A Review on Uv-Visible Spectroscopy

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Abstract*— The UV visible spectroscopy is used in analytical chemistry for quantitative determination of biological macromolecules, analytes & metal ions. 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 composition and structure of the materials can be examined using the spectrum. These conclusions have uses in academia, business, medical labs, and chemical examination of environmental samples. The pharmaceutical analysis comprises the procedure necessary to determine the "identity, strength, quality and purity" of such compounds. It also includes the analysis of raw material and intermediates during the manufacturing process of drugs. It is well known that the dissociation constant is a most important parameter in development and optimization of anew compound for effective formulation development.

Index Terms- UV spectroscopy, Instrumentation, Beer Lambert law, Choice of solvent, UV-VIS Spectrometer, UV-VIS Spectrum etc.

I. INTRODUCTION

UV spectroscopy is the absorption or reflectance spectroscopy of the ultraviolet and adjacent visible regions of the electromagnetic spectrum. It is also known as UV-visible spectrophotometry (UV-Vis or UV/Vis). Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation (EMR) absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. In other words, spectroscopy measures the changes in rotational, vibrational and /or electronic energies. 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). When the measurement of radiation frequency is done experimentally, it gives a value for the change of energy involved and from this one may draw the conclusion about the set of possible discrete energy levels of the matter. The ways in which the measurements of radiation frequency (emitted or absorbed) are made experimentally and the energy levels deduced from these comprise the practice of spectroscopy.

Principle:

The principle of UV visible spectroscopy is based on absorption of ultraviolet light or visible light by a chemical compounds, which gives spectra. When radiation induces an electronic transition in a molecule or ion's structure, the object will exhibit absorption in the visible or ultraviolet range. As a result, when a sample absorbs light in the ultraviolet or visible range, the molecules inside the sample experience a change in their electronic state. The interaction of light and matter is the foundation of spectroscopy. When matter absorbs light, it experiences excitation and de-excitation, which results in the formation of a spectrum.[3] When an electromagnetic wave strikes a material, phenomena such as transmission, absorption, reflection, and scattering can occur, and the observed spectrum depicts the interaction of wavelengths with discrete-dimensional objects such as atoms, molecules, and macromolecules. 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

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 probable and strong

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.
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

Laws :
Beer Lambert law :
Beer Lambert law states that, "The absorbance (A) of monochromatic beam is directly proportional to concentration (C) & Path length (l). A UV-Visible/NIR spectrophotometer calculates the transmittance, or amount of light transmitted through a sample, by dividing the intensity of incident light (I0) by the intensity of transmitted light (I). The regression line can also be used for determining concentration of a solution whose absorbance is obtained using a colorimeter/spectrophotometer.

$$A = \varepsilon C. L$$

Where,
A = Absorbance
ε = Molar absorption coefficient
C = Molar concentration
L = Path length.

II. TYPES OF SPECTROSCOPY

Spectroscopy can be conveniently divided into following types based on
1. Whether the study is made at atomic or molecular level.
a) Atomic Spectroscopy –where the changes in energy take place at atomic level.
Eg. Atomic absorption spectroscopy, Flame photometry –where either atomic absorption or atomic emission of radiation is being studied.
b) Molecular Spectroscopy –where the changes in energy take place at molecular level.
Eg. UV spectroscopy, Colorimetry, Infra-Red Spectroscopy, Fluorimetry – where the molecular absorption, emission or vibration is being studied.

2. Whether the study is based upon absorption or emission of EMR.
a) Absorption Spectroscopy –where absorption of radiation is being studied. Eg. UV spectroscopy, Colorimetry, Infra-Red Spectroscopy, NMR Spectroscopy, Atomic absorption Spectroscopy.
b) Emission Spectroscopy –where emission of radiation is being studied. Eg. Flame photometry, Fluorimetry.

3. Whether the study is at electronic or magnetic levels.
a) Electronic Spectroscopy Eg. UV spectroscopy, Colorimetry, Fluorimetry –where the study is done using electromagnetic radiation only (without the influence of magnetic field).
b) Magnetic Spectroscopy Eg. NMR Spectroscopy, ESR spectroscopy –where the study is done using electromagnetic radiation under the influence of magnetic field. The energy of a molecule can be due to electronic, vibrational or rotational energy. They are in the following ratio: Rotational energy: Vibrational energy: Electronic energy = 1: 100: 10,000 When any electromagnetic radiation is passed on to a molecule, the following energy changes take place in a molecule/atom which can be measured.

Electromagnetic Spectrum:
If we arrange all types of electromagnetic radiations in order of their increasing wavelengths, then portion above the visible region is called infrared while that below it is the ultra –violet region.

The visible spectrum (from violet to red) represents only a small portion of the electromagnetic spectrum.
Infra-red radiations have longer wave lengths and are thus less energetic.
microwaves have larges wavelengths and are used in telephone transmission.
Cosmic ray carry high energy while radio waves are energetic.
The arrangement of all types of electromagnetic radiations in order of their increasing wavelength or decreasing frequencies is known as complete electromagnetic spectrum.

a) -ray region: This lies between 0.02 to 1A0 . The gamma rays are shortest waves emitted by atomic nuclei, involving energy changes of 10-9 to 1011 Joules / gram atom.
b) X –ray region: This lies between 1 to 10 A0 . X-rays emitted or absorbed by movement of electrons close to the nuclei of relatively heavy atoms, involve energy changes of the order thousand kilo Joules.
c) Visible and Ultraviolet Region: these are further made up of the following regions:
Vacuum ultraviolet: 1 -180 nm
Ultraviolet: 80 – 400 nm
Visible: 400 – 750 nm
Types of Electronic Transitions: Four main types of electronic transitions are observed.
1) $\sigma \rightarrow \sigma^*$ transition
2) $\pi \rightarrow \pi^*$ transition
3) $n \rightarrow \sigma^*$ transition
4) $n \rightarrow \pi^*$ transition

1) $\sigma \rightarrow \sigma^*$ transition :
• σ electron from orbital is excited to corresponding anti-bonding orbital σ*.
• The energy required is large for this transition.
• e.g. Methane (CH4) has C-H bond only and can undergo $\sigma \rightarrow \sigma^*$ transition and shows absorbance maxima at 125 nm.

2) $\pi \rightarrow \pi^*$ transition :
• π electron in a bonding orbital is excited to corresponding anti-bonding orbital π*.
• Compounds containing multiple bonds like alkenes, alkynes, carbonyl, nitriles, aromatic compounds, etc undergo $\pi \rightarrow \pi^*$ transitions.
• e.g. Alkenes generally absorb in the region 170 to 205 nm.

3) $n \rightarrow \sigma^*$ transition :
• Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of $n \rightarrow \sigma^*$ transition.
• These transitions usually require less energy than $\sigma \rightarrow \sigma^*$ transitions.
• The number of organic functional groups with $n \rightarrow \sigma^*$ peaks in UV region is small (150 – 250 nm).

4) $n \rightarrow \pi^*$ transition :
• An electron from non-bonding orbital is promoted to anti-bonding π* orbital.
• Compounds containing double bond involving hetero atoms (C=O, C≡N, N=O) undergo such transitions.
• $n \rightarrow \pi^*$ transitions require minimum energy and show absorption at longer wavelength around 300 nm.

Instrumentation :

1. Light Source
2. Monochromator
3. Sample & reference cells
4. Detector
5. Recorder.

1. Light Source : The light source used must provide consistent & stable light.
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.

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

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

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

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

2) Monochromators:
• Monochromators are better and more efficient than filters in converting polychromatic light or heterochromatic light into monochromatic light.
• Monochromators are primarily designed for spectral scanning, i.e. a process of continuously varying the radiation wavelength over a considerable range.
• Mechanical construction of monochromators for UV, visible and IR radiation is similar in that all of them employ slits, lenses, mirrors, windows, and gratings or prisms.

Types of monochromators:
a) Prism monochromator
b) Grating monochromator
All Monochromator contain the following component parts:
- An entrance slit
- A collimating lens
- A dispersing device
- A focusing lens
- An exit slit

3. Sample & reference cells:
•The cuvette are generally made up of quartz & borosilicate.
• One beam pass through sample solution & second beam pass through reference solution.
•The cuvette are generally transparentThe cells or cuvettes are used for handling liquid samples.
• The cell may either be rectangular or cylindrical in nature.
• For study in UV region; the cells are prepared from quartz or fused silica whereas color corrected fused glass is used for visible region.
• The surfaces of absorption cells must be kept scrupulously clean.
• No fingerprints or a touch should be present on cells.
• Cleaning is carried out washing with distilled water or with dialcohol, acetone.

4. Detector :
•Device which converts light energy into electrical signals, that are displayed on readout devices.
• The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample
• The following types of detectors are employed in instrumentation of absorption spectrophotometer
Requirements of an ideal detector: -
• It should give quantitative responses.
• It should have high sensitivity and low noise level.
• It should have a short response time.
• It should provide signal or response quantitative to wide spectrum of radiation received.

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

b) Photo Tubes/ Photo emissive Tubes:
• Consists of a evacuated glass tube with a photocathode and a collector anode.
• The surface of photocathode is coated with a layer of elements like caesium, silver oxide or mixture of them.
• When radiant energy falls on photosensitive cathode, electrons are emitted which are attracted to anode causing current to flow.
• More sensitive compared to barrier layer cell and therefore widely used.

C)Photo Multiplier Tubes:

•The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons.
• In a vacuum tube, a primary photo-cathode is fixed which receives radiation from the sample.
• Some eight to ten dynodes are fixed each with increasing potential of75- 100V higher than preceding one.
• Near the last dynode is fixed an anode or electron collector electrode.
• Photo-multiplier is extremely sensitive to light and is best suited where weaker or lower adiation is received.

5. Recorder:
The recorder detect & record the data of the experiment. It also stores the data in computer when it connected to computer.

Advantage's of UV visible spectroscopy :
•Cost effective instrument
• Cover the entire of ultraviolet and visible
• It can be utilized in the qualitative and quantitative analysis
• The Derivative graph can be obtained by UV-VIS spectrophotometer
• It can be used in the degradation study of drug.
•The core advantage is the accuracy of the UV-VIS spectrophotometer
• The UV-VIS spectrometer is easy to handling and use
• Provide robust operation
• UV-VIS spectroscopy is simple to operate

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

Applications of UV visible spectroscopy :

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

• Quantitative Analysis of Pharmaceutical Compounds UV spectroscopy is widely employed for the quantitative analysis of pharmaceutical compounds, ensuring accurate concentration measurements [1

• 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.

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

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

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

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

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

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

•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].

•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.

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

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

•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.

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. If used with the right standard curve and applied to pure substances, UV-visible spectroscopy is a reliable, straightforward, and affordable approach for estimating the concentration of absorbing species. One of the crucial methods for analyzing the optical characteristics of PMCs is UV-Vis spectroscopy. It clarifies the relationship between the matrix and the nanofiller and examines how the nanofillers contribute to the enhancement of the properties of the nanocomposites. To assess the intended optical properties of nanofillers in a polymer matrix, UV-Vis spectroscopy is a crucial technique. The review paper contains all information about UV visible spectroscopy, its principle, theory, Instrumentation, advantages, Disadvantage's & its applications. The identification of impurities are carried out by using UV visible spectroscopy more accurately & UV visible spectroscopy is a very crucial spectroscopy.

REFERENCES

[1] Modern Chemical Techniques, Ultraviolet/visible Spectroscopy, The Royal Society of Chemistry, 102,103.

[2] International Journal of Pharmaceutical Research & Analysis Gandhimathi R. et al., 2012;2(2): 72-78 Pg no. 76.

[3] Sharma Y.R. Ultraviolet and visible spectroscopy in; Elementary Organic spectroscopy,1ed., S.Chand & Company Ltd ., New Delhi, 2004; 9-60 Basic UV-Vis Theory, Concepts and Applications, 7-10.

[4] Mend ham J., et al; Vogel,s Textbook of Quantitative Chemical Analysis. 6th ed. Pearson education, Singapore pvt.Ltd ., Delhi, 2003; 6(3): 3-8.

[5] Donald L. Pavia, Gary M. Lampman, George S.Kriz, IamesR.Vijaan. Spectroscopy. Third Edition, CBS Publishers, and Distributors, 1997.

[6] Clark BJ, Frost T, Russell MA, editors. UV Spectroscopy: Techniques, instrumentation and data handling. Springer Science & Business Media, 1993 Apr 30.

[7] Skoog, D.A. Holler, F.J., Nieman, D.A., Introduction to UV Spectroscopy in, principle of instrumental analysis, 5thed., Cole publication, 2004.

[8] Analytical process of drugs by ultraviolet (UV) spectroscopy – a review R. Gandhimathi*, S. Vijay Raj, M.P. Jyothirmaie *Department of Pharmaceutical Analysis, SreeVidyanikethan College of Pharmacy, Tirupathi-517102, Andhra Pradesh, India.

[9] Ganesh S, et al. "A Review on Advances in UV Spectroscopy", Research J. Science and Tech. 2020,12(1),47-51

[10] Amira H. Kamal, Samah F, et al. "A REVIEW ON UV SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS MULTICOMPONENT ANALYSIS", European Journal of Pharmaceutical and Medical Research, 2016,3(2), 348-360 15.M. Patil, "Advantages and Disadvantages of UV Visible Spectroscopy", NOV 2018, https://chrominfo.blogspot.com/2018/11/advantages-anddisadvantages-of-uv.html?m=1

[11] N Lavanya, et al. "Simultaneous Determination and Comparison of Sun Protection Factor of Various merchandised sunscreen formulations by using UV Spectrophotometer", Asian Journal of Pharmaceutical Analysis, 2022,12(2),111-11

[12] Sheffield Hallam University, UV-Visible Spectroscophy Instrumentation.

[13] Thermo Spectronic, Basic UV-Vis Theory, Concepts and Applications, 11- 12

[14] G. R. Chatwal, S. K. Anand Instrumental methods of chemical analysis, Himalaya Publishing House (1979).

[15] Sethi PD. Quantitative Analysis of drugs in pharmaceutical Formulations, 3rd ed., CBS Publishers and Distributors, New Delhi 1997.

[16] Beckett A.H., Stenlake J.B., Practical Pharmaceutical Chemistry; 4th ed. CBS Publisher and distributors delhi, 2001: 280-286.