International Journal of Pharmaceutics 391 (2010) 125–136

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Influence of polymer content on stabilizing milled amorphous salbutamol sulphate

P.N. Balani[a], S.Y. Wong[a], W.K. Ng[c], E. Widjaja[d], R.B.H. Tan[b,c,*], S.Y. Chan[a,**]

[a] Department of Pharmacy, National University of Singapore, Block S4, 18, Science Drive 4, Singapore 117543, Singapore
[b] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4, Engineering Drive, Singapore 117576, Singapore
[c] Crystallisation and Particle Sciences, Institute of Chemical and Engineering, Sciences, Agency for Science, Technology and Research, 1 Pesek Road, Jurong Island, Singapore 627833, Singapore
[d] Process Science & Modelling, Institute of Chemical and Engineering Sciences, Agency for Science, Technology and Research, 1 Pesek Road, Jurong Island,, Singapore 627833, Singapore

ARTICLE INFO

Article history:
Received 5 November 2009
Received in revised form 10 February 2010
Accepted 26 February 2010
Available online 6 March 2010

Keywords:
Salbutamol sulphate
Polyvinyl pyrrolidone K30
Co-milling
X-ray powder diffraction
Raman microscopy
Stability

ABSTRACT

The study investigates the influence of polyvinyl pyrrolidone (PVP) concentration on stabilizing the amorphous form of salbutamol sulphate (SS) before and after storage under ambient and elevated humidity conditions. Different mass ratios of SS and PVP (0–90 wt%) were co-milled using a planetary ball mill. X-ray powder diffraction (XRPD), high sensitivity differential scanning calorimetry (HSDSC), dynamic vapor sorption (DVS), infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and Raman microscopy (RM) were used to analyze the stability of the co-milled mixtures against heat and humidity treatments as well as storage at different humidity conditions. Prior storage, DSC and DVS analyses revealed that re-crystallization of amorphous SS was suppressed above PVP content of 33 wt%. Probable hydrogen bond interaction between SS and PVP was found in FT-IR analysis. XRPD diffractograms and SEM analysis showed stability against re-crystallization was achieved in the co-milled mixtures with a minimum PVP content of 80 wt% after storage. Homogeneous distribution of SS and PVP from RM analysis showed fine clustering of SS and PVP, suggesting the formation of an amorphous dispersion at molecular level. The results provide insights on the application of thermal and humidity treatments, accelerated stability testing and investigations on drug–excipient interactions to predict the minimum ratio of an excipient for stabilizing the amorphous state of a milled API.

1. Introduction

One of the major challenges of the pharmaceutical industry is to improve the water solubility of new chemical entities (NCEs) under development. Low water solubility and poor oral bioavailability affect the inherent efficacy of the NCEs (Vasconcelos et al., 2007). Hence, the amorphous form of the drug is desirable as it can have advantages of solubility, dissolution rate and better compression characteristics over the less soluble crystalline form (Yu, 2001), but not always. However, amorphous form of the drug is often associated with stability problems as it is thermodynamically unstable and tends to re-crystallize to stable but less soluble crystals under temperature and humidity stress encountered during accelerated storage conditions designed for testing pharmaceutical preparations (Vasconcelos et al., 2007). Thus, stabilization of an amorphous form of drug is particularly desirable for pharmaceutical products. For practical purposes, it is important to optimize a subtle balance between amorphization and stabilization (Watanabe et al., 2001), by application of techniques such as melt quenching, spray drying, melt extrusion to formulate stable amorphous drug–excipient dispersions (Miyazaki et al., 2004; Ghebremeskel et al., 2006; Pokharkar et al., 2006). Co-grinding or co-milling with excipients has also been recently studied to prepare amorphous materials that are more prone to degradation either by heat or solvent which preclude other preparation methods (Crowley and Zografi, 2002; Watanabe et al., 2003). Bahl and Bogner reported the improvement in the dissolution profile of indomethacin on co-grinding with silicates (Bahl and Bogner, 2008; Bahl et al., 2008). They previously reported the effects of humidity and drug–excipient ratios on the stability of amorphous indomethacin as a co-ground mixture (Bahl and Bogner, 2006). A stable amorphous indomethacin–silica alloy was formed due to plasticization of amorphous drug leading to mechanical transfer of drug to silicate, vapor phase mass transfer and water in facilitating particle–particle surface migration of

* Corresponding author at: Department of Chemical and, Biomolecular Engineering, National University of Singapore, Block E5, 4, Engineering Drive 4, #02-14, Singapore 117576, Singapore. Tel.: +65 65166360; fax: +65 67791936.
** Corresponding author. Tel.: +65 65162646; fax: +65 67791554.
E-mail addresses: chetanbh@nus.edu.sg (R.B.H. Tan), phacsy@nus.edu.sg (S.Y. Chan).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.02.029

the drug to the silicate. Other studies showed that the higher glass transition temperature and greater viscosity of polymers gave rise to a more stable amorphous drug mixture (Hancock et al., 1995; Hancock and Zografi, 1997). For instance, polyvinyl pyrrolidone (PVP), a hydrophilic polymer inhibited re-crystallization and stabilized the amorphous dispersions (Aso et al., 1996; Watanabe et al., 2003; Cirri et al., 2004). However, a major concern with the use of PVP is its hygroscopic nature. PVP has been reported to have an equilibrium moisture content of 27.8 wt% at 25 °C/75% RH and classified as "very hygroscopic" (Umprayn and Mendes, 1987). The hygroscopic nature of PVP could be detrimental when it is used to stabilize dispersions. Marsac et al. (2008) observed that 25 wt% PVP in the presence of water increased the nucleation rate of the solvent evaporated amorphous nifedipine–PVP molecular dispersions leading to re-crystallization. Vapor sorption studies indicated that pure amorphous nifedipine with a moisture uptake of around 1–2 wt% showed a significant increase in moisture uptake up to 6 wt% when PVP content was increased from 0 to 25 wt%. In an Atomic Force Microscopy study, Mahlin et al. (2006) found that the more PVP present in spray-dried lactose–PVP increased the propensity of moisture-induced re-crystallization. The glass transition temperature (T_g) of pure amorphous lactose was below 25 °C at 40% RH. This RH referred to as the critical crystallization RH increased steadily with more PVP in the composite mixture. PVP when used at high concentrations impaired the stability of co-formulated amorphous phase. On the other hand, Shakhtshneider's group (2007b) recently reported that lower PVP content resulted in less stable cryogenic co-ground mixture of indomethacin and PVP. Concentrations of PVP above 50 wt% was required to keep the amorphous indomethacin stable for 10 months. However, indomethacin–PVP solid dispersions prepared by solvent evaporation technique did not show any re-crystallization even at 20 wt% PVP. The authors suggested that amorphous forms prepared by grinding may contain crystalline seeds or nuclei which facilitated re-crystallization at low PVP concentrations.

Physical stabilization of the amorphous form is affected by several factors. Controlling molecular mobility, inter- and intramolecular interactions and variations in processing conditions play an important role (Bhugra and Pikal, 2008). With reference to drug–polymer systems, the stability of the amorphous form primarily depends on factors such as drug and polymer interaction, viscosity of polymer and glass transition temperature of the mixture (Choksi et al., 2008). In addition, the presence of polymer has been shown to increase the activation energy towards crystallization and could act as a physical barrier against crystallization. Although T_g serves as an important indicator of crystallization tendency, modes of molecular mobility not reflected by T_g should also be considered. This was clearly observed in case of amorphous solid dispersions of PVP with drugs such as nifedipine and felodipine, where T_g alone could not explain the reduced rate of crystallization in the presence of polymer and water (Marsac et al., 2008). On the other hand, PVP–indomethacin cryoground systems (Shakhtshneider et al., 2007b) were found to show an elevated T_g but at higher concentration of PVP (>50 wt%). Similar results were reported in case of PVP–piroxicam cryoground systems (Shakhtshneider et al., 2007a). Apart from factors discussed above, intra- and intermolecular hydrogen bonding interactions also play a key role in achieving stable amorphous dispersions. Miyazaki et al. (2004) attributed the ability of polyacrylic acid (PAA) to stabilize amorphous dispersions of acetaminophen to the strength of interaction between hydroxyl group of acetaminophen and the carboxyl group in PAA in comparison to PVP. Tang et al. (2002) investigated the differences in hydrogen bonding tendencies in the crystalline and amorphous states of seven dihydropyridines analogues. The authors concluded that for some compounds, hydrogen bonding was stronger in the crystalline state while for others, it was stronger in the amorphous state. A strong relation of hydrogen bonding in molecules to molecular mobility was also found in case of acetaminophen glass (Gunawan et al., 2006). This meant that the strength of the hydrogen bonding could significantly impact the structural relaxation time, i.e. the molecular mobility and hence impact the tendency to crystallize. Hence, a precise knowledge of such interactions could be useful if the acceptor or donor groups are targeted by polymeric excipients in order to achieve physical stabilization. Hydrogen bonding has also been reported in case of PVP–drug co-ground systems. Hydrogen bonding with PVP has played an important role in stabilizing amorphous co-ground systems of drugs such as indomethacin, piroxicam and sulphathiazole (Boldyrev et al., 1994; Shakhtshneider et al., 2007a,b).

However, investigations into the minimum ratio of PVP required to stabilize the amorphous form of milled pharmaceutical actives are lacking. Thus, the objective of this study is to understand the influence of the proportion of PVP for stabilizing amorphous co-milled SS before and after storage under ambient and elevated humidity conditions. The degree of crystallinity and the re-crystallization tendency of the co-milled mixtures were evaluated using X-ray powder diffractometry (XRPD), high sensitivity differential scanning calorimetry (HSDSC) and dynamic vapor sorption (DVS) respectively. Fourier transformed infrared spectroscopy (FT-IR) was used to study possible chemical interactions between the drug and polymer. Raman microscopy (RM) was utilized to map the spatial distribution of drug and excipient in the co-milled mixture. Scanning electron microscopy (SEM) analysis of co-milled mixtures kept under ambient and elevated humidity conditions (75% RH) was conducted to study the effect of re-crystallization on particle agglomeration and morphological changes.

Salbutamol sulphate (SS) is the model compound as a fully X-ray amorphous form could be obtained easily on ball milling (Balani et al., 2009), and this could readily undergo moisture-induced amorphous to crystalline transition at 60–70% RH (Balani et al., 2009; Brodka-Pfeiffer et al., 2003b). The stability of amorphous SS is dependent on storage conditions as the T_g of SS is influenced by relative humidity and temperature (Burnett et al., 2004). Brodka-Pfeiffer et al. (2003a,b) reported that the amorphous content of partially micronized SS decreased significantly from 7.7 to 2.5 wt% when stored for 24 h at ambient conditions of 25 °C and 45% RH. Storage at accelerated conditions of 40 °C and 75% RH resulted in complete re-crystallization within 5 h (Brodka-Pfeiffer et al., 2003a).

2. Materials

SS (≥99% purity) was purchased from Junda Pharmaceutical Co. Ltd. (Jiangsu, PR China). Polyvinyl pyrrolidone K-30 (PVP, M_w range 29,000–55,000, 99% pure) was purchased from (Sigma–Aldrich, Singapore). All other reagents and chemicals used were of analytical grade.

3. Methods

3.1. Ball milling

Sieved fraction (75–250 μm) of crystalline SS (cSS, non-milled) was prepared using Retsch Sieve Shaker (Retsch GmbH, Rheinische Straße, Haan, Germany) set at amplitude of 2.5 mm for 10 min. Milling of cSS was carried out using Fritsch Pulverisette 5 (Fritsch GmbH Pulverisette 5, Idar-Oberstein, Germany), a planetary ball mill equipped with stainless steel jar and balls (diameter 10 mm). As PVP is amorphous in nature, no prior milling was required. The mass ratio of ball to sample was kept at 50:1 (Parrott, 1974). The rotation speed was set at 300 rev min^{-1} and a milling duration of

60 min at room temperature (25 °C) was used for all samples. The milled SS (mSS) was characterized immediately after extracting the sample from the milling jars at the end of the milling process. Immediate characterization was needed due to the unstable nature of mSS.

3.1.1. Preparation of co-milled mixtures

The different drug to excipient ratios prepared for the study were: (5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 and 1:5, w/w) or (10–90 wt% PVP) of cSS with PVP. Different cSS:PVP ratios were mixed in a turbula mixer at 49 rpm for 20 min and mixtures were subsequently co-milled at room temperature (25 °C) in the planetary ball mill at conditions mentioned in the previous section of "Ball milling". The co-milled mixtures were characterized immediately after extracting the samples from the milling jars at the end of the milling process.

3.1.2. Preparation of physical mixtures

The physical mixtures of drug with PVP were prepared by mixing freshly milled drug (mSS) and PVP in a turbula mixer at 49 rpm for 20 min. Drug to excipient ratios were the same as those used in the preparation of co-milled mixtures. The physical mixtures were characterized immediately after extracting the samples from the glass jars at the end of the mixing process.

3.1.3. Storage conditions

Milled SS, co-milled mixtures of cSS:PVP and physical mixtures of mSS:PVP at different ratios were stored in desiccators at approximately 22 °C for 7 days. Storage humidity conditions of 15 and 75% RH were maintained using phosphorus pentoxide and standard hygrostatic NaCl solutions (OIML, 1996).

3.2. Characterization

3.2.1. XRPD

X-ray diffraction patterns of cSS, mSS, PVP, co-milled mixtures of cSS:PVP and physical mixtures of mSS:PVP were obtained using an X-ray powder diffractometer (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany), equipped with PSD Vantec-1 detector. Measurements were performed with monochromatized Cu Kα radiation (λ = 1.542 Å) over the reported (Columbano et al., 2002) angular range for SS from $2 < 2\theta < 40^\circ$ in step scan mode (step width 0.017°, scan rate 1° min^{-1}). Crystalline structures of the samples were verified by comparing to the standards reported in the Cambridge Structural Database.

3.2.2. HSDSC

HSDSC thermograms of mSS, PVP, co-milled mixtures of cSS:PVP and physical mixtures of mSS:PVP were analyzed using a Micro-DSC III (Setaram, Caluire, France). Approximately 20 mg of each sample was loaded into Hastelloy-made vessels (1 cm^3). Scanning was performed from 40 to 110 °C at a heating rate of 1 °C min^{-1}.

3.2.3. FT-IR

FT-IR absorbance spectra of cSS, mSS, co-milled mixtures of cSS:PVP and physical mixtures of mSS:PVP were obtained on a FT-IR spectrometer (Perkin–Elmer, 2000, MA, USA). Samples were prepared as a pellet in a KBR matrix. Thirty-two scans were collected for each sample with a spectral resolution of 4 cm^{-1}.

3.2.4. SEM

The particle morphology was examined by high resolution scanning electron microscopy (SEM) (JSM-6700F, JEOL Ltd., Tokyo, Japan) operating at 10 keV under secondary electron imaging (SEI) mode. Each sample was mounted on a carbon sticky tab and platinum coated for 1 min by a sputter coater (Cressington 208HR, Cressington Scientific Instruments Inc., Watford, UK), prior to analysis.

3.2.5. RM

Raman spectra of co-milled mixtures of cSS:PVP at ratios of 1:3, 1:5, 3:1 and 5:1 (w/w) as well as physical mixtures of mSS:PVP at ratio of 1:5 were measured using a Raman microscope (InVia Reflex, Renishaw) equipped with near infrared enhanced deep-depleted thermoelectrically Peltier cooled CCD array detector (576 × 384 pixels) and a high grade Leica 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. Raman point-by-point mapping with a step size of 5 μm in both the *x* and *y* directions was performed in an area of 100 μm × 100 μm. Measurement scans were collected using a static 1800 groove per mm dispersive grating in a spectral window from 700 to 1800 cm^{-1}. Since the collected raw Raman spectra were a combination of Raman scattering signals, spikes due to cosmic rays, and some autofluorescence background, spectral preprocessing was then carried out first in order to generate Raman spectral alone. Spikes were removed in the first step followed by baseline correction. The preprocessed Raman mapping data was then analyzed using the band-target entropy minimization (BTEM) algorithm (Widjaja et al., 2003) to reconstruct the pure component spectra (i.e. SS and PVP) from the spectra of the co-milled and the physical mixtures. BTEM algorithm is one of the self-modeling curve resolution (SMCR) techniques, which was developed to recover the pure component spectra of underlying constituents from a set of mixture spectra without recourse to any a priori known spectral libraries and can reconstruct pure component spectra of minor components (Li et al., 2002; Widjaja and Seah, 2008).

3.2.6. DVS

Sorption isotherms of co-milled mixtures of cSS:PVP were obtained using DVS (Advantage, Surface Measurement Systems, Alperton, UK). The humidity range was varied from 0 to 90% RH in steps of 10% RH at 22 °C. The instrument was operated at dm/dt mode to decide when equilibrium was reached, with a reported (Young and Price, 2004) dm/dt set at 0.002% min^{-1} within an interval of 5 min. All sample weights were approximately 10–12 mg.

4. Results and discussion

4.1. Effect of co-milling on crystallinity of SS

4.1.1. XRPD

To study the influence of co-milling SS with PVP on the crystallinity of SS, the XRPD plots of co-milled mixture of cSS:PVP and physical mixture of mSS:PVP at the same weight ratio of 1:1 were plotted together with those of cSS, mSS and PVP in Fig. 1. A broad diffusion amorphous halo at around 20° 2θ for mSS confirmed the X-ray amorphous state. In comparison, XRPD pattern of cSS matched very closely to the calculated pattern, based on the Cambridge Structural Database. PVP remained X-ray amorphous as suggested by amorphous halos. As expected, the characteristic peaks of SS were absent in all physical mixtures of mSS:PVP. Absence of crystalline peaks of SS in the diffraction pattern confirmed the amorphous nature of the co-milled mixtures. The concentration of PVP (10–90 wt%) had little effect on the crystallinity of co-milled mixtures as all were found to be X-ray amorphous (data not shown).

4.1.2. HSDSC

To study the thermal stability of co-milled samples, HSDSC analyses were conducted. The thermal characteristics of both milled

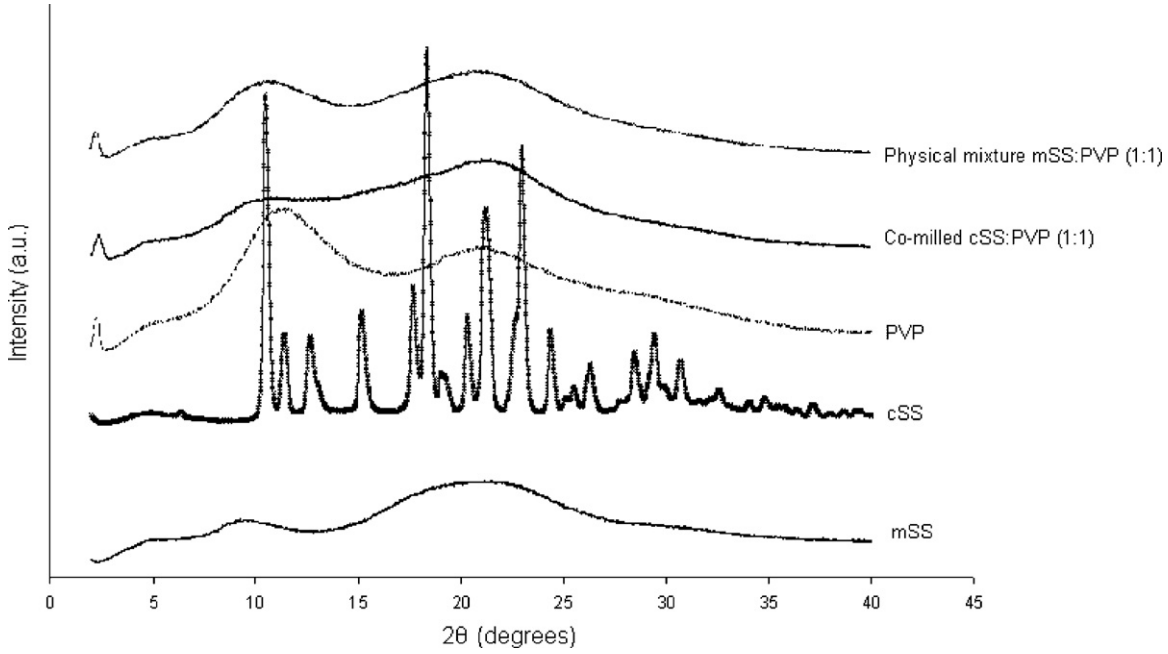

Fig. 1. Powder X-ray diffractograms of mSS, cSS, PVP, co-milled mixture of cSS:PVP 1:1 and physical mixture of mSS:PVP 1:1.

SS and PVP, and co-milled mixture of cSS:PVP and physical mixture of mSS:PVP at the same weight ratio of 1:1 are illustrated in Fig. 2. The DSC thermogram of milled SS showed a glass transition temperature (T_g) within the range of 60–65 °C, which agreed with that recently reported for spray-dried SS (Dhumal et al., 2009). As the temperature increased, molecules in the amorphous region gained sufficient energy and molecular mobility to overcome the activation energy barrier to re-crystallization. They rearranged themselves into their thermodynamically stable crystalline form (Burnett et al., 2004). The observed (re-crystallization induced) exothermal effect was shown with a peak at 104.15 °C with an apparent total heat of crystallization of 24.07 J/g. Pfeiffer and team also found a similar value for heat of crystallization for an amorphous form of SS obtained by ball milling in an agate mortar for 5 h (Brodka-Pfeiffer et al., 2003b). DSC thermogram of PVP alone did not show any heat transitions except a small change in baseline in range of 60–65 °C. This could be due to evaporation of absorbed water as previously reported for PVP (Friedrich et al., 2005). No shift in T_g of drug or presence of re-crystallization exotherm could be seen in the DSC thermogram of co-milled ratios of cSS:PVP (1:1–1:5). However at lower PVP contents of ratios 3:1 and 5:1 (cSS:PVP) re-crystallization exotherms could be seen. Re-crystallization exotherms as indicated by re-crystallization energies, were present in all physical mixture ratios (Table 1). Interestingly, distinct increases in the re-crystallization temperature in DSC thermogram of physical mixture mSS:PVP (1:1) (Fig. 2) and the re-crystallization energy of physical mixture mSS:PVP (1:1) were observed (Table 1). The re-crystallization energies increased with an increase in PVP content in all physical mixtures as seen in Table 1. A similar increase in crystallization temperature at PVP content of 20 wt% and below has been reported for indomethacin–PVP co-precipitates (Yoshioka et al., 1995). However, at the same time re-crystallization energies were found to decrease with increase in PVP content. It was also noted that the increase in re-crystallization energies was not proportional to the content of mSS in the physical mixtures (Table 1). Although; the reason for the increase in re-crystallization energies in DSC thermograms of physical mixtures was not clear, it could be reasonably said that the presence of PVP did have an effect on increasing re-crystallization temperature in the DSC thermogram of physical mixture mSS:PVP (1:1). Additionally, glass transition of drug in physical mixtures showed adherence to the Gordon–Taylor predicted equation (data not shown) only at PVP content of 25 wt% and below. Shakhtshneider's group (2007b) reported a similar observation in case of cryoground mixtures of indomethacin and PVP. The glass transition temperatures were not found to increase with PVP content as predicted by Gordon–Taylor equation. The non-ideal mixing of the system was explained in terms of molecular size differences between a macromolecule (PVP) and drug (indomethacin). An excess free volume will be introduced in the system due to large molecular size of PVP. The DSC results showed that co-milling of SS with PVP attributed thermal stability to the amorphous SS at ratio at PVP content of 33.3 wt% and above. Physical mixing with PVP at all ratios did not enhance the stability of the mixtures.

4.1.3. FT-IR

To evaluate possible intermolecular interactions in co-milled SS, spectra of individual components, physical and co-milled mixtures were recorded using FT-IR (Fig. 3a and b). In Fig. 3a, FT-IR spectrum of cSS was well characterized by the presence of peaks corresponding to secondary amine salt (C–N) stretch at 1616 and 1509 cm^{-1}

Table 1
Re-crystallization energies.

Components	Energy of re-crystallization (J/g)
mSS	24.07
Co-milled cSS:PVP (1:1)	NO[a]
Physical mixture mSS:PVP (1:1)	38.80
Co-milled cSS:PVP (1:2)	NO
Physical mixture mSS:PVP (1:2)	40.23
Co-milled cSS:PVP (1:3)	NO
Physical mixture mSS:PVP (1:3)	46.41
Co-milled cSS:PVP (1:5)	NO
Physical mixture mSS:PVP (1:5)	48.77
Co-milled cSS:PVP (2:1)	NO
Physical mixture mSS:PVP (2:1)	41.55
Co-milled cSS:PVP (3:1)	0.92
Physical mixture mSS:PVP (3:1)	39.50
Co-milled cSS:PVP (5:1)	0.45
Physical mixture mSS:PVP (5:1)	37.70

[a] Re-crystallization exotherm: not observed.

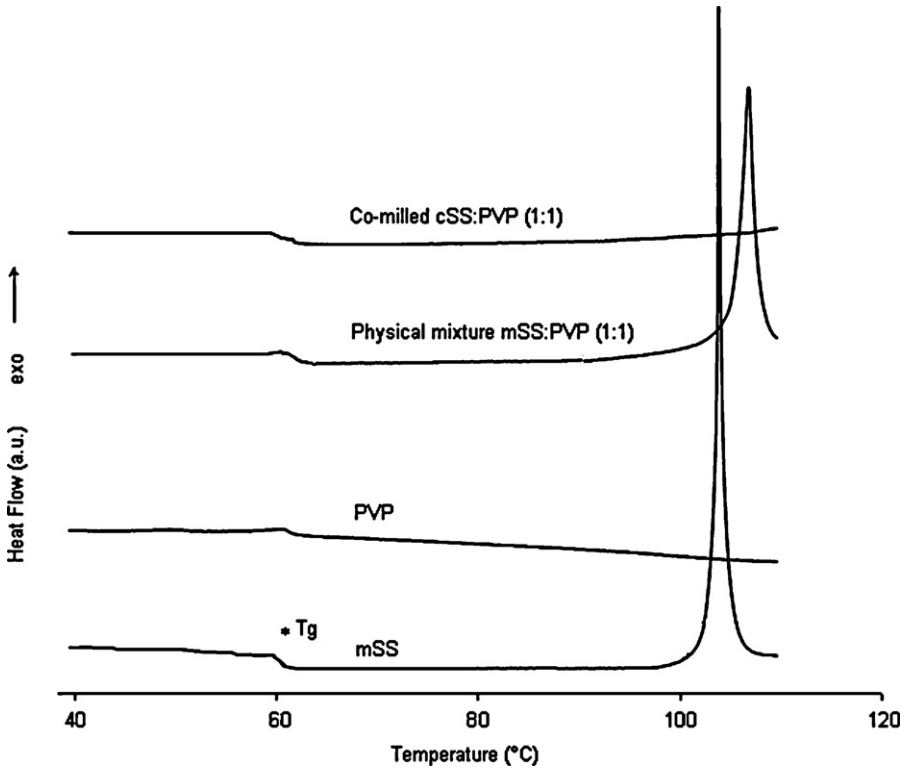

Fig. 2. HSDSC thermograms of mSS, PVP, physical mixture of mSS:PVP 1:1 and co-milled mixture of cSS:PVP 1:1.

apart from phenolic C–O stretching and secondary alcoholic C–O stretching at 1209 and 1085 cm^{-1} respectively (Geeta and Baggi, 1989; Hyvönen et al., 2005). Under mechanical activation, these peaks decreased in intensity as seen in spectra of milled SS. PVP showed the characteristic C=O stretching vibration at 1654 cm^{-1} (Shakhtshneider, 1997). Similar peaks were observed in the physical mixture of mSS:PVP (1:1), suggesting no interaction between SS and PVP in the physical mixture. Co-milled mixture at similar ratio exhibited distinct changes in the IR spectra. The characteristic phenyl stretch seen at 1209 cm^{-1} in the physical mixture ratio (1:1) had shifted to a lower wavenumber (~5 cm^{-1}) in case of the co-milled mixture. The secondary alcoholic C–O stretching peak at 1085 cm^{-1} was almost absent in the co-milled mixture spectra. The peak was quite evident in the physical mixture at similar ratio. The OH and NH stretching vibrations (>3000) of cSS while still vaguely evident in physical mixture samples could not be observed in co-milled mixture (lower PVP) (Fig. 3b). Moreover, the presence of a single peak at 1654 cm^{-1} corresponding to the carbonyl of PVP (Fig. 3a) in comparison to two distinct peaks at 1616 and 1654 cm^{-1} in the physical mixture (Fig. 3a) suggested that there was some chemical interaction between the components during co-milling. Banchero et al. (2009) have recently reported similar peak broadening in FT-IR spectra of PVP/piroxicam impregnated samples. Hydrogen bonds between the NH group of drug and the N or C=O functions of the PVP were strong enough to weaken the drug NH stretching resulting in a weak and broad peak that is completely covered by the bond stretches of the PVP. As reported earlier (Shakhtshneider, 1997; Chen et al., 2008) for SS–PVP composites, our results supported the notions that amino (NH) stretch of SS formed hydrogen bonds with C=O group of the polymer. At higher ratios of SS (SS:PVP) (≤50 wt% PVP), carbonyl peak of PVP at 1654 cm^{-1} (data not shown) did show increased absorbance in co-milled ratios in comparison to physical mixtures but did not change its position. Although an increase in intensity of SS peaks was observed in co-milled mixtures with higher ratios of SS, a change in peak broadening (>3000) as seen in Fig. 3b was not evident. This meant that, formation of a solid dispersion was uncertain in co-milled ratios containing less than 50 wt% of PVP. On the other hand, although increases in absorbance of SS peaks were less prominent in co-milled mixtures at higher ratios of PVP (≥50 wt%), the shifting of NH stretch to lower wavenumber was quite notable in comparison to physical mixture at similar ratios (data not shown). The results were found to agree with strong hydrogen bonding reported for PVP–felodipine dispersions (Konno and Taylor, 2006). Thus, FT-IR results further supported DSC results and indicated that the inter-molecular interactions between SS and PVP could have resulted in stable amorphous form of SS on co-milling with PVP (≥50 wt%).

4.1.4. RM

The collected Raman image data with co-milled mixtures of cSS:PVP at ratios of 1:3, 1:5, 3:1 and 5:1 (w/w) and physical mixture of mSS:PVP at ratio of 1:5 were preprocessed and analyzed using the BTEM method. Unlike other self-modeling curve resolution (SMCR) techniques, BTEM has been recently reported to be applicable in resolving pure component spectra of species present at sub-ppm levels both in non-reactive and reactive multicomponent liquid systems measured by FT-IR as well as in resolving pure component spectra of Raman data measured from solid-state chemical problems and biological materials (Widjaja and Seah, 2008). Fig. 4 shows the pure component reference spectra of cSS, PVP and the extracted reference spectra of physical mixture mSS:PVP (1:5) and co-milled mixture ratio of cSS:PVP (1:5) estimated by BTEM. The extracted pure component spectra estimate of co-milled mixture of cSS:PVP at ratio of 1:5 (Fig. 4d) exhibited combined peaks of SS and PVP which bore resemblance to Raman peaks for cSS (Fig. 4a) and PVP (Fig. 4b) respectively. A distinct spectral variation in the extracted pure component spectrum estimate was not evident which suggested that there could be composition co-linearity between the two components. On the other hand, BTEM analysis was able to resolve or separate out the pure component spectra of SS and PVP in the physical mixture of mSS:PVP at ratio

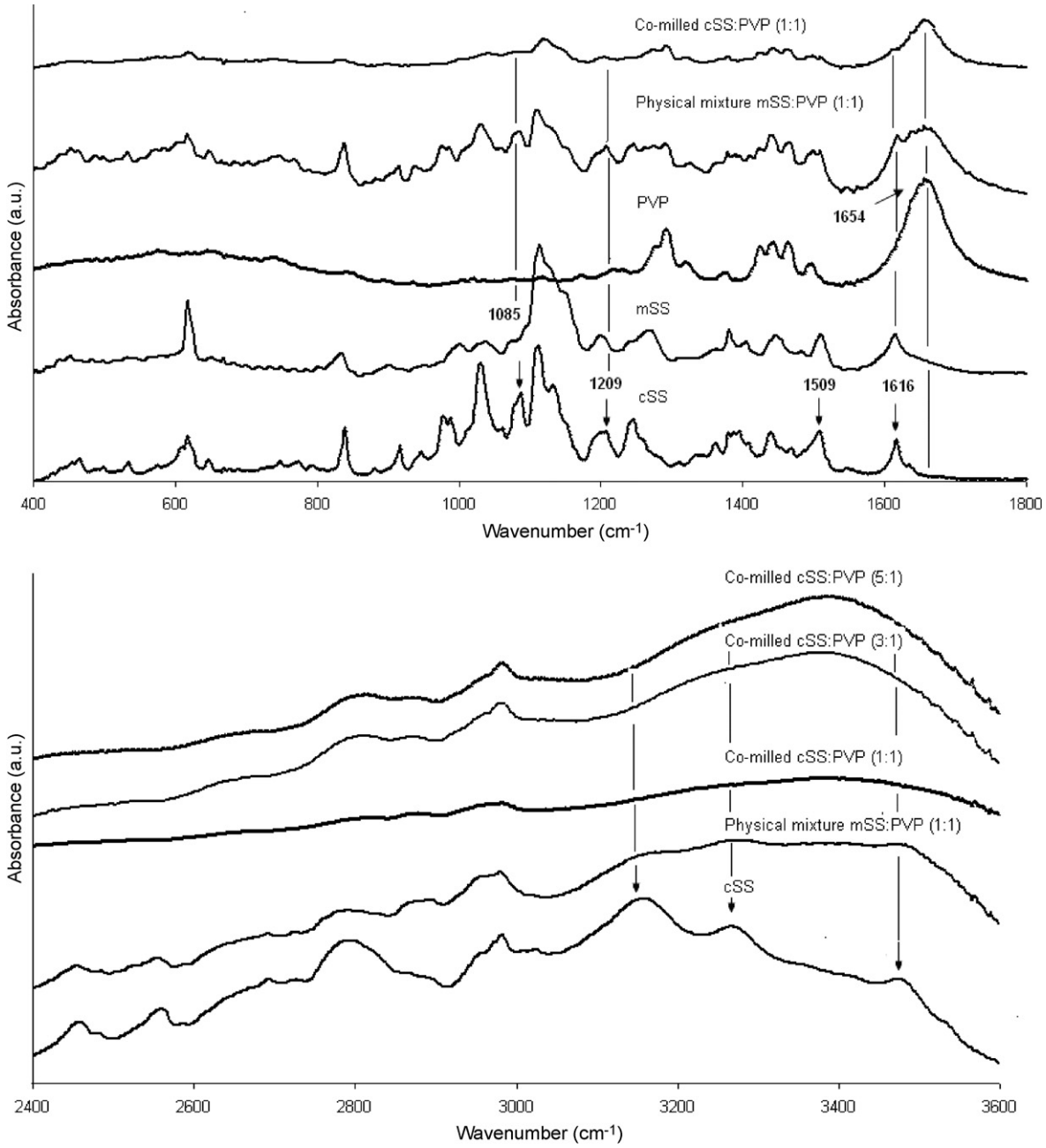

Fig. 3. (a and b) FT-IR spectra of cSS, mSS, PVP, physical mixture of mSS:PVP 1:1, co-milled mixtures of cSS:PVP (1:1, 3:1 and 5:1).

of 1:5 (Fig. 4c). This indicated that physical blending alone was not adequate in ensuring homogeneous distribution of SS. To further understand the difference in distribution behaviour of SS and PVP particles, spatial distributions of drug and polymer in physical mixture and co-milled samples were generated using Raman point-by-point mapping. The spatial distribution of SS and PVP in co-milled mixtures was generated by projecting the pure spectra reference of SS and PVP (measured from cSS and PVP samples) onto the measured Raman mapping data. As seen in Fig. 5, SS and PVP on co-milling were found to be very homogeneously distributed in the mapped areas down to 5 μm resolution at all ratios of cSS:PVP (3:1) and below. The mapping data of co-milled ratio of cSS:PVP (5:1) (Fig. 5e) indicated the presence of cSS along with a mixture of mSS and PVP by the reconstruction of pure component spectrum of cSS via BTEM analysis. The BTEM cSS spectrum estimate is similar to the pure spectra reference of cSS (Fig. 4a). It should be noted that the BTEM estimate of cSS (Fig. 4a) along with BTEM estimates of mSS and PVP (Fig. 4c) were combined together to generate spatial distribution of co-milled mixture of SS:PVP at ratio of 5:1 (Fig. 5e). The results suggested that the minute presence of crystalline SS could have contributed to the instability against re-crystallization. As comparison, a distinct presence of SS clusters was observed in the mapping data of physical mixture of mSS:PVP at ratio of 1:5 (Fig. 5b). The Raman results showed that at PVP concentrations of 25 wt% and above in co-milled mixtures, SS and PVP were uniformly dispersed at 5 μm × 5 μm scale, which supported the FT-IR results that interactions at molecular level were present.

4.2. Stability studies

To evaluate if co-milling improved the physical stability of amorphous SS, milled SS, co-milled and physical mixtures of SS:PVP

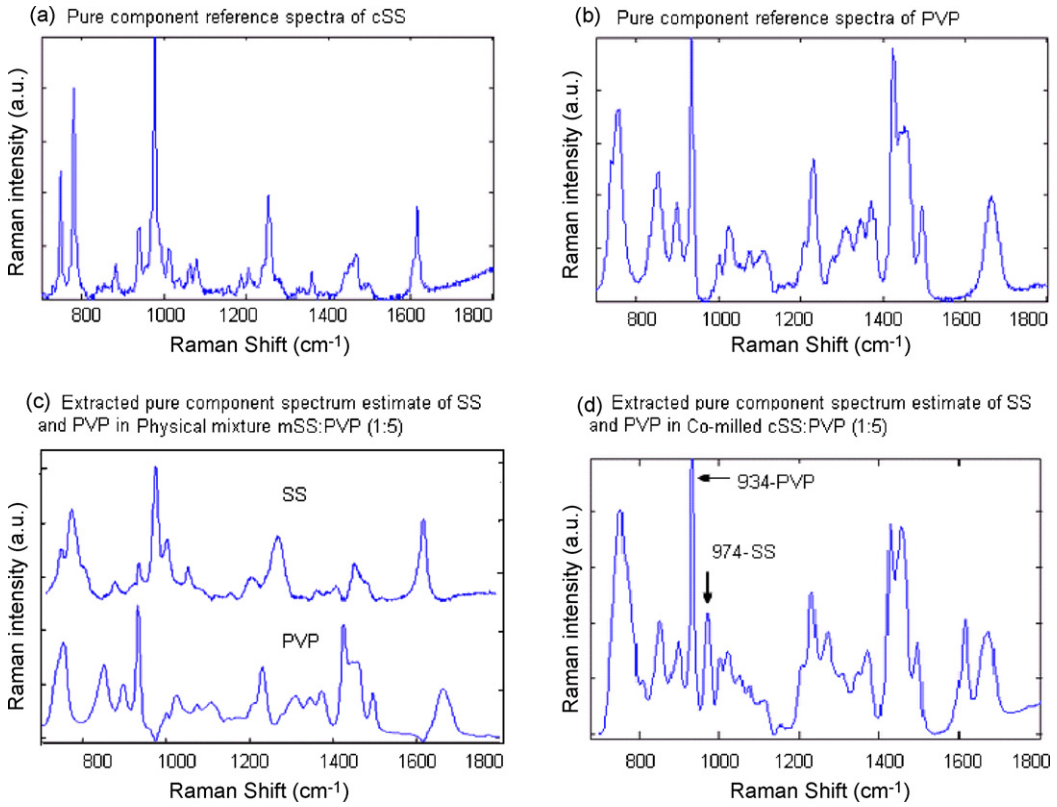

Fig. 4. Pure component Raman spectral estimates of SS and PVP obtained via BTEM from cSS, mSS, PVP, physical mixture of mSS:PVP (1:5) and co-milled mixture of cSS:PVP (1:5).

at all ratios were stored for 7 days (at 22 °C, 15% RH and 75% RH). The samples were analyzed in terms of crystallinity and morphology prior and after storage. Fig. 6 summarizes the XRPD patterns. mSS remained amorphous when stored at 15% RH. As reported in case of amorphous SS (Buckton et al., 1995; Columbano et al., 2002), vapor induced re-crystallization was clearly evident as indicated by the presence of crystalline peaks in XRPD pattern of mSS at 75% RH. A reported DVS study further confirmed the re-crystallization behaviour of mSS at elevated humidity range (Young and Price, 2004). At 75% RH, an expected occurrence of re-crystallization was also noted in all ratios of physical mixtures, which was indicated by an emergence of sharp crystalline peaks in the respective XRPD patterns. In contrast, co-milled mixtures at higher concentration of PVP, i.e. ≥80 wt% stored under elevated humidity conditions were found to be fully X-ray amorphous (Fig. 6). This was in agreement with the indomethacin–PVP amorphous dispersions prepared by cryogrinding (Shakhtshneider et al., 2007b) where more than 50% of PVP was required to stabilize the amorphous form of indomethacin–PVP cryoground mixtures stored for 10 months at ambient conditions. Presence of PVP has been shown to suppress re-crystallization (Watanabe et al., 2003; Cirri et al., 2004). Despite the earlier report that high concentration of hygroscopic PVP may not work as a stabilizer at elevated RH, the results demonstrated that stability of mSS at 75% RH was enhanced by PVP above 80 wt%. To evaluate the degree of crystallinity of co-milled ratios (1:1 and 1:3) stored at 75% RH, their XRPD diffractograms were compared with an X-ray amorphous physical mixture of SS:PVP (1:1) seeded with 1 wt% of crystalline SS. As seen in Fig. 6, principal diffraction peaks of SS in physical mixture ratio seeded with 1 wt% crystalline SS were found to be sharper than in the co-milled ratio 1:1. This suggested that after storage at elevated humidity conditions, co-milled mixture at PVP content of 50 wt% had low crystallinity while higher PVP ratios led to more enhanced stabilization in the amorphous form.

SEM is useful for visualizing changes in particle shape and surface in terms of milling time (Glushenkov et al., 2008) and storage of the milled material (Ng et al., 2008). Fig. 7a–k illustrates the SEM images of mSS, physical and co-milled mixtures of SS and PVP under both storage conditions. The sample mSS (Fig. 7a), which has similar morphology reported for spray-dried SS (Corrigan et al., 2006) appeared close to spherical on storage at 15% RH for 7 days. The appearance was most likely due to the formation of amorphous regions on the SS surface (Ambike et al., 2004). Spherical-like shape of mSS (Fig. 7a) was not retained after storage at 75% RH for 7 days (Fig. 7c). Fines appeared to agglomerate on irregularly shaped block-like structures, which tallied with the occurrence of re-crystallization shown in earlier XRPD results (Fig. 6a). Amorphous nature of the PVP particles was clearly evident with a rough and undulated surface suggesting a lack of arrangement in the structure of PVP (Fig. 7b). The appearance was different from reported SEM images (Marini et al., 2003) of an untreated PVP sample which is made up of round and smooth particles, characteristic of the glassy phase. SEM photomicrograph of co-milled mixture of cSS:PVP (1:1) stored under 15% RH (Fig. 7d) showed particle fusion with some of the SS particles retaining the spherical-like shape rather similar to mSS (Fig. 7a). Particle agglomeration and subsequent re-crystallization were evident in the SEM photomicrograph of co-milled mixture of cSS:PVP (1:1) stored under 75% RH (Fig. 7e). As expected, the spherical shape of the SS was relatively well-retained in the physical mixture of mSS:PVP(1:1) (Fig. 7f) stored at 15% RH, while particle agglomeration with no distinct retention of the spherical shape was seen in the sample stored under 75% RH (Fig. 7g). SEM photomicrographs of sample of co-milled cSS:PVP at a ratio of 1:4 stored at both 15% (Fig. 7h) and 75% RH (Fig. 7i) did not indicate any distinct morphological differences. They appeared similar to PVP stored under 15% RH (Fig. 7b) with particles in agglomerated form. Absence of block-like structures as seen in SEM photomicrograph of mSS stored under 75% RH

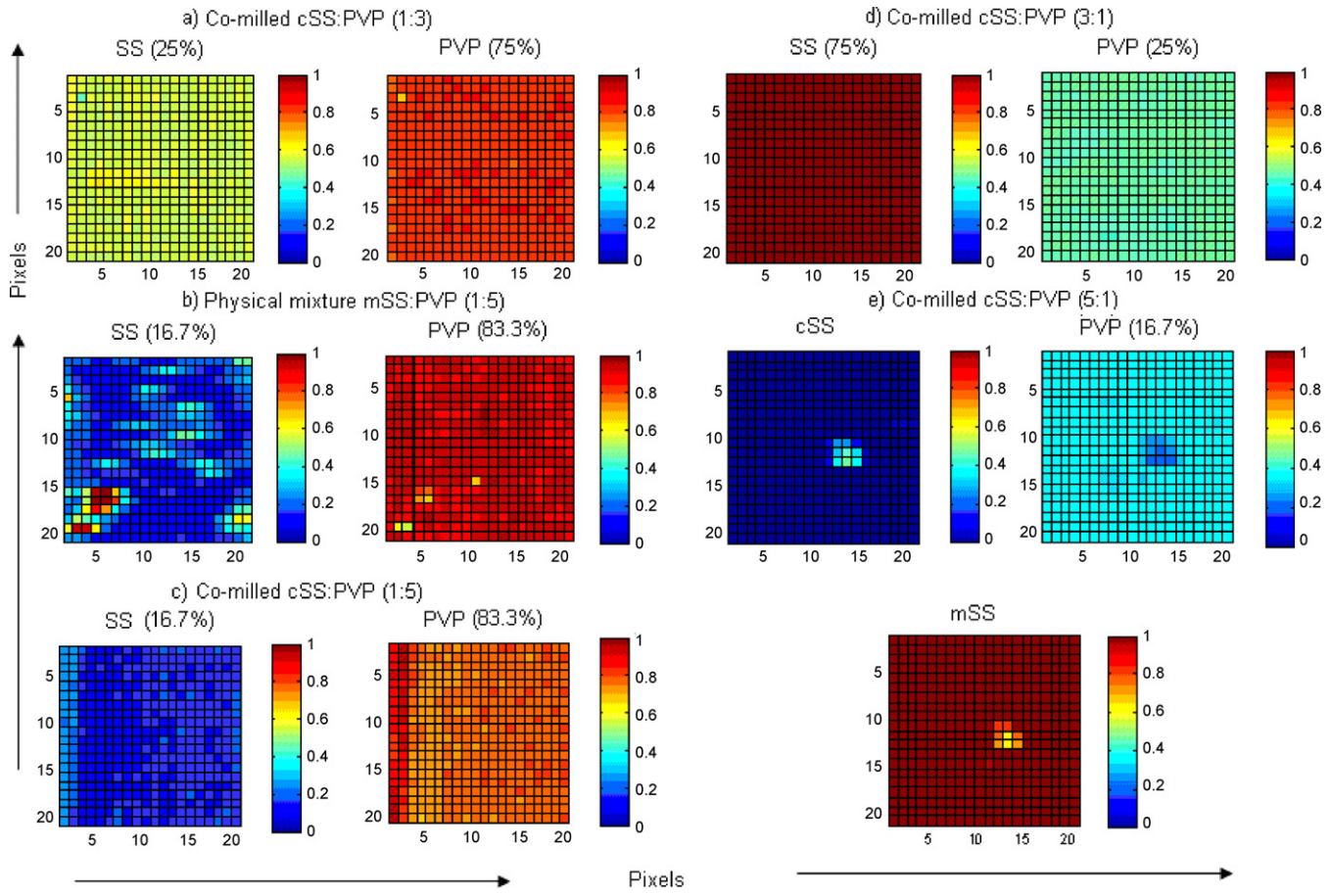

Fig. 5. Raman mapping score images obtained via BTEM from cSS, mSS, PVP, physical mixture of mSS:PVP (1:5) and co-milled mixtures of cSS:PVP (1:3, 1:5, 3:1 and 5:1). The axes of the score images are in pixels and can be directly correlated to distance by multiplying each pixel with 5 μm.

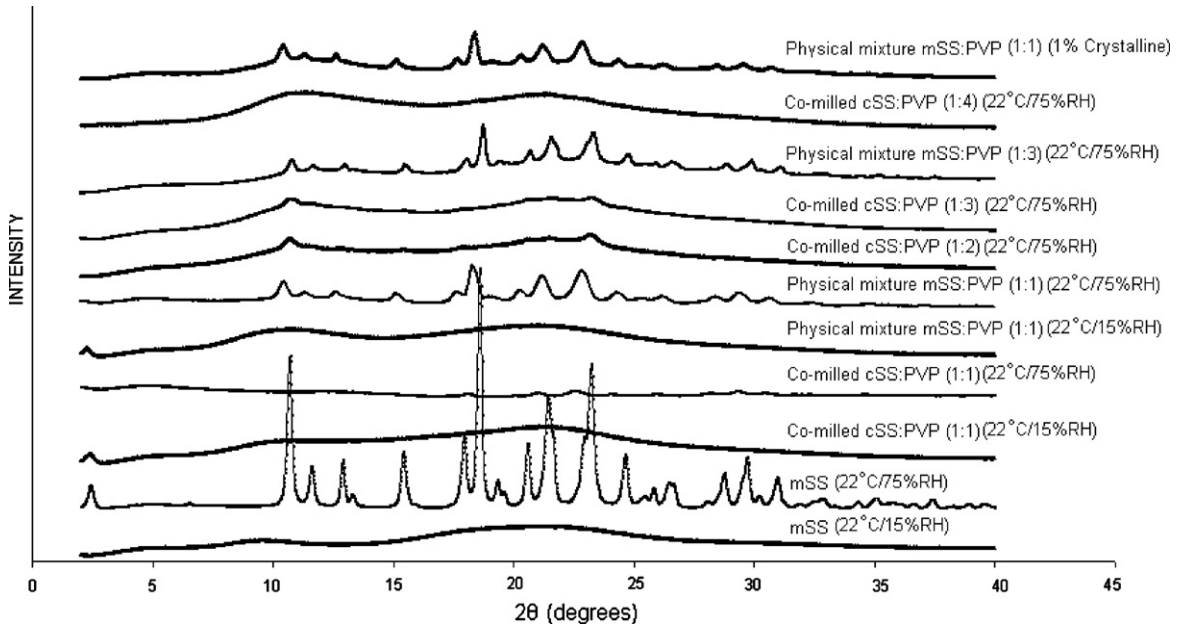

Fig. 6. Powder X-ray diffraction patterns of stability samples and a physical mixture of mSS:PVP (1:1) seeded with 1 wt% cSS.

(Fig. 7c) suggested that co-milled SS:PVP (at 1:4 ratio) could have formed a single solid phase. The spherical shape of the SS particles was found in the physical mixture of mSS:PVP (1:4) (Fig. 7j) stored under 15% RH. The physical mixture of mSS:PVP (1:4) stored under 75% RH (Fig. 7k) indicated presence of block-like structures with a glassy appearance. The glassy appearance could be attributed to presence of PVP, which has a tendency to absorb moisture at elevated humidity conditions. Conditioned PVP in a wet atmosphere for 3 h resulted in transformation of PVP into a glassy semisolid mass (Marini et al., 2003). This remained unchanged after an hour exposure at RH >90%. The block-like structures bore resemblance to the SEM photomicrograph of milled SS stored under 75% RH (Fig. 7c) indicating possible occurrence of re-crystallization. This was found to be consistent with XRPD pattern of the similar ratio stored at 75% RH (data not shown). SEM analysis was found to be consistent with XRPD results and further confirmed that co-milling of SS with 80 wt% or more PVP stabilized the amorphous form of SS even under elevated humidity conditions.

Earlier Raman results confirmed homogeneous distributions of PVP and SS on particle surfaces. However, when only milled SS was present in Fig. 7a and c, the amorphous surfaces came into close contact with each other, formed solid bridges via re-crystallization and led to particle fusion and growth (Young et al., 2007). Compared to Fig. 7h and i, where PVP is present at 80 wt%, no evident changes in morphology occurred in the absence of re-crystallization. Interdispersion of PVP among the SS could have helped to reduce these bridge formations.

To understand the role of moisture with respect to drug–polymer ratio in co-milled mixtures, DVS was used to estimate the moisture sorption behaviour of co-milled mixtures. As indicated in Fig. 8, an increase in moisture content of the co-milled mixtures was evident with a corresponding increase

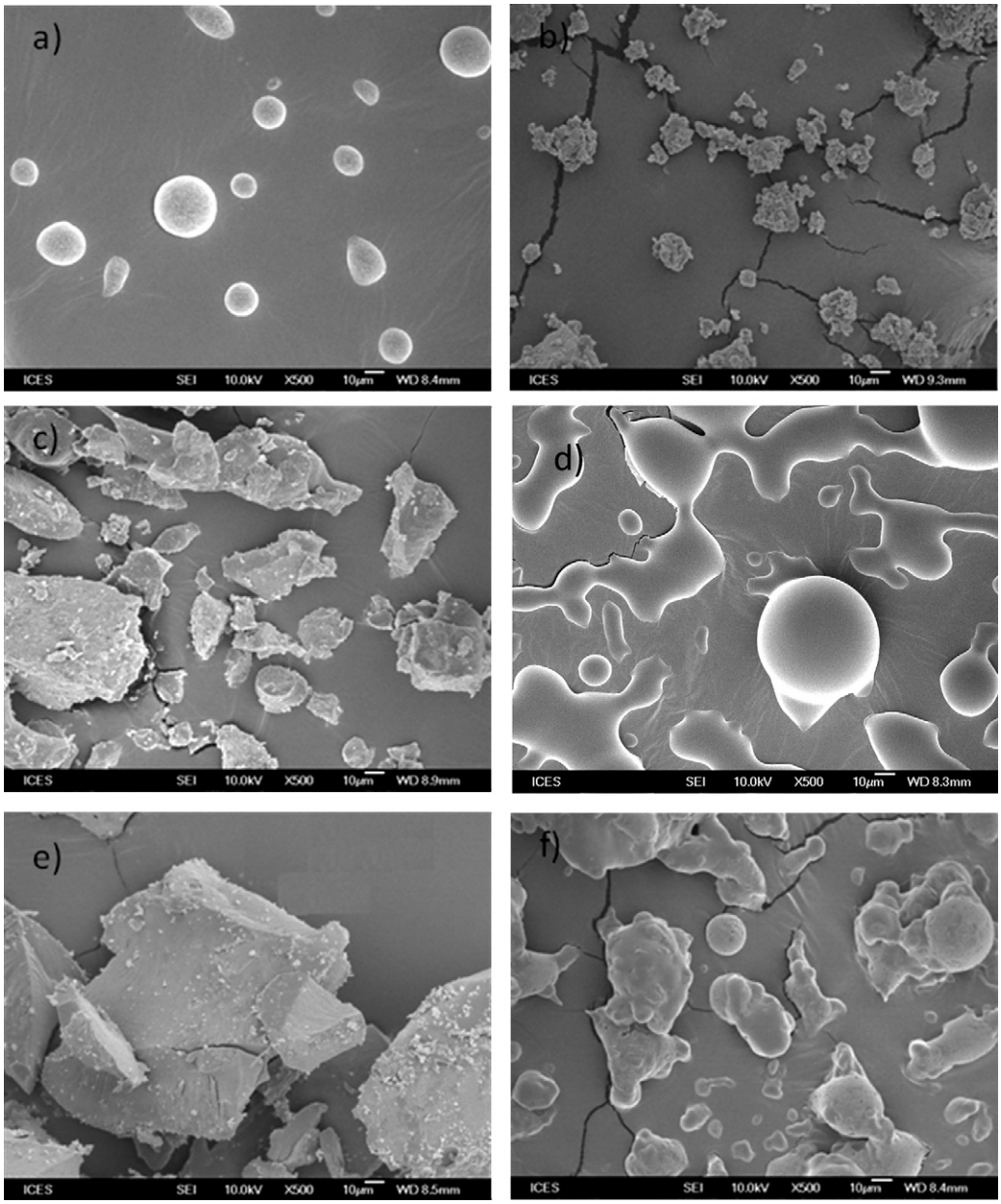

Fig. 7. SEM images of stability samples (a) mSS (22 °C/15% RH); (b) PVP (22 °C/15% RH); (c) mSS (22 °C/75% RH); (d) co-milled mixture of cSS:PVP 1:1 (22 °C/15% RH); (e) co-milled mixture of cSS:PVP 1:1 (22 °C/75% RH); (f) physical mixture of mSS:PVP 1:1 (22 °C/15% RH); (g) physical mixture of mSS:PVP 1:1 (22 °C/75% RH); (h) co-milled mixture of cSS:PVP 1:4 (22 °C/15% RH); (i) co-milled mixture of cSS:PVP 1:4 (22 °C/75% RH); (j) physical mixture of mSS:PVP 1:4 (22 °C/15% RH); (k) physical mixture of mSS:PVP 1:4 (22 °C/75% RH).

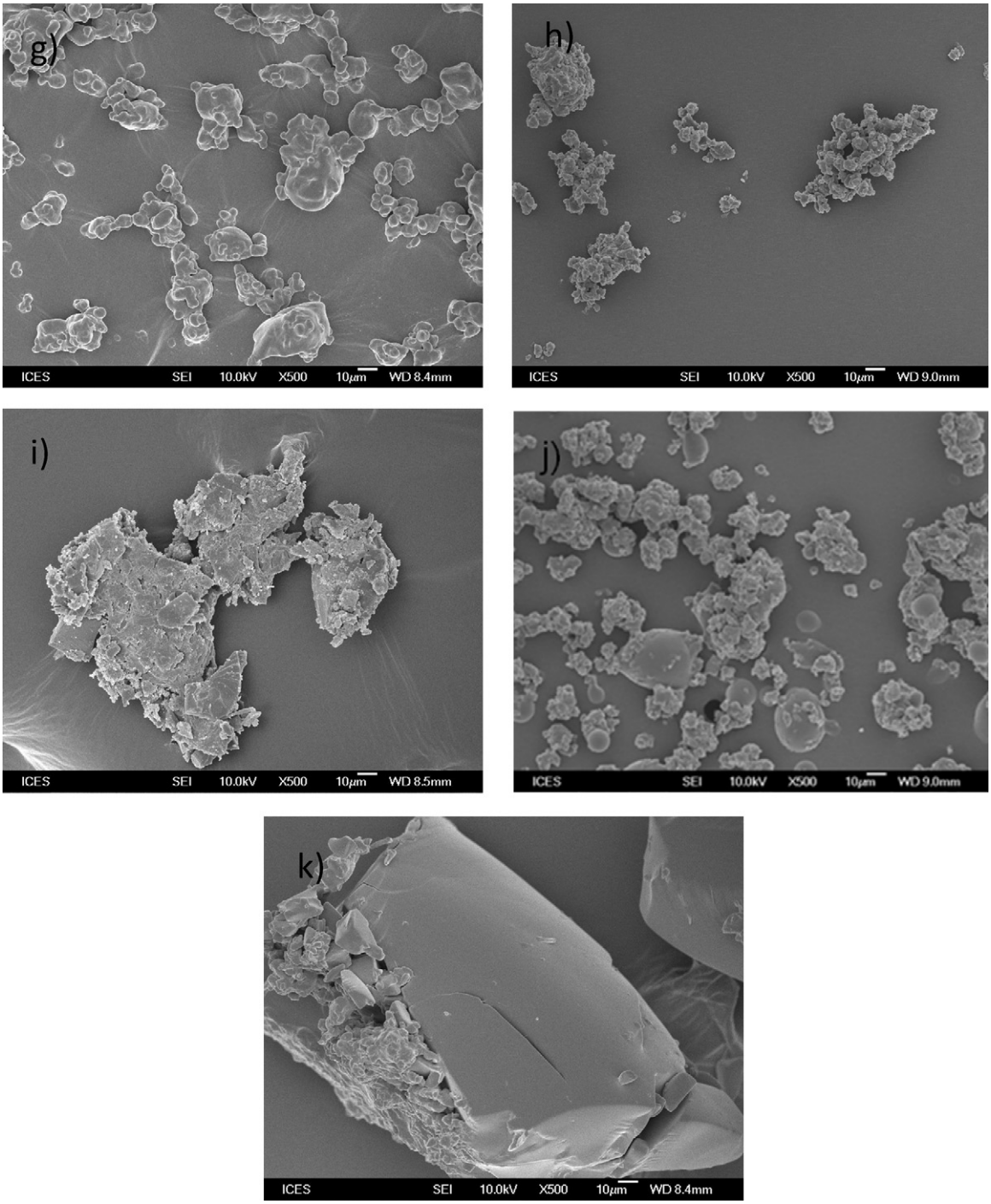

Fig. 7. (*Continued*).

in PVP content. However, high RH did not result in a steep mass loss, which typically represents a clear transition from amorphous to crystalline in DVS sorption profiles. However at high cSS:PVP ratios of (2:1 and 5:1), an inflexion is observed between 50 and 70% RH, which indicates some extent of re-crystallization. This suggests that a minimum PVP content may be required to retain the amorphous character of co-milled mixture at elevated RH. An important consideration here is the minimum amount of excipient needed to stabilize the drug in the amorphous phase on co-milling. As seen from XRPD results, co-milled ratios of 80 wt% or more of PVP stored under elevated humidity conditions for 7 days were found to be completely X-ray amorphous, with emergence of crystalline peaks in co-milled ratios of 80 wt% PVP. DVS profiles also registered some degree of re-crystallization at ratios containing less than 33.3 wt% PVP. The difference is not surprising as XRPD is more sensitive to crystallinity while DVS is more accurate for detecting amorphous content. This was observed by Bhugra and co-author (Bhugra and Pikal, 2008), who explained that the stability of drug polymer amorphous dispersions was due to two mechanisms based on the polymer concentration. At higher concentrations (25%), polymer could act as a diluent whereby the nucleation rate was reduced as the drug was diluted in the polymer and diffusion pathway for crystallization increased. In the present study, it could be reasonably inferred that polymer

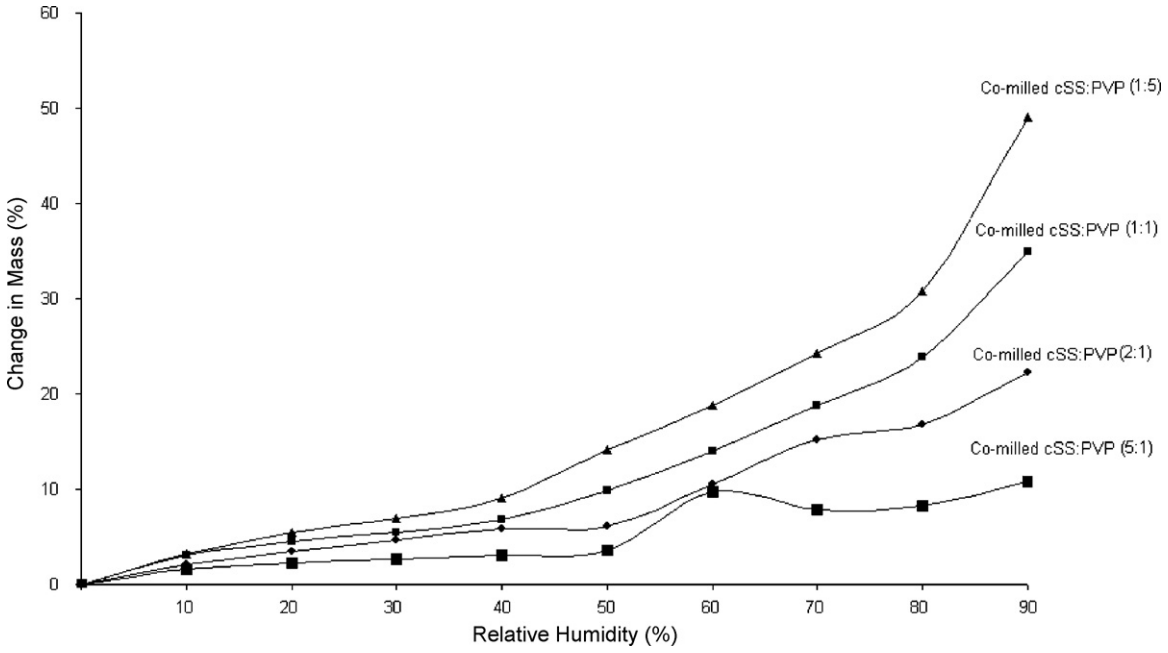

Fig. 8. Sorption isotherms of co-milled mixtures.

acted as a diluent as the co-milled mixtures at higher ratios of PVP were found to remain X-ray amorphous under elevated humidity conditions.

5. Conclusions

The present study was conducted to investigate the influence of additive PVP concentration on stabilizing the amorphous form of an inhaled drug, salbutamol sulphate, upon milling. Co-milling of SS with PVP content ranging from (0 to 90 wt%) resulted in fully amorphous materials. However, upon storage at 25 °C and elevated RH of 75 °C for 7 days, XRPD analyses showed that only SS co-milled with at least 80 wt% PVP content remained X-ray amorphous. In comparison, physical blending of milled SS with PVP did not hinder the re-crystallization process. Even at lower PVP content between 50 and 75 wt%, the crystallinity of stored samples remained low and below that of 1 wt% crystalline SS added to an amorphous physical mixture. Thermal treatment using DSC showed co-milled SS containing over 33 wt% PVP content was thermally stable with no re-crystallization exotherm detected. Similar to DSC, DVS analysis using humidity treatment did not detect any re-crystallization tendency at co-milled mixture with over 33 wt% PVP content. The differences in minimum PVP contents are attributed to firstly, XRPD samples were subjected to an extended exposure to elevated humidity of 1 week, which provided more time for re-crystallization to occur and secondly, XRPD can detect low degree of crystallinity while DSC and DVS are sensitive to low amorphous content. At crystallinity level of about 1%, XRPD is probably more sensitive in our study. In terms of particle morphology, SEM micrographs showed that no observable particle agglomeration or surface changes occurred at minimum PVP content of 80 wt%. FT-IR results supported the formation of hydrogen bonding between SS and PVP on co-milling. The extent of inter-molecular interactions increased with higher PVP content suggesting that they could contribute towards a stabilized amorphous phase even when stored at higher RH. RM showed that SS and PVP were homogeneously distributed (at minimum PVP content of 25 wt%) and could not be distinguished from each other even at 5 μm resolution. This evidence suggested that PVP could be dispersed in very fine clusters or at molecular interaction level, resulting in a very stable solid dispersion. In summary, a good understanding of the drug–excipient interactions, their susceptibilities to heat and humidity treatments, as well as stabilities upon storage under accelerated ageing is important for designing a stable amorphous formulation using co-milling.

Acknowledgements

This work was supported by the research scholarship of the National University of Singapore awarded to Mr. Prashant Balani and the research grant from Science and Engineering Research Council of A*STAR (Agency for Science, Technology and Research), Singapore. The authors would like to thank Mr. Lim Seng Chong, Mr. Benjamin Ang, Mr. Ng Junwei, Miss Tok Ai Tee, Miss Goh Xue Ping, Mr. Kwek Jin Wang of the Institute of Chemical and Engineering Sciences, A*STAR, Singapore for their contributions in conducting XRPD, DVS and SEM studies.

References

Ambike, A.A., Mahadik, K.R., Paradkar, A., 2004. Stability study of amorphous valdecoxib. Int. J. Pharm. 282, 151–162.

Aso, Y., Yoshioka, S., Kojima, S., 1996. Relationship between water mobility, measured as nuclear magnetic relaxation time, and the crystallization rate of amorphous nifedipine in the presence of some pharmaceutical excipients. Chem. Pharm. Bull. 44, 1065–1067.

Bahl, D., Bogner, R.H., 2006. Amorphization of indomethacin by co-grinding with neusilin US2: amorphization kinetics, physical stability and mechanism. Pharm. Res. 23, 2317–2325.

Bahl, D., Bogner, R.H., 2008. Amorphization alone does not account for the enhancement of solubility of drug co-ground with silicate: the case of indomethacin. AAPS Pharm. Sci. Tech. 9, 146–153.

Bahl, D., Hudak, J., Bogner, R.H., 2008. Comparison of the ability of various pharmaceutical silicates to amorphize and enhance dissolution of indomethacin upon co-grinding. Pharm. Dev. Technol. 13, 255–269.

Balani, P.N., Ng, W.K., Tan, R.B.H., Chan, S.Y., 2009. Influence of excipients in co-milling on mitigating milling-induced amorphization or structural disorder of crystalline pharmaceutical actives. J. Pharm. Sci., doi:101002/jps.21998.

Banchero, M., Manna, L., Ronchetti, S., Campanelli, P., Ferri, A., 2009. Supercritical solvent impregnation of piroxicam of PVP at various polymer molecular weights. J. Supercrit. Fluids 49, 271–278.

Bhugra, C., Pikal, M.J., 2008. Role of thermodynamic, molecular, and kinetic factors in crystallization from the amorphous state. J. Pharm. Sci. 97, 1329–1349.

Boldyrev, V.V., Shakhtshneider, T.P., Burleva, L.P., Severtsev, V.A., 1994. Preparation of the disperse systems of sulphathiazole-poly(vinylpyrrolidone) by mechanical activation. Drug Dev. Ind. Pharm. 20, 1103–1114.

Brodka-Pfeiffer, K., Häusler, H., Graβ, P., Langguth, P., 2003a. Conditioning following powder micronization: influence on particle growth of salbutamol sulphate. Drug Dev. Ind. Pharm. 29, 1077–1084.

Brodka-Pfeiffer, K., Langguth, P., Graβ, P., Häusler, H., 2003b. Influence of mechanical activation on the physical stability of salbutamol sulphate. Eur. J. Pharm. Biopharm. 56, 393–400.

Buckton, G., Darcy, P., Greenleaf, D., Holbrook, P., 1995. The use of isothermal microcalorimetry in the study of changes in crystallinity of spray-dried salbutamol sulphate. Int. J. Pharm. 116, 113–118.

Burnett, D.J., Thielmann, F., Booth, J., 2004. Determining the critical relative humidity for moisture-induced phase transitions. Int. J. Pharm. 287, 123–133.

Chen, R., Okamoto, H., Danjo, K., 2008. Preparation of functional composite particles of salbutamol sulfate using a 4-fluid nozzle spray-drying technique. Chem. Pharm. Bull. 56, 254–259.

Choksi, R.J., Shah, N.H., Sandhu, H.K., Malick, A.W., Zia, H., 2008. Stabilization of low glass transition temperature indomethacin formulations: impact of polymer-type and its concentration. J. Pharm. Sci. 97, 2286–2298.

Cirri, M., Mura, P., Rabasco, A., Ginés, J., Moyano, J., Gònzalez-Rodrìguez, M., 2004. Characterization of ibuproxam binary and ternary dispersions with hydrophilic carriers. Drug Dev. Ind. Pharm. 30, 65–74.

Columbano, A., Buckton, G., Wikeley, P., 2002. A study of the crystallization of amorphous salbutamol sulphate using water vapour sorption and near infrared spectroscopy. Int. J. Pharm. 237, 171–178.

Corrigan, D.O., Corrigan, O.I., Healy, A.M., 2006. Physicochemical and in vitro deposition properties of salbutamol sulphate/ipratropium bromide and salbutamol sulphate/excipient spray dried mixtures for use in dry powder inhalers. Int. J. Pharm. 322, 22–30.

Crowley, K., Zografi, G., 2002. Cryogenic grinding of indomethacin polymorphs and solvates: assessment of amorphous phase formation and amorphous phase physical stability. J. Pharm. Sci. 91, 492–507.

Dhumal, R.S., Biradar, S.V., Paradkar, A.R., York, P., 2009. Particle engineering using sonocrystallization: salbutamol sulphate for pulmonary delivery. Int. J. Pharm. 368, 129–137.

Friedrich, H., Nada, A., Bodmeier, R., 2005. Solid state and dissolution rate characterization of co-ground mixtures of nifedipine and hydrophilic carriers. Drug Dev. Ind. Pharm. 31, 719–728.

Geeta, N., Baggi, T.R., 1989. An improved spectrophotometric method for the determination of salbutamol sulfate with 3-methylbenzthiazolinone-2-hydrazone. Microchem. J. 39, 137–144.

Ghebremeskel, A.N., Vemavarapu, C., Lodaya, M., 2006. Use of surfactants as plasticizers in preparing solid dispersions of poorly soluble API: stability testing of selected solid dispersions. Pharm. Res. 23, 1928–1936.

Glushenkov, A.M., Stukachev, V.I., Hassan, M.F., Kuvshinov, G.G., Liu, H.K., Chen, Y., 2008. A novel approach for real mass transformation from V_2O_5 particles to nanorods. Cryst. Growth Des. 8, 3661–3665.

Gunawan, L., Johari, G.P., Shanker, R.M., 2006. Structural relaxation of acetaminophen glass. Pharm. Res. 23, 967–979.

Hancock, B.C., Zografi, G., 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. J. Pharm. Sci. 86, 1–12.

Hancock, B.C., Shamblin, S.L., Zografi, G., 1995. Molecular mobility of amorphous pharmaceutical solids below their glass transition temperatures. Pharm. Res. 12, 799–806.

Hyvönen, S., Peltonen, L., Karjalainen, M., Hirvonen, J., 2005. Effect of nanoprecipitation on the physicochemical properties of low molecular weight poly (L-lactic acid) nanoparticles loaded with salbutamol sulphate and beclomethasone diproprionate. Int. J. Pharm. 295, 269–281.

Konno, H., Taylor, L.S., 2006. Influence of different polymers on the crystallization tendency of molecularly dispersed amorphous felodipine. J. Pharm. Sci. 95, 2692–2705.

Li, C.Z., Widjaja, E., Chew, W., Garland, M., 2002. Rhodium tetracarbonyl hydride: the elusive metal carbonyl hydride. Angew. Chem. 41, 3784–3789.

Mahlin, D., Berggren, J., Gelius, U., Engström, S., Alderborn, G., 2006. The influence of PVP incorporation on moisture-induced surface crystallization of amorphous spray-dried lactose particles. Int. J. Pharm. 321, 78–85.

Marini, A., Berbenni, V., Moioli, S., Bruni, G., Cofrancesco, P., Margheritis, C., Villa, M., 2003. Drug–excipient compatibility studies by physico-chemical techniques. The case of indomethacin. J. Therm. Anal. Cal. 73, 529–545.

Marsac, P.J., Konno, H., Rumondor, A.C.F., Taylor, L.S., 2008. Re-crystallization of nifedipine and felodipine from amorphous molecular level solid dispersions containing poly(vinylpyrrolidone) and sorbed water. Pharm. Res. 25, 647–656.

Miyazaki, T., Yoshioka, S., Aso, Y., Kojima, S., 2004. Ability of polyvinylpyrrolidone and polyacrylic acid to inhibit the crystallization for amorphous acetaminophen. J. Pharm. Sci. 93, 2710–2717.

Ng, W.K., Kwek, J.W., Tan, R.B.H., 2008. Anomalous particle shift during post-milling storage. Pharm. Res. 25, 1175–1185.

Organisation Internationale de Métrologie Légale (OIML), 1996. The Scale of Relative Humidity of Air Certified Against Saturated Salt Solutions. OIML, p. R121.

Parrott, G.L., 1974. Milling of pharmaceutical solids. J. Pharm. Sci. 63, 813–829.

Pokharkar, V.B., Mandpe, L.P., Padamwar, M.N., Ambike, A.A., Mahadik, K.R., Paradkar, A., 2006. Development, characterization and stabilization of amorphous form of a low T_g drug. Powder Technol. 167, 20–25.

Shakhtshneider, T.P., 1997. Phase transformations and stabilization of metastable states of molecular crystals under mechanical activation. Sol. St. Ion. 101–103, 851–856.

Shakhtshneider, T.P., Danède, F., Capet, F., Willart, J.F., Descamps, M., Paccou, L., Myz, S.A., Boldyreva, E.V., Boldyrev, V.V., 2007a. Grinding of drugs with pharmaceutical excipients at cryogenic temperatures. Part II. Cryogenic grinding of piroxicam–polyvinylpyrrolidone mixtures. J. Therm. Anal. Chem. 89, 699–707.

Shakhtshneider, T.P., Danède, F., Capet, F., Willart, J.F., Descamps, M., Paccou, L., Surov, E.V., Boldyreva, E.V., Boldyrev, V.V., 2007b. Grinding of drugs with pharmaceutical excipients at cryogenic temperatures. Part II. Cryogenic grinding of indomethacin–polyvinylpyrrolidone mixtures. J. Therm. Anal. Chem. 89, 709–715.

Tang, X.C., Pikal, M.J., Taylor, L.S., 2002. A spectroscopic investigation of hydrogen bond patterns in crystalline and amorphous phases in dihydropyridine calcium channel blockers. Pharm. Res. 19, 477–483.

Umprayn, K., Mendes, R.W., 1987. Hygroscopicity and moisture adsorption kinetics of pharmaceutical solids: a review. Drug Dev. Ind. Pharm. 13, 653–693.

Vasconcelos, T., Sarmento, B., Costa, P., 2007. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov. Today 12, 1068–1075.

Watanabe, T., Wakiyama, N., Usui, F., Ikeda, M., Isobe, T., Senna, M., 2001. Stability of amorphous indomethacin compounded with silica. Int. J. Pharm. 226, 81–91.

Watanabe, T., Hasegawa, S., Wakiyama, N., Kusai, A., Senna, M., 2003. Comparison between polyvinylpyrrolidone and silica nanoparticles as carriers for indomethacin in a solid state dispersion. Int. J. Pharm. 250, 283–286.

Widjaja, E., Seah, R.K.H., 2008. Application of Raman microscopy and band-target entropy minimization to identify minor components in model pharmaceutical tablets. J. Pharm. Biol. Anal. 46, 274–281.

Widjaja, E., Li, C.Z., Chew, W., Garland, M., 2003. Band target entropy minimization. A general and robust algorithm for pure component spectral recovery. Anal. Chem. 75, 4499–4507.

Yoshioka, M., Hancock, B.C., Zografi, G., 1995. Inhibition of indomethacin crystallization in poly(vinylpyrrolidone) coprecipitates. J. Pharm. Sci. 84, 983–986.

Young, P.M., Price, R., 2004. The influence of humidity on the aerosolisation of micronized and SEDS produced salbutamol sulphate. Eur. J. Pharm. Sci. 22, 235–240.

Young, P.M., Chan, H., Chiou, H., Edge, S., Tee, T.H.S., Traini, D., 2007. The influence of mechanical processing of dry powder inhaler carriers on drug aerosolization performance. J. Pharm. Sci. 96, 1331–1341.

Yu, L., 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. Adv. Drug Del. Rev. 48, 27–42.