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Quantification of polymorphic impurity in an enantiotropic polymorph system using differential scanning calorimetry, X-ray powder diffraction and Raman spectroscopy

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ABSTRACT

The ability to detect and quantify polymorphism of pharmaceuticals is critically important in ensuring that the formulated product delivers the desired therapeutic properties because different polymorphic forms of a drug exhibit different solubilities, stabilities and bioavailabilities. The purpose of this study is to develop an effective method for quantitative analysis of a small amount of one polymorph within a binary polymorphic mixture. Sulfamerazine (SMZ), an antibacterial drug, was chosen as the model compound. The effectiveness and accuracy of powder X-ray diffraction (PXRD), Raman microscopy and differential scanning calorimetry (DSC) for the quantification of SMZ polymorphs were studied and compared. Low heating rate in DSC allowed complete transformation from Form I to Form II to take place, resulting in a highly linear calibration curve. Our results showed that DSC and PXRD are capable in providing accurate measurement of polymorphic content in the SMZ binary mixtures while Raman is the least accurate technique for the system studied. DSC provides a rapid and accurate method for offline quantification of SMZ polymorphs, and PXRD provides a non-destructive, non-contact analysis.

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1. Introduction

Polymorphism is an important concern in the pharmaceutical industry since different polymorphs of the same compound exhibit different physicochemical properties, such as density, habit, color, refractive index, solubility, dissolution rate, melting point, and mechanical properties, thereby influencing the stability and bioavailability of the formulated drug product. It is necessary to ensure that only the polymorph specified in the regulatory filing is present in a formulation so that the properties of the formulated product are not compromised by the unintended presence of the other polymorphic forms. In pharmaceutical manufacture, polymorphic impurities, i.e. the undesired polymorph(s), can be introduced as a result of incomplete conversion of the metastable to stable form during crystallisation in primary manufacture or during unit operations in secondary manufacture, e.g. milling and granulation.

A variety of techniques have been employed to characterize the solid forms of pharmaceuticals, including X-ray diffraction (XRD), thermal analysis (differential scanning calorimetry), solid state nuclear magnetic resonance (NMR) spectroscopy, optical microscopy and vibrational spectroscopy (including infrared (IR), Raman) (Brittain, 1999). Powder X-ray diffraction (PXRD) has always been the definitive test for the identification of polymorphs. However, its use for quantitative analysis is often marred by preferred orientation effects and the potential difficulty in separating diffraction peaks of a single component (Shah et al., 2006). Vibrational spectroscopic methods as alternatives to PXRD are useful in obtaining both structural information and quantitative polymorphic information (Stephenson et al., 2001). Raman spectroscopy, in particular, is potentially more powerful for quantitative analysis compared to IR because of its smaller bandwidths (Stephenson et al., 2001). Univariate calibration, i.e. the use of areas or heights of spectral bands or diffraction peaks, sometimes sufficed in providing satisfactorily accurate and precise quantification of polymorph composition (Langkilde et al., 1997; Roberts et al., 2002a,b; Tiwari et al., 2007; Varasteh et al., 2009). Often due to overlapping peaks or peak shifting, it is necessary to use multivariate analysis such as partial least square (PLS) regression and principal component regression (PCR) to improve the accuracy and robustness of the calibration model (Chieng et al., 2009; Heinz et al., 2007; Német et al., 2009; Pratiwi et al., 2002; Strachan et al., 2004).

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Despite the prevalent use of DSC for polymorphism studies, its use has been largely qualitative. Reports on polymorphic quantification using DSC are scarce. Tong et al. (2003, 2005) quantify trace levels of polymorphic impurity in salmeterol xinofoate powders using DSC. Their approach involved modeling the crystallization kinetics of both tested and reference drug materials in melts using a modified Avrami-Erofe'ev (AE) rate expression (Tong et al., 2003, 2005). Unfortunately the need for the modeling of crystallization kinetics and the empirical nature of the modified AE expression make this approach inconvenient and complicated for general applications. Recently McGregor and coworkers (McGregor and Bines, 2008; McGregor et al., 2004) made use of high-speed differential scanning calorimetry (Hyper-DSC™) to quantify polymorphs of carbamazepine and a Merck development compound. The common problem of concurrent recrystallization to an alternative crystal form and subsequent melting of the new form during heating at standard heating rates was overcome by using accelerated heating rates available by the Hyper-DSC. At such high heating rates, recrystallization was fully inhibited and the melting endotherms for the metastable form could be obtained. However, they were unable to achieve accurate quantification of low levels of polymorphic impurities due to partial recrystallization enabled by crystal seeding. Nevertheless, there is still potential for using DSC for accurate quantification of polymorphs. Therefore, in this study, we explore the use of DSC for the quantification of polymorphic impurity in binary polymorph mixtures and compare against PXRD and Raman spectroscopy. As far as the authors are aware, there are no previous reports that compare the performance of the three techniques for such an application.

The model compound in this study is sulfamerazine, a widely used sulfonamide antibacterial drug. Sulfamerazine has three polymorphs (Acharya et al., 1982; Caria and Mohamed, 1992; Hossain, 2006) and Form III was only found recently in 2006 (Hossain, 2006). Form I is the commercially available form. Form I and Form II are enantiotropic (Gu et al., 2001, 2002; Zhang et al., 2002) and the binary mixtures of these two forms are used in this study.

2. Materials and methods

2.1. Materials and polymorph preparation

SMZ (4-amino-N-[4-methyl-2-pyrimidinyl] benzenesulfonamide) was purchased from Sigma Co. (purity >99.9%). The raw material was Form I. Form II was prepared by suspending Form I in acetonitrile–water (80:20, v/v) for 3 days at room temperature (Gong et al., 2008). Since the raw material of SMZ bought from Sigma was in the form of fine powder, bigger and more well-defined crystals of Form I were prepared by dissolving 0.5 g of raw material at 40 °C in 20 g acetonitrile/water (80:20, v/v) followed by fast cooling to room temperature in 2 min. All solvents used, including water, were HPLC grade. Solvent mixtures were prepared by mixing two solvents of predetermined volume.

All binary mixtures of polymorphs were created by mixing the pure dry samples. As it is known that particle size affect PXRD and Raman measurements (Tiwari et al., 2007; Wang et al., 2002), the particle size of the samples used here was controlled to a small range. Pure Form I and Form II were ground separately and sieved to particle size between 38 μm and 45 μm (passed through B.S.S #350 and collected on B.S.S #440). Polymorphic purities of the ground and sieved Form I and Form II samples were verified by PXRD and Raman analysis (see Section 3.1). For PXRD and Raman analysis, mixed polymorph samples were prepared by mixing Form I and Form II manually for over 10 min. For DSC analysis, the two forms were individually weighed into the DSC pans.

2.2. Microscope

The morphologies of crystals were analyzed using an Olympus BX51 polarized light microscope.

2.3. Powder X-ray diffractometry (PXRD)

PXRD patterns were obtained using a Bruker D8-ADVANCE X-ray diffractometer (CoKα radiation). The voltage and current applied were 40 kV and 40 mA respectively. The scan range was from 5° to 35° 2θ at a scanning rate of 0.0167°/s. To prevent mixing inhomogeneity, sample rotation was used during X-ray spectrometric measurement. Each sample was repacked and reanalyzed by PXRD for five times.

2.4. Raman spectroscopy

Raman spectra were acquired using a Raman microscope (J.Y. Horiba LabRam HR UV–Visible–NIR) equipped with VIS, NIR, and a high stability BX41 Olympus microscope. The Raman scattering was excited with a 785 nm near infrared diode laser and a 50× objective lens was used to collect the backscattered light. Silicon was used as reference standard to monitor wavenumber accuracy. For the characterization of pure polymorph samples, a total of two scans were averaged for each sample over a range of 1800 cm^{-1} to 50 cm^{-1} at a resolution of 1 cm^{-1}. For mixed polymorph samples, Raman point-by-point mapping techniques were used instead. An area of 152 μm × 228 μm of the sample was imaged with step numbers of 15 and 25 in the *x* and *y* directions respectively. The imaging was performed by acquiring Raman spectrum at each pixel scanned within the area imaged. Scans were collected using a static 600 groove/mm dispersive grating in a spectral window from 100 to 1200 cm^{-1}, and acquisition time was 30 s with two scans.

2.5. Differential scanning calorimetry (DSC)

DSC measurements were performed using a PerkinElmer diamond hyper-DSC (PerkinElmer, Beaconsfield, UK). The instrument was calibrated for temperature and heat flow using indium as standards. The samples (2–5 mg) were placed in sealed aluminum pans under nitrogen purge at flow rate of 20 ml/min. The samples were heated from 40 °C at a heating rate ranging from 2 to 100 °C/min. As the melting temperature of Form I increased with increasing heating rates due to thermal lag effect (Vanden Poel and Mathot, 2006), the final heating temperature (240–255 °C) was adjusted depending on the heating rate in order to capture the whole melting event. Data acquisition and analysis were performed using the Pyris software. For enthalpy calculation, the start and end points for the integration of the thermal peak were identified by visual inspection.

2.6. Multivariate analysis

Partial least square (PLS) regression was used to build calibration models from PXRD and Raman spectra collected for SMZ polymorph mixtures at different polymorphic contents. The data was randomly divided into calibration set to build a quantification model and a prediction set to validate the model. Instead of using peak height or area of selected peaks, the PLS regression was performed over a spectral range selected that showed the biggest difference between Form I and Form II. The quality of the model was evaluated based on the correlation coefficient (R^2), test set validation coefficient (Q^2), root mean square error of calibration (RMSEC), and root mean square error of prediction (RMSEP). All computations were performed using MATLAB 7.9.0 and PLS_Toolbox 5.8.2 developed by Eigenvector Research Inc. (Manson, WA).

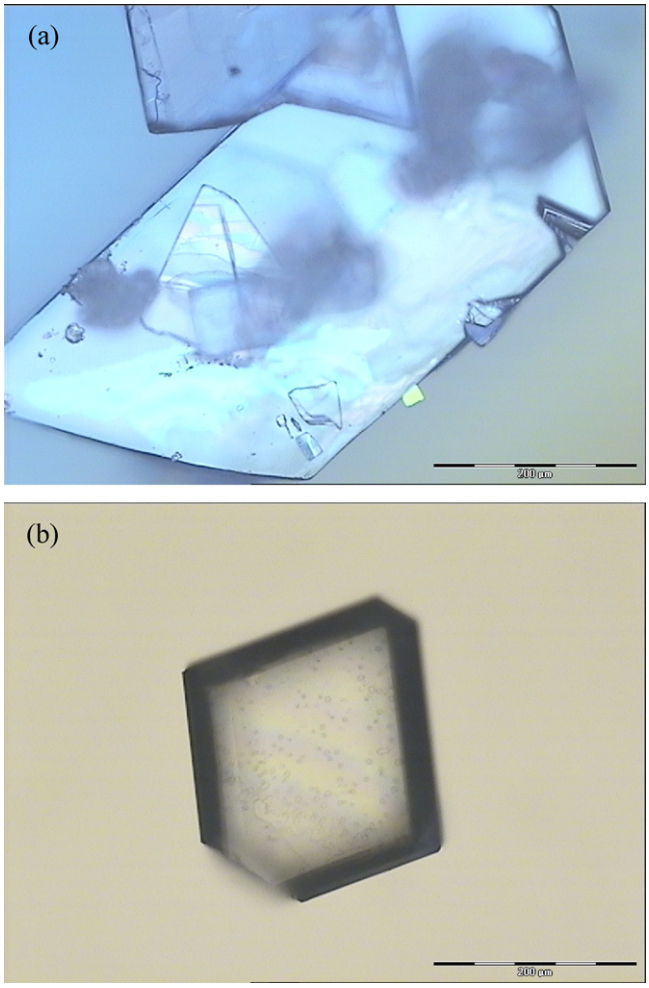

Fig. 1. Microscopic image of SMZ (a) Form I and (b) Form II.

3. Results and discussions

3.1. Characterization of SMZ polymorphs

Pictures of SMZ Form I and Form II crystals are shown in Fig. 1. Form I crystals are plate-like while Form II are prismatic in shape.

Fig. 2 compared our experimental PXRD patterns with the simulated patterns. The experimental patterns agreed well with the respective simulated patterns for Form I (refcode SLFNMA02) (Caria and Mohamed, 1992) and Form II (refcode SLFNMA01) (Acharya et al., 1982). Our PXRD characterization therefore showed that the Form I and Form II samples we prepared were pure and did not contain any detectable amount of the other polymorph.

Raman spectra of SMZ Form I and Form II are shown in Fig. 3 and they agreed well with the literature results (Cao et al., 2005). Form I and Form II showed very similar scattering throughout the frequency range scanned but could still be clearly differentiated from each other. Form I exhibited split peaks at 363 cm^{-1} and 380 cm^{-1} while Form II showed a single peak at 371 cm^{-1}. These peaks are attributed to the SO_2 scissoring vibration (Socrates, 2001). Similar difference was observed in the NH_2 scissoring vibrations (Woolfenden, 1977). Peak splitting was observed at 1628 cm^{-1} and 1641 cm^{-1} for Form I while a single peak was observed at 1647 cm^{-1} for Form II. Cao et al. (2005) attributed the peak splitting observed for Form I to the lower symmetry of its crystal structure. In addition, the polymorphs displayed different SO_2 symmetric stretching vibrations around 1150 cm^{-1}. Form I exhibited a peak at 1151 cm^{-1} while Form II showed a peak at 1144 cm^{-1}. Our Raman characterization therefore showed that the Form I and Form II samples we prepared were pure and did not contain any detectable amount of the other components.

The DSC thermograms for SMZ Form I and Form II at a heating rate of 10 °C/min are shown in Fig. 4. For SMZ Form I, only a single endothermic melting peak with an onset of 237.2 °C and an enthalpy of 149.5 J/g was observed at this heating rate. This single melting peak indicated the presence of only one polymorph and there were no polymorphic impurities within the sample and no phase transformation during heating. For SMZ Form II, an endothermic transition with an onset temperature of 167.7 °C with an enthalpy 10.04 J/g was observed first. This endothermic transition was due to the enantiotropic transformation from Form II to Form I (Caria and Mohamed, 1992; Zhang et al., 2002). A small but sharp endotherm with onset at 228 °C and an enthalpy of 0.2273 J/g then followed. This small endothermic was attributed to the melting of residual polymorph II that failed to convert Form I at lower temperatures (Zhang et al., 2002). A large endotherm due to melting of Form I was finally observed with an onset of 236.7 °C and an enthalpy 151.8 J/g. It has to be noted that no thermal degradation was observed in the DSC thermograms for all the samples within the temperature range scanned and at all the heating rates. This was because prior to DSC experiments, thermogravimetric analysis (TGA) (SDT2960 simultaneous DSC-TGA, TA instruments Co. Ltd.) was performed to identify the temperature at which the sample started to decompose. The upper limit of the DSC experiments at that particular heating rate was then set to below the decomposition onset temperature measured by TGA to avoid degradation occurring during DSC experiments.

McGregor and Bines (2008) have shown that at high enough heating rate the kinetics of melting transition of a low melting polymorph of a pharmaceutical compound could be altered such that concurrent exothermic recrystallization to the higher melting form was inhibited. This then allowed for direct measurement of the enthalpy of fusion of the lower melting polymorph and enabled the quantification of the polymorphic content in mixtures of the two polymorphs. The accuracy of this method is however hampered by crystal seeding and recrystallization of the higher melting polymorph from the melt due to the presence of that polymorph in the mixture prior to analysis. Therefore, in this work, we investigate the effect of heating rates on the thermal response of SMZ Form II and explore if an alternative method could be developed for more accurate quantification of polymorphic content. DSC measurements of Form II were performed at heating rates of 2, 5, 10, 40 and 100 °C/min. The resulting thermograms are shown in Fig. 5 and the data are summarized in Table 1. As heating rate decreased, the enthalpy of endothermic transition increased while the enthalpy of fusion for Form II decreased. The endothermic transition peak could clearly be identified at all the heating rates studied here which suggest that a heating rate higher than 100 °C/min would be required for complete inhibition of phase transformation. On the contrary, Form II melting peak became diminished as the heating rate decreased. Form II melting endotherm at 5 °C/min was so small that it could only be observed in the enlarged diagram in Fig. 5(b). Eventually Form II melting endotherm completely disappeared at heating rate of 2 °C/min (Fig. 5(c)). This suggests that the lower the heating rate, the more time Form II has to transform to Form I. This also explains the increase in Form I enthalpy of fusion with decreasing heating rate as more Form II was transformed to Form I during heating. Since complete transformation of Form II to Form I occurred at 2 °C/min, the heat of transformation at that heating rate could be suitable for the characterization of Form II content in mixtures of Forms I and II.

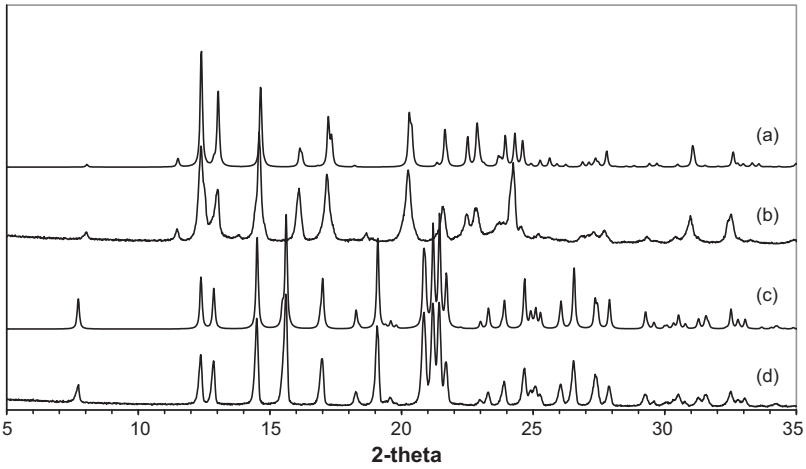

Fig. 2. PXRD patterns of SMZ Form I and Form II. (a) Simulated pattern of Form I, (b) experimental pattern of Form I, (c) simulated pattern of Form II, and (d) experimental pattern of Form II.

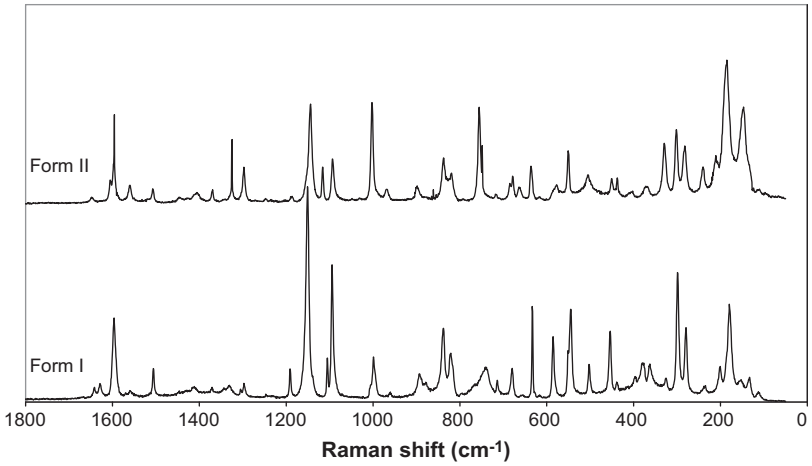

Fig. 3. Raman spectra of SMZ Form I and Form II.

3.2. Calibration models built using PXRD, Raman and DSC

It is known that PXRD diffractions can be affected by various factors such as type of sample holder, rotation of the sample, particle size, powder packing and preferred orientation effects (Roberts et al., 2002b; Tiwari et al., 2007). Therefore, all standard samples prepared were sieved and only particles in the size range of 38–45 μm were used. To avoid mixing inhomogeneity, sample rotation was used during PXRD acquisition. In addition, each standard sample was repacked after each acquisition and five PXRD diffractograms were acquired for each sample. The PXRD patterns of SMZ binary mixtures at different Form II contents are shown

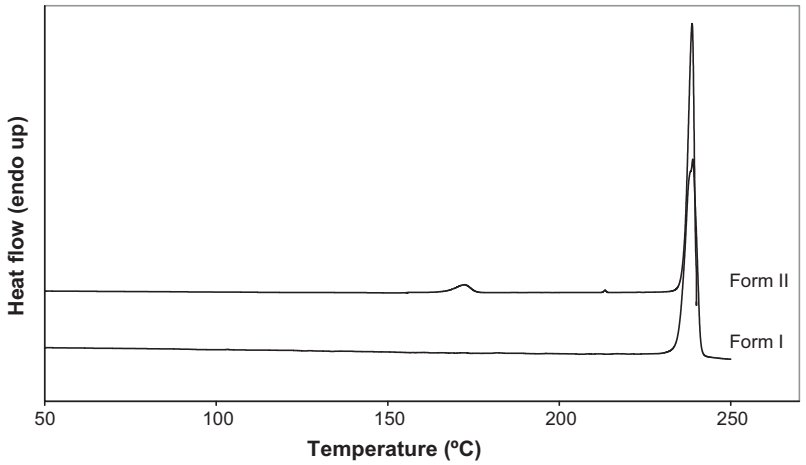

Fig. 4. DSC thermograms of SMZ Form I and Form II at 10 °C/min heating rate.

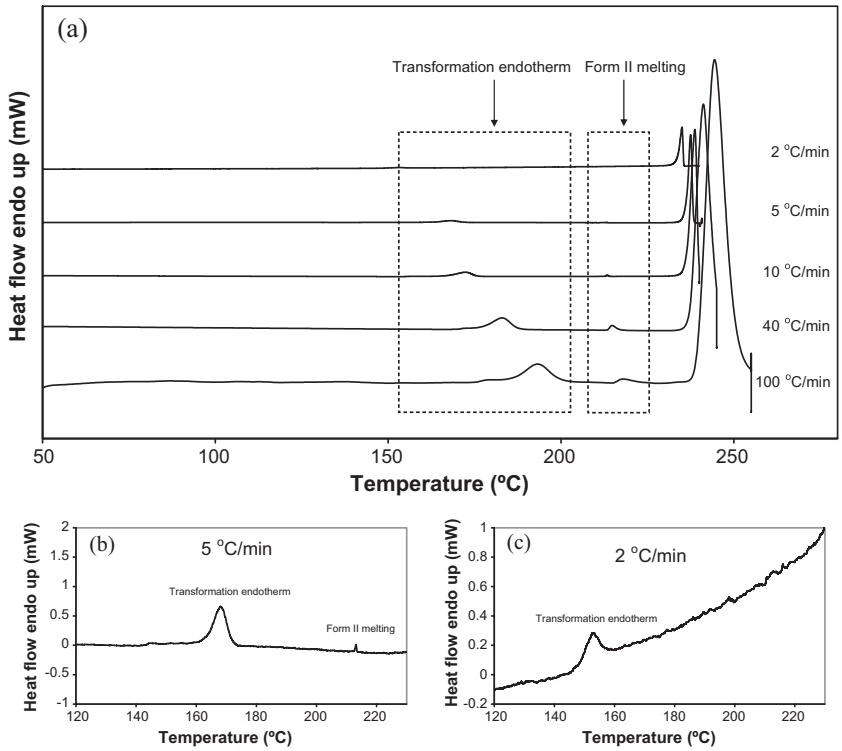

Fig. 5. DSC thermograms of SMZ Form II. (a) Stacked plot showing all heating rates studied. Enlarged plots at (b) 5 °C/min and (c) 2 °C/min.

Table 1
SMZ Form II onset temperature and enthalpy recorded from DSC.

Heating rate (°C/min)	Transformation from Form II to Form I		Residual Form II melting		Form I melting	
	Onset temperature (°C)	ΔH (J/g)	Onset temperature (°C)	ΔH (J/g)	Onset temperature (°C)	ΔH (J/g)
2	160.8	11.21	–	–	236.0	156.4
5	164.1	10.90	212.6	0.1996	236.2	153.7
10	167.7	10.04	212.8	0.2273	236.8	151.8
40	176.4	9.831	212.5	1.544	236.4	150.3
100	182.2	9.597	211.9	4.298	236.4	147.7

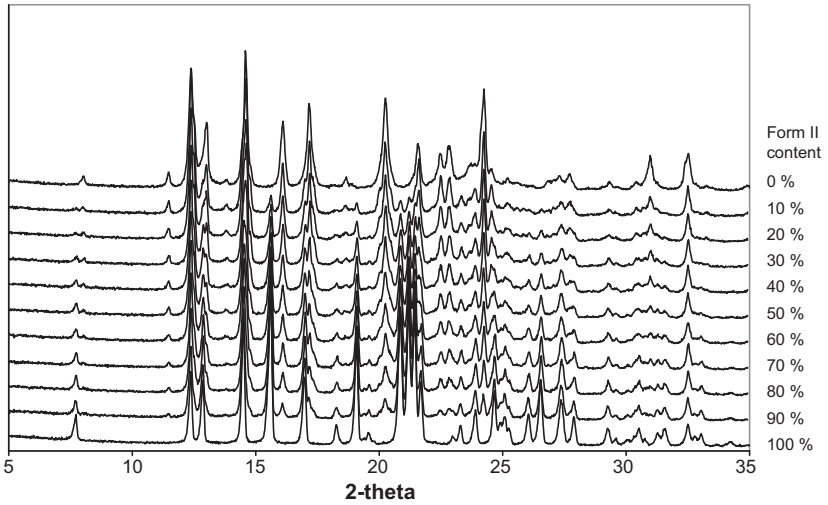

Fig. 6. PXRD diffractograms of different SMZ Form I and Form II binary mixtures.

Table 2
Performance of the PLS regression models using different preprocessing methods.

Methods	Baseline correction	Normalization	PLS factors	R^2	Q^2	RMSEC (%)	RMSEP (%)
PXRD	Yes	None	4	0.999	0.998	1.28	1.65
Raman	Yes	None	10	0.852	–	19.31	–
Raman	Yes	Yes	4	0.968	0.639	5.98	6.75

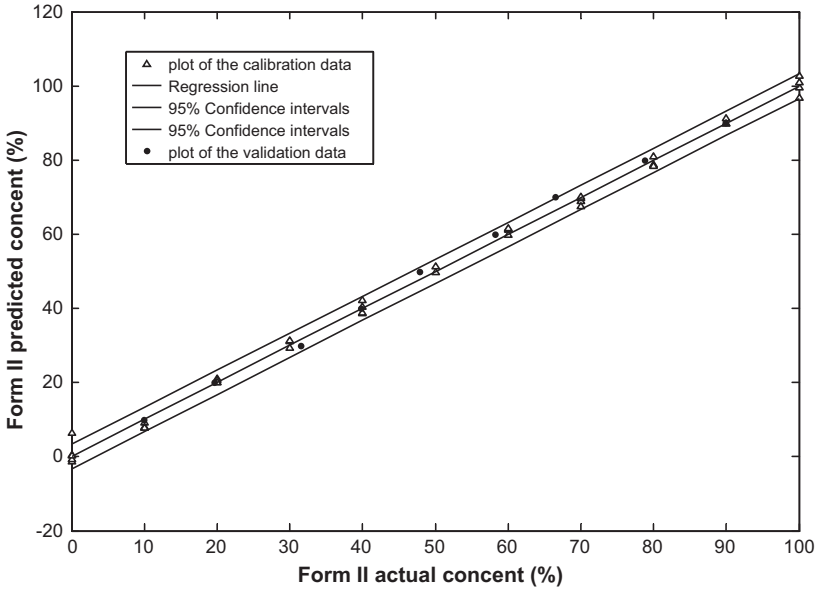

Fig. 7. Predicted vs. actual polymorphic content of Form II using PXRD. Regression line: $y = 0.9980x + 0.0948$, $R^2 = 0.999$.

in Fig. 6. As Form II content increased, the characteristic peaks of Form I at 11.5°, 16.1° and 22.5° 2θ decreased gradually while the characteristic peaks of Form II at 15.6°, 18.3°, 19.1° and 26.6° 2θ increased. Among the above-mentioned peaks, peaks located at 15.6°, 16.1°, 18.3° and 19.1° 2θ were very distinct and relatively strong. As such, the diffraction angle range between 14.9° and 19.3° 2θ was used to build the calibration model. A total of 55 PXRD diffractograms were acquired for the eleven standard samples. All the diffractograms were subjected to baseline correction in the selected range (14.9–19.3° 2θ) prior to PLS regression. During regression, 46 diffractograms were randomly selected for calibration and the remaining nine were used for validation. The predicted Form II contents from the PLS model are plotted against the actual Form II contents in Fig. 7. The entire validation dataset was within the 95% confidence intervals indicating reasonable reliability of our model. Table 2 summarizes the quality indicators of our calibration model.

Similarly, Raman spectra collected for the standard samples were analyzed and the calibration model was built using PLS regression. As discussed in the previous section, the polymorphs displayed different SO_2 symmetric stretching vibrations resulting in distinct and relatively strong peaks around 1150 cm^{-1}, therefore the spectral range of 1040–1175 cm^{-1} was chosen for calibration purpose. Prior to the data regression, the spectra were carefully examined and spikes due to cosmic rays and autofluorescence background were removed followed by baseline correction to each spectrum. The spectra acquired at 5% and 10% of Form II in binary mixtures were used for validation while the rest of the spectra were used for calibration. The calibration model built was unfortunately unsatisfactory as it required 10 PLS factors with an R^2 of 0.852 (see Table 2). The poor performance of this calibration model could be explained by the inherent shortcoming of the Raman microscope in mapping mode. The focal point of Raman laser could not be automatically adjusted to compensate for the inhomogeneity in sample height as the sample was moved from one point to the next during mapping, leading to variation in signal intensity as the surface of the loose binary mixture samples was not flat. To circumvent this problem, spectra were normalized prior to PLS regression. As seen in Table 2 and Fig. 8, the calibration model was much improved with normalization, requiring only 4 PLS factors with R^2 of 0.968. However, the performance of the Raman calibration model is obviously inferior to the PXRD model. There are data points outside the 95% confidence intervals and the 95% confidence internals is much wider than that of PXRD. The RMSEC and RMSEP values for the Raman model are significantly larger than the PXRD model.

As shown earlier, complete transformation of Form II to Form I could be achieved at slow heating rate of 2 °C/min in DSC. Therefore the transformation enthalpy obtained at 2 °C/min was used for calibration and the calibration curve is plotted in Fig. 9. The enthalpy of transformation from Form II to Form I showed good linear relationship to Form II content, giving a regression line with R^2 of 0.9896. This is in contrast to the calibration curve reported by McGregor and Bines (2008) which was highly nonlinear. Instead of using high heating rate to inhibit the recrystallisation of the lower melting polymorph, we approached in the opposite direction by using low enough heating rate to encourage complete transformation of the lower melting polymorph (Form II) to the high melting polymorph (Form I). As our method gives sufficient time for complete transformation, the problem of crystal seeding can be avoided.

3.3. Comparison of the three techniques

To compare the accuracy of the three techniques, three standard samples containing different amounts of Form II were prepared and analyzed. From Table 3, all three techniques were able to detect down to 3% of Form II in a binary mixture of Form I and Form II. Among them, PXRD provided the most accurate determination of the amount of Form II followed by DSC, while Raman mapping was the least accurate.

The poor performance by Raman mapping was mainly due to sample inhomogeneity and sample packing. Firstly, the polymorphs may be unevenly distributed in the mixture and since the laser only excites a small sampling area, the sampling area may not be representative for the overall content in the bulk sample. Secondly, the sample surface after packing onto the glass slide may be uneven as the two polymorphs have distinctly different habits (see in Fig. 1), leading to large intensity differences as the laser moved along the sample during mapping. Various previous studies (Heinz et al., 2007; Johansson et al., 2002; Német et al., 2009; Pellow-Jarman et al., 1996; Taylor and Zografi, 1997; Wang et al., 2002) have also identified and analyzed the possible sources of error related to quantification of binary mixtures of polymorphs

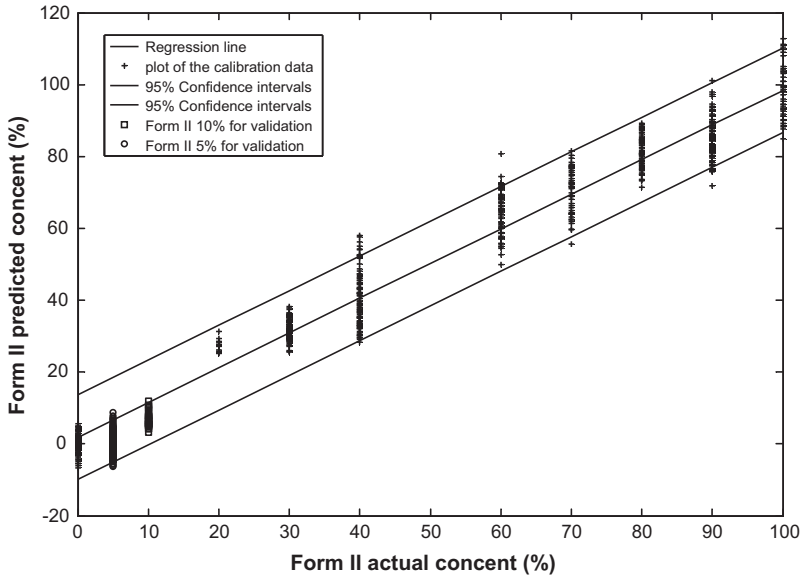

Fig. 8. Predicted vs. actual polymorphic content of Form II from Raman microscopy. Regression line: $y = 0.9962x + 1.73$, $R^2 = 0.9682$.

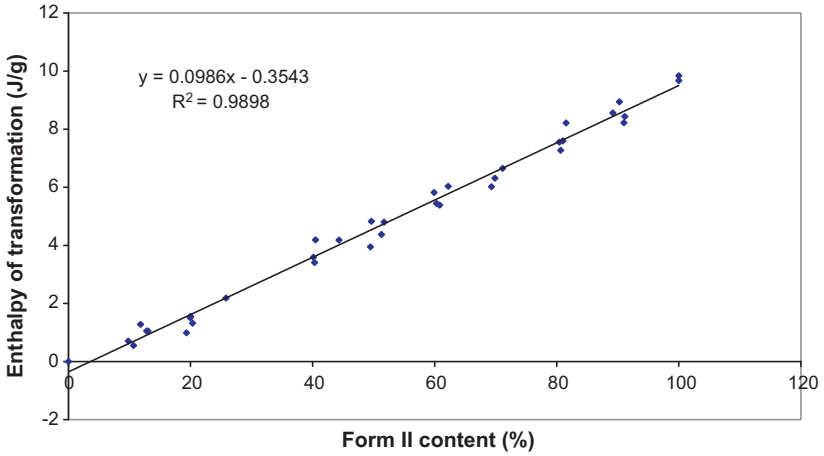

Fig. 9. Calibration curve of SMZ Form II from DSC.

using Raman spectroscopy. Despite the contradictory findings on the effect of particle size on the measured Raman intensity (Német et al., 2009), the general recommendation is to reduce the particle size and to ensure particle size uniformity. Recent report by Wang et al. (2002) supported this recommendation. In our case, the particle size has already been controlled to a small range of 38–45 μm and yet the Raman mapping results are still unsatisfactory. For better results, the particle size may have to be reduced further and a better packing method, such as that outlined by Német et al. (2009), may have to be adopted.

PXRD coupled with multivariate analysis showed slightly better accuracy compared to DSC in determining Form II content in the standard samples. However, similar to Raman, PXRD requires careful sample preparation (Roberts et al., 2002b; Tiwari et al., 2007). In contract, DSC appears to be a more convenient method since no special sample preparation is required and the calibration is simple and straightforward. This led to the question if PXRD would be capable of providing similar accuracy without the elaborate multivariate analysis. We built simple linear regression models using peak heights and peak areas for comparison and the results are also shown in Table 3. The details of the linear regression models are included in the supplementary information. It is obvious that PXRD with simple linear regression calibration models is incapable of providing proper determination of the polymorphic content in

Table 3
Comparison of the three techniques for the quantification of Form II content in binary mixtures.

Actual Form II content (%)	Form II content (%) determined by				
	DSC	PXRD (multivariate analysis)	PXRD (linear regression, height)	PXRD (linear regression, area)	Raman in mapping mode
3.07	5.42 ± 0.96	2.39 ± 0.38	−4.08 ± 2.14	−3.50 ± 0.87	10.55 ± 1.44
6.55	8.14 ± 1.46	7.90 ± 0.85	3.31 ± 2.69	3.67 ± 0.40	14.09 ± 2.42
10.00	9.5 ± 0.70	9.05 ± 0.45	15.65 ± 1.09	12.31 ± 0.78	12.81 ± 1.90

our system. Nonetheless, PXRD technique has the advantage of being non-destructive so the sample can still be used for further analysis after measurement by PXRD. As each compound has its own set of signature peaks, the interference from the presence of components, e.g. excipients, other than the API on the diffraction pattern should be minimal. Therefore PXRD is suitable for the analysis of formulated samples such as tablets or solid samples from various secondary manufacture operations. On the other hand, DSC could be a convenient way for ensuring the polymorphic purity of API samples from final stage purification process or before being transferred to secondary processing.

Despite the simplicity of the DSC method, there are potential drawbacks associated with our current approach. An obvious problem is that the presence of small amount of impurities other than the two polymorphs could produce unpredictable change to the phase transformation process or thermal events that interfere or overlap with the phase transformation peak, thus resulting in unreliable quantification of the polymorphic content. This also means that the DSC method will not be suitable for quantifying polymorphic content in formulated products that contain excipients and other additives. In addition, the current approach is unable to detect SMZ Form II when it falls below 3% because the transformation enthalpy is too low. This low sensitivity is due to the low heating rate employed in this work. DSC output is a function of heat flow per unit time. As the heating rate decreases, the input of heat flow per unit time decreases and hence the same heat flow occurs over a longer period of time. Therefore, the sensitivity becomes lower as the heating rate is decreased. That explains why the detection limit is not as low as 1% reported by McGregor et al. (2004). Nevertheless, our approach of using low heating rate circumvents the problem of partial recrystallization encountered in the previous work, yielding a linear calibration curve. The success of the current approach lies in the detection of the transformation enthalpy between SMZ Forms I and II. In other systems in which the transformation enthalpy cannot be easily detected, the melting enthalpy may have to be used for the calibration instead. For example, the solid state transformation of carbamazepine from Form III to Form I cannot be detected as any endothermic or exothermic event in the DSC thermograms regardless the heating rates used because the solid state transformation was isothermal (Behme and Brooke, 1991; O'Brien et al., 2004). In cases like carbamazepine, the high heating rate approach of McGregor and co-workers (McGregor and Bines, 2008; McGregor et al., 2004) would be more suitable. Therefore, our low heating approach and the high heating rate approach of McGregor and co-workers could be complementary depending on the type of polymorphic system under investigation.

4. Conclusion

Quantification of SMZ Form I and Form II in solid binary mixtures was studied using DSC, PXRD, and Raman microscope in mapping mode. Our DSC method made use of slow heating rate to facilitate complete transformation of Form I to Form II, thereby circumventing the problem of crystal seeding experienced by other authors (McGregor and Bines, 2008; McGregor et al., 2004). A linear calibration curve for DSC was successfully obtained using this approach. Comparing the three techniques, DSC and PXRD (with partial least square regression) are both capable of accurate quantification of polymorphic impurity for SMZ down to 3% of Form II in the binary mixtures. Raman spectroscopy in mapping mode was found to be the least accurate method for the model compound studied even when coupled with chemometric techniques. Due to the simplicity in the DSC calibration, it could be a convenient way for the checking of polymorphic purity of API samples from final stage purification or before being transferred to secondary processing. On the other hand, the non-invasive PXRD would be suitable for the analysis of solid dosage forms or solid samples from various secondary processes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.05.058.

References

Acharya, K.R., Kuchela, K.N., Kartha, G., 1982. Crystal structure of sulfamerazine. J. Cryst. Spectrosc. Res. 12, 369–376.
Behme, R.J., Brooke, D., 1991. Heat of fusion measurement of a low melting polymorph of carbamazepine that undergoes multiple-phase changes during differential scanning calorimetry analysis. J. Pharm. Sci. 80, 986–990.
Brittain, H.G., 1999. Methods for the characterization of polymorphs and solvates. In: Brittain, H.G. (Ed.), Polymorphism in Pharmaceutical Solids. Marcel Dekker, Inc., New York.
Cao, X., Sun, C., Thamann, T.J., 2005. A study of sulfamerazine single crystals using atomic force microscopy, transmission light microscopy, and Raman spectroscopy. J. Pharm. Sci. 94, 1881–1892.
Caria, M.R., Mohamed, R., 1992. Positive indentification of two orthorhombic polymorphs of sulfamerazine (C11H12N4O2S), their thermal analyses and structural comparison. Acta Crystallogr., Sect. B: Struct. Sci. 48, 492–498.
Chieng, N., Rehder, S., Saville, D., Rades, T., Aaltonen, J., 2009. Quantitative solid-state analysis of three solid forms of ranitidine hydrochloride in ternary mixtures using Raman spectroscopy and X-ray powder diffraction. J. Pharm. Biomed. Anal. 49, 18–25.
Gong, Y., Collman, B.M., Mehrens, S.M., Lu, E., Miller, J.M., Blackburn, A., Grant, D.J.W., 2008. Stable-form screening: overcoming trace impurities that inhibit solution-mediated phase transformation to the stable polymorph of sulfamerazine. J. Pharm. Sci. 97, 2130–2144.
Gu, C.H., Chatterjee, K., Young Jr., V., Grant, D.J.W., 2002. Stabilization of a metastable polymorph of sulfamerazine by structurally related additives. J. Cryst. Growth 235, 471–481.
Gu, C.H., Young, V., Grant, D.J.W., 2001. Polymorph screening: influence of solvents on the rate of solvent-mediated polymorphic transformation. J. Pharm. Sci. 90, 1878–1890.
Heinz, A., Savolainen, M., Rades, T., Strachan, C.J., 2007. Quantifying ternary mixtures of different solid-state forms of indomethacin by Raman and near-infrared spectroscopy. Eur. J. Pharm. Sci. 32, 182–192.
Hossain, G.M.G., 2006. A new polymorph of sulfamerazine. Acta Crystallogr., Sect. E: Struct. Rep. Online 62, o2166–o2167.
Johansson, J., Pettersson, S., Taylor, L.S., 2002. Infrared imaging of laser-induced heating during Raman spectroscopy of pharmaceutical solids. J. Pharm. Biomed. Anal. 30, 1223–1231.
Langkilde, F.W., Sjöblom, J., Tekensberg-Hjelte, L., Mrak, J., 1997. Quantitative FT-Raman analysis of two crystal forms of a pharmaceutical compound. J. Pharm. Biomed. Anal. 15, 687–696.
McGregor, C., Bines, E., 2008. The use of high-speed differential scanning calorimetry (Hyper-DSCTM) in the study of pharmaceutical polymorphs. Int. J. Pharm. 350, 48–52.
McGregor, C., Saunders, M.H., Buckton, G., Saklatvala, R.D., 2004. The use of high-speed differential scanning calorimetry (Hyper-DSCTM) to study the thermal properties of carbamazepine polymorphs. Thermochim. Acta 417, 231–237.
Német, Z., Demeter, A., Pokol, G., 2009. Quantifying low levels of polymorphic impurity in clopidogrel bisulphate by vibrational spectroscopy and chemometrics. J. Pharm. Biomed. Anal. 49, 32–41.
O'Brien, L.E., Timmins, P., Williams, A.C., York, P., 2004. Use of in situ FT-Raman spectroscopy to study the kinetics of the transformation of carbamazepine polymorphs. J. Pharm. Biomed. Anal. 36, 335–340.
Pellow-Jarman, M., Hendra, P.J., Lehnert, R.J., 1996. The dependence of Raman signal intensity on particle size for crystal powders. Vib. Spectrosc. 12, 257–261.
Pratiwi, D., Fawcett, J.P., Gordon, K.C., Rades, T., 2002. Quantitative analysis of polymorphic mixtures of ranitidine hydrochloride by Raman spectroscopy and principal components analysis. Eur. J. Pharm. Biopharm. 54, 337–341.
Roberts, S.N.C., Williams, A.C., Grimsey, I.M., Booth, S.W., 2002a. Quantitative analysis of mannitol polymorphs. FT-Raman spectroscopy. J. Pharm. Biomed. Anal. 28, 1135–1147.

Roberts, S.N.C., Williams, A.C., Grimsey, I.M., Booth, S.W., 2002b. Quantitative analysis of mannitol polymorphs. X-ray powder diffractometry-exploring preferred orientation effects. J. Pharm. Biomed. Anal. 28, 1149–1159.
Shah, B., Kakumanu, V.K., Bansal, A.K., 2006. Analytical techniques for quantification of amorphous/crystalline phases in pharmaceutical solids. J. Pharm. Sci. 95, 1641–1665.
Socrates, G., 2001. Infrared and Raman Characteristic Group Frequencies: Tables and Charts, 3rd ed. John Wiley & Sons Ltd., Chichester.
Stephenson, G.A., Forbes, R.A., Reutzel-Edens, S.M., 2001. Characterization of the solid state: quantitative issues. Adv. Drug Delivery Rev. 48, 67–90.
Strachan, C.J., Pratiwi, D., Gordon, K.C., Rades, T., 2004. Quantitative analysis of polymorphic mixtures of carbamazepine by Raman spectroscopy and principal components analysis. J. Raman Spectrosc. 35, 347–352.
Taylor, L.S., Zografi, G., 1997. Spectroscopic characterization of interactions between PVP and indomethacin in amorphous molecular dispersions. Pharm. Res. 14, 1691–1697.
Tiwari, M., Chawla, G., Bansal, A.K., 2007. Quantification of olanzapine polymorphs using powder X-ray diffraction technique. J. Pharm. Biomed. Anal. 43, 865–872.
Tong, H.H.Y., Shekunov, B.Y., Chan, J.P., Mok, C.K.F., Hung, H.C.M., Chow, A.H.L., 2005. An improved thermoanalytical approach to quantifying trace levels of polymorphic impurity in drug powders. Int. J. Pharm. 295, 191–199.
Tong, H.H.Y., Shekunov, B.Y., York, P., Chow, A.H.L., 2003. Thermal analysis of trace levels of polymorphic impurity in salmeterol xinafoate samples. Pharm. Res. 20, 1423–1429.
Vanden Poel, G., Mathot, V.B.F., 2006. High-speed/high performance differential scanning calorimetry (HPer DSC): temperature calibration in the heating and cooling mode and minimization of thermal lag. Thermochim. Acta 446, 41–54.
Varasteh, M., Deng, Z., Hwang, H., Kim, Y.J., Wong, G.B., 2009. Quantitative determination of polymorphic impurity by X-ray powder diffractometry in an OROS formulation. Int. J. Pharm. 366, 74–81.
Wang, H.L., Mann, C.K., Vickers, T.J., 2002. Effect of powder properties on the intensity of Raman scattering by crystalline solids. Appl. Spectrosc. 56, 1538–1544.
Woolfenden, R.D.G., 1977. Sulphamerazine. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances. Academic Press, New York.
Zhang, G.G.Z., Gu, C., Zell, M.T., Todd Burkhardt, R., Munson, E.J., Grant, D.J.W., 2002. Crystallization and transitions of sulfamerazine polymorphs. J. Pharm. Sci. 91, 1089–1100.