Chapter 2

Experimental Techniques

2.1. X-Ray Diffraction by Crystal

X-rays, discovered by Wilhelm Rontgen in 1895, are used to produce the diffraction pattern because their wavelength (I) is typically the same order of magnitude (1-100Å) as the spacing "d" between planes in the crystal. X-ray diffraction is an important phenomenon to study the internal structure of material by wave-material interaction. In 1913, the famous diffraction condition-Bragg's law (**Figure 2.1**) is established by W. L. Bragg and his father W. H. Bragg and which is given by

$$2d_{hkl}\sin\theta = n\lambda \quad \text{..... (1)}$$

Where, n = Order of reflection

d_{hkl} = Inter-planar spacing of the planes which makes an angle 'θ' with incident beam

λ = Wavelength of X-rays

2θ = Angle by which the Bragg reflection deviates from direct beam

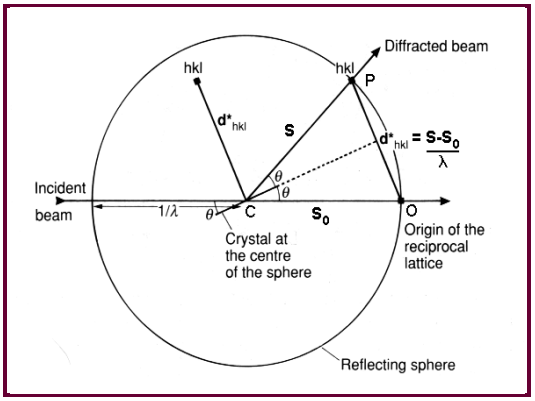

Figure 2.1 The "Ewald Sphere" construction

Note: The reciprocal lattice has its origin at O. The sphere of radius 1/λ passes through O. Its diameter is along the direction of the incident beam. If the reciprocal lattice point P lies on the surface of the sphere, then a reflected beam is directed parallel to CP **[117]**.

Diffraction: Whenever a wave interacts with an obstacle, diffraction occurs. In crystallography, the static structure factor is a mathematical description of how a material scatters incident radiation. The concept of atomic scattering factor (f) i.e. the efficiency of an atom to scatter which is defined by

_____ ……. (2)

is introduced by Bragg and f is directly related to atomic number of the atom, scattering direction for a given X-ray wavelength and structure factor (F) which is defined by

_____ ……. (3)

When a single crystal is exposed to X-rays, isolated spots are appeared on photographic film due to diffraction of X-rays at some specific Bragg angle. The arrangement of the spots on photograph is periodic i.e. in the form of lattice and it is known as 'reciprocal lattice'.

The resultant of the waves scattered by all the atoms in the unit cell, in the direction of the hkl reflection, is called the structure factor (F_{hkl}). The structure factor depends on both the position of each atom and its scattering factor.

$$F_{hkl} = \sum_{n=1}^{N} f_n \exp\left[2\pi i\left(hu_n + kv_n + lw_n\right)\right] \quad \text{..... (4)}$$

where f_n is the scattering factor of the j^{th} atom and x_j, y_j, and z_j are its fractional coordinates.

This series can be expressed in terms of sines and cosines (periodic nature of a wave) and is called a Fourier series. In a crystal with a center of symmetry and n unique atoms in the unit cell (the unique set of atoms is known as the asymmetric unit), the above equation simplifies to:

$$F_{hkl} = \sum_{n=1}^{N} f_n \cos 2\pi\left(hu_n + kv_n + lw_n\right) \quad \text{..... (5)}$$

The electron density distribution within a crystal can be expressed using a three-dimensional Fourier series.

$$\rho(xyz) = \frac{1}{V}\sum_{h}\sum_{k}\sum_{l} F_{hkl} e^{-2\pi i(hx+ky+lz)} \quad \text{..... (6)}$$

where ρ(x, y, z) is the electron density as a position x, y, z in the unit cell and V is the volume of the unit cell. The electron density is Fourier transform of the structure factor (and vice versa). If the structure factor is known, then it is possible to calculate the electron density distribution in the unit (atomic position).

2.2. Single Crystal

A crystal is a solid material whose constituent atoms, molecule, or ion are arranged in an orderly repeating pattern extending in all three spatial dimensions and unbroken to the edges of the sample, with no grain boundaries i.e. atomic arrays those are periodic in three dimension. In addition, crystals are used in industrial technological applications, especially in optics, in electronics, in materials science for the production of high strength material with low thermal creep, such as turbine blade **[118-119]**. A good quality of single crystal is pre-requirement for crystal structure determination by X-ray diffraction technique. The

X-ray diffraction technique involves recording of the intensity of the diffraction pattern, so better the crystal better is the data and more accurate is the structure exploring to the informations down to atomic level.

2.3. Reflux Method

Author has reported three silver complexes in the thesis which are synthesized by reflux method (**Figure 2.2**) which involves the condensation of vapour and the return of this condensate to the system from which it is originated. A liquid reaction mixture is placed in a round bottom flask which is open only at the top. The round bottom flask is connected to a condenser such that any vapors given off are cooled back to liquid, and fall back into the round bottom flask. The round bottom flask is then heated vigorously for the course of the reaction. The purpose is to thermally accelerate the reaction by conducting it at an elevated temperature.

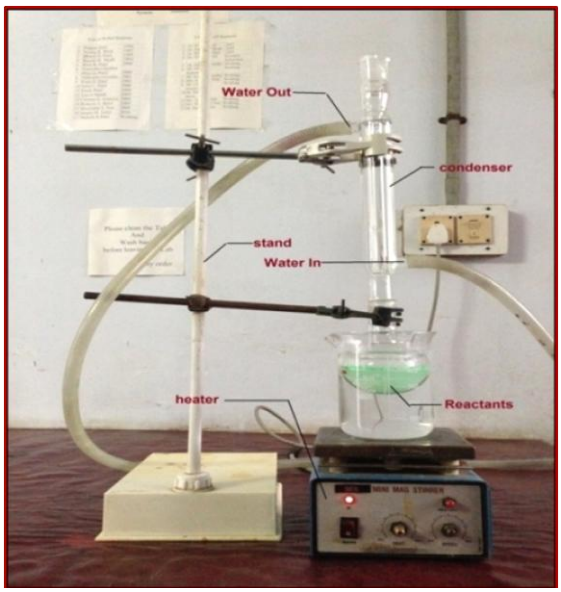

Figure 2.2 Experimental setup for reflux method

2.3.1. Synthesis of Metal Complex

The complexes are synthesized by the following simple reaction procedures: The sodium salt of sulfonamide (Na/HSmd) is dissolved in 25 mL of hot methanol and the aqueous solution of the respective metal salts (Silver salt in present study) is added slowly with constant stirring and the mixture is refluxed for 2-3 hours. The precipitate is filtered and washed with hot distilled water and methanol consecutively and dried in a desiccator over anhydrous $CaCl_2$. The complexes of silver are white powder.

Reaction scheme:

$$Ag^{1+} + 2(Na/HSmd) \longrightarrow Ag(Smd)_2 + 2Na^{+}/2H^{+}$$

The precipitate is used for further reaction with secondary ligand in pyridine solution to obtain the single crystals for the complexes.

$Ag(Smd)_2 + x\ L \longrightarrow Ag(Smd)_2(L)_x$

Where, L is any secondary ligand, bidentate when x = 1 and monodentate when x = 2.

All the complexes are stable in air and at room temperature. The complexes are insoluble in water, alcohol and most organic solvents but soluble in solvents like Dimethylformamide (DMF), pyridine, picoline and Dimethyl sulfoxide (DMSO).

2.4. Crystal Growth Techniques

Growth of crystal ranges from a small inexpensive technique to a complex sophisticated expensive process and the crystallization time ranges from minutes to months. Single crystals can be produced by the transport of crystal constituents in the solid, liquid or vapour phase. On the basis of this, crystal growth may be classified into three categories as follows,

1. Solid Growth - Solid-to-Solid phase transformation
2. Liquid Growth - Liquid to Solid phase transformation
3. Vapour Growth - Vapour to Solid phase transformation

Based on the phase transformation process, crystal growth techniques are classified as solid growth, vapour growth, melt growth and solution growth **[120]**. Growing good single crystals is an art **[121, 122]**. Solution growth techniques are often applied to fabricate high-quality single crystals which cannot be grown from their own melts, materials, which have high solubility and have variation in solubility with temperature, can be grown easily by solution method. There are two methods in solution growth depending on the solvents and the solubility of the solute.

1. High temperature solution growth
2. Low temperature solution growth

Different techniques are well known to grow the single crystal of organic compounds viz., slow evaporation, slow cooling, vapour diffusion, liquid diffusion, sublimation etc. Slow cooling is the simplest method for single crystal growth in which the saturated solution of the compound is heated to just its boiling point or just below it and allow it to cool to room temperature. It is the most widely used method for the growth of single crystals, when the starting materials are unstable at high temperature **[123]** and also which undergo phase transformation below melting point **[124]**. In vapor diffusion and liquid diffusion method, one solvent diffused into the other while precipitating the product that results into

deposition of crystals. In the sublimation method, compound is heated under vacuum and crystals are collected on cold-finger. Slow evaporation technique is used to grow the single crystal of significant biomolecules from different solvents like water, methanol, chloroform, ethanol, ethyl acetate.

2.4.1. Slow Evaporation Method

This is the simplest way to grow crystals and works best for compounds which are not sensitive to ambient conditions in the laboratory, the best quality of single crystals results under optimized conditions. The use of a range of temperatures may not be desirable because the properties of the grown material may vary with temperature. In the Slow evaporation method, crystals are grown from the saturated or nearly saturated solution of the compound prepared using different solvents or mixture of any two solvents with varying proportion and is allowed to stand without disturbance. Solvent evaporates slowly resulting in the precipitation of the compound in the form of single crystal.

2.4.2. Factors Influence the Crystal Growth

There are number of variables which can influence on crystallization of organic material like, the interaction of ion/molecule of the solute, the solubility of substance and thermodynamic parameters of the process; temperature, pressure and solvent concentration. Few of them are discussed here.

Sample Purity: The poor purity is one of the most common causes of unsuccessful crystallization.

Temperature and Viscosity of Solvent: The rate of change of temperature and concentration of solution can greatly influence crystallization. Increased volatility of the solvent raises the rate of increase in concentration of the solution, speeding up both nucleation and growth. The viscosity of the solvent directly affects the rate of crystal growth through its influence on the solute flux. Growth of crystals from solution is mainly a diffusion- controlled process; the medium must be less viscous to enable faster transport of the growth units from the bulk solution by diffusion.

Chemical Nature of Solution: The chemical nature of the solution also plays a leading role in the crystallization process. The nature of solute-solvent interaction influences the balance in stabilities of solid and solution and therefore, affects the energy of crystallization. If these interactions are strong, they may also act as a retarding force on nucleation and growth rates.

2.4.3. Choice of Solvent and Solubility

Growth from aqueous solution provides information on the fundamental process applicable to both low and high temperature solution growth method (Bordui 1987) **[125]**. A simple thumb rule in proper selection of a solvent is chemical similarity between the solvent and the compound to be grown. For example, crystal of nonpolar organic compounds can be easily grown from nonpolar organic solvent. Various experiments reveal that a solvent in which the compound has solubility between 10 and 60% at a given temperature is economically suitable for crystal growth. Very low and very high solubility of a solute provide low growth rates due to low solute concentration and increased viscosity, respectively. Organic solvents such as acetone, acetonitrile, hexane, toluene and acetic acid and alcohols such as ethanol, methanol and propanol are also used as solvent **[126]**. The change in morphology occurs because of the direct interaction of solvent molecules with the crystal surface itself and by the interaction of partially deprotonated molecule with the solvent **[127]**. Thus, solvent can play an indirect role in changing the morphology of a crystal. The solvent changes the structure of the solute molecule, which subsequently adsorb on several faces of the crystal, thereby blocking their normal growth. The effect of solvent on the growth rate of different faces is associated with selective adsorption of solvent molecule on them. The solvent has a strong influence on the habit of crystalline material because solvent molecule affects the growth rate of different faces appearing in the crystal morphology differently. If the solubility is too high, it is difficult to grow bulk single crystal and too small a solubility restrict the size and growth rate of the crystal. Solubility gradient is another parameter, which dictates the growth procedure.

2.5. X-ray Diffraction Study

2.5.1. X-ray Diffractometer

The schematic diagram of CCD diffractometer is shown in **Figure 2.3**. Three dimensional intensity data are collected on a four circle Kappa Apex-II CCD 4 single crystal X-ray diffractometer (**Figure 2.4**), installed at Department of Physics, Sardar Patel University. A diffractometer is an instrument, which measures the intensities of diffracted beam individually by counting the number of X-ray photons those arrived at a suitably placed detector. By recording the diffracted beam from X-rays hitting a single crystal, the unit cell can be determined from the position of the diffraction spots. The exact atomic position within the unit cell can then be determined from the intensity of the spots.

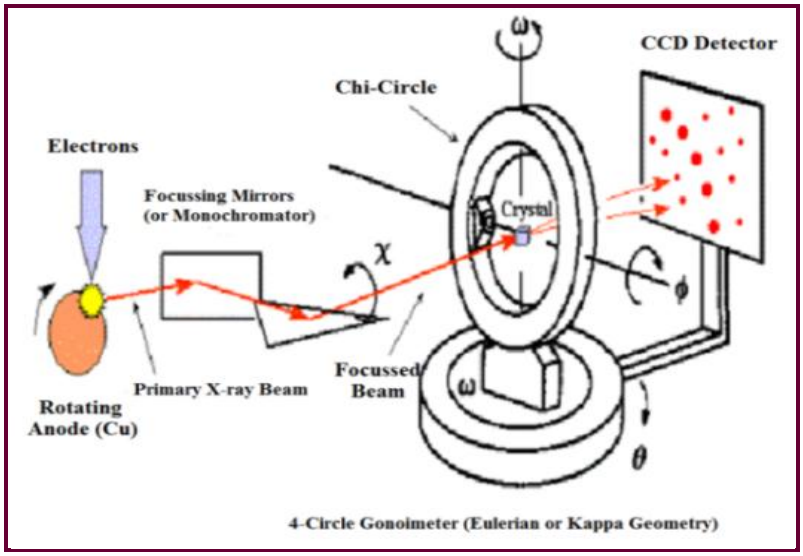

Figure 2.3 Schematic diagram of CCD 4 diffractometer

Figure 2.4 Bruker AXS Kappa Apex II CCD 4 diffractometer

The term four circle is referred to the number of rotational motions available the crystal orientation is defined by ϕ, χ, ω and the detector position is defined by the fourth angle 2θ. The detector is constrained to move in the horizontal plane containing the incident X-ray beam. The goniometer head, which carries the crystal, is mounted on the ϕ circle and ϕ is usually, indicates a rotation about the axis of goniometer head. The ϕ circle is carried on to the χ-circle, which in turn is carried on the ω-circle. The axes of these two circles are perpendicular to each other. The axis of ω-circle represents the axis about which the crystal rotates during measurement, the axis is always normal to the X-ray beam. The

Goniometer (**Figure 2.5**) allows flexible sample orientation and complete data collection to high theta value.

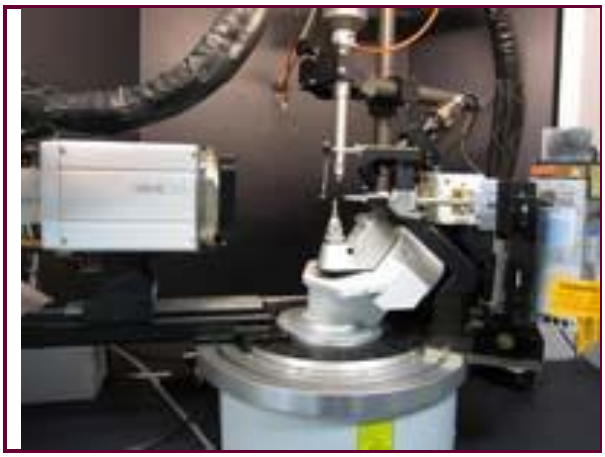

Figure 2.5 Photograph of the goniometer

2.5.2. Structure Solution and Refinement

Accurate and rapid measurement of the position and intensity of the hkl reflections is possible using single crystal X-ray diffractometer. The recording of diffracted beam does not record all the information about the diffracted X-ray but only the intensity. The detectors are not sensitive to the phase of the X-ray. Intensity is proportional to the square of the amplitude of the wave, thus the phase information is lost. The phase information is related to the atomic positions in a crystal structure. This is known as 'phase problem' in crystallography. If the Bragg angle of the reflections are measured and indexed, information on translation symmetry elements and symmetry information, the size of the unit cell are obtained. The intensities (and position) of the reflection are different and these can be quantified using a detector, CCD plate or scintillation counter and recorded electronically.

Collected data need to undergo some routine corrections, known as data reduction.

Lorentz- Polarization correction (L) relates to the geometry of the collection mode. The Lorentz factor or Lorentz term is an expression which appears in several equations in special relativity. It arises from deriving the Lorentz transformations. The name originates from its earlier appearance in Lorentzian electrodynamics–named after the Dutch Physicist Hendrik Lorentz. **Polarization correction (P)** allows the fact that a non-polarized X-ray beam may become partially polarized on reflection from the crystal.

Absorption correction is applied, particularly for inorganic structure, because large Z atom absorbs some X-ray rather than scatter. A correction for anomalous dispersion can be made when the wavelength of the incident X-ray is close to its absorption edge.

Extinction, which is an attenuation of the primary beam as it passes through a crystal, is first investigated by Darwin (1922), who has divided the phenomenon into two types, primary and secondary. The reduction of intensity with the depth of penetration of the primary beam due to the diversion of some of the energy (intensity) into the reflected beam is called secondary extinction.

2.5.3. Solving Single Crystal Structures

The structure factor (an intensity of a reflection) is dependent on both the position of each atom and its scattering factor. Knowing the atomic position, one can calculate the structure factor and from X-ray diffraction, we obtain the structure factor without phase information. When we take the square root of the intensity, we obtain the modulus of the structure factor, i.e. magnitude only and the sign of the structure factor is not known. We need phase information to calculate the electron density distribution and atomic positions. The square roots of the corrected data are taken to give a set of observed structure factors (F_{obs} or F_o). In order to calculate the electron density distribution in the unit cell, we need both the magnitude of the structure factors and the phase. There are four different methods commonly used to deduce the phase. The methods are

1. Direct method
2. Patterson method
3. Isomorphous replacement method
4. Anomalous scattering method

Direct method: It is used for crystal containing atoms with similar scattering properties. A mathematical probability for the phase values and electron density map of the unit cell are created to provide a starting point in the structure solution and refining process. In two dimensions, it is relatively easy to solve the phase problem directly, but not so in three dimensions. The key step is taken by Hauptman and Karle, who developed a practical method to employ the Sayre equation for which they are awarded the Nobel Prize (1985) in chemistry. The basic assumptions used to derive phase in direct method are

- Electron density is never negative.
- Electron density consists of discrete spherically symmetric atoms. The density map has high value at and near atomic position and has nearly zero value everywhere else.
- The method is very good with a few atoms i.e. about<100 and thus it is good for small molecular structure.

At present, direct method is the preferred method for phasing crystal of small molecule having up to 1000 atoms in the asymmetric unit. However, they are generally not feasible by themselves for larger molecule such as protein **[128]**.

Patterson method: Patterson introduced the method of determining inter-atomic distance from a Fourier transform of intensities in 1934 **[129]**. It relies on the presence of at least one (not many) heavy atom in the unit cell and is useful for solving inorganic structures. The family of method employed in structure determination to derive relationship between the scattering centers in a crystal lattice when the diffraction phases are unknown. They depend upon interpretation of the Patterson function

$$P(uvw) = (1 / V) \sum_{h} \sum_{K} \sum_{l} \{|F(hkl)|^2 \cos [2\pi(hu + kv + lw)]\} \quad \text{..... (7)}$$

to reveal inter atomic vector within the unit cell.

An electron density map can be constructed from an inverse Fourier transform of the structure factor of a wave diffracted from a crystal. Diffracted intensities can be measured directly, and are related to the square of the amplitude of the structure factor; but the diffraction phase cannot be determined by direct observation. The Patterson function represents a convolution of electron density with itself. It loses all phase information, but reduces to a function of $|F_{(hkl)}|^2$ alone, and is thus derivable from the measured intensities.

Without phase information, the Patterson map (i.e. the Patterson function evaluated at points u,v,w throughout the unit cell) may be interpreted as a map of vectors between the scattering atom. Vectors in a Patterson correspond to vectors in the real crystal cell, translated to the Patterson origin. Their weights are proportional to the product of electron densities at the tip of the vectors in the real cell.

The Patterson unit cell has the same size as the real crystal cell. The symmetry of the Patterson comprises the Laue point group of the crystal cell plus any additional lattice symmetry due to Bravais centering. The reduction of the real space group to the Laue symmetry is produced by the translation of all vectors to the Patterson origin and the introduction of a centre of symmetry. Nevertheless, if other techniques are used to establish the position of one atom, the Patterson function becomes useful in determining the location of other atoms.

2.5.4. Structure Refinement

After the atoms in a structure have been located, a set of structure factors, F_{calc} and F_o, are determined for comparison with the F_{obs} magnitudes. The inaccuracies inherent in the

observed structure factor magnitudes and phases imply that the first derived electron density map is not a totally accurate representation of the true structure.

The derivation of the trial structure

This is deduced from the first calculated electron density function using the observed structure factor magnitude with the observed phase. This is a crude model of the true structure.

Cyclic Fourier refinement

From the trial model, we calculate a set of structure factor, allowing for thermal effect. We then compute new Fourier synthesis using the observed structure factor magnitude with the calculated phase. The cyclic Fourier refinement processes primarily give us better value of the phase, and allow us to draw an electron density map with more accurately located atomic sites.

Difference Fourier synthesis

Having obtained a more reliable set of phase, we then calculate a Fourier synthesis using the quantities $|F_0|-|F_c|$. This gives a difference Fourier map which has certain features which enable us to refine our correct model still further. Specifically, a difference Fourier map is particularly useful for the more precise location of atomic position, for the identification of missing atoms and for refinement of thermal parameters.

Least squares refinement

This is a statistical treatment of our data so that we obtain a model which represents the best fit with the observed data. In the most sophisticated treatment, three positional and six thermal parameters are fitted for each atom.

Refinement based on F_o or F_o^2 data

Refinement may be carried out either as a function of error in the structure factor, $\Delta_1=|\,|F_0|-|F_c|\,|$ or in those of the intensities, $\Delta_2 =|F_o^2 - F_c^2|$. Until recently, nearly all refinements are carried out based on F_o, i.e. using $\Delta 1$. For such a refinement, very weak data give problem, since as a result of counting statistics, for very weak data, the background will occasionally be estimated to be stronger than the peak. This will result in a negative value for F^2 and so, for these data, no value of F_o can be directly calculated. To avoid this problem, it is customary to take an arbitrary values for F_o (e.g. $\sigma F_o/4$) for all "unobserved data", say data with $F_o^2 < \sigma(F_o^2)$, so that they can be used for relationship in direct method. This, of course, introduces a systematic error into the data set.

In order to indicate how well a structural model confirms to reality, so called residual or "R-factor" is evaluated. The "conventional" R-factor is

$$R = \frac{\sum_{hkl} \left| |F_0| - |F_c| \right|}{\sum_{hkl} |F_0|} \qquad \text{..... (8)}$$

When multiplied by 100% gives the average relative deviation between the observed and calculated structure factor as a percent. If weights are taken into consideration, the resulting R factor is usually large. The weighted R factor is directly related to the quantity that is minimized in the least squares refinement. It is defined depending on whether the refinement is based on F_o or on F_o^2.

$$\omega R = [\frac{\sum_{hkl} \omega \Delta_1^2}{\sum_{hkl} \omega F_0^2}]^{1/2} \qquad \text{..... (9)}$$

$$\omega R_2 = [\frac{\sum_{hkl} \omega \Delta_2^2}{\sum_{hkl} \omega (F_0^2)^2}]^{1/2} \qquad \text{..... (10)}$$

They are much more sensitive to small errors in the structure model, such as disorder or missing H atoms. A further index used to indicate the quality of a refinement is the goodness of fit, S given by

$$S = [\frac{\sum_{hkl} \omega \Delta^2}{(m-p)}]^{1/2} \qquad \text{..... (11)}$$

(m = number of reflections, p = number of parameters). For a correct structure with a suitable weighting scheme, S will have a value close to 1.

2.5.5. Interpretation and Presentation of Results

The interest in determining the structure of a molecule often stems from quantities which can be derived from the structural parameters. Prominent among these are the distance between two covalently bonded atoms, angle between pair of bond, torsion angle involving bonded atoms, conformation of the molecule and intra and intermolecular interactions.

Bond Length and Bond Angle: The distance measured between the accurately determined positional coordinate of the atoms joined together by single or multiple bonds given the bond length of the respective bond. Bond length is readily derived from the differences in the atomic coordinates: $\Delta x = x_2 - x_1$, $\Delta y = y_2 - y_1$, $\Delta z = z_2 - z_1$.The general equation for a triclinic bond length system and the corresponding standard deviation in it are given by the law of cosines in three dimension

$$D = \left[(\Delta x a)^2 + (\Delta y b)^2 + (\Delta z c)^2 - 2ab\Delta x \Delta y \cos\gamma - 2bc\Delta y \Delta z \cos\alpha - 2ca\Delta x \Delta z \cos\beta \right]^{1/2} \qquad \text{..... (12)}$$

Where, a, b, c, α, β, γ = Unit cell parameters.

The standard deviation in the bond length is calculated from

$$\sigma_D = \left[\begin{array}{l} (\sigma^2 x_1 + \sigma^2 x_2)\dfrac{(\Delta x - \Delta y\cos\gamma - \Delta z\cos\beta)^2}{D} + (\sigma^2 y_1 + \sigma^2 y_2)\dfrac{(\Delta y - \Delta x\cos\gamma - \Delta z\cos\alpha)^2}{D} + \\ (\sigma^2 z_1 + \sigma^2 z_2)\dfrac{(\Delta z - \Delta x\cos\gamma - \Delta y\cos\alpha)^2}{D} \end{array} \right]^{1/2} \quad \text{..... (13)}$$

Where, σx_1, σx_2 = standard deviation of atom 1 and 2 in x - co-ordinate

σy_1, σy_2 = standard deviation of atom 1 and 2 in y - co-ordinate

σz_1, σz_2 = standard deviation of atom 1 and 2 in z - co-ordinate

D = bond length

If the axes are orthogonal, equation (13) reduces to

$$\sigma_D = \left[(\sigma^2 x_1 + \sigma^2 x_2)\frac{(\Delta x)^2}{D} + (\sigma^2 y_1 + \sigma^2 y_2)\frac{(\Delta y)^2}{D} + (\sigma^2 z_1 + \sigma^2 z_2)\frac{(\Delta z)^2}{D} \right]^{1/2} \quad \text{..... (14)}$$

Bond angle is measured by angle subtended by two atoms joined to a third one. Bond angle is most readily calculated using the cosine law. The angle At2-At1-At3 in terms of the three inter-atomic distances d_{12}, d_{13} and d_{23} is:

$$\cos\phi At2, At1, At3 = \frac{d_{12}^2 + d_{13}^3 - d_{23}^2}{2d_{12} \times d_{13}} \quad \text{..... (15)}$$

Dihedral Angle and Torsional Angle: In discussing crystal structure, interplanar or dihedral angle, the angle between the normal to two planes, are often important. A torsion angle is a special case of dihedral angle. As shown **Figure 2.6**, the torsion angle for the atoms 1-2-3-4 is essentially the angle between the planes defined by atoms 1, 2 and 3 and 2, 3 and 4. Alternatively, consider the angle between the bonds 1-2 and 3-4 projected onto the plane normal to the bond 2-3, with the bond 1-2 toward the viewer (a Newman projection). The torsion angle is then the angle measured from atom 1 to atom 4. If this is clock wise, the torsion angle has a positive sign, otherwise negative. This sign does not change if the measurement is made 4-3-2-1 instead, but the torsion angle for the mirror image of the group has the opposite sign.

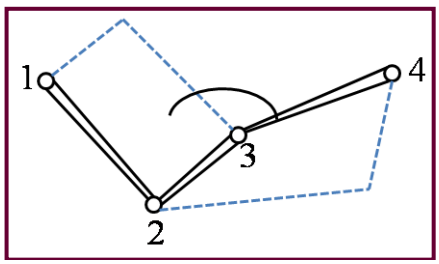

Figure 2.6 Torsional angle

The dihedral angle (θ) is the angle between the two planes defined by A-X-Y and X-Y-B in a molecule [A-X-Y-B] as shown in **Figure 2.7**.

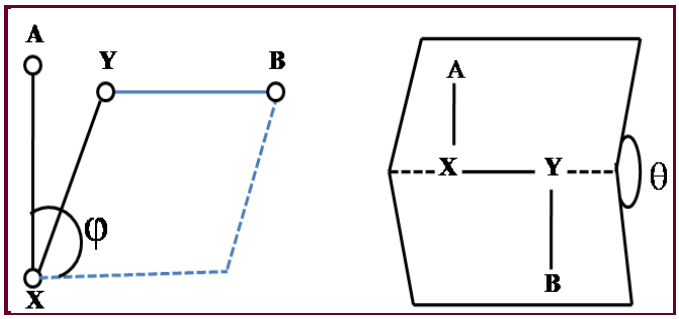

Figure 2.7 Dihedral angle

The standard error of the bond length, the most important error estimates in a structure, may be derived in a rather complicated way from the standard error of the positional parameter of both atoms and the orientation of the interatomic vector (Glusker and Trueblood, 1985; Stout and Jensen, 1989 **[130-131]**). A useful approximation may be obtained by estimating the isotropic error for each atom

$$[\sigma_l = (\sigma_x^2 a^2 + \sigma_y^2 b^2 + \sigma_z^2 c^2)^{1/2}] \qquad \text{..... (16)}$$

Then, for a bond:

$$\sigma_d = (\sigma_1^2 + \sigma_2^2)^{1/2} \qquad \text{..... (17)}$$

In a well determined light atom structure, the standard error for bond between C, N and O atom usually in the range 0.002-0.004 Å. For heavier atom, this may be smaller than 0.001 Å

Best Planes and Least-Square Plane: To determine whether or not the coordination of an atom is planar or whether the conformation of a ring is flat or puckered in the crystal structure, the best plane equation are worked out. For this purpose, a least square method can be used to calculate a plane which minimizes the sum of the square of the distance δ of the i^{th} defining atoms from it.

$$\sum_i \delta_i^2 = \min . \qquad \text{..... (18)}$$

The mean standard error of this "best" or "least square" plane is then

$$\sigma_p = [\sum_{i=3} \delta_i^2]^{1/2} \qquad \text{..... (19)}$$

indicates how well the conformation of the group of atom is described by a plane.

2.5.6. Intra and Intermolecular Interactions

Interactions between two or more molecules are called intermolecular interaction, while the interaction between the atoms within a molecule is called intramolecular interactions. Intermolecular interactions occur between all types of molecule or ion in all states of

matter. Depending on geometry and environmental condition, the hydrogen bond may be worth between 5 and 30 kJ/mole in thermodynamic terms. This makes it stronger than a van der Waals interaction, but weaker than covalent or ionic bond. The important property of an intermolecular interaction is its directionality. This characteristic is of obvious significance in crystal design because interaction directionality can be exploited to achieve specific and pre-desired intermolecular orientation. Isotropic interactions are the ones responsible for close packing and are mainly of the dispersion–repulsion type. They include the very common C···C, C···H and H···H interaction and purely ionic interaction. Anisotropic interaction has certain extra chemical attributes that arise from specific electronic distribution around atoms.

Hydrogen Bond Interactions: A hydrogen bond is the electrostatic attraction between polar group that occurs when hydrogen (H) atom bound to a highly electronegative atom such as nitrogen (N), oxygen (O) or fluorine (F) experiences attraction to some other nearby highly electronegative atom. Hydrogen bonding has been called the master-key of molecular recognition. It is the most reliable interaction in the toolkit of the crystal engineer. The X–H (donor) could become sufficiently polarized so that it would be attracted electrostatically to the electronegative Y (acceptor) atom. So a hydrogen bond is shown as X (δ–)–H (δ+)···Y (δ–)–Z. The electronegative atom forms a covalent bond with hydrogen covalent bond being directional in nature, results in a partial positive charge on the other side of hydrogen being small in a size compare to other atom. These classical hydrogen bond interaction O-H···O, N-H···O etc are well established and play a very significant role in stabilizing supramolecular structure of organic solid. However, in sulfonamide compound, along with the conventional hydrogen bond interaction due to the presence of donor and accepter atom, a set of somewhat weaker and less directional interactions such as C-H···O, C-H···N, C-H···π and π···π interaction have been recognized to play an important role in generating supramolecular structure. Several structural and energetic similarities have been observed between the conventional hydrogen bond and the dihydrogen bond. The C-H···O hydrogen bond is first identified by Sutar in 1962 **[132]**. The C-H···O bond though weak but attractive interaction with a long range distance character and occur within certain distance (C···O = 3.0-4.0Å) and angle (C-H···O =90˚-180˚) range **[133]**. Other types of weak interaction such as π···π interaction involving interaction between the π-electron system of aromatic, as well as that of aliphatic are generally called as π-complexes, are well established.

C-H···π Interaction: C-H···π interaction (**Figure 2.8**) is another type of C-H weak but important molecular force having a directional preference with the pointing towards the center of electron rich aromatic ring **[134]**. C-H···π interaction is the weakest hydrogen bond that operate between a soft acid C-H and a soft or intermediate base π system **[135]**. It has been recognized that this kind of weaker and softer interaction plays significant role in chemistry **[136]**, in self-assembly, and in chiral recognition. As it is non-polar and effective in water, the C-H···π interaction is also especially important in biological system **[137]**. The interacting distance usually falls shorter than the sum of vander Waals radius of the hydrogen atom and sp^2 carbon atom. The interaction is mainly due to charge transfer from the π system to the anti- bonding orbital of the C-H bond, N-H···π and O-H···π interactions which are also same type as C-H···π observed in many crystal structures. According to Malone et al., **[138]** there are, in total six possible forms of interactions between a hydrogen atom and an aromatic ring. The C-H···π is gaining attention because of its role as the driving force in determining crystal packing, molecular conformation. This interaction is characterized by an inter- or intra-molecular distance in the range of 2.6-3.0 Å. Furthermore, the C-H bond points close to the center of an aromatic ring and the angle between the C-H bond and the center of the aromatic ring is close to linearity **[139]** Despite being the weakest hydrogen bond, the C-H···π interaction can influence the molecular recognition pattern especially in a host-guest system **[140]**.

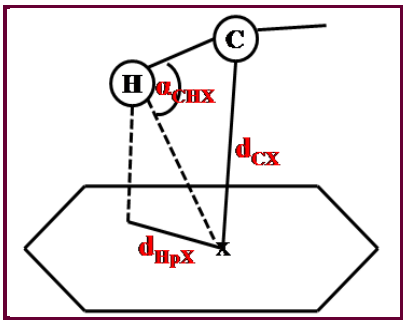

Figure 2.8 C-H… π interaction

π···π Interaction: In chemistry, π···π stacking interaction refers to attractive, noncovalent interaction between aromatic rings, since they contain π bonds. Just like in an electrostatic interaction where a region of negative charge interacts with a positive charge, the electron-rich π system can interact with another π system **[141]**. Non-covalent interactions involving π systems are pivotal to biological events such as protein-ligand recognition **[142]**. The interaction is important in nucleobase stacking within protein folding, template-directed synthesis, DNA and RNA molecules and molecular recognition. Attractive interactions between π-systems are one of the principal non-covalent forces governing

supramolecular architecture. The interaction found to influence the structure of protein, DNA, host-guest complex, solid material containing aromatic groups and also control the interaction of certain drug into DNA **[143]**. The simplest prototype of π···π interaction is considered as the benzene dimer. The simple picture of a π-system can be projected as a sandwich of the positively charged σ-framework between two negatively charged π-electron clouds and it accounts well for the observed interactions between π systems. Thus a face to face interaction is rare but the usual π-interaction is an off-set or slipped stacking i.e. the rings are displaced **[144].** A π···π interaction is defined as an interaction between two aromatic rings in which either (a) the angle between the ring planes is less than 30° and the distance between the ring centroids is less than 4.4 Å (face-to-face), or (b) the angle between the ring planes is between 60° and 120° and the distance between the ring centroids is less than 5.5 Å (edge-to-face) **[145]**. The two molecules forming π-complexes consist of a donor molecule with a low ionization potential so that an electron can be donated (a delocalized π- electron of the polycyclic aromatic hydrocarbon) to an acceptor molecule with a high affinity for electron, resulting in stack of alternating donor and acceptor molecule in the crystal structure. These interactions are characterized by short intermolecular distance between the centroid of the two rings perpendicular to the stacking direction and by 'off-set' distance, the distance of centroid of the one ring to the projection of perpendicular ring (**Figure 2.9**).

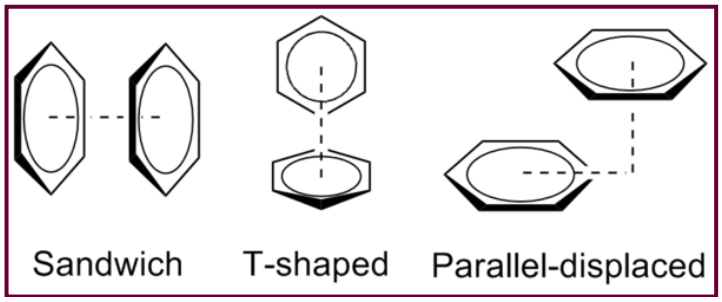

Figure 2.9 Three representative conformations of the benzene dimer

Intermolecular interactions, conventionally described by strong and directional N-H···O, N-H···N, O-H···O and O-H···N hydrogen bonds are the forces which chiefly governed the molecular assembly in a crystal. However, in molecule with an imbalance of hydrogen bond donors and acceptors, the deficiency in either donor-acceptor is fulfilled by other types of weak or less directional forces. Non-conventional hydrogen bond interactions such as C-H···O, C-H···N and C-H···π are of great importance in the absence of conventional hydrogen bond, as they play a significant role in molecular packing. Interactions involving the π-cloud in aromatic compounds also belong to this category. It

is in this context very relevant to invertible the role of these non-conventional interactions in supramolecular architecture of organic molecule.

2.6. CHN Analyzer

A CHN Analyzer is a scientific instrument which can determine the carbon (C), hydrogen (H), and nitrogen (N) elemental concentrations in a given sample. Most CHN systems also have the capability to measure Sulfur (S) and oxygen (O). Such analyzers usually use very small quantities, many times around 1 to 3 mg of the sample. Analytically, the analyzer uses a combustion process to oxidize substances into simple compound which are then quantified by thermal conductivity detection or infrared spectroscopy. Separation of the combusted elemental product is performed by adsorption/desorption or chromatography (restricted to smaller sample size) techniques. An elemental analysis for C, H, and N can be the most direct, fastest, and least ambiguous method for determining a copolymer or blend composition. The photograoh of CHN analyzer, at SICART, Vallabh Vidyanagar, is shown in **Figure 2.10**.

Figure 2.10 CHN analyzer

2.7. Infrared (IR) Spectrophotometer

Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the transition energy of the bond or group that vibrates. The energies are determined by the shape of the molecular potential energy surfaces, the mass of the atoms, and the associated vibronic coupling. The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample. When the frequency of the IR is the same as the vibrational frequency of a bond or collection of bond, absorption occurs. Examination of the transmitted light reveals how

much energy is absorbed at each frequency (or wavelength). This measurement can be achieved by scanning the wavelength range using a monochromator. Alternatively, the entire wavelength range is measured using a Fourier transform instrument and then a transmittance or absorbance spectrum is generated using a dedicated procedure. This technique is commonly used for analyzing sample with covalent bonds. Simple spectra are obtained from sample with few IR active bond and high level of purity. More complex molecular structures lead to more absorption band and more complex spectra. The mid-infrared, approximately 4000–400 cm^{-1} (2.5–25 μm) may be used to study the fundamental vibration and associated rotational-vibrational structure. A basic IR spectrum is essentially a graph of infrared light absorbance (or transmittance) on the vertical axis vs. frequency or wavelength on the horizontal axis. In the present study, the IR spectra are recorded on a Perkin Elmer FT-IR spectrometer GX spectrum using KBr pellets at SICART, Vallabh Vidyanagar (**Figure 2.11)**.

Figure 2.11 Infrared Spectrophotometer

2.8. Nuclear Magnetic Resonance (NMR) Spectrometer

Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy, is a research technique that exploits the magnetic properties of certain atomic nuclei. It determines the physical and chemical properties of atom or the molecule in which they are contained. Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure. For example, NMR can quantitatively analyze mixtures containing known compound. For unknown compounds, NMR can either be used to match against spectral libraries or to infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as

well as studying physical properties at the molecular level such as conformational exchange, phase change, solubility, and diffusion. The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned. The NMR spectrum consists of series of peaks which correspond to different applied field strength and each peak means a set of nuclei at the same magnetic environment. The technique is useful for structure identification of organic, inorganic and polymer compound. In the present study, ^{1}H NMR and ^{13}C NMR spectra are recorded on a Bruker Avance III spectrometer at SICART, Vallabh Vidyanagar (**Figure 2.12)**.

Figure 2.12 NMR Spectrometer

2.9. UV-Visible Absorption Spectrometer

Ultraviolet–visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived color of the chemical involved. Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transition from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. It measures the intensity of light passing through a

sample (I), and compares it to the intensity of light before it passes through the sample (I_o). The ratio I/I_o is called the transmittance, and is usually expressed as a percentage (%T). The absorbance, A, is expressed in terms of transmittance as follows:

$$A = -\log(\%T / 100\%)$$

In the present study, UV-Visible spectra are recorded on a Perkin Elmer Lambda 19 spectrophotometer at room temperature. The photograph of instrument at SICART, Vallabh Vidyanagar is shown in **Figure 2.13**.

Figure 2.13 Perkin Elmer Lambda 19 Spectrometer

2.10. Electrical Conductivitymeter

Conductivity (or specific conductance) of an electrolyte solution is a measure of its ability to conduct electricity. The SI unit of conductivity is siemens per meter (S/m). Conductivity is totally dependent upon concentration of ion. Conductivity is generally measured by conductivity meter which consists of conductance cells having electrode of platinum, this electrode is placed parallel at a fixed distance. As the ionization of solute is totally dependent on temperature, all the results are measured at 25˚C. Electrical Conductivity is determined by using EQ-660 Conductivity meter (**Figure 2.14)** at Department of Physics, Sardar Patel University.

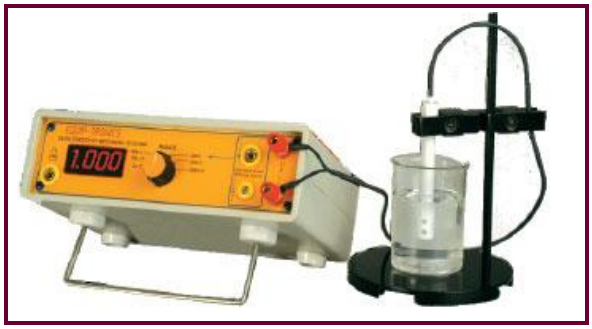

Figure 2.14 EQ-660 Conductivity meter

2.11. Thermogravimmetric Analyzer (TGA)

Thermogravimetric analysis (TGA) is a method of thermal analysis in which change in physical and chemical properties of material are measured as a function of increasing temperature (with constant heating rate), or as a function of time (with constant temperature and/or constant mass loss). TGA is commonly used to determine selected characteristic of material that exhibit either mass loss or gain due to decomposition, oxidation, or loss of volatiles. Common application of TGA are; (1) material characterization through analysis of characteristic decomposition pattern, (2) studies of degradation mechanism and reaction kinetic, (3) determination of organic content in a sample and (4) determination of inorganic content in a sample (e.g. ash), which may be useful to support predicted material structure or simply used as a chemical analysis. Characteristic thermogravimetric curves are given for specific material and chemical compound due to unique sequence from physicochemical reaction occurring over specific temperature range and heating rate. These unique characteristics are related to the molecular structure of the sample. The analyzer usually consists of a high-precision balance with a pan (generally platinum) loaded with the sample. That pan resides in a furnace and is heated or cooled during the experiment. A different process using a quartz crystal microbalance has been devised for measuring smaller sample on the order of a microgram. The sample is placed in a small electrically heated oven with a thermocouple to measure the temperature accurately. The atmosphere may be purged with an inert gas to prevent oxidation or other undesired reaction. A computer is used to control the instrument. Thermogravimetric analysis is made in nitrogen atmosphere using Seiko SII-EXSTAR TG/DTA-7200 (**Figure 2.15)** at Department of Physics, Sardar Patel University.

Figure 2.15 Thermogravimetric SII-EXSTAR TG/DTA-7200 analyzer

2.12. Microbiological Assays

Broth Dilution Method is used to evaluate the antibacterial activity. It is one of the non-automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agent that is needed to inhibit growth of specific microorganisms. The steps for performing the Micro broth dilution method are based on recommendation from the National Committee for Clinical Laboratory Standards **[146]**.
The standard strains used are *Escherichia Coli* MTCC 443, *Shigella Flexneri* MTCC 1457 as gram negative bacteria and *Staphylococcus Aureus* MTCC 96, *Bacillus Subtillis* MTCC 441 as gram positive bacteria. DMSO is used as diluent to get desired concentration of drug to test upon standard bacterial strains. Mueller Hinton Broth is used as Nutrient medium at 37°C to grow and dilute the drug suspension for the test bacteria. Inoculum's size for test strain is adjusted to 108 $CFU.ml^{-1}$ by comparing the turbidity. Each synthesized drug is diluted obtaining 2000 μg/ml concentration, as a stock solution. Serial dilutions are prepared in primary and secondary screening. In primary screening 1000, 500 and 250 μg/ml concentrations of the synthesized drug are taken. The active synthesized drugs, found in this primary screening, are further diluted to obtain 200, 100, 50, 25, 12.5 and 6.25 μg/ml, concentrations for secondary screening. MIC is the lowest concentration of a compound in DMSO that exhibits no visual growth of the organism in the culture tubes. Each of the above experiment is repeated thrice along with a control set using DMSO. The mean value obtained for three individual replicates are then used to calculate the growth inhibition zone of each sample. Antibacterial activity is carried out at Microcare Laboratory, Surat, Gujarat.

2.13. Computer Programs Used

- **WinGX-Version 1.80.05:** WinGX **[147]** is a MS-Windows system of program for solving, refining and analyzing single crystal X-ray diffraction data for small molecule. The WinGX suite is a coherent collection of windows programs for the solution, refinement and analysis of Single Crystal X-ray diffraction data for small molecule.

- **SHELXS-86/97 Program:** The SHELXS-86 program **[148]** is a powerful program for the determination of crystal structure. The SHELXS-86 program is primarily designed for the solution of 'small moiety' (1-200 unique atoms) structure from single crystal at atomic resolution, but are also useful for the location of heavy atom from macromolecular isomorphous or anomalous DF data. SHELXS is general and efficient for all space groups

in all settings, and there are no arbitrary limits to the size of problem which can be handled, except for the total memory available to the program. It is developed by G.M. Sheldrick of University of Gottingen, Germany (1986).

- **SHELX-97 Program:** This is the most upgraded version of the computer program package released by Prof. G. M. Sheldrick in the year 1997 for the crystal structure solution and refinement. SHELX-97 **[149]** contains six executable programs. These are SHELXS, SHELXL, CIFTAB, SHELXA, SHELXPRO and SHELXWAT. The SHELXL refinement program, which carries out full matrix least square refinement of the positional parameters and temperature factors, is used to refine the structure. It also calculates the torsional angle, least square plane, dihedral angle, hydrogen bond geometry etc.

- **ORTEP-3:** Ortep-3 for Windows is a version of the program ORTEP-III (1.0.3) **[150]**, which incorporates a Graphical User Interface to make the production of thermal ellipsoid plot much easier. Using this program, up to 1999 atoms may be drawn (maximum 500 atoms in the asymmetric unit). A number of options are provided to control the view direction.

- **PLATON:** PLATON **[151]** is a general crystallographic tool implementing a large variety of standard geometrical calculations, i.e. calculations of bond length, bond angle, torsional angle, plane and inter molecular contacts etc. either fully automatic or as specified. Molecular graphics program PLUTON, a completely redesigned and considerably expanded variety of the popular program PLUTON 78 by Motherwell and Clegg, is available as an option within PLATON. Most PLATON features complement those available in the widely distributed SHELX-1997 package.

- **PARST:** PARST is a system of computer routines written by M. Nardelli (1983, 1995) **[152]** for calculating molecular parameters from the result of crystal structure analysis. The program calculates least square planes, bond distance, bond angle, and dihedral angle formed by planes, intramolecular and intermolecular contact, possible hydrogen bond etc.

- **Mercury Program:** Mercury **[153]** offers a comprehensive range of tool for the 3D structure visualization and the exploration of crystal packing.

- **PublCIF:** The CIF standard is supported, maintained and developed by the International Union of Crystallography (IUCr) and most major journals require electronic

data deposition in cif format. Crystallographic Information File (cif) is the internationally agreed standard file format for information exchange in crystallography. PublCIF **[154]** enables users to validate cifs and ensure their files are format-compliant for deposition with journal and database or for storage in laboratory archives. Supplement the data in cif via two data entry wizards, one for publication details and the other for chemical, physical and crystallographic properties.

- **CHEMDRAW 7.0:** CHEMDRAW **[155]** for windows (version: 7.01. February, 2002) is a computer programme software from CambridgeSoft Corporation provides the chemical properties and information of the chemical structure drawn using it. It also provides IUPAC names to the chemical structure drawn as well as structures from the IUPAC names. This programme generates the .mol file from the chemical structure which can be used as an input file to the different software programme.

- **Crystal Explorer 3.1:** Crystal Explorer **[156]** is a full-featured molecular crystal visualization tool. Crystal Explorer provides a new way of visualizing interactions in molecular crystals using the full suite of Hirshfeld surface tools and ab initio quantum mechanical calculations. Structure of complex, which is imported from cif files with H–X bond length, set to neutron value of C–H = 1.083 Å, O–H = 0.983 Å and N–H = 1.009 Å.

- **Schrödinger:** Schrödinger is scientific leader software in computational chemistry, providing software solution and service for life science and material research. It aims to provide integrated software solution and to give power to researchers around the world to achieve their goal of improving human health and quality of life through advanced computational techniques. Hence to accelerate their research and development activities and make novel discoveries. **Dr. Richard A. Friesner** is a co-founder of Schrödinger, and a member of Schrödinger's Board of Directors who is Professor of Chemistry at Columbia University and Director of the Columbia Center for Biomolecular Simulation. Schrödinger has achieved breakthrough in quantum chemistry, molecular modeling, force field, molecular dynamic, protein structure determination, scoring and virtual screening.

- **JAGUAR:** JAGUAR **[157]** is a high performance ‘Ab initio and DFT’ package from Schrödinger software for both gas and solution phase simulation. It helps to calculate theoretically bond length, bond angle, torsional angle and dihedral angle. Computed bond length, bond angle, torsional angle and dihedral angle are compared with the experimental

X-ray data. Molecular orbital and orbital energies, charge distribution, dipole moment have been calculated. The input file for JAGUAR can be .cdx, .mol, .cif or .pdb etc.

- **GLIDE:** GLIDE **[158-160]** offers the full spectrum of speed and accuracy from high throughput virtual screening of millions of compound to extremely accurate binding mode prediction. The PDB file of structure is used for the docking study with different receptors using GLIDE module of Schrodinger software. The ligand molecule is subjected full minimization with OPLS_2005 force field using LigPrep module. The structure of receptors are imported and refined by Protein Preparation Wizard **[161]**. Energy minimization is done by using OPLS_AA force field and refinement is carried out until average mean square deviation of the non hydrogen atoms reached 0.3Å and resulting optimized structure is used for docking. Receptor grid is generated and enclosed by a box at the receptor residue. Finally, prepared ligand and receptor are docked by Ligand Docking utility.

- **CLP**: The Coulomb London Pauli (CLP) approach **[162]** is encoded in a program package for the calculation of intermolecular properties of molecular clusters or of condensed phases (liquid and crystal). The program is written in plain FORTRAN which can compile and operate on all types of computers. Units are angstrom for all length and coordinate (except fractional coordinate), and $kJmol^{-1}$ for all energies. The PIXELC **[109]** method has been applied to a number of problem in intermolecular interaction, and its performance is comparable to that of MP2 calculation on dimers and crystal lattice energies. The input file for PIXEL is .cif file.