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Quantifying amorphous content of lactose using parallel beam X-ray powder diffraction and whole pattern fitting

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Abstract

The objective of this study was to demonstrate the applicability of parallel beam X-ray powder diffraction (XRPD) and a new method for whole pattern fitting to the quantification of the residual amount of amorphous content in a pharmaceutical solid using lactose as a model system. Lactose monohydrate, prepared by slurry conversion of anhydrous lactose, was mixed with different amounts of amorphous lactose produced by lyophilization. X-ray powder diffractograms of each mixture were recorded and analyzed by whole pattern fitting using Percentage Crystallinity Determination Software from Kratos Analytical Inc. The polycapillary X-ray optic, which provides a parallel beam of X-radiation, has advantages over Bragg–Brentano Optics with respect to sample height artifacts. Significant shifts in peak position with changes in sample height of lactose monohydrate were observed using Bragg–Brentano Optics while no change was detected for the polycapillary X-ray optic. A technique to normalize all diffractograms to have the same total integrated intensity was necessary to eliminate tube fluctuation effects. After normalization, the amorphous content of lactose in the range of 1–10% was reproducibly predicted (small standard deviation between samplings) using whole pattern fitting. The limit of detection was calculated to be 0.37% amorphous content. The results indicated that parallel beam XRPD and whole pattern fitting can provide accurate analysis of relatively small amounts of amorphous content in pharmaceuticals compared to typical XRPD analysis.

Keywords: Amorphous; Lactose; X-ray powder diffraction; Parallel beam; Polycapillary X-ray optics; Whole pattern fitting

1. Introduction

Quantification of residual amorphous content is an important and difficult pharmaceutical analytical technique. Amorphous forms can be induced by various pharmaceutical processes, such as recrystallization, lyophilization, milling, and com-

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paction [1–4]. Amorphous fractions of a material might exist as a separate bulk phase or as disorder on crystalline surfaces [5,6]. The relatively high free energy of an amorphous form results in higher solubility [7], hygroscopicity [8], and often, lower chemical stability than the crystalline phase [3]. Many physical and chemical transitions are believed to originate in amorphous regions, including phase transformation, desolvation, and thermal reactions [9]. However, in some cases, it is desirable to produce and maintain some amorphous content to enhance dissolution and compactibility [10]. Whether or not the production of amorphous content is deliberate, it is difficult to control the event. The amount of amorphous content may be a contributor to batch-to-batch variation for drug substance and product properties and/or behavior. Usually, the amorphous to crystalline conversion varies with sample history and environmental condition. Therefore, quantifying the residual amount of the amorphous form in pharmaceuticals is very critical for quality and process control, and trouble-shooting.

Quantifying amorphous content remains one of the most challenging of pharmaceutical analyses. Gravimetric methods based on water vapor sorption are most sensitive and popular [4]. However, it is not feasible to use these methods if the crystalline material is also hygroscopic or the amorphous phase is non-hygroscopic (the use of alternate organic vapors is possible). The isothermal calorimetric technique seems to have a niche. It was reported that it could detect amorphous content below 1% [11]. But it requires that crystallization from the amorphous is kinetically favored and may be very time consuming. FT-Raman and Near-IR have been applied to detect the presence of amorphous phase [12,13], but they demand well-resolved bands in the spectra of amorphous and crystalline phases. To search for a fast and sensitive method, we pursued a combination of advanced X-ray powder diffraction (XRPD) methods and a new method for whole pattern fitting to quantify low amounts of amorphous content in drugs and/or excipients.

XRPD has been used for the measurement of crystallinity in many case-studies [4,14,15]. Traditionally, a couple of the strongest diffraction peaks are picked, and their integrated intensity is used to construct a calibration curve for quantification. The information for the amorphous phase is typically ignored. The detection limit for this method is about 10% of amorphous content [4]. No study we found attempts to use whole pattern fitting to quantify the amorphous content in pharmaceuticals. This new approach employs the diffractograms of the 100% crystalline and 100% amorphous forms to fit the diffractogram of the unknown sample. Since the information from the amorphous form in the mixture is used, the detection limit may be pushed down to 1%, which few analytical techniques can achieve.

The whole pattern fitting requires that the peak position and peak shapes for a specific phase be consistent. Otherwise, the prediction will not be reliable. It is well know that the Bragg–Brentano Optic gives excellent resolution but is very sensitive to sample displacement errors [16]. Minor changes in sample height will result in shifts of peak position, which may introduce significant errors in pattern fitting. Careful packing of a sample to be coplanar with the holder surface is necessary to minimize the peak shift. This makes it difficult to avoid pressing the sample, which may introduce preferred orientation. It is well recognized that preferred orientation is the biggest challenge for any kind of quantitative method based on XRPD [17]. The polycapillary optic has distinct advantages with respect to sample displacement artifacts. The polycapillary optic is also called a Kumakhov lens after its Russian inventor. It contains an array of hollow optical fibers [18]. X-Ray Optical Systems (Albany, NY) is the major vendor of the polycapillary optic. Their polycapillary optic system is able to receive X-rays over a large solid angle (4.2°) from the point source and carry the X-rays into a parallel beam with a 0.22° divergence by collimating both the axial and planar directions. The polycapillary optic has found application in macromolecular crystallography [19], stress and texture analysis [20], and micro X-ray fluorescence analysis [21]. There is no report demonstrating its advantage over Bragg–Brentano Optics in pharmaceutical analysis; this is shown in our case study on quantifying residual amorphous content with lactose as a model compound.

Lactose, as a diluent in tablets and capsules, is one of the most used pharmaceutical excipients [22]. The amount of amorphous form may affect the compressibility of lactose[10], and may impact on its use in dry powder inhalers. So it is very critical to know the amorphous content in the types of lactose from the vendors, and the lots. A fast, online or even at-line method would be very useful for industrial quality assurance and process control. Moreover, the amorphous form of lactose is relatively stable at ambient temperature and relative humidity less than 50%. All of these factors make lactose a good model for this method development.

2. Experimental section

2.1. Materials

Anhydrous lactose and acetone are from Mallinckrodt (Phillipsburg, NJ). Lactose monohydrate was prepared by slurry conversion of anhydrous lactose in double distilled water overnight at ambient temperature. The harvested lactose monohydrate was washed with an equal volume of acetone to prevent caking. It was then dried under ambient conditions overnight. The particle size was below 50 μm by optical microscopy. The material was assumed to be 100% crystalline.

Amorphous lactose was prepared by lyophilization of a 5% lactose solution. It was confirmed to be amorphous by XRPD. The amorphous material was stored in a desiccator over P_2O_5 prior to use. The water content is below 0.1% as measured by Karl Fisher.

2.2. Preparation of physical mixtures

Known ratios of amorphous and crystalline lactose were mixed in an agate mortar and pestle without grinding. The mixed sample with a total weight of 200 mg was transferred to an aluminum top-fill sample holder for XRPD analysis at ambient temperature and relative humidity less than 45%.

2.3. Differential scanning calorimetry

The DSC thermograms were recorded with a DSC 2920 (TA Instruments, New Castle, DE). The temperature and cell constant were calibrated with indium (m.p. 156.6°C). About a 3-mg sample was weighed and sealed hermetically in an aluminum pan. The heating profile was from 25 to 160°C at a rate of 5°C/min under a nitrogen purge at 25 ml/min. Data collection and analysis were conducted on the data station, Thermal Analyst (TA Instruments, New Castle, DE).

2.4. Thermogravimetric analysis

TGA was carried out using TGA 2050 (TA Instruments, New Castle, DE). All runs were performed in an open platinum pan with a nitrogen purge at 10 ml/min. A 10–15 mg sample was heated from 25 to 160°C at 10°C/min.

2.5. Karl Fisher titrimetry

The water content was determined using a Model 100 Titration Controller (Fisher Scientific), employing pyridine-free reagents.

2.6. XRPD

A Shimadzu XRD-6000 diffractometer was used to collect all diffractograms with either Bragg–Brentano or polycapillary optic (X-Ray Optical Systems, Albany, NY). Samples were filled in an aluminium holder and exposed to Cu–K_α radiation (40 kV, 40 mA). The scanning range was 10–30° 2θ and the rate was 2°/min with a 0.02° step size. The simulated powder pattern of lactose monohydrate was calculated from the published crystal structure by using *Cerius*^{2} (Molecular Simulations, INC.).

2.7. Whole pattern fitting

The diffraction pattern from a mixed amorphous crystalline material contains four distinctive 'frequency' components, which must be controlled before any reliable analysis can be performed. The highest 'frequency' component is the

measurement noise described by Poisson statistics for the random process of X-ray generation. The frequency of this noise contribution is directly related to the step size used during the measurement and has a contribution given by the square root of the actual measured signal.

The crystalline contribution is contained within the relatively sharp diffraction peaks and is the closest in 'frequency' to the measurement noise. In order to ensure that the noise and crystalline component can be cleanly differentiated, the measurement step size should be chosen such that there are at least ten steps across the crystalline diffraction peak. When there is a clear difference in 'frequency' between the crystalline peaks and the noise, the noise can be safely removed without biasing the crystalline contribution using traditional smoothing techniques or frequency filtering. The Shimadzu software uses Savitsky-Golay 2nd derivative digital filters to remove the noise contribution from the subsequent analysis.

At the low 'frequency' end, the data contains contributions from the incoherent background and the amorphous contribution. The incoherent background contribution is closely related to the electron density of the material being studied. As this may vary for some amorphous and crystalline components, the background contribution must be carefully analyzed prior to any removal. In practice it is difficult to remove the appropriate amounts of incoherent background without biasing the amorphous component. In the Shimadzu percentage crystallinity software the data is analyzed without removing the incoherent background low frequency component.

The quantification of the high 'frequency' crystalline component and the low 'frequency' amorphous component is based upon statistically fitting a standard 100% amorphous pattern and a standard 100% crystalline pattern to the measured data. Both of these standard reference patterns should be measured under exactly the same conditions using similar sample preparation techniques as the unknown sample being analyzed. In order to remove any random sample to sample variation that may still remain, all three data sets are normalized using Vainsteins law (see subsequent section). If there are electron density difference between the amorphous and crystalline components, however, then this normalization will introduce a fixed bias in the analysis. This systematic error can be easily allowed for by using regression calibration of a few known mixed amorphous-crystalline standards. A similar systematic error will be introduced if the 100% reference patterns are themselves mixed amorphous/crystalline systems. The regression calibration will also remove this bias. As a further refinement to improve the precision of the quantification, the data being analyzed is allowed some movement in 2θ to correct for any peak movement that may occur due to sample displacement. The degree of movement required is calculated by comparing the peak positions of the three strongest peaks from the 100% crystalline reference pattern to the equivalent peak positions in the measured data.

The actual pattern fitting is based on a simplex minimization method [25], which fits the standard reference patterns to the measured data. The simplex procedure fits differing amounts of the 100% amorphous reference data and the 100% crystalline reference data to the measured data set until a good fit is achieved. The fitting proceeds by moving the three vertices of the simplex about the simplex center in a minimization direction until all three of the simplex vertices lie within the final fitting criteria. Each vertex is defined by a fixed ratio of amorphous and crystalline contributions based upon the two reference patterns. Each vertex has a goodness of fit criteria describing how well the fixed ratio of amorphous and crystalline components describe the measured data. The goodness of fit criteria uses a combination of least squares minimization and Poisson statistics weighted least squares minimization. The least squares criteria minimizes the square of the difference between the calculated and measured data. The Poisson statistics weighting is achieved by dividing the difference squared by the square root of the average of the calculated and measured data. The Poisson statistics weighting is only required when the noise contribution to the measured data remains a significant component. For ideally smoothed data files, the straight least squares method is most applicable.

The analysis is initiated by providing the software the measured data from the unknown sample and the measured data sets from the two reference standards. Along with the three data sets, the final fitting criteria must be provided along with amorphous and crystalline percentage of the initial three simplex vertices. For the simplex method, the final criteria fitting is defined in terms of the acceptable difference between the least squares results calculated at the three simplex vertices. The output from the analysis is the final percentage of crystalline and amorphous contribution along with the resulting simulated diffraction pattern.

3. Results and discussions

3.1. Characterization of the reference materials

Fig. 1 shows the X-ray powder diffractograms of crystalline lactose monohydrate and amorphous lactose, which were used as reference materials in this study. The diffractogram of lactose monohydrate, prepared by slurry conversion, matches almost perfectly with the calculated pattern from the crystal structure (a and b in Fig. 1). The weight loss in TGA was about 5.013% and the water content measured by Karl-Fisher was 5.029%, which are consistent with the expected weight loss of 4.996% for the monohydrate. DSC analysis revealed that the amorphous material has a glass transition temperature at 117°C, which agrees well with the value published previously [23]. These analyses verify the forms of the starting phases.

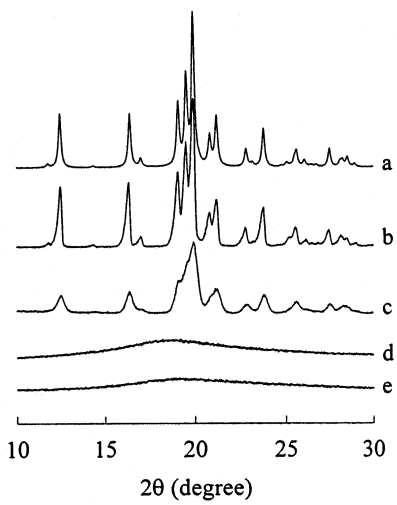

Fig. 1. X-ray powder diffraction of crystalline lactose monohydrate and amorphous lactose measured at Bragg–Brentano (B–B) optics and polycapillary optics. Key: (a) caculated pattern of lactose monohydrate; (b) pattern of lactose monohydrate in B–B optics; (c) pattern of lactose monohydrate in Polycapillary optic; (d) amorphous lactose in B–B optics; (e) amorphous lactose in polycapillary optic.

Fig. 1 also compares the diffractograms from Bragg–Brentano and polycapillary optics. The amorphous form shows a similar amorphous halo with both optics (d and e in Fig. 1). The difference in the intensity of the halo may be due to different instrument alignment. The amorphous halo was less intense and all crystalline peaks became broader and less intense in the polycapillary optic compared with the Bragg–Brentano optic (b and c in Fig. 1). Some well-resolved peaks with the Bragg–Brentano optic fuse into one peak with the polycapillary optic. (The resolution may be improved with the addition of custom Soler slits before the monochromater.)

3.2. Effect of sample displacement on the diffractogram of lactose monohydrate

The polycapillary optic is less sensitive to changes in sample height and roughness. In order to verify that in this study, 100, 200, or 300 mg of lactose monohydrate were packed evenly in the same sample holder. The 200 mg sample was coplanar with the holder surface, while the 100 mg sample was displaced -1 mm and the 300 mg sample was displaced $+1$ mm from the sample surface. The diffractograms from both optics were collected and are compared in Fig. 2. This demonstrates that the peak position for every peak of lactose monohydrate is consistent irrespective of the sample height changes for the polycapillary optic. However, all peaks shift with the Bragg–Brentano optic when the sample height varies. The results confirm that the polycapillary optic is superior to the Bragg–Brentano optic with respect to tolerance to reasonable variations in sample height.

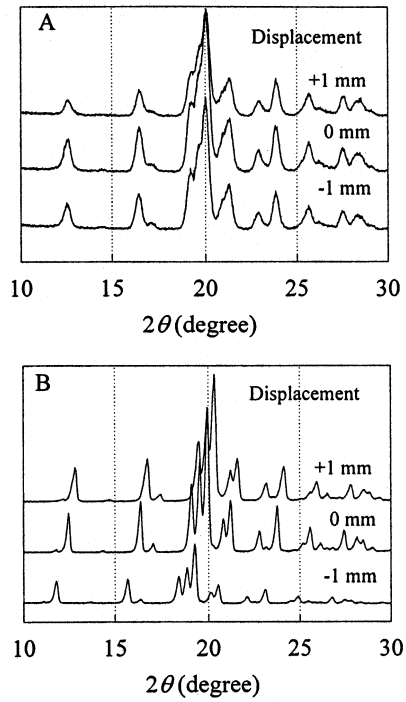

Fig. 2. Effect of sample displacement on the diffractogram of lactose monohydrate, using the polycapillary (A) and Bragg–Brentano (B) optics. One sample has the sample surface coplanar with the holder surface (0 mm). The other two are either higher (+ 1 mm) or lower (− 1 mm) than the holder surface.

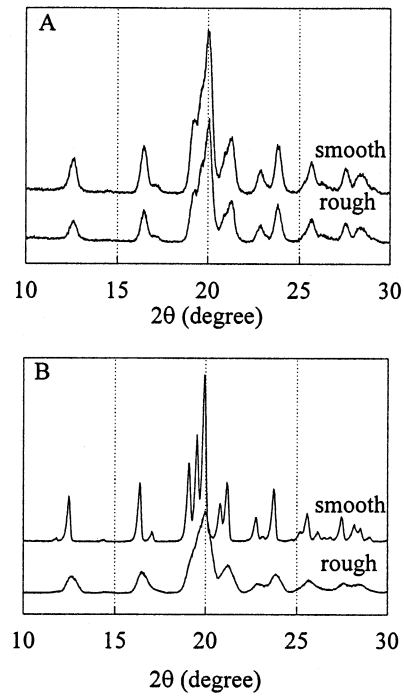

Fig. 3. Effect of sample roughness on the diffractogram of lactose monohydrate, using the polycapillary (A) and Bragg–Brentano (B) optics. The samples are either smooth or rough.

This advantage is substantiated in another investigation (Fig. 3). The 200 mg sample with a smooth surface, which was coplanar with the holder surface, was disrupted to make a rough surface containing grooves. The bottom of the grooves is about 1 mm below the holder surface and the top of the grooves is about 1 mm above the holder surface. The peak position and peak shape of the rough sample is nearly identical to those of the smooth sample in the polycapillary optic (Fig. 3). In the Bragg–Brentano optic, the rough sample has similar peak positions to the smooth sample, however, the peak shape changes dramatically: all peaks became broader and some neighboring peaks are fused together. This diffractogram is similar to that obtained from the polycapillary optic.

It seems that the diffractogram is an average of diffractograms from local differences in sample height caused by the roughness. This confirms that the polycapillary optic is more suitable for quantitative analysis based on whole pattern fitting, which requires consistent peak position and shape for good output. It is noteworthy that consistent peak position is also necessary for peak indexing and Rietveld fitting [24], which are used in phase identification and quantification.

The effect of sample preparation on peak intensity has not been discussed. Fig. 3 does indicate that the rough sample has decreased peak intensity for all peaks for the polycapillary optic. As

Table 1
The effect of roughness on the total XRPD integrated intensity of lactose monohydrate[a]

Optic	Smooth (%)	Rough (%)
Polycapillary	100	86 (10)
Bragg–Brentano	100	86 (7)

[a] The S.D. is shown in parentheses ($n = 4$).

listed in Table 1, the integrated intensity of all peaks in the rough sample is 14% less than the smooth sample. Similarly, the integrated intensity of all peaks decreases for the Bragg–Brentano optic when sample roughness changes. This indicates that the sample variation in height and roughness may yield a variation of peak intensity in the polycapillary optic. So a normalization technique is needed to minimize the variations from sample preparation.

3.3. Effect of normalization on the quantitative results

In addition to sample preparation, tube output fluctuation may affect the peak intensity for the polycapillary optic. It is instrument-dependent and related to the stability of the X-ray source. Usually, an external or internal standard is used to cancel out some variations. External standards may not be reliable if the fluctuation is time-dependent. Internal standards are better, however, to make a homogenous mix that does not segregate is challenging. In some cases, the use of an internal standard is not possible or appropriate as when analyzing intact drug products or monitoring dynamic change. A reliable normalization technique without an internal standard is very important for quantification purposes based on XRPD, whether dealing with polymorph or amorphous content measurement.

A unique normalization technique was pursued in this study. It is based on Vainshtein's law, which states 'Within identical regions of reciprocal space, the diffracted intensity from a material will be independent of its state of order'. This means that the integrated intensity diffracted by a 100% crystalline sample will be the same as that diffracted by a 100% amorphous sample within the same measurement range in reciprocal space (this makes sense if you consider that the total number of electrons has not changed). Based on this assumption, all samples with different levels of amorphous content can be normalized to the same integrated intensity of either 100% amorphous or 100% crystalline. This is an effective way to minimize the error from instrument drift from run-to-run or day-to-day. The normalization was performed by Percentage Crystallinity Software. To pursue the whole pattern fitting, a diffractogram of 100% crystalline lactose and a diffractogram of 100% amorphous lactose were selected. All diffractograms were normalized to have the same integrated intensity as the selected diffractogram of 100% crystalline lactose. For diffraction intensity for every data point, the normalization was carried out as following equation:

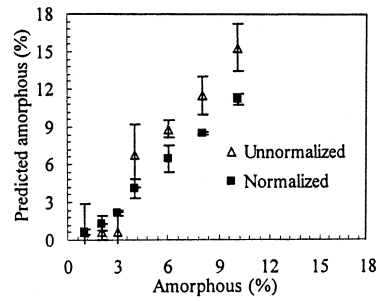

Fig. 4. The effect of normalization on the fitting result. Error bars show one S.D. ($n = 4$).

$$\text{Modified intensity} = \text{Original intensity} \times F$$

F = integrated intensity of 100% crystalline/integrated intensity of this diffractogram.

Fig. 4 shows the predicted results from whole pattern fitting for the samples with amorphous content from 1 to 10%. All diffractograms (intensity vs. 2θ) are smoothed before analysis. Each data point is an average of four samples from the same batch. Without normalization, the results varied due to instrument drift. The standard deviation for every sample is large and the overall predicted results are different from the experimental. The results were much better using the normalized data. The predicted results are much closer to experimental ones. The standard deviation from different samples from each batch is reduced. This demonstrates that the normalization is necessary for reliable fitting. It also indicates that the normalization technique is effective. With normalization, the data with or without smoothing gives very close results (Fig. 5). Although the reliability and standard deviation are slightly improved for the smoothed ones.

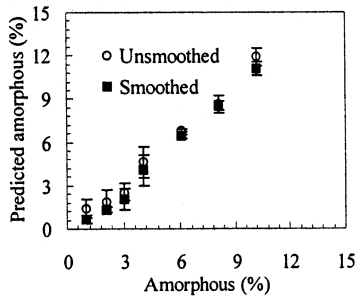

Fig. 5. The effect of smoothing on the fitting result. Error bars show one S.D. ($n = 4$).

3.4. Effect of different sampling on the quantitative results

After normalization, the whole pattern fitting process can differentiate samples with 1–2% difference in amorphous content. In order to test the robustness of the fitting method, another two groups of samples were analyzed on different days. The agreement among three groups is excellent as demonstrated in Fig. 6. The standard deviation for three distinct samples is very small. The correlation curve between the experimental and predicted value has very high linearity ($R^2 = 0.9958$). The limit of detection (LOD) was estimated by following equation [26]:

LOD = (3.3*S.D. of intercept)/Average slope.

The LOD is down to 0.37% amorphous content. So the quantitation limit (LOQ) is about 1% amorphous form according to following equation [26]:

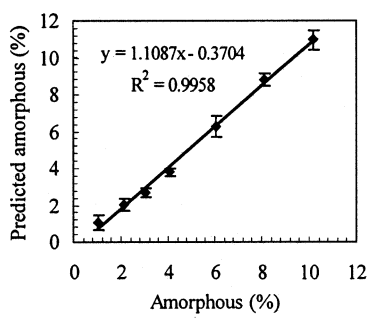

Fig. 6. The correlation curve between experimental data and predicted data from whole pattern fitting. Error bars show one S.D. ($n = 3$).

Table 2
Comparison of known amorphous content and the predicted value from whole pattern fitting[a]

Amorphous (%)	Caculated (%)
1	1.08 (0.39)
2.1	2.05 (0.35)
3.03	2.70 (0.24)
4.07	3.82 (0.20)
6.05	6.29 (0.58)
8.10	8.78 (0.32)
10.20	10.99 (0.50)

[a] The S.D. is shown in parentheses ($n = 3$).

LOQ = (10*S.D. of intercept)/Average slope.

Table 2 shows that the predicted and real amorphous values are in good agreement.

The unique normalization technique cancelled out some error from the instrument fluctuation. However, sample inhomogeneity remained a contributor to the measurement variation. Any quantitative method requires homogeneous mixing of calibration standards. However, making a uniform mix is challenging for solid samples, especially for the lyophilized samples. During XRPD experiments, the X-rays hit on the sample on a limited spot size, which varies with the measuring angle. The diffraction signal is from this sub-sampled volume under the spot. How representative this is of the whole sample relies on the homogeneity of mixing. It was found that different measurements of the same sample, which were not touched between measurements, gave a very small standard deviation compared to the remixing measurements (data not shown). This indicated that the quantification performance of this technique was limited by sample mixing. It was also the largest source of error in quantifying the crystallinity of indomethacin via Raman spectroscopy as discussed by Taylor et al. [12]. As shown in Figs. 4 and 6, the standard deviation between different samplings was close to that from different remixings. It further revealed that mixing was a more significant contributor for error than weighing. Sophisticated mixing strategies or efficient average technology from different

part of the sample could improve the detection limit.

Nevertheless, comparison of different technologies revealed that parallel beam XRPD combined with fitting is one of the best ways to quantify residual amorphous content, with regards to the detection limit and time of analysis (Table 3). Moreover, it is one of a few technologies that have potential to be developed online or at-line. So a combination of the polycapillary optic and whole pattern fitting improves a traditional technology, XRPD. Furthermore, in cases where a calibration curve may not be constructed, the techniques offer a less precise alternative to using only the 100% and whole pattern fitting. Similarly, this technology will be very useful to quantify residual amount of crystalline material in amorphous matrix. Relatively, this task is easier than quantifying residual amount of amorphous form because crystalline material displays characteristic peaks. So the detection limit can be pushed to lower than 0.37%.

4. Conclusions

The use of the polycapillary optic to generate the parallel beam X-ray is superior to the Bragg–Brentano optic with respect to its tolerance to the variation of sample preparation such as sample height and roughness. It is more suitable for use with whole pattern fitting for quantitative purposes as the peak position and shape are consistent, independent of sample variations. However, the sample height and roughness can affect the peak intensity for the polycapillary optic. Normalization is necessary to cancel out the sample variation and the X-ray tube fluctuation. A unique normalization technique, which makes all diffractograms have the same total integrated intensity in the same scanning range, was found to be very effective. With the help of normalization, the predicted value from whole pattern fitting is good. The variation between different samplings is small. The detection limit was calculated to be 0.37% amorphous content. This study demon-

Table 3
Comparison of different methods for quantifying amorphous content

Method	Measures	Destructive	Online	Time scale	Detection limit (%)
DSC	Heat of crystallization of amorphous form	Yes	None	10–30 min	10 [4]
Density	Difference in density between amorphous and crystalline forms	No	None	10–30 min	10 [4]
Traditional XRPD	Diffraction of crystalline form	No	Possible	10–20 min	10 [4]
Solution calorimetry	Difference in Heat of solution between crystalline and amorphous forms	Yes	None	0.5–1 min	1 [3]
Traditional gravimetry	Water sorption of amorphous form	No	None	24–48 h	1 [4]
FT-Raman	Difference in Raman spectra between crystalline and amorphous forms	No	Possible	5–10 min	1 [12]
Near-IR	Difference in Near–IR spectra between crystalline and amorphous form	No	Possible	5–10 min	1 [27]
Modulated DSC	Glass transition of amorphous form	No	None	1–2 h	0.90 [28]
Microcalorimetry	Crystallization of amorphous form	Yes	None	0.5–4 h	1 [29], 0.5 [30]
Solid-state NMR	Difference in NMR spectra between crystalline and amorphous forms	No	None	0.5–10 h	0.5 [30]
Our parallel beam XRPD	Difference in diffraction between crystalline and amorphous forms	No	Possible	10–20 min	0.35
Modified gravimetry	Crystallization of amorphous form	Yes	None	$<$100 min	0.10–0.30 [31]

strated that XRPD can be a very useful method for quantifying residual amorphous content in pharmaceuticals, if the parallel beam optic and advanced fitting software are used. The detection limit can be improved if imperfect mixing can be minimized or other average techniques can be applied.

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