GENERAL INTRODUCTION TO ELECTROMAGNETIC SPECTRUM AND MOLECULAR SPECTROSCOPY

Spectroscopy, Spectrometery and Spectrum:

Spectroscopy:

Interaction of electromagnetic radiation with matter is called spectroscopy. OR

"Spectroscopy deals with the transition induced in a chemical species by its interaction with the photons of electromagnetic radiation."

Spectroscopic methods are generally used to measure the energy difference between various molecular energy levels and to determine the atomic and molecular structures.

***Spectrometery*:**

The instruments used in such studies, called spectrophotometers, these are devices to measure the relative energy that is emitted, transmitted or reflected in the infrared, visible or ultraviolet regions, as a function of wavelength or wave number. Special devices are incorporated in these instruments for the automatic recording of spectra.

***Spectrum*:**

The spectrum of molecule is thus presented as continues graph obtained by plotting either absorption or transmittance of EMR as a function of wavelength or wave number over a particular range.

Nature of electromagnetic radiation and electromagnetic spectrum:

Light or EMR is a form of energy that is transmitted through space at a constant velocity of 3×10^8 ms^{-1}. These radiations are said to have dual nature exhibiting both wave and particle characteristics. The dual character is indeed useful for understanding the interactions of radiation with matter.

***Wave theory of electromagnetic radiations*:**

According to this theory, the electromagnetic radiation travel in the form of waves. This wave motion consists of oscillating electric and magnetic fields directed perpendicular to each other and perpendicular to the direction of propagation of the wave.

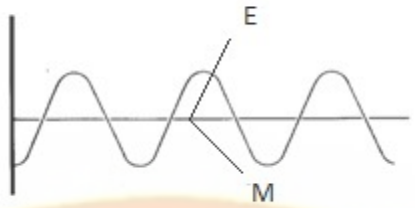


Fig: A beam of EMR, showing the electric (E) and magnetic (M) component.

In spectroscopic studies, the effects associated with the electrical component of the electromagnetic wave are important.

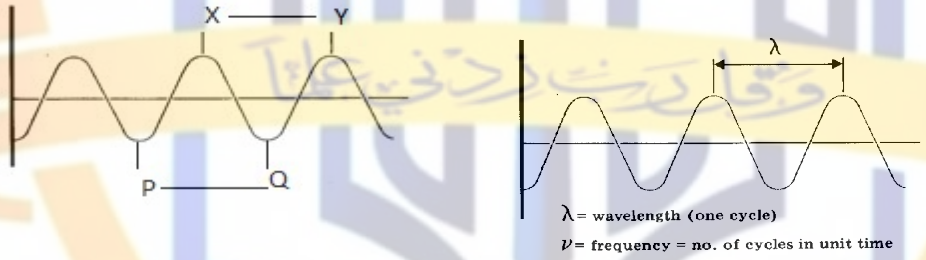


Fig: The electric component of a propagation light wave.

The points X, Y, P and Q on the wave represent the maximum disturbance in the electric field. Distance from the mean position is known as amplitude of the wave. The distance from the crest X to crest Y (or from valley P to valley Q) is the wavelength λ.

Frequency: The number of complete wavelength units passing through a given point per second is called frequency ν. These two quantities are related to each other by the equation

$$\nu = {}^{c}/_{\lambda} \qquad \text{equation (1)}$$

Where, C is velocity of electromagnetic wave. Since C is constant (3×10^8 ms^{-1} in vacuum) for all types of electromagnetic radiations, the above relation may be expressed as

$$\nu \alpha {}^{1}/_{\lambda} \quad \text{equation (2)}$$

Reciprocal of wavelength, i.e. ${}^{1}/_{\lambda}$ is called *wave number*, ν'.
So equation (1) may be written as

$$\nu = c\,\nu' \quad \text{equation (3)}$$

The wavelength is expressed in terms of centimeters (cm), meter (m), microns (μ) or micrometer (μm) or angstrom Å units.

The other commonly used unit is nanometer (nm) where

$1m = 10^{-9}\,m = 10^{-3}\,\mu m = 10$ Å

$1Å = 10^{-10}$ m , $1\mu m = 10^{-6}$ m, $1cm = 10^{-2}$ m, $1nm = 10^{-9}$ m, $1mm = 10^{-3}$ m

Frequency is measured as cycles per second (cps) called hertz (Hz). Bigger units are kilocycles (KHz) per second or megacycles per second (MHz).

$1KHz = 10^3$ Hz and $1MHz = 10^6$ Hz or cps

The ***wave number*** is the number of waves per unit distance and is expressed in units of cm^{-1} called "kaysers" (K), sometimes kilo kayser (KK) is also used;

$1KK = 1000\ k = 1000\ cm^{-1}$

Quantum theory of electromagnetic radiation:

Quantum theory describes the electromagnetic radiation consisting of stream energy packets called photon or quanta, which travel in the direction of propagation of the beam with the velocity of light. Thus, during emission or absorption of light by chemical species, the energy changes take place only discretely, always as integral multiple of small units of energy i.e. photon. The energy E of the photon is proportional to the frequency of radiation and is given by equation.

$$E = h\nu \quad \text{equation (4)}$$

Where h is plank's constant [6.625×10^{-27} erg second, 6.625^{-34} Joule second (Js)].

The energy of a photon is called quantum of energy and this depends only on the frequency but not on the intensity of radiation.

So equation (4) can also be written as

$E = h\nu = hc/\lambda = hc\nu'$ equation (5)

Electromagnetic spectrum:

The electromagnetic spectrum, for most spectroscopic purpose, is considered to consisting of the region of radiant energy ranging from wavelength of 10 meters to 1 X 10^{-12} centimeters. When a molecule absorbs electromagnetic radiation, it can undergo various types of excitation. This excitation may be electronic excitation, rotational excitation, excitation leading to a change in nuclear spin, excitation resulting in bond deformation and so on. If the energy available approaches the ionization potential of the molecule, an electron may be ejected and ionization may occur. Since each mode of excitation requires a specific quantity of energy, the different absorption appear in different regions of the electromagnetic spectrum

Different regions of electromagnetic spectrum			
Types of radiations	**wavelength**	**Wave number**	**Type of molecular spectrum**
Radio frequency	>100 mm	< 3 X10^{9} cm^{-1}	NMR(spin orientation)
Microwave	1 to 100 mm	10 to 0.1 cm^{-1}	Rotational
Far-IR	50 µm to 1mm	200 to 10 cm^{-1}	Vibrational fundamentals or rotational
Mid-IR	2.5 to 50 µm	4000 to 667 cm^{-1}	Vibrational fundamentals
Near-IR	780nm to 2.5 µm	(13 to 4) x10^{3} cm^{-1}	Vibrational (over tones)
Visible	380 to 780 nm	(2.6 to 1.3) x 10^{4} cm^{-1}	Electronic(valance orbital)
Near UV	200 to 380 nm	(5 to 2.6) x 10^{4} cm^{-1}	
Vacuum UV	10 to 200 nm	10^{6} to 5 x 10^{4} cm^{-1}	
X-rays	10 pm to 10 nm	10^{9} to 10^{6} cm^{-1}	Electronic (core orbital)
Gamma rays	10^{-10} cm	10^{10} cm^{-1}	Nuclear transition (excitated states of nuclei)
Cosmic rays	10^{-12} cm	10^{12} cm^{-1}	Nuclear transition (excitated states of nuclei)

Absorption of electromagnetic radiation by organic molecule:

When a molecule absorbs radiation, its energy increases in proportion to the energy of the photons as per the relation

$$E = h\nu = {}^{hc}/_{\lambda} \text{ equation (1)}$$

Since the energy absorbed by a molecule is quantized, there will not be continuous absorption by a molecule throughout a particular spectral range; instead the molecule will absorb those frequencies which will provide it with the exact quanta of energy necessary to raise its normal energy level to a higher level or levels. Thus, when an organic molecule interacts with electromagnetic radiation, it may change its energy from E_1 to E_2 by absorption of radiation of frequency ν, so that

$$E_2 - E_1 = nh\nu \text{ equation (2)}$$

Where n is an integer.

The lowest state of energy of an atom or molecule is called the ground state. By absorbing one quantum of energy, hν, the molecule is promoted to the next higher level and is said to be in excited state. Similarly, absorption of more energy in integral multiple of hν, will result in further excitation to higher energy levels.

Study of absorbed radiation from a continuous source that is utilized in raising the internal energy of a molecule constitutes absorption spectroscopy. After the absorption of energy, the excited species return to the ground state by emitting this energy as radiations. The study of this emitted radiation constitutes emission spectroscopy.

The portions of the electromagnetic radiation which do not satisfy relation (2) may either simply pass through the matter or undergo scattering or reflection with or without change of wavelength.

Types of molecular spectroscopy:

1. Infrared spectroscopy
2. Pure rotational (microwave) spectroscopy
3. Ultra violet and visible spectroscopy
4. Nuclear magnetic resonance spectroscopy
5. Electron spin resonance spectroscopy
6. Mass spectroscopy

Infrared spectroscopy (IR):

The absorption of infrared radiation leads to vibrational transition of the molecules. This absorption bands in this region correspond to fundamental vibrational frequencies of molecules. The energy involved is 4000 to 667 cm^{-1} for organic compounds (Mid IR).

Microwaves spectroscopy:

The transitions between rotational energy levels of a molecule occur in the micro waves region and contain a permanent dipole moment. For lighter molecules such as HCl, HF etc, the transition occur in far-infrared region. The energy involved is in the range from 10 - 0.1 cm^{-1}.

Ultraviolet and visible spectroscopy:

Branch of spectroscopy deals with transitions between the electronic energy level of a molecule brought about by absorption of ultra violet or visible radiation.

180 - 800 nm

Nuclear magnetic resonance (NMR):

NMR spectroscopy is concerned with the study of interaction of energy with spin-active nuclei. Spin active nuclei have permanent magnetic moments and quantized nuclear spin states.

Electron spin resonance (ESR) spectroscopy:

Electron spin resonance spectroscopy pertains to the study of atoms, ions, free radicals, and molecules possessing and odd number of electrons which display characteristic magnetic properties arising from the spin properties of unpaired electrons.

Ultraviolet/ Visible spectroscopy

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. Instruments that measure electromagnetic emissions are called spectroscopes or spectrograph. Those that measure electromagnetic absorption are called spectrophotometer. All spectroscopic instruments separate electromagnetic radiation into its components wavelengths to enable one to measure the intensity or strength of the radiation at each wavelength.

There are three kinds of emission spectra

1. Continues spectra, which are emitted by incandescent solids.
2. Line spectra, which are characteristic of atoms that have been excited and are emitting their excess energy.
3. Band spectra, which are emitted by excited molecules.

Electrons in an atom are normally in their lower energy states known as ground state. When sufficient energy is added, electrically or thermally, one or more electrons may be raised to higher energy states. When these electrons lose their energy and return to their ground state, they emit electromagnetic radiations. In returning to their ground state, they may do this in several discrete jumps or energy changes, emitting light of different wavelengths for each jump. When high energy excitation is used, more lines appear in the spectrum.

Radiant power (P):

The rate at which energy in a beam of radiation arrives at some fixed point. Intensity (I) is the same term.

Transmittance (T):

The ratio of the radiant power (P) in a beam of radiation after it has passed through a sample to the power of the incident beam (P_o). It is also referred to as percent T or T X 100

$$T= P/P_o$$

Absorbance (A):

Also called the optical density, and it is the logarithm (base 10) of the reciprocal of the transmittance (T)

$$A= \text{Log } 1/T = \text{Log } P_o/P$$

Where, P= radiation transmitted by the solution

P_o= radiation transmitted by the pure solvent

Molar Absorptivity (ε) :

(Also known as molar extinction coefficient)

The absorbance of a solution divided by the product of the optical path (l) in cm and the molar concentration (c) of the absorbing molecules or ions.

$$\varepsilon = A/lc$$

***Molecular orbital theory*:** Two atomic orbitals from the two bonding atoms combine to form one " bonding" molecular orbital of low energy and one "anti bonding" molecular orbital of very high energy(δ, δ^*, π, π^*). Sigma bonds are formed when there is "head-on" atomic orbital overlap and pi bonds are formed when there is parallel atomic orbital overlap. Valance electrons which are not participating in chemical bonding in molecules are referred to as non bonding or " n" electrons.

Ultraviolet and visible radiation absorption promotes the electronic transition

$$\delta \rightarrow \delta^*, n \rightarrow \delta^*, n \rightarrow \pi^*, \pi \rightarrow \pi^*$$

Above diagram shows that the ΔE values for transitions are in the order:

$$n \rightarrow \pi^* < \pi \rightarrow \pi^* < n \rightarrow \delta^* << \delta \rightarrow \delta^*$$

The energy required for the $\delta \rightarrow \delta^*$ transition is very large.

Chromophores:

Functional groups that absorb visible and/or U.V radiations are called charomophore, when they are bonded to a non bonding, saturated residue which possess no unshared, non bonding valance electron (e.g. a hydrocarbon chain)

Type	Example	Absorption bond, nm
Alkenes	$CH_2=CH_2$	165-193
Alkynes	$HC\equiv CH$	145-225
Aldehyde	CH_3CHO	180-290
Ketones	CH_3COCH_3	188-279
Carbonyl acid	CH_3COOH	208-210
Aromatics	C_6H_6	204-254

Auxochromes:

These are functional groups such as –OH, $-NH_2$ and -Cl which have nonbonding valance electrons and do not absorb radiations at wavelength > 200 nm.

Bathochromic shift or effect (red shift):

When an Auxochrome is attached to a chromophore, the chromophore absorption band typically shifts to longer wavelength and increase in intensity (i.e. the molar Absorptivity, ε_{max}, at the wavelength of maximum absorbance, λ_{max} increase).

Hypsochromic shift or effect (blue shift):

The hypsochromic effect is a shift of the absorption band to shorter wavelength; this effect is often noted when positive charge is introduced into the molecule and when changing from non polar to polar solvents.

Theory of spectrophotometery:

The radiant energy absorbed by a solution can be used to detect and identify the analyte quantitatively. This was recognized by Bouger's experiment.

He placed a series of identical containers; full of an absorbing material in a row and measured the amount of light falling on each cell and amount that passed through it. He assumed radiation falling upon the first cell had a value of 1.0; only 50% was transmitted by it. In the like manner only 25% of the original light could be detected after passing through two cells. To describe these observations quantitatively, transmittance was defined as

$$T = P / P_o$$

Where P is the amount of radiant energy transmitted by the cell and P_o is the amount of radiant energy falling upon the cell.

LAMBERT (1760) investigated the relationship between P and P_o and BEER extended the experiments to the solution and deduced their laws. The combinations of these two laws are known as Lambert-Beer law which forms the basis of spectrophotometery.

LAMBERT LAW

It be stated that amount of radiant energy transmitted decreases exponationally as the thickness of absorbing material (l) increases.

Mathematically it can represented as

$I = I^o e^{-kl}$

Now by taking ln on both sides above equation can be written as

$\ln I = \ln I^o \ln e^{-kl}$

As we know that natural logarithm (ln) and exponantional are opponent to each other and can be cancelled so above equation can also be written as,

$\ln I = \ln I^o - kl$

$\ln I - \ln I^o = -kl$

$\ln I^o - \ln I = kl$

$\ln(I^o/I) = kl$

Now if we convert the ln to $\log_{10}$, as we know that ln is equal to 2.303 log, so

$2.303 \log (I^o/I) = kl$

$\text{Log } (I^o/I) = kl/2.303$

As we know that k and 2.303 are constants, so if we replace these constant with another constant k′, the above equation can be written as

Log (I^o/I) = k′l OR A= k′l Lambert law equation (1)

According to above equation the absorbance is directly proportional to the path length.

BEER'S LAW

It can be stated that amount of radiant energy transmitted decreases exponationally as the concentration of absorbing material (c) increases.

Mathematically it can represented as

$I = I^o e^{-kc}$

Now by taking ln on both sides above equation can be written as

$\ln I = \ln I^o \ln e^{-kc}$

As we know that natural logarithm (ln) and exponantional are opponent to each other and can be cancelled so above equation can also be written as,

$\ln I = \ln I^o - kc$

$\ln I - \ln I^o = -kc$

$\ln I^o - \ln I = kc$

$\ln(I^o/I) = kc$

Now if we convert the ln to $\log_{10}$, as we know that ln is equal to 2.303 log, so

$2.303 \log (I^o/I) = kc$

$\text{Log } (I^o/I) = kc/2.303$

As we know that k and 2.303 are constants, so if we replace these constant with another constant k″, the above equation can be written as

Log (I^o/I) = k″c OR A= log 1/T OR A= k″c Beer law equation (2)

According to above equation the absorbance is directly proportional to the concentration of absorbing material.

On combining equation 1 and 2 we obtained Beer's law equation

A= k′l k″c Or A= k′ k″ cl

If we replace k′ k″ with new constant 'a'

A= acl

This is fundamental equation of spectrophotometery and is often called beer-lambert law equation. The value of "a" will depends upon the method of expression of the concentration. If C is expressed in moles/litter and "l" in centimeter then "a" will be replaced by ε which is called molar extinction coefficient (now commonly known as molar absorption coefficient). ε can thus be defined as reciprocal of thickness in centimeters of 1 molar solution required to reduce the light to $1/10^{th}$ of its intensity. When the molecular weight is not known, it is not possible to write molar extinction coefficient. In such cases an alternate way is to express the extinction coefficient as specific extinction coefficient $\varepsilon^{1\%}_{1cm}$, which may be defined as absorption per unit thickness (1cm) and unit concentration (1%).

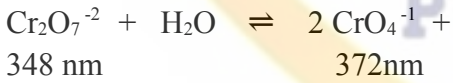
Transmittance is usually expressed as a range 0-100%, but absorbance has no units and varies from $0 - \infty$.

Deviations from the beer-lambert law:

There are no known exceptions to the Lambert law for homogenous samples. Beer's law is limiting case applicable only to dilute solutions and monochromatic radiation.

Apparent deviation may be summarized as follows.

1. At concentration greater than about 0.01M, refractive index changes and perturbing effect of solute molecules or ions on the charge distribution of their neighbors both affect the value of ε. Positive or negative deviation may result.
2. Solutes involved chemical equilibria i.e. dissociation, association or complex formation or in interaction with solvent molecules, may show marked spectral changes with concentration, e.g. dichromate and chromate ions in aqueous solution interconvert to a degree which is pH dependant.



Unless standards are prepared in buffered media, positive or negative deviations may result from measurements at 380 nm or 372 nm respectively. Alternatively, measurements can be made at the isobestic point i.e. where the absorbance curves of each form intersect, and absorbance is not a function of equilibrium concentrations but only of overall concentration. Solution of week acids and bases should also be measured at their isobestic point for the same reason.

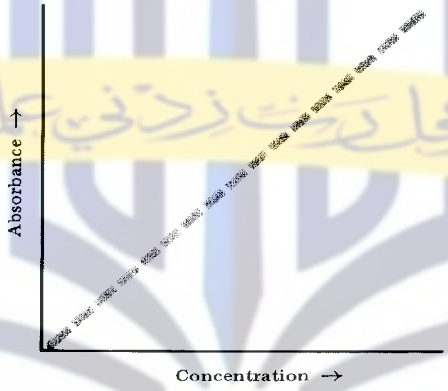
3. Negative deviations occur if the radiation used in polychromatic, as in the case of filter photometer.

4. Stray light passing through the optical system is generally not absorbed by the sample to the same extent as that of the selected wavelength, its presence leads to negative deviations.

Note: Stray light (radiation): stray radiation is that portion of the light reaching the detector that is not the desired wavelength, given by the portion of monochromator.

Use of the beer-lambert law:

Although applicable to measurements in all regions of electromagnetic spectrum, only in visible U.V and infrared spectrometery are quantitative measurements based on Beer-Lambert law used extensively. The usual procedure is to prepare a calibration graph or beer's law plot, by plotting absorbance against concentration for a series of standards. This should give a straight line passing through origin and slope equal to product ε l.



Measurements are generally made at a maximum wavelength in the absorbance curve to maximize sensitivity and to minimize errors in setting the instrument at chosen wavelength. This also minimizes apparent deviations from beer's law for incident radiation of wide band width. The concentrations of unknown can then be read directly from the graph or calculated using a factor i.e. the absorbance reading is divided by slop ε l.

The composition mixture of two or more absorbing materials can be established by measuring standards and samples at two or more wavelengths preferably corresponding to the absorbance maximum of each component. The concentration of components can be calculated from a set of simultaneous equations once the respective molar absorptivities at each wavelength are known e.g. for two components

$$A \text{ Mixture } = \varepsilon_1^{\lambda 1} c\, l + \varepsilon_2^{\lambda 1} c\, l \quad \text{at } \lambda 1$$

And A Mixture $= \varepsilon_1^{\lambda 2} c\, l + \varepsilon_2^{\lambda 2} c\, l$ at $\lambda 2$

The values of $\varepsilon_1^{\lambda 1}$, $\varepsilon_2^{\lambda 1}$ etc are calculated from calibration graphs for the separate components.

Conjugation effects:

Absorption bands due to conjugated chromophores are shifted to longer wavelength (bathochromic or red shift) and intensified relative to an isolated chromophore. The shift can be explained in terms of interaction or delocalization of the π and π* orbitals of each chrompphore to produce new orbitals in which the highest π orbital and the lowest π* orbitals are closer in energy for example the conjugation of two ethylene chromophores to form 1,3 butadiene. The π → π* transition in ethylene occurs at 165 nm with an ε value of 1500 whereas, 1,3 butadiene the values are 217 nm and 2100 respectively.

Fig. Effect of conjugation on absorption of ethylene.

If two unlike chromophores are conjugated and one group has non bonding electrons, the n → π* transition is also shifted bathochromically because the energy of the anti bonding orbital is lowered. Thus, the weak n→ π* band in a saturated carbonyl compound is shifted from below 300 nm to above 300 nm with an increase in ε.

Conjugation of additional chromophoric groups moves λ max progressively towards a visible region and increase ε. For example, tetradecahexane (6 double bonds) absorbs at the blue end of the visible region and appears yellow whilst with further conjugation, as in the carotenes (10 or more double bonds), the compound may appear orange, red, purple or even black.

Benzene can be regarded as a special case of the conjugated triene. It has a relatively weak π → π* band near 255 nm which shifts bathochromically and intensifies on chromophoric substitution. Of more important is that substitution produces a new and intense band between 200 and 300 nm which arises from a π → π* transition in the extended conjugated system.

Effect of auxochromes:

In general, auxochromic substitution of Chromophores causes Bathochromic shifts and increase in intensity for $\pi \rightarrow \pi^*$ transitions, and Hypsochromic shifts (to shorter wavelengths) for $n \rightarrow \pi^*$ transition. The shifts are explainable in terms of mesomeric (resonance) effects caused by interactions of lone pair electron associated with such Auxochromes as –OH, -Cl, $-NH_2$ with the π system of Chromophores. This leads to increase in the energies of π and π^* orbitals, the π being raised by more than the π^*, but leaves the energy of the non bonding orbital unchanged.

Solvent effects:

Absorption bonds arising from $n \rightarrow \pi^*$ transition suffer hypsochromic shifts on increasing the solvent polarity, whilst those of $\pi \rightarrow \pi^*$ transitions are shifted bathochromically. Explanations lie in the fact that the energy of non bonding orbital is lowered by hydrogen bonding in the more polar solvent thus increasing the energy of the $n \rightarrow \pi^*$ transition, but the energy of π^* orbital is decreased relative to the π orbital.

The position and intensities of the $\pi \rightarrow \pi^*$ bonds in such compounds as phenols and amines exhibit a marked sensitivity to pH changes because of changes in the interaction of non bonding electrons with the π system.

Instrumentation of ultraviolet – visible spectrophotometer

The essential components of spectrophotometer are:

1. Stable source of radiant energy
2. System of lenses , mirrors and slits, which define, collimate(make parallel) and focus the beam
3. Monochromator to resolve the radiation into component wavelengths or bands of wavelengths
4. Transparent container to hold the sample called cell
5. Radiation detector
6. Read out system

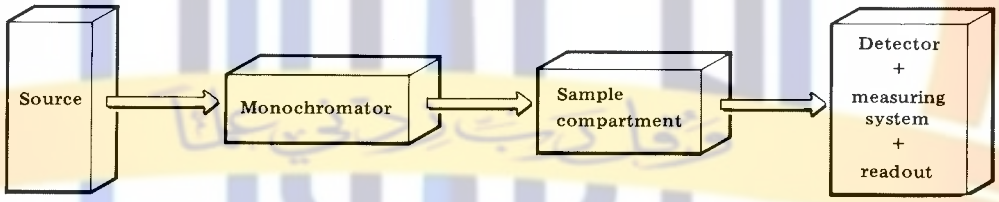


Fig: Block diagram of spectrophotometer

1. **Source of radiant energy:**

Source of radiant energy consist of materials that are excited to higher energy states by a high voltage electric discharge or by electrical heating. As the material return to low energy states or their ground states, they emit photons of characteristics energies corresponding to the energy difference between the excited and lower energy states.

Source of ultra violet radiation

The hydrogen lamp and deuterium are the most common sources of u.v radiation. They consist of a pair of electrodes which are enclosed in a glass tube provided with a quartz window and filled with hydrogen or deuterium gas at low pressure. When a stabilized high voltage is applied to electrodes, an electrode discharge occurs which excite other electrons in the gas molecules to high energy state. As the electrons return to their ground sate, they emit radiation which is continuous in the region roughly between 180 and 350 nm.

Source of visible radiation

A tungsten filament lamp is the most satisfactory and in expensive source of visible radiations. The filament is heated by a stabilized DC power supply, or by a storage battery. The tungsten filament emits continuous radiation in the region between 350 and 2500 nm.

2. Lenses and mirrors:

Radiation is collimated and focused by lenses and mirrors. Materials used for lenses must, of course, be transparent to the radiation being used.

3. **Monochromator:**

Monochromator resolves polychromatic radiation into its individual wavelengths and isolates these wavelengths into very narrow bands. The components of a monochromator include:

An entrance slit which admits polychromatic radiation from the source.

A collimating device, either a lens or a mirror.

A dispersion device, either a prism or grating, which resolve the radiation into component wavelengths.

A focusing lens or a mirror

An exit slit

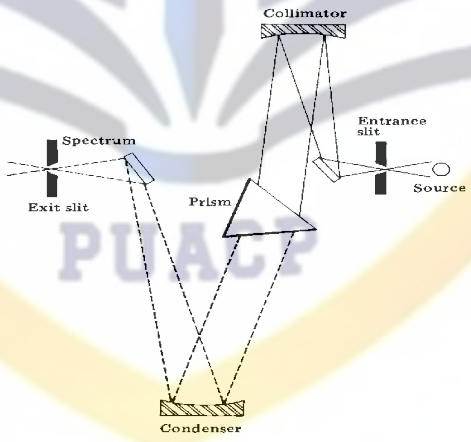


Fig: Prism monochromator

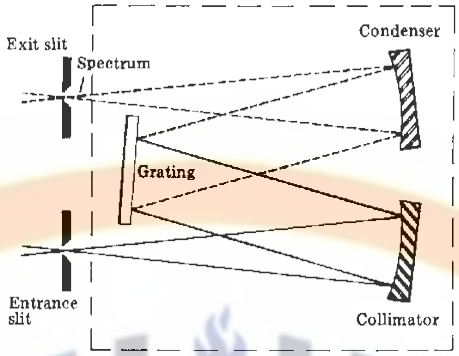


Fig: Diffraction grating Monochromator

When wave of an electromagnetic radiation strikes an obstacle that does not reflect or refract them, a change occurs in their amplitude or phase, this change is called **Diffraction.** A diffraction grating is a flat piece of metal, glass or plastic that has a great many parallel grooves ruled on it. Non visible electromagnetic radiations that are directed onto its surface are separated into their individual wavelength components.

4. Sample container or sample cell or cuvette

Samples to be studied in the ultraviolet or visible region are usually solutions and are put in cells or cuvettes. Quartz or fused silica cells are used in the ultraviolet region, while ordinary glass or more expensive quartz is used in the visible region. Cells with known width and optical length are used. Protect them from scratches. Avoid the use of abrasive cleaning agents, clean them well with soft cloths, avoid finger marks and lint or dirt and handle them only by the top edge when inserting them into the instrument. Because of additional reflection from the air to glass surface, empty cells transmit less radiation than do cells filled with reference standards, such as distilled water.

5. Ultraviolet and visible radiation detectors:

Ultraviolet and visible photons possess enough energy to cause photo ejection of electrons when they strike surfaces which have been treated with specific types of compounds. This process generates an electric current which is directly proportional to the radiant power of the absorbed

photons. Devices which employ these systems are called photoelectric detectors and are sub-classified as phototubes and photovoltaic cells.

Phototubes:

Phototube consists of :

a. An evacuated glass envelope (with a quartz window for use in ultraviolet region)
b. A semi-cylindrical cathode which has an inner surface coated with a compound with relatively loosely bound electrons, such as an alkali or alkaline earth oxide
c. A central metal wire anode

A potential difference of approximately 90 volts is applied across the electrodes. The radiation enters through the quartz window and strikes the photo emissive surface of the cathode. The photons are absorbed and transfer their energy to the loosely bound electrons of the surface material. The electrons escape from the surface and are collected at the anode causing the current to flow through the circuit. If the electron collection is essentially 100% efficient, the phototube current should be proportional to the radiant power of the incident radiation. However, the magnitude of the photocurrent also depends on the voltage applied to the electrodes and the wavelength of the incident radiation.

A small phototube current, known as "**dark current**" is observed even when there is no incident radiation on the phototube. This is a result of the random thermal emission of electrons from the cathode surface. The magnitude of dark current increases with increasing cathode surface area and increasing temperature.

Photomultiplier tubes*:*

In the discussion of phototubes we have indicated the mechanism by which an electron escapes from a photo emissive surface. If the ejected electron is accelerated by an electric field, it acquires more energy and if it strikes another electron active surface, it may transfer some of its energy, ejecting several more electrons. These electrons may in turn be accelerated to another

surface and produce even more electrons and so on. This is the principle of the photomultiplier tube. Each succeeding electron-active plate, or dynode, is at higher electrical potential and thus acts as an amplification stage for the original photon. After nine stages of amplification, the original photon has been amplified by a factor of approximately 10^6. In practice, photomultiplier tubes are used only for low radiant power levels, otherwise they exhibit great instability.

6. Read out systems:

Readout system composed of amplifier and meter or recorder. In modern equipment computer is attached with this system to save the results.

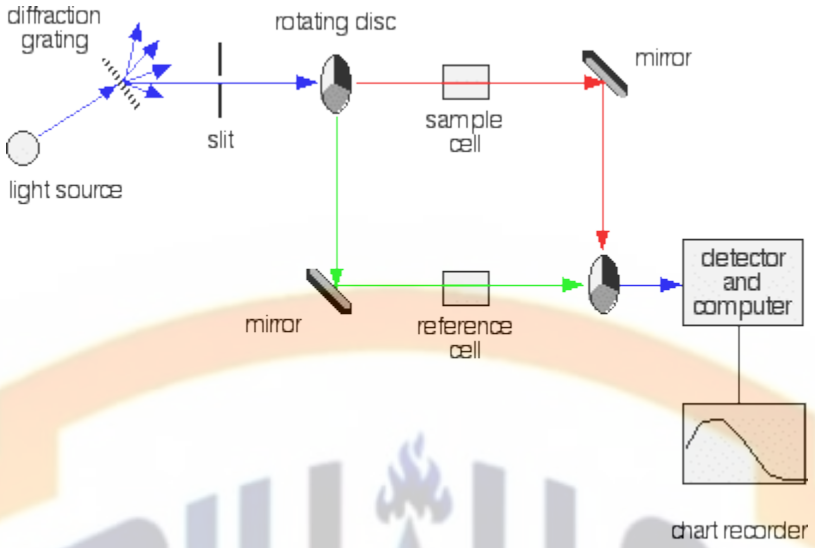
Single beam and double beam instruments:

Two basic instrument designs are in use in commercial spectrophotometer, one employing single beam only whereas the other provides a double beam.

Single beam operation:
A beam of radiation from the source enters the monochromator where it is dispersed by a prism or grating. As the dispersing element is rotated, the various resolved bands of radiation are focused at the exit slit. The radiation then passes through the cell and then on a detector. The single beam method requires stable, high quality components in the source, detector, and amplifier for high precision measurements. The instrumental parameters can fluctuate between the time of 100% T calibration with the blank and determination of the transmittance of the sample. Direct reading instruments using meters give immediate readout with an accuracy of ± 0.2 to 3% in transmittance. Unless, high accuracy is required, the meter readout instruments are quite satisfactory. Single beam instruments are simpler and less expensive than the double beam, but they are not readily adapted to recording because of the necessity of calibration at each wavelength.

Double beam operation:

Double beam instruments employ some type of beam splitter prior to the sample cells. One beam is directed through the “blank” cell (or reference cell) and the other beam through the sample cells. The two beams are then compared either continuously or alternately many times a second. In the double beam design, fluctuations in the source intensity, the detector response and amplifier gain are compensated for by observing the difference signal between the blank and the sample. Thus double beam instruments are more sophisticated electronically and mechanically than the single beam design and consequently are more expensive.



Qualitative analysis:

Visible and UV spectrophotometry are of secondary importance to other spectral methods for the identification and structural analysis of unknown compounds. This is a direct consequence of the broad bands and rather simple spectra, which make differentiation between structurally related compounds difficult. As an adjunct to infrared, magnetic resonance and mass spectrometry, however, it can play a useful role. It can be particularly helpful in conforming the presence of acidic or basic groups in a molecule from the changes in band position and intensity associated to changes in pH.

Quantitative analysis:

The use of visible and ultraviolet spectrophotometry for quantitative analysis by comparing the absorbance of standards and samples at a selected wavelength in perhaps the most widespread of all analytical techniques. It is also one of the most sensitive. The analysis of mixture of two or more components is facilitated by the additivity of absorbance. Quantitative methods based on the absorption of electromagnetic radiation involve measurement of the reduction in intensity of the radiation on passage through an absorbing medium i.e. the sample. For monochromatic, collimated radiation passing through a homogenous liquid sample, the reduction in the intensity of the incident radiation can be related to the concentration of absorbing species and to the thickness of the absorbing medium, both relations are described in Beer- Lambert law.

Applications of UV/visible spectrometry:

Quantitative analysis by visible and UV spectrometry is practiced by almost every analytical

laboratory at one time or another. Most inorganic, organic and biochemical substances can be determined either directly or after the formation of an absorbing derivative or complex. A selection of typical application is given in following table.

Element or compound determined	**Reagent**	**Example or application**
Fe	o-Phenanthroline	Natural water, petroleum products
Cu	Neocuproine	Minerals, alloys
Mn	Oxidation to MnO^-_4	Steels
Cr	Diphenylcarbazide	Alloys, minerals
Hg, Pb	Dithizone	Food products, fish
P, PO_4^{-3}	Reduction to molybdenum blue	Fertilizer residue, soils
F^-	Lanthanum alizarin complexone	Drinking water
Aspirin	-	Analgesic preparation
Paracetamol	-	Analgesic preparation
Vitamin A	Glycerol trichlorohydrin	Food stuffs
Sulphonamides	Diazo derivatives	Drug preparation

The technique is among the most sensitive and is predominantly used for the determination of minor, trace or ultra trace level constituents. Its applications to the determination of metals in trace amounts were particularly widespread because of the many intensely colored complexes known and the degree of selectivity introduced by proper choice of organic reagent and masking reactions or by solvent contraction.

Calibration of ultraviolet visible spectrophotometer:

1. Wavelength accuracy or control of wavelength/photometric control:

Wavelength accuracy is the closeness of the wavelength value reported by the instrument to the actual value. This check can be done using holmium perchlorate solution or holmium oxide glass. The measurements are taken in the following wavelengths, 241.15nm, 287.15nm, 361.5nm and 536.3 nm . Maximum tolerance in ultraviolet region is ± 1nm and ± 3nm in visible region.

2. Control of absorbance or photometric linearity:

This is a general check of the instruments performance to confirm that a solution known to

conform to the Beer- Lambert law will give a linear plot of absorbance verses concentration when measured by a spectrophotometer. For control of absorbance solution of potassium dichromate is used. 57 to 63 mg of $K_2Cr_2O_7$ dissolved in 1000 ml of 0.005 M H_2SO_4. Tolerance is ±0.01.

Wavelength	A(1%,1cm)	Max. tolerance
235nm	124.5	122.9 to 126.2
257nm	144.5	142.8 to 146.2
313nm	48.6	47.0 to 50.3
350nm	107.3	105.6 to 109.0

3. Limit of stray light:

For stray light determination 1.2% solution of potassium chloride is used. Absorbance greater than 2.0 at a wavelength between 198 nm and 20 2nm (200 nm usually used) when compared with water as reference liquid.

4. Resolution (for quantitative analysis):

The test solution is a 0.02% solution of toluene in n-hexane. It is recommended in literature that the absorbance ratio at the maximum around 269 nm and the minimum around 266 nm is greater than 1.5.