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Formation, Physical Stability and Quantification of Process Induced Disorder in Cryo-milled Samples of a Model Polymorphic Drug

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ABSTRACT

The formation and physical stability of amorphous sulfathiazole obtained from polymorphic forms I and III by cryo-milling was investigated by X-ray powder diffraction (XRPD) and near-infrared (NIR) spectroscopy. Principal component analysis was applied to the NIR data to monitor the generation of crystalline disorder during milling and to study subsequent recrystallization under different storage conditions. Complete conversion into the amorphous phase was observed for both forms. Upon storage under vacuum over silica gel for 14 days at 4 °C amorphous samples remained amorphous. However, under the same conditions at 22 °C, recrystallization occurred. Amorphous samples obtained from form I crystallized back to the original polymorph, while those prepared from form III partially crystallized to mixtures of polymorphs (I, II and III). Amorphous samples stored at ambient temperature and 35 % relative humidity absorbed moisture which facilitated crystallization to a mixture of polymorphs in both cases. Quantitative analyses of amorphous content in binary mixtures with forms I and III were carried out by XRPD and NIR spectroscopy combined with partial least squares regression. The calibration model had root mean square errors of prediction values of < 2.0 %, and was applied to quantify the extent of crystalline disorder during cryo-milling.

Keywords:

Amorphous; Near-infrared spectroscopy; X-ray powder diffractometry; Stability; Multivariate analysis

INTRODUCTION

Milling or grinding is one of the most common manufacturing procedures in the

pharmaceutical industry.[1-3] When milling a crystalline active pharmaceutical ingredient (API), the mechanical stress during the milling process cannot only change the particle size, surface area and crystallinity of the API but can also induce polymorphic transformations.[3-11] Solid-state polymorphic transitions and amorphization induced by milling have been reported for many pharmaceutical products such as gabapentin,[3] famotidine,[9] ranitidine hydrochloride,[10] and Fananserine.[11] Cryo-milling (Cryogenic grinding) seeks to operate ball milling, a high energy process, at low temperatures with the aid of cryogenic media such as liquid nitrogen.[12] This process represents an effective method of reducing the risk of recrystallisation of any amorphous materials produced due to the lower process temperatures involved compared to milling at ambient or higher temperatures, and it is often a more efficient way to prepare an amorphous API for those compounds which are disordered by milling.[13-17]

Amorphous drugs are important as their greater surface area and surface activity can lead to higher bioavailability[18,19] and attempts have also been made to use the amorphous form of a drug to circumvent patents which apply to a crystalline form.[20] However, due to the metastable nature of amorphous drugs, the amorphous regions or crystal defects created by milling are unstable and under a range of circumstances such as elevated temperature or relative humidity, they undergo structural changes or revert to the original form or to other stable crystalline forms.[21,22] Thus, it is necessary to monitor and quantify the amount of amorphous phase during processing. Various analytical techniques have been used for the detection and quantification of amorphous content in solid-state pharmaceuticals,[23-33] such as X-ray powder diffraction (XRPD),[25-27] isothermal microcalorimetry (IMC),[28] differential scanning calorimetry (DSC),[29,30] solid-state nuclear magnetic resonance (NMR)[25,30] and vibrational spectroscopy.[31-33] While XRPD is the classic technique for the analysis of the solid state it however suffers from various problems including the influence of preferred crystal orientation, packing and sample preparation parameters on measured intensity.[24] In contrast to XRPD, which probes the orderly arrangement of molecules in the crystal lattice, vibrational spectroscopy probes differences in chemical bonding

(usually H-bonding and other weak interactions) in the solid state of the API. With the help of a variety of chemometric and statistical techniques, a range of spectroscopic methods have been successfully used for the qualitative and quantitative analysis of pharmaceutical products. Among them, near infrared (NIR) spectroscopy has extensive applications in the characterization and quantitative analysis of polymorph mixtures. It is fast and non-destructive and requires minimal sample preparation. Furthermore, fiber optic probes coupled to the spectrometer allow for real-time in-process measurements. The combination of XRPD and NIR is therefore particularly effective for the analysis of polymorph mixtures.

Sulfathiazole (Fig. 1), a sulfa drug, exhibits at least five polymorphic forms. The main differences between the polymorphs of sulfathiazole lie in hydrogen bonding and the packing of the molecules in the crystal lattice.[34] The physicochemical properties of the five forms have been described in detail.[35-40] Relative thermodynamic stabilities at 0 K are generally accepted to follow the order of the densities of the structures, i.e. III ≈ IV > II > I > V, with form I being metastable at room temperature.[39,40] Shakhtshneider and Boldyrev reported the influence of mechanical treatment in a planetary ball mill on the polymorphic transformations of sulfathiazole and sulfathiazole – polyvinylpyrrolidone.[41-43] They found that mechanical treatment of sulfathiazole led to the partial amorphization and to form III - form I transformations. Aaltonen *et al*. have monitored the decrease in crystallinity, when crystalline sulfathiazole samples were milled at room temperature.[44] However, in contrast to Shakhtshneider and Boldyrev,[41,42] they did not observe any polymorph transformations in the XRPD patterns of samples subjected to mechanical treatment.

Lagas and Lerk prepared amorphous sulfathiazole by melt-quenching and Kordikowski *et al*. obtained a mixture of form I and amorphous sulfathiazole from acetone using supercritical CO_2.[35,45] Recently, the preparation of amorphous sulfathiazole by cryo-milling has been reported but no stability testing of the amorphous phase was described.[13]

In this paper we describe a detailed study of the effect of cryo-milling on two sulfathiazole polymorphic forms I and III. The physical stability of the amorphous

forms produced under different storage conditions has been investigated and the quantitative analysis of binary mixtures of amorphous sulfathiazole with crystalline forms I and III is also constructed to quantify the process induced crystalline disorder in the cryo-milled samples.

MATERIALS AND METHODS

Materials

Sulfathiazole (form III) was purchased from Sigma-Aldrich, and was used as received. Form I was prepared by heating commercial sulfathiazole in an oven for 30 min at 180 ºC.[37] The identity and purity of polymorphs I and III was confirmed by XRPD, differential scanning calorimetry (DSC) and NIR spectroscopy.

Methods

Cryo-milling procedure

For the milling experiments an oscillatory ball mill (Mixer Mill MM400, Retsch GmbH & Co., Germany) was used. A fresh 1 g batch of powder was used for each milling experiment. The powder sample was placed in a 25 mL stainless steel milling jar containing one 15 mm stainless steel ball. Milling jars were then sealed and immersed in liquid nitrogen for three minutes before milling at 25 Hz for up to 180 min. Re-cooling of the milling chambers for 2 min with liquid nitrogen was performed every 7.5 min. The average sample temperature measured at 7.5 min intervals was -10±2 ºC. Samples milled for 2, 4, 6, 7.5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 min were used to evaluate the effect of cryo-milling on the crystallinity of sulfathiazole.

Stability study of amorphous sulfathiazole

The stability of amorphous sulfathiazole obtained by cryo-milling of form I for 30, 45, 60, and form III for 60, 90, 120, 150 and 180 min was studied at 4 ºC and ambient temperature 22 (±2) ºC. Samples were kept under vacuum in a desiccator over silica gel and in a 35 (±5) % relative humidity (RH) chamber. XRPD and NIR

spectra were recorded at regular time intervals.

Preparation of binary mixtures of amorphous sulfathiazole with form I and form III

Mixtures containing 0 – 100 % amorphous phase were prepared by mixing known amounts of amorphous sulfathiazole and forms I or III in an oscillatory ball mill at 15 Hz for 1 min to ensure sample homogeneity.[16] Each sample was prepared in triplicate. All samples were analyzed immediately after mixing by XRPD and NIR spectroscopy. The amorphous sulfathiazole used to prepare these samples was obtained by cryo-milling form I for 60 min.

Analytical techniques

X-ray powder diffraction

X-ray powder diffraction data were collected on a Siemens D500 powder diffractometer which was fitted with a diffracted beam monochromator. Diffraction patterns were recorded between 5 and 40 ^{o} (2θ) using CuK_{α} radiation with steps of 0.05 ^{o} and with 2 seconds counting time per step. The Oscail software package was used to generate theoretical XRPD patterns of the sulfathiazole forms.[46]

Near-infrared spectroscopy

NIR spectra were collected in glass vials (15 mm × 45 mm) on a PerkinElmer Spectrum One fitted with an NIR reflectance attachment. Spectra were collected with interleaved scans in the 10000 – 4000 cm^{-1} range with a resolution of 8 cm^{-1}, using 32 co-added scans. Sample vials were shaken and repositioned between the triplicate measurements of each sample. The mean spectrum for each sample was used in the analysis.

Data analysis

Data analysis was carried out using the multivariate data analysis software The Unscrambler v.9.8 (Camo, Norway). The removal of unimportant baseline (offset)

interferences and scattering effects and the accentuation of the spectral signals of interest were effected using several different pre-processing methods. The methods used for NIR data included multiplicative scatter correction (MSC), standard normal variate (SNV), first derivative, and second derivative calculations. Savitzky-Golay first and second derivative calculations and smoothing were performed with a window size of 11 points and a second order polynomial. For the XRPD data, area normalization combined with first derivation was used. The gap and segment sizes were 9 and 1 respectively. Principal component analysis (PCA) was used to investigate the spectral variation during the cyro-milling and crystallization processes. A total of 33 samples were prepared for each data set (amorphous/form I and amorphous/form III). Both data sets were divided into a calibration set to build the model (17 samples) and a prediction set to test the model (16 samples). The spectroscopic data were subjected to mean centering prior to PCA and partial least squares (PLS) regression analysis. The optimal number of PLS factors was determined by using a leave-one-out cross validation procedure. The performances of the models were evaluated by using the correlation coefficient (R^2) of calibration and root mean square error (RMSE) of calibration (RMSEC), cross-validation (RMSECV) and prediction (RMSEP), defined as

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}}$$

where y_i is the reference value, $\hat{y}_i$ the calculated value and n is the number of samples.

RESULTS AND DISCUSSION

Effect of cryo-milling on sulfathiazole polymorphs

XRPD is the classic method for determining the crystallinity of a sample since XRPD patterns of amorphous material usually exhibit a diffuse halo without observable sharp peaks. The XRPD patterns of the cryo-milled sulfathiazole polymorphs forms I and III at various milling times are shown in Fig. 2. The 2θ peaks at 10.9, 16.0, 17.7, 18.9, 20.9 and 22.0° are specific to form I, while peaks at 15.4,

and 22.2° are specific to form III. The XRPD patterns of both forms showed a rapid decrease in the diffraction peak intensities within the first 30 min of milling. The diffraction peaks of form III have disappeared after 150 min, while in the case of form I, amorphisation was complete after 45 min. Thus, cryo-milling of both forms led to X-ray amorphous materials. No polymorphic conversion was observed during cryo-milling of forms I and III for up to 180 min. This suggested that the crystal to amorphous phase transformation was direct. The NIR spectra of the sulfathiazole polymorphs measured after various milling times are shown in Fig. 3. The changes observed in the NIR spectra were in agreement with the XRPD results. In the NIR spectra of form III the intensities of the bands around 6410, 6370, 4916, and 4900 cm^{-1} which are specific to form III decreased during milling. At the same time peaks at 6890, 6680, 5070, 5010 and 4530 cm^{-1} that are indicative of amorphous sulfathiazole increased in intensity. It is noted that for form III that decreasing intensity of NIR peaks around 6410 and 6370 cm^{-1} is a good indication of crystallinity decrease for sulfathiazole form III. The NIR spectrum of amorphous sulfathiazole is similar to that of form I. Nevertheless, the conversion of form I into the amorphous phase could be monitored by a general broadening of NIR bands and the disappearance of the peaks at 6152, 6104, 5080, 4900 and 4690 cm^{-1}. The peaks in the spectrum of amorphous sulfathiazole are broader than those of crystalline samples due to the greater range of molecular environments that are available in the amorphous state. Fig. 4 shows the PCA scores and the corresponding loadings plots of the milled samples obtained from the second-derivative NIR spectra in the regions 6980–5800 and 5130–4000 cm^{-1}. There are clear spectral differences between form III with form I and amorphous sulfathiazole, and the differentiation could be performed with the first two principal components (PCs), which summarize 98.5% of the variation in the second-derivative NIR spectra. It is clear that in both cases the relative amount of crystalline disorder or amorphous phase increases with milling time. Roughly, the transformation of form III to amorphous phase occurred in the direction of PC1, and the peaks at 6126 and 6110 cm^{-1} with form III are positively weighted in the corresponding loading. The score values of PC2 are a good indication of the

crystallinity change of form I, as evidenced by the PC2 loading plot which shows the characteristic peaks of form I at 6152, 5080, 4182 cm^{-1}.

However, conversion of form III into the amorphous phase required a longer milling time than the conversion of form I. For other pharmaceutical compounds, such as indomethacin (γ, α, and δ polymorphs)[14] and ranitidine hydrochloride (form 1 and form 2),[16] the milling time required for complete amorphisation is independent of the polymorphic form used as starting material. It has been suggested that the ease of milling crystalline powders to an amorphous form should be strongly related to molecular volume and the glass transition temperature T_g. The latter is expected to be directly related to lattice energy.[13] It is difficult to compare the molecular volumes and densities of the indomethacin polymorphs as those reported on the Cambridge data base have all been measured at different temperatures. However in agreement with prediction forms I and II of ranitidine hydrochloride have molecular volumes 437.8 and 437.7 $Å^3$ respectively and indistinguishable densities of 1.33 $Mg.m^{-3}$. On the other hand the very different results reported here for sulfathiazole are also in agreement with prediction as sulfathiazole forms I and III have molecular volumes of 169.5 and 162.4 $Å^3$ and lattice energies of -24.6 and -24.75 $kcal.mol^{-1}$ respectively.[47] Recent work has suggested that fully amorphous sulfathiazole was difficult to obtain either by extended milling times at room temperature or by spray drying methods. The material produced also showed very high instability and rapid recrystallization.[19] The results reported here clearly demonstrate the advantages of lower milling temperatures.

Stability study of amorphous sulfathiazole samples

The stability of amorphous sulfathiazole obtained by cryo-milling forms I and III was assessed by XRPD and NIR spectroscopy for samples stored under vacuum over silica gel at 4 °C and 22 °C and at 22 °C in a humidity chamber at 35% RH. Fig. 5 displays the XRPD patterns of the amorphous samples stored under the above conditions after a range of milling times. The XRPD patterns of form III samples milled for 60, 90 and 120 min which were stored at 4 °C showed sharp peaks of low

intensity (indicated in Fig. 5a(2)) after 14 days, indicating that some crystallization had occurred. Form I samples milled for 30 min and stored at 4 °C for 14 days also showed some crystallization back to form I with characteristic 2θ peaks at 16.0, 17.7, and 18.9 °. However, form I samples milled for 45 and 60min remained amorphous when stored at 4 °C. It thus appears that in the case of form I when dislocation is less complete after 30 min milling time the ordered material present has a catalytic effect on recrystallization.

In contrast amorphous samples prepared by milling both forms showed considerable recrystallization when stored for 14 days at 22 °C under vacuum (Fig. 5a (3)). The milled form I sample were found to have mostly crystallized back to form I while amorphous form III samples had partially recrystallized back to the mixtures of forms II and III with a small amount of form I. The broadened XRPD peaks observed in the latter case indicated some residual disorder. It is interesting that the starting crystal form affected the physical stability of milled amorphous materials. Other reports of the recrystalliztion behavior of amorphous forms produced by milling vary. For example Chieng et al.[16] have reported the spontaneous recrystallization of amorphous ranitidine hydrochloride back to the original crystal form only but that seeding with a different from could induce crystallization of that form, and Crowley and Zografi[14] who examined the recrystallization of amorphous indomethacin reported results that are similar to those reported here for sulfathiazole.

The recrystallization of an amorphized fraction can thus occur toward the starting form or toward another polymorph which is kinetically easier to reach.[11] Thus while amorphous samples may have an intricsic tendency to crystallize back to the initial state because of the presence of residual crystalline particles of starting form spontaneous or catalysed crystallization to other more stable forms is also possible. Relative thermodynamic stability of sulfathiazole polymorphs is proposed to be III ≈ IV > II > I.[39,40] Therefore according to Ostwald's Rule of Stages, amorphous sulfathiazole should initially produce form I as it is the least stable form, followed by a stepwise conversion, through the other metastable forms, to the thermodynamically most stable form.[34] Form I by its initial recrystallization back to form I is obeying the

Rule of Stages to some extent while amorphous form III shows a lesser effect in that it was found that the amount of form II increases with the cryo-milling time, as evidenced by an intensity increase in NIR bands at 6414 and 6112 cm^{-1} (Fig. 6).

Amorphous solids often readily absorb moisture. It is well established that water and other solvents absorbed by an amorphous solid lowers the glass transition temperature T_g of the solid, thus acting as plasticizers and increasing the molecular mobility and recrystallization rates.[14,48] The behaviour of amorphous samples stored under 35% RH at 22 ^oC for 7 days was also investigated. The rapid absorption of water is evident from the water peaks around 7070 and 5200 cm^{-1} in the NIR spectra (Fig. 7).[32,33] Furthermore, it was observed from Fig. 5 that the absorbed water promoted crystallization. Even more importantly, it was also found that the composition of the resulting crystalline material was not the same as samples stored under vacuum. As can be seen from Figs. 5 and 6, amorphous samples prepared from form I crystallized into the mixtures of forms II and IV with very small amounts of form I, while that from form III crystallized into the mixtures of forms I, II and III. The relative amount of form I is much lower in the milled form I samples, and higher in the milled form III samples compared to that of samples stored under vacuum over silica gel, as shown by the intensity of the XPRD 2θ peaks at 17.7 and 18.9 ^{o} and the shift in the band around 6900 cm^{-1} to 6878 cm^{-1} and the intensity changes to the peak at 5080 cm^{-1} in NIR spectra. The results shown in here are also in agreement with a recent report which described the effect of relative humidity on amorphous sulfathiazole prepared by spray drying.[49]

Figure 8 shows the PCA scores scatter plot obtained using the first and second PCs of the crystallized samples stored under different conditions using second-derivative NIR spectra in the 6980–5800 and 5130–4000 cm^{-1} regions. Two principal components summarized 87.6% of the variation in the spectral information. The PCA model allowed clustering of the crystallized samples based on storage conditions. It is also noted that the greatest differences arose from the amorphous sulfathiazole with other polymorphic forms (II, III and IV) in the direction of PC1. And the second PC2 accounted for variability in the transformation of amorphous to

form I. Thus, the score values of PC2 were a good indication of the percentage of form I in the samples. As can be seen in Fig. 8, the amount of form I is highest when the form I milled samples were stored under vacuum at 22 ^{o}C. In a similar way, the amount of the other two polymorphic forms is higher in the form I milled samples stored under 35% RH. As expected, a PCA with two components was not able to divide polymorphs II, III and IV because of the similarity of the NIR spectra of these three forms. The close similarity of the vibrational spectra of forms II, III and IV is a consequence of the fact that the H-bonding pattern in the crystal structure of form III in effect combines the H-bonding motifs of forms II and IV.[32] The present study suggests that the recrystallization process of the amorphized fraction of sulfathiazole is influenced by both storage conditions and cryo-milling time.

Quantification of amorphous content in binary mixtures by XRPD and NIR spectroscopy

XRPD is one of the most widely used techniques for the quantification of crystalline and amorphous content. A reduction in crystallinity results in a reduction in peak heights and areas and in the appearance of a "halo" in the diffractogram. In binary crystalline / amorphous mixtures of one form, the diffraction intensity is linearly related to the concentration of the crystalline phase. In the XRPD pattern of sulfathiazole form III, significant intensity variations are observed between the calculated and experimental patterns due to preferred crystal orientation effects.[35] Therefore, the 2θ regions used for the analysis must be carefully selected. 2θ peaks in the 21.0–23.0° range are most affected by preferred crystal orientation and this region is not analytically useful. The 2θ regions between 14.0–21.0, 23.5–29.0, and 14.0–29.0° were used to build calibration models for binary mixtures of amorphous sulfathiazole with form I and III. The best calibration model for the quantification of the amorphous phase in these two binary mixtures was obtained when area normalization was followed by first derivatization using the Gap-segment method. The RMSEC and RMSEP values were 1.58 and 1.22 % for the determination of amorphous content in amorphous / form I mixtures and 1.39 and 1.38% for the

determination of amorphous content in amorphous / form III mixtures. In both cases just one PLS factor was required.

As described above, NIR spectroscopy has been successfully used for polymorph screening and for the quantification of different crystalline polymorphic forms of sulfathiazole.[44,46] Sulfathiazole form III and amorphous sulfathiazole show clear spectral differences in the 7000–6200 cm^{-1} and 5150–4850 cm^{-1} ranges (Fig. 3), which encompass the region where the first overtones for the N-H stretching vibrations and the combination bands of N-H stretching and NH_2 bending vibrations are expected. The NIR spectra of amorphous sulfathiazole and form I clearly differ in the 6300–5810 cm^{-1} and 5150–4650 cm^{-1} regions. As mentioned above, peaks around 7070 and 5200 cm^{-1} are caused by water absorbed by amorphous sulfathiazole (Fig. 7). Thus, a combination of the 6980–5800 and 5130–4000 cm^{-1} ranges was chosen for the quantitative analysis of amorphous content in form I / amorphous and form III / amorphous binary mixtures. SNV correction was found to be the best pre-processing method (Table 1).

RMSEC and RMSEP values were 1.11 and 1.24 % for the determination of amorphous content in amorphous/form I mixtures and 1.18 and 1.18 % for the determination of amorphous content in amorphous/form III mixtures both with one PLS factor. NIR spectroscopy gave slightly better results than XRPD.

In a simple attempt to simulate the effect of the appearance of an unexpected polymorph in a real work situation the NIR calibration model derived for amorphous/form I was used to analyse the prediction sample set for amorphous/form III and *vica versa*. In both cases and despite the presence of increasing amounts of the wrong polymorph the predicted amorphous content remained within experimental error at100 %, see supporting information.

Comparison of XRPD and NIR methods for the quantification of crystalline disorder in the cryo-milled samples

The calibration models developed above were also used to monitor the conversion of form III and form I into the amorphous state during cryo-milling

without any extra milling or mixing of the samples. Fig. 9 shows the percentage of crystalline disorder *versus* milling time. Samples were more than 98.6% amorphous when form I was cryo-milled for 45 min or when form III was cryo-milled for 150 min. The crystalline disorder in samples of form III that were cryo-milled for 10 min was determined to be 59.9 ± 0.5 % when the calibration model developed for the NIR method was used. XRPD analysis of the same samples gave an amorphous content of 62.8 ± 0.7 %. NIR and XRPD analysis of samples of form I after 10 min cryo-milling predicted gave 85.9± 0.3 % and 82.3 ± 2.6 % crystalline disorder, respectively. The results showed that process induced crystalline disorder from form I was higher than that of form III at the same milling time. It is also evident from Fig. 9 that there is reasonably good agreement between the XRPD and NIR results when the form I samples were milled for more than 6 min and 10 min for form III samples. However, when the milling time is short, it appears that XRPD tends to give a higher percentage of crystalline disorder than NIR spectroscopy. For example after 4 min. milling time NIR predicted crystalline disorder of 33.7 ± 0.7 % in the form III sample, and XRPD gave 41.4 ± 1.3 %. These differences are not surprising as samples milled for short times will have a range of particle sizes and similar problems have also been observed for other systems.[32,50,51]

CONCLUSION

Both commercial sulfathiazole (form III) and form I could be made fully amorphous by cryo-milling for 150 and 45 min, respectively, as determined by XRPD and NIR spectroscopy. No polymorphic transformation was observed during the cryo-milling process. It was found that the recrystallization of the amorphous phase that was prepared from form III and kept under vacuum at 22 ^{o}C is more complicated than that of amorphous sulfathiazole obtained from form I. Water not only increased the crystallization rates of amorphous phase of sulfathiazole, but also influenced the type and the amount of sulfathiazole polymorphs formed. NIR spectroscopy combined with multivariate data analysis can detect and quantify the crystalline disorder generated by cryo-milling of both sulfathiazole forms I and III.

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FIGURE CAPTIONS

Figure 1: Chemical structure of sulfathiazole.

Figure 2: XRPD patterns of cryo-milled sulfathiazole polymorphs at various milling times.

Figure 3: NIR spectra of cryo-milled sulfathiazole polymorphs at various milling times.

Figure 4: Scores plot (a) and the corresponding loadings (b) of the second-derivative NIR spectra of the cryo-milled samples. The arrows represent the direction of increasing amorphous content with milling time.

Figure 5: XRPD patterns of (a) sulfathiazole sample form III and (b) form I after cryo-milled for the times indicated. (1) immediately after cryo-milling, (2) after storage for 14 days at 4 ^{o}C under vacuum, (3) after storage for 14 days at 22 ^{o}C under vacuum, (4) after storage for 7 days at 22 ^{o}C, 35% RH conditions.

Figure 6: NIR spectra of the sulfathiazole samples cryo-milled for the times indicated for storage (a) 14 days at 22 ^{o}C under vacuum; (b) 7 days under 22 ^{o}C, 35% RH condition.

Figure 7: NIR spectra of 60 min cryo-milled sulfathiazole form I stored under 22 ^{o}C, 35 % RH condition for 3 hours.

Figure 8: Scores plot of the second-derivative NIR spectra of the cryo-milled samples stored at different conditions.

Figure 9: Percentage of process induced crystalline disorder *vs.* cryo-milling time monitored by XRPD and NIR methods.

Figures

Figure 1

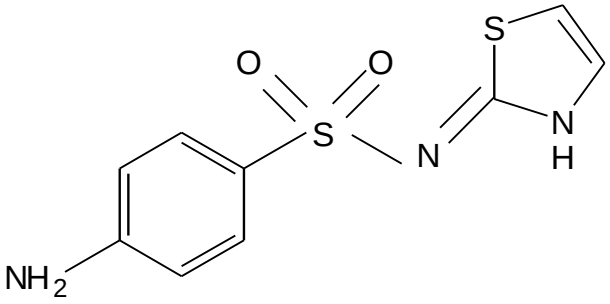


Figure 2

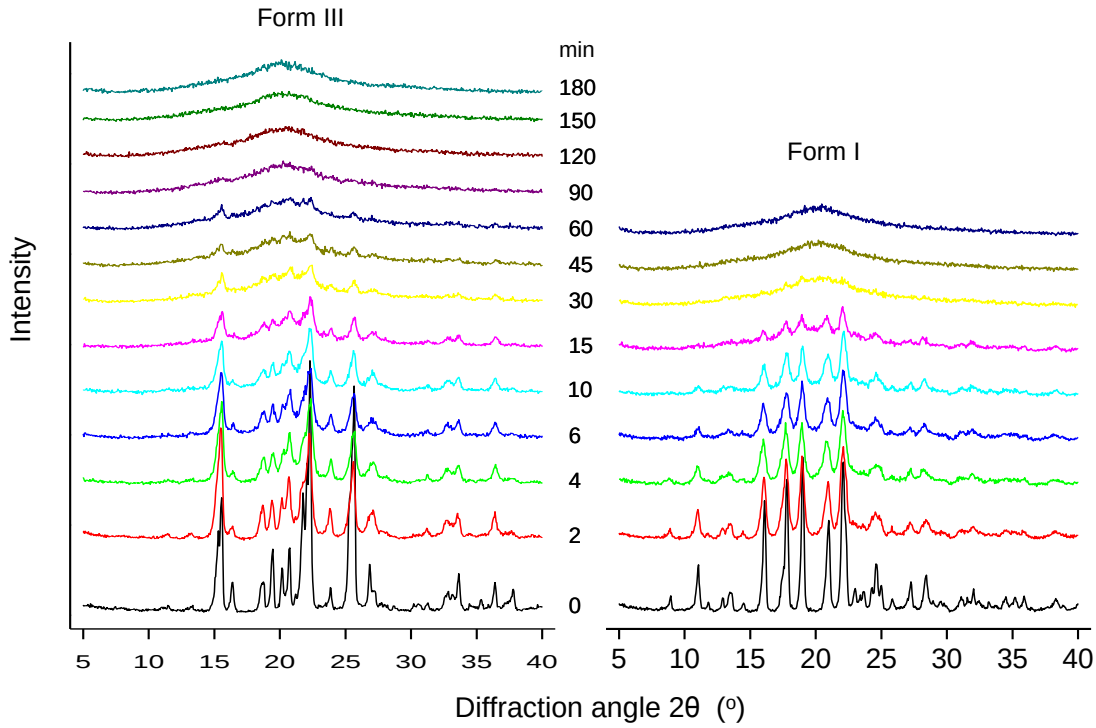
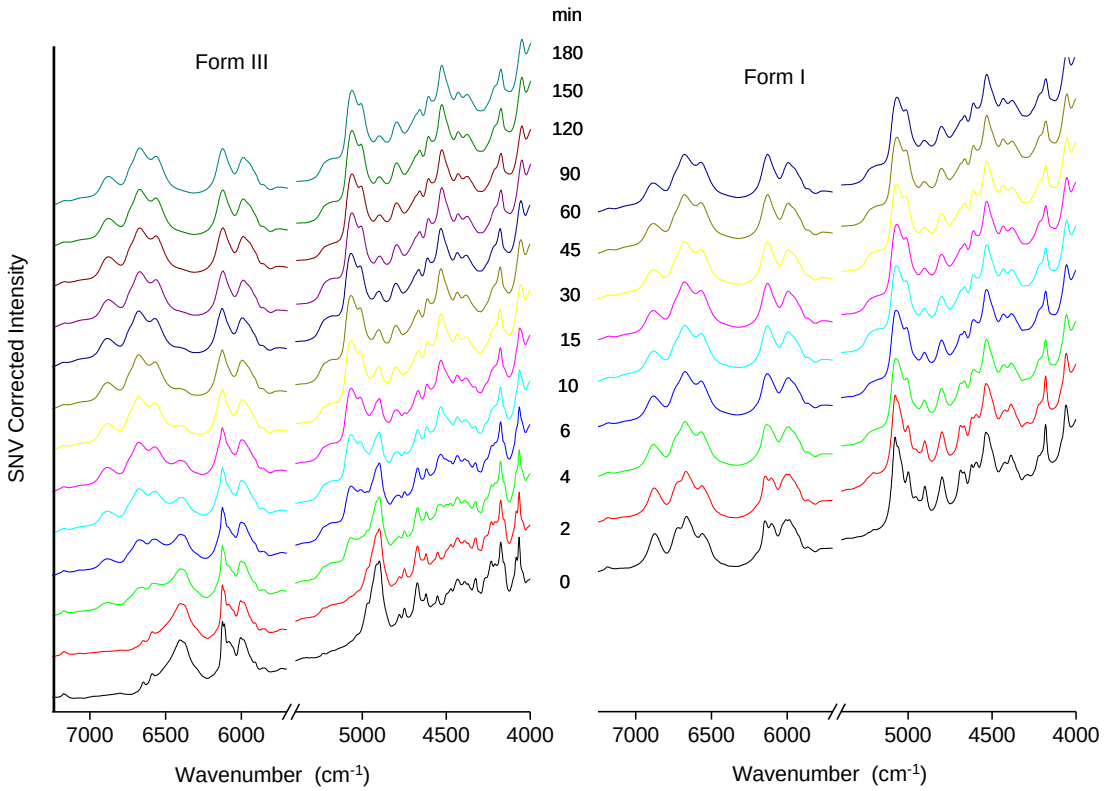


Figure 3



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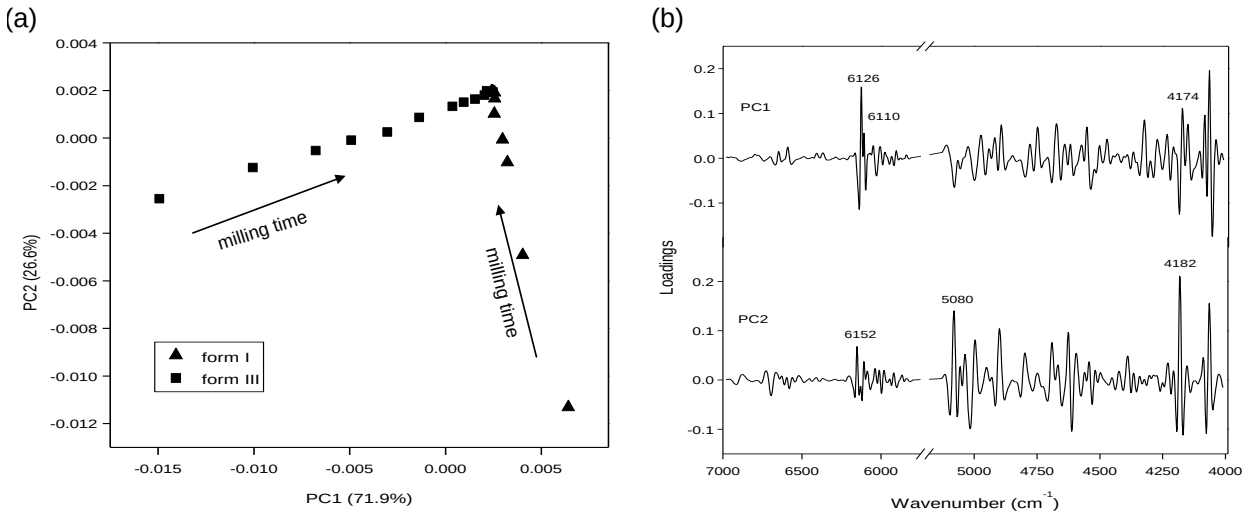

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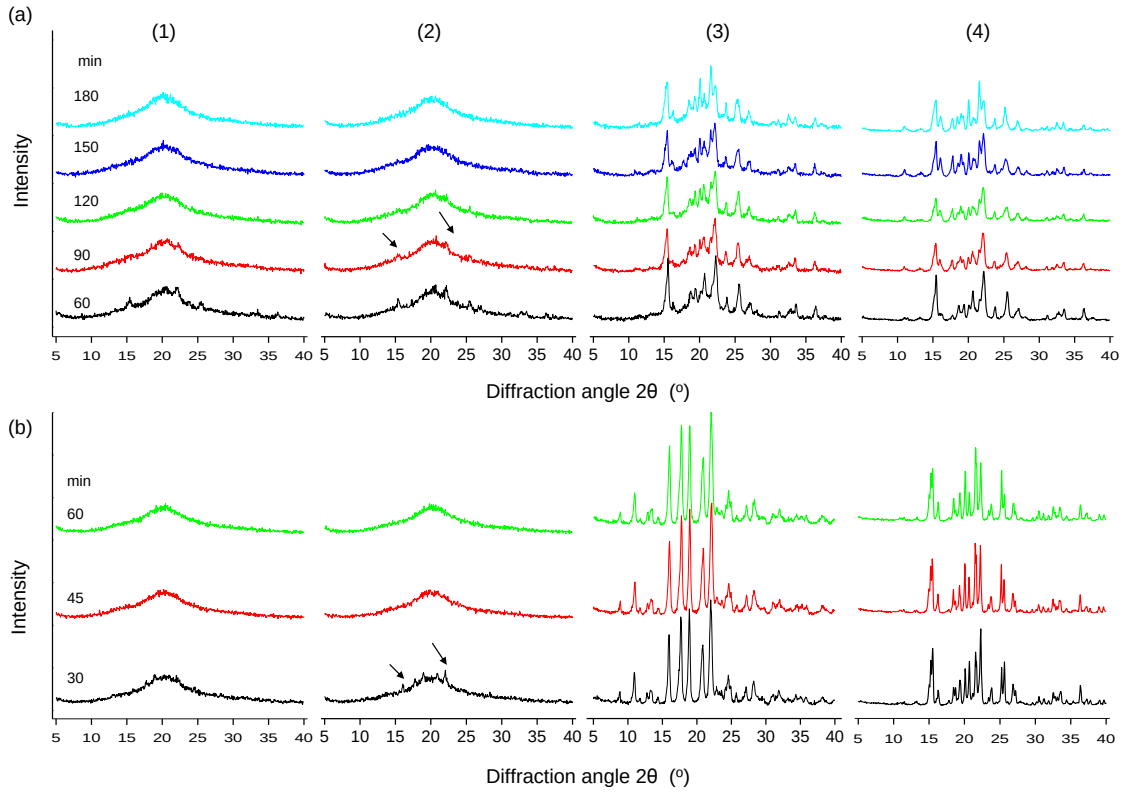

Figure 6

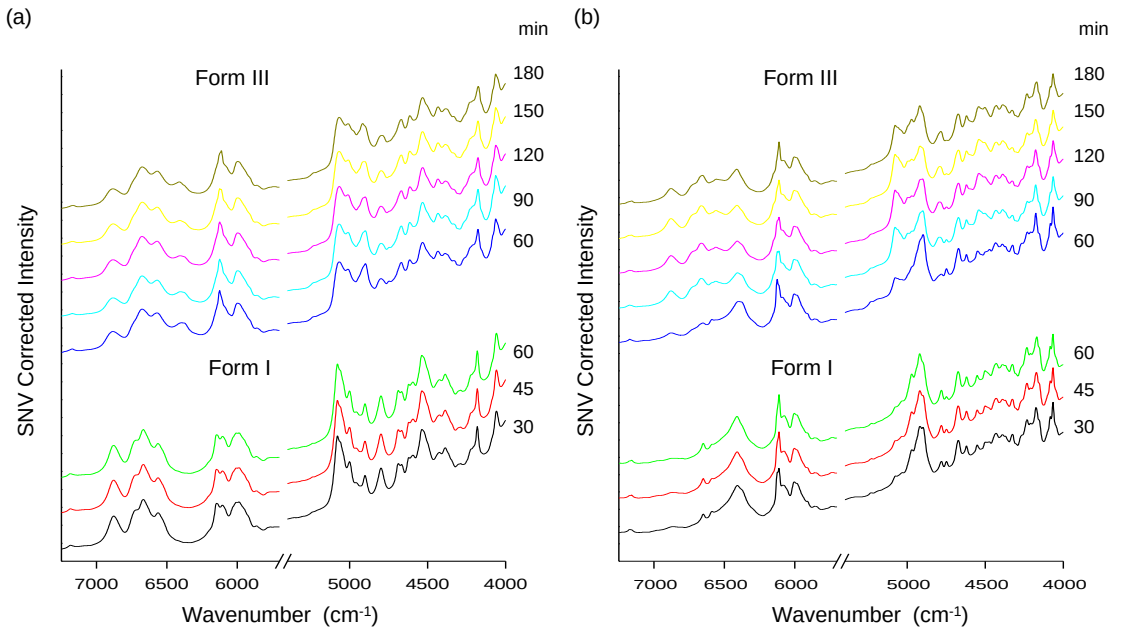

Figure 7

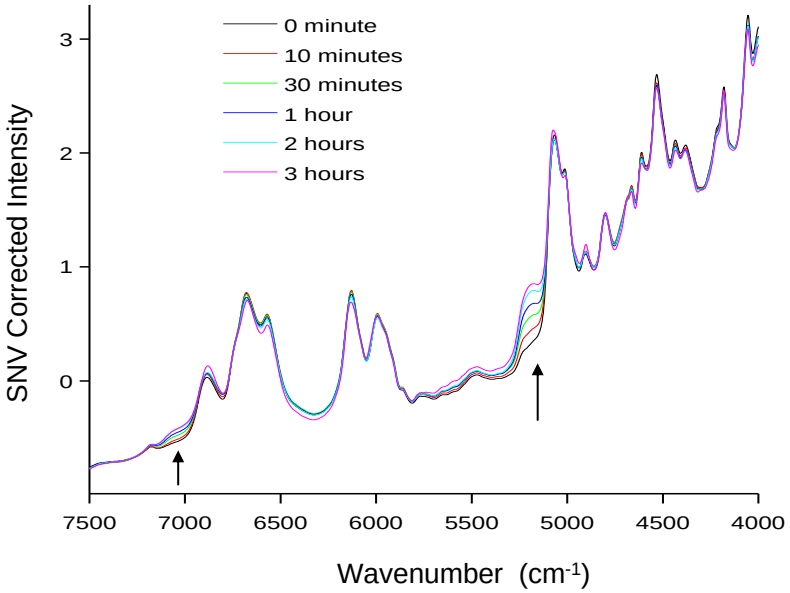

Figure 8

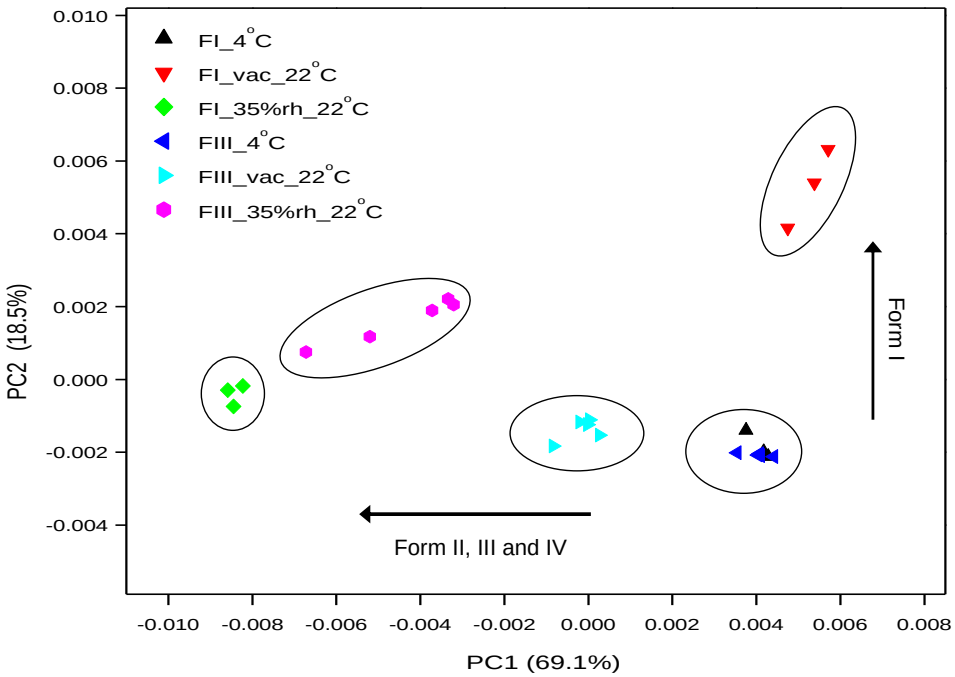

Figure 9

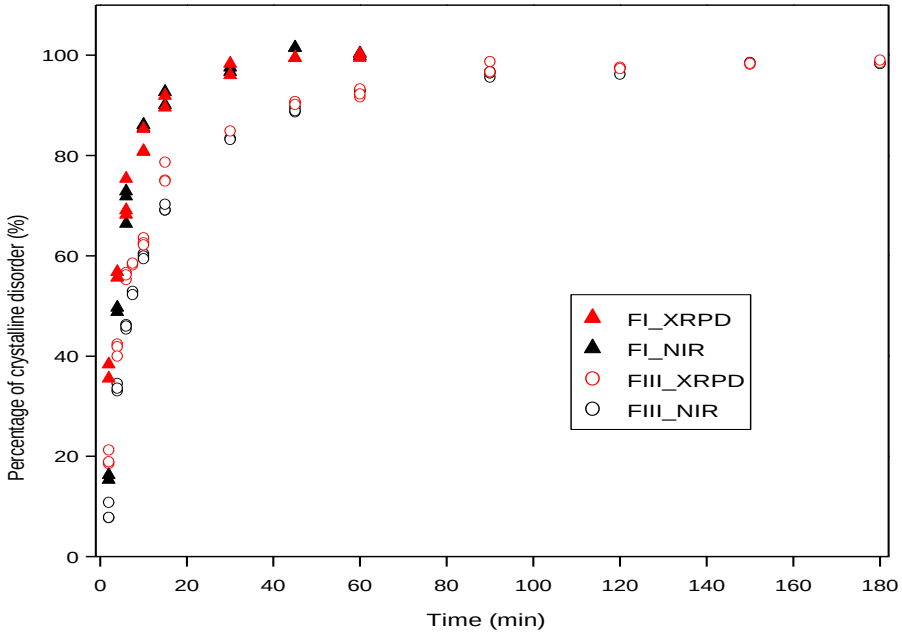

Table 1 Performance characteristics of multivariate XRPD and NIR methods.

Binary mixtures	Analytical methods	Pre-processing method	PLS factors	R^2	RMSEC (%)	RMSECV (%)	RMSEP (%)
Form I / Amorphous	XRPD	Area Normalization + 1st derivative	1	0.9976	1.58	1.80	1.22
	NIR	SNV	1	0.9988	1.11	1.31	1.24
Form III / Amorphous	XRPD	Area Normalization + 1st derivative	1	0.9981	1.39	1.59	1.38
	NIR	SNV	1	0.9987	1.18	1.35	1.18