UV-Spectroscopy- A Comprehensive Review

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Abstract- *One of the earliest instrumental techniques for analysis is UV-VIS spectroscopy. Many different types of materials can be characterized using UV-VIS spectroscopy. The UV-Vis delivers details based on the degree of absorption or transmittance of a varied wavelength of beam light and the various responses of samples. Radiant energy absorption by materials can be quantitatively described using the general law known as Beer's law. The UV-VIS spectrometer is simple to use and handle. Both qualitative and qualitative analyses can make use of it. The metal and metal oxide nanoparticles are typically characterized using wavelengths between 200 and 700nm. The intricate mechanism of complexation between templates, monomer, and cross-linker during polymerization can also be better understood with the aid of the UV / Vis spectrum. It is quick, simple, and affordable characterization and structure of materials can be examined using the spectrum. These conclusions have uses in academia, business, medical labs, and chemical examination of environmental samples.*

Keywords- UV- visible spectroscopy, optimization technique, electron transition, photomultiplier tube.

I. INTRODUCTION

Traditional practice has required analysts to quick analyses of complex samples, multicomponent formulations and bio-therapeutic products. Analytical methods can be characterized into two groups: classical methods and instrumental methods. An instrumental method refers to a procedure in which the signal is directly proportional to concentration of an analyte. Other techniques revealed by classical techniques include analyte separation, qualitative analysis, and quantitative analysis.[1,2] Spectroscopy and chromatography are the two main analytical techniques used widely to describe the strength, purity, and identify of the compound. When we compare the HPLC method with spectroscopy, spectroscopy is better of the nature of easy handling, less sample required, and economy.

SPECTROSCOPY:

Spectroscopy is the measurement and interpretation of EMR absorbed and emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. [1]

UV-VISIBLE SPECTROSCOPY:

Ultraviolet (UV) spectroscopy is a physical technique of optical spectroscopy that uses light in the UV, visible and near infrared ranges and it is based on Beer-Lambert's law, which states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length. The ultraviolet region ranges from 190nm-400nm and visible region ranges from 400nm-800nm. [1,2]

TYPES OF UV SPECTROSCOPIC ANALYTICAL TECHNIQUES:[3]

Following are the exclusive UV spectroscopic analytical techniques:

- Simultaneous equation technique.
- Difference spectrophotometry.
- Derivative spectrophotometry.
- Absorbance ratio spectra.
- Spinoff ratio spectra.
- Successive ratio-spinoff method.
- Q-absorbance ratio method.
- Absorptivity issue method.
- Twin wavelength approach.
- Absorption element approach.
- Multivariate chemometric method.
- Isosbestic point technique.

PRINCIPLE:

A molecule or ion will display re-tension in a noticeable or bright locale when radiation causes an electronic change inside its structure. Absorption of light by a sample changes the electronic state of a molecule in the UV or visible region. The energy provided by the light will force the electrons from their ground state orbitals to higher energy, energized state orbitals or anti-bonding orbitals. Possibly, three sorts of ground state orbitals might be involved:[4,5]

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

Also, two kinds of anti-bonding orbitals might be engaged with the progress:

1. σ^*(sigma star) orbital
2. π*(pi star) orbital

Excitation of bonding s electron to anti-bonding σ orbital referred as to σ to σ^* transition. Similarly, the transition of one electron of a lone pair (non-bonding electron pair) to an anti-bonding π orbital represents π to π*. On absorption of ultraviolet and visible light, following transition occurs:

1. σ to σ^*
2. n to σ^*
3. n to π*
4. π to π*

Both s to σ^* transitions require an excellent deal of energy and so occur in the far ultraviolet region or in a weakly region of 180-240nm. Consequently, saturated groups in the ordinary ultraviolet region do not exhibit strong absorption. Transitions of electrons from n to π* and π to π* in molecules with unsaturated centers, require less energy and occur at longer wavelengths. It will be seen that the wavelength of the maximum absorption and the intensity of absorption are determined by molecular structure. Transitions to π* anti-bonding orbitals which occur in the ultraviolet region for a particular molecule may take place in the visible region if the molecular structure is modified. Some inorganic compounds in solution also exhibit absorption in the visible region. Such compounds include incomplete inner electron shells (mainly transition metals). The ions of transition metals are complexed by hydration. Eg.$[Cu(H204)^{2+}]$. Electrons are transferred from one part of the system to another by absorbing visible light.[2]

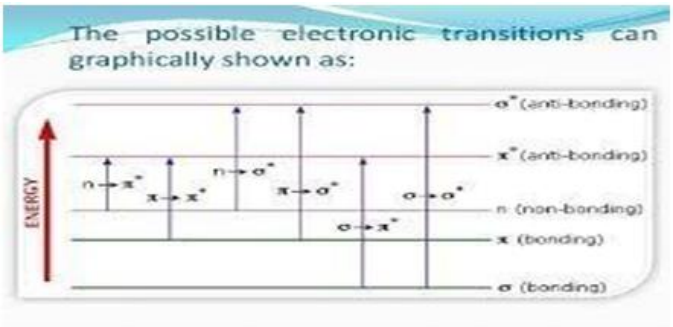

Figure-1:Electrontransitiongraphicallyrepresented.

ULTRAVIOLET ABSORPTION SPECTROPHOTOMETRY:[6,7,8,9,10,11]

Spectrophotometry is normally desired, in particular with the aid of using small-scale industries, because the price of the system is much lower and the renovation troubles are minimal. The evaluation technique is primarily based on measuring the absorption of a monochromatic light in the ultraviolet regionof the spectrum (200-400nm) by colourless compounds. The basic operational principle of a spectrophotometer disclosing the UV region consists of light of a specific wavelength passing through the sample cell with solvent and falling directly to the photoelectric cell, which transforms the radiant energy into electrical energy measured by a galvanometer. Ultraviolet-visible spectroscopy is employed to get the absorbance spectra of a compound in solution or as a solid form. Absorption of light energy or electromagnetic radiation by the compound or material, results in excitation of electrons from ground to the first singlet excited state. The energy and wavelength of electromagnetic radiation in the UV region is 1.5-6.2 EV and 800-200nm respectively. The fundamental principle of absorption spectroscopy is Beer-Lambert's Law.

A= abc

Where, A=absorbance

a= absorptivity
b= path length
c= concentration.

There are three types of absorbance instruments used to collect UV-visible spectra:

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

These instruments include a light source (typically a deuterium or tungsten light), a sample holder, and a detector, yet some have a filter for choosing one wavelength at a time. The single beam instrument consists of a monochromator or filter between the source and the sample, which analyse one wavelengthat a time (Fig-2). The double beam instrument has a single source and a monochromator, followed by a splitter and a series of mirrors to get the beam to a reference sample which is to be analysed, which permits more accurate results (Fig-3). The simultaneous instrument (Fig-4) consists of diode array detectors which allow it to detect the absorbance at all wavelength simultaneously. The simultaneous instrument is rapid and more efficient to handle.

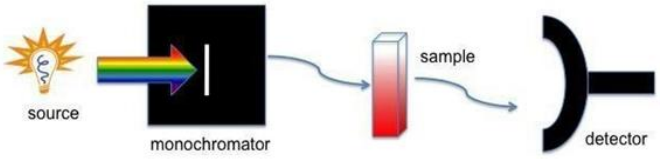

Figure2-IllustrationofasinglebeamUV-Visibleinstrument.

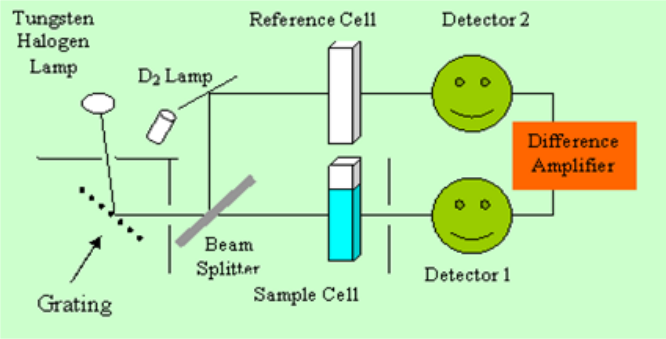

Figure3-IllustrationofadoublebeamUV-Visibleinstrument.

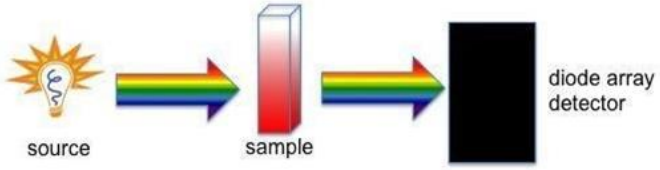

Figure4-IllustrationofasimultaneousUV-Visibleinstrument.

INSTRUMENTATION: [17]

The desired parameter in spectrophotometry is absorbance A, but it cannot be directly measured. In actual practice, values of the incident radiation power P_0, and the transmitted power P, or their ratio must be obtained. From these values and the relationship absorbance is determined.

$$A=\log \frac{P_0}{P}$$

Another type is a double beam spectrophotometer. The differences between single and double beam systems will be discussed at a later stage, here it is adequate to recognize that the components and their functions in the two designs are practically identical.

COMPONENTS:

1. Power supply
2. Source
3. Monochromators
4. Detectors
5. Sample cells
6. Recording system
   a) Amplifier
   b) Display

1.Power supply:

The power source performs the following function:

1. A transformer is used to reduce the line voltage to the instrument's operating level.
2. If the instrument needs direct current, it uses a rectifier to convert alternating current to it.
3. It eliminates ant ripple in the line voltage that might appear so that the source lamp and instrument receive a steady voltage.

2.Source:

The tungsten filament lamp is the most common source of visible radiation, being usable ain the 320-2500nm region, whereas the hydrogen lamp yields a continuous spectrum from 180to 375nm, the ultraviolet region. Deuterium lampsare more intense than those of hydrogen and cover the same wavelengths. This extra intensity may make it advantageous to substitute deuterium for hydrogen lamps. The lamps should be maintained at the proper power, adjusted and aligned with the optical path.

a) Radiation sources in ultraviolet spectrometers:

Generally, hydrogen or deuterium lamps are used as source of radiation in ultraviolet spectrometers. Sometimes xenon discharge lamps and mercury arc lamps are also used as source of radiation in ultraviolet spectrometer.

b) Hydrogen discharge lamps :

In such lamps hydrogen is stored under high pressure and electric discharge is passed through them. This excites hydrogen molecules which emit ultraviolet radiation. Since the pressure is high so, we get a continuous hydrogen spectrum. Such lamps are used in the range 3500-1100 A° (350-120 nm). These lamps are found to be robust and stable so these are widely used.

c) Deuterium lamps:

In such lamps deuterium (Heavy hydrogen) is used instead of ordinary hydrogen. The intensity of radiation emitted by such lamps is found to be 3-5 times more as compared to that in hydrogen lamps. These are more expensive than hydrogen lamps and are used when high degree of intensity is required.

d) Xenon discharge lamps :

In such lamps xenon is stored under pressure (10-30 atom). It contains two electrodes which are separated by a distance of 8mm. When a low voltage is applied, an electric arc is created between electrodes which produce ultraviolet light. The intensity of these ultraviolet radiations is much more than those of hydrogen lamps.

e) Mercury arc:

In this, high-pressure mercury vapours are stored, and mercury atoms are excited by an electric discharge. These radiations are considered as standard sources for ultraviolet experiment, but because of the presence of sharp lines or bands, this source is not suited for continuous spectral studies.

3.Monochromator :

The function of monochromators is to disperse the radiation according to wavelength. It has an entrance slit, a dispersing component, and an exit slit. By rotating the dispersing element, the nominal wavelength that passes through the exit slit can be changed. The dispersing element may be a prism or a grating, which are generally made of glass, quartz or fused silica. Glass has the highest resolving power but is not transparent to radiations between 200-300nm because of strong absorption by glass in this region. The use of quartz is wide in ultraviolet spectrometer because it is transparent throughout the entire ultraviolet range. Fused silica prisms are transparent in short wavelength region and are used it very intense radiation is to be produced.

A monochromator system consists of a minimum of two components.

1. A dispersion device, such as prism or grating, whose function is to spread out the various wavelengths of the incidence beam.
2. A series of slits, mirrors, and lenses whose function (among others) is to isolate specific wavelengths of the beam.

4.Detectors:

Following three types of detectors are commonly used:

a) Barrier layer cell
b) Photo cell
c) Photomultiplier tube

Photomultiplier tube:

This is generally used in ultraviolet spectrophotometers. A photomultiplier tube is an electron-multiplying amplifier. It consists of an evacuated tube containing one photo-cathode and 9-16 electrodes known as dynodes. Outer layer of each dynode is of Be-Cu, Cs-Sb or whatever comparative mater.

When incident radiations fall on the metal surface of a photo cathode, electrons get emitted which are attracted towards the first dynode. It results in emission of more electrons from the surface of first dynode. These are attracted by means of second dynode and electrons are emitted via second dynode. In this way, the process is repeated at all the dynodes until a shower of electrons reaches the collector. The number of electrons that reach the collector is a measure of the incident radiations on the detector. To get a steady signal dynodes are operated at optimum voltage. This is quite sensitive and gives an extremely fast response.

5.Sample cells:

These cells contain the sample. Commonly used cells are made up of quartz or fused silica. Such cells are generally available in matched pairs, so that one of these is used as a reference cell. The single beam spectrophotometer is a direct reading instrument but the double beam photometer is a null-type photometer. In the single beam system, it is necessary to obtain values of the incoming power P, and the transmitted power P.

6.Recording system:

Recording system receives signal from the photomultiplier tube and the recording is done by a recorder pen.

Tableno1:Illustratestherelationshipbetweenlightabsorption andradiation(nm).

ColourAbsorbed	ColourAbsorbed	AbsorbedRadiation(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

CHROMOPHORES :[18]

The presence of a certain functional group in many organic compounds causes them to absorb ultraviolet and visible radiation. Chromophores are the molecules that really absorb radiation. Some electronic transitions are predicted to

be statistically probable (said to be allowed, and these absorptions are powerful and typically have values in excess of 10,000), according to mathematical analyses of the energy levels of orbital systems. Other transitions have a probability of zero, are considered to be forbidden, and are not supposed to happen at all, but they frequently do, producing weak bands with values that infrequently go beyond 1000.

Depending on the substituents on the benzene ring, some particularly useful forbidden transitions occur:

d→d absorptions of transition metals;
n→π* absorptionofcarbonylgroupsatca280nm;

π→π*absorptionofaromaticcompoundsatca230–330nm.

AUXOCHROME: [18]

The color of a molecule generally absorbs poorly in the 200-800nm, but can be enhanced by groups called auxochromophores that affect the spectrum of the chromophore to which the molecule is attached. The main auxochromes are OH, NH2, CH3 and NO2, which can be acidic (phenolic) or basic in nature. The polarity of an auxochrome determines its actual impact on a chromohore. Groups like CH3- in general it is possible to predict the effects of nonpolar or weakly polar auxochromes, but it is difficult to predict the effects of highly polar auxochromes. In addition, the availability of non-bonding electrons that can undergo transitions also contributes significantly to the auxochrome effect. CH3CH2- and Cl have very small effects, usually small red-shifts of 5-10nm. Other groups such as –NH2 and –NO2 are very popular and completely alter the spectra of chromophores.

APPLICATIONS OF UV-VISIBLE SPECTROSCOPY: [13,14,15,16]

UV-visible spectroscopy has different application

1. Impurities detection
2. Structural elucidation of organic compounds
3. Qualitative analysis
4. Quantitative analysis
5. Chemical analysis
6. Dissociation constant of acids and bases
7. Quantitative analysis of pharmaceutical substance
8. Molecular weight determination
9. As HPLC detector
10. Deviations from the Beer-Lambert law.

ADVANTAGES OF UV-VISIBLE SPECTROMETERS:[19]

1. 1.The biggest advantage for chemists and astronomers who use UV-vis spectrometers is the accuracy of the device.
2. Simple and inexpensive instrumentation.
3. Most organic molecules absorb UV/Vis light.
4. In astronomy research, an UV/ Vis spectrophotometer helps the scientists to analyse the galaxies, neutron stars, and other celestial objects.
5. A UV spectrum can provide rich information of the velocity and the elements of an astronomical object.
6. In other industries, UV/Vis spectrophotometer also brought the high-tech spectral analysis possibilities.
7. General sense of food quality factors, such as colour, appearance, smell, taste, an UV/Vis spectrophotometer can further assist the food supplier an instrumentation ways of analysis by chemistry, biology.
8. Most of the ones used in chemistry are comparable in size to electron microscopes and require the same basic skills to use.
9. Because they are simple to operate, there is little chance of a UV-Vis spectrometer being used improperly.

DISADVANTAGES OF UV-VISIBLE SPECTROMETERS:[19]

1. 1.The main disadvantage of using a UV-Vis spectrometer is the time it takes to prepare to use one.
2. Because stray light reduces the linearity range and the absorbency of the substance it measures, it can affect the accuracy of spectra measurements made by UV-Vis spectrophotometers due to defective equipment design and other causes.
3. The filters are restricted to the visible region only.
4. They are not very good wavelength selectors.
5. They permitwide band width, hence they are not helpful for research. Therefore, there are more opportunities for Lambert Beer's law to be violated.
6. Additionally, the spectrometer's electrical circuit layout and detector circuit quality will influence how much noise is coupled into the measurement signal, decreasing measurement precision and lowering the instrument's sensitivity.
7. The region must be cleared of any visible light, electronic noise, or other external pollutants that could interfere with the spectrometer's reading.
8. If the space has been properly prepared ahead of time, UV-Vis spectrometers are simple to use and

give accurate results. However, if the area hasn't been adequately prepared, even a tiny amount of outside light or vibration from a tiny electronic equipment could obstruct the results of UV-Vis spectrometer.

9. Mixtures of molecules can be a problem due to overlap.
10. Spectra are not highly specific for particular molecules.

II. CONCLUSION

There are several biopharmaceutical products, complicated matrix samples, and multicomponent formulations, available on the market. However, UV spectrophotometric approaches for drug determination are simpler, quicker, chapter, and more accurate than other analytical techniques like chromatography and electrophoresis. Depending on the type of analysis, a specific approach can be selected from among the UV spectroscopic techniques. In comparison to other well-developed instruments like HPLC, UV-visible spectroscopy has more advantages in terms of robustness, ease of trouble shooting, and physiochemical interferences. Thus, the ideal method for an analyst to analyser the pharmaceutical industry is UV spectrophotometry.

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