FORMATION OF A PHYSICALLY STABLE AMORPHOUS DRUG COMPLEX
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# Table of Contents

## Chapter 1. Introduction

Introduction 6  
Gaps in Literature 8  
Conclusion 9  
References 11

## Chapter 2. Formation and Stabilization of Amorphous Solids

Introduction 13  
Stabilization of Amorphous Solids 14  
Using Silicates to Enhance Stability 15  
Analysis Techniques 17  
Conclusion 19  
References 22

## Chapter 3. Project Background

Scope of the Project 23  
Experimental  
  a. Materials 25  
  b. Methods 29  
Conclusion 33  
References 37

## Chapter 4. Griseofulvin

Introduction 38  
Cryo-milling 41  
Ball Milling 44  
Conclusion 48  
References 50

## Chapter 5. Astemizole and Pyrimethamine

Introduction 51  
Cryo-milling 54  
Ball Milling 57  
Conclusion 60  
References 62
Chapter 6. Sulindac

Introduction 63
Cryo-milling 65
Ball Milling 68
HME of Sulindac/Neusilin Mixtures 76
Physical Stability of Ball milled and HME Samples 80
Dissolution of Ball milled and HME Samples 82
Dissolution of Drug Product of Sulindac/Neusilin Complexes 84
Choice of Polymers to Stabilize the Amorphous Phase 88
Conclusion 90
References 92

Chapter 7. Conclusions and Future Considerations 95
References 100
Chapter 1. Introduction

Use of the amorphous phase to improve the oral bioavailability of poorly soluble drugs is well known. The amorphous phase is the higher energy form and as such offers the promise of greater solubility and faster dissolution rate which have the potential to increase bioavailability.\textsuperscript{1,2} However, amorphous materials are rarely used during drug development due to physical and chemical stability issues and processing difficulties.\textsuperscript{3} The amorphous form tends to be more chemically unstable than their crystalline counterparts\textsuperscript{4} although from a development standpoint the physical instability raises the most challenges. Physical instability leads to the transformation of the amorphous state to the thermodynamically favored crystalline state. A typical approach to improve the physical stability of amorphous pharmaceuticals is to combine them with inactive ingredients such as polymers to form amorphous solid dispersions. There are a number of reports describing the use of organic polymers to make amorphous solid dispersions. The solubility and dissolution advantage that can be obtained with these systems is seen both in vitro and in vivo.\textsuperscript{7-12} The organic polymers typically used are poly(vinyl pyrrolidone), polyethylene glycol, poly(methyl methacrylate) and cellulosic polymers. The exact mechanism of stabilization of the amorphous API by the organic polymer has not been fully determined. It has been attributed to a number of reasons. One is that intermolecular interactions between the polymer and the API are stabilized by hydrogen bonding. Another is that the production of single phase mixtures of the polymer and the API leads to increased glass transition temperature and polymers providing a diffusional
barrier to crystallization of the API. Although a number of mechanisms for stabilization of the amorphous API by the organic polymers have been described, they all depend to some extent upon the physical interaction between the polymer and the drug.

While there have been several studies done using organic excipients, the application of inorganic materials to improve the physical stability of amorphous API has not been explored in great detail. Bogner et al. have reported the amorphization of three acidic drugs (Ketoprofen, Naproxen, and Indomethacin) and the basic drug progesterone when co-ground in a ball mill with Neusilin US2, a synthetic magnesium aluminometasilicate. The amorphous state of the milled complex of all four drugs was found to be physically stable for up to 4 weeks when stored at 40°C/75%RH conditions. The physical stability of the amorphous phase of the acidic drug-Neusilin complexes were attributed to two different possible interactions. The first mechanism proposed was an acid-base reaction between the carboxyl group of the acidic drug with the surface hydroxyl groups in Neusilin. The second proposed mechanism is an ion-dipole interaction between the metal ions in Neusilin and the drug. The stability of the amorphous progesterone-Neusilin complex was attributed to hydrogen bonding between the carbonyl group of progesterone and the silanol group of Neusilin. Bogner et al. have also reported the dependence of amorphization kinetics of Indomethacin on the weight ratio of Indomethacin to Neusilin used during co-grinding. Watanabe et al. have reported the formation of amorphous Indomethacin when co-ground with silica in a vibration mill. Using $^{13}$C and $^{29}$Si solid state NMR they have concluded that the milling causes mechanochemical reactions between Indomethacin and the silanol groups on the surface of silica and the damaged siloxane bonds in the bulk of silica. Kinoshita et al.
have reported the improvement in oral bioavailability of a development drug TAS 301 when hot melt extruded with porous calcium silicate. The amorphous phase produced after extrusion was found to be physically stable for a period of two years at ambient conditions. The authors attributed the amorphous stability to the melt adsorption of the drug onto the porous silicate with the possibility of hydrogen bonding between the drug and the silanol groups on the surface of the silicate. Mallick et al. reported the formation of amorphous Ibuprofen when it was co-ground with Kaolin (hydrated aluminum silicate) in a ball mill. The amorphous complex was found to be physically stable at 40°C/75% RH for a period of 10 weeks. The physical stability was attributed to salt formation involving the carboxyl group of Ibuprofen which was determined using FTIR analysis. Inorganic silicates provide an alternate mechanism of stabilization of amorphous API through salt formation potential which is in contrast to organic polymers. Because there is the potential for stabilization using inorganic silicates there is the need to understand how salt formation of acidic drugs with inorganic silicates can be used to produce a physically stable amorphous phase and how the process can be scaled up to ultimately produce a marketable drug product.

**Gaps in the Literature**

There has been some investigation in utilizing silicates to increase stability in amorphous solids. One particular excipient, Neusilin US2 (Neusilin) has been used very successfully in the stabilization of solid amorphous drug material. Recent literature has shown that Neusilin in combination with an acidic solid amorphous drug promotes phase stability through complex formation between Neusilin and the acidic moiety when the
two are ground together\textsuperscript{[1-3]}. While the success of complex formation between the Neusilin and acidic drug has been well documented, the details of milling process, stability mechanisms, or whether the amorphization process can be scaled up beyond the bench need to be further investigated.

There has only been limited description of the types of mills used in these studies. In addition, milling conditions have not been fully described or addressed. No optimal milling process has been identified to maximize the amount of amorphization in a given sample. There has been no demonstration of the ability to scale up the amorphization process using the complex formation mechanism available with Neusilin and an acidic drug solid. Without information on whether the process can be scaled up beyond bench top applications there is no knowing whether this process would be feasible in making a marketable drug product. Because the end goal of pharmaceutical research is to develop a final drug product that will reach the market meeting the demands of processing and storage, scalability and stability are of the utmost importance.

**Conclusion**

Challenges arise in the use of amorphous drugs in the form of chemical and physical instability which can hamper their development if not make it impossible, altogether. However the advantages that can be had make the pursuit of a stable amorphous drug very attractive to pharmaceutical chemists. Increasing dissolution rate and bioavailability of a drug are primary goals in the development of new drugs resulting in new methods and materials being constantly explored. Organic excipients mixed with the amorphous form of a drug have been shown to help stabilize against crystallization by
various mechanisms. The advantage of the use of organic polymers is that the amorphous solid dispersions are able to maintain the original dissolution and solubility advantages of the original amorphous drug material. Several methods for the mechanism of stabilization of the amorphous drug material using organic polymers have been proposed. These include hydrogen bonding between the excipient and drug, steric hindrance caused by the polymer to prevent crystallization of the amorphous material and an increased glass transition temperature as a result of the production of a single phase mixture between the polymer and amorphous drug. The common characteristic of each proposed mechanism for stabilization is that they all require physical interaction between the amorphous material and the polymer.

Inorganic silicates have been used in the stabilization of amorphous drug material recently with growing frequency. Unlike organic polymers, inorganic silicates provide the possibility of stabilization of amorphous API through the mechanism of salt formation. There is a definite need to better understand how salt formation of acidic drugs with inorganic silicates can be manipulated to produce long term physically stable amorphous phases. If stabilization is accomplished then a process to scale up production of the amorphous form needs to be identified in order to ultimately produce a drug product. The scale up of the production process has not been determined and requires further investigation. While more work is currently being done in this area it has not yet been thoroughly explored and the reaction mechanisms between the inorganic excipients and amorphous drug have yet to be fully determined.
References


Chapter 2. Formation and Stabilization of Amorphous Solids

Introduction

Improvements in solubility and dissolution rate of crystalline drug compounds by various methods have been shown to increase the bioavailability of the drugs\textsuperscript{[2]}. Because of this, enhancing dissolution and bioavailability is a main area of focus for pharmaceutical scientists in formulation development. Use of the amorphous phase to improve the oral bioavailability of poorly soluble drugs is well known. The amorphous form of a compound is at a higher energy state than the crystalline form, which suggests it will provide greater solubility and faster dissolution rates and result in higher oral bioavailability. These advantages make the conversion from the crystalline form to amorphous desirable. However, chemical and physical stability issues often result in reversion to the crystalline state or a form change and limit the use of amorphous material in drug development. In spite of the difficulties in using amorphous drug material, the improvements in oral bioavailability make it an attractive option to try. The key in using amorphous material in drug product development is the stabilization of the amorphous form during all processing steps as well as for long term stability or shelf life of the final product itself.

A solid amorphous material is defined as having no long-range order in the atomic structure, while a solid material that does exhibit long-range order is defined as crystalline. Most crystalline solids can be made amorphous by using a melt-quench technique. This involves heating the crystalline material to where it is melted and
becomes a liquid, followed by rapidly cooling it, often with liquid nitrogen. Other reported methods that were utilized to effect the conversion from crystalline to amorphous form were milling\textsuperscript{[1]} and melt adsorption\textsuperscript{[5]}. Two milling methods that have been reported as successful techniques are cryo-milling under liquid nitrogen conditions and ball milling.

**Stabilization of Amorphous Solids**

Solid crystalline material is more thermodynamically stable than the amorphous form. The amorphous form when presented the appropriate conditions will crystallize but not necessarily back to the original crystalline form. Both the amorphous and crystalline forms have particular physical characteristics that can be either advantageous or detrimental depending on the intended use of the material. This is especially true when developing a drug product. The crystalline form will have a higher melting temperature, a slower dissolution rate and lower solubility than the amorphous form. It is more stable both physically and chemically. While these traits are favorable for long term stability of the material they are not always desirable when using these materials for drug applications. The physical traits of amorphous materials lend themselves more readily in drug development when attempting to maximize exposure. Higher solubility and faster dissolution rates can lead to greater bioavailability when dosed\textsuperscript{[2, 5]}. However, amorphous materials are rarely used when developing a drug product due to the decreased chemical and physical stability resulting in processing and storage challenges.
This instability can limit scale up and therefore commercialization of the drug in the amorphous form.

The most common method used in stabilizing solid amorphous pharmaceuticals is the addition of inactive excipients that allow for the formation of amorphous solid dispersions. The most frequently reported method in the literature is the use of organic polymers as the excipient. The most common of these used are poly (vinyl pyrrolidone), polyethylene glycol, poly (methyl methacrylate) and cellulosic polymers. The advantage of the use of organic polymers is that the amorphous solid dispersions are able to maintain the original dissolution and solubility advantages of the original amorphous drug material. Several methods for the mechanism of stabilization of the amorphous drug material using organic polymers have been proposed. These include hydrogen bonding between the excipient and drug, steric hindrance caused by the polymer to prevent crystallization of the amorphous material and an increased glass transition temperature as a result of the production of a single phase mixture between the polymer and amorphous drug. The common characteristic of each proposed mechanism for stabilization is that they all require physical interaction between the amorphous material and the polymer.

**Using Silicates to Enhance Stability**

Recently investigation into using inorganic excipients in the stabilization of amorphous drug material has become reported in the literature with growing frequency. While more work is currently being done in this area, it has not yet been thoroughly explored and the reaction mechanisms between the inorganic excipients and amorphous drug have yet to be fully determined.
Many of the most commonly reported inorganic excipients being investigated are silicates. Bahl et al. reports co-grinding with six different silicates in the amorphization of Indomethacin\textsuperscript{[6]}. These include Neusilin US2 (synthetic magnesium alumino-metasilicate), Veegum-F (magnesium aluminosilicate), Florite-F (calcium fluorine), calcium silicate, kaolin (hydrated aluminum silicate) and Aerosil-200 (fumed silica). Kinoshita et al. developed a hot melt extrusion (HME) process using calcium silicate in order to amorphize a crystalline drug sample\textsuperscript{[5]} and Bogner et al. have done extensive work co-grinding Neusilin US2 with various pharmaceutical compounds\textsuperscript{[1,3,4,6]}.

One of the more successful excipients utilized in stabilizing the amorphous form of a drug has been Nuesilin US2. Gupta et al. reported 1 month of physical stability of the amorphous form at 40°C/75% Relative Humidity (RH) for drugs co-ground with Neusilin US2\textsuperscript{[4]}. Bahl et al. reports not only 3 months of physical stability at 40°C/75% RH of amorphous Indomethacin co-ground with Neusilin but that the Indomethacin ground with Neusilin was amorphized in less time than when ground with the other silicates used in the same study. Silanols present on the surface of Neusilin make it a potential proton donor or proton acceptor due to the high hydrogen bonding potential of the silanols. This lead to the suggested stabilizing mechanisms of hydrogen bonding or acid-base reactions between the drug and silanols and possible ion-dipole interactions between the drug and metal ions at the surface of Neusilin. The same study reported the dependence of amorphization kinetics of Indomethacin on the weight ratio of Indomethacin to Neusilin used during co-grinding as well as the grinding time itself. Samples ground for longer periods of time resisted crystallization longer than the samples with shorter milling times\textsuperscript{[6]}. Gupta et al. reported that carboxylic acid containing drugs
form an amorphous salt when milled with Neusilin. The amorphous complex was found to be physically stable at 40°C/75% RH for a period of 4 weeks. The physical stability was attributed to salt formation involving the carboxyl group of the acidic drugs determined by FTIR data that showed an absence of the carbonyl peaks \[^{[4]}\]. The increased stability of the amorphous form and shorter milling times make Nuesilin an attractive candidate for further study.

Utilizing another silicate other than Neusilin, Kinoshita et al. have reported producing an amorphous phase of a development drug TAS 301 when hot melt extruded with porous calcium silicate. The amorphous phase produced after extrusion showed an improvement in oral bioavailability and was found to be physically stable for a period of two years at ambient conditions. The mechanism of stability of the amorphous form was attributed to the melt adsorption of the drug onto the porous silicate with the possibility of hydrogen bonding between the drug and the silanol groups on the surface of the silicate \[^{[5]}\].

Unlike organic polymers, inorganic silicates provide the possibility of stabilization of amorphous API through the mechanism of salt formation. There is a definite need to better understand how salt formation of acidic drugs with inorganic silicates can be manipulated to produce long term physically stable amorphous phases. If stabilization is accomplished then a process will need to be identified that can be scaled up to ultimately produce a drug product which is an avenue of investigation that has not been thoroughly explored.

**Analysis Techniques**
Analysis techniques such as X-Ray Powder Diffraction (XRPD), Differential Scanning Calorimetry (DSC) and Solid State Nuclear Magnetic Resonance (SSNMR) aid in determining the difference between the two forms. The most common use of XRPD in drug development is in the characterization of crystalline drug compounds. Each crystalline material analyzed will have a unique diffraction pattern displaying distinctive, sharp peaks in the X-Ray scan. Amorphous material will produce a broad background signal, also known as an amorphous halo. This diffraction pattern has no sharp peaks indicating a lack of crystalline lattice structure. However, when the crystal size is very small it can be difficult to determine between a truly amorphous material and the crystalline form. This is further complicated by the fact that a large proportion of the atoms are located at or near the surface of very small crystals. The smaller the crystal, the higher the surface area it has. A decrease in structural order can be seen due to interfacial effects and relaxation of the surface which can distort the atomic positions.

DSC analysis can give a more definitive answer in determining whether the sample is crystalline or amorphous. The DSC curve will show at what temperatures endothermic and exothermic events occur within the sample as it is being heated. If the sample is amorphous a glass transition, $T_g$, will be observed. The transition occurs as an amorphous solid is being heated and is observed as a step in the baseline signal of the DSC curve at a temperature lower than the melting point of the crystalline form. During this transition no formal phase change occurs. An amorphous sample will become less viscous as the temperature increases allowing the molecules to rearrange into a crystalline form. If this occurs an exothermic peak will be observed in the DSC signal which will then be identified as the crystallization temperature, $T_c$. As the temperature increases the
sample will ultimately reach its melting temperature, \( T_m \) which will appear as an endothermic event in the DSC signal and may or may not occur at the same temperature as the melting point of the original crystalline material. A melting point that is different from the original crystalline material indicates that the amorphous sample crystallized into a different form from that of the original material.

SSNMR can detect atomic differences within a group of molecules when the only difference present is in the immediate chemical environment of the group of atoms in question. When compared with the original crystalline material the amorphous form will be indicated by a chemical shift observed in the NMR spectra. This makes it a very useful technique in determining the amount or percentage of amorphous material present in a given sample. NMR data can also be utilized to determine the kinetics of the amorphization process. When the percent of amorphous material present in the sample is plotted against processing or milling time a picture of the kinetics of overall amorphous formation emerges. This information allows insight into the mechanism of the reaction especially where excipients used to increase stability are involved in the formation of the amorphous phase.

Utilizing the analytical techniques available with XRPD, DSC and SSNMR together presents a cohesive and more comprehensive understanding of the amorphization and stabilization process.

**Conclusion**

Increased solubility and dissolution rate are desirable characteristics inherent in the amorphous form of a crystalline drug. Due to the less stable nature of amorphous
material a reliable method for consistently generating the amorphous form and stabilizing it against crystallization is needed. Various methods have been demonstrated to produce the amorphous form from a crystalline drug. Melt-quenching is a common method to amorphize crystalline material although does not allow for the addition of an excipient and therefore is more difficult to stabilize. Cryo-milling under liquid nitrogen conditions and ball milling have both been shown to be reliable methods for producing amorphous material both with and without the addition of excipients. An optimized method of milling, whether utilizing an excipient or not, still remains to be identified. Further investigation into the development of a method for scaling up the production of amorphous material beyond the bench top is also needed.

Previous work done with both organic and inorganic excipients has demonstrated promising results in the stabilization of amorphous materials. The most commonly used excipients are organic polymers. The main advantage of this method is that the dissolution and solubility improvements of the original amorphous drug material are maintained in the amorphous solid dispersions. Stabilization is also increased with the addition of organic polymers with several different stabilization mechanisms proposed that all share a common characteristic. Each proposed mechanism for stabilization requires physical interaction between the amorphous material and the polymer. The most probable of the suggested mechanisms include hydrogen bonding between the excipient and drug, steric hindrance that prevents crystallization of the amorphous material and an increased glass transition temperature as a result of the production of a single phase mixture between the polymer and amorphous drug.
Inorganic silicates are being investigated more frequently as promising excipients because of a trend toward increased stabilization of amorphous material. In the case of Neusilin, a synthetic magnesium aluminometasilicate, it has been demonstrated to aid in the amorphization process resulting in a higher percentage of amorphous material formed as opposed to processing without an excipient. Suggested stabilizing mechanisms include hydrogen bonding or acid-base reactions between the drug and silanols and possible ion-dipole interactions between the drug and metal ions at the surface of Neusilin. The production of the amorphous phase of a development drug was demonstrated utilizing porous calcium silicate and a hot melt extrusion process. The extruded amorphous material showed an improvement in oral bioavailability and was found to be physically stable for a period of two years at ambient conditions. The proposed mechanism of stability was a melt adsorption of the drug onto the porous silicate with the possible addition of hydrogen bonding between the drug and the silanol groups on the surface of the silicate.

The investigation into the use of inorganic silicates with amorphous material has revealed a desirable advantage. Inorganic silicates present a stabilization mechanism through salt formation which is not possible with organic excipients. The manner in which salt formation of acidic drugs with inorganic silicates can be manipulated to produce long term physically stable amorphous phases requires further investigation. First the stabilization of the amorphous form of a crystalline drug must be accomplished. Once achieved a process will then need to be developed that allows for the scale up of the production of the stable amorphous material. Only this will ultimately produce a
marketable amorphous drug product and is an area of drug development that has not yet been fully explored.

References


Chapter 3. Project Background

Scope of the Project

Previous studies have shown that the most success at stabilizing an amorphous drug occurred when the drug was acidic. The stabilization is often attributed to interactions brought about by co-grinding between the carboxyl group of the drug and the excipient. In order to better determine a mechanism for stabilization three types of drugs were investigated; an acidic, a basic and a neutral drug. It has been indicated that the presence of an acidic group in the drug molecule may be required for a stable drug/excipient complex to form. If stabilization of either the basic or neutral drug were to occur alternative stabilization mechanisms could be discovered and explored.

In designing amorphization experiments the best possible milling method first must be identified. The method used must encompass the milling conditions that can produce Neusilin-drug complexes and the effect of the milling parameters must be determined. Once the best method is identified the milling process can be optimized resulting in a higher yield of the amorphous phase of the sample. The initial attempts at amorphization of crystalline drug were performed using a cryo-mill under liquid nitrogen conditions. Ball milling was the next technique investigated in order to determine if the milling process could be further optimized. All of the samples were milled both with and
without the addition of the excipient Neusilin. Milling times and techniques were
developed for optimization by analyzing milled samples at predetermined time points
using XRPD to determine the extent of amorphization taking place.

Once the milling method and technique had been optimized and samples were
found to be stable for more than a week the next step was to determine the reaction
mechanism. Because the formation of a complex between the drug and Neusilin is the
key to the stabilization of the amorphous form $^{13}\text{C}$ SSNMR analysis was used to further
understand the mechanism of complex formation. Samples milled under optimized
conditions with the addition of Neusilin were taken at various time points and analyzed as
a function of time. The data showed the percentage of amorphous conversion over
milling time as well as an indication of the reaction mechanism for complex formation.

The next step was to identify a scalable process that would result in the same
drug/Neusilin complex formation. This process would be used demonstrate the
feasibility of scaling up the production of the amorphous Neusilin-drug complexes. Hot
Melt Extrusion (HME) was found to produce the same amorphous drug/Neusilin
complexes as milling but with a higher percent of amorphous conversion. HME is a
scalable process that can be used at the commercial level. The process of complex
formation was not only scalable but was also improved upon.

Once the HME process was shown to be successful investigation of the stability
of the amorphous drug/Neusilin complex was begun. The dissolution profile of the HME
product was determined concurrently with the stability study of the samples. The HME
complex was found to have an improved dissolution profile over the crystalline drug as
well as the milled drug/Neusilin complex. Additionally it was found to be stable for a longer period of time than the milled samples.

The final step in the process was to determine the feasibility of formulating a drug product containing amorphous drug/Neusilin complexes. Tablets were made with the HME material using the same formulation recipe as is used in the commercially available tablets containing crystalline drug. An improvement in the dissolution profile of the tablet formulated using the amorphous HME material when compared to the tablet made with crystalline material would indicate the positive viability of using an amorphous drug complex in drug product development. The tablet made with HME amorphous material was found to have the same dissolution profile as the tablet made with crystalline material. An investigation into the excipients used found that Magnesium Stearate was causing crystallization of the amorphous complex when added to the tablet formulation. An excipient study was initiated to identify alternative excipients with heightened sensitivity toward preventing crystallization.

Experimental

Materials

Sulindac ((Z)-5-fluoro-2-methyl-1-[[p-(methylsulfinyl)phenyl]methylene]-1H-indene-3-acetic acid), an acidic non-steroidal anti inflammatory drug, was purchased from Toronto Research Chemicals (North York, Ontario) and was used asreceived. The chemical structure of Sulindac is shown in Figure 1. Griseofulvin (2S,6'R)- 7-chloro-2',4,6-trimethoxy- 6'-methyl- 3'H,4'H-spiro [1-benzofuran- 2,1'-cyclohex[2]ene]- 3',4'-
dione), a neutral oral anti-fungal drug was purchased from Sigma-Aldrich and used as received. The chemical structure of Griseofulvin is shown in Figure 2. Astemizole 1-[(4-fluorophenyl)methyl]-N-[1-[2-(4-methoxyphenyl)ethyl]-4-piperidyl]benzoimidazol-2-amine, a free-base antihistamine drug was purchased from Sigma-Aldrich. The chemical structure of Astemizole is shown in Figure 3. Neusilin US2 synthetic magnesium aluminometasilicate sample was obtained as a gift from Fuji Chemicals (Englewood, NJ). The chemical structure of Neusilin is shown in figure 4. Pyrimethamine 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine, a free-base is used as an antimalarial drug for both the prevention and treatment of malaria. The Pyrimethamine used was purchased from MP Biomedicals. The chemical structure of Pyrimethamine is shown in figure 5.

![Chemical structure of Sulindac](image)

Figure 1. Chemical structure of Sulindac
Figure 2. Chemical structure of Griseofulvin

Figure 3. Chemical structure of Astemizole
Figure 4. Chemical structure of Neusilin

Figure 5. Chemical Structure of Pyrimethamine
Methods

Preparation of melt quenched amorphous sample

The amorphous phase of Sulindac was generated by melt quenching. About 500mgs of Sulindac was held isothermally at 190°C (above its melting point) for 2 minutes in a glass vial. The vial was then immersed in liquid nitrogen to produce the amorphous phase of Sulindac. The amorphous material was recovered from the vial and was gently ground with a mortar and pestle to reduce its particle size. The ground amorphous material was stored over desiccant in a closed container.

Milling

A Retsch Ball Mill model #MM301 was used for room temperature milling with and without Neusilin. The samples to be milled were placed in a 25mL chamber with metal grinding balls at room temperature. The vibration frequency of the mill was set at 20Hz for all experiments. The samples were milled for varying lengths of time between 10 and 60 minutes. At pre-determined times points the solid sample was removed from the mill for further characterization to understand the kinetics of the mechanochemical reaction. Milling was continued until the sample was ascertained to be completely amorphous using X-ray powder diffraction and $^{13}$C solid state NMR.
A Spex SamplePrep Freezer Mill model #6770 was used to mill samples with and without Neusilin at liquid nitrogen temperatures. The milling time was set at 10 minute intervals. At the end of each interval the liquid nitrogen bath was refilled. The samples were milled for varying total times between 10 and 60 minutes in 10 minute intervals. At pre-determined time points the solid sample was removed from the mill for further characterization to understand the kinetics of the mechanochemical reaction. Milling was continued until the sample was ascertained to be completely amorphous using X-ray powder diffraction.

**X-Ray Powder Diffraction (XRPD)**

The diffractometer (PANalytical X’pert, Philips) was equipped with a CuKα source (\(\lambda = 1.54056 \text{ Å}\)) operating at a tube load of 45 kV and 40mA. The divergence slit size was 1/4°, while the receiving slit and the detector slit, were 5.0mm, and 0.1mm respectively. Small amount of sample was loaded onto Si 510 zero-background sample holder and scanned between 3 and 40° (2\(\theta\)) with a step size of 0.008 and a step time of 15.2 s/step in the continuous mode. Data was collected by a high-resolution sealed proportional detector. The Si (111) with a diffraction peak at 28.44° 2\(\theta\) was used as a standard to calibrate the instrument.
Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA)

DSC measurements were conducted in crimped Aluminum pans using a Q1000 (TA Instruments, Newcastle, DE) unit under 50mL/min N₂ purge. 2 to 3 mg samples were tested each time. Standard DSC scan using a heating rate of 10°C/min and modulated DSC scan using a heating rate of 5°C/min with modulation amplitude of 0.5°C within a period of 40 s were used. Indium was used as the calibration standard.

TGA measurements were conducted in Aluminum pans using a Q500 (TA Instruments, Newcastle, DE) unit under 50mL/min N₂ purge. A heating rate of 10°C/min was used for all TGA runs.

Solid State Nuclear Magnetic Resonance (SSNMR)

All SSNMR measurements were conducted using a Bruker DSX spectrometer operating at a $^1$H resonance frequency of 600MHz. A Bruker 4-mm double resonance magic angle spinning (MAS) probe head was used to record all NMR data. $^{13}$C NMR spectra were recorded using routine cross polarization sequence with sample spinning at 14 kHz. 512 to 1024 transients were collected for each sample for signal to noise averaging. $^1$H 90° pulse length of 2.5 µs and a cross polarization contact time of 2 ms were employed. $^1$H decoupling was achieved with a spinal 64 sequence using a pulse length of 5 µs. A recycle delay of 10 seconds was used for all samples. A total suppression of spinning sidebands (TOSS) sequence was appended to the CP sequence to
achieve a spectrum free of spinning sidebands. Natural abundance glycine with the carbonyl peak at 176.03ppm was used as the chemical shift reference.

**Hot Melt Extrusion (HME)**

The purpose of the HME experiments was to determine the scalability of producing stable Neusilin-drug complexes. Blends of Neusilin-drug in a 1:1 and 2:1 by weight ratios were extruded using a Prism PharmaLab 16mm Twin Screw Extruder. The extruder temperature was held at 200°C, the screw speed was 50 rpm, and the volumetric feed rate was set at 5%. Each run processed a total of 100g of material.

**Powder Dissolution**

Powder dissolution data were generated using a 6-channel pION µ-Diss Profiler (pION Inc., Woburn, MA) apparatus in 0.1 N HCl media with a stirring speed of 100 rpm at ambient conditions. The dissolution rate profiles of Sulindac and Sulindac-Neusilin complexes in the medium were monitored using an in-line UV detector employing fiber optic cables. The concentration profiles were calculated using a standard curve determined using a DMSO stock solution of the drug. Each sample was analyzed in triplicate and the average concentrations and standard deviations are reported. For sake of clarity not all data points collected are shown.
Stability Analysis

Samples ball-milled with and without Nuesilin and HME samples were stored in stability chambers at 25°C/60% RH and 40°C/75% RH conditions. Approximately 25mg of each sample was weighed into glass vials using a separate vial for each timepoint. Vials were closed for storage. Samples were assayed by XRPD and HPLC for each time point to determine both chemical and physical stability.

Tablet Formation

Tablets were made on a Carver Press using a direct compression method. The tablets were formulated using a blend of 80% Hot Melt Extruded 1:1 ratio of Sulindac to Neusilin, 9.5% Avicel PH 102, 9.5% Starch 1500, and 1% Magnesium Stearate. Each tablet had a total weight of 500mg.

Conclusion

Both cryo-milling and ball milling have been identified in various studies as reliable methods to produce the amorphous form of a drug when milled with and without excipients. The addition of excipients has been found to increase the likelihood of the
transition to the amorphous form. Addition of excipients, such as inorganic silicates like Neusilin, has also been demonstrated to assist in the stabilization of the amorphous form. The most likely mechanisms for stabilization of the amorphous drug/excipient complexes formed would best be determined by analysis using $^{13}$CSSNMR. The stability of the amorphous drug/Neusilin complex is attributed to hydrogen bonding and/or an acid-base reaction between Silanol groups on the surface of Neusilin and the acid moiety in Sulindac which could be clarified with the use of $^{13}$CSSNMR analysis.

DSC and TGA analysis would identify the glass transition and melt temperature of the amorphous material generated by ball milling. Comparison of the glass transition temperature with the relative milling temperature could either prove or disprove the idea that milling below or near the glass transition temperature results in a higher rate of amorphization.

XRPD is an invaluable tool in determining whether amorphization has occurred in a milled or HME sample. With a relatively quick analysis run, about 15 minutes, it can be determined whether a sample has become amorphous or not. A scan showing an amorphous halo with no crystalline peaks is the ideal outcome when attempting the amorphization of a crystalline compound. When peaks are still observed growing out of the halo, optimized milling or processing times can be developed to further the amorphization process allowing for a more reliable method for amorphization. On the other hand, when no amorphous halo is observed in the scan and crystalline peaks remain after substantial milling times XRPD can definitively determine that a sample is not going to amorphize and allow the researcher to move on.
The amorphization process investigated worked very well using bench top mills however not all bench top processes can be scaled up. If the process cannot be scaled up then it cannot be used for any commercial applications rendering it useless for development. Due to the results of the stability studies of the milled material it was determined that temperature during milling has a significant effect on the outcome of stability. Hot Melt Extrusion (HME) therefore was identified as a possible means to scale up production of the Sulindac/Neusilin complex. HME as a process has the possibility of amorphizing a greater percentage of the crystalline drug and may result in greater long term stability of the drug/Neusilin complex. This would result in amorphous samples of superior quality to what the ball mill produced and would identify HME as a viable method to scale up production enough to enable further development of the amorphous complex.

The formation of a useful drug product is the end goal for Pharmaceutical development. To this end a drug product containing the amorphous material would need to be compared to the commercially available drug product. For direct comparison tablets would be formulated containing the amorphous material using the same recipe as the commercially available tablet containing crystalline material. Dissolution experiments would clearly demonstrate any improvement in solubility and dissolution rate of the amorphous drug product over that of the crystalline drug product. Care would need to be taken in order to prevent crystallization of the amorphous form by the addition of excipients or by the pressure induced by the press to form the tablets.

Determining the stability of the amorphous samples generated is a key component for the development of a drug product using amorphous material. Samples stored in
stability chambers at accelerated conditions (40°C/75% RH) allow the samples to be stored for a shorter amount of time than they would otherwise be if held at room temperature conditions. This allows for a faster determination of the long term stability of the samples and drug product saving time during the investigation process.

The development of a drug product using the amorphous form of a crystalline drug promises to provide improvements upon the original drug product. It is known that an improvement in the dissolution can relate to an improvement in bioavailability. If the bioavailability were to show the expected improvement in the amorphous drug product, then drug load in the new product could be lower. This has the added benefit of lowering the production costs of the drug by lowering the amount of drug produced while keeping the efficacy of the drug product the same.


Chapter 4. Griseofulvin

Introduction

In formulation development enhancing dissolution and bioavailability is a main area of focus for pharmaceutical scientists. Improvements achieved in these areas by various methods have been shown to increase the bioavailability of crystalline drug compounds \(^2\). Amorphization of crystalline drug compound is a proven method and as such use of the amorphous phase to improve the oral bioavailability of poorly soluble drugs is well known. The main drawback to pursuing development of the amorphous form is the decrease in chemical and physical stability found after amorphization.

The higher energy state of the amorphous form of a crystalline compound suggests it will afford greater solubility and faster dissolution rates resulting in higher oral bioavailability. While these advantages make the conversion from the crystalline form to amorphous appear favorable chemical and physical stability issues do exist. The challenges to the stability of the amorphous form often result in reversion to the crystalline state or a form change. These issues are the main limiting factor in the use of amorphous material for drug development. In fact, amorphous materials are rarely used when developing a drug product due to processing and storage challenges caused by the decrease in chemical and physical stability. These issues can limit scale up and therefore
commercialization of the drug in the amorphous form. In spite of the challenges posed, promise of improvements in oral bioavailability make amorphization of a poorly soluble crystalline drug an attractive area for development. The main point in developing amorphous drug material for product development is the stabilization of the amorphous form during all processing steps, long term stability and shelf life of the final product.

The most successful studies aimed at the stabilization of an amorphous drug were achieved with an acidic drug combined with an inorganic excipient. The stabilization has been attributed to interactions between the carboxyl group of the drug and reactive sites on the excipient brought about by co-grinding, although no definitive explanation of the reaction mechanism has been developed. In an effort to establish a mechanism for stabilization three types of drugs were investigated; an acidic, a basic and a neutral drug. It has been suggested that the presence of an acidic group in the drug molecule may be required for a stable drug/excipient complex to form. If stabilization of either the basic or neutral drug were to occur alternative stabilization mechanisms could be determined and utilized.

This study investigated the amorphization and stabilization of a neutral drug compound. Griseofulvin is an orally administered anti-fungal drug marketed by Glaxo Laboratories as Grisovin. It is used in both humans and animals to treat fungal infections in the nails and skin, primarily ringworm. It has a melt temperature of 220°C with the onset temperature of glass transition at 48°C. Griseofulvin was chosen for investigation based on the structure as a neutral compound and that it is readily available for purchase.

Methods
Milling

A Retsch Ball Mill model #MM301 was used for room temperature milling with and without Neusilin. The samples to be milled were placed in a 25mL chamber with metal grinding balls at room temperature. The vibration frequency of the mill was set at 20Hz for all experiments. The samples were milled for varying lengths of time between 10 and 60 minutes. At pre-determined times points the solid sample was removed from the mill for further characterization to understand the kinetics of the mechanochemical reaction. Milling was continued until the sample was ascertained to be completely amorphous using X-ray powder diffraction and $^{13}$C solid state NMR.

A Spex SamplePrep Freezer Mill model #6770 was used to mill samples with and without Neusilin at liquid nitrogen temperatures. The milling time was set at 10 minute intervals. At the end of each interval the liquid nitrogen bath was refilled. The samples were milled for varying total times between 10 and 60 minutes in 10 minute intervals. At pre-determined time points the solid sample was removed from the mill for further characterization to understand the kinetics of the mechanochemical reaction. Milling was continued until the sample was ascertained to be completely amorphous using X-ray powder diffraction.

X-Ray Powder Diffraction (XRPD)

The diffractometer (PANanalytical X’pert, Philips) was equipped with a CuKα source ($\lambda = 1.54056$ Å) operating at a tube load of 45 kV and 40mA. The divergence slit size was $1/4^\circ$, while the receiving slit and the detector slit, were 5.0mm, and 0.1mm
respectively. Small amount of sample was loaded onto Si 510 zero-background sample holder and scanned between 3 and 40° (2θ) with a step size of 0.008 and a step time of 15.2 s/step in the continuous mode. Data was collected by a high-resolution sealed proportional detector. The Si (111) with a diffraction peak at 28.44° 2θ was used as a standard to calibrate the instrument.

**Cryo-milling**

Cryo-milling was the initial milling technique used to attempt the conversion of griseofulvin from the crystalline to the amorphous form. Griseofulvin was cryo-milled with no excipients for a total of 60 minutes. Samples were removed at 10 minutes and 60 minutes for XRPD analysis. When compared with the baseline scan of un-milled crystalline Griseofulvin it can be seen that over time some amorphization does occur. The baseline scan shows well defined, sharp peaks that are indicative of a crystalline structure. After 60 minutes of milling certain changes can be observed in those sharp peaks that were present in the base line scan. All have decreased in intensity and at some points they have disappeared entirely into the amorphous halo. An example is the peak that appears at 18.02° 2 Theta in the baseline scan. In the sample taken after 10 minutes of milling this peak is observed to be diminishing in intensity. The same peak observed in the sample analyzed after 60 minutes of milling is lost in the amorphous halo forming in between the still observable peaks. Although amorphization is beginning to occur it is not complete after 60 minutes of cryo-milling as shown in figure 1.
Figure 1. Baseline XRPD scan of Griseofulvin compared with XRPD scans of Griseofulvin cryo-milled at times of 10 and 60 minutes. No amorphous conversion observed.

Because cryo-milling Griseofulvin without excipients for 60 minutes failed to generate the amorphous form the same milling procedure was repeated but with the addition of Neusilin as an excipient in a 1 to 1 ratio of drug to Neusilin for a total volume of 1 gram of material. It has been shown in literature that co-grinding crystalline drug material with a silicate can increase amorphization of the drug [1]. The first sample taken at 35 minutes for XRPD analysis showed a greater degree of amorphous conversion than the Griseofulvin milled alone for 60 minutes. While the peak at 18.02° 2 Theta has disappeared completely, some crystalline peaks were still observed. A second sample was analyzed after 50 minutes of milling. The conversion to the amorphous form was found to be complete with no sharp peaks to indicate crystallinity observed within the amorphous halo as shown in figure 2.
After generation of the amorphous form of Griseofulvin was achieved by co-grinding with Neusilin, samples were taken and placed in two separate stability chambers. The storage conditions were 25°C/65% Relative Humidity (RH) and at 45°C/75% RH. After 24 hours samples were removed and analyzed using XRPD. Crystallization can be observed in both samples regardless of storage conditions. When compared to the baseline scan of un-milled crystalline Griseofulvin it is clear the amorphous material is crystallizing back to the original crystalline form as shown in figure 3. Although the addition of a silicate as an excipient did allow for the
amorphization of Griseofulvin to occur it did not stabilize the amorphous form against crystallization.

Figure 3. XRPD scan of 24 hour stability samples 1:1 Griseofulvin:Neusilin co-ground using cryo-mill. Storage conditions are 25°C/65% RH and 45°C/75% RH.

**Ball Milling**

Although amorphization was observed when Griseofulvin was cryo-milled with Neusilin the amorphous form was found to have crystallized within 24 hours and was therefore not stabilized. While it has been reported that amorphization occurs more easily when milling is performed at lower temperatures [2] it was decided to repeat the cryo-milling experiments using a ball mill where the milling temperature would be higher. It has been reported that the amorphous form of a drug compound can be
stabilized when it undergoes a melt-adsorption process with an excipient [3], something that would not occur under liquid Nitrogen conditions but may be induced at the elevated temperatures associated with ball milling.

Crystalline Griseofulvin was ball milled without an excipient for a total time of 60 minutes with samples removed at 40, 50 and 60 minutes for XRPD analysis. When compared to the baseline scan of un-milled crystalline Griseofulvin no amorphous conversion was observed at any time point. In fact, there is no indication of even partial amorphization. The XRPD data is identical for each time point and the baseline scan as shown in figure 4.

![Figure 4. Comparison of baseline XRPD scan of crystalline Griseofulvin to samples ball milled at 40, 50 and 60 minutes without excipient.](image)

The ball milling experiment was repeated under the same conditions using crystalline Griseofulvin with Neusilin as an excipient at a ratio of 1 to 1, drug to
excipient. Milling was allowed to continue for 60 minutes with no interruptions to take samples in order to ensure the increase of temperature needed for a melt-adsorption process to occur. After 60 minutes a sample was taken of the milled material for XRPD analysis. A high degree of amorphization was observed to have occurred. There was the hint of a peak in the amorphous halo at 16.76° 2 Theta which corresponds to an observed peak in the baseline scan of crystalline Griseofulvin as shown in figure 5.

Figure 5. Comparison of XRPD scans of crystalline Griseofulvin and amorphous Griseofulvin:Neusilin in a 1:1 ratio ball milled for 60 minutes.

The milled amorphous material was divided with equal portions put in separate stability chambers at conditions of 25°C/65% RH and 45°C/75% RH. After 24 hours
samples were taken and analyzed using XRPD. Crystalline peaks can be seen growing in the amorphous halo after 24 hours at both storage conditions. The addition of Neusilin aided in the amorphization of the crystalline material but stabilization was not achieved as shown in figure 6.

Figure 6. XRPD scans of 24 hour stability samples of ball milled Griseofulvin with Neusilin in a 1 to 1 drug to excipient ratio stored at 25°C/65% RH and 45°C/75% RH. Crystallization is observed in the samples.
Conclusion

In the griseofulvin samples milled without excipient a greater degree of amorphization was observed for the cryo-milling technique as compared to the ball milling technique. This finding correlates to a study conducted by Descamps, et. al, that milling at a lower temperature results in an easier amorphization process because the transformation takes place below the glass transition temperature \( (T_g) \) of the crystalline material. It is suggested that introduction of defects in the crystalline structure during the milling process induces progressive destabilization. When performed below the glass transition temperature an amorphous solid is formed\(^{[2]}\). Under liquid Nitrogen conditions the cryo-milling process temperature was well below the glass transition temperature of griseofulvin. This would explain the higher degree of amorphization seen in the cryo-milled samples that contained no excipient when compared to the samples ball milled with no excipient. When performed above the glass transition temperature it is predicted that the crystalline material will melt to a metastable liquid phase which would give the molecule enough freedom of motion to re-form into a crystalline structure.

The temperature increase during ball milling, while not measured is assumed to be higher than the glass transition temperature. Because the XRPD data for the griseofulvin ball milled without excipient shows no signs of amorphization it would seem this to be a safe assumption. This would also explain the high degree of amorphization observed in the samples ball milled with excipient because as Kinoshita et. al, reported, amorphization can occur in the presence of an excipient through a melt-adsorption process that can occur during processing\(^{[3]}\).
However, although the amorphization process is greatly enhanced with the addition of an excipient during ball milling it does not stabilize the amorphous form against crystallization. The rapid crystallization of the samples indicates that there is no reaction occurring between the drug and the excipient which would interfere with the crystallization process. The initial samples taken directly after ball milling may appear amorphous because a portion of the griseofulvin molecules are still in the metastable liquid state adsorbed on the surface of the excipient. As the sample rests it is possible that the excipient is not presenting enough steric hindrance to the amorphous form to prevent the molecules from reorganizing themselves into a crystalline structure. The lack of stability of the amorphous form of griseofulvin when co-ground with an excipient indicates that pursuit of the development of the amorphous form of a neutral drug compound would prove difficult and probably not feasible from an economic standpoint.
References


Chapter 5. Astemizole and Pyrimethamine

Introduction

The higher energy state of the amorphous form of a crystalline compound suggests it will provide improvements over the less soluble crystalline form. This includes greater solubility and faster dissolution rates which can result in higher oral bioavailability than is observed in the crystalline drug. Use of the amorphous phase to improve the oral bioavailability of poorly soluble crystalline drugs is well known. During development of a drug product these types of improvements are highly sought after and developed by pharmaceutical scientists as increased bioavailability can be used to enhance the performance of the final drug product.

While the advantages of increased solubility and dissolution make the conversion from the crystalline form to amorphous appear favorable, chemical and physical stability issues do exist. The challenges to the stability of the amorphous form often result in a form change or reversion to the crystalline state. The challenges to stability are the main limiting factor in the use of amorphous material for drug development. In fact, amorphous materials are only rarely used when developing a drug product due to processing and storage challenges caused by the decrease in chemical and physical stability. The areas that require the most attention in developing amorphous drug material for product development is the stabilization of the amorphous form during all processing steps, long term stability and shelf life of the final product. Although stabilization poses a challenging problem, the promise of improvements in oral
bioavailability make amorphization of a poorly soluble crystalline drug an attractive area for development.

Studies utilizing an acidic drug combined with an inorganic excipient demonstrated the most success at stabilization of an amorphous drug. The stabilization mechanism has been attributed to interactions between the carboxyl group of the drug and reactive sites on the excipient brought about by co-grinding. A mechanism for stabilization still needs to be established. Comparing amorphization and stabilization using three types of drugs; an acidic, a basic and a neutral drug will clarify by what mechanism stabilization occurs. It has been suggested that the presence of an acidic group in the drug molecule may be required for a stable drug/excipient complex to form. If stabilization of either the basic or neutral drug were to occur alternative stabilization mechanisms could be determined and utilized.

This study investigated the amorphization and stabilization of two basic drug compounds. Both astemizole and pyrimethamine were investigated due to limited availability of astemizole. At the conclusion of the cryo-milling experiments it was discovered that astemizole was no longer available for purchase. This may be caused by the fact that it has been withdrawn from the market due to rare but potentially fatal side effects.

Astemizole was a second generation antihistamine marketed under the brand name Hismanal. It has a melt temperature of 174°C with the onset temperature of glass transition at 45°C. This compound was chosen for this study based on the structure as a basic compound and the assumption that it would be readily available.
Pyrimethamine is marketed under the brand name Daraprim and is used to treat protozoal infections. It is also used for both the treatment and prevention of malaria and can be used in combination with Sulfadiazine for the treatment of toxoplasma gondii infections in immunocompromised patients, such as HIV-positive individuals. It has a melt temperature of 233°C. Glass transition onset temperature was not measured. This compound was chosen for this study based on the structure as a basic compound and that it is readily available for purchase.

Methods

Milling

A Retsch Ball Mill model #MM301 was used for room temperature milling with and without Neusilin. The samples to be milled were placed in a 25mL chamber with metal grinding balls at room temperature. The vibration frequency of the mill was set at 20Hz for all experiments. The samples were milled for varying lengths of time between 10 and 60 minutes. At pre-determined times points the solid sample was removed from the mill for further characterization to understand the kinetics of the mechanochemical reaction. Milling was continued until the sample was ascertained to be completely amorphous using X-ray powder diffraction and $^{13}$C solid state NMR.

A Spex SamplePrep Freezer Mill model #6770 was used to mill samples with and without Neusilin at liquid nitrogen temperatures. The milling time was set at 10 minute intervals. At the end of each interval the liquid nitrogen bath was refilled. The samples were milled for varying total times between 10 and 60 minutes in 10 minute intervals. At pre-determined time points the solid sample was removed from the mill for further
characterization to understand the kinetics of the mechanochemical reaction. Milling was continued until the sample was ascertained to be completely amorphous using X-ray powder diffraction.

**X-Ray Powder Diffraction (XRPD)**

The diffractometer (PANanalytical X’pert, Philips) was equipped with a CuKα source (λ = 1.54056 Å) operating at a tube load of 45 kV and 40mA. The divergence slit size was 1/4°, while the receiving slit and the detector slit, were 5.0mm, and 0.1mm respectively. Small amount of sample was loaded onto Si 510 zero-background sample holder and scanned between 3 and 40° (2θ) with a step size of 0.008 and a step time of 15.2 s/step in the continuous mode. Data was collected by a high-resolution sealed proportional detector. The Si (111) with a diffraction peak at 28.44° 2θ was used as a standard to calibrate the instrument.

**Astemizole and Cryo-Milling**

The initial milling technique used to attempt the conversion of Astemizole from the crystalline to amorphous form was cryo-milling. An XRPD baseline analysis of crystalline astemizole was taken to demonstrate crystallinity prior to milling. The baseline scan shows well defined, sharp peaks that are indicative of a crystalline structure. Astemizole was milled with no excipients for a total of 60 minutes. Samples were removed at 40, 50 and 60 minutes for XRPD analysis. When compared with the
baseline scan of un-milled crystalline astemizole it was observed that no amorphization had taken place. The data taken for the baseline scan appears identical to the data obtained at each time point as shown in figure 1.

![Figure 1. XRPD data for un-milled crystalline Astemizole and samples cryo milled for 40, 50 and 60 minutes. No amorphous conversion is observed.](image-url)

Astemizole was then cryo-milled with Neusilin in a 1 to 1 drug to excipient ratio with a total volume of 1 gram of material for a total time of 60 minutes. This has been reported in literature to be a reliable method for increasing the probability of amorphous conversion \(^\text{[1]}\) and had been demonstrated in previous experiments using Griseofulvin. Samples were not removed for analysis over the course of the milling process due to the lack of amorphous conversion after 60 minutes of milling without Neusilin. The XRPD
analysis done after 60 minutes of milling with Neusilin shows that a high degree of amorphization has taken place. See figure 2.

![XRPD data of astemizole cryo-milled for 60 minutes with Neusilin in a 1 to 1 drug to excipient ratio. Complete amorphization appears to have occurred.](https://example.com/figure2.png)

Figure 2. XRPD data of astemizole cryo-milled for 60 minutes with Neusilin in a 1 to 1 drug to excipient ratio. Complete amorphization appears to have occurred.

Once generation of the amorphous form of astemizole had been achieved by co-grinding with Neusilin, the milled material was divided into equal portions and placed in two separate stability chambers. The storage conditions were 25°C/65% Relative Humidity (RH) and at 40°C/75% RH. After 24 hours samples were removed and analyzed using XRPD. Crystallization can be observed in both samples regardless of storage conditions. When compared to the baseline scan of un-milled crystalline
astemizole it is apparent that the amorphous material is crystallizing back to the original crystalline form as shown in figure 3.

Figure 3. XRPD analysis of un-milled astemizole compared with 24 hour stability samples of astemizole cryo-milled with Neusilin in a 1 to 1 drug to excipient ratio stored at 25°C/65%RH and 40°C/75% RH. The amorphous form has crystallized to the original crystalline form.

PyrImethamine and Ball Milling

PyrImethamine was ball milled without excipient for a total of 65 minutes with a sample removed at 35 minutes for XRPD analysis. An XRPD baseline analysis of crystalline pyrimethamine was taken to demonstrate crystallinity prior to milling. The
baseline scan shows well defined, sharp peaks that are indicative of a crystalline structure. After 35 minutes of ball milling the XRPD scan shows a decrease in the intensity and sharpness of the crystalline peaks. This trend was observed to have continued in the sample analyzed after 65 minutes of milling. The broadening of the peaks and the decrease in intensity indicates some amorphization is occurring in the sample but is not complete after an hour as shown in figure 4.

Figure 4. XRPD analysis of un-milled crystalline pyrimethamine compared to ball milled samples without excipient at 35 and 65 minutes. Some conversion to the amorphous form has occurred.

The ball milling experiment was repeated with Neusilin added to the pyrimethamine to be co-ground in a 1 to 1 ratio of excipient to drug. One sample was removed after 35 minutes of milling for XRPD analysis with a second sample being analyzed after 65 minutes of milling. It can be observed that there is a greater degree of
amorphicity in the samples milled with Neusilin than without even after milling for only
35 minutes. However, complete amorphization has not taken place as indicated by the
presence of crystalline peaks at 12° and 18° 2 Theta. These peaks directly correspond to
the two peaks with highest intensity observed in the crystalline pyrimethamine baseline
XRPD data. This indicates that the milled samples contain crystalline material that is of
the same form as the original crystalline form prior to milling and no form conversion has
taken place. Because full amorphous conversion could not be achieved no samples were
placed on stability for further analysis as shown in figure 5.

![Figure 5](image.png)

**Figure 5.** Comparison of XRPD data of crystalline pyrimethamine prior to milling and
pyrimethamine co-ground with Neusilin in a 1 to 1 ratio for 35 and 65 minutes.
Complete amorphization has not occurred.
Conclusion

The astemizole samples cryo-milled without excipient demonstrated a much lesser degree of amorphization than was observed for the samples cryo-milled with Neusilin. The samples milled with Neusilin appeared to have undergone complete amorphous conversion. A greater degree of amorphization is also observed for the pyrimethamine co-ground with Neusilin using the ball mill. The greater degree of amorphization of a crystalline drug when co-ground with a silicate excipient supports the findings of Bahl et. al. [1]

When the two milling techniques are compared it is obvious that a greater degree of amorphization does occur at the lower temperature provided by the cryo-milling under liquid nitrogen conditions. This observation agrees with a study conducted by Descamps, et. al, that milling at a lower temperature results in a higher degree of amorphous conversion because the transformation takes place below the glass transition temperature \( T_g \) of the crystalline material. It is suggested that introduction of defects in the crystalline structure during the milling process induces progressive destabilization and when this process is performed below the glass transition temperature an amorphous solid is formed [2]. The cryo-milling process performed under liquid nitrogen conditions was conducted at a temperature well below that of the glass transition temperature of astemizole. This would explain the higher degree of amorphization seen in the cryo-milled samples that contained excipient when compared to the samples ball milled with excipient.

When performed above the glass transition temperature it is predicted that the crystalline material will melt to a metastable liquid phase which would give the molecule
enough freedom of motion to re-form into a crystalline structure. The temperature increase during ball milling, while not measured is assumed to be higher than the glass transition temperature but lower than the melting temperature. Because the XRPD data for the pyrimethamine ball milled without excipient shows no signs of amorphization or form change it would seem this to be a safe assumption. However, because complete amorphization did not occur in the samples ball milled with Neusilin which were shown to still contain detectable amounts of crystalline material, the metastable liquid phase of pyrimethamine was not achieved. Without a melt occurring during milling the melt adsorption process described by Kinoshita et. al, as a way to amorphize crystalline material would not apply [3]. This also helps explain why complete amorphization did not occur in the time allotted for the milling process. Possibly if the material were milled for a much longer time allowing for a greater rise in temperature a melt might occur inducing the melt-adsorption process and complete amorphization of the sample.

While the amorphization process is demonstrated to be greatly enhanced by the addition of an excipient during ball milling it has not been shown to stabilize the amorphous form against crystallization. The rapid crystallization of the one sample that was completely amorphized indicates that there is no reaction occurring between the drug and the excipient which would interfere with the crystallization process. The lack of stability of the amorphous form of astemizole when co-ground with Neusilin and the lack of complete amorphous conversion of pyrimethamine when co-ground with Neusilin indicates that the development of the amorphous form of a basic drug compound for commercial use would not be successful.
References


Chapter 6. Sulindac

Introduction

Use of the amorphous phase to improve the oral bioavailability of poorly soluble drugs is well known. From the standpoint of maximizing exposure the amorphous phase is of great interest for pharmaceutical scientists. It is at a higher energy and offers the promise of higher solubility and faster dissolution rate and which delivers the potential to increase bioavailability.\textsuperscript{1,2} During development of a drug product these improvements are highly sought after for development as increased bioavailability can be used to enhance the performance of the final drug product.

While the advantages of increased solubility and dissolution make the conversion from the crystalline form to amorphous appear favorable, chemical and physical stability issues as well as processing difficulties do exist.\textsuperscript{3} The amorphous form tends to be more chemically unstable than it’s crystalline counterparts.\textsuperscript{4} However from a development standpoint the physical instability is the most problematic. The challenges to the stability of the amorphous form often result in a form change or reversion to the crystalline state. A typical approach to improve the physical stability of amorphous pharmaceuticals is to combine them with inactive ingredients such as polymers to form amorphous solid dispersions. The challenges to stability are the main limiting factor in the use of amorphous material for drug development. In fact amorphous materials are only rarely used when developing a drug product due to the processing and storage challenges caused by the decrease in chemical and physical stability.
The areas that require the most attention in developing amorphous drug material for product development is the stabilization of the amorphous form during all processing steps, long term stability and shelf life of the final product. Although stabilization poses a challenging problem the promise of improvements in oral bioavailability make amorphization of a poorly soluble crystalline drug an attractive area for development.

Studies utilizing an acidic drug combined with an inorganic excipient demonstrated the most success at stabilization of an amorphous drug. The stabilization mechanism has been attributed to interactions between the carboxyl group of the drug and reactive sites on the excipient brought about by co-grinding. It has been suggested that the presence of an acidic group in the drug molecule may be required for a stable drug/excipient complex to form. A mechanism for stabilization still needs to be established.

In this study we report the production of the amorphous complex of an acidic drug, Sulindac with Neusilin US2 by cryo-milling, ball milling and hot melt extrusion. The physical/chemical stability and dissolution behavior of these amorphous complexes have been investigated. As a method of scaling up production hot melt extrusion was identified as a method capable of continuous production of the amorphous drug/excipient complexes. The nature of the mechanochemical reaction induced between Sulindac and Neusilin has been investigated using $^{13}$C SSNMR spectroscopy and the possible mechanism of amorphous stabilization is speculated.
Cryo-milling

Cryo-milling was the initial milling technique used to initiate the conversion of Sulindac from the crystalline to the amorphous form. Crystalline Sulindac was cryo-milled as received with no excipients in 10 minute intervals for a total milling time of 60 minutes. Samples were removed at 40, 50 and 60 minutes for XRPD analysis. The sample appears to be almost completely amorphous by 40 minutes with only the hint of crystalline peaks at 19.0, 21.8 and 24.6° 2 Theta. These peaks have completely disappeared into the amorphous halo by 60 minutes as shown in figure 1.

![Figure 1. XRPD data of unmilled and cryo-milled Sulindac with no excipients for 40, 50 and 60 minutes. Complete amorphization appears after 60 minutes of milling.](image)

The samples were placed in stability chambers in closed containers at 25°C/60% Relative Humidity and 40°C/75% Relative Humidity (RH). After 24 hours the samples were
removed and analyzed using XRPD to determine if the sample had remained in the amorphous form or crystallized. It was found that both samples had begun crystallization, although to different degrees. Peaks can be seen growing in the amorphous halo on the samples from both stability conditions as shown in figure 2.

![Figure 2. XRPD data of 24 hour stability samples of cyro-milled Sulindac with no excipients stored at 25°C/60% RH and 40°C/75% RH. Crystallization can be seen beginning in both samples.](image)

Sulindac was then cryo-milled with Neusilin in a 1 to 1 ratio by weight. Samples were milled in 10 minute intervals for a total time of 35 minutes with a sample removed for XRPD analysis after 20 minutes. At 20 minutes small peaks indicating the presence of crystalline material were still present at 18.6, 21.5 and 24.3° 2 Theta. By 35 minutes of milling time the peaks had become unresolved as shown in figure 3.
Figure 3. XRPD data of Sulindac cryo-milled with Neusilin in a 1 to 1 ratio by weight for 20 and 35 minutes. Amorphization is complete at 35 minutes.

The milled samples were put on stability at 25°C/60% RH and 40°C/75% RH. After 24 hours the first sample was analyzed using XRPD. The sample stored at 40°C/75% RH showed that crystallization had occurred but the sample at 25°C/60% RH showed no signs of crystallization. The sample on stability at 25°C/60% RH was found to maintain stability in the amorphous form ultimately for 4 months as shown in figure 4. Although stability for 4 months looked promising it was not long enough to justify further investigation of cryo-milled material for development as a drug product.
Figure 4. XRPD data of stability samples of Sulindac cryo-milled with Neusilin in a 1 to 1 weight ratio. Storage conditions at 25°C/60% RH and 40°C/75% RH for 24 hours and at 25°C/60% RH for 4 months. Stability in the amorphous phase is seen for 4 months at 25°C/60% RH.

**Ball milling**

Crystalline Sulindac was ball milled as received with no excipients investigate the effect of mechanical grinding on the crystalline phase. After 60 minutes of ball milling, no phase change was observed in the ground material. The XRPD pattern of the ground material conforms to the as-received form II material as shown in figure 5. A closer inspection of the XRPD pattern of the ground material shows that the diffraction peaks are broader than the as-received material indicating that crystallite size reduction and/or lattice distortions are introduced into the ground material. Descamps et. al have shown that the position of the temperature of milling with respect to the glass transition temperature will determine the outcome of the milling product.18,19
Figure 5. XRPD patterns of Sulindac (a) as-received and (b) ball milled for 60 minutes and (c) ball milled with Neusilin for 60 minutes.

For example when $\gamma$-Indomethcin (Tg~42 °C) is ground at room temperature in a ball mill the final product is a mixture of 50% $\alpha$-indomethacin and 50% amorphous phase. However when $\gamma$-indomethacin is ground at cryogenic temperatures complete amorphization is achieved.$^{20,21}$ These results show that when temperature of milling is close to the glass transition temperature either transition between polymorphic varieties or no change in the original form can be expected. However when the temperature of milling is far below the glass transition temperature complete amorphization can be expected as seen in the cryo milling data, figure 1. Our results with the ball milling of Sulindac can be explained on the basis of this scheme. Since ball milling at room temperature is close to the glass transition of Sulindac (~69 °C) no form conversion is
observed. It was also observed that cryogenic milling of Sulindac for 60 minutes converts the material into an amorphous phase. See figure 1. However this amorphous phase rapidly recrystallizes when stored at ambient conditions as shown in figure 2.

Sulindac was ball milled with Neusilin US2 in a 1 to 1 weight ratio for 60 minutes. The resulting mixture was confirmed to be amorphous by XRPD and the data are shown in Figure 5. This indicates that the addition of Neusilin to the mixture facilitates amorphous formation during ball milling while ball milling of the as-received crystalline material did not result in amorphous formation. Our results are consistent with that reported by Bogner et. al. for Indomethacin-Neusilin system. They observed that Neusilin promotes amorphous formation of Indomethacin during ball milling. Bogner et. al reported that it took nearly 10 days of co-grinding with Neusilin to achieve complete amorphization of Indomethacin. In our study complete amorphization of Sulindac (as determined by XRPD) was achieved in 60 minutes. This difference is most likely due to the different type of mill used in the two studies. For their study Bogner et. al. used a low energy jar milling while high energy ball milling was used in this study.

The ball milled amorphous complex was analyzed by modulated DSC and TGA. A glass transition temperature for the amorphous complex could not be detected in MDSC measurement. A clear glass transition was observed in DSC for amorphous Sulindac produced by melt quenching. Although XRPD indicates that both materials are amorphous the DSC traces are significantly different indicating substantial difference between the two amorphous phases. TGA analysis of the amorphous complex revealed a continuous weight loss of 4.1% from room temperature to 180°C associated with the
moisture loss from the sample. The ball-milled sample was analyzed by HPLC and was found to be free of chemical degradation.

In order to understand the nature of the amorphous complex formed by ball milling Sulindac with Neusilin, time course studies were initiated. Sulindac was ball milled with Neusilin in a 1 to 1 weight ratio for 5, 20, 40, and 60 minutes. The sample from each time point was analyzed by $^{13}$C SSNMR spectroscopy. Figure 6 shows the $^{13}$C CP/MAS/TOSS spectra of Crystalline and melt-quenched amorphous Sulindac. Some of the peaks in the spectra have been assigned based on comparison with solution NMR data. The spectrum of the amorphous sample shows broader peaks when compared to the peaks in the crystalline sample reflecting the broader distribution of molecular orientations in the amorphous phase. The peak labeled ‘4’ at 174.6ppm corresponding to the carboxyl group in the crystalline sample shows about a 3.2ppm upfield shift in the amorphous melt quenched sample. The carboxyl peak at 171.4ppm in the amorphous material compares well with a $^{13}$C shift of 171.5ppm measured in solution.\textsuperscript{22} As the carboxyl region of the spectra provides a clear distinction between the amorphous and the crystalline sample, it was selected to monitor the mechanochemical changes occurring during ball milling. The carboxyl region of $^{13}$C spectrum of Sulindac ball milled with Neusilin for different lengths of time is shown in Figure 7.
Figure 6. $^{13}$C CP/MAS/TOSS spectra of the crystalline and melt-quenched amorphous samples of Sulindac. The dotted line shows the change in chemical shift of the carboxyl group in going from the crystalline to amorphous sample.
Figure 7. Carboxyl region of the $^{13}$C CP/MAS spectra of crystalline Sulindac, melt-quenched Sulindac and Sulindac ball milled with Neusilin at a 1:1 weight ratio for times indicated in the figure. Ball milling progressively converts crystalline Sulindac ‘a’ into a mixture of amorphous Sulindac ‘b’ and amorphous Sulindac-Neusilin complex ‘c’. The dotted lines are provided to aid visualization of data.
Ball milling progressively converts crystalline Sulindac (peak labeled ‘a’)) into amorphous material. Two types of amorphous phases are generated upon ball milling (peaks labeled ‘b’ and ‘c’). The peak labeled ‘c’ corresponds to the amorphous melt quenched material. However, the peak labeled ‘b’ corresponds to a distinct second amorphous phase. This peak is associated with a complex formation between Neusilin and Sulindac. A number of mechanisms have been proposed in literature for this complex formation during co-grinding of Indomethacin with Neusilin. Watanabe et al. have proposed a hetero bridging bond formation between the carboxyl group of Indomethacin and surface silanol groups of Neusilin based on a number of experimental techniques. Bogner et al. have suggested that in addition to salt formation, ion-dipole interactions and/or hydrogen bonding between the carboxyl groups of Indomethacin and Silanol groups of Neusilin may also account for the changes observed in ATR-FTIR. Although the mechanism at this point is unclear, it is evident from the data presented some form of interaction between Sulindac and Neusilin occurs to provide a downfield chemical shift of the carboxyl group in the $^{13}$C SSNMR spectrum. This can be explained through salt formation and/or hydrogen bonding. However, bridging bond formation appears unlikely based on HPLC retention times being identical for the as-received crystalline material and the ball milled amorphous complex.

In order to understand the kinetics of amorphous complex formation as a function of ball milling time the $^{13}$C SSNMR data in the carboxyl region was deconvoluted to provide relative amounts of the amorphous Sulindac and amorphous Sulindac-Neusilin. This analysis assumes equal cross polarization efficiency for the carboxyl peak of crystalline and amorphous phases. The data are shown in Figure 8.

23 Bogner et al. have suggested that in addition to salt formation, ion-dipole interactions and/or hydrogen bonding between the carboxyl groups of Indomethacin and Silanol groups of Neusilin may also account for the changes observed in ATR-FTIR.
Figure 8. Percentage of amorphous phase produced as a function of ball milling time: (●) Overall amount of amorphous material, (△) amount of amorphous Sulindac, and (□) amount of amorphous Sulindac-Neusilin complex.

The overall kinetics of amorphous formation appears to be biphasic with the amount of amorphous formation increasing rapidly from 0 to 90% in the first 40 minutes then going to 100% from 40 to 60 minutes. Bogner et al. have observed similar biphasic kinetics for amorphous formation in their study of Indomethacin with Neusilin. The kinetics of amorphous Sulindac formation parallels that of the overall kinetics of amorphous formation between 0 and 40 minutes, however the amount of amorphous Sulindac decreases between 40 and 60 minutes. In contrast, the kinetics of amorphous complex formation increases linearly throughout the entire course of ball milling. The slowdown in kinetics of the overall amorphous formation in last 20 minutes of milling is attributed to the progressive conversion of the amorphous Sulindac into the amorphous Sulindac-Neusilin complex.
Hot melt extrusion (HME) of Sulindac-Neusilin mixtures

While ball milling provides a convenient route to generate small quantities of Sulindac-Neusilin amorphous complexes in the lab it is not a scalable process. HME was explored as a means of scaling up the production of these complexes. HME can be used as a mechano-chemical process because it allows close material packing with efficient mixing, milling, and temperature control. The advantage over more traditional technologies (e.g. ball milling) is that HME is a continuous process that is more easily scaled to pharmaceutically relevant batch sizes. Additionally HME has the advantage of being a ‘green chemistry’ technology that needs little or no solvents. The process can be readily monitored and controlled i.e. screw speed, feed rate, throughput, temperature, mixing, shear, and conveying ability. The HME screw design used for generating the amorphous complexes is shown in Figure 7(a). The screw design is customized to include several mixing and conveying elements.

$^{13}$C SSNMR data in the carboxyl region for samples made by HME are shown in Figure 7(b). HME was initially attempted at 150°C at two screw speeds 50 and 200 rpm to investigate whether the complex formation occurs below the melting point of Sulindac (184.6°C). Different screw speeds were used to change the residence time of the material in the extruder barrel. It is clear from the SSNMR data presented that no conversion was observed at this temperature irrespective of the screw speed used. However conversion of the crystalline material to the amorphous complex was observed when HME was conducted at a temperature of 200°C which was above the melting point of Sulindac. Thus, temperatures above the melting point of Sulindac are required to effect the conversion to the amorphous complex. Consistent with ball milling, HME
produced samples containing a mixture of the amorphous Sulindac (peak ‘b’) and amorphous Sulindac-Neusilin complex (peak ‘c’). Notably the amount of the complex formed was found to be greater than that achieved from ball milling for 60 minutes. The amount of complex formed remained about the same irrespective of the starting composition of Sulindac to Neusilin. This shows that Neusilin amounts greater than 1:1 do not serve to increase the amount of complex formed under these experimental conditions. About 100 grams of 1:1 and 1:2 complexes were produced using HME at 200 °C with a screw speed at 50 rpm. The sample was recovered as a powder from the HME apparatus. The HME samples were analyzed by HPLC and were found to be free of chemical degradation. HME thus provides an efficient and a convenient route to the continuous production of Sulindac-Neusilin amorphous complexes.
Figure 9. (a) HME screw design used to generate amorphous complexes. (b) Carboxyl region of the $^{13}$C CP/MAS spectra of HME samples. Peak labels: ‘a’ crystalline Sulindac, ‘b’ amorphous Sulindac, and ‘c’ amorphous Sulindac-Neusilin complex.
The surface area of crystalline Sulindac and Sulindac-Neusilin amorphous complexes as measured by Kr BET are shown in Table 1. The surface area of Neusilin US2 is consistent with that reported in product literature. The measured surface area of as-received crystalline Sulindac was 1 m$^2$/g which is consistent with what can be expected for an un-milled pharmaceutical compound. The 1:1 Sulindac-Neusilin amorphous complexes produced by either ball milling or by HME have a much lower surface area than expected average value of about 156m$^2$/g. This data indicates that complexation between Neusilin and Sulindac occurs at the surface of Neusilin. As an outcome of this solid state reaction the pores in Neusilin are no longer available for the Krypton gas to probe leading to a reduced surface area. The surface area for the HME sample is lower than that of the ball-milled sample showing higher extent of reaction for the HME sample. The surface area of 1:2 Sulindac-Neusilin amorphous complex produced by HME has a much higher surface area than the 1:1 complex. This surface area of 91m$^2$/g is closer to the expected average value of about 102m$^2$/g. This shows the addition of more Neusilin beyond the 1:1 ratio does not impact the extent of surface solid state reaction. The extent of reaction predicted by surface area results are in good agreement with those obtained by $^{13}$C SSNMR. Moreover this data also shows that Sulindac does not penetrate the pores of Neusilin and thereby does not get confined in the mesopores of Neusilin. This result is not surprising given the short residence time in the extruder. Thus, production of Sulindac-Neusilin using HME saturates complex formation at a 1:1 weight ratio and does not confine Sulindac to the pores of Neusilin.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface Area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neusilin</td>
<td>304.0</td>
</tr>
<tr>
<td>Crystalline Sulindac</td>
<td>1.0</td>
</tr>
<tr>
<td>Sul-Neu Ball milled 60 minutes</td>
<td>4.8</td>
</tr>
<tr>
<td>Sul-Neu 1:1 HME</td>
<td>2.2</td>
</tr>
<tr>
<td>Sul-Neu 1:2 HME</td>
<td>91.2</td>
</tr>
<tr>
<td>Sul-Neu 1:1 PhysMix</td>
<td>115.0</td>
</tr>
</tbody>
</table>

Table 1. Surface area of Sulindac/Neusilin samples measured using Kr-BET analysis.

### Physical stability of ball milled and HME samples

Figure 10 shows the XRPD patterns of 1:1 ball-milled (BM) and 1:1/1:2 HME samples as a function of time at 40°C/75% RH. The XRPD pattern for the time zero samples showed a broad halo without any characteristic reflections typical of the amorphous phase. The BM and HME samples remained amorphous after 3 and 4 months storage at 40°C/75% RH respectively. Moreover all samples were found to remain amorphous for more than a year at ambient conditions (data not shown). At the time points shown in Figure 10, the samples were analyzed by HPLC and were found to be free of chemical degradation. This demonstrates that either ball milling or hot melt extruding of Sulindac with Neusilin provides both physically and chemically stable amorphous phase.
Figure 10. XRPD samples of Ball milled (BM) and hot-melt extruded (HME) samples as a function of time at 40°C/75% RH. All samples remained amorphous for 3 to 4 months at accelerated stress conditions.

Our results are consistent with other observations in literature where acidic compounds such as Indomethacin and Aceclofenac showed good physical stability for periods of one to three months at 40°C/75% RH when co-ground with Neusilin. Moreover it has been shown that there is a reduction in the crystallinity of partially crystalline drug-Neusilin complexes upon storage at accelerated conditions. As it is thermodynamically impossible to convert a crystalline material to its amorphous counterpart spontaneously (without input of energy), such a conversion as described in literature must be mediated by a phase change involving Neusilin. This complex formation of acidic drugs with Neusilin helps the stabilization of amorphous phase. Thus
Neusilin provides additional avenue of stabilization when compared to organic polymer excipients.

**Dissolution of Ball milled and HME samples in 0.1N HCl**

Dissolution profiles of crystalline Sulindac and Sulindac-Neusilin amorphous complexes in 0.1N HCl are shown in Figure 11. Crystalline Sulindac is poorly soluble in the medium and reaches a solubility of 3μg/mL in about 60 minutes. In contrast the Sulindac-Neusilin amorphous complexes showed faster dissolution rate and higher solubility. The HME samples show a peak concentration and a plateau concentration. For 1:1 HME sample the peak concentration was 19.4μg/mL while the peak concentration for the 1:2 HME sample was 32.1μg/mL. The plateau concentration for both samples was around 13.2 to 13.4μg/mL. In contrast the ball milled 1:1 sample showed a peak concentration of 15.1μg/mL and a plateau concentration of 14.4μg/mL. The peak to plateau ratio follows the order 1:2 HME> 1:1 HME>1:1 ball milled. The peak concentration for all samples was attained within 10-17 minutes. Similar dissolution behavior has also been observed for Indomethacin-Neusilin amorphous complexes.25
Crystalline Sulindac did not show any appreciable improvement in dissolution or solubility when Neusilin was added to the dissolution media. The presence of Neusilin cannot explain the increased plateau concentration observed for the amorphous complexes. In order to understand the dissolution behavior the amorphous complexes were slurried in 0.1N HCl in a separate experiment. Solids recovered from the slurry after about 20 minutes showed conversion to the crystalline material by XRPD. Interestingly all samples had converted to the metastable anhydrous Form I (data not shown). It has been reported that Form I has a solubility five to seven fold higher than the more stable Form II at room temperature. During dissolution the conversion to the metastable polymorph occurs leading to a drop from the peak concentration towards the
plateau concentration which is about 5 fold higher than the plateau concentration observed for the more stable form II. The increase in the peak to plateau ratio for the 1:2 HME sample can be attributed to the microenvironmental pH increase provided by the excess Neusilin. In summary, the amorphous Sulindac-Neusilin complexes are able to provide enhanced dissolution rate and solubility when compared to the as-received crystalline material.

**Dissolution of drug product of Sulindac-Neusilin complexes**

For the solubility/dissolution advantage of the Sulindac-Neusilin complexes to be translatable to the clinic a viable drug product has to be demonstrated. To this end tablets were made from the 1:1 HME amorphous complex and these were compared with the commercial Sulindac tablets. The composition of the tablets is shown in Table 2.

<table>
<thead>
<tr>
<th>Component</th>
<th>Commercial tablet</th>
<th>1:1 HME tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (mgs)</td>
<td>Weight %</td>
</tr>
<tr>
<td>Sulindac</td>
<td>200</td>
<td>60.6</td>
</tr>
<tr>
<td>Neusilin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch 1500</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Avicel PH 102</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Total tablet weight</td>
<td>330</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2.** Compositions of commercial crystalline Sulindac and 1:1 Sulindac/Neusilin HME amorphous tablets. The exact weights of Starch, Avicel, and Magnesium Stearate used in the commercial tablet is not known. Neusilin was not used in the commercial tablet.
Although the total weight of the two tablets was different the amount of active drug in both tablets was kept the same. The dissolution comparison of the 1:1 HME and commercial tablets in 0.1N HCl is shown in Figure 12(a). Surprisingly, the tablets made from the 1:1 amorphous complex did not provide any dissolution advantage when compared to the commercial tablets made from crystalline material. The tablets made from amorphous complex were then analyzed by XRPD and were found to have crystallized into Form II thereby negating the solubility/dissolution advantage as shown in figure 12(b).

![Figure 12. (a) Tablet dissolution in 0.1 N HCl: (●) Commercial tablet containing Form II Sulindac and (◇) Tablet containing 1:1 HME Sulindac-Neusilin complex. (b) XRPD of tablet containing 1:1 HME Sulindac-Neusilin complex.](image)

The 1:1 HME drug product was analyzed nine months after manufacture. The data shows that while the amorphous drug substance is stable at ambient conditions the drug product becomes physically unstable. The crystallization in the drug product could
be due to (a) interaction with excipients, (b) compressive forces used in tabletting, or (c) a combination of excipient interaction and compression. A preliminary blending study of 1:1 HME powder with excipients and compression of either amorphous powder by itself or in combination with excipients did not produce the phase change to the crystalline substance although Magnesium Stearate containing blends showed trace amount of crystallinity. This data could not be unambiguously confirmed. Thus the change in crystallinity was attributed the propensity of the 1:1 HME sample to slowly crystallize over a period of time. In fact, after 15 months of storage at ambient conditions the 1:1 HME sample was found to have crystallized. However in the drug product crystallization occurs after nine months of storage at ambient conditions. Thus the crystallization is accelerated in the drug product. With crystallization occurring over such long periods it is difficult to identify the excipient responsible for promoting crystallization in the drug product.

It was observed that even after 17 months of storage at ambient conditions the 1:2 HME sample was still amorphous. Tablets were made with the 1:2 HME sample and were analyzed by dissolution. For exact comparison tablets were also made using the same 1:2 compositions with crystalline Sulindac and Neusilin. The composition of the tablets is shown in Table 3. The composition was changed to include stearic acid and HPMC. Stearic acid was chosen to replace Magnesium Stearate as the lubricant as it was suspected to promote crystallization of the amorphous complex. HPMC E4 was added as an agent to sustain super saturation. The ability of HPMC in maintaining super saturation is well documented.\textsuperscript{27-30} The tablets were confirmed to be amorphous and crystalline by XRPD. The data for the physical form in the tablets in presented in Figure 13(a).
Table 3. Compositions of crystalline and HME amorphous 1:2 Sulindac/Neusilin tablets.

<table>
<thead>
<tr>
<th>Component</th>
<th>1:2 crystalline tablet</th>
<th>1:2 HME tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (mgs)</td>
<td>Weight %</td>
<td>Weight (mgs)</td>
</tr>
<tr>
<td>Sulindac</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Neusilin</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Starch 1500</td>
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<td>10</td>
</tr>
<tr>
<td>Avicel PH 102</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>HPMC E4</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Total tablet weight</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 13. (a) XRPD of crystalline Sulindac, 1:2 Crystalline Sulindac/Neusilin tablet, and 1:2 HME amorphous Sulindac/Neusilin tablet. (b) Dissolution in 0.1 N HCl: (●)1:2 Crystalline Sulindac/Neusilin tablet and (◊)1:2 HME amorphous sulindac/Neusilin tablet.

The dissolution comparison between the 1:2 tablets made from crystalline and amorphous HME Sulindac-Neusilin complex are shown in Figure 13(b). The crystalline Sulindac tablet reached a final concentration of 5.7μg/mL with 9% release in 90 minutes which compares well with powder dissolution data of crystalline Sulindac. In contrast to
the 1:2 crystalline tablets, the 1:2 HME tablet reaches a concentration of 56µg/mL with 100% release in 90 minutes. It may be recalled from the powder dissolution data presented in Figure 11, 1:2 HME amorphous powder showed a peak concentration of 32.1µg/mL and a plateau concentration of 13.4µg/mL. The drop from the peak to the plateau was attributed to crystallization in the powder sample. Clearly the tablet made from 1:2 HME amorphous powder sample outperforms the commercial tablet while also overcoming the problem of crystallization seen in the 1:2 HME powder sample during dissolution. Excipients such as HPMC added to the tablet may have helped in maintaining the super saturation, thereby preventing crystallization of Sulindac in the dissolution medium. Judicious choice of excipients during tablet formulation can help in the realizing the full potential (in terms of solubility and dissolution) of amorphous acidic drug-Neusilin complexes.

**Choice of polymers to stabilize the amorphous phase**

Traditionally amorphous pharmaceutical solids have been stabilized using organic polymers. A number of mechanisms have been proposed by which organic polymers stabilize the amorphous pharmaceutical. However, all these mechanisms involve some form of physical interaction such as hydrogen bonding or Van-Der Waals forces. Moreover the polymer which typically has higher glass transition than the amorphous pharmaceutical, can serve as a diffusional barrier to recrystallization. The extent of phase mixing and the degree of physical interaction between the organic polymer and the amorphous pharmaceutical will determine the physical stability of the amorphous pharmaceutical.
Inorganic polymers such as silicates have also been reported to stabilize amorphous pharmaceuticals. In contrast to their organic counterparts, some inorganic polymers such as Neusilin US2 have the potential to form salts with acidic compounds in addition to other physical interactions such as hydrogen bonding and dipole-dipole interactions. As discussed in this paper this additional stabilization mechanism can impart greater solid state stability. For example amorphous Sulindac crystallizes within 24 hours at ambient conditions. Sulindac/Polyvinylpyrrolidone (1:1) amorphous dispersions crystallize within 2 weeks at 40°C/75% RH. However Sulindac-Neusilin amorphous mixtures at both 1:1 and 1:2 weight ratios are stable for up to 3 months at 40°C/75% RH. This additional stability imparted by Neusilin correlates with its potential to form amorphous salt complexes with acidic drugs. In addition Neusilin US2 is also an adsorbent for moisture because of its high surface area. As it is able to absorb a high levels of moisture it acts as moisture sink thereby protecting the amorphous API from the effects of moisture. These properties of Neusilin US2 make it an ideal choice for stabilizing amorphous acidic drugs. This fact has been demonstrated for many acidic API’s such as Indomethacin, Ketoprofen, Naproxen, Ibuprofen and Aceclofenac when they are co-ground with inorganic silicates.

However with neutral and basic drugs the situation with respect to Neusilin stabilization is less clear. Silicates have been reported to stabilize non-acidic drugs such as progesterone and TAS-301. The advantage of using silicates over organic polymers for non-acidic drugs is not obvious since stabilization with silicates will occur along the same lines as discussed for organic polymers. Again, for silicates containing other metal ions such as magnesium and aluminum like Neusilin, ion-dipole interactions may also
provide another mechanism for stabilization. In the case of non-acidic drugs the stability enhancement provided by Neusilin will have to be investigated on a case by case basis. However for the broad class of acidic drugs containing the carboxyl moiety, Neusilin or other similar silicates would be better choices to stabilize amorphous phase than organic polymers.

While advantages of using Neusilin-type silicates are apparent a mention must be made about the methods that are used to make these amorphous complexes at scale. Typically amorphous solid dispersions made using organic polymers can be scaled using processes that involve solvent such as spray drying, lyophilization, and solvent precipitation, or by dry process such as HME. However production of amorphous complexes with inorganic excipients can only be accomplished by a process such as HME. As mentioned in the preceding sections the stabilizing power of the complex is due to the ability of the silicate to form a salt with the API. This reaction is driven by employing temperatures close to the melting point of the API. As such only a method like HME can be used to produce large quantities of these complexes. While processes involving solvent like spray drying may be used to generate amorphous API/Neusilin mixtures they will not be able to generate the desired complex and thereby the physical stability of the amorphous material may be compromised.

Conclusions

The use of an inorganic magnesium aluminum metasilicate to stabilize the amorphous form of Sulindac was explored in this paper. Ball milling of crystalline
Sulindac with Neusilin in a weight ratio of 1:1 was able to produce an amorphous complex that was found to be physically and chemically stable at 40°C/75% RH for a period of 4 months. $^{13}$C SSNMR measurements of ball milled samples indicated the presence of two types of amorphous materials namely: amorphous Sulindac and amorphous Sulindac-Neusilin complexes. Moreover NMR measurements also indicate the formation of salt between Sulindac and Neusilin. The excellent physical stability of these complexes was attributed to this unique phenomenon of salt formation. HME was demonstrated as a method capable of producing these complexes at scale. In addition to the production of the amorphous complex, a viable tablet formulation was demonstrated with these complexes. The tablets made from the 1:2 Sulindac-Neusilin amorphous complex showed 100% release in 0.1N HCl medium while the corresponding tablet made from the crystalline material showed only a 9% release. It was shown that care must be employed while selecting the components of the formulation to make the drug product not only to maintain the physical stability of the amorphous complex but also to enhance the dissolution properties. While Neusilin clearly provides an advantage in stabilizing acidic drugs that contain the carboxyl moiety more work needs to be done to extend the utility of Neusilin to basic and neutral drugs.
References


Chapter 7. Conclusions and Future Considerations

The use of amorphous drugs for drug product development carries with it certain challenges namely, the chemical and physical instability that is the nature of amorphous material. With the challenges come some rewards in the form of increased dissolution rate and bioavailability. Manipulating these characteristics toward the improvement of drug performance during development are some of the primary goals of the pharmaceutical chemist. The pursuit of these goals results in the development new methods and materials being constantly explored.

Organic excipients mixed with the amorphous form of a drug have been shown to help stabilize against crystallization by various mechanisms.\textsuperscript{1-6} The main advantage to the use of organic polymers is that the amorphous solid dispersions are able to maintain the original dissolution and solubility advantages of the original amorphous drug material. Several methods for the mechanism of stabilization of the amorphous drug material using organic polymers have been proposed. These include hydrogen bonding between the excipient and drug, steric hindrance caused by the polymer to prevent crystallization of the amorphous material and an increased glass transition temperature as a result of the production of a single phase mixture between the polymer and amorphous drug.

Recent work has been done utilizing inorganic silicates in the stabilization of amorphous drug material. Inorganic silicates provide the possibility of stabilization of amorphous API through the unique mechanism of salt formation, a mechanism not available when using organic polymers. An interesting inorganic silicate Neusilin, a
synthetic magnesium aluminometasilicate, has been demonstrated to aid in the amorphization process resulting in a higher percentage of amorphous material formed as opposed to processing without an excipient. Hydrogen bonding or acid-base reactions between the drug and silanols and possible ion-dipole interactions between the drug and metal ions at the surface of Neusilin are the proposed mechanisms for stabilization.

Both cryo-milling and ball milling were found to produce the amorphous form of Sulindac when milled either with or without Neusilin however amorphous Sulindac milled without Neusilin crystallized within 24 hours. Both griseofulvin and astemizole resisted amorphization when cryo-milled without the addition of Neusilin. Griseofulvin was amorphized when ball milled with and without the addition of Neusilin yet all amorphous samples of griseofulvin crystallized within 24 hours. The addition of Neusilin was found to be a requirement for the amorphization of both griseofulvin and astemizole although astemizole was only cryo-milled and not ball milled. Due to the lack of availability of astemizole, pyrimethamine was chosen as a replacement as it is also a basic compound. Pyrimethamine was not amorphized during ball milling even with the addition of Neusilin. The amorphous Sulindac/Neusilin complex formed upon ball milling was found to have the longest term stability out of all samples generated. The amorphous griseofulvin/Neusilin samples and amorphous astemizole/Neusilin samples were found to crystallize within 24 hours of milling regardless of milling technique used. As a result, the amorphous Sulindac/Neusilin complex became the focus of the investigation.

The formation of a complex between the amorphous Sulindac and Neusilin upon milling was the cause for resulting stability of the amorphous form. The most likely
mechanisms for stabilization of the Sulindac/Neusilin complex were determined upon $^{13}$CSSNMR analysis and showed the formation of a salt between the amorphous Sulindac and Neusilin.

Bench top mills were very effective in the amorphization and complex formation of the Sulindac/Neusilin samples but this bench top process cannot be scaled up. Without the ability to scale up the batch size the process cannot be used for any commercial applications rendering it useless for development. Because the amorphous Sulindac/Neusilin samples generated from ball milling were more stable than the ones generated from cryo-milling it was determined that temperature during milling has a significant effect on the outcome of stability. Hot Melt Extrusion (HME) was identified as a possible means to scale up production of the amorphous drug/excipient complex. The HME extruded samples demonstrated not only a greater percentage of amorphization of the Sulindac but also greater long term stability of the Sulindac/Neusilin complex. Because the quality of the HME samples were superior to what the ball mill produced it was determined that HME was a viable method to scale up production enough to enable further development of the amorphous complex.

Because the formation of a useful drug product is the final goal for Pharmaceutical development a drug product was made using the HME complex. Tablets containing the HME complex were manufactured using the same recipe as commercially available Sulindac tablets marketed under the name Clinoril and use crystalline Sulindac. It was expected that the tablets using amorphous drug would show improved dissolution over the tablets using crystalline material. This did not turn out to be true. The dissolution profile was the same for both the crystalline and amorphous tablets. A study
was initiated to determine if the added excipients had contributed to the crystallization of the tablets containing the HME material. It was found that Magnesium Stearate was causing the amorphous HME Sulindac/Neusilin complex to crystallize prior to compression into tablets.

A new tablet blend was developed replacing the Magnesium Stearate with Stearic Acid and adding Hydroxypropylmethylcellulose as an additional excipient. The 1:1 HME Sulindac/Neusilin material was found to have crystallized during storage. It was replaced with 1:2 HME Sulindac/Neusilin material which proved to have remained amorphous. The experiment was run again and this time the tablets containing the HME amorphous Sulindac/Neusilin complex demonstrated a marked improvement in the dissolution rate over the tablets containing the crystalline material. Stability analysis of the tablets stored at both 5°C and 40°C/75% RH using XRPD shows that the tablets remained amorphous after 4 weeks.

The goals set for determining the feasibility of the use of amorphous drug material in a drug product were successfully met. Crystalline drug material was reliably amorphized using an optimized ball milling method and stabilized with the addition of the inorganic excipient, Neusilin. The scale up of the stable amorphous drug-excipient complex was achieved using a solventless HME process and tablets were produced using the amorphous material from the scaled up process. These tablets have demonstrated an improvement in the dissolution rate over the tablets made using crystalline drug material. The tablets made from the 1:2 Sulindac-Neusilin amorphous complex showed 100% release in 0.1N HCl medium while the corresponding tablet made from the crystalline
material showed only a 9% release and have remained stabilized against crystallization for one month at 5°C and 40°C/75%RH.

These results indicate that the development of a drug product using the amorphous form of a crystalline drug can not only be done but also promises to provide improvements upon the original drug product. It is known that an improvement in dissolution can relate to an improvement in bioavailability. If the bioavailability were to show the expected improvement in the amorphous drug product, then drug load in the new product could be lower. This has the added benefit of lowering the production costs of the drug by lowering the amount of drug produced while keeping the efficacy of the drug product the same. While Neusilin clearly provides an advantage in stabilizing acidic drugs that contain the carboxyl required for salt formation further investigation is required to extend the utility of Neusilin to basic and neutral drugs.
References


