CHAPTER 1
INTRODUCTION

1.1 FUNGICIDE

Fungicides are either chemicals or biological agents that inhibit the growth of fungi or fungal spores. Fungi can cause serious damage in agriculture. Fungicides are used both in agriculture (example: anilazine, benalaxyl, benomyl, flutolanil and mepronil) and to fight fungal infections in animals (example: clotrimazole) (Latijnhouwers et al., 2003). Fungicides can either be contact, translaminar or systemic. Contact fungicides are not taken up into the plant tissue and only protect the plant where the spray is deposited, translaminar fungicides redistribute the fungicide from the upper sprayed leaf surface to the lower unsprayed surface, systemic fungicides are taken up through roots from soil and redistributed through the xylem vessel to the upper parts of the plant. Fungicides help to increase the efficiency of production, by preventing or reducing damage to the growing crop or stored food and to improve the quality of the product by preventing rotting and a damaged appearance (Smart 2003).

1.2 CLASSIFICATION OF FUNGICIDES

The fungicides are classified by chemical structural grouping, they can be categorized agriculturally and horticulturally according to the mode of application. According to the origin of fungicides, fungicides are classified into two major groups.

1.2.1 Biofungicides

Biofungicides contain living microorganisms (bacteria, fungi) that are antagonistic to the pathogens that cause turf disease. Example: Ecoguard contains *Bacillus licheniformis*.

1.2.2 Chemically based fungicides

Synthesized from organic and inorganic chemicals, most of the fungicides that are sold throughout the world are chemically-based. They can be classified into three groups.

1.2.2.1 Chemical structure

All fungicides in a chemical group generally, control the same diseases. For example, the strobilurin fungicides provide good to excellent control of anthracnose, brown patch, gray leaf spot and summer patch (Burpee 2006).

1.2.2.2 Topical activity

Fungicides can be placed into one of the four groups based topical activity

(a) Contact fungicides

Contact fungicides act only on plant surfaces. They are not absorbed by leaves, stems or roots and cannot inhibit fungal development inside plants. Example: anilazine, dithiocarbamates, nitriles, phenylpyrolles, cyanoimidazoles.

(b) Localized penetrants

Localized penetrant fungicides are absorbed by leaves and move short distances within a treated leaf, they do not move from one leaf to another and they are not absorbed by roots. These fungicides inhibit fungi on treated plant surfaces and inside treated leaves. Example: dicarboximides, strobilurins.

(c) Acropetal penetrants

Acropetal penetrants can penetrate plants through roots and leaves. These fungicides are absorbed by the xylem and move upwards (acropetally) in plants. Acropetal penetrants inhibit fungi on and in treated plant surfaces and inside plant parts that lie above the treated surface. Example: benzimidazoles, triazoles, pyrimidines, carboximides, acylalanines, azoxystrobin and fluoxastrobin.

(d) Systemic fungicides

Systemic fungicides are the only fungicides that are absorbed into xylem and phloem and moves up and down in plants. These fungicides inhibit fungi on and in treated plant surfaces and inside plant parts that lie above or below the treated surfaces. Example: benalaxyl, benomyl, flutolanil and mepronil.

1.2.2.3 Mode of action

The body or thallus of most fungi exists as microscopic tubes called hyphae. A fungal cell contains many of the same organelles as other eukaryotes (Foster et al., 2010). Fungicides can be divided into 2 groups based on mode of action in fungal cells:

(a) Site-specific inhibitors

Site-specific inhibitors target individual sites within the fungal cell. Example: demethylation inhibitors fungicide, benzimidazole fungicide, carboxamides.

(b) Multi-site inhibitors

Multisite inhibitors target many different sites in each fungal cell. Example: nitrile fungicides, dithiocarbamates, peroxides.

1.3 AIMS AND OBJECTIVES OF RESEARCH WORK

The present investigation aims at the spectroscopic analysis of certain organic compounds with some specific biological importance. Structure-property relationships are qualitative or quantitative empirically defined relationship between molecular structure and observed properties. The work is substantially motivated on one hand by the fundamental theoretical importance of such systems and on the other hand by their relevant applicability on daily life. This work is aimed at investigating structure property relationship of fungicide molecules using FT-IR, FT-Raman, UV-visible, NMR, In-vitro and In-silico analysis. Computational chemistry tools can reliably predict interaction, energies, geometric structures and electronic properties. An important part of this work is the comparison between the quantum chemical calculations and the experimental data, in order to make unambiguous assignments of the various fundamental modes of vibration.

Structural elucidation and complete vibrational assignments of certain biologically active fungicidal compounds including Anilazine (Triazine fungicide), Benalaxyl (Phenylamide fungicide), Benomyl (Benzimidazole fungicide), Clotrimazole (Conazole fungicide), Flutolanil (Phenyl benzamide fungicide) and Mepronil (Phenyl benzamide fungicide) and their related compounds have been performed in this study. This work reveals an additional quantitative chemical knowledge of the formation, relative stability, bioactivity, geometry as well as more detailed insight into systemic difference between experimental and computational techniques.

1.4 RECENT TRENDS IN THE APPLICATION OF SPECTROSCOPY ON FUNGICIDE COMPOUNDS

Spectroscopy is used in almost every scientific research discipline, yet continuous improvements and refinements to the field are still enabling expansion into new industries. Perhaps the strongest trend of the past few years, as can be seen across numerous scientific fields and types of spectroscopy, is the push towards smaller, more efficient instrumentation that can perform measurements in situ, in vivo, and with minimal invasion (Nicole 2017). Vibrational spectroscopic techniques have an immediate appeal in the field of biology and medicine and have been used for the characterization of drugs and monitoring of the drug-delivery system (Singh et al., 2012).

FT-IR is already being used to identify microbes, but it has only recently been used to determine the species of fungi. Fungal infections in humans are difficult to treat, and the correct species must be identified for the correct treatment. The two fungi (C. neoformans and C. gatti) from the Cryptococus genus using attenuated total reflection FT-IR (ATR-FTIR) and multivariate analysis of the functional groups from each fungus (Fernanda et al., 2016). These two species cause infection of the central nervous system and the lungs when their spores are inhaled. This approach is faster and less labor intensive than traditional methods, and could be a cheap alternative to routine diagnostic analysis. Fungicides are widely applied agrochemicals and have been associated with developmental defects in amphibians; thus, it is important to determine chronic effects of environmentally relevant concentrations of such contaminants in target cells. Infrared (IR) spectroscopy has been employed to signature the biological effects of environmental contaminants through extracting key

features in IR spectra with chemometric methods. Herein, the Xenopus laevis (A6) cell line has exposed to low concentrations of carbendazim (benzimidazole fungicide) or flusilazole (triazole fungicide) either singly or as a binary mixture. Cells are then examined using attenuated total reflection Fourier transform IR (ATR-FTIR) spectroscopy coupled with multivariate analysis (Strong et al., 2016). Pesticides are natural or synthetic compounds, which are used to prevent, destroy or control diseases, pests and weeds or to adjust plants and insect growth. Recent technological advancements of Surface Enhanced Raman Spectroscopy (SERS) has promoted the creation of alternative detection techniques (Xu et al., 2017).

The rapid and sensitive detection of pesticide residue is essential for ensuring the food safety of consumers. However, there are many disadvantages for current approaches to detect pesticide residue treatment and low sensitivity. The meta material combines with Tera Hertz Time Domain Spectroscopy is a potential new approach for food quality and safety control (Qin et al., 2018). Pesticide resistance mechanisms can result in differences in pest metabolism. Near-infrared spectroscopy is a non-destructive technique that can reveal differences in the chemical composition of any target organism. This approach is principally used in clinical practice, where it has multiple applications, but it has also been shown to detect fungicide resistance rapidly and with high sensitivity (R4P network 2016).

Recently, the field of molecular spectroscopy has expanded into new and exciting biological, medical and sensing applications. This expansion results from both the improvements in existing instrumentation and the development of new techniques in the fields of Raman, fluorescence, and infrared spectroscopy. A broad

field encompassing several techniques, molecular spectroscopy has revealed fundamental chemical and biological information in a variety of applications. Although there are many different types of molecular spectroscopy, these process involve how analytes interact with light (Laura 2014).

1.5 SPECTROSCOPY

Spectroscopy is the interaction of light and matter that deals with a wide range of physical and chemical behaviour. It deals with the transition induced in a chemical species by its interaction with the photons of electro magnetic radiation (Jeanne 1999). It is used to refer the measurement of radiation intensity as a function of wavelength and correlated to the experimental techniques. Spectroscopy is a qualitative or quantitative tool of analysis for the determination of matter or physical process that involves the study of interaction between electromagnetic radiation and matter in the form of absorption, emission or scattering. The spectroscopic methods are used to identify the relation between molecular energy levels and the molecular structures. The combination of atoms into molecule leads to the creation of energy states and the transition between these states. The electronic excitations are studied using UV-visible spectroscopy. Distinct nuclear spin states and their energy separation are observed in NMR spectroscopy (Croush et al., 2007 & Smith 2011).

1.6 ELECTRO MAGNETIC RADIATION

Electromagnetic radiation is a form of energy that is produced by oscillating electric and magnetic disturbances or by the movement of electrically charged particles travelling through a vacuum or matter. The electric and magnetic fields come at right angles to each other and the combined wave moves perpendicular to both

magnetic and electric oscillating fields. The radiation is released as photons, which are bundles of light energy that travel at the speed of light as quantized harmonic waves. This energy is then grouped into categories based on its wavelength into the electromagnetic spectrum. These electric and magnetic waves travel perpendicular to each other and have certain characteristics including amplitude, wavelength, and frequency. When electromagnetic energy is released as the energy level increases, the wavelength decreases and frequency increases. Thus, electromagnetic radiation is then grouped into categories based on its wavelength or frequency into the electromagnetic spectrum. The different types of electromagnetic radiations shown in the electromagnetic spectrum consists of radio waves, microwaves, infrared waves, visible light, ultraviolet radiation, X-rays and gamma rays. The part of the electromagnetic spectrum that we are able to see is the visible light spectrum (Mc Quarrie et al., 1997). The names of different regions along with the order of the range of frequencies and wavelength are shown in figure 1.1

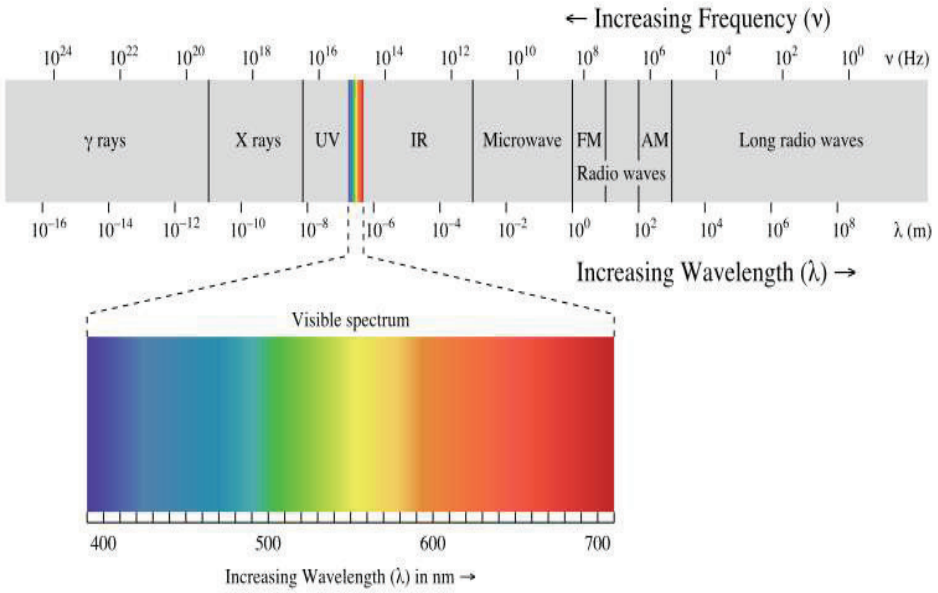

Figure 1.1 Electromagnetic spectrum

1.7 INFRARED SPECTROSCOPY

Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule. It is a form of vibrational spectroscopy which involves the absorption of infrared radiations on to the organic compounds. For identification of organic compounds the best region is between 400-4000cm^{-1} (Pavia et al., 2009 & Schradeer 2008).

1.7.1 Principle and selection rule of Infrared spectroscopy

Infrared radiation refers to the part of electro magnetic spectrum between the visible and micro wave region. The absorption of infrared radiation causes an excitation of molecule from a lower to a higher vibrational level, each vibrational level is associated with the rotational levels. Thus infrared spectra are considered as vibrational-rotational spectra.

For the absorption of quantum of radiation hν and the energy difference between the two states are represented by Ψ_i and Ψ_j. A molecule can absorb a photon of energy hν and corresponding transition states are $\Psi_i^*$and Ψ_j. The probability of transition from i^{th} state to j^{th} state is given by

$$\mu_{ij} = \int \Psi_i^* \mu \Psi_j \, d\tau \qquad \ldots\ldots (1.1)$$

$\mu\rightarrow$ 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, which can be expressed in Taylor series and is given by

$$\mu = \mu_0 + \left(\frac{\partial \mu}{\partial Q_k}\right) Q_k + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1.2)$$

Neglecting the higher terms and substituting (2) in (1)

$$\mu_i = \int \Psi_i^* \mu_0 \Psi_j \, d\tau + \int \Psi_i^* \left(\frac{\partial \mu}{\partial Q_k}\right) Q_k \Psi_j \, d\tau$$

$$= \mu_0 \int \Psi_i^* \Psi_j \, d\tau + \left(\frac{\partial \mu}{\partial Q_k}\right) \int \Psi_i^* \, Q_k \, \Psi_j \, d\tau \qquad \ldots\ldots (1.3)$$

The first term is vanished and second term is non zero. If $\left(\frac{\partial \mu}{\partial Q_k}\right)$ is finite at least for one component of dipole moment (ie.) for a mode of vibration to be IR active, the vibrational motion of the mode must change in dipole moment. Homo nuclear molecule has no dipole moment, so the vibrational spectra are only possible for hetero-nuclear molecule (Silverstein et al., 2005).

1.7.2 INFRARED SPECTROPHOTOMETER

The instrument that determines the absorption spectrum for a compound is called an Infrared Spectrophotometer. They are classified as Dispersive Infrared Spectrophotometer and Fourier Transform Spectrophotometer. Both of these types of instruments provide spectra of compounds in the common range of 400-4000 cm^{-1} (George et al., 1986).

1.7.2.1 Dispersive Infrared Spectrophotometer

Dispersive infrared instruments are sometimes called grating or scanning spectrophotometers. A dispersive infrared instrument also has source and mirrors. The source energy is sent through both the sample and the reference path, through the chopper to moderate the energy reaching the detector and directed to a diffraction grating. It separates range and directs each wavelength individually through a slit to the detector. Each wavelength is measured one at a time, with the slit monitoring the spectra bandwidth and the grating moving to select the wavelength being measured. Dispersion occurs when energy falling on the entrance slit is collimated on to the

dispersive element and the dispersed radiation is then reflected back to the exit slit. The width of the entrance and exit slits vary and are programmed to compensate for any variation of the source energy with wavenumber. In the absence of a sample, the detector then receives radiation of constant energy as the spectrum is scanned. Sources of infrared emission have included the Globar, which is constructed of silicon carbide. There is also the Nernst filament, which is a mixture of the oxides of Zirconium, Yttrium and Erbium. Most detectors have consisted of thermocouples of varying characteristics (Brittain et al., 1975 & Kiddes et al., 2002). Figure 1.2 shows the schematic diagram of the dispersive infrared spectrophotometer.

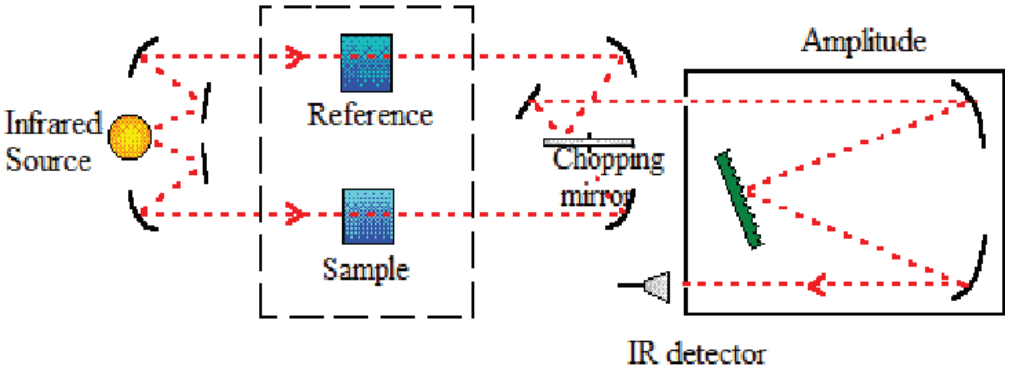

Figure 1.2 Schematic diagram of dispersive infrared spectrophotometer

1.7.2.2 Fourier Transform Infrared Spectrophotometer

The most modern infrared spectrophotometers operate on a different principle. The design of the optical pathway produces a pattern called an interferogram. An interferogram is essentially a plot of intensity versus time. A mathematical operation known as a fourier transform (FT) can separate the individual absorption frequencies from the interferogram, producing a spectrum virtually identical to that obtained with a dispersive spectrometer. This type of instrument is known as a Fourier transform infrared spectrophotometer.

The FT-IR uses an interferometer to process the energy send to the sample. In the interferometer, the source energy passes through a beam splitter, a mirror placed at a 45° angle to the incoming radiation, which allows the incoming radiation to pass through but separates it into two perpendicular beams, one undeflected, the other oriented at a 90° angle. One beam, the one oriented at 90° goes to a stationary or fixed mirror and is returned to the beam splitter. The undeflected beam goes to a moving mirror and is also returned to the beam splitter. When the two beams meet at the beam splitter, they recombine but the path length differences of the two beams cause both constructive and destructive interferences. The combined beam containing these interference patterns is called the interferogram. This interferogram contains all of the radiative energy coming from the source and has a wide range of wavelength. The interferogram generated by combining the two beams are oriented towards the sample by the beam splitter. As it passes through the sample, the sample simultaneously absorbs all the wavelengths that are normally found in its infrared spectrum. The modified interferogram signal that reaches the detector contains information about the amount of energy that has absorbed at every wavelength. The computer compares the modified interferogram to a reference laser beam to have a standard of comparison (Pavia et al., 2009). The schematic diagram of a Fourier Transform Infrared Spectrophotometer is shown in Figure 1.3.

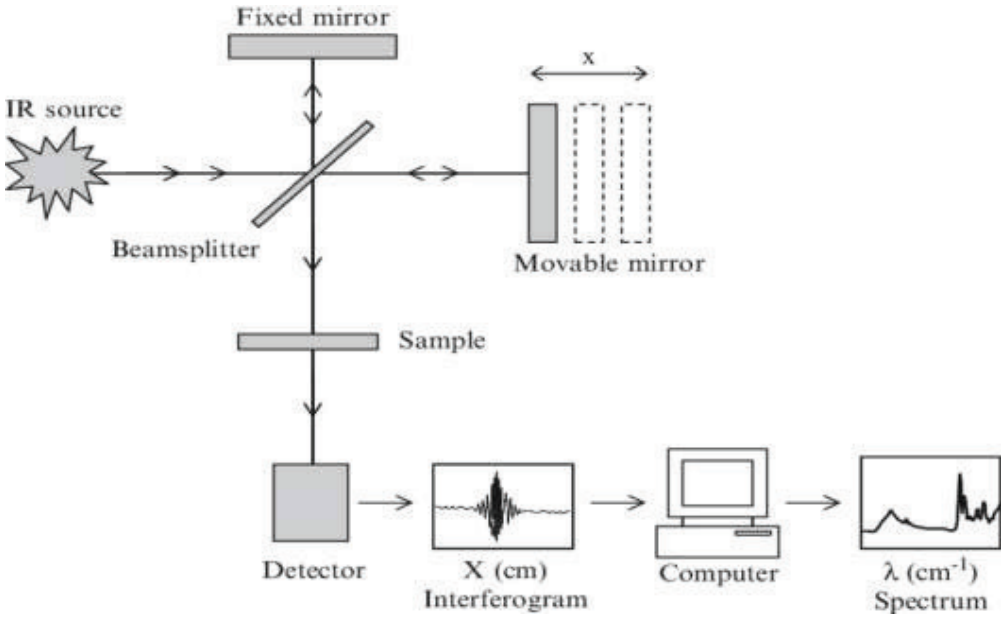

Figure 1.3 Schematic diagram of Fourier Transform Infrared Spectrophotometer

1.8 RAMAN SPECTROSCOPY

1.8.1 Classical theory of Raman effect

The classical theory is based on the wave theory of light. The classical theory of the Raman effect is based upon polarizability of molecules. The polarization P is proportional to the applied electric field.

$$P = \alpha E \qquad \ldots\ldots (1.4)$$

where α is the polarizability of the molecule.

According to the classical theory (Ian et al., 2001), when radiation of frequency 'υ_0' is allowed to fall on molecules, each molecule experiences a varying electric field. This field depends on the incident frequency is given by:

$$E=E_0\cos 2\pi \upsilon_0 t \qquad \ldots\ldots (1.5)$$

where 'E_0' is the vibrational amplitude. If the vibrational motion of the molecule 'q' is the normal coordinate associated with a particular mode of vibration frequency 'υ_m' of the molecule, the harmonic approximation of 'q' can be written as:

$$q = q_0 \cos 2\pi \upsilon_m t \qquad \ldots\ldots (1.6)$$

where 'q_0' is the amplitude of vibration.

For small vibrational amplitudes, the polarizability 'α' can be expanded using Taylor series as:

$$\alpha = \alpha_0 + \frac{\partial \alpha}{\partial q} q + \ldots\ldots\ldots\ldots \qquad \ldots\ldots (1.7)$$

Where ' α_0' is the polarizability at the equilibrium position. The term $\frac{\partial \alpha}{\partial q}$ is the rate of change of 'α' with respect to 'q', evaluated at the equilibrium position. The induced dipole moment is given by :

$$P = \alpha_0 E_0 \cos 2\pi \upsilon_0 t + \frac{1}{2} \frac{\partial \alpha}{\partial q} q_0 E_0 [\cos 2\pi (\upsilon_0 + \upsilon_m) t + \cos 2\pi (\upsilon_0 - \upsilon_m) t] \qquad \ldots\ldots (1.8)$$

The first term represents Rayleigh scattering and the second term represents anti-stokes ($\upsilon_0+\upsilon_m$) and stokes ($\upsilon_0-\upsilon_m$) lines of Raman scattering. For Raman active vibrations, the rate of change of polarizability should be nonzero, $\frac{\partial \alpha}{\partial q} \neq 0$. 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 frequency of the Raman lines $\upsilon_0 \pm \upsilon_m$, but it fails to predict correct intensities. Quantum mechanical theory is therefore introduced to predict the intensities of Raman lines.

1.8.2 Quantum theory of Raman effect

According to quantum theory, the Raman effect may be regarded as the collisions between the light photons and molecules of the substance. If ν is the velocity of the molecule before collision, m be the mass of the molecule before collision with photon. E_a be the energy of the molecule before collision and hν be the energy of the incident photon. ν be the frequency of the photon before collision. E_b be the energy after collision and ν' be the frequency of the molecule after the collision. The new energy state of the system is given by

$$E_a+\frac{1}{2} mv^2+ h\nu = E_b+\frac{1}{2} mv^2+ h\nu' \quad \text{..... (1.9)}$$

$$\nu' = \nu + \Delta\nu \quad \text{..... (1.10)}$$

If $E_a=E_b$, the frequency difference $\Delta\nu = 0$. It means that $\nu' = \nu$, and this refers to the unmodified line, where the molecule simply deflect the photon without receiving the energy from it. This collision is elastic and equal to Rayleigh scattering. If $E_a > E_b$, then $\nu' > \nu$ which refers to the anti stoke's line. It means that the molecule previously entered into the excited state and handover some of its intrinsic energy to the incident photon. Thus the scattered photon has greater energy. If $E_a < E_b$ then $\nu' < \nu$ this corresponds to stoke's line. The molecule has absorbed some energy from the photon and consequently the scattered photon will have lowest energy. Figure 1.4 represents the Rayleigh, Stoke's and anti stoke's scattering energy levels.

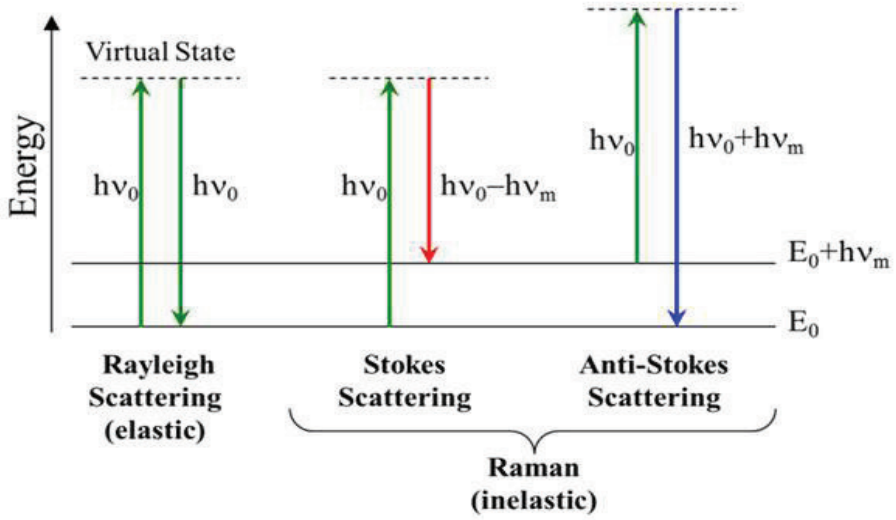

Figure 1.4 Rayleigh, Stoke's and anti stoke's scattering energy levels

1.8.3 FT-Raman Spectrophotometer

In the conventional Raman spectroscopy, the noise level of the photomultiplier detector is proportional to the square root of the light intensity striking it. Although the signal-to-noise (S/N) ratio increases as the square root of the bandwidth of the resolution elements, the use of a multiplex spectrophotometer (interferometer) allows all the scattered energy to bear on the detector simultaneously. The noise increases at the detector by the same amount as the S/N increase for multiplexing, thus cancelling out the two effects (Richard 2000). Conventional Raman spectroscopy measures intensity versus frequency or wavenumber. Alternatively, FT instruments measure the intensity of light of many frequencies simultaneously. The latter is often referred to as a time-domain spectroscopy. This spectrum is then converted into a conventional spectrum by means of Fourier transformation using a computer algorithm. The distinctive feature of the FT technique, like FT-IR, is that information for all the wavelengths fall on the detector at all time. This provides improved resolution, spectral acquisition times, and S/N ratios over conventional dispersive Raman spectroscopy.

1.8.3.1 Sources

FT-Raman instruments employ a CW Nd:YAG laser with an excitation at 1,064 nm (9,395cm^{-1}) as a source. The use of such a near IR laser suffers from a 16-fold reduction in signal as compared with a visible laser lasing at 514.5 nm because the cross section of Raman scattering follows the ν^4 relationship. The maximum power of the laser is as high as 10W, although less power (~ 1W) generally is used (John et al., 2003).

1.8.3.2 Filters

An important aspect of FT-Raman instrumentation is the necessity for optical filtering. The first task is to eliminate the stray light caused by the laser excitation because it will saturate the detector. The filtering must be capable of reducing the Rayleigh line, which is 10^6 times stronger than the stokes shifted lines in the Raman spectrum. In order to be sufficiently efficient and to obtain maximum information in the Raman measurement, the reduction of the Rayleigh line should be comparable with the strongest Raman line. Holographic notch filters have been most successful in eliminating light at the laser frequency from the scattered signal. These filters are fabricated by recording interference patterns formed between two mutually coherent laser beams on multiple film layers. Since all layers are recorded simultaneously within a thick stack, the optical density of the notch filter is high, and its spectral bandwidth can be extremely narrow. In addition, the layering profile is sinusoidal instead of square wave, thus holographic notch filters are free from extraneous reflection bands and provide significantly higher laser damage thresholds. Filters are also necessary to remove the optical output of the He-Ne laser (used for referencing) because the laser has optical axis collinear with the main laser source. Since the

detectors used in FT-Raman are sensitive to the He-Ne wavelength, the laser is a source of interference. Here, plasma emission filters can be used. The white light of the instrument is filtered with a near-IR cutoff filter. A final filter may be used in front of the detector.

1.8.3.3 Detectors

Raman signals are inherently weak, thus the problems involved with detection and amplification are severe. Most of the very early work has been performed with photographic detection using long exposure time. Furthermore, the time to develop photographic plates and examine them with a microphotometer rendered Raman spectroscopy unfit as a routine technique. This situation has changed considerably since the development of strong laser sources and sensitive detection techniques.

1.8.3.4 Photomultiplier tube

For spectrophotometers equipped with monochromators and single detectors, the Raman scattered light in the focal plane and exiting through the slits of the monochromator is collected and focused on a photomultiplier (PM) tube, which converts photons into an electrical signal. The PM tube consists of a photocathode that emits electrons when photons strike it; a series of dynodes, each of which emits a number of secondary electrons when struck by an electron; and an anode that collects these electrons as an output signal.

1.8.3.5 Charge-coupled device

In recent years, charge-coupled devices (CCDs) have been used increasingly in Raman spectroscopy. A CCD is a silicon-based semiconductor arranged as an array of photosensitive elements, each one of which generates photoelectrons and stores

them as a small charge. Charges are stored on each individual pixel as a function of the number of photons striking that pixel. On command, the charges from each row are shifted to the next higher row. Charges on pixels in the top row are shifted from left to right on request and read by a single analogue-to-digital converter. The charge transfer process is essentially noise-free, and almost all the noise contributed to the signal by the CCD is from the output stage, where the charge content of each bucket is measured. This is called the readout noise. The major advantages of the CCD relative to other multichannel detectors are the low readout noise, which makes optical intensification unnecessary, and high quantum efficiency and sensitivity in a wide wavelength range (120-1000 nm). Thus the CCD coupled with near-IR laser excitation (dye laser, diode laser) can be used to measure Raman spectra of fluorescent compounds.

1.8.3.6 Other detection devices

In FT-Raman spectroscopy, IR laser lines such as 1064nm line of the Nd:YAG laser are used for excitation. To detect such IR radiation, several detectors with the required sensitivity are available. Most of the FT-Raman instruments use indium-gallium-arsenide (InGaAs) detectors (John et al., 2003). For a detector 1 mm in size, a noise equivalent power (NEP) of or less is usually attained when the detector is cooled. This allows direct coupling of direct current (dc) to the first-stage amplifier and allows for both dc and alternating current (ac) components of the interferogram to be monitored. Several other detectors are available for use in the near-IR. Cooled PbS, Ge, InGaAs, InSb, and platinum silicide have been investigated as detectors for FT-Raman. The block diagram of the FT-Raman Spectrophotometer is shown in Figure 1.5.

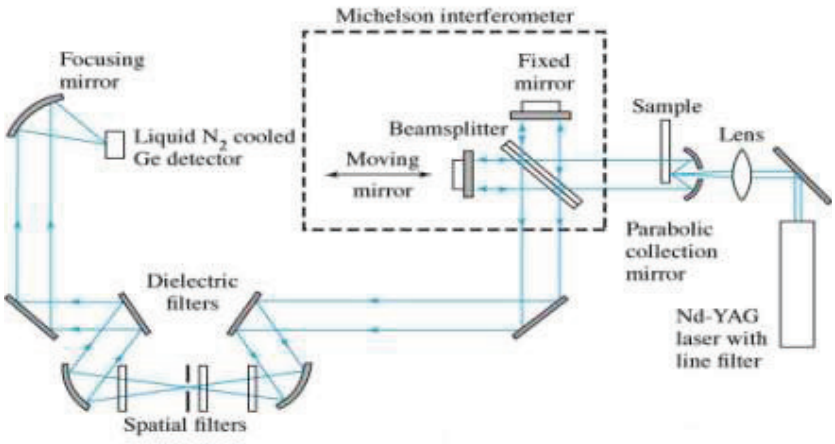

Figure 1.5 The block diagram of FT -Raman Spectrophotometer

1.9 UV-VISIBLE SPECTROSCOPY

UV-visible spectroscopy is a form of absorption spectroscopy which involves the absorption of ultra violet and visible radiations on to the organic compound. This causes molecules to undergo electronic excitations from one energy level to another energy level. Molecules containing σ electrons, π-electrons and n-electrons absorb energy in the form of ultra violet or visible light to excite their electrons to higher antibonding molecular orbitals. The absorption of radiation is characteristic and depends upon the nature of electrons in a molecule (Clark et al., 1993).

1.9.1 UV-visible Spectrophotometer

Today a wide range of instruments are available for making molecular absorption measurements in the UV-visible range. In a double beam spectrophotometer, the radiation coming from the monochromator is split into two beams with the help of a beam splitter. These are passed simultaneously through the reference and the sample cell. The transmitted radiations are detected by the detectors and the difference in the signal at all the wavelengths are suitably amplified and are sent for the output. Schematic diagram of UV-visible double beam spectrophotometer is shown in Figure 1.6.

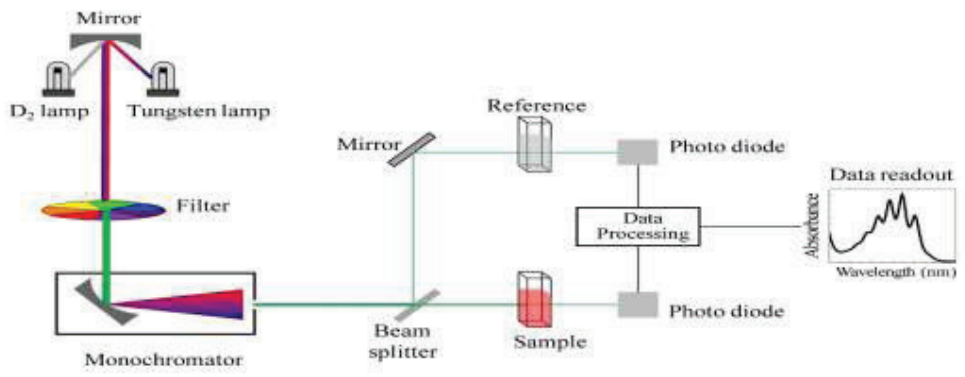

Figure 1.6 Schematic diagram of UV-visible double beam spectrophotometer

1.9.1.1 Radiation Sources

A spectrophotometric radiation source must provide a stable high energy output over a broad range of wavelengths. The sources should provide stable output over the entire UV-visible range (190-780 nm). For measurements in the UV region, electric discharge sources like hydrogen or a deuterium lamp are used. In these, the excitation of the gaseous molecules is brought about by the passage of electrons through the gas at low pressures. A hydrogen lamp is commonly used in the spectrophotometers and gives light in the wavelength region of 160-375 nm. The radiant power of the hydrogen lamp is low and these are replaced by deuterium lamps. The modern instruments use a tungsten filament lamp as the radiation source. This consists of a thin, coiled tungsten wire that is sealed in an evacuated glass bulb. This gives radiation in the range of 350-2200 nm. As the output depends on the voltage, the tungsten lamp is energised by the output of a constant voltage transformer (August 1971).

1.9.1.2 Wavelength Selectors

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

1.9.1.3 Detectors

The detectors are used to convert a light signal into an electrical signal which can be suitably measured and transformed into an output. The detectors used in most of the instruments generate a signal, which is linear in transmittance i.e. they respond linearly to radiant power falling on them. The transmittance values can be changed logarithmically into absorbance units by an electrical or mechanical arrangement in the signal read out device.

1.9.1.4 Signal Processing and Output Devices

The electrical signal from the transducer is suitably amplified or processed before it is sent to the recorder to give an output. The subtraction of the solvent spectrum from that of the solution is done electronically. The output plot between the wavelength and the intensity of absorption is the resultant of the subtraction process and is characteristic of the absorbing species (Howell et al., 1986 & Meehan et al., 1981).

1.10 NMR SPECTROSCOPY

Nuclear Magnetic Resonance (NMR) is a spectroscopic method, which gives information about the number of magnetically distinct atoms of the type being studied. The nuclei with the spin state of lower energy or aligned nuclei are induced to absorb energy of radio frequency range to change their spin orientation with respect to applied magnetic field (Keeler 2011).

1.10.1 Chemical Shift

Isotope nuclei of various functional groups well show their NMR signals with different abscissa because of their different σ values. A specific substance is selected as an internal standard, the peak for which is set as the origin of an NMR Spectral abscissa. The Chemical shift can be expressed as

$$\delta = \frac{\nu_{sample} - \nu_{standard}}{\nu_{standard}} 10^6 \qquad \text{..... (1.11)}$$

where ν_{sample} and $\nu_{standard}$ are the resonant frequencies of functional group and the standard. The standard reference substance is Tetra Methyl Silane (TMS) (Young 2005).

1.10.2 Fourier Transform NMR Spectrophotometer

A resolution spectrophotometer contains a complex collection of electronic equipment. The schematic representation of a nuclear magnetic resonance spectrophotometer is shown in Figure 1.7. The apparatus consists of the following essential components.

1.10.2.1 Magnet

An NMR spectrophotometer consists of a powerful magnet, and the associated electronics to control the properties of the magnet to create and to detect radiofrequency signals. Now most spectrophotometers use superconducting magnets to achieve field strengths which give proton resonances from 200-900 MHz. The magnetic field must be very stable over a period of hours and very homogeneous over the sample volume.

1.10.2.2 Probe

At the heart of an NMR spectrophotometer is the probe, which is a removable cylinder inserted into the centre of the magnet. The probe contains: the sample tube holder and air spinner outlets, the radiofrequency coils for signal detection, decoupler irradiation, and locking of the magnetic field, dewar, gas inlets and outlets for cooling and heating of the sample and the tuning coils for fine adjustments of the magnetic field. The very latest probes have the electronics for signal detection cooled to liquid helium temperatures (cryoprobes) to provide substantially improved sensitivity.

1.10.2.3 Detection of NMR Signals

The first generation of NMR spectrophotometers detected the NMR signals in the same way as it was done for the earlier spectroscopic methods such as IR and UV/VIS, the instrument scans through the frequency region of interest (or keeps the frequency constant and scans the magnetic field). When there is a frequency match, the transitions are detected by the spectrophotometer, and after signal processing, are plotted as an NMR spectrum. Advances in microwave electronics made possible a much more efficient way of detecting NMR signals in which frequencies are not scanned, but instead a very short powerful pulse is applied to the sample. The pulse is

short enough that its frequency is not well defined to within a few thousand Hertz, so it interacts with all of the nuclei of one isotope in the sample. The pulse duration is accurately specified so that the precession of the nuclei around the axis of the pulse corresponds to a well-defined angle. A Fourier transform is an operation, which converts functions from time to frequency domains. The usual frequency-domain spectrum can be obtained by computing the Fourier transform of the signal averaged free induction decay (FID) (Qunther 1995 & John 2009).

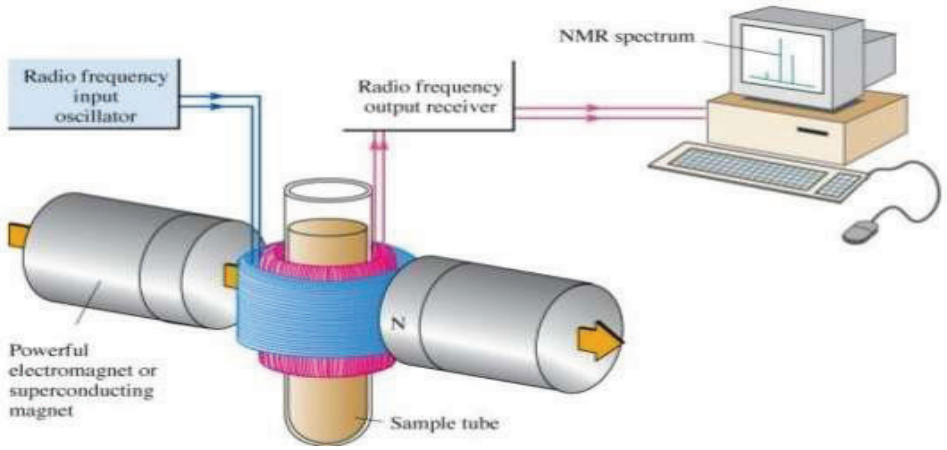

Figure 1.7 Schematic diagram of nuclear magnetic resonance spectrophotometer

1.11 COMPUTATIONAL CHEMISTRY

Computational chemistry is rapidly emerging as a subfield of theoretical chemistry, where the primary focus is on solving chemically related problems by calculations. The term computational chemistry is generally used when a mathematical method is sufficiently well developed that it can be automated for implementation on a computer. In computational chemistry, one particular important way is to model a molecular system prior to synthesizing that molecule in the laboratory. A second use of computational chemistry is in understanding a problem more completely (David 2001 & Leach 1996).

1.12 QUANTUM CHEMICAL COMPUTATION METHODS

1.12.1 Molecular Mechanics

The molecular mechanics energy expression consists of a simple algebraic equation for the energy of a compound. It does not use a wavefunction or total electron density. A set of equations with their associated constants is called a force field. The fundamental assumption of the molecular mechanics method is the transferability of parameters. The energy expression consists of the sum of simple classical equations. These equations describe various aspects of the molecule such as bond stretching, bond bending, torsion, electrostatic interaction, van der Waals forces and hydrogn bonding. Force fields differ in the number of terms in the energy expression, the complexity of these terms, and the way in which the constants are obtained. Since electrons are not explicitly included, electronic processes cannot be modeled (Jensen 1999).

1.12.2 Molecular Dynamics

Molecular dynamics is a simulation of the time-dependent behavior of a molecular system such as vibrational motion or Brownian motion. It requires a way to compute the energy of the system, most often using molecular mechanics calculation. This energy expression is used to compute the forces on the atoms for any given geometry (Grant et al., 1995).

1.12.3 Ab initio Methods

It is a quantum chemical calculation in which using a simple functional form for a function or finding an approximate solution to a differential equation. The most common type of ab initio calculation is called a Hartree- Fock (HF) calculation in

which the primary approximation is the central field approximation. This means that the coulombic electron-electron repulsion is taken to account by integrating the repulsion term. The second approximation HF calculation is due to the fact that the wavefunction must be described by some mathematical function, which is known exactly for only a few one electron system. The functions used most often are linear combination of Gaussian Type Orbitals (GTO). If the molecule has a single spin, then the same orbital spatial function can be used for both α and β spin electrons in each pair. This is called the restricted Hartree Fock method (HF) (Balbuena et al., 1999 & Veszprimi et al., 1999).

1.12.4 Semi Empirical Methods

Semi empirical calculations are setup with the same general structure as a HF calculation, in this there is a Hamiltonian and a wavefunction. Semi empirical calculations have been very successful in the description of organic chemistry, where there are only a few elements used extensively and the molecules are of moderate size. Some semi empirical methods have been devised specifically for the description of inorganic chemistry as well. The advantage of semi empirical calculations is that they are much faster than ab initio calculations (Sadlej 1985).

1.12.5 Density Functional Theory (DFT)

The energy of a molecule can be determined from the electron density instead of a wavefunction. The original theorem is applied only to find the ground state electronic energy of a molecule. A practical application of this theory was developed by Kohn and Sham who formulated a method similar in structure to the Hartree-Fock method. In this formulation, the electron density is expressed as a linear combination

of basis functions similar in mathematical form to HF orbitals. A determinant is then formed from there function called Kohn - Sham orbitals. It is the density from, this determinant of orbitals that is used to compute the energy (Levine 1991).

A density functional is then used to obtain the energy for the electron density. A functional is a function of a function. The exact density functional is not known. Therefore, there is a whole list of different functionals that may have advantages or disadvantages. Some of these functionals are developed from fundamental quantum mechanics and some are developed by parameterizing functions to best reproduce experimental results. One recent development in DFT is the advent of linear scaling algorithms. These algorithms replace the coulomb terms for distant regions of the molecule with multiple expansions. This results in a method with a time complexity of N for sufficiently large molecules. The most common linear scaling techniques are the fast multipole method (FMM) and the continuous fast multipole method (CFMM).

1.12.5.1. Kohn-Sham theory

The foundation for the use of DFT methods in computational chemistry is the introduction of orbitals, as suggested by Kohn and Sham (KS). The KS model is closely related to the HF method, sharing identical formulas for the kinetic, electron-nuclear and coulomb electron-electron energies. Assume for the moment a Hamiltonian operator of the form in eqn (1.12) with $0 \leq \lambda \leq 1$.

$$H_\lambda = T + V_{ext}(\lambda) + \lambda V_{ee} \qquad \ldots\ldots (1.12)$$

The exact kinetic energy can be calculated from the natural orbitals arising from the exact density matrix.

$$N_{elec} = \sum_{i=1}^{\infty} ni \qquad \text{..... (1.13)}$$

The exact density matrix is not known, a density can be written in terms of a set of auxillary one-electron functions, ie. orbitals.

$$\rho_{approx} = \sum_{i=1}^{Nelec} /\emptyset i/^2 \qquad \text{..... (1.14)}$$

The remaining kinetic energy is absorbed into an exchange-correlation term, and a general DFT energy expression can be written as

$$E_{DFT}[\rho] = T_s[\rho] + E_{ne}[\rho] + J[\rho] + E_{xe}[\rho] \qquad \text{..... (1.15)}$$

By equating E_{DFT} to the exact energy, this expression defines E_{xe}. It is the part that remains after subtraction of the non-interacting kinetic energy, and the E_{ne} and J potential energy terms

$$E_{xe}[\rho] = (T[\rho] - Ts[\rho]) + (E_{ee}[\rho] - J[\rho]) \qquad \text{..... (1.16)}$$

The first parenthesis in equation (1.16) may be considered as the kinetic correlation energy, while the last contains both potential correlation and exchange energy.

1.13 BASIS SET

The basis set commonly used in quantum mechanical calculations are composed of atomic functions. The atom centered functions used to describe the atomic orbitals are known as basis function and collectively form a basis set. There are two types of basis functions commonly used for electronic structure calculations, they are Slater Type Orbitals (STO) and Gaussian type orbitals (GTO). Slater type orbitals have the functional form as,

$$\chi_{\zeta, n, l,m(r,\theta,\varphi)} = N\, Y_{l,m}(\theta,\varphi)\, r^{n-1} e^{-\zeta r} \qquad \text{..... (1.17)}$$

Where N is a normalization constant and $Y_{l,m}$ are spherical harmonic functions. Slater type orbitals are primarily used for atomic and diatomic systems and in semi empirical methods. They can also be used with DFT methods that do not include exact exchange and the coulomb energy is calculated by fitting the density into a set of auxillary functions. Gaussian type orbitals cartesian co-ordinates are shown in equation (1.18)

$$\chi_{\zeta, n, l,m(r,\theta,\varphi)} = N\, Y_{l,m}(\theta,\varphi)\, r^{2n-2-l} e^{-\zeta r^2}$$

$$\chi_{\zeta,lx,ly,lz(x,y,z)} = N x^{lx} y^{ly} z^{lz} e^{-\zeta r^2} \qquad \text{..... (1.18)}$$

On the basis of type of function (STO/ GTO) and the location (nuclei) different types of basis sets are used. The smallest number of functions are possible in a minimum basis set. Double Zeta (DZ) type basis set is used for doubling of all basis functions. A variation of the DZ type basis only doubles the number of valence orbitals, producing split valence basis set. A doubling of the core orbitals can rarely be considered and the term DZ basis is also used for split valence basis set. The triple split valence basis set is used for six-s functions and three p-functions for the first row elements. For higher angular momentum functions polarization functions are used. For independent particle wavefunctions, where electron correlation is not considered, the first set of polarization functions is the most important and it is used for charge polarization effects. Polarization functions are added to chosen sp-basis, adding a single polarization function (p-functions on hydrogens and d-functions on heavy atoms) to the DZ basis set forms a Double Zeta Plus Polarization (DZP) basis set. If two sets of polarization functions are added to a Triple zeta SP basis, a triple zeta plus double polarization (TZ2P) type basis is obtained (Jenson 1999).

1.14 GEOMETRY OPTIMIZATION

Computing the geometry of a molecule is one of the most basic functions of a computational chemistry program. One way of defining the geometry of a molecule is by using a list of bond distances, bond angles and dihedral angles called a z-matrix. A z-matrix is a convenient way to specify the geometry of a molecule by hand. Many ab-initio and semi empirical programs optimize the geometry of the molecule by changing the parameters in the z-matrix. In general, this can be a very good way to change the geometry, because these parameters correspond to molecular motions similar to those seen in the vibrational modes. In order to have the advantages of a well constructed z-matrix is that how the geometry is defined, this system is called redundant internal coordinate. When redundant internal coordinates are used, the input geometry is first converted into a set of cartesian coordinates. The algorithm then checks the distances between every pair of atoms to determine the geometry of the molecule. This is the most efficient way to optimize geometry (Schelgel 1998 & Schlick 1998).

1.15 NORMAL COORDINATE ANALYSIS

The method used to calculate and interpret the frequencies of the bands observed in vibrational spectra of molecules or ions and to describe the type of vibration responsible for a certain bands has become known as normal coordinate analysis. The potential energy distribution (PED) provides one way to quantify the contribution of a certain internal coordinate to a normal coordinate. The different definitions have been used for the PED contribution of symmetry coordinate j to the normal coordinate k.

In the first definition, the contribution of the diagonal force constant F_{jj} is compared directly with the eigen value Δk°. It has the disadvantage that the sum of the PED contributions generally deviates from 100%, most severely when large off diagonal force constants are present. Second definition corrects this drawback by normalizing the sum of the diagonal contributions. The third definition explains the problem without normalization. Normal coordinates and harmonic frequencies may be calculated in a normal coordinate analysis provided that the equilibrium geometry and the force constant are known with sufficient accuracy (Peter Groner 2006).

1.16 POPULATION ANALYSIS

1.16.1 Natural Population Analysis

A method of natural population analysis has been developed to calculate atomic charges and orbital population of molecular wavefunctions in general atomic orbital basis sets. The natural analysis is an alternative to conventional mulliken population analysis and exhibit the numerical stability and explains the electronic distribution in compounds of high ionic character (Alan et al., 1985).

1.16.2 Molecular electrostatic potential

A significant part of the nonbonded interaction between polar molecules is described in terms of electrostatic interactions between fragments having an internal asymmetry in the electron distribution. The fundamental interaction is called molecular electrostatic potential (MESP) generated by one molecule and the charged particles of another. The ESP at position r is given as a sum of contributions from the nuclei and the electronic wavefunction (Williams 1991).

$$\emptyset_{ESP(r)} = \sum_{A}^{nuclei} \frac{ZA}{r-RA} - \int \frac{\psi\ r'\ 2\ dr\prime}{(r-r\prime)} \qquad \ldots\ldots (1.19)$$

1.16.3 Hirshfeld surface analysis

Hirshfeld charges are based on using atomic densities for partitioning the molecular electron density. The promolecular density is defined as the sum of atomic densities placed at the nuclear geometries in the molecule. The actual molecular electron density at each point in space is partitioned by weighting factors according to the promolecular contributions. Hirshfeld charges may be considered as a soft boundary version of the voronoi charges. The normal approach is to use spherically averaged ground state densities for neutral atoms, valence configuration may be considered (Hirshfeld 1977 & Guerra et al., 2003).

1.16.4 Natural Orbital Analysis

Natural orbits are the eigen functions of the first order reduced density matrix. The localization procedure allows orbitals to be defined as those centered on atoms and those encompassing pair of atoms these can be integrated to obtain charges on the atoms. Analysis of the basis function weights and nodal properties allows these transformed orbitals to be classified as bonding, anti bonding core and Rydberg orbitals (Hehre et al., 1986).

1.17 POTENTIAL ENERGY SURFACE SCAN ANALYSIS

The Potential Energy Surface (PES) is the most complete description of all conformers, isomers and energetically accessible motions of the system. Minima on this surface correspond to optimized geometries. The lowest energy minimum is called the global minimum. There can be many local minima, such as higher energy conformers or isomers. The transition structure between the reactants and the products of a reaction is a saddle point on this surface. The vibrational properties of the

molecule can be obtained from the PES. Molecular mechanics calculations are often used for examining possible conformers of a molecule. Semi empirical calculations can give a qualitative picture of a reaction surface. Ab initio methods must often be used for quantitatively correct reaction surfaces (Minkin et al., 1990 & Mezey 1987).

1.18 SOLVENT EFFECT

Solvent interactions can significantly affect the energy of the transition structure and only slightly change the transition-structure geometry. The presence of solvent, particularly a polar solvent, can also stabilize charge separation within the molecule. The quantum mechanical interaction between solute and solvent, which must be averaged over all possible arrangements of solvent molecules. There are several effects present in the region where the molecule meets the solvent shell. The cavitation energy is the energy required to push aside the solvent molecules, thus making a cavity in which to place a solute molecule. The force attracting the solute molecule to the solvent is called van der Waals force. The solvent molecules in the first shell can rearrange in order to maximize interactions with the solute (Schleht 1998 & Reichardt 1988).

1.19 FRONTIER MOLECULAR ORBITAL THEORY

Frontier Molecular Orbital (FMO) theory attempts to predict relative reactivity based on properties of the reactants. It is commonly formulated in terms of perturbation theory, where the energy change in the initial stage of a reaction is estimated and extrapolated to the transition state (Fleming 1976). The frontier molecular orbital theory represents the Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) pair of orbitals. The reaction of a

nucelophile involves the addition of electron to the reactant, i.e. interaction of the HOMO of the nucleophile with LUMO of the reactant. If there is more than one possible centre of attack, the preferred reaction mode is predicted to occur on the atom having the largest LUMO coefficient (Devaquet 1970).

1.19.1 Chemical descriptors

The electronic potential μ is represented as the first derivative of the energy with respect to number of electron, which in a finite difference version is given as the average of the ionization potential (IP) and electron affinity (EA) (Mulliken 1934).

$$-\mu = \chi = \frac{\partial E}{\partial N_{ele}} \approx \frac{1}{2}(IP+EA) \quad \text{..... (1.20)}$$

The second derivative of the energy is called hardness (Giju et al., 2005).

$$\eta = \frac{1}{2}(IP-EA) \quad \text{..... (1.21)}$$

The electrophilicity index measures the total ability to attract electrons (Parr et al., 1999) is defined as,

$$\omega = \mu^2/2\eta = \frac{IP+EA\ (IP+EA)}{4(IP-EA)} \quad \text{..... (1.22)}$$

Using Koopman's theorem, the hardness is related to the HOMO-LUMO energy difference. A hard molecule thus has a large HOMO-LUMO gap, and is expected to be chemically unreactive. Hardness is related to chemical stability. A small HOMO-LUMO gap indicates a soft molecule.

1.20 BIOLOGICAL ACTIVITY

Biological activity includes both desired properties, such as drug activity and undesired properties such as, toxicity. Such a prediction poses very difficult problems

due to the complexity of biological systems. Molecular simulation techniques used to predict how a compound will interact with a particular active site of a biological molecule. QSAR is a curve fitting technique used for creating an equation that predicts biological activity from the properties of the individual molecule only. Once this equation has been created using compounds of known activity, it can be used to predict the activity of new compounds (Lewis 1992).

1.20.1 Molecular Docking

An important example of a global optimization problem is determining the best alignment of two molecules with respect to each other, typically trying to fit a small molecule into large protein structure, this process is called docking. Given an X-ray structure of an enzyme, preferably with a bound ligand to identify the active site, the ligand can be removed, and virtual compounds may be docked into the active site to possibly identify new molecules with a stronger binding affinity. Since many drugs act by inhibiting specific enzymes, docking is an important element in drug design and leads optimization (Blaney et al., 1992).