Presentation topic –

Ultra-violate spectroscopy

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UV SPECTROSCOPY

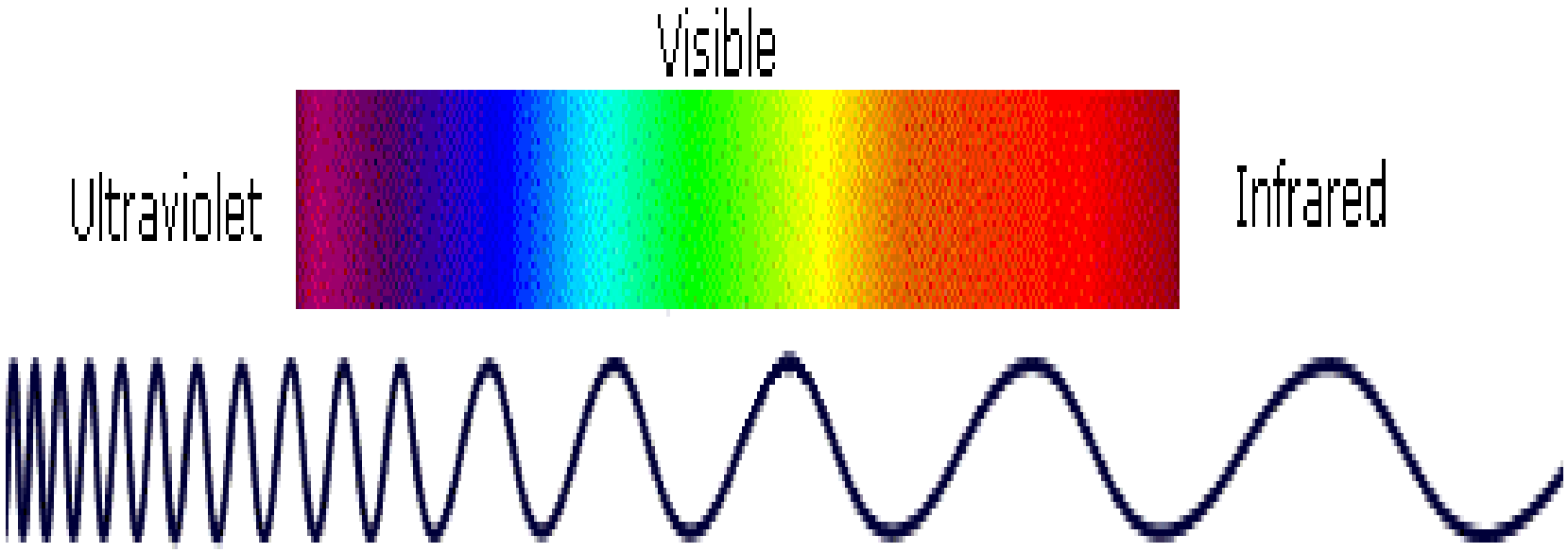
Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range.

Why we use UV spectroscopy ?

1. Detection of functional groups.
2. Detection of impurities
3. Qualitative analysis
4. Quantitative analysis
5. Single compound without chromophore
6. Drugs with chromophoric reagent
7. It is helps to show the relationship between different groups, it is useful to detect the conjugation of the compounds

UV RADIATION

The region beyond red is called infra-red while that beyond violet is called as ultra –violet. The wavelength range of uv radiation starts at blue



PRINCIPLE OF UV–VIS SPECTROMETRY

- Ultraviolet absorption spectra arise from transition of electron with in a molecule from a lower level to a higher level.

- A molecule absorb ultraviolet radiation of frequency (ϑ), the electron in that molecule undergo transition

from lower to higher energy level.

The energy can be calculated by the equation,

E=h erg

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$E_1 - E_o = h\vartheta$

Etotal=Eelectronic+Evibrotional+Erotational

The energies decreases in the following order:

Electronic Vibrational Rotational

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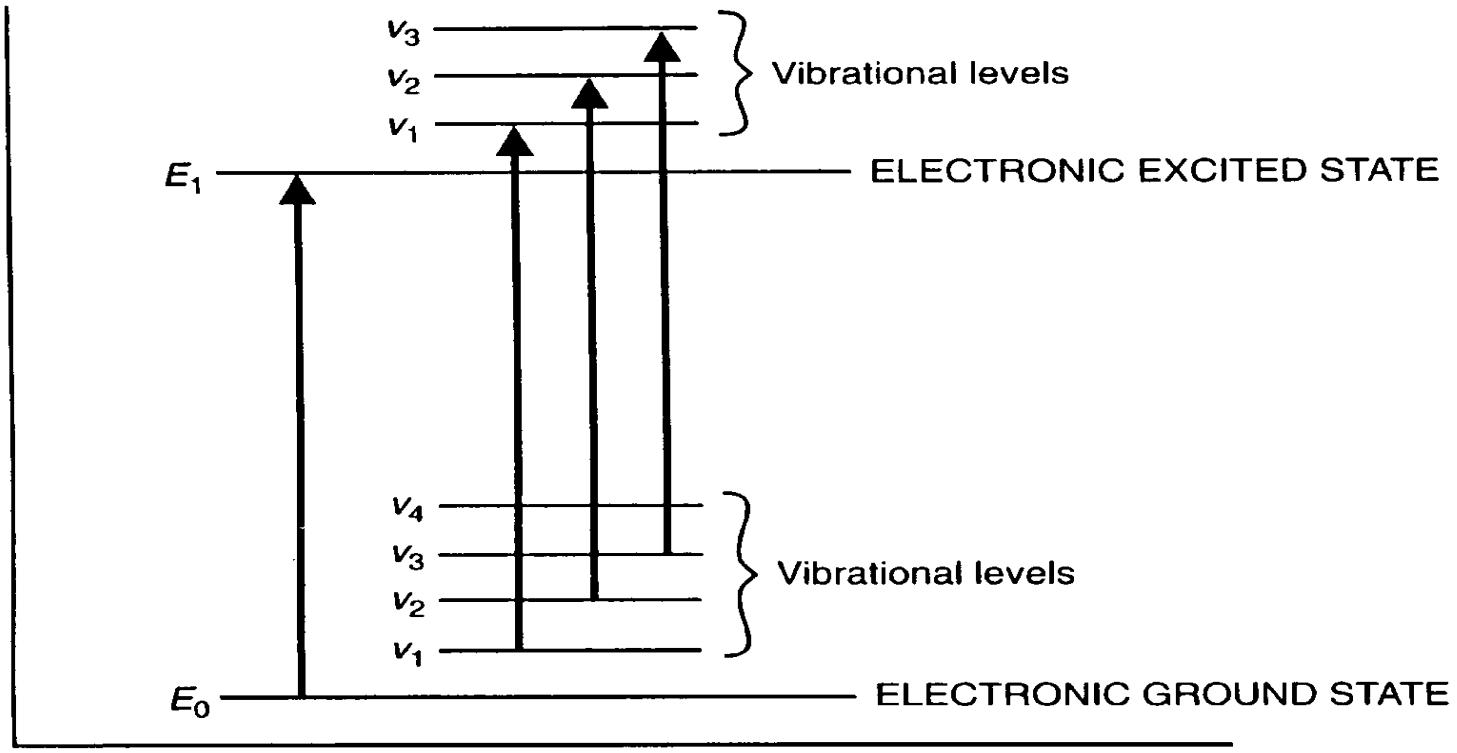

Thus the energy of the radiation in the visible range is generally: 36 to 72 kcal/mole while that in the ultraviolet range goes as high as 143 kcal/mole

THE ABSORPTION SPECTRUM

When a sample is exposed to light energy that matches the energy difference between a possible electronic transition within the molecule, a fraction of the light energy would be absorbed by the molecule and the electrons would be promoted to the higher energy state orbital. A spectrometer records the degree of absorption by a sample at different wavelengths and the resulting plot of absorbance (A) versus wavelength (λ) is known as a spectrum.

The significant features:

- λmax (wavelength at which there is a maximum absorption)
- ϵmax (The intensity of maximum absorption)

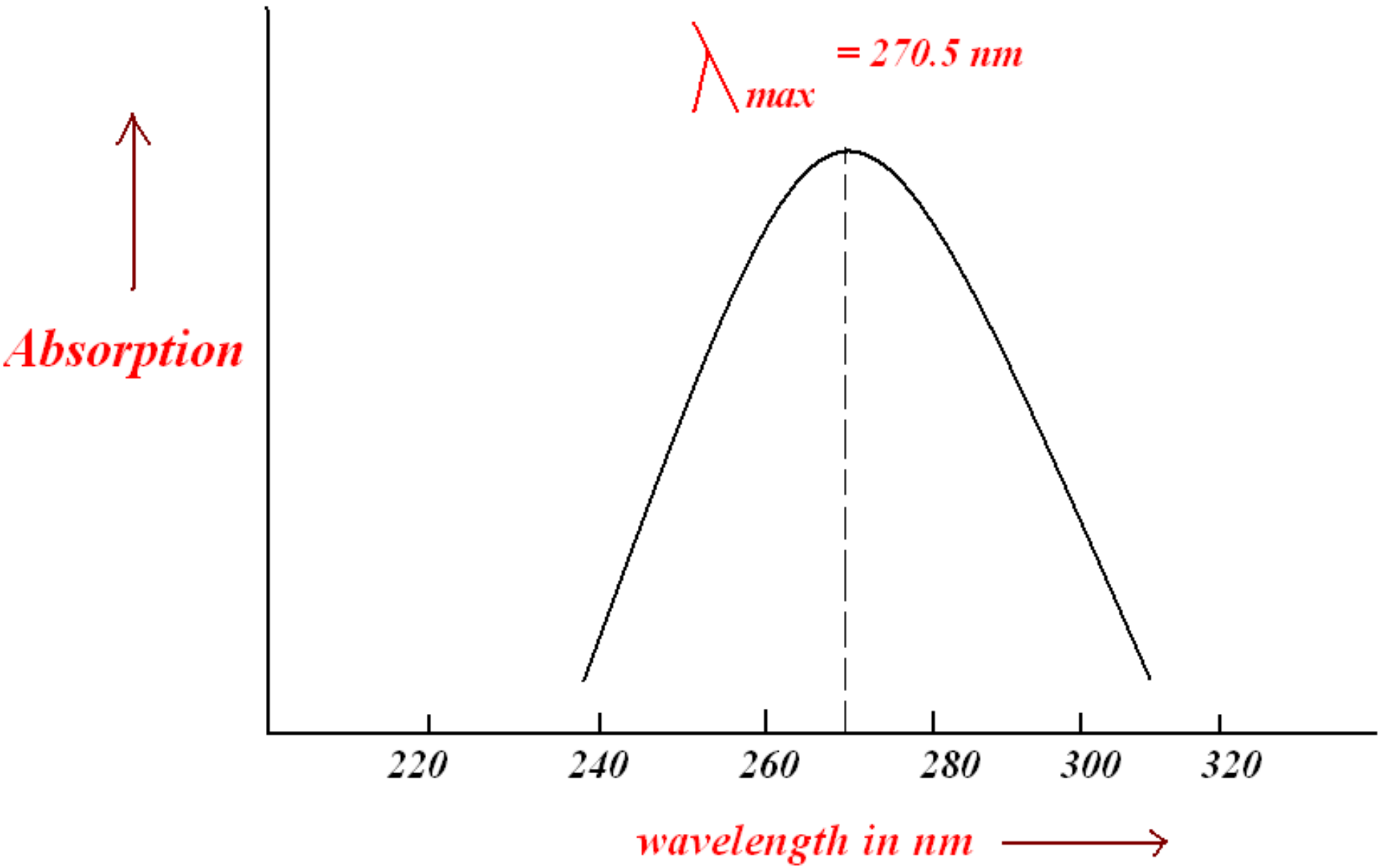

fig:- UV spectrum of acetone

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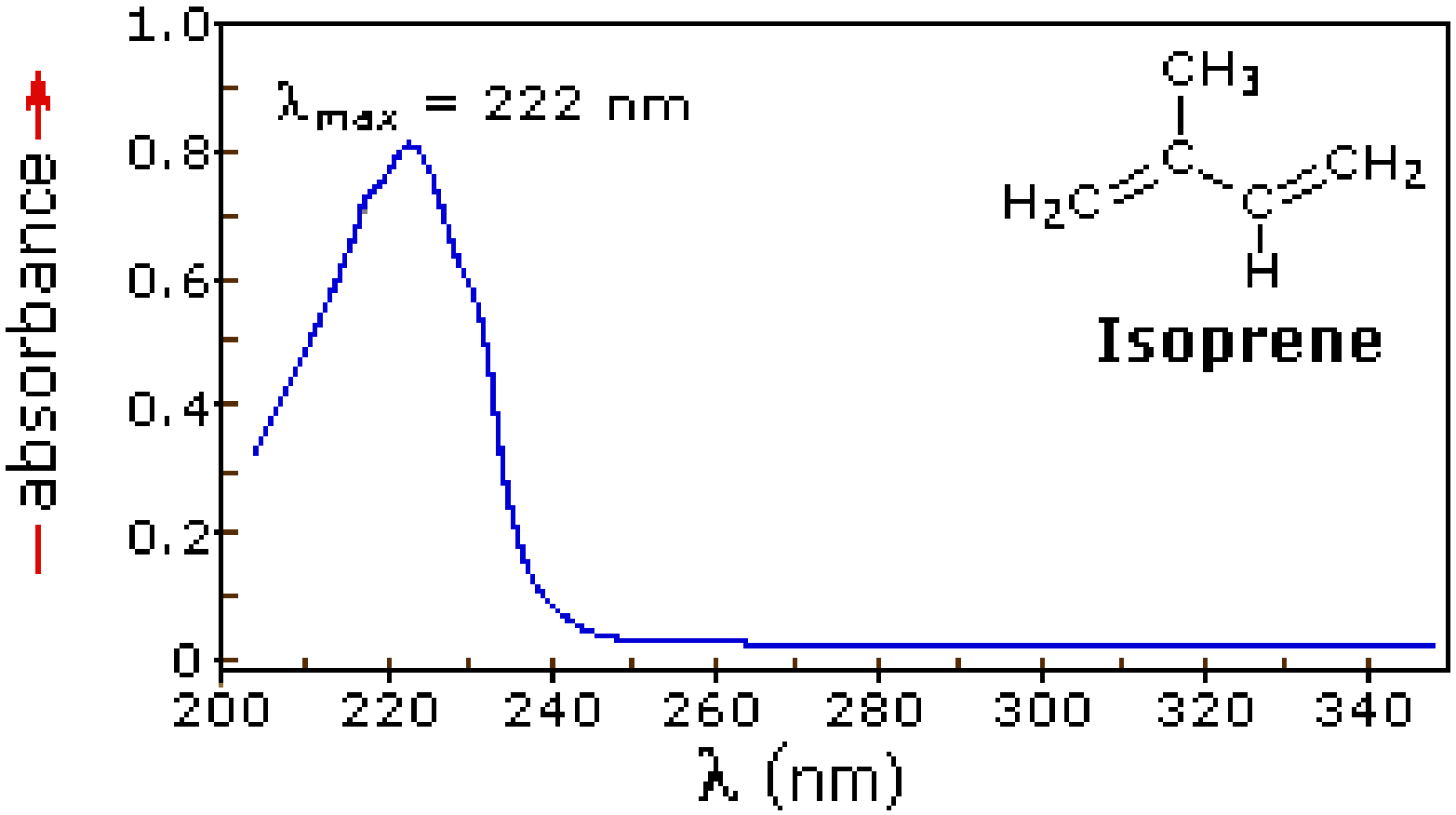
- UV-visible spectrum of isoprene showing maximum absorption at 222 nm.



Every time a molecule has a bond, the atoms in a bond have their atomic orbitals merged to form molecular orbitals which can be occupied by electrons of different energy levels. Ground state molecular orbitals can be excited to anti-bonding molecular orbitals.

These electrons when imparted with energy in the form of light radiation get excited from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) and the resulting species is known as the excited state or anti-bonding state.

TYPES OF TRANSITIONS:

In U.V spectroscopy molecule undergo electronic transition involving σ, π and n electrons.

- Four types of electronic transition are possible.

i. $\sigma \rightarrow \sigma^*$ transition
ii. $n \rightarrow \sigma^*$ transition
iii. $n \rightarrow \pi^*$ transition
iv. $\pi \rightarrow \pi^*$ transition

i. σ → σ* Transition :

- An electron in a bonding σ orbital of a molecule is excited to the corresponding anti-bonding orbital by the absorption of radiation.
- To induce a σ → σ* transition it required LARGE ENERGY.
- Ex: Methane
- Methane contain only single C-H bonds it undergo only σ → σ* transition only, it gives absorption maximum at 125nm.

ii. n→ σ* transition :

In this type saturated compounds containing atoms with unshared electron pairs are undergo n → σ* transition.

It require less energy than the σ → σ* type.

Most of the absorption peaks appearing below 200nm.

In the presence of polar solvents the absorption maximum tend to shift shorter wavelength

Ex: Water , ethanol.

- In this the peaks in U.V region relatively small.

Ex: Methlychloried , Oxygen, Nitrogen.

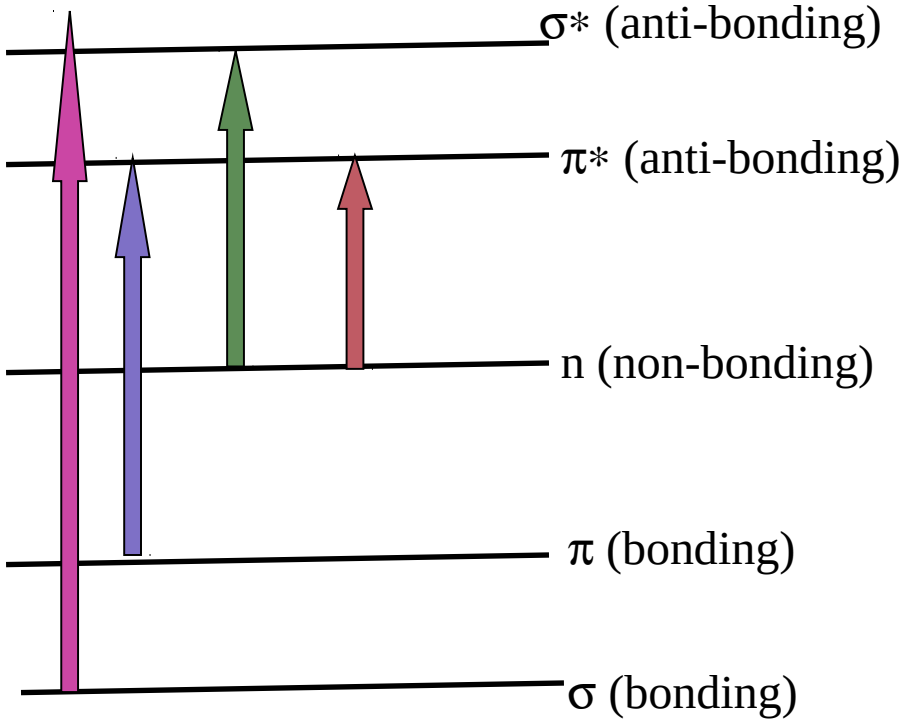
iii n → π* & π → π* transitions

Most organic compounds are undergo transitions for n → π* and π → π* transition.

- Because energies required for processes bring the absorption peaks into spectral region.
- Both transition require the presence of an unsaturated functional group to the ´∏´ orbitals.

- Ex: For π → π* ⊩ Alkenes, carbonyl compounds, alkynes

- For n → π* ⊩ carbonyl compounds.



Four types of transitions

$\sigma \rightarrow \sigma^*$

$\pi \rightarrow \pi^*$

$n \rightarrow \sigma^*$

$n \rightarrow \pi^*$

ABSORBANCE LAWS

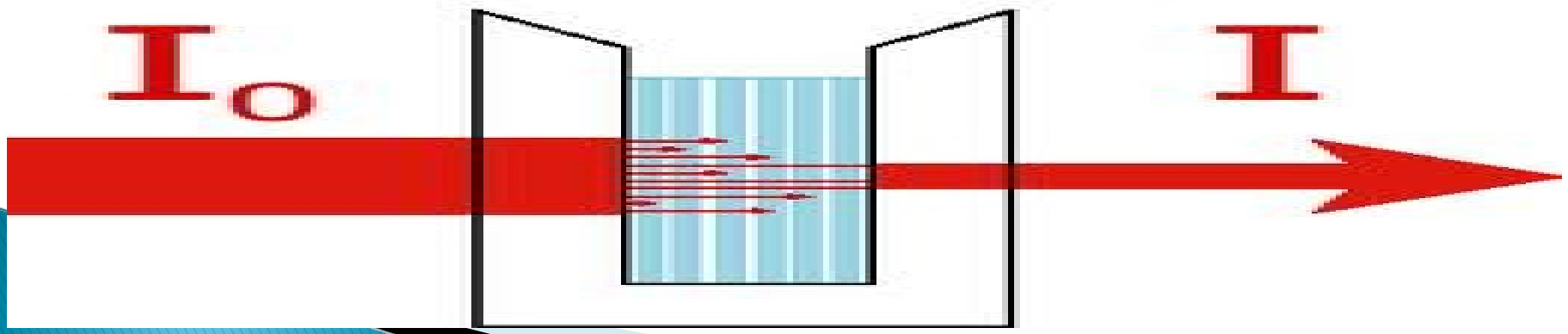
BEER'S LAW

" The intensity of a beam of monochromatic light decrease exponentially with the increase in concentration of the absorbing substance" .

Arithmetically;

$$-dI/dc \bar{\alpha} I$$

$$I = Io.\, e^{-kc} \text{ ----------eq (1)}$$



LAMBERT'S LAW

" When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light"

mathematically;

$$-dI/dt \propto I$$

$$-\ln . I = kt+b \text{ ----------eq(2)}$$

the combination of eq 1 & 2 we will get

$$A = Kct$$

$$A = \varepsilon ct \qquad (K=\varepsilon)$$

LIMITATION OF LAWS

- The real limitation of the beer's law is successfully in describing the absorption behavior of dilute solution only.
- In this regarding it may be considered as a limiting law.

- As degree of interaction depends upon the contraction, the occurrence of this phenomenon causes deviations from linear relationship between absorbance and contraction.

INSTRUMENTATION

Components of spectrophotometer

- Source
- Monochromator
- Sample compartment
- Detector
- Recorder

INSTRUMENTATION

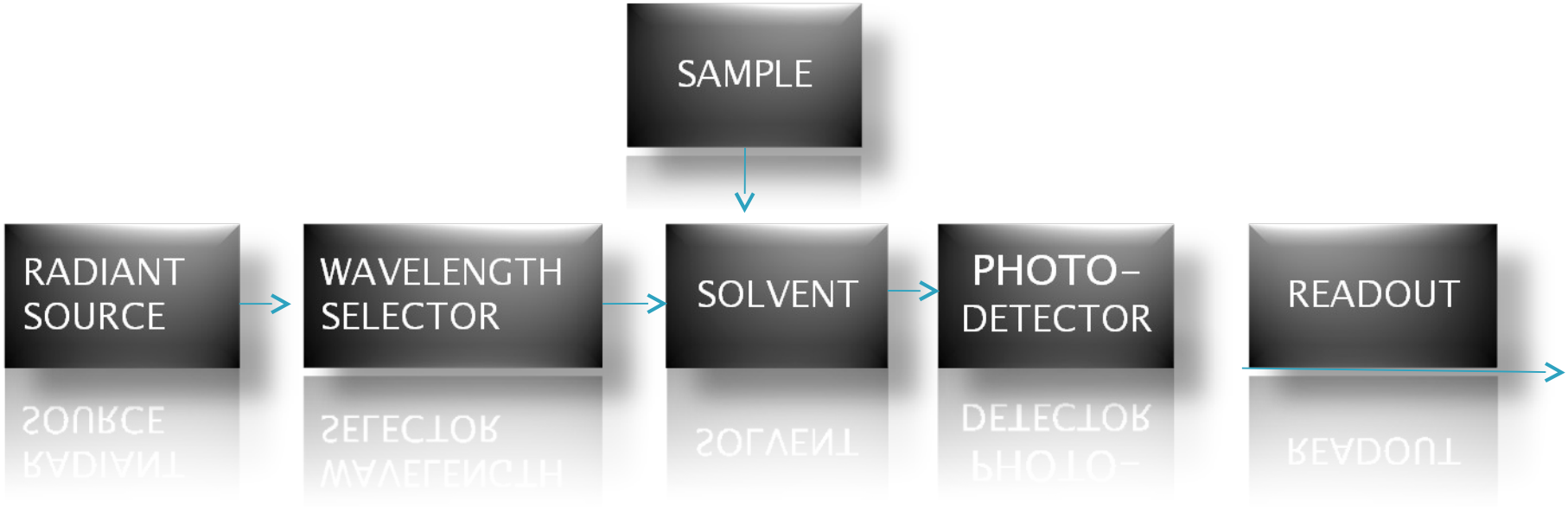


Fig.-block diagram of instrumentation of UV-spectrophotometer

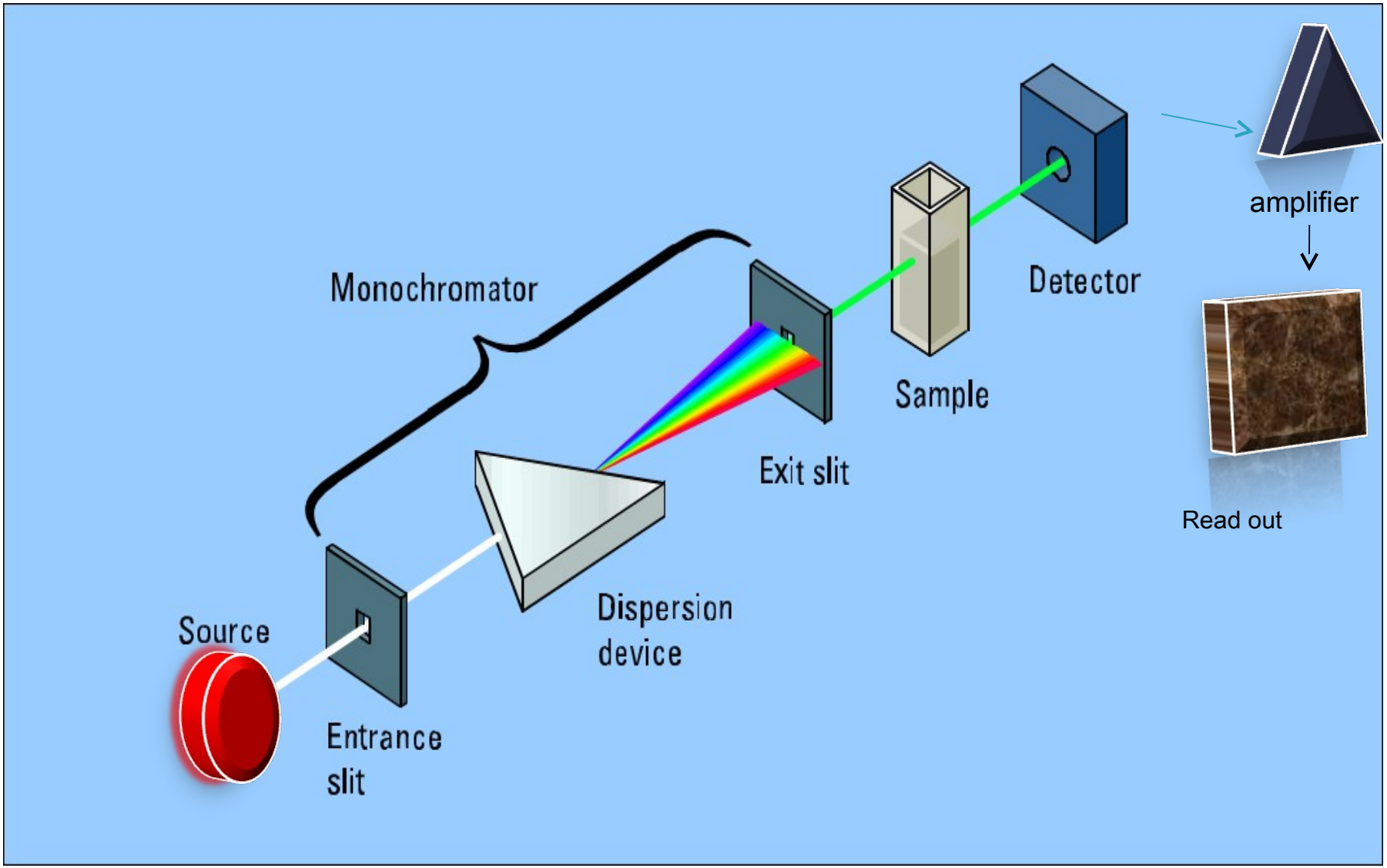


Fig:- block diagrammatic representation of UV-spectrophotometer

RADIATION SOURCE

It is important that the power of the radiation source does not change abruptly over its wavelength range. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum.

Both Deuterium and Hydrogen lamps emit radiation in the range 160 - 375 nm.

Problem-

- Due to evaporation of tungsten life period decreases.
- It is overcome by using tungsten-halogen lamp.
- Halogen gas prevents evaporation of tungsten.

RADIATION SOURCE

For ultra violet region-

Hydrogen discharge lamp

- consist of two electrode contain in deuterium filled silica envelop.

UV-Vis spectrophotometer have both deuterium & tungsten lamps.

- Selection of lamp is made by moving lamp mounting or mirror to cause the light fall on Monochromator.

Deuterium lamps:-

- Radiation emitted is 3-5 times more than the hydrogen discharge lamps.

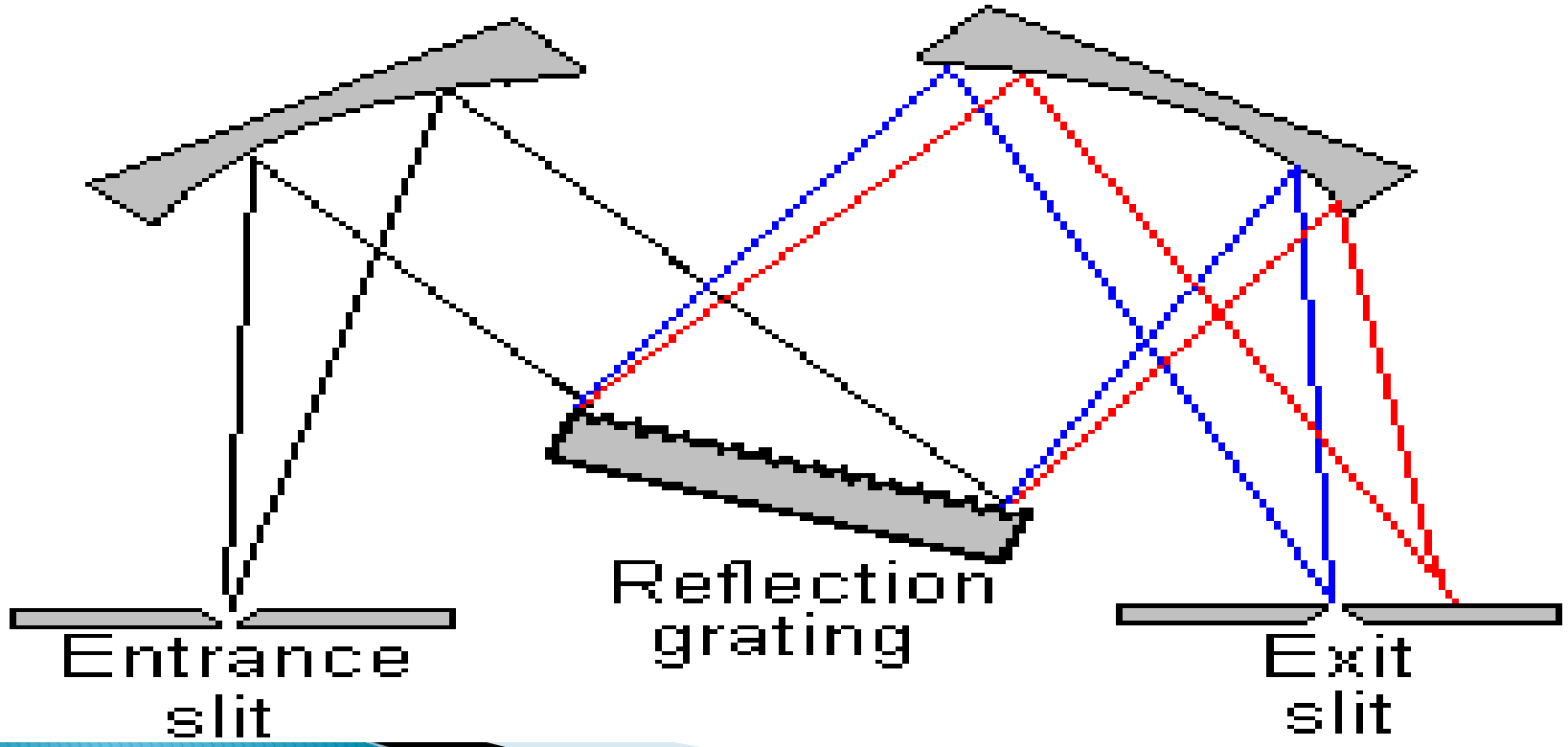
Xenon discharge lamp:-

- Xenon stored under pressure in 10-30 atmosphere.

FILTERS OR MONOCHROMATORS

All Monochromators contain the following component parts;

- An entrance slit
- A collimating lens
- A dispersing device (a prism or a grating)
- A focusing lens
- An exit slit



- Filters –

a)Glass filters- Made from pieces of colored glass which transmit limited wave length range of spectrum. Wide band width 150nm.

b)Gelatin filters- Consist of mixture of dyes placed in gelatin & sandwiched between glass plates. Band width 25nm.

c)Inter ferometric filters- Band width 15nm

- Prisms-

-Prism bends the monochromatic light.
-Amount of deviation depends on wavelength
-They produce non linear dispersion.

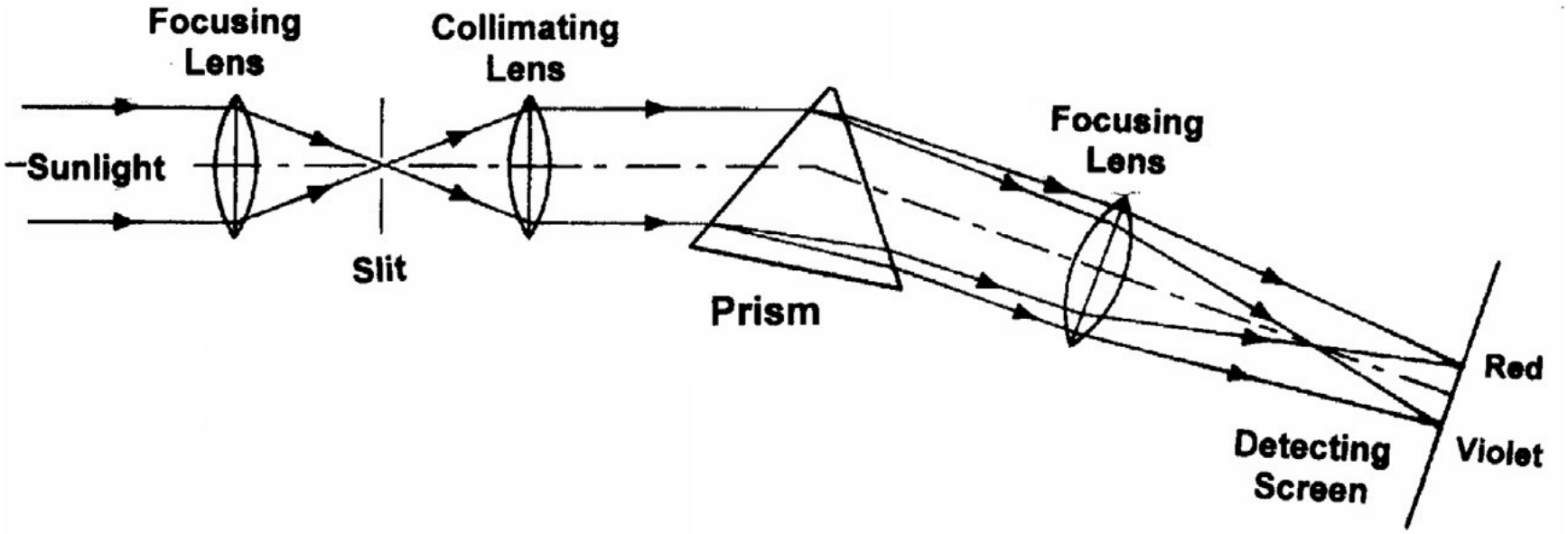


Fig.-mechanism of working of prism.

SAMPLE CONTAINERS OR SAMPLE CELLS

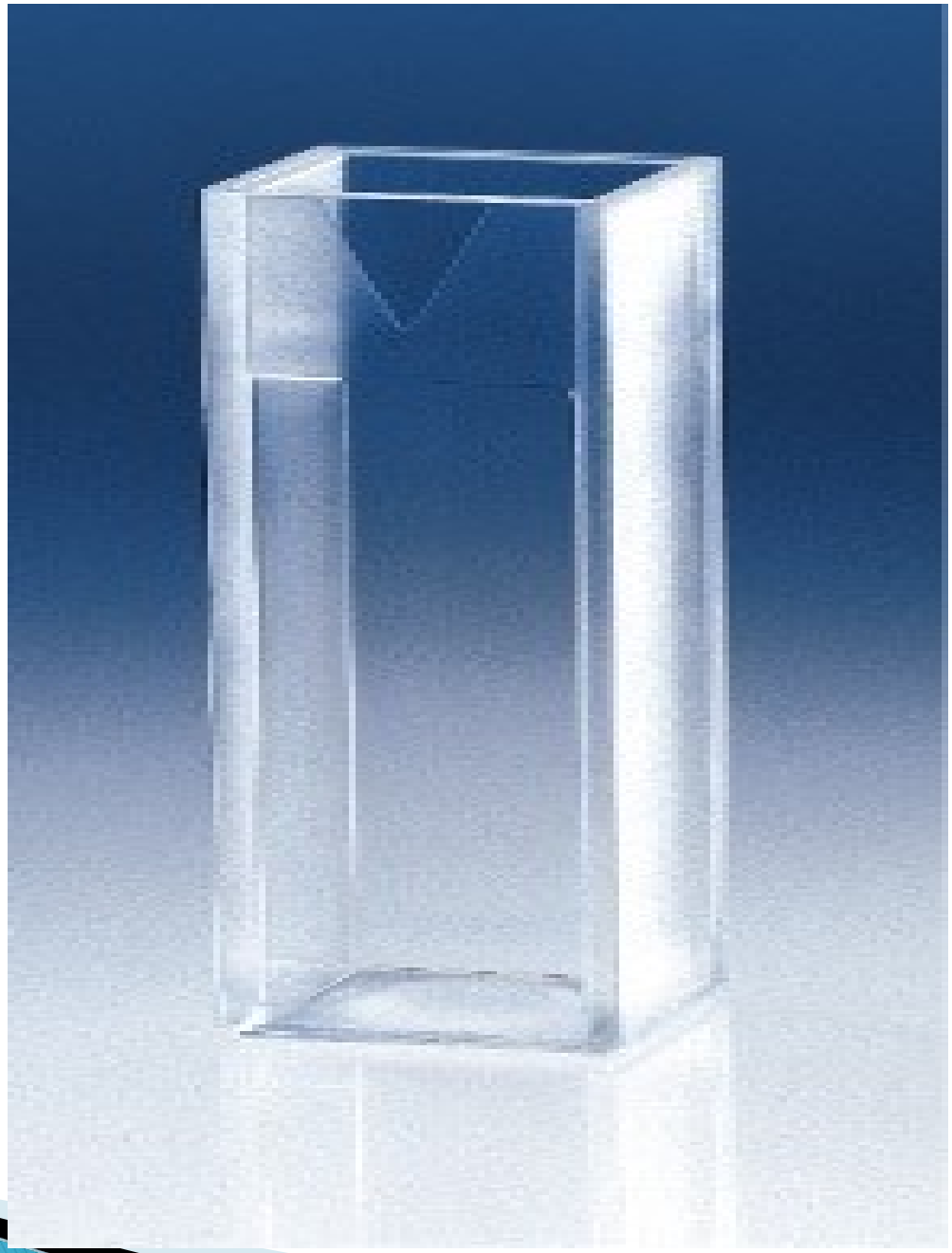
A variety of sample cells available for UV region. The choice of sample cell is based on

a) the path length, shape, size

b) the transmission characteristics at the desired wavelength

c) the relative expense

- The cell holding the sample should be transparent to the wavelength region to be recorded. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000nm. The thickness of the cell is generally 1 cm. cells may be rectangular in shape or cylindrical with flat ends.



DETECTORS

Three common types of detectors are used

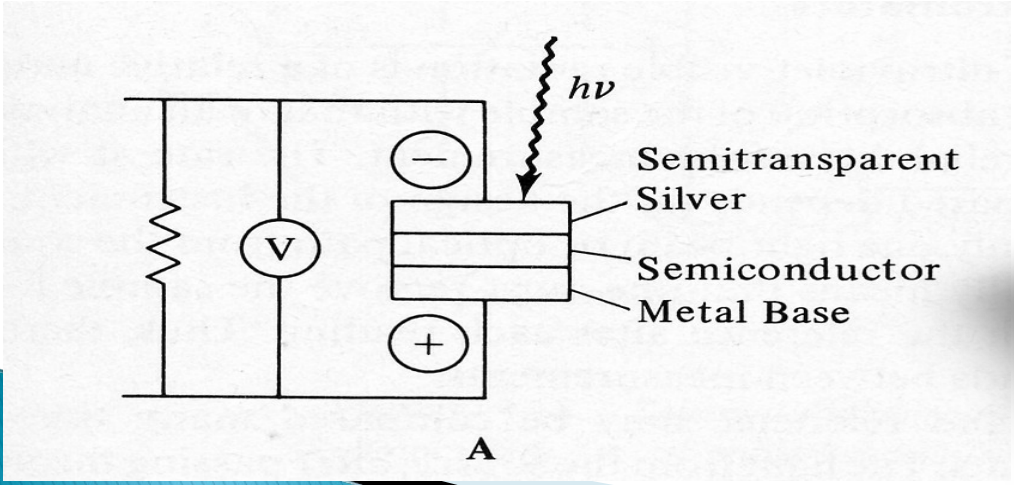
I. Barrier layer cell

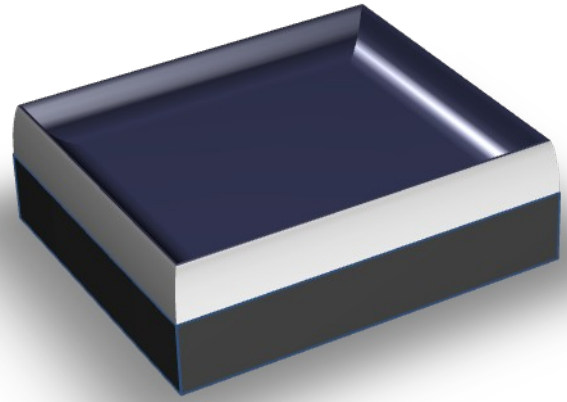
II. Photo cell detector

III. Photomultiplier , Photo voltaic cells

barrier layer cells

It consist of flat Cu or Fe electrode on which semiconductor such as selenium is deposited. on the selenium a thin layer of silver or gold is sputtered over the surface.





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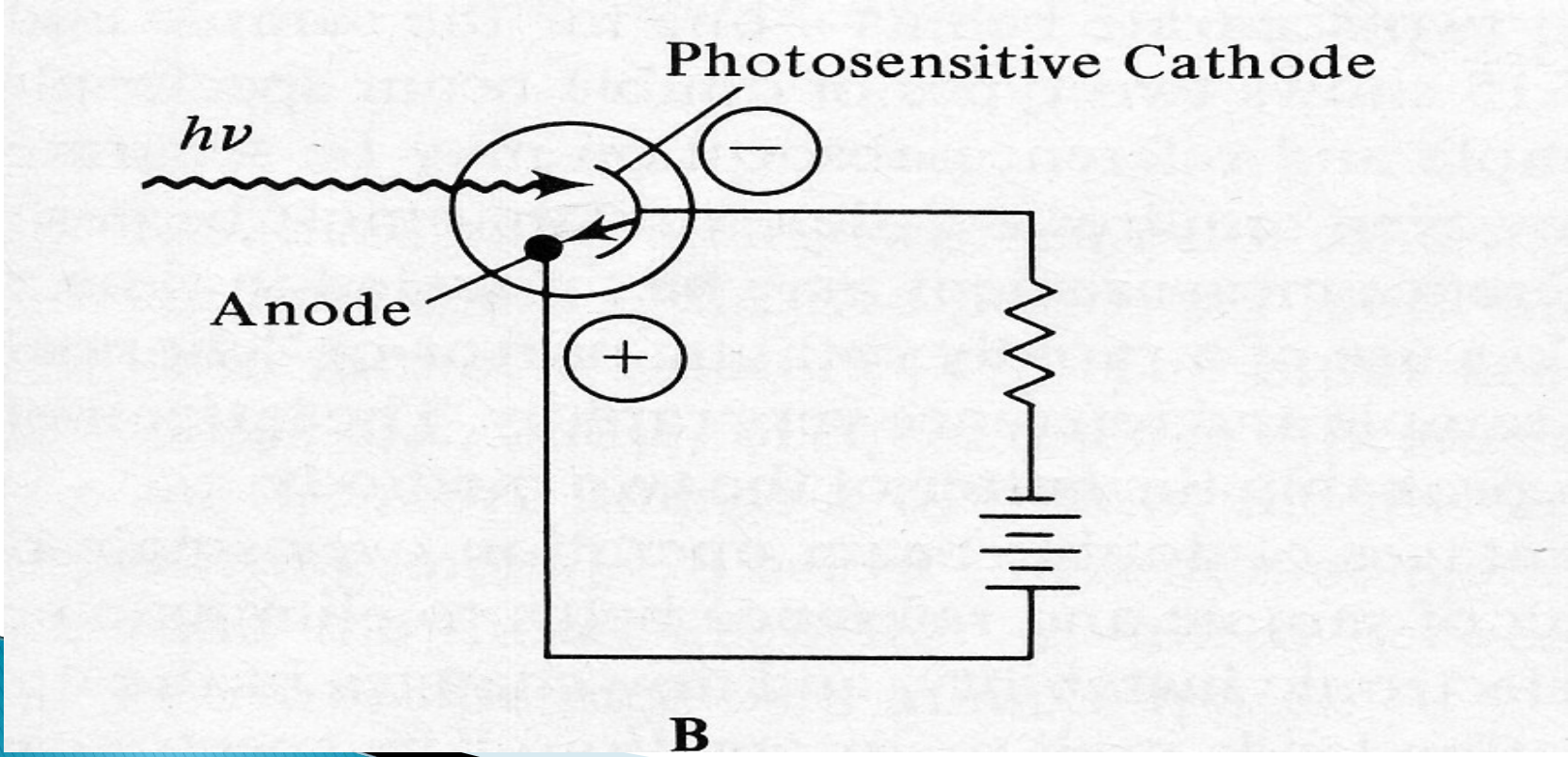
- Photomultiplier tube

  It is generally used as detector in UV- spectrophotometer It is the combination of photodiode & electron multiplier.

  It consist of evacuated tube contains photo- cathode. 9-16 electrodes known as dynodes.



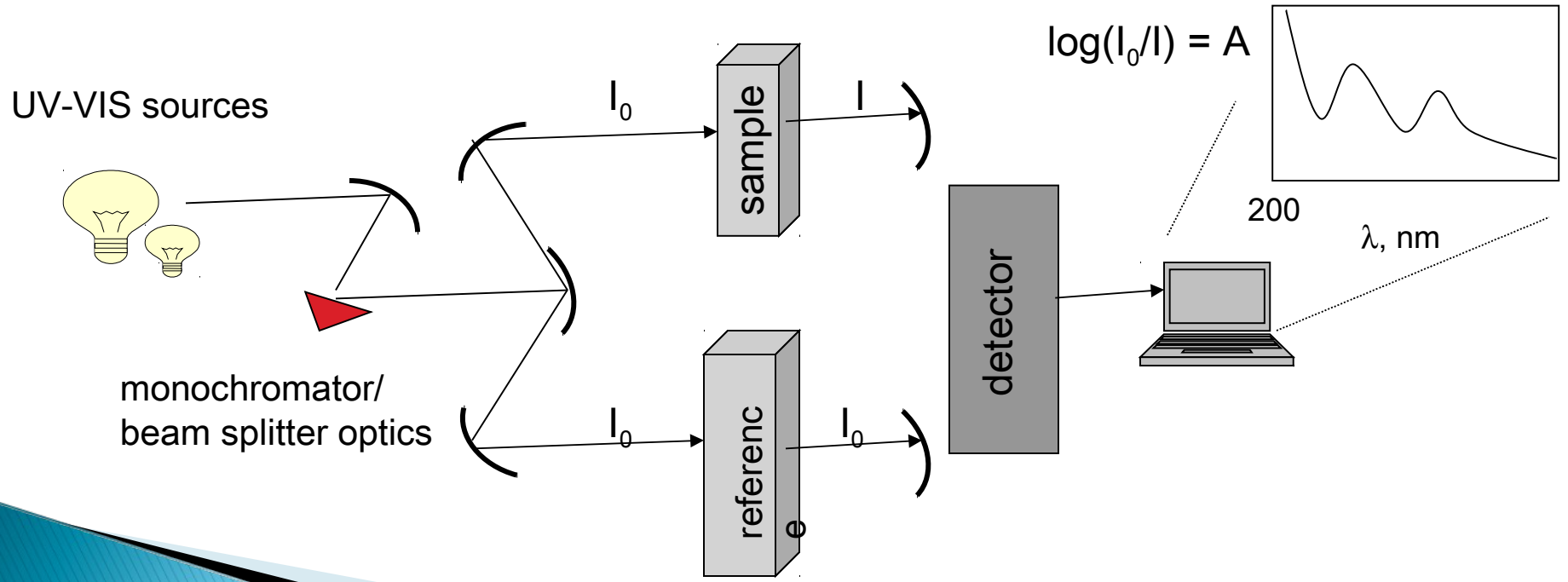
Recorder

DESCRIPTION OF UV– SPECTROPHOTOMETER

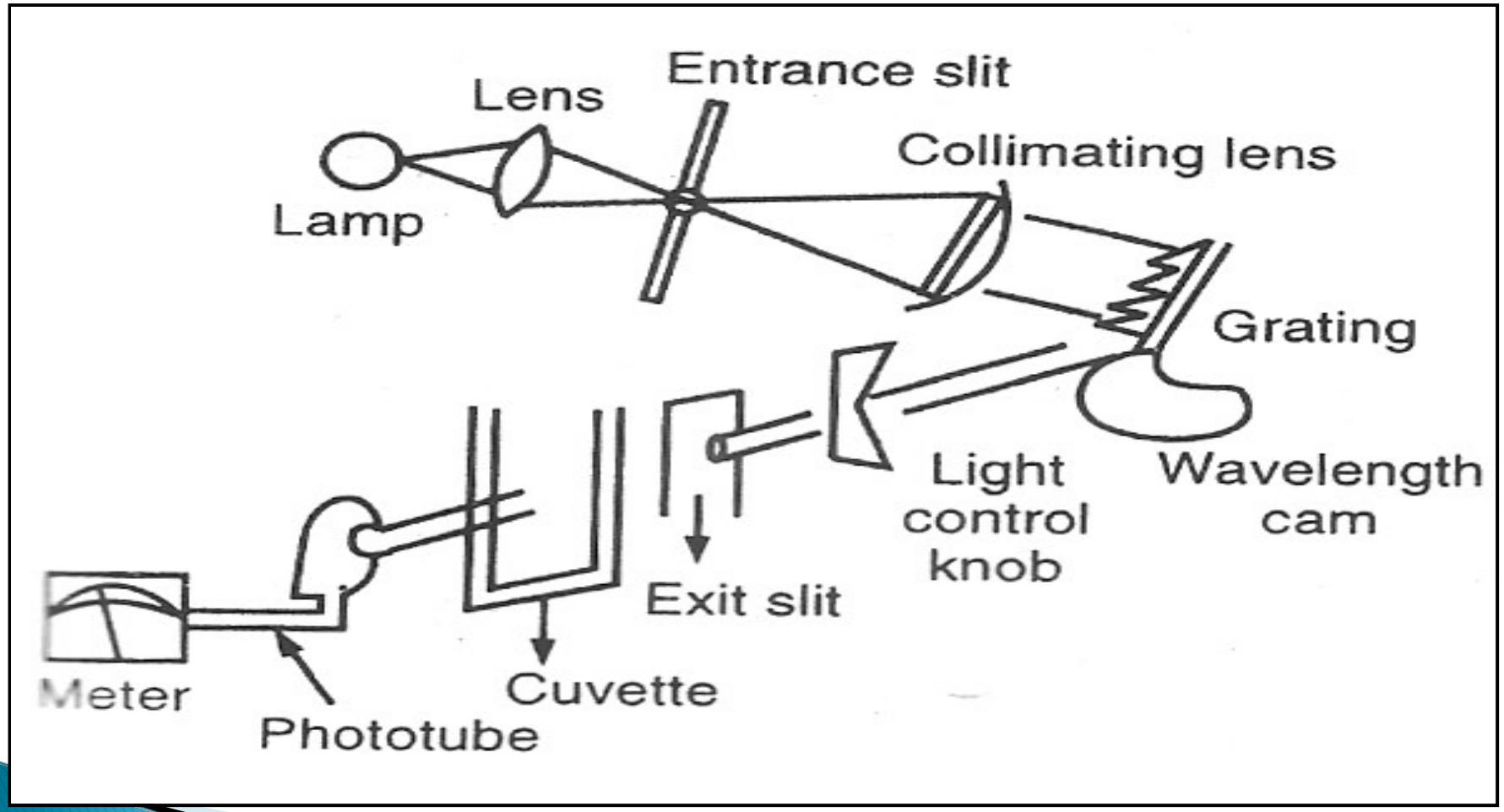
Advantage of double beam spectrophotometer:- It is not necessary to continually replace the blank with the sample or to adjust the auto zero.

The ratio of the powers of the sample & reference is constantly obtained.

It has rapid scanning over the wide wavelength region because of the above two factors.



Single beam spectrophotometer



Double beam colorimeter

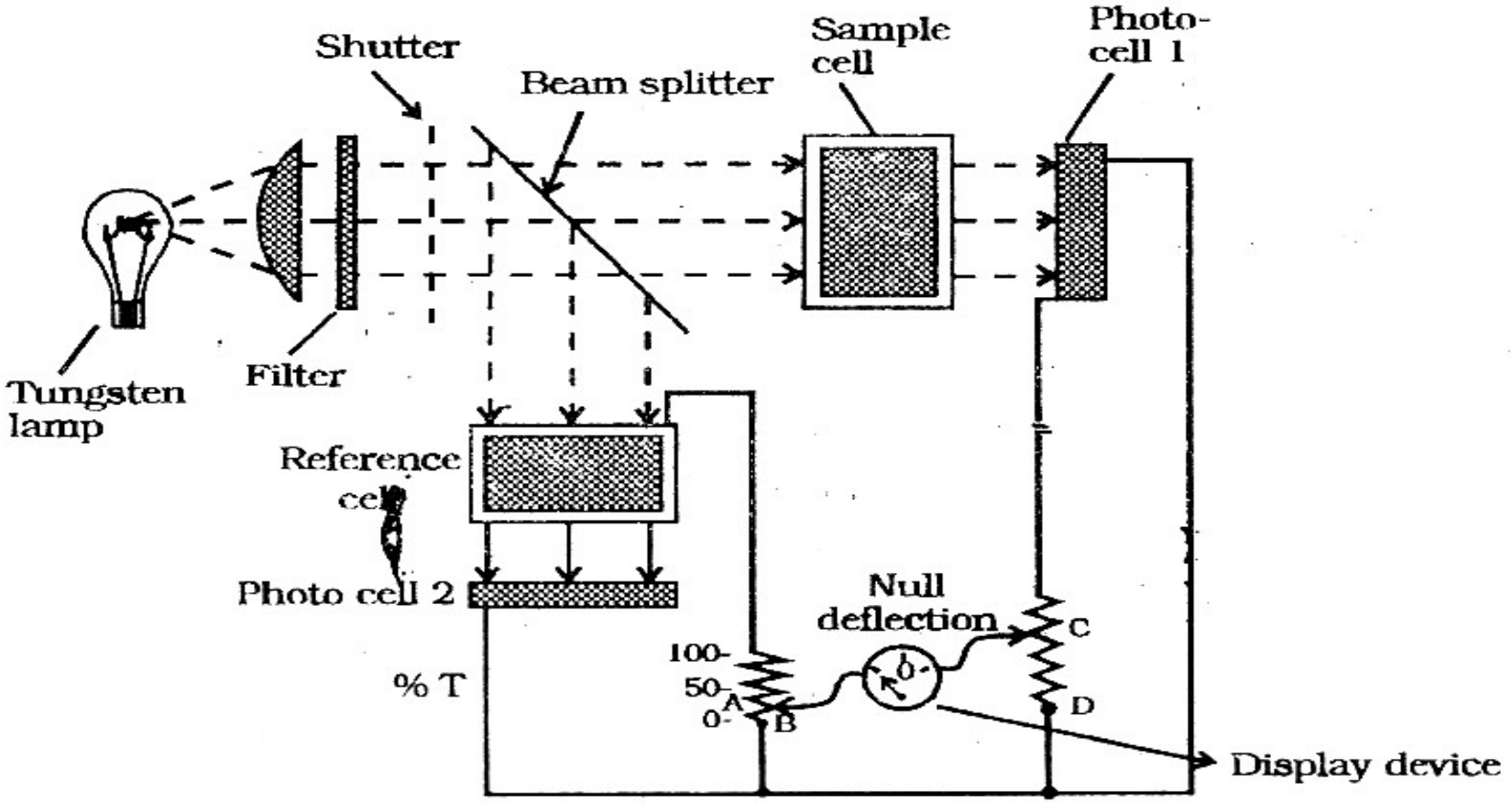


Fig 1.15 Double beam Colorimeter

CHROMOPHORE

- Any Functional group which is responsible for impairing colour to the compound is called as chromophore.

  Ex: NO2

- Covalently unsaturated groups responsible for the impairing of the colures.

  Ex: C=C, C=O

- Two types of chromophore

  a) Independent chromophore

  b) dependent chromophore

SIMPLE CHROMOPHORIC GROUPS

Groups	
C – C	1350
C = C	1900
C = O	1900 2800
O – H	1850
NO_2	2800
C_6H_5(PHENYL)	1950 2500

AUXOCHROME

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

Ex: -OH, -NH2, -OR groups

For example when the auxochrome –NH2 is attached to the benzene ring, it absorption changes from λmax 255 to 280nm.

TYPES

Two types

a. Bathochromic groups
b. Hypsochromic group

BATHOCHROMIC GROUPS

Those groups which deepen the colour of chromogen are called bathochromic groups.

Deepening of colour means displacement to longer wavelength.

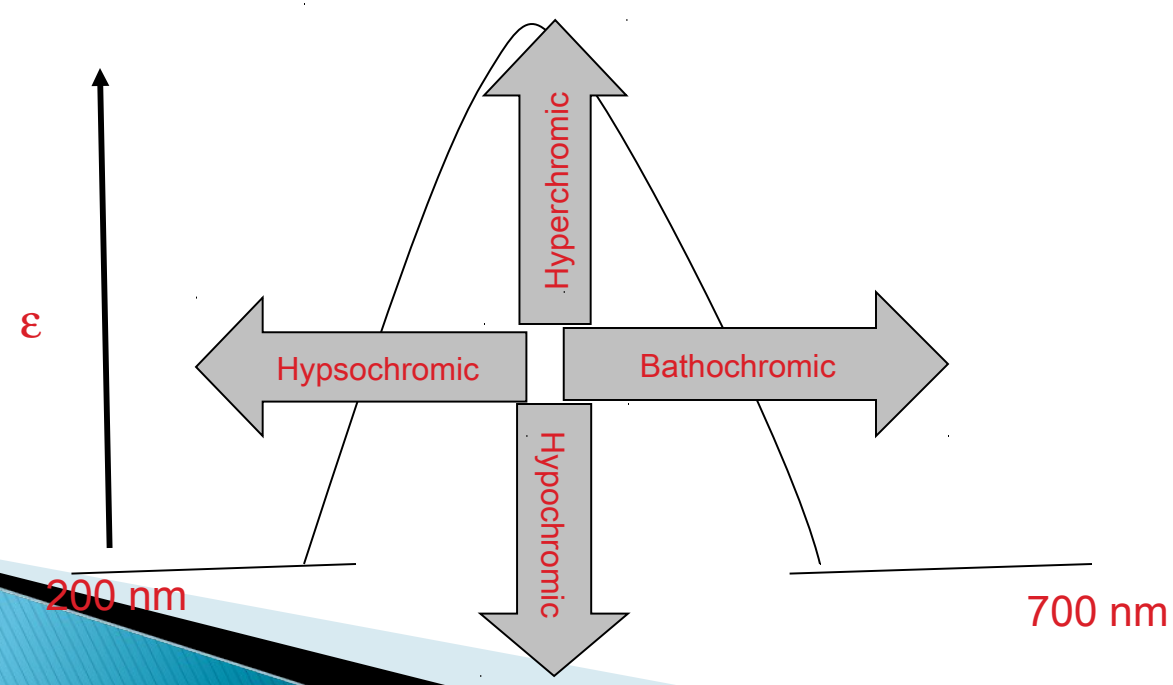
yellow→ orange → red → purple → violet→ blue → green

HYPSOCHROMIC GROUPS

Those groups which diminish or lighten the colour of the chromogen are called hypsochromic groups.

- They cause displacement to shorter wavelength.

  Ex:- acetylation of –OH or –NH2 groups, -OCOCH3 and –NHCOCH3



WOODWARD–FEISER RULE

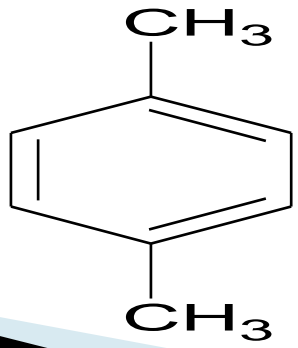
- It is used for calculating the absorption maxima
- Woodward (1941) gives certain rule for correlating λmax with the molecular structure

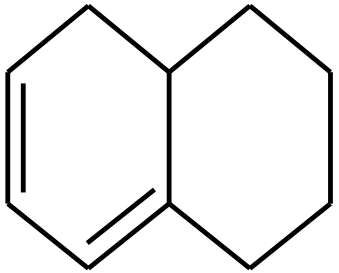
This rule for calculating λmax in conjugated dienes, trienes, polyenes.

- Homoannular dienes:-

  cyclic dienes having conjugated double bonds in the same ring.

  e.g.





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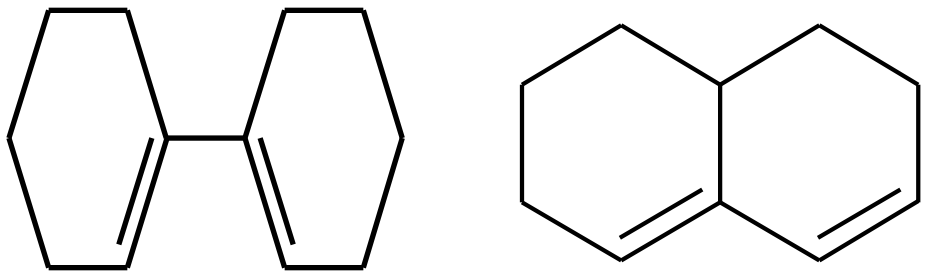
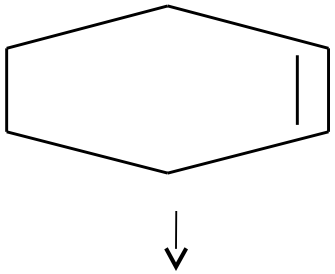
- Hateroannuler dienes



e.g. Heteroannuler dienes

- Endocyclic double bonds

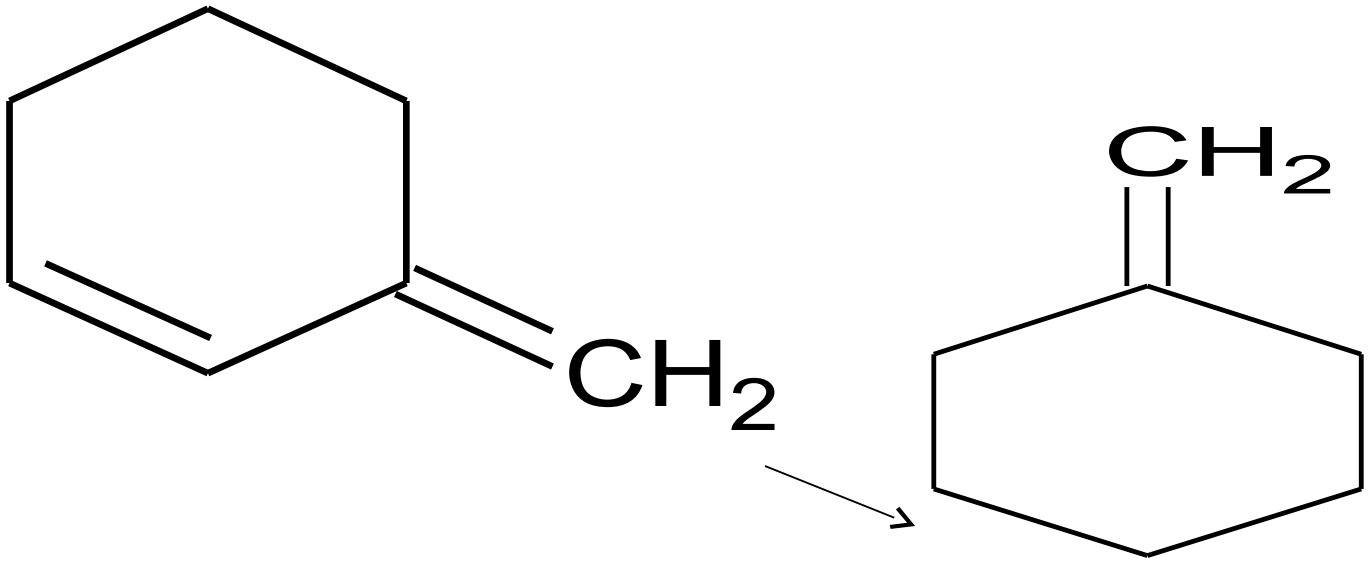
  it is the double bond present in ring as shown.



Endocyclic double bond

Exocyclic double bonds

double bond in which one of the double bonded atom is the part of ring system.



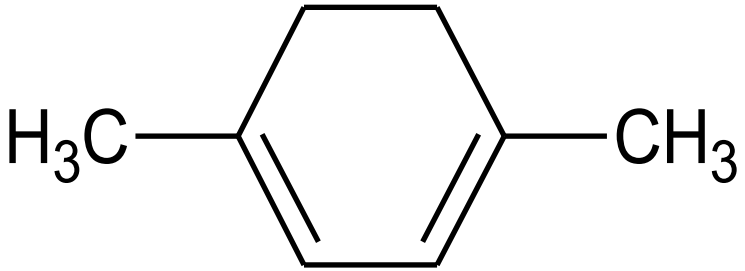
Exocyclic double bond

WOODWARD'S–FIESER RULE FOR CONJUGATED DIENES

- a)Parent values-
  1. acyclic & Hateroannuler conjugated dienes 215 nm
  2.Homoannular conjugated dienes 253 nm
  3.Acyclic trienes 245 nm

- b)Increments-
  1.Each alkyl substituent or ring residue 5 nm
  2.Exocyclic double bond 5 nm
  3.Double bond extending conjugation 30 nm
  4.auxochromes-
- -OR 6 nm
- -SR 30 nm
- -Cl , Br 5 nm
- -NR2 60 nm
- -OCOCH3 0 nm

1,4- dimethyl cyclohex-1,3,-diene



Parent value for Homoannular dienes = 253 nm

Two alkyl substituent's $2 \times 5 = 10$ nm

Two ring residues $2 \times 5 = 10$ nm

Calculated value = 273 nm

SOLVENT EFFECTS - INTENSITY

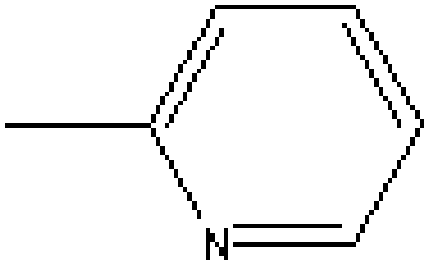
Solvents can induce significant changes in the intensity of peaks.

Hyperchromic – Increase in absorption intensity.

Hypochromic – Decrease in absorption intensity.

Absorption characteristics of 2-methylpyridine

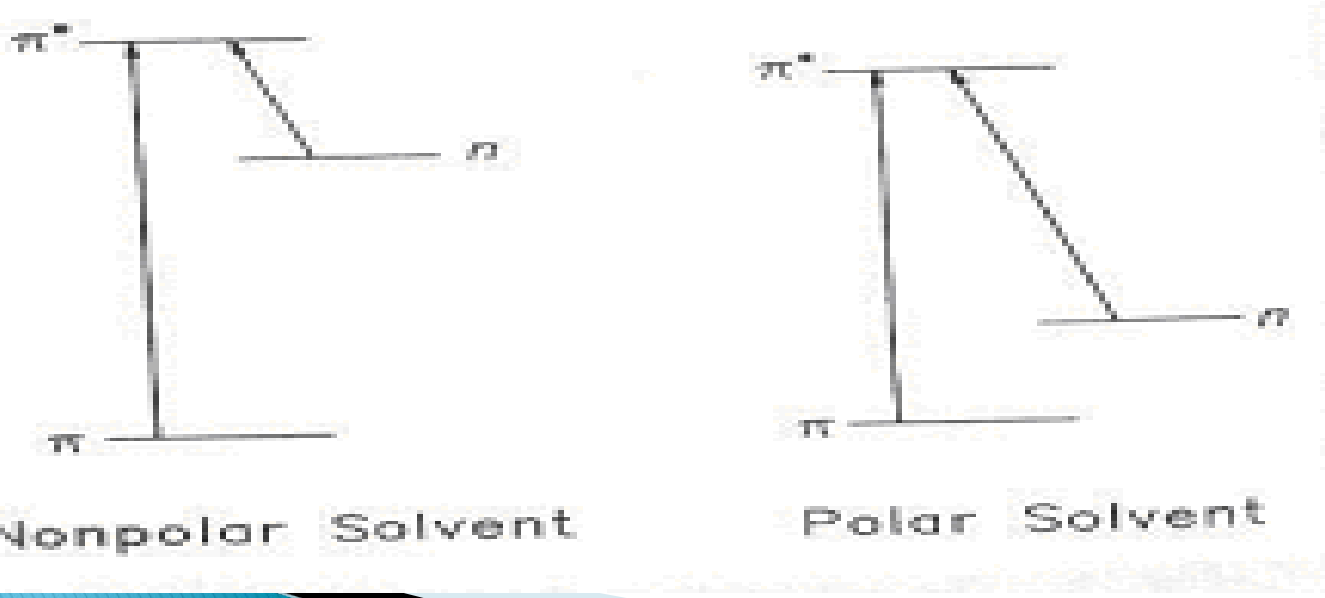
Solvent	λ_{max}	ε_{max}
Hexane	260	2000
Chloroform	263	4500
Ethanol	260	4000
Water	260	4000
Ethanol - HCl (1:1)	262	5200



SOLVENT EFFECTS

- π -> π* transitions leads to more polar excited state that is more easily stabilized by polar solvent associations (H-bonds). The π* state is more polar and stabilized more in polar solvent relative to nonpolar one, thus in going from nonpolar to polar solvent there is a red shift or bathochromic shift (increase in λ_{max}, decrease in ΔE).

- For n -> π* transition, the n state is much more easily stabilized by polar solvent effects (H-bonds and association), so in going from nonpolar to polar solvent there is a blue shift or hypsochromic shift (decrease in λ_{max}, increase in ΔE).



APPLICATIONS:

A. APPLICATIONS IN ORGANIC COMPOUNDS

1.It is helps to show the relationship between different groups, it is useful to detect the conjugation of the compounds

2.Detection of geometrical isomers, In case of geometrical isomers compounds, that **trans isomers** exhibits λmax at slightly longer wavelength and have larger extinction coefficient then the **cis isomers .**

3.Detection of functional groups, it is possible to detect the presence of certain functional groups with the help of UV Spectrum.

GENERAL APPLICATIONS:

1.Qualitative analysis, UV absorption spectroscopy can characterizes those type of compounds which absorb UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compound.

2. It is useful in Quantitative analysis of the compounds.

3. Detection of impurities, UV absorption spectroscopy is the one of the best method for detecting impurities in organic compounds.

Tautomeric equilibrium, UV spectroscopy can be used to determine the percentage of various keto and enol forms present in tautomeric equilibrium.

5. Chemical kinetics, UV spectroscopy can be used to study the kinetics of reactions.

6. Molecular weight determination, molecular weights of compounds can be measured by spectroscopy.

7. Analysis of inorganic compounds.

8. Measuring concentration of solution, absorption band can also used to determine the concentration of compounds in a solution.

9. Inorganic chemistry, absorption spectra have been used in connection with many problems in inorganic chemistry.

10. It is useful to determine the structure of the chloral.

QUALITATIVE ANALYSIS

Pharmacopoeial identification of drug
(1) By using absorbance & wavelength
(2) By taking absorption ratio
(3) Limit test (b)Structural analysis

Quantitative analysis

Quantitative analysis A)By using beer's law and using absorptivity value By using reference standard Multiple standard method

B)Single compound analysis direct analysis Using separation method After extraction after chromatographic separation Using column chromatography Using HPLC

Indirect analysis

a)Single compound without chromophore
b) Drugs with chromophoric reagent
1.For analyte which absorb weakly in UV region
2.For avoiding interference

3.Improve selectivity of assay
4. Determination of composition of complex Mole ratio method Continuous variation method (job curve method)
5 . Study of kinetics

Disadvantages:

- Samples should be in solution. Mixture of substances poses difficult to analyse and requires prior separation.
- Interference from the sample's matrix makes the measurement difficult .

THANK YOU