Solid-state amorphization of rebamipide and investigation on solubility and stability of the amorphous form

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ABSTRACT
Solid-state amorphization of crystalline rebamipide (RBM) was realized by ball milling and spray drying. The amorphous content of samples milled for various time was quantified using X-ray powder diffraction. Crystalline RBM and three amorphous RBM obtained by milling and spray drying were characterized by morphological analysis, X-ray diffraction, thermal analysis and vibrational spectroscopy. The crystal structure of RBM was first determined by single-crystal X-ray diffraction. In addition, the solubility and dissolution rate of the RBM samples were investigated in different media. Results indicated that the solubility and the dissolution rates of spray-dried RBM-PVP in different media were highly improved compared with crystalline RBM. The physical stabilities of the three amorphous RBM were systematically investigated, and the stability orders under different storage temperatures and levels of relative humidity (RH) were both as follows: spray dried RBM < milled RBM < spray dried RBM-PVP. A direct glass-to-crystal transformation was induced under high RH, and the transformation rate rose with increasing RH. However, amorphous RBM could stay stable at RH levels lower than 57.6% (25°C).

Introduction

Various solid forms of drugs can exhibit significantly different physicochemical properties, such as stability, solubility, dissolution rate and bioavailability, so they show varying qualities, therapeutic efficiencies and toxic side effects. Amorphous forms have recently received considerable attention for developing novel techniques or patterns to improve physicochemical properties of many poorly water-soluble drugs. Compared with the crystalline counterparts, the amorphous form lacks a long-range molecular order and exhibits higher Gibbs free energy, which translates to higher solubility, faster dissolution rate and greater bioavailability.

A number of methods can be used to induce amorphization of the crystalline forms, such as milling, comilling, melt quenching, spray drying, freeze drying and dehydration of crystalline hydrates. Milling is frequently used in the pharmaceutical industry to reduce the particle size of drugs and change the structural state of the material through an amorphization process, which can improve solubility and dissolution rate of the drugs further. Moreover, milling is an environment-friendly process to produce amorphous forms without using solvents or high-temperature melting. Spray drying can induce a solid-state transformation from crystalline starting material to amorphous product without destructive chemical change. In addition, in the presence of a polymer, the amorphous material can be stabilized and show an improved dissolution rate and absorption.

The therapeutic characteristics of the amorphous material generally cannot be guaranteed during storage, because amorphous drugs have a metastable nature and show a propensity to revert to the original form or recrystallize to other stable crystalline forms. Storage temperature can affect the stability of the amorphous drug through influencing its molecular mobility. Water is a ubiquitous plasticizer that can lower Tg of the system and enhance molecular mobility, and thus, it can induce recrystallization of amorphous drugs, which is an obstruction for ensuring the quality of solid-state pharmaceutical products. Moreover, both temperature and water vapor are unavoidable environmental stress during storage. Therefore, understanding the effects of temperature and water vapor on the physical stability of amorphous drugs is essential for the stability assessment and quality control of amorphous drugs.

Rebamipide [2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid] (RBM; Figure 1), is a potent antiulcer agent that is mainly used for the treatment of gastric ulcers, acute gastritis and exacerbated chronic gastritis. According to the US Food and Drug Administration, RBM is listed in the Biopharmaceutics Classification System Class IV because of its low aqueous solubility and low permeability. Thus, RBM possesses very low oral bioavailability under 10% and is used as a model drug to plan different strategies for improving bioavailability. The solubility and dissolution rate of amorphous RBM solid dispersion tablet, which was prepared by spray drying in combination with hydrophilic polymer (sodium alginate) and alkaliizer (sodium carbonate), were dramatically improved compared with crystalline RBM. This study aimed to investigate the effect of mechanical milling on the structure, solubility, and dissolution rate of RBM and compare the findings of milling with those of spray drying. The crystal structure of RBM was first disclosed by single-crystal X-ray diffraction (SXRD). The structural changes induced by milling were demonstrated using various analytical techniques. Solid-state transformations of RBM during milling were monitored at room temperature, and the amorphous content (%) of samples milled for various time was quantified using
X-ray powder diffraction (XRPD). Moreover, the solubility and dissolution rate of three amorphous RBM obtained by milling and spray drying in various media, and the stabilities of amorphous RBM under different temperatures and levels of relative humidity (RH), were investigated to evaluate the ability of the further application of amorphous RBM in the pharmaceutical industry.

Materials and methods

Materials

RBM (99% pure) was purchased from Yitai Technology Co., Ltd. (Shanghai, China). Ultrapure water (18 MΩ resistivity from a Millipore system) was used throughout the experiment. Dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) were of analytical grade and purchased from Kelong Co., Ltd. (Chengdu, China). Poly(vinylpyrrolidone) K30 (PVP) was purchased from Fengli Jingqiu Commerce and Trade Co., Ltd. (Beijing, China).

Preparation and milling of crystalline RBM

A single crystal of crystalline RBM was obtained by antisolvent diffusion (water). RBM was dissolved in DMSO (1 g/15 mL) in a beaker flask by heating and stirring to achieve complete dissolution. The solution was then cooled to room temperature and slowly diffused into the water. RBM crystals were placed in a transparent glass holder. Surface morphology was recorded by an optical microscope (CX21, Olympus, Tokyo, Japan) with a magnification of 40×. Photomicrographs were taken with a digital camera (DSC-HX-50, Sony, Tokyo, Japan).

Scanning electron microscopy (SEM)

The SEM micrographs were examined using a JSM-7500F scanning electron microscope at 5.0 kV. Electrically conductive samples were prepared by coating with a thin layer of gold in vacuum prior to examination.

XRPD

XRPD data were collected at room temperature using an X’Pert PRO diffractometer (PANalytical) with a PIXcel 1D detector and Cu Kα radiation (λ = 1.5406 Å, generator settings: 40 kV and 40 mA). Samples were loaded on a rectangular glass sample holder. Diffractograms were collected in 2θ range of 4–50°, with a step size of 0.01313° and a counting time of 30 ms/step.

SXRD

Single-crystal data were collected on an Oxford Diffraction Xcalibur Nova system with Mo Kα radiation (λ = 0.71073 Å) at 293.15 K. A suitable crystal was selected and held on Xcalibur Eos diffractometer. Cell refinement and data reduction were applied using the program of Olex236, and the structure was solved and refined using SHELX-97 programs37.

Differential scanning calorimetry (DSC)

Thermal transformations were determined by a differential scanning calorimeter Q200 (TA Instruments Co., New Castle, DE). Samples (3–5 mg) were exposed to a heating rate of 10 °C/min over a temperature of 30–400 °C under N2 purging (20 mL/min) in pierced aluminum pans. The instrument was calibrated for temperature and heat flow using indium standard.

Thermogravimetric analysis (TGA)

TGA was performed using TG209F1 Iris (NETZSCH, Germany) in aluminum crucibles at a heating rate of 10 °C/min from 30 °C to 800 °C under N2 purging (60 mL/min).

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were recorded on a Nicolet-6700 FT-IR (Thermo, Waltham, MA) spectrometer with a smart OMNI-sampler accessory. Each sample was mixed with 100-fold KBr to prepare the sheet and measured in transmission mode (a spectral range of 4000–400 cm−1, resolution of 4 cm−1 and 64 interferograms per spectrum).

Quantification of amorphous content

Samples of differing crystallinity were prepared by shaking mixtures of crystalline RBM and amorphous RBM in glass vials. The mixtures contained 0, 20, 40, 60, 80 and 100% amorphous RBM (w/w) were measured by XRPD to yield a calibration curve for further quantification studies, each measurement was performed in triplicate.
**Solubility studies**

Excess amounts of RBM samples were added to 5 mL of triple-distilled water, HCl (pH 1.2) and phosphate buffer (PBS, pH 6.8) in Erlenmeyer flasks. The suspensions were filtered through 0.45-μm filters and appropriately diluted after stirring for 72 h at constant temperature (37°C). The amount of dissolved RBM was quantified by UV-vis spectroscopy (TU-1901, Beijing) at 230 nm and 327 nm, and the solid residue was analyzed by XRPD. All solubility measurements were performed in triplicate.

**Dissolution studies**

Dissolution studies were conducted using a ZRC-8D dissolution tester (Tianjin Chuangxing Electronic, China) at 100 rpm and 37°C ± 0.5°C. RBM samples (100 mg) were added to 900 mL of triple-distilled water, HCl (pH 1.2) and PBS (pH 6.8). Approximately 5 mL of the samples was withdrawn using a syringe at 5, 10, 15, 20, 30, 60, 90, and 120 min. The samples were filtered through a 0.45-μm hydrophilic membrane filter, appropriately diluted and analyzed using a UV-vis spectrophotometer at 230 nm and 327 nm. All dissolution measurements were performed in triplicate.

**Stabilities of amorphous RBM**

The stability of amorphous RBM to temperature was investigated under different temperatures of -20, 4, 25 and 50°C (0% RH, achieved with P2O5 in sealed desiccators). The effect of water vapor on the stability of amorphous RBM was monitored under different RH levels of 11.3%, 32.8%, 43.2%, 57.6%, 75.3%, 84.3% and 97.3% in desiccators at 25°C, which were sealed using saturated salt solutions of LiCl, MgCl2, K2CO3, NaBr, NaCl, KCl and K2SO4, respectively.

**Results and discussion**

**Optical microscopy and SEM analysis**

Single crystals of RBM showed a colorless transparent rhomboid block-shaped crystal structure (Figure 2(a)). The surfaces of the blocky crystals were not smooth; some ups and downs of edges and corners on the surfaces were observed (Figure 2(b)). Commercial RBM showed a clavate structure with various dimensions (Figure 2(c)), and PVP showed an atypical spherical structure with hollows (Figure 2(d)). RBM obtained by milling was observed as irregularly shaped blocky particles (Figure 2(f)), while that acquired by spray drying showed spherical structure. The spherical structure of the spray dried RBM was complete and smooth (Figure 2(g)); however, the spray dried RBM-PVP showed an atypical spherical structure with hollows, which was smaller than PVP (Figure 2(h)).

**SXRD analysis**

The crystal structure of RBM was determined by SXRD for the first time. The crystallographic data and details of refinement are listed in Table 1. In the 3D packing of RBM, molecules were arranged in layers along the ab plane and the layers stretched in a positive and inverted “W” fashion (Figure 3(c)). Two kinds of intermolecular hydrogen bonds (O–H⋯O and N–H⋯O) were found in RBM (Figure 3(b)). These bonds contributed to the stability and periodicity of the RBM structure. All the hydrogen bonds were in the direction...
of the c-axis. The hydrogen atoms of carboxyl in RBM interacted with another molecule via the oxygen atoms of amide in the quinoline ring through O2–H2/C1/C1/C1O4 (2.513 Å, 165°) hydrogen bonds. The N2–H2A/C1/C1/C1/C1O3 (2.870 Å, 166°) hydrogen bonds formed between the carbonyl oxygen atoms of carboxyl and the hydrogen atoms of amide in the quinoline ring. Carboxyl and amide formed a six-membered ring under the influence of the two kinds of hydrogen bonds, and this ring was beneficial to the stability of the RBM crystal structure.

**XRPD analysis**

The XRPD patterns of crystalline RBM, PVP, the physical mixture, milled RBM, spray dried RBM, and spray dried RBM-PVP are illustrated in Figure 4(a). The single crystals of RBM were proven to share the same crystal form with commercial RBM by XRPD, which revealed numerous Bragg peaks, characteristic of crystalline materials. Thus, both commercial RBM and the single crystals were named as crystalline RBM. PVP showed no intrinsic peak obviously due to the amorphous nature of the polymer. In the XRPD pattern of the physical mixture, almost all of the characteristic peaks of crystalline RBM and the diffuse halos of PVP were observed. However, the XRPD patterns of milled RBM, spray dried RBM, and spray-dried RBM-PVP exhibited two large and diffuse halos characteristic of amorphous materials.

**DSC analysis**

Figure 4(b) showed the DSC curves of PVP and RBM samples. Crystalline RBM only showed an endothermic peak at 310.3 °C, which revealed its melting point. PVP showed no peak due to its amorphous nature. The physical mixture showed both the Cp jump of PVP and the melting peak of RBM. The first broad endotherm observed in milled RBM around 90 °C corresponded to the loss of free water molecules unavoidably caught by the powder during the extensive milling procedure. Milled RBM displayed a Cp jump characteristic of a glass transition at Tg = 140.3 °C (onset), which was followed by an exothermic recrystallization peak at 176.4 °C. This recrystallization and the Cp jump indicated that milled RBM was amorphous form12,16. The milled amorphous RBM recrystallized as crystalline RBM when heated to 180 °C (Figure S1), which showed a melting endotherm at lower temperature (302.2 °C). Thus, the shift of the melting peak was probably attributed to that crystallites after recrystallization were much smaller than that before milling. Spray dried RBM showed a glass transition at Tg = 88.3 °C (onset) and two exothermic recrystallization peaks at 137.9 °C and 142.3 °C. In addition, spray-dried RBM recrystallized to a new form (Form A) upon heating, which showed a melting peak at 296.4 °C (Figure S1). The DSC curve of the spray dried RBM-PVP indicated that the crystalline form of RBM was transformed into the amorphous form. In addition, the amorphous RBM spray dried with PVP did not recrystallized upon heating, which indicated that PVP stabilized the amorphous RBM. The wide

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**Table 1. Crystallographic parameters of RBM crystals.**

| Parameters          | RBM         |
|---------------------|-------------|
| Chemical formula    | C19H15ClN2O4 |
| Formula weight      | 370.78      |
| Crystal system      | Monoclinic  |
| Space group         | P21/c       |
| T (K)               | 293.15      |
| a (Å)               | 8.9919(6)   |
| b (Å)               | 28.987(2)   |
| c (Å)               | 9.0093(7)   |
| α (°)               | 90.00       |
| β (°)               | 97.113(6)   |
| γ (°)               | 90.00       |
| V(Å³)               | 2537.1(3)   |
| Z                   | 4           |
| ρcal (g/cm³)        | 0.971       |
| μ (mm⁻¹)            | 0.169       |
| Crystal size (mm³)  | 0.3 × 0.2 × 0.2 |
| F (000)             | 768.0       |
| 2θ for data collection (°) | 5.94 to 52.74 |
| Index ranges        | -11 ≤ h ≤ 11, -34 ≤ k ≤ 36, -12 ≤ l ≤ 12 |
| Reflns collected    | 20,595      |
| Unique reflns       | 5166        |
| Goodness-of-fit on F² | 0.961     |
| R₁, R(≥ 2(I))       | 0.0817      |
| wR² (all)           | 0.2452      |

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![Figure 3](image-url)
shape of the melting peak of crystalline RBM suggested that RBM underwent chemical decomposition accompanied with melting.

**TGA analysis**

TGA curves can provide information about mass reduction at each period (Figure 4(d)). Crystalline and milled RBM had no mass reduction before decomposition. PVP showed a weight loss of 10.38% before 105°C. With the effect of PVP, different levels of weight loss were observed in the TGA curves of the physical mixture and spray dried RBM-PVP. A weight loss of 8.45% was detected before 180°C for spray-dried RBM, which was attributed to the removal of DMF. Thus, spray dried RBM was the amorphous form of the DMF solvate of RBM. The weight loss of the RBM samples was observed at over 300°C, further illustrated the melting with decomposition of RBM, which was explained in DSC analysis. However, the initial decomposition temperature of the RBM samples were different (crystalline RBM > spray-dried RBM > milled RBM > spray-dried RBM-PVP). This finding suggested that commercial crystalline RBM was more thermally stable than those recrystallized from the three amorphous RBM upon heating.

**FTIR analysis**

The FTIR spectra of the samples are shown in Figure 4(c). Crystalline RBM showed a broad peak at 3450.8 cm⁻¹ and a small peak at 3274.8 cm⁻¹, which were attributed to the ν(N–H). The peaks observed at 1726.5 and 1645.4 cm⁻¹ for crystalline RBM were assigned to the ν(C=O) of the carboxyl and amide, respectively. PVP showed three peaks (3551.8, 3478.6 and 3415.3 cm⁻¹) at the range of 3600–3400 cm⁻¹ and a characteristic broad peak at 1640.3 cm⁻¹ corresponding to the ν(C=O). The physical mixture showed almost all of the characteristic peaks of the crystalline RBM and PVP. The ν(N–H) of milled RBM was observed at 3444.6 and 3277.2 cm⁻¹, and the ν(C=O) was located at 1723.2 and 1646.1 cm⁻¹. The N–H stretching vibrations of spray-dried RBM were observed at 3415.1, and 3269.6 cm⁻¹. In addition, its ν(C=O) of the carboxyl (1724.1 cm⁻¹) became weak, and its ν(C=O) of the amide (1651.4 cm⁻¹) became broad, which may be due to the effect of DMF. The IR spectrum of spray dried RBM-PVP included the characteristic peaks of RBM and PVP but was different from the physical mixture. The C=O stretching vibration of the carboxyl was concealed and a broad peak at 1657.9 cm⁻¹ corresponding to the ν(C=O) of the amide was observed.

**Amorphization of crystalline RBM by milling**

The crystal-to-glass conversion upon milling often requires a milling time of several hours to complete. The effect of milling time on the amorphization of RBM was monitored using XRPD and DSC (Figure 5).

The diffraction peaks decreased rapidly in the first 0.5 h of milling, and 51.34% of amorphous form was generated (Figure 5(a)). With extending milling time, the amorphization rate decreased, and complete amorphization occurred after milling for 4 h (Figure 5(b)). In addition, no new diffraction peak appeared throughout the entire milling process, suggesting that milling induced a direct crystal-to-glass transformation.

XRPD demonstrated that milling of crystals could remove all traces of crystallinity. However, the resulting material may not be an amorphous structure but a microcrystalline state, containing crystals so small that they pass the detection of XRPD. Therefore, DSC was used to distinguish between amorphous and microcrystalline states of RBM based on the presence or absence of glass transition. The glass transition jump and the recrystallization exothermic peak on DSC curves gradually became visible after 0.5 h (Figure 5(c)), which indicated that amorphous form was generated. The enthalpies of recrystallization became larger with the increase in milling time, which indicated that the degree of amorphization rose. In addition, the DSC curves remained approximately the same for milling time longer than 5 h, suggesting that complete amorphization was realized, which slightly deviated from the 4 h of the XRPD result (Figure 5(d)).
Quantification of amorphous content

Degree of crystallinity (C) is given by the following equation:

\[
C = \frac{I_c}{I_c + I_a} \times 100
\]

(1)

where \(I_c\) and \(I_a\) are intensities of the crystalline and amorphous regions, respectively. The intensity of the XRPD from the crystalline region of the sample \(I_c\) is proportional to the area under the sharp peaks above the background scattering, while the intensity of the XRPD from the entire sample \(I_c + I_a\) is proportional to the total area of the X-ray pattern. Thus, the amorphous content (A) of the sample can be calculated by the following equation:

\[
A = 100 - R_x \times 100
\]

(2)

where \(R_x\) is the ratio of the area under the crystalline peaks to the total area.

The \(R_x\) of samples were calculated using MDI jade 6.5 program. The calibration curve was obtained by plotting \(R_x\) vs A in mixtures contained 0, 20, 40, 60, 80 and 100% amorphous RBM (Figure 6). The amorphous content of the sample milled for various time was quantified using the calibration curve (Table 2).

Solubility studies

The solubility of the three amorphous RBM was improved at various degrees in all media (Figure 7). The solubility of milled RBM (39.0 µg/mL), spray dried RBM (51.0 µg/mL), and spray-dried RBM-PVP (478.9 µg/mL) in water was increased by about 2.5, 3.2 and 30.1 times, respectively, compared with crystalline RBM (15.9 µg/mL), respectively. Crystalline RBM showed very low solubility in pH 1.2 HCl (0.5 µg/mL) due to the carboxyl of RBM. Three amorphous RBM all showed an improved solubility, especially spray dried RBM-PVP. And the solubility of milled RBM (0.8 µg/mL), spray-dried RBM (1.3 µg/mL), and spray-dried RBM-PVP (127.1 µg/mL), was improved by 1.6, 2.6 and 254.2 times, respectively. For the solubility in pH 6.8 PBS, a 1.9-fold (2448.0 µg/mL) and a 3.9-fold (5067.9 µg/mL), was improved in milled and spray dried amorphous RBM, respectively, compared with crystalline RBM (1289.1 µg/mL).

These results indicated that amorphization of RBM could improve its solubility in different media at various degrees, which might provide a potential approach to improve the bioavailability of RBM.

Residual solid samples recovered in various media in solubility studies were analyzed by XRPD. All samples showed numerous XRPD patterns, amorphous content quantified using XRPD and DSC patterns at 100–200 °C of crystalline RBM milled for different time.
diffraction peaks, indicating that amorphous RBM reversed to crystalline RBM. Thus, the amorphous RBM samples lost some solubility advantages due to recrystallization. However, spray-dried RBM-PVP still showed highly improved solubility in the three media, which was ascribed to the effect of hydrophilic PVP.

**Dissolution studies**

The dissolution profiles of crystalline RBM and three amorphous RBM in different dissolution media are shown in Figure 8. Milled RBM showed the fastest dissolution rate in water, and the released RBM still increased after 120 min and reached 30.7%. In contrast, spray-dried RBM showed the slowest dissolution rate due to the static charges developed by spray drying, which caused the samples to aggregate to show decreased wettability and delayed dissolution rate. Crystalline RBM, spray-dried RBM and spray-dried RBM-PVP all reached equilibrium of dissolution after 90 min, and the released RBM were about 15.5, 10.0 and 20.0%, respectively.

When dissolved in HCl, the released RBM of crystalline RBM, milled RBM and spray-dried RBM were approximately 0.2% to 1.2%, owing to the very low solubility of RBM in the acidic condition. However, the dissolution equilibrium of the milled and spray-dried RBM were improved compared with crystalline RBM. The dissolution of spray dried RBM-PVP was highly enhanced, and the “Spring and Parachute” was observed. The “peak” dissolution (7.7%) appeared at 20 min, followed by a steep decline in the dissolved RBM due to recrystallization of amorphous RBM. But the released RBM was still 15 times of crystalline RBM at 120 min.

In the case of PBS, crystalline RBM, milled RBM and spray-dried RBM-PVP released approximately 85% of drug in 20 min.

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**Figure 7.** Solubility of crystalline RBM, milled RBM, spray-dried RBM and spray-dried RBM-PVP in different media (n = 3).

**Figure 8.** Dissolution curves of crystalline RBM, milled RBM, spray-dried RBM and spray-dried RBM-PVP in different media (n = 3).

**Figure 9.** XRPD patterns of milled amorphous RBM at different storage temperatures: (a) – 20, (b) 4, (c) 25 and (d) 50 °C (the curves from bottom to top: 1, 2, 3, 5, 8 and 12 weeks).
Figure 10. The XRPD patterns of initially milled amorphous RBM following equilibration at different RH conditions at 25 °C: (a) 11.3%, (b) 32.8%, (c) 43.2%, (d) 57.6%, (e) 75.3%, (f) 84.3% and (g) 97.3% RH (the curves from bottom to top: 5, 10, 15, 20, 25, 30 and 60 d).

Figure 11. SEM images of amorphous RBM storage at 97.3% RH for (a) 0, (b) 5, (c) 10, (d) 20, and (e) 30 d. (20,000×).
After 20 min, crystalline and milled RBM almost reached a dissolution equilibrium, while spray-dried RBM-PVP showed an increasing dissolution and reached an equilibrium of 99.9% at 90 min. Spray-dried RBM released more slowly than other samples within 60 min, but it exceeded crystalline and milled RBM subsequently and released 94.9% of drug after 120 min.

**Stability analysis**

Milled amorphous RBM was proven to be stable in three months at the four temperatures (Figure 9); no sharp diffraction peaks were detected in the XRPD patterns. Spray-dried RBM-PVP was also stable, because PVP stabilized the amorphous RBM, which was explained in DSC analysis. Spray-dried RBM was unstable at 50°C, which was transformed to mixtures of commercial RBM and Form A after 9 d (Figure S2). Combining with the DSC results, the stability order to temperature was spray-dried RBM < milled RBM < spray-dried RBM-PVP.

Milled amorphous RBM was relatively stable at low RH levels (lower than 57.6%); no peak was observed in the XRPD patterns up to 60 d (Figure 10). However, diffraction peaks with low intensity gradually appeared when stored at 75.3% RH for 25 d (Figure 10(e)), indicating that amorphous RBM transformed to crystalline RBM with a relatively slow conversion rate. No new diffraction peak was detected except for those in crystalline RBM, which indicated that a glass-to-crystal transformation was induced by high RH. Obvious diffraction peaks appeared at 15 d under 84.3% RH, while they appeared at 5 d under 97.3% RH. This phenomenon indicated that a higher RH resulted in a more rapid transformation rate, which was also confirmed by quantification experiment. After stored under 75.3%, 84.3% and 97.3% RH for 60 d, the amorphous content was 74.5%, 23.8% and 16.9%, respectively. Hence, milled amorphous RBM should be stored at RH levels lower than 57.6% to maintain its stability. Spray-dried RBM and spray-dried RBM-PVP were also transformed to crystalline RBM under high RH (Figure S3). The transformation rate of spray-dried RBM was faster than that of spray-dried RBM-PVP; diffraction peaks with high intensity were observed in spray-dried RBM after 9 d under 84.3% RH, and 12 h under 97.3% RH, while peaks observed in spray dried RBM-PVP after 9 d under 97.3% RH were still with very low intensity. Thus, the stability order to RH was spray-dried RBM < milled RBM < spray-dried RBM-PVP.

In addition, the effects of moisture sorption on the $T_g$ and the recrystallization of milled amorphous RBM under 97.3% RH were further investigated using XRPD and DSC (Figure S4). Results indicated that amorphous RBM recrystallized to crystalline RBM with low intensity just 24 h later. The $T_g$ of RBM decreased with increasing time under 97.3% RH (Figure S4(c)), which was due to the plasticizing of water.

The shape and surface morphology of milled amorphous RBM at 97.3% RH were recorded by SEM at 0, 5, 10, 20 and 30 d (Figure 11). Samples stored at 97.3% RH for 0 d showed an irregular granular shape with smooth surface and sharp edges. With prolonged storage time, the smooth surface became rough; the edges of the granules became blunt and ill defined; some granules even blended together and exhibited a layered structure. In addition, some small round and elliptical particles gradually grew from the rough surface, and even short rods were observed after 30 d. These particles and rods may be attributed to the residual nuclei of crystalline RBM, which recrystallized to crystalline RBM driven by growth.

**Conclusions**

The crystal structure of RBM was first determined by SXRD in this study. Three different amorphous forms of RBM were obtained by mechanical milling and spray drying, which were characterized by optical microscopy, SEM, XRD, DSC, TGA and FT-IR. The materials obtained by spray drying were amorphous form of DMF solvates of RBM. Milled and spray-dried RBM crystallized to two different forms of RBM upon heating. Some amorphous RBM lost part of advantages in solubility and dissolution because of the fast recrystallization to crystalline RBM. But compared with crystalline RBM, both the solubility and dissolution of the spray-dried RBM-PVP were highly improved in different media, which were ascribed to PVP. Both the stability orders to temperature and RH were spray-dried RBM < milled RBM < spray-dried RBM-PVP. Milled amorphous RBM and spray-dried RBM-PVP were stable at −20, 4, 25 and 50°C, while spray-dried RBM transformed to mixtures of commercial RBM and Form A only in 9 d at 50°C. The three amorphous RBM were stable under low RH (lower than 57.6%) and were transformed to crystalline RBM under high RH. The transformation rate was faster in higher RH. Thus, amorphous RBM should be stored at a relatively dry environment.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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**References**

1. Maddileti D, Swapna B, Nangia A. Tetramorphs of the antibiotic drug trimethoprim: characterization and stability. Crystal Growth Des 2015;15:1745–56.
2. Łaszcz M, Trzcińska K, Witkowska A, et al. Phase transition studies of dutasteride crystalline forms. CrystEngComm 2015;17:2346–52.
3. Du Y, Zhang H, Xue J. Vibrational spectroscopic study of polymorphism and polymorphic transformation of the anti-viral drug lamivudine. Spectrochim Acta A 2015;137:1158–63.
4. Lim RTY, Ng WK, Tan RBH. Dissolution enhancement of indomethacin via amorphization using co-milling and supercritical co-precipitation processing. Powder Technol 2013;240:79–87.
5. Lim RTY, Ng WK, Widjaja E. Comparison of the physical stability and physicochemical properties of amorphous indomethacin prepared by co-milling and supercritical anti-solvent co-precipitation. J Supercrit Fluid 2013;79:186–201.
6. Kanaujia P, Poovizhi P, Ng WK, et al. Amorphous formulations for dissolution and bioavailability enhancement of poorly soluble APIs. Powder Technol 2015;285:2–15.
7. Surwase SA, Iktenon L, Aaltonen J, et al. Polymer incorporation method affects the physical stability of amorphous indomethacin in aqueous suspension. Eur J Pharm Biopharm 2015;96:32–43.
8. Xia D, Wu JX, Cui F, et al. Solvent-mediated amorphous-to-crystalline transformation of nitrendipine in amorphous particle suspensions containing polymers. Eur J Pharm Sci 2012;46:446–54.

9. Murdande SB, Pikal MJ, Shanker RM, et al. Solubility advantage of amorphous pharmaceuticals: II. Application of quantitative thermodynamic relationships for prediction of solubility enhancement in structurally diverse insoluble pharmaceuticals. Pharm Res 2010;27:2704–14.

10. Chawla G, Gupta P, Thilagavathi R, et al. Characterization of solid-state forms of celecoxib. Eur J Pharm Sci 2003;20:305–17.

11. Caron V, Willart JF, Lefort R, et al. Solid state amorphization kinetic of alpha lactose upon mechanical milling. Carbohydr Res 2011;346:2622–8.

12. Willart JF, Durand M, Briggner LE, et al. Solid-state amorphization of linoprazan by mechanical milling and evidence of polymorphism. J Pharm Sci 2013;102:2214–20.

13. Bahl D, Bogner RH. Amorphization of indomethacin by co-grinding with neusilin US2: amorphisation kinetics, physical stability and mechanism. Pharm Res 2006;23:2317–25.

14. Bolla G, Mittapalli S, Nangia A. Pentamorphs of acedapsone. Crystal Growth Des 2014;14:5260–74.

15. Wlodarski K, Sawicki W, Paluch KJ, et al. The influence of amorphization methods on the apparent solubility and dissolution rate of tadalafl. Eur J Pharm Sci 2014;62:132–40.

16. Willart JF, Descamps M. Solid state amorphization of pharmaceuticals. Mol Pharm 2008;5:905–20.

17. Sussich F, Cesaro A. Trehalose amorphization and recrystallization. Carbohydr Res 2008;383:2667–74.

18. Macfionnhghale P, Hu Y, Gniado K, et al. Effects of ball-milling and cryomilling on sulfamerazine polymorphs: a quantitative study. J Pharm Sci 2014;103:1766–78.

19. Hu Y, Macfionnhghale P, Caron V, et al. Formation, physical stability, and quantification of process-induced disorder in cryomilled samples of a model polymorphic drug. J Pharm Sci 2013;102:93–103.

20. Xu K, Zheng S, Zhai Y, et al. Two solid forms of tauroursodeoxycholic acid and the effects of milling and storage temperature on solid-state transformations. Int J Pharm 2015;486:185–94.

21. Caron V, Tajber L, Corrigan Ol, et al. A comparison of spray drying and milling in the production of amorphous dispersions of sulfathiazole/polyvinylpyrrolidone and sulfadimidine/polyvinylpyrrolidone. Mol Pharm 2011;8:532–42.

22. Caron V, Hu Y, Tajber L, et al. Amorphous solid dispersions of sulfonanide/Soluplus® and sulfonamide/PVP prepared by ball milling. AAPS PharmSciTech 2013;14:464–74.

23. Zidan AS, Rahman Z, Sayeed V, et al. Crystallinity evaluation of tacrolimus solid dispersions by chemometric analysis. Int J Pharm 2012;423:341–50.

24. Singh A, Van den Mooter G. Spray drying formulation of amorphous solid dispersions. Adv Drug Deliv Rev 2016;100:27–50.

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

26. Balani PN, Ng WK, Tan RBH. Influence of excipients in co-milling on mitigating milling-induced amorphization or structural disorder of crystalline pharmaceutical actives. J Pharm Sci 2009;99:2462–74.

27. Bhugra C, Pikal MJ. Role of thermodynamic, molecular, and kinetic factors in crystallization from the amorphous state. J Pharm Sci 2008;97:1329–49.

28. Qi S, Avalle P, Saklatvala R, et al. An investigation into the effects of thermal history on the crystallisation behaviour of amorphous paracetamol. Eur J Pharm Biopharm 2008;69:364–71.

29. Li N, Taylor LS, Mauer LJ. The physical and chemical stability of amorphous (–)-epi-gallocatechin gallate: effects of water vapor sorption and storage temperature. Food Res Int 2014;58:112–23.

30. Lee SH, Bae JH, Park Y, et al. Sulfinic acid salts of donepezil and stabilization of amorphous donepezil via formation of amorphous salts. Crystal Growth Des 2015;15:3123–30.

31. Jondhale S, Bhise S, Pore Y. Physicochemical investigations and stability studies of amorphous glidace. AAPS PharmSciTech 2012;13:448–59.

32. Pradhan R, Tran TH, Choi JY, et al. Development of a rebamipide solid dispersion system with improved dissolution and oral bioavailability. Arch Pharm Res 2014;38:522–33.

33. Tung NT, Park CW, Oh TO, et al. Formulation of solid dispersion of rebamipide evaluated in a rat model for improved bioavailability and efficacy. J Pharm Pharmacol 2011;63:1539–47.

34. Cho HY, Yoon H, Park GK, et al. Pharmacokinetics and bioequivalence of two formulations of rebamipide 100-mg tablets: a randomized, single-dose, two-period, two-sequence crossover study in healthy Korean male volunteers. Clin Ther 2009;31:2712–21.

35. Park CW, Tung NT, Rhee YS, et al. Physicochemical, pharmacokinetic and pharmacodynamic evaluations of novel ternary solid dispersion of rebamipide with poloxamer 407. Drug Dev Ind Pharm 2013;39:836–44.

36. Dolomanov OV, Bournis LJ, Gidlea RJ, et al. OLEX2: a complete structure solution, refinement and analysis program. J Appl Crystallogr 2009;42:339–41.

37. Sheldrick GM. Crystal structure refinement with SHELXL. Acta Crystallogr C 2015;71:3–8.

38. Lu T, Chen C. Uncertainty evaluation of humidity sensors calibrated by saturated salt solutions. Measurement 2007;40:591–9.

39. Ondrusová D, Jona E, Šimon P. Thermal properties of N-ethyl-N-phenyl-dithiocarbamates and their influence on the kinetics of cure. J Therm Anal Calorim 2002;67:147–52.

40. Willart JF, De Gusseme A, Hemon S, et al. Direct crystal to glass transformation of trehalose induced by ball milling. Dev Ind Pharm 2013;31:271–39.

41. Willart JF, Carpenter L, Danede F, et al. Solid-state vitrification of crystalline griseofulvin by mechanical milling. J Pharm Sci 2012;101:1570–7.

42. Shah B, Kakumanu VK, Bansal AK. Analytical techniques for quantification of amorphous/crystalline phases in pharmaceutical solids. J Pharm Pharmacol 1977;29:684–5.

43. Bandarkar FS, Vavia PR. An optimized commercially feasible milling technique for molecular encapsulation of meloxicam in β-cyclodextrin. Drug Dev Ind Pharm 2011;37:1318–28.

44. Zimeri JE, Kokini JL. The effect of moisture content on the crystallinity and glass transition temperature of inulin. Carbohydr Polym 2002;48:299–304.

45. Chattoraj S, Bhugra C, Telang C, et al. Origin of two modes of non-isothermal crystallization of glasses produced by milling. Pharm Res 2012;29:1020–32.