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The effect of temperature and moisture on the amorphous-to-crystalline transformation of stavudine

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ABSTRACT

Stavudine is a nucleoside reverse transcriptase inhibitor active against HIV, and is known to exist in two polymorphic forms designated as forms I and II, and a hydrate form III. An amorphous solid of stavudine was successfully prepared and characterized during this investigation. A comprehensive evaluation of the stability of this amorphous solid showed that the amorphous solid transforms to either form II (anhydrous) or form III (hydrate) when exposed to temperature, in the absence or presence of moisture, respectively. The amorphous-to-hydrate transformation occurred at relatively low RH (>32%) and led to the formation of crystal aggregates of the hydrated form. Steady state growth rate analyses also showed that the amorphous-to-crystalline transformation occurs at a greater rate in the presence of moisture, compared to the transformation at the same temperature in a dry environment. Crystal growth studies showed that it is possible to stabilize the amorphous solid of stavudine against crystal transformations in the absence of moisture by coating it with poly(methyl methacrylate). However, this polymer coating could not prevent crystal growth from the amorphous solid during exposure to moisture.

1. Introduction

Stavudine, also known by the acronym d4T, is a nucleoside analog reverse transcriptase inhibitor (NRTI) active against HIV. Current WHO guidelines recommend a combination of two NRTI's and one non-nucleoside reverse transcriptase inhibitor (NNRTI) antiretroviral (ARV) drugs as a first-line regimen in resource-poor settings to treat HIV-1 infection (Marcelin et al., 2007). When several (of these) drugs, typically three or four, are taken in combination, the approach is known as highly active antiretroviral therapy, or HAART. Currently, only one WHO prequalified combination of NRTI/NNRTI is available as a fixed-dose combination (FDC), combining stavudine (30–40 mg), lamivudine (150 mg) and nevirapine (200 mg), and its use has been shown to be effective (Laurent et al., 2004, 2007; Duse et al., 2008).

Companies producing generic drugs were the first to market FDC ARV preparations containing stavudine, and these drugs have won broad acceptance in the developing world (Duse et al., 2008). In these regions, FDC products promote adherence, since they greatly reduce pill burden, dosing frequency and prescription errors. In fact, in Sub-Saharan Africa generic companies provide most of the drugs used and almost exclusively supply several fixed-dose combination drugs (Chien, 2007). Although there are always questions about the quality of generic products, several investigators have found that the amount of drug in generic tablets is similar to trade formulations (Penzak et al., 2003, 2004). However, this does not guarantee good dissolution and absorption when administered to patients.

In fact, several reports have shown that while the drug concentrations in generic products were similar to their trade counterparts, one or more of the drugs in the ARV FDC products did not achieved the strict definition of bioequivalence endorsed by the US FDA (GMR 90%, CI 80–125% for both C_{max} and AUC). For example, the Malawian antiretroviral program uses generic Triomune (stavudine, lamivudine, and nevirapine), and when tested, this product did not meet the strict definition of bioequivalence for these drugs (Hosseinipour et al., 2007). Patients taking Triomune had notably higher stavudine C_{max} values that could potentially influence toxicities such as neuropathy. The generic drug, unlike the trade version, is not coated and since stavudine is primarily absorbed in the proximal intestine, the authors speculate that the generic formulation may have dissolved faster, leading to increased drug absorption. This product was also tested in Uganda and although the pharmacokinetic profiles of generic and branded drugs were similar, the stavudine plasma concentrations were significantly lower for the generic formulation (Byakika-Kibwika et al., 2008).

In another study the pharmacokinetics of two generic FDCs for HIV-infected infants and children were tested (L'homme et al., 2007). Non-parametric statistical tests revealed no statistically significant differences in C_{max} and T_{max} of stavudine, lamivudine, and

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nevirapine between the two generic paediatric formulations and the branded products, but the AUC_{0-1} of stavudine and lamivudine differed significantly between the product formulated for babies and the branded product. This finding is important because a major challenge in delivering treatment includes the lack of paediatric ARVs that can be dosed in small children (Bowen et al., 2008). Worldwide, by the end of 2006, compared with 28% coverage for adults, only 15% of children with HIV that needed antiretroviral treatment were receiving it.

The reported variability in the bioavailability of stavudine in generic products is intriguing because it is a BCS Class I (highly soluble and highly permeable) drug that is eligible for the BCS-based biowaiver approach by the WHO. Perhaps the inconsistent pharmacokinetic parameters are related to the unique physicochemical properties of stavudine. Stavudine has two known monotropically related polymorphs, form I is the more stable least soluble (89 mg/ml) and form II is the metastable more soluble form (106 mg/ml) (Gandhi et al., 2000; Mirmehrabi et al., 2006a,b). A hydrated form with a stavudine:water stoichiometry of 3:1 (form III, solubility 90 mg/ml) and a few solvates has also been described (Gandhi et al., 2000; Radatus and Murthy, 2003). Stavudine is highly susceptible to chemical degradation by hydrolysis (Dunge et al., 2005; Kaul et al., 2005). During processing this low dose drug with needle shaped crystals, low bulk density, and cohesive nature, can decrease the content uniformity in dosage forms (Mirmehrabi et al., 2006a; Mohammed et al., 2008). Not much is reported about the formulation of stavudine products. Mohammed et al. (2008) reported the coprocessing of nevirapine and stavudine by spray drying. During spray drying, stavudine crystallized as form III. XRD and DSC analysis indicated a decrease in crystallinity of the spray-dried products.

In this study, we report the preparation and stability of an amorphous form of stavudine. The accidental incorporation of amorphous stavudine into a dosage form during large-scale manufacturing could adversely change the physicochemical properties of the drug and the final product and its bioavailability (Mohammed et al., 2008). Several circumstances can result in the formation of amorphous solids (Bauer-Brandl, 1996; Craig et al., 1999). These include pharmaceutical manufacturing processes such as, condensation from the vapor or precipitation from a solution (e.g. spray drying, freeze-drying, spin-coating and decompression of supercritical fluids), mechanical destruction of the crystalline structure (e.g. milling, wet granulation and desolvation) and quench cooling of the melt (Hancock and Zografi, 1997).

Amorphous solids are defined as structurally disordered systems that thermodynamically have an excess free energy compared to crystalline solids (Yu, 2001; Petit and Coquerel, 2006; Bhugra and Pikal, 2008). The increase in free energy enhances the solubility of the amorphous phase, and this can increase the bioavailability of the drug (Hancock and Parks, 2000). However, the increased free energy also results in a decrease in the physical and chemical stability of amorphous solids, especially when exposed to increased temperature and moisture. Frequently amorphous solids recrystallize into different polymorphic forms that are less soluble or degrade faster (Fukuoka et al., 1986; De Villiers et al., 1991; Andronis and Zografi, 1997, 2000; Guo et al., 2000). Perhaps an increase in non-crystalline content as seen during spray drying could explain some of the variability in bioavailability found among generic products containing stavudine (Hosseinipour et al., 2007; L'homme et al., 2007; Byakika-Kibwika et al., 2008; Mohammed et al., 2008).

For this reason, if the objective is not to increase the solubility of a drug, the accidental introduction of amorphous material should be avoided. This study reports the effect of changes in environmental conditions, increased temperature and moisture, on the amorphous-to-crystalline or hydrate transformation of stavudine, a water-soluble drug. It is hoped that the results presented in this study will highlight the importance of avoiding the accidental introduction of amorphous materials into products containing stavudine. In addition, the stability of the amorphous solid and a method that can be used to stabilize the glass against the effect of temperature is also reported.

2. Materials and methods

2.1. Materials

Stavudine, polymorph form I and form II, was purchased from Xiamen Mchem Laboratories Ltd. (China). Poly(methyl methacrylate) (PMMA) with an average molecular weight of 15,000 was purchased from Sigma–Aldrich Inc., St. Louis, MO. Toluene and tetrahydrofuran (THF) were purchased from Fisher Scientific, Fair Lawn, NJ, and all other solvents and reagents were analytical grade. Polymorphic forms I, II and III (a hydrated form with a stavudine:water stoichiometry of 3:1) were also prepared for comparison with the amorphous solid. These crystalline solids were obtained from recrystallization of the stavudine raw material form ethanol, methanol and milli-Q water, respectively (Gandhi et al., 2000).

2.2. Preparation and coating of amorphous stavudine

Both supercooling of the melt and spin-coating were used to prepare amorphous stavudine. For the first method, approximately 10 mg of stavudine was evenly spread out on a glass microscope slide, and heated to 168–172 °C on a Velp® Scientifica hotplate (Italy). The liquid/melt that formed was cooled by placing the microscope slide on a refrigerated block of aluminum. For the second method, a 2% (w/v) solution of stavudine in THF was prepared, and six drops of this solution was placed on a clean, round microscope cover glass with a diameter of 25 mm. The cover glass was spun at 3000 revolutions per minute for 2 min using a TC100 spin-coater (MTI Corporation, Richmond, CA). In order to coat the amorphous film with PMMA, an additional spin-coating step, using the same method, was carried out with a 3.5% (w/v) solution of PMMA in toluene. Toluene was used because stavudine is insoluble in this solvent and it should therefore not dissolve the formed stavudine film.

2.3. Characterization of the amorphous form and polymorphs

Thin-layer chromatography (TLC) was used to confirm the absence of thymine, the major degradation product of stavudine in the sample prepared from the melt (Kawaguchi et al., 1989; USP, 2007).

The amorphous solid, and the polymorphs, were characterized using various analytical techniques. X-ray powder diffraction (XRPD) patterns were recorded using a Bruker D8 Advanced diffractometer (Bruker, Germany), and the data were analyzed using the Eva software package. The instrumental setup was as follows: voltage, 40 kV; current, 40 mA; radiation source, Cu; divergence slit, 1 mm; anti-scatter slit, 0.1 mm; detector slit, 0.1 mm; scan range, from 2° to 40° 2θ, scan speed, 0.4° 2θ/min with a step size of 0.02° 2θ and a step time of 3.0 s. In order to observe the crystalline transformations with increasing temperature, variable temperature X-ray powder diffraction (VT-XRPD) analyses were performed on the amorphous solid using the abovementioned instrument and setup, with the addition of an Anton Paar TTK 450 low temperature camera (Anton Paar, Austria). Samples were heated at a rate of 1 °C/s and were held at each preset temperature for the duration of each scan. Diffraction patterns were obtained from 25 to 160 °C, with a sequential increase of 10 °C between successive scans.

Thermal analyses were performed using a TA Instruments DSC Q2000 (for differential scanning calorimetric analyses) and a SDT Q600 (for thermogravimetric analyses) with Universal TA Analysis 2000 software (New Castle, DE). Approximately 2–3 mg of each sample was placed in an aluminum pan and either covered with an aluminum lid (during DSC analyses) or left uncovered (during TGA analyses). Samples were scanned at a heating rate of 10 °C/min from 25 to 190 °C, with a nitrogen gas flow rate of 50 ml/min for DSC analyses and 100 ml/min for TGA analyses.

2.4. Vapor sorption and phase transformation analysis

The effect of moisture on the amorphous solid prepared from the melt was determined by placing ±25 mg of powdered sample in a platinum plated quarts weighing bowl and measuring the vapor sorption and desorption using a TA Instruments TGA Q5000 SA with Universal TA Analysis 2000 software (New Castle, DE). Samples were held at 25 °C whilst the humidity was sequentially increased from 0 to 90% relative humidity (RH), with a 10% RH increase between each successive step. Samples were held at each step until no significant weight change, defined as a weight change of less than 0.0005 mg within 240 min, was detected.

To investigate the crystal transformations of the amorphous solid with increasing temperatures and humidity, samples were placed in a VWR International vacuum oven (West Chester, PA) at 100 °C or 30 °C; 32% RH. The humidity was controlled by placing a saturated solution of $MgCl_2$ in a desiccator, which was kept in a temperature controlled oven (Fisher Scientific, Fair Lawn, NJ). The impact of small amounts of amorphous solid on the physical properties of crystalline form II was also investigated using XRPD and scanning electron microscopy (SEM). For this experiment physical mixtures of 5–20% (w/w) amorphous solid and raw material, form II, were prepared. Initial XRPD analyses were performed on these samples, they were then exposed to 30 °C; 43% RH for 5 days, after which a second XRPD analysis was performed. The samples were also observed using a Hitachi S-570 LaB_6 SEM (Schaumburg, IL). Samples were coated with a layer of gold/palladium using a SeeVac Auto Conductavac IV sputter coater (KDF Sputtering Systems, Rockleigh, NJ) before being imaged using SEM. In addition uncoated samples exposed to moisture were imaged with a field-emission environmental SEM (Quanta 200 ESEM, FEI Corporation, Hillsboro, OR, USA) in wet mode utilizing a Peltier stage for temperature control and high water vapor pressure in the specimen chamber for increasing the humidity up to 100%.

2.5. Crystal growth rate measurements

The growth rate of the crystals grown from the amorphous form was investigated using amorphous samples prepared by spin-coating. Spin-coating was used in order to ensure a uniform amorphous film thickness because this improved the microscopic observation and subsequent growth measurements of the samples. The influence of temperature and humidity on the growth rate was investigated using the following controlled environments: 30 °C, 0% RH; 40 °C, 0% RH; 50 °C, 0% RH; 30 °C, 32% RH and 30 °C, 43% RH. The temperature was maintained using a temperature controlled oven (Fisher Scientific, Fair Lawn, NJ) set at 30, 40 or 50 °C, respectively. The humidity was controlled by storing the samples at 30 °C in a desiccator, which contained either anhydrous $CaSO_4$ (0% RH), a saturated solution of $MgCl_2$ (32% RH) or a saturated solution of K_2CO_3 (43% RH). Both uncoated and samples coated with PMMA were studied. The center of each sample was observed for crystal growth using an optical polarizing microscope (Lomo, Northbrook, IL) with an attached camera (Ken-A-Vision, Kansas City, MO). The crystal growth measurements were determined by calculating the radius of a circle drawn around the edge of an observable crystal, using the Image J software package. This method was chosen since it could be used for both spherulites and non-spherical crystals.

3. Results and discussion

3.1. Amorphous solid characterization

Stavudine is a thymidine nucleoside with a chemical structure shown in Fig. 1 that is known to crystallize in different crystal forms (Gandhi et al., 2000; Mirmehrabi et al., 2006a). The stavudine available commercially is usually recrystallized from hot organic solvent solution, as the final step in the process to produce pure form I, which is preferred (Gandhi et al., 2000; Radatus and Murthy, 2003). If this is not done carefully, fast evaporation of the solvent could lead to an increase in the amorphous content of the final product. In this study, an amorphous form of stavudine was prepared by supercooling of the melt or spin-coating. TLC analyses confirmed that there was no thymine present in the amorphous samples prepared by supercooling of the melt of stavudine. The Rf value of the amorphous sample and the stavudine standard were 0.70, whilst the thymine standard had an Rf value of 0.65. Therefore, the melt method could successfully be used to prepare an amorphous solid of stavudine, since no detectable thymine or major degradation products were formed. The results obtained from the XRPD analyses, Fig. 1, of the three known polymorphic forms of stavudine agreed with the results reported in the literature. Form I has a unique diffraction peak at 19.1° 2θ, form II has unique peaks at 11.2° and 18.6° 2θ and form III has a unique peak at 15.5° 2θ (Gandhi et al., 2000; Mirmehrabi et al., 2006a). The amorphous solid showed no detectable diffraction peaks and only displayed a broad hump or amorphous halo.

The result of the DSC analysis of the amorphous solid (prepared by quench cooling of the melt) is shown in Fig. 2, and shows a glass transition (T_g) at 36.9 °C (onset temperature). The presence of a T_g is a characteristic feature of amorphous solids, and thus reiterates the amorphous nature of this solid (Craig et al., 1999). DSC analysis of the amorphous form also shows the presence of a unique recrystallization exothermic signal at 89.9 °C, whilst the results of DSC analyses of forms I and II show no exothermic signals, whereas form III has an exothermic signal at 141.9 °C (Gandhi et al., 2000; Mirmehrabi et al., 2006a). TGA analysis demonstrated an insignificant percentage weight loss of 0.45% between 25 and 120 °C for freshly prepared amorphous solid.

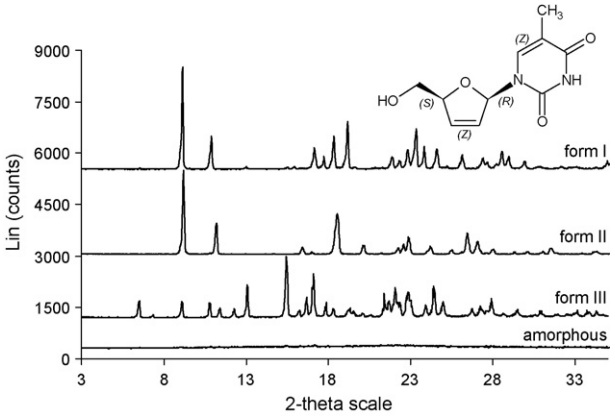

Fig. 1. XRPD patterns of two polymorphic forms, one hydrate and an amorphous form of stavudine.

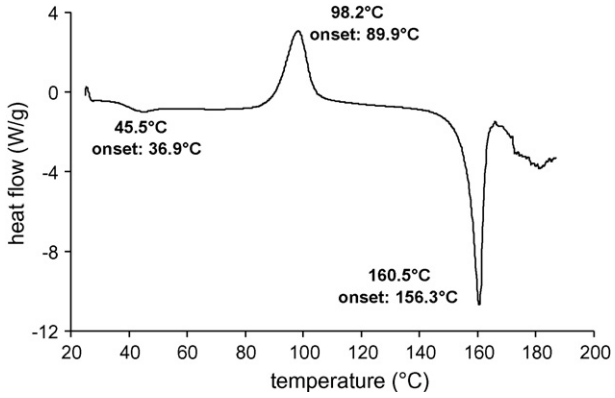

Fig. 2. DSC thermogram of amorphous stavudine (prepared by quench cooling of the melt), indicating a glass transition temperature at 36.9 °C (onset temperature), an exothermic recrystallization onset at 89.9 °C and melting onset at 160.5 °C.

3.2. VT-XRPD study of the amorphous-to-crystalline transformation

Initially, the transformation of the amorphous solid was investigated using VT-XRPD analysis. This method has been used successfully to study the polymorphic transformations of other polymorphic solids, most notably the transformation of polymorph C of the anthelmintic drug mebendazole (De Villiers et al., 2005). The results shown in Fig. 3 indicate that with an increase in temperature up to 65 °C at ambient humidity, the amorphous solid appears to transform to form III, a hydrated form of stavudine. The X-ray diffraction pattern of the sample at this temperature and the pattern of form III correspond because the unique peak for form III at 15.5° 2θ is present in both patterns. When the temperature was further increased to 140 °C, the sample appeared to transform mostly to form I with the appearance of the peak at 19.1° 2θ, shown in Fig. 3. This transformation was complete at 150 °C. However, the final diffraction pattern at 150 °C has a peak at 11.2° 2θ with a relative intensity (relative to the largest peak) of 12.8%. This small peak and the lack of a detectable peak at 18.6° 2θ indicate the presence of a small amount of form II. This transformation of form III to form I, containing trace amounts of form II, was also observed by Gandhi et al. (2000). VT-XRPD of the amorphous form therefore indicated that the sequence of transformation with an increase in temperature at ambient atmosphere, is thus: amorphous solid → form III → form I, containing trace amounts of from II.

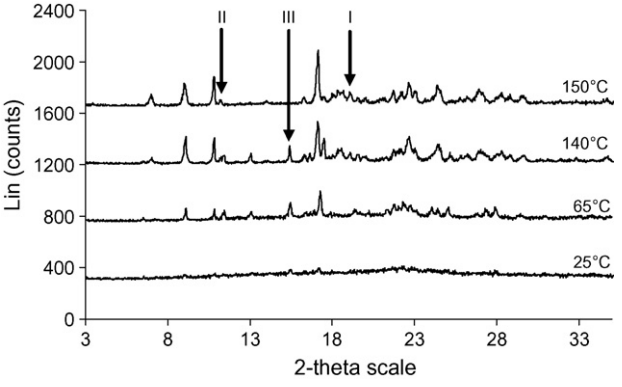

Fig. 3. VT-XRPD patterns of amorphous stavudine, showing the diffraction patterns at 25, 65, 140 and 150 °C. The arrows indicate the positions of the unique diffraction peaks of form I (19.1° 2θ), form II (11.2° 2θ) and form III (15.5° 2θ). Samples were held at each temperature for approximately 25 min (the duration of each analysis), and were exposed to ambient air.

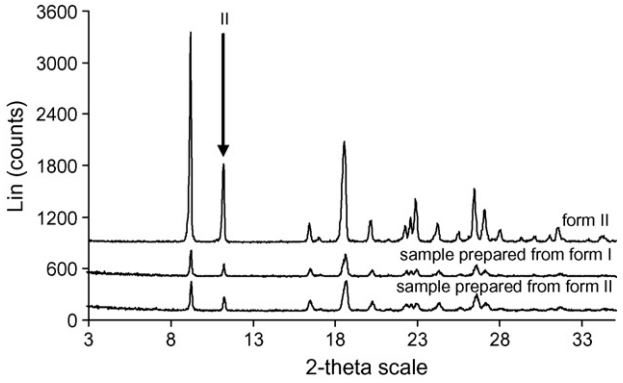

Fig. 4. XRPD patterns of the amorphous samples, stored at 100 °C for 1 h in a vacuum oven, prepared from form I and form II. The XPRD pattern of these samples corresponds to the pattern of form II, as shown. The arrow shows the unique diffraction peak of form II (11.2° 2θ).

3.3. Effect of moisture on amorphous-to-hydrate transformation

Moisture influences the stability of amorphous solids (Alneck and Zografi, 1990). The prefered transformation to form III, a hydrate, suggests that atmospheric moisture plays a significant role in the transformation of amorphous stavudine. In order to determine whether this was the case, amorphous samples were stored at 100 °C in a vacuum oven and analyzed after an hour. XRPD analysis, shown in Fig. 4, showed that the amorphous solid transformed to the more stable crystalline form II with a unique diffraction peak at 11.2° 2θ in the absence of moisture. This showed that form III did not form as an intermediate during the transformation in a moisture free environment. Surprisingly, there were also no diffraction peaks of form I detectable in the final pattern. To further explore this, samples were prepared by the melt method from both form I and form II. The thought was that if seeds of form I remain in the sample prepared from form I, this sample would transform

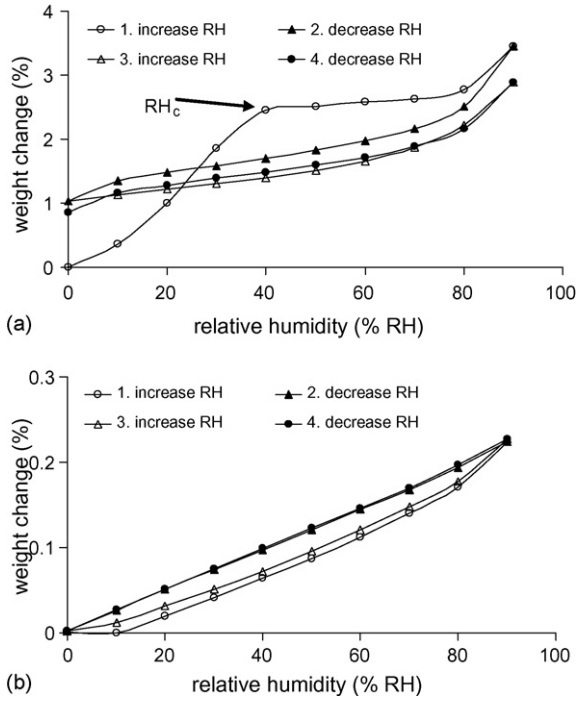

Fig. 5. DVS results for the (a) amorphous and (b) form II of stavudine. The RH_c of the amorphous solid is indicated on the graph. The numbers 1–4 indicate the order in which the humidity was increased and decreased.

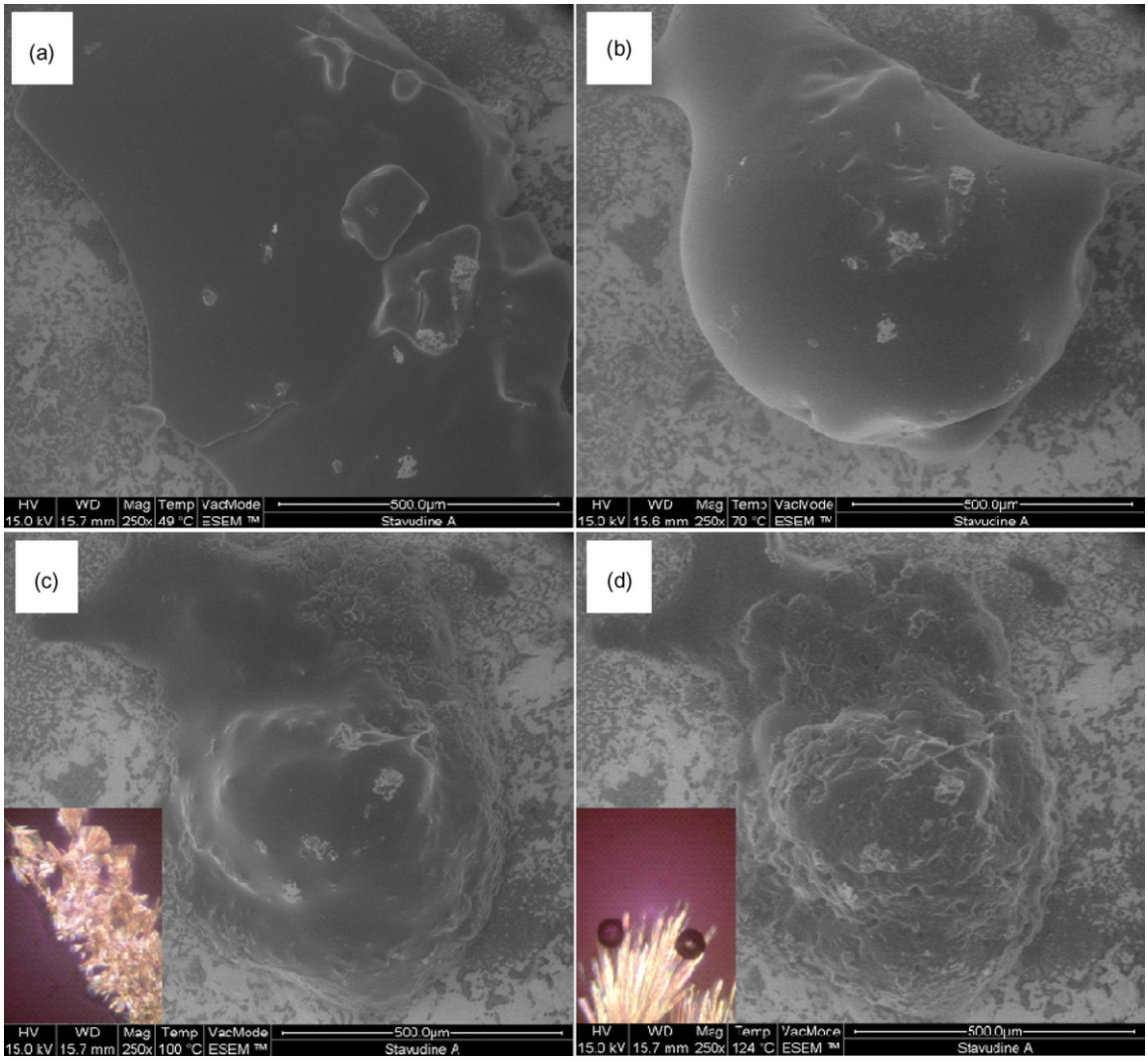

Fig. 6. ESEM images of amorphous stavudine exposed to RH > 75%: (a) 49 °C, (b) 70 °C, (c) 100 °C, and (d) 124 °C. Insert on photomicrographs (c) and (d) represents hot-stage microscope images at the same temperatures.

to form I (Mirmehrabi et al., 2006a; Chieng et al., 2008). Instead, the amorphous solids prepared from both form I and form II both transformed to form II at 100 °C in a vacuum oven (Fig. 4).

Moisture also has a plasticization effect on amorphous solids (Alneck and Zografi, 1990). Since this increases the molecular mobility and decreases the glass transition temperature of the solid, it could influence the crystal transformation (Alneck and Zografi, 1990; Burnett et al., 2004). When a solid undergoes an amorphous to crystalline transition in the presence of moisture, the water sorption capacity typically decreases or slows down, and the relative humidity at which this occurs is known as the crystallization or critical RH (RH_c) (Burnett et al., 2004). The results of the vapor sorption experiment of the amorphous solid, Fig. 5a, show that it absorbs moisture up to approximately 40% RH with a weight increase of approximately 2.5%. The weight of the sample remains reasonably constant with no significant weight change between 40 and 80% RH. Crystal transition is therefore complete at 40% RH. The moisture isotherm indicates that the amorphous solid transformed to a hydrate, form III, because the moisture uptake of 2.5% agrees with the theoretical water content of the hydrated form of stavudine of 2.67% reported in the literature (Gandhi et al., 2000; Mohammed et al., 2008). The results from the vapor sorption experiment of form II of stavudine are shown in Fig. 5b for comparison, and it shown no significant moisture adsorption or transformations.

The weight increase observed at 80–90% RH seen in Fig. 5a is attributed to the moisture sorption properties of form III. In order to confirm whether form III was indeed formed during the vapor sorption experiment, an XRPD and DSC analysis were performed on the recovered sample. The XRPD diffraction pattern of this solid, as well as the diffraction pattern of an amorphous sample that was stored at 30 °C, 43%RH for 5 days, corresponds to that of form III. The DSC thermogram of the vapor sorption sample had an exothermic peak at 141.1 °C. This value compares to the exothermic peak of 141.9 °C reported for form III (Gandhi et al., 2000). The XRPD, DSC and DVS results illustrate that the amorphous solid of stavudine transforms to the hydrated crystalline form III after exposure to moisture, and this occurs at a relative low RH between 20 and 40%.

It should be noted that during vapor desorption, the hydrated crystalline solid only lost 1–1.5% of the 2.5% water that it originally adsorbed. This suggests that the water is tightly bound to the crystal lattice via strong interactions (Gandhi et al., 2000). Although after desorption form III lost a significant portion of its moisture, the X-ray pattern did not show any transformation. Hydrates which desolvate yet retain their original crystal lattice are common (Stephenson et al., 1998). Therefore, form III seems to be an isomorphic desolvate because it retains the structure of its parent hydrated form. The molecular vacuum created by desolvation usually result in an extremely hygroscopic solid (Byrn et al., 1982; Stephenson et

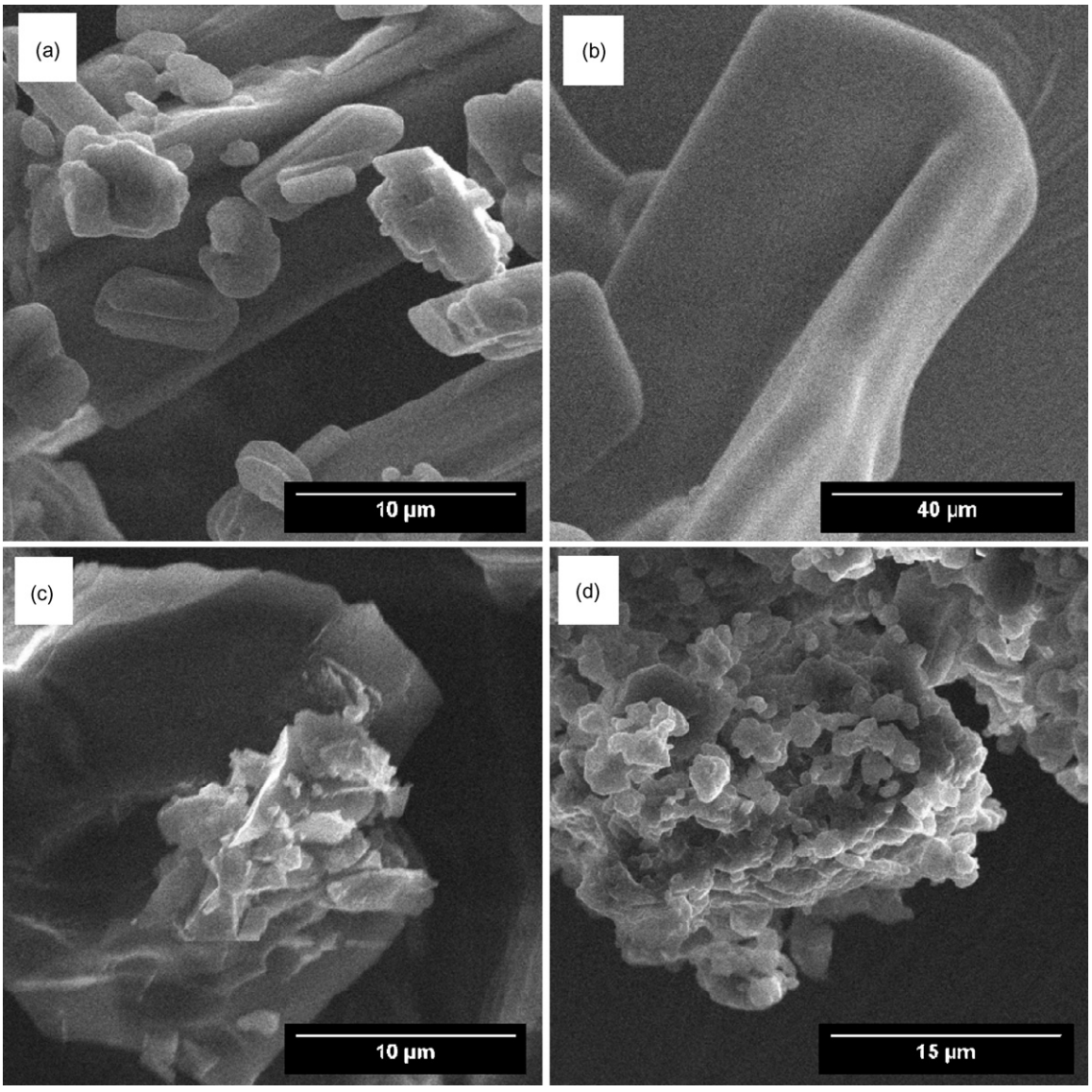

Fig. 7. Scanning electron micrographs of (a) stavudine form I, (b) the amorphous solid, (c) the amorphous solid after exposure to 30 °C, 43% RH for 5 days and (d) the crystalline raw material containing 20% (w/w) amorphous solid after exposure to 30 °C, 43% RH for 5 days.

al., 1998). However, although the partially dehydrated sample was rehydrated when exposed to an increase in RH (Fig. 5a), rehydration was completed at a much higher RH (80–90%) compared to the RH_c of 40% for the amorphous form.

The effect of moisture on the amorphous-to-hydrate transformation was also observed by ESEM and hot-stage microscopy (HSM), Fig. 6. In the ESEM when exposed to an increase in temperature while the RH was maintained >75%, it was observed that above the T_g, ~50 °C, the amorphous solid seems to dissolve in the condensed moisture from which form III crystallized between ~100 and 125 °C as an aggregated crystal, Fig. 6c and d. This observation corresponded with that seen by HSM.

3.4. Effect of increasing amorphous content on transformation to the hydrate

To further explore the effect that the amorphous-to-crystalline transformation could have on the stability and morphology of stavudine powder, increasing amounts of amorphous material, up to 20%, was intimately mixed with form II, and then stored at 30 °C; 43% RH for 5 days. The samples were studied using XRPD and SEM analyses. The amorphous material was mixed with form II because of its tendency to change to this form in a moisture free environment as shown in Fig. 4. XRPD analysis showed the appearance of the unique diffraction peak of form III at 15.5° 2θ in the diffraction pattern of the mixture containing 20% (w/w) amorphous solid. This indicated that even in the presence of form II, the amorphous solid transformed to the hydrated form when exposed to low moisture levels. In this study, form III was not detected in the samples containing 5 and 10% (w/w) amorphous solid, most probably due to the lack of sensitivity of the XRPD analysis.

These results show that there is an increase in the moisture content of non-hydroscopic crystalline stavudine depending on the amorphous content. For example if a 40 mg stavudine capsule contained 10% amorphous material which transformed to form III the moisture content of the capsule would increase by 0.1 mg or 0.25%. Similarly, if the amorphous content of the capsule were 40% the moisture content would increase by 0.4 mg or 1%. This can adversely change the content uniformity of stavudine capsules. Amorphous solids are generally less dense than its crystalline counterpart, having more "free" volume into which water vapor might be able to penetrate into more easily (Alneck and Zografi, 1990).

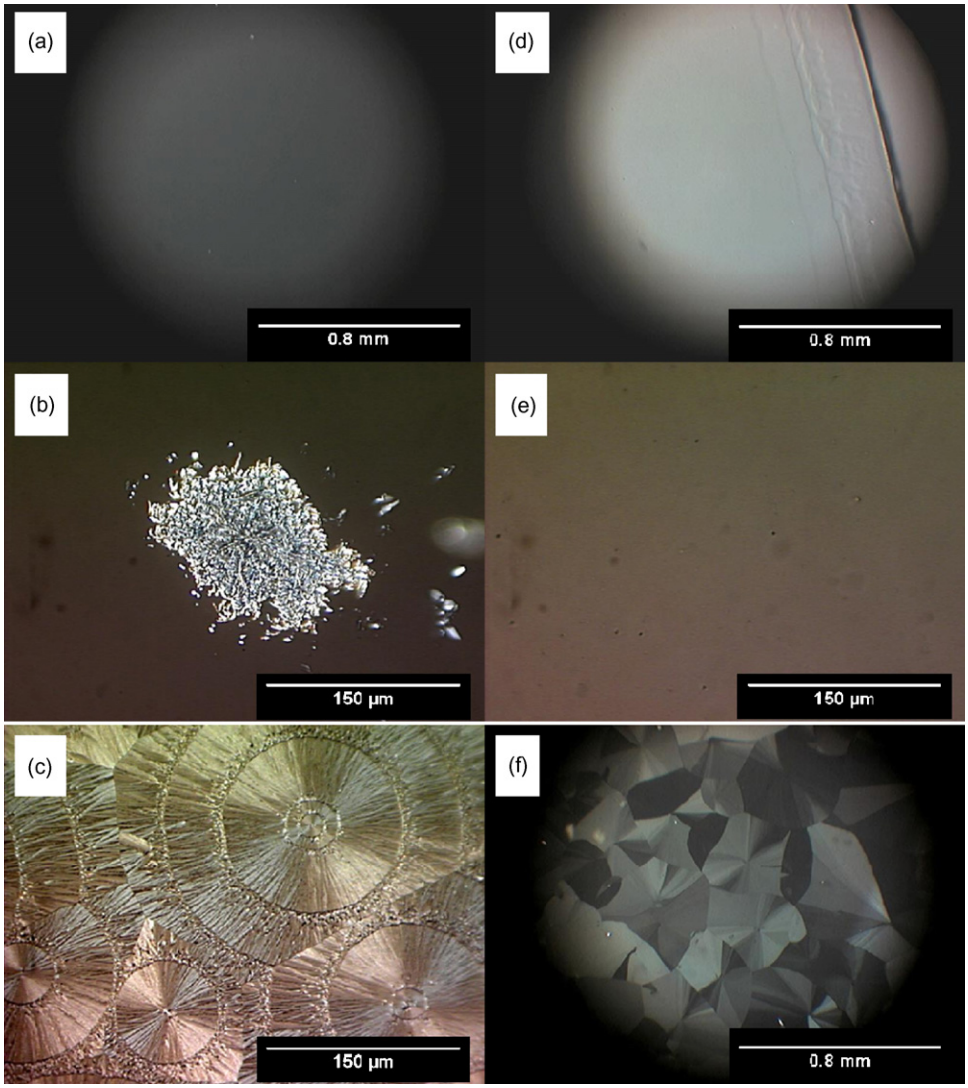

Fig. 8. The difference in crystal growth observed for uncoated amorphous samples: (a) initial, (b) stored at 50 °C under vacuum and (c) stored at 30 °C and 32% RH. Photomicrographs of PMMA coated samples: (d) spin coated PMMA with no drug exposed to 30 °C and 32% RH, (e) amorphous drug spin coated with PMMA stored at 50 °C under vacuum and (f) the same sample stored at 30 °C and 32% RH.

Thus assuming that the moisture is concentrated in the amorphous regions then the localized moisture content estimated from the DVS results would be around 2.5% (w/w). This is important since stavudine is soluble in water (80–100 mg/ml at 20–30 °C) and when the amorphous drug dissolves in the adsorbed water as shown by ESEM analysis, Fig. 6, this could lead to chemical decomposition (Mirmehrabi et al., 2006b). Dunge et al. (2005) reported the hydrolysis of stavudine in water at 80 °C and based on their findings the $t_{0.5}$ at this temperature is 30 min and $t_{0.9}$ only 5 min. Connors et al. (1986) reported that the activation energy for the hydrolysis of organic drug molecules is 19–20 kcal/mol and based on this estimation and the rate constant, k_1, at 80 °C, the $t_{0.5}$ and $t_{0.9}$ of an aqueous stavudine solution at room temperature, 25 °C, would be around 4 days and 14 h, respectively. These values corresponded to those reported by Kaul et al. (2005) for the hydrolysis of stavudine in an acid solution. This prediction show how unstable this drug is in water, a problem that could adversely affect the chemical stability of powders that contain amorphous stavudine.

Table 1
Crystal growth rates of amorphous samples, not coated with PMMA, that were exposed to various temperatures and humidities.

Storage conditions		Crystal growth rate, υ (m s^{-1})
Temperature (°C)	RH	
30	0	8×10^{-11}
30	32	2×10^{-9}
30	43	3×10^{-9}
40	0	9×10^{-10}
50	0	3×10^{-9}

3.5. *Effect of the amorphous-to-hydrate transformation on particle morphology*

SEM photomicrographs of form II, the amorphous solid, and the amorphous solid mixed with form II and stored at 30 °C; 43% RH for 5 days are shown in Fig. 7. Form I, form II, and form III of stavudine are rodlike, but depending on the operating conditions of the crystallization, the aspect ratios are different (Mirmehrabi et al., 2006a). These thin rods exhibited poor flow, Carr's index 24% and Hausner ratio 1.32, because of their shape and electrostatic charge (Mohammed et al., 2008). The form II used in this study was also rod like, separated particles, Fig. 7a, while the amorphous form did not have a distinct morphology, Fig. 7b. Fig. 9c and d shows the effect of moisture on the amorphous solid and the mixture.

The crystallization of form III from the amorphous solid produces crystal aggregates (see also Fig. 6). In the mixture, this aggregation leads to the formation of larger particles. It was difficult to distinguish forms II and III from one another in these crystal aggregates. The particles of form II alone stayed separated when exposed to moisture. A similar observation was made when amorphous lactose was exposed to increasing temperatures below its melting point (Takeuchi et al., 2000). This aggregation increases particle size, and with the increased moisture content of the raw material, this could adversely affect the powder flow properties, particle size analysis, and compression of the raw material (Dawoodbhai and Rhodes, 1989; Tan and Newton, 1990; De Villiers et al., 1993; De Villiers, 1995). In addition, since stavudine is formulated as either capsules or dry powder for suspension (USP, 2007) substantial changes in the physicochemical properties of the raw material could adversely affect the content uniformity of these dosage forms.

3.6. The crystal growth rate of the amorphous solid

Crystal growth rate analysis is a useful tool for determining the influence of temperature and humidity on amorphous solid transformations (Wu and Yu, 2006; Konno and Taylor, 2008). The amorphous-to-crystalline transformation for stavudine samples prepared by spin-coating can be studied under a polarizing microscope as shown in Fig. 8. Initially no growth is observed, Fig. 8a. The morphology of the crystals growing on the surface of amorphous stavudine exposed to moisture was different from those growing on samples stored under vacuum. This again showed that in the presence of moisture the amorphous-crystalline transformation favors the formation of form III, the hydrate (Fig. 8c), but when kept in a moisture free environment form II dominates (Fig. 8b). At 40 °C and 75% RH the amorphous-to-hydrate conversion was complete within 1 h.

In this study the steady state growth rate for amorphous samples stored at increased temperatures in the presence and absence of humidity, and of PMMA coated and uncoated amorphous samples was determined as described by Wu and Yu (2006). The results are shown in Fig. 9a–c and Table 1. Plots of crystal radius versus time, Fig. 9a, were linear: 30 °C, 0% RH, $r^2 = 0.9311$; 30 °C, 32% RH, $r^2 = 0.9979$; 30 °C, 43% RH, $r^2 = 0.9988$; 40 °C, 0% RH, $r^2 = 0.9721$; 50 °C; 0% RH, $r^2 = 0.9950$. Therefore, the slopes of the lines were used to calculate steady state growth rates, υ (m s^{-1}), listed in Table 1.

Growth rates increased linearly with an increase in temperature, and this increase was about 1 order of magnitude for each 10 °C increase in temperature. An Arrhenius plot of the growth rates vs. temperature (Fig. 9b) was linear ($r^2 = 0.9979$), and made possible the calculation of the activation energy: $Ea = 147.59$ kJ mol^{-1} and $\ln A = 35.38$. This activation energy is within the expected range (Byrn et al., 1999). However, a plot of growth rate as a function of increased RH at 30 °C, showed that moisture, even at relative humidities <43%, increased the growth rate by approximately two orders of magnitude compared to the growth rate at the same temperature in a dry environment. Exponential fitting of this curve achieved a greater correlation coefficient ($r^2 = 0.9980$) compared to linear fitting ($r^2 = 0.9620$) indicating that the growth rate increased exponentially with an increase in humidity. The increased growth rate caused by temperature alone approaches the rate in the humid environments, only when the temperature is increased to 50 °C. This indicates that relatively low levels of moisture increased the rate of transition from the amorphous to crystalline solid significantly more, compared to a dry environment, and increased temperature.

3.7. Stabilizing amorphous stavudine

Previous observations showed the enhanced surface crystallization of amorphous indomethacin (Wu and Yu, 2006) and nifedipine

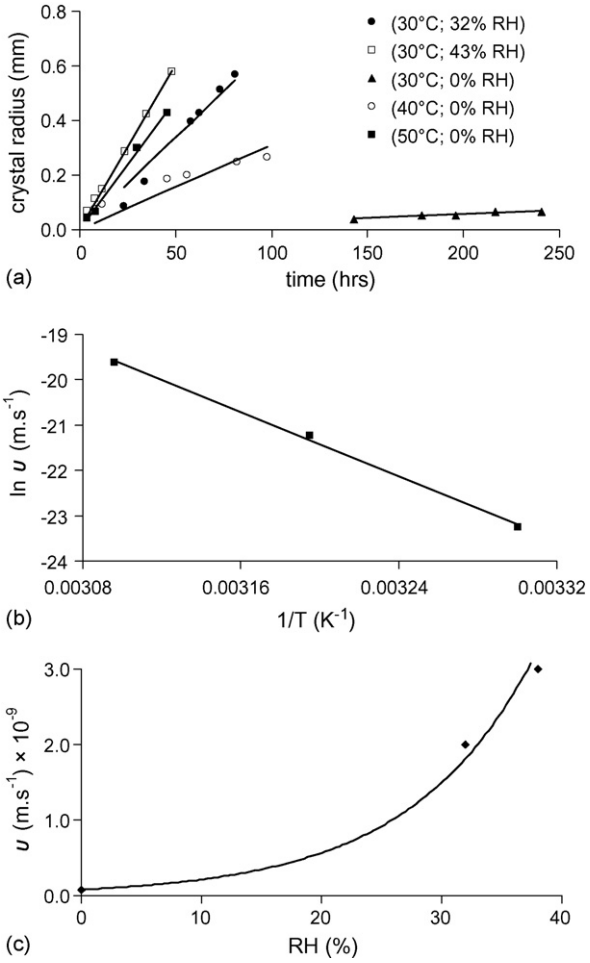

Fig. 9. (a) A plot of the crystal radius vs. time of uncoated amorphous stavudine showing the difference in growth rates (slopes) between samples stored in the absence and presence of moisture. (b) An Arrhenius plot of the natural logarithm of the crystal growth rate vs. the reciprocal of temperature for the samples stored in a dry environment. (c) Plot of crystal growth rate vs. relative humidity for samples that were stored at increasing RH.

(Zhu et al., 2008), and surface crystallization can be inhibited by polymer coatings using a unique process of layer-by-layer self-assembling (Wu et al., 2007). Based on these observations, an attempt was made to stabilize spin coated amorphous stavudine films by coating it with a polymer. Stavudine is highly soluble in water. This meant that the polymer, PMMA, used in the coating process had to be dissolved in toluene, an organic solvent in which the drug is not soluble. PMMA coats, with an approximate thickness less than 1 μm, were applied to the amorphous films by spin-coating. No crystal growth, Fig. 8d, was detected in thin films of the polymer exposed to moisture. In addition, no crystal growth was seen for the duration of this investigation for the PMMA coated samples that were exposed to 30, 40 and 50 °C and no moisture, Fig. 8e. The PMMA coating therefore stabilized the amorphous film in the absence of moisture. These results also indicated that crystallization of amorphous stavudine in the absence of moisture occurs at a greater extent at the surface and that bulk crystallization did not contribute to the crystal growth observed for these samples (Wu and Yu, 2006; Wu et al., 2007).

However, the growth rate of the PMMA coated samples that were exposed to moisture followed the same trend as the uncoated samples that were kept in humid environments. For example at 40 °C and 75% RH, crystallization was complete within 2 h. The PMMA coating did not stabilize the amorphous film against moisture,

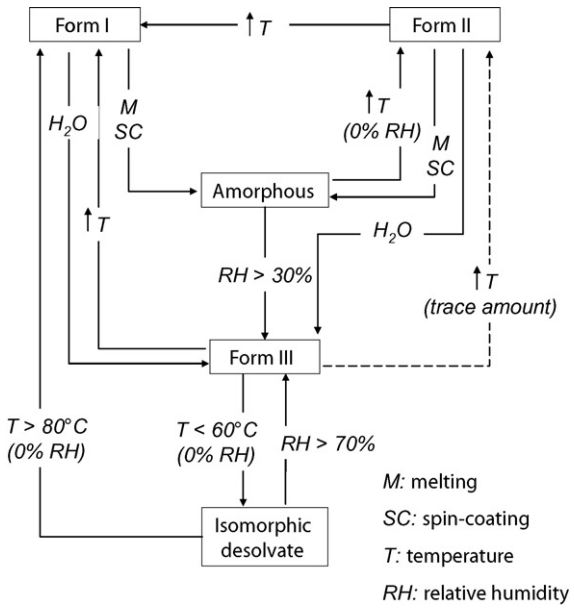

Fig. 10. A schematic representation that shows the interrelationship between the amorphous form and the solid state stability of stavudine crystal forms.

Fig. 8f, because spherulite crystal growth similar to that observed for uncoated samples, Fig. 8c, was observed. At 30 °C and 32% RH crystallization was completed within 110–120 h and at 30 °C and 43% RH with 90–100 h. This might be explained by the interactions between stavudine, PMMA and water. The stabilization of amorphous solids or films by polymers depends on the strength of the interactions between the amorphous material and the polymer (Konno and Taylor, 2006). These interactions can be either hydrogen bonding, ionic or van der Waals interactions (Konno and Taylor, 2006; Wu et al., 2007). It is possible that the interactions between PMMA and stavudine are weaker than between water and stavudine. As a result, stavudine molecules interact with water rather than with PMMA, leading to crystal growth in the presence of water. It is also possible that the PMMA coating is too porous to prevent the diffusion of water to the amorphous film, which would result in crystallization of the hydrate.

4. Conclusion

The polymorphic behavior and characterization of stavudine has been well studied and described in the literature. This information, as well as what has been determined during this study, is schematically represented in Fig. 10. It has been shown by Gandhi et al. (2000) that form II can be transformed to form I, and that form III (hydrate) transforms to the stable form I (anhydrous) above 80 °C. It was also shown by these authors, and it was confirmed during this study as well, that form III can dehydrate in a dry environment, without a change in the crystal structure (as confirmed by X-ray powder diffraction analysis). This thus points to the formation of an isomorphic desolvate from form III.

During this study an amorphous form of stavudine was prepared from both forms I and II using the spin-coating and melt methods. It was also shown that there is a moisture dependant amorphous-to-crystalline transformation that is possible for this solid state. In the presence of a relative humidity of greater than 30%, the amorphous form transforms to the hydrate of stavudine, whereas during exposure to only temperature, it transforms to form II (anhydrous). This is a significant observation, since it implies that the presence of traces of amorphous stavudine in commercial products can lead to possible instability due to the formation of the hydrate of stavudine, which could lead to increased degradation by hydrolysis. In the absence of moisture, the amorphous-to-crystalline transformation was inhibited by the application of a polymeric coating to the amorphous form, consisting of PMMA. However this coating could not prevent the crystallization of the amorphous form in a humid environment.

It is thus imperative to prevent the possible accidental formation of amorphous stavudine during the various stages of large-scale manufacturing, as this could adversely affect the shelf life of the dosage form. It can also possibly explain the variability seen in the pharmacokinetic parameters of generic products that limit the treatment efficiency of HIV infections.

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References

Alneck, C., Zografi, G., 1990. The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state. Int. J. Pharm. 62, 87–95.
Andronis, V., Zografi, G., 1997. Molecular mobility of supercooled amorphous indomethacin, determined by dynamic mechanical analysis. Pharm. Res. 14, 410–414.
Andronis, V., Zografi, G., 2000. Crystal nucleation and growth of indomethacin polymorphs from the amorphous state. J. Non-Cryst. Solids 271, 236–248.
Bauer-Brandl, A., 1996. Polymorphic transitions of cimetidine during manufacture of solid dosage forms. Int. J. Pharm. 140, 195–206.
Bhugra, C., Pikal, M.J., 2008. Role of thermodynamic, molecular, and kinetic factors in crystallization from the amorphous state. J. Pharm. Sci. 97, 1329–1349.
Bowen, A., Palasanthiran, P., Sohn, A.H., 2008. Global challenges in the development and delivery of paediatric antiretrovirals. Drug Disc. Today 13, 530–535.
Byakika-Kibwika, P., Lamorde, M., Kalemeera, F., D'Avolio, A., Mauro, S., Di Perri, G., Ryan, M., Mayanja-Kizza, H., Khoo, S., Back, D., Boffito, M., Merry, C., 2008. Steady-state pharmacokinetic comparison of generic and branded formulations of stavudine, lamivudine and nevirapine in HIV-infected Ugandan adults. J. Antmicrob. Chemother. 62, 1113–1117.
Burnett, D.J., Thielmann, F., Booth, J., 2004. Determining the critical relative humidity for moisture-induced phase transitions. Int. J. Pharm. 287, 123–133.
Byrn, S.R., Lin, C.T., Perrier, P., Clay, G.G., Sutton, P.A., 1982. Solid state photooxidation of 21-cortisol tert-butylacetate to 21-cortisone. J. Org. Chem. 47, 2978–2981.
Byrn, S.R., Pfeiffer, R.R., Stowel, J.G., 1999. Solid-State Chemistry of Drugs, 2nd ed. SSCI Inc., West Lafayette, IN, pp. 461–460.
Chien, C.V., 2007. HIV/AIDS drugs for sub-Saharan Africa: how do brand and generic supply compare? PLoS ONE 2, e278.
Chieng, N., Rades, T., Saville, D., 2008. Formation and physical stability of the amorphous phase of ranitidine hydrochloride polymorphs prepared by cryo-milling. Eur. J. Pharm. Biopharm. 68, 771–780.
Connors, K.A., Amidon, G.L., Stella, V.J., 1986. Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists, 2nd ed. Wiley Interscience, New York.
Craig, D.Q.M., Royall, P.G., Kett, V.L., Hopton, M.L., 1999. The relevance of amorphous state to pharmaceutical dosage forms: glassy drugs and freeze dried systems. Int. J. Pharm. 179, 179–207.
Dawoodbhai, S., Rhodes, C.T., 1989. The effect of moisture on powder flow and on compaction and physical stability of tablets. Drug Dev. Ind. Pharm. 15, 1577–1600.
De Villiers, M.M., 1995. Influence of the cohesive behaviour of micronized drug powders on particle size analysis. J. Pharm. Biomed. Anal. 13, 191–199.
De Villiers, M.M., Terblanche, R.J., Liebenberg, W., Swanepoel, E., Dekker, T.G., Song, M., 2005. Variable temperature X-ray powder diffraction analysis of the crystal transformation of the pharmaceutically preferred polymorph C of mebendazole. J. Pharm. Biomed. Anal. 38, 435–441.
De Villiers, M.M., Van Der Watt, J.G., Lötter, A.P., 1991. The interconversion of the polymorphic forms of chloramphenicol palmitate (CAP) as a function of temperature. Drug Dev. Ind. Pharm. 17, 1295–1303.
De Villiers, M.M., Van Der Watt, J.G., Lötter, A.P., 1993. Influence of surfactants and interactive mixing on the cohesive properties of a poorly wettable solid. Powder Technol. 75, 159–165.
Dunge, A., Sharda, N., Singh, B., Singh, S., 2005. Establishment of inherent stability of stavudine and development of a validated stability-indicating HPLC assay method. J. Pharm. Biomed. Anal. 37, 1115–1119.
Duse, A.G., Morar, A., Landman, I., Vermaak, W.J.H., Schoeman, H., Kruger, M.J., Janse van Rensburg, E., Luthy, R., Singh, S., 2008. Short-term effectiveness and safety of

HAART in the form of a generic fixed-dose combination of stavudine, lamivudine and nevirapine (Triviro) in HIV-1-infected adults in Zimbabwe. S Afr. J. HIV Med. 9, 51–56.
Fukuoka, E., Midori, M., Shigeo, M., 1986. Some physicochemical properties of glassy indomethacin. Chem. Pharm. Bull. 34, 4314–4321.
Gandhi, R.B., Bogardus, J.B., Bugay, D.E., Perrone, R.K., Kaplan, M.A., 2000. Pharmaceutical relationships of three solid state forms of stavudine. Int. J. Pharm. 201, 221–237.
Guo, Y., Byrn, S.R., Zografi, G., 2000. Physical characteristics and chemical degradation of amorphous quinapril hydrochloride. J. Pharm. Sci. 89, 128–143.
Hancock, B.C., Parks, M., 2000. What is the true solubility advantage for amorphous pharmaceuticals? Pharm. Res. 17, 397–404.
Hancock, B.C., Zografi, G., 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. J. Pharm. Sci. 86, 1–12.
Hosseinipour, M.C., Corbett, A.H., Kanyama, C., Mshali, I., Phakati, S., Rezk, N.L., Van der Horst, C., Kashuba, A.D.M., 2007. Pharmacokinetic comparison of generic and trade formulations of lamivudine, stavudine and nevirapine in HIV-infected Malawian adults. AIDS 21, 59–64.
Kaul, N., Agrawal, H., Paradkar, A.R., Mahadik, K.R., 2005. The ICH guidance in practice: stress degradation studies on stavudine and development of a validated specific stability-indicating HPTLC assay method. J. Chromatogr. Sci. 43, 406–415.
Kawaguchi, T., Fukushima, S., Ohmura, M., Mishima, M., Nakano, M., 1989. Enzymatic and chemical stability of 2′,3′-dideoxy-2′,3′-didehydropyrimidine nucleosides: potential anti-acquired immunodeficiency syndrome agents. Chem. Pharm. Bull. 37, 1944–1945.
Konno, H., Taylor, L.S., 2006. Influence of different polymers on the crystallization tendency of molecularly dispersed amorphous felodipine. J. Pharm. Sci. 95, 2692–2705.
Konno, H., Taylor, L.S., 2008. Ability of different polymers to inhibit the crystallization of amorphous felodipine in the presence of moisture. Pharm. Res. 25, 969–978.
L'homme, R.F.A., Dijkema, T., Warris, A., Van der Ven, A.J.A.M., Gibb, D.M., Burger, D.M., 2007. Pharmacokinetics of two generic fixed-dose combinations for HIV-infected children (Pedimune Baby & Pedimune Junior) are similar to the branded products in healthy adults. J. Antimicrob. Chemother. 59, 92–96.
Laurent, C., Kouanfack, C., Koulla-Shiro, S., Nkoue, N., Bourgeois, A., Calmy, A., Lactuock, B., Nzeusseu, V., Mougnutou, R., Peytavin, G., Liegeois, F., Nerrienet, E., Tardy, M., Peeters, M., Andrieux-Meyer, I., Zekeng, L., Kazatchkine, M., Mpoudi-Ngole, E., Delaporte, E., 2004. Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1-infected adults in Cameroon: open-label multicenter trial. Lancet 364, 29–34.
Laurent, C., Kouanfack, C., Koulla-Shiro, S., Njoume, M., Nkene, Y.M., Ciaffi, L., Brulet, C., Peytavin, G., Vergne, L., Calmy, A., Mpoudi-Ngole, E., Delaporte, E., 2007. Long-term safety, effectiveness and quality of a generic fixed-dose combination of nevirapine, stavudine and lamivudine. AIDS 21, 768–771.
Mirmehrabi, M., Rohani, S., Murthy, K.S.K., Radatus, B., 2006a. Polymorphic behavior and crystal habit of an anti-viral/HIV drug: stavudine. Cryst. Growth Des. 6, 141–149.
Mirmehrabi, M., Rohani, S., Perry, L., 2006b. Thermodynamic modeling of activity coefficient and prediction of solubility: part 2. Semipredictive or semiempirical models. J. Pharm. Sci. 95, 798–809.
Mohammed, G.A., Puri, V., Bansal, A.K., 2008. Coprocessing of nevirapine and stavudine by spray drying. Pharm. Dev. Technol. 13, 210–299.
Marcelin, A., Jarrousse, B., Derache, A., Ba, M., Dakouo, M., Doumbia, A., Haidara, I., Maiga, A., Carcelain, G., Peytavin, G., Katlama, C., Calvez, V., 2007. HIV drug resistance after the use of generic fixed-dose combination stavudine/lamivudine/nevirapine as standard first-line regimen. AIDS 21, 2341–2343.
Penzak, S.R., Acosta, E.P., Turner, M., Tavel, J.A., Masur, H., 2003. Analysis of generic nevirapine products in developing countries. JAMA 289, 2648–2649.
Penzak, S.R., Acosta, E.P., Turner, M., Tavel, J.A., Masur, H., 2004. Antiretroviral drug content in products from developing countries. Clin. Infect. Dis. 38, 1317–1319.
Petit, S., Coquerel, G., 2006. The amorphous state. In: Hilfiker, R. (Ed.), Polymorphism in the Pharmaceutical Industry. Wiley-VCH, Weinheim, pp. 259–285.
Radatus, B.K., Murthy, K.S.K., 2003. Process for the preparation of substantially pure stavudine and related intermediates useful in the preparation thereof. US Patent 6,635,753.
Stephenson, G.A., Groleau, E.G., Kleemann, R.L., Xu, W., Rigsbee, D.R., 1998. Formation of isomorphic desolvates: creating a molecular vacuum. J. Pharm. Sci. 87, 536–542.
Takeuchi, H., Yasuji, T., Yamamoto, H., Kawashima, Y., 2000. Temperature- and moisture-induced crystallization of amorphous lactose in composite particles with sodium alginate prepared by spray-drying. Pharm. Dev. Technol. 5, 355–363.
Tan, S.B., Newton, M.B., 1990. Powder flowability as an indication of capsule filling performance. Int. J. Pharm. 61, 145–155.
The United States Pharmacopeia, 2007. The United States Pharmacopeial Convention. The United States Pharmacopeia, Rockville, MD.
Wu, T., Yu, L., 2006. Surface crystallization of indomethacin below T_g. Pharm. Res. 23, 2350–2355.
Wu, T., Sun, Y., Li, N., De Villiers, M.M., Yu, L., 2007. Inhibiting surface crystallization of amorphous indomethacin by nanocoating. Langmuir 23, 5148–5153.
Yu, L., 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. Adv. Drug Deliv. Rev. 48, 27–42.
Zhu, L., Wong, L., Yu, L., 2008. Surface-enhanced crystallization of amorphous nifedipine. Mol. Pharm. 5, 921–926.