

The Basic Principle of UV Spectroscopy:

UV spectrophotometer principle follows the Beer-Lambert Law. This law states that whenever a beam of monochromatic light is passed through a solution with an absorbing substance, the decreasing rate of the radiation intensity along with the thickness of the absorbing solution is actually proportional to the concentration of the solution and the incident radiation.

This law is expressed through this equation:

$$A = \log \frac{I_0}{I}$$

Basing from the Beer-Lambert law, it has been established that the greater the number of the molecules that are capable of absorbing light at a certain wavelength, the greater the extent of the absorption of light.

UV-Vis is often called a general technique because most molecules will absorb in the UV-Vis wavelength range. The UV extends from 100–400 nm and the visible spectrum from 400–700 nm. The 100–200 nm range is called the deep UV.

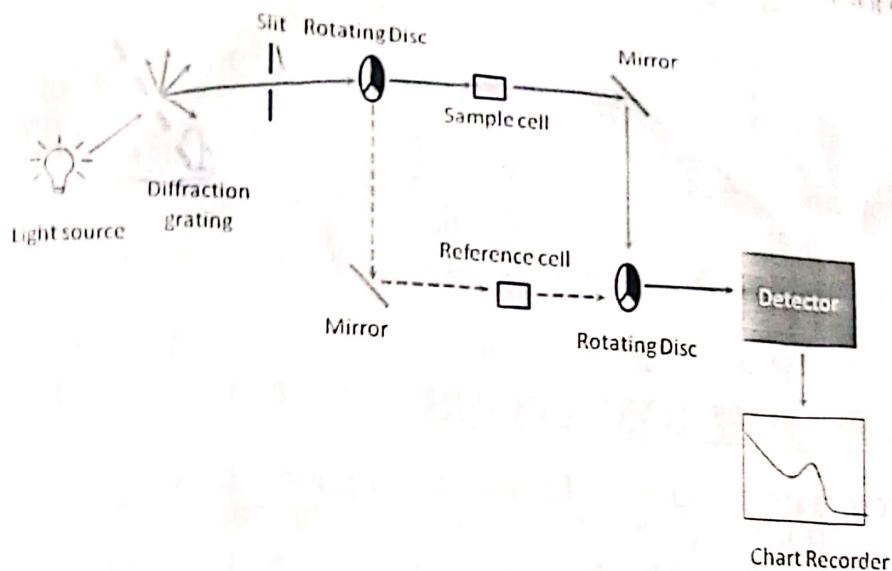
X-ray X-rays UV-visible Infrared microwave

INSTRUMENTATION

A spectrometer or spectrophotometer is an instrument that will resolve polychromatic radiation into different wavelengths and measure the light intensity at one or more wavelengths.

→ All spectrometers require:

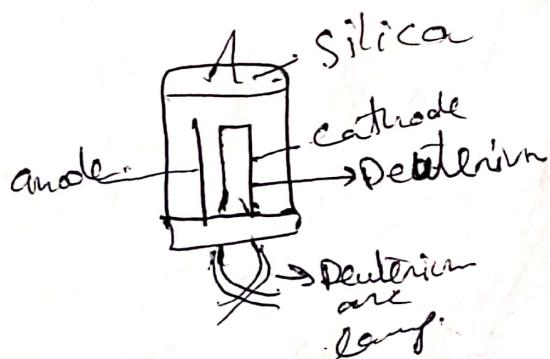
- 1) a source of continuous radiation over the wavelengths of interest,
- 2) a monochromator for dispersing the light into its component wavelengths and frequently, choosing a narrow band of wavelengths from the source spectrum,
- 3) a sample cell,
- 4) a detector, or transducer, for converting radiant energy into electrical energy, and
- 5) a device to read out the response of the detector.



I. SOURCES:

It is important that the power of the radiation source does not change abruptly over its wavelength range. The source should have a readily detectable output of radiation over the wavelength region for which the instrument is designed to operate. No source, however, has a constant spectral output.

- The most commonly used source for the visible region is a **Quartz tungsten–halogen (QTH) lamp**. The useful wavelength range is from about 325 or 350nm to 25 μm , so it can also be used in the near-ultraviolet and near-infrared regions. A stable, regulated power supply is required to power a spectrometer light source.
- LEDs are very power-efficient light sources. They provide approximately monochromatic light; a white LED is composed of a blue LED and a phosphor that emits primarily in the green but extends into red. The usable wavelength for a white LED is 425–700nm.
- For the ultraviolet region, a low-pressure **Deuterium discharge lamp** is generally used as the source. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon. This can be shown as;



UV-Vis SPECTROSCOPY

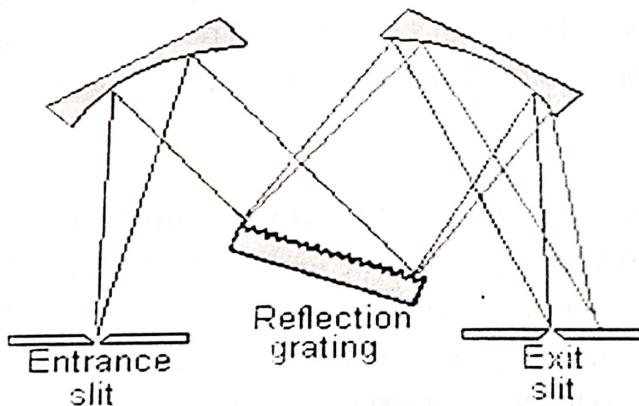
The deuterium continuum emission extends from 185 to 370nm but the lamp has useful spectral output out to 600nm. Ultraviolet sources must have a quartz window, because glass is not transparent to ultraviolet radiation.

II. MONOCHROMATORS

All monochromators 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.



A monochromator consists chiefly of a dispersing element to "separate" the wavelengths of the polychromatic radiation from the source. It additionally uses lenses or mirrors to focus the radiation, entrance and exit slits to reject unwanted radiation and help control the spectral purity of the radiation emitted from the monochromator.

There are mainly two types of dispersing elements, the prism and the diffraction grating. Various types of optical filters may also be used to select specific wavelengths.

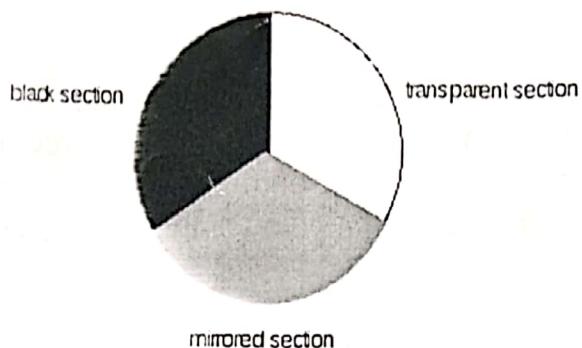
I. **Prisms:** When electromagnetic radiation passes through a prism, it is refracted because the index of refraction of the prism material is different from that of air. The index of refraction depends on the wavelength and, therefore, so does the degree of refraction. Shorter wavelengths are refracted more than longer wavelengths. The effect of refraction is to "spread" the radiation apart into different wavelengths. By rotation of the prism, different wavelengths of the spectrum can be made to pass through an exit slit and through the sample.

A prism works satisfactorily in the ultraviolet and visible regions and can also be used in the infrared region. However, because of its nonlinear dispersion, it works more effectively for the shorter wavelengths. Glass prisms and lenses can be used in the visible region, but quartz or fused silica must be used in the ultraviolet region. The latter can also be used in the visible region.

II. **Diffraction Gratings:** These consist of a large number of parallel lines (grooves) ruled on a highly polished surface such as aluminum, about 6000 to 12000 per millimeter for the ultraviolet and visible regions. The grooves act as scattering centers for rays impinging on the grating. The result is equal dispersion of all wavelengths of a given order, that is, linear dispersion.

→ The rotating discs

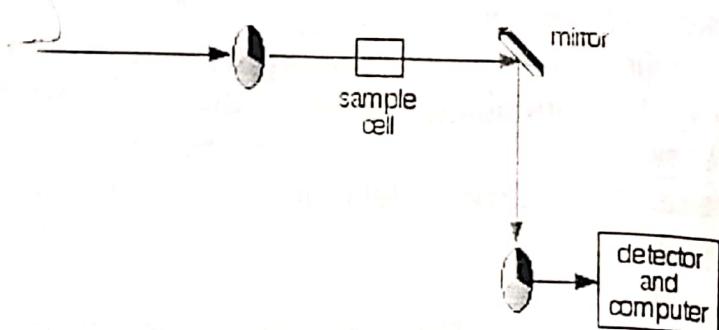
Each disc is made up of a number of different segments. Those in the machine we are describing have three different sections - other designs may have a different number.



The light coming from the diffraction grating and slit will hit the rotating disc and one of three things can happen.

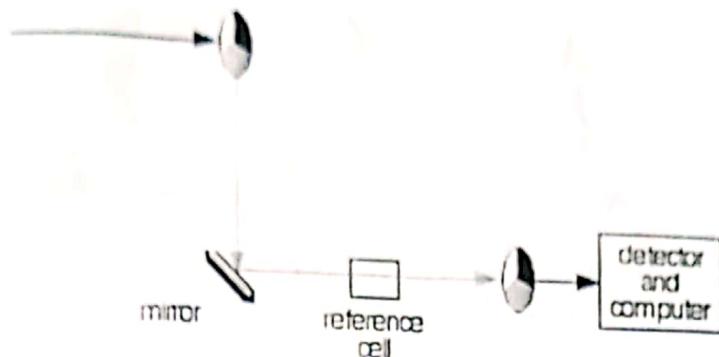
1. If it hits the *transparent section*, it will go straight through and pass through the cell containing the sample. It is then bounced by a mirror onto a second rotating disc.

This disc is rotating such that when the light arrives from the first disc, it meets the mirrored section of the second disc. That bounces it onto the detector. It is following the red path in the diagram:



2. If the original beam of light from the slit hits the *mirrored section* of the first rotating disc, it is bounced down along the green path. After the mirror, it passes through a reference cell (more about that later). Finally, the light gets to the second disc which is rotating in such a way that it meets the transparent section. It goes straight through to the detector.





3. If the light meets the first disc at the *black section*, it is blocked - and for a very short while no light passes through the spectrometer. This just allows the computer to make allowance for any current generated by the detector in the absence of any light.

III. SAMPLE CELLS

For liquids the sample is held in an optically flat, transparent container called a cell or **Cuvette**. The reference cell or cuvette contains the solvent in which the sample is dissolved and this is commonly referred to as the blank. The reference cell just contains the pure solvent.

The **choice of sample cell** is based on;

- a) the path length, shape, size
- b) the transmission characteristics at the desired wavelength
- c) the relative expense

The cell holding the sample (usually a solution) must, of course, be transparent in the wavelength region being measured. The cells for use in visible and ultraviolet spectrometers are usually cuvettes 1cm in pathlength (internal distance between parallel walls, the walls are typically 1 mm thick).

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.

IV. Detectors

In order to detect radiation, three *types of photosensitive devices* are

- Photovoltaic cells or barrier-layer cell
- Phototubes or photo emissive tubes
- Photomultiplier tubes

*silver
Selenium
Iron or*

Photovoltaic cell is also known as barrier layer or photonic cell. It consists of a metallic base plate like iron or aluminum which acts as one electrode. On surface, a thin layer of a semiconductor metal like selenium is deposited. Then the surface of selenium is covered by a very thin layer of silver or gold which acts as a second collector tube.

When the radiation is incident upon the surface of selenium, electrons are generated at the selenium-silver surface and the electrons are collected by the silver. This accumulation at the silver surface creates an electric voltage difference between the silver surface and the basis of the cell.

Phototubes are also known as photo emissive cells. A phototube consists of an evacuated glass bulb. There is light sensitive cathode inside it. The inner surface of cathode is coated with light sensitive layer such as potassium oxide and silver oxide.

When radiation is incident upon a cathode, photoelectrons are emitted. These are collected by an anode. Then these are returned via external circuit. And by this process current is amplified and recorded.

A Photomultiplier tube (PMT) is more sensitive than a phototube and is widely used for sensitive detection in visible and ultraviolet regions. It consists of a photo-emissive cathode, which the photon strikes, and a series of electrodes (dynodes), each at a more positive potential (50 to 90V) than the one before it. When an electron strikes the photo-emissive surface, a primary electron is emitted (this is the photoelectric effect). The primary electron released from the photo-emissive surface is accelerated toward the first **dynode**. The impact of the electron on the dynode surface causes the release of many secondary electrons which in turn are accelerated to the next electrode where each secondary electron releases more electrons, and so on; By this time, each original photon

dynode...? an intermediate electrode which emits additional electrons in photomultiplier similar to PMT

as produced 106 - 107 electrons, up to about 10 stages of amplification is common. The electrons are finally collected by the anode. The final output of the photomultiplier tube may, in turn, be further electronically amplified.

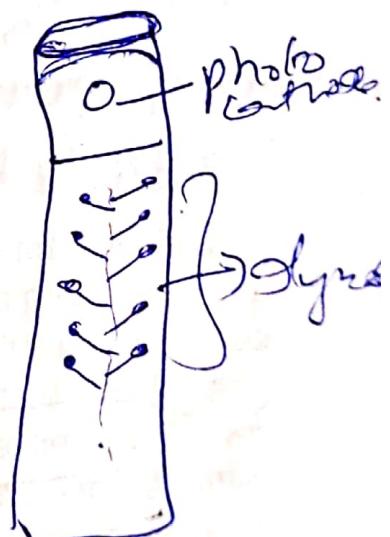
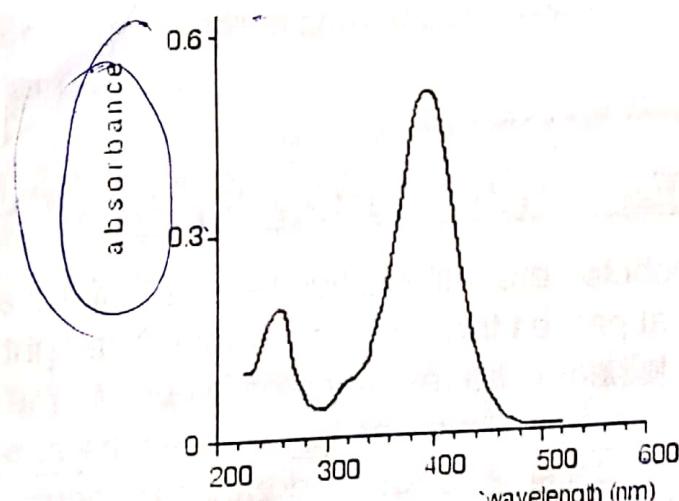
The detector converts the incoming light into a current. The higher the current, the greater the intensity of the light. For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured. This is usually referred to as I_0 - that's I for Intensity. The intensity of the light passing through the sample cell is also measured for that wavelength - given the symbol, I. If I is less than I_0 , then obviously the sample has absorbed some of the light. Absorbance is given by;

Current & Intensity

$$A = \log_{10} \frac{I_0}{I}$$

V. The chart recorder

Chart recorders usually plot absorbance against wavelength. The output might look like this:



Types of Instruments

There are many variations depending on the manufacturer, the wavelength regions for which the instrument is designed, the resolution required, and so on.

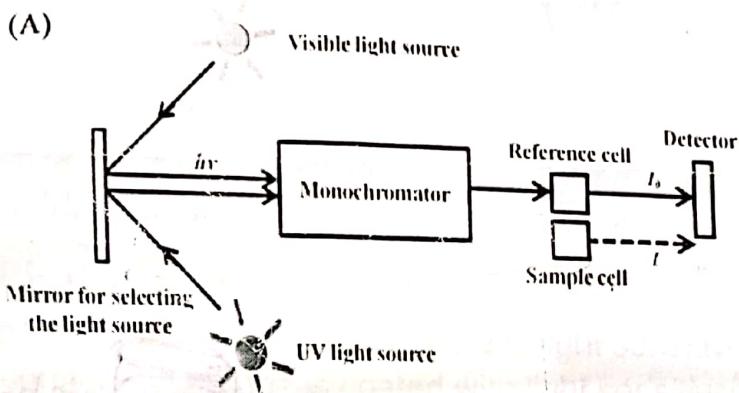
1. SINGLE-BEAM SPECTROMETERS

A single beam spectrophotometer utilizes one beam of light that passes through the sample and the intensity of the light reflected from a reference is measured without the sample. A single beam instrument records the ratio of the incident beam energy to the transmitted beam energy.

Benefits of Single Beam Design

- Less number of optical components results in improved light transmission and reduced noise
- High energy throughput due to non-splitting of source beam results in high sensitivity of detection

gives



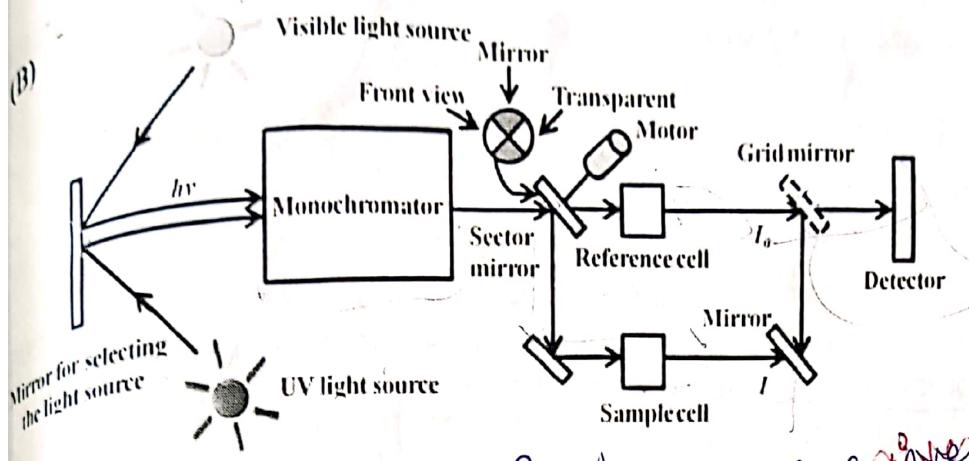
2. DOUBLE-BEAM SPECTROMETERS

A double beam spectrophotometer utilizes two beams of light: a reference beam and a sampling beam that passes through the sample. The light beam from the source is split into sample beam and reference beam by the mechanical chopper. The reference beam monitors the lamp energy whereas the sample beam reflects sample absorption. The observed absorbance measurement is the ratio of the sample and reference beams which are recombined before moving to the monochromator.

Benefits of Double Beam Design

- Correction of absorbance for solvent blank
- They can automatically compensate for absorbance by the blank, as well as for drifts in source intensity.

$$\text{absorbance} = \frac{\text{Sample}}{\text{reference}}$$



an atom or group whose presence gives possible
for colour of compound.

Applications of UV spectroscopy

Detection of functional groups-

UV spectroscopy is used to detect the presence or absence of chromophore in the compound. This technique is not useful for the detection of chromophore in complex compounds. The absence of a band at a particular band can be seen as an evidence for the absence of a particular group. If the spectrum of a compound comes out to be transparent above 200 nm than it confirms the absence of -
 a) Conjugation b) A carbonyl group c) Benzene or aromatic compound d) Bromo or iodo atoms.

II. Detection of extent of conjugation- double bond \propto long wavelength

The extent of conjugation in the polyenes can be detected with the help of UV spectroscopy. With the increase in double bonds the absorption shifts towards the longer wavelength. If the double bond is increased by 8 in the polyenes then that polyene appears visible to the human eye as the absorption comes in the visible region.

III. Identification of an unknown compound-

An unknown compound can be identified with the help of UV spectroscopy. The spectrum of unknown compound is compared with the spectrum of a

reference compound and if both the spectrums coincide then it confirms the identification of the unknown substance.

IV. Determination of configurations of geometrical isomers-

It is observed that cis-alkenes absorb at different wavelength than the trans-alkenes. The two isomers can be distinguished with each other when one of the isomers has non-coplanar structure due to steric hindrances. The cis-isomer suffers distortion and absorbs at lower wavelength as compared to trans-isomer.

V. Determination of the purity of a substance- Purity of a substance can also be determined with the help of UV spectroscopy. The absorption of the sample solution is compared with the absorption of the reference solution. The intensity of the absorption can be used for the relative calculation of the purity of the sample substance.

VI. Dissociation constants of acids and bases-

$$\text{PH} = \text{PKa} + \log [\text{A}^-] / [\text{HA}]$$

From the above equation, the PKa value can be calculated if the ratio of $[\text{A}^-] / [\text{HA}]$ is known at a particular PH, and the ratio of $[\text{A}^-] / [\text{HA}]$ can be determined spectrophotometrically from the graph plotted between absorbance and wavelength at different PH values.

VII. Chemical kinetics-

Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

VIII. Quantitative analysis of pharmaceutical substances

Many pharmaceutical compounds contain chromophores that make them suitable for analysis by UV/Vis absorption. Products that have been analyzed in this fashion include antibiotics, hormones, vitamins, and

analgesics. Many drugs are either in the form of raw material or in the form of formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at specific wavelength.

X. As HPLC detector-

A UV/Vis spectrophotometer may be used as a detector for HPLC. The presence of an analyte gives a response which can be assumed to be proportional to the concentration.

X. Forensic Applications-

UV/Vis molecular absorption is routinely used in the analysis of narcotics and for drug testing.

1 drug used
to
reduce
pain.

028121
~~Pharm~~
High performance
liquid chromatography