CHAPTER - 2

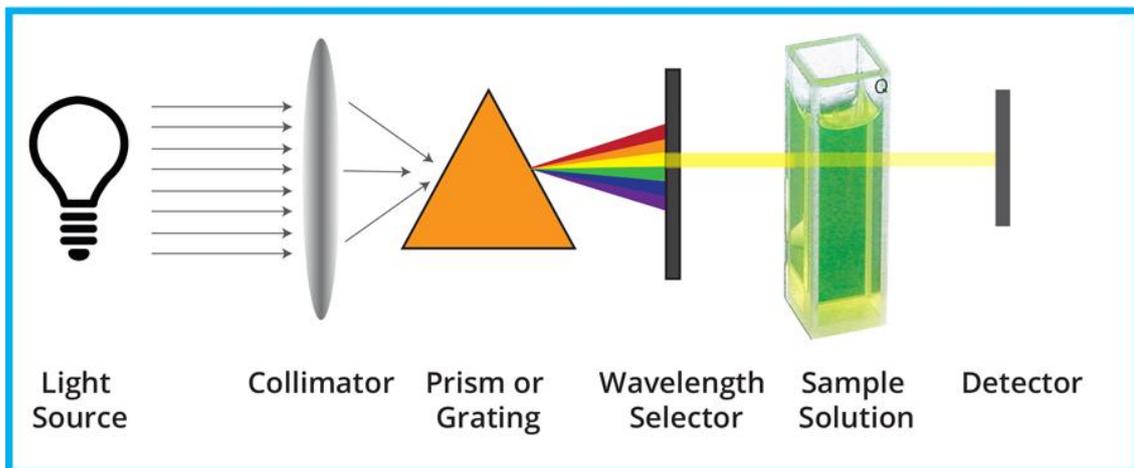Light Source — Collimator — Prism or Grating — Wavelength Selector — Sample Solution — Detector

INSTRUMENTATION AND SAMPLE HANDLING TECHNIQUES IN FT-IR, FT-RAMAN, UV-Vis AND NMR SPECTROSCOPY

CHAPTER - 2

2.1 INTRODUCTION

The infrared (IR) and Raman spectroscopy generally yield similar types of information. IR and Raman spectroscopic techniques from its early beginning continues to be a complementary fertile research tool in the hands of physicists and chemists. Often it is necessary to use several instrumental techniques to obtain the information need to solve an analytical problem. Analytical instrumentation plays an important role in the production and evaluation of new products.In recent years, Fourier transform (FT) spectroscopy techniques enjoy the advantages over the conventional IR and Raman spectroscopy due to the intensive interest created by offering some unique features.

The ultimate performance of infrared spectrometer is determined by measuring its signal-to-noise ratio (SNR). This is best achieved by FT-IR spectrometer.The improved SNRs available with FT-IR make it to become the instrument of choice for obtaining IR spectra. Frequency precision, best spectral subtractions and high resolution are little difficult to achieve with conventional Raman spectroscopy. Hence a new technique, in which the Raman module is an accessory to an FT-IR spectrometer, called FT-Raman spectroscopy. FT-IR and FT-Raman techniques have been profound to be helpful in elevating the spectral sensitivity and thus yielding novel information about the atomic or molecular species, or the functional group that is present in the sample.

Advanced spectrometers are generally attached with sophisticated computers and elevated energy sources like lasers, which approve spectrum storage and retrieval, repetitive scanning, scale expansion, spectral comparison, automatic control of slit and spectral simulation etc… These are below the control of a microprocessor and microcomputers. Accessories such as reflectance units, beam condensers, microcells and polarizers can normally be added to extend versatility or accuracy. In this chapter the instrumentation and sample handling techniques of FT-IR and FT-Raman spectrometers are presented.

The IR and Raman spectroscopic techniques are based on various principles. The intensity of the infrared spectrum depends on the magnitude of the dipole moment change where as the Raman intensity is dependent on the polarizability of the vibrating atom and their bonds. Hence the appearance of Raman band normally differs from the corresponding IR band. However the position the bands are usually in good agreement. The IR and Raman methods are used to study the materials existing in various states. The molecular vibrational frequencies obtained by these methods have been used in molecular mechanics and they yield considerable information about the interatomic forces in various molecules (Sindhu, 1985).

2.2 FOURIER TRANSFORM INFRARED SPECTROMETER

The discovery of the fast Fourier transform algorithm and the synchronized introduction of sophisticated computers allowed new generation of infrared instrumentation, known as Fourier transform infrared spectrometer. The Fourier transform infrared spectrographs have replaced the conventional infrared spectrographs. A FT-IR spectrometer consists of two parts: (a) an optical system which uses an interferometer and (b) a dedicated computer which stores data, performs computations on data and plots the spectra. A schematic diagram of the essential components of a FT spectrometer based on Michelson interferometer is shown in Figure 2.1. It consists of two perpendicular mirrors; one of which is a stationary mirror and other a movable mirror which can be displaced perpendicularly to the fixed mirror at a constant velocity. Between these two mirrors, the beam splitter is set at 45° from the original position of the movable mirror. A parallel beam of radiation from an infrared source is moved to the mirrors through the beam splitter. The beam splitter reflects about half of the beam to the permanent mirror which reflects it back to the beam splitter and transmits the other half to the changeable mirror that reflects it reverse to the beam splitter. The returning beams are split again and mixed about half going back to source and half passing through the sample compartment. The masterpiece of the beam splitter depends on the specific spectral region. For example, a beam splitter of germanium coated on KBr plate (substrate) is used in the mid-infrared region (4000–400 cm^{-1}),. Germanium reflects the radiation while KBr transmits most of the pleasing radiation. In the far

infrared region, germanium coated on CSI (800–200 cm^{-1}) or germanium coated on Mylar (polyethylene terephthalete) (650–10 cm^{-1}) are utilized as beam splitters. A thin ray of film of the beam splitter material is covered on an optically flat substrate.

The return beams from both the mirrors along the identical path length as their incident path are recombined into a single beam at the beam splitter. The path length of one of the return beams is altered in order to form phase difference to cause an interference pattern. The recombined radiation is then directed through the sample and focused on to the detector. The detector calculates the quantity of energy at distinct intervals of the movement of mirror.

The movable mirror can be moved in a range of about ± 5 cm. The mirror velocities from 5 to 0.05cm^{-1} are used. Interferometer instruments require detectors with response times short enough to detect and transmit rapid changes to the recorder. The detector involved in grouping with fast scanning interferometers in the mid-infrared region at optimum room temperature is triglycine sulfate with KBr windows as pyroelectric bolometer. It has a high response time. Other most general detectors used includes - thermocouples, bolometers and Golay detectors have minimum response time. The design of the Michelson interferometer is to create measurement in any infrared region possible by simply altering the beam splitter and the detector.
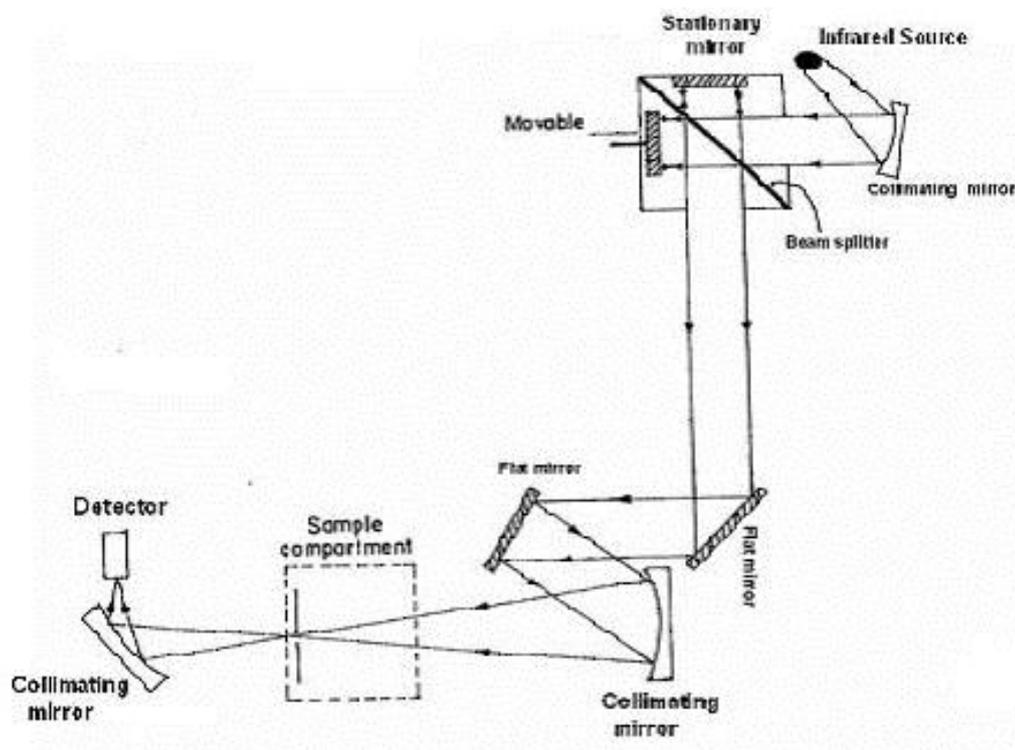
Figure 2.1 Block diagram of FT-IR spectrometer

2.2.1 Instrumentation

(a) Introduction

The basic components of an infrared spectrometer are shown in Figure 2.2. A source supplies radiation over the complete range of the infrared spectrum. The monochromator disperses the light and then selects a narrow wave number range. The detector actions the energy and converts it into an electric signal. This signal is enlarged additionally and registered by the recorder.

With recent improvements in instrumentation, the infrared region of the electromagnetic spectrum is now considered to cover the range from approximately 12500 - 10 cm^{-1} (1000 - 0.8μm). It is commonly subcategorized into three sections:mid infrared region(4000 – 400 cm^{-1}), near infrared (12500–4000 cm^{-1}) and the far infrared

(400–10 cm^{-1}). The mid infrared region is the one of the most widely used standard laboratory investigation as it coats almost all the vibrational and rotational transitions. The FT-IR spectra of most of the samples were recorded in mid and far IR regions and are used for the present study (Skoog., *et al.,* 1996; Willard *et al.,* 1996; Khandpur., 2002; Rubinson., 2000).
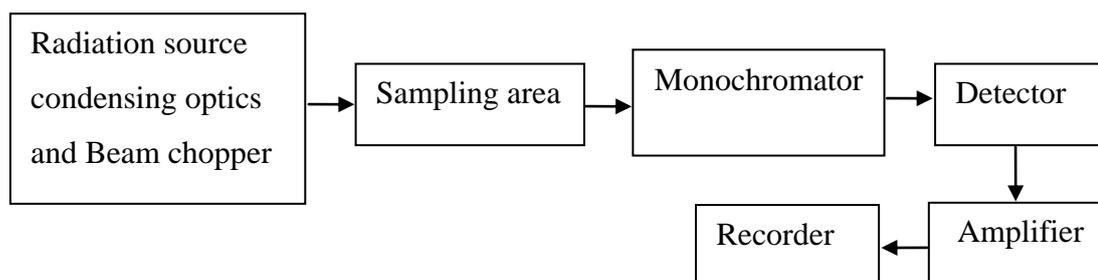

Figure 2.2 Schematic representation of the major components of a dispersive FT-IR spectrometer

(b) Source

Infrared radiation is produced by electrically heating a source, usually a Nernst filament or a Globar to 1800–1000$^{°}$C. The Nernst filament is made-up from a binder and oxides of thorium, cerium, zirconium and yttrium. The Globar is a small thin rod of silicon carbide commonly 5cm in length and 0.5 cm in diameter. The greatest radiation for the Globar takes place in the 5500–5000cm^{-1} region. Carbon arc, Nichrome wire, rhodium wire and tungsten filament lamp are used as a light source.

In a commercial infrared spectrometer either a nichrome wire or a platinum filament contained in a ceramic tube is generally used as infrared source for the range 4000-400 cm^{-1}.

(c) Monochromator

A monochromator is a way of untying wavelengths of the source radiation. The monochromator is used to separate polychromatic radiation into a suitable

monochromatic form (Rubinson, 2000). This is achieved by means of prisms or diffraction grating. Materials for prism construction, which are present most suitably in the infrared region are: Glass-SiO_2 [300 mμ to 2μ (5000 cm^{-1})], Quartz [800 mμ to 3μ (12500 to 3300 cm^{-1})], Lithium fluoride [(600 mμ to 6μ (1670 cm^{-1})], Calcium fluoride [(200 mμ to 9μ (1100 cm^{-1})], Cesium iodide-CsI [(38 to 10μ(260 cm^{-1})]. Sodium chloride-NaCl [(200 mμ to 14.5μ (625 cm^{-1})] and Potassium bromide-KBr [(25 to 10μ (400 cm^{-1})] and Cesium iodide-CsI [(38 to 10μ (260cm^{-1})].

An ideal prism instrument would consist of huge number of prisms that are made from different optical materials, so that each could be utilized in sequence in its effective region. A high resolution prism instrument consists of grouping of SiO_2, NaCl and KBr prisms. A Low-cost instrument uses a NaCl prism over the full range. They donate maximum resolution in the essential finger–print region.

A monochromator thus carries out three functions:

(i) It disperses the radiation in accordance to its wave number mechanism (ii) It restricts the radiation falling on the detector into a narrow wave number range, and (iii) It maintains the energy incident on the detector to just about constant level when no sample is present throughout the wave number range of the instrument. Some instruments uses a double monochromator ie., the exit slit of the first monochromator that serves as the entrance slit for the second monochromator. As a result, the spectrum obtained with spectrophotometers having double monochromator have higher resolution.

(d) Detectors

Detectors used in infrared spectrophotometers that generally transform the thermal radiant energy into electrical energy, which can then be plotted on a chart recorder. There are two types of the detectors are generally used:

i.   Thermal detector
ii.   Photon detector

1. Thermal Detector

Thermal Detector, in which the infrared radiation forms a heating effect that changes some physical property of the detector.

The detector mostly forms an electrical signal which is proportional to the intensity of the incident radiation over the whole spectral range of the instrument. The most advantageous features of the detectors are the nearness with which they move toward the behaviour of a black body, high sensitivity, high speed and robustness.

The infrared detectors may be selective or non-selective. The selective detectors are those whose response is noticeably reliant upon the wavelength of the occurrence of radiation. Few such examples of this type are photographic plates, photocells, photoconductive cells and infrared phosphors. The non-selective detectors are those whose response is directly proportional to incident energy but comparatively not dependent of wavelength. Common examples include thermocouples, bolometers and pneumatic cell. Detectors that are recently used are fabricated from crystals are termed as pyroelectrics and take very less time than other thermal detector. Hence with these crystals, radiation can be chopped at a higher rate. However, these are highly expensive and not extensively employed.

2. Photon Detector

Photon Detector, which utilize the quantum effects of the infrared radiation to alter the electrical properties of a semiconductor.

The more reliable infrared detectors implies on a quantum interaction between the incident photons and a semiconductor - the result producing electrons. This is the internal photoeffect. A satisfactorily energetic photon that hits an electron in the detector can elevate that electron from a non-conducting state to a conducting state. As conductors, electrons tend to contribute to the current flow in one of two ways, depending on the pattern of the semiconductor. These are called as photoconductive and photovoltaic cells.

Photoconductive cells most widely used are indium antimonide (InSb) or Hg-Cd-Te (MCT), which have high sensitivity and very fast response time in infrared detection. These detectors operate at liquid nitrogen and temperature which reduces noise. The radiant energy falling on the detector is transformed into measurable electrical energy.

Photovoltaic cellsare basically electrical resistors, which lowers its resistance in relation to the intensity of light striking their surface and are characterized by greater sensitivity and rapidity of response. They are constructed from a thin layer (0.1μ) of semiconductor such as lead sulphide and lead tin telluride. These detectors extend spectral sensitivity up to 6 and 3.5 μ respectively (Khandpur., 2002).

(e) Amplifiers and Recorders

The radiant energy perceived by the detector is transformed into measurable electrical signal and is augmented by the amplifiers. The amplified signal is registered by a recorder or a plotter. The recorder is determined with a speed which is synchronized with that of a monochromator, so that, the pen moving across the chart, records the transmittance of the sample as a function of the wavenumber.

(f) Beam Splitter

Beam splitters are built by transparent materials with refractive indices such that approximately 50% of the radiation is reflected and 50% is transmitted. Most commonly used material for the far-infrared region is made of a thin film of mylar sandwiched between two plates of a low refractive index solid. Thin films of germanium or silicon deposited on cesium iodide or bromide, sodium chloride, or potassium bromide are acceptable for the mid-infrared region.

2.3 SAMPLE HANDLING TECHNIQUES IN FT-IR SPECTROSCOPY

Sample handling is found to be an essential technique in infrared spectroscopy. There are different methods of sample preparation to enable almost any type of sample to be examined.

Some momentous problems arise when trying to build sample containers for vibrational spectrometry, because each and every material has some vibrational absorption. The material that has a low interference in the regions of interest is utilized as sample. The choice of material for IR spectroscopy is of solid potassium bromide plate. Such plates are formed in various ways. Polyethylene pellets are used for recording the IR spectra from a far region.

(a) Solids

Solids are sampled in a diverse variety of routes. If the sample is miscible, it may be dissolved and handled as for a liquid. Solid samples for which no solvent is suitable can be used for analysis by incorporating them into a pressed pellet of alkali halide, normally as potassium bromide. Sample is mixed with a weighed quantity of powdered potassium bromide and the mixture is admitted to a pressure of numerous tonnes in a dye, to make a high crystal clear plate or disc which can be inserted into the spectrophotometer.

The use of KBr removes the problems of additional bands due to mulling agent. KBr does not absorb infrared light in the region 15-2.5 μm and an entire spectrum of the sample is obtained.

Solid samples have also been investigated in the form of a thin layer deposited by sublimation or solvent evaporation on the surface of a salt plate. Other method, namely mulling has also been developed, in which the powdered sample is mixed to form a paste with small amount of heavy paraffin oil. The mull is sandwiched between the plates containing salt for calibration. Mulls are formed by grinding 5 to 2mg of finely powered sample in the presence of one or two drops of a heavy hydrocarbon oil

called Nujol (Skoog *et al*., 1996; Willard *et al*., 1996; Khandpur., 2002; Rubinson., 2000).

(b) Liquids

In most instances, the spectra of liquids are quantified in either a demountable type of cell or in a fixed thickness or sealed cells. The spectra of pure samples can be calculated as very thin films squeezed between two alkali halide windows of a demountable cell. This technique can able to form a film of thickness 0.01 mm or less. This procedure is more functional for qualitative work only because the sample thickness cannot be controlled by its own. Liquid cells contain a two alkali halide windows normally NaCl or KBr, estranged by a spacer of specific thickness made of Teflon or lead which confines the volume of the cell (Skoog *et al*., 1996; Willard *et al*., 1996; Khandpur., 2002; Rubinson., 2000).

(c) Gases

Absorption spectra of gases can be analyzed in a wide variety of gas cells ranging from a few centimeters to several meters that can be placed directly in the path of the infrared beam. The terminal walls of the cell are commonly made of sodium chloride which is transparent to infrared. The low frequency vibrational alterations in the gaseous phase often split the high frequency vibrational bands (Kaur., 2003).

(d) Solvents

Solvents of good infrared transparency over a convenient frequency range are available and the spectra of the sample dissolved in carbon tetrachloride and carbon disulphide supplies an entire range. Chloroform is reported to be a significant solvent and is habitually used because it shows absorptions though it is less symmetric molecule than carbon tetrachloride and carbon disulphide.

2.4 ADVANTAGES OF FT-IR TECHNIQUE

The major advantages of FT-IR spectroscopy are the superior ease and speed of measurement. The whole spectrum can be recorded within a matter of second using sophisticated computers. Recent developments in FT-IR spectrometers have thus, led to higher resolution, higher accuracy in frequency, total wavelength coverage and intensity measurements. It can also be involved in the characterization of all kinds of samples. In FT-IR method, the entire energy source passes through the instrument and the resolving power is constant over the complete spectrum. The signal to noise ratio (SNR) is also enhanced (Banwell., 1994). The smoothening of peaks and the vertical and horizontal expansion of selective region is also promising.

2.5 FT-RAMAN SPECTROSCOPY

The FT-Raman spectroscopy is made complicated with the advent of gas lasers and computers. The advantages of lasers are their high intensity, narrow band width, high monochromaticity, high resolution and coherence. From the time of invention of Raman Effect, both FT-IR and FT-Raman spectra of chemical compounds have been successfully used for the evaluation of molecular structure and also for the quick detection for the presence of any characteristic group in the compound as discussed in the literature reviews (Szymanski., 1969; Fateley., 1971; 1974). In order for a molecular vibration to be Raman active there must be an alteration in the polarizability of the molecule.

A significant merits of Raman spectra over infrared spectra lies in the fact that water does not cause interferences. Certainly, FT-Raman spectra can be found from an aqueous solution. A glass or quartz cells can be in use, thus preventing the difficulty in working with sodium chloride or other atmospherically unstable window materials. FT-Raman spectra are taken up by irradiating a sample with a strong laser source of visible or near-infrared monochromatic radiation. Unique advantage of FT-Raman over conventional dispersive Raman spectroscopy is its capacity to provide spectra that are commonly free of fluorescence interference. FT-Raman produces the wavelength

precision of FT-IR so that spectra may be co-added, resulting in a quick improvement in signal-no-noise ratio (SNR) performance. In this study, the spectral data were recorded on BRUKER IFS 66V, FT-IR with FRA 106FT- Raman spectrophotometer and the block diagram of the instruments is shown in Figure 2.3.
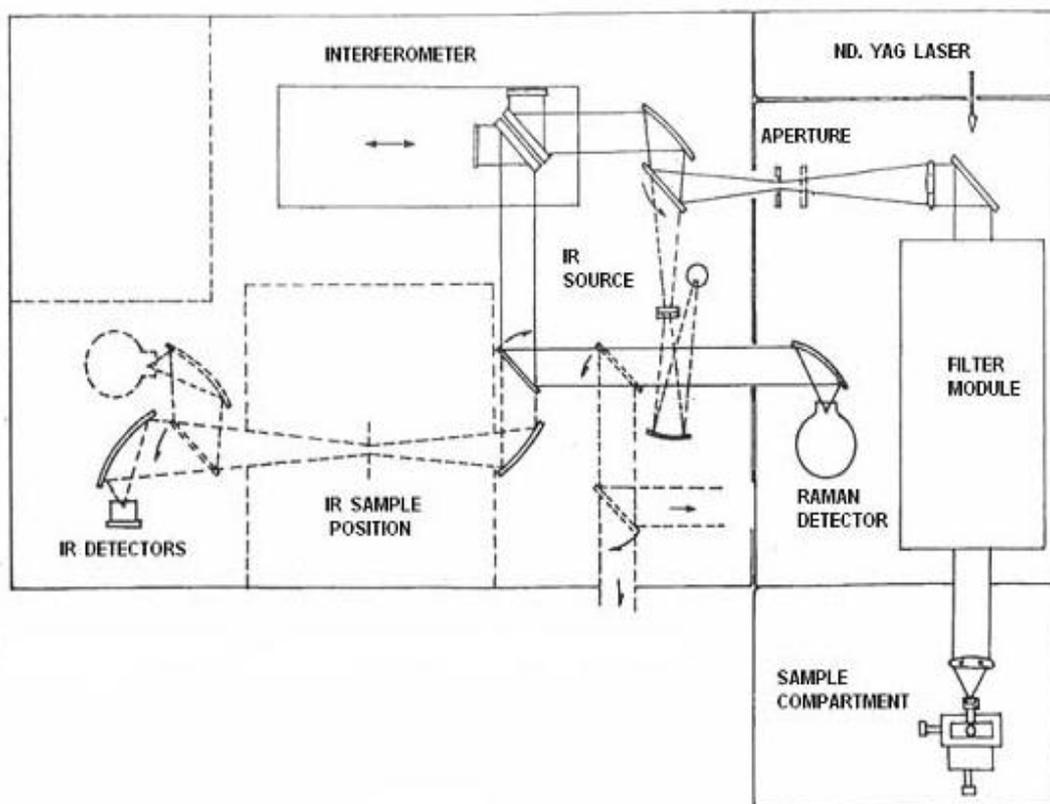

Figure 2.3 Block diagram of FT-Raman spectrometer

2.5.1 Instrumentation

(a) Introduction

In FT-Raman spectrometer, the sample is irradiated with a monochromatic light and the scattered light is found at right angles to the incident radiation of FT- Raman spectrometer which consists of various sources, a sample holder, a monochromator and a detector. Figure 2.4 depicts a schematic representation of a FT-Raman spectrometer.
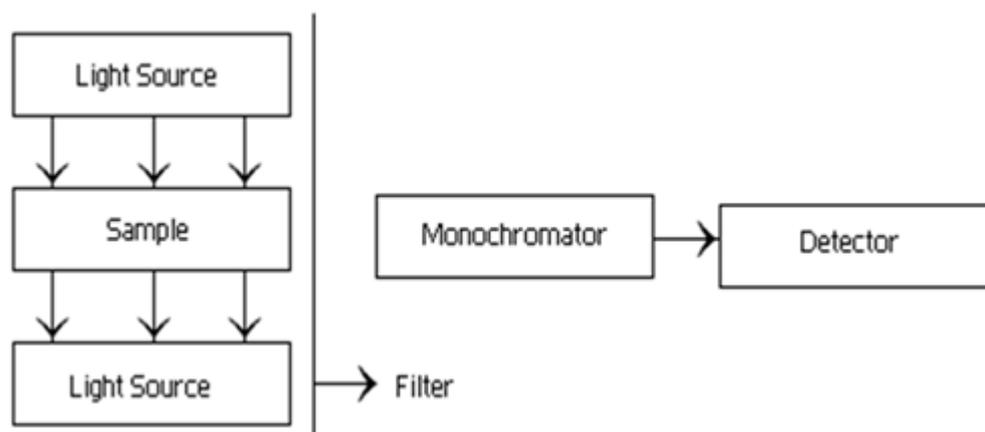
Figure 2.4 Schematic representation of major components of FT-Raman photo spectrometer

(b) Source

The sources are mainly used in modern Raman spectroscopy that are almost lasers because their elevated intensity is required to produce Raman scattering of adequate intensity to be calculated with a reasonable signal to-noise ratio (SNR). Five of the most general lasers along with their wavelength (nm) used for Raman spectroscopy are; Krypton ion (647.1 or 530.9 nm), Argon ion (514.5 or 488 nm), Diode laser (830 or 782 nm), Helium / Neon (632.8 nm) and Nd:YAG (1064 nm). Because of the intensity of Raman scattering, several of the fourth power frequency like, argon and krypton ion sources that emit in the blue and green region of the spectrum have merits over the other sources. Diode and Nd:YAG (Neodymium Yttrium Aluminium Garnet) laser which transmit near-infrared radiation are used as strong excitation sources. Near-infrared sources have two chief advantages over shorter wavelength lasers. The first is that they can be operated at much higher power (upto 50W) without causing photo decomposition of the sample. The second is that they are not energetic enough to colonize a potential number of fluorescence producing excited electronic energy states in most molecules. Subsequently, fluorescence is basically much less intense or non-existent with these lasers. The Nd:YAG line at 1064 nm is specifically effective in removing fluorescence. The two lines of the diode array laser at 830 nm and 782 nm also markedly minimize fluorescence in most cases.

The laser radiation is directed to the sample by means of a lens and a parabolic mirror and the scattered light from the sample is collected and transmitted to a beam splitter and to the moving mirrors. It is then transmitted through a number of dielectric filters and paid attention to a liquid nitrogen cooled detector.

(c) Filter

Liquid filters are placed between the source and the samples tube. Different types of filters are used for various excitation radiations. Filters that are made of quartz glass or nickel oxide glass is used for getting monochromatic radiations. The special functions of filters are-

i. To isolate a single exciting line
ii. To eliminate elevated energy radiation that might cause photo decomposition or fluorescence.
iii. To eliminate the continuous spectrum in the region engaged by the Raman lines.

(d) Monochromator

In the monochromator, both lenses and mirrors have been widely used. Most Raman spectrometers use a diffraction grating as the dispersing element. A grating instrument has a broad aperture and an average dispersion ratio. A double monochromator is used intensively to keep away from stray light problem and from scattering by dust particles in the sample.

(e) Detector

FT-Raman spectrum can be detected by a common spectrograph. Basically, there are two ways to detect and record the Raman lines. The easiest way is to gather the scattered light emerging through a glass window at the terminal end of the Raman sample tube. It is then made to pass through a prism or grating and then focused on a photographic plate. The plate is then developed and both the line frequencies and intensities can be evaluated using external equipments. Modern spectrometer which has

photo multiplier tubes which direct measurements and facilitate automatic scanning of a spectrum. The spectrum produced by the monochromatic light is passed through a slit which allows a narrow range of wavelength region to get through which is focused on to a photo multiplier type detector. This detector employs with an amplifier and a recorder. It directly supplies the Raman spectrum.

2.6 SAMPLE HANDLING TECHNIQUES IN FT-RAMAN SPECTROMETER

FT-Raman spectroscopic measurements for handling samples is as simpler than for infrared spectroscopy since glass can be utilized for lenses, windows and other optical components instead of the more delicate and atmospherically less stable crystalline halides. In addition, the laser source is easily focused on a small sample area and the emitted radiation efficiently focussed on slit. Consequently a very small sample can be investigated. In fact, a general sample holder for non-absorbing liquid sample is a glass melting-point of capillary action.

(a) Solids

The FT-Raman spectra of solids as polycrystalline material or as a single crystal can be recorded and medium like null, KBr or solvent is required. A few milligrams of the solid samples are needed. Solids can be tightly packed into a capillary tube as a powder. On a glass or silica fibre in a goniometer, the crystal is mounted. The spectra can be measured for different orientation of the crystal. For a single crystal, the Raman spectrum differs depending on the direction of the crystal axis, when polarized light is used as incident radiation. Raman spectra of adsorbed species can be recorded at various temperatures and pressures.

(b) Liquids

The spectrum of a liquid can be recorded as neat or in solution form and essentially 0.3 ml of a liquid may be needed. The sample could be taken in a glass or silica containers or capillaries. The spectrum can be qualitatively measured from the reaction vessel. As water is a universal solvent, it can be used for recording the Raman

spectra. Water absorbs powerfully in the infrared but it gives a reduced Raman scattering. Raman spectroscopy is thus a potential tool for studying water soluble biological materials.

(c) Gases

The FT-Raman spectra of gases are commonly weaker than those of liquids or solids and hence may need cells of larger path length. The gas may be filled in a glass or silica tube of 2 to 1 cm diameter. If the resolving power of the instrument is good and if the molecule has sufficiently low moment of inertia, the rotational fine structure may be found on either side of the Rayleigh line. Generally, a broad band contour may be observed.

The chief advantages of Raman spectroscopy is that it may be used for a wide variety of sizes and forms of the sample. The samples in the form of gas, liquid and solid states can be analyzed easily.

2.7 ADVANTAGES OF FT- RAMAN SPECTROSCOPY

The linear relationship of intensity versus concentration in the FT-Raman spectrum makes identification of chief components of a mixture easier than in infrared, where intensity is logarithmically correlated to concentration. Raman intensities are directly proportional to the concentration of the active species. In this respect, Raman spectroscopy is more closely resembles fluorescence than absorption, where the concentration intensity relationship is logarithmic.

Analysis of multi component mixtures, especially the conjugated systems, dienes, styrenes, cyclic paraffins etc., where the infrared spectra are often identical, and simpler with FT-Raman spectroscopy. FT-Raman spectroscopy has a potential advantages over infrared for the study of aqueous solutions, because infrared is not readily adaptable to the study of aqueous solutions. Raman spectroscopy can be readily employed for the analysis of organic compounds that holds polar groups but fluctuate in skeleton structures.

2.8 UV-Vis SPECTROSCOPY

UV-Vis spectroscopy employs ultraviolet and visible radiations for making the transitions between the electronic energy levels. It helps to identify the functional groups and nature of the bond in the organic compounds. It also provides the information about molecular structure and oxidation state (Bair, 1962).

2.8.1 Instrumentation

A broad range of instruments are accessible for making molecular absorption measurements in the UV-Visible range. In a double beam spectrometer, the radiation coming from the monochromator is split into two beams with the help of a beam splitter. These are approved simultaneously through the reference and the sample cell. The transmitted radiations are detected by the detectors and the difference in the signal at all the wavelengths is preferably amplified and sent for the output. UV-Vis double beam spectrometer is shown in Figure 2.5.

Figure 2.5 UV-Vis double beam spectrometer

2.8.2 Radiation Sources

A spectrometric radiation source must supply a constant high energy output over a broad range of wavelengths. The sources should supply stable output over the entire UV-Visible range 780–190 nm. The source of radiation commonly used for the UV and visible range are described below.

For measurements in the UV region, electric discharge sources like hydrogen or a deuterium lamp are used. In these, the excitation of the gaseous molecules is brought about by the passage of electrons through the gas at low pressures. A hydrogen lamp is generally used in the spectrophotometers and provides light in the wavelength region of 375–160 nm. The radiant power of the hydrogen lamp is low and these are replaced by deuterium lamps. The modern instrument uses a tungsten filament lamp as the radiation source. This consists of a thin, coiled tungsten wire that is preserved in an evacuated glass bulb. This gives radiations in the range of 2200-350 nm. As the output depends on the voltage, the tungsten lamp is energized by the output of a stable voltage transformer (Hell, 1971; Lewin, 1965).

2.8.3 Wavelength Selectors

Spectrometric measurements need a narrow band of wavelengths of light. This increases the selectivity and sensitivity of the instrument. In many applications, we need to continuously vary the wavelength over a distinct range. This can be achieved by using monochromators. Most modern instruments use monochromators that make use of a prism or diffraction grating as the dispersing medium.

(a) Prism Monochromators

The radiations of distinct colours having different wavelengths are refracted to different extent due to the difference in the refractive index of glass for different wavelengths. In a prism monochromator, a fine beam of the light from the source is obtained by passing through an entrance slit. It is then collimated on the prism with the aid of a lens. The refracted beams are then focused on an exit slit. The prism is then rotated in a predetermined way to supply the desired wavelength from the exit slit.

(b) Grating Monochromators

A grating is made by cutting or etching a series of tightly spaced parallel grooves on the smooth reflective surface of a solid material. The surface is made of reflection by the production of a thin film of aluminium on it and the etching is done with the aid of a

preferable shaped diamond tool. The intensity of radiation reflected by a grating varies with the wavelength.A wavelength of increased intensity being dependent on the angle from which the radiation is reflected from the surface of the line of the grating. In grating monochromator, a fine beam of the light from the source falls on a concave mirror through an entrance slit. Rotation of the grating in a predetermined way can be utilized to possess the desired wavelength from the exit slit.

2.8.4 Detectors

The detectors are used to transform a light signal into an electrical signal which can be highly measured and transformed into an output. The detectors utilized in most of the instruments produce a signal, which is linear in transmittance i.e. they react linearly to radiant power that falls on them. The transmittance values can be altered logarithmically into absorbance units by an electrical or mechanical arrangement in the signal read out device. There are three different types of detectors which are used in modern spectrophotometers.

(a) Phototube

A phototube comprises of a photoemissive cathode and an anode in an evacuated tube with a quartz window. These two electrodes are subjected to high voltage. When a photon enters into the tube and strikes the cathode, an electron is ejected and is attracted to the anode ensuing in a flow of current which can be amplified greatly and quantified.

(b) Photomultiplier Tube

A photomultiplier tube possesses a series of electrodes, called dynodes. The voltage of successive electrodes is essentially maintained at 50-90 V. An electron is emitted, when a radiation potentially falls on the cathode. This is accelerated towards the next photoemissive electrode by the potential difference between the two. Here, it analyzes many more secondary electrons. These, in turn are accelerated to the next electrode where each secondary electron releases more electrons. This process

progresses up to 10 stages of amplification. The final output of the photomultiplier tube yields a much larger signal than the photocell.

(c) Diode Array Detector

These detectors employ a huge number of silicon diodes oriented side by side on a single chip. When UV-Vis radiation falls on the diode and potentially increases their conductivity. This elevation in conductivity is proportional to the intensity of the radiation and can be readily quantified. Since, a large number of diodes can be arranged together, the intensity at a number of wavelengths can be calculated simultaneously.

2.8.5 Signal Processing and Output Devices

The electrical signal from the transducer is amplified or processed before it is sent to the recorder to yield an output. The subtraction of the solvent spectrum from that of the solution is done electronically. The output plot between the wavelength and the intensity of absorption is the resultant of the subtraction process and is characteristic of the absorbing species (Hargis, 1996).

2.8.6 SAMPLE HANDLING TECHNIQUES IN UV-Vis SPECTROSCOPY

The UV-Vis absorption spectra are generally determined either in vapour phase or in solution. In order to take the UV spectrum of the analyte is taken in a cell known as cuvette which is held transparent to the wavelength of light passing through it. Most of the spectrometers employ quartz cuvettes for both visible and UV region. Usually, square cuvettes having internal path length 1.0 cm are used, though cuvettes of much smaller path lengths say of 0.1 mm or 0.05 mm are also available. The sample whose spectrum is to be calculated is dissolved in a solvent that should be transparent in the UV region.

Hexane, ethanol, methanol and 1,4-dioxane are generally referred as solvents. In a typical measurement of a UV spectrum, the solution of the sample is used inside a suitable cuvette and the spectrum is run in the desired range of the wavelengths. The

absorption by the solvent, if any, is compensated by running the spectrum for the solvent alone in the similar/ identical cuvette and subtracting it from the spectrum of the solution. This yields the spectrum owing to the absorption species under solvent alone in the similar or identical cuvette and subtracting it from the spectrum of the solution.

2.9 NMR SPECTROSCOPY

The extensive chemical and biochemical applications of NMR lie in the elucidation of structure, identification of compounds, study of molecular conformation, dynamic processes and the interaction between molecules. NMR spectroscopy is a very powerful technique for studying the molecules at atomic level (Harris., 1996).

There are two different classes of NMR spectrometers, which includes - continuous wave spectrometers and pulse spectrometers (FT-NMR). Fourier transform (FT) spectroscopy is a common concept used to find very weak signals after isolating it from environmental noise. In case of FT-NMR measurements, nuclei in a magnetic field are found to vary with brief pulses of intense radio frequency radiation. After a impediment for minimal seconds, one more pulse is applied. The interruption is so chosen that during the interval between the two pulses all the excited nuclei lets go back to the ground state and the population variance is achieved. This relaxation is characterized by free induction decay (FID). This FID can be detected with a radio receiver coil which is perpendicular to the static magnetic field. Solitary coil is recurrently used both to supply pulse to the sample and also to perceive the FID signal.

The time domain decay signals (FIDS) from diverse range of consecutive pulses are added to enhance the signal to noise ratio. The resulting total data are then transformed to a frequency domain signal by Fourier transformation and consequently, digital filtering may be applied to the data to further increase the signal to noise ratio. The resulting frequency domain spectrum is comparable to the spectrum produced by a scanning continuous wave experiment. FT instruments have the estimable advantage of increased resolving power and wavelength reproducibility which makes potential for the analysis of complex spectra (Abraham *et al.,* 1978).

2.9.1 Instrumentation

FT-NMR spectrometer is shown in Figure 2.6. The major components of FT-NMR spectrometer are (i) a magnet (ii) a radiofrequency transmitter (iii) NMR probe (iv) a radiofrequency receiver and (v) a recording device (computer) (Derome, 1987).

Figure 2.6 FT-NMR spectrometer

2.9.2 Magnet

The magnet used in FT-NMR spectrometer is essential to provide strong, stable and highly homogeneous magnetic field. The advantages of using higher magnetic field are higher sensitivity, high spectral resolution, improving signal to noise ratio. In recent years, superconducting magnets (solenoids) operating at liquid helium temperature are employed. Strong magnetic field is formed with a sort of Dewar glass that requires to keep a low temperature, around 4K, in order to optimize the properties of superconductivity of the solenoid. The solenoid is produced by alloys based on niobium, which are immersed in a bath of liquid helium. The inner dewar is contained within an outer jacket of stainless steel or aluminium that contains liquid nitrogen. Nitrogen and helium should be refilled in the magnet periodically.

2.9.3 Radio Frequency Transmitter

By means of a pulse transmitter, the radiofrequency radiation is generated for the nucleus of interest. A waveform generator produces a continuous voltage oscillating sinusoidally at the required frequency. This output is chopped into pulses by a switch, which is opened and closed by a pulse generator. The pulses are largely amplified and sent to the NMR probe. In order to permit double resonance experiments of different types, a second rf source of variable frequency should be available. To record the spectra of nuclei other than ^{1}H using the instrument of magnetic field strength say 2.349 T, a diverse radio frequency source appropriate to the nucleus being examined is required. For example with a 2.349 T magnet, ^{1}H requires 100 MHz, but ^{19}F spectrum can be recorded using radiofrequency source at 94.07 MHz mean while ^{13}C spectrum requires 25.14 MHz source.

2.9.4 NMR Probe

The probe at the centre of the magnetic field is an electrical device with sample holder and contains the transmitter, receiver coils and a spinner. The radiofrequency is applied to the sample through the transmitter coil. The probe has the electronic equipment inside which is designed to detect very tiny NMR signal.

The central component of the probe is a wire that perceives the pulse from the transmitter and dissipates it into the sample. It also receives the signal from the sample and transfers it to the receiver circuit. At resonance, a voltage is induced in the receiver coil, which identifies the sample magnetization. The probe withholds and spins the sample about its vertical axis in order to average the outfield in homogeneities. An other frequency has to be transmitted and received to work the field/frequency lock.

2.9.5 Radio Frequency Receiver

The coil which is at right angles to the source coils and also sorrounds the sample is called receiver coil. It has been used to detect the radio frequency signal

produced by the resonating nuclei. The electrical signal produced in the coil must be amplified and it can be recorded with the computer.

2.9.6 Computer

A predominant feature of the FT-NMR spectrometer is a digital computer. All the functions of the spectrometer are under its control. It plays a key central role in the experimental setup. The computer controls both the radiofrequency transmitter and the receiver, stores and processes the incoming data and transfers the results to the recorder. The computer is also used to manipulate, compare, add, subtract or simulate spectra or to control the more complicated pulse sequences. Recently, the recording device is always a computer.

2.9.7 SAMPLE HANDLING TECHNIQUES IN NMR SPECTROSCOPY

The sample is taken in a thin walled 5mm diameter pyrex glass tube to which approximately 0.5 ml of the sample solution is added. Larger diameter tubes (10 mm) may be used for less sensitive nuclei so that more samples are present. The solution should fill the tube to a height of 3–2 cm. The sample size for routine ^{13}C and ^{31}P spectra about 100–50 mg and for ^{1}H and ^{19}F NMR spectra, usually 10–5 mg or 10 µl of the sample dissolved in 0.5 ml of the solvent.

An optimum concentration of 25–20 millimolar solution is recommended. Thus, ^{1}H NMR spectra can be readily measured at minimum concentrations.Where as an elevated concentration is necessary for recording ^{13}C NMR spectra.The reference compound is then added and the tube is spun at about 30 rps about its vertical axis to enhance the effective homogeneity even further. Although, superconducting magnet is operated at the temperature of liquid helium (4K), the sample itself is typically at room temperature.

Dilute solutions of the sample in inert solvents are utilized to acquire high resolution NMR spectra. Chemical shifts will depend on concentration, solvent and temperature, if there are solute-solvent interactions. For a proton spectrum deuterated solvents are most often used partly to avoid resonances from protons of the solvent

from swamping the proton spectrum of the compound. The most widely used solvents are CCl_4, $CDCl_3$, DMSO-d_6, CD_3CN, C_6D_6 and D_2O. Frequently, a mixture of solvents such as $CDCl_3$ and DMSO-d_6 may be used since it has dissolving properties and $CDCl_3$ is less viscous than DMSO-d_6.