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Brief Concept on UV-Visible Spectroscopy

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Abstract**—***A variety of multi-component formulations, bio-therapeutic medicines, and complicated matrix samples must be analyzed, therefore rapid and easy methods are needed. Several Ultraviolet (UV) spectrophotometric techniques are employed for this purpose. The notion of additivity, the concept of absorbance distinction, and process absorption spectra were the foundations upon which many forms of actinic radiation qualitative analysis methodologies were built. Information on coincidental equation methodology, distinction spectrophotometry, byproduct spectrophotometry, absorbance quantitative relation spectra, by-product quantitative relation spectra, successive quantitative relation by-product spectra, Q-absorbance quantitative relation methodology, absorption factor |physical property factor methodology, twin wavelength methodology, absorptivity methodology, and multivariate chemometrics are all part of this project's objectives. Here is a brief summary of the ideas, mathematical foundation, and a few uses for these square measure procedures*.

Keywords**—** *Spectrophotometry, Absorbance, Spectra*.

I. INTRODUCTION

Ultraviolet visible spectroscopy is often used in order to provide characterization data for a variety of parameters. Materials may be organic or organic, solid or liquid groups, such as organic molecules and functional groups. observed using UV-Visible spectroscopy, as can reflectance measurements for coatings, paints, textiles, Bioassay, separation kinetics, band gap measurements, etc.
Depending on the level of absorption or transmittance of a different wavelength of beam light and the various responses of samples.

Fig- UV- Visible Spectroscopy

II. PRINCIPLE

Beer-Lambert Law: According to the Beer-Lambert law, a solution's absorbance (A) is inversely related to the path length (b) and the concentration of the absorbing species (c) in the solution.
Molar absorptivity constant x length x concentration equals absorbance (A).

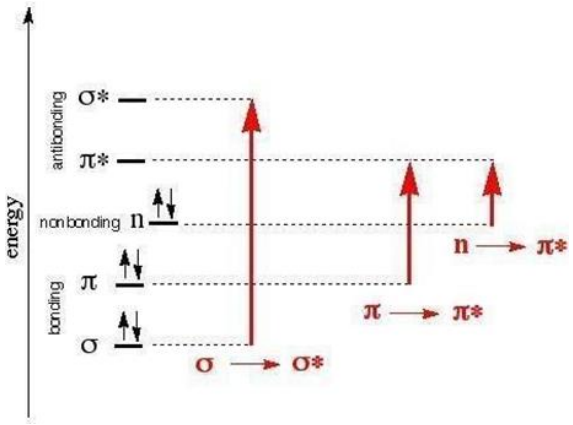
$$A=ab \quad C=A/ab$$

Where,
A = absorbance
Abs stands for molar absorptivity.B = Path length and
C = Concentration

III. ELECTRONIC TRANSITION

When a molecule or ion is exposed to ultraviolet or visible light, it undergoes an electronic transition in its structure. When a sample absorbs light in an ultraviolet or visible region, the electrons in the sample undergo a change in their electronic state. The energy provided by the light causes the electrons to move from their ground state orbital to higher energy orbital, excited state orbital, or anti bonding orbital.

When ultraviolet or visible light is absorbed by a molecule or ion, it undergoes the following electronic transitions:



In organic compounds, the majority of absorption spectroscopy involves base transitions of an electron in a bonding orbital to the corresponding anti-bonding orbital, which requires a large amount of energy. For instance, methane, which only has C-H bonds and can only undergo these transitions, has an absorbance maximum of 125 nm, while the absorption maxima due to these transitions are not visible in the typical UV-Vis spectra (200-700 nm). In organic compounds, the majority of absorption spectroscopy involves base transitions of an electron in a bonding orbital to the corresponding anti-bonding orbital, which requires a large amount of energy. For instance, methane, which only has C-H bonds and can only undergo these transitions, has an absorbance maximum of 125 nm, while the absorption maxima due to these transitions are not visible in the typical UV-Vis spectra (200-700 nm). Additionally, saturated compounds containing lone pairs of electrons are capable of n-to- pairs, which usually require less energy than the transition

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to the excited state. These transitions are initiated by light with a wavelength of 150-250 nm, and require an unsaturated group within the molecule to supply the electrons. The molar absorbance from one transition to the next is pretty low, usually ranging from 10 to 100 L mol-1 cms^{-1}. The transition from one to the other usually gives a molar absorbency between 1000 and 10000 L mol-1 cms^{-1}.[2]

IV. About Ultra Voilet rays-

When you project a white light into a prism or slit, it will scatter into spectrum. The lower frequency red light has less energy at one end of spectrum. The higher frequency purple light has richer energy at the other end of spectrum. From this side, it's the region of invisible electromagnetic radiation .In addition to ultraviolet, there are other high energy invisible lights as well. UV radiation can harm your skin when you're exposed to sun light. The International Organization for Standardization (ISO) defines ultraviolet as follows:

NUV, MUV, FUV, EUV.

Based on the wavelength (energy), we can divide the ultraviolet into 4 segments:

Ultra violet Segment	Wavelength Region(10nm- 400nm)
Extreme Ultraviolet **[EUV]**	10nm-121 nm
Far Ultraviolet **[FUV]**	122 nm-200 nm
Middle Ultraviolet **[MUV]**	200 nm-300 nm
Near Ultraviolet **[NUV]**	300 nm-400 nm

Note: The wave length of visible light region is 380nm– 760nm

V. According to BEER'S LAMBERTS LAW

Spectrophotometers that measure ultraviolet (UV) and non- irradiated (NIR) light can be usedto measure the amount of light that passes through a sample. The intensity of the incident light, I0, is converted into the intensity of the transmitted light, I, by dividing,

$$T = I/I0.$$

The energy difference between the two samples is associated with an optical phenomenon, which is determined by the Max Planck equation.

$$E=h\nu=hc/\lambda$$

Where,
E represents the energy required to reach the excited state,
H is Planck's constant,
V is the wave number,
C is the velocity of light, and
λ is the wavelength.

The Planck's equation indicates that the longer an optical phenomenon's wavelength is, the more energy is required to attract its electrons. Absorption bands are a reflection of the molecular structure of the sample and can vary in wavelength and concentration depending on molecular interactions and ambient conditions. These bands are typically broad and flat due to the numerous molecular wave levels that are associated with electronic energy levels. The relationship between transmission and absorption is further explained by the following equation.

$$Abs = 2\text{-}\log$$

$$I/I0 \cdot 100 = 2\text{-}\log (\%T)$$

Absorbance measurements are often based on the Beer-Lambert Law, which outlines the attenuation of light-weight supported by the materials through which it passes. This attenuation is directly related to the concentration of the unknown sample, and the transmission, or absorbance, of the sample is inversely related to c, the molar physical properties of the sample, and the length of the cuvette path, l, of the sample.

$$I = I0e\text{-}\varepsilon cl$$

Taking the power on each side and reworking the formula,

$$-\log I/I0 = \varepsilon cl$$

If the left aspect -log I/I0 is outlined because absorbanceThen

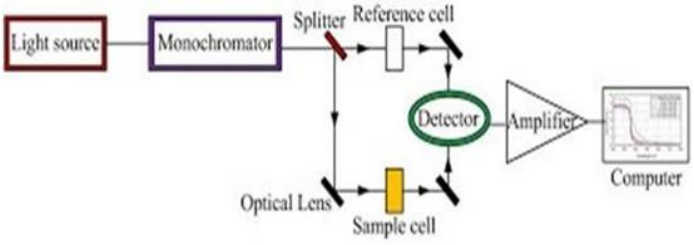
$$A= \varepsilon cl$$

The amount of sunlight that passes through a sample is based on the number of molecules it interacts with. If the sample is more concentrated, it has more molecules, and the absorbance is higher. The longer the gap is, the longer the cells' path length, and the more molecules interact, the higher the absorbance. To compare the absorbances of two solutions, you need to have a constant variable for normalization. You can also use the cell's absorbance to figureout the concentration of the sample. In order to figure out the concentration or path length of a sample, you need to have a constant variable. That constant is the molar property,which is the probability that an electronic transition will happen. Different molecules have different molar transitions with different strengths, so the molar property can change depending on which transition you're looking at, and it's wavelength-dependent.

VI. Instrumentation-

The Essential components of UV-Visible Spectrophotometerare follows:

- Sources (UV and visible light Source)
- Monochromator-
  - *Entrance slit.
  - *Collimator.
  - *Prism/grating.
  - *Collimator.
  - *Exit Slit.
- Sample containers (Cuvette)
- Detector.
- Amplifier and recorder.



1. Source- U.V. Spectroscopy necessitates the use of a

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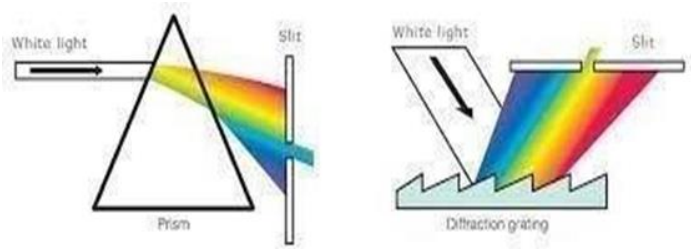
continuous source of radiation over a wide range of wavelengths. The following radiation sources are commonly used in U.V. spectro-photometry: hydrogen lamps, deuterium lamps, xenon lamps, and tungsten lamps. Hydrogen lamps are stable, robust, and emit radiation in wavelengths ranging from 160-380 nm, as they consist of hydrogen gas at high pressure through which electrical discharge is induced, resulting in the emission of radiation. Deuterium lamps are often used as U.V. sources and emit radiation in ranges from 160-450 nm, although they are more expensive than hydrogen lamps. Tungsten lamps are the most commonly used light source in spectrophotometry, and composed of tungsten filament in a glass envelope. Xenon lamps are discharge lights with a xenon glass- sealed bulb, with wavelengths ranging from 250-600 nm.

2. *Monochromator*- It is used for the change into Polychromatic light into Monochromatic light. Monochromatic light is produced by a mono chromator, which utilizes a collimated beam to remove unwanted wave lengths from radiation source light. The beam is then split into component wavelengths by a grating or prism, and radiation of a specified wavelength is then emitted through the exit slit when the dispersing element is moved or the exit slit is opened.

Types of monochromator:
Prism Monochromator
Grating Monochromator



3. *Sample Containers [Cuvette]*- A Cuvette is a sample container that is designed to be transparent to all wavelengths of light and is used for spectroscopic measurement. It is constructed of Quartz, with a square shape and a pathlength of 1 cm, and is suitable for wavelengths between 190 and 200 nm.
4. *Detectors*- Light energy is converted into electrical signals by the detector, which are shown on the readout device. The radiation is reflected off the detector and the intensity of the radiation absorbed by the sample is determined.

Type of detectors used in the instrumentation of absorption spectrophotometer:
Barrier layer cell / Photo voltaic cell. Photo tubes / Photo emissive tube.
Photo multiplier tube [4].
Silicon Photo diode/Photo sensor.

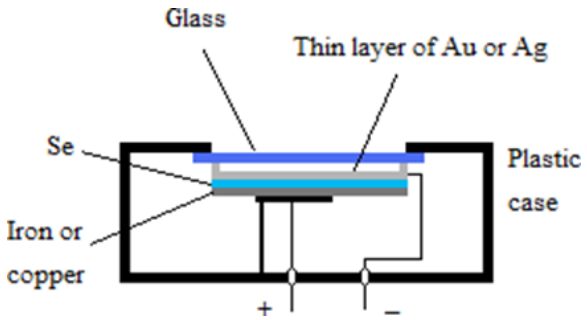


Fig- Barrier layer cell.

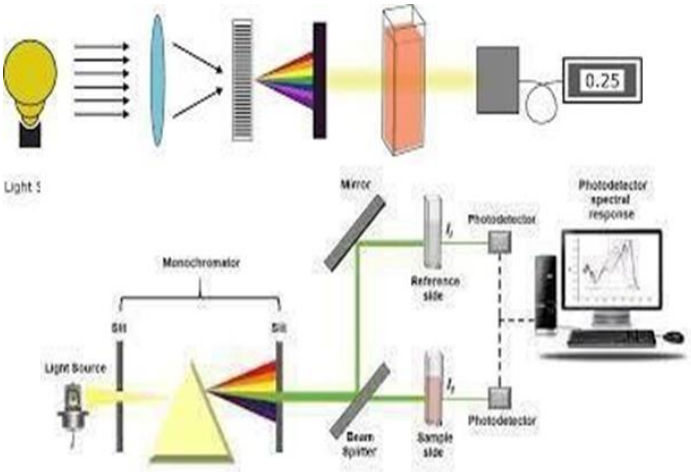
Types of UV-Visible Spectrometer-

The two types of absorbance instruments used to collect U.V.-Visible spectra are:

1. Single beam spectrometer.
2. Double beam spectrometer.

All of these instruments are equipped with a light source (typically a deuterium/tungsten lamp), a sample holder and detector. Some instruments have a filter to select a single wavelength at a time, while others have a mono chromator or filter between the source and sample to analyse a single wave length.

On the other hand, a double beam instrument has a single source and mono-chromator and then asplitter and series of mirrors to transfer the beam to the reference sample and to analyse the sample. This enables a more precise Mono-Chromator to be used between the sample and source; instead, the diode array detector allows the instrument to detect absorption at all wavelengths simultaneously. Simultaneous instruments are typically much more efficient and faster.



Applications-

- Identification of impurities.

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- Structural lucidity of organic compound.
- Detection of conjugation.
- Detection of functional group.
- Detection of geometrical isomer.
- Molecular weight determination.
- Difference of Cis – trans isomerism.[4]

Limitations-

- The absorption reported may be affected by scattering and reflection.
- Reaction with solvent.
- High concentration of the solvent affects charge distribution. The average distance between ions decreases, bringing particles closer together.
- The presence of stray light.

Calibration

Control of absorbance (measures of the quantity absorbed bythe system)

- ❖ Control of wavelength (measurement of lengthof the wave)
- ❖ Limit of stray light Resolution power(1stdeviation)
- ❖ Resolution power (2nddeviation)
- ❖ % transmittance cell[6]

VII. CONTROL OF ABSORBANCE

- Triturate and dry a quantity of potassium dichromate (AR Grade) byheating to constant wt. at 130° C.
- Preparation of 0.005M Sulphuric acid (H2SO4), dilute 0.3ml of H2SO4 to 1000ml withpurified water.
- Stock solution of K2Cr2O7 and then accurately weigh a quantity of about 60mg in to a100ml volumetric flask.
- Add 0.005M H2SO4 to dissolve and dilute up to the mark. Use this solution formeasurement of wavelength 430 nm.
- Determine the absorbance using 0.005M H2SO4 as a blank.[5]
- Calculate the value (1%,1 cm) as per the following formula-: Example

For 430 nm (1%,1cm) =Absorbance ×100 × 10 ÷ wt. of $K_2Cr_2O_7$ in mg

Check the value of A(1%,1cm) at each wave length againstthe acceptance criteria–

Wavelength	Acceptance criteria
235	122.9-126.2
257	142.8-146.8
313	47.0-50.3
369	105.6-109.0
430	15.7-16.1

Fig- Table for acceptance criteria

Control of wavelength

4 % w/v Holmium perchlorate solution in 1.4 percholic acid. Scan the solution using1.4 M percholic acid as a blank using the followinginstrument parameter and take the print.

Instrument parameter: -set the parameter as follows,

Start Wavelength	600nm
End Wavelength	200nm
Measurement Mode	Absolute
Scan Speed	Fast

Note down the maxima observed in the calibration sheet, Check the maxima observed against the acceptance and criteria given below–

Wavelength	Maximum Tolerance
241.15nm	240.15-241.15nm
287.15nm	286.15-289.15nm
361.50nm	360.50-362.50nm
486.00nm	483.00-489.00nm
536.30nm	533.30-539.30nm

Limit of Stray Light-

Measure the absorbance of 12% KCL solution in water between 195nm -220 nm using water as reference liquid and take the point. Absorbent should be greater than 2.0 at a wavelength between 198nm-202nm

Resolution Power [1st Derivation]-

Record the spectrum in the range 260nm - 275nm of 0.20% of v/v Toluene in hexane using, hexane in the reference cell and take the print.

Acceptance criteria- The ratio of the absorbance at the maximum at about 269nmto that at the minimum at about 226 nm is not less than 1.5.

Resolution Power [2nd Derivation]-

Scan and record the second deviation spectrum in the range 225 -275nm of 0.02% v/v toluene in methanol using methanol as the reference cell. After scan go to data processing click on derivative and put value 2 in order enter and again press then enter and take the print.

Acceptance criteria - A small negative extremum located between two large negative extreme at about 261.0nm and 268.0nm should be clearly visible the ratio A/B is not less than2.[6]

% Transmittance cell-Instrument parameter-

- Wave length program
- Wavelength {240,220,200nm}
- Number of cycle1
- Measurement mode %T

Link on auto zero with air blank in both the cell holder`s

i.e. (Bath with the empty cell holders).

Place on cell in the sample compartment with purified water using air blank as the reference and read the transmittance.

Next place the other cell in the sample compartment with purified water using air blank as reference and read the transmittance.

Record the transmittance at 200nm, 220nm, 240nm wavelength individually and take print.

Note: - Before using the new cell % of cell parameter to be performed and record shallbe maintained manually.

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Acceptance criteria- Transmittance for all the set of wavelengths should not less than 80%.

VIII. CONCLUSION

U.V. visible spectroscopy has a strong theoretical basis that allows for the development of more selective, effective, rapid and reproducible analytical techniques. Generally, there are two main measurement methods; quantitative (how many analytes are present in the sample) and qualitative (which analyte is present in the sample). An under-curve method states that the area under two points of the mixture spectrum is directly related to the concentration of a compound of interest. This is especially useful for compounds that do not have a sharp peak or broad spectrum. The pharmaceutical analysis performed by U.V visible spectroscopy includes the procedures needed to determine the identity, strength, quality, and purity of compounds.

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