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Crystal engineering of flavonoids

Padmini Kavuru

University of South Florida

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Crystal Engineering of Flavonoids

by

Padmini Kavuru

A thesis submitted in partial fulfillment
Of the requirements for the degree of
Master of Science
Department of Chemistry
College of Arts and Sciences
University of South Florida

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Dedication

To my husband, parents and in-laws
Acknowledgements

I would like to thank my advisor, Professor Michael J. Zaworotko, for the opportunity to conduct research under his supervision, and for his advice and guidance throughout the Graduate Program.

I would also like to thank Dr. Julie P. Harmon and Dr. Abdul Malik, my committee members, for their helpful comments and encouragements.

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CRYSTAL ENGINEERING OF FLAVONOIDS

PADMINI KAVURU

ABSTRACT

Crystal engineering is attracting attention in the pharmaceutical industry because the design of new crystal form of drugs can improve their stability, bioavailability and other relevant physical characteristic properties. Therefore, crystal engineering of nutraceuticals such as flavonoids by exploring their hydrogen bonding interactions can generate novel compounds such as pharmaceutical cocrystals. Flavonoids are polyphenolic secondary plant metabolites that are present in varying levels in fruits, vegetables and beverages. The “French paradox”, low cardiovascular mortality rate in spite of high intake of saturated fat among the Mediterranean populations made flavonoids an appropriate target for therapeutic researchers.

The work herein deals with the crystal engineering of two flavonoids, quercetin and hesperetin, which are already known to exhibit antioxidant properties and reduce cardiovascular effects in humans. However, they have limited bioavailability and poor water solubility. Several new forms of quercetin and hesperetin in the form of solvates and cocrystals were synthesized. These new crystal forms were characterized by various techniques: FT-IR, DSC (Differential Scanning Calorimetry), single X-ray diffraction, powder X-ray diffraction, TGA (Thermal Gravimetric Analysis) and
melting point. The new compounds were also studied via dissolution studies performed in 1:1 ethanol/water (V/V%). Thus, crystal engineering proves to be effective way to enhance the solubility and bioavailability of the target flavonoid molecules.
1. INTRODUCTION

1.1 Nutraceuticals

The term ‘Nutraceutical’ is an intermediate of nutrition and pharmaceutical.\(^1\) It is used to describe a medicinal or nutritional component of food, plant or naturally occurring material in purified or concentrated form claimed to have a medicinal effect on human health. Such foods are also called functional foods. It can also refer to individual chemical present in common foods. The position of nutraceuticals on the legislative grounds is marginal between pharmaceutics and food.

The term nutraceutical was coined by Dr. Stephen De Felice (founder and chairman of Foundation of Innovation in Medicine) in 1976.\(^1\) He defined it as:

“Nutraceuticals are ‘food, or parts of food, that provide medical or health benefits, including the prevention and treatment of disease’. Nutraceuticals include a wide range of products, such as polyphenols (Flavonoids), vitamins, oils from fish and flax seed, glucosamine, chondroitin, resveratrol, calcium-fortified juices etc.\(^1\)

1.2 Flavonoids

Flavonoids are naturally occurring polyphenolic compounds that are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine.\(^2\) These natural products were known for their beneficial effects on health long before flavonoids were isolated as
the effective compounds. There are more than 4000 varieties of flavonoids which have been identified till now and are considered responsible for the attractive colors of flowers, fruit, and leaves.\textsuperscript{3,4} These are responsible for many of the plant colors that dazzle us with their brilliant shades of yellow, red and orange. Many of the flavonoids are dietary antioxidants and constituents of medicinal herbs. These compounds were discovered in by a Hungarian scientist, Dr. Albert Szent-Györgyi in 1938, who discovered Vitamin C (Nobel laureate) by mistake. Rusznyák and Szent-Györgi (1936) suggested that flavonoids be known as vitamin P or vitamin C\textsubscript{2}.\textsuperscript{5-8} However, by the 1950’s the vitamin claim had been abandoned due to a lack of substantive evidence.

Flavonoids are also known as plant secondary metabolites which does not effect the normal growth, development or reproduction of organisms like the primary metabolites neither their absence result in immediate death.\textsuperscript{9} But, these compounds are used as defenses against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes (coloring agents, attractive smells, etc) and hence they are called as the “First Line of Defense”.\textsuperscript{10} The research on flavonoids took a rapid momentum with the discovery of the French paradox, i.e., low cardiovascular mortality rate observed in Mediterranean populations with red wine consumption and a high saturated fat intake.\textsuperscript{22,23} The flavonoids in red wine are responsible for this effect. In addition, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease. After a number of subsequent studies on animals it was found that flavonoid intake is inversely correlated with mortality due to coronary heart disease.\textsuperscript{11,12} These potential benefits are being used to promote the consumption of flavonoid-rich foods, beverages and dietary supplements.
1.3 Structure and classification

The basic structure of flavonoids is based on the 15 carbon skeleton, i.e. $C_6 - C_3 - C_6$. The two ‘C6’ represents the number of carbon atoms of the two phenyl groups and the C3 represents the number of carbon atoms that bridge the two phenyl rings by a linear three carbon chain.\(^7\)-\(^10\) Figure 1.1 explains the above description in most convenient way.

![Figure 1.1 Skeleton of Flavonoid](image)

In some of the flavonoids, the three carbon linear chain is replaced by a chromane ring and the position of ring could be at 2, 3 or at 4\(^{th}\) carbon atom. The overall structure now consists of three rings A, B and C. The position of the ring B varies from one class of flavonoid to the other.

![Figure 1.2 Structure of Flavonoid with the chromane ring](image)

In some of the flavonoids, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered ring. The oxygen bridge involving the central carbon atom ($C_2$) of the three carbon chain occurs in a limited number of cases resulting in a heterocyclic ring which is of the furan type.
Most of the flavonoids occur as glucosides in which the C6-C3-C6 aglycone part of the molecule is linked with different number of sugars. If the linkage of the sugar to the flavonoid aglycone is through an OH group then these are called as O-glycosylflavonoids and if the linkage is through C-C bond then these are called as C-glycosylflavonoids.\textsuperscript{17} The type of sugar and the position of sugars vary for different glycosides. These have a more complex structure than the phenolic skeleton materials.

Glycoside = Aglycone + Glycone (sugar)

The most common citrus flavanone-glycosides are rutin, natrirutin, naringin, hesperedin and neohesperidin. Naringin (in grapefruits) and hesperidin (in oranges) are the two major flavonoid-glycosides present in the citrus fruits, and are primarily concentrated in the peel and the tissue of the fruit.\textsuperscript{18} The table below represents the structures of various flavonoids.
Table 1.1 Structures of different of Flavonoids

<table>
<thead>
<tr>
<th>Anthocyanidins</th>
<th>Flavanols</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Anthocyanidins" /></td>
<td><img src="image2.png" alt="Flavanols" /></td>
</tr>
<tr>
<td>R1 = H; R2 = H: Pelargonidin</td>
<td>R1 = H: (+)-Catechin</td>
</tr>
<tr>
<td>R1 = OH; R2 = H: Cyanidin</td>
<td>R1 = OH, R2 = H: (-)-Epicatechin</td>
</tr>
<tr>
<td>R1 = H; R2 = OH: Delphinidin</td>
<td>R1 = OH: (+)-Epicatechin</td>
</tr>
<tr>
<td>R1 = OCH3; R2 = OH: Petunidin</td>
<td>R1 = OH: (-)-Epicatechin galactoside</td>
</tr>
<tr>
<td>R1 = OCH3; R2 = OCH3: Malvidin</td>
<td>R1 = H: (-)-Epicatechin galate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavonols</th>
<th>Flavonones</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Flavonols" /></td>
<td><img src="image4.png" alt="Flavonones" /></td>
</tr>
<tr>
<td>R1 = H; R2 = H: Kaempferol</td>
<td>R1 = H; R2 = OH: Naringenin</td>
</tr>
<tr>
<td>R1 = OH; R2 = H: Quercetin</td>
<td>R1 = OH; R2 = OH: Eriodictyol</td>
</tr>
<tr>
<td>R1 = H; R2 = OH: Myricetin</td>
<td>R1 = OH; R2 = OCH3: Hesperetin</td>
</tr>
<tr>
<td>R1 = OCH3; R2 = H: Isorhamnetin</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavones</th>
<th>Isoflavones</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="Flavones" /></td>
<td><img src="image6.png" alt="Isoflavones" /></td>
</tr>
<tr>
<td>R1 = H; R2 = H: Apigenin</td>
<td>R1 = H; R2 = H: Daidzein</td>
</tr>
<tr>
<td>R1 = OH; R2 = H: Luteolin</td>
<td>R1 = OH; R2 = H: Genistein</td>
</tr>
<tr>
<td></td>
<td>R1 = H; R2 = OCH3: Glycitein</td>
</tr>
</tbody>
</table>

Flavonoids are divided into various classes on the basis of their molecular structure.\(^{19}\) The six main groups of flavonoids are listed in Table 1.2, which includes the known members of
each group and the food sources in which they are present.

Table 1.2 Classification Flavonoids and their common food source

<table>
<thead>
<tr>
<th>Flavonoid Subclass</th>
<th>Dietary Flavonoids</th>
<th>Some Common Food Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanidins</td>
<td>Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin</td>
<td>Red, blue and purple berries, red and purple grapes, red wine</td>
</tr>
<tr>
<td>Flavonones</td>
<td>Hesperetin, Naringenin, Eriodictyol</td>
<td>Citrus fruits and juices, e.g., oranges, grapefruits, lemons</td>
</tr>
<tr>
<td>Flavonols</td>
<td>Quercetin, Kaempferol, Myricetin, Isorhamnetin</td>
<td>Widely distributed: yellow onions, scallions, kale, broccoli, apples, berries, teas.</td>
</tr>
<tr>
<td>Flavones</td>
<td>Apigenin, Luteolin</td>
<td>Parsley, thyme, celery, hot peppers,</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Daidzein, Genistein, Glycitein</td>
<td>Soybeans, soy foods, legumes</td>
</tr>
</tbody>
</table>

1.4 Bioactivity and bioavailability

Flavonoids are widely known for their antioxidant activity. Important effect of flavonoids is the scavenging of oxygen-derived free radicals. In vitro experimental systems showed that flavonoids possess anti-inflammatory, anti-allergic, antiviral, and anti-carcinogenic properties. An overview of the hypothetical links between the working mechanisms and clinical effects of flavonoids is given in Figure 1.5.
One clue to the health benefits of flavonoids comes from studies of the "French paradox". French eat almost four times more butter and three times more lard—and have higher cholesterol levels and blood pressures—than do Americans and yet the French are 2.5 times less likely than Americans to die of coronary heart disease. Many people have suggested that the liberal French consumption of red wine protects against coronary heart disease, apparently by lowering cholesterol levels or preventing abnormal blood clots. In fact, at least eight medical studies have found that a glass or two of wine daily protects against heart disease. But some studies have reported that red wine is better than white wine, suggesting that some of the benefits might be unrelated to the alcohol. The antioxidant property of flavonoids can be explained in the following way: Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of
the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive, according to the following equation: 22

\[
\text{Flavonoid (OH)} + R\cdot \rightarrow \text{Flavonoid (O\cdot)} + \text{RH (I)}
\]

where, \(R\cdot\) is a free radical and \(O\cdot\) is an oxygen free radical. Selected flavonoids can directly scavenge super oxides; whereas other flavonoids can scavenge the highly reactive oxygen derived radical called peroxynitrite. Epicatechin and rutin are also powerful radical scavengers. The scavenging ability of rutin may be due to its inhibitory activity on the enzyme xanthine oxidase. By scavenging radicals, flavonoids can inhibit LDL oxidation in vitro. 24 This action protects the LDL particles and, theoretically, flavonoids may have preventive action against atherosclerosis.

With the exception of flavanols (catechins and proanthocyanidins), flavonoids occur in plants and most foods as glycosides. Even after cooking, most flavonoid glycosides reach the small intestine intact. Only flavonoid aglycones and flavonoid glucosides (bound to glucose) are absorbed in the small intestine, where they are rapidly metabolized to form methylated, glucuronidated or sulfated metabolites. 25 Hollman and Katan suggested that the glycosylated forms of quercetin are absorbed more readily than are the aglycone forms; however, this has been questioned by other researchers. The role of flavonoid glycosylation in facilitating absorption is questioned by the fact that catechin, which is not glycosylated in nature, is absorbed relatively efficiently. As most of the flavonoids are poorly absorbed by the body and the major percentage is excreted out which, limits its bioavailability. Even though numerous strategies exist for enhancing the bioavailability of drugs with low aqueous solubility, the success of these approaches is not yet able to be guaranteed and is greatly dependent on the physical and chemical nature of the molecules being developed. Crystal
engineering offers a means to improved solubility and dissolution rate and stability, which can be adopted through an in-depth knowledge of crystallization processes and the molecular properties of active pharmaceutical ingredients.26

1.5 Crystal Engineering

Crystal engineering is described as the ‘exploitation of noncovalent interactions between molecular or ionic components for the rational design of solid-state structures that 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’.27 The noncovalent interactions which have been exploited in this field are hydrogen bonding, π…π, 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 photo chemistry and this is considered as the beginning era of crystal engineering.28 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 define crystal engineering as 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”.

Crystal engineering can be used to manipulate the solubility and dissolution rate of the active pharmaceutical ingredients (API) in the crystalline state without compromising with its
bioactivity. There are many possible ways that may be achieved from recent developments in
the study of molecular solids and reviews topical issues such as habit modification,
polymorphism, solvation, co-crystal formation and surface modification. Therefore,
particular attention is paid to the area of co-crystallization, which is an emerging area of
strategic importance to the pharmaceutical sector.\textsuperscript{27}

1.5.1 Supramolecular Chemistry

\textit{Supramolecular chemistry} is a relatively new field of chemistry which focuses literally on
going "beyond" molecular chemistry.\textsuperscript{29} The importance of supramolecular chemistry was
recognized by Donald J. Cram, Jean-Marie Lehn, and Charles J. Pedersen and for their work
in this area they were awarded Nobel Prize for Chemistry in 1987. The important
breakthrough that allowed the elucidation of the double helical structure of DNA occurred
when it was realized that there were two separate strands of nucleotides connected through
hydrogen bonds. The utilization of noncovalent bonds is essential to replicate because they
allow the strands to be separated and used to template new double stranded DNA. The
development of selective "host-guest" complexes, in which a host molecule recognizes and
selectively binds a certain guest, was cited as an important contribution. Unlike, the organic
synthesis that involves the making and breaking of covalent bonds to synthesize a desired
molecule, supramolecular chemistry utilizes far weaker and reversible noncovalent
interactions, such as hydrogen bonding, metal coordination, hydrophobic forces, van der
Waals forces, pi-pi interactions, sometimes electrostatic effects to assemble molecules into
multimolecular complexes. 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. \(^{30}\) 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 to cellular function. It also has application in green chemistry where reactions have been developed where the reactions take place in the solid state directed by non-covalent bonding. Such procedures are highly desirable since they reduce the need for solvents during the production of chemicals.

1.5.2 Supramolecular synthons

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 supermolecules, that is, crystals. A key aspect of crystal engineering is therefore the dissection of a target network into supramolecular synthons not the molecular synthons which connect the supramolecular synthons. \(^{31}\) Such a dissection simplifies the analysis of a target network and is important in crystal engineering because it recognizes the interchangeability of supramolecular synthons in a family of structures. \(^{32, 33}\) Supramolecular synthons are classified into two categories: *supramolecular homosynthons* and *supramolecular heterosynthons*. \(^{34}\) 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 \(^{35, 36}\) and amides \(^{37}\) whereas supramolecular heterosynthons include carboxylic acid···amide, \(^{38-42}\)
hydroxyl···pyridine, 43-45 and carboxylic acid···pyridine.46-49

(a)  ![Supramolecular homosynthons: Carboxylic acid homosynthon](image-a)
(b)  ![Supramolecular heterosynthons: Carboxylic acid···pyridine heterosynthon](image-b)

Figure 1.6 Supramolecular synths: (a) Carboxylic acid homosynthon, (b) Carboxylic acid···pyridine heterosynthon

The supramolecular homosynthons usually exists in structures of single-component compounds, for example the carboxylic acids exists as dimers. Where as, if multiple functional groups are present, a supramolecular heterosynthon is more likely to be formed as the formation of heterosynthons wins over the homosynthons when ever these two are competing with each other. When a supramolecular heterosynthon is formed between two functional groups that are located on different molecules, a multicomponent is formed.

1.5.3 Cocrystals

Cocrystals belong to the class of compounds which are long known but little studied. Earlier, cocrystals were known with different names like; molecular compounds, 50 organic molecular compounds, 51 addition compounds, 52 molecular complexes, 53 solid-state complexes, 54 or heteromolecular crystals. 55 In 1844, the first cocrystal of benzoquinone and hydroquinone was synthesized by Wohler, and then followed by studies of halogen derivatives of quinhydrone. 50,56 Until 1960 the structural information for the cocrystal was absent. The elucidation of DNA structure 57, 58 through X-ray analysis inspired many people to come out with numerous nucleobase complexes in 1950’s and 60’s 59-62 and then the term “cocrystal”
was first coined in this context \^63 later it was subsequently popularized by Etter.\^64 During this time, Hoogstein came up with the new base pair called “\textit{Hoogstein base pair}”, a cocrystal formed between 9-methyladenine and 1-methylthymine.\^61

![Figure 1.7 Crystal structure of the triclinic form of quinhydrone](image)

![Figure 1.8 The Hoogsteen base-pair](image)

The term cocrystal sounds very simple but the definition is often a topic of debate.\^65, 66 The Zaworotko research group defines cocrystal as: “\textit{A cocrystal is a multiple component crystal in which all components (molecule or ion and a molecular cocrystal former) are solid under ambient conditions when in their pure form}”. All components are solid under ambient conditions has important practical considerations, because synthesis of cocrystals can be achieved via the solid-state.\^67 Aakero\”y and co-workers,\^68 they defined cocrystals as (i) compounds constructed from neutral molecules; (ii) made from reactants that are solids at ambient conditions; and (iii) structurally homogeneous crystalline materials that contains at
least two neutral building blocks with a well-defined stoichiometry. A broad definition of a cocystal given by Dunitz: “a crystal containing two or more components together” would include molecular adducts, salts, solvates/hydrates, inclusion compounds, etc. What ever way people define the term cocystal, the field of cocystal is attaining the heights of excellence, and Figure 1.9 reveals the growing interest in the subject. Therefore, it is evident that cocrystals play a vital role in pharmaceutical science and elsewhere.

Figure 1.9 Occurrence of the term “cocrystal” in papers published between 1991 and 2007 (SciFinder search 2/2008).

1.5.4 Pharmaceutical cocrystals

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 it is the convenient and compact format 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. The solid form of the drug dictates the properties like stability, hygroscopicity, dissolution rate, solubility and bioavailability. Figure 1.10 represents the different ways in which drugs are administered. 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. Sometimes, the pharmaceutical developers come across a problem, that the most common polymorphic form of the drug is least stable. Therefore the current approaches to change the properties of APIs include the utilization of ionic salts, solvates, hydrates, and polymorphs. In this context pharmaceutical cocrystallization is potentially attractive for improving the material properties while leaving an API unaltered. The intellectual property implications of creating co-crystals are also highly relevant. A pharmaceutical co-crystal can described as “a multiple component crystal in which at least one component is molecular and a solid at room temperature (the co-crystal former) and forms a supramolecular synthon with a molecular or ionic API”. The first application of crystal engineering to generate of
pharmaceutical co-crystals was a series of studies by Whitesides et al. with the use of substituted barbituric acid, including barbital and melamine derivatives and generated a supramolecular ‘linear tape,’ ‘crinkled tape,’ and ‘rosette’ motifs.74-80

(a)                                                                                     (b)

Figure 1.11 Crystal packing of 1:1 co-crystals of 5, 5-diethylbarbituric acid (barbital)

To date, many pharmaceutical cocrystals have been reported in the scientific and patent literature and most of the cocrystals have been found to exhibit improved material properties like solubility, stability, bioavailability etc over the pure API. A few examples pharmaceutical cocrystals are: carbamazepine (CBZ), aspirin, profens, piracetam, caffeine, loracarbef, cephalexin, cefaclor, conazoles, topiramate, modafinil, phenytoin, olanzapine, nabumetone, fluoxetine, theophylline, sulfadimidine, trimethoprim, and paracetamol.81-97

(a)                                                                                     (b)

Figure 1.12 (a) Itraconazole•succinic acid cocrystal, (b) Carbamazepine•saccharin cocrystal

16
Therefore, the applications of concepts of supramolecular synthesis and crystal engineering to the development of pharmaceutical cocrystals offer many opportunities for the drug development and delivery. So it would not be exaggeration in saying that sooner or later pharmaceutical cocrystals will gain a broader foothold in drug formulation.
2. CSD ANALYSIS

2.1 Cambridge Structural Database

The Cambridge Structural Database (CSD) is an important product of the Cambridge Crystallographic Data Centre (CCDC), which was established by Olga Kennard at Cambridge University in 1965. It comprises software for database access, structure visualization and data analysis, and structural knowledge. It has a collection of bibliographic, chemical and crystallographic information for organic molecules and metal-organic compounds whose 3D structures have been determined using X-ray diffraction or neutron diffraction and the information is stored in form of a cif file (.cif). The collection also includes the results of single crystal studies powder diffraction studies which yield 3D atomic coordinate data for at least all non-H atoms. The crystal structure data arising from publications in the open literature and private communications via direct data deposition are also assimilated in the CSD.

The basic softwares that CSD relies on are: ConQuest (search and information retrieval), Mercury (structure visualization), Vista (numerical analysis) and PreQuest (database creation). The CSD helps to compare existing data with that obtained from crystals grown in their laboratories. It is evident from Figure 2.1 that there is a rapid growth of the CSD since 1970, and it is predicted growth would increase exponentially during the decade 2001-2010. Thus CSD is proves to be an inexhaustible source for crystallographers and interpretation of existing crystal structures with complete knowledge on interplay between
supramolecular synthons would help in designing new multicomponent crystals.

![Figure 2.1](image)

Figure 2.1 (a) Growth of the CSD since 1972, (b) Predicted growth of the CSD during the decade 2001 - 2010

2.2 CSD statistics for Phenols and Polyphenols

Flavonoids are known as polyphenolic compounds and to design new crystal forms (solvates, cocrystals etc) of these compounds it is necessary to know about the statistics of existing synthons of hydroxyl groups with other functional groups present in the CSD. Although phenols and alcohols have the hydroxyl group common in their structures as they differ in acidity. Phenols have relatively higher acidities due to the aromatic ring tightly coupling with the oxygen and a relatively loose bond between the oxygen and hydrogen. The acidity of the hydroxyl group in phenols is intermediate between that of aliphatic alcohols and carboxylic acids (their $pK_a$ is between 10 and 12). The polyphenols for the CSD search were recognized by the presence of more than one hydroxyl group per molecule as represented in the Figure 2.2.

![Figure 2.2](image)

Figure 2.2 Structure of polyphenol for the CSD search
All the searches were done by considering the constraints: no ions, only organics, R factor: \( \leq 7.5\% \) and structures with 3D coordinates. The statistics for the occurrence of various interactions of phenols and polyphenols with other functionalities in the CSD are tabulated below.

**Table 2.1 CSD statistics for Phenols and Polyphenols (January 2008 update)**

<table>
<thead>
<tr>
<th>Moieties present in a structure</th>
<th>No. Struc (Phenols)</th>
<th>Structures with synthon</th>
<th>No. Struc (Polyphenols)</th>
<th>Structures with synthon</th>
</tr>
</thead>
<tbody>
<tr>
<td>*OH (Phenols only)</td>
<td>6932</td>
<td>O-H\textsuperscript{•}OH (synthon I) 1320 / 6932 (19.0%)</td>
<td>1196 / 6932 (19.2%)</td>
<td>O-H\textsuperscript{•}OH 492 / 1196 (41.1%)</td>
</tr>
<tr>
<td>*OH &amp; CO (ket &amp; ald)</td>
<td>960</td>
<td>O-H\textsuperscript{•}CO (synthon II) 569 / 960 (67.0%) &amp; O-H\textsuperscript{•}OH (phenolic) 202 homosynthons (20.0%)</td>
<td>446</td>
<td>O-H\textsuperscript{•}CO 186 / 446 (41.7%) &amp; O-H\textsuperscript{•}OH (phenolic) 163 homosynthons (36.6%)</td>
</tr>
<tr>
<td>*OH &amp; N\textsubscript{arom}</td>
<td>566</td>
<td>O-H\textsuperscript{•}N\textsubscript{arom} (synthon III) 315 / 566 (59.3%) &amp; O-H\textsuperscript{•}OH (phenolic) 88 homosynthons (15.5%)</td>
<td>151</td>
<td>O-H\textsuperscript{•}N\textsubscript{arom} 128 / 151 (84.8%) &amp; O-H\textsuperscript{•}OH (phenolic) 46 homosynthons (30.5%)</td>
</tr>
<tr>
<td>*OH &amp; O (ether)</td>
<td>2391</td>
<td>O-H\textsuperscript{•}O (synthon IV) 278 / 2391 (11.6%) &amp; O-H\textsuperscript{•}OH (phenolic) 383 homosynthons (16.6%)</td>
<td>330</td>
<td>O-H\textsuperscript{•}O 66 / 330 (23.5%) &amp; O-H\textsuperscript{•}OH (phenolic) 131 homosynthons (39.7%)</td>
</tr>
<tr>
<td>*OH &amp; CONH\textsubscript{2} (1\textdegree amides)</td>
<td>85</td>
<td>O-H\textsuperscript{•}O=C (synthon V) 16 / 85 (%)</td>
<td>10</td>
<td>O-H\textsuperscript{•}O=C 3 / 10 (30%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OH\textsuperscript{•}NH\textsubscript{2} (synthon VI) 14 / 85, (16.5%) &amp; O-H\textsuperscript{•}OH (phenolic) 6 homosynthons (7.1%)</td>
<td></td>
<td>OH\textsuperscript{•}NH\textsubscript{2} 8 / 10 (80%) &amp; O-H\textsuperscript{•}OH (phenolic) 2 homosynthons (20%)</td>
</tr>
</tbody>
</table>

* Constrains: no ions, only organics, R factor: \( \leq 7.5\% \) and structures with 3D coordinates
* * represents phenolic –OH group
2.3 Interactions of phenolic -OH group with other functional groups

The CSD search reveals that there are 6232 hits for phenols and 1196 polyphenols (constraints: no ions, only organics, R factor: ≤ 7.5% and structures with 3D coordinates).

\[
\text{HO} \quad \text{O} \quad \text{H}
\]

Figure 2.3 Supramolecular phenolic OH OH homosynthon (Synthon I)

Out of 6232 phenols the OH…OH phenolic supramolecular homosynthon was found in 19.0% structures and out of 1196 polyphenols it was 41.1%. The bond range for synthon I was determined by the histogram generated by Vista software and it was found to be 2.5-3.07 Å (cut off range). This implies that the remaining structures form supramolecular heterosynthons in the presence of other competing groups and the Figure 2.4 represents the cut off range for synthon I.

![Histogram for synthon I](image)
The search for the entries with phenolic –OH group and the carbonyl group (aldehydes and ketones only) revealed that out of 960 hits 67.0% were found to have phenolic OH—CO supramolecular heterosynthon (synthon II) and only 20.0% have OH—OH phenolic supramolecular homosynthon (synthon I).

\[
\text{Figure 2.5 Supramolecular phenolic OH—CO heterosynthon (Synthon II)}
\]

In case of polyphenols the presence of synthon II was only 41.7% and 36.6% have synthon I out of 446 entries with polyphenols and carbonyl compounds. The cut off range for synthon II was determined as 2.5-3.10 Å as shown in Figure 2.6.

\[
\text{Figure 2.6 Histogram for synthon II}
\]

Another search for the occurrence of phenolic OH—N<sub>Ar</sub> supramolecular heterosynthon (synthon III) among the structures containing aromatic nitrogen with phenols and polyphenols was made.

\[
\text{Figure 2.7 Supramolecular phenolic OH—N<sub>Ar</sub> heterosynthon (Synthon III)}
\]
The search results in 566 hits for compounds containing aromatic nitrogen and phenols. Out of the 566 hits 59.3% were found to have synthon III and 15.5% synthon I. For polyphenols the percentage for the occurrence of synthon III is much higher than that of phenols. Out of 151 total structures containing polyphenols and aromatic nitrogen, 84.8% were found to have synthon III and 30.5% have synthon I. The distance range for synthon III was found to be 2.5-3.125 Å as shown in Figure 2.8.

![Figure 2.8 Histogram for synthon III](image)

The search for the phenolic OH···O supramolecular heterosynthon (synthon IV) for ether with phenols and polyphenols shows that ethers are not good hydrogen bond acceptors as other functional groups. Even though the occurrence ethers with phenols and polyphenols is high when compared to other functional groups, out of 2391 hits only 11.6% of the total structures are capable of forming synthon IV and 16.0% form synthon I.

![Figure 2.9 Supramolecular phenolic OH···O heterosynthon (Synthon IV)](image)
For polyphenols out of 330 hits 23.5% were found to have the synthon IV and 39.7% form synthon I. The distance range for the occurrence of synthon IV was found to be 2.55-3.07 Å.

The similar kind of search was made for the presence of amides with phenols and polyphenols was made for the occurrence of phenolic OH···CO (synthon V) and phenolic OH···NH (synthon VI) supramolecular heterosynthons. Out of 85 hits containing phenols and amides only 18.8% hits form the synthon V and 16.5% form synthon VI and only 7.1% of the total hits form synthon I.

There were only 10 hits for amides and polyphenols and out of which only 3 hits were found to form synthon V and 8 hits form synthon VI and only 2 hits were found to form synthon I.
The bond ranges for the synthons V and VI were found to be 2.6-3.0 Å, 2.75-3.175 Å and respectively as shown in Figure 2.12.

![Figure 2.12 Histogram for (a) Synthon V; (b) Synthon VI](image)

2.4 Discussions

Upon reviewing the statistics obtained from CSD searches, it is evident that aromatic nitrogen seems to be more prominent and competitive functional group to hydrogen bond with phenols and polyphenols. Even the carbonyls are good competitors for hydrogen bonding with the phenols and polyphenols. The data obtained from CSD statistics is useful in selecting the cocrystal formers (CCFs) that contains the competitive functional groups and are capable of forming hydrogen bond to the target nutraceuticals. Compounds containing aromatic nitrogen, carbonyl and amide functional groups are good candidates as CCFs for polyphenolic compounds such as flavonoids. Thus CSD proves to be very good tool for crystal engineering and would provide a valuable insight for crystal engineering strategies in generation of new multicomponent materials.
3. QUERCETIN

Quercetin (3, 3’4, 4’, 5-7-pentahydroxyflavone) is a bioflavonoid which is widely distributed in the plant kingdom. It is the most abundant of all the flavonoid molecules found in many often consumed foods including apples, citrus fruits, onions, tea, berries and vegetables, as well as many seeds, nuts, flowers, barks and leaves. It is also present in medicinal botanicals like Ginkgo biloba, Hypericum perforatum, Sambucus Canadensis and many others.

![Figure 3.1 Structure of Quercetin](image)

3.1 Description

Quercetin (Mol Wt = 302.2) is a bioflavonoid (pigment), found in almost all herbs, fruits, and vegetables. Bioflavonoids provide the body with anti-inflammatory and antioxidant protection, and quercetin is one of the most powerful and effective herbal anti-inflammatory, and antioxidant supplements in the market today. Quercetin has been shown to prevent the development of a variety of conditions related to inflammation by direct inhibition of several initial processes of inflammation and free-radical damage, including arthritis, allergies, macular degeneration, heart disease and various forms of
cancer.\textsuperscript{107} Quercetin also shows anti-tumor properties.\textsuperscript{108} The estimated average daily dietary consumption of quercetin is about 30 mg/day. However, the manufacturer’s recommended daily dose of over-the-counter quercetin supplements ranges from 400–1200 mg/day.\textsuperscript{109} There is no recommended standard dose for quercetin but there have been some occasional reports of nausea when taken as high doses in supplements.\textsuperscript{106} Quercetin exhibits poor solubility in water, it is practically insoluble in water but it is soluble in 100\% sodium hydroxide and methanol, but in its glycoside form (with a sugar attached), which is the common form of its occurrence in fruits and vegetables, is more soluble in the body.\textsuperscript{110} Some of the glycoside forms of quercetin are rutin and quercitrin.

\section*{3.2 Strategy for Crystal Engineering of Quercetin}

The quercetin molecule consists of three rings designated A, B and C respectively and is illustrated in Figure 3.2. The five hydroxyl groups, labeled as a-e can act as hydrogen bond acceptors and/or donors and the ether and carbonyl moieties are capable of serving as hydrogen bond acceptors.

![Figure 3.2 Representation of different rings in Quercetin](image)

Based on the CSD search as discussed in Chapter 2 for the occurrence of supramolecular
synthons between polyphenols and other functionalities, aromatic nitrogen, carbonyls and amides look more promising competitors for hydrogen bonding. To date, the crystal structure of pure quercetin is not known but there are two entries for quercetin dihydrate with ref codes FEFBEX\textsuperscript{111} and FEFBEX01\textsuperscript{112} and a formamide solvate (EVIIJUO)\textsuperscript{113} and other hydrates (LORKEI)\textsuperscript{114} in the CSD. Figure 3.3 represents the packing in the quercetin dihydrate. The hydrogen bonding for the dihydrates of quercetin with the ref codes FEFBEX and FEFBEX01 are same but the R factor for FEFBEX01 (R_{fac} 4.8) is better than FEFBEX (R_{fac} 9.5).

![Figure 3.3 Hydrogen bonding in Quercetin dihydrate](image)

In the dihydrate form quercetin crystallizes with two water molecules that participate in extended hydrogen bonding network through the crystal lattice. In the crystal structure quercetin exists as a dimer which is formed by the OH\textsubscript{b} and the adjacent carbonyl moiety and these are also involved in the intramolecular hydrogen bonding in the dimer (circled in Fig 3.3). All the hydroxyl and carbonyl groups and the water molecules participate in hydrogen bonding. All the three rings of quercetin molecule in the crystal lattice are not
planar. The rings A and C lie in the same plane whereas the ring B is not. The dihedral angle between the plane containing rings A and C and the plane containing ring B is 8.10° as shown in Figure 3.4. The overall hydrogen bonding leads to the stacking of infinite 2D sheets.

![Figure 3.4 Illustration of dihedral angle between the planes. (Red plane contains rings A, C and the blue plane contains ring B)](image)

3.3 Solvates of Quercetin

Three solvates of quercetin with pyridine, tetrahydrofuran (THF) and acetone namely I, II, III, were obtained by different methods. All the chemicals used during the preparation were purchased from Sigma Aldrich and used as received.

<table>
<thead>
<tr>
<th>Form</th>
<th>Solvate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Quercetin-Pyridine solvates</td>
</tr>
<tr>
<td>III</td>
<td>Quercetin-THF solvate</td>
</tr>
<tr>
<td>IV</td>
<td>Quercetin-Acetone solvate</td>
</tr>
</tbody>
</table>

Table 3.1 Solvates of Quercetin

3.3.1 Form I and Form II (Pyridine solvates of Quercetin)

*Synthesis:* 34 mg of quercetin dihydrate (purchased from Sigma Aldrich and used as received) was dissolved in 5ml of Pyridine; this solution was layered with 4 mL of water
and refrigerated. After 4 hours golden yellow crystals were produced (yield- 25mg). The crystallization experiment was conducted in an unmodified atmosphere and the solvents were dried by standard methods prior to use. mp-314 °C.

Figure 3.5 Single crystal of quercetin-pyridine solvate
The attempts to get pure crystals of quercetin using pyridine solvate resulted in two different forms of quercetin-pyridine solvate namely Form I and Form II. The Form I in due course of time transforms to Form II. The crystal structure of Form I reveals that two molecules of pyridine hydrogen bond to each molecule of quercetin. The quercetin molecules don’t exists as a dimer as found in its dihydrate form (FEFBEX01). But the quercetin molecules forms a cyclic pattern, described by Etter’s\textsuperscript{115} graph set R\textsuperscript{2} \_2 (8). The cyclic pattern includes heterosynthons such as OH…CO (D = 2.724 Å) and OH…OH (D = 2.914 Å). Where as the crystal structure Form II reveals that quercetin molecules exists as dimer as found in quercetin dihydrate (FEFBEX01). But the only difference being that in FEFBEX01 the dimer includes OH\textsubscript{b} and the carbonyl moiety whereas in Form II the dimer includes OH\textsubscript{c} and the carbonyl moiety (D = 2.749 Å) and the OH\textsubscript{b} is just involved in the intramolecular hydrogen bonding (D = 2.608(3) Å). Figure 3.6 represents the hydrogen bonding in Form I and Form II. The R\textsuperscript{2} \_2 (8) graph set in Form I and the quercetin dimer in Form II are circled in the figure.
The dihedral angle between the planes containing the rings A, C and ring B for form I and Form II are 21.11° and 23.71° respectively. These angles are higher than that is found in FEFBEX01 (8.10°). This indicates that quercetin molecules in Form I and II are not that planar as in FEFBEX01 (quercetin dihydrate).

The presence of quercetin-pyridine solvate in two different forms indicates that quercetin exhibits polymorphism.65 The CSD search has no entries for the crystal structure of polymorphic quercetin but the literature searches reveal that quercetin exhibits polymorphism.116-119 The Form II could be most stable than Form I because according to Ostwald’s step rule134,135 which states: “In general it is not the most stable but the least stable polymorph that crystallizes first”. In both the forms the pyridine molecules hydrogen bond to OHₐ and OHₑ of quercetin molecules. The hydrogen bond distances for the OHₐ···Nₐ and OHₑ···Nₐ in From I are 2.705 Å and 2.677 Å respectively whereas
for Form II it is 2.765 Å and 2.64 Å. The distances are in accordance with the distance ranges found in CSD as discussed in Chapter 2. Overall supramolecular hydrogen bonding in Form I results in 2D tape and in Form II it results in a 2D sheet. Desolvation occurs when the crystals are heated at 150°C for 10 min and they become amorphous. The amorphous powder could be pure quercetin as it decomposes at 316°C which coincides with the literature value.  

3.3.2 Form III (THF solvate of Quercetin)  

**Synthesis:** 34 mg of quercetin dihydrate (purchased from Sigma Aldrich and used as received) was dissolved in 5ml of THF; this solution was layered by 4 mL of hexane and refrigerated. After 4 days golden yellow crystals in the form of plates were produced (yield-20.5mg). The crystallization experiment was conducted in an unmodified atmosphere and the solvents were dried by standard methods prior to use. mp 316 °C.

![Figure 3.8 Single crystal of quercetin-THF solvate (Form III)](image)

The crystal structure reveals that there are three THF molecules in the asymmetric unit. Two of the THF molecules are involved in the formation of supramolecular network with the quercetin molecules and the third distorted THF molecule just act as a guest. All the hydroxyl and the carbonyl groups of quercetin and the THF molecules are involved in hydrogen bonding. In Form III also quercetin exists as a dimer (circle in Fig 3.9) with a
bond distance of 2.749(3) Å similar to that found in Form II (quercetin-pyridine solvate) with hydrogen bond distance of 2.749 Å (OH…CO). Figure 3.9 compares the hydrogen bonding in Form II and III. The dimer in Form III can also be described as $R_2^2(10)$ graph set.\textsuperscript{115}

![Figure 3.9 Comparison of hydrogen bonding in Form III (left) with that of Form II (right)](image)

Desolvation of Form III crystals occurs after 15-20 minutes when they are taken out from the mother liquor and leaves the system amorphous and the decomposition temperature of the powder coincides with that of quercetin.\textsuperscript{120}

### 3.3.3 Form III (Quercetin-Acetone solvate)

**Synthesis:** 34 mg of quercetin dihydrate and 13.3 mg of caprolactam were dissolved in 5ml of acetone and the refrigerated for 3 days. Golden yellow crystals of solvate III were obtained wherein quercetin and acetone were in 1:1 stoichiometric ratio (yield-20mg). All the chemicals used during the preparation were purchased from Sigma Aldrich and used as received. The crystallization experiment was conducted in an unmodified atmosphere and the solvents were dried by standard methods prior to use. mp 316°C.
The crystals of solvate III were obtained during an attempt to prepare the cocrystal of quercetin and caprolactam by using acetone as a solvent. In Form III also quercetin exists as a dimer, but this dimer is formed by OH\textsubscript{d} and OH\textsubscript{c} of quercetin molecule form strong hydrogen bond distance of 2.754 Å which falls well within the range for alcohol-alcohol supramolecular homosynthon as discussed in Chapter 2. Figure 3.11 represents the dimer that is formed in Form III. All hydroxyls and the carbonyl groups of quercetin participate in the hydrogen bonding. Further, OH\textsubscript{c} of both quercetin molecule hydrogen bonds with OH\textsubscript{d} of adjacent quercetin molecule with OH…OH interaction (D = 2.765 Å) and the OH\textsubscript{d} hydrogen bonds to the carbonyl group of another adjacent quercetin molecule and forms a strong OH…O hydrogen bond (D = 2.682 Å).
The OHa of each quercetin molecule is bifurcated in hydrogen bonding with acetone molecule forming OH− O hydrogen bond (D = 2.596 Å) and OH− OH supramolecular homosynthon with adjacent OHa quercetin molecules (D = 2.750 Å). All the hydrogen bond distances mentioned above fall well within the range of distances found in CSD as discussed in Chapter 2. The overall hydrogen bonding results in a crisscross grid network as represented in Figure 3.12.

3.3.4 Discussions

The attempts to obtain the pure crystals of quercetin from quercetin dihydrate resulted in the formation of new forms namely, Form I, Form II, Form III and Form IV. The Form I and Form II are the polymorphs of quercetin whereas, the Form III and IV are the solvates of THF and acetone respectively. The quercetin molecule exists as dimer in Form II and III similar to that of quercetin dihydrate (FEFBEX01)90 where as such kind of dimer is absent in Form I. Hydrogen bonding in Form I and II results in the formation of linear tapes where as the Form III results in cross grid network. Desolvation occurs for Form II after 5-10 minutes when the crystals are taken out of the mother liquor and an amorphous powder is obtained where as, the Form I and III are pretty stable in absence of mother liquor. In order to get rid of the solvent molecules in Form I and III, the crystals were heated at 150 and 55 °C respectively. The amorphous powders from all the three
forms were analyzed by DSC and melt temp separately and it was found that in all the three cases the powder decomposes at 328 °C, that exactly coincides with the decomposition temperature of quercetin. The amorphous form obtained could be pure quercetin, but this could not be confirmed as the crystals of pure quercetin were not produced by other means.

3.4 Cocrystals of Quercetin

Crystal engineering of quercetin lead to the generation of two novel cocrystals namely Cocrystal I and II with caffeine and isonicotinamide were obtained by slow evaporation. The functional groups that are more likely to undergo hydrogen boning in quercetin are the hydroxyls and the carbonyl. These functional groups were utilized to target the various functional groups in caffeine and isonicotinamide such as the nitrogen in the imidazole ring and the imide groups in caffeine and the aromatic nitrogen and the amide groups in isonicotinamide. The CSD searches (Chapter 2) reveal that the polyphenols are more likely to form supramolecular synthons with functional groups like carbonyl and aromatic nitrogen. The percentages obtained for OH⋯N_{arom} and OH⋯CO supramolecular heterosynthons in the CSD searches are 84.8% and 74.4% respectively.

3.4.1 Quercetin- Caffeine- Methanol cocrystal solvate

![Figure 3.13 The asymmetric unit of 1:1:1 cocrystal solvate of quercetin, caffeine and methanol](image)
**Synthesis:** 68 mg of quercetin dihydrate and 38 mg of caffeine were dissolved in approximately 5 mL of methanol and heated until a clear solution was obtained. Slow evaporation of this solution in refrigerator resulted in 1:1 crystals after 3 days (yield- 30 mg). All the chemicals used during the preparation were purchased from Sigma Aldrich and used as received. All crystallization experiments were conducted in an unmodified atmosphere and the solvents were dried by standard methods prior to use. mp: 246°C.

![Figure 3.14 Single crystal of quercetin-caffeine-methanol cocrystal solvate (Cocrystal I)](image)

The crystal structure reveals that the imide group and the aromatic nitrogen of caffeine are the functional group that interacts with the hydroxyl groups of quercetin. Caffeine molecules interact with quercetin molecules via the formation of $\text{OH}_c^{-}\cdot\cdot\cdot\text{N}_{\text{arom}}$ and $\text{OH}_a^{-}\cdot\cdot\cdot\text{CO}$ supramolecular heterosynthons. The former supramolecular heterosynthon is formed by the interaction of $\text{OH}_c$ quercetin and the aromatic nitrogen of the imidazole ring in caffeine ($D = 2.821(3) \ \text{Å}$) and the latter results due to the hydrogen bonding between $\text{OH}_a$ of quercetin and the CO moiety of the imide group of caffeine ($D = 2.716(3) \ \text{Å}$). The carbonyl in the caffeine molecule hydrogen bonds to the methanol molecule ($D=2.712(3) \ \text{Å}$). All the above distances are in accordance with the $\text{OH}^{-}\cdot\cdot\cdot\text{O}$ synthon and $\text{OH}_c^{-}\cdot\cdot\cdot\text{N}_{\text{arom}}$ distance ranges found in CSD as discussed in Chapter 2.
Adjacent quercetin molecules interact by utilizing some of the remaining hydrogen bonding sites as follows: trifurcation of the OH₅ moiety of quercetin molecules through supramolecular homosynthon and OH\(^{-}\)CO supramolecular heterosynthon. The OH\(^{-}\)OH supramolecular homosynthon is formed between the OH₅ of quercetin molecules and OH₆ and OH₇ of adjacent quercetin molecules (D = 2.774(3) Å, 2.847(3) Å respectively). OH₅ is also engaged in OH\(^{-}\)CO intramolecular heterosynthon (D = 2.602(3) Å). The bond distances fall in the range found in the CSD searches for the respective synthons as discussed in Chapter 2. These hydrogen bond interactions afford a supramolecular sheet that stacks up. These sheets are further connected via the interaction of methanol molecules with OH₆ moieties of quercetin molecules through OH\(^{-}\)OH supramolecular homosynthons (D = 2.633(3) Å). The overall effect of the hydrogen bonding results in the formation of a network where the methanol could be described as a guest although as shown in Figure 3.16.
3.4.2 Quercetin-Isonicotinamide

*Synthesis:* 67.6 mg of Quercetin dihydrate and 24.6 mg of isonicotinamide were dissolved in approximately 5 mL of methanol and heated until a clear solution was obtained. Slow evaporation of this solution in refrigerator resulted in 1:1 crystals after 2 days (yield-31.5mg). All the chemicals used during the preparation were purchased from Sigma Aldrich and used as received. The crystallization experiment was conducted in an unmodified atmosphere and the solvents were dried by standard methods prior to use. mp: 256-260°C.
The aromatic nitrogen and the carbonyl moiety of the amide group and the carbonyl group in quercetin acts as hydrogen bond acceptors where as all the hydroxyl groups of quercetin can act as hydrogen bond acceptors/donors. Isonicotinamide molecules interact with quercetin molecules via, CO…OH and NH…OH supramolecular heterosynthons and OH…OH supramolecular homosynthons. One of the two hydrogen atoms in the amino moiety of the isonicotinamide hydrogen bonds to the carbonyl group of adjacent quercetin molecules and the other hydrogen atom interacts with OH₃ of a different quercetin molecule giving rise to NH…CO (D = 3.018(4) Å) and NH…OH (D = 3.028(4) Å) supramolecular heterosynthons, respectively. These distances are in accordance with the distance ranges found in the CSD search for amides in Chapter 2. The carbonyl of the amide moiety hydrogen bonds to OHₑ quercetin molecules whereas the Nₐrom atom of the isonicotinamide molecule interacts with OHₐ of quercetin molecules and thereby generates CO…OH (D = 2.607(3) Å) and Nₐrom…OH (D = 2.684(3) Å) supramolecular heterosynthons respectively. The distances fall within the range found in CSD as discussed in Chapter 2.

Figure 3.19 Intermolecular interactions in the 1:1 Quercetin-Isonicotinamide co-crystal.

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The OHd & OHe moieties of two quercetin molecules and the amide moieties and the syn hydrogen atoms of the amino group of two isonicotinamide molecules form a four component assembly composed of two $R^2_2 (8)$ and one $R^2_2 (10)$ supramolecular synthons (Etter’s graph set). Both the patterns result in $R^4_4 (18)$ graph set as represented in Figure 3.6.

![Figure 3.20 Illustration of $R^4_4 (18)$ graph set in quercetin and isonicotinamide cocrystal.](image)

The $R^2_2 (8)$ graph set is formed by two quercetin molecules and one isonicotinamide molecules which includes OH$^--$CO supramolecular heterosynthon formed between OHe moieties and the carbonyl groups of isonicotinamide molecules ($D = 2.607(3)$ Å). The $R^2_2 (10)$ supramolecular synthon occurs between two quercetin molecules in which OH$^--$OH supramolecular homosynthons are formed between OH$_d$ & OH$_c$ groups of two quercetin ($D = 2.765(3)$ Å). The dimer $[R^2_2 (10)]$ is formed between two quercetin molecules remains intact and the exterior hydrogen bonding sites are utilized in the hydrogen bonding with N-H of isonicotinamide ($D = 3.028(4)$ Å). The bond distances found in the supramolecule are in accordance with the distances found in CSD for the respective synthons as discussed in Chapter 2.
3.4.4 Discussions

Crystal engineering has lead to the generation novel cocrystals of quercetin and thus confirms that flavonoids are capable of generating novel cocrystals with pharmaceutically acceptable molecules such as caffeine and isonicotinamide. As caffeine and isonicotinamide have no solubility problems whereas caffeine has problem with physical stability against hydration. The CSD reveals that there are 17 entries for the cocrystals of caffeine. There are also evidences which indicate that cocrystallization of caffeine with suitable cocrystal formers (CCF) overcome the hydration problem.\textsuperscript{121} Even isonicotinamide\textsuperscript{122} seems to be a good CCF as there are cocrystals of it in the literature. The CSD statistics as discussed in Chapter 2 reveals that aromatic nitrogen and carbonyl functional groups are good acceptors to hydrogen bond with the hydroxyls of polyphenolic compounds. Therefore caffeine and isonicotinamide (isomer of nicotinamide, known as niacin)\textsuperscript{123} stand as suitable CCFs for cocrystallization and are pharmaceutically acceptable molecules. Thus crystal engineering allows one to choose complementary components for polyphenolic compounds for designing supramolecules.
4. HESPERETIN

Hesperetin (RS-2,3-dihydro-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one) is a naturally occurring flavanone which is unique among the others as it is the only one which is faintly sweet while most of the flavonoids are tasteless or bitter. Hesperetin is the aglycone form of hesperedin which is abundantly present in citrus fruits. Animal studies have shown that hesperetin has very good anti-inflammatory properties.

![Structure of hesperetin](image)

Figure 4.1 Structure of hesperetin (hydrogen are not included)

4.1 Description

Hesperetin (Mol.Wt 302.27 g/mol) is a bioflavonoid that is present in the plants in its glycoside form (hesperedin). We have discussed earlier in Chapter 1, how glycoside forms of flavonoids are more soluble that the aglycone form. Hesperedin is more soluble than hesperetin because of the sugar ring attached to it. It is a phenolic antioxidant, antiallergic, and antimitagenic. It may scavenge the reactive oxygen species as superoxide anions and may protect against peroxidation. Studies on rats have shown that hesperetin helps in reducing the cholesterol by the inhibition of 3-hydroxy-3-ethylglutaryl coenzyme A. In vitro studies have also shown that hesperetin has some anti cancer activity too.
4.2 Strategy for Crystal Engineering of Hesperetin

Hesperetin has similar structure to that of quercetin, the difference being rings B and C. In ring B there is a methoxy group instead of a hydroxyl and saturated ring C. Figure 4.2 represents the structure of hesperetin with its 3 rings A, B, C. The hydroxyl groups are labeled a, b and c. The hesperetin molecule has two hydroxyl groups on ring A and one on ring B act as hydrogen bond acceptors and/or donors. Hesperetin has also has an ether and carbonyl (ketone) moieties on ring C that can act as hydrogen bond acceptors.

Figure 4.2 Illustration of rings in Hesperetin

The data available in the CSD reveals that there are two crystal structures of hesperetin discovered so far, one is the pure hesperetin (YEHROS)\textsuperscript{132} and the other is the monohydrate form (FOYTOC)\textsuperscript{133}

Figure 4.3 Hydrogen bonding in (a) Hesperetin (b) Hesperetin monohydrate

Figure 4.3 represents the hydrogen bonding in both the available forms of hesperetin as a
racemate and monohydrate. Hesperetin molecule in YEHROS is not a planar molecule as represented in Figure 4.4 (a).

(a) YEHROS

(b) FOYTOC

Figure 4.4 Illustration of the dihedral angles (blue plane contains rings A and C; red plane contains the ring C).

The two rings A and C lie in the same plane and the ring B lies in the other plane with a dihedral angle of 53.59°. Whereas, in FOYTOC hesperetin adopts almost a planar conformation while the conformation in YEHROS seems to be more favorable as there is less repulsive steric hindrance between the rings with a dihedral angle of 2.91° as represented in Figure 4.4 (b).133

4.2.1 Hesperetin-Isonicotinamide cocrystal (Cocrystal 3)

Figure 4.5 The asymmetric unit of hesperetin:isonicotinamide 1:1 cocrystal
**Synthesis:** 60 mg of Hesperetin and 24.6 mg were dissolved in approximately 5 mL of ethanol and heated until a clear solution was obtained. Slow evaporation of this solution in refrigerator resulted in 1:1 crystals after 5 days. All crystallization experiments were conducted in an unmodified atmosphere and the solvents were dried by standard methods prior to use. All chemicals were purchased from Aldrich and used as obtained. m.p 172-176°C.

![Figure 4.6 Single crystal of hesperetin-isonicotinamide cocrystal](image)

Crystallization of hesperetin with isonicotinamide resulted in a 1:1 cocrystal. In the isonicotinamide molecule, the aromatic nitrogen and the carbonyl moiety of the amide group act as hydrogen bond acceptors. The two hydrogen atoms of the amino group of the amide moiety act as the hydrogen bond donors. The X-ray crystal structure reveals the existence of a supramolecular homosynthon (amide dimer) formed by the interaction of two isonicotinamide molecules. This results in a graph set notation$^{115} \text{R}_2^2(8)$ with O---N$_{\text{arom}}$ interaction as represented in Figure 4.8 with bond distance of 2.868(3) Å that fall well with the range found in CSD in Chapter 2. The supramolecular synthon formed in the cocrystal includes OH---N hydrogen bond between the nitrogen atom of isonicotinamide and the OH$_a$ of the adjacent hesperetin molecule with an O---N bond distance of 2.623(2) Å. OH$_a$ is further bifurcated as it forms a hydrogen bond with the anti N-H of the isonicotinamide
dimer with an N---O bond distance of 3.031(3) Å. The amide dimer remains intact and as observed in the crystal structure of pure isonicotinamide.

![Figure 4.7 Overall hydrogen bonding in the Hesperetin-Isonicotinamide 1:1 cocrystal](image)

The hesperetin molecules lie opposite to each other around the crystallographic inversion center as represented in Figure 4.8. The adjacent hesperetin molecules interact via OH---CO utilizing the hydroxyl moieties on ring B with a bond distance of 2.720(2) Å. There is also intramolecular hydrogen bonding between the carbonyl (ketone) group on ring C and the OH<sub>c</sub> with a bond distance of 2.584(2) Å. All the hydrogen bond distances found in hesperetin and isonicotinamide falls well with in the expected as discussed in Chapter 2.

![Figure 4.8 Illustration supramolecular synthons in the cocrystal 3.](image)

Figure 4.7 represents the how the hesperetin and isonicotinamide molecules arrange
themselves in the crystal lattice. The hydrogen bonding in the cocrystal 3, the hesperetin molecules form a one dimensional tape along Z-axis and these tapes are connected through centrosymmetric amide dimers formed by isonicotinamide molecules forming a two dimensional sheets with cavities (31 x 10 Å$^2$).

Figure 4.9 The cavity formed in hesperetin:isonicotinamide cocrystal.

The cavities are filled by similar sheets and its eight fold interpenetrated. The overall hydrogen-bonding pattern is that of a zigzag 2-D sheet with interpenetration and it means that all hydrogen bond donors and acceptors in both molecules are satisfied as shown in Figure 4.10.

Figure 4.10 Illustration of 8-fold interpenetrated network formed in Cocrystal 3
The hesperetin molecules maintain the angular conformation similar to what is observed in pure hesperetin crystal structure (YEHROS). The dihedral angle observed for hesperetin molecules in the cocrystal between the planes containing rings A, C and ring B is greater by 24.2° than found YEHROS. The hesperetin molecules are more stable in angular conformation as the steric hindrance is reduced. Figure 4.11 represents the dihedral angle between the planes of the rings in hesperetin molecule.

![Figure 4.11 Illustration of dihedral angle in hesperetin molecules (red plane contains rings A and C; blue contains ring C)](image)

4.2.2 Hesperetin-Nicotinic acid cocrystals

![Figure 4.12 Hesperetin – Nicotinic acid 1:1 zwitterion cocrystal (Form I)](image)

**Synthesis:** 60 mg of Hesperetin and 24.6 mg of nicotinic acid were dissolved in approximately 5 mL of methanol and heated until a clear solution was obtained. Slow
evaporation of this solution in refrigerator resulted in 1:1 crystals after 5 days. All
crystallization experiments were conducted in an unmodified atmosphere and the solvents
were dried by standard methods prior to use. All chemicals were purchased from Aldrich and
used as obtained. m.p 198-204 °C.

Figure 4.13 Single crystals of hesperetin-NA cocrystals.

Crystallization of hesperetin with nicotinic acid results in two 1:1 cocrystals in which the
nicotinic acid exists as a zwitterionic state, Form I contains only one of the enantiomer of
R-hesperetin (Cahn-Ingold-Prelog priority rules) and converts to Form II which contains both
forms of (+) hesperetin (Cahn-Ingold-Prelog priority rules) crystallizes with nicotinic
zwitterion. As Form I contains only one of the enantiomer of hesperetin in the crystal
structure it is called as the racemic conglomerate and Form II is the racemate of hesperetin.
During the crystallization process both the cocrystals were generated in the same vial and the
reproduction of the Form I cocrystal was not achieved. This indicates that Form I is
kinetically more favored and Form II is thermodynamically. Figure 4.13 represents the
overall hydrogen bonding in cocrystal Form I. The crystal structure of Form I reveals the
existence of nicotinic acid (NA) as a zwitterionic species in the cocrystal, which is not an
amino acid. The zwitterion of NA can be confirmed by the following structural information:
a) The C-O bond distances in the carboxylate ion were found to be 1.241 Å 7& 1.250 Å and
the corresponding C-N-C (protonated pyridine) bond angle equal to 121.54°. While as, the C-O & C=O bond distances and the C-N-C bond angle in NA are 1.217Å & 1.289 Å and 117.65° respectively.

Figure 4.14 Overall hydrogen bonding in the hesperetin Form I cocrystal. (Blue color- R-Hesperetin)

Pure NA forms a linear tape where they are hydrogen bonded to each other in a head to tail fashion with a bond distance of 2.666 Å. Similar kind of tape is also present in Form I cocrystal with hydrogen bond distance of 2.604 Å. Figure 4.15 represents the tapes that are formed in NA in pure NA (a) and in Form I cocrystal (b) respectively.

Figure 4.15 Illustration of hydrogen bonds in NA: a) NA in pure form b) NA in the cocrystal, hesperetin molecules are deleted for clarity.
The CSD search for the cocrystals of NA reveals that there are only two entries with ref codes AWIDEB\textsuperscript{136} and SESLIM.\textsuperscript{137} The two cocrystals were made by using 3,5-dinitrobenzoic and 4 amino benzoic acid respectively. The crystal structure of SESLIM reveals that NA acid form a molecular tape similar to that found in pure NA and Form I cocrystal. The other modes by which the remaining hydrogen bonding sites are exploited includes O---O (D = 2.504 Å) interaction between NA and the OH\textsubscript{a} of hesperetin molecule. The hesperetin molecules are interconnected via OH…OH supramolecular hydrogen bonding (D = 2.867 Å) and forms linear tapes that sandwich the NA molecular tapes. There is weak hydrogen bonding between the OH\textsubscript{b} of hesperetin molecule with the ether group of the other hesperetin molecules with a bond distance of 3.001 Å. All the hydrogen bond distances fall in the ranges found in the CSD as discussed in Chapter 2. In Form I, the hesperetin changes in conformation adopts almost a planar conformation similar to that in hesperetin monohydrate (FOYTOC) with a dihedral angle of 4.68° between the planes containing the rings A, B and C. Figure 4.16 represents the dihedral angle.

Figure 4.16 Representation of dihedral angle between the rings. (red plane contains rings A and C, blue plane contains ring B)

The crystal structure of the Form II reveals (Cahn, Inngold and Prelog rules) that hesperetin crystallizes out as a racemate, is a result of thermodynamically favored product. The NA zwitterions hydrogen bond with each other in a head-to-tail fashion and thereby form chains
that are sandwiched by chains of hesperetin molecules. The crystal is still polar as the head-to-tail chains of NA zwitterions are parallel throughout the crystal structure. The three chains are linked by lateral hydrogen bonds and therefore form a network that could be described as a supramolecular tape as represented in Figure 4.17. The molecular structure of hesperetin as it exists in Form II reveals that one of the three hydroxyl groups engages in an intramolecular hydrogen bond with the carbonyl moiety. All the hydrogen bonds formed in both the forms have their distances that fall well within the bond ranges obtained in the CSD searches as discussed in Chapter 2.

![Figure 4.17 The hydrogen bonding in the hesperetin Form II cocrystal (yellow and maroon: NA molecules, (+) hesperetin: blue, (-) hesperetin: green)](image)

In Form II, the hesperetin maintains the almost planar conformation as found in Form I but it is slightly greater. In Form I it is 4.69° and in Form II it is around 11.38°.

![Figure 4.18 The dihedral angle formed between the planes of rings A, C and ring B in Form II](image)
4.2.4 Discussions

Crystal engineering has lead to the generation novel cocrystals of hesperetin with pharmaceutically acceptable molecules such as isonicotinamide and nicotinic acid (NA). For the first time crystal engineering has been applied to generate cocrystals of Flavonoids. As we discussed earlier in Chapter 2 that compounds containing aromatic nitrogen are more likely to form hydrogen bond with phenolic –OH groups. Both isonicotinamide and NA have aromatic nitrogen and make them good cocrystal formers for the cocrystallization of hesperetin. In addition to aromatic nitrogen isonicotinamide and NA have amide and acid groups respectively which are capable of forming hydrogen bonds with hesperetin. The CSD search reveals that there are almost 26 cocrystals of isonicotinamide. Among the 26 cocrystals three are with polyphenols such as phloroglucinol,\textsuperscript{138} hydroquinone,\textsuperscript{138} and resorcinol.\textsuperscript{138} NA has two cocrystals dinitrobenzoic\textsuperscript{136} acid and aminobenzoic acid.\textsuperscript{137} Thus isonicotinamide and NA seem to be good cocrystal former (CCF) for cocrystallization.
5. DISSOLUTION STUDIES OF COCRYSTALS

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 these are the convenient and compact format to store a drug. The absorption of all the forms of a drug depends on its physical properties. There are examples of drugs that are commercially available that suffer problems related to solubility, dissolution rate and absorption in the neutral form. These entire problems thus restrict the bioavailability of drugs. In this context the application of crystal engineering to the field of pharmaceutics provides a solution. It has intensified the research in the pharmaceutical industries as it offers a means to alter the solubilities of drugs by the discovery of new crystal forms thereby improving the bioavailability. The new forms include polymorphs, solvates, salts, hydrates and cocrystals. There are evidences where it is proved that drugs that are commercially available, such as fluoxetine HCl (Prozac®), Itraconazole (Sporanox®) with low solubility show a drastic improvement in the solubilities via cocrystallization. But not all cocrystals show improved properties, and they are not a panacea for all problems.

For example the cocrystals of Fluoxetine hydrochloride, an anti depressant drug, with a series of acids, has shown variations in its solubility when compared to the pure API. For fluoxetine HCl: succinic acid cocrystal an approximate 3-fold increase in the dissolution of the API relative to fluoxetine HCl, the cocrystal of fluoxetine HCl: benzoic acid dissolves at a rate that is approximately half of the rate for the API alone, while the dissolution rate for fluoxetine HCl: fumaric acid cocrystal is
roughly similar to that of fluoxetine HCl. These results reveal that, by cocrystallizing an API with different guest molecules, it is possible to increase or decrease the dissolution rate of the API or to leave the effective dissolution rate essentially unchanged. The Figure 5.1 represents the results of dissolution studies carried out for various cocrystals of fluoxetine HCl.

![Figure 5.1](image)

**Figure 5.1** Powder dissolution profiles for (1), (2), (3), and (4) in water at 20 °C.

Another example of API that forms cocrystals is Itraconazole (Sporanox), a low solubility drug that is available commercially in the amorphous form. The dissolution studies of the cocrystals of Itraconazole drug with carboxylic acids show a wide range of solubilities ranging from 4-20 fold than that of the crystalline form of API.

![Figure 5.2](image)

**Figure 5.2** Dissolution profiles into 0.1 N HCl at 25 °C for Sporanox beads and the cocrystals
The carbamazepine (CBZ): saccharin cocrystal is also an appropriate example in this context. CBZ, commercially available as Tegretol, is another example of low soluble drug.\textsuperscript{142} The CZB:saccharin cocrystal has better solubility when compared to CBZ dihydrate and pure CBZ. The cocrystal even offers more stability (cocrystal is more stable than pure CBZ for more than 24hrs) to CBZ in the cocrystal. Figure 5.3 compares the plasma concentration of CBZ and the cocrystal in fated beagle dogs.

![Figure 5.3 Average plasma time curves of carbamazepine concentrations (±SEM) from a cross-over experiment in fasted beagle dogs (n = 4) given oral doses of 200 mg of the active drug as Tegretol tablets and co-crystal](image)

5.1 Dissolution experiments of quercetin and hesperetin cocrystals

Dissolution is a characterization test commonly used by the pharmaceutical industry to guide formulation design and control product quality.\textsuperscript{143} It is also the only test that measures the rate of in vitro drug release as a function of time, which can reflect either reproducibility of the product manufacturing process or, in limited cases, in vivo drug release. The common analytical methods used for quantifying drug release in dissolution tests are: spectrophotometric, chromatographic, mass spectrometric, and
The UV/VIS spectrophotometric method has been the traditional analytical method for dissolution testing. A compound will exhibit absorption in the UV region if it contains one or more chromophores, such as aromatic nitro, azoxy, nitroso, carbonyl, or azo groups. For quantitation of any given drug, the desired absorption (A) should be 0.3–1.0. In the UV absorption spectra of flavonoid, there are generally two main absorption bands, band I (300–400 nm) and band II (240–300 nm) which are associated with the absorption of the cinnamoyl system and the absorption of the benzoyl moiety in the molecules respectively. The two absorption bands are more sensitive to the environment of pH due to the weak acidity of the phenolic hydroxyl groups of flavonoid. In the UV absorption spectra of hesperetin in ethanol, the main absorption peak is assigned as band II, centered in about 290 nm, and there is only a weak broad shoulder peak in about 330 nm. The reason for this is because the B ring of hesperetin is not conjugated with the carbonyl group in ring C. Whereas quercetin has two bands at 255 nm and 374 nm respectively. The band II for quercetin is broader than band I and this is due to the conjugation of ring B with ring C.

As quercetin and hesperetin did not show any UV absorbance spectrum in water raised a situation to look for a suitable solvent in which these compounds are soluble and suitable for the dissolution studies. Most of the flavonoids are soluble in alcohols; therefore a mixture of solvent system consisting of 1:1 ethanol/water (V/V%) was adopted in order to carry out the dissolution experiments. Figure 5.4 represents UV spectrum of quercetin and hesperetin in 1:1 ethanol/water (V/V%) solvent system.
For cocrystals 2, 3, 4 the dissolution were carried out in 50 % EtOH/Water (v/v) mixture. In order to avoid the interference from methanol in the quercetin:caffeine:methanol cocrystal solvate, methanol/water solvent system was employed. A 3:2 methanol/water (V/V%) solvent system was used for the dissolution of cocrystal 1. The dissolution study for cocrystal 1 was carried at 400nm as caffeine did not absorb at this wavelength and did not interfere with the determination of concentration of quercetin. In the similar way the wavelengths for the dissolution of cocrystals 2, 3, 4 (cocrystal 2 = 360nm, cocrystal 3 & 4 = 300nm) were selected in such way that the interference of the cocrystal formers was not observed while determining the concentration of respective flavonoids. The standard calibration curves all the cocrystals were plotted by preparing the standard solutions of the cocrystals in their respective solvent systems. For the bulk powder dissolution of cocrystals 1-4 the powder was sieved using ASTM standard sieve in order to get the particle sizes
ranging between 53 - 75 µm. All the experiments were carried at room temperature in 3 sets for each cocrystal in order to have reliability. Approximately 900mg of the sample was added to the beakers containing 80mL of solvent mixture and the resulting slurry was stirred at 200 rpm with the help of spinning vane on a stir plate. At regular intervals of time an aliquot was drawn out from the beaker and it was filtered using a 0.25 µm nylon filter. Appropriate amount of the resulting solution was diluted with the solvent mixture and then the UV absorption was recorded.

5.2 Dissolution study of Quercetin:Caffeine cocrystal (Cocrystal 1)

The dissolution studies of cocrystal 1 (solvated and desolvated forms) in water did not show any improvements in the solubility of quercetin. Therefore, the dissolutions of methanol solvate of quercetin-caffeine cocrystal (KP05) and desolvated cocrystal were carried out in 3:2 methanol/water (V/ V %) solvent system. The Figure 5.4 represents the comparison of the dissolution of quercetin dihydrate with the methanol solvate and desolvated cocrystals. It is evident from the Fig 5.5 that there is an approximate 6-fold improvement in the solubility of quercetin in the methanol solvate cocrystal and 21-fold improvement in the desolvated cocrystal in 3:2 methanol/water (V/ V %) solvent system when compared to quercetin dihydrate. The solubility of quercetin in the desolvated cocrystal (amorphous form) has much better solubility than the solvated cocrystal in the first hour. But, later (after 1 hour) the desolvated cocrystal converts to the solvated cocrystal as the solubility goes down and coincides with the solvated cocrystal. The dissolution experiment demonstrates that the pure cocrystal has better solubility than the solvated cocrystal. Thus, cocrystallization can tailor the solubility of a compound and could afford to improve the bioavailability.
5.2 Dissolution study of Quercetin-Isonicotinamide (Cocrystal 2)

The dissolution quercetin-isonicotinamide cocrystal (KP10) was carried out in 1:1 ethanol/water (V/V%) solvent system as there was no improvement in the solubility of quercetin. It is clear from the Figure 5.5 that the solubility of quercetin in the cocrystal reaches the maximum value within the first five minutes and then the solubility goes down and gets almost close to that of quercetin dihydrate. The solubility of pure quercetin throughout the experiment is almost constant this indicates that the solubility of pure quercetin reaches the saturation limit within five minutes. The dissolution of quercetin cocrystal with isonicotinamide reveals that the solubility of quercetin has been improved 6-folds in 1:1 ethanol/water (V/V%) solvent system.
Figure 5.6 Dissolution profiles of quercetin and quercetin-isonicotinamide (1:1) cocrystal in 1:1 EtOH/Water (V/V%) solvent mixture

5.3 Dissolution study of Hesperetin-Isonicotinamide (Cocrystal 3)

The dissolution of cocrystal 3 (KP15) was also carried out in 1:1 ethanol/water (V/V%) solvent system as it did not showed any improvement the solubility in water. The solubility of pure hesperetin remains constant through out the time. But the solubility of hesperetin in the cocrystal increases slowly reaches a maximum value after 40 min and later it deteriorates down, if extrapolated probably could reach to the saturated limit similar to that pure hesperetin. Figure 5.6 represents the comparison of the concentrations of pure hesperetin and hesperetin in the cocrystal plotted against time. This indicates that the solubility of hesperetin has been improved 10-12 fold via cocrystallization in 1:1 ethanol/water (V/V%) solvent system.
5.4 Dissolution study of Hesperetin-NA (Cocrystal 4)

The dissolution experiment of cocrystal 4 (KP22) reveals that the solubility of hesperetin in cocrystal increases tremendously from zero to 3.5 mg/mL of 1:1 ethanol/water (V/V%) solvent system within five minutes of the experiment. In the due course of time the solubility of hesperetin (cocrystal) decreases reaches around 2 mg/mL after 3 hrs was carried out in 1:1 ethanol/water (V/V%) solvent system. The Figure 5.7 represents the comparison of the dissolution of hesperetin with its cocrystals with isonicotinamide and nicotinic acid. The NA cocrystal shows an approximate 5-fold increase in the solubility of hesperetin.
5.5 Discussions

The application of crystal engineering to the field of nutraceuticals has been demonstrated by synthesizing the cocrystals with pharmaceutically acceptable molecules. Incorporating pharmaceutically acceptable molecules with the desired target nutraceuticals provides an opportunity to tailor the solubilities and thereby generating more chances to improve the bioavailability. Crystal engineering plays a vital role in setting up the strategies for selecting appropriate nutraceutical:guest combinations that could form complementary hydrogen bonds and producing the novel cocrystals.

In order to check whether the cocrystallization has altered the solubility of hesperetin and quercetin it was important do the dissolution experiments for the pure flavonoids as well as the cocrystals. But owing to the poor solubility of hesperetin and quercetin in water has forced to adopt a different solvent for the dissolution of the cocrystals.
As most of the flavonoids show good solubility in alcohols, 1:1 ethanol-water solvent system was adopted. It is evident from the dissolution experiments that all the cocrystals of hesperetin and quercetin showed an improved solubility. Thus confirming the fact that cocrystallization offers a potential means to tailor the physical properties of the target molecules in the solid state. As the solubility and bioavailability are interdependent, cocrystallization could be a better alternative to tailor the solubility, in return the bioavailability.
6. CONCLUSIONS AND FUTURE DIRECTIONS

The study of crystal engineering remained as an understudied subject until Schmidt’s work on topochemical reactions\textsuperscript{15} popularized it. Later it expanded to larger areas like Supramolecular Chemistry.\textsuperscript{16} In the field of pharmaceutics crystal engineering has excelled greatly as it offers a wide range of application to the drug discovery. It has lead to the discovery of new forms of drugs as solvates, hydrates, polymorphs\textsuperscript{58} and cocrystals\textsuperscript{54} which allows handling problems pertaining to the physiochemical properties of drugs such as hygroscopicity, solubility and bioavailability.

In case most of the APIs and nutraceuticals these properties can be tailored by cocrystallizing pharmaceutically acceptable molecules. In this regard, crystal engineering proves to be an efficient tool. It directs the one to select appropriate cocrystal formers to generate cocrystals by exploring the non-covalent interactions such as hydrogen bonding.

The flavonoids have many benefits to the human health; the problem lies with their solubility and bioavailability that limits their usage.\textsuperscript{2} Therefore, flavonoids have been selected for the research work. The results herein demonstrate the wide range application of crystal engineering to generate new forms of quercetin and hesperetin. The utilization of crystal engineering to synthesize cocrystals has been demonstrated using the cocrystals of quercetin and hesperetin with pharmaceutically acceptable molecules like caffeine, nicotinic acid and isonicotinamide. The work also developed new solvent systems for dissolutions where, the dissolutions studies in water are not possible. The application of such solvent
systems may not be allowed pharmaceutically but could act as templates for other cocrystals as the results indicate increase in the solubility of the quercetin and hesperetin via cocrystallization. These results could be the subject of future contributions.
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APPENDICIES
Appendix A:

1. Crystallographic data and Experimental data for the new crystal forms of Quercetin

<table>
<thead>
<tr>
<th></th>
<th>Form I</th>
<th>Form II</th>
<th>Form III</th>
<th>Form IV</th>
<th>Cocrystal 1</th>
<th>Cocrystal 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
<td>C_{25}H_{20}N_{2}O_{7}</td>
<td>C_{25}H_{20}N_{2}O_{7}</td>
<td>C_{27}H_{26}O_{10}</td>
<td>C_{16}H_{10}O_{8.75}</td>
<td>C_{24}H_{24}N_{4}O_{10}</td>
<td>C_{21}H_{16}N_{2}O_{8}</td>
</tr>
<tr>
<td><strong>m.p. (°C)</strong></td>
<td>316 (decomp)</td>
<td>316 (decomp)</td>
<td>318 (decomp)</td>
<td>316 (decomp)</td>
<td>246</td>
<td>256-260</td>
</tr>
<tr>
<td><strong>Mol. Wt.</strong></td>
<td>460.43</td>
<td>460.43</td>
<td>510.48</td>
<td>351.25</td>
<td>528.47</td>
<td>424.36</td>
</tr>
<tr>
<td><strong>Crystal System</strong></td>
<td>Monoclinic</td>
<td>Triclinic</td>
<td>Triclinic</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
<td>Triclinic</td>
</tr>
<tr>
<td><strong>Space Group</strong></td>
<td>P2(1)/c</td>
<td>P-1</td>
<td>P-1</td>
<td>C2/c</td>
<td>P2(1)c</td>
<td>P-1</td>
</tr>
<tr>
<td><strong>A (Å)</strong></td>
<td>16.727(7)</td>
<td>10.188(2)</td>
<td>9.0830(3)</td>
<td>38.192</td>
<td>10.315(2)</td>
<td>4.9780(10)</td>
</tr>
<tr>
<td><strong>B (Å)</strong></td>
<td>17.564(8)</td>
<td>10.224(2)</td>
<td>10.858(3)</td>
<td>3.6344</td>
<td>14.853(4)</td>
<td>12.636(3)</td>
</tr>
<tr>
<td><strong>C (Å)</strong></td>
<td>6.919(3)</td>
<td>12.082(3)</td>
<td>13.063(3)</td>
<td>21.227</td>
<td>15.229(4)</td>
<td>15.571(3)</td>
</tr>
<tr>
<td><strong>A (°)</strong></td>
<td>90</td>
<td>104.167(4)</td>
<td>102.344(5)</td>
<td>90</td>
<td>90</td>
<td>110.53(3)</td>
</tr>
<tr>
<td><strong>B (°)</strong></td>
<td>91.456(12)</td>
<td>109.081(3)</td>
<td>93.516(4)</td>
<td>104.57(3)</td>
<td>100.667</td>
<td>97.63(3)</td>
</tr>
<tr>
<td><strong>Γ (°)</strong></td>
<td>90</td>
<td>107.775</td>
<td>111.149(4)</td>
<td>90</td>
<td>90</td>
<td>99.39(3)</td>
</tr>
<tr>
<td><strong>Volume (Å³)</strong></td>
<td>2032.2(14)</td>
<td>1046.3(4)</td>
<td>1255.5(5)</td>
<td>2851.7(10)</td>
<td>2292.9(4)</td>
<td>885.7(3)</td>
</tr>
<tr>
<td><strong>calc density (mg/cm³)</strong></td>
<td>1.505</td>
<td>1.461</td>
<td>1.350</td>
<td>1.636</td>
<td>1.531</td>
<td>1.591</td>
</tr>
<tr>
<td><strong>Solvent</strong></td>
<td>Pyridine</td>
<td>Pyridine</td>
<td>THF</td>
<td>Acetone</td>
<td>Methanol</td>
<td>Methanol</td>
</tr>
</tbody>
</table>

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1.1 Experimental data for Form I and Form II

DSC thermogram, FT-IR spectrum and X-ray powder diffraction patterns of bulk sample (black) calculated from the single crystal structure of Form I (green) and Form II (red) and TGA.
1.2 Experimental data for Form III

DSC thermogram, FT-IR spectrum and X-ray powder diffraction patterns of bulk sample (red) and calculated from the single crystal structure (black) and TGA.
1.3 Experimental data for Form IV

DSC thermogram, FT-IR spectrum and X-ray powder diffraction patterns of bulk sample (red) and calculated from the single crystal structure (black) and TGA.
1.4 Experimental data for Cocrystal 1

DSC thermogram, FT-IR spectrum and X-ray powder diffraction patterns of bulk sample (red) and calculated from the single crystal structure (black) and TGA.
1.5 Experimental data for Cocrystal 2

DSC thermogram, FT-IR spectrum and X-ray powder diffraction patterns of bulk sample (red) and calculated from the single crystal structure (black).
Appendix B

2. Crystallographic data and Experimental data of the new crystal forms of Hesperetin

<table>
<thead>
<tr>
<th></th>
<th>Cocrystal 3</th>
<th>Cocrystal 4 (Form I)</th>
<th>Cocrystal 4 (Form II)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
<td>C_{22}H_{20}N_{2}O_{7}</td>
<td>C_{21}H_{16}N_{2}O_{8}</td>
<td>C_{21}H_{16}N_{2}O_{8}</td>
</tr>
<tr>
<td><strong>m.p. (°C)</strong></td>
<td>172-176</td>
<td>198-204</td>
<td>256-260</td>
</tr>
<tr>
<td><strong>Mol. Wt.</strong></td>
<td>424.40</td>
<td>424.36</td>
<td>424.36</td>
</tr>
<tr>
<td><strong>Crystal System</strong></td>
<td>Monoclinic</td>
<td>Orthorhombic</td>
<td>Triclinic</td>
</tr>
<tr>
<td><strong>Space Group</strong></td>
<td>P21/c</td>
<td>P2_{12}1_{2}</td>
<td>P-1</td>
</tr>
<tr>
<td><strong>a (Å)</strong></td>
<td>25.653(10)</td>
<td>6.411(3)</td>
<td>6.7055(15)</td>
</tr>
<tr>
<td><strong>b (Å)</strong></td>
<td>5.129(2)</td>
<td>12.340(6)</td>
<td>11.494(3)</td>
</tr>
<tr>
<td><strong>c (Å)</strong></td>
<td>14.880(6)</td>
<td>23.418(3)</td>
<td>12.402(3)</td>
</tr>
<tr>
<td><strong>α (°)</strong></td>
<td>90°</td>
<td>90°</td>
<td>89.635(4)°</td>
</tr>
<tr>
<td><strong>β (°)</strong></td>
<td>103.384(7)°</td>
<td>90°</td>
<td>85.483(4)°</td>
</tr>
<tr>
<td><strong>γ (°)</strong></td>
<td>90°</td>
<td>90°</td>
<td>86.623(4)°</td>
</tr>
<tr>
<td><strong>Volume (Å³)</strong></td>
<td>1904.7(13)</td>
<td>1852.6(15)</td>
<td>951.3(4)</td>
</tr>
<tr>
<td><strong>calc density (mg/cm³)</strong></td>
<td>1.480</td>
<td>1.525</td>
<td>1.485</td>
</tr>
<tr>
<td><strong>Solvent</strong></td>
<td>Methanol</td>
<td>Methanol</td>
<td>Methanol</td>
</tr>
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</table>
2.1 Experimental data of Cocrystal 3

DSC thermogram, FT-IR spectrum and X-ray powder diffraction patterns of bulk sample (red) and calculated from the single crystal structure (black).
2.2 Experimental data of Cocrystal 4 (Form I and Form II)

DSC thermogram, FT-IR spectrum and X-ray powder diffraction patterns of bulk sample (red), calculated from the single crystal of Form I (black) and calculated from the single crystal of Form II (Green).