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A REVIEW ON UV-VISIBLE SPECTROSCOPY

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ABSTRACT

Ultraviolet spectroscopy is a oldest instrument-based analytical technique, has shown crucial tool in numerous scientific areas. Beer's law, an important idea in UV spectroscopy, describes how samples absorb light. Both quantitative and qualitative analyses can be conducted effectively using UV spectroscopy. It gives precision in their result and used for the analysis of variety of sample compounds. It determines the Electromagnetic Radiation which is absorbed when molecules or atoms and ions of the sample move from one energy state to another energy state. The monochromator is used to convert the chromatic light into single desirable monochromatic light. Converts the light into electrical signal, and the detector convert the polychromatic light into electrical signal.

KEYWORDS: UV spectroscopy, Beer's law, Quantitative analysis.

INTRODUCTION

The study of interaction between light or electromagnetic radiation and matter is known as spectroscopy. UV spectroscopy is a powerful analytical Technique using light with the UV or visible region ranging wavelength from 200 to 800nm. This method is flexible, proficient of analysing both colourless compounds in uv range (400-200nm) and coloured compounds in apparent range (800-400)[1] UV spectroscopy is also called as electronic spectroscopy, this is rapid and simple technique, and relevant to minimal quantities of compounds. The fundamental law of spectroscopy process that supply the quantitative spectrophotometric analysis which is based on the Beer-Lambert's law[2] one of the first spectroscopic scientist was Johannes Marcus Marci of Kronland (1595-1667) in Eastern Bohemia. He illustrated interest in occurrence of performed and rainbow analysis to explain it.[3] The most important impact of such interaction is that power is absorb or released the matter in separate amounts is known as quanta.[5] This approach is commonly utilized in a wide variety of fundamental and applied applications.[6]

PRINCIPLE

The principle of UV visible spectroscopy is depended on absorption of the polychromatic light or visible light by a sample which gives spectra. When the sample is absorb the sample molecule goes to the singlet excited state from the ground state.[7] There are three possible categories of ground state orbit involve σ (bonding) molecular, π (bonding) molecular orbital and n (bonding) atomic orbital, moreover to two class of antibonding orbitals that may involved in the transition process: σ* (sigma star) orbital and π* (pi star) orbital. Note that there is no any antibonding orbital n* since the n electrons don't form bonds, and hence electronic transitions can take place by absorbing ultraviolet and visible light.[8]

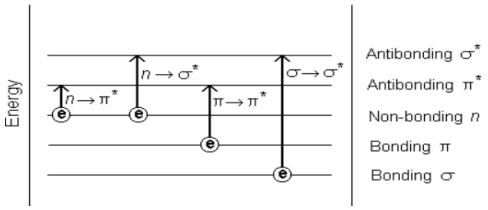

Figure 1: Electronic transition graphical representation.

ABSORPTION OF LIGHT: UV-visible spectroscopy works on the idea that when light shines on a material, the material reacts to it. This reaction helps us study and understand the substance.

ELECTROMAGNETIC SPECTRUM: Light is made of waves of different sizes uv-visible spectroscopy is a straightforward way to check how much light a sample absorbs in two ranges uv (200-400) nm and visible (400-800) nm this helps us figure out what the sample is made of its fast easy and useful for studying different substances.

ELECTRONIC TRANSITIONS: When a beam of light moves through a material, some wavelengths disappear because the substance takes in part of the light. This occurs when electrons inside the molecules gain energy from the incoming photons, causing them to jump from their ground states to higher-energy levels.

π → π Transitions: This is often seen in conjugated organic molecules, where electrons in a π-bond system absorb energy and move into a π* (anti-bonding) orbital.

n → π Transitions: This involves electrons that aren't part of any bond (called non-bonding or "n" electrons) getting excited into a π* (pi-star) anti-bonding orbital, which usually happens in molecules that have lone pairs.

ABSORPTION SPECTRA: An absorption spectrum is a graph that shows how much light a sample absorbs at different colours (wavelengths). The peaks on this graph happen where the electrons in the sample jump to higher energy levels. These peaks give clues about the structure of the molecules and the amount of the substance present in the sample.[32]

BEER-LAMBERT LAW

The Beer–Lambert Law clarify how the quantity of light absorbed by a solution is linked to the concentration of the absorbing category within it.

$A=\log_{10}(I0/I) = \varepsilon \cdot c \cdot l$

- A is the absorbance of the solution.
- I0 is the intensity of the incident light.
- I is the intensity of the transmitted light.
- ε is the molar absorptivity, which is a constant that indicates how strongly the substance absorbs light at a particular wavelength.
- c is the concentration of the analyte in the solution.
- l is the path length of the sample cell.

INSTRUMENTATION

There are two forms of UV spectrophotometer. They are basically:

- Single beam UV spectrophotometer (Figure 2)
- Double beam UV spectrophotometer (Figure 3)

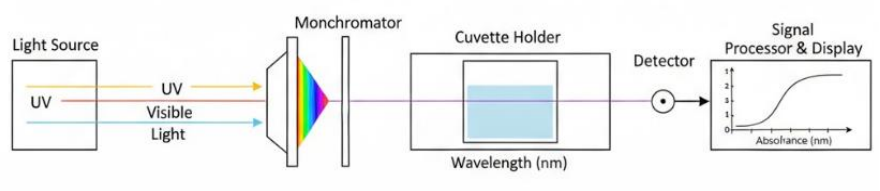

Figure 2: Single Beam UV spectrophotometer.

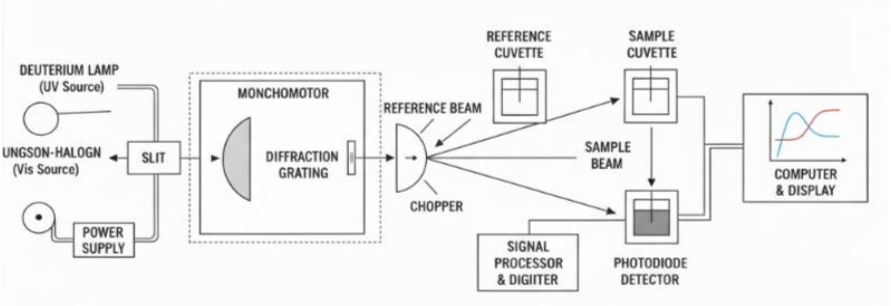

Figure 3: Double Beam UV spectrophotometer.

The basic instrumentation of UV spectrophotometer includes:

LIGHT SOURCE

Source of UV radiation: It's essential that the radiation source's power remains stable across its entire wavelength range. A continuous UV spectrum is created when deuterium or hydrogen is electrically excited at low pressure. This happens because the excited molecules break apart, forming two atoms and releasing a UV 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 since glass absorbs radiation of wavelengths below 350 nm.

Sources of visible radiation: The most common source of visible radiation is a tungsten filament lamp. This kind

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, which means that the voltage to the lamp must be very stable indeed for the energy output to remain stable. Electronic voltage regulators or constant-voltage transformers are used to achieve this stability.[1]

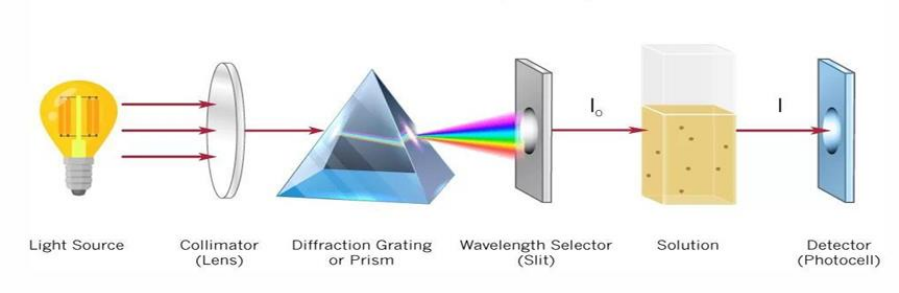

Figure 4: Instrumentation of UV Spectrophotometer.

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

The entry point for the multi-chromatic light radiation containing various wavelengths is the entrance aperture once inside the monochromator the optical path first collimates the beam making the light rays parallel this conditioned beam then impinges upon the dispersive medium either a grating or a prism the function of this element is to diffract or refract the light spreading the spectrum across space selection of the desired wavelength is accomplished by mechanically manipulating the system the dispersive component is rotated or the output slit is repositioned so that only the narrow spectral band of interest is aligned with and exits through the final exit slit.[1]

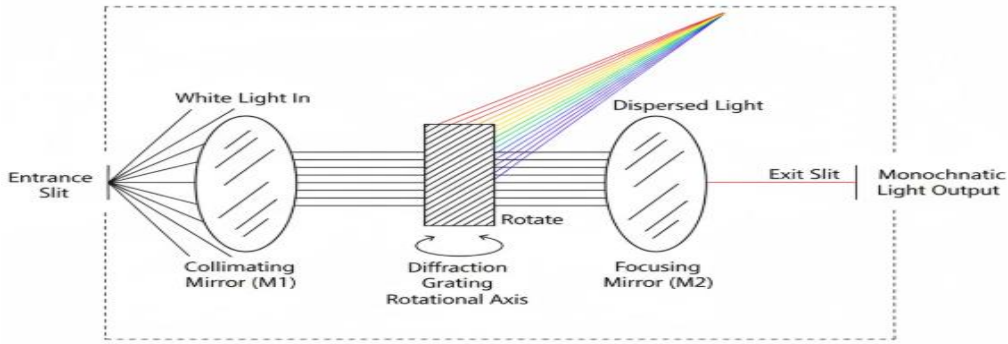

Figure 5: Turner Grating Monochromator.

Sample cell

The containers for the sample solution must be transparent for the radiation which will goes through it quartz or fused silica cuvettes are require for spectroscopy technique in the uv region these cells are also transparent in the visible region silicate glasses could be used for the manufacture of cuvettes for use between 350-2000 nm glass cuvettes should not be used in uv region because it may absorb uv radiation but may be used in visible region.[1]

Detector

The light signal gets converted into the electrical signal with the help of detector. It should give a straight response without any error over a wide range of low noise a sensitivity.

1. Photomultiplier tube: The Photomultiplier Tube stands out as one of the most highly sensitive and delicate instruments utilized in UV-visible spectrophotometers, with its primary function being to convert quantities of light energy into a significant, measurable electrical current. The PMT's operation relies on a multi-stage amplification process that enables the detection of very low light levels. The main structural components include a photocathode (a light-reactive surface that releases electrons when struck by photons), a sequence of dynodes (intermediate electrodes that emit multiple secondary electrons upon impact), and an anode (the final collector electrode). When light enters the PMT, it first hits the photocathode, triggering the release of electrons. These initial electrons are accelerated toward the first dynode, which is maintained at a high positive potential. Upon striking the first dynode, the process of secondary emission occurs, ejecting multiple new electrons. This electron multiplication is repeated at every succeeding dynode in the chain, causing the

number of electrons to escalate rapidly by the time they reach the anode, a single original photon can result in a cascade of millions of electrons. The accumulated electrons generate a measurable current that is proportional to the intensity of the light that initially entered the detector. This powerful amplification grants PMTs exceptional sensitivity, a fast response time, and makes them ideal for detecting weak UV and visible radiation signals.[1]

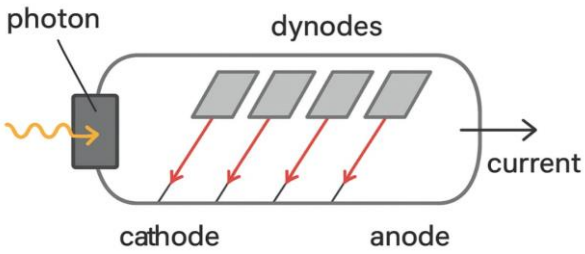

Figure 6: Photo multiplier tube.

2. Photo diode detector: A photodiode array works like a high-speed camera for measuring light. Instead of scanning one colour at a time, this detector uses a single chip lined with hundreds of tiny sensors usually 1,024 of them to capture the entire light spectrum all at once. Inside the instrument, the chip is placed exactly where the light beam spreads out, allowing it to record everything instantly. This speed makes it perfect for analysing liquids that flow by very quickly, such as the samples in HPLC (liquid chromatography) testing.[1]

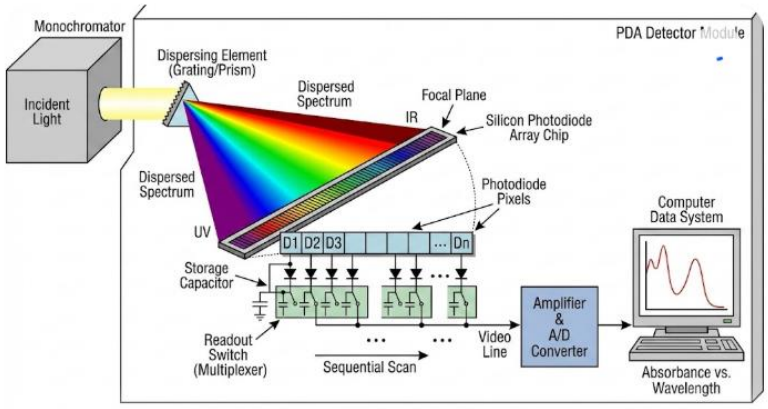

Figure 7: Photodiode array detector.

The advantages of UV-Visible spectroscopy

1. Cost-Effective Equipment
2. Simple Operation
3. Rapid Results
4. Non-Destructive
5. Minimal Sample Preparation
6. Minimal Sample Preparation
7. Quantitative Accuracy
8. Wide Range of Applications
9. Real-Time Monitoring
10. Qualitative Identification

Disadvantages of UV-Visible spectroscopy

1. Limited Selectivity (Interference)
2. Only Works for Liquid
3. Stray Light Errors
4. Limited Structural Information
5. Beer-Lambert Law Limitations
6. Solvent Interference
7. Sensitivity to pH Changes
8. Requires Clear Samples (No Turbidity)
9. Damage to Light-Sensitive Samples
10. Regular Maintenance Required

Applications

1. **Identification of impurities**: UV-Visible spectroscopy is a simple and reliable way to find out how much of a particular substance is present in a sample. It works by measuring how much light the substance absorbs and linking that value to its concentration. This relationship between absorbance and concentration is described by the Beer–Lambert law.
2. **Qualitative analysis:** Helps us figure out what a substance is by viewing closely at the precise light structure the absorbance spectrum it creates and then similar that pattern up with the known patterns of other substances.

3. **Ionization Constant of acid and bases:** This technique permits researchers to assess the force or weakness of an acid or base the Determination.
4. **Structural Explication of Organic composition:** The spectral data secured is exceptionally valuable because it immediately assists in explaining and confirming the structural arrangement of molecules.
5. **quantitative examination: UV**–visible is achieved by meticulously examine the extent to which the substance separates into ions dissociates once it is dissolved in a solvent.
6. **Molecular weight Determination:** While UV-visible spectroscopy is not the primary instrument for establishing the molar mass of a substance the structural insights derived from the spectra concerning chromophores and electronic configuration nonetheless yield ancillary data that can assist in the verification or estimation of the substances molecular weight.
7. **As HPLC detector :** Inside an HPLC configuration the UV-vis is a critical measuring component as the separated compounds leave the column it measures the absorbance of their light at pre- et wavelengths this absorbance data allows the overall system to output valid quantitative statistics of importance in calculating the concentration of each component within the sample under test.
8. **Deviations from Beer-Lambert Law:** The direct relationship assumed by the Beer-lambert law may break down cause deviations due to several factors including excessively high concentrations of the analyte the presence of non-uniform non-homogeneous solutions or the interference of unwanted light stray light in the instrument.[4]
9. **Chemical Analysis:** Measures the concentration and structure of chemicals in a extract.
10. **Quantitative Analysis of Pharmaceutical Substances:** Assures exact dosage and purity of pharmaceuticals.[4]

CONCLUSION

UV-visible spectroscopy remains one of the foundational yet versatile tools in analytical chemistry capable of conducting both quantitative and qualitative analyses with a high degree of precision based on the principles of electronic transitions and the beer-lambert law this technique enables one to determine analyte concentrations via the measurement of the electromagnetic radiation absorbed as molecules shift between energy states from stable light sources such as deuterium and tungsten lamps to advanced detectors the evolution of instrumentation has made this methodology increasingly useful with the use of modern detectors which include the very sensitive part and high-speed spectral data can be recorded correctly and rapidly while the accompanying disadvantages include interference from the solvent and stray light errors the inexpensive nature of this technique the fact that it is non-destructive and minimal sample preparations make it quite valuable in the final analysis its wide-ranging applications-from guaranteeing the purity of pharmaceuticals to acting as a critical detector in HPLC-confirm its enduring importance in scientific research.

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