Ultraviolet–visible spectroscopy
UV

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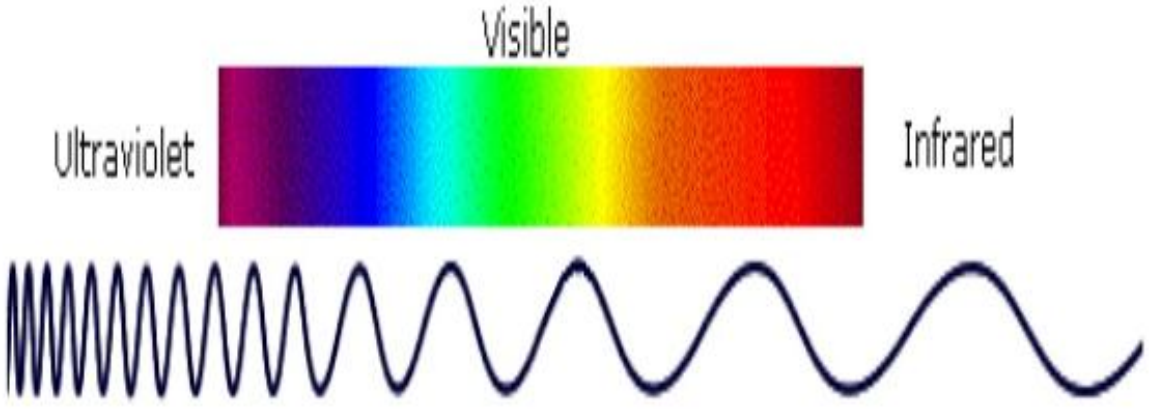
Ultraviolet–visible spectroscopy or **ultraviolet-visible spectrophotometry** : refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region.

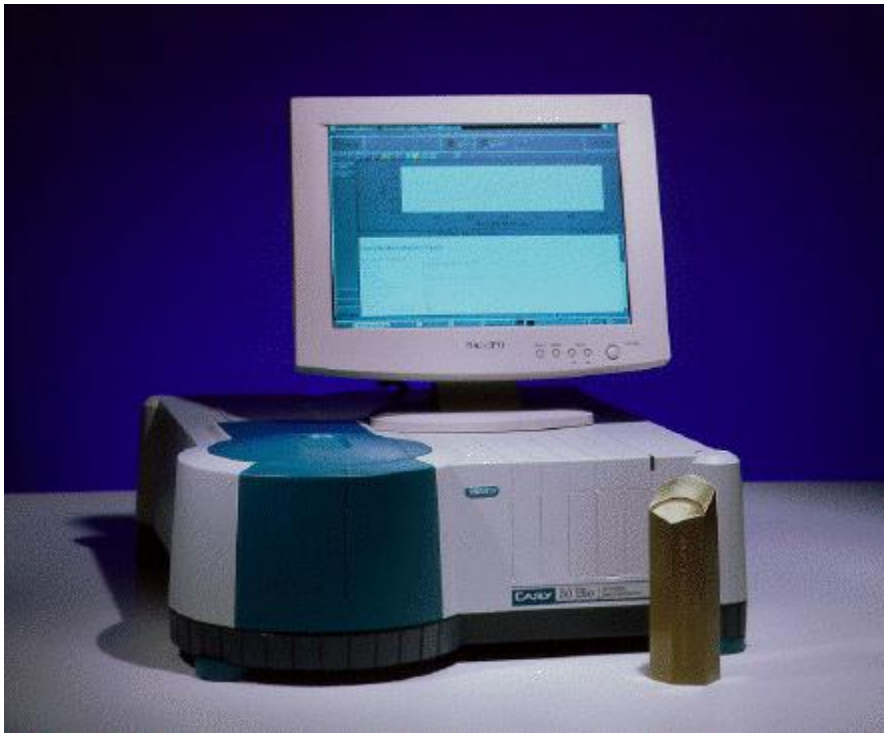
This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges.
The absorption or reflectance in the visible range directly affects the
perceived color of the chemicals involved.
In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

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 end of visible light(4000Å) & ends at 2000A.

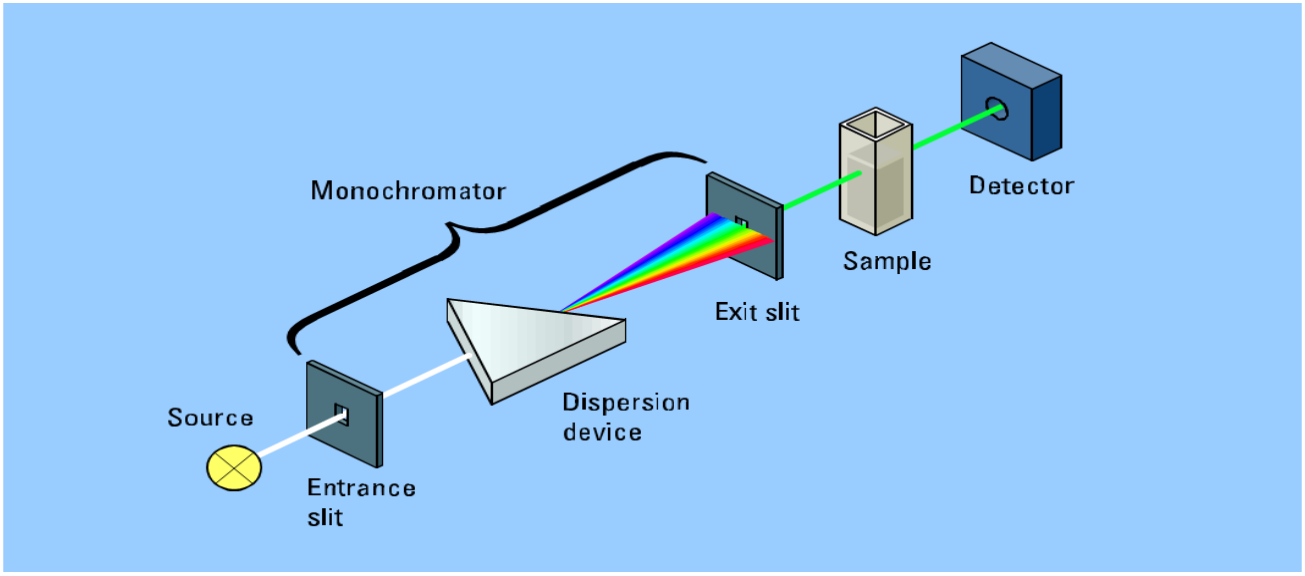


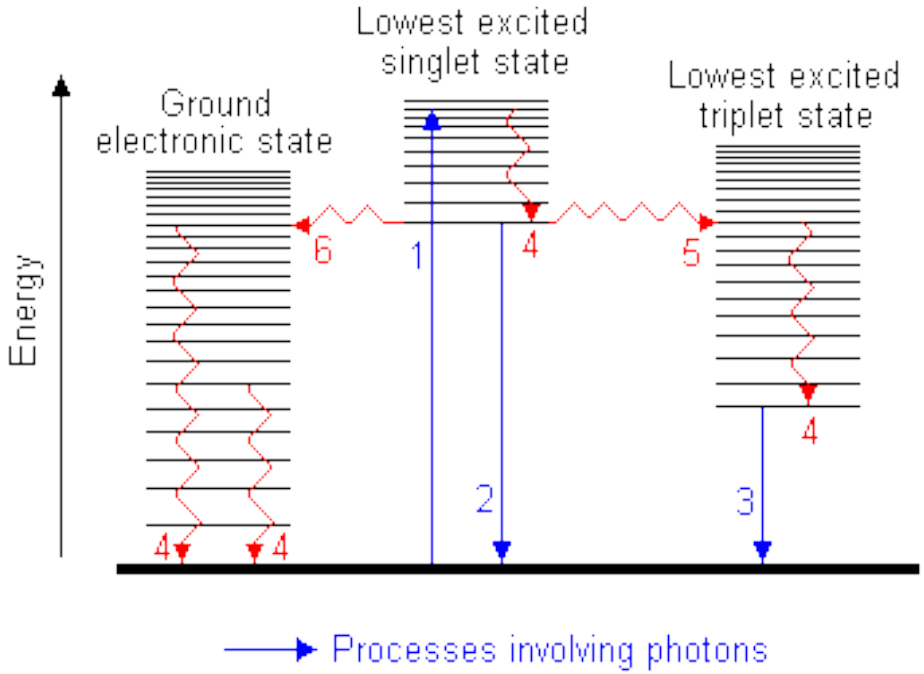

Dell CPU
Speaker
Cary 50 Spectrometer
Flat ScreenMonitor and Keyboard
HP Printer



Principle of ultraviolet-visible absorption :

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals.
The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb.





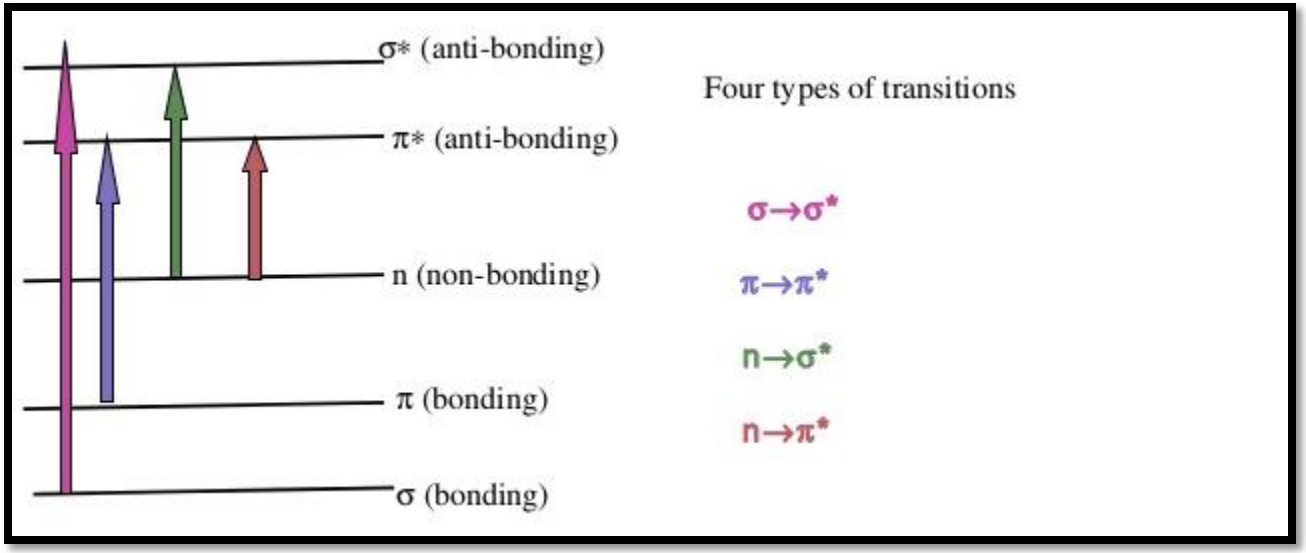
1. Absorption
2. Fluorescence
3. Phosphorescence
4. Vibrational relaxation
5. Intersystem crossing
6. Internal conversion

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 → σ^* transition
iii. n → π^* transition
iv. $\pi \rightarrow \pi^*$ transition



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;

$$-dI/dc \propto I$$

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

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;

$-dI/dt \propto I$

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

the combination of eq 1 & 2 we will get

$A = Kct$

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

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.

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

Components Of spectrophotometer :

- Source
- Monochromator
- Sample compartment
- Detector
- Recorder

INSTRUMENTATION

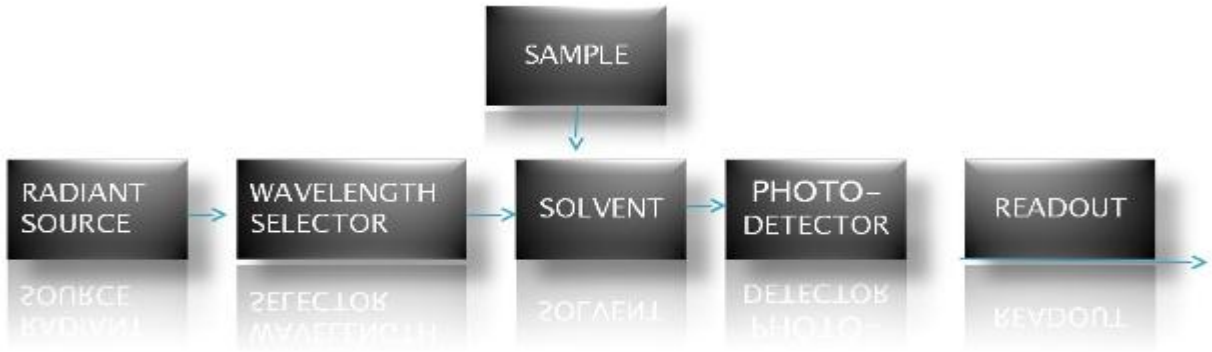
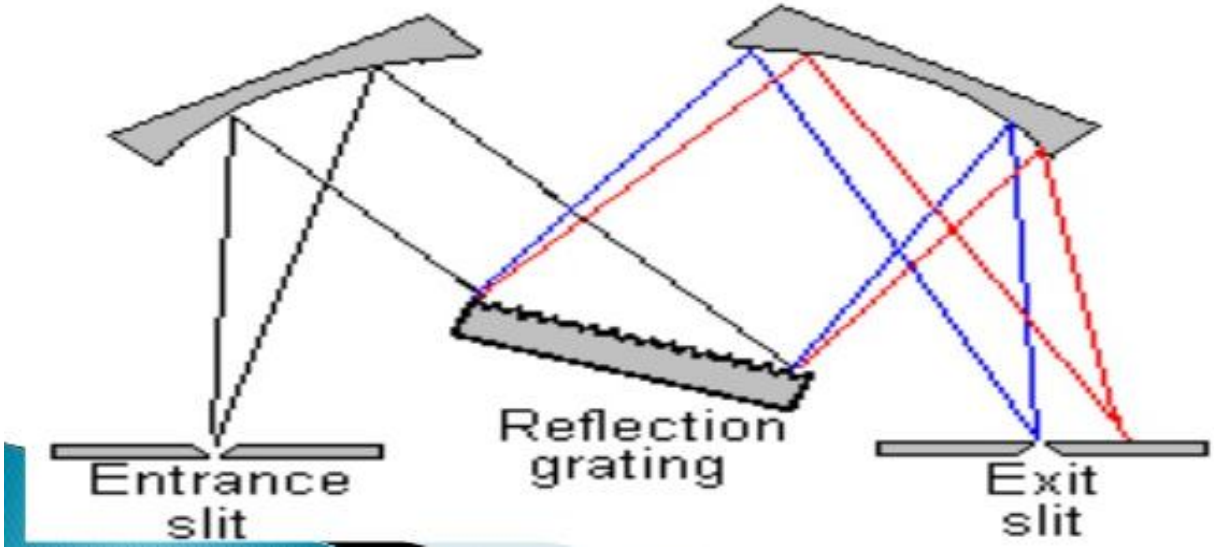


Fig.-block diagram of instrumentation of UV-spectrophotometer

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

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.

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.

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 .