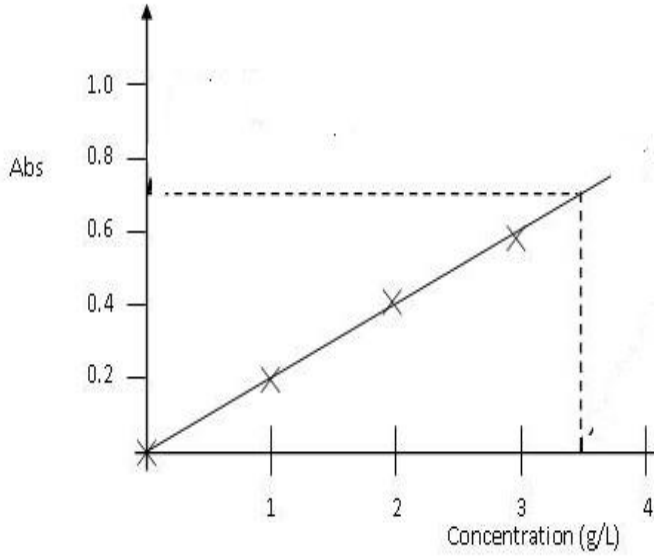
COLORIMETRY

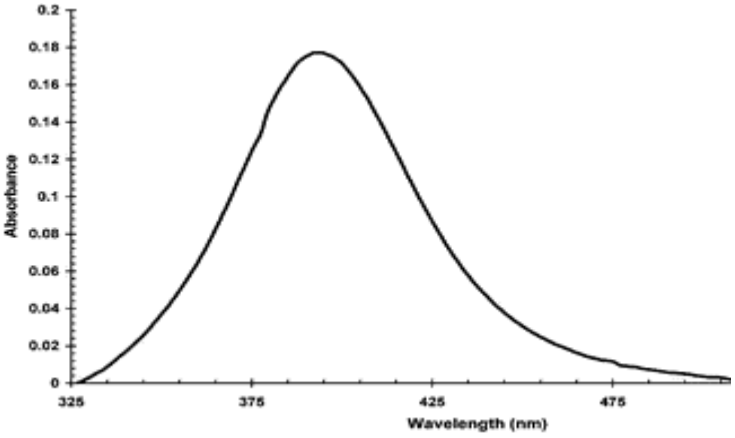
COLORIMETRY (400-800nm)

A colorimeter is a light-sensitive device used for measuring the transmittance and absorbance of light passing through a liquid sample. The device measures the intensity or concentration of the color that develops upon introducing a specific reagent into a solution. By knowing which wavelengths have passed through, the detector can also work out which colored wavelengths were absorbed. The colorimeter uses the Beer-Lambert law to detect the absorbance of the wavelength. Beer-Lamberts law is commonly written as:

$A = \varepsilon cl$

Where, A is the absorbance, Ɛ (epsilon) is the molar absorptivity, c is the concentration of the solution and l is the length that the light passes through (also known as the mean free path). Aside from this, if there is a continual changing of the solution, i.e. it is a reaction, then % of transmittance against time is generally used. The wavelength at which maximum absorbance of radiation take place is called as λmax. λmax is a qualitative aspect, useful in identifying the substance. λmax is not usually affected by concentration of the substance. The absorbance of a solution increases with increasing concentration but there is no change in λmax when concentration changes.





$A = 2 - \log_{10} \%T$

Converting absorbance to transmittance

Absorbance (optical density)	Light Transmittance %
0	**100%**
1	**10**
2	**1**
3	**0.1**
4	**0.01**
5	**0.001**
6	**0.0001**

Instrumentation

1- Source of light- The visible spectrum ranges from 400-800 nm.
Tungsten lamp 2- carbon arc lamp
2- Filter and monochromator- the source of light gives radiation from from 400-800 nm. This is polychromatic in nature. So filter convert polychromatic light in monochromatic light. Two type of filter are used.

ABSORPTION FILTER **2- INTERFERENCE FILTER**

ABSORPTION FILTER- These filter are made up of glass coated with pigments or they are made up of dyed gelatin. They absorb the unwanted radiation and transmit the rest of the radiation which is required for colorimetry. The filter can be selected according to the colour solution. If the colour of the solution is red we have use green filter and if red color we use green filter.

Si. No.	Color of the Solution	Colour Absorbed	Wavelength of Absorption
1.	Yellow to Green	Violet	400 nm – 435 nm
2.	Yellow to Orange	Blue	435 nm – 490 nm
3.	Red	Blue to Green	490 nm – 500 nm
4.	Purple	Green	500 nm – 560 nm
5.	Violet	Yellow to Green	560 nm – 580 nm
6.	Blue to Green	Yellow to Orange	580 nm – 650 nm
7.	Bluish Green	Red	650 nm – 700 nm

RED------GREEN BLUE---- ORANGE VIOLET------YELLOW

INTERFERANCE FILTER-

It has dielectric spacer made up of CaF2, MgF2. Between two parallel reflecting silver films. The mechanism is that, the radiation reflected by second film and the incoming radiation undergoes constructive interference to give a monochromator.

ULTRAVIOLET AND VISIBLE SPECTROSCOPY

This absorption spectroscopy uses electromagnetic radiations between 190 nm to 800 nm and is divided into the ultraviolet (UV, 190-400 nm) and visible (VIS, 400-800 nm) regions. Since the absorption of ultraviolet or visible radiation by a molecule leads transition among electronic energy levels of the molecule, it is also often called as electronic spectroscopy. The information provided by this spectroscopy when combined with the information provided by NMR and IR spectral data leads to valuable structural proposals.

NATURE OF ELECTRONIC TRANSITIONS

The total energy of a molecule is the sum of its electronic, its vibrational energy and its rotational energy. Energy absorbed in the UV region produces changes in the electronic energy of the molecule. As a molecule absorbs energy, an electron is promoted from an occupied molecular

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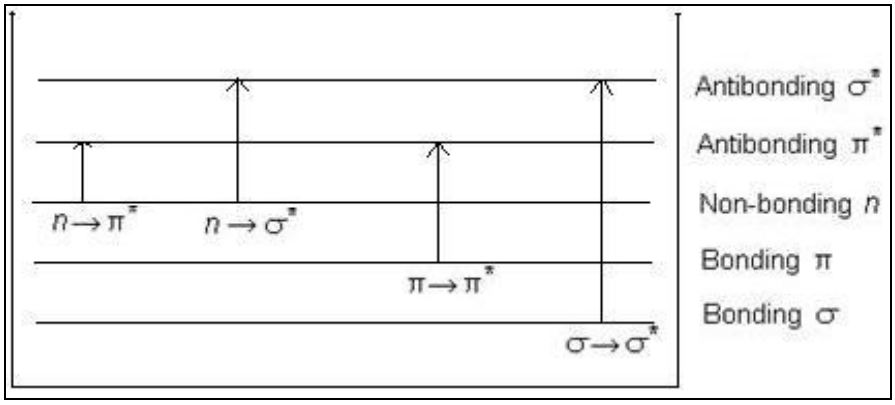
(1) $\sigma \rightarrow \sigma^*$ (2) $\pi \rightarrow \pi^*$ (3) $n \rightarrow \sigma^*$ (4) $n \rightarrow \pi^*$

Transition	Example	Cause/Source	λ (ηm)
$\sigma \rightarrow \sigma^*$	hexane	Alkanes (sat'd HC's)	< 200
$\pi \rightarrow \pi^*$	C_2H_4, 1,4-pentadiene	Unconjugated, unsaturated cpds.and conjugated systems	<200
$n \rightarrow \sigma^*$	CH_3NH_2, CH_3OH, CCl_4	Most heteroatom's in saturated cpds.	160 - 190
$n \rightarrow \pi^*$	ACETONE,	heteroatom's in unsaturated cpds.	> 250



$\sigma \rightarrow \sigma^*$-

This type of transition require the highest energy .this is observed with saturated hydro carbon .The peak does not appear in uv region but occur in vacuum region.(below 200nm) .it have lowest wavelength among all the transition.

Eg.-Methane, Ethane, Cyclo propane

$\pi \rightarrow \pi^*$-

Compound contain double or triple bond the energy requirement of this transition is between $n \rightarrow \sigma^*$& $n \rightarrow \pi^*$

Eg. Alkene, Alkyne, carbonyl

$n \rightarrow \sigma^*$

This transition occurs in saturated compound with hetero atom like S, O, N, Cl it require lesser energy when compare to $\sigma \rightarrow \sigma^*$ transition .Normally peak due to this transition occur from 180nm to 250nm**.**

Eg-CH_3Cl, H_2O, CH_3OH

$n \rightarrow \pi^*$-

Of all type of transition $n \rightarrow \pi^*$require the lowest energy (longer wave length) this type of transition can be seen in compounds where "n" electron (present in S, O, N, Cl) is present in a compound containing double or triple bond

Eg-Aldehyde ,ketone, nitro compound.

PRINCIPLES OF ABSORPTION SPECTROSCOPY : BEER'S AND LAMBERT'S LAW

The greater the number of molecules that absorb light of a given wavelength, the greater the extent of light absorption and higher the peak intensity in absorption spectrum. If there are only a few molecules that absorb radiation, the total absorption of energy is less and consequently lower intensity peak is observed. This makes the basis of Beer-Lambert Law which states that the fraction of incident radiation absorbed is proportional to the number of absorbing molecules in its path.

BEER`S LAW-

The intensity of beam of monochromatic light decreases exponentially with increasing in the concentration of the absorbing species arthmetically

$$-di/dc \propto I$$

$$-dI/dc = kI$$ (removing and introducing the constant of proportionality ''k'')

$$-dI/I = kdc$$
$$-\ln I = kc+b \quad \ldots\ldots(1)$$

(On integration ,b is constant of integration)

When concentration =0 there is no absorbance hence $I= I_0$ substituting in equation 1

$-\ln I_0 = kX0+b$, $-\ln I_0 = b$ putting the value of b in equation 1

$$-\ln I = kc - \ln I_0 \quad ,$$
$$\ln I_0 - \ln I = kc$$

ln I_0/I= kc **(**Since logA-logB= LogA/B)

$I_0/I = e^{kc}$ (removing natural logarithim)

$I/I_0 = e^{-kc}$ (making inverse on both side)

$I = I_0 e^{-kc}$……2 (equation for beers law)

LAMBERT`S LAW-

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

$- di/dt \propto I$

$- dI/dt = kI$ (removing and introducing the constant of proportionality ‘’k’’)

$- dI/I = kdt$
$-\ln I = kt+b$ ……(1)

(On integration ,b is constant of integration)

When concentration =0 there is no absorbance hence $I= I_0$ substituting in equation 1

$-\ln I_0 = kX0+b$, **$-\ln I_0 = b$** putting the value of b in equation 1

$-\ln I = kt - \ln I_0$,
$\ln I_0 - \ln I = kt$
ln I_0/I= kt **(**Since logA-logB= LogA/B)

$I/I_0 = e^{-kt}$ (making inverse on both side)

$I = I_0 e^{-kt}$…….3 (equation for Lamberts law)

Eq.2 & 3 can be combine to get

$I = I_0 e^{-kct}$
$I = I_0 10^{-kct}$ (Converting natural log to base 10)
$I/ I_0 = 10^{-kct}$

$I_0/I = 10^{kct}$ **(**inverse on both side**)**

log **I_0/I =kct** **(**Taking log on both side**)**…….(4)

Transmittance (**T)= I/I_0** & Absorbance **(A) = log 1/T**

Hence A=log 1/ I/I_0

A=log **I_0/I .....(5)**

Using Eq. 4 & 5 we can infer that

A=kct or A=∈ct

Mathematical Eq. for beers lambert law A=Absorbance
∈=Molicular extinction coefficient
C=conc. Of drug (MMol/L)
t =Path length

CHROMOPORE – Any group or part of a molecules that is responsible for characteristics absorption in the visible or uv region
Chromopore are covalentally unsaturated group/molecules
Eg.- >C=C< , N=N-Cl, NO_2
In nitro benzene nitro group is a chromophore which impart yellow colour in compound

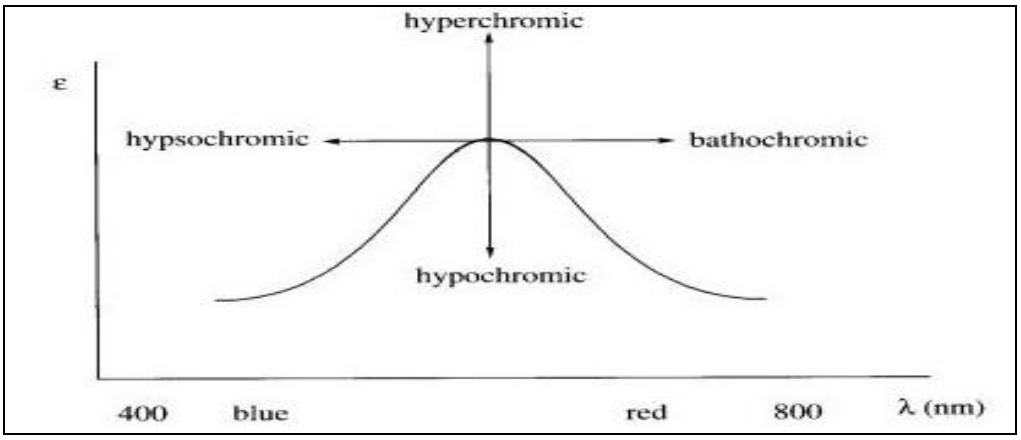
AUXOCHROME: The substituent's that themselves do not absorb ultraviolet radiations but their presence shifts the absorption maximum to longer wavelength is called auxochromes. The substituents like methyl, hydroxyl, alkoxy, halogen, amino group etc. are some examples of auxochromes.

A. BATHOCHROMIC SHIFT OR RED SHIFT: A shift of an absorption maximum towards longer wavelength or lower energy.

B. HYPSOCHROMIC SHIFT OR BLUE SHIFT: A shift of an absorption maximum towards shorter wavelength or higher energy.

C. HYPOCHROMIC EFFECT: An effect that results in decreased absorption intensity.

D. HYPERCHROMIC EFFECT: An effect that results in increased absorption intensity.



FACTORAFFECTING ABSORPTION

1-CONJUGATION-Conjugtion increase the λ toward higher value

Eg,- CH_3-CH =CH-CH =CH-CH_3 (λ = 217nm)

2-ALKYL HALLIDE- CH_3X With increase size of halogen atom (increase electro negativity) the λ is increases CH_3Cl has higher electro negativity & because of this non bonding electron (n electron) on chlorine atom are difficult to excite.
Higher energy (λ) CH_3Cl=172nm,, CH_3I=258nm where n electron on iodine are loosly bound

3-EFFECT OF HYDROGEN BONDING- Hydrogen bonding shift the uv absorbance to shorter wavelength hydrogen bonding cause needs for greater energy for transition.

Eg.-Amine absorb at higher wavelength than methanol
Greater hydrogen bonding more energy & decrease wave length ($E \propto 1/\lambda$)
CH_3OH =210nm (greater hydrogen bonding)
CH_3NH_2=240nm (lesser hydrogen bonding)

4-EFFECT OF ALKYL SUBSTITUTION- alkyl substitution of olefins move the absorption to longer λ.
Trance isomer absorb at longer λ with greater intensity than cis-isomer (effective π orbital over lapping in trans).

5-EFFECT OF SOVENT ON ∝ β UNSATURATED CARBONYL COMPOUND-

(A) n → π*- absorption band moves to shorter λ by increasing the polarity of solvent
In **n → π*** transition has ground state is more polar compared to excited state.

(B) π → π*- absorption bands move to longer λ by increasing the polarity the dipole dipole interaction with solvent molecule lower the energy of excited state more than ground state.
π*orbital get more hydrogen bonding with polar solvent it is because greater polarity of **π*** orbital compared to π orbital.

(C) n → σ*-absorption band move to shorter λ by increasing polarity.

6- BENZENE-
(A)-**alkyl substitution shift the absorption to longer λ slightly.**

Eg. Benzene **λ(280 nm) Toluene λ(285 nm)**

(B)**-non bonding (OH,OR,NH_2) shift the absorption to longer**

Benzene **λ(280 nm) Phenol (310nm)**

(C) M group para to +M group shift wavelength to higher value. As non bonding pair donator is effectively completed to the electron withdrawing. Eg. Para nitro aniline.

7- STERIO CHEMICAL FACTOR- As planarity decrease absorption shift to shorter wave length.eg biphenyl and 2-methyl biphenyl.

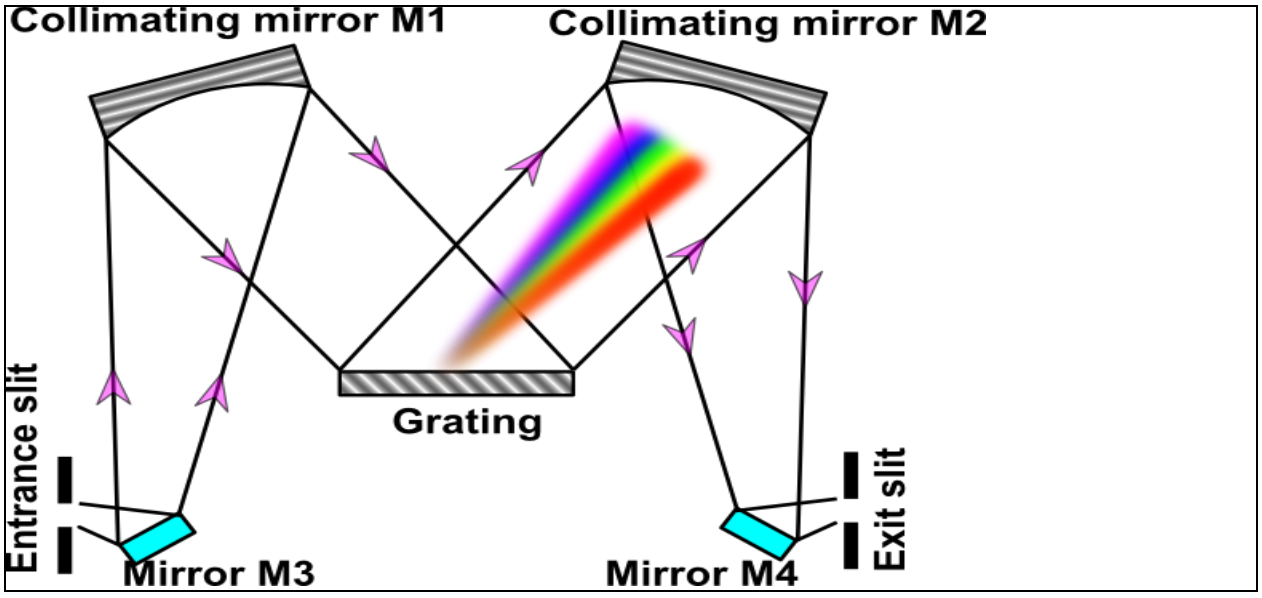
INSTRUMENTION

1- Light source 2- Monochromator 3- sample cell 4- solvent 5- detector 6- recorder

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 375nm.

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

All monochromators employ slits, mirrors, lenses, gratings or prisms.

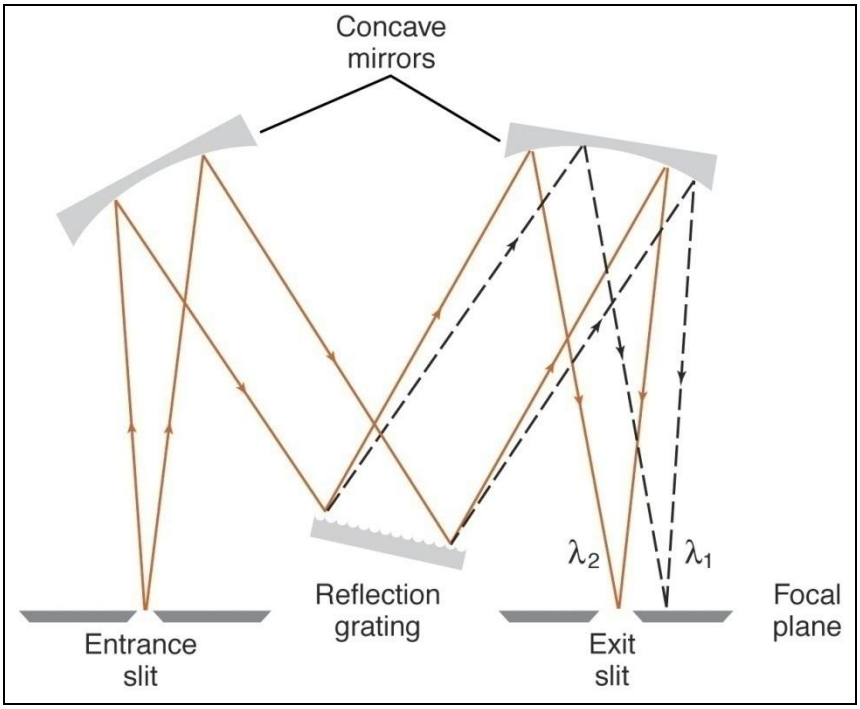


1-GRATING MONOCHROMATORS

Polychromatic radiationfrom the entrance slit is collimated (made into beam of parallel

rays) by a concave mirrors. these ray fall on reflection grating where upon different

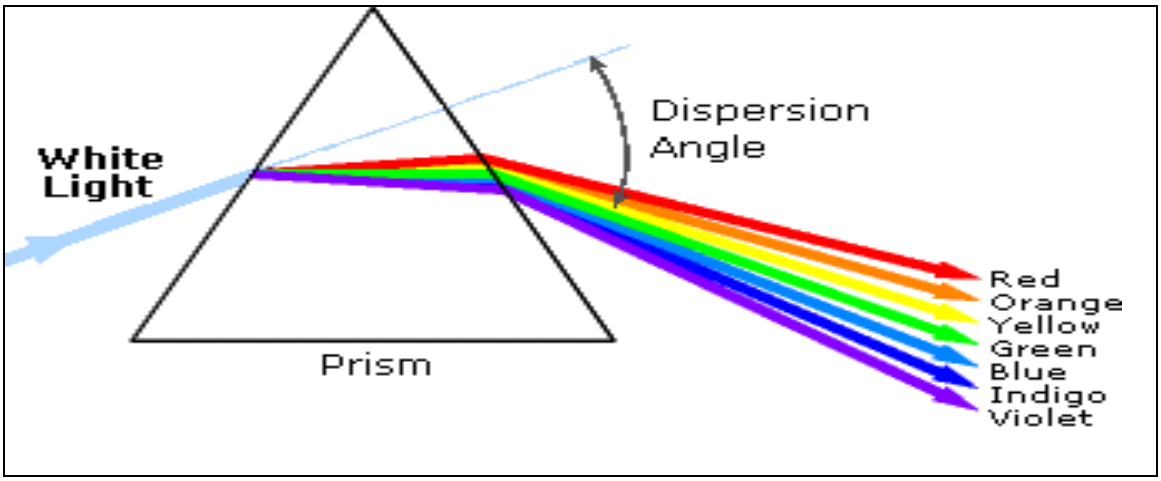
wavelength are reflected at different angles. The orientation of the reflection grating directs only one narrow band wavelength λ2, to the exit slit of the monochrmators.rotaion of the grating allows different wavelength λ1, to pass through the slit



The reflection grating monochromator Device consists of entrance and exit slits, mirrors, and a grating to disperse the light

2- PRISM MONOCHROMATORS

Dispersion by prism depends on refraction of light which is wavelength dependent. Violet color with higher energy (shorter wavelength) are diffracted or bent most While red light with lower energy (longer wavelength are diffracted or bent least As a result, the poly- chromatic white light is dispersed to its individual colors.



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 UVregion.

DETECTOR- The detectors are devices that convert radiant energy into electrical signal. Detector should be sensitive, and has a fast response over a considerable range of wavelengths. In addition, the electrical signal produced by the detector must be directly proportional to the transmitted intensity (linear response) Phototube emits electrons from a photosensitive, negatively charged cathode when struck by visible or UV radiation The electrons flow through vacuum to an anode produce current which is proportional to radiation intensity. 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.

Photon detector used mainly in u.v.vis. Near I.R.
Photo voltaic cell- Radiantenrgy produce current in a semiconductor.
Photo tube- Radiation cause emission of electron by the photoelectric effect.
Photomultiplier tube -Contain many photosensitive surfaces to multiply the produced by the photoelectric effect

1 PHOTO VOLTAIC CELL
2-PHOTO TUBE OR PHOTO EMMISIVE CELL
3-PHOTO MULTIPIER TUBE

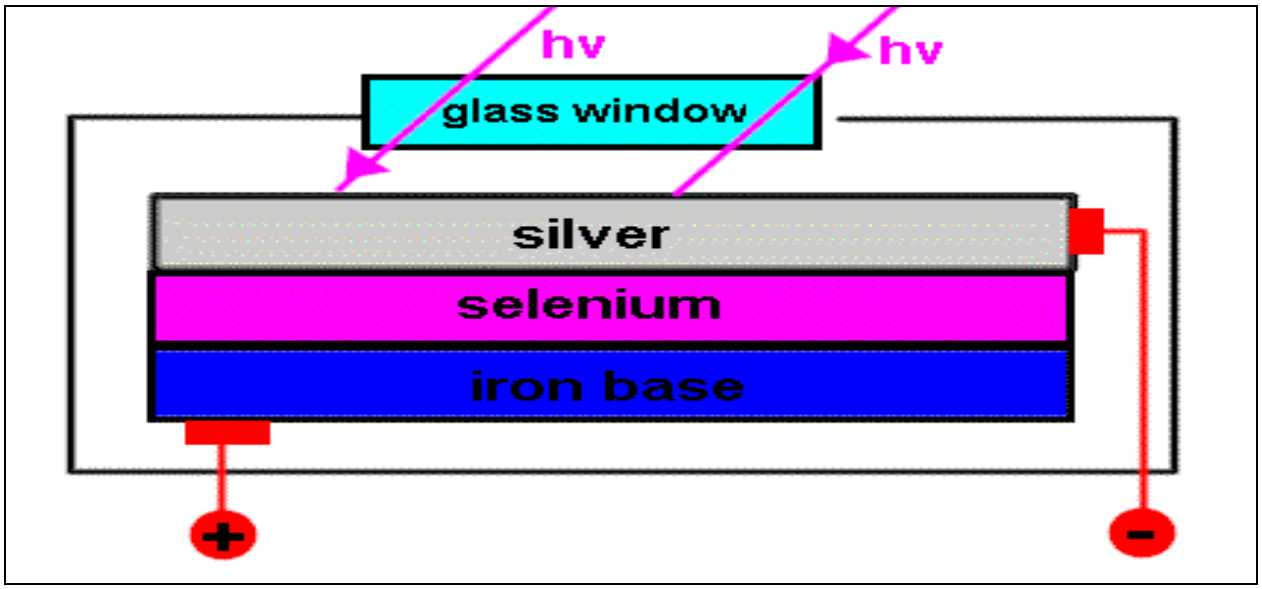
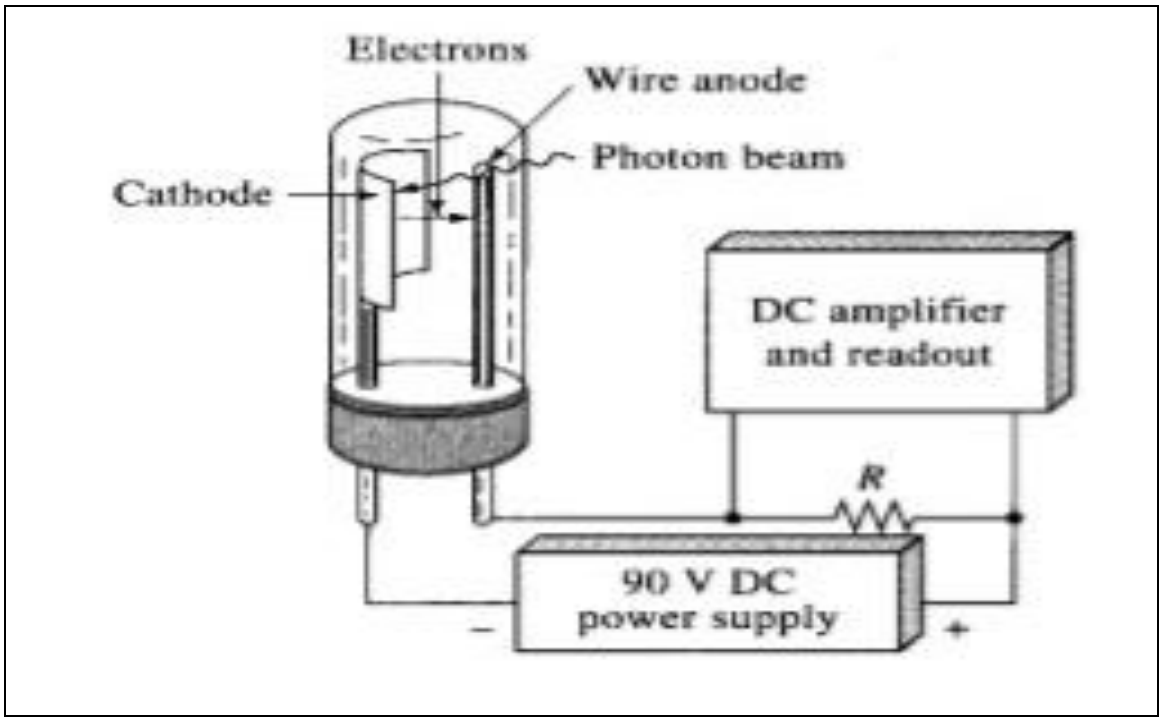


PHOTO VOLTAIC CELL

The detector has a thin metallic layer coated with silver or gold and act as an electrode. it has a metal base plate acts as another electrode . These two layer are separated by a semiconductor layer of selenium. When light radiation fall on selenium layer these electron become mobile and are taken up by the transparent metal layer. This creates a potential difference between the two electrodes and cause a flow of current. The flow of current cause deflection of the galvanometer which depend on the wavelength and intensity of radition.

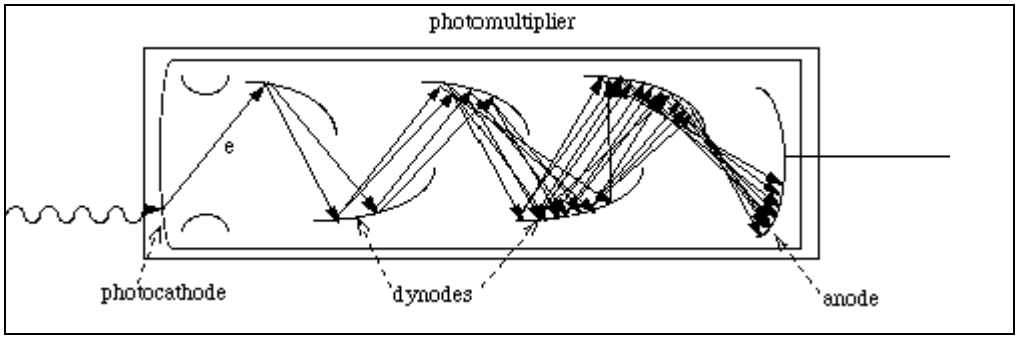
2-PHOTO TUBE OR PHOTO EMMISIVE CELL



This detector is composed of an evacuated glass tube which consists of a photo cathode and a collector anode. The photo cathode is coated with element of high atomic volume like cesium, potassium or silver oxide which can liberate electron when light radiation falls on it. The flow of electron towards anode produces a current proportional to the intensity of light radiation. The signal from the detector can also be amplified using an amplifier circuit.

PHOTO MULTIPIER TUBE

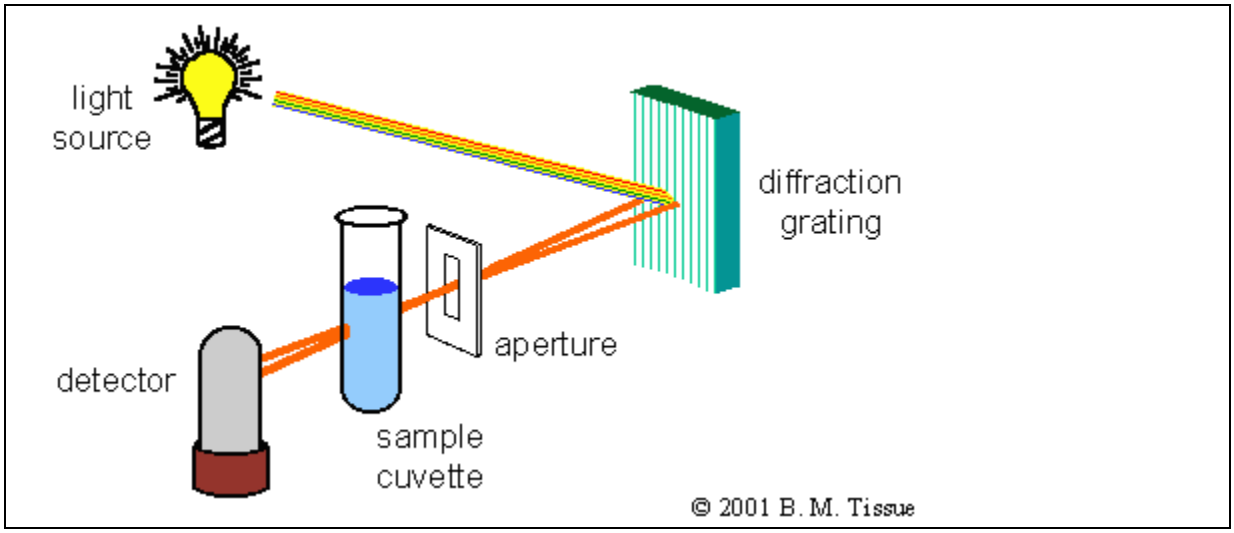
It is a very sensitive device in which electrons emitted from the photosensitive cathode strike a second surface called dynode which is positive with respect to the original cathode. Electrons are thus accelerated and can knock out more than one electrons from the dynode.
If the above process is repeated several times, so more than 10^6 electrons are finally collected for each photon striking the first cathode



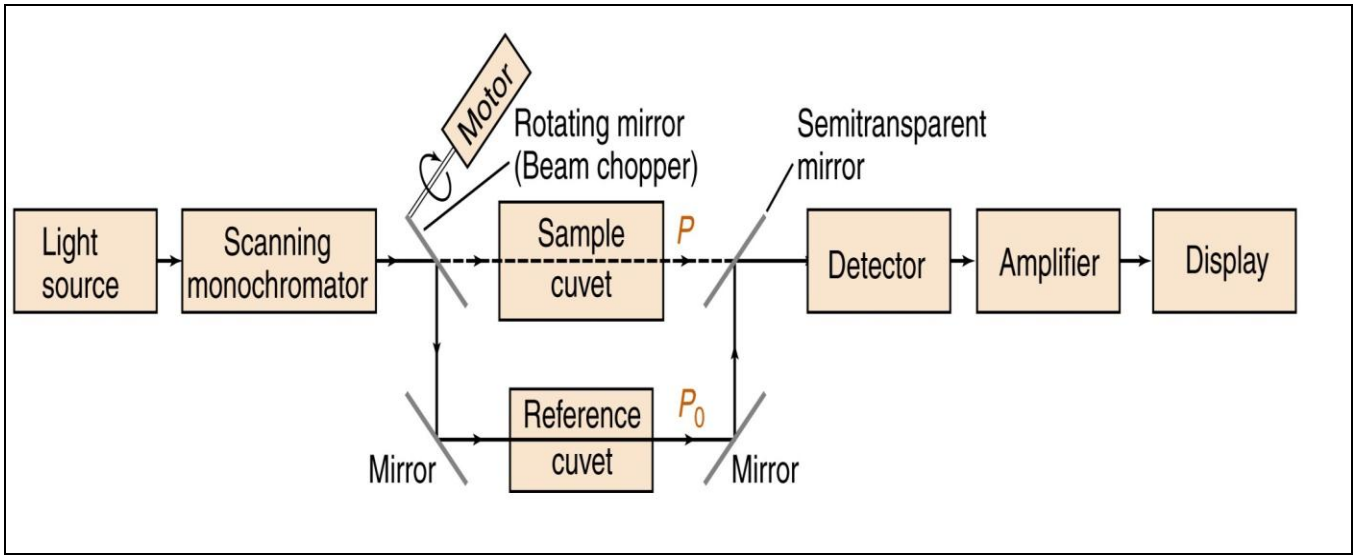
AMPLIFIER- The alternating current generated in the photocells is transferred to the amplifier. The amplifier is coupled to a small servometer. Generally current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear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

SINGLE BEAM SPECTRO PHTOMETER



(DOUBLE BEAM SPECTROPHOTOMETER)



In double beam arrangement, the light alternately passes through the sample and reference (blank), directed by rotating half-sector mirror (chopper) into and out of the light path. When light passes through the sample, the detector measures the P. When the chopper diverts the beam through the blank solution, the detector measures P_0. The beam is chopped several times per second and the electronic circuit automatically compares P and $P_{0\ to}$ calculate absorbancnce and Transmittance.

WOODWARD FIESER RULE

Woodward suggested empirical rules for predicting the absorption of open chain and six-membered ring dienes which have been later on extended to large number of dienes and trienes

Empirical Rules for Diene and Triene absorptions

Parent open chain / heteroannular diene	**214nm**
Homoannular diene	**253 nm**
Acylic trienes	**245 nm**
Butadiene / cyclic conjugated	**217 nm**
Increments for	
(a) each alkyl substituent or ring residue	**5 nm**
(b) double bond extending conjugation	**30 nm**
(c) exocyclic double bond	**5 nm**
(d) lone pair conjugation	
(i) O-C(=O)-R	**0 nm**
(ii) O-alkyl	**6 nm**
(iii) S-alkyl	**30 nm**
(iv) –Cl, -Br	**5 nm**
(v) NR2	**60 nm**

For example, here the absorption maxima for 2,4 hexadienes been calculated according to Woodward rules. The comparison of calculated λmax values with observed λmax values highlights the importance of these rules.

CH_3-CH =CH-CH =CH-CH_3 basic value =217

2 alkyl substituent 2x5= 10

Calculated value = 227

EMPIRICAL RULES FOR A, B-UNSATURATED KETONES AND ALDEHYDES ABSORPTION MAXIMA

Parent acyclic ketone or six-membered		215nm
Parent α, β-unsaturated pentanone		202nm
Parent α, β-unsaturated aldehyde		207nm
Increments for		
(a) a double bond extending conjugation		30 nm
(b) each alkyl group or ring residue	α	10 nm
	β	12 nm
	γ or higher	18 nm
(c) auxochrome		
(i) –OH	α	35 nm
	β	30 nm
	δ	50 nm
(ii) OCOR	α, β, δ	6 nm
(iii) OCH3	α	35 nm
	β	30 nm
	γ	17 nm
	δ	31 nm
(iv) Cl	α	15 nm
	β	12 nm
(v) Br	α	25 nm
	β	30 nm
(Vi) NR2	β	95 nm
(d) exocyclic double bond		5 nm
(e) Homocyclic diene		30 nm

APPLICATIONS OF UV SPECTROSCOPY

1-Molecular spectroscopy based upon UV-Vis radiation is used for identification and estimation of inorganic, organic and biomedical species. Molecular UV-Vis absorption spectrophotometry is employed primarily for quantitative analysis.

2. **Detection of extent of conjugation-** 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.

3. **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.

4. **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.

5. **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.