SPECTROSCOPY

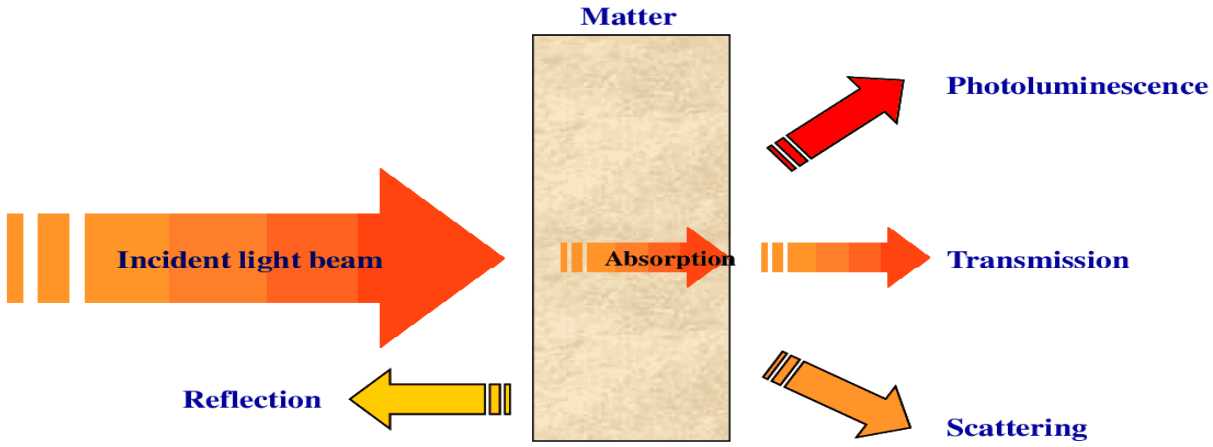
Dr. Fozia Batool
Assistant Professor
University of Sargodha

SPECTROSCOPY

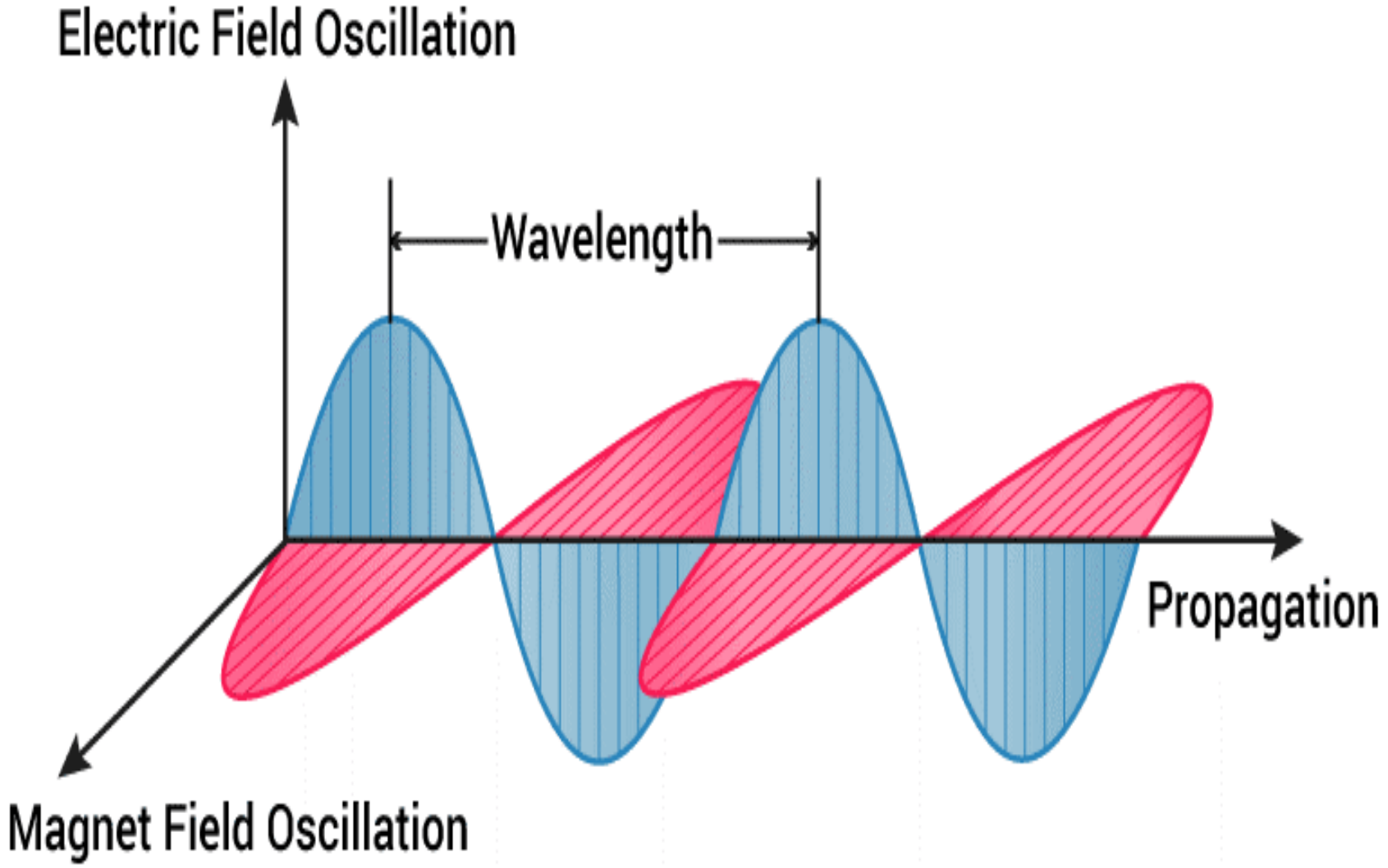
Principles of Spectroscopy

Interaction of radiation and matter

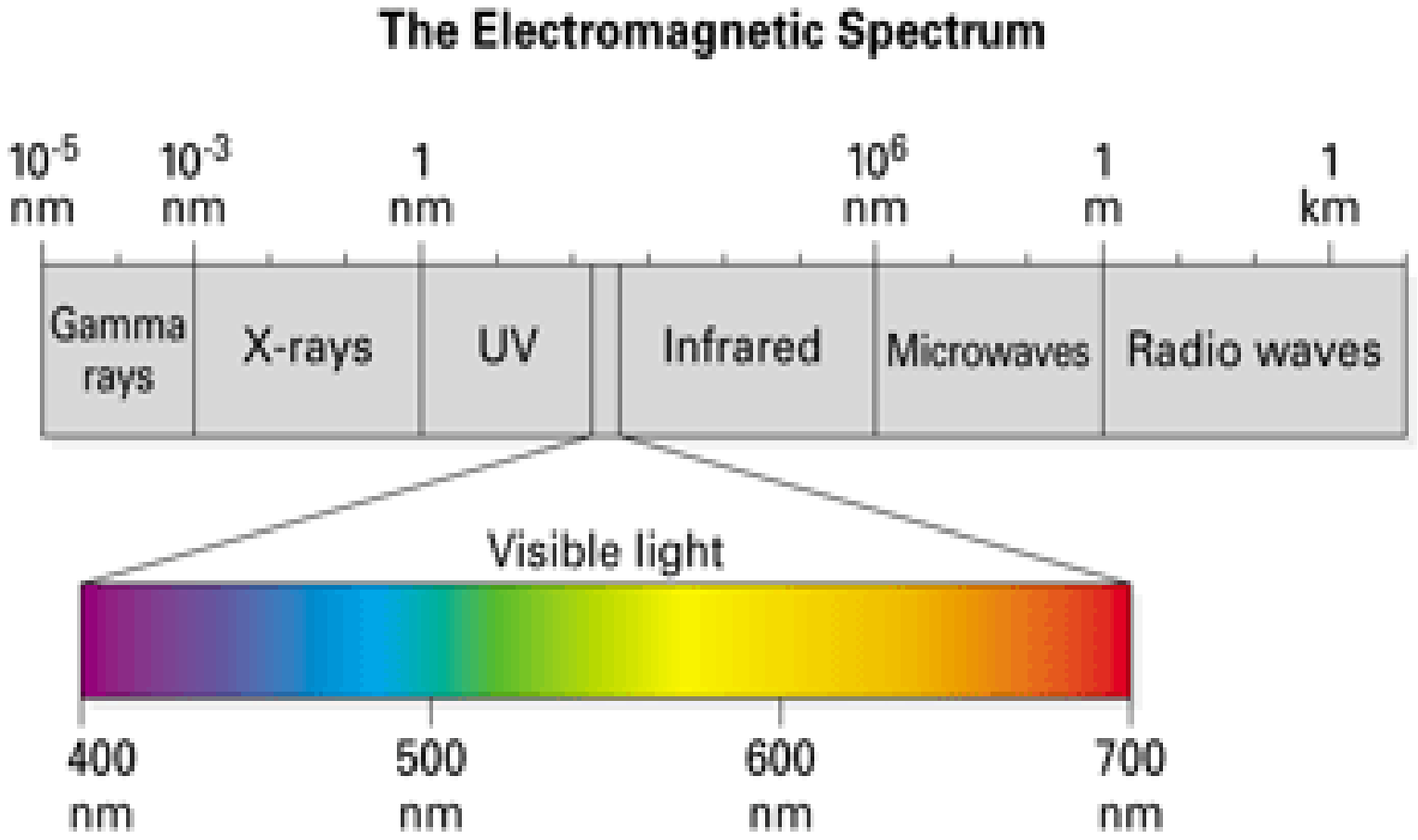
If matter is exposed to electromagnetic radiation, e.g. infrared light, the radiation can be absorbed, transmitted, reflected, scattered or undergo photoluminescence. Photoluminescence is a term used to designate a number of effects, including fluorescence, phosphorescence, and Raman scattering.



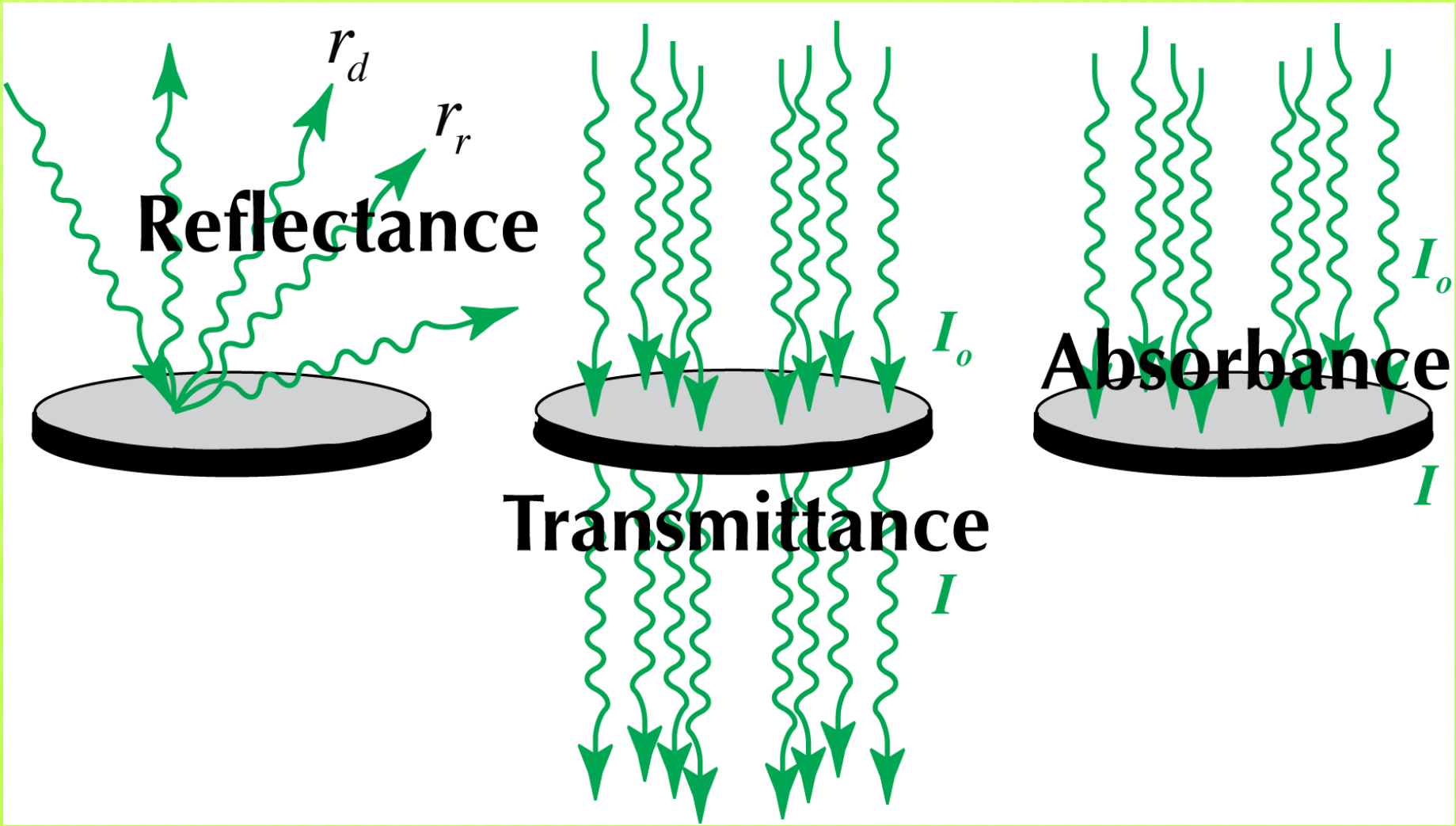
ELECTROMAGNETIC RADIATION



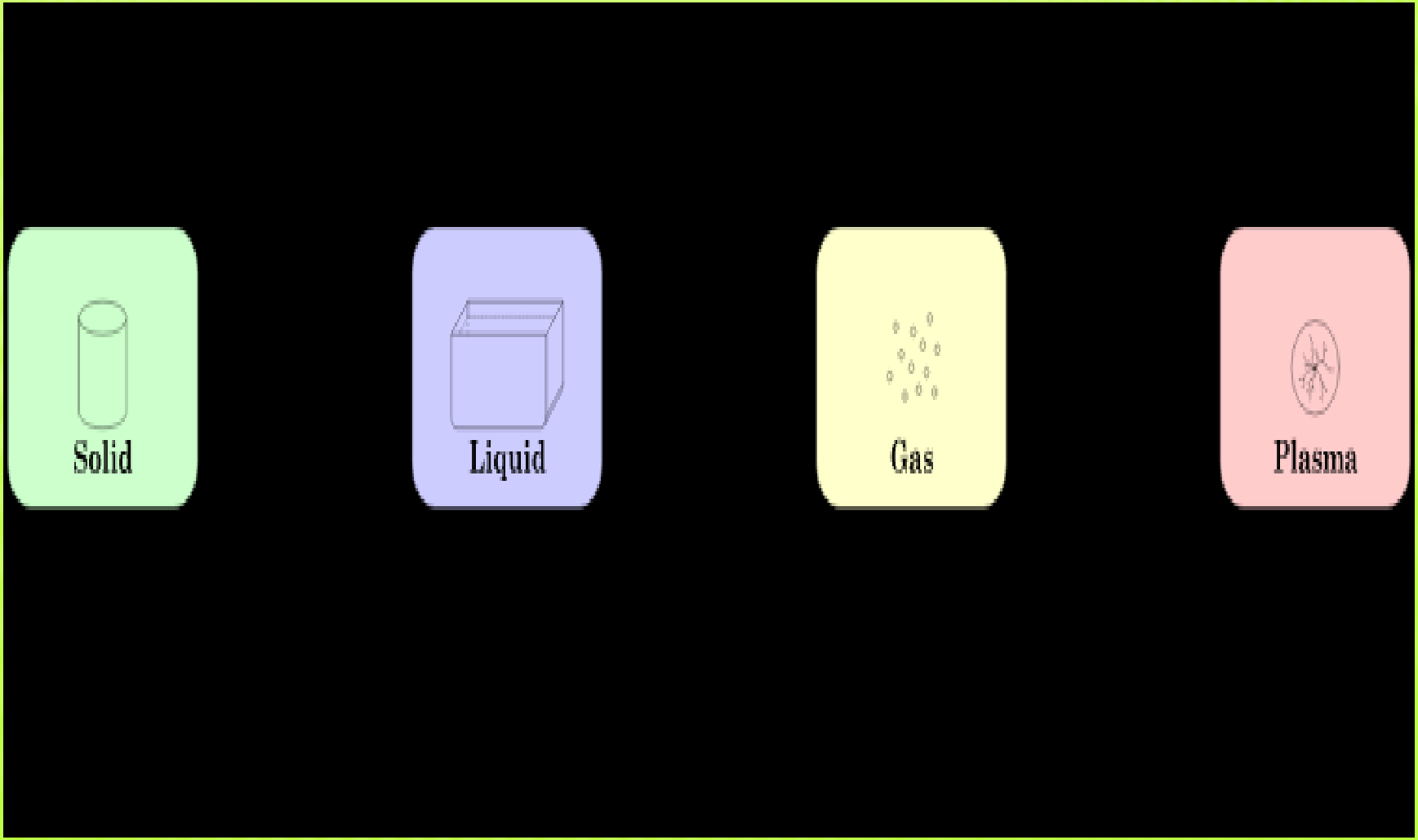
ELECTROMAGNETIC SPECTRUM



INTERACTION



MATTER



TYPES OF SPECTROSCOPY

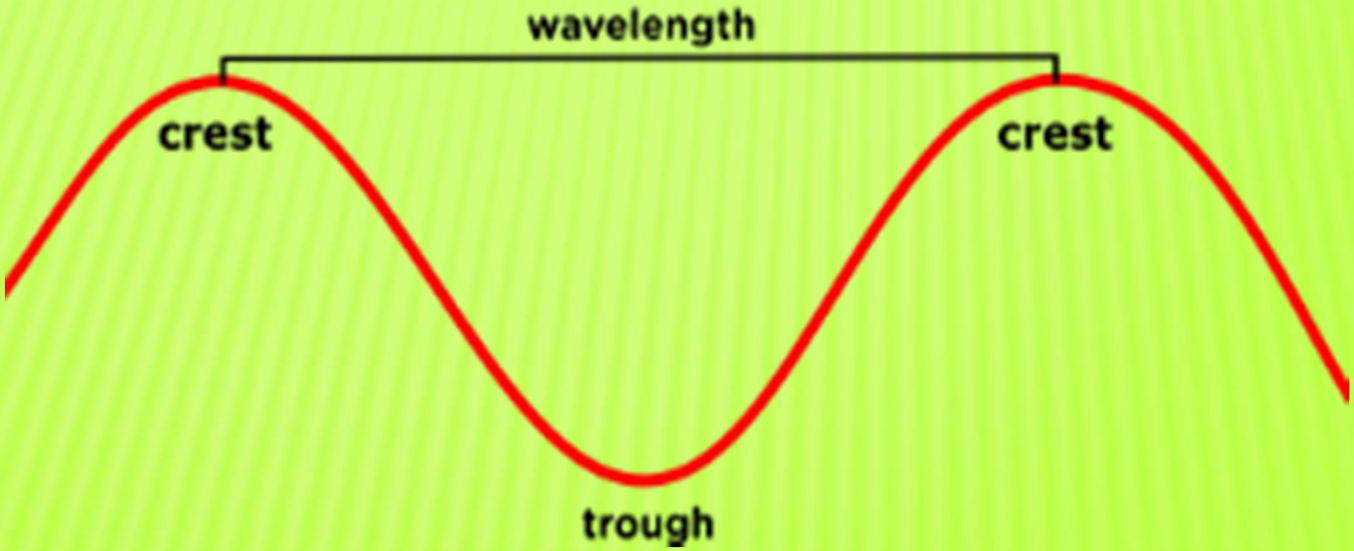
- Atomic Absorption Spectroscopy
- Atomic emission spectroscopy
- Attenuated Total Reflectance Spectroscope
- Electron Spectroscopy.
- Gamma-ray Spectroscopy.
- Infrared Spectroscopy.
- Laser Spectroscopy.
- Mass Spectrometry.
- Multiplex or Frequency-Modulated Spectroscopy
- Raman Spectroscopy
- X-ray Spectroscopy

SPECTROSCOPY

- WAVELENGTH = It is distance between two adjacent crest or trough. Units are m or nm
- FREQUENCY= It is number of waves passing through a point in unit time. Units are Hz
- WAVENUMBER= It is number of waves in unit distance. Units are m^{-1} or cm^{-1}

WAVELENGTH

- Distance between two adjacent crest or trough.



ENERGY

- $E = h\nu$
- where

  E = energy

  h = Planck's constant = 6.626×10^{-34} J·s

  ν = frequency

ENERGY

- $c = \lambda v$
- where
  c = speed of light = 3×10^8 m/sec
  λ = wavelength
  v = frequency
- Rearrange the equation to solve for frequency:
  $v = c/\lambda$

ENERGY

- Next, replace frequency in the first equation with c/λ to get a formula you can use:
  $E = h\nu$
  $E = hc/\lambda$

ENERGY

- If Wavelength is 633nm.
- $E = 6.626 \times 10^{-34}$ J·s x 3 x 10^{8} m/sec/ (633 nm x 10^{-9} m/1 nm)
  $E = 1.988 \times 10^{-25}$ J·m/6.33×10^{-7} m E = 3.14 x $^{-19}$ J

  Answer:

  The energy is 3.14 x $^{-19}$ J.

RELATIONSHIP OF UNITS

- $1nm = 10^{-9}m$
- $1m = 10^{9}\ nm$
- $1eV = 1.6022 \times 10^{-19}J$

PROBLEMS

- If a light of 500nm passes through a material, calculate energy in eV and J produced? Also calculate frequency and wave number?

SOLUTION

- Wavelength =500nm = 500×10^{-9}m
- $E = hc/\lambda$
- $E = 6.626 \times 10^{-34}$ J·s x 3 x 10^{8} m/sec/(500 x 10^{-9} m)
- 3.975×10^{-19} J

JOULE TO EV

- $E = 3.975 \times 10^{-19}$ J
- $1eV = 1.6022 \times 10^{-19}$J
- So,
- $E = 3.975 \times 10^{-19} / 1.6022 \times 10^{-19}$
- E = 2.480 eV

FREQUENCY AND WAVE NUMBER

- $v = c/\lambda$
- Wave number = $1/\lambda$

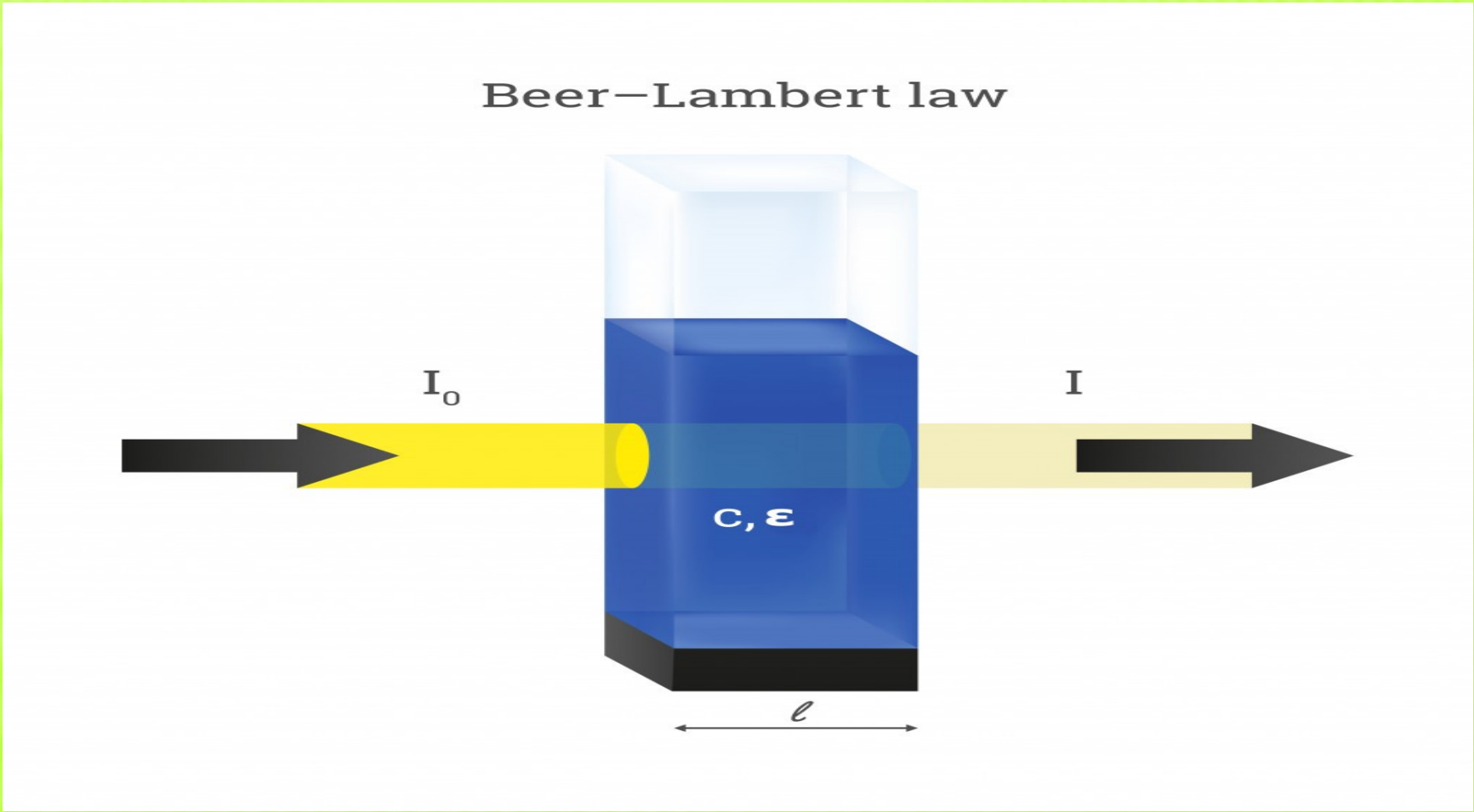
BEER-LAMBERT LAW

- Two separate laws governing absorption are usually known as lambert's law and beer's law, in the combined form they known as the beer's-lambert law.
- Lambert's Law: Lamberts law states that when monochromatic light passes through a transparent medium, the rate of decrease in intensity with the thickness of the medium is proportional to the intensity of the emitted light.

BEER-LAMBERT LAW

- According to Lambert,
- $A \alpha b$
- Where
- b = path length

BEER-LAMBERT LAW



BEER-LAMBERT LAW

- According to Beer-Lambert
- $A \alpha C.b$
- $A = \varepsilon.C.b$
- Where
- A = absorbance
- C = Molar Concentration
- b = Path length
- ε = Molar absorptivity

UNITS OF MOLAR ABSORPTIVITY

- $\varepsilon = A/C.b$
- $\varepsilon = 1x\ dm^3/mol\ x\ cm$
- $1\ dm^3 = 1000cm^3$
- $\varepsilon = 1000\ cm^3/mol.cm$
- $\varepsilon = 1000cm^2.\ mol^{-1}$

TRANSMITTANCE

- Transmittance: T = I / Io Eq. 1
- We can convert this ratio into a percentage by multiplying by 100 to get Percent Transmittance (%T):
- % Transmittance: I / Io X100
- Thus if the intensity of the light exiting our sample is 76 and the intensity of the light entering our sample is 100, then the Transmittance would be 0.76 and the % Transmittance would be 76%.

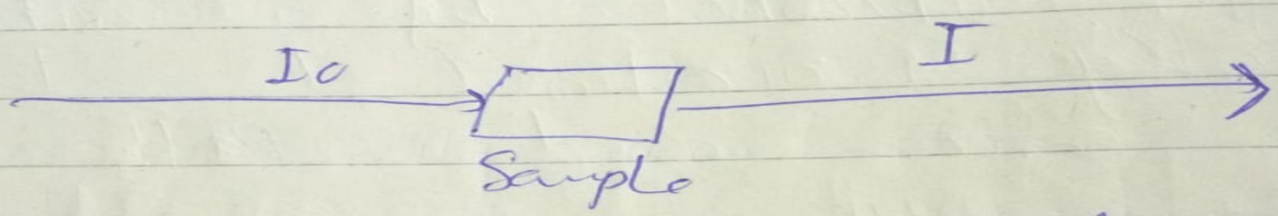
ABSORBANCE

- Absorbance: $A = -\log_1 T$

- Absorbance: $A = -\log_{10} I / Io$
- Or $A = \log_{10} Io/I$ Eq. 2
- Absorbance is a direct measure of how much light is absorbed by our sample. If you play with the formula in your calculator you will find that absorbance can take on values between 0 (at 100% Transmittance) and about 2.0 (at 1% Transmittance);

MAXIMUM AND MINIMUM TRANSMITTANCE

$$T = \frac{I}{I_0}$$

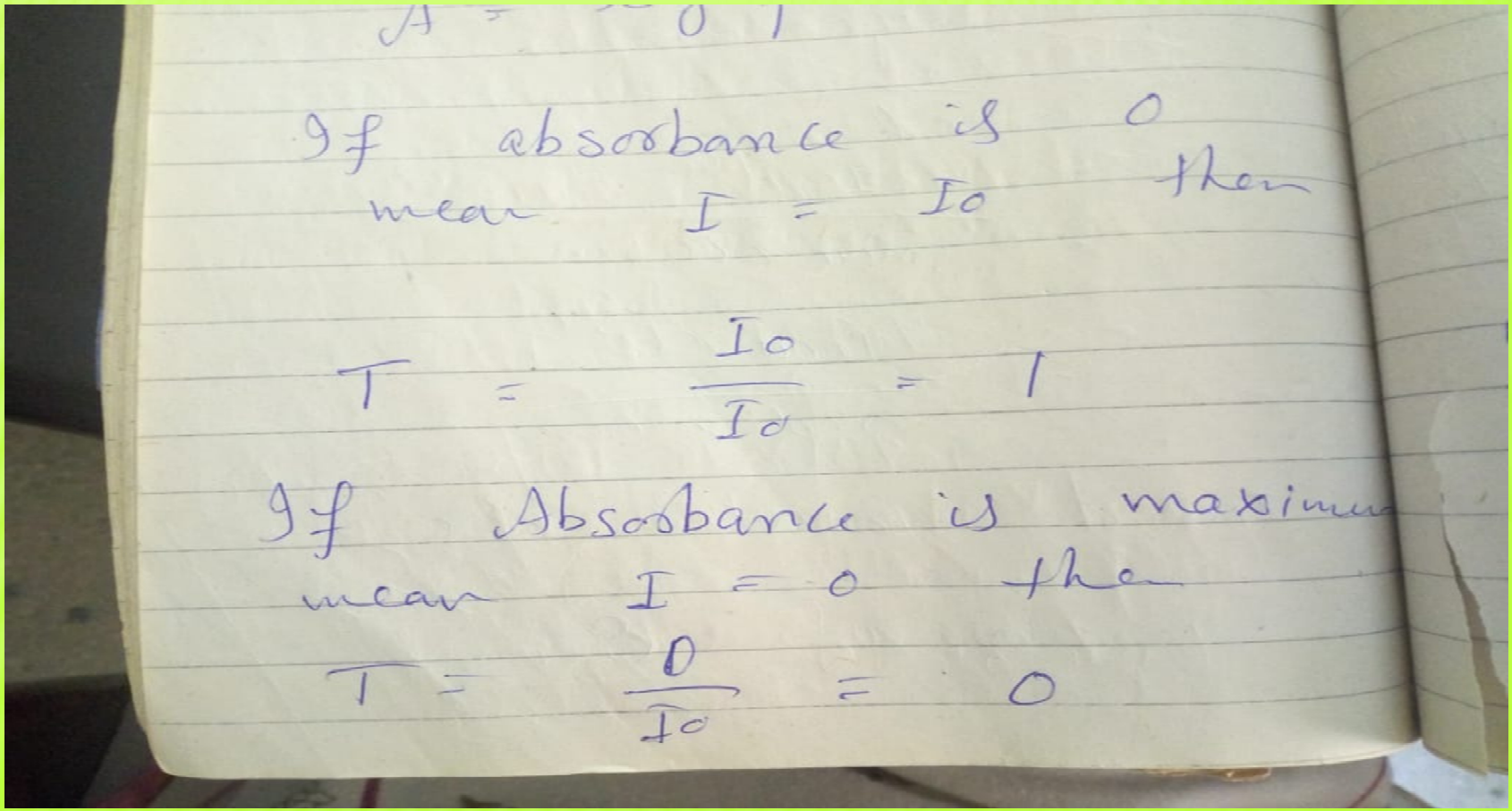


$$A = -\log T \quad \text{or} \quad \log \frac{1}{T}$$

$$A = \log \frac{I_0}{I} \quad \text{or}$$

$$A = \log \frac{1}{T}$$

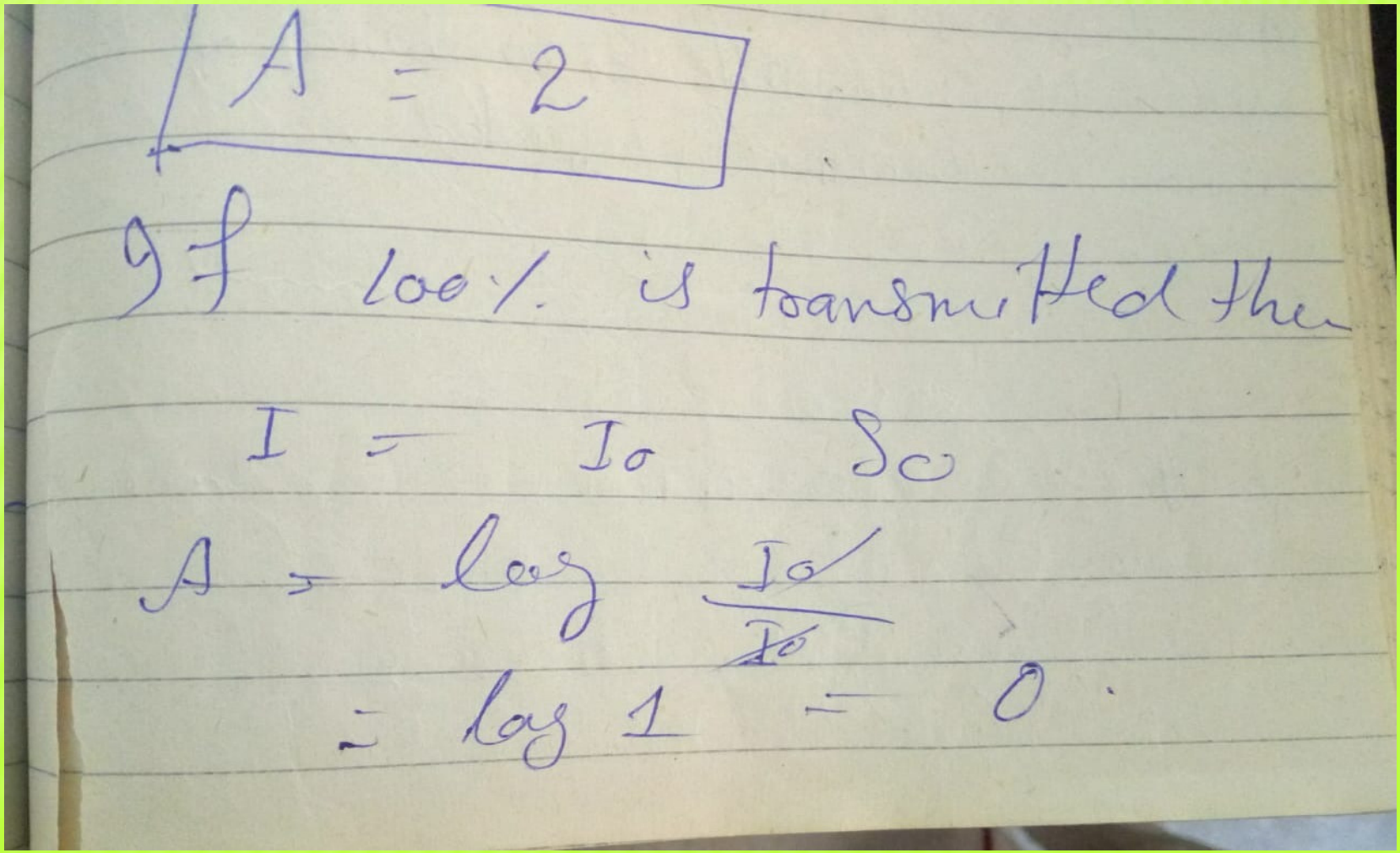
MAXIMUM AND MINIMUM TRANSMITTANCE



MAXIMUM AND MINIMUM ABSORBANCE

- If absorbance is maximum then,
- Transmittance will be minimum mean 100% light is absorbed. So we ca say,
- $I = Io/100$
- Putting this value of "I" in eq 2
- $A = \log_{10} Io/I$ eq 2
- $A = \log_{10} Io/Io \times 100$
- $A = \log_{10} 100 = 2$

MAXIMUM AND MINIMUM ABSORBANCE



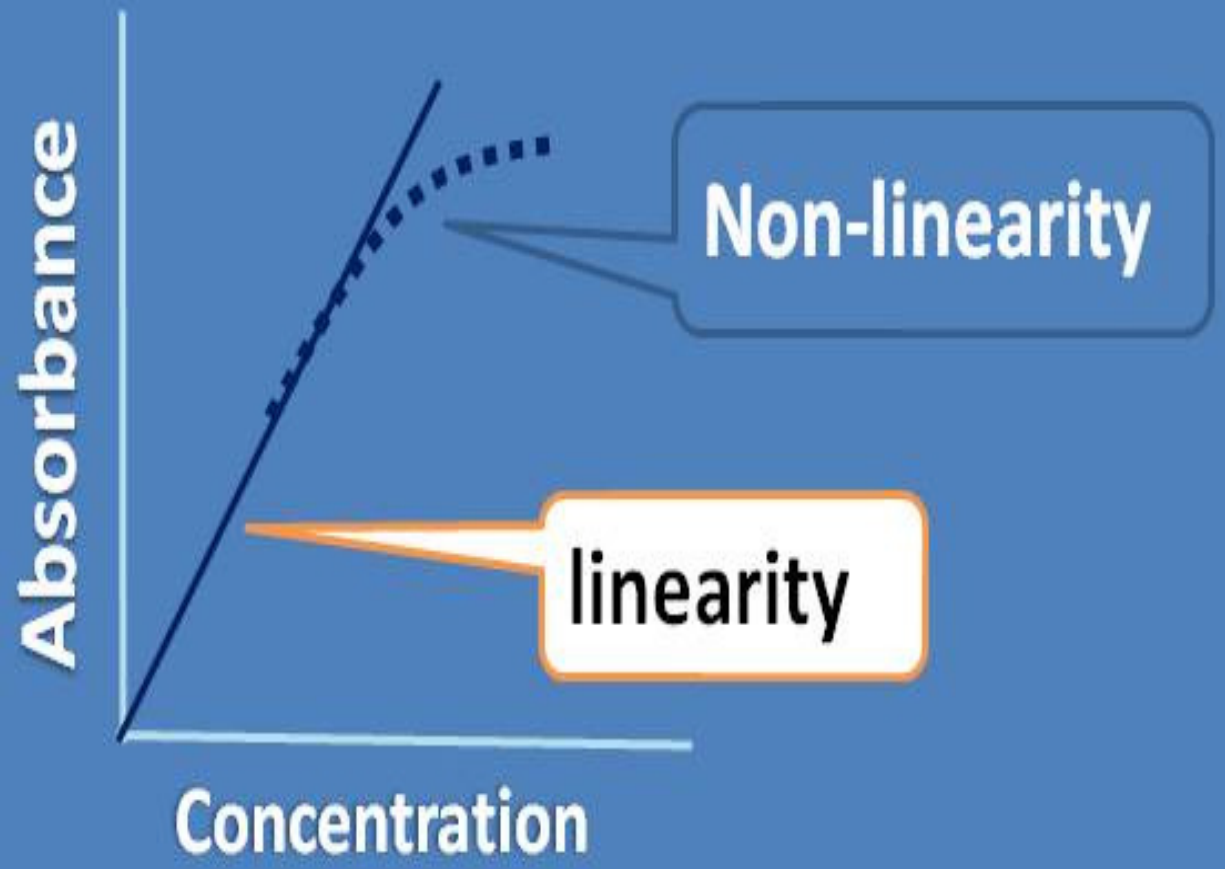
MAXIMUM AND MINIMUM A & T

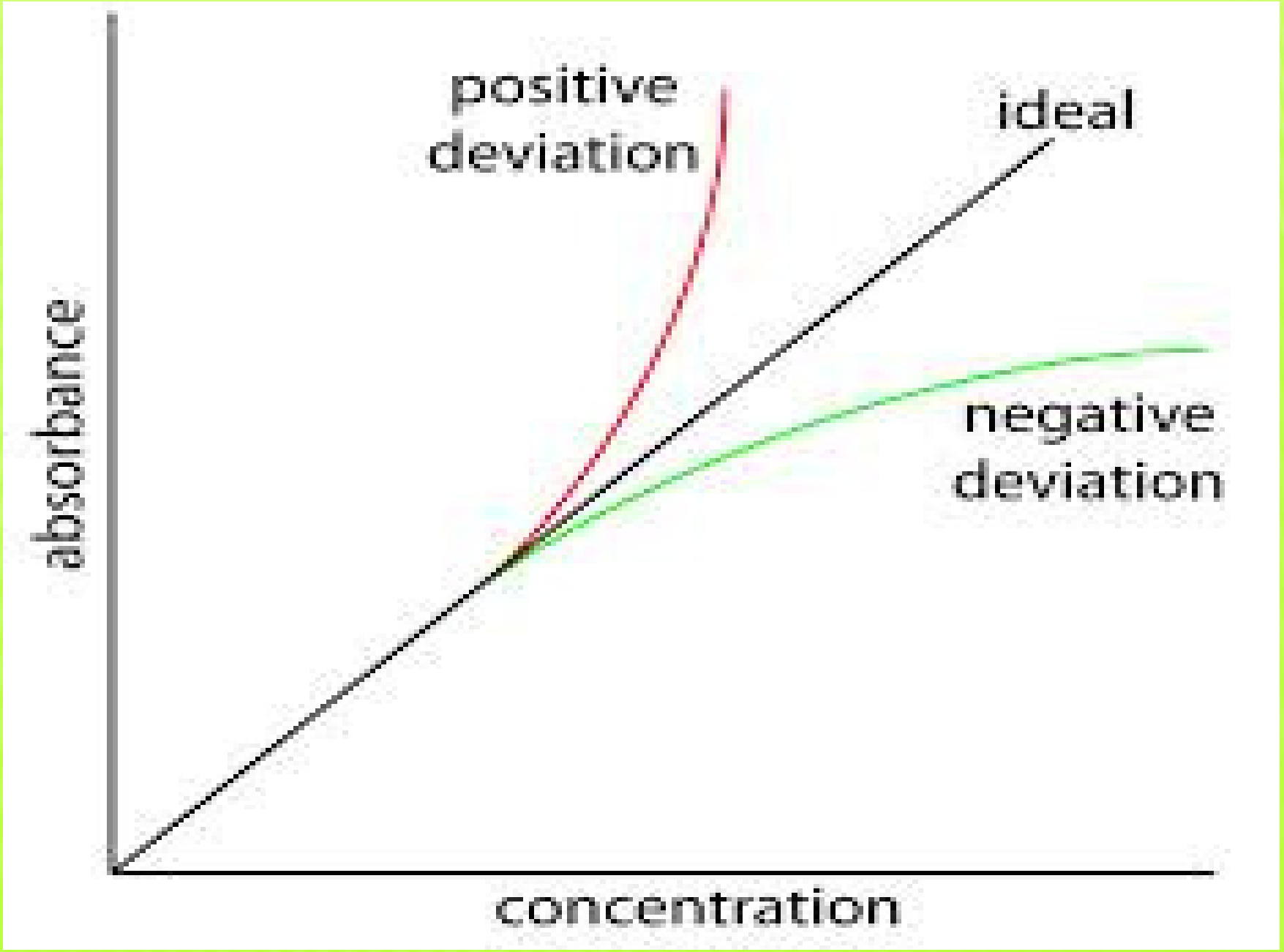
- So range of A IS 0-2
- Minimum A is 0
- Maximum A is 2
- Minimum T is 0
- Maximum T is 1 or 100%

DEVIATIONS FROM LAW

- Beer-Lambert law deviate from ideal behavior at high concentration of sample.
- Ideal behavior is increase in A with concentration in linear way.
- But at high concentration it will not happened.

Deviations from Beer-Lambert Law?




positive
deviation
ideal
negative
deviation
absorbance
concentration

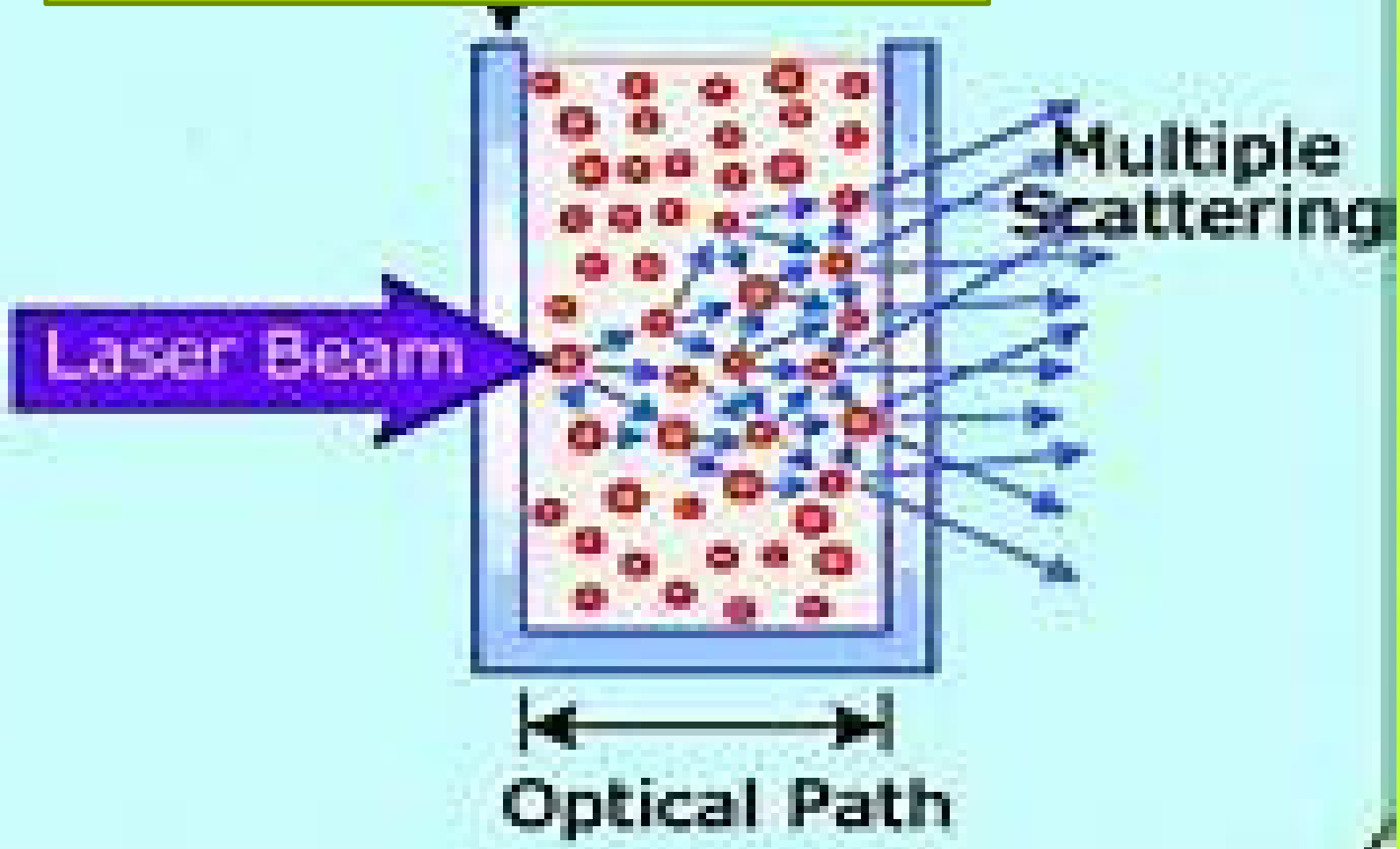
REASONS OF DEVIATION

- Beer-Lambert law is followed under some conditions and these conditions are,
- 1- Sample should not interact mutually. Each sample particle whether atom or molecule should behave independently.
- Sample solution should be completely clear so that no diffraction or scattering of light occure.

DEVIATION

- When concentration of sample is high in solution these conditions are not followed.
- At high concentration sample start interaction with each other and generate intermolecular forces.
- Due to high concentration scattering of light occurs.

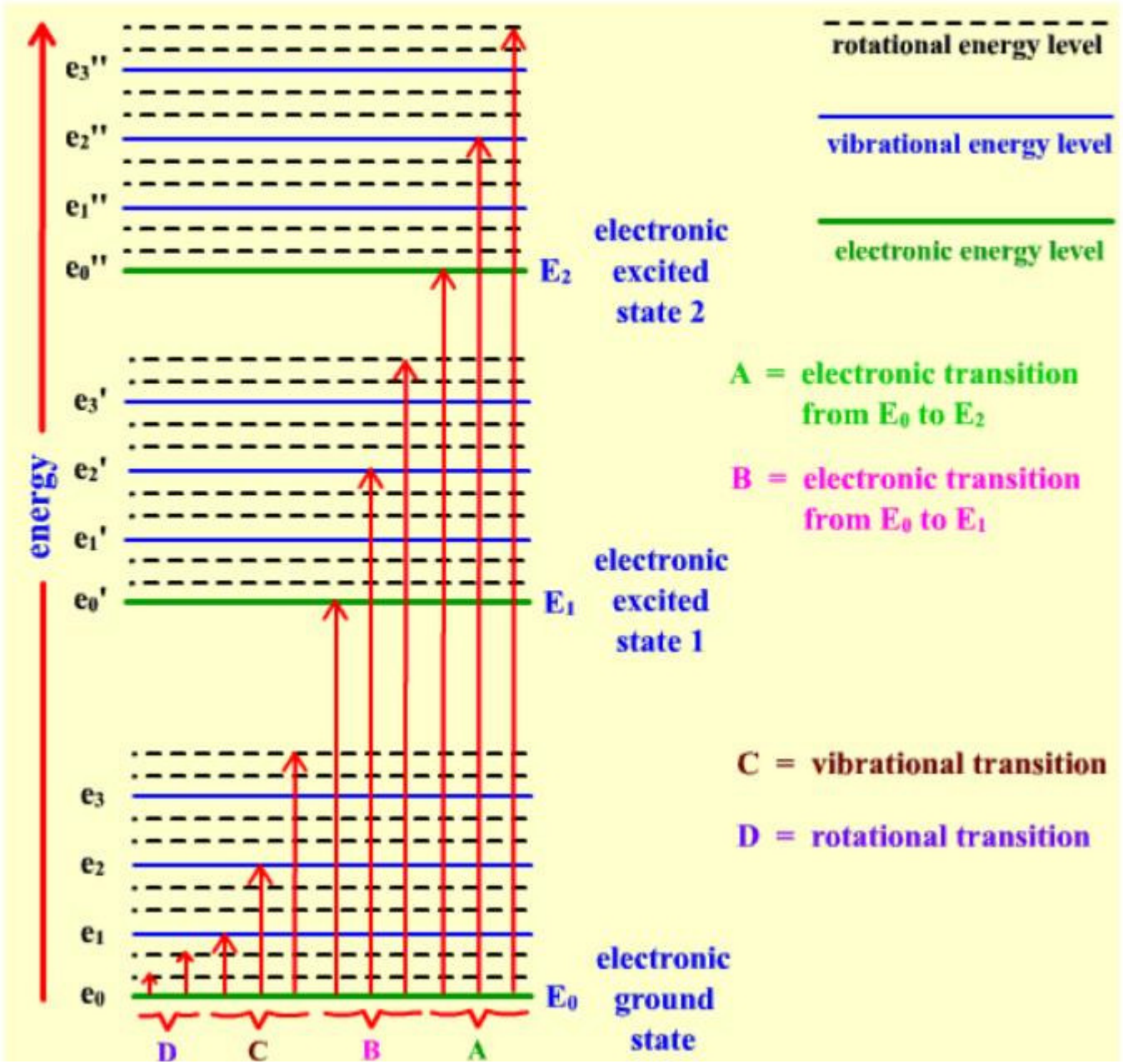
Deviation from Beer Lambert Law



MOLECULAR ENERGY LEVELS

- Electronic Energy Levels
- Vibrational Energy Levels
- Rotational Energy Levels

Origin of Electronic Spectra



ELECTRONIC TRANSITIONS (ET)

- ET occurs from lower electronic energy level to higher electronic energy level, or from vibrational or rotational of lower electronic to vibrational or rotational of higher electronic level.
- UV-Visible light is required for these transitions.

ELECTRONIC TRANSITIONS (ET)

- Molecular electronic transitions take place when electrons in a molecule are excited from one energy level to a higher energy level. The energy change associated with this transition provides information on the structure of a molecule and determines many molecular properties such as colour.

ELECTRONIC TRANSITIONS

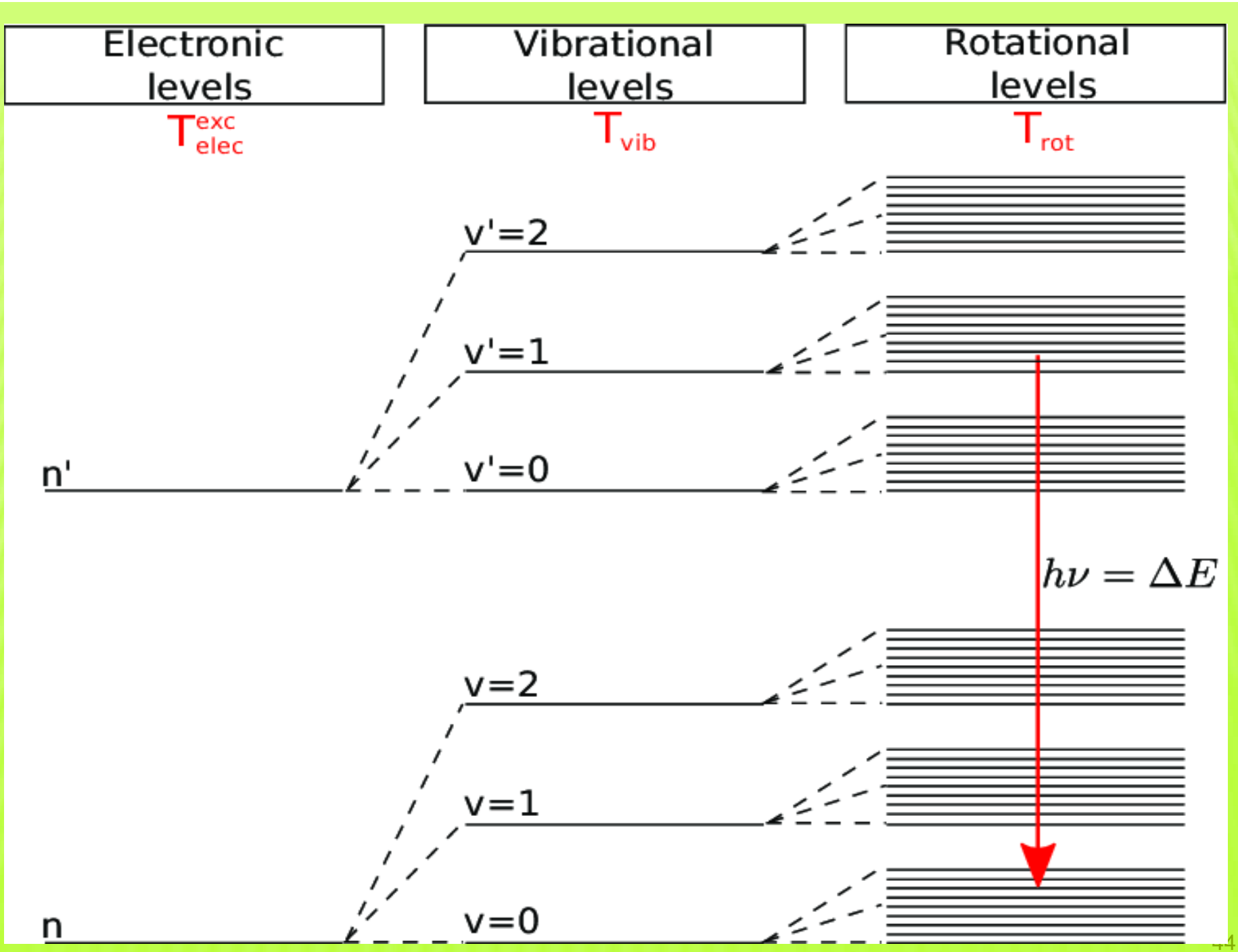
- The electronic transitions in organic compounds and some other compounds can be determined by ultraviolet–visible spectroscopy, provided that transitions in the ultraviolet (UV) or visible range of the electromagnetic spectrum exist for this compound.

VIBRATIONAL TRANSITIONS (VT)

- VT occurs from lower vibrational to upper vibrational, or from rotational of lower vibrational to rotational of upper vibrational.
- vibrational spectroscopy is a branch of molecular spectroscopy concerned with infrared and Raman spectra of molecules in the gas phase.
- IR radiations are required for these transitions.

ROTATIONAL TRANSITIONS (RT)

- RT occurs from one rotational to other rotational energy level.
- A rotational transition is an abrupt change in angular momentum.
- Microwaves are responsible for these transitions.

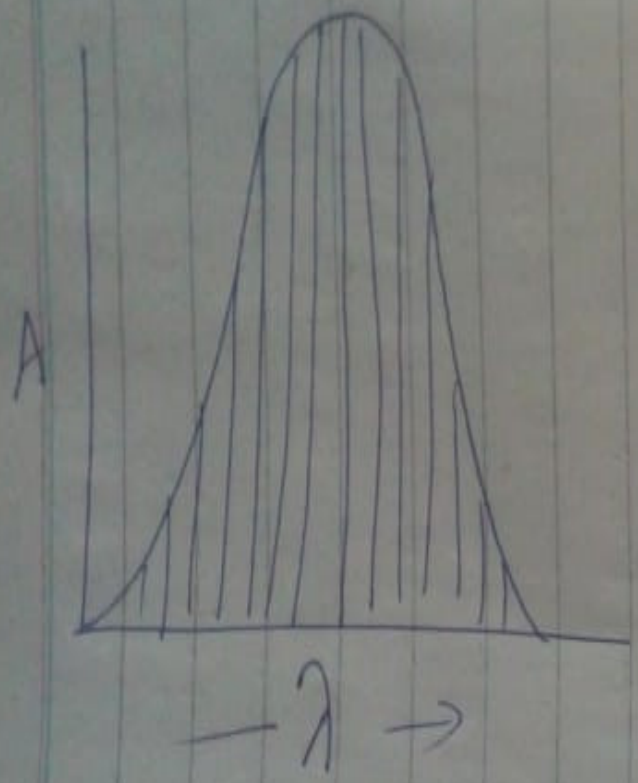

Electronic levels
T_{elec}^{exc}
Vibrational levels
T_{vib}
Rotational levels
T_{rot}
v'=2
v'=1
v'=0
n'
$h\nu = \Delta E$
v=2
v=1
v=0
n

SPECTRUM SHAPE

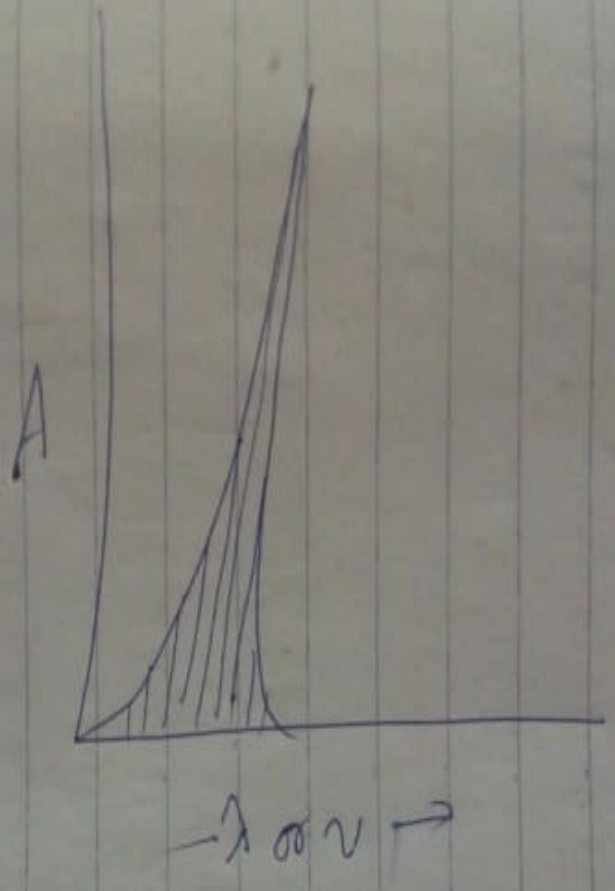
- Broad band for Electronic transitions. e.g. UV-Visb. Spectroscopy.
- Peak for Vibrational transitions.

e.g. IR Spectroscopy.

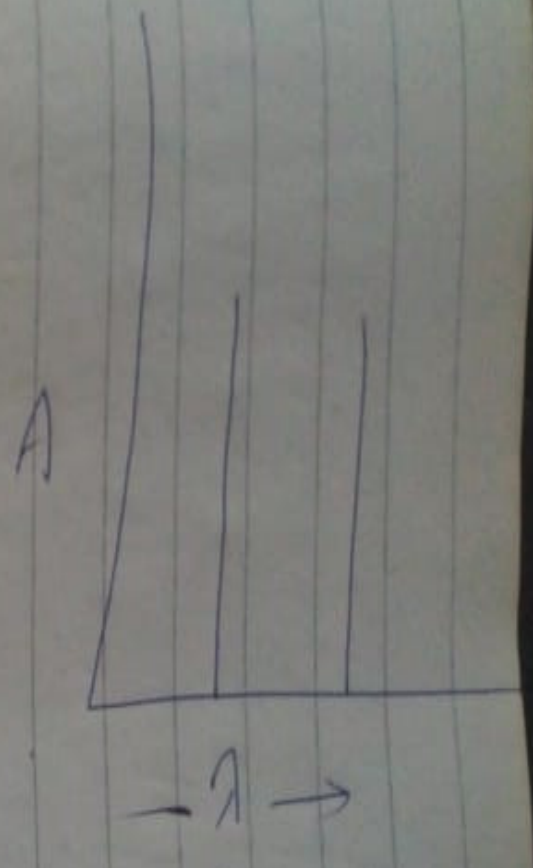
- Line for Rotational transitions.



Electronic Trans.



Vibrational Tran.



Rotational Trans.

UV-VISIBLE SPECTROSCOPY

Principle

- The UV radiation region extends from 10 nm to 400 nm and the visible radiation region extends from 400 nm to 800 nm.

  Near UV Region: 200 nm to 400 nm

  Far UV Region: below 200 nm

- Far UV spectroscopy is studied under vacuum condition.
- The common solvent used for preparing sample to be analyzed is either ethyl alcohol or hexane.

VACUUM UV REGION

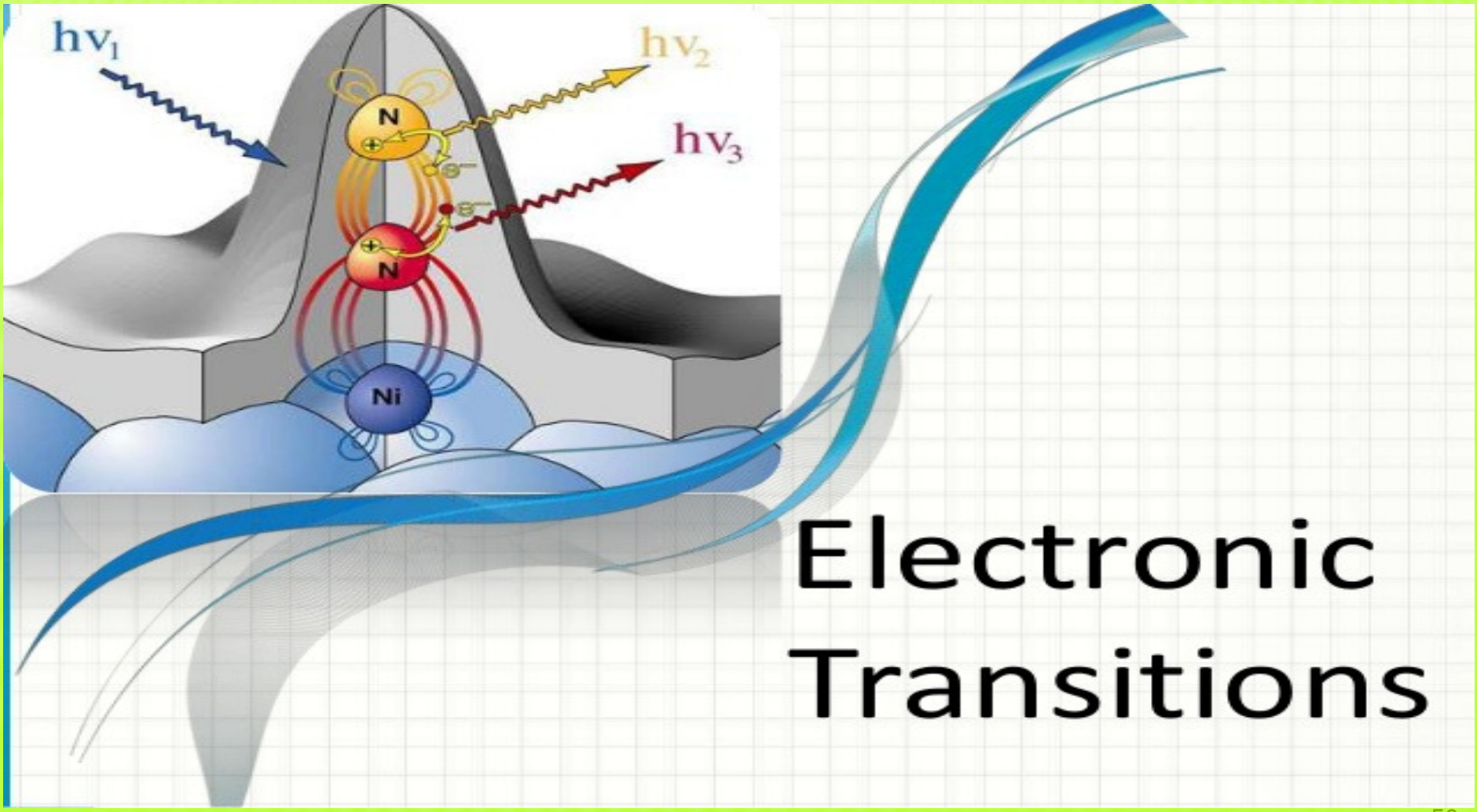
- 10-200nm light is absorbed by air (N_2 and O_2).

UV-VISIBLE SPECTROSCOPY

- Basic Principle

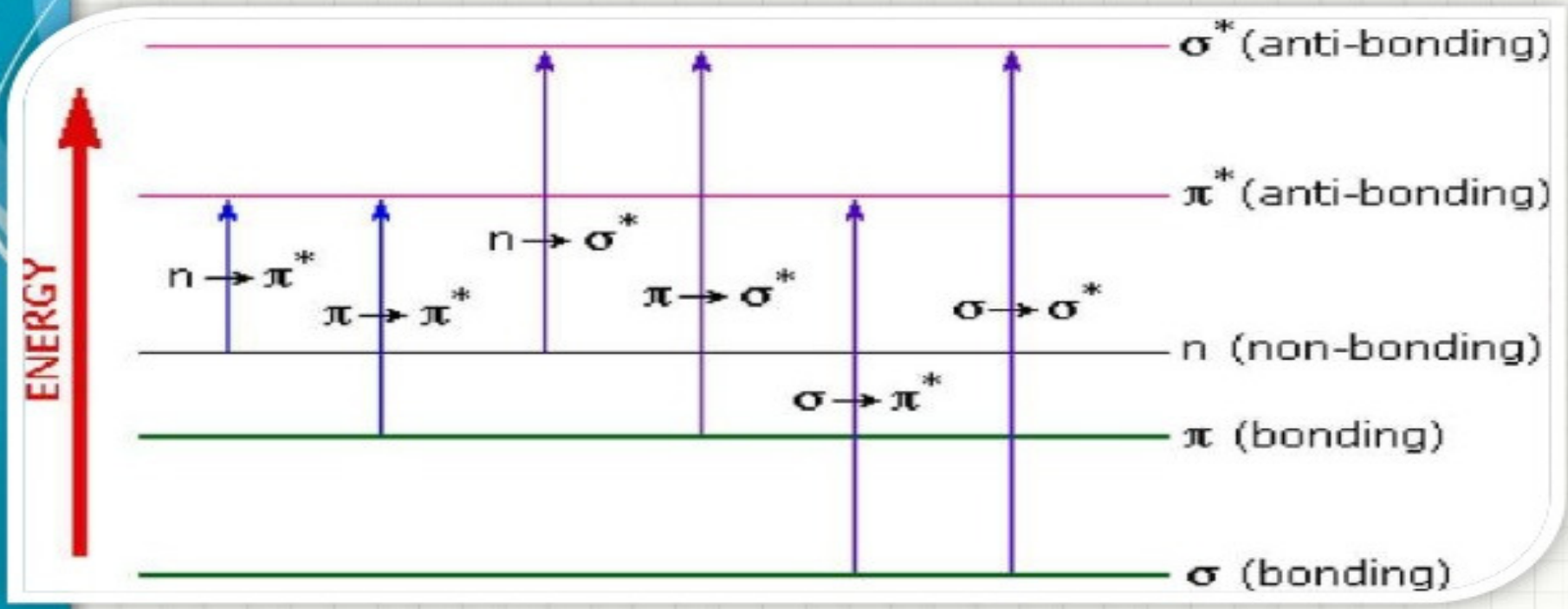
- Quantized absorption of UV-Visible radiations leading to electronic excitations.

TYPES OF ELECTRONIC TRANSITIONS



TYPES OF ELECTRONIC TRANSITIONS

The possible electronic transitions can graphically shown as:

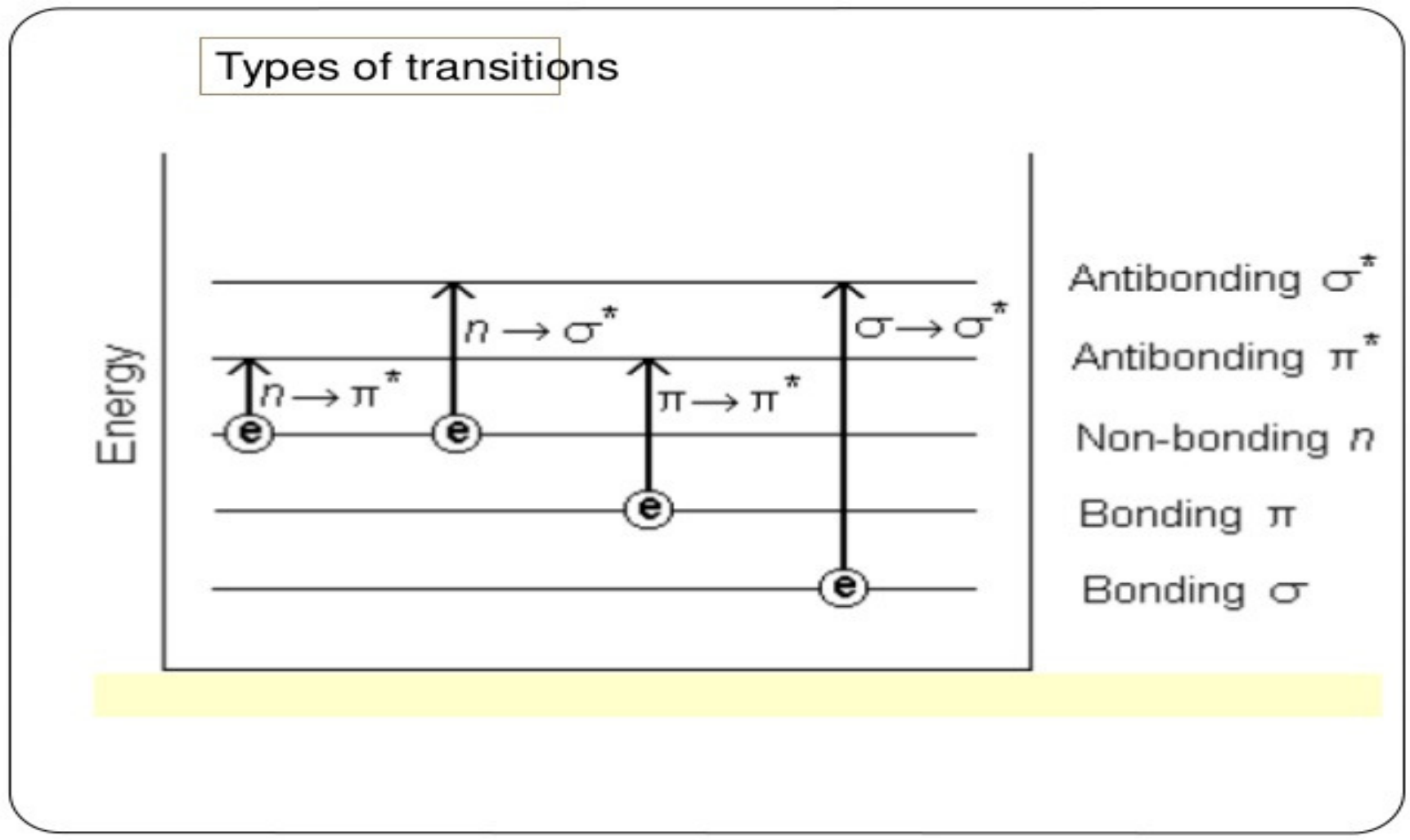


TYPES OF ELECTRONIC TRANSITIONS

The possible electronic transitions are

1. • $\sigma \rightarrow \sigma^*$ transition
2. • $\pi \rightarrow \pi^*$ transition
3. • $n \rightarrow \sigma^*$ transition
4. • $n \rightarrow \pi^*$ transition
5. • $\sigma \rightarrow \pi^*$ transition
6. • $\pi \rightarrow \sigma^*$ transition

TYPES OF ELECTRONIC TRANSITIONS



1 • 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 (CH_4) has C-H bond only and can undergo σ → σ* transition and shows absorbance maxima at 125 nm.

2 • $\pi \rightarrow \pi^*$ transition

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

3 • 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 requires less energy than σ → σ* transitions.
- The number of organic functional groups with n → σ* peaks in UV region is small (150 – 250 nm).

EXAMPLE

- CH_3OH
- C_2H_5OH

4 • 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 longer wavelength around 300 nm.

5 • $\sigma \rightarrow \pi^*$ transition

& • $\pi \rightarrow \sigma^*$ transition 6

- These electronic transitions are forbidden transitions & are only theoretically possible.
- Thus, n → π* & π → π* electronic transitions show absorption in region above 200 nm which is accessible to UV-visible spectrophotometer.
- The UV spectrum is of only a few broad of absorption.

- C=C
- C-C

Chromophore

The part of a molecule responsible for imparting color, are called as chromospheres.

OR

The functional groups containing multiple bonds capable of absorbing radiations above 200 nm due to n → π* & π → π* transitions.

e.g. NO_2, N=O, C=O, C=N, C≡N, C=C, C=S, etc

CHROMOPHORES

- Chromophores are any structural features which are responsible for absorption of light in UV-Visb. Region.

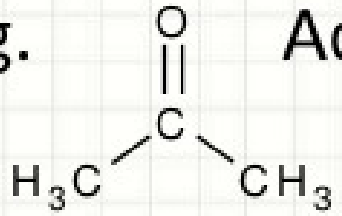
Chromophore

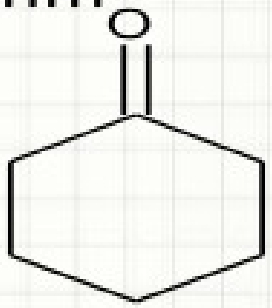
To interpretate UV – visible spectrum following points should be noted:

1. Non-conjugated alkenes show an intense absorption below 200 nm & are therefore inaccessible to UV spectrophotometer.

2. Non-conjugated carbonyl group compound give a weak absorption band in the 200 - 300 nm region.

Chromophore

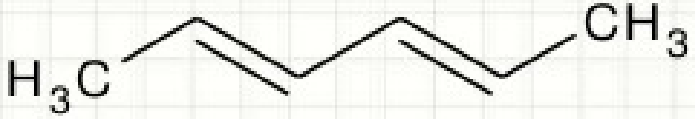
e.g.  Acetone which has λ_{max} = 279 nm

and that cyclohexane has λ_{max} = 291 nm. 

When double bonds are conjugated in a compound λ_{max} is shifted to longer wavelength.

e.g. 1,5 - hexadiene has λ_{max} = 178 nm

2,4 - hexadiene has λ_{max} = 227 nm

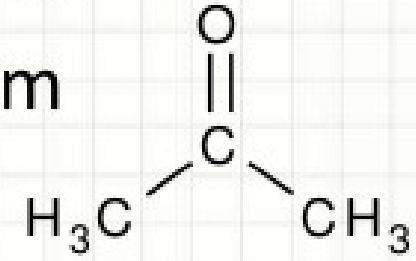


Chromophore

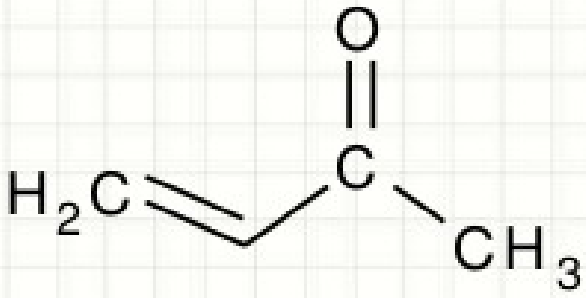
3. Conjugation of C=C and carbonyl group shifts the λ_{max} of both groups to longer wavelength.

e.g. Ethylene has λ_{max} = 171 nm

Acetone has λ_{max} = 279 nm



Crotonaldehyde has λ_{max} = 290 nm



Auxochrome

The functional groups attached to a chromophore which modifies the ability of the chromophore to absorb light , altering the wavelength or intensity of absorption.

OR

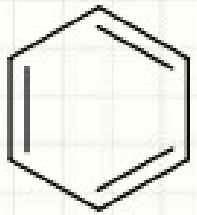
The functional group with non-bonding electrons that does not absorb radiation in near UV region but when attached to a chromophore alters the wavelength & intensity of absorption.

AUXOCHROMES

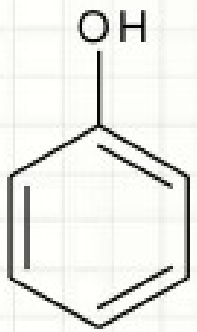
- Auxochromes are not chromophore but their presence with chromophore, shift the absorption towards longer wavelength.

Auxochrome

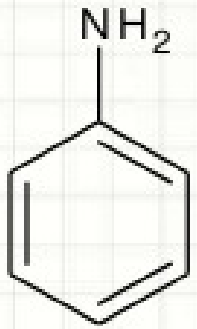
e.g. Benzene λ_{max} = 255 nm



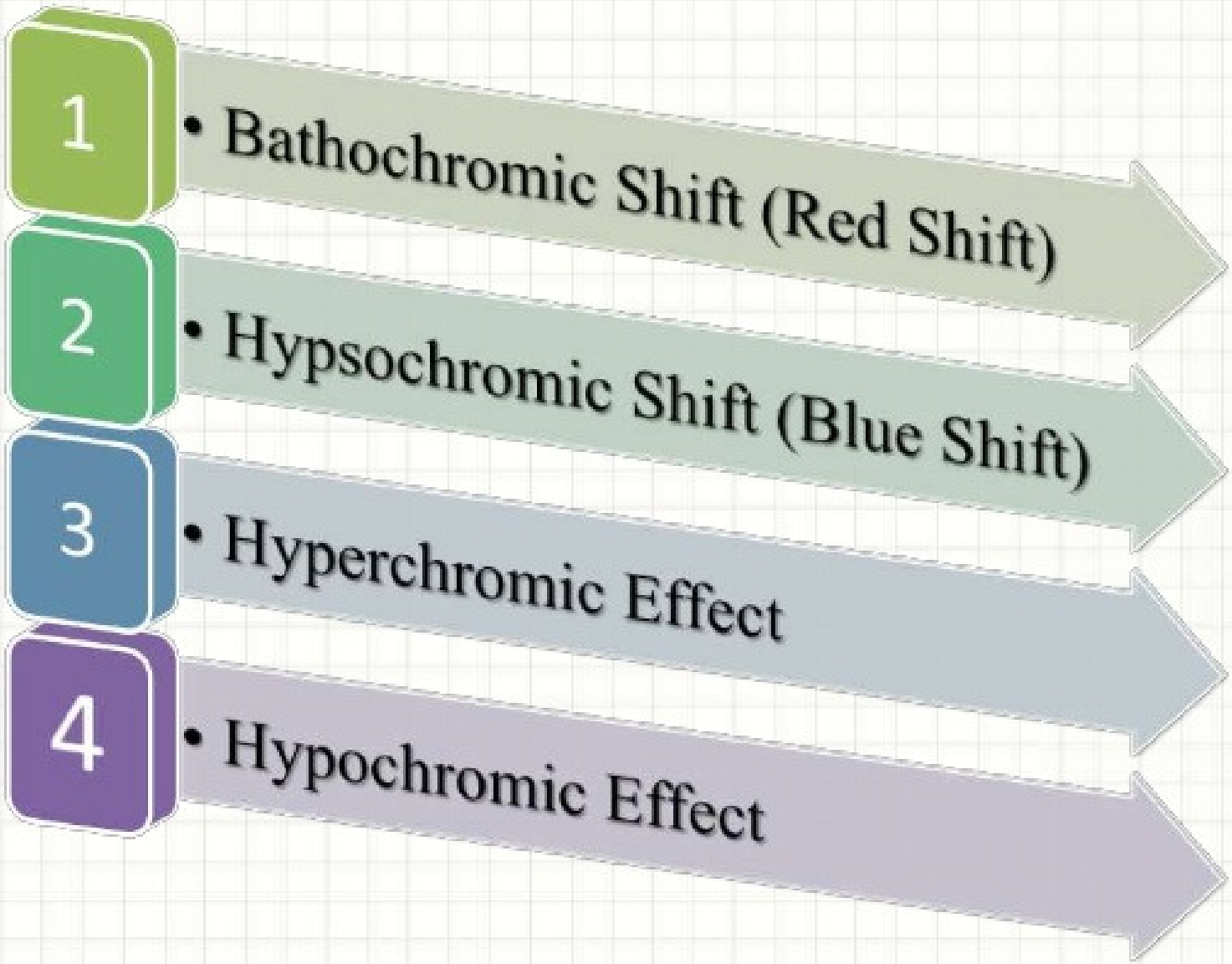
Phenol λ_{max} = 270 nm



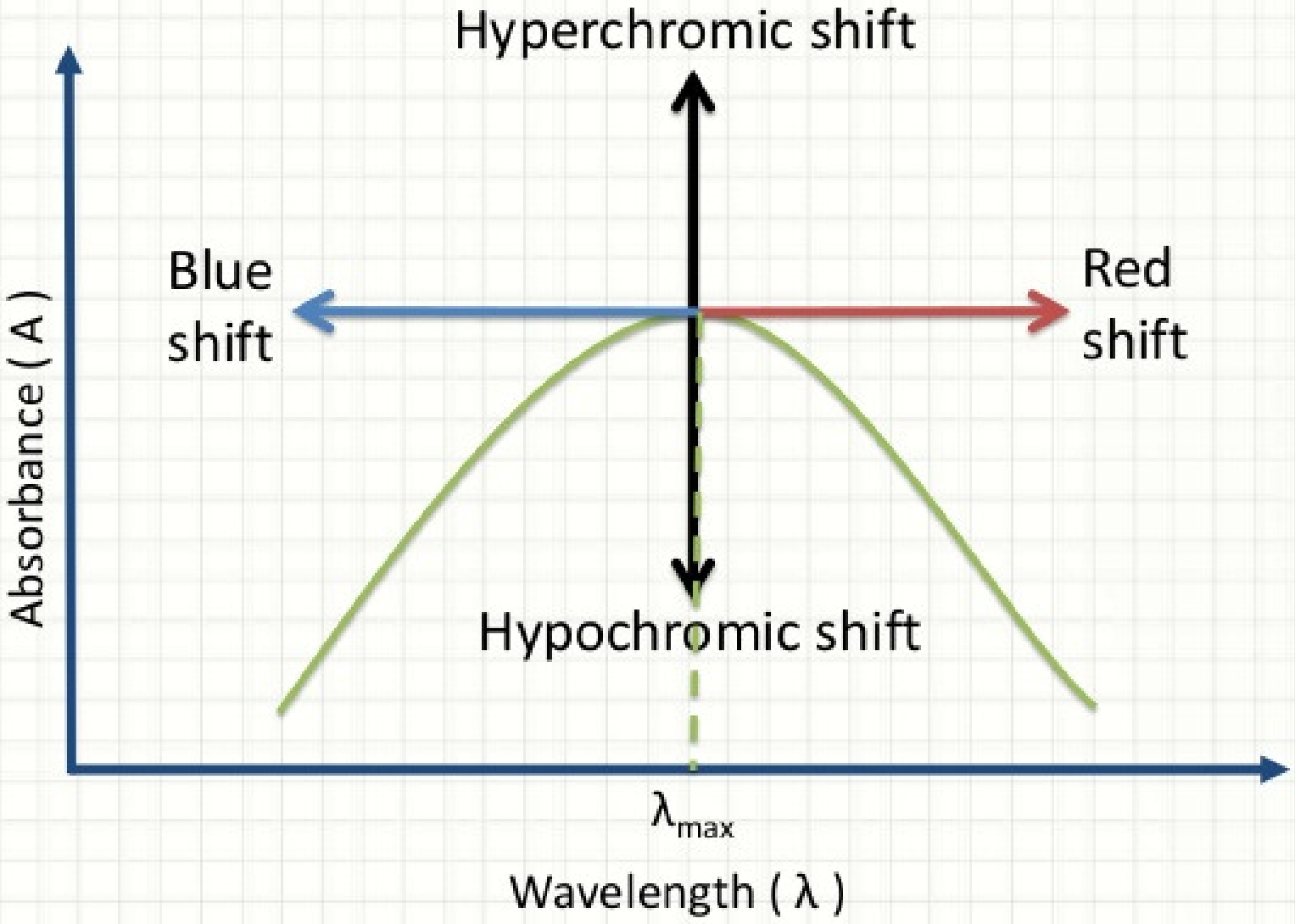
Aniline λ_{max} = 280 nm



Absorption & Intensity Shifts


1
• Bathochromic Shift (Red Shift)
2
• Hypsochromic Shift (Blue Shift)
3
• Hyperchromic Effect
4
• Hypochromic Effect

Shifts and Effects

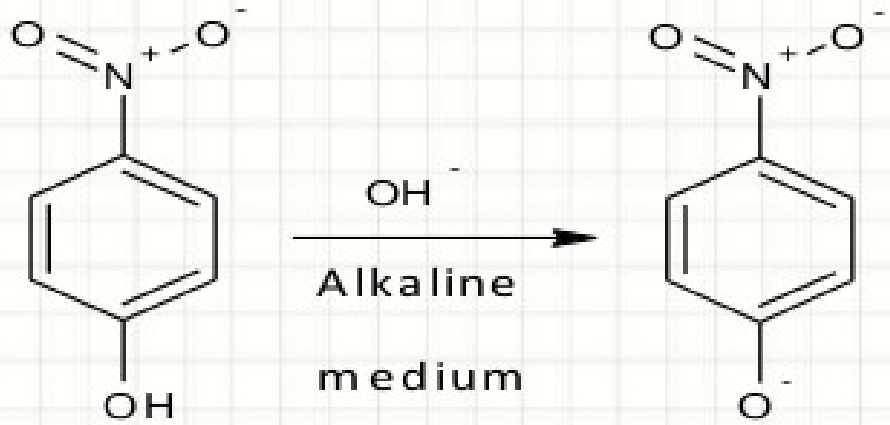


1 • Bathochromic Shift (Red Shift)

- When absorption maxima (λ_{max}) of a compound shifts to longer wavelength, it is known as bathochromic shift or red shift.
- The effect is due to presence of an auxochrome or by the change of solvent.
- e.g. An auxochrome group like –OH, $-OCH_3$ causes absorption of compound at longer wavelength.

1 • Bathochromic Shift (Red Shift)

- In alkaline medium, p-nitrophenol shows red shift. Because negatively charged oxygen delocalizes more effectively than the unshared pair of electron.



p-nitrophenol

λ_{max} = 255 nm λ_{max} = 265 nm

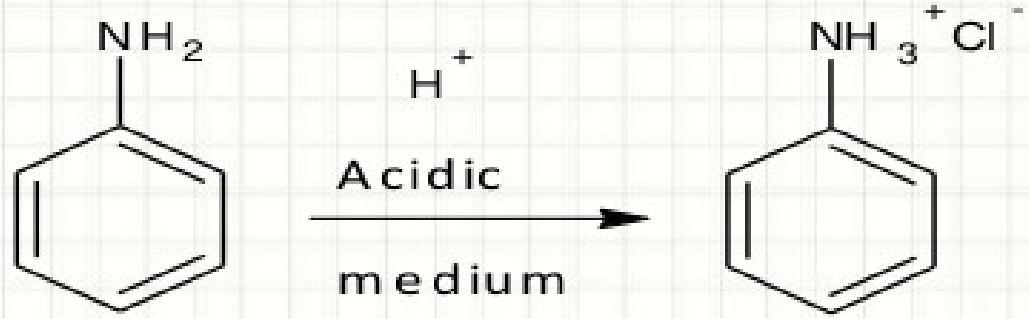
2 • Hypsochromic Shift (Blue Shift)

- When absorption maxima (λ_{max}) of a compound shifts to shorter wavelength, it is known as hypsochromic shift or blue shift.

- The effect is due to presence of an group causes removal of conjugation or by the change of solvent.

2 • Hypsochromic Shift (Blue Shift)

- Aniline shows blue shift in acidic medium, it loses conjugation.



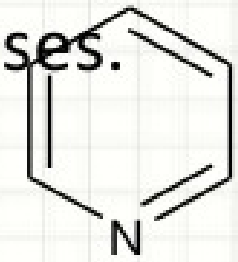
Aniline

λ_{max} = 280 nm

λ_{max} = 265 nm

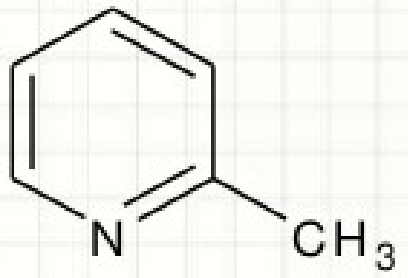
3 • Hyperchromic Effect

- When absorption intensity (ε) of a compound is increased, it is known as hyperchromic shift.
- If auxochrome introduces to the compound, the intensity of absorption increases.



Pyridine

λ_{max} = 257 nm

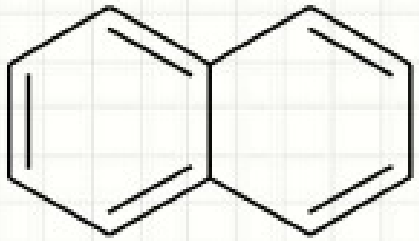


2-methyl pyridine

λ_{max} = 260 nm

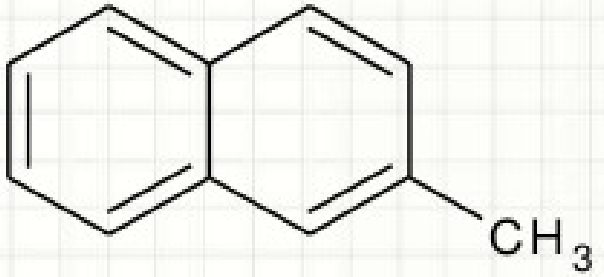
4 • Hypochromic Effect

- When absorption intensity (ε) of a compound is decreased, it is known as hypochromic shift.



Naphthalene

ε = 19000



2-methyl naphthalene

ε = 10250

ABSORPTION MAXIMA (Λ_{MAX})

- The absorption maximum (maxima is plural of maximum) of a compound is where the compound absorbs UV or visible light the most.

UV-visible spectrum

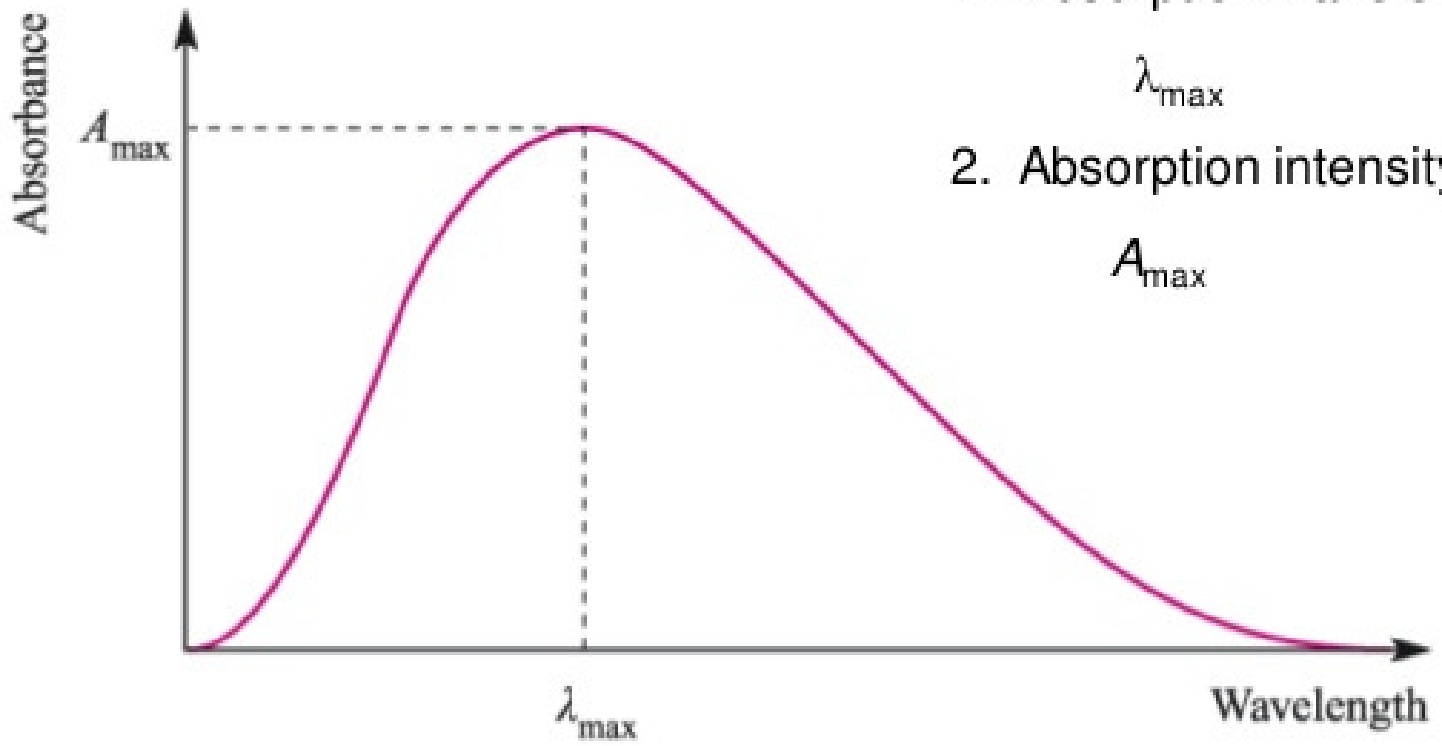
The two main properties of an absorbance peak are:

1. Absorption wavelength

   λ_{max}

2. Absorption intensity

   A_{max}



Housecroft and Sharpe, p. 466

INSTRUMENTATION

UV-VISIBLE SPECTROPHOTOMETER

INSTRUMENTS

- PHOTOMETER
- SPECTOPHOTOMETER
- COLORIMETER

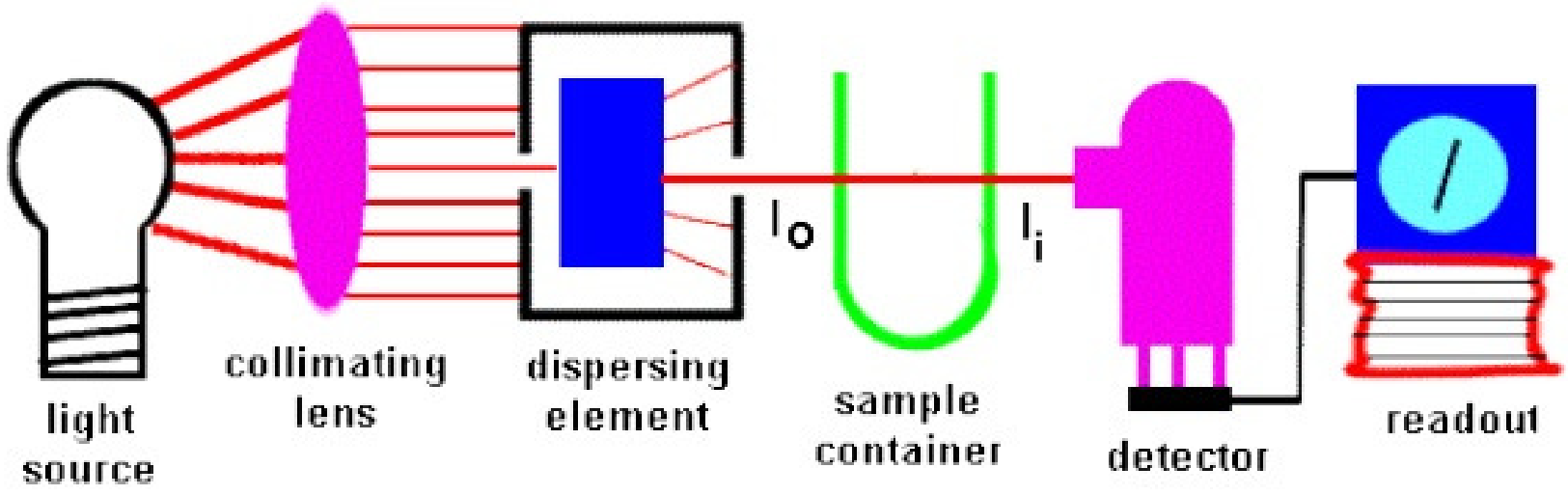
- **PHOTOMETER**: An instrument for measuring the intensity of light or the relative intensity of a pair of lights. Also called an illuminometer. It utilizes filter to isolate a narrow wavelength region.

- **SPECTOPHOTOMETER:** An instrument measures the ratio, or a function of the two, of the radiant power of two EM beams over a large wavelength region. It utilizes dispersing element (Prisms/Gratings) instead of filters, to scan large wavelength region.

- **COLORIMETER**: An instrument which is used for measuring absorption in the visible region is generally called colorimeter.

COMPONENTS OF UV-VIS SPECTROPHOTOMETER

- source of radiant energy.
- Collimating system.
- monochromator system.
- sample holder or container to hold sample.
- detector system of collecting transmitted radiation.
- suitable amplifier or readout device.


light source
collimating lens
dispersing element
I_o
I_i
sample container
detector
readout

SOURCE OF RADIANT ENERGY

REQUIREMENTS OF AN IDEAL SOURCE

- ✓ It should be stable and should not allow fluctuations.
- ✓ It should emit light of continuous spectrum of high and uniform intensity over the entire wavelength region in which it's used.
- ✓ It should provide incident light of sufficient intensity for the transmitted energy to be detected at the end of optic path.
- ✓ It should not show fatigue on continued use.

FOR VISIBLE RADIATION

TUNGSTEN HALOGEN LAMP

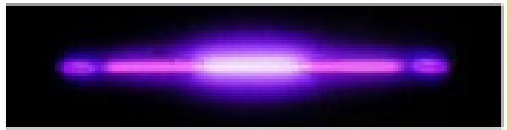
- Its construction is similar to a house hold lamp.
- The bulb contains a filament of Tungsten fixed in evacuated condition and then filled with inert gas.
- The filament can be heated up to 3000 k, beyond this Tungsten starts sublimating.
- It is used when polychromatic light is required. To prevent this along with inert gas some amount of halogen is introduced (usually Iodine).

- ✓ Sublimated form of tungsten reacts with Iodine to form Tungsten –Iodine complex.
- ✓ Which migrates back to the hot filament where it decomposes and Tungsten get deposited.
- ✓ DEMERIT:
- ✓ It emits the major portion of its radiant energy in near IR region of the spectrum.

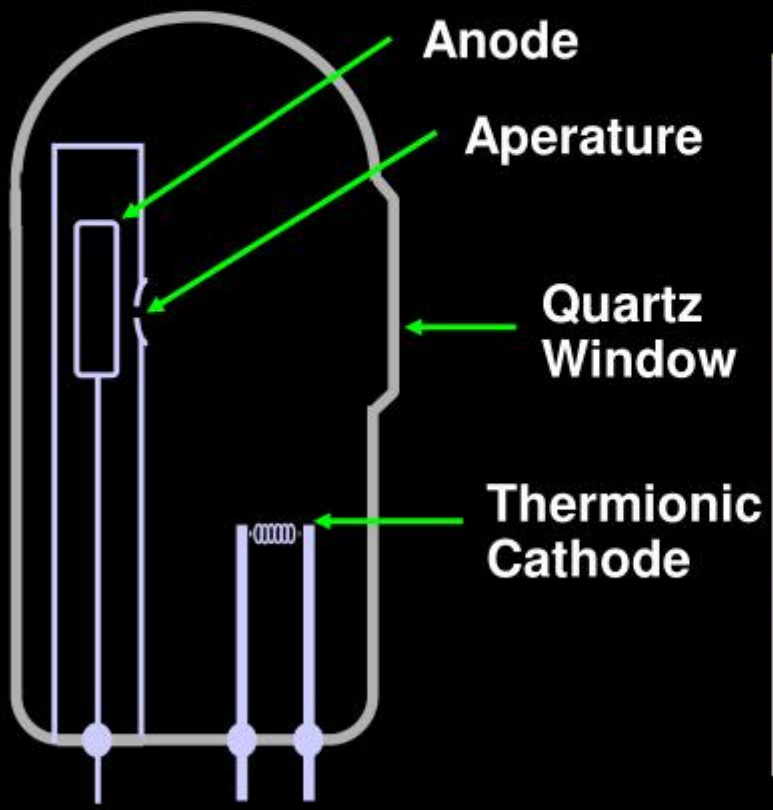
SOURCE FOR UV RADIATION

HYDROGEN DISCHARGE LAMP:

- In Hydrogen discharge lamp pair of electrodes is enclosed in a glass tube (provided with silica or quartz window for UV radiation to pass trough) filled with hydrogen gas.
- When current is passed trough these electrodes maintained at high voltage, discharge of electrons occurs which excites hydrogen molecules which in turn cause emission of UV radiations in near UV region.
- They are stable and robust.



Deuterium Lamp



- Used to measure non-atomic absorption
- Useful from 190 - 425nm
- Fill gas is Deuterium (D_2)
- Discharge current excites D_2 gas
- High light emission through discharge aperature

COLLIMATING SYSTEM

The radiation emitted by the source is collimated (made parallel) by lenses, mirrors and slits.

LENSES:

- Materials used for the lenses must be transparent to the radiation being used.
- Ordinary silicate glass transmits between 350 to 3000 nm and is suitable for visible and near IR region.
- Quartz or fused silica is used as a material for lenses to work below 300nm.

MIRRORS

- These are used to reflect, focus or collimate light beams in spectrophotometer.

- To minimize the light loss, mirrors are aluminized on their front surfaces.

SLITS:

- Slit is an important device in resolving polychromatic radiation into monochromatic radiation.
- To achieve this, entrance slit and exit slit are used.
- The width of slit plays an important role in resolution of polychromatic radiation.

WAVELENGTH SELECTION DEVICE

- Filters
- Prisms
- Gratings

FILTERS

- Filters are colored glass pieces.
- They allow only particular color to pass through them.
- Remaining part is absorbed by filters

FILTERS

Selection of filters is usually done on a compromise between peak transmittance and band pass width; the former should be as high as possible and latter as narrow as possible.

1. ***Absorption filters-*** works by selective absorption of unwanted radiation and transmits the radiation which is required.

   Examples- Glass and Gelatin filters.

Selection of absorption filter is done according to the following procedure:

- Draw a filter wheel.

- Write the color VIBGYOR in clockwise or anticlockwise manner, omitting Indigo.

- If solution to be analyzed is **BLUE** in color a filter having a complimentary color **ORANGE** is used in the analysis.

- Similarly, we can select the required filter in colorimeter, based upon the color of the solution.

MERITS:-

- Simple in construction
- Cheaper
- Selection of the filter is easy

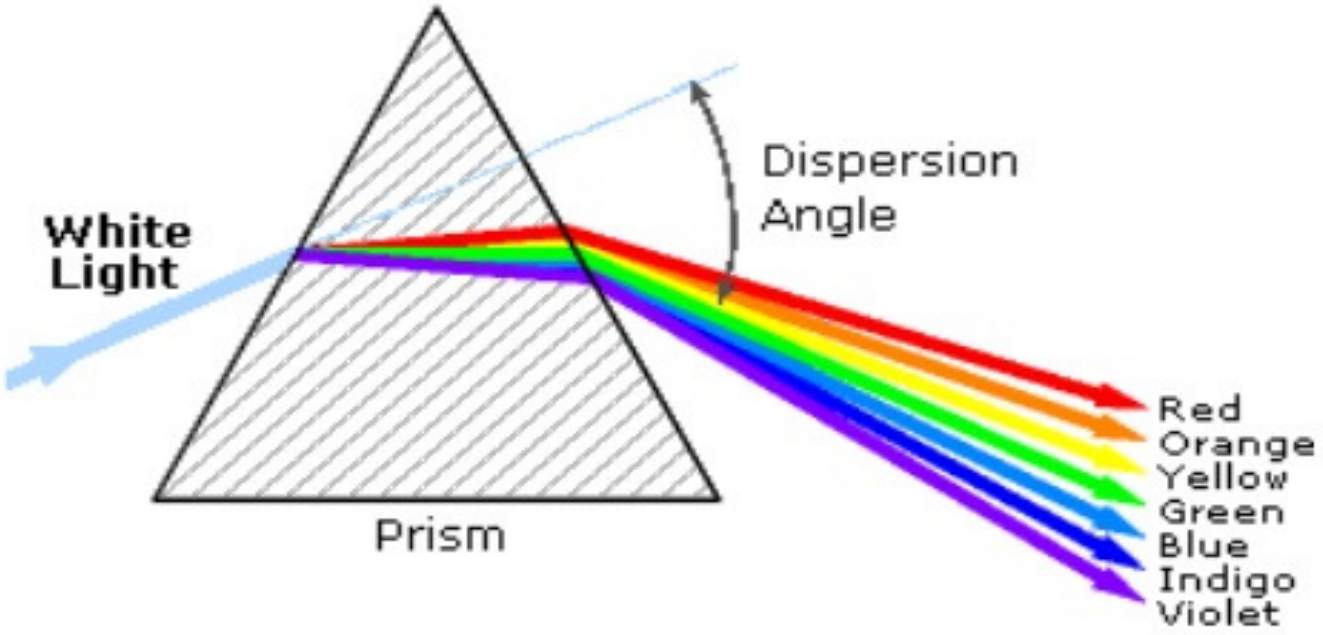
DEMERITS:-

- Less accurate
- Band pass (bandwidth) is more (±20-30nm) i.e. if we have to measure at 400nm; we get radiation from 370-430nm. Hence less accurate results are obtained.

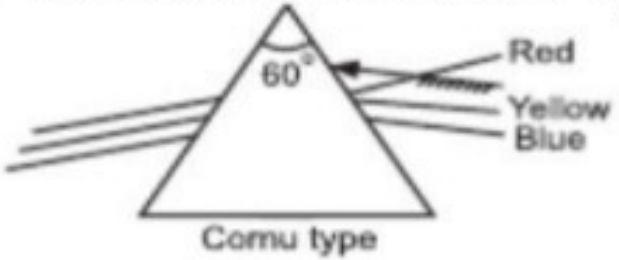
PRISM

- 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.

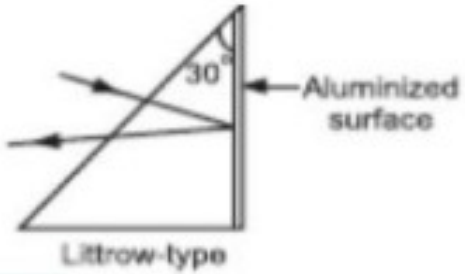
PRISM

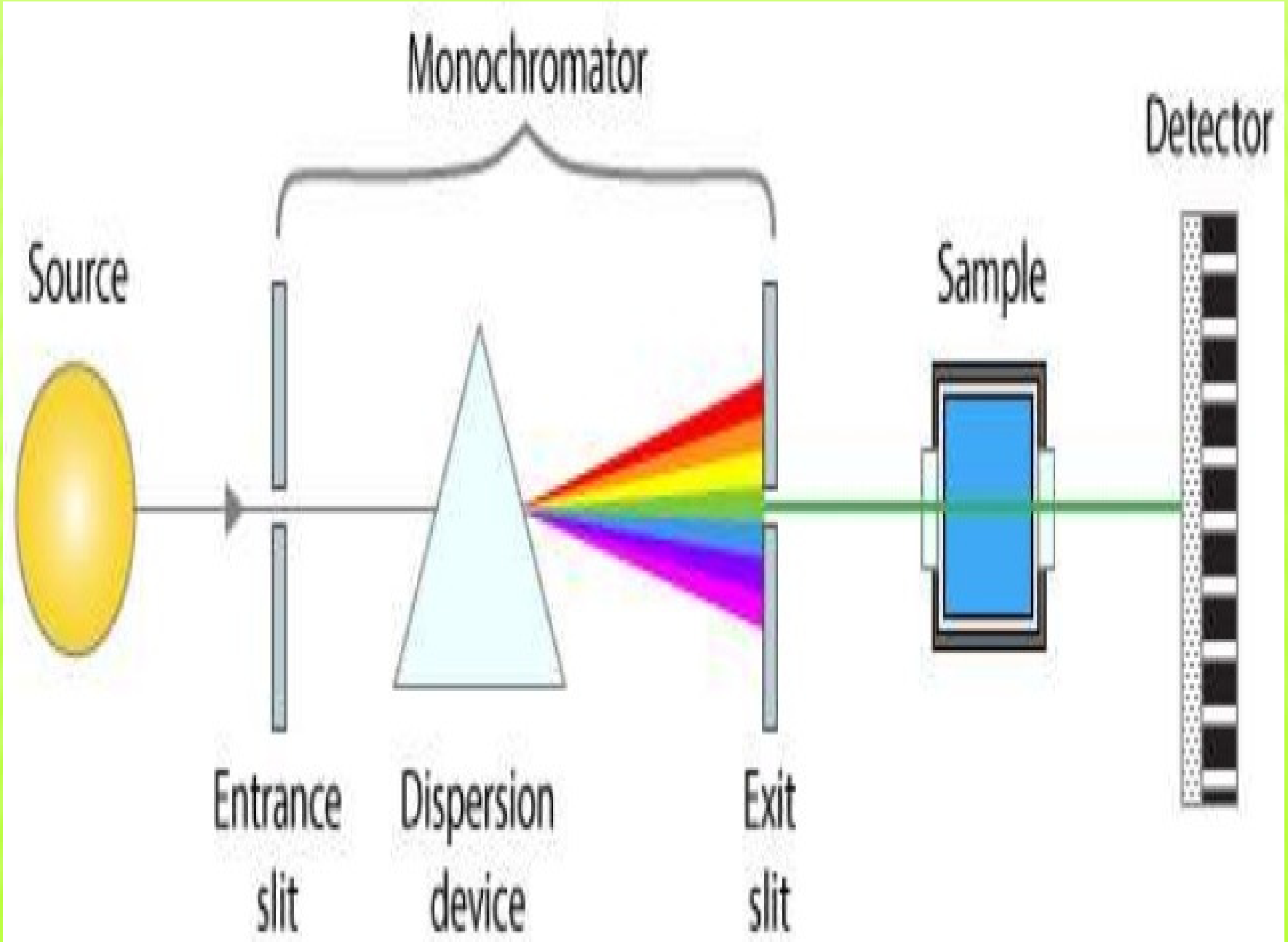

White Light
Dispersion Angle
Prism
Red
Orange
Yellow
Green
Blue
Indigo
Violet

- There are two types of mounting in an instrument one is called **'Cornu type'**(refractive), which has an optical angle of 60° and its adjusted such that on rotation the emerging light is allowed to fall on exit slit.



- The other type is called "**Littrow type**"(reflective), which has optical angle 30° and its one surface is aluminized with reflected light back to pass through prism and to emerge on the same side of the light source i.e. light doesn't pass through the prism on other side.




Monochromator
Detector
Source
Sample
Entrance slit
Dispersion device
Exit slit

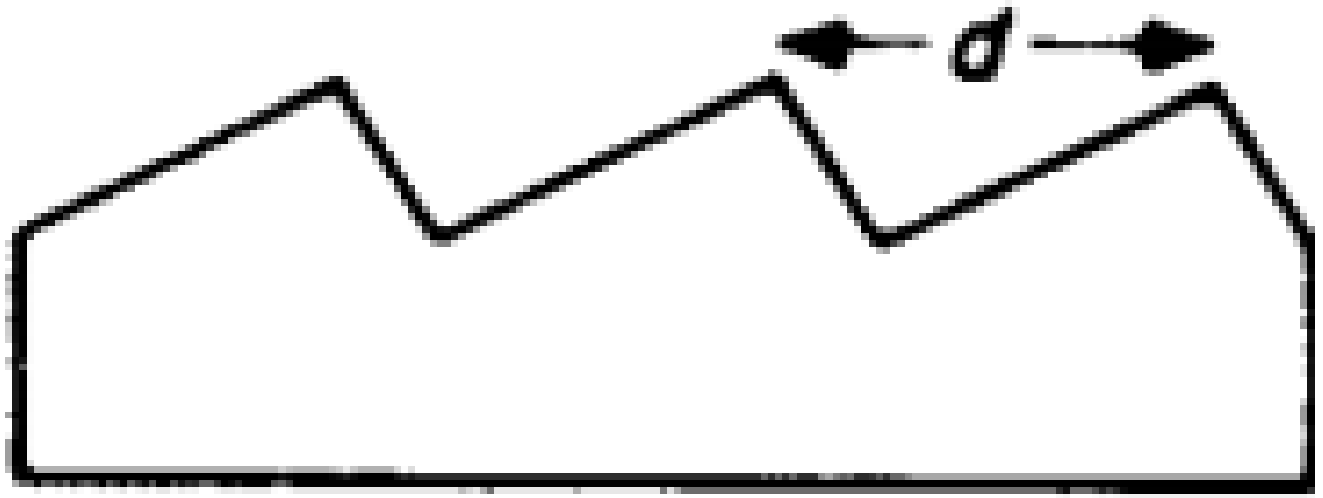
GRATINGS

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

1. Diffraction grating.
2. Transmission gratings.

CONSTRUCTION OF GRATINGS

- Gratings are made by drawing parallel grooves on glass plate.
- In old time gratings were made from diamond tipped tool to mark grooves on glass.
- Now a day grooves are marked by LASER.
- Such gratins are called Holographic gratings.

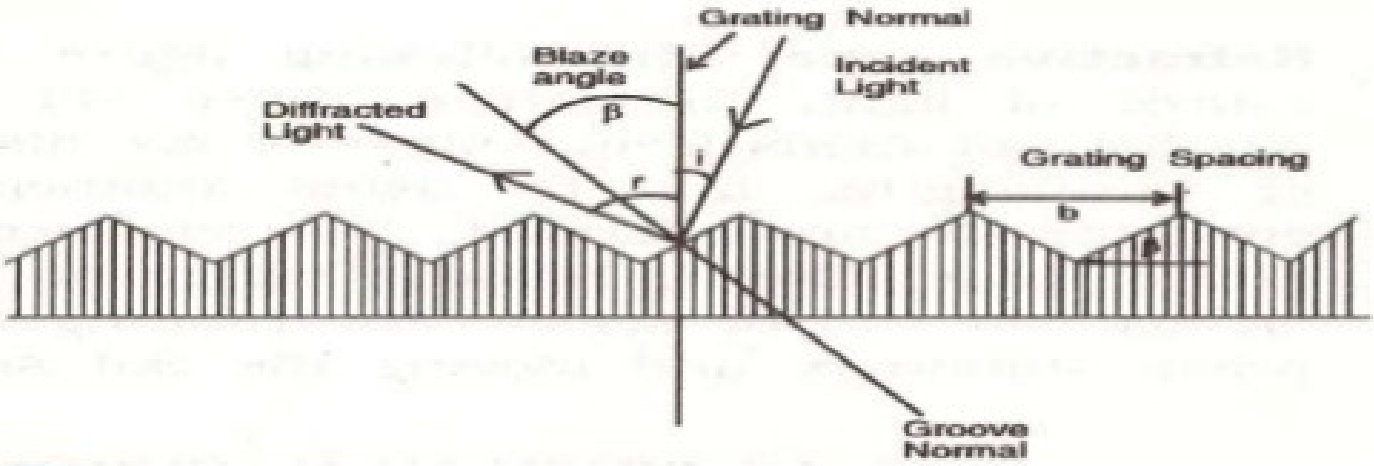


Enlarged side view

Diffraction Grating

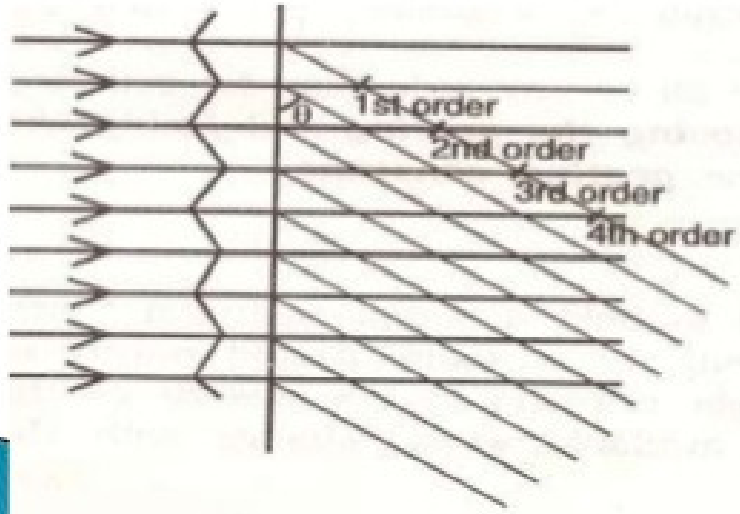
- More refined dispersion of light is obtained by means of diffraction gratings.
- These consist of large number of parallel lines (grooves) about 15000-30000/ inch is ruled on highly polished surface of aluminum.
- these gratings are replica made from master gratings by coating the original master grating with a epoxy resin and are removed after setting

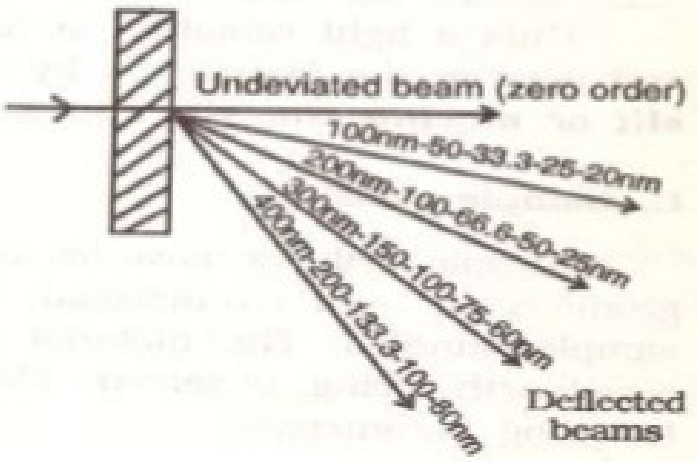
- To make the surface reflective, a deposit of aluminum is made on the surface. In order to minimize to greater amounts of scattered radiation and appearance of unwanted radiation of other spectral orders, the gratings are blazed to concentrate the radiation into a single order.



Transmission grating

- It is similar to diffraction grating but refraction takes place instead of reflection. Refraction produces reinforcement. this occurs when radiation transmitted through grating reinforces with the partially refracted radiation.





Advantages

- Grating gives higher and linear dispersions compared to prism monochromator.
- Can be used over wide wavelength ranges.
- Gratings can be constructed with materials like aluminium which is resistant to atmospheric moisture.
- Provide light of narrow wavelength.
- No loss of energy due to absorption.

Comparison	Prism	Grating
Made of	Glass-: Visible Quartz/fused silica-: UV Alkali halide:- IR	Grooved on highly polished surface like alumina.
Working Principle	Angle of Incident	Law of diffraction $n\lambda = d\ (\sin i \pm \sin\theta)$
Merits/demerits	➢ Prisms give non-liner dispersion hence no overlap of spectral order. ➢ It can't be used over consideration wavelength ranges. ➢ Prisms are not sturdy and long lasting.	➢ Grating gives liner dispersion hence overlap of spectral order. ➢ It can be used over considerable wavelength ranges. ➢ Grating are sturdy and long lasting

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 blotches should be present on cells.
- Cleaning is carried out washing with distilled water or with dilute alcohol, acetone.

Sample holder

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

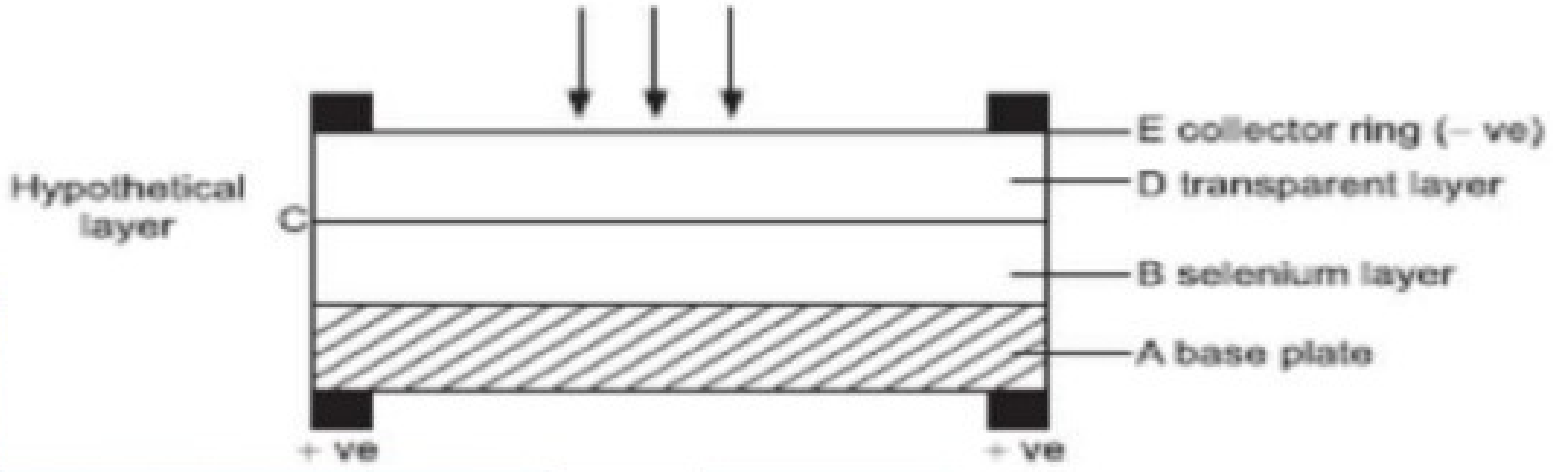
1. Barrier layer cell/Photovoltaic cell
2. Phototubes/ Photo emissive tube
3. Photomultiplier tube

Requirements of an ideal detector:-

- It should give quantitative response.
- It should have high sensitivity and low noise level.
- It should have a short response time.
- It should provide signal or response quantitative to wide spectrum of radiation received.

Barrier layer cell/Photovoltaic cell

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



- When light radiation falls on selenium layer, electrons become mobile and are taken up by transparent metal layer.
- This creates a potential difference between two electrodes & causes the flow of current.
- When it is connected to galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light falling on it.

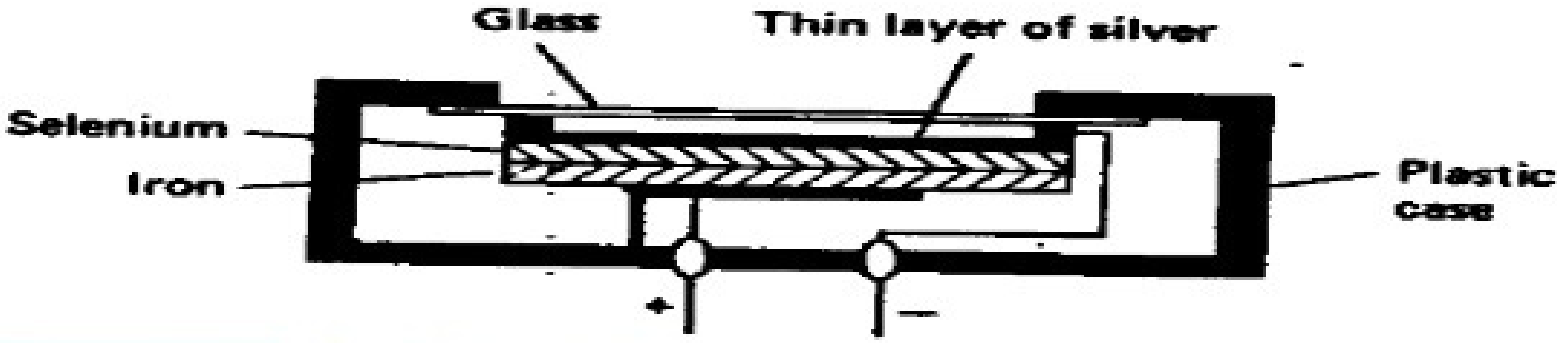


Photo Tubes/Photoemissive Tubes

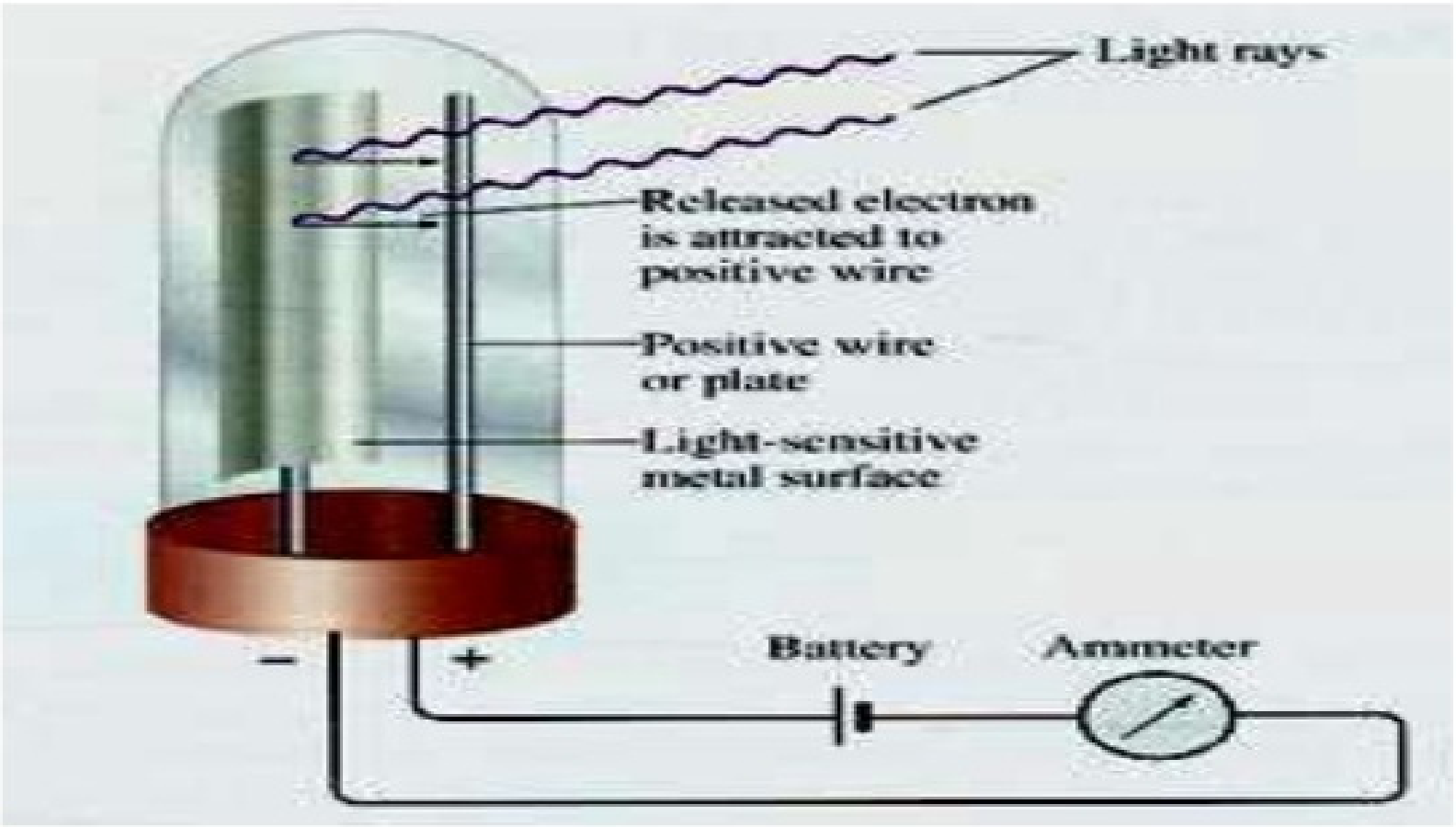
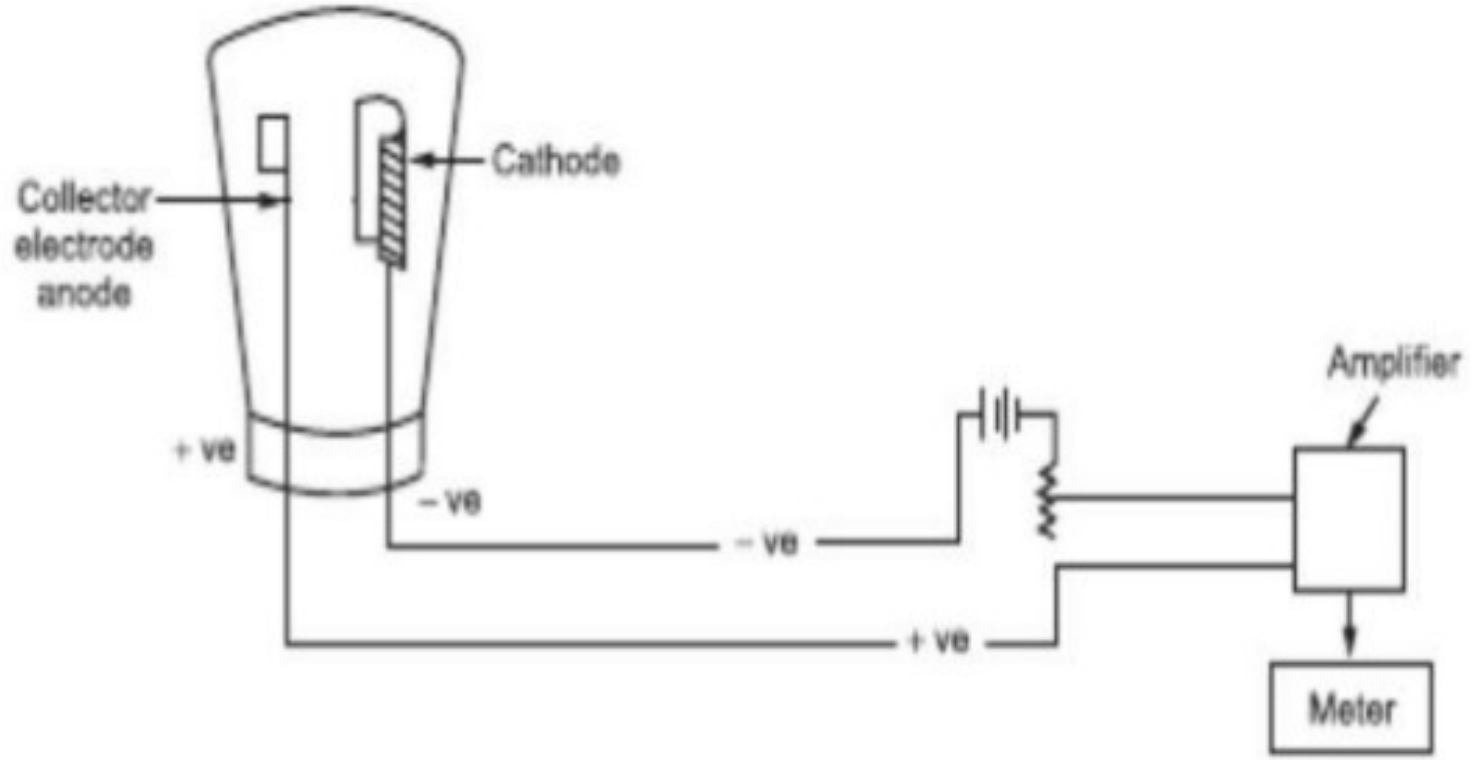


Photo tubes



42

Photo Tubes/Photoemissive Tubes

- Consists of a evacuated glass tube with a photocathode and a collector anode.
- The surface of photocathode is coated with a layer of elements like cesium, silver oxide or mixture of them.
- When radiant energy falls on photosensitive cathode, electrons are emitted which are attracted to anode causing current to flow.
- More sensitive compared to barrier layer cell and therefore widely used.

Photo Tubes/Photoemissive Tubes

- Consists of a evacuated glass tube with a photocathode and a collector anode.
- The surface of photocathode is coated with a layer of elements like cesium, silver oxide or mixture of them.
- When radiant energy falls on photosensitive cathode, electrons are emitted which are attracted to anode causing current to flow.
- More sensitive compared to barrier layer cell and therefore widely used.

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 of 75-100V 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 low radiation is received

Photo Multiplier Tubes

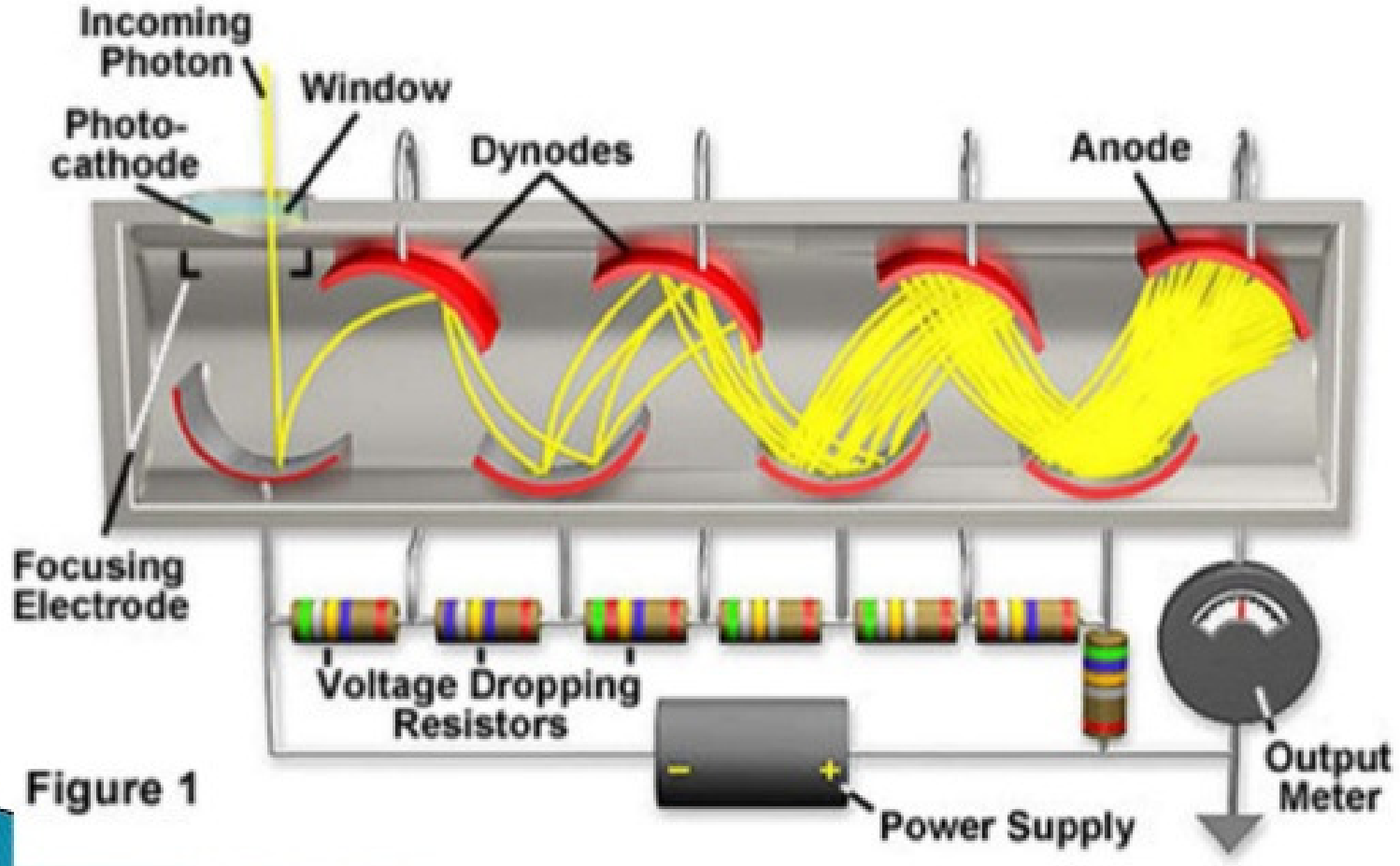


Figure 1

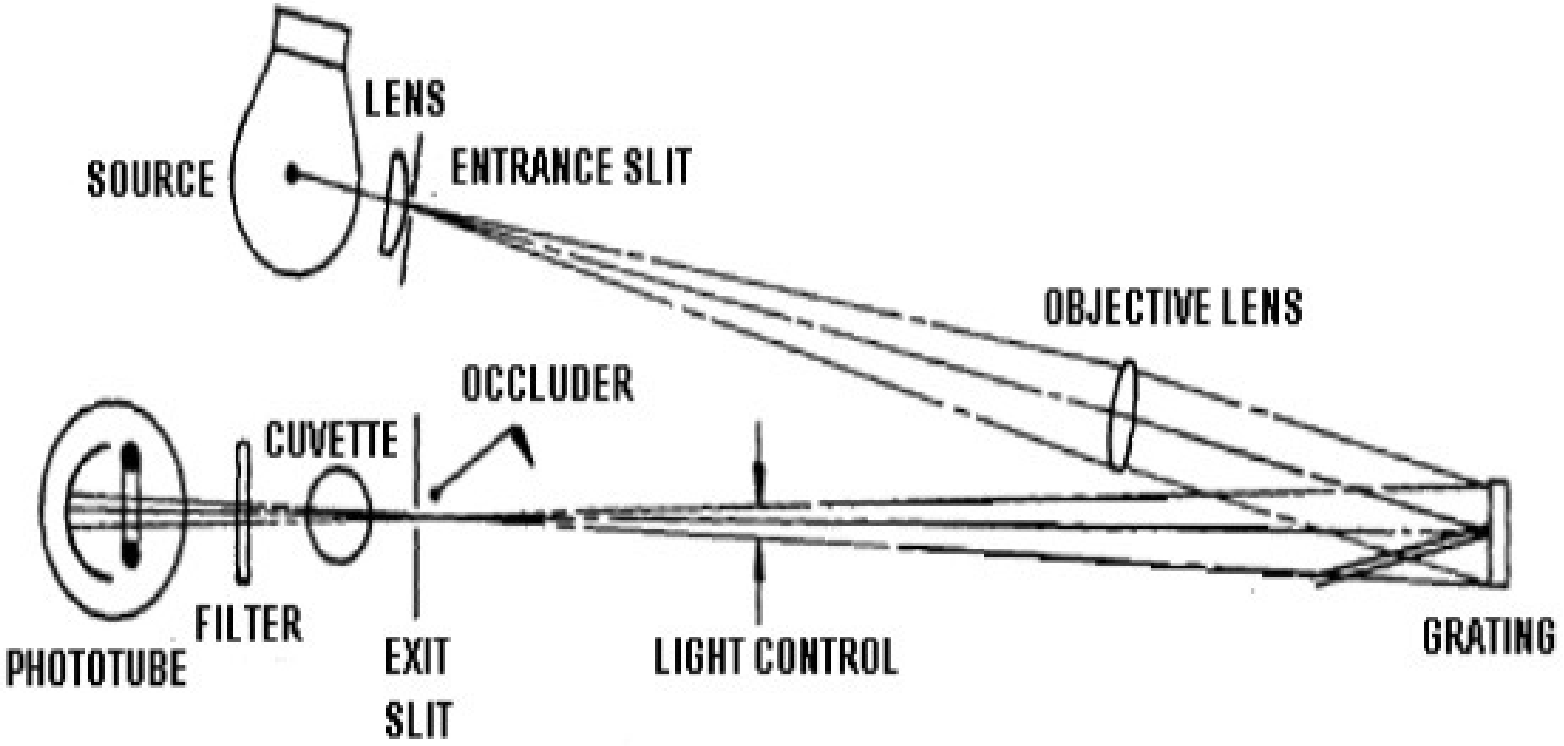
INSTRUMENT DESIGN

- Depending upon the monochromators (filters or dispersing device) used to isolate and transmit a narrow beam of radiant energy from the incident light determines whether the instrument is classified as Photometer or a Spectrophotometer.
- *Spectrophotometers* used here detects the percentage transmittance of light radiation, when light of certain intensity & frequency range is passed through the sample.
- Both can be a single beam or double beam optical system.

SINGLE BEAM SPECTROPHOTOMETER

- Light from the source is carried through lens and/or through aperture to pass through a suitable filter.
- The type of filter to be used is governed by the colour of the solution.
- The sample solution to be analysed is placed in cuvettes.

Single beam instrument

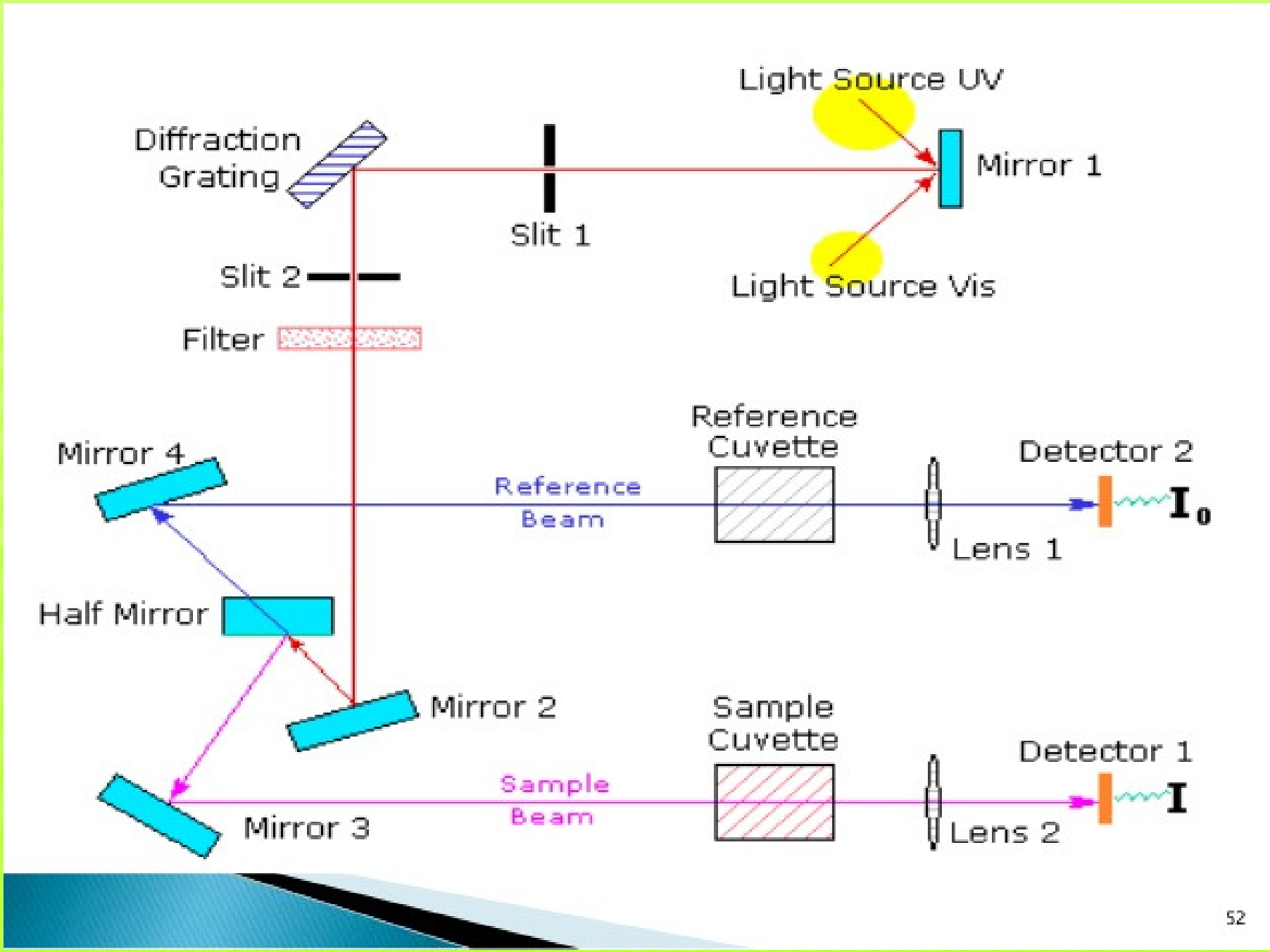


- After passing through the solution, the light strikes the surface of detector (barrier-layer cell or phototube) and produces electrical current.
- The output of current is measured by the deflection of needle of light-spot galvanometer or micro ammeter. This meter is calibrated in terms of transmittance as well as optical density. The readings of solution of both standard and unknown are recorded in optical density units after adjusting instrument to a reagent blank.

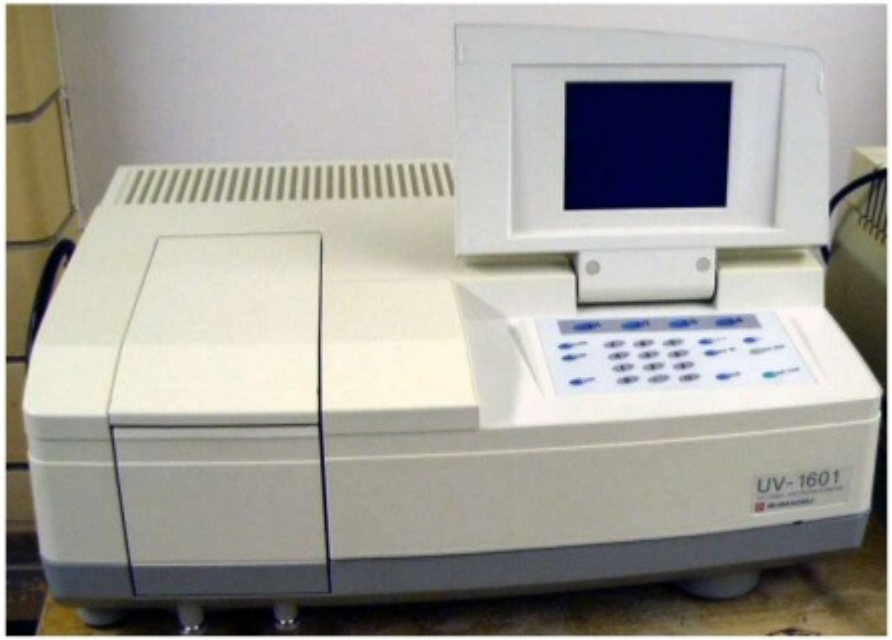
Single beam instrument

DOUBLE BEAM UV-VIS SPECTROPHOTOMETER

- Double beam instrument is the one in which two beams are formed in the space by a U shaped mirror called as beam splitter or beam chopper .
- *Chopper* is a device consisting of a circular disc. One third of the disc is opaque and one third is transparent, remaining one third is mirrored. It splits the monochromatic beam of light into two beams of equal intensities.


Light Source UV
Diffraction Grating
Slit 1
Mirror 1
Slit 2
Light Source Vis
Filter
Reference Cuvette
Mirror 4
Detector 2
Reference Beam
I_0
Lens 1
Half Mirror
Mirror 2
Sample Cuvette
Detector 1
Sample Beam
I
Mirror 3
Lens 2

Double Beam



53

Advantages of single & double beam spectrophotometer

Single beam-

- Simple in construction, Easy to use and economical

Double beam-

- It facilitates rapid scanning over wide λ region.
- Fluctuations due to radiation source are minimised.
- It doesn't require adjustment of the transmittance at 0% and 100% at each wavelength.
- It gives ratio of intensities of sample & reference beams simultaneously.

Disadvantages

Single beam

- Any fluctuation in the intensity of radiation sources affects the absorbance.
- Continuous spectrum is not obtained.

Double beam

- Construction is complicated.
- Instrument is expensive.

COMPARISON:

SL. NO	SINGLE BEAM INSTRUMENT	DOUBL BEAM INSTRUMENT
1.	Calibration should be done with blank every time, before measuring the absorbance or transmittance of sample	Calibration is done only in the beginning.

2	Radiant energy intensity changes with fluctuation of voltage.	It permits a large degree of inherent compensation for fluctuations in the intensity of the radiant energy.
3	It measure the total amount of transmitted light reaching the detector	It measures the percentage of light absorbed by the sample.

4	In single beam it's not possible to compare blank and sample together.	In double beam it's possible to do direct one step comparison of sample in one path with a standard in the other path.
5	In single beam radiant energy wavelength has to be adjusted every time.	In this scanning can be done over a wide wavelength region
6	Working on single beam is tedious and time consuming.	Working on double beam is fast and non tedious.

Applications of UV / Visible Spectroscopy

Applications

- Qualitative & Quantitative Analysis:
  - It is used for characterizing aromatic compounds and conjugated olefins.
  - It can be used to find out molar concentration of the solute under study.
- Detection of impurities:
  - It is one of the important method to detect impurities in organic solvents.
- Detection of isomers are possible.
- Determination of molecular weight using Beer's law.


90
Th
Thorium
119
A
Adamantium
7
N
Nitrogen
19
K
Potassium
39
Y
Yttrium
8
O
Oxygen
92
U
Uranium
PERIODICALLY, IT'S NICE TO BE
REMINDED THAT WE MATTER