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REVIEW ARTICLE

UV-Visible Spectroscopy: Conspectus

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ABSTRACT
UV-VIS Spectroscopy is the term used for the analytical estimation of the different types of the solvents and substances. It has been in use for the last 35 years and become the most important analytical instrument in the modern-day laboratory. Spectrophotometer is generally preferred by small-scale industries as the Instrument is inexpensive and the maintenance problems are minimal. The pharmaceutical analysis includes the procedure necessary to seethe "identity, strength, quality and purity" of compounds. It measures a large number of organic and inorganic compounds in a wide range of products and processes - in nucleic acids and proteins, foodstuffs, pharmaceuticals and fertilizers and also includes the raw material analysis and intermediates during the manufacturing process of drugs. The analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-400nm).

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1. Introduction

Definition: Spectroscopy is defined as study of interaction of electromagnetic radiation with matter. It 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 i.e. from ground state to excited state and excited to ground state.

Spectrum: It is a plot of the response as a function of wavelength or frequency is referred to as a Spectrum.

UV-VIS Spectroscopy: Ultraviolet (UV) spectroscopy is a physical technique and it is based on Beer-Lambert law. This law states that the absorbance of a solution is directly proportional to the concentration and path length. Thus, for a fixed path length, we can determine the concentration

of the absorber in a solution. It is necessary to find out the absorbance changes with concentration.

Principle of UV-Vis Spectroscopy: when a molecule or ion will exhibit absorption in the visible or ultraviolet region, radiation causes an electronic transition within its structure. Thus, the absorption of light by a sample within the ultraviolet or visible region is in the course of a modification in the electronic state of the molecules. It will promote electrons from bonding to antibonding orbitals.

Potentially, three types of ground state orbital may be involved:

1. σ (bonding) molecular
2. π (bonding) molecular orbital
3. n (non-bonding) atomic orbital

In addition, two kinds of antibonding orbital are also concerned with in the transition:

i) σ* (sigma star) orbital
ii) π* (pi star) orbital

A transition in which a bonding sigma electron is excited to an anti-bonding σ orbital is referred to as σ to σ* transition. Within the same means π to π* represents the transition of 1 electron of a lone pair (non-bonding electron pair) to an antibonding π orbital. Therefore, the subsequent electronic transitions will occur by the absorption of ultraviolet and visual light:

- ✓ σ to σ*
- ✓ n to σ*
- ✓ n to π*
- ✓ π to π*.

Both σto σ* and n to σ* transitions require a highest energy and therefore occur in the far ultraviolet region 180-240nm. Consequently, in ordinary ultraviolet region saturated groups do not exhibit strong absorption. Transitions from π to π* type occur in unsaturated centers of the molecule; they require less energy and occur at longer wavelengths than transitions to σ* anti-bonding orbital. Transitions to π* anti-bonding orbital that happens within the ultraviolet region for a specific molecule could place in the visible region, if the molecular structure is changed. Several inorganic compounds in solution also show absorption in the visible region. These include salts of elements with incomplete inner electron shells (mainly transition metals) whose ions are complexed by hydration.

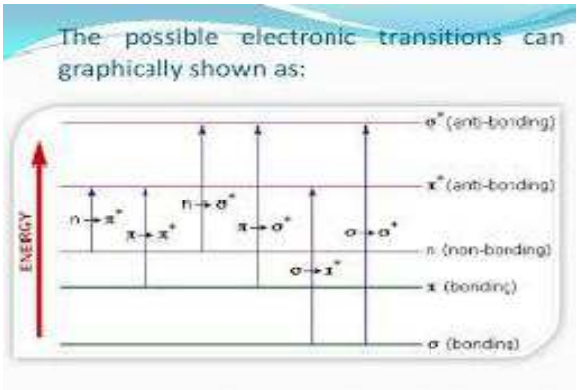

Fig 1: Electron Transition graphically represented

UV absorption spectrometry: The spectrometer is generally preferred by small scale industries. The method of study relies on measuring the absorption of amonochromatic light by colorless compounds in the near ultraviolet path of the spectrum i.e. 200-400nm. The light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer. Ultraviolet-visible spectrometry is employed to get the absorbance spectra of a compound in a solution. The energy for the electromagnetic spectrum covers 1.5 - 6.2 EV which relates to a wavelength range of 800 - 200 nm. The principle behind absorbance spectroscopy is Beer-Lambert Law.

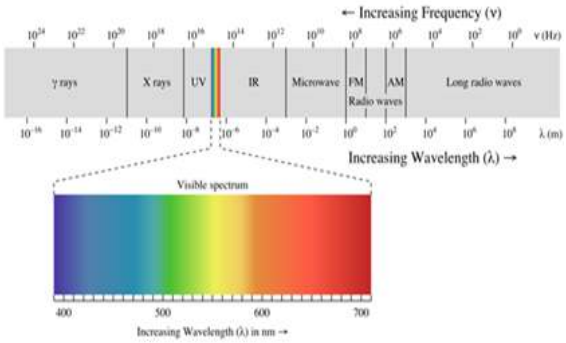

Fig 2: Electro Magnetic Radiation Ranges

For a single wavelength, A is absorbance, a is the molar absorptivity of the compound in solution (M-1cm-1), b is the path length of the cuvette or sample holder (usually 1 cm), and c is that the concentration of the solution (M).

$$A = a\ b\ c$$

Where,
A = Absorbance,
a = absorptivity,
b = path length,
c = concentration

$$C = A / a\ b$$

To collect UV-Visible spectra, three types of absorbance instruments are used:

1. Single beam spectrometer.
2. Double beam spectrometer.
3. Simultaneous spectrometer.

All these instruments have a Supply of light source (usually a deuterium or tungsten lamp), a sample holder and a detector, but some have a filter for selecting one wavelength at a time. The single beam instrument (Figure 3) contains a filter or a monochromator between the source and the sample to Investigate or analyze one wavelength at a time. The double beam instrument (Figure 4) has a single source and a monochromatic and then there is a splitter and a series of mirrors to get the beam to a reference sample and also the sample to be analyzed, this allows for more accurate monochromator between the sample and the source; instead, it has a diode array detector that allows the instrument to at the same time find absorbance in all wavelengths. The instrument is usually much faster and

more efficient, but all of these types of spectrometers work well (Figure5).

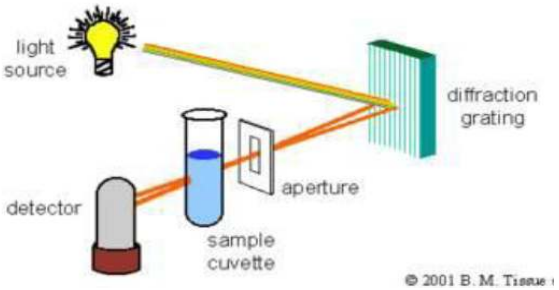

Fig 3: Illustration of Single Beam UV-Spectroscopy

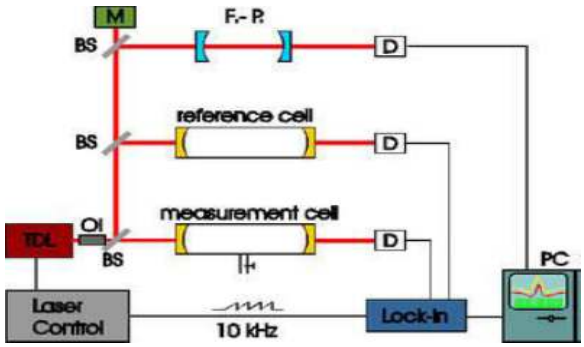

Fig 4: Illustration of Double Beam UV-Spectroscopy

2. Instrumentation

Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components.

- Source
- Monochromator
- Sample cell
- Detector
- Readout system
- Amplifier
- Display

Have a look at this schematic diagram of a double-beam UV-Vis spectrophotometer;

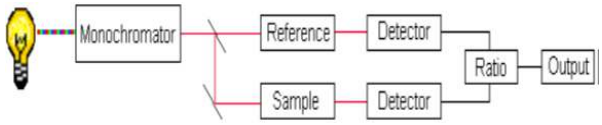

Fig 5: Schematic Diagram of UV-VIS Spectrophotometer

Sources of UV Radiation: The source of radiation should be continuous over its wavelength range. The electrical excitation of atomic number 1(Hydrogen isotope) 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 to 375 nm. Quartz cell must be used in these lamps, because glass absorbs UV radiation of wavelengths less than 350 nm.

Sources of visible radiation: The tungsten filament lamp is usually employed as a source of visible light. This type of lamp is employed within the wavelength range of 350 - 2500 nm. The energy emitted by a tungsten filament lamp is proportional to the fourthpower of the operatingvoltage. Electronic voltage regulators or constant-voltage transformers are used to ensure this stability.

The Monochromator or Wavelength selector:

Monochromator contain the following component parts;

- 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. This monochromator converts polychromatic light into monochromatic light. The beam iscollimated then strikes the dispersing component at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing component or the exit slit, radiation of only a particular wavelength leaves the Monochromator through the exit slit.

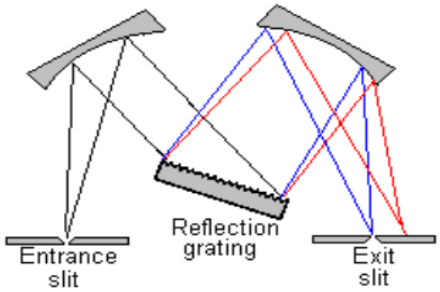

Fig 6: Grating Monochromator

Sample cell or Cuvette:

The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or amalgamated Silicon oxide cuvettes are needed for spectrometry within the actinic radiation region. These cells also are clear within the visible region. Silicate glasses may be used for the manufacture of cuvettes to be used between 350 and 2000 nm.

Detectors: A detector converts a lights signal into an electrical signal. Three types of detectors are usedBarrier layer cell

Photo tubes

Photo multiplier tubes:

Out of all Photo multiplier tubes are widely used.The photomultiplier tube could be an un remarkably used detector in UV-Vis spectrometry. It consists of a photograph emissive cathode (a cathode which emits electrons when struck by photons of radiation), several *dynodes* (which emit several electrons for each electron striking them) and an *anode*. A photon of radiation coming into the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the primary dynode (which is 90V a lot of positive than the cathode). The electrons strike the primary dynode, inflicting the emission of several electrons for each incident electron. These electrons are then accelerated

towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. Eventually, 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 terribly sensitive to UV and visual radiation. They have fast response times.

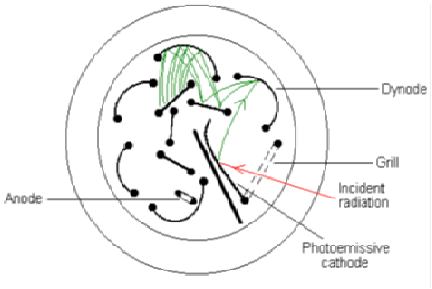

Fig 7: Photomultiplier tube

The linear photodiode array is an example of a *multichannel photon detector*. These detectors are capable of measuring all components of a beam of dispersed radiation at the same time. A linear photodiode array includes several little semiconducting material formed on a single silicon chip. Sensor elements on a chip, can be between 64 to 4096 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.

The use of the photodiode array is that it is positioned at the focal plane of the monochromator 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, like in an HPLC detector.

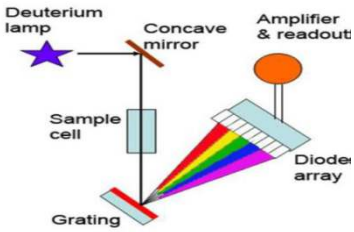

Fig 8: Photo diode array

Charge-Coupled Devices (CCDs) are similar to diode array detectors, but instead of diodes, they consist of an array of photo capacitors.

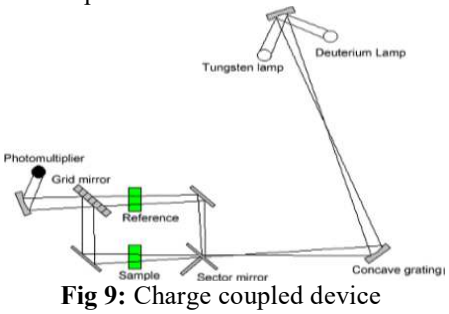

Fig 9: Charge coupled device

Chemical Origins:

When white light falls on a sample, Part of it is being absorbed and the rest is transmitted. The color of the sample is determined by the reflected light. If violet is absorbed, the sample appears yellow-green and if yellow is absorbed, the sample appears blue. The colors are described as complementary. A close relationship exists between the color of a substance and its electronic structure.

Table 1: Illustrates the relationship between light absorption and color

Color absorbed	Color absorbed	Absorbed Radiation(nm)
Violet	Yellow green	400-435
Blue	Yellow	435-480
Green blue	Orange	480-490
Blue green	Red	490-500
Green	Purple	500-560
Yellow green	Violet	560-580
Yellow	Blue	580-595
Orange	Green blue	595-605
Red	Blue green	605-750

Correlation of Molecular Structure and Spectra

Conjugation: π to π * transitions, when occurring in a molecule, give rise to absorptions of fairly low intensity. However, conjugation of unsaturated groups in a molecule produces a noteworthy result upon the absorption spectrum. The wavelength of maximum absorption moves to an extended wavelength and the absorption intensity might usually increase. Shift in λ_{max} towards longer wavelength is called Bathochromic shift or Red shift. Increase in the intensity of absorption is called hyper chromic effect. The same effect occurs with n to π electron group. In general, greater the length of a conjugated system in a molecule, the nearer the λ_{max} comes to the visible region. When such absorption happens, two types of groups can influence the resulting absorption spectrum of the molecule: chromophores and auxochromes.

Chromophores:

It is a region in the molecule where the energy difference between two separate molecular orbital falls within the range of the visible spectrum. Visible light that hits the chromophore can be absorbed by exciting an electron from its ground state to an excited state. Some of the important chromophores are: ethylene, acetylene, carbonyls, acids, esters and nitrile groups etc. A carbonyl group is an important chromophore, although the absorption of light by an isolated group does not give rise to any color in the ultra-violet spectroscopy.

Auxochrome:

Functional group with lone pair of electrons attached to a chromophorewhich modifies the ability of a chromophore to absorb light and intensity of wavelength. They themselves fail to produce color; but present along with the chromophores it intensifies the color. Examples include the hydroxyl group (−OH), the amino group ($-NH_2$), the aldehyde group (−CHO), and methyl

mercaptan group ($-SCH_3$).

Solvents:

The effect on absorption spectrum of a compound, when diluted in a solvent, will vary depending on the chemical structure. The interaction of non-polar solvents with non polar molecules shows least effect where as polar molecules exhibit quite dramatic differences when interacted with a polar solvent. The interaction between solute and solvent leads to absorption band broadening reduction ε max. For ionic forms care must be taken to avoid interaction between the solute and the solvent in acidic or basic conditions. Again care has to be taken to avoid interaction Where ever methodology needs buffering, solutions. Commercially available solvents are accompanied by their cut-off wavelengths, based on a 10mm path length. The commonly used solvents for absorption spectrometry are Water, 0.1N hydrochloric acid and sodium hydroxide.

Table 2: Commonly used solvents and their 'cut-off' wavelengths

Solvent	Cut – off (nm)
Methanol	210
Ethanol	205
Iso octane	202
chloroform	245
Benzene	280
Carbon tetrachloride	265

Applications of ultraviolet/visible spectroscopy: UV-Vis spectroscopy has many different applications

- It is used extensively in assaying rather than identification.
- Manganese in steel can be determined by firstly reacting the sample to get the metal into solution as a Manganate ion.
- The most useful piece of information from spectrum is the absorbance because if the absorption coefficient of the chromophore is known we can calculate the concentration of the solution, and mass of the metal in the sample.
- For drug metabolites, Samples are taken from various sites of the body and their solutions are analyzed to determine the amount of drug reaching the body.
- UV-Vis spectroscopy is used to calculate very small concentrations (of the order 0.0001 mol dm-3) with extreme accuracy.
- Impurities detection.
- Structural elucidation of organic compounds and Dissociation constant of acids and bases

3. Conclusion

UV-Visible Spectroscopy is more selective, efficient, fast and reproducible analytical method. There are two major measurement techniques; how much analyte is in the sample (quantitative analysis) and which analyte is in the sample (qualitative analysis).It is necessary to determine the "identity, strength, quality and purity" of compounds.

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