SECOND STAGE

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# Principles Of Instrumentation [photometer, Colorimetry and Spectrophotometry Components]

## Introduction

Photometry means "The Measurement Of Light"

Photometer & spectrophotometer are instruments used for this type of measurement, in which a photocell or photomultiplier tube is used to detect the amount of light that passes through a colored solution from a light source.

### Characteristics Of Light

1- Light is a form of electromagnetic energy that travels in waves.

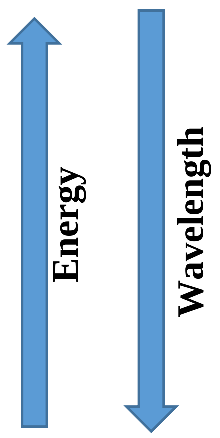
2- The wavelength of light is the distance between two beaks of the light wave, is inversely pro portional with its energy.

3- In the visible region, the color of light is a function of its wave length, increasing from violet towards the red color.

4- Objects that appear colored absorb light at particular wavelength and reflect the other parts of the visible spectrum resulting in many shades of color.

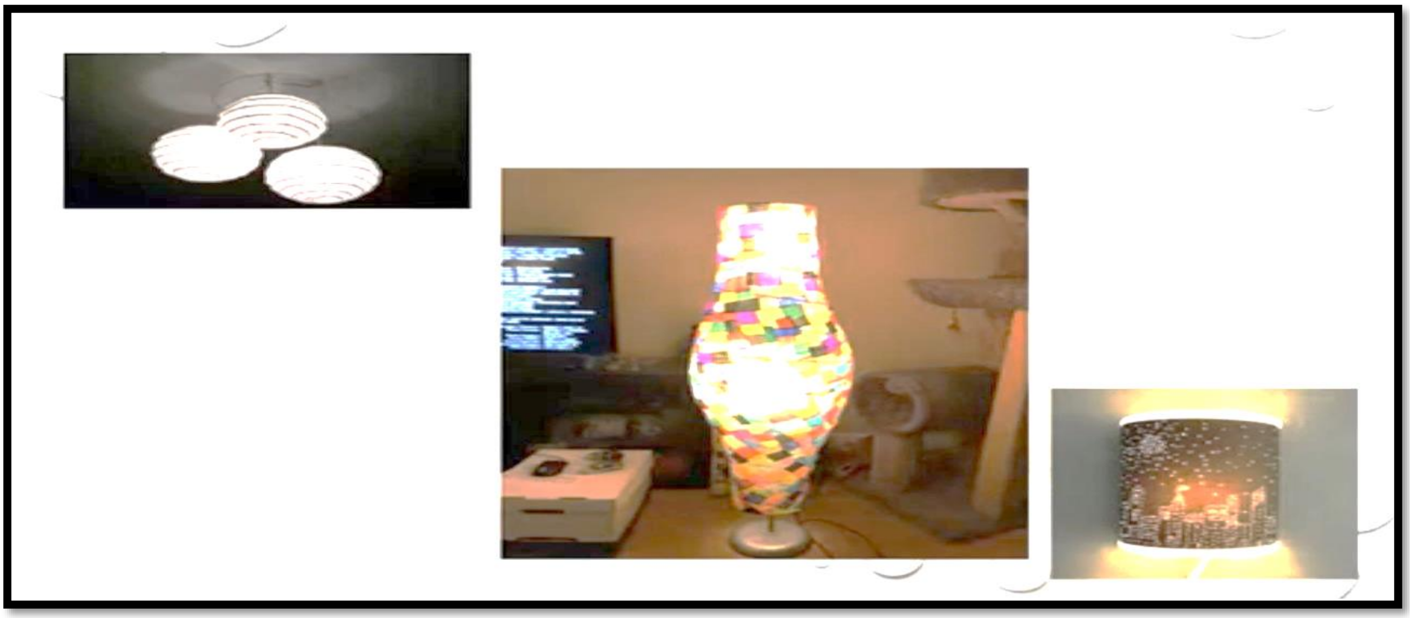
### wavelengths of various Types of radiation

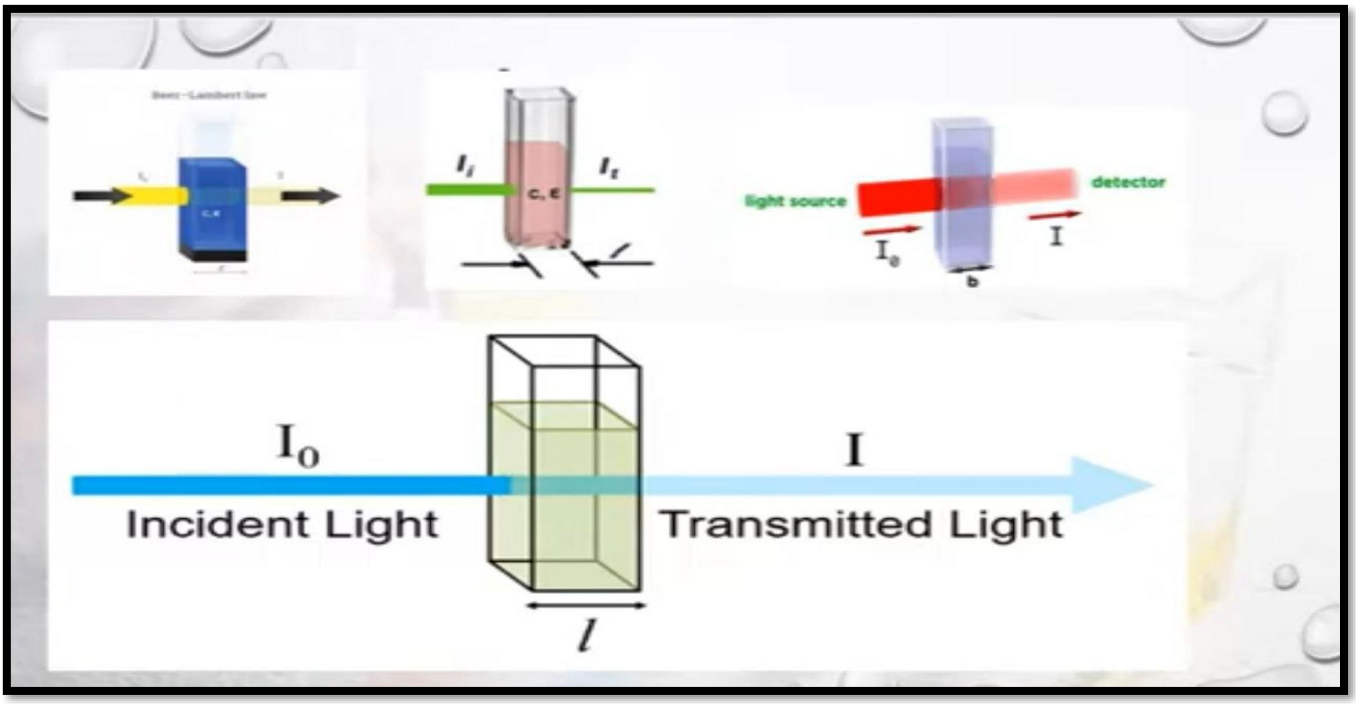
| Types of radiation | Approximately wavelength |
|---|---|
| Gamma | <0.1 |
| X-rays | 0.1-10 |
| Ultraviolet | < 380 |
| Visible | 380-750 |
| Infrared | > 750 |
| Radio-waves | $>25 \times 10^7$ |



## PRINCIPLE

- If a substance can be converted to a soluble, colored material, its concentration may be determined by the amount of color present in the solution.
- The greatest sensitivity is obtained when the light permitted to pass through the solution is of a particular wavelength. (The wavelength shows shows the maximum absorbance for the solution color).





### Example

- a substance that absorbs violet light at 400 nm reflects all other light and appears as yellow green.
- To measure the concentration of a blue solution, light is passed through it at about 590 nm. The amount of yellow light absorbed varies directly in proportion to the concentration of the blue substances in the solution.

The visible Spectrum

| Approximately Wavelength | Color Of Absorbed Light | Color Of Reflected Light |
|---|---|---|
| 400-435 | Violet | Green–Yellow |
| 435-500 | Blue | Yellow |
| 500-570 | Green | Red |
| 570-600 | Yellow | Blue |
| 600-630 | Orange | Green blue |
| 630-700 | Red | Green |

### Beer's law

When the light of an appropriate wavelength strikes a cuvette that contains a colored sample, some of the light is absorbed and the rest is transmitted through the sample to the detector. % percent transmittance which represents the proportion of light reaches the detector.

$$\%T = \frac{It}{Io} \times 100\%$$


Where:

Io: is the intensity of light striking the sample.

It : is the intensity of transmitted light.

## Beer's law

- If the concentration of a solution is increased, the It will decrease and then % T is decreased.
- The relationship between the concentration and %T is not linear, but if the logarithm of the %T is plotted against the concentration, a straight line is obtained
- The term absorbance is used to represent (-log % T).

$$A = -\log \% T = \frac{1}{\log \% T}$$


## Beer-Lambert Law

A=a b c

Which states that "the absorbance of a solution is directly proportional with the concentration of the dissolved substance" Where:

- A is the absorbance
- a is the molar absorptivity coefficient.
- b is the light bath through a solution.

For x substance:

(1) Abs(x) = a b Conc.(x)

For standard substance:

(2) Abs(st) = a b Conc.(st)

From 1, 2

$$\frac{\text{Abs. (x)}}{\text{Abs. (st)}} = \frac{\text{conc. (x)}}{\text{conc. (st)}}$$

$$\text{Conc. (X)} = \frac{\text{Abs.(X)}}{\text{Abs.(St)}} \times \text{Conc. (St)}$$

Then we can determine the concentration of x substance by measuring both sample and standard absorbance, which can be made by spectrophotometers.

## Requirements for the Beer's - Lambert's law to hold true

### 1.Solution Requirements

The solution must be the same through out the test time, and the molecules of which it is composed must not associate or dissociate at the time absorbance is being measured.

### 2. Instrument Requirement

- The instrument used in colorimetric tests must show satisfactory accuracy, sensitivity and reproducibility at the different wavelengths used.
- The cuvettes used in the instrument must be optically matched, free from scratches clean.

## UV-Visible Photometry

### Typical coloremetric instruments contain five components:

1. Stable source of radiation energy.

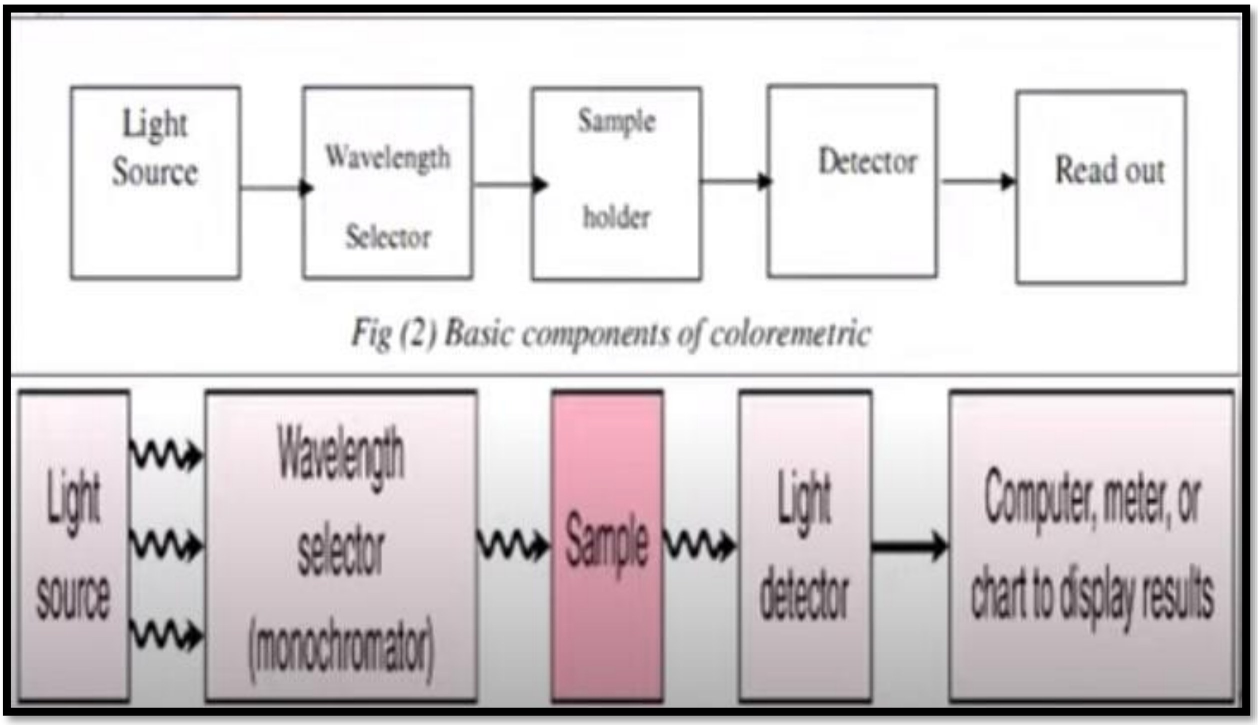
2. A device that isolates a restricted region of the spectrum for measurement.

3. A transparent container for holding the sample.

4. A radiation detector which converts radiant energy to electrical signals.

5. A signal processor and read out which displays the transudated signals, a meter scale, a digital meter or a recorder chart.

## UV-Visible Photometry




### 1. Radiation Sources

**In UV region:**

The most commonly used is deuterium lamp or hydrogen lamp. That produced light with (160-375) nm.

**In visible region:**

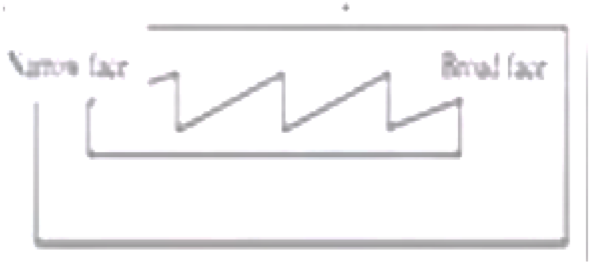
Tungeston filament lamp is the most commonly used and produces light at (350-2500) nm.

### 2. Wavelength Selectors

**A. Filters**

- may be formed of a transparent dielectric layer such as calcium fluoride, the thickness of this layer is controlled carefully and determines the wave length of transmitted light. Or formed of colored glass that absorbs certain portions of spectrum and transmits others, according to its color.

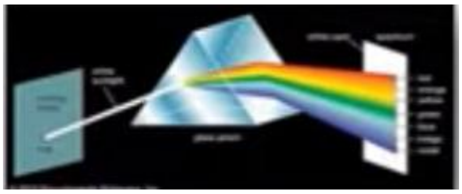
**B. Monochromators** Which may




### 1. Grating monochromator:

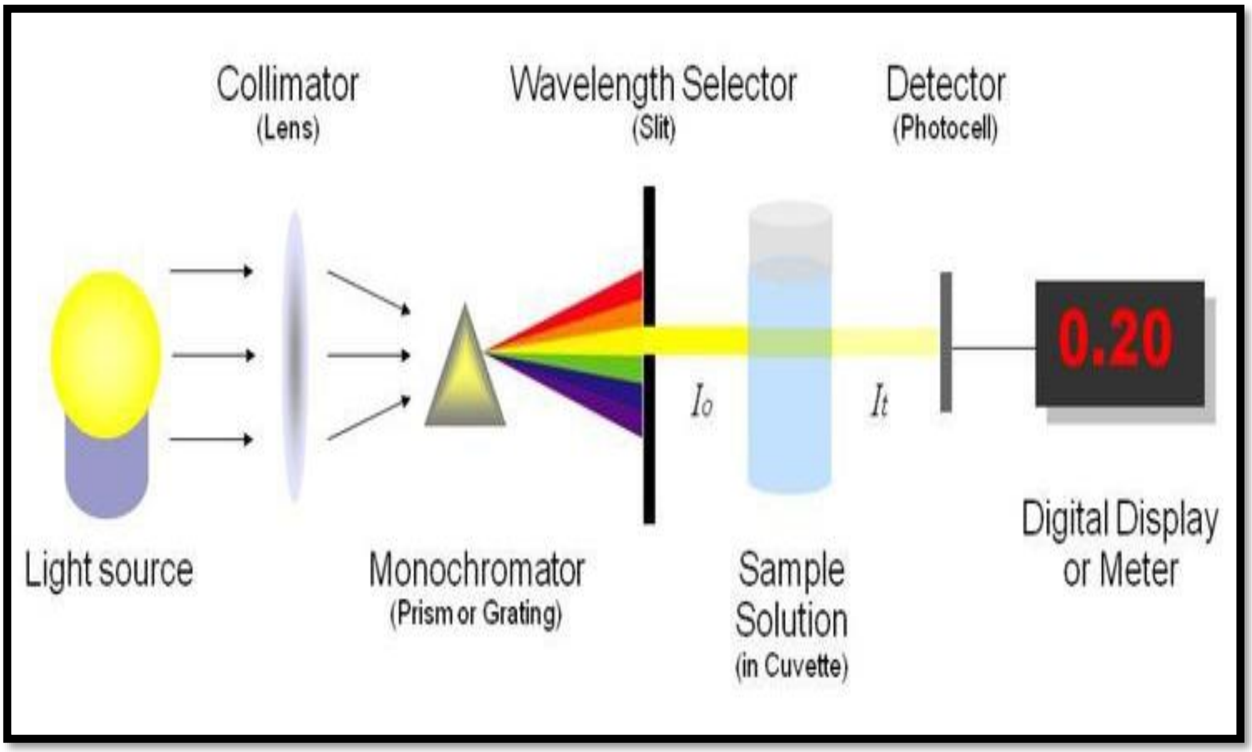
- A monochromatic is a device used in optics to select a range of the narrowest possible wavelengths from a polychromatic luminous beam.
- Grooves formed of relatively broad faces and narrow unused face

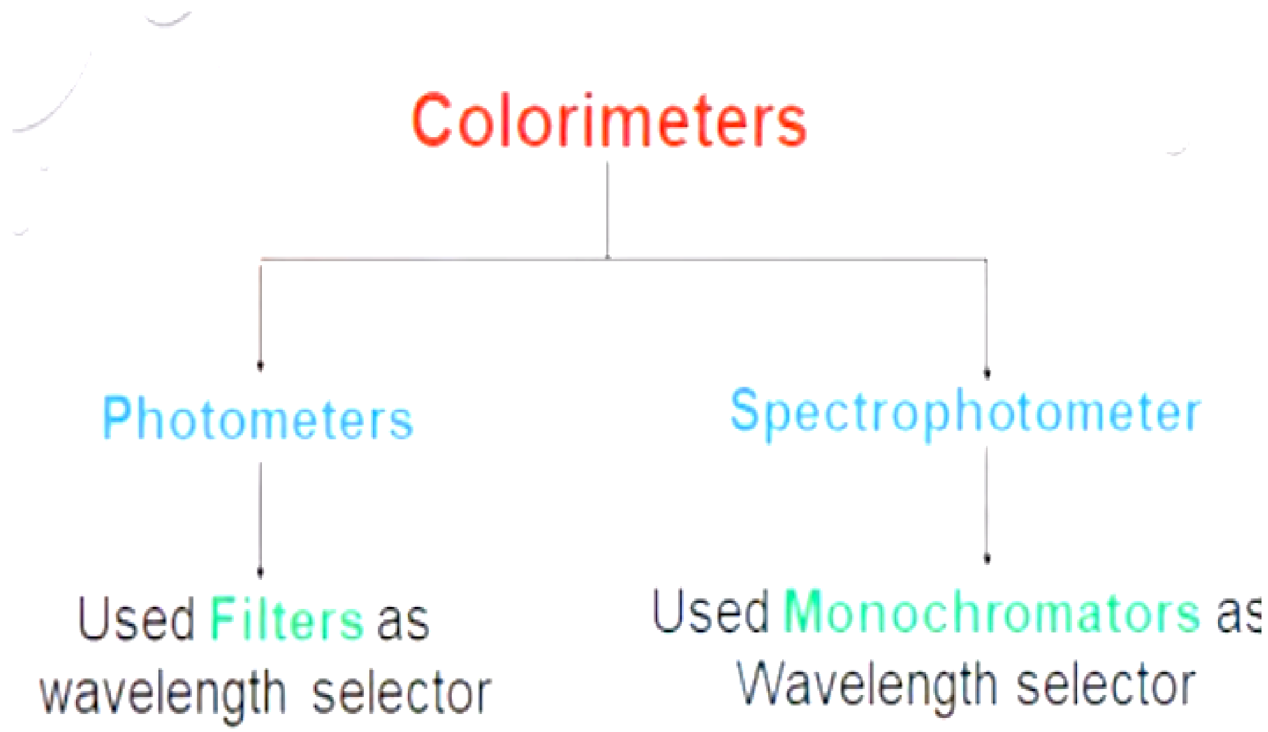
### 2. Prism



### Principle

- Suppose a radiation of 2 wave length enter from the entrance slit, they strike the mirror to be reflected on the dispersing element to produce angular dispersion of light which face a black surface and come out from the exit slit only, by moving monochromator, a specific wave length will pass from the exit slit.
- For prism by moving it only one $\lambda$ will exit.






### 3. Sample containers

- Cuvettes that hold the samples must be made of material that passes radiation in the spectral region of interest.

- Quartz or fused silica may be used in the spectral region (350-3000 nm), mean it may be used in the UV, visible and a part of infrared.

### 4. Radiation detectors and read out

| | |
|---|---|
| Phototubes | Photomultiplier Tube |
| Photoconductivity detector | silicon diode electrode |

One of them may be used to transform radian energy into electrical energy. Which may be measured by galvanometer or any read out device.

## Principle of phototube

Photo-emissive cathode that tends to emit electrons when irradiated. These electrons flow to the anode generating current.