

Solid-state Transformations of Sulfathiazole Polymorphs: the Effects of Milling and Humidity

Yun Hu¹, Boyan Li^{1,2}, Alan G. Ryder^{1,2}, Andrea Erxleben^{1,*}, Patrick McArdle^{1,*}, Åke C. Rasmuson³, B. Kieran Hodnett³

¹ *School of Chemistry, National University of Ireland, Galway, University Road, Galway, Ireland*

² *Nanoscale Biophotonics Laboratory, School of Chemistry, National University of Ireland, University Road, Galway, Ireland*

³ *Materials and Surface Science Institute, Department of Chemical and Environmental Sciences, University of Limerick, Ireland*

* Corresponding author. Tel.: +353 91 49 2487; Fax: +353 91 525700.

Email address: andrea.erxleben@nuigalway.ie (A. Erxleben)

p.mcardle@nuigalway.ie (P. McArdle).

Abstract

The effect of milling on the solid-state transitions of sulfathiazole polymorphs in the absence and presence of solvent and excipients was monitored by X-ray powder diffraction (XRPD), attenuated total reflectance infrared (ATR-IR) and near-infrared (NIR) spectroscopy. Sulfathiazole forms FII-FV undergo a transformation toward the metastable FI, which involves an intermediate amorphous stage upon milling at ambient temperature. Milling FIII with catalytic amounts of solvent converts FIII to FIV or to mixtures of FI and FIV depending on the solvent used. Pure FIV can be easily prepared from FIII by this method. The physical stability of pure sulfathiazole forms in the presence of different levels of relative humidity (RH) was also investigated. At low RH all sulfathiazole forms are stable but at RH levels above 70% FII, FIII and FIV remain stable while FI and FV transform to mixtures of FII and FIV without any apparent change in the external form of the crystals. Co-milling FIII with a range of excipients gave results which depended on the excipient used and co-milling with cellulose gave samples which had an amorphous content that was stable at 10% RH for at least nine months at ambient temperature.

Keywords:

Sulfathiazole; Milling; Polymorph transformation; Stability; NIR spectroscopy; X-ray powder diffraction; ATR-IR spectroscopy

1. Introduction

Milling or grinding processes are among the most common manufacturing procedures in the pharmaceutical industry and are typically thought of as particle size reduction processes. Milling is also often used to improve the dissolution properties and bioavailability of poorly water-soluble drugs by increasing their specific surface area in secondary pharmaceutical manufacturing [1-2]. However the high levels of mechanical energy generated during milling may cause structural changes in milled materials such as changes in crystal morphology, an increase in the number of crystal defects, a change in chemical stability, a polymorphic transformation and partial or complete amorphization. These changes clearly have the potential to lead to a significant change in the physicochemical properties of pharmaceutical products [2-7]. Since milling may affect the mechanical properties of pharmaceutical dosage forms an ability to control these transformations would be highly valuable.

It is well known that storage conditions, such as temperature, humidity or pharmaceutical excipients may affect the stability of amorphous and metastable crystalline forms [8]. In particular, hygroscopicity can have a significant impact on the physical and chemical stability of active pharmaceutical ingredients (APIs) [9]. Therefore, it is important to know the physicochemical stabilities of different forms and the kinetics of their transformation at various levels of relative humidity.

Sulfathiazole ($C_9H_9N_3O_2S_2$) (Figure 1), a sulfonamide antimicrobial agent, is known to have at least five polymorphic forms and a number of solvates [10-12]. The main differences between the polymorphs of sulfathiazole lie in hydrogen bonding and packing of the molecules in the crystal lattice [13]. There are some inconsistencies in the nomenclature of sulfathiazole forms in the literature and in this study the numbering of the polymorphic forms labeled FI–FV is in accordance with that used by the Cambridge Crystallographic Data Centre (CCDC) [14].

The influence of mechanical treatment in a planetary ball mill on the transformations of sulfathiazole polymorph III and sulfathiazole mixed with polyvinylpyrrolidone (PVP)

system has been reported [15-18]. However, apart from an increase in amorphous content, no transformations between sulfathiazole polymorphs by mechanical activation were observed in the X-ray powder diffraction (XRPD) analysis of the processed samples reported by Aaltonen et al. [19]. However, since those studies examined the effect of milling on just one or two polymorphic forms in this work the extent of polymorphic transformations on all five sulfathiazole forms during milling neat and with solvent and excipients is examined.

In our recent study, the physical stability of cryo-milled amorphous sulfathiazole samples was dependent not only on the initial polymorphic state but also on the storage conditions [20]. In this work the stability of the five sulfathiazole polymorphs under varying relative humidity (RH) levels of up to 98% is examined.

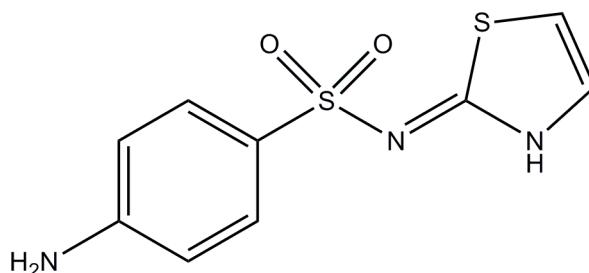


Figure 1 Molecular structure of sulfathiazole.

2. Material and methods

2.1. Materials

Sulfathiazole was purchased from Sigma-Aldrich with a purity of 98%, and was used as received. Millipore water was used. The solvents (HPLC grade) methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, acetonitrile, acetone, hexane, and ethyl acetate were obtained from Sigma-Aldrich. Spectrophotometric grade n-heptane was purchased from Fisher Scientific. Polyvinylpyrrolidone (PVP), monohydrate lactose, corn starch, microcrystalline cellulose, glucose, D-sorbitol, D-mannitol and glutaric acid were purchased from Sigma-Aldrich.

2.2 Methods

2.2 .1 Preparation of sulfathiazole polymorphs

There are numerous methods reported for the preparation of pure sulfathiazole forms however, Bakar et al. have recently reviewed the available methods and have selected a best method for the preparation of each form [21]. Commercial sulfathiazole was determined to be pure FIII and it was used as received. FI was prepared by heating commercial sulfathiazole samples in an oven for 30 min at 180 ° C as described in a previous study [11]. Crystals of FV were obtained by evaporating a boiling aqueous solution of sulfathiazole to dryness, followed by drying at 105 ° C [10,22]. FII was crystallized from acetonitrile using the method described by Bakar et al. [21]. It was discovered in this work that pure FIV can be selectively prepared by solvent drop milling of FIII (discussed below). The identification and confirmation of the stability of polymorphs FI–FV was carried out by XRPD, DSC, ATR-IR and NIR spectroscopy.

2.2.2 Milling experiments

Milling experiments were performed using an oscillatory ball mill (Mixer Mill MM400, Retsch GmbH & Co., Germany) using a 25 mL stainless steel milling jar containing one 15 mm diameter stainless steel ball at 25Hz. The experiments were carried out with 0.5g of sulfathiazole. Samples ground for 2.5, 5, 7.5, 10, 15, 30, 45, 60, 90 and 120 min were used to evaluate the effect of neat milling (NM) on the sulfathiazole polymorphs. The solvent-drop milling (SDM) or liquid-assisted milling (LAM) experiments were carried out by adding 0.025, 0.050 and 0.100 ml of a selected solvent to the sulfathiazole prior to milling. A total of 11 solvents were used in the SDM experiments and these solvents were water, methanol, ethanol, 1-propanol, 1-butanol, acetonitrile, acetone, hexane, n-heptane and ethyl acetate. The resulting powder samples were analyzed by XPRD, IR and NIR spectroscopy. Sulfathiazole FIII was also co-milled with excipients at a weight ratio of 1:1 for 15, 30, 60, 120 min. Pure FIV was obtained by milling 1.0 g sulfathiazole with ethanol (0.025 ml) in an oscillatory ball mill at 25 Hz for 30 min followed by storage in *vacuo* for 24 hours. In order to avoid possible over-heating of the samples, pause periods of 5 min were made after every 30 min of milling.

2.2.3 Stability study of sulfathiazole samples

The stability of the milled samples was studied at ambient temperature (22 ± 2 °C) under different relative humidity (RH) conditions ranging from 10 to 98% RH. RH values of 10, 43, 75, 85 and 98% were achieved in desiccators sealed using silica gel and using solutions of the salts K_2CO_3 , NaCl, KCl and K_2SO_4 [23]. Samples which had been milled for 60 and 120 min were exposed to the different RH environments and analyzed at regular time intervals by XRPD and IR and NIR spectroscopies.

2.3 Analytical techniques

2.3.1 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° (2θ) using CuK_{α} radiation with steps of 0.05° with a 2 seconds counting time per step. The Oscan software package was used to generate theoretical XRPD patterns of the sulfathiazole forms [24].

2.3.2 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 using interleaved scans in the 10000 – 4000 cm^{-1} range with a resolution of 8 cm^{-1} , using 32 co-added scans.

2.3.3 Attenuated Total Reflectance-Infrared Spectroscopy

ATR-IR spectra were recorded from 4000 to 650 cm^{-1} using a PerkinElmer Spectrum 400 (FT-IR/FT-NIR Spectrometer) with 32 accumulations at a resolution of 4 cm^{-1} . This instrument was equipped with a DATR 1 bounce Diamond/ZnSe Universal ATR sampling accessory.

2.3.4 Electron Microscopy

A JEOL CarryScope JCM-5700 scanning electron microscope was used to analyse the crystalline samples.

2.3.4 Data Analysis

Data analysis was carried out using the multivariate data analysis software The Unscrambler v.9.8 (Camo, Norway). Principal components analysis (PCA) was used to investigate the spectral variation during the milling process and stability of milled samples under various RH conditions. Standard normal variate (SNV) and second derivative pre-processing methods were sequentially applied to IR and NIR data. Savitzki-Golay second derivative calculations were performed with a window size of 11 points and a second order polynomial.

3. Results and Discussion

3.1 Characterization of sulfathiazole polymorphs

XRPD Patterns

It was of crucial importance in this study to be able to identify polymorph mixtures as accurately as possible and the crystal structures of the sulfathiazole polymorphs FI–FV were verified by comparing observed XRPD patterns with those calculated using the structures available on CCDC, Figure 2. On the whole, there was good agreement between the experimental and calculated patterns, however, there were some differences in intensities that are probably due to preferred orientation effects. The XRPD pattern of FI is distinguished by a 2θ peak at 10.9° , together with peaks at 16.0 , 17.7 , 18.8 , 20.9 and 21.9° . The peaks at 20.3 and 23.3° are specific for FV [10,21]. As is expected from their closely related crystal structures, the XRPD patterns of polymorphs FII, FIII, and FIV are very similar, but distinguishing peaks could still be identified for each form. FII has major peaks at 18.4 , 19.3 , 20.1 , 21.6° , and FIV has specific peaks at 20.7 , 22.2° . The XRPD patterns of FIII showed the intensity differences mentioned above and these preferred crystal orientation effects and minor variations in the unit cell have been

documented in the literature [10]. The presence of a triplet peak at 20.6 ° is indicative of the presence of FIII [14]. It has also been reported that it is difficult to obtain a pure sample of FIV and especially samples which have no trace of FII [14]. However, in this study it was found that pure FIV samples can be easily prepared using the SDM method and this is described below.

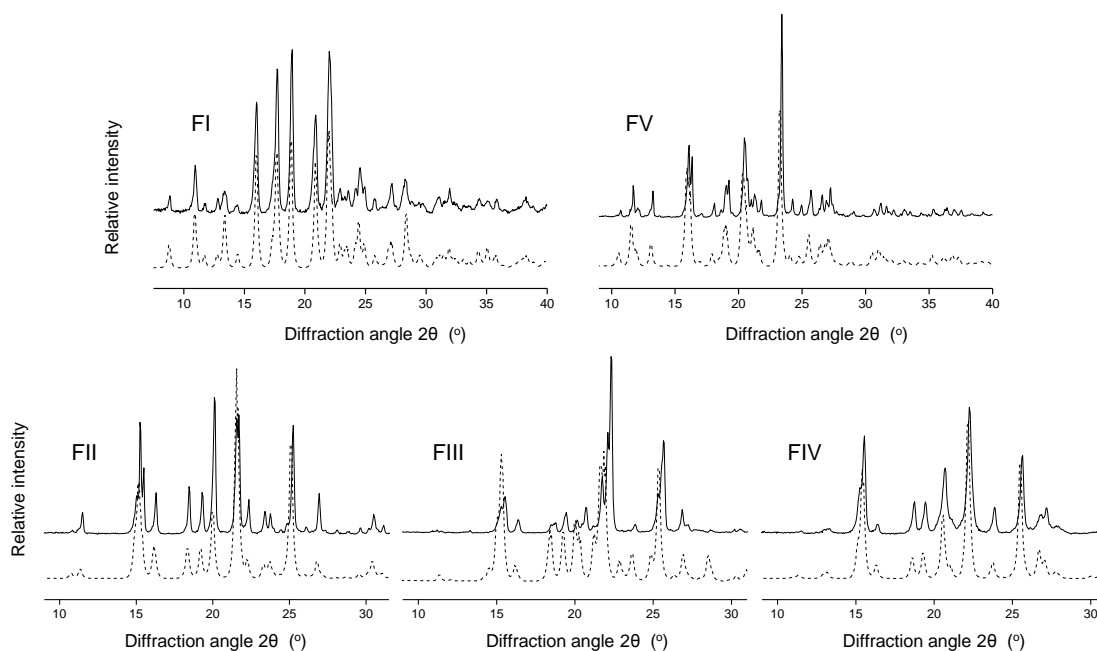


Figure 2 Theoretical (dashed traces) and experimental (solid traces) XRPD patterns of sulfathiazole forms FI–FV.

IR Spectra

The region of greatest interest in the IR spectra of crystalline and amorphous sulfathiazole is 3200–3500 cm^{-1} , as it is here that any differences in H-bonding will have their greatest effect, (Figure 3a). Each form has specific bands in the NH stretching region. The IR spectrum of amorphous sulfathiazole (FA) is most similar to that of FI, however the peaks are broader in the former. It is noted that a peak at 709 cm^{-1} is specific to FI and for FV bands were observed at 3443 and 3418 cm^{-1} . FII has a broad band at 3350 cm^{-1} and FIV has a small band shoulder at 3355 cm^{-1} . FIII has double overlapping bands at 3355 and 3346 cm^{-1} . Furthermore, the IR spectrum of FII shows a broad band at 1263 cm^{-1} , and a small band at 867 cm^{-1} . Thus it is possible to differentiate FII from FIII

and FIV.

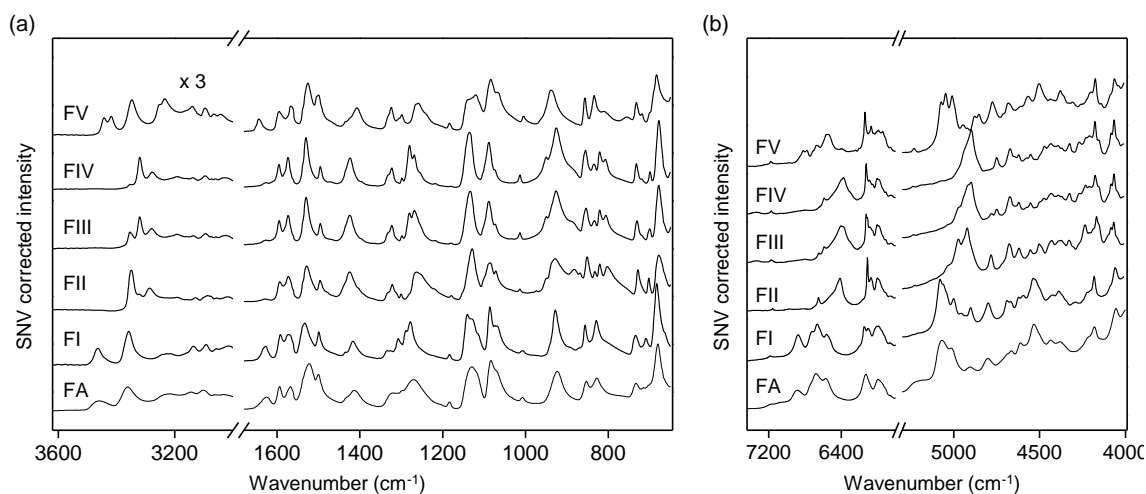


Figure 3 (a) ATR-IR and (b) NIR spectra of sulfathiazole amorphous (FA) and polymorphic forms FI–FV.

NIR Spectra

While IR and NIR spectroscopy can distinguish pure samples of the polymorphs with ease there can be considerable difficulty in the analysis of mixtures which contain FII, FIII and FIV. [19,24]. The NIR spectra of the five polymorphs and amorphous sulfathiazole differ most in the 7050–5820 cm^{-1} range which encompasses the region of the first overtones for the N-H stretching vibrations and in the region from 5150–4850 cm^{-1} where the combination bands of N–H stretching and NH_2 bending vibrations appear (Figure 3b). It is relatively easy to differentiate FI and FV. A broad band around 6880 cm^{-1} is characteristic of both FA and FI while the bands at 6820, 6778, 6138, 5046 and 4774 cm^{-1} are specific to FV. NIR spectra of FII and FIV show some clear differences, such as the bands at 6651, 6414, 6112, 4973, 4780 cm^{-1} for FII, and the bands at 6590, 6374, 6126, 4897 and 4749 cm^{-1} for FIV. This is due to the close similarity of their vibrational spectra as the H-bonding pattern of FIII in effect combines the H-bonding motifs of FII and FIV. Thus the IR and NIR spectra of FIII are virtually the sums of those of FII and FIV [13,25]. As will be seen below this problem is somewhat reduced in this work as mixtures containing FIII are not observed in most cases.

3.2 The effect of neat milling on forms I to V

Neat milling (NM) at room temperature was carried out on FI to FV for a range of times up to 120 minutes to gain an insight into mechanically activated polymorph conversion. The PCA scores and the corresponding loadings plots for the NIR spectra of the milled samples in the regions 6980–5800 and 5130–4000 cm^{-1} are shown in Figure 4. The clear spectral differences between FII, FV and FI/FA are reflected in the plot. The first two principal components (PCs) summarize 75.5% of the variation in the NIR spectra and again the amount of FI/FA increases with milling time. The transformation of forms (FII to FV) to FI/FA occurred in the direction of PC1, and the peaks at 6152, 6100, 5086 and 4186 cm^{-1} associated with FI and FA are positively weighted in the corresponding loading. The score values of PC2 differentiate the four forms, FII to FV, as shown by the PC2 loadings plot which shows the characteristic peaks of FV at 6138, 5046, 4774 and 4172 cm^{-1} . Similar PCA results were obtained using IR data (see supplementary information).

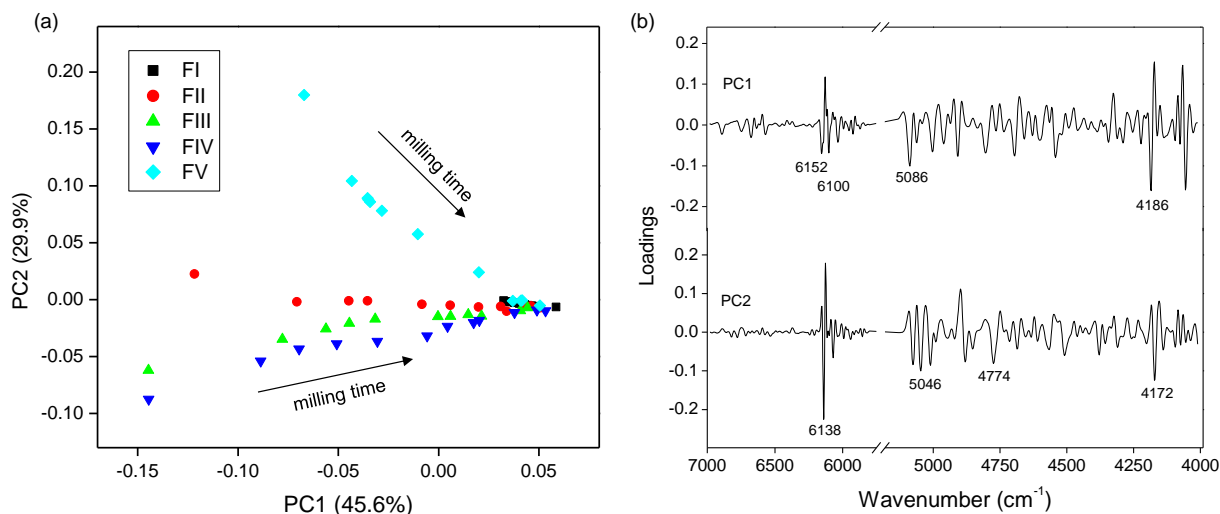


Figure 4 Scores plot (a) and the corresponding loadings (b) of the NIR spectra of the milled samples. The arrows represent the direction of milling time.

The NIR band at 6890 cm^{-1} and the IR band at 1628 cm^{-1} which are characteristic of FI/FA could also be used to monitor the polymorphic transformations which take place

on milling at room temperature. The results which illustrate the conversion of FI to FV to mixture of FI/ FA are shown in Figure 5. It is clear from the figure that the FI/FA component of the mixture increased rapidly in the first 30 min of milling time and that after 120 min the starting polymorph had been completely transformed to FI/FA. These findings show clearly that milling at room temperature led to the formation of FI/FA irrespective of the starting form.

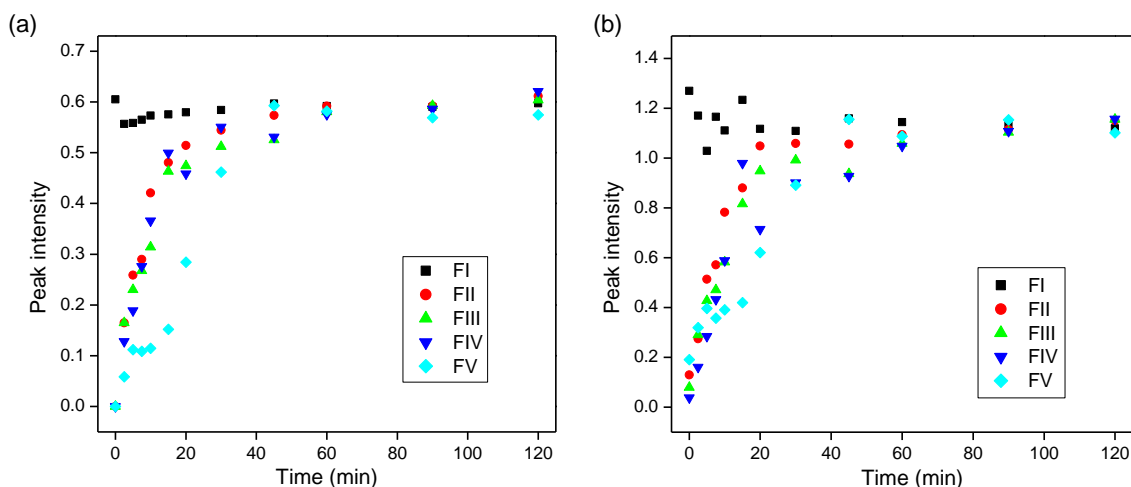


Figure 5 Peak intensity changes (a) of the NIR band at 6890 cm⁻¹, (b) and the IR band at 1628 cm⁻¹ with milling time.

Mechanical activation induced solid-state polymorphic transitions and amorphizations have been reported in many cases such as gabapentin [1], famotidine [26], fananserine [27] trehalose [28] and griseofulvin [29]. In general it is not clear if polymorphic transformations are direct crystal–crystal transformations or if an intermediate stage of transient amorphization or a transient unstable crystal phase is generated which is followed by a rapid recrystallization process to a known polymorph [27]. Several theories have been proposed to describe the mechanism of amorphization upon milling [5,27,30-32]. One widespread hypothesis suggests that amorphization is the result of local thermal melting that is followed by a rapid quench [30]. Another suggestion is that amorphization results from a lattice instability induced by a progressive static disordering of the initial crystals [4,27]. In addition, Fecht [31,32] has further suggested that the mechanism is associated with the accumulation of lattice defects in the

crystal resulting in an increase in the entropy of the crystal until it is above that of the liquid and if the temperature is below the glass transition temperature (T_g) the system effectively enters an amorphous state as if it were melting below the melting point. In this study no evidence for an unstable intermediate crystal form was found and transition through an amorphous form seems to be the most likely. In contrast to the present results cryo-milling of sulfathiazole led to complete amorphization as the recrystallization rate at low temperature is very slow [20].

3.3 Solvent-drop milling of sulfathiazole FIII

As a modification of the NM method, the SDM method can selectively control the polymorphic outcome with the addition of a minor quantity of a specific solvent to the material before milling. This has been successfully used in the preparation of a drug in a pure form and it is an environmentally friendly procedure [33-35]. The role of solvent is that of a crystallization catalyst and it is believed to function as a lubricant for molecular diffusion [36]. To the best of our knowledge, no study has reported the application of SDM to sulfathiazole.

In the present study, 11 solvents were used and samples of sulfathiazole FIII were milled for 30 min with different quantities of added solvent. The nonpolar solvents hexane and heptane gave mixtures of FI and FIV and the content of FI decreased with increasing amount of solvent. In the case of the polar solvents examined, the purity of the FIV obtained is strongly dependent on the amount of solvent used. A summary of the results obtained from SDM are presented in Table 1.

It is particularly interesting that FIII can be converted to pure FIV by milling with ethanol. It was further found that milling of all five forms with ethanol gave pure FIV. Thus, it is possible to prepare pure FIV in an environmentally friendly manner using the almost solvent-less SDM technique.

3.4 Co-milling sulfathiazole FIII in the presence of different excipients

Milling and mixing APIs with excipients is an important part of secondary processing in the pharmaceutical industry. API-excipient interactions can lead to the

stabilization or suppression of amorphization and polymorphic transitions of APIs. In this study, the effect of co-milling on the polymorphic transitions of FIII with eight excipients was investigated. Co-milling of FIII with the excipients examined gave three different outcomes as follows.

Co-milling FIII with corn starch, lactose monohydrate, glucose or mannitol gave a mixture of FI/FA. This result was identical to neat milling. The summarized results are presented in Table 2.

Milling FIII in the presence of sorbitol or glutaric acid gave FIV after 30 min milling. Extending the milling time in the presence of sorbitol to 60 min did not alter the result.

On co-milling FIII with PVP, FIII was converted to FA after milling for 30 min. It has been reported [17] that the appearance of a new band at 3220 cm^{-1} corresponds to the interaction of the sulfathiazole NH group with the PVP ketone oxygen in a mechanically activated sulfathiazole PVP mixture. However, this band comes from FA (Figure 6). None the less the IR bands of FA at 3460 and 3364 cm^{-1} do shift to the lower wavenumbers of 3450 and 3350 cm^{-1} in a PVP mixture and the PVP band at 1661 cm^{-1} also shows a small shift. These shifts suggest that there are interactions between the PVP C=O group and sulfathiazole N-H or NH_2 groups which stabilize FA [16]. FIII can also be transformed into FA after 120 min co-grinding with cellulose.

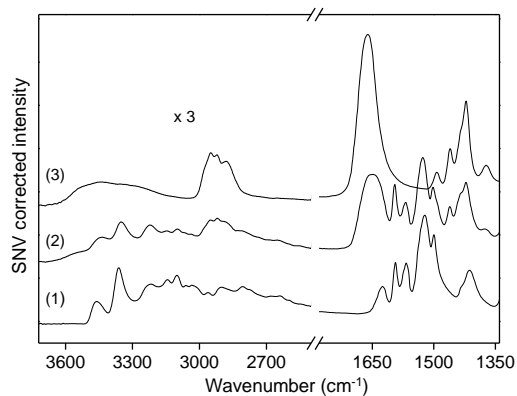


Figure 6 IR spectra of (1) melt-quenched sulfathiazole, (2) 60 min co-milled sulfathiazole-PVP (1:1) mixture, (3) 60 min milled PVP.

The first group of four excipients had no effect on outcome of the milling and thus there was no specific interaction between sulfathiazole and these excipients. The second group appeared to have the same effect as a drop of ethanol in the SDM experiments and it is reasonable that sorbitol and glutaric acid could be acting as lubricants as they are the excipients with the lowest melting points. It is only PVP and cellulose that stabilize FA. The stabilization of FA by PVP has been also been previously reported [16-18] and perhaps it is not surprising as PVP has also been found to stabilize the highly unstable amorphous naproxen [37].

3.5 Effect of humidity on sulfathiazole samples

3.5.1 Effect of humidity on sulfathiazole polymorph stability

The control of relative humidity is important in quality assurance of pharmaceutical solid dosage products and it is well established that humidity can induce polymorphic transitions in APIs [38-40].

Pure FI and FV were found to be relatively stable at 10% and 43% RH, in that no polymorphic changes were observed for up to two months. When the RH values were raised above 70% solid-state transformations of FI and FV were observed. It was possible to follow the rate of transformation using the NIR bands at 6872 and 6318 cm^{-1} for FI and FV respectively, Figure 7. It was clear that the rate of transformation increased with RH.

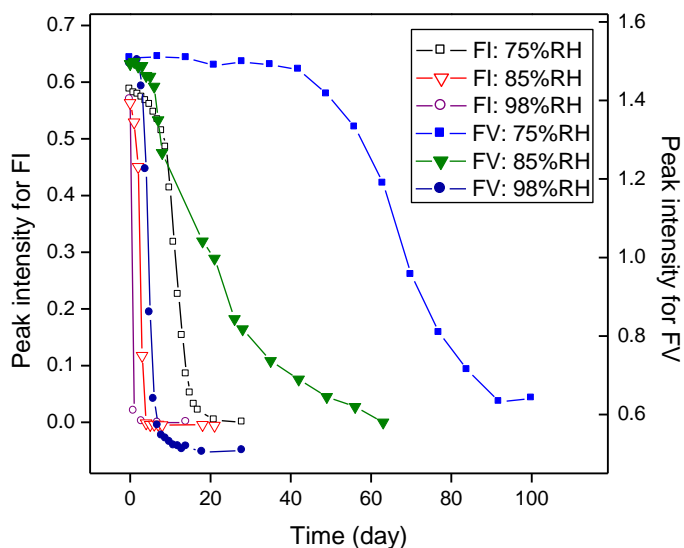


Figure 7 Intensity changes of NIR bands at 6872 cm⁻¹ for FI and 6318 cm⁻¹ for FV with time.

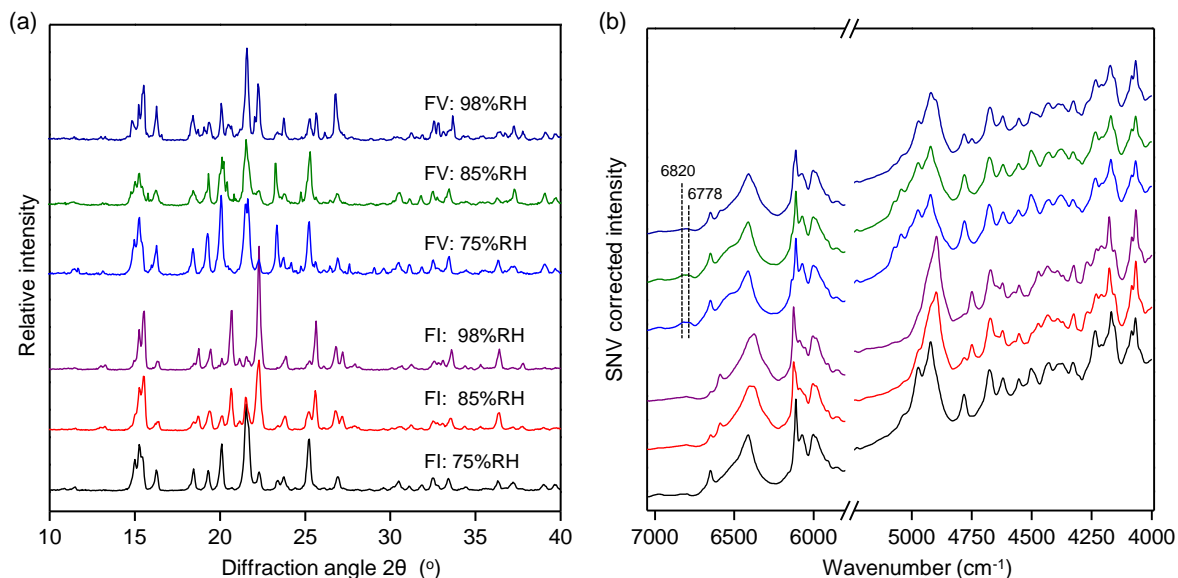


Figure 8 XRPD patterns (a) and NIR spectra (b) of FI and FV after exposure at different RH. The lines from bottom to top: FI at 75% RH for 28 days; FI at 85 % RH for 21 days; FI at 98% RH for 14 days; FV at 75% RH for 100 days; FV at 85% RH for 63 days; FV at 98% RH for 28 days;

The XRPD patterns and NIR spectra in Figure 8, show that in the presence of 75% RH, FI transformed to FII with a small amount of FIV (around 5%) in less than 28 days. At 85% RH, FI transformed to a mixture of FII and FIV which contained about 80% FIV in less than 21 days and at 98% RH FI transforms to a mixture of FII and FIV which is about 95% FIV within three days. Thus with increasing RH FI transforms more rapidly to mixtures of FII and FIV which range in composition from almost pure FII at 75% RH to almost pure FIV at 98% RH. In contrast the transformation of FV is a lot slower and at 75% RH transformation is to FII only and is 70-80% complete after 100 days. The amount of FV remaining is indicated by the NIR bands at 6820 and 6778 cm⁻¹, Fig. 8b. At 85% RH FV is also 70-80% converted after 63 days to FII with a small amount of FIV but at 98% RH FV is almost fully converted to FII and FIV (with approximately 30-40% FIV) after 28 days. Thus in summary FI and FV transform to mixtures of FII and FIV and the rate of transformation and the relative amount of FIV correlates with the RH value.

3.5.2 Effect of humidity on milled and co-milled sulfathiazole samples

The physical stability of sulfathiazole samples milled for 120 min, which are the mixture of FI and FA, was investigated at 10% RH up to 28 days, at 43% RH up to 56 days, and at 75 and 98 % RH for 14 days by using PCA method. As mentioned in our recent study [20], NIR bands around 7070 and 5200 cm^{-1} are caused by water absorbed by amorphous sulfathiazole. Thus, this region was excluded and a combination of the 6980–5800 and 5130–4000 cm^{-1} regions was chosen for PCA analysis. Figure 9a shows the PCA scores scatter plot obtained using the first and second PCs of the crystallized samples stored under different humidity conditions. The first two principal components summarized 97.6% of the variation in the spectral information. The PCA model allowed clustering of the crystallized samples based on relative humidity. The greatest differences arose from FA/FI with other polymorphic forms (FII, FIII and FIV) along PC1. At 10% RH the amorphous fractions in milled samples slowly recrystallized to FI as indicated in black dots, Fig. 9a. In contrast to the un-milled samples discussed in section 3.5.1., the milled samples at higher RH had a triplet peak at 20.6 ° which is characteristic of FIII and FIII/FIV is used to describe these samples [20]. Thus, at 43% RH the samples gradually recrystallized to mixtures of FII and FIII/FIV (red triangles) and the decrease in the intensity of the peak at 6880 cm^{-1} reflected the FA/FI decrease with time, (Fig. 9b). At 75 and 98% RH the same mixture of FII and FIII/FIV was also formed (green and blue squares) in a shorter time.

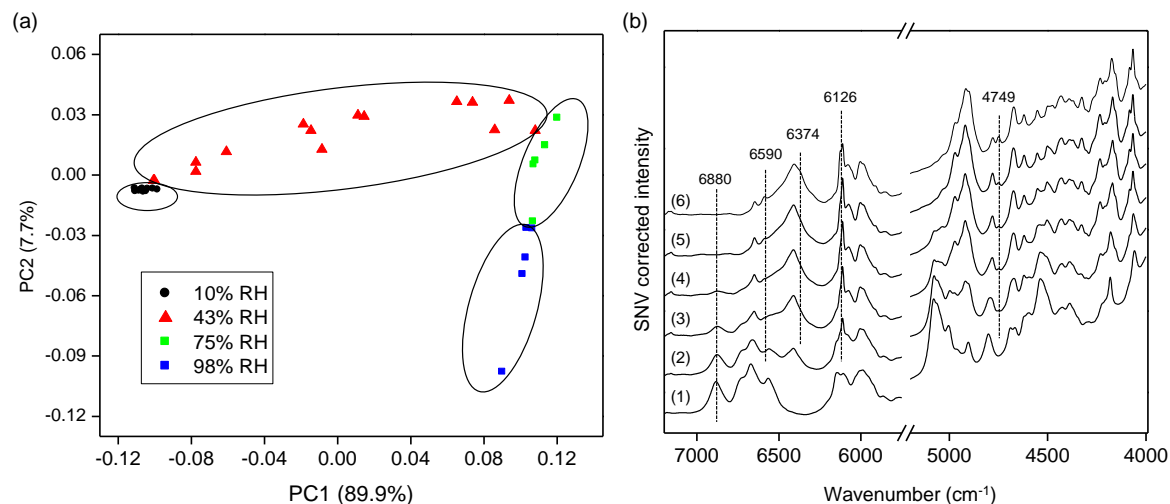


Figure 9 (a) Scores plot of the NIR spectra of the 120-min milled samples stored at different humidity conditions, (b) NIR spectra of 120-min milled form II samples stored (1) at 10% RH for 28 days; (2) at 43% RH for 14 days; (3) at 43% RH for 28 days; (4) at 43% RH for 56 days; (5) at 75% RH for 14 days; (6) at 98% RH for 14 days.

The second component, PC2, accounted for variability of FII and III/IV. The amount of FIII/IV is higher for the samples stored at 98% RH than at 75% RH, as demonstrated by the NIR bands of FIII/IV at 6590, 6374, 6126 and 4749 cm^{-1} .

The effect of RH levels on the stability of sulfathiazole samples co-milled with excipients was also examined. In the case of PVP, the amorphous phase of sulfathiazole remains amorphous at 10% and 43% RH for up to 28 days. When the RH was raised to 75%, the FA present showed some slight crystallization to a mixture of FII/FIII/FIV which could be detected by the presence of IR bands at 3355, 3346 and 3322 cm^{-1} . The amorphous phase in cellulose samples co-milled for 60 min re-crystallized to FI at 10 and 43% RH where the samples contained a mixture of FA and FI. However, cellulose containing samples co-milled for 120 min, in which sulfathiazole is fully amorphous, show that cellulose can stabilize the amorphous phase of sulfathiazole at 10% RH at ambient temperature at least nine months.

The amorphous content of the co-milled samples with the four excipients which showed less specific effects (corn starch, lactose, glucose and mannitol) usually crystallized to FI at 10 and 43% RH. However, the amorphous phase partly crystallized to

FII/FIII/FIV at 43% RH. The results are summarized in Table 3. It is interesting that in the presence of glucose pure FI was observed and was stable even at 75% RH for up to 1 month. The results clearly show that excipients co-milled with sulfathiazole can influence the stability of the forms observed at different RH levels.

3.6 The mechanism of humidity induced transformations

The specific NIR bands at 6872, 6112, and 6126 cm^{-1} for FI, FII and FIV were used to monitor the transformation at 85% RH, Figure 10. It is clear from these results that FI is transforming directly to FII and FIV and that FIV is not arising *via* the initial formation of FII.

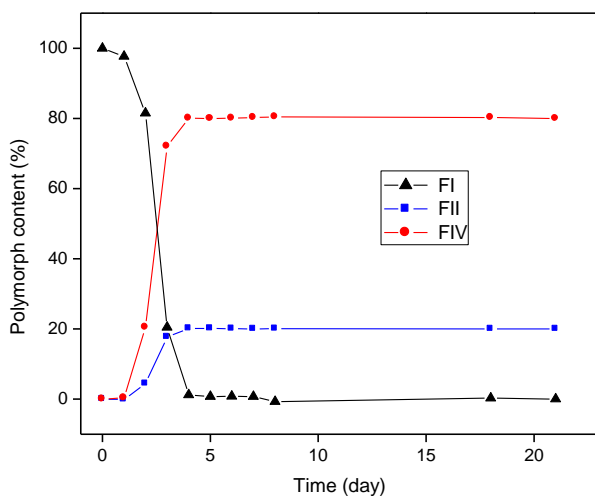


Figure 10 The time profile of the polymorph composition of FI samples at 85% RH.

In an attempt to shed further light on the mechanism of the humidity induced transformations scanning electron microscope images of the samples before and after exposure to the humid atmosphere were examined in an effort to decide between a direct crystal-crystal transition and a crystal-amorphous-crystal process. The images in Figure 11 show that the external form of the crystals does not change during the transformation. This observation would seem to favor a direct crystal-crystal transition. In an analysis of the crystal structures of the five forms using the *XPac* program Gelbricht et al. [41] have shown that the low density forms FI and FV have different structures that are unrelated to

those of FII, FIII and FIV. The latter three forms have structures based on the same mono-layer. FII and FIV have bi-layers which differ in a slip of the mono-layers while FIII is more complex in that it consists of the bi-layers of both FII and FIV. It is suggested that the transformations observed here involve the catalysed collapse of the low density form and simultaneous growth of either FII or FIV within a crystal leading to a product with little change in external shape. An intermediate amorphous phase would have been expected to lead to an extensive change in particle size. It is interesting that the form with the most complex structure, FIII, is not observed. The mechanisms for the formation of FII and FIV are probably simpler than that required for FIII. In fact FIII was rarely observed as a product of the neat milling experiments and it may be that the growth of FIII is assisted by the presence of a solution phase.

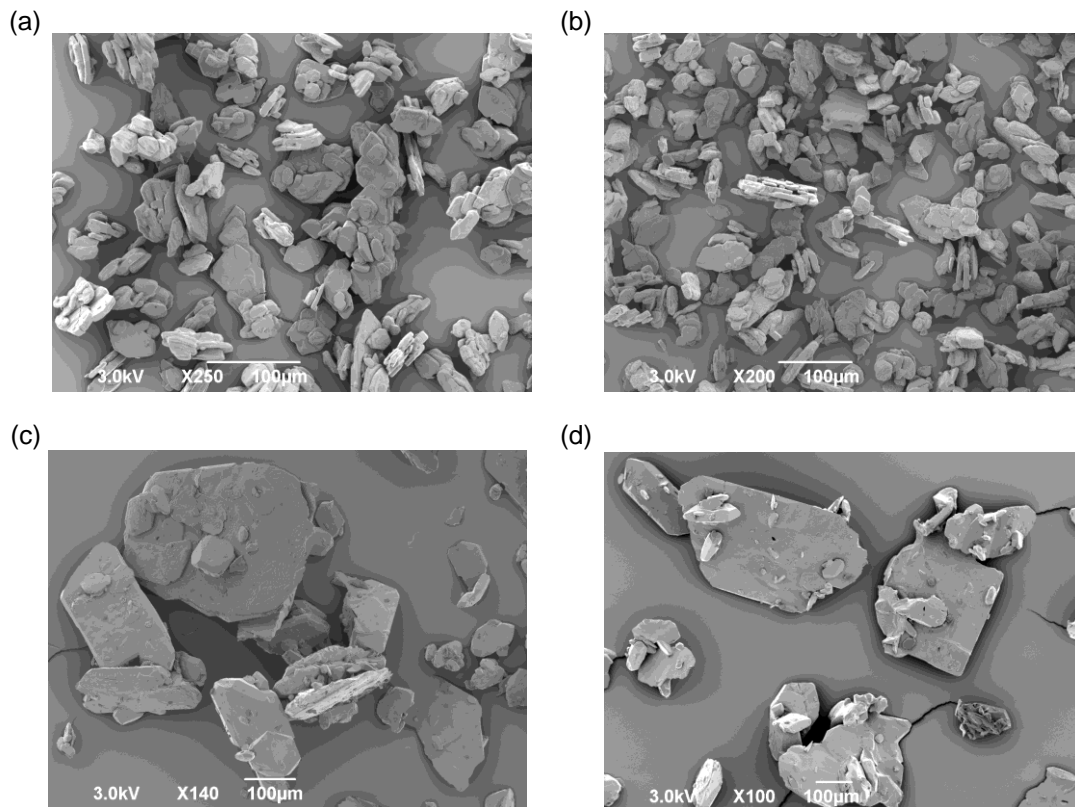


Figure 11 SEM images of FI (a) before, and (b) after exposure to 85% RH for 21 days and FV (c) before, and (d) after exposure to 98% RH for 28 days.

3.7 The most stable form under ambient conditions

Temperature induced solid state phase transitions among the five sulfathiazole polymorphs have been investigated by terahertz pulsed spectroscopy and differential scanning calorimetry (DSC) [25]. In that study FI was shown to be the most stable form at high temperature. FV melted at around 197 °C and recrystallized at a higher temperature to FI. The other three polymorphs FII, FIII and FIV converted to FI *via* a solid-solid phase transition at a temperature below 177 °C. Recently Munroe et al. investigated the relative stabilities using binary mixtures both in the solid state and in isothermal suspension equilibration experiments and concluded that at 50 °C the stability order was FI < FV <<< FIV < FII < FIII with only small differences between FII, FIII and FIV [14]. The transformations observed in this work at high humidity levels suggest that the stability order at ambient temperature is FI < FV <<< FII < FIV. It is not possible to include FIII in this order as it was not observed in the transformations of FI and FV. In the equilibration experiments mentioned above FIII and FIV did not equilibrate at ambient temperature thus it is still not possible to decide which of these forms is the most stable at room temperature [14]. It might appear that solvent drop milling at room temperature with a drop of ethanol which produced FIV from FIII suggests that FIV is the more stable form at ambient temperature, however, this conversion may proceed through the formation of FI/FA from which the generation of FIII, in the absence of a solution phase, appears impossible.

4. Conclusion

Milling and humidity can invoke polymorphic transformations of sulfathiazole as determined by XRPD, ATR-IR and NIR spectroscopy. Neat milling at ambient temperature gave a mixture of FI and FA after 120 min milling irrespective of the starting form. Pure FIV and its mixtures with other forms can be obtained by solvent drop milling of FIII. Co-milling of FIII in the presence of excipients gave results which were dependent on the excipient used. In the presence of elevated RH levels of up to 98% FII, FIII and FIV were stable. However, FI and FV transformed at RH levels above 70% to mixtures of FII and FIV by what appears to be a direct crystal to crystal transformation.

Samples co-milled with cellulose contained amorphous sulfathiazole which was stable at 10% RH and ambient temperature for at least nine months.

Acknowledgement

This work was supported by Science Foundation Ireland under Grant No. [07/SRC/B1158] as part of the Solid State Pharmaceutical Cluster (SSPC).

References

- [1] Lin, S.Y.; Hsu, C. H.; Ke, W. T. Solid-state transformation of different gabapentin polymorphs upon milling and co-milling, *Int. J. Pharm.* **2010**, *396*, 83–90.
- [2] Ng, W. K.; Kwek, J. W.; Yuen, A.; Tan, C. L.; Tan, R. Effect of milling on DSC thermogram of excipient adipic acid, *AAPS PharmSciTech* **2010**, *11*, 159-167.
- [3] Colombo I.; Grassi G.; Grassi M. Drug mechanochemical activation. *J Pharm Sci* **2009**, *98*, 3961–3986.
- [4] Willart, J. F.; Descamps, M. Solid state amorphization of pharmaceuticals, *Mol Pharm* **2008**, *5*, 905-920.
- [5] Planinšek, O.; Zadnik, J.; Kunaver, M.; Srčič, S.; Godec, A. Structural evolution of indomethacin particles upon milling: time-resolved quantification and localization of disordered structure studied by IGC and DSC, *J Pharm Sci* **2010**, *99*, 1968–1981.
- [6] Branham, M. L.; Moyo, T.; Govender, T. Preparation and solid-state characterization of ball milled saquinavir mesylate for solubility enhancement, *Eur. J. Pharm. Biopharm.* **2012**, *80*, 194–202.
- [7] Li, H.; Stowell, J. G.; He, X.; Morris, K. R.; Byrn, S. R. Investigations on solid–solid phase transformation of 5-Methyl-2-[(4-Methyl-2-Nitrophenyl)Amino]-3-Thiophenecarbonitrile, *J. Pharm. Sci.* **2007**, *96*, 1079–1089.
- [8] Raijada, D. K.; Singh S.; Bansal, A. K. Influence of Microenvironment pH, humidity, and temperature on the stability of polymorphic and amorphous forms of clopidogrel bisulfate, *AAPS PharmSciTech* **2010**, *11*, 197-203.
- [9] Uchida, H.; Yoshinaga, T.; Mori, H.; Otsuka, M. Application of a novel

- combination of near-infrared spectroscopy and a humidity-controlled 96-well plate to the characterization of the polymorphism of imidafenacin, *J Pharm Pharmacol*, **2010**, *62*, 1526–1533.
- [10] Anwar, J.; Tarling, S. E.; Barnes, P. Polymorphism of sulfathiazole, *J. Pharm. Sci.* **1989**, *78*, 337–342.
- [11] Apperley, D. C.; Fletton, R. A.; Harris, R. K.; Lancaster, R. W.; Tavener, S.; Threlfall, T. L. Sulfathiazole polymorphism studied by magic-angle spinning NMR, *J. Pharm. Sci.* **1999**, *88*, 1275–1280.
- [12] Bingham, A. L.; Hughes, D. S.; Hursthouse, M. B.; Lancaster, R. W.; Tavener, S.; Threlfall, T. L. Over one hundred solvates of sulfathiazole *Chem. Commun.* **2001**, 603–604.
- [13] Blagden, N.; Davey, R. J.; Lieberman, H. F.; Williams, L.; Payne, R.; Roberts, R.; Rowe, R.; Docherty, R. Crystal chemistry and solvent effects in polymorphic systems Sulfathiazole, *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 1035–1045.
- [14] Munroe Á.; Rasmuson, Å.C.; Hodnett, B. K.; Croker, D. M. Relative Stabilities of the Five Polymorphs of Sulfathiazole, *Cryst. Growth Des.* **2012**, *12*, 2825–2835.
- [15] Shakhshneider, T. P.; Boldyrev, V. V. Phase transformations in sulfathiazole during mechanical activation, *Drug Dev. Ind. Pharm.* **1993**, *19*, 2055–2067.
- [16] Shakhshneider, T. P. Phase transformations and stabilization of metastable states of molecular crystals under mechanical activation, *Solid State Ionics* **1997**, *101–103*, 851–856.
- [17] Boldyrev, V. V.; Shakhshneider, T. P.; Burleva, L. P.; Severtsev, V. A. Preparation of the disperse systems of sulfathiazole-polyvinylpyrrolidone by mechanical activation, *Drug Dev. Ind. Pharm.* **1994**, *20*, 1103–1114.
- [18] Caron, V.; Tajber, L.; Corrigan, O. I.; Healy, A. M. A comparison of spray drying and milling in the production of amorphous dispersions of sulfathiazole/polyvinylpyrrolidone and sulfadimidine/polyvinylpyrrolidone. *Mol. Pharm.* **2011**, *8*, 532–542.
- [19] Aaltonen, J.; Rantanen, J.; Siiriä, S.; Karjalainen, M.; Jørgensen, A.; Laitinen, N.;

- Savolainen, M.; Seitavuopio, P.; Louhi-Kultanen, M.; Yliruusi, J. Polymorph screening using near-infrared spectroscopy, *Anal. Chem.* **2003**, *75*, 5267–5273.
- [20] Hu, Y.; MacFhionnghaile, P.; Caron, V.; Tajber, L.; Healy, A. M.; Erxleben, A.; McArdle, P. Formation, physical stability and quantification of process induced disorder in cryo-milled samples of a model polymorphic drug, *J Pharm Sci* **2013**, *102*, 93-103.
- [21] Bakar, M. R. A.; Nagy, Z. K.; Rielly, C. D.; Dann, S. E. Investigation of the riddle of sulfathiazole polymorphism. *Int. J. Pharm.* **2011**, *414*, 86–103.
- [22] Chan, F. C.; Anwar, J.; Cernik, R.; Barnes, P.; Wilson, R. M. *Ab initio* structure determination of sulfathiazole polymorph V from synchrotron X-ray powder diffraction data, *J. Appl. Cryst.* **1999**, *32*, 436–441.
- [23] Lu, T.; Chen, C. Uncertainty evaluation of humidity sensors calibrated by saturated salt solutions, *Measurement*, **2007**, *40*, 591-599.
- [24] Hu, Y.; Erxleben, A.; Ryder, A. G.; McArdle, P. Quantitative analysis of sulfathiazole polymorphs in ternary mixtures by attenuated total reflectance infrared, near-infrared and Raman spectroscopy, *J. Pharm. Biomed. Anal.* **2010**, *53*, 412–420.
- [25] Zeitler, J. A.; Newnham, D. A.; Taday, P. F.; Threlfall, T. L.; Lancaster, R. W.; Berg, R. W.; Strachan, C. J.; Pepper, M.; Gordon, K. C.; Rades, T. Characterization of temperature-induced phase transitions in five polymorphic forms of sulfathiazole by terahertz pulsed spectroscopy and differential scanning calorimetry, *J. Pharm. Sci.* **2006**, *95*, 2486–2498.
- [26] Lin, S. Y.; Cheng, W. T.; Wang, S. L. Thermodynamic and kinetic characterization of polymorphic transformation of famotidine during grinding. *Int J Pharm* **2006**, *318*, 86–91.
- [27] Gusseme, A. D.; Neves, C.; Willart, J. F.; Rameau, A.; Descamps, M. Ordering and disordering of molecular solids upon mechanical milling: the case of Fananserine, *J Pharm Sci* **2008**, *97*, 5000-5012.
- [28] Willart, J. F.; Gusseme, A. D.; Hemon, S.; Odou, G.; Danede, F.; Descamps, M. Direct crystal to glass transformation of trehalose induced by ball milling. *Solid*

State Commun **2001**, *119*, 501–505.

- [29] Otte, A.; Zhang, Y.; Carvajal, M. T.; Pinal, R. Milling induces disorder in crystalline griseofulvin and order in its amorphous counterpart, *CrystEngComm* **2012**, *14*, 2560-2570.
- [30] Shakhshneider, T. P., Boldyrev, V. V. Mechanochemical synthesis and mechanical activation of drugs. In: Boldyreva, E.; Boldyrev, V. Reactivity of molecular solids. John Wiley & Sons, 271-312.
- [31] Fecht, H. J. Defect-induced melting and solid state amorphization. *Nature* **1992**, *356*, 133-135.
- [32] Grisedale, L. C.; Jamieson, M. J.; Belton, P. S.; Barker, S. A.; Craig, D. Q. M. Characterization and quantification of amorphous material in milled and spray-dried salbutamol sulfate: a comparison of thermal, spectroscopic, and water vapor sorption approaches, *J. Pharm. Sci.* **2011**, *100*, 3114–3129.
- [33] Trask, A. V.; Motherwell, W. D. S.; Jones, W. Solvent-drop grinding: green polymorph control of cocrystallisation, *Chem. Commun.* **2004**, 890– 891.
- [34] Trask, A.V.; Shan, N.; Motherwell, W. D. S.; Jones, W.; Feng, S.; Tan, R. B. H.; Carpenter, K. J. Selective polymorph transformation via solvent-drop grinding. *Chem. Commun.* **2005**, 880–882.
- [35] Aitipamula, S.; Chow, P. S.; Tan, R. B. H. Conformational polymorphs of a muscle relaxant, metaxalone, *Cryst. Growth Des.* **2011**, *11*, 4101–4109.
- [36] Braga, D.; Giaffreda, S. L.; Grepioni, F.; Pettersen, A.; Maini, L.; Curzi, M.; Polito, M. Mechanochemical preparation of molecular and supramolecular organometallic materials and coordination networks, *Dalton Trans.* **2006**, 1249–1263 .
- [37] Paudel, A.; Nes, E.; Mooter, G. V. Relating hydrogen-bonding interactions with the phase behavior of naproxen/PVP K 25 solid dispersions: evaluation of solution-cast and quench-cooled Films, *Mol. Pharm.* **2012**, *9*, 3301–3317.
- [38] Yoshinari, T.; Forbes, R. T.; York, P.; Kawashima, Y. Moisture induced polymorphic transition of mannitol and its morphological transformation, *Int. J. Pharm.* **2002**, *247*, 69–77.

- [39] Matsuo, K.; Matsuoka, M. Solid-State polymorphic transition of theophylline anhydrate and humidity effect, *Cryst. Growth Des.* **2007**, *7*, 411–415.
- [40] Bianco, S.; Caron, V.; Tajber, L.; Corrigan, O. I.; Nolan, L.; Hu, Y.; Healy, A. M. Modification of the solid-state nature of sulfathiazole and sulfathiazole sodium by spray drying. *AAPS PharmSciTech* **2012**, *13*, 647–660.
- [41] Gelbrich, T.; Hughes, D. S.; Hursthouse, M. B.; Threlfall, T. L. Packing similarity in polymorphs of sulfathiazole, *CrystEngComm*, **2008**, *10*, 1328–1334.
- [42] McArdle, P.; Hu, Y.; Lyons, A.; Dark, R. Predicting and understanding crystal morphology: the morphology of benzoic acid and the polymorphs of sulfathiazole, *CrystEngComm*, **2010**, *12*, 3119–3125.

Table 1. Solvent-drop milling of sulfathiazole FIII

Solvents	Polymorphs (Solvent: 0.025ml)	Polymorphs (Solvent: 0.050ml)	Polymorphs (Solvent: 0.100ml)
Water	FIV + FIII ^a	FIV + FIII	FIII
Methanol	FIV + FIII ^a	FIV + FIII	FIII
Ethanol	FIV	FIV	FIV + FIII ^a
1-propanl	FIV	FIV	FIV + FIII ^a
2-propanl	FIV + FI	FIV	FIV
1-butanoal	FIV	FIV	FIV + FIII ^a
Acetonitrile	FIV + FIII ^a	FIV + FIII	FIII + FIV
Acetone	FIV + FI	FIV + FI	FIV
Ethyl acetate	FIV + FI	FIV	FIV + FIII ^a
Hexane	FIV + FI	FIV + FI	FIV + FI
n-heptane	FIV + FI	FIV + FI	FIV + FI

^a the amount of FIII detected by NIR spectra is less than 10.0%.

Table 2. Sulfathiazole FIII co-milled with various excipients for different times

Excipients	Polymorphs (Milling time:15min)	Polymorphs (Milling time:30min)	Polymorphs (Milling time:60min)	Polymorphs (Milling time:120min)
PVP	FIII + FA	FA	FA	–
Cellulose	FIII+ FI + FA	FIII + FI + FA	FI + FA	FA
Corn starch	FIII + FI + FA	FIII + FI + FA	FIII + FI + FA	FI + FA
Lactose monohydrate	FIII + FA	FIII + FI + FA	FIII + FI + FA	FI + FA
Glucose	FIII + FI + FA	FI + FA	FI + FA	FI + FA
Mannitol	FIII + FA	FI + FA	FI + FA	FI + FA
Sorbital	FIII + FIV	FIV	FIV	–
Glutaric acid	FIII + FIV	FIV	–	–

Table 3. Composition of sulfathiazole FIII samples co-milled for 60 min and stored at different RH levels for 28 days.

Excipients	Relative humidity			
	10% RH	43% RH	75% RH	98% RH
PVP	FA	FA	FA + FII/FIII/FIV	N
Cellulose	FA + FI	FA + FI	FI + FII/FIII/FIV	FI + FII/FIII/FIV
Corn starch	FA + FI + FIII	FI + FIII	FI + FII/FIII/FIV	FI + FII/FIII/FIV
Lactose monohydrate	FA + FI + FIII	FI + FII/FIII/FIV	FI + FII/FIII/FIV	FII/FIII/FIV
Glucose	FA + FI	FI	FI	N
Mannitol	FA + FI	FI	FII/FIII/FIV	FII/FIII/FIV
Sorbital	FIV	FIV	N	N

FII/FIII/FIV: indicates a mixture FII, F III and FIV; N: an intractable paste formed.