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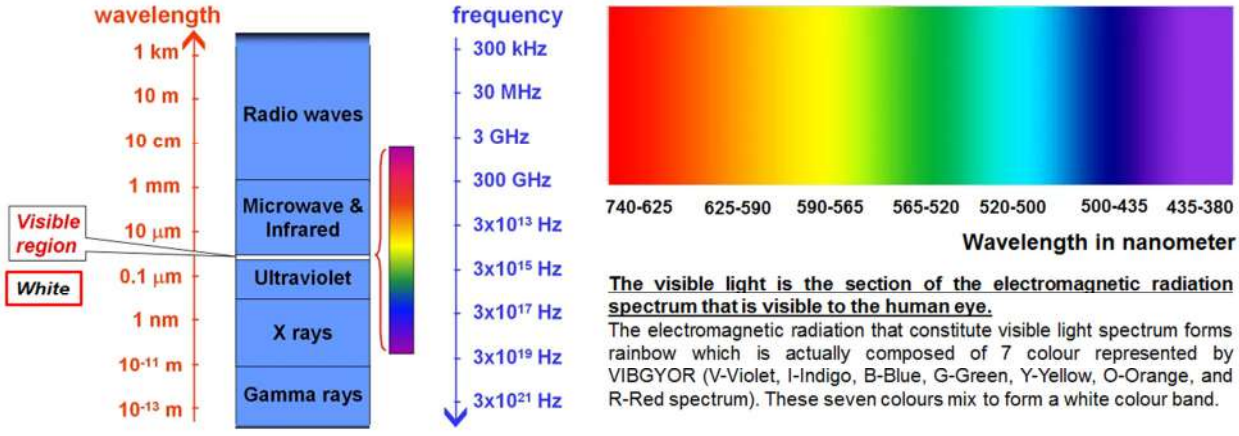
PRINCIPLES AND USES OF ANALYTICAL INSTRUMENT - SPECTROPHOTOMETER

A spectrophotometer is an instrument that is used to measure absorbance of radiant energy at various wavelengths. It is basically very similar to colorimeter except that it uses prism or diffraction gating to produce monochromatic light. Thus in this sense, a spectrophotometer has all the basic components of a photoelectric colorimeter with more sophistication. Furthermore, unlike to colorimeter, it can be operated in ultraviolet (UV) region, visible spectrum (VR) as well as infrared (IR) region of the electromagnetic spectrum. In other words, a colorimeter can measure absorbance of wavelength of only visible spectral ranges (VR), while a spectrophotometer measures absorbance of ultraviolet (UV), visible range (VR), and infrared (IR) ranges electromagnetic spectrum. Therefore, **spectrometers** or **spectrophotometers** are the instruments that are used to study the absorption or emission of electromagnetic radiation as a function of wavelength.

Electromagnetic radiation

The electromagnetic radiation is energy that is transmitted at the speed of light through oscillating electric and magnetic field. It is a form of energy that is all around us such as radio waves, microwaves, X-rays and gamma rays. Sunlight is also a form of electromagnetic radiation. The distribution of electromagnetic radiation according to frequency or wavelength is called as **electromagnetic spectrum**. Wavelength of electromagnetic radiation increases from 10^{-18} m to 100 km, and this corresponds to frequencies decreasing from 3×10^{26} Hz to 3×10^{3} Hz. Visible part of this electromagnetic spectrum is that we can detect with our eyes which makes up only a small fraction of this electromagnetic spectrum and is known as **visible light spectrum**.



In a vacuum, all electromagnetic radiation travels at the speed of light i.e. 299, 792, 458 m/s ($c = 3 \times 10^8 m/s$). Energy (E) can be associated with each region of the electromagnetic spectrum (EMS) using the equation:

$$\boldsymbol{E = hf}$$

Where ***'f'*** is the frequency and ***'h'*** is Planck's constant i.e. $\boldsymbol{h = 6.6260693(11)^{-34} Js}$

The table below lists typical wavelengths, frequencies and energies for different regions of the electromagnetic spectrum:

Region	**Wavelength**	**Frequency**	**Energy**
Hard Gamma	1×10^{-9} nm	3×10^{26} Hz	1.2×10^{12} eV
Gamma	1×10^{-6} nm	3×10^{23} Hz	1.2 GeV
Gamma/X-ray	0.001 nm	3×10^{19} Hz	12 MeV
X-ray	1 nm	3×10^{17} Hz	120 keV
X-ray/**Ultraviolet**	10 nm	3×10^{16} Hz	12 keV
Ultraviolet	100 nm	3×10^{15} Hz	1.2 keV
Visible (violet)	400 nm	7.5×10^{14} Hz	3.1 eV
Visible (red)	700 nm	4.3×10^{14} Hz	1.8 eV
Infrared	10000 nm	3×10^{13} Hz	0.12 eV
Microwave	1 cm	30 GHz	1.2×10^{-4} eV
Microwave/Radio	10 cm	3 GHz	1.2×10^{-5} eV
Radio	100 m	3 MHz	1.2×10^{-8} eV
Radio	100 km	3 KHz	1.2×10^{-11} eV

*Cosmos – Electromagnetic Spectrum (**https://astronomy.swin.edu.au/cosmos/E/Electromagnetic+Spectrum**)*

Since, electromagnetic waves are categorized according to their frequency ***'f'*** or, equivalently, according to their wavelength ***'ƛ'*** with the following equation:

$$\lambda = \frac{c}{f}$$

As described above in the table, visible light has a wavelength range from ~400 nm to ~700 nm. By this equation, we can easily calculate the wavelength of any electromagnetic radiation, if frequency is known.

Spectroscopy

This EM spectrum emitted by a source is analyzed by spectroscopy through a special device known as spectroscope. In other words, **spectroscope** or **spectrophotometer** is an instrument which is used to analyze EM radiation spectrum especially of ultraviolet, light and infrared ranges. Spectrophotometer transmits and receives light for the analysis of spectrum and therefore it is used to evaluate samples of test materials by passing light by means of the sample and studying the intensity of the wavelengths. It becomes possible due to the fact that different

samples absorbs the light differently and modify the light that is emitting from the test materials and therefore, by viewing the change in light conduct as it passes by way of the test materials; an investigator is able to obtain facts about the test materials like measurement of solute concentration. It was first developed by a Scientist named as Arnold J. Beckman at the National Technologies Laboratories (NTL) in 1940 and the device was then called as *Beckman DU Spectrophotometer*. The Spectrophotometer is used in many studies in biology, chemistry, physics and industrial laboratories. Generally, it is known as **Ultraviolet-Visible (UV-Vis) Spectroscopy**.

Principles

The Spectrophotometer is a much more elaborated version of a colorimeter. In a colorimeter, filters are used which allows a broad range of wavelengths to pass through, whereas in the spectrophotometer a prism (or) grating is used to split the incident beam into different wavelengths. By suitable mechanisms, waves of specific wavelengths are manipulated in a manner so that it falls on the test solution. The range of the wavelengths of the incident light can be as low as 1 to 2nm. The light coming from the test materials are detected on the basis of intensities with a charge-coupled device, and displayed the results as a graph on the detector and then on the display device. This is the basic Principle of spectrophotometry in biochemistry.
Therefore, it is based on the photometric technique which states that when a beam of incident light of intensity ***'I_0'*** passes through a solution, a part of the incident light is reflected ***'I_r'***, a part is absorbed ***'I_a'*** and rest of the light is transmitted ***'I_t'***.

$$\text{Thus, } \boldsymbol{I_0 = I_r + I_a + I_t}$$

In colorimeter and spectrophotometer, ***'I_r'*** is eliminated because the measurement of ***'I_0'*** and is sufficient to determine the ***'I_a'***. For this purpose, the amount of light reflected ***'I_r'*** is kept constant by using cells that have identical properties, and therefore ***'I_0'*** and ***'I_t'*** are then measured.

The mathematical relationship between the amount of light absorbed and the concentration of the substance can be shown by the two fundamental laws of photometry on which the Spectrophotometer is based that is Beer's Law and Lambert's Law.

Beer's Law: This law states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution. It can be represented by following formula;

$$\boldsymbol{Log_{10}\frac{I_0}{I_t} = a_s\, c}$$

Where, ***'a_s'*** is absorbency index and ***'c'*** is concentration of solution.

Lambert's Law: It states that the amount of light absorbed is directly proportional to the length and thickness of the solution under analysis.

$$\boldsymbol{A = Log_{10}\frac{I_0}{I_t} = a_s\, b}$$

Where, ***'A'*** is absorbance of test material, ***'a_s'*** is absorbance of standard, and ***'b'*** is the length/thickness of the solution.

Therefore, in combined form i.e. in Beer-Lambert's law, the equation becomes as follows;

$$A = Log_{10}\frac{I_0}{I_t} = a_s\, bc$$

If ***'b'*** is kept constant by applying cuvette or standard cell, then the same equation becomes as follows;

$$A = Log_{10}\frac{I_0}{I_t} = a_s\, c$$

The absorbency index ***'a_s'*** is then defined as

$$a_s = \frac{A}{Cl}$$

Where, ***'C'*** is the concentration of the absorbing material in ***gm/liter***, and ***'l'*** is the distance traveled by the light in solution in ***cm***.

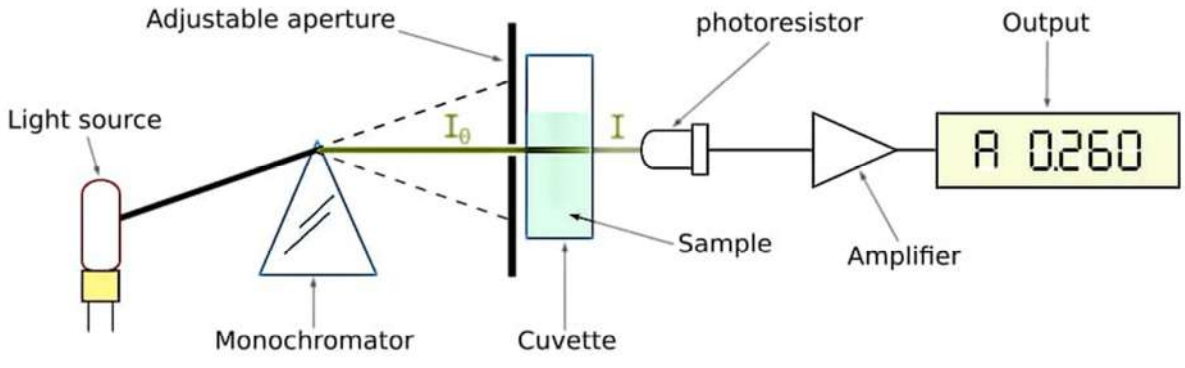
Simply, the Beer-Lambert's law states that the amount of light absorbed by a colour solution is directly proportional to the concentration of the solution and the length of a light path through the solution i.e.

$$A \propto cl \text{ or } A = \in cl$$

Where, ***'A'*** is the absorbance or optical density of solution, ***'c'*** is the concentration of solution, ***'l'*** is the path length of the solution, and ***'$\in$'*** is the absorption coefficient.

Types of Spectrophotometer

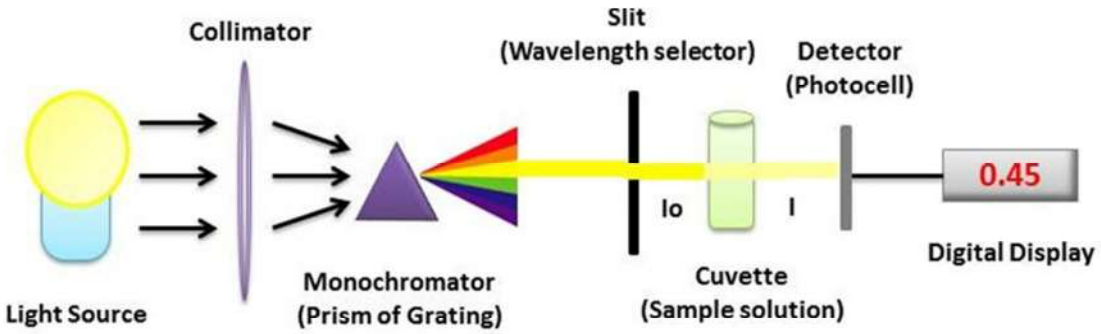
There are two types of spectrophotometers namely Single beam spectrophotometer, and Double beam spectrophotometer.



Single Beam Spectrophotometer

Single beam spectrophotometer: This type of spectrophotometer utilizes single beam of light and operates between 325nm to 1000nm wavelength. In single beam spectrophotometer, the light travels in one direction and the test solution and blank are read in the same.

Double beam spectrophotometer: This type of spectrophotometer utilizes two photocells, which splits the light from the Monochromator into two beams, out of which, one beam is used for reference and the other beam is used for sample reading. Uses of two beams or use of Monochromator eliminates the error which occurs due to fluctuations in the light output and the sensitivity of the detector. It operates between 185nm to 1000nm wavelength.



Double Beam Spectrophotometer

Basic instrumentation of a Spectrophotometer

A spectrophotometer is basically composed by following specialized instruments;

1. A stable and cheap radiant energy source of light
2. Wavelength selectors such as filters and a monochromator to break the polychromatic radiation into component wavelength (or) bands of wavelengths.
3. Transport vessels (cuvettes) to hold the sample
4. A photosensitive detector and an associated readout system.

The detail of these basic instrumentations of a spectrophotometer is following;

1. **Radiant Energy Sources:** Materials that can be excited to high energy states by a high-voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.
   - *i.* ***Sources of Ultraviolet radiation:*** The most commonly used sources of UV radiation are the hydrogen lamp and the deuterium lamp. Xenon lamp may also be used for UV radiation, but the radiation produced is not as stable as the hydrogen lamp.
   - *ii.* ***Sources of Visible radiation:*** "Tungsten filament" lamp is the most commonly used source for visible radiation. It is inexpensive and emails continuous radiation in the range between 350 and 2500nm. "Carbon arc" which provides more intense visible radiation is used in a few commercially available instruments.
   - *iii.* ***Sources of IR radiation:*** "Nernst Glower" and "Global" are the most satisfactory sources of IR radiation. Global is more stable than the nearest flower.

2. **Wavelength selectors:** Wavelength selectors in the spectrophotometer is of two types which are as follows;
   - ***i. Filters:*** Mostly gelatin filters are used. "Gelatin" filters are made of a layer of gelatin, coloured with organic dyes and sealed between glass plates.
   - ***ii. Monochromator:*** It resolves polychromatic radiation into its individual wavelengths and isolates these wavelenths into very narrow bands. The essential components of a monochromator are;
     - **a)** ***Entrance slip –*** admits polychromatic light from the source.
     - **b)** ***Collimating device –*** Collimates the polychromatic light into the dispersion device.
     - ***c) Wavelength resolving device*** like ***Prism*** (or) a ***Grating***[1].
     - **d)** ***A focusing lens*** (or) ***a mirror***.
     - **e)** ***An exit slip*** – it allows the monochromatic beam to escape.

The resolving elements such as PRISM and GRATING has much importance in spectrophotometry.

Prism: A prism is an optical device that disperses polychromatic light from the source into its constituent wavelengths by virtue of its ability to reflect different wavelengths to a different extent. The degree of dispersion by the prism depends on the optical angle of the Prism which is usually 60° and the material of which a prism is made. There are two types of Prisms employed in spectrophotometers commercially namely 60° Cornu quartz and 30° Littrow Prism.

Grating: As described in footnote, it is an optical element used to diffract light into several beams. It is used in the monochromator of spectrophotometers operating ultraviolet, visible and infrared regions.

3. **Sample containers:** Sample containers are also one of the parts of Spectrophotometer instrumentation. Samples to be studied in the ultraviolet (or) visible region are usually glasses (or) solutions and are put in cells known as "cuvettes".
   Cuvettes meant for the visible region are made up of either ordinary glass (or) sometimes Quartz. Most of the spectrophotometric studies are made in solutions, the solvents assume prime importance.
   The most important factor in choosing the solvent is that the solvent should not absorb (optically transparent) in the same region as the solute.
4. **Detection devices:** Most detectors depend on the photoelectric effect. The current is then proportional to the light intensity and therefore a measure of it. Important requirements for a detector includes the following;
   - High sensitivity to allow the detection of low levels of radiant energy
   - Short response time
   - Long-term stability
   - An electric signal which easily amplified for a typical readout apparatus.
5. **Amplification and Readout:** Radiation detectors generate electronic signals which are proportional to the transmitted light. These signals need to be translated into a form

[1] **Grating** or **diffraction grating** is an optical element or a component with a periodic structure that splits and diffracts light into several beams travelling in different directions.

easy to interpret. This is accomplished by using amplifiers, Ammeters, Potentiometers and Potentiometric recorders.

Working of the Spectrophotometer

Before using spectrophotometer, it requires being calibrated first which is done by using the standard solutions of the known concentration of the solute that has to be determined in the test solution. For this, the standard solutions are filled in the Cuvettes and placed in the Cuvette holder in the spectrophotometer that is similar to the colorimeter.

For spectrophotometric analysis of a test solution, there is a beam of light with a certain wavelength that specific for the assay is directed towards the solution. Before reaching the solution the ray of light passes through a series of the diffraction grating, prism, and mirrors. These mirrors are used for navigation of the light in the spectrophotometer and the prism splits the beam of light into different wavelength and the diffraction grating allows the required wavelength to pass through it and reaches the Cuvette containing the standard or Test solutions. When the monochromatic light (light of one wavelength) reaches the Cuvette, some of the light is reflected, some part of the light is absorbed by the solution and the remaining part is transmitted through the solution which falls on the photodetector system. The photodetector system measures the intensity of transmitted light and converts it into the electrical signals that are sent to the galvanometer. The galvanometer measures the electrical signals and displays it in the digital form. That digital representation of the electrical signals is the absorbance or optical density of the solution analyzed.

If the absorption of the solution is higher than there will be more light absorbed by the solution and if the absorption of the solution is low then more lights will be transmitted through the solution which affects the galvanometer reading and corresponds to the concentration of the solute in the solution. By putting all the values in the formula given below, one can easily determine the concentration of the solution.

$$\boldsymbol{A = \in cl}$$

⇒ In double beam spectrophotometers, the beam splitters are present which splits the monochromatic light into two beams one for the standard solution and the other for test solution. In this, the absorbance of Standard and the Test solution can be measured at the same time and any no. of test solutions can be analyzed against one standard. It gives more accurate and precise results, eliminates the errors which occur due to the fluctuations in the light output and the sensitivity of the detector.

$$A = \in cl$$

As described, for two solutions i.e. test and standard, $\in$ is constant l is also constant (using the same cuvette or standard cell)

$$A_T = C_T \qquad \text{..... (i)}$$

$$A_S = C_S \qquad \text{..... (ii)}$$

From (i) & (ii),

$$A_T \times C_S = A_S \times C_T$$

$$C_T = (A_T/A_S) \times C_S$$

Where,

C_T = Concentration of the Test solution

A_T = Absorbance/ Optical density of the test solution

C_S = Concentration of the standard

A_S = Absorbance / Optical density of the standard solution

Applications of Spectrophotometer

The spectrophotometer is commonly used for the determination of the concentration of colored as well as colorless compounds by measuring the optical density or its absorbance.

It can also be used for the determination of the course of the reaction by measuring the rate of formation and disappearance of the light absorbing compound in the range of the visible & UV region of electromagnetic spectrum.

By spectrophotometer, a compound can be identified by determining the absorption spectrum in the visible region of the light spectrum as well as the UV region of the electromagnetic spectrum.

Problems Related to Elctromagnetic Radiation and its Spectra

Problem - 1

Two microwave frequencies are authorized for use in microwave ovens, 900 and 2560 MHz. Calculate the wavelength of each.

Solution –

- Reasoning:
  For all electromagnetic waves in free space $\lambda f = c$.
- Details of the calculation:
  $\lambda = c/f$

  Wavelength of one microwave λ (900MHz) = $3 \times 10^8/900 \times 10^6 = 1/3$ m

  Wavelength of one microwave λ (2560 MHz) = $3 \times 10^8/2560 \times 10^6 = 11.7$ m

Problem - 2

Distances in space are often quoted in units of light years, the distance light travels in one year.
(a) How many meters is a light year?

(b) How many meters is it to Andromeda, the nearest large galaxy, given that it is 2.54×10^6 light years away?
(c) The most distant galaxy yet discovered is 12×10^9 light years away. How far is this in meters?

Solution –

- Reasoning:
  All electromagnetic waves in free have speed c.
- Details of the calculation:
  (a) 1 light year (ly) = distance light travels in one year
  $= (3 \times 10^8 \text{ m/s}) \times (365 \times 24 \times 3600 \text{ s}) = 9.46 \times 10^{15}$ m.
  (b) The distance to Andromeda is $2.54 \times 10^6 \text{ ly} \times 9.46 \times 10^{15} \text{ m/ly} = 2.4 \times 10^{22}$ m.
  (c) The distance to this galaxy is $12 \times 10^9 \text{ ly} \times 9.46 \times 10^{15} \text{ m/ly} = 1.14 \times 10^{26}$ m.

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