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Research Article

The Stabilization of Amorphous Zopiclone in an Amorphous Solid Dispersion

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Abstract. Zopiclone is a poorly soluble psychotherapeutic agent. The aim of this study was to prepare and characterize an amorphous form of zopiclone as well as the characterization and performance of a stable amorphous solid dispersion. The amorphous form was prepared by the well-known method of quench-cooling of the melt. The solid dispersion was prepared by a solvent evaporation method of zopiclone, polyvinylpyrrolidone-25 (PVP-25), and methanol, followed by freeze-drying. The physico-chemical properties and stability of amorphous zopiclone and the solid dispersion was studied using differential scanning calorimetry (DSC), infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), hot-stage microscopy (HSM), X-ray diffractometry (XRD), solubility, and dissolution studies. The zopiclone amorphous solid-state form was determined to be a fragile glass; it was concluded that the stability of the amorphous form is influenced by both temperature and water. Exposure of amorphous zopiclone to moisture results in rapid transformation of the amorphous form to the crystalline dihydrated form. In comparison, the amorphous solid dispersion proved to be more stable with increased aqueous solubility.

KEY WORDS: amorphous; fragile; solid dispersion; stability; zopiclone.

INTRODUCTION

Zopiclone is a hypnotic agent belonging to the cyclopyrrolone chemical group. Even though it is chemically unrelated to the benzodiazepines, it has a similar spectrum of activity (1,2). The chemical structure of zopiclone is illustrated in Fig. 1. Zopiclone is a racemic mixture of two enantiomers, in which only (S)-zopiclone, is psycho-active (4). Zopiclone is characterized as a poorly water-soluble drug; however, very limited information regarding the solubility is available, and no definite calculated values, to date, has been reported in literature. In a study conducted by Terblanche *et al*. (5), two distinct crystal forms of zopiclone have been reported. Form A, the true polymorph and form B, the dihydrate. Shankland *et al*., 2001, further reported three forms of zopiclone, namely: a monoclinic dihydrate (I), monoclinic anhydrous (II), and a non-centrosymmetric orthorhombic anhydrous structure (III). Shankland further describes the structural basis for the reversible transformation between form (I) and form (II) and provides evidence for an irreversible solid-state chiral separation in which the racemic crystal structure (II) loses its center of symmetry during a transformation to form (III) (4). Up to this point in time, not one amorphous form of zopiclone has been reported. During preliminary studies, it was observed that zopiclone quickly starts to degrade after melting was achieved (178°C=melting point of zopiclone raw material). Quench cooling of the melt resulted in either rapid recrystallization of the molten product or degradation of zopiclone immediately after melting. A dihydrate recrystallized from toluene was prepared, the subsequent dehydration of this form resulted in an anhydrous solid-state form of zopiclone with a melting point below that of commercially available zopiclone raw material. This anhydrous solid-state form was used to prepare amorphous zopiclone.

It is generally known that the amorphous solid-state form of a drug has an increased Gibbs free energy (ΔG), which results in a higher dissolution rate and improved solubility in comparison with their crystalline counterparts (6–8). On the other hand, this increased free energy also results in a decrease in the physical and chemical stability, especially when exposed to increased temperature and moisture (8–10). Recently, the Food and Drug Administration (FDA) suggests that the first choice for solving solubility challenges for oral delivery is leaning toward amorphous solid dispersion technologies (11). Amorphous solid dispersions have great potential to improve the solubility, dissolution rate, and bioavailability of poorly water-soluble drugs (1, 2). However, concerns of the solid dispersions include their kinetics of phase separation in the solid state, when in contact with the gastro-intestinal tract (GIT) fluid (11, 12), the likelihood of the amorphous drug substance undergoing crystallization during storage and the effect of moisture on storage stability (13).

Zopiclone has zero number of rotatable bonds in its chemical structure and is therefore said to have a rigid structure. According to literature, this together with the low molecular weight of zopiclone, suggests it to have a low glass-

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forming ability (GFA) (13). Considering all mentioned aspects of zopiclone, the successful preparation of amorphous zopiclone could result in an increased solubility and bioavailability of this drug, but the effect of stability needs to be considered (8). In this study, we report on the preparation of amorphous zopiclone through quench-cooling of an anhydrous form of zopiclone, as well as the preparation of a stable solid dispersion and the physico-chemical and recrystallisation properties thereof.

MATERIALS AND METHODS

Materials

Crystalline zopiclone raw material, anhydrous optically active racemic mixture (Fig. 1), was purchased from DB Fine Chemicals Pty Ltd (Rivonia, Johannesburg, South Africa). Milli-Q water with a resistivity of 18.2 MΩ cm^{-1} was used throughout this study, and all other reagents used were either of chromatography or analytical grade.

Preparation of Anhydrous Zopiclone

A dihydrate recrystallized from toluene was prepared (14). Approximately 1 g of zopiclone was added to 10 mL of toluene while stirring continuously and heating the solution to 60±5°C. A few drops of cold ultrapure water were subsequently added to the solution. The beaker containing the saturated solution was covered with Parafilm®. After slow evaporation of the toluene, crystals were obtained. The recrystallized material was removed from the solvent and dried on filter paper to ensure evaporation of surface solvent. The solid-state form was confirmed through differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), Karl Fischer (KF), and X-ray powder diffraction (XRPD) analyses. This dihydrate solid-state form was then dehydrated by drying the recrystallization product in a laboratory oven (Binder, Tuttlingen, Germany) at 80°C for 24 h. The anhydrous form was confirmed by means of thermal analysis i.e. a melting endotherm (±150°C, a recrystallization exotherm (±164°C), followed by a final melting endotherm (±180°C). Furthermore it was characterized by its characteristic XRPD (Bragg) peaks, which are seen at °2θ values of 5.66, 13.58, 16.07, 16.39, 20.32, 20.52, 24.98, 25.49, 26.30, and 27.14 values (15).

Preparation of Amorphous Zopiclone

The amorphous zopiclone was prepared by heating a small portion (approximately 500 mg) of the prepared anhydrous form to approximately 150°C and subsequently quenching it to room temperature on an aluminum surface. The amorphous habit was confirmed by XRPD, DSC, and visually through hot-stage microscopy (HSM).

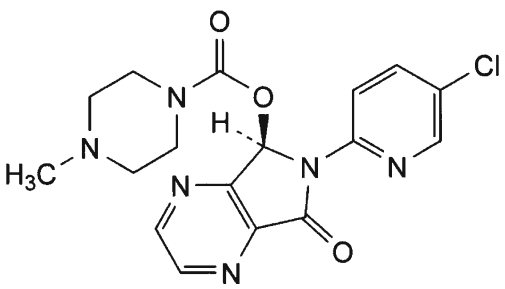

Fig. 1. The chemical structure of zopiclone (3)

Directly after preparation the purity of the zopiclone amorphous sample was confirmed by means of high-performance liquid chromatograpy (HPLC) analysis. HPLC analysis indicated that potency of the amorphous zopiclone was 99±1%. Furthermore, the optical rotation was determined in order to confirm the correct enantiomer.

Preparation of the Amorphous Solid Dispersion

The solid dispersion was prepared by a solvent evaporation method, followed by freeze-drying. A mixture consisting of 500 mg crystalline zopiclone and 500 mg polyvinylpyrrolidone-25 (PVP-25) were dissolved in 200 mL methanol through continuous stirring at ambient temperature. A clear solution was obtained. The resulting solution was rapidly evaporated, using a rotavapor (Bellingham and Stanley ADP 440, UK). The obtained dispersion was then quenched at −72°C for 12 h, using a Forma® Scientific Freezer, Inc. 938 series (Marjetta, Ohio,USA). Following this, the resulting dispersion was then loaded on a freeze-dryer shelf, VirTis SP Scientific Sentry 2.0 (Stone Ridge, New York, USA), and the dispersion was vacuum dried over night at −40°C and 100 mTorr. This procedure ensures that there is essentially no residual methanol and "freezing" the amorphous drug substance in the polymer matrix restricts molecular mobility and limits nucleation and crystal growth (14). The obtained solid dispersion showed no residual crystallinity detected by XRPD, HSM and DSC. It has been verified by HPLC that the preparation process does not induce detectable chemical degradation of the drug. HPLC potency analysis indicated that potency of the amorphous solid dispersion was 99%±1%. The optical rotation was also determined of zopiclone incorporated into the amorphous solid dispersion.

Differential Scanning Calorimetry (DSC)

A Shimadzu (Kyoto, Japan) DSC-60 instrument was used to record the DSC thermograms. Samples (3–5 mg) were accurately weighed and sealed in aluminum crimp cells with pierced lids. The samples were heated from 25°C to 250°C with a heating rate of 10°C/min and a nitrogen gas purge of 35 mL/min. The onset temperatures of the thermal events are reported. All analyses were performed in triplicate.

Thermogravimetric Analysis (TGA)

A Shimadzu (Kyoto, Japan) TGA-60 instrument was used to determine the percentage weight loss (%) of the zopiclone solid-state forms during heating. Samples (3–5 mg) were accurately weighed into open aluminum crucibles. The samples were heated from 25°C to 250°C with a heating rate of 10°C/min and a nitrogen gas purge of 35 mL/min. All analyses were performed in triplicate.

Infrared Spectroscopy

Infrared (IR) spectra were recorded using a Shimadzu IR Prestige-21 spectrophotometer (Kyoto, Japan) over a range of

400–4000 cm^{-1}. Potassium bromide (KBr) was used as a background. The diffuse reflectance method was implemented and involves grinding of approximately 1 mg of the sample with KBr and measuring its IR spectrum in a reflectance cell.

X-ray Powder Diffraction

Powder X-ray diffraction measurements were performed to confirm the crystalline or amorphous nature of the solid-state forms under investigation. A PANalytical (Almelo, Netherlands) Empyrean X-ray diffractometer with a PIXcel3D detector was used to record XRPD patterns at ambient temperature. Samples were evenly distributed on a zero background sample holder. The measurement conditions for all scans were set as follows: target, Cu; voltage, 40 kV; current, 40 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; scanning speed, 2°/min (step size, 0.02°; step time, 1.0 s).

For XRPD measurements where the recrystallization behavior of amorphous zopiclone was investigated during exposure to water the ASD was pre-mixed with a sufficient amount of distilled water to create a slurry. This slurry was quickly and evenly distributed on a zero background sample holder. The measurement was started immediately using the same conditions as mentioned above.

Optical Rotation

The optical rotations of the zopiclone solid-state forms under investigation were determined according to the method described by the British Pharmacopoeia (3). Approximately 200 mg of each of the respective solid-states were dissolved in *N,N*-dimethylformamide (Saarchem, Johannesburg, South Africa) and diluted in a volumetric flask to 20 mL using the same solvent. The optical rotation was measured with a Bellingham and Stanley ADP 440 polarimeter (Tunbridge Wells, UK). The angle of optical rotation for a racemate was given as between −0.05 and +0.05. Any values not within this range were an indication of possible enantiomeric resolution or separation.

Hot-Stage Microscopy

For HSM, a small amount of sample was placed on the center of the slide and viewed under the microscope. The presence or absence of amorphous material was evaluated by the observance of birefringence, under cross polarized light. TM analysis was performed with a Nikon Eclipse E4000 microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan) and a Linkam THMS600 heating stage equipped with a T95 LinkPad temperature controller (Surrey, England).

Scanning Electron Microscopy

Scanning electron microscopy (SEM) images of the solid-state forms were also obtained in order to observe possible morphological differences between the samples. For SEM analyses, samples were coated with a layer of gold/palladium using an Eiko Engineering Ion Coater IB-2 (Eiko Engineering, Ibaraki, Japan) and were subsequently imaged using a field-emission environmental FEI Corporation, Quanta 200ESEM (Hillsboro, Oregon, USA).

HPLC Analysis

HPLC analysis was done utilizing a Shimadzu (Kyoto, Japan) UFLC chromatographic system. The system consisted of a SIL-20AC auto-sampler fitted with a sample temperature controller, a UV/VIS Photodiode Array detector (SPD-M20A) and an LC-20AD solvent delivery module. The mobile phase consisted of a 0.018 M buffer, pH 4.55 with an ion-pairing agent (3.4 g/L) monosodium hexanesulfonate-acetonitrile-tetrahydrofuran (81:18:1, *v*/*v*/*v*). The mobile phase was filtered and degassed prior to use. A Luna C18 150×3.9-mm column was used with a flow rate set to 1.0 mL/min and a wavelength of 303 nm (16). Validation of this method provided a linear regression (r^2) of 0.9964.

Karl Fischer Titration

Karl Fischer titrations were performed on samples to determine the total moisture content. The instrument used was a Metrohm 870 KF Titrino Plus autotitrator (Herisau, Switzerland). It was calibrated using a predetermined mass of water (25–30 μL) and a Hydranal® water standard 10.0 (1 g contains 10.0 mg water (1%). Approximately 100 mg of each sample was used for the moisture determination. The titration experiment was performed in at least triplicate for each sample.

Vapor Sorption Analysis

The moisture sorption analyses were performed utilizing a VTI-SA vapor sorption analyzer (TA Instruments, New Castle, Delaware, USA). The microbalance was calibrated prior to each vapor sorption run with a 100 mg standard weight. The microbalance was set to zero prior to weighing of the sample into the stainless steel sample container. The sample was carefully placed into the sample holder, and care was taken to evenly distribute the sample. The percentage relative humidity/temperature program was set using TA Instruments Isotherm software. The %RH ramp was set from 5% to 95% RH, followed by a decrease in %RH from 95% to 5%. The last absorption phase was set to also ramp from 5% to 95% RH. The temperature was set at a constant 25°C throughout the %RH ramp. The program criteria were set to 0.0001% weight change or 2 min stability of weight gained or lost before the program would continue to the next set parameter.

Equilibrium Solubility Studies

Approximately 100 mg of crystalline zopiclone raw material, the quench-cooled amorphous form, and the amorphous solid dispersion were respectively weighed into test tubes (n=7); 10 mL distilled water (25°C) was pipetted into each test tube. The test tubes were sealed with Parafilm® and tightly capped in order to prevent any leakage, followed by affixing the test tubes to a rotating axis in a water bath set at 37°C. The axis was set to rotate at 54 rpm. Withdrawals were taken on 10, 20, 30, 60, 120, 180, 240, 480, and 5760 min for all three forms. From this data, it was calculated that a period of

24 h for both the raw material and the amorphous form was required to reach equilibrium solubility. For the solid dispersion, a period of 96 h was necessary.

Powder Dissolution Testing

A VanKel700 dissolution bath (Varian, Cary, USA) was used for dissolution testing. USP apparatus 2 (paddle) was set up at 37°C with a rotational speed of 100 rpm, 900 mL distilled water was added to each dissolution vessel. Approximately 200 mg of crystalline zopiclone (raw material) and 900 mg of the amorphous solid dispersion (weight equivalent to 200 mg zopiclone) were weighed into 10 mL test tubes, to which 100 mg sample and 50 mg glass beads, ≤106 μm (Sigma-Aldrich, South Africa) was added, respectively; 5 mL of dissolution medium (distilled water maintained at 37°C) was added to each test tube. The mixtures were agitated for a period of 120 s, using a vortex mixer. The resulting mixtures were transferred to each dissolution vessel; 5 mL of solution was withdrawn from each dissolution vessel at predetermined time intervals. The dissolution medium was not replaced after each withdrawal since a supersaturated solution is required to observe solution-mediated transformations (17). After withdrawal, the samples were filtered through a 0.45 μm PVDF filter into an HPLC vial. The filtered solutions were analyzed by HPLC.

RESULTS

Amorphous Zopiclone Prepared from Quench Cooling of the Melt

During preliminary studies for the preparation of amorphous zopiclone through the method of quench cooling of the melt, it was observed that zopiclone quickly start to degrade after melting was achieved at ≅178°C (the melting temperature of crystalline zopiclone raw material). Quench cooling indicated to be an ineffective method for the preparation of an amorphous form of zopiclone. Upon further investigation, it was decided to prepare an anhydrous form with a lower melting point as that of commercially available zopiclone and to subsequently melt and quench this form to prepare an amorphous form. Literature reports (14) a dihydrate recrystallized from toluene. The anhydrous form of zopiclone is then obtained through subsequent drying of this dihydrated form. The anhydrous form has a significantly lower melting point of 150.1°C, compared with that of zopiclone raw material (181.2°C) (Fig. 2). The water content of anhydrous zopiclone was determined to be 0.01%. The water content was determined by means of TGA and Karl Fischer determinations and is in good agreement with moisture determinations reported in literature (14,15).

Amorphous zopiclone was prepared as described above through the method of quench cooling of the melt of anhydrous zopiclone. Thermal analysis of the amorphous zopiclone showed a T_g of 56.7°C with a recrystallisation phase at 155.0°C and subsequent melting of the recrystallisation product at 177.5°C (Fig. 3b).

Figure 4a depicts the XRPD pattern obtained for zopiclone dihydrate while Fig. 4b shows the XRPD patterns of anhydrous zopiclone prepared as described above. The amorphous habit of the quench cooled zopiclone was confirmed by XRPD. Figure 4b, crystalline zopiclone clearly indicates the crystalline habit compared with the amorphous halo observed in Fig. 4a (amorphous zopiclone).

Physical and Chemical Stability of Amorphous Zopiclone

As mentioned earlier, the advantage of amorphous forms of drugs rests with the higher apparent solubility and faster dissolution rates which could lead to higher bioavailability. However, these advantages come at the cost of decreased physical and chemical stability in comparison with the crystalline form of the drug. This section will discuss the physicochemical properties of amorphous zopiclone.

The fragility parameter can be determined by measuring the temperature dependence of viscosity above T_g and by applying the Vogel-Tammann-Fulcher (VTF) equation. The VTF equation in its modified form describes the relationship between η and temperature (T) in the supercooled state:

$$\eta = \eta_0 \exp \frac{DT_0}{T - T_0} \tag{1}$$

where η_0 is assumed to be 10^{-5} Pa s for normal liquids, and T_0 (the ideal glass transition temperature) is between 20°C and 50°C lower than the measured T_g (18–20), and D is the strength parameter. A large D value (>30) represents a "strong" glass-forming behavior, while a low D value (<10) denotes a "fragile" glass-forming behavior. Fragility is a concept that may help explain the glass-forming ability and various other amorphous characteristics (18,21). Amongst various methods available to determine the fragility, the dependence of T_g on the heating rate (q), in DSC measurements, was selected, due to the fact that it does not necessitate precise thermodynamic parameters such as heat capacity (19, 20, 22). Subsequently, the fragility parameter (m) can be defined as:

$$m = \left(\frac{d \log \eta}{d\left(\frac{T_g}{T}\right)} \right) \tag{2}$$

and thus

$$m = \frac{\Delta H\eta^*}{(2.303\, RT_g)} \tag{3}$$

where $\Delta H\eta^*$ is the activation enthalpy for viscous flow and R is the gas constant (23–26). Using Eqs. (1)–(3), the following relationship between m and D can be derived:

$$m = \frac{D\,\frac{T}{T_0}}{(ln10)\left(\frac{T_g)}{T_0 - 1}\right)} 2 \tag{4}$$

Assuming that the viscosity at T_g is 10^{12} Pa s, bearing in mind that η_0 is 10^{-5} Pa s, then D can be expressed as:

$$D = \frac{666}{m - 17} \tag{5}$$

Where, 17 is equal to the order of magnitude change from T_g to η_0. It was not possible to make viscosity determinations. Therefore, the determination of m and D from viscosity

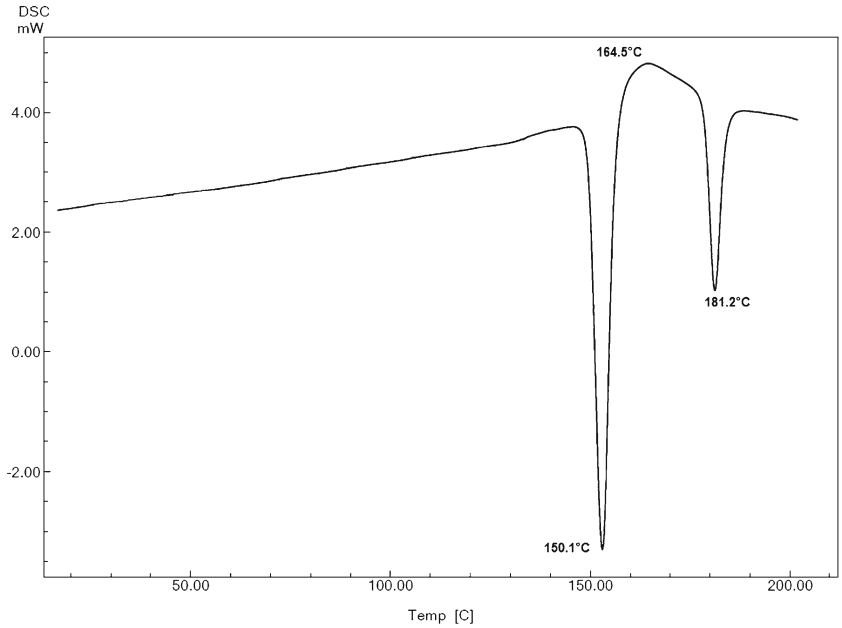

Fig. 2. The DSC thermogram obtained for anhydrous zopiclone that was prepared through the dehydration of zopiclone dihydrate

measurements was not possible; however, it was assumed that the activation enthalpy for viscous flow ($\Delta H\eta^*$) is equal to the activation enthalpy for relaxation (ΔH^*) (27). The T_g was determined for amorphous zopiclone using DSC analysis at heating rates of 2°C, 4°C, 6°C, 8°C, and 10°C.min^{-1}. From this, it was possible to calculate ΔH^* from the slope of the plot of ln heating rate (ϕ) vs. $1/T_g$ which subsequently enabled the calculation of m and D using Eqs. (3) and (5), respectively (27).

$$\frac{d\ln\phi}{d\left(\frac{1}{T}\right)} = \frac{-\Delta H^*}{R} \tag{6}$$

The ΔH^* for amorphous zopiclone was calculated to be 556.30 kJ.mol^{-1}, the fragility parameter (m) 89.80 and the strength parameter (D) 9.15. From the calculated value of D, it is clear that amorphous zopiclone, prepared through quench cooling of the melt, results in a fragile amorphous form. With the fragility and strength parameters known T_0 can be determined by applying Eq. (4). The T_0 of amorphous zopiclone was determined to be 7.48°C. Considering the determined parameters, it can be concluded that amorphous zopiclone prepared through quench cooling of the melt, resulted in a fragile glass with a relative low T_0. This suggests that amorphous zopiclone might be unstable and further processing techniques might induce recrystallization to the most stable form of zopiclone.

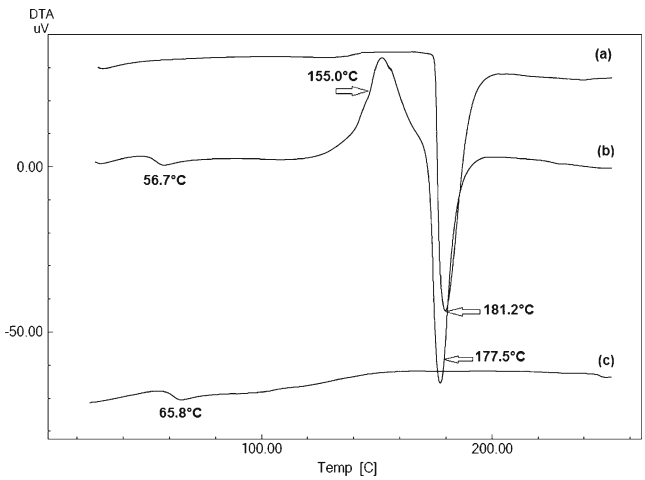

Fig. 3. The DSC thermograms of **a** commercially available crystalline zopiclone, **b** amorphous zopiclone, and **c** the amorphous solid dispersion

SEM images also showed the physical stability of amorphous zopiclone to be poor. Figure 5a exhibits the cubic/needle like morphology of zopiclone raw material. The smooth glassy surface of amorphous zopiclone is depicted in Fig. 5b; however, upon closer inspection one can observe the recrystallization of the amorphous form on the edges where agitation occurred. Mere handling and chipping of glassy zopiclone lead to surface recrystallization (Fig. 5c).

The influence of moisture on amorphous zopiclone was studied by means of moisture sorption analyses. Figure 6 depicts the moisture sorption isotherms obtained for amorphous zopiclone at ambient temperature. The first adsorption isotherm showed 30% RH to be the humidity that would allow sufficient plasticization which would lead to the subsequent recrystallization for amorphous zopiclone. The sudden loss in sample weight can be attributed to water desorption during crystallization. Therefore the relative humidity where crystallization will occur for amorphous zopiclone was determined to be 30% RH. In terms of stability, this is detrimental due to the fact that storage of amorphous zopiclone will have to be below 7.0°C and relative humidity of less than 30% RH. After the moisture sorption analysis, the sample was subjected to Karl Fischer and TGA analyses. It was determined that the sample contains 8.9% water, which correlates well with the

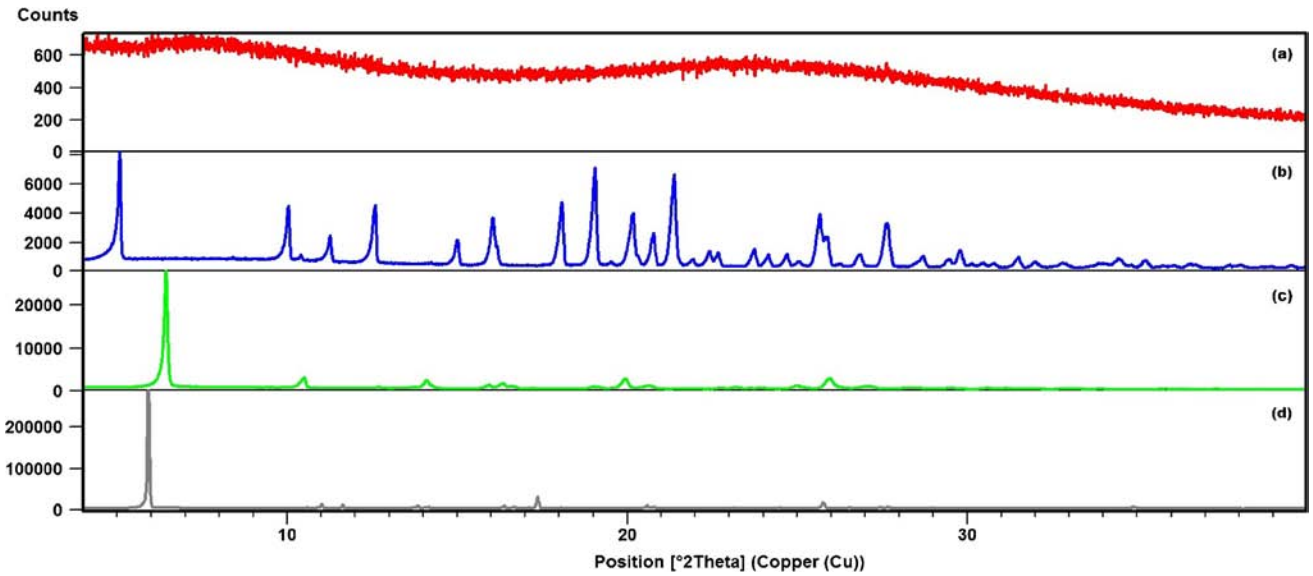

Fig. 4. Overlay of the XRPD patterns of **a** amorphous zopiclone prepared through quench cooling of the melt and **b** crystalline zopiclone raw material, **c** dihydrate prepared through recrystallization from toluene, and **d** anhydrous zopiclone obtained from the dehydration of the dihydrate prepared through the recrystallization of toluene

theoretically calculated water content of 8.8% for a zopiclone dihydrate. The crystallinity of the sample was furthermore confirmed by XRPD and it was evident that the XRPD pattern correlated with that obtained with the dihydrate form prepared through the recrystallization of zopiclone from toluene (Fig. 7a, b).

From the determined parameters and the influence of moisture on amorphous zopiclone, it is evident that the stability is a questionable factor. The rapid recrystallization of amorphous zopiclone upon exposure to moisture also indicated that a possible experimental determination of the solubility advantage of this solid-state form will not be viable. Although

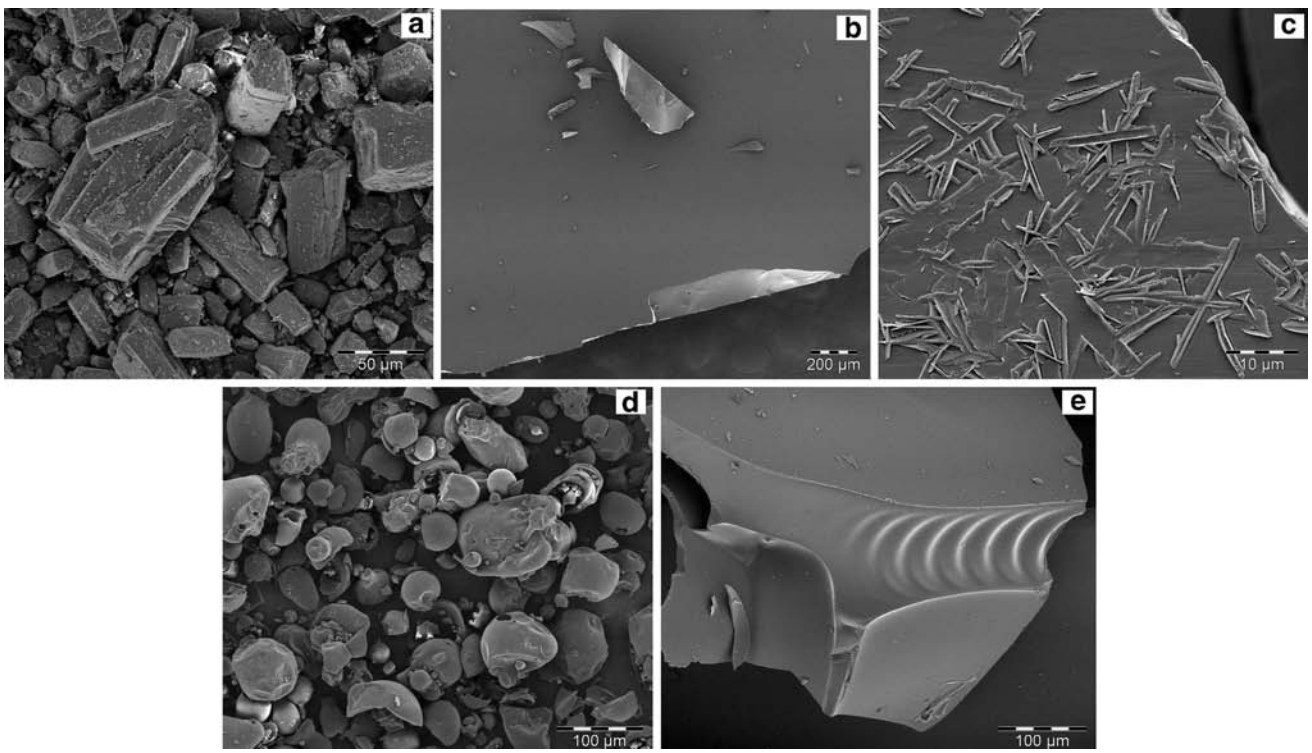

Fig. 5. SEM micrographs of **a** zopiclone raw material, **b** amorphous zopiclone prepared through quench cooling of the melt, **c** surface recrystallization of amorphous zopiclone upon agitation of the sample, **d** polyvinylpyrrolidone (PVP-25), and **e** the obtained amorphous solid dispersion

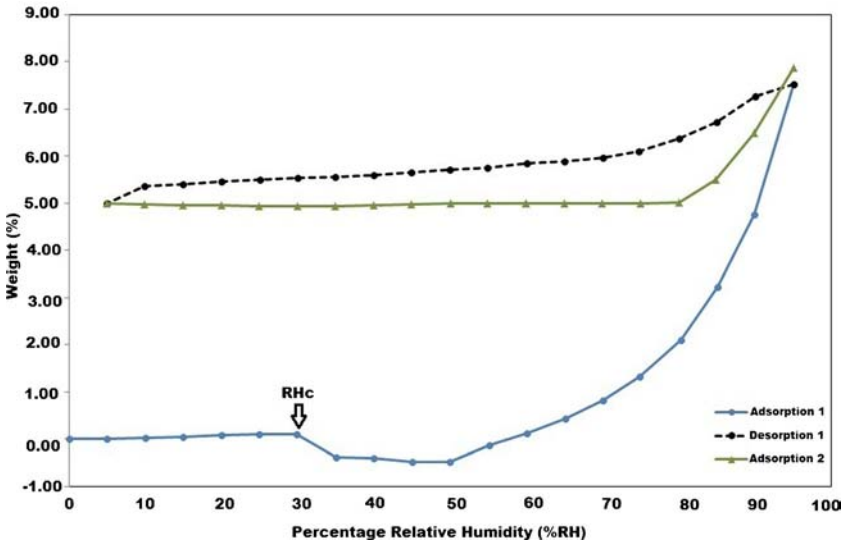

Fig. 6. Moisture sorption isotherms obtained for amorphous zopiclone. The isotherms were obtained at 25°C with humidity variation of 5–95% RH

it will not be possible to include amorphous zopiclone in any dosage form design processes, a stabilized amorphous form of this drug might lead to improved aqueous solubility. A thermodynamic approach, as reported by Hancock and Parks (7), was used to calculate the predicted or estimated enhancement in solubility of amorphous zopiclone. According to Hancock and Parks the solubility ratio of the two forms, σ^a/σ^c (amorphous/crystalline) at any temperature (T) is considered to be directly related to the difference in free energy (ΔG) between the amorphous and crystalline form (8).

$$\Delta G_T^{a,c} = -RT\, ln \frac{\sigma \frac{a}{T}}{\sigma \frac{c}{T}} \tag{7}$$

where R is the gas constant and T is the temperature at which solubility of the crystalline material was determined. In order to calculate the free energy difference it is necessary to determine the differences in entropy (S) and enthalpy (H) using Eq. (8).

$$\Delta G_T^{a,c} = \Delta H_T^{a,c} - \left(T \Delta S_T^{a,c}\right) \tag{8}$$

In order to apply Eq. (8), the entropy and enthalpy differences are calculated as follows:

$$\Delta H_T^{a,c} = \Delta H_f^c - \left(C_p^a - C_p^c\right)\left(T_f^c - T\right) \tag{9}$$

$$\Delta S_T^{a,c} = \Delta S_f^c - \left(C_p^a - C_p^c\right)\left(\ln\left(\frac{T_f^c}{T}\right)\right) \tag{10}$$

$$\Delta S_f^c = \frac{\Delta H_f^c}{T_f^c} \tag{11}$$

By applying this thermodynamic approach it was calculated that the amorphous solid-state form could possibly allow a 2.40-fold increase in the aqueous solubility of zopiclone. However, the neat amorphous form is significantly unstable and therefore a different approach is necessary to improve the aqueous solubility of this drug.

Determination of the Miscibility of Zopiclone and PVP-25

In order to overcome the stability issues described in the previous paragraphs, the preparation of an amorphous solid dispersion containing zopiclone was investigated. It was therefore imperative to determine the miscibility of zopiclone and PVP-25. It is common practice to presume complete miscibility of a drug and polymer if a single glass transition (T_g) is observed after all necessary processing techniques. However, it is noted that this is a mere assumption and the presence of a single T_g is not a fixed method to indicate complete miscibility of a drug-polymer mixture. Different ratios of crystalline zopiclone raw material and PVP-25 were prepared and the melting point depression was determined with an increase in the PVP-25 concentration. The following ratios of zopiclone and PVP-25 were prepared: 0.3:1, 0.5:1, 1:1, 2:1, and 3:1, it should be noted that the samples were not dried before DSC analysis. Figure 8 depicts the melting point depression obtained.

The Flory-Huggins interaction parameter (χ) is a useful method to determine the free energy involved in the mixing of two components. The melting point of a pure drug occurs at a temperature at which the chemical potential of the crystalline drug is equal to the chemical potential of the molten drug (29,30). Therefore, if the drug is miscible with a polymer, the chemical potential of the drug-polymer mixture must be less than the chemical potential of the pure amorphous drug. On the other hand, if the drug and polymer are immiscible no melting point depression is expected due to the fact that the

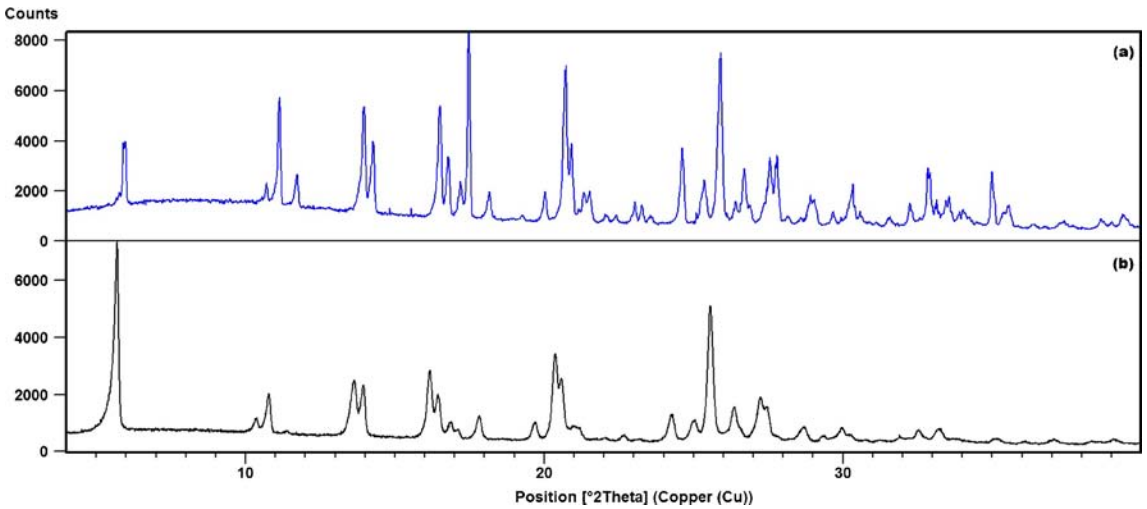

Fig. 7. Overlay of the XRPD patterns obtained for **a** zopiclone dihydrate obtained by recrystallization from toluene and **b** zopiclone dihydrate obtained through the recrystallization process of amorphous zopiclone upon exposure to relative high humidity conditions

chemical potential of the molten drug is unchanged by the presence of the polymer (29,30). In this study, the Flory-Huggins interaction parameter (χ) was determined for a zopiclone-PVP-25 mixture. Equation (12) shows the relationship between the melting temperature of the pure drug (T_M^{pure}), the depressed melting temperature of the pure drug (T_M^{mix}) and the Flory-Huggins interaction parameter (χ).

$$\frac{1}{T_M^{mix}} - \frac{1}{T_M^{pure}} = RT[n_1 \ln \Phi_1 + n_2 \ln \Phi_2] + n_1 \Phi_2 \chi_{12} \quad (12)$$

Where R is the gas constant, T is the absolute temperature, n_1 is the number of moles of drug, Φ_1 is the weighted volume fraction of the drug, n_2 is the number of moles of polymer, and Φ_2 is the weighted volume fraction of the polymer. For a 1:1 zopiclone/PVP-25 mixture, an interaction parameter (χ) of −0.2 was calculated. A negative or close to zero interaction parameter is an indication of a miscible system (30). It can therefore be deduced that for the combination of crystalline zopiclone with PVP-25 sufficient miscibility is obtained in a weight ratio of 1:1.

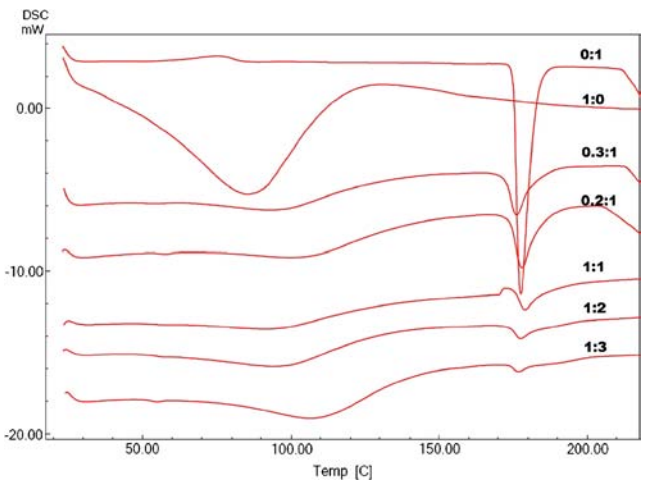

Fig. 8. Overlay of the DSC thermograms obtained during the miscibility determination of crystalline zopiclone and PVP-25. Ratios are denoted as PVP/zopiclone

Physical Characterization of a Zopiclone Amorphous Solid Dispersion

The physico-chemical properties of the amorphous solid dispersion (ASD) of zopiclone were investigated further. Figure 3 depicts the DSC thermograms obtained with crystalline zopiclone raw material, the neat amorphous zopiclone prepared through quench cooling of the melt and the zopiclone ASD. It is clear that an increase in temperature above the T_g of the ASD did not result in any recrystallization of zopiclone (Fig. 3c). Furthermore, the amorphous habit of the ASD was confirmed through XRPD analyses (Fig. 9c).

The morphology of the resulting ASD was also investigated by means of SEM. Figure 5 depicts SEM micrographs of the starting materials as well as the resultant ASD of zopiclone. The ASD exhibits a smooth "glassy" surface without any noticeable pores. The SEM results also confirm that a complete mixture of the two individual compounds was achieved during the preparation of the solid dispersion (Fig. 5e).

The amorphous habit is confirmed by the broadening of the peaks between 4000 and 3500, 3000 and 2500, and 2500 and 2000 cm^{-1} (Fig. 10b). Borea *et al.* and Bertolasie *et al.* reported that the C=O group of the pyrrolopyrazine ring is the main receptor binding site of zopiclone on the benzodiazepine receptor complex and that the C=Cl group of the chloropyridine

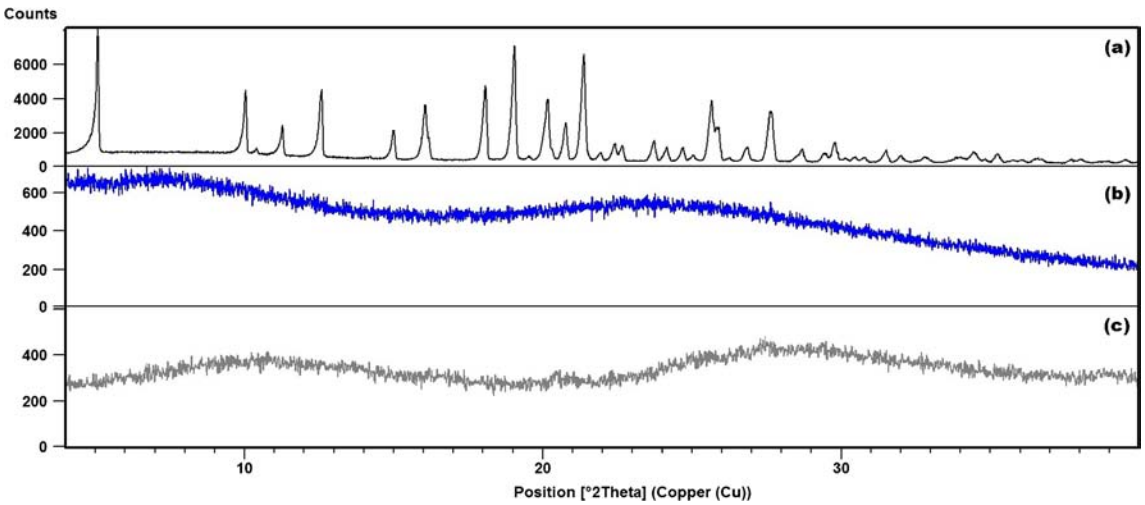

Fig. 9. Overlay of diffractograms obtained for **a** zopiclone raw material, **b** amorphous zopiclone, and **c** amorphous solid dispersion

ring and the 4-methyl-1-piperzaine carboxylate fragment are regions responsible for an increase in the agonistic properties of zopiclone (28,31). Table I summarizes the important functional groups and their characteristic absorption bands, all present in the ASD.

The Pharmaceutical Significance of a Zopiclone Amorphous Solid Dispersion

Moisture sorption analysis of the zopiclone ASD showed no recrystallization of zopiclone upon exposure of the sample to relative high humidity (95% RH) (Fig. 11). Furthermore, the moisture sorption data showed almost no hysteresis, therefore indicating that the relative high humidity did not cause any hydration and subsequent recrystallization of the amorphous drug. The amorphous habit of the ASD was also confirmed with XRPD after the moisture sorption analysis. These results indicate that this ASD of zopiclone will most likely show increased stability and resistance to crystallization of the amorphous drug.

XRPD experiments at ambient temperature were used to study the recrystallization tendency of the amorphous solid dispersion while exposed to a sufficient amount of water that will allow dissolution of the amorphous solid dispersion (Fig. 12). From these results it is clear that although the ASD is more stable than neat amorphous zopiclone, recrystallization of zopiclone still occur through the process of solution-mediated phase

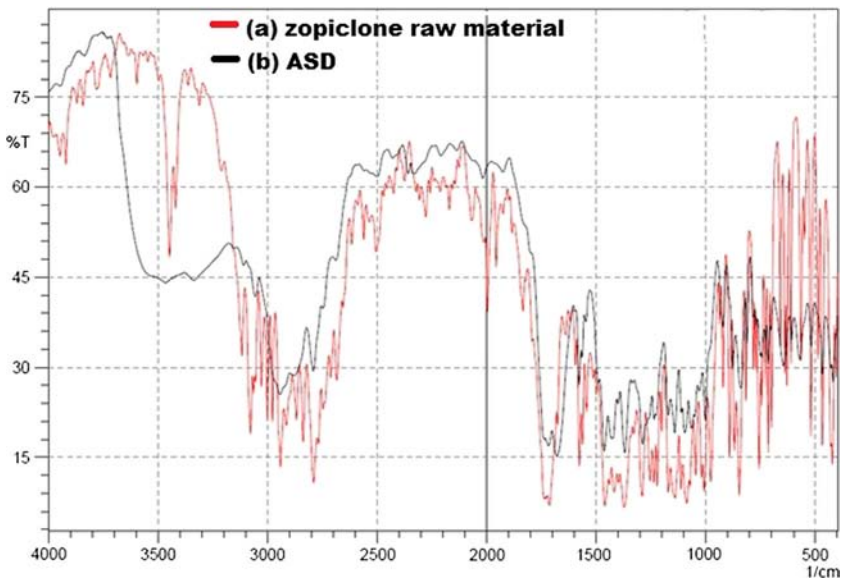

Fig. 10. Overlay of the IR spectra obtained for crystalline zopiclone **a** raw material and the **b** ASD

Table I. Summary of the Important Functional Groups, Their Characteristic Absorption Bands, and the Three Different Solid-State Forms of Zopiclone

Wavenumbers (cm^{-1})			
Group	C=Cl	C=O	O–C=O
Frequency range	700–800	1690–1760 1050–1300	1690–1760 10,500–1300
Zopiclone raw material	Present	Present	Present
Amorphous solid dispersion	Present	Present	Present

transformation. Solution-mediated phase transformation is a phenomena that occurs when a metastable form of a drug (i.e., amorphous form) dissolve in a solvent phase and from that solution a stable solid state form nucleates and grows (17,32). Considering this, it is clear that such a transformation will typically have a detrimental effect on the dissolution behavior of a drug due to the fact that the more thermodynamically stable a solid-state form of a drug is the less soluble it becomes. Aucamp *et al.* (32) also studied the phase transformation of amorphous roxithromycin through the solution-mediated mechanism by means of XRPD analysis. During this study, the detrimental effect that the recrystallization of the amorphous drug had on the dissolution rate and dissolved drug concentration was clearly demonstrated. It should however be mentioned that although recrystallization of the drug was evident from the XRPD analyses, this recrystallization process did not result in the crystal growth of a highly crystalline hydrated form of zopiclone. Comparison of the XRPD data (Fig. 12) showed that the peak intensities obtained after 210 min was significantly lower than that of zopiclone raw material. Therefore, it can be deduced that although recrystallization of the ASD was identified it occurs at a slow rate. However, since recrystallization of zopiclone ASD was identified with the XRPD analyses the effect of such a recrystallization on the dissolution behavior of the drug was imperative.

The dissolution studies were performed in distilled water at 37°C and compared with the aqueous solubility results obtained for crystalline zopiclone and the zopiclone ASD. Only water was used as a dissolution medium, since the study focused on the stability of the amorphous solid dispersion. Our objective was not to demonstrate the increase in solubility and dissolution rate the ASD holds, but rather to emphasize the fact that the ASD remains stable, even when exposed to water for prolonged periods.

The rationale for these dissolution parameters was to allow the study of the solution-mediated phase transformation of amorphous zopiclone to the stable crystalline form of zopiclone. The equilibrium solubility of crystalline zopiclone was determined to be 0.22 mg/mL, after 24 h. The amorphous solid dispersion showed a solubility of 0.63 mg/mL after 24 h, this is a 3-fold increase in the solubility of zopiclone. Figure 13 depicts the comparison of dissolution profiles of zopiclone amorphous solid dispersion (a) and the crystalline raw material (b) in distilled water at 37°C for a period of 24 h. The dissolution profile of the ASD shows almost complete dissolution of zopiclone

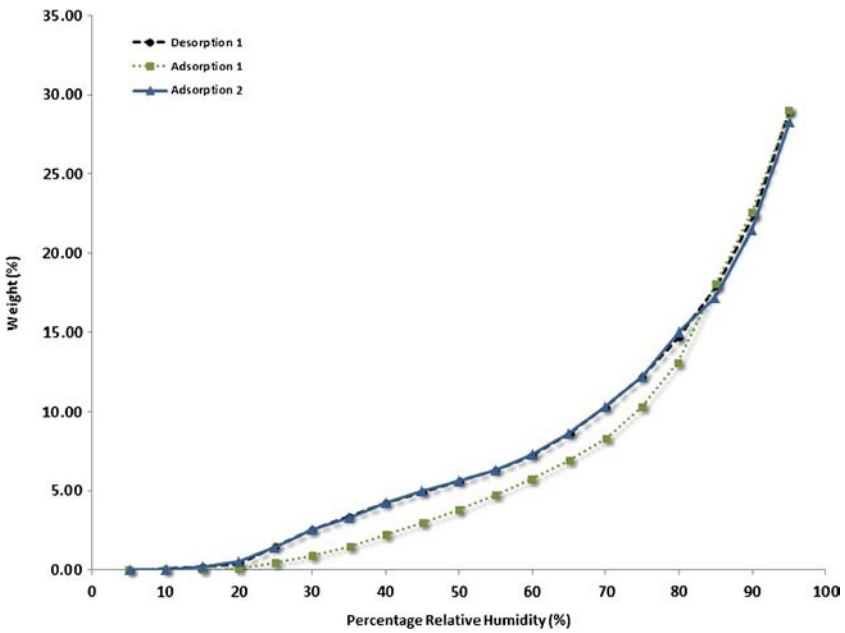

Fig. 11. Vapor sorption isotherms obtained for the amorphous solid dispersion of zopiclone at ambient temperature

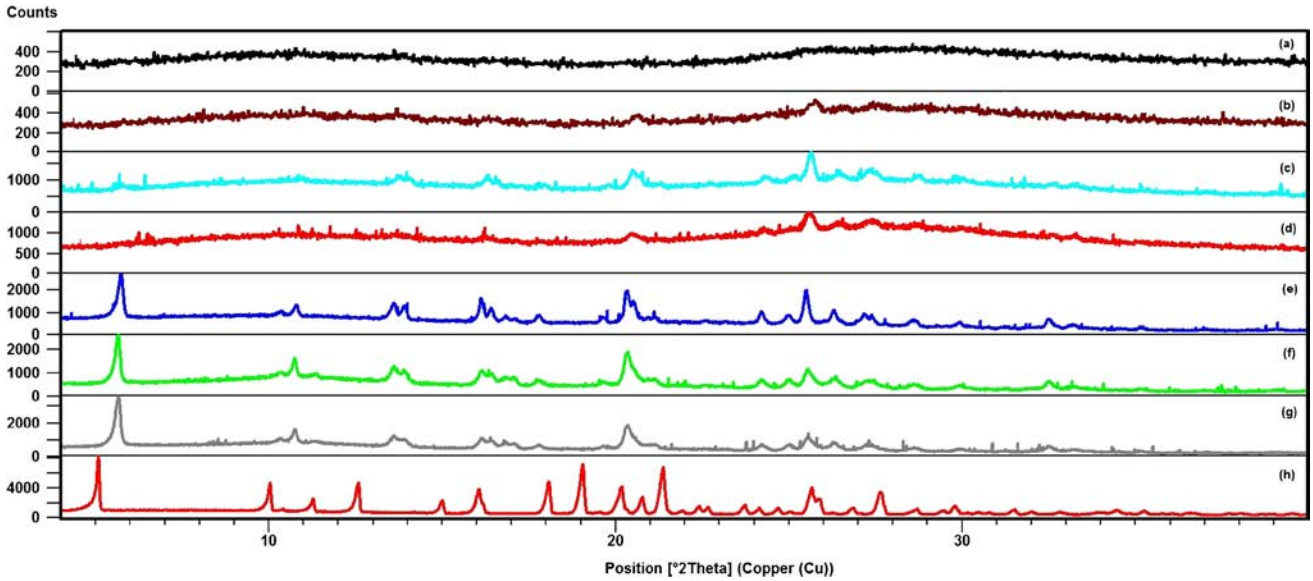

Fig. 12. XRPD overlay of the amorphous solid dispersion at **a** initial, **b** 30, **c** 60, **d** 90, **e** 120, **f** 150, and **g** 210 min and **h** reference diffractogram for crystalline zopiclone raw material

with a peak concentration of 0.56 mg/mL after 60 min in comparison with the lower dissolved concentration of 0.23 mg/mL obtained with crystalline zopiclone. This is a significant difference of 55.92%. The decrease in the dissolved concentration after 60 min is most probably due to solution-mediated phase transformation of the amorphous drug; however, it is clear that the transformation process is not a complete transformation since the dissolved concentration does not decrease to such an extent that it would correlate to the dissolved concentration of crystalline zopiclone. The question remains whether the resulting improved dissolution rate and higher drug solubility is truly due to the amorphous state in which the drug is captured or does the presence of the polymer play a more pronounced role?

The dissolution profile of the ASD indicates that over time the dissolved concentration decreases. It could be assumed that the concentration will decrease up to a point where it will become the same as that of zopiclone raw material; however, for the purpose of this study and considering the pharmaceutical relevance the dissolutions were not performed for a period longer than 24 h. The dissolution results are however in good correlation with the solution-mediated phase transformation identified by means of XRPD analyses. From these results, it is however clear that the presence of the polymer still inhibited the recrystallization tendency of amorphous zopiclone to such an extent that almost complete dissolution of the drug is achieved. Furthermore, it would be considered useful to study the dissolution behavior of the ASD in biorelevant media in order to ascertain the effect of

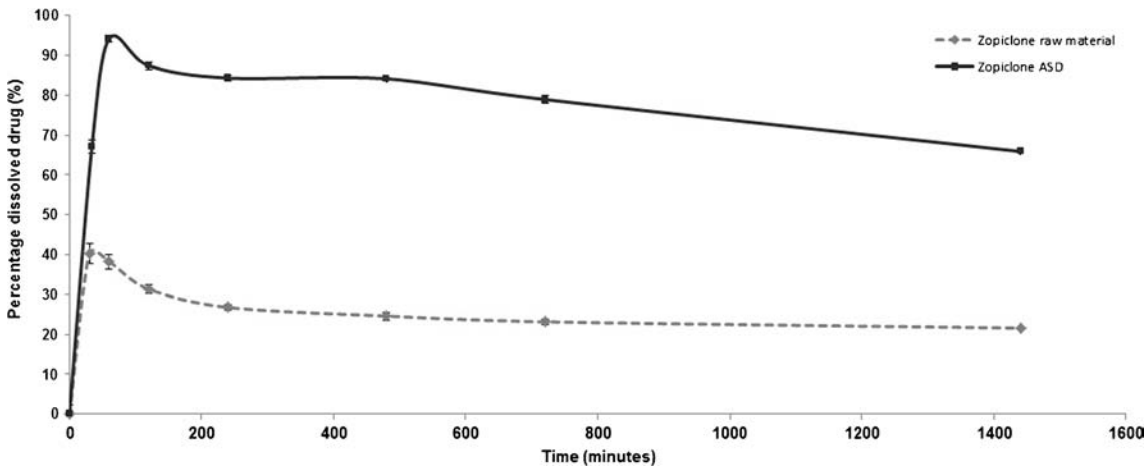

Fig. 13. Comparison of the dissolution profiles of zopiclone amorphous solid dispersion and crystalline zopiclone raw material in distilled water at 37°C

amorphicity and proven stability of the ASD on solubility and dissolution.

CONCLUSION

During this study, an amorphous form of zopiclone was prepared by the quench cooling of the melt of anhydrous zopiclone. However, the determined fragility and strength parameters indicated that the prepared amorphous form of zopiclone is extremely fragile. It was concluded that the stability of the neat amorphous form is influenced by temperature, moisture and agitation. Further studies focused on the preparation of a stable amorphous solid dispersion in order to address the stability issues identified with the neat amorphous solid-state form of zopiclone. The amorphous solid dispersion was successfully prepared by a solvent evaporation method of zopiclone, polyvinylpyrrolidone-25 (PVP-25) and methanol, followed by freeze-drying. The ASD of zopiclone showed improved stability due to the fact that even high relative humidity and an increase in temperature did not result in recrystallization of zopiclone. The ultimate advantage of this amorphous solid dispersion of zopiclone is found in the dissolution results. A 60% increase in the average percentage dissolved drug in water was observed. Furthermore, the presence of the PVP inhibited the recrystallization of a more stable solid-state form of zopiclone during dissolution studies. It is proposed that further studies will be necessary in order to investigate the influence of different polymers on amorphous solid dispersions of zopiclone. Furthermore, it would be pertinent to investigate the true role that the polymer plays when considering the enhancement of the dissolution behavior of zopiclone.

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REFERENCES

1. Dollery C. Therapeutic drugs, second edition. Edinburgh: Churchill Livingstone. 1999; 194–198.
2. Dundar Y, Boland A, Strobl J, Dodd S, Haycox A, Bagust A, *et al.* Newer hypnotic drugs for the short term management of insomnia: a systematic review and economic evaluation. Health Technol Assessment Pubmed Health. 2004;8(24):1–140.
3. British Pharmacopoeia 2014. http://www.pharmacopoeia.co.uk.nwulib.nwu.ac.za/bp2014/ixbin/bp.cgi. Accessed 20 Aug 2014.
4. Shankland N, David WIF, Shankland K, Kennedy AR, Frampton CS, Florence AJ. Structural transformations in zopiclone. Chem Commun. 2001;21:2204–5.
5. Terblanche RJ, Liebenberg W, de Villiers MM. Characterization of zopiclone crystal forms found among generic raw materials. Drug Dev Ind Pharm. 2000;26(5):531–7.
6. Aulton ME, Taylor K. Aulton's pharmaceutics: the design and manufacture of medicines. 4th ed. Spain: Harcourt Publishers Limited; 2013. p. 26–239. 145–148.
7. Hancock BC, Parks M. What is the true solubility advantage for amorphous pharmaceuticals? Pharm Res. 2000;17:379–404.
8. Aucamp M, Liebenberg W, Strydom SJ, van Tonder EC, de Villiers MM. Physiochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. AAPS PharmSciTech. 2012;13(2):467–76.
9. Hancock BC, Zografi G. Characteristics and significance of the amorphous state in pharmaceutical systems. J Pharm Sci. 1997;86(1):1–12.
10. Byrn SR, Pfeiffer RR, Stowel JG. Solid-state chemistry of drugs. 2nd ed. West Lafayette: SSCI Inc; 1999. 573p.
11. Brougha C, Williams RO. Amorphous solid dispersions and nano-crystal technologies for poorly water-soluble drug delivery. Int. J. Pharm. 2013; 157–167.
12. Huang Y, Daib W. Fundamental aspects of solid dispersion technology for poorly soluble drugs. APSB. 2013; 18–25.
13. Wang X, Michoel A, Van den Mooter G. Solid-state characteristics of ternary solid dispersions composed of PVP VA64, Myrj 52 and itraconazole. Int J Pharm. 2005;303:54–61.
14. Terblanche RJ. Crystal transformations of the cyclopyrrolone zopiclone a non-benzodiazepine sedative and hypnotic drug. Potchefstroom: NWU. (Thesis – MSc). 1999.
15. Terblanche RJ. Thermo-mechanical transformation of drug polymorphs and pseudopolynorphs characterised by differential scanning calorimetry and X-ray powder diffraction. PhD thesis, Potchefstroom: NWU. 2001.
16. Paw B, Misztal G. Determination of zopiclone in tablets by HPLC and UV-spectrophotometry. J Pharm Biomed. 2000;23:819–23.
17. Greco K, Bogner R. Solution-mediated phase transformation: significance during dissolution and implications for bioavailability. J Pharm Sci. 2012;101:2996–3018.
18. Senkov ON. Correlation between fragility and glass forming ability of metallic alloys. Phys Rev B. 2007;76:104202-1–6.
19. Kawakami K. Dynamics of ribavirin glass in sub-*T*g temperature region. J PhysChem B. 2011;115:11375–81.
20. Crowley KJ, Zografi G. The use of thermal methods for predicting glass-former fragility. Thermochim Acta. 2001;380:79–93.
21. Baird JA, van Eerdenbrugh B, Taylor LS. A classification system to assess the crystallization tendency of organic molecules from undercooled melts. J Pharm Sci. 2010;99:3787–806.
22. Kawakami K, Usui T, Hattori M. Understanding the glass-forming ability of active pharmaceutical ingredients for designing supersaturating dosage forms. J Pharm Sci. 2012;3239–3248.
23. Li Y, Han J, Zhang GGZ, Grant DJW, Suryanarayanan R. In situ dehydration of carbamazepine dehydrate: a novel technique to prepare amorphous anhydrous carbamazepine. Pharm Dev Tech. 2000;5(2):257–66.
24. Angell CA. Why C1=16–17 in the WLF equation is physical—and the fragility of polymers. Polym. 1997;38(26):6261–6.
25. Bohmer R, Angell CA. Correlations of the non-exponentially and state dependence of mechanical relaxations with bond connectivity in germanium-arsenic-selenium supercooled liquids. Phys Rev B. 1992;45(17):10091–4.
26. Angell, CA, Alba, C, Arzmanoglou, A, Bohmer, R, Fan, J, Lu, Q, Sanchez, E, Senapati, H, Tatsumisago, M. Slow processes in viscous liquids: stress and structural relaxation, chemical reaction freezing, crystal nucleation and microemulsion arrest, in relation to liquid fragility. Am. Inst. Phys. Conf. Proc. 992; (256): 1; 3–19.
27. Lu Q, Zografi G. Properties of citric acid at the glass transition. J Pharm Sci. 1997;86(12):1374–8.
28. Borea PA, Gilli G, Bertolasi V, Ferretti V. Streochemical feuters controlling binding and intrinsic activity properties of benzodiazepine-receptor ligands. Mol Pharm. 1987;31:334–44.
29. Flory PJ. Principles of polymer chemistry. Ithaca: Cornell University Press; 1953.
30. Marsac PJ, Li T, Taylor LS. Estimation of drug-polymer miscibility and solubility in amorphous solid dispersions using experimentally determined interaction parameters. Pharm Res. 2009;26(1):139–51.

31. Bertolasie V, Feretti V, Gilli G. Stereochemistry of benzodiazepine receptor ligands: possible role of C–H···x interactions in drug-receptor binding and crystal structures of CL218-872, zopiclone and DMCM. J Chem Soc Perkins Trans. 1990;2:283–9.

32. Aucamp ME, Stieger N, Barnard N, Liebenberg W. Solution-mediated phase transformation of different roxithromycin solid-state forms: Implications on dissolution and solubility. Int J Pharm. 2013;449:18–27.