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A review on UV-visible spectroscopy

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Abstract:

One of the earliest instrumental techniques for analysis is UV-VISIBLE spectroscopy. Many different types of materials can be characterized using UV-Visible spectroscopy. The UV-Vis delivers details based on the degree of absorption or transmittance of a varied wavelength of beam light and the various responses of samples. Radiant energy absorption by materials can be quantitatively described using the general law known as Beer's law. The UV-VIS spectrometer is simple to use and handle. Both qualitative and quantitative analyses can make use of it. The metal and metal oxide nanoparticles are typically characterized using wavelengths between 200 and 700 nm. The intricate mechanism of complexation between templates, monomer, and cross-linker during polymerization can also be better understood with the aid of the UV/Vis spectrum. It is quick, simple, and affordable characterization method. The composition and structure of the materials can be examined using the spectrum. These conclusions have uses in academia, business, medical labs, and chemical examination of environmental samples. The pharmaceutical analysis comprises the procedure necessary to determine the "identity, strength, quality and purity" of such compounds. It also includes the analysis of raw material and intermediates during the manufacturing process of drugs. It is well known that the dissociation constant is a most important parameter in development and optimization of anew compound for effective formulation development.

INTRODUCTION TO SPECTROSCOPY

- Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation (EMR) absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state.
- This change may be from Ground State to excited state or excited state to Ground state.

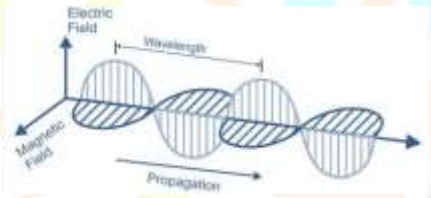
- At ground state, the energy of a molecule is the sum total of rotational, Vibrational and electronic energies.
- In other words, spectroscopy measures the changes in rotational, vibrational and /or electronic energies.

ELECTROMAGNETIC RADIATION:

- Electro Magnetic Radiation is made up of discrete particles called photons.
- EMR has got both wave characteristic as well as particle characteristics.
- This means that it can travel in vacuum also. The different types of EMR are Visible radiation, UV radiation, IR radiation, Microwaves, Radiowaves, X-rays, γ-rays or Cosmic rays.
- As these radiations have different wavelength or frequency or energy, they are conveniently named so.

Wave character of electromagnetic radiation:

EMR is having both electrical and magnetic field in space; hence this has got an electric component and magnetic component which oscillate perpendicular to each other and perpendicular to the direction of propagation of radiation.



- The energy of an electromagnetic radiation can be given by the following equation:

$$E = h\nu$$

Where E = Energy of radiation

h = Plank's constant (6.624×10^{-34} JSec)

ν = Frequency of radiation

Frequency = $\frac{c}{\lambda}$ or $\frac{\text{velocity of light in vacuum}}{\text{wavelength}}$

Hence $E = h\nu = \frac{hc}{\lambda} = hc\bar{\nu}$

Where $\bar{\ }$ = wavenumber

- the energy of a radiation depends upon frequency and wavelength of the radiation.

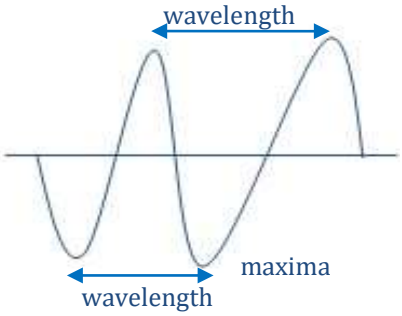
Parameters associated with EMR:

a) **Frequency:** Frequency is the number of complete wavelength units passing through a given point in unit time**.** Frequency is measured in Hz (Hertz) or cps (cycles per second)

The higher units used are 1 Kilohertz or 1KHz = 10^3 Hz;

1 Megahertz or MHz = 10^6Hz; 1 Fresnel = 10^{12} Hz

b) **Wavelength:** Wavelength is the distance between two successive maxima (or)



maxima minima or distance between two successive troughs or peaks.

Wavelength can be measured in metres, centimetres (cm or 10^{-2}m), millimetres (mm or 10^{-3}m), micrometres (μ or 10^{-6}m), nanometres (nm or 10-9m) or (Angstrom) (A or 10^{-10}m).

c) Wave Number = It is the number of waves per cm.

$$\bar{\nu} = \frac{1}{\lambda}$$

Wave number is expressed in cm^{-1} or Kayser.

Wave number is especially used in IR spectroscopy where small wavelength measurements are made, to differentiate frequency of vibrations in molecules.

d) **Velocity** (c): In contrast to frequency, the velocity is dependent upon the medium through which radiation is passing. It has the unit cm s^{-1} or ms^{-1}. The product of wavelength and frequency is equal to the velocity of the wave in the medium, i.e.

wavelength x frequency =velocity

or $$\lambda \times \nu = c$$

Relation between frequency, velocity and wavelength:

Frequency, $\nu = \frac{c}{\lambda}$ or $\lambda = \frac{c}{\nu}$

Wave number, $\bar{\nu} = \frac{\nu}{c}$ or $\nu = c\bar{\nu}$

Where c = Velocity, $\bar{\nu}$ = Wave number, ν = frequency and λ = wavelength.

Particle Properties of EMR:

EMR is emitted, propagated and absorbed in particles called Quanta or Photons of energy E. E is directly proportional to frequency of radiation Hence energy of an electromagnetic radiation can be given by the following equation: $\mathbf{E = h\nu}$

Where E = Energy of radiation

h = Plank's constant (6.624 x 10^{-34} JSec)

ν = Frequency of radiation

Frequency = $\frac{c}{\lambda}$ or $\frac{\text{velocity of light in vacuum}}{\text{wavelength}}$

Hence E = h$\nu = \frac{hc}{\lambda} = hc\bar{\nu}$

Where $\bar{\nu}$ = wavenumber

the energy of a radiation depends upon frequency and wavelength of the radiation.

TYPES OF SPECTROSCOPY:

Spectroscopy can be conveniently divided into following types based on

1. Whether the study is made at atomic or molecular level.

a) **Atomic Spectroscopy –**where the changes in energy take place at **atomic** level.

Eg. Atomic absorption spectroscopy, Flame photometry –where either atomic absorption or atomic emission of radiation is being studied.

b) **Molecular Spectroscopy –**where the changes in energy take place at molecular level.

Eg. UV spectroscopy, Colorimetry, Infra-Red Spectroscopy, Fluorimetry – where the molecular absorption, emission or vibration is being studied.

2. Whether the study is based upon absorption or emission of EMR.

a) Absorption Spectroscopy –where absorption of radiation is being studied.

Eg. UV spectroscopy, Colorimetry, Infra-Red Spectroscopy, NMR Spectroscopy, Atomic absorption Spectroscopy.

b) Emission Spectroscopy –where emission of radiation is being studied.

Eg**.** Flame photometry, Fluorimetry.

3. Whether the study is at electronic or magnetic levels.

a) Electronic Spectroscopy

Eg. UV spectroscopy, Colorimetry, Fluorimetry –where the study is done using electromagnetic radiation only (without the influence of magnetic field**).**

b) Magnetic Spectroscopy

Eg. NMR Spectroscopy, ESR spectroscopy –where the study is done using electromagnetic radiation under the influence of magnetic field.

The energy of a molecule can be due to electronic, vibrational or rotational energy. They are in the following ratio:

Rotational energy: Vibrational energy: Electronic energy = 1: 100: 10,000

When any electromagnetic radiation is passed on to a molecule, the following energy changes take place in a molecule/atom which can be measured.

If we pass	**Cosmic rays**	**Gamma rays**	**X –ray**	**Ultraviolet radiation Visible radiation**		**Infrared radiation**	**Micro waves**	**Radio waves**
							+ Magnetic field	
Changes take place		Within atomic nuclei	in inner electrons (K & L shells)	In Valence electrons		Molecular vibrations	Spin orientation of electrons (ESR spectra)	Spin orientation of Nuclei (NMR spectra)
Wave length region	10-9nm	0.2 - 1oA	1-10oA	200nm to 400nm	400nm to 800nm	2.5 μ to 25μ	0.1 cm – 100cm	1-1000m (>1000Km)

Electromagnetic Spectrum:

- The arrangement of all types of electromagnetic radiations in order of their increasing wavelength or decreasing frequencies is known as complete electromagnetic spectrum.
- The visible spectrum (from violet to red) represents only a small portion of the electromagnetic spectrum.
- If we arrange all types of electromagnetic radiations in order of their increasing wavelengths, then portion above the visible region is called **infra-red** while that below it is the **ultra –violet region.**
- Infra-red radiations have longer wave lengths and are thus less energetic.
- Cosmic ray carry high energy while radio waves are energetic.
- microwaves have larges wavelengths and are used in telephone transmission.

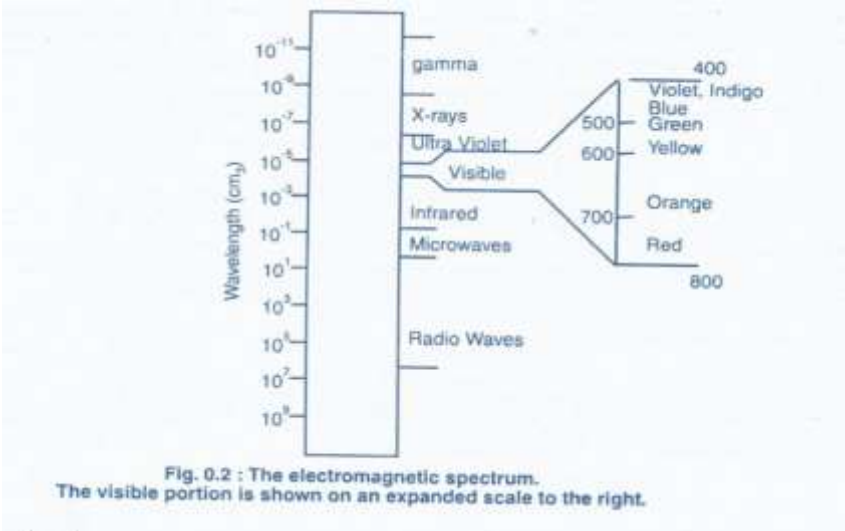


Fig. 0.2 : The electromagnetic spectrum.
The visible portion is shown on an expanded scale to the right.

The major characteristics of various spectrum regions are outlined as follows

a) **γ-ray region:** This lies between 0.02 to 1A^0. The gamma rays are shortest waves emitted by atomic nuclei, involving energy changes of 10^{-9} to 10^{11} Joules / gram atom.

b) **X –ray region:** This lies between 1 to 10 A^0. X-rays emitted or absorbed by movement of electrons close to the nuclei of relatively heavy atoms, involve energy changes of the order thousand kilo Joules.

c) **Visible and Ultraviolet Region:** these are further made up of the following regions:

Vacuum ultraviolet: 1 -180 nm
Ultraviolet: 80 – 400 nm
Visible: 400 – 750 nm

Colours of Visible Light

Colour	Wavelength, nm
Violet	400 – 435
Blue	435 – 480
Green –blue	480 – 490
Blue – Green	490 – 500
Green	500 – 560
Yellow – Green	560 -580
Yellow	580 – 595
Orange	595 – 610
Red	610 -750

d) Infrared region: this region has been further divided into the following sub-regions:

Infrared (near): 0.7 – 2.5 μ
Infrared: 2.5 – 15 μ
Far infrared: 15 – 200μ

Deviations from Beer's Law:

- A system is said to obey Beer's law, when a plot of Concentration Vs absorbance gives a straight line.

- The straight line is obtained by using line of best fit or method of least squares or by joining the maximum no of points in such a way that positive and negative errors are balanced or minimised.
- The regression line can also be used for determining concentration of a solution whose absorbance is obtained using a colorimeter/spectrophotometer.
- When a straight line is not obtained, that is a non-linear curve is obtained in a plot of concentration Vs absorbance, the system is said to undergo deviation from Beer's Law.
- Such deviation can be positive deviation or negative deviation.
- Positive deviation results when a small change in concentration produces a greater change in absorbance.
- Negative deviation results when a large change in concentration produces smaller change in Absorbance.
- It is normally seen that several system obey Beer's Law only in concentration range, above which may show deviation (eg) 10 - 50μg/ml, it may obey, but may exhibit deviation above 50μg/ml.

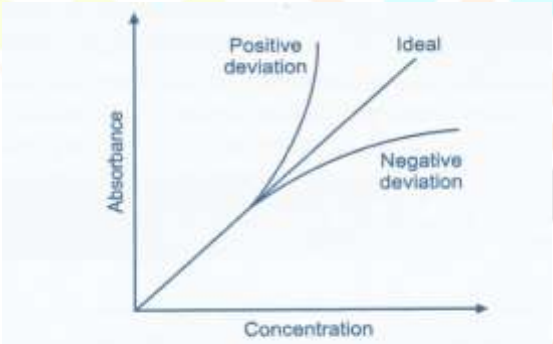


Fig. 1.4: Deviations from Beer-Lambert law

Several reasons for the observed deviations from Beer's law are as follows:

1. Instrumental deviations

   Factors like stray radiation, improper slit width, fluctuations in single beam and when monochromatic light is not used can influence the deviation.

2. Physicochemical changes in solution

   Factors like Association, Dissociation, ionisation (Change in pH), faulty development of colour

(incompletion of reaction), refractive index at high concentrations, can influence such deviations.

Absorption by organic molecules:

- All organic compounds are capable of absorbing electromagnetic radiation because all contain valence electrons that can be excited to higher energy levels, the excitation energies associated with electrons of single bonds are high, their absorption is found in vacuum UV region.
- The electrons that contribute to the absorption characteristics of an organic molecule are:

a) Bonding electrons: These electrons directly participate in bond formation.

b) Non-bonding electrons: Unshared outer electrons that are localized about atoms such as O, N, Halogens (X), S.

There are different types of electrons contributing electronic transition.

a) Sigma (σ) electrons

b) Pi (π) electrons

c) Non-bonding (n or p) electrons

a) Sigma (σ) electrons: Saturated bonds only contain sigma electrons which are held very tightly resulting in very high bond strengths. Hence, these require very high energy for excitation and compounds containing only σ bonds absorb only in vacuum UV region instead of near UV region, where most other chromophoric groups show absorption. This is because energy in the near UV region is not sufficient to excite the σ-electrons. Compounds containing only σ -electrons are useful as solvents for near UV region. **Example :** Saturated hydrocarbons, alkanes like n-hexane are used as solvents in UV region.

b) Pi (π) electrons: Pi electrons are present in unsaturated compounds like alkenes, alkynes and aromatic compounds. These are loosely held electrons localized in a direction perpendicular to the nuclear axis. The energy associated with near UV radiation can excite these electrons to higher energy levels. Examples include all alkenes, alkynes and aromatic compounds.

c) Non-bonding (n) electrons: Non-bonding electrons are less fimly held unshared outer electrons localized on hetero atoms such as oxygen, nitrogen, sulphur or halogens. These electrons are not involved in bonding between individual atoms within the molecules and require much lesser energy for excitation.

Hence, the energy required to excite electrons follows the order.

Sigma (σ) electron > Pi (π) electron > Non-bonding (n) electron.

Types of Electronic Transitions:

Four main types of electronic transitions are observed (fig.1.5). They are as follows:

1) $\sigma \rightarrow \sigma^*$ transition
2) $\pi \rightarrow \pi^*$ transition
3) $n \rightarrow \sigma^*$ transition
4) $n \rightarrow \pi^*$ transition

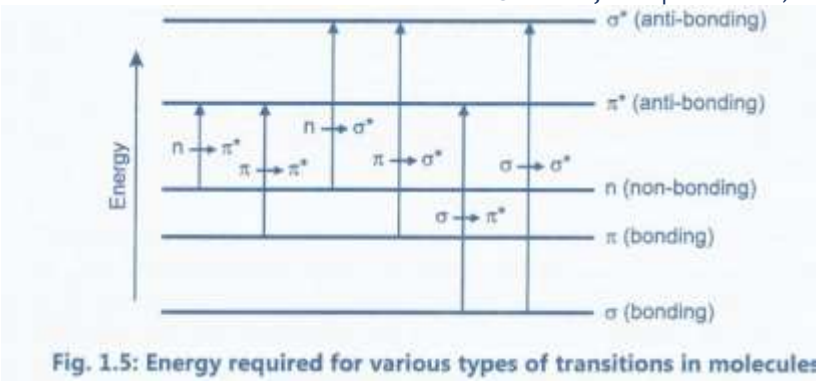


Fig. 1.5: Energy required for various types of transitions in molecules

1) **σ → σ* transition**
   - σ electron from orbital is excited to corresponding anti-bonding orbital σ*.
   - The energy required is large for this transition.
   - e.g. Methane (CH4) has C-H bond only and can undergo σ → σ* transition and shows absorbance maxima at 125 nm.

2) **π → π* transition**
   - π electron in a bonding orbital is excited to corresponding anti-bonding orbital π*.
   - Compounds containing multiple bonds like alkenes, alkynes, carbonyl, nitriles, aromatic compounds, etc undergo π → π* transitions.
   - e.g. Alkenes generally absorb in the region 170 to 205 nm.

3) **n → σ* transition**
   - Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of n → σ* transition.
   - These transitions usually require less energy than σ → σ* transitions.
   - The number of organic functional groups with n → σ* peaks in UV region is small (150 – 250 nm).

4) **n → π* transition**
   - An electron from non-bonding orbital is promoted to anti-bonding π* orbital.
   - Compounds containing double bond involving hetero atoms (C=O, C≡N, N=O) undergo such transitions.
   - n → π* transitions require minimum energy and show absorption at longer wavelength around 300 nm.

Concept of Chromophore and Auxochrome:

- Absorption of light in UV-visible region gives rise to absorption spectra.
- This spectrum arises due to transitions among electronic energy levels (Eo to E_1) of certain groups present in the molecule.
- This group of atoms which is responsible for the absorption in UV-visible region is known as the chromophore.
- Mostly the unsaturated groups, heteroatoms or heteroatoms containing lone pair of electrons are the possible chromophores.

- These chromophores show absorption in certain region of spectrum (Say from 400 to 200 am), however at particular wavelength it shows maximum absorption (say at 254 nm) and that wavelength is called as "Wavelength of maximum absorption." It is denoted by λmax.

Auxochrome:

- These groups do not absorb the radiations in UV-visible region, however when they are attached to chromophore they shift the absorption bands cither towards higher wavelengths or lower wavelengths i.e. they bring out the changes in absorption spection of chromophore when attached to it.
- These auxochromes generally have nonbonding valence electrons. Eg. –OH, - NH_2, -Cl etc.
- It must be remembered that auxochrome shows intense absorption in far UV region i.e.at wavelengths greater than 780 nm. In short it can be stated as auxochromes itself do not absorb the radiation in UV region but when attached to chromophore causes changes in its absorption spectrum.
- Thus, in UV-visible region only chromophore absorbs the radiation.

Spectral shift:

Bathochromic shift (Red shift):

- When an auxochrome is attached to the chromophore, sometimes the absorption band is shift is called as.
- Bathochromic shift or red shift.
- The term red is used as it has the higher wavelength.

Hypsochromic shift (Blue shift):

- When an auxochrome is attached to the chromophore, sometimes the absorption band is shifted towards shorter wavelength, this is called as.
- Hypsochromic shift or blue shift. The term blue is used as it has the shorter wavelength.

Hyperchromic shift:

- When an auxochrome is attached to the chromophore, sometimes the intensity of absorption band is increased; this is called as hyperchromic shift.
- This in hyperchromic shift the absorptivity of molecule increases.

Hypochromic shift

- When an auxochrome is attached to the chromophore, sometimes the intensity of absorption band is reduced; is called as hypochromic shift.
- This in hypochromic shift the absorptivity of molecule decreases.
- This altogether it can be specified that Bathochromic shift and Hypsochromic shift deals with shift of wavelength (λmax) that is shift of absorption bad whereas Hyperchromic shift and Hypochromic shift deals with intensity of absorbed radiation i.e. (Emax) or relative power of radiation.
- All these shift are summarized in the figure give below.

INSTRUMENTATION OF UV-VISIBLE SPECTROPHOTOMETRY:

Content

- Introduction
- Components of spectrophotometry.
- Instrument design.

INTRODUCTION

- Absorption spectrophotometry in the ultraviolet and visible region is considered to be one of the oldest physical methods for quantitative analysis and structural elucidation.
- Wavelength
- UV -200 -400nm
- Visible – 400 -800nm

Instruments

- Photometer
- Spectrophotometer
- Colorimeter

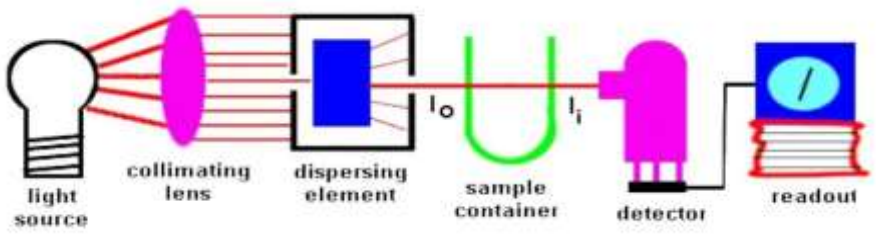
Photometer: An instrument for measuring the intensity of light or the relative intensity of a pair of light. Also called an illuminometer. It utilizes filter to isolate a narrow wavelength region.

Spectrophotometer: An instrument measures the ratio, or a function of the two, of the radiant power of two EM beams over a large wavelength region. It utilizes dispersing element (Prisms/Gratings) instead of filters, to scan large wavelength region.

Colorimeter: An instrument which is used for measuring absorption in the visible region is generally called colorimeter.

Components of UV-vis spectrophotometer

- Source of radiant energy.
- Collimating system.
- Monochromator system.
- Sample holder or container to hold sample.
- Detector system of collecting transmitted radiation.



Source of Radiant Energy:

Requirements of an Ideal Source

- It should be stable and should not allow fluctuations.

- It should emit light of continuous spectrum of high and uniform intensity over the entire wavelength region in which it's used.
- It should provide incident light of sufficient intensity for the transmitted energy to be detected at the end of optic path.

Source for Visible Radiation:

a) Tungsten Halogen Lamp

- Its construction is similar to a house hold lamp.
- The bulb contains a filament of Tungsten fixed in evacuated condition and then filled with inert gas.
- The filament can be heated up to 3000k, beyond this Tungsten starts sublimating.
- Sublimated form of tungsten reacts with Iodine to form tungsten-Iodine complex.
- Which migrates back to the hot filament where it decomposes and tungsten get deposited.
- **Demerit:**
- It emits the major portion of its radiant energy

Source For UV Radiation:

a) Hydrogen Discharge Lamp:

- In Hydrogen discharge lamp pair of electrodes is enclosed in a glass tube (provided with silica or quartz window for UV radiation pass through) filled with hydrogen gas.
- When current is passed through these electrodes maintained at high voltage, discharge of electrons occurs which excite hydrogen molecules which in turn cause emission of UV radiations in near UV region.
- They are stable and robust.

b) Xenon Discharge Lamp:

- It possesses two tungsten electrodes separated by some distance.
- These are enclosed in a glass tube (for visible) with quartz or fused silica and xenon gas is filled under pressure.

- An intense arc is formed between electrodes by applying high voltage. This is a good source of continuous plus additional intense radiation. Its intensity is higher than the hydrogen discharge lamp.

Demerit: The lamp since operates at high voltage becomes very hot during operation and hence needs thermal insulation.

c) Mercury Arc Lamp:

In mercury arc lamp, mercury vapor is stored under high pressure and excitation of mercury atoms is done by electric discharge.

Demerit:

Not suitable for continuous spectral studies,
(because it doesn't give continuous radiations).

Collimating System:

- The radiation emitted by the source is collimated (made parallel) by lenses, mirrors and slits.

Lenses:

- Materials used for the lenses must be transparent to the radiation being used. Ordinary silicate glass transmits between 350 to 3000 nm.

Mirrors:

- These are used to reflect, focus or collimate light beams in spectrophotometer.
- To minimize the light loss, mirrors aluminized on their front surfaces.

Slits:

- Slit is an important device in resolving polychromatic radiation into monochromatic radiation.
- To achieve this, entrance slit and exit slit are used.
- The width of slit plays an important role in resolution of polychromatic radiation.

Dispersive Devices (Wavelength Selection Devices):

Dispersive devices or wavelength selectors ae classified broadly into two categories:

1) Filters 2) Monochromators

1) Filters:

Filters are the devices which allow selective transmission of light of specific wavelengths. Selection of filters is usually done on a compromise between peak transmittance and band pass with.

Advantages: These are simpler in construction and cheaper in cost.

Disadvantages: Accuracy is very low.

Types of filters used in Spectroscopy:

1) Absorption filters 2) Interference filters

1) **Absorption filters –** Absorption filters are simple filters which Works by selective absorption of unwanted radiation and transmits the radiation which is required. Examples Glass and Gelatin filters**.**

a) Glass filter: It is made of solid sheet of glass that has been colored by pigments which Is dissolved or dispersed in the glass. The color in the glass filter are produced by incorporating metal oxides like (V, Cr, Mn, Fe, Ni, Co, Cu etc).

b) Gelatin filter: It is an example of absorption filter prepared by adding organic pigments; hence instead of solid glass sheets thin gelatin sheets are used. Gelatin filters are not use now days.

It tends to deteriorate with time and gets affected by the heat and moisture. The color of the dye gets bleached.

Selection of absorption filter is done according to the following procedure :

1) Draw a filter wheel.
2) Write the color VIBGYOR in clockwise or anticlockwise manner, omitting Indigo.
3) If solution to be analyzed is BLUE in color a filter having a complimentary color ORANGE is used in the anlysis.
4) Similarly, we can select the required filter in colorimeter, based upon the color of the solution.

Merits: -

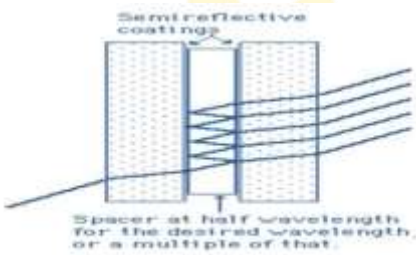
- Simple in construction
- Cheaper
- Selection of the filter is easy

Demerits: -

- Less accurate
- Band pass (bandwidth) is more (20 -30 nm) i.e. if we have to measure at 400nm; we get radiation from 370-430nm. Hence less accurate results are obtained.

Interference filters:

- Works on the interference phenomenon, causes rejection of unwanted wavelength by selective reflection.
- It is constructed by using two parallel glass plates, which are silvered internally and sepearted by thin film of dielectric material of different (CaF_2, SiO, MgF_2) refractive index. These filters have a band pass of 10-15nm with peak transmittance of 40-60%.



Merits: –

- It provide greater transmittance and narrower band pass (10 -15 nm) as compare to absorption filter.
- Greater the bandwidth definition, lower is the percentage transmittance through that filter.
- Inexpensive
- Additional filters can be used to cut off undesired wavelength.

Demerits: -

- Peak transmission is low, and becomes so when additional filters are used to cut off undesired wavelength.
- The band pass is only 10-15nm and hence higher resolution obtained with monochromators or gratings cannot be achieved.

2) Monochromators:

- Monochromators are better and more efficient than filters in converting polychromatic light or heterochromatic light into monochromatic light.
- Monochromators are primarily designed for spectral scanning, i.e. a process of continuously varying the radiation wavelength over a considerable range.
- Mechanical construction of monochromators for UV, visible and IR radiation is similar in that all of them employ slits, lenses, mirrors, windows, and gratings or prisms.

Types of Monochromators:

1) Prism monochromators 2) Grating monochromators

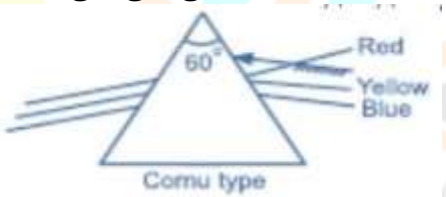
1) Prism monochromators :

- Prism is made from glass, Quartz or fused silica.
- Quartz or fused silica is the choice of material of UV spectrum.
- When white light is passed through glass prism, dispersion of polychromatic light in rainbow occurs. Now by rotation of the prism different wavelengths of the spectrum can be made to pass through in exit slit on the sample.
- The effective wavelength depends on the dispersive power of prism material and the optical angle of the prism.

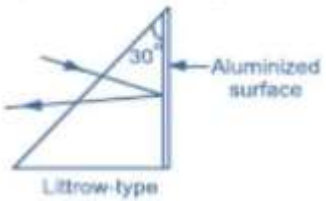
The two types of prisms available are:

a) Cornu Prism
b) Littrow prism.

a) **Cornu prism**(refractive): It has an optical angle of 60^0 and its adjusted such that on rotation the emerging light is allowed to fall on exit slit.



b) **Littrow prism (**reflective**):** It has optical angle 30^0 and its one surface is aluminized with reflected light back to pass through prism and to emerge on the same side of the light source that is light doesn't pass through the prism on other side.



2) Grating monochromators:

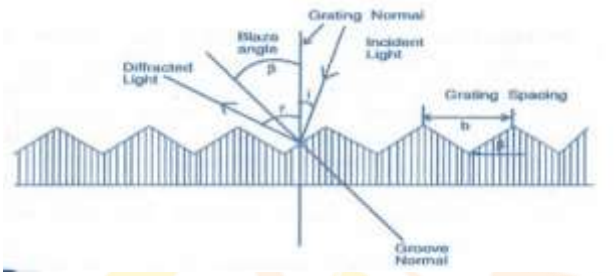
Are most effective one in converting a polychromatic light to monochromatic light. Asa resolution of +/- 0.1 nm could be achieved by using ratings, they are commonly used in spectrophotometers.

Gratings are of two types.

1) Diffraction grating.
2) Transmission gratings.

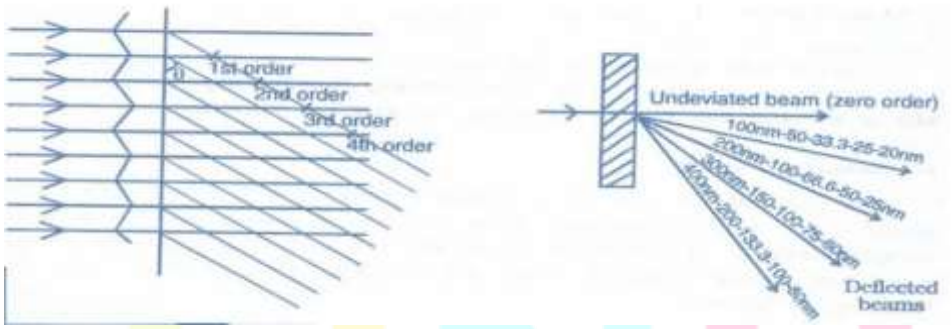
Diffraction Grating:

- More refined dispersion of light is obtained by means of diffraction ratings.
- These consist of large number of parallel lines (grooves) about 15000-30000/ inch is ruled on highly polished surface of aluminium.
- These gratings are replica made from master gratings by coating the original master grating with epoxy resin and are removed after setting.
- To make the surface reflective, a deposit of aluminium is made on the surface. In order to minimize to greater amounts of scattered radiation and appearance of unwanted radiation of other spectral orders, the gratings are blazed to concentrate the radiation into a single order.



Transmission grating:

- It is similar to diffraction grating but refraction takes place instead of reflection. Refraction produces reinforcement. This occurs when radiation transmitted through grating reinforces with the partially refracted radiation.



Advantages:

- Grating gives higher and linear dispersions compared to prism monochromator.
- Can be used over wide wavelength ranges.
- Gratings can be constructed with materials like aluminium which is resistant to atmospheric moisture.
- Provide light of narrow wavelength.

- No loss of energy due to absorption.

Comparison	Prism	Grating
Made of	Glass-: Visible Quartz/fused silica-: UV Alkali halide:- IR	Grooved on highly polished surface like alumina.
Working Principle	Angle of Incident	Law of diffraction $n\lambda = d(\sin i \pm \sin\theta)$
Merits/demerits	➢ Prisms give non-liner dispersion hence no overlap of spectral order. ➢ It can't be used over consideration wavelength ranges. ➢ Prisms are not sturdy and long lasting.	➢ Grating gives liner dispersion hence overlap of spectral order. ➢ It can be used over considerable wavelength ranges. ➢ Grating are sturdy and long lasting

Sample Holders/Cuvettes

- The cells or cuvettes are used for handling liquid samples.
- The cell may either be rectangular or cylindrical in nature.
- For study in UV region; the cells are prepared from quartz or fused silica whereas color corrected fused glass is used for visible region.
- The surfaces of absorption cells must be kept scrupulously clean.
- No fingerprints or a touch should be present on cells.
- Cleaning is carried out washing with distilled water or with dialcohol, acetone.

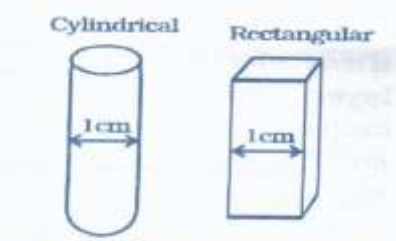


Fig 1.10. Types of sample cells

Detectors

- Device which converts light energy into electrical signals, that are displayed on readout devices.
- The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample
- The following types of detectors are employed in instrumentation of absorption spectrophotometer

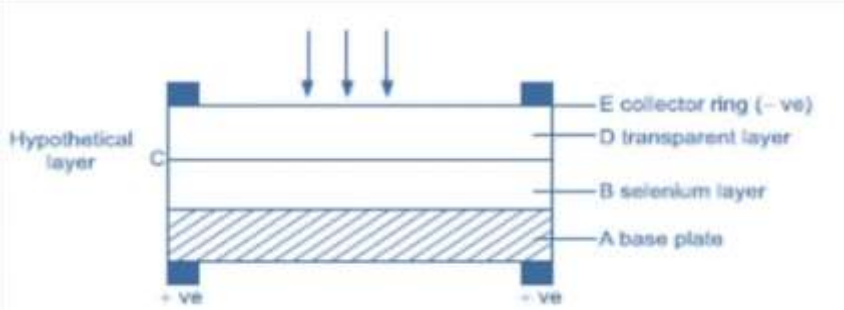
a) Barrier layer cell/Photovoltaic cell
b) Phototubes/Photo emissive tube
c) Photomultiplier tube.

Requirements of an ideal detector: -

- It should give quantitative responses.
- It should have high sensitivity and low noise level.

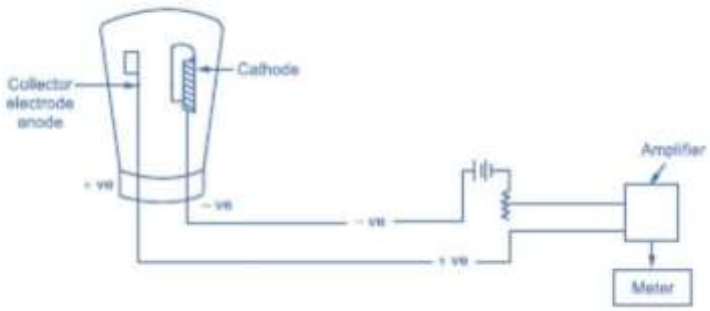
- It should have a short response time.
- It should provide signal or response quantitative to wide spectrum of radiation received.

a) Barrier layer cell / Photovoltaic cell:



- The detector has a thin film metallic layer coated with silver or gold and acts as an electrode.
- It also has a metal base plate which acts as another electrode.
- These two layers are separated by a semiconductor layer of selenium.
- When light radiation falls on selenium layer, electrons become mobile and are taken up by transparent metal layer.
- This creates a potential difference between two electrodes and causes the flow of current.
- When it is connected to galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light falling on it.

b) Photo Tubes/ Photo emissive Tubes:



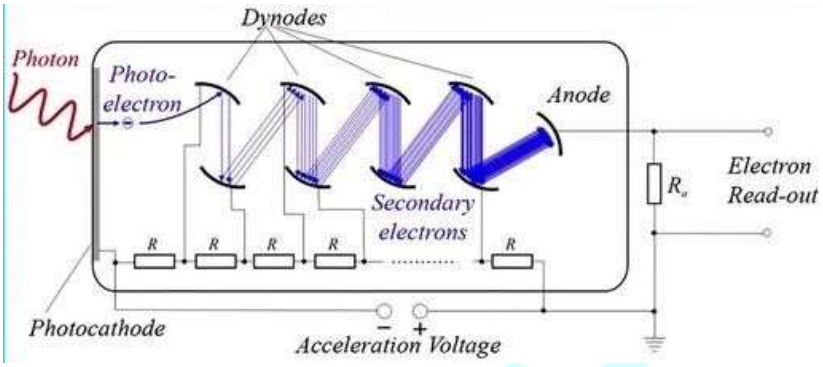
- Consists of a evacuated glass tube with a photocathode and a collector anode.
- The surface of photocathode is coated with a layer of elements like caesium, silver oxide or mixture of them.
- When radiant energy falls on photosensitive cathode, electrons are emitted which are attracted to anode causing current to flow.
- More sensitive compared to barrier layer cell and therefore widely used.

C) Photo Multiplier Tubes:

The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons.

- In a vacuum tube, a primary photo-cathode is fixed which receives radiation from the sample.

- Some eight to ten dynodes are fixed each with increasing potential of75-100V higher than preceding one.
- Near the last dynode is fixed an anode or electron collector electrode.
- Photo-multiplier is extremely sensitive to light and is best suited where weaker or lower adiation is received.



Instrument Design:

- Depending upon the monochromators (filters or dispersing device) used to isolate and transmit a narrow beam of radiant energy from the incident light determines whether the instrument is classified as Photometer or a Spectrophotometer.
- Spectrophotometers used here detects the percentage transmittance of light radiation, when light of certain intensity and frequency range is passed through the sample.
- Both can be a single beam or double beam optical system.

Single Beam Spectrophotometer:

- Light from the source is carried through lens and /or through aperture to pass through a suitable filter.
- The type of filter to be used is governed by the colour of solution.
- The sample solution to be analysed is placed in cuvette.

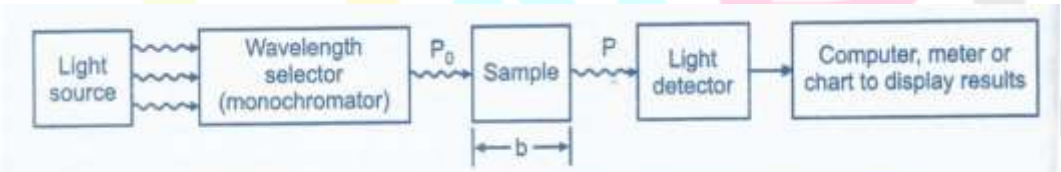


Fig. 1.21: Schematic diagram of single beam UV spectrophotometer

After passing through the solution, the light strikes the surface of detector (barrier-layer cell or phototube) and produces electical current.

- The output of current is measured by the deflection of needle of light-spot galvanometer or micro ammeter. This meter is calibrated in terms of transmittance as well as optical density.
- The readings of solution of both standard and unknown are recorded in optical density units after adjusting instrument to a reagent bank.

Double Beam UV-VIS spectrophotometer:

- Double beam instrument is the one in which two beams are formed in the space by a U-shaped mirror called as beam splitter or beam chopper.
- Chopper is a device consisting of a circular disc. One third of the disc is opaque and one third is transparent, remaining one third is mirrored. It splits the monochromatic beam of light into two beams of equal intensities.

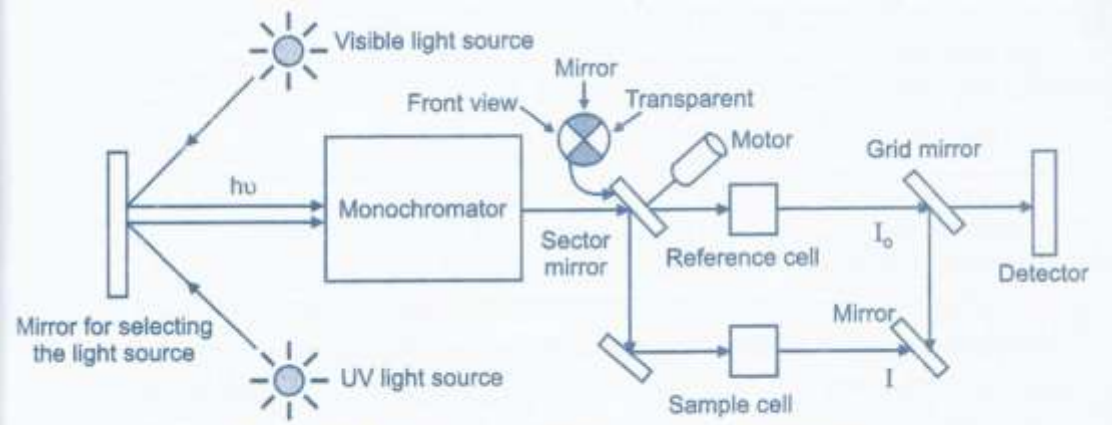


Fig. 1.22: Double beam UV-Vis spectrophotometer

Advantages: of single and double beam spectrophotometer

Single beam – Simple in construction, Easy to use and economical.

Double beam –

- It facilitates rapid scanning over wide λ region.
- Fluctuations due to radiation source are minimised.
- It doesn't require adjustment of the transmittance at 0% and 100% at each wavelength.

Disadvantages:

Single beam

- Any fluctuation in the intensity of radiation sources affects the absorbance.
- Continuous spectrum is not obtained.

Double beam

- Construction is complicated.
- Instrument is expensive.

Comparison:

Sr. No	Single Beam Instrument	Double Beam Instrument
1.	Calibration should be done with blank every time, therefore measuring the absorbance or transmittance of sample	Calibration is done only in the beginning.
2.	Radiant energy intensity changes with fluctuation of voltage.	It permits a large degree of inherent compensation for fluctuations in the intensity of the radiant energy.
3.	It Measure the total amount of transmitted Light reaching the detector	It measures the percentage of light absorbed by the sample.
4.	In single beam it's not possible to compare bank and sample together.	In double beam it's possible to do direct one step comparison of sample in one path with a standard in the other path.
5.	In single beam radiant energy wavelength absorption to be adjusted every time.	In this scanning can be done over a wide wavelength region.
6.	Working on single beam is tedious and time consuming.	Working on double beam is fast and non-tedious.

Choice of Solvent:

- The choice of the solvent to be used in ultraviolet spectroscopy is quite important.
- The first criterion for a good solvent is that it should not absorb ultraviolet radiation in the same region as the substance whose spectrum is being determined.
- Usually, solvents that do not contain conjugated systems are most suitable for this purpose, although they vary regarding the shortest wavelength at which they remain transparent to ultraviolet radiation.
- A second criterion for a good solvent is its effect on the fine structure of an absorption band.
- A non-polar solvent does not hydrogen bond with the solute, and the spectrum of the solute closely approximates the spectrum that would be produced in the gaseous state, in which fine structure is often observed.

- In a polar solvent, the hydrogen bonding forms a solute-solvent complex, and the fine structure may disappear.
- Table l, lists some common ultraviolet spectroscopy solvents and their cut off points or minimum regions of transparency.
- Of the solvents listed in Table l, water 95% ethanol, and hexane are most commonly used.
- Each is transparent in the regions of the ultraviolet spectrum in which interesting absorption peaks from sample molecules are likely to occur.

Table l: solvent used

Solvents	Wavelength	Solvent	Wavelength
Acetonitrile	190 nm	n-Hexane	201 nm
Chloroform	240 nm	Methanol	205 nm
Cyclohexane	195 nm	Iso-octane	195 nm
1,4 – Dioxane	215 nm	Water	190 nm
95% Ethanol	205 nm	Trimethyl phosphate	210 nm

Applications of UV Spectroscopy:

1) **Structure Elucidaiton of Organic Compounds:**

UV spectroscopy is useful in the structure elucidation of organic molecules, presence or absence of unsaturation and presence of hetero atoms.

i) **Effect of conjugation:**

- Extended conjugation causes a shift in the absorption maximum (λmax) towards longer wavelengths.
- On the other hand, saturation of double bonds or reduction of the compound gives opposite effect, that is hypsochromic shift.
- More extended the conjugation (i.e., greater is the number of conjugated double bonds) in a molecule, greater will be its absorption maximum, $\lambda_{max.}$

ii) Effect of geometrical isomerism:

- Geometric isomerism has a significant effect on UV absorption (e.g. cis-and trans-isomerism).
- The trans-isomers generally absorb at longer wavelengths compared to the cis-isomers due to increase in the chromophoric length.

iii) Effect of number of rigs:

- Fusion of two or more benzene rings results in a bathochromic shift of benzenoid bands.

- For example, benzene has an absorption maximum at 261 nm, naphthalene (having two rights) at 312 nm and anthracene (three rigs) at 375 nm.

iv) Effect of substituents:

- As number of alkyl substitutions in the compound increase, it leads to bathochromic shift of the chromophoric band.
- For example, phenolic –OH groups cause marked bathochromic shift of the benzen band (at around 255 nm) to 280nm and the shift depends upon the pH of the medium.

2) **Qualitative Analysis:**

UV absorption spectroscopy can characterized by all those compounds which are capable of absorbing UV radiation. It is useful for the detection of certain chromophoric functional groups:

- Carbonyl compounds show a weak absorption band at 280-290 nm, which is displaced towards shorter wavelength in polar solvents.
- As discussed previously, differentiation between compounds may be possible based on possible effects of conjugation on absorption wavelength.
- Vitamin A_1 shows one band; whereas Vitamin A_2 shows two bands.
- Bathochromic shift is observed from 326 nm, in Vitamin A_1 to 351 nm in Vitamin A_2.
- Intense UV absorption of many ketosteroids is entirely due to the presence of a conjugated enone system.
- Thus, λ_{max} is same for all the compound which don't possess any extended conjugation.

3) **Quantitative Analysis:**

- Compounds, which absorb UV radiations can be quantitatively determined by UV absorption spectroscopy based on their intensity of absorption at a selected wavelength.
- Qualitative analysis establishes the chemical identity of the species in the sample, whereas, quantitative analysis determines or indicates the amount of each substance or analyte in a sample.
- Two methods are used in quantitative determination of analytes:

i) Direct methods:

- Spectrophotometric analysis of all drugs containing one or more chromophoric groups is possible.
- Many inorganic species also absorb in UV or visible regions and are amenable to direct determination e.g.

nitrites, nitrates, chromates, molecular iodine, ozone, etc. Common methods followed. Are;

a) Using standard absorptivity $(E^{1\%}_{1cm})$ values

b) Single standard or direct comparison method

c) Calibration curve method or multiple standard method.

ii) Indirect methods:

- These may be applicable to substances which are themselves non-absorbing in UV or visible regions, but can be reacted selectively with certain reagents to yield products which are strong chromophores.
- Successful application of this method requires that the selected reaction must proceed to completion.
- Typical inorganic reagents include thiocyanate ion for molybdenum, iron and cobalt; anion for H2O2 for determination of titanium, vanadium; iodide ion for bismuth, palladium and tellurium.
- Organic reagents include o –phenanthroline for determination of iron, N, N-dimethyl glyoime for determination of nickel, diethyl dithiocarbamate for copper determination and diphenyl dithiocarbazone for estimation of lead.

4) Analysis of Mixtures (Multicomponent Systems):

Simultaneous Multicomponent Method:

- This method is based on the fact that the absorption intensity is proportional to the number, type and location of chromophoric structures in the molecule.
- This method is based on absorbance measurements at two wavelengths.
- Two dissimilar chromophoric substances must have differing absorbance values corresponding to at least two (or more) wavelengths.
- If measurements are done separately on solutions of these two individual components at two such wavelengths (say, $\lambda_1$and λ_2), a pair of simultaneous equations may be obtained from which the two unknown concentrations may be determined.
- Firstly, two points are selected on the wavelength scale, where the ratio of the molar absorptivity for the two components is maximum.
- Then, the molar absorptivites for both the components ($\varepsilon_1$and ε_2) are obtained at both the wavelengths.
- For example, ε_1 (λ_1) and ε_1 (λ_2) are molar absorptivity of component 1 at wavelengths λ_1 and λ_2 respectively.
- Similarly, ε_2 (λ_1), ε_2 (λ_2) are molar absorption of component 2 at both the wavelengths $\lambda_1$and λ_2 respectively.

conclusion:

❖ UV-visible spectroscopy, a simple, rapid, precise and highly accurate method for quantitative estimation is in great use now a day. Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands by calculating and plotting one of the mathematical derivatives of a spectral curve. Therefore the derivative spectra (first to fourth-order) of the mixtures were checked to select a suitable spectrum to be used for the simultaneous determination of the components.

- Derivative techniques in spectroscopy often offer a powerful tool for a resolution enhancement, when signal overlaps or interference exists. Several specific signals were singled out for the components in the spectra of different derivative orders but the first-order derivative spectra seemed to be generally the most suitable for analytical aim.
- A derivative spectrum shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the max of the individual bands. Secondly, DS discriminates in favor of substances of narrow spectral bandwidth against broad bandwidth substances. All the amplitudes in the derivative spectrum are proportional to the concentration of the analyte provided that Beer's law is obeyed by the fundamental spectrum.

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