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REVIEW ARTICLE ON UV-VISIBLE SPECTROSCOPY IN PHARMACEUTICAL ANALYSIS METHODOLOGIES AND APPLICATIONS

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Abstract:

UV-Visible spectroscopy refers to the analytical examination of various solvents and compounds. The analysis involves measuring the absorption of monochromatic light by colourless and coloured molecules in the region of the spectrum 200–400 nm and 400 to 800nm respectively, the principle of the technique is based on the electronic transitions of molecules, where photons in the UV and visible regions promote electrons from lower energy orbitals to higher ones. The methodology involves passing a beam of light through a sample and measuring the intensity of light before and after it interacts with the sample. The resulting absorbance spectra can be utilized to identify compounds, quantify concentrations using Beer-Lambert law, and assess chemical reactions. Factors such as the solvent, pH, and temperature can affect the absorption characteristics, making careful experimental design crucial. Recent advancements in UV-visible spectroscopy include the development of more sensitive detectors, integration with other analytical techniques, and the ability to conduct real-time measurements, broadening its applicability in research and industry.

This technique used to determine the "identity, strength, quality, and purity" of such compounds UV-visible spectroscopy is a widely employed analytical technique used to measure the absorbance or transmittance of ultraviolet and visible light by the sample. This technique is essential in various fields, including chemistry, biochemistry, and environmental science, as it provides valuable insights into the electronic structure of compounds, concentration analysis, and chemical kinetics.

This abstract encapsulates the significance and versatility of UV-visible spectroscopy as an essential tool in scientific analysis (1)

***Keywords**: UV-Vis Spectroscopy, Optimization Techniques, Electrochemical Methods.*

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INTRODUCTION:

UV-Visible spectroscopy is an analytical technique that measures the amount of discrete wavelengths of ultraviolet (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's composition, potentially providing information about its constituents and their concentrations.

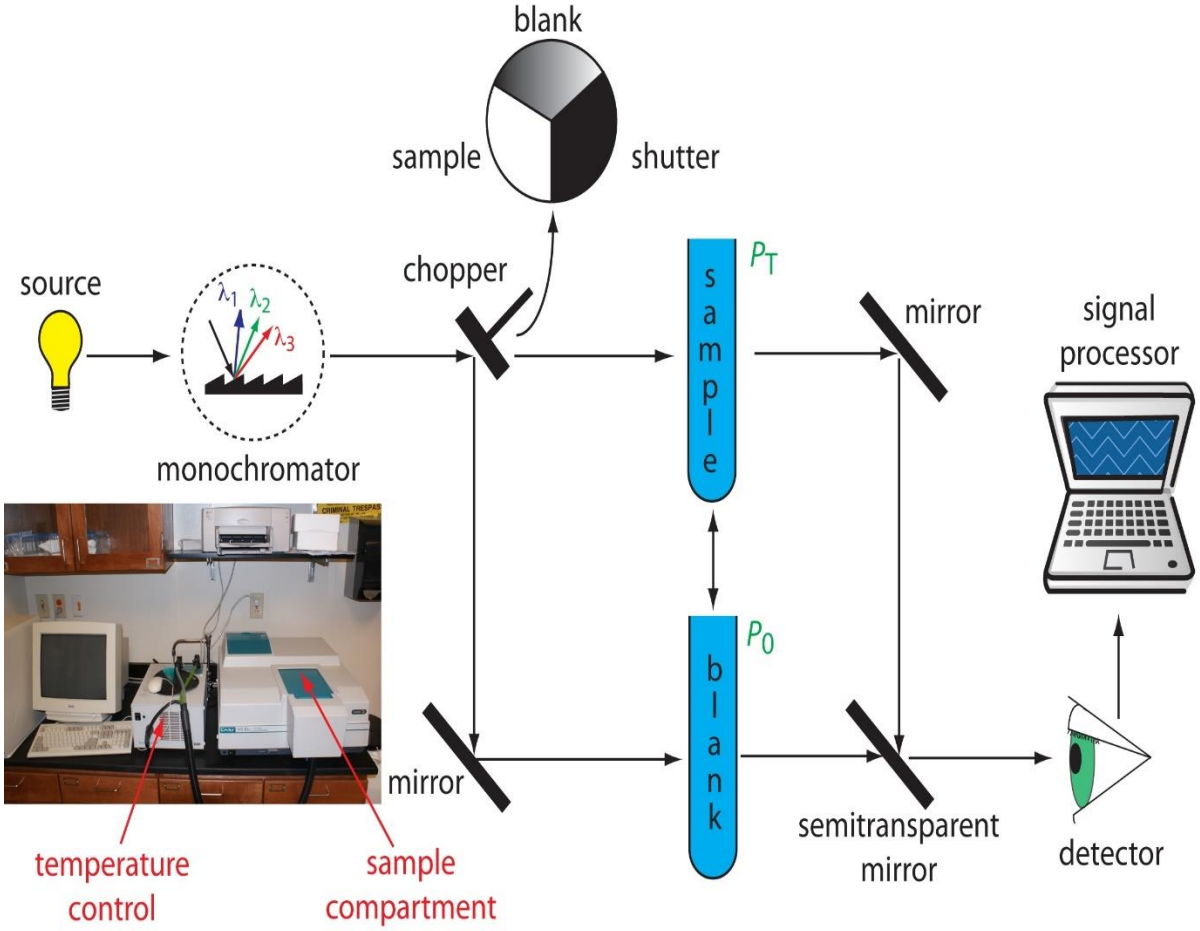

Figure 1: UV visible spectroscopy

Since this spectroscopy technique relies on the use of light, it is important to consider the properties of light first. Light possesses a certain amount of energy that is inversely proportional to its wavelength. Thus, shorter wavelengths of light carry more energy, while longer wavelengths carry less. A specific amount of energy is required to promote electrons in a substance to a higher energy state, which we detect as absorption. Electrons in different bonding environments within a substance require varying amounts of energy to be promoted to a higher energy state. This variability explains why the absorption of light occurs at different wavelengths in different substances.

Humans can perceive a spectrum of visible light, ranging from approximately 380 nm, which appears violet, to about 780 nm, which appears red. UV light has wavelengths shorter than visible light, extending down to approximately 100 nm. Therefore, light can be described by its wavelength, which is useful in UV-Vis spectroscopy for analysing or identifying different substances by locating the specific wavelengths corresponding to maximum absorbance (see the Applications of UV-Vis Spectroscopy section).

Figure 2: UV visible spectroscopy

This discussion refers to absorption spectroscopy and reflectance spectroscopy, particularly in the ultraviolet (UV) range and the entire adjacent visible region of the electromagnetic spectrum. Being relatively inexpensive and easy to implement, this methodology is widely used in various applied and fundamental applications. The only requirement is that the sample must absorb in the UV-Vis region, meaning it should be a chromophore. Absorption spectroscopy is complementary to fluorescence spectroscopy. Parameters of interest, in addition to the wavelength of measurement, include absorbance (A), transmittance (%T), reflectance (%R), and their changes over time.

A UV-Vis spectrophotometer is an analytical instrument that measures the amount of ultraviolet (UV) and visible light absorbed by a sample. This technique is widely employed in chemistry, biochemistry, and other fields to identify and quantify compounds in a variety of samples.

UV-Visible spectrophotometers operate by passing a beam of light through the sample and measuring the amount of light absorbed at each wavelength. The amount of light absorbed is proportional to the concentration of the absorbing compound in the sample [(2)]

Beer's Law

the Beer-Lambert Law, describes the relationship between the absorbance of light by a substance in solution and its concentration. It provides a quantitative relationship that is fundamental in fields such as chemistry, biochemistry, and environmental science, particularly in UV-Visible Spectroscopy**.**

A=ε CA = \Var epsilon CA=ε C

Where:

- AAA = Absorbance
- CCC = Concentration of the solution
- var epsilon= Molar absorptivity or attenuation coefficient

Lambert's Law

Lambert's Law, established by Johann Heinrich Lambert, states that absorbance is directly proportional to the path length of the light through the sample. This relationship can be represented as [(3)]

A= ε lmA = \Var epsilon l A=ε l

Where:

- AAA = Absorbance
- LLL= Length of the sample through which light passes
- E\ var epsilon = Molar absorptivity or attenuation coefficient

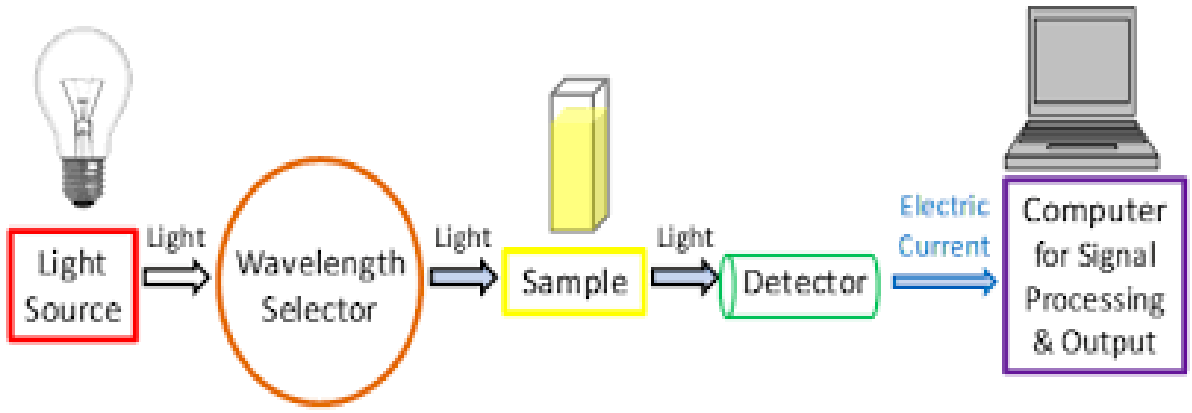

Figure 3: light source

UV-Vis Spectroscopy Principle

When a specific wavelength of light strikes a molecule, that molecule becomes excited. This process involves an electron transitioning from the ground (lower) energy state to a higher energy state. When an electron is excited, it absorbs light energy because electrons in a lower energy orbital require energy to move to a higher energy level.

Energy is neither created nor destroyed; it can only be transformed from one form to another. When electromagnetic radiation (EMR) in the UV-Vis range (200–800 nm) passes through a sample, only light possessing the precise amount of energy needed for electronic transitions will be absorbed, as the energy levels of matter are quantized.

If energy is absorbed, the intensity of the transmitted light is reduced. The energy absorbed by the electrons corresponds to the energy difference between the two energy levels involved in the transition.

During this interaction with electromagnetic radiation, the resulting spectra are called absorption spectra. This phenomenon is often referred to as electron spectroscopy. Conversely, when electrons in a higher energy level return to the ground energy level, the resulting spectra are called emission spectra.

UV-Visible spectroscopy (UV-Vis) is a simple yet efficient laboratory method to determine concentration of a substance within a larger substance. A spectrophotometer, the machine that allows for this procedure, measures how much light chemical compounds absorb and records the wavelengths transmitted by the compounds, producing distinct spectra to do this, the machine sends out a beam of light to a cuvette (a translucent container) filled with the desired substance. The cuvette absorbs only specific wavelengths depending on the concentration of the solution. The wavelengths that do not get absorbed pass through the sample, through a small opening, and are finally diffracted onto a photoluminescence detector, yielding a particular pattern that varies with each compound [(4)]

Types of UV-Visible Spectroscopy

Ultraviolet-Visible (UV-Vis) spectrophotometry is a pivotal technique in analytical chemistry that employs various types of spectrometers to analyse samples. There are primarily two types of UV-Visible Spectrophotometers:

1. Single Beam UV-Visible Spectrophotometer

As the name suggests, a single beam UV-Vis spectrophotometer utilizes a single beam of light. The process begins with the incident light emanating from the source, which is subsequently passed through a monochromator. This monochromatic light then traverses a slit and passes through the sample solution. During this phase, a portion of the incident light is absorbed by the sample, while the remainder is transmitted.

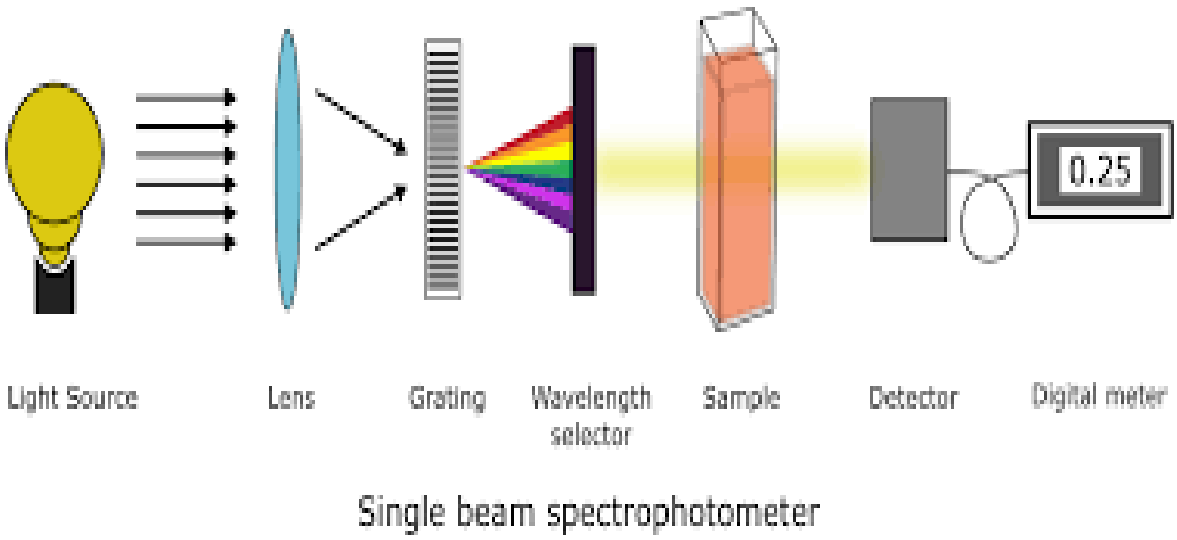

Figure 4: Single beam

The transmitted light is then detected by a specialized detector. Following detection, the light is amplified and recorded, culminating in its display on an appropriate readout device. A spectrum is then plotted, and the λ max (wavelength of maximum absorbance) is identified.

The integral components of a single beam UV-Vis spectrophotometer include:

- Light source
- Lens
- Gratings
- Wavelength selector
- Sample container (cuvette)
- Detector
- Digital meter or recorder

2. Double Beam UV-Visible Spectrophotometer

The foundational instrumentation of both single and double beam spectrophotometers remains largely analogous. However, the distinguishing feature of a double beam UV-Vis spectrophotometer is its ability to simultaneously direct the beam of incident light toward both the reference and the sample cuvettes.

The incident light is divided and channelled towards the reference and sample cuvettes in tandem. The beams that are refracted or transmitted are subsequently detected by two separate detectors. It's important to note that a double beam UV-Vis spectrophotometer requires two detectors, which are crucial for measuring the absorbance of the test sample by comparing it to the reference.

Additionally, a double beam UV-Vis spectrophotometer necessitates a stabilized voltage supply to ensure accurate and consistent readings. [5]

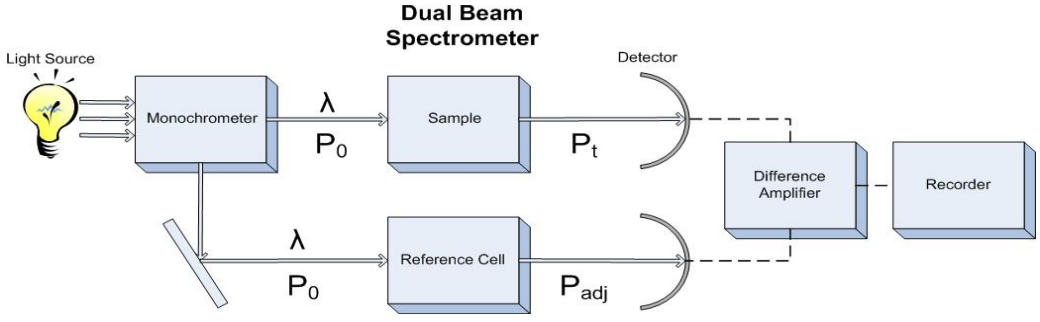

Figure 5: Double Beam spectrometer

UV visible spectroscopy instrumentation and working Spectroscopy is a powerful analytical technique used to measure the absorption of ultraviolet (UV) and visi ble light by a sample1. Here's a breakdown of the inst rumentation and how it works:

Instrumentation
The following elements make up instruments used to measure the absorption of ultraviolet or visible light:

1. Source (UV and visible)
2. Monochromator or filter
3. Sample cells or containers
4. Detector

1. RADIATION SOURCE
It is crucial that the radiation source's power does not fluctuate drastically across the spectrum of its wavelengths. A continuous UV spectrum is produced when deuterium or hydrogen is electrically excited under low pressure. This process involves the formation of an excited molecular species, which splits into two atomic species and an ultraviolet photon.

Lamps made of deuterium and hydrogen produce radiation between 160 and 375 nm. These lamps must use quartz cuvettes and windows since glass absorbs light with wavelengths shorter than 350 nm. The following are various sources of UV radiation:

1. Deuterium lamp
2. Hydrogen lamp
3. Tungsten lamp
4. Xenon discharge lamp
5. Mercury arc lamp

DEUTERIUM LAMP
A deuterium lamp, also known as a D2 lamp, is a light source that emits intense ultraviolet (UV) radiation and is commonly used in UV-visible spectroscopy:

- UV output

Deuterium lamps produce a high intensity of UV radiation, down to 160 nanometres (nm) for some models. They emit little visible and infrared radiation.

- Stability

Deuterium lamps are stable and have a long lifetime, typically around 2,000 hours.

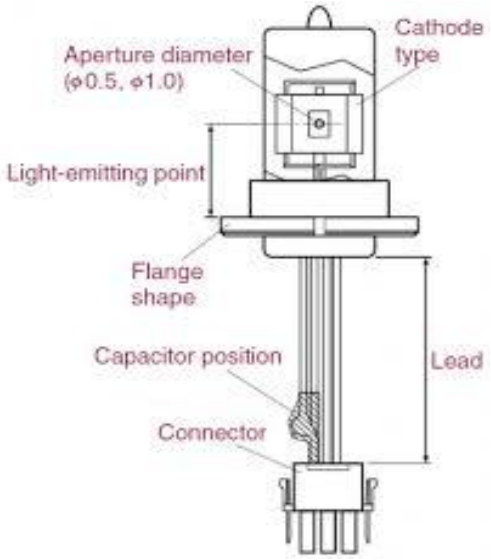

Figure 6: Deuterium lamp

Figure 7: Deuterium lamp 2

HYDROGEN LAMP
A hydrogen discharge lamp is a type of lamp used in UV-visible spectroscopy that emits UV radiation:

- How it works: A hydrogen lamp contains hydrogen gas under high pressure, and an electric discharge passes through the lamp to excite hydrogen molecules. These excited molecules then emit UV radiation.
- Wavelength range: Hydrogen lamps emit radiation in the range of 160–375 nanometre's (nm).
- Advantages: Hydrogen lamps are stable, robust, and widely used.

Figure 8: Hydrogen Deuterium lamp

TUNGSTEN LAMP

A tungsten lamp is a common light source used in UV-visible spectroscopy because it emits light over a broad range of wavelengths, including the visible region:

- Wavelength range: Tungsten halogen lamps emit light from 320–1100 nanometre's (nm), which includes the visible region of 380–700 nm
- Light source type: Tungsten halogen lamps are a type of gas-discharge light source, similar to deuterium lamps
- Construction: Tungsten halogen lamps consist of a tungsten filament in a glass or quartz envelope with a small amount of iodine vapor
- Cost: Tungsten halogen lamps are a low-cost optical source
- Polarization: Light from a tungsten lamp is randomly polarized and incoherent

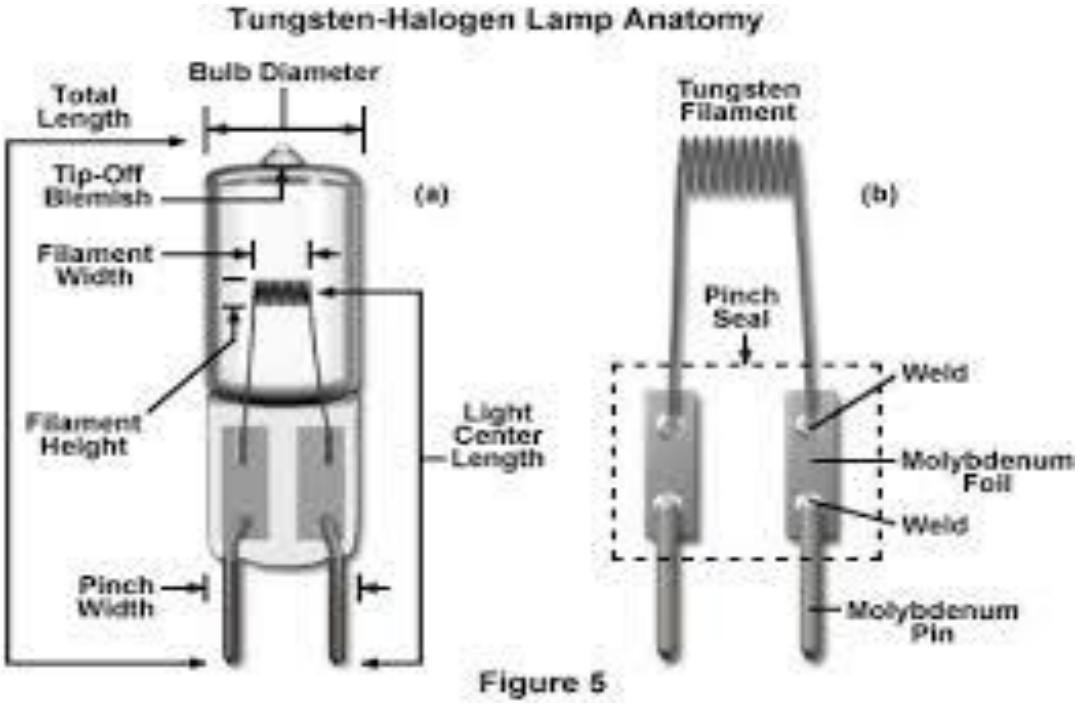

Figure 9: Tungsten Halogen lamp

Various visible radiation sources include:

1. Tungsten lamp
2. Mercury vapor lamp
3. Carbon arc lamp

2. FILTERS OR MONOCHROMATORS

All monochromators contain the following components:

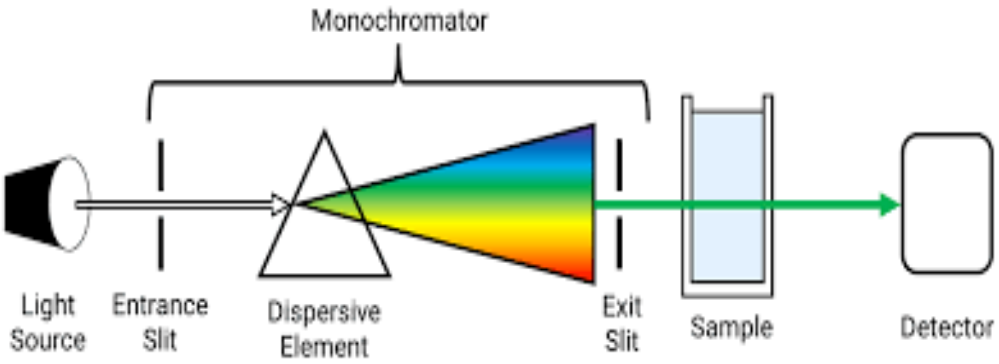

Figure 10: Filters and Monochromators

- An entrance slit
- A collimating lens
- A dispersing device (a prism or a grating)
- An exit slit
- A focusing lens

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

3. SAMPLE CELLS OR CONTAINERS

Numerous UV-transparent sample cells are available. The selection of a sample cell depends on factors such as path length, shape, size, transmission properties at the chosen wavelength, and relative cost. The sample-holding cell must be transparent to the wavelength range being measured. For UV spectroscopy, quartz or fused silica cuvettes are necessary. Cuvettes made from silicate glasses are suitable for use between 350 and 2000 nm. Typically, a cell is 1 cm thick and can be cylindrical with flat ends or rectangular in shape.

4. DETECTORS

Photovoltaic cells, also known as barrier-layer cells, consist of a metallic base plate made of iron or aluminium with a thin layer of a semiconductor metal, such as selenium, deposited on its surface. A very thin film of silver or gold is also present on the surface of the selenium, which serves as an additional collector. When radiation strikes the surface of selenium, electrons are produced. This accumulation at the silver surface generates an electric voltage difference between the silver surface and the cell's base.

Phototubes, also known as photo emissive cells, are made up of a glass bulb that has been evacuated and contains a light-sensitive cathode. A light-sensitive coating, such as potassium oxide or silver oxide, is applied to the inner surface of the cathode. When radiation strikes the cathode, photoelectrons are released and collected using an anode, which amplifies the current and records it.

The photomultiplier tube is a popular detector in UV spectroscopy. It consists of an anode, a photo emissive cathode (which emits electrons when exposed to photons of light), and several dynodes (which emit multiple electrons for each photon that strikes them). When a photon of radiation enters the tube and hits the cathode, it releases several electrons. These electrons are propelled toward the first dynode, which is at a voltage of 90V more positive than the cathode. For each electron that strikes the first dynode, several new electrons are released. These electrons are then accelerated to the second dynode, and the process continues, leading to the collection of electrons at the anode. Each initial photon can generate 10^6 to 10^7 electrons. Amplification and measurement are performed on the resulting current. Photomultipliers are highly sensitive to UV and visible light, and they have fast response times. However, they can only measure low-power radiation, as intense light can damage them. [6]

Working

1. Illumination: The light source emits a beam of UV and visible light. The light source can be a deuterium lamp for UV light and a tungsten-halogen lamp for visible light.
2. Monochromation: The monochromator, which contains a diffraction grating or a prism, separates the light into its component wavelengths. This allows the selection of a specific wavelength to pass through the sample.
3. Sample Interaction: The selected light passes through the sample solution in a quartz cuvette. The sample absorbs some of the light, and the remaining light reaches the detector.
4. Detection: The detector, which can be a photodiode or a photomultiplier tube, measures the intensity of the transmitted light.
5. Signal Processing: The detector signal is amplified and processed by the instrument's electronics.
6. Absorbance Calculation: The instrument calculates the absorbance using the Beer-Lambert law, which relates absorbance to the concentration of the absorbing species in the sample.

Components and Their Functions

1. Light Source: Provides a continuous spectrum of UV and visible light.
2. Monochromator: Selects a specific wavelength of light to be used in the measurement.
3. Sample Holder: Holds the sample in a cuvette, usually made of quartz for UV measurements.
4. Detector: Measures the intensity of the transmitted light.

5. Amplifier and Recorder: Amplifies the detec tor signal and records the absorbance values. (7)

Applications of UV-Visible Spectroscopy

- Detection of Impurities
  UV absorption spectroscopy is one of the most effective methods for determining impurities in organic molecules. Additional peaks observed in the UV spectrum can indicate the presence of impurities in the sample, which can then be compared to those of standard raw materials. By measuring absorbance at specific wavelengths, impurities can be accurately detected.
- Quantitative Analysis
  UV absorption spectroscopy is widely used for the quantitative determination of compounds that absorb UV radiation. This is achieved using Beer's Law, which is expressed as:

  A=log⁡(I0It) =−log⁡(T)= ε c BA = \log\left(\frac{I_0} {I_ t} \right) = -\log(T) = \ Var epsilon c BA=log (ItI0) =−log(T)= ε C b

  Where:

  - AAA = Absorbance
  - I0I_0I0 = Intensity of the incident light
  - ITTL = Intensity of the transmitted light
  - TTT = Transmittance
  - E\ var epsilon = Extinction coefficient
  - ccc = Concentration of the solution
  - BBB = Path length of the cell used in the UV spectrophotometer
- QualitativesAnalysis
  UV absorption spectroscopy can characterize compounds that absorb UV radiation. Identification is achieved by comparing the absorption spectrum of the sample with The kinetics of chemical reactions can also be studied using UV spectroscopy. UV radiation is passed through a reaction cell, allowing the observation of changes in absorbance over time.
- This technique can be used to detect the presence or absence of functional groups in a compound. The absence of a band at a particular wavelength serves as evidence for the lack of a specific functional group.
- Substances Many drugs exist either as raw materials or in formulated products. They can be assayed by preparing a suitable solution of the drug in a solvent and measuring the absorbance.

2. Structure Elucidation of Organic Compounds

- UV spectroscopy is useful in elucidating the structure of organic molecules, particularly in determining the presence or absence of unsaturation and the presence of heteroatoms. By analysing the location and combination of peaks in the spectrum, one can conclude whether the compound is saturated or unsaturated and whether heteroatoms are present.
- Qualitative Analysis
- UV absorption spectroscopy can characterize compounds that absorb UV radiation. Identification is accomplished by comparing the absorption spectrum with those of known compounds. This technique is particularly useful for characterizing aromatic compounds and aromatic olefins.
- Dissociation Constants of Acids and Bases
- The dissociation constant (Pak) can be determined using the equation:
- From this equation, the Pak value can be calculated if the ratio of [A−]/[HA][A^-]/[HA][A−]/[HA] is known at a particular ph. This ratio can be determined spectrophotometrically from a graph plotted between absorbance and wavelength at different pH values.

Chemical Kinetics

- The kinetics of reactions can also be studied using UV spectroscopy. UV radiation is passed through the reaction cell, and changes in absorbance can be observed over time.
- Quantitative Analysis of Pharmaceutical Substances
- Many drugs exist in either raw material form or as formulations. They can be assayed by preparing a suitable solution of the drug in a solvent and measuring the absorbance at a specific wavelength. For example, diazepam tablets can be analysed using 0.5% H2SO4 in methanol at a wavelength of 284 nm.
- Molecular Weight Determination
- The molecular weights of compounds can be measured spectrophotometrically by preparing

suitable derivatives of these compounds. For instance, to determine the molecular weight of an amine, it can be converted into amine picrate. A known concentration of amine picrate is then dissolved in one litre of solution, and its optical density is measured at λ max = 380 nm. Afterward, the concentration of the solution in grams per mole per litter can be calculated using the appropriate formula. [8]

ADVANTAGES OF UV VISIBLE SPECTROSCOPY

- Sensitivity: UV-Vis Spectroscopy can detect very low concentrations of analytes, often in the micromolar or even nanomolar range. This sensitivity makes it useful for analysing trace compounds in complex mixtures.
- Simplicity and Speed: The technique is straightforward and typically requires minimal sample preparation. Measurements can be performed quickly, allowing for high-throughput analysis in laboratories.
- Wide Range of Applications: UV-Vis Spectroscopy can be used to analysed a variety of substances, including organic and inorganic compounds, biological macromolecules, and even nanoparticles. It is useful in fields such as pharmaceuticals, environmental monitoring, and food safety.
- Quantitative Analysis: The Beer-Lambert Law, which relates absorbance to concentration, allows for quantitative analysis of samples. This makes
- UV-Vis Spectroscopy an essential tool for determining the concentration of unknown samples.
- Non-destructive: Most UV-Vis spectroscopy methods are non-destructive, meaning that the sample can often be recovered for further analysis or use after measurement.
- Information on Molecular Structure: UV-Vis Spectroscopy provides insights into electronic transitions in molecules, helping to deduce information about their structure, such as the presence of conjugated systems and functional groups.
- Cost-effective: Compared to other analytical techniques like mass spectrometry or NMR spectroscopy, UV-Vis Spectroscopy instruments are generally more affordable and require less maintenance.
- Portable Equipment: Many UV-Vis spectrophotometers are available in compact, portable designs, making them suitable for fieldwork and on-site analysis.
- Real-time Monitoring: UV-Vis Spectroscopy can be used for real-time monitoring of reactions, making it valuable in kinetic studies and process optimization.
- Compatibility with Various Sample States: The technique can analyse solids, liquids, and gases, enhancing its versatility in different analytical scenarios.
- Rapid Analysis: UV spectroscopy offers a convenient and fast analytical method, making it suitable for high-throughput analysis scenarios, such as in the pharmaceutical industry and environmental monitoring.
- Non-destructive Approach: This technique does not alter or damage the sample, making it ideal for analyses that require preserving sample integrity.
- Cost-Effectiveness: UV spectrometers are relatively inexpensive and easy to maintain, enabling widespread use [9]

- **DIS ADVANTAGES**
- Limited to UV-Vis Active Compounds: Only substances that absorb UV or visible light can be analysed, excluding non-absorbing compounds.
- Solvent Interference: The choice of solvent can affect the results, as solvents may absorb light in the UV-Vis range, leading to potential inaccuracies.
- Sensitivity to Environmental Conditions: Factors such as temperature, pH, and ionic strength can influence absorbance and lead to variability in results.
- Calibration Requirement: Regular calibration is necessary for accuracy, and failure to calibrate properly can result in misleading data.
- Limited Structural Information: UV-Vis Spectroscopy mainly provides information about electronic transitions and does not give detailed insights into molecular structure.
- Path Length Limitations: High concentrations can cause saturation, making it difficult to obtain accurate absorbance readings due to the dependence on path length.
- Deviation from Beer's Law: At high concentrations, deviations from Beer's Law can occur due to phenomena like scattering or aggregation, affecting concentration determinations.
- Overlapping Absorption Peaks: In complex mixtures, overlapping peaks can complicate the analysis, making it challenging to identify individual components.
- Cost of Advanced Equipment: While basic UV-Vis Spectrophotometers are relatively affordable,

advanced models with better specifications can be expensive.

- Limited Dynamic Range: The dynamic range may be restricted compared to other analytical techniques, impacting the analysis of samples with very high or low concentrations.
- Limited to Transparent Samples: Highly turbid o r opaque samples can scatter light, interfering wit h measurements.
- Interference: Substances that absorb at similar w avelengths can cause overlaps and inaccuracies.
- Concentration Limits: Very high concentrations can lead to non-
  linearity in absorbance measurements.
- Sample Preparation: Some samples may require extensive preparation to fit within the optimal ra nge for measurement.
- Solvent Effects: The choice of solvent can affect the absorption spectrum, sometimes complicatin g the analysis.
- Not Specific: While it's great for quantification, i t might not be as specific in identifying unknown compounds compared to other techniques like m ass spectrometry. [10]

VALIDATION

- Linearity
- Limits of detection and quantification (LOD and LOQ)
- Specificity and selectivity
- Precision
- Accuracy
- Robustness
- Range
- Ruggedness
- Stability

Linearity and Range

- Linearity refers to the ability of a method to yield test results that are directly proportional to the analyte concentration within a specified range. The range is defined as the interval between the upper and lower levels of analytes that have been demonstrated to be determined with precision, accuracy, and linearity using the described method. Standard solutions at concentrations of 3, 6, 9, 12, and 15 µg/ml were selected to assess the linearity range. A calibration curve was plotted using the concentration of the standard solution versus absorbance, and the regression equation was calculated. The least squares method was employed to determine the slope, intercept, and correlation coefficient.
- **Selectivity and Specificity**
- The selectivity and specificity of the method were evaluated by measuring the analyte of interest in the presence of other components, such as excipients in the micro sponges. A placebo solution was prepared by adding polymers like Eudragit RS 100, Eudragit RSPO, and plasticizers like polyethylene glycol and propylene glycol to methanol. The standard, placebo, placebo with the analyte, and test preparations were analysed as per the method to identify any interference from the placebo with the absorbance of lornoxicam.
- **Detection and Quantification Limits**
- The Limit of Detection (LOD) represents the lowest amount of analyte in the sample that can be detected, while the Limit of Quantification (LOQ) represents the lowest amount of analyte that can be quantitatively determined. LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve:
- LOD=3.3σS\text {LOD} = \frac {3.3\sigma} {S}LOD=S3.3σ LOQ=10σS\text{LOQ} = \frac{10\sigma}{S}LOQ=S10σ
- Where σ\sigma is the standard deviation and SSS is the slope of the calibration curve.
- **Accuracy**
- The accuracy of the proposed method was assessed through recovery studies at three different levels: 80%, 100%, and 120%. Recovery studies involved adding a known amount of standard solution of lornoxicam to a pre-analysed micro sponge solution.
- **Precision**
- The resulting solutions were then re-analysed using the proposed method. The total amount of drug found and percentage recovery were calculated. Precision was evaluated at three different levels: repeatability, intra-day precision, and inter-day precision, covering the concentration range. For repeatability, the absorbance of a 6 µg/ml lornoxicam solution was measured six times, and the percentage relative standard deviation (% RSD) was calculated. Intra-day precision involved analysing six replicate standard solutions on the same day, while inter-day precision was assessed by analysing a series of standard solutions over three consecutive days using the proposed UV-spectrophotometric method.
- **Robustness**
- The evaluation of robustness should be considered during the development phase and depends on the specific procedures. Deliberate

variations in method parameters were introduced. If measurements are susceptible to variations in analytical conditions, those conditions should be controlled, or a precautionary statement should be included in the procedure. In this study, the absorption maxima were increased and decreased by 1 nm, and the process was carried out for a 9 µg/ml standard solution. The % RSD was calculated. The proposed method was found to be simple, sensitive, accurate, precise, economical, and rapid for the estimation of lornoxicam in micro sponges. The method was validated in accordance with ICH guidelines (Q2 (R1)).

- **ANALYTICAL METHOD VALIDATION**
- Analytical methodologies are essential for the development of new medications, formulation and Pre formulation research stability studies, and quality control inspections. This strategy must be straightforward, precise, accurate, cost-effective, and user-friendly. It is necessary to conduct method validation both during development and in use. Analytical validation refers to the process of assessing and substantiating that an analytical method successfully achieves its intended objective

- ELEMENTS OF VALIDATION
- **Design Qualification (DQ)**
- Design qualification ensures that the design of the apparatus conforms to Good Manufacturing Practice (GMP) requirements. The design principles should guarantee that GMP objectives are fulfilled. It is essential to review the mechanical drawings and design specifications provided by the equipment manufacturer.
- Installation Qualification (IQ)
- Installation qualification is a critical process that must be performed on newly constructed or modified equipment, systems, and structures. The certification for installation should encompass the following critical areas:
- Examination of the installations of instrumentation, infrastructure, services, and equipment.
- Collection of the provider's operational guidelines, maintenance specifications, and calibration requirements.
- Verification of construction materials, maintenance sources, and spare parts.
- **Operational Qualification (OQ)**
- This stage follows IQ and encompasses assessments developed based on knowledge of the processes, systems, and instruments that establish minimum and maximum operational thresholds. The term "worst-case conditions" may occasionally be used to describe this concept.
- **Performance Qualification (PQ)**
- Following the conclusion of IQ and OQ, the next qualification to be accomplished is PQ. PQ should include:
- Investigations involving prototypes, substitutes, or Modelled products.
- Developing an understanding of the facilities, systems, apparatus, and processes that yield these outcomes.
- Tests that possess upper and lower bound criteria.

- **TYPES OF VALIDATION**
- Prospective
  This involves implementing written evidence to support the claims or capabilities of a system in accordance with a predetermined strategy. This validation is performed before the release of new products.
- Retrospective
  This is the generation of written evidence that substantiates the claim or performance of a system, derived from an examination and evaluation of available data. This is achieved by utilizing manufacturing, testing, and control data collected on a previously released product.
- Concurrent
  Concurrent validation involves the process of utilizing data generated during system construction to provide written substantiation of what a system does or purports to do. [11]

CONCLUSION:

UV-Visible spectroscopy is a powerful analytical technique used to identify and quantify substances based on their absorption of ultraviolet and visible light. By measuring the absorbance of a sample at specific wavelengths, we can determine various properties such as concentration, structure, and the presence of certain functional groups.

The technique is widely used because it is relatively quick, non-destructive, and requires minimal sample preparation. The results provide insights into the electronic transitions of molecules, especially those with conjugated double bonds, aromatic systems, and other chromophores.

In summary, UV-Visible spectroscopy is essential in fields like chemistry, biochemistry, environmental science, and pharmaceuticals, making it an invaluable tool for both qualitative and quantitative analyses. [12]

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