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Effect of wet milling process on the solid state of indomethacin and simvastatin

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ABSTRACT

The aim of this study was to investigate the effect of wet milling on the solid state of indomethacin (IMC) and simvastatin (SIM). Wet milling was performed using high pressure homogenization (HPH). Polyvinylpyrrolidone-K25 (PVP) and poloxamer 407 (P407) were used as suspension stabilizers. Samples were characterized before and after wet milling using particle size analyzer, scanning electron microscopy (SEM), infrared (IR) spectroscopy and modulated temperature differential scanning calorimetry (MTDSC) techniques. After wet milling of IMC, physical appearance and IR spectra indicated surface amorphization; however, the solid state of SIM remained unaffected. MTDSC could not detect surface amorphization in IMC, suggesting that if present, it was only at very low levels. These results are in contradiction to the previous reports where dry milling of IMC and SIM resulted in amorphization of crystalline particles. Moreover, cryogrinding of IMC in the absence of water resulted in an amorphous form while presence of water using the same cryogrinding conditions resulted in a solid state similar to that obtained after wet milling. These results signify the role of water in inhibiting the amorphization during wet milling of crystalline drugs.

1. Introduction

The literature reports that 30–40% of the newly developed molecules have severe water insolubility problems, which lead to poor bioavailability and high dropout rate from the development program (Lipinski, 2000; Lipinski et al., 2001). Nanosizing can be used to increase the solubility, dissolution rate and oral absorption of poorly water soluble compounds. It is a robust approach with scale up feasibility as exemplified by marketed formulations (Rapamune® (Wyeth), Emend® (Merck), Tricor® (Abbott Laboratories) and Megace® ES (Bristol-Myers Squibb)) (Van Eerdenbrugh et al., 2008). Nanosizing is generally performed by one of the two wet milling processes; high pressure homogenization (HPH) and media milling. During these processes, mechanical energy is applied to generate a sufficient strain on the solid particles resulting in the disruption of the crystal lattice and particle fracture. An important mechanism of particle size reduction is through cavitation (generated during HPH) where high temperature (up to 5000 °C) and pressure (500 atm) can develop (Kipp, 2004). Under the stress conditions generated during mechanical energy input there is a possibility of physical or chemical change in the crystalline solid, including polymorphic transitions, chemical degradation, complexation or formation of an amorphous phase (Crowley and Zografi, 2002).

The transformation of crystalline to amorphous phase after mechanical energy input has been described by mechanical and thermodynamic mechanisms of destabilization (Crowley and Zografi, 2002). The mechanical mechanism describes the destabilization of crystal lattices as a function of pressure applied; above a critical pressure the resulting increased lattice vibrations would destabilize the crystal lattice. According to the thermodynamic mechanism, the number of defects increases with the increase in pressure and transformation occurs above a critical defect concentration, where the amorphous phase is more stable than the disordered crystal.

Generation of amorphous regions in crystalline nanoparticles is undesirable due to the high reactivity of the amorphous phase (resulting from high molecular mobility), which may favor chemical degradation or particle aggregation, leading to a decreased stability of the final product (Buckton and Darcy, 1999). Moreover, the amount and location of the amorphous content is unpredictable. The presence of water during wet milling may have a considerable impact on the solid state transformation. Such an impact can be reduction of the glass transition temperature (T_g; leading to reduced bulk amorphization) of the amorphous phase generated during the process, enhanced polymorphic transformation or chemical degradation.

The aim of this work was to investigate solid state transformation in indomethacin (IMC) and simvastatin (SIM) during the HPH process. IMC (Fig. 1) is a nonsteroidal anti-inflammatory agent. It has a molecular weight of 357.79 g/mol and is practically insoluble (<0.01 μg/ml) in water (Merck Index, 2006). Three polymorphs of

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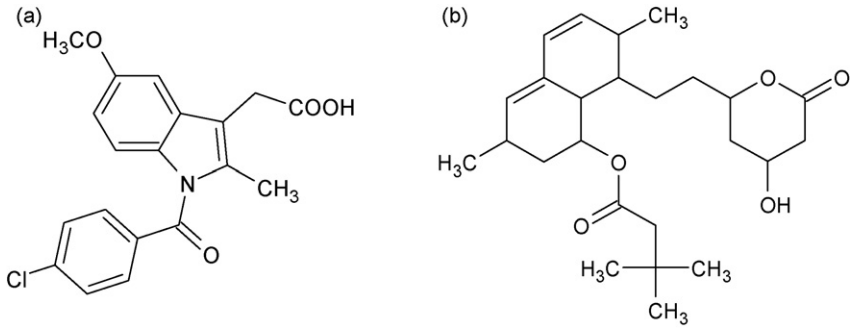

Fig. 1. Structure of (a) IMC and (b) SIM.

IMC, α, γ and δ, have been reported, of which γ is most stable (in dry state) followed by α and δ (Wu and Yu, 2006). The effect of grinding and mechanical pressure on the solid state properties of IMC has been investigated in various studies (Crowley and Zografi, 2002; Patterson et al., 2005; Okumura et al., 2006; Otsuka et al., 1986; Otsuka and Kaneniwa, 1988). It was reported that solid state milling of IMC at cryogenic and room temperatures in the absence or presence of stabilizers such as PVP, Neusilin US2 resulted in an amorphous form (Bahl and Bogner, 2006; Shakhtshneider et al., 2007). The amorphous form exhibits (T_g) at 43–45 °C (Crowley and Zografi, 2002; Patterson et al., 2005). Crystallization from amorphous IMC is temperature dependent and temperatures below T_g favor the formation of γ polymorph, whereas the α polymorph crystallizes at temperatures above T_g. The melting points (T_m) of α and γ polymorphs are 155 and 161 °C, respectively and exhibit monotropic polymorphism (Yoshioka et al., 1994). SIM (Fig. 1) is a cholesterol lowering agent. Its molecular weight is 418.57 g/mol and it is practically insoluble in water (6.3 μg/ml) (Graeser et al., 2008). It exists as a stable crystalline form which has a T_m of 135–138 °C. Similar to IMC, solid state milling of SIM results in the amorphous form that has T_g around 27–29 °C (Graeser et al., 2008). IMC and SIM were selected because of their poor water solubility and availability of extensive literature regarding their solid state characterization.

In the current study, Polyvinylpyrrolidone-K25 (PVP) and poloxamer 407 (P407) were used as suspension stabilizers at ratios of 2:1 and 1:1, respectively, to that of the drug. Different techniques were used to detect the solid state changes. SEM and polarized light microscopy (PLM) were used to study properties associated with the particulate level. Intra- and intermolecular interactions were studied using IR spectroscopy and MTDSC. IR spectroscopy was performed using the attenuated total reflection (ATR) sampling technique. In this technique the sample is placed in contact with a crystal, which has a high refractive index (Bugay, 2001). The IR beam passes through the crystal and penetrates the surface of the sample to give spectral information of the surface. The advantages of this technique over the conventional IR technique (alkali halide pellet preparation) are that sample preparation is not required and samples can be analyzed in the presence of water. Moreover, when the IR beam is transmitted through the crystal it penetrates only from 0.1 to a few μm, and is therefore suitable for surface characterization studies (Bugay, 2001). Throughout this manuscript the term 'wet milling' has been used synonymously with 'HPH'.

2. Materials

IMC, SIM and P407 were purchased from Sigma (USA). PVP was received as gift sample from International Specialty Products (USA). Acetonitrile and methanol (HPLC grade) were purchased from Ajax Fine Chemicals (Australia); and trifluoroacetic acid was purchased from Scharlau Chemie (Spain). All other reagents, chemicals and solutions used were of analytical grade.

3. Methods

3.1. Preparation of nanosuspensions

HPH was performed using a piston gap high pressure homogenizer, Emulsiflex C-3 (Avestin Inc., Canada) attached to a heat exchanger (Avestin Inc., Canada). PVP and P407 were used as stabilizer at 2% and 1% (w/v) concentration, respectively, in deionized water. For each variable, the batches were prepared in triplicate.

3.1.1. Pre-milling

Separately, IMC and SIM were dispersed in the stabilizer solution (batch size 50 ml) at a 1% (w/v) concentration and pre-milled using an ultra-turrax homogenizer (IKA Werke GmbH and Co., Germany). Precautions were taken to avoid any generation of air bubbles during pre-milling. After 30 min of pre-milling 25 ml was separated as a pre-milled sample and the rest (25 ml) was used for HPH.

3.1.2. HPH

A 25 ml pre-milled sample was added to the sample chamber of the high pressure homogenizer. Pre-homogenization (10 cycles) was performed at 500 bar followed by 10 cycles at 1000 bar. HPH was performed at 1500 bar (20 cycles) followed by 20 cycles at 1750 or 2000 bar. The study on the effect of temperature on the solid state of model drugs was also performed at five temperatures, 2, 10, 20, 30 and 40 °C.

The temperature was controlled using a heat exchanger. The heat exchanger consisted of a cylindrical jacketed assembly through which water was continuously recirculated at a predetermined temperature using a controlled temperature water circulator (Julabo Labortechnique, GmbH, Germany). This heat exchanger was attached to the sample outlet tube in the homogenizer.

3.2. Characterization

Different techniques were used for the characterization of the drugs. To evaluate the effect of wet milling on solid state of drugs, pre-milled samples (obtained after dispersing the drug in the aqueous phase with ultra-turrax homogenizer) were compared to the milled samples (obtained after HPH). In addition, samples were also taken out at different stages of the process for characterization.

3.2.1. Particle size analysis

Particle size analysis of the nanosuspension formulations was performed by laser diffraction using a Mastersizer 2000 (Malvern Instruments, UK). The formulations were added drop-wise to the sample dispersion unit containing water (saturated with IMC) as a dispersant. The laser obscuration range was always maintained between 10 and 20%. A refractive index value of 1.5 was used for particle size analysis. The analysis was performed in triplicate for each formulation and the average values of volume distribution were

used for analysis. Average particle size was expressed as d50 (50% of the particles by volume below certain size). d90 values represent 90% of the particles by volume below a particular size.

3.2.2. Physical appearance

To evaluate the physical appearance, pre-milled and milled samples were filtered through 0.20 μm nylon filter membranes and the precipitate was vacuum dried at 30 °C. The dried residue was observed for any colour change before and after wet milling and pictures were obtained using a Nikon coolpix 4500 camera (Nikkon Co., Japan).

3.2.3. SEM and PLM

The morphological evaluation of the particles in the pre-milled and milled suspension samples was conducted by SEM. The samples were placed on carbon specimen holders and air dried. The samples were then coated with platinum in a sputter coater (Polaron SC 7640) and observed using a scanning electron microscope, Philips XL30S FEG (Philips, Netherlands).

For PLM, a drop of the suspension (milled or pre-milled) was placed on a clean glass slide and covered carefully with a coverslip. PLM was performed using brightfield Leica DMR polarized light microscope (20× normal or 100× oil immersion lens, 10× objective) (Leica, GmbH, Germany). Pictures were taken using a Nikon coolpix 4500 camera.

3.2.4. Chemical degradation

To assess the chemical degradation post-wet milling, high pressure liquid chromatography (HPLC) was used. Pre-milled and milled samples (40 μl) were dissolved in acetonitrile in triplicate, and 20 μl of each sample was injected onto the HPLC column for analysis. To determine the degradation, the percentage area of drug peak was compared against total area of all the peaks in the chromatogram. Any decrease in the percentage area of the drug peak in the pre-milled or milled sample was considered as degradation. Before injecting the samples, a blank (without drug) was always injected onto the HPLC column under the same conditions as that used for the sample analysis. The blank consisted of surfactant solution alone (without drug) and was prepared by HPH.

An Agilent series 1200 liquid chromatograph (Agilent Corporation, Germany) comprising of a quaternary pump, an autosampler and photodiode array detector were used with data acquisition by Chemstation (Agilent Corporation, Germany). Chromatographic separation was performed using a Nova-pak® C_{18} analytical column (300 mm × 3.9 mm, particle size 4 μm) from Waters, USA and a C_{18} precolumn of the same packing (4.0 mm × 3 mm).

The mobile phase, comprising acetonitrile and water (60:40, v/v) (with 0.1% trifluoroacetic acid in both phases), was used at an isocratic flow rate of 1 ml/min for the analysis of IMC. For the analysis of SIM, a mobile phase comprising acetonitrile and water (80:20, v/v) (with 0.1% trifluoroacetic acid) was used under the same conditions as that for IMC. A sample volume of 20 μl was injected onto the HPLC column and analysis was carried out at a wavelength of 264 and 238 nm for IMC and SIM, respectively.

3.2.5. IR spectroscopy

The IR spectra of suspension samples were recorded on a Bruker Tensor 37 (Bruker Optik, GmbH, Germany) spectrometer with MIRacle Micro ATR (Attenuated Total Reflectance) attachment with diamond crystal. For recording of spectra, a drop of suspension was placed on the diamond crystal and compressed lightly using the pressure clamp. Scanning was performed in the 4000–400 cm^{-1} region with a resolution 4 cm^{-1}, from 64 parallel scans. IR spectra of the samples were recorded following ultra-turrax and HPH at 500, 1000 and 1500 bar. Spectra were also recorded after milling of the samples at different temperatures (2, 10, 20, 30 and 40 °C). The reported spectra were obtained by subtracting the spectrum of the pure solution without drug from that of the nanosuspension. Data was analyzed using OPUS software (Bruker Optik GmbH, Ettlingen, Germany) version 6.5.

3.2.6. MTDSC

For MTDSC, the pre-milled and milled suspension samples were vacuum filtered through 0.2 μm nylon filter membrane and the precipitate was vacuum dried at 30 °C. After 30 min, the dried precipitate was collected in glass vials. For MTDSC of pure drugs no sample processing was done. Samples (2.5 ± 0.1 mg) were heated at 2 °C/min with modulation parameter of 0.6 °C every 40 s above the melting point of the drug on a Q1000 Tzero™ module DSC instrument (TA Instrument, USA). The instrument was calibrated for enthalpy and heat capacity using indium and sapphire, respectively. Data was analyzed using Universal Analysis software (TA Instruments, USA) version 4.1D. Melting points are reported as onset temperature and all the samples were analyzed in triplicate. The data is reported as mean ± SD. Statistical analysis of the MTDSC results was performed by SigmaStat 3.5 using Student's *t*-test and a p value <0.05 was considered statistically significant.

4. Results and discussion

4.1. HPH—processing, particle size and morphology

The diameter of the homogenization valve is very small (25 μm) and therefore a suspension containing large particles or a highly concentrated suspension can cause blockage of the homogenizer. To prevent the blockage, pre-milling of the suspension was done using ultra-turrrax homogenizer. When pre-milling was not sufficient to prevent the blockage, the concentration of the drug in the suspension was adjusted. Based upon the trial runs conducted, 1% (w/v) concentration of IMC and SIM, respectively, did not cause any blockage of the homogenizer. Further, the generation of air bubbles during pre-milling was minimized by altering the speed of ultra-turrax homogenizer.

HPH of IMC resulted in significant reduction of the particle size (Fig. 2). As compared to the particle size of pre-milled IMC, HPH resulted in over 70% of the particles below 2 μm size (Table 1). Moreover, when PVP was present as stabilizer ~90% of the particles were below 2 μm size as compared to ~71% obtained in the presence of P407 as stabilizer.

Similar to IMC, particle size of SIM was reduced after HPH (Fig. 2). However, when compared with IMC, percentage of SIM particles below 2 μm was more in the presence of P407 as compared to PVP (Table 1).

SEM micrographs of air dried samples of IMC and SIM nanosuspensions are shown in Fig. 3. In addition to particle size analysis, the SEM micrographs further provided the evidence that HPH resulted in significant reduction of particle size. Moreover, as compared to

Table 1
Effect of high pressure homogenization and stabilizer type on the particle size distribution of IMC and SIM.

Samples	Size distribution (μm)			% <2 μm
	d10	d50	d90	
Pre-milled IMC	0.16 ± 0.02	11.77 ± 0.37	32.84 ± 2.70	13 ± 1.80
Milled IMC + PVP*	0.08 ± 0.01	0.20 ± 0.04	2.37 ± 0.17	90 ± 0.91
Milled IMC + P407*	0.17 ± 0.03	1.46 ± 0.07	3.96 ± 0.14	71 ± 1.77
Pre-milled SIM	3.02 ± 0.08	7.23 ± 0.09	70.20 ± 2.07	26 ± 1.24
Milled SIM + PVP**	0.36 ± 0.03	2.34 ± 0.10	16.76 ± 0.41	51 ± 0.81
Milled SIM + P407**	1.14 ± 0.06	1.94 ± 0.24	3.31 ± 0.26	70 ± 0.61

Data shown are the mean values and standard deviation obtained from the particle size analysis of three independently prepared suspensions. (*) and (**) are significant difference in the % of particles <2 μm; $p < 0.05$ (*t*-test).

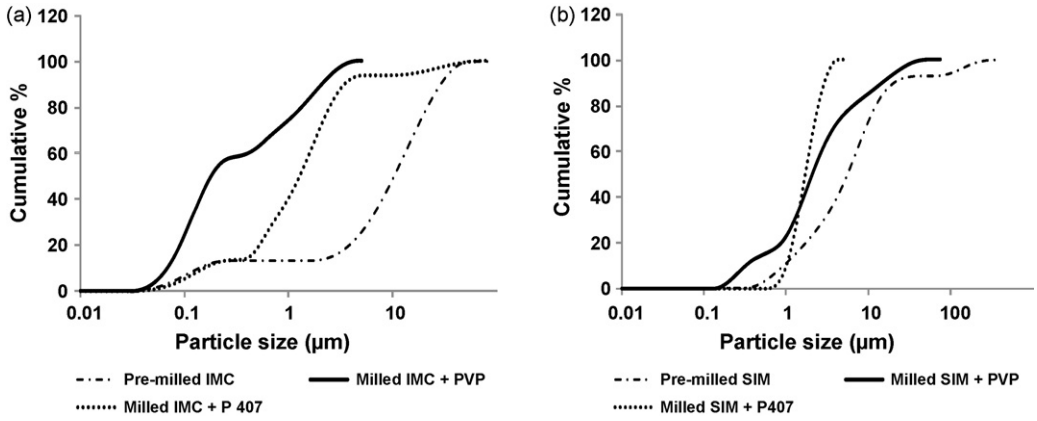

Fig. 2. Particle size distributions of (a) IMC and (b) SIM before and after high pressure homogenization (PVP: Polyvinyl pyrrolidone-K25; P407: Poloxamer 407). Data shown are the mean values obtained from the particle size analysis of three independently prepared suspensions.

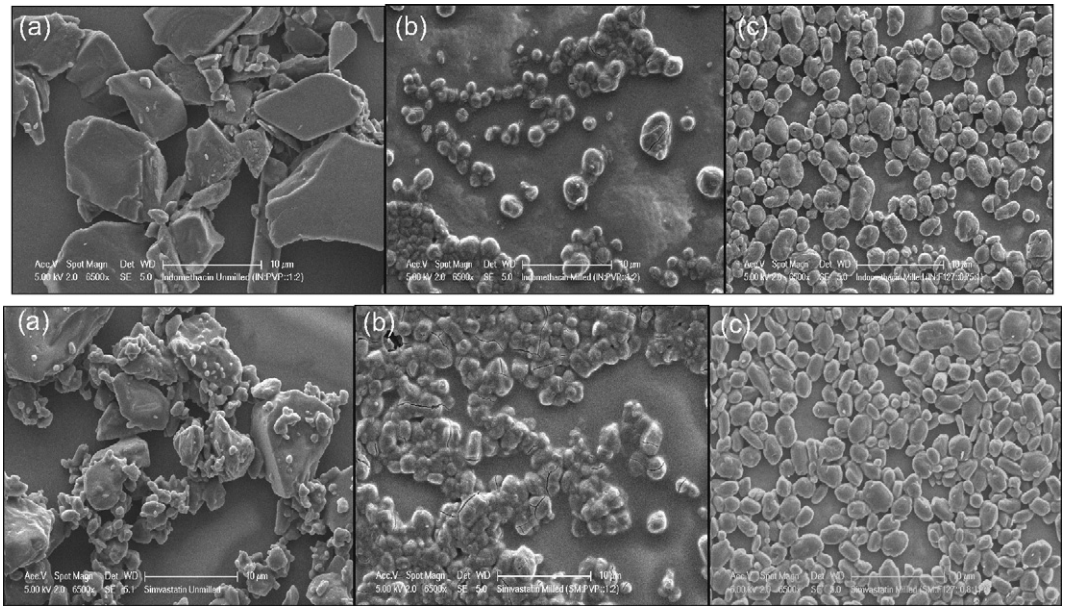

Fig. 3. SEM micrographs of IMC (above) and SIM (below): (a) pre-milled, (b) milled with PVP and (c) milled with P407; scale bar = 10 μm.

the pre-milled particles, the majority of the milled particles were round in shape (without any sharp edges) and in contact with each other owing to the presence of stabilizer coating on the surface. Particles coated with PVP showed a uniform and a shiny film on their surface whereas P407 coating was uniform.

It was evident from particle sizing and SEM micrographs that HPH resulted in the generation of a significant percentage of submicron particles. Therefore, the next step was to characterize the effect of HPH on the solid state of IMC and SIM.

4.2. HPH—solid state characterization

HPLC analysis of the milled samples of IMC and SIM indicated no degradation after HPH. Other than drug peaks, no extra peaks were observed in the chromatograms (100% peak area for drug) and peak purity analysis across the drug peaks showed UV spectra of a single component only.

4.2.1. Visual observation and PLM

Post wet milling, the colour of IMC changed to yellow (Fig. 4). However, pre-milled samples did not show any change in colour and a white colour similar to the unmilled IMC (as received from sup-

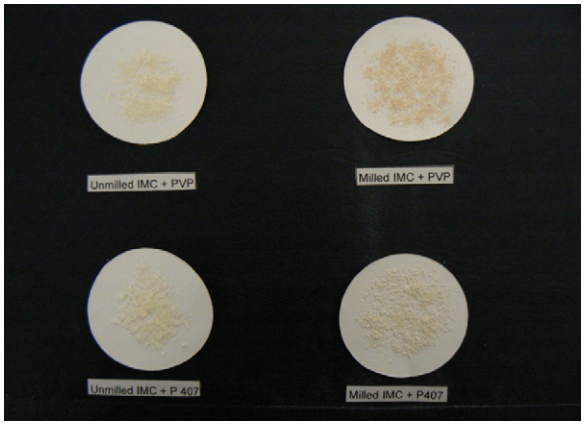

Fig. 4. IMC milled with PVP showed change in colour (white to yellow) after high pressure homogenization. With P407, intensity of yellow colour was less compared with PVP.

plier) was observed. The intensity of the colour change in the milled samples varied with the stabilizer type. When compared with samples containing P407, the intensity of yellow colour was high in the milled samples containing PVP. Milled SIM samples also showed a colour change in the presence of PVP, however the intensity of the yellow colour was much less when compared with milled IMC samples. Milling of SIM in the presence of P407 did not change the colour and a white colour similar to pre-milled SIM was obtained.

Sheth et al have previously reported that the formation of amorphous phase following cryogrinding of crystalline piroxicam was accompanied by a change in colour from white to yellow (Sheth et al., 2005). Shakhtshneider et al have reported a similar yellow colour for amorphous IMC produced by cryogrinding (Shakhtshneider et al., 2007). This change in the colour of the organic molecules after the application of mechanical stress has been termed as 'mechanochromism' (Sheth et al., 2005). In the case of piroxicam, mechanochromism and amorphization were related to the occurrence of an intermolecular proton transfer in the zwitterionic structure of piroxicam molecules; and a similar mechanism was suggested for change in colour of amorphous IMC following cryogrinding (Sheth et al., 2005). Further, the intensity of the yellow colour of amorphous phase depends upon the proportion of the zwitterionic species. For piroxicam, the amorphous phase consisted majorly of neutral molecules and only 8% deprotonated species (enolate ion); however, this percentage was sufficient to impart a yellow colour to the amorphous sample (Sheth et al., 2005).

It appears that the change in colour of IMC from white to yellow following HPH could be due to the formation of amorphous phase. During the process, amorphous IMC can interact with PVP through intermolecular proton transfer between the OH group (proton donor) of the carboxylic acid function of IMC and amide carbonyl of the PVP (proton acceptor) (Taylor and Zografi, 1997). However, it is unlikely that this interaction would take place in the bulk of the particles since PVP monomers are expected to be present on the solid–liquid interface. Therefore, at this stage it is speculated that the yellow colour and hence the amorphization is limited to the surface of IMC particles.

A similar intermolecular proton transfer can also take place between the OH group in SIM and the C=O group in PVP (Ambike et al., 2005), thereby conferring a yellow colour to the milled SIM samples. The lesser intensity of the yellow colour in the milled SIM samples could be due to the weak nature of the intermolecular interaction.

Despite the indication, taken from the altered physical appearance of milled IMC and SIM particles, that surface amorphization has occurred, the post-wet milling PLM analysis of the suspension showed birefringence in the samples (Fig. 5), which is an indicator of crystallinity. However, there is a possibility of the occurrence of birefringence (strained birefringence) in the amorphous samples as well (Patterson et al., 2007), and therefore, amorphization of IMC and SIM particles after HPH cannot be completely ruled out.

4.2.2. IR spectroscopy

Unmilled IMC was characterized as received and the IR spectra showed it to be γ IMC (Fig. 6a) as reported in the literature (Taylor and Zografi, 1997). The spectra showed the presence of strong bands at 1712 and 1688 cm^{-1} corresponding to asymmetric acid C=O of a cyclic dimer and benzoyl C=O, respectively. When compared with pre-milled IMC, the IR spectra of milled IMC showed changes in the peak shapes in the 1300–1200 cm^{-1} region (Fig. 6a). These changes were observed irrespective of the type of stabilizer. Similar changes in the peak shapes were observed when the IR spectra of IMC milled with PVP at different homogenization pressures and temperatures were compared (indicated with arrows) (Fig. 6a). Comparison of the carbonyl stretching frequencies with those reported in the literature (Taylor and Zografi, 1997) for IMC polymorphs indicated absence of any polymorphic transformation or formation of amorphous IMC.

However, overlaid spectra of carbonyl stretching region of IMC milled with PVP showed a shift towards higher frequency bands (Fig. 6b). This shift in IR frequency occurs as a result of increase in the stretching force constant of C=O bond (Pavia et al., 2001). It is also an indicator of a stronger carbonyl bond. This increase in the C=O bond strength following wet milling can be explained by

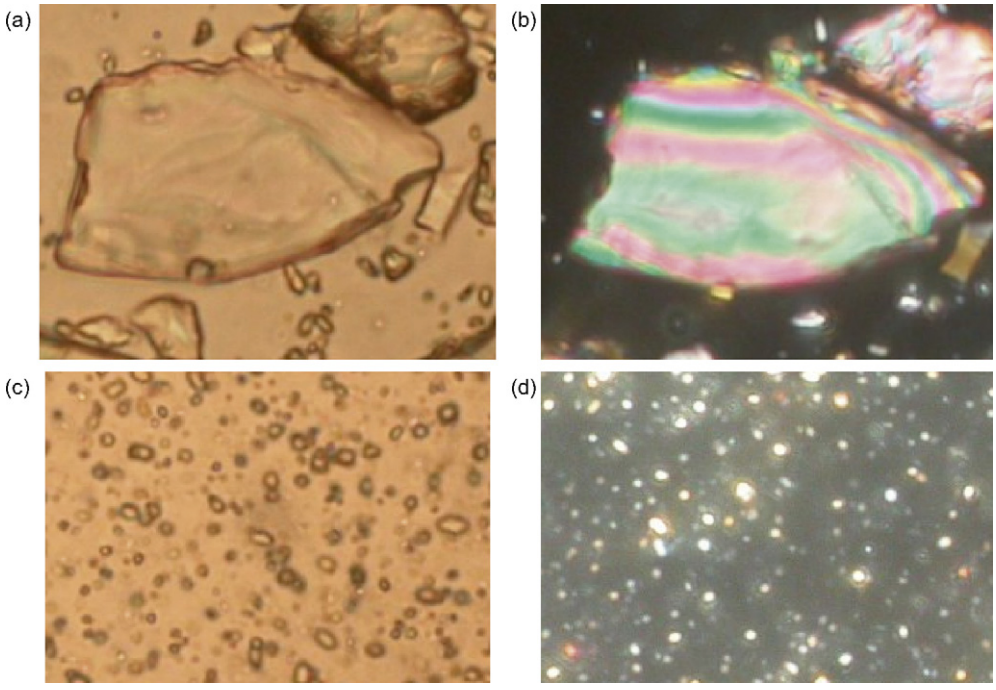

Fig. 5. Light microscopy pictures of IMC pre-milled (top) and milled (bottom) samples using PVP as a stabilizer: (a) normal light, pre-milled, (b) polarized light, pre-milled, (c) normal light, milled and (d) polarized light, milled.

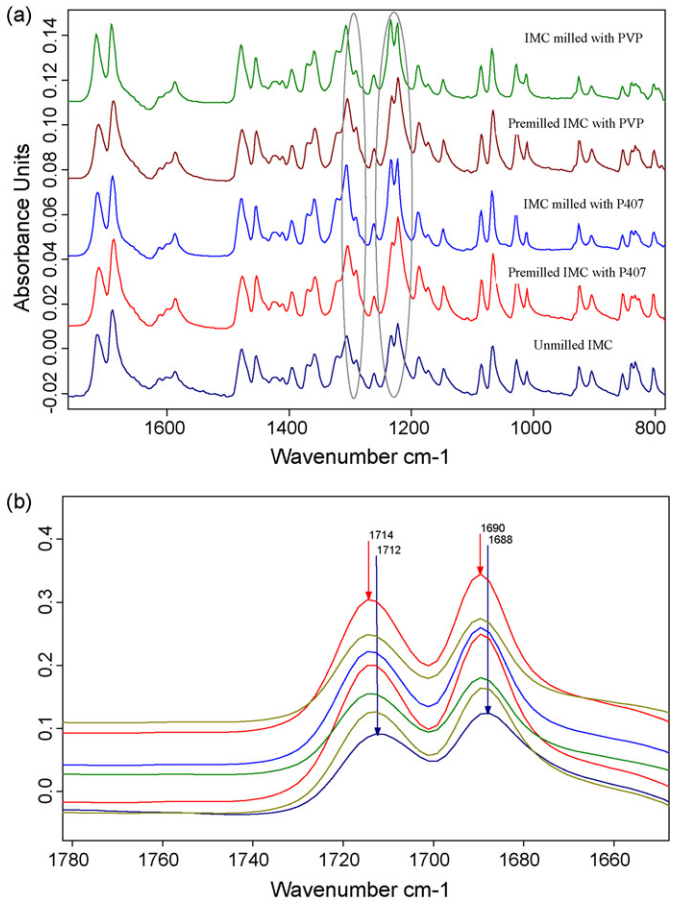

Fig. 6. IR spectra of IMC showing (a) change in the 1300–1200 cm^{-1} region and (b) milled IMC with PVP as a stabilizer at different homogenization pressures and temperatures showing peak shift. From bottom to top (a) pre-milled IMC; (b) IMC milled at 1000 bar, 2 °C; (c) IMC milled at 1500 bar, 2 °C; (d) IMC milled at 1500 bar, 10 °C; (e) IMC milled at 1500 bar, 20 °C; (f) IMC milled at 1500 bar, 30 °C; (g) IMC milled at 1500 bar, 40 °C. Shifts were observed in the spectra following high pressure homogenization. The peaks at 1712 and 1688 cm^{-1} were shifted to 1714 and 1690 cm^{-1}, respectively.

considering the effect of intermolecular interaction of amorphous IMC and PVP on the IMC dimer.

In γ IMC, molecules exist as dimers (Fig. 7), where, two IMC molecules are associated via intermolecular hydrogen bonding between their carboxylic acid functional groups (Taylor and Zografi, 1997). High mechanical energy input during wet milling may induce surface amorphization and lead to disruption of the dimers in the presence of PVP. Since, the OH group of the carboxylic acid function of IMC is involved in intermolecular hydrogen bonding with PVP (Taylor and Zografi, 1997) (Fig. 7) the dimer disruption will then lead to an increase in the availability of free C=O and hence results in an increase in its bond strength. Moreover, the formation of intermolecular bond between PVP and IMC also stabilizes the amorphous phase present on the surface of the particles against recrystallization.

It appears that during wet milling surface amorphization is induced in the milled IMC particles. In contrast to milled IMC, no differences in the peak positions and the shape were observed in the IR spectra of milled SIM (data not shown). This indicates that SIM particles were crystalline following wet milling. Although peak shifts were observed for the milled IMC samples in the presence of PVP as stabilizer, the magnitude of the peak shift was not significant suggesting that the surface amorphization may be present at very low levels.

Table 2
Thermodynamic parameters for IMC samples derived from MTDSC measurements.

Samples	T_m (°C)	ΔH_m (J/g)
IMC unmilled	158.8 ± 0.9	101.6 ± 1.2
IMC-PVP pre-milled*	158.4 ± 0.4	90.3 ± 1.1
IMC-PVP milled*	157.0 ± 0.6	83.3 ± 1.3
IMC-P407 pre-milled	158.6 ± 0.3	100.4 ± 0.9
IMC-P407 milled	157.6 ± 0.6	97.2 ± 2.8

IMC: Indomethacin; PVP: Polyvinyl pyrrolidone-K25; P407: Poloxamer 407.
* Significant difference in ΔH_m; $p = 0.028$.

4.2.3. MTDSC

MTDSC is a suitable technique for the detection of low levels (<5%) of amorphous content in the samples (Guinot and Leveiller, 1999). Therefore, to confirm the results (surface amorphization) obtained from IR spectroscopy, MTDSC was utilized.

In a suspension, the stabilizer is present (i) in the aqueous phase (dissolved) and (ii) on the particle surface (adsorbed). After vacuum drying of a suspension, the excess stabilizer in the dried formulation may influence the thermodynamic transitions of the drug during an MTDSC run. Therefore, to remove the unadsorbed stabilizer, the suspension was filtered and the precipitate (consisting of drug and adsorbed stabilizer) was used for MTDSC studies.

The MTDSC thermograms of the pre-milled and milled IMC (Fig. 8a) and SIM (Fig. 8b) samples did not show the presence of glass transition event or recrystallization exotherm as determined from reversing and non-reversing heat flow signals. Endothermic peaks corresponding to the melting of γ IMC and SIM were observed in thermograms (Fig. 8a and b). The absence of T_g in the thermogram indicated that either the milled samples were crystalline or the amorphous content in milled samples was too low to be detected by MTDSC. MTDSC technique utilizes the bulk (entire) sample for analysis and amorphous content present on the surface of crystalline particle becomes a small part of the total signal (Buckton and Darcy, 1999) and hence may not be detected when present in low level.

Although surface amorphization could not be detected, the thermodynamic parameters derived for melting transition were different for unmilled, pre-milled and milled samples (Tables 2 and 3). The T_m of the milled IMC was lower when compared with the pre-milled IMC. Broadening of the melting peak of the milled samples was also observed. At the same time, the melting enthalpy (ΔH_m) of the unmilled, pre-milled and milled samples was different.

The above-mentioned changes in the thermodynamic parameters of the drugs could be due to the presence of stabilizer in the pre-milled and milled samples. Although the excess stabilizers present in the suspensions were removed by vacuum filtration before MTDSC, the stabilizer that was adsorbed on the particles can still affect the T_m. During an MTDSC run, if the stabilizer has lower melting point than the drug, it will melt first. If the drug is miscible in the melt, it may decrease the T_m of the drug. Moreover, T_m is a colligative property and hence miscibility of the drug in the stabilizer, during an MTDSC run, will decrease the T_m of the drug (Chokshi

Table 3
Thermodynamic parameters for SIM samples derived from MTDSC measurements.

Samples	T_m (°C)	ΔH_m (J/g)
SIM unmilled	139.3 ± 1.0	70.8 ± 0.8
SIM-PVP pre-milled*	138.4 ± 0.8	71.2 ± 0.9
SIM-PVP milled*	136.7 ± 0.7	54.9 ± 3.2
SIM-P407 pre-milled	138.9 ± 0.5	69.1 ± 1.1
SIM-P407 milled	138.9 ± 0.7	67.4 ± 1.5

SIM: Simvastatin; PVP: Polyvinyl pyrrolidone-K25; P407: Poloxamer 407.
* Significant difference in ΔH_m; $p = 0.02$.

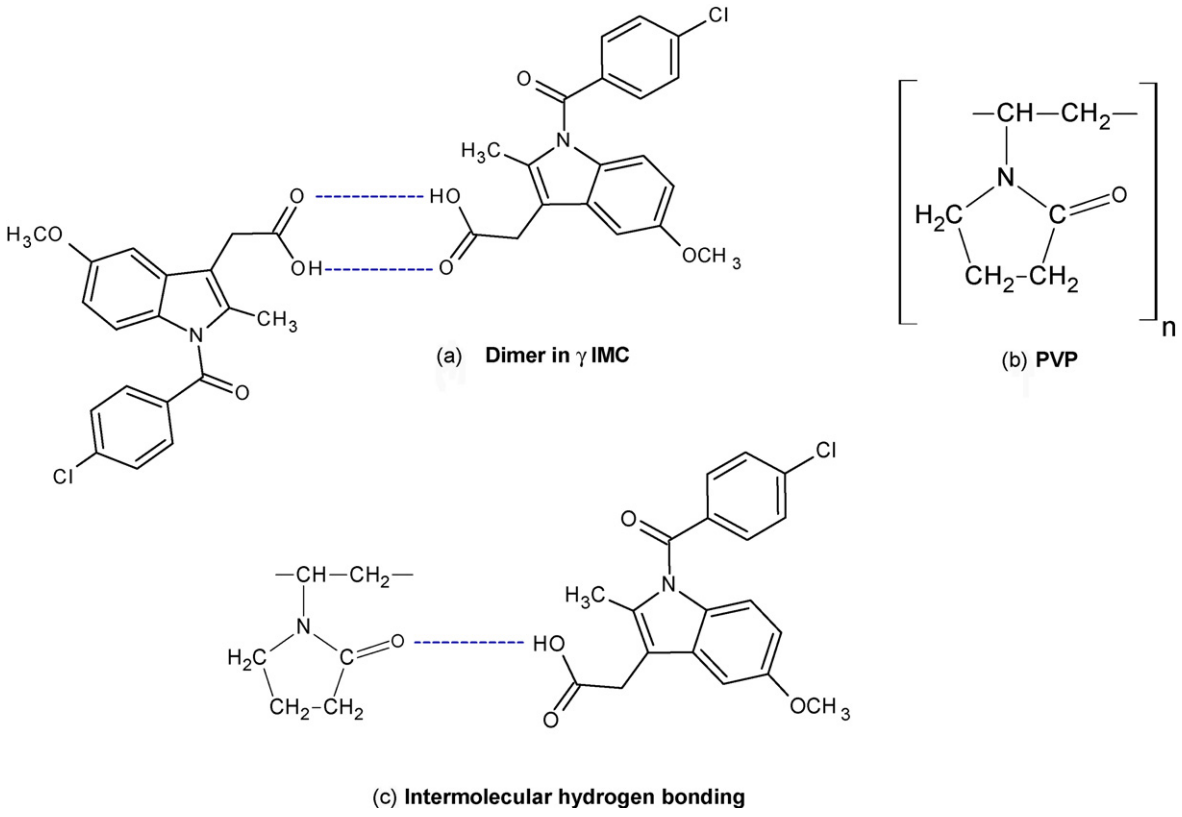

Fig. 7. Dimer in γ IMC and intermolecular hydrogen bonding between IMC and PVP.

et al., 2005). When the drug is completely miscible in the stabilizer, the decrease in T_m will depend upon the amount of stabilizer present in the sample.

Previously, wet milled freeze dried nifedipine samples showed a broadening of melting peak and a decrease in T_m when compared with the unmilled drug (Hecq et al., 2005). These results were attributed to the miscibility of nifedipine in hydroxyl propyl methyl cellulose which was used as stabilizer during milling. In a similar way, PVP and P407 present in the milled samples may affect the endothermic transition of milled IMC and SIM.

Further, the enthalpy value obtained from MTDSC is directly proportional to the amount of drug in each sample. Considering that some amount of adsorbed stabilizer was present in the milled sample, this could have affected the weight of the drug used for MTDSC. As a result, although, the total weight of the samples was uniform (2.5 mg), the actual weight of the drug in each sample was different. This could be the reason for a difference in enthalpy values obtained in unmilled, pre-milled and milled samples.

From solid state characterization studies, it appears that following HPH there are changes in the solid state of IMC but not in SIM. Physical appearance and IR spectroscopy studies indicated that the changes are limited to the surface of the IMC particles (possible surface amorphization). However, complete amorphization of the drugs was not observed as reported previously after cryogrinding of IMC and SIM (Crowley and Zografi, 2002; Graeser et al., 2008).

Absence of bulk amorphization during HPH signifies the role of water in the solid state transformation. To further investigate this aspect, cryogrinding of IMC was performed with and without water. For this experiment, an impact mill (Model 6750, SPEX CertiPrep, Metuchen, NJ) was used. It consisted of stainless steel cylindrical vessel immersed in liquid nitrogen. To perform grinding, a stainless steel rod was placed inside the vessel and vibrated using a magnetic coil. IMC (150 mg) was added to the vessel and grinding was performed for 30 min at an impact frequency of 10 cycles per second for a 2 min period separated by a 2 min cool-down period. After grinding, sample was transferred to a glove bag purged with nitrogen gas and allowed to warm to room temperature. For grinding in the presence of water, 0.2 ml of water was added to the vessel along with IMC and the above-mentioned procedure was performed. All the samples were prepared in triplicate and characterized by IR spectroscopy.

Cryogrinding of γ IMC in the absence of water resulted in transformation to amorphous form (Fig. 9). The IR spectra showed characteristic peaks of amorphous IMC at 1735 cm^{-1} (shoulder), 1710 cm^{-1} (strong) and 1688 cm^{-1} (strong), as reported in the literature (Taylor and Zografi, 1997). Crowley and Zografi (2002) have reported similar results where cryogenic grinding of γ IMC resulted in the formation of amorphous phase.

In the presence of water, cryogrinding did not produce amorphous phase and IR spectra similar to γ IMC were observed (Fig. 9). Moreover, the spectra showed similarities to that obtained after HPH at 1500 bar. Changes in 1300–1200 cm^{-1} region and band shifts to higher frequency were observed as noted previously for samples milled by HPH.

One possibility is that water with a T_g of −130 °C would plasticize the amorphous phase formed during cryogrinding and decrease the T_g of amorphous IMC much below the room temperature leading to rapid recrystallization before the analysis. Previously Crowley and Zografi (2002) reported similar results where cryogrinding of IMC solvates (methanol and t-butanol) did not produce amorphous phase and plasticizing effect was suggested by Crowley et al. to be the reason for absence of amorphous phase.

Another possibility is that the mechanical energy input during cryogrinding is not sufficient to destabilize the crystal (or to increase the crystal defects beyond a particular concentration), in the presence of water molecule. Prior to grinding IMC and

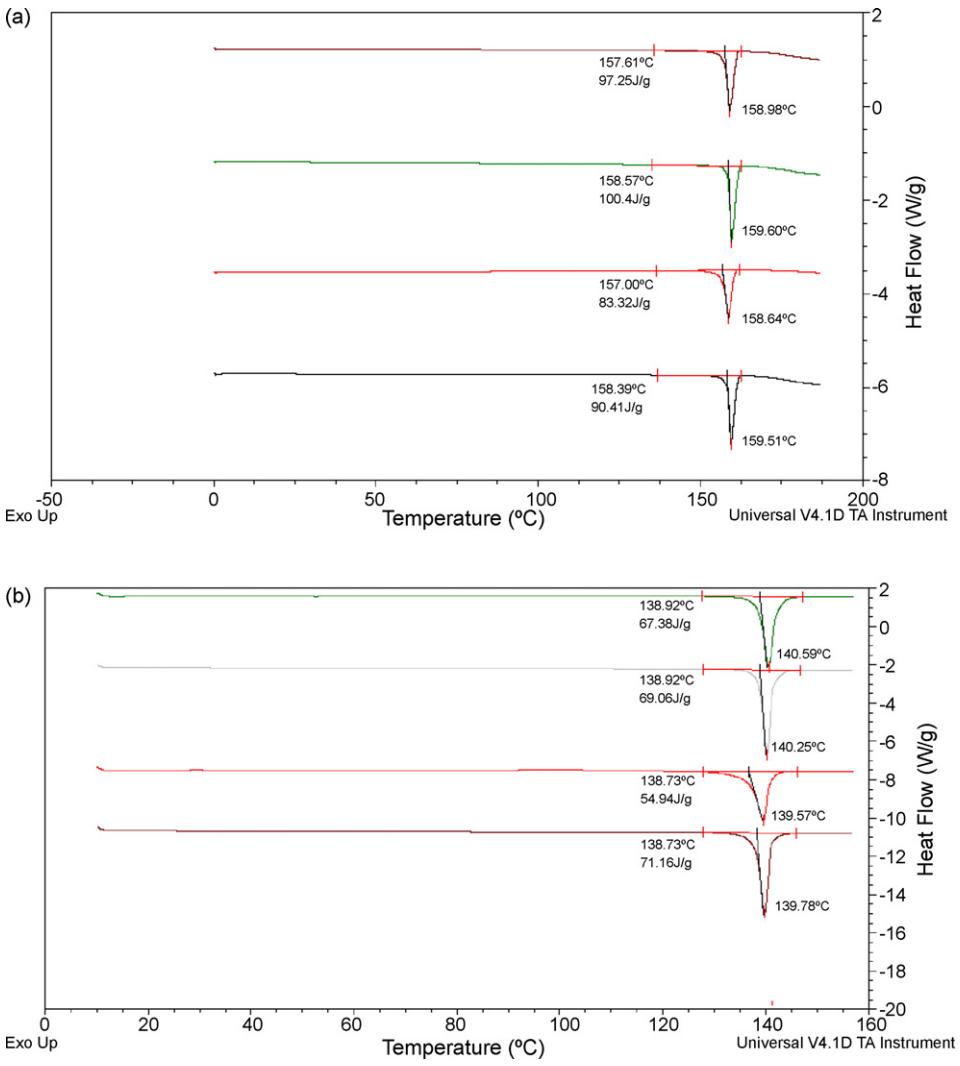

Fig. 8. MTDSC thermograms (total heat flow) of (a) IMC and (b) SIM. The four thermograms in (a) and (b) represent samples milled with P407, pre-milled with P407, milled with PVP and pre-milled with PVP (top to bottom).

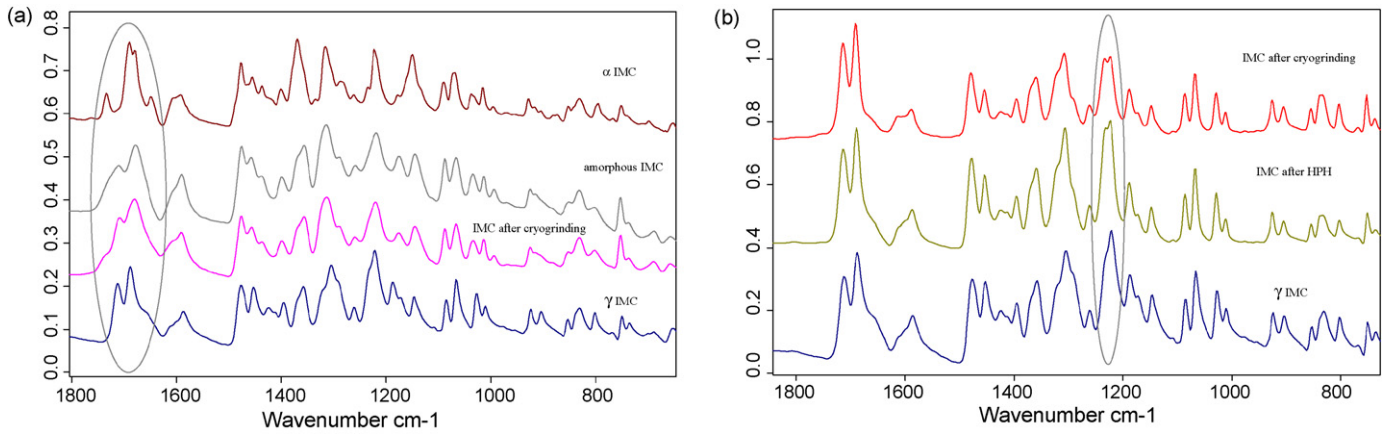

Fig. 9. IR spectra of IMC after cryogrinding (a) in the absence of water and (b) in presence of water. IR spectra of α, γ and amorphous IMC have been shown for comparison in (a).

water are under cryogenic conditions. Therefore, during grinding, the mechanical energy is utilized to destabilize the lattice of both IMC (ΔH fusion ~100 J/g) and ice (ΔH fusion ~330 J/g). This would leave insufficient energy for the formation of IMC amorphous phase.

5. Conclusion

In conclusion, post HPH there was no chemical degradation as indicated by HPLC analysis. Solid state characterization by visual observation and IR spectroscopy indicated surface amorphization

in the milled IMC and SIM crystalline particles. Surface amorphization could not be detected by MTDSC and therefore it is possible that the amorphization is induced at very low levels (<1%) and only on the surface.

In general, bulk amorphization of a crystalline drug may not be favored during wet milling due to the presence of water. However, there is a possibility of polymorphic transformation, during wet milling, in those cases where a metastable form is used as a starting material. In the present study, for both IMC and SIM, most stable polymorphic forms were used, and therefore, polymorphic transitions were not observed.

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