INFRA RED (IR) SPECTROSCOPY

Infrared (IR) spectroscopy is one of the most common and widely used spectroscopic techniques. Absorbing groups in the infrared region absorb within a certain wavelength region. The absorption peaks within this region are usually sharper when compared with absorption peaks from the ultraviolet and visible regions. In this way, IR spectroscopy can be very sensitive to determination of functional groups within a sample since different functional group absorbs different particular frequency of IR radiation. Also, each molecule has a characteristic spectrum often referred to as the fingerprint. A molecule can be identified by comparing its absorption peak to a data bank of spectra. IR spectroscopy is very useful in the identification and structure analysis of a variety of substances, including both organic and inorganic compounds. It can also be used for both qualitative and quantitative analysis of complex mixtures of similar compounds.

Infrared spectroscopy (IR spectroscopy) is that type of spectroscopy which deals with the infrared region of the electromagnetic spectrum; that is light with a longer wavelength and lower frequency than visible light. IR is one of the types of absorption spectroscopy.

The term "infra red" covers the range of the electromagnetic spectrum between 0.78 and 1000 µm. In the context of infra red spectroscopy, instead of wavelength, measurements are made in "wavenumbers" ($\bar{\upsilon}$), which have the unit cm^{-1}.

$$\text{Wavenumber } (cm^{-1}) = 1 / \text{Wavelength in centimeters}$$

$$\text{Wavenumber } (cm^{-1}) = 10^4 / \text{Wavelength in } (\mu m)$$

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

Region	**Wavelength range** (µm)	**Wavenumber range** (cm^{-1})
Near	0.78 - 2.5	12800 – 4000
Mid	2.5 – 16	4000 – 625
Far	16 – 50	625 - 200

The most useful I.R. region is the middle region which lies between 4000 – 625 cm^{-1}.

Theory of IR absorption

Infra red (IR) spectroscopy deals with the interaction between a molecule and radiation from the IR region of the EM spectrum (IR region = 4000 - 400 cm^{-1}). IR radiation causes the excitation of the vibrations of covalent bonds within that molecule. Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the frequency of the bond or group that vibrates.

In general terms it is convenient to split an IR spectrum into two approximate regions:

- 4000-1000 cm^{-1} known as the **functional group region**, and
- < 1000 cm^{-1} known as the **fingerprint region**

There are three main processes by which a molecule can absorb radiation and each of these routes involves an increase of energy that is proportional to the light absorbed. The first route occurs when absorption of radiation leads to a higher rotational energy level in a rotational transition. The second route is a vibrational transition which occurs on absorption of quantized energy. This leads to an increased vibrational energy level. The third route involves electrons of molecules being raised to higher electron energy, which is the electronic transition. It's important to state that the energy is quantized and absorption of radiation causes a molecule to move to a higher internal energy level. This is achieved by the alternating electric field of the radiation interacting with the molecule and causing a change in the movement of the molecule. There are multiple possibilities for the different possible energy levels for the various types of transitions.

The energy levels can be rated in the following order: electronic > vibrational > rotational. Each of these transitions differs by an order of magnitude. Rotational transitions occur at lower energies (longer wavelengths) and this energy is insufficient and cannot cause vibrational and electronic transition but vibrational (near infra-red) and electronic transitions (ultraviolet region of the electromagnetic spectrum) require higher energies.

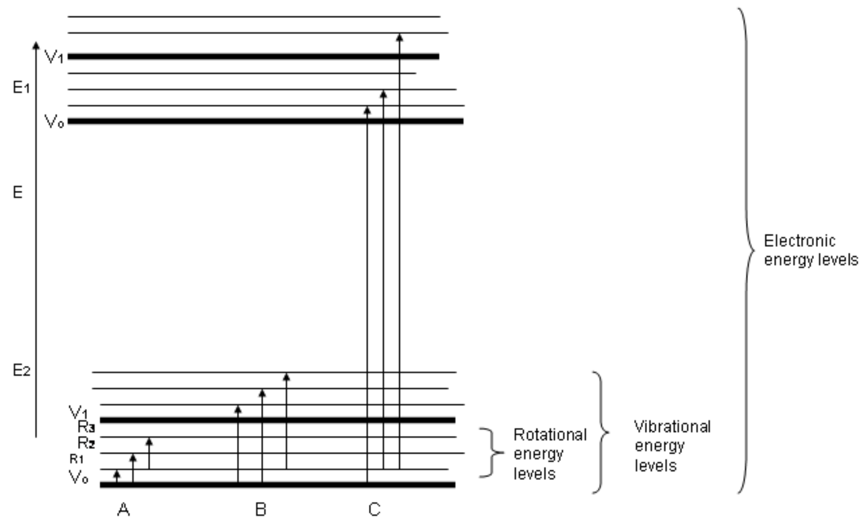


Figure: Energy levels for a molecule. Possible transitions that occur: (A): Pure rotational Transitions, (B) rotational-Vibrational Transitions, (C) Rotational-Vibrational-Electronic Transitions

The energy of IR radiation is weaker than that of visible and ultraviolet radiation, and so the type of radiation produced is different. Absorption of IR radiation is typical of molecular species that have a small energy difference between the rotational and vibrational states. A criterion for IR absorption is a net change in dipole moment in a molecule as it vibrates or rotates. Using the molecule HBr as an example, the charge distribution between hydrogen and bromine is not evenly distributed since bromine is more electronegative than hydrogen and has a higher electron density. HBr thus has a large dipole moment and is thus polar. The dipole moment is determined by the magnitude of the charge difference and the distance between the two centers of charge. As the molecule vibrates, there is a fluctuation in its dipole moment; this causes a field that interacts with the electric field associated with radiation. If there is a match in frequency of the radiation and the natural vibration of the molecule, absorption occurs and this alters the amplitude of the molecular vibration. This also occurs when the rotation of asymmetric molecules around their centers results in a dipole moment change, which permits interaction with the radiation field.

Molecules such as O_2, N_2, Br_2, do not have a changing dipole moment (amplitude nor orientation) when they undergo rotational and vibrational motions, as a result, they cannot cannot absorb IR radiation.

MODES OF MOLECULAR VIBRATIONS

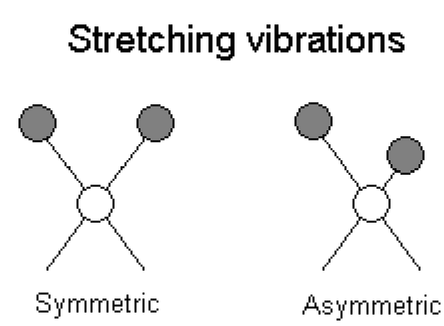
The positions of atoms in molecules are not fixed; they are subject to a number of different vibrations. Vibrations fall into the following two main categories:

a) **STRECTCHING VIBRATIONS**

Stretching vibrations involve the change in inter-atomic distance along bond axis and no change in angles takes place. Stretching vibrations are further divided in to two types:

Symmetric Stretching vibrations are those in which atoms go away from the central atom and come back to central atom at the same time.

Asymmetric Stretching vibrations are those in which if one atom goes away from the central atom then the other atom comes back to central atom at the same time.



b) **BENDING VIBRATIONS**

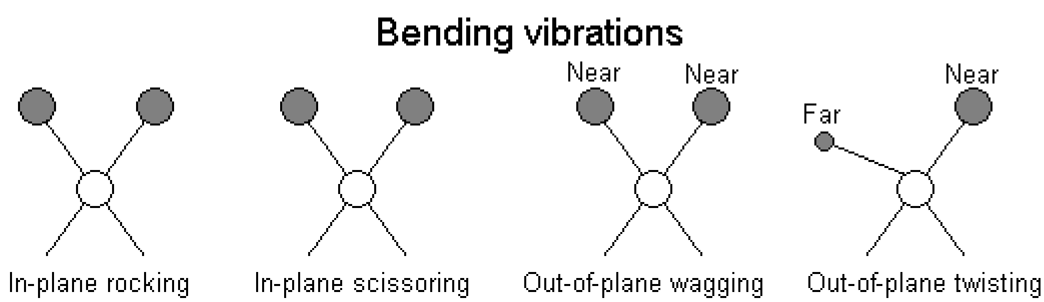
In bending vibrations the change in angles, between two atoms, takes place but no change in inter-atomic distance is involved. Bending vibrations are divided in to two types:

In-Plane bending vibrations are those in which only two planes are involved and have the following two types:

> ***Scissoring Vibrations*** are those in which two bonds come close to one another and then go away from one another just like scissor.
>
> ***Rocking Vibrations*** are those in which both the bonds move in one direction and then go back simultaneously.

Out of plane bending vibrations are those in which atoms also involve third plane during vibration and have the following two types:

> ***Wagging vibrations*** are those in which both the bonds come out of plane and go back of the plane simultaneously.
>
> ***Twisting vibrations*** are those in which if one bond comes out of plane the other goes back of the plane and vice versa.



Vibrational frequencies for stretching bonds in molecules are related to the strength of the chemical bonds and the masses of the atoms.

As stated earlier, molecular vibrations consist of stretching and bending modes. A molecule consisting of (N) number of atoms has a total of 3N degrees of freedom, corresponding to the Cartesian coordinates of each atom in the molecule. In a non-linear molecule, 3 of these degrees of freedom are rotational, 3 are translational and the remainder is fundamental vibrations. In a linear molecule, there are 3 translational degrees of freedom and 2 are rotational. This is because in a linear molecule, all of the atoms lie on a single straight line and hence rotation about the bond axis is not possible. Mathematically the normal modes for a linear and non linear can be expressed as

Linear Molecules: (3N - 5) degrees of freedom

Non-Linear molecules: (3N - 6) degrees of freedom

Some General Trends:

- Stretching frequencies are higher than corresponding bending frequencies (it is easier to bend a bond than to stretch or compress it.)
- Bonds to hydrogen have higher stretching frequencies than those to heavier atoms.
- Triple bonds have higher stretching frequencies than corresponding double bonds, which in turn have higher frequencies than single bonds.

Absorption bands in the 4000 to 1450 cm^{-1} region are usually due to stretching vibrations of diatomic units, and this is sometimes called the **group frequency** region.

Since most organic compounds have C-H bonds, a useful rule is that absorption in the 2850 to 3000 cm^{-1} is due to sp^3 C-H stretching; whereas, absorption above 3000 cm^{-1} is from sp^2 C-H stretching or sp C-H stretching if it is near 3300 cm^{-1}.

Typical Infrared Absorption Frequencies

	Stretching Vibrations			Bending Vibrations		
Functional Group/ Class	**Range (cm^{-1})**	**Intensity**	**Assignment**	**Range (cm^{-1})**	**Intensity**	**Assignment**
Alkanes	2850-3000	Str	CH_3, CH_2 & CH 2 or 3 bands	1350-1470 1370-1390	Med Med	CH_2 & CH_3 deformation CH_3 deformation

				720-725	Wk	CH_2 rocking
Alkenes	3020-3100 1630-1680 1900-2000	Med Var Str	$=C-H$ & $=CH_2$ (usually sharp) C=C (symmetry reduces intensity) C=C asymmetric stretch	880-995 780-850 675-730	Str Med Med	$=C-H$ & $=CH_2$ (out-of-plane bending) cis-RCH=CHR
Alkynes	3300 2100-2250	Str Var	C-H (usually sharp) C≡C (symmetry reduces intensity)	600-700	Str	C-H deformation
Arenes	3030 1600 & 1500	Var Med-wk	C-H (may be several bands) C=C (in ring) (2 bands) (3 if conjugated)	690-900	Str-med	C-H bending & ring puckering
Alcohols & Phenols	3580-3650 3200-3550 970-1250	Var Str Str	O-H (free), usually sharp O-H (H-bonded), usually broad C-O	1330-1430 650-770	Med Var-wk	O-H bending (in-plane) O-H bend (out-of-plane)
Amines	3400-3500 (dil. soln.) 3300-3400 (dil. soln.) 1000-1250	Wk Wk Med	N-H (1°-amines), 2 bands N-H (2°-amines) C-N	1550-1650 660-900	Med-str Var	NH_2 scissoring (1°-amines) NH_2 & N-H wagging (shifts on H-bonding)
Aldehydes & Ketones	2690-2840 (2 bands) 1720-1740 1710-1720 1690 1675 1745 1780	Med Str Str Str Str Str Str	C-H (aldehyde C-H) C=O (saturated aldehyde) C=O (saturated ketone) aryl ketone α, β-unsaturation cyclopentanone cyclobutanone	1350-1360 1400-1450 1100	Str Str Med	α-CH_3 bending α-CH_2 bending C-C-C bending
Carboxylic Acids & Derivatives	2500-3300 (acids) overlap C-H 1705-1720	Str Str	O-H (very broad) C=O (H-bonded)	1395-1440	Med	C-O-H bending

	(acids) 1210-1320 (acids) 1785-1815 (acyl halides) 1750 & 1820 (anhydride) 1040-1100 1735-1750 (esters) 1000-1300 1630-1695 (amides)	 Med-str Str Str Str Str Str Str	 O-C (sometimes 2-peaks) C=O C=O (2-bands) O-C C=O O-C (2-bands) C=O (amide I band)	1590-1650 1500-1560	Med Med	N-H (1°-amide) II band N-H (2°-amide) II band
Nitriles **Isocyanates, Isothiocyanates, Diimides, Azides & Ketenes**	2240-2260 2100-2270	Med Med	C≡N (sharp) -N=C=O, -N=C=S -N=C=N-, $-N_3$, C=C=O			

The measurement of the amount of light absorbed as a function of the wavenumber or frequency generates a spectrum.

Sample preparation for IR spectroscopy

Gaseous samples require a sample cell with a long path-length (typically 5–10 cm), to compensate for the diluteness.

Liquid samples can be sandwiched between two plates of a salt (commonly sodium chloride, or common salt, although a number of other salts such as potassium bromide or cesium iodide also used). The plates are transparent to the infrared light and do not introduce any lines onto the spectra.

Solid samples can be prepared in a variety of ways.

One common method is to crush the sample with an oily mulling agent (usually Nujol) in a marble or agate mortar, with a pestle. A thin film of the mull is smeared onto salt plates and measured.

The second method is to grind a quantity of the sample with a specially purified salt (usually potassium bromide) finely. This powder mixture is then pressed in a mechanical press to form a translucent pellet through which the beam of the spectrometer can pass.

A third technique is the "cast film" technique, which is used mainly for polymeric materials. The sample is first dissolved in a suitable, non hygroscopic solvent. A drop of this solution is deposited on surface of KBr or NaCl cell. The solution is then evaporated to dryness and the film formed on the cell is analyzed directly. Care is important to ensure that the film is not too thick otherwise light cannot pass through. This technique is suitable for qualitative analysis.

It is important to note that spectra obtained from different sample preparation methods will look slightly different from each other due to differences in the samples' physical states.

Instrumentation of IR spectrometer

Development of IR Spectrometers

Up till FTIR spectrometers, there have been three generations of IR spectrometers.

The *first generation* IR spectrometer was invented in late 1950s. It utilizes prism optical splitting system. The prisms are made of NaCl. The requirement of the sample's water content and particle size is extremely strict. Further more, the scan range is narrow.

The *second generation* IR spectrometer was introduced to the world in 1960s. It utilizes gratings as the monochrometer. But low sensitivity, low scan speed and poor wavelength accuracy rendered it out of date.

The invention of the *third generation* IR spectrometer, Fourier transform infrared spectrometer, marked the abdication of monochrometer and the prosperity of interferometer. With this replacement, IR spectrometers became exceptionally powerful.

Dispersive IR Spectrometers

Generally, dispersive spectrometers have a double-beam design with two equivalent beams from the same source passing through the sample and reference chambers as independent beams. These reference and sample beams are alternately focused on the detector by making use of an optical chopper, such as, a sector mirror. One beam will proceed, traveling through the sample, while the other beam will pass through a reference species for analytical comparison of transmitted photon wavefront information.

The basic components of a dispersive IR spectrometer include a radiation source, monochromator, and detector.

Radiation sources

The common IR radiation sources are inert solids that are heated electrically to promote thermal emission of radiation in the infrared region of the electromagnetic spectrum.

The Nernst glower

The Nernst glower is constructed of rare earth oxides in the form of a hollow cylinder. Platinum leads at the ends of the cylinder permit the passage of electricity. Nernst glowers are fragile. They have a large negative temperature coefficient of electrical resistance and must be preheated to be conductive. The Nernst glower can reach temperatures of 2200 K.

The globar source

A globar is a rod of silicon carbide (5 mm diameter, 50 mm long) which is electrically heated to about 1,500 K. Water cooling of the electrical contacts is needed to prevent arcing.

Chopper

The two beams are reflected to a chopper which is rotating at a speed of 10 rotations per second. This chopper makes the reference and the sample beam to fall on the monochromator grating alternately.

Monochromator

The monochromator is a device used to disperse or separate a broad spectrum of IR radiation into individual narrow IR frequencies. After the incident radiation travels through the sample species, the emitted wavefront of radiation is dispersed by a monochromator (gratings and slits) into its component frequencies. A combination of prisms or gratings with variable-slit mechanisms, mirrors, and filters comprise the dispersive system.

Detectors

Detectors are devices that convert the analog spectral output into an electrical signal. These electrical signals are further processed by the computer using mathematical algorithm to arrive at the final spectrum. The detectors used in IR spectrometers can be classified as either photon/quantum detectors or thermal detectors.

Thermocouple

A thermocouple consists of a pair of junctions of different metals; for example, two pieces of bismuth fused to either end of a piece of antimony. The potential difference (voltage) between the junctions changes according to the difference in temperature between the junctions.

Pyroelectric detectors

Pyroelectric detectors consists of a pyroelectric material which is an insulator with special thermal and electric properties. Triglycine sulphate is the most common material for pyroelectric infrared detectors. Unlike other thermal detectors the pyroelectric effect depends on the rate of change of the detector temperature rather than on the temperature itself. This allows the pyroelectric detector to operate with a much faster response time and makes these detectors the choice for Fourier transform spectrometers where rapid response is essential.

Photoconducting detectors

Photoconducting detectors are the most sensitive detectors. They rely on interactions between photons and a semiconductor. The detector consists of a thin film of a semiconductor material such as lead sulphide, mercury cadmium telluride or indium antimonide deposited on a nonconducting glass surface and sealed into an evacuated envelope to protect the semiconductor from the atmosphere. The lead sulphide detector is used for the near-infrared region of the spectrum. For mid- and far-infrared radiation the mercury cadmium telluride detector is used. It must be cooled with liquid nitrogen to minimize disturbances.

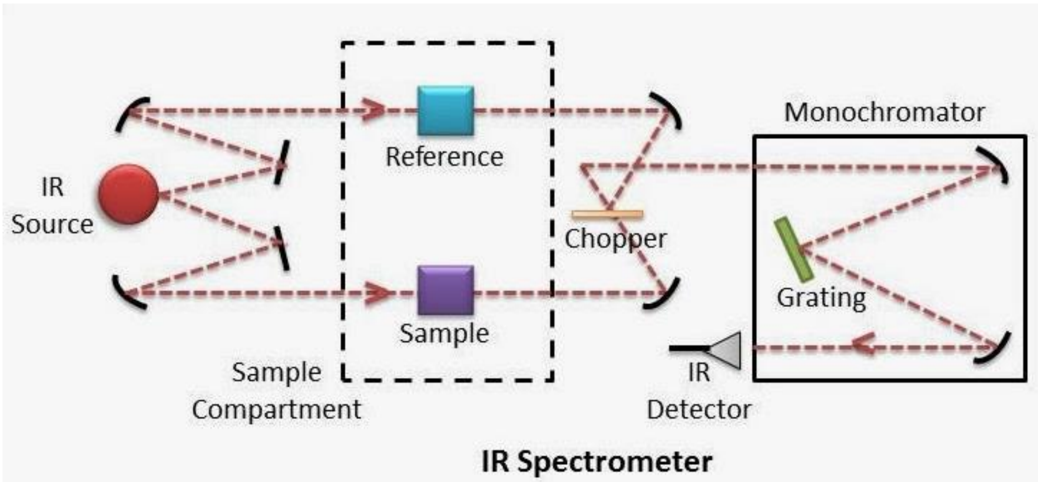


Figure. Simplified representation of a dispersive IR spectrometer.

FTIR Spectrometers

The Components of FTIR Spectrometers

A common FTIR spectrometer consists of a source, interferometer, sample compartment, detector, amplifier, A/D convertor, and a computer. The source generates radiation which passes the sample through the interferometer and reaches the detector. Then the signal is amplified and converted to digital signal by the amplifier and analog-to-digital converter, respectively. Eventually, the signal is transferred to a computer in which Fourier transform is carried out.

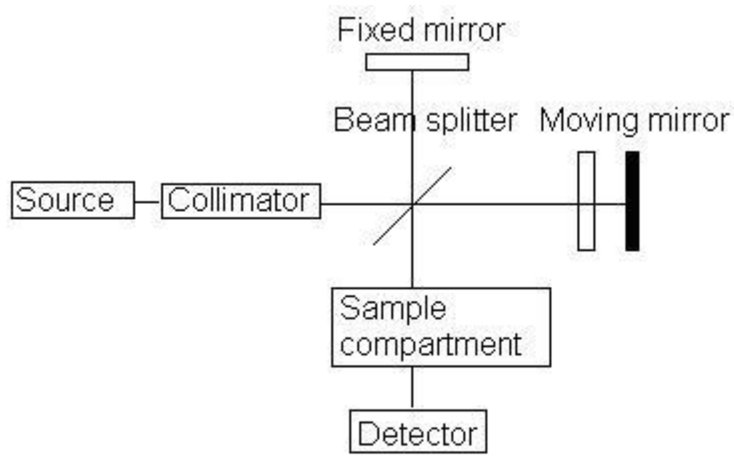


Figure. Block diagram of an FTIR spectrometer

The major difference between an FTIR spectrometer and a dispersive IR spectrometer is the Michelson interferometer.

Michelson Interferometer

The Michelson interferometer, which is the core of FTIR spectrometers, is used to split one beam of light into two so that the paths of the two beams are different. Then the Michelson interferometer recombines the two beams and conducts them into the detector where the difference of the intensity of these two beams is measured as a function of the difference of the paths. **Figure 3** is a schematic of the Michelson Interferometer.

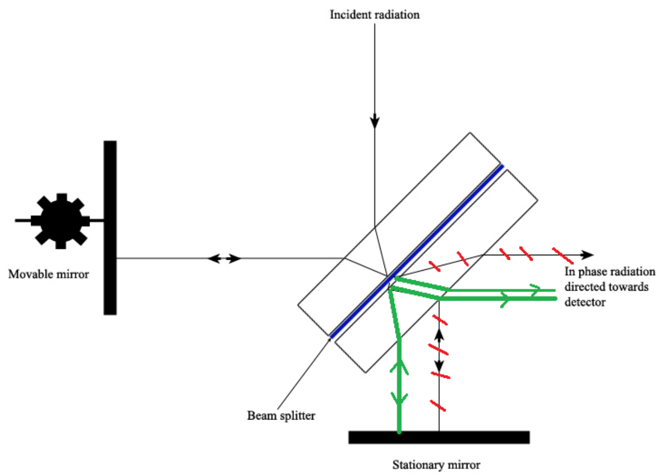


Figure. *Schematic of the Michelson interferometer*

A typical Michelson interferometer consists of two perpendicular mirrors and a beamsplitter. One of the mirror is a stationary mirror and another one is a movable mirror. The beamsplitter is designed to transmit half of the light and reflect half of the light. Subsequently, the transmitted light and the reflected light strike the stationary mirror and the movable mirror, respectively. When reflected back by the mirrors, two beams of light recombine with each other at the beamsplitter.

If the distances travelled by two beams are the same which means the distances between two mirrors and the beamsplitter are the same, the situation is defined as zero path difference (ZPD). But imagine if the movable mirror moves away from the beamsplitter, the light beam which strikes the movable mirror will travel a longer distance than the light beam which strikes the stationary mirror. The distance which the movable mirror is away from the ZPD is defined as the mirror displacement. The extra distance is defined as the optical path difference (OPD).

It is well established that when OPD is the multiples of the wavelength, constructive interference occurs because crests overlap with crests, troughs with troughs. As a result, a maximum intensity signal is observed by the detector. In contrast, when OPD is the half wavelength, destructive interference occurs because crests overlap with troughs. Consequently, a minimum intensity signal is observed by the detector.

Pharmaceutical applications of IR spectroscopy

- ✓ Infrared spectroscopy is widely used in both research and industry as a simple and reliable technique for measurement, quality control and dynamic measurement.
- ✓ It is also used in forensic analysis in both criminal and civil cases, enabling identification of polymer degradation for example.
- ✓ By measuring at a specific frequency over time, changes in the character or quantity of a particular bond can be measured. This is especially useful in measuring the degree of polymerization in polymer manufacture.
- ✓ IR spectroscopy is also used to monitor the drug synthesis. This can be done by monitoring the progress of reactions.
- ✓ Infrared spectroscopy has been highly successful for applications in both organic and pharmaceutical chemistry.
- ✓ The infrared spectrum of a molecule is a useful chemical tool. It can be used to determine the types of functional groups in a molecule and intensity of the peaks provide analytical measure of the amount of substance present.
- ✓ Further, the position of the peaks gives information about the vibrational frequencies and the intensity of the peaks gives information about the symmetry of the vibrations. All of this information is extremely useful in characterizing drug molecules.
- ✓ Because the IR spectrum of each molecule is unique, it can serve as a signature or fingerprint to identify the molecule. This feature, along with the fact that it is a non-destructive technique, have made infrared spectroscopy a valuable method in chemical analysis.
- ✓ Areas in which it is used extensively include pharmaceutical analysis, quality control in industrial processes, environmental chemistry, geology and astronomy.

- ✓ By comparing the spectrum of the sample under study, with library of spectra of known compounds, identity and purity of sample can easily be established. This procedure is common in environmental and forensic analyses.
- ✓ IR spectroscopy is a non-destructive method for the analysis of cells, tissues and fluids that has been used to detect different diseases and stages of malignancy in infected prostate, cervical and colon tissues. But methods to process the cells before analysis are not standard throughout different laboratories, which can make the results difficult to interpret by non-specialists in hospitals.