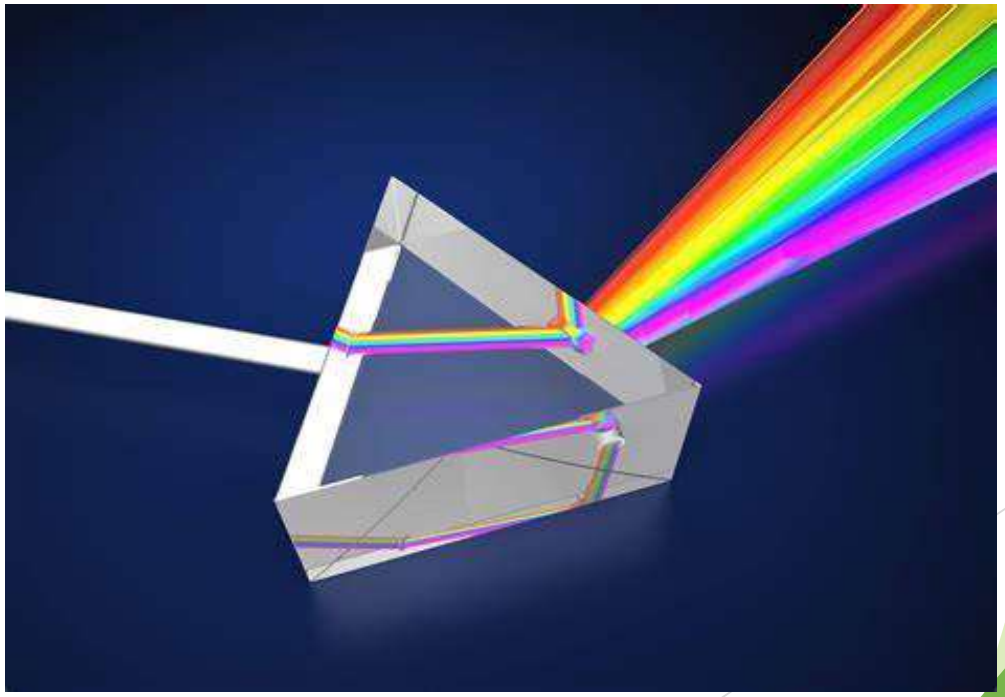
Spectroscopic Techniques and Applications

UNIT-II



PTU-SYLLABUS

Unit II Spectroscopic techniques and applications Electronic spectroscopy: Principle and instrumentation, electronic transitions, Chromophores and auxochromes, factors affecting the value of max and intensity of spectral lines. Fluorescence and its applications in medicine. Vibrational and rotational spectroscopy of diatomic molecules: selection rules, expression for energies. Nuclear magnetic resonance (1H NMR): Principle, instrumentation, chemical shift, coupling (spin-spin coupling), splitting of peaks, interpretation of 1H NMR of simple molecules; Applications of spectroscopy

Spectroscopy

Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter. It is a powerful tool available for the study of atomic and molecular structure and it is also used in the analysis of most of the samples.

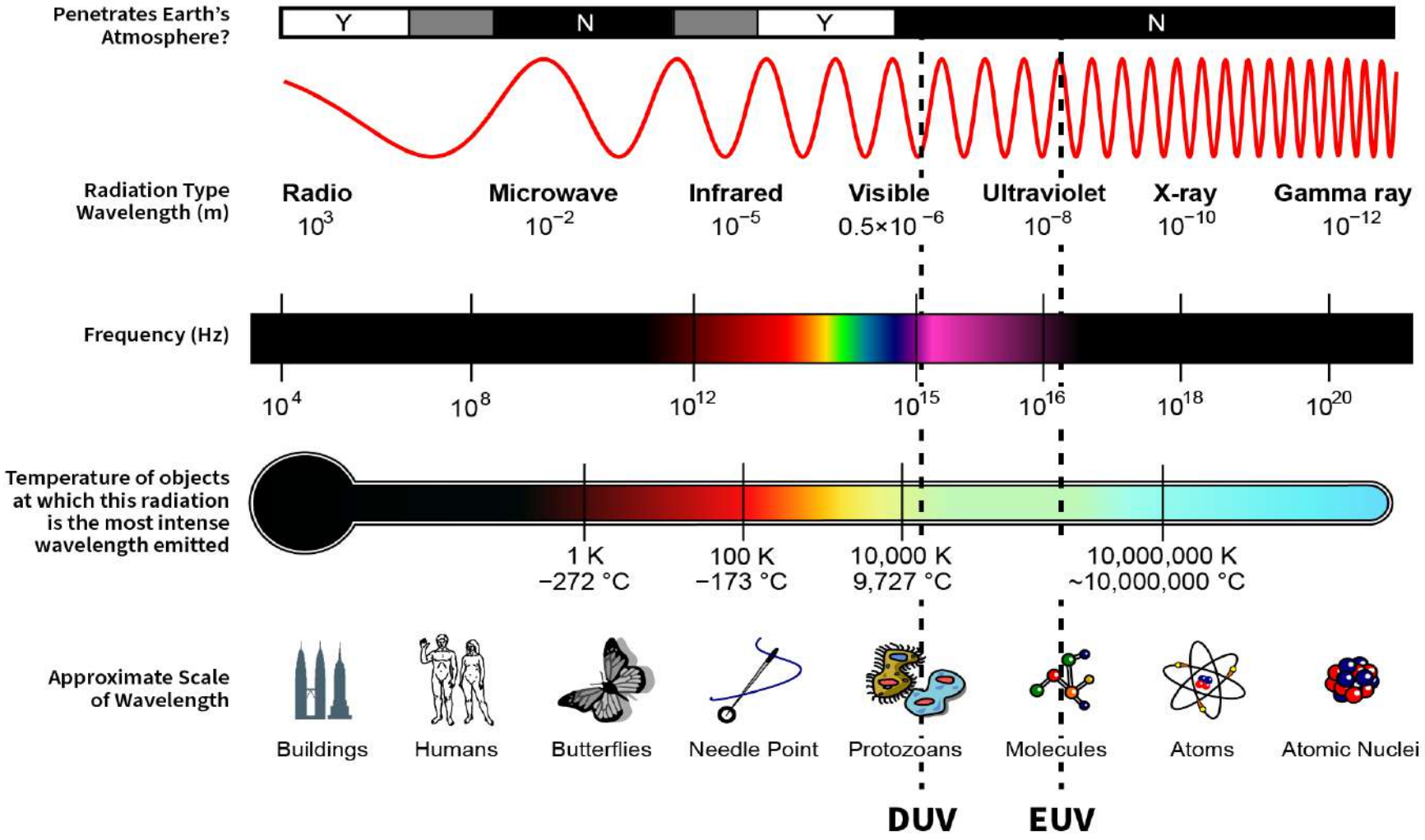
Types of Spectroscopy:-

The study of spectroscopy is divided into two types. They are,

1. Atomic spectroscopy

2. Molecular spectroscopy.

ELECTROMAGNETIC SPECTRUM



Types of Spectroscopy

- **Atomic Spectroscopy**

  It deals with the interactions of electromagnetic radiation with atoms.

- **Molecular Spectroscopy**

  It deals with the interaction of electromagnetic radiation with molecules.

Selection Rules

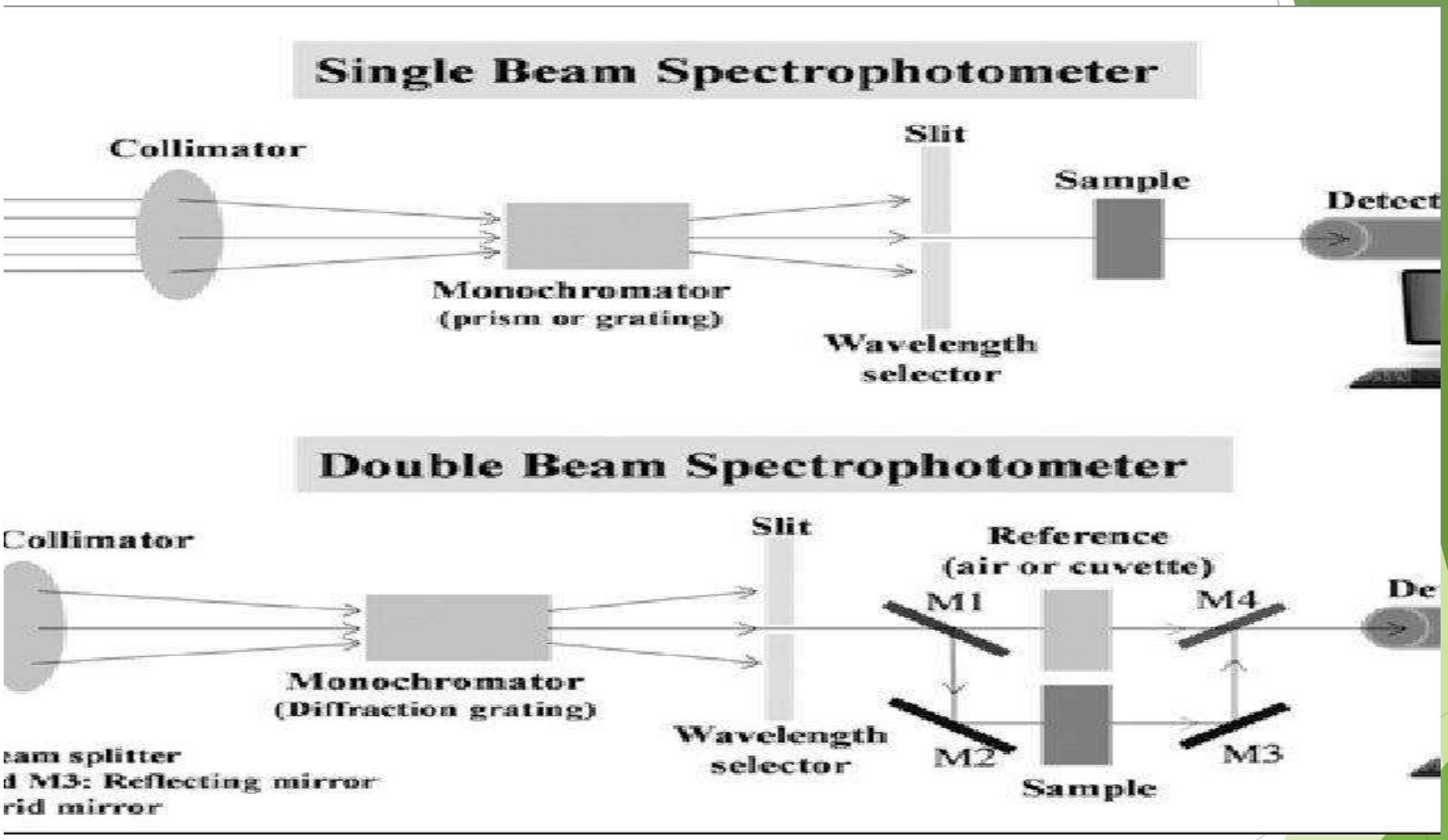
The Selection rules are used to find out whether transitions are allowed or forbidden. The Selection rules to be followed depend upon the type of transition.

1.For Vibrational transitions selection rule is $\Delta v=+1$, $\Delta V= -1$ where v is the vibrational quantum number. further v= + 1 corresponds to absorption and v= -1 corresponds to emission.

2.For rotational transitions , the selection rule is $\Delta J=+1$, $\Delta J= -1$,where j is rotational quantum number. further J= + 1 corresponds to absorption and J= -1 corresponds to emission.

3.For electronic transitions in atomic spectra, selection rule is is $\Delta l=+1$, $\Delta l= -1$ where l is the azimuthal quantum number. Further $\Delta l= + 1$ corresponds to absorption and $\Delta l= -1$ corresponds to emission.The transitions which are obtained by following selection rules are called allowed transitions. The transitions which violate selection rules are called forbidden

UV/VISIBLE SPECTROPHOTOMETER



Applications of UV/Visible spectrum.

UV spectroscopy is an important tool in analytical chemistry. The other name of UV (Ultra-Violet) spectroscopy is Electronic spectroscopy as it involves the promotion of the electrons from the ground state to the higher energy or excited state. Information can be obtained from UV-Visible spectrum describe below

1 Detection of functional groups- UV spectroscopy is used to detect the presence or absence of chromophore in the compound.

2. Detection of extent of conjugation- The extent of conjugation in the polyenes can be detected with the help of UV spectroscopy.

3. Identification of an unknown compound

4. Determination of configurations of geometrical isomers

5. Determination of the purity of a substance

Auxochrome and Chromophore

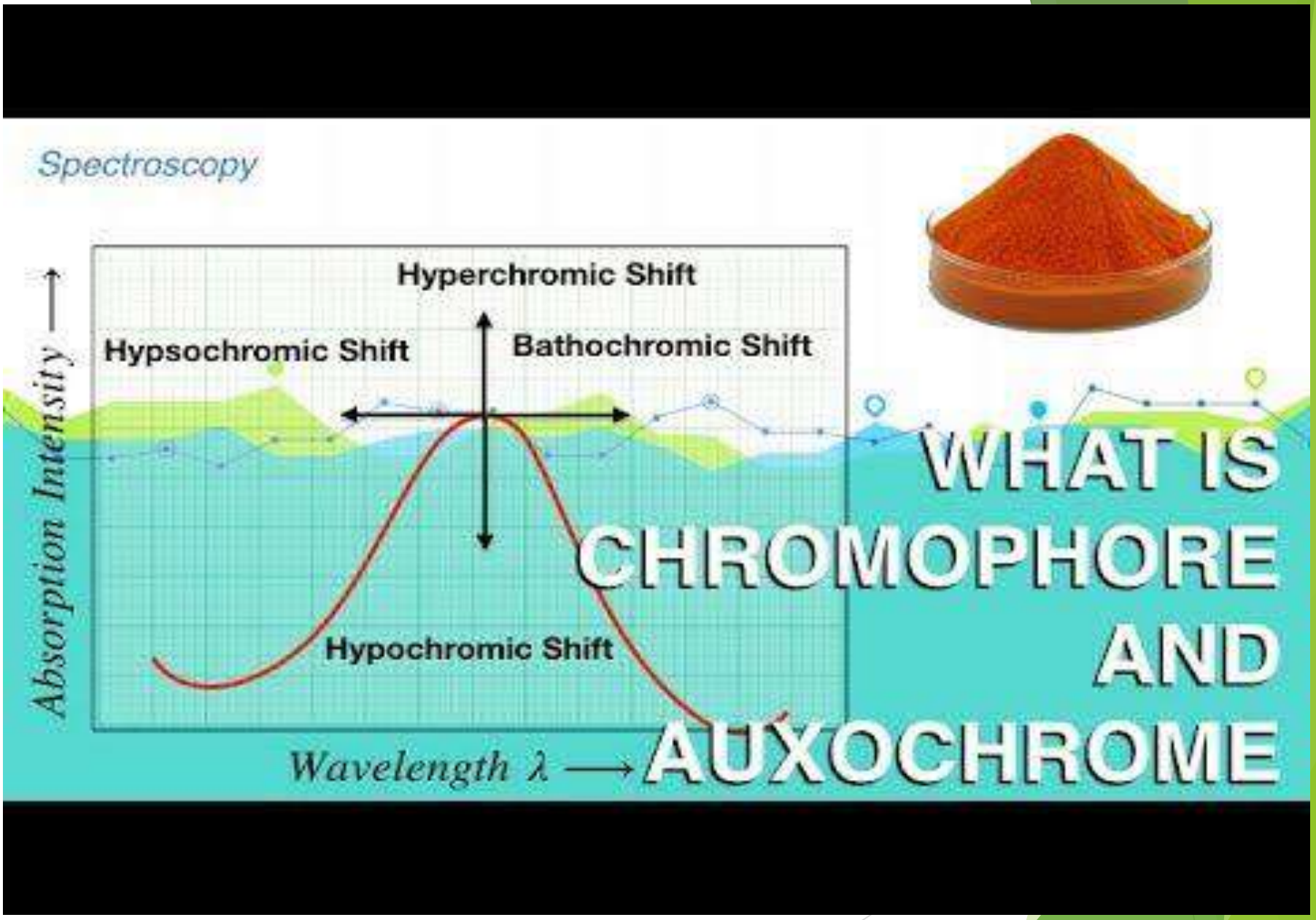
An **auxochrome** (Greek auxo "to increase" and *chrōma*: "colour") is a group of atoms attached to a chromophore which modifies the ability of that chromophore to absorb light. Examples include the hydroxyl group (-OH), the amino group ($-NH_2$), the aldehyde group (-CHO), and the methyl mercaptan group (-SMe).

Chromophore : A chromophore is defined as functional group which shows a characterstick absorption in UV-visible region and which may or may not imparts colour to compound.

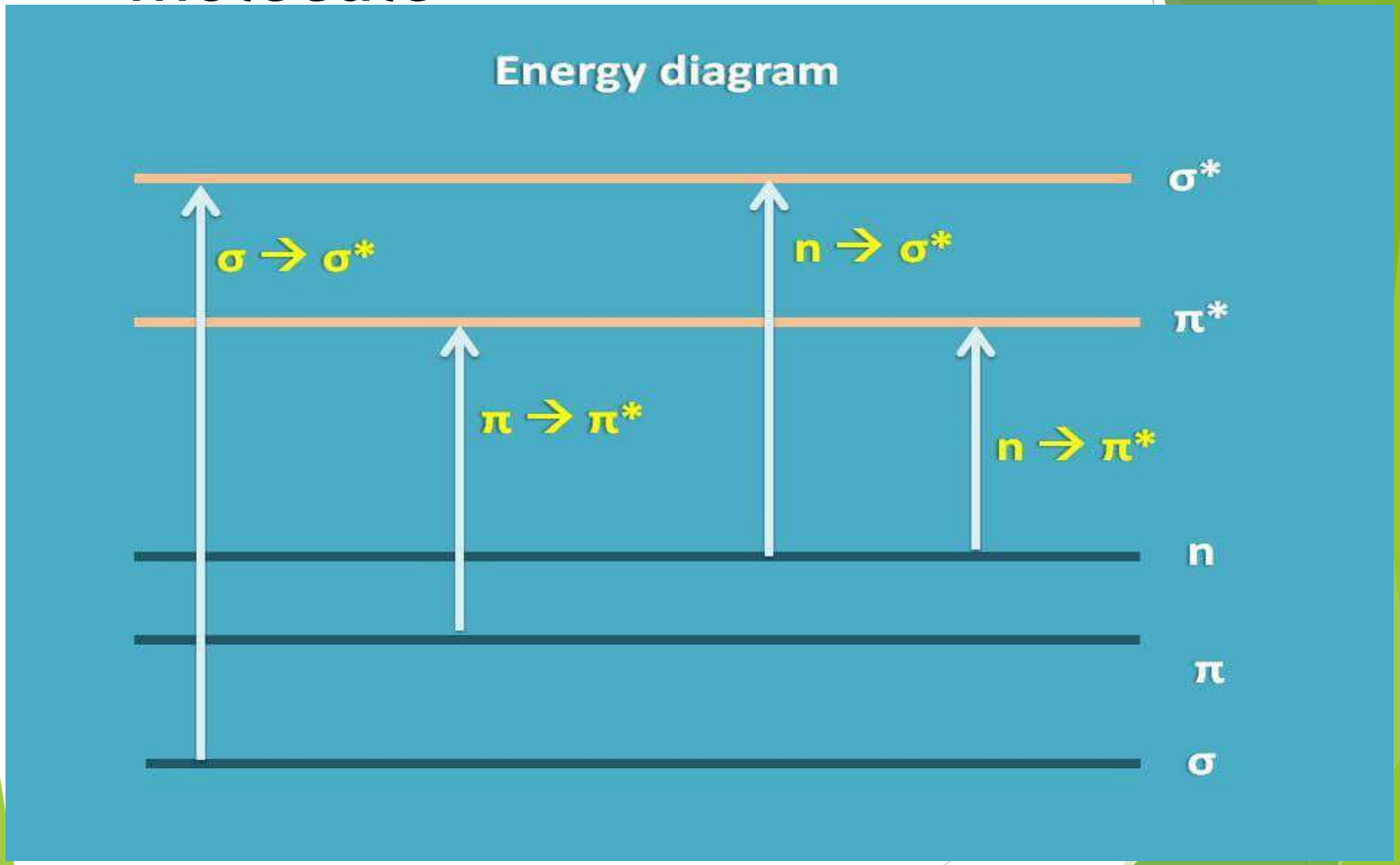
Bathochromic and Hypsochromic shift?

Bathochromic Shift : It is an effect by virtue of which the absorption maximum is shifted towards longer wavelength due to the presence of an auxochrome or by change of solvent.

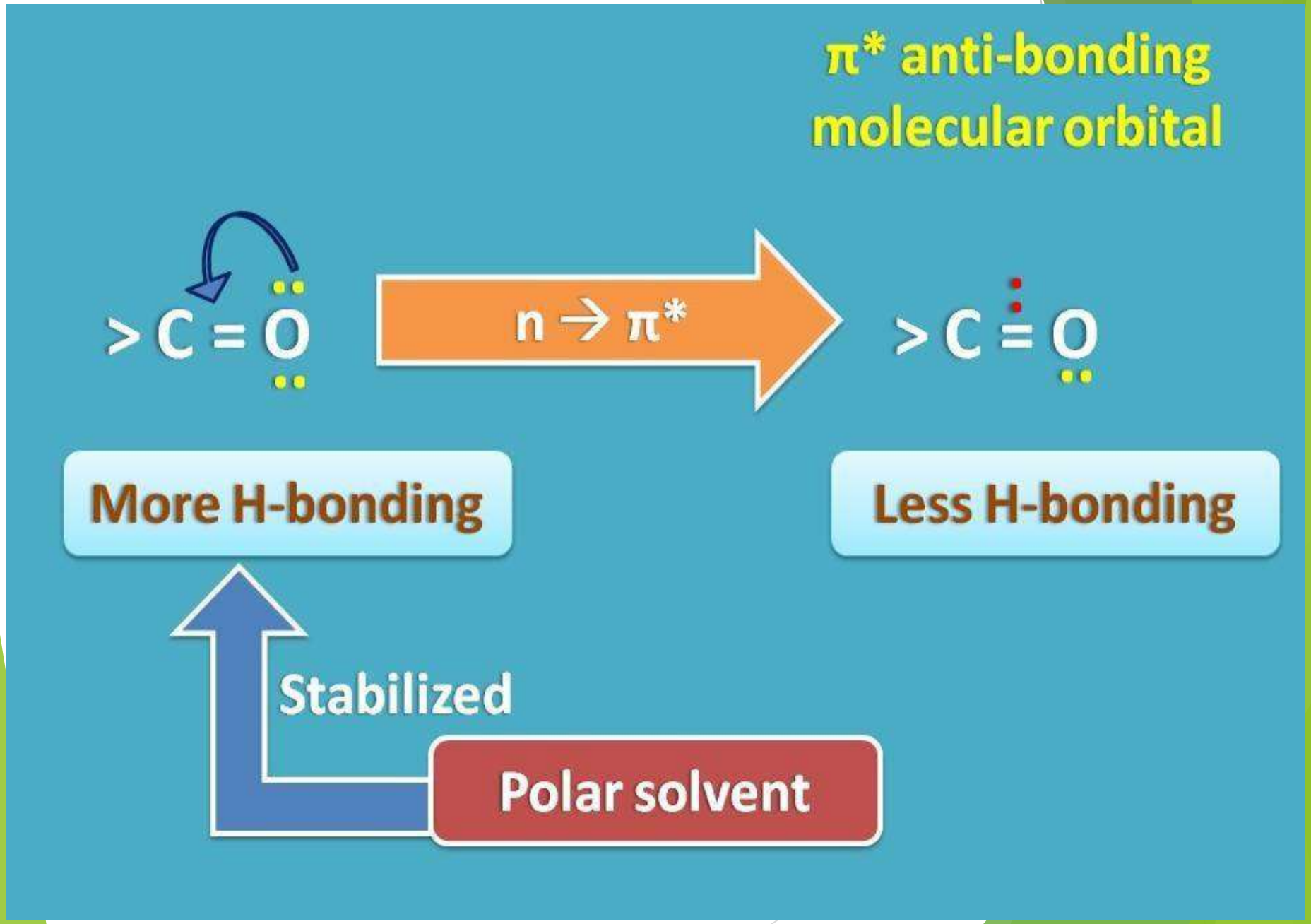
Hypsochromic shift : It is an effect by virtue of which the absorption maximum is shifted towards shorter wavelength.


Spectroscopy
Hyperchromic Shift
Hypsochromic Shift
Bathochromic Shift
Hypochromic Shift
Absorption Intensity
Wavelength λ
WHAT IS
CHROMOPHORE
AND
AUXOCHROME

Electronic Transitions in a molecule



EFFECT OF SOLVENT ON ELECTRONIC TRANSITIONS



1. $n \rightarrow \pi^*$ transition – shown by unsaturated molecule having hetero atoms like N, O, & S. It occurs at longer wavelength with low intensity. Eg. Aldehyde & ketone.

2. $\sigma \rightarrow \sigma^*$ transition - occur in the compound in which all the electrons are involved in single bond and there are no lone pair of electrons. The energy required for this is very large (120 – 136 nm). Eg. Saturated hydrocarbons.

3. $n \rightarrow \sigma^*$ transition – occur in the saturated compounds having lone pair of electrons. This occurs at longer wavelength (180 – 200 nm). Eg. Trimethylamine

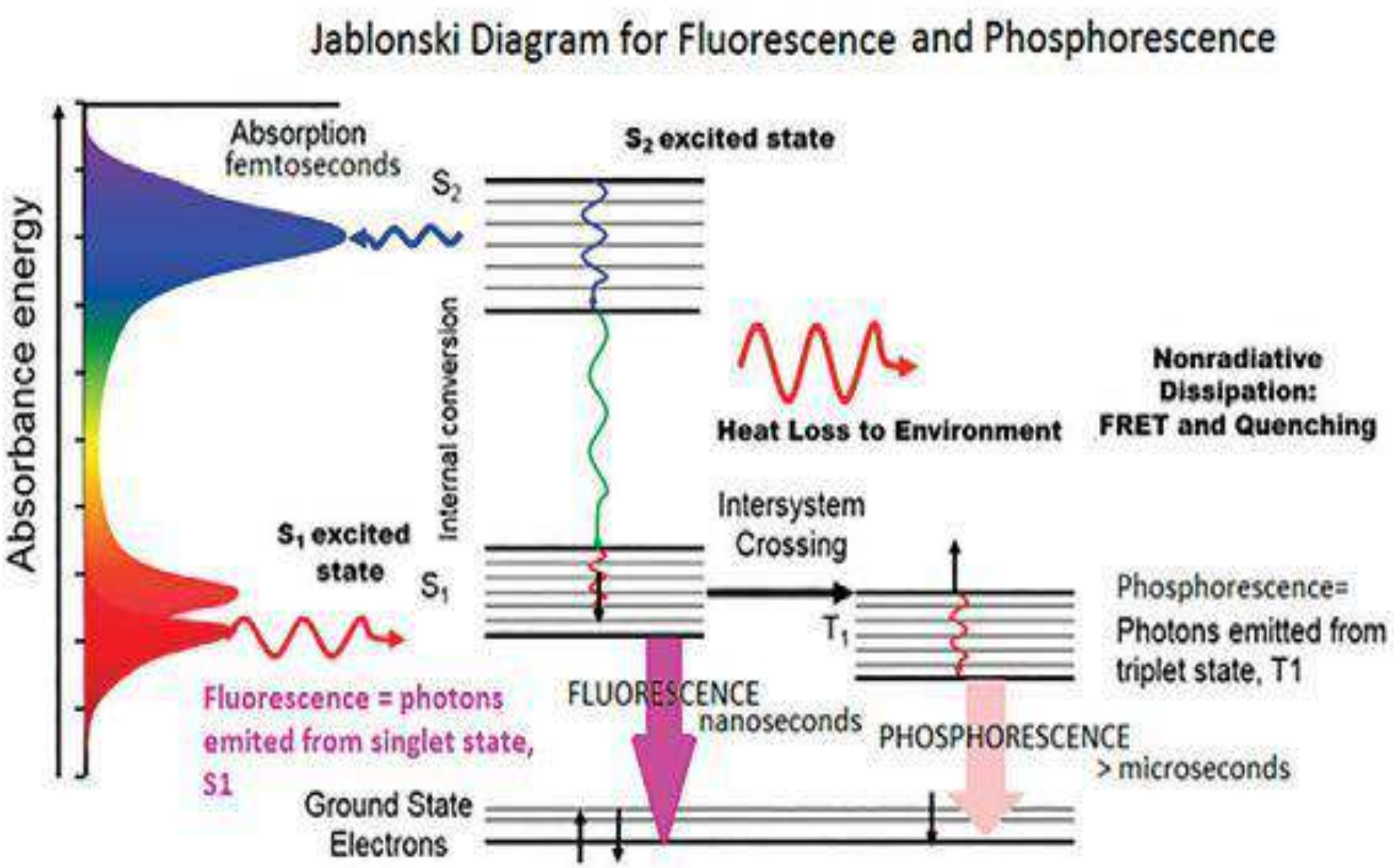
4. $\pi \rightarrow \pi^*$ transition – occur in molecule having a π electron system. Eg. Ethylene.

Allowed transitions are $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$ and $\pi \rightarrow \pi^*$. These transitions give rise to strong absorption bands but the energy involved is higher than for $n \rightarrow \pi^*$ transition.

Forbidden transition is $n \rightarrow \pi^*$. It give rise to band with low intensity.

Order of decreasing energy for the absorption is $\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$

FLOURESCENCE



APPLICATIONS OF FLOURECENCE SPECTROSCOPY

Applications

- Used in both qualitative and quantitative(major) estimation.
- Assays of vitamin B in food stuff ,NADH, hormones, drugs, pesticides, Carcinogens, chlorophyll, cholesterol, metal ions etc.
- enzyme assays and kinetic analysis.
- Protein structure analysis.
- Membrane structure analysis.
- Microspectrofluorimetry (used to detect malignant cell in biopsy tissue)
- FACS.

Infra-Red spectroscopy

It deals with Vibration modes present in a molecule. IR region is divided in to three regions

1.Near IR-4000-1500

2.Middle IR-1500-600

3.Far IR-600-50

The functional group region runs from 4000 $cm^{-1}$to 1500 cm^{-1}, and the fingerprint region from 1500 cm^{-1} to 667 cm^{-1}. In the fingerprint region, the spectra usually consist of bending vibrations within the molecule. The pattern of peaks is more complicated, and it is much more difficult to pick out individual bonds in this region. The fingerprint region is important because each different compound produces its own unique pattern of peaks (**like a finger print**) in this region.

Vibrational Modes

1) In case of linear molecule, Translational degree of freedom =3

Rotational degree of freedom= 2

So Vibrational degree of freedom = 3N-5

CO_2 is a linear molecule and the degrees of freedom of a linear molecule = 3N– 5

where N = no. of atoms in the molecule, In CO_2, O = C = O, N = 3

Vibrational degree of freedom = 3 ×3 - 5 = 4

2) In case of Non linear molecule, Translational degree of freedom =3

Rotational degree of freedom = 3

So Vibrational degree of freedom = 3N-6

SO_2 is a nonlinear molecule and the degrees of freedom of a non-linear molecule = 3N-6

As N= 3 in SO2, **Therefore Vibrational degree of freedom = 3x 3-6 =3**

3) CH_4 is a nonlinear molecule and the degrees of freedom of a non-linear molecule = 3N-6

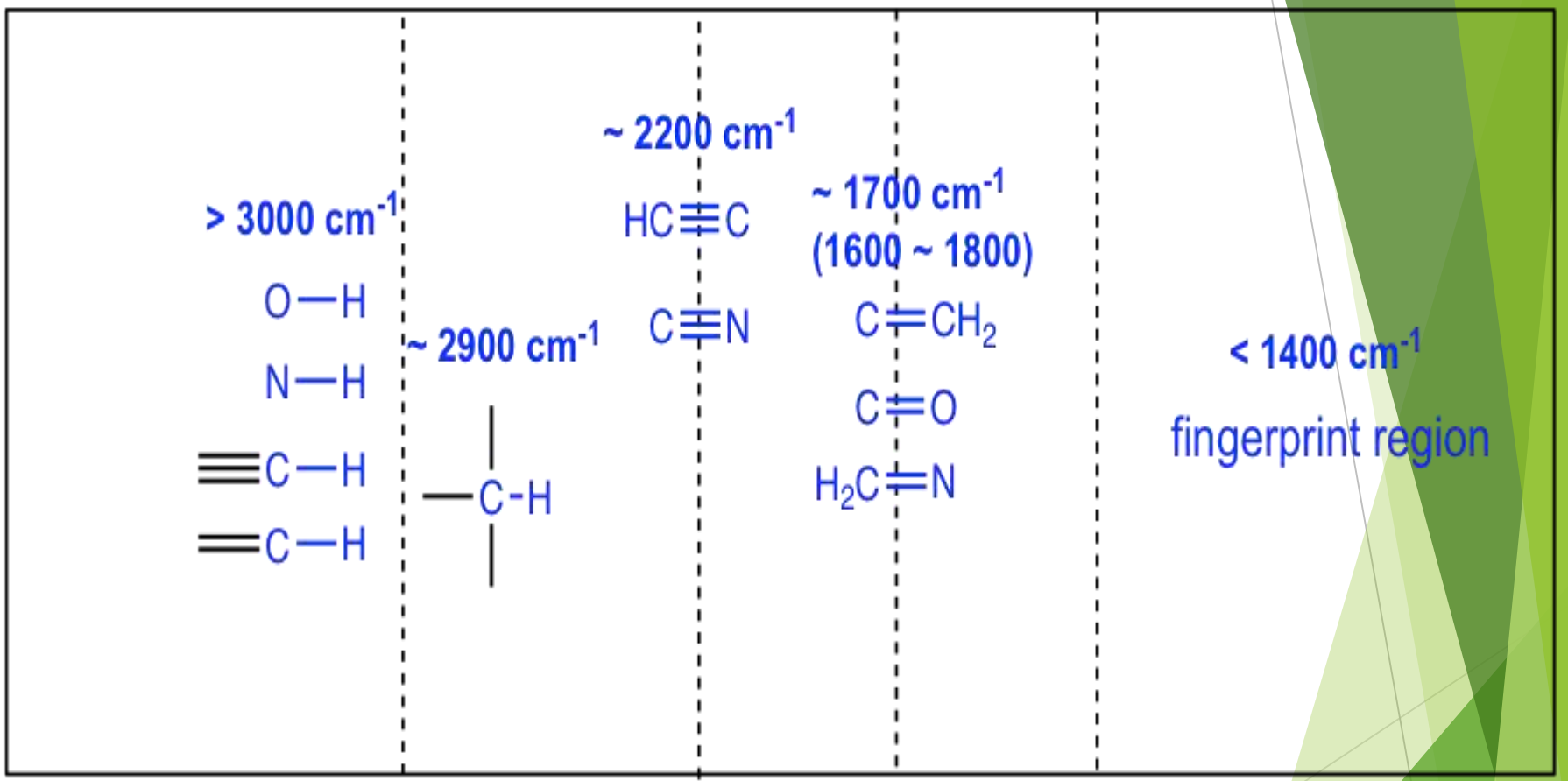
as N = 5 in CH_4, **Therefore Vibrational degree of freedom = 3x 5-6 =9**

IR ACTIVE AND INACTIVE MOLECULES

H_2, HCl, CH_4, CO_2, H_2O

HCl, CH_4, CO_2, H_2O these molecules will show IR spectra because these possess change in dipole moment when undergo asymmetric stretching. HCl is a polar molecule and CO_2, CH_4, H_2O possess dipole moment on absorption of IR radiation.

REGIONS OF IR SPECTRA



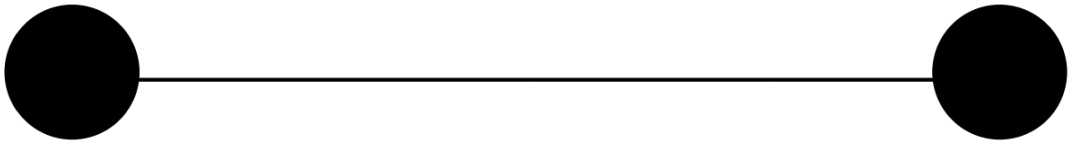
$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

$\bar{\nu}$ = wave number, in cm^{-1}, corresponding to the vibrational frequency of the bond

c = speed of light in cms^{-1}

K = force constant in $dynescm^{-1}$ (a measure of bond strength. The stronger the bond, the larger the K.)

μ = reduced mass in $gatom^{-1}$



atom 1

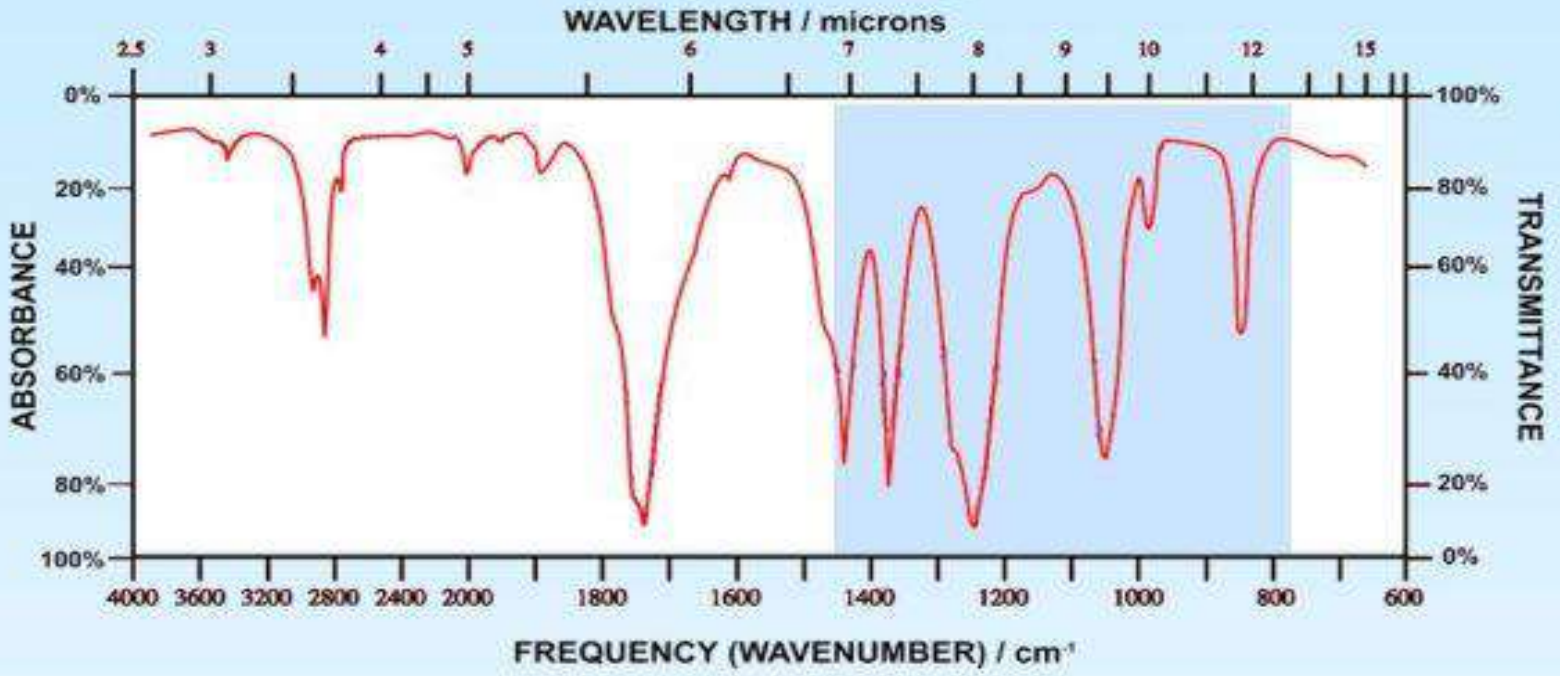
mass = m_1 g

atom 2

mass = m_2 g

$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$

Fingerprint region



- organic molecules have a lot of C-C and C-H bonds within their structure
- spectra obtained will have peaks in the 1400 cm^{-1} to 800 cm^{-1} range
- this is referred to as the "fingerprint" region
- the pattern obtained is characteristic of a particular compound the frequency of any absorption is also affected by adjoining atoms or groups.

APPLICATIONS OF IR SPECTROSCOPY

- Identification of functional groups & structure elucidation of organic compounds.
- Quantitative analysis of a number of organic compounds.
- Study of covalent bonds in molecules.
- Studying the progress of reactions.
- Detection of impurities in a compound.
- Ratio of cis-trans isomers in a mixture of compounds.
- Shape of symmetry of an inorganic molecule.
- Study the presence of water in a sample.
- Measurement of paints and varnishes.

Nuclear Magnetic Resonance

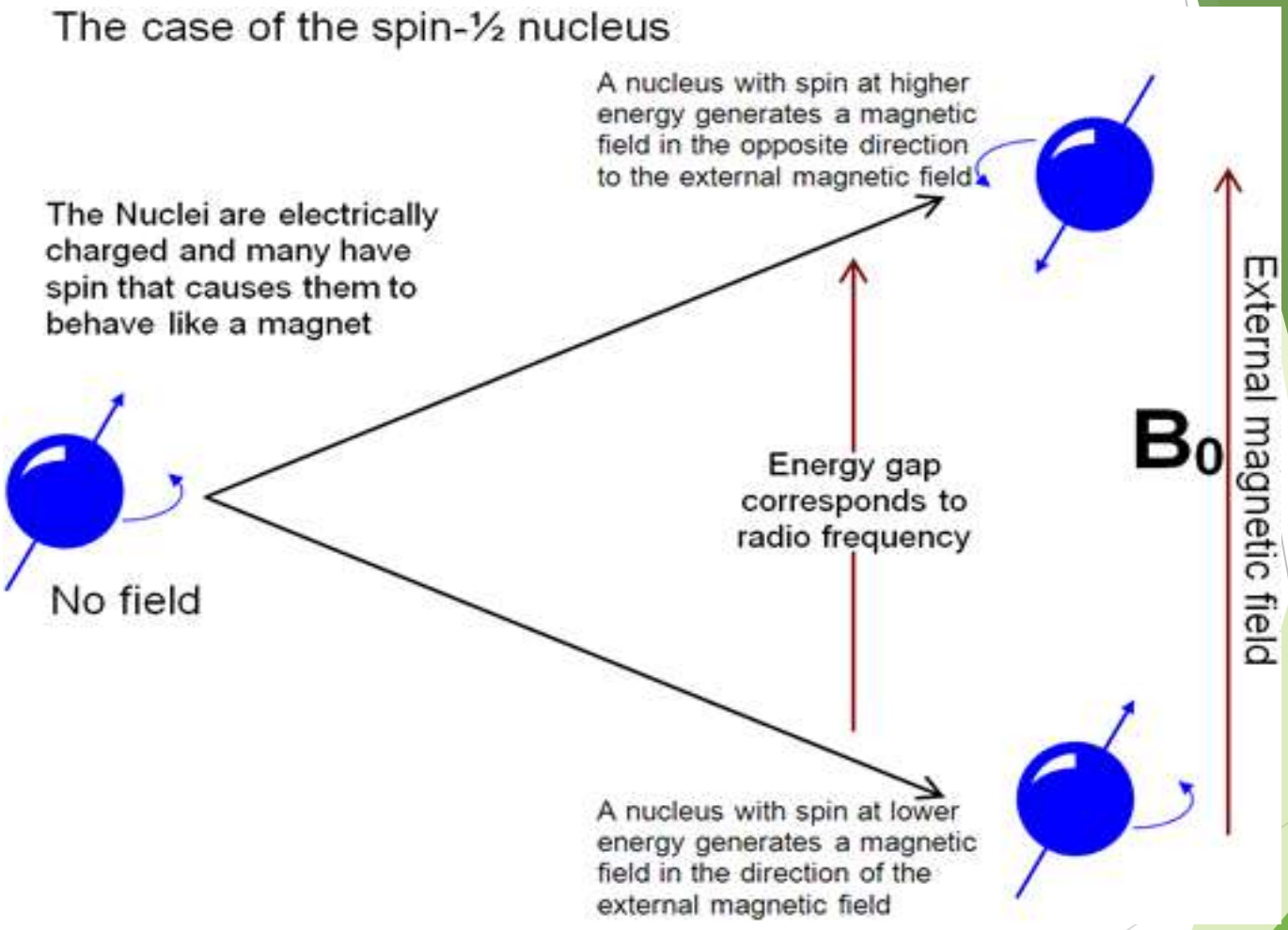
The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the ground energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its ground level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

The rules for determining the net spin of a nucleus are as follows:

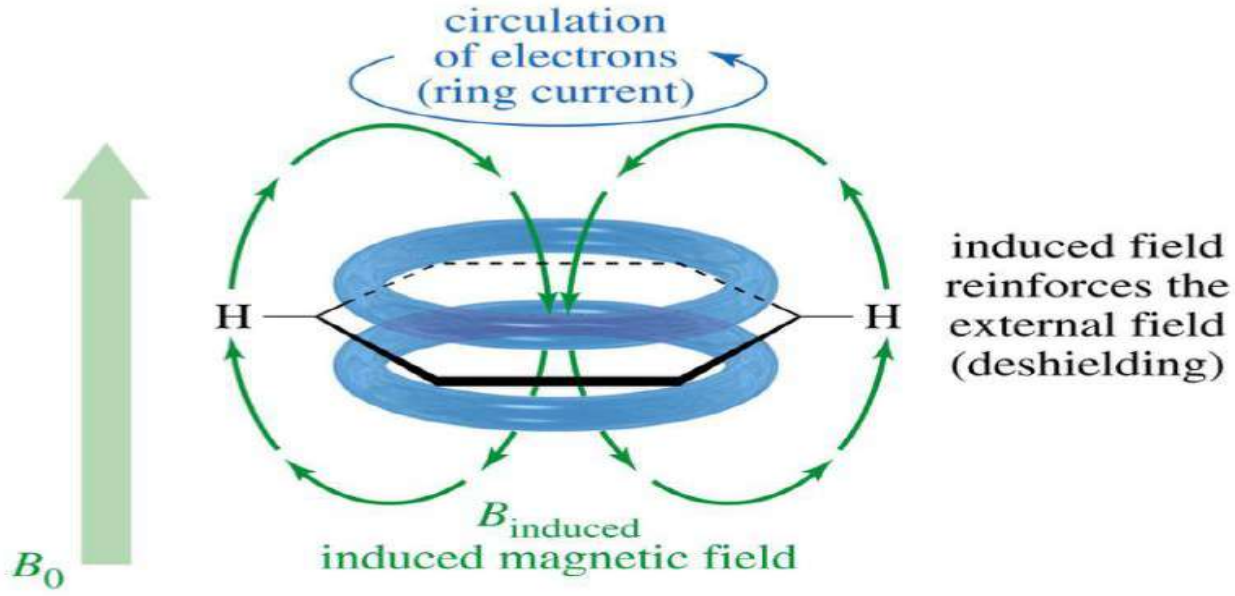
1.If the number of neutrons **and** the number of protons are both even, then the nucleus has NO spin.

2.If the number of neutrons **plus** the number of protons is odd, then the nucleus has a half-integer spin (i.e. 1/2, 3/2, 5/2)

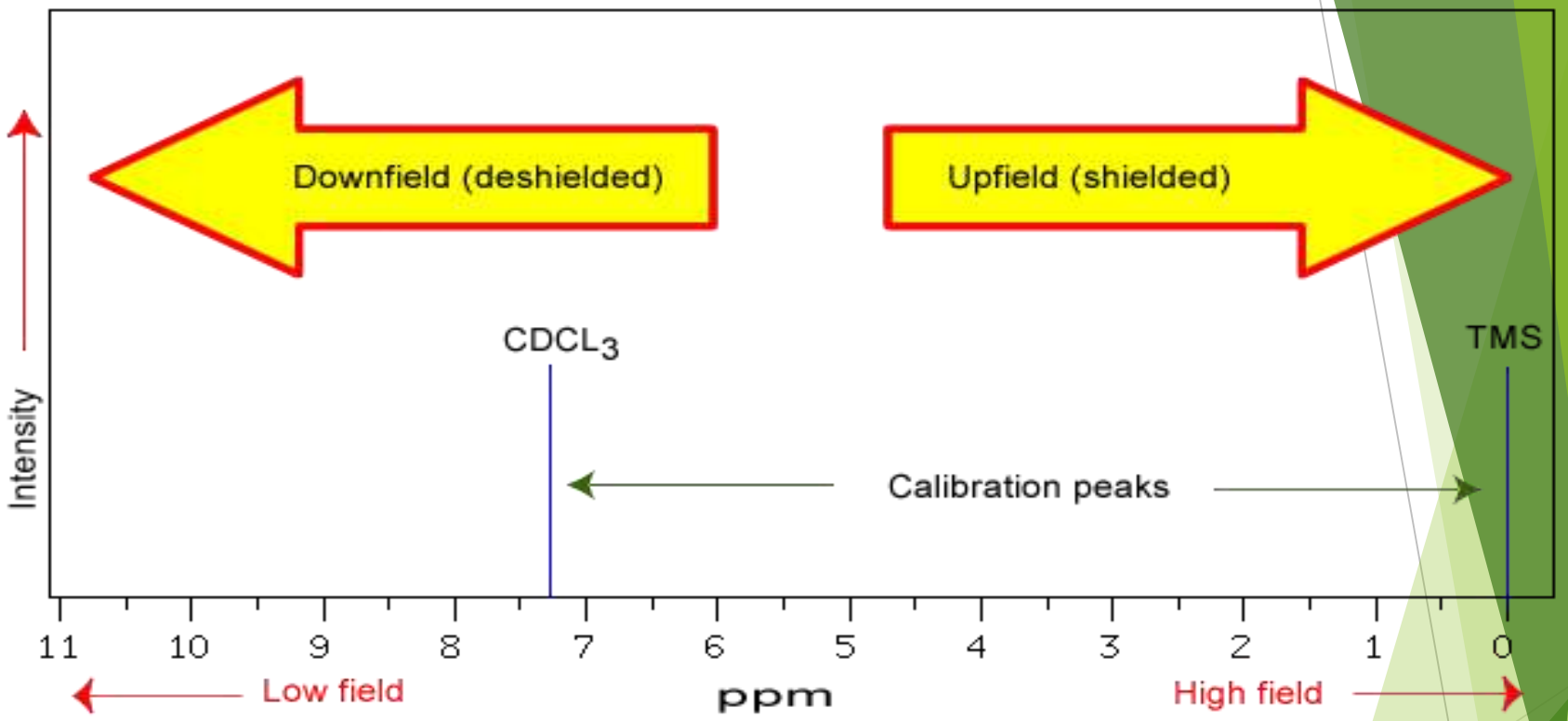
3.If the number of neutrons **and** the number of protons are both odd, then the nucleus has an integer spin (i.e. 1, 2, 3)


The case of the spin-½ nucleus
A nucleus with spin at higher energy generates a magnetic field in the opposite direction to the external magnetic field
The Nuclei are electrically charged and many have spin that causes them to behave like a magnet
Energy gap corresponds to radio frequency
B0
External magnetic field
No field
A nucleus with spin at lower energy generates a magnetic field in the direction of the external magnetic field

Diamagnetic Anisotropy
Shielding and Deshielding



CHEMICAL SHIFT



Tetra methyl silane

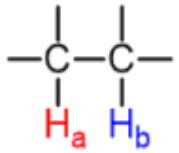
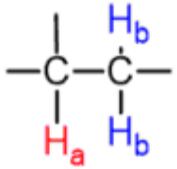
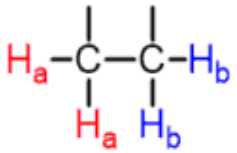
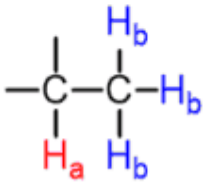
TMS is used as standard reference compound in 1H NMR. Chemical shift of protons in different compounds are measured relative to standard reference compound TMS. TMS i.e. tetra methyl silane used as reference compound in recording 1H NMR.

REASONS:

1. TMS contains 12 H atoms which are equivalent , so show intense sharp signal at low concentrations.
2. TMS is chemically inert and miscible with almost all organic solvents.
3. TMS has low boiling point(27° C), so it can be removed from compound easily after taking the NMR spectrum.

Summary of Signal Splitting Patterns in ^{1}H NMR Spectroscopy

The pattern is that n protons split the signal into n+1 peaks, which is known as the **n+1 rule**.

Multiplicity	N +1	H_a	Signal	H_b	N +1	Multiplicity
Doublet	1+1 = 2				1+1 = 2	Doublet
Triplet	2+1 = 3				1+1 = 2	Doublet
Triplet	2+1 = 3				2+1 = 3	Triplet
Quartet	3+1 = 4				1+1 = 2	Doublet

NMR INSTRUMENTATION