UV spectroscopy is an important tool in analytical chemistry. The other name of UV (Ultra-Violet) spectroscopy is Electronic spectroscopy as it involves the promotion of the electrons from the ground state to the higher energy or excited state.

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Absorption of the ultra-violet radiations results in the excitation of the electrons from the ground state to higher energy state. The energy of the ultra-violet radiation that are absorbed is equal to the energy difference between the ground state and higher energy states ($\delta E = hf$).

Generally, the most favoured transition is from the highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO). For most of the molecules, the lowest energy occupied molecular orbitals are s orbital, which correspond to sigma bonds. The p orbitals are at somewhat higher energy levels, the orbitals (nonbonding orbitals) with unshared paired of electrons lie at higher energy levels. The unoccupied or antibonding orbitals (π and σ) are the highest energy occupied orbitals.

In all the compounds (other than alkanes), the electrons undergo various transitions. Some of the important transitions with increasing energies are: nonbonding to π, nonbonding to σ, π to π, σ to π and σ to σ.

Principle:

Spectrophotometry is a technique that uses the absorbance of light by an analyte (the substance to be analyzed) at a certain wavelength to determine the analyte concentration. UV/VIS (ultra violet/visible) spectrophotometry uses light in UV and visible part of the electromagnetic spectrum. Light of this wavelength is able to effect the excitation of electrons in the atomic or molecular ground state to higher energy levels, giving rise to an absorbance at wavelengths specific to each molecule. When a beam of radiation (light) passes through a substance or a solution, some of the light may be absorbed and the remainder transmitted through the sample. The ratio of the intensity of the light entering the sample (Io) to that exiting the sample (I) at a particular wavelength is defined as the transmittance (T). The absorbance (A) of a sample is the negative logarithm of the transmittance.

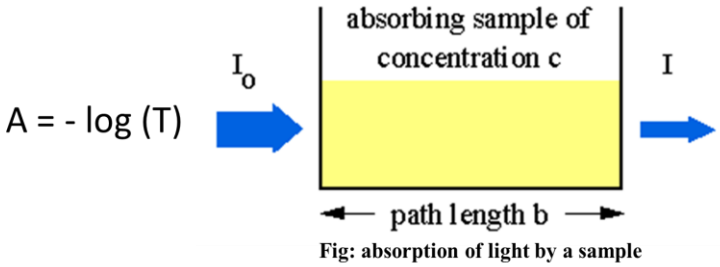


Fig: absorption of light by a sample

UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

The expression of Beer-Lambert law is-

$A = \log (I_0/I) = Ecl$

Where, A = absorbance

I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

C = molar concentration of solute

L = length of sample cell (cm.)

E = molar absorptive

From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV spectroscopy.

Prerequisite for Beer Lambert law:

- ✓ The absorbing medium must be homogeneous in the interaction volume
- ✓ The absorbing medium must not scatter the radiation – no turbidity;
- ✓ The incident radiation must consist of parallel rays, each traversing the same length in the absorbing medium;
- ✓ The incident radiation should preferably be monochromatic

Instrumentation and working of UV spectroscopy:

Instrumentation and working of the UV spectrometers can be studied simultaneously. Most of the modern UV spectrometers consist of the following parts-

Light Source- Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region. Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

Monochromator- Monochromators generally composed of prisms and slits. The most of the spectrophotometers are **double beam spectrophotometers**. The radiation emitted from the

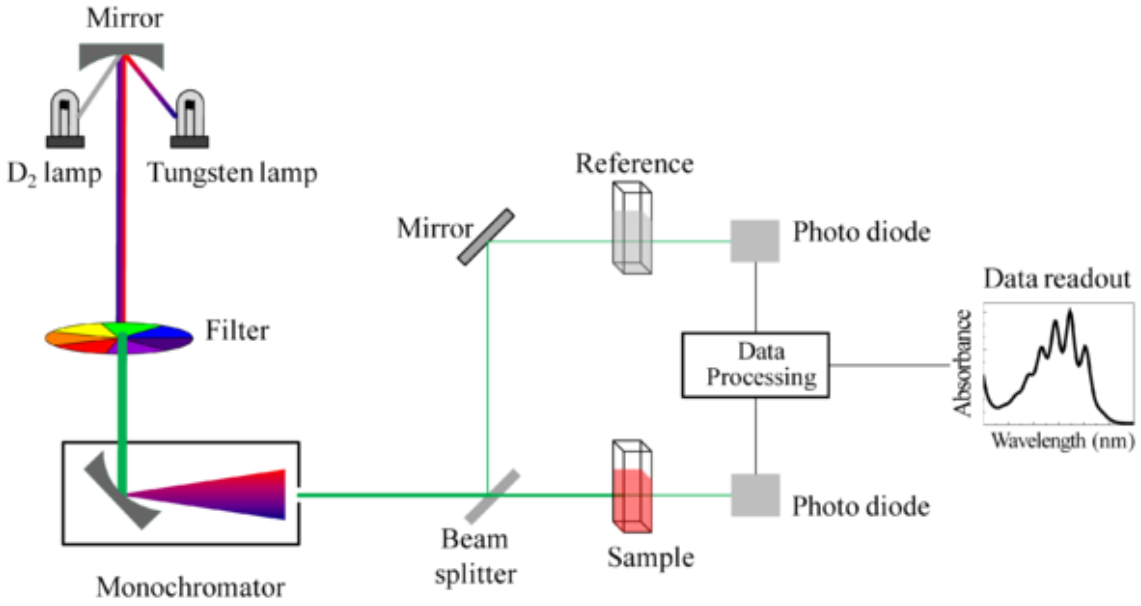
primary source is dispersed with the help of rotating prisms. The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelength to pass through the slits for recording purpose. The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

Sample and reference cells- One of the two divided beams is passed through the sample solution and second beam is passé through the reference solution. Both sample and reference solution are contained in the cells. These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

Detector- Generally two photocells serve the **purpose of detector in UV spectroscopy**. One of the photocell receives the beam from sample cell and second detector receives the beam from the reference. The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.

Amplifier- The alternating current generated in the photocells is transferred to the amplifier. The amplifier is coupled to a small servometer. Generally current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.

Recording devices- Most of the time amplifier is coupled to a pen recorder which is connected to the computer. Computer stores all the data generated and produces the spectrum of the desired compound.



Applications of UV spectroscopy:

Detection of Impurities:

UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected.

Benzene appears as a common impurity in cyclohexane. Its presence can be easily detected by its absorption at 255 nm.

Structure elucidation of organic compounds:

UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms.

From the location of peaks and combination of peaks, it can be concluded that whether the compound is saturated or unsaturated, hetero atoms are present or not etc.

Quantitative analysis:

UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is based on Beer's law which is as follows.

$$\mathbf{A = \log I_0 / I_t = \log 1/ T = - \log T = abc = \varepsilon bc}$$

Where ε is extinction co-efficient, c is concentration, and b is the length of the cell that is used in UV spectrophotometer.

Other methods for quantitative analysis are as follows.

a) Calibration curve method

b) Simultaneous multicomponent method

c) Difference spectrophotometric method

d) Derivative spectrophotometric method

Qualitative analysis:

UVabsorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds.

UV absorption spectroscopy is generally used for characterizing aromatic compounds and aromatic olefins.

Dissociation constants of acids and bases:

PH = PKa + log $[A^-]$ / [HA]

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

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.

Quantitative analysis of pharmaceutical substances:

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.

Diazepam tablet can be analyzed by 0.5% H2SO4 in methanol at the wavelength 284 nm.

Molecular weight determination:

Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.

For example, if we want to determine the molecular weight of amine then it is converted in to amine picrate. Then known concentration of amine picrate is dissolved in a litre of solution and its optical density is measured at λmax 380 nm. After this the concentration of the solution in gm moles per litre can be calculated by using the following formula.

$$C = \frac{\log I_0 / I_t}{\varepsilon_{max} \times l}$$

"**C**" can be calculated using above equation, the weight "**w**" of amine picrate is known. From "**c**" and "**w**", molecular weight of amine picrate can be calculated. And the molecular weight of picrate can be calculated using the molecular weight of amine picrate.

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. For more accurate results, the instrument's response to the analyte in the unknown should be compared with the response to a standard; as in the case of calibration curve.