"A Review on UV Visible Spectroscopy"

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ABSTRACT:

One of the earliest instrumental techniques for analysis is UV-VIS spectroscopy. Many different types of materials can be characterized using UV-Vis 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.

KEYWORDS: UV-Visible Spectroscopy, UV-VIS Spectrometer, UV-VIS Spectrum

INTRODUCTION TO SPECTROSCOPY:[1]

Spectroscopy as a science began with Isaac Newton splitting light with a prism and was called optics. Therefore, it was originally the study of visible light which we call color that later under the studies of James Clerk Maxwell came to include the entire electromagnetic spectrum. Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter. The most important consequence of such interaction is that energy is absorbed or emitted by the matter in discrete amounts called quanta. The absorption or emission processes are known throughout the electromagnetic spectrum ranging from the gamma region (nuclear resonance absorption or the Mossbauer effect) to the radio region (nuclear magnetic resonance). When the measurement of radiation frequency is done experimentally, it gives a value for the change of energy involved and from this one may draw the conclusion about the set of possible discrete energy levels of the matter. The ways in which the measurements of radiation frequency (emitted or absorbed) are made experimentally and the energy levels deduced from these comprise the practice of spectroscopy.
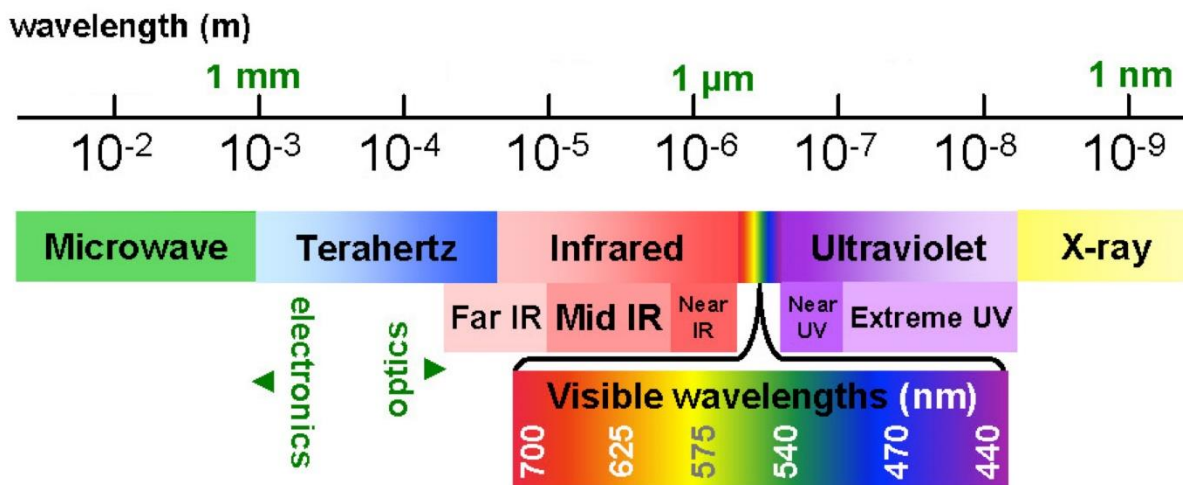
Figure 1: spectroscopy

Spectroscopy is one of the most powerful tools available for the study of atomic and molecular structure and is used in the analysis of a wide range of samples. The study of spectroscopy can be carried out under following heads.

1) Atomic spectroscopy

This deals with the interaction of electromagnetic radiations with atom which are most commonly in their lowest energy state called the ground state. The electronic absorption of electromagnetic radiation can occur only if the photon has an energy which is equal to the energy difference between two quantized energy levels, i.e.,

$$\Delta E = h \, v$$

where AE is the energy difference between two quantum levels and v is the frequency of photon which can result in the electronic excitation. Applications of electronic spectroscopy in the field of chemistry are few. However, its importance has increased by the development of lasers.
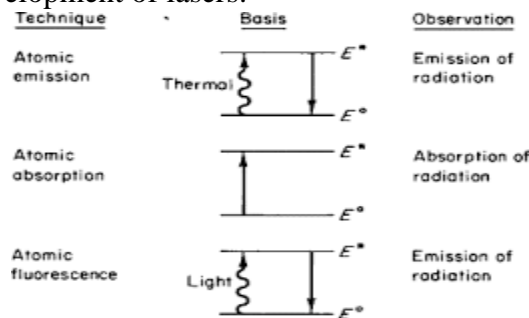

Figure 2: atomic spectroscopy

2) Molecular Spectroscopy

This deals with the interaction of electromagnetic radiation with molecules. This results in transitions between rotational and vibrational energy levels in addition to electronic transitions. As a result, the spectra of molecules are much more complicated than those of atoms. Molecular spectra extend from the visible through infrared into the microwave region. Current interest in molecular spectroscopy is very great because the number of known molecules are extremely large as compared with free atoms.

The various types of spectra given by molecular species, the regions in which these spectra lie and the energy changes that takes place in the molecules on absorption of radiation, are listed below.

a) Rotational (Microwave) Spectra

These spectra arise due to transitions between the rotational energy levels of a gaseous molecule on the absorption of radiations falling in the microwave region These spectra are shown by molecules which possess a permanent dipole moment, e.g., HCI, CO, HO vapor, NO, etc.

b) Vibrational and Vibrational-Rotational (Infrared) Spectra

These spectra arise due to transitions induced between the vibrational energy levels of a molecule on the absorption of radiation belonging to the infrared region. IR spectra are shown by molecules when vibrational motion is accompanied by a change in the dipole moment of the molecule. These spectra occur in the spectral range of 500-4000 cm^{-1}.

c) Raman Spectra

Raman spectra is related to vibrational and/or rotational transitions in molecules but in a different manner. In this case, what we measure is the scattering and not the absorption of radiation. An intense beam of monochromatic radiation in the visible region is allowed to fall on a sample and the intensity of scattered light is observed at right angles to the incident beam. Most of the scattered light has the same frequency as the incident beam (this is called Rayleigh scattering). Raman spectra are observed in the visible region, viz., 12,500-25,000 cm^1.

d) Electronic Spectra

Electronic spectra arise due to electronic transitions in a molecule b absorption of radiations falling in the visible and ultraviolet regions. While electronic spectra in the visible region span 12,500-25,000 cm, those in the ultraviolet region span 25,000-70,000 cm. As electronic transitions in a molecule are invariably accompanied by vibrational and rotational transition the electronic spectra of molecules are highly complex.

e) Nuclear magnetic resonance

NMR spectra arise due to transitions induced between the nuclear spin energy levels of a molecule in an applied magnetic field. NQR spectra arise due to the transitions between the nuclear spin energy of a molecule arising from the interaction of the unsymmetrical charge distribution in nuclei with the electric field gradients (EFG) which arise from the bonding and non-bonding electrons in the molecule, NMR and NQR spectra span the radiofrequency regions, viz., 5-100 MHz.

f) Mossbauer Spectra

Also called Nuclear Gamma Resonance Fluorescence (NRF) Spectra Mossbauer spectra is a type of nuclear magnetic resonance spectra. However, while NMR spectra arise due to absorption of low energy photons of frequency around 60 MHz, Mossbauer spectra result from absorption of high energy y-photons of frequency around 1013 MHz by the nuclei. Gamma ray spectra have been used specifically for the study of compounds of iron and tin. In this case, y-radiations from 57Co-source are allowed to fall on a sample in which the iron nuclei are in an environment identical with that of the source atoms. This gives arise to the resonant absorption of y-rays. The splitting in Mossbauer lines are found to be of the same order as in NMR spectroscopy.

UV SPECTROSCOPY:

❖ Introduction:[2]

UV-Vis (ultraviolet visible) spectroscopy is frequently used to provide characterization data for a variety of materials. Inorganic or organic, solid or liquid groups, such as organic molecules and functional groups, can be observed using UV-Visible spectroscopy, as can reflectance measurements for coatings, paints, textiles, biochemical analysis, dissolution kinetics, band gap measurements, etc. Depending on the degree of absorbance or transmittance of a different wavelength of beam light and the various responses of samples, the UV-Vis provides these details.

1.1. Electromagnetic Spectrum

The ability of electromagnetic radiation to discretely interact with atoms and molecules and produce distinctive absorption or emission profiles is essential for spectroscopic activities. The wavelength of electromagnetic radiation is the characteristic that governs the perceived color spectrum. The visible section of the electromagnetic spectrum is that portion of the spectrum that the human eye can see. These visible wavelengths span a region between 400 and 800 nm.

A specific wavelength or color of visible light corresponds to the optical density when it is measured with spectrophotometers. This light is absorbed, vanishes, and becomes invisible. The approximate complimentary connection between the light wavelengths that are absorbed and those that are transmitted is shown in Figure. For instance, the complimentary color of light, orange, would be strongly absorbed by a blue substance.
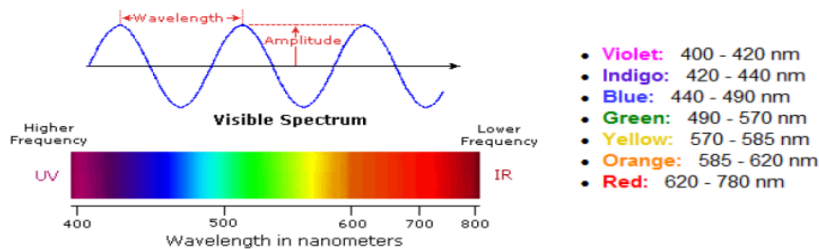
**Figure 3:- sine wave representation of electromagnetic radiation and electromagnetic spectrum**

The distance between neighbouring peaks or troughs is known as the wavelength. The following straightforward equation can be used to define the wavelength, of EMR as a function of its frequency v, and the speed of light c

$$v = \frac{c}{\lambda} \tag{1}$$

EMR has both particle and wave behaviour (the dual nature of light), and the relationship between energy and the wavelength of such a particle, a photon, is given by the equation

$$E = hc/\lambda * 10^9 \tag{2}$$

Where c is the speed of light in a vacuum (2.998×10^8 ms-1), c is the Planck's constant (6.63×10^{-34} Js), E is the photon's energy, and is the wavelength in nm.
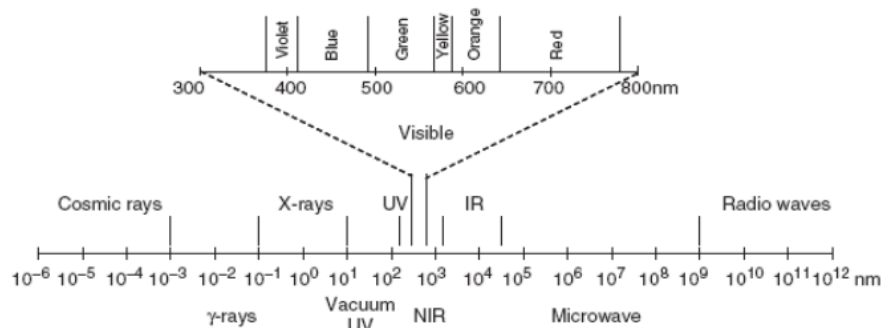
Figure 4:- The electromagnetic spectrum

Beer's Law:

The intensity of the incident radiation (Io) will be higher than the emerging radiation when a beam of electromagnetic radiation passes through an absorbing material (I). The general rule known as Beer's law can be used to quantitatively describe how radiant energy is absorbed by materials.

According to Beer's law, the amount of radiation that is absorbed (absorbance, A) or transmitted by a solution or medium is inversely related to the amount of the absorbing substance that is present, c (moles per liter), and the length of the radiation's passage through the sample, b. (centimeters).

Therefore, a plot of absorbance against concentration should result in a line that is straight and has a slope equal to Єb, passing through the origin.

$A = - \log (I/Io) = \text{\euro}bc$

where $\text{\euro} = k/2.303$

The molar absorptivity is a constant ϵ that is unaffected by concentration or path length.

If the route length and molar absorptivity are known, Beer's law equation can be used to calculate the concentration of an organic molecule by locating its highest absorbance in the UV-Vis absorption spectrum.

❖ Principle of Uv-Vis Spectroscopy:[3]

When radiation induces an electronic transition in a molecule or ion's structure, the object will exhibit absorption in the visible or ultraviolet range. As a result, when a sample absorbs light in the ultraviolet or visible range, the molecules inside the sample experience a change in their electronic state. Electrons will be promoted from their ground state orbital to a higher energy, excited state orbital by the energy from the light. or anti-bonding orbital. Potentially, three types of ground state orbitals may be involved.

1) σ (bonding) molecular

2) π (bonding) molecular orbital

3) n (bonding) atomic orbital

In addition, two types of anti-bonding orbitals may be involved in the transition

1) σ* (sigma star) orbital

2) π* (pi star) orbital

There is no such thing as an n* anti-bonding orbital as the n electrons do not form bonds). Thus, the following electronic transitions can occur by the absorption of ultraviolet and visible light.

1) σ to σ*

2) n to σ*

3) n to π*

4) π to π*

Due to their high energy requirements, the σ to σ* and n to σ* transitions both take place in the far ultraviolet area or sporadically in the range of 180–240 nm. Saturated groups consequently do not show high absorption in the common UV range. In contrast to transitions to the π* anti-bonding orbital, transitions from then to the π* and to π* type occur in molecules with unsaturated centers. They need less energy and take place at longer wavelengths. It will soon be clear that molecule structure controls both the absorption's maximum wavelength and its intensity. If a molecule's chemical structure is changed, transitions to the π* anti-bonding orbital that normally take place in the UV range could very well occur in the visible region. Many inorganic compounds in solution also show absorption in the visible region. These include salts of elements with incomplete inner electron shells (mainly transition metals) whose ions are complexed by hydration. Such absorptions arise from a charge transfer process, where electrons are moved from one part of the system to another by the energy provided by the visible light.[36]
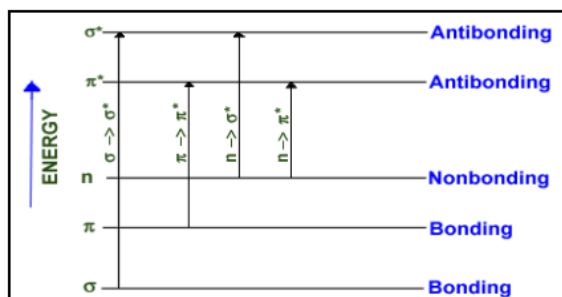
Figure 5 :- Electron Transition graphically represented.

❖ Instrumentation:[3,4]

The Essential components of UV-VIS Spectrophotometer are as follows:

1. Sources (UV and visible)

2. Monochromator

3. Sample containers (Cuvette)
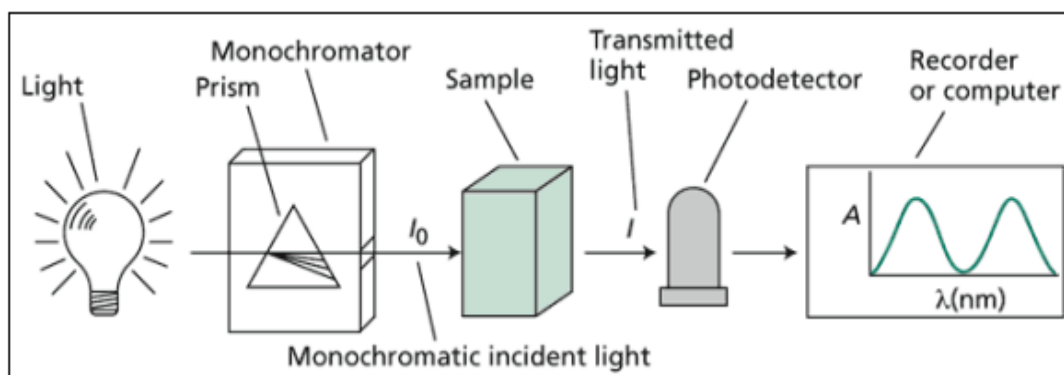
4. Detector

5. Amplifier and recorder

Figure 6: - Schematic diagram of UV Spectrophotometer

1. SOURCES:

A continuous source, or one that produces radiation at a variety of wavelengths, is necessary for UV-Vis Spectroscopy. Assorted UV radiation sources include the following:

1) Hydrogen lamp:

Hydrogen lamps are reliable, steady, and continuously emit radiation between 160 and 380 nm. It consists of hydrogen gas at high pressure, which causes an electrical discharge. The excited hydrogen molecules produce radiation.

2)Deuterium lamp:

A gas discharge lamp called a deuterium lamp is frequently employed as a UV source. It emits radiation in the 160–450nm range. It costs more than a hydrogen lamp.

3)Tungsten lamp:

The most typical light source utilized in spectrophotometers is the tungsten lamp. With a wavelength range of roughly 330 to 900 nm, it comprises of a tungsten filament encased in a glass envelope and is utilized for the visible spectrum.

4)Xenon discharge lamp:

A xenon lamp is a discharge light source that contains xenon gas inside a bulb. Radiation from xenon ranges from 250 to 600 nm.

2. MONOCHROMATOR:

By filtering out undesirable wavelengths from the radiation source light, a monochromator creates monochromatic light. Through the entrance slit, multi-wavelength polychromatic light enters the monochromator. Following collimation, the beam is directed at an angle toward the dispersion component. The grating or prism separates the beam's wavelengths into their individual components. Only radiation of a specific wavelength exits the monochromator through the exit slit when the dispersing element or the exit slit are moved.

Types of monochromators:

1) Prism monochromator
2) Grating monochromator

All Monochromator contain the following component parts:

- An entrance slit
- A collimating lens
- A dispersing device
- A focusing lens
- An exit slit

Radiation with many wavelengths, or polychromatic radiation, enters the monochromator through the entrance slit. After being collimated, the beam angles toward the dispersing component. The grating or prism separates the beam's wavelengths into their individual components. Only radiation of a specific wavelength exits the monochromator through the exit slit by changing the dispersing element or the exit slit.
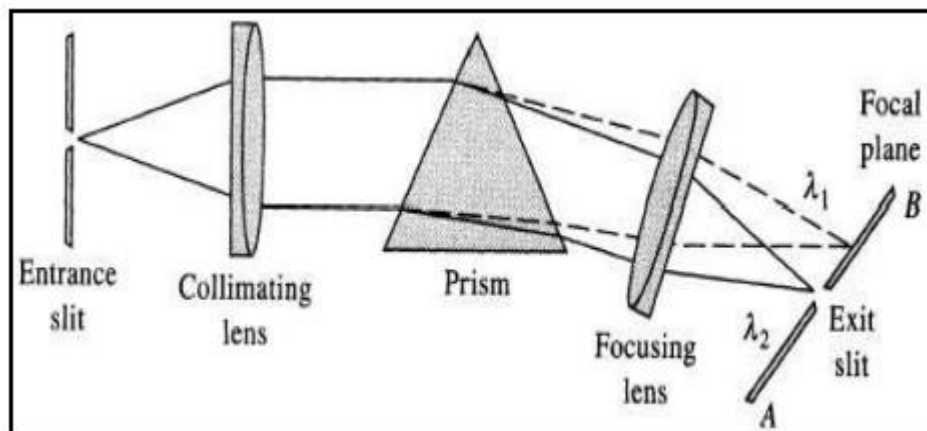

Figure 7: - Prism monochromator

3.SAMPLE CONTAINERS (CUVETTE):

Cuvettes are sample containers that are transparent to all wavelengths of light flowing through them and are used to hold samples for spectroscopic measurements. The cuvette is composed of quartz, is square in shape, has a 1 cm route length, and may be utilized for wavelengths between 190 and 200 nm.

4. DETECTORS:

Light energy is converted by detectors into electrical impulses that are read out by readout devices. The transmitted radiation strikes the detector, determining the amount of radiation absorbed by the sample. The absorption spectrophotometer's apparatus uses the following types of detectors.

Types of Detectors:

1. Barrier layer cell/Photovoltaic cell

2. Phototubes/ Photo emissive tube

3. Photomultiplier tube

❖ Different UV spectrophotometric multicomponent analysis methods[5]:

1.Simultaneous equation method:

If a sample contains two absorbing drugs (x and y) each of which absorbs at the λ max of the other, it may be possible to determine both drugs by the technique of simultaneous equation (Vierordt's method) provided that certain criteria apply.

The information required is

• The absorptivities of x at λ1 and λ2, ax1 and ax2 respectively

• The absorptivities of y at λ1 andλ2, ay1 and ay2 respectively

• The absorbance of the diluted samples at λ1 and λ2, A1 and A2 respectively.

Let C_x and C_y be the concentration of x and y respectively in the diluted samples. Two equations are constructed based upon the fact that at λ1, the absorbance of the mixture is the sum of the individual absorbance of x and y.

$$A_1 = a_{x1}\ b\ C_x + a_{y1}\ b\ C_y \quad \text{..............(1)}$$
$$A_2 = a_{x2}\ b\ C_x + a_{y2}\ b\ C_y \quad \text{..............(2)}$$

For measurements in 1 cm cells, b =1cm. Rearrange Eq.(2)

$$C_y = \frac{(A_2 - a_{x2}\ C_x)}{a_{y2}} \quad \text{..............(3)}$$

Substituting for Cy in eq. (1) and rearranging gives

$$C_x = \frac{(A_2 a_{y1} - A_1\ a_{y2})}{(a_{x2} a_{y1} - a_{x1} a_{y2})} \quad \text{..............(4)}$$
$$C_y = \frac{(A_1 a_{x2} - A_2 a_{x1})}{(a_{x2} a_{y1} - a_{x1} a_{y2})} \quad \text{..............(5)}$$

Criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested that place limits on the relative concentrations of the components of the mixture. The criteria are that the ratio and should lie outside the range 0.1-2.0 for the precise $\frac{\overline{A2/A1}}{a_{x2}/a_{x1}}$ $\frac{a_{y2}/a_{y1}}{A2/A1}$ determination of y and x respectively. These criteria are satisfied only when the λmax of the two components reasonably dissimilar and if the two components do not interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorbances. Simultaneous equation method was developed for simultaneous determination of several mixtures, e.g., atenolol and indapamide, and dexibuprofen and paracetamol.

2.Difference spectrophotometry:

The selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferents may be markedly improved by the technique of difference spectrophotometry. The essential feature of this method is that the measured value is the absorbance difference (ΔA) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics.

3. Derivative spectrophotometry (DS):

DS involves the conversion of a normal spectrum (fundamental, zero-order spectrum) to its first, second or higher derivative spectra by differentiating absorbance of the sample with respect to wavelength(λ). The differentiation of zero-order spectrum can lead to separation of overlapped signals, elimination of background caused by presence of other compounds in a sample, improvement of resolution of mixtures as it enhances the detectability of minor spectral features, and enhancement of sensitivity and specificity.

4.Absorbace ratio spectra method:

Consider a mixture of two compounds x and y. The absorption spectrum of the mixture "measured in 1 cm cell" is defined by the equation

$$A_M = a_x\,C_x + a_y\,C_y$$

Where; AM is the absorbance of the mixture, a_x and a_y are the molar absorptivities, Cx and Cy are the concentrations of x and y, respectively. If the absorbance of the mixture is divided by the absorbance of a standard solution of x (its absorbance $A^0{}_x = a_x\,C^0{}_x$), the following equation results

$$\frac{A_M}{A^0_X} = \frac{C_X}{C^0_X} + \frac{A_y}{A^0_X}$$

The ratio is $\frac{C_X}{C^0_X}$ a constant value which can be eliminated by taking the difference in absorbance ratio amplitudes between two wavelengths λ 1 and λ 2.

5.Derivative ratio spectra method:

This simple spectrophotometric method, developed by Salinas et al. is based on the derivation of the ratio spectra for resolving binary mixtures. It permits the use of the wavelength of highest value of analytical signals with several maxima and minima, which give an opportunity for the determination of active compounds in the presence of other compounds and excipients which could possibly interfere in the assay.

6.Double divisor ratio spectra derivative method:

This method is based on the use of the derivative of the ratio spectrum obtained by dividing the absorption spectrum of the ternary mixture by a standard spectrum of a mixture of two of the three compounds in the mixture, and the measuring at either the maximum or minimum wavelengths. It can only be used for the mixtures that the ratio of the concentrations of two interfering compounds (used as double divisor) is known. In other words, the

ratio of the concentrations of two interfering compounds should be the same in calibration, prediction and unknown samples.

7.Successive ratio - derivative spectra method:

This method is used for simultaneous determination of the three compounds in ternary mixtures without need to know the ratio of concentration of species.

8.Q-absorbance ratio method:

This method also termed "**absorption ratio method**" is a modification of the simultaneous equation's method. According to this method, the ratio of absorbance at any two wavelengths for a substance, which obeys Beer's law, is a constant value independent of the concentration and path length. This constant is termed as "Hufner's Quotient" or Q-value. The method involves the measurement of absorbance at two wavelengths, one being the λ max of one of the components (λ 2) and the other being a wavelength of equal absorptivity of the two components (λ 1), called the iso-absorptive point. The concentration of each component can be calculated by mathematical equations

$Cx = (Qm-Qy/Qx-Qy) * A/a1$ ………………… (1)

$Cy = (Qm-Qx/Qy-Qx) * A/a2$ ………………… (2)

where; Cx and Cy are the concentrations of x and y respectively, A is absorbance of sample at isoabsorpitive wavelength and a1 and a2 are the absorptivity of x and y respectively at isoabsorpitive wavelength.

9.Isosbestic " isoabsorptive" point method:

Erram and Tipnis developed the isosbestic point method. This technique can be used only if the spectra of the same concentration of the two studied drugs cross at a point called isosbestic or isoabsorptivity point. At the isosbestic point both drugs have equal absorptivities and their mixture acts as a single component and gives the same absorbance as pure drug.

10.Absorpitivity factor method:

The absorptivity factor (modification of the classical isoabsorpitive method) is applied for the analysis of binary mixture if only there is a large difference in the absorptivity between both drugs, so there is no occurrence of an isoabsorptive point.

In isoabsorptive technique the spectra of the same concentration of the two studied drugs should cross at a point called isoabsorptivity point at which they have equal absorptivities while in absorpitivity factor method the crossing point did not occur at equal concentration. Crossing point is obtained only between different concentrations of the two drugs at which the absorptivities of the two drugs are not equal but they are equal to the inverse of the ratio of the used concentrations.
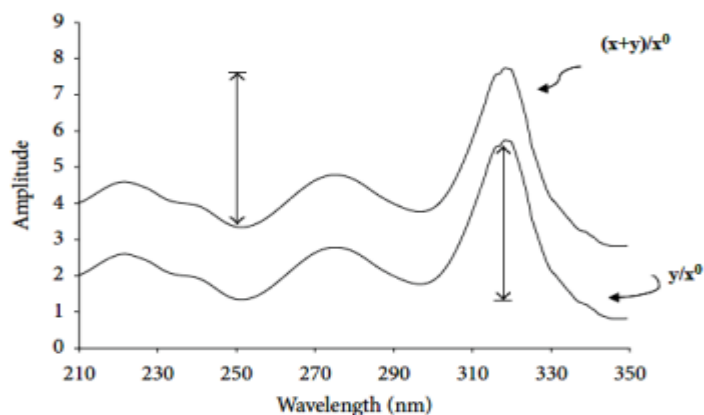
Figure 8 :- Zero order spectra of 8 µg. mL-1 salmeterol and 16 µg. mL-1 fluticasone showing the absorptivity factor points.

11.Dual wavelength method:

Dual wavelength method "also known as two wavelengths method" facilitates analyzing a component in presence of an interfering component by measuring the absorbance difference (ΔA) between two points in the mixture spectrum. In this method (Fig. 4); one of the drugs is considered as a component of interest and the other drug is considered as an interfering component and vice-versa. The basis for such method is the selection of two wavelengths where the interfering component shows the same absorbance (ΔA equals zero) whereas the component of interest shows significant difference in absorbance with concentration. ΔA between two points on the mixture spectra is directly proportional to the concentration of the component of interest independent of interfering component. This method was used for simultaneous determination of different drugs, e.g., atenolol and indapamide, drotaverine and aceclofenac, atorvastatin and ezetimibe, chlorpheniramine and phenylpropanolamine, dexketoprofen and tramadol.
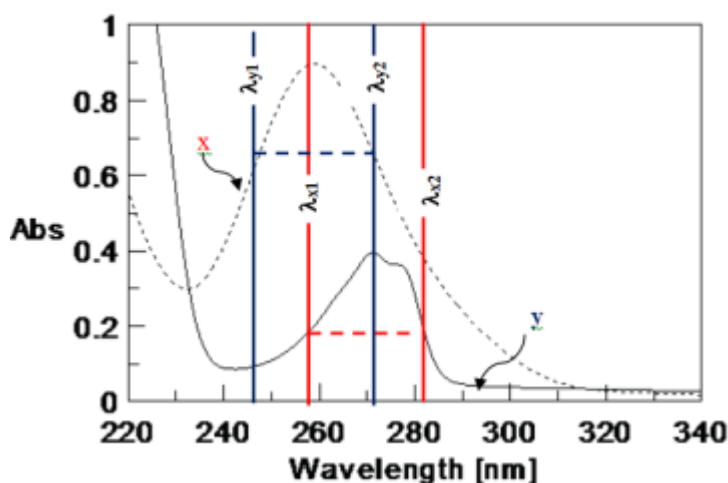
Figure 9 :- Selection of wavelengths for dual wavelength method.

12.Mean centering of the ratio spectra:

This method is applied for further improvement of the selectivity to resolve the overlap present between drugs in binary and ternary mixtures. This eliminates the derivative step and therefore the signal-to-noise ratio is enhanced.

13.Absorption factor method (AFM):

This method describes the analysis of a binary mixture where the two components x and y have overlapped spectra. Y shows interference at λ max of x, while x shows no interference with y at another wavelength (λ 2).

14.Multivariate chemometric methods:

Chemometrics, in the most general sense, is the art of processing data with various numerical techniques in order to extract useful information. Drug separation, identification, determination and validation have been studied using chemometrics. Chemometrics recognizes that it is often better to measure many nonselective signals and then combine them in multivariate model (multivariate analysis), whereby multiple variables are considered simultaneously. A multivariate measurement is defined as one in which multiple measurements are made on a sample of interest. So, more than one variable or response are measured for each sample. Multivariate methods include multiple linear regression (MLR) methods and factor-based methods.

❖ ADVANTAGES:[6]

- The core advantage is the accuracy of the UV-VIS spectrophotometer
- The UV-VIS spectrometer is easy to handling and use
- Provide robust operation
- UV-VIS spectroscopy is simple to operate
- Cost effective instrument
- Cover the entire of ultraviolet and visible
- It can be utilized in the qualitative and quantitative analysis
- The Derivative graph can be obtained by UV-VIS spectrophotometer
- It can be used in the degradation study of drug
- Only possible for the analytes which have a chromophore

❖ DISADVANTAGES:[6]

- Only those molecules are analyzed which have chromophores
- The results of the absorption can be affected by pH, temperature, contaminants, and impurities.
- Only liquid samples are possible to analyze
- It takes time to get ready to use it
- Cuvette handling can affect the reading of the sample

❖ APPLICATIONS:[3]

UV –vis spectroscopy has many different application

1. Detection of impurities

2. Structural elucidation of organic compounds

3. Quantitative analysis

4. Qualitative analysis

5. Chemical analysis

6. Quantitative analysis of pharmaceutical substance

7. Dissociation constant of acids and bases

8. Molecular weight determination

9. As HPLC detector

10. Deviations from the Beer-Lambert law

CONCLUSION:

If used with the right standard curve and applied to pure substances, UV-visible spectroscopy is a reliable, straightforward, and affordable approach for estimating the concentration of absorbing species. One of the crucial methods for analyzing the optical characteristics of PMCs is UV-Vis spectroscopy. It clarifies the relationship between the matrix and the nanofiller and examines how the nanofillers contribute to the enhancement of the properties of the nanocomposites. To assess the intended optical properties of nanofillers in a polymer matrix, UV-Vis spectroscopy is a crucial technique. The polymer nanocomposites with some optically responsive nanofillers, such as metals, semiconductor nanocrystals, and nano oxides, are characterized using the UV-Vis spectroscopic approach in order to produce functional materials with significant technological applications. UV-Vis Spectroscopy (or Spectrophotometry) is a quantitative technique used to measure how much a chemical substance absorbs light. This is done by measuring the intensity of light that passes through a sample with respect to the intensity of light through a reference sample or blank.

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