SPECTROSCOPIC TOOLS USED FOR STRUCTURAL ELUCIDATION

Spectroscopy deals the qualitative and quantitative determination of structural and chemical composition of organic and biomolecular samples. Qualitative method yields informations about the atomic or molecular species or the functional groups that exist in a sample. In contrast, quantitative method provides numerical information about the relative amount of one or more of the components present. Analytical methods are often classified as being either classical or instrumental.

In the classical method, the analyte components are separated by precipitation, extraction or distillation and then treated with reagents that yielded products that could be recognized by their colours, solubilities, melting and boiling points etc. Comparable to the classical method, instrumental method converts an analytical signal in to a spectrum. The position, height, width, and pattern of bands carry informations about the sample that can be extracted for in-depth understanding.

This chapter emphasizes the spectroscopy instrumentations used and the interpretation methods adopted for systematic identification of compounds. Instrumentations used for structural optimization are:

(i) Raman spectroscopy
(ii) Infra red spectroscopy
(iii) Surface enhanced Raman spectroscopy
All these spectroscopic techniques are efficient and widespread method of structural investigations of molecules and crystals which deals with the interaction of electromagnetic radiation with matter and can be used to derive valuable information regarding molecular structures, nature of chemical bonding, vibrational interactions, conformation analysis, symmetry of molecular groups or ions, feature of bonding, H-bonding, the nature of the co-ordination between atoms [1-14] etc.

2.1 RAMAN SPECTROSCOPY

Molecules are made up of atoms bound together by chemical bonds and the bond angles are not rigid which may flexibly vary depending on the molecular environment. Since molecules behave as coupled harmonic oscillators, photons can interact with molecules and they begin to vibrate. In addition to vibration, interacted photons are inelastically scattered and have frequencies lower and higher than the incident frequency, attributed to the excitation (or relaxation) of vibrational modes of a molecule as predicted theoretically by Smekal in 1923 [15]. In continuation, Raman and Krishnan first experimentally demonstrated the phenomenon and reported in 1928, as inelastic scattering effect which was characterized by “its feebleness in comparison with the ordinary scattering” [16]. This “feeble” phenomenon is then named as Raman scattering and now it becomes the top most spectroscopy tool for the determination of the vibrational normal modes of molecules and crystals.

In Raman spectroscopy, a sample is irradiated with intense laser beam \( (\nu_0) \) and the scattered light is observed perpendicular to the incident beam. Intense scattered light of same frequency as the incident beam \( (\nu_0) \) is termed as
Rayleigh scattering. Weak scattering of light \((10^{-6} \text{ times as incident beam})\) and has frequencies \(v_0 \pm v_m\) are Raman lines, where \(v_0 - v_m\) and \(v_0 + v_m\) are called Stokes and anti-Stokes lines respectively. Raman spectrum is a plot of intensity of scattered light versus energy difference. Since, the energy difference between the incident photon and the Raman scattered photon is equal to the energy of vibration of the scattering molecule, obtained spectra can be interpreted for the determination of molecular structures based on the frequency shift, polarization of lines, vibrational selection rules and symmetry of vibration.

2.1.1 Classical Theory of Raman Scattering

An insight into the phenomenon of Raman Scattering is possible on the basis of classical considerations. Accordingly, molecules placed in an electric field undergo polarization due to which the negatively charged electron cloud is being attracted towards the positive pole and the positively charged nuclei get attracted towards the negative pole. The polarization 'P' so induced is proportional to the applied electric field 'E':

\[
P = \alpha E
\]

where, \(\alpha\) is the constant of polarizability.

According to classical theory, when radiation of frequency '\(v_0\)' is allowed to fall on molecules, each molecule experiences a varying electric field [17]. This field depends on the incident frequency, which is expressed as:

\[
E = E_0 \cos 2\pi v_0 t
\]

where, '\(E_0\)' is the vibrational amplitude.
If the vibrational motion of the molecule ‘q’ is in the normal co-ordinate associated with a particular mode of vibration frequency \( \nu_m \) of the molecule, the harmonic approximation of ‘q’ can be written as:

\[
q = q_0 \cos 2\pi \nu_m t
\]  

(2.3)

where, ‘\( q_0 \)’ is the amplitude of vibration.

For small vibrational amplitudes, the polarizability ‘\( \alpha \)’ can be expanded using Taylor series as:

\[
\alpha = \alpha_0 + \left( \frac{\partial \alpha}{\partial q} \right)_0 q + ...
\]  

(2.4)

where, ‘\( \alpha_0 \)’ is the polarizability at the equilibrium position. The term \( \left( \frac{\partial \alpha}{\partial q} \right)_0 \) is the rate of change of ‘\( \alpha \)’ with respect to ‘\( q \)’, evaluated at the equilibrium position.

The induced dipole moment is therefore:

\[
P = \alpha_0 E_0 \cos 2\pi \nu_0 t + \frac{1}{2} \left( \frac{\partial \alpha}{\partial q} \right)_0 q_0 E_0 \left[ \cos 2\pi (\nu_0 + \nu_m) t + \cos 2\pi (\nu_0 - \nu_m) t \right]
\]  

(2.5)

The first term represents Rayleigh scattering and the second term represents anti-Stokes \( (\nu_0 + \nu_m) \) and Stokes \( (\nu_0 - \nu_m) \) lines of Raman scattering. For Raman active vibrations, the rate of change of polarizability should be non zero, \( \left( \frac{\partial \alpha}{\partial q} \right)_0 \neq 0 \).

Thus a molecular vibration will be Raman active only if it causes a change in a component of polarizability either in magnitude or in direction.
Though the classical theory correctly describes the frequencies of the Raman lines $v_0 \pm v_m$, it fails to predict correct intensities. Quantum mechanical theory is therefore introduced to predict the intensities of Raman lines [18].

2.1.2 Quantum Theory of Raman Scattering

In explaining Raman scattering using quantum hypothesis, incident radiation of frequency $'v_0'$ is considered as a stream of particles undergoing collision with sample molecules. Perfect elastic collision will not exchange any energy between the photons and the molecules, but, inelastic collision will exchange energy between the two. Molecules that participates in the collision process either give up or lose energy equal to the energy difference $'\Delta E'$ between any two of its allowed states. If the molecule gains energy, the scattered photons will have energy $h(v_0 - v_m)$ [Stokes lines]. On the other hand, if the molecule loses energy, the scattered photons will have energy $h(v_0 + v_m)$ [anti-Stokes line]. The energy level diagram of the entire transitions is shown in Fig. 2.1.

![Energy level diagram showing Rayleigh, Stokes and anti-Stokes lines](image)

**Fig. 2.1 Energy level diagram showing Rayleigh, Stokes and anti-Stokes lines**
Incident radiation make an upward transition to a virtual state of the system corresponds to a combined state of the molecules and radiations [19]. Most of the molecules of the system return to the original state from the virtual state resulting Rayleigh scattering. However, a very small fraction returns to states of higher and lower energies giving rise to Stokes and anti-Stokes lines respectively.

2.1.3 Raman Selection Rules

The intensity of a Raman band is proportional to the square of the transition moment [20], which is given in terms of induced polarization ‘P’ as:

$$\int \psi_n^* P \psi_m \, d\tau = E \int \psi_n^* \alpha \psi_m \, d\tau$$  \hspace{1cm} (2.6)

where, ‘$$\psi_m$$’ and ‘$$\psi_n$$’ are the time independent wavefunctions of the states and the integral is extended over the whole range of co-ordinates.

Since the electric polarizability of a molecule is a function of all the normal vibrational co-ordinates, ‘$$\alpha$$’ may be expanded as a Taylor series with respect to these co-ordinates. On neglecting higher powers:

$$\alpha = \alpha_0 + \sum_k \left[ \left( \frac{\partial \alpha}{\partial Q_k} \right)_0 Q_k \right]$$  \hspace{1cm} (2.7)

where, ‘$$\alpha_0$$’ is the polarizability tensor in the equilibrium configuration and

$$\left( \frac{\partial \alpha}{\partial Q_k} \right)_0$$

is the derived polarizability at the equilibrium configuration for the $$k^{th}$$ normal mode.

Induced polarization thus gives extra terms to explain Raman lines in terms of the normal modes such as $$k^{th}$$ mode.
The first term accounts for the Rayleigh scattering and the second term is responsible for Raman scattering. It is evident that a particular normal vibrational frequency is allowed in Raman effect only if \( \frac{\partial \alpha}{\partial Q_{k}} \) is non zero, otherwise, Raman effect is forbidden.

The magnitude of the intensity of a permitted Raman transition is determined from \( P_{nm}^2 \) and is given by:

\[
I_{nm} = \frac{64\pi^2}{3c^2} (\nu_0 + \nu_{nm})^4 P_{nm}^2
\]  

(2.9)

where, 'c' is the velocity of light, '\( \nu_0 \)' is the incident frequency and '\( \nu_{nm} \)' is the magnitude of vibrational frequency.

The total intensities due to a transition between a state 'n' and a higher state 'm', containing 'N_n' and 'N_m' molecules respectively are given by:

\[
I_{\text{Stokes}} = \frac{64\pi^2}{3c^2} (\nu_0 + \nu_{nm})^4 N_n P_{nm}^2
\]  

(2.10)

\[
I_{\text{Anti-Stokes}} = \frac{64\pi^2}{3c^2} (\nu_0 + \nu_{nm})^4 N_m P_{nm}^2
\]  

(2.11)

Intensity of the Raman lines are frequency dependent and are related by [13, 21]:

\[
\frac{I_{\text{Stokes}}}{I_{\text{Anti-Stokes}}} = \frac{\nu_{nm}}{\nu_0 + \nu_{nm}} (\nu_0 + \nu_{nm})^4 N_m P_{nm}^2
\]  

(2.12)

It is evident that Stokes lines are more intense than that of the anti-Stokes lines.
2.1.4 Polarizability Tensor and Polarizability Ellipsoid

Intense laser beam (electric field) persuades the molecule to endure distortion, since the positively charged molecules are shifted in the direction of the field and electrons in the opposite direction, producing an induced dipole moment. In matrix form, the polarizability tensor can be written in terms of the components of the field vectors as:

\[
\begin{pmatrix}
    p_x \\
p_y \\
p_z
\end{pmatrix} = \begin{pmatrix}
    \alpha_{xx} & \alpha_{xy} & \alpha_{xz} \\
    \alpha_{yx} & \alpha_{yy} & \alpha_{yz} \\
    \alpha_{zx} & \alpha_{zy} & \alpha_{zz}
\end{pmatrix} \begin{pmatrix}
    E_x \\
    E_y \\
    E_z
\end{pmatrix}
\] (2.13)

The polarizability tensor provides valuable information regarding the polarization properties of vibrational modes [22] and the dependence of vibrational intensity on polarization direction [23]. Observed transition is Raman active only if any of the components in the polarizability tensor has to be changed during vibration. For normal Raman scattering, polarizability tensor should be symmetric, i.e., \( \alpha_{xy} = \alpha_{yx}, \alpha_{xz} = \alpha_{zx} \) and \( \alpha_{yz} = \alpha_{zy} \).

Polarizability ellipsoid is another valuable tool for predicting the polarization properties of internal vibrations of sample molecules [24]. A plot representing \( \frac{1}{\sqrt{\alpha_i}} \), where \( \alpha_i \) is the value of \( \alpha \) in \( i^{th} \) direction from the centre of gravity of a molecule in all directions, a three dimensional surface is obtained, which is the polarizability ellipsoid. During normal vibration, the change in size, shape or orientation of the polarizability ellipsoid represents Raman active mode. Whenever the size of the ellipsoid changes, the diagonal elements \( \alpha_{xx}, \alpha_{yy} \) and \( \alpha_{zz} \) change simultaneously. The diagonal elements change with different rates for
any modification in the shape of polarizability ellipsoid and the off diagonal elements vary for orientational changes.

2.1.5 Depolarization Ratio

In addition to intensity and frequency information, Raman measurements provide an additional parameter, the depolarization ratio that is useful in determining the structure of molecules. Depolarization ratio can be obtained by considering a molecule irradiated by a plane polarized light from Y-direction whose electric vector 'E' is in the Z-direction (Fig. 2.2). The scattered beam by the molecule is observed in the X-direction and \( I_Y \) (\( I_{\text{parallel}} \)), \( I_z \) (\( I_{\text{perpendicular}} \)) are respectively the minimum and maximum intensity observed through the analyzer. Then depolarization ratio is given by,

\[
\rho = \frac{I_{\text{perpendicular}}}{I_{\text{parallel}}} \tag{2.14}
\]

which measures the degree of polarization. Theoretically, depolarization ratio is given by [25]:

\[
\rho = \frac{3g^s + 5g^a}{10g^o + 4g^s} \tag{2.15}
\]

where,

\[
g^0 = \frac{1}{3} [\alpha_{xx} + \alpha_{yy} + \alpha_{zz}]^2
\]

\[
g^a = \frac{1}{3} [(\alpha_{xx}-\alpha_{yy})^2 + (\alpha_{yy}-\alpha_{zz})^2 + (\alpha_{zz}-\alpha_{xx})^2] + \frac{1}{2} [(\alpha_{xy} - \alpha_{yx})^2 + (\alpha_{yz} + \alpha_{zy})^2 + (\alpha_{zx} + \alpha_{xz})^2]
\]

\[
g^a = \frac{1}{2} [(\alpha_{xy} - \alpha_{yx})^2 + (\alpha_{yx} - \alpha_{zy})^2 + (\alpha_{sx} - \alpha_{zx})^2]
\]
For normal Raman scattering, the polarizability tensor is symmetric and therefore, $g_0^0 = 0$. For totally symmetric vibrations, $g_0^0 > 0$ and $g^s \geq 0$. The vibration is polarized and '$p$' takes the value ranges with $0 \leq p \leq \frac{3}{4}$. The nonsymmetric vibrations are depolarized and therefore, $g_0^0 = 0$ and $g^s > 0$, then $p = \frac{3}{4}$. Calculated depolarization ratio with all these values provide valuable information regarding the symmetry of vibration which helps in band assignments and vibrational analysis [26, 27].

![Fig. 2.2 Depolarization measurements](image)

2.1.6 FT-Raman Instrumentation

Raman spectrometers basically employ one of the two techniques either dispersion or Fourier Transform (FT) for the collection of spectral data. Dispersive Raman spectrometers produce interfacing fluorescence due to incorporation of impurities with the samples. Furthermore, the cost of this type of equipments and its maintenance are quite high. So FT-Raman spectrometer replaces the dispersive Raman without producing sample fluorescence. Sample is irradiated with longer wavelength excitation laser, so the virtual state is lower and it will
overlap an upper electronic state. This greatly reduces fluorescence interferences and therefore Raman signals are free from distortions. Conventional Raman spectroscopy measures intensity versus frequency. But, FT Raman is a time domain spectroscopy which measures the intensity of light of many frequencies simultaneously. This spectrum is then converted to conventional spectrum by applying Fourier transform. The block diagram of the FT-Raman spectrometer is shown in Fig. 2.3. The three basic spectrometer components in an FT system are source, interferometer and detector.

![Schematic diagram of an FT-Raman spectrometer](image.png)

**Fig. 2.3 Schematic diagram of an FT-Raman spectrometer**

Lasers are commonly used as radiation sources in FT-Raman spectrometer. They are highly directional and intense, allowing one to record the spectrum down up to 10 cm\(^{-1}\) from the exciting line. The fluorescence is also expected to be less, as most of the lasers operate at lower frequencies. The commonly used sources are He-Ne laser (632.8nm), Argon ion laser (488 and
514.5nm), Krypton laser (647.1, 568.2, 530.8, 520.8, 482.5, 476.2nm) and Ar-Kr mixed laser (488, 514.5, 647.1 nm). In certain cases, red radiation is preferred to reduce fluorescence and decomposition of the sample.

The FT-Raman spectrometer has an interferometer to produce an interferogram, which “encodes” the unique frequencies of the Raman scattering into a single signal. The interferometer employs a beam splitter optimized for near-infrared (NIR) radiation, which divides the incoming Raman signal and scatter into two optical beams, one transmitted and one reflected. The reflected beam travels to and reflects off a flat fixed mirror. The transmitted beam also travels to and reflects off a flat moving mirror, which has a constant frequency and fixed motion. The two beams recombine at the beam splitter. Depending on their path difference, the two beams constructively and destructively interfere with each other. Constant frequency and fixed motion of the moving mirror modulates the interference pattern. The resulting interferogram has the unique property that every data point (a function of the moving mirror position) has information about every frequency of the Raman scatter collected from the sample. The signal can be measured very quickly, making signal averaging fast and accurate.

Detector detects the interferogram signals and fed to the computer. It is the time domain spectrum and records the detector response changes versus time within the mirror scan. If the sample happens to absorb the signal at this frequency, the amplitude of the sinusoidal wave is reduced by an amount proportional to the amount of sample in the beam. The interferogram contains the information over the entire Raman region to which detector is responsive. The required Raman shift is obtained after calibrating the original data with respect to
the known wavenumber relationship. The plot represents the wavenumber and
the relative scattering intensity in photon counts.

2.1.7 Sample Handling

In Raman spectroscopy, the sample can be used as such, which is simple
and is the main advantage over other spectroscopic techniques. Polycrystalline
sample are finely powdered and filled in a capillary tube and placed in the sample
compartment [28,29]. For moisture sensitive samples, the ampule is first filled
with the sample. It is then evacuated, sealed and placed in the sample
compartment. To handle minute quantity of sample, it is dissolved in a low boiling
solvent, taken in a fine capillary tube and then the solvent is allowed to
evaporate.

2.1.8 Fluorescence

Sample may absorb radiation and re-emit it instantaneously \(10^{-5}\) sec as
fluorescence. The intensity is \(10^4\) times greater as compared to Raman signal
and hence Raman signals may be obscured by strong fluorescence bands [30].
An FT-Raman instrument using near IR source can effectively reduce
fluorescence. By exposing the sample to 200-500 mW laser for 30-60 min and
subsequent cooling before recording the spectra reduce the fluorescence.

2.1.9 Signal to Noise Ratio and Fast Fourier Transform

Various methods have been employed to improve the signal to noise ratio
(S/N) in Raman spectra, which may be low due to low scattering intensities from
the sample or low signals in certain spectral region caused by detector fall off
[31]. Smoothing routines like Fast Fourier Transform (FFT) have been conventionally used to improve signal to noise ratios [32]. In this method, the spectral data are Fourier transformed and is truncated to remove higher frequencies, since spectral noise is strong at higher frequencies. However, the low frequency information is retained. Finally, reverse transform is taken and the noise has been removed.

2.1.10 Scattering Cross Section

The scattering cross section for Raman scattering is very low of the order of $1 \times 10^4$, which may limit the detection capability of the spectrometer. In order to enhance capability, laser beam is focused on an area of $5 \times 10^{-7} \text{m}^2$ containing approximately $10^{15}$ scattering molecules [33]. The problem of low scattering cross section is effectively reduced by increasing the number of scattering molecules and by increasing the intensity of incident light which in turn enhances the scattered intensity. However in surface enhanced Raman scattering technique, Raman signal is enhanced by $10^6$ times due to the absorbed molecules on rough metallic surface.

2.2 INFRARED SPECTROSCOPY

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR
spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification. The advent of Fourier Transform (FT) instrumentation made significant impact with regard to rapid scanning, high resolution, high sensitivity and better signal to noise ratio. Now the computer averaging technique is possible by the introduction of powerful computers to increase the number of scans and thus increases the signal to noise ratio. Also, the operations like the subtraction of the spectrum of reference sample from the spectrum of mixture and curve fitting routines are possible with FT-IR spectrometers [34,35].

2.2.1 IR Frequency Range and Spectrum Presentation

IR absorption information is generally presented in the form of a spectrum with wavenumber as the x-axis and absorption intensity or percent transmittance as the y-axis. The transmittance spectra provide better contrast between intensities of strong and weak bands because transmittance ranges from 0 to 100%, whereas absorbance ranges from infinity to zero. The analyst should be aware that the same sample will give quite different profiles for the IR spectrum, which is linear in wavenumber, whereas the IR plot is linear in wavelength. It will appear as if some IR bands have been contracted or expanded.

The most frequently used region is the mid IR region, between 4000 and 400 cm\(^{-1}\) (2.5 to 25 µm). The complexity of infrared spectrum in the 1450 cm\(^{-1}\) to 600 cm\(^{-1}\) region makes it difficult to assign all the absorption bands, because of the unique pattern found here and it is called the finger print region. But the absorption bands in the 4000 cm\(^{-1}\) to 700 cm\(^{-1}\) region are usually due to the
stretching and bending vibrations of the diatomic units and is often called group frequency region \[36\]. The far IR requires the use of specialized optical materials and sources. It is used for analysis of organic, inorganic, and organo-metallic compounds involving heavy atoms (mass number over 19). It provides useful information to structural studies such as conformation and lattice dynamics of samples. Near IR spectroscopy needs minimal or no sample preparation. It offers high-speed quantitative analysis without consumption or destruction of the sample. This instrument can often be combined with UV-visible spectrometer and coupled with fiber optic devices for remote analysis. Near IR spectroscopy has gained increased interest, especially in process control applications.

2.2.2 Theory and Selection Rules

When infrared radiation is allowed to fall on a molecule, the system absorbs energy, causing the excitation of the molecule to higher vibrational levels. The molecules absorbing a quantum of energy give rise to bands, characteristic of the molecule from about 200 to 4000 cm\(^{-1}\) \[37\]. Each functional group absorbs characteristic frequencies of infrared radiation uniquely. The fact that many functional groups can be identified by their characteristic vibrational frequencies makes the infrared spectra one of the most reliable methods for understanding the structure of molecules \[38-41\].

In IR spectroscopy, the necessary condition for the absorption of a quantum of radiation \(hv\) by the molecule should be equal to the energy difference between two states represented by the wavefunctions \(\psi_i\) and \(\psi_j\) respectively. The transition between these states under the influence of
electromagnetic radiation depends on the interaction of the electric field of the radiation with the electric dipole moment of the molecule [42].

According to quantum mechanical theory of a molecular system, the probability of transition from a state ‘i’ to the state ‘j’ is proportional to the square of the transition moment.

\[ \mu_{ij} = \int \psi_i^* \mu \psi_j \, d\tau \]  

(2.16)

where, ‘\( \mu \)’ is the dipole moment of the molecule.

The dipole moment of a molecule is a function of the normal coordinates ‘\( Q_k \)’ of the vibrational mode and can be expanded in a Taylor series as:

\[ \mu = \mu_0 + \left( \frac{\partial \mu}{\partial Q_k} \right)_0 Q_k + ..... \]  

(2.17)

On neglecting higher terms:

\[ \mu_{ij} = \mu_0 \int \psi_i^* Q_k \psi_j \, d\tau + \left( \frac{\partial \mu}{\partial Q_k} \right)_0 \int \psi_i^* Q_k \psi_j \, d\tau \]  

(2.18)

The first term vanishes (Orthogonality condition) and the conditions for the second term to be nonzero are:

(i) \( \left( \frac{\partial \mu}{\partial Q_k} \right)_0 \) must be finite at least for one component of the dipole moment.

That is, for a mode of vibration to be infrared active; the vibrational motion of that mode must give rise to change in dipole moment.

(ii) The integral \( \int \psi_i^* Q_k \psi_j \, d\tau \) must be finite, which is possible only if the vibrational quantum number change \( \Delta \nu = \pm 1 \) under harmonic approximation and for anharmonic oscillator \( \Delta \nu = \pm 1, \pm 2, \pm 3 \ldots \) [43,44].
In addition, the symmetry of the molecule also restricts the activity of vibrations. Homo-nuclear diatomics have no dipole moment and also they have no change in dipole moment during vibration. Hence vibrational spectra are observable only in the case of hetero-nuclear diatomics. Vibrational spectra are usually studied in absorption and hence \( \Delta \nu = \pm 1 \), are the important selection rule [11,42,45].

2.2.3 FT-IR Instrumentation

In Fourier Transform Infrared (FT-IR) spectrometer, spectrum of a sample is obtained by recording an interferogram with an interferometer, which measures all infrared frequencies simultaneously. Then the spectrometer digitizes the interferogram, performs the FT function and outputs the spectrum. The main parts of the FT-IR spectrometer are, radiation source, interferometer and detector. A simplified optical layout of a typical FT-IR spectrometer is illustrated in Fig. 2.4.

The common radiation source for FT-IR spectrometer is an inert solid heated electrically from 1000°C to 1800°C. Three popular types of sources are Nernst glower, Globar and Nichrome coil. They all produce continuous radiations, but with different radiation energy profiles. The source is more often water-cooled in FT-IR instruments to provide better power and stability.

Interferometer produces interference signals, which contain infrared spectral information generated after passing through the sample. The most commonly used interferometer is a Michelson interferometer. It consists of three active components: a moving mirror, a fixed mirror, and a beam splitter. The two mirrors are perpendicular to each other. The beam splitter is a semi reflecting
device and is often made by depositing a thin film of germanium onto a flat KBr substrate. Radiation from the broadband IR source is collimated and directed into the interferometer and impinges on the beam splitter. At the beam splitter, half the IR beam is transmitted to the fixed mirror and the remaining half is reflected to the moving mirror. After the divided beams are reflected from the two mirrors, they are recombined at the beam splitter. Due to changes in the relative position of the moving mirror to the fixed mirror, an interference pattern is generated. When the mirror is moved at a constant velocity, the intensity of radiation reaching the detector varies in a sinusoidal manner to produce the interferogram output as shown in Fig. 2.4.

Interferometer output is a time domain spectrum which is a record of the detector response changes versus time within the mirror scan. If the sample
happens to absorb at this frequency, the amplitude of the sinusoidal wave is reduced by an amount proportional to the amount of sample in the beam. The interferogram contains more information over the entire IR region to which the detector is responsive. A mathematical operation known as Fourier transformation converts the interferogram (a time domain spectrum displaying intensity versus time within the mirror scan) to the final IR spectrum. When the interferogram signal is transmitted through or reflected off of the sample surface, the specific frequencies of energy are adsorbed by the sample due to the excited vibration of functional groups in molecules. The infrared signal after interaction with the sample is the unique characteristic of the sample. The beam finally arrives at the detector and is recorded [46].

The two most popular detectors for a FT-IR spectrometer includes deuterated triglycine sulfate (DTGS) and mercury cadmium telluride (MCT) crystals. The DTGS detector is a pyroelectric detector that delivers rapid responses because it measures the changes in temperature rather than the value of temperature. The MCT detector is a photon (or quantum) detector that depends on the quantum nature of radiation and also exhibits very fast responses. The detected interferogram cannot be directly interpreted. It has to be "decoded" with a well-known mathematical technique in terms of Fourier Transformation. The computer can perform the Fourier transformation calculation and present an infrared spectrum, which plots absorbance (or transmittance) versus wavenumber [47].

The final transmittance/absorbance spectrum should be devoid of all instrumental and environmental contributions and only present the features of the sample. When a background and sample spectra are obtained, their contributions
to the spectrum will ratio out exactly and their bands will not occur, but reveal the contributions of the sample only.

2.2.4 Sample Preparation

Samples for FT-IR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride (salt) which is transparent to infrared light. The drop forms a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) to form a very fine powder. This powder is then compressed into a thin pellet of specific dimension and then it can be analyzed. Alternatively, solid samples can be dissolved in a solvent such as methylene chloride and the solution is placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate. Films can be placed in an attenuated total reflectance cell for recording the spectrum.

2.3 IR AND RAMAN SPECTROSCOPY-COMPLEMENTARY PRINCIPLES

Infrared absorption and Raman scattering are two different physical processes. The studies in one field frequency supplement or confirm the information derived from the other. These informations are summarized as mutual exclusion principle.

2.3.1 Mutual Exclusion Principle

The vibrations of the molecules may cause both infrared absorption and Raman scattering, limited by the selection rules. For a molecule having centre of symmetry, the IR active vibrations will be Raman inactive and vice versa. This is known as mutual exclusion principle [13,48]. The totally symmetric vibrations are
always Raman active. However, the vibrations will be strong in IR, if the bond is ionic and strong in Raman if the bond is covalent. For covalent bonds, the relative Raman intensities for stretching vibrations of C-C, C=C and C≡C bonds are in the ratio 1:2:3 nearly.

The Raman spectra of the sample in aqueous solution can be recorded without major interference of water vibrations. However, IR spectrum suffers from strong absorption of water. The Raman spectra of hygroscopic compounds can be recorded in sealed glass tubing. This is not possible in IR, since glass tubing absorbs infrared radiation. The IR and Raman measurements thus complement each other. To obtain the complete vibrational picture of a molecule, both the techniques are needed. This exhibits their togetherness in elucidating the structural informations of complex molecules.

2.3.2 Information Derived from IR and Raman Spectroscopy

Much information has been derived from both IR and Raman spectroscopy, since vibration of the molecule may cause both infrared absorption and Raman scattering. The symmetric as well as the bonding nature provide informations regarding vibrational spectra.

Symmetric vibrations are always Raman active. Ionic bonding molecules are strongly IR active, where as covalent bonding molecules are strongly Raman active. From these informations, one can identify the type of bonding available in a particular molecular system using vibrational analysis. Information related to symmetry of vibration is usually obtained from the depolarization ratio, which is available in Raman spectra alone.
Since laser beam is used in Raman spectra, a small quantity of sample is enough to record the spectrum. Moreover, samples in aqueous solution can be recorded without major interference from water molecules. However in Raman spectroscopy, local heating, photodecomposition and fluorescence are the common consequences of the laser source that masks weak Raman lines. Resolving power of the Raman instrument is low in the UV-visible region, where the rotation-vibration spectrum is observed. Since both IR and Raman techniques are unique in nature, the complete vibrational picture of the molecule is obtained by interpreting both the spectra.

2.4 SURFACE ENHANCED RAMAN SPECTROSCOPY

In normal Raman scattering technique, the scattering cross section is small for most of the molecules. The fluorescence in molecules of interest further compromises detectability, since the luminescence emission obscures weak signals. These problems have been reduced to a great extent by modernization of instrumental components like lasers, imaging detectors, counting electronics and digital spectral processing. The major break through in Raman detection capability took place with the discovery of surface enhanced Raman spectroscopy (SERS), when anomalous scattering intensity of pyridine absorbed on roughened silver electrode was reported in 1974 [49].

Since then, surface enhanced Raman scattering has evolved as an important laser spectroscopic characterization technique, applied to biomedically significant molecules to study structural-functional properties. Enormous enhancement of Raman signals from molecules adsorbed on rough metallic surface and quenching of fluorescence have made SERS a very attractive
technique over normal Raman spectroscopy [50]. SERS is therefore widely used to elucidate information about the behavior of biomolecules adsorbed by the metal surfaces, orientation of adsorbed species and the changes in the orientation induced by external factors [51-53]. Further researches showed that the Raman scattering cross section is enhanced $10^5 - 10^6$ times as a result of surface adsorption [54].

2.4.1 Theoretical Background

The explanation to enormous enhancement of Raman scattering cross section of the molecules adsorbed on rough metallic surface is based on electromagnetic (EM) and charge transfer (CT) theories [55]. Accordingly, the Raman Stokes component of dipole moment is given by:

$$\mu_{RS} = \alpha_{RS} E(r, \omega) \exp \left[ -i(\omega - \omega_s) t \right]$$

(2.19)

where, $\alpha_{RS}$ is the Raman Stokes polarizability, $E(r, \omega)$ is the local field intensity, $\omega$ is the incident laser frequency and $\omega_s$ is the frequency of molecular vibrations.

The combination of electromagnetic and chemical effect between the molecule and the surface will enhance the total cross section. The electromagnetic effect acts through the local field and the chemical effect through the molecular polarizability. The contribution of each mechanism to the total enhancement depends on the optical and chemical properties of the surface, type of molecules absorbed, sample preparation and excitation characteristics.

Electromagnetic enhancement results due to surface plasmon resonance when laser beam falls on the rough metallic spheres. The incident field $E_0(\omega)$
induces a dipole moment $P(\omega)$ at the centre of the sphere. This will cause surface polarization resulting in the creation of large local field near the surface [56]. In turn, the induced dipole moment creates a field $E(r,\omega)$ at the molecular location $(r)$ and therefore the molecule experiences an effective field given as:

$$E_{\text{eff}}(r,\omega) = E_0(r, \omega) + E(r,\omega)$$  \hspace{1cm} (2.20)

When the molecules cover the entire metallic sphere, the enhancement factor is given by:

$$G = |(1+2g)(1+2g^0)|^2$$  \hspace{1cm} (2.21)

where, ‘$g$’ and ‘$g^0$’ are the values of function $\{(\varepsilon-1)/(\varepsilon+2)\}$, calculated respectively at incident and scattered frequencies. ‘$\varepsilon$’ is the ratio of the complex dielectric constant of the material of the particle to that of the surrounding medium. The best SERS spectrum will be resulted if the frequency of the emitted radiation matches with the plasma resonance frequency of the colloidal particle.

Chemical enhancement results due to adsorption that induces modifications in the polarizability [57,58]. Adsorption may be due to physisorption and chemisorption. In physisorption, the molecular dipole moment induces an image dipole of opposite polarity on other side of the surface. The two dipoles attract each other resulting dipole-dipole interaction, causing an association between the molecule and the surface. In chemisorption, a weak chemical bond is formed between the molecule and the metal. The possibility of chemisorption will be high, if the molecule has lone pair of electrons. A weak bond is formed when the molecule is chemisorbed, but stronger than the physisorbed one. Chemisorbed molecule will give metal-adsorbate stretching band in the low frequency region.
Due to adsorption, a charge transfer occurs between the ground state and excited states. In the ground state charge transfer, the charge transfer takes place between the molecule in the ground state and empty state in the metal. The charge transfer is modulated by the molecular vibration and the corresponding polarizability change will cause the enhancement of the associated vibrational mode. In the case of excited state charge transfer, the electron below the Fermi level in the metal is excited to an unoccupied state of the adsorbed molecule. Resonance can occur when the energy of the incident photon equals the energy of the charge transfer transition.

### 2.4.2 Selection Rules

In SERS, selection rules are based on the adsorbed molecules on a colloidal metal sphere. Spectroscopists use a set of thumb rules to explain SERS spectra instead of the rigid set of selection rules used previously [59]. Accordingly, vibrations involving larger components of dipole moment normal to the surface will be enhanced more. When a molecule is adsorbed flat, the out of plane bending modes are more enhanced. For the vertical orientation of the molecule, the in-plane bending modes are enhanced. When the in-plane and out-of-plane bending modes are equally enhanced, the molecule will have a tilted orientation. Also, the vibrations involving atoms which are directly bonded to metal surface or very close to the metal surface will be enhanced more. When an asymmetric stretching mode is enhanced, the shift from the normal frequency is large, but the frequency shift is small for symmetric mode.

For each adsorbate, there is an optimum concentration at which the enhancement is high. At this stage, a monolayer of adsorbate is formed on the
metal surface. When concentration increases, multilayer is formed and bulk properties will be resulted.

2.4.3 SERS Microprobe

SERS spectra are usually recorded with the help of the instrument used for obtaining normal Raman spectra. The laser power is maintained below 50mW to prevent photo destruction of samples by employing multichannel detection system. Detailed information of biomedical problems can be obtained by incorporating a microscope in the Raman scattering spectrometer. This enables the study of the surface of supramolecular biological complexes. Focusing the laser beam with the help of the objective lens of the microscope into an extremely small spot makes it possible to obtain the SERS spectra of very small volumes of matter. As the beam is scanned over the surface, the two dimensional spatial distribution of SERS of each wavenumber is obtained. The possibility of detection and identification of biomolecules on surfaces made it a very sensitive spectroscopic method of surface science and trace analysis.

2.4.4 Sample Preparation and Recording of Spectrum

In SERS experiments, different types of metal surfaces are used such as cold deposited metal films, metal electrodes and metal sols. Metal sols are normally used for sample preparation. They are the particles of diameter in nm, prepared by reducing the metal salt in solution by suitable reducing agent. The most common substrate used for SERS are the colloidal suspensions of Ag and Au particles (~5-20 nm in diameter).
Silver hydrosols can be prepared by two methods [60]. In the first method, the silver sol is prepared by reducing silver nitrate (AgNO$_3$) solution with sodium borohydride. The resulting solution is yellowish in colour and shows an absorption peak at 398 nm. This method allows the SERS study of biomolecules at concentrations down to $10^{-9}$ mol, with small amount of sample ($1\,\mu$l). In the second method, silver nitrate is reduced using sodium citrate resulting in silver hydrosol. The sol is greenish yellow in colour and shows absorption peak at 420 nm.

Spectral analysis appears to be a powerful tool in the investigation of the adsorption behavior of molecules on the surface of SERS active metals because the vibrations of molecules may change significantly at solid-liquid interface [61,62]. It is possible to determine the geometry of the adsorbate and the groups being bonded to the surface. The groups that are directly involved in adsorption in general could be recognized by sufficiently large ‘$\Delta \nu$’ values and also by enhancement of the intensity [63]. The latter parameter increases more significantly if the vibrational mode of the adsorbate has a large polarizability component perpendicular to the surface. From SERS spectra, the information on the strength of adsorbate-surface interaction and the information of the adsorbed molecule on the substrate can be obtained.

2.4.5 Applications of SERS

Surface enhanced Raman spectroscopy has been found to be very useful for trace analysis of biomolecules. Strong intensification of the Raman signal, reproducibility and stability over time finds application for the determination of biomolecules in low concentrations within nano structured substrates [63,64]. SERS can be applied for the identification of pharmacologically active substances.
in their qualitative and quantitative analysis and structural determination [65-69]. The interaction of organic and inorganic molecules with metal surfaces can be investigated by means of SERS, which are of considerable importance in coordination chemistry and possible application in corrosion protection [70]. The capability of detecting molecules such as crystal violet at very low concentrations makes SERS a promising tool for monitoring the illicit use of the compound in aquaculture industry [71].

Surface enhanced Raman spectroscopy can also be engaged in the field of art/archaeology for the detection of dye stuffs, high purity ceramics, colour pigments, etc. [72-74]. Anthrax and glucose sensors have been developed and their activities have been investigated by surface enhanced Raman spectroscopy [75,76]. In view of its enormous applications even down to the molecular level, it is reasonable to infer that SERS is far superior to the conventional Raman spectroscopy.

2.5 GEOMETRICAL FEATURES AFFECTING VIBRATIONAL MODES

Since molecules are harmonic oscillators, any disturbance will lead to deviation from its equilibrium state. This leads to vibration of molecules in such a way that the motion can be considered to be a superposition of a number of simple harmonic vibrations, called normal modes of vibration. Some of the factors that affect the normal modes of vibrations are detailed below.

2.5.1 Group Frequencies

Group frequencies are vibrations that are associated with certain structural units and appear in fairly constant region in the spectrum [77,14]. They are
described in terms of the motions that the nuclei in a structural group in the molecule undergoes vibration. The approximate constancy of the position of group frequencies forms the basis for the structural analysis of compounds. The relative constancy of spectral positions of group frequencies depends on the following factors.

(i) Masses of atoms of the molecule
(ii) Force constants of bonds between the atoms.
(iii) Symmetry of bonds between atoms.
(iv) Interaction of vibrations

When these factors remain reasonably constant for related vibrations in a series of compounds, a characteristic group frequency will be found. Factors affecting group vibrations are:

(i) Internal factors involving changes in atomic mass, vibrational coupling, resonance field effects, hydrogen bonding, bond angle strain etc.
(ii) External forces involving physical state (gas, liquid, solid, solution, solvent and concentration) and temperature. Often one factor is isolated from the rest, so that its influence upon one particular group frequency can be studied [14].

2.5.2 Anharmonicity

For small oscillations of atoms in molecules, the potential energy at equilibrium configuration, neglecting higher order terms can be expressed as:

\[ V = \frac{1}{2} \sum_i \sum_j \left( \frac{\partial^2 V}{\partial q_i \partial q_j} \right) q_i q_j \]  

(2.22)
This is the harmonic term. But in real systems, the contribution of higher order terms to the potential function becomes appreciable [14]. The deviation of potential harmonic form causes violation of selection rule, $\Delta n=\pm 1$, leading to mechanical anharmonicity. This will induce simultaneous transitions of more than one modes, giving rise to combination band. This will also cause unequal spacing of energy levels and activation of overtones. The electrical anharmonicity is caused by the non linear change in polarizability or dipole moment.

2.5.3 Overtones and Combinations

While deriving the selection rules for Raman and IR spectra, the normal vibrational modes are assumed to be harmonic in which case only fundamental modes would be expected. When anharmonicities in potential energy and polarizability or electric dipole moment are taken into account, overtones and combination bands are also permitted. In Raman spectra, such bands are very weak and less in number when compared with the IR spectra.

The anharmonic terms in potential energy lead to the mechanical anharmonicity. Due to this, an overtone may occur if the fundamental is active and combinations which involve at least one active fundamental are allowed. Anharmonic terms in polarizability or electric dipole moment contribute to electrical anharmonicity. In this case, the overtones and combinations are active even if the fundamental vibration is not active [36,78,79]. The electrical anharmonicity may be present even if the mechanical anharmonicity is absent.
2.5.4 Intermolecular Hydrogen Bonding

Hydrogen bonding is a donor-acceptor interaction involving hydrogen atom [33]. To form a hydrogen bond D-H· · ·A, the electronegativity of ‘D’ relative to ‘H’ should be greater enough to withdraw electrons, leaving the protons unshielded. To interact with ‘D-H’, the acceptor ‘A’ should have lone pair electrons or polarizable \( \pi \)-electrons. The intermolecular hydrogen bonding produces geometrical perturbations and causes changes in the vibrational characteristics.

The hydrogen bond causes the shifting of D-H stretching wavenumbers to lower values (red shift) which is followed by band broadening and increase in intensity, depending upon the strength of hydrogen bonding. Opposite behavior is observed for C-H· · ·O interactions for which the C-H stretching wavenumber increases followed by the decrease of C-H bond distance, known as ‘blue shifting of hydrogen bond’ [33].

The general trend of hydrogen bonding interaction is to shift the D-H deformation band to higher frequency. Some new bands corresponding to H· · ·A stretching and bending may appear in the low wavenumber range. Small shift in vibrational frequencies for modes involving hydrogen bond acceptor atom can also be observed. Since the D-H stretching modes are pure and relatively free from vibrations of molecules, the vibrational spectroscopy provides a powerful technique to understand the nature and strength of hydrogen bonding.

2.5.5 Intramolecular Hydrogen Bonding

Intramolecular hydrogen bonding is formed between donor and acceptor atoms in the same molecule, when the molecular configuration brings them within the hydrogen bond geometry [80,81]. Intramolecular hydrogen bonds are formed
in all three phases, but in crystalline state, their strength competes with that of intermolecular hydrogen bonds. The carbohydrates are rich in intramolecular hydrogen bonding due to the presence of hydroxyl groups attached to the adjacent carbon atoms. The vibrational spectral consequences of such interactions can be noticed in the spectra, such as the appearance of H· · ·A stretching band and the shifting of D-H stretching band position.

2.5.6 Back Donation

When the orbital of a bond is in the trans position with the lone pair of the adjacent electronegative atom, the electronic charge is back donated from the lone pair to the σ* orbital [82]. This will increase the electronic charge in the orbital. This will make the bond weaker, decreases the stretching force constant of the bond, increases the bond distance and the stretching vibrational intensity.

2.5.7 Induction

The presence of an electron attracting constituents attached to the carbon atom in the molecule causes the shifting of bonding electron pair, inducing polarity in the carbon atom as well as on the substituents [83]. The electron attracting substituents develops negative charge in it and produces negative inductive effect, whereas the electron releasing substituents causes positive inductive effect.

In C-H bond, stronger polarization occurs due to induction in molecules and the presence of electronegative atom. This effect reduces the distance of CH bond from the electronegative atom. Stronger polarization makes the charge on
hydrogen atom to increase. The CH stretching force constant increases whereas the CH bond distance and CH stretching intensity decreases.

2.5.8 Hyperconjugation

It is the interaction of orbital of a single bond with the $\pi$-orbital of an adjacent double/triple bond. In CH bonds, the hyperconjugation causes the releasing of electronic charge from a $\sigma_{CH}$ to the C-C bond [83]. As a result, C-C bond length decreases and the bond strength increase. Due to the release of electronic charge from $\sigma_{CH}$, the hydrogen becomes more acidic due to increase of charge on hydrogen. This will increase CH bond ionicity and CH bond strength. But due to the decrease of electronic charge in $\sigma_{CH}$ bonding orbital, there should be decrease in CH bond strength. Due to suitable balance between these two opposing effects, the bond length and spectral characteristics of CH bonds of the hyper conjugated systems do not show direct correlation with the charge on hydrogen.

2.5.9 Conjugation

The conjugation effect operates on systems with conjugate $\pi$-bonds, alternate ‘$\sigma$’ and ‘$\pi$’ bonds, in which the electron displacement is relayed through the $\pi$-electron system. The vibrational spectral influence of conjugation differs for various systems.

For an aliphatic conjugation of carbon-carbon double bonds, the splitting of C=C stretching band is resulted [84]. The conjugation of the carbonyl group causes appreciable shift in C=O stretching band position and hence most of the ketones produce C=O absorption band in the lower wavenumber range, 1680-
For unsaturated aldehydes containing double bond in the ‘α’ or ‘β’ positions show a fall in the carbonyl frequency due to conjugation. Aromatic ring in conjugation with aldehyde groups also show marked effect on band position and hence C=O absorption occurs in the range 1710 – 1695 cm$^{-1}$ [36,85].

### 2.5.10 Non Bonded Interactions

The most important geometrical characteristics of non-bonded interaction are the non bonded distance, which is less than the sum of the Van der Waals radii [86]. These ‘short contact’ interactions may be attractive or Van der Waals repulsive. The Van der Waals repulsion between two neighboring hydrogen atoms in a molecule causes twisting of phenyl ring as well as the rotation of methoxy groups [87,88]. The vibrational spectral consequence of the above types of interaction is to shift the vibrational stretching frequencies for the bands that involves in non-bonded interaction.

### 2.5.11 Intramolecular Charge Transfer

Compounds having electron donor and electron acceptor at the end positions of the π-conjugated system is related to the existence of large intramolecular charge transfer (ICT) responsible for second order NLO activity [89]. The ICT causes the electron releasing effect in the acceptor moiety, affecting the spectral modes. In C=C stretching mode, the acceptor subunit occurs at lower wavenumber compared to the corresponding mode of the donor subunit [90]. For the conjugated path, ICT induces large variation in the dipole moment as well as in the molecular polarizability simultaneously during vibrations. This produces the IR and Raman activity for the same mode and
hence comparable intensities of IR and Raman bands arise from the vibrations of conjugated system [90]. The electron donating effect of donor unit also causes wavenumber shifting for the vibrations of the donor group.

2.6 RECENT ADVANCES IN VIBRATIONAL SPECTROSCOPY

2.6.1 IR and RAMAN Spectroscopy

Vibrational (infrared and Raman) spectroscopy has played and is playing a very important role, as it is one of the most efficient method of molecular structure analysis, with the help of which direct information about the chemical compounds can be obtained. Among the rapidly increasing chemical, physical and biological investigations, the number of molecular structure studies of organic and semi-organic compounds is also growing year by year. Recent rapid development in vibrational spectroscopy has greatly encouraged its application to analytical chemistry, pharmaceutical industry, tissue research, biomedical research and applications, forensic science, diagnostics and environmental science [91-95]. This is mainly due to the recent developments in instrumentation and the easiest sample handling practice of the IR and Raman spectroscopy tools.

Spectroscopic method of identifying compounds is a suitable method for probing relationship between structure, dynamics and function of biomolecules. The investigation of chemical compounds has focused fundamentally on the chemical bond between the metal and its environment. Mink et al. [96] have studied the chemical bond between the metal and its ligands through FT-IR and Raman analysis. A new and simple normal-coordinate treatment using rigid body approximation for n-complexes has been suggested to calculate metal-ligand force constants. Moreover, degree of substitution and the structural changes can
also be determined by IR and Raman spectroscopy [97]. In fast reactions (microwave enhanced), usually the determination of optimal endpoint fails because the conventional analytical methods are too slow. However, Heller et al. [98] have established a fast method using FT-IR spectroscopy to monitor the reaction control analysis within less than 1 min. With the aid of Raman spectroscopy, modification of porphyrin structures results in various prophyrin-binding modes to nucleic acids which serve as appropriate model to identify DNA-related anticancer action of drugs were confirmed [99]. Cooling the samples allowed characterizing solid oligonucleotides such as dimers, trimers and pentamers of cytidine in the IR range of the out-of-plane bending molecular modes. Well-resolved NH\textsubscript{2} bending mode should provide important information on the exact chemical form and hydrogen bonding interactions of cytidine amine groups. IR spectroscopy is suggested as a practical analytical tool to validate and characterize synthetic DNA bases and oligonucleotides [100].

Identification of cells, tissues, bacteria by spectroscopy tools at a single cell level lead to quantification of diagnosis. Brain tissue is characterized by high lipid content. Its content decreases and the lipid composition changes during transformation from normal brain tissue to tumors. Therefore, the analysis of brain lipids might complement the existing diagnostic tools to determine the tumor type and tumor grade [101]. Micro Raman spectra of single yeast cells are used for the analysis of hierarchical cluster to identify single cells during the lag phase of its growth [102,103]. Study on pigmented bacterium using Raman spectral investigations have been reported for cell population heterogeneity [104].

The medical applications of infrared technology involve a variety of tasks: oncology, pain, vascular disorders, arthritis, rheumatism, surgery, tissue viability,
dermatological disorders, monitoring the efficiency of therapeutic drugs, etc. This technique is suitable to identify viruses with cervical screening methods [105] and to detect the damage cells of lungs [106]. Spectroscopic studies can also be extended to the study of Intracranial tumors [107] and the molecular changes associated with glioma tissues [108]. Today, infrared imaging is proving its ability to assist the surgeon in making real time decisions during the open heart surgery procedure. It gives valuable information during the operation by providing important information regarding coronary flow, coronary anatomy and myocardial perfusion and function [109]. Alterations in the lipid and protein components have been found to be responsible for the change in the Raman spectra of erythrocyte membranes upon varying pH and temperature, whereas specific changes in the C-H stretching region are reported to be related to the alterations in the integral protein environment [110]. Metabolic fingerprinting has been forwarded as a potential tool for disease diagnosis using infrared and Raman spectroscopies, which involves rapid, high-throughput global analysis to discriminate between samples of different biological status or origin [111]. Broad ranges of vibrational spectroscopic applications with the focus on imaging and fiber-optical methods are discussed to study and characterize diseases, tumors and other pathologies [112]. Vibrational spectroscopy is increasingly used for the investigation of biological tissues: spectral analysis reveals hidden information at microstructural and molecular levels. Moreover, in vivo and ex vivo applications have established the potential of these techniques for detecting and diagnosing different types of lesions. For in vivo diagnosis, Raman spectroscopy permits a non-destructive analysis, unhindered by the aqueous nature of tissues. It does not require any previous sample preparation or labeling. In addition, it is a rapid technique.
enabling real-time spectral acquisition. In 1997, M.G. Shim and B.C. Wilson [113] described a fiber-based Raman system and presented in vivo spectra from buccal cheek epithelium, human skin, fingernail and tooth. Since then, several other in-vivo studies have been done on atherosclerotic plaque [114], skin [115], colon and esophagus [116].

Nano researchers can now exploit the key tools of Raman to probe the composition, band gap and particle size of the new generation functional nanomaterials [117-119]. Electrochemical polymerization of 2, 2'-bithiophene (BTh) on single-walled carbon nanotube (SWCNT) films has been studied by Raman scattering and infrared absorption spectroscopy. Systematic peaks in specified location indicate the charge transfer process, functionalization and steric hindrance produced by the nanotube binding [120]. Nanoparticles of metals, oxides and sulfides have been developed and used as catalysts for hydrocarbon conversion, partial oxidation and combustion reactions. Similar to metal nanoparticles, polymers can be synthesized and confirmed by FT-IR and Raman spectroscopy [121]. Raman studies of nanocrystalline titanium oxide prepared by sol-gel method revealed peak shift and broadening which were attributed to the confinement of phonons in the anatase crystallites [122]. Ablation of mixture targets of perylenetetracarboxylic dianhydride (PTCDA) with cobalt powder is carried out using the third harmonic beam of an Nd:YAG laser to obtain thin films consisting of various sizes of nanoparticles. FT-IR and Raman spectroscopic studies are used to confirm final fragments without the anhydride groups of PTCDA [123]. Fourier transform infrared microspectroscopy was used to nondestructively measure stress in ceramics by inspecting external reflectance over spatial areas as small as 100 µm [124].
Raman and infrared spectroscopy are two complementary spectroscopic techniques that can produce fast, efficient and accurate detection of the pigments and/or binders used in automotive topcoats. Besides the industrial importance, the analysis of automotive topcoats is of great significance for forensic science, especially for the investigation of hit-and-run accidents [125]. Raman, infrared and XRD analysis have been applied to the examination of deterioration on historical monuments [126]. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was used for the examination of historic blue pigments and blue tempera paintings commonly found on works of arts [127]. Recent developments in spectroscopic techniques have made them popular for solid phase analysis; they are fast, accurate and suitable for real-time measurements during processing, and further, they can be used to obtain structural understanding of solid forms [128]. During the manufacturing process of clinical drug candidates, foreign materials may be introduced into biopharmaceutical products and result in non-conformance incidents. The purpose of forensic analysis is to identify the foreign materials and find their root causes. FTIR microspectroscopy plays a key role in forensic analysis by providing information on the functional groups present [129]. Motor gasoline adulteration can be identified using FTIR spectroscopy and multivariate calibration without the need for using chromatographic separation or other expensive instruments [130].

Industrial applications of IR and Raman spectroscopy have been of intense interest due to introduction of respective instrumentation. The utilization of online spectroscopic methods for the development and the monitoring of chemical processes are demonstrated to obtain the information about a reaction system faster and more efficiently than with conventional methods. This allows
the rapid optimization of safe chemical processes and provides information for process control [131]. Raman spectroscopy is also found useful in petroleum industry [132], in refining of metal ores [133], and in paper industry [134]. It is also found useful in sea and fresh water analysis [135], to determine the pesticides in fruits and vegetables [136,137]. Corrosion and film formation over surfaces were identified by the electrochemical analysis by engaging Raman instrumentation [138].

2.6.2 Surface Enhanced Raman Spectroscopy

SERS has become a useful tool for probing the behavior of molecules adsorbed on metal surfaces. In order to achieve large SERS enhancements, molecules must be adsorbed on the surface of efficient substrates. Several studies demonstrating highly reproducible SERS data have been recently reported [139-141]. Silver sols results generally in surface enhanced Raman intensities that exhibit limited reproducibility. Nanostructured gold surfaces form SERS-active substrates that exhibit both high sensitivity and improved reproducibility.

Surface enhanced Raman spectroscopy has been found to be very useful for trace elemental analysis. It is often applied to investigate biomolecules at very low-concentrations [142]. Simple fabrication procedures for the preparation of Ag/Au nanoalloys on Si/SiO$_x$ substrates, with tunable plasmon resonances have been reported [143]. The mechanism and kinetics of the nano-alloy formation and its optical properties were studied by SERS. Surface enhanced Raman scattering was employed to study phospholipid molecules adsorbed on nanometre-sized Au structures [144]. Howes et al. [145] studied the influence of pH and anions on the
adsorption mechanism of the widely used antibiotic rifampicin on silver colloids. Observed spectrum revealed the formation of an ion pair between rifampicin and Cl\textsuperscript{−} anions adsorbed on the Ag surface.

By imposing selection rules on SERS, predictions about the bonding situations, geometry and orientation of the species can be made. Therefore SERS are engaged for structural investigations of biological molecules [59,146]. SERS study on N-acetyllalanine monolayer, self assembled on a silver surface was performed by Yang et al. [147]. The monolayer of N-acetyllalanine on the metallic surface served as biocompatible interface for biosensing. The usefulness of aluminium and nanoporous alumina platforms for detecting soft biospecies ranging from bacterial spores to protein markers have been confirmed using SERS [147]. Quantification of pathogenic organism has also been investigated [148,149]. S. Basu et al. [150] reported the interaction between nanoscale silver particles and various DNA bases (adenine, guanine, cytosine, and thymine), which are used as molecular linkers because of their biological significance. In colloidal solutions, the color of silver nanoparticles may range from red to purple to orange to blue, depending on the degree of aggregation as well as the orientation of the individual particles within the aggregates.

SERS of the plant materials like alfalfa seeds, green tea leaves, carrots and red cabbage on colloidal gold and silver surfaces was reported by Zeiri [151]. Observed SERS bands were attributed to nicotinamide adenine dinucleotide and other adenine-containing materials. Additional bands were assigned to flavins, lipids and other biocomponents. The capability to detect molecules such as crystal violet at very low concentrations make SERS sensor, very promising for monitoring the diffuse illicit use of the molecule in aquaculture industry [152].
SERS study of red dye laccaic acid (highly fluorescent anthraquinone dye), which is of great importance in the history of early textile and lake pigments, has been performed by Canamares and Leona [153]. Interaction between the red dye and ovalbumin has been studied with SERS and the interaction is found weak which takes place through alizarin in neutral form [154].

Yuan et al. [155] reported a new method of detecting heavy metal ions by SERS. Manimaran and Jana [156] reported on the synthesis of 2-5 nm size gold nanoparticles labels for SERS-based immunoassay shown to detect protein molecules. Observations of single molecule detection on thionine and its dynamic interactions on aggregated gold nanoparticle clusters using SERS and the observed Raman intensities were found to be independent of the size of the Au particles [157]. Kudelski [158] discussed some aspects of SERS temporal fluctuations by analyzing the intense spectra of hydrogenated amorphous carbon deposited on Silver. The influence of some electrolytes on the SERS spectral fluctuations was also discussed. Strained silicon used as a substrate for metal-oxide-semiconductor (MOS) transistors have been analyzed for applications in many areas of science and technology [159].

Vibrational spectroscopic investigations can also be extended to arts and archeology for non-destructive identification of glass beads, pearls, porcelain, pottery, textiles, ceramics, shards, paintings including wall and rock paints, frescos, medieval miniatures, contemporary prints, corrosion product, musical instruments and fossils bones [160]. Advances both in technology and instrumentation in vibrational spectroscopy will find new and unimaginable frontier research areas in the coming years.
Infrared and Raman spectroscopy represents one of the most useful tools for obtaining information about the structure and properties of molecules from their vibrational transitions, particularly about the strength of bonds in molecules. Theoretical simulations can certainly assist in obtaining a deeper understanding of the vibrational spectra of complex molecules. Comparison of the experimental and quantum chemically calculated Raman wavenumbers allow for a reliable assignment of the experimentally observed Raman bands, leading to a detailed understanding of the geometrical and electronic structure of the investigated molecular compounds. This thesis covers a summary of research work carried out on the quantum chemical simulations and vibrational spectral investigations of certain complex molecules and novel organic nonlinear optic materials with specific properties applicable to medical/biological and for optical computing/communication. The organic and semiorganic molecules investigated are the following.

The non-linear optically active compound p-hydroxybenzoic acid is widely used as a preservatives in food, cosmetic and pharmaceutical products. Acute, subchronic, and chronic studies in rodents indicate that PHBA is practically non-toxic. They are rapidly absorbed, metabolized and excreted. The ability of para-hydroxybenzoic acid to trans-activate the estrogen receptor in vitro increases with alkyl group size [161]. Previous reports are not available with respect to their structural confirmation using vibrational spectral analysis and density functional theoretical methods. However, thermal studies of the material have been carried out to estimate the vapor pressure characteristics using the Antoine equation as the analytical tool [162]. Lack of spermatotoxic effects of methyl and ethyl esters
of p-hydroxybenzoic acid was confirmed in rats by analyzing the secretion of sex hormones [163]. *Ab initio* calculation (AM1 and MNDO-PM3) of the rotational barriers in PHBA was performed to yield a very important and practical result for modeling [164]. F. Rodante and G. Pistoia have studied the enthalpic and entropic contributions to substituent effects for the ionization of *meta-* and *para-* hydroxybenzoic acids in water-dimethylsulfoxide mixtures at 25°C [165, 166].

The pharmaceutically important non-linearly active compound para-bromo acetanilide, possesses pain relieving activity by blocking the pulse, dissipating along the nerve fiber [167] and has the potential role in the energy transport in biological system [168]. The proton transfer along intermolecular hydrogen bonding using *ab initio* computations has recent applications [169]. The restricted rotation about the carbonyl-nitrogen bonds of acetanilides has been investigated by means of high performance liquid chromatography [170]. Conformations of acetanilide were studied by ultraviolet photoelectron spectroscopy [171]. The photoelectron spectroscopy of acetanilide has been used to investigate the intermolecular vibration and internal rotation of methyl group [172]. The dynamic location in crystalline acetanilide has been analyzed using neutron scattering and computer simulation [173]. The N-H· · ·C=O hydrogen bond plays a key role in determining the conformations of proteins [174]. Every protein molecule consists of a series of secondary amide linkages and hence special attention has been devoted from both experimental and theoretical point of view [175].

Caffeic acid (3,4-dihydroxycinnamic acid), one of the most common phenolic acids, frequently occurs in fruits, grains and dietary supplements. These compounds have attracted great attention in medical research because of their anti-inflammatory and anti-mutagenic effects and antitumoral activity in different
cancer cells [176,177]. Ethyl-3-(3,4-dihydroxyphenyl)-2-propenoate prepared from the Knoevenagel condensation reaction of 3,4-dihydroxybenzaldehyde and monoethyl malonate is almost a planar molecule [178]. Photo induced coupling and polymorphism of cinnamic acid compounds have been studied using vibrational spectroscopy, NMR and SERS [179-181]. Recent efforts have been focused to develop organic molecules with large molecular nonlinear optical (NLO) response (β, first hyperpolarizability), improved optical transparency, and good thermal stability [182]. The π-electron cloud movement from donor to acceptor makes the molecule highly polarized. Moreover, the degrees of electronic charge delocalization along the charge transfer axis and by the low band gaps make the molecule to have high polarization. The efforts towards deeper knowledge about the relationships between molecular architecture, nonlinear response, and hyperpolarizability, using vibrational spectra of the molecules, can lead to discovery of new efficient materials for technological applications.

Sarcosine, a N-methyl derivative of glycine belongs to a group of biologically important compounds. Sarcosine and other related amino acids are known to accelerate the rate of photosynthesis [183]; in addition, they are also important biological intermediators [184,185]. The spectral properties [186,187] and crystal structure [188,189] of such compounds and their derivatives have been extensively studied. Many studies have been reported about the IR spectral investigations of Sarcosine [190-194]. Savtos et al. [195] discussed the NH stretching vibrations of the metal complexes of this ligand. The Raman and IR spectroscopy of Sarcosine ligand with calcium chloride has been reported by Chen et al. [196]. Nakamoto et al. [197] reported the relation between the
carboxyl stretching frequencies and the strength of the metal-oxygen interactions about some metal complexes of both ligands. However, no reports related to the study of sarcosinium oxalate monohydrate have been reported so far.

Compounds derived from amino acids, often exhibiting weak Van der Waals interactions and hydrogen bonds, possess a high degree of delocalization and hence are expected to be more non-linear than their inorganic counter parts [198,199]. Furthermore, amino acids have peculiar physical and chemical properties which are attributed to the presence of an H-atom-donor carboxylic acid group (-COOH) and an H-atom-acceptor amino group (-NH₂). Due to this dipolar nature, amino acids and related compounds often have physical properties which make them potential candidates for non-linear optical activity [200]. The geometry of glycylglycine (Gly-Gly) has been investigated by X-ray and neutron diffraction at room temperature [201-203]. The X-ray charge densities have been used in the calculation of intermolecular interactions and lattice energies in the crystal of Gly-Gly [204]. The interaction between Gly-Gly and polyoxometalates has been examined for an understanding of their anti tumour and anti HIV activity [205,206].

Proteins are macromolecular polymers composed of amino acids as the basic unit that contain carbon, hydrogen, oxygen, and nitrogen. The amino acid L-histidine has been extensively studied because of the ability of its imidazole moiety to act as a proton donor, a proton acceptor and a nucleophilic reagent [207-209]. The metal complexes with several imidazole-containing ligands have also been widely studied as structural and model compounds of metal active sites [210,211]. L-Histidinium Dihydrogenmonophosphate Monohydrate crystallize in noncentrosymmetric space groups and therefore exhibits high NLO properties
due to the presence of imidazole group in addition to amino-carboxylate groups. The structure has been solved by Blessing [212] and IR spectrum by Espinosa et al. [213]. The crystal has 2024 independent reflections with \( \text{C}_6\text{H}_{10}\text{N}_3\text{O}_2^+ \) groups perpendicular to the b-axis alternate with \( \text{H}_2\text{PO}_4^- \) groups. The \( \text{H}_2\text{PO}_4^- \) groups are linked by hydrogen bonds to form infinite chains, which are themselves interconnected with the histidine planes through several other hydrogen atoms [214].

2.8 CONCLUSION

This chapter elaborates the spectroscopic tools used for vibrational analysis of organic and biomolecules with respect to structural optimization. It reports the extensive study of vibrational spectral features using infrared, Raman and surface enhanced Raman spectrometers. Explanations related to normal modes of vibrations, infrared absorption, Raman scattering, factors affecting vibrational spectra and the recent advances in the field of vibrational spectroscopy are summarized systematically.