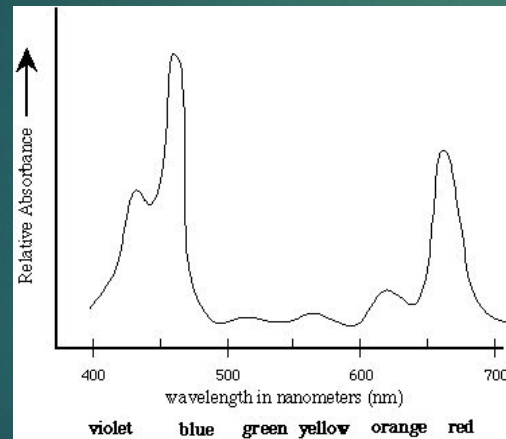




# Applications

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- Measure the absorption of the unknown
- from the standard plot, read the related concentration

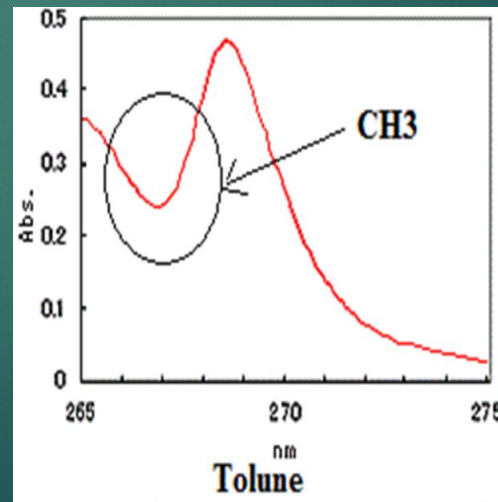


# Applications

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## 5. Detection of Functional Groups

↳ Absence of a band at particular wavelength regarded as an evidence for absence of particular group



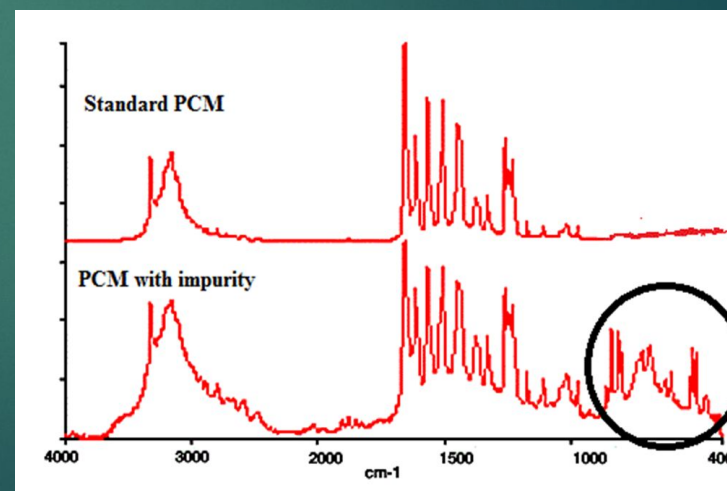
# Applications

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## 2. Detection of Impurities

UV absorption spectrophotometry is one of the best methods for determination of impurities in organic molecules.

Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material.



# Applications

## 6. Molecular weight determination

- Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable **derivatives** of these compounds.
- For example, if we want to determine the molecular weight of amine then it is converted in to ***amine picrate***.

## Flame Emission Spectrophotometer

### PRINCIPLE:

- Atoms of many metallic element, when given sufficient energy, emit this energy at a wave length characteristic for that element.
- A specific amount of energy is absorbed by an orbital electron. These electrons being unstable in high energy state, release this energy as photons of a particular wavelength.

## Flame Emission Spectrophotometer

- Used for the quantitative measurement of sodium and potassium in the body fluids.
- Also used for measurement of lithium, usually used in the treatment of psychiatric disorders, for therapeutic monitoring.



# Summary

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- ∪ The spectrophotometer technique is to measure **light intensity** as a function of wavelength.
- ∪ It does this by:
  1. **diffracting** the light beam into a spectrum of wavelengths
  2. **direct** it to an object
  3. **receiving** the light reflected or returned from the object
  4. **detecting** the intensities with a charge-coupled device
  5. **displaying** the results as a graph on the detector and then the display device .[1],[2]

# Summary

## Applications

- ❑ Quantitative estimation of biological constituents
- ❑ Detection of functional groups
- ❑ Detection of impurities
- ❑ Molecular weight determination

A photograph of a white lighthouse with a red lantern room, situated on a rocky cliff. The scene is captured at sunset, with a warm orange and yellow glow on the horizon and the lighthouse's walls. The foreground is dominated by dark, jagged rocks. In the background, there are evergreen trees and a view of the ocean with distant islands under a clear blue sky. The text "Thank you" is overlaid in a large, white, sans-serif font in the upper right quadrant of the image.

Thank you