3 Spectroscopy Techniques and Applications

"The past, like the future, is indefinite and exists only as a spectrum of possibilities."

—Stephan Hawking

CHAPTER OUTLINE

3.1 INTRODUCTION

Spectroscopy is the branch of science which deals with trasnsitions occurring in a molecule when it interacts with electromagnetic radiations. Our knowledge about molecular structure is derived indirectly from different spectroscopic techniques. It is not possible to see atoms and molecules even with the help of most powerful microscope because of their infinitesimal small dimensions.

As atoms give line spectra and each atom gives its characteristic spectrum, so the molecular spectra depend upon the nature of atoms involved and also upon the arrangement of the atoms in the molecules. *Atomic spectra is due to electronic transitions only i.e., electrons shift from one energy level to another. But in molecular spectra, rotational and vibrational trasitions also take place in addition to electronic transitions and this spectra is therefore, much complicated than atomic spectra.*

The molecular spectra is of much help to a chemist in elucidating the structure of molecules.

3.2 ELECTROMAGNETIC SPECTRUM

Th visible light represents only a small part of the entire electromagnetic spectrum, which extend from high energy cosmic rays to low energy radio or radar waves. The complete electromagnetic spectrum

gives the arrangement of various types of electromagnetic radiations in order of their increasing (or decreasing) wavelengths or frequencies (Fig. 3.1).

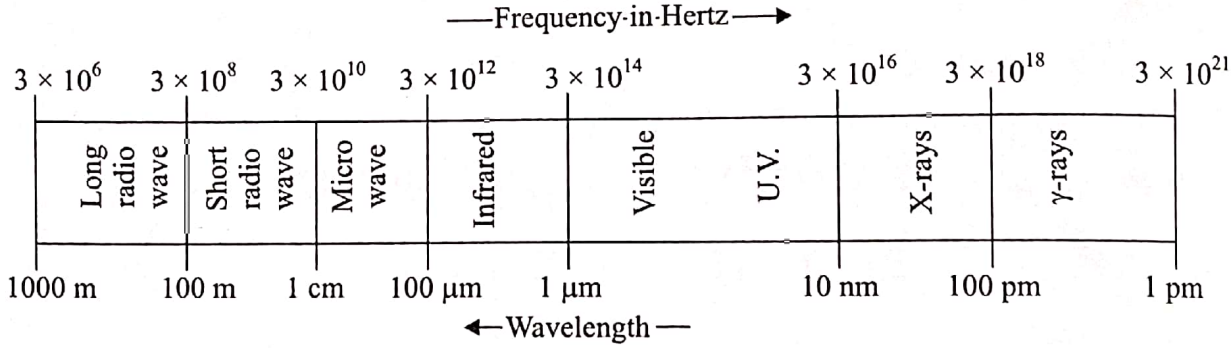


Fig. 3.1 Electromagnetic spectrum

Table 3.1. Illustrates the various regions into which electromagnetic radiation has been divided. It also includes the type of spectra that can be studied in each region of electromagnetic radiation.

Table 3.1. The Regions of Electromagnetic Radiations

Electromagnetic radiation	*Frequency (ν)*	*Wavelength (λ)*	*Spectrum*
Radio frequency region	$3 \times 10^6 - 3 \times 10^{10}$ Hz	10 m – 100 m 100 m – 1 cm	Nuclear magnetic resonance Electron spin resonance
Microwave region	$3 \times 10^{10} - 3 \times 10^{12}$ Hz	1 cm – 100 μm	Microwave (rotational) spectroscopy
Infra-red region	$3 \times 10^{12} - 3 \times 10^{14}$ Hz	100 mm – 1 μm	Infared (vibrational) spectroscopy
Visible and Ultraviolet region	$3 \times 10^{14} - 3 \times 10^{16}$ Hz	1 μm – 10 nm	Utra-violet visible (electronic) spectroscopy
X-ray region	$3 \times 10^{16} - 3 \times 10^{18}$ Hz	10 nm – 100 pm	Photoelectron spectroscopy
γ-ray region	$3 \times 10^{18} - 3 \times 10^{20}$ Hz	1 pm – 100 pm	Enegy changes involve the rearrangement of nuclear particles

$1\ \mu m = 10^{-4}$ cm; $1\ nm = 10^{-9}$ m = 10 Å

3.3 ATOMIC AND MOLECULAR SPECTROSCOPY

3.3.1 Atomic Spectroscopy

It is obtained by the interaction of electromagnetic spectrum with atoms. Each atom gives its own characteristic spectrum in the form of lines. It is, therefore referred to as **line spectrum** also. It can be obtained either by absorption or by emission of radiation. Depending upon the mode of formation, atomic spectrum is called absorption or emission spectroscopy.

The characteristic line spectrum obtained in case of an atom is due to the electronic trasnsitions between the energy levels.

3.3.2 Molecular Spectroscopy

A molecule can absorb or emit energy as a result of transitions between different electronic energy levels. From Fig. 3.2, it is evident that each **electronic level** consits of a number of **vibrational sub-levels** represented by $v = 0, 1, 2, 3$ etc., and further each vibrational level consists of a number of **rotational sub-levels** represented by $j = 0, 1, 2, 3$ etc., v and j are called *vibrational and rotational quantum numbers* respectively. However, a molecule can absorb a quantum of energy and increase its vibrational energy and rotational energy also. The quanta associated with these three different types of energy levels namely, *electronic, vibrational and rotational* are of very different energy. The energies of the quanta that are absorbed or emitted by a molecule can be measured by spectroscopic means.

The molecular spectra arise due to changes in rotational, vibrational and electronic energies possessed by molecules. The total energy of a molecule, according to **Born-Oppenheimer approximation,** *is given by:*

$$E = E_{tr} + E_{rot} + E_{vib} + E_{el} \quad \text{where } E_{el} >>> E_{vib} >> E_{rot} > E_{tr}$$

where E_{tr}, E_{rot}, E_{vib} and E_{el} represent translational, rotational, vibrational and electronic energies respectively. The translationl energy is not quantised, whereas all the other energies are quantised. Since the translational energy is negligibly small, we can write the above approximation as:

$$E = E_{rot} + E_{vib} + E_{el}$$

responding to the three types of energies possed by molecules three quantum numbers namely, roational quantum number (j), vibrational quantum number (v), and electronic quantum number have been assigned.

A diagram illustrating the distribution of different energy levels is shown in Fig. 3.2. Two electronic level n'' and n' are shown. The lower level n'' has a number of vibrational energy level denoted by the vibrational quantum number $v'' = 0, 1, 2, \ldots$ and each vibrational level has its own rotational energy levels represented by the rotaional quantum number $J'' = 1, 2, \ldots$. In the same way, the upper electronic level n' has vibrational level $v' = 0, 1, 2, \ldots$, and vibrational level has rotational energy levels $J' = 1, 2, \ldots$.

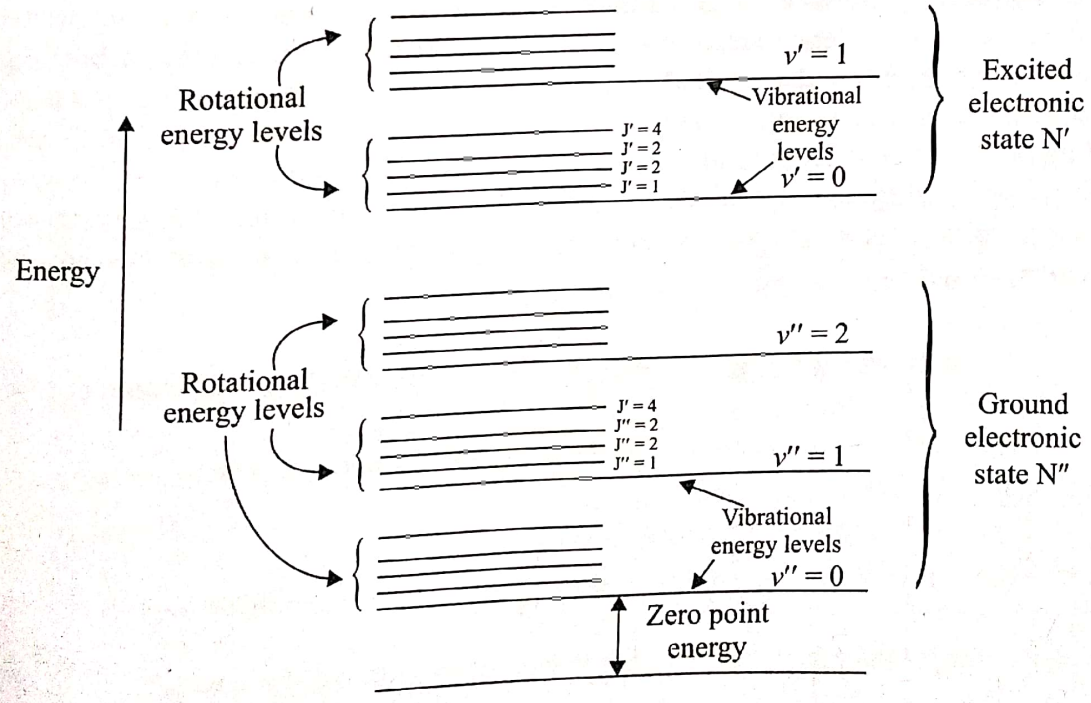


Fig. 3.2 Energy level diagram showing the various molecular energies

Transitions between vibrational levels within the same electronic level give rise to spectra which are called **vibrational spectra.** In the near infra-red, transitions between different rotational levels within the same vibrational level are responsible for **rotational spectra** in the far infra–red or microwave region. Infact a transition between two electronic level is usually accompanied by changes in the vibrational and rotational quantum numbers, so that electronic spectra are really electonic vibration-rotational spectra. In the same way, vibrational spectra are really **vibrational-rotational spectra** and are normally given the name. However, very small changes are required for transitions between rotational level alone. Hence the spectra corresponding to the smallest changes in energy are thus called **pure rotational spectra.**

The study of molecular spectra gives information about the structure and properties of molecules such as bond lengths, bond angles, bond strength, shapes, dipole moments etc.

3.4 ULTRAVIOLET AND VISIBLE SPECTROSCOPY

To understand why some compounds are colored and some are not, and to determine the relationship of conjugation to color, we must make accurate measurements of light absorption at different wavelength in and near the visible part of the spectrum. The visible region of the spectrum comprises photon energies of 36 to 72 kcal/mole, and the near ultraviolet region, out to 200 nm, extends this energy range to 143 kcal/mole. Ultraviolet radiation having wavelengths less than 200 nm is difficult to handle, and is seldom used as a routine tool for structural analysis.

The energies noted above are sufficient to promote or excite a molecular electron to a higher energy orbital. Consequently, absorption spectroscopy carried out in this region is also called **electronic spectrocopy.**

3.4.1 Electronic Transition

According to the Molecular orbital theory, when a molecule is excited by the absorption of energy (UV or visible light), its electron are promoted from a bonding to an antibonding orbitals. When a molecule is formed then valence electron taking part in bond formation exist in bonding orbital. Electrons forming sigma (σ) bond are called σ electrons and those which participate in π bond (pi) are called π electrons. Unshared electrons in a molecule which do not take part in any bond formation exist as non bonding orbitals. Such electrons are called *n*-electrons, non bonding electrons. There are higher energy levels termed as antibonding orbitals which are associated with σ and π bond as σ* (sigma star) and π* (pi-star) orbitals. As *n* electrons do not take parts in bond formation thus is no antibonding orbital associated with it.

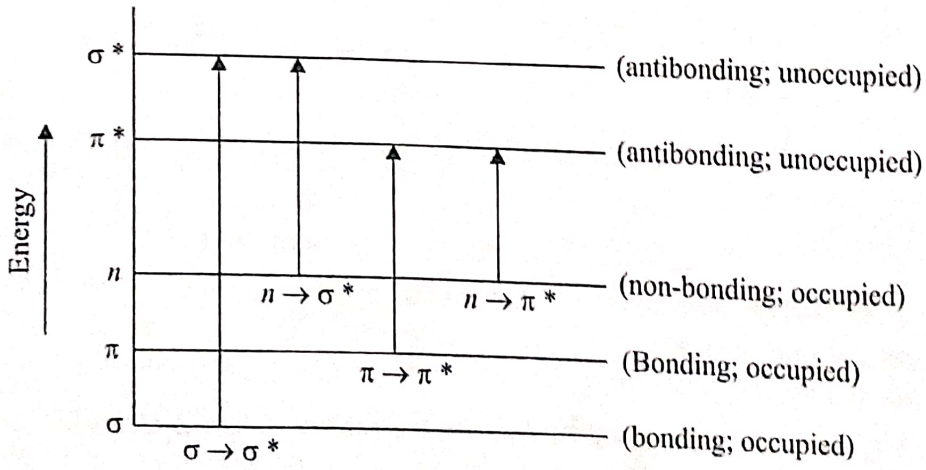


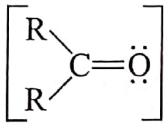
Fig. 3.3 Relative energies required for various type of electronic transition.

A schemeatic arrangement showing the energy levels of all these orbitals is shown in Fig. 3.3.

(*i*) ***Transition between bonding orbitals and antibonding orbitals,*** **e.g., $\sigma \rightarrow \sigma^*$ or $\pi \rightarrow \pi^*$. This excited state closely resembles the polar character.**

(*a*) These are high energy transition as σ bond are generally strong bonds. Saturated hydrocarbons like methane, propane etc. shows such high energy transitions.

(*b*) **$\pi \rightarrow \pi^*$ transition:** Molecules having unsaturation show this kind of transition and absorb in ultraviolet or visible region for eg. alkenes, alkynes, carbonyl compounds etc.

(*ii*) ***Promotion of non-bonding electron*** (*i.e.*, unshared pair *n*) into or antibonding π, *i.e.* $n \rightarrow \sigma^*$ or $n \rightarrow \pi^*$. These involve transitions of non-bonding lone pair of hetero atom (*e.g.*, oxygen, nitrogen) to an empty anti-bonding molecular orbital. The absorption bands for such transitions occur in longer wavelength with low intensity. Compounds like saturated alkyl halides, alcohols, ethers aldehydes, ketones amines shows $n \rightarrow \sigma^*$ transition. Compounds like saturated aldehydes or ketones  also show $n \rightarrow \pi^*$ transitions.

(*iii*) Transition between bonding (occupied) σ and π orbitals to antibonding (unoccupied) σ^* and π^* orbitals *i.e.*, $\sigma \rightarrow \pi^*$ and $\pi \rightarrow \sigma^*$ can also take place. *According to selection rules these raditions are forbidden and therefore have weak intensities.*

All the above four type of electronic transitions have been summed up in Fig. 3.3. The relative energies required for these transitions have also been shown. The energy required is maximum for $\sigma \rightarrow \sigma^*$ transition and minimum for $n \rightarrow \pi^*$ transitions. The energy requirements in the decreasing order of these transitions is given below:

$$\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$$

3.4.2 Selection Rules

We know that atomic spectra as well as molecular spectra are obtained due to transitions taking place between energy levels. To make the spectral study simpler and easy interpretation of the data obtained from it, certain restrictions are imposed on quantum numbers during transitions, which are known as 'Selection Rules'. If a spectral transition is as per the selection rule, it is called as allowed transition.

If these rules are not followed, the transition can not take place and it is called a **forbidden** *transition. Example of forbidden transitions are from s to s orbital and from p to p orbital. For these transitions* $\Delta l = 0$. *In general allowed transition are intense strong where as* **forbidden transition** *are weak.*

The selection rules to be followed depend upon the type of transition (in case of molecular spectra). The selection rules are generally expressed in terms of changes in quantum number for the allowed transitions. For example:

(*i*) In generally $\Delta l = \pm 1$, where *l* is called azimuthal quantum number. Transitions from *s* to *p* orbital and from *p* to *q* orbital are allowed transitions, because $\Delta l = \pm 1$.

(*ii*) For the pure rotational transition, the selection rule is $\Delta J = \pm 1$, where J represents rotational quantum number, ($\Delta J = +1$ corresponds to absorption and $\Delta J = -1$ corresponds to emission).

(*iii*) For pure vibrational transition, the selection rule is $\Delta v = \pm 1$, where *v* represents the vibrational quantum number.

Thus

(*a*) The transition which involve a change in the spin quantum number of an electron during the transition do not occur. Thus singlet-triplet transitions and forbidden.

(*b*) The transitions between orbitals of different symmetry do not occur for e.g. $n \rightarrow \pi^*$ transition is symmetry forbidden.

3.4.9 Fluorescence and Phosphorescence

Fluorescence: Certain substance absorb photo-energy and get excited *i.e.*, the electrons are raised from lower enrgy levels to higher energy level, but their excited molecule immediately or instantaneously re-radiates or emits part of the absorbed energy at a greater wavelength. *Such substances which absorb photo-energy and emit a part of it immediately at a greater wavelength with a short time (10 sec.) are called* ***Fluorescent Substances*** *and this phenomenon is called* ***Fluorescence.*** This process is instantaneous and starts immediately after the absorption of light and stops the moment the radiation is cut off.

Examples of fluorescence are solutions of fluorescein and eosin. When their solution are placed in light, they show fluorescence from green to violet colour; uranyl sulphate, chloropyll (green colouring matter of plants) fluorospar (CaF_2) and petroleum also show this phenomenon. It may be pointed that different substances shows the phenomenon of fluorescence with different wavelengths. Thus, fluorospar show fluorescence with blue light, chlorophyll with red light, uranium glass with green light and so on.

Phosphorescence: In this process the absorbed light energy raises the electron to a higher level

Both fluorescence and phosphorescence emit radiations of shorter frequencies or larger wavelength than the incident radiation (or exciting radiation) because some part of radiation absorbed by the molecule is dissipated in the form of heat during the non-radiative transitions.

3.4.12 Applications of Fluorescence in Medicines

Clinically compatible time-resolved fluorescence spectroscopy and imaging systems developed by several research groups have shown that fluorescence can be employed to know about the diseased tissues in patients. It has also been investigated that the fluorescence lifetime contrast can be used in tissue characterization and diagnosis. Systematic studies of the fluorescence decay characteristics in patients are helpful for a complete and accurate clinical diagnosis.

Some important applications in the field of medicine are listed below:

(*i*) The analysis of the concentration of riboflavin (Vitamin B_2) in chloroform has been carried out.

(*ii*) Use of fluorescent microscopes and fluoroscope used in X-ray diagnosis help in testing the condition of food stuff and detecting ring worms etc.

(*iii*) Fluorescence is helpful in tissue characterization and diagnosis.

(*iv*) Applications of time resolved fluorescence to diagnosis of pathologic conditions in humans such as:

(*a*) diagnosis of cancer of the gastrointestinal (GI) tract, bronchi/lung, skin, head and neck, and brain;

(*b*) ophthalmic pathologies; and

(*c*) atherosclerotic cardiovascular disease.

(*v*) The potential role of autofluorescence is in the diagnosis of head and neck tumors including the cancer of the oral cavity through a variety of spectroscopic and imaging techniques.

(*vi*) Autofluorescence technique has been used in the treatment of primary brain tumors.

(*vii*) Several fluorescence techniques have been employed for the characterization of skin. Analysis of skin physiology, optical biopsy of skin, and detection of dermatological disorders including fungal infections, skin age, hair pigment, and cancer has been carried out by researchers in the field.

(*viii*) Time-resolved Fluorescence has been applied in the detection of eye diseases and research.

Applications of fluorescence in other fields are as under:

(*i*) For lighting purposes in fluorescent tubes: Mercury arc producing large proportion of ultra violet light is used in a tube coated with fluorescent salts which gives visible light.

(*ii*) By mixing fluorescent dyes with coloured paints, the fluorescence of the dye helps the light reflected by the paint to produce extra-ordinary brightness and luster. These materials are used for road signs. For example, Brilliant Sulpho Flavine FF and Rhodamine 6G dispersed in a special kind of plastic are used for this purpose.

(*iii*) In industry for testing and identifying materials, *e.g.* in rubber industry.

(*iv*) In television–the cathode stream of the photoelectric effect is made visible in the cathode ray tube by adding ZnS to which a little Ni is added to cut off phosphorescence which otherwise makes the picture blurred.

(*v*) A fluorescent dye is used as whitener for washing cloth when mixed with washing powder.

3.5 INFRA-RED SPECTROSCOPY

Infra-red spectrum provides enormous information about the structure of a compound. It is an important tool in the hands of a chemist to confirm the structure of known as well as unknown *organic compounds*. The structure of any newly synthesized compound is established using this unique technique. The absorption of Infrared radiations causes the vibration of various bonds in the molecule to stretch and bend with respect to one another.

The spectroscopic technique involving transitions in the vibrational energy levels due to absorption of Infrared radiations by molecules is defined as IR Spectroscopy. It is called vibrational spectroscopy because the transitions take place between the vibrational energy levels. It is called IR spectroscopy since the frequency of the radiations absorbed lie in the IR region of electromagnetic spectrum.

In Infra-red radiations are capable to induce transitions between vibrational and rotational levels as each vibrational level is associated with rotational level of a molecule. Hence it is also called **rotational-vibrational spectroscopy.**

It is useful to divide the infra red region into three sections; *near*, *mid* and *far infra* red:

Region	*Wavelength range (mm)*	*Wavenumber range (cm^{-1})*
Near IR	0.78 – 2.5	12800 – 4000
Mid IR	2.5 – 50	4000 – 200
Far IR	50 – 1000	200 – 10

The most useful IR region lies between 4000 – 670 cm^{-1}. Photon energies associated with this part of the infrared (from 1 to 15 kcal/mole) are not large enough to excite electrons, but may induce vibrational excitation of covalently bonded atoms and groups. The covalent bonds in molecules are like stiff springs that can be stretched and bent. Hence, 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.

3.5.1 Theory of Infra-red Ab...

3.6 NUCLEAR MAGNETIC RESONANCE (NMR)

Nuclear magnetic resonance involves the interaction between oscillating electro-magnetic radiation and the magnetic properties of certain magnetic nuclei notably the nucleus of hydrogen atom– **the proton.**

Examples of magnetic isotopes are ^{13}C, ^{1}H, ^{19}F, ^{14}N, ^{17}O, ^{31}P, and ^{22}S. If a nucleus is not magnetic, it can't be studied by nuclear magnetic resonance spectroscopy.

NMR war idependently discovered in 1946 by the American physicists **F. Bloch** and **E.M. Purcell**; they shared Nobel Prize in Physics (1952). The first application of NMR to chemistry was made in 1951 when NMR spectrum of ethanol was recorded, since then NMR technique has almost been completely monopolized by chemists, especially organic chemists and biochemists for structural elucidation.

NMR spectroscopy is based on the measurement of absorption of electromagnetic radiations in the radio frequency region from roughly 4 to 900 MHz. Absorption of radio waves in the presence of magnetic field is accompanied by a special type of nuclear transition, and for this reason, such type of spectroscopy is known as ***Nuclear magnetic resonance (NMR) spectroscopy.***

Since in early sixties, the growth of NMR has been nothing short of an explosion and has led to highly sophisticated instrumental techniques which in turn have led to complex organic structural investigations and the elucidation of mechanism and stereochemistry.

3.7 MAGNETIC RESONANCE IMAGING (MRI)

MRI is an important technique for the diagonsis of soft tissues of human body. It is based on the priciple of nuclear magnetic resonance. MRI can be used to discriminate between healthy and diseased tissues. It makes use of the fact that protons present within water, fats, lipids etc., come to resonance at

a given frequency. The images of the various body parts can be easily generated because human body contains 75% water and each water molecule has two hydrogen nuclei. The distribution of water, fats and lipids etc., changes due to various diseases in body. So MRI can detect the body part affected by the disease.

In order to get an image in MRI, instead of applying a uniform field, a varying magnetic field is applied across the body part. The protons in different regions will come to resonance at different frequencies and the intensity of signal will be proportional to the number of protons at each magnetic field. If body part is rotated into a different orientation, another projection can be determined. Then, the data is combined to get a three dimensional image of the body part. In brief, image of a body part can be constructed by scanning a slice of particular body part and then various slices can be combined by computer to get the final image.

Applications of MRI

(*i*) MRI needs very small time and can be used for observing the function of myocardium and various heart diseases.

(*ii*) It can identify the regions of excessive fat deposition in various organs and in blood vessels.

(*iii*) Sodium (^{23}Na) MRI can be used for the analysis of blood.

(*iv*) Fluorine (^{19}F) MRI can be used for determination of concentration of fluorine in various body parts.

Although various other nuclei present in body such as phosphorus (^{31}P), Fluorine (^{19}F), Sodium (^{23}Na), Oxygen (^{17}O) can be used for MRI but they are less abundant in the body and have very weak magnetic moment, so they do not give sharp images. Moreover, the time required for construction of image is large.

3.7.1 Surface Characterisation Techniques

These techniques analyse the patterns produced when a sample is illuminated by X-rays and causes deflections. Diffraction patterns provide the atomic structure of molecules such as powders, small molecules or large molecules like protein crystals. Diffraction can be used to measure strains in materials under load, by monitoring changes in the spacing of atomic planes. Scattering experiments are used to determine the molecular structure of non-crystalline materials, including complex biological samples and polymers.

The interface between one material and another in composites, for instance, glass, fibre and resin or thermal barrier coating etc., is critical to the performance of the product in operation. The analytical techniques suggested by **Lucideon** are used to understand the nature and functionality of material interfacial interactions in terms of their composition and shape. Chemical analysis of materials can tell what is present, where it is and how much is there, for all the elements and molecular fragments as large as 10000 mass units. Let us discuss the chemical analysis techniques and physical surface profiling methods and their application to the characterisation of various composite materials.

The main diffraction and scattering techniques employed under the category of ***Synchrotron Techniques*** are given below:

1. Macromolecular Crystallography (MX)
2. Grazing Incidence X-ray Diffraction (GIXD)
3. Powder Diffraction
4. X-Ray Reflectivity (XRR)
5. Scattering-SAXS and WAXS

6. X-ray Photoelectron Spectroscopy (XPS) in Surface Chemical Analysis
7. Time-of-Flight Secondary Ion Mass Spectrometry (ToFSIMS) in Surface Characterization
8. Adhesion Failure Analysis of Composite Materials
9. Dynamic Secondary Ion Mass Spectrometry (DSIMS)
10. 3D Profilometry Using White Light Interferometry (3DP)

The details of the above methods in brief are given below:

Before taking up the brief details of above noted techniques, let us know the meaning of Synchrotron Technique, because most of the above noted technologies utilise this technique.

These techniques include the measurements that can be carried out using the initial suitable beamlines and it employed in case of the following four main categories of technologies.

(*i*) **Diffraction/scattering** for crystallography.

(*ii*) Spectroscopy for analysis of chemical composition and specification in the bulk material and at surfaces, down to nanometre level.

(*iii*) Polarimetry for measuring the shape of complex molecules, such as proteins, and the properties of magnetic materials.

(*iv*) **Imaging** for highly detailed imaging of small animals, and ultimately humans, down to the substructure of biological and physical materials, using light from infrared to hard X-rays.

INFORMATION WINDOW

UV-visible spectroscopy

Spectroscopy

1. Molecular spectra arises due to change in rotational, vibrational and electronic energies possessed by molecules and the total energy of molecule is given Born-oppenheimer approximation which is given by

$$E = E_{vib.} + E_{rot.} + E_{tran.} + E_{elec.}$$

2. In electronic spectroscopy UV range is 200-400 nm and visible rang is 400-800 nm.
3. UV spectra of organic compounds consists of bond (and not sharp peaks) which is due to solvent-solute interactions. Vibrational and rotational effects are superimposed on electronic transitions resulting into bands.
4. Hydrogen bonding shifts the UV absorptions to shorter wavelengths of high frequencies.
5. There are four main types of electronic transitions and in terms of decreasing energy requirement, arranged as below:

$$\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$$

$\rightarrow$ decreasing energy.

6. For simple study of spectra and its easy interpretation of data certain restrictions are imposed on quantum numbers during transition which are called as "selection rules".
7. Transitions as per selection rules are allowed transitions. If these rules are not followed, then transition will not take place and is called forbidden transition.
8. Chromophores are isolated covalently bonded group that shows a characteristic absorption in the ultraviolet or visible region.
9. Auxochromes it self does not act as Chromophore but their presence brings shifts of absorption band towards the red end of spectrum (longer wavelength).
10. Bathochromic effect shift the absorption maximum towards longer wavelength in the presence of auxochrome or change of solvent. Increase in conjugation also causes bathchromic effect.
11. **Hypsochromic shift or effect:** Due to this absorption maximum is shifted to shorter wavelength. This happens when conjugation is reduced or polarity of solvent is changed.
12. **Hyperchromic effect:** It is an effect due to which intensity of absorption maximum increases.
13. **Hypochromic effect:** When intensity of absorption maximum decreases then it is called hypochromic effect.
14. Franck-Condon principle states that when electronic transition takes place so rapidly that a vibrating molecule does not change its internuclear distance during transition because electron moves much faster then the nuclei and thus the nuclei does not change their position and velocities.
15. **Fluorescence:** When substance absorbs photo-energy and emit a part of it immediately at a greater wavelength are called fluorescent substances and phenomenon is called fluoresence.
16. **Phosphoresence:** It is slow fluoresence. In this phoenomenon the emission of light of different wavelength continues even when source of radiation is removed.
17. Spin multiplicity of a state is given by $(2S + 1)$ where S is total electron spin.

IR Spectroscopy

18. Since the frequence of radiation absorbed lies in the IR region of electromagnetic spectrum, it is called IR spectroscopy.

    It is also called as vibrational spectroscopy because transition takes place between vibrational energy levels.

19. For a molecule to absorb IR radiation, vibration and rotation in a molecule must cause net change in the dipole moment of a molecule.
20. IR spectra is given by molecules with permanent dipole moment and polyatomic molecules with or without dipole moment.
21. When IR is passed through sample, increase in vibrational and rotational energies result in the excitation of bond deformations called as stretching and bending vibrations.
22. Stretching vibrations can be symmetric or Asymmetric type, *i.e.,* movement of atoms in a molecule w.r.t. central atom is either in same direction or in different direction.
23. Various stretching and bending vibration of a band occur at certain quantised frequencies.
24. Bending vibration may be Scissoring, Rocking, Wagging and Twisting.
25. **Hooke Law:** According to this law the restoring force (F) is directly proportional to distance for displacement of atoms from their mean positions *i.e.,* $F = -kx$.
26. Fundamental bands as related to degree of freedom. Total degree of freedom of a molecule is $3n$ where n is the number of atoms is a molecules.
27. Usual analysis of region of IR spectra varies from 4800 – 600 cm^{-1}. The region from 4000 to 1500 cm^{-1} is characterised as functional group region and below 1500 cm^{-1} as finger print region.
28. Presence of electron denoting group and conjugation lowers the wave number of absorption while the presence of electron attracting groups raise the wave number of absorption.
29. Hydrogen bonding lowers the wave number of absorption. This is significant in case of inter molecular hydrogen bonding along with broadening of band.

Nuclear Magnetic Resonance (NMR)

30. NMR spectroscopy involves the interaction between oscillating electromagnetic radiation and the magnetic properties of certain magnetic nuclei like the proton of the Hydrogen atom.
31. In NMR absorption of radio waves in the presence of magnetic field is accompanied by nuclear transition and that is why it is known as Nuclear Magnetic Resonance spectroscopy (NMR).
32. Spin quantum number for the nucleus contour values 0, $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2 etc., depending upon the number of neutrons and protons having parallel and antiparallel spins.
33. When sum of proton and neutrons is even, mass number and atomic number is also even, then nuclear spin is zero.
34. For nuclei in which the sum of protons and neutrons is even but number of proton is odd (Mass number is even and atomic number is odd) then nuclear spin is integral.
35. For nuclei in which sum of proton and neutron is odd, (Mass number is odd) then nuclear spin is always odd integral multiple of $\frac{1}{2}$.

36. Larmor frequency or precessional frequency is frequency of precession and is identical to transition frequency. It is equal to the number of revolution per second made by magnetic moment vector of the nucleus around external field.
37. Relaxation process involves non-radiative transitions by which a nucleus in an upper transitions state returns to lower spin state.
38. Broadening of a signal is directly proportional to the life time of the absorbing nuclei in excited state.
39. **Chemical shift:** It is the difference in the resonance frequency of a given proton compared to that of Methyl protons of TMS under experimental conditions. It is independent of operating frequency of instruments but depends upon the nature of the solvent.
40. **Shielding Effect:** When effective magnetic field experienced by the nucleus is less than that of the applied field, the nucleus is said to be shielded and signal appears at up field *i.e.*, lower value of δ(delta).
41. **Deshielding effect:** When effective magnetic field experienced by the nucleus is more than the applied field, then the nucleus is said to be deshielded. Deshielding of protons is caused when it is surrounded by electronegative groups or atoms for such protons signal appears at down field *i.e.*, higher value of δ(delta).
42. **Spin-spin Spliting:** When there is magnetically non-equivalent protons then splitting occurs between nuclei with different chemical shift. The coupling interaction takes place between neighbouring protons through bonds and results in the splitting of spectral lines. If environment is stereochemically different then coupling also takes place between protons on the same carbon atom.
43. **Protons Equivalence:** Chemically equivalent protons are also Magnetically equivalent and possess same frequency. All three protons of Methyl group are magnetically equivalent because as a result of the rotation around C—C bond, all the three protons have the same time averaged chemical environment and hence the same resonance frequencies. The chemically equivalent protons are stereochemically equivalent which may be looked at by labelling the hydrogen atoms in a compound as enantiotopic diastereotopic.
44. **Coupling Constant:** It is a measure of the coupling interaction between the nuclei. The spacing between the lines with in a coupled multiplet is constant. This constant distance, called as coupling constant is denoted by 'J' and is expressed in Hertz (Hz).
45. Multiplicity of Signal is give by $n + 1$ rule, where n = number of protons on the adjacent atoms.

Magnetic Resonance Imaging (MRI)

46. MRI is an important technique for the diagnosis of soft tissues of human body based on principle of NMR.
47. Surface characterization technique are the methods by which the properties associated with the surface of the materials are determined for *e.g.*, surface area, surface roughness, pore size and reflectivity, all constitute surface characteristics.
48. **Macromolecular Crystallography (X):** It is the most powerful technique for determining three dimensional structures of large biological molecules such as proteins. It is also known as protein crystallography.
49. **Grazing Incidence X-Ray Differaction:** This is an ideal tool to determine the morphology of novel materials in realistic operating conditions for *e.g.*, electronic, magnetic and chemical behaviour are determined by those technique.