# SPECTROSCOPY

Spectroscopy deals with the transitions that a molecule undergoes between its energy levels upon absorption of suitable radiation as determined by quantum mechanical selection rules. It is the study of electromagnetic radiation scattered, absorbed or emitted by molecules.

The structure of organic compounds can be determined by the study of their interaction with light (electromagnetic radiation). Spectroscopic techniques are non-destructive and generally require small amounts of sample. They help in fast analysis of sample.

Light is uniform or homogeneous in color, it is actually composed of a broad range of radiation having wavelengths in the ultraviolet (UV), visible and infrared (IR) portions of the spectrum. Different regions of the electromagnetic spectrum provide different kinds of information as a result of such interactions. Light may be considered to have both wave-like and particle-like characteristics. Like all wave characteristics, it is characterized by a wavelength or frequency.

Wavelength is defined, as the distance between adjacent peaks (or troughs), and may be designated in meters, centimeters or nanometers (10^{-9} meters).
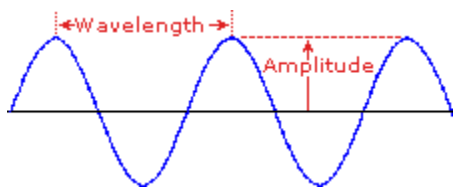
- **Violet:** 400 - 420 nm
- **Indigo:** 420 - 440 nm
- **Blue:** 440 - 490 nm
- **Green:** 490 - 570 nm
- **Yellow:** 570 - 585 nm
- **Orange:** 585 - 620 nm
- **Red:** 620 - 780 nm

Frequency is the number of wave cycles that travel past a fixed point per unit of time, and is usually given in cycles per second, or hertz (Hz).

Frequency and wavelength are inversely related but has a direct relationship to energy.

$\nu = c/\lambda$ ν=frequency, λ=wavelength, c=velocity of light (c=$3 \cdot 10^{10}$ cm/sec)

$\Delta E = h\nu$ E=energy, ν=frequency, h=Planck's constant (h=$6.6 \cdot 10^{-27}$ erg sec)

Visible wavelengths cover a range from approximately 400 to 800 nm. The longest visible wavelength is red and the shortest is violet.
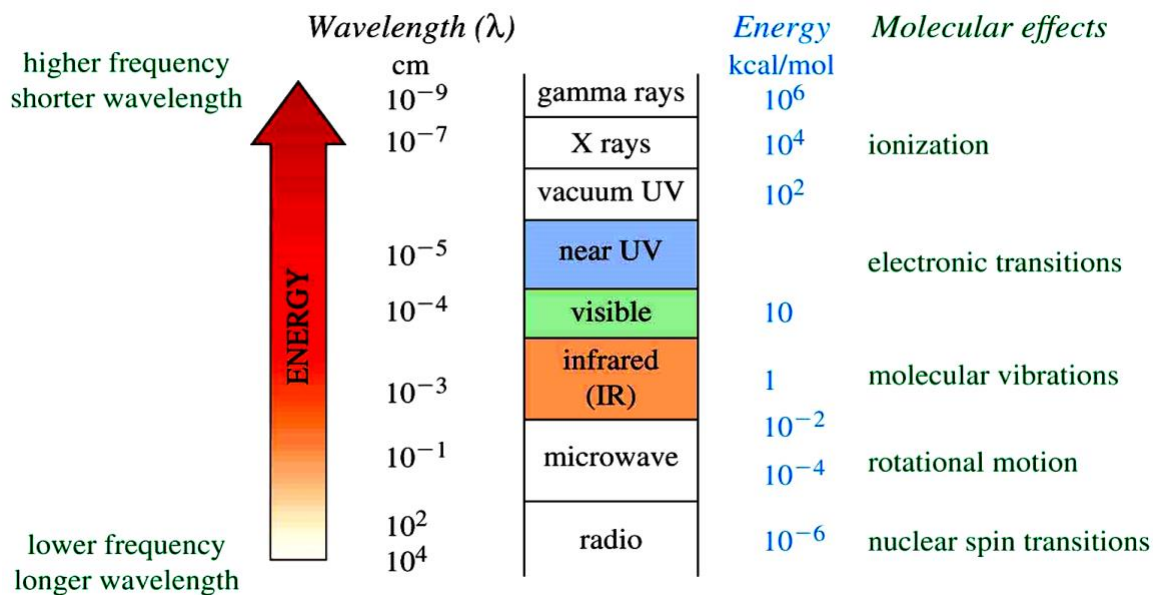
The Electromagnetic Spectrum

The visible spectrum constitutes but a small part of the total radiation spectrum. Most of the radiation that surrounds us cannot be seen, but can be detected by dedicated sensing instruments. This electromagnetic spectrum ranges from very short wavelengths (including gamma and x-rays) to very long wavelengths (including microwaves and broadcast radio waves). The following chart displays many of the important regions of this spectrum, and demonstrates the inverse relationship between wavelength and frequency.
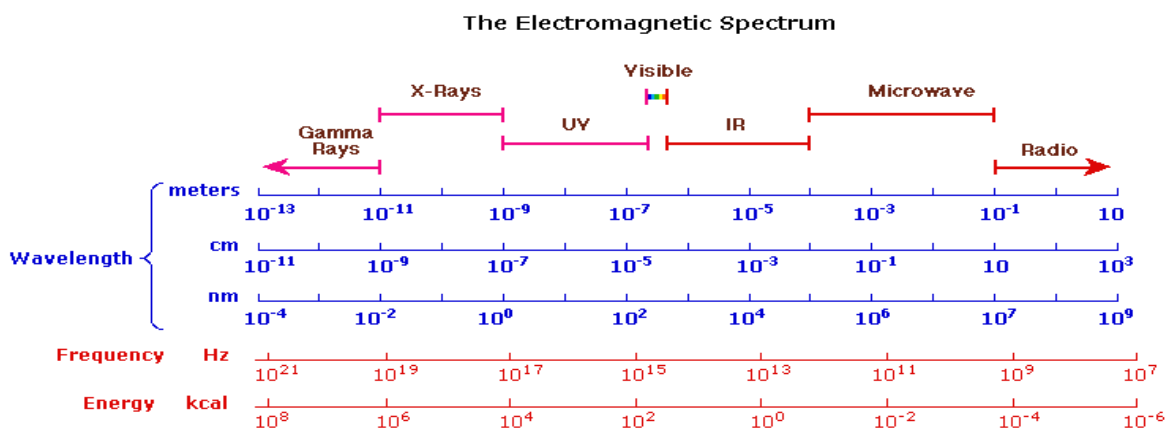
$\upsilon = c/\lambda$ υ=frequency, λ=wavelength, c=velocity of light (c=3•10^{10} cm/sec)

$\Delta E = h\upsilon$ E=energy, υ=frequency, h=Planck's constant (h=6.6•10^{-27} erg sec)

Effect of electromagnetic radiation on molecules

Spectra	Transitions	Region of electromagnetic spectrum
Infrared	Between the vibrational energy levels	Infrared (500-4000cm^{-1})
UV-Vis	Between the electronic energy levels	Visible (12,500-25,000cm^{-1}) UV (25,000 – 70,000cm^{-1})
NMR	Between the magnetic energy levels of nuclei	Radiofrequency

UV-Vis. Spectroscopy

- ➢ It involves transitions of electrons within a molecule from a lower to higher energy level or vice-versa by the absorption or emission of energy or radiations.
- ➢ The frequency of the radiations fall in the UV-Visible range of the electromagnetic spectrum. The Visible range is 12,500-25,000cm^{-1} (400-750nm) and the UV region is 25,000-72,000 cm^{-1} (190-400 nm)
- ➢ They have limited use in sample identification but are highly useful in quantitative measurements.

For quantitative measurement,

The concentration of an analyte in solution can be determined by measuring the absorbance at a specific wavelength and applying the Beer Lambert law.

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (A) versus wavelength.

Beer Lambert law: $A = \varepsilon\, C\, l$

Where A= absorbance (no units, since $A = \log_{10} P_0 / P$); C= sample concentration in mole/L, l= length of light passing through the sample in cm; ε is the molar absorbtivity with units of L mol^{-1} cm^{-1}

The law tells us that absorbance depends on the total quantity of the absorbing compound in the light path through the cuvette. If we plot absorbance against concentration, we get a straight line passing through the origin (0,0).
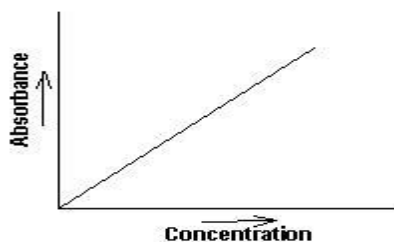
Principle of UV-Vis Spectroscopy:

Electronic transitions

The absorption of UV or visible radiation corresponds to the excitation of outer most valence electrons. When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state. In a molecule, the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level. Thus each electronic level in a molecule is associated with a number of vibrational sub-level (with smaller energy separation) and each vibrational sub-level is again associated with a number of rotational sub-levels (with still smaller energy separation.

Thus in its transition to a higher energy level, an electron can go from any of the sub-levels (corresponding to various vibrational and rotational states) in the ground state to any of the sub-levels in the excited state (diag below). The spectrum of a molecule containing these chromophores is complex. This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines. This appears as a continuous absorption band.
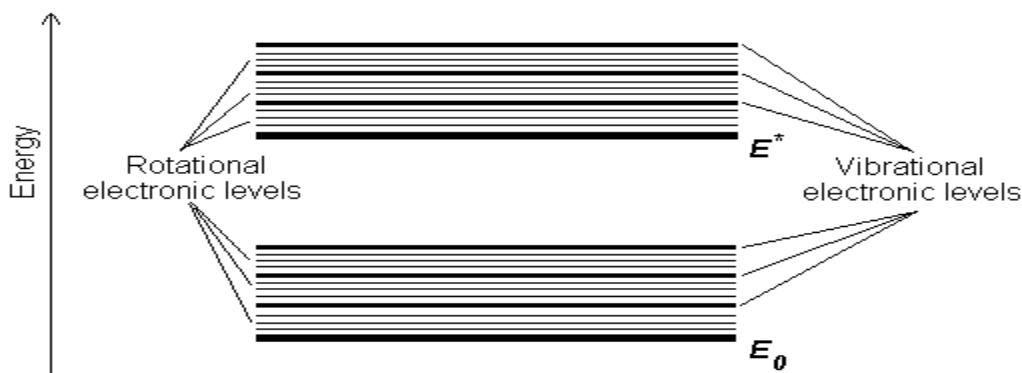

Absorbing species containing π, σ, and n electrons
Absorption of ultraviolet and visible radiation in organic molecules is restricted to certain functional groups (chromophores) that contain valence electrons of low excitation energy.

Classification of electronic transitions:

1. **σ → σ* transition:** As (σ) electrons are held more firmly in the molecule, this transition takes place in the UV region.

2. **Π → Π* transition:** This transition takes place in the near UV and the Vis region.

3. **n → n* transition:** This transition takes place in the Vis region.

The relative energies of the molecular orbitals showing such transitions are shown:
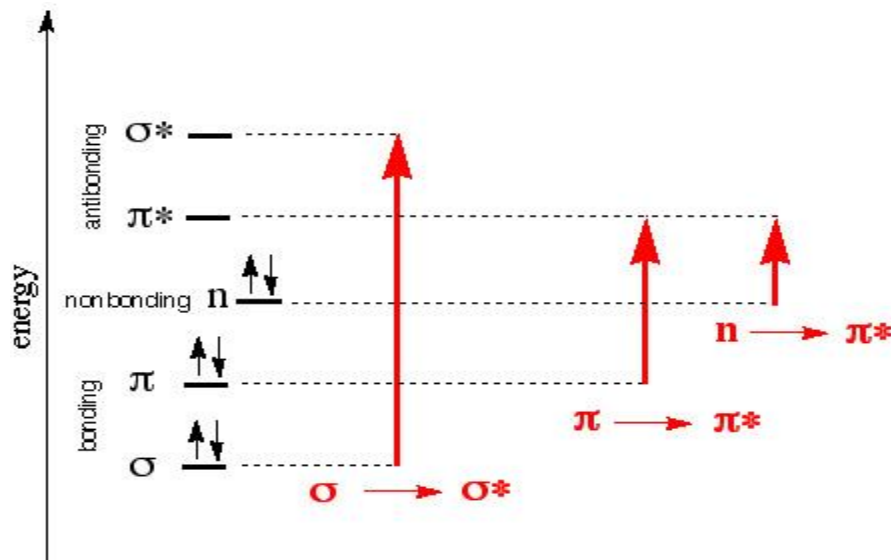
The position of bands for different electronic transitions is summarized:

Organic compound	Electronic transitions	Position of bands
Alkanes	**σ → σ* transition**	150 nm (requires high energy which does not lie in the UV region
Alkenes	**Π → Π* transition**	170-190nm
Aliphatic ketones	**n to Π* transition**	280 nm (forbidden and of low intensity)
	n to σ* transition	185 nm (allowed and of high intensity)
	Π to Π* transition	160 nm (allowed and of high intensity)
Conjugated dienes	**Π to Π* transition**	217 nm (transition is of low energy and is due to conjugation)

Chromophores: A chromophore is the part of a molecule responsible for its color. The color arises when a molecule absorbs certain wavelengths of visible light and transmits or reflects others. The chromophore is a region in the molecule where the energy difference between two different molecular orbitals falls within the range of the visible spectrum. Visible light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state.

Auxochrome: An auxochrome is a group of atoms attached to a chromophore which modifies the ability of that chromophore to absorb light. They themselves fail to produce the colour; but when present along with the chromophores in an organic compound intensifies the colour of the chromogen.

Examples include the hydroxyl group (-OH), the amino group (-NH2), and an aldehyde group (-CHO).

Terminology for absorption shifts

The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species. Peaks resulting from $n \rightarrow \pi^*$ transitions are shifted to shorter wavelengths (*blue shift*) with increasing solvent polarity. This arises from increased solvation of the lone pair, which lowers the energy of the *n* orbital. Often (but *not* always), the reverse (i.e. *red shift*) is seen for $\pi \rightarrow \pi^*$ transitions. This is caused by attractive polarization forces between the solvent and the absorber, which lower the energy levels of both the excited and unexcited states. This effect is greater for the excited state, and so the energy difference between the excited and unexcited states is slightly reduced - resulting in a small red shift. This effect also influences $n \rightarrow \pi^*$ transitions but is overshadowed by the blue shift resulting from solvation of lone pairs.
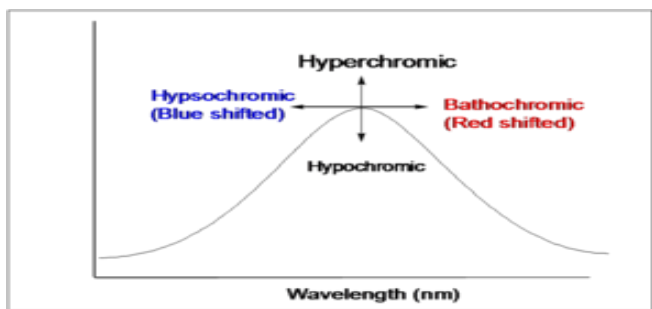
Bathochromic: a shift of a band to lower energy or longer wavelength (often called a red shift).

Hypsochromic: a shift of a band to higher energy or shorter wavelength (often called a blue shift).

Hyperchromic: an increase in the molar absorptivity.

Hypochromic: a decrease in the molar absorptivity.

Applications of UV-Vis. Spectroscopy

1. For the identification of aromatic compounds and conjugated dienes or other olefins.

2. Detection of impurities

3. Quantitative determination of analyte

4. Determination of molecular weight

5. Study of kinetics of chemical reaction

6. Dissociation constant for weak acids and bases can be determined.

Infra-Red Spectroscopy

Theory or Principle of Infrared Absorption Spectroscopy

a. IR photons have low energy (1-15kcal/mol); hence are not large enough to excite electrons (electronic transitions do not occur) but may induce vibrational excitation of covalently bonded atoms and groups in organic molecules.

b. The covalent bonds in molecules are not rigid sticks or rods, but are more like stiff springs that can be stretched and bent. Thus, in addition to the facile rotation of groups about single bonds, molecules experience a wide variety of vibrational motions, characteristic of their component atoms. Consequently, virtually all organic compounds will absorb infrared radiation that corresponds in energy to these vibrations.

c. There are three types of molecular transitions that occur during IR irradiation:

   1) Rotational transitions:

   2) Vibrational-rotational transitions

   3) Vibrational transitions

d. Conditions for IR absorbance to occur:

   ➢ The frequency of vibration must satisfy the equation: $E = h\nu$.

   ➢ The intensity of absorption must be proportional to square of the rate of change of dipole.

   ➢ The natural frequency of vibration of the molecule must be equal to the frequency of incident radiation.

   ➢ Changes in the vibration must stimulate changes in the dipole moment of the molecule. Hence Symmetric molecules (or bonds) do not absorb IR radiation since there is no dipole moment.

   Thus Infra red inactive molecules-

   a. do not show vibrational spectra.

   b. Do not have a permanent dipole moment

   c. Do not exhibit a change in dipole moment

   d. Eg - O_2, H_2, N_2 etc

Infra red active molecules –

a. Show vibrational spectra

b. Have a permanent dipole moment

c. Exhibit a change in the dipole moment

d. CO_2 is a molecule which does not have a permanent dipole moment but still is IR active.

e. The IR region is divided into three regions: the near, mid, and far IR. The mid IR region is of greatest practical use to the organic chemist. This is the region of wavelengths between 3 x 10^{-4} and 3 x 10^{-3}cm. In wave numbers, the mid IR range is 4000–400 cm^{-1}. An increase in wave number corresponds to an increase in energy.

f. The different Vibrational Modes occurring as a result of IR absorption:

i. **Stretching** -the rhythmic movement along a bond axis with a subsequent increase and decrease in bond length

Symmetric   Asymmetric
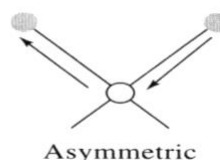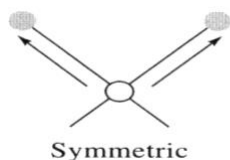
ii. **Bending** - a change in bond angle or movement of a group of atoms with respect to the rest of the molecule.

In-plane rocking   In-plane scissoring

Out-of-plane wagging   Out-of-plane twisting
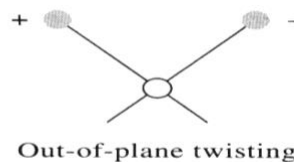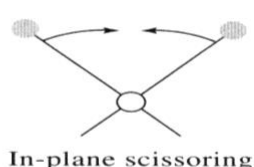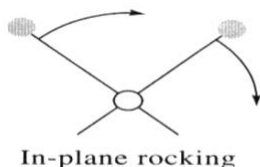
g. It is not necessary that all fundamental vibrations will exist in the IR spectrum. Some vibrations are inadequate and are called degenerate bonds. Since different bonds and functional groups absorb at different wavelengths, an IR spectrum is used in the identification of a compound or establishing the structure of an unknown substance.

Regions of IR spectra

1. **Functional group region**: is the high frequency region between **5000-1300cm^{-1}**. It is particularly useful in the identification of important functional groups present in the organic molecule.

2. **Finger print region:** is the middle frequency region between **1300-650cm^{-1}**. This region helps in the identification of unknown organic substance. No two compounds, however closely related can have identical or superimposable bands in this region.

Applications of IR Spectroscopy

1. **Identification of an unknown compound**
2. **Identification of functional groups in organic molecules**.
3. **To distinguish between inter and intra molecular hydrogen bonding**
4. **To determine the structure of an organic compound**

# NMR-Spectroscopy (Nuclear Magnetic Resonance Spectroscopy)

➢ The nuclei of many elemental isotopes have a characteristic spin (I).

➢ Some nuclei have integral spins (e.g. I = 1, 2, 3 ....), some have fractional spins (e.g. I = 1/2, 3/2, 5/2 ....), and a few have no spin, I = 0 (e.g. ^{12}C, ^{16}O, ^{32}S, ...).

➢ Isotopes of particular interest and use to organic chemists are ^{1}H, ^{13}C, ^{19}F and ^{31}P, all of which have I = 1/2.

The theory behind NMR comes from the spin, I of a nucleus. Just as electrons have a +1/2, -1/2 spin, certain nuclei also experience charged spins that create a magnetic field (called the magnetic moment), which allows chemists to study them using NMR. Nuclei with even numbers of both neutrons and protons experience NO spin and nuclei with odd numbers of both neutrons and protons have integer spins. Nuclei that have the sum of protons and neutrons equal to an odd number (like ^{1}H and ^{13}C) have half-integer spins. When there is no external or applied magnetic field (B_0), the nuclear spins orient randomly; however, when there is an applied magnetic field, the nuclei orient themselves with or against the larger applied field. The α-spin state is parallel to the applied force and has lower energy than the β-spin state that is antiparallel to the applied force. The energy difference (ΔE) between the α- and β -spin states depends on the strength of the applied magnetic field. The greater the strength of the applied magnetic field, the greater is the ΔE between the between the α- and β -spin states. This (ΔE) is ~0.02 cal mol^{-1}, which lies in the radio frequency region. The emitted energy in this region produces an NMR signal.
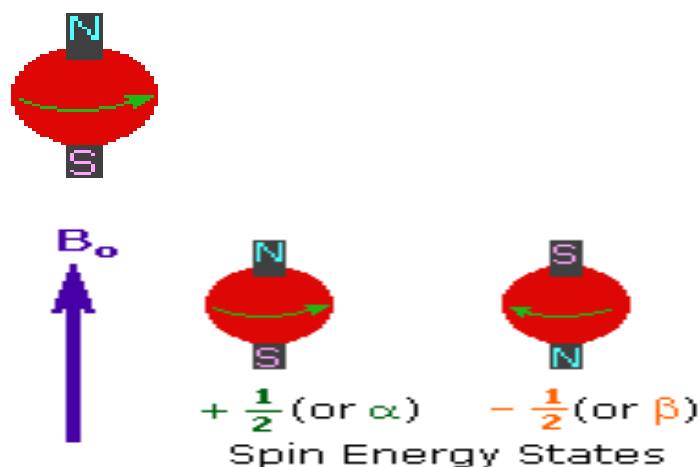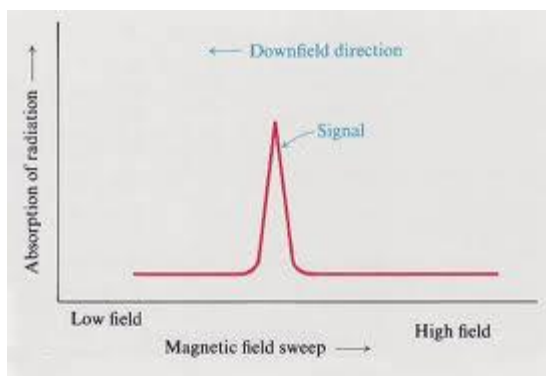
Addition of energy results in a spin flip
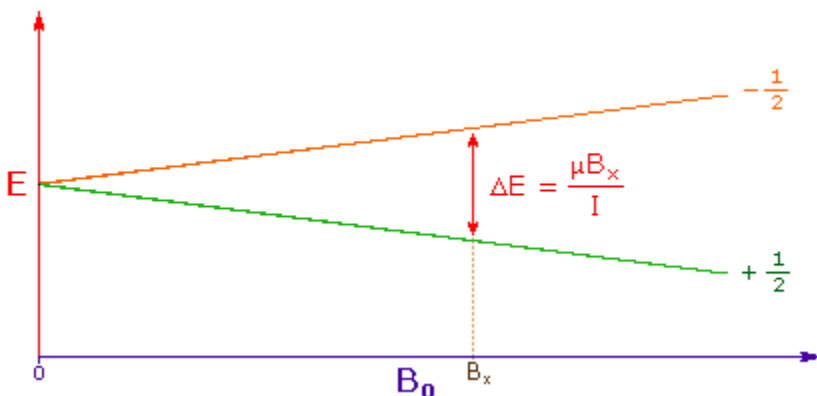
As the applied magnetic field increases, so does the energy difference between α- and β - spin states

NMR Spectrum

The NMR spectrum is a plot of the intensity of NMR signal versus the magnetic field (frequency) in reference to a standard (TMS).

NMR Applications

Today, NMR has become a sophisticated and powerful analytical technology that has found a variety of applications in many disciplines of scientific research, medicine, and various industries. Modern NMR spectroscopy has been emphasizing the application in biomolecular systems and plays an important role in structural biology. With developments in both methodology and instrumentation in the past two decades, NMR has become one of the most powerful and versatile spectroscopic techniques for the analysis of biomacromolecules, allowing characterization of biomacromolecules and their

complexes. Together with X-ray crystallography, NMR spectroscopy is one of the two leading technologies for the structure determination of biomacromolecules at atomic resolution. In addition, NMR provides unique and important molecular motional and interaction profiles containing pivotal information on protein function. The information is also critical in drug development.