Crystal Engineering of Nutraceutical Cocrystals

by

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Dedication

For my husband, family and friends
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Crystal Engineering of Nutraceutical Cocrystals

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ABSTRACT

The work presented herein focus upon crystal engineering of nutraceutical cocrystals. Cocrystals are considered unique solid dosage form which has many advantages over other traditionally known solid forms. Furthermore, cocrystals have proven to improve stability, solubility and bioavailability of Active Pharmaceutical Ingredient (API) as shown in the case of carbamazepine and other APIs in previous studies.

Crystal engineering is commonly used to design new solid forms based on the bases of supramolecular chemistry. In this study, crystal engineering based on intensive Cambridge Structural Database (CSD) analysis used to predict and design new cocrystals of targeted nutraceuticals. Two nutraceuticals were selected for this study; resveratrol and citric acid. The rationale behind selecting resveratrol was to improve its solubility and, accordingly, bioavailability. On the other hand, citric acid is known as a highly soluble and safe nutraceutical, and thus it can be used as a coformer. Five new cocrystals were prepared and characterized using a variety of techniques that include single crystal X-ray
diffraction (XRD), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), FT-IR, and thermo-gravimetric analysis (TGA). Most of the reported cocrystals were obtained using different techniques; solvent slow evaporation, mechanichemical approach, slurry, and from melt. Moreover, dissolution test has been performed on resveratrol and two of its cocrystals, using UV-vis spectrophotometer, where the data demonstrate that through cocrystallization with different cocrystal formers, solubility of resveratrol could be greatly modified, and further controlled.

The polymorphism phenomenon is encountered, and accordingly addressed, herein where four novel polymorphs were obtained during cocrystallization attempts. Polymorphism has a significant importance in industry, in general, and in pharmaceutical industry, in particular, due to the vast differences in physical properties of polymorphs. Furthermore, the study of polymorphism provides valuable information essential to understand how different crystal forms are attained.
Chapter 1. Introduction

1.1. Crystal Engineering

The term “crystal engineering” was first introduced by Pepinsky¹ in 1955, but it was not widely employed until the pioneering works of Schmidt² in topochemical reactions of cinnamic acids. Later, Desiraju defined crystal engineering as “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”³.

Thus, crystal engineering is based on the concept of forming new solids with desired properties, using noncovalent interactions, such as hydrogen or ionic bonding. Understanding of these interactions can be employed to obtain novel molecular entities based on the elements of design in supramolecular chemistry.

The early beginnings of supramolecular chemistry started with works of Johannes Diderik van der Waals in 1873. However, it was not until 1987 when the field gained a lot of attention mainly due to the 1987 Nobel Prize in chemistry awarded to Jean-Marie Lehn, Donald J. Cram, and Charles J. Pedersen as a result of their works in supramolecular chemistry. Supramolecular chemistry is unique in that it relies only on relatively weak forms of interactions. Thus, no covalent interactions are involved in supramolecular chemistry. Therefore it is commonly known as “chemistry beyond the
molecules”. Of particular interest among non covalent interactions to supramolecular chemistry are hydrogen bonding, electrostatic interactions, metal coordination, and van der Waals interactions.

The large range of non-covalent intermolecular interactions include electrostatic interactions (ion-ion, ion-dipole and dipole-dipole interactions), coordination bonds, hydrogen bonds, halogen bonds, \(\pi-\pi\) stacking and Van der Waals interactions.

The concept of molecular-recognition could be considered to have emerged in 1890 when Fischer suggested "lock and key" model for enzyme-substrate interactions. However, the definition of hydrogen bonding could be traced back to Latimer and Rodebush in 1920.\(^4\)

One important aspect of the weak intermolecular interactions involved in supramolecular chemistry is the reversibility of the relatively weak bonding interactions involved. This allows molecules to self-assemble and further to self-correct the overall structure.

The term Self-assembly refers to processes in which a disordered system of pre-existing components forms an organized structure or pattern as a consequence of specific, local interactions among the components themselves, without external direction.

The molecular recognition between two molecules is accomplished through complimentary interactions of specific functional groups in the two molecules. Such specific complimentary interacting parts of the two molecules are usually referred to as supramolecular synthons. The term “synthon” was introduced by Corey in 1967.\(^5\)
A broader definition of supramolecular synthons made by Desiraju et al. where supramolecular synthons could be visualized as “structural units within supermolecules which can be formed and/or assembled by known or conceivable synthetic operations involving intermolecular interactions”.6

Supramolecular synthons can then be classified to either supramolecular homosynthons or supramolecular heterosynthons7,8. Supramolecular homosynthons are those synthons formed between the same functionalities, while supramolecular homosynthons are those formed between the different functionalities.

Examples of the two types are shown in Figure 1.1, where two functionalities are complementary to each other and can be assembled through two points of recognitions to form supramolecular homosynthons or heterosynthons.

![Figure 1.1. Supramolecular homosynthons (left) and supramolecular heterosynthons (right)](image)

Many case studies of supramolecular homosynthons or heterosynthons were recently presented in research. Examples of supramolecular homosynthons can be seen in carboxylic acid dimers10 and amide dimers.11 Supramolecular homosynthons can be seen in carboxylic acid···amide12, carboxylic acid···N\textsubscript{arom}13, and phenol···N\textsubscript{arom}.14
Years ago, Feynman\textsuperscript{15} asked his famous question “What would the properties of materials be if we could really arrange the atoms the way we want them?”. However, advances in field of crystal engineering might be the answer to that question.

In this context, we have to note that cocrystals are amenable for design using crystal engineering principles, which are based on supramolecular chemistry.

The research presented herein is based on exploiting the concept of donor-acceptor molecular recognition of complementary functional groups to form novel supermolecules, namely; nutraceutical cocrystals.

1.2. Cambridge Structural Database

As we discussed earlier, understanding of supramolecular interactions is the key to crystal engineering of novel crystalline entities which could be achieved through careful inspection of existing hydrogen bond interactions in previously reported supermolecules, hence facilitating the supramolecular retrosynthesis.\textsuperscript{16-18}

Cambridge Structural Database (CSD), the product of Cambridge Crystallographic Data Center (CCDC)\textsuperscript{19}, is invaluable tool to study a wide collection of reported crystal structures that enables identification of the occurrence frequency of distinct supramolecular synthons.

CCDC began its activities in 1965, led by Dr. Olga Kennard at Cambridge University, as a depository of crystal structures for organic and metal-organic small molecules studied by X-ray or neutron diffraction techniques. Since its beginning, the
number of reported crystalline structures is exponentially increasing over the years. The last reported number of entries is 423752, according to August 2008 update.

![Figure 1.2. Growth of the CSD since 1972](image)

Thus, CCDC is the center for archiving these crystal structures which could be easily visualized, classified and analyzed through an integral collection of software application impeded into the CSD program. This collection contains the following softwares: search and information retrieval (ConQuest), structure visualization (Mercury), numerical analysis (Vista), and database creation (PreQuest).

According to Kennard et al., about twenty years after the first release of CSD, “The systematic analysis of large numbers of related structures is a powerful research technique, capable of yielding results that could not be obtained by any other method”.

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5
Thus, CSD is considered as an invaluable research tool, which can be used for design and prediction of new crystalline entities.

1.3. Cocrystals

The term cocrystal is used to describe “a long known but little-studied”\(^{22}\) class of crystalline compounds.

For years, many terms have been used to describe cocrystals. Some of these terms are: molecular compounds\(^{23}\), addition compounds\(^{24}\), organic molecular compounds\(^{25}\), molecular complexes\(^{26}\), solid-state complexes\(^{27}\), and heteromolecular crystals.\(^{28}\)

Even recently, there is still a debate around the most appropriate name for this class of crystalline compounds.\(^{29,30}\)

However, Dr. Zaworotko research group defines cocrystal as “a multiple component crystal in which all components are solid under ambient conditions when in their pure form. These components co-exist as a stoichiometric ratio of a target molecule or ion and a neutral molecular cocrystal former(s)”.\(^{27}\) This definition makes clear difference between cocrystals and salts; where in cocrystal at least one of the molecules is neutral, while in slats both molecules are ionized. The definition also excludes hydrates and solvates.
Figure 1.3. Various possible crystalline forms for an API: (a) pure API; (b) polymorph of API; (c) clathrate hydrate/solvate of API; (d) hydrate/solvate of API; (e) salt of API; (f) pharmaceutical cocrystal

As we mentioned earlier, cocrystals are a long known class of crystalline compounds. This can be seen from works of Wohler in 1844, when he synthesized the first cocrystal between benzoquinone and hydroquinone. However, the crystal structure was determined later by Sakurai in the 1960’s.

Figure 1.4. Crystal structure of quinhydrone (CSD refcode: QUIDON)
The first scientific use of cocrystals was reported in work of Hoogsteen\textsuperscript{34,35}, when he studied the base pairing in the cocrystal of 9-methyladenine and 1-methylthymine, in the contest of DNA base pairing. However, cocrystals were popularized latter by work of Etter\textsuperscript{36}.

![Figure 1.5. The Hoogsteen base pairing in the cocrystal of 9-methyladenine and 1-methylthymine (CSD refcode: MTHMAD)](image)

The term “pharmaceutical cocrystals” is used to describe “A multiple component crystal in which at least one component is molecular and a solid at room temperature (the cocrystal former) and forms a supramolecular synthon with a molecular or ionic API”\textsuperscript{8}. Many pharmaceutical cocrystals are currently synthesized, where they represent a novel class of APIs, which has more advantages compared to traditionally known crystalline forms, such as; salts, solvates, hydrates, and polymorphs.

However, pharmaceutical cocrystals have shown to improve physical properties of APIs, such as solubility and therefore bioavailability. Two APIs\textsuperscript{37} and two of their cocrystals were studied in animal models.
Carbamazepine (Tegretol®) is an anti-epileptic agent, which has many drawbacks\textsuperscript{38,39}, such as; narrow therapeutic window, autoinduction of metabolism and dissolution-limited bioavailability, and high tendency to polymorphism. Tegretol® and carbamazepine: saccharin cocrystals\textsuperscript{9} were tested in dog model to compare carbamazepine bioavailability in both. As shown in figure 1.6., it is found that the cocrystal shows higher plasma levels than that of Tegretol®.

![Figure 1.6. Comparing average plasma concentrations versus time of carbamazepine in Tegretol® and that in carbamazepine: saccharin (cocrystal 1)\textsuperscript{37}](image)

Another case study\textsuperscript{40} was performed using 2-[4-(4-chloro-2-fluorophenoxy) phenyl] pyrimidine-4-carboxamide, a low solubility, sodium channel blocker. 2-[4-(4-chloro-2-fluorophenoxy) phenyl] pyrimidine-4-carboxamide and its glutaric acid
cocrystal were tested in dog model. As shown in figure 1.7, it is found that the cocrystal shows higher plasma levels than that of pure API.

![Graph comparing plasma concentrations](image)

**Figure 1.7. Comparing average plasma concentrations versus time of 2-[4-(4-chloro-2-fluorophenoxy) phenyl] pyrimidine-4-carboxamide and that in glutaric acid cocrystal**

It can be concluded from the above discussion that cocrystal is a promising class of crystalline drugs, which can afford some advantages over the other traditional forms.

1.4. *Crystal Forms of Drugs and Biopharmaceutical Classification System*

Over the years, different routes were developed for drug administration, such as oral, topical, and parenteral. Orally administered drugs constitute about 85% of the most sold drugs in the USA and Europe. Moreover, orally administered drugs are available in
different solid or liquid dosage forms amongst which oral solid dosage forms are the most common among other available dosage forms.

The active pharmaceutical ingredients (APIs) in solid dosage forms could either be present as amorphous or crystalline forms. As shown in figure 1.3., there are different possible crystalline forms for an API such as: pure API, polymorph, hydrate, solvate, salt, or cocrystal. In all of the aforementioned cases, crystallinity of API directly affects its physical properties, mainly solubility and dissolution rate.

The Biopharmaceutical Classification System (BCS) is a recently developed guidance which “correlates in vitro drug product dissolution and in vivo bioavailability”.

The BCS predicts gastrointestinal drug absorption based on dissolution and permeability data. In this system, two parameters, namely; solubility and permeability are used to classify oral drugs into four classes, as shown in figure 1.8.

![Figure 1.8. The four classes of APIs based on BCS](image)
Those two parameters are defined by FDA as following: a drug substance is considered HIGHLy SOLUBLE when the highest dose strength is soluble in \( \leq 250 \text{ ml} \) water over a pH range of 1 to 7.5. Furthermore, a drug substance is considered HIGHLy PERMEABLE when the extent of absorption in humans is determined to be \( \geq 90\% \) of an administered dose based on mass-balance or in comparison to an intravenous reference dose.

According to this classification system, about 70\% of commercially available drugs have inherently low aqueous solubility, which therefore limit their bioavailability. It becomes evident that drug bioavailability is mainly affected by its solubility, or more specifically, solubility of its solid form. Therefore, as cocrystal solid forms were demonstrated to enhance solubility of APIs, cocrystal solid forms represent interesting targets for studies concerning enhanced bioavailability of solid APIs.
Chapter 2. Nutraceutical Cocrystals

2.1. Introduction

For many years, food has been used for more than its nutritional value. It is well known that ancient civilizations used herbal products as a remedy for many diseases. The term “pharmacognosy” was first used by Schmidt in 1811, which is derives from the Greek words pharmakon (drug), and gnosis or "knowledge". It is defined latter by The American Society of Pharmacognosy to describe “the study of the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources.” However, formulation of a specific term to describe food or its active ingredients, which have medicinal values, was eagerly required.

The term ‘Nutraceutical’ was coined by Dr. Stephen De Felice, founder and chairman of Foundation of Innovation in Medicine, in 1976 to describe “food, or parts of food, that provide medical or health benefits, including the prevention and treatment of disease”.

It is believed that nutraceuticals can have a rule in preventing and treating many diseases; starting from common cold, weight problems and ending with cardio vascular diseases and even cancer.

Nutraceuticals include many food and food products, including vitamins, soy products, glucosamine, chondroitin, and many polyphenols and flavonoids (resveratrol,
ellagic acid, and quercetin). The vast majority of nutraceuticals are extracted from plant origins; such as fruits, vegetables, roots, and rhizomes. However, many nutraceuticals are derived from animal origins; vitamins and amino acids.

Nutraceuticals can be used as targets for cocrystal formation because many of them have major problems with solubility and bioavailability, and cocrystal can solve those problems, as discussed earlier in section 1.1.3. Furthermore, nutraceutical cocrystals are patentable as they meet the criteria required for patents.47

Nutraceuticals have a big market currently and annual growth rates of nutraceuticals are predicted to increase. This rate expected to be up to 25% for some products.44 However, nutraceutical cocrystals can offer an opportunity for exponential growth of nutraceuticals market.

2.2. Resveratrol

2.2.1. Introduction

Resveratrol \([\text{trans}-3,5,4'\text{-trihydroxystilbene}]\) is a nutraceutical, extracted mainly from skin of red grapes. It can also be obtained from other dietary sources as shown in table 2.1.
Table 2.1. Different sources of resveratrol

<table>
<thead>
<tr>
<th>Source</th>
<th>Trans-resveratrol concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red wines</td>
<td>0.1–14.3 mg/L</td>
</tr>
<tr>
<td>White wines</td>
<td>&lt;0.1–2.1 mg/L</td>
</tr>
<tr>
<td>Ports and sherries</td>
<td>&lt;0.1 mg/L</td>
</tr>
<tr>
<td>Grapes</td>
<td>0.16–3.54 µg/g</td>
</tr>
<tr>
<td>Dry grape skins</td>
<td>24.06 µg/g</td>
</tr>
<tr>
<td>Red grape juices</td>
<td>0.50 mg/L</td>
</tr>
<tr>
<td>White grape juices</td>
<td>0.05 mg/L</td>
</tr>
<tr>
<td>Cranberry juice</td>
<td>0.2 mg/L</td>
</tr>
<tr>
<td>Blueberries</td>
<td>32 ng/g</td>
</tr>
<tr>
<td>Peanuts</td>
<td>0.02–1.92 µg/g</td>
</tr>
</tbody>
</table>

Resveratrol attracted a lot of attention when Jang et al.\textsuperscript{46} published their paper in 1997, reporting anticarcinogenic activity of resveratrol. Since that significant discover, research on resveratrol biological activity has greatly increased, which is reflected as exponential increase in the number of scientific publications concerning resveratrol, as seen in figure 2.1.
Figure 2.1. PubMed search for word ‘resveratrol’ shows exponential increase in the number of resveratrol related publications over years\textsuperscript{48}

However, medical evidences have shown that resveratrol can participate in preventing and treating many diseases\textsuperscript{48} such as; cancer, cardiovascular diseases, myocardial infarction, brain damage…etc. It also plays an important role in supporting immunity and reducing stress.

There are many mechanisms\textsuperscript{48} proposed to participate in resveratrol biological activity. Anticarcinogenic activity originates from its antioxidant properties, in addition to its ability to inhibit cyclooxygenase (COX1 and COX2), ornithine decarboxylase, and angiogenesis. Moreover, resveratrol activity against heart diseases originates from its antioxidant properties, in addition to its ability to inhibit platelet aggregation and its vasodilation effect. Furthermore, various species treated with resveratrol had shown
lifespan extensions. Up to 70% increase in lifespan in some species was reported, but unfortunately this effect is unknown in mammals.

However, administer of enough quantity of resveratrol is required to get those beneficial effects. This means that resveratrol has to reach to certain blood level, i.e. it has to be bioavailable. Unfortunately, resveratrol bioavailability is low because of two factors. First, solubility of resveratrol is low as 0.019 mg/ml. Second, oral resveratrol undergoes first pass effect, thus, it is predisposed to metabolism by cytochrome P450. Hence, higher doses are required which has two main drawbacks. Toxic effects are reported upon administering 1 or more g per kg (body weight). Furthermore, estimated cost of administering enough doses is about $6,800 based on annual consumption of 2.7 kg.

Interestingly, Baur et al commented on this situation as following “Therefore, blocking the metabolism of resveratrol, developing analogues with improved bioavailability, or finding new, more potent compounds that mimic its effects will become increasingly important”. Hence, resveratrol is a valid target for cocrystallization and the following sections describe how cocrystals of resveratrol were designed and the subsequent observed effect(s) on resveratrol solubility.

However, CSD search for resveratrol reveals one crystal structure of trans-resveratrol, while no cocrystals were reported on CSD (August 2008 update).
2.2.2. CSD Analysis

As we discussed earlier in section 1.2.2., CSD search can serve as a tool to predict and design new cocrystals. Therefore, detailed CSD search conducted herein using Conquest v. 1.10, Aug 2008 update. Filters applied to the searches to limit the results according to the following criteria: 3D coordinates determined, R factor < 7.5%, no ions and only organics. The alcohols were excluded from the search due to the difference between acidity of phenols and alcohols which therefore affects their supramolecular interactions. The results are summarized in table 2.2. were raw data indicates results in presence of competitive hydrogen bonding groups while refined data is that in absence of any competitive groups.

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Raw data</th>
<th>Refined data</th>
<th>Bond distance range (Å)</th>
<th>mean σ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH···OH</td>
<td>1045/7069 (14.8%)</td>
<td>129/216 (59.7%)</td>
<td>2.504-3.069</td>
<td>2.817(3)</td>
</tr>
<tr>
<td>Primary amide</td>
<td>26/81(32%)</td>
<td>6/6 (100%) 6/6 (100%)</td>
<td>2.607-3.497</td>
<td>2.944(57)</td>
</tr>
<tr>
<td>OH···CONH₂</td>
<td>29/81(35.8%)</td>
<td>2.765-3.162</td>
<td>3.018(13)</td>
<td></td>
</tr>
<tr>
<td>OH···NH₂CO</td>
<td>6/6 (100%)</td>
<td>6/6 (100%)</td>
<td>2.765-3.162</td>
<td>3.018(13)</td>
</tr>
<tr>
<td>secondary amide</td>
<td>24/52 (46.1%)</td>
<td>10/10 (100%)</td>
<td>2.603-2.767</td>
<td>2.683(11)</td>
</tr>
<tr>
<td>OH···CO OH···NH</td>
<td>23/52 (44.2%)</td>
<td>7/10 (70%)</td>
<td>2.892-3.489</td>
<td>3.271(47)</td>
</tr>
<tr>
<td>OH···CO</td>
<td>619/1653 (37.5%)</td>
<td>162/247 (65.6%)</td>
<td>2.525-3.098</td>
<td>2.776(4)</td>
</tr>
<tr>
<td>OH···Nprom</td>
<td>338/595 (56.8%)</td>
<td>107/130 (82.3%)</td>
<td>2.515-3.114</td>
<td>2.750(3)</td>
</tr>
</tbody>
</table>

CSD Conquest v. 1.10, Aug 2008 update, organics only, 3D coordinates, R<7.5%, no ions
CSD search reveals 7069 entries with phenolic OH. In presence of competitive groups, 1045 (14.8%) entries exhibit OH···OH supramolecular homosynthon. The percentage increases to 59.7% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.2. and it reveals bond distance range of 2.504-3.069 Å (mean = 2.817(3) Å).

![Figure 2.2. Histogram for OH···OH homosynthon](image)

Furthermore, several CSD searches were carried out to investigate supramolecular heterosynthons. CSD search for structures with both phenolic OH and primary amide reveals 81 entries. In presence of competitive groups, 26 (32%) entries exhibit OH···CONH₂ supramolecular heterosynthons while 29 (35.8%) entries exhibit OH···NH₂CO supramolecular heterosynthons. The percentage increases to 100%, for both OH···CONH₂ and OH···NH₂CO, in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.3. and it reveals bond distance range of 2.607-3.497Å (mean = 2.944(57) Å) and 2.765-3.162 Å (mean = 3.018(13) Å), for OH···CONH₂ and OH···NH₂CO, respectively.
Another CSD search reveals 52 entries with both phenolic OH and secondary amide. In presence of competitive groups, 24 (46.1%) entries exhibit OH···CONH supramolecular heterosynthons while 23 (44.2%) entries exhibit OH···NHCO supramolecular heterosynthons. The percentages increase to 100% for OH···CONH and 70% for OH···NHCO, in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.4. and it reveals bond distance range of 2.603-2.767 Å (mean = 2.683(11) Å) and 2.892-3.489 Å (mean = 3.271(47) Å), for OH···CONH and OH···NHCO, respectively.
To investigate supramolecular heterosynthons between phenolic OH and aldehyde or ketone, carbonyl group is used as a representative for the two later groups. CSD search reveals 1653 entries with both phenolic OH and carbonyl group. In presence of competitive groups, 619 (37.5%) entries exhibit OH···CO supramolecular heterosynthons. The percentages increase to 65.6% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.5. and it reveals bond distance range of 2.525-3.098 Å (mean = 2.776(4) Å).
Another CSD search reveals 595 entries with both phenolic OH and aromatic nitrogen, \( N_{\text{arom}} \). In presence of competitive groups, 338 (56.8\%) entries exhibit OH\( \cdots N_{\text{arom}} \) supramolecular heterosynthons. The percentages increase to 82.3\% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.6. and it reveals bond distance range of 2.515-3.114 Å (mean = 2.750(3) Å).

![Figure 2.6. Histogram for OH\( \cdots N_{\text{arom}} \)](image)

2.2.3. Experimental

All the chemicals used during the preparation were obtained from commercial sources and used as received. All crystallization experiments were conducted in an unmodified atmosphere and the solvents were distilled prior to use.

2.2.3.1. Resveratrol-caprolactam cocrystals (DA182901 and DA182902)

22.8mg (0.100 mmol) of resveratrol and 22.6mg (0.200 mmol) of \( \varepsilon \)-caprolactam were dissolved in 5ml of hot acetone. The solution was allowed to slowly evaporate at room temperature and pale yellow crystals of DA182901 were harvested after one day
(yield = 36.2mg, 0.0796 mmol, 79.7%).

DA182901 can also be obtained through solvent drop grinding of trans-resveratrol (22.8mg, 0.100 mmol), ε-caprolactam (22.6mg, 0.200 mmol) and acetone (20µl) for 5 min in an agate pestle and mortar. DA182901 can also be obtained from slurry of 1:2 of trans-resveratrol and ε-caprolactam in acetone. (Melting point = 107°C).

After few weeks of first report of DA182901, crystals of DA182901 were transformed to DA182902 crystals (Melting point = 113°C). However, DA182901 were not obtained after appearance of DA182902 crystals.

![Image](image_url)

**Figure 2.7. Single crystal of resveratrol-caprolactam cocrystal (DA1829)**

### 2.2.3.2. Resveratrol-flavone cocrystals (RESVONE 01 and RESVONE02)

11.4 mg (0.050 mmol) of trans-resveratrol and 22.2 mg (0.100 mmol) of flavone were dissolved in 1ml of acetone to result in a clear solution. The solution was allowed to slowly evaporate at room temperature resulting in yellow glassy material and a white precipitate. Re-dissolving in hot 1ml of ethyl acetate then allowing the solvent to slowly evaporate at room temperature resulted in pale yellow crystals of RESVONE01 that were harvested after one day (yield = 22.5mg, 0.0334 mmol, 67%).
RESVONE01 can also be obtained from slurry of trans-resveratrol (114 mg, 0.500 mmol), flavone (222mg, 1.00 mmol)) and 2 ml acetone, which was stirred for five hours then filtered. The filtrate was allowed to slowly evaporate at room temperature to obtain crystals of RESVONE01.

RESVONE01 can also be obtained by grinding of trans-resveratrol (11.4mg, 0.050 mmol) and flavone (22.2mg, 0.100 mmol) for 5 min in an agate pestle and mortar using either 20µl of acetone, ethyl acetate or equal mixture of acetone and ethyl acetate. (Melting point = 160°C).

Crystals of RESVONE02 were reported during preparation of RESVONE01 crystals. RESVONE02 crystals appeared once and then disappeared. Efforts to prepare RESVONE02 resulted always in obtaining RESVONE 01 crystals.

Figure 2.8. Single crystals of resveratrol-flavone cocrystal (RESVONE01)

2.2.3.3. Resveratrol and 4,4'-dipyridyl cocrystal (DA4)

22.8 mg (0.100 mmol) of resveratrol and 15.6 mg (0.100 mmol) of 4,4'-dipyridyl were dissolved in 6ml of acetone by heating on a hotplate until a clear solution was
obtained. The solution was allowed to slowly evaporate at room temperature and pale yellow crystals of DA4 were harvested after one day (yield = 34mg, 0.0368, 88.5%).

DA4 can also be obtained through solvent drop grinding of 22.8 mg (0.100 mmol) of resveratrol, 15.6mg (0.100 mmol) of 4,4’-dipyridyl and acetone (10ul) for 10 min in an agate pestle and mortar. DA4 can also be obtained from slurry of resveratrol (228mg, 1 mmol), 4,4’-dipyridyl (156mg, 1 mmol) and 4 ml acetone. (Melting point = 236 °C).

![Single crystals of resveratrol and 4,4'-dipyridyl (DA4)](image)

Figure 2.9. Single crystals of resveratrol and 4,4'-dipyridyl (DA4)

2.2.4. Results and discussion

2.2.4.1. Resveratrol-caprolactam cocrystals (DA182901 and DA182902)

![Molecular structure](image)

Figure 2.10. The molecular structure of trans-resveratrol molecule shows labeled rings and phenolic OH groups

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The single crystal x-ray analysis of DA1829 crystals reveals that it forms a 1:2 ratio cocrystal between *trans*-resveratrol and ε-caprolactam. *Trans*-resveratrol molecules form head-to-tail chains through phenolic supramolecular homosynthons, OH···OH (D = 2.7905(12) Å). Each *trans*-resveratrol molecule is hydrogen bonded to three molecules of ε-caprolactam through the following synthons: OHₐ···CO, OHₐ···CO, and OHₐ···NH (D = 2.5725(13), 2.6587(12), and 2.9299(14) Å respectively). One of the ε-caprolactam molecules is further linked to another ε-caprolactam molecule to form a centrosymmetric dimer, NH···CO (D = 2.8871(14) Å). The whole structures shows extended sheets of *trans*-resveratrol and ε-caprolactam molecules. Those sheets are further extended to form three dimensional structures.

![Figure 2.11. Comparing the asymmetric unit of two polymorphs of 1:2 cocrystal of *trans*-resveratrol and ε-caprolactam, DA182901 (left) with that of DA182902 (right)](image)

The single crystal x-ray analysis of DA182902 crystals reveals that it forms a 1:2 ratio cocrystal between *trans*-resveratrol and ε-caprolactam. Each *trans*-resveratrol molecule is hydrogen bonded to four molecules of ε-caprolactam through the following
synthons: OH\textsubscript{a}···CO, OH\textsubscript{a}···NH, OH\textsubscript{b}···CO, OH\textsubscript{c}···NH and OH\textsubscript{c}···CO (D = 2.690(12),
2.992(12), 2.705(14), 2.896(13), and 2.639(14) Å). The whole structures shows extended
sheets of trans-resveratrol and ε-caprolactam molecules. Those sheets are further
extended to form three dimensional structures.

Figure 2.12. Comparison of hydrogen bonding in DA182901 (up) and DA182902 (down)

Comparing the two polymorphs, DA182901 shows both supramolecular
homosynthons and supramolecular heterosynthons in the same crystal structure. On the
other hand, DA182902 shows only supramolecular heterosynthons in its crystal structure.
Based on CSD search for phenols, OH···OH supramolecular homosynthons were observed in 14.78% of the entries in presence of other competitive groups. On the other hand, in absence of competitive groups, OH···CONH supramolecular heterosynthons were reported in 100% of the entries, while OH···NHCO were reported in 70% of the entries. Thus, crystal structure of DA182902 was more expected than that of DA182901. All observed hydrogen bonding distances are within the accepted range based on CSD search discussed in section 2.2.2.

There is also a major difference between the dihedral angles of the two aromatic ring planes of resveratrol. That dihedral angle is 15.41° in DA182901 while it becomes smaller as 5.89° in DA182902. The latter value is close to that angle in DALGON which is 5.33°.

This case of polymorphism originates from flexibility of trans-resveratrol molecule, thus, it can be considered as conformational polymorphism. At the same time the two polymorphs adopt different packing, so, it can also be considered as packing polymorphism.

Efforts to reproduce DA182901 crystals resulted in obtaining crystals of DA182902 which indicates that DA182902 is the most stable form. The difficulty to obtain DA182901 crystals may result from contamination by DA182902 seeds. This indicates that DA182902 is the thermodynamically favored while DA182901 is the kinetically more favored form.
2.2.4.2. Resveratrol-flavone cocrystals (RESVONE 01 and RESVONE 02)

Figure 2.13. Molecular structures of \textit{trans}-resveratrol molecule (left), and that of flavone (right)

The single crystal x-ray analysis of RESVONE01 crystals reveals a 1:2 ratio cocrystal between \textit{trans}-resveratrol and flavone, respectively. The structure exhibit molecules of \textit{trans}-resveratrol bridged through hydrogen bonding to flavone molecules, whereas flavone molecules aligned perpendicular to \textit{trans}-resveratrol molecules. The hydrogen bond interactions between \textit{trans}-resveratrol and flavone molecules occur through the phenolic OH and CO of resveratrol and flavone, respectively, with OH···CO distances of 2.715(11), 2.721(12), and 2.759(12) Å. The structure also contains two flavone molecules involved in $\pi$-$\pi$ interaction with a head-to-tail arrangement, centroid-to-centroid distance of 3.680 Å. Weak hydrogen bonding between phenolic OH and CH of \textit{trans}-resveratrol molecules are present, OH···CH (D = 3.413 and 3.498 Å).

Figure 2.14. Comparing the asymmetric unit of two polymorphs of 1:2 cocrystal of \textit{trans}-resveratrol and flavone, RESVONE01 (left) with that of RESVONE02 (right)
The single crystal x-ray analysis of RESVONE02 crystals reveals a 1:2 ratio cocrystal between trans-resveratrol and flavone, respectively. The hydrogen bond interactions between trans-resveratrol and flavone molecules occur through the phenolic OH and CO of resveratrol and flavone, respectively, with OH···CO distances of 2.773(6), 2.737(4), and 2.736(4) Å. The structure also contains two flavone molecules involved in π-π stacking with a head-to-tail arrangement, centroid-to-centroid distance of 3.675 Å. The overall structures show alternating molecules of trans-resveratrol and flavone whereas flavone molecules aligned perpendicular to trans-resveratrol molecules.

Figure 2.15. Comparison of hydrogen bonding in RESVONE01 (left) and RESVONE02 (right)

Figure 2.16. Comparison of hydrogen bonding pattern in RESVONE01 (left) and RESVONE02 (right)
It is observed that in both structures, oxygen ether did not involve in any hydrogen bonding interactions. Furthermore, phenolic supramolecular homosynthon did not observe in any of the two polymorphs. Thus, supramolecular heterosynthons were dominant over supramolecular homosynthons. This is consistent with CSD search for phenols which reveals that in absence of competitive groups, OH···CO supramolecular heterosynthons are observed in 65.59% of entries. Furthermore, OH···OH supramolecular homosynthons were not expected as their occurrence reported only in 14.78% entries, in presence of competitive groups. Moreover, all observed hydrogen bonding distances are within the accepted range based on CSD search discussed in section 2.2.2. Thus, the structures reported herein are considered to be expected based on CSD search.

This case of polymorphism can be explained in terms of packing and variations in dihedral angles because trans- resveratrol and flavone are both flexible molecules.

The dihedral angle of the two aromatic ring planes of trans- resveratrol is 7.46° in RESVONE01 while it becomes larger as 12.49° in RESVONE02. The earlier value is close to that angle in DALGON which is 5.33°.

On the other hand, the dihedral angles between chromone and phenyl rings, in flavone molecules, are also different in the two polymorphs. The angles values are 6.65° and 3.79° in RESVONE01. On RESVONE02, the values are 9.42° and 13.17°.

Since this case of polymorphism originates from flexibility of trans- resveratrol and flavone molecules, thus, it can be considered as conformational polymorphism. At
the same time the two polymorphs adopt different packing, so, it can also be considered as packing polymorphism.

Efforts to reproduce RESVONE02 crystals resulted in obtaining crystals of RESVONE01 which indicates that RESVONE01 is the most stable form. The difficulty to obtain RESVONE02 crystals may result from contamination by RESVONE01 seeds. This indicates that RESVONE01 is the thermodynamically favored while RESVONE02 is the kinetically more favored form.

2.2.4.3. Resveratrol and 4, 4’-dipyridyl cocrystal (DA4)

The single crystal x-ray analysis of DA4 crystals reveal that it forms a 2:3 ratio cocrystal between resveratrol and 4,4'-dipyridyl.

![Figure 2.17. The asymmetric unit of DA4](image)

Two sets of tetrameric units are formed between *trans*-resveratrol and 4,4'-dipyridyl molecules forming OH···N_{arom} supramolecular heterosynthons through the two phenolic OH groups of resveratrol located at meta position to each other and aromatic nitrogen atom of 4,4'-dipyridyl. These tetrameric units are linked through OH···N_{arom} hydrogen bond formed between the third phenolic OH group of resveratrol and aromatic
nitrogen atom of 4,4'-dipyridyl forming spiral tapes. The hydrogen bond distances between the phenolic OH···aromatic nitrogen (OH···N_{arom}) are as following: 2.804(5), 2.707(4), 2.850(5), 2.762(5), 2.725(5), and 2.726(5) Å.

![Figure 2.18. Two sets of tetrameric units in DA4](image)

It is observed that phenolic supramolecular homosynthons did not observe in DA4. Thus, supramolecular heterosynthons were dominant over supramolecular homosynthons. This is consistent with CSD search for phenols which reveals that in absence of competitive groups, OH···N_{arom} supramolecular heterosynthons are observed in 82.31% of entries. Furthermore, OH···OH supramolecular homosynthons were not expected as their occurrence reported only in 14.78% entries, in presence of competitive
groups. Moreover, all observed hydrogen bonding distances are within the accepted range based on CSD search discussed in section 2.2.2. Thus, DA4 crystal structure is considered to be expected based on CSD search.

Furthermore, the observed structure was expected based on Aoyama et al.\textsuperscript{50} and MacGillivray et al.\textsuperscript{51-53} work on [2+2] photodimerization in case of resorcinol and 4,4’-bpe derivatives, were OH···N\textsubscript{arom} supramolecular heterosynthons were occasionally observed.

**Figure 2.20. [2+2] photodimerization of 2 (4,4’-bpe) and 2(resorcinol)\textsuperscript{51}**

Based on Schmidt work, parallel olefins separated by < 4.2 Å should be used for photodimerization.\textsuperscript{54} However, 4,4’-dipyridyl molecules in DA4 are parallel and separated by 3.916Å and 3.764Å.

In fact, there are no olefins in the case presented herein. But the ability of this cocrystal to exhibit this topology and distances predict that cocrystallization of trans-resveratrol with other derivatives of 4,4'-dipyridyl may result in cocrystals which become suitable for photodimerization.
2.2.5. Dissolution test

As discussed earlier in section 1.4., biopharmaceutical classification system (BCS) considers aqueous solubility as one of the factors that directly affect drug bioavailability. However, the vast majority of APIs tend to have low aqueous solubility, therefore, improving their solubility is necessary to improve their bioavailabilities.

Aqueous solubility could be enhanced by particle size reduction, lyophilization, additives, and forming new crystalline forms such as new polymorphs, salts, or cocrystals.

Several studies of pharmaceutical cocrystals demonstrated that solubility of a particular API could be controlled via cocrystallization. Fluoxetine hydrochloride (Prozac®), a commonly used antidepressant, has been studied in this context. Aqueous dissolution rate of three cocrystals of fluoxetine HCl were determined and compared to that of fluoxetine HCl. As shown in figure 2.24., alteration of dissolution rate of fluoxetine HCl has been observed. Fluoxetine HCl: succinic acid cocrystal exhibits 2-fold increase in dissolution rate, while the rate decreases to the half in case of fluoxetine HCl: benzoic acid cocrystal. In addition, a slight increase in dissolution rate has been reported in case of fluoxetine HCl: fumaric acid cocrystal. Thus, it becomes evident that dissolution rate of an API can be modified, and further controlled, by cocrystallization. Furthermore, this case could be considered as an obvious illustration of how cocrystals can afford higher aqueous dissolution rates comparing to salts.
Another case study on dissolution rate was performed on itraconazole (Sporanox®), an antifungal drug with low aqueous solubility, which is marketed in the amorphous form. Figure 2.25. shows the dissolution profiles of itraconazole and four of its cocrystals, which reveals up to 20-fold increase of itraconazole dissolution rate, while cocrystallized with organic acids, compared to that of amorphous itraconazole.
However, no reports exist in current literature concerning dissolution studies of nutraceutical cocrystals, in general, and *trans*-resveratrol, in particular. Therefore, we report herein a dissolution study of *trans*-resveratrol and two of its cocrystals. UV-vis spectrophotometer was employed in this study due to presence of two aromatic rings in *trans*-resveratrol which represent the chromophores.

Since dissolution rate can greatly be affected by particle size, all samples were sieved using ASTM sieve in order to keep particle sizes between 53µm-75µm. Because of the inherent low solubility of resveratrol in water, aqueous medium was not suitable to conduct the intended investigation. Moreover, 25% ethanol: water solvent system resulted in poor dissolution of the solids. In a 50% aqueous ethanol (v/v), satisfactory dissolution profiles were obtained and thus this solvent system was employed in this study.

Since both *trans*-resveratrol and flavone have closely similar conjugated systems, i.e. chromophores, there was an interference between their UV absorption spectra. However, that interference was avoided by measuring absorbance at 345 nm where absorbance of *trans*-resveratrol only could be recorded in this region.

Calibration plot for *trans*-resveratrol was created using a range of known concentrations of *trans*-resveratrol and recording the corresponding absorbance at 345 nm. Furthermore, little shift in absorbance profile of *trans*-resveratrol was detected in case of its cocrystals, which could be explained as a result of interactions between *trans*-resveratrol and its coformers. Thus, additional calibration plots were created for each cocrystal where the concentration of resveratrol in each case is known based on the
composition in the single crystal structure. *Trans*-resveratrol constitutes 50.2 wt% and 34.0 wt% in the case of *trans*-resveratrol-caprolactam (DA182902) and *trans*-resveratrol-flavone (RESVON01) cocrystals, respectively.

All the experiments were carried out at 296 K, and three sets of each sample were performed to keep the accuracy.

Using stir bars, the samples were stirred at 125 rpm for four hours. Fixed amounts of the aliquot were drawn at various time intervals, and filtered using 0.25 mm syringe filters equipped with 0.45 µm nylon membranes. Proper dilutions were performed to obtain acceptable absorption values, and then UV absorbance was recorded. Finally, concentration of *trans*-resveratrol was calculated using calibration plots and dilution factors. Finally, dissolution profiles were created using time versus concentration of *trans*-resveratrol, as shown in figure 2.26.

![Resveratrol Dissolution](image)

**Figure 2.23. Dissolution profiles of *trans*-resveratrol and its cocrystals**

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The dissolution profiles indicate a 4-fold increase of trans-resveratrol dissolution rate in case of trans-resveratrol: caprolactam cocrystal (DA182902). On the other hand, trans-resveratrol: flavone cocrystal (RESVONE01) has a low dissolution rate.

This can be explained in terms of relative solubility\(^5^7\) and trends in melting points. Relative solubility could be calculated as a ratio of solubility of the cocrystal former to that of the drug in the same solvent system.

It is believed that substance with high melting point has strong self interactions, and therefore, it will not dissociate easily, and in other words, it will not dissolve easily. The opposite is true for low melting point substance.

It is found that relative solubility of trans-resveratrol: caprolactam cocrystal is higher than that of trans-resveratrol: flavone cocrystal. This is in agreement with trends in melting points, trans-resveratrol: flavone cocrystal is higher than that of trans-resveratrol: caprolactam cocrystal. These results are in agreement with Rodriguez-Hornedo et al.\(^5^7\)

In summary, cocrystallization could be used to control solubility of nutraceuticals, either by increasing or decreasing their solubilities.

\textbf{2.2.6. Conclusion}

The choice of trans-resveratrol as a target for cocrystallization attempts conducted in this study is justified due to the rapidly growing interest in current literature establishing desirable pharmacological properties of resveratrol and, equally important, the known poor solubility and bioavailability of resveratrol. The principles and concepts of crystal engineering were implemented in developing a suitable strategy to produce
cocrystals of resveratrol. Through a statistical analysis of CSD, phenols, in general, were found to be able to participate in hydrogen bond interactions forming supramolecular heterosynthons with a variety of functional groups including secondary amide, aromatic nitrogen, and carbonyl. Therefore, molecules that contain these functionalities were employed as cocrystal formers in the design strategy for resveratrol cocrystallization. Three cocrystals of trans-resveratrol are presented herein and it is found that they can be obtained by different cocrystallization techniques. Interestingly, two cases of polymorphism in cocrystals are reported herein. CSD search for polymorphic cocrystals shows only 40 cases, which represents 1.9% of the total number of cocrystals available on CSD (August 2008 update).

Moreover, dissolution test was performed on resveratrol and two of its cocrystals, using UV-vis spectrophotometer, where the data demonstrate that through cocrystallization with different cocrystal formers, dissolution rate of resveratrol could be greatly modified, and further controlled.

In conclusion, cocrystals are amenable to design and prediction using basics of crystal engineering and supramolecular chemistry, where CSD is considered as invaluable tool in this context. Overall, nutraceutical cocrystals represent viable and readily accessible crystalline forms to enhance, and further control, the solubility of nutraceuticals.
2.3. Citric acid

2.3.1. Introduction

Citric acid [2-hydroxy-1,2,3-propanetricarboxylic acid] is a nutraceutical, exists in a variety of fruits. However, it is mainly extracted from citrus fruits as it constitutes about 8% of their dry weight.\(^5\)

![Figure 2.24. Molecular structure of citric acid](image)

Because of its antioxidant properties, citric acid is widely used as a natural food preservative. It also plays an important role in biochemistry as an intermediate in citric acid cycle. However, citric acid has many uses and applications in industry, in general, and in pharmaceutical industry, in particular since it has a particular value as an excipient.\(^6\) It is widely used as flavoring and stabilizing agent in many pharmaceutical preparations. The characteristic effervescence of antacids is a result of combining bicarbonate or carbonates with citric acid. Moreover, citric acid is used in many cases to form salts of APIs.

Furthermore, U.S. Food and Drug Administration (FDA) consider citric acid as a safe substance; therefore, it is a member of EAFUS list (Everything Added to Food in the United States).\(^7\) It also has high LD50 (3000 mg/kg in rat). In addition to its safety, citric acid is considered to be highly soluble in water (1330mg/ml).
Thus, citric acid is safe, highly soluble, and abundant in low cost, therefore, it could be considered as a valid cocrystal former.

However, CSD search for citric acid reveals a two polymorphs of unhydrous citric acid and a crystal structure of citric acid monohydrate (CSD refcodes: CITRAC10, CITRAC11 and CITARC, respectively). Four crystal structures were reported as cocrystals formed between citric acid and the following cocrystal formers: 2,5-bis(4-Pyridyl)-1,3,4-oxadiazole, betaine, theophylline monohydrate, and caffeine (CSD refcodes: MEBRUH, XOBHIF, KIGKAN, and KIGKER). (CSD August 2008 update).

### 2.3.2. CSD analysis

As we discussed earlier in section 1.2.2., CSD search can serve as a tool to predict and design new cocrystals. Therefore, detailed CSD search conducted herein using Conquest v. 1.10. Filters applied to the searches to limit the results according to the following criteria: 3D coordinates determined, R factor < 7.5%, no ions and only organics. The search included acids and alcohols because they are the targeted functional groups in citric acid. Interestingly, both acids and alcohols can serve as hydrogen bond donors or acceptors. Phenols were excluded from the search for alcohols due to the difference between acidity of phenols and alcohols which therefore affects their supramolecular interactions. The results are summarized in tables 2.3. and 2.4. were raw data indicates results in presence of competitive hydrogen bonding groups while refined data is that in absence of any competitive groups.
2.3.2.1. CSD statistics for acids

Citric acid molecule contains three carboxylic acid groups. Those groups can serve as hydrogen bond donors, OH, and acceptors, CO. The results of CSD search for carboxylic acids are summarized in table 2.3. and it is based on 5690 entries.

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Raw data</th>
<th>Refined data</th>
<th>Bond distance (Å)</th>
<th>mean σ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH···N arom</td>
<td>457/607 (75.29%)</td>
<td>119/126 (94.44%)</td>
<td>2.510-2.827</td>
<td>2.652(2)</td>
</tr>
<tr>
<td>COOH···Cl</td>
<td>171/267 (64.04%)</td>
<td>51/51 (100%)</td>
<td>2.772-3.238</td>
<td>3.001(4)</td>
</tr>
<tr>
<td>COOH···P=O</td>
<td>68/122 (56%)</td>
<td>17/17 (100%)</td>
<td>2.417-2.852</td>
<td>2.579(6)</td>
</tr>
<tr>
<td>COOH···OH</td>
<td>OH···COOH 523/1176 (44.47%)</td>
<td>OH···HOOC 501/1176 (42.60%)</td>
<td>2.607-3.000</td>
<td>2.414-2.989</td>
</tr>
<tr>
<td></td>
<td>173/230 (75.22%)</td>
<td>186/230 (80.87%)</td>
<td></td>
<td>2.790(3)</td>
</tr>
<tr>
<td></td>
<td>2.607-3.000</td>
<td>2.414-2.989</td>
<td></td>
<td>2.790(3)</td>
</tr>
<tr>
<td>COOH···CONH₂</td>
<td>78/177 (44.07%)</td>
<td>10/19 (52.63%)</td>
<td>COOH···CONH₂ (2.501-2.713)</td>
<td>2.583(5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CONH₂···COOH (2.809-3.247)</td>
<td>2.957(9)</td>
</tr>
<tr>
<td>COOH···COOH</td>
<td>Dimers 1785/5690 (31.37%)</td>
<td>Catemers 156/5690 (2.74%)</td>
<td>2.536-2.983</td>
<td>2.649(1)</td>
</tr>
<tr>
<td></td>
<td>Dimers 384/474 (81%)</td>
<td>Catemers 35/474 (7.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.536-2.983</td>
<td>2.649(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOH···CO</td>
<td>147/597 (24.62%)</td>
<td>67/178 (37.64%)</td>
<td>2.451-2.863</td>
<td>2.689(5)</td>
</tr>
</tbody>
</table>

CSD Conquest v. 1.10, Jan 2008 update, organics only, 3D coordinates, R<=7.5%, no ions

CSD search reveals 607 entries with both COOH and N arom. In presence of competitive groups, 457 (75.29%) entries exhibit COOH···N arom supramolecular heterosynthons. The percentage increases to 94.44% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.28. and it reveals bond distance range of 2.510-2.827 Å (mean = 2.652(2) Å).
Another CSD search for structures contain both COOH and Cl\(^-\) reveals 267 entries. In presence of competitive groups, 171 (64.04%) entries exhibit COOH\(\cdots\)Cl\(^-\) supramolecular heterosynthons. The percentage increases to 100% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.29. and it reveals bond distance range of 2.772-3.238Å (mean = 3.001(4) Å).

Figure 2.25. Histogram for COOH\(\cdots\)N\(_{\text{arom}}\)

Figure 2.26. Histogram for COOH\(\cdots\)Cl\(^-\)
Another CSD search for structures with both COOH and P=O reveals 122 entries. In presence of competitive groups, 68 (56%) entries exhibit COOH···P=O supramolecular heterosynthons. The percentage increases to 100% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.30, and it reveals bond distance range of 2.417-2.852 Å (mean = 2.579(6) Å).

![Figure 2.27. Histogram for COOH···P=O](image)

Another CSD search for structures with both COOH and alcoholic OH reveals 1176 entries. In presence of competitive groups, 523 (44.47%) entries exhibit OH···COOH supramolecular heterosynthons while 501 (42.60%) entries exhibit OH···HOOC supramolecular heterosynthons. In absence of competitive functional groups, the percentage increases to 75.22% and 80.87%, for OH···COOH and OH···HOOC, respectively. The histogram for bond distance range is shown in figure 2.31, and it reveals bond distance range of 2.607-3.000Å (mean = 2.790(3) Å) and 2.414 Å-2.989 Å (mean = 2.655(3) Å), for OH···COOH and OH···HOOC, respectively.
Another CSD search for structures with both COOH and primary amide reveals 177 entries. In presence of competitive groups, 78 (44.07%) entries exhibit COOH···CONH$_2$ supramolecular heterosynthons. The percentage increases to 52.63%, in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.32. and it reveals bond distance range of 2.501-2.713 Å (mean = 2.583(5) Å) and 2.809-3.247 Å (mean = 2.957(9) Å), for COOH···CONH$_2$ and CONH$_2$···COOH, respectively.

Figure 2.29. Histogram for COOH···CONH$_2$ (left) and CONH$_2$···COOH (right)
For search of carboxylic acid supramolecular homosynthons, CSD search reveals 5690 entries with carboxylic acid. In presence of competitive groups, 1784 (31.35\%) entries exhibit acid dimers while 156 (2.74\%) entries exhibit acid catemers. In absence of competitive functional groups, the percentages increase to 81\% and 7.4\%, for acid dimers and catemers, respectively. The histogram for bond distance range is shown in figure 2.33, and it reveals bond distance range of 2.536-2.983 Å (mean = 2.649(1) Å).

![Histogram for carboxylic acid dimers](image)

**Figure 2.30. Histograms for carboxylic acid dimers**

To investigate supramolecular heterosynthons between carboxylic acids and aldehydes or ketones, carbonyl group is used as a representative for the two later groups. CSD search reveals 597 entries with both COOH and CO. In presence of competitive groups, 147 (24.62\%) entries exhibit COOH···CO supramolecular heterosynthons. The percentage increases to 37.64\% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.34, and it reveals bond distance range of 2.451-2.863 Å (mean = 2.689(5) Å).
2.3.2.2. CSD statistics for alcohols

Citric acid molecule contains one alcoholic OH. This group can serve as hydrogen bond donor and acceptor. The results of CSD search for carboxylic acids are summarized in table 2.4. and it is based on 25035 entries. Phenols were excluded from this search because the difference between acidity of alcohols and phenols, as discussed earlier.

Table 2.4. CSD statistics for alcohols

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Raw data</th>
<th>Refined data</th>
<th>Bond distance (Å)</th>
<th>mean σ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH···CT</td>
<td>389/529 (75.53%)</td>
<td>0</td>
<td>2.853-3.496</td>
<td>3.116(4)</td>
</tr>
<tr>
<td>OH···CONH₂</td>
<td>144/252 (57.14%) OH···CONH₂</td>
<td>50/54 (92.59%) OH···CONH₂</td>
<td>2.607-2.997</td>
<td>2.759(6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.990(6)</td>
</tr>
<tr>
<td>OH···CONH₂</td>
<td>141/252 (55.95%) OH···NH₂CO</td>
<td>49/54 (90.74%) OH···NH₂CO</td>
<td>2.737-3.198</td>
<td>2.990(6)</td>
</tr>
<tr>
<td>OH···CONH₂</td>
<td>141/252 (55.95%) OH···NH₂CO</td>
<td>49/54 (90.74%) OH···NH₂CO</td>
<td>2.737-3.198</td>
<td>2.990(6)</td>
</tr>
<tr>
<td>OH···NH₂CO</td>
<td>49/54 (90.74%) OH···NH₂CO</td>
<td>2.737-3.198</td>
<td>2.990(6)</td>
<td></td>
</tr>
<tr>
<td>OH···NH₂CO</td>
<td>49/54 (90.74%) OH···NH₂CO</td>
<td>2.737-3.198</td>
<td>2.990(6)</td>
<td></td>
</tr>
<tr>
<td>OH···P=O</td>
<td>202/368 (54.89%)</td>
<td>32/48 (66.67%)</td>
<td>2.429-2.899</td>
<td>2.714(4)</td>
</tr>
<tr>
<td>OH···N₂⁺</td>
<td>457/930 (49.14%)</td>
<td>70/102 (68.63%)</td>
<td>2.590-3.099</td>
<td>2.821(4)</td>
</tr>
<tr>
<td>OH···COOH</td>
<td>COOH···COOH</td>
<td>523/1176 (44.47%) COOH···HOOC</td>
<td>2.607-3.000</td>
<td>2.790(3)</td>
</tr>
<tr>
<td></td>
<td>COOH···COOH</td>
<td>523/1176 (44.47%) COOH···HOOC</td>
<td>2.607-3.000</td>
<td>2.790(3)</td>
</tr>
<tr>
<td></td>
<td>501/1176 (42.60%)</td>
<td>1694/3886 (43.59%)</td>
<td>552/851 (64.86%)</td>
<td>2.413-3.098</td>
</tr>
<tr>
<td>OH···CO</td>
<td>1694/3886 (43.59%)</td>
<td>552/851 (64.86%)</td>
<td>2.413-3.098</td>
<td>2.825(2)</td>
</tr>
<tr>
<td>OH···OH</td>
<td>5184/18475 (28.06%)</td>
<td>824/1055 (78.10%)</td>
<td>2.510-3.070</td>
<td>2.797(5)</td>
</tr>
</tbody>
</table>

CSD Conquest v. 1.10, Jan 2008 update, organics only, 3D coordinates, R<=7.5%, no ions
CSD search for structures contain both OH and Cl\textsuperscript{–} reveals 529 entries. In presence of competitive groups, 389 (75.53%) entries exhibit OH···Cl\textsuperscript{–} supramolecular heterosynthons. Interestingly, in absence of competitive functional groups, no entries with both OH and Cl\textsuperscript{–} were found. The histogram for bond distance range is shown in figure 2.35. and it reveals bond distance range of 2.853-3.496Å (mean = 3.116(4) Å).

![Figure 2.32. Histogram for OH···Cl\textsuperscript{–}](image)

Another CSD search for structures with both OH and primary amide reveals 252 entries. In presence of competitive groups, 57.14% of the entries exhibit OH···CONH\textsubscript{2} while 55.95% exhibit OH···NH\textsubscript{2}CO supramolecular heterosynthons. In absence of competitive functional groups, the percentages increase to 92.59% and 90.74%, for OH···CONH\textsubscript{2} and OH··· NH\textsubscript{2}CO, respectively. The histogram for bond distance range is shown in figure 2.36. and it reveals bond distance range of 2.607-2.997Å (mean = 2.759(6) Å) and 2.737 -3.198 Å (mean = 2.990(6) Å), for OH···CONH\textsubscript{2} and OH··· NH\textsubscript{2}CO, respectively.
Another CSD search for structures with both OH and P=O reveals 368 entries. In presence of competitive groups, 202 (54.89%) entries exhibit OH···P=O supramolecular heterosynthons. The percentage increases to 66.67% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.37 and it reveals bond distance range of 2.429-2.899Å (mean = 2.714(4) Å).

Another CSD search reveals 930 entries with both OH and N\textsubscript{arom}. In presence of competitive groups, 457 (49.14%) entries exhibit OH···N\textsubscript{arom} supramolecular heterosynthons. The percentage increases to 68.63% in absence of competitive functional
groups. The histogram for bond distance range is shown in figure 2.38. and it reveals bond distance range of 2.590-3.099Å (mean = 2.821(4) Å).

Figure 2.35. Histogram for OH···$N_{\text{arom}}$

To investigate supramolecular heterosynthons between alcoholic OH and aldehydes or ketones, carbonyl group is used as a representative for the two later groups. CSD search reveals 3886 entries with both OH and CO. In presence of competitive groups, 1694 (43.59%) entries exhibit OH···CO supramolecular heterosynthons. The percentage increases to 64.86% in absence of competitive functional groups. The histogram for bond distance range is shown in figure 2.39. and it reveals bond distance range of 2.413-3.098 Å (mean = 2.825(2) Å).

Figure 2.36. Histogram for OH···CO
CSD search reveals 18475 entries with alcoholic OH. In presence of competitive groups, 5184 (28.06%) entries exhibit OH···OH supramolecular homosynthons. The percentage increases to 78.10% in absence of competitive functional groups. Bond distance range is found to be 2.510-3.070 Å (mean = 2.780(5) Å).

2.3.3. Experimental

All the chemicals used during the preparation were obtained from commercial sources and used as received. All crystallization experiments were conducted in an unmodified atmosphere and the solvents were distilled prior to use.

2.3.3.1. Citric acid-iso-nicotinamide cocrystal (DA005)

38.4 mg (0.200 mmol) of citric acid and 73.2 mg (0.600 mmol) of iso-nicotinamide were dissolved in 5ml of hot methanol until a clear solution was obtained. The solution was allowed to slowly evaporate at room temperature and colorless crystals of DA005 were harvested after one day (yield = 106.8mg, 0.257 mmol, 95.7%). (Melting point = 148°C).

Figure 2.37. Single crystals of citric acid-iso-nicotinamide cocrystal (DA005)
2.3.3.2. Dihydrate of citric acid- iso-nicotinic acid cocrystal (DA02)

76.8mg (0.400 mmol) of citric acid and 49.2mg (0.400 mmol) of iso-nicotinic acid were dissolved in 5ml of methanol by heating on a hotplate, filtered and allowed to slowly evaporate at room temperature. Colorless crystals of DA02 were harvested after one day (yield = 114.5mg, 0.241 mmol, 90.9%). (Melting point = 187°C).

![Figure 2.38. Single crystals of citric acid- iso-nicotinic acid dihydrate cocrystal (DA02)](image)

2.3.4. Results and discussion

2.3.4.1. Citric acid- iso-nicotinamide cocrystal (DA005)

The single crystal x-ray crystal structure of DA005 indicates that molecules of iso-nicotinamide form hydrogen bonds with molecules of citric acid with a stoichiometry of 2:1. The asymmetric unit of DA005 contains two crystallographically independent iso-nicotinamide molecules and a citric acid molecule.

![Figure 2.39. The asymmetric unit of DA005](image)
Each molecule of citric acid is linked to three molecules of *iso*-nicotinamide, $\text{OH}_{\text{acid}} \cdots \text{N}_{\text{arom}}$ ($D = 2.521(3)$ Å) $\text{NH}_{\text{amide}} \cdots \text{CO}_{\text{acid}}$ ($D = 2.832(5)$ and $2.905(5)$ Å). There is further an intramolecular hydrogen bonding in citric acid molecules, $\text{OH}_{\text{acid}} \cdots \text{OH}_{\text{alcohol}}$ ($D = 2.902(2)$ and $2.937(4)$ Å).

Two *iso*-nicotinamide molecules form noncentrosymmetric amide dimer through, $\text{NH}_{\text{amide}} \cdots \text{CO}_{\text{amide}}$ ($D = 2.894(2)$ and $2.905(5)$ Å). Each of the aromatic nitrogen atoms of *iso*-nicotinamide are further included in a hydrogen-bond interaction with terminal carboxylic acid group of a citric acid molecule $\text{OH}_{\text{acid}} \cdots \text{N}_{\text{arom}}$ ($D = 2.521(3)$ and $2.625(2)$ Å) to form zigzag tapes. These tapes are connected through the middle carboxylic acid moieties of citric acid molecules.

![Figure 2.40. Crystal packing in DA005](image)

Based on CSD search discussed earlier, raw search is applicable in this case rather than refined search because we have four groups which can serve as hydrogen bonding donors or acceptors. Certainly, there are three functional groups which can act as hydrogen bonding donors or acceptors; COOH, alcoholic OH, and primary amide. On the other hand, $\text{N}_{\text{arom}}$ can act as hydrogen bonding acceptor only.
However, all interactions reported in DA005 were expected based on CSD search (raw data), and this can be explained as following: COOH···Narom supramolecular heterosynthons were observed in 75.29% of entries, COOH···CONH₂ were observed in 44.07% of entries, and COOH···OHₐlc were observed in 42.60% of entries. All observed hydrogen bonding distances are within the accepted range based on CSD search discussed in sections 2.3.2.1 and 2.3.2.2.

On the other hand, two supramolecular heterosynthons were not observed while they have chance to appear based on CSD search. Those are HOOC···OHₐlc (44.47%), and OHₐlc···Narom (49.14%).

In summary, COOH supramolecular heterosynthons were more expected than COOH···COOH supramolecular homosynthons, and this is the case reported herein.

2.3.4.2. Dihydrate of citric acid- *iso*-nicotinic acid cocrystal (DA02)

The single crystal x-ray structure analysis of DA02 reveals that the asymmetric unit contains a dihydrate of 1:2 cocrystal of citric acid and *iso*-nicotinic acid, where *iso*-nicotinic acid molecules are present in zwitterionic form.

![Figure 2.41. The asymmetric unit of DA02](image)
Each molecule of citric acid is linked to two molecules of *iso*-nicotinic acid, $\text{OH}_{\text{acid}}\cdots\text{CO}$ and $\text{OH}_{\text{alcohol}}\cdots\text{CO}$ ($D = 2.641(2)$ and $2.645(2)$ Å respectively). The indicated molecule of citric acid is further linked to three water molecules, $\text{OH}_{\text{acid}}\cdots\text{OH}_{\text{water}}$ ($D = 2.556(2)$ and $2.621(2)$ Å), $\text{OH}_{\text{alcohol}}\cdots\text{OH}_{\text{water}}$ ($D = 2.676(2)$ Å), and $\text{OH}_{\text{water}}\cdots\text{CO}_{\text{acid}}$ ($D = 2.991(2)$ Å). The two zwitterions of *iso*-nicotinic acid molecules constitute molecular tapes through the formation of charge assisted $\text{NH}^+\cdots\text{CO}$ supramolecular heterosynthons ($D = 2.668(2)$ and $2.676(2)$ Å). Such tapes are running in anti-parallel fashion and are connected by two water molecules, $\text{OH}_{\text{water}}\cdots\text{CO}$ ($D = 2.683(2)$ and $2.740(2)$ Å) and $\text{OH}_{\text{water}}\cdots\text{OH}_{\text{water}}$ ($D = 2.707(3)$ Å). The citric acid molecules connect such anti-parallel tapes through hydroxyl and carboxylic acid moieties forming sheets.

![Figure 2.42. Crystal packing in DA02](image)

This zwitterionic structure is based on charge-assisted hydrogen bonding rather than neutral hydrogen bonding interactions. This makes it a unique structure and thus, CSD search discussed earlier is not applicable to this case. It is known that charge-
assisted hydrogen bonding interactions are stronger than neutral hydrogen bonding interactions, which make distances shorter in the earlier case than the latter one. Thus, CO$^-$ is stronger than neutral CO and this explains dominance of charge-assisted hydrogen bonding over neutral hydrogen bonding in this crystal structure.

Furthermore, water molecules are able to act as hydrogen bonding donors or acceptors. Hence, they played important rule in sustaining the crystal structure as they involved in four out of eight of the hydrogen bonding interactions exhibited herein.

To confirm that iso-nicotinic acid molecules in DA02 are zwitterions, further study of the crystal structure has to be performed. In addition to IR spectroscopy, two measurements can be used for this purpose, C-O distances and C-N-C angles of pyridines.$^{61-66}$

![Figure 2.43. Comparison of the C-N-C angle of the pyridine and the C-O distances of the carboxylic acid Moieties in DA02 molecules (left) and iso-nicotinic acid (right)](image)

It is known that in neutral carboxylic acid, C-O distance is longer than that of C=O. In case of carboxylate, no C=O is present, and thus the distance of both C-O moieties are in between those of C-O and C=O. Moreover, C-N-C angle is larger in protonated pyridine than those of neutral ones.
Table 2.5. Comparison of the C-N-C angle of the pyridine and the C-O distances of the carboxylic acid Moieties in DA02 molecules (left) and iso-nicotinic acid (right)

<table>
<thead>
<tr>
<th>Analyzed crystallographic parameter</th>
<th>Zwitterionic molecules</th>
<th>iso-nicotinic acid molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-O distances</td>
<td>1.260 Å, 1.270 Å, 1.244 Å, 1.236 Å</td>
<td>1.215 Å, 1.294 Å</td>
</tr>
<tr>
<td>C-N-C angles</td>
<td>121.91°, 121.81°</td>
<td>118.91°</td>
</tr>
</tbody>
</table>

Furthermore, ΔpKa [pKa(base)-pKa (acid)] is traditionally used in pharmaceutical industry as an indicator of salt formation. It is known that ΔpKa >3 is usually associated with salt formation. If the value of ΔpKa is between 2 and 3, formation of either a salt or a cocrystal cannot be predicted. However, ΔpKa for citric acid and pyridyl moiety of iso-nicotinic acid = 4.88-2.93= 1.95 which does not meet that criterion for salt formation. However, it is clear that iso-nicotinic acid molecule has both acidic and basic moieties. Thus, it might tend to present as zwitterion. Moreover, we can also consider presence of COOH and Naron in para position as a factor which might stabilize the zwitterions in this structure.

However, CSD search for iso-nicotinic acid reveals one neutral crystal structure and there are no cocrystals of iso-nicotinic acid. Thus, no more information about iso-nicotinic acid cocrystals can be obtained.
2.3.5. Conclusion

Citric acid was chosen for cocrystallization based on many of its characteristics such as safety, low cost, high solubility, and its abundance in various pharmaceutical preparations. Two functionalities were used as a target for crystal engineering of citric acid; carboxylic acid and alcoholic OH. Therefore, CSD analysis was conducted and it suggested that functionalities like aromatic nitrogen and primary amide could be considered as viable candidates.

Two novel cocystals of citric acid were obtained and fully characterized using various analytical techniques. Interestingly, a hydrate of zwitterionic cocystal is reported, and this remarkable situation was confirmed by some crystallographic parameters.

In conclusion, cocrystals are amenable to design and prediction using basics of crystal engineering and supramolecular chemistry, where CSD is considered as an invaluable tool in this context.

2.4. Flavanone single crystal

Single crystal of flavanone [2,3-Dihydroflavone] is reported for first time during an attempt to cocrystallize trans-resveratrol and flavanone.

![Molecular structure of flavanone](image)

Figure 2.44. The molecular structure of flavanone
2.4.1. Experimental

112 mg (0.499 mmol) of flavanone were dissolved in 5ml of acetone and heated on a hotplate. The solution allowed to slowly evaporating at room temperature.

2.4.2. Result and discussion

The crystal structure of flavanone is monoclinic with one molecule of flavanone in the asymmetric unit.

![Figure 2.45. The asymmetric unit of flavanone](image)

The crystal structure does not show strong hydrogen bonding since flavanone does not have strong hydrogen bond donors, although some forms of weak hydrogen bonding were observed, \( \text{CH}\cdots\text{CO} (D = 3.437 \text{ and } 3.476 \text{ Å}) \) and \( \text{CH}\cdots\text{O} (D = 3.475 \text{ Å}) \). The structure sustained by \( \text{CH}-\pi \) interactions, \( \text{CH}\cdots\text{centroid} (D = 3.604 \text{ and } 3.834 \text{ Å}) \) which lead to herringbone pattern as shown in figure 2.49.
2.4.3. Conclusion

Single crystal of flavanone is reported for first time during an attempt to cocrystallize \textit{trans}-resveratrol and flavanone. In the crystal structure, weak hydrogen bond interactions, CH···CO, CH···O and electrostatic CH-π interactions are observed.

Flavanone could be considered as relatively safe; LD50 is 75 mg/kg (bird-wild bird species), therefore, it can be used as a cocrystal former.
Chapter 3. Polymorphism

3.1. Introduction

The term Polymorphism (Greek: *poly* = many, *morph* = form) was first introduced around 1822 by Mitscherlich, while the earliest report of the phenomenon is most probably found in the works of Klaproth (1788). Klaproth reported two different phases of calcium carbonate, calcite and aragonite. The phenomenon, for molecules and complexes, is closely related to allotropism, in elements, where different structural forms of the same element can exhibit quite different physical properties. Allotropism is best exemplified by diamond, graphite, and fullerenes, three allotropes of carbon, Figure 3.1.

![Allotropes of carbon](image)

Figure 3.1. Allotropes of carbon diamond (left), graphite (middle), and fullerene (right).
The stunning case of highest number of polymorphs is the case of ROY\textsuperscript{69,70} [5-Methyl-2-[(2-nitrophenyl) amino]-3-thiophencarbonitrile]. It named ROY because the crystal colors of its polymorphs are red, orange, and yellow. ROY which has eight polymorphs and it is an ideal example of conformational polymorphism as discussed in section 3.1.3.

![Figure 3.2. The molecular structure of a red polymorph of ROY (CSD refcode: CAXMEH03)](image_url)

A polymorph is defined by McCrone (1965) as “a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state”.\textsuperscript{71}

While this definition might imply that structures where molecules exhibit different conformations are not considered polymorphs, McCrone pointed out that polymorphs will be essentially the same in liquid and vapor states but different in crystal structure.\textsuperscript{72} However, this later “safe” definition of polymorphs can accommodate conformational polymorphs.
As the number of published crystal structures has grown exponentially since the original works of Klaproth, the number of polymorphs has also increased. According to McCrone, all compounds are polymorphic, and the number of known polymorphs of a single compound is proportional to time and effort spent researching this compound.71

Other terms related to polymorphism are commonly used in literature. One of these terms is “pseudopolymorphism”, which is used to describe solvates and hydrates. The use of this term was a matter of a recent scientific debate. Rogers70, Seddon73, and Bernstein74 opposed its use on the basis of being a misleading and an ambiguous term where they recommended journal editors to eliminate it from current usage. However, Desiraju69,75 argued that despite its ambiguity it has to be retained because it is too common and it has a scientific value. Nangia76 also agrees on retaining its usage as it is clear enough to all scientists and he pointed out its scientific and legal importance.

3.1.1. Significance of polymorphism to pharmaceutical industry

Polymorphism has significant applications in pharmaceutical industry. The choice of the right polymorph is critical during formulation of pharmaceutical preparations.77 As we pointed out earlier, polymorphs exhibit significantly different physical properties, it is widely recognized that different polymorphic forms of the same active pharmaceutical ingredient (API) exhibit different stabilities, solubilities, melting points, processabilities, bioavailabilities, particle flow, etc.77 Therefore, it becomes evident that physical properties of APIs can be optimized through control, and further design, of suitable polymorphs. A striking example for this phenomenon is demonstrated in the case of
paracetamol. Two polymorphs of paracetamol form I\textsuperscript{50} and II (Haisa 1974), reported by Haisa et al., figure 3.3., exhibit widely different compressibility. This difference in compressibility greatly affects processing of the drug, and thus represents a good example of the effect of polymorphism on targeted physical properties in API formulation. The difference in compressibility between the two polymorphs could directly be linked to the difference in the underlying packing patterns in the two polymorphs. In polymorph I, hydrogen-bonded paracetamol molecules crystallize in puckered hydrogen-bonded sheets, resisting compressibility as the sheets do not slip easily over each other. On the other hand, paracetamol molecules in form II exhibit flat sheets that can slip easily over each other, and thus demonstrate better compressibility.

![Figure 3.3. Paracetamol polymorphs, (left) form I showing puckered hydrogen-bonded sheets, and (right) form II showing flat sheets. (CSD refcodes: HXACAN01 and HXACAN08, respectively)](image)

Effect of polymorphism on bioavailability can be demonstrated by the case of chloramphenicol palmitate. Chloramphenicol is an antimicrobial agent which has a strong bitter taste and therefore it cannot be administered orally. Edgerton\textsuperscript{78} has synthesized a tasteless chloramphenicol palmitate which is a poorly soluble ester. It does not dissolve in
mouth while administered orally and it undergoes hydrolysis in the small intestine to form active chloramphenicol. The rate of ester hydrolysis, which is the rate of absorption determinant factor, depends on the selection of the right polymorph as chloramphenicol palmitate exhibits polymorphism.

Aguiar et al. carried out an experiment to study the absorption of two polymorphs of chloramphenicol palmitate A and B. Varied concentrations of A and B were administered orally by human volunteers and urine and blood samples were collected over a 24 hr period. Urinary excretion data are shown in Figure 3.4, which demonstrate that concentration of chloramphenicol equivalent increases as concentration of form B increases. Blood concentrations of free chloramphenicol were also plotted versus percent concentration of form B. This shows that chloramphenicol blood serum levels proportionally increases by increasing concentration of polymorph B and this linear relationship is shown in figure 3.5. This study reveals that API absorption is directly related to the type and concentration of the polymorph.

Figure 3.4. Chloramphenicol urinary excretion rates after oral administer of chloramphenicol palmitate with varying percents of polymorph B: M, 0%; N, 25%; O, 50%; P, 75%; and L, 100%

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One important aspect of polymorphism in pharmaceuticals is susceptibility of polymorphs to interconvert, where many consequences can result from conversion of the kinetically favored form to the thermodynamically stable form either during the manufacturing processes or upon storage (shelf life). This may result in precipitation of the low solubility stable form which may lead to pharmaceutically unacceptable preparations. Consequently, interconversion into the low solubility, thermodynamically stable, form may limit pharmacological utility of the API. Furthermore, as the solubility of API is greatly affected by particle size, precipitation and subsequent particle growth may lead to variations in particle size distribution in a given formulation, which subsequently affect the solubility of an active ingredient.

In their review, Haleblian and McCrone provided many examples for the consequences of phase interconversion in pharmaceutical industry. Considering parenteral preparations, precipitation may result in significant particle growth which may affect the syringibility of the product. Precipitation in suspensions might lead to caking,
which could be significant enough to affect the API uniformity and furthermore might prevent resuspension by shaking.

Other formulations as creams and suppositories can also significantly be affected by phase conversion. In those cases, it is essential to determine the solubility of the API in its vehicle as phase interconversion may result in nucleation which could lead to pharmaceutically and cosmetically unacceptable preparations.

In case of creams, use of an unsuitable polymorph might lead to phase conversion and precipitation of a more stable phase. Significant particle growth could arise in the vehicle yielding grainy particles, which might result in cosmetically unacceptable creams in which the active ingredient is unevenly distributed.

Some suppository bases are polymorphic and the selection of an appropriate polymorph could affect the melting characteristics of the preparation. The low melting phase may become softer or even liquefy during the shelf life. Being very soft may preclude the ability to administer that dosage form. On the other hand, formulation of suppositories with a high melting phase could result in a hard product which may not melt upon administration. This position could be demonstrated by Theobroma oil as a suppository base exhibiting polymorphism. The metastable $\alpha$-form has a melting point of 30°C, while the thermodynamically stable $\beta$-form has a higher melting point. Using the appropriate method of manufacture is essential to permit the crystallization of the more stable, higher melting point $\beta$-form.

From the above discussion, it can be seen that investigation of polymorphism and selection of the suitable polymorph is critical in pharmaceutical industry. Therefore,
polymorphism can be seen as an opportunity to tailor tune preparations. Since the thermodynamically stable form has lower solubility than the metastable form, retaining the metastable form can improve the efficacy of some pharmaceutical preparations.\textsuperscript{77}

### 3.1.2. Polymorphism and patents in pharmaceutical industry

As we mentioned previously, polymorphs are different in their crystal structures and thereby they can vary in their physical properties. Hence, discovery of a new polymorph of an API can be considered as an opportunity to improve its physical characteristics. Furthermore, the new form may be considered as a new invention and thus it might be awarded a patent.

There are three criteria to patent a new entity. It has to be novel, useful and non-obvious. New polymorphic form is novel as it has a distinct solid-state structure. It might be useful if it provides improved physical properties of a certain API. It is also non-obvious since many efforts to predict new polymorphic crystal structures are not successful so far.\textsuperscript{70}

According to the Oxford English Dictionary, patent is defined as “a license to manufacture, sell, or deal in an article or commodity, to the exclusion of other persons; in modern times, a grant from the government to a person or persons conferring for a certain definite time the exclusive privilege of making, using, or selling some new invention”. This definition implies that the purpose of the patent is to prevent other producers from making, using or selling the invention covered by the patent, and thereby many legal consequences can result upon patent infringement.
One of the well-documented legal cases of patented polymorphic drugs is the case concerning ranitidine hydrochloride (Zantac®) which is used for treatment of peptic ulcer. Ranitidine hydrochloride was patented directly after discovery in 1977 by Glaxo Group in its initial polymorph, later designated polymorph I.

![Molecular structure of ranitidine](image)

**Figure 3.6. Molecular structure of ranitidine**

Polymorph I was prepared by dissolving ranitidine base in methylated spirit containing hydrogen chloride followed by adding ethyl acetate to the solution. This process had several disadvantages. Hydrogen chloride is corrosive and supplied as compressed gas which is not easy to handle. Moreover, the solution of hydrogen chloride in methylated spirit and ethyl acetate has to be freshly prepared. Furthermore, since the industrial production requires preparation of large quantities of the hydrogen chloride in methylated spirit, handling the hydrogen chloride caused serious problems.\(^{85}\)

Consequently, Glaxo worked on developing alternative methods for ranitidine hydrochloride preparation. In 1980, Glaxo succeeded to prepare ranitidine hydrochloride using concentrated hydrochloric acid instead of hydrogen chloride gas and thus they avoided the consequences of using hydrogen chloride gas. The new procedure also allowed using a single solvent rather than the mixture of methylated spirit and ethyl acetate, which can be easily recovered after preparation. Furthermore, the product found to be less hygroscopic than the earlier one.
During characterization of the later product, it found to have different IR and XRPD than the earlier product and thus Glaxo concluded that it is a different polymorph of ranitidine hydrochloride, designated form II.

Given the advantages of form II over form I, Glaxo decided to use form II as the active ingredient of Zantac®. Appropriate commercial procedure for form II preparation has been developed and new patent application has been filled by Glaxo.

As Zantac® sales reached around $3.5 billion annually by 1991; other pharmaceutical firms looked forward to take a share of these profits by marketing form I of Zantac® in 1995, the expiration date of the first patent. Novopharm Ltd. chemists worked on preparation of form I but they ended with form II. Novopharm claimed that since form II produced by following the production procedure in the first patent, therefore, the product was always form II and the second patent is invalid. Thus, Novopharm sought to market form II and filled an abbreviated new drug application at the FDA. Novopharm notified Glaxo that the second patent is invalid and therefore Glaxo sued Novopharm for infringement of the second patent. Glaxo proved by evidence that following the procedure described in the first patent leading to form I rather than form II and thus, the court decided that the second patent is valid.

Thereafter, Novopharm worked faithfully and succeeded in producing form I of ranitidine hydrochloride, and filled an abbreviated new drug application. Glaxo sued Novopharm claiming that the product is a mixture of forms I and II, rather than a pure form I. Novopharm proved that the product is 99% form I with about 1% impurities
which may include form II and therefore the court allowed Novopharm to market the product.

From the above discussion it is clear that the consequences of obtaining a new form of polymorphic drug can go beyond the direct effects on physical properties to be a source of litigation.

3.1.3. Conformational polymorphism

The term conformational polymorphism was first introduced by Corradini (1973) to describe the phenomenon in which flexible molecules crystallize in different conformations. From conformational perspective, organic molecules can be seen as rigid or flexible. Rigid molecules can adopt packing polymorphism while flexible molecules might be able to adopt conformational polymorphism. This phenomenon can be explained in the light of the intramolecular rotation energy about single bonds. Since this energy is relatively low, 1-3 kcal mol$^{-1}$, molecules with torsional degrees of freedom can adopt several conformations in solutions and thus they might be able to crystallize in different conformations.

It is also important to notice that the activity of the biological molecules and APIs depends mainly on their conformations, thus, investigation of different conformational polymorphs is essential in drug development.
3.1.4. Disappearing polymorphs

Bernstein et al.\textsuperscript{89} used the term “disappearing polymorphs” to describe the situations when a particular polymorph is no longer obtained. This phenomenon is widely recorded in literature where it is difficult to reproduce a metastable form after nucleation of a thermodynamically more stable form.
The crystallization process composed of formation of nuclei followed by their
growth. The nucleation step is the determinant step in crystallization. One of the
commonly used techniques to induce growth of a certain polymorph is by intentional
seeding. In some cases seeding might happen unintentionally and lead to formation of
undesirable polymorph. This unintentional seeding may result from contamination with
seeds of the more stable form. The area affected by contamination might be small as a
one lab but it can expand to a town, country or in some cases it might lead to “universal
seeding”.

Since different forms of a polymorphic compound can be produced by variations
in some experimental conditions as temperature and pressure, Jacewicz and Nayler argued that disappearing of a polymorph is temporary and that a given form can reappear
by finding the appropriate experimental conditions. They concluded that “Any authentic
crystal form should be capable of being re-prepared, although selection of the right
conditions may require some time and trouble”.

The phenomenon of disappearing polymorphism can also be explained by
applying Ostwald rule of stages which explains how interconvert happens in case of
polymorphism. The rule states that during crystallization of a polymorphic compound,
the least stable form crystallizes first followed by more stable phases. The conversion of
the metastable form to the more stable form occurs step by step to result finally in
crystallization of the most stable form.
3.2. Experimental

All the chemicals used during the preparation were obtained from commercial sources and used as received. All crystallization experiments were conducted in an unmodified atmosphere and the solvents were distilled prior to use.

3.2.1. Salicylamide polymorph II (DA008)

![Molecular structure of salicylamide]

Figure 3.8. Molecular structure of salicylamide

13.7 mg (0.0999 mmol) of salicylamide and 112.8 mg (0.300 mmol) of riboflavin were dissolved in 5ml of methanol by heating on a hotplate, filtered and allowed to slowly evaporate at room temperature. The crystals of DA008 were obtained after two days.

3.2.2. Iso-nicotinamide polymorph III (DA16712)

![Molecular structure of iso-nicotinamide]

Figure 3.9. Molecular structure of iso-nicotinamide
36.6 mg (0.300 mmol) of iso-nicotinamide and 11.4 mg (0.0499 mmol) of *trans*-resveratrol were dissolved in 5ml of ethyl acetate by heating on a hotplate (60°C) for 12 hours followed by gradual cooling and allowed to slowly evaporate at room temperature. Crystals of DA16712 were obtained after one day.

3.2.3. *Trans*-resveratrol polymorph II (DA2116)

![Molecular structure of trans-resveratrol](image)

Figure 3.10. Molecular structure of *trans*-resveratrol

11.4 mg (0.0499 mmol) of *trans*-resveratrol were dissolved in 5ml of acetonitrile or ethyl acetate and heated on a hotplate. The solution allowed to slowly evaporating at room temperature. Crystals of DA2116 were obtained after one day.

3.2.4. Citric acid monohydrate polymorph II (C-azod)

76.8 mg (0.400 mmol) of citric acid and 23.2 mg (0.200 mmol) of azodicarbonamide were dissolved in 5ml of methanol by heating on a hotplate, filtered and allowed to slowly evaporate at room temperature. Crystals of C-azod were obtained after two days.
3.3. Results and Discussion

3.3.1. Salicylamide polymorph II (DA008)

Salicylamide [2-hydroxybenzamide] is an active ingredient in BC Powder®, which is over-the-counter pain relief product. The CSD search shows one crystal structure of salicylamide (SALMID) with no polymorphic structures. However the presence of the dihedral angle of the amide-benzene ring planes suggests that polymorphism of salicylamide may occur.

The crystals of DA008 were obtained during an attempt to prepare a cocrystal of riboflavin and salicylamide using methanol as a solvent. The crystal structure of DA008 consists of two crystallographically-independent molecules of salicylamide in the asymmetric unit. The two molecules differ mainly in the dihedral angle of the amide-benzene ring planes, 15.90° and 2.51°, in molecules A and B, respectively. The two molecules form noncentrosymmetric amide dimers with bond distances (NH$_2$···CO) of 2.867(6) and 2.910(6) Å. The amide molecules do not form catemers because the amide carbonyl is involved in intramolecular hydrogen bonding with the phenolic OH, OH$_a$···CO$_a$ (D = 2.511(5) Å) and OH$_b$···CO$_b$ (D = 2.497(5) Å), while the amide amine is involved in intermolecular hydrogen bonding with the neighbor phenolic OH, OH$_a$···NH$_2a$ (D = 2.891(6) Å). Chains of salicylamide molecules result from phenolic OH and amide NH$_2$ (D = 2.891(6) Å) intermolecular H-bond interactions of molecules A.
The structure shows sheets formed by amide dimers of molecules A and B. Molecules of A are further linked where π-π stacking occurs between the aromatic rings (face-to-face interactions) with interplanar distance of 3.480 Å. Further interactions towards neighboring sheets occur through CH-π interactions between molecules A and B, CH\textsubscript{a}···centroid\textsubscript{b} (D = 3.781 Å). Molecules B are further connected to molecules B in the other sheets through CH\textsubscript{b}···CO\textsubscript{b} with a distance of 3.267 Å. The overall hydrogen bonding results in sandwich herringbone pattern as represented in Figure 3.13.
Figure 3.12. Comparison of hydrogen bonding in DA008 (up) to that in SLMID (down)

The crystal structure of SLMID is monoclinic with one molecule of salicylamide in the asymmetric unit. The dihedral angle of the amide-benzene ring planes is 4.62°. The salicylamide molecules form centrosymmetric amide dimers with bond distances (NH$_2$···CO) of 2.940 Å. The amide molecules do not form catemers because the amide carbonyl is involved in intramolecular hydrogen bonding with the phenolic OH, OH···CO (D = 2.492 Å), while the amide amine is involved in intermolecular hydrogen bonding with the neighbor phenolic OH, OH···NH$_2$ (D = 2.812 Å). Chains of salicylamide molecules result from phenolic OH and amide NH$_2$ (D = 2.812 Å) intermolecular H-bond interactions of salicylamide molecules. The sheets are linked
through CH-\(\pi\) stacking, CH···centroid (\(D = 3.721\) Å) and through \(\pi\)-\(\pi\) stacking with interplanar distances of 3.261 and 3.346 Å. The overall hydrogen bonding results in herringbone pattern as shown in figure 3.16.

Figure 3.13. Illustration of the sandwich herringbone pattern in DA008 (left) and the herringbone pattern in SALMID (right)

Figure 3.14. Illustration of face to face interactions in DA008 (left) and that in SALMID (right)

The CSD search shows that 16.32% (39/239) of primary aromatic amide structures are polymorphic. This polymorphism originates mainly from their flexibility due to the dihedral angle of the amide-aromatic ring planes. Since polymorphism in most
of these cases result from variation in conformation rather than the packing, those structures exhibit conformational polymorphism. The histogram shown in figure 3.15 reveals the dihedral angle of polymorphs of primary aromatic amides and it shows 32 structures with minimum of 0.681°, maximum of 75.833°, and the mean is 24.944°. Furthermore, the CSD search for the dihedral angle of primary aromatic amides shows 228 structures with minimum of 0.034 and maximum of 86.334°, while the mean is 21.536°. The histogram of the dihedral angle is shown in figure 3.16. The dihedral angles of molecule B in DA008 and that of SALMID are within the range of majority of the CSD entries, while that of molecule A in DA008 does not locate in the average value. However, the dihedral angle of molecule A of DA008 is still has accepted value.

![Histogram for the dihedral angle of polymorphs of primary aromatic amides](image)

**Figure 3.15. Histogram for the dihedral angle of polymorphs of primary aromatic amides**
As mentioned earlier, the CSD search reveals 239 crystal structures with primary aromatic amides. 144 entries (60.25%) exhibit amide dimers with bond range of 2.705-3.189 Å (mean 2.927 Å). Amide catemers were observed in 177 entries (74.06%) with bond range of 2.596-3.242 Å (mean 2.940 Å). The histograms are shown in figures 3.17. and 3.18., respectively. This indicates that amide catemers are more dominant than amide dimers in primary aromatic amides. However, no amide catemers were observed in any of the two polymorphs because the amide carbonyl is involved in intramolecular hydrogen bonding with the phenolic OH. Hence, there are no available amide moieties to form catemers.

Further CSD analysis shows 26 structures with both primary aromatic amide and phenol. Only five structures do not contain any competitive hydrogen bonding donors or acceptors, namely HXBNZM, HXBNZM01, SALMID, SALMID01, and VIDMAX. The phenolic OH in all of those structures is bifurcated in hydrogen bonding with amide moieties forming OH···NH and OH···CO supramolecular heterosynthons which is also
observed in DA008 and SALMID. All hydrogen bonding distances are within the distance ranges discussed above and that discussed in Chapter 2. concerning phenol hydrogen bonding interactions.

Figure 3.17. Histogram for primary aromatic amide dimers

Figure 3.18. Histogram for primary aromatic amide catemers

Crystals of DA008 were obtained concomitantly with SALMID crystals, and they transformed later to SALMID crystals. Efforts to reproduce DA008 crystals resulted in obtaining crystals of SALMID which indicates that SALMID is the more stable form.
The difficulty to obtain DA008 crystals may result from contamination by SALMID seeds. This indicates that SALMID is the thermodynamically favored while DA008 is the kinetically more favored form according to Ostwald rule of stages. This hypothesis can be supported by the fact that DA008 has the highest Z’ value among the known structures of salicylamide.

This case of polymorphism originates from flexibility of salicylamide molecule, thus, it can be considered as conformational polymorphism. At the same time both polymorphs adopt different packing, so, it can also be considered as packing polymorphism.

3.3.2. Iso-nicotinamide polymorph III (DA16712)

Iso-nicotinamide [pyridine-4-carboxamide] is a nicotinamide analogue has shown that it can enhance the activity of antitumor drug, 2-amino-l,3,4-thiadiazole.

The CSD search shows two polymorphs of iso-nicotinamide (CSD refcodes: EHOWIH01 and EHOWIH02). Both structures are monoclinic and they differ in the dihedral angle of the amide-benzene ring planes, the number of molecules in the asymmetric unit and in their hydrogen bonding patterns.

The crystals of DA16712 were obtained during attempts to prepare cocrystal of trans-resveratrol and iso-nicotinamide using ethyl acetate as a solvent. The crystal structure of DA16712 is orthorhombic with one molecule of iso-nicotinamide in the asymmetric unit. The dihedral angle of the amide-benzene ring planes is 31.75°. The iso-nicotinamide molecules are extended through amide catemers with bond distances (NH₂···CO) of 3.031(3) Å. The iso-nicotinamide molecules are further forming head to
tail chains through amide NH₂···N_{arom} (D = 2.995(3) Å). The overall structure shows herringbone pattern sustained by CH-π interactions, CH···centroid (D = 3.664 Å).

Crystals of EHOWIH01 were prepared by recrystallization of iso-nicotinamide from methanol, nitrobenzene or nitromethane. The crystal structure of EHOWIH01 is monoclinic with one molecule of iso-nicotinamide in the asymmetric unit. The dihedral angle of the amide-benzene ring planes is 32.41°. The iso-nicotinamide molecules form centrosymmetric amide dimers with bond distances (NH₂···CO) of 2.9366(16) Å. The amide molecules are further extended through amide catemers with bond distances (NH₂···CO) of 2.9354(14) Å. The iso-nicotinamide molecules form extended sheets sustained by weak hydrogen bonding interactions between aromatic CH and adjacent aromatic nitrogen, CH···N_{arom} (D = 3.4275(19) and 3.4790(19) Å), and by π-π stacking with interplanar distances of 3.477Å.
Figure 3.20. Comparison of hydrogen bonding in DA16712 (up), EHOWIH01 (middle), and in assemblies composed of molecules A and B respectively in EHOWIH02 (down)

Crystals of EHOWIH02 were prepared by recrystallization of *iso*-nicotinamide from different solvents; ethanol, water, THF, dioxane, etc. The crystal structure of
EHOWIH02 is monoclinic with two molecules of iso-nicotinamide in the asymmetric unit. The two molecules differ mainly in the dihedral angle of the amide-benzene ring planes, 25.44° and 24.82°, in molecules A and B, respectively. Each of the two molecules give rise to an independent assembly, each contain the same hydrogen bonding pattern with subtle variations in bond distances. The iso-nicotinamide molecules are extended through amide catemers with bond distances NH$_{2a}$···CO$_a$ (D = 2.934(2) Å) and NH$_{2b}$···CO$_b$ (D = 2.947(3) Å). The iso-nicotinamide molecules are further forming head to tail chains through amide amine and aromatic nitrogen, NH$_{2a}$···N$_{aroma}$ (D = 2.982(3) Å) and NH$_{2b}$···N$_{aromab}$ (D = 2.974(3) Å). The overall structure shows herringbone pattern sustained by CH-π interactions, CH$_a$···centroid$_b$ (D = 3.902 Å) and by π-π stacking between B molecules with interplanar distances of 3.539 Å.

![Figure 3.21. Illustration of the herringbone pattern in DA16712 (left), sheets of EHOWIH01 (middle), and the herringbone pattern in EHOWIH02 (right)](image)

In contrast to salicylamide polymorphs, all three polymorphs of iso-nicotinamide exhibit amide catemers because there is no interference with such hydrogen bonding pattern. This can be predicted from CSD analysis discussed earlier in section 3.3.1. However, amide dimers were observed in EHOWIH01 in addition to amide catemers.
This is also anticipated since occurrence of amide dimers is reported in 60.25% of crystal structures of primary aromatic amides in CSD.

Further CSD analysis reveals 99 structures with both primary aromatic amide and aromatic nitrogen. 22 of those structures do not contain any competitive hydrogen bonding donors or acceptors, and 72.73% (16/22) exhibit amide NH\_2···N\_arom supramolecular heterosynthons. This is in accordance with reported structures of DA16712 and EHOWIH02. However, this was not the case in EHOWIH01 crystal structure. Overall, all hydrogen bonding distances and dihedral angles are located within the expected range based on the CSD search discussed earlier in section 3.3.1.

Efforts to reproduce DA16712 crystals resulted in obtaining crystals of EHOWIH01 which indicates that EHOWIH01 is more stable form. The difficulty to obtain DA16712 crystals may result from contamination by EHOWIH01 seeds. This indicates that EHOWIH01 is the thermodynamically favored while DA16712 is the kinetically more favored form.

This case of polymorphism originates from flexibility of iso-nicotinamide molecule, thus, it can be considered as conformational polymorphism. At the same time all polymorphs adopt different packing, so, it can also be considered as packing polymorphism.

3.3.3 Trans-resveratrol polymorph II (DA2116)

Trans-resveratrol [trans-3,5,4'-trihydroxystilbene] is a nutraceutical with antioxidant properties and many promising applications in medicine as discussed in
section 2.2.1. The CSD search shows one crystal structure of trans-resveratrol (DALGON) with no polymorphic structures. However the presence of the dihedral angle of the two aromatic ring planes suggests that polymorphism of trans-resveratrol may occur.

The crystal structure of DA2116 is monoclinic with two molecules of trans-resveratrol in the asymmetric unit. The dihedral angles of the two aromatic ring planes are 8.51° and 2.17°. Each A molecule of trans-resveratrol is hydrogen bonded to other six molecules of molecules B of trans-resveratrol through supramolecular homosynthons, OH\textsubscript{A}···OH\textsubscript{B}. Two of these molecules are almost on the same plane with the indicated trans-resveratrol molecule while the other four are aligned perpendicular to that plane. The bond distances are: OH\textsubscript{Aa}···OH\textsubscript{Bb} (D = 2.668(2) Å), OH\textsubscript{Aa}···OH\textsubscript{Bc} (D = 2.706(2) Å), OH\textsubscript{Ab}···OH\textsubscript{Ba} (D = 2.710(2) Å), OH\textsubscript{Ab}···OH\textsubscript{Bb} (D = 2.677(2) Å), OH\textsubscript{Ac}···OH\textsubscript{Ba} (D = 2.715(2) Å), and OH\textsubscript{Ac}···OH\textsubscript{Bc} (D = 2.709(2) Å). In the crystal structure, sheets of molecules A and B, alternatively stacked, with interplanar distance of 3.210 Å are observed.

The crystal structure of DALGON\textsuperscript{75} is monoclinic with one molecule of trans-resveratrol in the asymmetric unit. The dihedral angle of the two aromatic ring planes in DALGON is 5.33°. Each molecule of trans-resveratrol is hydrogen bonded to other six molecules of trans-resveratrol through supramolecular homosynthons, OH···OH. Two of these molecules are almost on the same plane with the indicated trans-resveratrol molecule while the other four are aligned perpendicular to that plane. The bond distances are: OH\textsubscript{a}···OH\textsubscript{b} (D = 2.685 Å), OH\textsubscript{a}···OH\textsubscript{c} (D = 2.687 Å), OH\textsubscript{b}···OH\textsubscript{b} (D = 2.727 Å), and
OH$_3$···OH$_3$ (D = 2.754 Å). The molecules are further connected through π-π stacking with interplanar distance of 3.305 Å.

**Figure 3.22. Comparison of hydrogen bonding in DA2116 (left) and that of DALGON (right)**

**Figure 3.23. Illustration of the packing in DA2116 (left) and that in DALGON (right)**

From the above discussion, it is clear that both DA2116 and DALGON adopt the same hydrogen bonding pattern with subtle variations in hydrogen bond distances. All hydrogen bonding distances are within the expected range based on CSD analysis discussed in section 2.2.2. However, the presence of OH···OH supramolecular homosynthons was expected due to the absence of any other moieties which can participate in hydrogen bonding. Furthermore, CSD search reveals that in absence of any other competing hydrogen bonding moieties, 59.72% out of 216 entries shows OH···OH supramolecular homosynthons.
Since *trans*-resveratrol is a flexible molecule, both structures differ mainly in their conformations, and this is an obvious example of conformational polymorphism.

### 3.3.4. Citric acid monohydrate polymorph II (C-azod)

Citric acid is a nutraceutical with antioxidant properties and many pharmaceutical and industrial applications as discussed in section 2.3.1. Citric acid can present as anhydrous [2-hydroxy-1,2,3-propanetricarboxylic acid] or monohydrate [2-hydroxy-1,2,3-propanetricarboxylic acid monohydrate]. However, CSD search reveals two polymorphs of citric acid anhydrate and only one structure of citric acid monohydrate. We record herein a new polymorph of citric acid monohydrate (C-azod).

The crystal structure of C-azod is triclinic with one molecule of citric acid and one molecule of water in the asymmetric unit. Each molecule of citric acid forms two distinct centrosymmetric acid dimers with two citric acid molecules, \(\text{OH}\cdots\text{CO} (D = 2.625(5)\ \text{and}\ 2.645(5)\ \text{Å})\). The indicated molecule is hydrogen bonded to two water molecules, acidic \(\text{OH}_{\text{acid}}\cdots\text{OH}_{\text{water}} (D = 2.675(5)\ \text{and}\ 3.035(8)\ \text{Å})\), and \(\text{OH}_{\text{alcohol}}\cdots\text{OH}_{\text{water}} (D = 3.024(7)\ \text{Å})\). There is intramolecular hydrogen bonding between \(\text{OH}_{\text{alcohol}}\cdots\text{CO}_{\text{acid}} (D = 2.903(8)\ \text{Å})\). The overall structure shows sheets of citric acid and water molecules linked to other sheets through the same hydrogen bonding patterns in addition to \(\text{OH}_{\text{alcohol}}\cdots\text{CO}_{\text{acid}} (D = 2.875\ \text{Å})\).

The crystal structure if CITARC is orthorhombic with one molecule of citric acid and one molecule of water in the asymmetric unit. Trimers formed of two citric acid molecules with one water molecule were observed, \(\text{OH}_{\text{acid}}\cdots\text{CO}_{\text{acid}} (D = 2.755\ \text{Å})\),
OH_{acid}···OH_{water} (D = 2.623 Å), and OH_{water}···CO_{acid} (D = 2.761 Å). That water molecule is further linked to the citric acid molecule in the next layer, OH_{acid}···OH_{water} (D = 2.695 Å). The alcohol moieties also share in connecting the layers, OH_{alcohol}···CO_{acid} (D = 2.765 Å) and OH_{alcohol}···OH_{water} (D = 2.768 Å). There is intramolecular hydrogen bonding between OH_{acid}···CO_{acid} (D = 3.020 Å). The overall structure shows sheets of citric acid and water molecules connected to other sheets through the same hydrogen bonding patterns.

Figure 3.24. Comparison of hydrogen bonding in C-azod (left) and that of CITARC (right)

Comparing the two structures, both structures form two dimensional sheets which extend to form three dimensional structures. But it is obvious that C-azod crystal structure is mainly controlled by acid dimers, while water molecules are present as guest molecules. On the other hand, water molecules in CITARC share with two molecules of citric acid to form trimers. However, based on CSD analysis discussed in section 2.3.2., acid dimer occurs in 31.35% of CSD entries in presence of other competing groups. Furthermore, OH_{alcohol}···CO_{acid} supramolecular heterosynthons is reported in 44.47% of CSD entries in presence of other competing groups. This supramolecular heterosynthons
observed in the two polymorphs of citric acid monohydrate. Moreover, CSD search reveals 5050 structures of organic hydrates, 43 (0.85%) of those structures exhibit polymorphism. On the other hand, polymorphism in single component structures is more common, 1.4% (1882/134,086).

Since the main difference between the two polymorphs is in their packing, this is considered as a case of packing polymorphism.

3.4. Conclusion

The four cases of polymorphism described in this chapter demonstrate two widely encountered types of polymorphism, namely conformational and packing polymorphism. Molecular flexibility in salicylamide, iso-nicotinamide, and trans-resveratrol resulted in isolation of conformational polymorphs while citric acid monohydrate exhibits packing polymorphism.

Generally, new polymorphs were obtained by serendipity, but systematic study of polymorphism can also be performed by varying some factors such as; temperature, pressure, solvent, and additives.

Polymorphism has a significant importance in industry, in general, and in pharmaceutical industry, in particular, due to the vast differences in physical properties of polymorphs. Furthermore, the study of polymorphism provides valuable information essential to understand how different crystal forms are attained.

Moreover, Aakeroy et al. argued that polymorphic compounds are considered as good cocrystal formers since they have synthons flexibility.
References


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Appendices
Appendix 1. Experimental data

The experiments were performed using DSC (TA instrument 2920), FT-IR (Nicolet Avatar 320 FTIR, solid state), X-ray powder diffraction (Bruker AXS D8, Cu radiation), and TGA (STM6000).

1.1. Experimental Data for DA182901

DSC thermogram, FT-IR spectrum, and X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up).
1.2. Experimental Data for DA182902

DSC thermogram, FT-IR spectrum, X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up), and TGA.
1.3. Experimental Data for RESVONE 01

DSC thermogram, FT-IR spectrum, X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up), and TGA.
1.4. Experimental Data for RESVONE 02

X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up).

1.5. Experimental Data for DA4

DSC thermogram, FT-IR spectrum, X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up), and TGA.
1.6. Experimental Data for DA005

DSC thermogram, FT-IR spectrum, X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up), and TGA.
1.7. Experimental Data for DA02

DSC thermogram, FT-IR spectrum, X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up), and TGA.
1.8. Experimental Data for flavanone

DSC thermogram, FT-IR spectrum, X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up), and TGA.
1.9. Experimental Data for DA16712

DSC thermogram and X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up).
1.10. Experimental Data for DA2116

DSC thermogram, FT-IR spectrum, X-ray powder diffraction patterns of bulk sample (down) and calculated from the single crystal structure (up), and TGA.
Appendix 2. Crystallographic data

2.1. Crystallographic data for *trans*-resveratrol-caprolactam polymorphic cocrystal (DA182901 and DA182902)

<table>
<thead>
<tr>
<th>Identification code</th>
<th>DA182902</th>
<th>DA182901</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C₂₆H₃₄N₂O₅</td>
<td>C₂₆H₃₄N₂O₅</td>
</tr>
<tr>
<td>Formula weight</td>
<td>454.55</td>
<td>454.55</td>
</tr>
<tr>
<td>Temperature</td>
<td>293(2) K</td>
<td>100(2) K</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Orthorhombic</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td><em>P</em>na2₁,</td>
<td><em>P</em>-1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unit cell dimensions</th>
<th>DA182902</th>
<th>DA182901</th>
</tr>
</thead>
<tbody>
<tr>
<td>a = 12.6665(10) Å, α = 90°</td>
<td>a = 8.1803(2) Å, α = 114.627(2)°</td>
<td></td>
</tr>
<tr>
<td>b = 8.2167(5) Å, β = 90°</td>
<td>b = 13.0557(3) Å, β = 106.532(2)°</td>
<td></td>
</tr>
<tr>
<td>c = 22.7749(19) Å, γ = 90°</td>
<td>c = 13.4915(5) Å, γ = 96.5990(10)°</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volume</th>
<th>2370.3(3) Å³</th>
<th>1209.76(6) Å³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z, Z'</td>
<td>4, 1</td>
<td>2, 1</td>
</tr>
</tbody>
</table>

Final R indices [I>2σ(I)]

<table>
<thead>
<tr>
<th>DA182902</th>
<th>DA182901</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁ = 0.0638, wR₂ = 0.1471</td>
<td>R₁ = 0.0403, wR₂ = 0.1239</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R₁ = 0.0954, wR₂ = 0.1729</td>
</tr>
</tbody>
</table>
2.2. Crystallographic data for *trans*-resveratrol-flavone polymorphic cocrystal (RESVONE 01 and RESVONE 02)

<table>
<thead>
<tr>
<th>Identification code</th>
<th>RESVONE01</th>
<th>RESVONE02</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical formula</strong></td>
<td>C_{44}H_{32}O_{7}</td>
<td>C_{44}H_{32}O_{7}</td>
</tr>
<tr>
<td><strong>Formula weight</strong></td>
<td>672.70</td>
<td>672.70</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>293(2) K</td>
<td>293(2) K</td>
</tr>
<tr>
<td><strong>Wavelength</strong></td>
<td>1.54178 Å</td>
<td>1.54178 Å</td>
</tr>
<tr>
<td><strong>Crystal system, space group</strong></td>
<td>Monoclinic, <em>P</em> 2(_1)</td>
<td>Orthorhombic, <em>P</em> c a 2(_1)</td>
</tr>
<tr>
<td><strong>Unit cell dimensions</strong></td>
<td>a = 7.8768(7) Å, (\alpha = 90^\circ)</td>
<td>a = 12.3620(5) Å, (\alpha = 90^\circ)</td>
</tr>
<tr>
<td></td>
<td>b = 23.796(3) Å, (\beta = 101.380(6)^\circ)</td>
<td>b = 19.0460(5) Å, (\beta = 90^\circ)</td>
</tr>
<tr>
<td></td>
<td>c = 9.2317(8) Å, (\gamma = 90^\circ)</td>
<td>c = 14.4410(8) Å, (\gamma = 90^\circ)</td>
</tr>
<tr>
<td><strong>Volume</strong></td>
<td>1696.4(3) Å(^3)</td>
<td>3400.1(2) Å(^3)</td>
</tr>
<tr>
<td><strong>Z, Z'</strong></td>
<td>2, 1</td>
<td>4, 1</td>
</tr>
<tr>
<td><strong>Goodness-of-fit on F(^2)</strong></td>
<td>1.004</td>
<td>0.946</td>
</tr>
<tr>
<td><strong>Final R indices [I&gt;2(\sigma)(I)]</strong></td>
<td>R1 = 0.0549, wR2 = 0.1224</td>
<td>R1 = 0.0445, wR2 = 0.0985</td>
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</tbody>
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2.3. Crystallographic data for *trans*-resveratrol and 4,4'-dipyridyl cocrystal(DA4)

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<th>Identification code</th>
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<tr>
<td><strong>Empirical formula</strong></td>
<td>C_{58}H_{48}N_{6}O_{6}</td>
</tr>
<tr>
<td><strong>Formula weight</strong></td>
<td>925.02</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>298(2) K</td>
</tr>
<tr>
<td><strong>Wavelength</strong></td>
<td>1.54178 Å</td>
</tr>
<tr>
<td><strong>Crystal system</strong></td>
<td>Triclinic</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>(P)-1</td>
</tr>
<tr>
<td><strong>Unit cell dimensions</strong></td>
<td>a = 9.4975(11) Å, (\alpha = 91.319(7)^\circ)</td>
</tr>
<tr>
<td></td>
<td>b = 10.3208(13) Å, (\beta = 95.689(6)^\circ)</td>
</tr>
<tr>
<td></td>
<td>c = 26.494(3) Å, (\gamma = 108.619(6)^\circ)</td>
</tr>
<tr>
<td><strong>Volume</strong></td>
<td>2444.8(5) Å(^3)</td>
</tr>
<tr>
<td><strong>Z, Z'</strong></td>
<td>2, 1</td>
</tr>
<tr>
<td><strong>Data / restraints / parameters</strong></td>
<td>7642 / 0 / 826</td>
</tr>
<tr>
<td><strong>Goodness-of-fit on F(^2)</strong></td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Final R indices [I&gt;2(\sigma)(I)]</strong></td>
<td>R1 = 0.0691, wR2 = 0.1817</td>
</tr>
</tbody>
</table>
2.4. Crystallographic data for flavanone single crystal

<table>
<thead>
<tr>
<th>Identification code</th>
<th>da2720_0m</th>
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</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C_{15}H_{12}O_{2}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>224.25</td>
</tr>
<tr>
<td>Temperature</td>
<td>293(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>1.54178 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P2_1/n</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td></td>
</tr>
<tr>
<td>a = 10.598(5) Å, α = 90°</td>
<td></td>
</tr>
<tr>
<td>b = 5.543(3) Å, β = 92.479(10)°</td>
<td></td>
</tr>
<tr>
<td>c = 19.105(9) Å, γ = 90°</td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>1121.3(9) Å³</td>
</tr>
<tr>
<td>Z, Z'</td>
<td>4, 1</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>0.795</td>
</tr>
<tr>
<td>Final R indices</td>
<td></td>
</tr>
<tr>
<td>[I&gt;2sigma(I)]</td>
<td>R1 = 0.0470, wR2 = 0.1072</td>
</tr>
</tbody>
</table>

2.5. Crystallographic data for citric acid- *iso*-nicotinamide cocrystal, DA005 (left)
and that of citric acid- *iso*-nicotinic acid cocrystal dihydrate, DA02 (right)

<table>
<thead>
<tr>
<th>Identification code</th>
<th>DA005</th>
<th>da02m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C_{18}H_{20}N_{4}O_{9}</td>
<td>C_{18}H_{22}N_{2}O_{13}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>416.22</td>
<td>474.38</td>
</tr>
<tr>
<td>Temperature</td>
<td>178(2) K</td>
<td>100(2) K</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>Cc</td>
<td>P-1</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a = 31.39(2) Å, α = 90°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b = 5.319(4) Å, β = 101.243(14)°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c = 11.762(8) Å, γ = 90°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>1926(2) Å³</td>
<td>1030.1(7) Å³</td>
</tr>
<tr>
<td>Z, Z'</td>
<td>4, 1</td>
<td>2, 1</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.352</td>
<td>0.984</td>
</tr>
<tr>
<td>Final R indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[I&gt;2sigma(I)]</td>
<td>R1 = 0.0830, wR2 = 0.1931</td>
<td>R1 = 0.0438, wR2 = 0.1057</td>
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</table>
Appendix 3. Comparing crystal structures of new polymorphs to that of known polymorphs

3.1. Comparing crystal structures of salicylamide polymorphs

<table>
<thead>
<tr>
<th>Dihedral angles</th>
<th>Salicylamide form I (SALMID)</th>
<th>Salicylamide form II (DA008)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.62°</td>
<td>15.90° and 2.51°</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hydrogen bonding</th>
<th>Amide dimers (2.940 Å), Intramolecular hydrogen bonding (2.492 Å), OH···NH$_2$ (2.812 Å), CH···π stacking (3.721 Å), π -π stacking (3.261 and 3.346 Å)</th>
<th>Amide dimers (2.867 and 2.911 Å), Intramolecular hydrogen bonding (2.512 Å and 2.497 Å), OH···NH$_2$ (2.891 Å), CH···π stacking (3.781 Å), CH$_b$···CO$_b$ (3.267 Å), π -π stacking (3.480 Å)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Space group</th>
<th>$I2/a$, monoclinic</th>
<th>$P2_1/n$, monoclinic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>$Z'$</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>a (Å)</td>
<td>12.920</td>
<td>6.483(5)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>4.980</td>
<td>15.735(11)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>21.040</td>
<td>12.632(9)</td>
</tr>
<tr>
<td>$a$ (°)</td>
<td>90.00</td>
<td>90.00</td>
</tr>
<tr>
<td>$β$ (°)</td>
<td>91.80</td>
<td>100.335</td>
</tr>
<tr>
<td>$γ$ (°)</td>
<td>90.00</td>
<td>90.00</td>
</tr>
<tr>
<td>Volume (Å$^3$)</td>
<td>1353.08</td>
<td>1267.68</td>
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</table>
3.2. Comparing crystal structures of *iso*-nicotinamide polymorphs

<table>
<thead>
<tr>
<th></th>
<th><em>Iso</em>-nicotinamide form I (EHOWIH01)</th>
<th><em>Iso</em>-nicotinamide form II (EHOWIH02)</th>
<th><em>Iso</em>-nicotinamide form III (DA16712)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dihedral angles</strong></td>
<td>32.41°</td>
<td>25.44° and 24.82°</td>
<td>31.75°</td>
</tr>
<tr>
<td><strong>Hydrogen bonding</strong></td>
<td>Amide catemers (2.935 Å), Amide dimers (2.937 Å), CH···N_{arom} (3.427 and 3.479 Å), π−π stacking (3.477 Å)</td>
<td>Amide catemers (2.935 and 2.947 Å), NH_{2···N_{arom}} (2.982 and 2.974 Å), CH-π interaction (3.902 Å), π−π stacking (3.539 Å)</td>
<td>Amide catemers (3.031 Å), NH_{2···N_{arom}} (2.995 Å), CH-π stacking (3.664 Å)</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td><em>P</em>\text{2}_1/c, monoclinic</td>
<td><em>P</em>\text{2}_1/c, monoclinic</td>
<td><em>P</em>\text{bca}, orthorhombic</td>
</tr>
<tr>
<td><strong>Z</strong></td>
<td>4</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Z’</strong></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>a (Å)</td>
<td>10.1756(11)</td>
<td>15.735(3)</td>
<td>10.1725(6)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>5.7319(6)</td>
<td>7.9976(18)</td>
<td>7.4507(6)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>10.0340(10)</td>
<td>9.885(3)</td>
<td>15.9013(9)</td>
</tr>
<tr>
<td>α (°)</td>
<td>90.00</td>
<td>90.00</td>
<td>90.00</td>
</tr>
<tr>
<td>β (°)</td>
<td>98.042(7)</td>
<td>105.586(17)</td>
<td>90.00</td>
</tr>
<tr>
<td>γ (°)</td>
<td>90.00</td>
<td>90.00</td>
<td>90.00</td>
</tr>
<tr>
<td>Volume (Å³)</td>
<td>579.483</td>
<td>1198.21</td>
<td>1205.2</td>
</tr>
</tbody>
</table>
3.3. Comparing crystal structures of *trans*-resveratrol polymorphs

<table>
<thead>
<tr>
<th></th>
<th><em>Trans</em>-resveratrol form I (DALGON)</th>
<th><em>Trans</em>-resveratrol form II (DA2116)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihedral angles</td>
<td>5.33°</td>
<td>8.51° and 2.17°</td>
</tr>
<tr>
<td>Hydrogen bonding</td>
<td>OH···OH (2.685, 2.687, 2.727, and 2.754 Å), π−π stacking (3.305 Å)</td>
<td>OH···OH (2.668, 2.706, 2.710, 2.717, 2.715, and 2.709 Å), π−π stacking (3.210 Å)</td>
</tr>
<tr>
<td>Space group</td>
<td><em>P 2_1/c</em>, monoclinic</td>
<td><em>P 2_1/n</em>, monoclinic</td>
</tr>
<tr>
<td>Z</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Z’</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>a (Å)</td>
<td>4.3791(5)</td>
<td>8.6551(3)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>9.2158(11)</td>
<td>9.1847(3)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>26.681(3)</td>
<td>26.6412(9)</td>
</tr>
<tr>
<td>α (°)</td>
<td>90.00</td>
<td>90.00</td>
</tr>
<tr>
<td>β (°)</td>
<td>92.748(2)</td>
<td>93.090(2)</td>
</tr>
<tr>
<td>γ (°)</td>
<td>90.00</td>
<td>90.00</td>
</tr>
<tr>
<td>Volume (Å³)</td>
<td>1075.52</td>
<td>2114.75(12)</td>
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</table>
3.4. Comparing crystal structures of citric acid monohydrate polymorphs

<table>
<thead>
<tr>
<th>Identification code</th>
<th>Citric acid monohydrate form I (CITARC)</th>
<th>Citric acid monohydrate form II (C-azod)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen bonding</td>
<td>Acid dimers (2.625 and 2.645 Å), OH\text{acid}···OH\text{water} (2.675 and 3.035 Å), OH\text{alcohol}···OH\text{water} (3.024 Å), OH\text{alcohol}···CO\text{acid} (2.903 and 2.875 Å)</td>
<td>OH\text{acid}···CO\text{acid} (2.755 and 3.020 Å), OH\text{acid}···OH\text{water} (2.623 and 2.695 Å), OH\text{water}···CO\text{acid} (2.761 Å), OH\text{alcohol}···CO\text{acid} (2.765 Å), OH\text{alcohol}···OH\text{water} (2.768 Å)</td>
</tr>
<tr>
<td>Space group</td>
<td>$P\text{ 2}_1\text{ 2}_1\text{ 2}_1$, orthorhombic</td>
<td>$P\text{-1}$, triclinic</td>
</tr>
<tr>
<td>$Z$</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>$Z'$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>a (Å)</td>
<td>6.297(3)</td>
<td>6.781(3)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>9.319(3)</td>
<td>7.630(4)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>15.398(3)</td>
<td>8.788(4)</td>
</tr>
<tr>
<td>$\alpha$ (°)</td>
<td>90</td>
<td>93.041(10)</td>
</tr>
<tr>
<td>$\beta$ (°)</td>
<td>90</td>
<td>106.201(8)</td>
</tr>
<tr>
<td>$\gamma$ (°)</td>
<td>90</td>
<td>100.819(10)</td>
</tr>
<tr>
<td>Volume (Å$^3$)</td>
<td>903.581</td>
<td>426.1(4)</td>
</tr>
</tbody>
</table>