Crystallization Studies of Epigallocatechin Gallate

by

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Dedication
To Sadguru Shivananda Murthy Garu, and my Family
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ABSTRACT

Flavonoids are a long and well-known class of natural products. Their potential health benefits can be attributed to their antioxidant activity, and modulation of cell signaling pathways. Green tea, one of the most widely consumed beverages, consists of flavonoids such as catechins and tannins. Epigallocatechin gallate (EGCG) is the major catechin of green tea and exhibits multiple health benefits due to its antioxidant nature. The radical scavenging activity of EGCG is attributed to its structure. Therefore, a study on molecular features of EGCG would provide valuable information on structural modifications, which may change the physiochemical properties such as bioavailability and solubility.

Although flavonoids are abundant and commercially available, they are difficult to purify and crystallize. In this respect, crystallizing EGCG was challenging. By exploring different techniques, EGCG was crystallized. Here in this study, one new form of EGCG and two solvates, acetonitrile and nitrobenzene, have been synthesized and structurally characterized by differential scanning calorimetry (DSC), infrared (IR) and powder X-ray diffraction (PXRD). The crystal structures were solved by single-crystal X-ray diffraction and a detailed description of synthesis and about the supramolecular synthons that exist in these crystal forms are given.
1. INTRODUCTION

1.1 Flavonoids

1.1.1 History

Flavonoid compounds, which are ubiquitous in nature, occupy a prominent position among the plant phenols. Though flavonoids are present in other parts of plants, many flavonoids are easily recognized as flower pigments in most angiospermic plants. Flavonoids are distributed in higher plants, bacteria, algae, fungi, and animals. The earliest suggestion that substances existed in nature that would eventually be recognized as flavonoids can be traced back to the 17th century. In 1682 Nehemiah Grew discussed the differences between the solubility properties of pigments, which can be recognized as the starting point for naturally occurring compounds. Flavonoids are responsible for coloration of the plants. Autumn coloration of leaves has attracted the attention of many workers for years. Anthocyanin pigments have played a major role in the history of naturally occurring compounds.

The term “flavone” first appeared in a paper by Kostanecki and Tambor (1895). In 1893 De Larie and Tiemann obtained the first isoflavone from the rhizomes of “Iris Florentina”. The first flavanone recognized as naturally occurring was butrin and the first isoflavanoid discovered structurally is “Prunetin” described by Finnamore (1910).
St. Von Kostanecki first established the basic structure of the flavones and synthesized a number of natural compounds. In a continuation to these studies, Herzig and A.G Perkin determined the positions of attachment of sugar residues in the glycosides. Out of all the flavonoids, anthocyanins are the first compounds which were studied in detail. This is due to their wide abundance in nature as a coloring compound in plants.

1.1.2 Structure

Flavonoids are based on a 15 carbon atom skeleton. A simple way to describe the flavonoid skeleton is, two phenyl rings connected by three carbon-bridges. It can also be represented as \( \text{C}_6 - \text{C}_3 - \text{C}_6 \).

![Figure 1.1: Skeleton of Flavonoid](image1)

The chemical structure of the flavonoids are based on a \( \text{C}_{15} \) skeleton with a chromane ring bearing a second aromatic ring B in position 2,3 or 4.

![Figure 1.2: Structural representation of Flavonoids](image2)
In certain flavonoids the C ring is either in an open form or replaced by a five membered ring (eg: aurones).

1.1.3 Classification of Flavonoids

![Chalcone](image1)

Figure 1.3: Chalcone

![Flavone](image2)

Figure 1.4: Skeleton of Flavone

![Quericetol](image3)

Figure 1.5: Quericetol

![Flavanone](image4)

Figure 1.6: Skeleton of Flavanone

![Anthocyanins](image5)

Figure 1.7: Anthocyanins

![Isoflavonoid](image6)

Figure 1.8: Skeleton of Isoflavonoid
Flavonoids are classified according to the substitution pattern of the C-ring. The oxidation state of the heterocyclic ring and the position of the B ring are also considered in classifying flavonoids. There are 6 major sub groups of flavonoids. 3

Chalcones, (Fig: 1.3) the first sub class of flavonoids contain three carbons – bridge in open form. 5 Flavones (Fig: 1.4) are based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Generally, found in herbaceous families like Labiatae, Umbellifereae, and Compositae etc. 3, 4 Flavanols (Fig: 1.5) contain a hydroxyl group at C-3 position (Eg: Quericetol). In flavanone (Fig: 1.5) the hydroxyl group at C-3 position is absent. Anthocyanins (Fig: 1.6) are considered as major class of pigments. Isoflavanoid (Fig: 1.7) are isomeric to flavones by virtue of their having the B-ring attached to position-3 rather than position-2 as in flavones. 1, 3 Neoflavonoids (Fig1.8) contain B-ring in 4th position. Derived from the 4-phenylcoumarine (4-phenyl-1, 2-benzopyrone) structure. 3, 6
1.1.4 Occurrence and distribution of Flavonoids

Although flavonoids are widely distributed in nature and in foods, they lack uniform distribution throughout the plant kingdom. Flavonoid compounds occur in all parts of the higher plants: roots, stems, leaves, flowers, pollen, fruit, seeds, wood and bark. Ferns contain many flavonoid compounds of the types found in flowering plants. Different food items contain varying concentrations of flavonoids. Teas, fruits, and dark chocolates contain moderate to high concentrations of flavonoids. Vegetables like broccoli and some fruit juices like cranberry and orange provide low levels of flavonoid content. Black and oolong tea contents have high contents of derived tannins.

1.1.5 Extraction and Purification

Different parameters effect the extraction of the flavonoids from plant materials; their chemical nature, storage time and conditions, and the presence of interfering substances. The solubility of the flavonoids depend upon the polarity of the solvent used, degree of polymerization, interaction with other food constituents and formation of insoluble complexes. There is no uniform or completely satisfactory procedure that is suitable for extraction of all flavonoids or specific type of flavonoids in plant materials. The most commonly used solvents in extraction of these compounds are methanol, ethyl acetate, acetone, and water, their combinations are also used. Propanol and dimethylformamide, are also used but to a lesser extent. Extraction period usually varies from 1 min to 24 hrs.
Extraction of polyphenols from food constituents also depends on sample-solvent ratio. The Flavonoid extracts can be partially purified, using ion exchange resins. Extracts can also be purified and fractioned by using solid phase extraction or solid phase microextraction (SPME), and column chromatography. Different columns are used for extraction of flavonoids, like C-18 column, Sephadex column etc. Recently counter current chromatography (CCC) has been used as an alternative to liquid chromatography for fractionation of various flavonoids. High speed centrifugal countercurrent chromatography is used in the separation of flavonoids like tea catechins, anthocyanins, theaflavins, etc.  

Quantification of flavonoids is carried out by different spectroscopic techniques. Gas Chromatography (GC) and high performance liquid chromatography (HPLC) are widely used in separation and quantitation of flavonoids. Structure elucidation is carried out by a combination of GC or HPLC with mass spectrometry and also various other relevant techniques.

1.1.6 Uses

Due to their beneficial health effects, flavonoids have garnered more interest in the past 2 decades. It was initially hypothesized that pharmacological effects of flavonoids would be related to their antioxidant activity, but the available evidence from cell culture experiments suggested that modulation of cell signaling pathway by flavonoids was attributed to their most of the biological effects. Studies in cell cultures have suggested that flavonoids may affect the chronic disease by inhibiting the kinases.
Flavonoids have a wide range of beneficial health effects such as, reducing the risk of cancer,\textsuperscript{116,117} anti-inflammatory,\textsuperscript{118} reducing the risk of developing diabetes mellitus,\textsuperscript{119} infertility,\textsuperscript{120} antichoolesterolemia,\textsuperscript{121} antiatherosclerosis,\textsuperscript{121} antiulcer, ability to inhibit human platelet aggregation,\textsuperscript{123} reducing the skin wrinkling, and also in reducing the risk of cardiovascular diseases.\textsuperscript{124} Flavonoids are most widely used as dietary supplements.

### 1.2 Green Tea

#### 1.2.1 History

Green tea one of the most widely consumed beverages in the world is an aqueous infusion of dried leaves of “Camellia Sinensis”. The birth place of green tea is in Asia. Tea plants grow only in warm climates. Although abundant foliage is produced by the tea plant, only the two leaves and the buds at each young shoot are picked for tea. The type of tea produced depends on the way in which leaves are processed: green, oolong, black. Green tea is the least processed tea; it is made by steaming the harvested leaves, rolling them and then spreading them out to dry until they become crispy (14).

The chemical contents of green tea include; flavonoids such as catechins and tannins, free amino acids, caffeine, ascorbic acid, saponins, and unsaturated fatty acids.\textsuperscript{15, 16} Catechins constitute a major component of green tea, which include Epicatechin (EC), Epigallocatechin (EGC), Epicatechin gallate (ECG), Epigallocatechin gallate (EGCG). These compounds constitute 30% of the chemical composition of green tea.\textsuperscript{12, 13} The most active component of green tea is EGCG, which constitutes 9-13% weight of green tea. It possesses a much greater antioxidant activity than the other catechins, and plays an
important role in the prevention of cancer and cardiovascular diseases.\textsuperscript{17}

A literature Search through Scifinder reveals that there are more than 8000 citations related to chemistry, bioactivity, production and potential health benefits of green tea. Out of 8000, 4000 references are related to EGCG and other natural products of green tea.\textsuperscript{18}

![Structure of EGCG](image)

\textbf{Figure 1.10 : Structure of EGCG}

\subsection*{1.2.2 Green tea production and trade}

World wide annual production of tea is estimated around 1 million tonnes and 70 percent of it is black tea and remaining is green tea. The world’s major producers of green tea are China, Japan, Korea, Taiwan, and Indonesia. China accounts for 65 percent of world production and 85 percents of world exports of green tea, where as Japan accounts for 20 percent of world production and five percent of world exports. United States of America and Canada are the major export destinations of green tea. It is also exported to Germany, United Kingdom, and Saudi Arabia.\textsuperscript{11}
1.2.3 Extraction and purification of green tea.

Various methods are used in extracting and purifying green tea. Most of these methods are patented. As EGCG is the principal active ingredient of green tea, most of the methods in extracting green tea are concentrated on extracting the pure EGCG. In most of the methods, polyphenols of green tea are extracted by solvent extraction, using different solvents like water, ethanol, diethyl ether, ethyl acetate, acetone etc. Purification of green tea is performed by using chromatographic techniques. Different types of columns are used in the separation of polyphenols, like Sephadex LH 20 column, Silica gel column, C-18 attached silica monolith microcolumns, and Lignocellulose column. Different lengths and particle size of HPLC columns are used for separation of catechins. Weakly basic anion exchange resins are also used in the separation of polyphenols. Polyphenols of green tea are also separated by Reverse phase liquid chromatography, and High speed counter current chromatography. During the purification process the time and temperature at which brewing is performed, is also considered (higher the brewing time higher the concentration of the catechins in green tea). 70°C is recognized as the optimum temperature for achieving the maximum concentration of polyphenols.

EGCG is also synthesized chemically, by the transformation of retero-chalcones into 1, 3-diarylpropene which are then subjected to asymmetric dihydroxylation. The resulting diarylpropane-1, 2-diols serve as Chiron’s for essentially enantiopure flavan-3-ols.
1.2.4 Bioactivity

Various diseases like cardiovascular diseases and cancer are considered to be caused by the activity of oxygen radicals; hence they are expected to be prevented by antioxidative compounds. It is well recognized that tea catechins (+) catechin (+C), (-)-epicatechin (-EC), (-) epigallocatechin (-EGC), (-) – epigallocatechin (-EGC), (-) - Epigallocatechin gallate show potent antioxidant activity. \(^{73, 74}\) Epidemiological studies have shown that the intake of tea catechins decrease the risk of coronary heart disease, stroke and cancer. \(^{75-77}\) Hence analysis of radical scavenging activity of the phenolic compounds present in different types of teas (green tea, oolong tea, black tea) has gained in importance. Different techniques such as the oxygen electrode method, \(^{69}\) High performance liquid chromatography, \(^{70}\) Electron spin resonance spectrometry, \(^{71}\) Nuclear magnetic resonance \(^{72}\) etc are used to analyze the scavenging activity of catechins on free radicals.

As mentioned earlier, epigallocatechin gallate (EGCG), the most abundant catechin in tea catechins, is shown to have the most effective scavenging activity among the tea catechins. \(^{67}\) The ortho trihydroxy group in the B ring and the galloyl moiety attached at 3\(^{rd}\) position contribute to the strong scavenging activity of Epigallocatechin gallate. \(^{68}\)
To elucidate the molecular mechanism underlying the radical scavenging and antioxidant activities of antioxidants, (+)-catechin was reacted with 1, 1- diphenyl-2-picrylhydrazyl (DPPH; stable free radical) as a model reaction. The reaction mixture is analyzed by C13 NMR. Two new carbonyl peaks were detected in the spectra, and the disappearance of characteristic peaks of the B ring was also observed, suggesting that two hydroxyl groups in the B- ring are more important in radical scavenging activity among the 4 phenolic hydroxyl groups of catechin and the B ring was changed to a quinone structure. Signals ascribable to A and C ring remained unchanged. 72
Experimental results have shown that the galloyl moiety in epigallocatechin gallate is more important in radical scavenging activity than the hydroxyl groups on the B ring.  

Therefore catechins are useful in the prevention of diseases caused by oxygen radicals. Consumption of green tea can decrease the cholesterol absorption, can decrease the body weight by interfering with sympathoadrenal system, inhibit the low density lipid
oxidation (LDL), antithrombotic activities and decrease the systolic and diastolic blood pressure. Catechins may ameliorate the impairments or injuries caused by intracellular active oxygen (Senile disorders), and might become useful for protecting human from Alzheimer’s disease.

EGCG, the most abundant catechin of green tea catechins, has several health benefits. However the beneficial effects of EGCG on human diseases are inconclusive, therefore epidemiological studies on protective action of green tea and EGCG yet to be explored

1.3 Crystal engineering

1.3.1 Introduction

Crystal engineering “the design of solids” is target oriented and property directed synthesis of molecular crystals. Traditionally, crystal engineering focuses on the relationship between the structure and properties of solids. Currently this concept is expanded into diverse areas such as material chemistry, supramolecular chemistry, molecular recognition and biology.

The term “Crystal engineering” was introduced by Pepinsky (1955) in the context of crystallization of organic ions with metal complexes. Schmidt applied the concept of crystal engineering in the context of solid state photodimerisation reactions. Modern Crystal engineering can be described as an interdisciplinary subject, owing to its implications for organic, inorganic, metal organic, theoretical and materials chemistry. A more useful definition for crystal engineering is provided by Desiraju, who illustrates
crystal engineering as a field with broader discipline “designing crystals with desired properties”.

Definition

“Crystal engineering is the understanding of intermolecular interactions in the context of crystal packing and in the utilization of such understanding in the design of new solids with desired physical and chemical properties”. 62

Gautam Desiraju.

The concept of crystal engineering has been successfully exploited in the synthesis of porous solids 82 and ion exchange materials. 83 Crystal engineering strategies are applied in the design of pharmaceuticals, 38 photographic materials, 84 and in novel optical, electronic and magnetic materials.

1.3.2 Pharmaceutical Crystal Forms

The majority of the drug substances are solid dosage forms, most of which contain active ingredient in a crystalline state. The inherent stability of crystalline materials and the well established impact of crystallization process on purification and isolation have made the chemists and the engineers to deliver the pharmaceutically active compounds in crystalline form. 31 The physical properties of the crystal such as purity, size and shape are determined by the operating conditions of crystallization process, in turn these properties determine the efficiency of the operations filtration, drying, formulating and also product effectiveness such as bioavailability and shelf-life. Drug dissolution and toxicity are affected by solid state phase and purity of the product, this leads to batch to
batch uniformity and consistency. Therefore, improved control of crystallization processes can achieve better crystal product quality, shorter process time and elimination of compromised batches.  

Pharmaceutical crystals may be chiral or achiral and they may exist in different forms, salts, solvate or hydrate and polymorphs. The existence of a crystal in different crystalline phases results in polymorphs. Solvates are “Molecular complexes that have incorporated the crystallizing solvent molecule in their crystal lattice”. When a crystal lattice incorporates water, it is designated as a hydrate. Approximately one third of pharmaceutical substances are capable of forming crystalline hydrates. Solvates and hydrates demonstrate different solubilities and dissolution rates when compared to their unsolvated counter parts. The stability of the hydrates and solvates at different temperatures and different vapor pressure, differ from their unsolvated forms. These differences can influence the stability of the pharmaceutical substances under different storage conditions.

The physical and chemical properties of a crystal depend upon the arrangement of the molecules in it and the physiochemical properties of the solid drug can affect its performance. Thus a study of the crystalline state can lead to an understanding of the drug properties which are important for preformulation and formulation. Different crystal packing changes the periodicity of the molecules which may in turn change physical properties of various crystal forms. Therefore, different solid forms can show different physical and chemical properties. Pharmaceutical drugs with different solid
forms can lead to phase transformations during processing and formulation, such as in theophylline, carbamazepine, Phenobarbital, lactose etc. The stability and bioavailability of the drug are affected by these phase changes. Therefore, an understanding in relationship between the solid state properties and crystal structures can be utilized for optimizing formulation strategies and in designing suitable stability protocols. Different solid forms of excipients, used in pharmaceutical formulations, can also affect the final physical form of a tablet.

Opposite enantiomers of chiral drugs, show different pharmacological, toxicological, pharmacodynamic and pharmacokinetic properties. This is due to the molecular environment, in which these solids exist. The presence of ions in pharmaceutical salts influences the physiochemical properties of the crystals, like dissolution rate, stability, solubility and hygroscopicity.

The structure of the crystal also affects mechanical properties, for example the intermolecular hydrogen bonds in theophylline monohydrate results in higher mechanical strength and less brittle than the anhydrous form of theophylline. Similarly, the presence of water molecules in the monohydrate of 4-hydroxy benzoic acid facilitates its plastic deformation. Therefore, it is possible to predict the mechanical properties from its crystal structure.

Different crystal forms of drug substances with different physiochemical properties can also affect scale up and transfer from laboratory quantities and procedure through pilot plant and full production. The characterization of crystal forms plays a major role in quality control and regulatory processes.
Physiochemical properties including mechanical properties of crystalline drug are determined by its molecular arrangement, packing, conformation and intramolecular interactions in its lattice. These physiochemical properties affect the pharmaceutical properties of the drug product. In order to predict the properties of pharmaceutical crystals a thorough understanding of the underlying crystal structure is desirable.  

1.3.3 Patent Relevance of Crystal Forms

“Patents are a mechanism for promoting research for society’s benefit: the right to exclude others from practicing a patented invention affords an economic incentive to the inventor, while the limited term of the exclusionary right ultimately delivers the invention into the public domain”.  

Andrew V. Trask

Patent can be defined as “A license conferring the sole right to manufacture, sell, or deal in a product or commodity; (now) spec. a license from a government conferring for a set period the sole right to make, use, or sell some process or invention”.

Oxford English Dictionary

Criteria for obtaining a patent:

A product or process must have novelty (not part of the “state of the art”), utility (must serve some worthwhile practical use), and must not be obvious (competent but without imagination). A product must be of human ingenuity, and should contain an enabling description.

M.J. Zaworotko
Newness of the drug product can be attributed not only for a new bioactive chemical form, but also for a different solid state form (polymorphic, amorphous or chiral), combination with other solids (carriers, coating, excipients), and different delivery method or even for a different proportion of the drug in this combination are patented.

Crystallization methods for a particular solid state form, new drugs, related manufacturing, and formulation aspects are protected by patents.

Examples

- Co-crystallization process (patent number; WO0053283, Authors; Reuter Karl, Reuter Chemische Apparatebau)

- A process for preparation of urea complexes of vitamin E and its esters (Patent number; IN182620, Authors; Bajaj Vikas; Madan Anil Kumar)

- High-throughput formation, identification, and analysis of diverse solid-forms (patent number; 20030162226, Authors; Cima, Michael J.; Levinson, Douglas; Lemmo, Anthony V.; Galakatos, Nicholas; Putnam, David A.)

- Novel Cnazole crystalline forms and related processes, pharmaceutical compositions and methods (patent number; WO03101392, Remenar Julius; MacPhee Michael; Peterson Matthew Lynn; Morissette Sherry L; Almarsson Orn)

Crystal forms of the chemical substances are patented on their powder X-ray diffraction pattern, because the powder pattern of a crystalline form is recognized as a finger print of it. Chemical substances can exist in more than one crystal form; this phenomenon is referred to as polymorphism. As different polymorphs of the same drug can exhibit
widely different solubility and bioavailability properties, polymorphism became an immediate concern for pharmaceutical industries during patent filing. A polymorph of a drug can be entitled to a different patent protection, as it has a different legal entity. For example, the two polymorphs of Zantac (ranitidine hydrochloride) are patented by Glaxo-Wellcome.

Ranitidine hydrochloride

Figure 1.14: Form I

Figure 1.15: Form II

Powder pattern for polymorphs of ranitidine

Figure 1.16: Powder X-ray diffraction of form I and II
As methods in generating the new patents guarantee the future financial security of the company, a complete screening and characterization of single crystal forms and development of corresponding crystallization techniques should be carried out as early as possible.
2. CSD STATISTICS

2.1 Cambridge Structural Database (CSD)

Cambridge Crystallographic Data Centre (CCDC) was established by Olga Kennard at Cambridge University in 1965. Cambridge structural data base is the principal product of the CCDC. The CSD comprises software for database access, structure visualization, data analysis, and structural knowledge bases derived from the scientist’s world wide. It records X-ray and neutron diffraction data of organic and metal organic compounds. It also records bibliographic, chemical and crystallographic information of these compounds. Crystallographic information includes single crystal studies and powder diffraction studies. Crystal structure data arising from the publications in the open literature and from private communications are also included in CSD. CSD software includes ConQuest (search program), Mercury (visualiser), Vista (statistical analysis), and Prequest (database creation) (33).

2.2 Supramolecular chemistry

“Supramolecular chemistry”\(^9\) which is also known as “chemistry beyond the molecule”\(^1\) is based on the selective recognition of molecules which interact via non covalent forces to form well organized assemblies. During the 1960’s and 1970’s much of the supramolecular chemistry research, involved in host guest systems\(^1\) for selective
binding of small alkali metal cations by macrocyclic receptors. The fascination for molecular recognition phenomenon inspired chemists in further exploration of supramolecular systems in the context of weak intermolecular interactions such as hydrogen bonds. 92-98

2.3 Supramolecular synthons

The term synthon was introduced by Corey in 1967 (99). Corey defined synthon as; “…… Structural units within the molecules can be formed or assembled by known or conceivable synthetic operations”. The term synthon is traditionally used in representing the key structural features in target molecule in organic synthesis.

Crystal engineering has emerged around the idea of establishing and later utilizing the intermolecular interactions to govern the crystal packing with reasonable predictability. In this respect the term supramolecular synthon was introduced by Desiraju in 1995. 34 It is defined as “a structural unit within the supermolecule which can be formed and/or assembled by known or conceivable intermolecular interactions”. Supramolecular synthons are also called as motifs 100 or patterns. 101 They can also be regarded as regions within a crystal structure where the recognition between constituent functional groups occurs. 102 Supramolecular synthons can be separated in to two distinct categories: Supramolecular homosynthon; which results from interaction of identical self-complementary functionalities 35 and supramolecular heterosynthon which results from interaction of different but complementary functionalities35. Examples of supramolecular homosynthons include carboxylic acid and amide dimmers, 103,104 while supramolecular
heterosynthon include acid-pyridine, \textsuperscript{35} acid –amide \textsuperscript{105-108} and hydroxyl- pyridine.\textsuperscript{109-111}

Figure 2.1 represents the acid-acid dimer (homosynth) and acid – pyridine (heterosynth).

![Figure 2.1 Examples of supramolecular homosynthon (left) and supramolecular heterosynthon (right)](image)

Supramolecular homosynthons tend to be observed in single component crystals, but their existence can also be observed in multiple component crystals in which each component has at least one identical functional group. Furthermore when multiple functional groups are present the formation of a supramolecular heterosynthon is possible. Supramolecular heterosynthon can exist between the functional groups located on two different molecules with same chemical make up or on the two different molecules with different chemical make up. Interpretation of existing crystal structures with complete knowledge on interplay between supramolecular synthons would help in designing new multicomponent crystals.

2.4 CSD Statistics for Polyphenols
The polyphenolic nature of the flavonoids provoked an interest to conduct a survey on intermolecular interactions of phenols and polyphenols in the CSD. Phenols and polyphenols are evaluated by considering the following constraints, no ions, only
organics, R factor: \( \leq 0.075 \), structures containing 3D coordinates. As EGCG contains ether, ester and alcohol groups, while the two known solvates of it contain acetonitrile and nitrobenzene, studies are conducted considering these functional groups.

There are 6,032 phenols in CSD as of May 2007. Out of 6,032 phenols 1,005 are polyphenols.

![Structure of polyphenol considered in the CSD search](image)

**Figure 2.2: Structure of polyphenol considered in the CSD search**

![Synthons I, II, and III](image)

**Figure 2.3: Synthon I; OH---OH, Synthon II OH---CO, Synthon IIIOH----O(ether) interactions, (R:\( \text{C}_6\text{H}_5\text{OH} \))**
In CSD analysis, the frequency of occurrence of supramolecular synthons hydroxyl to hydroxyl (synthon I), hydroxyl to carbonyl of ester (synthon II) and hydroxyl to ether (synthon III) in phenols and polyphenols is evaluated. In order to determine appropriate distance ranges, within which homo and heterosynthons exist, distance histograms were generated. Based on visual inspection of the resulting interactions in the crystal structures included in the histograms, the lower and higher cut offs for hydrogen bond distances were determined. The histograms (Fig 0.2) revealed that hydroxyl to hydroxyl synthon (I) occurs within a distance range of 2.6-3.0 Å. Supramolecular synthons II (hydroxyl to carbonyl of an ester) and III (hydroxyl to ether) exist within a range of 2.56-2.94 Å and 2.5-3.0 Å respectively.

Figure 2.4: Histograms of contacts for supramolecular synthons I (OH---OH), II (OH---CO) and (OH---O) III
Similarly hydroxyl to cyano group Synthon (synthon IV as represented in figure 2.5) and hydroxyl to nitro group synthon (synthon V as represented in figure 2.5) were evaluated. Histograms revealed the distance ranges within which these synthons occur are 2.7- 3.2 Å and 2.78 - 3.06 Å respectively.

Figure 2.6 : Histograms of contacts for supramolecular synthons IV
<table>
<thead>
<tr>
<th>Synthon I</th>
<th>Phenols</th>
<th>Polyphenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>(OH---OH)</td>
<td>836 hits/6,032</td>
<td>385 hits/1,005</td>
</tr>
<tr>
<td></td>
<td>14.8%</td>
<td>38.3%</td>
</tr>
</tbody>
</table>

Table 2.1: Frequency of occurrence of synthon I in phenols and polyphenols

<table>
<thead>
<tr>
<th>Phenol + Ester</th>
<th>OH--CO (ester) 2.56-2.94Å</th>
<th>Phenol+ ether (ether) 2.5-3.0 Å</th>
<th>Phenol + Cyano group 2.7-3.2Å</th>
<th>OH---NO 2.78-3.06Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>799 hits/6,032 phenols</td>
<td>225 hits/799</td>
<td>2,074 hits/6,032</td>
<td>74 hits/6,032</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>191 hits/1,005 polyphenols</td>
<td>81 hits/191</td>
<td>281 hits/1,005</td>
<td>31 hits/1,005</td>
</tr>
</tbody>
</table>

Table 2.2: Frequency of occurrence of synthons from II to V in phenols and polyphenols

Table 2.2 represents the frequency of occurrence for synthons II to V in the presence of the respective functional groups with phenols and polyphenols. Though the presence of ethers with phenols and polyphenols is high in comparison with other functional
groups, the occurrence of synthon III is relatively lower compared to the other synthons.

<table>
<thead>
<tr>
<th>Supramolecular synthons</th>
<th>D----A (Å)</th>
<th>Mean (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH----OH</td>
<td>2.6 -3.0 Å</td>
<td>2.80</td>
</tr>
<tr>
<td>OH---CO (ester)</td>
<td>2.56-2.94</td>
<td>2.771</td>
</tr>
<tr>
<td>OH---O (ether)</td>
<td>2.5-3.0</td>
<td>2.805</td>
</tr>
<tr>
<td>OH----CN (cyano)</td>
<td>2.7-3.2</td>
<td>2.873</td>
</tr>
</tbody>
</table>

Table 2.2.3: Distance ranges within which different synthons exists

This analysis has revealed that the frequency of occurrence of hydroxyl to hydroxyl synthon (synthon I) is higher in phenols (14.8%) and polyphenols (38.3%), when compared to other synthons (II, III, IV and V). The prevalence of the specific supramolecular synthons in these crystal structures would provide a valuable insight for crystal engineering strategies in generation of new multicomponent materials comprised of polyphenols.
3. EXPERIMENTAL DATA

Some solvents were obtained from Sigma Aldrich. Distillation of few solvents was performed in the lab itself, such as methanol, ethanol, dichloromethane, chloroform. Distilled water is used. EGCG was provided by Dr. Roland Shytle.

3.1 Infrared Spectroscopy (IR)

A Nicolet Avtar 320 Fourier transform Infrared spectrometer was used for collecting the IR spectra of the samples (sample concentration 2mg). The spectra were measured over the range of 4000 – 400 cm\(^{-1}\). Data were analyzed by using EZ Omnic software.

3.2 Differential Scanning Calorimetry (DSC)

Thermal analysis of the samples was performed on TA instrument DSC 2920 equipped with liquid nitrogen cooling. The samples of crystals (2mg-7mg) were placed in aluminum pans, and were scanned at 10° C/min in the range 25° - 350° C under a dry nitrogen atmosphere (flow rate 70ml/min). The data were managed by TA universal analysis.

3.3 Powder X-ray Diffraction (PXRD)

PXRD patterns were collected on Bruker AXS D8 powder diffractometer with a Cu Kα radiation (1.54056 Å). The tube voltage and amperage were set at 50kV and 40mA, respectively. Each sample was scanned between 3 and 40° in 2θ with a step size of 0.02°.
The experimental PXRD patterns and calculated PXRD patterns from single crystal structures were compared to confirm the composition of materials.

3.4 Single Crystal X-Ray Crystallography

Crystals of forms II, III and IV were examined under a microscope and suitable single crystals were selected for X-ray analysis. Data were collected on a Bruker–AXS SMART APEX CCD diffractometer with monochromatized Mo Ka radiation (\(\lambda = 0.71073 \text{ Å}\)) connected to KRYO-FLEX low temperature device. Data were collected at 100 K.

Lattice parameters were determined from least square analysis, and reflection data were integrated using the program SAINT. Lorentz and polarization corrections were applied for diffracted reflections. In addition, the data was corrected for absorption using SADABS. Structures were solved by direct methods and refined by full matrix least squares based on \(F^2\) using SHELXTL. All non-hydrogen atoms were refined with anisotropic displacement parameters. All H-atoms bonded to carbon atoms, except methyl groups, were placed geometrically and refined with an isotropic displacement parameter fixed at 1.2 times \(U_q\) of the atoms to which they were attached. N or O bonded protons, as well as H-atoms of methyl groups, were located from Fourier difference map and refined isotropically based upon the corresponding N, O or C atom (\(U(H)=1.5U_q(N, O)\)).
3.5 Form I

90% pure EGCG provided by Dr. Roland Douglas Shytle is considered as form I. Up to the date the reported form of egcg in literature is form I. This is confirmed by PXRD. 

3.5.1. Physical properties

Melt-Temp: 220° C, Soluble in ethanol, methanol, ethyl acetate, acetonitrile, acetic acid, formic acid. It is insoluble in chloroform, cyclohexane, toluene, and hexane. Egcg is sensitive to light, so it is stored at temp 2°- 8°C.

3.5.2 IR of Form I

The IR of form I match with the IR found in literature. 

![Figure 3.1: IR of Form I](image)
3.5.3 DSC of Form I

Phase transitions are observed at 126.43°C and 227.71°C. DSC matches with the DSC in the literature. 88
3.5.4 Powder X-ray Diffraction of EGCG form I


Figure 3.4: PXRD of Form I

Figure 3.5: PXRD of Form I from literature
3.6 Form II

3.6.1 Synthesis

65mg of 90% pure epigallocatechin gallate (0.141 moles) dissolved in 1ml of acetonitrile (99% pure) is layered on 2.5 ml of dichloromethane and allowed to stand in refrigerator. After 72 hours colorless platelet crystals (Form II) were observed. An increase of 3-fold and 5-fold in contents has shown same results. Form II was also crystallized from acetonitrile and chloroform.

3.6.2 Physical properties

Melt- Temp: 235-238°C. Solubility properties are observed to be similar to that of Form I. Crystals are stable up to four days at temperature 2° to 8° C. Upon heating at 120° C for twenty minutes, solvent is evaporated, and Form II is converted to Form IV.

3.6.3 IR of Form II

IR of form II shows O-H stretch 3323.8 cm\(^{-1}\), C=O stretch at 1605 cm\(^{-1}\), and aromatic carbon stretch at 742.4 cm\(^{-1}\).

![Figure 3.6: IR of Form II](image-url)
3.6.4 DSC of Form II

Phase transitions are observed at 101.43° C and 252.71°C.

![Figure 3.7: DSC of Form II](image)

3.6.5 TGA of Form II

Below 100° C 16.68 % weight loss is observed. 20.20 % weight loss is observed between 230° C.

![Figure 3.8: TGA of Form II](image)

35
3.6.6 Powder X-ray Diffraction of form II

The experimental PXRD matches with the calculated PXRD as shown in fig 3.11. Major peaks, experimental (calculated): 7.12 (7.12), 7.8 (7.92), 8.46 (8.56), 13.04 (13.3), 15.24 (15.28), 23.54 (23.86), 25.92 (25.44).

Figure 3.9: Experimental PXRD of Form II

Figure 3.10: Calculated PXD of Form II
3.6.7 Picture of Single crystal

Figure 3.12: Single crystal of Form II
3.6.8. Single Crystal X-ray Crystallography Data

Molecular formula: C_{24}H_{21}N_{11}O_{11}; Formula weight: 499.42; Crystal system: Monoclinic; Space group: C2; Unit cell dimensions: a = 22.876(3) Å, b = 14.9054(17) Å; c = 15.8886(18) Å, α =90°; β =102.688(2)°; γ = 90°; Volume: 5285.4(10)Å³; Z=8; Temperature:100(2) K; Density (calculated): 1.433 Mg/m³ ; λ(Mo-Kα): 0.71073 Å ;
Reflections measured: 12985; Independent reflections: 10165 [R(int) = 0.0277]; Final R indices [I>2sigma(I)]: R1 = 0.0831, wR2 = 0.1993; R indices (all data): R1 = 0.1013, wR2 = 0.2144

3.7 Form III

3.7.1 Synthesis

In a typical reaction, a reaction tube with 6ml of dichloromethane is taken, and 1ml of nitrobenzene is layered on it. 200mgs (0.436mmoles) of 90% pure EGCG dissolved in 2.5ml of acetonitrile is layered on nitrobenzene. Reaction test tube is left on refrigerator, after 24 hours yellow needle like crystals were observed

3.7.2 Physical properties

Melt Temp: 235 °-238°; Solubility properties are similar to that of Form I. Form III crystal are more stable compared to Form II crystals at temp 2°- 8° C. Upon heating to 120° C for twenty five minutes, Form III converts to Form IV.
3.7.3 IR of Form III

3272.3 cm\(^{-1}\) (O-H stretch), 1681.33 (N-O stretch) are observed.

Figure 3.13: IR of form III

3.7.4 DSC of Form III

Phase transitions are observed at 140.10\(^{\circ}\)C, 169.26 \(^{\circ}\)C, and 253.52\(^{\circ}\)C.

Figure 3.14: DSC of Form III
3.7.5 TGA of Form III

11.98 % of weight loss is observed at 131° C, 25.31 % of weight loss is observed at 211.36° C is observed.

![TGA of Form III](image)

Figure 3.15: TGA of Form III

3.7.6 Powder X-ray Diffraction of Form III

The experimental PXRD matches with the calculated PXRD as shown in fig 3.18. Major peaks, Experimental (calculated): 13.4 (13.4), 15.4 (15.4), 19.4 (19.44), 24.3 (24.4), 27.8 (27.65), 28.5 (28.5), 31.3 (31.3), and 37.6 (37.5)

![Experimental PXRD of Form III](image)

Figure 3.16: Experimental PXRD of Form III
Figure 3.17: Calculated PXRD of Form III

Figure 3.18: Calculated Vs Experimental PXRD of Form III
3.7.7. Picture of single crystal

Figure 3.19: Single crystal of Form III

3.7.8. Single Crystal X-ray Crystallography Data

Molecular formula: C$_{28}$ H$_{25}$ N O$_{14}$; Formula weight: 599.49; Crystal system: Orthorhombic; Space group: P2(1)2(1)2(1); Unit cell dimensions: a = 13.2070(14) Å, b = 13.2134(14) Å, c = 14.5781(16) Å, α = β = γ = 90°; Volume: 2544.0(5) Å$^3$; Z=4; Temperature: 100(2) K; Density (calculated): 1.565 Mg/m$^3$; λ(Mo-Kα): 0.71073 Å; Reflections measured: 11442; Independent reflections: 4964 [R(int) = 0.0608]; Final R indices [I>2σ(I)]: R1 = 0.0605, wR2 = 0.1126; R indices (all data): R1 = 0.0787, wR2 = 0.120
3.8. Form IV

3.8.1 Synthesis

Form IV is obtained by heating form II and III at 120° for 20-25 minutes. Form IV crystals: EGCG (45mg, 0.098mmoles of form IV powder obtained by heating form III at 120° C for 25 minutes) is dissolved in 1ml of acetonitrile (99%pure, Aldrich). The solution is layered on 2.5ml dichloromethane (distilled and stored over molecular sieve) and seeded with form IV crystals obtained by heating form III (at 120° C for 25 minutes) and was allowed to stand in refrigerator. After one week colorless needle like crystals were observed.

3.8.2. Physical Properties

Melt-Temp: 232°-235°. Solubility properties are similar to that of form I.

3.8.3. IR of Form IV

3466.81 cm⁻¹ (O-H stretch), 1606.19 (C=O) stretch are observed.

![Figure 3.20: IR of Form IV](image)
3.8.4 DSC form IV

Phase transition is observed at 250.08 °C.

![Figure 3.21: DSC of Form IV](image)

3.8.5 TGA form IV

30.02 % of weight loss is observed at 251.78 °C.

![Figure 2.22: TGA of Form IV](image)
3.8.6 Powder X-ray Diffraction of form IV

The experimental PXRD matches with the calculated PXRD as shown in fig 3.24.

Major peaks (experimental): 13.9 (13.84), 17.2 (17.12), 21.2 (21.16), 23.6 (23.64), 26.5 (26.6), 29.0 (29.16), and 31.5 (31.7).
3.8.7 Picture of Single Crystal

Figure 3.24: Calculated Vs experimental PXRD of Form IV

Figure 3.25: Single crystal of Form IV
3.8.8 Single Crystal X-ray Crystallography Data

Molecular formula: $\text{C}_{22}\text{H}_{18}\text{O}_{11}$; Formula weight: 458.36; Crystal system: Monoclinic;

Space group: $\text{P2}(1)$; Unit cell dimensions: $a = 13.006(10)$ Å, $b = 5.686(4)$ Å; $c = 13.089(10)$ Å, $\alpha = 90^\circ; \beta = 107.062(11)^\circ; \gamma = 90^\circ$; Volume: $925.4(12)\text{Å}^3$; $Z=2$;

Temperature: 100(2) K; Density (calculated): 1.645 Mg/m$^3$; $\lambda$(Mo-Kα): 0.71073 Å;

Reflections measured: 2623; Independent reflections: 2550 [R(int) = 0.0312]; Final R indices [I>2σ(I)]: $R_1 = 0.0641$, $wR_2 = 0.1494$; R indices (all data): $R_1 = 0.0800$, $wR_2 = 0.1674$
4. RESULTS AND DISCUSSION

4.1 Structure Description

EGCG structure; EGCG consists of 4 rings (A, B, C, D) with 8 hydroxyl groups, as shown in Fig.4.1. In all forms of EGCG the A and C ring lie in one plane while rings B and D lie in different planes with different dihedral angles. EGCG makes full use of all hydroxyl groups to form different synthons such as hydroxyl to hydroxyl, hydroxyl to ether, hydroxyl to carbonyl of ester, hydroxyl to cyano and hydroxyl to nitro.

![Figure 4.1: Structure of EGCG](image)

4.1.1 Form II

The crystal structure of form II was obtained from Single crystal X-ray diffraction as described in the experimental section. Form II solvate of acetonitrile, crystallizes in the
monoclinic C2 space group with two egcg and two acetonitrile molecules and some disordered solvent molecules in asymmetric unit. Analysis of the structure of form II indicates the presence of four type’s synthons. They are hydroxyl to hydroxyl (OH---OH), hydroxyl to ether (OH---O), hydroxyl to cyano group (OH---CN), and hydroxyl to carbonyl of ester (OH---CO) synthons.

The OH---OH hydrogen bond exists in between, the two hydroxyl groups of the D ring of molecule I and one hydroxyl group of B ring of molecule II. The OH---OH hydrogen bond is observed between hydroxyl group on the B ring of molecule I and two hydroxyl groups of D ring of molecule II and also in between the hydroxyl group on the A ring of molecule I and the hydroxyl group of A ring on molecule III. The hydrogen bond distances in synthon OH---OH are 2.999Å, 2.733Å, 2.753Å, 2.838Å, and 2.603Å. The distances are consistent with the CSD analysis (2.6 – 3.0Å).

![Figure 4.2: Representation of OH---OH synthon in Form II](image)
The OH---O (ether) hydrogen bond exists between the oxygen of the C ring and the hydroxyl of the D ring. The hydrogen bond distance in OH---O synthon is 3.026Å which is consistent with the CSD analysis (2.5-3.0Å).

![Figure 4.3: Representation of OH---O synthon in form II]

The OH---CO (ester) hydrogen bond exists between the ester carbonyl of molecule I and the hydroxyl on the D ring of molecule II and also with the two hydroxyl groups on the B ring of molecule II. The hydrogen bond distances in synthon OH---CO are 2.847Å, 2.924Å, and 2.680Å. The distances are consistent with the CSD analysis (2.56-2.94Å).
The OH---CN hydrogen bond exists between the hydroxyl group on the B ring and cyano group of acetonitrile molecule. The hydrogen bond distance in the synthon OH---CN is 2.898Å which is consistent with the CSD analysis (2.7-3.2 Å).

Figure 4.4: Representation of OH---CO synthon in form II

Figure 4.5: Representation of OH---CN synthon in form II
Acetonitrile molecules are embedded in between egcg molecules and sustained by hydrogen bonding.

Figure 4.6: Solvent entrapment in form II

Unit cell packing of form II includes 8 egcg molecules, 8 acetonitrile molecules, and disordered solvent molecules.

Figure 4.7: Unit cell packing of form II
4.1.2 Form III

Form III solvate of nitrobenzene crystallizes in orthorhombic P2\(_1\) space group with one egcg molecule, one nitrobenzene and one water molecule in asymmetric unit. Analysis of form III crystal indicated the presence of four types of synthons. They are hydroxyl to hydroxyl (OH---OH), hydroxyl to carbonyl of ester (OH---CO), hydroxyl to nitro (OH---NO) and hydroxyl to oxygen in water (OH---O).

Figure 4.7 represents the OH----OH interactions of molecule I and molecules II, III, IV and V. One of the hydroxyl group on the A ring of molecule I and hydroxyl groups on the B and D ring of molecule V form hydrogen bond. The other hydroxyl group of A ring in molecule I forms hydrogen bond with the hydroxyl group on the B ring of molecule II. The hydroxyl group on the B ring (of molecule I) forms hydrogen bond with hydroxyl group on the B ring of molecule III. The two hydroxyl groups on D ring (molecule I) form hydrogen bond with hydroxyl group on the A ring of molecule IV and also with hydroxyl group on the B ring. The hydrogen bond distances in the synthon OH----OH are 2.910Å, 2.827Å, 2.831Å, 2.827Å, 2.910Å, and 2.982Å. The distances are consistent with the CSD analysis (2.6-3.0Å).
The OH---CO (ester) hydrogen bond exists between the carbonyl of the EGCG molecule and the hydroxyl group on the A ring of another EGCG molecule. The hydrogen bond distances in OH—CO synthon is 2.671Å which is consistent with the CSD analysis (2.56-2.94Å).
The OH---NO hydrogen bond is observed between the hydroxyl group of B ring and nitro group of nitrobenzene. Hydrogen bond distance in the synthon OH---NO is 2.773Å which is consistent with the CSD analysis (2.7- 3.2Å).

Figure 4.10: Representation of Synthon OH---NO in Form III

The OH—O hydrogen bond is observed between the hydroxyl groups on B ring and oxygen of water molecule. The hydrogen bond distances in the synthon OH---O (water) are 2.837Å, 2.957Å, and 2.610Å.

Figure 4.11: Representation of Synthon OH---O in Form III
Nitrobenzene molecule is sandwiched between two egcg molecules and sustained by hydrogen bond.

Figure 4.12: Solvent entrapment in form III

Unit cell of form III contain 4 egcg molecules, 4 nitrobenzene molecules and 4 water molecules

Figure 4.13: Unit cell packing of Form III
4.1.3 Form IV

Form IV of egcg crystallizes in monoclinic P2 (1) space group with one egcg molecule in asymmetric unit. Form IV exhibits three types of synthons. They are OH---OH, OH---CO (ester), and OH----O (ether).

![Representation of Form IV structure](image)

Figure 4.14: Representation of Form IV structure

The OH---OH hydrogen bond in form IV exists between the hydroxyl groups on A ring of molecule I and hydroxyl group on B and D rings of molecules VI, and IV respectively. The hydroxyl groups on the D ring of molecule I exhibits OH---OH hydrogen bond with hydroxyl group on the A and B rings of molecules IV and III respectively. The hydroxyl group on the B ring of molecule I exhibits the OH---OH hydrogen bond with hydroxyl group on the A ring of molecule II. The hydrogen bond distances in the synthon OH---OH are 2.801 Å, 2.697Å, 2.696Å, and 2.960Å. The distances are consistent with the CSD analysis (2.6 -3.0 Å).
Figure 4.15: Representation of OH---OH synthon in Form IV

The OH—O (ether) hydrogen bond exists between the hydroxyl group of the B ring and the oxygen in the C ring of another molecule. The hydrogen bond distance of synthon OH---O is 2.875Å which is consistent with the CSD analysis (2.5-3.0Å).

Figure 4.16: Representation of OH—O synthon in Form IV
The OH---CO (ester) interaction is observed between the carbonyl of molecule I and the hydroxyl groups on the B and D rings of molecules II and III respectively. The hydrogen bond distances in the synthon OH----CO are 2.813Å, 2.839Å, and 2.879Å. The distances are consistent with the CSD analysis (2.56-2.94Å).

Figure 4.17: Representation of OH—CO synthon in FormIV
Unit cell of form IV contain two egcg molecules

Figure 4.18: Unit cell packing of Form IV

4.2 Conformational analysis

Conformational flexibility in the EGCG molecule was previously analyzed by proton NMR. It includes the orientation of the linkage between B, D and C ring owing to the puckering of C ring. The conformational changes in C ring with respect to the B and D rings, gives rise to 4 conformers (A, B, C, and D).

Conformer A
- \( R_1 = \text{Pseudoequitorial} \)
- \( R_2 = \text{Pseudo axial} \)

Conformer B
- \( R_1 = \text{Pseudo axial} \)
- \( R_2 = \text{Pseudoequitorial} \)

Conformer C
- \( R_1 = \text{Pseudoequitorial} \)
- \( R_2 = \text{Pseudoequitorial} \)

Conformer D
- \( R_1 = \text{Pseudo axial} \)
- \( R_2 = \text{Pseudo axial} \)
The pseudoequitorial and pseudoaxial positions of the B and D rings with respect to the C ring in forms II, III, and IV suggest that they exist as conformer A. The puckering of the C ring generates the pseudoequitorial and pseudoaxial positions of rings B and D.

The two chiral centers (C1 and C2) on C ring exists in S, S, for form II and R, R for forms III, IV respectively.
4.3 Discussion

Although flavonoids are abundant and commercially available they are difficult to purify and crystallize. In this respect developing new crystal forms of EGCG was challenging. Different crystallization techniques were explored in crystallizing EGCG, such as solvent evaporation, vapor diffusion, slow cooling, and solvent layering. Solvents used in these techniques were ethanol, methanol, toluene, cyclohexane, hexane, 1, 4-dioxane, dimethylsulfoxide, dimethylformamide, chloroform, dichloromethane, acetonitrile, nitrobenzene, benzene, and water. Solvent layering technique was found to be the most successful technique in crystallizing EGCG. As mentioned in chapter 3, two solvates and a new form of EGCG were obtained by solvent layering technique.

Liquid chromatography- Mass spectrometry (LC-MS) was used to evaluate the purity of crystals of the two solvates and the new form of EGCG. The LC-MS results have revealed the presence of impurities. An impurity with mass/charge ratio 443 (retention time 13 minutes), was observed in all three forms.

![Figure 4.20: LC-MS analysis of 90% pure EGCG](image-url)
Figure 4.21: LC-MS analysis of Form II

Figure 4.22: LC-MS analysis of Form III
Figures 4.20-4.23 represents the LC-MS analysis of all four forms of EGCG. LC-MS Data: Retention time; EGCG: 7.609 (Mol.wt of EGCG: 458.4), Impurity: 13.039 minutes.

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Bulk</th>
<th>Form II</th>
<th>Form III</th>
<th>Form IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.609</td>
<td>459 (M+1)</td>
<td>459 (M+1)</td>
<td>459 (M+1)</td>
<td>459 (M+1)</td>
</tr>
<tr>
<td></td>
<td>481 (M+23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.039</td>
<td>443 (M+1)</td>
<td>443 (M+1)</td>
<td>443 (M+1)</td>
<td>443 (M+1)</td>
</tr>
<tr>
<td></td>
<td>481 (M+38)</td>
<td>506 (M+63)</td>
<td>481 (M+38)</td>
<td>523 (M+80)</td>
</tr>
<tr>
<td></td>
<td>506 (M+63)</td>
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Table 4.1: Representing the components eluted at different retention times
Here in table 4.1, bulk represents the 90% pure EGCG. LC-MS results have revealed that from all four forms, EGCG was eluted after 7 minutes and the impurities after 13 minutes. Identities of impurities were not determined.

Though crystallization techniques could not produce 100% pure EGCG, a careful analysis of the existing supramolecular synthons in the new crystal forms can provide an educated guess to choose other molecule with complementary components when designing co-crystals. Considering the CSD analysis (chapter 2), a complete knowledge on interplay between the supramolecular synthons (OH—OH, OH---CO, OH---O, OH---CN, OH---O) would help to develop a strategy in choosing the co-crystal formers.
5. CONCLUSION

In summary the study presented herein involves the synthesis of different crystal forms of EGCG with limited CSD analysis on supramolecular synthons that exist in these crystal forms. In crystallizing EGCG the technique known as solvent layering was found to be the most successful technique. Two solvates, with acetonitrile and nitrobenzene, and one new form of EGCG is reported. A total of five types of synthons exist in the crystal forms of EGCG. Synthons I (OH---OH), and II (OH---CO (ester)) exist in all three forms (II, III and IV). The absence of synthon III (OH---O (ether)) in form III suggests that the occurrence of an OH---O (water) synthon in the presence of a competing acceptor, ether moiety, is relatively high. Synthons IV (OH---CN) and V (OH---NO) exists in form II and form III respectively. All the five synthons occur within the distance ranges which are consistent with the CSD analysis.

Though EGCG shows profound beneficiary effects on health, it suffers from the problem of poor bioavailability. This study, on structural features of EGCG, could potentially provide valuable information for structural modifications, which may change the physiochemical properties of EGCG. One of the future aspects of this study could be the design of co-crystals of EGCG. A brief description for co-crystals is provided here.

A broad definition for co-crystal is given by Dunitz as “Crystals containing two or more components together”. 112 In a more specific way others 113,114 defined co-crystal as “a multiple component crystal formed between compounds that are solid under ambient
conditions: at least one component is molecular and forms a supramolecular synthon with the remaining components”. Co-crystals exhibit different physiochemical properties (such as bioavailability, solubility) from their starting materials, due to being new and distinct solid state structures.

To design co-crystals of EGCG co-crystal formers can be chosen based on CSD analysis. A small survey which represents the competitiveness between aromatic nitrogen and the carbonyl of an ester group to form a hydrogen bond with the hydroxyl moiety on phenols is conducted in the CSD. Results have revealed that 36 hits were present containing hydroxyl, ester, and aromatic nitrogen groups. Out of these 36 hits, 14 hits contain the OH---N (arom) synthon, 7 hits contain the OH---CO (ester) synthon, and 4 hits contain the OH---OH synthon. All three synthons occur within the distance range of 2.5-3.0 Å (OH---N (arom)), 2.56-2.94 Å (OH---CO (ester)) and 2.6-3.0 Å (OH---OH) respectively. The distances are estimated from histograms generated by the CSD. This study has revealed that the occurrence of the OH---N (arom) synthon is relatively more favored than other synthons. Therefore compounds like caffeine and theobromine (which contain aromatic nitrogen) could potentially act as good co-crystal formers.
REFERENCES

1. Bhom, A.B.; Introduction to flavonoids; Overseas Publishers Association; 1998

2. Geissman, T. A.; Chemistry of Flavanoid compounds; Pergamon Press LTD.; 1962

3. Harborne, J.B.; Mabry T.J.; Mabry, H.; The Flavonoids; Academic Press; 1975


6. Friedli, L.G; “Introduction and Classification of Flavanoids”; URL
   http://www.friedli.com/herbs/phytochem/flavonoids.html#intro


11. DAFF; Processing of Green tea”; URL


14. Mateljan, G.; “Green tea”; URL


17. Yixin, Z.; “Green tea extract: Epigallocatechin gallate”; URL


33. Cambridge structural Database; URL
http://www.ccdc.cam.ac.uk/products/csd/


51. Grant, D.J.W.; Brittain H.G.; *Drugs and pharm.sci.*, **1999**, 95, 1.


66. CSD search parameters: ConQuest Version 1.7, May 2007 release, organic compounds with 3D coordinates determined, and R < 7.5%


URL

89. Hara, Y.; 1986; Patent number: 4,613,672.

Supramolecular Science, 1996, 2, 175.


95. Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.;


115. Pauling, L.; “Micronutrient research for optimum health”; URL

   [http://lpi.oregonstate.edu/infocenter/phytochemicals/flavonoids/](http://lpi.oregonstate.edu/infocenter/phytochemicals/flavonoids/)


120. Cheng, I.F; Breen, K.; *2000, 13, 77.

121. Sakata, K; Hirose, Y; Qiao, Z; Tanaka, T; Mori, H.; *Cancer Lett, 2003, 199, 139.

