Crystal Engineering of Multiple Component Crystal Forms of Active Pharmaceutical Ingredients

by

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Dedication

For Bryana and Izabelle
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Abstract

Enhancing the physicochemical properties of solid-state materials through crystal engineering enables optimization of these materials without covalent modification. Cocrystals have become a reliable means to generate novel crystalline forms with multiple components and they exhibit different physicochemical properties compared to the individual components. This dissertation exemplifies methodologies to generate cocrystals of active pharmaceutical ingredients (API’s) based upon knowledge of supramolecular interactions (supramolecular synthons), while focusing on enhanced delivery through \textit{in vitro} and \textit{in vivo} processes with both salts and cocrystals respectively.

The utility of mechanochemistry involving small amounts of an appropriate solvent, or solvent drop grinding (SDG), has been shown to reliably reproduce cocrystals with the anti-convulsant carbamazepine that were originally obtained by solution crystallization. This technique has been confirmed as a reliable screening method using solvents in which both components exhibit some solubility. The benefits of this technique lie in the time and cost efficiency associated with it as well as its inherently small environmental impact making it a “Green” method. SDG was also used as an efficient way to discover cocrystals of the anti-inflammatory meloxicam with carboxylic acids after analysis of existing reports and the analysis of structural data from the Cambridge Structural Database (CSD) to guide the choice of coformer. It has been shown that SDG can be used to screen for cocrystalline forms that are also obtainable by solution
crystallization which is important in later stage development and manufacturing including but not limited to large scale up processes. Single crystals suitable for single crystal X-ray diffraction were obtained with meloxicam and two of the coformers, fumaric and succinic acid. Some of the meloxicam cocrystals exhibited enhanced pharmacokinetic (PK) profiles in rats exemplifying significantly higher serum concentrations after only fifteen minutes and consistently higher exposure over the time studied while others maintained lower exposure. This reveals that cocrystals can fine tune the PK profile of meloxicam in order to reduce or enhance exposure.

Two different sulfonate salts, 4-hydroxybenzenesulfonate (p-phenolsulfonate) and 4-chlorobenzenesulfonate, of the anti-spastic agent (R,S) baclofen were developed by strategically interrupting the intramolecularly stabilized zwitterionic structure of baclofen. This zwitterionic structure results in low solubility associated with physiological pH required for intrathecal administration. Structural data for both salts in the form of single crystal X-ray diffraction data was successfully obtained. Solubility based on baclofen was assessed and shown to increase in pure water and at pH’s 1 and 7. Only the 4-chlorobenzenesulonate salt maintained an increased solubility over two days at pH 7 making it a viable candidate for further study in terms of intrathecal administration. During crystallization experiments with (R,S) baclofen two polymorphic forms of the baclofen lactam were generated, Forms II and III. Both forms are conformational polymorphs confirmed by single crystal X-ray diffraction and Form II has a Z’ of 4 with an unusual arrangement of enantiomers.
Chapter 1: Introduction

1.1 Supramolecular Chemistry

1.1.1 van der Waals Forces

When two atoms come together to form a molecule (e.g. N₂) they are held together by a covalent bond, which can be described as sharing of electron density that is delocalized over the entire molecule resulting in a strong chemical bond.¹ The interaction of atoms or molecules with one another is the fundamental basis for the physical state in which they exist under ambient conditions and ultimately affects their physicochemical properties. Johannes Diderik van der Waals was born in 1837 in the Netherlands and was one of the first professors of physics at the University of Amsterdam, then called Athenaeum Illustre of Amsterdam. During van der Waals’s work towards understanding the different phases of physical chemistry he published the van der Waals equation of state that contained a constant related to the strength of attraction between species.² The idea of intermolecular attractive forces were considered earlier by Borelli and Jurin with respect to capillary action where they explain their results by attractive forces between the molecules of the tube and the liquid.³ Once the existence of these forces was accepted, explanations were sought after. Debye suggested molecules have a deformable distribution of charge and are therefore this distribution is not rigid and can be polarized in an external electromagnetic field resulting in attractive forces if the field is non-uniform.⁴ For molecules with dipoles and quadrupoles in the gaseous state at low
temperature Keesom’s alignment effect was developed, which says that as a molecule rotates and moves in space it will frequently collide with another molecule resulting in the molecules positioning in such a way that they are caught in an attractive position.\(^5\) Debye’s theory fills in the gaps for the persistence of van der Waals forces at higher temperatures not accounted for by Keesom. For nonpolar molecules the very weak attractive van der Waals forces can be explained by London Dispersion forces which are related to the deformable distribution of charge mentioned earlier, whereby temporary very weak dipoles are formed resulting in electrostatic attraction.\(^3\) This work led to the measurement and reporting of van der Waals volumes and radii in 1964 once X-Ray diffraction data of crystalline materials became sufficiently available and as of 2010 the manuscript has been cited 10,767 times.\(^6\) X-ray diffraction is now considered the “gold standard” for the characterization of solid-state materials. van der Waals equation of state has been updated several times to account for progress in the field of physical chemistry to its current status as the Elliott, Suresh, Donohue equation of state.\(^7\) These forces can be summed up as weak non-directional electrostatic interactions usually less than 5 kJ/mol.\(^8\)

1.1.2 Coordination Chemistry

The stronger side of electrostatic interaction involves charged species i.e. ionic bonds and coordination bonds also known as coordinate covalent. Coordination chemistry involves a metal ion coordinating with a donating ligand. The ligand in this case can be organic and can be negatively charged, as in the case of a carboxylate, or contain a lone pair of electrons, as in the case of the nitrogen atom of 4, 4′-bipyridine. For the neutral pyridine molecule there is a dipole – ion interaction with the positive charge on the metal, which may be considered a coordinate covalent bond. These
interactions can lead to discrete structures, polymers, and three-dimensional (3D) networks depending on the different coordination geometries of the metal employed but are not considered completely covalent in nature because they are reversible.9 These coordination polymers can have porosity or cages similar to zeolites and when organic ligands are used they are termed metal organic frameworks (MOFs).10

1.1.3 Hydrogen Bonding

A hydrogen bond (H-Bond) can be described as an exaggerated dipole – dipole interaction in which a hydrogen atom is attached to an electronegative atom and is attracted to another dipole in close proximity, in what is termed a donor (D)– acceptor (A) relationship, where the atom covalently linked to the proton (H) is the donor. The relationship can be generally described by, D-H•••A. Linus Pauling described the H-bond as electrostatic in nature early on and he believed the donor atom and the acceptor atom needed to be sufficiently electronegative in order to have enough electrostatic attraction to result in a bond.11 H-bonds are strong and directional by nature compared to van der Waals forces with bond energies ranging from 4 – 120 kJ/mol.8 The weaker end of the H-bond energy spectrum merges into van der Waals forces where there lies a grey area between the two.12

1.1.4 Supramolecular Chemistry and its Biological Significance

Other intermolecular interactions include C-H•••π, π•••π stacking, and cation•••π interactions.8 All of these intermolecular interactions including those mentioned in the two previous sections can lead to self assembled aggregates called supermolecules, where there lies a host guest type of interaction.13 The term supermolecule dates back to 1949.
when the term “übermolekeln” was used for an intermolecular complex.\textsuperscript{14} This self assembly based on intermolecular interactions is termed supramolecular chemistry by Jean-Marie Lehn which he defined as follows, “\textit{Supramolecular chemistry may be defined as chemistry beyond the molecule, bearing on the organized entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces.}”\textsuperscript{15} Supramolecular chemistry could be traced back to investigations into receptor binding in biological systems when a “lock and key” model was described by Emil Fischer.\textsuperscript{16} H-bonding is the lead intermolecular force directing self assembly involving organic compounds and has been described as the “masterkey interaction” in supramolecular chemistry.\textsuperscript{8} Each of the intermolecular interactions described can affect supramolecular self assembly and their bond energies are compared in Table 1.1. Although H-bonds are usually the primary directing interaction for self assembly, other weaker intermolecular interactions can serve to stabilize the resulting structure.

\textbf{Table 1.1: Selected bond energies for comparison.}

<table>
<thead>
<tr>
<th>Bond Type</th>
<th>Bond Energy (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covalent</td>
<td>150-450</td>
</tr>
<tr>
<td>Ionic</td>
<td>100-350</td>
</tr>
<tr>
<td>Ion - Dipole</td>
<td>50-200</td>
</tr>
<tr>
<td>H-Bond</td>
<td>4-120</td>
</tr>
<tr>
<td>(\pi) - Cation</td>
<td>5-80</td>
</tr>
<tr>
<td>Dipole-Dipole</td>
<td>5-50</td>
</tr>
<tr>
<td>(\pi) (-\pi) Stacking</td>
<td>&lt;50</td>
</tr>
<tr>
<td>van der Waals</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>
Using nature as a template for instruction and molecular recognition, the field of supramolecular chemistry has included research involving crown ethers\textsuperscript{17-18}, host guest systems\textsuperscript{19-20}, cryptands\textsuperscript{21-22}, and cavitands.\textsuperscript{23} One of the most important discoveries in biological sciences was the determination of the structure of deoxyribonucleic acid (DNA),\textsuperscript{24} which consists of nucleotide polymers connected via phosphodiester backbones that H-bond between base pairs creating a double helix. This genetic material is necessary for cellular replication and the polymeric strands must be unwound in order to be decoded and translated into instructions for cellular components, therefore a reversible supramolecular process is necessary to read the DNA. H-bonding and supramolecular recognition are necessary for many biological functions including but not limited to receptor binding, immunological responses, protein folding and function, and coordination bonds responsible for hemoglobin’s ability to transport oxygen.\textsuperscript{8}

1.2 Crystal Forms

1.2.1 Single Component Molecular Crystals

Molecules tend to arrange themselves into regular repeating units in the solid state creating a 3D molecular array which is defined as a crystal. If there is no regular repeating unit the solid is termed amorphous, for example glass. Amorphous compounds are known to be unstable and reactive compared to their crystalline counterparts.\textsuperscript{25} An example of a molecular crystalline substance is table sugar which consists of sucrose crystals. The regular repeating 3D unit of a crystal is termed the unit cell and the way molecules arrange themselves inside the unit cell is referred to as crystal packing. Crystals can vary in size from a few nanometers to meters\textsuperscript{26} and exhibit a particular shape.
or morphology. The points in the unit cell that define where molecules reside can be referred to as the crystal lattice. The crystal lattice can be simplified to a 3D box with points inside representing lattice points and if those points were spheres then different centering within the box will determine where other points can reside. There are 7 ways in which a lattice can arrange in the unit cell, called a crystal system, and when combined with different lattice centerings the result is 14 Bravais Lattices (infinite set of points generated by discrete translations) and within these there are only 230 possible arrangements called space groups, which are based on mathematically generated symmetry operations.\(^2^5\) The study and determination of crystal structures comprises the field of crystallography and \(Z\) is defined as the number of formula units in the unit cell, while \(Z'\) is the number of formula units in the crystallographic asymmetric unit. \(Z'\) can be strictly defined as the number of formula units in the unit cell divided by the number of independent general positions.\(^2^7\) Covalent network solids containing only one type of atom, diamond for example, should also be mentioned, however, due to the scope of this manuscript they are not discussed further.

1.2.2 Salts

A crystalline salt is an ionic solid involving a charge-charge interaction between ions of opposite charge. Organic and inorganic salts, like sodium chloride composed of alternating sodium and chloride ions, are held together solely by very strong electrostatic interactions. An organic salt can result from ionizable functional groups or in combination with permanent charge states as in a quarternary nitrogen atom carrying a positive charge. Primary, secondary, or tertiary ammonium salts are examples of charge
assisted H-bonds\textsuperscript{28}, which typically contain ion-dipole electrostatic interactions with bond energies between those of ionic bonds and H-bonds as exemplified in Table 1. Inorganic ions can also readily pair with organic ions as is the case of pharmaceutical hydrochloride salts.

1.2.3 Other Multiple Component Crystals: Solvates, Hydrates, and Cocrystals

When more than one entity or molecule crystallizes together in a stoichiometric ratio it is referred to as a multiple component crystal. When a single component crystalline material has solvent as part of the crystalline arrangement in a stoichiometric ratio the binary crystal is termed a solvate and when water is the solvent involved the crystalline form is referred to as a hydrate. In these instances the solvent helps stabilize the overall packing of the unit cell. The term cocrystal is used very loosely depending on the scientific field it applies to and in terms of solid-state chemistry there is current debate about how narrowly or broadly one defines a cocrystal.\textsuperscript{29-34} For this work a narrow definition of cocrystal is applied: a stoichiometric multiple component crystal that is formed between two compounds that are solids under ambient conditions in which at least one cocrystal former (second molecule) is molecular and forms a supramolecular synthon (specific intermolecular interaction to be discussed further in section 1.4.2) with the remaining cocrystal former(s).\textsuperscript{35-36}

One of the first uses of the term cocrystal in the context of solid-state chemistry dates back to 1967 describing the H-bonded complex between 9-methyladenine and 1-methylthymine, first reported by Hoogsteen.\textsuperscript{37-38} Cocrystals as defined here began to appear in the literature in 1844 via a grinding experiment by Friedrich Wöhler who
prepared the 1:1 quinone:hydroquinone cocrystal by “kugelchen” or little ball likely referring to a ball mill type of apparatus. Cocrystals were not named as such early on and over the years many cocrystals were discovered and reported but labeled under different nomenclature including molecular complexes, addition compounds, organic molecular compounds, heteromolecular crystals, solid-state complexes, and molecular compounds. Both single component and multiple component crystalline materials can have the same building blocks arranged in a different manner considered a separate crystalline entity of the same molecules or a polymorph.

1.2.4 Polymorphism

The generally accepted definition of polymorphism is the ability of a compound to exist in more than one crystalline state. Bernstein, however, provides a more narrow definition limited by what he considers as “safe” criterion for classifying polymorphic systems which states, “classification of a system as polymorphic would be if the crystal structures are different but lead to identical liquid and vapor states”. Pseudopolymorphism is a term McCrone proposed which he defines as, “a convenient term to use to describe a variety of phenomena sometimes confused with polymorphism. They include desolvation, second-order transitions (some of which are polymorphism), dynamic isomerism, mesomorphism, grain growth, boundary migration, re-crystallization in the solid state and lattice strain effects.” A prototypical example of a polymorphic system would be 5-methyl-2-[(2-nitrophenyl)-amino]-3-thiophenecarbonitrile, also known as ROY, which exhibits red, orange, and yellow polymorphs. For organic crystals there are two main types of polymorphism; packing polymorphism, which refers
to the 3D arrangement of individual molecules with respect to one another, and conformational polymorphism, which involves molecules that can adopt different geometries through rotation about single bonds, bond stretching/compressing, and bending of bond angles. Conformational polymorphism began to appear in the literature in the 1970’s. Different molecular geometries that lead to conformational polymorphism in order to satisfy a particular solid-state arrangement under a particular set of physical conditions can often lead to structures with more than one symmetry independent molecule, $Z' > 1$. It should also be noted that intramolecular and intermolecular interactions can affect both conformation and packing characteristics and therefore also play a role in polymorph control. There are two types of polymorphic systems with respect to polymorphic transformations, monotropic and enantiotropic. Considering for the sake of discussion that there are two polymorphs, A and B, monotropic systems represent a situation where once A has converted to B the transformation is irreversible, while enantiotropic systems are reversible. According to McCrone’s statement, “the number of forms known for a given compound is proportional to the time and money spent in research on that compound,” there are virtually endless possibilities and likely there is great validity in this statement, however, existing literature and databases do not represent the full scope of polymorphs discovered since many structures may not be of interest and not reported, therefore it is difficult to confirm. Polymorphism as a whole is an important phenomenon with respect to intellectual property and physicochemical properties especially with respect to pharmaceutical science.
1.3 Solid-State Characterization

X-ray diffraction is the “gold standard” for solid-state characterization but multiple other complimentary techniques such as attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), Raman spectroscopy, solid-state nuclear magnetic resonance (SS NMR), microscopy, differential scanning calorimetry (DSC), and thermal gravimetric analysis (TGA) are used in conjunction. X-ray diffraction is well suited for solid phase characterization due to the X-ray region of electromagnetic radiation containing wavelengths smaller than the diameter of an atom since the principles of optics dictate that the wavelength of light used to observe an object must be smaller than the object itself. This is only part of the story however because no lens exists that can focus X-rays and therefore creating a direct image is not possible. Since X-rays have the ideal wavelength for reflecting off of atoms they can be directed at a crystal resulting in scattering of the radiation in a specific manner called a diffraction pattern. This was first interpreted by William H. Bragg in 1913 and he developed the Bragg equation which remains the basis for this technique, Eq. 1.1.

\[ n\lambda = 2d \sin (\theta) \]  

Eq.(1.1)

This equation states that n, an integer multiple of the wavelength, \( \lambda \), equals twice the distance, d, between planes of atoms multiplied by the sine of the angle of incidence, while the intensity of diffraction is determined by the electron density of the atom.
This technique is used for single crystal analysis resulting in a single crystal structure with the aid of mathematical analysis and is also amenable to powders in which case it is known as powder X-ray diffraction (PXRD). PXRD usually only provides a diffraction pattern and not a single crystal structure but in some cases where the diffraction pattern intensity is large enough the structure can be delineated mathematically from the pattern.56

The other techniques mentioned each have their own advantages in diagnosing a solid phase. ATR-FTIR utilizes absorption of infrared light based on vibrational intramolecular and intermolecular movement which results in a spectral output. New intermolecular interactions between molecules results in shifting of peaks or even new peaks. The other spectroscopic techniques, SS NMR and Raman spectroscopy (measurement of scattering of infrared light), will also result in form specific spectra and similarly, significant new intermolecular interactions in a new solid-state phase will cause shifts in the spectrum. DSC is a valuable tool for determining heats of fusion and resultant enthalpies associated with a specific crystal form and can also be applied to study polymorphic behavior such as reversible phase transitions and the presence of impurities. TGA is ideal for studying solvates and hydrates as it determines weight loss over time upon heating which can delineate the stoichiometry of solvent present and the qualitative strength of the interaction. Microscopy is useful in determining crystal habit/morphology, twinning in some cases, and with the use of a polarizer, whether the crystal is polar. Hot-stage microscopy coupled with other techniques is useful for determining polymorphic transformations and single crystal to single crystal transitions.46
1.4 Crystal Engineering, Supramolecular Synthons, and the Cambridge Structural Database

1.4.1 Background

Designing crystalline materials for specified applications via manipulation of intermolecular interactions has been established as the field of crystal engineering. The term crystal engineering was introduced by Pepinsky in 1955 when he revealed controllable unit cell dimensions and symmetries with metal organic complexes. This nomenclature seemed to be ahead of its time since the development of crystal structure determination, or crystallography, as a field was still young. In 1959 the Hoogsteen base pair cocrystal structure emerged between 9-methyladenine and 1-methylthymine, which was paramount to understanding DNA which utilized Watson-Crick base pairing. It wasn’t until 1971 that the elegance behind supramolecular chemistry and the possibility of engineering crystalline forms came to fruition in the literature with Schmidt’s report on photodimerization in the solid state with cinnamic acids further studied by MacGillivray in 2000 utilizing a cocrystal template as depicted in Figure 1.1.

![Figure 1.1: Single-crystal to single-crystal photodimerization within a cocrystal.](image)

Figure 1.1: Single-crystal to single-crystal photodimerization within a cocrystal.
Guatem Desiraju defined crystal engineering in 1989 as, “the understanding of intermolecular interactions in the context of crystal packing and the utilization of such understanding in the design of new solids with desired physical and chemical properties.”

The importance of supramolecular chemistry was well defined at this point and more attention was paid to understanding intermolecular interactions and how to subsequently manipulate those interactions in crystalline materials. Margaret Etter helped to develop the field in the late 1980’s and 1990’s by studying cocrystallization and analyzing H-bond patterns, which she termed “motifs,” that helped develop graph set analysis to define specific H-bonded motifs. Etter’s contributions helped establish the foundation for the current rapid growth of supramolecular chemistry and in 1991 she proposes three general rules with regards to H-bonding; 1) all good proton donors and acceptors are used, 2) six membered ring intramolecular H-bonds form before intermolecular H-bonds, and 3) the best donors and acceptors left after intramolecular H-bonding will be used for intermolecular H-bonding. She includes the following statement with regards to her work foreshadowing the importance of physicochemical property enhancement through supramolecular chemistry, “We have also shown that hydrogen-bonded molecular aggregates can have unexpected properties as a result of the collective behavior of these weakly bound molecules.”
1.4.2 Supramolecular Synthons

As mentioned in section 1.2.3 supermolecules interact with one another via supramolecular synthons which are defined by Desiraju as, “*structural units within supermolecules which can be formed and/or assembled by known or conceivable synthetic operations involving intermolecular interactions.*” Typically complimentary H-bonds act as the supramolecular synthon, although other intermolecular interactions are also included. There are two classifications of supramolecular synthons based on complimentarity and homogeneity, which are referred to as supramolecular homosynthons and supramolecular heterosynthons. Figure 1.2 below depicts examples of each with respect to carbamazepine (CBZ), an anti-convulsant API.

Figure 1.2: a) Amide•••Amide supramolecular homosynthon between CBZ molecules. b) Acid•••Amide supramolecular heterosynthon between aspirin and CBZ.
When there is self complimentarity between functional groups the result is a supramolecular homosynthon and when different functional groups compliment each other the result is a supramolecular heterosynthon. It should be noted that the term supramolecular mixed homosynthon can be used in the instance where two different carboxylic acids interact via an acid•••acid supramolecular homosynthon. Examples of supramolecular homosynthons are amide•••amide and acid•••acid dimers. Carboxylic acid dimers have been mentioned in the literature as early as 1897 when the association of acetic acid in benzene was recognized. Later work by F. T. Wall in the early 1940’s reported the association of benzoic, m-toluic, and o-toluic acid with themselves in benzene as individual solutions confirmed by vapor pressure osmometry.

Supramolecular heterosynthons can be exemplified by acid•••amide, acid•••aromatic nitrogen (N_{arom}), alcohol•••amine, and alcohol•••N_{arom}. With crystal engineering in mind it was important to determine which supramolecular synthons would persist over others. Synthon competition was assessed by including the presence of other functionalities in order to establish which interactions take precedence in order to determine the hierarchy. Cocrystals have been particularly important in delineating these hierarchies with respect to alcohols, carboxylic acids, N_{arom}, cyanos, and amides. Once Etter established some ground rules in terms of H-bonding patterns others started to experiment with multiple functional groups present at the same time during crystallization. Christer Aakeröy studied whether or not the strongest acid would interact with the strongest base and then if the next strongest acid went with the next strongest base and so on within their chosen set of molecules. He also studied ternary
cocrystals by employing molecules with carboxylic acid, N_{arom}, and amide functionality on the same molecule resulting in acid•••N_{arom} and acid•••amide supramolecular heterosynthons.\textsuperscript{96, 98-99} More reports on this subject soon followed where Bis reveals that alcohols prefer to interact with N_{arom} while in the presence of cyano groups.\textsuperscript{100} Shattock then determined that carboxylic acids in the presence of N_{arom} will prefer the supramolecular heterosynthon over the homosynthon and that the same theme holds true for alcohols in the presence of N_{arom}.\textsuperscript{101} This study also attempted to distinguish between which supramolecular heterosynthon is preferred, however, the findings indicate that both heterosynthons occurred for the limited data set utilized leaving this particular piece of the hierarchy open for debate for now. Charge assisted H-bonds are also categorized as supramolecular synthons\textsuperscript{28, 102} and are also important in terms of crystal engineering.

It has been observed that the carboxylate•••weakly acidic hydroxyl supramolecular heterosynthon is persistent in a set of fifteen zwitterionic cocrystals with nutraceuticals.\textsuperscript{103-106} Figure 1.3 below depicts an earlier example of the carboxylate•••weakly acidic hydroxyl supramolecular heterosynthon between L-sarcosine and L-ascorbic acid which has been deposited into the Cambridge Structural Database (CSD).\textsuperscript{107}

![Figure 1.3: Carboxylate•••Weakly Acidic Hydroxyl supramolecular heterosynthon, CSD Refcode SERASC10 (Serine•••Ascorbic Acid).](image)
Other supramolecular synthons include charge assisted H-bonds with other inorganic ions such as phosphate,\textsuperscript{108} interactions with chlorine of a hydrochloride salt (cocrystal of a salt),\textsuperscript{109} and also halogen bonding, Figure 1.4.\textsuperscript{110-116}

![Figure 1.4: Halogen bonded chain between tetrafluoro-1,4-diiodobenzene and piperazine, CSD Refcode DIVCUH.](image)

1.4.3 The Cambridge Structural Database

The CSD represents a large computerized archive of X-ray and neutron diffraction data of small (less than 500 non-H atoms) organic and metal organic molecules that began in 1965.\textsuperscript{117-118} This database package of software was developed by the Cambridge Crystallographic Data Centre (CCDC) which includes software to visualize and analyze molecular interactions including but not limited to bond distances, bond angles, symmetry elements, histogram generation, and 3D networks. The CSD contains 523,834 entries as of August 2010 and this vast array of structural data has become an integral tool for crystal engineering. One simply needs to draw out a proposed interaction of interest and a plethora of information is likely to be discovered with statistical significance depending on the frequency of occurrence. Each entry is labeled with a six letter code, refcode, which may have numbers attached for repeat entries. The
robustness of supramolecular synthons can be evaluated if enough data on that particular interaction is available. Database mining a very useful tool with regards to solid-state chemistry since one can evaluate a very large pool of data not otherwise available from one source. Indeed, much of the work devoted to understanding supramolecular synthons and their hierarchies mentioned above was supplemented and even guided by CSD analysis and it has now become routine that the initial approach to a crystal engineering experiment involves analysis of existing structural data.

Crystal engineering has led to the discovery of materials with distinct applications such as porous materials, non-linear optics, pharmaceuticals, and photographic materials. An example of the power of the CSD is represented in Figure 1.5, in which the average donor-acceptor distance of the carboxylic acid•••N_arom supramolecular heterosynthon is revealed to be 2.639 Å through histogram generation.

Figure 1.5: D-H•••A (D•••A distance) in Ångstroms for the carboxylic acid•••N_arom supramolecular heterosynthon. CSD parameters; Aug. 2010 Update, Only Organics, R ≤ 0.075, 3D Coordinates Determined, and as drawn in white box.
1.4.4 Crystal Structure Prediction

Crystal form screening, polymorph control, and molecular design would be immensely facilitated if a solid-state chemist could accurately predict the crystalline structure of organic molecular crystals. In general, computational approaches search for the most thermodynamically stable structure. As remarked by Maddox, “One of the continuing scandals in the physical sciences is that it remains in general impossible to predict the structure of even the simplest crystalline solids from a knowledge of their chemical composition,”¹²⁷ it remains true that complete and reliable prediction of a crystal structure has not been achieved.⁴⁵,¹²⁸-¹³⁵ The CCDC held the first crystal structure prediction workshop in 1999 to evaluate the current state of computational methods and the first “blind test” was initiated in which different computational groups were given a molecular diagram and asked to predict the crystal structure.¹²⁸ There have been four blind tests to date and the results are increasingly promising as the fourth test concluded with dramatic improvements in the success rate of prediction.¹²⁸,¹³⁴,¹³⁶-¹³⁷ The fourth test included fourteen groups with four targets in their sites. Thirteen successful predictions prevailed overall and for each target at least two groups were successful, while only one group got all four as their first choices. There is a long way to go but progress in this area is encouraging.
1.5 Pharmaceutical Cocrystals

1.5.1 Synthesis

Pharmaceutical cocrystals are defined as multiple component crystals in which at least one component is molecular and a solid at room temperature (the coformer), and forms a supramolecular synthon with a molecular or ionic active pharmaceutical ingredient (API). Cocrystals and pharmaceutical cocrystals are identical in terms of synthetic routes. Traditional techniques for crystallization apply where supersaturation and subsequent nucleation from solution remains to be the most widely accepted method and in the case of multiple component crystals the supramolecular synthon between individual molecules helps determine the initial aggregate that leads to cocrystal growth. Grinding two solids together, or mechanochemistry, to create a new crystalline phase is perhaps the simplest way to generate a multiple component stoichiometric adduct or cocrystal. It is ideal in terms of cost, environmental impact, and difficulty, and furthermore, the addition of small amounts of solvent during the grinding process was shown to dramatically increase the kinetics of cocrystal formation in certain cases. Grinding can be done by hand with a mortar and pestle or mechanically with a ball mill or mixer mill. The melt/cool method involves melting one component followed by addition of the second component thereby dissolving it followed by controlling the rate of cooling and may be performed in absence of unstable or high melting compounds. Slurry methods, which can be facilitated by sonication, are useful as well and represent a method somewhere in between solvent drop grinding and evaporative solution based techniques. Reaction crystallization is a solution based technique that has been specifically applied to cocrystal formation on the premise of manipulating ternary phase
Reaction crystallization relies upon the cocrystal having a lower solubility in the solvent system involved resulting in precipitation of the cocrystal. Supercritical carbon dioxide can also be used as a unique solvent system to produce cocrystals provided the components are soluble. Less common techniques involve vapor diffusion and layering with different solvents/solutions which are more common for proteins and MOFs respectively. Each of the methods described above can produce pharmaceutical cocrystals and in some cases they will reach the same crystalline form, while in others they may produce polymorphs or even novel forms. Crystal engineering with pharmaceuticals via the supramolecular synthon approach is successful due to the occurrence of functional groups with H-bonding capability, which is related to natures use of supramolecular chemistry to regulate biological functions and indeed APIs contain lots of H-bonding functionalities, see Table 1.2.

Table 1.2: Occurrence of H-Bonding functional groups in the top 100 prescription APIs.

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>% of Top 100 APIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>39</td>
</tr>
<tr>
<td>Tertiary Amine</td>
<td>37</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>35</td>
</tr>
<tr>
<td>Ether</td>
<td>33</td>
</tr>
<tr>
<td>Secondary Amine</td>
<td>31</td>
</tr>
<tr>
<td>Carboxylic Acid</td>
<td>30</td>
</tr>
<tr>
<td>Ester</td>
<td>22</td>
</tr>
<tr>
<td>N-hetom</td>
<td>12</td>
</tr>
<tr>
<td>Secondary Amide</td>
<td>11</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>3</td>
</tr>
</tbody>
</table>
1.5.2 History

The existence of pharmaceutical cocrystals goes very far back in time, however, their importance has only recently been appreciated as highlighted by Zaworotko and Shan as, “long known but little studied.”\textsuperscript{138} It should be noted that the following is not a comprehensive timeline but rather a description of important events that led to the current state of pharmaceutical cocrystals. One of the first appearances of pharmaceutical cocrystals in the literature resides in a French patent describing molecular complexes between barbiturates and amino pyridines.\textsuperscript{150} Barbiturates are central nervous system depressants and years later, 1968 – 1974, more complexes with barbiturates surfaced.\textsuperscript{151-154} Almost concurrently theophylline cocrystals with chlorosalicylic acid and sulfathiazole are reported by Shefter.\textsuperscript{155-156} Theophylline acts as a bronchodilator and sulfathiazole is an antimicrobial so it seems highly likely that synergism was factor of interest for bacterial lung infections. These APIs seemed to have drawn attention to pharmaceutical cocrystals, although they were seen as complexes at the time, which remains a correct chemical description. Since then other theophylline, sulfa drug, and barbiturate pharmaceutical cocrystals continued to be reported throughout the 1980’s early 1990’s.\textsuperscript{157-163} Caira reported complexes with sulfonamides including benzoic acids and salicylic acids as coformers with synergism in mind.\textsuperscript{164-165} In 1992 Zerkowski and Whitesides report a complex between melamine and cyanuric acid which would later become very important.\textsuperscript{166} In 2007 there was a pet food recall due to Chinese protein export contamination in which melamine:cyanuric acid cocrystals crystallized out in the kidneys of animals causing death in many instances.\textsuperscript{167} It is known that cyanuric acid can be produced by hydrolysis of melamine, which was added to the pet food to falsely
mimic proteinogenic amino acids resulting in an inflated protein content analysis. In 2008
this same phenomenon caused the Chinese baby milk scandal that caused thousands of
Chinese babies to get sick while some even lost their lives.$^{168-169}$ Reverting back to 1993
the highly prescribed antidepressant Depakote®, which was actually a cocrystal of salt
between two sodium hydrogen divalproate oligomers, was patented due to its superior
stability compared to the free acid or the monomeric sodium salt, Figure 1.6.$^{170}$

![Figure 1.6: Sodium hydrogen divalproate oligomer.](image)

Throughout the 1990’s and the early 2000’s more examples of pharmaceutical
cocrystals continued to emerge as the advent of crystal engineering began to take hold.$^{68,}
171-178$ In 2003 and 2004 the importance of these pharmaceutical compositions began to
take hold in the patent landscape with the realization that the physicochemical properties
of a molecule can be significantly changed without covalently changing the molecule.$^{179-}
182$ A seminal article by Zaworotko in 2003 sparked lots of interest in what is now the
most studied API in terms of pharmaceutical cocrystals, the anticonvulsant CBZ. The
interest in CBZ grew rapidly and has resulted in the production and analysis of dozens of
multiple component forms.$^{145, 183-188}$ Later in the year Zaworotko also reported multiple
component pharmaceutical phases based on the carboxylic acid···N$_{\text{arom}}$ supramolecular
synthon with 4,4'-bipyridine and the non-steroidal anti-inflammatories (NSAID’s) aspirin, ibuprofen and flurbiprofen. It wasn’t until 2004 that the term pharmaceutical cocrystal is coined by Almarsson and Zaworotko and used from there on out.

1.5.3 Crystal Form Impact and Physicochemical Property Manipulation

“Over 90% of all pharmaceutical products, such as tablets, aerosols, capsules, suspensions, and suppositories contain drug in particulate, generally crystalline form.”

Unique crystalline forms tend to exhibit distinctive physicochemical properties effecting the dissolution, manufacturing, physical stability, permeability, and oral bioavailability of an API. Aqueous solubility of APIs is indispensable for their optimal bioavailability and efficacy. The rate limiting step for absorption of an API in an oral dosage form can be the dissolution of that API in the gastrointestinal (GI) tract. Some of the earliest dissolution experiments were conducted and published by Arthur A. Noyes and Willis R. Whitney in 1897 where they proposed that the rate of dissolution is proportional to the difference of concentration at time t and the saturation solubility governed by Eq. (1.2) below.

\[
\frac{dx}{dt} = C(C_S - x) \quad \text{Eq.(1.2)}
\]

In Eq.(1.2) C denotes a constant, x denotes the concentration at time t, and \( C_S \) denotes the solubility. The constant, C, in this equation assumes that all systems behave similarly however each individual compound will have different rates of diffusion based on its respective physical properties including but not limited to electrostatics. This
mathematical model for their observation as a general law was modified by Bruner and Tolloczko in 1900\textsuperscript{194} to include surface area (S) and then further by Nernst and Brunner in 1904\textsuperscript{195-196} to include a diffusion coefficient (D), the thickness of the diffusion layer (h), and the volume of the dissolution medium (V) as shown in Eq. (1.3).

\[
\frac{dx}{dt} = \frac{DS}{Vh} (C_S - x) \quad \text{Eq. (1.3)}
\]

The advent of this equation was guided by Fick’s second law which predicts how diffusion changes the concentration field i.e. changes in the concentration gradient.\textsuperscript{197} These relationships are related to how kinetics can affect solubility and will dictate how fast an API will dissolve and subsequently be absorbed.

The Biopharmaceutics Classification System (BCS) rates APIs on a scale from class I - IV based on solubility and permeability\textsuperscript{198} and when a compound is deemed class II, for example, it is said to have high permeability and low solubility. Class II compounds represent a situation where absorption is primarily limited by the rate of dissolution and since in Eq. (3) x is negligible compared to $C_S$, sink conditions are created. It could also be inferred that the dissolution rate is more important than the thermodynamic solubility since absorption prevents saturation in GI fluids not to mention dissolution must occur before the thermodynamic solubility can be reached. The FDA guidance on BCS class designation is highlighted on the next page,\textsuperscript{149} although it has recently been suggested by Zaki and Bergström that modification of this classification system may be necessary to reflect \textit{in vivo} behavior more accurately.\textsuperscript{199}
• Class I - High Permeability, High Solubility
  Class II - High Permeability, Low Solubility
  Class III - Low Permeability, High Solubility
  Class IV - Low Permeability, Low Solubility

• A drug substance is considered HIGHERLY SOLUBLE when the highest dose strength is soluble in \( \leq 250 \) ml water over a pH range of 1 to 7.5.

• A drug substance is considered HIGHERLY 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.

• A drug product is considered to be RAPIDLY DISSOLVING when \( \geq 85\% \) of the labeled amount of drug substance dissolves within 30 minutes using USP (US Pharmacopeia) apparatus I or II in a volume of \( \leq 900 \) ml buffer solutions.

When salt formation, amorphous dispersions, particle size reductions, and formulation changes fail to improve solubility and subsequent bioavailability the more recent technology of pharmaceutical cocrystallization can be employed. Amorphous compositions are typically more soluble than their crystalline counterparts but are much less stable in the solid state as they tend to revert to more thermodynamically stable crystalline compositions,\(^{192}\) therefore their use to improve solubility is not desired if other crystalline options exist. Dissolution rate can be either increased or decreased depending on the particular crystalline form.\(^ {143}\) In 2003 Transform Pharmaceuticals, Inc. reported cocrystals between cis-itraconazole, an anti-fungal, and carboxylic acids with improved dissolution profiles compared to pure API and a comparable profile of corystals with L-malic acid or L-tartaric acid against the amorphous marketed form (Sporanox) in 0.1 N HCl at 25° C, Figure 1.7.\(^ {200}\)
In 2004 Childs in conjunction with SSCI Inc. divulged the first pharmaceutical cocrystal with a hydrochloride salt, fluoxetine HCl with carboxylic acids, with improved intrinsic dissolution profiles, Figure 1.8a. Another important part of that study was the appearance of the “spring and parachute” behavior of certain cocrystals where an initial spike in the dissolution profile is followed by a decrease toward the concentration of the pure API indicating dissociation of the cocrystal, Figure 1.8b. Dissolution enhancement through pharmaceutical cocrystallization continued to be studied and reports have continuously surfaced since 2003. Other important physical properties like stability to humidity and improved compressibility have also been demonstrated with pharmaceutical cocrystals.
Figure 1.8: a) Intrinsic dissolution profiles for fluoxetine HCl pharmaceutical cocrystals in water at 10° C. b) Spring and parachute of fluoxetine HCl:succinic acid during powder dissolution in water at 20° C.
Pharmaceutical cocrystals represent an opportunity to diversify the number of crystal forms of a given API and in turn fine tune or even customize its physicochemical properties without the need for chemical (covalent) modification. There is no longer any doubt that cocrystals can change the physicochemical properties of a given API, however, the way these changes affect the pharmacokinetic (PK) profile is not predictable or fully understood due in part to the limited number of animal studies reported in the literature. In fact, there are only thirteen case studies in the literature to date, including patent literature, which report pharmaceutical cocrystal PK studies in animals.

In 2005 Transform Pharmaceuticals, Inc. applied for two patents, both of which included animal PK data on pharmaceutical cocrystals. One for modafinil (antinarcoleptic) revealed a cocrystal with malonic acid to show an increase in $C_{\text{max}}$ (maximum plasma concentration) and AUC (area under the curve) of about 43% and 23% respectively when compared to the marketed form, Figure 1.9.²²⁰

![Figure 1.9: Plasma concentration over time for the modafinil:malonic acid pharmaceutical cocrystal over 24 hrs compared to pure API.](image)
The other provided data for itraconazole cocrystals with tartaric acid revealing an even greater enhancement reflected by an approximate increase in both $C_{\text{max}}$ and AUC of 80% with a reduction of $T_{\text{max}}$ (time to reach $C_{\text{max}}$) by 80% when compared to the marketed version which is an amorphous dispersion coated on beads. This work was followed by McNamara in 2006 when a sodium channel blocker was cocrystallized with glutaric acid. The resulting cocrystal showed a ca. 14-fold increase in plasma $C_{\text{max}}$ when compared to the parent API at 50 mg/kg dose in dogs, Figure 1.10.

![Figure 1.10: Plasma concentration of the glutaric acid pharmaceutical cocrystal over time in dogs at 50mg/kg oral dosing compared to parent API, Open Circles = Cocrystal, Filled Circles = Pure API.](image)

Also in 2006 Variankaval in association with Merck & Co., Inc. published a phosphodiesterase 4 inhibitor cocrystal with L-tartaric acid. Due to the lack of single crystal X-ray diffraction data, the determination of cocrystal rather than salt was based upon $\Delta pK_a$ and solid state NMR data. They reported plasma concentrations compared to the parent API in rhesus monkeys revealing an approximate 15-fold increase in $C_{\text{max}}$ and
23-fold increase in AUC. In 2007 Chen in association with Merck & Co., Inc. reported the first inorganic acid cocrystal in which they claim solubility of > 250 mg/mL and excellent in vivo performance, however they show no data to back up such claims. 2007 also produced an example in dogs where the CBZ:saccharin cocrystal was slightly better than the marketed form of CBZ, Figure 1.11.

Figure 1.11: Plasma concentration in dogs for the CBZ:saccharin pharmaceutical cocrystal in a capsule compared to the marketed form immediate release tablet at a 200 mg dose equivalent for CBZ.

In 2008 Bak produced an example in which an API: excipient interaction that led to the discovery of a cocrystal between sorbic acid and a vanilloid receptor 1 antagonist, AMG 517, that proved to have about an 8-fold increase in C\text{max} and AUC compared to the parent API in rats. A patent application in 2008 revealed a fumaric acid cocrystal of tenofovir disoproxil, a reverse transcriptase inhibitor, which showed bioequivalence to the marketed form in rats. 2009 was equally uneventful in producing PK data on cocrystals since only one patent application, which originated in Europe as EP 2009010 A1, described a C-glycoside derivative - L-proline cocrystal for the treatment of diabetes that
had better efficacy in mice.\textsuperscript{223} 2010 has continued to produce animal studies and Cheney contributed to this area with a case study of lamotrigine salts and cocrystals where the cocrystals decreased the serum concentration in rats.\textsuperscript{143} Also in 2010 Stanton provides us more animal studies with AMG 517 cocrystallized with carboxylic acids and their corresponding amides in rats via oral gavage at 100 mg/kg resulting in 2.4 – 7.1 fold increase for all cocrystals in plasma AUC over six hours compared to the free base.\textsuperscript{206} An interesting part of this study was the concomitant measurement of former concentration as part of their powder dissolution measurements where increases in benzoic acid and benzamide concentrations nicely complimented the decrease in the free base, API, concentration implying that the cocrystal dissociates over time, Figure 1.12.\textsuperscript{206}

![Figure 1.12: Powder dissolution profiles over 4 hrs for AMG 517 pharmaceutical cocrystals. AMG 517= triangles, benzoic acid = open squares, benzamide = filled squares, cinnamic acid = open circles, cinnamide = filled circles.](image)

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Finally in 2010 the indomethacin:saccharin pharmaceutical cocrystal was analyzed in dogs at pH 1.2 and 7.4, which ended up being comparable to the marketed form.\textsuperscript{216} These examples provide a solid foundation for pharmaceutical cocrystals as a tool to fine tune the physicochemical properties of APIs without making or breaking a covalent bonds with the possibility of enhancing the PK performance.

1.5.4 Intellectual Property

As mentioned in section 1.5.2 patent activity on pharmaceutical cocrystals began to increase after the realization in 2003 that physicochemical properties could be modified via cocrystallization and that these new crystalline forms are new compositions of matter with unpredictable new physicochemical properties enabling them as patentable forms. This new intellectual property caught the attention of the pharmaceutical industry very rapidly and caused an increase in patent activity.\textsuperscript{179-182, 224-225} Pharmaceutical cocrystals offer the advantage of life cycle management on old API’s as well as improved clinical performance. Similar to salt screening, cocrystal screening has now become another tool for preformulation chemists to search for novel crystalline forms with novel physicochemical properties expanding the scope of pipeline production. There are two examples of how important crystal forms are to intellectual property and while they are not cocrystals they still represent the importance of crystalline forms in general to the pharmaceutical industry. The first is the case of ranitidine.

Ranitidine HCl, marketed as Zantac®, was developed in the 1970’s by Allen and Hanburys Ltd. as part of the Glaxo group, now GlaxoSmithKline (GSK), for antagonism of the histamine H\textsubscript{2} receptor for the treatment of ulcers. The hydrochloride salt was
discovered and patented by Glaxo in 1978. In 1980 a batch was found to have different PXRD and IR data and this new form was called form II for which two patents were granted in 1985 and 1987. Before expiration of the form I patent generic companies attempted to produce form I to be ready to market it by replicating example 32 of the form I patent but they claimed this example led to form II and therefore the form II patent is invalid but Glaxo wins in litigation proving form I can be made by example 32 and claims form II seeds were present in the generic companies attempts. The generic companies then continue to pursue form I and Glaxo sues, still claiming form II is present, but this time they lose, however the litigation took till 1998 allowing Glaxo to continue to make money on form I longer than they should have. At that time this was the highest grossing API pulling in billions of dollars.

The second is the case of ritonavir, an HIV protease inhibitor marketed by Abbott Laboratories. In 1996 ritonavir was marketed as Norvir® and was available as an oral liquid or a semi-solid capsule. Both of these formulations contained an aqueous ethanolic solution because the solid-state form was not bioavailable. During research and development only one crystal form was discovered and 240 lots of the capsules were made without any problems. By the middle of 1998 however, some lots had failed dissolution testing due to a new polymorph with very reduced solubility, which was characterized and found to be a conformational polymorph.
This new, more stable but less soluble, form II of ritonavir then popped up throughout the manufacturing process putting a halt to the old formulation. This cost Abbott about $250 million dollars and lots of bad publicity. These examples of crystal form control highlight the importance of extensive polymorph screens and, in the case of ritonavir, even a liquid formulation needs solid-state form screening in order to avoid potential precipitation.

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Chapter 2: Mechanochemistry: Solvent Drop Grinding vs. Solution Evaporation for the Synthesis of Pharmaceutical Cocrystals Involving Carbamazepine

2.1 Background

Carbamazepine (CBZ) is an API with the brand name Tegretol® that is used mainly as an anticonvulsant but also as an analgesic and is known as 5H-dibenzo[b,f]azepine-5-carboxamide. Oral dosage forms consist of tablets or a suspension with eighty percent bioavailability despite its low aqueous solubility, however, the absorption is slow in humans with a variable half life of twenty five to sixty five hours. CBZ represents a microcosm of the challenges and opportunities related to crystal forms of APIs since it exhibits polymorphism\(^1\) to the extent of eight different polymorphs\(^2-10\) and readily forms a less soluble dihydrate when exposed to moisture.\(^11\) The amide functionality predisposes this molecule to various H-bonding possibilities. Therefore, it should not be surprising that CBZ was an early candidate for cocrystallization studies\(^12-13\) and it is one of the few APIs for which there is published data concerning bioavailability of a cocrystal, the 1:1 cocrystal of CBZ and saccharin. This cocrystal does not form a hydrate and it exhibits improved bioavailability in dogs when compared to Tegretol® tablets, Figure 1.1.\(^14\) In fact, it has been proposed that the CBZ:Nicotinamide cocrystal exhibits a 152-fold increase in aqueous solubility compared to the dihydrate.\(^15\)
Zaworotko\textsuperscript{12,16} and others\textsuperscript{17} have reported that CBZ forms supramolecular adducts with a wide range of complementary cocrystal formers and a recent report addressed the preparation of CBZ cocrystals through four different methodologies, including solvent drop grinding (SDG).\textsuperscript{17} The research herein addresses SDG as a methodology for the reproduction of cocrystals with CBZ that have been previously reported via solution crystallization using and a wide range of organic coformers and solvents with varying functionalities.

Mechanochemistry in its earliest incarnation, grinding of solids, is a technique that dates back thousands of years in that tribal medicine utilized something similar to a mortar and pestle to process medicinally beneficial plants\textsuperscript{18} and even today a mortar and pestle symbolizes pharmacies and schools of pharmacy. To the best of my knowledge, the first report of mechanochemistry in the scientific literature appeared over 160 years ago in 1844 when Wöhler prepared the 1:1 quinone•hydroquinone cocrystal by “kugelchen”.\textsuperscript{19} In 1893 cocrystals of charge transfer complexes were studied and they were also prepared via grinding of solids.\textsuperscript{20} Today solid state grinding represents an attractive alternative to solution processes because it is an inherently “Green” approach to synthetic chemistry in that it offers a facile and low or no waste methodology for discovery or processing of new or existing compounds. Recent interest in grinding and cocrystals can be traced to the 1980’s, when Etter \textit{et al.} demonstrated that dry grinding, also referred to as neat grinding, represents a viable methodology to prepare cocrystals of, for example, methyladenine and methylthymine.\textsuperscript{21-29} An important refinement to grinding came when a small but controlled amount of solvent was added during the grinding process. SDG was reported by Shan \textit{et al.} in 2002 and it became evident that the
The kinetics of cocrystal formation can be significantly enhanced through SDG. SDG can offer other advantages over solution crystallization since dissolution of both cocrystal formers is not required, solvent interactions that might interfere with solute-solute interactions are more limited and we are taken to a region of the ternary phase diagram that might favor cocrystals over starting materials. Indeed, in certain cases SDG can produce cocrystals that are not readily obtainable through solution crystallization and SDG can be employed for control over polymorphism in caffeine:glutaric acid cocrystals. Two concomitant polymorphs of the caffeine:glutaric acid cocrystal result from solution but SDG with a non-polar solvent afforded only form I whereas a polar solvent afforded only form II. SDG has been used in other situations to control polymorphism. Whereas there are numerous examples of cocrystals made by SDG that have also been grown from solution and characterized by single crystal X-ray diffraction, a direct comparison of the efficacy of SDG vs. solution crystallization over a wide range of cocrystal formers has not been systematically studied. This is addressed herein through studying cocrystals with the range of supramolecular synthons involving CBZ, Figures 2.1 and 2.2.

Figure 2.1: Chemical diagram of CBZ.
2.2 Experimental Details

2.2.1 Materials

Reagents were obtained from commercial sources and used as received. Solvents were obtained from commercial sources and distilled before use.

2.2.2 Methods

Cocrystals were characterized by infrared spectroscopy (IR), X-ray powder diffraction (PXRD), and single crystal X-ray analysis where applicable. IR data was collected using a Nicolet Avatar 320 FTIR instrument. PXRD data was collected using a Bruker AXS D8 discover X-ray diffractometer equipped with GADDS™ (General Area Diffraction Detection System), a Bruker AXS HI-STAR area detector at a distance of
15.05 cm as per system calibration, a copper source, an automated x-y-z stage, and a 0.5 mm collimator. Data were collected over a 3.0-40.0 2θ range at a step size of 0.05 2θ.

SDG: Cocrystal formers were subjected to grinding with an agate mortar and pestle for 4 minutes and thereafter characterized by IR and PXRD. In the event that partial conversion was achieved a ball mill, SPEX 8000M Mixer/Mill, was used for 2 increments of 10 minutes with addition of solvent prior to each increment. The same stoichiometric ratios that the cocrystals exhibited after solution crystallization were used for grinding unless otherwise specified. The volumes of solvents used during SDG were as follows: methanol (MeOH) 20 μL, ethylacetate (EtOAc) 20 μL, dimethylsulfoxide (DMSO) 4 μL, water 5 μL, toluene (Tol) 20 μL, cyclohexane (Cychex) 20 μL, chloroform 20 μL, dimethylformamide (DMF) 4 μL. Each solvent was used for each cocrystal experiment.

Experimental details for each of the single crystal crystallizations have been previously published and the SDG experiments were conducted as described earlier. The following cocrystals were studied in terms of SDG: 2:1 cocrystal of CBZ and 4,4’-bipyridine, 1; hydrate of the 1:1 cocrystal of CBZ and 4-aminobenzoic Acid, 2; 1:1 cocrystal of CBZ and 2,6-pyridinedicarboxylic Acid, 3; 2:1 cocrystal of CBZ and benzoquinone, 4; 2:1 cocrystal of CBZ and terephthalaldehyde, 5; 1:1 cocrystal of CBZ and saccharin, 6; 1:1 cocrystal of CBZ and nicotinamide, 7; 1:1 cocrystal of CBZ and aspirin, 8.
2.3 Results and Discussion

2.3.1 Reproducibility

The reliability of SDG to reproduce cocrystals of CBZ that were first prepared by slow evaporation was systematically analyzed. Cocrystal formers contained a wide range of functional groups which seemed to have no effect on reproducibility. Cocrystals 1-8 were all reproduced with at least one solvent, Table 2.1. The 100% rate of success for producing cocrystals obtained by solution further confirms the reliability of SDG for reproducing a specific form in a controlled fashion with little waste although it remains to be seen if SDG and slurrying complement one another. Indeed, a recent study of slurrying using ethanol effected polymorphic transformation of the CBZ:isonicotinamide cocrystal.44 Experimental and calculated PXRD patterns and IR spectra for 1-8 are presented in Appendix 1.
2.3.2 Solvent Choice

Solvents with a range of polarity from non-polar to polar extremes were employed. Organic solvents like Cyclohex and Tol represent the non-polar side while water and DMSO represent the polar side. The middle of the range was covered with solvents like MeOH and EtOAc. The solvents used for single crystal growth were not always used in this screen however that could be useful in further studies. In the cases where that solvent was used the results were a positive match. DMF and DMSO appear particularly well suited for SDG. Given that DMF is slightly less prone to solvate formation when compared with DMSO\textsuperscript{45}, it would probably be the solvent of choice if only one SDG experiment were to be conducted.

Table 2.1: Summary of SDG results for Cbz: Red = starting materials, Green = cocrystal formed, and Hyd = hydrate.

* Indicates Unconverted Starting Materials by Powder X-Ray Diffraction.

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<th>Cocrystal Former</th>
<th>CHCl\textsubscript{3}</th>
<th>Water</th>
<th>DMF</th>
<th>DMSO</th>
<th>MeOH</th>
<th>Cyclohex</th>
<th>Toluene</th>
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However, in the case of 8 only DMSO reproduced the cocrystal form obtained from solution. Nevertheless, it is clear that there is an underlying trend: the more soluble the API and the cocrystal formers are in the solvent used for SDG, the more likely it is that the cocrystal will be produced.

2.3.3 Utility as a Screening Technique

This set of SDG experiments involved more than one supramolecular synthon and is perhaps more representative of an industrial situation for which crystal form screening would probably be conducted with multiple cocrystal formers and solvents. Our results complement those of Childs et al.,\textsuperscript{17} who utilized SDG, evaporation, Sonic Slurry\textsuperscript{TM}, and “reaction crystallization” to screen for novel forms of CBZ with pharmaceutically accepted carboxylic acids while simultaneously screening for polymorphic behavior. Using sonication for cocrystal formation is a relatively recent technique which was introduced into the literature by Bucar et al.\textsuperscript{46} While SDG can be used for polymorph screening, other techniques have also been employed: supercritical fluid,\textsuperscript{47} reaction crystallization,\textsuperscript{17} and Sonic Slurry\textsuperscript{TM}.\textsuperscript{17}

2.4 Conclusions

SDG is indeed a viable technique to generate cocrystals since all 8 cocrystals that were prepared from solution were reproduced, often times with multiple solvents. Alternative methodologies which are also solvent based include crystallizations at high pressure\textsuperscript{48} and slurries\textsuperscript{49} but, as for slow evaporation, the volume of solvent necessary to perform these techniques is usually significant. Grinding or milling is typically used for particle size reduction as a means of improving the dissolution rate of APIs and there also
exists a correlation between particle size reduction and bulk properties such as flowability, bulk density, mixing ability etc.\textsuperscript{50} However, cocrystals are new compositions of matter and the thermodynamic solubility of the API is therefore not only affected by particle size dynamics. Therefore, given that the newest generation of APIs tends to exhibit an increasing tendency towards low water solubility, i.e. they are BCS class II drugs,\textsuperscript{51} the generation of pharmaceutical cocrystals could have important implications for drug development. That the cocrystals of this study and, by implication, cocrystals in general may be synthesized via SDG is therefore relevant to both discovery and processing of cocrystals. In addition, SDG provides an eco-friendly alternative to the use of relatively large amounts of solvent. In summary, SDG is a broadly useful, inexpensive, and “Green” approach to cocrystal formation.

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3.1 Background

Traditional routines are resistant to change in the pharmaceutical industry but during the 1990’s a major shift revolutionized the in vitro screening process from aqueous based manual screening to high throughput screening in the name of efficiency and hopes of increased pipeline opportunities. The high throughput process began with the utilization of dimethylsulfoxide (DMSO) as a medium which led to numerous hits exhibiting very low aqueous solubilities.\(^1\) As a result the majority of API’s currently in development exhibit aqueous solubilities less than 0.1 mg/mL.\(^2\) Meloxicam, 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a non-steroidal anti-inflammatory drug (NSAID) originally developed by Boehringer Ingleheim in 2000. It is used for the following indications; rheumatoid and osteoarthritis,\(^3\) postoperative pain\(^4-5\) and fever.\(^6\) Oral doses comes as 7.5 or 15 mg tablets or a 7.5mg/mL suspension (no greater than 15 mg per day). It exists as a yellow solid that is practically insoluble in water\(^7\) and is considered a Class II drug (i.e. low solubility and high permeability) by the Biopharmaceutics Classification System (BCS).\(^8\) Meloxicam has variable aqueous solubility related to its pH dependent ionization states. Under acidic conditions meloxicam is present in solution in its cationic form, while in basic solutions it is present in its anionic form. When the molecule is neutral in charge it will either be in its zwitterionic or enolic form depending on the polarity of the solvent.\(^9-10\) The different
ionization states of meloxicam are shown in Figure 3.1. Due in part to its low solubility under acidic conditions the $T_{\text{max}}$ (time to reach maximum concentration) of meloxicam in the human body is typically four to six hours, while it can take more than two hours for the drug to reach its therapeutic concentration in humans.$^{11}$

![Figure 3.1 Meloxicam and its ionization states.](image_url)

The slow onset of meloxicam prevents it from potential application for the relief of mild to medium level acute pain. To accelerate its onset of action, various complexes of meloxicam have been prepared and evaluated with respect to aqueous solubility, including cyclodextrin inclusion complexes,$^{12}$ various solvates,$^{7}$ ethanolamine,$^{13}$ ammonium and sulfate salts,$^{9}$ or metal complexes with potassium and calcium.$^{14}$ Preparation of polymorphic crystal forms of meloxicam$^{10}$ has also been attempted,
although unsuccessfully, to improve its dissolution profile.\textsuperscript{15-16} In spite of all the efforts that have been taken, a faster onset oral dosage form for meloxicam (i.e. 30 minutes or less) does not exist at this time.

Unique crystalline forms tend to exhibit distinctive physicochemical properties effecting the dissolution, manufacturing, physical stability, permeability, and oral bioavailability of an API.\textsuperscript{17-19} A typical crystal form selection process comprises two stages of development after a target API has been selected: discovery of as many pharmaceutical crystal forms as possible and examination of the physicochemical properties of the newly discovered crystal forms. When salt formation, amorphous dispersions, particle size reductions, and formulation changes fail to improve solubility and subsequent bioavailability the more recent technology of pharmaceutical cocrystallization can be employed. A given API may form cocrystals with numerous pharmaceutically acceptable and/or approved materials, and these cocrystals could exhibit enhanced solubility\textsuperscript{20-23} and/or stability to hydration or compressibility.\textsuperscript{24} At the stage of crystal form discovery, two primary approaches are used. The more straightforward approach is largely based on trial-and-error (e.g. high throughput crystal form screening) and has been implemented to discover crystal forms including, but not limited to, salts,\textsuperscript{18} hydrates,\textsuperscript{25} solvates,\textsuperscript{26} and, more recently, cocrystals.\textsuperscript{27,28-29} The alternative approach for crystal form discovery is the supramolecular synthon approach,\textsuperscript{30} which recognizes supramolecular synthons\textsuperscript{31} as a design tool and can be more selective, time-efficient and cost-effective. The supramolecular synthon approach uses crystal engineering\textsuperscript{32-40} to carefully analyze the relevant supramolecular arrangements that an API might exhibit by utilizing the Cambridge Structural Database (CSD),\textsuperscript{41} and
effectively prioritize all possible guest molecules for crystal form screening. Such an
approach can be generally effective but has found particular success in generating
pharmaceutical cocrystals.\textsuperscript{30, 42}

Pharmaceutical cocrystals represent an opportunity to diversify the number of
crystal forms of a given API and in turn fine tune or even customize its physicochemical
properties without the need for chemical (covalent) modification. There is no longer any
doubt that cocrystals can change the physicochemical properties of a given API, however,
the way these changes affect the pharmacokinetic (PK) profile is not predictable and is
not yet fully understood due in part to the limited number of animal studies reported in
the literature.\textsuperscript{23, 43-51} These examples, discussed in Chapter 1, do however provide a solid
foundation for pharmaceutical cocrystals as a tool to fine tune physicochemical and
subsequent PK properties of API’s without making or breaking a covalent bond.
While the work presented herein was being performed a report on cocrystals of
meloxicam with carboxylic acids by grinding surfaced.\textsuperscript{52}

Compared to other drugs in the same class, such as piroxicam, meloxicam is
preferred due to its ability to selectively inhibit cyclooxygenase-2 (COX-2).\textsuperscript{53-54} The only
difference between these two API’s is a pyridine ring on the amide linkage for piroxicam
versus a thiazole ring on meloxicam. Between a previous report on piroxicam cocrystals
with carboxylic acids\textsuperscript{55} and CSD analysis, pharmaceutical cocrystallization of meloxicam
with carboxylic acids\textsuperscript{56} represents a promising approach to diversify the crystal form
portfolio to be used to improve the relevant aqueous solubility and accelerate the onset of
action for acute mild to medium level pain relief. Indeed a recent report on PK analysis in
rats for an aspirin:meloxicam cocrystal resulted in a 44 fold increase in kinetic solubility
and a 4.4 fold increase in bioavailability. That study utilized previously reported
toxicological information resulting from co-administration of meloxicam and aspirin. The
rate limiting step in absorption and subsequent bioavailability of a BCS class II API is
solubility since as it dissolves and readily absorbs due to its high permeability sink
conditions are created. Synthesis and rat PK analysis for a set of meloxicam
pharmaceutical cocrystals is presented with this in mind.

3.2 Experimental Details

3.2.1. Materials

Meloxicam was purchased from Jai Radhe Sales, India with a purity of 99.64%
and was used without further purification. All other chemicals were supplied by Sigma-
Aldrich and used without further purification.

3.2.2 Methods

Meloxicam was reacted with 11 selected coformers: fumaric acid, succinic acid,
maleic acid, malonic acid, gentisic acid, 4-hydroxybenzoic acid, adipic acid, (+)-
camphoric acid, glycolic acid, DL-malic acid, and α-ketoglutaric acid. All coformers
produced at least one meloxicam cocrystal except α-ketoglutaric acid. The
cocrystallization attempts resulted in 10 cocrystals (1 – 10), many of which were
prepared via multiple synthetic techniques including solvent-drop grinding and
slurrying. Single crystals suitable for X-ray diffraction were successfully prepared for
two of the new cocrystals (1 & 2).
Synthesis of meloxicam:fumaric acid (2:1) cocrystal (1) – (a) solvent-drop grinding – 0.088 g (0.250 mmol) meloxicam was ball-milled with 0.015 g (0.129 mmol) of fumaric acid and 50 μL of THF for 30 minutes, generating 1 in ca. 100% yield; (b) slurry – 0.880 g (2.50 mmol) meloxicam and 0.150 g (1.29 mmol) of fumaric acid were slurried in 3 mL of THF overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was filtered and washed with THF. 1 was isolated in ca. 81% yield; (c) solution crystallization – 0.100 g (0.284 mmol) meloxicam and 0.330 g (0.284 mmol) of fumaric acid was dissolved in 9 mL of a THF and left to slowly evaporate resulting in single crystals of 1 (ca. 31% yield).

Synthesis of meloxicam:succinic acid (2:1) cocrystal (2) – (a) solvent-drop grinding – 0.088 g (0.250 mmol) meloxicam was ball-milled with 0.015 g (0.127 mmol) of succinic acid and 50 μL of THF for 30 minutes, generating 2 in ca. 100% yield; (b) slurry – 0.880 g (2.50 mmol) meloxicam and 0.150 g (1.27 mmol) of succinic acid were slurried in 3 mL of THF overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was filtered and washed with THF. 2 was isolated in ca. 78% yield; (c) solution crystallization – 0.100 g (0.284 mmol) meloxicam and 0.017 g (0.142 mmol) of succinic acid was dissolved in 10 mL of 1:1 THF and left to slowly evaporate. Single crystals of 6 (ca. 55% yield) grew concomitantly with meloxicam form I and succinic acid.

Synthesis of meloxicam:maleic acid (1:1) cocrystal (3) – (a) solvent-drop grinding – 0.175 g (0.498 mmol) meloxicam was ball-milled with 0.058 g (0.498 mmol) of maleic acid and 40 μL of THF for 30 minutes, generating 3 in ca. 100% yield; (b) slurry – 0.750 g (2.13 mmol) meloxicam and 0.248 g (2.13 mmol) of maleic acid were slurried in 2 mL of THF overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was
filtered and washed with the same solvent employed for the slurry. 3 was isolated in ca. 92% yield. 3 can also be synthesized via ethyl acetate slurry.

**Synthesis of meloxicam:malonic acid (1:1) cocrystal (4)** – (a) solvent-drop grinding – 0.175 g (0.498 mmol) meloxicam was ball-milled with 0.052 g (0.498 mmol) of malonic acid and 40 μL of THF for 30 minutes, generating 4 in ca. 100% yield; (b) slurry – 0.900 g (2.56 mmol) meloxicam and 0.266 g (2.56 mmol) of malonic acid were slurried in 2 mL of THF overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was filtered and washed with THF. 4 was isolated in ca. 88% yield.

**Synthesis of meloxicam:gentisic acid (1:1) cocrystal (5)** – (a) solvent-drop grinding – 0.175 g (0.498 mmol) meloxicam was ball-milled with 0.077 g (0.498 mmol) of gentisic acid and 40 μL of chloroform or THF for 30 minutes, generating 5 in ca. 100% yield; (b) slurry – 0.850 g (2.41 mmol) meloxicam and 0.373 g (2.41 mmol) of gentisic acid were slurried in 2 mL of chloroform overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was filtered and washed with the same solvent employed for the slurry. 5 was isolated in ca. 85% yield. 5 can also be synthesized via slurry in ethyl acetate.

**Synthesis of meloxicam:4-hydroxybenzoic acid (1:1) cocrystal (6)** – solvent-drop grinding – 0.175 g (0.498 mmol) meloxicam was ball-milled with 0.069 g (0.498 mmol) of 4-hydroxybenzoic acid and 40 μL of THF for 30 minutes, generating 6 in ca. 100% yield.

**Synthesis of meloxicam:adipic acid (2:1) cocrystal (7)** – (a) solvent-drop grinding – 0.088 g (0.250 mmol) meloxicam was ball-milled with 0.018 g (0.123 mmol) of adipic acid and 50 μL of THF for 30 minutes, generating 7 in ca. 100% yield; (b) slurry – 0.880
g (2.50 mmol) meloxicam and 0.180 g (1.23 mmol) of adipic acid were slurried in 3 mL of THF overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was filtered and washed with THF. 7 was isolated in ca. 80% yield.

**Synthesis of meloxicam:DL-malic acid (2:1) cocrystal (8)** – (a) solvent-drop grinding – 0.088 g (0.250 mmol) meloxicam was ball-milled with 0.017 g (0.127 mmol) of DL-malic acid and 50 μL of THF for 30 minutes, generating 8 in ca. 100% yield; (b) slurry – 0.880 g (2.50 mmol) meloxicam and 0.170 g (1.27 mmol) of DL-malic acid were slurried in 3 mL of THF overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was filtered and washed with THF. 8 was isolated in ca. 79% yield.

**Synthesis of meloxicam:(+)-camphoric acid (3:2) cocrystal (9)** – (a) solvent-drop grinding – 0.233 g (0.663 mmol) meloxicam was ball-milled with 0.886 g (0.042 mmol) of DL-malic acid and 50 μL of chloroform for 30 minutes, generating 9 in ca. 100% yield; (b) slurry – 0.700 g (1.99 mmol) meloxicam and 0.266 g (1.33 mmol) of (+)-camphoric acid were slurried in 4 mL of chloroform overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was filtered and washed with chloroform. 9 was isolated in ca. 91% yield.

**Synthesis of meloxicam:glycolic acid (1:1) cocrystal (10)** – (a) solvent-drop grinding – 0.175 g (0.498 mmol) meloxicam was ball-milled with 0.038 g (0.498 mmol) of glycolic acid and 40 μL of chloroform for 30 minutes, generating 10 in ca. 100% yield; (b) slurry – 0.950 g (2.70 mmol) meloxicam and 0.206 g (2.70 mmol) of glycolic acid were slurried in 2 mL of ethyl acetate overnight sealed under ambient conditions at ca. 250 rpm. The resulting solid was filtered and washed with ethyl acetate. 10 was isolated in ca. 92% yield.
Crystal Form Characterization

Quality single crystals for X-ray diffraction were obtained for two compounds, 1 and 2. Attempts to crystallize 3-10 did not afford crystals suitable for single crystal X-ray crystallographic analysis. Single crystal analysis for 1-2 was performed on a Bruker-AXS SMART APEX CCD diffractometer with monochromatized Cu Kα radiation (\( \lambda = 1.54178 \, \text{Å} \)). Data for 1 - 2 were collected at 100 K. Lattice parameters were determined from least-squares analysis, and reflection data were integrated using SAINT.60 Structures were solved by direct methods and refined by full matrix least squares based on \( F^2 \) using the SHELXTL package.61 All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms bonded to carbon, nitrogen, and oxygen atoms 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. Hydrogen atoms bonded to methyl groups were placed geometrically and refined with an isotropic displacement parameter fixed at 1.5 times \( U_q \) of the carbon atoms.

**Powder X-Ray Diffraction (PXRD):** 1-10 were characterized using a D-8 Bruker X-ray Powder Diffractometer using Cu Kα radiation (\( \lambda = 1.54178 \, \text{Å} \)), 40 kV, 40 mA. Data were collected over an angular range of 3 ° to 40 ° 2\( \theta \) value in continuous scan mode using a step size of 0.05 ° 2\( \theta \) value and a scan rate of 5 °/min.

**Calculated PXRD:** Calculated PXRD diffractograms were generated from the single crystal structures of 1-2 using Mercury 2.2 (Cambridge Crystallographic Data Centre, UK) for the following complexes and compared to the pattern obtained for the corresponding bulk sample.
**Differential Scanning Calorimetry (DSC):** DSC was performed on a Perkin Elmer Diamond Differential Scanning Calorimeter with a typical scan range of 25 °C – 280 °C, scan rate of 10 ºC/min, and nitrogen purge of ca. 30 psi.

**Fourier Transform Infrared Spectroscopy (FT-IR):** FT-IR analysis was performed on a Perkin Elmer Spectrum 100 FT-IR spectrometer equipped with a solid-state ATR accessory.

**Pharmacokinetic Study**

Eight week old male Sprague-Dawley rats with a jugular vein catheter were purchased from Charles River Laboratories, Inc. and housed in a temperature-controlled room for at least 48 hours before the PK study. All animal experiments in the present study were approved by the Institutional Animal Care and Use Committee (IACUC). The rats, n = 5, were fasted over night and weighed immediately before dosing. Ten mg/kg of meloxicam or its equivalent (for cocrystals) were prepared, sieved to a particle size range between 53 and 75 μm, and suspended in 1 ml of 5% polyethylene glycol 400 (PEG 400) with 95% methylcellulose solution (weight percentage) and administered in a single dose suspension via oral gavage in a single dose. Serial blood samples (0.2 mL) were obtained from the catheter at 0, 0.25, 0.5, 0.75, 1, 2, and 4 hours after oral administration. Blood samples were centrifuged with an Eppendorf Centrifuge at 3000 rpm, 4 ºC, for 10 min in order to obtain serum samples. All serum samples were stored at -80 ºC for subsequent HPLC analysis.
HPLC Analysis.

A 12.5 µg/mL piroxicam methanol solution was used as the internal standard (IS). 50 µL of animal plasma sample was transferred into an individual Eppendorf microcentrifuge tube and 200 µL IS working solution was added. Each Eppendorf microcentrifuge tube was hand shaken well and the sample was allowed to sit for 20 min. Each Eppendorf microcentrifuge tube was shaken again and the sample was transferred into a 0.2 µm Nylon-66 Microfilterfuge tube (Rainin, Oaklong, CA), and spun at 10,000 rpm for 4 min. Clear methanol solutions (200 µL or less) with meloxicam were separated from serum proteins and 160 µL of clear methanol solution was transferred into individual HPLC vials. HPLC analysis was carried out on a Perkin Elmer Instruments LLC comprising the following units: Series 200 Gradient Pump; 785A UV/VIS Detector; Series 200 Autosampler; NCI 900 Network Chromatography Interface and 600 Series Link. The machine was operated by Total Chrome Workstation (Perkin Elmer Instruments LLC). Sample holder temperature was at 4°C and a 250 x 4.6 mm x 1/4” Microsorb-MV 300-5 C-18 column was used. The analytes were eluted with a mixture of phosphate buffer (pH 3.0) and methanol (1/1, v/v). The temperature of the column was set at 40 °C with a flow rate of 1 mL/min, an injection volume of 20 µL, and absorbance was measured at 360 nm.

PK Parameters and Statistical Analysis

Microsoft Excel 2007 was used to process the PK data and generate statistics, one way analysis of variance (ANOVA) was used.
3.3 Results and Discussion

3.3.1 Cambridge Structural Database Analysis

As previously mentioned, a key step in generating pharmaceutical cocrystals is to analyze the target API from a crystal engineering perspective, i.e. to evaluate how the target molecule would form supramolecular synthons. This methodology partitions the target molecule by its functional groups and statistically examines the percentage of occurrence of supramolecular homo- and heterosynthons for these functional groups. The targeted supramolecular synthons are typically sustained via hydrogen bonds as they are strong and directional in nature. This method is particularly beneficial as most API’s tend to be rich in functional groups that are capable of forming strong hydrogen bonds.

Polymorphic form I of meloxicam indicates that meloxicam molecules form supramolecular chains that are sustained by sulfonyl-amide and thiazole-alcohol supramolecular heterosynthons, as shown in Figure 3.2. The chains are held together by various weak interactions, stacking along the a-axis in a slipped fashion. Thus for meloxicam cocrystallization, one or all of these supramolecular synthon motifs must be interrupted.

![Figure 3.2: Meloxicam supramolecular chains sustained by sulfonyl:amide dimers and thiazole-alcohol supramolecular synthons, CSD Refcode SEDZOQ.](image)

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A CSD analysis\textsuperscript{64} was conducted to examine the occurrence of supramolecular synthon formation. First a search for an amide-thiazole (5-membered ring, containing a nitrogen and a sulfur atom, linked to a primary amide) was conducted resulting in zero entries therefore the structure was narrowed down to an amino-thiazole functionality (5-membered ring, containing a nitrogen and a sulfur atom, linked to a primary amine) resulting in five entries. When this structure was searched in the presence of a carboxylic acid, primary amide, or alcohol moiety the result was zero entries. The search was then narrowed further employing a simple thiazole (5-membered ring containing one nitrogen atom and one sulfur atom). The reliability of supramolecular heterosynthon versus homosynthon formation between a thiazole and a carboxylic acid, primary amide, and alcohol were then examined in the CSD.

Due to the inability of the thiazole to form a supramolecular synthon with itself; only the homosynthon formation of the carboxylic acid, primary amide, and alcohol moieties in the presence of a thiazole was examined. Any conclusions from the limited data set might not be statistically significant due to the low number of hits for each search, however, heterosynthon formation was favored for carboxylic acids and alcohols, Table 3.1.
### Table 3.1: CSD statistics for thiazole supramolecular synthons.\(^{64}\)

<table>
<thead>
<tr>
<th>Search Type</th>
<th>Total # of Entries</th>
<th>Homosynthon (%) of total</th>
<th>Heterosynthon (%) of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazole (N as acceptor) - Acid</td>
<td>22</td>
<td>31.8</td>
<td>40.9</td>
</tr>
<tr>
<td>Thiazole (S as acceptor) - Acid</td>
<td>22</td>
<td>31.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Thiazole (N as acceptor) - Amide</td>
<td>15</td>
<td>60</td>
<td>13.3</td>
</tr>
<tr>
<td>Thiazole (S as acceptor) - Amide</td>
<td>15</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Thiazole (N as acceptor) - Alcohol</td>
<td>80</td>
<td>7.5</td>
<td>10</td>
</tr>
<tr>
<td>Thiazole (S as acceptor) - Alcohol</td>
<td>80</td>
<td>7.5</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Based on the preference of supramolecular heterosynthon formation between thiazoles and carboxylic acids, a meloxicam cocystal screen with acidic coformers that are generally recognized as safe (GRAS) or pharmaceutically acceptable and/or approved was conducted. The focus of the study was rather narrow in scope and did not include coformers that only possessed alcohol moieties despite the potential for interaction based upon the CSD statistics. Meloxicam was thereby reacted with fumaric acid, succinic acid, maleic acid, malonic acid, gentisic acid, 4-hydroxybenzoic acid, adipic acid, (+)-camphoric acid, glycolic acid, DL-malic acid, and α-ketoglutaric acid. All coformers except α-ketoglutaric acid produced at least one cocystal.
The cocrystallization attempts resulted in 10 crystal forms; namely, meloxicam:fumaric acid cocrystal, meloxicam:succinic acid cocrystal, meloxicam:maleic acid cocrystal, meloxicam:malonic acid cocrystal, meloxicam:gentisic acid cocrystal, meloxicam:4-hydroxybenzoic acid cocrystal, meloxicam:adipic acid cocrystal, meloxicam:(+)camphoric acid cocrystal, meloxicam:glycolic acid cocrystal, and meloxicam:DL-malic acid cocrystal. Table 3.2 contains chemical diagrams of the cocrystal formers, melting points, and pK$a$ information.
Table 3.2: Molecular diagrams, $pK_a$ information, and melting points for meloxicam and cocrystals 1 - 10.

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecular structure</th>
<th>$pK_a$</th>
<th>$\Delta pK_a$</th>
<th>Ratio Meloxicam : Coformer</th>
<th>MP of coformer (°C)</th>
<th>MP of cocrystal (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam</td>
<td>See Figure 3.1</td>
<td>4.18</td>
<td>N/A</td>
<td>N/A</td>
<td>254</td>
<td>N/A</td>
</tr>
<tr>
<td>Fumaric Acid (1)</td>
<td><img src="image" alt="Fumaric Acid" /></td>
<td>3.1</td>
<td>1.08</td>
<td>2:1</td>
<td>287</td>
<td>240</td>
</tr>
<tr>
<td>Succinic Acid (2)</td>
<td><img src="image" alt="Succinic Acid" /></td>
<td>4.19</td>
<td>-0.01</td>
<td>2:1</td>
<td>185</td>
<td>226</td>
</tr>
<tr>
<td>Maleic Acid (3)</td>
<td><img src="image" alt="Maleic Acid" /></td>
<td>1.93</td>
<td>2.25</td>
<td>1:1</td>
<td>137</td>
<td>192</td>
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<tr>
<td>Malonic Acid (4)</td>
<td><img src="image" alt="Malonic Acid" /></td>
<td>2.83</td>
<td>1.35</td>
<td>1:1</td>
<td>134</td>
<td>164</td>
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<td>Gentisic Acid (5)</td>
<td><img src="image" alt="Gentisic Acid" /></td>
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<td>1.20</td>
<td>1:1</td>
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<td>237</td>
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<tr>
<td>4-Hydroxybenzoic Acid (6)</td>
<td><img src="image" alt="4-Hydroxybenzoic Acid" /></td>
<td>4.58</td>
<td>-0.40</td>
<td>1:1</td>
<td>214</td>
<td>209</td>
</tr>
<tr>
<td>Adipic Acid (7)</td>
<td><img src="image" alt="Adipic Acid" /></td>
<td>4.42</td>
<td>-0.24</td>
<td>2:1</td>
<td>152</td>
<td>209</td>
</tr>
<tr>
<td>DL-Malic Acid (8)</td>
<td><img src="image" alt="DL-Malic Acid" /></td>
<td>3.40</td>
<td>0.78</td>
<td>2:1</td>
<td>131</td>
<td>215</td>
</tr>
<tr>
<td>(+)-Camphoric Acid (9)</td>
<td><img src="image" alt="(+)-Camphoric Acid" /></td>
<td>4.70</td>
<td>-0.52</td>
<td>3:2</td>
<td>183</td>
<td>212</td>
</tr>
<tr>
<td>Glycolic Acid (10)</td>
<td><img src="image" alt="Glycolic Acid" /></td>
<td>3.83</td>
<td>0.35</td>
<td>1:1</td>
<td>75</td>
<td>163</td>
</tr>
</tbody>
</table>
3.3.2 Crystal Structure Descriptions

**Meloxicam:Fumaric Acid (2:1) Cocrystal, 1**

The asymmetric unit of the meloxicam:fumaric acid cocrystal (1) contains two meloxicam molecules and one fumaric acid molecule which crystallizes in the space group $P\bar{1}$. In 1, the meloxicam dimer persists and links to adjacent dimers via fumaric acid molecules, creating an infinite supramolecular chain, Figure 3.3. The supramolecular heterosynthon comprising carboxylic acid and thiazole/NH moieties is a two point recognition observed between fumaric acid and meloxicam. Both O-H···N and NH···O hydrogen bonds are observed [O22-H22O···N3: O···N 2.68(3) Å, H···N 1.821 Å, O-H···N 174.39 °; N2-H2···O21: N···O 2.857(4) Å, H···O 1.976 Å, N-H···O 160.49 °]. The supramolecular chains of meloxicam and fumaric acid exhibited in 1 present as stacked layers, Figure 3.4.

![Figure 3.3: Supramolecular synthons observed in Meloxicam:Fumaric Acid (2:1), Cocrystal 1.](image)
Meloxicam:Succinic Acid (2:1) Cocrystal, 2

Preparation of the meloxicam:succinic acid cocrystal (2) by solvent-drop grinding has been recently reported but without determination of its crystal structure. The calculated PXRD of 2 based upon this single crystal structure data matches that of the previously reported PXRD. 2 crystallizes in the space group $P\bar{1}$ with the asymmetric unit containing one meloxicam molecule and half a succinic acid molecule. The crystal structure of 2 reveals that the meloxicam dimers are associated with adjacent dimers by succinic acid molecules forming infinite supramolecular chains, Figure 3.5. Similar to previous meloxicam cocrystal structures, the primary intermolecular interactions of 2 are hydrogen bonds between meloxicam and succinic acid via the carboxylic acid to thiazole/NH supramolecular heterosynthon. The OH···N and NH···O=O hydrogen bonds are involved [O7-H7···N3: O···N 2.863(4) Å, H···N 1.847 Å, O-H···N 173.63 °; N2-H2···O6: N···O 2.849(4) Å, H···O 1.993 Å, N-H···O 164.32°]. The supramolecular chains of meloxicam and succinic acid in 2 are reminiscent of the supramolecular chains.
found in 1, Figure 3.5. As shown in Figure 4.6, supramolecular chains of succinic acid and meloxicam stack with an interplanar spacing of 3.386 Å. As a result of overall packing comparison between meloxicam cocrystal structures, it is observed that the crystal structures of 1 and 2 are isostructural. Table 3.3 lists the crystallographic details for 1 and 2.

Figure 3.5: Supramolecular synthons observed in Meloxicam:Succinic Acid (2:1), Cocrystal 2.

Figure 3.6: Supramolecular layers stacking in Meloxicam:Succinic Acid (2:1), Cocrystal 2.
Table 3.3: Crystal structure parameters for 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>(C\textsubscript{14}H\textsubscript{13}N\textsubscript{3}O\textsubscript{4}S\textsubscript{2})\textsubscript{2} C\textsubscript{4}H\textsubscript{4}O\textsubscript{4}</td>
<td>(C\textsubscript{14}H\textsubscript{13}N\textsubscript{3}O\textsubscript{4}S\textsubscript{2})\textsubscript{2} C\textsubscript{4}H\textsubscript{6}O\textsubscript{4}</td>
</tr>
<tr>
<td>Formula weight</td>
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<td>820.88</td>
</tr>
<tr>
<td>Crystal System</td>
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<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P\textsubscript{i}</td>
<td>P\textsubscript{i}</td>
</tr>
<tr>
<td>a (Å)</td>
<td>7.145(5)</td>
<td>7.2315(4)</td>
</tr>
<tr>
<td>b (Å)</td>
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<td>8.4994(5)</td>
</tr>
<tr>
<td>c (Å)</td>
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<td>14.9383(8)</td>
</tr>
<tr>
<td>α (°)</td>
<td>82.250(9)</td>
<td>82.741(4)</td>
</tr>
<tr>
<td>β (°)</td>
<td>81.368(9)</td>
<td>80.061(3)</td>
</tr>
<tr>
<td>γ (°)</td>
<td>70.519(9)</td>
<td>70.313(4)</td>
</tr>
<tr>
<td>Vol (Å\textsuperscript{3})</td>
<td>848.1(7)</td>
<td>849.21(8)</td>
</tr>
<tr>
<td>D\textsubscript{cal} (g cm\textsuperscript{-3})</td>
<td>1.603</td>
<td>1.605</td>
</tr>
<tr>
<td>Z</td>
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<tr>
<td>T (K)</td>
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<td>100</td>
</tr>
<tr>
<td>R\textsubscript{1}</td>
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<td>0.0463</td>
</tr>
<tr>
<td>wR\textsubscript{2}</td>
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<td>0.0845</td>
</tr>
<tr>
<td>GOF</td>
<td>0.995</td>
<td>0.995</td>
</tr>
</tbody>
</table>

3.3.3 Meloxicam Crystal Forms: Cocrystals or Salts?

Pharmaceutical cocrystals and salts are well defined and are typically classified as distinct subsets of crystal forms. Whether the meloxicam crystal forms generated by the 10 coformers in this study are cocrystals or salts has also been addressed. For crystal forms with single crystal XRD data (i.e. 1-2), the conclusion that 1-2 are cocrystals was drawn in a relatively simple and reliable manner. However, it was less straightforward to
identify whether 3-10 are cocrystals or salts. The $pK_a$ values for meloxicam are 1.09 and 4.18. The value of 1.09 is associated with the enolic OH group while the value of 4.18 is linked to the nitrogen atom on the sulfathiazole ring. The enolic OH is much less accessible from a crystal engineering perspective as it is involved in intramolecular hydrogen bonding to the neighboring ketone or NH moieties. In contrast, the nitrogen atom on the thiazole ring is the primary target for cocrystal or salt formation, as it could potentially sustain a supramolecular synthon with various hydrogen bond donors.

$\Delta pK_a$ is widely accepted as a guideline to predicting whether a salt or cocrystal will form. It is generally considered that, if $\Delta pK_a < 0$, the resulting compound will be a cocrystal, whereas the result is typically a salt if $\Delta pK_a > 3$. For the region of $\Delta pK_a$ between $0 < \Delta pK_a < 3$, our ability to predict whether the resulting complex will be neutral or charged is limited. Indeed, ca. half of the $\Delta pK_a$ values reported herein fall into the range of $0 < \Delta pK_a < 3$ so how can one identify whether 3-10 are cocrystals or salts, especially where the FT-IR spectra may not provide adequate information?

The $\Delta pK_a$ value of cocrystal 1, which has structural data, was used as a reference to determine whether 3-10 are cocrystals or salts. As shown in Table 3.2, 1 possesses a $\Delta pK_a$ of 1.08 and proton transfer was not observed between meloxicam and fumaric acid. Although the molecular arrangement of various coformers may have an influence to the electron distribution and protonation of meloxicam, with the single crystal XRD data of 2, it is reasonable to assert that all but one of the coformers involved in this study with $\Delta pK_a$ values close to or less than 1.08 would potentially produce a cocrystal rather than a salt with meloxicam. Based on this, with the exception of 3, all crystal forms prepared in this study can be identified as meloxicam cocrystals. Since 3 remained questionable
due to a $\Delta pK_a$ of 2.25 further investigation into the FT-IR spectrum was performed to help identify the position of the proton. The carbonyl group of maleic acid in 3 exhibits a distinct peak at 1716 cm$^{-1}$, indicating that the carboxylic acid group is neutral rather than negatively charged. Therefore, proton transfer does not occur between meloxicam and maleic acid and it is deemed a cocrystal in agreement with Myz et al.\textsuperscript{52}

3.3.4 Cocrystal Stoichiometries

Cocrystals 3 - 5 and 7 - 10 were prepared from both solution and solid-state grinding methods, while 6 was only produced by solvent drop grinding. The absence of structural data for 3 - 10 is the result of unsuccessful solution-based growth attempts for single crystals. Nevertheless, polycrystalline powders of 3-10 were characterized by PXRD, FT-IR, and DSC, see Appendix 2. As mentioned in section 3.1 Myz et al. reported cocrystals of meloxicam by grinding with succinic acid (1:1) and maleic acid (1:2) but the stoichiometric ratio presented here for the succinic acid cocrystal, 2 here, was 2:1 and for maleic cocrystal, 3 here, 1:1. Furthermore, no single crystal data was produced by Myz, but only FT-IR and PXRD data. Even without the single crystal XRD data, the stoichiometries of 3-10 were determined by DSC or NMR in compliment with FT-IR and PXRD data as exemplified by 3. Based on the potential supramolecular interactions of meloxicam and the lack of ratio in the previous report, the most likely stoichiometry of meloxicam and maleic acid in 3 was proposed to be either 1:1, 2:1, or 1:2 (API:former). In order to determine the stoichiometry of 3, ethyl acetate slurries of physical mixtures of meloxicam and maleic acid in molar ratios of 2:1, 1:1, and 1:2 were performed at room temperature overnight. From each slurry experiment, the solid
crystalline powder was separated, washed with ethyl acetate and dried for characterization. The absence of pure maleic acid, water, or ethyl acetate was confirmed based on the DSC analysis, Figure 3.8, upon all three solid powders from the slurries. PXRD characterization indicated that the solids generated from the 1:1 and 1:2 slurries were identical to the cocrystal form from the initial solvent drop grind experiment conducted at a 1:1 ratio. In contrast, the 2:1 slurry produced a physical mixture of meloxicam form I and the cocrystal as confirmed by PXRD, Figure 3.7. $^1$H NMR (nuclear magnetic resonance) analysis (400 MHz, d$_6$-DMSO) on solids from the 1:1, 2:1, and 1:2 slurries confirmed that the stoichiometry of meloxicam and maleic acid in 3 is 1:1. PXRD, NMR, FT-IR, and DSC data can be found in Appendix 2.

![PXRD patterns for Meloxicam:Maleic Acid slurries.](image)

**Figure 3.7: PXRD patterns for Meloxicam:Maleic Acid slurries.**
Figure 3.8: DSC of Meloxicam:Maleic Acid slurries.
3.3.5 *In vivo* Performance via Rat Pharmacokinetic Studies

The PK profiles of 10 meloxicam:carboxylic acid cocrystals and pure meloxicam were determined and evaluated with respect to serum concentration in male Sprague-Dawley rats over four hours via a single oral dose of 10 mg/kg equivalent of meloxicam, Figure 3.9. The time versus concentration profile for many of the crystal forms continued to increase throughout most of the study. Cocrystals 7 and 8 had serum concentrations that were still increasing at four hours. An intravenous (IV) leg was not conducted therefore bioavailability was not obtainable, however, C\text{max}, T\text{max}, and AUC over four hours are shown in Table 3.4. Cocrystals 2, 4, and 9 decreased T\text{max} significantly over the time of the study, although, since pure API and two of the cocrystals are still increasing at four hours this information could be misleading with respect to long term action.

In terms of onset of action the fifteen minute data point is intriguing as it reveals that seven of the cocrystals reach higher serum concentrations; 6 is 2.73 fold higher, 3 is 2.42 fold higher, while 5 and 9 are ca. 2 fold higher. This could result in faster therapeutic levels in clinical studies and warrants further investigation. The C\text{max} values over four hours increased for five of the cocrystals compared to meloxicam with 7 and 8 reaching ca. 25% higher values. AUC values increased for five of the ten cocrystals (2, 3, 6, 7, and 8) with the largest differences attributed to 2 and 6.
Figure 3.9: Serum concentration for meloxicam and meloxicam cocrystals in rats over 4 hours.
Table 3.4: Pharmacokinetic data for meloxicam and meloxicam cocrystals.

<table>
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<tr>
<th>Compound</th>
<th>Melody</th>
<th>Fumaric (1)</th>
<th>Succinic (2)</th>
<th>Maleic (3)</th>
<th>Malonic (4)</th>
</tr>
</thead>
<tbody>
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<td>Time (min)</td>
<td>Mean (µg/mL)</td>
<td>Std. Dev.</td>
<td>Mean (µg/mL)</td>
<td>Std. Dev.</td>
<td>Mean (µg/mL)</td>
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<tr>
<td>15</td>
<td>14.83</td>
<td>0.76</td>
<td>6.69</td>
<td>0.34</td>
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<td>9.69</td>
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<td>1.14</td>
<td>30.23</td>
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<td>4995.38</td>
<td>9030.40</td>
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<tr>
<td>AUC</td>
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<tr>
<td>Cmax (µg/mL)</td>
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<th>DL-malic (8)</th>
<th>(+)Camphoric (9)</th>
<th>Glycolic (10)</th>
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<td>Time (min)</td>
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<td>Std. Dev.</td>
<td>Mean (µg/mL)</td>
<td>Std. Dev.</td>
<td>Mean (µg/mL)</td>
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<td>8354.86</td>
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<tr>
<td>AUC</td>
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<td></td>
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</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>33.45</td>
<td>40.86</td>
<td>47.85</td>
<td>49.72</td>
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<tr>
<td>Tmax (min)</td>
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<td>240</td>
<td>240</td>
<td>240</td>
<td>60</td>
<td>240</td>
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</tbody>
</table>
The therapeutic concentration of meloxicam is achieved after ca. 2 hours in humans for the currently marketed oral dosage form and for comparison purposes the concentration of pure meloxicam as determined herein at 2 hours, 30.19 μg/mL, will be used as a reference for further discussion although it should be noted that efficacy was not assessed here. Cocrystals 1, 4, 5, 9, and 10 had serum concentrations equal to or less than pure meloxicam at 2 hours, while 2, 3, and 6 - 8 were greater. Figure 3.9 indicates five cocrystals have outperformed pure API at the two hour point. 2 achieved the highest serum concentration at 2 hours (44.02 μg/mL), while 10 had the lowest concentration (20.77 μg/mL). The meloxicam:fumaric acid cocrystal, 1, has an interesting PK profile in that it is relatively linear over 4 hours making it suitable for possible application as a controlled release form.

Melting point is sometimes related to solubility due to the strength of the interactions involved in the crystalline lattice. The lower the melting point of a substance the easier it may be to dissolve that substance in GI fluids therefore correlations between cocrystal melting points and serum concentrations, AUC’s, and coformer melting points were investigated. It was found that only the correlation between cocrystal and coformer melting points exhibited a weak linear trend with an R² value of 0.636. Predicted aqueous solubilities were also used to investigate correlations with the PK data and no correlations were found. The lack of correlations with the PK data suggests that other physiological processes besides dissolution may be exerting their effects.
3.4 Conclusions

Nine meloxicam:carboxylic acid cocrystals have been discovered and the previously reported maleic acid cocrystal ratio was determined. Most of them can be synthesized by solvent drop grinding and slurry methods. Two of them, 1 and 2, produced single crystals suitable for X-ray diffraction resulting in reliable structural data. These two cocrystals are isostructural which is not surprising considering the only difference between these two coformers is a double bond. Interestingly two of the diacids here produced 1:1 stoichiometries while five others produced 2:1 stoichiometries. In the case of 3 this could be explained by intramolecular hydrogen bonding due to the cis conformation of maleic acid resulting in the H-bonding functionalities being less available to partake in supramolecular synthons.

Five of the cocrystals performed worse than pure meloxicam in PK analysis in rats and five others performed better with respect to $C_{\text{max}}$ and AUC. Cocrystal 8 achieved a 26.2% increase in $C_{\text{max}}$ compared to pure meloxicam and 2 achieved a 36.4% increase in AUC compared to pure meloxicam. At fifteen minutes seven of the cocrystals reached a higher serum concentration with 6 exhibiting a 2.73 fold increase, which could lead to faster onset in terms of therapeutic levels in clinical studies and warrants further investigation of this and the other crystal forms that out performed meloxicam at this time point.

As an oral dosage form reaches the gastrointestinal (GI) tract, it must first be dissolved in GI fluids before it can be absorbed. During this process many different physiological molecules such as enzymes, hormones, second messengers, and
immunological compounds may be present and actively interfering. Undoubtedly these physiological factors will also affect the absorption of the API. Since meloxicam is highly permeable (BCS class II) solubility is the limiting factor for absorption. However, permeability may also be affected by the use of cocrystals. In this case that effect may be insignificant or overlooked due to its inherently high permeability. It is well accepted that cocrystals represent a way to affect the solubility of an API, but how this affects the in vivo performance of that API remains to be fully understood or predictable.

3.5 References


(4) Thompson, J. P.; Sharpe, P.; Kiani, S.; Owen-Smith, O., British Journal of Anaesthesia 2000, 84, 151.


(52) Myz, S. A.; Shakhtshneider, T. P.; Fucke, K.; Fedotov, A. P.; Boldyreva, E. V.; Boldyrev, V. V.; Kuleshova, N. I., *Mendeleev Communications* 2009, 19, 272.

(53) Burdan, F., *Toxicology* 2005, 211, 12.


(61) Sheldrick, G. M. *SHELXTL*, University of Göttingen: Germany, 1997.

(62) It must be noted that the CSD structures have not been systematically populated with respect to balanced representation of the various functional groups. Although the supramolecular synthon approach has been successfully used in the various pharmaceutical cocrystal studies, the CSD statistical result here could be biased.


(64) CSD version 5.31 (Aug 2010 Update) Search Parameters: 3D coordinates, $R \leq 0.075$, and only organics. If contacts between donor and acceptor are used they were defined to be between 2 and 3.5 Å.


(70) Identification of cocrystals based on ΔpKa values is straightforward but also could be less rigorous. However, confirmation of relevant proton locations in 3-10 remains inconclusive using available characterization techniques.


(72) *Scifinder Scholar* 2007.
Chapter 4: Crystalline Forms of (R,S) Baclofen: A Zwitterionic Active Pharmaceutical Ingredient

4.1 Background

Oral dosage forms approved by regulatory agencies, such as the Food and Drug Administration (FDA), are typically stable crystalline forms whether they be in tablet, capsule, or suspension form. Different crystalline forms include, but are not limited to, salts, hydrates, solvates, and, more recently, cocrystals. Novel crystalline forms can tune solubility by changing the thermodynamic properties of the solid which is of seminal importance when considering oral dosage forms. Polymorphs, different packing arrangements of the same molecule, can also exhibit different solubilities although those differences are usually small due to the nature of small differences in crystal packing. However, in some rare cases of conformational polymorphism the difference in solubility can be very substantial as in the case of ritonavir since the crystal packing is significantly different. Improving aqueous solubility through salt formation has been the gold standard approach for the pharmaceutical industry when low solubility, ionizable, APIs are involved. Crystal packing of a salt is usually very different than that of the conjugate acid or base which leads to different physicochemical properties such as rate of dissolution. Ionized molecules are generally far more water soluble than their neutral counterparts because they have a large increase in dipole moment which provides the basis for this approach. Not including APIs which are peptide hormones, antibodies,
proteins, polymeric, inorganic, or greater than 1000 Da in molecular weight, there were 1356 compounds listed in the FDA’s Orange Book of approved drug products at the end of 2006. Of that total, 51.4% are salts with the majority of these salts formed from basic APIs where the most prevalent counterion is chloride (53.4% of these compounds). The most prevalent cation for acidic APIs was sodium (75.3% of these compounds). Logic dictates these as the most obvious choices since they are already prevalent in the human body. Different counterions will dictate different crystalline forms which will present a range of physicochemical properties as in the case of diclofenac where the sodium salt is used for delayed release tablets and the potassium salt for immediate release tablets since the two have significantly different dissolution rates.

The focus of this study is a low solubility zwitterionic API known as (R,S) baclofen or (R,S) β-amino-γ-(p-chlorophenyl)-butyric acid. Baclofen is an anti-spastic agent developed in the early 20th century for epilepsy that mimics γ-aminobutyric acid (GABA) and acts as an agonist for GABA b receptors which results in the reduction of excitatory neurotransmitter effects. It is a lipophilic derivative of GABA that can permeate the blood brain barrier which is delivered as the racemate. However, the R enantiomer is more active since it can attain the same conformation as GABA. It was quickly determined that it had minimal effects on epilepsy but was recognized for its ability to reduce spasticity in certain patients. Baclofen is most widely used by patients with spasticity related problems like cerebral palsy, dystonia, and trigeminal neuralgia but is also currently being investigated for numerous other indications like spinal cord injuries, binge eating, alcohol dependence, and opiate addiction.
There are oral and injectable dosage forms available at a maximum dose of 80 mg per day. Novartis marketed baclofen as Lioresal® and Medtronic has devised an intrathecal infusion pump for patients with severe spasticity.27

Zwitterions are neutral amphoteric molecules that carry both a formal positive and negative charge. In an aqueous solution the ionization state is determined by the pH value. Amphoteric molecules in water have an isoelectric point (pI), which is the pH at which the zwitterionic conformation is stabilized. Under normal circumstances if the pH is lowered below the pI then the molecule is protonated with a net positive charge and the opposite is true if the pH rises above the pI. With these pH changes the molecule becomes polarized and more soluble in water. Baclofen has a unique conformationally stabilized zwitterionic structure through intramolecular charge assisted H-bonding, Figure 4.1, that survives at a pH range of 5 - 8.5 creating a low solubility throughout this pH range.27

![Figure 4.1: Baclofens zwitterionic structure.](image-url)
Currently baclofen precipitates out at concentrations above 2 mg/mL in water or at pH conditions near the pI, 6.7, which can cause the intrathecal pump mentioned earlier to clog or create inaccurate dosing.\textsuperscript{27} By manipulating the crystalline form of baclofen it is possible to increase the concentration in the infusion pump to allow for less physician visits involving surgical refills and reduce the risk of precipitation and subsequent overdose if the pump clogs up and bursts.

The degree of proton transfer is the distinguishing factor between cocrystals and salts and is affected by the ionization constants\textsuperscript{28}, however, in the case of gabapentin, a zwitterionic API similar to baclofen, a multiple component crystalline form exists with 4-hydroxybenzoic acid where there appears to be partial proton transfer due to a disordered proton on a special position crystallographically with 50% occupancy.\textsuperscript{29} This is a rare case which could potentially complicate intellectual property (IP) arguments and patentability, however such rare cases cannot be ignored due to mounting evidence of the stability of such structures including but not limited to the conclusion of Mohamed \textit{et al.} 2009 where computational modeling revealed that a significant energy penalty is reduced when the presence of a disordered acidic proton is indicated.\textsuperscript{30} The study herein compares different multiple component crystal forms of (R,S) baclofen as sulfonate salts, on the basis of aqueous solubility under different pH conditions. The sulfonic acids used are depicted in Figure 4.2, which were chosen in order to break the interaction represented in Figure 4.1 with the thought that a strong enough acid would protonate the carboxylate. Structural comparisons, dissolution profiles in pure water, pH 1, and pH 7, and solubilities are discussed.
4.2 Experimental Details

4.2.1 Materials

(R,S) Baclofen anhydrous was purchased from Spectrum and was used without further purification. All other chemicals were supplied by Sigma-Aldrich and used without further purification.

4.2.2 Methods

**Synthesis of (R,S) Baclofen monohydrate (1):** (R,S) Baclofen was dissolved in 10% methanol, 0.68% acetic acid water with heat and left to evaporate. After one week colorless plates and needles were obtained with ca. 95% yield.

**Synthesis of (R,S) Baclofen:p-Phenolsulfonate (2):** (R,S) Baclofen was dissolved in a 65% by weight aqueous p-phenolsulfonic acid solution with heat to nearly saturated and then allowed to cool to room temperature. Colorless needles were obtained after cooling down in ca. 91% yield.
Synthesis of (R,S) Baclofen:4-Chlorobenzenesulfonate (3): (R,S) Baclofen and 4-chlorobenzenesulfonic acid were dissolved in water with heat at a 1:1 stoichiometric ratio and the resultant solution was left to evaporate affording colorless plates and needles after 5 days in ca. 95% yield. Synthesis of 4 and 5 are discussed in section 4.5.2.

Crystal Form Characterization

**Single-Crystal X-ray Diffraction:** Single crystals were obtained for 3 compounds. Single crystal analysis for 1-5 was performed on a Bruker-AXS SMART APEX CCD diffractometer with Mo Kα radiation (λ = 0.71073 Å) connected to a KRYO-FLEX low-temperature device and collected at 100 K for 1-4 and 223 K for 5. Lattice parameters were determined from least-squares analysis and reflection data were integrated using SAINT. Structures were solved by direct methods and refined by full matrix least squares based on F^2 using the SHELXTL package. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms bonded to carbon, nitrogen, and oxygen atoms 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. Hydrogen atoms bonded to methyl groups were placed geometrically and refined with an isotropic displacement parameter fixed at 1.5 times U_q of the carbon atoms.

**Powder X-Ray Diffraction (PXRD):** Powders were characterized by a D-8 Bruker X-ray Powder Diffractometer using a Cu Kα radiation (λ = 1.54178 Å), 40kV, 40mA. Data was collected over an angular range of 3° to 40° 2θ value in continuous scan mode using a step size of 0.05° 2θ value and a scan speed of 5.0 °/min.
**Calculated PXRD:** Calculated PXRD diffractograms were generated from the single crystal structures using Mercury 1.5 (Cambridge Crystallographic Data Centre, UK) for 1-5 for comparison to the bulk sample.

**Differential Scanning Calorimetry (DSC):** Thermal analysis was performed on a TA Instruments DSC 2920 Differential Scanning Calorimeter. Aluminum pans were used for all samples and the instrument was calibrated using an indium standard.

For reference, an empty pan sealed in the same way as the sample was used. Using inert nitrogen conditions, the samples were heated in the DSC cell from 30°C to the required temperature (melting point of the cocrystal) at a rate of 5°C/min unless otherwise specified.

**Fourier Transform Infrared Spectroscopy (FT-IR):** FT-IR analysis was performed on a Perkin Elmer Spectrum 100 FT-IR spectrometer equipped with a solid-state ATR accessory.

**Thermogravimetric analysis (TGA):** A Perkin Elmer STA 6000 Simultaneous Thermal Analyzer was used to conduct thermogravimetric analysis. Open alumina crucibles were used to heat the samples from 30°C to the required temperature at 10 °C/min scanning rate under nitrogen stream.

**Dissolution and Solubility**

**Particle Size:** Crystals were ground up and sieved to maintain a particle size range between 53 and 75 μm.
High Performance Liquid Chromatography (HPLC): Analysis was performed on a Shimadzu Prominence HPLC system comprising the following units: an SIL 20AHT autosampler; a SPD 20A UV/vis Detector; a CBM 20A Communications Bus Module; LC20 AT Liquid Chromatograph; DGU 20A5 Degasser. The system was at room temperature and a flow rate of 1 mL/min was used. The column was a Thermo Scientific Hypersil ODS C-18 (100 _ 4.6 mm _ 5μm). The mobile phase consisted of a mixture of 0.01 M phosphate buffer (pH 3.5) with acetonitrile (4/1, v/v).

Dissolution Study: Dissolution studies were performed on pure (R,S) baclofen, 2 and 3 allowing for the salt forms to be compared against the original API. Deionized water, pH 1 aqueous solution (0.1 N HCl, 37º C), and a 0.8 M sodium phosphate buffer pH 7 were used all at 37º C. The dissolution study was conducted using an excess of free-flowing solid in solution in 25 mL of solvent; for (R,S) baclofen 175 mg/beaker was used in water, 875 mg/beaker was used in 0.1 M HCl, and 150 mg/beaker was used in pH 7 buffer; for 2, 3 g of solid was used in water, 3.5 g of solid was used in 0.1 M HCl, and 250 mg/beaker was used in pH 7 buffer; for 3, 188 mg of solid was used in water and 425 mg of solid was used 0.1 M HCl; The slurries were stirred with a magnetic stir bar at a rate of ca. 200-300 rpm. Aliquots were filtered with 0.45 μm filters after 1, 5, 10, 15, 30, 60, 120, 240, 480, 720, and 1440 min (2880 min also for pH 7). The resulting solution was processed and the concentration of baclofen was measured using HPLC. The pH values of the resulting solutions and crystal forms of the solid in those solutions were also determined. The experiment was done in triplicate to allow for statistical analysis.33

pH Determination: pH was determined using a VWR SympHony pH meter model SP70P with a digital readout.
Photographs

(R,S) Baclofen monohydrate (1): 1 was viewed with a fully automated, upright Zeiss Axio-Imager Z.1 microscope with a 20x/0.70NA dry objective.

(R,S) Baclofen:p-Phenolsulfonate (2): 2 was viewed with a fully automated, upright Zeiss Axio-Imager Z.1 microscope with a 20x/0.70NA dry objective, and Nomarski DIC contrasting prisms. Z-stacks of images were created at 0.5 micron step sizes using the AxioCam MRm CCD camera and Axiovision version 4.6.02 software suite. Images were then 3-dimensionally reconstructed using the iso-surface technique in Bitplane’s (Zurich, Switzerland) Imaris software version 5.0.3.

(R,S) Baclofen:4-Chlorobenzenesulfonate (3): 3 was viewed with an Olympus MIC-D digital microscope.

4.3 Results and Discussion

4.3.1 Pure Baclofen

The hydrochloride salt of (R) baclofen which crystallizes in the P2₁2₁2₁ space group was deposited into the Cambridge Structural Database (CSD) in 1982.¹⁶ Each baclofen molecule is protonated by HCl to have a neutral carboxylic acid group and an ammonium group which counterbalances the chloride anion. The ammonium group is involved in charge assisted hydrogen bonding with three adjacent chloride anions and the carbonyl of another baclofen molecule whose carboxylic OH group also interacts with
one of the same chloride anions that interacts with the ammonium. A bilayer sheet exists parallel to the ab plane and these sheets interdigitate in a staggered fashion with each other through C-H···II interactions stacking along the c axis, Figure 4.3.

![Supramolecular arrangement of (R) Baclofen HCl from the CSD Refcode CRBMZC10.](image1)

Figure 4.3: Supramolecular arrangement of (R) Baclofen HCl from the CSD Refcode CRBMZC10.

(R,S) Baclofen monohydrate crystals (1) were grown but provided poor diffraction data and a suitable/publishable structure was not obtained. Colorless plates (Figure 4.4) and needles were simply too small, too thin, or twinned. PXRD data matched the previously published data on the monohydrate.34

![Single Crystals of (R,S) Baclofen monohydrate, 1, 20x magnification.](image2)

Figure 4.4: Single Crystals of (R,S) Baclofen monohydrate, 1, 20x magnification.
4.3.2 Sulfonate Salts of (R,S) Baclofen and their Crystal Structure Descriptions

The pKₐ values for (R,S) baclofen are 3.87 and 9.62 for the carboxylic acid and amino functional groups respectively.²⁷ It is generally accepted as a guideline that the difference in the pKₐ of the base minus the pKₐ of the acid must be less than zero if the desired outcome is a neutral complex (i.e. cocrystal).³⁵-³⁶ To generate a salt one would select two molecules with a difference in pKₐ of three or more units.¹¹, ³⁷ For the region in between (ΔpKₐ 0-3) the ability to predetermine whether the resulting complex will be neutral or charged is difficult.²⁹-³⁰, ³⁸ It should also be noted that pKₐ is a solution-based measurement and does not always translate to solid state chemistry. The pKₐ values and the ΔpKₐ values for the individual components of the supramolecular assemblies studied herein are provided in Table 4.1 and imply salt formation in each case.

Table 4.1: pKₐ and ΔpKₐ values.

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>pKₐ</th>
<th>ΔpKₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baclofen</td>
<td>9.62²⁷</td>
<td>NA</td>
</tr>
<tr>
<td>p-Phenolsulfonic Acid</td>
<td>-2.19³⁹</td>
<td>11.81</td>
</tr>
<tr>
<td>4-Chlorobenzenesulfonic Acid</td>
<td>-0.83⁴⁰</td>
<td>10.45</td>
</tr>
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</table>

(R,S) Baclofen:p-Phenolsulfonate monohydrate, 2: 2 crystallizes in the P2₁/n space group and contains one molecule of baclofen, p-phenolsulfonate, and water in the asymmetric unit, Figure 4.5b. An inversion center lies between R and S baclofen molecules and each baclofen contains charge assisted hydrogen bonds from the ammonium group to three different p-phenolsulfonate molecules via oxygen bonded to
sulfur [N11-H11A···O22: N···O 2.829(8) Å, H···O 2.159 Å, N-H···O 156.7 °; N11-
H11B···O22: N···O 2.887(8) Å, H···O 2.201 Å, N-H···O 129.2 °; N11-H11C···O23:
N···O 2.832(9) Å, H···O 2.062 Å, N-H···O 148.1 °]. The carboxylic OH group on
baclofen acts as a donor to a water molecule which then inturn hydrogen bonds to three
different p-phenolsulfonate molecules creating a 3D network, Figure 4.6, [O11-
H11A···O1S: O···O 2.705(8) Å, H···O 1.899 Å, O-H···O 160.0 °; O24-H24···O1S: O···O
2.964(9) Å, H···O 2.160 Å, O-H···O 160.1 °; O1S-H1SA···O23: O···O 2.712(7) Å, H···O
1.655 Å, O-H···O 179.0 °; O1S-H1SB···O24: O···O 3.066(9) Å, H···O 2.057 Å, O-H···O
143.6 °]. The packing arrangement is similar to (R) baclofen HCl in the sense that
staggered interdigitating phenyl rings are evident.

Figure 4.5: a) Single Crystals of Baclofen:p-Phenolsulfonate monohydrate, 2.41
b) Asymmetric unit for 2.

114
(R,S) Baclofen:4-Chlorobenzenesulfonate monohydrate, 3: This sulfonate salt crystallizes in the C2/c space group as needles, Figure 4.7, and contains one molecule of baclofen, 4-chlorobenzenesulfonate, and water in the asymmetric unit. Each different proton on the ammonium group interacts with three different 4-chlorobenzenesulfonate molecules via oxygen [N1-H1A⋯O23: N⋯O 2.911(2) Å, H⋯O 2.099 Å, N-H⋯O 111.2 °; N1-H1B⋯O22: N⋯O 2.810(2) Å, H⋯O 1.984 Å, N-H⋯O 158.4 °; N1-H1C⋯O21: N⋯O 2.828(2) Å, H⋯O 2.034 Å, N-H⋯O 155.9 °]. The carboxylic acid group of an (R) baclofen molecule interacts with two different water molecules via the carbonyl, [O2⋯H41B-O41: O⋯O 2.769 (2) Å, H⋯O 2.003 Å, O⋯H-O 160.41 °] and the OH group [O1-H1O⋯O41: O⋯O 2.535(2) Å, H⋯O 1.660 Å, O-H⋯O 169.4 °]. Those water molecules then interact in the same manner with an (S) baclofen molecule, Figure 4.8. There are chains created by (R) baclofen molecules interacting by charge assisted hydrogen bonding with 4-chlorobenzenesulfonate molecules along the b axis that are
connected to similar chains made by (S) baclofen molecules via water in the direction of the c axis. These stacked chains create sheets parallel to the bc plane that stack along the a axis, Figure 4.9, with 2\(\text{1}\) screw axis along the b axis in between the sheets.

Table 4.2 contains crystallographic information pertaining to 2-3. Indeed the strategy to use a strong acid to disrupt the intramolecular interaction for baclofen was successful. TGA’s for 1-3 can be seen in Appendix 3 and are relatively consistent with one water for each. All of them indicate that the water molecules are in channels due to the water coming off below 100° C,\(^{42}\) which is confirmed by the structures of 2 and 3. The water is held less tightly for 3 since in the TGA weight loss begins the moment heating begins from ambient temperature.

Figure 4.7: Single Crystals of baclofen:4-chlorobenzenesulfonate monohydrate, 3.
Figure 4.8: Supramolecular interactions within the unit cell for baclofen:4-chlorobenzenesulfonate monohydrate, 3.

Figure 4.9: Overall supramolecular packing motif for baclofen:4-chlorobenzenesulfonate monohydrate, 3, looking down the b axis.
Table 4.2: Crystal structure parameters for salts 2-3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C_{10}H_{13}ClNO_{2} C_{6}H_{5}O_{4}S H_{2}O</td>
<td>C_{10}H_{13}ClNO_{2} C_{6}H_{4}O_{3}SCl H_{2}O</td>
</tr>
<tr>
<td>Formula weight</td>
<td>405.89</td>
<td>424.33</td>
</tr>
<tr>
<td>Crystal System</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P2_{1}/n</td>
<td>C2/c</td>
</tr>
<tr>
<td>a (Å)</td>
<td>13.837(9)</td>
<td>29.694(4)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>5.584(3)</td>
<td>5.610(8)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>23.750(13)</td>
<td>23.438(3)</td>
</tr>
<tr>
<td>α (°)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>β (°)</td>
<td>102.381(14)</td>
<td>101.453(2)</td>
</tr>
<tr>
<td>γ (°)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Vol (Å³)</td>
<td>1792.5(19)</td>
<td>3826.8(9)</td>
</tr>
<tr>
<td>D_{cal} (g cm⁻³)</td>
<td>1.504</td>
<td>1.473</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Z'</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>3273</td>
<td>8778</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>2231</td>
<td>4303</td>
</tr>
<tr>
<td>Temperature (K)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>R₁</td>
<td>0.0829</td>
<td>0.0424</td>
</tr>
<tr>
<td>wR₂</td>
<td>0.2044</td>
<td>0.1071</td>
</tr>
<tr>
<td>GOF</td>
<td>0.98</td>
<td>1.046</td>
</tr>
</tbody>
</table>
4.3.3 Pure Water Dissolution

In order to dissolve a solid into any solvent system the lattice energy of the solid must be overcome in order for solvent molecules to interact and solvate each individual molecule within the solid. Melting point is sometimes indicative of a general solubility trend when dealing with non-ionizable compounds but in the case of salts this can be misleading since a salt will typically have a high melting point and a high aqueous solubility as in the case of sodium chloride whose melting point is ca. 800° C with an aqueous solubility of 360 mg/mL. The melting points for the multiple component crystalline forms studied here are shown in Table 4.3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Melting Point of Supramolecular Complex (°C)</th>
<th>Melting Point of Former (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baclofen</td>
<td>NA</td>
<td>206-208</td>
</tr>
<tr>
<td>1</td>
<td>NA</td>
<td>198.37</td>
</tr>
<tr>
<td>2</td>
<td>181.47</td>
<td>6.4</td>
</tr>
<tr>
<td>3</td>
<td>190.61</td>
<td>102</td>
</tr>
</tbody>
</table>

Dissolution profiles were measured at 37° C in order to mimic physiological conditions although it should be pointed out that these were not USP (United States Pharmacopeia) validated protocols. The particle size was controlled by sieves with a range of 53 – 75 μm for all dissolution profiles in this and the next two sections. The goal of these dissolution experiments herein were aimed at understanding the effect of different pH environments with regard to oral and intrathecal dosing. Figures 4.10 and 4.11 show the twenty four hour and one hour dissolution profiles for baclofen, 2, and 3 in pure water. Ranking the forms from lowest to highest solubility in water results in 3 at 3.88 mg/mL, baclofen at
5.01 mg/mL, and 2 at 60.22 mg/mL. The standard deviation for these profiles is low and 2 is the clear winner if 1st prize is for the greatest solubility. 2 reached a value ca. 20 times higher than pure baclofen partially due to the decrease in pH to 2.40 initially and 1.86 at twenty four hours associated with the dissolution of the form since baclofen is more soluble under these pH conditions. Table 4.4 includes the pH values recorded. Interestingly 3 retained a slightly lower solubility compared to baclofen even over a full day despite the fact that the pH decreased to < 3. Both salts did not have greater solubility in water which is somewhat surprising considering the sustained lower pH of both salt forms in water. It should be pointed out that the only difference between 2 and 3 is an alcohol on the aromatic ring of p-phenolsulfonate in 2, which is a chloride in 3. The hydrogen bonding capability in 2 fueled by the strong dipole moment for an alcohol is likely the cause for such a dramatic difference in solubility with a minor difference in molecular structure due to solvation by water.

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>pH Initial</th>
<th>pH 24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baclofen</td>
<td>5.70</td>
<td>6.58</td>
</tr>
<tr>
<td>Bac:p-Phenolsulfonate</td>
<td>2.40</td>
<td>1.86</td>
</tr>
<tr>
<td>Bac:4-Chlorobenzenesulfonate</td>
<td>2.96</td>
<td>2.71</td>
</tr>
</tbody>
</table>
Figure 4.10: 24 hour dissolution profiles in water at 37° C.

Figure 4.11: 1 hour dissolution profiles in water at 37° C.
PXRD data was collected on the solid remaining after dissolution, Appendix 3, for baclofen, 2, and 3. For baclofen the powder was partially amorphous and exhibited peaks primarily from the original anhydrous material and also 1, which is expected since the formation of the hydrate can take days at 25°C. For 2 the post dissolution PXRD pattern matched the salt as indicated by the dissolution profile remaining stable. For 3 the post dissolution PXRD pattern also matched the salt as indicated by no change in the dissolution profile where it could have been possible to precipitate out 1.

4.3.4 pH 1 (0.1 N HCl) Dissolution

The dissolution profiles in 0.1 N HCl are shown in Figures 4.12 and 4.13 for twenty four hours and 1 hour respectively. The order of lowest to highest solubility is the same as in water however the differences between the forms are not the same. The solubilities from the low end are; 3 at 9.36 mg/mL, baclofen at 30.79 mg/mL, and 2 at 59.16 mg/mL. Each solubility dramatically increased in 0.1 N HCl, when compared to water, except for 2 which was not significantly different. As it is in the single crystal structures, in a solution at a pH of 1 baclofen will be protonated leading to a neutral carboxylic acid and an ammonium cation, which in turn causes the molecule to be polar and therefore much more water soluble since the zwitterionic structure is defeated. This phenomenon explains the increases in solubility under low pH conditions. The solubility of 2 remained virtually the same compared to the water dissolution since the pH was decreased to < 2 in water after twenty four hours. The pH values for powder dissolution in 0.1 N HCl are listed in Table 4.5.
Table 4.5: pH values for 0.1 N HCl dissolution.

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>pH Initial</th>
<th>pH 24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baclofen</td>
<td>3.17</td>
<td>3.23</td>
</tr>
<tr>
<td>Bac:p-Phenolsulfonate</td>
<td>1.20</td>
<td>0.97</td>
</tr>
<tr>
<td>Bac:4-Chlorobenzenesulfonate</td>
<td>1.22</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Figure 4.12: 24 hour dissolution profiles in 0.1 N HCl at 37° C.
Figures 4.12 and 4.13 are more representative of an environment that an oral dosage form would experience since the stomach contains HCl and the average human body temperature is 37º C. The equilibrium solubility has not been reached in 0.1 N HCl for baclofen at one day since the curve continues to climb slightly. Interestingly the post dissolution solids contained the same phases as in water, as shown in Appendix 3, for each compound except for baclofen which was considered more crystalline due to the absence of the amorphous hump between 5 and 20º 2 theta. Also, it should be pointed out that the HCl salt of baclofen was not indicated by any of the PXRD data.

4.3.5 pH 7 Sodium Phosphate Buffer Dissolution

Intrathecal administration is currently used for baclofen in cases of severe spasticity when the patient is unresponsive to oral therapy or central nervous system (CNS) side effects, like sedation, become too great, like sedation for example. Use of an
implanted pump allows for a large reduction of dose while maintaining efficacy with reduced side effects, however, surgically implanted pumps have their own inherent risks which is why they are reserved for patients that do not derive sufficient benefit from oral therapy. Plasma levels associated with intrathecal administration are 100 times lower than those obtained by oral doses. Current dosing for maintenance therapy associated the implanted pumps ranges from 12 - 2003 μg/day. Current refill intervals last ca. sixty to ninety days and Albright et al. acknowledges the importance of increasing the solubility to reduce pump refill intervals which is highlighted in the intrathecal package insert, “certain populations of patients require higher daily doses of baclofen, and obtaining a desirable length of time between refills for these patients becomes difficult.”

Compounding the problem, strict aseptic conditions must be used during refills to avoid bacterial infection. Therefore improvement of the solubility could reduce the frequency of refills and overall healthcare costs by reducing physician visits. With this in mind solubility of the baclofen salts discovered was tested in a pH 7 buffer since the pH of the cerebral spinal fluid (CSF) is 7.30 to 7.36 and the current formulation for intrathecal administration comes in the pH range of 5 - 7.

The solubilities from lowest to highest were as follows; 4.10 mg/mL for baclofen, 5.51 mg/mL for baclofen:p-phenolsulfonate, and 5.81 mg/mL for baclofen:4-chlorobenzenesulfonate. The dissolution profiles, Figures 4.14 and 4.15 for twenty four hours and 1 hour respectively, reveal the salts having relatively low increases compared the other solvent systems tested.
2 exhibits a profile with an initial boost in solubility that is lost as the concentration is moves toward that of pure baclofen after two days. Two day time courses were employed for this solvent system since equilibrium was not reached for baclofen after one day in 0.1 N HCl.

Figure 4.14: 24 hour dissolution profiles in pH 7 buffer at 37° C.

Figure 4.15: 1 hour dissolution profiles in pH 7 buffer at 37° C.
While baclofen seems to have reached equilibrium, 3 appears to be still increasing after two days which is surprising considering the small difference in molecular structure between 2 and 3. The interaction between ions is clearly stronger with respect to 3 at pH 7, which could be related to solvation related to the polar nature of the hydroxyl group on 2 instead of the chlorine on 3. Both salts resulted in ca. 66% increase in solubility compared to baclofen after one hour although it is not known why between five and ten minutes 3 remained constant. It is possible that this was experimental error although each of the three samples agreed as indicated by the very small error bars. Compound 3 has the best performance of the two salts since it maintains a higher solubility and the concentration of API does not come down although it should be noted that longer studies would be necessary to test the ability of this form to sustain long periods of time in an intrathecal implanted pump. A pH of 7 remained constant throughout the entire dissolution experiment and interactions with buffer are also likely affecting the results. Both the formation of phosphate salts and solute-solute interactions between all the components present can affect solubility. Further studies would be necessary to optimize buffer concentration, which could be lowered since the current approved formulation for intrathecal administration exhibits a pH range of 5 - 7. The PXRD of the solid remaining after the dissolution experiment can be referred to in Appendix 3. For baclofen the PXRD pattern has an amorphous hump and peaks corresponding to 1 and baclofen. PXRD data for 2 primarily matches 1 that is slightly mixed with baclofen. For 3 the PXRD pattern is not what one would expect after looking at the dissolution profile. Since the profile does not decrease towards baclofen's value it would be expected that the pattern would match the original salt as it does in the other
two solvent systems tested, however, the pattern is consistent with 1 containing some amorphous content. This could mean that the sustained solubility for baclofen could be related to excess 4-chlorobenzenesulfonic acid that is present while 1 is precipitating out or phosphate salt formation could be occurring. The excess powder present that is amorphous could be the source of baclofen fueling this trend. It would be useful to further experiment with 3 to determine if at a certain concentration known to be soluble would result in 1 precipitating out over time.

4.4 Conclusions for Baclofen Salts

pH has a tremendous effect on the solubility of baclofen but predicting such an affect for baclofen sulfonate salts becomes very difficult if not impossible. Physiologically speaking some level of aqueous solubility is necessary for an API to be effective as a therapeutic agent. pH has a clear effect on ionizable APIs which becomes more complicated when zwitterions are involved. The more ionization states a molecule possesses the more difficult predicting solubility becomes, but it is possible to control which ionization state the API exhibits in the solid state through crystal engineering which could be translated to solution. The existing literature shows salts, in general, will have a larger increase in aqueous solubility and that cocrystals are more amenable to crystal engineering, however, in this case using the strategy of utilizing a strong acid to disrupt the uniquely stabilized zwitterionic structure of baclofen was successful. The solubility difference pertaining to the sulfonate salts was dramatic in water and at pH 1 which maintained a trend of lowest to highest solubility of 3, baclofen, then 2. Although a twenty fold increase in solubility was achieved with 2 in those solvent systems and p-
phenolsulfonic acid (median lethal dose (LD$_{50}$) = 1900 mg/kg in rats) has been used in dental applications implying some level of safety,$^{46-47}$ the solubility was not as dramatically increased or stable at pH 7. For intrathecal administration 2 is not a useful candidate since the pH range of 5-7 must be maintained without precipitation of the API as 1. 3 was the most interesting since it retained a lower solubility in water and at pH 1 compared to baclofen but maintained the highest solubility at pH 7. This ca. 66% increase in solubility compared to pure baclofen makes 3 a promising candidate to study further and could lead to fewer refills for intrathecal pumps reducing surgery associated risks and potential precipitation at concentrations above 2 mg/mL, which can clog the pump leading to an underdose or overdose due to bursting. The safety of sulfonates in CSF is beyond the scope of this work but potential therapeutic benefits may exist. 4-Chlorobenzenesulfonic acid has an LD$_{50}$ > 500 mg/kg in rats but toxicological studies would be necessary upon further development once the physicochemical properties are completely understood. There is a concern of pH reduction in CSF with 3, however, this can be controlled either by the natural CSF buffering capabilities$^{45}$ which utilize endogenous bicarbonate or by addition of a bicarbonate buffer to the intrathecal formulation.

4.5 Baclofen Lactam Polymorphism: High and Low Z’ Structures

4.5.1 Background

Z’ can be strictly defined as the number of formula units in the unit cell divided by the number of independent general positions.$^{48}$ In 2006 structures with a Z’ > 1 accounted for 8.8% of the CSD$^{49}$. Despite their low frequency of occurrence there has
been recent interest concerning structures with $Z' > 1$ with some debate over their occurrence in the CSD\textsuperscript{50} and why they occur.\textsuperscript{48, 51-54} Whether high $Z'$ prime structures are simply kinetic structures which have not reached thermodynamic equilibrium or are just the best option energetically is an important question, however, a CSD analysis by Steed along with other reports claim that some high $Z'$ structures are in fact the most stable form\textsuperscript{51-52, 55}, which has been supported by computational modeling.\textsuperscript{56-57} It is recognized that high $Z'$ structures occur more frequently for homochiral molecules such as steroids and nucleosides\textsuperscript{53} and also for chiral space groups.\textsuperscript{48} On rare occasions chiral, or Sohnke, space groups can be assigned to racemates, \textit{i.e.} kryptoracemates, which are likely to contain pseudosymmetric elements between enantiomers.\textsuperscript{54}

The appearance of pseudosymmetry in structures with $Z' > 1$ has been shown to occur 27\% of the time, where as for $Z' = 2$ structures it occurs 83\% of the time.\textsuperscript{48, 57-58} Pseudosymmetry, which can be described as molecules or intermolecular aggregates coming very close to a symmetry element without actually fulfilling it is likely influenced by what has been called “frustration” between favorable packing and highly directional (strong) supramolecular synthons.\textsuperscript{55} It would not be surprising that molecules of a certain shape and size simply cannot satisfy either demand completely, which could result in a compromise between the two, leading to more than one symmetry equivalent in the asymmetric unit and indeed Steed has highlighted the factors that may lead to a high $Z'$ structure\textsuperscript{48}; 1) irregular, non-self complementary shape 2) small number of strong intermolecular interacting functionalities 3) frustration between overall packing and strong intermolecular interactions and 4) strong self complimentary functionality with a
resolved chiral center. Also, when there are multiple energetically favorable conformations for a molecule a higher tendency toward polymorphism and \( Z' > 1 \) structures would be expected. There are a multiple examples containing polymorphic systems where at least one of the polymorphs have a \( Z' > 1 \) \cite{51-52, 56, 59-64}, but there are also cases where each structure in a polymorphic set contains a \( Z' > 1 \), as in the case of cholesterol \cite{48, 65}.

A case of polymorphism including a high \( Z' \) structure for (R,S) baclofen lactam, or (R,S) 4-(4-chlorophenyl)-2-pyrrolidone, Figure 4.16, is presented herein. Baclofen lactam is a dehydration product of baclofen and can be isolated as a synthetic intermediate on the way to baclofen \cite{66} or used for the synthesis of a proposed GABAAergic prodrug. \cite{67} It has also been shown that a lyophilized formulation with baclofen containing polyvinylpyrrolidone K30 (PVP K30), a hydrophilic excipient used as a wet binder or disintegrant, had a significant effect on the degradation of baclofen to the lactam \cite{68}. Form I of (R,S) Baclofen lactam has been previously deposited in the CSD (refcode ZUWKOR) and exhibits \( Z' = 1 \). \cite{69} The R form of baclofen lactam prepared by resolution using (2R, 3R)-(+-)-tartaric acid was also reported in the same article as Form I. It is reported herein how attempts to prepare single crystals of (R,S) baclofen or cocrystals of (R,S) baclofen afforded two new polymorphs of the lactam, Forms II and III.
4.5.2 Synthesis

Form II, was prepared by dissolving 300mg of (R,S) baclofen in hot 3-bromopyridine and the solution was left at room temperature to evaporate. Large colorless blocks were present after 128 days.

Form III, was prepared by dissolving a 1:2 molar ratio of glutaric acid (92.75mg, 0.702mmol) to (R,S) baclofen (300mg, 1.40mmol) in hot dimethylformamide (DMF) and the solution was left to evaporate at room temperature. Colorless plates and blocks were present after 38 days.

4.5.3 Cambridge Structural Database Statistics

The CSD was surveyed for the occurrence of high Z’ structures and the statistics are presented in Table 4.6. Z’ > 1 structures account for 8.94% of all structures and 11.90% of organics. These values are consistent with the of 8.8% and 11.5%, respectively, provided by Steed in 2006. Steed also reported that 14.6% of crystals that
adopt chiral space groups exhibit high Z’ values, as might be expected.\textsuperscript{70} As Z’ increases
the occurrence of structures drops off considerably and only 0.08% of structures exhibits
Z’ > 4.

**Table 4.6: CSD statistics for Z’ > 1.\textsuperscript{71}**

<table>
<thead>
<tr>
<th>Z’ value</th>
<th>No restrictions</th>
<th>% of total</th>
<th>Only organics</th>
<th>% of only organics</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1</td>
<td>46827</td>
<td>8.94</td>
<td>26706</td>
<td>11.90</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>4868</td>
<td>0.93</td>
<td>3133</td>
<td>1.40</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>2542</td>
<td>0.49</td>
<td>1731</td>
<td>0.77</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>441</td>
<td>0.08</td>
<td>322</td>
<td>0.14</td>
</tr>
<tr>
<td>≥4</td>
<td>2531</td>
<td>0.48</td>
<td>1726</td>
<td>0.77</td>
</tr>
<tr>
<td>=4</td>
<td>2090</td>
<td>0.39</td>
<td>1404</td>
<td>0.63</td>
</tr>
</tbody>
</table>

A search was undertaken with greater restrictions (3D coordinates determined, R
\leq 0.05, only organics, and Z’ \geq 4) to get a more detailed perspective of Z’ \geq 4 structures,
Table 4.7. This afforded only 175 hits amounting to 0.17% of the total amount of entries
with these same restrictions. This value is much lower than the 0.77% in Table 4.6 for Z’
\geq 4 (only organics) indicating that as higher standards are applied the occurrence of high
Z’ structures is even less likely than indicated by the raw search. Each of the 175 entries
in Table 4.7 was analyzed for chiral centers, for racemates, and for kryptoracemates. It
was found that 23.30% of Z’ \geq 4 structures are racemates whereas only 3.43% were
kryptoracemates, which has been reported to be very rare.\textsuperscript{54}
Table 4.7: CSD Refined statistics for $Z' \geq 4$.

<table>
<thead>
<tr>
<th>Total</th>
<th>% out of 104,996</th>
<th># Racemates (% of total)</th>
<th>#Kryptoracemates (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>175</td>
<td>0.17</td>
<td>41 (23.30)</td>
<td>6 (3.43)</td>
</tr>
</tbody>
</table>

4.5.4 Crystal Structure Descriptions

**Form II (4)**

Form II is a racemate that crystallizes in $P$-1 with four molecules in the asymmetric unit. The asymmetric unit is depicted in Figure 4.17 where the bc plane is the plane of the page. The four different molecules in the asymmetric unit are labeled A – D. Each molecule forms an amide dimer with another baclofen lactam molecule but not necessarily with the opposite enantiomer. A (proton at the chiral center is coming toward you), B, and D are in the R configuration, while C is in the S configuration.

![Figure 4.17: 50% probability ORTEP diagram for the asymmetric unit of Form II.](image)
The hydrogen bond distances and angles are presented in Table 4.8. A is paired with another R molecule (R-R dimer) [N41-H41⋯O61: N⋯O 2.849(4) Å, H⋯O 1.92 Å, N-H⋯O 170.0 °], B is paired with C, an S molecule (R-S dimer) [N1-H1⋯O21: N⋯O 2.919(4) Å, H⋯O 2.09 Å, N-H⋯O 163.1°; N21-H21⋯O1: N⋯O 2.827(4) Å, H⋯O 1.97 Å, N-H⋯O 177.6 °], and D is paired with another R molecule (R-R dimer) [N61-H61⋯O41: N⋯O 2.925(4) Å, H⋯O 1.96 Å, N-H⋯O 158.2 °]. These hydrogen bond distances are close to that of Form I (2.925 Å), the primary amide dimer (2.95 Å, see Appendix 3), and the secondary amide dimer which includes lactams (2.87 Å, see Appendix 3). The B-C (R-S) dimer appears to have a pseudosymmetric center of inversion. There are centers of inversion on the corners of the unit cell, the middle of each axis, the center of the unit cell, and at the center of each face. These inversion centers are between dimeric pairs of S-S and R-R or R-S and R-S. It is rather unusual that the R-S pairing is maintained but not around an inversion center as is typical of racemates. Figure 4.18 highlights the odd pairing of enantiomers. Selected bond angles and distances are provided in Table 4.9. The bond distances and angles around the stereogenic carbon atom for A - D range as follows; A: 1.512(5) – 1.536(5) Å and 101.4(3)° – 118.4(3)°, B: 1.515(4) – 1.560(5) Å and 102.0(3)° – 118.0(3)°, C: 1.463(6) – 1.537(5) Å and 104.5(3)° – 117.3(3)°, and D: 1.516(5) – 1.556(5) Å and 103.0(3)° – 118.2(3)°. Each symmetry independent molecule varies slightly from the next but C, which is the only S molecule in the asymmetric unit, has a relatively short C-C bond between the chiral carbon and the carbon connected to nitrogen in the five member ring. This could be a result of packing forces.
Figure 4.18: Pairing between R and S baclofen lactam molecules in Form II.

Form III (5)

Form III crystallizes in $P2_1/c$ and like form I only has one molecule in the asymmetric unit. This low $Z'$ polymorph is a racemate and contains the amide dimer observed in Forms I and II with hydrogen bonding parameters as follows; [N1-H2N⋯O1: N⋯O 2.915(3) Å, H⋯O 1.91 Å, N-H⋯O 168.6 °], Table 4.8. The amide dimer is depicted in Figure 4.19.
These hydrogen bond distances are close to that of Form I (2.925 Å), the primary amide dimer from a CSD survey (2.95 Å, see Appendix 3), and the secondary amide dimer from a CSD survey which includes lactams (2.87 Å, see Appendix 3). The bond angles and distances, Table 4.8, around the chiral carbon atom range from 103.0(3) – 115.5(3)° and 1.512(4) - 1.540(4) Å respectively. The bond distances and angles are comparable to Forms I and II. The torsion angle (C3-C4-C7-C10) of 123.2(3)°, Table 4.9, is smaller than that in Form II but larger than that in Form I. The puckering of the five member ring is reminiscent of Form II but the planes of the rings are closer to perpendicular. There is an inversion center between R and S molecules and a 21 screw axis along b. Form III was obtained in the presence of glutaric acid during an unsuccessful cocrystallization attempt with (R,S) baclofen. The heat involved most likely caused the dehydration of baclofen to the lactam and the presence of glutaric acid served as an accidental additive affecting nucleation of Form III. Additives have been shown to affect the outcome of crystallizations, for example there have been failed cocrystal attempts where the cocrystal former has impacted polymorphism and even induced high Z’ structures. Crystallographic data for Forms II and III can be found in Table 4.10.
Table 4.8: Selected bond distances and angles for Forms II (4) and III (5) [Å and °].

<table>
<thead>
<tr>
<th>Bond lengths (Å)</th>
<th>Bond angles (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Form II</strong></td>
<td></td>
</tr>
<tr>
<td>C(4)-C(7)</td>
<td>1.515(4)</td>
</tr>
<tr>
<td>C(7)-C(8)</td>
<td>1.539(5)</td>
</tr>
<tr>
<td>C(7)-C(10)</td>
<td>1.560(5)</td>
</tr>
<tr>
<td>C(24)-C(27)</td>
<td>1.537(5)</td>
</tr>
<tr>
<td>C(27)-C(30)</td>
<td>1.463(6)</td>
</tr>
<tr>
<td>C(27)-C(28)</td>
<td>1.528(5)</td>
</tr>
<tr>
<td>C(44)-C(47)</td>
<td>1.517(5)</td>
</tr>
<tr>
<td>C(47)-C(48)</td>
<td>1.516(5)</td>
</tr>
<tr>
<td>C(47)-C(50)</td>
<td>1.556(5)</td>
</tr>
<tr>
<td>C(64)-C(67)</td>
<td>1.512(5)</td>
</tr>
<tr>
<td>C(67)-C(68)</td>
<td>1.526(5)</td>
</tr>
<tr>
<td>C(67)-C(70)</td>
<td>1.536(5)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Form III</strong></td>
<td></td>
</tr>
<tr>
<td>C(4)-C(7)</td>
<td>1.512(4)</td>
</tr>
<tr>
<td>C(7)-C(10)</td>
<td>1.538(4)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.9: Selected torsion angles (°) for Forms I, II (4), and III (5).

**Form II**

| A                | C(45)-C(44)-C(47)-C(48) | 171.7(3) |
| B                | C(5)-C(4)-C(7)-C(8)     | 175.7(4) |
| C                | C(25)-C(24)-C(27)-C(28) | 144.9(4) |
| D                | C(65)-C(64)-C(67)-C(68) | 167.3(4) |

**Form III**

| C(3)-C(4)-C(7)-C(8) | -118.3(3) |
| C(3)-C(4)-C(7)-C(10) | 123.2(3) |
Table 4.10: Crystallographic data for forms II (4) and III (5).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Form II</th>
<th>Form III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C$<em>{10}$H$</em>{10}$ClNO</td>
<td>C$<em>{10}$H$</em>{10}$ClNO</td>
</tr>
<tr>
<td>Formula Weight</td>
<td>195.64</td>
<td>195.64</td>
</tr>
<tr>
<td>Crystal System</td>
<td>Triclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space Group</td>
<td>P-1</td>
<td>P2$_1$/c</td>
</tr>
<tr>
<td>a (Å)</td>
<td>9.7937(10)</td>
<td>10.936(4)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>13.2904(14)</td>
<td>8.932(4)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>16.2963(17)</td>
<td>9.878(4)</td>
</tr>
<tr>
<td>α (°)</td>
<td>69.985(2)</td>
<td>90</td>
</tr>
<tr>
<td>β (°)</td>
<td>79.546(2)</td>
<td>90.202(8)</td>
</tr>
<tr>
<td>γ (°)</td>
<td>68.724(2)</td>
<td>90</td>
</tr>
<tr>
<td>V (Å$^3$)</td>
<td>1853.2 (3)</td>
<td>964.9(7)</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Z’</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>D (g cm$^{-1}$)</td>
<td>1.402</td>
<td>1.347</td>
</tr>
<tr>
<td>Temperature (K)</td>
<td>100 (2)</td>
<td>223(2)</td>
</tr>
<tr>
<td>θ range (°)</td>
<td>1.33 - 28.28</td>
<td>1.86 to 28.28</td>
</tr>
<tr>
<td>Limiting indices</td>
<td>-10 ≤ h ≤ 13, -17 ≤ k ≤ 16, -20 ≤ l ≤ 13</td>
<td>-13 ≤ h ≤ 12, -8 ≤ k ≤ 11, -12 ≤ l ≤ 12</td>
</tr>
<tr>
<td>Reflns measd</td>
<td>9112</td>
<td>4571</td>
</tr>
<tr>
<td>Reflns unique / R(int)</td>
<td>7342, 0.0189</td>
<td>2114, 0.0394</td>
</tr>
<tr>
<td>Reflns observed</td>
<td>5157</td>
<td>935</td>
</tr>
<tr>
<td>T(min, T(max)</td>
<td>0.361, 1.000</td>
<td>0.781, 1.000</td>
</tr>
<tr>
<td>Goodness of fit on F$^2$</td>
<td>1.021</td>
<td>0.970</td>
</tr>
<tr>
<td>Completeness to θ</td>
<td>28.28°, 79.8%</td>
<td>28.28°, 88.7%</td>
</tr>
<tr>
<td>R$_1$, w R$_2$ [I&gt;2sigma(I)]</td>
<td>R1 = 0.0699, wR2 = 0.1632</td>
<td>R1 = 0.0574, wR2 = 0.1343</td>
</tr>
</tbody>
</table>
4.5.5 Conclusions for Baclofen Lactam Polymorphs

Forms II and III of (R,S) baclofen lactam both came serendipitously. Form II came from an attempt to grow single crystals of pure (R,S) baclofen where the applied heat in the presence of 3-bromopyridine caused dehydration of baclofen to the lactam and subsequent crystallization. Form II crystals did not form for 128 days therefore whether or not this is a kinetic product remains to be seen and requires further study. It is possible however that a high Z’ structure can be the most stable form. 55-57 One factor previously reported to lead to a high Z’ structure, having a small number of strong intermolecular functionalities, is confirmed for Form II. This high Z’ polymorph contains a peculiar arrangement of enantiomers and has larger torsion angles than the other polymorphs. Z’ ≥ 4 structures only account for a mere 0.48% of the CSD and any further discoveries of high Z’ structures will add to the understanding of why and how these solid-state arrangements come about.

4.6 References Cited


(33) It is noted that this dissolution study did not use the standard USP apparatus but it is believed that our dissolution study produced reasonably comparable results.


(39) EPA, Initial Risk-Based Prioritization of High Production Volume (HPV) Chemicals Hydroxybenzenesulfonic Acid (CASRN 1333-39-7) 2009.

(40) Scifinder Scholar 2007.

(41) Samples were viewed with a fully automated, upright Zeiss Axio- ImagerZ.1 microscope with a 20x /0.70NA dry objective, and Nomarski DIC contrasting prisms. Z-stacks of images were created at 0.5 micron step sizes using the AxioCam MRm CCD camera and Axiovision version 4.6.02 software suite. Images were then 3-dimensionally reconstructed using the iso-surface technique in Bitplane’s (Zurich, Switzerland) Imaris software version 5.0.3. .

(43) Sodium Chloride Material Safety Data Sheet, J.T. Baker.


(70) Brock, C. P.; Dunitz, J. D., *Chemistry of Materials* 1994, 6, 1118.

(71) CSD Conquest 1.12 (August 2010 update) 523,834 total entries. Search parameter: organics only 224,378 total entries.

(72) CSD Conquest 1.12 August 2010 update. Search parameters: 3D coordinates, \( R \leq 0.05 \), only organics, and \( Z' \geq 4 \) total entries 104,996. One duplicate removed, KOVBIG.
Chapter 5: Summary and Future Directions

5.1 Summary

Crystal engineering has become another tool for solid-state chemists in general and biological sciences in particular to control self assembly of molecules with biological activity and is based upon understanding strong intermolecular interactions. Utilizing highly directional and strong hydrogen bonding can be used to affect physicochemical properties of materials, especially crystalline pharmaceutical formulations, with emphasis upon solubility and subsequent delivery of therapeutic compounds.

This contribution has exemplified methodologies to generate new multiple component crystalline forms of pharmaceuticals with new properties such as solubility and pharmacokinetics. Mechanochemistry with the addition of small amounts of solvent, or solvent drop grinding (SDG), has been shown to be consistent with traditional solution based crystal growth for the generation of cocrystals with carbamazepine. Dimethylformamide and dimethylsulfoxide have been shown to be the most effective solvents to grind with and subsequently produce the same cocrystal obtained by solution crystallizations. Solvents in which both molecules employed, API and cocrystal former, have some solubility have also been shown to successfully generate cocrystalline forms via SDG.
This method is not only “Green” by nature, using microliter amounts of solvent, but also inexpensive and time efficient. These properties make it attractive for crystal form screening and the patentability of new crystalline forms drives the pharmaceutical industry’s recent interest in this technique.

SDG was also successfully used to generate cocrystals with meloxicam, a commonly prescribed NSAID for mild to moderate pain. The use of this medicine for acute pain could exist if a faster onset formulation was developed. With this in mind ten carboxylic acid cocrystals were synthesized, many by slurry methods as well, and their respective pharmacokinetics in rats was assessed with a single dose administration via oral gavage as a suspension. Structural data in the form of single crystal X-ray diffraction data was produced for two of the cocrystals, meloxicam:fumaric acid and meloxica:succinic acid. These two structures were isostructural which is not surprising considering these two coformers only differ by a double bond. Five of these cocrystals outperformed meloxicam in terms of AUC and $C_{\text{max}}$. In terms of a faster onset the earliest time point recorded was fifteen minutes and the meloxicam:4-hydroxybenzoic acid cocrystal exhibited a 2.73 fold increase in serum concentration. The solubility of a largely prescribed anti-spastic agent, baclofen, has been affected by the generation of two sulfonate salts, one with p-phenolsulfonic acid and one with 4-chlorobenzenesulfonic acid. The current intrathecal formulation has limited solubility, 2 mg/mL, at the pH range necessary for perispinal administration. This limits the length of time between surgically implanted pump refills and any concentration greater than that will lead to precipitation of baclofen monohydrate over time. Dissolution profiles were generated in three different solvent systems at 37° C, two of which were pH controlled. Pure water powder
dissolution resulted in acidic pH and a 20 fold increase in solubility compared to pure baclofen for the p-phenolsulfonate salt after twenty four hours with a dramatic increase after only one minute. The 4-chlorobenzenesulfonate salt maintained a slightly lower solubility over one day compared to baclofen in water although the pH remained less than three throughout the study similar to the p-phenolsulfonate salt. For pure baclofen the pH range sustained was 5.70 – 6.58 over twenty four hours in water and it is known that a zwitterionic state is maintained at this pH range. In 0.1 N HCl the pH remained close to one for both salts and the order of least soluble to most soluble remained the same as in water, which is unsurprising since an acidic pH was maintained. There was also a 20 fold increase in solubility with the p-phenolsulfonate salt, however, pure baclofen was much more soluble at this pH due to protonation resulting in disruption of the zwitterionic state bringing baclofen to its cationic form. The 4-chlorobenzensulfonate salt was one third lower in solubility when compared to baclofen. In pH 7 sodium phosphate buffer the order of highest to lowest solubility changed to the 4-chlorobenzenesulfonate salt, p-phenolsulfonate salt, then baclofen. The magnitude of change was much less dramatic and only the 4-chlorobenzensulfonate salt maintained higher solubility than baclofen, which was still increasing after forty eight hours. The p-phenolsulfonate salt decreased towards pure baclofen making it unsuitable for further development. More experiments are necessary to determine if the 4-chlorobenzensulfonate salt is safe for intrathecal administration and stable over long periods of time without precipitation of baclofen monohydrate.
Baclofen lactam polymorphs, Forms II and III, were discovered during crystal growth experiments with pure baclofen and cocrystallization attempts with glutaric acid respectively. Both forms are conformational polymorphs while Form II has a high $Z'$ of four. It was found that only 0.39% of entries in the CSD have a $Z' = 4$. The conformational flexibility of baclofen lactam enables Form II to have this high $Z'$ and an odd pairing of enantiomers including the presence of pseudosymmetry is exhibited. Form II was the result of dehydration of baclofen upon crystallization experiments targeted at growing single crystals of pure baclofen. Form III was the result of a failed cocrystallization experiment with glutaric acid and, similarly to Form II, the dehydration of baclofen to the lactam resulted from heating the solution. Supramolecular synthons for Forms II and III are consistent with Form I exhibiting the amide dimer.

Multiple component pharmaceutical crystal forms have been shown to be produced by multiple methods and these new crystalline entities can have tunable physicochemical properties that can lead to tunable pharmacokinetics. These conclusions have added tools to basic pharmaceutical sciences and shown that solid-state forms can be engineered. New multiple component crystalline forms can help remedy problems such as solubility, stability, and bioavailability and even extend the life cycle of currently marketed API’s with new patent protection.
5.2 Future Directions

Supramolecular chemistry has grown as a field impacting both academia and industry. It is now well accepted that self assembly of organic and inorganic compounds can be rationally designed and controlled based upon the understanding of supramolecular interactions. Manipulation of intermolecular forces to obtain desirable characteristics has expanded its presence to include multiple facets of industrial application. Pharmaceutical sciences in particular has taken a deep interest in optimizing and developing novel crystalline forms of active pharmaceutical ingredients (API’s) without the use of covalent changes in order to retain desired pharmacological action while improving clinical performance. The future of this area will be more focus on understanding how the physicochemical properties of novel crystalline materials are impacted based of the parameters chosen for design, i.e. which salt or cocrystal former will lead to what change. In terms of pharmaceuticals, in vitro – in vivo correlations need much more time and effort to understand how the bioavailability and pharmacological action will be affected. Utilizing synergistic multiple component pharmaceutical crystal forms could lead to lower doses and a subsequent reduction in adverse events. To understand these effects more animal studies across multiple species will be required to harness the full potential of multiple component pharmaceutical crystal forms including but not limited to salts and cocrystals.

This manuscript has shown that SDG can be a useful technique for cocrystal screening with two BCS class II API’s. In the future this technique can be utilized much more frequently and even routinely for crystal form discovery. It is likely that the crystal forms discovered will also be amenable to later stage development and processing. This
is important with respect to traditional large scale batches for the production of kilogram quantities. The fact that multiple solvents can produce the same cocrystal provides the chemical engineers more options in the event that a particular solvent is considered dangerous or simply fails to be reproducible upon scale up. This method is attractive and could become routine due to its cost effectiveness, eco-friendliness, and time efficiency.

Two BCS class II compounds have been shown to be amenable to cocrystal formation through SDG while corresponding solution based methods produced the same cocrystals. Beyond that meloxicam cocrystals have been shown to have tunable PK properties compared to the pure API. A carbamazepine cocrystal has also been shown by others to have improved PK performance therefore BCS class II compounds in general could be future targets for cocrystallization experiments where the goal is tunable PK performance and potentially tunable clinical performance. Future work could be aimed at using more animal models with various API’s in this class and a broader choice of coformer to systematically assess this technology for BCS class II compounds as a group.

For the zwitterionic API baclofen the use of strong acids has been used to disrupt a conformationaly stabilized zwitterionic state and change its pH dependent solubility. In particular it has been revealed that the problem of limited solubility at physiological pH can be increased with this strategy. Future work should be done to see if this rationale can be used for other zwitterionic API’s with similar limitations. Thereafter or concurrently the in vitro data presented here should be tested for correlations with in vivo testing.
By learning from and complimenting nature’s template of non-covalent intermolecular interactions the field of cocrystallization will become more and more important as the number of non-ionizable lipophilic drug candidates persists. Few cocrystal formulations are currently marketed as such and the development of scale up and processing will become the next challenge for this area. It is also possible that polypeptide based API’s may be stabilized to degradation before they reach their targets by manipulating strong hydrogen bonding interactions. Other unstable formulations may also benefit from non-covalent modifications. The application of novel drug delivery and tunable physicochemical properties could eventually reach a level of predictability in order to customize PK profiles for a particular need whether it be controlled release or targeted delivery.
Appendices

Appendix 1: Experimental Data for Carbamazepine

Figure 1.1A: FT-IR data for SDG with Carbamazepine and 4,4’-Bipyridine.
Figure 1.2A: PXRD data for SDG with Carbamazepine and 4,4'-Bipyridine.
Figure 1.3A: FT-IR data for SDG with Carbamazepine and 4-Aminobenzoic Acid.

Figure 1.4A: PXRD data for SDG with Carbamazepine and 4-Aminobenzoic Acid.
Figure 1.5A: FT-IR data for SDG with Carbamazepine and 2,6-Pyridinedicarboxylic Acid.

Figure 1.6A: PXRD data for SDG with Carbamazepine and 2,6-Pyridinedicarboxylic Acid.
Figure 1.7A: FT-IR data for SDG with Carbamazepine and Benzoquinone.

Figure 1.8A: PXRD data for SDG with Carbamazepine and Benzoquinone.
Figure 1.9A: FT-IR data for SDG with Carbamazepine and Terephthalaldehyde.

Figure 1.10A: PXRD data for SDG with Carbamazepine and Terephthalaldehyde.
Figure 1.11A: IR data for SDG with Carbamazepine and Saccharin.

Figure 1.12A: PXRD data for SDG with Carbamazepine and Saccharin.
Figure 1.13A: Ft-IR data for SDG with Carbamazepine and Nicotinamide.

Figure 1.14A: PXRD data for SDG with Carbamazepine and Nicotinamide.
Figure 1.15A: IR data for SDG with Carbamazepine and Aspirin.

Figure 1.16A: PXRD data for SDG with Carbamazepine and Aspirin.
Appendix 2: Experimental Data for Meloxicam

**Figure 2.1A:** Melting point correlation between meloxicam cocrystals and coformers.

**Figure 2.2A:** PXRD data for 1.
Figure 2.3A: FT-IR data for 1.

Figure 2.4A: DSC data for 1.

Mel MP = 254°C
Fumaric MP = 287°C
Cocrystal MP = 237°C
Figure 2.5A: PXRD data for 2.

Figure 2.6A: FT-IR data for 2.
Mel MP = 254° C
Succinic MP = 185° C
Cocrystal MP = 226° C

Figure 2.7A: DSC data for 2.

Figure 2.8A: FT-IR data for 3.
Figure 2.9A: $[{}^1H{}]$ NMR Spectrum For 1:1 Meloxicam:Maleic Acid Slurry in EtOAc.
Figure 2.10A: $^1$H NMR Spectrum For 1:2 Meloxicam:Maleic Acid Slurry in EtOAc.
Figure 2.11A: [1H] NMR Spectrum For 2:1 Meloxicam:Maleic Acid Slurry in EtOAc.
Figure 2.12A: PXRD data for 4.

Figure 2.13A: FT-IR data for 4.
Mel MP = 254° C  
Malonic MP = 134° C  
Cocrystal MP = 164° C
Figure 2.16A: FT-IR data for 5.

Figure 2.17A: DSC data for 5.
Figure 2.18A: PXRD data for 6.

Figure 2.19A: FT-IR data for 6.
Figure 2.20A: DSC data for 6.

Mel MP = 254° C
4HBA MP = 214° C
Cocrystal MP = 207° C

Figure 2.21A: PXRD data for 7.
Figure 2.22A: FT-IR data for 7.

Figure 2.23A: DSC data for 7.
Figure 2.24A: PXRD data for 8.

Figure 2.25A: FT-IR data for 8.
Figure 2.26A: DSC data for 8.

Figure 2.27A: PXRD data for 9.
Figure 2.28A: FT-IR data for 9.

Figure 2.29A: DSC data for 9.
Figure 2.30A: PXRD data for 10.

Figure 2.31A: FT-IR data for 10.
Figure 2.32A: DSC data for 10.

Mel MP = 254° C
Glycolic MP = 75° C
Cocrystal MP = 163° C
Appendix 3: Experimental Data for Baclofen and Baclofen Lactam

Figure 3.1A: TGA for (R,S) Baclofen Monohydrate.

Figure 3.2A: DSC for (R,S) Baclofen Monohydrate.
Figure 3.3A: TGA for (R,S) Baclofen:p-Phenolsulfonate.

Figure 3.4A DSC for (R,S) Baclofen:p-Phenolsulfonate.
Figure 3.5A: TGA for (R,S) Baclofen:4-Chlorobenzenesulfonate.

Figure 3.6A: DSC for (R,S) Baclofen:4-Chlorobenzenesulfonate.
Figure 3.7A: PXRD data for baclofen dissolution.

Figure 3.8A: PXRD data for Baclofen:p-Phenolsulfonate dissolution.
Figure 3.9A: PXRD data for Baclofen:4-Chlorobenzenesulfonate in pH 7 sodium phosphate buffer.

Figure 3.10A: Histogram for amide-amide dimer, 1st contact. CSD version 5.31 Aug. 2010 update with search parameters; 3D coordinates, organics, and R ≤ 0.05. Contact between donor and acceptor defined as 2.7 – 3.3 Å.
Figure 3.11A: Histogram for amide-amide dimer, 2\textsuperscript{nd} contact. CSD version 5.31 Aug. 2010 update with search parameters; 3D coordinates, organics, and R ≤ 0.05. Contact between donor and acceptor defined as 2.7 – 3.3 Å.

Figure 3.12A: Histogram for secondary amide- secondary amide dimer (includes lactams), 1\textsuperscript{st} contact. CSD version 5.31 Aug. 2010 update with search parameters; 3D coordinates, organics, and R ≤ 0.05. Contact between donor and acceptor defined as 2.5 – 3.1 Å.
Figure 3.13A: Histogram for secondary amide- secondary amide dimer (includes lactams), 2nd contact. CSD version 5.31 Aug 2010 update with search parameters; 3D coordinates, organics, and R ≤ 0.05. Contact between donor and acceptor defined as 2.5 – 3.1 Å.
About the Author

David R. Weyna received his Bachelor of Science degree in chemistry with a concentration in biochemistry in 2001 from the University of North Carolina at Wilmington in Wilmington, North Carolina. While at UNCW undergraduate research was supported by the Charles Cahill Award. This undergraduate research, regarding the synthesis of a Succinyl Phosphate analog, was published in 2007 in a peer reviewed journal. Two years were then spent at the Medical University of South Carolina in the biomedical sciences doctoral program focusing on pharmaceutical sciences. He then transferred to the University of South Florida (USF) under the advisory of Dr. Michael Zaworotko to continue doctoral studies. While at USF pharmaceutical cocrystallization and other multiple component pharmaceutical crystal form discovery was pursued with emphasis on physicochemical property and pharmacokinetic enhancement. During 2006 and 2007 his research was presented at meetings of the American Chemical Society. Also in 2007 he began work at Thar Pharmaceuticals Inc. concurrently while finishing the second half of the doctoral program at USF with particular focus on pharmaceutical cocrystal discovery and clinical improvement of active pharmaceutical ingredients. He is a co-inventor on thirteen patent applications and his work has been published in the following peer reviewed journals; Phosphorus, Sulfur, Silicon, and Related Elements, Molecular Pharmaceutics, Crystal Growth and Design, and the Journal of Pharmaceutical Sciences.