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REVIEW ARTICLE

A Review on UV-visible spectroscopy

Alekhya Mandru [1]*, Jyothi Mane [2], Ramya Mandapati [3]
[1]*Student, B.Pharmacy, Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli, Andhra Pradesh, India*
[2]*Student, B.Pharmacy, Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli, Andhra Pradesh, India*
[3]*Student, B.Pharmacy, Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli, Andhra Pradesh, India*

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Abstract: UV spectroscopy, as one of the earliest instrumental techniques for analysis, has proven to be a versatile and indispensable tool in various scientific domains. Its capability to distinguish different types of materials makes it a valuable asset in analytical chemistry. This method is commonly employed to ascertain the identity, strength, quality, and purity of diverse samples. The fundamental principle of UV spectroscopy lies in the absorption of specific wavelengths of light by samples, and it provides valuable insights into the responses of substances to this absorption. The application of Beer's law, a universal principle in UV spectroscopy, elucidates the absorption of radiant energy by samples. Notably, this method is characterized by its accuracy, simplicity, and a broad spectrum of applications, including drug discovery, structural elucidation of organic molecules, molecular weight determination, and the detection of impurities. Both quantitative and qualitative analyses can be conducted effectively using UV spectroscopy. The equipment operates within a wavelength range of 200nm to 800nm, allowing the analysis of both colorless and colored compounds in both the UV and visible regions. In summary, UV spectroscopy stands as a robust analytical technique with wide-ranging applications, making it a cornerstone in scientific research and analysis.

Keywords: UV Spectroscopy; Beer's Law; Analytical Chemistry; Molecular Characterization; Quantitative Analysis

1. Introduction

Spectroscopy involves the study of interaction between light or electromagnetic radiation and matter. This interaction manifests a crucial phenomenon where 'energy is either absorbed or emitted by the matter in discrete amounts termed quanta.' Across the electromagnetic spectrum, spanning from gamma rays to radio waves, absorption and emission processes unfold, each offering unique insights into the nature of the studied materials [1, 2].

Among the various spectroscopic techniques, UV spectroscopy emerges as a powerful analytical tool, utilizing light within the UV or visible region with wavelengths ranging from 200 to 800 nm. This technique stands versatile, capable of analyzing both colorless compounds in the UV range (400-200 nm) and colored compounds in the visible range (800-400 nm). In essence, UV spectroscopy quantifies the discrete wavelengths of UV or visible light absorbed or transmitted by a sample in comparison to a reference or blank. This property, intricately linked to the sample's composition, holds the potential to unveil details about the sample's constituents and their concentrations. The outcomes of UV spectroscopy are graphically represented as spectra, providing a visual and quantitative depiction of the spectral data. This review aims to comprehensively explore the principles, applications, and advancements in UV spectroscopy, shedding light on its significance in diverse scientific domains. Through a systematic examination of the literature, we intend to elucidate the pivotal role of UV spectroscopy in analytical chemistry, molecular characterization, and quantitative analysis, offering a nuanced understanding of its contributions to contemporary scientific endeavors [3,4].

2. UV Spectroscopy

2.1. Principle

UV absorption spectra arises from the transition of electrons within a molecule or an ion from a lower to a higher energy level and the UV emission spectra arise from the reverse type of transition. The UV radiation has sufficient energy to promote or excite the valence electrons in a molecule or an ion from a ground state orbital to a higher energy level, excited state orbital or anti bonding orbital which can be detected as absorption [5].

Chromophores: Many organic molecules absorb ultraviolet/visible radiation and this is usually because of the presence of a particular functional group. The groups that actually absorb the radiation are called chromophores. Some electronic transitions are statistically

* Corresponding author: Alekhya Mandru

probable and strong. Other transitions have a probability of zero and are said to be forbidden but 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 [6].

*Auxochromes: The Colour of a molecule may be intensified by groups called auxochromes which generally do not absorb significantly in the 200-800nm region, but will affect the spectrum of the chromophore to which it is attached. The most important auxochromic groups are OH, NH2, CH3 and NO2 and their properties are acidic (phenolic) or basic. The actual effect of an auxochrome on a chromophore depends on the polarity of the Auxochromes, e.g. groups like CH3-. In general, it should be possible to predict the effect of non-polar or weakly polar auxochromes, but the effect of strongly polar auxochromes is difficult to predict.

*Solvents: The effect on the absorption spectrum of a compound when diluted in a solvent, will vary depending on the chemical structures involved. Generally, non-polar solvents and non-polar molecules show the least effect. However, polar molecules exhibit quite dramatic differences when interacted with a polar solvent. The interaction between solute and solvent leads to absorption band broadening and a consequent reduction in structural resolution and ε max. Thus care must be taken to avoid an interaction between the solute and the solvent. Water and 0.1N solutions of HCl and NaOH are commonly used solvents for absorption spectrometry. Methodology requires buffering, solutions have to be non-absorbing and generally both the composition and pH will be specified. For reactions in the 4.2 to 8.8 pH region, mixtures of 0.1N dihydrogen sodium phosphate and 0.1N hydrogen disodium phosphate are generally used [7]

Potentially, there are three ground state orbitals involved. They are: σ (bonding) molecular orbital, π (bonding) molecular orbital and n (non bonding) atomic orbital Similarly, the anti-bonding orbitals involved are: σ* orbital and π* orbital. There is no such thing as an n* anti-bonding orbital as the n electrons do not form bonds [8].

Then, the possible electronic transitions in uv and visible regions are as follows:

1. σ to σ*

2. n to σ*

3. n to π*

4. π to π*

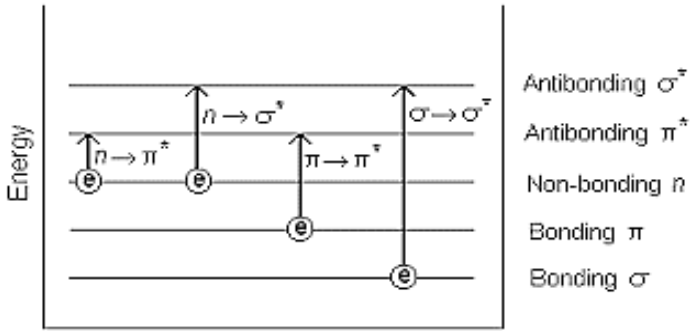

Figure 1 Electronic transitions

2.1.1. Beer Lambert's Law

Different molecules absorb different amounts of energy and this can be represented as a graph of absorbance on the y-axis and wavelength on x-axis. This graph is typically referred to as an absorption spectrum. We can determine the λmax (wavelength at which the sample is showing maximum absorbance) of the sample and thereby the concentration It is mainly based on the principle of Beer-Lambert's Law which states that 'the absorbance of a sample is directly proportional to the concentration of the absorbing species and the path length [9].

$$A = \varepsilon cl$$

Where, A – Absorbance, ε - molar extinction coefficient, c - concentration of the absorbing species, l - path length

For a fixed path length, it can be used to determine the concentration of an absorber in a sample. Thus, it is necessary to know how rapidly the absorbance changes with the concentration

2.2. Instrumentation

There are two types of absorbance instruments to collect uv absorption spectra. They are:

• Single beam UV spectrophotometer (Figure 2)

• Double beam UV spectrophotometer (Figure 3)

The single beam instrument has a filter or a monochromator between the source and the sample to analyze one wavelength at a time. The double beam instrument has a single source and a monochromator and then there is a splitter and a series of mirrors to get the beam to a reference sample and the sample to be analysed, this allows for more accurate analysis. The simultaneous instrument, double beam UV spectrophotometer is usually much faster and more efficient [10].

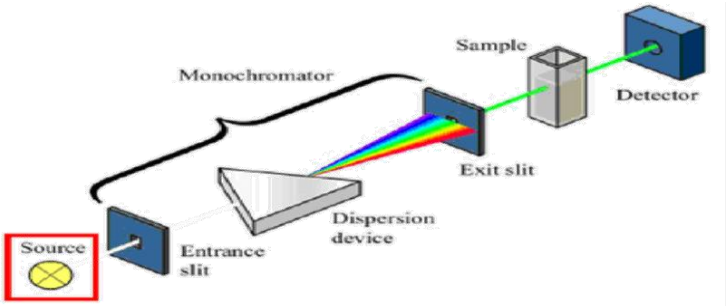

Figure 2. Single Beam UV spectrophotometer

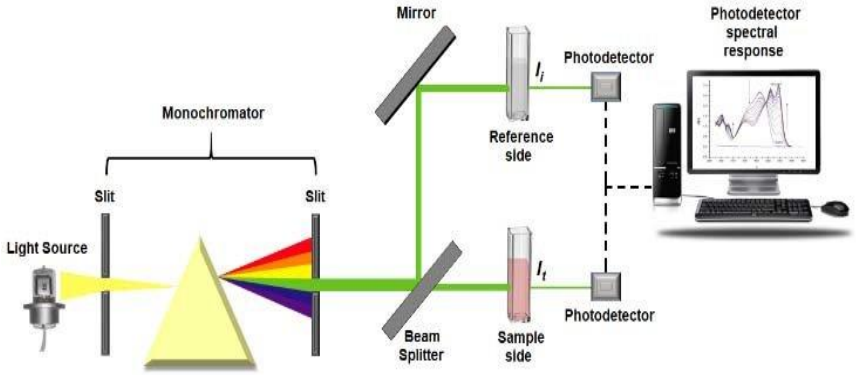

Figure 3. Double Beam UV spectrophotometer

The basic instrumentation (as shown in Figure 4) of a UV spectrophotometer includes:

2.2.1. Light source

Sources of UV radiation: It is important that the power of the radiation source does not change abruptly over its wavelength range. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves the formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon.

Both deuterium and hydrogen lamps emit radiation in the range 160 - 375 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used because glass absorbs radiation of wavelengths less than 350 nm.

Sources of visible radiation: The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of 350 - 2500 nm. The energy emitted by a tungsten filament lamp is proportional to the fourth power of the operating voltage. This means that for the energy output to be stable, the voltage to the lamp must be very stable indeed. Electronic voltage regulators or constant-voltage transformers are used to ensure this stability [11].

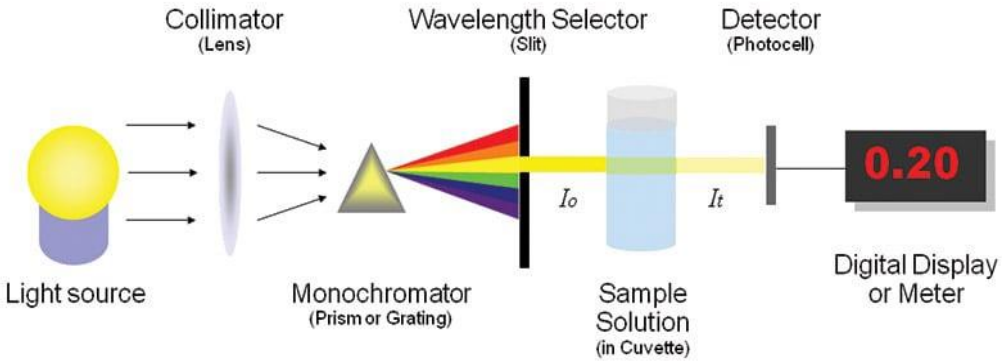

Figure 4. Instrumentation of UV Spectrophotometer

2.2.2. Monochromator (Wavelength selector)

All Monochromators contain the following components;

• An entrance slit

• A collimating lens

• A dispersing device (usually a prism or a grating)

• A focusing lens

• An exit slit

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only particular wavelength leaves the Monochromator through the exit slit [12]

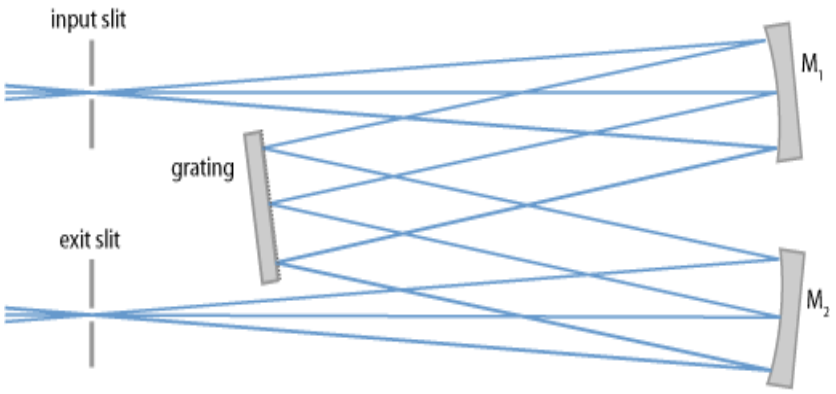

Figure 5. Turner Grating Monochromator

2.2.3. Sample cell

The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm. Glass cuvettes should not be used in uv region as they may absorb uv radiation but can be used in visible region.

2.2.4. Detector

A detector converts a light signal into an electrical signal. It should give a linear response over a wide range of low noise and high sensitivity.

1. Photomultiplier tube detector: The photomultiplier tube (Figure 6) is a commonly used detector in UV-Vis spectroscopy. It consists of a photoemissive cathode (a cathode which emits electrons when struck by photons of radiation), Anodes (which emit several electrons for each electron striking them). A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first Anode (which is 90V more positive than the cathode). The electrons strike the first anode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second anode, to produce more electrons which are accelerated towards the anode. The electrons are collected at the anode. By this time, each original photon has produced 106-107 electrons. The resulting current is amplified and measured. Photomultipliers are very sensitive to UV - visible radiation. They have fast response times [13].

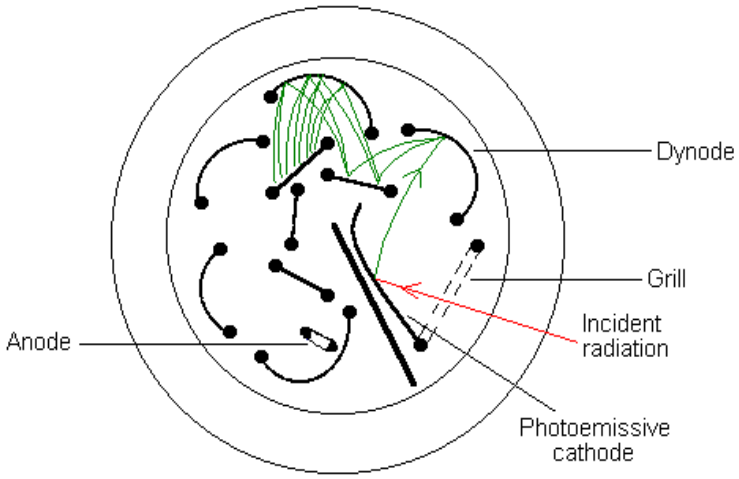

Figure 6: Photo multiplier tube

2. Photo diode detector: The Photodiode detector (Figure 7) is an example of a multichannel photon detector. These detectors are capable of measuring all elements of a beam of dispersed radiation simultaneously. A linear photodiode array comprises of many small silicon photodiodes formed on a single silicon chip. There can be between 64 to 4096 sensor elements on a chip, the most common being 1024 photodiodes. For each diode, there is also a storage capacitor and a switch. The individual diode-capacitor circuits can be sequentially scanned. In use, the photodiode array is positioned at the focal plane of the monochromator (after the dispersing element) such that the spectrum falls on the diode array. They are useful for recording UV-Vis absorption spectra of samples that are rapidly passing through a sample flow cell, such as in an HPLC detector [14].

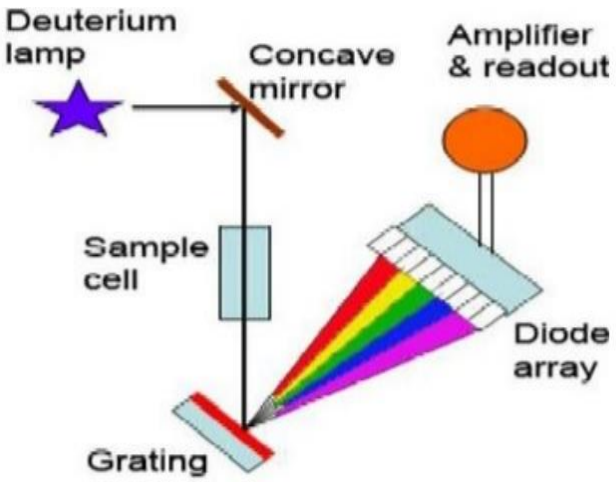

Figure 7: Photodiode array detector

3. Applications

3.1. Structural Elucidation of Organic Compounds

UV spectroscopy serves as a valuable tool for unraveling the structural details of organic compounds through the analysis of their UV absorption patterns [15].

3.2. Determination of Molecular Weight

The technique is employed for the precise determination of molecular weights, aiding in the characterization of molecules based on their UV absorption characteristics.

3.3. Detection of Impurities

UV spectroscopy is instrumental in detecting impurities within substances, ensuring the purity and quality of compounds.

3.4. Dissociation Constant of Acids and Bases

It is utilized to determine the dissociation constants of acids and bases, providing insights into their chemical properties.

3.5. DNA and RNA Analysis

UV spectroscopy plays a crucial role in the analysis of DNA and RNA, facilitating the study of nucleic acid structures and concentrations.

3.6. Bacterial Culture

The technique finds application in the analysis of bacterial cultures, offering insights into microbial growth and metabolism.

3.7. Quantitative Analysis of Pharmaceutical Compounds

UV spectroscopy is widely employed for the quantitative analysis of pharmaceutical compounds, ensuring accurate concentration measurements [16].

3.8. Qualitative and Quantitative Analysis

It is used for qualitative analysis, allowing the identification of substances based on their unique UV absorption patterns. UV spectroscopy contributes to the assay of medicinal substances, ensuring the efficacy and quality of pharmaceutical formulations.

3.9. Detection of Functional Groups

The technique is applied to detect specific functional groups within molecules, aiding in the identification of chemical moieties.

3.10. Used in Chemical Kinetics

UV spectroscopy finds utility in chemical kinetics studies, providing real-time insights into reaction mechanisms.

3.11. As HPLC Detector

UV spectroscopy serves as a detector in High-Performance Liquid Chromatography (HPLC), enhancing its capabilities in compound separation and analysis.

3.12. Beverage Analysis

UV spectroscopy is applied in the analysis of beverages, ensuring quality control and adherence to standards.

3.13. Evaluation of Raw Materials

It contributes to the evaluation of raw materials in various industries, ensuring the quality and integrity of starting materials.

3.14. In Drug Discovery

UV spectroscopy plays a vital role in drug discovery, aiding in the screening and characterization of potential therapeutic compounds.

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4. Conclusion

In conclusion, UV spectroscopy stands as a pivotal and indispensable characterization technique, offering profound insights into the properties of diverse samples through the analysis of their interaction with electromagnetic radiation. This method, characterized by its ease of use, simplicity, accuracy, validity, and cost-effectiveness, emerges as a valuable analytical tool, particularly in determining the concentration of absorbing species when applied to pure compounds with appropriate standard curves. Its widespread application spans across fundamental scientific disciplines such as chemistry, pharmaceuticals, physics, and material science. Significantly, UV spectroscopy serves as a fundamental tool, enabling the exploration and analysis of the composition, physical structure, and electronic structure of matter at the atomic and molecular levels. In essence, its contributions resonate across various fields, solidifying its position as a cornerstone in contemporary scientific research and analysis.

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