CHAPTER – I

INTRODUCTION

1.1. Chemical Crystallography

The knowledge of accurate molecular structure is a prerequisite for drug design and structure-based functional studies to aid the development of effective therapeutic agents and drugs. Crystallography is the experimental science of the arrangement of atoms in solids. It provides a reliable answer to many structure-related questions from global fields to atomic details of bondings. Crystallographic methods depend on the analysis of the diffraction patterns of a sample targeted by a beam of X-rays, neutrons, or electrons. These three types of radiations interact with the specimen in different ways. Because of these different forms of interactions, the three types of radiations are suitable for different crystallographic studies. However, X-rays are the most commonly used in crystallographic studies.

Chemical crystallography is expected to pave paths into nano sciences, reaction pathways and also, above all, make significant contributions to biological science\textsuperscript{1a,lb}.

1.2. Supramolecular chemistry

Supramolecular chemistry is relatively a new field of chemistry which focuses literally on going “beyond” molecular chemistry\textsuperscript{2}. D. J. Cram, J. M. Lehn, and C. J. Pedersen recognized the importance of supramolecular chemistry and for their work in this area they were awarded Nobel Prize for Chemistry in 1987. Unlike the organic synthesis which utilizes covalent bonds to synthesize a desired molecule, supramolecular chemistry utilizes far-weaker and reversible non-covalent interactions such as hydrogen bonding, metal coordination, hydrophobic forces, van der Walls
forces, $\pi-\pi$ interactions and sometimes electrostatic effects to assemble molecules into multimolecular complexes. Among these different types of non-covalent interactions, the hydrogen bonding plays a very important role in supramolecular organization.

Various fields that are classified under supramolecular chemistry include molecular self-assembly, molecular recognition, host-guest chemistry, mechanically-interlocked molecular architectures, and dynamic covalent chemistry\(^3\). It is important for the development of new pharmaceutical therapies by understanding the interactions at a drug binding site. In addition, supramolecular systems have been designed to disrupt protein-protein interactions that are important for cellular function. It also has applications in green chemistry.

### 1.2.1. Application of Supramolecules

Molecular devices are functional materials that are structurally precise down to the molecular level and are constructed using the concepts of supramolecular chemistry. Super molecules capable of electron conduction and electrical switches\(^5\) (molecular electronic devices), supermolecules that can be used for information processing and calculations (molecular computer) and supermolecules that move, rotate and catch targets (molecular machines) are introduced as examples of molecular devices\(^6\). Well-defined molecular assemblies provide useful devices for direction controlled information transfer. These examples suggest that supramolecular chemistry will be the main tool used in the development of biotechnology and nanotechnology which are predicted to revolutionize our lifestyles and economics in the near future\(^7\).
1.3. Hydrogen bonding

The hydrogen bond was discovered almost 100 years ago, but still it is a topic of vital scientific research. The reason for this long-lasting interest lies in the eminent importance of hydrogen bonds for the structure, function, and dynamics of a vast number of chemical systems which range from inorganic to biological chemistry. The diverse scientific branches involved are mineralogy, material science, general inorganic and organic chemistry, molecular medicine and pharmacy.

The interest in this subject is rapidly developing as shown in the Fig. 1.1. The number of references in chemical abstracts under the heading ‘The Hydrogen Bond’ has increased progressively. It is reasonable to predict that a paper on hydrogen bonding is now being published every half an hour of the working day, somewhere in the world.

**Figure 1.1** Number of papers abstracted by *Chemical Abstracts* under the subject index with “Hydrogen Bonding”
Studies carried out in the period 1939-1953 showed strong evidence that the principal cohesive forces between the subunits of biological structures were hydrogen bonds. These were recognized as stronger than van der Waals forces and more directional leading to compounds with high melting points and generally harder crystals. The significance of hydrogen bonding in biological structure was realized by the two major discoveries, the α-helix and β-pleated sheet structure of protein architecture in 1951 and the Watson-Crick base-pairing in the DNA double helix in 1953. All subsequent structural research on proteins and nucleic acids has reinforced the concept that although hydrogen bonds are weak interactions, they are the most important atomic interactions, determining the three-dimensional folding of biological macromolecules. Life would be impossible without hydrogen bond!

Hydrogen bond is a weak electrostatic chemical bond which forms between covalently bonded hydrogen atoms and a strongly electronegative atom with a lone pair of electrons. Because of the strong and highly directional nature of hydrogen bond, it has been described as the ‘master key interaction’ in the areas of supramolecular chemistry, material science and biological recognition. Hydrogen bonding occurs between a proton donor group D-H and a proton acceptor group A. The D-H…A interaction is called a ‘hydrogen bond’ (bond energy 1-40 Kcals/mol).

George Jeffrey has an in-depth study of hydrogen bonds and classified them into three general categories as (i) very strong, (ii) moderate/strong, and (iii) weak, according to the energy of interaction. General properties of these three types of hydrogen bonds are tabulated in Table 1.1. These three divisions are mainly for our convenience, since there is very much a ‘continuum of the hydrogen bonds’ with the weakest strong bond being of comparable energy to the strongest medium bond and so on. According to the suggestion made by Hamilton and Ibers, the distance of the
atoms, which is less than the sums of their van der Waals radii, is a sufficient, but not a necessary condition for hydrogen bond formation\textsuperscript{18}.

Table 1.1 Properties of Hydrogen Bonds

<table>
<thead>
<tr>
<th>Properties</th>
<th>Very Strong</th>
<th>Moderate</th>
<th>Weak Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bond Energy((kcal/mol))</td>
<td>15−40</td>
<td>4−15</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Examples</td>
<td>[F─H…F]⁻</td>
<td>O─H…O=C</td>
<td>C─H…O</td>
</tr>
<tr>
<td></td>
<td>[N─ H…N]⁺</td>
<td>N─H…O=C</td>
<td>O─H…π</td>
</tr>
<tr>
<td></td>
<td>[P─ OH…O=P]</td>
<td>O─H…O─H</td>
<td>Os─H…O</td>
</tr>
<tr>
<td>Relative frequency shift in IR</td>
<td>&gt;25%</td>
<td>5-25%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Bond lengths</td>
<td>H…A ≈ X─H</td>
<td>H…A &gt; X─H</td>
<td>H…A &gt;&gt; X─H</td>
</tr>
<tr>
<td>Lengthening of X─H (Å)</td>
<td>0.05−0.2</td>
<td>0.01−0.05</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>D (X…A) range (Å)</td>
<td>2.2−2.5</td>
<td>1.5−2.2</td>
<td>3.0−4.0</td>
</tr>
<tr>
<td>d (H…A) range (Å)</td>
<td>1.2−1.5</td>
<td>1.5−2.2</td>
<td>2.0−3.0</td>
</tr>
<tr>
<td>Bonds shorter than vander Waals radii</td>
<td>100%</td>
<td>Almost 100%</td>
<td>30−80%</td>
</tr>
<tr>
<td>θ(X─H…A) range (Å)</td>
<td>175−180</td>
<td>130−180</td>
<td>90−180</td>
</tr>
<tr>
<td>kT (at room temperature)</td>
<td>&gt;25</td>
<td>7−25</td>
<td>&lt;7</td>
</tr>
<tr>
<td>Effect on crystal packing</td>
<td>Strong</td>
<td>Distinctive</td>
<td>Variable</td>
</tr>
<tr>
<td>Utility in crystal engineering</td>
<td>Unknown</td>
<td>Useful</td>
<td>Partly useful</td>
</tr>
<tr>
<td>Covalency</td>
<td>Pronounced</td>
<td>Weak</td>
<td>Vanishing</td>
</tr>
<tr>
<td>Electrostatics</td>
<td>Significant</td>
<td>Dominant</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

As hydrogen bonds are of long range interactions, a group X─H can be bonded to more than one acceptor (A) at the same time. If there are two or more acceptors in a
hydrogen bond then that is called a bifurcated or trifurcated respectively, hydrogen bond (Figure 1.2).

![Different hydrogen bond patterns](image)

**Figure 1.2** Different hydrogen bond patterns

In many supramolecular self assembling structures, the individual components are held together by arrays of double [for instance AT (Adenine-Thymine) base pair; Figure 1.3a], triple [for instance GC (Guanine-Cytosine) base pair; Figure 1.3b] quadruple¹⁹ and quintuple²⁰ hydrogen bonds. Whitesides et al.²¹-²⁴ extensively investigated the hydrogen-bonded adduct of melamine and cyanuric acid forming rosettes (cyclic hexamer) and tape motifs having multiple hydrogen bond network (Figure 1.4).

![Double and triple hydrogen bonds in DNA base pairs](image)

**Figure 1.3** Double and triple hydrogen bonds in DNA base pairs

(a) AT pair, (b) GC pair.
Figure 1.4 Rosette and tape motifs in the solid state structure of melamine: cyanuric acid

Hydrogen bonding is probably the most widely used interaction to generate supramolecularly organized organic systems with a variety of novel structural features\textsuperscript{25-29}. Such supramolecularly organized organic solids show unique chemical and physical\textsuperscript{30-32} properties including porosity\textsuperscript{33-35}. Hydrogen bonding has been effectively used to predict and design supramolecular assemblies in one and two dimension\textsuperscript{36-41}. Hydrogen bonded systems generated from organic cations and anions are of special interest since they would be expected to show stronger hydrogen bonds than neutral molecules and enable the use of simple acid-base chemistry to tune donor and acceptor properties of the counterions.
1.3.1. Graph Set Analysis

Etter, Bernstein, and coworkers have introduced a language based upon graph-theory for describing and analyzing hydrogen bond networks in three-dimensional solids.\(^{42-44}\) Perhaps, the most remarkable feature of the graph set approach to analysis of hydrogen bond patterns is the fact that even complicated networks can be simplified to combinations of four simple patterns. Each pattern specified by a designator: chains (C), rings (R), intramolecular hydrogen-bonded patterns (S), and other finite patterns (D).

The graph set descriptor is given as

\[ G^a_d(n) \]

Where \( G \) = descriptor referring to one of the four patterns of hydrogen bonding.

\( a = \) hydrogen bond acceptor

\( b = \) hydrogen bond donor

\( n = \) degree (number of atoms) of the pattern.

These four patterns and their descriptors are best illustrated by the following examples figure 1.5 and 1.6

\[ C(4) \]

\[ S(6) \]

\[ D \]

\[ R^2(8) \]

**Figure 1.5** Some examples of the four basic descriptors used to define hydrogen bond patterns
A structure containing only one type of hydrogen bond is referred to as motif and will have one or more graph set descriptors associated with it. In more complex cases, where there is more than one motif in the structure, it is possible to assign graph set descriptors for each motif individually as if the others were not present. Such descriptors are termed as first level graph sets (N1). Second level (N2) and third level graph sets (N3) are used for structures having two (Figure 1.7) and three types of motifs respectively.

**Figure 1.6** Graph set assignments for the binary level α-glycine

**Figure 1.7** Examples of first (N1) and second level (N2) graph sets
Graph set analysis of hydrogen bonding is a useful tool for the diagnosis, analysis, understanding, and prediction of supramolecular aggregates dominated by hydrogen bonds. It is very much helpful in distinguishing polymorphs\textsuperscript{45,46}.

1.4. Crystal Engineering

Crystal engineering is a flourishing field of research in modern chemistry, practiced by scientists interested in modeling, design, synthesis, and application of crystalline solids. It is a sub-discipline of supramolecular chemistry, dealing with the construction of crystalline materials from molecules or ions by the exploitation of non-covalent interactions\textsuperscript{10}. These materials might exhibit interesting electrical, magnetic and optical properties. It is also recognized that it is becoming increasingly evident that the specificity, directionality, and predictability of intermolecular hydrogen bonds can be utilized to assemble supramolecular structures of, at the very least, controlled dimensionality\textsuperscript{47}. The non-covalent interactions which have been exploited in this field are hydrogen bonding, $\pi\ldots\pi$, dipole interactions, ionic bonds, hydrophobic interactions, London dispersion forces etc.

The term ‘crystal engineering’ was coined by Pepinsky in 1955, but it was popularized by the work of Schmidt in solid state photochemistry and this is considered as the beginning era of crystal engineering\textsuperscript{48}. By his work it was clear that the chemical and physical properties of the crystalline solids are dependent on the arrangement of the molecules in the crystal lattice.

Gautam Desiraju, in this context, defined crystal engineering as given below. “Crystal engineering is the understanding of intermolecular interactions in the context of crystal packing and in the utilization of such understanding in the design of new solids with desired physical and chemical properties”.

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What best the chemists can do is to find recurring packing patterns adopted by certain functional groups and rely on the robustness of such motifs to create new solid structures. The repetitive units of these small-sized hydrogen-bonded motifs are supramolecular synthons\textsuperscript{49} and they hold the key to crystal engineering. The design of a number of supramolecular architectures, layers, ribbons, rosettes, rods, tubes, sheets and spheres can be achieved through N-H---O and O-H---O hydrogen bonds. Furthermore, the diversity of the physicochemical properties that are associated with crystal engineered materials can be exploited in many areas as exemplified by co-crystals, which have relevance to host-guest chemistry\textsuperscript{50}, Non-Linear Optics (NLO)\textsuperscript{51}, organic-conductors\textsuperscript{52}, photographic materials\textsuperscript{53}, pharmaceuticals\textsuperscript{54-57}, and solid state organic chemistry\textsuperscript{58}.

1.5. History and Definitions of Co-crystals

Co-crystals belong to the class of compounds which are long known but little studied. Co-crystals have been the subject of research for the past 165 years. Earlier, co-crystals were known with different names like molecular compounds\textsuperscript{59}, organic molecular compounds\textsuperscript{60}, addition compounds\textsuperscript{61}, molecular complexes\textsuperscript{62}, solid-state complexes\textsuperscript{63} or hetromolecular crystals\textsuperscript{64}. Wholer synthesized the first co-crystal of quinhydrone (Figure 1.8) in 1844 and several related co-crystals were made from halogenated quinones\textsuperscript{59,65}. 
The structural information for the co-crystal was absent until 1960. The elucidation of DNA structure\textsuperscript{66,67} through X-ray analysis inspired many people to synthesize numerous nucleobase complexes in 1950’s and 60’s\textsuperscript{68-71}. The term “co-crystal” was first coined\textsuperscript{72} in this context and then it was popularized by Etter\textsuperscript{73}, one of the more prolific researchers of the era.

During this time, Hoogstein synthesized the new base pair called “Hoogstein base pair” (Figure 1.9), a co-crystal formed between 9-methyladenine and 1-methylthymine\textsuperscript{70}.

\textbf{Figure 1.8} Crystal structure of the triclinic form of quinhydrone

\textbf{Figure 1.9} Hoogstein base-pair
Though the term co-crystal sounds very simple, the definition is often a topic of debate\textsuperscript{74,75}.

Stahly\textsuperscript{76} defined co-crystal as “a crystalline structure with unique properties that is made up of two or more components. A component may be an atom, ionic compound, or molecule”.

The Zaworotko research group\textsuperscript{77} defines co-crystals as “a multiple component crystal in which all components (molecule or ion and a molecular co-crystal former) are solid under ambient conditions when in their pure form”.

Aakeroy and coworkers\textsuperscript{78} defined co-crystals as (i) compounds constructed from neutral molecules (ii) structurally homogenous crystalline materials that contain at least two neutral building blocks with a well-defined stoichiometry and (iii) compounds made from reactants that are solids at ambient conditions.

Dunitz\textsuperscript{79} gave a broad definition to co-crystal as ‘a crystal containing two or more components together’, and thus co-crystal encompasses molecular compounds, molecular complexes, solvates inclusion compounds, channel compounds, clathrates, and possibly a few other types of multi-component crystals.

Whatever way people define the term co-crystal, the field of co-crystal is attaining the heights of excellence and Figure 1.10 reveals the growing interest in the subject.

Therefore, it is evident that co-crystals play a pivotal role in many industries such as pharmaceutical, textile, paper, chemical processing, photographic, propellant and electronic.
1.5.1. Synthon Approach to Co-crystal Design

Crystal engineering utilizes an empirical knowledge of the non-covalent forces that trigger the formation of supramolecular synthons\textsuperscript{80}, which are subsequently exploited to form compounds with desired physical and chemical properties. Supramolecular synthons are the smallest structural units within which is encoded all the information inherent in the mutual recognition of molecules to yield solid state super molecules, that is, crystals. The key aspect of crystal engineering is, therefore, the dissection of a target network into supramolecular synthons. The understanding of supramolecular synthons, their geometries, and their frequency of occurrence in the presence of other hydrogen bonding groups is a must for the rational design and supramolecular synthesis of novel co-crystals. Hydrogen-bonded supramolecular synthons are commonly used in crystal engineering of co-crystals due to their relatively high strength, predictability, ubiquity, and directionality\textsuperscript{81-85}.

**Figure 1.10** Occurrence of the term “co-crystal” in titles and abstracts of papers published during 1991–2007.
Supramolecular synthons are classified into two categories: supramolecular homosynthons and supramolecular heterosynthons\textsuperscript{86}. The supramolecular homosynthons are formed between the same, self-complementary functional groups and supramolecular heterosynthons are formed between different but complementary functional groups. Examples of supramolecular homosynthons include the dimers formed between carboxylic acids and amides\textsuperscript{80,87,88}, whereas supramolecular heterosynthons include carboxylic acid…amide\textsuperscript{89-93}, hydroxyl…pyridine\textsuperscript{94-96} and carboxylic acid…pyridine\textsuperscript{97-100}(Figure 1.11).

The supramolecular homosynthons usually exist in structures of single-component compounds. For example, the carboxylic acids exist as dimers, whereas, if multiple functional groups are present, a supramolecular heterosynth is more likely to be formed, as the formation of heterosynthons wins over the homosynthons whenever these two are competing each other. When a supramolecular heterosynthon
is formed between two functional groups that are located on different molecules, a multi component is formed.

Co-crystals are ideally suited to study the competition between different supramolecular heterosynthons\textsuperscript{81}. In general, co-crystals are formed by supramolecular heterosynthons rather than supramolecular homosynthons. According to ‘co-crystal approach’, a co-crystal will be produced only if the favoured supramolecular heterosynthon is formed between the reacting molecules. Conversely, a co-crystal is not expected to form if a dominant supramolecular heterosynthon can occur between the functional groups present in the same component.

Synthons explain molecular recognition when molecules assemble into supramolecules/co-crystals and illustrate the conceptual similarities between retrosynthetic phenomenon and supramolecular assembly\textsuperscript{101}. Synthon-based co-crystal design has become increasingly important to the synthesis of new co-crystals.

### 1.5.2. Pharmaceutical Co-crystals

Most of the ‘Active Pharmaceutical Ingredients (APIs)’ are administered in the solid state as part of an approved dosage type, like tablets, capsules etc as they are the convenient and compact formats to store a drug. APIs can exist in a variety of distinct solid forms, where each form may display unique physicochemical properties such as hygroscopicity, morphology and most importantly solubility. Physicochemical properties of a solid form depend on the arrangement of molecules in the crystal lattice\textsuperscript{102}. The solid form of the drug dictates the properties like stability, hygroscopicity, dissolution rate, solubility and bioavailability. Figure 1.12 represents the different ways in which drugs are administered\textsuperscript{103}. 
This makes the pharmaceutics to look for a crystal form with the best properties for therapeutic use and manufacturability. Therefore, whatever form of drug is selected, it must be amenable to handling and processing and as a drug it must be effective and safe.

Though co-crystals were discovered in the 19th century, the pharmaceutical industry has only been recently attracted towards the potential applications \(^{57, 104-107}\) of co-crystals. This is exemplified by the growing number of related publications and conferences and the number of single crystal structures deposited in the Cambridge Structural Database System (CSDS).

Pharmaceutical co-crystals are crystalline molecular complexes that contain therapeutically active molecules. They represent a new paradigm in API formulation
that might address important intellectual and physical property issues in the context of drug development and delivery\textsuperscript{108}. Properties essential to the bio-availability such as solubility, physical stability, hygroscopicity, and dissolution rate have been demonstrated in case of co-crystallization\textsuperscript{109}. Co-crystal formation does not require an ionizable center on the pharmaceutical molecule, as in the case for salt formation. In contrast to the limited number of salt-forming counter ions available today, there exists a relatively large number of biologically non-toxic co-crystallizing molecules available for use in complexation with pharmaceuticals. These pharmaceuticals include food ingredients and additives, vitamins, nutrients, pharmaceutical excipients, biomolecules, and other drug molecules.

Pharmaceutical co-crystallization is potentially attractive for improving the material properties while leaving an API unaltered. The inherent advantages of pharmaceutical co-crystallization indicate a significant impact on the development of future medicines. A pharmaceutical co-crystal might also be used to isolate or purify an API during manufacturing and the co-crystal former may be discarded before formulation.

The first application of crystal engineering to generate pharmaceutical co-crystal was a series of studies by Whitesides \textit{et al}\textsuperscript{110-116} with the use of substituted barbituric acid, including barbital and melamine derivatives and they generated supramolecular ‘linear tapes’, ‘crinkled tapes’, and ‘rosette’ motifs.

To date, many pharmaceutical co-crystals have been reported in the scientific and patent literature and most of the co-crystals have been found to exhibit improved material properties like solubility, stability, bioavailability \textit{etc.} over the pure API. Pharmaceutical co-crystals have been described for many drugs such as
acetoaminophen, aspirin, ibuprofen, caffeine, cephalexin, fluoxetine, theophylline sulfadimidine, trimethoprim, paracetamol etc\textsuperscript{117-133}.

Co-crystallization can also be used for chiral resolution. APIs, which are obtained only in the amorphous form, may be crystallized as co-crystals. For example, itraconazole, an extremely water insoluble antifungal drug that is marketed in the amorphous form has been made into co-crystals with certain 1,4-dicarboxylic acids. (Figure 1.13)

![Chemical Diagram](image1)

(a)

![Crystal Packing Diagram](image2)

(b)

**Figure 1.13** Structure of itraconazole: succinic acid co-crystal (a) chemical diagram (b) crystal packing diagram
The applications of concepts of supramolecular synthesis and crystal engineering to the development of pharmaceutical co-crystals offer many opportunities for the drug development and delivery. So it would not be an exaggeration in saying that sooner or later pharmaceutical co-crystals will gain a broader foothold in drug formulation.

1.5.3. Polymorphism

Polymorphism[134-140] is the phenomenon wherein the same chemical compound exists in different crystal forms. In the initial days of crystal engineering, polymorphism was not properly understood. Today, it is one of the most exciting branches of the subject. Polymorphism in multi-component crystal is gaining interest in the recent times in the context of pharmaceutical co-crystals. Polymorphs have different stabilities and may spontaneously convert from a metastable form (unstable form) to the stable form at a particular temperature. In addition, they exhibit different melting points and solubility which affect the dissolution rate of drug and thereby, its bioavailability in the body. Co-crystal polymorphs suggest additional options to modify properties, increase patent protection, and improve marketed formulations. Aswini Nangia[137] schematically represented the polymorphic forms of a rigid molecule (Figure 1.14).

![Figure 1.14 Schematic representation of polymorph I and polymorph II for a rigid molecule](image)

Figure 1.14 Schematic representation of polymorph I and polymorph II for a rigid molecule
1.5.4. Pharmaceutical Co-crystals as Intellectual Property

Compared to other classes of solid forms, co-crystals possess particular scientific and regulatory advantages. These advantages are intellectual property issues which give unique opportunities and challenges to co-crystals. Researchers reported the importance regarding patents on pharmaceutical co-crystals to the pharmaceutical industry. The value of co-crystals to the pharmaceutical industry should become clearer, mainly with respect to several relevant legal and regulatory issues, as products containing co-crystal technology should enter into the market through pharmaceutical development pipelines.

A novel polymorph can offer an opportunity in terms of better physicochemical performance and new product development. Physicochemical properties of a compound can differ critically from one form to another. Hence inducing and controlling a specific polymorph are very important in the chemistry of pharmaceuticals, explosives, pigments etc.

Polymorphism tends to be prominent in molecules that contain multiple, hydrogen-bonding moieties, thereby forming multiple supramolecular synthons, and/or conformational flexibility as exemplified by ROY and in a number of APIs such as aspirin, piracetam, and virazole. In addition, simultaneous crystallization of polymorphs, known as concomitant polymorphism, can occur under certain conditions.

Polymorphism occurs in single component crystals, salts, and solvates and is exhibited by most APIs. It is worthy to note that the number of polymorphs of a co-crystal was more than the number of polymorphs of its parent API.

Two different polymorphic forms of paracetamol are reported by Oswald et al. Carbamazepine-nicotinamide co-crystal and carbamazepine-saccharin co-crystal
were found to exist in two polymorphic forms. Co-crystal polymorphs of carbamazepine and isonicotinamide having 1:1 stoichiometry were reported and they were formed through a solvent-mediated transformation process upon suspending a dry mixture of the pure crystalline components in ethanol. Two polymorphs of a co-crystal between 2-ethoxybenzamide and saccharin sustained by a carboxamide-imide heterosynthon involving two N-H---O hydrogen bonds were prepared and structurally characterized by single crystal X-ray diffraction. The metastable form alone was formed in the grinding experiment, whereas both polymorphs were reported by solution crystallization.

Polymorphs are considered important in the pharmaceutical industry in view of their effectiveness in the drug. For example, out of its four polymorphic forms, only one form of cortisone is stable in water.

1.6. Crystal Growing and Crystal Quality

The aim is to grow single crystals of suitable size in at least two of the three dimensions. The size of the crystals can be influenced by a number of factors such as the solubility of the sample in the chosen solvent, the number of nucleation sites and time. A solvent should be chosen to facilitate the sample moderately soluble. The crystal-growing vessel should be clean, because dust provides numerous nucleation sites and may initiate interfering crystal growth. It is important to avoid disturbance of the vessel. Vibration or frequent movement tends to lead to poor quality crystals.

The most promising crystals are transparent and sharp-edged with the preferred dimensions. Acceptable crystals may be produced serendipitously from the preparative route. Different colours or shapes of crystals may indicate unreacted
starting material or by-products. If the crystal is not of sufficient quality, it may be necessary to use a different crystallization technique.

Producing good quality crystals of a suitable size is the first and most important step in determining any crystal structure. Crystallization is the process of arranging atoms or molecules that are in a fluid or solution state into an ordered solid state. Co-crystallization is a deliberate attempt at bringing together different molecular species within one periodic crystalline lattice without making or breaking covalent bonds. Recrystallization and co-crystallization processes (Figure 1.15) are, in essence, only distinguishable by their intents. The goal of recrystallization is a homomeric product, whereas the co-crystallization procedure strives for a heteromeric product. Crystallization process occurs in two steps, nucleation and growth. Nucleation may occur at a seed crystal. In the absence of seed crystals, it usually occurs at some particle of dust or at some imperfection in the surrounding vessel. Crystals grow by the ordered deposition of material from the fluid or solution state to a surface of the crystal.

![Figure 1.15 Schematic representation of recrystallization and co-crystallization](image)

1.6.1. Crystallization Methods

There are numerous ways to grow crystals. The choice of method depends greatly upon the physical and chemical properties of the sample. For solution methods
of crystallization, the solubility of the sample in various solvent systems must be explored. If heating methods are selected for growing crystals, the thermal stability and melting point of the sample should be determined.

1.6.1.1. Evaporation Method

Evaporation is by far one of the easiest methods for crystallizing organic and organometallic small molecule compounds. The choice of solvent is very important, because it can greatly influence the mechanism of crystal growth and the solvent may be incorporated into the crystalline lattice. It is customary to screen a large number of solvents or solvent mixtures to find the best conditions for crystal growth. The rate of crystal growth can be slowed either by reducing the rate of evaporation of the solvent or by cooling the solution. Formation of only a few rosette-shaped masses is an indication of an insufficient number of nucleation sites. The number of nucleation sites may be increased either by seeding the solution or by scratching the exposed surfaces of the glass vessel.

1.6.1.2. Liquid and Vapor Diffusion Methods

Liquid and vapor diffusion methods are often tried when evaporation methods do not immediately succeed. Both methods require finding two solvents or solvent mixtures in which the compound is soluble in one system but insoluble in the other. The two solvent systems should be immiscible or nearly immiscible for liquid diffusion and should be miscible for vapor diffusion. Crystal growth may be slowed somewhat by cooling the apparatus. Liquid diffusion usually requires that the less dense solvent system be carefully layered on top of the more dense system. The
sample can be dissolved in either solvent system. Crystals grow at the interface between the solutions.

When compounds precipitate immediately upon being formed, it is possible to slow down the reaction and thus grow larger crystals by putting the reactants in different liquid layers which are separated by a third solvent layer that is not miscible with either of the layers or with the sample. The top layer should be added very slowly to assure a minimum of mixing of the layers.

Vapor diffusion is carried out by dissolving a small amount of the sample in a small vial, then placing this inner vial inside a larger vial that contains a small volume of a solvent system in which the sample is insoluble. The outer vial is then sealed. Vapor from the solvent of the outer vial then diffuses into the solution in the inner vial, causing the compound to grow crystals. The vertical surfaces of the inner vial should not touch the outer vial to keep the outer solution from rising by capillary action and filling the inner vial.

1.6.1.3. Thermal Gradient Method

Thermal gradient methods usually produce very high quality crystals. Such methods include slow cooling of sealed, saturated solutions, refluxing of saturated solutions, sublimation, and zonal heating. Zonal heating is used primarily for crystallizing solid solutions or mixtures. Small crystals may sometimes be grown larger by zonally refluxing a supersaturated solution.

Sublimation may be carried out in a variety of tubes or vessels. Sealed vessels offer an advantage for sublimation in that the chamber may be evacuated or a partial pressure of some inert gas may be introduced before sealing the sample in the apparatus. Sublimation methods consistently produce very high quality crystals.
Larger crystals may be grown either by decreasing the thermal gradient or by cyclic heating and cooling of the sample.

1.6.1.4. Gel diffusion Method

Some compounds, which precipitate as very small crystals immediately upon synthesis, are extremely insoluble. Suitable crystals of these compounds can often be prepared by greatly decreasing the rate at which the reactants combine by making the reactants diffuse through a gel barrier. This is often carried out by forming a gel in a U-tube and introducing the reactants in the two separate ends of the tube. Such methods usually take weeks to months to produce good quality crystals depending on the rate of diffusion of reactant through the gel.

1.6.1.5. Choice of Solvents and Counter ions

There are a number of solvents and counter ions that are commonly found to be disordered in crystal structures and thus should be avoided if possible. The solvents giving the most trouble are petroleum ether, mixed hydrocarbons like hexane or kerosene, and halogenated hydrocarbons such as dichloromethane and chloroform. Often these solvents occupy sites in a crystal structure that are larger than the solvent molecule. The halogenated solvents are particularly troublesome because the disorder includes heavier atoms. Better choices of solvents are benzene, xylene, primary and secondary alcohols, and tetrahydrofuran. The counter ions most likely to cause difficulties are Bu₄N⁺, BF₄⁻, and PF₆⁻. Some alternative counter ions that are usually ordered are triflate, BPh₄⁻, (Ph₄P)₂N⁺, and Ph₄As⁺.
1.6.2. Solvent free Methods

There are some methods in which we could use less or no solvents. These methods, a green route to crystal engineering and polymorph, are used for making hydrogen bonded adducts or co-crystals\(^\text{154}\).

1.6.2.1. Grinding or Ball Milling

In this method, the two solid components are ground together with a ball mill with or without a small amount of solvent\(^\text{155, 156}\). Etter and collaborators investigated the formation of hydrogen bonded co-crystals by grinding of the solid components, even in the presence of a third solid component\(^\text{157}\). This grinding method was used for the interconversion of one polymorph to another\(^\text{158}\). This method is not popular in academic research laboratories and is often dismissed as ‘non-chemical’, even though it is commonly used at industrial level mainly with inorganic solids and materials\(^\text{159}\).

1.6.2.2. Kneading and Oligo Diffractrometry

Another process which can be applied in small scale research laboratory is the so called ‘kneading’\(^\text{160}\) the use of small amounts of solvent or of a liquid reactant to accelerate the solid state reactions carried out by grinding or milling. Kneading has been described as a sort of solvent catalysis of the solid state processes, where the small amount of solvent provides a lubricant for molecular diffusion. This method is commonly employed in the preparation of cyclodextrin inclusion compounds. Single crystals of gases, liquids and their co-crystals can be obtained by an \textit{in situ} crystallization technique, called oligo diffractrometry. Nevertheless, this does not mean that synthesis of co-crystals is routine.
1.7. Introduction to the Theory and Experimental Techniques in X-ray Crystallography

Crystal structure analysis via X-ray diffraction is an analytical technique, which uses the diffracted X-ray intensities from the crystal. The main theme of single crystal X-ray structure analysis in crystallography is to locate the exact position of atoms in the unit cell. Among the various techniques available for molecular structure determination, X-ray crystallography provides unambiguous information about the structure of molecules. The central concept in this method is the binding forces that keep the atoms and molecules together in a crystal. So the ultimate aim of X-ray crystallography is to understand the nature of this force and thus to predict the chemical and physical properties of the substance through X-ray diffraction. Precisely, it gives the information about the geometrical parameters such as bond length, bond angle, torsion angle and thermal parameters of the atoms in a molecule. Moreover, the intra- and intermolecular bonding can also be determined.

1.7.1. Single Crystal X-ray Diffraction

To determine the structure using single crystals, one should know the experimental procedures of single crystal X-ray diffractometry like data collection, the theoretical background of the techniques of structure solution and refinement. Here the pattern produced by the diffraction of X-rays through the closed spaced lattice of atom in the crystal is recorded and analyzed to reveal the nature of the lattice.

The spacing in the crystal lattice can be determined using the Bragg’s law.

\[ n\lambda = 2dsin\theta \]

where, \( n = 1, 2, 3 \ldots \)
d = interplanar spacing

θ = angle of diffraction

λ = wavelength of incident radiation.

1.7.2. Experimental

1.7.2.1 Selection of Single Crystals

To evaluate the quality and appropriate size of crystalline samples, the samples should be examined under low power magnification (10X to 40X). Good crystals usually have smooth flat faces and sharp edges. The selected crystal should show no obvious external twinning (e.g. reentrant faces or different parts of the crystal extinguishing at different rotation angles under a polarizing microscope). The crystal chosen for analysis needs to be large enough to produce an adequate diffraction pattern and, at the same time, as small as possible to minimize absorption problems. The calculation of structure factor amplitudes assumes that the crystal is being completely bathed in a uniform beam of X-rays. Since the uniform region of the X-ray beam is about 0.5 mm in diameter, this is taken as the maximum dimension of any crystal. For most samples, a minimum dimension of 0.1 mm is needed to produce adequate X-ray scattering. Compounds with few atoms or very heavy atoms can have all three dimensions toward the small end of this 0.1 to 0.5 mm range. The best crystals for compounds with many light atoms should have all three dimensions toward the large end (0.4-0.5 mm) of this range.

1.7.2.2. Crystal Mounting

Crystal mounting must be rigid to hold the sample in the same orientation and must minimize the amount of extraneous material that is in the incident and diffracted
beam paths. The sample support is usually made from an amorphous material such as glass that is held in a metal pin and clamped on a goniometer head. Solid glass fibers may be used; however, fibers pulled from glass tubing are actually small capillary tubes and are more rigid than solid glass fibers. These narrow tubes also place less non-crystalline material in the X-ray beam path than solid fibers. Air stable crystals are usually glued (using epoxy, Elmers/water, Duco/amyl acetate, etc.) to the end of a glass fiber. The sample should be mounted with its smallest surface on the end of the glass fiber to minimize absorption effects and to minimize background scattering from the sample mount. Mildly air unstable compounds can be coated with epoxy or an inert viscous material such as Paratone N™ or Krytox™ oil. These mountings are usually carried out in an inert atmosphere such as a dish filled with argon gas. The crystal is prevented from decomposition during data collection by cooling the sample in a chilled, inert (nitrogen) gas stream.

1.7.2.3. Unit Cell Parameters

The structural units which are termed unit cells are repeated regularly and indefinitely in three dimensions in space. The shape of the unit cell is described by six parameters. These six parameters are three axial lengths, designated as a, b and c and three inter axial angles α, β and γ. The unit cell has a definite volume V, which contains the atoms and molecules necessary for generating the crystal. Amorphous solids lack the long range order present in crystals\textsuperscript{161}.

Each crystal can be classified as a member of one of seven possible crystal systems or crystal classes that are defined by the relationship between the six cell parameters. The structure of the given crystal may be assigned to one of the 230 space groups. Certain space groups occur more frequently than others. According to the
Cambridge Structural Data Base\textsuperscript{162}, about 76\% of all organic and organo metallic compounds crystallize in only 5 space groups, \textit{P2\textsubscript{1}/C, P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}, P-1, P2\textsubscript{1}} and \textit{C\textsubscript{2}/C}.

\subsection*{1.7.2.4. Intensity Data Collection}

Two general methods are available for intensity data collection.

(i) Photographic method in which the diffracted beam is detected by the photographic film and then the intensity information is collected from the film.

(ii) Quantum counting device that measures the number of photons directly (Diffractrometer or Counter).

Three decades ago photographic film method\textsuperscript{163} was the only possible method for intensity data collection. Today’s data collection is executed by automated diffractometer. Figure 1.16 represents an X-ray diffractometer.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Bruker_Apex-II_CCD_Area_Detector.png}
\caption{Bruker’s Apex-II CCD Area Detector}
\end{figure}
1.7.2.5. Data Reduction and Structure Solution

The procedure followed to obtain the relative structure factor amplitudes from the raw integrated intensities is called data reduction.

The structure of the molecule is obtained by a Fourier transform of the observed amplitude $F_{hkl}$. The main theme of single-crystal analysis in crystallography is to locate the exact position of atoms in the unit cell. If the structure factors and phases are known, electron density of the unit cell can be calculated.

1.7.2.6. Absorption Correction

Some absorption will take place if an X-ray is transmitted through a crystal i.e. reduction in intensity. The fraction of radiation transmitted

$$ T = \frac{I}{I_0} \exp (-\mu t) $$

where $I_0$ is the intensity of the beam, $I$ is the transmitted intensity through the crystal, $t$ is the thickness of the crystal $\mu$ is the linear absorption coefficient (mm$^{-1}$).

1.7.3. Objectives of the Present Work

1. To develop well-defined supramolecular structures via multiple hydrogen bonds by self-assembly of components containing complementary array of hydrogen bonding sites.
2. To use pyridine-2,3-dicarboxylic acid, $N$-phenyl anthranilic acid, $o$-formyl phenoxy acetic acid, $p$-formyl phenoxy acetic acid, $p$-formyl phenoxy propionic acid, eosin, and bases DABCO, 4,4'-bipyridine, stien, and caffeine in view of their hydrogen bonding ability.
3. To record IR, $^1$H NMR, TGA/DTA, elemental analysis and single crystal XRD for the co-crystals obtained.
4. To analyze hydrogen bonding interactions, π-π interactions and other non-covalent interactions involved in the synthesized co-crystals.

5. To study the structural organization in these materials by applying Etter’s graph set notations to the various types of hydrogen bonding interactions involved.

6. To study the antimicrobial and antifungal activities of the synthesized materials.

7. To study the antioxidant activities of some of the synthesized materials.
References


