POWDER X-RAY DIFFRACTION DETECTION OF CRYSTALLINE PHASES IN AMORPHOUS PHARMACEUTICALS

B. A. Sarsfield,¹ M. Davidovich,¹ S. Desikan,¹ M. Fakes,¹ S. Futernik,¹ J. L. Hilden,¹ J. S. Tan,² S. Yin¹, G. Young¹, B. Vakkalagadda, and, K. Volk¹

¹Bristol Myers Squibb Co., New Brunswick, NJ
²Purdue University, West Lafayette, IN

ABSTRACT
Amorphous materials are often produced in the pharmaceutical industry. Materials in the amorphous phase are less thermodynamically stable than any crystalline form, leading to a tendency for the amorphous materials to transform to a known or potentially unknown crystalline phase. The time scale for any transformation is also unknown and can, in part, be evaluated via stability studies. The detection of crystalline phases in mixed systems is often performed by powder X-ray diffraction. In this work, a limit of detection PXRD method was developed to detect low levels of crystalline material in an amorphous material. Method development activities focused on defining the method requirements, determining the sample and standard material characteristics, understanding beam penetration, sample cell design, sample preparation, and data analysis. Validation is also discussed briefly.

INTRODUCTION
Amorphous materials are often used in pharmaceuticals and offer several advantages. Amorphous materials can be naturally occurring, such as insulin, large molecule excipients (mostly polymers), and some small drug molecules that are difficult to crystallize. The pharmaceutical industry often engages in costly high energy processes, such as lyophilization, high energy milling, melt extrusion and coprecipitation with polymers to obtain an amorphous material. The desired benefits of amorphous drugs include higher rate of solution and higher kinetic solubility.² Improved bioavailability of poorly soluble drugs is an especially desired consequence of higher kinetic solubility. Several additional excellent references exist which discuss the use and characteristics of amorphous materials in pharmaceuticals.

The use of amorphous materials in any field is associated with some challenges. A very significant challenge is that the amorphous phase is thermodynamically unstable compared to any crystalline phase of the same material. The transformation from amorphous to a crystalline phase is dependent on diffusion properties of the amorphous material, as well as the kinetics of nucleation and growth of the crystalline phase. Additionally, the identity of the resultant crystalline phase(s) cannot be absolutely predicted. First, though the identity of the most stable crystalline form of a drug is sought, it is possible that the most stable form is not yet identified. Second, there is no guarantee that the amorphous phase will transform to the most stable crystalline phase.

MATERIALS AND METHODS
Instrumentation: PXRD patterns of amorphous and amorphous/crystalline mixes were collected on either of two instruments, both of which used the Bragg-Brentano configuration, a copper X-ray tube and a point detector. The Bruker D8 Advance configuration included power of 40 kV x 40 mA, 1.0 mm divergence slit, 1.0 mm anti-scattering slit, 0.1 mm detector slit and 0.6 mm receiving slit. The Philip X’Pert configuration included power of 45 kV x 40 mA, automatic
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The divergence slit, and 0.2 mm receiving slit. The scan 2theta ranges, step size and time per step are discussed in the Method Development section.

Materials and Sample Preparation: Methods discussed in this report were developed for several amorphous Active Pharmaceutical Ingredients (API). This report will focus on Amorphous Material A, which is a blend of 40% API/60% excipients. To determine the limit of detection in each case, blends using the amorphous material and its related most stable known crystalline API phase, Form 1, were prepared directly on the sample holder using the top-fill method. All standard mixes are expressed as %crystalline phase/total API. For example, a 2.5% crystalline Form 1 standard contains 2.5% Form 1 per total API, which is equivalent to 1.0% Form 1 per total API/excipient blend. The flat plate sample holders’ outer dimensions and outer materials varied to fit the Bruker D8 or the Philips X’Pert. All sample holders were prepared with flat 511 Si inserts to assure sample depth of 0.5 mm, except where inserts were not used as discussed in the Method Development section. The Form 1 sample was assumed to represent a 100% crystalline phase upon positive comparison to the simulated powder patterns (based on single crystal X-ray results). The amorphous materials were assumed to be 100% amorphous if no crystalline peaks were observed. Amorphous Material A was stored at 4°C in chambers containing Drierite.

METHOD DEVELOPMENT
Method development begins with defining the requirements and the information desired from the technique. The purpose of the method discussed here is to demonstrate that the test samples contain amorphous material without evidence of crystalline materials, within the limits of detection. A “low” limit of detection is desired, with the eventual desire to relate degree of crystallinity to product performance, such as dissolution rate. The development of a technique to understand the structure (truly amorphous and/or crystallites too small to be detected by X-ray) of the amorphous material is not a goal of this method, and would require more advanced instrumentation and possibly the use of a synchrotron. The method, including interpretation of the powder patterns, must be able to be run and analyzed reproducibly by several scientists, and possibly may need to be transferred to a production site.

A full understanding of the material to be tested is also important. The test samples are defined as containing API that is amorphous by X-ray. There is a possibility that some of the samples will also contain API that has transformed to a crystalline phase. Thus, the expected PXRD pattern will be an amorphous halo, sometimes with peaks due to crystalline excipients. The limit of detection was determined using the most stable crystalline phase. One assumption is that the amorphous-to-crystalline transition can be simulated with the physical mixture of Form 1 added to the Amorphous Material A. This assumes that any preferred orientation in the physical mixture is similar to the naturally occurring crystallization of Form 1 in Amorphous Material A. Since there is no guarantee that the most stable known phase will be the result of the transition from the amorphous phase, the method must be general enough to pick up any new crystalline peaks. The kinetics of the amorphous-to-crystalline transition are unknown and likely to depend on temperature, humidity and physical treatment such as grinding.

The standard samples are defined as amorphous by X-ray and/or the related crystalline form. Preparing standard samples to determine the limit of detection introduced a sample uniformity
challenge that needed to be handled. To reduce errors from sample loss, standards were prepared by adding Form 1 to Amorphous Material A directly on the sample holder. The instrument and sample holder configuration can also be selected to reduce sample uniformity issues. The first selection is for an instrument with a wide beam so that the largest sample surface can be analyzed. The second is to understand beam penetration into the sample. For an ideal crystalline material, the diffracted intensity, $I$, is related to the incident intensity, $I_o$, by the Beer-Lambert law,

\[
I = I_o \exp(-\mu t), \quad \text{with} \quad \mu = \tau + \sigma
\]

where $\mu$ is the total linear absorption coefficient (cm$^{-1}$) for the incident beam, $t$ is the thickness of a homogeneous, isotropic material with surfaces normal to the beam direction and more than covering the total beam cross-section, $\tau$ is the photoelectric absorption (fluorescence), $\sigma$ is the total linear scattering coefficient (Compton and Rayleigh scattering). This can be rewritten in terms of the mass absorption coefficient, $\mu/\rho$ (g/cm$^2$), which are tabulated values$^7$ for each atom at the wavelengths of several radiation sources. With the assumption of a homogeneous sample and the use of Bragg-Brentano geometry, the intensity of the X-rays diffracted by the surface layer which has thickness, $x$, is equal to

\[
I(x) = I_1 \left[1 - \exp\left(-\frac{\mu}{\rho} \rho_A \cdot x \cdot \frac{2}{\sin \theta}\right)\right]
\]

where $I_1$ is the intensity collected by the detector on a semi-infinite sample (infinite thickness), $\rho_A$ is the density of the packed sample and $\theta$ is the angle of incidence in the Bragg-Brentano geometry. Considering that the analyzed thickness corresponds to the layer which gives significant fraction of the signal, it is possible to estimate the analyzed depth. In the case of amorphous material A, the chemical composition (no heavy atoms), density packed on the X-ray sample holder and the percentage of each element in the sample were used to calculate beam penetration. The AbsorbDX$^8$ software was used to calculate depth for 90% contribution to the diffracted beam. Figure 1 shows the results, which indicate that a crystalline material with the composition of amorphous material A would have less than 1 mm penetration depth at 24°2θ.
The calculated penetration depth information was used as a guide, but was not expected to be accurate since the sample materials were not pure crystalline materials, homogeneous, nor isotropic. Tests were performed to determine the depth of penetration so that the appropriate sample depth, especially for standard preparation, could be designed. Instead of thoroughly mixing the 2.5% Form 1 with the API/polymer blend, 2.5% Form 1 was layered in the sample holder at 1.0 mm depth, 0.5 mm depth and at the top surface. Figure 2, which demonstrates the effect of depth of penetration, shows that Form 1 can be detected when at 0.5 mm depth, but not at 1.0 mm depth. The powder pattern for 2.5% Form 1 on the top surface is not shown in Figure 2 because it exactly overlaps the 0.5 mm depth powder pattern. This led us to use sample holders with 511 Si inserts to bring all samples to 0.5 mm depth, which allowed the full depth of the sample to be analyzed by the beam.

Figure 2: Effect of depth of crystalline Form 1 in Amorphous Material A/polymer blend. 2.5% Form 1 was layered at 1.0 mm depth (bottom), 0.5 mm depth (top).

The scan range, scan step size, time per step and the analysis method were evaluated simultaneously. The scan ranges were selected based on strong peak positions and limiting interference from excipients, which lead to scan ranges set to 12° to 22° 2θ for Form 1 and Amorphous Material A. This allowed the appearance of multiple peaks from the most likely crystal form and a large enough range to allow detection of a form other than the most stable known form. The scan step size and time per step were set to optimize signal/noise, with the optimum appearing to be 0.04° 2θ step size and 30 seconds per step.

Several data analysis methods were evaluated to obtain a reproducible analysis technique to observe the limit of detection (LOD) of a crystalline Form 1 in Amorphous Material A. With the noise generated by the diffuse disorder scattering from the amorphous material, reproducible interpretation of a peak is difficult, but necessary for a validated method. The FDA published guidelines for LOD methods, which suggested LOD can be determined by visual evaluation, signal-to-noise evaluation and Standard Deviation of the Response and Slope. This last method is not possible for this case because the signal is not linear with the degree of crystallinity. Also, as pointed out previously, the method must be general enough to detect any peak, not just the peaks from the most stable known form. This means that peak detection at known 2θ values was not appropriate, though this is the most common method of analysis and was certainly used for method development and identifying limit of detection standards. Often, a sufficiently trained scientist can recognize a peak, which would make the visual evaluation possible. However, validation of that visual recognition is close to impossible. Therefore, a numeric approach to
analysis must be taken. Jade, a program which reads several PXRD file formats, was used for the analyses. Smoothing algorithms, background subtraction and peak finding routines were evaluated. The Fast Fourier Transform (fft) smoothing algorithms work well with crystalline materials but tend to introduce low intensity peaks for an amorphous material, which lead to problems distinguishing real peaks from fft-generated peaks. The traditional smoothing methods, including the Sovitsky-Golay least squares smoothing algorithm, tended to reduce the detectability of low intensity peaks. Background subtraction techniques often did not adequately describe the background of a halo. This version of Jade allows the scientist to select the background more accurately, but again, that lead to validation and repeatability concerns. The analysis that appeared most reproducible was a simple peak find routine, with a parabolic filter, variable filter length, summit peak location, 0.1% intensity cutoff, 1.0 2θ range to find background, and 7 points for the background average. Normally, a threshold of 3σ is used for peak detection, but 5σ was required due to the occasional interference of noise from the amorphous halo.

The limit of detection of the known most stable crystalline form for each system was determined by making mixes of Form 1 in the Amorphous Material A. The limit of detection was determined to be 2% by observing that 2.0% Form 1 was repeatedly detected, but that lower amounts were not reproducibly detected. To assure that 2% Form 1 could always be detected, the source intensity was varied by decreasing the source energy (Figure 3). An external standard, the Quartz 20.9° 2θ peak was also monitored. As can be seen from Figure 3, Form 1 could not be detected at the lowest source energy. The value of the Quartz 20.9° 2θ peak at 30 mA was set as the lower limit for source intensity, and a Quartz external standard is run as a system suitability test with every daily set of sample analyses.

![Figure 3: System Suitability. Effect of source energy on Form 1 detection at 40 mA(top), 35 mA(2nd), 30 mA(3rd) and 25 mA(bottom)](image)

Demonstrating that the method will work reproducibly is an important step to completing method validation. In the pharmaceutical industry, the FDA has prepared guidelines for method validation. Most of the guidelines were initially based on chromatography method development, but many of the concepts, such as specificity, linearity, precision, accuracy, sensitivity and robustness, can be used for other methods, including PXRD methods.

Specificity is the ability to measure a specific analyte free from interference of other components. In this case, the method was developed to test the 2θ range where the amorphous
halo or excipients provide the least interference while crystalline Form 1 presence is obvious. The analysis method was not designed to be specific, in that it is designed to pick up any peak associated with a known or unknown crystal form.

Linearity is defined as showing direct and proportional response to changes in concentration. The interferences by diffuse disorder scattering changes with the relative amounts of amorphous/crystalline content, \(^{11}\) which interferes with linearity. In practice, we found that the response is not linear near the limit of detection, which is an expected result. Therefore, linearity is not a parameter that is tested to validate this limit of detection PXRD method. Accuracy is also a concept that is not applicable to this method. Since there is no linearity, we are not using this method to predict the amount of Form 1 present; we are simply determining that the amount of crystalline phases present are either greater than or less than the limit of detection.

Precision, which is the closeness of agreement among a series of measurements, is a parameter that should be evaluated for this technique. In this case, standards at the limit of detection (LOD) are run repeatedly and are demonstrated to consistently detect peaks of Form 1. The related phenomenon, robustness, is often defined as the capacity of a method to remain unaffected by small, deliberate variations in the method. In regards to robustness, we demonstrated that the amorphous material A was not induced to form crystalline API by grinding or holding the sample for several days beyond the normal analysis time. We also demonstrate that additional grinding of a standard LOD mix results in a sample from which Form 1 can still be detected.

**CONCLUSIONS**

The method described in this report was developed to determine if Amorphous Material A has crystalline character that is detectable by PXRD. The most stable known form, Form 1, was used to prepare standards to determine its limit of detection, though the method was also designed to detect peaks from other crystal forms. This method, and similar methods for other amorphous materials, have been used for formulation feasibility and stability studies. In the case of amorphous materials, stability studies are especially important because, besides the possibility of crystallization, the amorphous materials are also more hygroscopic and often less chemically stable than related crystalline forms. The methods have also been used to demonstrate that the materials tested in clinical studies have amorphous character and no detectable crystalline character. Other methods are required to quantify the amount of crystallinity detected or to characterize the X-ray amorphous material.

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10. Jade Version 6.1, Materials Data Inc, Redwood, CA