



Spectrophotometry :

Instruments & Applications



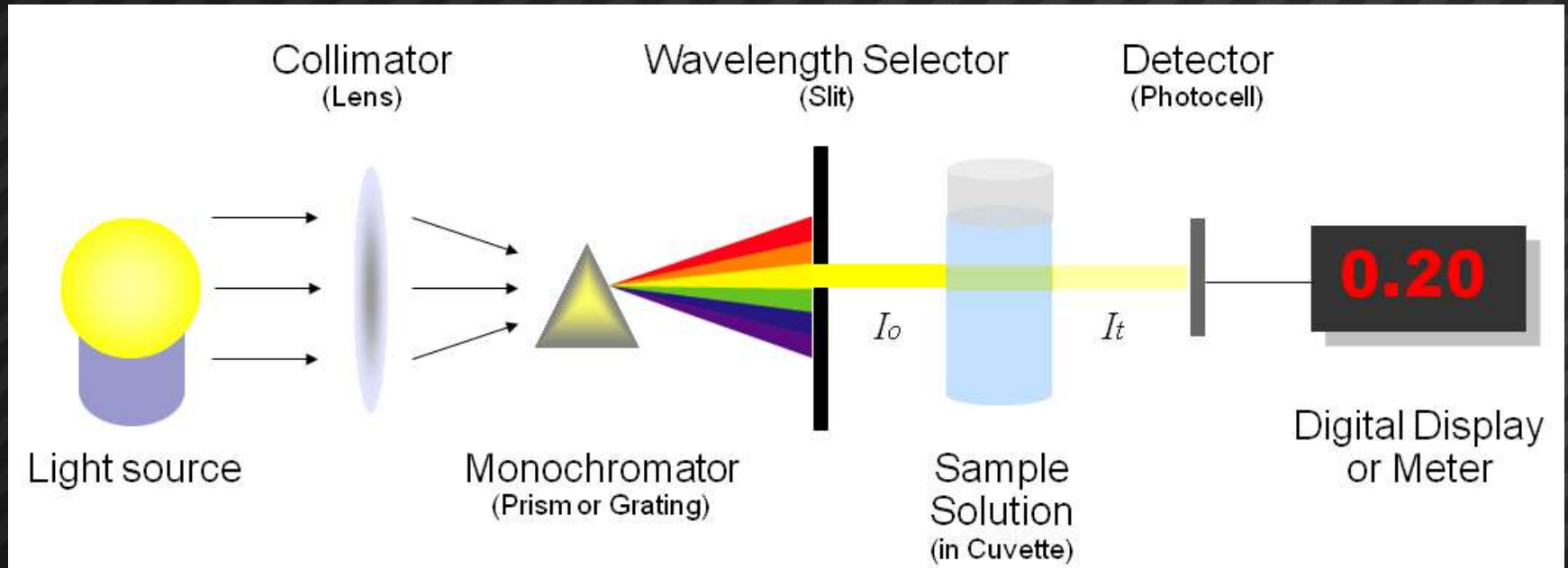
Content

- Introduction
- Beer-Lambert law
- Instruments
- Applications

Introduction

- The spectrophotometer technique is to measures **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]

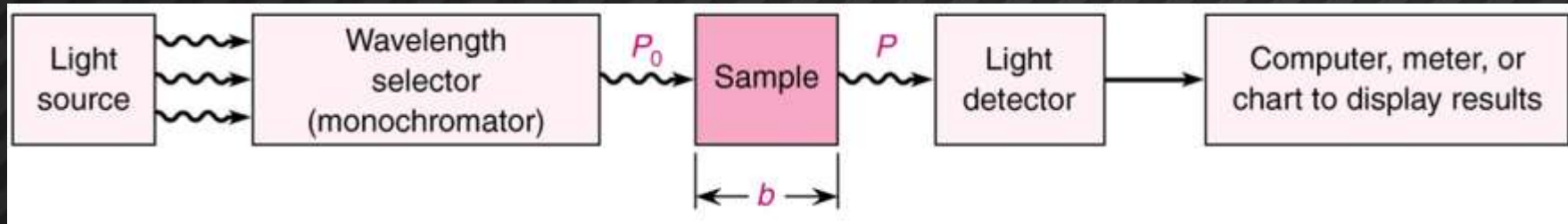
Introduction



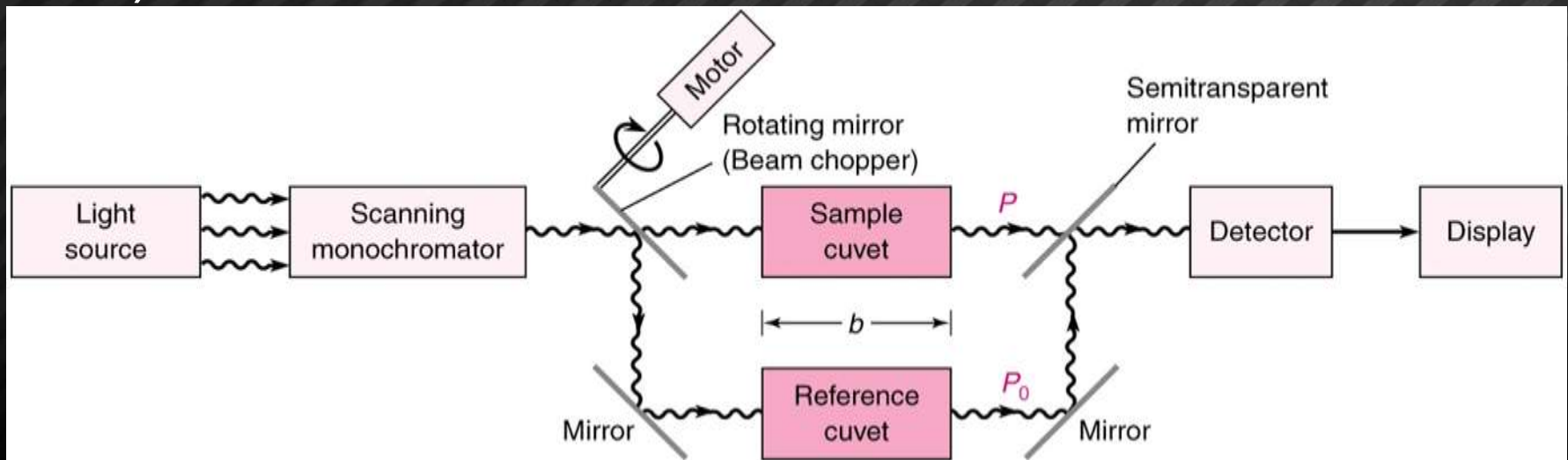
Introduction

- Spectrophotometer:

- a) Single-beam

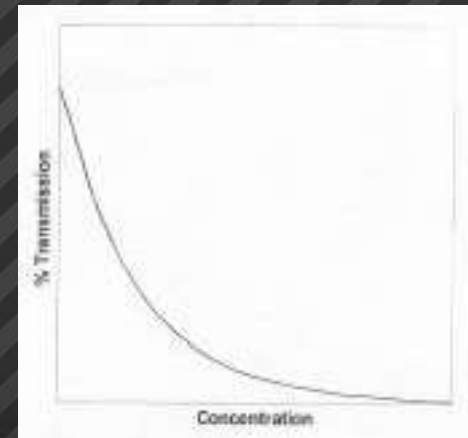
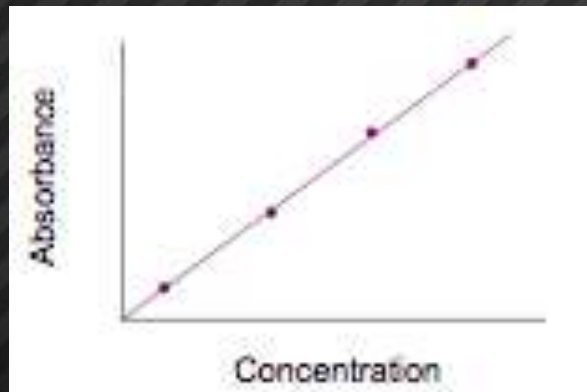


- b) Double-beam



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.
- As Concentration increases, light Absorption increases, **linearly**, As Concentration increases, light Transmission decreases, **exponentially**. [3]



Beer-Lambert law

- Light Absorbance: $(A) = \log (I_0 / I) = \epsilon LC$

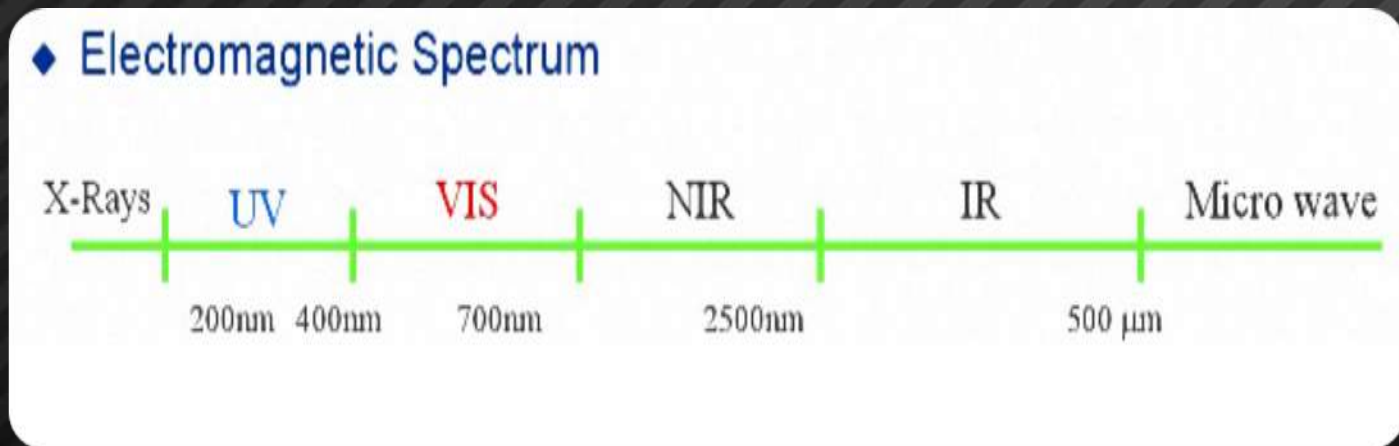
- Light Transmission $(T) = I/I_0 = 10^{-\epsilon CL}$

- I_0 : Light Intensity entering a sample
- I : Light Intensity exiting a sample
- C : The concentration of analyte in sample
- L : The length of the light path in glass sample cuvette
- ϵ : a constant for a particular solution and wave length

[5]

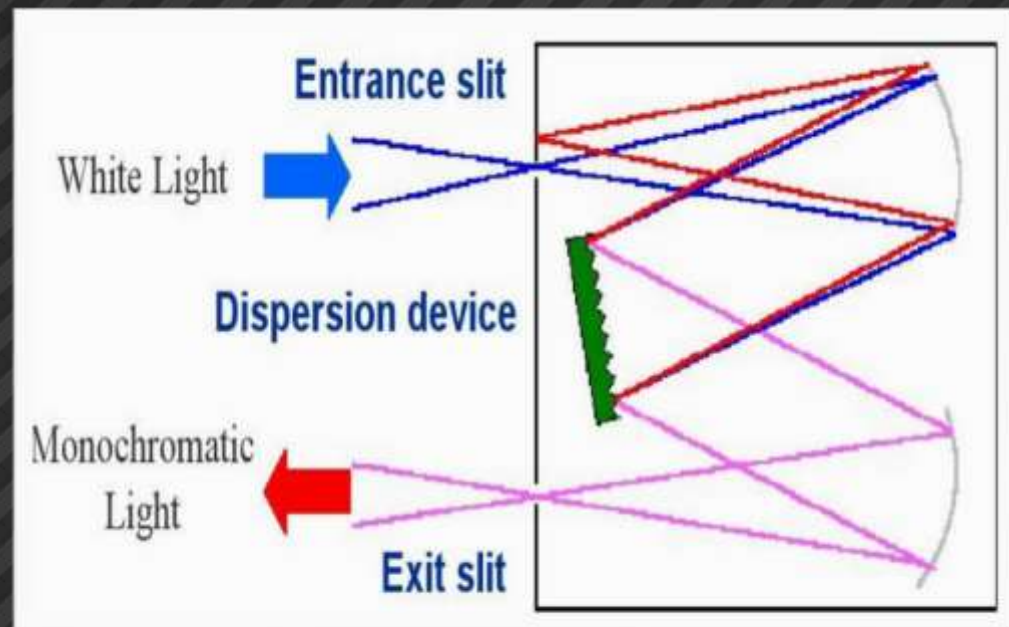
Instruments

- **Light source:** provide a sufficient of light which is suitable for marking a measurement.
- The light source typically yields a high output of polychromatic light over a **wide range** of the spectrum.[4]



Instruments

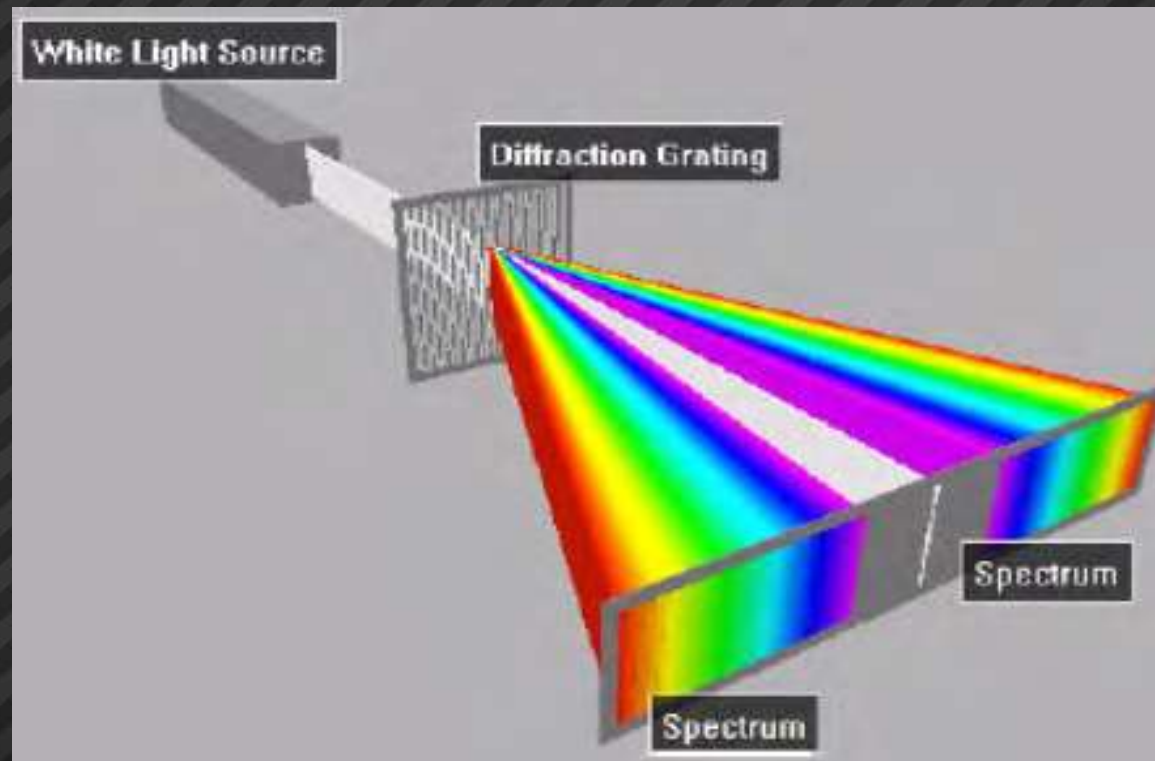
- **Monochromator** : Accepts **polychromatic** input light from a lamp and outputs **monochromatic** light.
- Monochromator consists of these parts:
 - I. Entrance slit
 - II. Collimating lens or mirror
 - III. Dispersion element
 - IV. Focusing lens or mirror
 - V. Exit slit [6]



Instruments

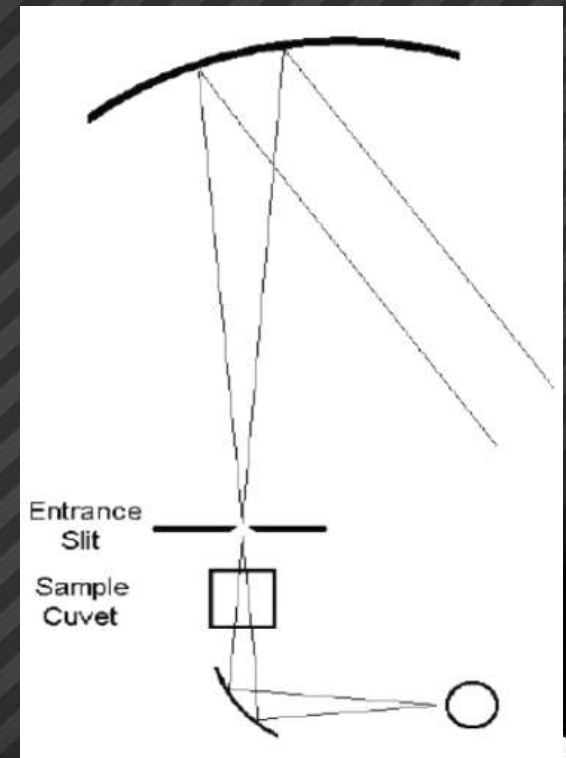
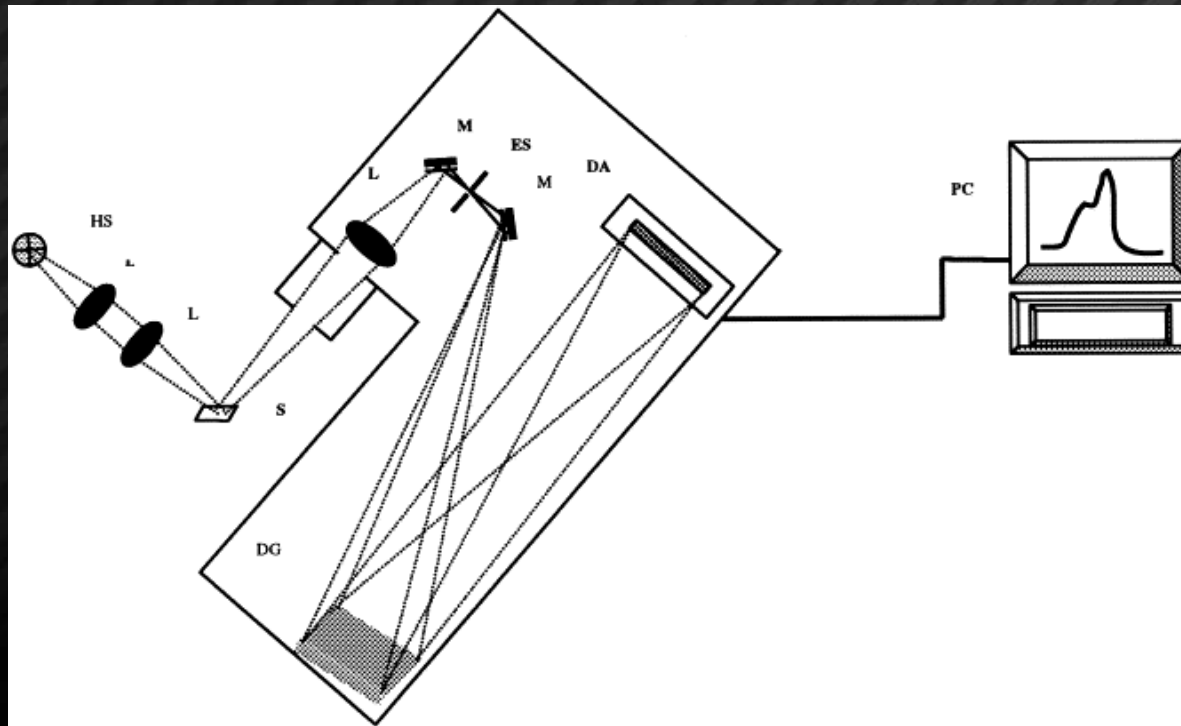
- **Dispersion devices:** A special plate with hundreds of **parallel grooved lines**.
- The grooved lines act to **separate** the white light into the visible light spectrum.

The more lines
the smaller
the wavelength
resolution.[5]



Instruments

- **Focusing devices:** Combinations of lenses, slits, and mirrors.
- relay and focus light through the instrument.[2]



Instruments

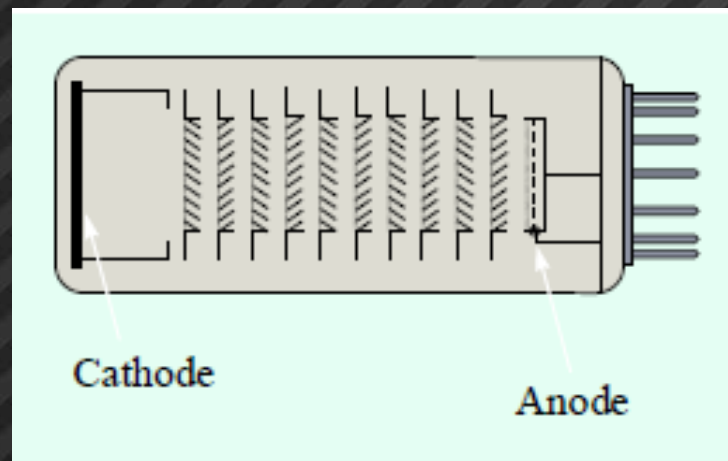
- **Cuvettes:** designed to hold samples for spectroscopic experiments. made of **Plastic**, **glass** or optical grade **quartz**
- should be as **clear** as possible, without impurities that might affect a spectroscopic reading.[2]



Instruments

- **Detectors:** Convert radiant energy (photons) into an **electrical signal**.

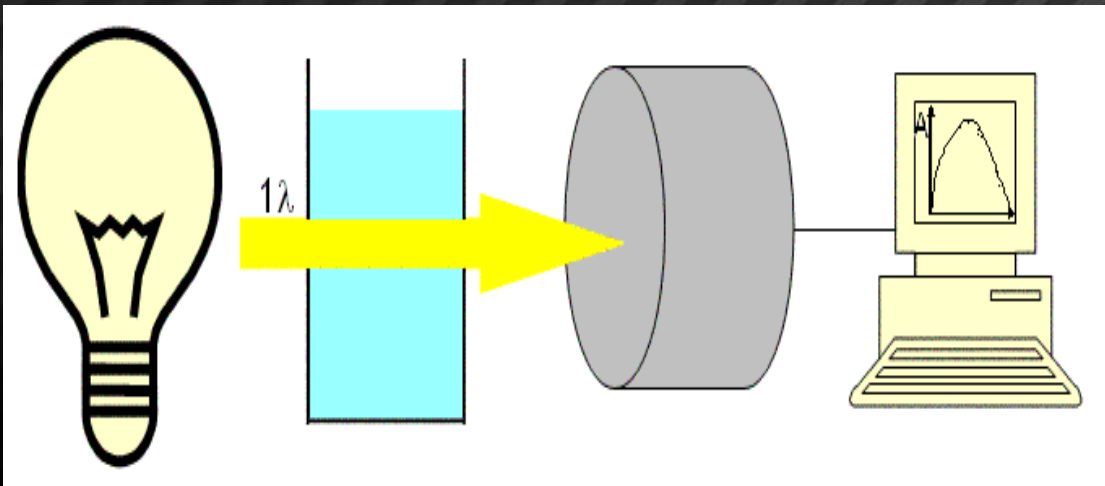
The **photocell** and **phototube** are the simplest photodetectors, producing current proportional to the intensity of the light striking Them .[1],[2]



The photomultiplier tube detector

Instruments

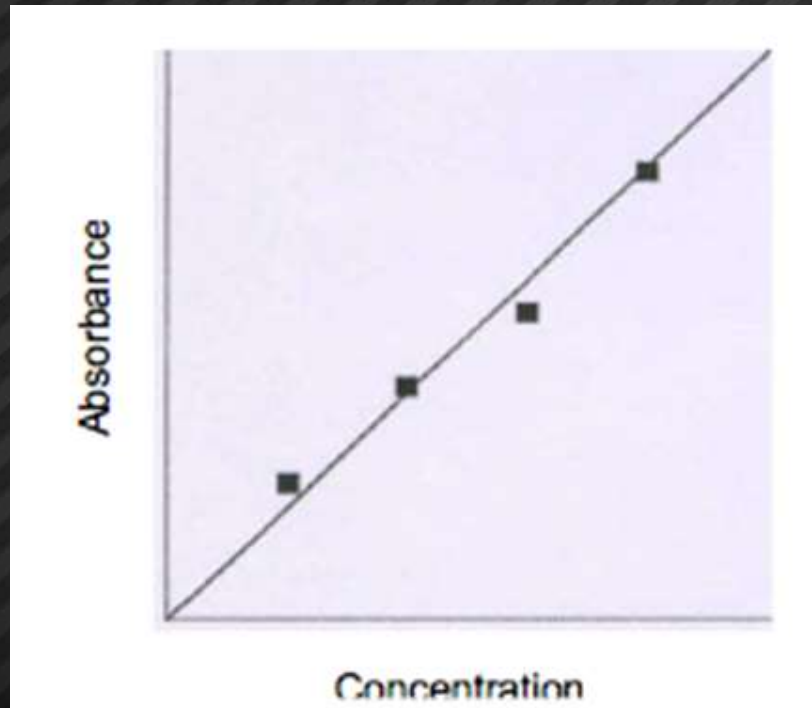
- **Display devices:** The data from a detector are displayed by a readout device, such as an **analog meter**, a light beam reflected on a scale, or a digital display, or **LCD**.
- The output can also be transmitted to a **computer** or **printer**. [3]



Applications

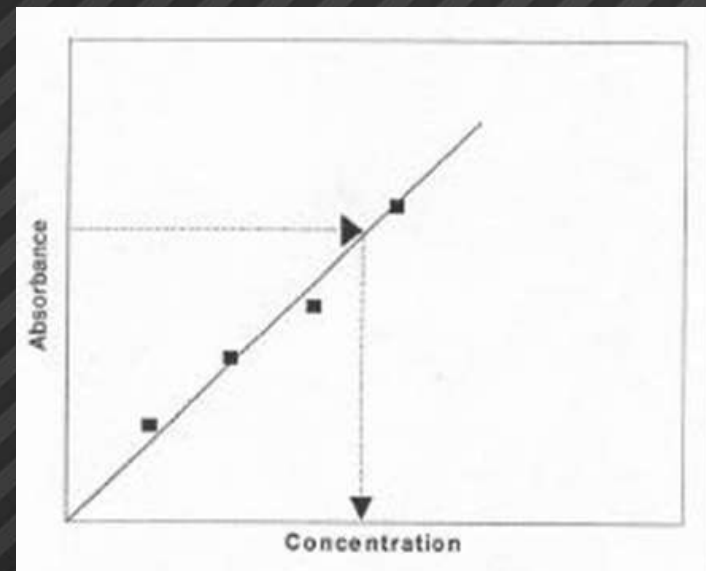
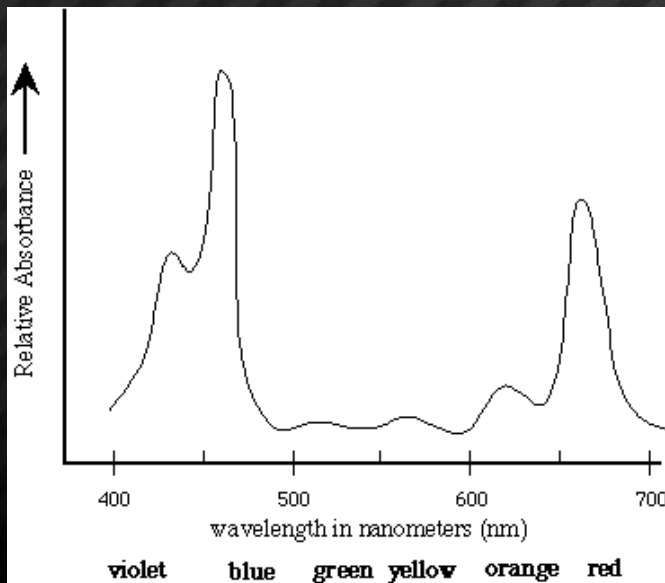
1. Concentration measurement

- Prepare samples
 - Make series of standard solutions of known concentrations
- [4]



Applications

- Set spectrophotometer to the λ of maximum light absorption
- Measure the absorption of the unknown, and from the standard plot, read the related concentration[4]

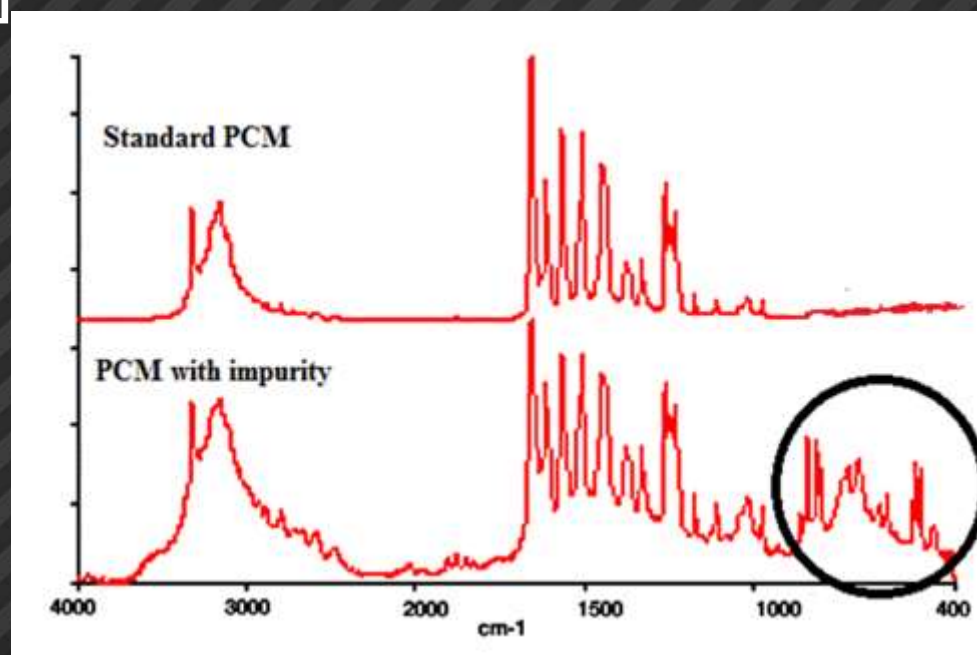


Applications

2. Detection of Impurities

- UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. [7]

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



Applications

3. Structure elucidation of organic compounds.

- From the location of **peaks** and combination of peaks UV spectroscopy elucidate **structure** of organic molecules:
 - the presence or absence of **unsaturation**,
 - the presence of **hetero atoms**.^[7]

Applications

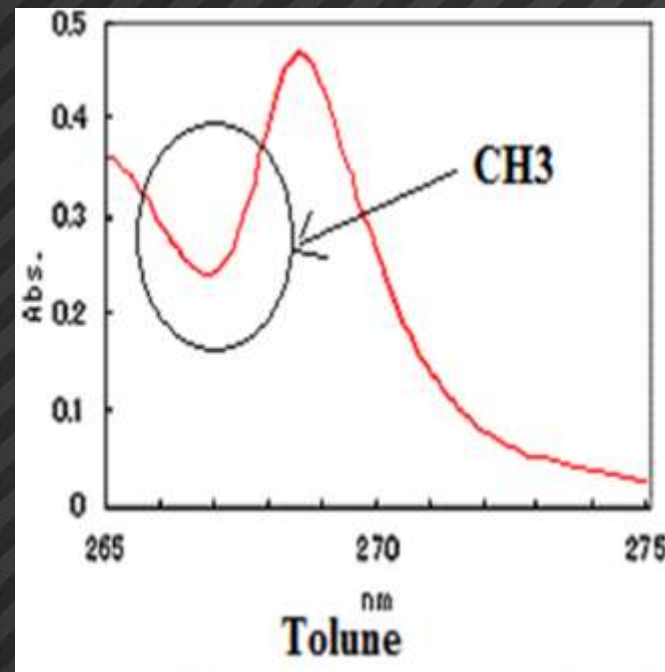
4. Chemical kinetics

- Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the **absorbance changes** can be observed.[7]

Applications

5. Detection of Functional Groups

- Absence of a band at particular wavelength regarded as an evidence for absence of particular group [5]



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***. [7]

References

- [1] The principles of use of a spectrophotometer and its application in the measurement of dental shades[2003]
- [2] Chapter 11 Spectrophotometer
- [3] Fundamentals of UV-visible spectroscopy, Tony Owen, 1996
- [4] UV/Visible Spectrophotometer, Mecasys Co., Ltd. , Mar2006
- [5] Spectrophotometry FUNDAMENTALS (Chapters 17, 19, 20), Dr. G. Van Biesen, Win2011
- [6] <http://www.bio.davidson.edu>
- [7] UNIT: Spectrophotometry, Clinical Chemistry Lab Manual