e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

UV-VISIBLE SPECTROSCOPY: A COMPREHENSIVE REVIEW OF PRINCIPLES, INSTRUMENTATION, AND APPLICATIONS

N. Gopinath*1, Parimala Vudikala*2

*1,2Joginpally BR Pharmacy College, Moinabad, Telangana, India.

DOI: https://www.doi.org/10.56726/IRJMETS69728

ABSTRACT

For the past 37 years, UV-Visible spectroscopy has been the term used for the analytical evaluation of various solvents and compounds. UV-Visible spectrometers are commonly used by small businesses due to their lower cost and maintenance requirements. This analytical method involves measuring the amount of monochromatic light absorbed by colorless materials in the 200–400 nm range of the near-ultraviolet spectrum. Pharmaceutical analysis encompasses the procedures required to determine the "identification, strength, quality, and purity" of these substances. It also includes the study of raw materials and pharmaceutical manufacturing. This study explores the various applications of UV spectroscopy both qualitatively and quantitatively.

I. INTRODUCTION

This investigation explores molecular absorption across the electromagnetic spectrum from 190 to 800 nm, specifically analyzing the ultraviolet range of 190-400 nm and the visible range of 400-800 nm [1]. The thickness (b) and concentration (c) of a homogeneous solution influence the amount of monochromatic radiation that passes through it in a cell. It represents the incident radiation intensity, while I indicate the transmitted radiation intensity [2].

SPECTROSCOPY: involves studying matter's properties by examining how it interacts with various forms of radiation, primarily electromagnetic radiation from the spectrum. [3]

SPECTROMETRIC TECHNIQUES: Encompass a wide array of analytical methods grounded in atomic and molecular spectroscopy. Spectrometry and associated methods involve measuring radiation intensity using a photoelectric transducer or other electronic devices.[4]

UV-VIS SPECTROSCOPY- UV-Vis spectroscopy is an analytical technique that measures the number of discrete wavelengths of UV or visible light absorbed by or transmitted through a sample compared to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration.[5] Ultraviolet and visible absorption spectroscopy is the measurement of the absorption of monochromatic radiation by the solution of chemical substances in the range of 185nm380nm and 380nm-780nm of spectrum spectroscopy.[6]

Fig 1: Uv-visible spectrophotometers [7]

II. PRINCIPLE

The principle of UV-visible spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, producing different spectra. Spectroscopy is based on the interaction between light and matter. When matter absorbs light, it becomes excited and de-excited, resulting in the production of a spectrum. When matter absorbs ultraviolet radiation, the electrons it contains become excited. This causes them to jump from a ground state (an energy state with an associated amount of energy) to an excited state (an energy state

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

with a relatively large amount of energy associated). It is important to note that the difference in the energies of the electron's ground state and excited state is always equal to the amount of ultraviolet or visible radiation that it absorbs.[8]

Electronic transition:

Electronic transition: An item will show absorption in the visible or ultraviolet spectrum when radiation causes an electronic transition in the structure of a molecule or ion. Therefore, the molecules within a sample change their electronic state when they absorb light in the visible or ultraviolet spectrum. The energy from the light will cause electrons to move from their ground state orbital to an excited state orbital, which is more energy. or orbital anti-bonding

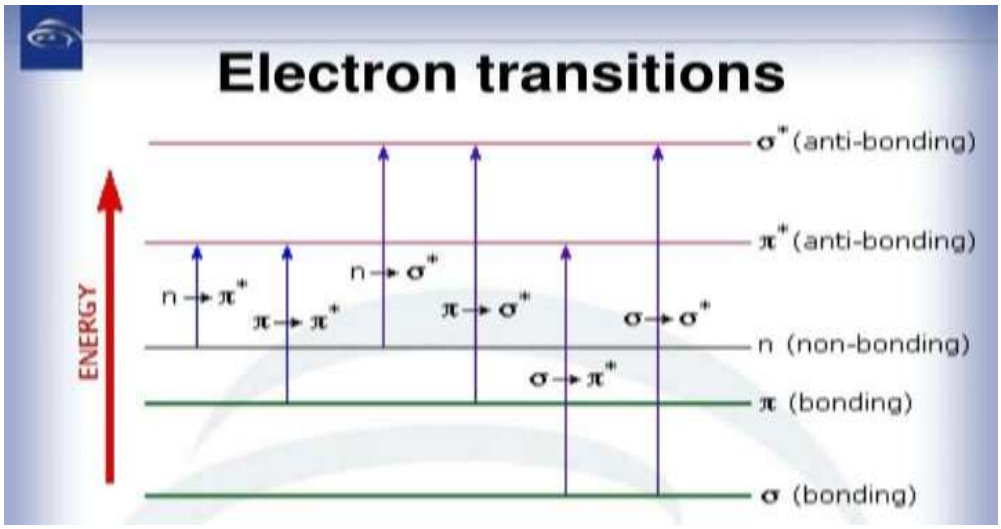

Fig 2: Electronic transition in molecular orbital.

Three different kinds of ground-state orbitals could be at play.

1) σ molecular bonding

2) The molecular orbital π (bonding)

3) n atomic orbital (bonding)

Furthermore, the transition may involve two different kinds of anti-bonding orbitals.

1) The orbital of σ* (sigma star)

2) π* orbital of a pi star

Since n electrons do not establish bonds, there is no such thing as a n* anti-bonding orbital. Therefore, the absorption of visible and ultraviolet light might result in the following electronic transitions.

1) from σ to σ*

2) from n to σ*

3) from n to π*

4) from π to π*

The σ to σ* and n to σ* transitions occur in the far ultraviolet region or occasionally in the 180–240 nm range because of their high energy requirements. As a result, saturated groups do not exhibit strong absorption in the typical UV spectrum. Transitions from then to the π* and π* type happen in molecules with unsaturated centers, as opposed to transitions to the π* anti-bonding orbital. They occur at longer wavelengths and need less energy. It will soon become evident that the structure of molecules determines the maximum wavelength and intensity of absorption. Transitions to the π* anti-bonding orbital, which typically occur in the UV range, may occur in the visible region if a molecule's chemical structure is altered. The visible spectrum is also where a lot of inorganic substances in solution exhibit absorption. These comprise salts of elements (mostly transition metals) having incomplete inner electron shells, whose ions undergo hydration-induced complexation. These absorptions result from a charge transfer mechanism, in which the energy from the visible light moves electrons from one area of the system to another. [9]

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

The energy order of different types of electronic transitions is as under.

$\sigma\text{-}\sigma^* > n\text{-}\sigma^* > \pi\text{-}\pi^* > n\text{-}\pi^*$

The Beer-Lambert law is used to study this occurrence.

The Beer-Lambert equation states that the absorbance (A) of a solution is inversely linked to the path length (b) and the concentration of the absorbing species (c) in the solution [10].

Molar absorptivity constant X cell length X concentration = absorbance A

C=A/ab

A=abc

where A stands for molar absorptivity, b for path length, and c for concentration in the context of absorbance.

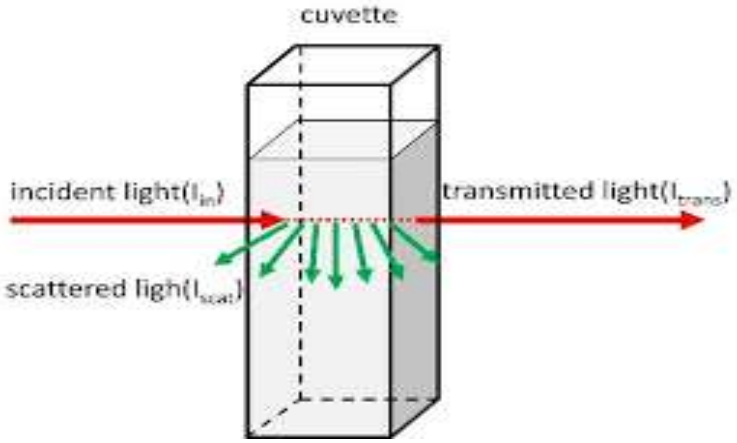

Fig 3: Absorbance, transmittance, turbidity. [11]

Lambert law:

Lambert's law implies that the absorbance 'A' of incident monochromatic light is directly proportional to the path length (cell length) 'ℓ'. This means that equal portions of absorbing material absorb equal fractions of incident light.

$A \propto \ell$

Beer law:

Beer law implies that the absorbance 'A' of incident light or electromagnetic radiation is directly proportional to the concentration 'c' of solution. This law gives the quantitative relationship between the intensity of radiation and the concentrations of chemical species.

$A \propto c$

Beer-lambert law:

Collectively Beer and Lambert's laws state that the absorbance 'A' of an incident monochromatic beam is directly proportional to concentration 'c' of the solution and path length 'ℓ'.

The rate of decrease in intensity of monochromatic light is proportional to the thickness of medium 'ℓ' and concentration 'c' of absorbing substance in dilution.

$A \propto c \,.\, \ell$

$A = \varepsilon . c.\ell$

Where,

ε is the molar absorptivity coefficient constant.

Or

ε is the absorbance for a solution of concentration 1mole/dm^{-3} and a path length of 1cm.

Absorbance:

It is the ratio of the intensity of incident electromagnetic radiation from the source to that of refracted electromagnetic radiation detected by the detector.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

Mathematical representation:

$A = \text{Log } \ell_o / \ell$

$A = \varepsilon . c . \ell$

So,

$\text{Log } \ell_o / \ell = \varepsilon . c . \ell$

Transmittance:

It is the ratio of the power of electromagnetic radiation leaving the sample P_t to that of the incident radiation on the sample from the source.

Mathematical representation in terms of transmittance

$T = P_t / P_o$

$\% T = T \times 100$

$\% T = P_t / P_o \times 100$

Conversion of absorbance to transmittance

$A = -\log T$

$A = -\log P_t / P_o$ (Since $T = P_t / P_o$)

$A = \log P_o / P_t$

$A = 2 - \log T \%$

$\% T = \text{antilog } (2 - A)$

Lambert-Beer Law's drawbacks:

1. A monochromatic light source is required.
2. This is only applicable to diluted solutions; it is not appropriate for concentrated solutions.

The dissociation of weak acids happens as dilution increases.

3. The conjugate base and the weak acids are brought into balance. The absorbance of the conjugate base (A^-) and the acid (HA) cannot be equal. Because of this, weak acidic solutions are not entirely covered by this law.

III. INSTRUMENTATION OF UV-VISIBLE SPECTROSCOPY

The essential components of a UV-Vis spectrophotometer are as follows.

1. Sources (UV- Visible)
2. Monochromator
3. Sample containers (cuvette)
4. Detector
5. Amplifier and recorder [12]

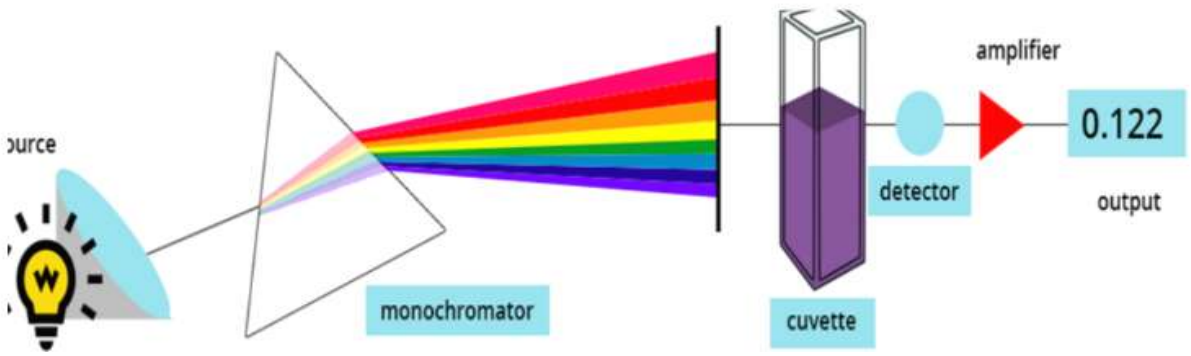

Fig 4: Schematic diagram of UV spectrophotometer [13]

1. Sources: UV-Vis spectroscopy requires a continuous source or one that emits radiation at several wavelengths. There are numerous sources of UV radiation, including the following:

2. Lamp for hydrogen discharge

3. Lamp made of deuterium

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

3. An LED light
4. A xenon discharge light source

1. Hydrogen discharge lamp: This type of light stores hydrogen gas at a comparatively high pressure. The lamp will produce excited hydrogen molecules that emit ultraviolet light when an electric discharge is conducted through it. Instead of emitting a straightforward hydrogen spectrum, the hydrogen in a hydrogen lamp emits a continuum due to the high pressure. The lamps are widely utilized, sturdy, and stable.

2. Deuterium lamps: It is also called a D2 lamp with a wavelength range of 190nm-370nm. If deuterium is replaced by hydrogen, the intensity of emitted radiation is 3 to 5 times the intensity of a hydrogen lamp. Due to its high temperature, quartz is used for it. These are more expensive than the hydrogen lamp, but it is used when high intensity is necessary. The lifespan of a deuterium lamp is 1000 hours.

3. Led lamp: LED lamps do not require a monochromator because they produce a single wavelength of light. LED lamp has a very long life and the cost is low. [14]

4. Xenon discharge lamp: In a Xenon lamp, a steady state can be reached in a short period whose wavelength ranges from 190nm-1100nm in UV and VISIBLE Spectrums. In the lamp, xenon gas is stored under pressure in the range of 10-30 atmospheres. It consists of two tungsten electrodes which are separated by about 8mm. When a low voltage is supplied, an intense arc is formed between two tungsten electrodes which produces ultraviolet light. The intensity of the ultraviolet produced is greater than the hydrogen lamp. When compared to deuterium (or) halogen lamps, xenon lamp has a longer life span and it is expensive. [15,16]

2. The monochromator:

A monochromator removes undesirable wavelengths from the light originating from the radiation source to produce monochromator light.

The entrance slit allows multi-wavelength polychromatic light to enter the monochromator. After collimation, the beam is directed toward the dispersion component. The prism or grating splits the beam's wavelengths into their constituent pieces. Only a specific wavelength of radiation contains the monochromator. through the exit slit when moving the exit slit or dispersing element. [17]

- The wavelength selector, or monochromator The following components are present in all monochromators.
- A focusing lens
- A collimating lens
- A dispersing apparatus (often a prism or grating)
- An exit slit [18]

Through the entrance slit, polychromatic radiation—radiation with many wavelengths—enters the monochromator. The beam angles in the direction of the dispersing element after collimation. The beam's wavelengths are divided into their constituent parts by the prism or grating. When the dispersing element or exit slit is changed, only a certain wavelength of radiation leaves the monochromator through the exit slit [19].

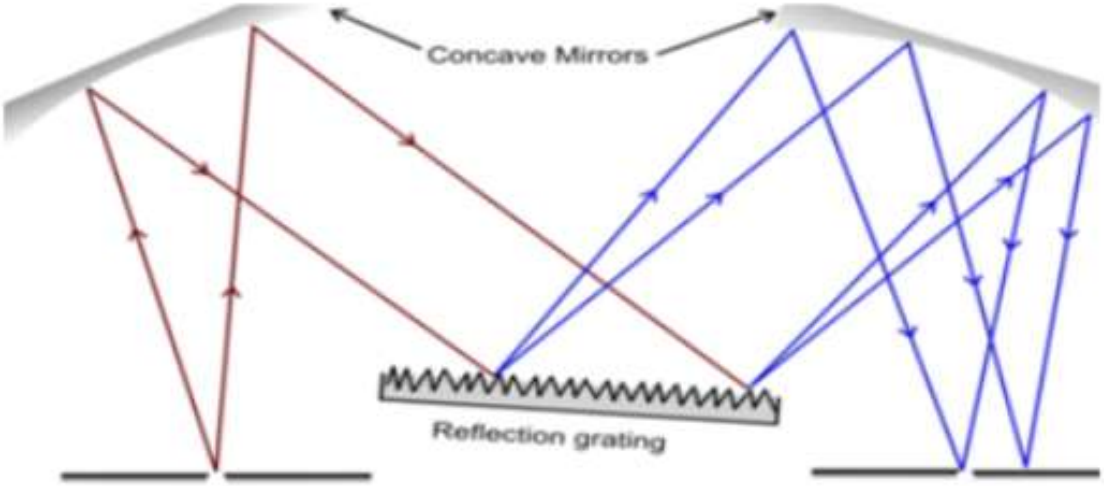

Fig 5: Schematic diagram of a grating monochromator. [20]

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

3. Sample cell: The containers that hold the sample and reference solution need to be transparent to permit the passage of radiation. For UV spectroscopy, it is essential to use quartz or fused silica cuvettes. Furthermore, these cells exhibit transparency in the visual spectrum. Silicate glasses can be employed to produce cuvettes suitable for wavelengths ranging from 350 to 200 nm [21].

Fig 6: Sample solution in cuvette [22]

4. Detector:

A photodetector is a semiconductor device that converts light energy into electrical energy. it is made up of a straightforward P-N junction diode and is intended to function when reverse-biased. As photons approach the diode, the photodiode absorbs them and produces electricity

Types of detectors:

1. Barrier layer cell/photovoltaic cell
2. Phototubes/photo emissive tube
3. Photomultiplier tube [23].

1. Barrier layer cell/photovoltaic cell:

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

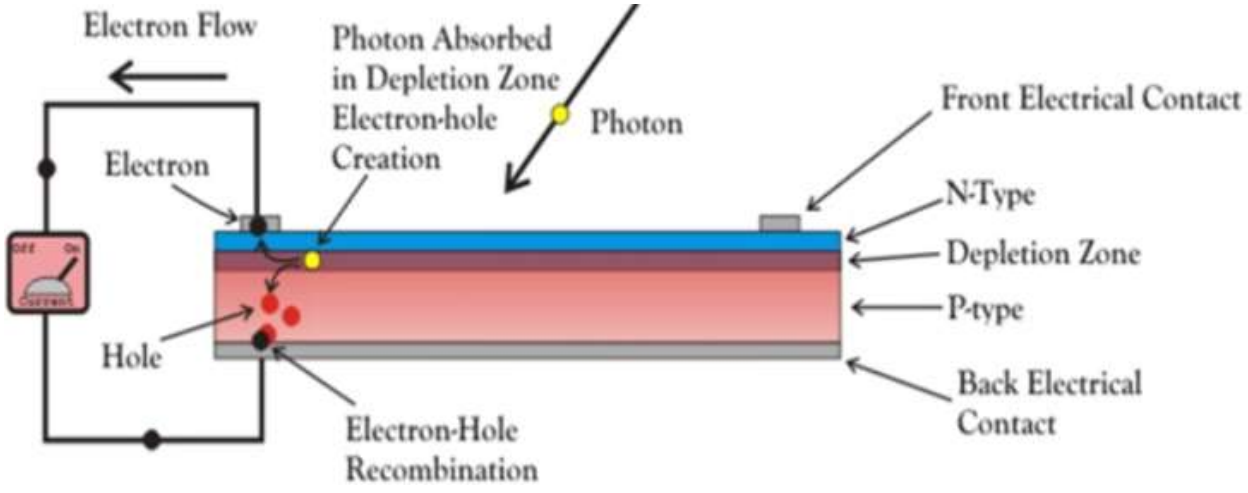

Fig 7: Photovoltaic cell [24]

2. Phototubes/photo emissive tube:

Construction: It consists of a spherical-shaped vacuum bulb containing a photo-emissive cathode and anode. The inner surface of the cathode mounted inside the bulb is coated with photosensitive material like cesium oxide, potassium oxide, or silver oxide. It has an anode to attract the electrons.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

Working: When the radiant energy falls on photosensitive cathode, electrons are emitted which are attracted towards anode causing current to flow. It is more sensitive as compared to barrier layer cell and hence widely used. Disadvantage: It produces dark current which attributed due to scattered electron emission from photosensitive cathode due to stray radiation striking its surface.

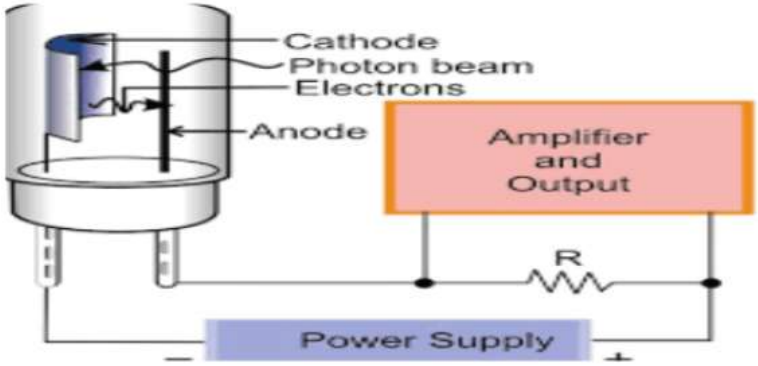

Fig 8: Phototubes/photo emissive tube [25]

3. Photomultiplier tube:

Principle: The principle employed in the detector is the multiplication of photoelectrons by the secondary emission of electrons.

Construction: It consists of a vacuum tube in which a primary photocathode coated with photosensitive materials like cesium oxide, potassium oxide, or silver oxide is fixed which receives radiation from the sample. Some eight to ten dynodes are fixed each with an increasing potential of 75-100 V higher than the preceding one which acts as electron-active surfaces. Near the last dyanode is fixed an anode which acts as an electron collector electrode.

Working: When the radiant energy falls on a photocathode coated with photosensitive material, electrons are released. These released electrons when come in contact with another electron-active surface i.e. dyanode, generate more and more electrons, and a large current is generated by photomultiplication. [26]

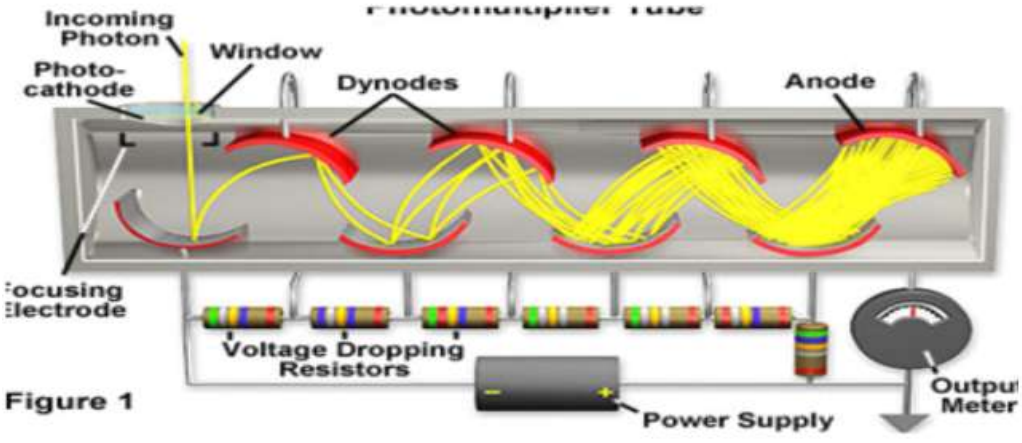

Fig 9: Photomultiplier tube [27]

Shifting of absorption band and change in intensity:

Chromophores: The chromophore is an atom or group of atoms that are responsible for the absorption of UV-visible radiation.

Types of chromophores: There are two types of chromophores:

1. Chromophores that can only contain π electrons. They undergo π-π^* transitions only e.g. ethylenic group (C ═C) and acetylenic group (C ≡ C) etc.
2. Chromophores that contain π as well as n (nonbonding) electrons. This type of chromophore contains lone pair(s) of electrons. so they are responsible for two types of transitions i.e. n-π^* and π-π^* e.g. nitro group (-NO_2), azo group (-N═N-), nitro group (-NO_3), carbonyl group (>C═O), nitrite group (-ONO).

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

3. Many organic molecules absorb ultraviolet/visible radiation and this is usually because of the presence of a particular functional group. Chromophores are the groups that absorb the radiation.
4. Mathematical treatments of the energy levels of orbital systems suggest that some electronic transitions are statistically probable (said to be allowed, and these absorptions are strong and tend to have ε values above 10,000). Other transitions have a probability of zero – they are not expected to occur at all – and are said to be forbidden but they frequently do occur, to give weak bands with ε values that rarely exceed 1 000.
5. Some particularly useful forbidden transitions are- d→d absorptions of transition metals; the n→π * absorption of carbonyl groups at ca 280 nm; and the π→π* absorption of aromatic compounds at ca 230–330 nm, depending on the substituents on the benzene ring. [28,29]

Auxochromes:

1. The hue of a molecule can be enhanced by certain groups known as auxochromes, which typically do not absorb significantly within the 200-800nm range but influence the spectrum of the attached chromophore.
2. The primary auxochromic groups include OH, NH2, CH3, and NO2, each exhibiting acidic (phenolic) or basic characteristics. The specific impact of an auxochrome on a chromophore is influenced by the polarity of the auxochrome; for example, groups like CH3-.
3. Generally, one can anticipate the effect of non-polar or weakly polar auxochromes, whereas predicting the influence of strongly polar auxochromes is more challenging. Additionally, the availability of non-bonding electrons which may enter into transitions also contributes greatly to the effect of an auxochrome. CH3CH2- and -Cl have very little effect, usually a small red shift of 5-10nm. Other groups such as -NH2 and -NO2 are very popular and completely alter the spectra of chromophores. [30]

There exist four types of shifts corresponding to auxochromes:

1. Bathochromic shift (redshift)
2. Hypsochromic shift (blueshift)
3. Hyperchromic shift
4. Hypochromic shift

1. Bathochromic shift or redshift

The bathochromic shift is the change of position of the absorption band towards the longer wavelength. This change occurs in the presence of auxochrome or a change in the solvent.

2. Hypsochromic shift or blue shift

The hypsochromic shift is the change in the position of the spectral band towards a shorter wavelength. It occurs due to the removal of conjugation or a change in the polarity of the solvent.

3. Hyperchromic shift

Hyperchromic shift is the increase in the intensity of the absorption band i.e. ε_{max}. A hyperchromic shift occurs due to the presence of an auxochrome.

4. Hypochromic shift

A hypochromic shift is a decrease in the intensity of the absorption i.e. ε_{max} band.

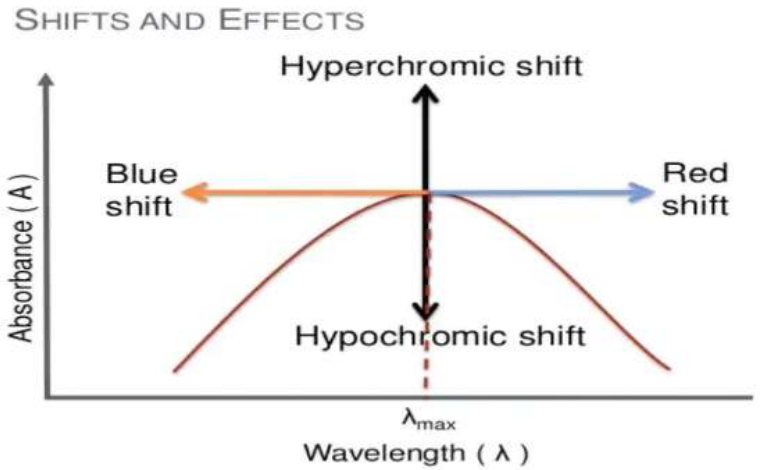

Fig 10: Spectral shift [31]

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

TYPES OF SPECTROPHOTOMETER:

As stated below, there are two varieties of spectrophotometers:

1. Single Beam Spectrophotometer

2. Double Beam Spectrophotometer

1. Single-Beam Spectrophotometer:

INTRODUCTION:

A single-beam spectrophotometer is an analytical device that allows all light waves emitted from the light source to pass through the sample. As a result, the measurements are based on the intensity of light that goes through the sample.

These single-beam spectrophotometers are compact and have a simpler optical design.

Definition:

A single-beam spectrophotometer uses a single ray of light that travels through the sample, measuring the intensity of light reflected from a reference that does not include the sample.

Instrumentation:

A single-beam spectrophotometer consists of several key components:

Light source: Spectrophotometers typically utilize three different types of light sources to generate light at varying wavelengths. The most frequently employed light source for the visible spectrum is a tungsten lamp. For ultraviolet radiation, the hydrogen lamp and the deuterium lamp are commonly used. The Nernst filament or globar serves as the most effective source of infrared (IR) radiation.

Monochromator: It is made up of an entrance slit and a dispersive element, which can be either a prism or a diffraction grating. To choose a specific wavelength, the light from the source is separated using a prism or diffraction grating.

Sample holder: Test tubes or cuvettes are utilized to contain the colored solutions, and they are typically constructed from glass suitable for visible wavelengths.

Photodetector system: When light strikes the detector system, it generates an electric current that corresponds to the reading on the galvanometer.

Measuring device: The current produced by the detector is sent to the measuring device, which is the galvanometer. The meter's reading is directly related to the intensity of the light. [32]

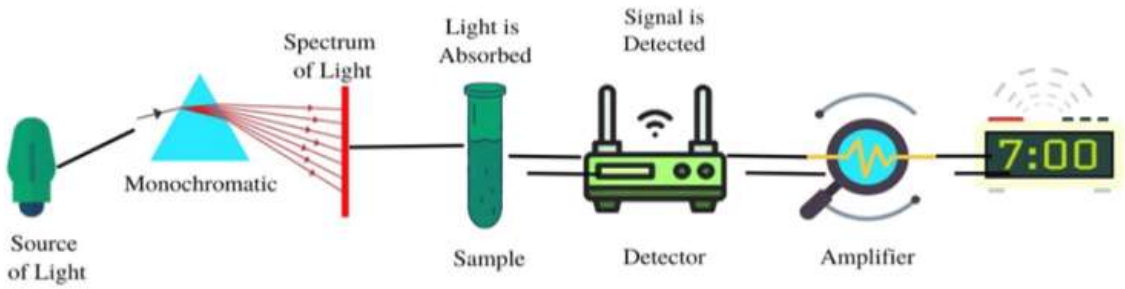

Figure 11: Single Beam Spectrophotometer [33]

Procedure for single beam spectrophotometer:

Calibration: Before using a spectrophotometer, it is essential to calibrate the device. Standard solutions that contain a known concentration of the solute whose concentration will be examined in the test sample are used to do this. To achieve this, the cuvettes are filled with the standard solution and then inserted into the cuvette holder of the spectrophotometer, which closely resembles a colorimeter.

A route to monochromator: The solution is targeted by a laser beam with a specific wavelength that is customized for the study. The solution is the target of this laser beam. Before reaching the solution, a light beam will pass via a prism, a diffraction grating, and a series of mirrors. The diffraction grating permits the required wavelength to pass through it and reach the cuvette, which may hold the test or reference solutions. The prism divides the light beam into its constituent wavelengths. The purpose of these mirrors is to guide the light inside the spectrophotometer. After analyzing the reflected light, it compares the results to a benchmark solution that has already been constructed.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

Detection system: The monochromatic light undergoes transmission, absorption, and reflection processes when it enters the cuvette. More specifically, the solution absorbs a portion of the light and reflects another portion. The photodetector device then records the light that makes it through the solution. After measuring the transmitted light's intensity, this system transforms it into electrical impulses, which are subsequently sent to the galvanometer.

Current measurement: Electrical signals are measured using a galvanometer, which displays the data digitally. The electrical signals in the examined solution are digitally represented by its absorbance or optical density.

Solution Concentration: A higher absorption means that more light will be absorbed by the solution, and a lower absorption means that more light will be transmitted through the solution, It represents the solute concentration in the solution and affects the galvanometer reading. One can quickly ascertain the solution's concentration by entering all of the values into the Beer-Lambert law formula. [34]

2. Spectrophotometer with double beams:

A double-beam optical system is used in many UV-Vis systems. The monochromator's light is divided into two beams in the double beam type: a reference beam and a sample beam. An optical component, such as a revolving wheel with a mirrored section or a beam splitter, which is a half-silvered mirror, is typically used to split the light. Separate optical pathways allow each beam to access the sample chamber. The reference/blank and sample can be measured simultaneously because there are two beams with the same wavelengths available. This implies that any instrument variations can be instantly adjusted for in the sample measurement. A very accurate measurement is produced by this real-time adjustment. [35]

What is the name of the double-beam spectrophotometer?

The device is called a "double-beam" spectrophotometer because it employs two light beams:

1. Reference beam: To track the energy of the bulb, the beam is sent via the reference standard.

2. Sample beam: To reflect the sample's absorption, the previously described beam passes through the sample medium.

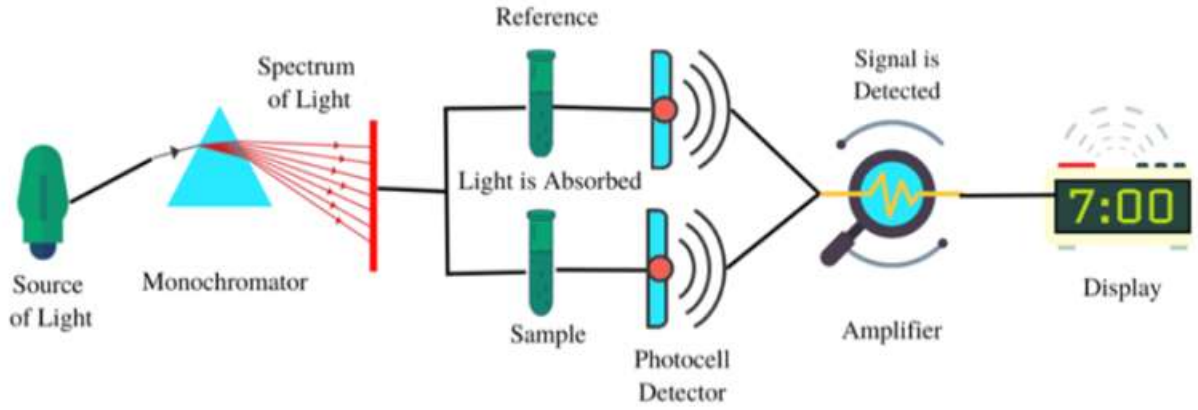

Fig 12: Double-beam spectrophotometer [36]

The double-beam spectrophotometer's: drawings show how a half mirror is used by the mechanical chopper to split the energy from the light source. As a result, two distinct beams are produced, one aimed at the sample side and the other at the reference side. This arrangement gives an instant point of comparison by enabling the simultaneous analysis of the reference and the sample. The color of the sample can be determined by measuring absorbance as a function of wavelength using an infrared thermometer and photometers in a dual-beam spectrophotometer. The ratio of the sample beam to the reference beam is used to calculate absorbance.

In other words, after excitation, the light beams may recombine before reaching a monochromator unit connected to the optical apparatus. In some situations, spectrophotometry makes use of two monochromators. The dispersion, reflection, and refraction of light are the basic ideas that underpin a typical double-beam spectrophotometer. By using these techniques, the results of analytes present in a biological material can be calculated based on their absorption properties.

Working principle:

The basic operational principle of a double-beam spectrophotometer is based on the behavior of light ray reflection and transmission. The main component in both single and double-beam spectrophotometers is the

light source, which produces intense light beams. The qualities of these light beams depend on the specific wavelength being analyzed, which can include electrically generated ultraviolet and visible light, as well as infrared light.

The monochromator efficiently separates specific light wavelengths from the wider spectrum produced by the light source. The wavelength selection is determined by the spectral characteristics of the light beams that pass through the double-beam spectrophotometer. The light wavelength utilized in the instrument is based on the absorbance characteristics of the analyte being analyzed. Monochromators are designed primarily to allow the transmission of polychromatic light through the instrument's entry slit. Additionally, they enable a single wavelength of monochromatic light to exit the device through the exit slit.

This intriguing and highly concentrated light beam consists of a relatively narrow segment of the electromagnetic spectrum. The width of the wavelengths and the electromagnetic band-pass associated with those wavelengths are determined by the slit methods or apertures of the monochromator connected to the spectrophotometer. The light beams in both single and double-beam spectrophotometers can be adjusted using these monochromators, which feature customizable settings. In this scenario, the transmission and reflection processes are enhanced by the properties of the light rays

In a double-beam spectrophotometer, the light source is employed within the instrument's design to illuminate the samples with light beams. During the spectrometry process, the light rays are deflected at a 90-degree angle. This process includes the rotation of a disk that comprises three separate panels within the spectrophotometer. One part of the device allows the unobstructed passage of the light beam through the disk without any obstruction. Another section features a mirrored surface, while the third section is coated in black. The sample cells receive direct illumination from the light beam as it passes through the disk panels. If the sample in question is liquid or gaseous, it is contained in a cuvette made of a transparent substance that exhibits no absorption of light rays within the spectral range relevant to the double-beam spectrophotometer. [37]

Benefits of UV visible spectroscopy:

- It is an affordable instrument.
- It encompasses the full range of ultraviolet and visible light.
- It serves both qualitative and quantitative analysis purposes.
- A derivative graph can be generated using a UV-VIS spectrophotometer.
- It is applicable for studying the degradation of drugs.
- The primary benefit is the precision offered by the UV-VIS spectrophotometer.
- Operating the UV-VIS spectrometer is straightforward and user-friendly.
- It ensures reliable performance.
- UV-VIS spectroscopy is easy to use.

Drawbacks of UV-visible spectroscopy:

- Only liquid samples can be analyzed.
- Preparation time is required before it can be used.
- Handling of the cuvette may influence the sample readings.
- Only molecules containing chromophores can be assessed.
- Absorption results may be influenced by factors such as pH, temperature, contaminants, and impurities. [38]

Applications for UV-Vis Spectroscopy:

Verifying the concentration and integrity of DNA and RNA is the main goal of DNA and RNA analysis, which is essential for subsequent processes like sequencing. It guarantees the purity or contaminancy of the DNA or RNA samples that are ready for sequencing.

The 260 nm/280 nm absorbance ratio is essential for demonstrating protein contamination in nucleic acids since pure DNA has an absorbance ratio of 1.8 and pure RNA has a ratio of 2. RNA and DNA have different absorbance ratios (2.15 to 2.50) at 260 nm and 230 nm. [39]

Bacterial culture:

- Bacterial culture frequently makes use of UV-visible spectroscopy. Using a wavelength of 600 nm, OD measurements are frequently and rapidly performed to track growth and estimate cell concentration.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:03/March-2025 Impact Factor- 8.187 www.irjmets.com

- 600 nm is widely employed and favored due to the optical properties of bacterial culture conditions in which they are cultivated and to avoid harming the cells in circumstances where they are necessary for continuing investigation.

Beverage analysis:

Another popular use of UV-visible spectroscopy is the identification of specific chemicals in beverages. Legal restrictions on caffeine levels must be met, and UV light can help with quantification.

Using UV-visible absorbance for quality control, it is simple to identify some groups of colored compounds, such as anthocyanin, which is present in blueberries, raspberries, blackberries, and cherries, by comparing their recognized peak absorption wavelengths in wine.

Cut-off Wavelength of Solvents:

Each solvent has its absorbance cutoff wavelength. The solvent itself absorbs light below this wavelength. Know the solvent's cutoff and the location of the analytes' absorption when selecting one. Select an alternative solvent if the wavelengths are near to one another. The UV cutoff wavelengths of popular solvents are shown in the list below.

Table 1: Solvent cut-off wavelength [40]

Solvent	UV cutoff (nm)
Acetonitrile	190
Water	190
Cyclohexane	195
Isooctane	195
n-hexane	201
Ethanol (95%)	205
Methanol	205
Trimethyl phosphate	210
Acetone	220
Chloroform	240
Xylene	280

IV. CONCLUSION

In summary, UV spectroscopy is an essential characterization method that provides deep insights into the properties of various samples by analyzing their interaction with electromagnetic radiation. When utilized with an appropriate standard curve and applied to pure substances, UV-visible spectroscopy serves as a dependable, simple, and cost-effective method for determining the concentration of absorbing species. One of the vital techniques for exploring the optical properties of PMCs is UV-visible spectroscopy, which elucidates the relationship between the matrix and the nanofiller while evaluating how the nanofillers enhance the properties of nanocomposites. To evaluate the desired optical characteristics of nanofillers within a polymer matrix, UV-visible spectroscopy is an essential technique. The review paper encompasses comprehensive details about UV-visible spectroscopy, including its principles, theoretical foundations, instrumentation, advantages, disadvantages, and applications. UV-visible spectroscopy allows for more precise identification of impurities. Furthermore, UV-visible spectroscopy is an essential method in the field of spectroscopy. [41]

V. REFERENCES

[1] Jeffery GH, Bassett J, Mendham J, Denney RC. Vogel's textbook of quantitative chemical analysis. Edition: 5th, ELBS with long man, Singapore, 3-11.
[2] Beckett & Stenlake Practical pharmaceutical chemistry Fourth Edition,1995
[3] https://www.researchgate.net/publication/ 337674152_UVVISIBLE_SPECTROMETRY
[4] https://www.researchgate.net/publication/ 337674152_UVVISIBLE_SPECTROMETRY

[5] https://www.technologynetworks.com/ana lysis/articles/uv-vis-spectroscopyprinciple-strengths-and-limitations-andapplications-349865
[6] Indian pharmacopoeia 2018,volume 1,8th edition effective from 1 st january 2018,page no183-184
[7] https://labindia-analytical.com/uploads/new-product/product_1737352810_cDJ65.png
[8] https://byjus.com/chemistry/principle-of-uv-visible-spectroscopy/
[9] G Verma, DR. M Mishra, "Development and Optimization of UV VIS Spectroscopy-A Review", World Journal of Pharmaceutical Research, 2018,7(11),1170-1180
[10] Rifino CB. Pharmaceutical Process Validation, Switzerland, Edition,2003:2:1-43.
[11] https://www.staff.u-szeged.hu/~aszucs/12Coagulation.pdf
[12] Patel D, Panchal D, Patel K. prof Dalwadim, Dr Upadhyay U. Review on uv visible spectroscopy. International Journal of Creative research Thoughts (IJCRT),2022:(10):2320-2882.
[13] https://chimactiv.agroparistech.fr/en/bases/spectroscopie-uv/theorie-illustree
[14] Smit Patel, Aniket Raulji, Diya Patel, Diya Panchal, Prof. MitaliDalwadi, Dr. Umesh Upadhyay. A Review on UV Visible Spectroscopy. Published by the International Journal of Pharmaceutical Research and applications, 2022.
[15] Smit Patel, Aniket Raulji, Diya Patel, Diya Panchal, Prof. MitaliDalwadi, Dr. Umesh Upadhyay. A Review on UV Visible Spectroscopy. Published by the International Journal of Pharmaceutical Research and applications, 2022.
[16] Gurdeep R. Chatwal, Sham K. Anand. Instrumental methods of chemical Analysis: Ultraviolet spectroscopy. Mumbai; Himalaya Publishing House Pvt Ltd, 2017; 5.
[17] UV-spectrophotometric method for simultaneous Estimation of metoprolol and amlodipine in bulk and Their formulation. International Journal of Biological & Pharmaceutical Research,2011:2(2):50-54.
[18] Brown, Chris W. "Ultraviolet, visible, near-infrared spectrophotometers." Ewing's Analytical Instrumentation Handbook, Fourth Edition. CRC Press,2019:117-128.
[19] Verma, Govinda, Manish Mishra. "Development and optimization of UV-Vis spectroscopy-a review." World Journal of Pharmaceutical Research,2018:7(11):1170- 1180.
[20] https://www.expertsmind.com/topic/grating-monochromators/schematic-diagram-of-a-grating-monochromator-913503.aspx
[21] Modern Chemical Techniques, Ultraviolet/visible Spectroscopy, The Royal Society of Chemistry, 102, 103.
[22] https://www.rakuten.com.tw/shop/dglife/product/100000008512878/
[23] Donald L. Pavia Gary M. Lampman, George S, Kriz Iames R, Vijaan. Spectroscopy. Third Edition, CBS Publishers, and Distributors, 1997.
[24] https://www.electrical4u.com/solar-cell/
[25] https://egyankosh.ac.in/bitstream/123456789/81744/1/Unit-12.pdf
[26] Modern Chemical Techniques, Ultraviolet/visible Spectroscopy, The Royal Society of Chemistry, 102,103.
[27] https://evidentscientific.com/en/microscope-resource/knowledge-hub/digital-imaging/concepts/photomultipliers
[28] Gurdeep R. Chatwal, Anand Sharma Instrumental method of Analysis 5th edition, Himalaya publishing house PVT.LTD, 2002 pp 2.566-2.587
[29] Skoog, D.A. Holler, F.J., Nieman, D.A., Introduction to UV Spectroscopy in, principle of instrumental analysis, 5thed., Cole publication, 2004.
[30] Sharma Y.R. Ultraviolet and visible spectroscopy in; Elementary Organic spectroscopy, 1 ed ., S.Chand & Company Ltd ., New Delhi, 2004; 9-60.
[31] https://www.facebook.com/solutionpharmacychannel/posts/the-shift-of-absorption-maximum-towards-longer-wavelength-is-calleda-bathochromi/1170054356813583/
[32] https://www.studocu.com/row/document/government-college-women-university-faisalabad/inorganic-chemistry/single-and-double-beam-spectrophotometre/15742585
[33] https://scienceinfo.com/single-beam-spectrophotometer-limitations/
[34] https://scienceinfo.com/single-beam-spectrophotometer-limitations/

[35] https://www.agilent.com/cs/library/primers/public/primer-uv-vis-basics-5980-1397en-agilent.pdf
[36] https://scienceinfo.com/double-beam-spectrophotometer/
[37] https://scienceinfo.com/double-beam-spectrophotometer/
[38] https://microbenotes.com/uv-vis-spectroscopy/
[39] Sethi PD. Quantitative Analysis of drugs in pharmaceutical Formulations, 3rd ed., CBS Publishers and Distributors, New Delhi 1997.
[40] https://www.slideserve.com/merlo/chapter-25-instruments-for-optical-spectrometry-powerpoint-ppt-presentation
[41] Beckett A.H., Stenlake J.B., Practical Pharmaceutical Chemistry; 4th ed. CBS Publisher and distributors delhi, 2001: 280-286.