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REVIEW ON QUALITATIVE AND QUANTITATIVE ANALYSIS OF DRUGS BY UV-VISIBLE SPECTROPHOTOMETER

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Abstract:

One of the earliest instrumental techniques for analysis is UV-VIS spectroscopy. Many different types of materials can be characterised using UV-VIS spectroscopy. The UV-VIS delivers data based on the degree of absorption or transmittance in a varied wavelength of light and responses of sample. The fundamental principle of operation UV-VIS spectrophotometer is Beer's- Lambert's law. The UV-VIS spectrophotometer is simple to use and handle and also less expensive. The analysis of sample is done at the wavelengths between 200nm-700nm. This review's goal is to provide information on the principle, instrumentation and mainly provides information regarding the methodology used for the qualitative and quantitative analysis of drugs by using UV-VIS spectroscopy. 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 materials and intermediates. This method is quick, simple and affordable. Here is a quick overview of the theories, instrumentation and methodology used for the qualitative and quantitative analysis of drug samples using UV-VIS spectroscopy.

Keywords: *UV-VIS spectroscopy, Spectroscopy, Analysis of drugs.*

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INTRODUCTION TO SPECTROSCOPY:

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principle from various branches of science like chemistry, physics, microbiology, nuclear science, electronics etc. pharmaceutical analytical techniques are applied mainly in two areas viz qualitative analysis and quantitative analysis, although these are several other applications. Drugs and pharmaceuticals are chemicals or like substances which are of organic, inorganic or other origin whatever may be the origin. We use some property of the medicinal agent to measure them qualitatively or quantitatively.

VARIOUS ANALYTICAL TECHNIQUES

1) SPECTRAL METHOD**:** Where we use light absorption or emission characteristics of drugs. Examples UV-spectroscopy, IR-spectroscopy, NMR-spectroscopy, fluorimetry etc.
   a) UV-Spectroscopy**:** the UV and visible spectrophotometry also called electronic spectroscopy, involves the measurement of energy absorbed when electrons are promoted from the ground state to the higher energy state.
   b) IR-Spectroscopy**:** Infrared spectrophotometry is one of the most power full analytical techniques which provide a wealth of information on transition between vibrational and rotational energy levels in molecules. It elucidates structure of unknown compound.
   c) NMR-Spectroscopy**:** NMR involves transition of a nucleus from one spin state to another with the resultant absorption of electromagnetic radiation by spin active nuclei when they are placed in a magnetic field.
   d) Fluorimetry**:** Absorption UV or visible radiation cause transition of electron from singlet ground state to singlet excited state, as these state is not stable it emits the energy in the form of UV or visible radiation and returns to singlet ground state. Principle of this technique is to study or measurement of emitted radiation.

2) CHROMATOGRAPHIC METHOD**:** Chromatographic method where we use affinity or partition coefficient difference between drugs. Example TLC, HPLC, paper chromatography etc.
   a) Thin Layer Chromatography**:** It is a technique used to isolate non volatile mixture.the principle of separation is adsorption. It consist of two phases stationary phase and mobile phase.
   b) High pressure liquid chromatography: it is a form of liquid chromatography used to separate compound dissolved in a solution. It is characterized by use of high pressure to push a mobile phase solution through column of stationary phase for separation of complex mixture.
   c) Paper chromatography: it is defined as a technique in which the analysis of unknown substance is carried out mainly by flow of solvents especially in designed filter paper. It is of two type ; paper absorption chromatography and paper partition chromatography. The principle of separation is mainly partition rather than adsorption

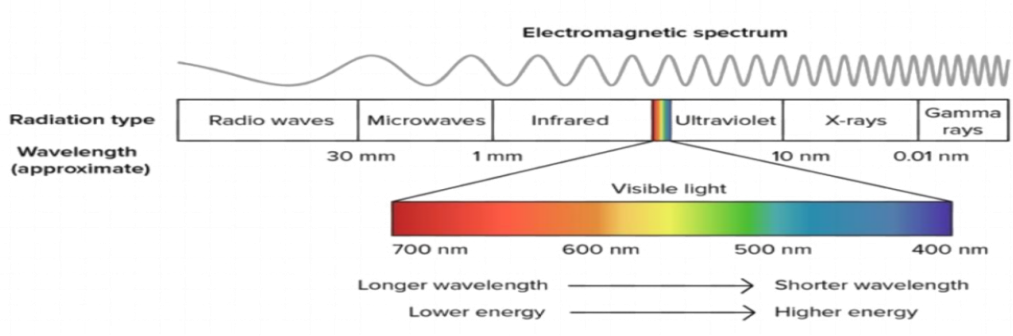
3) ELECTRO ANALYTICAL TECHNIQUE: it is based on the electro chemical property of drug. Eg; potentiometry, conductometry , polarography, amperometry, electrophoresis etc.

4) MISCELLANIOUS TECHNIQUES**:** like conventional titrymetric method, polarimetric method, physical method, radioactive methods, biological and microbiological methods

THEORY ASPECTS OF UV-VISIBLE SPECTROSCOPY

Spectroscopy is the measurement and interpretation of electromagnetic 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 changes maybe from ground state to excited state or excited state to ground state. At ground state the energy of the molecule is the sum total of rotational, vibrational and/or electronic energies.

Electromagnetic radiation is made up of discrete particles called photons. EMR has got both wave characteristics 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, gamma rays or cosmic rays. As these radiations have different wavelength or frequency or energy, they are conveniently named so.



- ❖ Frequency: It is the number of complete wavelength unit passing through a given point in unit time. Frequency is measured in Hz.
- ❖ Wavelength: It is the distance between two successive maxima or minima or distance between two successive troughs or peaks. It can be measured in meters, centimeters, millimeters, nanometers or angstrom.
- ❖ Wave number: It is the number of waves per cm. it is expressed in cm^{-1} or Kayser.

UV-Vis spectroscopic methods are used to identify, characterize, and quantify an extraordinary wide range of molecular compounds. It can be used for non-destructive measurements, such as determining the sugar, lipid, protein content of foodstuffs and for identifying medicinals.

PRINCIPLE

- VISIBLE SPECTROSCOPY/ COLORIMETRY: It is concerned with the visible region which ranges from 400nm-800nm. Colored substance will absorb radiation in this wavelength region. Colored substances, absorb light of different wavelength in different manner and get an absorption curve. From this absorption curve the wavelength at which maximum absorption of radiation is obtained called 'λ max'.

'λ max ' is unique and this is a qualitative aspect, useful in identifying the substance. There is no change in λ max when concentration changes.

- UV SPECTROSCOPY: It is concerned with the study of absorption of UV radiation which ranges from 200nm-400nm. Colourless compounds absorb UV radiation. Any molecule has either n, π or sigma or a combination of these electrons. These bonding (sigma and π) and non-bonding (n) electrons absorbs the characteristic radiation and undergo transition from ground state to excited state. By the characteristic absorption peaks, the nature of the electron present and hence the molecular structure can be elucidated.

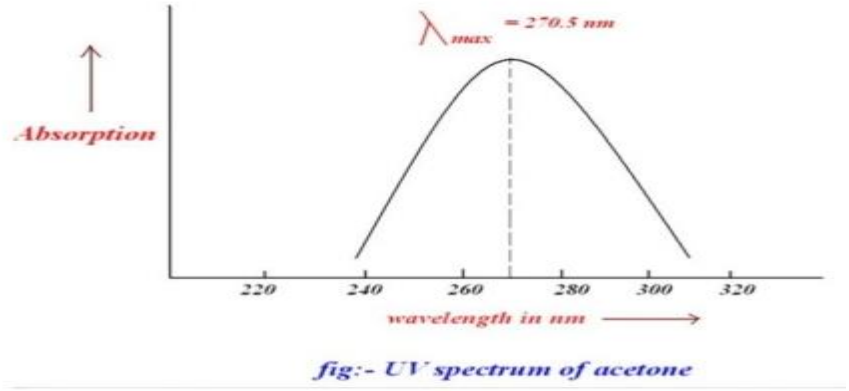


fig:- UV spectrum of acetone

Certain spectrophotometric terms and symbols:

*a) Radiant power (*P*):* It is the rate at which energy is transported in a beam of radiant energy that is radiant flux.

*b) Transmittance (*T*):* It is ratio of the radiant power transmitted by the sample (P) to the radiant power incident on the sample (P_0), both being measured at the same spectral position and with the same slit width. Thus transmittance T is defined by $\frac{P}{P_0}$. The alternate of transmittance is the name transmission.

*c) Absorbance (*A*):* It is the logarithm to the base 10 of reciprocal of the transmittance. A= log10 ($\frac{1}{T}$) = log T. It can also expressed as A= log $\frac{P_0}{P}$. The alternate of this term is extinction or optical density (D).

*d) Absorptivity (*a*):* It is the ratio of the absorbance of the product of concentration and length of optical path. It is a constant characteristics of (a= $\frac{A}{bc}$) substance and wavelength. The alternate of this term is extinction coefficient or absorbance index.

e) Molar absorptivity (ϵ*):* The absorptivity expressed in units of litre or (mole cm) is called molar absorptivity. Here the concentration is in the mol/litre and cell length in cm. Thus molar absorptivity ϵ = AM/ bc. The alternate term for molar absorptivity is molar extinction coefficient or molar absorbance index.

*f) Path length (*b*):* It is the internal cell length expressed in cm.

*g) Millimicrons (*mμ*):* It is a unit of length equal to 1000 of a micron. One micron= 10^{-6} metre.

h) Spectral bandwidth: The range of wavelengths of radiant energy emerging from the exit slit of the monochromator.

i) Visible or white light: Radiant energy which is perceived by the normal human eye (approx 380-780mμ).

*j) Slit width (*SW*):* It is mechanical distance (mm) between the sides of the narrow aperture which permits radiant energy to enter and to leave the monochromator. Spectral slit width is the width of the image of the exit slit along the wavelength scale. The effective slit width is the width of the image at which that is the intensity is half of the maximum.

LAWS GOVERNING ABSORPTION OF RADIATION

The two laws related to the absorption of radiation are:

1. Beer's law (related to concentration of absorbing species)
2. Lambert's law (related to thickness or path length of absorbing species)

These two laws are applicable when there is no reflection/scattering of light are takes place.

Beer's Law:

Beer's law states that the intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically.

$$I = I_0 e^{-kc}$$

Lambert's Law:

The rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light.

Mathematical equation for Beer's-Lambert's law:

$$A = \epsilon ct$$

Where A= Absorbance or optical density

ϵ = Molecular extinction coefficient

c = Concentration of drug (mmol/lit)

t= Path length (normally 10 mm or 1cm)

ϵ can also expressed as; $\epsilon = E_{1cm}^{1\%} \times \frac{molecular\ weight}{10}$

Where, $E_{1cm}^{1\%}$ means the absorbance of 1% w/v solution, using a path length of 1cm.

Electronic transitions and excitation process by UV radiation

It was stated earlier that π, σ and n electrons are present in a molecule and can be excited from ground state by the absorption of UV radiation. The required for excitation for different transitions are: $n \rightarrow \pi^* < \pi \rightarrow \pi^* < n \rightarrow \sigma^* < \sigma \rightarrow \sigma^*$ of these transitions $n \rightarrow \pi^*$ required lowest energy and $\sigma \rightarrow \sigma^*$ requires the highest energy for excitation in the UV region.

After absorption of UV radiations these electronic structures have greater or lesser polar character than in ground state.

Polar solvent shifts $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ to shorter wavelengths and $\pi \rightarrow \pi^*$ to longer wavelength.

Types of electronic transitions:

1. $n \rightarrow \pi^*$: the peaks due to this transition is also called as R-bands. Type of peak can be seen compounds where n electron (present in S,O,N or halogen) is present in a compound containing double bond or triple bond eg: aldehydes or ketones, nitro compounds.
   - Without double or triple bond peaks occurs between 270-300nm
   - When separated by 2 or more single bond peaks occurs between 300-350nm.

   The presence of $n \rightarrow \pi^*$ transition can be identified easily by comparing the UV spectrum of the substance with the spectrum recorded in the acid solution of the same substance. If $n \rightarrow \pi^*$ has been present in an acid solution, the band disappears.

2. $\pi \rightarrow \pi^*$: This type of transition gives rise to B, E, and K bands. The energy required for these transitions is between $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$.

TYPES	DUE TO
B- bands (benzenoid bands)	Aromatic and hetero atromatic systems.
E- bands (ethylenic bands)	Aromatic systems
K- bands ($\pi \rightarrow \pi^*$)	Conjugated systems.

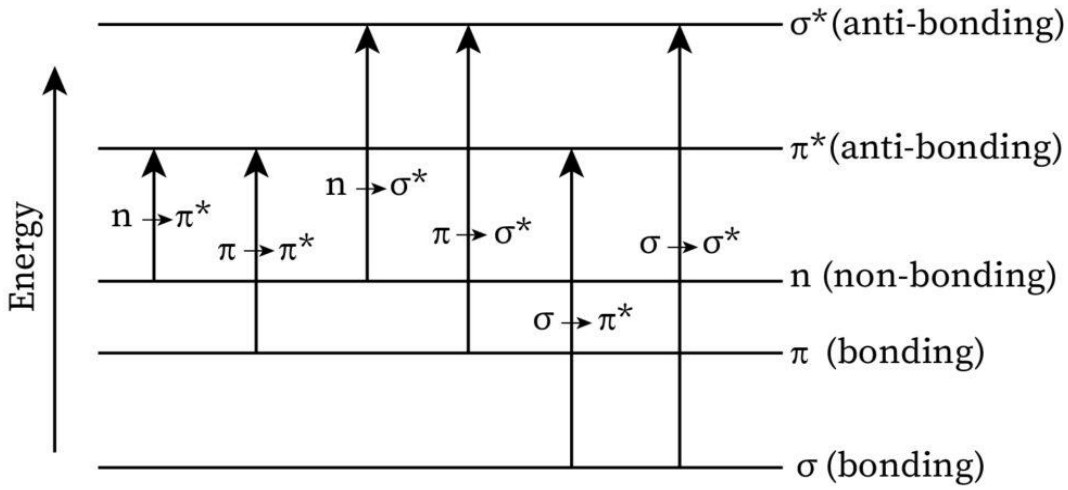
3. $n \rightarrow \sigma^*$: this transitions occurs in saturated compounds, with hetero atoms like S,N,O or halogens. It requires lesser energy when compared to $\sigma \rightarrow \sigma^*$ transitions. Peaks due to this transition occurs from 180nm-250nm.as these peaks are observer at the lower end of the UV spectrum, it can be called as end absorption.

Compounds with $n \rightarrow \sigma^*$ transitions:

4. $\sigma \rightarrow \sigma^*$: This type of transitions requires the highest energy. This is observed with saturated compounds

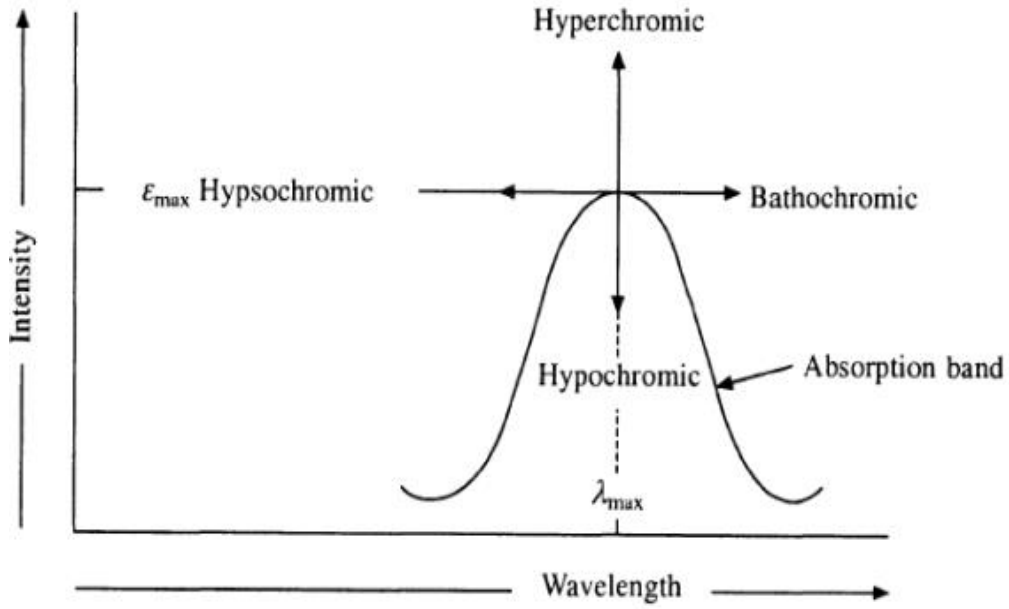
Compound	λ max
Methylene chloride	173nm
Water	191nm
Methanol	203nm

(hydrocarbons). The peaks do not appear in UV region, but occur in vaccum UV or for UV region that is 125-135nm. Example: methane (122nm), ethane (135nm), propane (135nm).
Cyclohexane (195nm) can be used as non-polar solvent, as it does not give solvent peak.



Certain Terms Used in Electronic Spectroscopy:
Chromophore
A covalently unsaturated group responsible for absorption in the UV or visible region is known as a chromophore. For example, C = C, C = O, C= N, N = N, NO2 etc.
Auxochrome
A covalently saturated group which, when attached to a chromophore, changes both the wavelength and the intensity of the absorption maximum is known as auxochrome, e.g. NH2 ,OH, SH, halogens etc. Auxochromes generally increase the value of λmax as well as εmax by extending the conjugation through resonance. These are also called colour enhancing groups. An auxochrome itself does not show absorption above 200 nm.

ABSORPTION AND INTENSITY SHIFTS
Bathochromic Shift or Effect.
The shift of an absorption maximum to a Ionger wavelength (Fig. 2.5) due to the
presence of an auxochrome, or solvent effect is called a bathochromic shift or red shift.



Hypsochromic Shift or Effect

The shift of an absorption maximum to a shorter wavelength is called hypsochromic or blue shift (Fig. 2.5). This is caused by the removal of conjugation or change in the solvent polarity.

Hyperchromic Effect.

An effect which leads to an increase in absorption intensity εmax is called hyperchromic effect (Fig. 2.5). The introduction of an auxochrome usually causes hyperchromic shift.

Hypochromic Effect

An effect which Ieads to a decrease in absorption intensity εmax is called hypochromic effect (Fig. 2.5). This is caused by the introduction of a group which distorts the chromophore.

Solvent Effects

Since the polarity of a molecule usually changes with electronic transition, the position and the intensity of absorption maxima may be shifted by changing solvent polarity.

(i) π→π*Transitions (K-Bands)

Owing to the non-polar nature of hydrocarbon double bonds, the π→π* transitions of alkenes, dienes and polyenes arenot appreciably affected by changing solvent polarity. The π→π* transitions of polar compounds, eg: saturated as well as a, α, ß-unsaturated carbonyl compounds are shifted to longer wavelengths and generally towards higher intensity with increasing solvent polarity.

(ii) B-Bands : These bands also originate from π→π* transitions, and their position and intensity are not shifted by changing solvent polarity except in case of heteroaromatic compounds which show a marked hyperchromic shift on increasing solvent polarity.

(iii) n→π* Transitions (R-Bands)

It has been found that an increase in solvent polarity usually shifts n→ π* transitions to shorter wavelengths (higher energy). For example, acetone shows λmax 279 nm in hexane, whereas in water it shows λmax 264.5 nm. This can be explained on the basis that the carbonyl group is more polar in the ground state than in the excited state Thus, dipole-dipole interaction or hydrogen bonding with a polar solvent lowers the energy of the ground state more than that of the excited state resulting in the hypsochromic shift in case of unconjugated as well as conjugated carbonyl compounds with increasing solvent polarity.

(iv) n→α* Transitions

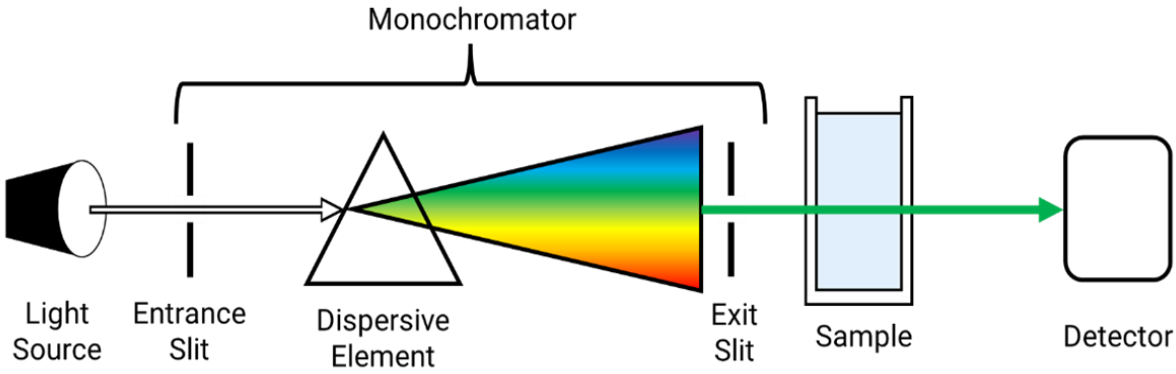
These transitions are affected by solvent polarity, especially by solvents capable of forming hydrogen bond. Alcohols and amines form hydrogen bonds with protic solvents. Such associations involve non-bonding electrons of the heteroatom. The involvement of nonbonding electrons in hydrogen bonding lowers the energy of the n orbital, and thus the excitation of these electrons requires greater energy resulting in the hypsochromic shift with increasing polarity. It has been found that an increase in solvent polarity usually shifts n→π* and n→α* bands toshorter wavelengths, and π→π* bands of polar compounds to longer wavelengths.

INSTRUMENTATION OF UV-VISIBLE SPECTROPHOTOMETER

A standard or a series of standards must always be available. Although visual colorimetric comparison described above are applicable for many routine determinations, the use of photoelectric filter photometers and spectrophotometers is now so

widespread in modern analytical technology that is extremely important to consider the later methods of measuring sample solution. They include:

a) An intense source of radiant energy.
b) A filter or monochromator to isolate the wavelength region to be used in irradiating the sample solution.
c) A pair of cuvettes one for sample, and the other for the blank or reference solution.
d) A photometer, comprising a photoelectric detector which converts radiant energy to electrical energy and a meter to indicate the resulting electric current.



Fundamental components:

Radiation source or Light source: Any lamp source which gives adequate intensity of radiation over the entire wavelength region can be used. The requirements are:

- Should provide continuous radiation.
- The source must generate beam with sufficient power for ready detection and measurement.
- It should be stable.

For visible spectroscopy: Tungsten lamp, Carbon arc lamp

For UV spectroscopy: Hydrogen discharge lamp, Deuterium lamp

Filter or monochromators: Filters isolate a wider band than the monochromators, but some interference filters have a narrower half intensity band width than some monochromators. Filters are of two types:

- Absorption filters: These filters are made up of glass, coated with pigments or they are made up of dyed gelatin. They transmit only the required radiation.
- Interference filters: This filter is otherwise known as Fabry-Perot filter. These filters rely interference phenomenon at desired wavelength, thus permitting rejection of unwanted radiation by selective reflection and producing narrow bands. It consist of transparent calcium fluoride or magnesium fluoride solution that occupies the space between two semi transparent metallic films coated inside surface of two glass plate.

Monochromators: It is better and more efficient than filters in converting a polychromatic light or heterochromatic light into a monochromatic light. A monochromator has the following units:

- Entrance slit (to get narrow source)
- Collimator (to render light parallel)
- Grating or prism (to disperse radiation)
- Collimator (to reform the images of entrance slit)
- Exit slit (to fall on sample cell)

Cuvettes or Sample cell: It is used to hold a sample solution geometry and material varies with the instrument and nature of sample handled. The volume of the cell varies like small volume cells 0.5 or less ml. having path length of 1cm and the shape may be cylindrical or rectangular.

Detectors: Detectors used in UV or visible spectrophotometers can be called as photometric detectors. In these detectors the light energy s converted into electrical signal which can be read or recorded.

Commonly used detectors are:

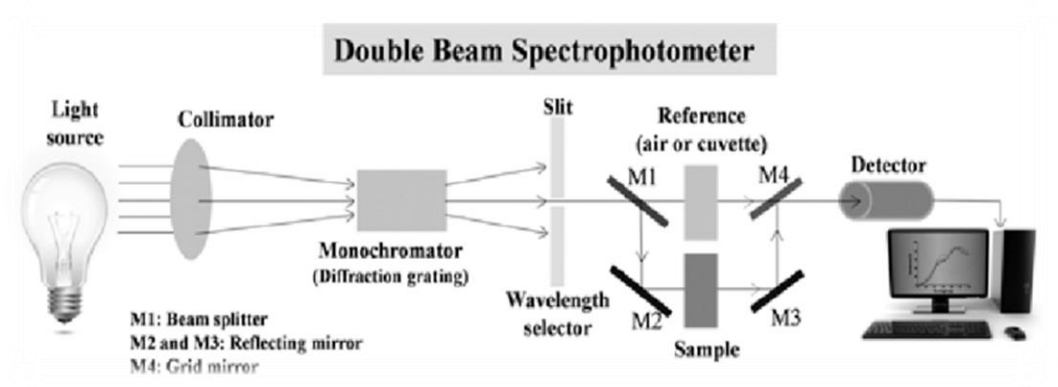
- Barrier layer cell or photo voltaic cell
- Photo tubes or photo emissive cells
- Photo multiplier cells

TYPES OF UV-VISIBLE SPECTROPHOTOMETERS

❖ Single Beam Spectrometer:
As the name suggests, these instruments contain a single beam of light. The same beam is used for reading the absorption of the sample as well as the reference. The radiation from the source is passed through a

filter or a suitable monochromator to get a band or a monochromatic radiation. It is then passed through the sample (or the reference) and the transmitted radiation is detected by the photodetector. The signal so obtained is sent as a read out or is recorded.

- Double Beam Spectrophotometer:
  In a double beam spectrometer, the radiation coming from the monochromator is split into two beams with the help of a beam splitter. These are passed simultaneously through the reference and the sample cell. The transmitted radiations are detected by the detectors and the difference in the signal at all the wavelengths is suitably amplified and send for the output.



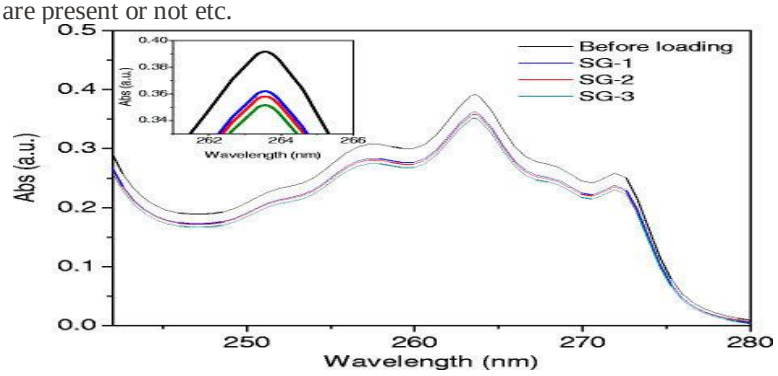
APPLICATIONS

1) Detection of Impurities
   UV absorption spectroscopy is one of the best method for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of the standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected.

2) Structure elucidation of organic compounds
   UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of heteroatoms. From the location of the peaks and combination of peaks, it can be concluded that wheather the compound is saturated or unsaturated, heteroatoms are present or not etc.

3) Quantitative ananlysis
   UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is based on beers law which is as follows
   $A=\log Io/It = \log I/T = -\log T = abc = \varepsilon bc$
   Where: ε - is extinction co-efficient, c- is the concentration and b- is the length of the cell hat is used in UV spectrophotometer

4) Qualitative Analysis
   UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds.



5) Chemical kinetics
Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.
6) Detection of functional groups
This technique is used to detect the presence or absence of functional group in the compound. Absence of a band at particular wavelength regarded as an evidence for absence of particular group.
7) Quantitative analysis of pharmaceutical substances
Many drugs are either I the form of raw material or in the form of formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at specific wavelength.
8) Examination of polynuclear hydrocarbons
Benzene and polynucleear hydrocarbons have characteristic spectra in ultraviolet and visible region. Thus identification of polynuclear hydrocarbons can be made by comparison with the spectra of known polynuclear compounds. Polynuclear hydrocarbons are the hydrocarbon molecule with two or more closed rings, examples are naphthalene, $C_{10}H_8$, with two benzene rings side by side, or diphenyl $(C_6H_5)_2$, with two bond connected benzene rings. Also known as *polycyclic hydrocarbon*

9) Molecular weight determination
Molecular weight of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.
For example, if we want to determine the molecular weight of amine then it is converted into amine picrate. The known concentration of amine picrate is dissolved in a litre of solution and its optical density is measured at λmax 380nm.
After this the concentration of the solution in gm moles per litre can be calculated by using the following formula.

$$C = \frac{\log I_0 / I_t}{\varepsilon_{max} \times 1}$$

"C" can be calculated using above equation, the weight 'w' of amine picrate is known. From 'C' and 'w', molecular weight of amine picrate can be calculated. And the molecular weight of picrate can be calculated using the molecular weight of amine picrate.

10) AS HPLC DETECTOR
A UV/Visible spectrophotometer may be used as a detector for HPLC.

HOW TO OPERATE UV VISIBLE DOUBLE BEAM SPECTROPHOTOMETER

STANDARD OPERATING PROCEDURE FOR DOUBLE BEAM UV SPECTROPHOTOMETER
MODEL: systronics double beam spectrophotometer 2203
Objective: to describe the operation of uv - visible double beam spectrophotometer
PROCEDURE:

1. Before starting the measurement check whether the cuvette chamber is empty.
2. Switch ON the instrument by pressing the red switch on the back.
3. Switch ON UV or Visible lamp by pressing F1 or F2.
4. Allow a warm up period of 30 minutes.
5. During the period the instrument will initialized and auto calibration will be made.
6. Select the conditions by pressing the appropriate number assigned for the criteria like single or multiple wavelength etc.
7. Press '1' to select absorbance.
8. Clean the cuvettes with distilled water and fill one with blank solution and the other with sample solution.
9. Wipe them with soft wipes and place them inside the cuvette chamber and close the chamber.
10. Press F1 to take reading and enter the number of readings and cell number in which the cuvette is placed.
11. The absorbance of the sample will be displayed on the display panel.
12. After the measurement is over, the cuvettes are removed and cleaned well.
13. The chamber is closed and UV or visible lamp is switched OFF by pressing F1orF2.
14. Switch OFF the instrument by pressing red switch on the back.

CALIBRATION AND VALIDATION OF UV-VISIBLE SPECTROSCOPY

CALIBRATION:

- Calibration is an operation which is performed to assure that the instrument readings are accurate with reference to the established standards.
- The aim of the calibration program is to ensure that all measuring and testing equipment included in the program are calibrated within the manufacturers accuracy specifications or the tolerance required for the application.

Calibration of UV-VIS spectrophotometer involves following parameters:

➢ *Calibration for wavelength accuracy.*
➢ *Calibration for absorbance measurement.*
➢ *Gratings performance or stray light test.*
➢ *Resolution power.*

❖ Calibration for wavelength accuracy:
- Take two empty cuvettes, into one cuvette add two drops of benzene and drain it.
- Place the cuvette in sample cell holder with benzene vapors occupied in it.
- Place the empty cuvette in reference cell holder.
- Scan it in between 240-270nm.
- The maximum wavelenght should be at 253.9 ± 0.51 nm.
- Absorbance should be below 1 (one).

❖ Calibration for absorbance measurement:
- Prepare 60ppm of potassium dichromate by using 0.01M sulfuric acid as solvent.
- Place the solution in sample cell holder.
- Place blank 0.01M sulfuric acid in reference cell holder.
- Measure the absorbance values at different λmax values.

❖ Gratings performance or stray light test:
- Prepare 1.2% potassium chloride solution in distilled water.
- Distilled water is taken as reference.
- Place the potassium chloride solution in sample cell holder.
- Absorbance is measured at 195-220nm.
- Absorbance is must be greater than 2 at 198

❖ Resolution power:
- When prescribed in a monograph, record the spectrum of a 0.02% v/v solution of toluene inhexane .
- The ratio of the maximum absorbance at about 269 nm to that at the minimum absorbance at about 266 nm should not be less than 1.5 unless otherwise specified in the monograph

VALIDATION

Validation is a fundamental requirement under Good Laboratory Practices and it is equally important to maintain documentary records of all such activities. It is mandatory to validate the performance parameters as suggested. Besides these some additional performance tests wil ensure results that would be acceptable in any other laboratory.

Validation of UV-visible spectroscopy involves the following parameters:

➢ Wavelength Accuracy:- Wavelength accuracy establishes the closeness of the recorded wavelength to the true value. It can be established by using either absorption or emission standards.

Absorption standards: Holmium oxide glass or Quartz filters provides a quick check on wavelength accuracy. The filters provide only sharp absorbing bands in the visible region. In order to establish wavelength accuracy over both UV and visible region of solution holmium oxide in perchloric acid is recommended in pharmacopoeias. Another reference standard recommended in pharmacopoeia is didymium glass.

Emission Standards: The deuterium lamp gives a sharp emission line at 656.1nm and a weaker line at 486.0nm. By switching off the visible position of either or both of the lines can establish the wavelength accuracy. Mercury lamp offers more lines in the UV region in comparison to the deuterium lamp but the deuterium lamp is more convenient to use as an emission standard.

Resolution:- Resolution tell us how well two closely appearing absorbance bands are resolved by the spectrophotometer. For the purpose, a solution of 0.2%w/v of toluene in hexane is used. The ratio of peak at 269nm to trough at 266nm should be equal to or greater than 1.5.

Photometric Accuracy:- Metal quartz filters have been used for keeping check on photometric or absorbance accuracy. Such filters are convenient to use but lead to complications due to back reflection caused by the reflective coating applied to the quartz plate.

A potassium dichromate solution offers reliable absorbance accuracy standard which is recommended by Pharmacopoeia's. Potassium dichromate is hygroscopic so it should be dried before weighing. 57.0 to 63.0mg of the solid is diluted to 1000ml with 0.005M sulphuric acid and absorbance is recorded at 235,257,313 and 350nm. Since 2005 an additional reference wavelength of 430nm has been included. The dilution in such case is made 100ml instead of 1000ml.

Stray light:- Stray light results from presence of other wavelength besides the wavelength isolated by the monochromator. It is an important performance specification as it can lead to noticeable interference particularly at high absorbance values.

As per European pharmacopoeia specification the absorbance of a 12g/l solution of potassium chloride solution at 198n should be 2 or higher.

Additional test parameters:- In addition to the stipulated tests additional tests can be performed and these provide useful information on overall performance of the instrument and its component parts. Such tests can include absorbance stability, baseline stability, and noise at zero or at other specified absorbance values. It is equally important that solvents from reliable sources of good quality are

used and optically matched pair of absorption cells are used for conducting validation studies.

DRUG ANALYSIS BY USING UV -VISIBLE SPECTROPHOTOMETER

CHOICE OF SOLVENT

Solubility of a drug is one of its important physic-chemical properties. The solvent system should provide homogenous solution where by all the solute substance under investigation is completely dissolved. Every solvents have a cutoff wavelength. That is the wavelength below at which the solvent itself absorbs all the light. If the cutoff wavelength off the compound and solvent is close, choose a different solvent. Solvent should not absorb light in the region where our sample is absorbing light, else peak will overlap. Solvents can be of two types, polar and non-polar. If possible avoid the use of polar solvents as they interact with chromophoric group. Polar solvents form smooth curved structure and non-polar solvent forms fine structure. Solvent should not interact chemically with the sample, because even a small hydrogen bond may affect the peak position and its width.

In order acquire a UV-Vis spectrum, the solid particles has to be completely dissolved in the suitable solvent. Note that not every solvent is suitable for spectroscopic analysis. Generally spectrograde solvents are used that have undergone special purification steps to remove trace impurities and other compounds.

SELECTION OF CONCENTRATION

UV-visible spectroscopy is an absorption technique, where Beer's Lambert's law is applied. It is only valid at low concentration ($<10^{-4}$ mol/L). At higher concentrations, negative deviations is observed. Another factor is the detector of instrument that only works linear within a certain range as well. Meaningful absorbance should be between 0.1-1, the concentration has to be low enough, so that the maximum absorbance should not exceed A=1.

SAMPLE CELLS

It is a must to take care of the cuvette is to be positioned in the same direction of the light source placed in the holder for the measurements. This avoids the error that occurs due to optical effects and provides identical optical effects for both reference and sample. For UV spectroscopy cuvette made up of quartz is used and for visible spectroscopy cuvette made up of glass is used. The cuvettes are fabricated with windows through which the light passes. This window is to be cleaned properly with a lint free tissue. The presence of dust or finger prints on the cuvette windows result in additional absorbing components and hence provides in accurate results. It is a must to take additional care to clean the cuvettes before and after use.

WAVELENGTH SELECTION

Ultraviolet-visible spectrophotometers use a light source to illuminate a sample with light across the UV to the visible wavelength range (190-900nm). It depends on the color of the solution. The best wavelength to use is the one with the highest molar absorptivity (λ_{max}), provided there are no interfering substances that absorb at the same wavelength. Absorbance measurements are carried out at fixed wavelength.

To select the suitable wavelength for analysis, follow the steps given below

- Prepare a standard solution of the analyte.
- Select the light source according to the color of the solution.
- Insert the solution into the instrument.
- Run a full scan of the wavelength in the range from 200-900nm.

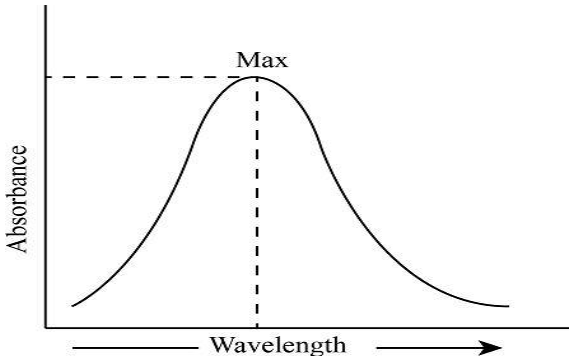
The suitable wavelength is the one that is responsible for the highest absorbance.

DETERMINATION OF MAXIMUM WAVELENGTH (λ_{max})

λ_{max} is the wavelength at which highest absorbance is produced.

To determine the λ_{max} follow the steps given below;

- Weigh accurately the required quantity of the analyte.
- Prepare a standard solution of the analyte using suitable solvent.
- Undergo dilutions until attaining the desired concentration (10µg/ml)
- Switch on the spectrophotometer and allow stabilize for 30 minutes.
- Switch on the suitable lamp and insert the sample into the instrument.
- Measure the absorbance of the solution from wavelength range from 200-800nm corresponding to the solution.
- Plot a graph between wavelength on x-axis and absorbance on y-axis. From this the maximum wavelength is obtained.



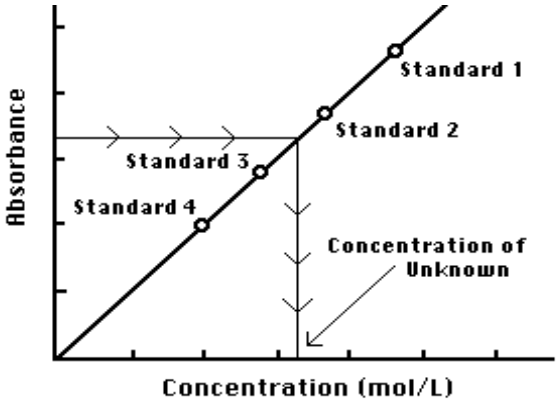
MEASURING ABSORBANCE BY SINGLE AND MULTIPLE WAVELENGTH ANALYSIS

Single wavelength analysis:
Single wavelength analysis is done to measure the absorbance of single componentat single wavelength.To measure on single wavelength, select the conditions by pressing the appropriate number assigned for the criteria and enter the desired wavelength.

Multiple wavelength analysis:
Multiple wavelength analysis is done to measure the absorbance of single or mulitiple component present in a complex mixture at multiple wavelengths.To measure on multiple wavelength, select the conditions by pressing the appropriate number assigned for the criteria and enter the desired wavelengths.

DETERMINING CONCENTRATION OF A DRUG SAMPLE
The concentration of the unknown sample can be determined by using various method on UV-visible spectroscopy as follows:

I. GRAPHICAL METHOD
In this method plotting curve using concentration versus absorbance value of six or more standard solutions. A straight line is drawn either through maximum number of points. This method is called as line of best fit. This line may or may not pass through origin. From the absorbance of the sample solution and calibration curve, the concentration the drug can be calculated.



II. SINGLE COMPONENT METHOD
In this method the concentration of a standard solution of known concentration and a sample solution is measured. The concentration of unknown can be calculated using the formula

$$\frac{A_1}{A_2} = \frac{C_1}{C_2}$$

Where A1, A2 - Absorbance of standard and sample
C1, C2 - Concentration of standard and sample.

III. ABSORPTIVITY METHOD
This method is used when reference standard is not available. The estimations is done by using the formula:
A= εbc Where A- the absorbance of the analyte
ε - molar absorptivity coeffiecient ($E_{1cm}^{1\%}$)
b - pathlength
c - concentration
ε can be get from the standard pharmacopoeias and standard analytical texts.

IV. SIMUTANEOUS MULTICOMPONENT METHOD
If a mixture of two components 'x' and 'y' are present, by measuring the absorbance of the mixture at two different wavelengths we can estimate the concentrations.

$$C_x = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

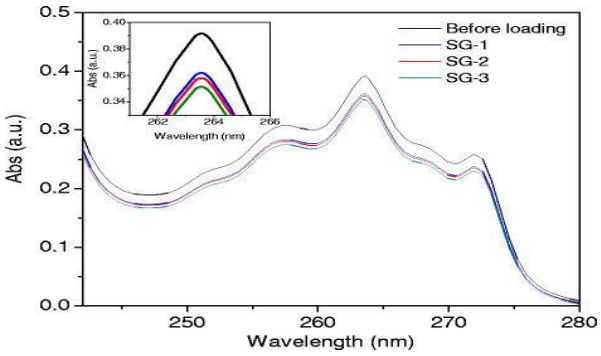
$C_y = \frac{A_1\, ax_2 - A_2\, ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$

Where, C_X and C_y - concentration of 'x' and 'y'
A_1 and A_2 – absorbance of at λ_1 and λ_2
ax_1 and ax_2 - absorptivity of 'x' at λ_1 and λ_2

ay_1 *and* ay_2 - absorptivity of 'y' at λ_1*and* λ_2

QUALITATIVE ANALYSIS

UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds.



CONCLUSION:

From the above we can conclude that by using UV-visible spectroscopy we are able to done the quantitative determination of different analytes. UV-visible spectroscopy is a valid, simple and cost effective.

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