

# Surface Interactions and Microstructural Characterization of API Subjected to Cryogenic Milling

DIANA GUZMAN-VILLANUEVA<sup>1</sup>, DEA HERRERA-RUIZ<sup>2</sup>, M. TERESA CARVAJAL<sup>1</sup>

<sup>1</sup>Department of Industrial and Physical Pharmacy, School of Pharmacy, Purdue University, 575 Stadium Mall Drive, West Lafayette, IN 47907.

<sup>2</sup>Dea Herrera-Ruiz, Facultad de Farmacia, Universidad de Morelos, UAEM, Cuernavaca, Morelos, México.

## INTRODUCTION

Use of crystalline active pharmaceutical ingredients (API) in solid oral dosage forms are a preferable form in the pharmaceutical industry because of good stability compared to amorphous. However, a large number of crystalline APIs has poor solubility being the rate limited for the bioavailability<sup>1</sup>, this has become a challenge in pharmaceutical research to come out with creative solubilization strategies to enhance dissolution and hence bioavailability. One strategy is the particle size reduction to improve the dissolution rate by increasing the surface area of materials; therefore drugs with poor water solubility are often micronized. This approach offers advantages for both oral delivery and pulmonary drug administration.

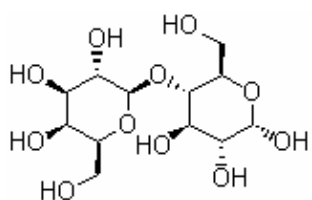
There are different methods used to reduce the particle size the solids in pharmaceutical manufacturing, being the milling of larges particles a common way, but depending on the product to be milled and the required final particle size, the drug would be size reduced by pressure, friction or impact<sup>2</sup>. Although the milling is process frequently used in the course of drug formulation, one main concern is that the milled materials present characteristics such as poor control not only in the particle size, shape and electrostatic charge but on the surface interactions, microstructural and physicochemical properties. These in turn are responsible for the functionality and the stability upon storage of the materials.

During the course of the milling process, molecular compounds are subjected to a large variety of perturbations such as mechanical stress; the high energy input can cause a disruption of the crystal lattice such as formation of disordered amorphous solids or the creation of defect which alter the physicochemical properties of pharmaceutical solids with an increased chemical reactivity<sup>3</sup>. All these transformations have a direct influence on the stability and functionality of

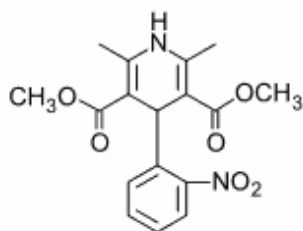
the compounds, due to thermodynamically activated solids can result in a greater susceptibility to spontaneous recrystallization during storage. For these reasons, there is need to study the microstructure from pharmaceutical solids when they are subjected to high energy milling processes.

## METHODS

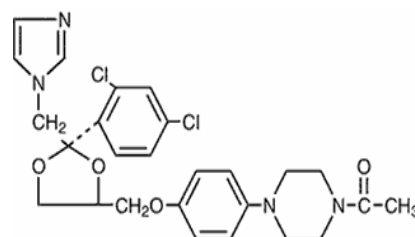
In this research, Nifedipine (NDP), Ketoconazole (KCZ), were chosen as a model drugs along with a common excipient: Lactose (LAC) widely used in the food and pharmacy industries.



$\alpha$ -Lactose



Nifedipine



Ketoconazole

These compounds were subjected to cryogenic milling and stored at low relative humidity in desiccators until ready to be analyzed. Cryogenic milling was performed using a method employed by Feng et al<sup>4</sup>. Samples were milled for 10 y 30 min periods. Thermal and surface characterization was investigated. The structure of milled materials was compared with their respective counterparts of un-milled (crystalline) and amorphous (quench melt) using techniques such as Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction (PXRD), Optical Microscopy (OM) and Inverse Gas Chromatography (IGC).

### Differential Scanning Calorimetry (DSC)

A Q10 DSC was used and the samples were analyzed in hermetic aluminum pans. To calibrate high purity indium was used. Nitrogen was used as purging gas. Approximately 3 mg of samples were weighed and heated at 10 °C/min until reach the melting point of the drugs, samples were carried out in triplicate. Different mixtures of crystalline, milled and amorphous samples were analyzed on the same DSC instrument under the same conditions and data were collected.

## Powder X-Ray Diffraction (PXRD)

Samples were analyzed using a Siemens X-ray diffractometer. All samples (un-milled, milled and quench melt) were placed in a circular silicon holder and then scanned from 4 to 40° 2θ with a scan rate of 4°/min. The patterns obtained were compared to each other with their respective crystalline and quench melt form.

## Optical Microscopy (OM)

A BH-2 Olympus Microscope was used to determine the presence of birefringence or double refraction as indicative of crystallinity in milled and un-milled samples. Approximately 2 mg of samples were placed at slide glasses and then were directly observed by optical microscope using a 10X-40X magnification in a black background.

## RESULTS AND DISCUSSION

PXRD of un-milled samples were compared with the calculated patterns from the Cambridge Structural Database. These data indicate that un-milled samples correspond to the pure crystalline forms (Figure 1). The reduction in the intensity of crystallization peaks of NDP was confirmed by PXRD analysis. The PXRD of milled and quench melt samples also was

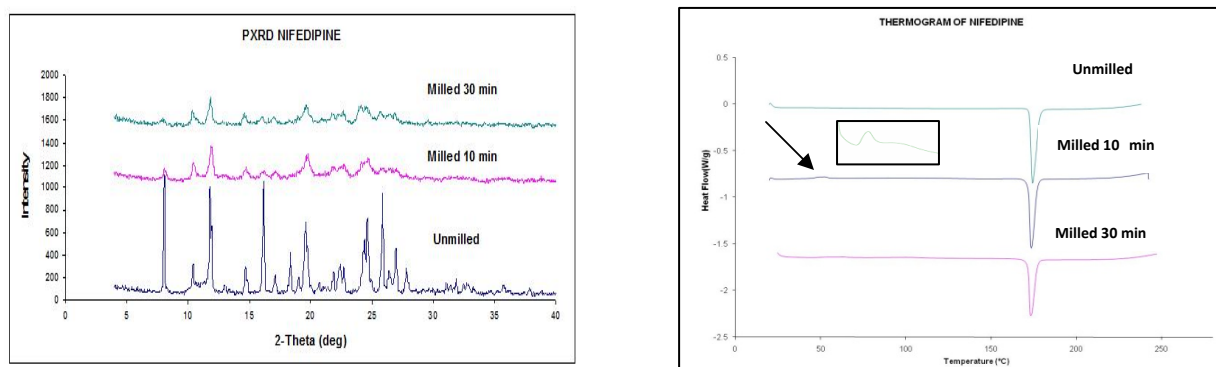


Figure 1. PXRD patterns and DSC thermograms from Nifedipine

compared and they show a different behavior. Quench melt shows the absence of crystallization peaks as was expected. DSC measurements were performed to study how the thermal properties are affected by cryogenic milling. Thermograms were obtained from un-milled

crystalline samples, amorphous quench melt and samples subjected to different time periods. The thermograms show consistent melting points for crystalline materials and glass transition and crystallization events for amorphous materials. DSC measurements of un-milled, milled and quench melt NDP samples presented the consistent melting point peak. The melt quench sample also show the glass transition temperature  $\sim 38$  °C as well as the crystallization event  $\sim 75$ °C respectively. However, the thermograms of milled samples 10 and 30 min show a new exothermic event approximately at  $55$ °C overlapping the glass transition temperature. These thermograms are completely different to amorphous samples (Figure 1). The new exothermic event is only showed in milled samples. Both milling times present the sample this event being more prominent at 30 min. Therefore, the presence of exothermic peaks is a consequence of milling process. These exothermal peaks cannot be categorized as a crystallization peaks produced as a result of an amorphous state due to the absence of glass transition temperature but the creation of defects in the crystalline lattice which translates in the reduction of crystallinity. Furthermore, NDP milled show a decrease in the melting point as well as heat of fusion. These denote the presence of defect in the milled samples (Figure 1).

Under the microscope the un-milled and milled samples of NDP samples show a decrease of birefringence (Figure 2) and hence a decrease of crystallinity as shown by PXRD. However, milled samples still present broad regions with a high birefringence. That means that cryogenic milling reduces the crystallinity having the three-dimensional array of the crystalline lattice. In contrast, KCZ shows a different behavior. The thermograms of 10 and 30 min milling times show the characteristic events of an amorphous state. KCZ presents a glass transition temperature at  $\sim 36$  °C. The crystallization peak was showed at  $\sim 50$  °C for both times. The crystallization peak was increased as the milling time was longer. The analysis of PXRD of KCZ milled sample resulted in characteristic amorphous patterns without the presences of peaks (Figure not shown). These patterns denote a total loss of crystallinity compared with the un-milled materials. That shows that cryogenic milling is able to change the initial state of KTZ sample and produce the absence of long- range and three-dimensional molecular array characteristic of the crystalline state. Nevertheless, in the microscopy studies is observed a significant loss of birefringence at 10 min but there is still the presence of crystalline material. A total loss of birefringence at 30 min is noted (See figure 2). The results obtained show that the amorphous state take place due to the accumulation of perturbation resulting in a transformation

from crystalline lattice to an amorphous state as is increasing the milling time. Therefore, the high mechanical energy input for the cryogenic milling not only generate the fragmentation of larger particles to reduce the particle size but it produce disruptions which alter the periodicity that characterizes the crystalline state, creating a disordered material.

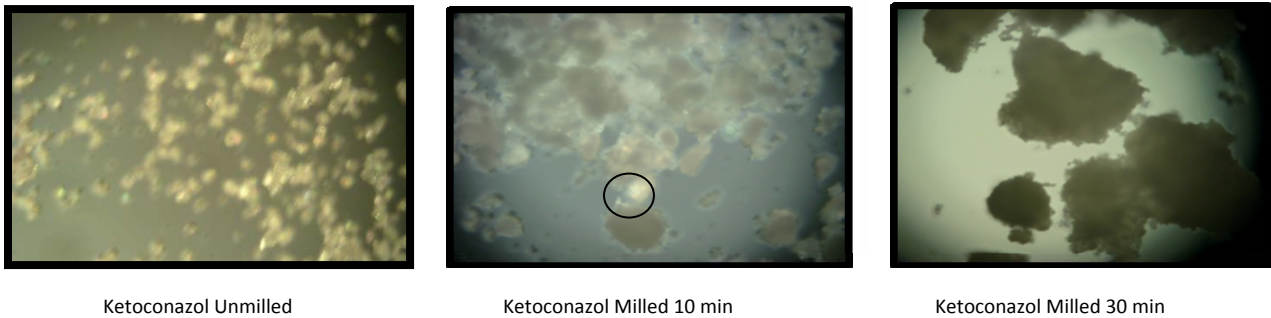


Figure 2. Presence of crystalline material at after milling Ketoconazole showed by birefringence.

Similar behavior to that of the Ketoconazole is observed in LAC milled sample, a progressive deterioration of the crystalline lattice that ends with the accumulation of dislocations causing overall perturbations which contribute to the formation of amorphous regions as the milling time is increased. DSC measurements of un-milled LAC show the presence of melting point and dehydration events at  $\sim 219^\circ\text{C}$  and  $\sim 150^\circ\text{C}$  respectively. However, after 10 min milling LAC show the formation of two endothermic peaks approximately at dehydration and melting point events which correspond to the presence of a mixture of  $\alpha$  and  $\beta$ -LAC. In contrast, the DSC thermogram of LAC milled 30 min show a thermal transition associated with the formation of an amorphous state represented for a sharp crystallization peak at  $\sim 50^\circ\text{C}$  (Figure 3).

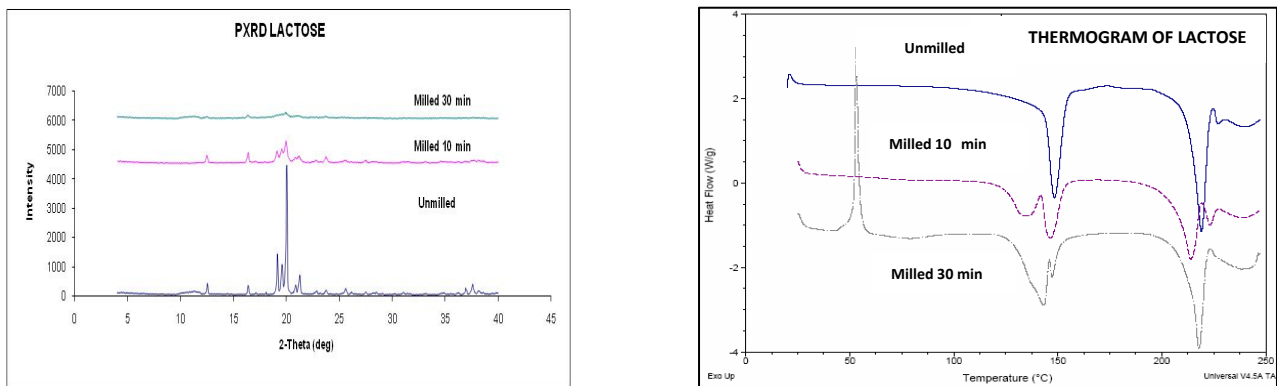


Figure 3. PXRD patters and DSC thermograms for Lactose.

The impact of the cryogenic milling in LAC samples is markedly observed by the reduction in the intensity of crystallization peaks in PXRD LAC patterns and by the loss of birefringence in microscopy studies.

## CONCLUSIONS

The techniques used have demonstrated the physical transformation of the materials after being subjected to cryogenic milling. The presence of different events: *disorder*, showed in several orders of magnitude by LAC, the formation of *crystal defect* by NDP and the creation of *amorphous states* by KTZ. All of them manifest different behaviors according to their capacity of control the high mechanical energy input during milling process. Crystal defects were characterized by the presence of new exothermal peaks in milled samples which overlap the glass transition region present in the amorphous form by the DSC and as reduction of crystallinity in PXRD patterns. The amorphous form was represented by glass transition temperature and crystallizations peaks as typically reported in the literature. This work clearly demonstrated that cryogenic milling induced physical transformations in the crystalline lattice. The surface of the milled material is left with high surface free energy compared to that of the un-milled material. It is expected that the milled material will be prompt to interact more easily than the un-milled material. Work is still underway to understand the properties that govern these energetic states in order to take them into consideration when carrying out stability and performance studies.

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