CHAPTER – 2
EXPERIMENTAL METHODS

Vibrational spectroscopy provides information used for the qualitative and quantitative purposes. The vibrational spectrum of the compound is its unique physical property and is characteristic of its molecular structure. IR spectra tend to highlight vibrations involving polar groups and Raman spectra tend to highlight vibrations involving non-polar groups. This chapter explains the experimental methods carried out in the present work, namely the vibrational and electronic spectroscopic techniques.

2.1 Crystallization

The organic compounds chosen for study in powder form were crystallized by slow evaporation method. The samples is dissolved in the corresponding solvent in the required stoichiometric ratio forms the mixture solution. The solution is left for evaporation at room temperature. After suitable period of time crystals of the organic compounds were obtained.

2.2 Molecular vibrations

A molecule containing N atoms has 3N degrees of freedom. The translational and rotational movement of a non-linear molecule uses six of the 3N degrees of freedom leaving 3N-6 fundamental vibrations. In a linear molecule there is no rotation about the bond axis leaving 3N-5 fundamental vibrations. N-atomic molecule has N-1 bonds and N-1 bond stretching vibrations. Remaining 2N-5 for non-linear and 2N-4 for linear are bending vibrations of the atoms. These stretching and bending vibrational motions are known as normal modes of vibration of the molecule. These

normal vibrations can be symmetric or antisymmetric vibrations in which all the atoms oscillate with the same frequency.

2.3 Infrared spectroscopy

Infrared spectroscopy involves the study of the interaction of infrared radiation with molecules. IR radiation range is generally classified into three regions: near-IR region is 14000–4000 cm^{-1}, the mid-IR region is 4000–400 cm^{-1}, and the far-IR region is 400 –50 cm^{-1}. IR spectroscopy measures transitions between molecular vibrational energy levels as a result of absorption of mid-IR radiation. Two important components to the IR absorption process are the radiation frequency and the molecular dipole moment. The interaction of the radiation with molecules is possible when the specific oscillating radiation frequency matches the natural frequency of a particular normal mode of vibration. The molecular vibration must cause a change in the dipole moment of the molecule, in order for energy to be transferred from the IR photon to the molecule via absorption. The selection rule for IR spectroscopy requires a change in the dipole moment during the vibration to be IR active. The IR absorption process involves absorption of energy by the molecule if the vibration causes a change in the dipole moment, resulting in a change in the vibrational energy level.

The IR spectrum is obtained by plotting the intensity (absorbance or transmittance) versus the wavenumber, which is proportional to the energy difference between the ground and the excited vibrational states. The relationship $A = \log(1/T)$ is used to convert spectra between absorbance and transmittance. According to Beer-Lambert's law [30], as concentration is linearly proportional to absorbance, quantitative analysis needs absorbance spectrum whereas transmittance spectrum is most suited for qualitative analysis.

2.3.1 Selection rules

The interaction of the electric field of the electromagnetic radiation with the dipole moment of the molecule determines the transition between the states. Quantum mechanical theory of time development of a molecular system envisages that the probability of a transition from a state i to state j is proportional to the square of the transition moment [31].

$$\mu_{ij} = \int \psi_i^* \mu \psi_j d\tau \qquad \text{..... (2.1)}$$

where μ is the operator for the dipole moment of the molecule. The dipole moment of a molecule is a function of the normal coordinate Q_k of the vibrational mode and can be obtained as in eq. (2.2).

$$\mu = \mu_0 + \left(\frac{\partial \mu}{\partial Q_k} \right)_0 Q_k + ... \qquad \text{..... (2.2)}$$

substituting the value of μ in eq. (2.1) and neglecting higher order terms,

$$\mu_{ij} = \mu_0 \int \psi_i^* \psi_j d\tau + \left(\frac{\partial \mu}{\partial Q_k} \right)_0 \int \psi_i^* Q_k \psi_j d\tau \qquad \text{..... (2.3)}$$

The first term vanishes due to orthogonality condition and the second term of eq. (2.3) to be nonzero is $\partial\mu/\partial Q_k)_0$ must be finite at least for one component of the dipole moment. A change in the dipole moment of the vibrational motion is required for the vibration to be IR active. Also $\int \psi_i^* Q_k \psi_j d\tau$ must be finite, only if the vibration quantum number change $\Delta v = \pm 1$ under harmonic approximation. The symmetry of

the molecule also restricts the activity of vibrations. Vibrational spectra are observed only for heteronuclear diatomics since homonuclear diatomics have no dipole moment and hence no change in dipole moment during vibration.

2.3.2 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a simple mathematical technique to resolve a complex wave into its frequency components. The Fourier transform converts the time domain spectrum to a frequency domain spectrum rapidly with incredible resolution and signal to noise ratio. The advantages of Fourier transform spectroscopy over dispersive instruments were high speed data collection, increased resolution, lower detection limits and greater energy throughput.

The basic components of a single beam Fourier transform infrared spectrometer are IR radiation source, Michelson interferometer and detector which are shown in Fig. 2.1. The most common mid-infrared source used in FT-IR spectrometers is Globar, heated silicon carbide rod. Other sources used in early FT-IR instruments are nichrome coil and Nernst glower. The usual source used for near-infrared spectrometry is QTH (quartz-tungsten-halogen) lamps. High pressure mercury lamps are the standard source used in far-infrared FT-IR spectrometry [32].

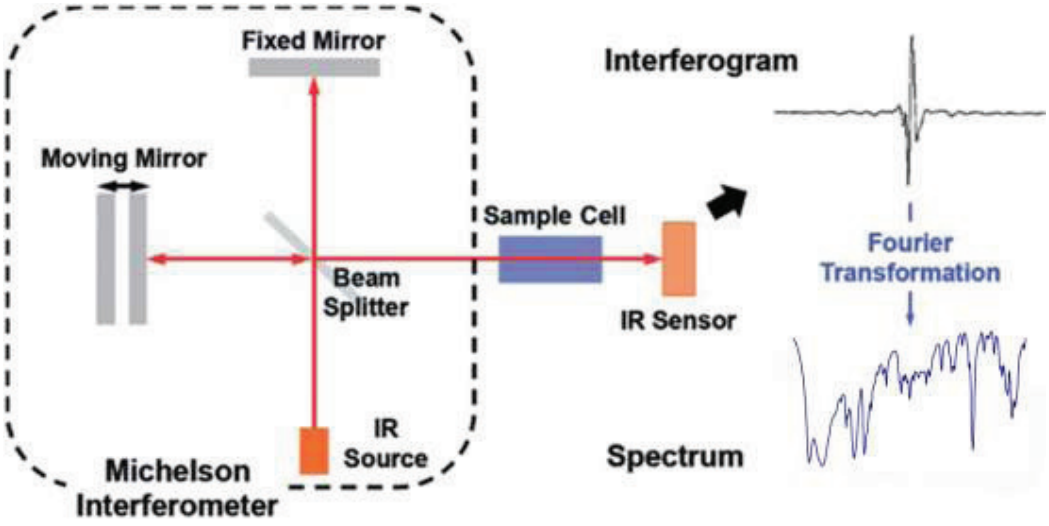

Fig. 2.1 Instrumentation of single beam Fourier transform infrared spectrometer.

The Michelson interferometer is a device that can divide a beam of radiation into two beams and then recombine the two beams after a path difference has been introduced. It consists of two mutually perpendicular plane mirrors (moving mirror and fixed mirror). Beamsplitter is at the bisection of fixed mirror and movable mirror, where a collimated beam of radiation from the source is partially reflected towards the moving mirror and partially transmitted towards the fixed mirror. When the beams return from the mirrors to the beamsplitter, they interfere. After the recombination, the beam is again partially reflected and partially transmitted by the beamsplitter. The reflected beam is then passed through the sample and focused on the detector. The intensity of each beam passing to the detector and returning to the source depends on the path difference between the beams due to interference. The intensity variation as a function of the path difference provides the spectral information in a FT-Spectrometer. The variation of intensity of the beam emerging from the interferometer versus optical path difference is the interferogram [33]. The interferogram is complex and a computer is required to perform the Fourier transform.

Infrared detectors can be divided into two types: thermal detectors and quantum detectors. Detectors that operate by sensing the temperature variation of the absorbing sample is thermal detectors. Recently the FT-IR instuments are equipped with DLATGS (deuterated l-alanine-doped triglycine sulfate) detector. The method of detecting IR radiation depends on the interaction of radiation with the electrons in a solid, causing the electrons to be excited to a higher enegy state. The detectors working on this principle are called quantum detectors. The most popular detector used for mid-IR spectrometry is mercury cadmium telluride (MCT).

2.3.3 Sample preparation

The sample used for recording an IR spectrum can be in any state: solid, liquid or gas. Most popular techniques for handling solid samples were KBr pelleting and mull method [34]. In KBr pellet technique, a milligram of powder sample is mixed with 100 mg of dried KBr powder. Potassium bromides have transparent nature when sufficient pressure is applied. The mixture is then pressed in a die and a transparent pellet is obtained. This pellet is kept in the path of the radiation and the spectrum of the sample is recorded. IR spectra of solid samples that are not pelleted in KBr are prepared with mull method. In this technique, mulls are formed by grinding powdered sample with one or two drops of hydrocarbon oil (Nujol). The resulting mull is examined as a film between flat rock salt plates. IR spectra of liquid samples are obtained by placing a drop of the pure liquid between two NaCl plates, and the plates are then placed in the path of the beam. The IR spectrum of gas sample can be obtained by permitting the sample to expand in the cylindrical cell (sample compartment). Longer path lengths provide more exposure of gas sample to the IR radiation.

2.4 Raman Spectroscopy

The phenomenon of inelastic scattering (Raman spectroscopy) was first postulated by Smekal and first observed experimentally by Raman and Krishnan. Raman effect arises when the incident light interacts with the molecule and causes the electrons to polarize releasing light immediately as scattered radiation [35]. The frequency of the inelastically scattered radiation is lower or higher than the frequency of the incident light. Raman spectroscopy being a non-destructive analytical technique, it finds vast range of applications in the field of biology, pharmaceutical industry, art and archaeology, electronics, polymer composites, forensic analysis etc. Raman spectroscopy is unique in that it is applicable to the samples in the solid, liquid and gaseous states. Quantum energy transitions for Rayleigh and Raman scattering is shown in Fig. 2.2.

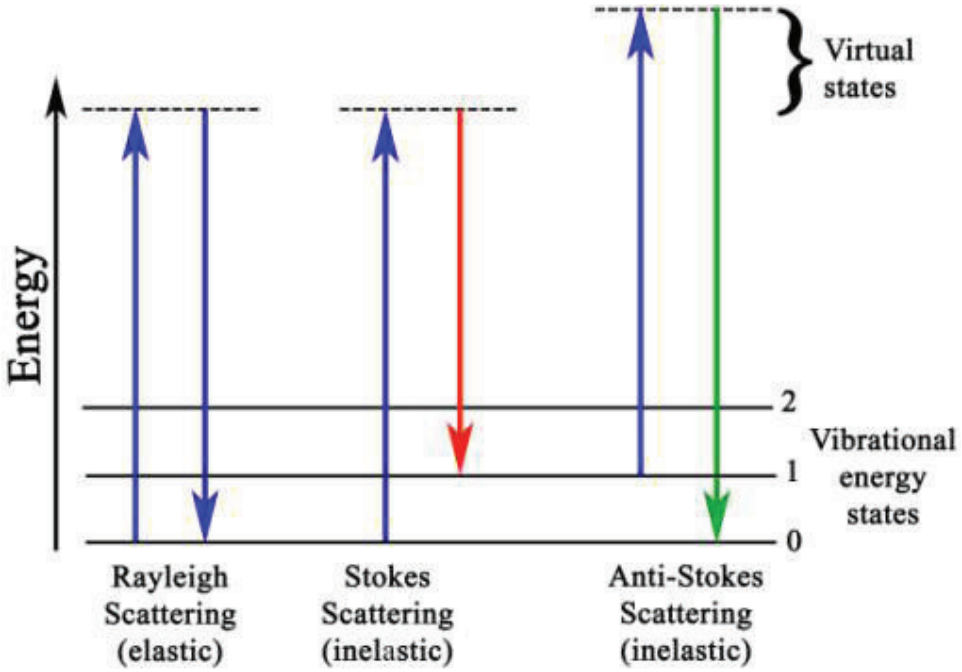

Fig. 2.2 Quantum energy transitions for Rayleigh and Raman scattering.

2.4.1 Quantum theory of Raman effect

In the quantum theory, radiation is a stream of particles called photons which undergoes collision with molecules. When the photon of incident beam collides with the molecule, the molecule will be excited to the virtual state and remains there for a very short period. Most of the molecules return to the original state from the virtual state giving the Rayleigh scattering. If there is no exchange of energy between photon and molecule, the collision is perfectly elastic. However, energy is exchanged between photon and molecule during collision known as inelastic collision. The molecule can gain or lose energy equal to the energy difference ΔE between two of its allowed states. If the molecule gains energy ΔE, the photon will be scattered with energy $h\nu - \Delta E$ (Stokes' line) and if the molecule loses energy ΔE, the energy of scattered radiation will be $h\nu + \Delta E$ (anti-Stokes' line). Since the molecular energy is increased in Stokes' scattering, Stokes' lines is more intense than anti-Stokes' line [36].

2.4.2 Selection rules

The selection rules for the transition can be elucidated from the calculation of the matrix elements of the polarizability given in eq. (2.4) [37].

$$p^{\nu'\nu''} = |E|\alpha_0 \int \psi'^* \psi'' dx + |E|\alpha_1 \int x\psi'^* \psi'' dx \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.4)$$

Here α is considered as varying with displacement $x = r - r_e$ linearly as $\alpha = \alpha_0 + \alpha_1 x$. The first integral is zero except for $\nu' = \nu''$ due to the orthogonality of the eigen functions which gives the undisplaced line. The second term has non-zero values when $\nu' = \nu'' \pm 1$ or $\Delta\nu = \pm 1$. Thus for harmonic oscillator approximation, the frequency shift is observed as similar to that of infrared spectrum. In the case of

anharmonic oscillator, the frequency shifts due to transitions $\Delta\nu = \pm 2, \pm 3...$ etc. are also possible with decreasing intensity as that of infrared selection rule.

2.4.3 Fourier transform Raman Spectroscopy

The instrumentation of Raman spectroscopy consists of a laser source, a sample illumination system and a suitable spectrometer. The most common lasers used in Raman spectroscopy are Argon ion (488 or 514.5 nm), Krypton ion (530.9 or 647.1 nm), Helium-neon (632.8 nm), Diode (785 or 830 nm) and Nd-YAG (1064 nm) laser. Sample handling in Raman spectroscopy is much simpler than in IR spectroscopy because glass can be used for windows, lenses and other optical components instead of NaCl and KBr. Gas samples are normally sealed in small capillary tubes and the laser beam is focused on it. Liquids can be sealed in ampoules, glass tubes or capillaries. Aqueous solutions can be studied by Raman spectroscopy, which is a major advantage over IR spectroscopy. Solid samples are finely powdered and filled in small cavity or capillaries to acquire a Raman spectrum [34].

The basic components of Fourier transform Raman spectrometer are laser source, Michelson interferometer and detector which are shown in Fig. 2.3. Like FT-IR spectrometer, the Fourier transform Raman instrument uses a Michelson interferometer. A continuous-wave Nd-YAG laser of 1064 nm wavelength used eliminates fluorescence and photodecomposition of samples. The radiation from the laser passes through the sample and then into the interferometer, consisting of the beamsplitter and the fixed and movable mirrors. The stray light and the Rayleigh scattering from the interferometer is then extensively filtered. After passing through the filters, the radiation is focused onto a liquid N_2 cooled Ge detector [34].

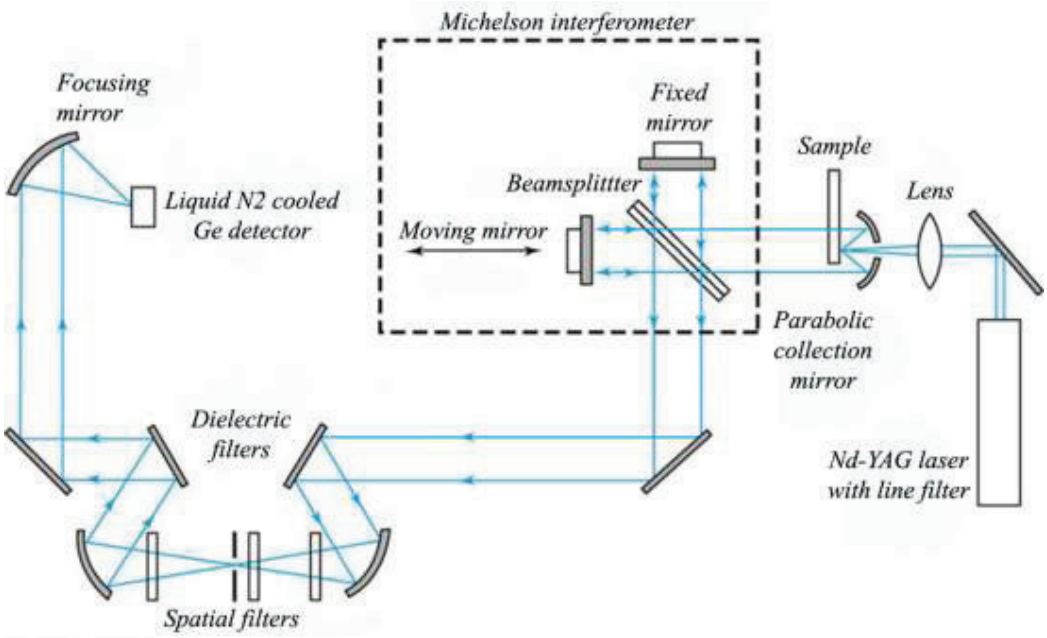

Fig. 2.3 Instrumentation of Fourier transform-Raman spectrometer.

2.5 Factors affecting vibrational spectra

2.5.1 Hydrogen bonding

Hydrogen bonding is a donor (A–H) – acceptor (B) interaction involving hydrogen atoms forming the bonding A–H···B. To interact with the donor (A–H) the acceptor (B) must have lone-pair electrons. Hydrogen bonding is an essential component of the structure and functioning of biological molecules. Hydrogen bond is classified into two types, intramolecular and intermolecular. When donor and acceptor groups are on the same molecule then it is intramolecular hydrogen bonding and intermolecular is when they are on different molecules. In A–H···B hydrogen bonding A–H is the covalent bond, H···B is the hydrogen bond length and A···B is the hydrogen bond distance. The hydrogen bond angle A–H···B is ~180° for stronger bonds whereas for moderate and weak hydrogen bonds it is bent from linearity.

2.5.2 Hyperconjugation

Hyperconjugation is the interaction of electrons in the σ orbital of the C–H bond and the π orbital of the C–C bond [38] to give an extended molecular orbital that increases the stability of the system. Hyperconjugation causes the electron density flow from σ(C–H) to the vacant p orbital, thus decreasing the single C–C bond length and increasing bond strength.

2.5.3 Inductive effect

Inductive effect is an electronic effect which arises due to the difference in the electronegativity of atoms. In a covalent bond, the electron pair forming the σ bond between unlike atoms, is never shared absolutely equally between the two atoms. The electron density tends to be attracted more towards the more electronegative atom of the two. For example, in an alkyl chloride, the electron density tends to be greater nearer chlorine than carbon, as chlorine is more electronegative than carbon [39]. Electron withdrawing and donating functional groups produce negative and positive inductive effect.

2.6 Electronic Spectroscopy

Electronic spectroscopy is the absorption of photons from ultraviolet and visible region of the electromagnetic spectrum by the molecules involving transfer of electrons in the electronic energy state. The UV-visible spectral region is divided into three sub-domains: near UV (185-400 nm), visible (400-700 nm) and very near infrared (700-1100 nm) [40].

2.6.1 Principle

The interaction of photons from the UV-vis (Ultraviolet-visible) region with the molecules of the sample gives rise to UV-vis absorption spectroscopy. As the molecule absorbs photons from the source, the electronic energy gets modified thus altering both rotational and vibrational energy of the molecule. UV-vis spectroscopy is the measure of the transmittance T or the absorbance A of sample of path length b. The transmittance T given in eq. (2.5) is a measure of the attenuation of a beam based upon the comparison between the intensities of the transmitted light (I) and the incident light (I_0).

$$T = \frac{I}{I_0} \qquad \text{..... (2.5)}$$

The absorbance A is defined by Beer's law:

$$A = -\log T = \varepsilon bc \qquad \text{..... (2.6)}$$

In eq. (2.6), ε is the molar absorptivity and c is the concentration of analyte. UV-visible spectrum is the graph usually obtained by plotting the transmittance or absorbance versus wavenumber in nanometers.

2.6.2 Electronic transitions

The transition of electrons to the anti-bonding orbitals due to the absorption of UV or visible radiation is as shown in Fig. 2.4. Valence electrons are generally found in σ bonding orbitals, π bonding orbitals and non-bonding orbitals (lone pair electrons). When the sample absorbs electromagnetic radiation of correct frequency, the transition of electrons from these bonding orbitals to antibonding orbitals (σ^* and π^*) occurs. The mostly observed absorptions were $\sigma \rightarrow \sigma^*, n \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions.

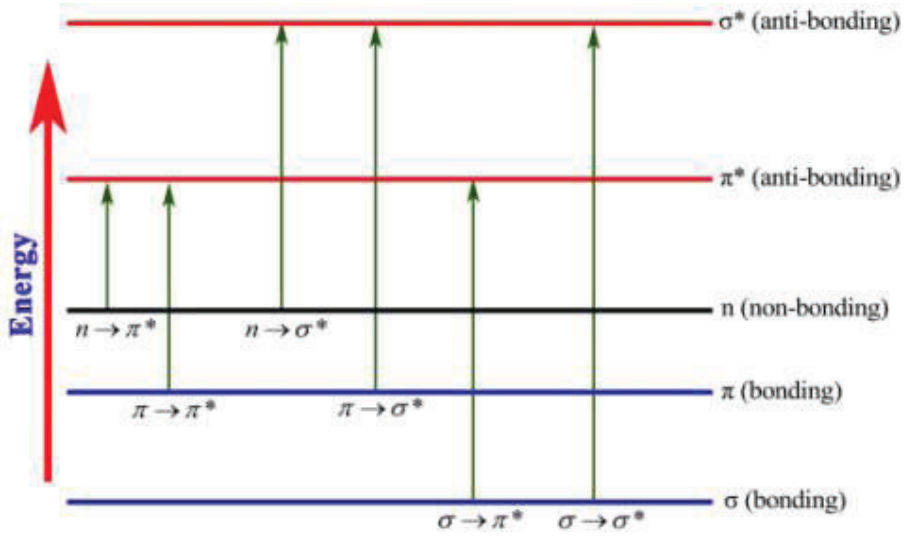

Fig. 2.4 Electronic transitions.

2.6.3 Instrumentation

The UV-visible spectrophotometer is an instrument for making molecular absorption measurements in the ultraviolet and visible regions. The source, dispersive device and the detection system forms the important spectrophotometer components which are as shown in Fig. 2.5. A hydrogen or deuterium discharge lamps for ultraviolet (185-400 nm) region, a tungsten filament lamps or tungsten-halogen lamps (also called quartz-halogen lamps) for visible region (400-700 nm) and a xenon arc lamp is used for entire region (185-1100 nm).

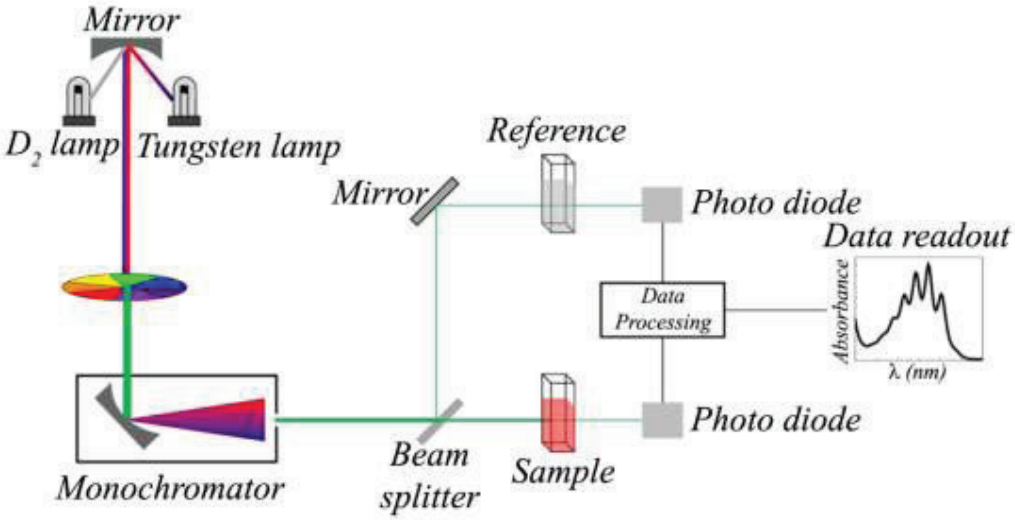

Fig. 2.5 Schematic of the double beam spectrophotometer for the UV-visible region.

The dispersion device selects the particular wavelength from the broadband radiation of the source. Prisms and holographic gratings are the commonly used dispersion devices in UV-visible spectrophotometer. The light emitted by the source falls on the filter. The filter is chosen such that it mostly matches the wavelength of maximum absorption. In general, the most suitable filter will be the colour complement of the solution being analyzed. The light from the filter is dispersed through either a plane or concave grating which forms part of a monochromator assembly. After exiting the monochromator assembly, the radiation is split into two beams by the beamsplitter [41]. One beam passes through the sample cell while the other passes through the reference cell.

The detector compares the two intensities transmitted by the sample and reference cell for the same wavelength. It converts the incoming light into an electrical signal. High spectral sensitivity, good wavelength response, fast response time and high signal to noise ratio are the properties of a good detector. The

commonly used detectors are photomultiplier tubes, photodiodes and Charged Coupled Device (CCD) [42]. The sample beam and the reference beam were detected by two photodiode detectors. The computer connected to the detector does the data processing and plots the absorbance against wavelength (nm) in the UV and visible range of the electromagnetic spectrum.