Chapter

UV-Visible Spectroscopy for Colorimetric Applications

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Abstract

UV-visible spectroscopy is an interpretive skill that amplitude the variety of different wavelengths of UV or visible light, which are captivated by or transferred via a pattern new assessment to an implication or blank constituent. This asset is encouraged by way of the pattern combination, doubtlessly subject to network on what is within the representative and at what attention. Because this spectroscopy execution confides on the control of mild. Therefore, illuminate can be described by its wavelength, which can be useful in UV-visible spectroscopy to analyse or identify different substances.

Keywords: detectors, filters, monochromators, sources, UV-visible spectroscopy

1. Introduction

The analytical chemistry is based on the quality of colour in coloured solution, we observe the colour, the colour's depth, or intensity. These observations led to the technique called colorimetry, the colour of a solution identify species while the intensity of the colour depends on identifying the concentration of the species present. The important and sensitive colour tests have been developed for the detection and determination of a wide range of chemical species, both inorganic and organic in nature, this used the development of visible and ultraviolet spectrometer [1].

The wavelength range of UV radiation starts at 400 nm, the blue end of visible light, and ends at 200 nm. The radiation has sufficient energy to excite electrons. When light passes through the solution and emerges as red light, then the solution is red. Because the solution has allowed the red component of white light to pass through, whereas if the solution has led the red component of white light to pass through because it has absorbed the complementary colours, yellow and blue [2].

If the solution has more concentration, more yellow and blue light is absorbed, and more intensely red solution appears to the eye. There is a difficulty in comparing the intensity of the two colours. The wavelength range of UV radiation starts at the end of visible light of 400 nm and ends at 800 nm [3]. The atoms or molecules have sufficient energy to excite valence electrons. Visible light starts the wavelength from 800 to 400 nm.

2. Theory

2.1 Electronic excitation in molecules

The atoms are held strongly by sharing electrons in a molecule. The electron in a molecule moves in molecular orbitals at discrete energy levels. When the energy of the electrons is at a minimum, the molecules are in the lowest energy state or ground state. The molecules can absorb radiation and move to a higher energy state or excited state. The movement of electrons from a higher energy state is called electronic excitation [4]. The frequency captivates or effuse by a molecule and the power is related by, $\Delta E = h\gamma$. The amount of energy required is based upon the variation in energy linking the ground state E_0 and the excited state E_1 of the electron. It is stated as $\Delta E = E_1 - E_0 = h\gamma$

where, E_1 is the energy of the excited state.

E_0 is the energy of the ground state.

The full strength of a molecule is the same as the sum of electronic, vibrational, and rotational electricity. The importance of the energies decreases inside the following order: E_{elec}, E_{vib}, and E_{rot}. Ultraviolet energy is computed, the assimilation spectrum arising from a single electronic transition must contain a single discrete line. However, an awesome line is not obtained because digital absorption is superimposed upon rotational and vibrational sublevels. Suppose of complex molecules in conjugation with an excess of two atoms, discrete bands merge to bring about broad absorption bands or "band envelops" [5]. Three distinct types of electrons are involved in organic molecules. They are as follows:

i. σ electrons: Electrons associated with the single bonds are known as σ electrons. Electrons are involved in saturated bonds, such as those between carbon and hydrogen-like C-C, C-H, O-H. As the amount of energy required to excite electrons in σ bonds is much more than that produced by UV light. Example: Hexane C_6H_{14}.

ii. π electrons: Electrons are involved in a double and triple bond that is involved in unsaturated hydrocarbon-like alkenes, alkynes, conjugated olefins, and aromatic compounds.

iii. n electrons: Electrons that are not involved in bonding between atoms or molecules. Organic compounds containing nitrogen, oxygen, sulphur or, halogens.

2.2 Electronic transition in organic molecule

A rule to predict how molecules undergo a transition is given by Quantum mechanics. Some transitions are "allowed" while others are "Forbidden."

i. **$\sigma \rightarrow \sigma^*$:** Orbitals are conserved; therefore, two molecular orbitals are formed, a sigma bonding orbital and a higher energy sigma antibonding orbital. The antibonding orbital is denoted by σ*. The energy difference between σ and σ* is equal, denoted by ΔE. The compounds in which all valence electrons are involved in the single bond formation such as saturated hydrocarbon show absorption in far UV radiation below 190 nm.

ii. **$n \rightarrow \sigma^*$:** the transition takes place in saturated compounds containing one hetero atom with unshared pair of electrons (n electron). The compounds

which undergo these transitions are saturated halides, alcohols, ethers, aldehydes, ketones, amines, etc. This transition requires less energy. In saturated alkyl halides, the energy required for such a transition decreases with the increase in the size of the halogen atom.

In methyl chloride and methyl iodide due to the electronegativity of chlorine atom, the n electrons on chlorine atom are comparatively difficult to excite, whereas the methyl iodide is 258 nm as n electrons on iodide atom are loosely bound.

iii. $\pi \rightarrow \pi^*$: The transitions occur in unsaturated compounds that contain double and triple bonds and in aromatics. The excitations of π electron require smaller energy and hence transitions of this type occur at a longer wavelength. π electron of a double bond is excited to π^* orbital. The compounds that undergo are alkenes, alkynes, carbonyl compounds, cyanides, azo compounds, etc.

iv. $n \rightarrow \pi^*$: the compounds with a functional group such as C=O, C=S, C=N undergo $n \rightarrow \pi^*$. This type of transition requires the least amount of energy. The compounds like nitrogen, oxygen, Sulphur, halogen atom especially Br and I in UV/visible region undergo transition with nonbonded electrons [6]. The electronic transitions are shown in **Figure 1**.

2.2.1 Beer's and Lambert's law

There are two laws related to the absorption of radiation [7].

$$I = Ia + It$$

I = Intensity of incident light.
Ia = Intensity of absorbed light.
It = Intensity of transmitted light.

2.2.2 Beer's law

The intensity of a beam of monochromatic light drops exponentially with expanding in the concentration of absorbing species arithmetically.

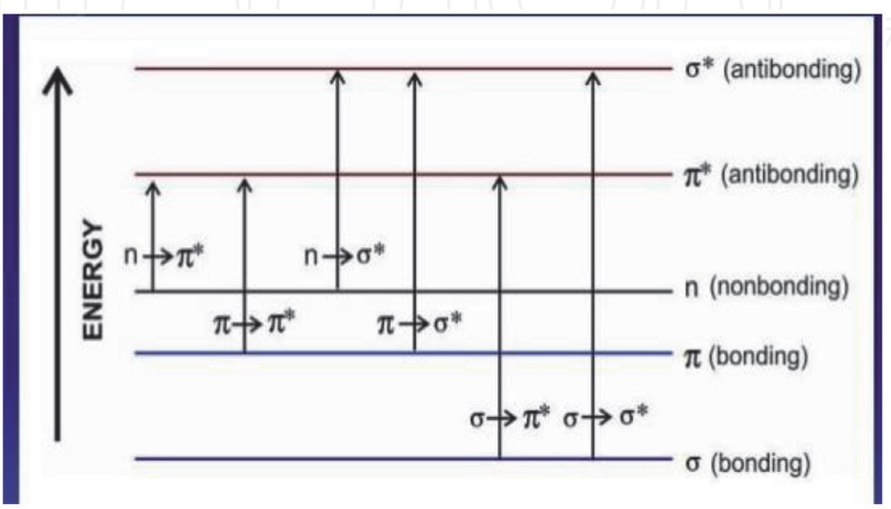

Figure 1.
The electronic transition.

$$-\frac{dI}{dc} \propto \mathrm{I} \left(\text{the decline in the intensity of incident light, with concentration, C is proportional to the strength of incident light, I}\right)$$

$$-\frac{dI}{dc} = \mathrm{K\,I} \left(\text{eliminate and introducing constant proportionality K}\right)$$

$$-\frac{dI}{dc} = \mathrm{K\,dc} \left(\text{rearranging terms}\right)$$

$$-\ln\ \mathrm{I} = \mathrm{Kc} + \mathrm{b} \left(\text{on integration}\right) \tag{1}$$

When concentration, C = 0, there is no absorbance I = I_0.
Exchange in Eq. (1).

$$-\ln\ \mathrm{I_0} = \mathrm{K} \times 0 + \mathrm{b}$$

$$-\ln\ \mathrm{I_0} = \mathrm{b}$$

Substitute the value of -ln I_0 = b in Eq. (1).

$$-\ln\ \mathrm{I} = \mathrm{Kc} - \ln\ \mathrm{I_0}$$

$$-\ln\ \mathrm{I_0} - \ln\ \mathrm{I} = \mathrm{Kc}$$

$$\ln\ \mathrm{I_0/I} = \mathrm{Kc}\ (\text{Since logA} - \log\ \mathrm{B} = \log\ \mathrm{A/B}) \tag{2}$$

$$\mathrm{I_0/I} = e^{Kc}\ (\text{separate natural logarithm})$$

$$\mathrm{I/I_0} = e^{-Kc}\ (\text{reversed on both sides})$$

2.2.3 Lambert's law

The rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light.

$$-\frac{dI}{dt} \propto \mathrm{I} \left(\text{the decline in the intensity of incident light, with concentration, C is proportional to the intensity of incident light, I}\right)$$

$$-\frac{dI}{dt} = \mathrm{K\,I} \left(\text{separate and introducing constant proportionality K}\right)$$

$$-\frac{dI}{I} = \mathrm{K\,dt} \left(\text{reposition terms}\right)$$

$$-\ln\ \mathrm{I} = \mathrm{Kt} + \mathrm{b} \left(\text{on integration}\right) \tag{3}$$

When concentration, t = 0, existent is never absorbance I = I_0.
Substituting in Eq. (3).

$$-\ln\ \mathrm{I_0} = \mathrm{K} \times 0 + \mathrm{b}$$

$$-\ln\ \mathrm{I_0} = \mathrm{b}$$

Substitute the rate of -ln I_0 = b in Eq. (1).

$$
\begin{aligned}
&-\ln\ \mathrm{I} = \mathrm{Kt} - \ln\ \mathrm{I}_0 \\
&-\ln\ \mathrm{I}_0 - \ln\ \mathrm{I} = \mathrm{Kt} \\
&\ln\ \mathrm{I}_0/\mathrm{I} = \mathrm{Kt}\ (\text{Since } \log\mathrm{A} - \log\ \mathrm{B} = \log\ \mathrm{A}/\mathrm{B}) \\
&\mathrm{I}_0/\mathrm{I} = e^{Kt}\ (\text{removing natural logarithm}) \\
&\mathrm{I}/\mathrm{I}_0 = e^{-Kt}\ (\text{Inverse on bilateral})
\end{aligned} \tag{4}
$$

Combine and equate Eqs. (3) and (4)

$$
\begin{aligned}
\mathrm{I}/\mathrm{I}_0 &= e^{-Kct} \\
\mathrm{I} &= Ioe^{-Kct} \\
\mathrm{I} = Io10^{-Kct}\ &(\text{Converting natural logarithm to base 10\&K} = \mathrm{K} \times (0.4343) \\
\mathrm{I}/Io &= 10^{-Kct}\ (\text{reposition terms}) \\
\mathrm{Io}/\mathrm{I} &= 10^{Kct}\ (\text{reverse on both sides}) \\
\mathrm{Log\ Io}/\mathrm{I} &= \mathrm{Kct}\ (\text{Taking log on both sides})
\end{aligned} \tag{5}
$$

Transmittance (T) = I/Io and Absorbance (A) = log 1/T.
Hence $\mathrm{A} = \log\ \frac{1}{I/Io.}$

$$\mathrm{A} = \log\ \mathrm{Io}/\mathrm{I} \tag{6}$$

Substitutes Eq. (6) in Eq. (5)

$$
\begin{aligned}
\mathrm{A} &= \mathrm{KCt}\ (\text{Instead of K, we write } \varepsilon) \\
\mathrm{A} &= \varepsilon\mathrm{ct}
\end{aligned}
$$

Where, A = Absorbance or optical density or extinction coefficient
ε = molecular extinction coefficient
C = Concentration of drug (mmol/lit)
T = pathlength (1 cm)
ε can also expressed as

$$\varepsilon = E\sum_{1cm}^{1\%} xMolecularweight/10$$

Where $E\sum_{1cm}^{1\%}$ means the absorbance of 1% W/V solution using a path length of 1 cm.

2.3 Deviation from Beer's and Lambert's law

Beer and Lambert's law is found to be obeyed by the system if a straight line passes through the origin and a graph is plotted between absorbance and concentration.

But there is always a deviation from the linear relationship between the absorbance and concentration particularly at higher concentration, and hence the absorption curve changes with the change in concentration of the solution. The deviation may be positive or negative, if the resulting curve is concave upwards it is called positive deviation. If the resulting curve is concave downwards it is called negative deviation, which is depicted in **Figure 2** [8].

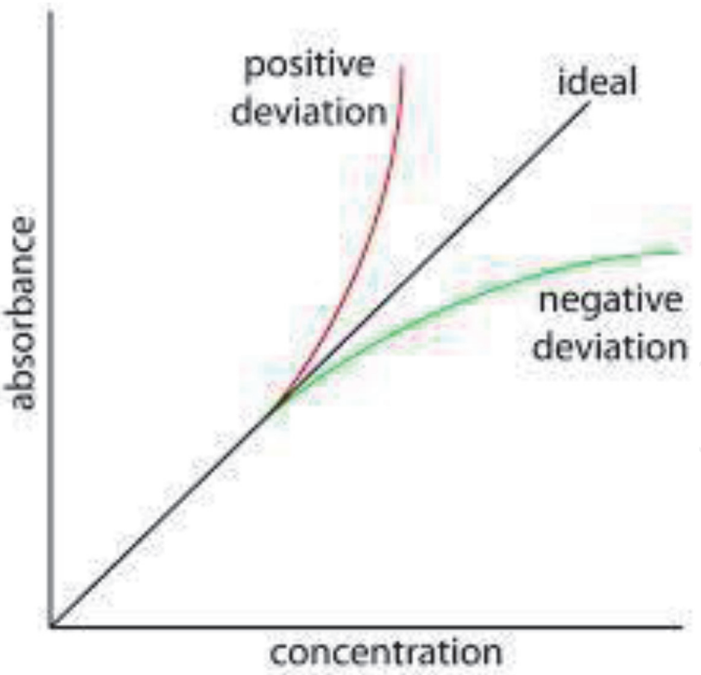

Figure 2.
Deviation from Beers & Lamberts law.

2.4 The reason for changing deviation from Beer's law

2.4.1 Instrumental deviation

Factors like stray radiation improper slit width, fluctuations in single and when monochromatic light is not used.

2.4.2 Physiochemical changes in solution

i. The law does not hold if the substance ionises, dissociates, or associates in solution. Since the nature of the ionised species in solution varies with the concentration.

Example: Benzoic acid in benzene is associated to form dimer and hence deviation occurs.

i. Potassium dichromate in high concentration exists as orange solution (λmax −450 nm). But on dilution, dichromate ions are dissociated into chromate ions, which are yellow-coloured (λmax −410 nm).

ii. When sufficient time is not allowed for making absorbance measurement or when the reading is made when the colour has faded away due to instability of colour, deviation can occur due to incomplete reaction.

iii. If a solute forms complexes, the composition and extent of complexation depend upon the concentration.

iv. A large number of electrolytes may shift the λmax and change the extinction coefficient.

v. If the change in concentration causes significant alterations in the refractive index, then deviations from the law.

vi. Changes in pH with a change in concentration of solute may cause deviation.

vii. The presence of impurities that fluoresce or absorb at the required absorption, the wavelength may cause deviation.

3. Effects of solvents UV spectra

Chromophore: the term chromophore is used to denote a functional group or presence of some structural feature that gives colour to a compound [9].

Example: Nitro group is a chromophore because its presence in a compound gives the yellow colour to the compound. It can be defined as any group which exhibits absorption of electromagnetic radiation in the visible or ultraviolet region. It may or may not impart any colour to the compound. Some of the important chromophores are ethylenic, acetylenic, carbonyls, acids, esters, nitrile group, etc.

There are two types of chromophores. The chromophore in which the group contains π electrons and they undergo n → π* transitions, the compounds like ethylene, acetylene, etc.

The other type of chromophore contains both π electrons and n (non-bonding) electrons. This type of chromophore undergoes two types of transitions, π → π* and n → π* and examples include carbonyls, nitriles, azo compounds, and nitro compounds.

3.1 Changes in position and intensity of absorption

For isolated chromophore groups such as >C=C < and -C ≡ C-, absorption takes place in the far ultraviolet region which cannot be easily studied.

But the role of absorption is maximum and the intensity of absorption can be edited in exceptional approaches by some structural adjustments or change of solvent.

3.1.1 Bathochromic shift or redshift

It involves the shift of absorption most in the direction of longer wavelength because of the presence of certain groups such as OH and NH_2 called auxochromes or by change of solvent. A Bathochromic shift is also produced when two or more chromophores are present in conjugation in the molecule.

Example: Ethylene shows π → π* transition at 170 nm, whereas 1,3 -butadiene (where two double bonds are in conjugation) shows λmax at 217 nm.

3.1.2 Hypsochromic or blue shift

The shift of absorption maximum towards shorter wavelength and may be by the removal of conjugation or by change of solvent. The absorption shift towards a shorter wavelength is also called the blue shift.

Example: Aniline shows maximum absorption at 280 nm, because the pair of electrons on the nitrogen atom is in conjugation with the π bond system of the benzene ring. In acidic solution, a blue shift is caused and absorption takes place at a shorter wavelength 200 nm. The electron pair is no longer present and hence conjugation is removed.

Hyperchromic effect: The effect is due to an increase in the intensity of absorption and it is brought about by the introduction of an auxochrome.

Hypochromic effect: It involves a decrease in the intensity of absorption and is brought by groups that are able to distort the geometry of the molecule.

Auxochrome: It is a group that itself does not act as a chromophore but when attached to a chromophore it shifts the adsorption maximum towards a longer wavelength along with an increase in the intensity of absorption.

3.2 Instrumentation

The various components of a UV-VIS spectrophotometer are as follows [3]:

1. Radiation source
2. Monochromators
3. Detector
4. Recording system
5. Sample cells
6. Matched cells
7. Power supply

4. Radiation source

In UV-VIS spectrophotometer, the normally pre-owned radiation is preferred to assets the hydrogen or deuterium lamps, the xenon discharge lamps, and mercury arcs. In all the assets, agitation is carried out by means of transient electrons through gasoline and those impacts in the midst of electron and gas molecules may bring about digital, vibrational, and rotational elation in the fume's particle [10].

The following are requirements of a radiation source:

1. It must be stable.
2. It must be sufficient intensity for the transmitted energy to be detected at the end of the optical path.
3. It must supply continuous radiation over the entire wavelength.

4.1 Tungsten lamp

The function is similar to an electric light bulb. It is a tungsten filament heated electrically to white heat. The structure is depicted in **Figure 3**.

4.1.1 Disadvantage

1. The intensity of radiation at a short wavelength < 350 nm is small.
2. To maintain a constant intensity, the electrical current to the lamp must be controlled.

Figure 3.
Tungsten lamp.

4.1.2 Advantage

The lamps are generally stable, robust, and easy to use.

5. Hydrogen discharge lamps

Hydrogen gas is stored under relatively high pressure. When an electric discharge is passed through the lamp, excited hydrogen molecules will be produced which emit UV radiations. Hydrogen lamps cover the range of 3500–1200A°. These lamps are stable, robust, and widely used.

Hydrogen discharge lamp consists of hydrogen gas under relatively high pressure through which there is an electrical discharge. The hydrogen molecules are excited electrically and emit UV radiation. The high pressure brings many collisions between the hydrogen molecules, resulting in pressure broadening. This causes the hydrogen to emit a continuous broadband rather than a simple hydrogen line spectrum. It is stable, robust, and widely used. It is more expensive is the disadvantage.it is depicted in **Figure 4**.

6. Deuterium lamp

It is used in place of hydrogen, the intensity of radiation emitted is 3–5 times the intensity of a hydrogen lamp of comparable design. It is more expensive than a hydrogen lamp. But it is used when high intensity is required. It is represented in [11] **Figure 5**.

7. Xenon discharge lamp

Xenon gas is stored under pressure in the range of 10–30 atmospheres. The xenon lamp possesses two tungsten electrodes separated by about 8 mm. When an

Figure 4.
Hydrogen discharge lamp.

Figure 5.
Deuterium lamp.

Figure 6.
Xenon discharge lamp.

intense arc is formed between two tungsten ultraviolet light is produced. The structure is depicted in **Figure 6**.

8. Mercury arc

Mercury vapour under high pressure, the excitation of mercury atoms is done by electric discharge. It is not suitable for continuous spectral studies because of the presence of sharp lines or bands. It is depicted in **Figure 7**.

9. Monochromators

The monochromator is used to disperse the radiation. The essential elements of a monochromator are:

- Entrance Slit (to get narrow source)

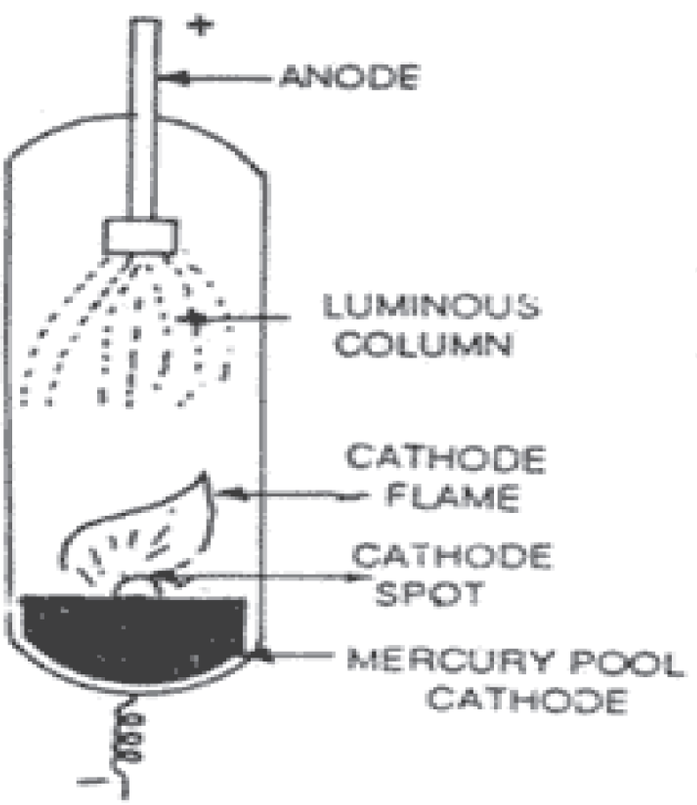

Figure 7.
Mercury arc.

- Collimator (to render light parallel)
- Grating or Prism (to disperse radiation)
- Collimator (to reform the images of entrance slit)
- Exit slit (to fall on sample cell)

Monochromators are better and more efficient than filters in converting polychromatic light or heterochromatic light into monochromatic light. The structure is depicted in **Figure 8**.

9.1 Prism

The prism disperses the light radiation into individual colours or wavelengths. These are found in expensive instruments. The bandpass is lower than that of filters and hence it has better resolution and is depicted in **Figure 9**.

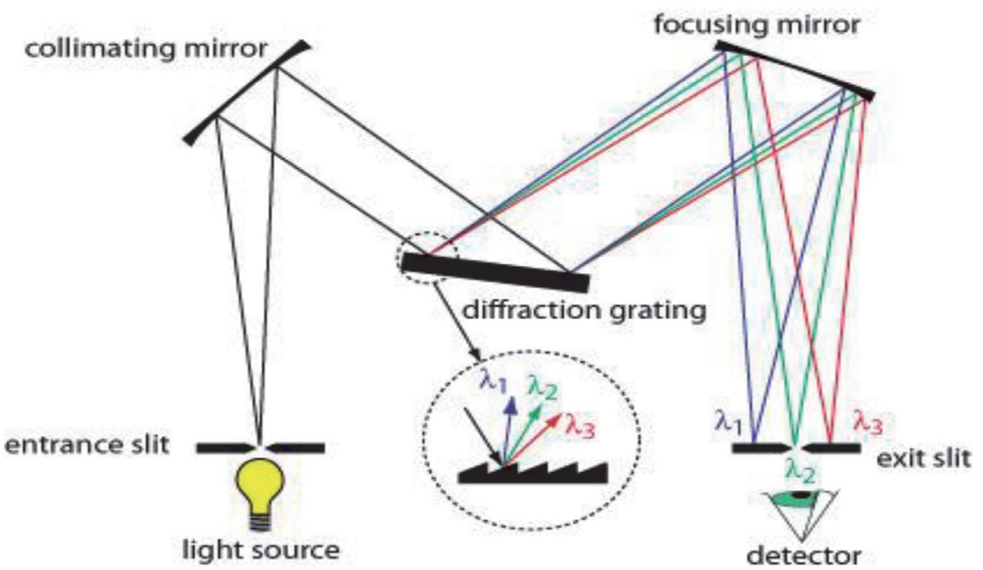

Figure 8.
Monochromators.

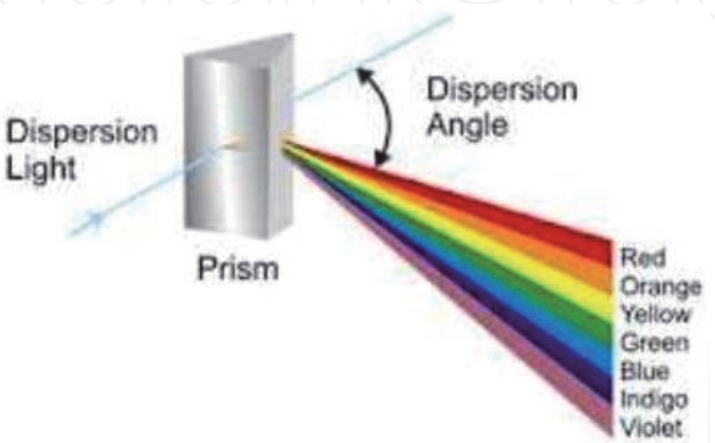

Figure 9.
Prism.

The two types of the prism are:

1. Refractive

2. Reflective

They undergo dispersion giving wavelengths that do not overlap and the disadvantage is they give non-linear dispersion.

9.1.1 Refractive type

The sources of light, through the entrance slit falls on a collimator. The parallel radiations from the collimator are dispersed into distinctive colorations or wavelength, and through the use of any other collimator, the pix of the front slit is reformed. The reformed ones will be both violet, indigo, blue, green, yellow, orange, or pink. The desired radiation on go-out slit may be decided on with the aid of rotating the prism or by way of preserving the prism stationary and transferring the exit slit which is depicted in **Figure 10**.

9.1.2 Reflective type

The dispersed radiation gets reflected and can be collected on the same side as the source of light.

9.1.2.1 Grating

Grating are the most efficient ones in converting a polychromatic to monochromatic light. Two types of the grating are diffraction and transmission.

9.1.2.2 Diffraction grating

A grating consists of a large number of parallel lines (grooves) ruled on a highly polished surface such as alumina, generally, 15,000–30,000 lines per square inch

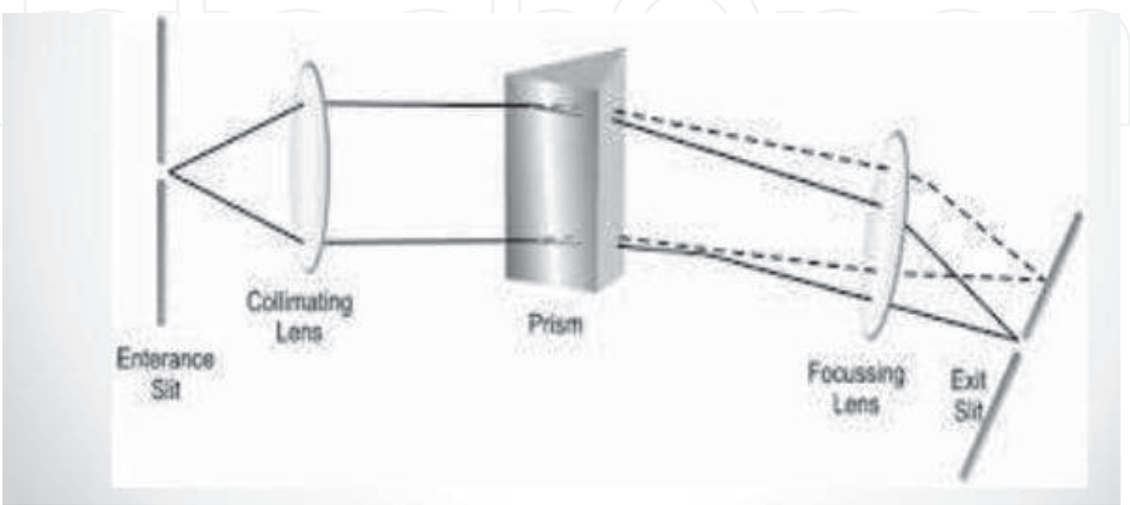

Figure 10.
Refractive prism.

are drawn. When light rays have impinged on the grating, its grooves act as scattering centres for light rays. The light is diffracted or reinforcement takes place. Grating are difficult to be prepared. The replica grating is prepared from an original grating. This is done by coating the original grating with a film of an epoxy resin, which after setting is removal to yield a replica (**Figure 11**).

$$m\lambda = b\ (\text{Sin } i \pm \text{Sin } r)$$

λ = wavelength of light produced
b = grating spacing
i = angle of incidence
r = angle of reflection
m = order

9.1.2.3 Transmission grating

Refraction takes place instead of reflection. The wavelength of radiation produced by transmission grating can be expressed by the following equation, the structure is depicted in **Figure 12**.

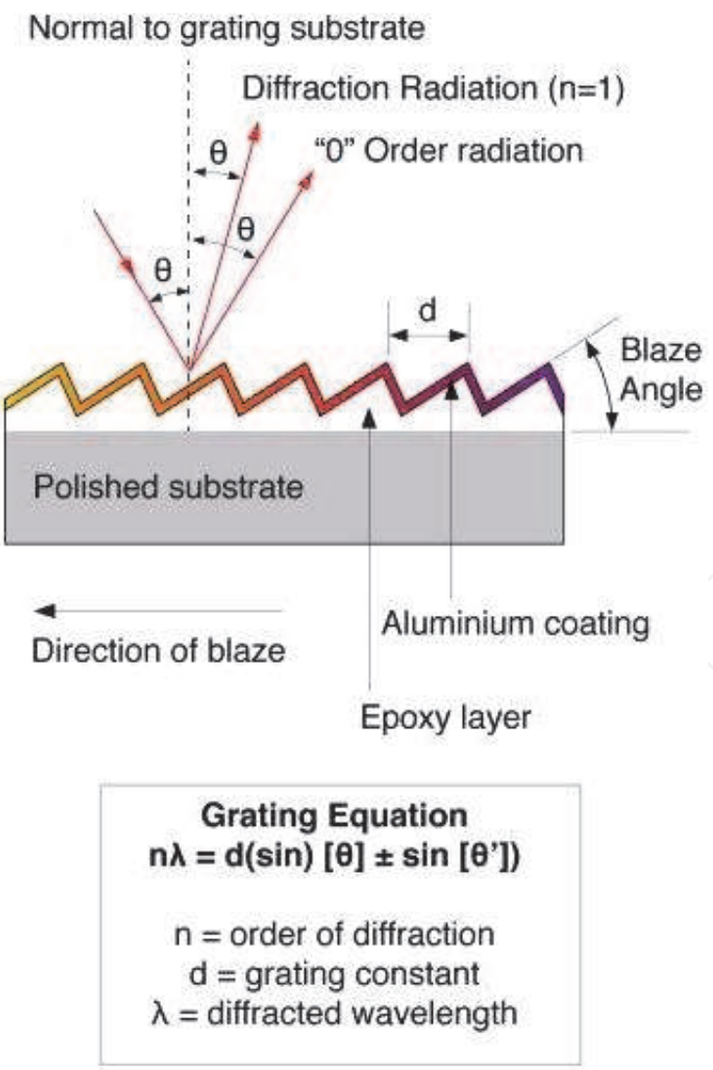

Figure 11.
Diffraction grating.

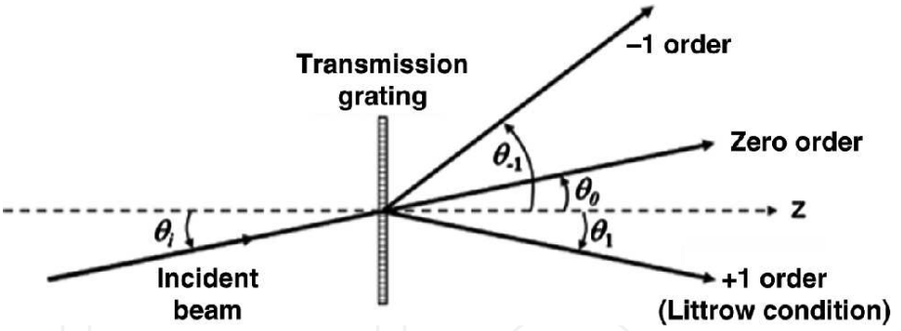

Figure 12.
Transmission grating.

$$\lambda = \left(\frac{d\sin\Theta}{m}\right)$$

λ = wavelength of radiation
d = 1/lines per cm
m = order no. (0, 1, 2, 3, ... etc.)
Θ = angle of deflection
d = 1/2000 = 0.0005 = 5×10^{-4}
Θ = 6.89°

10. Detector

Whilst a radiation is passed via a pattern cellular, part of its miles being absorbed by means of the pattern solution and rest is being transmitted. The transmitted radiation falls on the detector and the intensity of absorbed radiation can be decided.

10.1 Barrier layer or photovoltaic cell

The barrier mobile includes a semiconductor, consisting of Selenium that is deposited on a sturdy steel base, inclusive of iron. A completely skinny sheet of silvery or aurelia is stammer ended the surface of the semiconductor to behave as collector electrode. The emission falling at the floor yield electron at the selenium silver interfaces. A barrier exists between the selenium and iron, which rule out the electrons against streaming into iron. The electrons are collected on the silver surfaces. The buildup of electrons on the silver surfaces produces an electric voltage distinction between the silver surfaces and the base of the mobile.

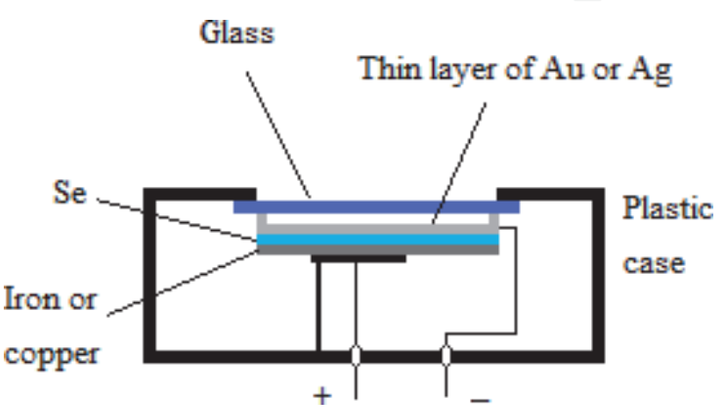

Figure 13.
Barrier layer or photovoltaic cell.

If the peripheral circuit secures a low resistance, a photocurrent will glide, that is precisely equivalent to the intensity of the incident radiation beam. It is holed directly to micrometre or galvanometer to read its output (**Figure 13**) [12].

10.2 Phototubes or photoemissive cell

It consists of a high-sensitive cathode in the form of a half-cylinder of metal is contained in an evacuated tube. The inside surface of the photocell is coated with a light touchy layer. While the mild is incident upon a photocell, the floor coating emits electron. Those are attracted and amassed by an anode. The modern-day, that is created among the cathode and anode, is seemed as a measure of radiation falling at the detector. A phototubes is greater touchy than photovoltaic cellular due to the fact excessive diploma of amplification can be used (**Figure 14**) [13].

10.3 Photomultiplier tubes

A photomultiplier tube is a combination of a photodiode and an electron-multiplying amplifier. A photomultiplier tube consists of an evacuated tube that contains one photo-cathode and 9–16 electrodes referred to as dynodes. The surface of each dynode is Be-Cu, Cs-Sb.

While radiation falls on a metallic floor of a photocathode, it emits electrons. The electrons are attracted towards the primary dynode that is kept of a fine voltage. While the electron strikes the primary dynode which is saved at a wonderful voltage. Whilst the electron strikes the first dynode, extra electrons are emitted with the aid of the floor of the dynode; these emitted electrons are then attracted via a second dynode, wherein comparable sort of electron emission take area. The technique is repeated over all of the dynodes gift in the photomultiplier tube until a bath of electrons reaches the collector. The range of electrons accomplishing the collector is the degree of the depth of light falling at the detector (**Figure 15**).

11. Recording system

The signal from the detector is finally by the recording system. The recording is done by recorder pen.

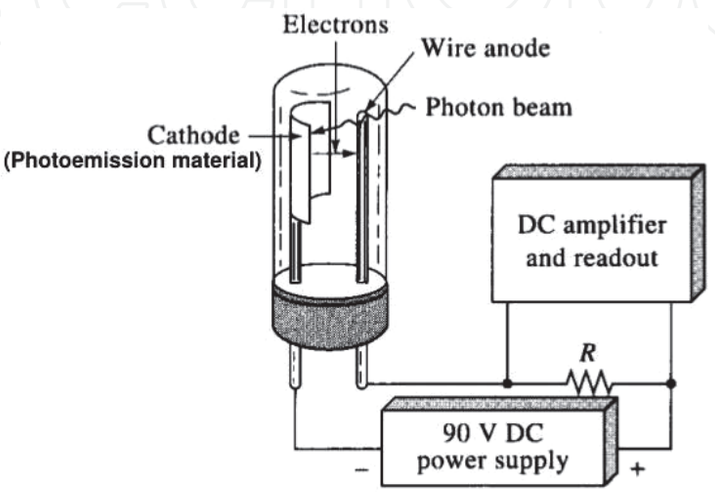

Figure 14.
Phototubes or photoemissive cell.

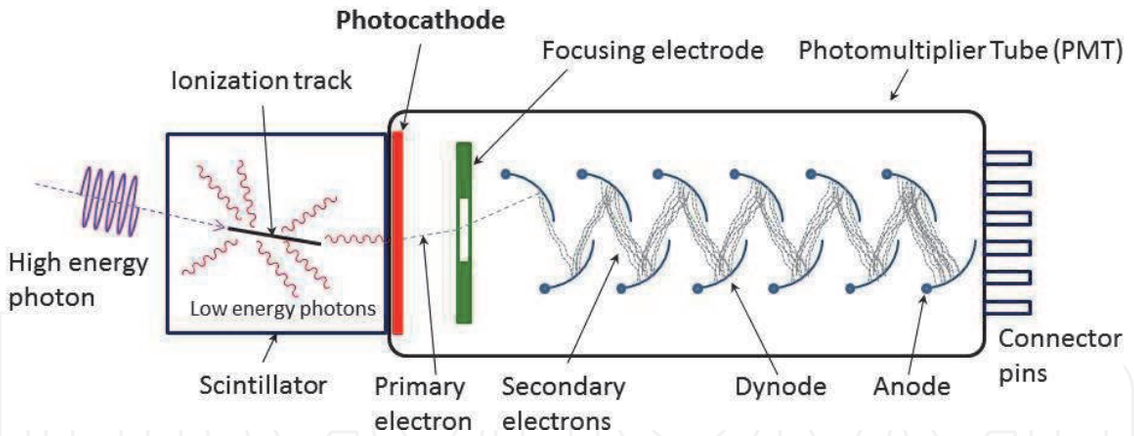

Figure 15.
Photomultiplier tubes.

12. Sample cells

The cells must contain:

i. Uniform in construction

ii. Material used for construction should be inert to solvents.

iii. They must transmit light of the wavelength.

The commonly used cells are made of quartz or fused silica (**Figure 16**) [13].

13. Matched cells

When double beam is used, two cells are needed, one for the reference and one for the samples. It is normal for the absorption by the cells to differ slightly. This causes a small error and can lead to analytical error, so matched cells are used. When the same matched cells are used, absorption is equal.

14. Power supply

It decreases the line voltage of the instrument. The converts A.C to D.C. It smooths out many ripples which may occur in the line voltage.

Figure 16.
Sample cells.

15. Types of spectrophotometers

15.1 Single-beam system

UV radiation is delineated by using the source. A convex lens accumulates the beam of emission and focal point it on the inlet splinter. The inlet splinter allows light from the source to bypass, however blocks out stray radiation. The light then reaches the monochromator, which splits it up consistent with wavelength. The exit splinter is positioned to permit mild of the required wavelength to skip thru. The chosen radiation passes through the pattern cells to the detector, which measures the depth of the radiation attaining it.

Next, to differentiate the depth of radiation preparatory to stop after it passes through the pattern, it is feasible to degree several radiation is absorbed by the pattern on the unique wavelength used. The output of the detection is commonly recorded on graph paper.

The drawback is that estimates the whole quantity of mild accomplishing particular detector, as opposed to particular proportion wrapped. The source of intensity may vary with changes in line voltage. For example, when the line voltage decreases, the intensity of the light coming from the source may decrease. The single-beam spectrophotometer is depicted in **Figure 17**.

15.2 Double-beam system

The radiation from the supply is authorised to skip thru a reflect device to the monochromator. The activity of the monochromator is to permit a slender variety of wavelengths to skip continuously an go-out slit. The radiation popping out of the monochromator through the go-out slit is received via the rotating zone which divides the beam into, one glancing through the reference and the opposite through the sample cellular. After glancing through the sample and reference mobile, the light beams are focussed onto the detector.

The yield of the detector is hooked up towards a development touchy amplifier which reciprocates to any trade-in transmission through sample and reference. The segment empathetic amplifier transmits the indicators to the recorder that is accompanied with the aid of the motion of the pen or chart. The chart drive is to integrate the rotation of the prism and for this reason, the optical density or transmission of the pattern is set down as a characteristic of wavelength.

The advantage is not necessary to continually replace the blank with the sample or to zero adjust at each wavelength. The ratio of the powers of the sample and

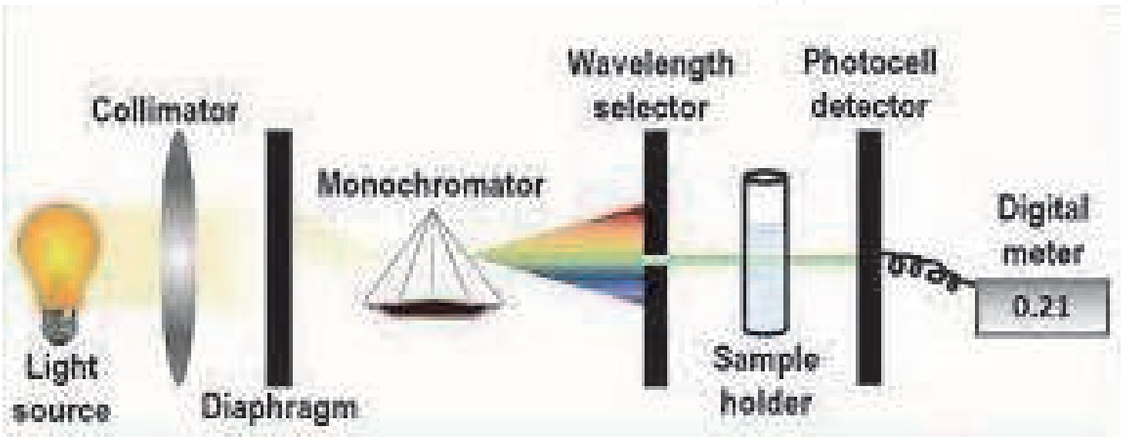

Figure 17.
Single beam spectrophotometer.

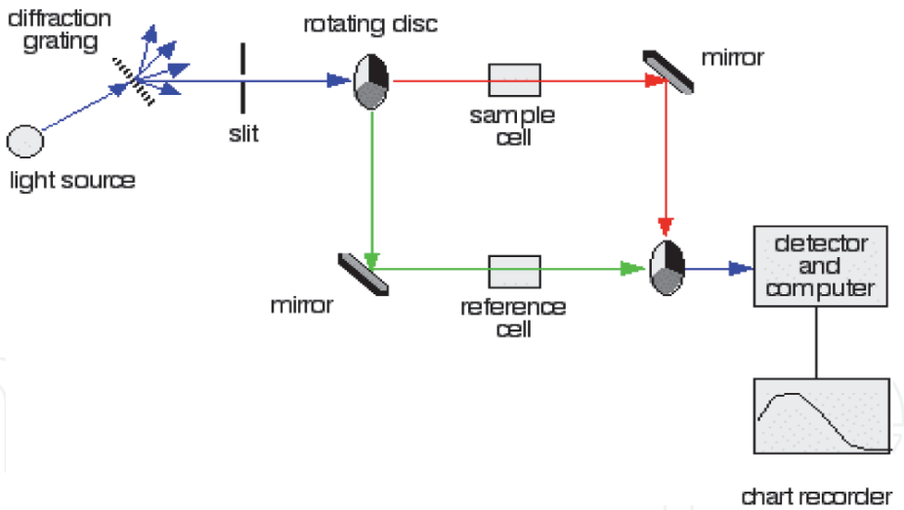

Figure 18.
Double beam spectrophotometer.

reference beams is constantly obtained and used. Any error due to variation in the intensity of the source and fluctuation in the detector is minimised (**Figure 18**).

16. Applications

16.1 Detection of conjugation

It enables to identify the relationship between the exceptional groups, especially with appreciate to conjugation can be among or extra carbon–carbon (double or triple) bonds, between carbon–carbon and carbon–oxygen double bonds and between double bonds and at an aromatic ring [11].

16.2 Detection of geometrical isomers

The trans isomers exhibit λmax at slightly longer wavelength and feature larger extinction coefficients than the Cis isomers. Examples Stilbenes in trans isomers show λmax at 294 nm, while the λmax Cis isomer has 278 nm. Detection of functional groups: to detect the presence of certain functional groups is possible, like conjugation, carbonyl group, and benzene ring.

Molecular weight determination: the molecular weight is determined. For example, the molecular weight of any amine is converted into amine picrate. Then a known concentration of amine picrate is dissolved in a litre of solution and its optical density is measured at λmax at 380 nm.

16.3 Dissociation constants of acids and bases

Consider an Acid (HA), it undergoes dissociation in water to form H_3O^+ and A^-, i.e.,

$$HA + H2O \rightarrow H_3O^+ \text{ and } A^-$$

16.4 Tautomeric equilibrium

To determine the percentage of various keto and enol forms present in a tautomeric equilibrium. Example: Ethyl acetoacetate in keto form has λmax 275 nm and ε = 16. This has only weak n → π* band of the isolated carbonyl group. The enol

form has λmax 244 nm and ε = 16,000, we can measure the proportions of tautomers present in ethyl acetoacetate.

16.5 Detection of impurities

The presence of impurities can be determined by the additional peaks and can be compared with that of standard raw material.

16.6 Structural elucidation

The presence or absence of unsaturation, the presence of heteroatom like S, O, N or halogen can be determined.

It is used to find out the percentage purity of samples from the formulations or raw material.

17. Conclusion

Ultra violet/visible spectroscopy is an analytical technique that is used to determine qualitatively and quantitatively for the estimation of different ions. It is a powerful technique for resolution enhancement when signal overlaps or interference occurs. This technique may also be used in many other industries. For example, measuring a colour index is useful for monitoring transformer oil as a preventative measure to ensure electric power is being delivered safely.

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