



DEVELOPMENT, OPTIMIZATION AND VALIDATION OF UV VISIBAL SPECTROPHOTOMETRY – RECIVEW

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Article Received on 22/04/2020

Article Revised on 13/05/2020

Article Accepted on 02/06/2020

ABSTRACT

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near infrared ranges. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration. [UV-VIS Spectroscopy is the term used for the analytical evaluation of the different types of the solvents and substances. Spectroscopy is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of a spectrum (200-400nm). 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 a new compound for effective formulation development.

KEYWORDS: UV- VIS Spectroscopy, Optimization Techniques, Electrochemical methods.

INTRODUCTION

Spectroscopy: Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation [EMR] absorbed and emitted when the molecules or atoms or ions of a sample move from one energy states to another energy states.^[1]

UV-VIS Spectroscopy: Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near-infrared ranges and it is based on Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length. Thus, for a fixed path length, it can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration, UV-VIS spectroscopy has been in general use for the last 37 years and over this period its become the most important analytical instrument in the modern day laboratory. In many application, other techniques could be employed

but none rival UV-VIS spectroscopy for its simplicity, versatility, accuracy, speed, and cost-effectiveness.^[1,2]

Principle of Uv-Vis Spectroscopy: A molecule or ion will exhibit absorption in the visible or ultraviolet region when radiation causes an electronic transition within its structure. Thus, the absorption of light by a sample in the ultraviolet or visible region is accompanied by a change in the electronic state of the molecules in the sample. The energy supplied by the light will promote electrons from their ground state orbital to higher energy, excited state orbital or anti-bonding orbital. Potentially, three types of ground state orbitals may be involved.^[3,4]

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

In addition, two types of anti-bonding orbitals may be involved in the transition

- i) σ^* (sigma star) orbital.
- ii) π^* (pi star) orbital.

There is no such thing as an n^* anti-bonding orbital as the n electrons do not form bonds). Thus the following electronic transitions can occur by the absorption of ultraviolet and visible light.

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

Both σ to σ^* and n to σ^* transitions require a great deal of energy and therefore occur in the far ultraviolet region or weakly in the region 180-240nm. Consequently, saturated groups do not exhibit strong absorption in the ordinary ultraviolet region. Transitions from n to π^* and π to π^* type occur in molecules with unsaturated

centers, they require less energy and occur at longer wavelengths than transitions to σ^* anti-bonding orbital. It will be seen presently that the wavelength of maximum absorption and the intensity of absorption are determined by molecular structure. Transitions to π^* anti-bonding orbital which occurs in the ultraviolet region for a particular molecule may well take place in the visible region if the molecular structure is modified. Many 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. Such absorptions arise from a charge transfer process, where electrons are moved from one part of the system to another by the energy provided by the visible light.^[1]

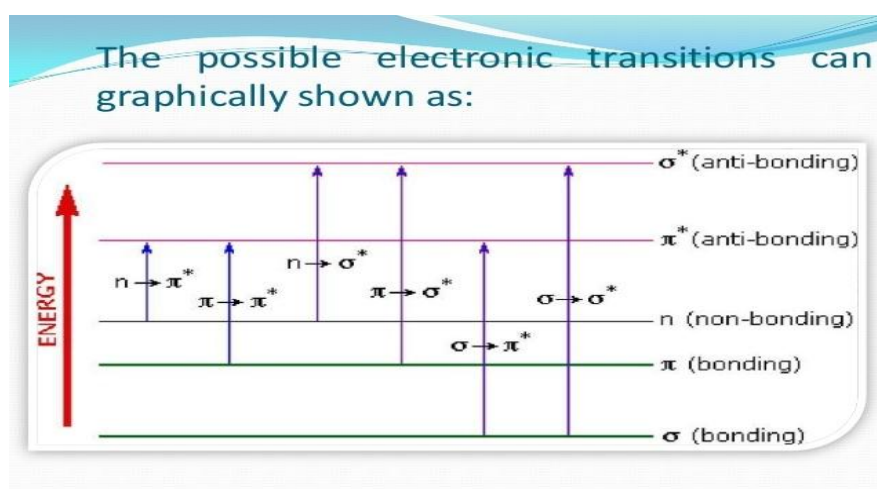


Fig 1: Electron Transition graphically represented.

Ultraviolet Absorption Spectrophotometry:^[5-8,13,14]

Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of the spectrum (200-400nm). The fundamental principle of operation of spectrophotometer covering UV region consists in that 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 spectroscopy is used to obtain the absorbance spectra of a compound in solution or as a solid. What is actually being observed spectroscopically is the absorbance of light energy or electromagnetic radiation, which excites electrons from the ground state to the first singlet excited state of the compound or material. The UV-visible region of energy for the electromagnetic spectrum covers 1.5 - 6.2 eV which relates to a wavelength range of 800 - 200 nm. The Beer-Lambert Law is the principle behind absorbance spectroscopy.

$$A = a bc$$

Where,

A = Absorbance,

a = absorptivity,

b = path length,

c = concentration.

There are two types of absorbance instruments used to collect UV-Visible spectra.

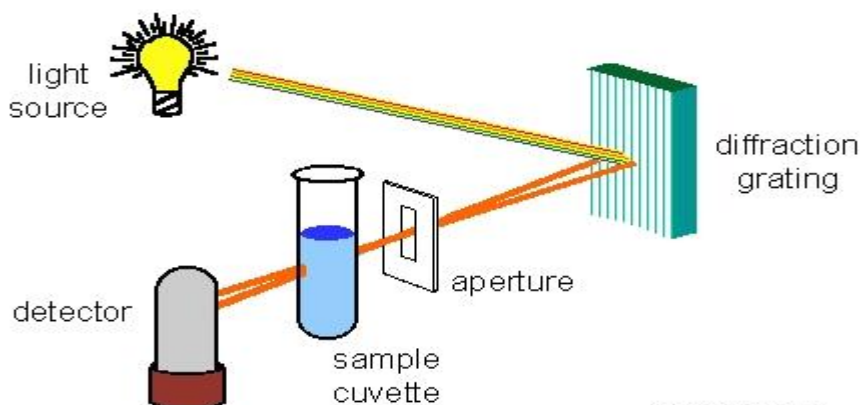
1. Single beam spectrometer

2. Double beam spectrometer

All of these instruments have a 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 2) has a filter or a monochromator between the source and the sample to analyze one wavelength at a time. The double beam instrument (Figure 3) has a single source and a Monochromator and then there is a splitter and a series of mirrors to get the beam to a reference sample and the sample to be analysed, this allows for more accurate Monochromator between the sample and

the source; instead, it has a diode array detector that allows the instrument to simultaneously detect the

absorbance at all wavelengths. The simultaneous instrument is usually much faster and more efficient.



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Fig 2: UV-Spectroscopy Single Beam.

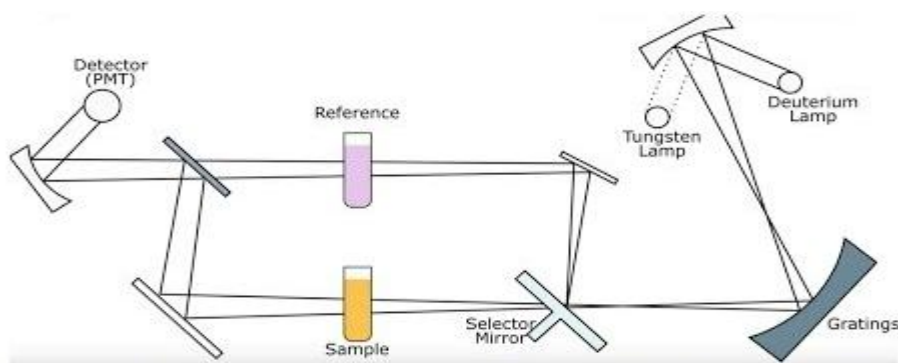
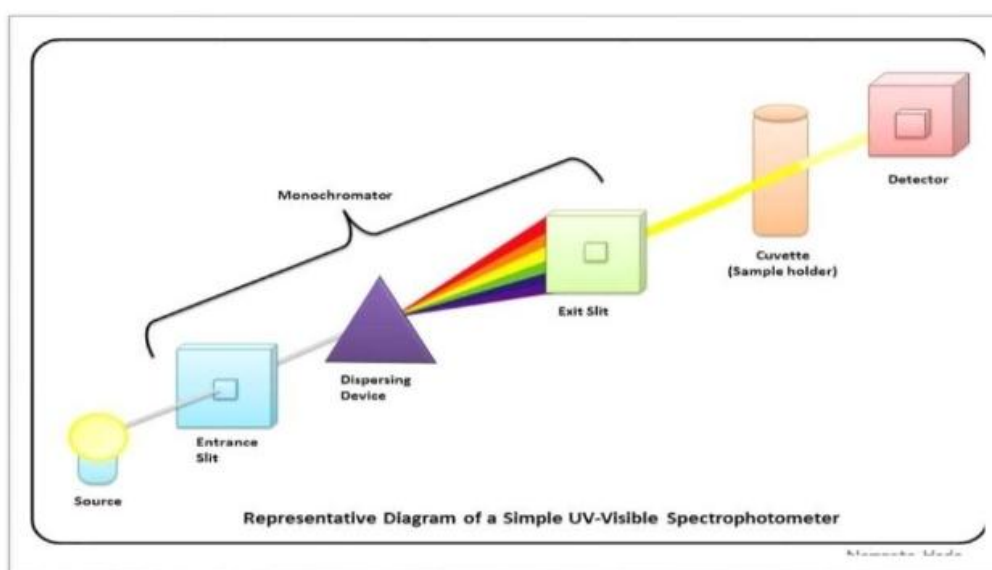


Fig. 3: UV-Spectroscopy Double Beam.

Instrumentation

At this schematic diagram of a double-beam UV-Visible Spectrophotometer.

Instrumentation



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Fig 4: Instrumentation of UV-Spectroscopy.

Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components.^[1,3,5,6,7,8]

1. Source
2. Monochromator
3. Sample cell
4. Detector
5. Readout system
 - a. Amplifier
 - b. Display

Sources

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.

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 because glass absorbs radiation of wavelengths less than 350 nm.

Sources of visible radiation: The tungsten filament lamp is commonly employed as a source of visible light.

This type 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. This means that for the energy output to be stable, the voltage to the lamp must be very stable indeed. Electronic voltage regulators or constant-voltage transformers are used to ensure this stability.

The Monochromator (Wavelength selector) All Monochromator contain the following component parts;

1. An entrance slit
2. A collimating lens
3. A dispersing device (usually a prism or a grating)
4. A focusing lens
5. An exit slit

Polychromatic radiation (radiation of more than one wavelength) enters the Monochromator through the entrance slit. The beam is collimated and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the Monochromator through the exit slit.

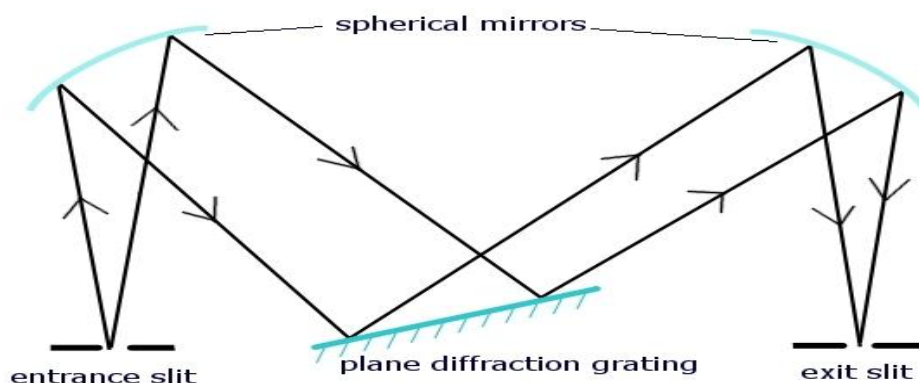


Fig. 5. Turner grating Monochromator.

Sample cell^[13]: The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.

Detectors: A detector converts a light's signal into an electrical signal. It should give a linear response over a wide range of low noise and high sensitivity.

1. Photomultiplier tube detector
2. Photodiode detector

1. The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy. It consists of a photoemissive cathode (a cathode which emits electrons when struck by photons of radiation), Anodes (which emit several electrons for each electron striking them). A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first Anode (which

is 90V more positive than the cathode). The electrons strike the first anode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second anode, to produce more electrons which are accelerated towards the anode. The electrons are collected at the anode. By this time,

each original photon has produced 10^6 - 10^7 electrons. The resulting current is amplified and measured. Photomultipliers are very sensitive to UV - visible radiation. They have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation.

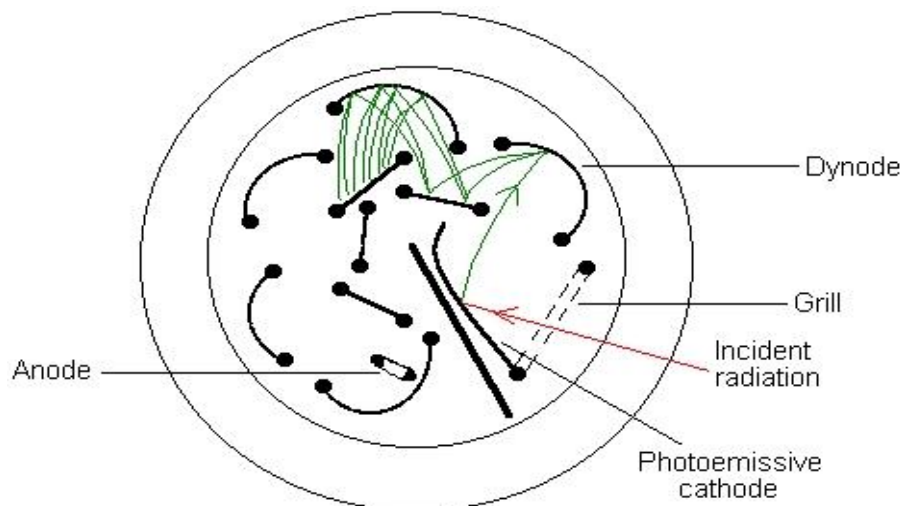


Fig. 6. Cross section of a photomultiplier tube.

2. The Photodiode detector: It is an example of a multichannel photon detector. These detectors are capable of measuring all elements of a beam of dispersed radiation simultaneously. A linear photodiode array comprises many small silicon photodiodes formed on a single silicon chip. There can be between 64 to 4096 sensor elements on a chip, 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. In use, the photodiode array is positioned at the focal plane of the monochromator (after the dispersing element) 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, such as in an HPLC detector.

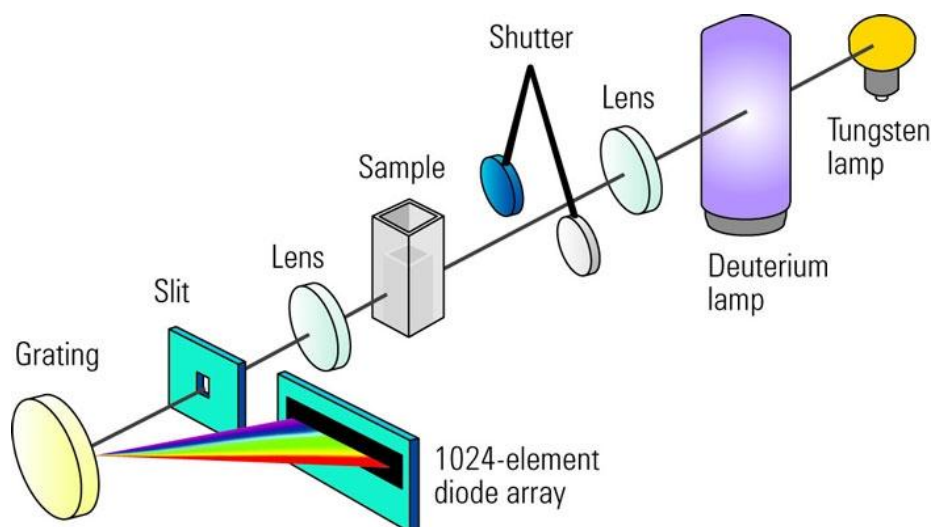


Fig 7: Photodiode array.

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

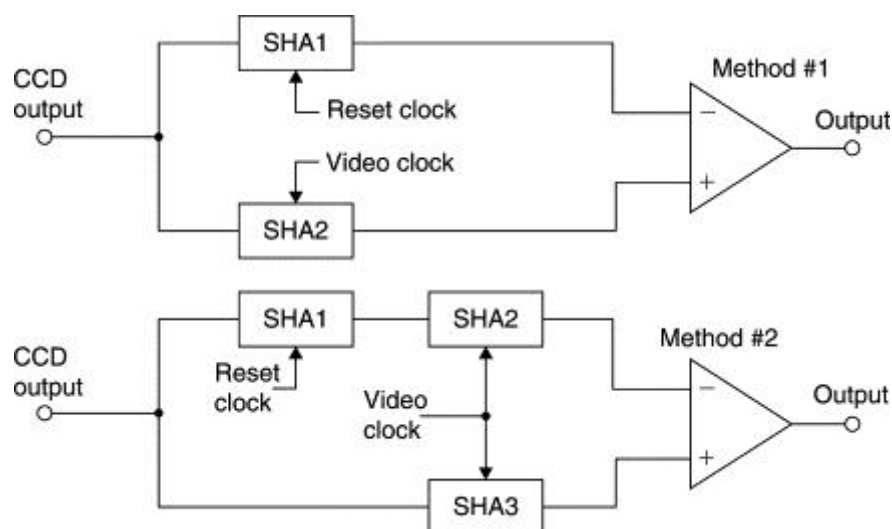


Fig 7: Charge Coupled Device.

Table. 1: Illustrates the relationship between light absorption and radiation (nm).

Colour Absorbed	Colour 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

Chromophores^[1,3,10] 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. Mathematical treatments of the energy levels of orbital systems suggest that some electronic transitions are statistically probable (said to be allowed, and these absorptions are strong and tend to have ϵ values in excess of 10 000). Other transitions have a probability of zero – they are not expected to occur at all – and are said to be forbidden but they frequently do occur, to give weak bands with ϵ values that rarely exceed 1 000. Some particularly useful forbidden transitions are- $d \rightarrow d$ absorptions of transition metals; the $n \rightarrow \pi^*$ absorption of carbonyl groups at ca 280 nm; and the $\pi \rightarrow \pi^*$ absorption of aromatic compounds at ca 230–330 nm, depending on the substituents on the benzene ring.

Auxochromes^[1,3,10] 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. The actual effect of an

auxochrome on a chromophore depends on the polarity of the Auxochromes, e.g. groups like CH₃-. In general, it should be possible to predict the effect of non-polar or weakly polar auxochromes, but the effect of strongly polar auxochromes is difficult to predict. In addition, the availability of non-bonding electrons which may enter into transitions also contributes greatly to the effect of an auxochrome. CH₃CH₂- and -Cl have a very little effect, usually a small red shift of 5-10nm. Other groups such as -NH₂ and -NO₂ are very popular and completely alter the spectra of chromophores.

Solvents^[1,3,5] The effect on the absorption spectrum of a compound when, diluted in a solvent, will vary depending on the chemical structures involved. Generally speaking, 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. Ionic forms may also be created in acidic or basic conditions. Thus care must be taken to avoid an interaction between the solute and the solvent. Commercially available solvents of 'spectroscopic purity' are accompanied by their cut-off wavelengths, based on a

10mm path length. Water and 0.1N solutions of hydrochloric acid and sodium hydroxide are commonly used solvents for absorption spectrometry. Again care has to be taken to avoid interaction. Where methodology requires buffering, solutions have to be non-absorbing and generally, both the composition and pH will be specified. However, if this information is not available lists can be found in the literature. For reactions in the 4.2 to 8.8 pH region, mixtures of 0.1N dihydrogen sodium phosphate and 0.1N hydrogen disodium phosphate are generally used.

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

Solvent	Cut-off (nm)
Is-octane	202
Ethyl alcohol	205
Cyclohexane	200
Acetone	325
Tetrachloroethylene	290
Benzene	280
Carbon tetrachloride	265
Chloroform	245
Ethyl ether	220
Isopropyl alcohol	210
Methyl alcohol	210

Applications of UV- Vis Spectroscopy^[9,10,11,12]

UV –vis spectroscopy has many different application

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

CONCLUSION

UV-Visible Spectroscopy is based on a firm theoretical basis, more selective, efficient, fast and reproducible analytical methods can be developed. In general terms, there are two major measurement techniques; how much analyte is in the sample (quantitative analysis) and which analyte is in the sample (qualitative analysis). An area under curve method is “the area under two points on the mixture spectra is directly proportional to the concentration of the compound of interest” particularly suitable for the compounds where there is no sharp peak or broad spectra are obtained. The pharmaceutical analysis by UV-Visible Spectroscopy comprises the procedures necessary to determine the “identity, strength, quality and purity” of compounds.

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