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Quantitative analysis of polymorphs in binary and multi-component powder mixtures by near-infrared reflectance spectroscopy

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Abstract

Near-infrared reflectance spectroscopy was employed to quantify polymorphs in binary and multi-component powder mixtures. Sulfamethoxazole (SMZ) forms I and II were used as model polymorphs for this study. The instrument reproducibility, method error, precision, and limits of detection and quantification of the method were assessed. Physical mixtures of the polymorph pair were made by weight, ranging from 0 to 100% SMZ form I in II. Near-infrared spectra of the powder samples contained in glass vials were obtained over the wavelength region of 1100–2500 nm. A calibration plot was constructed by plotting SMZ form I weight percent against a ratio of second derivative values of $\log(1/R')$ (where R' is the relative reflectance) versus wavelength. The coefficients of determination, R^2, were generally greater than 0.9997 and standard errors were low for all the systems. Instrument error was assessed by analyzing a sample 10 times without perturbation. Method error was assessed in the same manner except the sample was re-mixed between analyses. A precision study was conducted by analyzing aliquots from a larger homogeneous sample. Limits of detection (LOD) and quantification (LOQ) were determined from the standard deviation of the response of the blank samples (100% SMZ form II, undiluted or diluted with 60% lactose). These limits were subsequently validated with independent samples. The results show that polymorphs can be quantified in binary and multi-component mixtures in the 2% polymorph composition range. These studies indicate that NIRS is a precise and accurate quantitative tool for determination of polymorphs in the solid-state, is comparable to other characterization techniques, and is more convenient to use than many other methods.

Keywords: Polymorphs; Near-infrared reflectance spectroscopy; Binary and multi-component mixtures; solid-state characterization

1. Introduction

The importance of polymorphism in pharmaceutical systems and its impact on drug physicochemical properties has been extensively reviewed

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(Haleblian and McCrone, 1969; Haleblian, 1975). Processing methods such as milling, compression (Ibrahim et al., 1977), and spray-drying can cause crystal form transitions, and drying processes associated with unit operations may cause desolvation (York, 1983). Incorporation of processed or 'activated' bulk drug substance in dosage forms may lead to crystal form transitions as a result of interaction with excipients or environmental conditions (Takenaka et al., 1980, 1981). In some instances it may be necessary to monitor and quantify solid-state changes in the intact dosage form.

Although a wide variety of physical characterization methods are available to quantify crystal forms in binary mixtures (Guillory and Erb, 1985; Suryanarayanan, 1989; Suryanarayanan and Wiedmann, 1990; Giron, 1995; Findlay and Bugay, 1998; Salari and Young, 1998), very few have been applied to multi-component mixtures. The feasibility of using NMR to quantify polymorphs in the presence of additional formulation components has been demonstrated (Saindon et al., 1993; Rohrs et al., 1999) and X-ray powder diffraction (XRPD) has been used to quantify actives in a formulation (Suryanarayanan and Herman, 1991). However, quantification of crystal forms within a matrix by XRPD remains difficult because overlapping peaks can confound the analysis. The investigation of additional methods of solid-state analysis for use in formulations is therefore of value.

We have previously investigated the use of near-infrared reflectance spectroscopy (NIRS) for quantifying crystal forms of drugs and excipients in binary mixtures (Luner et al., 2000). Accurate and precise quantification over a wide range of composition was demonstrated with four different crystal form pairs, ranging from polymorphs to hydrates. NIRS has been applied successfully to the quantitative analysis of drugs in various dosage forms. These and other applications of NIRS as well as the fundamental aspects of the technique have been reviewed (Honigs, 1985; Ciurczak, 1987; Blanco et al., 1998). Because near-infrared absorbances are 10–1000 times weaker than their fundamental bands in the mid-infrared, absorbances caused by the matrix components do not completely overshadow bands of the analyte(s) and it is possible to extract the relevant chemical information from the spectrum of a multi-component system. The feasibility of using NIRS to quantify polymorphic forms of a drug in a tablet matrix has been reported (Gimet and Luong, 1987). A variety of factors associated with the solid dosage form can influence near-infrared (NIR) spectra (Murray and Williams, 1990; Drennen et al., 1991; Yoon et al., 1998). Because solid dosage forms usually contain a large proportion of excipients, application of any physical characterization method for crystal forms requires that quantification be achieved at a significant level of dilution with excipient(s). As an extension of our previous work with binary mixtures of crystal forms and to gain a more fundamental understanding of dilution on NIRS analysis, we have studied the effect of dilution with additional components on the quantification of binary mixtures of polymorphs. In this paper we compare the quantitative results for binary mixtures of polymorphs to those in the presence of diluents composed of near-infrared absorbers and non-absorbers in powder systems. The objectives of this study were to: (i) assess the impact of dilution on quantification of polymorphs; (ii) compare simple calibration methods in these systems; and (iii) assess the major sources of error in the technique. The study was conducted using NIRS as a primary technique, analogous to how XRPD or NMR would be used for quantitative physical characterization.

2. Materials and methods

2.1. Materials

Sulfamethoxazole (SMZ) forms I and II were used as a model polymorph system. SMZ was obtained from Sigma Chemical Company (St. Louis, MO). Sodium chloride, microcrystalline cellulose (Avicel PH101, FMC BioPolymer, Philadelphia, PA) and spray-dried lactose monohydrate (Foremost, Foremost Farms, Baraboo, WI) were used as diluents and were pharmaceutical grade. Isopropanol (HPLC grade) was used for recrystallizing SMZ.

2.2. Methods

2.2.1. Preparation and characterization of crystal forms

SMZ forms I and II were prepared as previously described (Luner et al., 2000). The polymorphs obtained were characterized using XRPD and thermal analysis. Prior to characterization, all materials were sieved with a sonic sifter (Allen-Bradley, Milwaukee, WI) and a size fraction of 38–106 μm was used. A Siemens Model D5000 X-ray diffractometer with Diffrac^{plus} Eva (Version 2.2, through Bruker AXS Inc., Madison, WI) software was used for the analyses. Diffraction patterns were collected over the range of 10–60° 2θ at a rate of 2° 2θ per minute (40 kV, 30 mA). Differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA) were performed using a Perkin-Elmer DSC-7 and TGA-7, respectively (Perkin-Elmer Corp., Norwalk, CT). The thermal analysis and X-ray diffraction results closely corresponded to data obtained in the literature (Hartauer et al., 1992) and were previously reported (Luner et al., 2000), verifying the identity of the polymorphs. The powder diffraction patterns also matched those reported in the ICDD database (Organic Databook, 1989). Weight loss determined by TGA was less than 0.2% for both polymorphs, indicating only minute amounts of residual solvent or moisture present.

SMZ forms I and II were stable under ambient conditions and did not undergo polymorphic conversion after long term storage as determined by XRPD. It has also been shown that grinding of either form does not produce any change in the DSC thermograms relative to unground samples (Hartauer et al., 1992) and SMZ form I does not undergo polymorphic transition on comminution (Yang and Guillory, 1972; Chan and Doelker, 1985). The sample preparation and the mixing process for NIRS studies were deemed insufficient to induce conversion over the time span of the measurements.

2.2.2. NIRS sample preparation and analysis

Physical mixtures of the polymorph pair were made by weight using a five-place analytical balance (Model AG 245, Mettler-Toledo Inc., Switzerland) over a wide range of composition (0–100% SMZ form I). Samples were weighed directly into glass vials (14 mm diameter × 45 mm length) and weights were kept consistent for each set of samples at approximately 300 mg. Sodium chloride, Avicel, lactose and a 1:1 mixture of Avicel and lactose were used as diluents. They were added step-wise to the binary mixtures resulting in dilutions ranging from 10 to 95%. Diluted samples ranged in weight from 330 to 6000 mg. Near-infrared spectra of the powder samples contained in glass vials were collected using a Foss NIRSystems Model 5000 monochromator equipped with a Rapid Content Sampler™ (Foss NIRSystems, Silver Springs, MD) over the wavelength region of 1100–2500 nm. Each spectrum was collected using 32 co-added scans. Three spectra were collected and subsequently averaged for each sample. The samples were mixed between collection of each spectra for approximately 60 s using a vortex mixer. Spectra were analyzed using Vision™ software (Version 2.11, Foss NIRSystems, Silver Springs, MD).

For data analysis, the second derivative of the response, $\log(1/R')$, (where R' is the ratio of the reflected intensity of the sample to that of a nominally absorbing ceramic reference plate), versus wavelength was calculated using a progressive second order finite difference method with a segment size of 10 and gap size of 0. The second derivative value at a single wavelength was normalized using the second derivative value at a second wavelength (univariate method). Calibration plots were constructed using an inverted least squares regression method where the constituent value, c, is a linear function of the response, R_i, at some wavelength, λ_i, so that,

$$c = K(0) + K(1) \times R_i.$$

In addition, multiple linear regression was performed using the second derivative values at three wavelengths and the constituent value, c, was calculated from

$$c = K(0) + K(1) \times R_i + K(2) \times R_{ii} + K(3) \times R_{iii},$$

where $K(0)$, $K(1)$, $K(2)$ and $K(3)$ are the regression coefficients. A partial least squares regression model with a maximum of four factors was also

used to quantify the constituent value c. NIRS predicted compositions were calculated for each theoretical value and plotted against the theoretical composition using the univariate, MLR or PLS methods. Analytical wavelengths were chosen on the basis of a combination of high correlation coefficient, sensitivity, and accurate validation. In addition, for the univariate method, wavelengths were chosen on the basis of interpretable differences in the spectra. The denominator wavelength was chosen by the software, but constrained to ± 200 nm of the analytical wavelength for most of the systems studied.

2.2.3. Mixing uniformity

A study was conducted to establish whether the procedure used for physically mixing the samples in glass vials achieved mixing uniformity using a qualitative analysis method for comparison of spectra. Correlation value (Clancy, 1988) was used to determine whether there were significant differences among the spectra as a function of mixing. This was done by layering the pre-mixed polymorph pair between the diluent and acquiring spectra after each mixing cycle of 5 s. The spectrum of the sample that had been subjected to the most mixing cycles was used as a reference. The spectrum of the sample at intermediate mixing cycles was compared to it to determine how many cycles were required so that the correlation value was independent of the mixing cycle. SMZ forms I and II and the diluent lactose were used for the mixing study. The correlation values after 3, 4, 5 and 6 mixing cycles were 0.999, 0.999, 1.000 and 1.000. These data indicated that the correlation values of the spectra after 3 mixing cycles did not change appreciably and the sample had been mixed homogeneously. Subsequently, all the samples were mixed for approximately 60 s. As the densities of the polymorphs were similar and the particle size range was controlled, segregation and de-mixing were unlikely to occur.

2.2.4. Limits of detection and quantification

The magnitude of the analytical background response was measured by analyzing blank samples ten times with mixing in between and calculating the standard deviation of the predicted composition. The aim of this study was to estimate LOD and LOQ of SMZ form I in II, thus the blank samples consisted of 100% SMZ form II, either undiluted or diluted with 60% lactose. The predicted composition was calculated using the univariate, MLR or PLS models. The standard deviation of the blank response multiplied by a factor, either 3 or 10, provides an estimate of the LOD and LOQ, respectively. These theoretical limits were validated by the analysis of a suitable number of samples prepared at compositions near these values (US Pharmacopeia XXIV, 2000).

2.2.5. Instrument reproducibility, method error and precision

Instrument reproducibility was determined by assaying one sample ten times without perturbation and calculating the percent relative standard deviation (%RSD). Method error was assessed in the same manner except the samples were remixed in between collecting spectra. Precision was determined by analyzing multiple aliquots of a larger homogeneous sample and calculating the %RSD of the response.

3. Results and discussion

3.1. Spectral and calibration studies

Quantitative analysis of polymorphic forms using spectroscopic methods requires that the spectrum of each polymorphic form be characteristically different and that these differences are retained in the presence of any diluents. The SMZ polymorphs have distinctly unique NIR spectra (Fig. 1). Addition of diluents effectively reduces the magnitude of the absorbances due to the crystal forms. When a non-absorber such as sodium chloride is added, it dilutes the sample and thus the radiation penetrates further into the sample. The diffusely reflected light from the analytes reaching the detector decreases, resulting in a reduction of signal, but this does not affect the shape of the spectra appreciably (Fig. 2). When an absorbing diluent such as lactose is added, it provides a background which is incorporated in the overall spectrum (Fig. 3). This occurs because

the radiation entering the sample is absorbed by both the diluent and the analytes and the remainder is reflected back. The analyte peaks may be attenuated, overlapped or obscured by the diluent spectrum and hence the peaks are not as clearly resolvable. Nevertheless, the underlying features affecting the shape of the spectrum of each polymorph are retained in both cases. The differences are more pronounced in the second derivative spectra (Fig. 4). A second derivative transformation was applied to the reflectance data because it resolves broad, overlapping peaks, and normalizes the baseline shifts (Kirsch and Drennen, 1995). For the univariate linear regression analysis, the ratio of the second derivative response at two

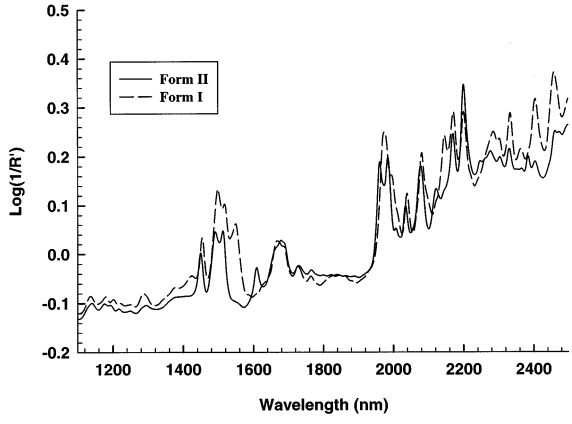

Fig. 1. Near-infrared spectra of SMZ form I and II.

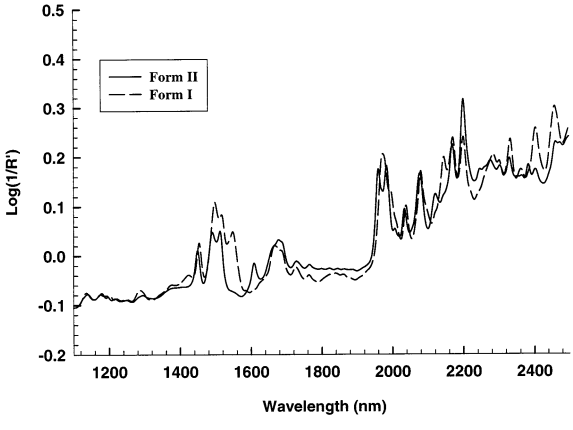

Fig. 2. Near-infrared spectra of SMZ form I and II diluted with 50% sodium chloride.

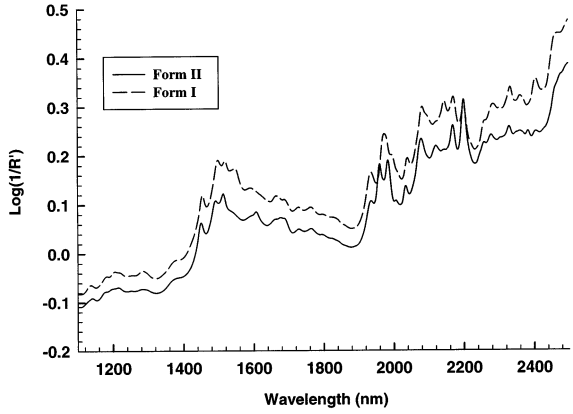

Fig. 3. Near-infrared spectra of SMZ form I and II diluted with 60% lactose.

wavelengths can reduce the effect of pathlength differences caused by variations in particle size and packing (Honigs, 1985).

The first objective was to confirm the ability of NIRS to quantify polymorphs in the presence of a diluent. To study this, binary mixtures of two polymorphs were first made over the 0–100% composition range and calibration models developed based on those spectra. Subsequently, those same binary mixtures were diluted with either a NIR non-absorbing powder (sodium chloride) or a NIR absorbing powder (Avicel, lactose, or a 1:1 mixture of both) in a step-wise fashion achieving dilutions ranging from 10 to 95%. Spectra were obtained at each dilution level and calibration models developed. The regression statistics (coefficient of determination, R^2, and standard error of calibration, SEC) for the binary mixtures were then compared to those of the diluted systems. The numerator wavelength was held constant at 1608 nm. However, the denominator, or normalizing wavelength was allowed to 'float' for the different dilution levels. There was no systematic relationship between the normalizing wavelength and the dilution levels. A unique band at 1610 nm is seen for form II, which results from a N–H moiety being strongly hydrogen bonded in form II (Luner et al., 2000). Univariate calibrations using this band provided very good results. It is not surprising that the denominator wavelength changes considering that the effective pathlength

and scattering properties of the powder bed are altered by the presence of the absorbing matrix. Wavelengths chosen were also selected on the basis of the best R^2 and SEC values.

For the MLR method, wavelengths at 1668, 1304, and 1414 nm were used to quantify binary mixtures. These wavelengths correspond to regions where the first overtone C–H stretch, first overtone C–H combinations, and the first overtone N–H stretch occur, respectively. Although water absorbs around 1414 nm, the polymorphs demonstrated no significant weight loss by TGA implying no interference from water on the calibration model. These wavelengths are in regions where there are distinct differences in the reflectance spectra of the polymorphs. With these same wavelengths it was possible to perform calibration and validation with the mixtures diluted with 60% lactose. However, wavelengths at 1302, 1726, and 1970 nm provided better results. The first two wavelengths correspond to the C–H vibrations observed for the binary system. The band at 1970 nm corresponds to the combination N–H stretch/NH_2 bending region (Workman, 1996) which is solely due to the polymorphs. Addition of the diluent (lactose) to the binary mixtures may interfere with the C–H bands of the polymorphs due to its own absorbance in the C–H region. Consequently, the calibration wavelengths needed for optimal minimization of the overall error are different for the ternary system. The inclusion of wavelengths corresponding to the diluent in the calibration model may serve to normalize its affect on the spectrum.

The binary mixtures had the lowest SEC values (Table 1). The regression statistics (not shown) were not greatly influenced by increasing dilution with either a NIR non-absorber or a NIR absorber. It was possible to obtain high coefficients of determination and relatively low SEC values with each of the calibration models at the highest dilution levels (Table 1). It is interesting to note that the presence of an absorbing diluent does not have a more substantial effect on the resultant calibration than a non-absorbing diluent (NaCl). This may be due to the reasons stated earlier in the discussion regarding the features of the analyte being superimposed on the background provided by the diluent. The results indicate that NIRS is sensitive to the polymorphic forms and the diluents do not ameliorate the chemical information needed to distinguish the two polymorphs. A comparison of the results for the undiluted and diluted systems using the univariate calibration model are shown (Fig. 5). These results indicate that it is possible to rationally select a wavelength or region that corresponds to an intrinsic difference in the two polymorphs that relates to their crystalline structures even with the interfering effects of a NIR absorber.

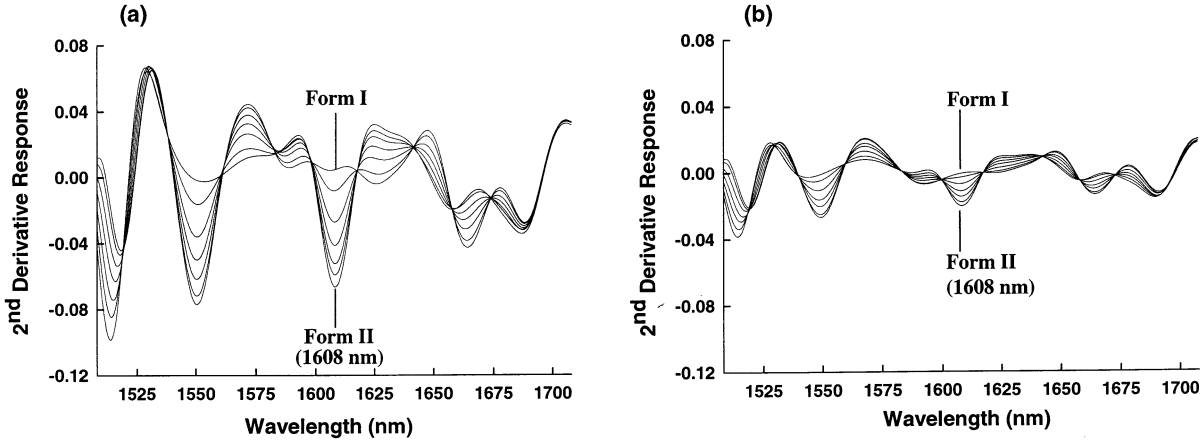

Fig. 4. The second derivative spectra of SMZ form I and II: (a) binary mixture; (b) diluted with 60% lactose. Each spectrum represents decreasing percent form I in II from top to bottom at the indicated wavelength.

Table 1
Near-infrared calibration results for SMZ form I and II in binary and multi-component mixtures

Composition	Univariate		MLR[a]		PLS[b]	
	R^2	SEC[c]	R^2	SEC	R^2	SEC
Binary mixture	0.9999	0.48	1.0000	0.12	1.0000	0.19
Binary mixture diluted with:						
95% Sodium chloride	0.9998	0.54	1.0000	0.14	0.9999	0.52
90% Avicel	0.9959	2.70	0.9974	2.81	0.9991	2.02
90% Lactose	0.9997	0.79	0.9999	0.43	1.0000	0.22
80% Avicel:Lactose (1:1)	0.9999	0.31	0.9999	0.67	0.9999	0.71

[a] Multiple linear regression with three wavelengths.
[b] Partial least squares regression with four factors.
[c] Standard error of the calibration (as % polymorph composition).

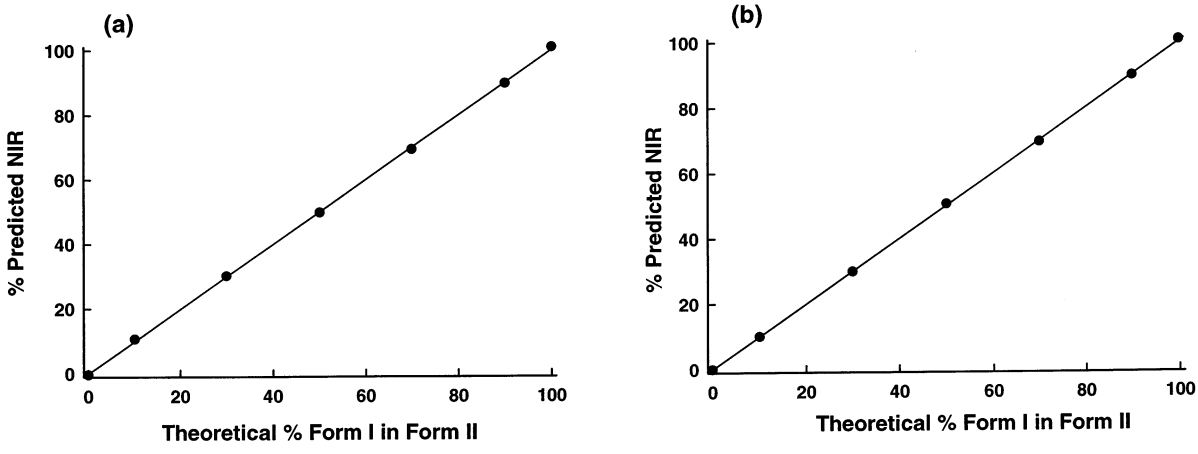

Fig. 5. Percent theoretical versus percent predicted by NIRS for SMZ form I in II: (a) binary mixture ($R^2 = 0.9998$, SEC = 0.63 at 1608/1784 nm); (b) diluted with 60% lactose ($R^2 = 0.9999$, SEC = 0.41 at 1608/2060 nm). Solid lines represent the data fit with a linear regression model.

3.2. Validation studies

To further test the predictive ability of the models, independent samples were prepared over the whole composition range of crystal form (diluted and undiluted) and used as validation samples (Tables 2 and 3). The SMZ binary system validated well and low absolute errors were obtained. Comparison of these binary system results to those in Table 3 where these same binary mixtures were diluted with 60% lactose shows that the presence of the diluent does not have a large effect on the ability to quantify the constituents. Although there is some deterioration in performance due to dilution, noted as an increase in absolute error, the results are very good for a solid-state method, especially considering the extreme range of composition studied. The largest increase in error was incurred with the PLS model.

The standard error of the calibration (SEC) and standard error of the prediction (SEP) are used as estimators of the accuracy of NIRs methods (Honigs, 1985). The SEC statistic is the standard deviation for the residuals due to differences between the actual and the predicted values for samples within the calibration set. The SEP is similar to the SEC except that it is applied to the

samples within the validation set (Workman, 1992). Thus, the SEP gives a better indication of the accuracy of the NIRs analysis for unknown samples. Close correspondence of the SEC and SEP indicates good predictive ability of the calibration model. The SECs and SEPs for the undiluted and diluted (60% lactose) polymorph pair were compared using three different calibration methods (Table 4). In all instances the SEPs are relatively low and comparable to the correspond-

Table 2
Validation results for binary mixtures of SMZ form I and II

SMZ form I actual %	Univariate		MLR		PLS	
	1608/1784 nm		1668, 1304, 1414 nm		2 factors	
	Predicted	Absolute error	Predicted	Absolute error	Predicted	Absolute error
0	0.56	0.56	0.18	0.18	−1.53	−1.53
30	30.00	0.00	29.57	−0.43	31.29	1.29
50	49.97	−0.03	50.14	0.14	52.98	2.98
70	68.73	−1.27	68.00	−2.00	69.35	−0.65
100	100.96	0.96	97.86	−2.14	100.26	0.26

Table 3
Validation results for mixtures of SMZ form I and II in the presence of 60% lactose

SMZ form I actual % (form I relative to form II)	Univariate		MLR		PLS	
	1608/2060 nm		1302, 1726, 1970 nm		3 factors	
	Predicted	Absolute error	Predicted	Absolute error	Predicted	Absolute error
0	0.40	0.40	−1.32	−1.32	−3.52	−3.52
30	30.07	0.07	28.80	−1.20	28.33	−1.67
50	49.97	−0.03	49.88	−0.12	46.35	−3.65
70	69.59	−0.41	70.06	0.06	68.32	−1.68
100	100.51	0.51	103.65	3.65	104.71	4.71

Table 4
Standard error of calibrations and predictions for binary and multi-component mixtures

SMZ form I in II	Univariate		MLR[a]		PLS[b]	
	SEC[c]	SEP[d]	SEC	SEP	SEC	SEP
Binary mixture[e]	0.63	0.75	0.05	1.31	1.17	1.63
With 60% Lactose[f]	0.41	0.34	0.57	1.82	0.59	3.27

[a] Multiple linear regression with three wavelengths.
[b] Partial least squares regression with maximum four factors.
[c] Standard error of the calibration (as % polymorph composition).
[d] Standard error of the prediction (as % polymorph composition).
[e] Calibration parameters same as in Table 2.
[f] Calibration parameters same as in Table 3.

Table 5
Validation results for calculated LOD and LOQ of SMZ form I in binary and multi-component mixtures

Binary mixture results (calculated LOD = 0.3%, LOQ = 1%)				Ternary mixture results (calculated LOD = 5.9%, LOQ = 19.6%)			
Actual % form I	Mean predicted	S.D.	%RSD	Actual % form I[a]	Mean predicted	S.D.	%RSD
1.0	1.69	0.175	10.3	0.4	2.65	0.340	12.8
2.0	2.44	0.167	6.8	0.8	3.21	0.392	12.2
5.0	5.28	0.096	1.8	2.0	4.19	0.330	7.9
10.0	9.45	0.389	4.1	4.0	5.75	0.263	4.6
				6.2	6.63	0.346	5.2

[a] Relative to total sample weight.

ing SECs. However, the difference between the SECs and SEPs is less in case of univariate models as compared to MLR or PLS models. Moreover, the SEPs obtained with the univariate calibration method are lower than those obtained using MLR or PLS for all systems studied. Because these systems were uncomplicated (i.e., binary and ternary powder mixtures), matrix effects may not be prevalent. Consequently, co-linearity and non-linearity in the data may be minimal and the simplest modeling procedures (univariate and sometimes MLR) are adequate to explain a greater amount of variation in the data.

3.3. Limits of detection and quantification

The univariate models were used for the calculation of limits of detection and quantification as they provided better results than MLR and PLS models. LOD and LOQ for SMZ form I (in the presence of form II) were calculated using the 1608/1784 nm calibration model. The calculated LOD and LOQ for the binary mixture were 0.3 and 1%, respectively. Several samples were prepared near the LOQ and subsequently analyzed. It can be concluded from these results (Table 5) that the LOQ lies between 2 to 5% on the basis of the 0–100% calibration range for the binary system. The calculated values of LOD and LOQ for SMZ form I in the presence of form II diluted with 60% lactose were 5.9 and 19.6%, respectively, using the 1608/2060 nm calibration model. These calculated limits were unusually high considering the samples at lower levels calibrated well. Additional samples were analyzed and the results (Table 5) show that the practical LOQ is between 2 to 4% for the multi-component mixtures on the basis of a %RSD limit in the 5% region. A consistent over estimation was observed for these results. This is because the calibration model was developed for a wide range of composition (0–100%) and the validation of LOQ dealt with the lower end of the calibration model. This type of bias was not seen in the validation results of ternary mixtures (Table 3) at higher composition levels.

The cause of the discrepancy between the calculated and practical limits for the multi-component system is not completely understood but may reflect the presence of matrix effects that are not adequately normalized using the univariate method. The traditional approach to calculation of LOD and LOQ on the basis of detection of signal over noise may not be appropriate for the ternary systems because there is always signal generated by the placebo (diluent and the single polymorph) and the presence of the analyte (the other polymorph) also alters the characteristic response of the placebo. Additionally, the limits have been calculated using an extremely broad range calibration and it is likely that limits calculated using a smaller range would produce more realistic results. Overall, the practically determined limits verify that NIRS can be used to determine small quantities of polymorphs in sample matrices with acceptable accuracy and precision. Moreover, the limits are comparable to other solid-state characterization methods for binary systems.

3.4. Instrument reproducibility, method error and precision

To assess the potential primary sources of error related to dilution inherent to the method, studies of the instrument reproducibility, method error and method precision were conducted. Instrument reproducibility and method error were evaluated using samples from the validation sets containing 30% or 50% of one polymorph form in the other, either as a binary mixture or with 60% lactose. The %RSD for instrument reproducibility was less than 0.5% in all systems except for MLR with the ternary system (Table 6). Comparison of the instrument reproducibility to the method error gives an indication of the error introduced by mixing, sample orientation, and packing effects (Table 7). As expected, method errors were larger overall and ranged from approximately 0.5–1.4% as calculated using the univariate method. For the binary systems there was only a small increase in the %RSD for method error determined with the univariate and MLR calibration models. This indicates that variation due to sample presentation

Table 6
Instrument reproducibility results for SMZ form I and II in binary and multi-component mixtures

System	Polymorph composition	Predicted % polymorph composition					
		Univariate		MLR[a]		PLS[b]	
		Mean (S.D.)	%RSD	Mean (S.D.)	%RSD	Mean (S.D.)	%RSD
Binary[c]	30% form I	30.09 (0.12)	0.40	29.61 (0.13)	0.43	31.91 (0.06)	0.17
	50% form I	49.14 (0.07)	0.13	49.72 (0.07)	0.15	51.36 (0.05)	0.09
With 60% Lactose[d]	30% form I	30.96 (0.15)	0.50	29.66 (0.38)	1.30	24.68 (0.06)	0.23
	50% form I	49.92 (0.04)	0.08	49.96 (0.45)	0.92	48.58 (0.05)	0.11

[a] Multiple linear regression with three wavelengths.
[b] Partial Least squares regression with maximum four factors.
[c] Calibration parameters same as in Table 2.
[d] Calibration parameters same as in Table 3.

Table 7
Method error results for SMZ form I and II in binary and multi-component mixtures

System	Polymorph composition	Predicted % polymorph composition					
		Univariate		MLR[a]		PLS[b]	
		Mean (S.D.)	%RSD	Mean (S.D.)	%RSD	Mean (S.D.)	%RSD
Binary[c]	30% form I	30.30 (0.22)	0.72	29.65 (0.29)	0.98	33.31 (0.68)	2.05
	50% form I	49.49 (0.42)	0.86	50.39 (0.66)	1.31	54.65 (1.55)	2.83
With 60% Lactose[d]	30% form I	29.92 (0.42)	1.39	27.36 (0.39)	1.43	25.37 (1.22)	4.82
	50% form I	50.04 (0.28)	0.56	49.69 (0.45)	0.92	45.54 (0.43)	0.96

[a] Multiple linear regression with three wavelengths.
[b] Partial least squares regression with maximum four factors.
[c] Calibration parameters same as in Table 2.
[d] Calibration parameters same as in Table 3.

effects in the binary mixtures is relatively small. However, in the presence of the diluent there is an appreciable change in the method error values, albeit, still within the range of acceptable error. The presence of the diluent adds a component of variability due to mixing, packing, and other particulate effects associated with scattering.

In the assay of powders, a major potential source of error is sampling variability (Bugay et al., 1996). To estimate the effect of sampling variability a precision study was conducted using five aliquots obtained from a larger homogeneous sample of the binary and multi-component mixtures and analyzed. The %RSDs calculated by the univariate method were 1.67% for both the binary and multi-component mixtures. These values are in the same range as those for method error. It is important to understand the relationships between each of the analytical performance parameters to assess the total error of the method. Error associated with instrument reproducibility is incorporated in the method error. Similarly, method error contributed to error in the precision study. The slightly higher values for the precision error indicate a small contribution of sampling error. In some cases sampling error could be considerably greater and may be the major contribution to error. The relative magnitude of the errors (using univariate method) were consistent with the relationship among the three sources of error evaluated. On the basis of this study, it is reasonable to expect a minimum overall error (independent of sampling, day-to-day, and operator error) in the range of 1–2% for polymorph quantification in binary or multi-component systems using the sample-in-vial method and this detector configuration, provided samples are in a suitable region of the calibration range. Additional optimization of sample presentation (Yoon et al., 1998) and minimization of other sources of variability (Borer et al., 1998) may potentially result in increasing sensitivity and reducing error. NIRs is amenable to a variety of sampling modes, some of which allow for greater sampling volume permitting a better representation of the whole sample and have been used in high-precision assays (Plugge and Van Der Vlies, 1996).

4. Conclusions

A quantitative diffuse reflectance near-infrared method was investigated as a primary technique to determine polymorphs in binary and multi-component powder mixtures. It was possible to develop univariate calibrations based on bands unique to the two polymorphs and attributable to fundamental differences in their molecular arrangement in the crystals. In addition, these bands were unrelated to those of the diluents and provided for excellent calibration and validation. Calibration methods using MLR and PLS were also successful, however, not with the same level of interpretation. Evaluation of the errors related to the NIRS technique show that method error is the major source of variability, however, overall error is in the range of 1–2% for the binary and multi-component systems. The validated limits of detection and quantification demonstrate that NIRS can be used to determine polymorphs in binary and multi-component mixtures in the range of 2–5% polymorph composition even at significant levels of dilution. The studies conducted show that NIRS has significant potential for use as a non-destructive, rapid, precise and accurate quantitative solid-state characterization method in formulations. Relatively low error was incurred using NIRS, and quantitatively it compares favorably to other solid-state characterization methods.

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