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Application of Partial Least-Squares (PLS) modeling in quantifying drug crystallinity in amorphous solid dispersions

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ABSTRACT

Among the different experimental methods that can be used to quantify the evolution of drug crystallinity in polymer-containing amorphous solid dispersions, powder X-ray diffractometry (PXRD) is commonly considered as a frontline method. In order to achieve accurate quantification of the percent drug crystallinity in the system, calibration curves have to be constructed using appropriate calibration samples and calculation methods. This can be non-trivial in the case of partially crystalline solid dispersions where the calibration samples must capture the multiphase nature of the systems and the mathematical model must be robust enough to accommodate subtle and not so subtle changes in the diffractograms. The purpose of this study was to compare two different calculation and model-building methods to quantify the proportion of crystalline drug in amorphous solid dispersions containing different ratios of drug and amorphous polymer. The first method involves predicting the % drug crystallinity from the ratio of the area underneath the Bragg peaks to total area of the diffractogram. The second method is multivariate analysis using a Partial Least-Squares (PLS) multivariate regression method. It was found that PLS analysis provided far better accuracy and prediction of % drug crystallinity in the sample. Through the application of PLS, root-mean-squared error of estimation (RMSEE) values of 2.2%, 1.9%, and 4.7% drug crystallinity was achieved for samples containing 25%, 50%, and 75% polymer, respectively, compared to values of 11.2%, 17.0%, and 23.6% for the area model. In addition, construction of a PLS model enables further analysis of the data, including identification of outliers and non-linearity in the data, as well as insight into which factors are most important to correlate PXRD diffractograms with % crystallinity of the drug through analysis of the loadings.

1. Introduction

One potential method to increase the bioavailability of an active pharmaceutical ingredient (API) with low aqueous solubility is to deliver the drug in the amorphous form intimately mixed with a polymer, forming what is commonly referred to as an amorphous solid dispersion (Chiou and Riegelman., 1971; Leuner and Dressman, 2000; Serajuddin, 1999; Ford, 1986). In such cases, the solubility advantage can only be maintained so long as conversion to the thermodynamically more stable crystalline form can be prevented. However, the free energy of the API in the amorphous solid dispersion is typically higher than that of the corresponding crystalline form; in other words, the API is above its "solubility limit" in the polymer matrix (Tao et al., 2009; Marsac et al., 2006b, 2009). As a result, API crystallization cannot be retarded indefinitely, and may proceed to varying degrees in the solid dispersion during storage (Rumondor et al., 2009b). It is thus desirable to be able to quantify the relative amounts of amorphous and crystalline API in this type of solid dispersion.

Different experimental methods have been utilized to quantify the degree of crystallinity in pure API systems, or for quantification of the different crystalline phases present in polymorphic mixtures. Such methods include differential scanning calorimetry (DSC), infrared and Raman spectroscopy, and powder X-ray diffractometry (PXRD) (Clas et al., 1995; Taylor and Zografi, 1998; Pan et al., 2006; Shah et al., 2006; Seyer and Luner, 2001; Lehto et al., 2006; Bertacche et al., 2006; Bai et al., 2004; Yoshioka et al., 1994, 1995; Suda et al., 2008; Chieng et al., 2009). In addition, quantitative determination of crystalline API in the presence of other components has also been reported (Suryanarayanan and Herman, 1991; Moore et al., 2008, 2009). However, quantitative determination of the amount of crystalline API present in an otherwise amorphous molecular-level solid dispersion system (where the drug and the polymer are intimately mixed) has not been widely reported. The goal of this work was thus to develop methods to quantify API crys-

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tallinity in such solid dispersion systems. PXRD was selected as the analytical method of choice, since this method is well-suited for identifying the presence of crystalline materials, and detection limits of 5% crystallinity or lower have been reported when mixtures of amorphous and crystalline APIs are analyzed using this method (Fix and Steffens, 2004; Nunes et al., 2005; Chen et al., 2001; Yoshioka et al., 1994, 1995; Otsuka and Kaneniwa, 1988; Surana and Suryanarayanan, 2000). In addition, Partial Least-Squares (PLS) multivariate modeling was applied to the X-ray data, to evaluate if more accurate quantification can be achieved as compared to more traditional PXRD peak area- or height-based methods.

The model amorphous solid dispersion system chosen for the study was comprised of the drug, felodipine (FEL), and the polymer, poly(vinylpyrrolidone) (PVP). This system is known to form an intimately mixed amorphous solid dispersion (Marsac et al., 2006a, 2006b, 2008, 2010; Konno and Taylor, 2006).

2. Materials and methods

2.1. Materials

Dichloromethane (ChromAR grade) and ethanol (200 proof) were obtained from Mallinckrodt Baker, Inc., Paris, KY, USA, and PHARMCO-AAPER, Brookfield, CT, USA, respectively. Felodipine (FEL) was a generous gift from AstraZeneca, Södertälje, Sweden, while poly(vinyl pyrrolidone) PVP K29-32, was purchased from Sigma–Aldrich Co., St. Louis, MO, USA. Prior to use, PVP was kept in desiccators filled with P_2O_5 for at least 1 week to remove any moisture.

2.2. Experimental methods

In order to build a robust calibration curve, it is necessary to prepare meaningful calibration samples which encompass the features of a partially recrystallized solid dispersion. It is recognized that the X-ray scattering pattern of an amorphous molecular-level solid dispersion system cannot be described by a linear combination of the diffractograms of pure amorphous systems (Rumondor et al., 2009a; Newman et al., 2008). Due to intimate drug–polymer mixing, changes in the shape of the halo of an amorphous molecular-level solid dispersion may occur, which need to be captured in the calibration samples. Thus the calibration samples must contain the amorphous solid dispersions mixed with crystalline felodipine rather than simply mixing amorphous and crystalline felodipine with the polymer.

To produce the most representative calibration samples, amorphous molecular-level solid dispersions were prepared by dissolving the desired ratios of crystalline FEL and PVP in a common solvent, 1:1 (weight basis) mixture of ethanol and dichloromethane. The solvent was then removed by rotary evaporation (Brinkman Instruments, Westbury, NY, USA), followed by overnight storage of the resultant solid under vacuum. Subsequently, the material was gently ground to a powder using a mortar and pestle in a controlled relative humidity environment (less than 10%). After confirming that the samples were X-ray amorphous using PXRD, an appropriate amount of crystalline FEL was added, and then each mixture was milled using a cryogenic grinding mill (Freezer/Mill model 6750, SPEX Certiprep, Methucen, NJ, USA) at two impacts per second for a total milling time of 4 min. The resulting fine powder was passed through a 150-μm sieve. Samples containing three PVP loadings (25%, 50%, and 75%, w/w) and six levels of drug crystallinity (0%, 10%, 25%, 50%, 75%, 90% drug, w/w) were prepared. For each data point, three independent replicate samples were prepared.

PXRD analysis was performed using a Shimadzu XRD-6000 (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a Cu-Kα source and set in Bragg-Brentano geometry. The scan range was set between 5° and 35° 2θ, and the scan speed was set to 8°/min with a 0.04° step size. Before each day of measurement, the accuracy of the 2θ angle was checked by verifying that the [1 1 1] peak of a Si-standard sample is located between 28.423° and 28.463°. Three consecutive readings were taken from each sample, and the diffractograms were overlaid to verify that drug crystallization did not occur during analysis.

Three samples containing 25% PVP (w/w) were also separately prepared with 50% of the FEL added as crystalline powder. These samples were cryo-milled for total milling times of 2, 4, and 6 min, and were analyzed using PXRD and infrared (IR) spectroscopy to ensure the milling procedure did not result in significant sample alterations. IR spectra of the samples were obtained in absorbance mode using a Bio-Rad FTS 6000 FT-IR spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA) equipped with a globar infrared source, KBr beamsplitter, and DTGS detector. The spectra of samples milled for 2, 4, and 6 min (obtained using a Golden Gate™ MkII ATR with diamond top-plate, Specac Inc., Woodstock, GA, USA) overlapped with one another. The absence of an increase in the relative intensity of peaks attributable to FEL-PVP specific interactions as mentioned by Marsac et al. (2006a) indicates that the milling process did not induce detectable formation of amorphous molecular-level solid dispersions. An absorbance peak due to crystalline felodipine was also clearly visible in all samples and no intensity change was observed with milling time. PXRD diffractograms of the same samples were also almost identical. A slight decrease in the height of Bragg peaks characteristic of crystalline FEL was detected from the diffractograms, indicating the milling process may have introduced some crystalline defects. However, the difference in calculated crystallinity between samples milled for 2 and 6 min was less than 2%, well within the quantification errors of the calculation methods used.

3. Results and discussion

3.1. Data pretreatment

In order to accurately quantify the amount of crystalline FEL in the samples, the diffractograms were first normalized against the photon intensity of the Si-standard sample recorded on the same day. In addition, the diffractograms were normalized to unit area. Following these steps, a set of normalized diffractograms resulted for each drug-to-polymer weight ratio. As the fraction of crystalline FEL in the samples was increased, the relative intensity of Bragg peaks was increased compared to the relative intensity of the amorphous halo, as shown in Fig. 1.

3.2. Felodipine crystallinity determination based on the relative height of the Bragg peaks

Assuming that the experimentally measured crystalline and amorphous X-ray intensities are proportional to the crystalline and amorphous fractions of the samples, % crystallinity of FEL in the samples can be estimated using the following equation (Shah et al., 2006; Nunes et al., 2005):

$$\%\ \text{crystallinity} = \frac{100A_c}{A_c + A_a} \quad (1)$$

Here, A_c and A_a represent the respective area contributions from the crystalline and amorphous phases of the sample to the diffractograms. Although peak and halo intensities are sometimes used instead of areas, for this study, the comparison of the areas was used to minimize variations due to lattice strain and particle size.

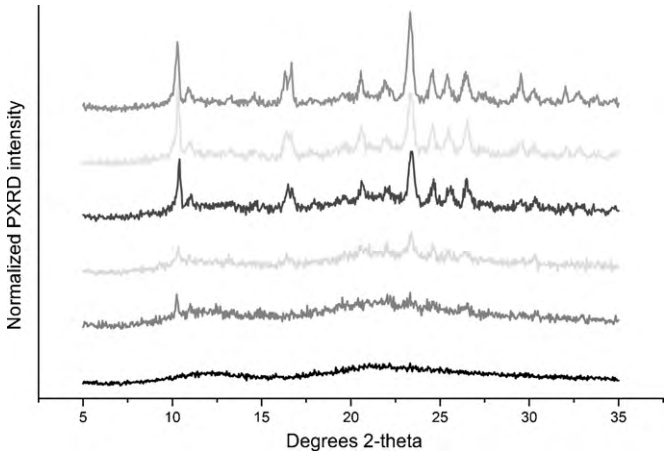

Fig. 1. PXRD diffractograms of solid dispersions containing 50% PVP and 50% FEL with (from top to bottom) 90%, 75%, 50%, 25%, 10%, and 0% of the FEL added as crystalline powder. Similar profiles were obtained with samples containing 25% and 75% PVP (results not shown).

Using the collected data, the total area underneath the diffractograms ($A_c + A_a$) was calculated between 5° and 35° 2θ using the trapezoidal rule, applied with Microsoft® Office Excel 2003 SP2 (Microsoft Corporation, Redmond, VA, USA). In order to separate contributions from the amorphous halo and the crystalline Bragg peaks, Fourier-transform (FT) filtering was applied to the diffractograms using Origin® v7 SR2 (OriginLab Corporation, Northampton, MA, USA). The area underneath the Bragg peaks (A_c) was then calculated separately from the overall area. The results, shown as linear correlations between actual and predicted % crystallinity, are plotted in Fig. 2, along with the 95% upper and lower prediction bands (De Gryze et al., 2007).

As can be seen from Fig. 2, a large scatter in the predicted % crystallinity was observed, resulting in relatively large prediction errors. This was especially apparent as the fraction of the polymer in the sample was increased, and the relative contribution from the Bragg peaks to the total area was decreased, and is reflected in the decreasing goodness-of-fit (R^2) and increasing root-mean-squared error of estimation (RMSEE) values (see Table 1). In addition, deviations of the calculated slopes (m) from unity were observed, indicating poor overall correlation between actual and predicted % crystallinity values. Different variations of calculation methods based on the area underneath the diffractograms were also attempted. For example, correlations were attempted between % crystallinity of FEL and the total area underneath the Bragg peaks, as well as various different area normalization methods. None of the methods attempted improved the prediction. In addition, the linearity between actual and predicted % crystallinity was lost, such that the RMSEE values was unacceptably high (>20%) at either extreme of drug crystallinity values.

One possible cause for the relatively large prediction errors is poor signal-to-noise ratio in the diffractograms. In PXRD measurements, increasing signal-to-noise ratio can be achieved, for example, by reducing the scan rate, effectively increasing sample exposure time to the X-ray beam. However, preliminary experiments of reducing the scan rate to 2 or 4°/min, conducted with mixtures of amorphous and crystalline nifedipine showed increases in the intensity of the Bragg peaks obtained from three consecutive readings (data not shown). These results suggest that application of X-ray energy to amorphous samples at lower PXRD scan speed induced drug crystallization within the samples. Other

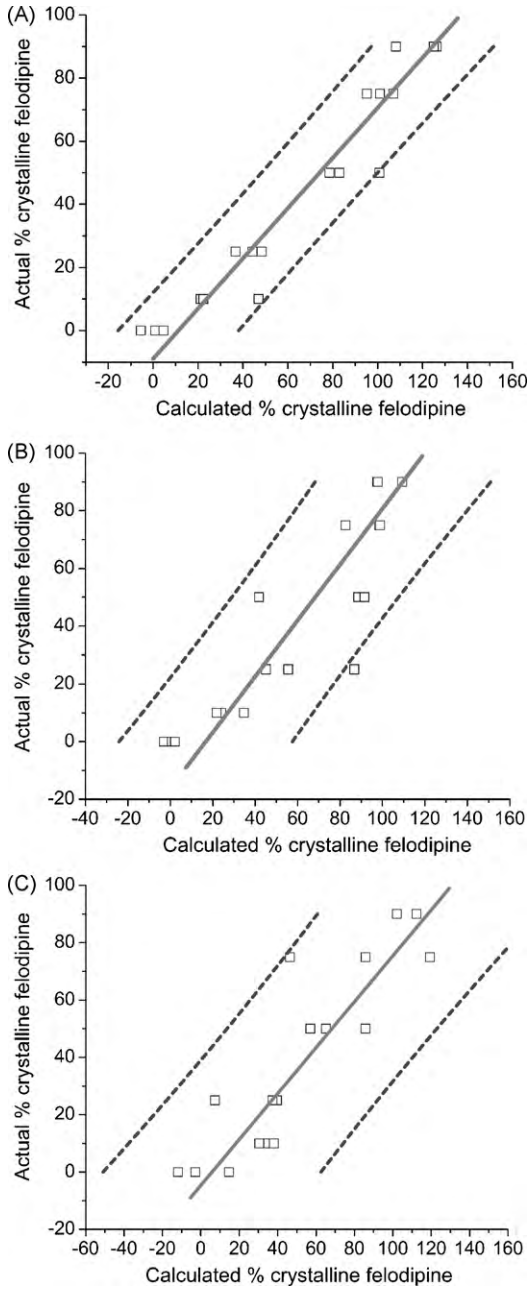

Fig. 2. Actual and predicted % FEL crystallinity in FEL-PVP samples containing (a) 25%, (b) 50%, and (c) 75% PVP (dry weight basis), as calculated from proportional amorphous and crystalline area contributions (see Eq. (1)).

Table 1
Slope and goodness-of-fit results from predicted versus actual values of % FEL crystallinity calculated using the area or PLS methods.

% PVP	Slope (m)		Goodness-of-fit (R^2)		RMSEE	
	Area calculation	PLS model	Area calculation	PLS model	Area calculation	PLS model
25	1.26	0.998	0.93	0.996	11.2	2.2
50	1.03	0.997	0.80	0.997	17.0	1.9
75	1.25	0.984	0.75	0.978	23.6	4.7

Table 2
Correlation values between PXRD diffractograms and % FEL crystallinity (R^2Y) as calculated from the PLS models.

Loading	25% PVP		50% PVP		75% PVP	
	R^2Y	R^2Y cumulative	R^2Y	R^2Y cumulative	R^2Y	R^2Y cumulative
1	0.872	0.872	0.948	0.948	0.511	0.511
2	0.119	0.992	0.0465	0.994	0.448	0.958

factors that may have introduced errors included lack of sample uniformity, and significantly different proportionality constants for the amorphous and the crystalline phases of the samples.

3.3. Partial Least-Squares (PLS) model

In order to improve the prediction of % crystalline FEL present in the samples, a Partial Least-Squares (PLS) model was constructed using SIMCA-P+ v.11 software (Umetrics Inc., Kinnelon, NJ, USA). For the data collected, two PLS components were found sufficient to fit the data without over-fitting. However, examination of the normal probability plots generated by this method showed non-linearity in the data, especially for samples containing 25% and 50% PVP. In order to address this problem, additional models were attempted following log transformation, and an improved model was generated. The summary of the model statistics are shown in Table 2, while the loadings are as shown in Figs. 3–5 for samples containing 25%, 50%, and 75% PVP, respectively.

From Figs. 3–5, it can be seen that the intensities of the Bragg peaks and the amorphous halo were the most important factors in the PLS model for predicting % crystallinity of FEL in the samples, as seen from the loading weights for the first PLS component. This result is in agreement with expectations. The amorphous halo contribution to the loading weights is negative while the contribution of the Bragg peaks is positive, essentially showing that as the fraction of the crystalline FEL in the calibration samples was increased at the same drug-to-polymer ratio, the intensity of the Bragg peaks increased, while the intensity of the amorphous halo decreased. As such, the total diffracted intensity recorded will be preserved, in agreement with Vainshtein's law (Chen et al., 2001).

Analyses of the loading weights for the second PLS components show other factors affecting FEL crystallinity prediction models that were not captured in the loading weights for the first PLS components; these are interpreted as shifts in both the Bragg peaks and the amorphous halo, as well as slight peak broadening of the Bragg peaks. Several potential explanations can be offered to describe the origin of these changes. The first is that the shifts and broadening in Bragg peaks may be caused by experimental inaccuracies. While care has been taken to collect the best quality diffractograms, experimental and sample limitations may have contributed to shifts observed. The second is the presence of strain within the crystal lattice, as well as the presence of small crystallites, which may have been introduced by the cryo-milling process.

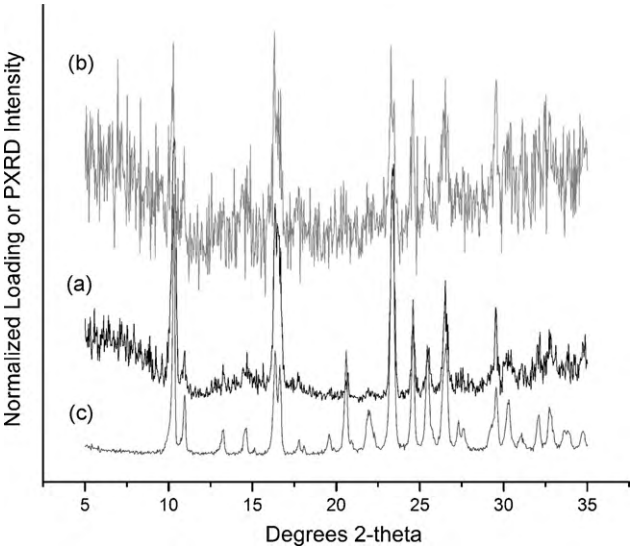

Fig. 4. Loading weights for the first (a) and second (b) PLS components of the model created using data containing 50% PVP. The PXRD diffractogram of crystalline FEL (c) is also included for comparison.

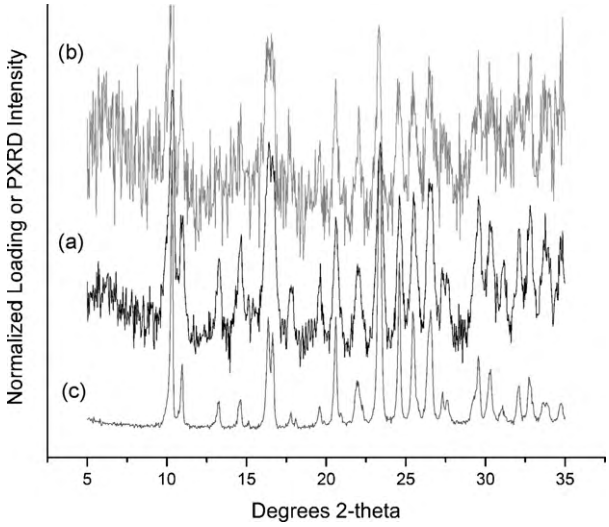

Fig. 3. Loading weights for the first (a) and second (b) PLS components of the model created using data containing 25% PVP. The PXRD diffractogram of crystalline FEL (c) is also included for comparison.

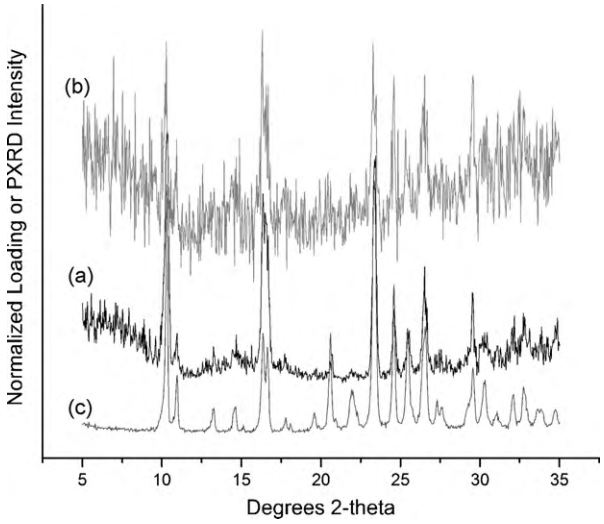

Fig. 5. Loading weights for the first (a) and second (b) PLS components of the model created using data containing 75% PVP. The PXRD diffractogram of crystalline FEL (c) is also included for comparison.

The third is the presence of intimate mixing in the amorphous solid dispersion samples as previously mentioned.

In general, it was found that % FEL crystallinity predictions generated by the PLS models were much better than the predictions made with Eq. (1). This can be seen from Table 1 from the better goodness-of-fit (R^2) and decreased root-mean-squared error of estimation (RMSEE) values for the PLS method compared to the area method. The improved ability to estimate the extent of crystallinity using the PLS method is particularly apparent if results for the dispersion containing 75% PVP are compared. Here the RMSEE value decreases from 23.6% to 4.7% for the area and PLS methods, respectively. This result indicates that the PLS method can estimate a crystallinity level of around 1.2% of the total mass of the dispersion (i.e. 4.7% of the 25% mass of drug present). This level of estimation is comparable with results obtained for crystallinity detection in blends of pure crystalline and amorphous material acquired using a laboratory diffractometer (Surana and Suryanarayanan, 2000). In addition, even when considerably more drug is present in the dispersion, i.e. for the dispersion containing only 25% PVP, the PLS method yields a much improved RMSEE value of 2.2% relative to a value of 11.2% for the area method. The prediction bands of the plots of actual versus predicted values were also much narrower (see Fig. 6). The prediction band for samples containing 75% PVP was larger compared to samples containing 25% and 50% PVP (see Fig. 6c), most likely as a result of lower sensitivity caused by reduced intensity of the Bragg peaks in these samples. However, a significant improvement from Fig. 2c was still observed.

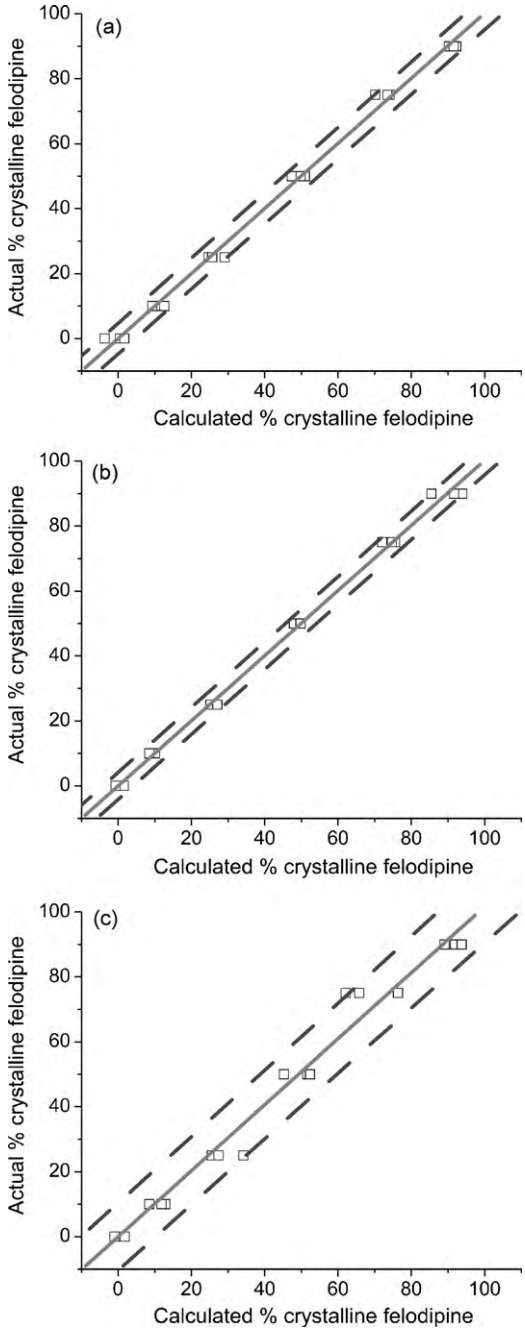

Fig. 6. Actual and predicted % FEL crystallinity in FEL-PVP samples containing (a) 25%, (b) 50%, and (c) 75% PVP (dry weight basis), as calculated from the PLS models.

4. Conclusions

Results obtained show that drug crystallinity in amorphous molecular-level solid dispersions containing felodipine and PVP can be well predicted through the application of PLS modeling, while standard methods involving the calculation of area underneath the Bragg peaks yielded worse calibration models. RMSEE values for the PLS models were 2.2%, 1.9%, and 4.7% drug crystallinity for samples containing 25%, 50%, and 75% amorphous polymer, respectively, as compared to values ranging from 11% to 24% crystallinity for the PXRD area-based method. It is thought that the improved prediction of the PLS method results from subtle variations in the diffractograms arising from factors such as the presence of small crystallites or strains in the crystals, and intimate drug–polymer mixing, which are captured in the loading weights for the second PLS component. Moreover, the PLS method requires less subjective and time intensive data analysis, since it can be performed on the data without the need to separate the contributions from the amorphous halo and the Bragg peaks.

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