Impurity-Coformer Cocrystals and/or Complexes and their Use in Separations

by

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Submitted to the Department of Chemical Engineering in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

at the

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ABSTRACT

Separation processes are of great importance in many industries, especially in those that produce highly regulated products. Crystallization is commonly used as a purification technique in many industries, but can have two drawbacks: the first is the reduced selectivity when a structurally similar impurity is incorporated into the crystal lattice of the target being crystallized; second is increased process time and cost related to filtration and drying, a particular issue for intermediates that are crystallized and need to be re-dissolved in a subsequent step. The aim of this thesis is to develop separation processes to enhance the selectivity along with minimization of solids handling. Three different approaches were studied: (1) the separation of impurities from solution by selective impurity cocrystal formation where the cocrystal has a lower solubility than that of the impurity alone; (2) the use of coformers to form impurity-coformer complexes in solution followed by the crystallization of the desired compound; and (3) the selective adsorption of the impurity in solution using functionalized self-assembled monolayers on gold surfaces.

All three approaches were built on the concept of “molecular recognition”. In the first approach, the impurity was crystallized in its cocrystal form by the addition of a coformer while the target remained solubilized for downstream processing. The feasibility of this process was assessed using ketoprofen/ibuprofen as the model target/impurity system. A strategy was established for selecting the optimal coformer, concentration of the coformer, and solvent for the separation process. The amount of ibuprofen was decreased from 6 wt% to 2.5 wt%.

In the second approach, impurity-coformer complexes that could no longer fit into the crystal lattice of the target compound were formed by the addition of coformers. The feasibility of this process was examined using three systems: benzamide/benzoic acid, cinnamamide/cinnamic acid, and amoxicillin trihydrate/4-hydroxyphenylglycine system. Using the two model systems (benzamide/benzoic acid and cinnamamide/cinnamic acid), we demonstrated the feasibility of reducing the amount of the impurity substituting into the target crystal lattice by adding coformers that could form cocrystal with the impurity but not with the target compound. In these cases we knew in advance that cocrystals of the impurity with particular coformers would form. The impurity content in the target crystals was approximately 20% less using the coformer than
without the coformer. We then tested this method using the amoxicillin trihydrate (AMCT)/4-hydroxyphenylglycine (4HPG) system for which we had no advance knowledge of coformers that could form cocrystals with 4HPG. In this case we were able to identify coformers that substantially reduced the impurity content in amoxicillin crystals. Their purities were even superior to the purity that would be obtained from two crystallizations of the initial solution. A clear correlation between the level of complexation and the purification results was shown in this system.

The goal of the third method was to adsorb the impurity in solution selectively using functionalized self-assembled monolayers on gold surfaces. Gold surfaces were functionalized using thiols with different tail groups that could form hydrogen bonds with a functional group on the impurity. Three target/impurity systems and two thiols were studied using this approach. Despite the reasonable concept and experimental design, large standard deviation between the experiments performed under same conditions was observed. No significant separation results were obtained.

Thesis Supervisor:

Allan S. Myerson
Title: Professor of Chemical Engineering
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1. INTRODUCTION

In many industries, separation processes are employed to recover and purify intermediates and final products. In industries that manufacture strictly regulated products with high quality, such as the biopharmaceutical and food industries, it is essential to develop efficient and economical separation processes to meet their needs. To do so, it is necessary for these processes to have high selectivity (purity), capacity (maximum applicable amount of material) and recovery (yield). Liquid-liquid extraction, chromatography, and crystallization are the most commonly used separation processes in these industries. Each process has its advantages and disadvantages and is applied based on specific needs.

Liquid-liquid extraction is operated based on differences in the distribution coefficients of different compounds in immiscible solvents. It is affected by the operating temperature and the phase behavior of the solvents, which change with the presence of different solutes and diluents. This process has been used extensively to refine petroleum and intermediates in the food and biopharmaceutical industries. Commercialized liquid-liquid extraction processes usually involve multiple stages (up to thousands of stages) to achieve the required selectivity and productivity. It is known (1) to be the most economic process to separate a group of similar compounds; (2) to have high concentrations of desired compounds; and (3) to be applied to separate temperature sensitive compounds, for example, biologically active compounds that could lose selectivity or decompose at elevated temperature. However, it cannot separate a group of similar compounds into individual species and hence cannot be applied to systems in which the impurity is structurally similar to the target compound.
Chromatography is another commonly used separation technology. It is based on the different affinity of different compounds to the chromatography resins. The process is designed by selecting the proper resin, column, and mobile phases. It has been developed to manufacture biopharmaceuticals and food products and for analytical use. Besides the usual high-performance liquid chromatography (HPLC), alternatives like gas chromatography (GC), thin-layer chromatography (TLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE), and countercurrent chromatography have been developed for different applications. Its advantages include (1) excellent assay precision (±0.5%) and (2) wide applicability and commercial availability for most applications. However, it can be time-consuming to develop an appropriate resin and can become difficult when large diameter columns are required on the industrial scale. In addition, it is hard to recover dilute effluent streams. Most importantly, it is costly to apply chromatography in an industrial process.

Crystallization is often used in the intermediate and final stages of separation and purification in the biopharmaceutical, food, and many other industries. This process can determine the purity and physical properties of the product, such as its crystal morphology, size distribution, and crystal structure. It can also affect the flowability, filterability, tableting behavior, bioavailability, and stability of the product. To control, optimize, and develop crystallization processes, it is essential to understand the thermodynamic properties and kinetics of the process and of the product. Compared to liquid-liquid extraction and chromatography, crystallization has a better balance between cost and selectivity. However, it can potentially have two drawbacks: first, the crystallization process can be time-consuming and costly when filtration and drying are
required, a particular issue when the intermediate is first crystallized and then re-
dissolved for subsequent steps; second, when a structurally similar impurity is present in
solution, the selectivity can be lower than desired if an impurity is incorporated into the
crystal lattice of the target compound. In these cases, additional crystallizations, which
reduce the impurity content but sacrifice the yield, are often performed.

This thesis focuses on developing new processes to separate structurally similar
impurities from target compounds while (1) avoiding re-dissolution of the target
compound and (2) enhancing the selectivity of the crystallization in the presence of
structural similar impurities. Three processes were studied: (1) separating impurities
from solution by selectively forming impurity cocrystals with low solubility; (2)
preventing the impurity from incorporating into the crystal lattice of the target
compound by selectively forming impurity-coformer complexes in solution, and (3)
selectively adsorbing impurities in solution using self-assembled monolayers (SAMs) on
gold surfaces.

The first approach aims to purify intermediates and involves the formation and
crystallization of an impurity-coformer cocrystal. A coformer that can form a cocrystal
with only the impurity but not with the target is chosen and added to the impurity/target
mixture. The impurity-coformer cocrystal is chosen because it has low solubility in the
solution mixture. The impurity cocrystal can then be crystallized from solution,
removing the impurity and leaving the target molecule in solution.

To develop this separation process, it is necessary to learn: (1) how to select
cofomers that meet our criteria, (2) how much coformer should be added, and (3) what
solvent should be used. A strategy established for finding the optimal coformer,
concentration of the coformer, and solvent for a specific intermediate/impurity system is presented. The first step is to search through the Cambridge Structural Database (CSD) to find promising coformers that form cocrystals with the impurity but not with the intermediate. We are interested in reported heterosynthons where one component is a functional group on the impurity; any coformer with a complementary functional group that encourages the formation of a heterosynthon with the impurity is thus a candidate. That is, the potential coformers include, but are not limited to, the ones known to form cocrystals with the impurity. Second, experiments are performed to confirm that the chosen coformers could selectively cocrystallize out the impurity from an impurity/intermediate mixture. Then, the solubilities of the impurity and its cocrystals are measured. The cocrystal solubility must be significantly lower than that of the impurity alone and should show the potential to meet the separation standard (~10% impurity level). If this criterion is met, then the coformer and solvent are deemed effective. Otherwise, a different coformer and solvent pair is tested by measuring the impurity and cocrystal solubilities. This process is repeated to establish the optimal coformer and solvent. Finally, phase solubility diagrams (PSD) of impurity concentration as a function of coformer concentration were constructed to determine the optimal coformer concentration yielding the lowest impurity concentration after cocrystal formation. In this work, ibuprofen (IBU) and ketoprofen (KETO) were chosen as the model impurity and target intermediate. We demonstrated the possibility to separate impurities from solution using selective impurity formation. The effectiveness of the design strategy to optimize the separation process was also validated.
The second approach targets systems in which the target compound is crystallized in the presence of an impurity that incorporates in the crystalline lattice. It is often possible for an impurity to substitute into the crystal lattice of the target compound because of their structural similarity.\textsuperscript{4,5} In this approach, a coformer that can form cocrystals with the impurity but not with the target compound is added to the impurity/target mixture to form an impurity-coformer complex in solution. We postulate that (1) the coformers that can form cocrystals with the impurity should form complexes with the impurity in solution and (2) because of steric effects, the impurity-coformer complexes would no longer fit into the crystal lattice of the target compound. By adding coformers, the amount of impurity available to incorporate in the crystalline lattice is reduced and thus the crystalline product obtained should have an increased purity.

To confirm that the purification was due to the complexation in solution, we estimated the binding constants of the complexes and used them as indicators of the level of complexation. Phase solubility diagrams for cocrystal systems were used to find the stoichiometry and the binding constants for these complexes in equilibrium with cocrystals.\textsuperscript{6-11} With different combinations of cocrystal stoichiometry, complex stoichiometries, and the binding constants of the complexes, the solubility of the cocrystal is a unique function of the coformer concentration.\textsuperscript{7,8} Benzoic acid/ benzamide (BA/BAM) and cinnamic acid/cinnamamide (CA/CAM) were chosen as our model impurity/target systems. We were able to demonstrate the possibility of purifying structurally similar compounds by adding coformers that can form cocrystals with the impurity.
This approach was further studied using a real target/impurity system, amoxicillin trihydrate (AMCT)/4-hydroxy-D-phenylglycine (4HPG), for which no cocrystals of the impurity compound had been reported. A cocrystal screen of a series of coformers was conducted with both the impurity and the target compound using solid state grinding. Coformers that could form cocrystals with 4HPG but not with AMCT were selected for further study. Separation experiments were performed with the presence of these selected coformers. Our results further demonstrated the effectiveness of purifying the target by adding coformers that can form cocrystals with the impurity.

The third approach has a similar separation mechanism to the first two: to choose a functional group that can form hydrogen bonding with the functional group on the impurity but not with the functional group on the target. Thiols with different functional groups were chosen and attached to gold surfaces. Ideally, these functionalized gold surfaces should selectively adsorb the impurity and leave a purified solution. Three impurity/target compound systems were studied: IBU/KETO in toluene, BA/BAM in ethanol and CA/CAM in ethanol. Two thiol molecules were chosen to bind the impurity selectively: 2-mercapto benzimidazole and 4-mercapto pyridine. After initial promising results shown in previous work, carefully repeated experiments show no significant separation in all three systems. We concluded that this result is presumably due to the lack of SAMs selectivity for impurities.

In this work, we successfully demonstrated the possibility to (1) separate the impurity from solution using selective impurity cocrystal formation and (2) purify structurally similar compounds by adding the proper coformers. The effectiveness of the second approach was further verified using the AMCT/4HPG system. With these two
approaches, we can successfully (1) decrease the usage of multi-stage crystallization and re-dissolution steps and (2) enhance the selectivity of separation methods.
2. BACKGROUND

2.1 Definition of Cocrystals

The definition of cocrystals has been debated for over 160 years. At first, cocrystals were defined as homogeneous crystalline materials composed of at least two components held together through non-covalent interactions. These components could be solid, liquid, gas, or a combination of all. The most common non-covalent interactions are ionic, hydrogen bonding and van der Waals forces. In 2005, Aakeröy suggested cocrystals are “made from reactants that are solids at ambient temperature”.\textsuperscript{12} This definition eliminates the compounds classified as clathrates and inclusion compounds (where the guest molecules are solvents and gas). In addition, salts are considered as a different class of materials. Therefore, most people classify the most common multi-component crystalline materials into three categories: salts, solvates (or hydrates), and cocrystals (Figure 2-1).\textsuperscript{13} Solvates (or hydrates) are active pharmaceutical ingredients (APIs) and solvents (or water) held together through non-covalent interactions while all components in salts and cocrystals are solid. In addition, while the components in salts are the API and its counter ion, cocrystals are composed of neutral molecules. In our research, we define cocrystals as a homogeneous crystalline material composed of a neutral target and a neutral coformer held together through non-covalent bonds.
2.2 Applications of Cocrystals

Pharmaceutical companies are interested in cocrystal engineering because of two main reasons. First, the physicochemical properties of APIs can be modified while the intrinsic activities of these drug molecules remain the same. For example, the solubility and bioavailability of many APIs can be enhanced by forming their cocrystals. In addition, cocrystals can help achieve a high dissolution rate comparable to that of an amorphous compound while maintaining the chemical and physical stability of the crystalline form. Second, the intellectual property protection of existing APIs can be extended by forming their cocrystals. Pharmaceutical companies used to extend the life cycle of an existing drug by trying to find its polymorphs. However, the number of polymorphs for an API is limited while the number of potential cocrystals is larger. Therefore, to extend the life cycle of an existing drug, designing its cocrystals is potentially a more efficient way than trying to find its polymorphs. Cocrystallization is also considered an environmentally friendly way to produce crystals because it does not require as much solvent as does the traditional crystallization (i.e. by grinding, see...
section 2.4). Cocrystallization also has the potential to be used in separation processes. We can purify the API by adding its coformers and then discard these coformers before the tablet formation. It is essential to understand the cocrystallization process so we can design cocrystals with desired properties.

Urbanus et al. demonstrated its potential as a separation technique by removing the product, cinnamic acid, from a fermentation reaction broth. The concentration of cinnamic acid was decreased below the limiting concentration by forming its cocrystal with 3-nitrobenzamide. Although this study looked at the cocrystal formation of the desired product, there is little to no work in the literature concerning the selective cocrystal formation of an impurity from an impurity/target mixture.

2.3 Design of Cocrystals

A typical cocrystal design process involves three steps: coformer selection, computational analysis, and cocrystal characterization. For a target API, we are interested in coformers with functional groups that can interact (i.e. form hydrogen bonds) with the functional groups on the API. Common functional groups, such as carboxylic acids, amides, and alcohols, are typically found to interact with one another in cocrystals (Table 2-1). The most common intra/intermolecular interaction found in cocrystals is hydrogen bonding. Etter has studied hydrogen bonds in cocrystals and use them as design elements. Instead of studying hydrogen bonds from an energy viewpoint, she analyzed the cocrystal patterns as a result of intra/intermolecular interactions and established general rules for hydrogen bonding. The three most important rules are listed in Table 2-2.
Table 2-1 Common Functional Groups Found in Cocrystals.\textsuperscript{16}

<table>
<thead>
<tr>
<th>Functional groups</th>
<th>Typical supramolecular synthons used in crystal engineering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxylic acid</td>
<td><img src="image1" alt="Diagram" /></td>
</tr>
<tr>
<td>(e.g., acetic acid, adipic acid, benzoic acid, fumaric acid, maleic acid, malonic acid)</td>
<td></td>
</tr>
<tr>
<td>Amides</td>
<td><img src="image2" alt="Diagram" /></td>
</tr>
<tr>
<td>(e.g., nicotinamide and urea)</td>
<td></td>
</tr>
<tr>
<td>Alcohols</td>
<td><img src="image3" alt="Diagram" /></td>
</tr>
</tbody>
</table>

Table 2-2 Hydrogen Bond General rules.\textsuperscript{17}

1. All good proton donors and acceptors are used in hydrogen bonding.
2. If six-membered ring intramolecular hydrogen bonds can form, they will usually do so in preference to forming intermolecular hydrogen bonds.
3. The best proton donors and acceptors remaining after intramolecular hydrogen-bond formation form intermolecular hydrogen bonds to one another.

In addition, she demonstrated the selectivity of hydrogen bonding in cocrystallization by using pyridines as an example.\textsuperscript{17} She cocrystallized 4-phenylpyridine and ethyl isonicotinate from a mixture of two carboxylic acids with...
different pKa values and found that they only selectively cocrystallized with the carboxylic acid with the smaller pKa (Table 2-3). The selectivity was demonstrated with a mixture of 3, 4-dinitro-and 3, 5-dinitrobenzoic acids, whose difference in pKa is only 0.04. Similarly, Seaton et al. used Hammett constants to design acid/acid cocrystals. Hammett constants are used to describe the electron withdrawing ability of the substituents on benzoic acid derivatives. The more acidic the hydrogen, the larger the Hammett constant. This study found that the greater the difference in Hammett constants, the greater the chance these two acids would form cocrystals. By understanding hydrogen bonding, we can select coformers for a target API. After potential coformers are selected, we can search the target API in the Cambridge Structural Database (CSD) and see if it has known cocrystals or heterosynthons that include the functional groups on selected coformers. With this list of selected coformers, we can perform experiments to confirm if they can form cocrystals with the target API. Finally, we can characterize these cocrystals for their physical and chemical properties. With this cocrystal design process, we can design cocrystals for a target API efficiently.
Table 2-3 Selectivity in Cocrystallization of Pyridines with Mixture of Carboxylic Acid.17

<table>
<thead>
<tr>
<th>Pyridines in Cocrystal</th>
<th>Acid in Cocrystal</th>
<th>ΔpKa</th>
<th>Uncomplexed Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Pyridine 1" /></td>
<td><img src="image2.png" alt="Acid 1" /></td>
<td>2.35</td>
<td><img src="image3.png" alt="Uncomplexed Acid 1" /></td>
</tr>
<tr>
<td><img src="image4.png" alt="Pyridine 2" /></td>
<td><img src="image5.png" alt="Acid 2" /></td>
<td>0.74</td>
<td><img src="image6.png" alt="Uncomplexed Acid 2" /></td>
</tr>
<tr>
<td><img src="image7.png" alt="Pyridine 3" /></td>
<td><img src="image8.png" alt="Acid 3" /></td>
<td>0.41</td>
<td><img src="image9.png" alt="Uncomplexed Acid 3" /></td>
</tr>
<tr>
<td><img src="image10.png" alt="Pyridine 4" /></td>
<td><img src="image11.png" alt="Acid 4" /></td>
<td>0.04</td>
<td><img src="image12.png" alt="Uncomplexed Acid 4" /></td>
</tr>
</tbody>
</table>
2.4 Methods to Prepare Cocrystals

Different methods have been used to produce cocrystals. Sheikh et al. did a survey on published research (Figure 2-2) and found that the three most common ways are solution cocrystallization (including slow solvent evaporation, cooling and anti-solvent), solid state grinding (including dry grinding and grinding with solvent-drop addition) and cocrystallization from melts.\(^\text{19}\)

![Figure 2-2 Break Down of Techniques Used for Cocrystallization in Open Literature.\(^\text{19}\)](image_url)

We can produce cocrystals from melt using the Kofler method, which involves four steps: (1) the two components are heated at a controlled rate; (2) the component with the lower melting point melts and dissolves the other component to create a mixing zone; (3) the sample is placed under polarizing microscope while being cooled and the sample crystallizes inside the mixing zone to form cocrystals; (4) if you increase the temperature, the eutectic compositions melt and create a liquid phase.\(^\text{20}\)

We can also grind the API with a coformer to form cocrystals. Etter used dry grinding as a technique to study hydrogen bonding in cocrystals.\(^\text{17}\) She and Adsmond
also found that dry grinding can not only reproduce the cocrystals obtained from solution cocrystallization but can also produce new cocrystals.\textsuperscript{21} They found that for the 2-aminopyrimidine and succinic acid system, while solution cocrystallization can only produce the 1:1 cocrystal, dry grinding can produce both 1:1 and 2:1 cocrystals. Recently, studies have shown that adding drops of proper solvent during grinding can help accelerate the cocrystallization process. Shan \textit{et al.} used cyclohexane-1,3cis,5cis-tricarboxylic acid (CTA) as an example.\textsuperscript{22} Originally, it took an hour for 4,4’-bipyridine to form cocrystals with CTA partially. By adding 0.05 ml methanol, cocrystallization reached complete conversion in 20 minutes. Similarly, the process of cocrystallizing CTA and 4,7-phenanthroline (fPh) was accelerated by adding methanol. Complete conversion was reached in 5 minutes.

Solution cocrystallization is another commonly used technique to produce cocrystals. Ling and Baker crystallized the derivatives of quinhydrone by slow evaporation of the solvent and by producing a supersaturated solution through cooling.\textsuperscript{23} These techniques are still commonly used to produce cocrystals. It is also common to utilize phase diagrams, such as ternary phase diagrams and phase solubility diagrams, to aide solution cocrystallization. We will discuss the details of these phase diagrams in section 2.5.

\textbf{2.5 Thermodynamics of Cocrystals: Ternary Phase Diagrams and Phase Solubility Diagrams}

For separation purposes, it is essential to produce cocrystals by adding the minimum amount of coformers to (1) trigger cocrystallization and (2) reach the highest separation efficiency. It is also important to understand how the API solubility is affected
by various amounts of coformers present in solution. Ternary phase diagrams have been used to study the composition of API in its pure state and in its cocrystal state at a constant temperature. A typical ternary phase diagram for a cocrystal system at a constant temperature is shown in Figure 2-3. Points S, A, B, and C represent the pure solvent, the pure API, the pure coformer, and the pure cocrystal respectively. Points D and E represent the solubilities of the API and of the coformer, respectively, in the specific solvent. Points F and G are eutectic points where the liquid phase is in equilibrium with two solid phases (A and C) in the ternary phase diagram at a given temperature and pressure. The solubility of the API at various amounts of the coformer present in the solvent is presented by line DF. Similarly, the solubility of the coformer at various amounts of the API present in the solvent is presented by line EG.

![Figure 2-3 A Typical Ternary Phase Diagram for a Cocrystal System.](image)

Based on the nature of the system (the relatively solubility of the API and of the coformer), we can divide the phase diagram into two categories: congruent and incongruent (Figure 2-4). In a congruent system (Figure 2-4 (a)), if the starting material is the pure cocrystal, no phase change would be observed during the process of adding solvent to dissolve the cocrystal (start from point C and move to point S along line CS).
On the other hand, in an incongruent system, if we add the solvent to the pure cocrystal, we would observe a phase change. As shown in Figure 2-4 (b), we would observe the formation of both the API (A) and the cocrystal (C) followed by the formation of the pure API (A) solid phase. It is important to construct the phase diagram of the interested cocrystal system to determine the best separation condition.

![Diagram](image)

(a) Congruent System  
(b) Incongruent System

Figure 2-4 Congruent (a) and Incongruent (b) Systems.

Although it is labor intensive to construct a ternary phase diagram, the information it can provide is quite useful. The construction of a ternary phase diagram includes two steps: identifying all solid phase regions and determining the liquidus curve for each single solid phase region. Ternary mixtures with various compositions are equilibrated at a set temperature and the resulting solid phase is analyzed by x-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC). Various API/coformer stoichiometries are chosen to spread across the entire phase diagram and the absolute solid amounts are enough to form slurries. After all solid phase regions are identified, it is necessary to determine the composition of the saturated liquid equilibrated with the pure solid phase. The composition can be measured by high-
performance liquid chromatography (HPLC) or by gravimetric analysis. Eutectic points are the intersection points of the liquidus curves. Chadwick et al. have used ternary phase diagram construction to discover new cocrystal forms. The ternary phase diagram for urea/glutaric acid in water was constructed (Figure 2-5) and a new stable polymorph of 1:1 urea:glutaric acid cocrystal was found in addition to the known 2:1 urea:glutaric acid cocrystal.24 To understand a cocrystal system from a thermodynamic viewpoint, it is necessary to construct a ternary phase diagram.

Figure 2-5 Ternary Phase Diagram of Urea/Glutaric acid in Water at 25°C in mass%.

Since we intend to use cocrystallization in a separation process, it is important to study the temperature effect on the cocrystal system. Therefore, instead of the traditional ternary phase diagram, we adopted the phase solubility diagram, which has been introduced as a graphical tool to present the ternary phase diagram in a x-y format. A typical phase solubility diagram is shown in Figure 2-6 where [A] and [B] represent the concentration of the API and the coformers respectively.6 The solid line is the solubility of the cocrystal at various concentrations of B and the dotted line is the solubility of A at
various concentrations of B. This phase solubility diagram is based on the following assumptions: (1) A is less soluble than B, (2) A is less soluble than the cocrystal in stoichiometric solutions (with respect to the cocrystal), (3) no complexation or ionization of cocrystal components occurs in solution, and (4) the solubility of A is independent of the concentration of B in solution. Four regions are separated by the solubility curve of A and the A-B cocrystal. In region I, A is supersaturated but the A-B cocrystal is undersaturated. Hence, we have pure A solids in this region. If the concentration of B is increased, then both A and the A-B cocrystal become supersaturated in region II. In region III, both A and the A-B cocrystal are undersaturated while in region IV, A is undersaturated and the A-B cocrystal is supersaturated. The pure A-B cocrystal can be obtained in this region. The intersection of the two solubility curves is $[B]_T$, where the solubility of A equals the solubility of the A-B cocrystal. The path x to y shows the phase transition when the concentration of B is increased. The point x represents the starting point where both A and the A-B cocrystal are undersaturated. After the concentration of B is increased to the point y, the chemical potential difference between point y and z drives the equilibrium to point z’ by forming A-B cocrystals. With the phase solubility diagram, we can plot multiple solubility curves of the impurity at different temperatures in the same diagram. This method helps us to envision the temperature effect. In addition, it is straightforward to calculate the amount of coformers we need to add to the solution to achieve the desired separation. In addition, the temperature effect on the cocrystal system can be presented and the heating/cooling path can be illustrated.
Figure 2-6 A Typical Phase Solubility Diagram.\textsuperscript{6}
2.6 Scale Up of the Cocrystallization Process

To incorporate cocrystallization into the continuous manufacturing process, we need to understand the criteria to scale up a cocrystallization process. As we mentioned before, slow evaporation and grinding are the most common methods to produce cocrystals.\textsuperscript{19} They are useful in cocrystal screening but their scalability is limited. On the other hand, solution cocrystallization (by cooling or adding anti-solvent) can be incorporated into the continuous manufacturing process at a large scale. Sheikh \emph{et al.} used carbamazepine and nicotinamide as an example to demonstrate the scaling up strategy.\textsuperscript{19} They were able to conduct a process at a 1 L scale with a yield of more than 90\% and a 14 L/kg throughput. For a specific API/coformer system, the scaling up strategy includes three steps: selecting a solvent, constructing a phase solubility diagram to identify the thermodynamically stable regions and the saturated liquid curves, and understanding the kinetics of the system. The following criteria of the solvents are recommended: (1) coformers should have higher solubility than the API in the solvent and (2) the critical concentration of the coformer at the operating temperature should be significantly different from the solubility of the coformer.\textsuperscript{19} A solvent with these properties provides the widest pure cocrystal phase and the highest throughput. In a continuous separation process, the solvent is constrained to be the solvent used in the upstream synthesis process. However, we can add other solvents to form a solvent mixture to achieve the desired solvent properties. After we select a solvent (or solvent mixture), we can construct the phase diagram for the API/coformer/solvent system.\textsuperscript{25} The phase diagram can help us identify saturated liquid curves and stable solid regions. We also need to study the kinetics of the system, including nucleation and crystal growth.
to develop seeding strategies and other parameters that may affect the cocrystallization process. Once we have all the information, we can start to design the process. A typical scale-up cocrystallization process is illustrated in Figure 2-7. First, we need to make a saturated solution of coformers at the harvest temperature ($T_{\text{harvest}}$, shown in blue). Then we heat the solution up to the temperature where the coformer concentration is just above the critical concentration. This temperature is the on-set temperature ($T_{\text{on-set}}$) at which the process is going to be operated (shown in red). At this temperature, the API is added to give us the maximum throughput. After the cocrystallization process, we can add the anti-solvent to wash these cocrystals and hence remove them from the process. For our separation purpose, it is essential to design the process with consideration of the temperature effect.

![Figure 2-7 Proposed Process Trajectory (Thick Grey Arrow) in Three-Dimensional Space Comprising Temperature, [Coformer] and [API].](image-url)
3. THE SEPARATION OF IMPURITIES FROM SOLUTION BY SELECTIVE IMPURITY COCRYSTAL FORMATION

3.1 Introduction

In this chapter, we demonstrate the possibility to separate impurities from solution by selective impurity cocrystal formation. Ibuprofen (IBU) and ketoprofen (KETO) were chosen as the model impurity/target system. Our strategy is to select coformers capable of significantly decreasing the solubility of the impurity through cocrystal formation, allowing for the removal of the impurity and for the retention of the target in solution. To achieve this, coformers that can selectively form cocrystals with the impurity but not with the target must be chosen. In addition, the impurity cocrystal must exhibit decreased solubility compared to the impurity alone. The operating conditions for the separation process, such as the coformer concentration and the solvent system that result in the lowest impurity concentration, must be determined.

A strategy was established for finding the optimal coformer, concentration of the coformer, and solvent for a specific impurity/target system. The first step is to search through the Cambridge Structural Database (CSD) to find promising coformers that form cocrystals with the impurity but not with the target. We are interested in reported heterosynthons where one component is a functional group on the impurity; any coformer with a complementary functional group that encourages the formation of a heterosynthon with the impurity is thus a candidate. That is, the potential coformers include, but are not limited to, the ones known to form cocrystals with the impurity. Second, experiments are performed to confirm that the chosen coformers could selectively crystallize out the impurity in its cocrystal form from an impurity/target
mixture. Then, the solubilities of the impurity and its cocrystals are measured. The cocrystal solubility must be significantly lower than that of the impurity alone and should show the potential to meet the separation standard (~10% impurity level). If this criterion is met, then the coformer and solvent are deemed effective. Otherwise, a different coformer and solvent pair is tested by measuring solubilities of the impurity and cocrystal. This process is repeated to establish the optimal coformer and solvent. Finally, phase solubility diagrams (PSD) of the impurity concentration as a function of the coformer concentration are constructed to determine the optimal coformer concentration yielding the lowest impurity concentration after cocrystal formation.

4,4'-bipyridine (BIPY) was chosen to be a potential coformer. To select the optimal solvent, solubilities of KETO, IBU, and the IBU-BIPY cocrystal were measured in two solvents: ethyl acetate (EtOAc) and a 50% water/ethanol (H₂O/EtOH) mixture. The results suggested that a 50% H₂O/EtOH mixture is a better solvent than EtOAc. Separation experiments were performed in both solvents and the results are presented in Table 3-2. The results confirm that the 50% H₂O/EtOH mixture is the optimal solvent. Phase solubility diagram data for the IBU-BIPY system were measured to find the optimal BIPY concentration. Two approaches were investigated to improve the separation result: the addition of cooling process and the use of nicotinamide (NCT) as the coformer. Neither showed improvement for the separation process.

3.2 Materials and Methods

3.2.1 Materials

Ibuprofen (IBU, ACS reagent, ≥99.5%) was purchased from Sigma-Aldrich. Ketoprofen (KETO, ≥98%), 4,4'-bipyridine (BIPY, 98%), nicotinamide (NCT, ≥99.5%,
HPLC grade), and ethyl acetate (EtOAc, CHROMASOLV® Plus, for HPLC, 99.9%) were purchased from Sigma-Aldrich and were used as received. Anhydrous ethanol (200 proof) was USP grade and was purchased from VWR. Acetonitrile (ACN, CHROMASOLV® for HPLC >99%) and water (H₂O, CHROMASOLV® Plus for HPLC) were purchased from Sigma-Aldrich and used for HPLC.

3.2.2 System selection

IBU and KETO were chosen as the model impurity and target, respectively. They are both well-studied pharmaceutical compounds and are structurally similar (Figure 3-1).

![Figure 3-1 Structures of (a) Ketoprofen and (b) Ibuprofen.](image)

A search of the CSD for potential cocrystals of IBU led to the selection of BIPY (Figure 3-2) as a coformer. A pre-existing cocrystal of IBU and BIPY can be found in the CSD (refcode: HUPPAJ). The cocrystal has a 2:1 stoichiometry (IBU:BIPY). A second search of the CSD found that there is no known cocrystal of KETO and BIPY. We then demonstrated the selective cocrystal formation of IBU from an IBU/KETO mixture using BIPY by preparing slurries of (a) 2:1 IBU:BIPY, (b) 2:1 KETO:BIPY and (c) 2:2:1 KETO:IBU:BIPY. After allowing these slurries to reach equilibrium, the solid phase was then filtered and analyzed using x-ray powder diffraction (XRPD). The diffraction data were compared to the simulated powder pattern, HUPPAJ, and
confirmed that (a) the known IBU-BIPY cocrystal was obtained, (b) an amorphous solid phase was observed, and (c) the selective cocrystal formation of IBU was achieved. The comparisons are shown in Figure 3-3, Figure 3-4, and Figure 3-5, respectively.

![Diagram of 4,4'-bipyridine]

Figure 3-2 The Structure of 4,4'-bipyridine.
Figure 3-3 Comparison between the Powder Pattern of the Experimental Solid (Solid Line) and the Simulated Powder Pattern of the 2:1 IBU-BIPY Cocrystal (Dotted Line).

Figure 3-4 Comparison between the Powder Pattern of the Experimental Solid (Solid Line) and the Powder Pattern of KETO (Dotted Line).
Following the same procedure, NCT (Figure 3-6) was found in the CSD to form a cocrystal with IBU. The cocrystal (refcode: SODDIZ) has a 1:1 stoichiometry (IBU:NCT). A second search of the CSD found that there is no known cocrystal of KETO and NCT. We then demonstrated that (a) the known IBU-NCT cocrystal was obtained, (b) no KETO:NCT cocrystal was formed, and (c) the selective cocrystal formation of IBU was achieved. The comparisons are shown in Figure 3-7, Figure 3-8, and Figure 3-9, respectively.
Figure 3-6 The Structure of NCT.

Figure 3-7 Comparison between the Powder Pattern of the Experimental Solid (Solid Line) and the Simulated Powder Pattern of the 1:1 IBU-NCT Cocrystal (Dotted Line).
Figure 3-8 The Comparison between the Powder Pattern of the Experimental Solid (Solid Line) and the Powder Pattern of the KETO (Dotted Line).

Figure 3-9 The Comparison between the Powder Pattern of the Experimental Solid (Solid Line) and the Powder Pattern of the 1:1 IBU: NCT cocrystal (Dotted Line).
To select the optimal solvent for the separation process, we measured the solubilities of KETO, IBU, and the IBU-BIPY cocrystal in ethyl acetate and in mixtures of H₂O/EtOH mixture, at various temperatures following the procedure described in section 3.2.3.

3.2.3 Solubility measurements

Solubilities were measured using both a Thermofisher Clarity solubility station, described by Yi et al., and HPLC. To measure the solubility of the target compound using HPLC, the compound of interest was added to the proper solvent to make supersaturated solutions. The slurry was stirred with magnetic stir bars in 20 ml glass vials overnight at a constant temperature maintained using a circulating water bath to reach equilibrium. The liquid phase was filtered using 0.45 μm PTFE syringe filters and diluted using the same solvent as the HPLC mobile phase. The concentration of the liquid phase was determined using HPLC (detailed method described in section 3.2.6). The solid phase was collected using vacuum filtration with filter papers and dried at room temperature overnight. These dried solids were confirmed to be the desired polymorph, if any, using XRPD.

3.2.4 Separation experiments

Separation experiments were performed in both EtOAc and a 50% H₂O/EtOH mixture. The initial solution contained a saturated amount of the target (KETO) at 20°C and the impurity (IBU) at an equimolar ratio. The amount of coformer added was the stoichiometric amount to form the cocrystal with the impurity. That is, the impurity to coformer ratio was the same as the stoichiometry used to make the impurity cocrystal. The solution was stirred overnight in a water bath at 20°C. The solids obtained from the
crystallization were analyzed using XRPD and the compositions of the liquid phase were
determined using the high performance liquid chromatography (HPLC) method
described in 3.2.6.

3.2.5 Phase solubility diagram construction

Different compositions of A and B solids (A-B combinations: IBU-BIPY and
IBU-NCT) were added to a 50% H₂O/EtOH mixture to make supersaturated solutions
with respect to all components and the cocrystal. The slurries were stirred with magnetic
stir bars in 20 ml glass vials overnight at a constant temperature (20°C) maintained using
a circulating water bath to reach equilibrium. The liquid phase was filtered using 0.45
µm PTFE syringe filters and diluted using the same solvent as the HPLC mobile phase
(30/70 water/acetonitrile with 0.1% trifluoroacetic acid). The concentration of the liquid
phase was determined using the HPLC method described in section 3.2.6. The solid
phase was collected using vacuum filtration with filter papers and dried at room
temperature overnight. The dried solids were confirmed to be the desired cocrystal using
X-ray powder diffraction.

3.2.6 High-Performance Liquid Chromatography

The HPLC instrument (Agilent 1260 Infinity) was equipped with a UV diode
array detector (Agilent Technologies G1315D). The column used was a YMC-Pack
ODS-A 150×4.6 mm I.D. column packed with 3 µm particles with 12 nm pore size
(YMC America Inc.). The maximum wavelength for absorbance was set at 230 nm. The
concentrations were analyzed using a 5 min isocratic method with a 30/70
water/acetonitrile mobile phase containing 0.1% trifluoroacetic acid.
3.2.7 X-ray Powder Diffraction

X-ray powder diffraction patterns were obtained using a PANalytical X'Pert PRO Theta/Theta powder X-ray diffraction system using a monochromatic CuK\textsubscript{α} radiation source with nickel filter (\(\lambda = 1.5418 \text{ Å}\)) generated at 45 kV and 40 mA, using an X'Celerator high-speed detector. The intensities were measured at 2-theta values from 5° to 40° at a continuous scan rate of 5°/min. Aluminum sample holders with a zero background silicon plate were used to carry out the measurements.

3.3 Results and Discussions

3.3.1 Solvent selection

As described in section 3.2.2, BIPY and NCT were found as two potential coformers. In this project, BIPY was used to demonstrate the strategy designed to select the optimal coformer and solvent pair and the optimal coformer concentration. NCT was later investigated as a potentially better coformer.

To find the optimal solvent, the solubilities of KETO, IBU, and the IBU-BIPY cocrystal were measured in both EtOAc and H\textsubscript{2}O/EtOH mixtures. The solubility of IBU was reduced by a factor of 8 from 478.64(±0.15) mg/g to 57.62(±0.15) mg/g by forming the BIPY cocrystal in EtOAc. However, despite the large decrease of IBU solubility, the IBU concentration was still too high to meet the separation standard. Solubilities of all components in H\textsubscript{2}O/EtOH mixtures with the concentration varying from 100% water to 50% water were measured (Figure 3-10). Since IBU is sparingly soluble in water and soluble in ethanol, the desirable IBU solubility can be found by tuning the solvent composition. The solubility of IBU decreased the most when it formed a cocrystal in the 50% H\textsubscript{2}O/EtOH mixture (Table 3-1). Hence, the 50% water/ethanol mixture was chosen.
to be our solvent. Indeed, the solubility of IBU decreased by a factor of 9 from 54.50(±0.056) mg/g to 5.90(±0.012) mg/g by forming the BIPY cocrystal.
Figure 3-10 Solubilities of IBU, KETO, BIPY, and the IBU Cocrystal in Different Solvent (H₂O/EtOH) Combinations.

Table 3-1 Comparison of IBU Solubility and its Solubility in Cocrystal in Different Solvent (H₂O/EtOH) Combinations.

<table>
<thead>
<tr>
<th>Water Content</th>
<th>IBU Solubility (mg/ g solvent)</th>
<th>IBU Solubility in Cocrystal (mg/ g solvent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>0.10±0.016</td>
<td>0.29±0.024</td>
</tr>
<tr>
<td>90%</td>
<td>0.10±0.013</td>
<td>0.57±0.082</td>
</tr>
<tr>
<td>80%</td>
<td>0.30±0.01</td>
<td>0.71±0.04</td>
</tr>
<tr>
<td>70%</td>
<td>1.38±0.023</td>
<td>1.35±0.012</td>
</tr>
<tr>
<td>60%</td>
<td>12.65±0.047</td>
<td>1.98±0.042</td>
</tr>
<tr>
<td>50%</td>
<td>54.50±0.056</td>
<td>5.90±0.012</td>
</tr>
<tr>
<td>40%</td>
<td>175.84±0.038</td>
<td>31.36±0.093</td>
</tr>
</tbody>
</table>
3.3.2 Separation experiments

Separation experimental conditions and results are shown in Table 3-2. In these experiments, a saturated amount of KETO at 20°C and IBU at an equimolar ratio was contained in the initial solution. BIPY was added at a 2:1 ratio to IBU to achieve the separation. The concentration of IBU, upon cocrystal formation, decreased from 149.10(±0.80) mg/g to 103.87(±1.47) mg/g in EtOAc while it decreased from 68.60(±1.20) mg/g to 28.04 (±0.23) mg/g in the 50% H₂O/EtOH mixture. Thus, both the solubility and separation experiments indicate that the 50% H₂O/EtOH mixture is a more effective solvent than EtOAc for the IBU/KETO system.

Table 3-2 Conditions of Separation Experiments.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Initial [KETO] (mg/g)</th>
<th>Initial [IBU] (mg/g)</th>
<th>Final [IBU] (mg/g)</th>
<th>Final [BIPY] (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOAc</td>
<td>184.89±0.37</td>
<td>149.10±0.80</td>
<td>103.87±1.47</td>
<td>11.29±0.56</td>
</tr>
<tr>
<td>50% H₂O/EtOH</td>
<td>85.34±0.66</td>
<td>68.60±1.20</td>
<td>28.04±0.23</td>
<td>4.23±0.14</td>
</tr>
</tbody>
</table>

As shown in the separation experiments, the final IBU concentration is higher than the IBU concentration indicated by the solubility measurements. This increase was due to the presence of KETO. The solubility of the cocrystal was measured as a function of KETO concentration, and the results are shown in Figure 3-11. The effective IBU concentration increased linearly with increasing KETO concentration, indicating that the presence of the intermediate can significantly decrease the impurity cocrystal solubility.
Figure 3-11 The Solubility of the Cocrystal (as Indicated by the Effective IBU Concentration) as a Function of KETO Concentration.

3.3.3 Phase solubility diagram

To find the lowest IBU concentration that can be obtained by adding the coformer, we constructed the PSD (Figure 3-12). The lowest IBU concentration achieved was 3.40(±0.39) mg/g for the 1:1 IBU:BIPY stoichiometry. Importantly, this result demonstrated that the solution stoichiometry resulting in the lowest impurity concentration (1:1 IBU:BIPY) does not have to be the same as the stoichiometry of the cocrystal (2:1 IBU:BIPY in our case).
3.3.4 Other improvements

In the separation experiment, the lowest IBU concentration we could achieve was 28.04(±0.23) mg/g in a 50% H₂O/EtOH mixture by forming its cocrystal with BIPY; however, the final BIPY concentration in solution was 4.23(±0.14) mg/g. To improve the separation results obtained in the previous experiments, it is necessary to decrease the effective IBU solubility. Potential methods include the cooling of the resulting solution from previous separation experiments or by the formation of a different IBU cocrystal whose solubility is lower than that of the IBU-BIPY cocrystal.

To evaluate the first approach, both the KETO solubility and the effective IBU solubility in the IBU-BIPY cocrystal at various temperatures were measured. By cooling the resulting solution from previous separation experiments, both KETO and the IBU-
BIPY cocrystal crystallize. Although the concentration of IBU decreased in solution, the yield of KETO decreased as well. Cooling would only be worth pursuing when the solubility of the cocrystal decreases faster than does the solubility of KETO. Figure 3-13 shows that the solubility of the cocrystal decreases much slower than the solubility of KETO does with decreasing temperature. Hence, in this case, cooling is not an effective way to improve the separation results.

Figure 3-13 KETO Solubility and Effective IBU Solubility in Its Cocrystal at Various Temperatures.
To evaluate the second approach, a low solubility coformer, nicotinamide (NCT), was investigated. However, despite its low solubility compared to BIPY, its cocrystal with IBU had a higher solubility (lowest at 147.50(±2.30) mg/g solvent according to Figure 3-14) than that of the IBU-BIPY cocrystal does. Hence, it cannot be used to improve the separation results.

![Graph](image)

Figure 3-14 Effective IBU Solubility in the IBU-NCT Cocrystal at Various NCT Concentrations in the 50% H₂O/EtOH Mixture at 20°C.

3.3.5 Summary

IBU and KETO were chosen as the model impurity and target, respectively. BIPY was chosen as the coformer to crystallize IBU from a IBU/KETO mixture selectively. By measuring the solubilities of all components, we found that a 50% H₂O/EtOH mixture is a better solvent than EtOAc. This conclusion was later confirmed in the separation experiments. The IBU concentration was decreased from 68.60(±1.20) mg/g to 28.04(±0.23) mg/g in the 50% H₂O/EtOH mixture, and 4.23(±0.14) mg/g of BIPY remained in solution. The presence of KETO increased the cocrystal solubility
from 5.90(±0.012) mg/g to 28.04(±0.23) mg/g. A PSD of IBU and BIPY was constructed to find that the lowest IBU concentration (3.40(±0.39) mg/g) can be found at a 1:1 IBU:BIPY stoichiometry. Two attempts (a cooling process and the use of NCT as an alternative coformer) were made to improve the separation result achieved using BIPY in the 50% H2O/EtOH mixture. The cooling process was not adapted because the KETO solubility decreased much faster than the cocrystal solubility did with decreasing temperature. NCT was determined to be a less ideal coformer since its cocrystal has a much higher solubility than the BIPY cocrystal does.

3.4 Conclusions

Using IBU and KETO as our model compounds, we showed that the impurity in a solution can be removed by its cocrystal formation. A workflow was established to choose the optimal coformer, concentration of the coformer, and solvent for the specific impurity/target system. In the separation experiment, the IBU concentration was reduced to 28.04(±0.23) mg/g in a 50% H2O/EtOH mixture by forming its cocrystal with BIPY; however, the final BIPY concentration in solution was 4.23(±0.14) mg/g. For an effective separation method, the impurity and coformer concentrations in the final solution must be reduced to a lower level.
4. THE PURIFICATION OF STRUCTURALLY SIMILAR COMPOUNDS BY THE FORMATION OF IMPURITY COMPLEXES IN SOLUTION

4.1 Introduction

In this approach, we investigated the possibility to purify structurally similar compounds by the formation of impurity complexes in solution. Two well-studied systems in the field of tailor-made additives, the benzamide/benzoic acid (BAM/BA) system and the cinnamamide/cinnamic acid (CAM/CA) system, were chosen as our model systems. Tailor-made additives are typically designed to alter properties of active pharmaceutical ingredients (API) such as morphologies. They can also be used as nucleation promoters and growth inhibitors. Tailor-made additives are designed (or selected) to be structurally similar to the API. Because of the structural similarity, it is easy for a tailor-made additive to substitute into the API crystal lattice and disturb the growth of the API crystal when the API was crystallized from an API/impurity mixture. Berkovitch-Yellin et al. used BA to change the morphology of BAM. The low impurity level (reported between 0.5% to 1%) made the BAM/BA system a good system for the purpose of this work. Similarly, CAM/CA was chosen to be our second system because of its similarity to the BAM/BA system. The structures of BAM, BA, CAM, and CA are shown in Figure 4-1. Both systems were investigated for purification.

![Figure 4-1 Structures of Benzamide, Benzoic Acid, Cinnamamide, and Cinnamic Acid (from Left to Right).](image-url)
The aim of this work was to prevent the impurity from substituting into the crystal lattice of the target compound. To achieve this, we added a coformer that could form a cocrystal with the impurity to the impurity/target mixture. In the 1950s, Higuchi conducted research on complexes formed in solution by caffeine. The stoichiometries of these complexes and the mechanisms of complex formation were studied. It is proven that complexation can affect solid properties. In our work, we assumed that (1) coformers that could form cocrystals with the impurity have a high probability of forming complexes with the impurity in solution and (2) because of steric effects, the impurity complexes would no longer fit into the crystal lattice of the target compound. By adding coformers, the amount of the impurity incorporated into the crystal lattice would decrease, thereby purifying the compound of interest.

To confirm that the purification was due to the complexation in solution, we estimated the binding constants of the complexes and used them as indicators for the level of complexation. Phase solubility diagrams for cocrystal systems were used to find the stoichiometry and the binding constants for these complexes in equilibrium with cocrystals. With different combinations of the cocrystal stoichiometry, complex stoichiometries, and the binding constants of the complexes, the solubility of the cocrystal is a unique function of the coformer concentration. Nehm et al. showed that when compound A forms a 1:1 cocrystal with coformer B in the absence of complex formation, the solubility product can be expressed as:

\[ K_{sp} = [A][B] \]  

where \( K_{sp} \) is the solubility product of the AB cocrystal, and \([A]\) and \([B]\) are the concentrations of both cocrystal components at equilibrium. The solubility product can
be calculated from the plot of [A] versus $\frac{1}{[B]}$. If there is 1:1 complex formation in solution, the total concentration of A, $[A]_T$, can be expressed as:

$$[A]_T = \frac{K_{sp}}{[B]_T - K_{11}K_{sp}} + K_{11}K_{sp}$$

(4-2)

where $[B]_T$ is the total concentration of B and $K_{11}$ is the binding constant of the 1:1 AB complex. Assuming $K_{11}K_{sp} << [B]_T$, the total concentration of A, $[A]_T$, can be expressed as:

$$[A]_T = \frac{K_{sp}}{[B]_T} + K_{11}K_{sp}$$

(4-3)

Similarly, if A and B form a 2:1 cocrystal, the solubility of this binary cocrystal $A_2B$ in a pure solvent where the cocrystal components do not ionize or form complexes in solution is given by the equilibrium reaction:

$$A_2B_{solid} \rightleftharpoons 2A_{solution} + B_{solution}$$

(4-4)

The equilibrium constant for this reaction is given by:

$$K_{sp} = \frac{aA^2aB}{aA_2B}$$

(4-5)

Assuming that the activity of the solid and the activity coefficients are 1, the cocrystal solubility product can be expressed as:

$$K_{sp} \approx [A]^2[B]$$

(4-6)

Therefore, if the $A_2B$ cocrystal is dissolved in pure solvent and there is no further ionization or complex formation in solution, then the plot of $[A]_T^2$ against $\frac{1}{[B]_T}$ would be linear with a slope of $K_{sp}$.

If a 1:1 complex forms in solution, the equilibrium reactions become:
\[ A_2B_{\text{solid}} \rightleftharpoons 2A_{\text{solution}} + B_{\text{solution}} \]  \hspace{1cm} (4-4)
\[ A_{\text{solution}} + B_{\text{solution}} \rightleftharpoons AB_{\text{solution}} \]  \hspace{1cm} (4-7)

The solubility product remains the same as that given in Eq. (4-6) and the binding constant for the 1:1 complex can be expressed as:

\[ K_{11} = \frac{[AB]}{[A][B]} \]  \hspace{1cm} (4-8)

The mass balances of A and B in solution are:

\[ [A]_T = [A] + [AB] \]  \hspace{1cm} (4-9)
\[ [B]_T = [B] + [AB] \]  \hspace{1cm} (4-10)

If a 2:1 complex forms in solution, the equilibrium reactions are:

\[ A_2B_{\text{solid}} \rightleftharpoons 2A_{\text{solution}} + B_{\text{solution}} \]  \hspace{1cm} (4-4)
\[ 2A_{\text{solution}} + B_{\text{solution}} \rightleftharpoons A_2B_{\text{solution}} \]  \hspace{1cm} (4-11)

The equilibrium constants are expressed as Eq. (4-6) and as:

\[ K_{21} = \frac{[A_2B]}{[AB][A]} \]  \hspace{1cm} (4-12)

The mass balances of A and B are:

\[ [A]_T = [A] + 2[A_2B] \]  \hspace{1cm} (4-13)
\[ [B]_T = [B] + [A_2B] \]  \hspace{1cm} (4-14)

If both the 1:1 and the 2:1 complexes are formed in solution, then the equilibrium reactions are:

\[ A_2B_{\text{solid}} \rightleftharpoons 2A_{\text{solution}} + B_{\text{solution}} \]  \hspace{1cm} (4-4)
\[ A_{\text{solution}} + B_{\text{solution}} \rightleftharpoons AB_{\text{solution}} \]  \hspace{1cm} (4-7)
\[ AB_{\text{solution}} + A_{\text{solution}} \rightleftharpoons A_2B_{\text{solution}} \]  \hspace{1cm} (4-15)
The equilibrium constants are expressed as Eq. (4-6), Eq. (4-8), and (4-12).

The mass balances of A and B are now:

\[
[A]_T = [A] + [AB] + 2[A_2B] \tag{4-16}
\]

\[
[B]_T = [B] + [AB] + [A_2B] \tag{4-17}
\]

In the case where A and B form 2:1 cocrystals, the binding constants cannot be solved analytically. Instead, they can be fitted using \([A]_T\) and \([B]_T\) obtained experimentally. Phase solubility diagram data measured for a cocrystal system with a known cocrystal stoichiometry can be used to determine if the cocrystal components form complexes in solution as well as the binding constants of the complexes.

The following coformers were chosen to form complexes with the impurities, BA and CA: (1) isonicotinamide (INA), (2) 2-amino-4,6-dimethylpyrimidine (DMP), and (3) dimethylglyoxime (DMG). Separation experiments were performed and the purities of the target compounds, BAM and CAM, under different experimental conditions were measured. The results are presented in Table 4-2, Table 4-3, and Table 4-4, respectively. Two systems showed promising purification results: (1) BA-DMG and (2) CA-DMP. Phase solubility diagram data for these two systems were measured and used to find the binding constants of the complexes formed in solution. Phase solubility diagram data for other two systems that did not show significant purification were measured as comparisons. The purification results were explained using the level of complexation in solution.

In this work, we were able to purify BAM and CAM by adding coformers reported to form cocrystals with the impurities, BA and CA, respectively. These coformers were confirmed to form complexes with the impurities and hence prevent the
impurities from substituting into the crystal lattice. These findings have practical applications in the development of purification methods.

4.2 Materials and Methods

4.2.1 Materials

Benzoic acid (BA, ACS reagent, ≥ 99.5%), benzamide (BAM, 99%), trans-Cinnamic acid (CA, ≥ 99%), cinnamamide (CAM, predominately trans, 97%), 2-amino-4,6-dimethylpyrimidine (DMP), dimethylglyoxime (DMG, ACS reagent) and isonicotinamide (INA, lot no. BCBD6627V, 99%) were purchased from Sigma-Aldrich and were used as received. Anhydrous ethanol (200 proof) was USP grade and was purchased from VWR. Methanol (MeOH, CHROMASOLV® for HPLC ≥99%) and water (H₂O, CHROMASOLV® Plus for HPLC) were purchased from Sigma-Aldrich and used for HPLC.

4.2.2 Coformer selection

A search in CSD was conducted to select the coformers reported to form cocrystals with the impurities, BA and CA. This search led to the selection of three coformers: (1) isonicotinamide (INA), (2) 2-amino-4,6-dimethylpyrimidine (DMP), and (3) dimethylglyoxime (DMG). Their structures are shown in Figure 4-2. The reference codes of these reported cocrystals in CSD are presented in Table 4-1.
Figure 4-2 Structures of (a) Isonicotinamide (INA), (b) 2-amino-4,6-dimethylpyrimidine (DMP), and (c) dimethylglyoxime (DMG).

Table 4-1 Reference Codes of the Reported Cocrystals.

<table>
<thead>
<tr>
<th></th>
<th>INA</th>
<th>DMP</th>
<th>DMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>BUDWEC</td>
<td>VOCZET, VOCZET01, VOCZET02, VOCZET03</td>
<td>NABCAV, NABCAV01</td>
</tr>
<tr>
<td>CA</td>
<td>LUNMAI</td>
<td>DAYWAC</td>
<td>NABCEZ</td>
</tr>
</tbody>
</table>

These six cocrystals were made successfully. Four cocrystals (BA-INA, BA-DMP, CA-INA, and CA-DMP) were prepared using solution crystallization with the reported cocrystal stoichiometry (1:1 molar ratio). BA-DMG and CA-DMG cocrystals were prepared using a different stoichiometry (1:1 molar ratio) from the cocrystal stoichiometry (2:1 molar ratio) because of the low solubility of DMG in ethanol. The obtained cocrystals were confirmed to be the reported ones using X-ray powder diffraction (XRPD).
4.2.3 Crystallization experiments

Three sets of experiments were performed to determine if the coformer could be used to purify the target compound. In the first experiment, BAM and CAM were crystallized with coformers to determine if the coformers substitute into their crystal lattices. The coformers incorporated into the target crystal lattices were no longer investigated further. In the second experiment, BAM and CAM were crystallized with only the impurities, BA and CA, respectively, to examine how much impurity was incorporated into the target crystal lattices. In the third experiment, BAM and CAM were crystallized with both the impurity and the coformers that were not excluded after the first experiment to examine the purities of BAM and CAM after the addition of coformers. The initial solution contained the saturation concentration of the target compound at 40°C and the impurity at weight ratios of 1:9 and 1:4 to the target compound. The amount of coformer added was the stoichiometric amount to form the cocrystal with the impurity. That is, the impurity to coformer ratio was the same as the stoichiometry used to make the impurity cocrystal. The solution was heated in a water bath to 50°C until the target compound dissolved. The solution was cooled to 30°C at a rate of 1°C/min and then to 20°C at a rate of 1°C/6 min. The solids obtained from the crystallization were collected and washed using iced ethanol. The purities of the resulting products were determined using high performance liquid chromatography (HPLC).
4.2.4 Phase solubility diagram construction

The same procedure as described in section 3.2.5 was followed. Different compositions of A and B solids (A-B combinations: BA-DMP, BA-DMG, CA-DMP, CA-INA) were added to ethanol to construct phase solubility diagrams.

4.2.5 High Performance Liquid Chromatography (HPLC)

The same HPLC instrument and column as those described in section 3.2.6 were used. The maximum wavelength for absorbance was set at 230 nm. The concentrations were analyzed using a 5 min isocratic method with a 30/70 water/methanol mobile phase containing 0.1% trifluoroacetic acid.

4.2.6 Model fitting

Different approaches were used to calculate the binding constants of the complexes for the cocrystal systems. For each 1:1 cocrystal system, the plot of the total impurity concentration versus the reciprocal of the total coformer concentration was analyzed using least squares regression. If linear dependence was shown, we could find the solubility product, $K_{sp}$ from the slope and the binding constant for the 1:1 complex, $K_{11}$, from the intercept. An intercept that was not significantly different from 0 indicated that there was no complexation and that $K_{11}$ was 0. Otherwise, the binding constants of the complexes, $K_{11}$, could be determined using Eq. (4-3). If no linear dependence was shown, then Eq. (4-2) was used to find the solubility product and the binding constant of the complex. Eq. (4-2) can be rearranged and expressed as:

$$([A]_T \times [B]_T) = K_{11} K_{sp} ([A]_T + [B]_T) + (K_{sp} - K_{11}^2 K_{sp}^2)$$  \hspace{1cm} (4-18)
The plot of \([A]_T \times [B]_T\) versus \([A]_T + [B]_T\) was thus used to find the solubility product and the binding constant using Eq. (4-19) and (4-20).

\[
K_{sp} = \text{intercept} + \text{slope}^2 \tag{4-19}
\]

\[
K_{11} = \frac{\text{slope}}{K_{sp}} \tag{4-20}
\]

For the 2:1 cocrystal system, the solubility product and binding constants could not be found using linear regression. Instead, the phase solubility diagram data obtained for the BA-DMG system was fitted to four different models: the 2:1 cocrystal system with no complexation, the system with the 1:1 complex in solution, the system with the 2:1 complex in solution, and the system with both 1:1 and 2:1 complexes, respectively.

In the first model, the cocrystal components do not ionize or form complexes in solution. For a given \(K_{sp}\), the total concentrations of \(A\), \([A]_T,\text{fitted}\), were calculated using Eq. (4-6) and the experimentally measured concentrations of \(B\). \([A]_T,\text{fitted}\), were then compared to the total concentrations of \(A\) obtained experimentally, \([A]_T,\exp\). The method of least squares was applied to find the best solution, or the solution with the minimum sum of squared errors, which was defined as:

\[
\text{Sum of Squared Errors} = \sum([A]_{T,\exp} - [A]_{T,\text{fitted}})^2 \tag{4-21}
\]

Various \(K_{sp}\) values (from 0 to 10 in steps of 0.0001) were examined to find the best \(K_{sp}\) value that minimized the sum of squared errors. This range was chosen based on the estimation using Eq. (4-6) and the fact that it should be \(\geq 0\).

The second, third and forth models were fitted in the same way. In the second model, the 2:1 cocrystal is in equilibrium with the 1:1 complex in solution. Given \(K_{sp}\) and \(K_{11}\), \([A]_T,\text{fitted}\) were calculated using Eq. (4-6), (4-8), (4-9), and (4-10) and \([B]_T,\exp\).
Various values of $K_{sp}$ (from 0 to 10 in steps of 0.0001) and $K_{11}$ (from 0 to 100 in steps of 0.0001) were examined to find the optimal ones that minimized the sum of squared errors defined in Eq. (4-21). The range for $K_{11}$ was chosen based on literature values and the fact that it should be $\geq 0$. In the third system where only the 2:1 complex is in equilibrium with the 2:1 cocrystal, we calculated $[A]_T, \text{fitted}$ using Eq. (4-6), (4-12), (4-13), (14), and $[B]_T, \text{exp}$. Various values of $K_{sp}$ (from 0 to 10 in steps of 0.00001) and $K_{21}$ (from 0 to 100 in steps of 0.0001) were examined to find the optimal ones that minimized the sum of squared errors defined in Eq. (4-21). The range for $K_{21}$ was chosen based on literature values and the fact that it should be $\geq 0$. Similarly, in the fourth system where the 2:1 cocrystal is in equilibrium with both 1:1 and 2:1 complexes, we calculated $[A]_T, \text{fitted}$ using Eq. (4-6), (4-8), (4-12), (4-16), (4-17), and $[B]_T, \text{exp}$. $K_{sp}$, $K_{11}$, and $K_{21}$ values that give the minimum sum of squared error were found after various $K_{sp}$ values (from 0 to 10 in steps of 0.0001), $K_{11}$ values (from 0 to 100 in steps of 0.0001), and $K_{21}$ values (from 0 to 100 in steps of 0.0001) were tested, respectively. A Matlab program was written to perform the method of least squares on the four models.

**4.3 Results and Discussions**

4.3.1 Purification results

The purities of BAM obtained from the crystallization experiments are presented in Table 4-2. The results using INA are not shown in the table because it substituted into the BAM crystal lattice and therefore was not used to purify BAM. The original mass percentage of BA in the BAM crystal was $0.35(\pm 0.033)$ wt%. Once DMP was added (1:1 molar ratio of BA:DMP), the amount of BA in the BAM crystal decreased to $0.32(\pm 0.049)$ wt% with $0.087(\pm 0.02)$ wt% of DMP present in the BAM crystals. With
the addition of DMG (1:1 molar ratio of BA:DMG), the amount of BA in the BAM crystals decreased to 0.28(±0.025) wt% with no trace of DMG detected. The effect of the coformer stoichiometry was studied by varying the amount of DMG (1:1.5 and 1:2 molar ratios of BA:DMG). With the addition of 1:1.5 and 1:2 molar ratios of DMG, the amount of BA decreased to 0.32(±0.024) wt% and 0.31(±0.029) wt%, respectively. To determine whether the decreases are statistically significant at a 95% confidence interval, all of the amounts of BA after the addition of coformers were compared to the original amount of BA using a t-test. The decreases of the amount of BA after the addition of DMG at all molar ratios were determined to be statistically significant (p=0.00005359 for the 1:1 molar ratio, p=0.04717 for the 1:1.5 molar ratio, and p=0.01899 for the 1:2 molar ratio). On the other hand, the decrease of the amount of BA after the addition of DMP was not statistically significant (p=0.1204). In addition, a t-test was performed to determine whether the amount of BA decreases with an increasing amount of DMG added. The amounts of BA with the addition of 1:1.5 and 1:2 molar ratios of DMG were compared to that with the addition of a 1:1 molar ratio of DMG, respectively. The results showed that increasing the amount of DMG did not statistically significant decrease the amount of BA substituted into the crystal lattice (p=0.9908 for 1:1.5 molar ratio and p=0.9694 for the 1:2 molar ratio).
Table 4-2 Purities of Benzamide in Different Crystallization Experiments.

(The Initial Solution contained a 9:1 Weight Ratio of BAM:BA.)

<table>
<thead>
<tr>
<th></th>
<th>BA (wt%)</th>
<th>DMP (wt%)</th>
<th>DMG (wt%)</th>
<th>Decreased (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAM/BA</td>
<td>0.35±0.033</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>With DMP</td>
<td>0.32±0.049</td>
<td>0.087±0.020</td>
<td>-</td>
<td>8.18</td>
</tr>
<tr>
<td>With 1x DMG</td>
<td>0.28±0.025</td>
<td>-</td>
<td>0.00</td>
<td>18.79</td>
</tr>
<tr>
<td>With 1.5x DMG</td>
<td>0.32±0.024</td>
<td>-</td>
<td>0.00</td>
<td>7.71</td>
</tr>
<tr>
<td>With 2x DMG</td>
<td>0.31±0.029</td>
<td>-</td>
<td>0.00</td>
<td>11.00</td>
</tr>
</tbody>
</table>

The purities of CAM obtained from the crystallization experiments are shown in Table 4-3. The original mass percentage of CA in CAM crystal was 0.85(±0.078) wt%. Once DMP was added (1:1 molar ratio of CA:DMP), the amount of CA in CAM crystal decreased to 0.65(±0.101) wt%. After the addition of INA (1:1 molar ratio of CA:INA), the amount of CA decreased to 0.76(±0.142) wt%. However, the addition of DMG increased the amount of CA incorporated into CAM crystal lattices to 0.92(±0.153) wt%. No trace of coformers was detected in any resulting CAM solids. A t-test determined that the amount of CA that decreased with the addition of coformers was statistically significantly at a 95% confidence interval. The amounts of CA decreased statistically significantly with the addition of DMP (p=0.009485) but not significantly with the addition of DMG (p=0.7684) and INA (p=0.1471).
Table 4-3 Purities of Cinnamamide in Different Crystallization Experiments.

(The Initial Solution Contained a 9:1 Weight Ratio of BAM:BA.)

<table>
<thead>
<tr>
<th></th>
<th>CA (wt%)</th>
<th>Decreased (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM/CA</td>
<td>0.85±0.078</td>
<td></td>
</tr>
<tr>
<td>With DMP</td>
<td>0.65±0.101</td>
<td>23.79</td>
</tr>
<tr>
<td>With DMG</td>
<td>0.92±0.153</td>
<td>-7.88</td>
</tr>
<tr>
<td>With INA</td>
<td>0.76±0.142</td>
<td>10.93</td>
</tr>
</tbody>
</table>

The effect of the initial impurity concentration was investigated using the CAM/CA system. CA was added at a weight ratio of 1:4 to CAM. DMP (1:1 molar ratio of CA:DMP) was added to purify CAM crystals. The purities of CAM obtained from the crystallization experiments are shown in Table 4-4. The original mass percentage of CA in the CAM crystals was 2.01(±0.21) wt%. Once DMP was added (1:1 molar ratio of CA:DMP), the amount of CA in the CAM crystals decreased to 1.58(±0.20) wt%. No trace of DMP was detected in any resulting CAM solids. A t-test determined that the amount of CA decreased with the addition of coformers statistically significantly at a 95% confidence interval (p=0.03045).
Table 4-4 Purities of Cinnamamide in Different Crystallization Experiments.

(The Initial Solution Contained a 4:1 Weight Ratio of BAM:BA.)

<table>
<thead>
<tr>
<th>CAM/CA</th>
<th>2.01±0.21</th>
</tr>
</thead>
<tbody>
<tr>
<td>WITH DMP</td>
<td>1.58±0.20</td>
</tr>
</tbody>
</table>

To summarize, two systems showed statistically significant increases of the purities of the compounds of interest: the BA-DMG system and the CA-DMP system. The addition of other coformers did not have a significant effect on the amount of impurities incorporated into the crystal lattices of the target compounds. In the BA-DMG system, the amount of BA did not decrease with an increasing amount of DMG added.

4.3.2 Complexation measurement

To examine whether the level of complexation has a correlation to the purification results, we estimated the binding constants of the complexes in our systems of interest. For the BAM/BA system, DMG was an effective coformer for BAM purification. Since BA and DMG are known to form a 2:1 cocrystal, the stoichiometries and the binding constants of complexes were determined using the method described above for 2:1 cocrystals. Model 3 had a large error (0.4393) compared to other models (0.0065 for model 1 and 0.018 for model 2 and 4) and was not considered. The facts that $K_{21}$ was determined to be 0 in model 4 and that the values of $K_{sp}$ and $K_{11}$ were identical in model 2 and 4 ($K_{sp}=0.4413$ and $K_{11}=0.3469$) indicated that the assumption of having a 2:1 complex did not change the behavior of model 2. That is, no 2:1 complex was
present in solution and model 4 was not considered. Model 1 had a smaller sum of square error than model 2 did. However, it is highly unlikely to form 2:1 cocrystals without complex formation in solution. Therefore, model 2 was the reasonable model that fitted best to the experimental data.

The BA-DMP system was also examined for complexation behavior. BA and DMP are known to form a 1:1 cocrystal. Following the method described above for systems forming 1:1 cocrystals, the total impurity concentrations (BA) were plotted against the reciprocal of the total coformer concentrations (DMP). Linear regression with least squares was applied and no linear dependency was shown ($R^2 = 0.84234$). Linear regression was again applied using Eq. (4-18). The results are shown in Figure 4-3. The solubility products and the binding constants were calculated using Eq. (4-19) and (4-20) with the slope and the intercept obtained from Figure 4-3. The solubility product was 0.0053 and the binding constant for the 1:1 complex was 6.67.

![Figure 4-3 Linear Regression Results of the BA-DMP System.](image)
For the CAM/CA system, the addition of DMP significantly decreased the amount of CA incorporated into CAM crystal lattices. CA and DMP are known to form 1:1 cocrystals and Eq. (4-1) was used to perform linear regression and no linear dependence was shown ($R^2=0.25146$). Linear regression was applied using Eq. (4-2) and the results are presented in Figure 4-4. $K_{sp}$ (0.0018) and $K_{11}$ (64.86) were calculated using Eq. (4-19) and (4-20).

![Figure 4-4 Linear Regression Results of the CA-DMP System.](image)

The complexation behavior in the CA-INa system was examined as a comparison to that in the CA-DMP system. Linear regression using Eq. (4-1) was performed because CA forms 1:1 cocrystals with INa. A linear dependence was shown in Figure 4-5 ($R^2=0.98$). Examination of the linear regression analysis revealed that the y-intercept was not statistically different from zero ($p=0.762$). This fact suggested that no complex was formed in solution. Based on Eq. (4-1), the solubility product of the CA-INa system was the slope of the regression line, 0.023. The solubility products and
the binding constants for the 1:1 complex for the BA-DMG, BA-DMP, CA-DMP, and CA-INA systems are summarized in Table 4-5.

![Graph showing the relationship between total CA concentration in equilibrium with the cocrystal, CA-INA, and the reciprocal of total INA concentration in ethanol at 20°C. The equation for the line is y = 0.0231x - 0.0018 and R² = 0.98223.]

Figure 4-5 Total CA Concentration in Equilibrium with the Cocrystal, CA-INA, as a Function of the Reciprocal of Total INA Concentration in Ethanol at 20°C.

Table 4-5 Solubility Products and Binding Constants for (1) BA-DMG, (2) BA-DMP, (3) CA-DMP, and (4) CA-INA.

<table>
<thead>
<tr>
<th></th>
<th>BA-DMG</th>
<th>BA-DMP</th>
<th>CA-DMP</th>
<th>CA-INA</th>
</tr>
</thead>
<tbody>
<tr>
<td>K&lt;sub&gt;sp&lt;/sub&gt;</td>
<td>0.4413</td>
<td>0.0053</td>
<td>0.0018</td>
<td>0.023</td>
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<tr>
<td>K&lt;sub&gt;11&lt;/sub&gt;</td>
<td>0.3469</td>
<td>6.67</td>
<td>64.86</td>
<td>0</td>
</tr>
</tbody>
</table>

4.3.3 Discussions

Results for the BAM/BA system did not show a positive correlation between the complexation level in solution and the purification results. The addition of DMG decreased the amount of BA the most but the binding constant of the BA-DMG complex was smaller than that of the BA-DMP complex. This result can be explained by the fact
that DMP substituted into BAM crystal lattices. As shown in Table 4-2, after the addition of DMP, DMP was detected in CAM crystals (0.087±0.020 wt%). It is likely that some BA-DMP complexes substituted into BAM crystal lattices. Therefore, despite the high complexation level in the BA-DMP system, the purity of BAM was enhanced the most with the addition of DMG.

A positive correlation was found between the level of complexation in solution and purification results in the CAM/CA system. The amount of CA decreased the most with the addition of DMP. On the other hand, the addition of INA did not decrease the amount of CA significantly. In addition, the binding constant of the CA-DMP complex was much larger than that of the CA-INA complex. We can conclude that for the CAM/CA system, the more complexes formed in solution, the better the purification results are.

This positive correlation was observed when we compared the results of the two systems that showed significant decreases: the BAM/BA system with the addition of DMG and the CAM/CA system with the addition of DMP. Although the original impurity levels were different in the BAM/BA and CAM/CA systems, we can compare purification results by calculating the decreases of impurity amounts in moles/g solids. The decrease of the CAM-CA-DMP system was $1.3 \times 10^{-5}$ moles/g solids while the decrease of the BAM-BA-DMG system was $5.3 \times 10^{-6}$ moles/g solids. The binding constant of CA-DMP complex was significantly larger than that of the BA-DMG complex. The level of complexation can be used to explain purification results in different API/impurity systems.
The complexation in purification experiments was not measurable using spectroscopy methods (including raman spectroscopy, ultraviolet–visible spectroscopy, and infrared spectroscopy) and nuclear magnetic resonance (NMR). For spectroscopy methods, impurity peaks overlap with coformer peaks and it is difficult to identify the contribution from the impurity, the coformer, and their complex. For NMR measurements, the complexation was not observed due to the concentration limit allowed in NMR. Additionally, the solvent we used in purification experiments, ethanol, is a polar solvent that can interfere with hydrogen bond formation between the impurity and the coformer. Hence, binding constants were the best indicators for the level of complexation in solution even if we only considered the interaction between the impurity and the coformer.

4.3.4 Summary

BAM/BA and CAM/CA were chosen as our model systems. A search in the CSD led to the selection of INA, DMP, and DMG as coformers. For the BAM/BA system, the addition of DMG decreased the amount of BA in the BAM crystal from 0.35(±0.033) wt% to 0.28(±0.025) wt%. The amount of BA did not decrease with an increasing amount of DMG added. For the CAM/CA system, the addition of DMP decreased the amount of CA incorporated into CAM crystal lattices from 0.85(±0.078) wt% to 0.65(±0.101) wt%. We estimated the binding constants in four systems: BA-DMG, BA-DMP, CA-DMP, and CA-INa to indicate the complexation level in solution. However, the decrease of the impurity level cannot be solely explained by the amount of impurity–coformer complexes formed in solution. It is premature to use the complexation behavior as a predictive tool to select a coformer for purification purposes.
4.4 Conclusions

In this project, we presented the purification results and complexation behaviors of the BA/BAM system and CA/CAM system after adding coformers. The results demonstrated the possibility of purifying structurally similar compounds by adding coformers to their solutions. The addition of DMG significantly decreased the amount of BA in BAM crystals. The purity of CAM also increased significantly after the addition of DMP. While the purification can be explained by the impurity-coformer complex formation in solution, the addition of coformers has other effects that prevent impurities from substituting into crystal lattices of the target compound.
5. THE PURIFICATION OF AMOXICILLIN

5.1 Introduction

In Chapter 4, we investigated the possibility of purifying structurally similar compounds using selective impurity complex formation in solution followed by crystallization of the target compound. We postulated that coformers that can form cocrystals with the impurity are more likely to form complexes with the impurity in solution and thus the impurity complex is too bulky to fit into the crystal lattice of the target crystal. Therefore, by adding coformers that can form cocrystals with the impurity but not with the target to mixtures of the target and impurity, we could prevent impurities from substituting into the target crystal lattice. For the two systems we studied (benzamide/benzoic acid and cinnamamide/cinnamic acid), we had knowledge of the impurity cocrystal formation in advance and were able to demonstrate the potential feasibility of purifying mixtures of structurally similar compounds by adding coformers to their solutions. The correlation between the level of complexation and the purification, however, needs additional experimental verification. In this chapter, a real drug/impurity system, amoxicillin trihydrate/4-hydroxyphenylglycine (AMCT/4HPG) was chosen to evaluate the practical use of the proposed method and its mechanism further.

Amoxicillin trihydrate (AMCT) is one of the major β-lactam antibiotics, which are widely used against a broad spectrum of bacteria. AMCT has a high solubility, high absorption rates and high stability under acidic conditions. In addition, at the same dosage, the blood level of AMCT is twice as high as that of ampicillin. It was first brought to the market in 1972 in the trihydrate form. Amoxicillin-clavulanate was later
introduced as a combination antibiotic as the clavulanate enhances the potency of amoxicillin.\textsuperscript{40-42}

Like other β-lactam antibiotics, AMCT was originally produced by a semisynthetic route. The first generation of the AMCT industrial process was based on the Dane salt route (Figure 5-1), whose yield could go above 90%. However, the reaction demands low temperature (\(-30^\circ\text{C}\)), protection, deprotection and activation steps and the usage of several undesirable reagents and solvents (e.g. \(\text{CH}_2\text{Cl}_2\)). Consequently, the amount of waste generated from this process is substantial.\textsuperscript{40}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5-1.png}
\caption{The Industrial Process for AMCT (the Dane Salt Route).}
\end{figure}

With increasingly tight environmental regulations, the need to replace the Dane Salt route with an enzymatic synthesis has increased. The enzymatic process takes fewer steps than the chemical synthetic route does and can be completed in aqueous solutions, at neutral pH, and at ambient temperature. Two approaches to produce β-lactam antibiotics have been studied: the thermodynamically controlled approach and the kinetically controlled approach. Figure 5-2 is the comparison of these two approaches in penicillin synthesis. In the thermodynamically controlled approach, the side chain reacts with 6-aminopenicicnic acid (6-APA), with penicillin G Acylase (PGA) as the catalyst, to form the antibiotic, with water as a side product. The main limitation of this approach is that both the carboxylic acid group on the side chain and the amine group on 6-APA
need to be neutral for the reaction to occur. However, under the active pH range of PGA, only few side chains meet this criterion. Attempts were made to overcome this limitation (different enzymes and different solvents). However, as of today, the kinetically controlled approach is the main interest and most promising replacement for the chemical synthetic route. In the kinetically controlled approach, the side chain derivative reacts with 6-APA to form the antibiotic, with PGA as the catalyst. However, since PGA can be both a transferase and a hydrolase, it hydrolyzes the side chain derivative and the product, the antibiotic, while catalyzing the main reaction. Many studies have been performed to optimize the reaction conditions for the maximum yield, selectivity (synthesis-to-hydrolysis ratio), and productivity. 40,43

![Chemical structures](image)

(a)

![Chemical structures](image)

(b)

Figure 5-2 (a) Thermodynamically Controlled and (2) Kinetically Controlled Synthesis of Semi-synthetic Penicillins with PGA as the Catalyst.
The kinetically controlled synthesis of AMCT is presented in Figure 5-3. This synthesis has become commercially feasible as the cost of suitable enzymes in robust and immobilized forms decreases. In this synthesis, 4-hydroxyphenylglycine methyl ester (HPGM) reacts with 6-APA to produce AMCT. Two hydrolysis reactions occur at the same time. In the first hydrolysis, the reactant, HPGM is hydrolyzed by PGA in the presence of water to form 4-hydroxyphenylglycine (4HPG) and methanol. In the second hydrolysis, the product, AMCT, is hydrolyzed by PGA in the presence of water to form 4HPG and 6-APA. Many studies have been done on the enzymatic synthesis of AMCT to maximize the yield, selectivity, and productivity. Various studies have been done on the effects of reactant concentrations, enzyme concentrations, the enzyme inhibitor and temperatures. Despite all efforts to try to remove it, 4HPG is inevitably present as an impurity in the synthesis of AMCT.

Figure 5-3 Enzymatic Synthesis of Amoxicillin using PGA as the Catalyst.
The importance of separating AMCT from its degradation products has been addressed in the literature. It has been shown that the degradation products can inhibit the nucleation process of AMCT, which is similar to the negative influence of impurities on the nucleation and growth rate in ampicillin crystallization. Factors affecting the amount of impurities incorporated into AMCT crystal lattice were also studied. The pH of the AMCT crystallization process can change the amount of 4HPG incorporated into AMCT crystal lattices. As the process pH increases, the solubility of 4HPG increases, and the powder pH of AMCT increases. Therefore, the amount of 4HPG in AMCT decreases as the process pH increases. The other factor studied was the presence of degradation products in the AMCT crystallization process. It was shown that the purities of AMCT crystallized with the presence of degradation products, whether at high or low concentration, were at least as pure as the standard material. It is known that USP grade AMCT should contain no more than 1% of D-hydroxyphenylglycine. When AMCT was synthesized through the Dane salt route, crystallization was used to purify and obtain the final products. As the interest in enzymatic synthesis grows, it is necessary to develop other purification methods, since different amounts of 4HPG can be incorporated into AMCT in different synthetic routes. Many chromatographic methods were developed to separate AMCT from 4HPG. However, in industrial processes, chromatography is often not desirable due to its high cost. Multi-stage recrystallization is often used to enhance the purity of the product but the yield can be sacrificed in the process. It is important to develop a separation method to separate AMCT from 4HPG without sacrificing the yield.
The goal of this work is to prevent 4HPG from substituting into the crystal lattice of AMCT by forming 4HPG complexes in solution. Without advance knowledge of cocrystal formation of 4HPG, a workflow was established to select the optimal coformer and the optimal amount coformer needed to achieve the best purification. Compounds with functional groups that could form hydrogen bonds with functional groups on 4HPG were ground with 4HPG and AMCT to find coformers that could form cocrystals with 4HPG but not with AMCT. We determined if the cocrystal was successfully formed by comparing the powder pattern of the resulting solid to that of the individual components. Eleven coformers were found to meet this criterion. AMCT was crystallized with both 4HPG and these coformers and the purities of the resulting products were evaluated using HPLC. The results are presented in Table 5-7. The four coformers that decreased the amount of incorporated 4HPG the most were 2-picolinic acid, L-lysine, L-leucine, and L-isoleucine. The amount of these coformer added was varied to (1) find the optimal amount of coformer that achieved the best purification, and (2) examine the correlation between the level of complexation and the purification results. The results are shown in Figure 5-6.

In this work, we were able to purify AMCT by adding coformers that would form cocrystals with 4HPG. Better purification was achieved using our proposed method than two crystallizations from the initial solution. A clear correlation between purification results and the amount of complexes formed was observed.
5.2 Materials and Methods

5.2.1 Materials

Amoxicillin trihydrate (AMCT) was purchased from Alfa Aesar and used as received. 4-hydroxyphenylglycine (4HPG, ≥98%), and all coformers (Table 5-1, Table 5-2, and Table 5-3) were purchased from Sigma-Aldrich and used as received. Hydrochloric acid concentrate (to produce a liter of 1.0M HCl) was purchased from Sigma-Aldrich and measured into a 1 L volumetric flask, which was then filled with HPLC grade water to the mark. Sodium hydroxide was purchased from Sigma-Aldrich and used to prepare a 5.0M solution. Potassium phosphate monobasic (for HPLC, ≥99.5%) and dibasic (for HPLC, ≥99.0%) were purchased from Sigma-Aldrich. Water (H$_2$O, CHROMASOLV® Plus for HPLC), methanol (MeOH, CHROMASOLV® for HPLC ≥99%) and acetonitrile (ACN, CHROMASOLV® for HPLC ≥99%) were purchased from Sigma-Aldrich and used as received.

5.2.2 Coformer selection

To find compounds with the potential to form cocrystals with 4HPG, we needed to find synthons in which one functional group could interact with a functional group on 4HPG (the carboxylic acid group or the amine group). Forty-seven compounds were chosen for screening based on this criterion. The entire list can be found in Table 5-1, Table 5-2, and Table 5-3. These compounds can be categorized as compounds with functional groups encouraging the formation of synthons with the carboxylic acid group on 4HPG (Group I, with amide, primary, secondary, and tertiary amine groups, Table 5-1), the amine group on 4HPG (Group II, with carboxylic acid group, Table 5-2), and both groups on 4HPG (Group III, with both carboxylic acid and amine group, Table 5-
3). Solid-state grinding was performed to select coformers that could form cocrystals with 4HPG but not with AMCT. We ground a 1:1 4HPG:coformer mixture using a mortar and pestle with few drops of water and measured the powder pattern of the resulting solid using x-ray powder diffraction (XRPD). The powder pattern was then compared to the powder patterns of the individual components. If new peaks were observed, the coformer was then selected to continue to the next step. Powder patterns of the thirteen resulting solids that show new peaks can be found in Appendix I. Two of them (4HPG ground with Lysine and Urea at a 1:1 molar ratio, respectively) are shown as examples in Figure 5-4 and Figure 5-5. In the following step, the coformer was ground with AMCT at a 1:1 molar ratio. Eleven compounds were found to have the potential to form cocrystals with 4HPG but not with AMCT (Table 5-4).
Table 5-1 Compounds with Amide, Primary, Secondary, and Tertiary Amine Groups (Group 1).

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecular Weight</th>
<th>Structure</th>
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<tr>
<td>1,3-diethylurea</td>
<td>116.16</td>
<td><img src="" alt="structure" /></td>
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<tr>
<td>1,1-diethylurea</td>
<td>116.16</td>
<td><img src="" alt="structure" /></td>
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<td>Urea</td>
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<td>4-benzyloxy-2(1H)-pyridone</td>
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## Table 5-1 Compounds with Amide, Primary, Secondary, and Tertiary Amine Groups (Group I) (Cont’d)

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<td>5-bromo-2(1H)-pyridone</td>
<td>174</td>
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<td>2-hydroxypyridine</td>
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<td>2-imidazolidone</td>
<td>86.09</td>
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<td>3-nitrobenzamide</td>
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<tr>
<td>4-chlorobenzamide</td>
<td>155.58</td>
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Table 5-1 Compounds with Amide, Primary, Secondary, and Tertiary Amine Groups (Group I) (Cont’d).

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<td>Imidazole</td>
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Table 5-1 Compounds with Amide, Primary, Secondary, and Tertiary Amine Groups (Group I) (Cont'd).

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Table 5-1 Compounds with Amide, Primary, Secondary, and Tertiary Amine Groups (Group I) (Cont’d).

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<td>4-phenylpyridine</td>
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Table 5-2 Compounds with Carboxylic Acid Group (Group II).

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Table 5-3 Compounds with Both Carboxylic Acid and Amine Groups (Group III).

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Table 5-3 Compounds with Both Carboxylic Acid and Amine Groups (Group III) (Cont’d).

<table>
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<th>Name</th>
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<th>Structure</th>
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| L-threonine| 119.12           | ![Molecular Structure](image)
| L-tryptophan| 204.23           | ![Molecular Structure](image)
| L-histidine| 155.15           | ![Molecular Structure](image)
| L-isoleucine| 131.17           | ![Molecular Structure](image)
| L-valine   | 117.15           | ![Molecular Structure](image) |
Figure 5-4 Comparison between the Powder Pattern of the Cocrystal/4HPG/L-lysine Mixture and the Powder Patterns of the Individual Components.

Figure 5-5 Comparison between the Powder Pattern of the Cocrystal/4HPG/Urea Mixture and the Powder Patterns of the Individual Components.
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</tr>
<tr>
<td>L-isoleucine</td>
<td><img src="image11" alt="L-isoleucine" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.2.3 Separation experiments

Three sets of experiments were performed to determine if the coformer could be used to purify AMCT. In the first experiment, AMCT was crystallized with 4HPG, to examine how much 4HPG was incorporated into the AMCT crystal lattice (product 1). In the second experiment, AMCT was crystallized with both the impurity and the coformer to examine the amount of 4HPG incorporated after the addition of the coformer (product 2). In the third set of experiments, product 1 was crystallized in fresh solvent to examine the amount of 4HPG incorporated after two crystallizations from the initial solution (product 3). In the initial solution, 1.67g of AMCT and 4HPG at 1:10 and 1:5 4HPG:AMCT weight ratios (0.167g and 0.334g, respectively) were dissolved in 100 ml of 1M HCl. Coformers were added at a 1:1 4HPG:coformer molar ratio. A 0.45 μm PTFE syringe filter was used to remove undissolved solids. The pH of the solution was then adjusted to 4.7 using 5M NaOH followed by a 1-hour wait. The solids obtained from the crystallization were collected and washed using 2 ml of a 15:85 water/isopropanol mixture. The solids were examined using x-ray powder diffraction and confirmed to be AMCT. The amount of 4HPG in resulting products was determined using high performance liquid chromatography (HPLC).

5.2.4 High-Performance Liquid Chromatography

The HPLC instrument (Agilent 1260 Infinity) was equipped with a UV diode array detector (Agilent Technologies G1315D). The column used was an Agilent ZORBAX Bonuss-RP 150×4.6 mm I.D. column packed with 5 μm particles (Agilent). The maximum wavelength for absorbance was set at 230 nm. The concentrations were analyzed using a 35 min gradient elution program (Table 5-5).\(^\text{48}\)
5.3 Results and Discussions

5.3.1 Coformer selection

Of the 47 compounds we screened, 13 could form cocrystals with 4HPG. Carbamazepine and 1,2-bis(4-pyridyl)ethane were able to form cocrystals with AMCT in addition to with 4HPG and therefore were eliminated from the final list of eleven coformers (Table 5-4). Of the 47 compounds, there were 31, 6, and 10 in Groups I, II, and III, respectively. Several factors were examined to determine their effects on the cocrystal formation: the molecular weight of the compound, the functional group(s) on the compound, and whether the compound had a phenyl ring or not. The results are summarized in Table 5-6. For the molecular weight, it was found that 28 compounds had a molecular weight lower than 150. Ten of these compounds could form cocrystals with 4HPG. Out of the 19 compounds with a molecular weight greater than 150, only three could form cocrystals with 4HPG; that is, if the compound had a molecular weight lower
than 150, it had a higher chance of forming cocrystal with 4HPG (35.71% versus 15.79%). When we examined the functional group(s), it was observed that no compound in Group II could form cocrystals with 4HPG. The probability for a compound in Group I and III to form cocrystals with 4HPG was 25.9% (8 out of 31 compounds) and 50% (5 out of 10 molecules), respectively; that is, a compound with functional groups that could form hydrogen bonds with both the carboxylic acid and amine groups on 4HPG had a better chance of forming cocrystals with 4HPG. Finally, we examined the correlation between having a phenyl ring in the molecule and its likelihood of forming cocrystals with 4HPG. The probability of having a phenyl ring and forming cocrystals with 4HPG was 35.29% (6 out of 17 compounds); the probability of not having a phenyl ring but forming cocrystals with 4HPG was 23.33% (7 out of 30). This result did not indicate any clear correlation.
Table 5-6 Numbers of Coformers in Different Groups Characterized by Molecular Weight (MW), Structures, and the Existence of Phenyl Ring.

<table>
<thead>
<tr>
<th>Group</th>
<th>MW &lt; 150</th>
<th>MW &gt; 150</th>
<th>Total #</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of Coformers</td>
<td># of Compounds</td>
<td># of Coformers</td>
</tr>
<tr>
<td>Group I</td>
<td>5</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>no phenyl ring</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>w/ phenyl ring</td>
<td>3</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Group II</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>no phenyl ring</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>no phenyl ring</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>w/ phenyl ring</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Grand Total</td>
<td>10</td>
<td>28</td>
<td>1</td>
</tr>
</tbody>
</table>

5.3.2 Separation experiments

Separation experiments were performed at a 1:10 4HPG:AMCT weight ratio. If coformers were added, a 1:1 4HPG:coformer molar ratio was used. The amounts of 4HPG incorporated into AMCT obtained from the crystallization experiments are
presented in Table 5-7. The mass percentage of 4HPG in AMCT crystals after the initial crystallization was 0.98(±0.071) wt%. After a second crystallization (using fresh solvent), the amount of 4HPG decreased to 0.42(±0.053) wt%. With the addition of 2-imidazolidone, urea, and L-methionine, the amount of 4HPG incorporated into AMCT crystal lattices after the initial crystallization increased to 2.45(±0.035) wt%, 1.52(±0.094) wt%, and 1.28(±0.053) wt%, respectively. The addition of 1,1-diethylurea, imidazole, 2-hydroxypyridine, and 5-bromo-2(1H)-pyridone decreased the amount of 4HPG after an initial crystallization to 0.94(±0.069) wt%, 0.72(±0.009) wt%, 0.6(±0.048) wt%, and 0.36(±0.035) wt%, respectively. The best separation was obtained from four compounds: 2-picolinic acid, L-lysine, L-isoeucine, and L-leucine where the amount of 4HPG in the AMCT crystal after one crystallization decreased to 0.17(±0.0056) wt%, 0.17(±0.0049) wt%, 0.15(±0.0023) wt%, and 0.12(±0.097) wt%, respectively.
Table 5-7 Amounts of 4HPG in AMCT from Crystallization Experiments with a 1:10 Weight Ratio of 4HPG:AMCT.

<table>
<thead>
<tr>
<th></th>
<th>Amount of 4HPG (%)</th>
<th>Decreased (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only 4HPG</td>
<td>0.98±0.071</td>
<td></td>
</tr>
<tr>
<td>Second crystallization</td>
<td>0.42±0.053</td>
<td>57.14</td>
</tr>
<tr>
<td>2-Imidazolidone</td>
<td>2.45±0.035</td>
<td>-150</td>
</tr>
<tr>
<td>Urea</td>
<td>1.52±0.094</td>
<td>-55.1</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>1.28±0.053</td>
<td>-30.6</td>
</tr>
<tr>
<td>1,1-Diethylurea</td>
<td>0.94±0.069</td>
<td>4.1</td>
</tr>
<tr>
<td>Imidazole</td>
<td>0.72±0.009</td>
<td>26.5</td>
</tr>
<tr>
<td>2-Hydroxypyridine</td>
<td>0.6±0.048</td>
<td>38.8</td>
</tr>
<tr>
<td>5-Bromo-2(1H)-pyridone</td>
<td>0.36±0.035</td>
<td>63.3</td>
</tr>
<tr>
<td>2-Picolinic acid</td>
<td>0.17±0.0056</td>
<td>82.7</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.17±0.0049</td>
<td>82.7</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>0.15±0.0023</td>
<td>84.7</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>0.12±0.0097</td>
<td>87.8</td>
</tr>
</tbody>
</table>

To investigate the effect of the initial impurity concentration on the amount of impurity incorporated into the crystal lattice of the target compound, 4HPG was added at a weight ratio of 1:5 to AMCT. The amount of 4HPG in AMCT crystals was 1.13(±0.077) wt%. Compared to the result where the initial molar ratio was 1:10, the amount of 4HPG did not increase significantly.
5.3.3 The effect of varying the amount of the coformer added

We also investigated the effect of varying the amount of coformer added on the purification results using the four compounds that purify AMCT the most: 2-picolinic acid, L-lysine, L-isoleucine, and L-leucine. For each coformer, four coformer-to-4HPG molar ratios \( r \) were studied: \( r=0.1, 0.5, 1, \) and \( 1.5 \). Purification results in terms of the amount of 4HPG incorporated into the AMCT crystal lattice are shown in Figure 5-6. Two trends were observed. In the 2-picolinic acid and L-lysine systems, no significant differences were observed for \( r=0.1 \) (0.92(±0.0069) wt% and 0.89(±0.074) wt%, respectively.) The amount of incorporated 4HPG decreased dramatically when \( r=0.5 \) (0.16(±0.0084) wt% and 0.15(±0.0048) wt%, for 2-picolinic acid and L-lysine, respectively) and no further decrease was observed with the increasing amount of the coformer added (for \( r=1 \), 0.17(±0.0056) wt% and 0.17(±0.0049) wt%, for 2-picolinic acid and L-lysine, respectively; for \( r=1.5 \), 0.15(±0.0075) wt% and 0.18(±0.0087) wt%, for 2-picolinic acid and L-lysine, respectively). A different trend was observed in the L-leucine and L-isoleucine systems. In the L-leucine system, the amount of 4HPG decreased from 0.87(±0.057) wt% to 0.12±(0.00097) wt% when \( r \) increased from 0.1 to 1. Similarly, the amount of 4HPG decreased from 0.79±(0.083) wt% to 0.15±(0.0023) wt% with increasing \( r \) (from 0.1 to 1) in the L-isoleucine system. The additional coformer (\( r=1.5 \)) did not further decrease the amount of 4HPG in AMCT crystals (0.13(±0.011) wt% for the L-leucine system and 0.12(±0.0046) wt% for the L-isoleucine system).
To summarize, 47 compounds were selected and examined using solid-state grinding. Eleven were found to form cocrystals with 4HPG but not with AMCT. Separation experiments were performed and the addition of four compounds (2-picolinic acid, L-lysine, L-leucine, and L-isoleucine) decreased the amount of 4HPG incorporated into AMCT crystal lattices the most. By varying the amount of the coformer added, the optimal coformer-to-4HPG molar ratio was found to be 0.5 for the 2-picolinic acid and L-lysine systems and to be 1 for the L-leucine and L-isoleucine systems. Further addition of coformers beyond a certain point did not have a significant effect on the amount of 4HPG incorporated into the crystal lattices of AMCT.

5.3.4 Discussions

Three characteristics of compounds were examined to find their effects on the formation of 4HPG cocrystal: molecular weight, different types of functional groups, and the presence of a phenyl ring in the chemical structure. It was found that if the compound had a molecular weight smaller than 150, it had a higher chance of forming

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Figure 5-6 The Effect of Coformer Amount Added on the Amount of 4HPG Incorporated into AMCT Crystal Lattices.
cocrystal with 4HPG. This observation can be explained by the steric effect. The greater the molecular weight, the larger the molecule. Hence, the steric effect would hinder the interaction between the functional groups on the coformer candidate and on 4HPG during the formation of a crystalline material. It was found that compounds with both carboxylic acid and amine groups had a high probability of forming cocrystals with 4HPG. This observation fits the general rules for hydrogen bonding in cocrystals established by Etter.\textsuperscript{17} She analyzed the cocrystal patterns as a result of intra/intermolecular interactions and found that six-membered ring hydrogen bonds formed between different molecules have a higher priority than non-cyclic hydrogen bonding. Therefore, when the coformer functional groups can form cyclic hydrogen bonds with the carboxylic acid and amine group on 4HPG, they have a higher chance of forming cocrystals with 4HPG. As for the phenyl ring, no clear correlation was observed between the presence of a phenyl ring and the cocrystal formation. Intra/intermolecular $\pi$-$\pi$ interactions between two phenyl rings either on the same or different components have been found in cocrystals.\textsuperscript{51,52-56} However, compared to hydrogen bonds, $\pi$-$\pi$ interactions are weak interactions and should never be used as the main design principle. Sometimes the presence of phenyl rings can have a steric effect and prevent two components from forming cocrystals. We can conclude that the steric effect and functional groups on coformer candidates are the two most important factors when we design cocrystals.

For the eleven coformers with which separation experiments were performed, 2-picolinic acid, L-lysine, L-leucine, and L-isoleucine enhanced the AMCT purity the most. Compared to all other coformers whose functional group could only interact with
one functional group on 4HPG, these compounds have functional groups that can interact with both functional groups on 4HPG to form cyclic hydrogen bonds. According to Etter’s hydrogen bonding rules, cyclic hydrogen bonding is always preferred to single hydrogen bonding. We assumed that the stronger the coformer interacts with 4HPG in the solid state, the higher the probability that the coformer would form complexes with the impurity in solution; and therefore, high amounts of complexes must form between these four coformers and 4HPG. High levels of complexation contributed to the enhancement of purification.

The purification results obtained from a single crystallization reduced the impurity concentration to as low as 0.12(±0.0097) wt% from 0.98(±0.071) wt% when no coformer was added. In addition, crystals made from a second crystallization without the coformer were still significantly less pure than crystals made from a single crystallization with the coformer. This result indicates the potential usefulness of the method. The most common way to improve product purity is to recrystallize the compound of interest. However, by doing so, the yield is sacrificed. In contrast, in our separation method, in addition to the high purity gained, the yield was not sacrificed. Our proposed separation method thus has the potential to be applied for expensive products when low yield is unacceptable.

We can correlate the level of complexation to the purification results with the addition of various coformer amounts. At r=0.1, no significant decrease was observed in any system. This could be due to the lack of complex formation. For the 2-picolinic acid and L-lysine systems, the best purifications were achieved at r=0.5 and additional coformers did not further decrease the amount of 4HPG in AMCT crystal lattices. This
fact suggested that the level of complexation increased when \( r \) was increased from 0.1 to 0.5 and that the maximum level of complexation was achieved at \( r=0.5 \). The optimum coformer-to-4HPG molar ratio for these two systems was at \( r=0.5 \) since we could achieve the best purification without adding excess amounts of coformers. For the L-leucine and L-isoleucine systems, the amount of 4HPG decreased with increasing \( r \) (from 0.1 to 0.5 and 1), indicating that the level of complexation increased with the increasing amount of coformers added. The fact that no further purification was observed when the additional coformer was added (after \( r=0.5 \) for 2-picolinic and L-lysine systems and after \( r=1 \) for L-leucine and L-isoleucine systems.) implied that the maximum level of complexation was achieved.

5.3.5 Summary

With these observations, we verified the practical use of our proposed separation method with AMCT/4HPG as our model system. General rules for cocrystal formation were established. Separation results with various amounts of coformers suggested that the purification was due to the interaction between the coformer and 4HPG, and the level of complexation in solution.

5.4 Conclusions

In this work, we evaluated the practical use of the proposed separation method with a real drug/impurity system. Without advance knowledge of cocrystal formation of 4HPG, coformers were identified to reduce the amount of 4HPG incorporated into AMCT crystals greatly. Forty-seven compounds were selected because they had functional groups that could form synthons with functional groups on 4HPG. Eleven were confirmed to form cocrystals with 4HPG but not with AMCT using solid-state
grinding. Separation experiments were performed and four compounds were found to decrease the amount of 4HPG incorporated into AMCT crystal lattices the most: 2-picolinic acid, L-leucine, L-isoleucine, and L-lysine. The amounts of 4HPG incorporated in AMCT in these four systems were lower than that for samples of AMCT produced through two crystallizations. The optimal amount of coformers needed to achieve the best purification was found. We were able to correlate purification results to the level of complexation by varying the amount of coformers added.
6. THE SEPARATION OF IMPURITIES FROM SOLUTION USING SELF-ASSEMBLED MONOLAYERS (SAMS) ON GOLD SURFACES

6.1 Introduction

In this work, we applied the same principle, molecular recognition, to separate the impurity from the target. However, instead of focusing on the molecular recognition between the impurity and the coformer, we now focus on the interaction between the impurity and a functionalized surface.

The aim of this work is to separate the impurity from the target using functionalized surfaces that can selectively adsorb the impurity in solution. The surfaces were functionalized so that their functional groups had the potential to form hydrogen bonds with the functional group on the impurity but not with the functional group on the target. Therefore, we hypothesized that when a functionalized surface immersed into an impurity/target mixture would selectively bind the impurity and leave the target in solution. We functionalized the gold surfaces by depositing self-assembled monolayers on them. Self-assembled monolayers (SAMs) refer to the spontaneous adsorption of molecules from solution to form an oriented monolayer film on a surface. SAMs consist of a head group, an alkyl chain and a tail group (Figure 6-1). The head group is adsorbed onto substrates followed by the formation of covalent bonds; the alkyl chains then slowly arrange into an ordered monolayer. The tail group is then exposed to the solid-liquid or solid-vapor interfaces and hence functionalizes the surface. Common head groups include thiols, silanes, and phosphonates and they can be deposited on
different substrates. In our work, thiols (organosulfur compounds) were chosen because of their high structural order, their flexibility in the structure of functional groups exposed at the solid-vapor or solid-liquid interface, and the ease of preparation and analysis. Many studies have been done on the preparation and structural characterization of thiols. It was found that by varying the length of the alkyl chain and the head group, tail group and solvent, the chemistry, structure, and properties of the surface could be controlled. The structures of SAMs were studied using transmission electron microscopy and diffraction techniques, reflection infrared spectroscopy, scanning tunneling microscopy, x-ray photoelectron spectroscopies, ellipsometry and wetting. It is a particular interest to relate the microscopic structure of SAMs to their physical, chemical, and biological properties (wettability, corrosion resistance, adhesive strength, and biocompatibility). With control over the wettability, adhesion, tribology, and corrosion, SAMs have been commonly used as photoresists, in promoting adhesion, in microelectronics, photochemical and electrochemical processes, and in modeling biological interfaces. Studies have also shown that functionalized SAMs on gold surfaces can selectively bind the target that is recognized by the functional group on the surface.

Figure 6-1 Representation of SAMs on the Substrate.
To achieve our goal, we manufactured gold surfaces with different functional groups and evaluated their abilities to adsorb an impurity from solution selectively. Two thiol molecules, 4-mercapto pyridine and 2-mercapto benzimidazole, were used to manufacture functionalized gold surfaces. Three systems, IBU/KETO in toluene, BA/BAM in ethanol, and CA/CAM in ethanol were chosen to evaluate the performance of the functionalized gold surfaces. Separation experiments were performed and the purities of the resulting solution were measured using HPLC. The performances of these gold surfaces were determined using the impurity-to-target concentration ratio in solution over time. If the gold surfaces could selectively adsorb the impurity, the impurity-to-target concentration ratio in solution would be lower than that in the initial solution and would decrease over time until the gold surfaces became saturated and no longer of adsorbing any more molecules. After that point, the impurity-to-target concentration ratio should remain the same. The results are presented in Figure 6-3, Figure 6-4, Figure 6-5, Figure 6-6, and Figure 6-7. Large variances were observed and no significant results were obtained. Because the concentration of the impurity and the target were too low in the wash, the impurity-to-target ratio absorbed on the gold surfaces (that ideally should be higher than that in the initial solution), could not be used to determine the performance of the gold surfaces.

In this work, we evaluated the potential of purifying impurity/target mixtures using functionalized SAMs on gold surfaces. For the three systems studied, large variances between the repeats were observed and no significant separation was obtained. Possible modifications to the apparatus, experimental setup, and system selection are
suggested and the amount of future work needed to verify the possibility of this separation method is significant.

6.2 Materials and Methods

6.2.1 Materials

Benzoic acid (BA, ACS reagent, ≥ 99.5%), benzamide (BAM, 99%), trans-cinnamic acid (CA, ≥ 99%), cinnamamide (CAM, predominately trans, 97%), ibuprofen (IBU, ≥ 98%), ketoprofen (KETO, ≥ 98%), 4-mercapto pyridine (≥ 95%), and 2-mercapto benzimidazole (≥ 97%) were purchased from Sigma-Aldrich and were used as received. Gold coated test slides (float glass size: 1" x 3" x .040", coating: 50 Å of titanium followed by 1,000 Å gold, ≥ 99.9%) were purchased from emf.

Sulfuric acid (95-98%, ACS reagent) and hydrogen peroxide (30 wt% in water, ACS reagent) were purchased from Sigma-Aldrich and used to prepare piranha solution. Anhydrous ethanol (200 proof) was USP grade and was purchased from VWR. Methanol (MeOH, CHROMASOLV® for HPLC ≥ 99%), acetonitrile (ACN, CHROMASOLV® for HPLC ≥ 99%) and water (H₂O, CHROMASOLV® Plus for HPLC) were purchased from Sigma-Aldrich and used for HPLC.

6.2.2 System selection

Three model systems, IBU/KETO in toluene, BA/BAM in ethanol, and CA/CAM in ethanol were chosen because they were studied in previous approaches. Toluene was chosen to be the solvent because it is a non-polar solvent that does not interfere with the hydrogen bond formation between the functionalized SAMs and the impurity. It was not used for the BA/BAM and CA/CAM system because all compounds were only sparingly soluble in it. Two thiol molecules, 4-mercapto pyridine and 2-
mercapto benzimidazole were chosen to synthesize SAMs on gold surfaces. Their structures are shown in Figure 6-2. The secondary and tertiary amine group on 2-mercapto benzimidazole and the tertiary amine group on 4-mercapto pyridine have the potential to form hydrogen bonds with the carboxylic acid groups on IBU, BA and CA. Although they have similar functional groups, the location of the functional group in these two thiol molecules is different. While the tertiary amine group on 4-mercapto pyridine is exposed on the gold surface, the secondary and tertiary amine groups on 2-mercapto benzimidazole are close to the gold surfaces and hindered by a phenyl ring. It is our interest to see if the steric effect would affect the performance of the functionalized gold surfaces.

![Figure 6-2 Structures of (a) 2-mercapto benzimidazole (b) and 4-mercapto pyridine.](image)

6.2.3 SAM preparation

Piranha solution (3:1 v:v sulfuric acid: hydrogen peroxide) was made by slowly adding hydrogen peroxide to sulfuric acid and allowing the mixture to reach room temperature over the course of an hour. Gold surfaces were immersed into the piranha solution for an hour to clean and then washed with water and isopropanol using a squirt bottle. These gold surfaces were blow-dried using a nitrogen gun and were subsequently ready for the thiol deposition.
Two thiols, 4-mercapto pyridine and 2-mercapto benzimidazole, were dissolved in ethanol to prepare 10mM solutions. Clean gold surfaces were immersed into thiol solutions for thiol deposition and the solution was degased overnight with N\textsubscript{2}. The gold surfaces were washed with ethanol and blow-dried with a nitrogen gun, after which they were functionalized and ready for use.

6.2.4 Selectivity and separation experiments

Staining dishes that could hold 20 slides were purchased from VWR and used to hold functionalized gold slides. Different functionalized gold slides were aligned on the slide holder and immersed into a target/impurity mixture for various time periods. Samples were taken every 10-30 mins for HPLC analysis as follows. Gold surfaces were gently washed with ethanol first to remove the solution retained on the surface. The wash was recollected to avoid the loss of the impurity or the target compound. The amount of solvent used to wash gold surfaces was recorded. The gold surfaces were then placed in ethanol and sonicated for 20 mins to remove the bound impurity or the target compound. Finally, gold surfaces were regenerated after they were washed using ethanol and dried with N\textsubscript{2}.

6.2.5 High-Performance Liquid Chromatography

For the IBU/KETO system, the method described in section 3.2.6 was followed. For the BA/BAM and CA/CAM system, the method described in section 4.2.5 was followed.
6.3 Results and Discussions

6.3.1 Separation experiments

Various factors, including the functional groups on the thiol molecules and the experimental time, were studied to determine their effects on the purification results. Large variances were observed among separation experiments with the same conditions (the same impurity/target system, the same functionalized gold surfaces, and the same time period). A set of separation results of three systems, IBU/KETO in toluene (over 150 mins), BA/BAM in ethanol (over 8 hours), and CA/CAM in ethanol (over 8 hours) using 4-mercapto pyridine and 2-mercapto benzimidazole chips are shown in Figure 6-3 to Figure 6-7. A t-test was performed to decide if a significant decrease was present in the impurity-to-target concentration ratio over time. Large variances between repeats were observed and no significant decrease was obtained over a long time period (from 150 minutes to 8 hours).
Figure 6-3 Separation Results of IBU/KETO in Toluene Using 4-mercapto Pyridine Chips.

Figure 6-4 Separation Results of BA/BAM in Ethanol Using 4-mercapto Pyridine Chips.
Figure 6-5 Separation Results of BA/BAM in Ethanol Using 2-mercapto Benzimidazole Chips.

Figure 6-6 Separation Results of CA/CAM in Ethanol Using 4-mercapto Pyridine Chips.
6.3.2 Discussions

The large variability among the purification results could be due to the evaporation of solvents from the sample. Despite the fact that the apparatus was sealed using parafilm, it was observed that evaporation still occurred during the sampling stage and over time. Modifications should be made to the apparatus to prevent evaporation. The fact that no significant purification was shown can be due to several reasons. First, the solvent used in the BA/BAM and CA/CAM systems, ethanol, can interfere with the hydrogen bond formation between the impurity and the functionalized gold surfaces. We suggest choosing systems with non-polar solvents in which both the impurity and the target are soluble. Second, the surface area of these functionalized surfaces is limited. Modifications should be made to increase the surface area dramatically to achieve detectable purification. Third, we can conclude that the lack of purification was due to
the lack the selectivity of the functionalized gold surfaces. It is suggested to choose thiol molecules that can form stronger hydrogen bonds with the functional group on the impurity.

6.3.3 Summary

For the three systems we studied, we did not observe any repeatable promising purification. Despite our attempts to modify the apparatus, the experimental setup, or the solvent, this separation method was unsuccessful. Several suggestions were made for future research.

6.4 Conclusions

We investigated the possibility of separating impurities from the solution using functionalized SAMs on gold surfaces. Three impurity/target compound systems: IBU/KETO in toluene, BA/BAM in ethanol and CA/CAM in ethanol were studied. Two thiol molecules were chosen to bind the impurity selectively: 2-mercapto benzimidazole and 4-mercapto pyridine. After initial promising results shown in previous work, carefully repeated experiments show no significant separation in all three systems. We concluded that this result was likely due to solvent evaporation, solvent interference, the limited surface area, and the lack of selectivity of the SAMs for the impurities.
7. CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

This thesis focuses on the study and optimization of separation processes that aim to (1) avoid crystallization, filtration and re-dissolution of the target compound by the selective removal of the impurity from solution and (2) enhance the selectivity of the crystallization in cases where the impurity substitutes into the crystalline lattice by the complexation of impurities in solution. We targeted the separation of structurally similar compounds and studied three approaches: (1) selective impurity cocrystal formation; (2) selective impurity complex formation in solution; and (3) selective adsorption of impurity from solution using functionalized self-assembled monolayers (SAMs) on gold. All approaches were designed based on molecular recognition, utilizing the differences between the functional groups on the impurity and on the target compound.

In the first approach, a coformer that can form cocrystals with the impurity but not with the target compound was added to the impurity/target mixture. The impurity cocrystal precipitates since it has a reduced solubility compared to the impurity itself. The purified solution can then go for downstream processing. A strategy was established to choose the optimal coformer, concentration of the coformer, and solvent for a specific impurity/target system and was demonstrated using the ibuprofen/ketoprofen system. While it was found that the amount of ibuprofen in solution decreased significantly, the amount remaining was still larger than desired. In addition, the coformer was present in the final purified solution. Several attempts to decrease the amount of the impurity and the coformer in solution further were made but none showed significant improvement.
The second approach targets systems where the impurity is incorporated into the crystal lattice of the target compound. A coformer that can form a cocrystal with the impurity but not with the target compound was added to the impurity/target mixture to form complexes with the impurity and hence prevent its incorporation into the target compound. Three systems were studied to demonstrate the effectiveness of this approach and to show the correlation between the level of the complexation and the purification results. The first two systems were benzoic acid/benzamide and cinnamic acid/cinnamamide. In each system, the target and the impurity are structurally similar compounds where the impurity incorporates into crystal lattices of the target compound. In both systems, particular coformers were known to form cocrystals with the impurity. It was found that the structurally similar compounds could be purified by adding these coformers that could form cocrystals with the impurity. However, no clear correlation between the level of complexation and the purification results were found. A real drug/impurity system, amoxicillin trihydrate/4-hydroxyphenylglycine for which no coformer was known to form cocrystals with the impurity was used to examine the practical use of this approach further, the work flow that would be employed, and the purification that might be obtained due to the complex formation in solution. The purification results after the addition of coformers were better than that obtained after two crystallizations. Hence, this method has been shown to be successful with potential practical use. In addition, it was found that by increasing the amount of the coformer added, the amount of the impurity in target crystals decreased with an optimal amount for each of the successful coformers. This result implies that the more complexes are formed, the less incorporation of the impurity into target crystal lattices occurs.
The third approach was to adsorb impurities using functionalized gold surfaces selectively. Thiol molecules with functional groups that could form hydrogen bonds with the functional group on the impurity were used to functionalize the gold surfaces. We hypothesized that by immersing these gold surfaces in solution, the impurity would selectively adsorb onto the surfaces and thus be removed from solution. In the three systems studied, large variances were observed and no significant purification was obtained, which is likely due to the lack of selectivity of the functionalized gold surfaces.

### 7.2 Future Work

In this study, we successfully developed two potential separation methods that could be used for intermediate and final product purifications. Though both showed the potential for practical usage, improvements can be made to accelerate the coformer selection process and enhance the purification further.

The current coformer selection process includes (1) a search in the CSD for reported heterosynthons which include the functional group on the impurity, (2) solid-state grinding to find compounds that can potentially form cocrystals with the impurity, (3) solution crystallization to make pure cocrystals and (4) solubility measurement to determine the solubility of all components. This process is very labor-intensive and involves a significant amount of trial and error. A computational method is desired to predict whether a compound can form cocrystals with the impurity and the solubility of the impurity cocrystal. For example, it could be possible to predict the formation of cocrystals between two components by calculating the energy decrease when two
components interact with each other. It is also possible to predict the cocrystal solubility by applying thermodynamic models.

For the first approach, more research should be done to decrease the amount of the impurity and the coformer left in the solution further. The amount of the impurity and the coformer left in the solution is limited by the solubility of the impurity cocrystal, which is affected by the operating solvent and temperature. A tradeoff between the yield and the purity was observed in our system when a cooling process was applied. However, the operating conditions would be system specific. It is suggested that a combination of cooling and the addition of anti-solvent should be investigated for potential improvements.

For the second approach, it is desired to quantify the level of complexation in solution and to use that information as a tool to predict the purification results. Attempts were made to quantify the level of complexation using different spectroscopies (including raman spectroscopy, ultraviolet–visible spectroscopy, and infrared spectroscopy) and nuclear magnetic resonance (NMR) but none were successful due to difficulty in deconvoluting individual contributions, solvent interference, and instrument limitation. Investigation into new technologies would be of great help.
8. REFERENCES


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9. APPENDIX I.

9.1 X-ray Powder Diffraction Patterns for 4HPG Cocrysalts

In this appendix, we presented the powder patterns of thirteen cocrystal/impurity/coformer mixture obtained by grinding 4HPG with coformer at a one-to-one molar ratio. Arrows in the graph indicate new peaks that belong to the cocystal.

Figure 9-1 Comparison between the Powder Pattern of the Cocrysal/4HPG/1,1-diethylurea Mixture and the Powder Patterns of the Individual Components.
Figure 9-2 Comparison between the Powder Pattern of the Cocrystal/4HPG/1,2-bis(4-pyridyl)ethane Mixture and the Powder Patterns of the Individual Components.

Figure 9-3 Comparison between the Powder Pattern of the Cocrystal/4HPG/2-hydroxypyridine and the Powder Patterns of the Individual Components.
Figure 9-4 Comparison between the Powder Pattern of the Cocrystal/4HPG/2-imidazolidone Mixture and the Powder Patterns of the Individual Components.

Figure 9-5 Comparison between the Powder Pattern of the Cocrystal/4HPG/2-picolinic acid Mixture and the Powder Patterns of the Individual Components.
Figure 9-6 Comparison between the Powder Pattern of the Cocrystal/4HPG/5-bromo-2(1H)-pyridone Mixture and the Powder Patterns of the Individual Components.

Figure 9-7 Comparison between the Powder Pattern of the Cocrystal/4HPG/Carbamazepine Mixture and the Powder Patterns of the Individual Components.
Figure 9-8 Comparison between the Powder Pattern of the Cocrystal/4HPG/L-isoleucine Mixture and the Powder Patterns of the Individual Components.

Figure 9-9 Comparison between the Powder Pattern of the Cocrystal/4HPG/L-leucine Mixture and the Powder Patterns of the Individual Components.
Figure 9-10 Comparison between the Powder Pattern of the Cocrystal/4HPG/L-lysine Mixture and the Powder Patterns of the Individual Components.

Figure 9-11 Comparison between the Powder Pattern of the Cocrystal/4HPG/L-methionine Mixture and the Powder Patterns of the Individual Components.
Figure 9-12 Comparison between the Powder Pattern of the Cocrystal/4HPG/Urea Mixture and the Powder Patterns of the Individual Components.

Figure 9-13 Comparison between the Powder Pattern of the Cocrystal/4HPG/Imidazole Mixture and the Powder Patterns of the Individual Components.