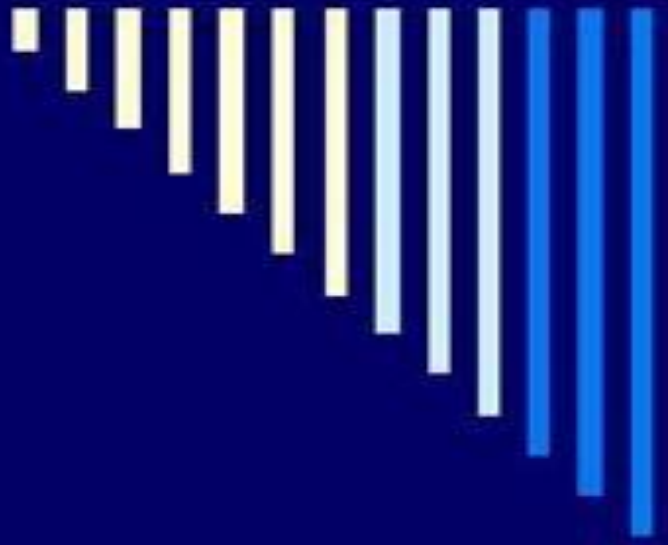


UV-Visible spectroscopy

Dr. BASMA M. ABD RAZIK

Advance pharmaceutical analysis:

- The aim of this course to teach the students how to identify organic compounds from the:

synergistic information afforded by the combination of mass (MS), infrared (IR), nuclear magnetic resonance (NMR), and ultraviolet (UV) spectra. Essentially, the molecule is perturbed by these energy probes and the molecule's responses are recorded as spectra.

The pharmaceutical analyses remain unchanged, but remarkable evolution of instrumentation has been done.

In comparison, ultraviolet spectrometry has become relatively less useful for our purpose. NMR, without question, has become the most sophisticated tool available to the organic chemist, in comparison; ultraviolet spectrometry has become relatively less useful for our purpose.

Mass spectrometry	Molecular size and formula
Infrared spectroscopy	Functional groups
NMR spectroscopy	Map of carbon–hydrogen framework

Absorption of UV-Visible light is chiefly caused by electronic excitation; the spectrum provides limited information about the structure of the molecule.

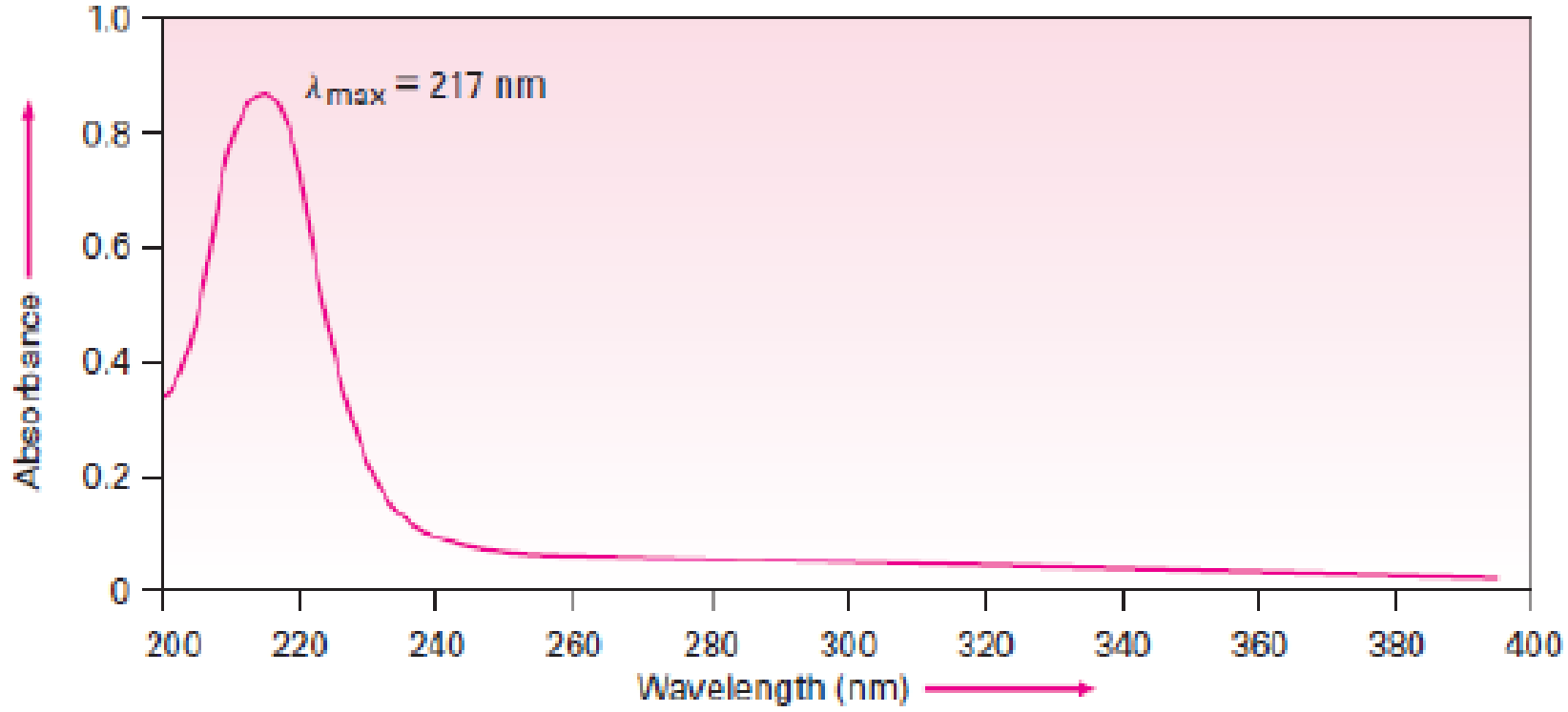


Figure 14.12 The ultraviolet spectrum of 1,3-butadiene, λ_{max} = 217 nm.

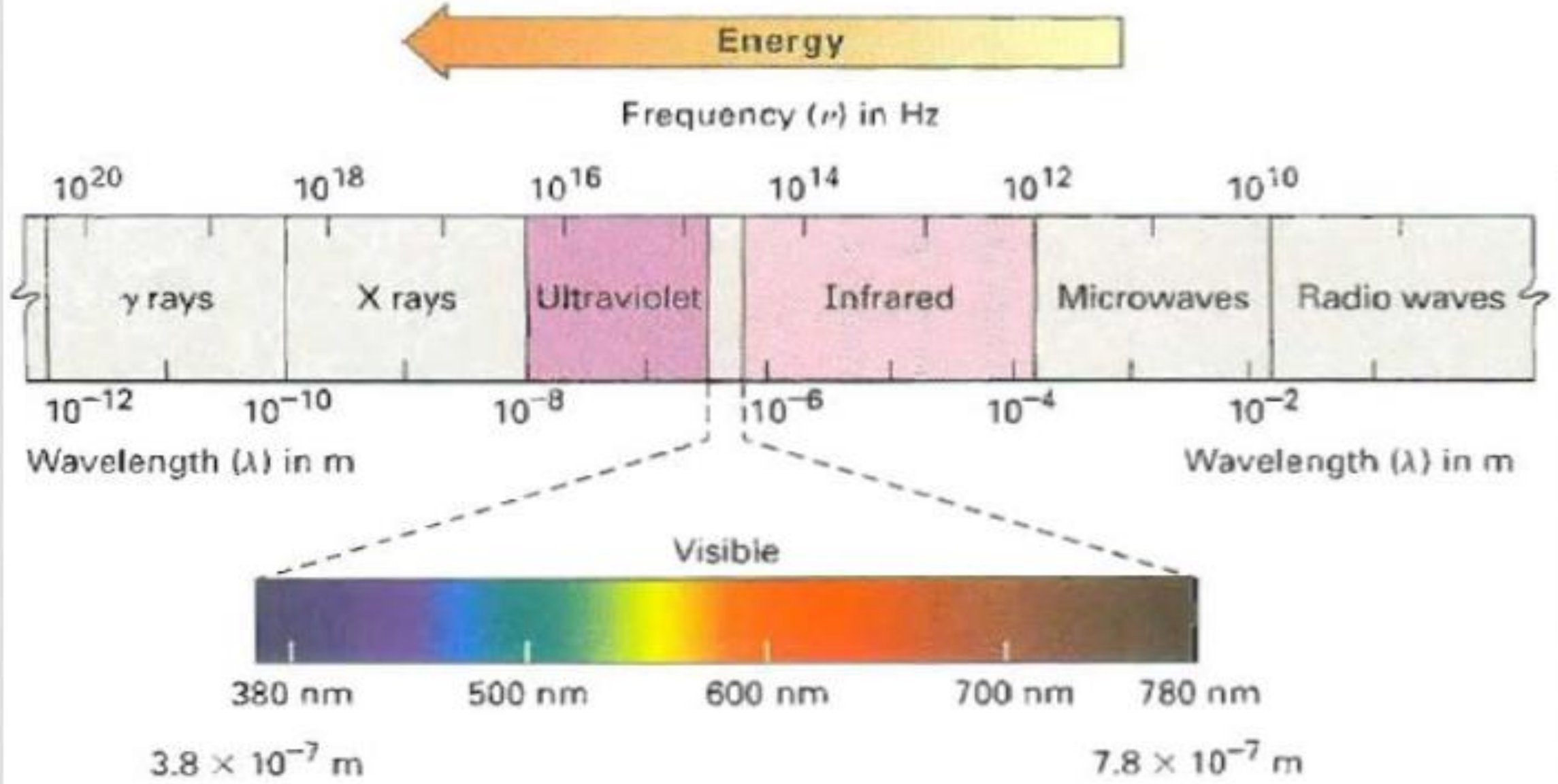
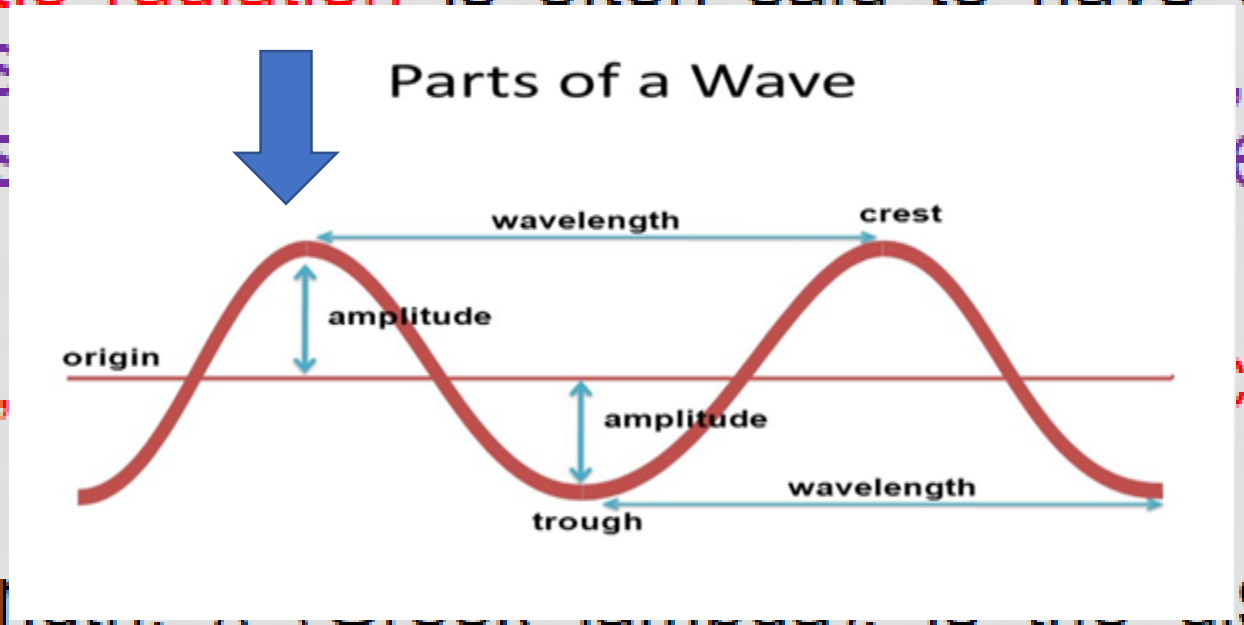


Figure 12.10-The electromagnetic spectrum covers a continuous range of wavelengths and frequencies, from radio waves at the low-frequency end to gamma rays at the high- frequency end. The

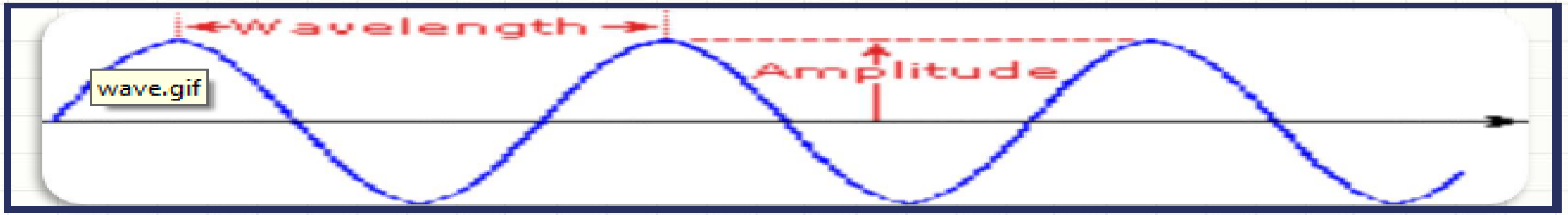
Electromagnetic radiation is often said to have dual behavior. In some respects , called a *photon*, yet in other res e.



Like all waves, cterized by:

- The wavelength, λ (Greek lambda), is the distance from one wave maximum to the next.
- The frequency, υ (Greek nu), is the number of waves that pass by a fixed point per unit time, usually given in reciprocal seconds (s^{-1}), or hertz, Hz (1 Hz = s^{-1}).
- The amplitude is the height of a wave, measured from midpoint to peak.

Electromagnetic Radiation



The various forms of electromagnetic radiation differ in their frequency and, therefore, their energy. The energy of electromagnetic radiation can be calculated in electron volts from the following equation:

- The relationship between wavelength & frequency can be written as:

$$c = \nu \lambda$$

- As photon is subjected to energy, so

$$\varepsilon = h\nu = \frac{hc}{\lambda}$$

where h = Planck's constant (6.62×10^{-34} J · s = 1.58×10^{-34} cal · s).

C= speed of the light (3* 10^{10}) cm /second

V = the frequency (hertz), and λ is the wavelength (cm).

UV / VISIBLESPECTROSCOPY

Principles of Spectroscopy

Principles of Spectroscopy

- The principle is based on the measurement of spectrum of a sample containing atoms / molecules.

- Spectrum is a graph of intensity of absorbed or emitted radiation by sample verses frequency (ν) or wavelength (λ).

- Spectrometer is an instrument design to measure the spectrum of a compound.

Principles of Spectroscopy

1. Absorption Spectroscopy:

- An analytical technique which concerns with the measurement of absorption of electromagnetic radiation.

- e.g. UV (185 - 400 nm) / Visible (400 - 800 nm) Spectroscopy, IR Spectroscopy (0.76 - 15 µm)

Principles of Spectroscopy

2. Emission Spectroscopy:

- An analytical technique in which emission (of a particle or radiation) is dispersed according to some property of the emission & the amount of dispersion is measured.

- e.g. Mass Spectroscopy

Interaction of EMR with Matter

Interaction of EMR with matter

1. Electronic Energy Levels:

- At room temperature the molecules are in the lowest energy levels E_0.

- When the molecules absorb UV-visible light from EMR, one of the outermost bond / lone pair electron is promoted to higher energy state such as E_1, E_2, ...E_n, etc is called as electronic transition and the difference is as:

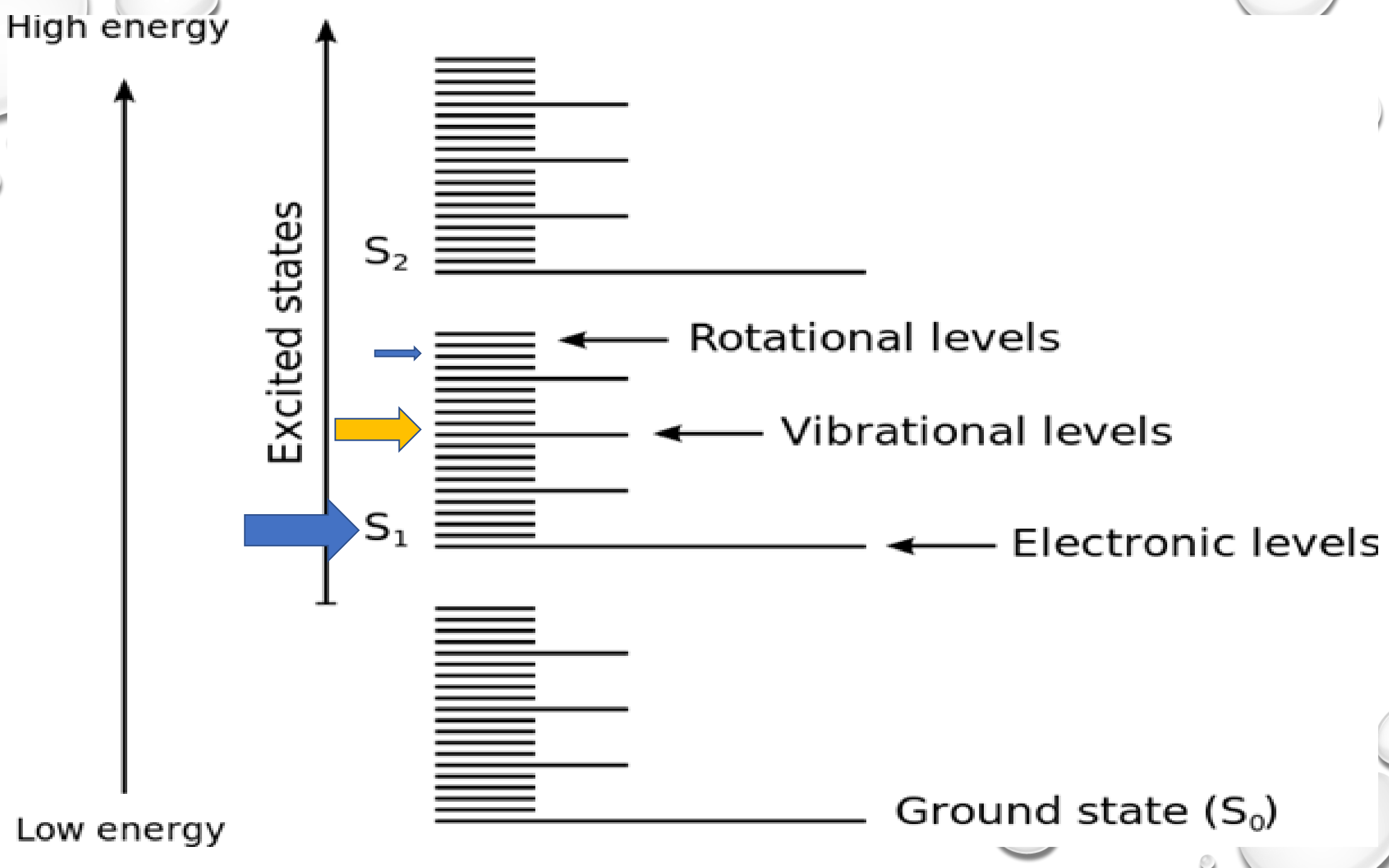
$$\Delta E = h\nu = E_n - E_0 \quad \text{where } (n = 1, 2, 3, \ldots \text{ etc})$$

$$\Delta E = 35 \text{ to } 71 \text{ kcal/mole}$$

Interaction of EMR with matter

2. Vibrational Energy Levels:

- These are less energy level than electronic energy levels.
- The spacing between energy levels are relatively small i.e. 0.01 to 10 kcal/mole.
- e.g. when IR radiation is absorbed, molecules are excited from one vibrational level to another or it vibrates with higher amplitude.


High energy
Low energy
Excited states
S_2
S_1
Rotational levels
Vibrational levels
Electronic levels
Ground state (S_0)

Interaction of EMR with matter

3. Rotational Energy Levels:

- These energy levels are quantized & discrete.
- The spacing between energy levels are even smaller than vibrational energy levels.

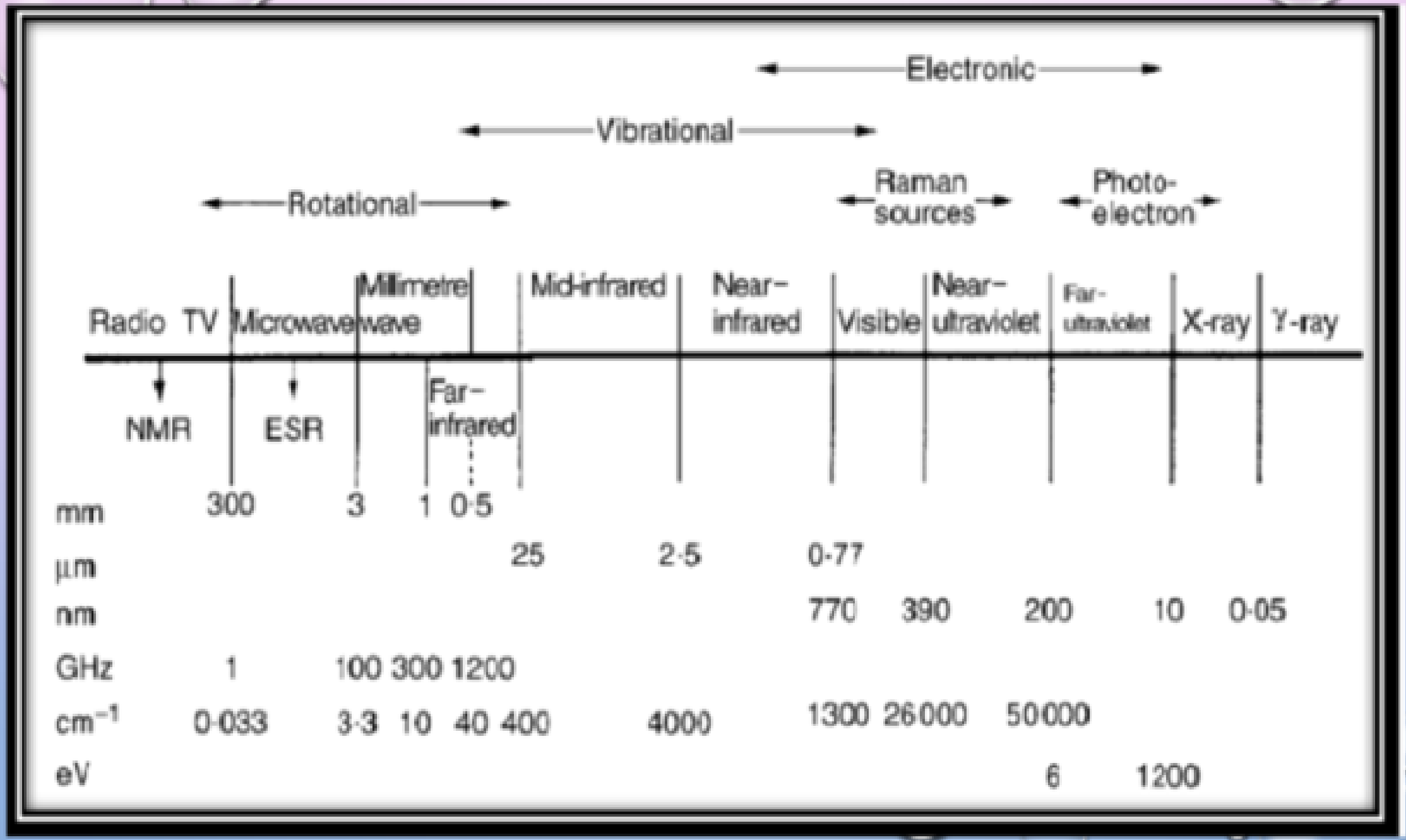
$$\Delta E_{rotational} < \Delta E_{vibrational} < \Delta E_{electronic}$$

Principles of uv –visible spectroscopy

- 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 95%, or hexane.

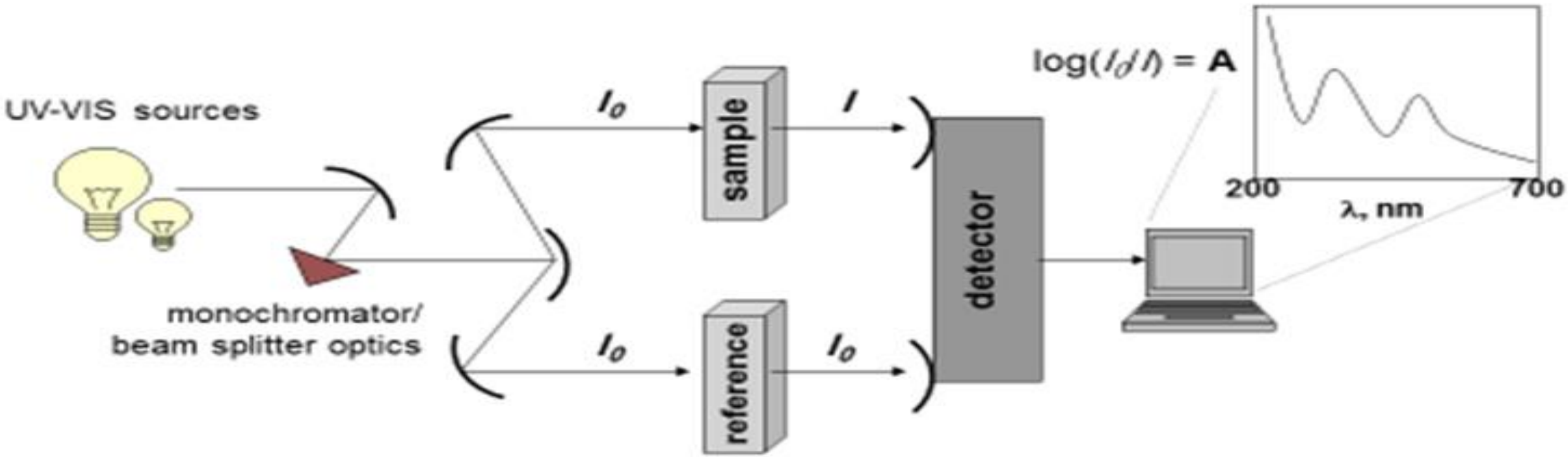

Electronic
Vibrational
Rotational
Raman sources
Photo-electron
Radio
TV
Microwave
Millimetre wave
Mid-infrared
Near-infrared
Visible
Near-ultraviolet
Far-ultraviolet
X-ray
γ-ray
NMR
ESR
Far-infrared
mm
300
3
1
0·5
μm
25
2·5
0·77
nm
770
390
200
10
0·05
GHz
1
100
300
1200
cm⁻¹
0·033
3·3
10
40
400
4000
1300
26000
50000
eV
6
1200

II. Instrumentation and Spectra

A. Instrumentation

1. The construction of a traditional UV-VIS spectrometer is very similar to an IR, as similar functions – sample handling, irradiation, detection and output are required

2. Here is a simple schematic that covers most modern UV spectrometers:



II. Instrumentation and Spectra

A. Instrumentation

3. Two sources are required to scan the entire UV-VIS band:
   - Deuterium lamp – covers the UV – 200-330
   - Tungsten lamp – covers 330-700

4. As with the dispersive IR, the lamps illuminate the entire band of UV or visible light; the monochromator (grating or prism) gradually changes the small bands of radiation sent to the beam splitter

5. The beam splitter sends a separate band to a cell containing the sample solution and a reference solution

6. The detector measures the difference between the transmitted light through the sample (I) vs. the incident light (I_0) and sends this information to the recorder

II. Instrumentation and Spectra

B. Instrumentation – Sample Handling

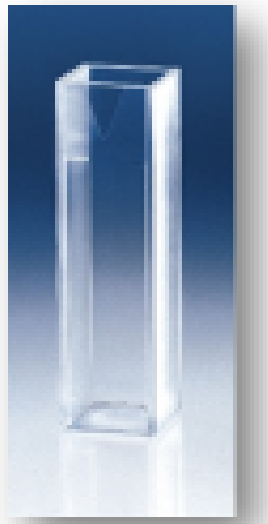
1. Virtually all UV spectra are recorded solution-phase

2. Cells can be made of plastic, glass or quartz

3. Only quartz is transparent in the full 200-700 nm range; plastic and glass are only suitable for visible spectra

4. Concentration (we will cover shortly) is empirically determined

A typical sample cell (commonly called a *cuvet*):



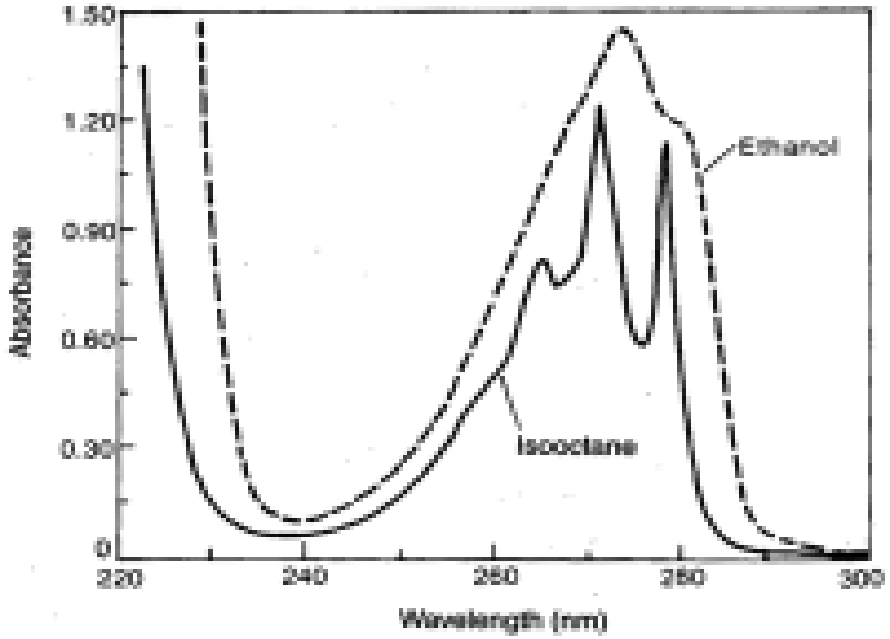
II. Instrumentation and Spectra

B. Instrumentation – Sample Handling

7. Additionally solvents must preserve the fine structure (where it is actually observed in UV!) where possible

8. H-bonding further complicates the effect of vibrational and rotational energy levels on electronic transitions, dipole-dipole interacts less so

9. The more non-polar the solvent, the better (this is not always possible)



The useful information obtained from the UV _Visible spectrum of any compound are:

1. The wave length of maximum absorption λmax .
2. The intensity of absorption.

The compound should be dissolved in some suitable solvent that doesn't itself absorb light in the region under investigation.

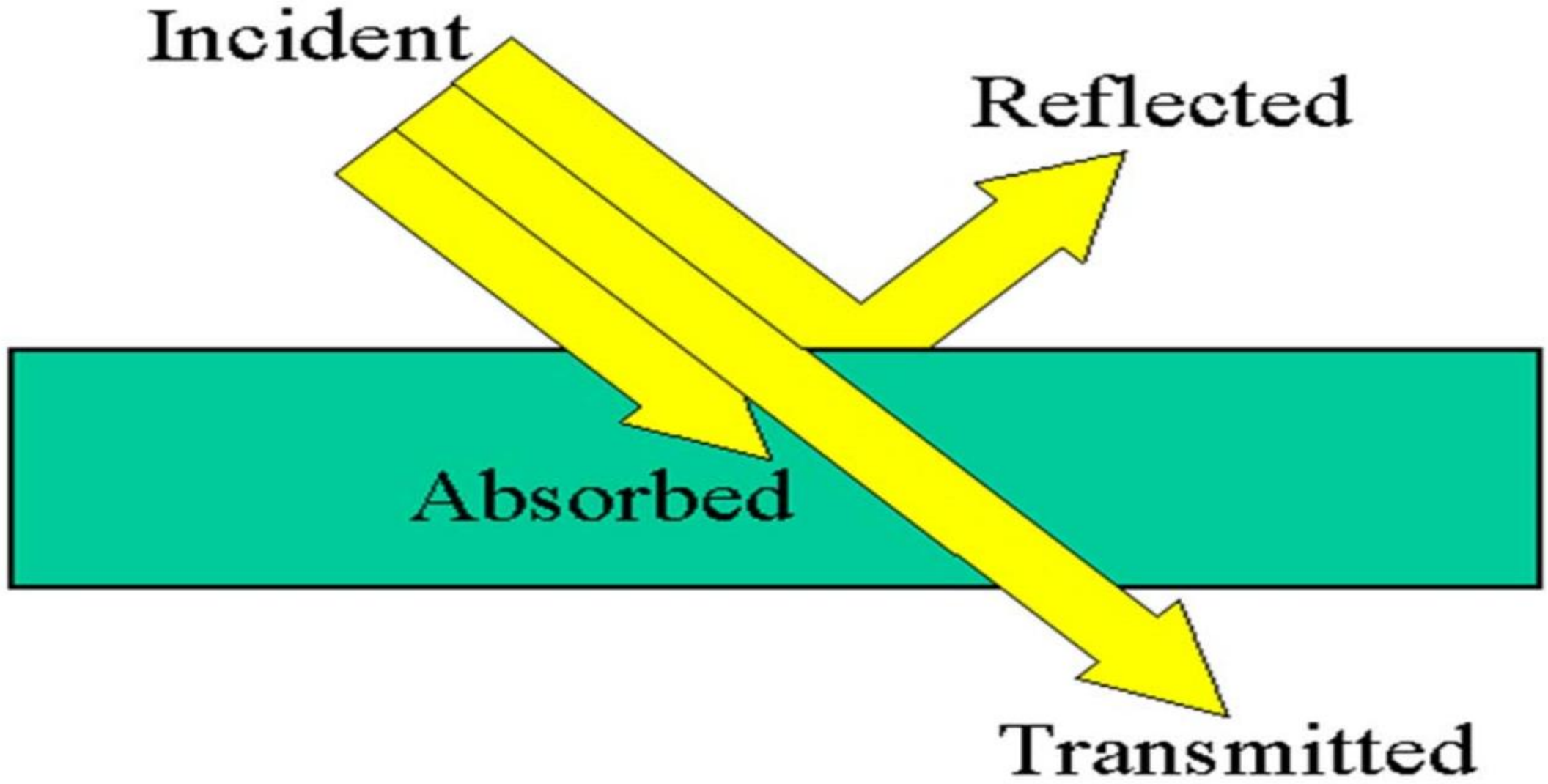
The position of the absorption peaks of a compound may be shifted if different solvents are used.

However, the λmax for non polar compounds is generally the same in alcohol and hexane, while λmax for polar compounds is usually shifted dependent on the polarity of the solvent used

THEORY INVOLVED

- When a beam of light falls on a solution or homogenous media ,a portion of light is reflected ,from the surface of the media, a portion is absorbed within the medium and remaining is transmitted through the medium.
- Thus if I_0 is the intensity of radiation falling on the media
- I_r is the amount of radiations reflected,
- I_a is the amount of radiation absorbed &
- I_t the amount of radiation transmitted then

$$I_0 = I_r + I_a + I_t$$


Incident
Reflected
Absorbed
Transmitted

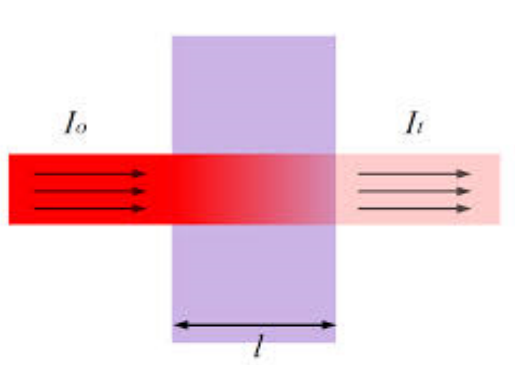
- The absorbance A or optical density is given by:
- $A = \log I_0 / I$
- The range of absorbance commonly recorded is 0 to 2
- Transmittion is the ratio of the transmitted light to incident light: $T = I / I_0$
- $A = \log 1/T$

ABSORPTION LAWS

- Lambert's law
- Beer's law
- Beer-lambert's law

- **Lambert's law: the intensity of the transmitted light decreases as the thickness of the layer increases**
- **Beer's law: the absorption is proportion to the numbers of the absorbing molecules.**

- Beer's law doesn't hold over the entire concentration range, it's operate in very dilute solution only.
- At concentrated solution we have +ve or _ve deviation which may be due to:



1. Association of the molecules.
2. The formation of the complex
3. Change in the refracting index of the solution.

These laws can be represented by this relationship:

The measurement of light absorption by a solution of molecules is governed by the Beer-Lambert Law, which is written as follows:

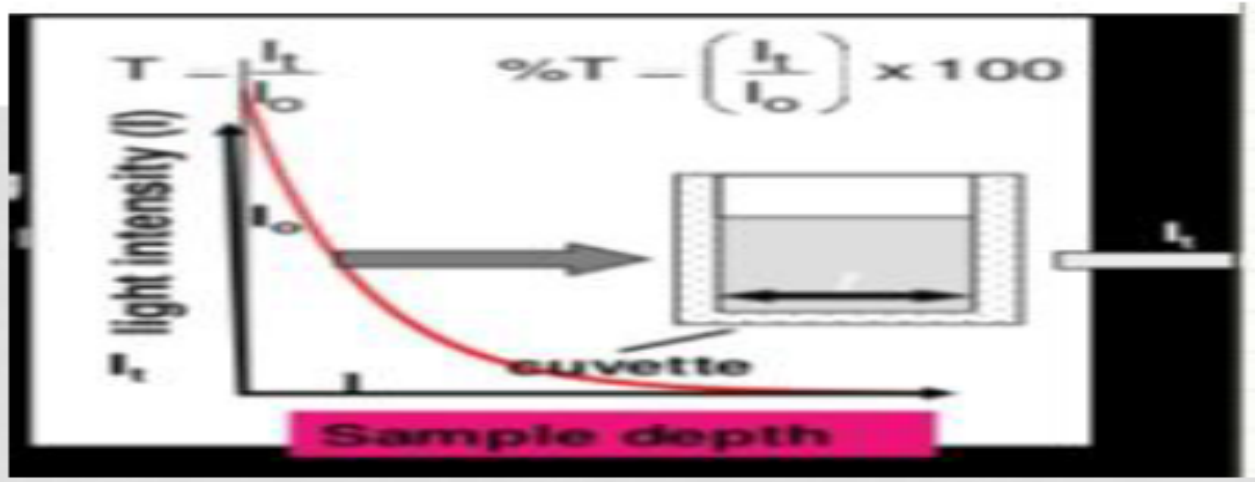
$$\log I_o/I_t = A = \varepsilon\, b\, c$$

where I_o is the intensity of incident radiation, I_t is the intensity of transmitted radiation; A is known as the absorbance and is a measure of the amount of light absorbed by the sample; ε is a constant known as the molar extinction coefficient and is the absorbance of a 1M solution of the analyte, b is the pathlength of the cell in cm, usually 1 cm and c is the concentration of the analyte in moles liter^{-1}.

Calculate the percentage of the incident radiation absorbed by a sample with an absorbance of (i) 2; (ii) 0.1.

Answers: (i) 99.0%; (ii) 20.6%

$E_{1cm}^{1\%}$?



In pharmaceutical products, concentrations and amounts are usually expressed in grams or milligrams rather than in moles and thus for the purposes of the analysis of these products, the Beer-Lambert equation is written in the following form:

$$A = A\,(1\%,\ 1\ \text{cm})\ \text{b}\ \text{c}$$

A is the measured absorbance; A (1%, 1 cm) is the absorbance of a 1% w/v (1 g/100 ml) solution in a 1 cm cell; b is the pathlength in cm (usually 1 cm); and c is the concentration of the sample in g/100 ml. Since measurements are usually made in a 1 cm cell the equation can be written:

$$\text{c} = \frac{A}{A\,(1\%,\ 1\ \text{cm})}$$ which gives the concentration of the analyte in g/100 ml

BP monographs often quote a standard A (1%,1 cm) value for a drug which is to be used in its quantitation.

What are the concentrations of the following solutions of drugs in g/100 ml and mg/100 ml?

(i) Carbimazole, *A* (1%, 1 cm) value = 557 at 291 nm, measured absorbance 0.557 at 291 nm.
(ii) Hydrocortisone sodium phosphate, *A* (1%, 1 cm) value 333 at 248 nm, measured absorbance 0.666 at 248 nm.
(iii) Isoprenaline, *A* (1%,1 cm) value = 100 at 280 nm measured absorbance 0.500 at 280 nm.

Answers: (i) Carbimazole 0.001 g/100 ml, 1 mg/100 ml; (ii) Hydrocortisone sodium phosphate 0.002 g/100 ml, 2 mg/100 ml, isoprenaline; (iii) 0.005 g/100 ml, 5 mg/100 ml

Applications in pharmaceutical analysis

- A robust, workhorse method for the quantification of drugs in formulations where there is no interference from excipients.
- Determination of the p*K*a values of some drugs.
- Determination of partition coefficients and solubilities of drugs.
- Used to determine the release of drugs from formulations with time, e.g. in dissolution testing.
- Can be used to monitor the reaction kinetics of drug degradation.
- The UV spectrum of a drug is often used as one of a number of pharmacopoeial identity checks.

Strengths

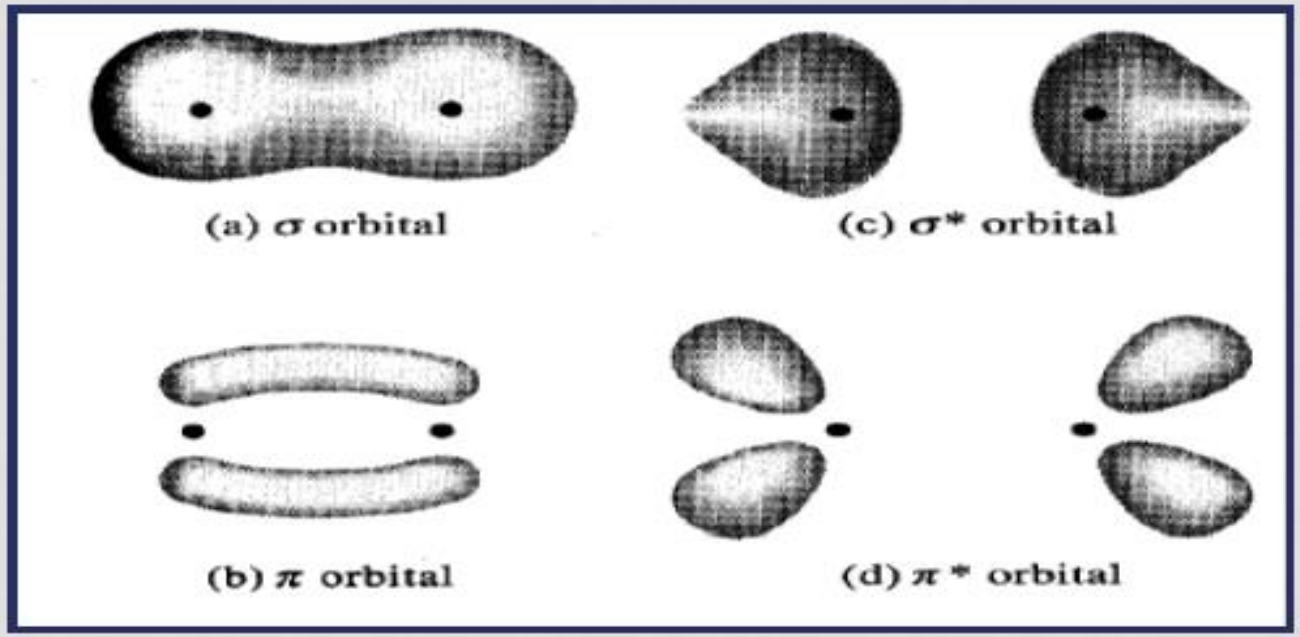
- An easy-to-use, cheap and robust method offering good precision for making quantitative measurements of drugs in formulations
- Routine method for determining some of the physico-chemical properties of drugs which need to be known for the purposes of formulation
- Some of the problems of the basic method can be solved by the use of derivative spectra.

Limitations

- Only moderately selective.The selectivity of the method depends on the chromophore of the individual drugs, e.g a coloured drug with an extended chromophore is more distinctive than a drug with a simple benzene ring chromophore
- Not readily applicable to the analysis of mixtures.

- The absorption of light energy by organic compounds in the UV–visible region involves promotion of electron in δ, π, & n–orbitals (non-bonding or n electrons that are not directly involved in bonding and are mainly located in atomic orbitals of O, S, N, & halogens) from the ground state to higher energy states.
- Higher energy states are described as Molecular orbitals that are vacant in the ground or unexcited state and are commonly called "antibonding orbitals"
- The antibonding orbital associated with δ bond is called δ* orbital and that associated with π bond is called π*orbital.
- As the n electrons don't forms bond there are no antibonding orbitals associated with them they use δ* and π*orbital.

- Ground states excited states



Only the valence electrons are excited or presented in antibonding orbitals

THE POSSIBLE ELECTRONIC TRANSITIONS CAN GRAPHICALLY SHOWN AS: