CHAPTER 2

Experimental Techniques
# Chapter 2

**Experimental Techniques**

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2.1 Single Crystal Growth

2.1.1 Single Crystal

A single crystal is a material in which the crystal lattice of the entire sample is continuous and unbroken to the edges of the sample, with no grain boundaries. i.e. atomic arrays those are periodic in three dimensions, with repeated distances and outstanding advantages of single crystals [80-81]. A good quality of single crystal is pre-requisite 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.

2.1.2 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, hours, days and to months. Single crystals may 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 [82]. Growing good single crystals is an art. This is clearly expressed in one of the text book on crystal growth [83, 84]. 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. They are

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 temperatures [85] and also which undergo phase transformations below melting point [86]. In vapor diffusion and liquid diffusion method, one solvent diffuse into the other while precipitating the product that results into depositing crystals. Sublimation is different than above listed methods of crystal growth. In this method compound is heated under vacuum and crystals are collected on cold-finger. Slow evaporation technique is used to grow the single crystals of these heterocyclic compounds from different solvents like water, methanol, chloroform, ethanol, ethyl acetate.

2.1.3 Slow Evaporation Method

In the Slow evaporation method, crystals are grown from the saturated or nearly saturated solution of the compound, prepared using different solvents like methanol, ethanol, acetonitrile, ethyl acetate or mixture of any two solvents with varying proportions and is allowed to stand without disturbance. Solvent evaporates slowly resulting in the precipitation of the compound in the form of single crystal. It is the best way to grow single crystals by solution technique. Growth of good quality single crystals by slow evaporation techniques require optimized conditions. The use of a range of temperatures may not be desirable because the properties of the grown material may vary with temperature. There are so many factors affecting on the mechanism of crystallization like, the interaction of ions/molecules of the solute, the solubility of substance and thermodynamic parameters of the process; temperature, pressure and
solvent concentration. The choice of the solvent is very important because it can greatly influence the mechanism of crystal.

2.1.4 Choice of Solvent and Solubility

Water is a popular solvent for the growth of a variety of inorganic and organic compounds. A simple thumb rule in proper selection of a solvent is chemical similarity between the solvent and the compound to be grown. For example, crystals of nonpolar organic compounds can be grown easily from nonpolar organic solvents. Chemical similarity also determines solute solubility in the 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 [87]. A solvent can also play an indirect role in changing the morphology of a crystal. The solvent changes the structure of the solute molecules, which subsequently adsorb on several faces of the crystal, thereby blocking their normal growth. The solvent has a strong influence on the habit of crystalline materials because solvent molecules affect the growth rates of different faces appearing in the crystal morphology differently. Solution is a homogeneous mixture of a solute in a solvent. Solute is the component, which is present in a smaller quantity. If the solubility is too high, it is difficult to grow bulk single crystals and too small a solubility restrict the size and growth rate of the crystals. Solubility gradient is another parameter, which dictates the growth procedure.

2.1.5 Factors Influence the Crystal Growth

There are number of variables which influence on crystallization of organic materials.
❖ Sample purity: The poor purity is one of the most common cause of unsuccessful crystallization and at the same time, the selection of a proper solvent for a given crystallization is difficult to predict.

❖ Selection of a proper solvent: A mixture of two or more solvents is occasionally found to possess the best properties for a particular crystallization purpose. To reduce the solubility of the solute, another solvent is added to a solution causing its precipitation and maximize the yield of the product, however it is expected that both the liquids must completely mix with one another.

❖ Temperature: Other environmental variables which influence crystallization include the rates of change of temperature and concentration of solution.

❖ Viscosity of solvent: The viscosity of the solvent directly affects the rate of crystal growth through its influence on the solute flux. Increased volatility of the solvent raises the rate of increase in concentration of the solution, speeding up both nucleation and growth.

❖ Chemical nature of solution: The chemical nature of the solution also plays a leading role in the crystallization process. The nature of solute-solvent interactions 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.

2.2 Density measurement

Crystal density is usually measured by flotation method, in which the crystal is made to float on a mixture of two liquids. Knowledge of crystal density also helps to establish crystal composition. The proportions of the liquid are adjusted until the crystal remains suspended in the medium. The pair of liquid often used is benzene-carbon tetrachloride (C_6H_6-CCl_4, 0.87 & 1.59 Mgm\(^{-3}\)), bromoform-benzene (CHBr_3-C_6H_6) and aqueous solution of potassium iodide/methyl iodide. The following equation is used to work out the crystal density,
The Aqueous potassium iodide solution is used to float the crystals. Density of the crystal can also be theoretically computed, once the lattice parameters are known using the following equation

\[ \rho_{\text{cut}} = \frac{M \times 1.6604 \times Z}{V} \]

Where, 
- \( Z \) = No. of molecules per unit cell
- \( V \) = Volume of the unit cell
- \( M \) = Molecular weight

The above equation can be used to determine 'Z', the no. of molecule per unit cell. Crystal density is measured for all the crystals and the result is summarized in each chapter.

### 2.3 X-ray diffraction study

#### 2.3.1 X-Ray Diffraction by Crystal

X-rays are discovered by (Wilhelm Röntgen) in 1895. X-rays are used to produce the diffraction pattern because their wavelength (\( \lambda \)) 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-Braggs' law (Figure 2.1) is established by father and son W. H. Bragg and W. L. Bragg which is given by

\[ 2d_{\text{hkl}} \sin \theta = n\lambda \]  

Where,
- \( n \) = Order of reflection
- \( d_{\text{hkl}} \) = inter-planar spacing of the planes which makes an angle ‘\( \theta \)’ with incident beam
- \( \lambda \) = wavelength of X-rays
- 2\( \theta \) = angle by which the Bragg reflection deviates from direct beam
Figure 2.1 The "Ewald Sphere" construction. The reciprocal lattice has its origin at O. The sphere of radius $1/\lambda$ 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.

**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 structure factor is particularly useful tool in the interpretation of interference patterns obtained by X-ray, electron and neutron diffraction experiments. The concept of atomic scattering factor ($f$) i.e. the efficiency of an atom to scatter which is defined by

$$f = \frac{\text{Amplitude of the wave scattered by an atom}}{\text{Amplitude of the wave scattered by an electron}}$$

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

$$F = \frac{\text{Amplitude of the wave scattered by a unit cell}}{\text{Amplitude of the wave scattered by an electron}}$$

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.

\[
F_{hkl} = \sum_{n=1}^{N} f_n \exp \left\{ 2 \pi i (u_n + k v_n + l w_n) \right\}
\]

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:

\[
F_{hkl} = \sum_{n=1}^{N} f_n \cos 2\pi (u_n + k v_n + l w_n)
\]

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

\[
\rho(xyz) = \frac{1}{V} \sum_h \sum_k \sum_l F_{hkl} e^{-2\pi i (hx + ky + lz)}
\]

where \( \rho(x, y, z) \) 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 positions).

### 2.3.2 X-ray Diffractometer

The X-ray diffractometer is an instrument, which measures the intensities of diffracted beams 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 positions of the diffraction spots. The exact atomic position within the unit cell can then be determined from the intensity of the spots. Single crystal X-ray diffractometer can determine crystal structure with detailed informations about bond lengths and bond angles. The term four circle is referred to as the number of rotational motions available to the crystal orientation defined by φ, x, ω and the detector position defined by the fourth angle 2θ. The detector is constrained to move in the horizontal plane containing the incident X-ray beam. We have used Bruker Kappa Apex II diffractometer (Figure 2.2) for intensity data collection of the single crystal. The Goniometer (Figure 2.3) allows flexible sample orientation and can complete data collection to high theta values.

Figure 2.2 Bruker AXS Kappa Apex II diffractometer
2.4 Structure solution and refinement

Accurate and rapid measurement of the position and intensity of the hkl reflections is possible using single crystal X-ray diffraction. The methods available to detect X-ray do not record all the informations about the diffracted X-ray, only the intensity. The detectors are not sensitive to the phase of the X-rays. 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 angles of the reflections are measured and indexed, the size of the unit cell, information on translation symmetry elements and symmetry information are obtained. The intensities (and position) of the reflections 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.

❖ The Lorentz 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.
❖ The Polarization correction (P) allows the fact that a non-polarized X-ray beam may become partially polarized on reflection from the crystal.
❖ An absorption correction is applied, particularly for inorganic structures, because large Z atoms absorb some X-rays 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, was first investigated by Darwin (1922), who 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.

2.4.1 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 positions, one can calculate the structure factors, but with X-ray diffraction, we measure the structure factors and from them the atomic positions. The phase problem occurs, since when we take the square root of the intensity, we obtain the modulus of the structure factor, i.e. the magnitude and not the sign of the structure factor is known. The phase information is lost and we need that 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 \( F_{\text{obs}} \) or \( F_0 \). 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 phases. The methods are

1. Direct methods
2. Patterson method
3. Isomorphous replacement method
4. Anomalous scattering method
**Direct methods**: It is used for crystals 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 were awarded the 1985 Nobel prize in Chemistry. The basic assumptions used to derive phases in direct methods 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 values 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 methods are the preferred method for phasing crystals of small molecules having up to 1000 atoms in the asymmetric unit. However, they are generally not feasible by themselves for larger molecules such as proteins [88].

**Patterson method**: Patterson introduced the method of determining inter-atomic distances from a Fourier transform of intensities in 1934 [89]. It relies on the presence of at least one (not many) heavy atoms in the unit cell and is useful for solving inorganic structures.

The family of methods employed in structure determination to derive relationships between the scattering centres in a crystal lattice when the diffraction phases are unknown. They depend upon interpretation of the Patterson function

\[
P(uvw) = \frac{1}{V} \sum \sum \sum |F(hkl)|^2 \cos [2\pi(hu + kv + lw)]
\]

\[h \ k \ l\]

to reveal inter atomic vectors within the unit cell.
An electron density map can be constructed from an inverse Fourier transform of the structure factors of a wave diffracted from a crystal. Diffracted intensities can be measured directly, and are related to the square of the amplitudes of the structure factors; but the diffraction phases 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 atoms. 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 tips 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 locations of other atoms.

2.4.2 Structure Refinement

After the atoms in a structure have been located, a set of structure factors, $F_{\text{calc}}$ and $F_c$, are determined for comparison with the $F_{\text{obs}}$ 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 process by which we obtain an electron density map in which we have more confidence is known as Refinement.
❖ The derivation of the trial structure
This is deduced from the first calculated electron density function using the observed structure factor magnitudes with the observed phases. This is a crude model of the true structure.

❖ Cyclic Fourier refinement
From the trial model, we calculate a set of structure factors, allowing for thermal effects. We then compute new Fourier synthesis using the observed structure factor magnitudes with the calculated phases. The cyclic Fourier refinement processes primarily give us better values of the phases, 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 phases, 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 positions, 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 $F_0$ or $F_0^2$ data
Refinement may be carried out either as a function of errors in the structure factors, $\Delta_1 = |F_0| - |F_c|$ or in those of the intensities, $\Delta_2 = |F_0^2 - F_c^2|$. Until recently, nearly all refinements are carried out based on $F_0$, i.e. using $\Delta_1$. For such a refinement, very weak data give problems, 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 $F_0$ can be directly calculated. To avoid this problem, it is customary to take an arbitrary values for $F_0$ (e.g. $\sigma F_0/4$) for all “unobserved data”, say data with $F_0^2 < \sigma[F_0^2]$, so that they can be used for relationship in direct methods. 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 residuals or “R-factors” are evaluated. The “conventional” R-factor is

$$R = \frac{\sum_{hk} |F_h| - |F_c|}{\sum_{hk} |F_h|}$$

When multiplied by 100% gives the average relative deviation between the observed and calculated structure factors as a percent. This is always quoted in the literature, even when the refinement was not based on $F_0$. 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. The changes, if any show whether changes in the structure model are actually meaningful. Unfortunately differences in weighting schemes make it difficult to use for comparing one structure with another. It is defined differently, depending on whether the refinement is based on $F_0$ or on $F_0^2$.

$$\omega R = \left[ \frac{\sum_{hk} \omega \Delta_1^2}{\sum_{hk} \omega F_0^2} \right]^{1/2} \quad \text{and} \quad \omega R_2 = \left[ \frac{\sum \omega \Delta_2^2}{\sum \omega (F_0^2)^2} \right]^{1/2}$$

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

$$S = \left[ \frac{\sum_{hk} \omega \Delta^2}{(m - p)} \right]^{1/2}$$

($m =$ number of reflections, $p =$ number of parameters). The difference $m - p$ gives the over determination of the structure. For a correct structure with a suitable weighting scheme, $S$ will have a value close to 1.
2.5 Intra and Intermolecular interactions

Interactions between two or more molecules are called intermolecular interactions, while the interactions between the atoms within a molecule are called intramolecular interactions. Intermolecular interactions occur between all types of molecules or ions in all states of matter. Hydrogen bonding interaction is comparatively stronger than most other intermolecular forces but weaker than covalent bond and ionic bond interactions.

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 orientations. Interactions are termed as being isotropic (lacking directionality) or anisotropic (having directionality). 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 interactions and purely ionic interactions. Anisotropic interactions have certain extra chemical attributes that arise from specific electronic distributions around atoms. Hydrogen bonding is the most important directional interaction in molecular crystals. It is the anisotropic character of interactions in a crystal structure that allows one to suggest design strategies for crystals of related molecules.

2.5.1 Hydrogen Bond interactions

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 typical hydrogen bond can be represented as an interaction between a donor X-H and an acceptor Y-Z. The hydrogen bond is then written as X-H...Y-Z with the three dots signifying the bond. Scientists have been studying hydrogen bonds for just under 100 years. Only in such a case it is felt that the X-H could become sufficiently polarized so that it would be attracted electrostatically to the electronegative Y atom. So a hydrogen bond is shown as X (δ−)−H (δ+)...Y (δ−)−Z. It is always known, however, that there was some small
amount of covalent character in a hydrogen bond. Generally, it was observed that in a hydrogen bond of the type X–H···Y–Z, the distance between the elements X and Y is much shorter than the sum of their van der Waals radii. As the name hydrogen bond, one part of the bond involves a hydrogen atom which is attached to strongly electronegative hetero atoms, such as O, N which are the strong H-bond donor. These electronegative atoms form 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, hydrogen can attach a lone pair of electron from an electronegative atom which becomes hydrogen-bond accepter. These classical hydrogen bonds O-H···O, N-H···O etc are well established and play a very significant role in stabilizing supramolecular structure of organic solids. However, in heterocyclic compounds, the absences of conventional hydrogen bond donor and accepter, a set of somewhat weaker and less directional interactions such as C-H···O, C-H···π, π···π have been recognized to play an important role in generating supramolecular structure.

C-H···O interactions have electrostatic character with a long range distance fall-off. The length of a C-H···O bond depends on both the acidity of the C-H group and the basicity of the oxygen atom. The more acidic C-H groups form shorter C-H···O hydrogen bonds. The more weakly acidic C-H donors form long hydrogen bonds, whose length may exceed the van der Waals separation distance. In practice, many longer C-H···O contacts (C···O 3.50–4.00 Å) have angular characteristics and effects on crystal structures that resemble the shorter contacts (3.00–3.50 Å). The C-H···O bond is not a van der Waals contact but is primarily electrostatic, falling off much more slowly with distance and hence viable at distances that are equal to or longer than the van der Waals limit. Even long C···O separations (~4.00 Å) may need to be considered; these contacts may have their origin in certain preferred orientations of molecules as they approach each other during crystallization. Accordingly, the study of weak
hydrogen bonds is important in crystal engineering. Typical C–H···O hydrogen bond angles (θ) occur in the range 100–180°. They cluster around 150–160° for reasons stated earlier. The length of a C–H···O bond correlates inversely with the angle. This is an essential criterion for hydrogen bonding. Most strong hydrogen bonds cluster in small length and angle ranges. Weak hydrogen bonds like C–H···O are found in larger length and angular ranges because they are weak: they can be distorted by other forces in the crystal. While C–H···O hydrogen bonds are widespread, the complementary O–H···π interactions are rare because carbon is not as electronegative as oxygen and also because carbon atoms are not often situated in sterically unhindered positions, unlike carbonyl and ethereal oxygen atoms that permit easy access by C–H groups to form C–H···O hydrogen bonds. C–H···π interactions are even weaker than O–H···π and C–H···O hydrogen bonds because both the donor and acceptor fragments are weakly polarized. There is no well accepted convention in which C–H···π interactions are accepted as hydrogen bonds. When the donors and acceptors are somewhat stronger, as in say acetylene and other alkynes, the definition of a C–H···π geometry as a hydrogen bond is somewhat less contentious. The C-H...O hydrogen bond was first identified by Sutar (1962) [90]. The C-H...O bond though weak and attractive interaction with a long range distance character and occur within certain distances (C...O = 3.0-4.0Å) angle (C-H...O =90°-180°) ranges, Taylor and Kennart (1982) [91]. It is electrostatic in nature, and resembles C-H...O and N-H...O hydrogen bonds in its geometrical properties, including the property of directionality on the donor as well as on the acceptor side of the contact. The C-H...O bond termed as C-H interaction and is observed widely among crystal structures those contain comparatively more number of oxygen atoms but relatively few proton groups.

The significance of these C-H...O interaction in a particular structure increases with their number relative to the stronger O-H...O and N-H...O interactions. Other types of weak interaction such as π-π
interaction involving interaction between the \( \pi \)-electron systems of aromatic, as well as that of aliphatic are generally called as \( \pi \)-complexes, are well established.

### 2.5.2 C-H\( \cdots \pi \) interaction

C-H\( \cdots \pi \) interactions are the weakest hydrogen bonds that operate between a soft acid C-H and a soft or intermediate base \( \pi \) system [92]. It has been recognized that this kind of weaker and softer interaction plays significant roles in various chemistry [93], self-assembly, and chiral recognition. As it is non-polar and effective in water, the C-H\( \cdots \pi \) interaction is also especially important in biological systems [94]. C-H... \( \pi \) interactions (Figure 2.4) is another type of C-H weak important molecular force having a directional preference with the pointing towards the center of electron rich aromatic ring [95]. The interacting distances usually fall 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 \( \pi \) system to the anti- bonding orbital of the C-H bond, N-H...\( \pi \) and O-H...\( \pi \) interactions which are also same type as C-H...\( \pi \) observed in many crystal structures. According to Malone et al., [96] there are, in total six possible forms of interactions between a hydrogen atom and an aromatic ring.

![Figure 2.4 C-H\( \cdots \pi \) interaction](image)
2.5.3 π - π Interactions

In Chemistry, π-effects or π-interactions are a type of non-covalent interaction that involves π systems. 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 a metal (cationic or neutral), an anion, another molecule and even another π system [97]. Non-covalent interactions involving π systems are pivotal to biological events such as protein-ligand recognition [98]. π-π interaction or aromatic-aromatic interactions are one of the important non-covalent intermolecular forces similar to that of hydrogen bonding. Attractive interaction 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 complexes, solid materials containing aromatic groups and also control the interaction of certain drugs into DNA [99]. The simplest prototype of π-π interactions are 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. It is a π-σ attraction rather than π-π electronic interaction which leads to favorable interactions. 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 (Figure 2.5).

![Model of bonding in benzene](image)

**Figure 2.5 Model of bonding in benzene**
The two molecules forming \( \pi \)-complexes consist of a donor molecule with a low ionization potential so that an electron can be donated (a delocalized \( \pi \)-electron of the polycyclic aromatic hydrocarbon) to an acceptor molecule with a high affinity for electrons, resulting in stacks of alternating donor and acceptor molecules are found in the crystal structures. 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 one ring to the projection of perpendicular ring. The most important amongst these are those with the \( \pi \)-cloud of the phenyl rings, Y-H...ph (aromatic hydrogen bonds) which occur in many chemical and biological systems.

### 2.5.4 Interaction involving halogen atoms

Halogen bonding is the attractive donor-acceptor interaction involving an atom possessing one or more lone pair of electrons (such as O, N or S which donate electrons, Lewis base) and a halogen atom (Cl, Br or I functioning as Lewis acid accepting the lone pair of electrons). Like hydrogen bond, in which H-atom covalently bonded to an electronegative atom approached another electronegative atom in halogen bond, the halogen atom bonded to an atom approaches a more electro negative atom. The stronger the electron withdrawing environment around the halogen atoms, higher is their ability to be engaged in halogen bonding interaction. The term halogen bonding was introduced by Dumas et al., in 1983 [100], latter used by several others [101-103]. Halogen (F, Cl, Br and I) can form short non bonded contacts both with electron acceptor as well as electron donors [104]. Halogen atoms have an anisotropic (non spherical) charge distribution in it (except fluorine, extreme electronegative and limited Polarizability). Few well recognized weak intermolecular interaction involving halogens: The C-hal...\( \pi \) type interaction was first reported by Dastidar [105]. A systematic Cambridge crystallographic data base and protein data bank study on intra and intermolecular geometries of
C-hal...π contacts have been carried out [106]. C-hal...π interactions are highly directional and though relatively weak but the directional nature of the hal...π contacts influence the packing of organic molecular crystals and provide a new tool for crystal engineering of both homo molecular and supramolecular aggregation of organic solids [107]. C-H...X interactions are real and do play an important role in molecular aggregation and useful for prediction of crystal structure C-H...x (x = F, Cl, Br, I) type interactions are observed to occur wherever halogen acts as hydrogen bond acceptor.

Intermolecular interactions, conventionally described by strong and directional N-H...O, C-H...O and O-H...N hydrogen bonds are the forces which predominantly governed the molecular assembly in a crystal. However, in molecules 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, C-H...π, C-H...hal (Cl, Br, I or F) are of considerable importance in the absence of conventional hydrogen bond, as they play an important role in molecular packing. Interactions involving the π-cloud in aromatic compounds also belong to these category, several types of aromatic X-H...π, X-hal...π and X-H...hal interactions have been characterized in many molecular assembly [108]. Potential importance of all these interaction generating supramolecular assembly with novel properties is the driving force behind exploring these interactions. It is in this context very relevant to invertible the role of these non-conventional interactions in supramolecular architecture of organic molecules and the work presented in the thesis form, author has made an attempt along this direction. Ten significant heterocycles derivatives are investigated by X-ray diffraction technique and the roles of non-covalent interactions involved there have been thoroughly analyzed.
2.6 Study of antimicrobial activity

To correlate the structure-function relationship of a particular molecule, the compound has been tested for its biological response; especially antimicrobial activity. The history of antimicrobials begins with the observation of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacteria failed to grow was that the other bacteria are producing an antibiotic. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health [109]. Nowadays gonorrhea, strep throat or pneumonia most of these infections can be easily cured with a short course of antimicrobials.

Estimation and detection of inhibitory or stimulatory of compounds by using microorganisms as test culture is defined as Bioassay.

Test cultures used for bioassay should possess following qualities:

- It should be genetically stable.
- It should respond in a graded manner only to the compound to be assayed and not to other materials present in solution.
- It should be aerobic or facultative aerobic.
- It should grow fast on assay medium.
- It should grow at pH that does not affect stability or toxicity of material under assay.
- It should grow in such a way that results can be interpreted properly.

Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods [110-111] are divided into types based on the principle applied in each system. They include:

<table>
<thead>
<tr>
<th>Diffusion &amp; Dilution (Minimum Inhibitory Concentration)</th>
<th>Diffusion Dilution</th>
<th>E-Test method</th>
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</thead>
<tbody>
<tr>
<td>Stokes method</td>
<td>Broth dilution</td>
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<tr>
<td>Test method</td>
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<tr>
<td>Kirby-Bauer method</td>
<td>Agar Dilution</td>
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There are three types of bioassay techniques:

(i) Multidisc agar cup method
(ii) Cylinder method
(iii) Disc method

Among these methods, Multidisc agar cup method is used to investigate the biological response of the title compounds.

**Multidisc agar cup method**

Principle: When an antibiotic is added in agar cup (made in previously inoculate with test organism) the radial diffusion of an antibiotic through the agar produce a concentration gradient. Thus zones of inhibition are formed. The diameters of zones of inhibition suggest the degree of sensitivity of test organism towards the antibiotics.

**Disc diffusion methods**

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS [112]. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective. Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC's and the results have corroborated with clinical data. Based on study results NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

**2.6.1 Procedure for Performing the Disc Diffusion Test**

*Growth Method*
The growth method is performed as follows

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.

2. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours)

3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

**Direct Colony Suspension Method**

- As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from an 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.

- This approach is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae*, and streptococci, and for testing staphylococci for potential methicillin or oxacillin resistance.

**Inoculation of Test Plates**

- Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
• The dried surface of a Müller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.

• The lid may be left agar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

**Application of Discs to Inoculated Agar Plates**

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.

2. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of *Haemophilus* spp., streptococci and *N. gonorrhoeae*, the plates should not be incubated in an increased CO₂ atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO₂ will significantly alter the size of the inhibitory zones of some agents.

**2.7 Computer Programs Used**

*WinGX* Version 1.80.05

The *WinGX* suite [113] is a coherent collection of (publically available crystallographic) programs for the solution, refinement and analysis of single crystal X-ray diffraction data for small molecules.
**SHELXS-86/97 Program**

The SHELXS-86 program [114] is primarily designed for the solution of 'small moiety' (1-200 unique atoms) structures from single crystal at atomic resolution, but is also useful for the location of heavy atoms 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 problems 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 [115] contains six executable programs. These are SHELXS, SHELXL, CIFTAB, SHELXA, SHELXPRO and SHELXWAT. The refinement program SHELXL carries out full matrix least squares refinement of the positional parameters and temperature factors. It also calculates the torsion angles, least squares planes, dihedral angles, hydrogen bond geometry etc.

**Mercury Program**

Mercury [116] offers a comprehensive range of tools for the 3D structure visualization and the exploration of crystal packing.

**ORTEP-3**

Ortep-3 for Windows is a version of the program ORTEP-III (1.0.3) [117], which incorporates a Graphical User Interface to make the production of thermal ellipsoid plots much easier.

**PLATON**

PLATON [118] is a general crystallographic tool implementing a large variety of standard geometrical calculations, i.e. calculations of bond lengths, bond angles, torsion angles, planes, inter molecular contacts etc. either fully automatic or as specified. Most PLATON features complement those available in the widely distributed SHELX-
1997 package. Molecular graphics program PLUTON, a completely redesigned and considerably expanded variety of the popular program PLUTO 78 by Motherwell and Clegg, is available as an option within PLATON.

**PARST Program**

PARST is a system of computer routines written by M. Nardelli [119] for calculating molecular parameters from the results of crystal structure analysis. The program calculates bond distances, bond angles, least squares planes and dihedral angles formed by planes, intramolecular and intermolecular contacts, possible hydrogen bonds etc.

**PubCIF**

Crystallographic Information File (CIF) is the internationally agreed standard file format for information exchange in crystallography. The CIF standard is supported, maintained and developed by the International Union of Crystallography (IUCr) and most major journals require electronic data depositions in CIF format.

Although the CIF provides an excellent architecture for archiving and accessing crystallographic data electronically, it is still perhaps rather 'unfriendly' with respect to human-readability and manual manipulation, e.g. for a non-crystallographer who wishes to publish a paper in a journal that only accepts CIF submissions of structure reports.

publCIF [120] enables users to validate CIFs and ensure their files are format-compliant for deposition with journals and databases or for storage in laboratory archives. Supplement the data in your CIF via two data entry wizards, one for publication details and the other for chemical, physical and crystallographic properties.

**CHEMDRAW 7.0**

CHEMDRAW [121] for windows (version: 7.01. February, 2002) is a computer programme software from CambrigdeSoft 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 PASS programme.

**PASS Programme**

'PASS' (Prediction of Activity Spectra for Substances) predicts simultaneously several hundreds of biological activities (pharmacological main and side effects, mechanisms of action, mutagenicity, tetratogenicity and embryotoxicity) with mean accuracy of prediction about 85% on the basis of the compound's structural formula. So that we can use PASS for the prediction of the biological activity spectrum for existing compounds and compounds, which are only planned to synthesize. The concept of Biological Activity Spectrum served as a basis for developing PASS [122] software product.

**Gaussian 09 software**

Gaussian is a computer program for Computational Chemistry initially released in 1970 by John Pople's use of orbitals to speed up calculations compared to those using orbitals, a choice made to improve performance on the limited computing capacities of then-current computer hardware for Hartree-Fock calculations. The current version of the program is Gaussian 09 [123]. Originally available through the Quantum Chemistry Program Exchange, it was later licensed out of Carnegie Mellon University, and since 1987 has been developed and licensed by Gaussian, Inc.

Gaussian quickly became a popular and widely-used electronic structure program. Prof. Pople and his students and post-docs were among those who pushed the development of the package, including cutting-edge research in quantum chemistry and other fields.