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A REVIEW ON UV- VISIBLE SPECTROSCOPY

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ABSTRACT

UV-Visible Spectroscopy is the earliest instrumentation techniques for analysis/ evaluation of different types of solvents and substances. In pharmaceutical sector UV-Visible spectroscopy is a fundamental technique for quantifying the concentration of active pharmaceutical ingredients (APIs) in formulations. It comprises the necessary to determine the identity, strength, quality, purity of compounds.

The method of analysis is based on measuring the absorption of monochromatic light by colourless compounds is near UV path of spectrum (200-400nm). Radiant energy absorption by materials can be quantitatively described using the general law 'Beers Law'.The application of Chemometrics in combination with UV spectroscopy for the assay of APIs, Impurities, adulteration issues and degradation products present in pharmaceutical dosage form. [1, 2]

KEYWORDS: *UV-Visible Spectroscopy, spectra, Chemometrics, APIs degradation products, Pharmaceutical dosage forms, Beers Law.*

INTRODUCTION

- **Spectroscopy**

Spectroscopy is a technique capable of identifying chemicals and performing quantitative analysis by utilizing the emissions or absorption spectra of different substances. It is the measurements and interpretation of Electro Magnetic Radiation (EMR) absorbed and emitted when molecule or atoms or ions of a sample move from one energy to other energy states.[3]

- **Types of Spectroscopy**

1) Atomic Spectroscopy

It is determination of elemental composition by its electromagnetic or mass spectroscopy.

- E= h v
- E= energy difference between two quantum levels.

V= frequency of photon which can result in electronic excitation.

2) Molecular Spectroscopy:

This deals with the interaction of electromagnetic radiation with molecules .

3) Microwave spectra: Spectra are shows by molecules which passes dipole moment (ex-HCL,NO ,etc.)
4) Vibrational-Rotational (IR spectra) :

These spectra occur in the spectral range of 500-4000cm-1 . These spectra arise due to transitions induced between the Vibrational energy levels of a molecule absorption of radiation belonging to IR region.

5) Raman Spectra: It observed in the visible region viz:12500-25000cm-1.Related to Vibrational-rotational transition in molecule but in different manner.
6) Electronic Spectra : Electronic spectra in visible region span 12500-25000cm.It arise due to electronic transitions in a molecular absorption of radiation falling in visible and ultraviolet regions.

UV -Visible Spectroscopy

It also known as UV-Visible spectroscopyoscopy. It has been in general use for last 37 year's and over this period it's become the most important analytical instrument in modern day laboratory. UV- Visible spectroscopy is widely utilized analytical technique in various fields, offering insights into molecular structure, concentration, electronic transition of compounds, particularly in both pharmaceutical and bio-allied sciences. [4, 5]

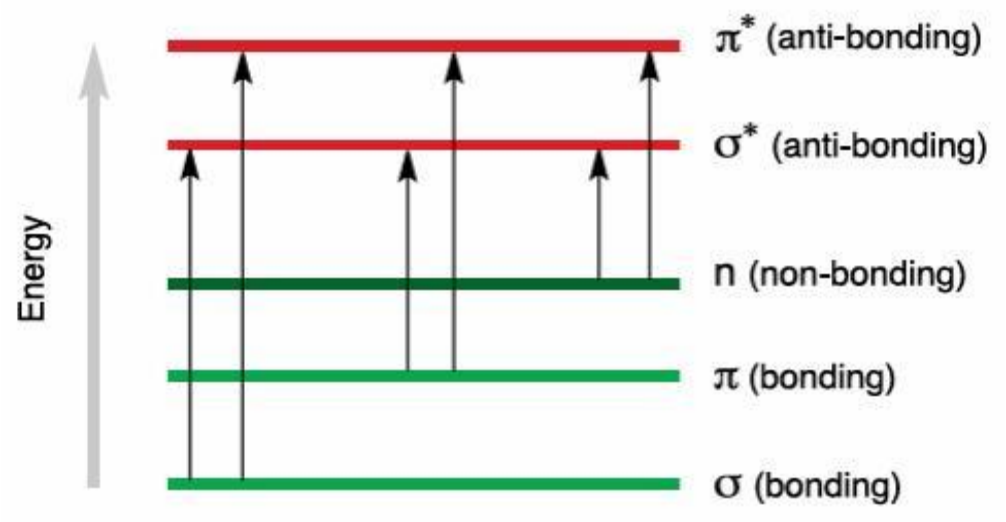
Principle Of UV-Visible Spectroscopy

UV absorption spectra arises from the transition of electrons within a molecules or an ion from a higher energy level and the UV emission spectra arise from the reverse type of transition. The UV radiation has sufficient energy to promote or excited the valence electrons in a molecule or an ion from ground state orbital to higher energy level, excited state orbital or anti bonding or orbital which can detected as absorptions.[6, 7]

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The Possible Electronic Transitions in UV and visible region are as follows:

1) σ→ σ* transitionπ
2) π → π* transition
3) n → σ* transition
4) n→ π* transition



(Fig. 1.Energy Required For Various Electronic Transition)

- σ → σ* transition
  σ electron from orbital is excited to corresponding anti-bonding orbital σ*.The energy required is large for this transition. e.g. Methane (CH4) has C-H bond only and can undergo σ → σ transition an
  n d shows absorbance maxima at 125 nm.

- π → π* transition
  π electron in a bonding orbital is excited to corresponding anti-bonding orbital π*. Compounds multiple bonds like alkenes, alkynes, carbonyl, nitriles, aromatic compounds, etc undergo π → π* transitions. e.g. Alkenes generally absorb in the region 170 to 205 nm.

- n → σ* transition
  Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of n → σ* transition. These transitions usually require less energy than σ → transitions. The number of organic functional groups with n → σ* . Peaks in UV region is small (150 – 250 nm).

- n→ π* transition
  An electron from non-bonding orbital is promoted to anti-bonding π* orbital. Compounds containing double bond involving hetero atoms (C=O, C≡N, N=O) undergo such transitions.
  n → π* transitions require minimum energy and show absorption at a longer wavelength around 300 nm. [8, 9]

Beer's law

Beer's law states that the absorbance 'A' of a substance in solution is directly proportional to the concentration of solution. When a beam of monochromatic radiation is passed through the absorbing medium, then the decrease in intensity of radiation is directly proportional to the concentration of solution.

A ∝ c

where, (a= absorbance; c= concentration).

❖ **Absorbance analysis can be classified into two different classes:**

I. Photometer
II. Spectrophotometer

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- **Photometer**

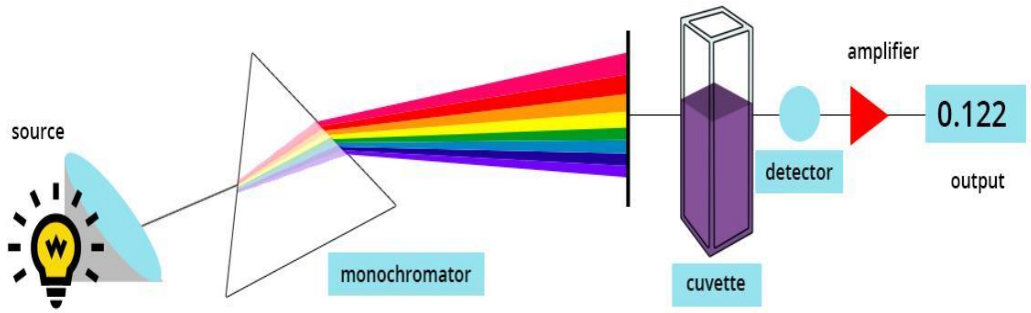
The photometer is a simple and relatively inexpensive tool for performing absorption analysis. In this instrument, filters are used to select wavelength. They can only detect a single wavelength at a time, have high throughout energy due to the simple optics and good signal to noise ratio.[10, 11]

Types of Photometer
a) Visible Photometer
b) Ultraviolet Photometer
c) Probe-Type Photometer

- **Spectrophotometer**

a) Single -Beam Instruments for the Ultraviolet-Visible Region:

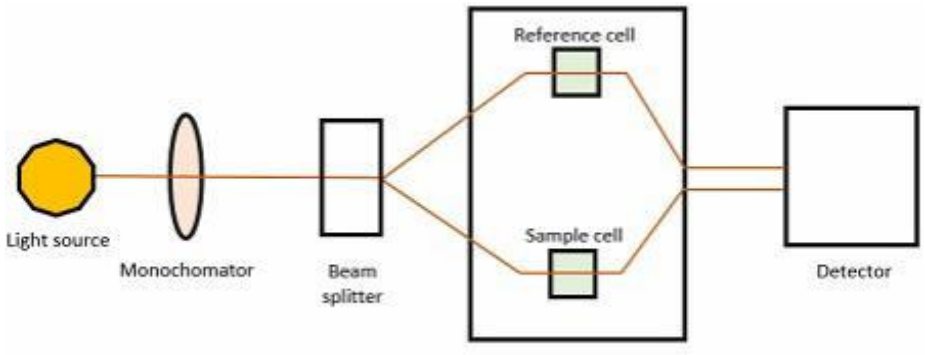
Single beam instrument can be used for both ultraviolet and visible measurements. The lower wavelength extremes for the instruments vary from 190-210 nm and upper from 800-1000 nm. It equipped with interchangeable tungsten and hydrogen Or deuterium lamps. Instrument employs Photomultiplier tubes or Photodiodes as detectors and gratings for dispersion. Digital readout meter is available in all UV -Visible spectrophotometer. [12, 13]



(Fig. 2. UV -Spectroscopy Single Beam)

b) Double Beam Instruments for the Ultraviolet-Visible Region

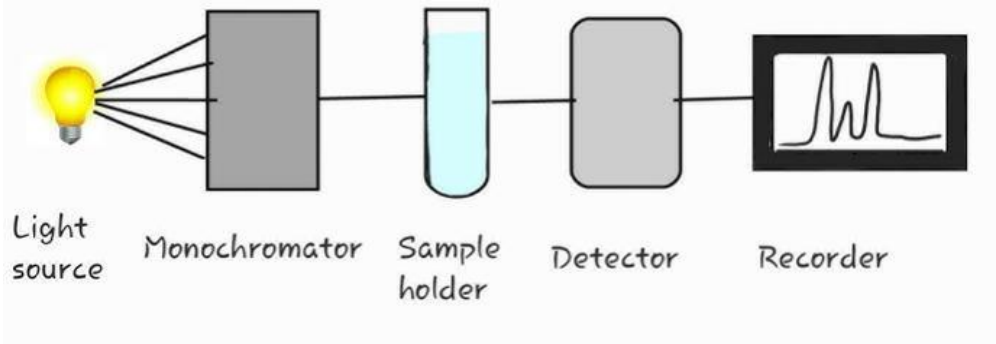
These instruments are more expensive as compared with single-beam spectrophotometer. Radiation from one of the sources passes through an entrance slit into the grating monochromator. After exiting the monochromator the radiation is split into two beams by chopper. The chopper contains a transparent segment and a mirrored segment. After passing through the cell, the beams are recombined by the second chopper and strike the photomultiplier tube at different times. [14,15]



(Fig. 3.UV -Spectroscopy Double Beam)

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Instrumentation



(Fig.4. Instrumentation of UV Spectroscopy)

Components of UV Visible Spectroscopy

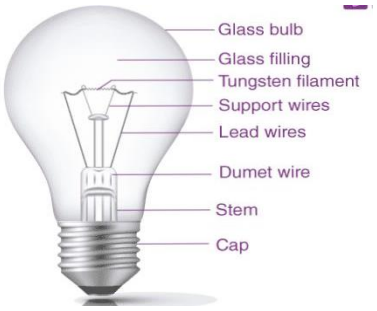
- Source
- Monochromator
- Sample cell
- Detector
- Read out system
    a) Amplifier
    b) Display
    c)

➢ **Sources**

A continuous source, or one that emits radiation at a variety Of wavelengths, is necessary for UV- Vis spectroscopy. It is important that the power of the radiation source does not change abruptly over its wavelength range.. The mechanism for this involves the formation of an excited molecular species, which breaks up to give two atomic species and an UV photon.
The following are many sources of UV radiation:

1. Tungsten filament lamp
2. Deuterium
3. Hydrogen lamp
4. Mercury arc lamp
5. Xenon discharge lamp

▪ **Tungsten Filament lamp**



(Fig.5. Tungsten Filament lamp)

It also known as Quartz Halogen Lamp .
The most typical light source utilized in spectrophotometers is the tungsten lamp. With a Wavelength range of roughly 330 to 900 nm, it comprises of a tungsten filament encased in a glass envelope and is utilized for the visible spectrum. [16, 17]

▪ **Hydrogen Lamp**

The light spectrum emitted by hydrogen lamps is constant, dependable, and ranges from 160 to 380 nm. It is made up of high-pressure hydrogen gas, which results in an electrical discharge. The hydrogen molecules that are excited create radiation.[19]

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- **Deuterium Lamp**

Its wavelength range is 190nm – 370nm.It also known as a D2 lamp. A typical deuterium lamp lifespan of about 1000 hours.[18]



(Fig.6. Deuterium Lamp)

- **Mercury Arc Lamp**

In mercury arc lamp, mercury vapour is stored under high pressure and excitation of mercury atoms is done by electric discharge.[20]

- **Xenon Discharge Lamp**

It possesses two tungsten electrodes separated by some distance. Radiation from xenon ranges from 250-460.

➢ **The Monochromator (Wavelength selector)**

All 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

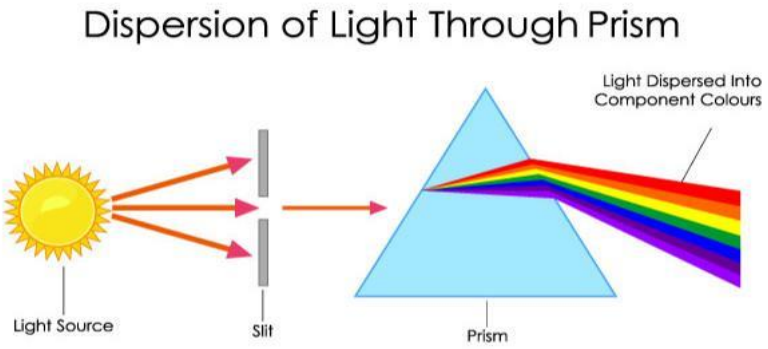
Polychromatic radiation (radiation of more than one wavelength) enters the Monochromator through the entrance slit. The beam is collimated and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.[21]

Types of Monochromators

1. Prism Monochromators
2. Grating Monochromators

1) Prism Monochromators

Prism is made from glass, Quartz or fused silica. Quartz or fused silica is the choice of material of UV spectrum. When white light is passed through glass prism, dispersion of Polychromatic light in rainbow occurs. Now by rotation of the prism different wavelengths of the spectrum can be made to pass through in exit slit on the sample. The effective wavelength depends on the dispersive power of prism material and the optical angle of the prism.



(Fig.7. Prism Monochromators)

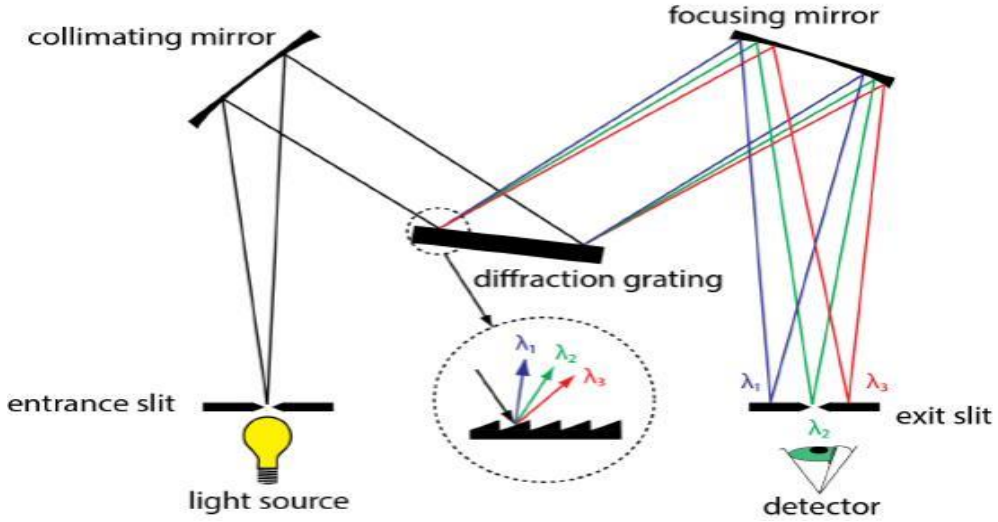
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2) Grating Monochromators

Are most effective one in converting a polychromatic light to monochromatic light . As a resolution of +/- 0.1 nm could be achieved by using ratings, they are commonly used in spectrophotometers.

Gratings are of two types.

- Diffraction grating.
- Transmission gratings.



(Fig.8. Grating Monochromators)

➢ **Sample Holders/Cuvettes**

• The cells or cuvettes are used for handling liquid samples.
•The cell may either be rectangular or cylindrical in nature.
• For study in UV region; the cells are prepared from quartz or fused silica whereas color corrected fused glass is used for visible region. The surfaces of absorption cells must be kept scrupulously clean.
• No fingerprints or a touch should be present on cells.
• Cleaning is carried out washing with distilled water or with dialcohol, acetone.

➢ **Detectors**

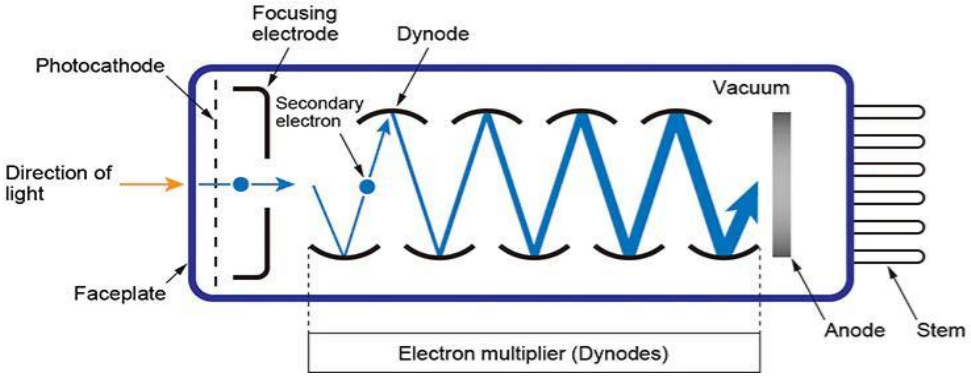
• Device which converts light energy into electrical signals, that are displayed on readout devices. The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample .
• The following types of detectors are employed in instrumentation of absorption spectrophotometer .[22]

1. Photomultiplier
2. Phototube /Emissive Cell Detector
3. Photodiode Array
4. Barrier Layer Cellar/Photovoltaic Cell

1) Photo Multiplier Tubes

The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons. In a vacuum tube, a primary photo-cathode is fixed which receives radiation from the sample. Some eight to ten dynodes are fixed each with increasing potential of75-100V higher than preceding one. Near the last dynode is fixed an anode or electron collector electrode. Photo-multiplier is extremely sensitive to light and is best suited where weaker or lower radiation is received.

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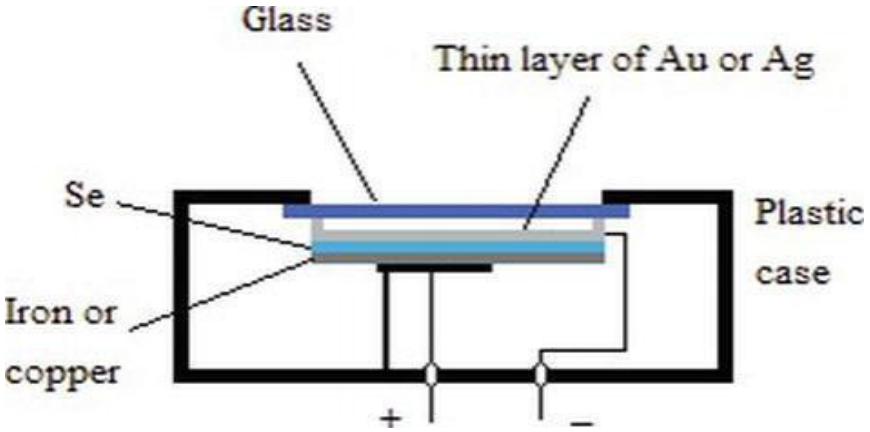


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(Fig.9. The Photomultiplier Tube)

2) Barrier Layer Cell / Photovoltaic Cell

It consists of a thin film metallic layer coated with silver or gold and acts as an electrode. It also has a metal base plate made up of iron which acts as an electrode. These two layers are separated by a semiconductor layer of selenium.

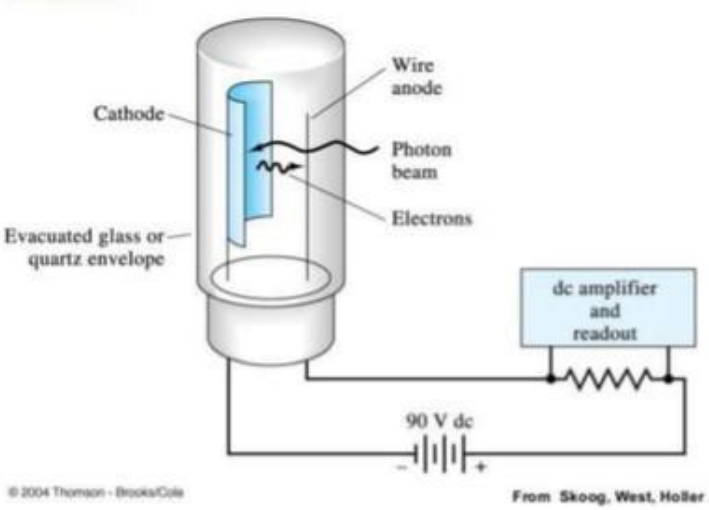


(Fig.10. Barrier Layer Cell / Photovoltaic Cell)

3) Phototubes/Photo Emissive Tubes

It consists of spherical shaped vacuum bulb containing photo emissive cathode and anode. The inner surface of cathode mounted inside bulb is coated with photosensitive material like cesium oxide, potassium oxide or silver oxide. It has anode to attract the electrons.

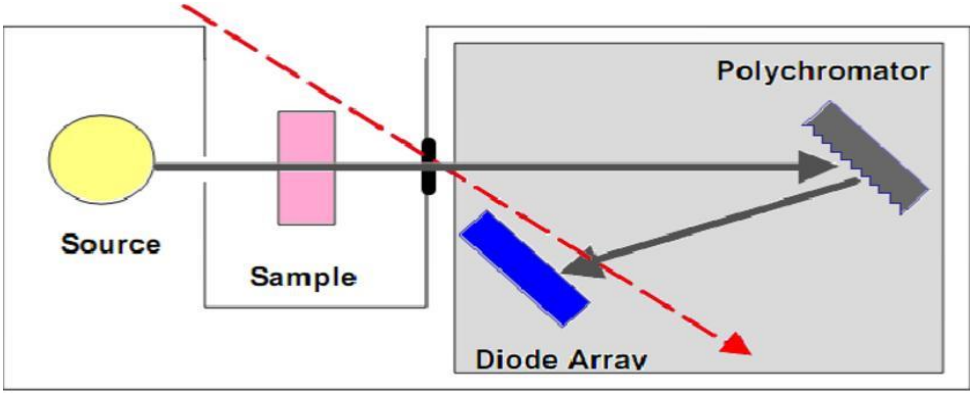
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(Fig.11. Phototubes/Photo Emissive Tubes)

4) Photodiode Array/Multichannel

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.12.Photodiode Array/Multichannel)

Applications Of UV Visible Spectroscopy [23]

1. Structure Elucidation of Organic Compounds.
   I. Effect of conjugation.
   II. Effect of geometrical isomerism.
   III. Effect of number of rigs.
   IV. Effect of substituents.

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

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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). 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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